Out of the Fog - Applying biomolecular methods to explore the south Scandinavian Stone Age

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Front Cover: Japetus Steenstrup & Frants Jacobsen in their teens walking through the Flommen meadows at Sorø, carrying tennis rackets. Painted in 1840-45 by Hans Georg Harder. Currently exhibited at Sorø Kunstmuseum.

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“*Everything from the earliest heathen period hangs before us as if in a thick fog. In an unmeasurable period of time...we know it is older than Christendom, but if by few years or a few hundred years - even maybe over a thousand years - older, is sheer guesswork and at best only likely hypotheses*' (Nyerup 1806:1, translation by Rowley-Conwy 2004)

English Abstract

The present thesis is composed of three projects, which share the theme of using minimally destructive state-of-the-art scientific methods to study objects from the Early Mesolithic and the transition to the Neolithic in Southern Scandinavia.

Firstly, Zooarchaeology by Mass Spectrometry (ZooMS), protein sequencing by liquid chromatography tandem mass spectrometry (LC-MS/MS) and direct accelerator mass spectrometry (AMS) radiocarbon dating were used to interrogate barbed bone points. The results reveal that these iconic artefacts can be used as proxies for species utilisation and availability. Significantly, a radiocarbon hiatus at 10.2 ka was discovered which implies a depeopling event occurred.

The second project focuses on very rare osseous finger ring. Direct dating of 70 proximal finds from the site places the ring at the cusp of the Neolithic. ZooMS and LC-MS/MS revealed that the ring is made from red deer (*Cervus elaphus*), and the generated proteome may give an indication of the skeletal element from which it was made.

The last study utilises a novel finds category (masticated birch pitch) as a new source of ancient human DNA. Shotgun sequencing generated an entire human genome (2.3x) of the person who chewed the pitch. Interesting, she showed genetic affinity to Western Hunter-Gatherers and not Neolithic farmers. Additionally, metagenomics allowed the investigation of her oral microbiome, latent viral diseases and diet.

Dansk Abstract

Følgende afhandling er bygget op omkring tre projekter. Et fællestræk for dem alle er, at minimal prøvetaging af arkæologisk materiale bliver kombineret med state-of-the-art metoder for at besvare spørgsmål om henholdsvis tidlig Mesolitikum, samt overgangen til Neolitikum i Sydskandinavien. Det første projekt bruger Zooarchaeology by Mass Spectrometry (ZooMS), protein sekventering vha. væskekromatografi med tandem massespektrometri (LC-MS/MS) og accelerator masse spektrometri (AMS) kulstof 14-datering til at undersøge tandede benspidser. Resultatet har afsløret at disse ikoniske genstande kan bruges som proxy, og viser en forhistorisk udvælgelse af specifikke arter samt forekomsten af disse i forskellige perioder. En væsentlig overraskelse blev observeret i dateringerne, der viste en hiatus omkring 10.2 ka og varende 600 år. Noget der måske vidner om en affolknings situation.

Det andet projekt fokuserer på en sjælden ossøs finger ring. Datering af 70 genstande rundt omkring findestedet, placerer ringen ved overgangen eller i starten af Tidlig Neolitikum. ZooMS og LC-MS/ MS afslørede at den var lavet af kronhjort (*Cervus elaphus*), og det efterfølgende genererede proteom gav mulige indikationer på hvilket skeletelement den var lavet af.

Det sidste studie undersøgte mulighederne for at sekventere gammelt DNA fra et stykke tygget birke beg, der var dateret til Tidlig Neolitikum. Shotgun sekventering gjorde det muligt at generere et komplet menneske genom (2.3x) af den kvinde, som havde tygget begen. Af signifikant betydning var, at hun viste genetisk tilhørsforhold til gruppen af Vestlige Jærger-Samlere og altså ikke Neolitiske bønder. Desuden, var det muligt med metagenomisk undersøgelse at kortlægge hendes orale mikrobiom, latente virale sygdomme, samt indikationer på fødeindtag.

Photos in thesis

Figures in thesis

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Fig. 2: Excavation at Mullerup in 1900. George F. L Sarauw sitting on a wooden box overseeing the excavation, probably done by locals (Negative1240: Mullerup, Maglemose 1900. Drøsselbjerg Parish, Løve Shire, Holbæk Amt.

Fig. 3: Excavation photo from Sværdborg mose in 1917-18 (Negative5130: Sværdborg bog. Sværdborg parish, Hammer Shire. 1917-18. Cultural layer in trench IC2).)

Statement of Authorship

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, Universities. All sources are acknowledged as References.

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Of my colleagues, I would like to send out a massive thank you to especially Alberto Taurozzi and Liam Lanigan (both Uni. Copenhagen), for helping out with everything from proofreading manuscripts to events on tasting fermented *Humulus lupulus* beverages. I would also like to thank Meaghan Mackie, for her tireless assistance on interpreting protein data, the same goes for Kristine Kurzow Richter (Uni. York and Max Planck Institute, Jena). Of friends, a big thanks are due to Søren Anker Sørensen (Museum Lolland-Falster) for accepting hour long phone calls, for sharing knowledge about the very old past and for providing access to material analysed in this thesis. Arne Sjöström (Uni. Lund) if it weren't for all your excavations in Rönneholms Mosse (starting in 2009), then I wouldn't be where I am today - thank you! You are also thanked for providing food and shelter whenever I visit you, for sharing your immense knowledge about peat bogs and Mesolithic archaeology and letting me sample all the bone points you have found. Anders Fischer (Kalundborg), I am incredibly grateful that you saw some talent in me, that you invited to the SPLASH-COS project - this was a big eye-opener. You are also thanked for taking me on recognisance at Tybrind Vig, to Øresø Mølle, for sharing your knowledge about the Stone Age as well a lot of hitherto unpublished information. Lastly, also for sending me on excavation at Havnø where I met two people, I today call my friends. Harry Robson (Uni. York) and Kurt Gron (Uni. Durham), you are both incredible human beings, and have been of significant importance to my academic career, as well as a source to hangovers. Mads Lou Bendtsen (The National Museum of Denmark) is thanked for help with layout and good discussions about archaeology over over-fermented beverages. Svein V. Nielsen (Uni. Oslo) is also thanked for great discussions and assistance with 14C dates.

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Authorship contributions

Everything apart from the manuscripts (Chapters 3-5), was written solely by Theis Zetner Trolle Jensen.

Chapter 3: Barbed bone point chronology reveals a radiocarbon hiatus at 10.2 ka during the Early Mesolithic in southern Scandinavia.

Theis Zetner Trolle Jensen, Arne Sjöström, Anders Fischer, Erika Rosengren, Liam Thomas Lanigan, Ole Bennike, Kristine Kurzow Richter, Kurt J. Gron, Meaghan Mackie, Morten Fischer Mortensen, Katrine Højholt Iversen, Alberto John Taurozzi, Jesper Olsen, Hannes Schroeder, Nicky Milner, Mikkel Sørensen, Matthew James Collins

TZTJ conceived the study. TZTJ collected and prepared samples. TZTJ and JO interpreted the radiocarbon data. TZTJ, KKR, MM, AJT and LTL, analysed samples for MALDI-TOF-MS and LC-MS/MS. KG interpreted the isotope data. AF and MFM provided unpublished radiocarbon dates. TZTJ wrote the manuscript with input from AS, AF, ER, TL, OB, KG, MS, MJC and the remaining authors.

Chapter 4: The biomolecular characterisation of a finger ring contextually dated to the emergence of the Early Neolithic from Syltholm, Denmark

Theis Zetner Trolle Jensen, Meaghan Mackie, Alberto John Taurozz, Liam Thomas Lanigan, Carsten Gundelach, Jesper Olsen, Søren Anker Sørensen, Matthew James Collins, Mikkel Sørensen, Hannes Schroeder

TZTJ and SAS initiated the study. SAS provided samples and contextual information. CG and TZTJ conducted the Micro CT scan and interpretations. Radiocarbon dates were produced and interpreted by JO and TZTJ. TZTJ performed the ZooMS experiments. TZTJ, MM, AJT, LTL and HS collected, prepared, and ran samples for MALDI-TOF-MS and LC-MS/MS. TZTJ, MM, AJT, and LTL interpreted data and wrote the paper, under the supervision of HS, MJC, and MS. All authors reviewed the manuscript.

Chapter 5: A 5700 year-old human genome and oral microbiome from chewed birch pitch

Theis Z. T. Jensen, Jonas Niemann, Katrine Højholt Iversen, Anna K. Fotakis, Shyam Gopalakrishnan, Åshild J. Vågene, Mikkel Winther Pedersen, Mikkel-Holger S. Sinding, Martin R. Ellegaard, Morten E. Allentoft, Liam T. Lanigan, Alberto J. Taurozzi, Sofie Holtsmark Nielsen, Michael W. Dee, Martin N. Mortensen, Mads C. Christensen, Søren A. Sørensen, Matthew J. Collins, M. Thomas P. Gilbert, Martin Sikora, Simon Rasmussen, Hannes Schroeder

TZTJ and HS designed and led the study. SAS provided the sample for analysis. MCC and MNM performed the FTIR and GC-MS analyses. MWD performed the radiocarbon dating. TZTJ, MHSS and MRE generated the genetic data. TZTJ, JN, KHI, AKF, SG, AJV, MWP, SHN, MEA and HS analysed the genetic data. TZTJ, JN, KHI, AKF, SG, AJV, MWP, MEA, LTL, AJT, MJC, MTPG, MS, SR and HS interpreted the results. TZTJ and HS wrote the manuscript with input from JN, KHI, and the remaining authors.

Fig. 1. Sites mentioned in the thesis (not including individual papers). 1: Syltholm, 2: Rönneholms mosse, 3: Ertebølle, 4: Mullerup, 5: Bare Mosse, 6: Sværdborg, 7: Bloksbjerg, 8: Kongemosen, 9: Blak, 10: Holmegaard mose, 11: Klosterlund, 12: Barmose, 13: Skottemarke, 14: Ulkestrup Lyng, 15: Koelbjerg, 16: Hammelev, 17: Ageröd, 18: Nørre Lyngby, 19: Flaadet, 20: Fredbogård, 21: Lundby mose, 22: Haväng, 23: Pilhaken. 24: Fogense Enge. 25: Lollikhuse. 26: Dværgebakke. 27: Ringsjöholm.

1.1 Aims and objectives

Modern peat bog excavations are relatively rare and most commonly ‘rescue’ in nature. Those that have been previously conducted have yielded considerable archaeological material, informing us on topics such as chronology, typology and vegetation history. Whilst the Stone Age in Scandinavia is considered well studied this accumulation of knowledge has come at a cost. Early radiocarbon dating was highly destructive, didn’t take into account modern contaminants e.g. humic acids and produced ‘broad dates’. Later, various stable isotope measurements also proved to be quite damaging. More recently, DNA studies, have necessitated the removal and destruction of whole teeth and/or all petrous bones. This problem has resulted in deteriorated skeletal material from an already very finite source - something that is very deleterious to future research. The present thesis, therefore, attempts to leverage technological and analytical advances to maximise data acquisition from rare south Scandinavian material in a minimally destructive manner. Attempts were made to maintain the exhibition potential of the archaeological artefact or object, by using minimal starting material. Specifically, this information was used to investigate; animal exploitation for artefact production, chronology of the Maglemose period, and population genetics of the Early Neolithic.

Three studies and the research questions

The first innovative study (Chapter 3) focuses on Maglemose bone points from Denmark and southern Sweden. The motivation for the work comes from the author's own work at archaeological bog excavations in primarily Sweden. Since it is impossible to identify bone points to species by visual observations, other methods needed to be used. Also, the absolute age of them has so far been guesswork.

The following questions form the basis of the study:

1) Can ZooMS (Zooarchaeology by Mass Spectrometry), be used to observe species selection for different types of bone points?
2) Can ZooMS analysis distinguish species that are genetically close; but represent fundamentally different environments and hunting strategies, such as red deer (*Cervus elaphus*) and elk (*Alces alces*)?
3) Is it possible to obtain dates of bone points by sampling minimal amounts (c. 100 mg) of bone (much less than standard studies)?
4) Can successful AMS dates obtained on bone points, aid in the construction of a more robust typochronology?

The second approach (Chapter 4) investigates how much information can be generated by using non-destructive X-ray microtomography and minimally destructive molecular methods on a rare finger ring from Syltholm (Fig. 1), Denmark. The motivation stems from the author's own participation in the excavation where the ring was found, as well as a sparsity of comparative material, which only constitutes a ring fragment and ring preform from the shell midden at Nederst in Jutland. These two artefacts are believed to be both derived from the tusks of wild boar. Therefore, the following questions are asked about the Syltholm ring:

1) Can MicroCT determine whether it is dental tissues or bone or antler?
2) Can AMS dates made on material found near the ring be used as an estimation for an indirect age of the ring?
3) Can minimal sample amount (10 mg) be used for ZooMS for species determination, with the remaining collagen being used for LC-MS MS protein sequencing in order to characterize the substrate proteome?

The third study (Chapter 5) examines the possibilities of new techniques on novel sources of potential human aDNA, in this case a chewed piece of birch pitch. The material is a piece of potentially chewed pitch and also comes from the Syltholm excavations. The motivation stems from the author's own participation in the excavation where the pitch was found, as well as the author's own hypothesis of the possible preservation of ancient DNA in chewed pitch. This idea was conceived in 2009 when just such a piece of masticated pitch was recovered from excavations at Rönneholms peat bog in Scania (Fig. 1), where the author was working at the time. In 2009, next generation sequencing was just becoming an established part of ancient molecules exploration. To the author, it seemed only logical that DNA from saliva would be trapped in this hydrophobic substance. For the following chapter, the following questions are asked:

1) Is it possible to extract, amplify, and sequence ancient DNA from chewed pitch. Can it act as an alternative to human bone, teeth, or dental calculus?
    a) Is it possible to determine genetic affiliation, sex, and phenotypic traits of the individual?
2) Can chewed pitch provide details of the oral microbiota, that due to their extraneous nature, should be different to the long-term reservoir of dental calculus?

Chapter 2: Introduction to the Stone Age of Southern Scandinavia and biomolecular archaeology

2.1 Introduction

Although the present thesis focuses on the utilisation of molecular methods to answer specific archaeological questions, the research chapters focus on different time periods within the Stone Age, primarily the Maglemose period, and to some degree on the Ertebølle/Funnelbeaker transition. At its core, this study is focused on combining modern molecular methods with the archaeological material from the most distant past of Denmark; and to a lesser degree, Southern Sweden. By first focusing on the lithic chronologies of the Maglemose period, later chapters are better contextualised. An overview of material culture and subsistence strategies of the Maglemose and Funnelbeaker cultures is provided, as well as, a brief introduction to the debate on the transition to the Neolithic. Additionally, a description of the Holocene climate is presented for the time period of 11 to 5.5 ka, as this is the time span which directly relates to the artefacts investigated in this thesis. The last part gives a general overview of biomolecular archaeology carried out in Southern Scandinavia.

2.2 On the history of research into the Stone Age of Southern Scandinavia and the emergence of chronologies and environmental archaeology

> "*Everything from the earliest heathen period hangs before us as if in a thick fog. In an unmeasurable period of time*"' (Nyerup 1806:1, translation by Rowley-Conwy 2004).

The above quote is by librarian Rasmus Nyerup, and relates to his thoughts on how to establish and order prechristian (hedenold) archaeological remains in a museum, thereafter, to be known as the Danish National Museum in Copenhagen. He wanted to put everything of "old" or "hedenold" into a separate room, but of the age he notes nothing is certain:

> "*...we know it is older than Christendom, but if by few years or a few hundred years - even maybe over a thousand years - older, is sheer guesswork and at best only likely hypotheses*' (Nyerup 1806:1, translation by Rowley-Conwy 2004).

The bombardment of Copenhagen by the English fleet in 1807, the subsequent war with England and the state bankruptcy, postponed the opening of the museum (Glenthøj 2007,84). At the same time, the historian Vedel Simonson noted in a series of books on the Danish history, that the people of "old" Scandinavia fashioned stone weapons to protect themselves from wild animals, and later copper was discovered and used, and finally iron (Simonsen 1813). This is the first testimony to an actual classification of raw materials. However, it took a few years for an actual scientific division of the antiquities to appear. This first division was established by a Dane, and it is arguably one of the most important contributions to establishing a chronological sense of time in the nascent field of archaeology. Christian Jürgensen Thomsen; from the National Museum in Copenhagen, arranged the archaeological remains of stone, bronze, and iron in the museum's collection into categories, and thus created the "Three-age system". His notion was based on the assumption that neither of the three materials were encountered in high frequencies with one another (Thomsen 1836), and thus prehistory could be divided into three main categories: The Stone, Bronze, and Iron Age. Several sources indicate that, besides being heavily influenced by classical writings, Thomsen was also inspired by the Swedish archaeologist Magnus Bruzelius, who had in early 1800, classified a two-period system based on stone and bronze (Gräslund 1987).

Meanwhile, other researchers had already started to establish museum collections based on the Three Age System even before his work was published (Gräslund1987,18) subsequently in his seminal work: "Ledetråd til nordisk Oldkyndighed" (translation: *Guide to Northern Archaeology*). This work was used as a template for an exhibition at the National Museum. Around this time the young archaeologist Jens Jacob Asmussen Worssae, was the first to move away from using folklore and myths to explain the past (Kristiansen 2002), and also expanded on Thomsens work (Worsaae 1843).

The same happened within the natural sciences, where a confrontation took place at this time between "old" and "new" paradigms. It was during this period that interdisciplinary archaeology came into being. For instance, George Forchhammer believed a catastrophic deluge had washed over Denmark from Sweden, and this resulted in the pine trees being laid down in westwards direction in some bog deposits (Forchhammer 1835; 1844). The aspiring young scientist Japetus Steenstrup instead conducted extensive research in various forest bogs, proving to his mentor Forchhammer, that the trees were actually overturned in all directions, and likely represented the deliberate felling of woodland by humans (Steenstrup 1842). The work is regarded as a pioneering study of vegetational development of the forest bogs. In this work, Steenstrup also mentions that bones from various extinct and cold-adapted animals; such as, aurochs, elk and reindeer (*Bos primigenius* and *Rangifer tarandus*) might be indicative of a colder climate in the past. Furthermore, he proposes that Europe, or perhaps the whole world, was at some point in time covered by ice (Steenstrup 1842, 120). At the same time in Lund, Sweden, the natural historian Sven Nilsson, published a large synthesis of different artefacts based on the three age system, but it was not merely a description of finds, but instead a comparative study utilising the new field of ethnography as a way to explain their usage (Nilsson 1843). This was seminal work for the time, and formed the basis for archaeological research that would first be revisited in the 1970s with the introduction of "New Archaeology" or processual archaeology.

The next significant topic of research was on Stone Age shell middens or *køkkenmøddinger,* found along many parts of the Danish coastline (H Andersen 1960; SH Andersen 1989, SH Andersen and KL Rasmussen 1991; SA Sørensen 1992; SH Andersen 2007; Milner, Craig and Bailey 2007). In 1837, the National Museum received some worked pieces of stone and deer antler, that had been excavated from a layer of oyster shells in a forest near the manor of Krabbesholm, Jutland. Prior to this point no one knew that these heaps of shells were actually refuse created by human occupation. In 1847, Japetus Steenstrup was the first investigator to make a stratigraphic recording that associated anthropogenic flint objects with shell accumulations. Consequently, it was decided to set up an interdisciplinary commission, the "*Leijre kommission*", which sat out to examine and explore the so-called "hævede havstokke" (translated; raised wave barriers), which were heaps of bivalves found at several places in Denmark. What followed was a series of investigations in 1848 conducted by the "*Leijre kommission*" (in retrospect named The First Kitchen Midden Commission), due to the presence of several of these shell heaps around Roskilde Fjord. The Leijre commission consisted of an interdisciplinary group of researchers who were to investigate other examples of these 'shell deposits'. This group of researchers consisted of non-other than the founders of 'archeological scientific investigations': Steenstrup, Forchhammer, and Worsaae.

At first, they disagreed about whether or not, the middens were made by humans. At the first meeting in The Royal Danish Academy Of Sciences And Letters, Forchhammer, stated, that the areas with shell banks had risen over time (Forchhammer and Steenstrup 1848, 66) (i.e. isostatic rebound). Steenstrup later noted that several bones from animals not present in contemporary Denmark were found (i.e. bear and turtle) (Forchhammer and Steenstrup 1848, 62–74). This work was ahead of its time, since it involved an interdisciplinary group of researchers tasked with answering specific questions on Danish prehistory (Andersen 2000). A couple of years later, a consensus was finally reached: these remains were indeed anthropogenic (Steenstrup 1852).

Despite this conclusion, several points of disagreement later created substantial and confrontational debate regarding the shell middens, notably between Japetus Steenstrup and J. J. A. Worsaae. The first disagreement related to age, specifically in comparison to the megalithic tombs (dolmens). Secondly, there was a difference of opinion regarding who was the first to acknowledge the shell middens as human made (Sørensen 1899). Steenstrup thought that the shell middens and the dolmens were coeval, whereas Worsaae, correctly stated that the shell middens predated the dolmens. This was the first subdivision of the Stone Age, and the first time the terms "Older Stone Age" and "Younger Stone Age" were introduced (Worsaae 1859, 105). The Leire commission was active from 1848 to 1854 and was soon after disbanded after completing their objective, i.e. establishing the nature of the shell middens.

The coming years saw a significant change in archaeological practice, now established as a professional scientific discipline (Kristiansen 2002). After Worsaae died in 1885, Steenstrup resurrected their old dispute regarding the relative age of dolmens and shell middens, which yet again led to a heated debate against Worsaaes'points ofview (Undset 1889). At this time Sophus Müller; who had already published several volumes from 1888 to 1895 on the ordering of Danish antiquities (*Ordning af Danmarks Oldsager* (*Ordering of Danish Antiquities*), entered the debate. To end the dissension with Steenstrup (and others); and preserve Worsaae's legacy once and for all, Müller in 1893 initiated 'The Second Kitchen Midden Committee'. One of the excavations was located in the large shell midden at Ertebølle, which would become the *locus classicus* coining the last period in the Mesolithic. The monumental work on these shell middens was published in 1900 (Madsen et al. 1900). This remains a definitive and brilliant piece of archaeological investigation, which in the end confirmed Worsaae's subdivision of the Stone Age (Madsen et al. 1900). However, much of our understanding of the Middle Stone Age (the Mesolithic) was still lacking. For example, in Scandinavia it was not known if there was anything that preceded the Ertebølle period, but a major breakthrough was soon to take place.

2.3 On the placing of settlements in the thick fog of time

By the end of the 19th century, most Stone Age localities were still poorly contextualised in time and space. There was a need for a system in which the sites could be better placed so that their history could be better understood.

One of the first systems to appear was when the Norwegian botanist Axel Blytt (Blytt 1876) and Swedish geologist Rutger Sernander (Sernander 1908b) introduced the climatic periods used today; i.e. Boreal, Atlantic, Subboreal, Subboreal and Subatlantic. Their study of macrofossil remains found in peat bogs, were used to infer climatic histories in the early Holocene, and is today referred to as the Blytt-Sernander system (the Preboreal was later added by Fægriin (1940)). The system became widely used, but was also found to have some pitfalls. One of the biggest discoveries related to use of the Blytt-Sernander system actually led to the discovery of pollen analysis. Faced with the issue of unclear stratigraphy in the form of *Sphagnum* peat at one part of the Lerbäck bog, which could not be placed in the Blytt-Sernander system, the Swedish geologist Lennart von Post, counted grains of pollen and thus invented the study of palynology (von Post 1916; Gron and Rowley-Conwy 2018).

On the Danish side of the strait, the geologist Knud Jessen adopted the system and later developed the pollen zones used today (i.e.; I - IX). It was also realised that the Baltic Sea had gone through several stages of fluctuating sea-levels. The Baltic Ice Lake, which was the freshwater lake created by the melting glaciers, was first recognised in 1910 (Munthe 1910), the following Yoldia Sea stage was named after the marine mollusc *Yoldia arctica* (now *Portlandia arctica*) (Munthe 1900). In turn, the Ancylus lake stage was named after the freshwater mollusc *Ancylus fluviatilis* (Munthe 1910) and the preceding Littorina Sea stage was named after the gastropod *Littorina littorea* (Lindström 1886). These stages in the Quaternary geological history in Southern Scandinavia, gave interdisciplinary archaeologists some tools in which to temporally-assign the various sites.

Fig. 2. Excavation at Mullerup in 1900. George F. L Sarauw sitting on a wooden box overseeing the excavation, probably done by locals (Permission to publish granted by The National Museum of Denmark).

In 1900, George F. L Sarauw was contacted by M. J. Mathiassen (father to Therkel Mathiassen - introduced below) a schoolteacher in Mullerup. A pupil of his had called his attention to worked bones (in fact barbed bone points) that peat cutters had unearthed in the Maglemose bog, near Mullerup (Fig. 1). Sarauw, a natural scientist, who was also part of the Second Kitchen Midden Committee, soon started to excavate the site under the auspices of the National Museum (Fig. 2). This was a major breakthrough, because Sarauw realised that the Mullerup site represented habitation that predated the shell middens, and that it should be placed in the later part of the Ancylus stage (Sarauw 1903, 164). Although, this was the first Early Mesolthic Maglemosian site that had been dug, it would take almost 30 years before the period was formally designated the Maglemose culture (Clark 1936).

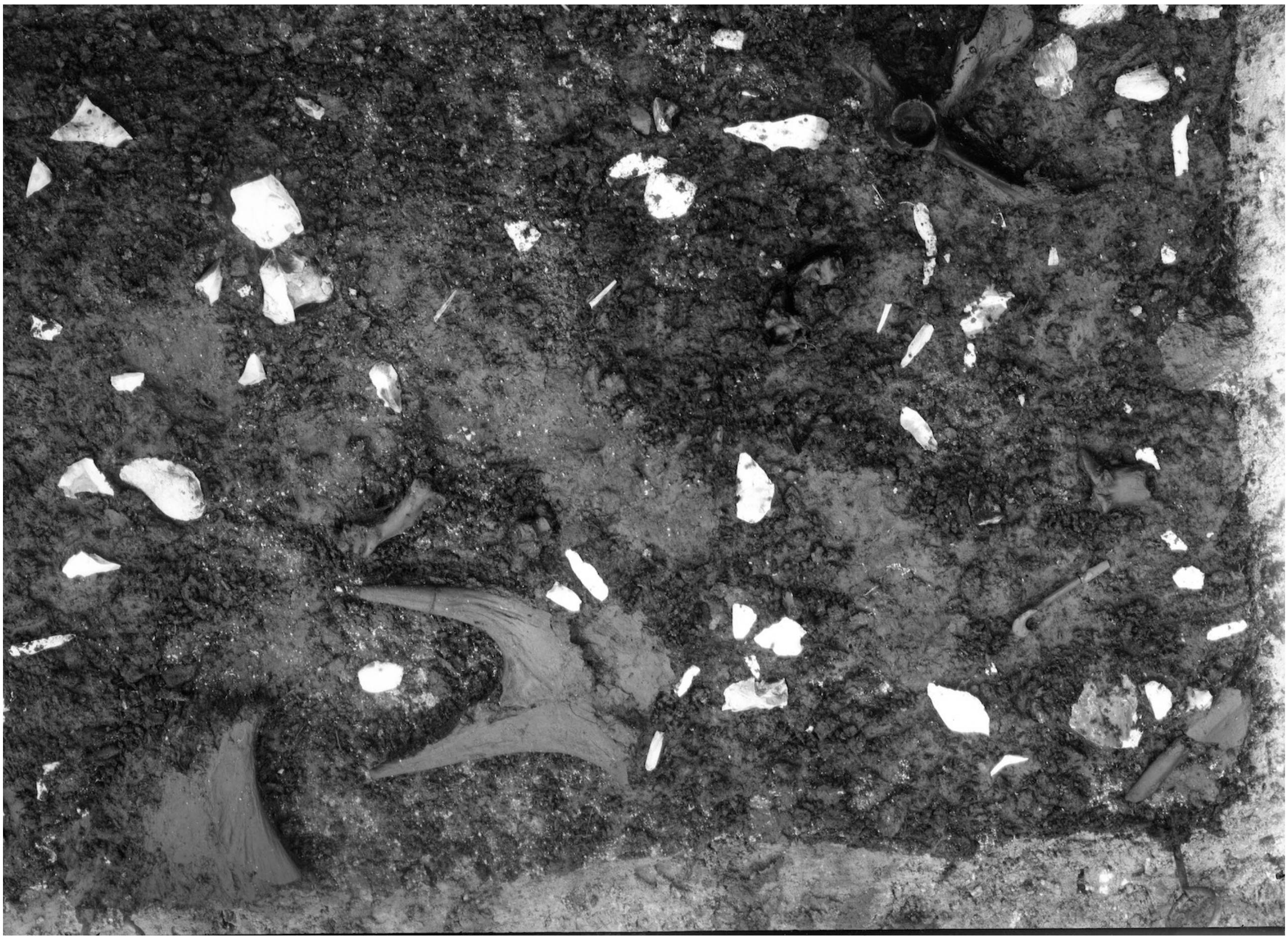

Fig. 3. Excavation photo from Sværdborg mose in 1917-18. The photo depicts the extraordinary preservation at many bog sites before drainage - a situation frozen in time. Pieces of elk antler and animal bones lies scattered on the prehistoric settlement surface. Knapped flint such as three Sværdborg triangles and a broken fish hook are lying where it was presumably left 8,000 years ago (Permission to publish granted by The National Museum of Denmark).

After Sarauws excavation at Mullerup, several of the classic Maglemose settlements were uncovered in Denmark. A common feature of these sites from eastern Denmark was that organic remains (i.e. bone and antler, and sometimes wood) were excellently preserved. The reason is that they were uncovered in connection with peat cutting below normal ground water level.

The primary reason for increased peat cutting was a severe lack of fuel during the World Wars. In 1907, Bare Mosse in Scania (Fig. 1) was also uncovered, and although bones were not preserved, it showed that the same cultural entity was also present there as the Maglemose people at Mullerup (Sernander 1908a). A few years later, in 1919, Friis-Johansen published his works on the Sværdborg settlement (Fig. 1) in the Lundby peat bog (Fig. 1, 3). Here he was able to correlate the age of occupation with Mullerup (later part of the Ancylus stage), based on the presence of tree species (*Pinus* sp.), which were present at both sites (Johansen, Jessen and Winge 1919, 223). The Sværdborg site would later become a *locus* site for pressure blade technology (i.e. Sørensen 2006).

At this point it was still not entirely known which cultural entity inhabited the period between the Maglemose and the Ertebølle period. In 1924; the amateur archaeologist and lawyer, Erik Westerby published his results from Bloksbjerg (Fig. 1) near Klampenborg. Westerby thought the inventory from Bloksbjerg was so distinct from other assemblages, and argued that the Bloksbjerg assemblage filled the gap between the Maglemose and the Ertebølle period and thus mertited for the period to be named after the location (Westerby 1927,197). Westerby was also the first 'archaeologist' to use the term "Mesolithic" in Denmark.

However, the cultural entity "Bloksbjerg" was not readily accepted (Broholm 1924,189), and was later renamed the to Ældre Kystkultur (translation: older coastal culture) by Vebæk (1938). Meanwhile, similar remains had been uncovered in Jutland, and yet another cultural term introduced for the lithic remains in Jutland. In the 1930's, Mathiassen had analysed a large collection of finds from around the Gudenå river in Jutland. In his publication he introduces the "Gudenå-culture" (Mathiassen 1937a). However, the finds associated with this 'culture' mostly derived from unstratified layers, and were later shown to be of mixed character from several periods (Rysgaard, Rasmussen and Riede 2016). The confusion was pronounced in terms of what this *culture* or period actually was. The breakthrough finally came with the excavation of the Kongemose (Fig. 1) *locus classicus* at Sandlyng in Åmosen by Svend Jørgensen in the 1940s (Jørgensen 1956a). The confusion lasted, until Johannes Brøndsted in *Danmarks Oldtid bind I* coined the 'Kongemose culture' (Brøndsted 1957, 101). However, the earliest part of the Kongemose culture, was still unknown, and it took many years of research into bridging the Maglemose and Kongemose cultures. Finally, this was achieved with the discovery of the submerged site at Blak (Fig. 1) in the Roskilde Fjord by Søren Anker Sørensen (1996).

In the periods before the discovery and the coining of the Kongemose culture, excavations resumed to undercover other Maglemose sites. Other key settlements were uncovered in the Lundby bog near Sværdborg, as well as in the nearby bog at Holmegaard (Fig. 1) (Broholm 1924). Soon after, Denmark's to date oldest settlement was discovered at Klosterlund (Fig. 1) in Jutland (Mathiassen 1937b). Unfortunately, no bones were preserved at Klosterlund, and it would take many years for the first Preboreal settlement with preserved organic remains to be discovered at sites such as: Barmose and Skottemarke (Fig. 1)(Sørensen 1978; Johansson 1990).

Another significant find that was uncovered during the 1940s fuel crisis, whereby peat cutting activity was much increased, were the two Maglemose huts at Ulkestrup Lyng (Fig. 1), in Store Åmose. The two huts were in the last part of the Maglemose period, placed close to each other amongst the reeds. Large parts of the structure was preserved, as well as abundant refuse deposits along the prehistoric waterfront. It took nearly forty years before these important remains were published by the excavator Knud Andersen and colleagues (Andersen, Jørgensen and Richter 1982). Subsequently, additional sites with hut remains were also uncovered; for instance, at Sværdborg and Holmegaard (Becker 1945; Petersen 1972). For Sværdborg I it also took nearly forty years for dissemination (Henriksen 1976).

Human skeletal remains from this period are almost non-existent. At present, there are only two burials dated to the Maglemose period from Denmark. One is the Koelbjerg *woman* (Fig. 1)(Bröste and Fischer-Møller 1943), which was placed in the Preboreal through pollen analysis (Troels-Smith 1943). However, recent aDNA sequencing has revealed that the individual was actually a male (Hansen *et al.* 2017). The other is the cremation burial at Hammelev (Fig. 1), which was, by chance, found among the post holes in an Iron Age house (Eriksen and Andersen 2016).

2.4 Towards the integration of unstable isotopes to place settlements in time and space

In 1952, the first radiocarbon laboratory outside of the United States, was established in Copenhagen, Denmark (Anderson, Levi and Tauber 1953). The person who took charge of the laboratory was a young chemistry engineer named Henrik Tauber. The first material to be measured on the instrument, was reported by botanist Johannes Iversen, who, with this new technology was now able to place the Late Glacial Allerød interstadial (warm period) in chronological time (Iversen 1953). Thereafter, a steady stream of measurements was reported from Tauber. Several of the previously excavated sites were radiocarbon dated, and absolute chronologies started to appear (Tauber 1972).

However, at that time, conventional radiocarbon dating was based on liquid scintillation counting, and most often, the material dated came from "bulk" samples. This produced great uncertainties, and although the dates are usable today (by using present day calibration curves i.e. IntCal13 (Reimer et. al. 2013)), they are less accurate than Accelerator Mass Spectrometry (AMS). Further, contamination of humic acids has resulted in radiocarbon dates that have since proven to be too young (i.e. Johansson 1990; Fischer 1996). The filtering of humic acids was not routinely performed in the old Copenhagen lab (prior to K-2127), and most dates produced some should be treated with some caution (mentioned in Chapter 3, SI 2.1).

2.5 The post 1940's Chronology of the Maglemose culture in Southern Scandinavia

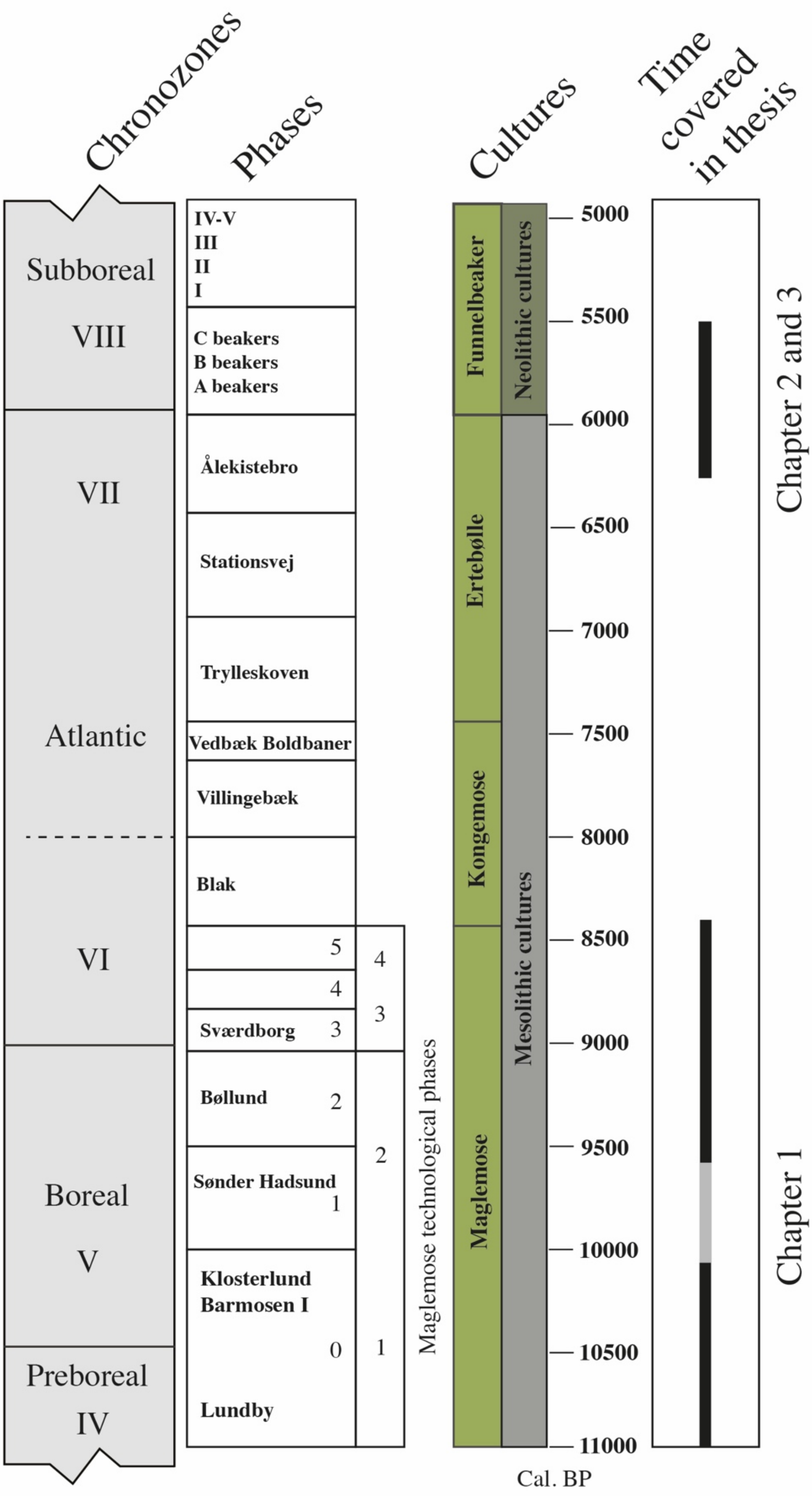

Fig. 4. *Chronozones and archaeological development during the period covered in the thesis. Chronozones, archaeological phases and cultures (modified after Petersen (1999) and Sørensen (2006)), and periods covered in present thesis.*

On the basis of the earlier scientists' discovery that, the Stone Age was indeed the oldest period of human occupation in Southern Scandinavia, and the recognition that the kitchen middens were human made, attempts to further subdivide the various periods was attempted. The first rigorous attempt to subdivide the various Mesolithic sites to create a relative chronology was done by Therkel Mathiassen in (1943, 143). By counting the lithic remains from the settlements and linking these with the palynological data of the sites, he was able to place most of the type sites in time and space. What largely confused the final result was that at that time, the Kongemose culture (Gudenå culture) had at that point not been recognised, and, as mentioned above, that several of the sites were consisted of stratigraphically mixed Maglemose and Kongemose occupations (Mathiassen, Troels-Smith and Degerbøl1943,162). At the same time, Carl Axel-Althin published his work on Mesolithic settlements in Scania (Althin 1954). His work focused almost largely on the lithic remains from Ageröd (Fig. 1). Strangely, Althin did not include any palynological analysis to try resolve the geological history of the sites; something that was also noted by Clark in his review of Althin's thesis (Clark 1955). In 1953, Carl Johan Becker proposed that the Maglemose culture should be divided in to five phases based on variation in the lithic assemblages (Becker 1953). Later, Erik Brinch Petersen expanded on Becker's typology by dividing the period into six phases (phase 0-5) (Fig. 4) (Petersen 1973). Still later, Axel Johansson (Johansson 1990) added an additional series of periods to the beginning of the Maglemosian cultural epoch. This typochronology prevailed for many years until Mikkel Sørensen, on the basis of in-depth technological flint studies, further refined the chronology of the Maglemose culture (Fig.4) (Sørensen 2006). A local chronology has also been proposed by Claudio Casati and Lasse Sørensen for Bornholm (Casati and Sørensen 2005).

3.1 The environment of Southern Scandinavia

An introduction to the Holocene environment of southern Scandinavia is of great significance as it had significant impact on humans, animals, and vegetation (i.e. Fig. 5). With the work carried out in the present thesis, Chapter 5 deals with the effect of how and if climate may have caused a change in material culture around 10,000 cal BP.

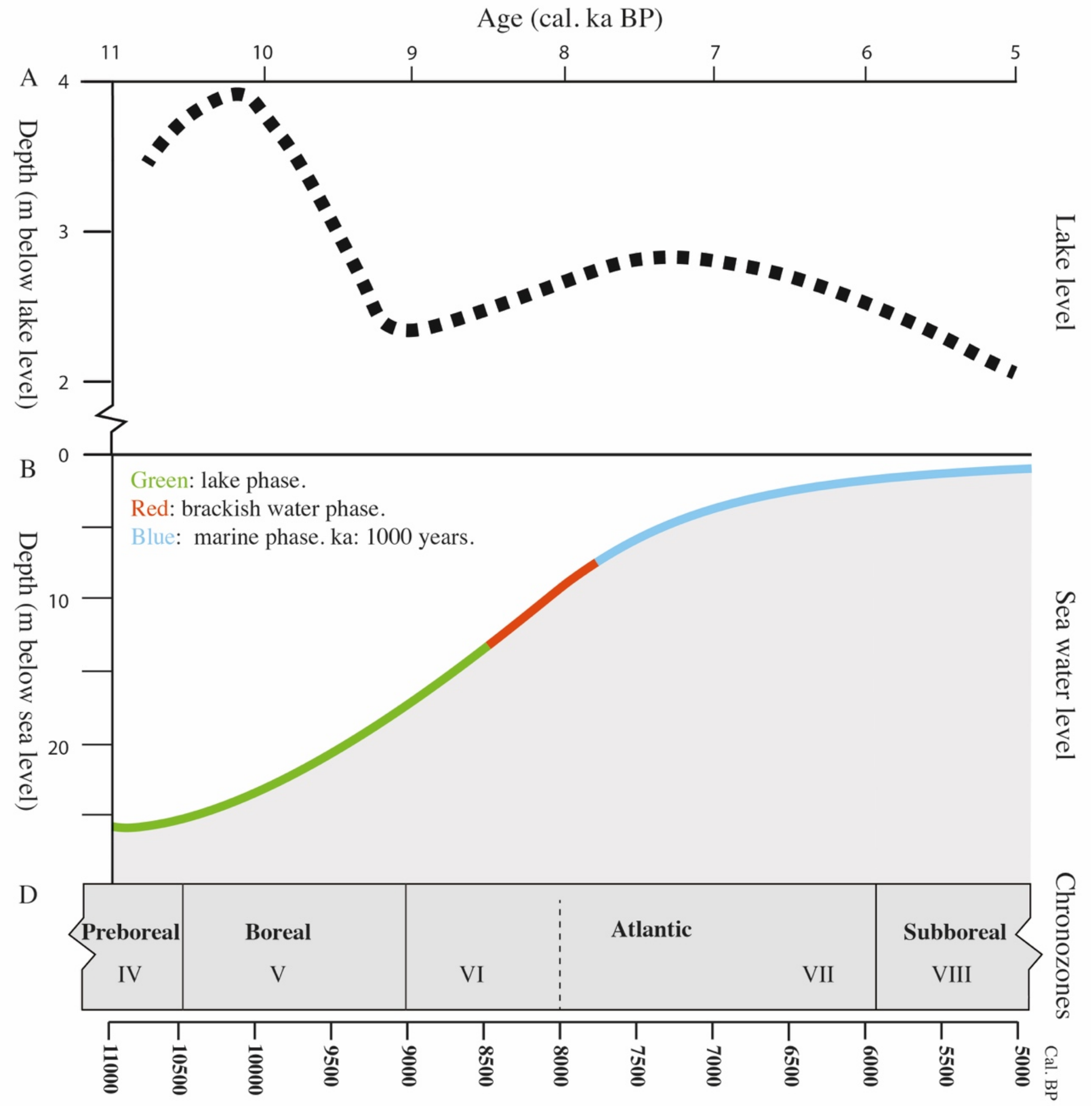

Fig. 5. *Hydrological development during the period covered in the thesis. A) lake level reconstruction in Lake Igelsjön in Southern Sweden (redrawn after Digerfeldt et al. (2013), B) sea-level reconstruction in Lillebælt, Denmark (modified after Bennike and Jensen (2011)) D) chronozones (modified after Petersen (1999).*

3.2 The Preboreal period (c. 11,350 – 10,500 BP)

As temperatures increased and the Weichselian glaciers retreated, a proglacial freshwater lake was established in the Baltic: the Baltic Ice Lake. At ca. 11,700 cal. Years BP the lake was dammed up. Subsequently, the water level suddenly dropped because the ice sheet margin retreated from Mt. Billingen in south-central Sweden. The drainage event led to a brackish- water low-stand: the Yoldia Sea Stage. The Yoldia Sea shore level was located 25 m below the level of the Baltic Ice Lake. During this low stand, the Øresund became dry land and a land bridge formed between Denmark and Sweden, and the island of Bornholm was connected to Germany (Björck 1995). At c. 10,700 BP isostatic rebound in south-central Sweden meant that the connection between Kattegat and the Baltic Basin became dry land, and the Baltic Basin became a fresh lake again; this stage is known as the Ancylus Lake Stage (Berglund et al. 2005). Throughout the period, there is also marked fluctuations in water levels in the inland lakes (i.e. Jørgensen 1963; Digerfeldt 1988) (Fig. 4). What is now the North Sea, was once a great low-lying land mass termed Doggerland, that connected Great Britain to northwestern Europe (Reid 1913). Over time, this large landmass was also gradually being inundated due to rising sea levels (Behre 2007).

The climate during the Preboreal can be characterised by a steady increase in temperature (Brown et al. 2012), although the climate was generally unstable. Several fluctuations in temperature have been observed in the δ^{18}O signals in the ice cores from Greenland and other records. The most pronounced of these climatic events occurred during the Preboreal Oscillation(s) that took place around 11,430 – 11,270 cal year BP. These events resulted in a significant drop in temperature, probably related to an outburst of water from the massive glacial Lake Agassiz in western Canada (Björck et al. 1997; Rasmussen et al. 2006, 2007; Fiłoc et al. 2018; Klotsko, Driscoll and Keigwin 2019). After this event, the prevailing Danish forest-tundra landscape shifted rapidly to a forested landscape. The forests were dominated by birch (*Betula pubescens*) and pine (*Pinus sylvestris*) and included thermophilous shrubs and herbs (Iversen 1973).

At the beginning of the Preboreal, the mammalian fauna consisted of a mixture of cold and warm adapted species. Of the cold adapted species, reindeer (*Rangifer tarandus*) was present throughout most of the Preboreal. The youngest specimen is dated to the transition to the following Boreal period (Aaris-Sørensen, Mühldorff and Petersen 2007/6). However, this date derives from the Nørre Lyngby (Fig. 1) reindeer antler axe, which after four dating attempts has produced wide ranging ages (Fischer et al. 2013).

Other species adapted to an open environment, such as the wild horse (*Equus ferus*) and European bison (*Bison bonasus*), were only present for a very limited period of time (Ekström 1993; Aaris-Sørensen 2009). Ungulates such as; aurochs (*Bos primigenius*), Eurasian elk (*Alces alces*), red deer (*Cervus elaphus*), and wild boar (*Sus scrofa*) were also present during the Preboreal (Aaris-Sørensen 2009). The same faunal composition was present in Scania, as continued immigration of terrestrial mammals was possible due to the continued land bridge between Zealand and Scania (Liljegren and Ekström 1996).

Habitation sites from the period are relatively rare, and typically devoid of preserved organic material. One major exception is bone points thought to be used for spearfishing (see Chapter 3). Of the sites with some organic material in the form of faunal remains are: Barmosen (Fig. 1) (Johansson 1990) in southern Zealand; Flaadet (Fig. 1) on Langeland (Skaarup, Fredskild and Møhl 1979); Fredbogård II (Fig. 1) in northern Zealand (Casati 2018), Lundby Mose (Fig. 1) (Jessen et al. 2015) and potentially Øresø Mølle (Fig. 1) in Lille Åmose (currently under excavation). For sites with lithic remains only, see Sørensen et al. (2018).

3.3 The Boreal period (c. ca.10,500 – 9,000 BP)

At the beginning of the Boreal period, isostatic uplift created a new lake, the fresh-water Ancylus Lake, as mentioned above. In the southern part of the Ancylus Lake, a marked transgression occurred, which peaked at c. 10,300 cal. Year BP (Bennike and Jensen 1998; Hansson et al. 2017). The location of the drainage outlet has yet to have been firmly established, but has been proposed via the Dana river (Darss

Sill, Fehmarn Belt, and then through the Great Belt to Kattegat), through Øresund or lake Vänern (Bendixen et al. 2015). Due to relative sea level rise in Kattegat, the Baltic gradually became marine. The end of the Boreal chronozone also marks the transition to the succeeding Littorina Sea, (named after the *Littorina littorea* mollusc), which was characterised by a steady rise in sea level during the early part. The temperature also kept rising during the Boreal, but a sudden climate anomaly appears at c. 8,200 cal BP, the so called 8.2 ka event (Björck et al. 1997; Rasmussen et al. 2006, 2007; Hede et al. 2010; Bjerring et al. 2013). More enigmatic with regards to how it may have affected flora, fauna and humans, is a climate anomaly, centered around 10,300 cal BP, which is characterised as a short cooling event that affected the entire Northern Hemisphere (Björck et al. 2001). This event is discussed in Chapter 3, and is surmised as a potential contributing factor for a hiatus in radiocarbon dated remains from eastern Denmark and Scania.

During the Boreal, hazel (*Corylus avellana*) dominated the forests, other broadleaved trees started to appear such as oak (*Quercus robur*), ash (*Fraxinus excelsior*), alder (*Alnus glutinosa*), lime (*Tilia cordata*), and elm (*Ulmus* spp.) (Aaby 1994; Blankholm 1996). Based on the appearance of thermophilous species such as ivy (*Hedera helix*) and mistletoe (*Viscum album*), as well as the European pond tortoise (*Emys orbicularis*), it can be inferred that the mean summer temperature was at least 18 °C (Troels-Smith 1960a; Gotfredsen 1998).

The faunal diversity increased significantly during this period. Mammals such as elk and aurochs were still present, but other species appeared, such as the apex predators lynx (*Lynx lynx*), grey wolf (*Canis lupus*), fox (*Vulpes vulpes*), wild cat (*Felis silvestris*), pine marten (*Martes martes*), badger (*Meles meles*), polecat (*Mustela putorius*) and otter (*Lutra lutra*) (Blankholm 1996; Aaris-Sørensen 2009).

3.4 The Atlantic period (c. 9,000 – 5,900 BP)

From the preceding Boreal period, sea levels started rising although the timing of the exact transition from the Ancylus Lake to the Littorina Sea is still debated (Björck 2008). The early part of the Littorina Sea Stage, is marked by pulses of brackish water, and around 8,500 saline water penetrated into the Baltic region (Bennike et al. 2012). During the Atlantic period, a marked increase in aquatic productivity is seen (Björck 2008), which in combination with high water temperatures permitted oysters (*Ostrea edulis*) to thrive in Danish waters. During this period; due to rising relative sea-level, Denmark became an archipelago and became separated from Scania. Apart from the cooling event at 8.2 ka (Rasmussen et al. 2007), the average temperature increased during this period also (Brown et al. 2012). Evidence for this can be seen in faunal remains from thermophilous species not currently found in the region today, such as the European pond tortoise (*Emys orbicularis*) and the Dalmatian pelican (*Pelecanus crispus*) (Degerbøl and Krog 1951; Hatting 1963).

The early part of the Atlantic period is marked by an increase of climax trees such as elm, oak, alder and lime (*Ulmus, Quercus, Alnus* and *Tilia*). These genera seems to have gradually replaced birch, pine and hazel (*Betula, Pinus* and *Corylus*). Gradually, the forest became denser and darker and turned into the Climax forest or Stable Primeval Forest, as evidenced by a decline of light-demanding plants (Jørgensen 1963, 33; Iversen 1973). The 8.2 ka event mentioned above, seems to have retarded vegetation and specific species for a short while. At Højby Sø, a sudden decline in thermophilous tree pollen is seen, which is dated to c. 8,250 – 7,900 cal BP. Pollen records from the lake show the presence of macrophyte remains, which are indicative of increased precipitation. After 7,900 cal BP, the aforementioned species started to dominate again (Rasmussen et al. 2008). Results from Åmosen appear to show similar patterns, but here the 8.2 event is only dated by pollen analyses (Jørgensen

1963, 72). A generally rich terrestrial and aquatic biotope appears to be continuous with the preceding period. However, some mammal species seem to disappear soon after 8,000 BP from the newly created islands. In depth studies of faunal remains from Kongemose and Ertebølle sites on Zealand, revealed an absence of brown bear (*Ursus arctos*), European polecat (*Mustela putorius*), European badger (*Meles meles*), lynx (*Lynx lynx*), elk and aurochs (Aaris-Sørensen 1980).

4.1 Stone Age Cultures of the earliest Mesolithic and Neolithic in Southern Denmark

4.2 Maglemose period

The Maglemose culture (c. 10.900 – 8.400 cal BP) represents the earliest permanent presence of hunter-gatherers in Southern Scandinavia after the last Ice Age. The culture can be considered as an inland culture as almost all of the sites from this period have been uncovered bogs. Seasonal indications from dental eruption in hunted prey, have revealed a predominantly spring/summer period of occupation at these sites (Rowley-Conwy 1993). This raises the question: where were the winter occupations? The Maglemose coastline now lies under water due to the continued Holocene sea level rise, but movement to the former coastline is one explanation for a lack of winter sites. However, only few submerged sites have been found to be dated to the Maglemose. One such site is located off the SW coast of Sweden, at Haväng, where archaeological remains, and fishing installations has been found along a now submerged river system (Hansson et al. 2018). Pilhaken in Øresund, is probably also a Mesolithic settlement located in the underwater Øresund river (Rausing and Larsson 1977). Recently, focused efforts to identify submerged Maglemose sites have proven successful (Astrup 2018, 103ff). Although closely connected to the sea, neither of these are coastal sites, nor is it known whether any of these sites constitute winter habitations.

The hunting strategies in the Maglemose period largely reflected in the faunal assemblages uncovered at the peat bog sites. Zooarchaeological evidence shows a predominance of large ungulates such as; aurochs, Eurasain elk, red deer, roe deer and wild boar, but also small fur bearing mammals, as well as, a large variety of birds (i.e. Degerbøl 1943; Leduc 2012). Waterfowl and fish were also frequently exploited, which is evidenced by the presence of bird bones in the faunal remains, as well as the presence of bone points for spearfishing. The archaeological presence of abundant bone points is suggestive of artefacts lost during fishing in ancient lakes (i.e. Andersen 1983; Johansson 2006 and Chapter 3). The lithic phases phases of the Maglemose traditionally follows Petersens 0-5 phase system (Petersen 1973).

Sørensens most recently subdivided the lithic knapping technology exercised during the Maglemose period into four techno-groups based on the *chaîne opératoire*. The four techno-groups groups incorporate Petersens old phases 0-5. The first techno-groups: 1 and 2 (phase 0,1 and 2) utilise direct percussion technique to make large and crude blades. However, the transition to techno-group 3 and 4 (phase 3, 4 and 5) exhibits a leap in technological refinement, by introducing pressure technology which creates delicate microblades. This technological advance is thought to arrive in Denmark around 9,000 years ago (Sørensen 2006) but originated somewhere in Siberia or China around 20,000 years ago (Desrosiers 2012), and then subsequently spread westwards into Scandinavia (Sørensen 2012; Sørensen et al. 2013; Sørensen 2018). Chapter 3 touches upon the possibility that the introduction to pressure blade technology arrived synchronously with larger-barbed bone points during the mid-Boreal period.

Human remains and actual burials from the Maglemose period are rare. The only real burials, are represented by the cremation burial at Hammelev in southern Jutland (Eriksen and Andersen 2016), and the “water burial” at Koelbjerg (Bröste and Fischer-Møller 1943).

The Maglemose is followed by the Kongemose period (c. 8.400 – 7.400 cal BP), which is represented by handle cores and very exquisite macro blade technology. The economy now focusing more on the coastal area and shell-middens start to appear (Sørensen 1996; Jørgensen 1956a, 1956b). The succeeding Ertebølle period (c. 7.400 – 5.900 cal BP) is the last period of the Mesolithic. During the Ertebølle period, eustacy and isostacy caused parts of Denmark’s Ertebølle sites to either become submerged or rise above sea-level. The coast was fully exploited as seen by the massive shell-middens, as well as $\delta^{13}C/\delta^{15}N$ values from human bone collagen reflecting almost entirely marine food consumption (e.g. Andersen 2007; Fischer et al 2007). Communal burial grounds also start to appear in the Kongemose and Ertebølle periods (Petersen 2015, p.19ff), and ceramics are introduced in the Ertebølle period (Povlsen 2013).

4.3 The transition from hunter-gatherer to farmer

Research on the mechanisms behind the transition from hunting and gathering to farming in Southern Scandinavia has received a lot of attention. One reason for this, is the abundant number of sites and material culture dated to the transition, but also that the area can be seen as a culturally and geographically constrained bottleneck. Traditional archaeological research has, as a starting point, sought to prove or disprove a cultural shift when several cultural features suddenly appear in a new area. This can be a sudden change in burial or building customs, ritual activities and/or new tools. Most of the above mentioned aspects appear almost synchronously around 6,000 BP in Southern Scandinavia, and three main theories have been put forward: i.e. migration, indigenism and integrationism (e.g. Mathiassen 1940; Becker 1947; Troels-Smith 1953; Fischer 2002; Ammerman 2003; Rowley-Conwy 2011). With the migration theory, it is argued that the introduction of farming, was brought from abroad by immigrant farmers that either displaced or acculturated with the indigenous Ertebølle people. By contrast, indigenism can be seen as the Ertebølle people being the active agents in establishing agriculture autonomously. Integrationism can be seen as a somewhat a mix between migration and indigenism (Sørensen 2014a, 12 and references herein; Gron and Sørensen 2018).

4.4 Funnelbeaker culture

The Funnelbeaker culture is the first Neolithic culture in Southern Scandinavia, it is preceded by the Ertebølle the last of the Mesolithic cultures. Nothing from this study dated from this earlier culture.

The change from an economy entirely based on hunting, gathering, and fishing, was suddenly replaced with a completely new subsistence strategy. The different aspects of, when, how, and why, has been discussed for more than 150 years (Mathiassen 1940; Becker 1947; Troels-Smith 1960b; Zvelebil and Rowley-Conwy 1984; Kristiansen 2002; Sørensen 2014a) and a thorough review is outside the scope of this thesis. While material culture changed with the transition to the Neolithic, the introduction of domesticated crops and animals are the key hallmarks of Neolithisation (Zvelebil 1998). The first signs of livestock and domesticated cereals appear in southern Scandinavia at c. 5.950 cal BP, in the form of cattle and sheep/goat (*Bos taurus* and *Ovis capra/Capra hircus*) and domesticated cereals such as wheat and barley (*Triticum* sp. and *Hordeum* sp.) respectively (i.e. Fischer 2002; Sørensen and Karg 2012; Sørensen 2014a, 72ff). During this time, there is ample evidence of intense forest clearing in Denmark (Andersen 1993), but not Scania (Regnell and Sjögren 2006). This clearance of forest is almost certainly related to increasing pasturage for domestic animals, and landing for sowing cereals.

This transition period also saw a change to the lithic toolkit, whereby the predominance of flake and core axes, was replaced with polished thin-butted axes (Nielsen 1977). Simple dwelling structures, such as the 'Ertebølle huts', were also replaced by two-aisled houses (Nielsen 1997). Monumental constructions in the form of; long barrows, caused way enclosures, and megalithic tombs, also appear during the early part of the Neolithic (Madsen 1979).

Despite a shift to agriculture, aquatic resources were still being exploited to a significant degree. On several of the stratified shell middens (i.e. Bjørnsholm and Norsminde), there is ample evidence for the continued exploitation of fish and molluscs (Andersen 1989, 1991). Also, the vast majority of fish weirs have been directly radiocarbon dated to periods within the Neolithic (Pedersen 1997; Pedersen, Fischer and Gregory 2017; Jensen et al. 2018).

5.1 Biomolecular analysis of the Southern Scandinavia Stone Age

5.2 Stable Isotopes

The first use of $\delta^{13}C$ to infer dietary preferences in Prehistory, was done by Henrik Tauber in (Tauber 1981). His seminal work presented the idea that $\delta^{13}C$ could be used as an indicator to distinguish terrestrial vs. marine food consumers. Tauber showed that there was a significant decrease in $\delta^{13}C$ after the introduction to agriculture, and based on these data noted, that people in the Mesolithic predominantly consumed marine foods, whereas people in the preceding Neolithic period mostly consumed terrestrial foods. This was in good agreement with the general idea of a massive shift in subsistence economy. Studies of the $\delta^{13}C$ ratios from Prehistoric domesticated dogs (*Canis familiaris*), showed that they consumed similar foodstuffs as their human owners on either side of the transition (Noe-Nygaard 1988). The analysis of both dogs and humans has been covered in depth by Fischer and others to infer mobility and dietary practices (i.e. Fischer et al. 2007a, 2007b). However, by coupling the archaeological evidence to isotopes, the shift in dietary preferences from marine to terrestrial may not have been as dramatic as the isotopes imply (Milner et al. 2004). This is further evidenced by the discovery of fish-traps several places in Denmark, all dated to the Neolithic (Fischer 2007; Jensen et al. 2018).

Later isotope studies focused on a wide variety of mammals from Åmosen, which showed pronounced differences in their isotopic values. This probably reflects different feeding regimes, as well as, the relative forest cover known as the canopy effect (Noe-Nygaard 1995; Noe-Nygaard, Price and Hede 2005). More recently, analysis has been extended to eel bones from in-land and coastal sites, to elucidate whether the values reflected marine or brackish origin. Their results showed that the eels had carbon/nitrogen values reflecting residency (Robson et al. 2012). Gron and Rowly-Conwy (2016) analysed carbon and nitrogen on a large set of domesticated and wild fauna from the Early Neolithic. They found that Early Neolithic cattle were feeding in an open environment, which argues for forest clearance.

Stable isotope studies have also been conducted on charred cooking residues in pottery (i.e. Arrhenius and Lidén 1984; Jennberg 1984; Andersen and Malmros 1985), primarily to discern the fraction of marine to terrestrial fraction of the foods used in cooking. However, these studies have later been recognised to be biased due to potential reservoir effects from marine and freshwater environments (Fischer and Heinemeier 2003; Philippsen and Meadows 2014).

Other isotopes have also been explored, such as sulphur ($\delta^{34}S$), but without baseline data to compare against, the results were somewhat inconclusive (Craig et al. 2006). Recently, Gron et al. showed cattle were moved around in the Neolithic based on strontium isotope $^{87}Sr/^{86}Sr$ ratios obtained from domestic cattle teeth. Most notably, some were probably moved over sea by boat (Gron et al. 2016).

5.3 Proteins

The analyses of ancient proteins has only scarcely been utilised on Stone Age material in Southern Scandinavia (i.e. Arrhenius 1984; Arrhenius and Lidén 1984), and the most current data will be presented in Chapter 1 and 2 of this thesis. From other periods, several studies have been conducted on faunal remains (bone and skin) from the Viking age and Medieval periods (Brandt et al. 2014; Brandt, Haase and Collins 2018). Several further pieces of faunal remains have also been analysed during the course of this PhD, with the intention of their future publication. These are also case studies, but diverse in terms of material and age. One study focuses on the application of ZooMS and LC-MS/MS protein sequencing of a decorated piece of bone found at Fogense Enge, Funen, dated to the Allerød Interstadial – making it the oldest piece of art in Scandinavia. Another project used ZooMS to prove or disprove previous visual identification of Holocene antlers identified as reindeer (*Rangifer tarandus*). One final case study used LC-MS/MS on tooth enamel from the Late Mesolithic shell midden at Lollikhuse, Zealand, for species identification and sexing. This specific piece has been proposed to be an indication of early domesticates on Zealand; however, based on morphological observations, a *cervid* identification cannot be discounted.

5.4 Lipids

Cooking vessels have proved to be remarkable receptacles of ancient biomolecules in the forms of fats and waxes from food contents preserved on the inner surfaces (Craig et al. 2004). With the use of gas chromatography mass spectrometry (GC-MS), it has become possible to characterise various lipid biomarkers such as pine resin (Heron and Pollard 1988), epicuticular plant waxes (Evershed, Heron and Goad 1991), beeswax (Evershed et al. 1997) and aquatic foods (Lucquin et al. 2016). Two pivotal studies by Craig *et al.* (2007, 2011) have revealed the continued presence of aquatic remains from the charred food crusts in Neolithic bog pots (votive pots placed in lakes presumably as offerings). This study was in stark contrast to the notion that Neolithic farmers completely abandoned the utilisation of marine resources (Tauber 1981; Richards, Schulting and Hedges 2003). And recently, Ertebølle ceramics from Syltholm were analysed by GC-MS. Biomarkers from aquatic and ruminant fats was observed. Interestingly, potential milk fats could were detected, however, the authors are hesitant to conclude the find as definitively milk, as a mixture of ruminant and aquatic lipids can result in false positives (Papakosta, Oras and Isaksson 2019). This seems like a valid explanation, although milk consumption would be an interesting observation.

5.5 Ancient DNA

Hitherto, very little work on ancient DNA has been carried out for the south Scandinavian Stone Age, although this will undoubtedly change over the coming years.

By having little or no ancient DNA to compare against, the first studies were predominantly compared against modern populations.

Some of the first studies to explore the use of ancient DNA were predominantly focused on whether the Pitted Ware Culture (PWC), a group existing in parallel with Neolithic farmers (TRB for *Trichterbecher Kultur*), and how or if, these two groups were related. The first study was done by Malmström *et al* (2009), where the main hypothesis was to establish if there was a genetic relationship between the PWC and TRB individuals. None were found, and it was therefore interpreted as evidence for population replacement, since the PWC were more genetically related to extant eastern Baltic populations (Malmström et al. 2009). Another point arguing for discontinuity, was that nine out of ten individuals of the PWC were lacking the lactase persistence genotype (i.e. inability to breakdown the lactose from nonhuman milk in adulthood) (Malmström et al. 2010). However, as noted elsewhere, the ability to be able to enzymatically breakdown milk sugars, does not say much about dairy ingestion, as dairy products can be consumed if processed to remove the milk sugar lactose (Gron and Rowley-Conwy 2018), for instance by cheese-making or fermentation (Alm 1982).

Another study, by Skoglund et al. (2012) analysed ancient DNA from three individuals from the Neolithic Pitted Ware Culture and one Neolithic farmer. They showed that the single Neolithic farmer shared the majority of its ancestry with southern Europeans, and thus argues for genetic discontinuity. A later study expanded more or less on the former, but also included a single Mesolithic individual and sequenced parts of their nuclear DNA (Skoglund et al. 2014). They found that there were still differences between the PWC and TRB, but that there also seemed to be some genetic admixture, notably between the TRB individuals from Gökhem and hunter-gatherers. They also showed that the PWC had markedly lower genetic diversity, indicating a smaller and more restricted group (Skoglund et al. 2014). This is perhaps not surprising, as the PWC individuals came from an island in the Baltic. They were also able to infer phenotypic traits of two of the individuals, where one of the PWC individuals from Ajvide, had carried the ancestral allele at polymorphism SLC45A2 which is indicative of dark pigmentation. In contrast, the Gökhem 2 individual carried the derived allele, associated with light pigmentation (Skoglund et al. 2014).

In general, aDNA is an incredibly powerful analytical method, and allows investigation of population histories, phenotypic traits, genetic affinities, and kinship to name a few. However, most of these objectives are only achievable by sequencing nuclear DNA.

While all these studies are of high quality, and were indeed technological breakthroughs, there are some inherent problems associated with them. One is, that the sample sizes were very limited, and only mtDNA was sequenced. Another is, that only one individual from the Mesolithic was actually sequenced. Lastly, it is also of the utmost importance that there is synergy between the two disciplines (archaeology – genetics) i.e. the archaeology is fundamental to the contextualisation of genomic data.

In Skoglund et al. (2012), the authors sought to ‘close the case’ regarding the Neolithic transition. However, this complex event can not be solved with only four to eleven mtDNA genomes. Many more individuals from relevant time periods would need to have their entire genomes sequenced if the evidence is to be considered robust in light of the 150 years of archaeological research on this particular topic, something that has previously been touched upon by Mikkel Sørensen (2014).

A recent study conducted on 24 individuals interred in Megalithic tombs across Europe (including previously studied individuals from Gökhem), showed that the individuals buried in these tombs were of pronounced Neolithic ancestry, but that some of the males showed admixture with hunter-gatherers and also that the interred individual were also predominantly males (Sánchez-Quinto et al. 2019). The term “Scandinavian hunter-gatherer” (SHG) was established by sequencing seven individuals from the middle Mesolithic site of Motala in Sweden. Here it was shown that the Motala individuals represented a mixture of two groups, namely the Western Hunter-Gatherers (WHG) and the “Ancient North Eurasian” (ANE) (Lazaridis et al. 2014).

Günther et al. (2018) sequenced seven individuals from the Early Mesolithic in Sweden and Norway, and showed all the individuals had mixed ancestry of both continental Western Hunter-Gatherer (WHG) and Eastern Hunter-Gatherer groups. This fits well with previous archaeological studies that proposed that the spread of pressure blade technology came from present day Russia, and entered the Scandinavian peninsula around 10,000 years ago (Sørensen et al. 2013). The latest study on Early Mesolithic ancient DNA, is by Kashuba et al. (2019). Similar, to what is presented in Chapter 5, they also extracted DNA from chewed birch pitch. Their material derived from the coastal site of Huseby Klev, and corroborated what was shown by Günther *et al.* (2018), that some of the earliest Scandinavians, were admixed with ANE. This aligns with the presence of pressure blade lithic technologies from the site.

Paradoxically, only two Stone Age aDNA studies have been published on Danish material. The first was done by Melchior *et al.* (2010) who pyrosequenced mtDNA from 56 individuals spanning all archaeological time periods. They found that individuals from the late Neolithic (*n* = 2) and Bronze Age (*n* =1) carried the U haplogroup, which is argued to be associated primarily with hunter-gatherers. This led to the reasoning that there was no support for a population replacement by Neolithic farmers (Melchior et al. 2010). The second study sequenced the Early Mesolithic Koelbjerg woman, and showed that 'she' was actually a male (Hansen et al. 2017).

Fig. 6 Uffe Seneca and Egon Iversen (Columbus) with their hands full of bone points found during field recognisance in 1989. Due to the removal of several metres of peat, large areas of present- day agricultural fields constitute what was once the bottom of Mesolithic lakes used for fishing (photo Anders Fischer).

Chapter 3: Barbed bone point chronology reveals a radiocarbon hiatus at 10.2 ka during the Early Mesolithic in southern Scandinavia

Draft manuscript.

Barbed bone point chronology reveals a radiocarbon hiatus at 10.2 ka during the Early Mesolithic in southern Scandinavia

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Abstract

The extensive peat bogs of southern Scandinavia have yielded rich Mesolithic archaeological assemblages, which has informed prehistoric studies for more than a century. Central to this has been the first recognisably Mesolithic culture, the Maglemose, first described in 1903 which has become a yardstick against which all other early Mesolithic cultures have been compared. Despite the excellent preservation of organic material, we have for the first time conducted a combined investigation of the typology, species composition and absolute chronology of Maglemose bone points. A demonstrable and significant change in barb morphology can be directly linked to a significant paucity of finds in Southern Scandinavia around 10.2 cal ka BP. Peptide mass fingerprinting (ZooMS) reveals that the majority of bone points are made from cervids and bovines. The ribs of bovines; for instance, are more frequently utilised following the hiatus. Furthermore, the marked change in barbed bone point morphology coincides with a change in lithic technology. This change in material culture has been shown to arrive archaeologically with eastern pioneers and colonisations through Fennoscandinavia. We therefore propose that the Maglemose Culture in southern Scandinavia is fundamentally divided into an Early Complex (11.6 - 10.4 kyr) and a Late Complex (10.4- 8.5 kyr): the former archaeologically characterised by percussion blade production and "fine-barbed bone points" and the latter archaeologically characterised by the innovations of pressure-blade production and "larger barbed bone points". Finally, through these integrated analyses we are able to show that a single artefact type can be used as a proxy for human populations.

Introduction

The temporal division of the Stone Age of Southern Scandinavia is one of the oldest cultural chronologies in the world. It was initially based upon a combination of archaeological, geological, and palaeobotanical observations. This chronological assessment was subsequently refined by a seriation of lithic artefacts from occupation sites that were dated palaeobotanically. Christian Jürgensen Thomsen was the first to establish the three period paradigm of the Stone, Bronze and Iron Age model (Thomsen 1836). Soon after, the 'First Kitchen Midden Commission' was able to conclude that ancient shell accumulations had an anthropogenic origin (Steenstrup 1852). In 1859, based on this research Jens. J.A. Worsaae divided the Stone Age into an "older" and a "younger" period thus, indirectly recognising a hunter-gather (Mesolithic) and an agricultural (Neolithic) Stone Age (1861). The older stage was subsequently termed Ertebølle Culture after a key site, excavated by the 'Second Kitchen Midden Commission' (Madsen et al. 1900). An even older stage of the North European Stone Age, The Maglemose Culture (11 - 8 ka BP), was soon after defined by Georg F. L. Sarauw, based on his seminal excavating at the Mullerup site in the bog called Maglemose (Sarauw 1903). The term Mesolithic was first applied in Southern Scandinavia by Erik Westerby (1927). During the mid 20th century the first detailed typochronologies of the Maglemose culture, based on lithic seriation, were established and subsequently refined (Becker 1951; Petersen 1966, 1973; Skaarup, Fredskild and Møhl 1979; Johansson 1990; Sørensen 2006).

The biological and geological record of the transition from the Late Glacial to the Early Holocene are manifested by a dramatic change in vegetation due to warming of the climate (Jessen et al. 2015). With the warming vast amounts of buried dead ice gradually melted forming small water filled depressions (kettle holes) especially in the eastern Denmark. The landscape in the early Holocene were therefore characterised by numerous shallow lakes and ponds in a relatively open birch and pine dominated woodland. (Mortensen et al. 2014). This was to become indispensable hunting and fishing grounds for the first Maglemosian people living in Southern Scandinavia.

Throughout the Early Mesolithic barbed bone points were frequently lost in connection with spearfishing in the lakes, which were gradually overgrown with peat. Fuel shortages, especially during the First and Second World Wars, resulting in industrialised peat extraction in peat bogs, caused these points to be uncovered and recognised for what they were (Sarauw 1903; Broholm 1924; Mathiassen, Troels-Smith and Degerbøl 1943).

The barbed bone points came to be closely associated with the Maglemose Culture. However, despite a long history of research on the typology of these characteristic items (i.e Clark 1936; Verhart 1990; Larsson, Sjöström and Nilsson 2019) their chronological placement has largely been indirectly deduced from stratigraphy and pollen dating (Broholm 1924; Andersen 1983). Species identification from bone points has previously been inferred based upon such evidence as bone debitage from habitation sites, from the absence of specific skeletal elements in an assemblage, and on specific osteological metrics of the points themselves (i.e. Sarauw 1903; David 1999 p.278; Leduc 2012). However, more often than not, the exact metrics used to identify a specific species are not provided. Based on these types of analyses, the majority of the Maglemosian bone points were thought to have been made from both ribs and long bones of "large ungulates", translating to aurochs, elk, red and roe deer (David 2019).

We conducted an analysis of 120 bone points from Southern Scandinavia (Fig. 1 and SI 1). By combining morphological typology, proteomic, isotopic, and radiocarbon analyses, we are able to assess the selection of raw materials and to date typological variation. This is the first attempt to investigate whether a single artefact type can be used as a proxy for human populations. We demonstrate that such comprehensive analyses can unravel information about species dynamics, culturally entrenched manufacture, and perhaps population mobility. Such integrated analyses provide a robust new framework for Maglemose chronology including changes in lithic manufacture.

Results

Bone points

In the present study, the neutral term 'barbed bone point*'* is used as a descriptor of the artefact under consideration, as opposed to functional laden descriptors, such as; leister- points or prongs. While the latter idioms are not inaccurate *per se,* they denote that the bone points are part of a composite system in which more than one barbed bone point is fastened to a shaft and their functional usage (Andersen 1983, 159). Their function was undoubtedly fishing gear, but evidence for composite leisters is only circumstantial (i.e. the proposed leister sets from Fulge Å and Mosegården III in Åmosen (Andersen 1983, 165)).

Local typochronologies have been proposed for two sites in Sweden, i.e. Rönneholms mosse (Larsson, Sjöström and Nilsson 2019) and Motala (Gummesson and Molin 2019). But since the present study comprise material from a much wider geographical region, including Rönneholm, it is presently not possible to sort the bone points into specific types, valid for the whole geographical range of the study. This may very well be possible for the Danish material, if the focus is on a restricted area. To not bias our study we therefore, only considered two main traits; i.e. fine-barbed bone points and larger-barbed bone points.

Radiocarbon dating

Prior to this study, only four bone points from Denmark had been directly radiocarbon dated (Fischer 1996, 2003). We submitted a further 23 bone points for radiocarbon dating. Two failed due to low collagen yields. 21 returned dates spanning the Preboreal, Boreal, and the beginning of the Atlantic chronozones, while two turned out to have C/N values bordering the accepted and should be treated with caution (C/N values >3.6, see Table 1). We merged our successfully dated bone point results with 19 dates from Sweden published by Larsson *et al.* (2019), the five published by Fischer (1996, 2003) and six previously unpublished dates from NW Zealand (1, Sønderød; 2, Mørke Enge; 1, Ulkestrup Øst IV; 1 Ulkestrup Lyng) and a slotted bone point from Fugle Å. The total of 51 AMS dates were separated into two phase models corresponding to morphology (i.e. fine-barbed vs. large-barbed) and calibrated into cal. years BP in OxCal v.4.3 (Ramsey 2017).

There is a clear temporal divide between the two morphologically distinct types of bone points. The fine-barbed bone points are confined to the mid-late Preboreal and cover the beginning of the Boreal (11,212 - 10,157 cal BP). Between the fine-barbed bone points and the larger-barbed bone points, there is an apparent hiatus in the radiocarbon ranges lasting nearly 600 years. The larger-barbed bone points are confined to the end of the Boreal and the beginning of the Atlantic chronozones (9,658 - 8,413 cal BP). In order to explore this hiatus, we further summed the AMS dates and performed a kernel density estimation (KDE) simulation to explore periods of activity. There does seem to be age correlated tendencies of certain bone points from both regions, which could indicate morphological patterns in time and space.

To further explore the presence or absence of animal species available for the production of barbed points, published and unpublished radiocarbon dates from remains of Eurasian elk (*Alces alces*; $n = 73$), red deer (*Cervus elaphus*; $n = 33$), and bovines (*Bos primigenius* and *Bison bonasus*; $n = 73$) from the study area were compiled. The results indicate declining populations of all the species around c. 10 cal. ka BP (See SI Fig. 23 and SI 2.2) in eastern Denmark. It should be noted that a corresponding decline was not visible in the dates from southern Sweden. Lastly, we also gathered radiocarbon dates from material associated with habitation. Here, there also seems to be a gap in dates from habitation sites in Eastern Denmark, but not in SW Sweden (see SI Fig. 24, and SI 2.3).

Isotopes

Twenty-three carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) bone collagen isotope ratios were obtained. Using ZooMS eight of these were attributed to aurochs or bison (henceforth referred to as bovines) and eleven to Eurasian elk or red deer (henceforth referred to as cervids) (Fig. 3). All samples fell within the acceptable atomic C:N range of 2.9-3.6 (DeNiro 1985), indicating a low likelihood of diagenesis. However, four of these $\delta^{13}C$ values (Ox-38342, 38343, 38337, 38341), dating to the period prior to the hiatus, diverge from the main dataset and must be treated with caution. In a broader context, and with available contemporary archaeological northern European large herbivores, these values are substantially more negative (Bocherens et al. 2015). Both the cervids ($\delta^{13}C$ -24.3‰ and lower) and bovine sample (-25.1‰) are lower than values obtained on the same taxonomic groups for the entirety of the Eurasian Holocene (Hofman-Kamińska et al. 2019). Substantial divergence from the main dataset is not typically a reason for caution; however, there are no recorded archaeological $\delta^{13}C$ values from northern Europe lower than -24.31‰ for the taxa in question (dIANA database, https://oasisnorth.org/diana.html, accessed 3/9/2019). For these reasons, these four values will not be discussed further, but we acknowledge that they are exceptionally low (but see SI 7 for extended discussion).

The remaining bovine (*n*=11) and cervid (*n*=8) $\delta^{13}C$ values range from -24.3 to -21.7‰ (mean -23.2‰) and -23.6 to -20.8‰ (mean -21.9‰) respectively. $\delta^{15}N$ values have a broader range, with bovine and cervid values between 1.5 to 6.3‰ (mean 4.8‰) and 1.7 to 7.2‰ (mean 4.3‰). There is a net decrease in average $\delta^{13}C$ before and after the hiatus in both bovines (-21.7 to -23.4‰) and cervids (-21.4 to -22.5‰), whilst average bovine $\delta^{15}N$ increases (2.7 to 4.8‰) and average cervid $\delta^{15}N$ decreases (5.2 to 3.4‰). In comparison with 329 well-dated large herbivore bone collagen carbon and nitrogen isotope values (Fig. 3) from across northern Europe recently published by (Hofman-Kamińska et al. 2019) there is a similar net decrease in $\delta^{13}C$ on either side of our apparent hiatus. However, our $\delta^{15}N$ data do not clearly relate to any long-term processes as proposed for the North European material by (Hofman-Kamińska et al. 2019), and no consistent inter-taxonomic trends are discernable (Fig. 3).

Proteins

120 barbed bone points were analysed by ZooMS (2016) (see SI 3.4.1 online resource for all identifications). They turned out to derive from three groups of animals: 77 from cervids (*Cervus elaphus*/*Alces alces*), 40 from bovines (*Bos primigenius* or *Bison bonasus*) and 3 from brown bear (*Ursus arctos*) (Fig. 4).

In the same way that we cannot distinguish between *B. primigenius* and *B. bonasus* (Buckley and Collins 2011), *C. elaphus* and *A. alces* could not be discriminated using the published markers of Welker *et al.* (2016). However, collagen sequence analysis revealed five single amino acid polymorphisms (SAPs) between red deer and elk resulting in five potential tryptic peptides (biomarkers) (SI Table 1 and SI 4.1). In three of these biomarkers one or both sequences also matched 100% to environmental bacterial sequences as identified using BLASTp and were discarded (see SI 4.2).

LC-MS/MS analyses of four reference samples (two of red deer and two of elk) confirmed that the two remaining biomarkers can be used to discriminate between the two species. A2T66 (Red deer - **S**GETGASGPPGFAGEK; Elk - **T**GETGASGPPGFAGEK) was present in the LC-MS/MS data, but peaks corresponding to the masses of these peptides were not visible in any of the MALDI spectra, making them useful for discrimination by LC-MS/MS, but not ZooMS. A2T66/67 (Red deer - GETGPAGR**P**GEVGPPGPPGPAGEK; Elk - GETGPAGR**A**GEVGPPGPPGPAGEK) contains a SAP after a tryptic cut site. A proline after a tryptic cut site is known to increase the frequency of missed cleavages at the site (Rodriguez et al. 2008). This is observed in the LC-MS/MS data with a high percentage of missed cleavages in the red deer peptide, but none in the elk peptide. In the MALDI spectra, the mass corresponding to the missed cleavage of the red deer tryptic peptide (m/z 2216) is visible and does not correspond to the mass of any known common contaminants. The red deer sequence for this region is identical to the aurochs sequence and m/z 2216 is also present in the aurochs ZooMS spectra. Therefore, we use m/z 2216 as a biomarker for red deer after previously published markers have grouped the samples into the red deer/elk taxonomic group. However, peaks corresponding to the masses of the same peptide for elk were not visible in any of the MALDI spectra so we cannot correspondingly confirm that samples not having the m/z 2216 are elk using ZooMS. Of the 77 bone points identified as either red deer or elk, 22 could thereby be assigned to red deer.

In addition, we also sequenced four bone points by LC-MS/MS, two from the Preboreal (VHM13821 and A37811) and two from the Boreal (A40894 and A42422), looking for the key tryptic peptides we identified above (see SI 3.5). The two samples from the Preboreal have the diagnostic peptides for elk while the two from the Boreal have the diagnostic peptides for red deer and show the characteristic red deer mass in their corresponding MALDI spectra.

Discussion and conclusion

Bone points and species utilised

Our protein mass spectrometry results reveal differences between fine-barbed (i.e. Preboreal and Early Boreal) and larger-barbed points (i.e. Late Boreal). Fine-barbed points (*n* = 45) are predominantly manufactured from cervid long bones (see fig. 3). As there is evidence for the presence of other large ungulates (aurochs, bison, reindeer and horse) in Southern Scandinavia at this time (Ekström 1993; Aaris-Sørensen, Mühldorff and Petersen 2007/6; Aaris-Sørensen 2010a), this preference for cervids suggests deliberate choice amongst the early Maglemosian hunter-gatherers, especially if procurement was associated with increased costs - i.e., search time - potentially reflecting smaller population sizes in the other species. On the other hand, it may also simply be a reflection of the species availability at the time. Since similar fine-barbed bone points dated to the Late Glacial have been recovered from the bottom of the North Sea and in Central Continental Europe, this type can belong to an earlier tradition (Gramsch 2004).

Following the proposed radiocarbon hiatus, the morphology changes to predominantly larger and a more varied barb design. In the large-barbed bone point (*n* = 75) assemblage, the number of bone points made of bovine ribs increase significantly (Fig. 4). In addition, bone points manufactured from brown bear appear for the first time. All confirmed bison remains from Denmark and southern Sweden have been radiocarbon dated to no later than the Preboreal (Ekström 1993; Aaris-Sørensen 2010b). This seemingly leaves aurochs as the sole persisting bovine species and assumed source of bone points produced after the Preboreal.

Only 3 out of 42 ribs used to manufacture points were from cervids, the remainder (93%) were made from aurochs. The bone points made from the ribs of aurochs, represent the very last remnants of this species before their disappearance on the Danish islands c. 9000 cal BP (data from Ulkestrup Lyng; (Aaris-Sørensen 1980; Noe-Nygaard, Price and Hede 2005). The selection of aurochs rather than cervid ribs probably reflects its preferable mechanical properties (see SI). It could therefore be argued that selection of the raw materials by Maglemosian hunter-gatherers was based upon practical considerations, rather than spiritual (see McGrath et al. 2019).

The dates of the majority of the large-barbed bone points fall within the Maglemose period, while seven have radiocarbon ranges spanning a few hundred years into the Kongemose period (c. 8,500 - 7,400 cal BP), after which simple bone points without barbs seems to have been preferred (at least in central Scania) (e.g. Sjöström 2011, 2014). The transition from large-barbed bone points to simple bone points, seems to be synchronous with the arrival of trapezoid lithic armaments (Fischer 1994; Sjöström 1997). This change in material culture may reflect a change of economy and seasonal rounds of the local population: coastal and inland groups merging within Southern Scandinavia as sea levels rose; seasonal spearfishing in the lakes losing its importance by communities that relocated their demographic centers to the coasts during the Kongemose Period (e.g. Fischer 1997; Fischer et al. 2007).

A potential cause for the hiatus

Several local studies have revealed climatic fluctuations of the Northern Hemisphere during the Early Holocene including lower temperatures and increased precipitation (Bond et al. 1997; Brathauer et al. 2000; Björck et al. 2001; Dahl et al. 2002; Nesje, Dahl and Bakke 2004; Bakke, Dahl and Nesje 2005; Nesje 2009; Berner, Koç and Godtliebsen 2010; Balascio and Bradley 2012).

The hiatus, centered around 10 cal. ka BP, seen in the barbed bone point dates from Southern Scandinavia, seems to be synchronous with similar events in other parts of Europe. In the Eastern

Fennoscandian a c. 200 years long gap seems to occur shortly after 10.3 cal. ka BP, which is suggested to be a climatic backdrop that lead to a decline in local population (Manninen, Tallavaara and Seppä 2018). The region was probably recolonised after 10.1 cal. ka BP. In Germany, there also seems to be a gap in the radiocarbon dates performed on human skeletal remains (Grünberg 2016), and a decline in radiocarbon dates is also observed in north-western Europe (Crombé 2019). Interestingly, in north-western Europe it is hypothesised that human populations declined during this period, due to increased droughts and severe wildfires (Marlon et al. 2013; Crombé 2019).

To our knowledge, no evidence of a similar cool interval or increase in precipitation between c. 10 to 10.3 cal. ka BP has been reported for Southern Scandinavia. This may be due to a lack of high-resolution studies of sediments from this period coupled with a sampling bias and further complicated by contemporaneous erosion events (Jørgensen 1963; Noe-Nygaard 1995; Noe-Nygaard et al. 1998). High-resolution studies of sediment cores conducted in southern Sweden, however, does not show an increase in precipitation in contemporary lake levels (e.g. Gedda 2001; Digerfeldt et al. 2013). Conversely, they indicate the lowest water levels of the entire Holocene during the early Boreal (Gaillard 1985; Gedda 2001; Digerfeldt et al. 2013), as well as sporadic forest fires (Olsson et al. 2010) The observed process of lakes drying and filling up with organic sediment is believed to have begun earlier in eastern Denmark than in southern Sweden (Welinder 1978). The northern advancement of hazel at this time (e.g. Jessen et al. 2015) is believed to have been facilitated by the markedly lowered water levels in the lakes and fens (Ekström 1993).

Pollen analytical correlations from the Aamose basin on Zealand and Rönneholms mosse in Scania, also revealed several dramatic water-level changes that occurred during the Early Holocene (Jørgensen 1963; Noe-Nygaard 1995). These studies are largely based on pollen zoning. During the transition from the Preboreal to the Boreal, a brief lowering of the water-levels took place yet again (Nilsson 1935). In Aamosen, this regression lead to erosion of the littoral zone and redeposition of sediments elsewhere (Noe-Nygaard et al. 1998). The reason for these water-level fluctuations are difficult to determine with certainty. One factor during the Early Preboreal could be the melting of stagnant ice; whereby static glaciers melt *in situ*. Temperature oscillations could also account for some of these alterations, with dryer climate resulting in increased evaporation. In addition, beavers can engineer water levels with their dams, resulting in significant changes; not just fluctuations during construction, but also during periodic dam bursts, albeit at a local scale.

Potential impact on humans - bust and boom?

As shown in fig. 2, there is a gap in radiocarbon dates from c. 10.200 - 9.600 cal BP in the dated barbed bone points. This gap also divides some of the *classic* Maglemosian habitation sites in Eastern Denmark (see Supplementary Figure 24, and SI 2.1). A similar gap in radiocarbon dates of mammalian remains further indicates a decline in fauna in Eastern Denmark, but interestingly not in Scania (see Supplementary Figure 23, and SI 2.2). This gap in radiocarbon dates during the Early Holocene might be interpreted as evidence for a decline in human habitation within eastern Denmark. Several factors could have caused a small and confined human population to disappear from the archaeological record; i.e. epidemics, warfare, changes in subsistence strategy, climate and migration. However, most of these factors are difficult to tie to the disappearance of bone points in the South Scandinavian lakes. One hypothesis is that this radiocarbon hiatus reflects an exodus of humans from the lakes of Zealand and Scania in a bust cycle. This would have resulted in a boom elsewhere, e.g., in Northern Germany and along the coasts of western Sweden, where sites and assemblages can be certainly dated to this period (see below). Other explanations for the hiatus could concern climatic, environmental, and taphonomic factors. A series of studies have demonstrated fluctuations in water levels in lakes during the Boreal period, generally showing that water levels dropped significantly during the early-mid Boreal period (i.e. Digerfeldt et al. 2013). This drop in water levels could have dried out the lakes for a period in eastern Denmark. These dry conditions would almost certainly have impacted the fishing opportunities for the human population, but also the preservation of organic material.

From Denmark there is weak evidence for human habitation during the ‘bone point hiatus’. The ^{14}C dates from the two habitation sites of Draved and Klosterlund in Jutland fall before and during the hiatus (Tauber 1966, 1973). However, these radiocarbon dates are conventional and from the very early days of radiocarbon dating when samples were not cleaned for secondary humic acids. A factor that can often result in misleadingly young dates (Fischer 2002). Five AMS dates from bone harpoon points date to the hiatus in southern Scandinavia. These are from Tunebjerg Øst (9050 ± 40 ^{14}C BP) and Trunderup Mose (8845 ± 60 ^{14}C BP), both sites on Funen (Andersen and Petersen 2009), from Rönneholms mosse in Scania (8610 ± 90 ^{14}C BP) (Larsson 1996) and from Vallensgaard Mose on the island of Bornholm in the East Baltic Sea (9250 ± 60 ^{14}C BP, 8875 ± 65 ^{14}C BP) (Sørensen and Casati 2015). It must be noted that Bornholm was at this time a part of continental Europe. The presence of harpoon bone points during the Mesolithic has been strongly tied to the hunting of marine mammals, although, the fact that they ended up in lakes may indicate a more varied use (Petersen 2009).

On the other hand, habitation sites from the Swedish west coast; Huseby Klev and Balltorp, are dated to the bone point hiatus in southern Scandinavia (Nordqvist 1995, 2000; Johansson 2014). The subsistence at these sites was focused mainly on marine mammals, marine fish, and coastal birds (Boethius 2018). The coastlines of the Early Holocene in southern Scandinavia became submerged during the Atlantic period due to rising sea levels (see SI 5), meaning that Early Mesolithic coastal sites are rarely located and excavated in these regions (Astrup 2018; Fischer and Petersen 2018). Consequently, it is not possible to study and compare coastal settlements which could corroborate a shift in economic strategy and subsistence on a regional scale during the Early Mesolithic in southern Scandinavia.

Human exploitation of marine resources along coastal environments in southern Scandinavia can be inferred from carbon and nitrogen values of collagen from human remains. Individuals from Køge Sønakke off the coast of eastern Zealand in Denmark (Fischer et al. 2007), Österöd (Ahlström and Sjögren 2007) and Huseby Klev (Nordqvist 2000; Ahlström 2003) from the south western coast of Sweden are contemporaneous with the identified hiatus. These skeletal remains have yielded δ^{13}C and δ^{15}N values consistent with partially or fully marine diet (SI 2.3). If a majority of lakes in eastern Denmark did dry up in this period, it is conceivable that humans in the area moved out, although we do not have much evidence for coastal activity from the study area, apart from the inferred stable isotopes values. The presence and continued use of fine-barbed bone points during the hiatus in Northern Germany (Cziesla and Pettitt 2003; David 2019), could indicate that this region did not experience a severe population decline - or may even have received immigrants from Southern Scandinavia.

When large-barbed bone points appear following the hiatus (at 9658 - 9495 cal BP), they show markedly different morphological traits, from their predecessors. This new bone point morphology seemingly appeared only a few decades before the first indications of pressure blade industry in Denmark at c. 9000 cal BP (Sørensen 2018). However, based on a previously unpublished radiocarbon date performed on a slotted bone implement (Clarks Type B1 or B2) from Fugle Å, near Ulkestrup Lyng in Store Åmose (AAR-11949, 8360 ± 55 ^{14}C BP) (see Fig. 2 and 3c), this pushes the pressure flaking industry a few hundred years backwards, and is now synchronous with the emergence of large-barbed bone points. The pressure blade industry is thought to have originated at around 20 ka BP in the area of present day Siberia/Northern China from where it spread westward to western Russia (Desrosiers 2012), and subsequently into northern Fenno-Scandinavia (Sørensen 2012; Sørensen et al. 2013; Sørensen 2018). Results from archaeological studies on the spread of pressure blade technology fits well with genetic studies from Norway and Sweden. These have shown that the individuals involved in the spread of pressure blade technology, were genetically admixed between Western Hunter-Gatherers and Eastern Hunter-Gatherers (Günther et al. 2018), and thus indicate migration routes westwards from Russia into Scandinavia. Most recently, reanalyses of lithic remains attributed to the oldest sequence at Huseby Klev (deep pit) revealed the presence of pressure blade technologies, which are securely dated to the hiatus period and the decades after (10,040–9610 cal BP). Moreover, the same study also extracted aDNA from chewed pitch, and revealed that the people chewing pitch were of genetically admixed ancestry (Kashuba et al. 2019). This leap in technological advancement coupled with a changed bone point morphology and large scale regional analysis of radiocarbon dates from Western Sweden and Central Scandinavia (Damlien, Kjällquist and Knutsson 2018) favours the spread of this technology through Scania and into present day Denmark.

In summary, we have shown that bone points without direct provenance form excellent repositories of information. Radiocarbon dates revealed a hiatus separating the two classified types of bone points at c. 10.2 cal BP. The hiatus span c. 600 years and effectively divides the Maglemose period into two complexes, based on radically different technologies. The introduction of pressure blade technology is synchronous with the emergence of large-barbed bone points after the hiatus and may indicate transmission of influences from Sweden. Species identification by ZooMS and LC-MS/MS indicates that a conscious selection was carried out in regards to the preferred skeletal elements, rib or long bone, from specific species for manufacture.

Material and methods

The bone points analysed ($n = 120$) all derive from southern Scandinavia (Denmark and southernmost Sweden). The bone points were, based on barb morphology and skeletal element used in their manufacture (i.e. long bone versus rib), and to some extent species, divided into two groups (see SI 1). A subset of the bone points were further submitted for AMS radiocarbon dating ($n = 23$), proteomic analysis to determine the species of each artefact ($n = 120$), and carbon and nitrogen isotopic analyses ($n = 19$). This has resulted in five groups with eight subtypes extending from the Preboreal into the Atlantic Period, corresponding to the Maglemose culture and slightly beyond, into the subsequent Kongemose cultural period.

Radiocarbon dating

We submitted bone powder or bone fragments (average weight 100 mg) of 23 bone points from Denmark for AMS dating at the Oxford Radiocarbon Accelerator Unit based on their typological grouping. Of the 23, 21 were successfully dated and were subsequently merged with 24 published AMS dates (Fischer 1996, 2003; Larsson, Sjöström and Nilsson 2019) as well as five unpublished dates from Zealand. Two artefacts (FP1469: 9375 ± 45 ^{14}C BP, 9208 ± 55 ^{14}C BP, and Brokøb B: 7940 ± 65 ^{14}C BP, 7890 ± 65 ^{14}C BP) were dated twice.

The total of 51 AMS dates were then calibrated to cal. years BP in OxCal v.4.3 using IntCal3 calibration curve (Ramsey 2009, 2017). We also compiled 50 published and unpublished radiocarbon dates performed on charcoal and bone associated with habitation sites, and three dates on human remains not associated with habitation (SI 2.3), and 118 published and unpublished radiocarbon dates from faunal remains (elk $n = 33$, red deer $n = 12$, auroch and bison $n = 73$) spanning the Maglemose period to infer presence or absence (SI 2.2). We applied Bayesian phase modelling on the dates from habitations, assuming coeval age of habitation events.

Isotopes

Samples with the prefix "OxA" were analysed at the University of Oxford's Radiocarbon Accelerator Unit. Stable isotope measurements for samples OxA-38,090, 38,092, 38,199, P46206, 38,665, and X-2807-20 were performed on a Fisons NA2000 elemental analyser coupled to a PDZ Europa 20-20 Isotope Ratio Mass Spectrometer. The remaining samples were analysed on a Sercon elemental analyser connected to a Sercon 20-22 isotope ratio mass spectrometer. All samples were measured relative to an in-house l-alanine standard routinely externally characterised by third parties.

Samples with the prefix "Ua" were analysed at The Tandem Laboratory at Uppsala University also marked with an asterisk * in table 1. Isotope ratios were determined on a Elementar ISOTOPE select elemental analyser coupled to a Elementar isoprime precisION isotope ratio mass spectrometer. Measurement accuracy was checked against internal sorghum flour, wheat flour, and protein standards, which were calibrated against international standards IAEA-C7 and IAEA-C6 as well as an internal laboratory standard.

Proteomics

We performed ZooMS on 120 bone points from Denmark and Scania in southern Sweden using protocols from (Buckley et al. 2009; van Doorn, Hollund and Collins 2011) (see SI 3.4.1). Mass spectrometry was conducted on a Bruker MALDI-TOF-MS/MS instrument in reflector mode to acquire spectra from 800-3500 m/z. Taxonomic identification was completed using published markers

(Welker et al. 2016). As red deer (*Cervus elaphus*) and European Elk (*Alces alces*) cannot be distinguished with published markers (Kirby et al. 2013), we compared their collagen (COL1α1 and COL1α2) sequences and identified five single amino acid polymorphisms (SAPs). We then analyzed these SAAPs to see if they provided unique tryptic markers for ZooMS analysis (see SI 4.1).
To confirm our candidate ZooMS marker four reference samples (two from each species) were sequenced using LC-MS/MS (see SI 3.6). In addition, we sequenced the tryptic peptides from four bone points, two from the Preboreal and two from the Boreal (see SI 3.6).

Author contributions statement

TZTJ conceived the study. TZTJ collected and prepared samples. TZTJ and JO interpreted the radiocarbon data. TZTJ, KKR, MM, AJT and LTL, analysed samples for MALDI-TOF-MS and LC-MS/MS. KG interpreted the isotope data. AF and MFM provided unpublished radiocarbon dates. TZTJ wrote the manuscript with input from AS, AF, ER, TL, OB, KG, MS, MJC and the remaining authors.

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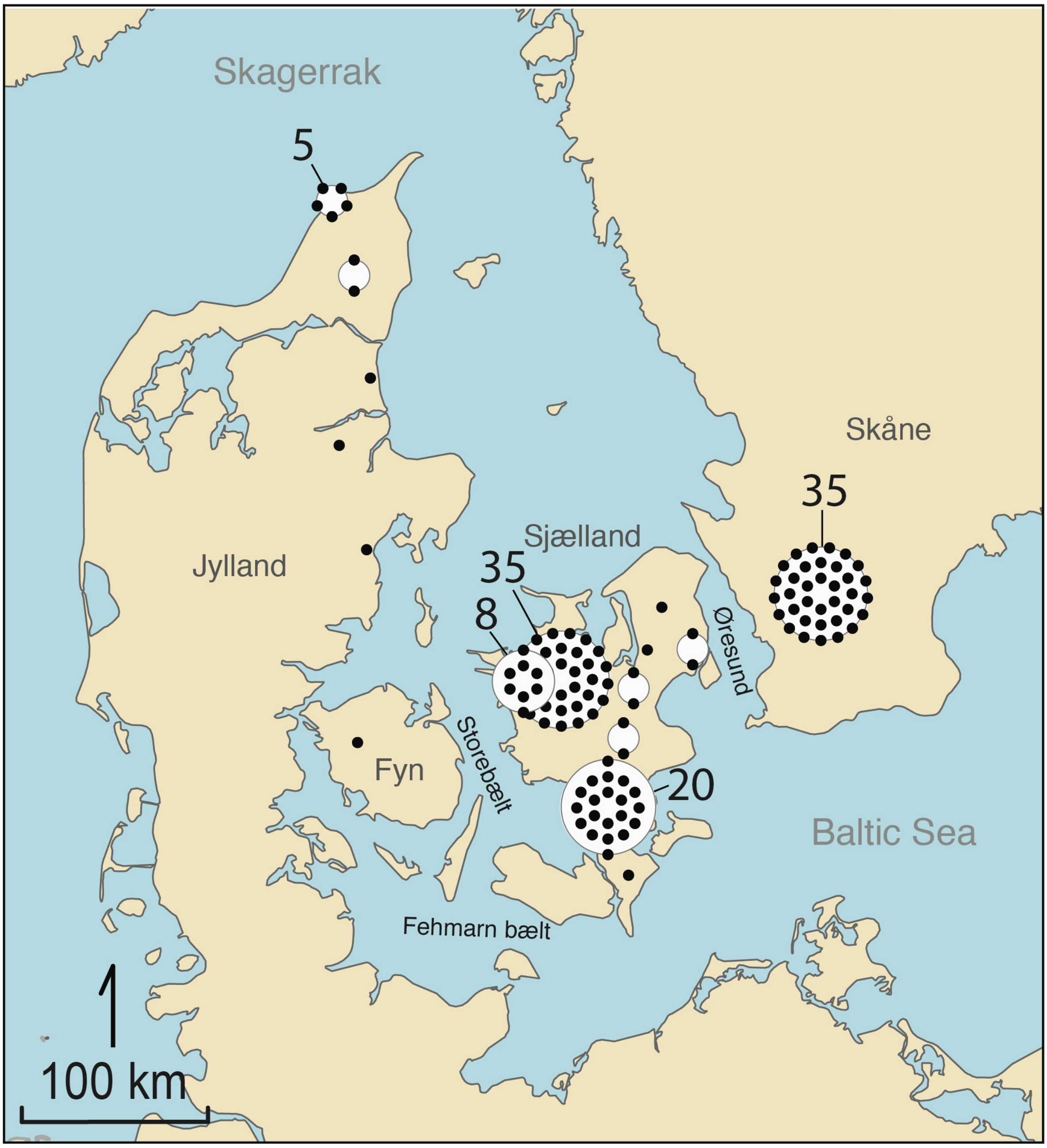


Fig. 1. Overview of approximate find spots for barbed bone points. Data points dispersed to avoid overlap (see SI 3.4.1 for more information on each artefact).

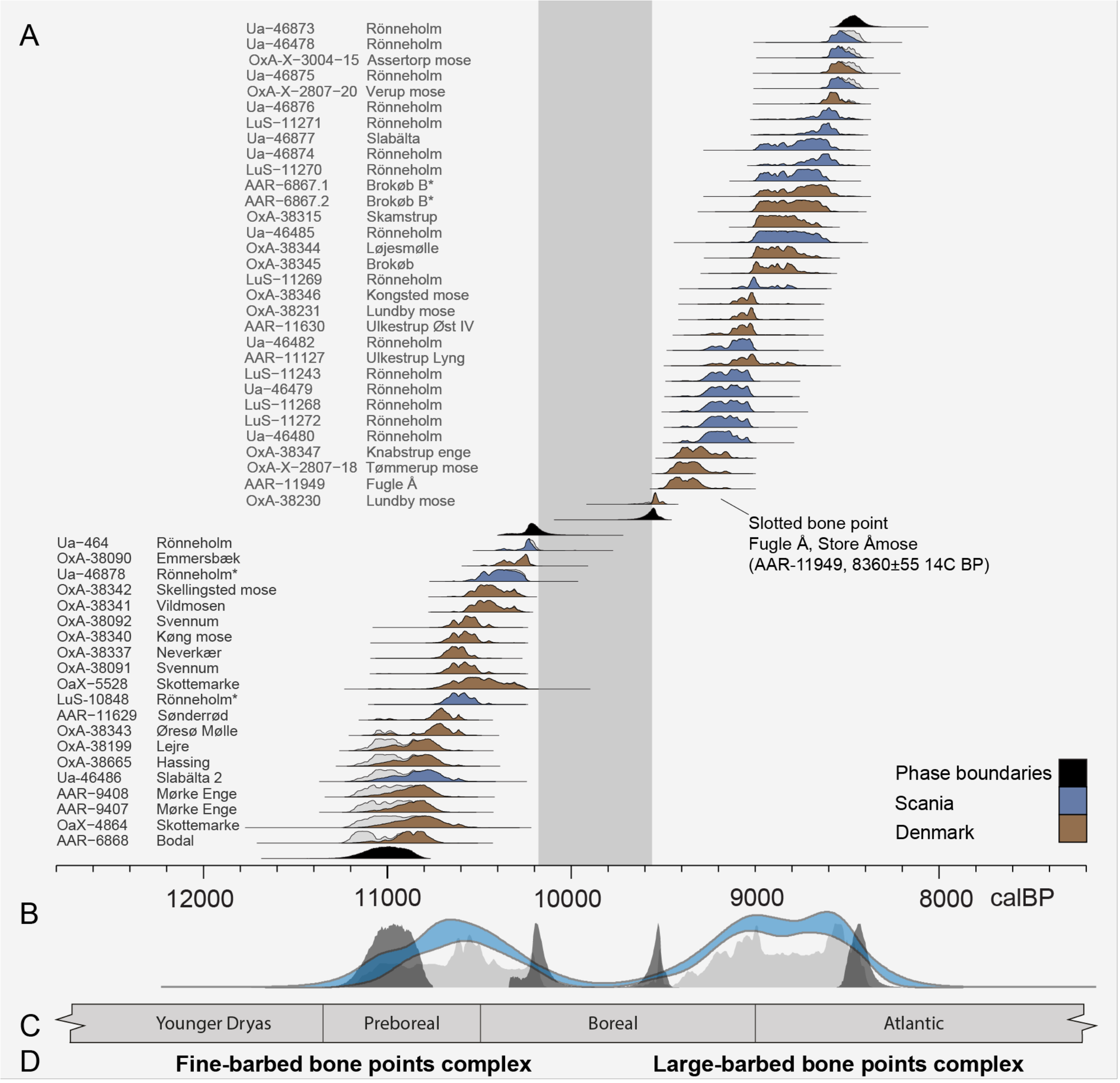


Fig. 2. A) Bayesian model assuming two phases, performed on 51 bone points. Double dates on same artefact marked with *. Carbon distributions colored to denote finding place or phase boundaries, B) Summed radiocarbon dates of bone points (shown in light gray), KDE to visualise activity and hiatus (light blue), boundaries marked in dark gray, C) chronozones (Preboreal onset after Jessen et al (2015), D) the two bone point complexes separated by hiatus.

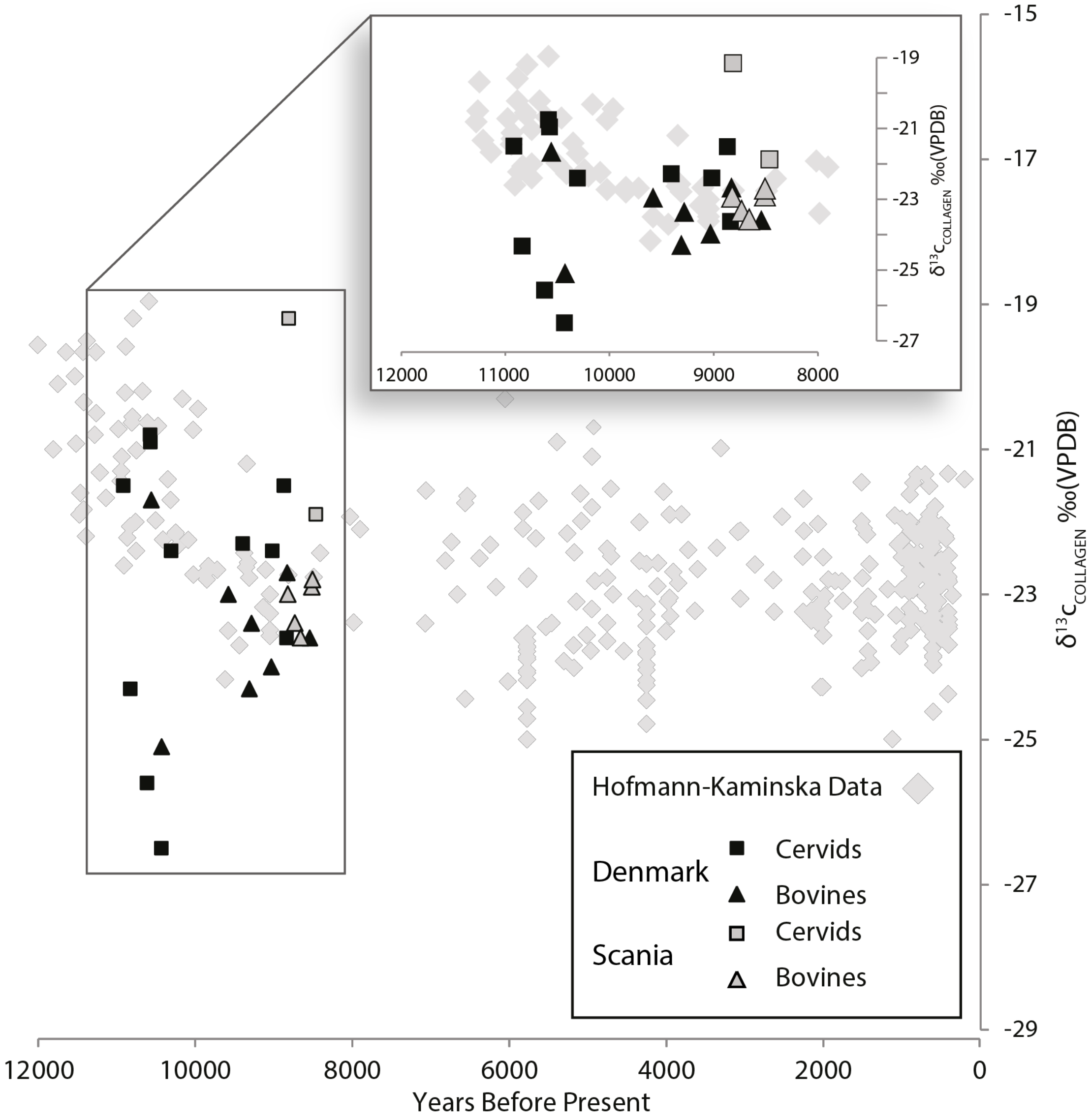


Fig. 3. Collagen $\delta^{13}C$ measurements obtained from bone points from Denmark and Rönneholm, plotted against data from Hofmann-Kamińska et al. (2019).

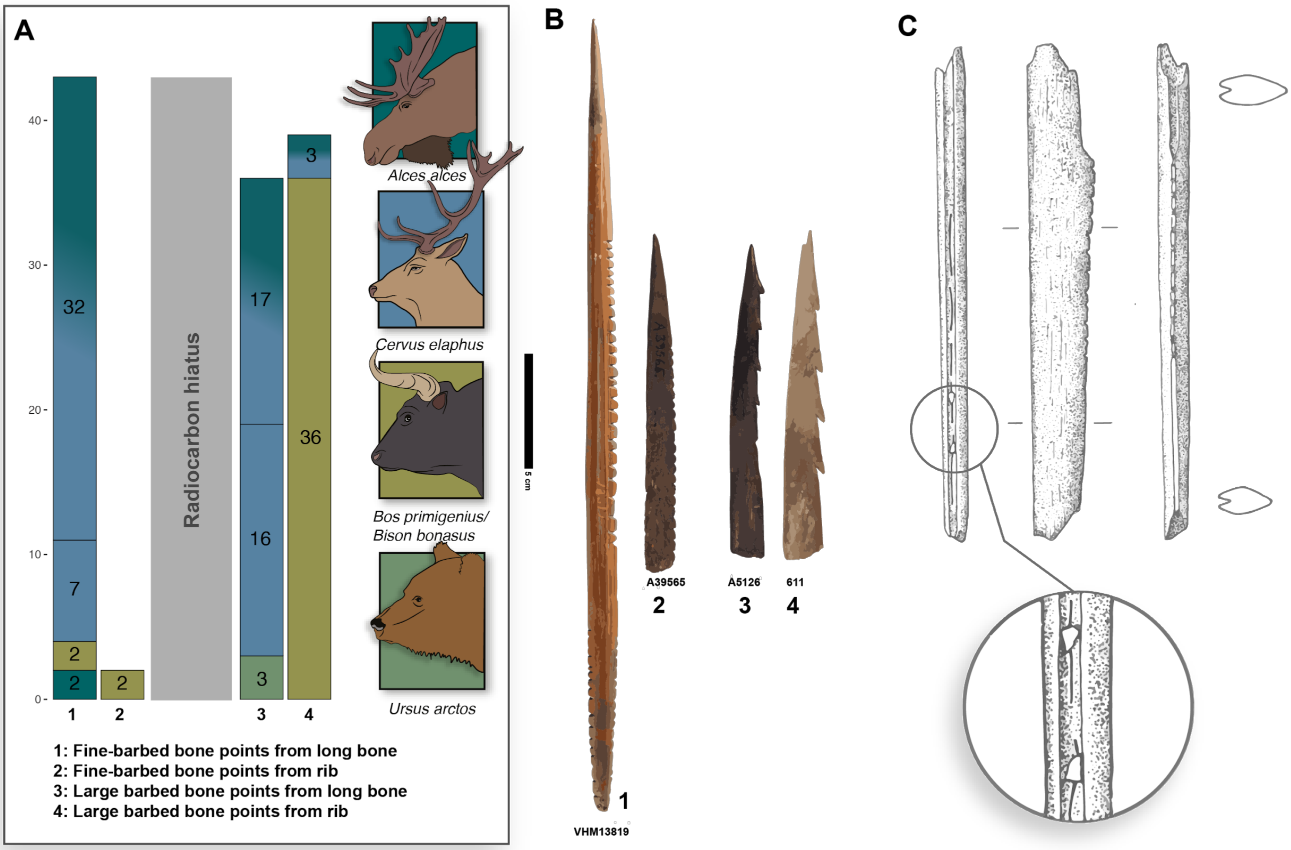


Fig. 4. A) Histogram of summed protein mass spectrometry identifications separated by radiocarbon hiatus. Each column refers to fine-barbed bone points made of respectively long bone and rib. Colours refer to stylized animal portraits. Gradient colours (blue vs. green) indicate either red deer or elk (see SI 3.4.1 for individual identifications), B) Selected bone points of each class, C) slotted bone point from Fulge Å, Store Åmose (drawn by Kurt Petersen, Kalundborg Museum in cooperation with Anders Fischer).

Table 1. List of 51 radiocarbon dates and carbon/nitrogen values used in this study. 14C ages calibrated to cal. years BP in OxCal v.4.3 using IntCal3 calibration curve (Ramsey 2009, 2017). Asterisk (*) after site name denotes double date performed on the same artefact. Asterisk after reference denotes remeasurements performed for this study of of δ^{13}C, δ^{15}N and C:N ratios conducted at the Uppsala Ångström Laboratory on material previously dated by AMS.

Lab Nr	**Site**	**Sample**	**14C yrs BP**	**Age cal. BP (95.4%)**	**δ^{13}C**	**δ^{15}N**	**CN**	**Reference**
AAR-6868	Bodal	AFi I 050700	9660±75	11212-10767	-	-	-	Fischer 2003
OaX-4864	Skottemarke	A20371	9570±100	11195-10603	-	-	-	Fischer 1996
AAR-9407	Mørke Enge	Lyster 10	9605±65	11175-10742	-	-	-	This study
AAR-9408	Mørke Enge	Lyster 7	9595±65	11171-10736	-	-	-	This study
Ua-46486	Slabälta 2	Sla2	9546±76	11161-10660	-	-	-	Larsson et al. 2019
OxA-38665	Hassing	A16966	9557±63	11143-10695	-22.3	3	3.4	This study
OxA-38199	Lejre	A37811	9555±55	11125-10702	-21.5	3.8	3.4	This study
OxA-38343	Øresø Mølle	A52426	9481±47	11070-10584	-24.3	2	3.4	This study
AAR-11629	Sønderød	A44205	9468±35	11061-10585	-	-	-	This study
LuS 10848	Rönneholms mosse*	FP1469	9375±45	10719-10444	-	-	-	Larsson et al. 2019
OxA-5528	Skottemarke	A20364	9310±90	10717-10252	-	-	-	Fischer 1996
OxA-38091	Svennum	VHM14923	9365±45	10709-10438	-20.8	5.5	3.6	This study
OxA-38337	Neverkær	A39316	9391±37	10708-10518	-25.6	5.5	3.2	This study
OxA-38340	Køng mose	534	9360±44	10703-10436	-20.9	4.4	3.5	This study
OxA-38092	Svennum	VHM21070	9345±40	10685-10430	-21.7	2.7	3.2	This study
OxA-38341	Vildmosen	A4763	9272±45	10575-10288	-26.5	3.4	3.4	This study
OxA-38342	Skellingsted mose	A39565	9261±46	10567-10282	-25.1	4.6	3.3	This study
Ua-46878	Rönneholms mosse*	FP1469	9208±55	10512-10244	-22.9	3.1	3	Larsson et al. 2019*
OxA-38090	Emmersbæk	VHM13821	9120±45	10405-10205	-22.4	7.2	3.4	This study
Ua-464	Rönneholms mosse	FP985	9054±47	10366-10157	-	-	-	Larsson et al. 2019
OxA-38230	Lundby mose	487	8592±40	9658-9495	-23	1.5	3.3	This study
AAR-11949	Fugle Å	N/A	8360±55	9499-9147	-	-	-	This study
OxA-X-2807-18	Tømmerup mose	A45173	8335±50	9476-9143	-24.3	4.4	3.5	This study
OxA-38347	Knabstrup enge	A45770	8290±43	9428-9136	-23.4	4	3.4	This study
Ua-46480	Rönneholms mosse	FP982	8223±43	9395-9028	-	-	-	Larsson et al. 2019
LuS-11272	Rönneholms mosse	FP1488	8205±45	9295-9023	-	-	-	Larsson et al. 2019
LuS-11268	Rönneholms mosse	FP1466	8195±50	9294-9015	-	-	-	Larsson et al. 2019
Ua-46479	Rönneholms mosse	FP923	8191±46	9280-9020	-	-	-	Larsson et al. 2019
LuS-11243	Rönneholms mosse	FP1589	8185±45	9272-9019	-	-	-	Larsson et al. 2019
AAR-11127	Ulkestrup Lyng	KAM-18325	8095±65	9256-8774	-	-	-	This study
Ua-46482	Rönneholms mosse	FP1198	8145±48	9255-9000	-	-	-	Larsson et al. 2019
AAR-11630	Ulkestrup Øst IV	A47608	8124±44	9243-8991	-	-	-	This study
OxA-38231	Lundby mose	490	8105±40	9243-8815	-24	4.3	3.5	This study
OxA-38346	Kongsted mose	A40894	8101±42	9243-8795	-22.4	3	3.4	This study
LuS-11269	Rönneholms mosse	FP1470	8065±45	9121-8775	-	-	-	Larsson et al. 2019
OxA-38345	Brokøb	A42422	8021±44	9020-8724	-21.5	1.7	3.5	This study
OxA-38344	Løjesmølle	A5126	7999±43	9009-8663	-23.6	3.6	3.5	This study
Ua-46485	Rönneholms mosse	FP1312	7966±73	9008-8610	-			Larsson et al. 2019
OxA-38315	Skamstrup	A44121	7975±39	8997-8655	-22.7	6.3	3.4	This study
AAR-6867.2	MC I 081183	Brokøb B	7940±65	8993-8609	-	-	-	Fischer 2003
AAR-6867.1	MC I 081183	Brokøb B	7890±65	8984-8562	-	-	-	Fischer 2003
LuS-11270	Rönneholms mosse	FP1483	7925±45	8980-8609	-	-	-	Larsson et al. 2019
Ua-46874	Rönneholms mosse	FP1204	7847±53	8973-8483	-23.4	6,0	3	Larsson et al. 2019*
Ua-46877	Slabälta	Sla1	7890±65	8969-8562	-23	5.4	3	Larsson et al. 2019*
LuS-11271	Rönneholms mosse	FP1487	7835±45	8850-8479		-	-	Larsson et al. 2019
Ua-46876	Rönneholms mosse	FP1247	7820±50	8765-8455	-23.6	5	3	Larsson et al. 2019*
OxA-X-2807-20	Verup mose	A44111	7790±45	8647-8435	-23.6	5.2	3.4	This study
Ua-46875	Rönneholms mosse	FP1220	7748±49	8602-8421	-22.9	5.4	3.1	Larsson et al. 2019*

OxA-X-3004-15	Assertorp mose	A42424	7738±54	8600-8415	-26.2	3.8	3.9	This study
Ua-46478	Rönneholms mosse	FP762	7745±42	8594-8430	-	-	-	Larsson et al. 2019
Ua-46873	Rönneholms mosse	FP746	7720±54	8592-8413	-	-	-	Larsson et al. 2019

Supplementary

SI 1. Bone points

Theis Zetner Trolle Jensen, Arne Sjöström, Morten Fischer Mortensen & Anders Fischer

The majority of bone points from Denmark are stray finds, found in connection with peat cutting during the great World Wars or from field surveys in the cut down bogs. Only a small portion derives from targeted excavations. Therefore, provenance is rarely exact and site descriptions can often be vague or in most cases entirely absent. A majority of these chance finds derive from large bog complexes from Zealand. These two bog complexes Little- and Greater Åmosen, will be briefly introduced in, as well as, the bog Rönneholms mosse in Scania. Photos of the bone points and their approximate provenance are visualised in Supplementary Figures 1-22 (Photos by Theis Zetner Trolle Jensen & Arne Sjöström).

SI 1.2. Åmosen, Zealand, Denmark

The Åmose (translated: river bog) is the largest peat bog on the Danish islands and the largest lowland mire (filled in lake system) in the country covering 36 km² (Andersen, 1983). The Åmose encompasses two major parts: Lille Åmose (Little Åmose), and Store Åmose (Greater Åmose), and the two comprises the richest area in NW Europe in terms of Mesolithic and Neolithic inland finds (Fischer, 1999). The finds were in general incredibly well preserved, but due to severe drainage events the following years, has led to the peat combusting.

During the 1940s and 1950s peat cutting was intensified due to fuel shortage, and a magnitude of well preserved settlements was uncovered. Barbed bone points has been found nearly all over the bog, and reflects the intense fishing activity carried out during especially the Mesolithic (Mathiassen, Troels-Smith and Degerbøl, 1943).

The Aamose basins have a long and complex developmental history, which has been the focus of dozens of geological and palaeoecological investigations over the last 100 years (eg. (Jørgensen, 1963; Noe-Nygaard, 1995). One prominent feature is the dramatic water level changes that occurred during the Early Holocene. The first big change happened in the Early Preboreal, when the water level in Major Aamose decrease as much as 4 metres, and the only preserved sediments from the Early Preboreal are confined to the deepest areas of the basin (Noe-Nygaard et al., 1998). Although a subsequent water level rise occurred, the lake surface remained relatively low. During the transition from the Preboreal to the Boreal, a brief lowering of the water levels took place yet again. This regression lead to erosion of the littoral zone and redeposition of sediments elsewhere. As a result of the lowering of the lake surface during the Holocene it is common place to find lake shore settlements of the Kongemose, Ertebølle and Funnel Beaker periods located on top of Maglemosian 'fishing grounds' (i.e. lake bottom sediments rich in Early Mesolithic bone points.

A later regression happened around the transition from the Boreal to the Early Atlantic period, leading to severe erosion of the littoral Boreal sediments. This caused a hiatus of 200-300 years in the sedimentary record; based on samples from several locations in the bog, during the Late Boreal.
The reason for these water level fluctuations are difficult to determine with certainty. One factor could be the melting of local stagnant ice, as well as temperature oscillations. In addition, beavers can engineer water levels with their dams, resulting in significant changes, not just fluctuations during construction, but also during periodic dam bursts.

SI 1.3. Rönneholms mosse, Scania, Sweden

The peat bogs Rönneholms mosse and the Ageröds mosse together form a 12 km² large bog area, separated by the Rönne river, northwest of the lake Ringsjön. Before the peat bogs formed in the Atlantic and Subatlantic chronozones, the area constituted a shallow lake that was part of a larger lake Ringsjön (c. 50 km²).

The first finds in the bog area were found in the beginning of the 20th century in connection to peat extraction and trenching. Several Mesolithic sites, mainly dated to the Maglemose and Kongemose cultures, were excavated in the 1940s at the former shorelines of the ancient lake and on peat islands in Ageröds mosse (Althin, 1954). Excavations at several of the sites were continued in the 1970s and the 1980s (Larsson, 1983, 1978). A few barbed bone points were found at the sites, but since they are not radiocarbon dated, they have not been included in the study. Only two points from Ageröds mosse have been dated, found at the recently excavated bog site Slabälta (Sjöström, 2013). Since the peat extraction in Ageröd was conducted in the first half of the 20th century, in a relatively large-scale by digging deep trenches, there is no data whether finds like fishing tools were found outside the settlement areas in this part of the bog.

In Rönneholms mosse, peat has been commercially extracted by horizontal and mechanical stripping, thus exposing settlements and other remains within a large area on an annual basis. It has therefore been possible to study the geological development and the human activity in this part of the bog area in detail. Archaeological remains have been found over the course of 26 years of excavations and field recognisance conducted by Arne Sjöström of Lund University.

In the southern part of the ancient lake, in the Rönneholm basin, a layer of lime gyttja was deposited during the Preboreal and Boreal chronozones. There are very few signs of fishing activities in this part of the ancient lake during this period. Only a few barbed bone points and pine torches, dated to the Late Preboreal, have been found in the lime gyttja, and there are no signs of net fishing in the layer. However, it should be noted that that the peat extraction field in Rönneholms mosse is located a few hundred meters from the presumed Preboreal shoreline and that other conditions may occur closer to land.

When the algae gyttja began to deposit on top of the lime gyttja, in the Late Boreal and Early Atlantic, there was a dramatic increase of human activity in the Rönneholm basin. Thousands of single finds and over 100 small campsites have been found scattered all over the basin in this layer, mostly dated to the Early Atlantic. Thousands of net sinkers of stone show that net fishing was common. Also numerous bone points were found in the algae gyttja during the extensive surveys at the Rönneholm bog (Larsson, Sjöström and Nilsson, 2019).

The radiocarbon dated barbed bone points from the bog area indicate that the use of this tool type in central Scania ended in the transition from the Maglemose to Kongemose culture. Finds of other tool types made of bone and numerous sites in the bog, dated to the Kongemose culture, shows that the disappearance of barbed bone points was not caused by poor preservation conditions. In the Kongemose spearfishing was most likely performed with leisters with wooden prongs and simple bone points without barbs. The Scanian study was based on a rather uniform assemblage of 35 barbed bone points from the Rönneholm and Ageröd peat bogs.

The geological development of the Rönneholm-Ageröd basin has been studied by Tage Nilsson (1935, 1964a, 1964b, 1967). The Rönneholm basin has only been partially studied by Nilsson in 1935, but since both the basins form the same lake during early postglacial time the detailed studies from the Ageröd basin can partially be applied to the Rönneholm basin.

Nilsson presented a hypothetical curve of the water level fluctuations, without absolute levels. He found that the water level in the lake was relatively high in the beginning of the Preboreal and that it dropped during the period and Early Boreal to form a low stand in the middle of the Early Boreal. In the transition to the Late Boreal a water level rise occurred, before the level dropped to a post glacial minimum level during the end of the Late Boreal. In the transition to the Early Atlantic the water level rose to a high level before it dropped again at the end of the period to form a longer low water stand during the Late Atlantic(Nilsson, 1964a, 1967).

It is difficult to determine the time length, the levels and the cause of the low water periods that Nilsson presented. The archaeological excavations in Rönneholms Mosse have shown that there have been several short periods with extreme low stands in the Rönneholm basin during the Early Atlantic, since small campsites from the same period have been found in the gyttja layers all over the basin. These short low water periods, when the basin was more or less dried out, have not been confirmed in the geological record. In spite of these extreme low water stands there have been activities with leister fishing in the lake. This is known by numerous single finds of leisters points situated at the same levels as the sites. This contradiction can probably be explained by extreme yearly changes in water level in the shallow lake during the Early Atlantic. A similar settlement pattern; with sites situated far out in the lake, has not been seen for the low water periods during the Boreal chronozone.

SI 2.1 Radiocarbon dates from Preboreal, Boreal and Early Atlantic in Southern Scandinavia

Theis Zetner Trolle Jensen, Jesper Olsen, Anders Fischer

We collected radiocarbon dates from Maglemose habitation as well as from faunal remains.
This was done to infer the presence or absence during the 10.2k bone point hiatus. However, caution should be exercised when using these dates as proxies for presence or absence, or using them at all. Firstly, most of the published dates are done by conventional radiocarbon dating over several decades ago. Secondly, because the majority was performed on charcoal that can reflect long age spans due to the "old wood effect", and thirdly, not all present day purification steps were routinely performed decades ago (i.e. removal of humic acids). Removal of humic acids from the collagen fraction was not routinely performed in the old 14C laboratory in Copenhagen. Removal of humic acids only became standard sample preparation from laboratory number K-2127 onwards (Henrik Tauber pers. comm. to Anders Fischer 1998; (Fischer, 2002, p.359). As a consequence, many of these samples should be suspected to have produced dates that are misleadingly young due to infusion of humic washed down from stratigraphically younger deposits.

SI 2.2 Fauna

We collected (*n* = 118) radiocarbon dates from the Maglemose period performed on faunal remains of the four main species observed by ZooMS in the bone point assemblage (i.e. elk, red deer, aurochs, and bison) from the Preboreal, Boreal, and Early Atlantic chronozones. No radiometric dating has been performed on brown bear from the Maglemose period. We performed a Bayesian phase model for each of the taxonomic groups within each region, we then summed the radiocarbon and performed KDE to indicate activity for all dates from a given region (see SI 2.2.1, and Supplementary Figure 23).

Online resources:

SI 2.2.1 Spreadsheet containing radiocarbon dates of faunal remains from Southern Scandinavia.
http://doi.org/10.5281/zenodo.3483215

SI 2.3 Habitation

We collected published and unpublished ($n = 24$) radiocarbon dates conducted on material (bone or charcoal) associated with habitation sites in Denmark. We performed a simple Bayesian model for each site creating phase models, assuming that the dates from each of the sites are of coeval age. We also summed the radiocarbon dates in a KDE model to visualise activity. In addition, we also included dates ($n = 22$) from the southwestern coast of Sweden, from Balltorp and Huseby Klev. Lastly, three dates performed on three isolated human remains (two burials and dredged off the coast) not associated was also included. The human remains was reservoir corrected based on their $\delta^{13}C$ values.

The habitation sites included are from Denmark: Barmose I, Klosterlund, Mullerup, Holmegaard, and Ulkestrup in Denmark. As well as Balltorp and Huseby Klev in Sweden (see SI 2.3.1, and Supplementary Figure 24).

Online resources:

SI 2.3.1 Spreadsheet containing radiocarbon dates associated with habitations.
http://doi.org/10.5281/zenodo.3483217

SI 3. Collagen sequencing and spectra analysis

Theis Zetner Trolle Jensen, Kristine Korzow Ricther & Meaghan Mackie

SI 3.1. Sampling

The sampling and protein extraction of the bone points was conducted in the palaeoproteomics laboratories at BioArCh, University of York, United Kingdom and Centre for GeoGenetics, University of Copenhagen, Denmark. An average of 20 mg of bone was either cut off or drilled to produce bone powder. The blade or drill was cleaned in 5% bleach followed by 80% ethanol between sampling. For the reference samples, two elk samples were obtained from the Zoological Department, University of Copenhagen, and two red deer specimens from morphologically distinguishable bone fragments were taken from ongoing Syltholm excavations, near Rødbyhavn, Denmark.

SI 3.2. Collagen Extraction

Two different extraction protocols were tested for the samples from these locations. 120 samples were tested (41 by Extraction 1 and 79 by Extraction 2). The reason was due to the discovery that one gave greater peptides yields, than the other. Extraction 1 provided better quality spectra (higher intensity and better resolution) and allowed a higher throughput of samples. It was therefore used for all subsequent samples. However, both methods allowed for species identification of the samples and the results are combined.

Extraction 1 followed a minimally destructive protocol by van Doorn *et al.* (2011). The samples were incubated in 100µL of 50 mM ammonium bicarbonate solution (NH_4HCO_3) pH 8.0 (AmBic) for 16 hours at ambient temperature. They were then vortexed for 15 seconds and centrifuged at 13,000 rpm for 1 min, the supernatant was discarded. 100µL of AmBic was added to the samples, followed by incubation for one hour at 65°C to gelatinise the collagen.

Extraction 2 followed a modified destructive protocol by Buckley *et al. (2009)*. The samples were demineralized in 250µL of 0.6M HCl at 4°C for approximately a week, with the acid changed daily. They were then vortexed for 15 seconds and centrifuged at 13,000 rpm for 1 min. The supernatant was discarded and the samples were rinsed three times with 250µL of AmBic. Finally 100µL of AmBic was added to the samples, followed by incubation for one hour at 65°C to gelatinise the collagen.

SI 3.3. Enzymatic Digestion and peptide clean-up

For all samples, 50µL of each extraction was transferred to a separate 1.5mL Eppendorf tube, 1µL of sequence grade Trypsin (0.4 µg/µl) (Promega) was added to each followed by incubation at 37°C for c. 16 hours.

Additionally, the four reference samples *Alces alces* (P220 and P221), *Cervus elaphus* (X4787 and X4997) which were were extracted using the Extraction 2 protocol were also enzymatically digested with several other enzymes to increase the sequence coverage for collagen. After gelatinization, the reference samples were dried using a vacuum centrifuge and resuspended in 100µL Tris-HCl, pH 8. 50µL was transferred to a new 1.5mL tube and incubated with 1µL of elastase (0.4 µg/µl) at 37°C for c. 16 hours. The remaining 50µL was incubated with 1µL of chymotrypsin (0.4 µg/µl) at 25°C for c. 16 hours.

After digestion, the extractions were centrifuged at 13,000 rpm for 1 min before acidification to <pH 2 using 5% (vol/vol) Trifluoroacetic acid (TFA, Sigma Aldrich). Purification was performed using C18 reverse phase resin ZipTip® pipette tips (EMD Millipore) according to the manufacturer's instructions. Peptides were eluted in 50µL.

SI 3.4. MALDI-TOF-MS

Peptide eluates of the trypsinated collagen were co-crystallised with α-cyano-4-hydroxycinnamic acid (Sigma Aldrich) matrix solution (50% ACN, 0.1% TFA (vol/vol)) at a ratio of 1:1 (1µL:1µL). Samples were spotted in triplicate with calibration standards onto a 384 spot ground steel MALDI target plate (Bruker). Samples were run on a Bruker Ultraflex III MALDI TOF/TOF mass spectrometer (Centre for Excellence in Proteomics at the University of York, United Kingdom) with a Nd:YAG smart beam laser, with a SNAP averaging algorithm used to obtain monoisotopic masses (C 4.9384, N 1.3577, O 1.4773, S 0.0417, H 7.7583). The MALDI was run in reflector mode over an m/z range of 800–3200. The generated spectra were converted to txt files and analysed using mMass v.5.5.0 (Strohalm et al., 2010). The triplicate raw files were averaged, and then peak picked with a S/N threshold of 4. The nine published biomarkers (Buckley et al., 2009; Kirby et al., 2013) were used to identify the spectra to taxonomic group. An additional biomarker was discovered (see SI 4.1) at m/z 2216 which was used to identify red deer.

Online resources:
SI 3.4.1 Spreadsheet information on bone points as well as identified unique peptides.
http://doi.org/10.5281/zenodo.3483220

SI 3.1.2 MALDI-TOF-MS raw data from 120 bone points
http://doi.org/10.5281/zenodo.3628020

SI 3.5. nLC-MS/MS

For the four reference samples, the different digestion elutions were dried using a vacuum centrifuge and resuspended in 50µL 80% ACN, 0.1% formic acid (FA). 10µL of each digestion was combined in a 96 well plate (one well per sample).

For the four bone points (VHM13821, A37811, A40894, A42422), the trypsin digested and eluted peptides were measured by NanoDrop spectrophotometry (Thermo Scientific, Wilmington, DE, USA) for protein concentration. The volume required for approximately 1.5 µg of peptide per sample was placed in separate wells in a 96-well plate and topped up to 30 µL using 40% ACN, 0.1% FA. The 96 well plate was vacuum centrifuged at 45ºC until approximately 3 µL was left in the wells, and the samples were then rehydrated with 10 µL of 0.1% TFA, 5% ACN. The samples were analyzed on anEASY-nLC 1200 (Proxeon, Odense, Denmark) coupled to a Q-Exactive HF (reference) or HF-X (bone points) (Thermo Scientific, Bremen, Germany) at the Novo Nordisk Foundation Center for Protein Research, the University of Copenhagen. The parameters used were as previously published (Mackie et al., 2018) with 5µl of sample injected.

SI 3.6. Analysis of nLC-MS/MS data

MaxQuant (v.1.6.2.6a or v.1.6.3.4) (Cox and Mann, 2008) was used to search the resulting raw files. The database contained a) elk collagen type I sequences, b) red deer collagen type I sequences, and d) common contaminants (from MaxQuant). Species diagnostic peptides found in bone points and reference material is shown in Supplementary Table 2. In addition, the four bone points were run against the soil bacteria *Klebsiella pneumoniae* proteome (see Section 3). MaxQuant parameters were: digestion mode semi-specific for trypsin, to account for possible additional hydrolytic cleavages occurring during diagenesis; variable modifications - oxidation (M,P), acetyl (N-term), deamidation (N,Q), pyro-Glu (E,Q); minimum score for unmodified and modified peptide searches was 60. The remaining parameters were set to the program defaults. Deamidation was assessed using publicly available code (Mackie et al., 2018).

SI 4. Database Creation and Hypothetical Marker Determination

Kristine Korzow Richter, Theis Zetner Trolle Jensen & Meaghan Mackie

SI 4.1. Elk and red deer collagen sequences and theoretical markers

The Collagen type I sequences from elk come from previously published sequences (Welker et al., 2016), with missing amino acids substituted with ones from the same positions based on the *Bos taurus* sequence (COL1A1 P02453 and COL1A2 P02465) (SI 4.1.2). The collagen type 1 sequences from red deer comes from the red deer genome. At the time of analysis the collagen type I sequences were not annotated. Therefore a blastx search of the *Bos taurus* collagen sequences against the entire red deer genome (Bioprojects: PRJNA324173) was used to find the location of the collagen genes (COL1A1 - chromosome 5 MKHE01000005.1:143374497-143390891; COL1A2 - chromosome 18 CM008025.1:14219961-14252107). In order to make sure all of the exons were aligned appropriately, the DNA sequence covering the entire gene was aligned to the corresponding *Bos taurus* genes which have annotated exons available on NCBI using Geneious Prime 2019.03 (https://www.geneious.com). The introns were removed and the resulting sequence translated and aligned to the *Bos taurus* and elk collagen sequences. The aligned sequences were then used to identify potential SAPs and the corresponding tryptic peptides that distinguished red deer and elk.

The tryptic peptides that contained SAPs were then searched against the non-redundant protein sequences using blastp. Any of the tryptic peptides which matched with 100% identity to a non-collagen sequence were removed from the list of biomarkers.

Online resources:

SI 4.1.2 FASTA file containing concatenated COL1a1 and COL1a2 sequence data of the complete collagen sequence of red deer, as well as elk COL1a1 and COL1a2 from Welker *et al. (2016)* and domestic cow from Uniprot (COL1A1 P02453 and COL1A2 P02465).
http://doi.org/10.5281/zenodo.3483213

SI 4.2. Possible soil bacteria

In order to confirm that the key peptides which distinguished elk and red deer were not soil contamination, the raw files from four bone points were also searched against the proteome of the soil bacteria *Klebsiella pneumoniae* (NCBI:txid573). This is due to the theoretically *Cervus elaphus* specific peptide GAPGPDGNNGAQGPPGPQGVQGGK occuring in all sequenced bone points with conflicting species specific markers. When searched with blastp, it matches the collagen-like protein (WP_139109296.1) of *K. pneumoniae*. The search against the proteome of *K. pneumoniae* gave evidence of additional proteins from this species are present, indicating that this peptide *could* be derived from soil contamination and this peptide was, therefore, not used for species identification. In addition, another species specific peptide GDVGSPGR for *Cervus* and GDIGSPGR for *Alces*, was found after a blastp search to match many different potential soil bacteria, and was also not used for species specificity (see SI Table 1).

SI 4.3. Deamidation

Deamidation levels of asparagine (Asn) and glutamine (Gln) amino acid residues were examined to assure protein authenticity. Asn and Gln naturally deamidate over time, influenced by factors such as temperature and moisture. In this case we compared the levels of deamidation of the collagen and other assumed authentic proteins (such as COL3, the bovine version being in MaxQuant's default contaminant list) with those detected and known to be common laboratory contaminants. In all cases, the peptides from the samples showed much more deamidation than the modern contaminants, supporting their authenticity (Fig 24 and 25). While still low, the deamidation rates of the contaminates varies a lot more than the samples, probably due to the much lower contamination peptide counts that the calculations are based on.

SI 5. Water Level Fluctuations in Danish/German/Swedish Straits

Ole Bennike

During the final drainage of the Baltic Ice Lake at about 11 700 cal. years BP, the water level dropped by c. 25 m over a few years (Björck, 1995). The water level in the Danish/German/Swedish straits reached a low stand during the early Preboreal, following this drainage event, peat bogs, local lakes, and forested areas became widespread in the straits. As the relative sea level in Kattegat began to rise, a fjord with brackish water formed in the northern Øresund. The ongoing eustatic sea-level rise led to increased salinity and the fjord became larger. However, the threshold in Øresund, with a present threshold depth of 7 m, was probably not flooded until at about 8000 cal. years BP (Bennike et al., 2012).

In the Great Belt, the oldest dated marine shells gave an age of ca. 8100 cal. years BP (Bennike et al., 2004). In the Little Belt, the oldest shell date is ca. 7700 cal. years BP (Bennike and Jensen, 2011) and in Mecklenburg Bay the oldest reported age is ca. 7600 cal. years BP. However, it appears that water level began to rise long before these ages, which resulted in the formation of large lakes. The youngest lake deposits in the Little Belt are dated to ca. 8500 cal. years BP, at this time the lake level was ca. 13 m below sea level. The lake phase was followed by a brackish-water phase that lasted ca. 800 years, during this time period the salinity was too low for marine molluscs to live in the Little Belt.

In the central parts of the Great Belt, between Zealand and Funen, fluvial deposits have been dated to about 10650 to 10250 cal. years BP (Wiberg-Larsen et al., 2001). The fluvial phase was followed by a lake phase that lasted until ca. 8800 cal. years BP. During this phase, the lake gradually became larger and larger and flooded wide areas, this was followed by a brackish-water phase. The development of Mecklenburg Bay and the Femern Belt was probably similar to the development in the Little Belt, but a river probably also existed in Femer Belt as in the Great Belt, as depicted on palaeogeographical maps by Jensen et al. (2002).

SI 6. Bone Density - ribs vs. long bones

Kurt Gron & Theis Zetner Trolle Jensen

The skeletal element of heavily worked artefacts made from either ribs or long bones are easily identified, due to gross morphological differences in shape, structure, and thickness. However, the reasons as to why humans preferred one over the other remains unclear.

This is because any *a priori* assumptions about choice of raw material for the manufacture of bone points almost certainly ignore various knowable and unknowable factors related to suitability for purpose. In the first instance, long bones and elements of the axial skeleton, such as ribs, likely have very different mechanical qualities, mostly predicated on differences in Bone Mineral Density (BMD, (Lam, Chen and Pearson, 1999). Long bones of the appendicular skeleton, for example, are generally denser than ribs, vertebrae, and other irregular-shaped elements more common in the axial skeleton; a fact often reflected in their differential survival in the archaeological record (Gron, 2015). This density will certainly affect their malleability if treated, so depending on the desired result, ribs may have been more appropriate than other elements for this particular purpose, despite the apparent strength advantage of long bones; ribs and other skeletal elements can be straightened if heated and soaked (Osipowicz, 2007). There is also a degree of intra- and inter-taxonomic variability even within a single skeletal element. For example, ribs from the anterior part of the trunk have a different form than those from the posterior part. Furthermore, the same rib will have a different curvature in animals of different sizes, so that a red deer and an auroch of similar body size may have similarly curved ribs, whilst two animals of the same taxon might have divergent element morphology if there are significant differences in the circumference of the rib cage. It is almost certain that multiple considerations were taken into account when selecting raw material for the manufacture of the bone points, and these considerations were likely independent of any consistent preference for one taxon, one element, or one part, of a bone than another.

SI 7 Extended; Potential Causes for Absence of Bone Points

Theis Zetner Trolle Jensen, Liam Lanigan, Erika Rosengren, & Kurt Gron

Based on southern Swedish lake sediment cores (Gaillard, 1985; Gedda, 2001; Digerfeldt et al., 2013), the Boreal may have been particularly dry and saw the lowest water levels of any point during the Holocene. This evaporation of lakes with sediment infill may have started even earlier in eastern Denmark (Welinder, 1978). Palynological records also provide corroboration that lake levels shifted appreciably during the Early Holocene (Jørgensen, 1963; Noe-Nygaard, 1995), and around the start of the Boreal period. Changes in vegetation are evidenced by a northern expansion of hazel forest, likely taking advantage of now reduced lacustrine coverage to colonise former lake beds. Lower concentrations of stagnant ice during the Boreal period, could go some way to explain a drier climate. Other factors include a warming climate and the role of 'biological engineers' such as beavers.

For species that are associated with wetland habitats, such as elk and aurochs, these changes in conditions would have almost certainly had a detrimental effect. We must be aware; however, that the lack of dated animal remains from this period could possibly be a matter of preservation, and/or other types of sample biases (see (Newell, 1990). Since wetlands constitute the optimal conditions for bone preservation, a dearth of remains may reflect subsequent challenging taphonomic conditions. Several analyses of osteological remains have lent support to an impoverishment of faunal material on Zealand (Magnell, 2006, p.80 and cited references).

Alternatively, palaeogeographical changes may help explain the 10 ka hiatus, with Zealand, Lolland, and Falster being separated from the rest of Denmark at about 10 cal. ka BP due to a rising shore level (Bennike and Jensen, 2011). At this time, large lakes also began forming in the Femern Belt and the Great Belt (Bennike et al., 2004). Despite this, it is likely that a land bridge still connected Zealand to southern Sweden (Bennike et al., 2012). Contemporary animal populations in Scania are still accounted for in the archaeological record, so it can be claimed that inundation of the sounds is not a viable explanation for why the large mammals seemingly did not migrate to or from eastern Denmark. Finally, at c. 8,000 cal BP several large ungulates and carnivores disappeared altogether from eastern Denmark (Aaris-Sørensen, 1980), possibly as a result of rising sea-levels (Littorian transgression) turning Denmark (apart from Jutland) into an archipelago (Christensen, 1995).

Despite the small sample size, the stable carbon isotope data is consistent with niche partitioning between species, as well as signs of environmental change, although the value obtained from a single bovine individual seem to indicate that it was exploiting a similar feeding niche to the cervids. Before ca. 10 ka BP, the $\delta^{13}C$ values vary considerably, but after 9,5 ka BP, decreasing $\delta^{13}C$ values indicate an environmental shift, from an open environment to a more closed setting, probably in part due to the canopy effect (see (Noe-Nygaard, 1995). The $\delta^{13}C$ values may therefore indicate that the mammals were feeding within an increasingly forested landscape after 9,5 ka BP. This probably reflects animals favouring the edges of wetlands and forest clearings. Within this setting, niche partitioning based on the preferred feeding ecology of the taxa results in higher cervid $\delta^{13}C$ values than the bovines (Hofmann 1989).

Following the hiatus, the manufacture of bone points changes considerably. Could this be a reflection of this greater environmental shift? There is a more varied species selection and choice of raw material. This may reflect changing habitat that favoured these species, as open hazel-pine (*Corylus* and *Pinus*) forests were replaced after the hiatus with more closed and darker forests dominated by lime, oak and elm (*Tilia* sp., *Quercus* sp., and *Ulmus* sp.) (Iversen, 1973). Conversely, it could reflect a shift in hunting strategy to the more localized environment, although there is some $\delta^{13}C$ overlap before and after the hiatus in the radiocarbon dates. Wear on the teeth of aurochs from this period suggests an abrasive diet, consistent with a major proportion of their diet being based on grasses (Schulz and Kaiser, 2007). The vegetational development might increasingly have restricted grazing species to the fringes of the forest (i.e. the 'marginal-effect') making them especially vulnerable to human hunting (Lepiksaar, 1986).

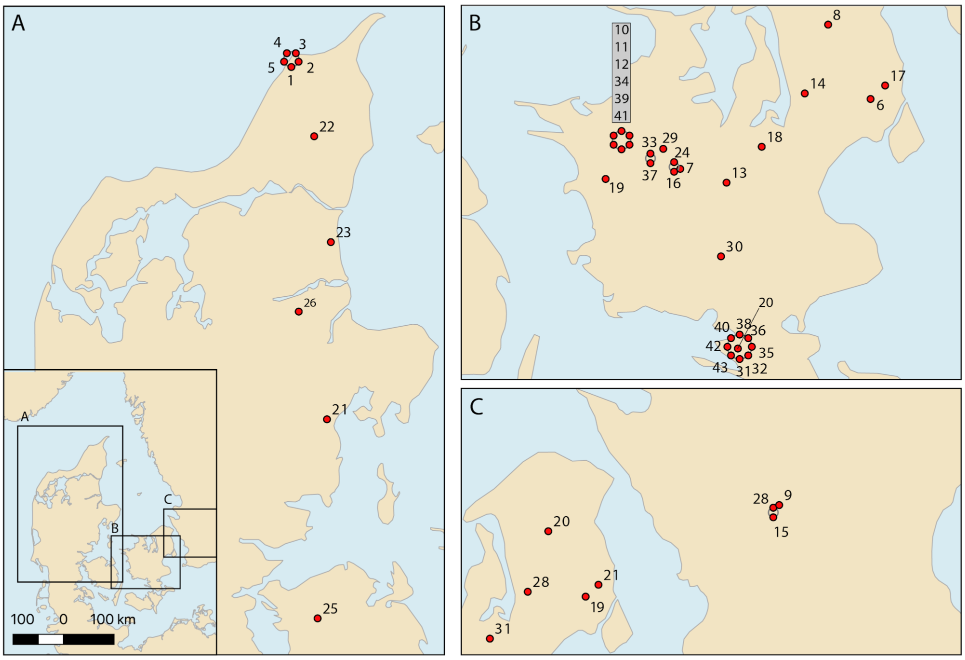
There is no appreciable change in $\delta^{15}N$ before or after the hiatus, but there is a rather large range of variation in the $\delta^{15}N$ values obtained herein. $\delta^{15}N$ values record the animals' dietary protein through the filter of its behavioural ecology, and therefore will at least in-part reflect available browsing and grazing environments. The taxa represented within the resolution of our ZooMS results encompass a broad range of preferred feeding habitats and feeding types, including bulk feeders, intermediate feeders, and concentrate selectors (Hofmann, 1989). These feeding types are at least in part influenced by the animals' digestive systems, a factor known to affect $\delta^{15}N$ values in herbivores of different gut complexities (Bocherens et al. 1996). Our $\delta^{15}N$ values are much more variable (Bovine, 4.8‰ and Cervid, 5.5‰) than values for single herbivore taxa in multiple environments (ca. 2-3‰) (Gron et al., 2016), and therefore probably reflect both physiological differences in addition to reflecting any environmental change. This relationship between high variability of nitrogen levels alongside landscape changes remains uncertain, and possibly indicates erroneous results for a few samples although all C/N values were within the accepted. More intriguingly, the unexpected results seen in a few samples might be indicative of physiological stress due to a disruption of 'normal' ecological conditions leading to unusual resource exploitation by local fauna.

Supplementary Figures

Supplementary Figure 1. Photos of bone points. 1: VHM13819, 2: VHM13818, 3: VHM13820, 4: VHM13821, 5: VHM13822, 6: A38560, 7: A20181, 8: A8524, 9: SLA2, 10:A52427, 11:52492, 12: 52454, 13: A50653, 14: A42153, 15: FP985, 16: A42156

Supplementary Figure 2. Photos of bone points. 17: A20416, 18: A37811, 19: A42792, 20: 442, 21: A8565, 22: VHM14923, 23: A4763, 24: A42924, 25: A39316, 26: A16966, 27: A9055

Supplementary Figure. Photos of bone points. 28: FP1469, 29: Ulk3, 30: A42381, 31: 534, 32: 432, 33: Skellingsted2, 34: A52426, 35: 546, 36: 438, 37: Skillingsted3, 38: 612, 39: A52445, 40: 562, 41: 52494, 42: 616, 43: 573



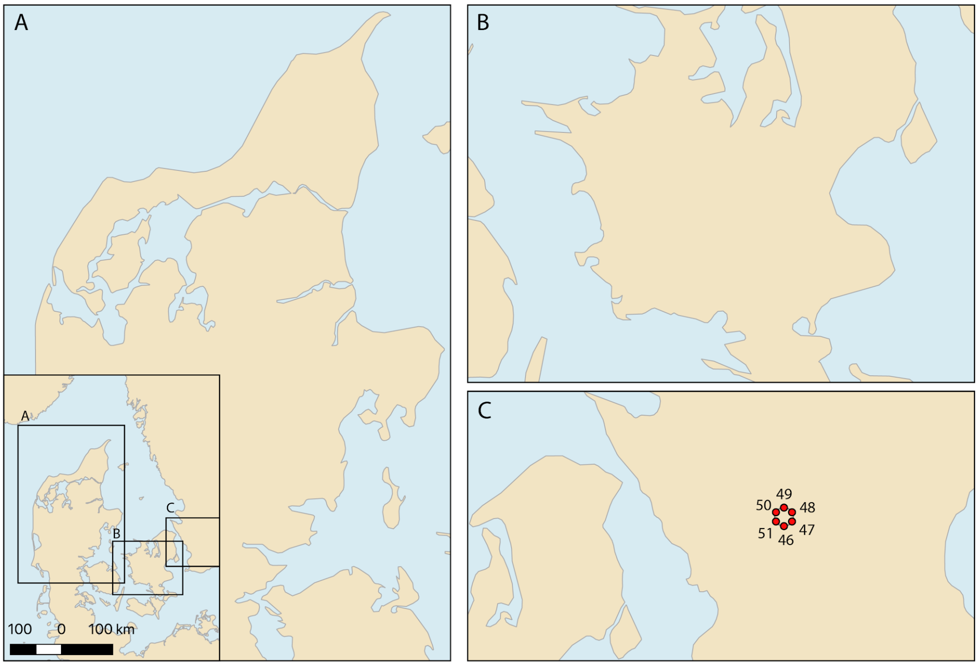
1	Emmersbæk	VHM13819
2	Emmersbæk	VHM13818
3	Emmersbæk	VHM13820
4	Emmersbæk	VHM13821
5	Emmersbæk	VHM13822
6	Mose Holmene	A38560
7	Assentorp	A20181
8	Bag gasværket	A8524
9	Slabälta	Sla2
10	Øresø Mølle	A52427
11	Øresø Mølle	A52492
12	Øresø Mølle	A52454
13	Skee mose	A50653
14	Gundsømagle	A42153
15	Rönneholms Mosse	FP985
16	Garbølle mose	A42156
17	Nymose	A20416
18	Lejre	A37811
19	Ravnsmose	A42792
20	Køng mose	442
21	Aarhusegnen	A8565
22	Svennum mose	VHM14923
23	Vildmosen	A4763
24	Assertorp mose	A42924
25	Neverkær	A39316
26	Østerkær	A16966
27	N/A	A9055
28	Rönneholms Mosse	FP1469
29	Ulkestrup area	Ulk3
30	Torpe mose	A42381
31	Køng mose	534
32	Lundby mose	432
33	Skillingsted	Skillingsted2
34	Øresø Mølle	A52426
35	Køng mose	546
36	Køng mose	546
37	Skillingsted	Skillingsted3
38	Køng mose	612
39	Øresø Mølle	A52445
40	Køng mose	562
41	Øresø Mølle	A52494
42	Køng mose	616
43	Køng mose	573

Supplementary Figure. Overview of approximate provenance of bone points 1-43. 27 not shown due to no provenance.

Supplementary Figure. Photos of bone points. 44: A39565, 45: VHM21070

Supplementary Figure. Overview of approximate provenance of bone points 44-45.

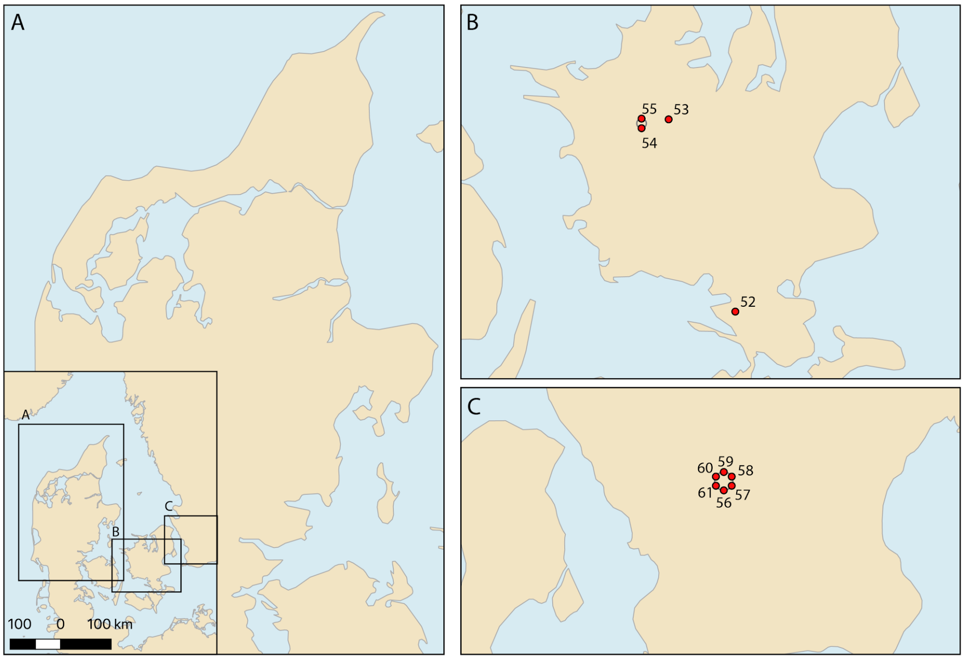
Supplementary Figure. Photos of bone points. 46: FP1507, 47: 923, 48: 1492, 49: 1488, 50: 982, 51: 1483



46	Rönneholms Mosse	FP982
47	Rönneholms Mosse	FP1492
48	Rönneholms Mosse	FP1483
49	Rönneholms Mosse	FP1507
50	Rönneholms Mosse	FP1488
51	Rönneholms Mosse	WP923

Supplementary Figure. Overview of approximate provenance of bone points 46-51.

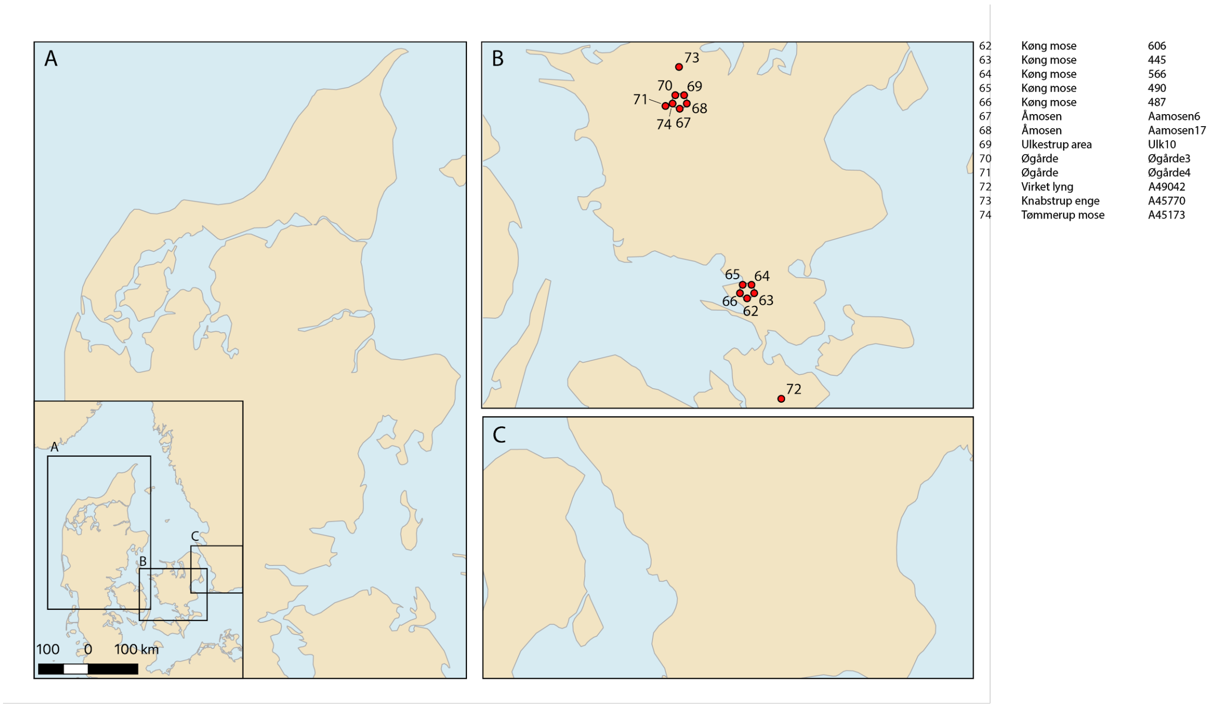
Supplementary Figure. Photos of bone points. 52: A40894, 53: 544, 54: FP1589, 55: Aamosen7, 56: FP1151, 57: FP1198, 58: FP132, 59: Brokøb B, 60: FP762, 61: FP1423



52	Køng mose	544
53	Åmosen	Aamosen7
54	Brokøb (Åmosen)	Brokøb B
55	Kongsted mose	A40894
56	Rönneholms Mosse	FP1589
57	Rönneholms Mosse	FP1198
58	Rönneholms Mosse	FP132
59	Rönneholms Mosse	FP1151
60	Rönneholms Mosse	FP762
61	Rönneholms Mosse	FP1423

Supplementary Figure. Overview of approximate provenance of bone points 52-61.

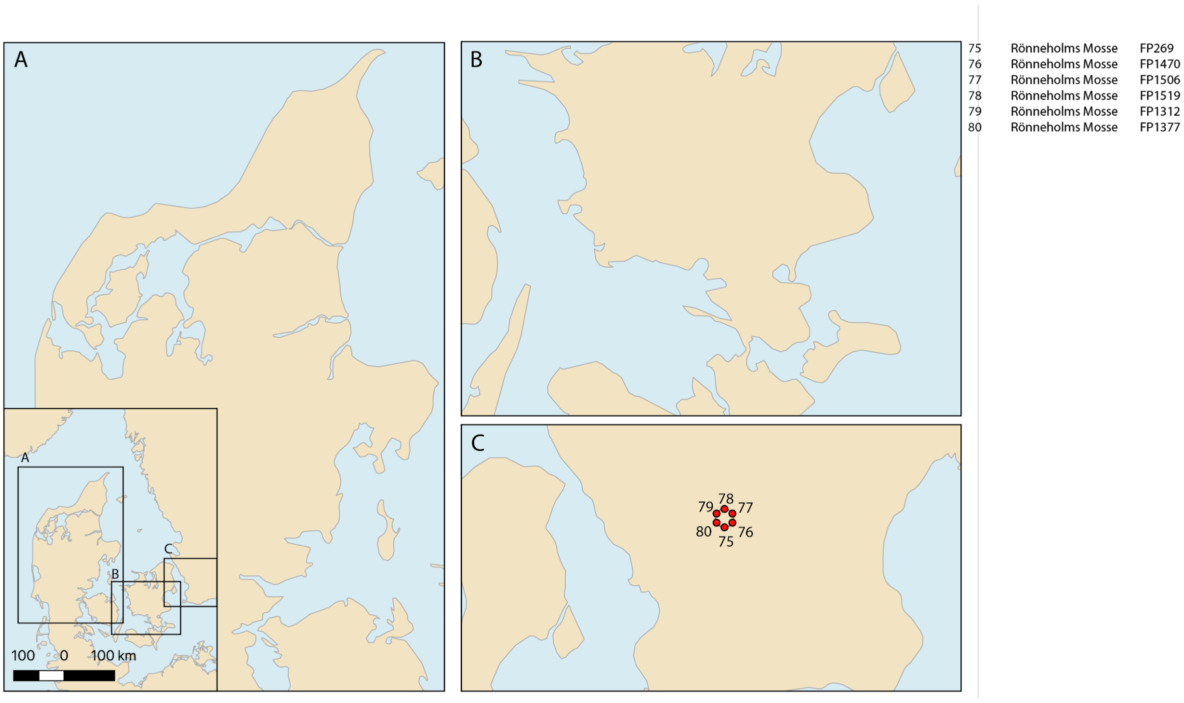
Supplementary Figure. Photos of bone points. 62: A49042, 63: A45770, 64: 487, 65: 490, 66: 566, 67: 445, 68: 606, 69: Aamosen17, 70: Øgårde4, 71: Aamosen6, 72: Ulk10, 73: Øgårde 3, 74: A45173.



62	Køng mose	606
63	Køng mose	445
64	Køng mose	566
65	Køng mose	490
66	Køng mose	487
67	Åmosen	Aamosen6
68	Åmosen	Aamosen17
69	Ulkestrup area	Ulk10
70	Øgårde	Øgårde3
71	Øgårde	Øgårde4
72	Virket lyng	A49042
73	Knabstrup enge	A45770
74	Tømmerup mose	A45173

Supplementary Figure. Overview of approximate provenance of bone points 62-74.

Supplementary Figure. Photos of bone points. 75: FP1516, 76: FP1377, 77: FP1470, 78: FP1516, 79: FP269, 80: FP1506.



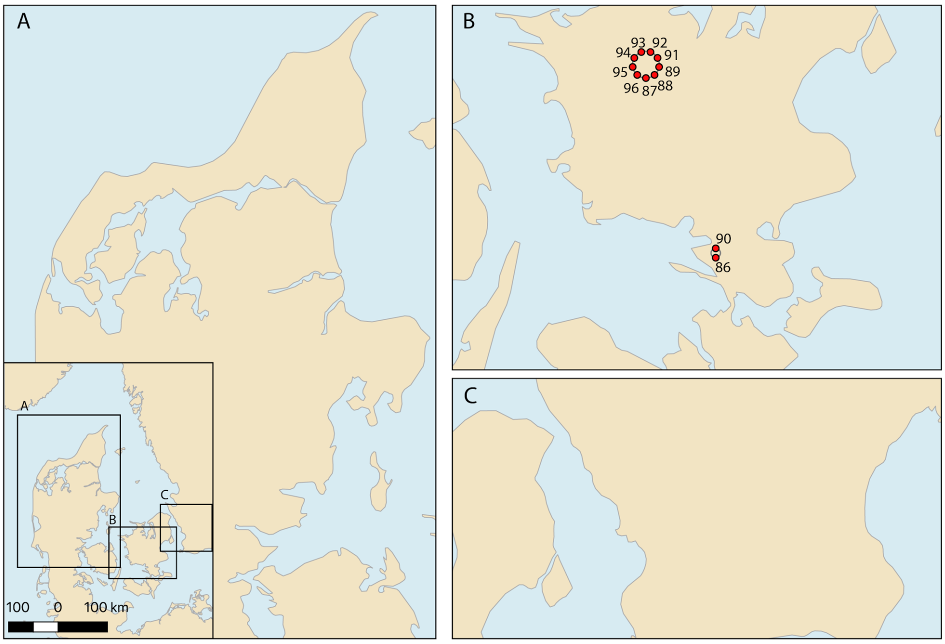
75	Rönneholms Mosse	FP269
76	Rönneholms Mosse	FP1470
77	Rönneholms Mosse	FP1506
78	Rönneholms Mosse	FP1519
79	Rönneholms Mosse	FP1312
80	Rönneholms Mosse	FP1377

Supplementary Figure. Overview of approximate provenance of bone points 75-80.

Supplementary Figure. Photos of bone points. 81: A42422, 82: A44112, 83: A31548, 84: A5126, 85: A42461

Supplementary Figure. Overview of approximate provenance of bone points 81-85
.

Supplementary Figure 17. Photos of bone points. 86: 587, 87: Aamosen1, 88: A44121, 89: Skellingsted1, 90: 617, 91: Øgårde1, 92: Aamosen15, 93: Aamosen14, 94: Ulk12, 95: Ulk11: 96: Aamosen10.



86	Køng mose	587
87	Åmosen	Aamosen1
88	Åmosen	A4412
89	Åmosen	Skillingsted1
90	Køng mose	617
91	Åmosen	Øgårde1
92	Åmosen	Aamosen15
93	Åmosen	Aamosen14
94	Åmosen	Ulk12
95	Åmosen	Ulk11
96	Åmosen	Aamosen10

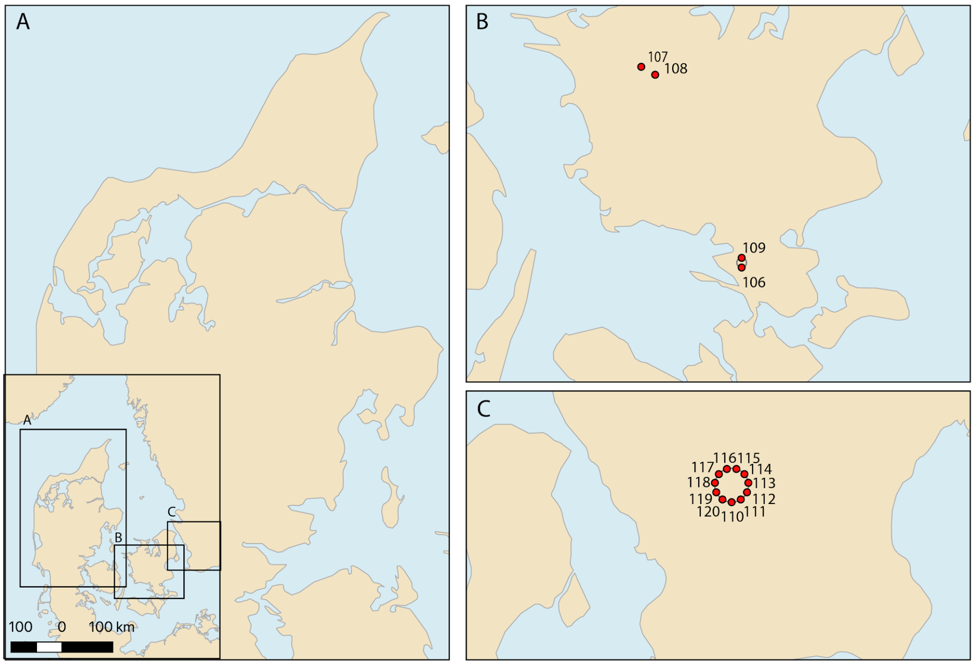
Supplementary Figure 18. Overview of approximate provenance of bone points 86-96.

Supplementary Figure 19. Photos of bone points. 97: A14632, 98: Ulk1, 99: A31546, 100: FP1006, 101: A39208, 102: Sla1-2, 103: A38552, 104: FP37, 105: Aamosen18.

	N/A	A14632
98	Ulkestrup area	Ulk1
99	Barmosen	A31546
100	Rönneholms Mosse	FP1006
101	Porsmose	A39208
102	Slabälta	Sla1-2
103	Åmosen	A38552
104	Rönneholms Mosse	FP37
105	Åmosen	Aamosen18

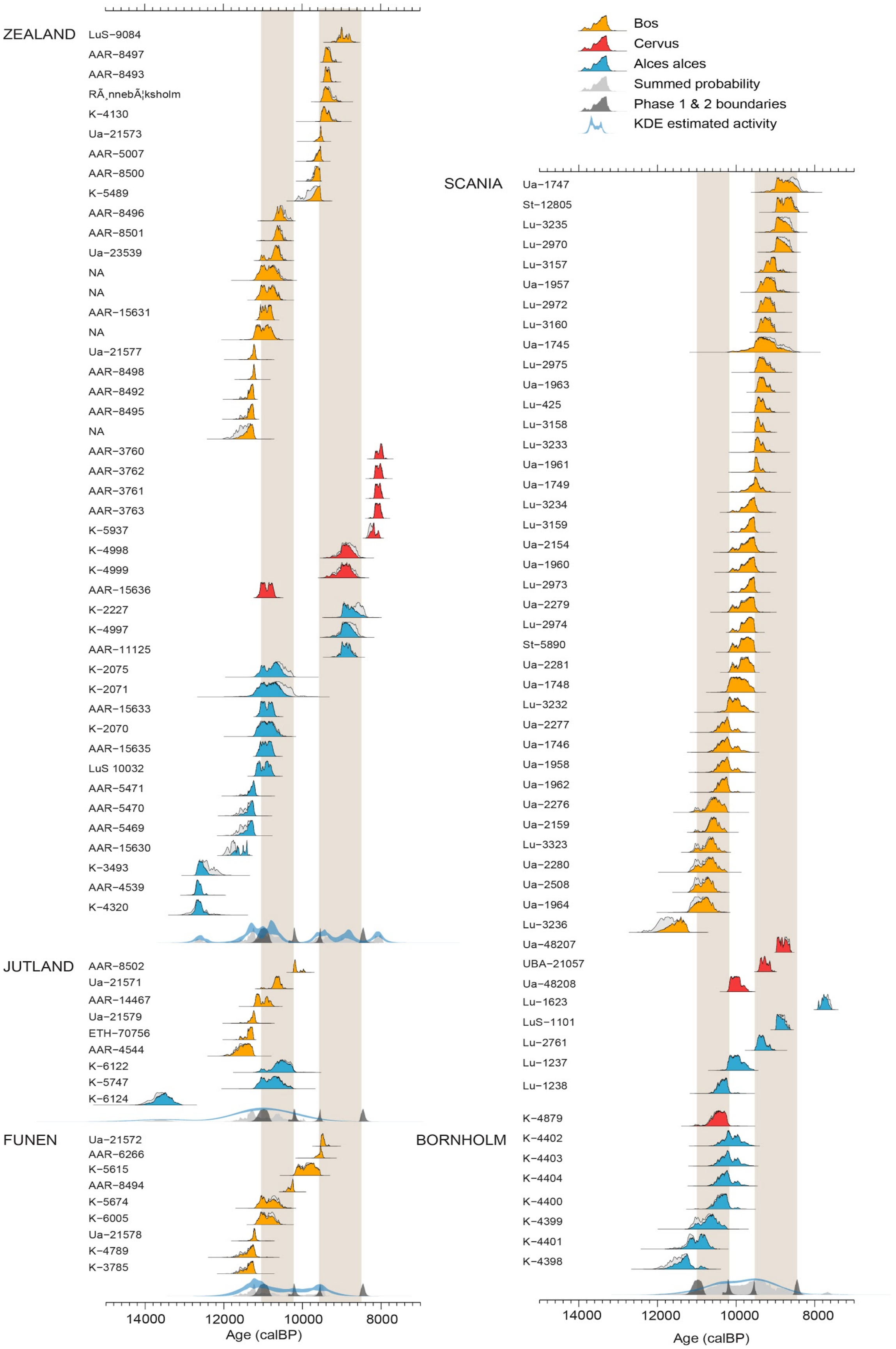
Supplementary Figure 20. Overview of approximate provenance of bone points 98-105. 97 not shown on map due to no provenance.

Supplementary Figure 21. Photos of bone points. 106: 715, 107: A42424, 108: A44111, 109: 427, 110: FP746, 111: FP1204, 112: FP1627, 113: FP1247, 114: FP1220, 115: FP1536, 116: FP107, 117: FP482, 118: FP1501, 119: FP1445, 120: FP956.

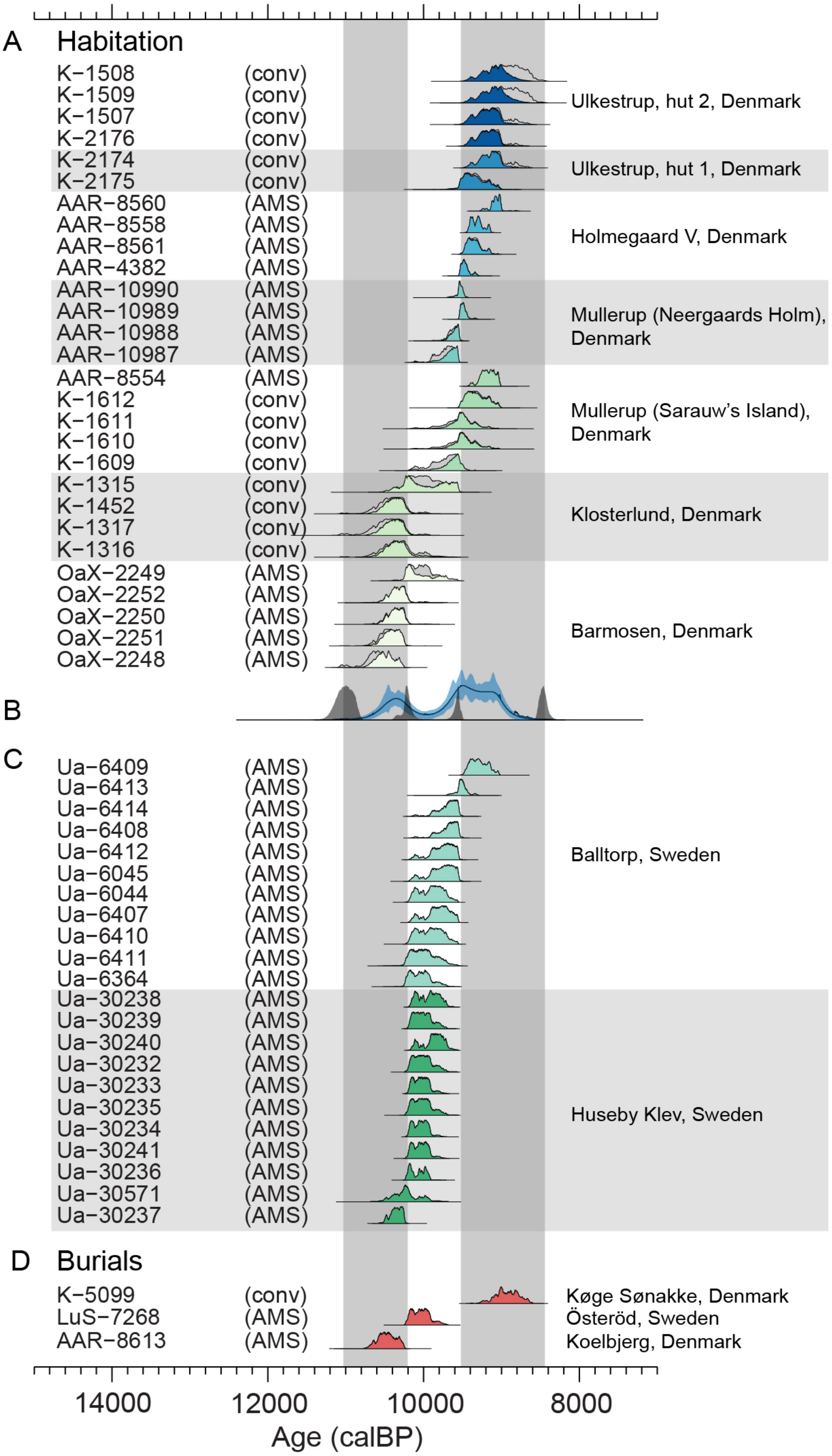


106	Køng mose	715
107	Brokøb	A42424
108	Verup (Åmosen)	A44111
109	Lundby mose	427
110	Rönneholms Mosse	FP746
111	Rönneholms Mosse	FP482
112	Rönneholms Mosse	FP1536
113	Rönneholms Mosse	FP1501
114	Rönneholms Mosse	FP1445
115	Rönneholms Mosse	FP1204
116	Rönneholms Mosse	FP1220
117	Rönneholms Mosse	FP1247
118	Rönneholms Mosse	FP956
119	Rönneholms Mosse	FP1627
120	Rönneholms Mosse	FP107

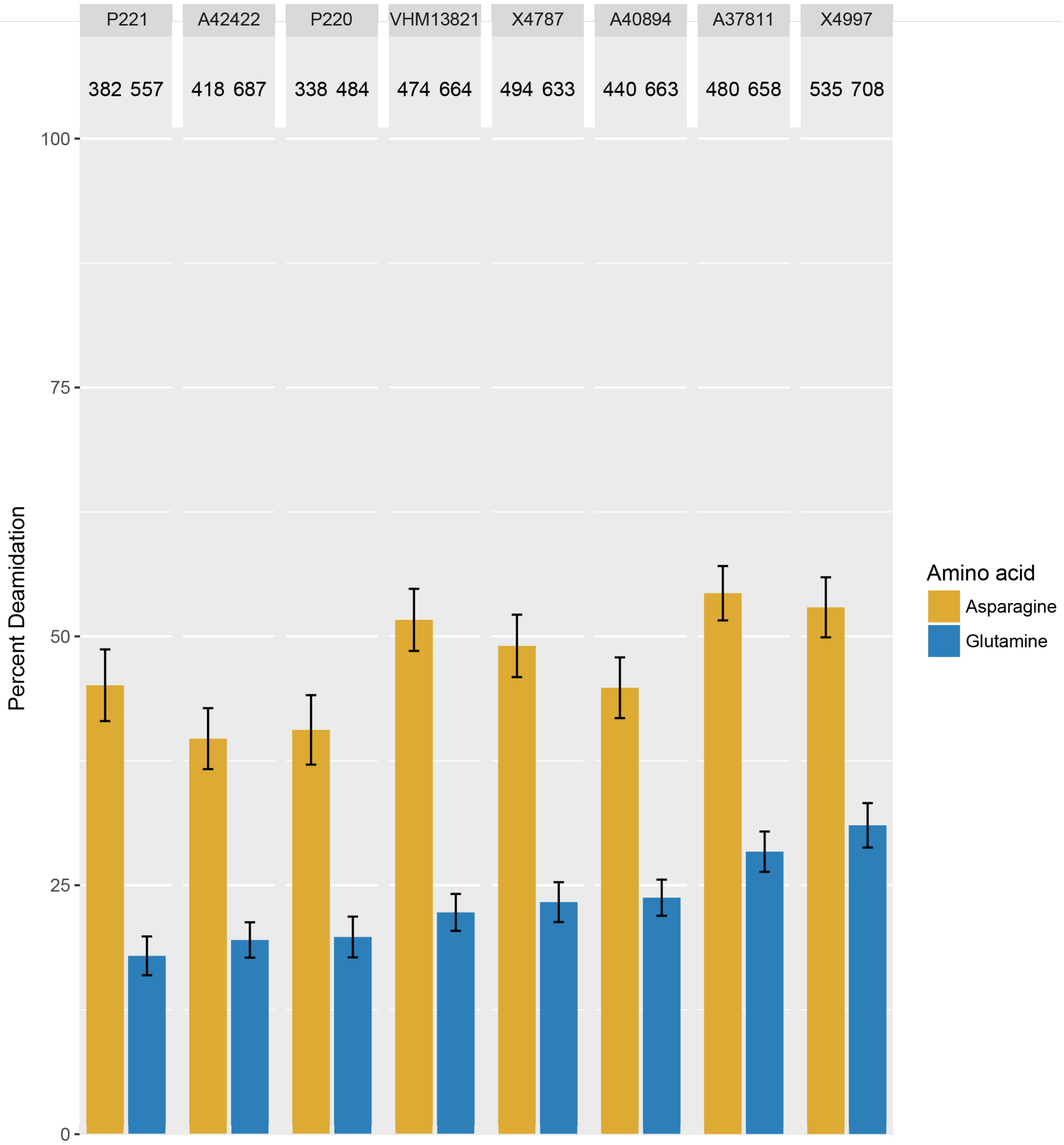
Supplementary Figure 22. Overview of approximate provenance of bone points 106-120.



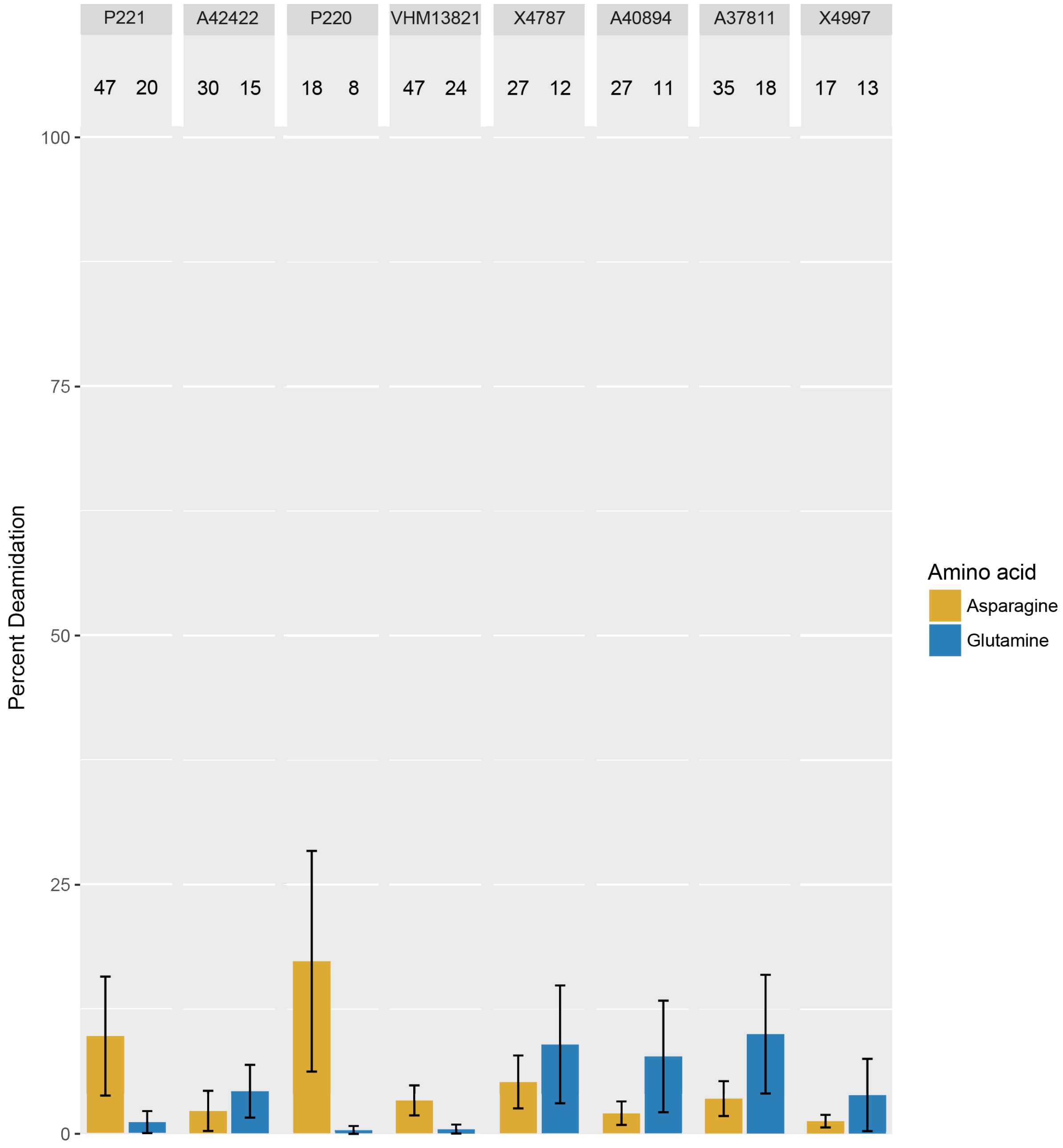
Supplementary Figure 23. Radiocarbon dates of faunal remains. Elk (blue), red deer (red), aurochs and bison (orange). Phase model applied to each taxonomic class by region. KDE model under each region denotes activity.



Supplementary Figure 24. Radiocarbon dates from the Early Mesolithic in Southern Scandinavia. A) radiocarbon dates from Denmark associated with habitation, B) KDE model showing activity, C) radiocarbon dates from Sweden associated with habitation, D) burials from contexts not associated with habitation (nb. Køge Sønakke may not represent a burial, as the remains were dredged).



Supplementary Figure 23. Overall percentage of deamidation for asparagine (N) and glutamine (Q) residues for the authentic bone proteins from the bone points (A37811, A40874, A42422, and VHM13821) and the standard elk (P220, P221) and red deer (X4787, X4997) samples. Error bars represent standard deviation and numbers above each bar represent the number of peptides the calculation is based on.



Supplementary Figure 24. Overall percentage of deamidation for asparagine (N) and glutamine (Q) residues for the contaminant bone proteins from the bone points (A37811, A40874, A42422, and VHM13821) and the standard elk (P220, P221) and red deer (X4787, X4997) samples. Error bars represent standard deviation and numbers above each bar represent the number of peptides the calculation is based on.

Supplementary Tables

Supplementary Table 1 Unique tryptic peptide sequences between red deer and elk collagen type 1

Marker Name	Location	Species	Sequence	Blast search matches	LC-MS/MS Marker	ZooMS Marker
A1T66/67	733-756 COL1a1	elk	GETGPAGR**A**GEVGPPGPPGPAGEK	only collagen	yes	no
		red deer	GETGPAGR**P**GEVGPPGPPGPAGEK	only collagen		yes - missed cleavage before P at m/z 2216
A1T74	849-855 COL1a1	elk	DGSPG**P**K	KFY28177.1 (*Pseudogymnoascus sp*), VDL62941.1 (*Hymenolepis diminuta*)	no	no
		red deer	DGSPG**A**K	collagen and fish proteins		
A2T40	430-443 COL1a2	elk	GAPGPDGNNGAQGPPG**L**QGVQGGK	only collagen	no	no
		red deer	GAPGPDGNNGAQGPPG**P**QGVQGGK	WP_139160575.1 (*Acinetobacter baumannii*), WP_139109296.1 (*Klebsiella pneumoniae*)		
A2T51	574-582 COL1a2	elk	GD**I**GSPGR	WP_026910359.1 (*Patulibacter minatonensis*), RIK66337.1 (*Planctomycetes bacterium*)	no	no
		red deer	GD**V**GSPGR	MAW74128.1 (*Gemmatimonadetes bacterium*), OTB08864.1 (*Hypoxylon sp.*)		
A2T66	741- 757 COL1a2	elk	**T**GETGASGPPGFAGEK	only collagen	yes	no
		red deer	**S**GETGASGPPGFAGEK	only collagen		

Supplementary Table 2 Species diagnostic peptides found in bone points and reference material. Based on search of LC-MS/MS raw files in MaxQuant (v. 1.6.3.4). 2 spectra in A40894 and 1 in A42422 hit *Alces alces* specific peptides; however, the specific amino acids are not individually covered by b or y ions. Due to the relatively small abundance of these peptides compared to the *Cervus elaphus* peptides found in these samples, these hits are most likely false positives possibly due to unknown modifications or come from sample cross-contamination.

*Based on best spectra if multiple
**Identified by MaxQuant's second peptide search
X- species specific amino acid
Q - deamidation
(gl)- glu -> pyro-Glu

Sample	Sequence (No. of Hydroxyproline)	Length (aa)	Missed cleavages	Mass (Da)	Charge	MQ score*	No. of Matched spectra
P220 - Elk (*Alces alces*) reference material	AGEVGPPGPPGPA (2)	13	0	1133.5353	1	194.90	1
	AGEVGPPGPPGPAGEK (1)	16	0	1431.6994	2	136.82	1
	AGEVGPPGPPGPAGEK (2)	16	0	1447.6943	2	257.75	4
	AGEVGPPGPPGPAGEK (3)	16	0	1463.6892	2	179.72	2
	AGEVGPPGPPGPAGEKG (2)	17	1	1504.7158	2	174.05	1
	AGEVGPPGPPGPAGEKGAPG (3)	20	1	1745.8220	2	166.62	1
	AGEVGPPGPPGPAGEKGAPG (4)	20	1	1761.8170	2	162.72	1
	AGEVGPPGPPGPAGEKGAPGADGPA (2)	25	1	2141.0025	2	333.13	1
	AGEVGPPGPPGPAGEKGAPGADGPA (3)	25	1	2156.9974	2	329.16	2
	AGEVGPPGPPGPAGEKGAPGADGPA (4)	25	1	2172.9924	2	306.72	1
	EGLRGPRGDQGPVGRT (0)	16	3	1650.855	2	191.69	1
	EGLRGPRGDQGPVGRT (0)	16	3	1651.839	2	137.81	1
	GDQGPVGRT (0)	9	1	886.41446	2	93.467	1
	GPRGDQGPVGRT (0)	12	2	1195.6058	2;3	136.86	2
	GPRGDQGPVGRT (0)	12	2	1196.5898	2;3	155.26	2
	GRAGEVGPPGPPGPAGEK (2)	18	1	1660.8169	2	202.63	1
	TGETGASGPPGFAGEK (1)	16	0	1477.6685	2	260.81	1
P221 - Elk (*Alces alces*) reference material	AGEVGPPGPPG (2)	11	0	965.44543	2	102.30	1
	AGEVGPPGPPGPAGEK (1)	16	0	1431.6994	2	169.82	1
	AGEVGPPGPPGPAGEK (2)	16	0	1447.6943	2	237.08	3
	AGEVGPPGPPGPAGEK (3)	16	0	1463.6892	2	150.65	2
	AGEVGPPGPPGPAGEKG (2)	17	1	1504.7158	2	184.43	1
	AGEVGPPGPPGPAGEKGAPG (2)	20	1	1729.8271	2	217.57	1
	AGEVGPPGPPGPAGEKGAPG (3)	20	1	1745.8220	2	145.81	1

	AGEVGPPGPPGPAGEKGAPG (4)	20	1	1761.8170	2	166.62	1
	AGEVGPPGPPGPAGEKGAPGADGPA (2)	25	1	2141.0025	2	328.92	1
	AGEVGPPGPPGPAGEKGAPGADGPA (3)	25	1	2156.9974	2	261.91	2
	AGEVGPPGPPGPAGEKGAPGADGPA (4)	25	1	2172.9924	2	303.44	1
	EGLRGPRGDQGPVGRT (0)	16	3	1650.855	2	199.30	1
	EGLRGPRGDQGPVGRT (0)	16	3	1651.839	2;3;4	138.97	3
	(gl)-EGLRGPRGDQGPVGRT (0)	16	3	1633.8285	3	105.77	1
	GDQGPVGRT	9	1	886.41446	2	100.02	1
	GPRGDQGPVGRT (0)	12	2	1195.6058	2;3	197.94	2
	GPRGDQGPVGRT (0)	12	2	1196.5898	2	160.91	1
	GRAGEVGPPGPPGPAGEK (2)	18	1	1660.8169	2	215.15	1
	GPRGDQGPVGRTGETGASGPPGF (1)	23	2	2169.0199	3	96.881	1
	TGETGASGPPGFAGEK (1)	16	0	1477.6685	2	273.02	1
	TGETGASGPPGFAGEK (2)	16	0	1493.6634	2	122.86	1
X4787 - Red Deer (*Cervus elaphus*) reference material	GETGPAGRPGE (1)	11	1	1042.468	2	106.40	1
	GETGPAGRPGEVGPPGPPGPA (3)	21	1	1900.8915	2	220.85	1
	GETGPAGRPGEVGPPGPPGPAGEK (3)	24	1	2215.0505	2;3	326.78	3
	GETGPAGRPGEVGPPGPPGPAGEK (4)	24	1	2231.0455	2;3	219.95	2
	GETGPAGRPGEVGPPGPPGPAGEKG (3)	25	2	2272.072	3	100.23	1
	GRPGEVGPPGPPGPAGEK (3)	18	1	1702.8275	2;3	278.30	4
	GRPGEVGPPGPPGPAGEK (4)	18	1	1718.8224	2	147.35	1
	PGEVGPPGPPGPAGEK (3)	16	0	1489.7049	2	257.16	3
	PGEVGPPGPPGPAGEK (4)	16	0	1505.6998	2	222.20	2
	SGETGASGPPGF (1)	12	0	1078.4567	2	171.75	1
	SGETGASGPPGFAGEK (0)	16	0	1447.6579	2	174.90	1
	SGETGASGPPGFAGEK (1)	16	0	1463.6529	2	234.79	2
	SGETGASGPPGFAGEK (2)	16	0	1479.6478	2	99.752	1
	GPRGDQGPVGRSGETGASGPPGF (1)	23	2	2155.0043	3	136.96	1
X4997 - Red Deer (*Cervus elaphus*) reference material	EGLRGPRGDQGPVGRS (0)	16	3	1636.8394	3	82.709	1
	EGLRGPRGDQGPVGRS (0)	16	3	1637.8234	3	72.341	1
	GETGPAGRPG (1)	10	1	913.42536	2	98.629	1
	GETGPAGRPGEVGPPGPPGPAGEK (2)	24	1	2199.0556	3	107.09	1
	GETGPAGRPGEVGPPGPPGPAGEK (3)	24	1	2215.0505	2;3	272.19	3
	GETGPAGRPGEVGPPGPPGPAGEK (4)	24	1	2231.0455	2;3	315.58	2

	GRPGEVGPPGPPGPAGEK (3)	18	1	1702.8275	2;3	278.68	2
	GRPGEVGPPGPPGPAGEK (4)	18	1	1718.8224	2;3	139.66	2
	PGEVGPPGPPGPAGEK (2)	16	0	1473.71	2	245.85	1
	PGEVGPPGPPGPAGEK (3)	16	0	1489.7049	2;3	295.20	5
	PGEVGPPGPPGPAGEK (4)	16	0	1505.6998	2	237.67	2
	SGETGASGPPGFAGEK (0)	16	0	1447.6579	2	177.10	1
	SGETGASGPPGFAGEK (1)	16	0	1463.6529	2	234.63	2
	SGETGASGPPGFAGEK (2)	16	0	1479.6478	2	143.97	1
	GPRGDQGPVGRSGETGASGPPGF (1)	23	2	2155.0043	3	175.33	1
A37811 - Preboral bone point (*Alces alces*)	AGEVGPPG (1)	8	0	698.32352	2	62.338	1
	AGEVGPPGP (1)	9	0	795.37628	2	67.741	1
	AGEVGPPGPPG (2)	11	0	965.44543	2	124.34	1
	AGEVGPPGPPGPA (2)	13	0	1133.5353	2	137.62	1
	AGEVGPPGPPGPAG (2)	14	0	1190.5568	2	117.04	1
	AGEVGPPGPPGPAGEK (1)	16	0	1431.6994	2	178.36	1
	AGEVGPPGPPGPAGEK (2)	16	0	1447.6943	2;3	259.47	7
	AGEVGPPGPPGPAGEK (3)	16	0	1463.6892	2	189.43	6
	AGEVGPPGPPGPAGEK (4)	16	0	1479.6842	2	103.44	2
	AGEVGPPGPPGPAGEKG (2)	17	1	1504.7158	2	85.536	1
	AGEVGPPGPPGPAGEKG (3)	17	1	1520.7107	2	117.20	1
	AGEVGPPGPPGPAGEKGA (3)	18	1	1591.7478	2	143.42	1
	RAGEVGPPGPPGPAGEK (3)	17	1	1619.7903	3	69.74	1
	TGETGASGPPGFAGEK (1)	16	0	1477.6685	2	243.40	1
	TGETGASGPPGFAGEK (2)	16	0	1493.6634	2	128.28	2
	TGETGASGPPGFAGEKG (1)	17	1	1534.69	2	153.75	1
A40894 - Boral bone point (*Cervus elaphus*)	ETGPAGRPGEVGPPGPPGPAGEK (4)	23	1	2174.024	3	78.837	1
	GETGPAGRPG (1)	10	1	913.42536	2	69.905	1
	GETGPAGRPGE (1)	11	1	1042.468	2	136.63	1
	GETGPAGRPGEV (1)	12	1	1141.5364	2	72.848	1
	GETGPAGRPGEVG (1)	13	1	1198.5578	2	199.68	2
	GETGPAGRPGEVGP (1)	14	1	1295.6106	2	223.87	1
	GETGPAGRPGEVGPP (2)	15	1	1408.6583	2	173.37	1
	GETGPAGRPGEVGPPG (2)	16	1	1465.6797	2	114.24	1
	GETGPAGRPGEVGPPGP (2)	17	1	1562.7325	2	122.52	1
	GETGPAGRPGEVGPPGPPG (3)	19	1	1732.8016	2	218.11	2
	GETGPAGRPGEVGPPGPPGPAG (3)	22	1	1957.913	2	283.10	1

	GETGPAGRPGEVGPPGPPGPAGEK (3)	24	1	2215.0505	2;3	285.34	3
	GETGPAGRPGEVGPPGPPGPAGEK (4)	24	1	2231.0455	2;3	276.03	2
	GETGPAGRPGEVGPPGPPGPAGEK (5)	24	1	2247.0404	3	179.50	5
	GETGPAGRPGEVGPPGPPGPAGEKG (4)	25	2	2288.0669	3	143.25	1
	GPAGRPGEVGPPGPPGPAGEK (4)	21	1	1943.9337	3	123.53	1
	GRPGEVGPPGPPGPAGEK (4)	18	1	1718.8224	3	67.995	1
	PAGRPGEVGPPGPPGPAGEK (4)	20	1	1886.9123	3	121.63	1
	PGEVGPPGPPG (3)	11	0	1007.456	2	95.352	1
	PGEVGPPGPPGPAGEK (2)	16	0	1473.71	2	132.99	1
	PGEVGPPGPPGPAGEK (3)	16	0	1489.7049	2	257.76	3
	PGEVGPPGPPGPAGEK (4)	16	0	1505.6998	2	236.11	3
	RPGEVGPPGPPGPAGEK (4)	17	1	1661.8009	3	63.374	1
	TGPAGRPGEVGPPGPPGPAGEK (4)	22	1	2044.9814	3	109.39	1
	SGETGASGPPGFAGEK (0)	16	0	1447.6579	2	134.56	1
	SGETGASGPPGFAGEK (1)	16	0	1463.6529	2	223.31	4
	SGETGASGPPGFAGEKG (1)	17	1	1520.6743	2	104.49	1
A42422 - Boral bone point (*Cervus elaphus*)	GETGPAGRPGE (1)	11	1	1042.468	2	145.89	1
	GETGPAGRPGEVG (1)	13	1	1198.5578	2	179.42	1
	GETGPAGRPGEVGP (1)	14	1	1295.6106	2	103.35	1
	GETGPAGRPGEVGPPG (2)	16	1	1465.6797	2	106.26	1
	GETGPAGRPGEVGPPGP (2)	17	1	1562.7325	2	130.79	1
	GETGPAGRPGEVGPPGPPG (3)	19	1	1732.8016	2	225.33	2
	GETGPAGRPGEVGPPGPPGPAG (3)	22	1	1957.913	2	314.55	1
	GETGPAGRPGEVGPPGPPGPAGEK (3)	24	1	2215.0505	3	210.32	4
	GETGPAGRPGEVGPPGPPGPAGEK (4)	24	1	2231.0455	2;3	293.20	5
	GETGPAGRPGEVGPPGPPGPAGEK (5)	24	1	2247.0404	3	142.32	1
	GRPGEVGPPGPPGPAGEK (4)	18	1	1718.8224	3	64.249	1
	PAGRPGEVGPPGPPGPAGEK (3)	20	1	1870.9173	3	65.425	1
	PGEVGPPGPPG (3)	11	0	1007.456	2	99.375	1
	PGEVGPPGPPGPAG (3)	14	0	1232.5673	2	153.08	1
	PGEVGPPGPPGPAGEK (2)	16	0	1473.71	2	164.68	1
	PGEVGPPGPPGPAGEK (3)	16	0	1489.7049	2	297.50	3
	PGEVGPPGPPGPAGEK (4)	16	0	1505.6998	2	222.20	2
	RPGEVGPPGPPGPAGEK (4)	17	1	1661.8009	3	63.145	1

	SGETGASGPPGFAGEK (0)	**16**	**0**	**1447.6579**	**2**	**168.02**	**1**
	SGETGASGPPGFAGEK (1)	**16**	**0**	**1463.6529**	**2**	**231.67**	**4**
	SGETGASGPPGFAGEK (2)	**16**	**0**	**1479.6478**	**2**	**100.47**	**1**
VHM13821 - Preboral bone point (*Alces alces*)	**AGEVGPPG** (1)**	**8**	**0**	**698.32352**	**1**	**85.306**	**1**
	AGEVGPPGPPG (2)	**11**	**0**	**965.44543**	**2**	**120.45**	**2**
	AGEVGPPGPPGPA (2)	**13**	**0**	**1133.5353**	**2**	**110.67**	**1**
	AGEVGPPGPPGPAGEK (1)	**16**	**0**	**1431.6994**	**2**	**147.10**	**1**
	AGEVGPPGPPGPAGEK (2)	**16**	**0**	**1447.6943**	**2;3**	**248.56**	**7**
	AGEVGPPGPPGPAGEK (3)	**16**	**0**	**1463.6892**	**2;3**	**172.60**	**7**
	AGEVGPPGPPGPAGEK (4)	**16**	**0**	**1479.6842**	**2**	**154.67**	**3**
	AGEVGPPGPPGPAGEKG (2)	**17**	**1**	**1504.7158**	**2**	**158.34**	**1**
	AGEVGPPGPPGPAGEKG (3)	**17**	**1**	**1520.7107**	**2**	**135.26**	**2**
	TGETGASGPPGFAGEK (0)	**16**	**0**	**1461.6736**	**2**	**173.76**	**1**
	TGETGASGPPGFAGEK (1)	**16**	**0**	**1477.6685**	**2**	**250.67**	**3**
	TGETGASGPPGFAGEK (2)	**16**	**0**	**1493.6634**	**2**	**162.68**	**3**

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Chapter 4: The biomolecular characterization of a finger ring contextually dated to the emergence of the Early Neolithic from Syltholm, Denmark

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Research

The biomolecular characterization of a finger ring contextually dated to the emergence of the Early Neolithic from Syltholm, Denmark

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We present the analysis of an osseous finger ring from a predominantly early Neolithic context in Denmark. To characterize the artefact and identify the raw material used for its manufacture, we performed micro-computed tomography scanning, zooarchaeology by mass spectrometry (ZooMS) peptide mass fingerprinting, as well as protein sequencing by

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liquid chromatography tandem mass spectrometry (LC-MS/MS). We conclude that the ring was made from long bone or antler due to the presence of osteons (Haversian canals). Subsequent ZooMS analysis of collagen I and II indicated that it was made from *Alces alces* or *Cervus elaphus* material. We then used LC-MS/MS analysis to refine our species identification, confirming that the ring was made from *Cervus elaphus*, and to examine the rest of the proteome. This study demonstrates the potential of ancient proteomics for species identification of prehistoric artefacts made from osseous material.

1. Introduction

Several excavations at Syltholm near Rødbyhavn on the island of Lolland, Denmark, have revealed an exceptionally well-preserved archaeological assemblage belonging to the Ertebølle (*ca* 7350–5950 cal BP) and Early Funnel Beaker periods (*ca* 5950–4750 cal BP) [1]. Among other things, the assemblage contains numerous artefacts made from organic material, such as wood, bone and antler, as well as several exotic objects, including a T-shaped antler axe [2], a Danubian shaft-hole axe made of amphibolite as well as pieces of Arkadenrand-type ceramics. These finds suggest connections with Neolithic societies of northern Germany and central Europe. One of the more spectacular finds from one Syltholm site (906-II) is one half of an osseous finger ring found in 2014 at the northernmost section of this site (figure 1).

The finger ring (find no. X2784) is broken, but is otherwise perfectly preserved and displays excellent handicraft, design and finish (figure 1*d*). It measures 2.4 cm in diameter, large enough to suggest that it might have been worn by an adult male. The exterior is finely polished, with only microscopic scratches and no use-wear visible, while the interior still shows well-preserved traces of carving, suggesting that it was either barely worn, or that it broke during manufacture. The ring was found in a layer containing a large amount of wooden artefacts, which have been directly dated to between *ca* 6300 and 5500 cal BP (table 1 and figure 2), spanning the period(s) of activity at the site. The ring itself was found close to a broken wooden spear made of ash (X4955, table 1), which yielded a date of 5983–5750 cal BP, and while we were unable to obtain a direct date for the ring itself (due to sampling limitations), we propose that these two contextually associated artefacts are coeval.

Finger rings made of osseous material first appear in large quantities during the Anatolian Neolithic [3], and later over a large area of southern and central Europe. On the Iberian peninsula, numerous finger rings attributed to the Neolithic Cardial culture are known [4–7]. Further north, bone rings are present in deposits from the Rubané culture, e.g. at Mulhouse-Est and in the wider Alsace area (Linear band ceramic (LBK)) [8]. Sporadic occurrences of rings appear from the LBK/Rubané periods and subsequent periods in north and central Europe. In The Netherlands, at Ypenburg 4, a bone ring was found in a child's burial dated to the Middle Neolithic [9]. In northern Germany, rings were found at the sites of Oldenburg Dannau LA 191 and at Wangels, both are dated to the Middle Neolithic (S. Hartz 2018, personal communication). In addition, a limited number of rings dated to the Danish and Swedish Early Neolithic have been found, predominantly in dolmens [10,11]. These artefacts are unlikely to be finger rings due to the large shank depth. The ring from Syltholm is the only example known from the Early Neolithic in Denmark, apart from another broken ring from the shell-midden site at Nederst in Jutland, which was found alongside a ring-preform. Both Nederst artefacts were manufactured from wild boar (*Sus scrofa*) tusk [12], as a thin layer of enamel is visible on the preform surface [12] (E. Kannegaard 2018, personal communication).

The composition of the Syltholm ring is not as readily identifiable as the Nederst ring, and we therefore carried out a series of analyses to identify and characterize the raw material used for its manufacture. X-ray micro-computed tomography (micro-CT) imaging was performed to create high-resolution scans of the ring, while zooarchaeology by mass spectrometry (ZooMS) peptide mass fingerprinting and liquid chromatography tandem mass spectrometry (LC-MS/MS) protein sequencing were used for species identification and further characterization. ZooMS is often chosen for archaeological research because it can provide a rapid, cost effective species identification for samples containing collagen (i.e. bone, antler and skin) [13,14]. However, at present ZooMS is unable to separate the two species of cervids *Cervus elaphus* (red deer, hereafter referred to as *Cervus*) and *Alces alces* (European elk or North American moose, hereafter referred to as *Alces*). Therefore, we also used LC-MS/MS protein sequencing to refine and confirm species identification. We demonstrate the potential of a combined approach for the analysis of prehistoric artefacts made from osseous material and add to the understanding of this hitherto under-studied finds category.

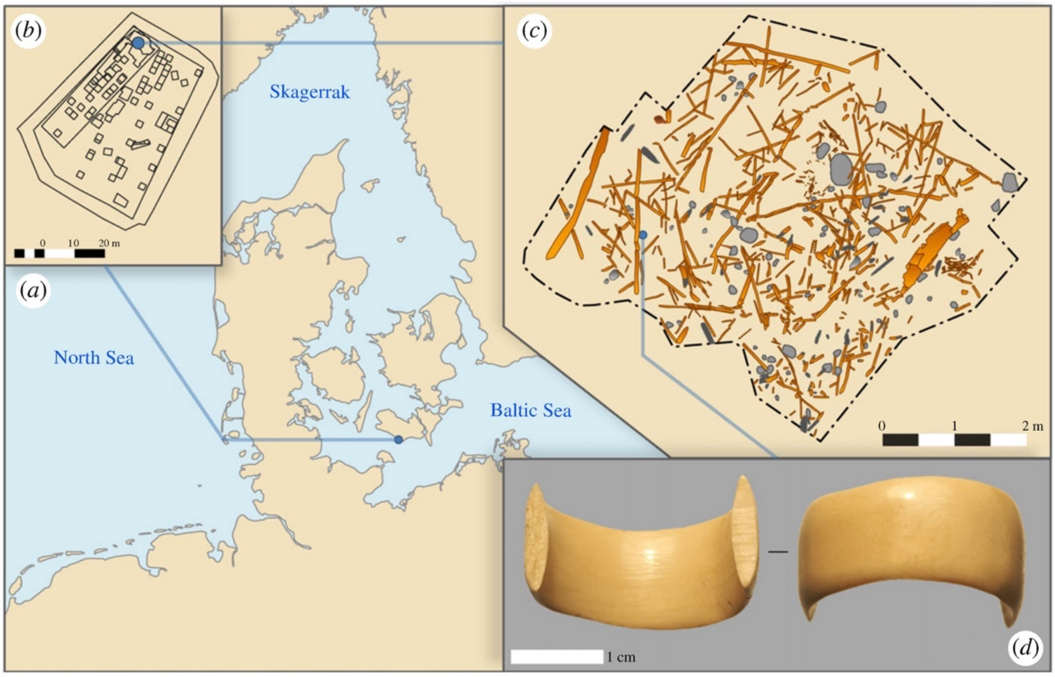


Figure 1. (*a*) Location of the site on the southern part of Lolland, Denmark. (*b*) Overview of site MLF906-II where the ring was found in the northern part. (*c*) Digitized archaeological wood and stones found in a small section of the site, from where the ring was found. Digitization based on seven three-dimensional models obtained by Structure from Motion. (*d*) Photograph of the ring.

2. Material and methods

2.1. The ring in context

Up until 1872 Syltholm was submerged, but after severe flooding on Lolland a reclamation project was undertaken. The area was dammed, thus preserving the inundated Stone Age landscape below. Around 7000 m^2 of prehistoric seabed was exposed at the sites MLF906-I and MLF906-II, with the underlying landscape located 1.50–3.00 m below the surface. By the end of the excavation campaign, a total of 80 000 m^2 will have been excavated (at all 20 sites) out of *ca* 187 hectares that will become a construction site for the Femern Belt connection [15]. During the Late Mesolithic and Early Neolithic, MLF906-II was located in a shallow brackish lagoon protected from the open sea to the south by shifting sandy barrier islands. Preservation of the site varies according to the degree of shelter provided by the barrier islands; however, the preservation of organic material (wood, bone and antler) is generally very good. Out of the 20 sites excavated to date, the majority of *in situ* finds were uncovered in the reed zone along the banks of the littoral lagoon. The finds were deposited in a coarse brown gyttja varying in thickness (10–50 cm), with no apparent stratigraphy. The layer on top of the gyttja is a clearly defined transgression horizon consisting of sand, shells and vast amounts of water rolled artefacts, suggesting an erosive milieu. Above this layer follows a layer of detritus gyttja, with no archaeological remains, and then a thick layer of sand.

2.2. Radiocarbon dating

Direct dating of the ring was not possible due to sampling limitations. However, 70 radiocarbon dates of various artefacts from the site (table 1) were commissioned as part of the wider project carried out by the Museum Lolland-Falster. The radiocarbon measurements were carried out at the Aarhus AMS Centre (AARAMS) at the University of Aarhus, Denmark. Wood samples were pretreated using a standard acid–base–acid procedure (1 M HCl for 1 h at 80°C, followed by 1 M NaOH for 3 h also at 80°C, and finally left overnight in 1 M HCl at room temperature). The pretreatment for bone samples followed a modified Longin procedure [16–18]. Bone minerals were dissolved using 1 M HCl at 4°C for several days followed by removal of humic substances using 0.2 M NaOH, also at 4°C. Subsequently, the extracted collagens were gelatinized in 0.01 M HCl at 58°C overnight. The collagen extracts were

Table 1. List of radiocarbon dates. Due to the rarity of the artefact, 15 AMS dates were extracted from the excavations AMS database, from an area surrounding the findspot of the ring, to infer a contemporaneous date of the ring. The material dated is primarily wood, apart from a harpoon made of roe deer (*Capreolus capreolus*) antler.

finds no.	material	lab no.	δ^{13}C (‰ VPDB)	age (^{14}C years BP)	age cal BP (2σ, modelled)
X4633	harpoon, type C (bone)	AAR-24615	−24.1	5412 + 29	6288–6183
	boundary (start)				6165–5960
X8506	ash spear	AAR-26285	−27.2	5308 + 24	6105–5946
X5616	log boat	AAR-26291	−28.4	5241 + 35	6096–5915
X4281	paddle shaft	AAR-26272	−27.0	5193 + 24	5990–5913
X10213	ash spear	AAR-26283	−27.0	5148 + 26	5986–5765
X4955	ash spear	AAR-24619	−26.0	5128 + 35	5983–5750
X8823	ash spear	AAR-25106	−27.0	5110 + 28	5923–5751
X2697	bow	AAR-22731	−29.8	5045 + 25	5896–5730
X10244A	paddle shaft	AAR-26284	−24.4	5041 + 25	5895–5724
X8844	ash spear	AAR-26280	−26.6	5025 + 27	5893–5662
X8732	stake	AAR-25100	−29.3	5024 + 27	5892–5662
X8643	ash spear	AAR-26279	−25.6	5015 + 31	5892–5657
X5617	fish trap	AAR-26271	−27.6	4960 + 30	5744–5610
X4282	ash spear	AAR-26278	−27.0	4909 + 25	5710–5595
X4303	paddle shaft	AAR-26273	−26.1	4887 + 24	5655–5593
	boundary (end)				5649–5512

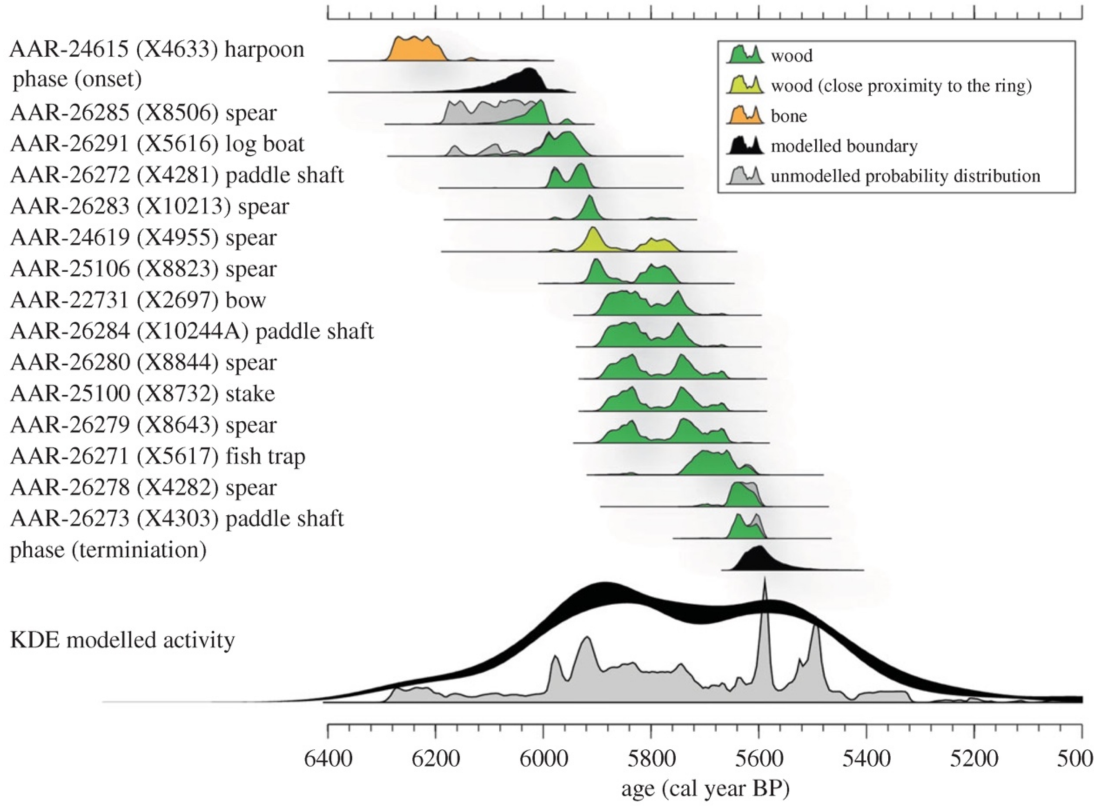


Figure 2. Probability distributions of the 15 radiocarbon samples found in close proximity to the ring. The coloured probability distributions are the result of a simple Bayesian model assuming all samples to originate from the same phase of activity. Onset and termination of the phase are indicated with black probability distributions. The light green probability distribution is the date we propose for the ring as well, based on the proximity of the spear to the ring. KDE model of all 70 ^{14}C dates indicating a single inferred period of archaeological activity at site in general.

ultra-filtered and the resulting greater than 30 kDa collagen fraction was used for radiocarbon analysis. The uncalibrated dates were calibrated using Oxcal v.4.3 and the IntCal13 calibration curve [19,20] and are listed in electronic supplementary material, SI 2 [21].

2.3. Imaging/computed tomography scanning

Micro-CT was used to examine the ring using the commercial Zeiss Xradia410 versa system. The ring was rotated 360° in 1601 steps taking a picture at each step using a pre-voltage of 80 kV and a power of 10 W. Two measurements with different pixel resolutions were performed at 32.3 and 13.5 µm. The three-dimensional volume was reconstructed using the software provided with the instrument system 'Reconstructor', which is based on a Feldkamp–Davis–Kress algorithm [22]. The resulting three-dimensional volumes are cylinders with a diameter and height of 3.2 cm and 1.35 cm, respectively, corresponding to the different pixel resolutions, containing different amounts of the object. Visualization was performed using Avizo 9.7 (Thermo Fisher Scientific). The volume investigated with high-resolution X-ray micro-CT has been segmented into elements of the bone (shown in transparent grey) and porosity in the bone (shown in blue). The different levels of blue are a result of the amount of transparency.

2.4. Sampling, protein extraction, enzymatic digestion and peptide purification

The artefact surface was decontaminated using 5% bleach followed by 80% ethanol and subsequently 11 mg of bone from one of the fracture planes of the ring was removed using a sterile scalpel. The sampling and protein extraction of the ring were conducted in the dedicated clean laboratories facilities at the Centre for GeoGenetics, University of Copenhagen, Denmark.

To explore the potential for proteomically discriminating between *Cervus* antler and bone in ancient samples, a sample each of both modern *Cervus* bone and antler were collected from the Zoological Museum of Denmark. These samples were taken from a specimen that was defleshed by heating it in water for 3 days at *ca* 65°C. This experimentally heated extant sample is more comparable to the ring sample than fresh bone or antler. The two reference samples from *Cervus* antler and bone (weighing approx. 15 mg) were subsequently sampled and extracted in a dedicated proteomics laboratories at the Section for Evolutionary Genomics, University of Copenhagen.

The protein extractions were based on a minimally destructive protocol published by van Doorn *et al.* [23] with the following modifications: The samples were incubated in 100 µl of 50 mM NH_4HCO_3 (Sigma) for 16 h at ambient temperature. Samples were then agitated using a vortex mixer for 15 s before centrifugation at 13 000 r.p.m. for 1 min, the supernatant was discarded. This step acts as a wash to limit contamination from the burial environment. After, two different extractions were performed for the ring (Extraction 1 and 2), while the reference samples were extracted according to the Extraction 1 protocol. Extraction 2 was performed to remove humic acids that could have contaminated the artefact. Extraction 1: 100 µl of 50 mM NH_4HCO_3 was added to the sample before incubation at 65°C for 1 h, the supernatant (Extraction 1) was collected. Extraction 2: the remaining sample was washed three times with 100 µl of 0.1 M NaOH at 4°C and subsequently incubated in 100 µl of 50 mM NH_4HCO_3 at 65°C for 1 h, the supernatant (Extraction 2) was collected. Fifty microlitres of each extraction were transferred to a separate 1.5 ml Eppendorf tube, 1 µl of sequencing grade trypsin (0.4 µg $µl^{-1}$) (Promega) was added to each followed by incubation at 37°C for 16 h. After digestion, the extractions were centrifuged at 13 000 r.p.m. for 1 min before acidification to less than pH 2 using 5% (vol/vol) trifluoroacetic acid (TFA, Sigma Aldrich). Purification was performed using C18 reverse phase resin ZipTips (PierceTM) according to the manufacturer's instruction, and the peptides were eluted with 50 µl of 50% acetonitrile (ACN) (Sigma Aldrich)/0.1% TFA (vol/vol).

2.5. ZooMS peptide mass fingerprinting

Peptide eluates of the ring were co-crystallized with α-cyano-4-hydroxycinnamic acid (Sigma Aldrich) matrix solution (50% ACN/0.1% TFA (vol/vol)) at a ratio of 1 : 1 (1 µl : 1 µl). Mass spectrometry was performed using a Bruker Ultraflex III (Bruker Daltonics) matrix-assisted laser desorption/ionization time of flight mass spectrometer (MALDI-TOF-MS) run in reflector mode with laser acquisition set to 1200 and acquired over an *m*/*z* range of 800–3200. The generated spectral output was converted to TXT and was analysed using the open-source software mMass v.5.5.0 [24]. The triplicate raw files were merged, and then peak picked with an S/N threshold of 4. MALDI-TOF-MS was performed at Centre for Excellence in Proteomics at the University of York, UK.

2.6. Liquid chromatography tandem mass spectrometry

The leftover peptide eluates of the ring sample were evaporated to dryness using a vacuum concentrator (Eppendorf, Hamburg, Germany), and transferred to the Novo Nordisk Foundation Center for Protein Research, University of Copenhagen for LC-MS/MS analysis on a EASY-nLC 1200 (Proxeon, Odense, Denmark) coupled to a Q Exactive HF-X (Thermo Scientific, Bremen, Germany). The dried peptides from the two ring extractions were resuspended in 100 µl of 80% ACN and 0.1% formic acid (FA), and 15 µl of each of the two ring sample extractions were combined. The combined sample was vacuum centrifuged at 45°C until approximately 3 µl was left, and was then rehydrated with 10 µl of 0.1% TFA, 5% ACN. Protein concentration of elutions was measured by UV absorbance at 205 nm using a Nanodrop (Thermo, Wilmington, DE, USA). The volume required for approximately 2 µg of protein per sample was placed in separate wells on a new 96-well plate and topped up to 30 µl using 40% ACN and 0.1% FA. They were then vacuum centrifuged and resuspended as above, with 5 µl of sample analysed by LC-MS/MS. The LC-MS/MS parameters were the same as previously used for palaeoproteomic samples [25], in short: MS1: 120 k resolution, maximum injection time (IT) 25 ms, scan target 3E6. MS2: 60 k resolution, top 10 mode, maximum IT 118 ms, minimum scan target 3E3, normalized collision energy of 28, dynamic exclusion 20 s, and isolation window of 1.2 *m*/*z*.

The Thermo RAW files generated were then searched using the software MAXQUANT (v. 1.6.2.6a or v. 1.6.3.4) [26]. The database was prepared using previously published type 1 collagen sequences from *Cervus elaphus* and *Alces alces* [27] (table 2). Missing amino acids in the *Alces* and *Cervus* collagen sequences were substituted with ones from the same positions from the *Bos taurus* sequence (obtained from UniProt, 20-07-18; electronic supplementary material, SI 1). Furthermore, in order to identify proteins in addition to collagen type 1, all protein sequences available for *Cervus elaphus* were downloaded from UniProt (20-07-18). Unfortunately, no other proteins for *Alces* have, thus far, been uploaded to UniProt. MaxQuant settings were as follows. Digestion mode was set to semispecific for Trypsin, to account for possible

Table 2. Identified *Cervus elaphus* peptides based on published collagen sequences from [27].

sequence	length	missed cleave	Da	*Q*	MQ score	matched spectra
PGEVGPPGPPGPAGEK	16	0	1441.7201	2	218.62	11
GETGPAGRPGEVGPPGPPGPAGEK	24	1	2167.0658	2;3	276.46	9
PGEVGPPGPPGPAGEKGAPGAD	22	1	1909.917	2	196.48	1
GAPGPDGNNGAQGPPGPQGVQGGK	24	0	2112.9937	2;3	319.91	9
SGETGASGPPGFAGEK	16	0	1447.6579	2	169.23	5

additional hydrolytic cleavages occurring during diagenesis. Variable modifications were: oxidation (M), Acetyl (Protein N-term), Deamidation (NQ), Gln → pyro-Glu, Glu → pyro-Glu and Hydroxyproline. Fixed modifications were: Carbamidomethyl (C). The remaining settings were set to the program defaults, apart from Min. score for unmodified and modified peptides searches, which were both set to 60. Proteins were considered confidently identified if at least two razor+unique peptides covering distinct areas of the sequence were recovered (a razor peptide is a peptide which is assigned to the matching protein group with the highest number of peptide identifications and those uniquely assigned to that protein group). MS/MS spectra were assessed manually for confident identification, and peptides from the *Cervus elaphus* Uniprot protein database were searched against the NCBI database using the BLASTp tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi, [28]) to determine species specificity. In addition, the samples were searched against the MaxQuant contaminant database that identifies proteins which may be present due to sample handling and laboratory analysis. Any protein not considered authentic to the ring (i.e. keratins from skin, bovine serum albumin (used as a laboratory standard)) was not included in further analysis except as a comparison for deamidation levels. Deamidation was assessed using publicly available code [25].

3. Results

3.1. Radiocarbon dates

The 15 calibrated radiocarbon dates, retrieved from close proximity to the ring, revealed an age distribution spanning the Late Ertebølle and the Early Neolithic periods (table 1). A harpoon (X4633) made from roe deer (*Capreolus capreolus*) antler produced the oldest date. The date of this artefact is in good agreement with directly and indirectly dated specimens of similar typology and raw material [29]. The collagen yield of the harpoon sample was 5.1% and it yielded stable isotope values ($\delta^{13}C = -24.1‰$, $\delta^{15}N = 4.4‰$ and $C/N = 3.3$) consistent with already published archaeological roe deer values in Denmark [30]. The remaining dated material was not typologically dated to a specific cultural period. The dates demonstrate that the site was frequented both before and after the Mesolithic/Neolithic transition, indicating that the site was continuously occupied during this transitional period. The usage period of the site was estimated using a simple Bayesian model assuming all finds to originate from a single phase of activity (figure 2). The onset of activity at the site is estimated to be *ca* 6060 cal BP (6173–5962 2σ) and the activity is suggested to end *ca* 5590 cal BP (5649–5512 2σ). The harpoon was excluded from the model due a low statistical agreement in the Bayesian model [17]. The reason for this is unknown as all collagen quality parameters are within expected ranges; however, it is possible that the harpoon was redeposited. The ring dates to the main occupation period of the site (*ca* 6060 cal BP–*ca* 5590 cal BP). The ash (*Fraxinus excelsior*) spear found closest to the ring returned a date of 5983–5750 cal BP (5128 ± 35 ^{14}C yr BP). Due to their close contextual and stratigraphic association, we propose that the ring has a similar date, but we cannot rule out that the ring is Late Mesolithic. However, as shown in the kernel density estimation (KDE) model, which includes an additional 55 dates from the surrounding area, activity at the site in general peaks during the Early Neolithic (figure 2).

3.2. Imaging/computed tomography scanning

Micro-CT imaging revealed the presence of many evenly spaced circular pores (figure 3*a–d*). The frequency and morphology of these pores indicate that they are osteons or Haversian canals.

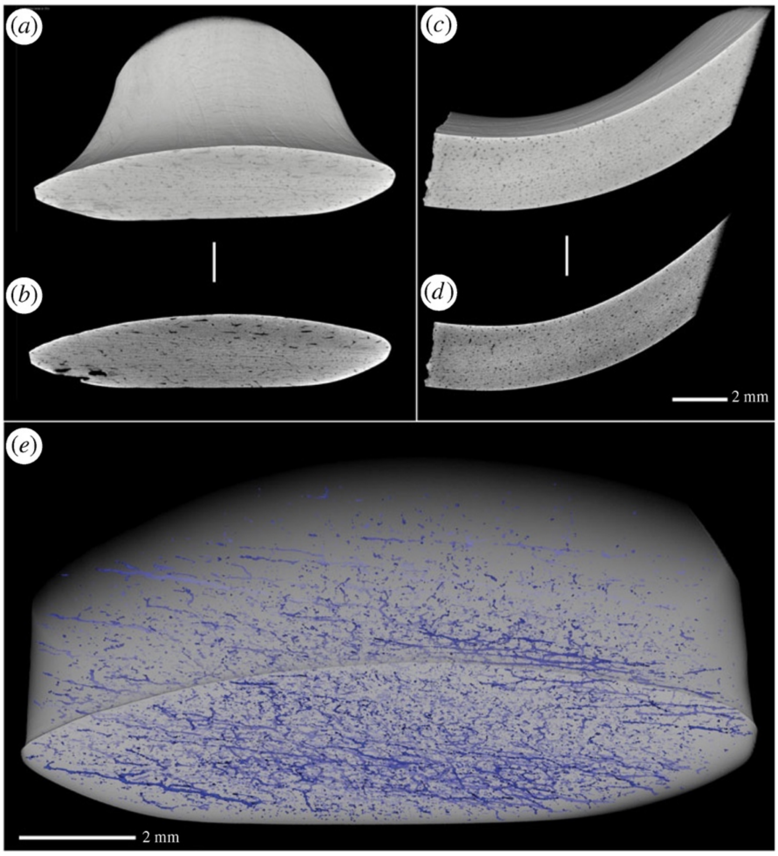


Figure 3. Micro-CT scan. (*a*) Full field of view of volume rendering, (*b*) transverse slice showing a few small black pores assumed to be Volkmann's canals, (*c*) cut along the middle of the volume rendering, (*d*) slice along showing several small black pores assumed to be osteons, (*e*) network of osteons arranged longitudinally and Volkmann's canals aligned perpendicular to the latter (in blue).

Additionally, Volkmann's canals, which are perpendicular connections between Haversian canals [31], can be seen in figure 3*e* and in the rendered supplementary animation (electronic supplementary material, video S1). The diameter of osteons in antler ranges from 100 to 225 µm, similar to those found in bovine femur [32]. By contrast, enamel only contains nanopores, and tubules in dentine and ivory (mostly composed of dentine) are approximately 1–2 µm in diameter [33]. We therefore conclude that the ring was manufactured from antler or bone, rather than enamel or dentine. Unfortunately, distinguishing archaeological antler from bone, where only the compact bone is present, using micro-CT scanning is problematic and inconsistent [34]. This is because the identification is based on subtle size differences in the diameter of the osteons, which are affected by diagenesis [34,35].

3.3. ZooMS peptide mass fingerprinting

To identify the species used to manufacture the Syltholm ring, we performed ZooMS peptide mass fingerprinting by MALDI-TOF-MS on the two extracts. The spectral outputs revealed a series of isotope distributions corresponding to the mass of tryptic peptide products within a range of 1105–3101 Da. Peptide masses previously reported to be unique for *Cervus* and *Alces* collagen 1 (COL1) were observed with high intensity [27,36] (figure 4). However, these two closely related species cannot be separated at present using ZooMS.

The two extraction methods showed some differences in terms of peptide count as well as intensity. Extraction 1 showed greater intensity peaks of lower molecular weight peptides than extraction 2. However, spectral data from extraction 2 showed greater intensity of a large portion of the higher molecular weight peptides. The reason for this small difference remains unknown. Given the spectral similarity between the two extractions, it is likely that the artefact was not contaminated with humic acids from the environment (figure 4).

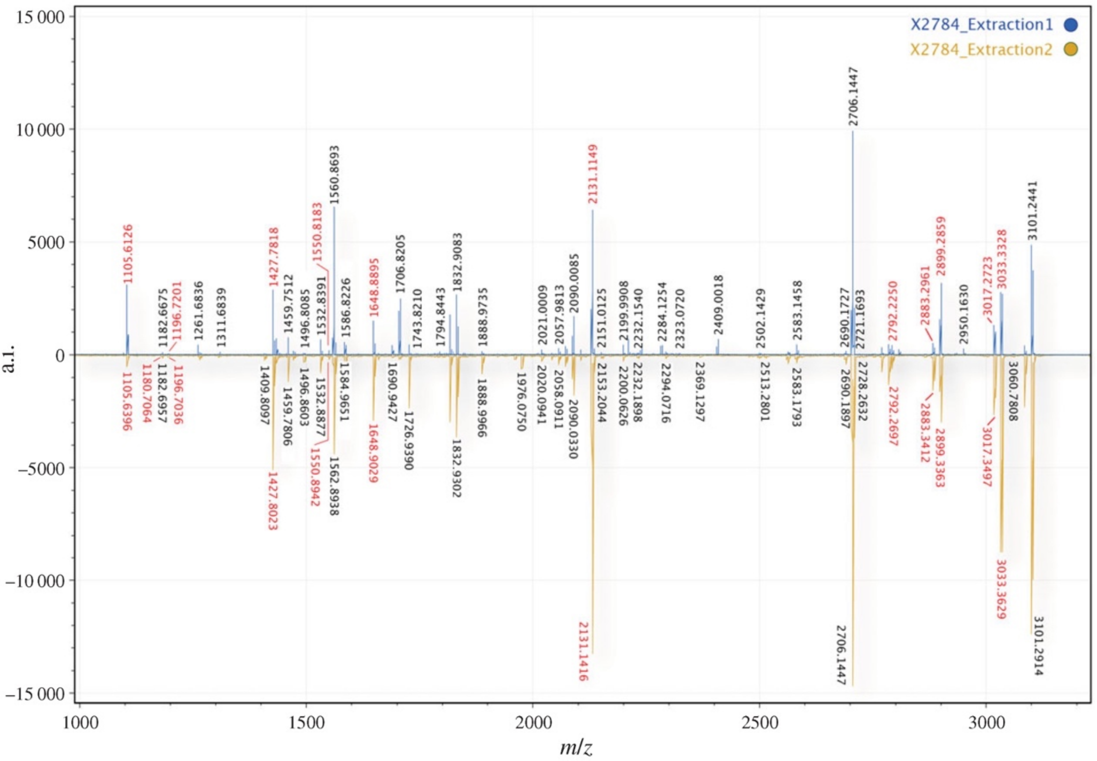


Figure 4. ZooMS results. MALDI-TOF-MS spectral output visualized in mMass (v.5.5.0) Extraction 1 and 2 flipped. Peptides unique to *Cervus* and *Alces* are highlighted in red.

3.4. Cervidae species identification by Liquid chromatography tandem mass spectrometry

In order to differentiate between *Cervus* and *Alces*, we performed LC-MS/MS analysis of the combined protein extracts. While the collagen type 1 (α1 and α2) chains in *Cervus* and *Alces* are highly conserved, three known SAPs (single amino acid polymorphisms) exist and can be used to separate these species. The sites are position 741 P (*Cervus elaphus*) or A (*Alces alces*) on the α1 chain, and 454 P (*Cervus elaphus*) or I/L (*Alces alces*), and 749 S (*Cervus elaphus*) or T (*Alces alces*) on the α2 chain. Using a MaxQuant search of our custom database, we identified peptides that map uniquely to the *Cervus* sequence at all three positions (two of which are shown in figure 5 as well as in table 2).

3.5. Additional proteins detected in the Syltholm ring

In addition to COL1, we identified 14 other endogenous proteins using LC-MS/MS excluding contaminants (electronic supplementary material, SI 3). These were additional collagens (collagen type 3 α1 (COL3A1), collagen type 11 (COL11A2) and collagen type 12 α1 (COL12A1)), blood proteins, such as immunoglobulin gamma-1 heavy chain (IGHG1), serum albumin (ALB), apolipoprotein A-I (APOA1), and nucleobindin 1 (NUCB1), and additional extracellular matrix proteins, such as osteocalcin (BGLAP), alpha 2-HS glycoprotein (AHSG), pigment epithelium-derived factor/serpin family F member 1 (SERPINF1), thrombospondin 1 (THBS1), biglycan (BGN), secreted phosphoprotein 2 (SPP2) and periostin (POSTN). In some cases, these proteins were identified specifically to *Cervus* (AHSG, IGHG1) or *Cervus*/*Odocoileus virginianus* (white-tailed deer) (SERPINF1, APOA1) when compared to all publicly available sequences for these proteins (which do not include sequences specific to *Alces*). Since white-tailed deer are native to the Americas, they can be excluded from species identification in this context. However, since the corresponding *Alces* sequences are not available, it cannot be ruled out that these may also match this species, and therefore the results reflect current data availability. For the other identified proteins, we recovered peptides that were less species-specific (electronic supplementary material, SI 3).

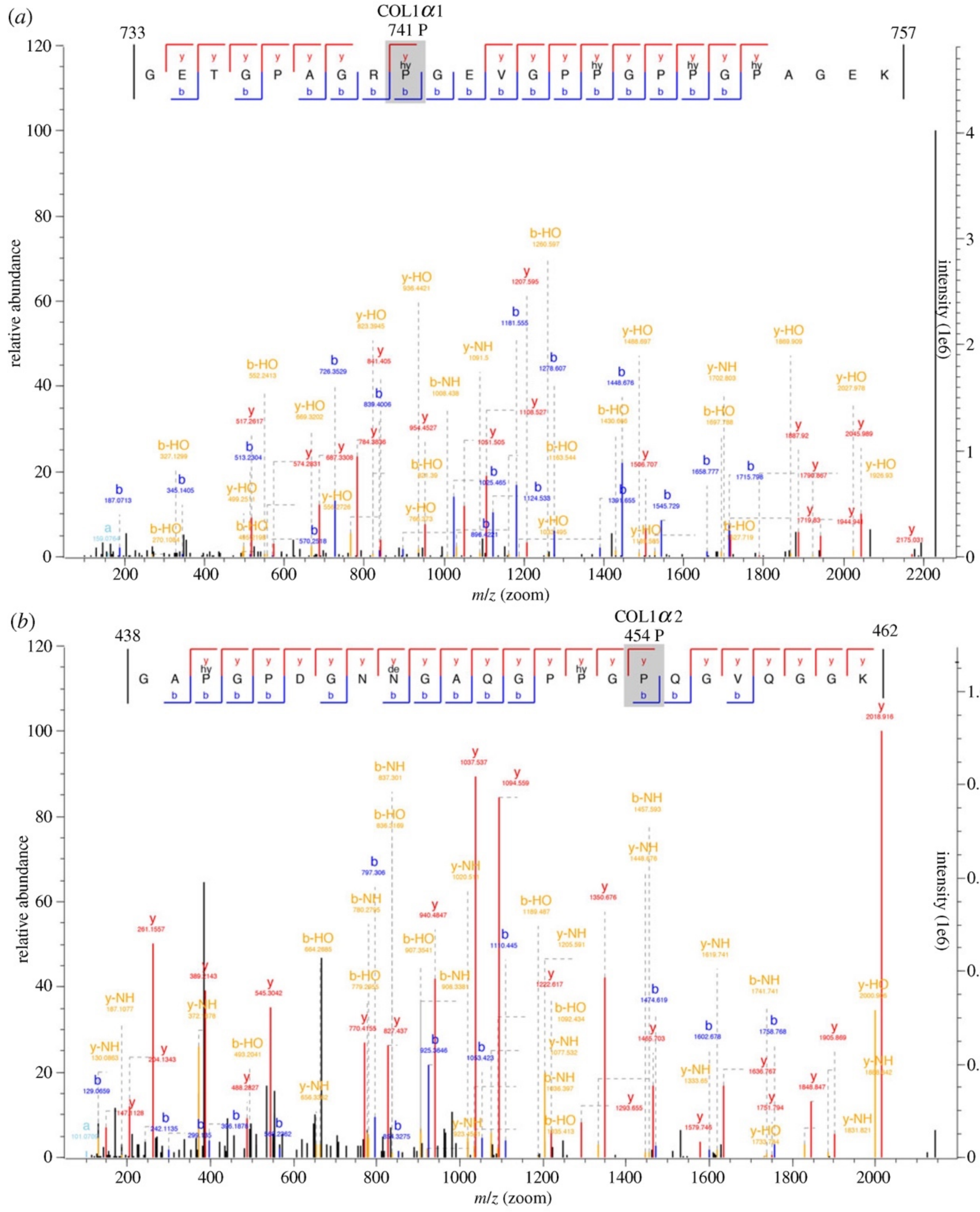
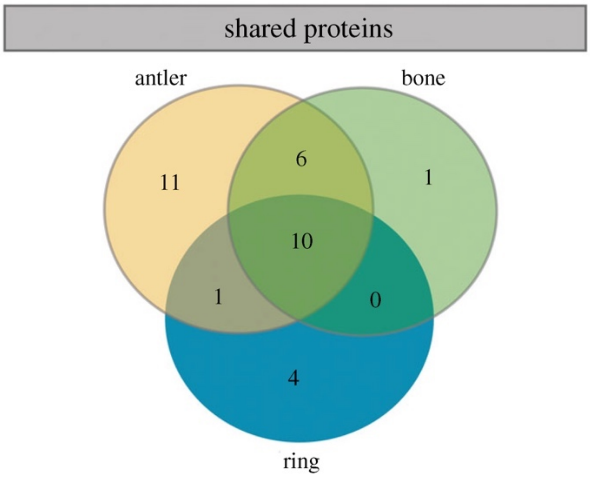


Figure 5. Example of two peptides (*a*,*b*) from the MS/MS output from MaxQuant, containing SAPs unique for *Cervus* (marked in grey). Panel (*a*) is located on the collagen 1 α-1 sequence, while (*b*) is located on the collagen 1 α-2 and both can confidently, based on the y and b ion series, be identified as *Cervus*.

3.6. Modern bone and antler proteomes

The experimentally heated extant bone and antler samples yielded 18 and 29 proteins, respectively (electronic supplementary material, SI 4). Even though they were modern samples, the same requirement of at least two razor+unique peptides for identification was followed. Both samples unsurprisingly contained collagens, namely COL1, COL3A1 and collagen type 5 α1 (COL5A1). Blood proteins were highly represented, especially in the antler. AHSG, SERPINF1, ALB, NUCB1 and tetronectin (CLEC3B) were found in both the antler and bone, whereas haemoglobin (HBB), antithrombin-III/serpin family C member 1 (SERPINC1), vitronectin (VTN), immunoglobulin lambda-1 light chain (IGL), serpin family D member 1 (SERPIND1) and APOA1 were found in the antler sample only. In addition, at least nine extracellular proteins were found in both the bone and antler samples: lumican (LUM), decorin (DCN), chondroadherin (CHAD), olfactomedin-like protein 1



samples	total	proteins
antler/bone/ring	10	BGN, ALB, BGLAP, COL3A1, COL1, NUCB1, THBS1, SPP2, SERPINF1, AHSG
antler/bone	6	ANXA2, DCN, CLEC3B, OLFML 1, LUM, CHAD
antler/ring	1	APOA1
antler	11	SPARC, COL5A1, HBB, SERPINC1, SERPIND1, IGL, VTN, S100A12, PCOLCE, S100A8, PGLYRP1
bone	1	COL5A2
ring	4	COL12A1, IGHG1, COL11A2, POSTN

Figure 6. Venn diagram demonstrating 1 protein shared between the ring and antler, and 0 proteins shared between the ring and bone.

(OLFML1), annexin A2 (ANXA2), BGN, BGLAP, SPP2 and THBS1. The antler sample also contained evidence of five other extracellular proteins: S100 calcium-binding proteins A8 and A12 (S100A8, S100A12), osteonectin (SPARC), procollagen C-endopeptidase enhancer (PCOLCE) and peptidoglycan-recognition protein (PGLYRP1). In general, there is significant overlap in the proteomes of all three samples, i.e. 10 proteins present in all samples. The sample with the most unique proteins is the antler (11 proteins), followed by the ring (four proteins) and the bone sample has only a single unique protein. The plasma protein APOA1 was uniquely recovered in our extractions of the ring and the heated modern *Cervus* antler. This is also visualized in a Venn diagram (figure 6). No unique proteins were recovered between only the ring and heated modern *Cervus* bone.

3.7. Liquid chromatography tandem mass spectrometry protein authentication

To assess the authenticity of the proteins recovered, we examined the deamidation patterns of asparagine (Asn/N) and glutamine (Gln/Q). Here, we observed a much higher deamidation rate in the ring sample. On average, the ring sample expressed 57.5% (SD 3.3%) Asn and 26.1% (SD 1.9%) Gln deamidation, while the contaminants showed 5.7% (SD 2.3%) and 0.6% (SD 0.6%), respectively (figure 7). This damage process occurs naturally over time, and while confounded by chemical and environmental factors (such as pH, temperature and humidity [37,38]), these results indicate that the proteins examined in our analysis are likely not preserved well enough to be modern contamination, due to the observed greater amount of damage detected when compared to known contaminant proteins. Additionally, we examined deamidation of the heated modern reference samples, which also have considerably less deamidation than the bone ring. The levels of deamidation present in the modern antler and bone are mostly of the faster reacting asparagine, and may have been caused by the heating step of the defleshing process they underwent before becoming a part of the museum's reference collection (figure 7).

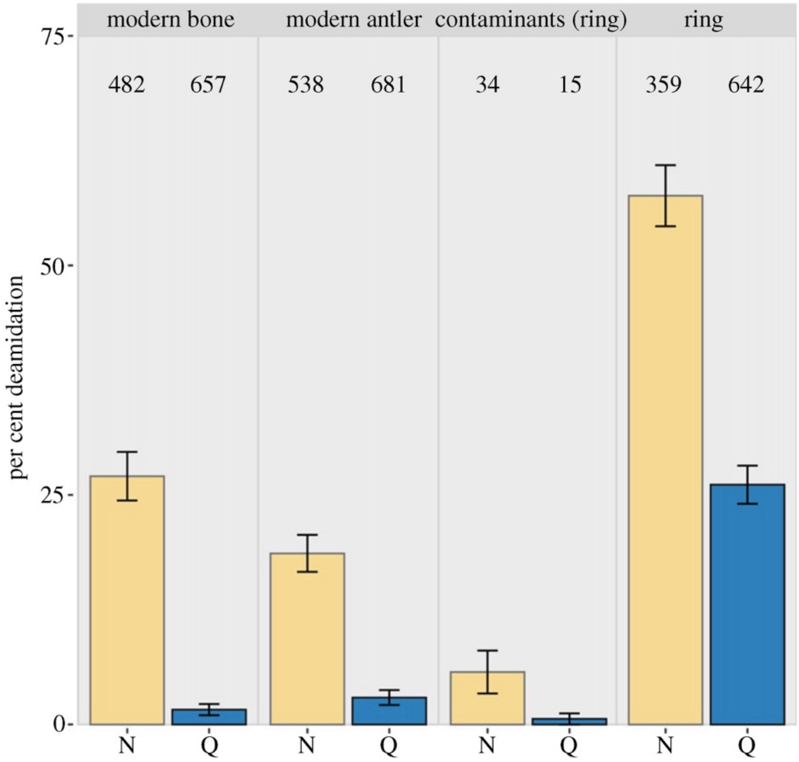


Figure 7. Deamidation comparison of the ring, heated modern reference samples as well as contaminant proteins. Numbers in each column denote number of peptides used for the calculation. Deamidated asparagine (N) and glutamine (Q) residues from the ring and known contaminant proteins, showing a considerably higher deamidation rate in the sample, which is evidence of authentically ancient proteins.

4. Discussion

Skeletal fragments and heavily worked artefacts often lack morphological osteological landmarks, and are nearly impossible to identify to the species level, let alone to skeletal element using osteological analysis. With the increased availability of advanced analytical techniques, molecular level resolution can be used to answer archaeological questions such as resource exploitation, manufacturing technology and trade.

4.1. The Syltholm finger ring is made from *Cervus* antler or bone

Bayesian modelling of 15 radiocarbon dates, obtained from the immediate proximity to the ring was used to indirectly date the ring to the Early Neolithic. Additionally, KDE modelling using a total of 70 dates confirmed that while the site was frequented in the Mesolithic the most intense activity was in the Early Neolithic. Micro-CT was employed to determine the skeletal element used to make the ring. Enamel and bone/antler are easily distinguished from one another by non-destructive micro-CT through visualization of Haversian canals, which are absent in enamel and dentine (including ivory) [35]. The scans (figure 3) clearly show the presence of osteons; therefore, we can exclude enamel/dentine as the raw material.

Having identified the material as deriving from bone or antler, ZooMS was performed to obtain species identification. The collagen I and II peptide mass fingerprint revealed that the ring was manufactured from either *Cervus* or *Alces*. At present, it is not possible to discriminate between some closely related species such as these, using ZooMS, due to the conserved nature of collagen I and II. A positive identification of *Alces* would imply that either an *Alces* bone/antler or the ring itself was imported. However, given the choice between the two species indicated by ZooMS, *Cervus* would be the most likely candidate. *Alces* disappeared at some point in time in this area due to rising sea-levels (Littorina transgressions (starting *ca* 8400 BP)) that effectively turned Denmark into an archipelago, whereas *Cervus* was still abundant in Denmark [39] at that time. *Alces* material could have been introduced to the site through trade; however, no evidence of this species has been recovered at

Sylthom from this period. LC-MS/MS protein sequencing was used to refine our ZooMS species identification. Three peptides with SAPs specific to *Cervus* were observed, confirming it as the source species.

4.2. Antler or bone?

Having identified the species from which the ring was crafted, we aimed to discern the skeletal element used, in an attempt to gather more information about the manufacturing process. As mentioned above, it is generally not possible to differentiate between archaeological antler and bone using micro-CT scanning due to bone diagenesis. Morphologically, it is more likely that antler was used, as the cross section of a mature antler tine is approximately the right size and shape of the Syltholm ring, aiding the manufacturing process. By contrast long bone is less circular, necessitating additional work to achieve a ring shape. Antler is also less energy consuming to attain, since it is shed yearly and does not require hunting an animal. Therefore, without biomolecular analysis, antler could be the most likely originating tissue for the ring.

At present, there has not been enough proteomic analysis of the differences in protein presence and expression levels between antler and bone. The two tissues are very similar and most variance could be down to quantitative differences between proteins instead of simply presence or absence. We attempted to investigate this further by generating 'reference' proteomes of *Cervus* antler and bone to which the ring could be compared (electronic supplementary material, SI 4). Due to the small proteome recovered from the ring, it would be inappropriate to compare this dataset against a modern proteome. To this end, we selected a *Cervus* specimen that had been experimentally heated [40] and performed the same extraction protocol that was used for the ring sample.

Unfortunately, comparison of the ring proteome versus the antler and bone did not enable us to confidently assign the ring to either. Due to the limitations of this study, we could not perform a quantitative analysis, partly due to methodology and partly due to the limited recovery of proteins of interest. To our knowledge, there has been no quantitative proteomic comparison between these two tissues, neither with modern nor archaeological samples [41]. Stéger *et al.* [41], however, did quantitatively examine modern *Cervus elaphus* antler and bone for differences in gene expression. They found that the expression of eight genes were 10–30-fold times more expressed in the ossified portion of antler than in skeletal bone from the same individual. Four of these proteins (COL1, COL3, BGLAP and SPARC) were also found in the ring sample. These are all proteins associated with skeletal development, and can be found in both antler and bone. However, it is unsurprising that bone development proteins are more abundant in antler as it is the fastest growing mammalian tissue [42] due to yearly regeneration. While our analyses were not quantitative, it is not out of the question that more abundant proteins would be more likely to be recovered, especially from a proteome depleted sample (due to taphonomic processes). Other proteins recovered from the Syltholm ring are associated with bone formation and mineralization, such as: AHSG [43], SPP2 [44] and POSTN [45]. Collagens type 3, 11 and 12 were recovered, all of which are associated with collagen formation in growing bone [46,47] and present in the growing antler [48,49]. Normally, COL11 and 12 are associated with cartilage, not mineralized bone [47,50,51], but are considered abundant in antler [48,49]. COL11 and COL12 have been recovered from archaeological bone (e.g. [52,53]), but Sawafuji *et al.* [53] have shown that the protein abundance score of COL12 decreases in older human individuals compared to those younger, correllating with the relative amount of bone growth.

We also discovered multiple blood proteins in the ring (IGHG1, ALB, APOA1 and NUCB1). Growing antlers are a highly vascular tissue and contain at least twice as much blood at their peak growth as ovine rib bones, which decreases as the antler ossifies [54]. APOA1, a major component of plasma high-density lipoprotein shown to be linked to osteoblastgenesis and bone synthesis [55], was the only protein uniquely recovered between the modern antler and the ring, although it has also been found in archaeological bone [52,53] and could represent missed recovery in the bone sample. It is of note that APOA1 was only detected by Sawafuji *et al.* [53] in the infant remains studied [53], indicating association with bone formation. It, along with SERPINF1, POSTN and THBS1, has been implicated in axon/nerve growth in growing antlers [56]. SERPINF1 and THBS1 have also been implicated in the stimulation and remodelling of vasculature in antler cartilage, respectively [56,57]. However, these proteins are also recovered from bone samples, albeit highly associated with bone growth and remodelling: SERPINF1 being expressed by osteoblasts during endochondrial bone formation [58], POSTIN is highly expressed in the periosteum and highly active during bone growth and contributes to changes in bone diameter and cortical thickness [45], and THBS1 is implicated in the remodelling of bone, maintenance of bone mass and fracture healing [59,60].

Therefore, we suggest that the proteins recovered, especially those related to increased bone growth and high vascularization, are consistent with a possible tissue identification of antler, suggested based on the ease of manufacture of this item from antler. However, there is not sufficient evidence to rule out bone either. We greatly encourage more research be undertaken to confirm the proteomic differences between antler and skeletal bone, as it would be valuable for future palaeoproteomic studies and the understanding of archaeological worked ossified objects.

4.3. Species identification sheds light on resource exploitation and manufacturing processes

The only coeval ring we know of from the Danish area is the ring from the Nederst shell-midden, on the Djursland peninsula of Jutland (approx. 200 km away). The Nederst ring is considerably smaller (Ø = 1.5 cm) and thus may have been made to fit an adult female or a child. As mentioned earlier, Kannegaard [12] suggests that the raw material used for manufacture was wild boar canine. From the same site, a small rectangular enamel/dentine disc was also found, with a semicircle removed by carving [12], thus showing that manufacturing took place at the midden. At another shell-midden, Stubbe Station, Jutland, two wild boar tusks with transverse saw marks were found. One shows a hole drilled into it, which seems to have cracked the tusk longitudinally [61]. A possible manufacturing process (or *chaîne opératoire*) of dentine/enamel rings can be suggested from these finds: (i) extracting the tusk of a wild boar, (ii) drilling a hole of desired size into the tusk, (iii) transverse sawing on either side to liberate a rectangular preform, (iv) removing the corners of the rectangle and making it circular, (v) grinding and polishing of the exterior. Since no waste material has so far been found from osseous finger ring production in Syltholm, its *chaîne opératoire* cannot be established at present. However, one can imagine that production of such a ring would require: (i) obtaining an antler or bone of a *Cervus*, (ii) transverse sawing to obtain a rough-out, (iii) scraping off the velvet bone for antler; more intensive circular shaping for bone, (iv) removal of the interior trabecular tissue using a flint borer and (v) polishing of the exterior.

Apart from being broken, the ring was well preserved and does not show any evidence of use-wear, which suggests that it was produced in the vicinity of the site. Based on the limited evidence presented here, it may be the case that there were geographically distinct *chaîne operatoires* for ring production during the transition period; the eastern part favouring rings made from antler tines or bone, whereas the western part favoured tusks. While it cannot be ruled out that the Syltholm ring was not manufactured at the site (trade item), there is evidence of regional differences in manufacturing techniques and resource exploitation in Denmark on both sides of the transition. During the Ertebølle period, bone combs, large circular bone rings cut from the scapulae of aurochs (*Bos primigenius*), extinct on Zealand during this period, are almost exclusively found in the western part of Denmark [62], whereas Limhamn and Oringe axes and adzes are mostly found in the eastern part and in Scania [63,64]. During the Early Neolithic, Volling-type ceramics are only found in the western part, whereas Svaleklint ceramics cluster in eastern areas [65].

5. Conclusion

This study presents the analysis of a unique artefact, an osseous finger ring, from the period of the Neolithization of northern Europe, contextually dated to the cusp of the Neolithic. We demonstrate the potential of combining several analytical methods on highly worked archaeological osseous artefacts to obtain a plethora of information even from small quantities of sample. The tomographic analysis revealed a matrix of osteons thus assigning the skeletal element to bone/antler, not enamel/dentine as used for the coveal and proximal Nederst ring. Peptide mass fingerprinting and protein sequencing revealed that the species used to manufacture the Syltholm ring was *Cervus elaphus*. Unfortunately, we were not able to determine if the ring was made of bone or antler. Nonetheless, this study demonstrates how ancient proteomics can still help identify and characterize the source of osseous material used in the manufacture of artefacts, which in turn can be used to infer regional differences in manufacturing processes and resource procurement.

Data accessibility. The mass spectrometry data for both ZooMS and LC-MS/MS have been deposited on the PRIDE Archive [66] with the dataset identifier PXD011811.

Authors' contributions. T.Z.T.J. and S.A.S. initiated the study. S.A.S. provided samples and contextual information. C.G. and T.Z.T.J. conducted the micro-CT scan and interpretations. Radiocarbon dates were produced and interpreted by J.O. and T.Z.T.J. T.Z.T.J. performed the ZooMS experiments. T.Z.T.J., M.M., A.J.T. and L.T.L. collected, prepared, and ran samples for MALDI-TOF-MS and LC-MS/MS. T.Z.T.J., M.M., A.J.T., L.T.L. and H.S. interpreted data and wrote the paper, with critical input from M.J.C. and M.S. All authors reviewed and approved the manuscript.

Competing interests. We declare we have no competing interests.

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Supplementary

SI 1: FASTA file of *Cervus elaphus* and *Alces alces* collagen.fasta
https://tinyurl.com/qnhmmkc

SI 2: Radiocarbon dates from Syltholm 906-II
https://tinyurl.com/tdpyxy9

SI 3: Protein recovery from the ring
https://tinyurl.com/vp7lmnd

SI 4: Protein Recovery of Modern Antler (P29) and Modern Bone (P55) Reference Material https://tinyurl.com/s6su9jm

SI Video: MicroCT imaging of the osseous ring from Syltholm
https://tinyurl.com/sdlkc5b

Chapter 5: A 5700 year-old human genome and oral microbiome from chewed birch pitch

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A 5700 year-old human genome and oral microbiome from chewed birch pitch

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The rise of ancient genomics has revolutionised our understanding of human prehistory but this work depends on the availability of suitable samples. Here we present a complete ancient human genome and oral microbiome sequenced from a 5700 year-old piece of chewed birch pitch from Denmark. We sequence the human genome to an average depth of 2.3× and find that the individual who chewed the pitch was female and that she was genetically more closely related to western hunter-gatherers from mainland Europe than hunter-gatherers from central Scandinavia. We also find that she likely had dark skin, dark brown hair and blue eyes. In addition, we identify DNA fragments from several bacterial and viral taxa, including Epstein-Barr virus, as well as animal and plant DNA, which may have derived from a recent meal. The results highlight the potential of chewed birch pitch as a source of ancient DNA.

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Birch pitch is a black-brown substance obtained by heating birch bark and has been used as an adhesive and hafting agent as far back as the Middle Pleistocene[1,2]. Small lumps of this organic material are commonly found on archaeological sites in Scandinavia and beyond, and while their use is still debated, they often show tooth imprints, indicating that they were chewed[3]. Freshly produced birch pitch hardens on cooling and it has been suggested that chewing was a means to make it pliable again before using it, e.g. for hafting composite stone tools. Medicinal uses have also been suggested, since one of the main constituents of birch pitch, betulin, has antiseptic properties[4]. This is supported by a large body of ethnographic evidence, which suggests that birch pitch was used as a natural antiseptic for preventing and treating dental ailments and other medical conditions[3]. The oldest examples of chewed pitch found in Europe date back to the Mesolithic period and chemical analysis by Gas Chromatography-Mass Spectrometry (GC-MS) has shown that many of them were made from birch (*Betula pendula*)[3].

Recent work by Kashuba et al[5]. has shown that pieces of chewed birch pitch contain ancient human DNA, which can be used to link the material culture and genetics of ancient populations. In the current study, we analyse a further piece of chewed birch pitch, which was discovered at a Late Mesolithic/Early Neolithic site in southern Denmark (Fig. 1a; Supplementary Note 1) and demonstrate that it does not only contain ancient human DNA, but also microbial DNA that reflects the oral microbiome of the person who chewed the pitch, as well as plant and animal DNA which may have derived from a recent meal. The DNA is so exceptionally well preserved that we were able to recover a complete ancient human genome from the sample (sequenced to an average depth of coverage of 2.3×), which is particularly significant since, so far, no human remains have been recovered from the site[6]. The results highlight the potential of chewed birch pitch as a source of ancient human and non-human DNA, which can be used to shed light on the population history, health status, and even subsistence strategies of ancient populations.

Results

Radiocarbon dating and chemical analysis. Radiocarbon dating of the specimen yielded a direct date of 5,858–5,661 cal. BP (GrM-13305; 5,007 ± 11) (Fig. 1b; Supplementary Note 2), which places it at the onset of the Neolithic period in Denmark. Chemical analysis by Fourier-Transform Infrared (FTIR) spectroscopy produced a spectrum very similar to modern birch pitch (Supplementary Fig. 4) and GC-MS revealed the presence of the triterpenes betulin and lupeol, which are characteristic of birch pitch (Fig. 1c; Supplementary Note 3)[3]. The GC-MS spectrum also shows a range of dicarboxylic acids and saturated fatty acids, which are all considered intrinsic to birch pitch and thus support its identification[7].

DNA sequencing. We generated approximately 390 million DNA reads for the sample, nearly a third of which could be uniquely mapped to the human reference genome (hg19) (Supplementary Table 2). The human reads displayed all the features characteristic of ancient DNA, including (i) short average fragment lengths, (ii) an increased occurrence of purines before strand breaks, and (iii) an increased frequency of apparent cytosine (C) to thymine (T) substitutions at 5′-ends of DNA fragments (Supplementary Fig. 6) and the amount of modern human contamination was estimated to be around 1–3% (Supplementary Table 3). In addition to the human reads, we generated around 7.3 Gb of

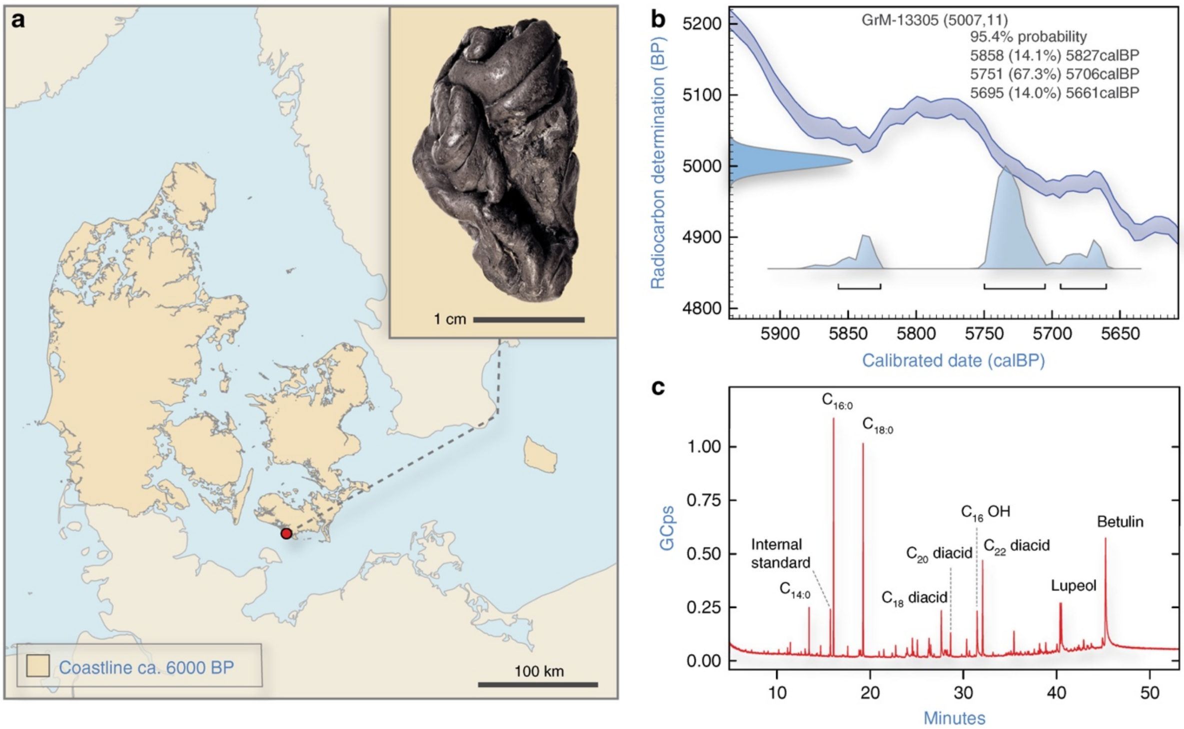

Fig. 1 A chewed piece of birch pitch from southern Denmark. (**a**) Photograph of the Syltholm birch pitch and its find location at the site of Syltholm on the island of Lolland, Denmark (map created using data from Astrup[78]). (**b**) Calibrated date for the Syltholm birch pitch (5,858–5,661 cal. BP; 5,007 ± 7). (**c**) GC-MS chromatogram of the Syltholm pitch showing the presence of a series of dicarboxylic acids (Cxx diacid) and saturated fatty acids (Cxx:0) and methyl 16-Hydroxyhexadecanoate ($C_{16}OH$) together with the triterpenes betulin and lupeol, which are characteristic of birch pitch[3].

sequence data (68.8%) from the ancient pitch that did not align to the human reference genome.

DNA preservation and genome reconstruction. With over 30%, the human endogenous DNA content in the sample was extremely high and comparable to that found in well-preserved teeth and petrous bones[8]. We used the human reads to reconstruct a complete ancient human genome, sequenced to an effective depth-of-coverage of 2.3×, as well as a high-coverage mitochondrial genome (91×), which was assigned to haplogroup K1e (see Methods). To further investigate the preservation of the human DNA in the sample we calculated a molecular decay rate (k, per site per year) and find that it is comparable to that of other ancient human genomes from temperate regions (Supplementary Table 3).

Sex determination and phenotypic traits. Based on the ratio between high-quality reads (MAPQ ≥ 30) mapping to the X and Y chromosomes, respectively[9], we determined the sex of the individual whose genome we recovered to be female. To predict her hair, eye and skin colour we imputed genotypes for 41 SNPs (Supplementary Data 1) included in the HIrisPlex-S system[10] and find that she likely had dark skin, dark brown hair, and blue eyes (Supplementary Data 2). We also examined the allelic state of two SNPs linked with the primary haplotype associated with lactase persistence in humans and found that she carried the ancestral allele for both (Supplementary Data 1), indicating that she was lactase non-persistent.

Genetic affinities. We called 593,102 single nucleotide polymorphisms (SNPs) in our ancient genome that had previously been genotyped in a dataset of >1000 present-day individuals from a diverse set of Eurasian populations[11], as well as >100 previously published ancient genomes (Supplementary Data 3). Figure 2a shows a principal component analysis (PCA) where she clusters with western hunter-gatherers (WHGs). Allele-sharing estimates based on f_4-statistics show the same overall affinity to WHGs (Fig. 2b). This is also reflected in the *qpAdm* analysis[12] (see Methods) which demonstrates that a simple one way model assuming 100% WHG ancestry cannot be rejected in favour of more complex models (Fig. 2c; Supplementary Table 6). To formally test this result we computed two sets of D-statistics of the form D(Yoruba, EHG/Barcın; test, WHG) and find no evidence for significant levels of EHG or Neolithic farmer gene flow (Supplementary Fig. 7; Supplementary Tables 7, 8).

Metataxonomic profiling of non-human reads. To broadly characterise the taxonomic composition of the non-human reads in the sample, we used MetaPhlan2[13], a tool specifically designed for the taxonomic profiling of short-read metagenomic shotgun data (see Methods; Supplementary Data 4). Figure 3a shows a principal coordinate analysis where we compare the microbial composition of our sample to that of 689 microbiome profiles from the Human Microbiome Project (HMP)[14]. We find that our sample clusters with modern oral microbiome samples in the HMP dataset. This is also reflected in Fig. 3b which shows the order-level microbial composition of our sample compared to two soil samples from the same site and metagenome profiles of healthy human subjects at five major body sites from the HMP[14], visualised using MEGAN6[15].

Oral microbiome characterisation. To further characterise the microbial taxa present in the ancient pitch and to obtain species-specific assignments we used MALT[16], a fast alignment and taxonomic binning tool for metagenomic data that aligns DNA sequencing reads to a user-specified database of reference sequences (see Methods; Supplementary Data 5). As expected, a large number of reads could be assigned to oral taxa, such as *Neisseria subflava* and *Rothia mucilaginosa*, as well as several bacteria included in the red complex (i.e. *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*) (see Table 1). In addition, we recovered 593 reads that were assigned to Epstein–Barr virus (Human gammaherpesvirus 4). We validated each taxon by examining the edit distances, coverage distributions, and post-mortem DNA damage patterns (see Supplementary Note 5).

Pneumococcal DNA. We also identified several species belonging to the Mitis group of streptococci (Table 1), including

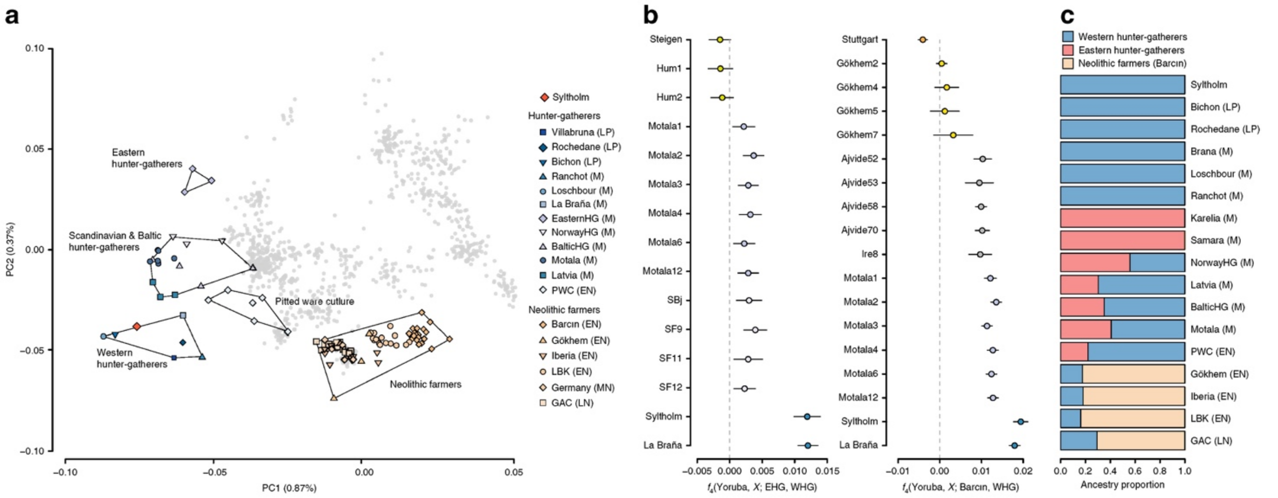

Fig. 2 Genetic affinities of the Syltholm individual. **a** Principal component analysis of modern Eurasian individuals (in grey) and a selection of over 100 previously published ancient genomes, including the Syltholm genome. The ancient individuals were projected on the modern variation (see Methods). **b** Allele-sharing estimates between the Syltholm individual, other Mesolithic and Neolithic individuals, and WHGs versus EHGs and Neolithic farmers, respectively, as measured by the statistic f_4(Yoruba, X; EHG/Barcın, WHG). **c** Ancestry proportions based on *qpAdm*[12], specifying WHG, EHG, and Neolithic farmers (Barcın) as potential ancestral source populations. *PWC* Pitted Ware Culture, *LBK* Linearbandkeramik, *GAC* Globular Amphora Culture, *LP* Late Paleolithic, *M* Mesolithic, *EN* Early Neolithic, *MN* Middle Neolithic, *LN* Late Neolithic. Data are shown in Supplementary Tables 4–6.

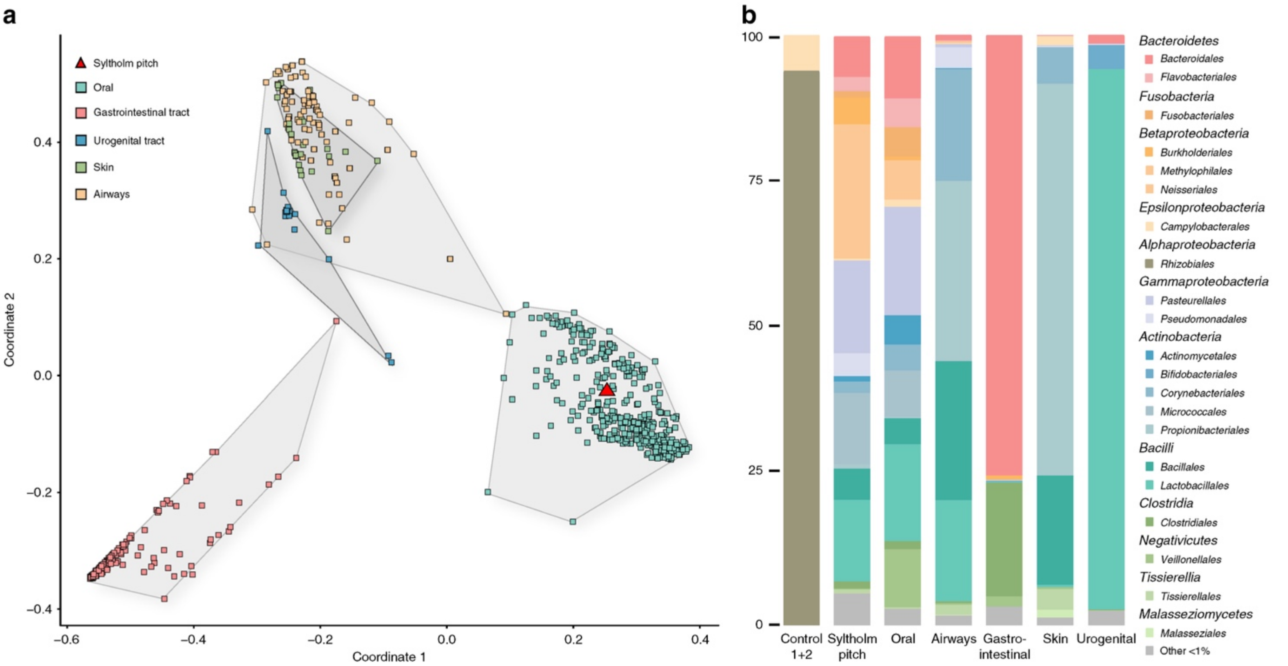

Fig. 3 Metagenomic profile of the Syltholm birch pitch. **a** PCoA with Bray-Curtis at genera level with 689 microbiomes from HMP[14] and the Syltholm sample (see Methods). **b** Order-level microbial composition of the Syltholm sample compared to a control sample (soil) and metagenome profiles of healthy human subjects at five major body sites from the HMP[14], visualised using MEGAN6[15].

Streptococcus viridans and *Streptococcus pneumoniae*. We reconstructed a consensus genome from the *S. pneumoniae* reads (Fig. 4) and estimated the number of heterozygous sites (2,597) (see Methods) which indicates the presence of multiple strains. To assess the virulence of the *S. pneumoniae* strains recovered from the ancient pitch, we aligned the contigs against the full Virulence Factor Database[17] in order to identify known *S. pneumoniae* virulence genes (see Methods). We identified 26 *S. pneumoniae* virulence factors within the ancient sample, including capsular polysaccharides (CPS), streptococcal enolase (Eno), and pneumococcal surface antigen A (PsaA) (see Supplementary Data 6).

Plant and animal DNA. Lastly, we used a taxonomic binning pipeline specifically designed for ancient environmental DNA[18] to taxonomically classify the non-human reads in the sample that mapped to other Metazoa (animals) and Viridiplantae (plants). We only parsed taxa with classified reads accounting for >1% of all reads in each of the two kingdoms and a declining edit distance distribution after edit distance 0 (Supplementary Data 7). We then validated each identified taxon as described above (see Supplementary Note 5). Using these criteria, we identified DNA from two plant species in the ancient sample, including birch (*Betula pendula*) and hazelnut (*Corylus avellana*). In addition, we detected over 50,000 reads that were assigned to mallard (*Anas platyrhynchos*).

Discussion

We successfully extracted and sequenced ancient DNA from a 5700-year-old piece of chewed birch pitch from southern Denmark. In addition to a complete ancient human genome (2.3×) and mitogenome (91×), we recovered plant and animal DNA, as well as microbial DNA from several oral taxa. Analysis of the human reads revealed that the individual whose genome we recovered was female and that she likely had dark skin, dark brown hair and blue eyes. This combination of physical traits has been previously noted in other European hunter-gatherers[19–22], suggesting that this phenotype was widespread in Mesolithic Europe and that the adaptive spread of light skin pigmentation in European populations only occurred later in prehistory[23]. We also find that she had the alleles associated with lactase non-persistence, which fits with the notion that lactase persistence in adults only evolved fairly recently in Europe, after the introduction of dairy farming with the Neolithic revolution[24,25].

From a population genetics point of view, the human genome also offers fresh insights into the early peopling of southern Scandinavia. Recent studies of ancient hunter-gatherer genomes from Sweden and Norway[23] have shown that, following the retreat of the ice sheets around 12–11 ka years ago, Scandinavia was colonised by two separate routes, one from the south (presumably via Denmark) and one from the northeast, along the coast of present-day Norway. This is supported by the fact that hunter-gatherers from central Scandinavia carry different levels of WHG and EHG ancestry, which reached central Scandinavia from the south and northeast, respectively[23]. Although we only analysed a single genome, the fact that the Syltholm individual does not carry any EHG ancestry confirms this scenario and suggests that EHGs did not reach southern Denmark at this point in prehistory.

The Syltholm genome (5700 years cal. BP) dates to the period immediately following the Mesolithic-Neolithic transition in Denmark. Culturally, this period is marked by the transition from the Late Mesolithic Ertebølle culture (c. 7300–5900 cal. BP) with its flaked stone artefacts and typical T-shaped antler axes, to the early Neolithic Funnel Beaker culture (c. 5900–5300 cal. BP) with its characteristic pottery, polished flint artefacts, and domesticated plants and animals[26]. In Denmark, the transition from hunting and gathering to farming has often been described as a relatively rapid process, with dramatic shifts in settlement patterns and subsistence strategies[27]. However, it is still unclear to what extent this transition was driven by the arrival of farming communities as opposed to the local adaptation of farming practices by resident hunter-gatherer populations.

Table 1 List of non-human taxa identified in the Syltholm pitch, including the 40 most abundant oral bacterial taxa, viruses, and eukaryotes. Bacteria in the red complex are denoted with an asterisk. Depth (DoC) and breadth of coverage (>1x) were calculated using BEDTools[72]. Deamination rates at the 5′ ends of DNA fragments were estimated using mapDamage 2.0.9[59]. -Δ% refers to the negative difference proportion introduced by Hübler *et al*[79]. (see Supplementary Note 5).

Species	Reads	Fragment length (bp)	DoC	SD DoC	>1x (%)	C-T 5′(%)	−Δ%
Bacteria							
Neisseria subflava	308,732	56	7.5	6.2	83.7	14.5	0.9
Rothia mucilaginosa	296,610	52	6.9	5.6	82.3	14.0	0.9
Streptococcus pneumoniae	176,782	57	4.7	6.3	65.7	13.8	0.9
Neisseria cinerea	153,683	58	4.9	5.1	71.7	15.1	1.0
Lautropia mirabilis	117,040	53	2.0	1.9	71.9	13.0	1.0
Neisseria meningitidis	100,540	51	2.3	4.3	42.4	14.9	0.9
Aggregatibacter segnis	95,670	58	2.8	2.8	73.3	14.5	0.9
Neisseria elongata	68,407	54	1.6	1.9	67.6	15.1	0.9
Prevotella intermedia	65,324	56	1.2	1.4	55.0	16.2	0.9
Streptococcus sp. ChDC B345	52,614	61	1.6	2.7	50.3	13.8	0.9
Streptococcus sp. 431	43,787	59	1.2	1.9	47.5	13.6	0.8
Aggregatibacter aphrophilus	43,231	56	1.1	1.6	50.4	15.0	0.8
Streptococcus pseudopneumoniae	38,832	61	1.1	2.4	34.9	14.4	0.9
Capnocytophaga leadbetteri	36,461	59	0.9	1.1	49.8	14.0	0.8
Corynebacterium matruchotii	36,070	52	0.7	0.9	44.0	13.0	1.0
Gemella morbillorum	32,284	63	1.2	1.5	56.4	16.3	1.0
Streptococcus viridans	27,840	60	0.8	1.5	36.5	14.5	1.0
Neisseria gonorrhoeae	27,704	53	0.7	2.0	21.3	15.0	1.0
Neisseria sicca	27,290	57	0.6	1.4	22.5	13.7	0.9
Fusobacterium nucleatum	26,783	64	0.8	1.1	47.8	14.1	0.9
Prevotella fusca	26,295	57	0.5	0.7	34.6	15.7	1.0
Kingella kingae	25,811	55	0.7	1.0	44.2	14.4	1.0
Ottowia sp. 894	25,425	52	0.5	0.7	34.6	14.4	1.0
Streptococcus sp. NPS 308	24,937	59	0.8	1.4	37.5	14.3	0.8
Actinomyces oris	24,029	52	0.4	0.7	29.8	12.7	1.0
Streptococcus australis	23,777	60	0.7	1.3	31.5	13.8	1.0
P. propionicum	22,864	50	0.3	0.6	26.8	13.2	0.9
Haemophilus sp. 036	19,707	62	0.7	1.5	28.4	14.5	1.0
*Porphyromonas gingivalis**	17,651	55	0.4	0.7	32.2	17.2	1.0
Capnocytophaga gingivalis	16,734	58	0.3	0.6	27.1	15.0	1.0
Neisseria polysaccharea	14,442	57	0.4	1.4	15.0	15.8	1.0
*Tannerella forsythia**	14,187	55	0.2	0.5	19.8	15.3	1.0
Streptococcus sp. A12	13,232	59	0.4	0.9	24.9	14.6	0.9
Capnocytophaga sputigena	12,587	58	0.2	0.5	19.9	14.7	0.9
Neisseria lactamica	11,971	56	0.3	1.0	14.2	14.2	0.8
*Treponema denticola**	11,379	59	0.2	0.5	19.5	14.0	0.8
Rothia dentocariosa	10,944	54	0.2	0.5	20.0	13.6	1.0
Tannerella sp. HOT-286	10,397	53	0.2	0.5	15.7	14.0	1.0
Actinomyces meyeri	10,105	51	0.3	0.5	21.3	14.0	1.0
Filifactor alocis	9,948	61	0.3	0.6	25.6	15.0	1.0
Viruses							
Epstein-Barr virus	593	51	0.2	0.4	13.3	17.8	1.0
Eukaryotes							
Anas platyrhynchos	55,986	51	<0.1	0.05	0.2	15.6	1.0
Corylus avellana	8,615	55	<0.1	0.04	0.1	19.7	1.0
Betula pendula	3,291	54	<0.1	0.02	<0.1	16.1	1.0

Our analyses have shown that the Syltholm individual does not carry any Neolithic farmer ancestry, suggesting that the genetic impact of Neolithic farming communities in southern Scandinavia might not have been as instant or pervasive as once thought[28]. While the mtDNA we recovered belongs to haplogroup K1e, which is more commonly associated with early farming communities[29–31], there is mounting evidence to suggest that this lineage was already present in Mesolithic Europe[32–34]. Overall, the lack of Neolithic farmer ancestry is consistent with evidence from elsewhere in Europe, which suggests that genetically distinct hunter-gatherer groups survived for much longer than previously assumed[35–37]. These WHG "survivors" might have triggered the resurgence of hunter-gatherer ancestry that is proposed to have occurred in central Europe between 7000 and 5000 BP[12].

In addition to the human data, we recovered ancient microbial DNA from the pitch which could be shown to have a human oral microbiome signature. Previous studies[38–40] have demonstrated that calcified dental plaque (dental calculus) provides a robust biomolecular reservoir that allows direct and detailed investigations of ancient oral microbiomes. However, unlike dental calculus, which represents a long-term reservoir of the oral microbiome built up over many years, the microbiota found in ancient mastics are more likely to give a snapshot of the species active at the time. As such, they provide a useful source of information regarding the evolution of the human oral microbiome that can complement studies of ancient dental calculus.

The majority of the bacterial taxa we identified (Table 1) are classified as non-pathogenic, commensal species that are considered to be part of the normal microflora of the human mouth

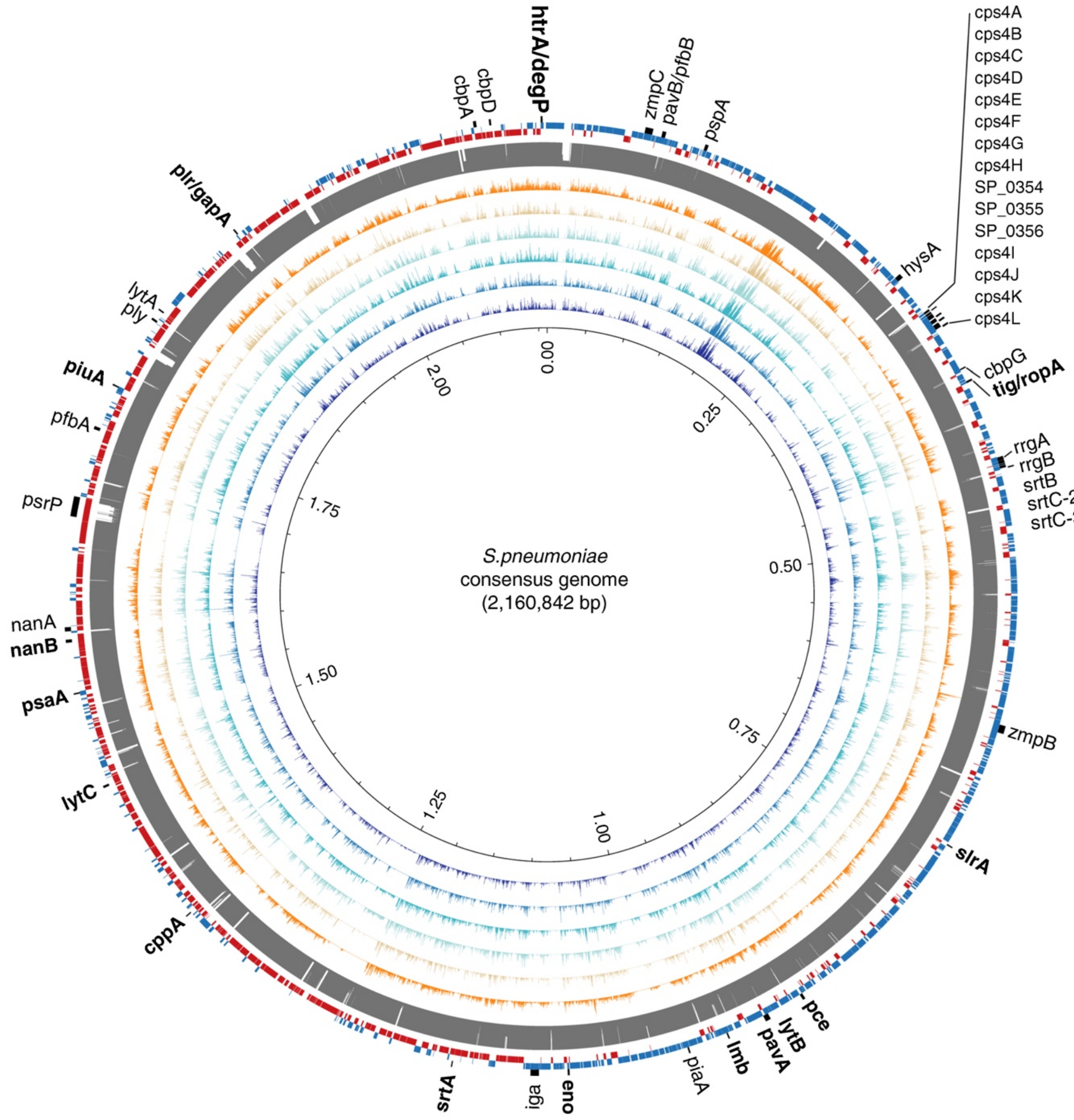

Fig. 4 *Streptococcus pneumoniae* consensus genome reconstructed from metagenomic sequences recovered from the ancient pitch. From outer to inner ring: *S. pneumoniae* virulence genes (black, shared genes are shown in bold); *S. pneumoniae* coding regions on the positive (blue) and negative (red) strand; mappability (grey); sequence depth for the Syltholm pitch (orange), HOMP sample SRS014468 (light brown), SRS019120 (light blue), SRS013942 (turquoise), SRS015055 (blue), and SRS014692 (dark blue). Sequence depths were calculated by aligning to the *S. pneumoniae* TIGR4 reference genome and visualised in 100 bp windows using Circos[73].

and the upper respiratory tract, but may become pathogenic under certain conditions. In addition, we identified three species (*Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*) included in the so-called red complex, a group of bacteria that are categorised together based on their association with severe forms of periodontal disease[41]. Furthermore, we identified several thousand reads that could be assigned to different bacterial species in the Mitis group of streptococci, including *Streptococcus pneumoniae*, a major human pathogen that is responsible for the majority of community-acquired pneumonia which still causes around 1–2 million infant deaths worldwide, every year[42].

S. pneumoniae has a remarkable capacity to remodel its genome through the uptake of exogenous DNA from other pneumococci and closely related oral streptococci[42]. Understanding this process and the distribution of pneumococcal virulence factors between different strains can help our understanding of *S. pneumoniae* pathogenesis. We identified 26 *S. pneumoniae* virulence factors within our ancient sample, including several that are involved in host colonisation (e.g. adherence to host cells and tissues, endocytosis) and the evasion and subversion of the host's immune response (Supplementary Data 6). While more research is needed to fully understand the evolution of this important human pathogen and its ability to cause disease, our capacity to recover virulence factors from ancient samples opens up promising avenues for future research.

In addition to the bacterial taxa, we identified 593 reads that could be assigned to the Epstein–Barr virus (EBV). Previous studies[43,44] have demonstrated the great potential of ancient DNA for studying the long-term evolution of blood borne viruses. Formally known as Human gammaherpesvirus 4, EBV is one of the most common human viruses infecting over 90% of the world's adult population[45]. Most EBV infections occur during childhood and in the vast majority of cases they are asymptomatic or they carry symptoms that are indistinguishable from other mild, childhood diseases. However, in some cases EBV can cause infectious mononucleosis (glandular fever)[46] and it has also been associated with various lymphoproliferative diseases, such as Hodgkin's lymphoma and hemophagocytic lymphohistiocytosis, as well as higher risks of developing certain autoimmune diseases, such as dermatomyositis and multiple sclerosis[47,48].

Lastly, we identified several thousand reads that could be confidently assigned to different plant and animal species, including birch (*B. pendula*), hazelnut (*C. avellana*), and mallard (*A. platyrhynchos*). While the presence of birch DNA is easily explained as it is the source of the pitch, we propose that the hazelnut and mallard DNA may derive from a recent meal. This is supported by the faunal evidence from the site, which is dominated by wild taxa, including *Anas* sp. and hazelnuts[6,49]. In addition, there is evidence from many other Mesolithic and Early Neolithic sites in Scandinavia for hazelnuts being gathered in large quantities for consumption[50]. Together with the faunal evidence, the ancient DNA results support the notion that the people at Syltholm continued to exploit wild resources well into the Neolithic and highlight the potential of ancient DNA analyses of chewed pieces of birch pitch for palaeodietary studies.

In summary, we have shown that pieces of chewed birch pitch are an excellent source of ancient human and non-human DNA. In the process of chewing, the DNA becomes trapped in the pitch where it is preserved due to the aseptic and hydrophobic properties of the pitch which both inhibits microbial and chemical decay. The genomic information preserved in chewed pieces of birch pitch offers a snapshot of people's lives, providing information on genetic ancestry, phenotype, health status, and even subsistence. In addition, the microbial DNA provides information on the composition of our ancestral oral microbiome and the evolution of specific oral microbes and important human pathogens.

Methods

Sample preparation and DNA extraction. We sampled c. 250 mg from the specimen for DNA analysis. Briefly, the sample was washed in 5% bleach solution to remove any surface contamination, rinsed in molecular biology grade water and left to dry. We tested three different extraction methods using between 20–50 mg of starting material: For method (1), 1 ml of lysis buffer containing 0.45 M EDTA (pH 8.0) and 0.25 mg/ml Proteinase K was added to the sample and left to incubate on a rotor at 56 °C. After 12 h the supernatant was removed and concentrated down to ~150 µl using Amicon Ultra centrifugal filters (MWCO 30 kDa), mixed 1:10 with a PB-based binding buffer[51], and purified using MinElute columns, eluting in 30 µl EB. For method (2) the sample was digested and purified as above, but with the addition of a phenol-chloroform clean-up step. Briefly, 1 ml phenol (pH 8.0) was added to the lysis mix, followed by 1 ml chloroform:isoamyl alcohol. The supernatant was concentrated and purified, as described above. For method (3) the sample was dissolved in 1 ml chloroform:isoamylalcohol. The dissolved sample was then resuspended in 1 ml molecular grade water and purified as described above. DNA extracts prepared using a Proteinase K-based lysis buffer followed by a phenol-chloroform based purification step produced the best results in terms of the endogenous human DNA content (see Supplementary Table 1); however, following metagenomic profiling the extracts were found to be contaminated with *Delftia* spp., a known laboratory contaminant[52]. The contaminated libraries were excluded from metagenomic profiling.

Negative controls. We included no template controls (NTC) during the DNA extraction and library preparation steps. The NTCs prepared with the additional phenol-chloroform step were also found to be contaminated with *Delftia* spp., suggesting that the contaminants were introduced during this step. In addition, we included two soil samples from the site, weighing c. 2 g each, as negative controls. DNA was extracted as described above using 3 ml EDTA-based lysis buffer followed by 9 ml 25:24:1 phenol:chloroform:isoamyl alcohol mixture to account for the larger amount of starting material. The sequencing results are reported in Supplementary Table 1.

Library preparation and sequencing. 16 µl of each DNA extract were built into double-stranded libraries using a recently published protocol that was specifically designed for ancient DNA[53]. One extraction NTC was included, as well as a single library NTC. 10 µl of each library were amplified in 50 µl reactions for between 15 and 28 cycles, using a dual indexing approach[54]. The optimal number of PCR cycles was determined by qPCR (MxPro 3000, Agilent Technologies). The amplified libraries were purified using SPRI-beads and quantified on a 2200 TapeStation (Agilent Technologies) using High Sensitivity tapes. The amplified and indexed libraries were then pooled in equimolar amounts and sequenced on 1/8 of a lane of an Illumina HiSeq 2500 run in SR mode. Following initial screening, additional reads were obtained by pooling libraries #2, #3, and #4 in molar fractions of 0.2, 0.4, and 0.4, respectively and sequencing them on one full lane of an Illumina HiSeq 2500 run in SR mode.

Data processing. Base calling was performed using Illumina's bcl2fastq2 conversion software v2.20.0. Only sequences with correct indexes were retained. FastQ files were processed using PALEOMIX v1.2.12[55]. Adapters and low quality reads ($Q < 20$) were removed using AdapterRemoval v2.2.0[56], only retaining reads >25 bp. Trimmed and filtered reads were then mapped to hg19 (build 37.1) using BWA[57] with seed disabled to allow for better sensitivity[58], as well as filtering out unmapped reads. Only reads with a mapping quality ≥30 were kept and PCR duplicates were removed. MapDamage 2.0.9[59] was used to evaluate the authenticity of the retained reads as part of the PALEOMIX pipeline[55], using a subsample of 100k reads per sample (Supplementary Fig. 6). For the population genomic analyses, we merged the ancient sample with individuals from the Human Origin dataset[11] and >100 previously published ancient genomes (Supplementary Data 1). At each SNP in the Human Origin dataset, we sampled the allele with more reads in the ancient sample, resolving ties randomly, resulting in a pseudohaploid ancient sample.

MtDNA analysis and contamination estimates. We used Schmutzi[60] to determine the endogenous consensus mtDNA sequence and to estimate present-day human contamination. Reads were mapped to the Cambridge reference sequence (rCRS) and filtered for MAPQ ≥ 30. Haploid variants were called using the *endoCaller* program implemented in Schmutzi[60] and only variants with a posterior probability exceeding 50 on the PHRED scale (probability of error: 1/100,000) were retained. We then used Haplogrep v2.2[61] to determine the mtDNA haplogroup, specifying PhyloTree (build 17) as the reference phylogeny[62]. Contamination estimates were obtained using Schmutzi's *mtCont* program and a database of putative modern contaminant mitochondrial DNA sequences.

Genotype imputation. We used ANGSD[63] to compute genotype likelihoods in 5 Mb windows around 43 SNPs associated with skin, eye, and hair colour[10] and lactase persistence into adulthood (Supplementary Data 2). Missing genotypes were imputed using impute2[64] and the pre-phased 1000 Genome reference panel[65], provided as part of the impute2 reference datasets. We used multiple posterior probability thresholds, ranging from 0.95 to 0.50, to filter the imputed genotypes. The imputed genotypes were uploaded to the HIrisPlex-S website[10] to obtain the predicted outcomes for the pigmentation phenotypes (Supplementary Data 3).

Principal component analysis. Principal component analysis was performed using smartPCA[66] by projecting the ancient individuals onto a reference panel including >1000 present-day Eurasian individuals from the HO dataset[11] using the option lsq project. Prior to performing the PCA the data set was filtered for a minimum allele frequency of at least 5% and a missingness per marker of at most 50%. To mitigate the effect of linkage disequilibrium, the data were pruned in a 50-SNP sliding window, advanced by 10 SNPs, and removing sites with an R^2 larger than 0, resulting in a final data set of 593,102 SNPs.

***D*- and *f*-statistics**. *D*- and *f*-statistics were computed using *AdmixTools*[67]. To estimate the amount of shared drift between the Syltholm genome and WHG versus EHG and Neolithic farmers, respectively, we computed two sets of f_4-statistics of the form f_4(Yoruba, *X*; EHG/Barcın, WHG) where "*X*" stands for the test sample. Standard errors were calculated using a weighted block jackknife. To confirm the absence of EHG and Neolithic farmer gene flow in the Syltholm genome and to contrast this result with those obtained for other Mesolithic and Neolithic individuals from Scandinavia, we computed two sets of *D*-statistics of the form *D*(Yoruba, EHG/Barcın; *X*, WHG) testing whether "*X*" forms a clade to the exclusion of EHG and Neolithic farmers (represented by Barcın), respectively.

qpAdm. Admixture proportions were modeled using *qpAdm*[12], specifying Mesolithic Western European hunter-gatherers (WHG), Eastern hunter-gatherers (EHG) and early Neolithic Anatolian farmers (Barcın), as possible ancestral source populations. We present the model with the lowest number of source populations that fits the data, as well as the model with all three admixture components (see Supplementary Table 6). When estimating the admixture proportions for WHGs and EHGs, the test sample was excluded from their respective reference populations.

MetaPhlan. We used MetaPhlan2[13] to create a metagenomic profile based on the non-human reads (Supplementary Data 4). The reads were first aligned to the MetaPhlan2 database[13] using Bowtie2 v2.2.9 aligner[68]. PCR duplicates were removed using PALEOMIX filteruniquebam[58]. For cross-tissue comparisons 689 human microbiome profiles published in the Human Microbiome Project Consortium[14] were initially used, comprising samples from the mouth ($N=382$), skin ($N=26$), gastrointestinal tract ($N=138$), urogenital tract ($N=56$), airways and nose ($N=87$). The oral HMP samples consist of attached/keratinised gingiva ($N=6$), buccal mucosa ($N=107$), palatine tonsils ($N=6$), tongue dorsum ($N=128$), throat ($N=7$), supragingival plaque ($N=118$), and subgingival plaque ($N=7$). Pairwise ecological distances among the profiles were computed at genus and species level using taxon relative abundances and the vegdist function from the vegan package in R[69]. These were used for principal coordinate analysis (PCoA) of Bray–Curtis distances in R using the pcoa function included in the APE package[70]. Subsequently, we calculated the average relative abundance of each genus for each of the body sites present in the Human Microbiome Project and visualised the abundance of microbial orders of our sample and the HMP body sites with MEGAN6[15].

MALT. To further characterise the metagenomic reads we performed microbial species identification using MALT v. 0.4.1 (Megan ALignment Tool)[16], a rapid sequence-alignment tool specifically designed for the analysis of metagenomic data. All complete bacterial ($n=12{,}426$) and viral ($n=8094$) genomes were downloaded from NCBI RefSeq on 13 November 2018, and all complete archaeal ($n=280$) genomes were downloaded from NCBI RefSeq on 17 November 2018 to create a custom database. In an effort to exclude genomes that may consist of composite sequences from multiple organisms, the following entries were excluded:

GCF_000922395.1 uncultured crAssphage
GCF_000954235.1 uncultured phage WW-nAnB
GCF_000146025.2 uncultured Termite group 1 bacterium phylotype Rs-D17

The final MALT reference database contained 33,223 genomes and was created using default parameters in *malt-build* (v. 0.4.1). The sequencing data for the ancient pitch sample, two soil control samples and associated extraction and library blanks were de-enriched for human reads by mapping to the human genome (hg19) using BWA aln and excluding all mapping reads. Duplicates were removed with seqkit v.0.7.1[71] using the 'rmdup' function with the '–by-seq' flag. The remaining reads were processed with *malt-run* (v. 0.4.1) where BlastN mode and SemiGlobal alignment were used. The minimum percent identity (–minPercentIdentity) was set to 95, the minimum support (–minSupport) parameter was set to 10 and the top percent value (–topPercent) was set as 1. Remaining parameters were set to default. MEGAN6[15] was used to visualise the output '.rma6' files and to extract the reads assigned to taxonomic nodes of interest for our sample. A taxon table of the raw MALT output for all samples and blanks, as well as species level read assignments to bacteria, archaea and DNA viruses for the ancient pitch sample are shown in Supplementary Data 5, where reads listed are the sum of all reads assigned to the species node, including reads assigned to specific strains within the species. Reads assigned to RNA viruses were not considered for further analyses, since our dataset consisted of DNA sequences only. Due to the limited number of reads assigned to archaeal species (Supplementary Data 5), we did not consider Archaea in downstream analyses of species identification. To validate the microbial taxa, we aligned the assigned reads to their respective reference genomes and examined the edit distances, coverage distributions, and post-mortem DNA damage patterns (see Supplementary Note 5).

Pneumococcus analysis. We reconstructed a *S. pneumoniae* consensus genome (Fig. 4) by mapping all reads assigned to *S. pneumoniae* by MALT[16] to the *S. pneumoniae* TIGR4 reference genome (NC_003028.3). To investigate the presence of multiple strains we estimated the number of heterozygous sites using samtools[57] mpileup function, filtering out transitions, indels, and sites with a depth of coverage below 10. Coverage statistics of the individual alignments ($\mathrm{MQ} \geq 30$) were obtained using Bedtools[72] and plotted using Circos[73] in 100 bp windows. Mappability was estimated using GEM2[74] using a k-mer size of 50 and a read length of 42, which is comparable to the average length of the trimmed and mapped reads in the ancient pitch. Virulence genes were identified by assembling the ancient *S. pneumoniae* MALT extracts into contigs using megahit[75]. The contigs were aligned against known *S. pneumoniae* TIGR4 virulence genes in the Virulence Factor Database[17] (downloaded 22/11–2018) using BLASTn[76]. Only unique hits with a bitscore >200, >20% coverage, and an identity >80% were considered as shared genes (Supplementary Data 6).

To identify all streptococcus virulence factors in the ancient pitch, we aligned the contigs against the full Virulence Factor Database[17] (downloaded 22/11–2018) using BLASTn[76] and the same filtering criteria as described above (Supplementary Data 6). To validate the approach we repeated the analysis with five modern oral microbiome samples (SRS014468; SRS019120; SRS013942; SRS015055; SRS014692) from the Human Microbiome Project (HMP)[14] using only the forward read (R1) (Supplementary Data 6). We find that the number of virulence genes we recovered directly correlates with sequencing depth (Supplementary Fig. 16).

Holi. For a robust taxonomic assignment of reads aligning to Metazoa (animals) and Viridiplantae (plants), all non-human reads were parsed through the 'Holi' pipeline[18], which was specifically developed for the taxonomic profiling of ancient metagenomic shotgun reads. Each read was aligned against the NCBI's full Nucleotide and Refseq databases (downloaded November 25th 2018), including a newly sequenced full genome of European hazelnut (*Corylus avellana*, downloaded April 10th 2019)[77]. The alignments were then parsed through a naive lowest common ancestor algorithm (ngsLCA) based on the NCBI taxonomic tree. Only taxonomically classified reads for taxa comprising ≥1% of all the reads within the two kingdoms and a declining edit distance distribution after edit distance 0 were parsed for taxonomic profiling and further validation. To validate the assignments, we aligned the assigned reads to their respective reference genomes and examined the edit distances, coverage distributions, and post-mortem DNA damage patterns (see Supplementary Note 5; Supplementary Data 7).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The sequencing reads are available for download from the European Nucleotide Archive under accession number PRJEB30280. All other data are included in the paper or available upon request.

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Author contributions

T.Z.T.J. and H.S. designed and led the study. S.A.S. provided the sample for analysis. M.C.C. and M.N.M. performed the FTIR and GC-MS analyses. M.W.D. performed the radiocarbon dating. T.Z.T.J., M.H.S.S. and M.R.E. generated the genetic data. T.Z.T.J., J.N., K.H.I., A.K.F., S.G., Å.J.V., M.W.P., S.H.N., M.E.A. and H.S. analyzed the genetic data. T.Z.T.J., J.N., K.H.I., A.K.F., S.G., Å.J.V., M.W.P., M.E.A., L.T.L., A.J.T., M.J.C., M.T.P.G., M.S., S.R., and H.S. interpreted the results. T.Z.T.J. and H.S. wrote the manuscript with input from J.N., K.H.I., and the remaining authors.

Competing interests

The authors declare no competing interests.

Additional information

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Supplementary Information for

A 5,700 year-old human genome and oral microbiome from chewed birch pitch

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Supplementary Note 1. Site description

Theis Z. T. Jensen and Søren A. Sørensen

Syltholm is located in the southern part of Lolland near Rødbyhavn in Denmark (Supplementary Fig. 1). The site covers ca. 187 hectares of land, which up until 1872 was open water. After a series of floods, a reclamation project was undertaken to dam up the area, thus preserving the inundated landscape below[1]. In 2012, Museum Lolland-Falster initiated large scale geological surveys and subsequent archaeological excavations in the area due to the upcoming establishment of the Fehmarn Belt fixed-link tunnel connecting Denmark to Germany. Several sites were selected for full-scale excavations, based on coring, landscape topography as well as investigative excavations. Excavation of the former fjord was significantly constrained by high groundwater levels, which preclude the initial use of mechanical excavators. This was solved by localised drainage for several months[2,3]. To date a total of 21 excavations have been completed. They vary in terms of age, finds intensity as well as preservation of organic material. The vast majority of the site spans from the Late Mesolithic Ertebølle to the Middle Neolithic Funnel Beaker periods. During the time of occupation the area would have been a shallow brackish lagoon protected from the open sea to the south by shifting sandy barrier islands. Human activity in this coastal environment in prehistory is reflected primarily by the finding of large numbers of organic and inorganic artefacts and thousands of faunal remains, many of which include cut-marks[3,4].

In the area of the site where the chewed birch pitch (Supplementary Fig. 2) was found (MLF906-I-II), the stratigraphy consists of 1) a top layer is a heterogeneous matrix of sand separated by thin sections of gyttja, ca. 1-1,5 m in thickness, which indicates several storm events, 2) a thin (5-10 cm) transgression horizon of coarse drift gyttja containing large amounts of molluscs as well as *ex situ* water rolled artefacts of flint and organic material, indicating an erosive milieu, 3) a layer (10-80 cm) of coarse brown gyttja where large amounts of *in situ* organic and inorganic archaeological artefacts and other material was uncovered, indicating a sheltered environment, and 4) a bottom layer of glacial till, which consists of blue clay. The glacial topography consists of several small depressions, which in the Ertebølle and Neolithic periods would, at certain places, gradually have been filled with organic matter forming gyttja.

Several hundred unpublished AMS dates from MLF906-II, including the ones presented in this manuscript (Supplementary Fig. 3) indicate that the area was frequented by people more or less continuously from the Late Mesolithic until Middle Neolithic. Continued artefact depositions seem to have been carried out at the site, as dates obtained from organic material, such as bone, antler or wood, found in small confined areas span nearly 1,000 years. During the earlier (Mesolithic) phase, the deposits are dominated by wild taxa, including red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), and pig (*Sus sp.*), as well as ox (*Vulpes vulpes*), otter (*Lutra lutra*), and wildcat (*Felis silvestris*), although large numbers of domestic dog (*Canis familiaris*) remains have also been found[5]. From around 5,800 BP other domesticated species (e.g. *Bos taurus*) also start to appear, but keep being dominated by wild taxa (see Supplementary Fig. 3).

Supplementary Note 2. Radiocarbon dating

Michael W. Dee

Radiocarbon dating was performed on ca. 10 mg of the birch pitch, following an acid-base-acid pretreatment[6]. First, the sample was treated with 4% HCl (80°C) and then rinsed to neutrality with ultra-pure water. Second, a basic solution 1% NaOH (RT) was applied, and the reaction vessel rinsed again to neutrality. Finally, a further acid step was applied using 4% HCl (80°C) to ensure no atmospheric CO_2 absorbed during the alkaline phase remained in the reaction vessel. After a last rinse to neutrality, the product was thoroughly air dried. An aliquot of ca. 4 mg was then weighed into a tin capsule for combustion in an Elementar IsotopeCube NCS Elemental Analyser (EA). The EA was coupled to an Isoprime 100 Isotope Ratio Mass Spectrometer, which allowed the $\delta^{13}C$ value of the sample to be measured, as well as a fully automated cryogenic system that trapped the liberated CO_2 into an airtight vessel. The vessel was manually transferred to a vacuum manifold, where a stoichiometric excess of $H_2(g)$ (1: 2.5) was added, and the sample $CO_2(g)$ reduced to graphite over an Fe(s) catalyst. The graphite was pressed into a cathode for radioisotope analysis in an MICADAS IonPlus accelerator mass spectrometer. The MICADAS generated an estimate of the ^{14}C:^{12}C ratio that was close to ±1‰, and from this data, and in accordance with all standard operations and conventions, the ^{14}C date (in yrs BP) was calculated. Thc calendar date range (years cal BP) was computed using the calibration program OxCal (v 4.3)[7].

Inaccuracy in ^{14}C dating largely arises from processes that occur before the sample reaches the laboratory. Misassociation of sample and context, or factors which can make substances ‘appear older’ such as marine/dietary reservoir effects or material reuse, are the most common. Enriching effects, which would cause the age to be too young, are negligible in the natural world. However, human error in the ^{14}C laboratory can introduce both younger or older contamination. To guard against such sources of inaccuracy, the radiocarbon laboratory in Groningen regularly analyzes reference samples, including full pretreatments and measurements on materials of independently known age.

Supplementary Note 3. FTIR and GC-MS analysis

Martin N. Mortensen and Mads C. Christensen

For the FTIR analysis ca. 1 mg of sample was ground with KBr (Fischer Scientific, IR Grade), pressed into a pellet and measured on a Perkin Elmer Spectrum 1000 FT-IR spectrometer. The FTIR spectra for the Syltholm pitch and a modern birch sample are shown in Supplementary Fig. 4. For the GC-MS analysis, ca. 0.5 mg sample was hydrolysed in methanolic KOH (Merck) and extracted with GC-grade tert-Butyl methyl ether (MTBE) after acidification. The extract was methylated using diazomethane (Sigma-Aldrich)[8]. 1 µl of this solution was injected on a Bruker SCION 456 GC-TQMS system equipped with a Programmable Temperature Vaporizer that was held at 64°C for 0.5 min, raised to 315°C at 200°C min^{-1} and held at that temperature for 40 min. The split ratio was high during the first 0.5 min and then switched to 5. The GC column was a Restek Rtx-5 capillary column (30 m, 0.25 mm ID, 0.25 µm) and the He flow rate was 1 cm^{3} min^{-1}. The GC oven temperature was held at 64°C for 0.5 min, then raised to 190°C at 10°C min^{-1} and then onto 315°C at 4°C min^{-1} and held at that temperature for 15 min. The EI (electron ionisation) ion source was held at 250°C and the ionisation potential was -70 eV. The mass spectrometer was operated in the full scan mode from *m*/*z* 45 to *m*/*z* 800. The GC-MS chromatograms for the Syltholm pitch and the betulin and lupeol references are shown in Supplementary Fig. 5.

Supplementary Note 4. Decay rate estimate

Morten E. Allentoft and Hannes Schroeder

To investigate the rate of human DNA degradation in the ancient pitch sample we examined the DNA read length distributions of the mapped reads, using a previously published method[9]. The distribution follows a typical pattern of degraded DNA with an initial increase in the number of reads towards longer DNA fragments, followed by a decline. We observe that the declining part of the distribution follows an exponential decay curve (R^2=0.99), as expected if the DNA had been randomly fragmented over time. Deagle et al.[10] showed that the decay constant (λ) in the exponential equation represents the fraction of broken bonds in the DNA strand (the damage fraction) and that 1/λ is the average theoretical fragment length in the DNA library. By solving the equation, we obtain a DNA damage fraction (λ) of 3.4%, which corresponds to a theoretical average fragment length (1/λ) of 29 bp (Table S2). We note that this is not directly comparable to the observed average length, which is affected by lab methods and sequencing technology. If the DNA is found in a stable matrix long term DNA fragmentation can be expressed as a rate and the damage fraction (λ, per site) can be converted to a decay rate (*k*, per site per year), when the age of the sample is known. By applying an estimated age of 5,700 years for the Syltholm pitch, the corresponding DNA decay rate (*k*) is 5,96^{-06} breaks per bond per year, which corresponds to a molecular half-life of 1,162 years for a 100 bp DNA fragment. This means that after 1,162 years (post cell death), each 100 bp DNA stretch will have experienced one break on average. This estimated rate of DNA decay for the pitch sample seems within the expected age for DNA preserved in a stable matrix in a temperate climate zone. For example, the rate is close to that observed in the La Braña sample[11], preserved at similar temperatures as the pitch sample (Supplementary Table 2). By contrast, the DNA decay in human remains from warmer climates is much faster[12]. Although these calculations are only based on a single sample, the results suggest that ancient mastics provide remarkable conditions for molecular preservation.

Supplementary Note 5. Analysis of metagenomic reads

Jonas Niemann and Hannes Schroeder

Accurate taxonomic classification of complex metagenomic datasets can be challenging, especially if closely related species are present in the sample or as environmental contaminants[13]. Additionally, robust classification can be complicated if reference databases are incomplete or sequencing effort is insufficient. A further complication is that, in some cases, reference databases contain poor quality genomes with contaminant sequences, which can lead to incorrect assignments[14,15]. While specific pipelines for the taxonomic classification of ancient metagenomic datasets have been developed[16,17], further validation is often necessary to exclude the possibility of false positive (misidentified) assignments. Methods used for validation include confirming the presence of ancient DNA damage patterns, evaluating edit distances, and assessing coverage distributions[18,19].

To test the robusticity of our pipelines[16,17], we performed two in silico experiments using archaeological and environmental samples as controls. First, we ran MetaPhlan2[20] and MALT[17] on two soil samples from the site and show that they have a completely different microbial composition from the ancient pitch (Fig. 4; Supplementary Data 4; Supplementary Data 5). We then ran Holi[16] on the same controls and, using the same criteria as for the ancient sample, did not retrieve any reads that could be assigned to the eukaryotic taxa we identified in the ancient pitch (Supplementary Data 7). Second, we ran the Holi pipeline[16] on a previously published dataset[12] generated from an ancient tooth (~33 million reads with an average length of 69 bp) to test whether some of our results might be false positives resulting from reference genomes being contaminated with DNA from other species, especially human DNA. Using the same criteria as the ones we applied in the present study, we did not identify any of the taxa we identified in the ancient pitch.

Independent validation of taxonomic assignments

To validate the taxonomic assignments of the metagenomic reads recovered from the ancient pitch, we aligned the assigned reads to their respective reference genomes and examined the edit distances, coverage distributions, and post-mortem DNA damage patterns[18,19]. For the bacterial taxa identified by MALT, we chose to further investigate bacterial species with ≥10,000 assigned reads (including strain specific reads). We then aligned the taxon-specific MALT extracts to their respective reference genomes that we obtained from the NCBI assembly database (Supplementary Data 5). The sequences were aligned using *bwa aln*[21] and PCR duplicates were removed using Picard Tools v.2.13.2[22]. MapDamage v.2.0.9[23] was used to estimate deamination rates (Supplementary Fig. 8). The breadth and depth of coverage were calculated with *bedtools* v.2.27.1[24] and visualised with *Circos* v.0.69-6[25] using a window size of 100 bp (Supplementary Fig. 9). Edit distances for all reads and filtered for PMD score ≥1 were extracted from the bam files with samtools view[21] and PMDtools[26] and plotted in R v.3.4.1[27] (Supplementary Fig. 10). The negative difference proportion (-Δ%) was calculated using only reads with PMD score ≥1. This metric was first introduced by Hübler et al.[19] and is a measure of the decline in the edit distance distribution, with a -Δ% value of 1 indicating a strictly declining distribution. Correct taxonomic assignments generally result in a continuously declining edit distance distribution, which reflects the fact that most of the aligned reads show no or only few mismatches, mostly resulting from aDNA damage or divergence of the ancient genome from the modern reference. By contrast, mapping to an incorrect reference tends to result in an increased number of mismatches, which is reflected in the edit distance distribution[19]. For the microbial taxa, we report species-specific assignments with a -Δ% value >0.8 to account for the possibility of cross-alignments due to horizontal gene transfer and the presence of closely related microbial species in the sample.

The Human Oral Microbiome Database (HOMD) was referred to in order to classify bacterial species as belonging to the human oral/respiratory microbiome or as environmental. Of the 64 most abundant bacterial species identified in the ancient pitch (Supplementary Data 5), four are known contaminants originating from lab reagents (*Delftia* spp.), which are also evident in the extraction blanks (Supplementary Data 5), while seven (*Pseudomonas stutzeri*, *Hydrogenophaga sp.* RAC07, *Leptospira alstonii*, *Ramlibacter tataouinensis*, *Thalassolituus oleivorans*, *Achromobacter spanius*, *Pseudomonas aeruginosa*) are likely derived from the environment. None of these 11 species showed the characteristic damage patterns of ancient DNA and were, therefore, not included in further analyses. The remaining 53 bacterial species are predominantly found in the oral cavity and the upper respiratory tract (see Table 1; Supplementary Data 5).

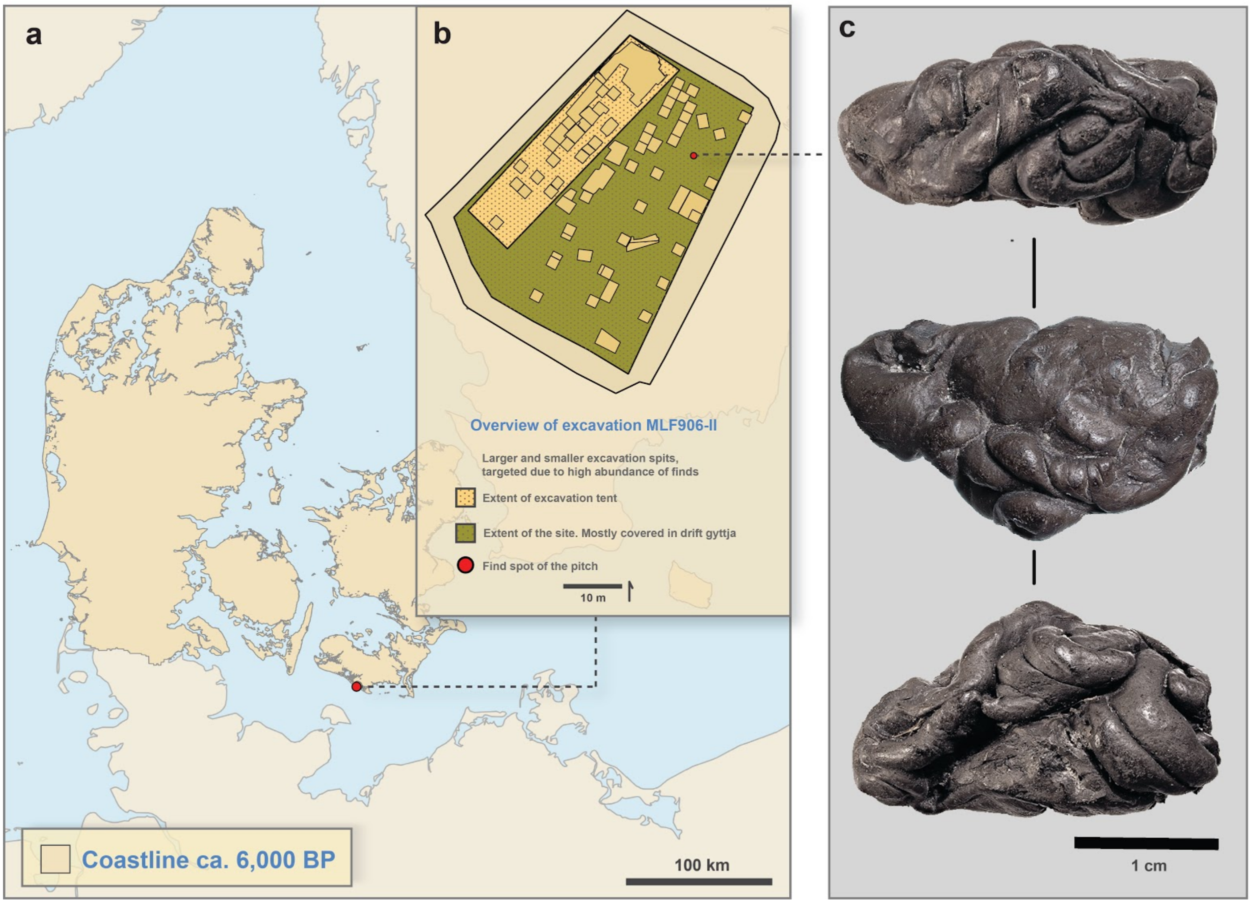
Among the viral species identified we chose to further authenticate reads assigned to the Epstein-Barr virus (*Human gammaherpesvirus 4*) (Supplementary Fig. 11), since it is the only non-bacteriophage viral taxon to which ≥200 reads were assigned. Viruses have considerably smaller genomes than bacteria and were therefore subject to a lower threshold of assigned reads.

The plant and animal taxa identified by Holi[16] were validated by evaluating sequence identity through edit distance distributions, evenness of coverage, and the presence of post-mortem DNA damage patterns as described above after extracting family level reads for each taxon and aligning them to their respective reference genome. For taxa with low coverage, we used bedtools[24] to calculate the proportion of mapped bases with a coverage >1× as an alternative way of assessing evenness of coverage (Supplementary Data 7). Using these criteria, we identified four taxa (*Anas platyrhynchos, Anser cygnoides, Betula pendula, Corylus avellana*) which showed characteristic ancient DNA damage patterns and a strictly declining edit distance distribution (Supplementary Fig. 12-14 and Supplementary Data 6). However, further analysis using mitochondrial (mtDNA) genomes as reference yielded only 291 reads aligning to the *A. cygnoides* mtDNA in contrast to 2,541 for the *A. platyrhynchos* (mallard) mtDNA, with >99% of bases covered and nearly 10× average depth of coverage (Supplementary Data 6). Furthermore, the edit distance distribution for the *A. cygnoides* mtDNA (Supplementary Fig. 13) is not declining, suggesting a poor match. We therefore excluded *A. cygnoides* as a likely false positive assignment.

As a further validation step and to assess whether reads from multiple taxa might have been misassigned to a single species, we examined the number of multiallelic sites in the 10× haploid mallard mtDNA. In haploid genomes (i.e. bacterial genomes or mtDNA), the vast majority of variable sites should be monoallelic, so that a large number of multiallelic sites might be indicative of multiple species or strains being present[13]. To assess the allele frequency distribution for the 10× mallard mtDNA, we rescaled the base qualities of the mallard mtDNA reads according to their likelihood of being damaged using mapDamage v.2.0.9[23] and called variants using samtools[21] mpileup function using a minimum depth of 10. The allele frequency distribution follows a normal distribution with a mean of ~0.5 indicating the presence of two haplotypes (Supplementary Fig. 15). This was confirmed by visual inspection of the alignment in IGV v.2.3.9[28]. However, rather than indicating the presence of two different taxa, we believe that this might indicate the presence of two individuals and it is not inconceivable that two or more individuals were consumed. This is supported by the fact that the only other Anatidae species with a significant number of reads identified by Holi[16] was the swan goose (*A. cygnoides*). However, as discussed above, we excluded this taxon based on the poor level of sequence identity with the *A. cygnoides* mtDNA as evident in the edit distances (Supplementary Fig. 13). We were unable to evaluate haploidy for the two plant taxa (*Betula pendula* and *Corylus avellana*) since the depth of coverage of the chloroplast DNA was too low.

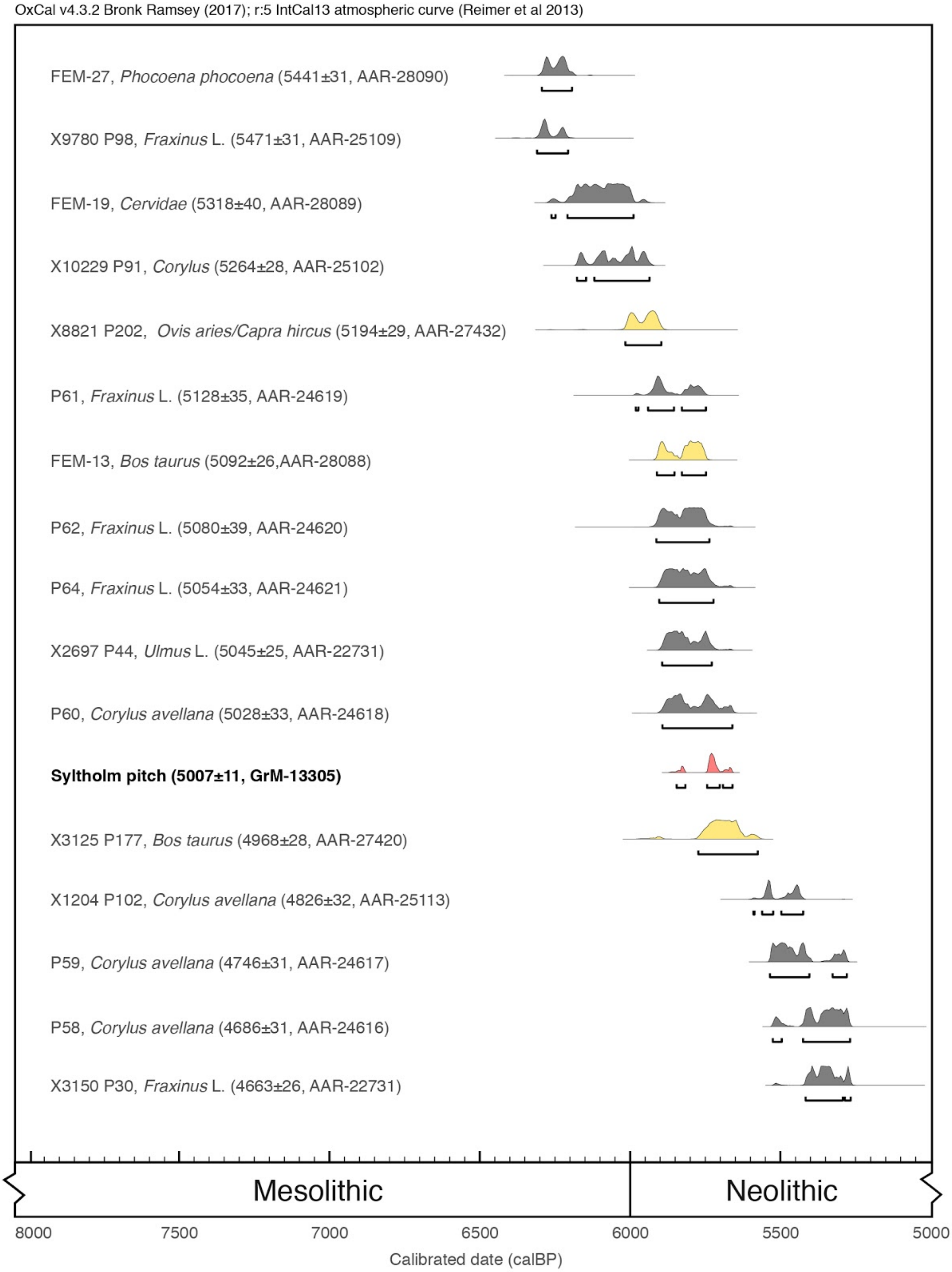
We also identified 3,213 reads that could be assigned to the human endoparasite *Spirometra erinaceieuropaei* (tapeworm). However, although the reads appear to be ancient, coverage was not even (>60% of mapped bases >1× despite an average depth of coverage of only 0.000025×) suggesting that they are likely false positive alignments perhaps due to the presence of contaminant (human) sequences in the reference (Supplementary Data 7). Recent studies[15] have shown that public genome assemblies of parasitic worms can be contaminated with DNA from the host species, other species that are commensal in the host, or laboratory contaminants, highlighting the need for curating public reference genome databases[29].

Lastly, we acknowledge that it is possible that some of the eukaryotic taxa we report (e.g. mallard) may have come from the environment as opposed to the diet. However, since the vast majority of the DNA we retrieved from the ancient pitch appears to be endogenous (i.e. either human or from the oral cavity), we find this to be unlikely and we believe that it is more likely that the taxa we report derived from the diet.

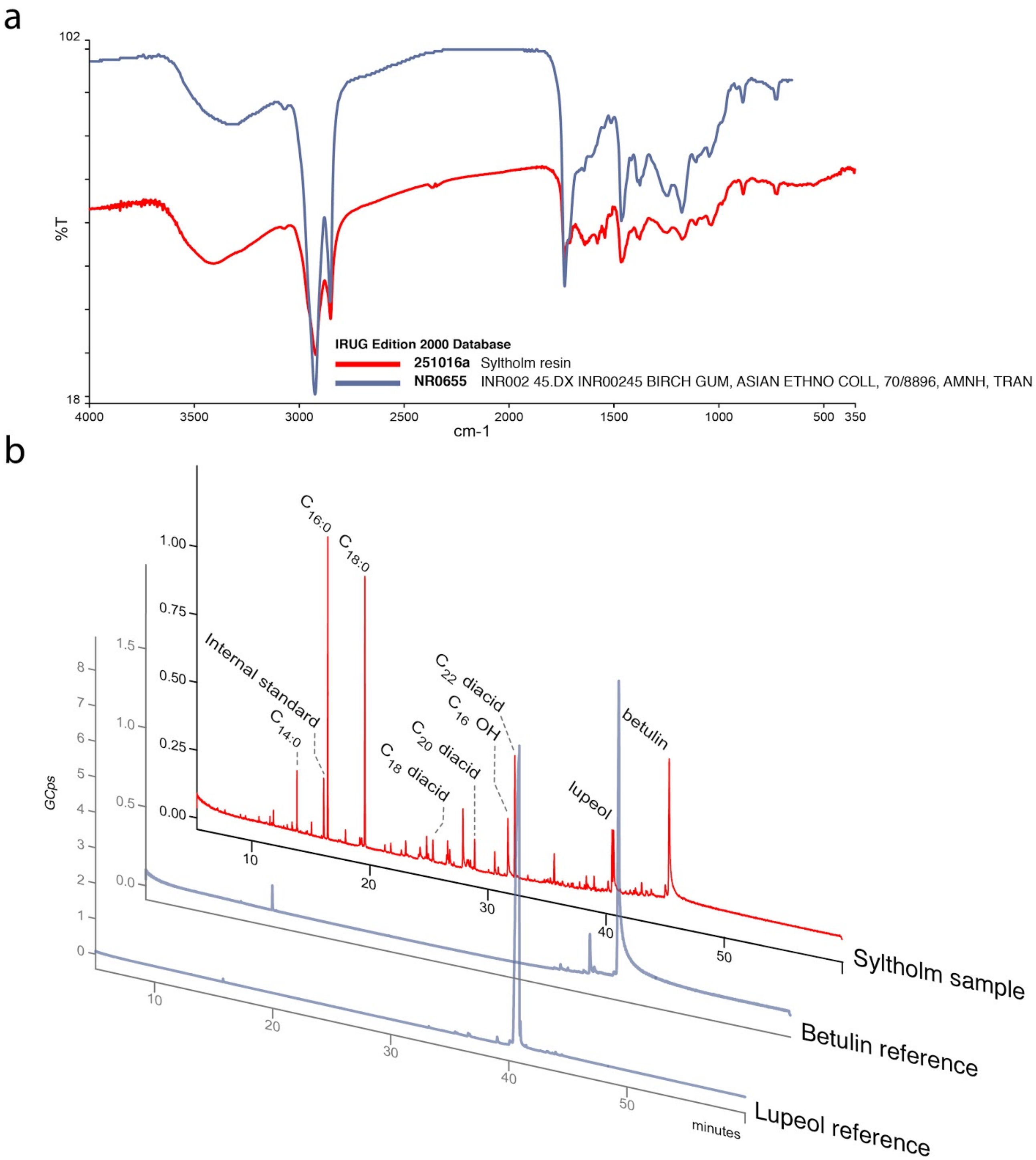


Supplementary Figure 1. a, Map of Denmark showing the location of Syltholm on the island of Lolland (map created using data from Astrup[30]). **b,** GIS site plan of the excavation and findspot of the birch pitch. **c,** photograph of the birch pitch.

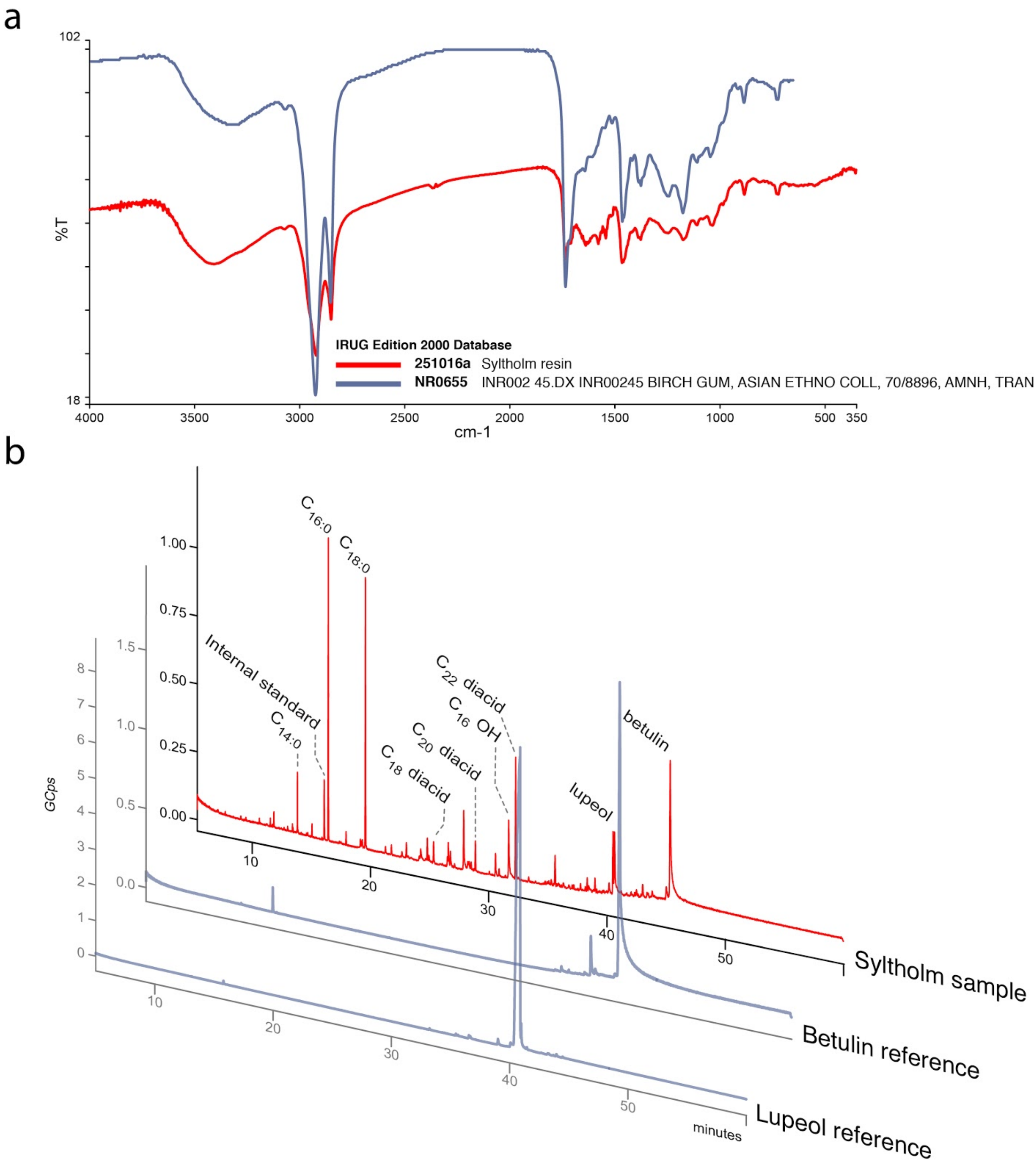
Supplementary Figure 2. Close-up photograph of the Syltholm pitch.



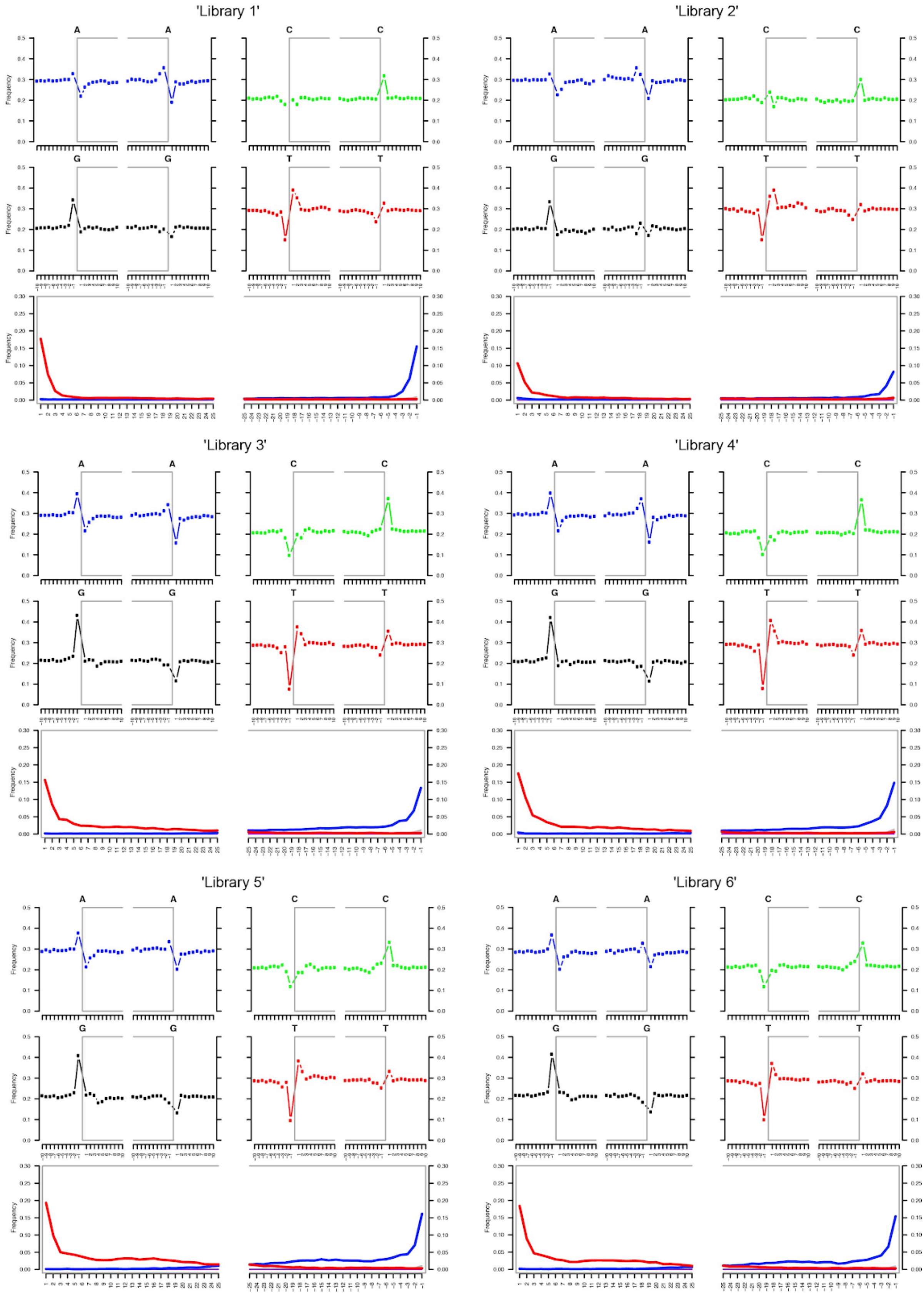
Supplementary Figure 3. Radiocarbon chronology for Syltholm site MLF906-II based on a series of 17 calibrated radiocarbon dates, including the birch pitch (marked in red). Samples from domesticated species are marked in yellow.



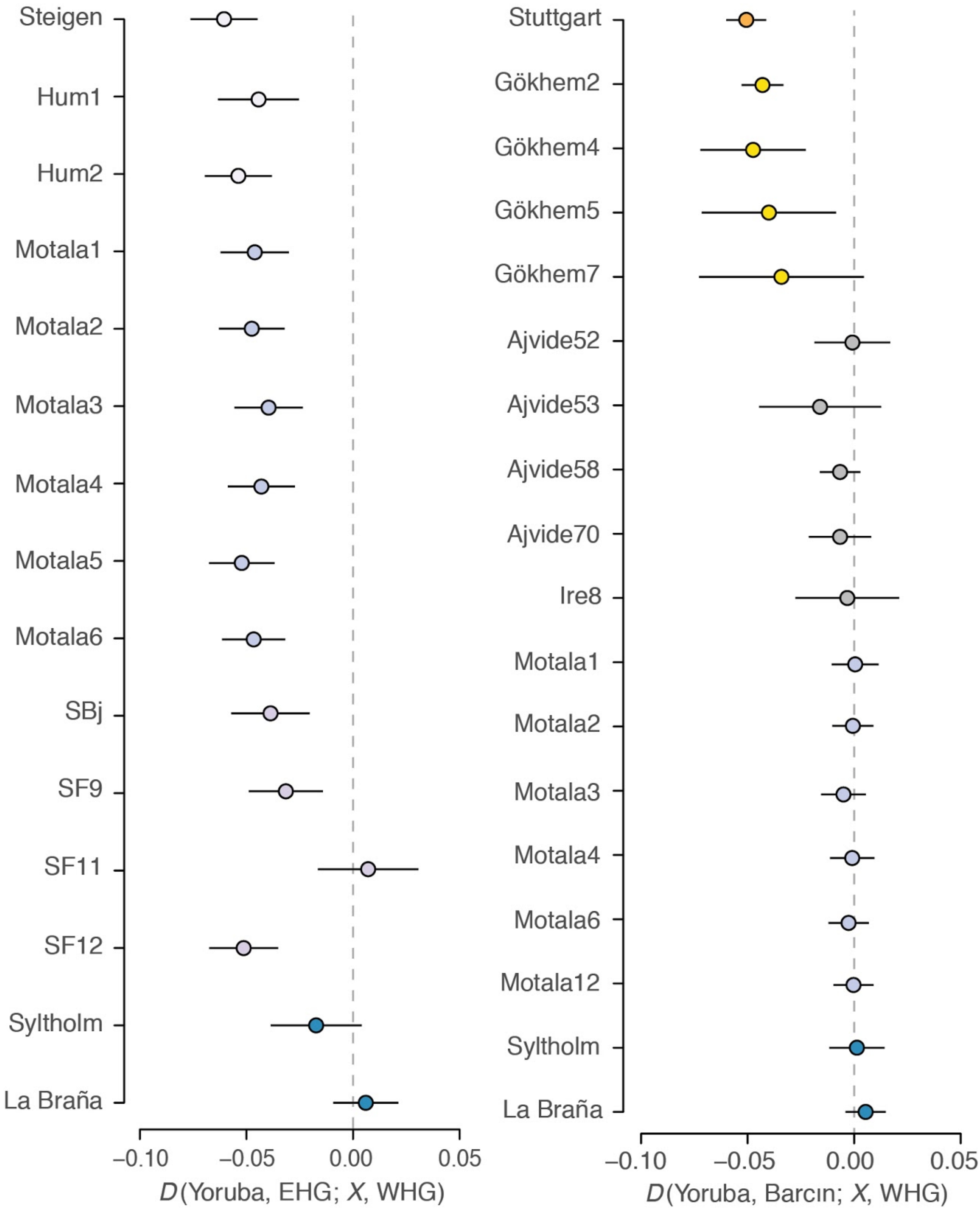
Supplementary Figure 4. FT-IR spectra of the Syltholm pitch and a modern birch sample.



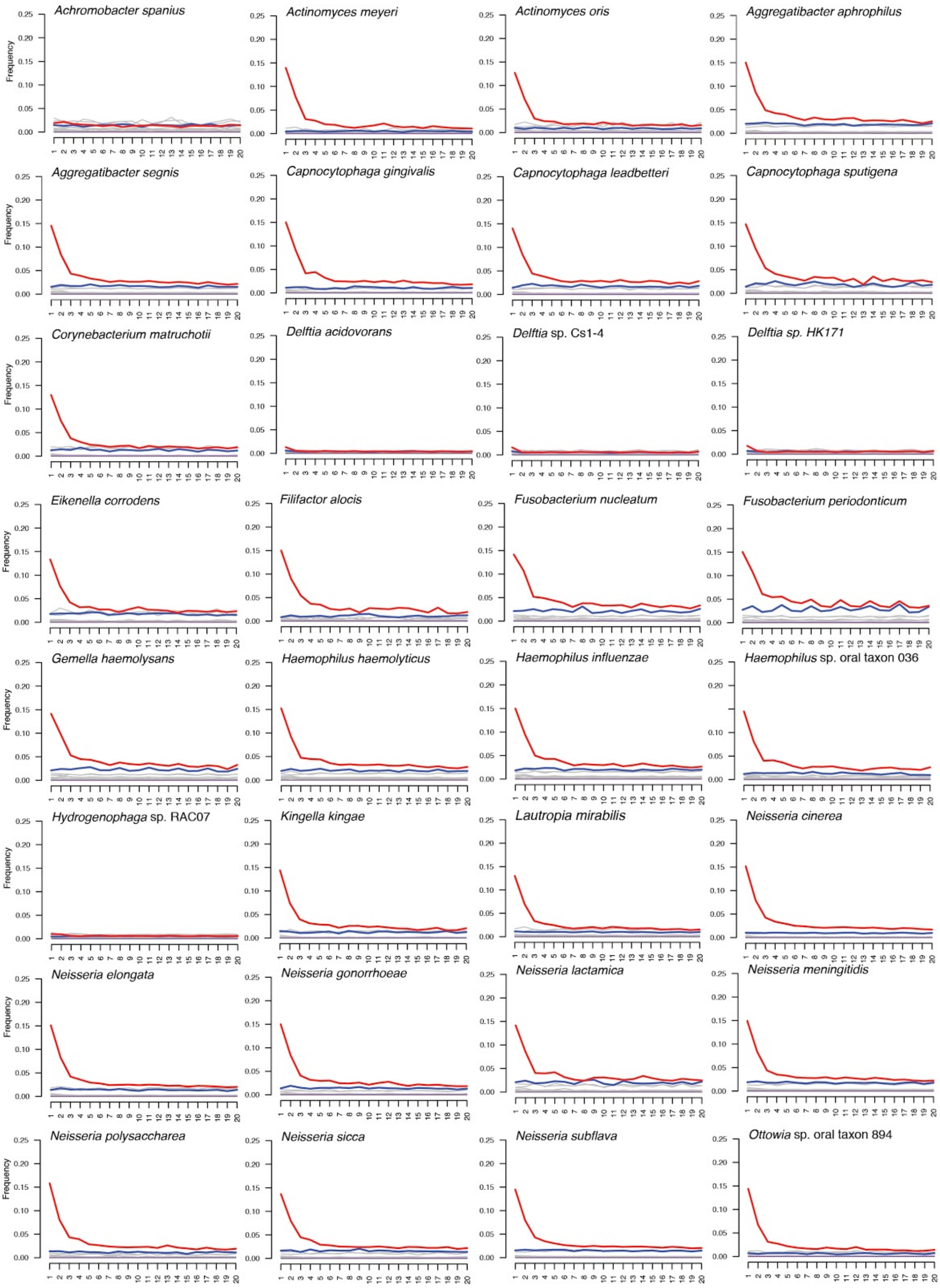
Supplementary Figure 5. GC-MS chromatograms of the Syltholm sample (back), betulin reference (middle) and lupeol reference (front).



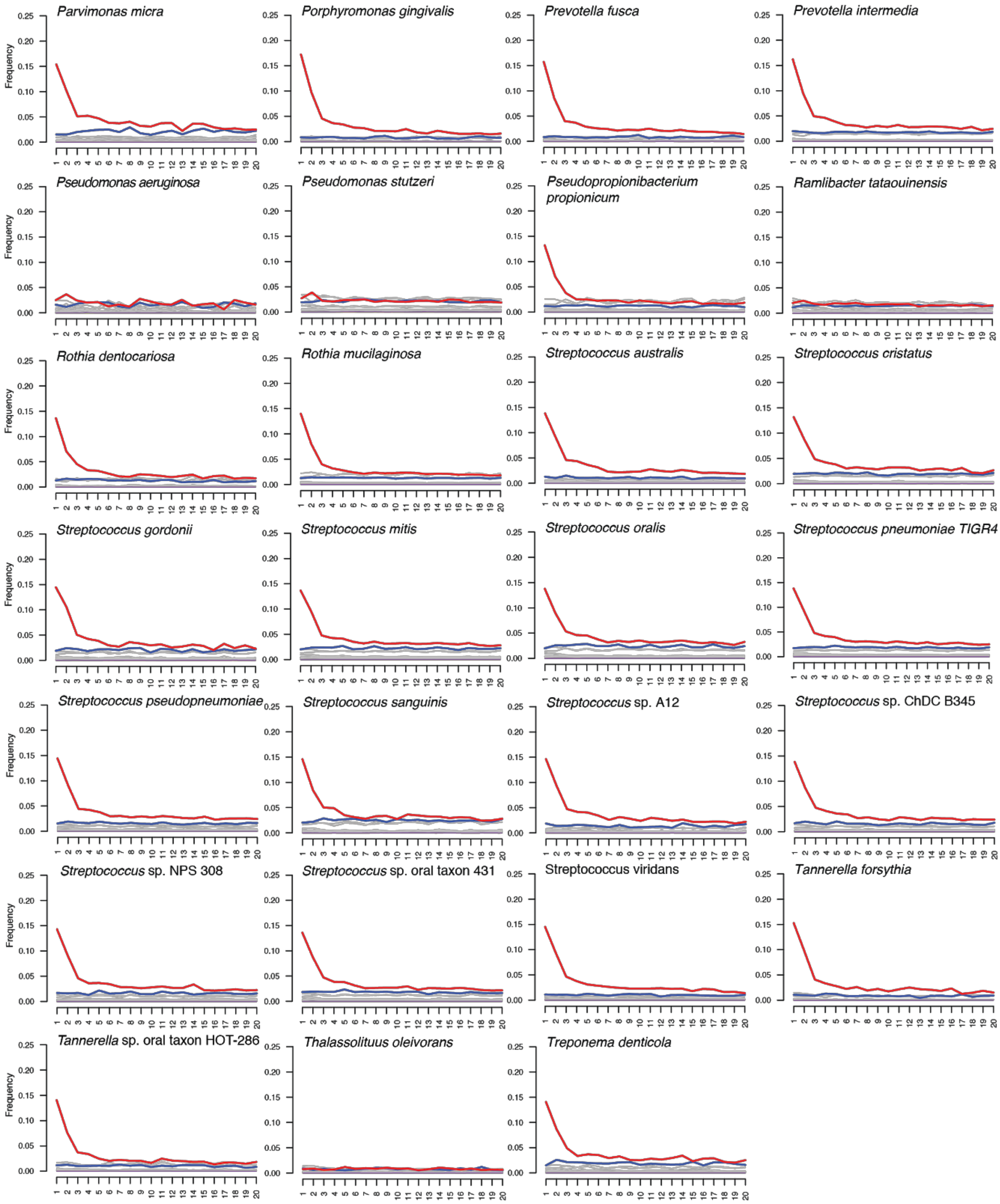
Supplementary Figure 6. MapDamage[23] plots for reads mapping to the human reference genome (hg19), by library.



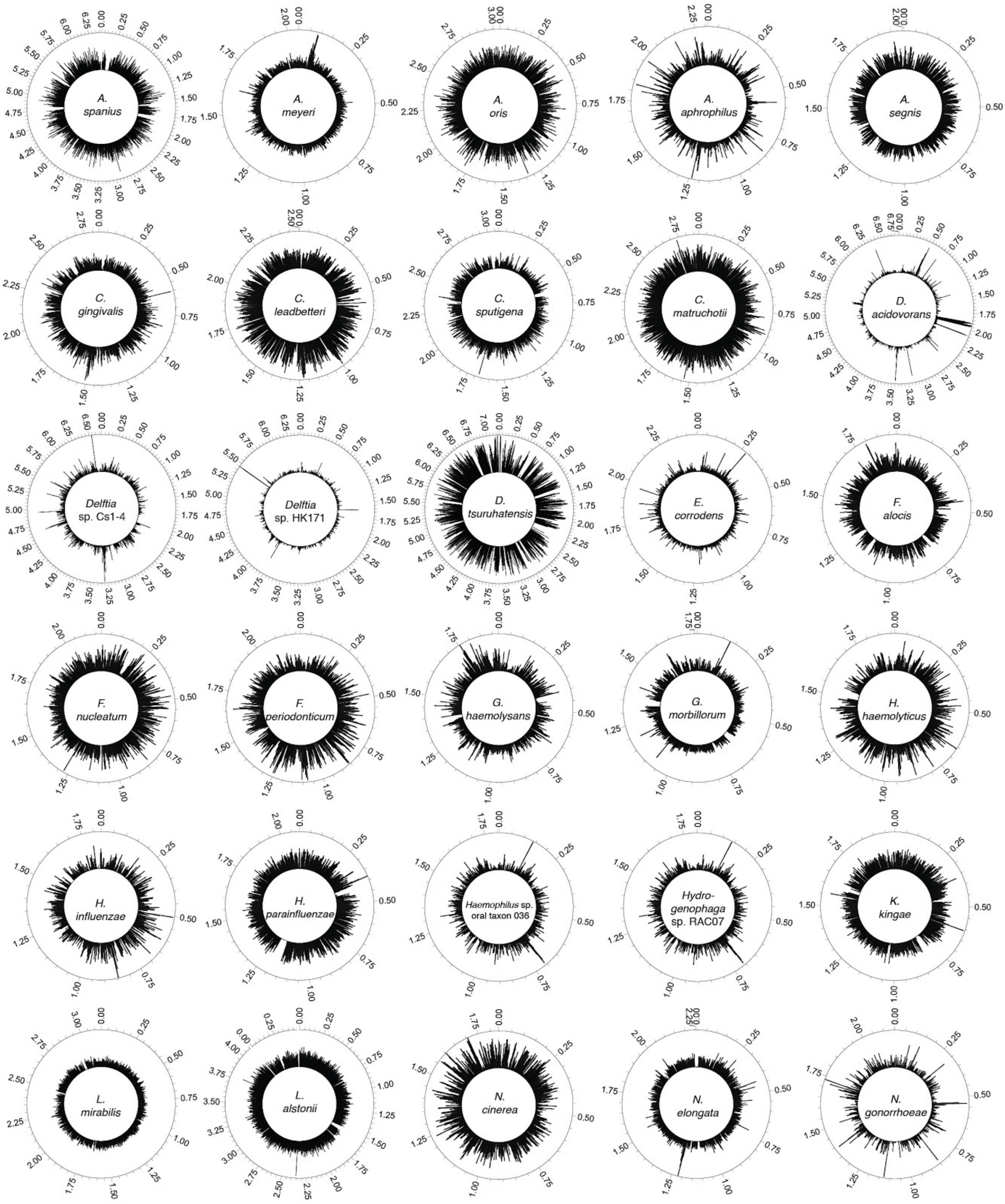
Supplementary Figure 7. *D*-statistics of the form *D*(Yoruba, EHG/Barcın; *X*, WHG) testing whether "*X*" forms a clade with WHG to the exclusion of EHG and Neolithic farmers (represented by Barcın), respectively. Error bars show three block-jackknife standard errors. Data are shown in Supplementary Tables 7 and 8.


Achromobacter spanius
Actinomyces meyeri
Actinomyces oris
Aggregatibacter aphrophilus
Aggregatibacter segnis
Capnocytophaga gingivalis
Capnocytophaga leadbetteri
Capnocytophaga sputigena
Corynebacterium matruchotii
Delftia acidovorans
Delftia sp. Cs1-4
Delftia sp. HK171
Eikenella corrodens
Filifactor alocis
Fusobacterium nucleatum
Fusobacterium periodonticum
Gemella haemolysans
Haemophilus haemolyticus
Haemophilus influenzae
Haemophilus sp. oral taxon 036
Hydrogenophaga sp. RAC07
Kingella kingae
Lautropia mirabilis
Neisseria cinerea
Neisseria elongata
Neisseria gonorrhoeae
Neisseria lactamica
Neisseria meningitidis
Neisseria polysaccharea
Neisseria sicca
Neisseria subflava
Ottowia sp. oral taxon 894

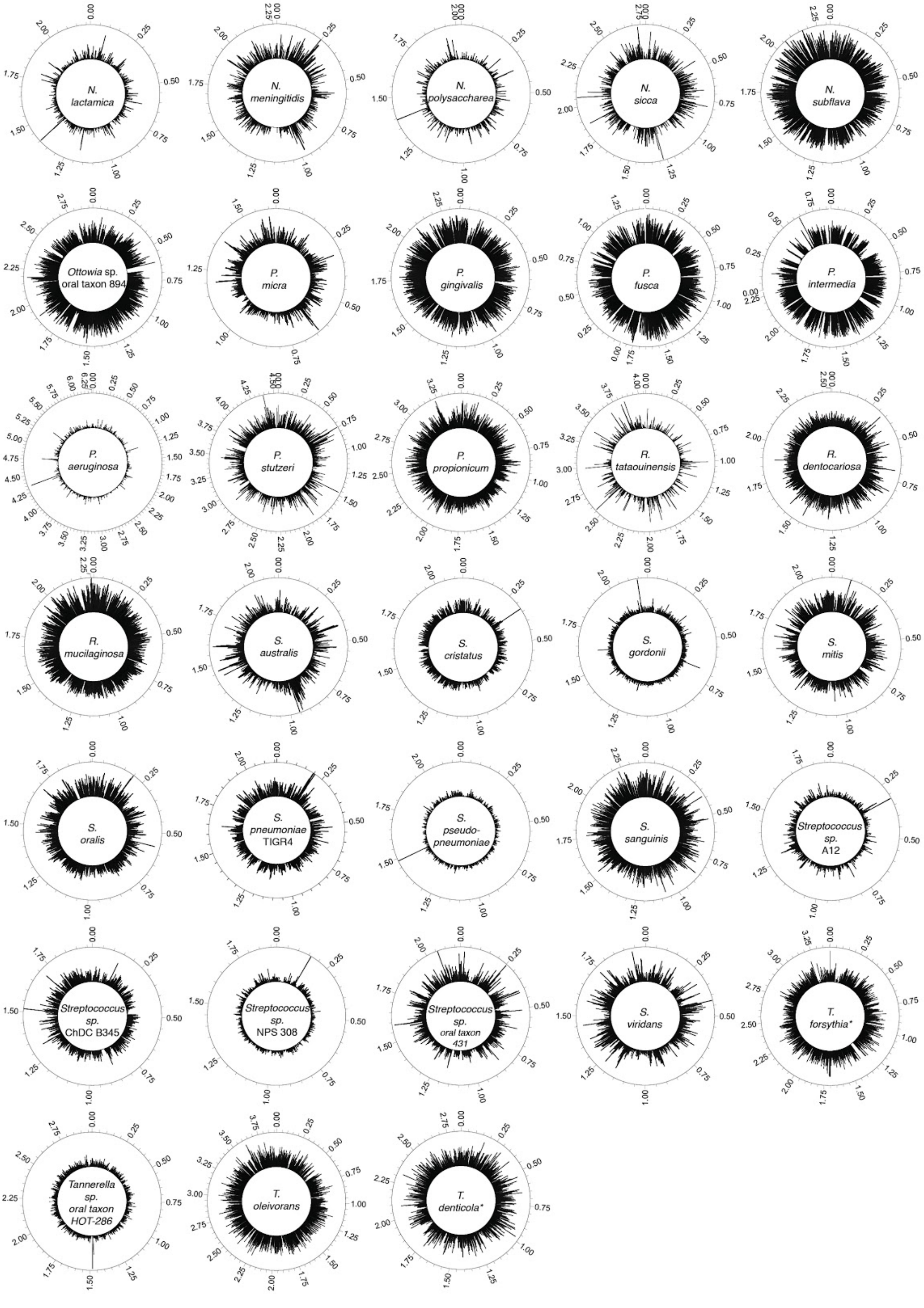
Supplementary Figure 8. MapDamage[23] plots for bacterial taxa with >10,000 assigned reads recovered from the Syltholm pitch.



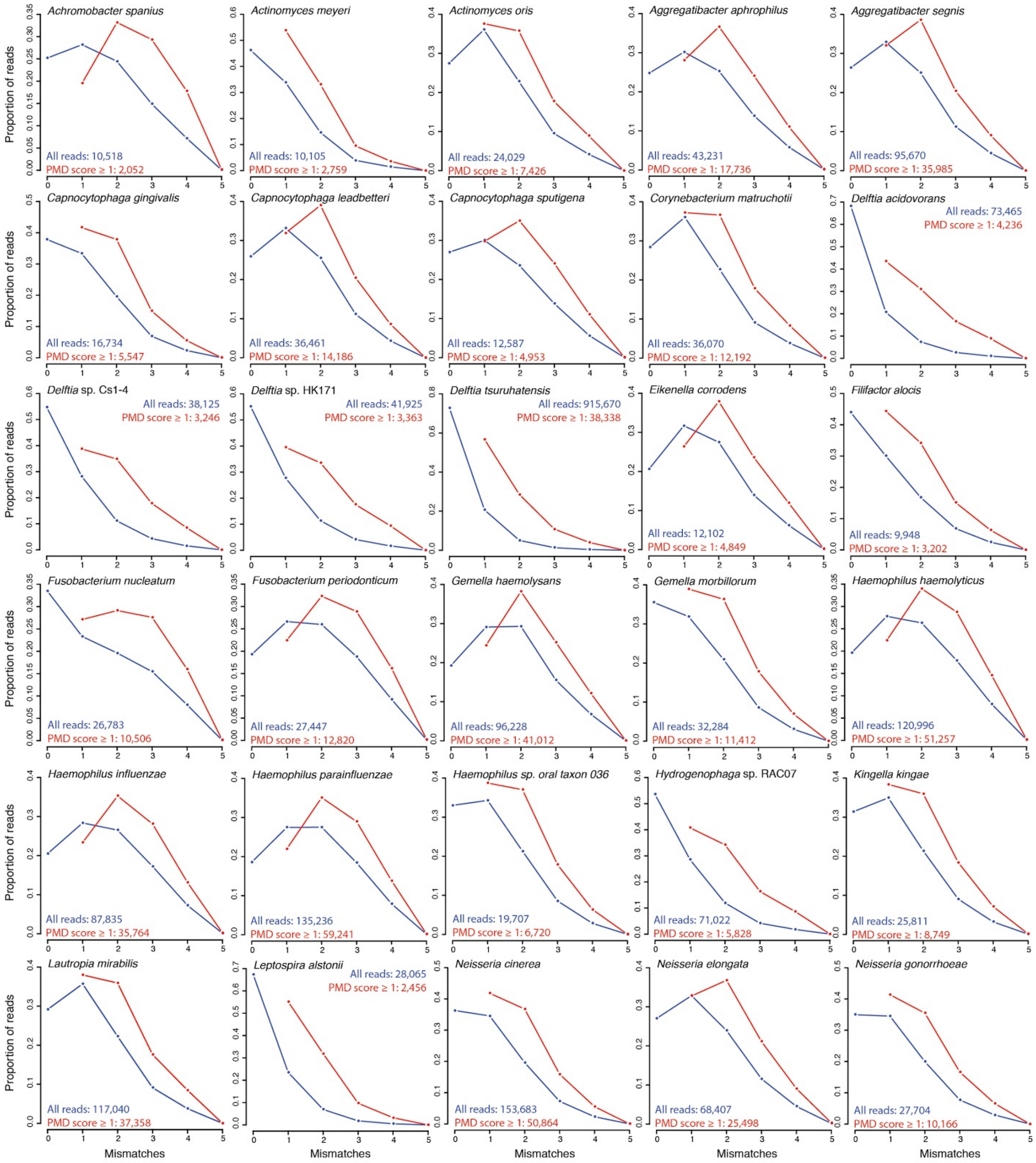
Supplementary Figure 8 ctd. MapDamage[23] plots for bacterial taxa with >10,000 assigned reads recovered from the Syltholm pitch.



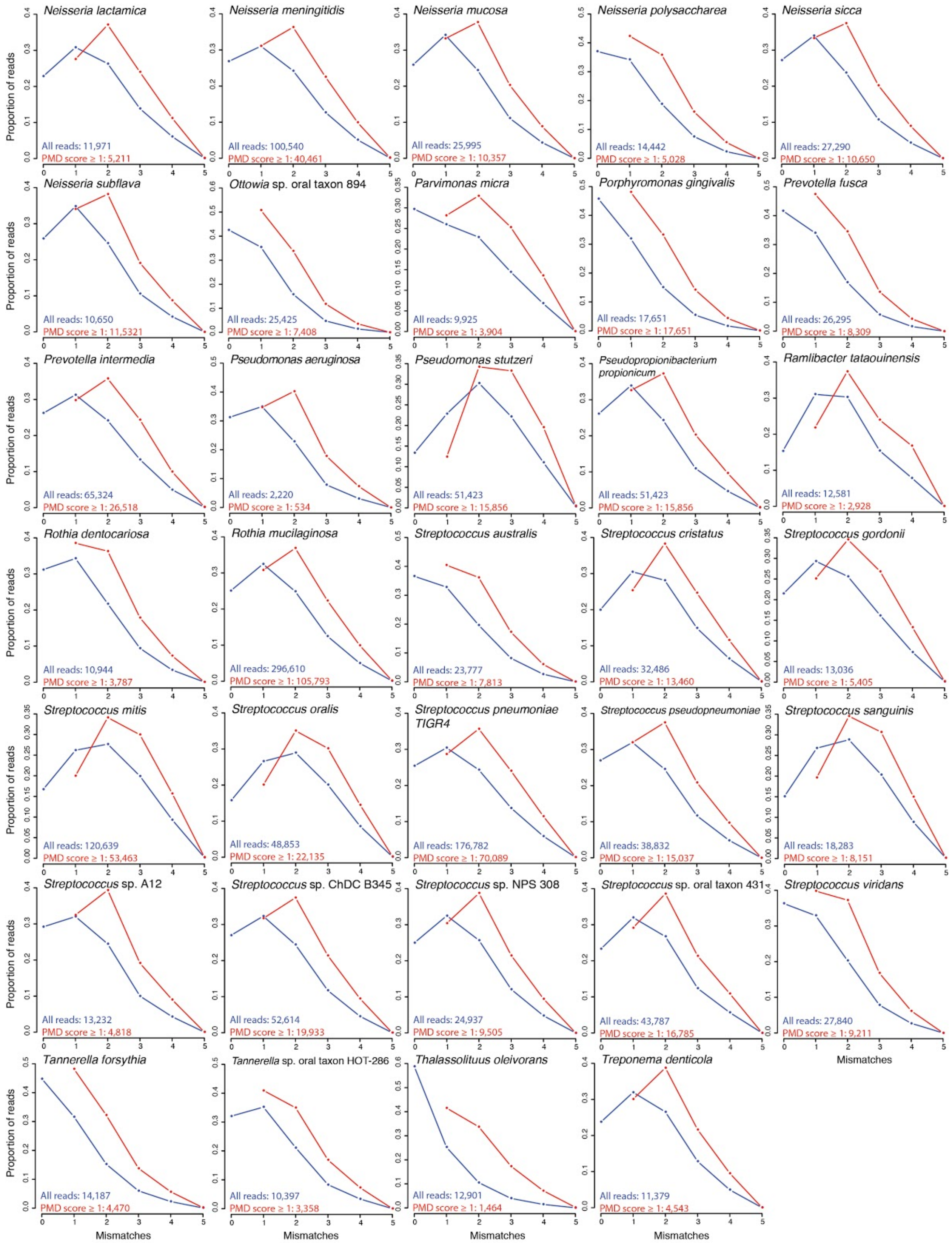
Supplementary Figure 9. Coverage plots for bacterial taxa recovered from the Syltholm pitch.



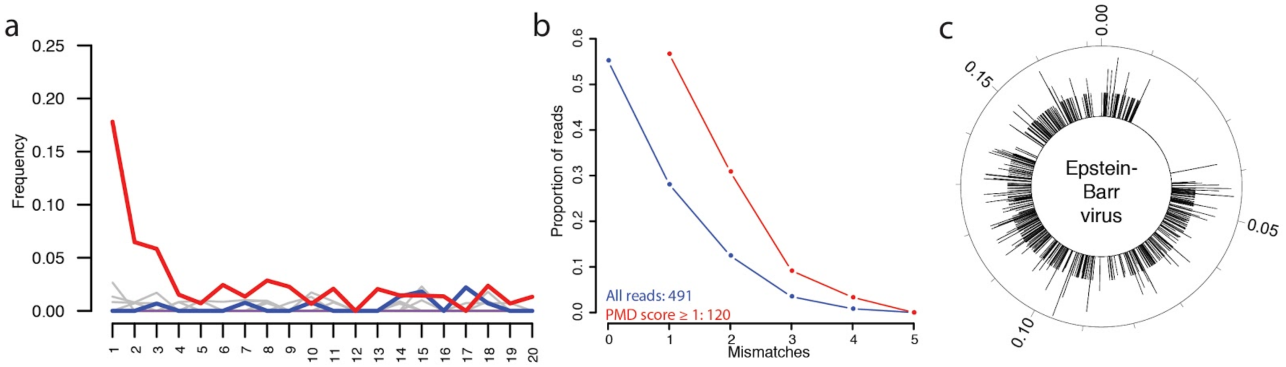
Supplementary Figure 9 ctd. Coverage plots for bacterial taxa recovered from the Syltholm pitch.



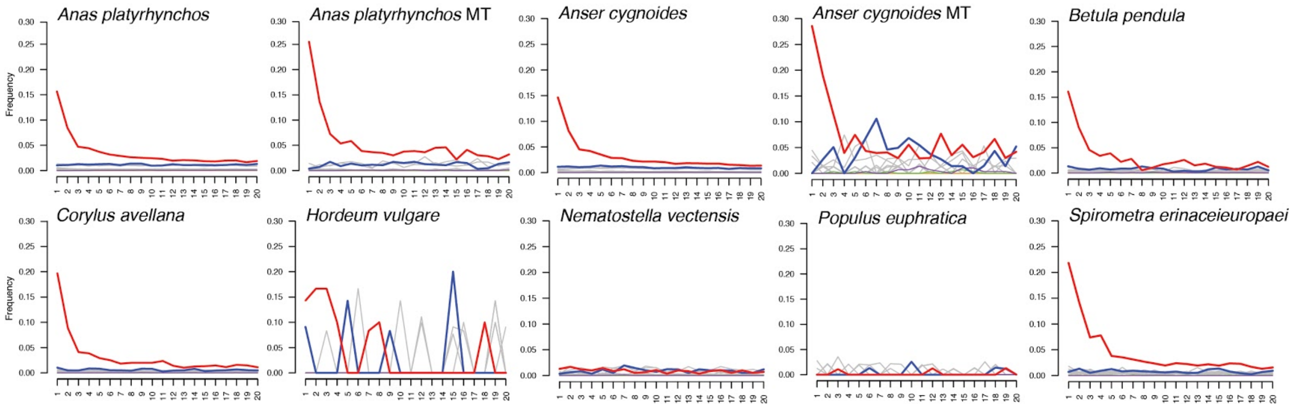
Supplementary Figure 10. Edit distance distributions of all reads (blue) and reads filtered for post-mortem damage (PMD≥1) (red) for bacterial taxa with >10,000 assigned reads recovered from the Syltholm pitch.



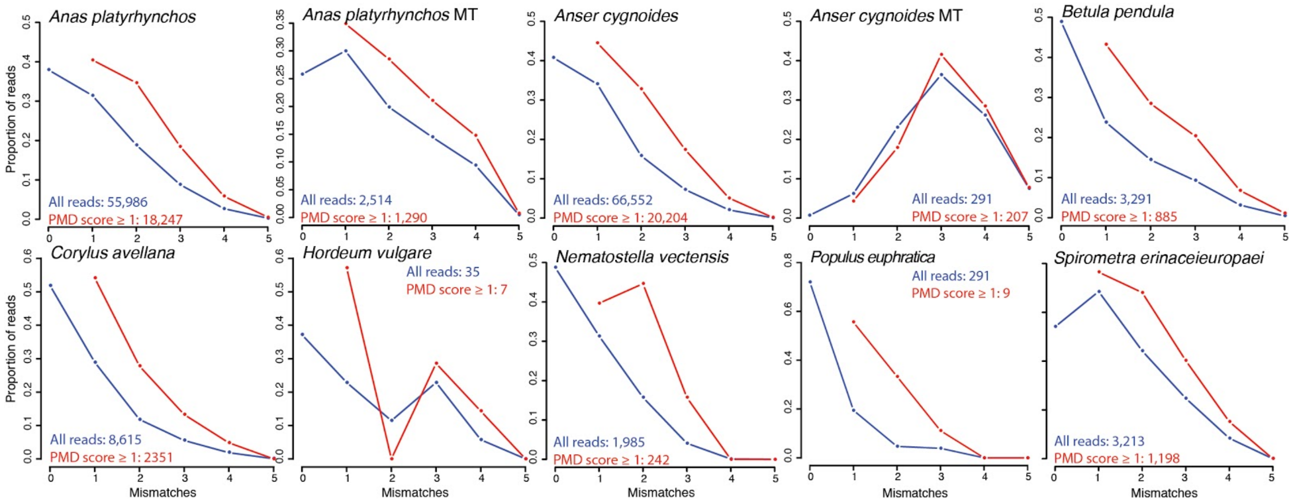
Supplementary Figure 10 ctd. Edit distance distributions of all reads (blue) and reads filtered for post-mortem damage (PMD≥1) (red) for bacterial taxa with >10,000 assigned reads recovered from the Syltholm pitch.



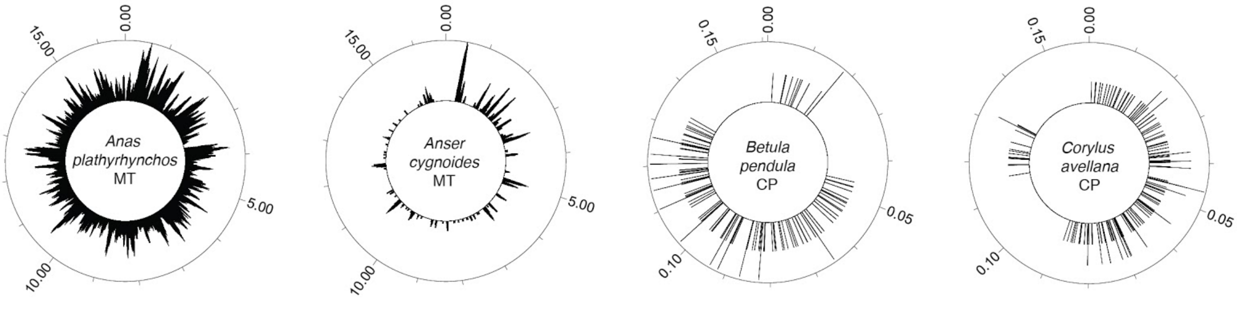
Supplementary Figure 11. MapDamage[23] plot (a), edit distance distribution (b), and coverage plot (c) for reads mapping to Epstein-Barr virus.



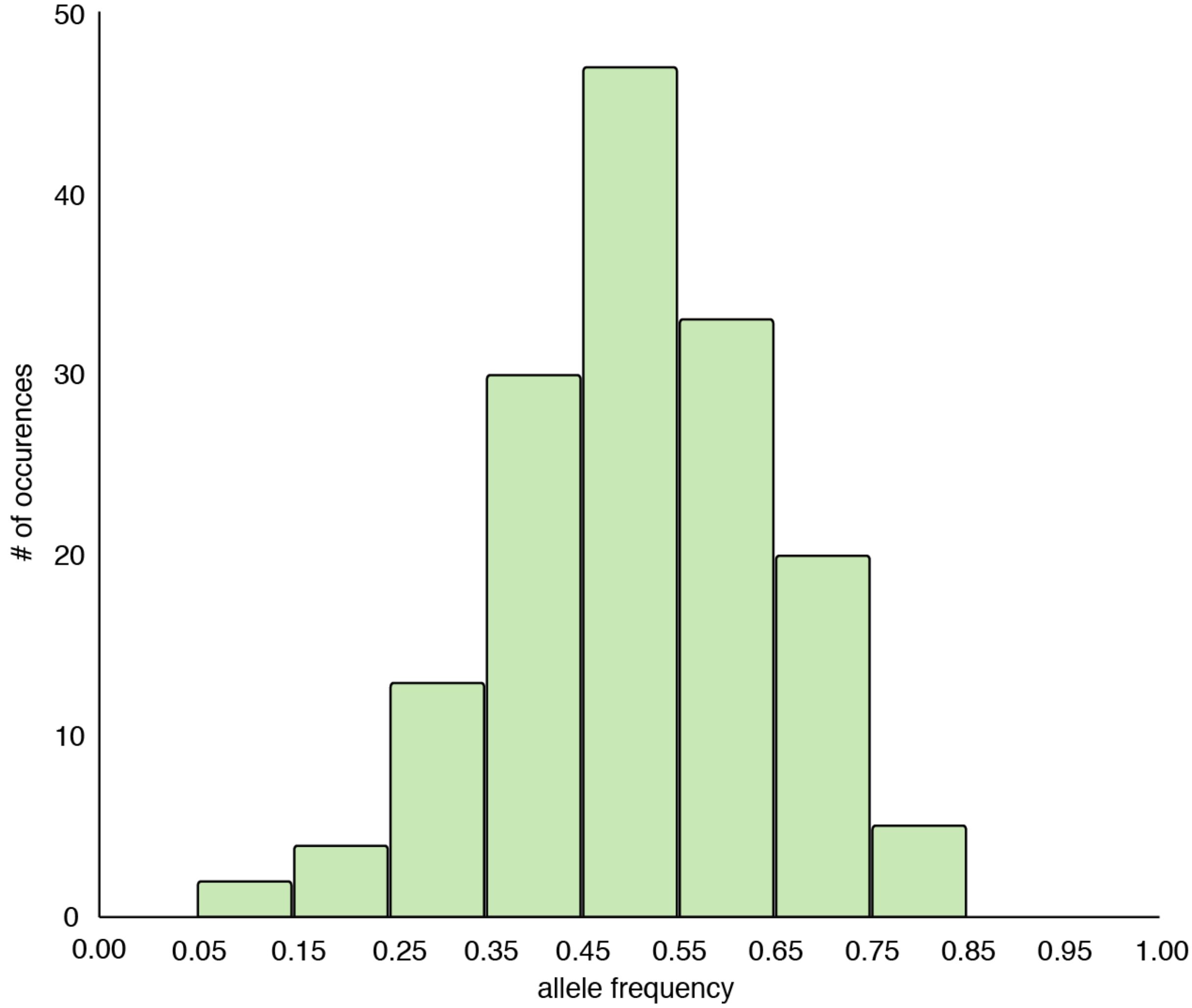
Supplementary Figure 12. MapDamage[23] plots for reads mapping to Metazoa (animals) and Viridiplantae (plants) in the ancient pitch sample. Note the absence of characteristic ancient DNA damage patterns for poplar (*Populus euphratica)*, starlet sea anemone (*Nematostella vectensis)* and barley (*Hordeum vulgare*).



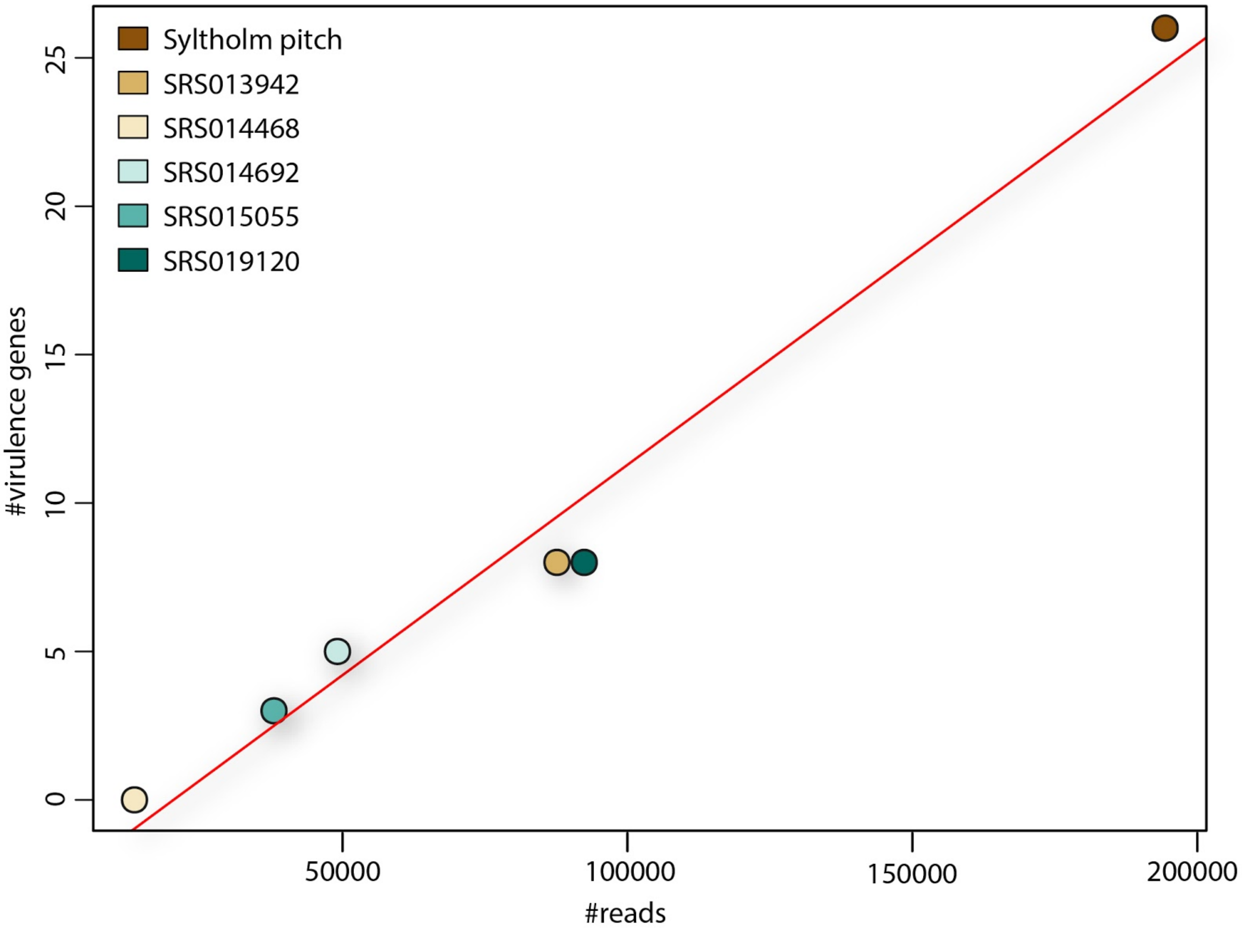
Supplementary Figure 13. Edit distance distributions of all reads from the ancient pitch assigned to Metazoa (animals) and Viridiplantae (plants). Reads filtered for post-mortem damage (PMD≥1) are shown in red.



Supplementary Figure 14. Coverage plots for eukaryotic taxa in the ancient pitch sample with more than 100 reads aligning to the chloroplast/mitochondrial genome. For poplar (*Populus euphratica)*, starlet sea anemone (*Nematostella vectensis)* and the tapeworm (*Spirometra erinaceieuropaei*) no fragment aligned to its cpDNA or mtDNA, while for barley (*Hordeum vulgare*) only 15 fragments aligned to its cpDNA. The gaps in the chloroplast DNA (cpDNA) represent inverted repeats, which are very similar to each other, although not completely identical.



Supplementary Figure 15. Allele frequency distribution of single nucleotide variants in the 10× mallard (*A. platyrhynchos*) mtDNA genome recovered from the ancient pitch. The symmetric distribution suggests the presence of two haplotypes present in equal abundance.



Supplementary Figure 16. The number of virulence genes identified in the ancient pitch sample and five human oral microbiome samples from the HMP[31].

Supplementary Table 1. Screening results for six different DNA extracts from the Syltholm pitch, extraction blank, and two soil control samples from the site.

sample	**weight**	**method**	**yield (ng)**[1]	**hg19 reads**[2]	**% dupl.**[3]	**% end.**[4]	**fragment length**[5]	**C-T 5' (%)**[6]
1	54 mg	1	4.7	449,096	9.1	3.7	56.1	17.4
2	52 mg	1	17.3	2,189,982	44.1	24.1	55.4	10.4
3	44 mg	2	6.8	2,754,931	6.3	56.5	59.9	15.0
4	48 mg	2	6.1	3,895,487	9.0	55.1	59.8	17.0
5	32 mg	3	0.3	63,390	57.8	1.6	62.3	19.3
6	24 mg	3	0.3	144,681	46.5	4.3	64.5	18.5
Control 1	~2 g	2	61.4	450	46.4	<0.1	50.8	18.8
Control 2	~2 g	2	58.2	401	50.2	<0.1	46.0	8.3
NTC	N/A	2	N/A	140	72.0	0.5	57.1	13.9

[1] Total DNA yields (ng) measured using the Agilent 4200 TapeStation; [2] Number of reads that could be uniquely mapped to the human reference genome (hg19) after removing duplicates; [3] Fraction of duplicate reads in the sample (in percent); [4] Endogenous human DNA content (in percent); [5] Average fragment length (in bp); [6] Deamination rate at 5' ends of DNA fragments (in percent)

Supplementary Table 2. Deep-sequencing results for the Syltholm pitch.

hg19 reads[1]	end. content[2]	fragment length[3]	C-T 5’[4]	mtDNA contamination[5]	>1X[6]	DoC[7]	mtDNA hg[8]
120,585,267	31.2%	59.9 bp	16.2%	1-3%	78.9%	2.3x	K1e

[1] Number of reads that could be uniquely mapped to the human reference genome (hg19) after removing duplicates and filtering for mapping quality (MAPQ ≥30); [2] Endogenous human DNA content (in percent); [3] Average fragment length (in bp); [4] Deamination rates at 5’ ends of DNA fragments (in percent); [5] MtDNA based contamination estimates determined using Schmutzi[34]**;** [6] Genome coverage (in percent); [7] Average depth of genome coverage; [8] Mitochondrial DNA haplogroup.

Supplementary Table 3. Molecular decay rates (*k*, per site per year) for the Syltholm genome and other previously published ancient genomes from different contexts[11,12,32,33].

Sample	Age (yrs BP)	Temp. (°C)	λ	*k*	*k*, 100 bp	half-life (yrs), 100 bp
Taino (The Bahamas)	1,000	20	0.016	1.60^{-05}	1.60^{-03}	434
Syltholm (Denmark)	5,700	8.5	0.034	5.96^{-06}	5.96^{-04}	1,162
La Braña (Spain)	7,500	8.1	0.033	4.40^{-06}	4.40^{-04}	1,576
Kennewick (WA, USA)	9,000	12.5	0.017	1.89^{-06}	1.89^{-04}	3,670
Anzick (MT, USA)	12,785	4.8	0.018	1.41^{-06}	1.41^{-04}	4,916

Supplementary Table 4. *F*-statistics of the form f_4(Yoruba, *X*; EHG, WHG) measuring the amount of shared genetic drift between different ancient genomes (*X*), EHG and WHG.

Pop2 (X)	**f_4-stat**	**SE**	**Z**	**BABA**	**ABBA**	**SNPs**
Syltholm	0.011917	0.000698	17.063	6,281	4,903	115,687
La Braña	0.012022	0.000525	22.894	29,695	23,219	538,716
Hum1	-0.001431	0.000644	-2.224	9,966	10,262	207,167
Hum2	-0.001152	0.000592	-1.947	26,029	26,646	536,119
Steigen	-0.001494	0.000565	-2.645	20,418	21,047	421,170
Motala1	0.00216	0.000575	3.755	17,719	16,950	355,954
Motala2	0.003681	0.000546	6.747	22,397	20,771	441,690
Motala3	0.002856	0.000529	5.396	13,191	12,427	267,396
Motala4	0.003171	0.000578	5.484	22,361	20,955	443,456
Motala6	0.002229	0.000554	4.023	18,922	18,073	380,891
Motala12	0.002848	0.000545	5.223	25,448	24,005	506,761

Supplementary Table 5. *F*-statistics of the form f_4(Yoruba, *X*; NEO, WHG) measuring the amount of shared genetic drift between different ancient genomes (*X*), WHG, and Neolithic farmers (represented by Barcın).

Pop2 (*X*)	**f_4-stat**	**SE**	**Z**	**BABA**	**ABBA**	**SNPs**
Syltholm	0.019419	0.000586	33.11	7,292	4,941	121,065
La Braña	0.017952	0.000436	41.145	34,006	23,859	565,167
Motala1	0.012127	0.000476	25.488	20,388	16,010	361,017
Motala2	0.013533	0.000425	31.864	25,789	19,700	449,904
Motala3	0.011315	0.00042	26.961	14,803	11,765	268,527
Motala4	0.012719	0.000444	28.662	25,548	19,828	449,704
Motala6	0.012387	0.000425	29.156	21,691	16,930	384,398
Motala12	0.012751	0.000426	29.936	29,548	22,926	519,374
Ajvide52	0.010292	0.0007	14.711	3,011	2,450	54,498
Ajvide53	0.009491	0.001117	8.5	920	764	16,417
Ajvide58	0.009906	0.000441	22.476	29,847	24,550	534,726
Ajvide70	0.01018	0.00057	17.861	5,297	4,330	95,022
Ire8	0.009695	0.000916	10.588	1,315	1,082	23,981
Gökhem2	0.000697	0.000428	1.629	21,220	20,929	418,556
Gökhem4	0.001914	0.000943	2.029	1,078	1,038	20,804
Gökhem5	0.001455	0.001157	1.258	704	685	13,614
Gökhem7	0.00348	0.001569	2.218	378	351	7,621
Stuttgart	-0.004073	0.000368	-11.065	26,734	29,024	562,246

Supplementary Table 6. Admixture proportions based on *qpAdm*[35] analysis, specifying western hunter-gatherers (WHG), eastern hunter-gatherers (EHG), and Neolithic farmers (Barcın) as ancestral source populations.

test population	reference population	admixture proportion	n SNPs	chi square	tail prob
Bichon (LP)	WHG	1.000	374,266	3.52	0.74
	EHG	0.000			
	Barcın	0.000			
Rochedane (LP)	WHG	1.000	113, 744	6.72	0.35
	EHG	0.000			
	Barcın	0.000			
La Braña (M)	WHG	1.000	538,715	7.15	0.31
	EHG	0.000			
	Barcın	0.000			
Loschbour (M)	WHG	1.000	544,933	9.79	0.13
	EHG	0.000			
	Barcın	0.000			
Ranchot (M)	WHG	1.000	200,185	4.02	0.67
	EHG	0.000			
	Barcın	0.000			
Syltholm	WHG	1.000	115,800	6.34	0.39
	EHG	0.000			
	Barcın	0.000			
Karelia (M)	WHG	0.000	294,370	11.15	0.08
	EHG	1.000			
	Barcın	0.000			
Samara (M)	WHG	0.000	294,370	11.15	0.08
	EHG	0.100			
	NF	0.000			
NorwayHG (M)	Barcın	0.441	558,124	3.10	0.68
	EHG	0.559			
	Barcın	0.000			
Latvia (M)	WHG	0.697	560,151	4.49	0.48
	EHG	0.303			
	Barcın	0.000			
BalticHG (M)	WHG	0.649	562,935	3.46	0.63
	EHG	0.351			
	Barcın	0.000			
Motala (M)	WHG	0.593	545,689	4.83	0.44
	EHG	0.407			
	Barcın	0.000			
PWC (EN)	WHG	0.780	523,969	3.14	0.68
	EHG	0.220			
	Barcın	0.000			
Gökhem (EN)	WHG	0.175	407,865	2.68	0.75
	EHG	0.000			
	Barcın	0.825			
Iberia (EN)	WHG	0.180	557,569	2.98	0.70
	EHG	0.000			
	Barcın	0.820			
LBK (EN)	WHG	0.162	563,150	2.61	0.76
	EHG	0.000			
	Barcın	0.838			
GAC (EN)	WHG	0.293	563,197	9.72	0.08
	EHG	0.000			
	Barcın	0.707			

Supplementary Table 7. *D*-statistics of the form *D*(Yoruba, EHG; *X*, WHG) testing whether "*X*" forms a clade with WHG to the exclusion of EHG.

Pop3 (*X*)	***D*-stat**	**SE**	**Z**	**BABA**	**ABBA**	**SNPs**
Sylthom	-0.0173	0.007118	-2.432	4,736	4,903	115,687
La Braña	0.006	0.005081	1.176	23,498	23,219	538,716
Motala1	-0.0461	0.005356	-8.611	15,456	16,950	355,954
Motala2	-0.0475	0.005144	-9.232	18,888	20,771	441,690
Motala3	-0.0396	0.005355	-7.387	11,481	12,427	267,396
Motala4	-0.043	0.005249	-8.19	19,228	20,955	443,456
Motala6	-0.0522	0.005122	-10.192	16,280	18,073	380,891
Motala12	-0.0466	0.004941	-9.427	21,868	24,005	506,761
SBj	-0.0387	0.006137	-6.308	7,622	8,236	174,952
SF9	-0.0315	0.005791	-5.44	12,895	13,734	293,510
SF11	0.0071	0.007857	0.91	3,261	3,214	69,375
SF12	-0.0513	0.005394	-9.516	24,421	27,064	561,611
Hum1	-0.0443	0.006341	-6.99	9,391	10,262	207,167
Hum2	-0.0538	0.005228	-10.299	23,924	26,646	536,119
Steigen	-0.0605	0.00524	-11.544	18,646	21,047	421,170

Supplementary Table 8. *D*-statistics of the form *D*(Yoruba, Barcın; *X*, WHG) testing whether "*X*" forms a clade with WHG to the exclusion of Neolithic farmers (represented by Barcın).

Pop3 (*X*)	***D*-stat**	**SE**	**Z**	**BABA**	**ABBA**	**SNPs**
Syltholm	0.0013	0.004313	0.307	4,954	4,941	121,065
La Braña	0.0054	0.003145	1.716	24,118	23,859	565,167
Ajvide52	-0.0008	0.005938	-0.128	2,447	2,450	54,498
Ajvide53	-0.016	0.009548	-1.673	740	764	16,417
Ajvide58	-0.0066	0.003166	-2.08	24,229	24,550	534,726
Ajvide70	-0.0066	0.004872	-1.361	4,273	4,330	95,022
Ire8	-0.0032	0.00813	-0.395	1,075	1082	23,981
Gökhem2	-0.043	0.003261	-13.183	19,203	20,929	418,556
Gökhem4	-0.0474	0.008241	-5.749	944	1,038	20,804
Gökhem5	-0.04	0.010509	-3.809	632	685	13,614
Gökhem7	-0.0341	0.012892	-2.648	328	351	7,621
Motala1	0.0005	0.003661	0.145	16,027	16,010	361,017
Motala2	-0.0006	0.00322	-0.19	19,676	19,700	449,904
Motala3	-0.005	0.003485	-1.445	11,647	11,765	268,527
Motala4	-0.0009	0.003462	-0.255	19,793	19,828	449,704
Motala6	-0.0026	0.003151	-0.833	16,841	16,930	384,398
Motala12	-0.0003	0.003127	-0.093	22,912	22,926	519,374
Stuttgart	-0.0506	0.003118	-16.231	26,228	29,024	562,246

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Chapter 6 Discussion and conclusions

This thesis has attempted to clarify some fundamental questions about the South Scandinavian Stone Age. It is based on the same impetus that the earliest scientists became known for, namely interdisciplinarity between archaeology and the natural sciences.

6.1 Ephemeral hunter-gatherers

With the study of Maglemose bone points, it has been shown that this category of objects, often without precise provenance, can be used as proxies to answer questions such as: chronology, species composition, and the possible movements of people. This study has created a chronological/cultural framework onto which settlement sites can be in-directly dated/placed, based on the finds of the categorised bone points. Creating a detailed typology based on several types (similar to Larsson et al (2019)) was not possible with the material at hand. The large study area revealed too much variation. This variation can not just assigned to a degree of altered shape due to taphonomy, but probably regional differences, even in a confined region such as Zealand. However, it was certainly possible to show that two major groups of bone points. One being the fine-barbed bone points while the other being the large-barbed bone points.

The hiatus where there is an absence of radiocarbon dates cannot be explained, but possible hypotheses can be postulated. As discussed in Chapter 3, it seems that the Early Boreal was a period with very low water-levels in the lakes; however, most of this data stems from palynological investigations that was done without the integration of radiocarbon dates. If the water-levels in the lakes were so low that inland fishing became almost impossible, it seems reasonable to suggest that people would emigrate. It remains to be seen how this happened. Did they leave Scandinavia, and move to the present-day Northern Germany, where the fine-barbed bone points were still in use during the hiatus? Or did they move to the coastal areas, that are now largely submerged in Southern Scandinavia? Evidence for and against is vague, due to the limited sites with dateable organic material.

6.2 Syltholm – transition in action?

With the study of the finger ring from Syltholm 906-II, an attempt was made to retrieve as much information about this rare object as possible through minimally destructive sampling. Based on 70 radiocarbon dates from the site, it is inferred that the ring likely dates from the beginning of the Neolithic period, and that activity at the site peaked during the Neolithic (Fig. 7).

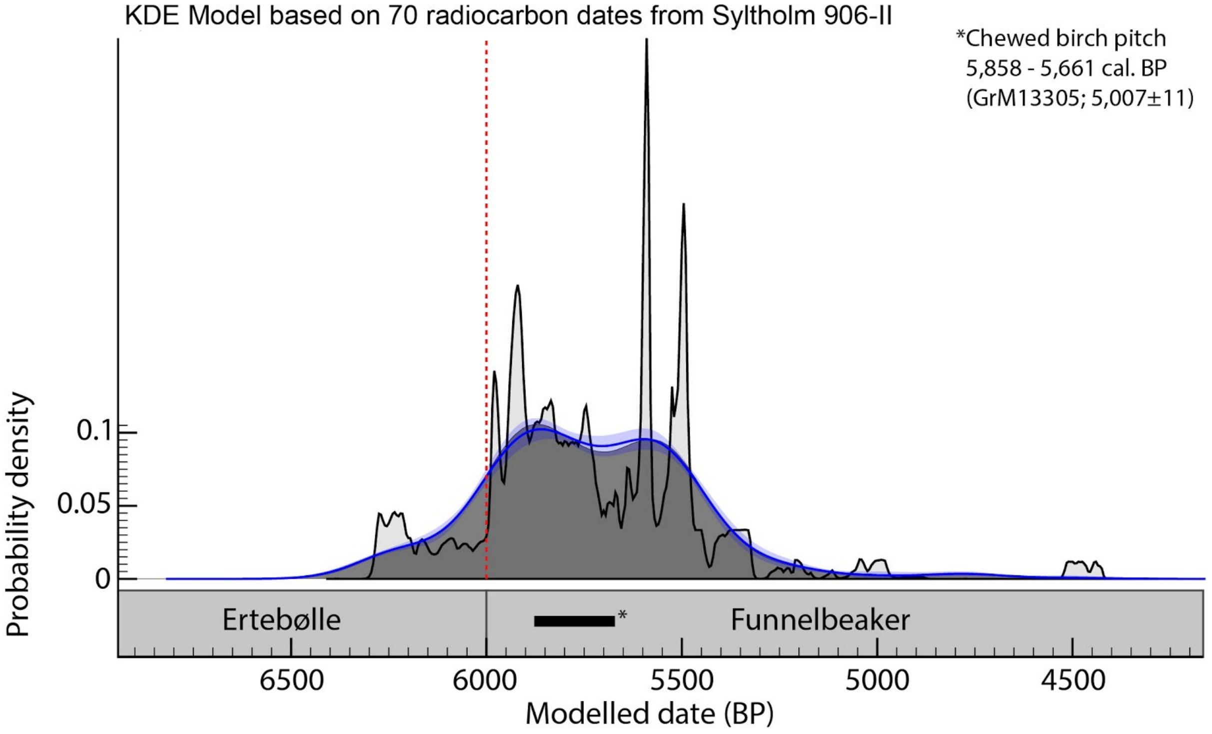

Fig. 7 Kernel density model (KDE) (Ramsey 2017) of 70 summed radiocarbon dates from Syltholm 906-II, used to visualise activity at the site. The calibrated chewed birch pitch marked as a black bar in the Funnelbeaker panel. The radiocarbon dates derive from Chapter 4.

Proteomic analyses revealed that it was made of red deer, a common ungulate at the time and at the site. In terms of the raw material, the sequencing of the ring proteome, revealed differences compared to modern red deer bone, but similarities compared to modern red deer antler, which may indicate the raw material. This tentative conclusion needs further testing. From the same site as the ring, large amounts of endogenous ancient DNA were extracted from a chewed piece of birch pitch. The person who chewed it was female and of 100% Western Hunter-Gatherer ancestry. She likely had dark skin and hair, and blue eyes, which is a common trait for this genetic group (Olalde et al. 2014). Additionally, she was not lactase persistent, and thus probably had difficulties digesting raw milk. Further, saliva contained in the pitch revealed a snapshot of her oral microbiota. Of note was the presence of the commensal *Streptococcus pneumoniae* but which was revealed to carry fewer virulence genes than modern strains. Another capture was the classification of eukaryotic taxa deriving from potential foodstuffs such as; mallard (*Anas platyrhynchos*) and hazelnuts (*Corylus avellana*). These findings are unique as they display a snapshot of likely a single day, compared to for instance dental calculus, which reflect a reservoir of DNA accumulated over many years.

Remarkably, the chewed piece of pitch was directly dated to the Early Neolithic. While this is only one piece in a larger puzzle dealing with the transition from hunting and gathering to farming, it does indicate that the transition may not have been as rapid as proponents of the migration theory assumed.

Fig. 8. Indications of 'dualism' in the Early Neolithic. The majority of faunal remains at Syltholm 906 derived from wild taxa. Left: red deer antler with braincase. Right: transverse arrowhead made from a polished Neolithic axe found in the 'mandible trench' (Photos by Theis Zetner Trolle Jensen).

In general, Syltholm must be regarded as a special place. Not only because of the high intensity of finds and the especially well-preserved organic material, but because depositions took place at a very specific place in a specific landscape. It is thus indicative of passed down knowledge, across the transition i.e. the same group of people.

One such unique place, is a small depositional area (c. 25 m^2) coined the "mandible trench". During excavation, c. 50 deposited mandibles from taxa, including wildcat (*Felis silvestris*), otter (*Lutra lutra*), fox (*Vulpes vulpes*), red deer, pig (*Sus* sp.), roe deer (*Capreolus capreolus*), domestic dog (*Canis familiaris*) were uncovered. Alongside these remains were a multitude of special artefacts such as, a piece decorated wood, dozens of tinder fungi (*Fomes formentarius*), a t-shaped antler (Mesolithic), a *Rosenkrans* antler axe (Neolithic) and a decorated red deer antler staff (Mesolithic). The period(s) of activity at this site span the Middle Ertebølle to the Early Neolithic. By expanding the excavations out a few dozen metres from the "mandible trench," Neolithic artefacts became more concentrated. Several ceramic vessels and domestic cattle skulls were encountered; which were placed deliberately in the shallow water, as well as a multitude of wooden artefacts rammed down into the glacial till (S. A. Sørensen 2016), and quern stones were dragged from dryland into the wetland. The inhabitants frequenting the site, can be argued to have had *a foot in both worlds (Fig.8)*.

Zvelebil and Rowley-Conwy originally proposed three different stages to the Neolithic transition (1984). Recently, Gron and Sørensen, expanded and adjusted these to four phases- first a contact/scouting phase (from c. 6,400 BP), then an introduction phase (from c. 6,000 BP), followed by negotiation phase (c. 6,000–5,700 BP), and finally a homogenisation phase (after c. 5,700 BP) (Gron and Sørensen 2018). Syltholm fits well within Gron and Sørensens' negotiation phase, where local hunter-gatherers are in direct contact with the farming population. Whether Early Neolithic Syltholm represents a last hunter-gatherer stronghold comprising a relict hunter-gatherer population is impossible to tell. What we are able to show is that the indigenous hunter-gatherers may have played a significant role in the adaptation of agriculture in Southern Scandinavia.

7.1 Conclusions

The overarching aim of this thesis: to use minimally destructive state-of-the-art analytical techniques, to explore the Stone Age archaeologically has been achieved. In Chapter 3 it was shown that the collagen content of barbed bone points was well preserved. This enabled identification to genus or species taxonomic level of all 120 bone points analysed. In terms of the species identifications, a novel biomarker capable of distinguishing red deer from elk was discovered and validated. This missed cleaved peptide with the mass of 2216 m/z was proven to be specific for red deer and does indeed gets ionized by MALDI-TOF-MS, whereby it is useful for identifying red deer. Unfortunately, the corresponding miss-cleaved peptide for elk, could not be detected by MALDI-TOF-MS.

The well-preserved nature of the bone points, demonstrated by ZooMS, enabled low amounts of bone or bone powder to be used for AMS dating. An unexpected and key finding of this thesis was the discovery of a hiatus at c. 10.2 ka lasting nearly 600 years. This was discovered by merging the radiocarbon dates from the bone points with published and unpublished data. Interestingly, this hiatus coincides with the technological development of large-barbed bone points which replace fine-barbed bone points i.e. fine- barbed bone points belongs to the earliest part of the Preboreal/Early Boreal chronozones, whilst large-barbed bone points appear after the hiatus and span the Late Boreal/ Atlantic chronozones. The change in bone point technology is also synchronous with technological advances of lithic industries. Taken together these observations may indicate two different groups present in the Maglemose landscape or various degrees of vertical or horizontal transmission between the two. Frustratingly, it was not possible to build a robust typochronology, due to the large geographical area covered. However, creating typologies based on absolute dates of bone points spanning the study area may be possible in the future.

Two manuscripts were devoted to Neolithic objects, both found at Syltholm in Denmark. In Chapter 4, a rare broken finger ring was revealed to be of either antler or bone, using non-destructive MicroCT imaging. Since a direct AMS date of the object was not possible due to sampling limitations, 70 AMS dates was used to infer the activity at the site, placing the ring at the cusp of the Early Neolithic. ZooMS and LC-MS/MS sequencing on only 10 mg of bone revealed that the ring was made from red deer. A partial proteome was generated for the ring along with reference material from modern antler and bone, in an attempt to determine the skeletal element used to manufacture the ring. Although the plasma protein APOA1 was only found in the ring and the reference antler, and not in bone it was not possible to assign antler as the substrate with any confidence.

The other study based on Syltholm material showed the successful extraction and aDNA sequencing from a piece of chewed pitch. The amount of endogenous DNA retrieved was comparable to well preserved petrus bone, and shows the potential for this material as a new source of aDNA especially from periods where human remains are limited in number. A human genome was sequenced to an average depth of coverage of 2.3x. The individual was a female and likely had dark skin, dark hair and blue eyes. Furthermore, she showed genetic affinity to Western Hunter-Gatherers. A direct AMS date of the pitch places it at c. 200 years into the beginning of the Early Neolithic. This key finding has huge implications for our understanding of the Mesolithic to Neolithic transition as it categorically demonstrates that Mesolithic ancestry persisted into the Neolithic. Surprisingly, a partial oral microbiome was generated that clusters with modern saliva samples and further, reads mapping to mallard and hazelnut probably reflect food consumption.

8.1 Future Directions

There are still great opportunities to do biochemical analysis on organic material from the Stone Age of Denmark.

The first chapter in the present thesis, showed a pronounced hiatus during the Maglemose period. The hiatus effectively divides the Maglemose period into two technocomplexes, i.e.; one using fine-barbed bone points and percussion blade production, the other using large-barbed bone points and pressure blade production. Unfortunately, we do not have high-resolution environmental data, to show exactly what happened during this period. The period is seemingly gone, as most sediment cores assumed to cover the hiatus show marked signs of erosion (pers. comm. Morten Fischer Mortensen). Therefore, it would be of great interest if targeted high-resolution approaches were carried out in bogs or lakes, to capture this elusive period. By this means, it may be possible to ascertain the cause for the lack of the bone points in the archaeological record at around 10.2 ka. Was it an extreme lowering of water levels, was it a climate change that forced people and animals out, or none of those? Another issue is the fact that, nearly all research focused on the Maglemose, as of today, is still based on conventional dating made decades ago; therefore, modern AMS dates from several of the original locus sites would be invaluable to attaining a better chronology for this period.

In regard to Chapter 2. The project showed some promise of potentially discriminating antler from bone. It would therefore be interesting to sequence tryptic peptides from a greater number of cervid bones and antlers, to elucidate whether separation between the two substrates is possible.

Finally, the present author, and one of his supervisors (Dr. Hannes Schroeder), received a generous grant last year to continue the work on chewed birch pitch. It is intended that this work starts in early 2020. In addition to the extraction and sequencing of DNA, it is of great interest to develop an extraction-protocol whereby it is also possible to isolate proteins or peptides from birch pitch. By also being able to sequence proteins, it will be possible to further verify eukaryotic and prokaryotic taxa first determined by DNA sequencing. As for the bacteria, it is possible to quantify the relative abundance of said taxa with methods, such as: TMT (Tandem Mass Tag) labeling or LFQ (label free quantification). This could possibly be used to quantify commensal bacteria, and to investigate the evolution of virulence factors. For example, we know that the female who chewed the birch pitch had *Streptococcus pneumoniae* as part of her oral flora, a species that while commensal, has pathogenic potential, but not whether it was pathogenic at the time it was trapped in the pitch. With sequenced peptides, it will also be possible to verify whether; for example, the mallard DNA is derived from meat or eggs. Intake of milk can be confirmed by protein sequencing, since milk consists primarily of the protein casein.

Access to chewing gum from periods older than the early Neolithic period has already been secured, so now a new combined 'omics' protocol has to be established. Here, pieces with tooth with imprints from the early Mesolithic sites Barmosen I and Dværgebakke at Bølling Sø will be examined. These sites represent a period when human bones are largely absent (the only exceptions are Koelbjerg and the cremation grave at Hammelev). In addition, pieces from Rönneholm's Mosse as well as Ringsjöholm in Scania will hopefully be included. These two sites date to early Kongemose culture. With the bone point hiatus in mind, it would be exciting to explore whether there is genetic continuity or not between the hunters who used fine-barbed bone points and percussion techniques, to those who made barbed bone points and pressure blade techniques. Was it two genetically different groups, or was it local development?

Lastly, when several individuals have been sequenced, it may be possible to imply kinship. The two chewing gums from Barmosen I are, on the basis of the dental impressions, inferred to have been chewed by two different individuals aged 7-8 years and 11 years respectively (Johansson 1990). It would be a beautiful story if the two individuals were siblings who, 10,300 years ago, stayed at the Barmosen site.

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Other published works during the PhD.

The following section comprises published work during the three-year research period. The publications are listed in chronological order.

Article published in Oceans of Archaeology

Jensen, TZT and Fischer, A (2018) Gifts from the sea – inland indications of early prehistoric activity at now submerged coasts. In: Fischer, A. and Pedersen, L. (Eds). Oceans of Archaeology. 101. Jysk Arkæologisk Selskabs Skrifter. pp.30–41.

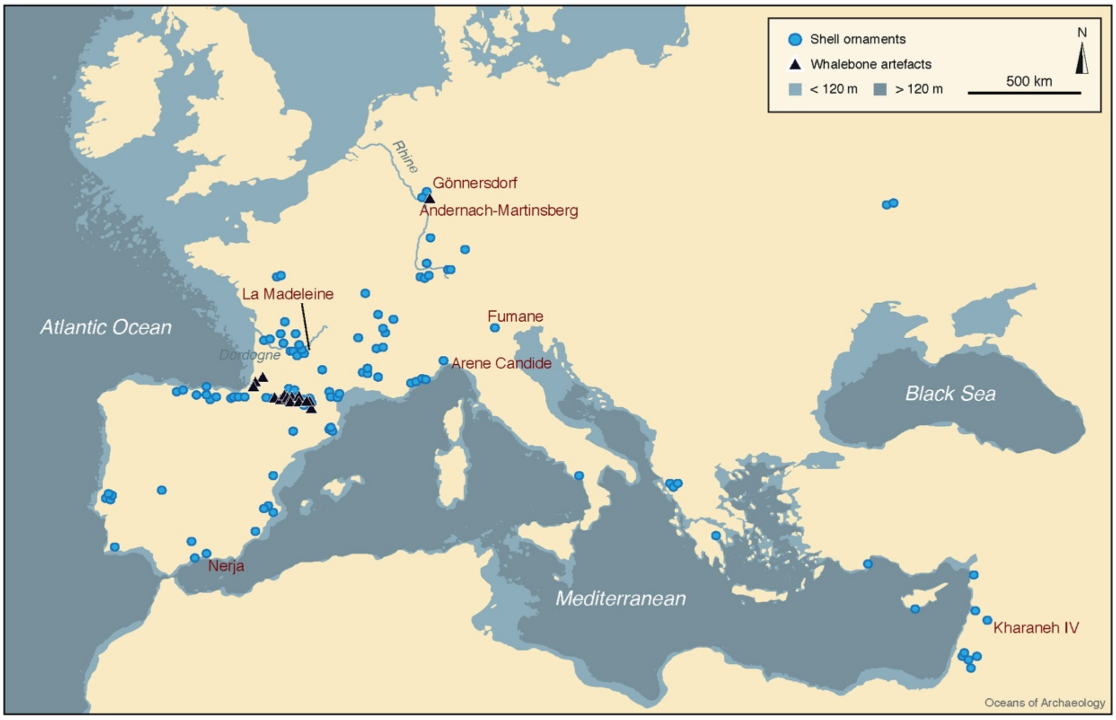

Fig. 1. Exports from sunken coasts: find spots with c. 45,000-12,000 year old shell ornaments and weapons made from bones of marine animals. These items come from the contemporaneous sea and bear witness to human coastal activity during periods when the land surface in western and southern Europe extended to include the pale blue areas of the map. Two major rivers are indicated as possible transport corridors. Illustration credits at the end of the chapter (cf. Ch. 6.2).

2.2 Gifts from the sea – inland indications of early prehistoric activity at now submerged coasts

Theis Zetner Trolle Jensen and Anders Fischer

Abstract

There are numerous records from inland sites in Europe and the Levant of early prehistoric ornaments and hunting weapons made from shells and bones of marine animals. Some have been found as much as 2000 km from the present-day coast. Many cluster along navigable rivers, perhaps implying a capacity for water travel at that time. These inland finds indicate extensive human activity along the now submerged beaches of the Atlantic, the Mediterranean and the Black Sea during the last c. 400,000 years of the Stone Age. Remarkably, the latest ice age, not least the Last Glacial Maximum between c. 30,000 and 15,000 years ago, is heavily represented. This was a time when the sea level was as much as c. 130 m lower than that of today and from which we, as yet, have no direct evidence of coastal settlement.

Ephemeral shores

Archaeologists and anthropologists have for decades published their thoughts on the significance of coasts in early human evolution (e.g. Sauer 1962; Bailey & Parkington 1988; Cleyet-Merle & Madelaine 1995). But archaeology has not been able to test these hypotheses against a diverse record from early prehistoric sites located in the immediate vicinity of contemporaneous sea shores. This evidence has, by and large, disappeared beneath the waves and is now located so far from land (Fig. 1), and at such great depths, that it has, until very recently, been out of practical reach for archaeologists (Fig. 2; cf. Chs. 1.2 and 5.1).

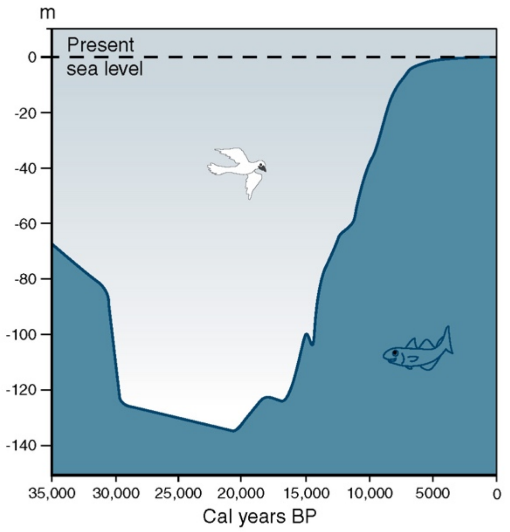

Fig. 2. Sea-level change over the last 35,000 years. For most of the time, the water level was significantly lower than now. Finds from sites above present sea level presented in this chapter (cf. Chs. 2.1 and 2.3) indicate that humans were active along the coasts throughout this period, which implies that we can expect traces of habitation to be present as deep as c. 130 m below current sea level.

Fig. 3. Skeleton of a young man buried around 28,000 years ago in the Arene Candide cave in northwest Italy. Several hundred beads lie around his skull, most of which are made from the shells of marine mollusc (i.e. Cyclope neritea *and* Cypraea *sp.). Archaeologists have given him the name 'The Prince', due to the grandeur of his grave goods (Cardini 1942). The isotope composition of his skeleton suggests that 20-25% of his protein intake came from the sea (Pettitt et al. 2003), the surface of which, at that time, was located more than 100 m below its present level.*

However, an extensive record from archaeological localities on land, as much as 2000 km from the contemporaneous coast, indirectly illuminates the subject (e.g. Fig. 1). It bears witness to human activity along coastlines that now lie deeply submerged beneath the surface of the sea. The inland sites, which provide the basis for such a conclusion, date mostly from the era of modern humans (*Homo sapiens sapiens*), but some also extend further back in time. The finds from these sites show that people exploited the sea for food and that shells of marine molluscs were widely used for symbolic purposes.

During the coldest period of the last ice age (about 30,000 to 15,000 years ago), when the sea level was between c. 100 and 130 m below that of today (Fig. 2) (Lambeck et al. 2014), Europe's coasts were the point of departure for extensive exports of objects such as exquisite sea shells and large spear heads made of whalebone, some of which reached the very heart of the continent (Fig. 1). No detailed and complete database exists with respect to these finds, even though the subject has been touched upon several times in recent decades (e.g. Bailey & Parkington 1988; Fischer 1995; Bicho et al. 2011). The present chapter should be seen primarily as an appetiser.

Kitchen middens and isotopes reveal consumption of seafood

Archaeologists have long been aware that marine food sources were exploited during various early prehistoric periods, although the degree of dependency on these resources has proved difficult to establish. Unequivocal evidence is provided by the numerous accumulations of marine mollusc shells ('kitchen middens') found along prehistoric coasts across much of the world (Erlandson 2001). In Europe, the phenomenon is widespread and has been particularly well researched along the Atlantic façade (Fischer & Kristiansen 2002; Milner et al. 2007; Gutiérrez-Zugasti et al. 2011). Kitchen middens here mostly date from the period 7000-5000 cal BP, by which time millennia of postglacial sea-level rise had come to a halt (Fig. 2). As a consequence, traces of coastal settlement from this period are generally located above present sea level, easily accessible to terrestrial archaeologists.

On some stretches of coast, where deep water extends in close to the present-day shore, kitchen midden deposits of earlier date have been encountered in coastal caves. For example, in northwest Italy and in the Iberian Peninsula, there are habitation layers in caves containing variable quantities of marine mollusc shells that are as much as 35,000 years old (Kuhn & Stiner 1998; Stiner 1999; Gutiérrez-Zugasti et al. 2011; Douka & Spinapolice 2012).

Another direct kind of evidence showing that people subsisted on food of marine origin is provided by the isotope composition ($\delta^{13}C$ and $\delta^{15}N$) of human bone collagen (Chs. 3.2 and 6.1). Once again, the period c. 7000-5000 year ago is most comprehensively represented (e.g. Lubell et al. 1994; Richards & Hedges 1999; Schulting & Richards 2001, 2002; Fischer et al. 2007; Schulting 2011). However, secure isotope-based evidence of substantial consumption of marine food extends more than 9000 years back in time, and possibly as much as 28.000 years (Fig. 3). Other indisputable traces of human links with the sea extend back considerably further, as will appear from the following.

Modern humans – exports of marine shells to the interior

Humans closely related to the present population of the continent began to migrate into Europe about 45,000 years ago (Higham et al. 2011, 2014), and from this point onwards thousands of ornaments made from marine mollusc shells appear in the terrestrial archaeological record. The following examples merely represent a small selection of this geographically very widespread phenomenon. They are taken from the Levant and Europe and are mentioned in chronological order.

The Fumane cave in Italy has been occupied by modern humans since about 35,000 years ago. They left behind more than 700 marine mollusc shells representing over 70 different species. Remarkably, all of these were gathered on the contemporaneous coast, located several hundred kilometres away (Fig. 1). The majority are from the colourful marine snail red dwarf turban (*Homalopoma sanguineum* and *Cyclope neritea*) (Gurioli et al. 2003; Bertola et al. 2013).

Another piece of indirect evidence relating to the coastal activities of ice age humans comes from the 20,000 year old inland locality of Kharaneh IV in Jordan, where two burnt-down huts have been excavated. More than a thousand marine mollusc shells were deposited on top of the ashes and red ochre was scattered at the site – apparently in a symbolic act (Fig. 4). The shells originate from the Mediterranean and the Red Sea, respectively c. 130 and 270 km distant (Richter et al. 2011; Maher et al. 2012).

Just as remarkable is the ice age locality of La Madeleine in inland France – another cave site with traces of human activity during the last ice

Fig. 4. Marine mollusc shells with drilled perforations – evidence of ritual activities at the settlement of Kharaneh IV in Jordan. The shells were gathered on the coast at a time when the global sea level was around 130 m lower than that of today.

age (Ch. 6.1). Here, the skeleton of a child of about five years of age was excavated. The youngster had been buried with no less than 1557 mollusc shells (Fig. 5) (Capitan & Peyrony 1928). Of these, 1314 were of the long, conical tusk shell (*Dentalium* sp.). Isotope analyses of the shells indicate that they were gathered along the Atlantic coast of the time, located more than 150 km away (Vanhaeren et al. 2004) – and the journey would have been even longer if made by boat along the Dordogne river and its tributaries.

The fourth and final example is a rather macabre find from Große Ofnet-Höhle in southern Germany. Here, archaeologists found 34 human skulls, buried in two pits (Fig. 6). The skulls are c. 8000 years old and show signs of having been severed from the body. Around and on top of them lay c. 4250 snail shells, which possibly formed part of conspicuous head ornaments. Most of the shells originate from freshwater habitats and fossil outcrops. But, five examples of the lustrous brown and white dove shell (*Columbella rustica*) are from the contemporaneous Mediterranean, i.e. way behind the Alpine Mountains and at least 500 km distant as the crow flies (Schmidt 1912; Hedges et al. 1989; Eriksen 2002; Orschiedt 2005).

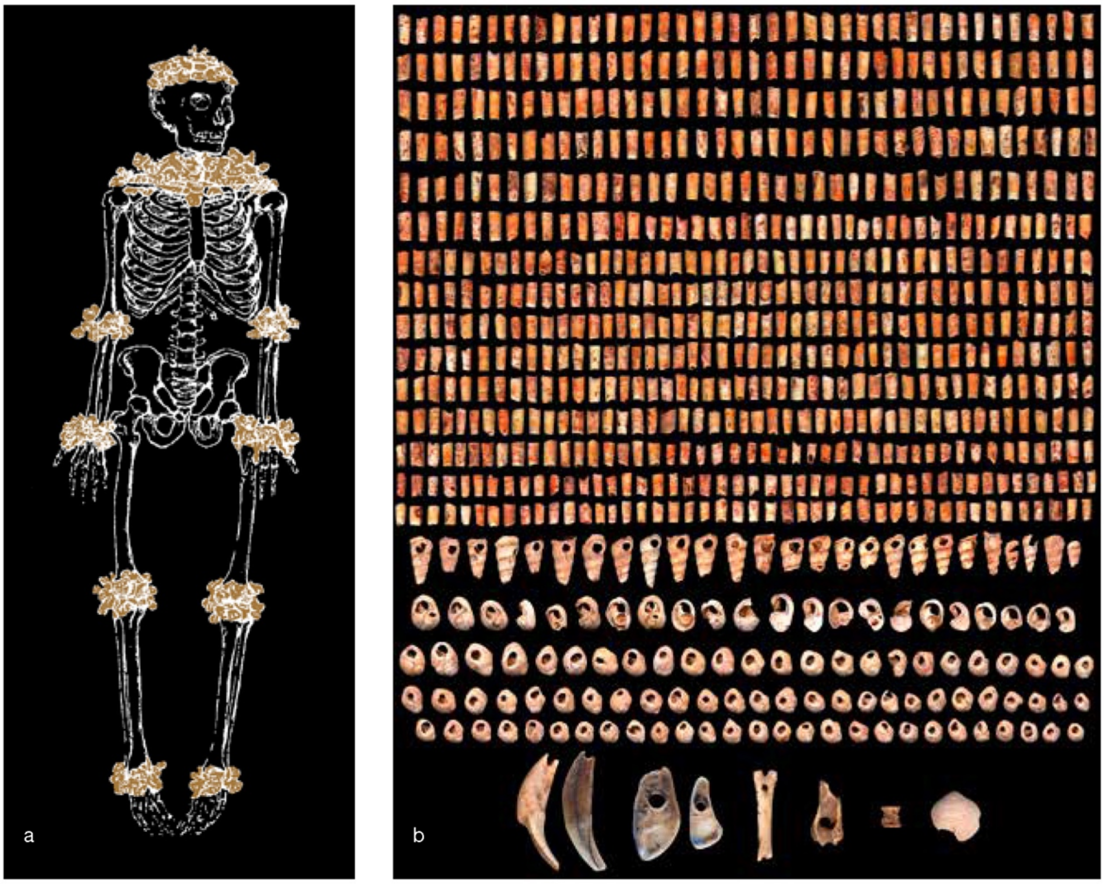

Fig. 5. Ice age grave in La Madeleine cave in France (a) and a selection of the numerous beads made of marine shells etc. that surrounded the child skeleton. The beads were probably sewn on to the young individual's clothing as decoration.

Fig. 6. Head burials at Groβe Ofnet-Höhle in Germany. Together with the 34 human skulls were large numbers of snail shells, including five marine examples imported from the Mediterranean coast, which was c. 500 km away and at that time had its surface about 15 m below today's level.

The attractive shells of marine invertebrates continued to have aesthetic and/or symbolic importance for several millennia after the ice age. A classic example is provided by ornaments or amulets made from the relatively large shells of the thorny oyster (*Spondylus gaederopus*). From 8500 to 4000 years ago, these shells were distributed from their place of origin in the Aegean Sea via a system of gift-giving (Shackleton & Renfrew 1970; Windler 2013), in which the Danube river possibly played a role as a transport corridor (Fig. 7). Even though countless of them must have disintegrated over time, impressive numbers have been recorded at localities across the Balkans and into central and western Europe – around 2000 km from their place of origin. This suggests a considerable level of human activity along the shores of the Aegean Sea at the time. Apart from areas with major tectonic activity, the coastline of this period is now located as much as 20 m below present sea level (Pirazzoli 1991; Lambeck & Bard 2000; Lambeck et al. 2004; Brückner et al. 2010). Traces of early prehistoric coastal activity associated with the collection of the raw material for this pan-European gift-exchange network are therefore largely to be found on the seabed.

Exports of whalebone tools to the interior

The testimony provided by marine mollusc shells is supported by a further group of finds: tools made of whalebone. Some have been found so far from the sea that it must be seen as impossible for these animals to have swum to the vicinity of the places where these artefacts were discovered (Fig. 1). A foreshaft for a harpoon or a spear made of whalebone was found at Andernach-Martinsberg, next to the Rhine in Germany. This demonstrates contact with the sea about 15,000 years ago, when the distance to the nearest marine shore was c. 1000 km, and considerably greater if the journey was undertaken by boat on the river (Fig. 1). The geographical clustering of finds of contemporaneous shell ornaments along major European rivers can be taken as an indication of the existence of this form of transport, even though remains of boats of such early date have yet to be found (Ch. 2.3). Long-distance contacts are further underlined by the discovery of a necklace made from the shells of marine molluscs at the Andernach-Martinsberg site (Langley & Street 2013).

Fig. 7. Distribution of sites with ornaments made of c. 7500-7000 year old Spondylus *shells, bearing witness to an extensive pan-European gift-giving network. Its point of departure is to be found along the contemporaneous coasts of the Aegean Sea, which now lie below present sea level.*

There are numerous records of similar whalebone points from approximately coeval inland sites across southern France (Pétillon 2013) (Fig. 1), demonstrating the existence of a network of contacts linking the continental interior and coastal areas of southwest Europe at this time, when the sea level was around 100 m lower than at present (Lambeck et al. 2014).

Indirect evidence that humans living in the vicinity of the ice age coasts actually exploited whales comes from c. 14,000 year old stowaways discovered at a settlement in the Nerja cave in Spain. Remains were found here of whale barnacles (*Tubicinella major* and *Cetopirus complanatus*) – small crustaceans that live most of their lives attached to whales. Presumably the huge host animals were stranded on the beach, where people subsequently removed the meat and took it back to the cave (Álvarez-Fernández et al. 2013).

The marine fauna in ice age art

A final source of indirect evidence of human activities along now submerged early prehistoric coasts is figurative art, in the form of cave paintings, rock carvings and decorated portable artefacts, dating from the end of the last ice age (dividing sheet for Part 2) (e.g. Clottes et al. 1992; Serangeli 2001; Bosinski & Bosinski 2009). All three categories include motifs, which show that the artists were familiar with the appearance and behaviour of the marine fauna (Fig. 8; cf. Ch. 5.2.2).

At the aforementioned Andernach-Martinsberg locality, fragments were found of a piece of slate engraved with a naturalistic image of a seal (Bosinski & Bosinski 2009). Twelve similar depictions of seals – together with a number of marine mollusc shells – were found at the approximately coeval locality of Gönnersdorf, only 2 km from Andernach-Martinsberg (Fig. 8) (Bosinski & Bosinski 2009; Street et al. 2012). At both localities the engravings were on slate that appears to have been obtained from the nearby river (Stapert & Terberger 1989). The obvious question then is whether these artists could have spent time on the coast and, during their stay there, learned to depict seals in this exceptionally realistic way (cf. Langley & Street 2013).

Early humans were also active at the coast

One of Europe's earliest indications of human activity by the sea is from a c. 400,000 year old site located above sea level at Terra Amata in France, reported more recently from Gorham's Cave and Vanguard Cave (Fa 2008; Stringer et al. 2008).

In addition, there are several localities dating from the period c. 130,000-115,000 BP, when the sea had approximately the same level as today, and for a while even exceeded the current level by up to 4 m (Ch. 1.2 Fig. 2) (Kopp et al. 2013; Dahl-Jensen et al. 2013). These sites, in Belgium, Italy, France, Spain, Portugal and Greece, lie immediately above present sea level (e.g. Coutard et al. 2005; Monnier 2006; Douka & Spinapolice 2012; Harvati et al. 2013) and suggest extensive activity at the coast at the time of Neanderthal occupation (cf. Benito et al. 2016).

Intense activity along the now submerged coast

Both modern humans and their predecessors were clearly active along the seashore. This is well illustrated by direct records from near-coastal Stone Age localities from periods when the sea level was approximately the same as today (c. 130,000-115,000 and 7000-5000 years ago, respectively) (Ch. 1.2 Fig. 2).

Much indirect evidence available from inland sites shows that similar close links between people and the coast also existed during early prehistoric periods when the sea level was considerably lower than at present. Archaeological sites on land therefore hold the promise that extensive traces of early coastal settlement are to be found on the shelf of the European continent and associated islands at depths as much as 130 m below present sea level.

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Fig. 1: Concept: Theis Z.T. Jensen and Anders Fischer. Data from Álvarez-Fernández 2002, 2010 and 2012, Benazzi et al. 2011), Colonese et al. 2011, Eriksen 2002, Langley & Street 2013, Maher et al. 2012, Peresani et al. 2013, Pétillon 2013, Rodríguez-Hidalgo et al. 2010. Cf. Ch. 6.2.
Fig. 2: Drawn on the basis of Lambeck et al. 2014. Concept: Anders Fischer.
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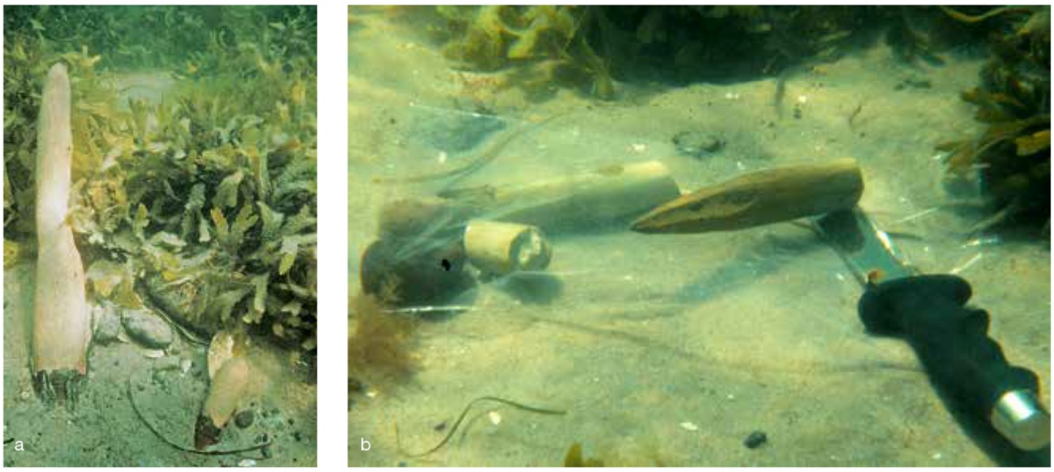

Fig. 1. How old are these two hazel rods projecting from the seabed (a)? At a first glance they had a recent appearance with a pale colour and fresh-looking bark, still with a green interior. But when dug out of the seabed, their lower ends showed cut marks (b), apparently made with a flint axe, which indicated a much older origin. A radiocarbon date answered the question: they are from c. 4900 BP. This result, and the site's remarkable extent and density of wooden remains, led to further investigations – and the undertaking of several further radiocarbon analyses (see below and Ch. 3.2.2). The Nekselø site, Denmark. Photos: Anders Fischer 1990.

6.1 Radiocarbon dates for submarine and maritime finds from early prehistory

Anders Fischer and Theis Zetner Trolle Jensen

Abstract

The Oceans of Archaeology volume makes intensive use of radiocarbon dates from sites in Europe and the Levant. The present chapter presents these ages and provides information and comments suitable for their future use and critical evaluation. The dates are given in radiocarbon years and as calibrated calendar ages. It is estimated that several of the ages for Palaeolithic and Mesolithic human skeletal remains are subject to a marine or freshwater reservoir effect, giving results that are up to several hundred years too old. This is based on stable isotope values (δ13C and δ15N) for the dated bones. Radiocarbon ages that may be misleading due to a high biological sample age or contamination from preservative substances are indicated. Most of the radiocarbon dates are for material recovered from sites located on the seabed, but there are also some finds relating to early prehistoric coastal activity and watercraft technology that have been found above present-day sea level.

Radiocarbon dates

Absolute dates form the chronological backbone of the *Oceans of Archaeology* volume. In most cases, these are based on radiocarbon assays (^{14}C measurements) performed on samples of wood or bone collagen. In the present chapter, dates are given as conventional radiocarbon ages in ^{14}C years before present (AD 1950) including the statistical uncertainty – 1 σ standard deviation. Additionally, they are listed in calibrated calendar years before present ('cal BP'; BP = Before Present = before AD 1950). The calibration is based on the IntCal13 curve, OxCal version 4.2.4 (Ramsey 2009), and the dates are listed in two ways: first as the 95.4% confidence intervals (2 σ standard deviation), secondly as single digit values representing the statistically most probable age, i.e. the median probability age in calendar years before present, rounded to the nearest hundred (Fig. 2).

Table 1 presents data from early prehistoric submarine sites in Europe and Israel mentioned in the book. They include all human skeletons for which a radiocarbon date and/or a stable isotope measurement are available. The table also lists several dates for sea shells etc. from European and Jordanian sites on land. They were imported from now inundated sea-shores and are fundamental to the discussions on early prehistoric coastal activity and navigation presented in Chapters 2.2 and 2.3.

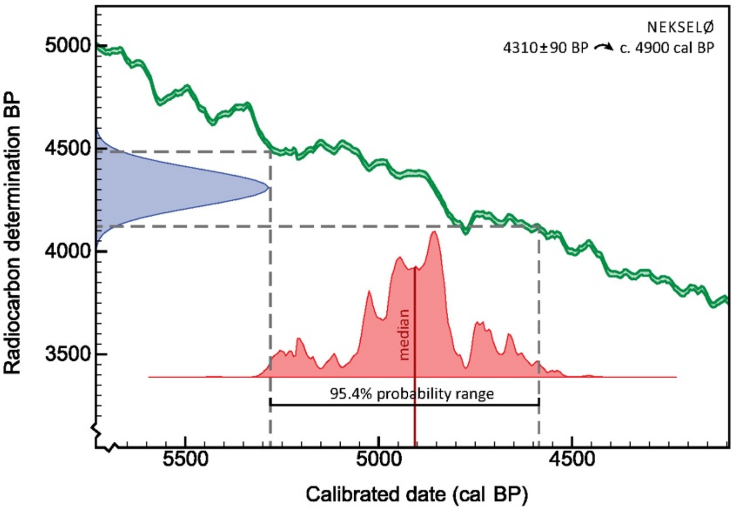

Fig. 2. Calibrating the date 4310±90 ^{14}C years BP from the Nekselø fish-weir complex (Fig. 1). Its probability distribution in radiocarbon years and in calendar years is shown in blue and red, respectively. In the table below, the calibrated date is presented in two ways: as the 95.4% probability range (5280–4586 cal BP) and as the statistically most probable age (the median value = 4905 cal BP), rounded to the nearest hundred (i.e. 4900). The green wiggled line is the calibration curve, based on radiocarbon measurements of tree rings of known absolute age. Graphics based on OxCal 4.2.4.

Aquatic food and the reservoir effect

In some cases, radiocarbon dates do not represent the true ages of the samples analysed. This occurs for instance when a date is obtained for a bone from a species of aquatic animal or from a human who has consumed aquatic food. In such instances, the measured age may be up to several hundred years older than that given by a radiocarbon date for contemporaneous terrestrial material. The chronological displacement derives from marine and freshwater food chains, whereby these organisms have obtained some of their carbon from sources other than from the contemporaneous atmosphere – for instance the deep ocean or fresh water containing fossil carbon dissolved from marine limestone (e.g. Olsson 1996; Olsen et al. 2009).

There are examples of radiocarbon ages for early prehistoric skeletons from lake and riverine environments in Europe that are as much as 800 years older than ages obtained for associated grave goods made from bones of terrestrial mammals (e.g. Lanting & van der Plicht 1998; Bonsall et al. 2004; Olsen et al. 2010; Wood et al. 2013). Dates for bones of Mesolithic humans who acquired most of their diet from the marine environment will typically be as much as 400 years too old (Stuiver et al. 1986), while ages obtained for bones of maritime-adapted Palaeolithic humans may, in some cases, show even greater displacement (cf. Butzin et al. 2005; Ascough et al. 2005).

Stable isotope values (δ^{13}C and δ^{15}N) for human bones (cf. Fig. 3) are frequently used as indicators of the reservoir effect (e.g. Arneborg et al. 1999; Cook et al. 2001; cf. Wood et al. 2013). For example, it can be concluded that the Mesolithic humans listed below from the sites of Hummervikholmen in Norway (δ^{13}C ~ -13‰) obtained a very high proportion of their protein diet from marine species (cf. Fischer et al. 2007b). Conversely, a human whose remains were recovered from the southern North Sea, offshore from the Netherlands (δ^{13}C -24.1‰), clearly acquired a very high proportion of his or her diet from freshwater species. Consequently, radiocarbon dates obtained for these individuals are subject to a significant influence from the reservoir effect.

Practical information and data evaluation

Table 1 provides information that can aid in identifying the relevant dating laboratory, dating method and museum collection for each individual date.

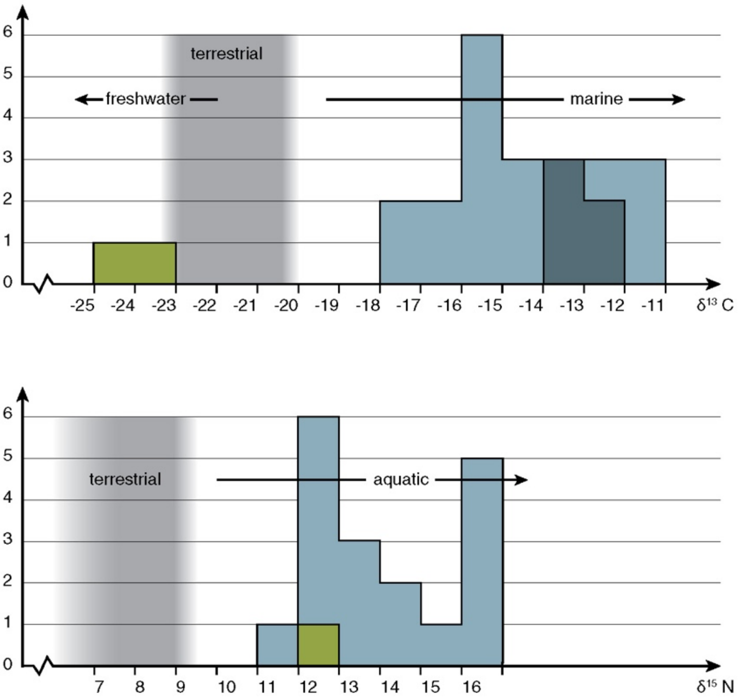

Fig. 3. Marine and freshwater food formed a significant part of the diet of inhabitants of northern European landscapes that are now submerged. This is indicated by stable isotope values for their bones. Consequently, due to reservoir effects associated with aquatic food, radiocarbon dates for these bones may be several hundred years too old. The Mesolithic individuals found in the Norwegian (dark blue blocks) and Danish (light blue blocks) sectors, lived in coastal environments, and their $\delta^{13}C$ *values indicate a diet rich in marine food. Moreover, their relatively high* $\delta^{15}N$ *values reveal a preference for high trophic level species – large fish or marine mammals. On the other hand, the two Early Mesolithic individuals from the Dutch sector (green blocks), who inhabited a riverine lowland, have* $\delta^{13}C$ *values indicative of a dependence on freshwater food. The grey zones indicate the stable isotope values that would theoretically be seen in humans who acquired all their protein from Mesolithic terrestrial mammals in Denmark (cf. Fischer et al. 2007b).*

Most have been obtained via the AMS method, which only requires relatively small samples (e.g. Figs. 4-5). Where the information is available, the table also provides the depths from which underwater finds were recovered, measured in relation to present-day average sea level. This information is considered important, not least in relation to studies of sea-level change. Table 1 also provides information on the nature of the material dated, whether charcoal, bone etc.

In most cases the biological ages of the samples are negligible when compared to the statistical uncertainty of the dates. Charcoal can be an exception when it results from the burning of 'old wood', for example driftwood from arctic or subarctic environments, where such material can survive for hundreds of years (cf. Vilhjálmsson 1991; Dyke & Savelle 2000). Similar problems are encountered with ages obtained for the inner heartwood of long-lived trees such as pine, oak and olive (Fig. 7). An example of the latter may exist within the group of dates from the Cosquer Cave, which appear mainly to have been obtained for remains of pine (*Pinus sylvestris*) (Clottes et al. 2005, 185-187). In addition, there is a possibility at this site that the artists who painted the walls reused ancient charcoal (perhaps thousands of years old) they picked up from the cave floor.

Contamination of samples is a critical factor that should also be considered. It may result from the use of preservative materials in archaeological conservation and it can also occur due to a natural influx of carbon from plant rootlets, humic acids etc. A potential problem of this kind has been identified in the present collection of dates pertaining to a piece of a worked antler found in Husum Harbour, Germany. Based on its shape and partially preserved natural surface, the object has long been assumed to derive from reindeer (*Rangifer tarandus*) and was consequently considered to be of Late Palaeolithic date (see references in Fletcher 2015). However, reindeer disappeared

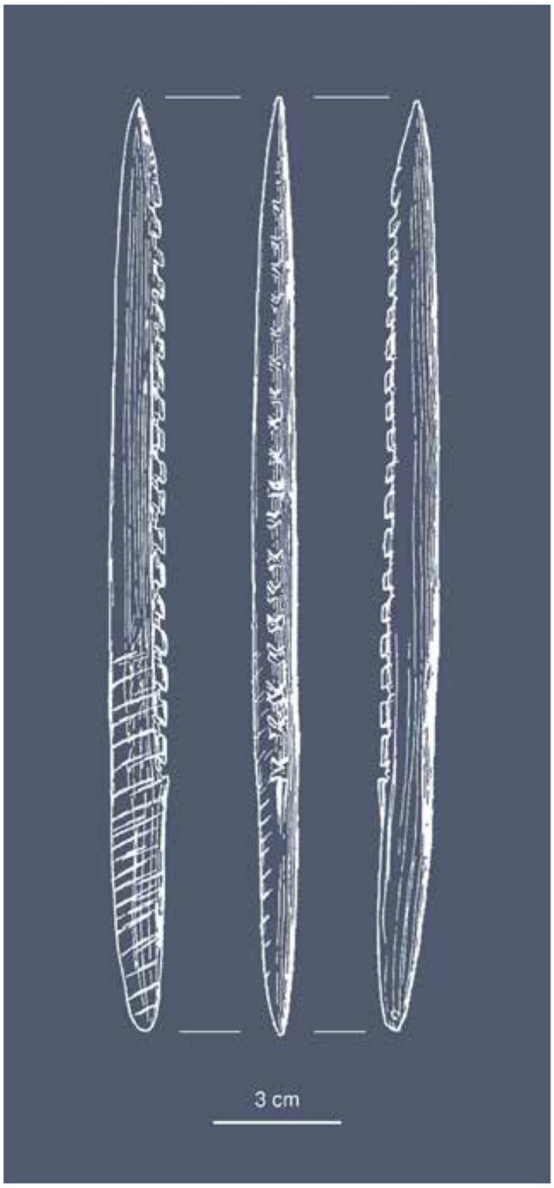

Fig. 4. Barbed antler point from the Leman and Ower Banks, dated to c. 13600 cal BP. When brought to the surface by an English trawler in 1931, it was embedded in a lump of peat, which indicates that it was originally lost while fishing in a now inundated wetland. Reproduced from Clark and Godwin 1957. © The Prehistoric Society; published by Cambridge University Press, reproduced with permission.

from the local fauna several millennia before the date of c. 6000 years BP that resulted from a radiocarbon analysis of the specimen (cf. Aaris-Sørensen 2009). It should therefore be considered whether the date represents a mixture of carbon from a > 10,000 year old antler and (more) recent preservative material (cf. the example of the reindeer antler artefact from Nørre Lyngby, presented in Fischer et al. 2013). The preservation material applied to the Husum artefact may include waterglass (sodium silicate) and linseed oil, which were favoured substances for such purposes in the 19th century, when the item was found (in 1881).

However, the dating laboratory has put much effort into removing the preservative material from the sample (personal communication Sönke Hartz, Archäologisches Landesmuseum, Schloss Gottorp, June 2014 and October 2015). An alternative explanation is that the species identification was incorrect and the antler is of a red deer (*Cervus elaphus*). This conclusion is supported by the fact that the artefact was found together with other cultural material which, from a typological point of view, would fit perfectly with the radiocarbon date of c. 6000 cal BP, when red deer was a common species in the local fauna. But it may also be that both the radiocarbon date and the species identification are correct, in which case this find would represent an archaeologically very important example of an import object from the extreme north of Europe, where reindeer were present at that time.

Fig. 5. Drowned pine trees, found 5 km off Læsø, Denmark. They stood in their original growing positions, exposed by water movements that had removed sand and peat which previously covered and protected them. Photo: Jan Hammer Larsen, Coastal Museum of Northern Jutland 2012.

*Fig. 6. Exposure to seawater initiated a gradual process of biological degradation of the tree stumps off Læsø (Fig. 5). Numerous burrows of the shipworm (*Teredo navalis*) practically hollowed out the interior of one of them (a). A radiocarbon date for another stump (b) revealed an age of c.10.400 BP, making it the oldest rooted tree stump yet found on the Danish seabed. About 35 tree rings were evident in the interior of the latter stump. The outer rings were unusually narrow – probably reflecting the gradual flooding of its root system as the sea level rose. Photos: Jan Hammer Larsen, Coastal Museum of Northern Jutland (a), and Anders Fischer (b).*

Table 1. Radiocarbon dates and associated practical information of importance for their further use.

Site name	Material dated	Age in ^{14}C years BP	Most probable calendar age BP
Dates from on-land sites relating to now submerged landscapes			
Arene Candide, Italy	Bone, Human (*Homo sapiens*)	23440±190	27600
Cap Blanc, France	Bone, human (*Homo sapiens*)		
Duruthy, France	Bone, human (*Homo sapiens*)		
Cingle del Mas Nou, Spain	Bone, human (*Homo sapiens*)	6925±35	7800
Cingle del Mas Nou, Spain	Bone, Human (*Homo sapiens*)	6897±34	7700
Coves de Santa Maria, Spain	Bone, human (*Homo sapiens*)	8690±50	9600
Coves de Santa Maria, Spain	Bone, human (*Homo sapiens*)	8283±37	9300
Coves de Santa Maria, Spain	Bone, human (*Homo sapiens*)		
Coves de Santa Maria, Spain	Bone, human (*Homo sapiens*)		
Fumane, Italy	Charcoal	42750±700	46100
Fumane, Italy	Charcoal	42000±750	45400
Fumane, Italy	Charcoal	38950±550	42900
Fumane, Italy	Charcoal	38550±550	42600
Fumane, Italy	Charcoal	36450±400	41100
Fumane, Italy	Charcoal	33000±400	37200
Große-Ofnet Höhle, Germany	Cranial bone, human (*Homo sapiens*)	7560±110	8400
Große-Ofnet Höhle, Germany	Cranial bone, human (*Homo sapiens*)	7520±20	8300
Große-Ofnet Höhle, Germany	Cranial bone, human (*Homo sapiens*)	7480±80	8300
Große-Ofnet Höhle, Germany	Cranial bone, human (*Homo sapiens*)	7450±80	8200
Große-Ofnet Höhle, Germany	Cranial bone, human (*Homo sapiens*)	7360±80	8200
Huseby Klev, Sweden	Bone, human (*Homo sapiens*)	9105±100	10300
Huseby Klev, Sweden	Bone, human (*Homo sapiens*)	9040±80	10200
Huseby Klev, Sweden	Bone, human (*Homo sapiens*)	8965±75	10100
Kharaneh IV, Jordan	Charcoal (Chenopodiaceae)	15890±90	19200
Kharaneh IV, Jordan	Charcoal (Dicotyledon)	15770±80	19000
Kendrick's Cave, Wales	Bone, human (*Homo sapiens*)	12090±90	13900
Kendrick's Cave, Wales	Bone, human (*Homo sapiens*)	11930±90	13800
Kendrick's Cave, Wales	Bone, human (*Homo sapiens*)	11880±90	13700
Kendrick's Cave, Wales	Bone, human (*Homo sapiens*)	11760±90	13600
La Madeleine, France	Cranial bone, human (*Homo sapiens*)	10190±100	11900
La Rochette 1, France	Bone, human (*Homo sapiens*)	23630±130	27700
Riparo Mochi, Italy	Charcoal	36350±260	41000
Riparo Mochi, Italy	Shell (*Trochus* sp.)	36320±270	41000
Riparo Mochi, Italy	Shell (*Cyclope neritea*)	26140±110	30500
Riparo Mochi, Italy	Shell (*Cyclope neritea*)	24600±100	28600
Stora Bjers, Sweden	Bone, human (*Homo sapiens*)	7970±80	8800
Stora Förvar, Sweden	Bone, human (*Homo sapiens*)	7830±90	8600
Dates from on-land sites relating to early prehistoric navigation			
Hotiza, Slovenia	Oak wood (*Quercus*), log boat	7325±70	8100
Husum Harbour, Germany	Antler, reindeer (*Rangifer tarandus*) or perhaps red deer (*Cervus elaphus*); worked; saturated in preservative material	5262±31	6000
Lago di Bracciano, Italy	Wooden pole near stern of in situ log boat	6565±64	7500

Calendar age BP (2 σ interval)	δ^{13}C (‰)	δ^{15}N (‰)	Depth below present sea level (m)	Dating lab. no.	Museum inventory no. etc.	Chapter reference	Literature reference
27899–27338	-17.6	12.4		OxA-10700		2.2	Pettitt et al. 2003
c. 12.000	-18.8					2.2	Hayden et al. 1987
c. 11.150	-19.4					2.2	Hayden et al. 1987
7835–7680	-17.5	9.5		OxA-V-2360-29	S-EVA 9089	2.2	Salazar-García et al. 2014
7797–7666	-18.4	7.9		OxA-V-2360-28	S-EVA 9084	2.2	Salazar-García et al. 2014
9705–9540				Beta 244010		2.2	Salazar-García et al. 2014
9415–9137	-17.8	9.9		OxA-V-2360-26	S-EVA 9051	2.2	Salazar-García et al. 2014
	-18.1	9.4			S-EVA 7691	2.2	Salazar-García et al. 2014
	-18.0	8.8			S-EVA 7692	2.2	Salazar-García et al. 2014
47683–44821				LTL-376A	A9	2.2	Peresani et al. 2008
46985–44082				LTL-378A	A11- nn	2.2	Peresani et al. 2008
43835–42109				OxA-572A	A9 - 31-41-51	2.2	Peresani et al. 2008
43436–41856				LTL-574A	A9 - 70b	2.2	Peresani et al. 2008
41813–40258				LTL-573A	A9I - 86	2.2	Peresani et al. 2008
38346–36223				OxA-8021	A4II - 95	2.2	Peresani et al. 2008
8589–8170				OxA-1575	Skull 32	2.2	Hedges et al. 1989
8506–8172				OxA-1573	Skull 3	2.2	Hedges et al. 1989
8425–8065				OxA-1574	Skull 34	2.2	Hedges et al. 1989
8408–8051				OxA-1572	Skull 10	2.2	Hedges et al. 1989
8347–8014				OxA-1571	Skull 8	2.2	Hedges et al. 1989
10562–9933	-15.6			Ua-6411		2.2	Ahlström 2003
10409–9914	-15.2			Ua-6410		2.2	Ahlström 2003
10249–9792	-15.6			Ua-6407			Ahlström 2003
19445–18926				OxA-22273	Above structure 1's floor	2.2	Maher et al. 2012
19234–18333				OxA-22274	Below structure 1's floor	2.2	Maher et al. 2012
14172–13754	-17.7	13.9		OxA-6114	60	2.2	Richards et al. 2005
14023–13556	-18.0	13.4		OxA-7004	59	2.2	Richards et al. 2005
13962–13483	-17.9	13.8		OxA-7003	57	2.2	Richards et al. 2005
13765–13431	-18.1	13.7		OxA-7002	69	2.2	Richards et al. 2005
12377–11398				GifA-95457	Infant	2.2	Gambier et al. 2000
27962–27516	-17.1	11.2		OxA-11053		2.2	Trinkaus et al. 2009; Orschiedt 2002
41549–40389				OxA-19569	RM-1, G 60	2.2	Douka et al. 2012
41538–40344				OxA-20000	Mochi 51 (2003), I 5	2.2	Douka et al. 2012
30817–30042				OxA-19729	Mochi 46, (1997) H 2	2.2	Douka et al. 2012
28874–28380				OxA-19800	Mochi 15, D 29	2.2	Douka et al. 2012
9014–8602	-17.8			Ua-10426		2.2	Ahlström 2003
8977–8430	-17.7			Ua-13406		2.2	Ahlström 2003
8319–8005				Z-2294 +2349			Lanting 1998
6171–5932			2	KIA-17652	KS11320	2.3	Tromnau 1987; Fletcher 2015
7577–7330				R-2561		2.3	Lanting 1998

Site name	Material dated	Age in ^{14}C years BP	Most probable calendar age BP
Lystrup 2, Denmark	Lime wood (*Tilia* sp.), log boat	6550±105	7500
Margrethes Næs I, Denmark	Lime wood (*Tilia* sp.), log boat	6530±105	7400
Margrethes Næs III, Denmark	Lime wood (*Tilia* sp.), fragment of log boat with (repair) holes	6070±105	6900
Nandy 1, France	Pine wood (*Pinus sylvestris*), log boat	8060±55	9000
Nandy 2, France	Pine wood (*Pinus sylvestris*), log boat	7990±55	8900
Noyen-sur-Seine, France	Pine wood (*Pinus sylvestris*), log boat	7960±100	8800
Pesse, Netherlands	Pine wood (*Pinus sylvestris*), coarsely hollowed-out trunk, considered to be a log boat	8760±145	9800
Stralsund boat 3, Germany	Lime wood (*Tilia* sp.), log boat	5901±34	6700
Stralsund boat 2, Germany	Lime wood (*Tilia* sp.), log boat	5853±34	6700
Stralsund boat 1, Germany	Maple wood (*Acer* sp.), log boat	5040±26	5800
Dates from submarine sites			
Denmark			
Argusgrund	Charcoal, oak (*Qurcus* sp.), 15 fragments	6840±105	7700
Argusgrund	Charcoal, lime (*Tilia* sp.), 66 fragments	6790±105	7600
Argusgrund	Charcoal, oak (*Tilia* sp.), 51 fragments	6930±105	7800
Argusgrund	Bone, human (*Homo sapiens*), 18-20 years old	7080±75	7900
Argusgrund	Bone, human (*Homo sapiens*), 18-20 years old		
Argusgrund	Bone, human (*Homo sapiens*), 2-3 years old		
Argusgrund	Bone, human (*Homo sapiens*), 2-3 years old		
Argusgrund	Bone, human (*Homo sapiens*), 4-5 years old		
Argusgrund	Bone, human (*Homo sapiens*), adult		
Argusgrund	Bone, human (*Homo sapiens*), adult		
Blak II	Bone, human (*Homo sapiens*)	7440±90	8300
Dogger Bank	Antler, red deer (*Cervus elaphus*), made into pressure flaker	8020±110	8900
Fløjstrup Skov	Bone, terrestrial mammal, possibly ulna of elk (*Alces alces*) or red deer (*Cervus elaphus*); fragmented in prehistory, possibly intentionally	8038±31	8900
Kalundborg Fjord, off Byens Mose	Rooted stump of oak (*Quercus*), exposed in a submerged forest	6835+-55	7700
Kalø Vig I	Hazel rod (*Corylus avellana*), possibly part of fish weir	7550±40	8400
Korsør Glasværk	Bone, human (*Homo sapiens*)	6180±95	7100
Korsør Nor inhumation	Bone, human (*Homo sapiens*)	6263±36	7200
Køge Havn	Hazel wood (*Corylus avellana*), part of fish weir	7450±32	8300

Calendar age BP (2 σ interval)	δ¹³C (‰)	δ¹⁵N (‰)	Depth below present sea level (m)	Dating lab. no.	Museum inventory no. etc.	Chapter reference	Literature reference
7611–7266				K-6012		2.3	Andersen 1996; Christensen 1997
7596-7256			1.5±0.1	K-5599		2.3	Myrhøj & Willemoes 1997; Fischer & Pedersen 1997
7238–6675			1.5±0.1	K-5596			Myrhøj & Willemoes 1997; Fischer & Pedersen 1997
9128–8725				Arc-1197		2.3	Lanting 1998
9008–8649				Arc-1196		2.3	Lanting 1998
9085–8550				Arc-1197		2.3	Lanting 1998
10192–9531				GrN-486		2.3	Lanting 1998
6795–6653	-28.0		2.6±0.4	KIA-24236	2001/2168-0204	2.3.2	Lübke 2005; Klooß & Lübke 2009; Klooß 2014
6747–6563	-26.1		2.6±0.4	KIA-24235	2001/2168-0246	2.3.2	Lübke 2005; Klooß & Lübke 2009; Klooß 2014
5922–5612	-25.4		2.6±0.4	KIA-24234	2001/2168-0247	2.3.2	Lübke 2005; Klooß & Lübke 2009; Klooß 2014
7930-7510	-22.7		5.2±0.2	K-4382		Fig. 7	Malmros 1987; Fischer et al. 2007a
7850-7462	-23.0		5.2±0.2	K-4383		Fig. 7	Malmros 1987; Fischer et al. 2007a
7951-7593	-23.1		5.2±0.2	K-4384		Fig. 7	Malmros 1987; Fischer et al. 2007a
8028-7726	-14.3	12.8	5±1	K-4354	AS 7/01		Fischer et al. 2007a
	-14.5	13.4	5±1	AAR-8858			Fischer et al. 2007a
	-15.1	16.9	5±1	AAR-8856		3.2 Fig. 8a	Fischer et al. 2007a
	-15.2	16.9	5±1	BCH198:32a+b		3.2 Fig. 8a	Fischer et al. 2007a
	-15.2	14.8	5±1	AAR-8857			Fischer et al. 2007a
	-16.3	12.7	5±1	F 58-54			Fischer et al. 2007a
	-17.3	13.5	5±1	AAR-8859			Fischer et al. 2007a
8403-8046	-17.9	12.8	3.5±1	Ka-6454			Sørensen 1996; Fischer et al. 2007b
9249–8593	-22.8		35±5	AAR-3046	FHM 3363A	5.1 Fig. 3	Heinemeier & Rud 1997, 302; Andersen 2005
9021–8777	-22.8		13.3±0.7	AAR-21074	NMU 1719X24	4.2.1	Dencker & Jensen 2000
7786-7585			2	AAR-8167		Figs. 5-6 and Ch. 6.2 Fig. 1	
8422-8220			6,4±0.2	AAR-8415			Fischer & Hansen 2005
7287-6798	-11.3	14.2	3.3±1	K-4185			Schilling 1997; Fischer et al. 2007b
7269-7028	-15.9	12.3	1.9±0.2	AAR-10246			Schilling 1997; Fischer et al. 2007b
8348-8189	-33		8.7	AAR-24741	VIR 2769; X479	4.1.1 Fig. 3	Personal communication Klara Fiedler and Frederik D. Hyttel, Viking Ship Museum, Roskilde, and Claudia Baittinger, National Museum of Denmark 2016

Site name	Material dated	Age in ^{14}C years BP	Most probable calendar age BP
Køge Sønakke	Bone, human (*Homo sapiens*)	8250±85	9200
Læsø, Als Dyb	Rooted pine stump (*Pinus sylvestris*), exposed in a submerged forest. Represents the c. 11 most recent tree rings	9206±41	10400
Møllegabet	Bone, human (*Homo sapiens*)	6170±85	7100
Nekselø	Hazel wood (*Corylus avellana*), part of fish weir	5560±80	6400
Nekselø	Hazel wood (*Corylus avellana*), part of fish weir	5440±70	6200
Nekselø	Hazel wood (*Corylus avellana*), part of fish weir	4770±85	5500
Nekselø	Lime wood (*Tilia* sp.), part of fish weir	4750±95	5500
Nekselø	Hazel wood (*Corylus avellana*), part of fish weir	4700±80	5400
Nekselø	Lime wood (*Tilia* sp.), part of fish weir	4660±65	5400
Nekselø	Hazel wood (*Corylus avellana*), part of fish weir	4660± 65	5400
Nekselø	Hazel wood (*Corylus avellana*), part of fish weir	4620±75	5400
Nekselø	Hazel wood (*Corylus avellana*), part of fish weir	4600±70	5300
Nekselø	Hazel wood (*Corylus avellana*), part of fish weir	4520±65	5200
Nekselø	Hazel wood (*Corylus avellana*), part of fish weir	4520±70	5200
Nekselø	Birch wood (*Betula* sp.), part of fish weir	4470±65	5100
Nekselø	Hazel wood (*Corylus avellana*), part of fish weir	4440±90	5100
Nekselø	Hazel wood (*Corylus avellana*), part of fish weir	4420±65	5000
Nekselø	Hazel wood (*Corylus avellana*), part of fish weir	4370±115	5000
Nekselø	Hazel wood (*Corylus avellana*), part of fish weir	4309±75	4900
Nekselø	Hazel wood (*Corylus avellana*), part of fish weir	4320±115	4900
Nekselø	Hazel wood (*Corylus avellana*), part of fish weir	4310±90	4900
Ronæs Skov	Lime wood (*Tilia* sp.), fragment of log boat	5230±60	6000
Rønsten	Bone, mandible, human (*Homo sapiens*)	7542±44	8400
Rønsten	Hazel rod (*Corylus avellana*), possibly part of fish weir	6980±70	7800
Rønsten	Hazel rod (*Corylus avellana*), possibly part of fish weir	6950±65	7800
Rønsten	Hazel rod (*Corylus avellana*), possibly part of fish weir	6580±65	7500
Solrød	Antler, elk (*Alces alces*), made into axe-like implement; no sign of preservative material	9800±80	11200
Solrød Strand	Bone, reindeer (*Rangifer tarandus*), marrow split	12238±46	14100
Solrød Strand	Antler, reindeer (*Rangifer tarandus*), worked	12170±45	14100
Solrød Strand	Antler, reindeer (*Rangifer tarandus*), worked	12140±110	14000
Torskegrund	Antler, red deer (*Cervus elaphus*), made into 'pick axe'	8124±23	9100
Tybrind Vig, young	Bone, human (*Homo sapiens*)	6905±55	7700
Tybrind Vig, adult	Bone, human (*Homo sapiens*)	6820±55	7700
Tybrind Vig, mother	Bone, human (*Homo sapiens*)	6740±80	7600
Tybrind Vig, child	Bone, human (*Homo sapiens*)		
France			
Cosquer Cave	Charcoal, in wall painting ('Oval symbol')	28370±440	32300

Calendar age BP (2 σ interval)	δ^{13}C (‰)	δ^{15}N (‰)	Depth below present sea level (m)	Dating lab. no.	Museum inventory no. etc.	Chapter reference	Literature reference
9437–9021	-16.8	12.4	8±2	K-5099	NMI 148/51; AS 7/61	3.2 Fig. 4a	Fischer et al. 2007b
10495-10444	-20.8		2.2±0.5	AAR-16017		Figs. 5-6	Personal communication underwater archaeologist Jan Hammer Larsen 2009 and wood anatomist Thomas Bartholin 2011
7265-6805	-12.6	15.7	4.8				Skaarup & Grøn 2004; Fischer et al. 2007b
6527–6195				K-6208		3.2.2	Fischer 2007
6397–6006				K-6207		3.2.2	Fischer 2007
5648–5317				K-6114		3.2.2	Fischer 2007
5710–5290				K-6112		3.2.2	Fischer 2007
5601–5145				K-6113		3.2.2	Fischer 2007
5585–5087				K-5959		3.2.2	Fischer 2007
5585–5087				K-5957		3.2.2	Fischer 2007
5580–5050				K-6473		3.2.2	Fischer 2007
5576–5045				K-5960		3.2.2	Fischer 2007
5445–4894				K-5955		3.2.2	Fischer 2007
5445–4891				K-5961		3.2.2	Fischer 2007
5305–4880				K-5958		3.2.2	Fischer 2007
5305–4858				K-6117		3.2.2	Fischer 2007
5286–4860				K-5956		3.2.2	Fischer 2007
5316-4628				K-6116		3.2.2	Fischer 2007
3323–2675				T-18053		3.2.2	Fischer 2007
5286–4710				K-6115		3.2.2	Fischer 2007
5280–4586				K-5643		3.2.2	Fischer 2007
6185–5900	-27.1		2	AAR-5462	FHM 3705 QQ	2.3 Fig. 4	Heinemeier & Rud 2001; Andersen 2009
8420–8211	-15.0	11.8		AAR-11355	AS 15/06	3.2 Fig. 8	
7942–7680				AAR-4622	EBM 232X71	1.2 Fig. 2c	Fischer 2007, Fig. 5.5 upper
7932–7671				K-6967	EBM 232X70	3.2 Fig. 5e	Fischer 2007
7585–7334				AAR-4621	EBM 232X30		Fischer 2007, Fig. 5.5 lower
11598–10812	-19.45			AAR-7905	A 52834	3.2 Fig. 4c	
14328–13981	-19.7		8±3	AAR-18733	P62/2013KMG	3.2.1	
14207–13873	-18.5		8±3	AAR-18732	P63/2013KMG	3.2.1	
14454–13736	-19.3		8±3	AAR-1036	MD 65e	3.2.1	Petersen & Johansen 1993
9122–9007	-22.6		30±10	AAR-18732		3.2 Fig. 2b	
7915-7622	-11.1	16.3	3.0±0.5	AAR-9342			Fischer et al. 2007b; Uldum 2011
7783-7576	-11.5	16.3	3.0±0.5	AAR-9341			Fischer et al. 2007b; Uldum 2011
7732-7459	-15.7	13.4	2.7±0.3	K-3558		Division sheet for Part 1; 3.2 Fig. 6	Fischer et al. 2007b; Andersen 2013,56
	-14.7	16.4	2.7±0.3	BCH198:27a+b		Division sheet for Part 1; 3.2 Fig. 6	Fischer et al. 2007b; Andersen 2013, 56
33462–31373				GifA-96074		5.2.1	Valladas et al. 2005

Site name	Material dated	Age in ^{14}C years BP	Most probable calendar age BP
Cosquer Cave	Charcoal, in wall painting (‘Hand 19’)	27740±410	31700
Cosquer Cave	Charcoal, in wall painting (‘Bison 2’)	27350±430	31300
Cosquer Cave	Charcoal, on cave floor (by ‘Feline’)	27870±470	31200
Cosquer Cave	Charcoal, in wall painting (‘Hand MR7’)	27110±430	31100
Cosquer Cave	Charcoal, in wall painting (‘Hand MR7’)	27110±400	31100
Cosquer Cave	Charcoal, on cave floor (by ‘Great Auk’)	26360±440	30500
Cosquer Cave	Charcoal, in wall painting (‘Bison 2’)	26250±350	30500
Cosquer Cave	Charcoal, in wall painting (‘Hand 12’)	24840±340	28900
Cosquer Cave	Charcoal, in wall painting (‘Horse 5’)	24730±300	28800
Cosquer Cave	Charcoal, on cave floor (by ‘Bison 1’)	20370±260	24500
Cosquer Cave	Charcoal, in wall painting (‘Horse 7’)	19720±210	23700
Cosquer Cave	Charcoal, in wall painting (‘Giant Deer 1’)	19340±200	23300
Cosquer Cave	Charcoal, in wall painting (‘Deer’)	19290±340	23300
Cosquer Cave	Charcoal, in wall painting (‘Feline’)	19200±240	23100
Cosquer Cave	Charcoal, in wall painting (‘Horse 1’)	18940±250	22800
Cosquer Cave	Charcoal, in wall painting (‘Horse 1’)	18820±310	22700
Cosquer Cave	Charcoal, in wall painting (‘Bison 1’)	18530±190	22400
Cosquer Cave	Charcoal, in hearth	18400±440	22200
Cosquer Cave	Charcoal, in wall painting (‘Bison 1’)	18010±200	21800
Cosquer Cave	Charcoal, in wall painting (‘Star symbol’)	17800±160	21500
Saint-Jean-le-Thomas	Fish weir	3750±40	4100
Saint-Jean-le-Thomas		3740±35	4100
Saint-Jean-le-Thomas		3720±30	4100
Saint-Jean-le-Thomas		3715±30	4000
Saint-Jean-le-Thomas		3705±40	4000
Saint-Jean-le-Thomas		3690±35	4000
Saint-Jean-le-Thomas		3690±30	4000
Saint-Jean-le-Thomas		3680±40	4000
Saint-Jean-le-Thomas		3680±30	4000
Saint-Jean-le-Thomas		3660±40	4000
Saint-Jean-le-Thomas		3650±40	4000
Saint-Jean-le-Thomas		3640±30	4000
Saint-Jean-le-Thomas		3635±40	4000
Saint-Jean-le-Thomas		3635±40	4000
Saint-Jean-le-Thomas		3630±40	4000
Saint-Jean-le-Thomas		3625±35	4000
Saint-Jean-le-Thomas		3615±36	4000
Saint-Jean-le-Thomas	Fish weir	3590±40	3900
Germany			
Norderhever	Bone, aurochs (*Bos primigenius*), votive deposition	3845±30	4300
Israel			
Atlit-Yam	Wood	7880±55	8700
Atlit-Yam	Wood	7755±55	8500
Atlit-Yam	Wood	7605±55	8400
Atlit-Yam	Wood	7465±50	8300
Atlit-Yam	Wood	7460±55	8300
Kfar Samir	Carob wood (*Ceratonia siliqua*), wooden bowl	7230±80	8100
Kfar Samir	Olive pulp (*Olea europaea*)	6500±70	7400
Kfar Samir	Basketry, possibly for olive oil extraction	6420±120	7300

Calendar age BP (2 σ interval)	δ^{13}C (‰)	δ^{15}N (‰)	Depth below present sea level (m)	Dating lab. no.	Museum inventory no. etc.	Chapter reference	Literature reference
32766–31014				GifA-96073		5.2.1	Valladas et al. 2005
32425–30730				GifA-95195		5.2.1	Valladas et al. 2005
33048–31033				GifA-92350		5.2.1	Valladas et al. 2005
32049–30445				GifA-92409		5.2.1	Valladas et al. 2005
31885–30531				GifA-92491		5.2.1	Valladas et al. 2005
31195–29586				GifA-95349		5.2.1	Valladas et al. 2005
31053–29659				GifA-96069		5.2.1	Valladas et al. 2005
29701–28136				GifA-95358		5.2.1	Valladas et al. 2005
29480–28122				GifA-9672		5.2.1	Valladas et al. 2005
25255–23916				GifA-92348		5.2.1	Valladas et al. 2005
24256–23191				GifA-98186		5.2.1	Valladas et al. 2005
23833–22804				GifA-95135		5.2.1	Valladas et al. 2005
24025–22489				GifA-98198		5.2.1	Valladas et al. 2005
23710–22539				GifA-92418		5.2.1	Valladas et al. 2005
23479–22364				GifA-92416		5.2.1	Valladas et al. 2005
23492–22022				GifA-92417		5.2.1	Valladas et al. 2005
22840–21921				GifA-92492		5.2.1	Valladas et al. 2005
23369–21186				Ly-5528		5.2.1	Valladas et al. 2005
22355–21303				GifA-92419		5.2.1	Valladas et al. 2005
21957–21051				GifA-96075		5.2.1	Valladas et al. 2005
4235-3984				Ly-12462		5.2	Billard et al. 2013
4228-3982				Ly-3117		5.2	Billard et al. 2013
4150-3980				Ly-6425		5.2	Billard et al. 2013
4150-3977				Ly-6424		5.2	Billard et al. 2013
4154-3921				Ly-13287		5.2	Billard et al. 2013
4147-3915				Ly-5379		5.2	Billard et al. 2013
4145-3926				Ly-6729		5.2	Billard et al. 2013
4145-3899				Ly-13295		5.2	Billard et al. 2013
4139-3914				Ly-6729		5.2	Billard et al. 2013
4139-3875				Ly-13286		5.2	Billard et al. 2013
4089-3865				Ly-13291		5.2	Billard et al. 2013
4082-3870				Ly-730		5.2	Billard et al. 2013
4084-3845				Ly-13234		5.2	Billard et al. 2013
4084-3845				Ly-13236		5.2	Billard et al. 2013
4083-3841				Ly-13292		5.2	Billard et al. 2013
4078-3841				Ly-13237		5.2	Billard et al. 2013
4074-3836				Ly-13463		5.2	Billard et al. 2013
4066-3728				Ly-13290		5.2	Billard et al. 2013
4406-4154	-20.2			KIA-20952			Kühn 2007
8977-8555			10±2	RT-2489	Well 66		Galili & Rosen 2011
8628-8420				RT-2495, 2493	Well 66		Galili & Rosen 2011
8540-8336			14±1.5	RT-2477, 2478	Well 11		Galili & Rosen 2011
8376-8187			14±1.5	RT-2475	Well 11		Galili & Rosen 2011
8379-8181			14±1.5	RT-2479	Well 11		Galili & Rosen 2011
8275-7872			0.3±0.25	RT-1360			Galili & Sharvit 1995; Galili et al. 1997; Galili & Rosen 2011
7560–7273			0.3±0.25	BETA-82715	Installation 6		Galili et al. 1997
7568–7027				RT-855	Installation 8		Galili et al. 1997; Galili & Rosen 2011

Site name	Material dated	Age in ^{14}C years BP	Most probable calendar age BP
Kfar Samir	Olive pit (*Olea europaea*)	6290±60	7200
Kfar Samir	Olive pit (*Olea europaea*)	6210±150	7100
Kfar Samir	Olive pit (*Olea europaea*)	6210±80	7100
Kfar Samir	Olive pit (*Olea europaea*)	6100±60	7000
Kfar Samir	Olive pit (*Olea europaea*)	6080±70	6900
Kfar Samir	Olive pit (*Olea europaea*)	5870±70	6700
Kfar Samir	Olive pit (*Olea europaea*)	5790±55	6600
Kfar Samir	Olive pit (*Olea europaea*)	5630±55	6400
Kfar Samir	Olive pit (*Olea europaea*)	5630±55	6400
Netherlands			
Boven Noord Hinder Putten/Eurogeul	Bone, human (*Homo sapiens*), mandible fragment	10070±50	11600
Eurogeul	Antler, red deer (*Cervus elaphus*), humanly modified	8070± 50	9000
Maasvlakte 2	Bone, human (*Homo sapiens*), cranial fragment	8565±45	9500
North Sea	Bone, human (*Homo sapiens*), mandible	8370±50	9400
Southern North Sea, west of Brown Bank	Mammal bone, aurochs (*Bos primigenius*)?; richly decorated with engraved zig-zag ornaments	11560±50	13400
North Sea, Southern Bight	Bone, human (*Homo sapiens*), mandible	9870±70	11300
North Sea, Southern Bight	Bone, humerus, wild boar (*Sus scrofa*)	9450±70	10700
North Sea, Southern Bight	Bone, human (*Homo sapiens*), cranium	8340±130	9300
Southern North Sea	Bone, human (*Homo sapiens*), cranial bone	9640±40	11000
Norway			
Hummervikholmen	Bone, human (*Homo sapiens*)	8700±70	9700
Hummervikholmen	Bone, human (*Homo sapiens*), individual 2	8635±75	9600
Hummervikholmen	Bone, human (*Homo sapiens*), individual 1	8600±95	9600
Hummervikholmen	Bone, human (*Homo sapiens*)	8455±75	9500
Hummervikholmen	Bone, human (*Homo sapiens*)	8095±55	9000
Kirkehavn	Bone (?) of marine mammal	8980±75	10100
Sweden			
Haväng	Hazel (*Corylus avellana*), part of fish weir	8086±60	9000
Haväng	Antler, elk (*Alces alces*), manufactured into axe-like implement	7975±35	8900
UK			
Leman and Ower Banks	Antler, red deer (*Cervus elaphus*), leister prong	11740±150	13600

Calendar age BP (2 σ interval)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Depth below present sea level (m)	Dating lab. no.	Museum inventory no. etc.	Chapter reference	Literature reference
7413–7013				BETA-82844	Installation 6		Galili et al. 1997; Galili & Rosen 2011
7424–6751				BETA-82846	Installation 6		Galili et al. 1997; Galili & Rosen 2011
7288–6897				BETA-82847	Installation 6		Galili et al. 1997; Galili & Rosen 2011
7162–6798				BETA-82843	Installation 7		Galili et al. 1997; Galili & Rosen 2011
7162–6783				BETA-82845	Installation 7		Galili et al. 1997; Galili & Rosen 2011
6877–6497				RT-1930	Installation 6		Galili et al. 1997; Galili & Rosen 2011
6729–6466				RT-1898	Installation 6		Galili et al. 1997; Galili & Rosen 2011
6531–6299				RT-1929	Installation 7		Galili et al. 1997; Galili & Rosen 2011
6531–6299				RT-1929A	Installation 7		Galili et al. 1997; Galili & Rosen 2011
11940–11343				GrA-42700	4513		Storm 2010
9130–8768				GrA-22999			Mol et al. 2006
9607–9475	-23.51	12.73	26±5	GrA-57501			Weerts et al. 2015
9495–9267				GrA-11642			Glimmerveen et al. 2006
13480–13285				GrA-28364	Private collection Jan Glimmerveen		Mol et al. 2008, 160-161
11604–11179				GrA-23205			Glimmerveen et al. 2006
11076–10510				UtC-7886	NO.2684		Glimmerveen et al. 2006
9535–9025							Glimmerveen et al. 2006
11186–10787	- 24.1			Utc-3750	NO.1063		Glimmerveen et al. 2006; personal communication Klaas van der Borg July 2015
9911–9534	-12.6			TUa-2107			Nymoen & Skar 2011
9887–9489	-13.3			TUa-2106			Nymoen & Skar 2011
9893–9439	-13.4			TUa-1257			Nymoen & Skar 2011
9547–9298	-12.9			TUa-2108			Nymoen & Skar 2011
9250–8777	-13.6			TUa-2105			Nymoen & Skar 2011
10261–9795	-16.6			TUa-1583			Nymoen & Skar 2011
9250–8770	-29.2			Ua-38145		3.1.1 and front cover	Nilsson 2012
8996–8658			6	LuS-11011	HAV42	3.1.1	Personal communication Arne Sjöström May 2016
13945–13281				OxA-1950		Fig. 4	Bonsall & Smith 1990

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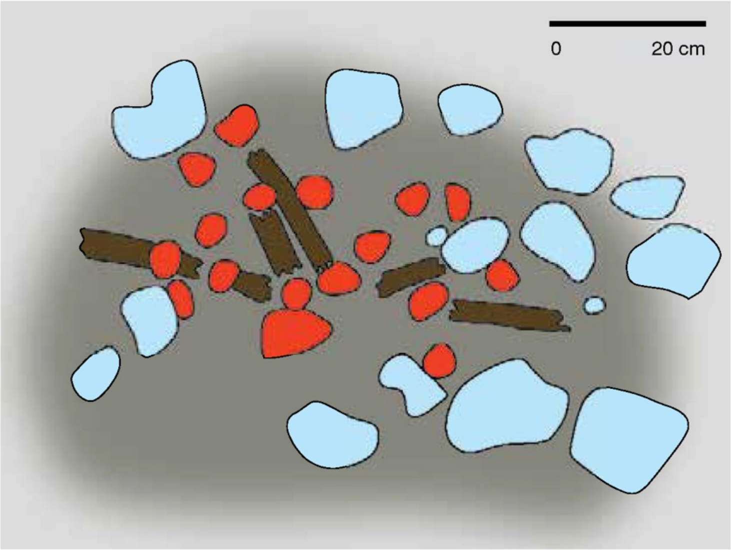

Fig. 7. When archaeologists excavated this hearth at the settlement at Argusgrund, now located 5 m below present sea level, they found ash (grey), charred branches (black) and fire-brittled flint (red) still in situ, surrounded by stones (blue). Three samples of charcoal gave radiocarbon dates within the interval c. 8000-7500 cal BP. The slight variation between the dates probably reflects the presence of old wood in the burnt material. Drawing based on a field sketch by Per Smed 1984.

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Jensen, TZT (2019) Per Persson, Felix Riede, Birgitte Skar, Heidi Mjelva Breivik and Leif Jonsson, eds. Ecology of Early Settlement in Northern Europe: Conditions for Subsistence and Survival (The Early Settlement of Northern Europe 1. Sheffield & Bristol: Equinox, 2018, 490pp., 49 tables, 156 figs, ISBN 9781781795156). - Kjel Knutsson, Helena Knutsson, Jan Apel and Håkon Glørstad, eds. Technology of Early Settlement in Northern Europe: Transmission of Knowledge and Culture (The Early Settlement of Northern Europe 2. Sheffield & Bristol: Equinox, 2018, 330pp., 14 tables, 98 figs, ISBN 9781781795163). - Hans Peter Blankholm, ed. Early Economy and Settlement in Northern Europe. Pioneering, Resource Use, Coping with Change (The Early Settlement of Northern Europe 3. Sheffield & Bristol: Equinox, 2018, 418pp., 22 tables, 113 figs, ISBN 9781781795170). European Journal of Archaeology, 22(3), pp. 415-421

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Book Reviews

Per Persson, Felix Riede, Birgitte Skar, Heidi Mjelva Breivik and Leif Jonsson, eds. *Ecology of Early Settlement in Northern Europe: Conditions for Subsistence and Survival* (The Early Settlement of Northern Europe 1. Sheffield & Bristol: Equinox, 2018, 490pp., 49 tables, 156 figs, ISBN 9781781795156).

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Hans Peter Blankholm, ed. *Early Economy and Settlement in Northern Europe. Pioneering, Resource Use, Coping with Change* (The Early Settlement of Northern Europe 3. Sheffield & Bristol: Equinox, 2018, 418pp., 22 tables, 113 figs, ISBN 9781781795170).

Until now, the Early Mesolithic in Northern Europe has been rather obscured by a fragmented archaeological record. What is known is that pioneers from the Central European as well as the Eurasian plains migrated into a completely new and quite barren landscape, just after the glaciers retreated. These nomadic hunter-gatherers faced some of the same challenges as we do today, such as climatic warming and rising sea-levels. The Preboreal—the time period just after the Younger Dryas—saw fluctuating temperatures, leading into increasingly warm subsequent geological periods. The floral and faunal composition gradually changed from taxa adapted to the previous harsh conditions of the Late Glacial, to a more continental one, completely replacing the old. However, much of this information is based on fragmentary evidence, especially when it comes to the preservation of organic material—one reason being that large parts of Sweden and most of Norway lack limestone deposits and are rich in acidic soils not conducive to preservation.

The Early Settlement of Northern Europe, edited by Glørstad, is a collection of articles forming a trilogy of hefty books. Each volume focuses on different subjects related to the Early Holocene in northern Europe. The forty articles total 1238 pages, supplemented by 367 figures and eighty-five tables. Together these books describe in detail the colonization of northern Europe in the first millennia of the Holocene, and how pioneers settled in this virgin landscape after the retreat of the Weichselian ice sheets, coping with changes including exposure to a new landscape, new flora and fauna, new raw material for tool production, and encounters with other groups of hunter-gatherers, all of which forced them to adapt.

Volume 1, *Ecology of Early Settlement in Northern Europe: Conditions for Subsistence and Survival*, is edited by Persson, Riede, Skar, Breivik, and Jonsson. The major themes are ecology and the conditions for subsistence and survival. Chapter 1 (Skar & Breivik) provides an introduction to the subject, alongside an overview of the following articles.

Chapters 2–8 discuss migration into the Skagerrak-Kattegat region and the establishment of permanent settlements in this area. Jonsson (Ch. 2) gives an introduction to the subfossil record in the Skagerrak area, and infers what was probably present in southern Norway based on finds from Huseby Klev in Sweden, since organic material is not preserved from the Early Holocene in Norway. He further delivers a substantial critique of Boethius' research on the same site (see Ch. 5) and the latter's interpretation of several aspects related to taphonomy, stratigraphy, and taxonomic identification at the site. But how did people reach the location in the first place, and how was hunting practiced? Schmitt (Ch. 3) argues that the Hensbacka travelled by boat, and uses shoreline displacement to date the Hensbacka sites, which lack organic material for radiocarbon dating while Cziesla (Ch. 4) presents a new take on the use of biserial harpoons and Lyngby axes, proposing that they may have been used for hunting seal as opposed to reindeer. As noted above, Boethius (Ch. 5) also presents material from Huseby Klev, Sweden—one of the only coastal Early Mesolithic sites included in these books at which organic material was preserved—arguing that a human-induced marine mammal collapse led to an intensification of fishing in this early period. Mansrud and Persson (Ch. 6) build an argument for a marine subsistence economy in the Skagerrak region in the Early and Middle Mesolithic, based on settlement locations near to the prehistoric coastline, as well as giving an introduction to Middle Mesolithic fish hook technology. Mjærum (Ch. 7) discusses the killing of large ungulates along natural chokepoints in the area around Røyrtjønna in Oppland county from the Middle Mesolithic onwards. The last article in this section, by Persson (Ch. 8), builds on Boaz's settlement intensity model (1999), which argues that the presence or absence of settlements can be inferred based on the presence or absence of radiocarbon dates. In light of this, Persson adds 105 AMS dates conducted on burnt bone from inland locations in Norway to Boaz's dataset. Based on the fluctuations observed in the sum of a total of 364 radiocarbon dates, Persson argues that the inland population decreased around c. 8300 cal BC before increasing at around 6900 cal BC, and gives indications as to what might have caused these differences.

Chapters 9–13 deal with the Early Mesolithic in the Baltic region, more specifically Sweden. Hallgren (Ch. 9) presents a very small Preboreal settlement uncovered at the Motala site in western Sweden. Moving further east, Petterson and Wikell (Ch. 10) present firm evidence of seal hunters island-hopping around the Södertorn archipelago, and also of the use of seal fat as a fuel. Keeping with the theme of seal hunting, Apel and Storå (Ch. 11) apply a behavioural ecology approach to uncover how new arrivals adjusted to hunting on Gotland, and how these early mariners colonized the Baltic islands, from a protein cost-benefit perspective. Thereafter, Boethius (Ch. 12) presents the evidence for large-scale fisheries and the potential harvest of forty-eight tonnes of fish during the habitation of Norje Sunnansund. The chapter concludes with a discussion of how the scale of marine exploitation reflects a highly structured society. A little further south, Nilsson, Sjöstrom, and Persson (Ch. 13) present the submerged and extraordinarily well-preserved Early Mesolithic landscape at Haväng off the coast of Blekinge, as well as some of the oldest wooden fish weirs in the world.

The remaining four chapters deal with the environmental conditions and land use in the North Sea area. Svendsen (Ch. 14) surveys twelve municipalities for Early Mesolithic Fosna sites, and based on this asserts that the Early Mesolithic population of western Norway combined their heavy reliance on seal hunting with ventures into the mountains to hunt reindeer. A potential caveat to these explanations is that there are no organic materials preserved and therefore all conclusions are inferred. However, Rosvold and Breivik (Ch. 15) do utilise organic material in the form of a partial skeleton of a bearded seal (*Erignathus barbatus*) dated to the Preboreal Oscillation cold event. Even though it is of a palaeontological nature, the find can still be used as a proxy for biotope and climate reconstruction of the Early Mesolithic environment, indicating it was rich in benthic organisms but also featured drift ice serving as breeding, resting, and foraging grounds for these ice-obligates. The last two chapters focus on the northerly British isles. Wickham-Jones and colleagues (Ch. 16) explore the landscape above and beneath the waves of Orkney by using a multidisciplinary approach to model and predict where settlement occurred. Wicks and Mithen, with contributions from others (Ch. 17), conclude Volume 1 by exploring the 'settling of the unknown' by early mariners at the Isle of Coll in Fiskary Bay (Inner Hebrides). Based on Bayesian modelling of radiocarbon dates, they argue for three occupation periods, before the island was potentially deserted, perhaps due to the 8.2ka cold event. The chapter finishes with a description and analysis of site finds, i.e. lithics, pollen, faunal remains, and archaeobotanical analysis.

Volume 2, *Technology of Early Settlement in Northern Europe: Transmission of Knowledge and Culture* is edited by Knutsson, Knutsson, Apel, and Glørstad. The focus of this book is almost entirely on lithic technology. The first article (Ch. 1), by the editors, is an overview of the articles to come and how they seek to answer questions related to the transmission or adaptation of technology and communication based on ideas rooted in sociological theory.

Chapters 2–4 discuss lithic manufacture and raw material acquisition during the Palaeolithic-Mesolithic transition in the context of the pioneers. Manninen and colleagues (Ch. 2), explore the impact of pioneers migrating into the 'virgin' landscape of north-eastern Fennoscandia. Of particular interest is the technological flexibility displayed by these early colonizers to make use of the very different raw materials that were available. Grużdź (Ch. 3) presents a technological overview of Post-Swiderian blade technology in Poland based on refitting of opposed platform cores, while Berg-Hansen (Ch. 4) gives an impressive overview of the technological shift in core and blade production from the Ahrensburgian to the Early Mesolithic in Scandinavia and northern Germany.

Chapters 5–10 are focused on the Mesolithic. Damlien and colleagues (Ch. 5) build on a previous study (Sørensen et al., 2013), but widen the resolution by including thirty-two new sites and 117 radiocarbon dates. The results show that the pressure blade technology originated somewhere in Eurasia in the Upper Palaeolithic and then spread into western Scandinavia and moved south taking multiple routes. The article also provides a seven-page appendix of radiocarbon dates used in the article. Adamczyk presents a technological study of Early Mesolithic sites from the Polish island of Wolin (Ch. 6), and how these different groups adapted their technological solutions to suit the raw material available. Sørensen (Ch. 7) delivers a pan-south Scandinavian overview of blade technology during the Maglemosian phase 3, arguing that pressure blade technology was probably not fully developed during this period, and most likely came from the north. Chapter 8 by Eymundsson and colleagues presents an analysis and classification of axes from the Early to Middle Mesolithic around the Oslofjord area. The only article involving organic material is presented by David and Kjällquist (Ch. 9). They argue that the recently excavated site at Norja Sunnansund in south-eastern Sweden might represent the route by which slotted bone technology reached the Danish Maglemosian area in the Late Boreal chronozone. The final article in the volume is by Guinard (Ch. 10), who also deals with the spread of the pressure blade technology, but in this case focusing on Sweden. It is suggested that the technology spread along the rivers and south into Scania and Denmark, but that the transmission might have occurred via groups moving up from southern Scandinavia and making contact with those groups already possessing the technology.

Volume 3, *Early Economy and Settlement in Northern Europe: Pioneering, Resource Use, Coping with Change* is edited by Blankholm. The focus is on the economy and settlement patterns of early Holocene pioneers of northern Scandinavia. Blankholm (Ch. 1) sets the scene for the book, reviewing the articles to come and in the process emphasizing that the early pioneers were exercising a maritime way of life, based on the fact that the majority of sites are located close to the prehistoric coastline. This volume is loosely organized geographically from north to south.

Kleppe (Ch. 2) discusses the colonization of northern Norway. Based on radiocarbon dates, lithics, vegetational data, and geology, he argues for different routes into northern Norway, namely both along the west coast and through Russia into Fennoscandia. Gjerde and Skandfer (Ch. 3) document the remains of substantial house pits at Tønsnes in northern Norway dated to around 8000 BP; due to the size of the dwellings the authors argue this is evidence for winter habitation and thus year-round settlement. Blankholm (Ch. 4) takes an unconventional approach to settlement patterns, using GIS and neural networks to predict the location of settlement sites on the Varanger peninsula. Several potential sites were suggested, and fifty-six new sites were found and verified through land surveys. Pioneer settlement in northern Sweden is scrutinised by Östlund (Ch. 5), who presents the three sites Aareavaara, Kangos, and Dumpokjauratj. Based on radiocarbon dates as well as topography, he argues that the three sites' uniformity in regards to topographic position in the landscape may serve as a guide for finding new sites in this vast area. Rankama and Kanpaanpää (Ch. 6) revisit the theme of eastern influence with reports on the missing piece in the jigsaw puzzle, in terms of an eastern influence in northern Sweden at the site of Sujala, which exhibits all the traits associated with Post-Swederian lithics technology.

Moving further south, to south Central Norway, Breivik and Bjerck (Ch. 7) give a synopsis of the existing archaeological record of 244 documented sites dated to the Early Mesolithic. They list different topics associated with these sites, such as age, location, settlement structure, and lithic tool tradition. The article also contains a twelve-page appendix with short descriptions of sites with dwelling structures and associated metadata. Fretheim and colleagues (Ch. 8) present another comparative analysis of Early Mesolithic dwellings, using a recently excavated dwelling structure at Mohalsen 2012-II, Vega, Norway, as the focus of the study. An overview of twenty-two known examples of such dwelling structures is also included as an appendix. Continuing southwards, to the west coast of Norway, Åstveit (Ch. 9) gives an introduction to the geological setting, climate, and shoreline displacement curves in Møre and Romsdal, Sogn and Fjordane, and Hordaland; as well as what was probably present in terms of fauna. He then presents six thoroughly excavated sites as case studies for comparison, before discussing different sites affected by the *Tepes* transgression and the difficulties of obtaining reliable radiocarbon dates from this region. Bang-Andersen (Ch. 10) discusses the settling of the interior and coastal region of south-western Norway. The article focuses on two site complexes in the mountains of south-western Norway, namely Myrvatn and Fløyrlivatn, the earliest known habitation sites in Norway. The chapter concludes with some interesting thoughts by the author regarding the possibility of a Late Glacial presence on the Jæren moraine plain in south-western Norway, which is known to have been ice-free during the Allerød interstadial. The article also includes an appendix of the hitherto excavated Preboreal sites of this region. Next, Dugstad (Ch. 11) presents a case study of five Early Mesolithic sites excavated on the island of Hundvåg, north of Stavanger. The author argues for a more complex social organization than is usually assumed for the Early Mesolithic, based on the distribution of lithics as well as evidence for an apprentice flint knapper on the site. Chapter 12 (Damlien & Solheim) provides an introduction to the Early and Middle Mesolithic of Eastern Norway. Based on recent excavations, the authors present arguments for similarities between the Early Mesolithic population and the Maglemose 0 and Hensbacka groups. Furthermore, they argue that there was an earlier presence in the interior than previously suggested, thus indicating that permanent occupation was established as soon as the glaciers retreated. The final article of the volume and the series is by Nyland (Ch. 13) and concerns the Early Mesolithic site of Pauler 2, south-eastern Norway. The author argues that Pauler 2 might be a special site, in the sense that only flint was used as raw material as opposed to other non-perishable sedimentary cryptocrystalline materials. Significantly, three 'needle points' made of flint were discovered which have no parallels in Early Mesolithic Norway. These aspects could perhaps point towards people frequenting Pauler from southern Scandinavia or Germany.

This trilogy represents an incredible collection of articles focused on the time just after the Weichselian Ice Age. The three books contain a plethora of information regarding flora, fauna, geology, and technology, stemming from decades of excavation and of research on the vast body of material unearthed, especially in Norway. But is it worth splitting this into three volumes, considering that each costs £110 to £135?

At first glance, the books look appealing: the layout is consistent, they are furnished with a thorough index, and are richly illustrated. However, the graphics, whilst plentiful, are of low resolution. Indeed, it looks as if a substantial amount of the graphical content has been downscaled; this is problematic for the reader as some details are almost impossible to read.

Volume 1 is the most diverse in terms of national input, and the only one to deal with the available organic material (apart from Vol. 2, Ch. 9), which is crucial to understanding how these early pioneers exploited and lived off the available biotopes. In the foreword of this volume, Skar and Breivik note that:

> '… organic material, a large proportion of the material record is long gone, leaving us with indirect sources for understanding the life-worlds of these early colonizers. Many authors in this volume demonstrate that the absence of hard archaeological data can be liberating—it forces us to squeeze the juice

> out of what data we may have, and dares us to create holistic accounts of past life even when the empirical base is fragmentary' (Vol. 1, p. 2).

This statement holds true for the other volumes. Organic material from the Early Mesolithic in northern Europe is almost non-existent north of Gothenburg in Sweden, mostly due to acidic soils (Vol. 1, Ch. 15). The majority of the papers (basically Volumes 2 and 3) are thus almost entirely focused on non-perishable materials and a lot of indirect inferences regarding species availability. That being said, it is impressive just how much information the authors are able to 'squeeze' out of the data, which from a Danish perspective seems quite limited.

That said, when faunal remains are available, in the case of Huseby Klev in Sweden, it seems to provoke a one-sided academic dispute between Jonsson (Vol. 1, Ch. 2) and Boethius (Vol. 1, Ch. 5). This takes the form of a sustained and unjustified attack by the former on the latter's faunal identifications and main interpretations. The fact that Jonsson is an editor of this volume, and Boethius is seemingly unaware of the criticism that precedes his work, makes this confrontation all the more distasteful. Presenting these disagreements in a debate-article format would have been much more agreeable and would have allowed both sides to present their case and accompanying evidence in an unbiased way (see e.g. Åstveit, 2014).

Several of the articles in Volume 2 deal with questions of lithic technology, particularly the use of the pressure technique to make blades. This innovation is argued to have been developed in the Far East, likely present-day Siberia, Mongolia, or northern China, some 20,000 years ago (Inizan, 2012), and then to have spread westwards and entered Fennoscandia right after the ice retreated. This is also in line with newly available genetic data (Günther et al., 2018). Volume 3 deals with the remarkable presence of *in situ* dwelling structures in Norway, which are abundant. In some areas such remains are immediately visible on the ground, as soil build up is extremely slow and they have not been affected by ploughing.

There are a few puzzling aspects of this trilogy, however: why are there only three articles devoted to continental northern Europe? Material is present at least from the interior peat bogs in Denmark and Germany, even with good organic preservation, from the Boreal and onwards (Sørensen et al., 2018), albeit less abundant than further north, since the coastal Early Mesolithic of present-day Denmark and northern Germany is largely located on the seabed. Or is it because the material from the south Scandinavian landmasses is to some extent 'covered' by previous works (Eriksen, 2006)?

The trilogy represents an almost overwhelming amount of information. Keeping track of this can at times almost exhaust the reader, for instance regarding the different lithic technologies first moving in one direction, then the other, and then mixing. Most chapters range from twenty to twenty-five pages, which in some cases seems a bit excessive. However, an overall picture does start to emerge: pioneers migrated northwards from Central Europe immediately after the ice lost its grip. This push was largely driven by exploitation of the rich biotope that was and is the sea, and the speed by which this happened must have required seaworthy vessels. As northern Scandinavia and Fennoscandia became ice-free, groups following the Post-Swederian tradition migrated into this area from present-day Russia, with a new flint technology that gradually made its way down south. Whether it was the same people that reached present-day Denmark during the Middle Mesolithic, or whether the latter came through the Baltic countries, remains open to debate.

So, where does this leave us? The books are certainly an important contribution to the settlement of northern Europe, but were three books really necessary? With such a high price tag for each book, it seems to be a very narrow audience that will read them. That being said, I certainly learned a lot from these works, which do represent the current knowledge of most of this large area, albeit perhaps with too much emphasis on the north-western part of the Scandinavian peninsula.

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Hingley, R. *Londinium: A Biography. Roman London from Its Origins to the Fifth Century*. (London: Bloomsbury Academic, 2018, 381pp, 75 illustr., pbk, ISBN 978-1-3500-4729-7)

In dedicating this book to Ralph Merrifield, Richard Hingley acknowledges the contribution Merrifield made to the study of Roman London, writing detailed works of synthesis which drew together many disparate strands of evidence to form a complex but accessible narrative (1983). As Hingley notes in his introduction, this approach to publication has ceased to be a common undertaking, with

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RESEARCH ARTICLE

Radiocarbon dating and isotope analysis on the purported Aurignacian skeletal remains from Fontana Nuova (Ragusa, Italy)

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Abstract

Proving voyaging at sea by Palaeolithic humans is a difficult archaeological task, even for short distances. In the Mediterranean, a commonly accepted sea crossing is that from the Italian Peninsula to Sicily by anatomically modern humans, purportedly of the Aurignacian culture. This claim, however, was only supported by the typological attribution to the Aurignacian of the lithic industries from the insular site of Fontana Nuova. AMS radiocarbon dating undertaken as part of our research shows that the faunal remains, previously considered Aurignacian, actually date to the Holocene. Absolute dating on dentinal collagen also attributes the human teeth from the site to the early Holocene, although we were unable to obtain ancient DNA to evaluate their ancestry. Ten radiocarbon dates on human and other taxa are comprised between 9910–9700 cal. BP and 8600–8480 cal. BP, indicating that Fontana Nuova was occupied by Mesolithic and not Aurignacian hunter-gatherers. Only a new study of the lithic assemblage could establish if the material from Fontana Nuova is a mixed collection that includes both late Upper Palaeolithic (Epigravettian) and Mesolithic artefacts, as can be suggested by taking into account both the results of our study and of the most recent reinterpretation of the lithics. Nevertheless, this research suggests that the notion that Aurignacian groups were present in Sicily should now be revised. Another outcome of our study is that we found that three specimens, attributed on grounds both of morphological and ZooMS identifications to *Cervus elaphus*, had $\delta^{13}C$ values significantly higher than any available for such species in Europe.

Introduction

The question of voyaging at sea in the Mediterranean during the Palaeolithic is still open, even when it comes to the Upper Palaeolithic [1]. One of the few claims for a sea crossing around

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the time of the first arrival of anatomically modern humans to Europe, coinciding with the inception of the Aurignacian culture, is that made on the basis of the Sicilian site of Riparo di Fontana Nuova. In his seminal book titled *The Making of the Middle Sea*, Broodbank [2] states that this claim possesses "one advantage over earlier claims of island occupation in the Mediterranean: it indisputably happened". This view is shared by most authors that have dealt in detail with the issues of prehistoric voyaging at sea in the Mediterranean Sea [3–4]. The cultural and chronological attributions of the site to the Aurignacian, however, solely hinge on chrono-typological considerations resulting from the study of the lithic assemblage recovered during unsystematic excavations [5–12]. A more recent revision of the lithic assemblage from Fontana Nuova has typologically attributed it to the closing stages of the Upper Palaeolithic, specifically to the Final (or Late) Epigravettian [13]. None of the above-mentioned studies included absolute dating, which we have undertaken as part of the research presented here.

The site and its lithic assemblage

The first archaeological activity at the site of Riparo di Fontana Nuova (Fig 1) was the unsystematic excavation conducted by a local nobleman, Vincenzo Grimaldi di Calamenzana, sometime before January 1914. In that month, in fact, he donated the lithic assemblage to the Archaeological Museum in Syracuse, having reburied the skeletal remains [14]. Following World War II, after Luigi Bernabò Brea was appointed 'Soprintendente delle Antichità per la Sicilia Orientale', the collections of the Museum were reorganized and, in the process, the boxes containing the lithic material were retrieved. This 're-discovery' pushed Bernabò Brea to search for the site and in 1949 he was able to locate the spoil heap of Baron Calamenzana's excavation, retrieving the unstratified skeletal finds as a record of what had been present at the site. During his visit, Bernabò Brea was also able to observe the remnants of the original stratigraphic section at Fontana Nuova, observing that it consisted of three distinct layers and concluding that the prehistoric material from the spoil heap must all have originated from the discrete dark middle layer containing charcoal and bone fragments. Bernabò Brea [14] published the first description of the material, leaving open the issue of its exact chronology, attributing the assemblage broadly to the Upper Palaeolithic. The importance of Fontana Nuova for the study of the Sicilian Upper Palaeolithic started with the first attribution of its lithic assemblage to the Aurignacian by Laplace in his seminal research on the Italian lithic assemblages [5]. The French archaeologist observed the peculiarity of the complex ("plus aurignacoïde qu'aurignacien") and justified the absence of diagnostic bone tools by suggesting that the material culture was compatible with a relatively evolved phase of the Aurignacian ("en l'absence de toute forme osseuse caractéristique, pouvoir être attribué à une phase relativement évoluée de l'Aurignacien").

The most detailed analysis of the lithic assemblage recovered at Riparo di Fontana Nuova is the one by Gioia [7], reviewed in 1996 by the same researcher [12], using a statistical analysis based on the typological list proposed by de Sonneville-Bordes & Perrot [15]. The total number of pieces examined was 212 in the first paper and 224 in the second, given that an additional 12 lithics were found among the faunal remains. The lithic assemblage is composed of 60.7% retouched tools, 33.5% by unretouched blades and flakes, and 5.8% cores. Overall, the lithic industry is markedly laminar and endscrapers are the main typological group (40.4%), which purportedly includes several Aurignacian types, such as keeled endscrapers, nosed endscrapers, endscrapers on an Aurignacian blade [12]. The assemblage has also been reported to include blades with scalar Aurignacian retouch and strangulated blades. These interpretations have led Chilardi et al. [12] to confirm the broad scheme proposed by Laplace [5]. However, Gioia [7] disagreed with the hypothesis put forward by Laplace [5] according to which the lithic industries from Fontana Nuova belonged to a peripheral and regional kind of

Fig 1. Location of Riparo di Fontana Nuova in south-eastern Sicily. Maps modified from: https://commons.wikimedia.org/wiki/File:Regione_Siciliana_topographic_map-blank.svg# and https://commons.wikimedia.org/wiki/File:Blank_map_of_Europe_(polar_stereographic_projection)_cropped.svg.

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Aurignacian. In fact, by comparing the techno-complex with some of the most typical industries of the Périgord (e.g. Caminade Ouest, Abri Cellier, La Ferrassie) and the few known for the Italian Peninsula, Gioia [7] placed it in phase I of the Aurignacian and connected it directly with the techno-complexes of human groups in France, hypothesizing a rapid movement of this culture from there to Italy [12]. The purported Aurignacian chronology of Fontana Nuova was also supported by arguing that its faunal assemblage differed from that of other Upper Palaeolithic (but in that case Epigravettian) sites on Sicily, which all post-date the Last Glacial Maximum (hereafter LGM). Chilardi et al. [12] argued that the absence of *Equus hydruntinus*, an equid that arrived on the island from southern Italy at the time of its land-bridge connection with Calabria [16], was proof that Riparo di Fontana Nuova was occupied before the LGM. The investigations on the different find categories conducted by Chilardi et al. [12] also concluded that the lithic assemblage, faunal and human remains all suggest that the site was not intensively or repeatedly occupied much over time, and was probably a short-term encampment.

The Aurignacian attributions of the lithic industries from Fontana Nuova were justified by the absence of microliths, backed points and blades (recurrent elements in Sicilian Epigravettian and Mesolithic lithic complexes), and by the presence of middle and large size pieces [12], as well as of what are regarded as diagnostic Aurignacian blades and scrapers. The first to doubt the attribution to the Aurignacian was Palma di Cesnola [9,10], not only because of the features of the assemblage, but also because of the patchy distribution of Aurignacian sites in southern Italy. Originally, Martini [17] was more inclined to accept the first attribution by Laplace [5], agreeing that this lithic industry was a regional and peripheral version of the classic French Aurignacian. In a more recent paper though [13], the Aurignacian attribution was rejected for the first time in favour of a much younger chronology, due to the very close resemblance of the lithic complex from Fontana Nuova with that of Grotta delle Uccerie (Favignana), which has been dated to 13191±120 (LTL1517A) and 12933±75 BP (LTL1518A) [13]. Martini and colleagues have pointed out that the Fontana Nuova complex is missing both the stylistic and structural traits typical of the Italian Aurignacian and that the lithics that have been interpreted as Aurignacoid elements (e.g. sub-carinated scrapers, blades with sinuous margins) are indeed elements compatible with the early (phase 1) Late Epigravettian of Sicily.

The chronological attributions of the lithic assemblage from Riparo di Fontana Nuova have all simply been based on chrono-typological criteria and none of them have been supported by absolute dating of the faunal and human remains, which have been so far considered coeval with the lithic assemblage (e.g. [12]). The main contribution of this research is to provide absolute dates on the skeletal remains.

Materials and methods

A total of 25 human (repository numbers 10207, 10210, 10211) and other faunal remains (no repository numbers available), listed in detail in the Supporting Information (S1 Table), were sampled for the present study at the Museo Archeologico Regionale 'Paolo Orsi' in Syracuse (Italy), where they remain available to other researchers. All necessary permits were obtained for the described study, which complied with all relevant regulations. As the attempt to extract collagen by Chilardi et al. [12] had failed, we exercised great caution in selecting the specimens to pretreat. It should be noted (as can be seen in the photos provided in the Supporting Information) that the preservation is quite homogeneous and that none of the specimens bears traces of consolidants or solvents. A detailed taphonomic study of the small faunal assemblage has not been undertaken, because according to Chilardi et al. [12] the bone surfaces are poorly preserved. Nevertheless, many of the bones in the assemblage bear traces of burning and green fractures, resulting from dismemberment and extensive processing of the carcasses.

Isotopes analyses and AMS radiocarbon dating

Bone pretreatment methods for isotope analyses and AMS radiocarbon dating are those established by Talamo and Richards [18] and the extractions were conducted at the Max Planck Institute for Evolutionary Anthropology in Leipzig (MPI-EVA, lab Code R-EVA).

The two human teeth were micro-CT scanned before sampling at the Department of Human Evolution of the above-mentioned institution. The three-dimensional digital images resulting from the scans are shown in the Supporting Information (S1 and S2 Figs).

The outer surface of the samples was first cleaned with a shot blaster and then 500mg of bone was taken. The samples were decalcified in 0.5M HCl at room temperature until no CO_2 effervescence was observed, usually for about 4 hours. 0.1M NaOH was added for 30 minutes to remove humics. The NaOH step was followed by a final 0.5M HCl step for 15 minutes. The resulting solid was gelatinized following Longin [19] at pH3 in a heater block at 75°C for 20h. The gelatine was then filtered in an Eeze-Filter (Elkay Laboratory Products (UK) Ltd.) to remove small (>80 μm) particles. The gelatine was then ultrafiltered with Sartorius "Vivaspin Turbo" 30 KDa ultrafilters [20]. Prior to use, the filters were cleaned to remove carbon containing humectants [21]. The samples were lyophilized for 48 hours.

The isotope analyses were performed on a Thermo Finnigan Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS) coupled to a Flash 2000 EA. Stable carbon isotope ratios are expressed relative to the VPDB (Vienna Pee Dee Belemnite) standard and stable nitrogen isotope ratios relative to AIR (Atmospheric N_2). The analytical error is 0.2‰ (1σ). Collagen from all 10 samples that yielded well-preserved extracts were sent to the Klaus Tschira Laboratory of the Curt-Engelhorn-Zentrum Archaeometrie in Mannheim (MAMS) for AMS radiocarbon dating, which was performed using the Mini Radiocarbon Dating System (MICADAS) [22]. The results of the AMS radiocarbon dating are reported in Table 1.

The animal remains sampled belong to *Cervus elaphus* (n = 16), *Bos primigenius* (n = 3) and *Sus scrofa* (n = 3), as listed in greater detail in the Supporting Information (S1 Table). Of these, only 8 specimens yielded extracts, all of which can be considered well-preserved collagen (Table 1), according to the quality criteria proposed by van Klinken [23]. All well-preserved extracts from the fauna were used for ZooMS (Zooarchaeology by Mass Spectrometry),

Table 1. Isotopic, elemental analyses and radiocarbon dates.

MPI-laboratory number	species	element	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	%C	%N	C:N	% collagen yield	AMS radiocarbon laboratory code	^{14}C date (BP)
R-EVA-1862	*Cervus elaphus*	humerus	-16.1	6.0	40.7	14.6	3.3	4.3	MAMS-30404	8285 ± 20
R-EVA 1865	*Cervus elaphus*	scafocuboid	-19.8	5.5	35.6	12.8	3.2	4.0	MAMS-30405	8699 ± 22
R-EVA-1866	*Cervus elaphus*	radius	-21.0	5.4	34.8	12.5	3.3	2.1	MAMS-30406	8701 ± 22
R-EVA-1871	*Sus scrofa*	metacarpal III	-20.6	4.9	36.5	13.0	3.3	5.1	MAMS-30407	7775 ± 20
R-EVA-1877	*Cervus elaphus*	tibia	-15.9	6.2	38.8	13.9	3.3	3.5	MAMS-30409	8680 ± 21
R-EVA-1878	*Cervus elaphus*	tibia	-20.3	5.9	40.3	14.5	3.2	4.6	MAMS-30410	8597 ± 22
R-EVA-1880	*Cervus elaphus*	tibia	-16.8	6.1	39.4	14.0	3.3	2.5	MAMS-30411	8793 ± 22
R-EVA-1881	*Cervus elaphus*	phalanx I	-19.5	6.2	37.6	13.6	3.2	5.4	MAMS-30412	8720 ± 22
R-EVA-1895	*Homo sapiens*	molar	-19.3	11.9	42.2	15.2	3.2	4.1	MAMS-30660	8675 ± 25
R-EVA-1896	*Homo sapiens*	premolar	-19.4	12.0	40.2	14.0	3.3	2.2	MAMS-30663	8658 ± 25

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values, elemental compositions, carbon-to-nitrogen ratios and AMS radiocarbon dates of the collagen from well-preserved skeletal remains recovered at Riparo di Fontana Nuova. The *C. elaphus* tibia belong to three clearly different individuals, given that they are all right side specimens. The state of preservation and size of the *C. elaphus* humerus suggest tentatively that a fourth individual may be represented in our isotopic dataset. The samples were pretreated at the Max Planck Institute for Evolutionary Anthropology (Leipzig) and dated at the Klaus Tschira Laboratory of the Curt-Engelhorn-Zentrum Archaeometrie in Mannheim (MAMS).

https://doi.org/10.1371/journal.pone.0213173.t001

according to the methods described below. The three human (*Homo sapiens*) specimens sampled include a cranial (parietal) fragment and two maxillary teeth (a premolar and a molar). According to Chilardi et al. [12], all human skeletal remains from Riparo di Fontana Nuova may have belonged to a single adult individual. The cranial fragment (R-EVA 1883) did not yield an extract. The teeth (R-EVA 1895 and R-EVA 1896) have extracts compatible with well-preserved collagen according to the criteria proposed by van Klinken [23], given that their elemental (%C, %N) and isotopic (δ^{13}, δ^{15}N) compositions, C:N ratios and yields fall within biogenic ranges (Table 1).

aDNA

Ancient DNA (aDNA) extractions and pre-PCR steps were carried out in clean room facilities dedicated to aDNA research at the Ancient DNA Laboratory of the Max Planck Institute for the Science of Human History (MPI SHH) in Jena. Tooth fragments of samples R-EVA 1895 and R-EVA 1896 were used for the analyses. All procedures followed the guidelines on contamination control in aDNA studies [24,25]. The teeth were UV-radiated to remove potential contaminants prior to drilling. Fifty milligrams of powder were used for extraction following a silica-based protocol [26]. Negative controls were included at all steps.

Double-stranded DNA sequencing libraries (UDGhalf) were prepared for each sample according to an established protocol for multiplex high-throughput sequencing [27]. Sample-specific indices were added to both library adapters via amplification with two index primers. The libraries were sequenced on 1/50 of a lane on the HiSeq 3000 (2x75 bp) at the MPI SHH in Jena, using the HiSeq v4 chemistry and the manufacturer's protocol for multiplex sequencing.

The adapter sequences were removed and overlapping paired-end reads were merged with ClipAndMerge, which is a module of the EAGER pipeline [28]. Mapping of the adapter-clipped and merged FASTQ files to the human reference genome hg38 was done using BWA [29] using a reduced mapping stringency of "-n 0.01" and the mapping quality parameter "q 30".

ZooMS

Eight faunal remains, sampled for AMS and isotope analyses were selected for ZooMS (Zooarchaeology by Mass Spectrometry) for species identification, to further strengthen the argument for intrapecies differences in the isotopic composition of *C. elaphus*.

Peptide extraction for ZooMS was carried out at Centre for GeoGenetics, Natural History Museum, University of Copenhagen, Denmark and MALDI-TOF-MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) analysis was subsequently done at the Centre for Excellence in Proteomics at the University of York (United Kingdom).

An average of 16.1 mg of bone was cut off of the selected bone samples using a circular saw-blade attached to a Dremel saw. To circumvent cross-sample contamination the blade was rinsed in 10% bleach and 70% ethanol in between samples.

Prior to protein extraction, each bone chip was incubated in 100 μL of 50 mM ammonium bicarbonate solution (NH_4HCO_3) pH 8.0 (AmBic) for 16 hours at ambient temperature. The samples were then vortexed for 15 seconds and centrifuged at 13,000 rpm for 1 min, and the supernatant discarded. To remove potential humic acids (which interfere with the MALDI--TOF-MS) 100 μL of NaOH was added and the samples were centrifuged at 13,000 rpm for 1 min, the supernatants were discarded and the bone fragments were washed three times in AmBic to neutralize pH. After the final wash the samples were incubated in 100 μL AmBic at 65˚C for 60 minutes to gelatinize the collagen. 50 μL of each supernatant was transferred into a new 1.5 mL eppendorf tube (labelled "extraction"). After cooling to ambient temperature, 1 μL of sequence grade Trypsin (Promega) was added to each of the extractions which were then incubated at 37°C for 16 hours. Following trypsin digestion, the extractions were centrifuged at 13,000 rpm for 1 min and 1 μL of 5% Trifluoroacetic acid (henceforth, TFA) was added to inactivate the trypsin. Peptides were then desalted and isolated using C18 reverse phase resin ZipTips (Pierce), and subsequently eluted in 50 μL of 50% acetonitrile (ACN)/0.1% TFA (vol/vol). 1 μL of the eluted peptides were spotted in triplicate on a ground steel plate using α-cyano-4-hydroxycinnamic acid as the matrix solution [1% in 50%ACN/0.1% TFA (vol/vol/vol)] in a ratio of 1:1 with eluate. Spots were left to dry for three hours. Mass spectrometry was performed using a Bruker Ultraflex III (Bruker Daltonics) MALDI-TOF-MS instrument in reflector mode with laser acquisition set to 1200. The generated spectral output was analysed using the open-source software mMass v.5.5.0 (www.mmass.org) and peptides were identified based on published unique marker ions [30,31].

Results

Radiocarbon dating

The isotopic values and the C:N ratios of all the Fontana Nuova collagen samples fall within acceptable ranges according to standard quality criteria [23]. Moreover, the collagen yields are between 2.1% and 5.4% (Table 1), thus well above the minimum acceptable value (1%). Based on these results, we can confirm the good quality of all the extracts and the validity of all the AMS radiocarbon dates reported in this paper.

We constructed a Bayesian model grouping together all the ^{14}C dates as a single phase (Fig 2), given the lack of stratigraphic information. The two humans are in red within the modelled sequences. To build the model, all the ^{14}C ages were calibrated with the IntCal13 calibration curve [32] using OxCal v4.3 [33]. Calibrated dates are given in cal BP, with both the 68.2% and 95.4% probability range in Table 2. In this way, we provide estimates for the start and end of Fontana Nuova phase within the model, represented by the different start and end boundaries (Fig 2 and Table 2).

The 2σ calibrated age ranges are comprised between 9910–9700 cal BP (R-EVA 1880: 8793 ±22 BP) and 8600–8480 cal BP (R-EVA 1871: 7775±20 BP), which means that they are all early Holocene (and not Late Pleistocene). In addition, it should be noted that, with the exception of two specimens (i.e. R-EVA 1862 and 1871), all other samples have overlapping calibrated age ranges (the overlap is marginal in the case of sample R-EVA 1880, which is the oldest specimen dated in this study). The two human teeth (R-EVA 1895 and 1896) have yielded practically identical dates (respectively 8675±25 BP and 8658±25 BP) and can be considered fully contemporary, which supports the hypothesis proposed by Chilardi et al. [12] (on the basis of their morphology) that these two specimens may belong to the same individual. The start and end boundaries, thus, indicate that Fontana Nuova was surely occupied during the early Holocene, when Sicily was inhabited by Mesolithic, and not Upper Palaeolithic, hunter-gatherers. The human teeth are contemporary with Mesolithic phase II at Grotta dell'Uzzo, whilst the most recent sample (R-EVA 1871) dates to between the late stages of Mesolithic II and the so-called Mesolithic-Neolithic transition [34,35].

Stable isotope analysis

The animal bones that yielded well-preserved collagen are 7 *C. elaphus* specimens, attributable to at least three individuals, and 1 *S. scrofa* specimen (Table 1 and Fig 3). Four red deer and the wild boar specimens have δ^{13}C values comprised between -21.0‰ and -19.5‰, which is compatible with the isotopic composition of environments dominated or characterized exclusively by the presence of C_3 plants. The mean δ^{13}C and δ^{15}N values for the four red deer alone (given

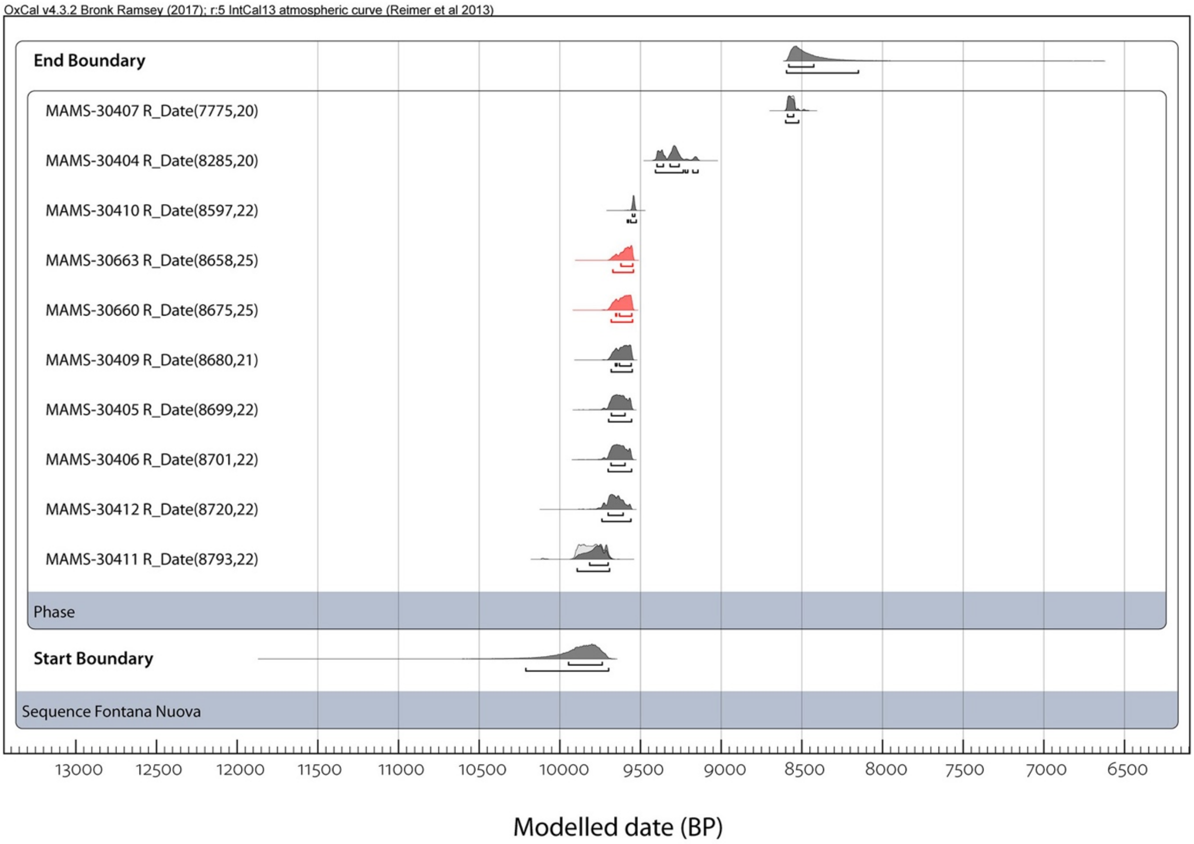

Fig 2. Radiocarbon dates. Plot of the modelled calibrated ages of the ten collagen extracts from human and other faunal skeletal remains recovered at Fontana Nuova. The two human teeth have probability distributions marked in red (MAMS-30660 and MAMS-30663).

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that as discussed above the *S. scrofa* is later than the humans analyzed) are respectively -20.2 ±0.7‰ and 5.8±0.4‰. The $\delta^{15}N$ of the only *S. scrofa* specimen for which data are available has a lower value than red deer, which suggests that the wild boar in question subsisted on plants and did not have a truly omnivorous diet. However, the fact that the single wild boar sample for which we have data is not contemporary to the humans precludes us from attempting to reconstruct the importance of *S. scrofa* relative to *C. elaphus* in the diet of the occupants of Fontana Nuova.

Three specimens identified as *C. elaphus* both on morphological [12] and proteomic grounds, however, have significantly higher $\delta^{13}C$ values between -16.8‰ and -15.9‰ (mean = -16.3‰ ± 0.5‰). The $\delta^{15}N$ values of these specimens are all quite similar (range = 0.2‰) and comprised between 6.0‰ and 6.2‰ (mean = 6.1 ± 0.1‰). This evidence suggests that the red deer in question, which as mentioned above may be represented by three or possibly four individuals, fed on a mixed diet of C_3 and C_4 plants and that there may have been an extremely large variability in carbon isotope ratios in early Holocene SE Sicily. Red deer are intermediate feeders, given that they are mainly browsers but can obtain around a third of their food from grasses, sedges and forbes [37]. This may explain the isotope composition of specimens R-EVA 1862, 1877 and 1880, although it should be noted that at no other Late Pleistocene or

Table 2. Modelled radiocarbon dates.

Fontana Nuova	Unmodelled (BP)				Modelled (BP)			
	from	*to*	*From*	*to*	*from*	*to*	*from*	*to*
	68.2%		*95.4%*		*68.2%*		*95.4%*	
End Boundary					**8580**	**8430**	**8600**	**8150**
MAMS-30407 (7775,20)	8590	8550	8600	8480	8590	8550	8600	8520
MAMS-30404 (8285,20)	9400	9260	9410	9140	9400	9260	9410	9140
MAMS-30410 (8597,22)	9550	9540	9580	9530	9550	9540	9580	9530
MAMS-30663 (8658,25)	9620	9550	9670	9550	9620	9550	9670	9540
MAMS-30660 (8675,25)	9650	9550	9680	9550	9650	9560	9680	9550
MAMS-30409 (8680,21)	9660	9560	9680	9550	9660	9560	9680	9550
MAMS-30405 (8699,22)	9680	9560	9700	9560	9680	9600	9700	9560
MAMS-30406 (8701,22)	9680	9570	9700	9560	9680	9600	9700	9560
MAMS-30412 (8720,22)	9700	9610	9740	9560	9700	9610	9740	9560
MAMS-30411 (8793,22)	9890	9750	9910	9700	9820	9700	9890	9690
Start Boundary					**9950**	**9740**	**10210**	**9700**

Calibrated AMS ^{14}C dating of the Fontana Nuova site (unmodelled). Modelled calibrated boundaries and ages of the site performed using OxCal 4.3 [33] with the IntCal13 curve [32].

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early Holocene Mediterranean site have *C. elaphus* individuals with such high δ^{13}C values been encountered (e.g. [35,36,38–45]).

This is also not the case of other prehistoric specimens from across Europe, even though the whole range of environments in which red deer can be found have been sampled [46–48]. The high δ^{13}C values measured on the bone collagen of R-EVA 1862, 1877 and 1880 are also significantly higher than the range for modern *C. elaphus* [49]. In fact, specimens that lived on the Scottish island of Rum had values no higher than -21.2‰, which actually was interpreted as indicating seaweed consumption. Given that the three above-mentioned red deer bones have δ^{13}C values averaging -16.3‰, this would be indicative of a consumption of marine foodstuffs that is unknown for these cervids. Moreover, had the red deer specimens with enriched ^{13}C consumed seagrasses or seaweed we would expect them to have had different δ^{15}N values relative to their conspecifics, which is not the case. Other possible plants that may have been consumed by the *C. elaphus* in question are sedges and forbes or grasses (including weeds such as *Cynodon dactylon*) [45] with C_4 photosynthetic pathway, neither of which have been attested for prehistoric wild mammals on the northern side of the Mediterranean basin. Similarly, high δ^{13}C values have been recorded in modern *C. elaphus* from North America, albeit from metabolically more active tissues than bone collagen (i.e. muscle) and not even in tissues with more intermediate turnover times such as the hoof [50,51]. These carbon isotope compositions are attained in C_4 grasslands, but even in such cases deer prefer feeding on C_3 plants [50], with possible increases in C_4 plant consumption during warm periods of the year [51]. Further research is needed to establish whether south-eastern Sicily and southern Europe were characterized by the presence of C_4 grasslands and, if so, how *C. elaphus* may have adapted to such conditions. Another theoretically possible interpretation of the high δ^{13}C values is that the red deer in question were not local and had been introduced from parts of the Mediterranean Basin where C_4 plants were widely present even in prehistoric times, although it is not clear why this should have been done given the abundance of this ungulate in Sicily during the early Holocene.

The dentinal collagen extracted from the two human teeth has very similar isotopic compositions (Table 1; Fig 2), suggesting that they may belong to the same individual, as

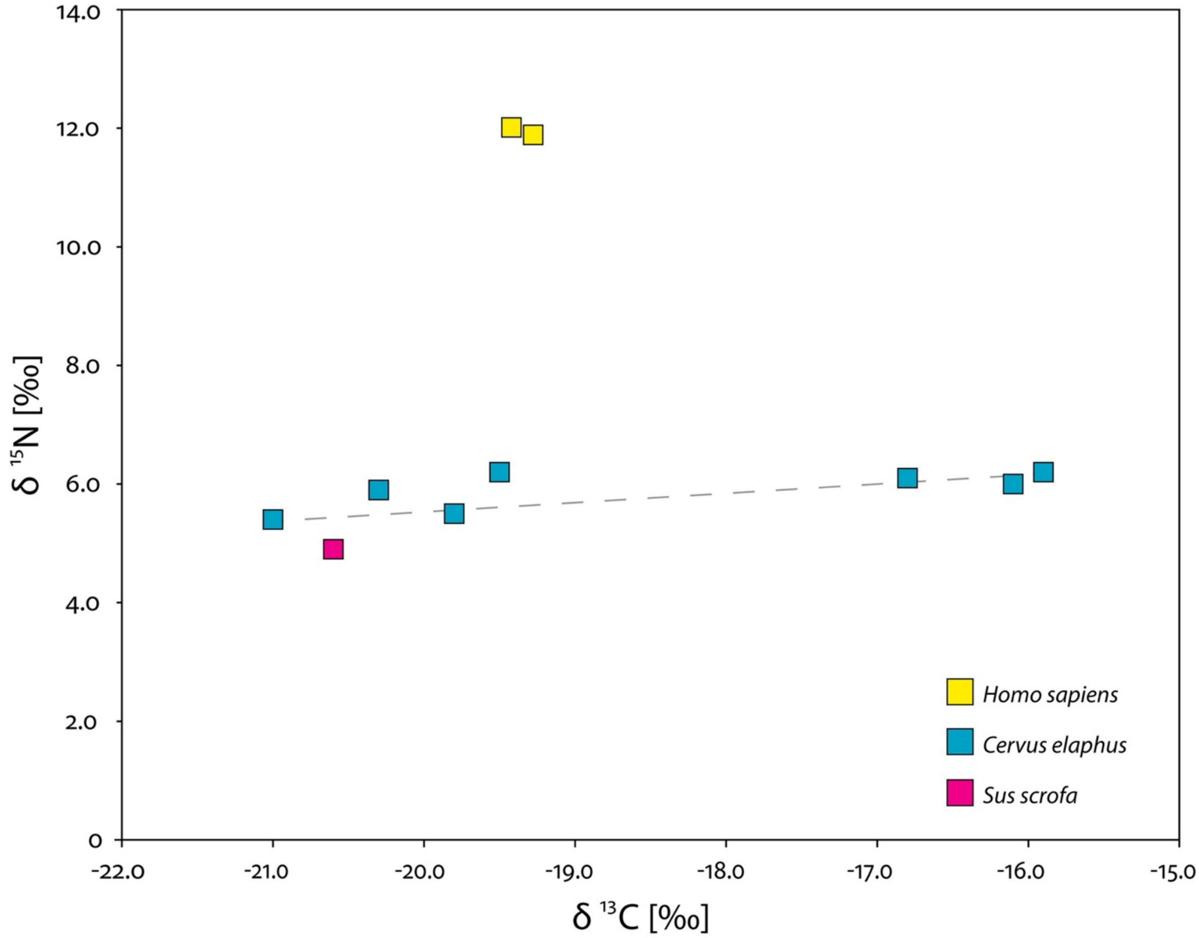

Fig 3. Isotope analyses. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope composition of collagen extracted human and faunal remains recovered at Riparo di Fontana Nuova. The x-axis covers most of the variation in $\delta^{13}C$ values recorded in Mediterranean contexts from fully terrestrial to marine [36].

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hypothesized by Chilardi et al. [12]. The $\delta^{13}C$ values are enriched by around 1.0‰ relative to the mean (= 20.2 ± 0.6‰) for the faunal specimens typical of environments dominated by C_3 plants. The $\delta^{15}N$ values are around 6.2‰ higher than the mean for the four deer specimens contemporary to the humans and typical of environments dominated by C_3 plants. These offsets suggest that the meat of terrestrial animals, such as *C. elaphus* (and possibly *S. scrofa*), were the main sources of dietary protein.

However, given that especially the nitrogen isotope values are offset by more than what is generally thought to be the case for a consumer-prey relationship (e.g. [52]), it is likely that animal resources other than those for which isotope values are available may have contributed around a tenth or more to the diet. One possible species that may have been consumed by the hunter-gatherers of Fontana Nuova is *Bos primigenius*, which in the Mediterranean context is known to have had higher $\delta^{15}N$ values than *C. elaphus* (e.g. [47]). Other possible foods that may result in higher $\delta^{15}N$ values are aquatic resources, such as freshwater and/or marine fauna. As in the case of the Mesolithic hunter-gatherers of Grotta dell'Uzzo [35], however, these are unlikely to have represented important foodstuffs in absolute terms. Overall, the isotopic composition of the two human teeth is typical of Late Pleistocene and early Holocene Mediterranean hunter-gatherers who relied heavily on terrestrial animal protein and partly on aquatic foods (e.g. [35,36,40,41,53]).

aDNA

4.5 and 5.8 million raw reads were generated respectively for samples R-EVA 1895 and R-EVA 1896. We could not identify any authentic human sequences for sample R-EVA 1895. Six reads could be aligned to the human mitochondrial genome and 429 reads to the nuclear genome of sample R-EVA 1896. The low content of authentic human DNA in sample R-EVA 1986 did not allow any further analysis.

ZooMS

Peptide mass fingerprinting by ZooMS followed established protocols. Seven (i.e. R-EVA 1862, 1866, 1871, 1877, 1878, 1880, 1881) samples produced spectra that enabled species identification that were in agreement with morphological examination as part of traditional zooarchaeological analysis (see Table 3). One sample (R-EVA 1865) did not contain preserved peptides to allow for species identification. Six samples were identified as red deer and one as wild boar (spectral triplicate raw data has been uploaded to ADS: https://doi.org/10.5284/1049180). ZooMS is not currently able to distinguish between red deer (*Cervus elaphus*) and European elk (*Alces alces*). This is because the amino acid sequences of their collagen triple helices are almost indistinguishable, and the few peptides displaying differences do not ionize under the current experimental conditions of the MALDI-TOF-MS. However, since elk was not part of the endemic fauna of Sicily during this period [54], the bones in question can be attributed to red deer with confidence.

Discussion

The main outcome of our study is to have clarified the chronology of Fontana Nuova, demonstrating that the faunal and human remains, used by Chilardi and colleagues to back up their attribution of the site to the Aurignacian [12], actually date to the Holocene (9900–8500 cal. BP), when Sicily was occupied by Mesolithic hunter-gatherers. An attribution of the lithic assemblage to the Aurignacian can now be rejected, not only due to the results of our radiocarbon dating, but also given that the lithic assemblage from this site has been re-assigned to the Late Epigravettian by Lo Vetro and Martini [13]. In fact, the finds only include 2 strangulated blades (both not intact, which makes their typological attribution more speculative) and 6 Aurignacian blades [7], representing a small fraction of the overall complex (ca. 6%), and do not include any animal bone tools, typical of the early Upper Palaeolithic culture in question. Given the similarity between Late Epigravettian and Mesolithic ('Undifferentiated Epigravettian') industries in Sicily (where some of the techno-typological features of the latter are rooted in the Epigravettian tradition) [13,55] and in the absence of a new study of the lithic assemblage, it is not possible to exclude that the site of Riparo di Fontana Nuova, as many others on the island, was occupied by hunter-gatherers of both cultures. However, this hypothetical possibility is not the most parsimonious interpretation of the radiocarbon data available for the site, because it would imply that: (1) its small and seemingly discrete assemblage is mixed, and that (2) collagen is preserved by chance only on bones of Holocene/Mesolithic age, whilst the remains that did not yield collagen are Late Pleistocene/Palaeolithic, or that (3) the lithics are mixed, but the fauna is not. Based on our experience on other prehistoric sites on Sicily, the kind of differential preservation of collagen necessary to support these hypotheses has never

Table 3. ZooMs analyses.

Sample name	Laboratory number (R-EVA)	Visual ID	Element	weight mg	P1	A1	A2	B	C	P2	D	E	F1	F2	G1	G2	Barcode ID
Fon-02	1862	*Cervus elaphus*	humerus	16.2	1105.5	trace	1196.6	1427.7	1550.7	1648.8	2131.1	trace	2883.3	2899.3	3018.*	3034.3*	*Cervus elaphus*
Fon-05	1865	*Cervus elaphus*	scafocuboid	12.2	-	-	-	-	-	-	-	-	-	-	-	-	NoID
Fon-06	1866	*Cervus elaphus*	radius	11.1	1105.7	1180.6	1196.6	1427.7	1550.7	1648.8	2131.1	trace	2883.3	2899.3	3018.2*	3034.2*	*Cervus elaphus*
Fon-11	1871	*Sus scrofa*	Metacarpal III	23.5	1105.5	-	trace	1453.8	1550.8	1648.8	2131.1	-	-	-	-	-	*Sus scrofa*
Fon-17	1877	*Cervus elaphus*	tibia	17.1	1105.5	1180.5	1196.5	1427.7	1550.7	1648.8	2131.1	-	2883.3	trace	3019.3*	-	*Cervus elaphus*
Fon-18	1878	*Cervus elaphus*	tibia	13.2	1105.5	1180.6	1196.6	1427.7	1550.8	1648.8	2131.1	-	2883.3	2899.3	3019.3*	3034.3*	*Cervus elaphus*
Fon-20	1880	*Cervus elaphus*	tibia	24.7	1105.5	-	1196.5	1427.7	1550.7	1648.8	2131.1	-	2883.2	-	-	-	*Cervus elaphus*
Fon-21	1881	*Cervus elaphus*	phalanx I	10.8	1105.5	1180.6	1196.6	1427.7	1550.7	1648.8	2131.1	trace*	2883.3	trace	3019.2*	3034.4*	*Cervus elaphus*

Unique species identification peptides observed by MALDI-TOF-MS.

*Deamidated. 1 Da mass shift.

https://doi.org/10.1371/journal.pone.0213173.t003 been recorded at sites occupied in both periods. Moreover, these hypothetical explanations contrast both with Bernabò Brea's on-site observation that all the material originated from the discrete middle layer [14] and with the nature of the material culture and skeletal assemblages suggesting that Fontana Nuova was occupied infrequently and short-term [12]. The fact that all but two of the calibrated age ranges for the specimens dated in this study overlap at 2σ and that the overall modelled age range covers only a period of between 2060 and 1100 calibrated years is fully compatible with an infrequently occupied short-term site.

Overall, there are no more grounds to support the interpretation that Riparo di Fontana Nuova was the southernmost Aurignacian site in Europe [12], as well as the notion arising from it that early Upper Palaeolithic hunter-gatherers crossed the Strait of Messina [2,56]. Paradigm-shifting attributions such as the one made for Fontana Nuova should be based on solid evidence and chronological frameworks, which is not the case in point, especially considering that there are no other Aurignacian sites on Sicily or on any other Mediterranean island. The oldest absolutely-dated archaeological sites in Sicily are, thus, Riparo del Castello (OxA-10040 13485 ±80, 16,900–15,600 cal. BP 2σ) [57] and Grotta delle Uccerie (LTL1517A 13191 ±120) [58], given that the earliest date for Acqua Fitusa has been questioned [59]. The oldest directly-dated human remains, on the other hand, are from Grotta Addaura Caprara (KIA-36055 12890 ±60 BP: 15,950–15,007 cal. BP 2σ) and Grotta di San Teodoro (ETH-34451 12580 ±130 BP: 15,232–14,126 cal. BP 2σ) [40]. This implies that the only undisputed evidence for an Upper Palaeolithic occupation of Sicily is represented by post-LGM, Epigravettian sites and even the plausible hypothesis that the peopling of Sicily may have occurred at the time of the emergence of the land bridge between the island and Calabria (around 21.5–20.0 ka cal. BP) [16] requires evidence in the form of archaeological sites earlier than Riparo del Castello and Grotta delle Uccerie.

A second outcome of our study is to have generated a small isotope dataset that allows us to attempt a broad reconstruction of Mesolithic diets in south-eastern Sicily. The carbon isotope composition of the two analysed human teeth is similar to that of other Mesolithic individuals from Sicily [35,36,41], demonstrating that Holocene hunter-gatherers on the island had a subsistence that was heavily based on hunting and on the consumption of the meat of terrestrial mammals. The only difference between the isotope composition of the teeth from Fontana Nuova and of other Mesolithic human remains from Sicily is represented by their high nitrogen values, which may indicate some reliance on aquatic foods or a different nitrogen isotope baseline in this part of the island.

The possibility of different isotopic baselines should also be considered in attempts to explain the high carbon isotope values on three *C. elaphus* specimens, for instance in connection to short-term climatic and environmental instability attested in Sicily during the early Holocene [60]. This hypothesis can probably be discounted, given that one of the deer samples (R-EVA 1862) does not overlap chronologically with the other two and that a similar hypothesis would thus imply an anomalous niche partitioning by red deer. In fact, the high carbon isotope ratios of R-EVA 1862, 1877 and 1880 may be indicative either of consumption of C_4 plants from extensive grasslands (the presence of which is not attested in south-eastern Sicily), of forbes, sedges and wetland C_4 vegetation (although it is not clear where such environments would have been located in the case of Fontana Nuova) or of their non-local origin. In the absence of human intervention in deer feeding, the first two scenarios would, as mentioned above, imply an unprecedented degree of niche partitioning within the same species, which is not likely in nature. In the latter case, it is possible that the deer originated from regions with a relatively high proportion of C_4 grasses, which are more common around the eastern or southern coasts of the Mediterranean Sea, as hypothesized for an ovicaprid with similar isotopic values (δ^{13}C: -16.2‰; δ^{15}N: 6.3‰) from the site of Grotta d'Oriente on Favignana in western

Sicily [41]. Another working hypothesis is that the three bones in question belonged to individuals that had been fed by humans, as part of proto-breeding of *C. elaphus*, which is something that has been speculatively claimed only for protohistoric times in Sicily [61]. This has also been hypothesized in the case of cervids in Cyprus [62], where it has been demonstrated that ungulates were introduced by boat in efforts by foragers to maintain viable stocks for hunting [63]. However, given that *C. elaphus* was common in Sicily during the early Holocene, it is not clear why these animals should have been introduced. To date the only prehistoric ungulates from southern European contexts that have been found to have $\delta^{13}C$ values as high as the three deer specimens from Fontana Nuova are domestic cattle specimens from the Late Neolithic site of Makriyalos in northern Greece [45]. Recent analyses on *Bos taurus* from this site have shown that some individuals acquired large proportions of their feed from pastures rich in C_4 vegetation that may have included weeds such as *Cynodon dactylon* or, more likely, plants in marshy coastal environments enriched in ^{13}C. In the case of the cattle from Makriyalos, their feeding areas were influenced by human management, whilst it is not clear why, if the ^{13}C-enriched *C. elaphus* were actually feeding in coastal marshes, this behavior has not been documented in any of their conspecifics elsewhere in the Mediterranean or further afield.

Conclusions

The AMS radiocarbon dates on collagen from ten skeletal remains retrieved at Riparo di Fontana Nuova assign both the fauna and humans to the Holocene. As these were used by Chilardi et al. [12] to argue an early Upper Palaeolithic occupation, we believe that the attribution of the site to the Aurignacian should be discarded. A late Upper Palaeolithic (i.e. Late Epigravettian) occupation cannot be discounted outright, although hypothesizing it requires a non-parsimonious explanation: either that the lithic assemblage is mixed but the bones are not or that by chance collagen was only preserved on bones of Holocene age. There are, thus, no longer credible claims for early Upper Palaeolithic sea crossings to large Mediterranean islands, which has important implications for our knowledge of prehistoric voyaging in this enclosed sea. As far as Sicily is concerned, our findings hopefully signal the end of speculation on the peopling of the island based on materials recovered from undocumented contexts and poorly-dated sites. Only new surveying and excavation campaigns will, thus, enable us to explore further when the largest Mediterranean island was first settled by anatomically modern humans.

Supporting information

S1 Table. Human and faunal skeletal remains sampled for isotopic and radiocarbon analyses.
(DOCX)

S1 Fig. Three-dimensional digital model of the right maxillary M2.
(JPG)

S2 Fig. Three-dimensional digital model of the left maxillary P3.
(JPG)

S3 Fig. *Cervus elaphus*, humerus (Fon-1), before the sampling.
(JPG)

S4 Fig. *Cervus elaphus*, humerus (Fon-2), before the sampling.
(JPG)

S5 Fig. *Cervus elaphus*, humerus (Fon-3), before the sampling.
(JPG)

S6 Fig. *Cervus elaphus*, fermur (Fon-4), before the sampling.
(JPG)

S7 Fig. *Cervus elaphus*, scaphucuboid (Fon-5), before the sampling.
(JPG)

S8 Fig. *Cervus elaphus*, radius (Fon-6), before the sampling.
(JPG)

S9 Fig. *Cervus elaphus*, ulna (Fon-7), before the sampling.
(JPG)

S10 Fig. *Cervus elaphus*, humerus (Fon-8), before the sampling.
(JPG)

S11 Fig. *Sus scrofa*, metatarsal II (Fon-9), before the sampling.
(JPG)

S12 Fig. *Sus scrofa*, metacarpal II (Fon-10), before the sampling.
(JPG)

S13 Fig. *Sus scrofa*, metacarpal III (Fon-11), before the sampling.
(JPG)

S14 Fig. *Bos primigenius*, cuneiform (Fon-12), before the sampling.
(JPG)

S15 Fig. *Bos primigenius*, vertebra (Fon-13), before the sampling.
(JPG)

S16 Fig. *Bos primigenius*, molar (Fon-14), before the sampling.
(JPG)

S17 Fig. *Cervus elaphus*, molar III (Fon-15), before the sampling.
(JPG)

S18 Fig. *Cervus elaphus*, molar I or II (Fon-16), before the sampling.
(JPG)

S19 Fig. *Cervus elaphus*, tibia, right (Fon-17), before the sampling.
(JPG)

S20 Fig. *Cervus elaphus*, tibia, right (Fon-18), before the sampling.
(JPG)

S21 Fig. *Cervus elaphus*, tibia, right (Fon-19), before the sampling.
(JPG)

S22 Fig. *Cervus elaphus*, tibia, right (Fon-20), before the sampling.
(JPG)

S23 Fig. *Cervus elaphus*, phalanx I (Fon-21), after the sampling.
(JPG)

S24 Fig. *Cervus elaphus*, calcaneus (Fon-22), after the sampling.
(JPG)

S25 Fig. *Homo sapiens*, parietal fragment (Fon-23), before the sampling.
(JPG)

S26 Fig. *Homo sapiens*, molar II (Fon-24), before the sampling.
(JPG)

S27 Fig. *Homo sapiens*, premolar III (Fon-25), before the sampling.
(JPG)

S1 Appendix. OxCal code.
(DOCX)

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