

University of Tartu
Institute of History and Archaeology
Department of Archaeology

Report on the analysis of microremains from Estonian Bronze Age human dental calculus

Agnes Unt

Tartu 2025

Contents

1. Data description	3
2. Methods.....	4
3. Results: a detailed description of microremains	8
3.1. Kingdom Plantae	9
3.2. Kingdom Animalia.....	15
3.3. Kingdom Fungi	17
3.4. Non-living nature	18
References.....	19

1. Data description

This dataset represents the results of dental calculus analysis from three individuals' teeth buried at Jõelähtme cemetery during the Bronze Age in Estonia. The dataset accompanies the article "Segregated food culture? Bronze Age (1250–500 cal BCE) dietary practices in Northern Estonia", submitted for publication in the journal *Archaeological and Anthropological Sciences*. The authors of the article are Mari Tõrv (corresponding author), Shidong Chen, Agnes Unt, Kristiina Johanson, Eve Rannamäe, Liivi Varul, Sandra Sammler, Holar Sepp, Valter Lang, Andres Tvauri, Siim Salmar, John Meadows and Ester Oras.

These analysed samples were:

- K1–3 Jõelähtme (AI 5306: 9D4 1; grave 1: 3)
- K2–3 Jõelähtme (AI 5306; grave 2: 3)
- K7–2 Jõelähtme (AI 5306; grave 7:2, cist (3))

2. Methods

In microfossil analysis it is important to monitor and prevent contaminants. The method for decontamination and extraction for using ancient dental calculus was adapted from MacKenzie et al. (2021), Cristiani et al. (2018; 2016), and Warinner et al. (2014).

The laboratory is a sealed room that is regularly checked for airborne pollutants before dental calculus extraction and mounting processes.

- A clean slide without a cover slip with 2–3 drops of a 1:1 solution of glycerol-distilled water mixture was left in the room for one day.
- This slide was analysed by light microscopy with magnifications up to 400x for any pollutants in the room.

The analyst wore no natural fibres, no makeup and shoes only worn inside the building. Their hair was covered, and they wore powder-free nitrile gloves. To understand the possible debris from the analyst's clothes and shoes, samples were taken from these parts using Sellotape; this was then stuck to a clean slide and analysed under light microscopy.

Prior to sampling, the teeth were photographed from every side, weighed before extraction and then soaked in ultrapure water in a sealed 10 ml test tube for 2–3 days to loosen the soil and the calculus. The calculus was then transported onto a clean Petri dish and a picture of the soil-covered calculus was taken before the cleaning process began. The cleaning process was done under a Nexius Zoom Evo stereomicroscope with magnifications up to 110x. For the cleaning, both, a 0.06 M and a 0.1 M HCl acid was used. The use of either solution was decided depending on the nature of the soil and the calculus. An acupuncture needle was dipped in the solution and a small part of the acid was put onto the calculus to decontaminate it and get rid of soil and other dirt adhering to it. The analyst applied the acid onto the calculus, watched dirt dissolve and once it had sufficiently dissolved, put ultrapure water onto the acid to neutralise it. The calculus was scraped clean of any dirt visible through the stereomicroscope. The acupuncture needle was held firmly from close to the tip to maintain some rigidity as it can be too bendy to work with if held from the top. A clean sewing needle was also used when needed.

Table. Teeth from Jöelähtme cemetery used for the microfossil analysis of dental calculus.

Context	Photo before the extraction	Photo after the extraction
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K1-3:

Jöelähtme (AI
5306: 9D4 1;
grave 1: 3)

Lower C, right

Weight of dry
bone (g): 1.4819

DC (g): 0.0025



K2-3:

Jöelähtme (AI
5306; grave 2: 3)

Lower M1, left

Weight of dry
bone (g): 1.3711

DC (g): 0.0012



K7-2:

Jöelähtme (AI
5306; grave 7:2,
cist (3)

Lower M2, left

Weight of dry
bone (g): 2.5468

DC (g): 0.0019



Once the calculus was clean, a picture was taken. A clean 2 ml Eppendorf tube was weighed before the calculus was put inside of it. For the extracting of calculus from the tooth, a sterile scalpel was used to scrape the calculus off. It was then carefully transported into the Eppendorf and the clean calculus was weighed.

Dissolving the outer layer of the calculus was done to get rid of any remaining dirt that may remain on the calculus.

1. A drop of HCl (either 0.06 M or 0.1 M, depending on the calculus) was put in a 2 ml Eppendorf with a clean pipette containing the cleaned dental calculus. It was left for 5 minutes and then held with tweezers in the sonicator for 30 seconds to loosen it up.
2. As much liquid as could be extracted was pipetted out and put in a separate clean 2 ml Eppendorf for future reference (see below for the mounting process).
3. A few drops of HCl were added to the original Eppendorf containing the clean dental calculus to begin the decalcifying stage.

The decalcifying stage varies in duration. Sonicating may help loosen and dissolve the calculus, but ultimately the acid is what is needed. Seeing as the calculus neutralises the acid, oftentimes a few drops will not be enough. Therefore, the neutralised calculus-acid mixture is pipetted out and mounted on microscope slides. This is the solution that contains the microfossils. The mounting process goes as follows:

1. Clean pipettes (rinsed with ultrapure water before use) are prepared, the work area is covered with aluminium foil. Microscope slides are taken out of their plastic cases and rinsed with ultrapure water to make sure no debris is sticking to it. A fresh solution of 50-50 glycerol-water is made. The cover slips are prepared, and the analyst is wearing appropriate clothing (see above for a detailed description).
2. As much liquid as possible is extracted from the 2 ml Eppendorf containing the neutralised calculus and HCl. This is pipetted onto a previously cleaned microscope slide and a drop of the glycerol-water mixture is added to allow for the rotation of the microfossils under light microscopy. The slide is then sealed with a cover slip.
 - a. It is important to note that no large visible chunks of dental calculus should end up on the microscope slide – in such a case the microfossils are still entrapped, and they cannot be analysed by light microscopy this way. Large chunks need to be kept in the Eppendorf and gradually dissolved by adding more HCl after mounting.

The slides were scanned with an Olympus BX51 microscope with magnifications up to 400x. Polarised light was also used. All debris relating to diet and environment was recorded and photographed. Microfossils could be rotated by tapping gently on the cover slip of the slide. Some microfossils will eventually overlap, for this tapping and zooming were used to make sense of the microfossils.

3. Results: a detailed description of microremains

The microfossils are categorised by kingdoms; debris from non-living nature are categorised separately. Within each kingdom, a categorisation based on the possibility of deliberate consumption is made. As no species level identification is present, the nature of the debris allowed to differentiate between possible deliberate and non-deliberate consumption. This distinction is made by the likelihood of a microfossil's origin into dental calculus. The identifications will rely on publications (see e.g., Chantran and Cagnato 2021; MacKenzie et al. 2021; Henry et al. 2020; Radini et al. 2017; Cristiani et al. 2016; Hardy et al. 2016; Leonard et al. 2015; Tromp and Dudgeon 2015) and UT plant reference collections.

The distinction between deliberate and non-deliberate consumption (Table 1) is more apparent with starch granules than with many other microremains. Numerous edible parts of plants contain nutritious starch granules. Therefore, it is likely that past humans ate these plant parts, and starch granules found their way to dental calculus. Recent studies have, however, shown that starch granules may enter the mouth from other activities than eating, such as grinding (Delaney et al. 2023). Distinguishing if a starch granule entered the mouth via eating or grinding is nearly impossible.

With other microremains such as burnt debris, the distinction is even harder to make. Burnt debris may come from different Kingdoms of Life and may therefore end up embedded in calculus via different ways – perhaps from working near fire and inhaling smoke or from charred pieces of food. Minerals (i.e. non-living nature) may originate from the soil found on uncleaned food; fungi may end up in dental calculus by way of plant pathogens; from the soil on uncleaned food. Fibres have a good chance of making it to dental calculus by way of crafts (MacKenzie et al. 2021, 5).

Particles measuring up to 70 µm in size can be inhaled (King Se et al. 2010, 297). Therefore, many of the microremains could have been incorporated into the dental calculus matrix through breathing, not only through food. This hypothesis of the mouth trapping debris by inhalation has been given in other publications as well (Laurence et al. 2011; Hardy et al. 2016; Radini et al. 2017; Radini et al. 2023). It ought to be noted that it is rare to see particles in dental calculus that measure more than 200 µm (Anita Radini, pers. comm. in April 2023).

Table 1. All found microremains from Jõelähtme dental calculus.

Kingdom	Deliberate/non-deliberate	Sample name	k2-3	k7-2	k1-3	Total	% of total
Problematic debris	Non-deliberate consumption	Burnt debris	present	20	11	32	14.1%
Non-living nature	Non-deliberate consumption	Mineral	3	2	6	14	6.2%
Fungi	Non-deliberate consumption	Fungi	11	8	1	20	8.8%
Plantae	Deliberate consumption	Triticeae starch	2	0	1	3	1.3%
Plantae	Deliberate consumption	Fabaceae starch	1	0	1	2	0.9%
Plantae	Non-deliberate consumption	Burnt phytolith	1	1	0	2	0.9%
Problematic debris	Deliberate consumption	Cf flesh	1	0	1	2	0.9%
Plantae	Non-deliberate consumption	Fibre	3	3	4	10	4.4%
Plantae	Non-deliberate consumption	Plant cell	2	1	3	6	2.6%
Plantae	Deliberate consumption	Starch	14	7	6	27	11.9%
Fungi	Non-deliberate consumption	Yeast?	1	0	0	1	0.4%
Plantae	Non-deliberate consumption	Unidentified plant debris	6	5	1	12	5.3%
Animalia	Non-deliberate consumption	Mouth (epithelium) cells	10	4	3	17	7.5%
Fungi	Non-deliberate consumption	Hypa	4	2	1	7	3.1%
Plantae	Non-deliberate consumption	Phytolith	1	2	0	3	1.3%
Plantae	Non-deliberate consumption	Plant hair	2	3	1	6	2.6%
Animalia	Non-deliberate consumption	Insect parts	1	2	0	3	1.3%
Plantae	Non-deliberate consumption	Conifer wood	0	2	1	3	1.3%
Plantae	Non-deliberate consumption	Wood	1	5	10	16	7.0%
Fungi	Non-deliberate consumption	Spore	3	2	1	6	2.6%
Plantae	Non-deliberate consumption	Plant epithelium	0	2	0	2	0.9%
Problematic debris	Non-deliberate consumption	Ambiguous debris	2	4	0	6	2.6%
Plantae	Non-deliberate consumption	Burnt wood	0	2	0	2	0.9%
Animalia	Deliberate consumption	Cf bone	2	1	0	3	1.3%
Bacteria	Non-deliberate consumption	Bacteria	4	1	10	15	6.6%
Plantae	Non-deliberate consumption	Oxalate	1	0	0	1	0.4%
Plantae	Non-deliberate consumption	Pollen	1	0	0	1	0.4%
Animalia	Non-deliberate consumption	Feather	1	0	0	1	0.4%
Plantae	Deliberate consumption	Compound starch	0	1	0	1	0.4%
Plantae	Non-deliberate consumption	Microcharcoal	1	0	0	1	0.4%
Non-living nature	Non-deliberate consumption	Salt crystal	2	0	0	2	0.9%
						Total	227

3.1. Kingdom Plantae

Starch was found from all three sampled teeth. They were described and identified based on their shape, surface features, their extinction cross under cross-polarised light, measurements and characteristics of their hilums (Table 2). The ancient starches were routinely compared to modern reference material.

Table 2. Morphological features of starch granules, adopted from Radini (2016) and MacKenzie et al. (2021).

Type	Morphology	Identification and references
1	Bimodal distribution with A- and B-type granules. Shape round to oval, granules are simple. Cracks and fissures may be visible on the surface. Hilum is central, extinction cross under cross polarised light appears clear and X-shaped. Lamellae visible.	Triticeae (MacKenzie et al. 2021; Cristiani et al. 2016; BeMiller and Whistler 2009; Henry et al. 2009; Winton and Moeller 1906)
2	Round, elliptical, kidney- or oval-shaped simple granules. Sunken hilum in the centre, granules often have cracks and fissures. Extinction cross with possibly multiple ‘arms’. Lamellae visible.	Fabaceae (Punia et al. 2019; Cristiani et al. 2016; Henry et al. 2009; Ratnayake et al. 2001; Winton and Moeller 1906)
3	Polyhedral compound granules. Extinction cross and a central hilum. Lamellae are not visible.	Compound starch – possibly <i>Avena</i> (Gismondi et al. 2018; Matsushima 2015; Mariotti Lippi et al. 2015)
4	Other	Most likely these are starches from wild gathered plants. Possible taxa may be <i>Quercus</i> or the likes of <i>Typha latifolia</i> .

Bimodal distribution of smaller and larger lenticular and round granules is characteristic of the Triticeae tribe (Winton 1906: 64, 69). The possible cultivated Triticeae plants could have been from the genera *Hordeum*, *Triticum* and *Secale* (Figure 1). Kidney or oval-shaped starch (Figure 2) belongs to the family Fabaceae. These can be grown as food (*Vicia faba*, *Pisum sativum*), Fabaceae plants also grow as weeds of crops; in addition, these can be eaten raw for example as a snack (Cristiani et al. 2016). Compound starch (Figure 3) is found in many plants, both cultivated (*Avena* – see for example Mariotti Lippi et al. (2015)) and in foraged foods, for example *Phragmites australis* and many other Poaceae plants, (see supplementary materials of Cagnato et al. 2021; Matsushima 2015, 427). Compound starch granules can be found in wild plants like *Trapa natans* (see more in Henry 2020, 99) as well.

Avena spp. plants were most likely a weed of the cultivated crops during the Bronze Age in Estonia (Kriiska et al. 2020, 177). The category of ‘other’ (Figure 4–5) was created due to a lack of reference data to be able to correctly identify these granules. Based on existing reference collection data, many of these granules seemed to be of wild gathered plant origin, possibly belonging to the genus *Quercus*. A similarity to the starch granules of *Typha latifolia* was noted as well.

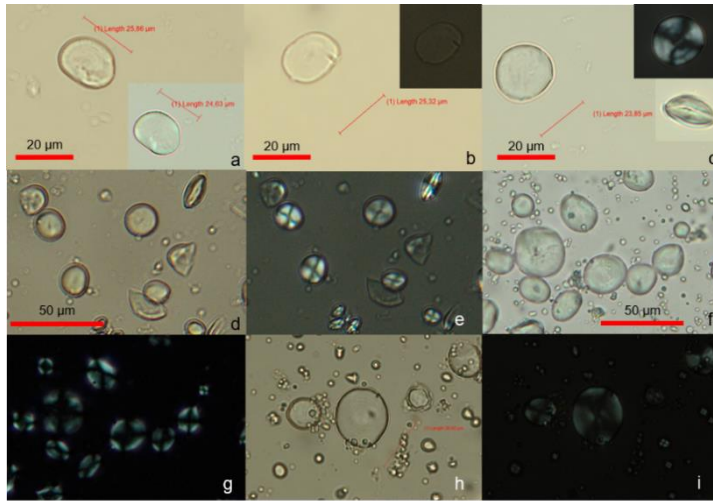


Figure 1. a, b, c – Triticaceae starch granules from the archaeological samples. D, e – *Triticum aestivum* starch from modern reference collection; f, g – *Hordeum vulgare* starch from modern reference collection; h, i – *Secale cereale* starch from modern reference collection. Note the A- and B-type starches in the modern reference images.

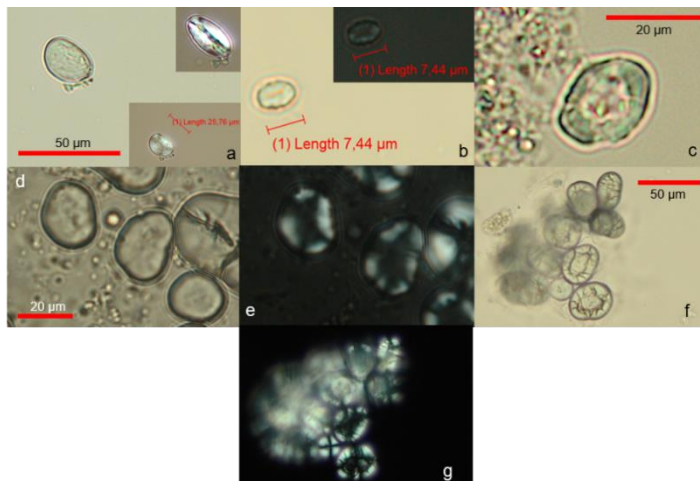


Figure 2. Fabaceae starch granules from the archaeological samples (a–c) and modern reference material (d–g); a – Fabaceae starch granule, above: the same Fabaceae starch granule when turned. b – poorly preserved possible Fabaceae starch granule; c – poorly preserved Fabaceae starch granule; d, e – *Pisum sativum* starch granules; f, g – *Vicia faba* starch granules.

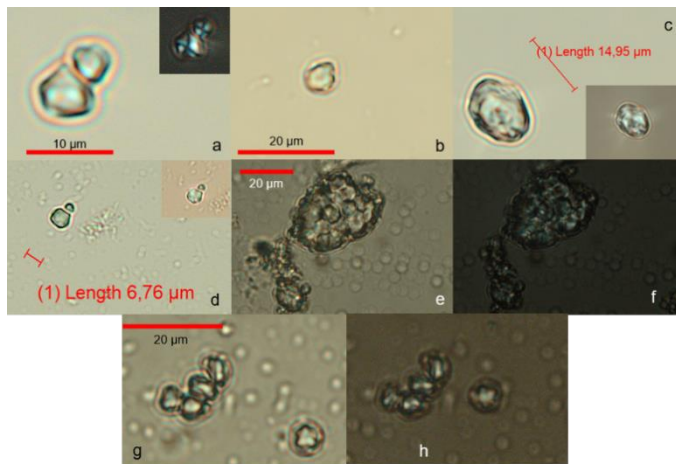


Figure 3. Compound starches (a–d) from archaeological samples; (e–h) modern reference material. a – compound starch granule; b – cf *Avena* starch granule (no polarised picture available); c – cf polyhedral starch granule; d – polyhedral starch granule; e–h – *Avena sativa* starch granules in a cluster, rhombous sides showing.

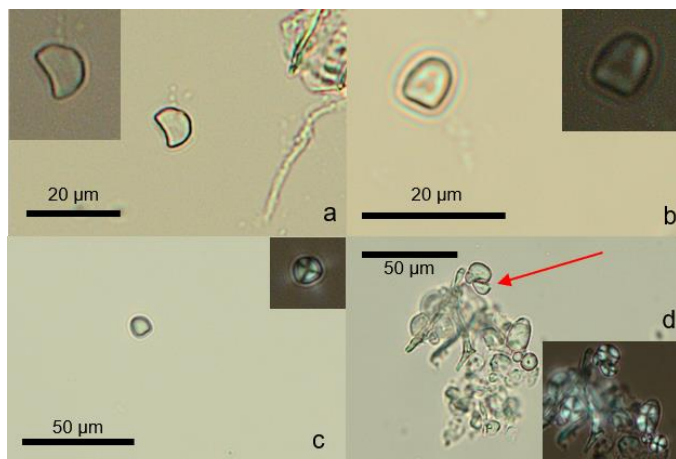


Figure 4. ‘Other’ starch granules, archaeological (a–b), reference collection (c–d): a – semilunar starch granule, showing barely any residual birefringency; b – semilunar starch granule; c – modern *Typha latifolia* starch granule; d – *Quercus* starch granules, note the semilunar one.

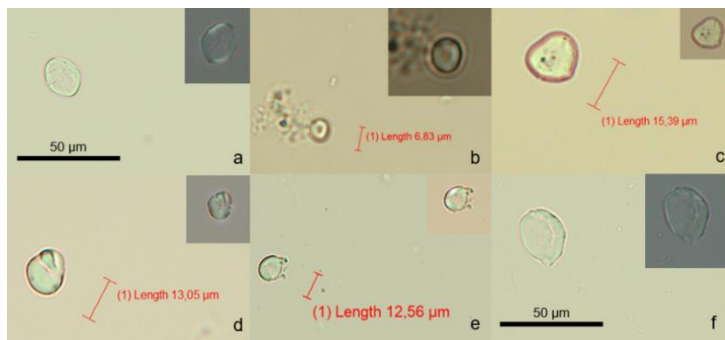


Figure 5. ‘Other’ unidentified starch granules: a – showing residual birefringency; b – very little residual birefringency; c – does not show any polarising effects under cross-polarised light; d – starch with defect; e –

starch granule; f – swollen starch.

Only two phytoliths were found (Figure 6). The elongated sinuated edge burnt phytolith could be a very damaged and burnt dendritic, however, the researchers did not have enough confidence in the material to state this. Dendritic phytoliths are found in the glumes of *Triticeae* plants, they are in many cases indicative of Triticeae plants having been eaten (see for example Tubb et al. 1993). As the state of preservation in the phytolith seen below, no such claims can be made at this time. Elongated phytoliths are common in plants (see for example (Lu et al. 2006)) and do not ascertain to any specific species. Piperno (2006, 121, 145) states that polyhedral phytoliths are produced in the stems, leaves and glumes of cereals such as wheats and barleys. Novello and Barboni (2015: 6) found polyhedral phytoliths from many different grass plants. See more on identifying phytoliths from Shillito (2013).

Wood was identified to division level – conifers. The small, bordered pits were visible (Figure 7), making it possible to identify it (see also Radini et al. 2016). One single pollen granule was found (Figure 8), it was identified as conifer pollen (see for comparison Owens et al. 1998: Fig 1. (b)). Fibres from plants are a common find in archaeological dental calculus. Fibres may be indicative of crafts (for example Buckley et al. 2014, 8), processing raw material, oral hygiene or from the remains of food (Hardy et al. 2016, 133). See more on identifying fibres Bergfjord and Host (2010). The fibres in the analysed dental calculus showed signs of damage (Figure 9).

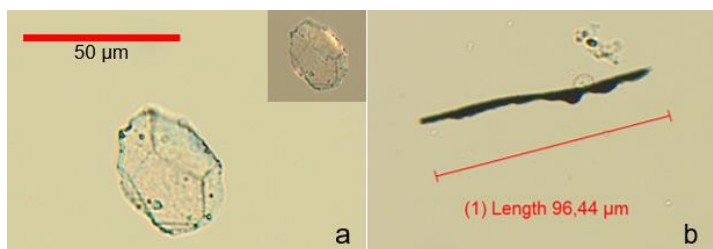


Figure 6. Phytoliths: a – possible polyhedral psilate; b – elongated, with a sinuated edge, burnt. Scale bar 50 microns.

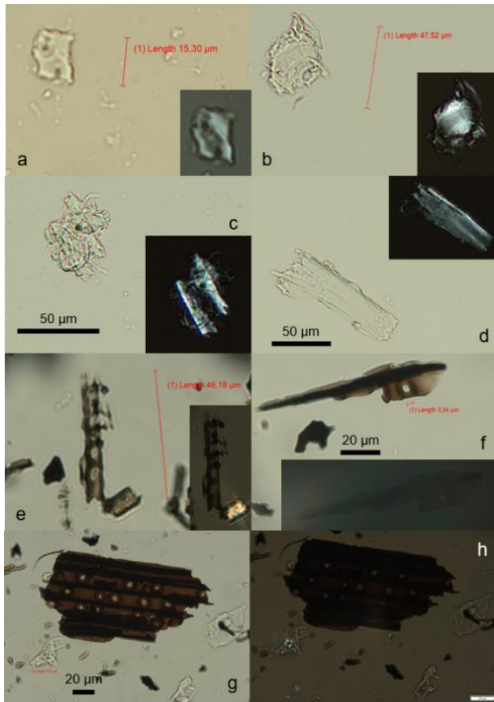


Figure 7. Woods from archaeological samples (a-d) and modern reference data (e-h): a – conifer wood; b – similar feature under cross-polarised light to starches – cross; c – processed wood; d – wood; e – burnt *Picea abies*, pits visible; f – burnt *Pinus sylvestris* wood pit measured; G, h – *Pinus sylvestris* burnt wood, larger chunk, small pit shows features under cross-polarised light.

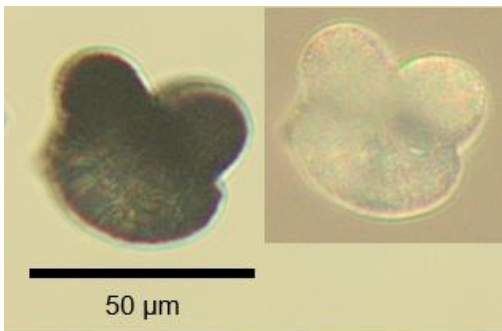


Figure 8. Conifer pollen.

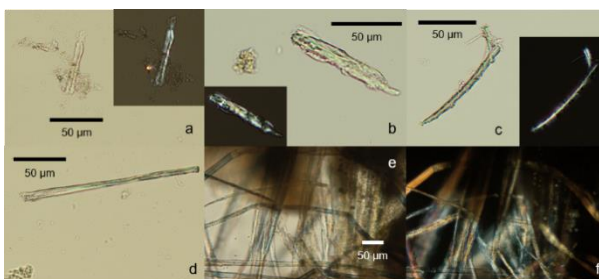


Figure 9. Fibres from archaeological samples: a – fibre, note the undissolved dental calculus near the fibre. b, c, d – fibres; e, f – fibres from reference collection, withered *Urtica*.

3.2. Kingdom Animalia

Some possible bone fragments were found (Figure 10). These can be interpreted as ending up in food as a by-product of eating meat or fish.

It is natural to assume that insects were not unknown to people in the Bronze Age. Therefore, the presence of insect debris is to be expected. Insect remains are discussed in Henry (2020, 290), MacKenzie et al (2021, 6) and in Hardy et al.'s (2016) supplementary materials. Henry (2020, 290) states that large insect body parts such as thoraxes, legs, wings, mandibles and heads allow for identification of taxa. Seeing as the Jöelähtme samples yielded only small fragments, no identification was possible (Figure 11). One microfossil was reminiscent of a butterfly (see for comparison Hardy et al. (2016, 132)).

Epithelial cells are found in many animal tissues (Figure 12). Epithelial mouth cells are associated with the development of dental calculus (Tinanoff and Gross, 1976). Epithelial cells are shed from the inner lining of the mouth and can end up in dental calculus. These cells can also hold DNA. Examples of these cells can be seen in Blondiaux and Charlier (2008, 8).

One possible fragment of a feather was found (Figure 13). Feathers in dental calculus are discussed in Henry (2020: 294) and in MacKenzie et al. (2021, 6).

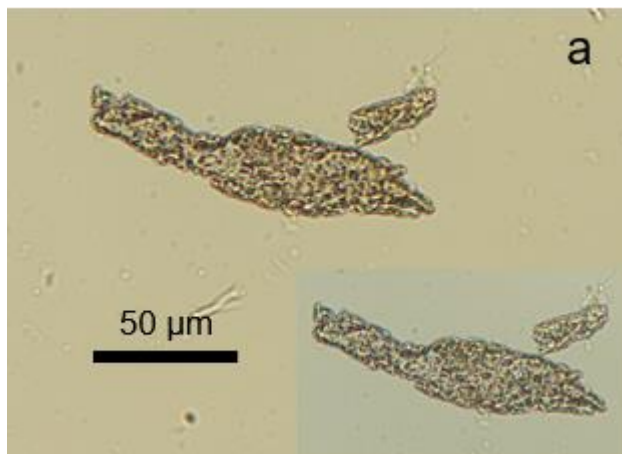


Figure 10. a – cf bone fragment, lower under cross polarised light with a long exposure time.

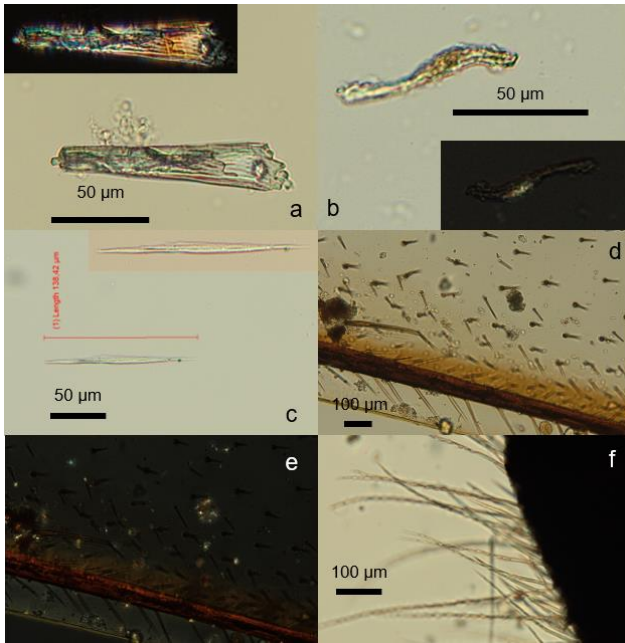


Figure 11. Insect remains from dental calculus (a–c), reference material (d–f): a – cf insect part, b – possible insect hair; c – cf butterfly debris, polarising picture with long exposure time; d – modern reference insect wing and hairs visible; e – insect wing and hairs under cross-polarised light; f – insect hairs visible.

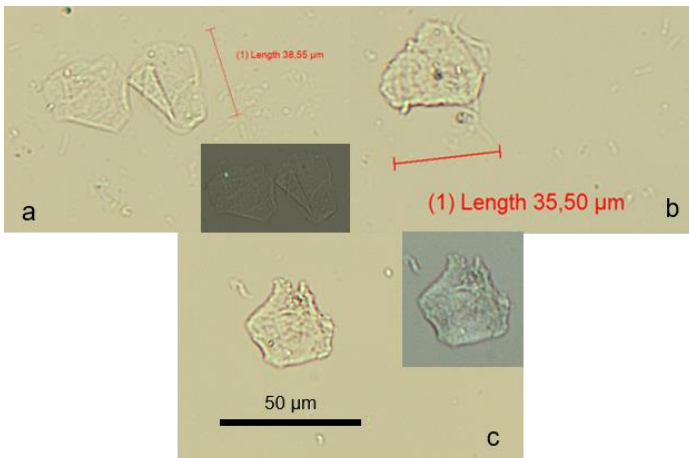


Figure 12. Epithelial mouth cells from dental calculus. a, b, c – cf epithelial mouth cells.

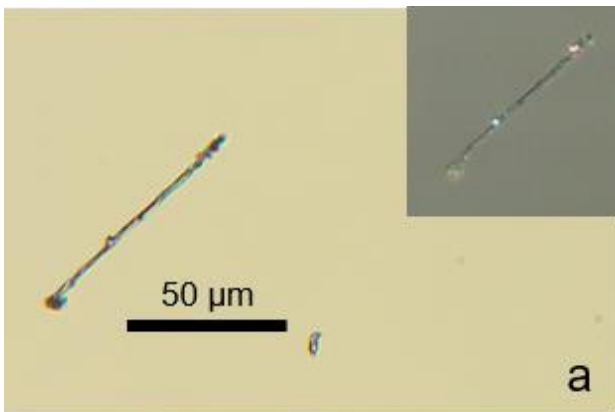


Figure 13. a – cf feather.

3.3. Kingdom Fungi

Even though fungi have their own Kingdom of Life, distinguishing fungal matter to taxonomic ranks is difficult due to the remains looking very similar to one another. This difficulty is discussed in MacKenzie et al. (2021, 7). Fungal debris in dental calculus can inform us of environmental conditions, it can even be indicative of plant pathogens such as *Alternaria* (MacKenzie et al. 2021, 7) and shed light on people's hygiene and health conditions (Afonso-Vargas et al., 2015). Intentionally consumed fungus has been found in dietary studies as well (Power et al. 2015). The only identifiable fungal debris (Figure 14) were Glomeromycota type remains (see for comparison Thangavelu and Raji 2016: Fig 2; Błaszowski et al. 2023). A possible identification of *Alternaria*-type fungal debris can be seen in the figure below (4) (see for comparison Woudenberg et al. 2015).

One possible particle of yeast was found (Figure 15). See for comparison (Volkov 2015: Figure 1).

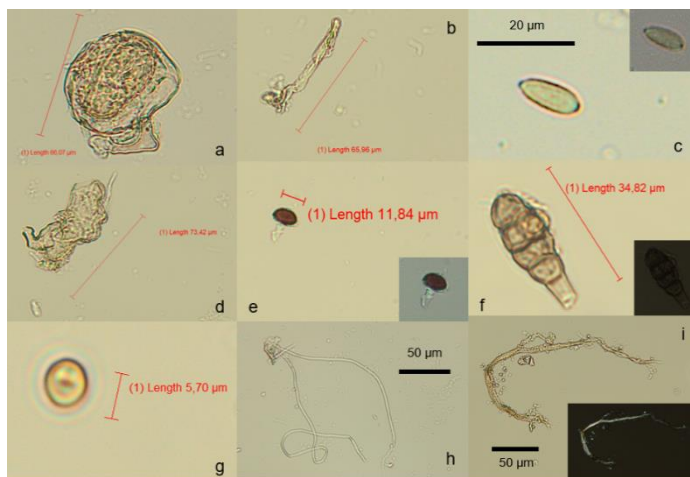


Figure 14. Fungi from archaeological samples (a–i): a – Glomeromycota-type fungal fruiting body; b, c, d – fungal matter. e – dark spore; f – cf *Alternaria*-type fungal spore. g – spore; h – cf hypha. i – cf hypha.

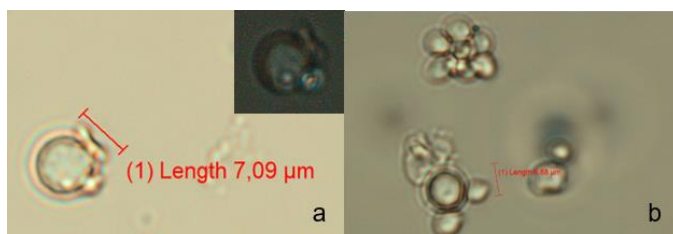


Figure 15. a – cf yeast from archaeological sample; b – yeast from reference material.

3.4. Non-living nature

This debris consists of minerals (Figure 16). Minerals may end up in dental calculus from the surrounding environment, e.g., from the soil or dust (Radini et al. 2017).

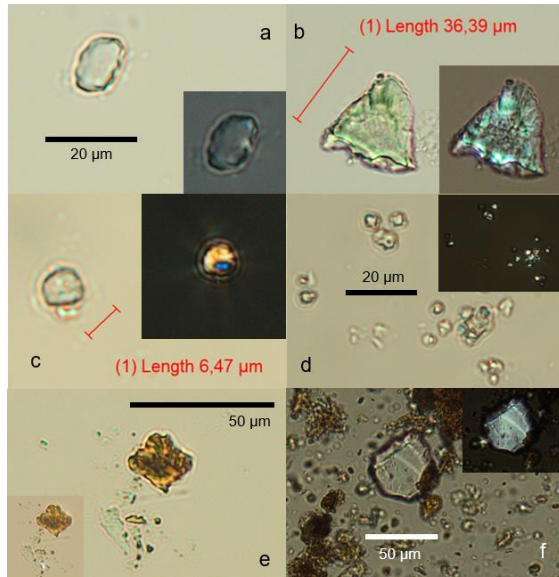


Figure 16. Minerals from archaeological samples (a–e), modern reference material (f): a – mineral; b – mineral showing signs of possible percussion. c – mineral; d – cluster of minerals; f – modern soil showing mineral components.

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