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Report on the analysis of microremains from the food crust samples of the pottery from Estonian Bronze Age sites.

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Contents:

1. Data description 1

2. Methods 1

2.1. Sampling of pottery food crust 1

2.2. Identification of microfossils from food crust 2

3. Results of the food crust analyses 2

4. Detailed analysis of microremains present in the food crust samples 2

4.1. Phytoliths 2

4.2. Wood remains 5

4.3. Calcium oxalate crystals 6

4.4. Starch grains 6

4.5. Pollen grains 7

4.6. Processed and burnt plant matte 7

4.7. Fungal remains 8

4.8. Other remains 9

5. References 10

1. Data description

Food crust samples from 9 potsherds from one Bronze Age fortified settlement and four Bronze Age burial sites were chosen for microfossil analysis.

The samples were:

* Iru fortified settlement (3 samples): AI 4051: 234, AI 4051: 491, AI 5302: 153
* Iru Bronze Age burial site, stone-cist grave no (1 sample): AI 4811: 40
* Muuksi Bronze Age burial site, stone-cist grave no 71 (3 samples): AI 4980: 58, AI 4980: 386, AI 4980: 854
* Jaani at Väo Bronze Age burial site (2 samples): AI 5220: 90, AI 5220: 91

2. Methods

2.1 Sampling of pottery food crust

All samples were prepared at the University of Tartu's department of archaeology where devoted facilities for archaeobotanical research have been created with dust monitored and blanks with mounting media to control contamination were prepared on regular basis.

Most of the sherds were quite poor of food crust, except for AI 4051: 491 (Iru settlement). As it was not possible to obtain soil samples from the excavation area, contamination risk from the environment was reduced by removing the top until no evidence of soil, meaning that the deepest part of the crust was scraped. In cases of very poor food crust, I might have scraped the pottery matrix, which would explain the larger presence of mineral grit in some samples (e.g., AI 5220: 90).

Samples were scraped from the food crust with a clean disposable scalpel. Sterile powder-free nitrile gloves and a lab coat was worn during sampling and mounting of slides. Around 1 mg of crust powder was used from each analysed sherd and put in clean Eppendorf vials. If need be, the powder was further homogenised with a disposable polypropylene micropestle. The further preparation followed the established protocols (e.g., Lucarini et al. 2016; Lucarini and Radini 2020). Up to 0.2 ml of clean Milli-Q ultrapure water was added to each vial. The vials were sonicated in Bandelin Sonorex ultrasonic bath for 3 minutes. One drop (approximately 0.05 ml) of the sample was put on a microscope slide and a drop of ultrapure water and glycerole mixture (1:1) was added to facilitate the observation of microfossils as glycerol allows the rotation and thus the proper identification of 3D shapes of microfossils. New disposable pipettes were used for each sample to ensure that no cross-contamination could occur. A coverslip was placed on top of the slide and kept in place by applying nail polish tothe corners. All slides were viewed immediately after mounting.

The entire area underneath the cover glass was examined by means of Olympus BX51 transmitted light compound microscope, at magnifications of 200x, 400x and 500x and complemented by

cross-polarized light. The finds were recorded, described and documented by means of photographs. The identification process was semiquantitative, meaning that most microfossils observed (starch granules, phytoliths, pollen granules) were counted, and their reported data represents absolute numbers, but fungal remains were marked as present/not present.

2.2. Identification of microfossils from food crust and dental calculus

The identification of the micro remains was conducted by using published resources and reference collections. A reference collection created and preserved at the University of Tartu was used to identify the microfossil remains found in dental calculus and food crust. The reference collection was created in a clean laboratory, mainly consists of Estonian plants, grown and used during the Bronze Age and associated with food and crafts during the time period. Fish and meat remains, fibres, fungal remains and possible lab contaminants were also included in the reference collection. The modern material was at times processed (ground, cooked) during experimental work, reflecting different ways in which past humans could have used them. The reference collection is being improved. For additional reference material we were given full access to the reference collection created by Dr Anita Radini (currently at University College Dublin, previously at the University of York). Such a reference collection is one of the largest built for the identification of microfossils of different nature from stone tools, dental calculus and pottery residue. It contains plants from North Europe, the Mediterranean region and North Africa, as well as processed and chewed food specimens and nondietary references (see more for example Cristiani et al. 2016). Published works were consulted to help identify phytoliths, starches and other plant parts (e.g., Winton 1906; Barboni et al. 2007; An 2016; Copeland and Hardy 2018; Danu et al. 2018; Gao et al. 2018; An and Xie 2022). The identifications of phytolith morphotypes follow the ICPN 2.0 (Neumann et al. 2019). For further identification of cereal phytoliths, publications by Ball et al. (1999, 2009, 2017) were used.

3. Results of the food crusts analysis

The numerical results have been presented as a separate Excel file in this dataset (Table 1. The results of the food crust analysis of pottery sherds from Estonian Bronze Age sites).

4. Detailed analysis of microremains present in the food crust samples

Six different types of plant microremains were identified in the food crust samples: phytoliths, starch grains, pollen gains, calcium oxalate crystals, wood tissue and other plant tissue. In addition, other types of microremains were found: diatoms, fungal remains, bone, flesh and oil. Also, possible fragment of a fish scale was identified.

4.1. Phytoliths

Of elongated phytolith morphotypes (Fig. 1), the most numerous were elongated psilate (n = 63) and elongated verrucate (n = 70) types. Of grass silica short cell phytoliths (GSSCP) rondels (n = 22), trapeziform sinuate (n = 13) (Fig. 4: 3, 4) and parallelepipedal (n = 15) morphotypes were most frequent. Of diagnostic phytolith morphotypes, elongate psilate and elongate verrucate types occur in all samples. These are primarily formed in the epidermis of grasses (Rudall et al. 2014).

Phytoliths of elongate dendritic/dentate morphotype (n = 9) (Fig. 2) only occurred in four samples. These form in the inflorescences of grasses and are associated with the consumption of domesticated cereals (Danu et al. 2018). Some of the elongated verrucate and psilate phytoliths were burnt.

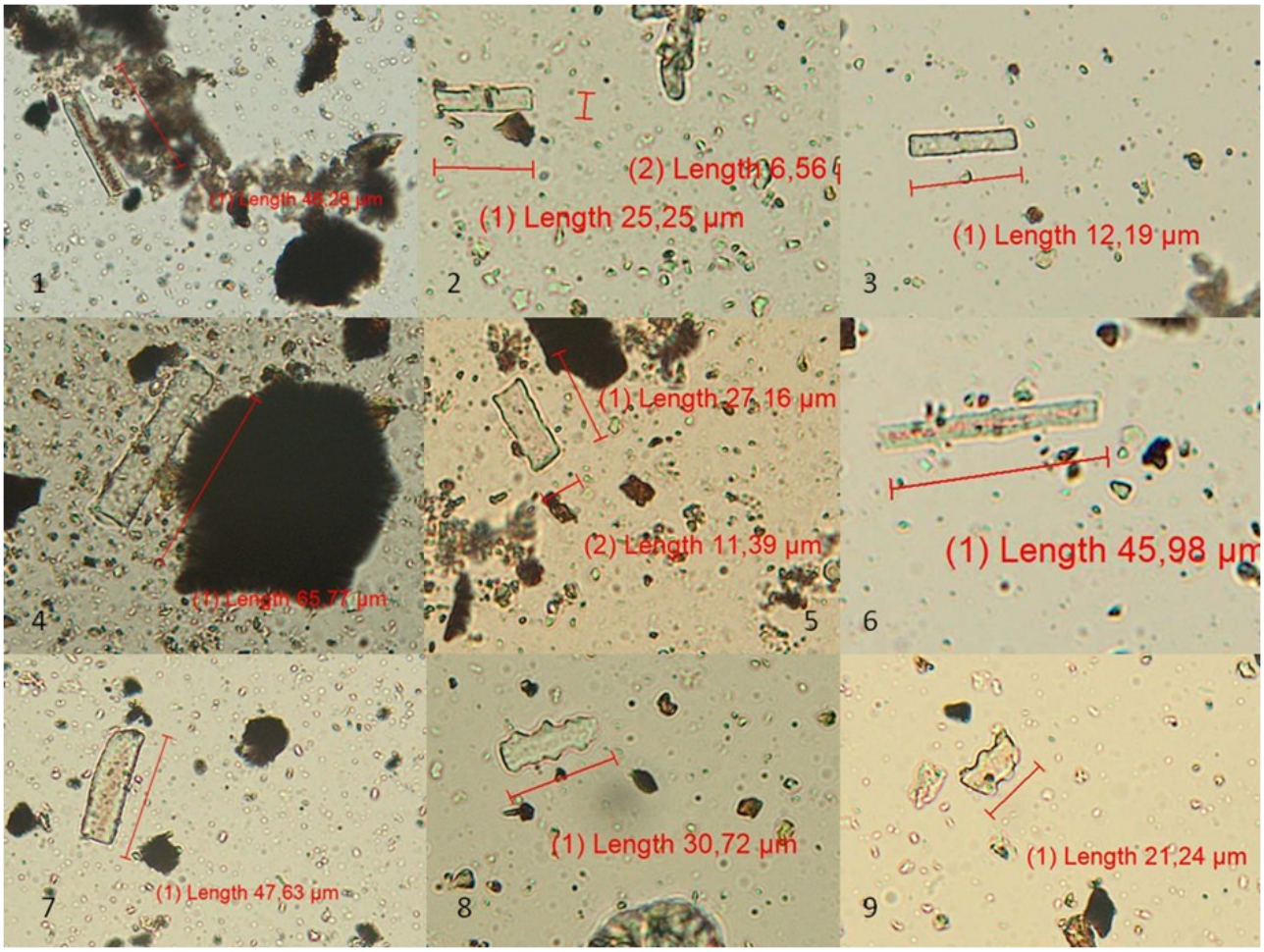

Fig. 1. Elongated phytolith morphotypes from the samples: elongated psilate (1 – AI 4980: 854, 2 – AI 4051: 491), elongated verrucate (3 – AI 4811: 40, 4 – AI 4051: 491, 5 – AI 4051: 491, 6 – AI 4980: 58, 7 – AI 4980: 854), elongated sinuate (8 - AI 5220: 90, 9 – AI 5220: 91).

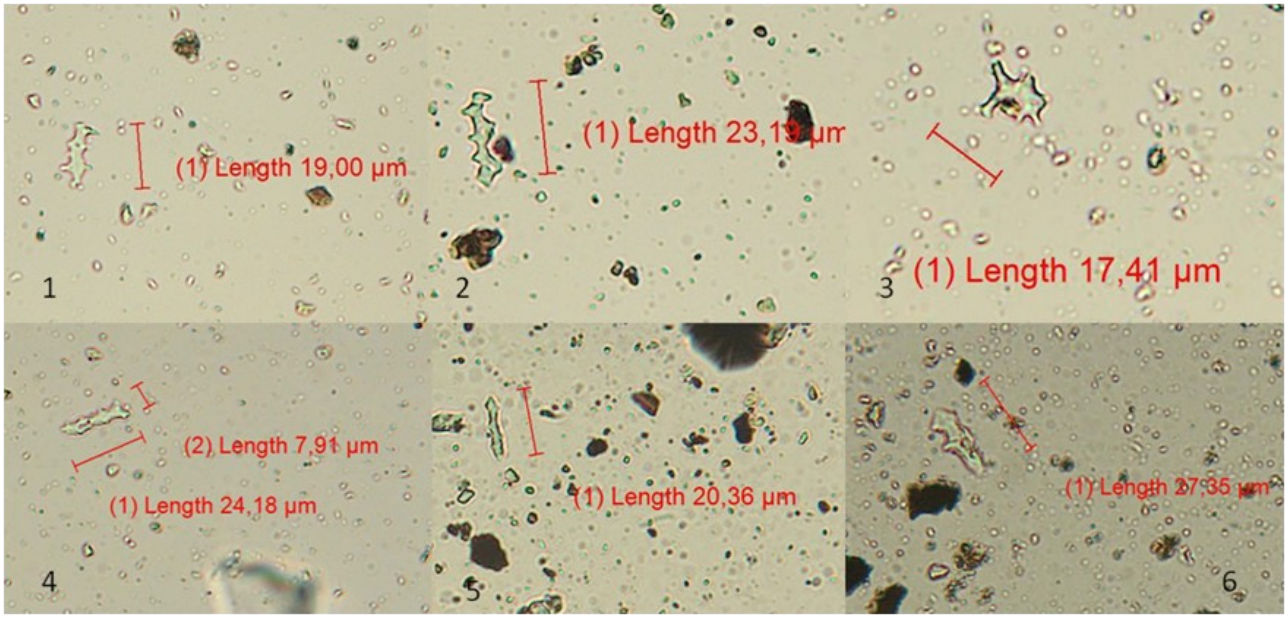

Fig. 2. Elongated dendritic phytoliths: 1, 2, 3, 4 – AI 5302; 153, 6 – AI 4980: 854.

Among the GSSCPs (Fig. 3, 4), rondels (Fig. 3: 1–4) and trapeziform sinuate (Fig. 4: 3, 4) types are frequently associated with the Pooideae subfamily (e.g., Barboni et al. 2007; Danu et al. 2018). The bilobates (n = 10) (Fig. 3, 5–7) mainly occur in the Panicoideae and Arundinoideae subfamilies (Danu et al. 2018). Of non-grass morphotypes, the blocky type was the most common (n = 85). These are likely associated with conifers (An 2016; Gao et al. 2018). Spheroid ornate phytoliths (n = 9) are diagnostic to different dicotyledons (Danu et al. 2018; An and Xie 2022).

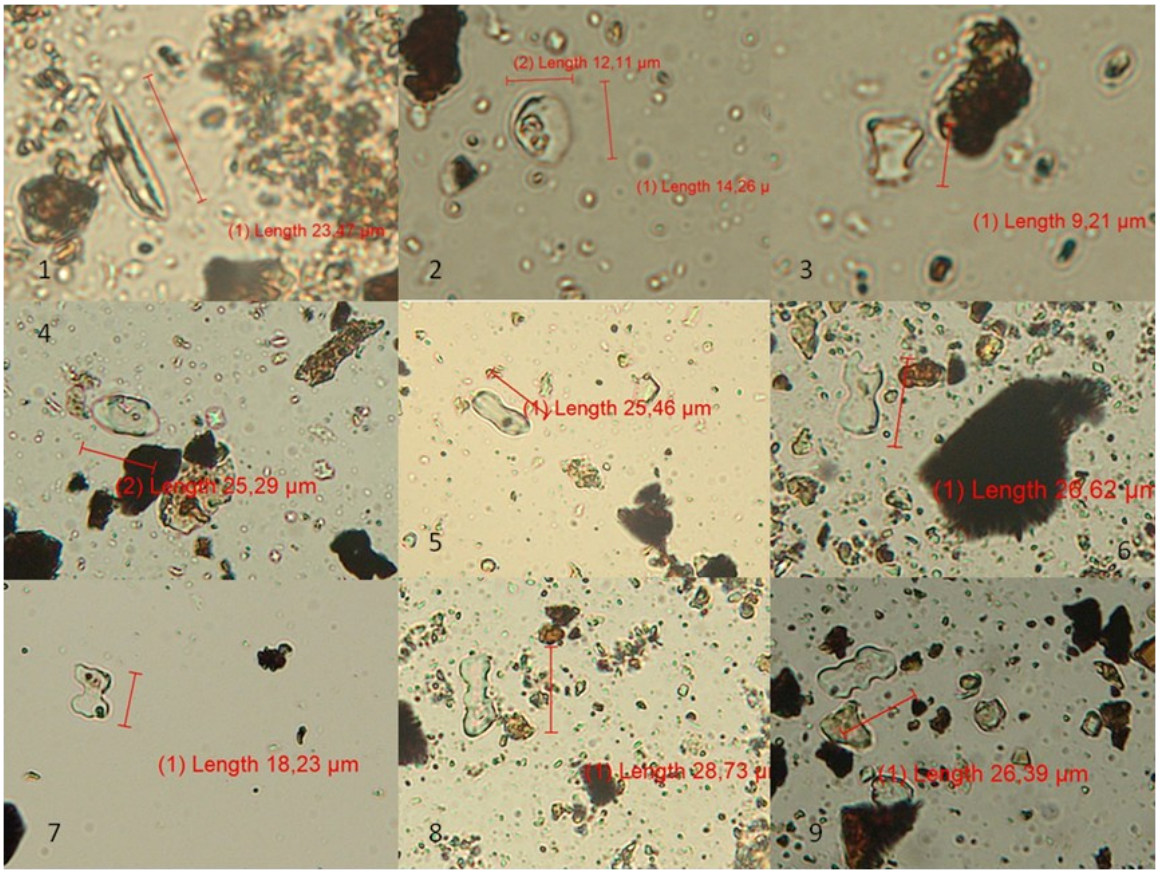

Fig. 3. GSSCP morphotypes: rondels (1 – AI 4051: 491, 2 – AI 5220: 90, 3 – AI 5220: 90, 4 – AI 4980: 386), bilobates (5 – AI 5220: 91, 6 – AI 4051: 491, 7 - AI 4051: 234), polylobates (8 – AI 4051: 491, 9 – AI 4980: 386).

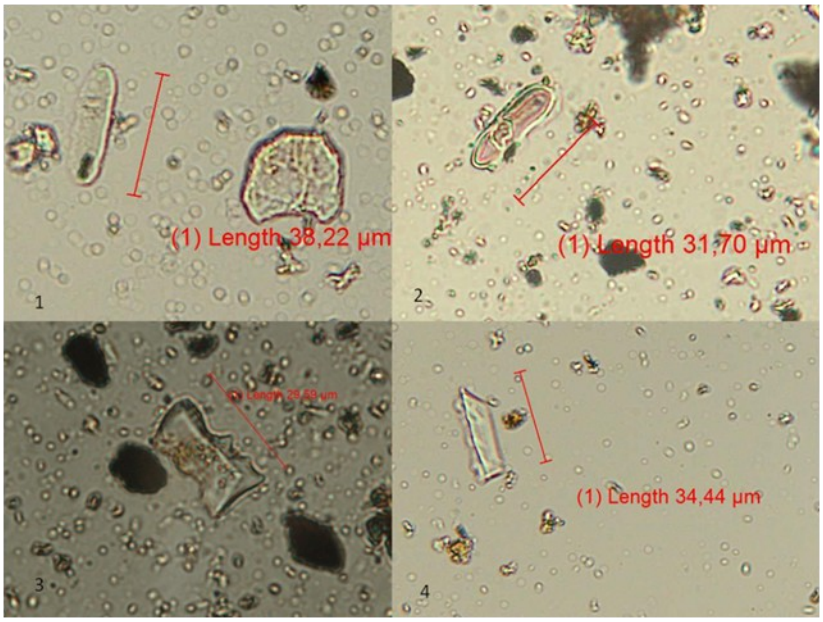

Fig. 4. GSSCP morphotypes: trapezoid ovate (1 – AI 4980: 58; 2 – AI 4980: 854), trapezoid sinuate (3 – AI 4980: 854, 4 – AI 4980: 58).

A few silica skeletons (Fig. 5) associated with fragments of the silicified epidermis of grasses were identified. One represents the elongate sinuate type, and two represent elongate dendritic type (Fig. 5: 1); these could refer to grass inflorescence, possibly the husk of cereal seed. One consists of two elongate verrucate phytoliths and is possibly part of the culm or leaves of grasses (Danu et al. 2018). The phytolith selection shows the definite presence of grasses, while the elongate dendritic phytoliths are clear indicators of cereal consumption.

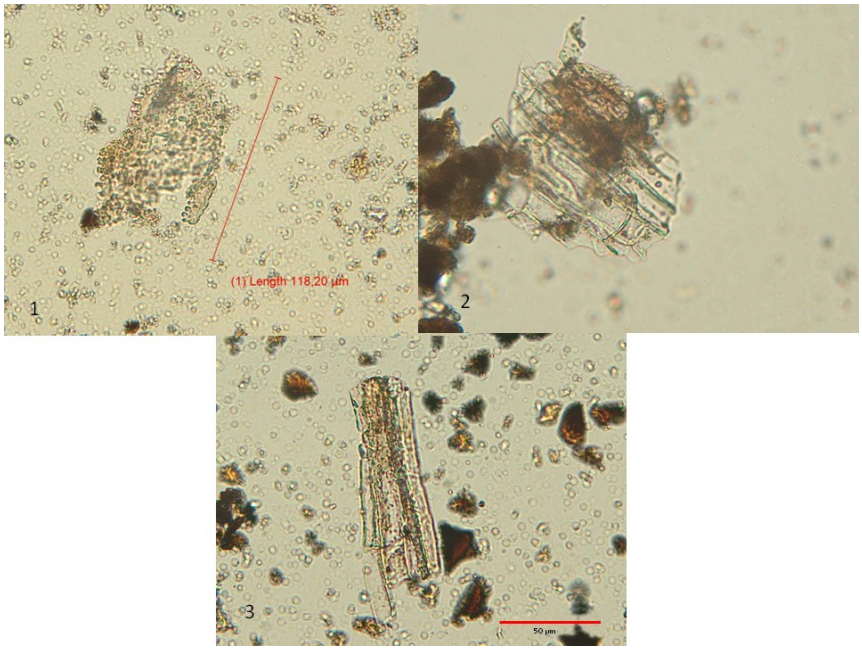

Fig. 5. Silica skeletons observed in the samples: elongated dendritic (1 – AI 4051: 449), elongated psilate (2 – AI 4811: 40), elongated verrucate (3 – AI 4980: 386).

4.2. Wood remains

Wood remains were found in all analysed samples. Among these (n = 66) were bundles of tissues as well as wood pits (Fig. 6: 2). Both conifers (e.g., Fig. 6: 1–4) and deciduous (Fig. 6: 5) trees were identified. The first was indicated by distinctive wood pits, blocky phytoliths and prismatic oxalate crystals present in many samples.

Fig. 6. Wood remains: 1 – AI 4980: 386, 2 – AI 4980: 58, 3 – AI 5302: 153, 4 – AI 4980: 386, 5 – AI 4051: 491, 6 – AI 4980: 386, 7 – AI 5220: 90, 8 – AI 4811: 40.

4.3. Calcium oxalate crystals

In addition to the mentioned microfossils, the samples also contained small cubic and hexagonal crystals that may have formed in the course of crystallisation during cooking processes. Oxalate crystals can form, e.g., during the steeping, mashing and fermentation of cereals (Dietrich et al. 2012, 687). There is, however, insufficient material to speculate about the pre-processing of the foodstuff in the food crust. Several druse-shaped crystals (Fig. 7: 1, 2) present in a single Iru settlement sample (AI 4051: 234) may also indicate some fruits, although, in general, oxalates cannot be considered diagnostic.

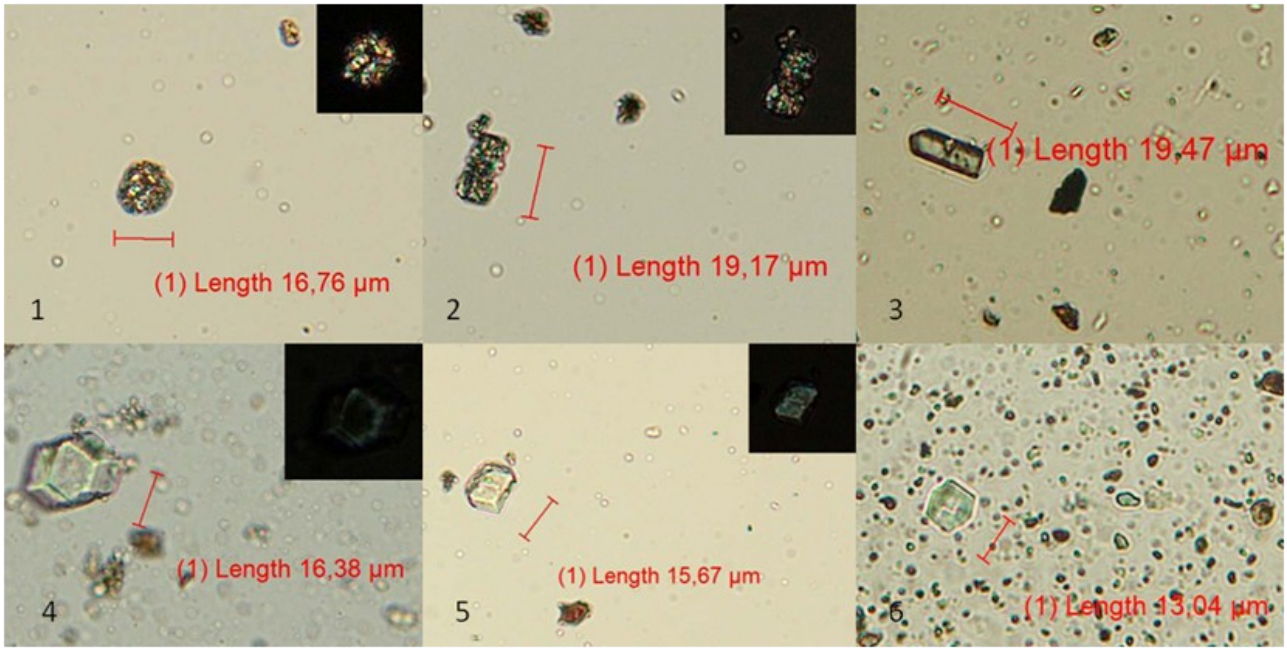

Fig. 7. Druses (1, 2 – AI 4051: 234), cubic phytoliths or calcium oxalates (3 – AI 5220: 90, 4 – AI 4980: 854, 5 – 4051: 234, 6 – AI 4980: 854)

4.4. Starch grains

Only single starch granules (4 in 3 samples) were found. Two (1, 2) of these are similar to the Triticeae starches (Fig. 8: 1–2), while a cluster of three (4) is reminiscent of *Panicum* starch (e.g. Yang et al. 2012). One remained (3) unidentified. However, there are very few phytoliths in the sample that would be indicative of *Panicum* species, and whereas there are not millet finds from this early period in Estonia so far, the *Panicum* remains to be confirmed in the future.

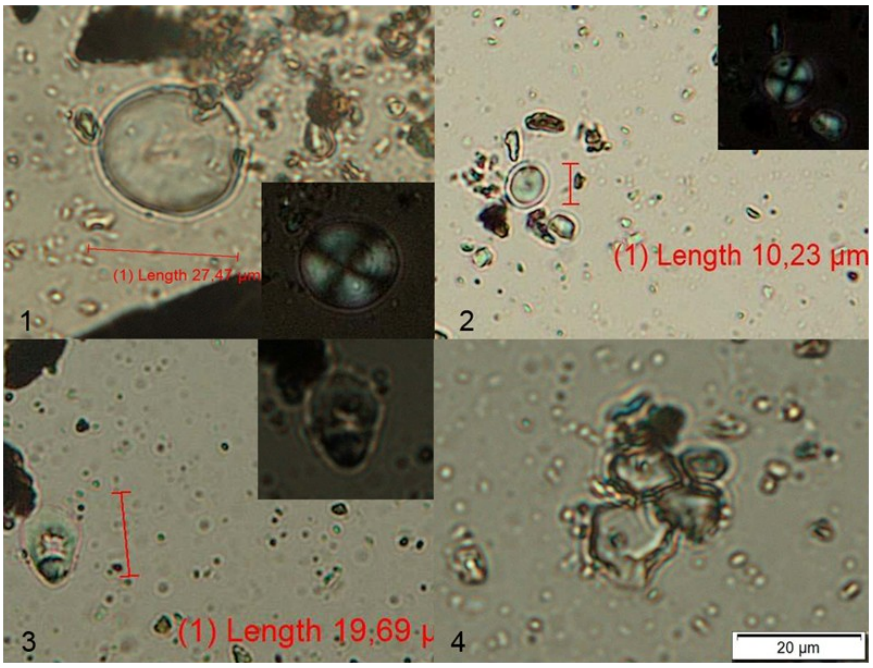

Fig. 8. Starch grains (1 – AI 4051: 491; 2 – AI 4980: 58, 3, 4 – AI 4980: 854).

4.5. Pollen grains

Only a few pollen grains were recorded. Unfortunately, these could not be more identified with confidence, but one could be that of Poaceae (Fig. 9: 1).

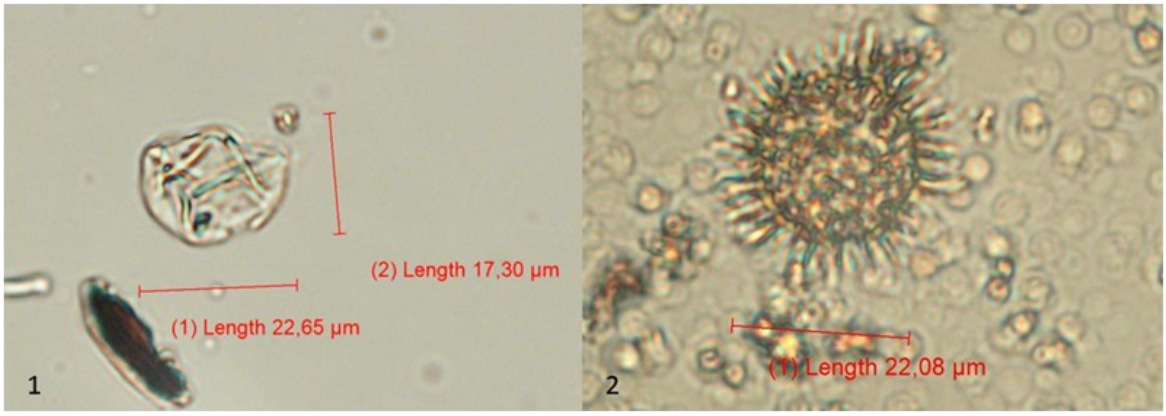

Fig. 9. Pollen grains: 1 – 4051: 234, 2 – AI 4051: 491.

4.6. Processed and burnt plant matter

Under this category, processed and sometimes clearly burnt plant matter was recorded. Here, the cell structure characteristic to plants, was visible, but they were not teated as silica skeletons, because there was still burned organic material visible.

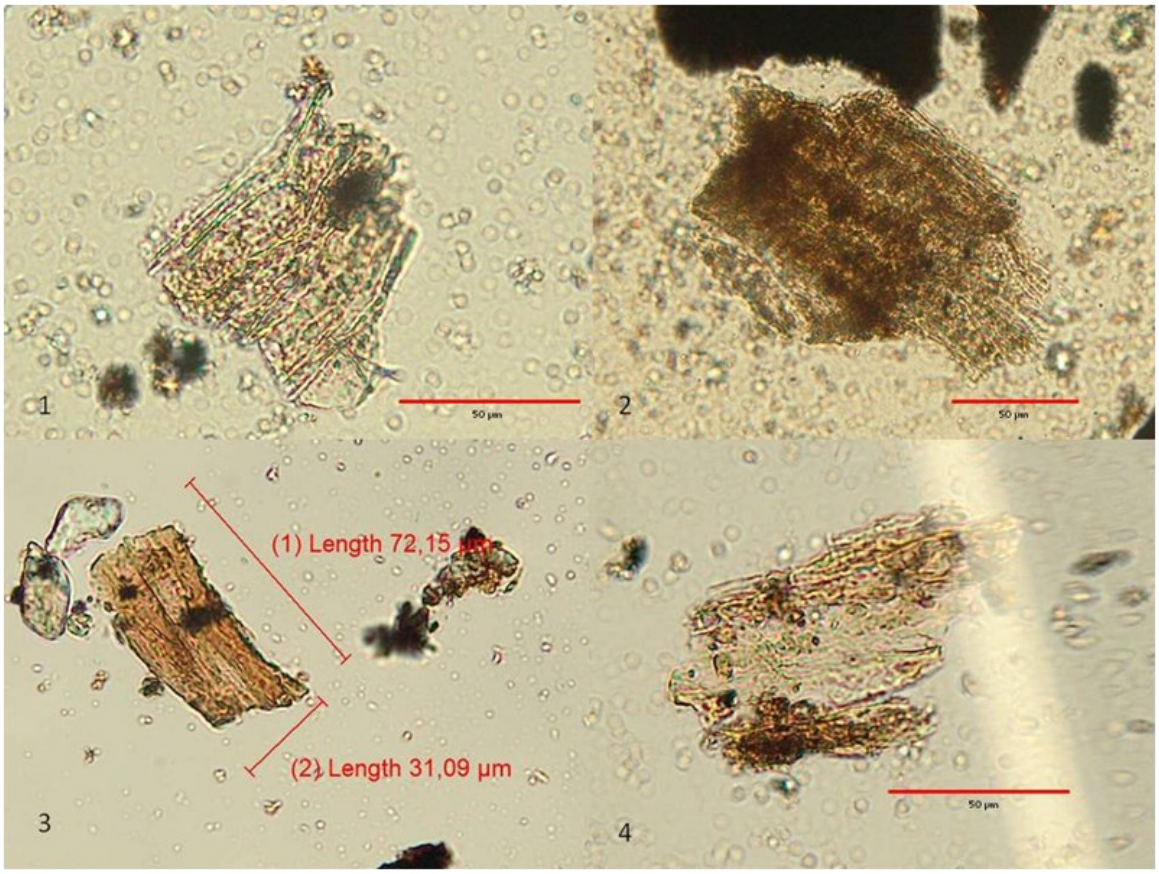

Fig. 10. Different processed plant matter: 1, 2 – AI 4051: 491; 3, 4 – AI 4980: 58.

4.7. Fungal remains

Fungal remains were detected in eight samples and mostly represented the hyphae and spores of Glomeromycota type (Fig. 11, 1–3). These are numerous in some samples (e.g., AI 4980: 58, AI 4980: 386, and AI 4051: 491), but in others (e.g., AI 4051: 234) only hyphae are present or no remains from the fungus detected (AI 5302: 153). Glomeromycota exists in many soils in symbiosis with the roots of plants. Their presence in food crusts could refer to cooking insufficiently washed vegetables. However, six samples contained remains of another type of fungi: e.g., a few spores of Curvularia, Alternaria or Stemphylium type fungi were detected at Iru (AI 4051: 491). All are common phytopathogens (Funnell-Harris et al. 2013; Marin-Felix et al. 2019); Alternaria can be associated with cereal crops, while Stemphylium creates yield loss in lentils, but also fruits (Das et al. 2019; Sharma and Joshi 2021).

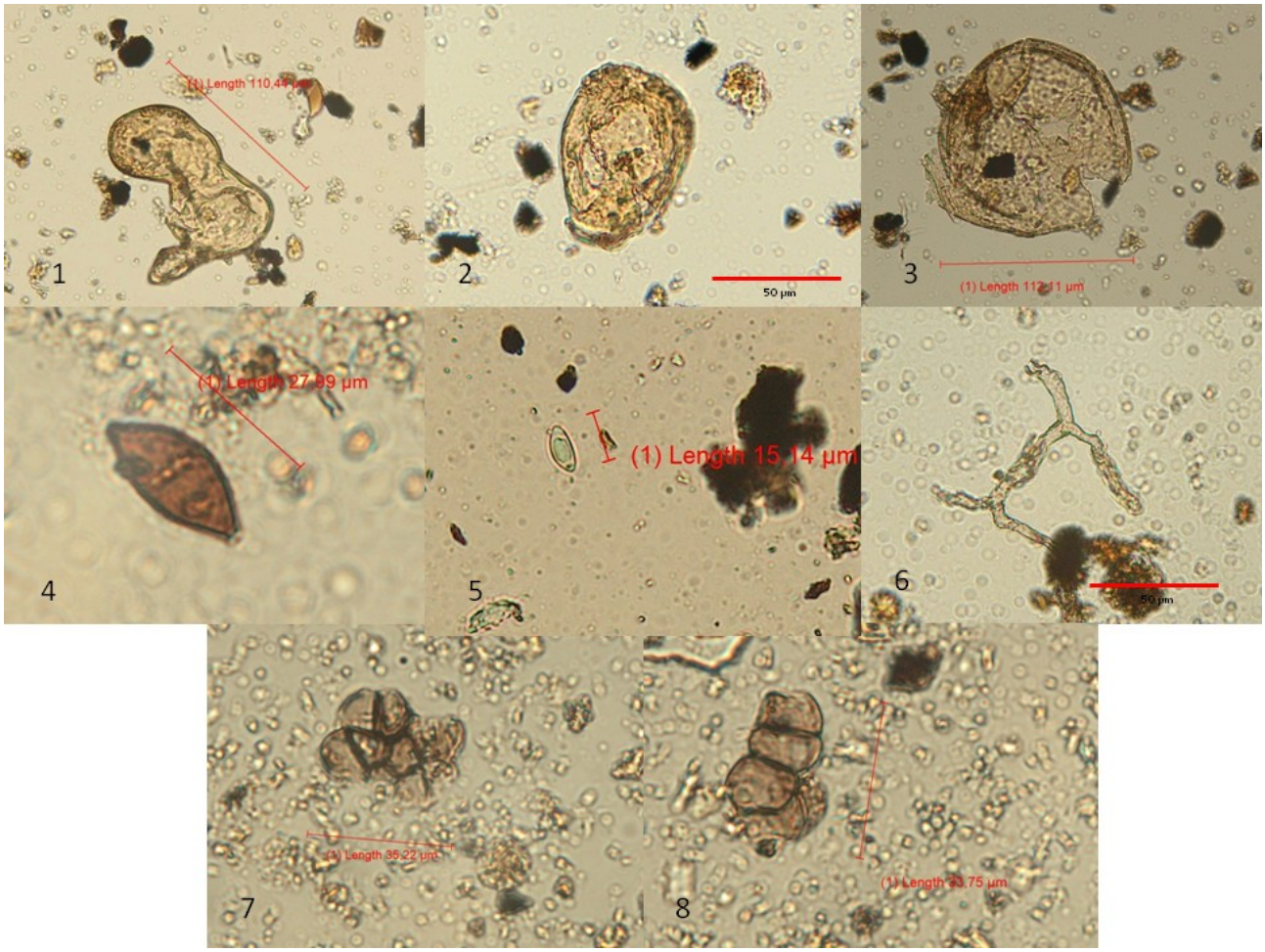

Fig. 11. Fungal remains: Glomeromycota type fungal spores (1 – AI 5220: 91, 2 – AI 4980: 58, 3–5220: 91), Glomeromycota type fungal hyphae (6 – AI 5220: 90), cf. Alternaria/Curvularia/ Stemphylium type fungal spores (7, 8 – AI 4051: 491), unidentified fungal spores (4 – AI 4051: 491; 5 – AI 4980: 386).

4.8. Other remains

In addition to different plant and fungal remains, all the samples contained debris, that were difficult to identify, but with the help of the comparative collection, bone (Fig. 12) and possible burnt flesh (Fig. 13) particles were suggested. Naturally, this kind of interpretation should be treated with caution as no published sources exist on bone and flesh in food crust and only few experiments of cooking and charring bone and flesh have been conducted so far by the autor to be more confident.

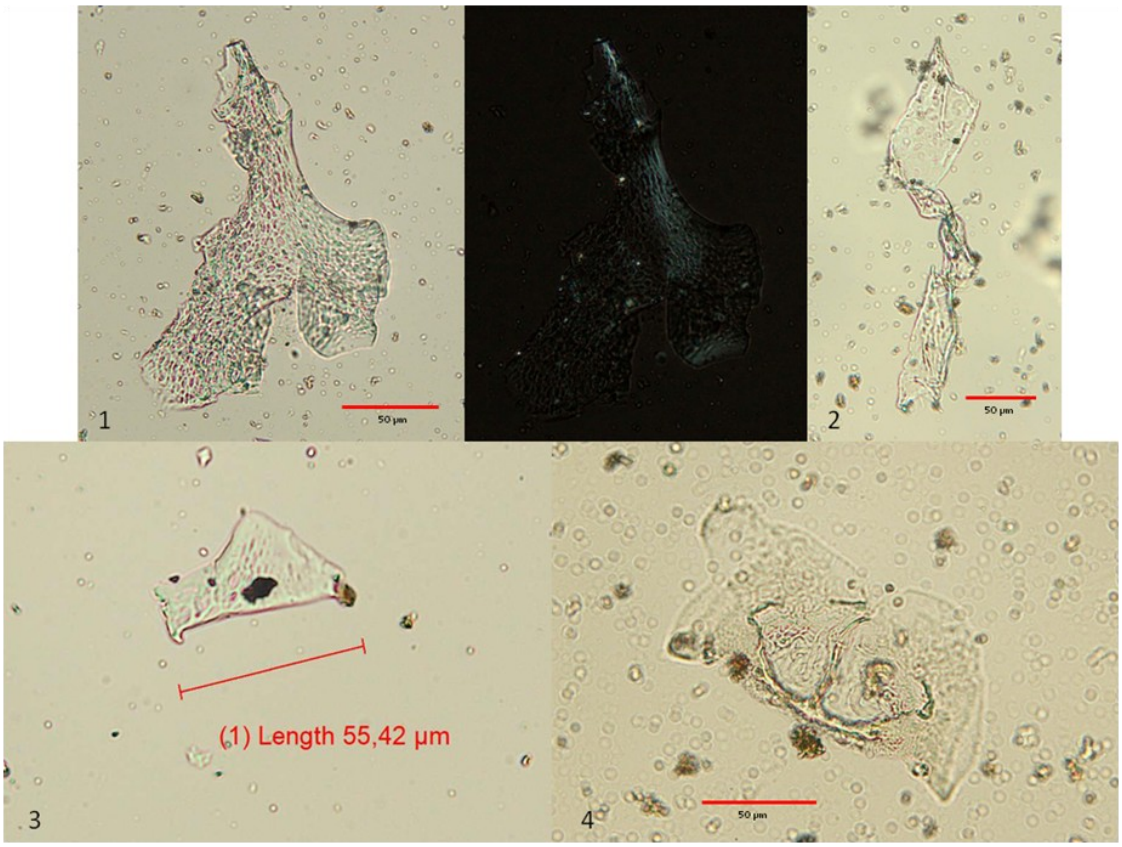

Fig. 12. Possible bone debris: 1 – AI 4980: 58, 2 – AI 4980: 386, 3 – AI 4051: 234, 4 – AI 5220: 91

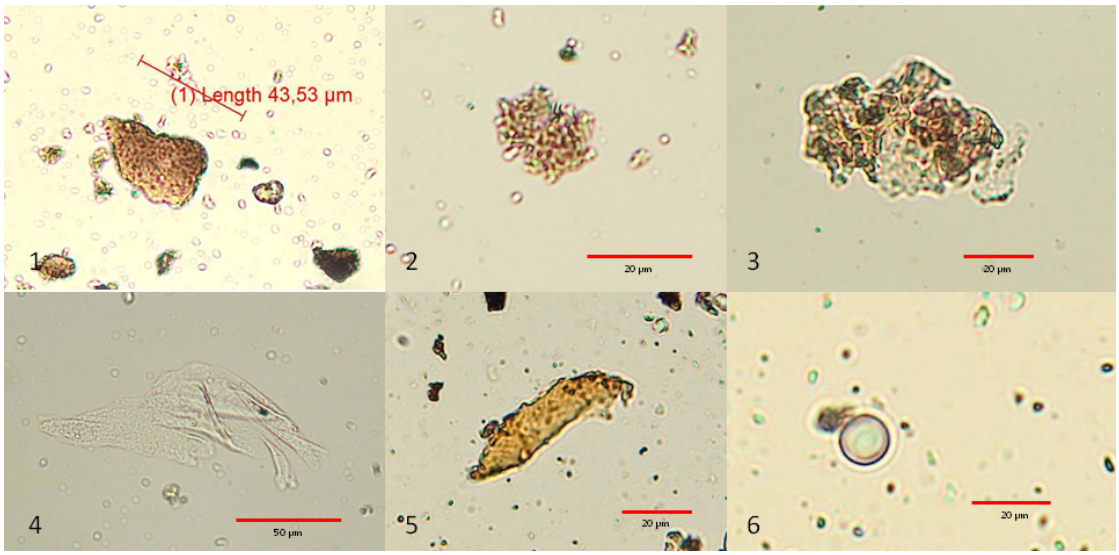

Fig. 13. Unidentified cf. burnt flesh particles (1, 2 – AI 5302: 153, 3–5 – AI 4980: 58) and oil drop (AI (AI 5220: 91).

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