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Phytolith analysis at the Neolithic tell Vrbjanska Čuka in Pelagonia (North Macedonia)

Master thesis

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Annotation:

The thesis deals with an analysis of phytoliths, microscopical residues of plants. The analysis was applied in order to gain a new archaeobotanical data on the Neolithic period at the archaeological site Vrbjanska Čuka in Pelagonia, North Macedonia. The results brought new insights into a plant management and related activities on the site, as well as contributed to an understanding of a past environment of the early agricultural society.

Prohlašuji, že jsem autorkou této kvalifikační práce a že jsem ji vypracovala pouze s použitím pramenů a literatury uvedených v seznamu použitých zdrojů.

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Kristýna Budilová

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Contents

1. Introduction

Reconstructing the environmental settings of settlement sites and understanding the plant management of early farming communities are issues of key importance when dealing with the Neolithic Balkans. However, available archaeobotanical data are rather scarce in this region and the activities of bioarchaeologists have mostly been oriented towards the thematic pioneer research of Neolithic archaeobotany and palaeoeconomy (Beneš et al. 2018). To move beyond a focus on economic concerns, an application of phytolith analysis on specific archaeological contexts of Neolithic settlement site is aimed to bring a new perspective on past human-plant relationship as well as the environmental ground of a specific site. For a combined view, allowing a better understanding of Neolithic communities and their surrounding environment, it is advisable to use phytolith analysis as a starting point in situations where classical analysis of plant macroremains does not yield reliable results due to the fragmentary preservation of the material (Wade et al., 2021).

Phytoliths (from Greek *phyton*-plant, *lithos*-stone) are microscopical particles precipitated in plant tissues as infills of cells, intercellular space, or features of epidermis (Piperno, 2006). They can appear as crystals of calcium oxalate, calcium carbonate (both in form of microscopic needles), or as hydrogenated silicic acid (H_4SiO_4), amorphous biogenetic Opal-A, which forms in a shape of the plant's anatomy and can sustain in such a state for million years, bearing information about the existence of the material in the past; when recovered from soil depositions, phytoliths can be reversely determined and attributed to a certain organ (root, stem, leaf, inflorescence, fruit) or a specific family and/or genus of the plant; it is, however, necessary to note that not all the plant families produce diagnostic phytoliths. Also, similar shapes can be found in different plants, while at the same time, one plant can generate a variety of distinct morphotypes. Most of the valuable shapes are to be found in families such as Poaceae (grasses), Cyperaceae (sedges), Bromeliaceae (bromelias), Arecaceae (palms), Cucurbitaceae (pumpkins), Musaceae (bananas) and others (Piperno, 2006).

In archaeology, analysis of opal phytoliths is slowly becoming a common praxis, due to a bursting development and application of the discipline in the last decades, contributing significantly to our understanding of human subsistence strategies through time and reconstruction of their natural, later on, anthropogenic environments (Neumann et al., 2017; Shillito, 2013). Especially in research of the Neolithic, phytoliths are widely used to detect cereal processing residues, offering an additional view on the agricultural practices performed through spaces, the more in dry sediments, where the seeds are usually preserved only when exposed to burning and pollen grains are not usually preserved at all.

To put in some examples centred on the research area (and the Neolithic), phytoliths were used to enlighten the use of space in a Neolithic village of Makri in northern Greece (Tsartsidou et al, 2009), cereal processing at a site Palimbela in Greek Macedonia (Tsartsidou et al., 2020), palaeoenvironments and site formation processes at a settlement Dispillio on shores of lake Kastoria (Karkanas et al.,2011), domestic and ritual use of plants in a Neolithic cave Alepotrypa in southern Peloponnese (Ntinou & Tsartsidou, 2017), salt exploitation in Romania (Danu et al., 2020) or food habits during a Mesolithic/Neolithic transition on Lepenski Vir (Jovanović et al., 2021). Deeper to the past, phytolith analysis contributed to the understanding of the Palaeolithic environment (and plant use) by Theopetra cave in Thessaly (Tsartsidou et al., 2015) or by a Pleistocene elephant butchery site at Marathousa (Field et al., 2018). Also, phytolith analysis of a Pikermi formation by Athens brought some first evidence of a savannah environment of possibly the first hominins in Europe during a Messinian age (Böhme et al., 2017).

1.1 Objectives of the theses

1) The thesis aims to present new phytolith data from the Neolithic levels at Vrbjanska Čuka archaeological site in Pelagonia and investigate how these data reflect deposition and post-deposition processes to provide a background for the further investigation of the taphonomic issues and research biases.
2) Main objective of the study is to provide another proxy for the archaeobotanical reconstruction of plant management on the Neolithic site and possibly to discover some differences in spatial organisation of activities related to food preparation, cooking or storage, in and around one building at the tell.
3) Much has been written on settings of the community in wetlands and phytolith analysis is expected to provide information on the environment around the settlement and bring more evidence on the exploitation of possible wetland resources, such as reed or any other plants related to wetter conditions.

1.2 The archaeological background

A subject of this study is the Neolithic period in the valley of Pelagonia in North Macedonia (Fig. 1), where the first farmers founded settlements preferably along wetlands at the end of the 7th and beginning of the 6th millenium BCE, (Naumov, 2016). Vrbjanska Čuka site is attributed to the Velušina-Porodin cultural group, which has its eponymous sites some 40 km south of the city of Bitola and is characterized by the presence of a fine white-on-red painted pottery, anthropomorphic house models, clay figurines, stamp seals or altars (Naumov, 2015).

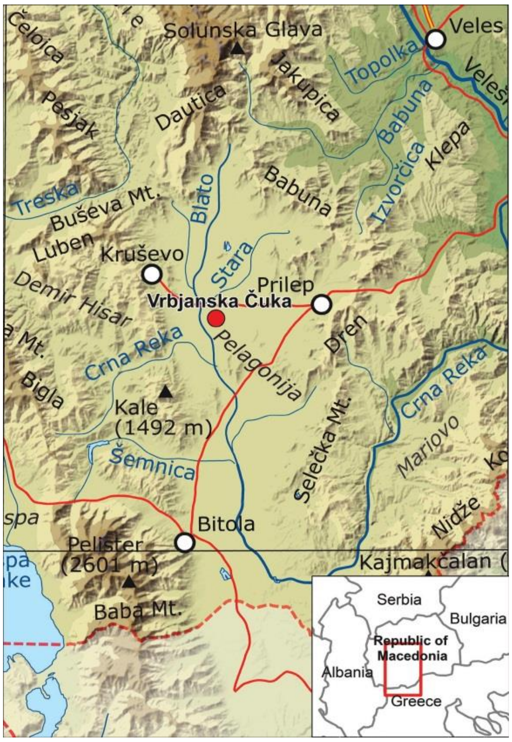

Fig. 1: Map of southern Balkan with a depiction of Pelagonia (map by Jiří Bumerl).

After a series of excavations and geomagnetic prospections of uncovered parts of the tell of Vrbjanska Čuka, it is known that people here settled in rectangular houses with walls constructed of wattle and daub, organised in a small village) surrounded by a ditch, creating a tell 3,5 m high and 180 m in diameter, with approximately 1,5 m of the Neolithic stratigraphy (Naumov et al., 2021).

Archaeobotanical analyses already delivered evidence of agricultural practices by recovering remains of cultivated plants with prevailing einkorn (*Triticum monoccocum*), followed by emmer (*Triticum dicoccum*), both naked and hulled barley (*Hordeum vulgare*, *H. distichon/vulgare*) and of pulses pea (*Pisum sativum*), lentil (*Lens culinaris*) and bitter vetch (*Vicia ervilia*); from wild, people collected bramble (*Rubus fruticous*), hazelnut (*Corylus avellana*), sloe (*Prunus spinosa*), elderberry (*Sambucus sp*.), wild strawberry (*Fragaria vesca*) and crab-apple (*Malus sylvestris)* (Antolín et al., 2020, for more see Beneš et al., 2018 and Vychronová, 2022). Cereals were harvested close to the ground, using sickles with serrated cutting edge, which is quite common type of the tool in Central and Eastern Mediterranean (Mazzuco et al., 2022; Naumov et al., 2021).

Interestingly, results of testing pottery sherds from Vrbjanska Čuka for lipid molecules suggested that the first farmers prepared food by mixing leafy plants and carcass fat from cattle, sheep or goat, possibly in the form of stew; domesticated animals are indeed represented by cattle (*Bos taurus*), sheep (*Ovis aries*), goat (*Capra hircus*), pig (*Sus domesticus*) and dog (*Canis familiaris*), accompannied by bones of wild species such as deer (*Cervus elaphus*), red deer (*Capreolus capreolus*), fox (*Vulpes vulpes)*, indetermind birds, fishes (Cyprinidae and Salmonidae), rodents, lizards and frogs, and a large amount of probably consumed river mussels (mostly *Unio crassus*) (Stojanovski et al., 2020). Of the activities non-related to food production, quartz and flint flaking was detected by micro-refuse analysis (Antolín et al., 2021).

1.3 Environmental settings of the site

The site Vrbjanska Čuka lies the north of an elongated valley which is part of a Pelagonian massif, consisting mostly of Proterozoic and Paleozoic metamorphic rocks and Proterozoic carbonate rocks (Petrušev et al., 2021). The valley was created as a tectonic depression in the Middle Miocene and was gradually transformed to a freshwater lake, which started to outflow in the Aegean sea around 1-0,85 Ma ago, the site thus lies on layers of Miocene, Pliocene and Pleistocene sediments, with the uppermost layers composed of eluvial and aeolian deposits (Arsovski, 1991 in Naumov et al., 2021). Yearly precipitation in the region of Prilep is about 570 mm, average temperature is slightly above 10°C and the climate is considered modified continental or moderate continental, under conditions typical for arid or semi-arid areas (Puteska et al., 2015). The site is located on the edge of alluvia, created by Blato river, at 600 m.a.s.l., a bit north bordered by a current wetland of the same name, where smaller streams meet to form the river. Geographer Jovan Trifunoski roughly divided Pelagonia to three biological zones: 1. woodlands at the slopes of the valley (oak forests above 900 m and a scrub vegetation under this line; 2. edges and the plain under steppe vegetation; 3. zone of frequent flooding with flora and fauna associated with water (Trifunoski, 1998 in Murgoski, 2016)

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Since no information on recent vegetation around Vrbjanska Čuka is provided by the aforementioned published archaeobotanical works nor by other publications centred on the wetland environment (e.g. Naumov, 2016), the following paragraphs aim to enlighten a bit a nature of the area, where botanical data are not easily accessible, if they exist at all.

Potential natural vegetation on the plain consists of Albanian – Macedonian - Greek periodically moist mixed downy oak forests with species such as *Quercus pubescens*, *Q. pedunculiflora*, *Ulmus minor*, *Fraxinus angustifolia s.l.*, with *Phillyrea latifolia*, *Luzula forsteri* and C*repis reuteriana*, bordered at the east by Albanian – Macedonian - Greek mixed Oriental hornbeam-downy oak forests (*Quercus pubescens*, *Q. virgiliana*, *Carpinus orientalis*) with *Symphytum ottomanum*, partly with *Phillyrea latifolia*, *Quercus coccifera*, *Asparagus acutifolius* (EuroVegMap). At the southern end of Mt Babuna Forests on a way to

Pletvar pass (997 m), forests of *Tilia argentea*, deciduous oaks and *Fagus sylvatica* were noted in 1839 by Auguste Grisebach (Strid, 2000).

In an attempt to basically describe at least some features of the current vegetation surrounding the archaeological site, my observations and collected plants, determined with a help of Milan Štech and Jan Novák, are presented. Starting at the wet area by the Slavej industrial zone (north of Vrbjanska Čuka), there were small patches of alkalic swamps with tall stands of *Juncus* sp. and *Bulboschoenus maritimus* agg.. Right on the tell in a shallow sand pit, the wetland vegetation consisted mostly of *Typha* sp., *Beckmannia eruciformis* and *Polypogon monspeliensis*; a deeper pit just by the excavation trench was overgrown by *Salix* and pond turtles (*Emys orbicularis*) used to dwell in. The tell itself was overgrown by an unconsolidated field of *Triticum aestivum*, in which the most notable weeds in a mid-summer were *Consolida regalis*, *Ferrulago campestre* and *Papaver* sp.. A tall elm tree (*Ulmus sp.*) grew on a tell by route, where also many ruderal species were spotted(e.g. *Verbena officinalis*, Chenopodiaceae, *Artemisia vulgaris*). In a northwestern part, *Tilia argentea* grew solely and at the same place quite a large meadow with a dominant *Beckmania eruciformis* stretched, a vegetation which has not yet been recorded in this area (Dítě et al., 2012, Eliáš et al., 2013); it is interesting from archaeobotanical point of view because the grass can be used as a fodder. A single plant was previously reported by a Czech botanist Eduard Formánek over 100 years ago from Rosoman by Gradsko (Vandas & Formánek, 1909).

West of the Neolithic tell there are currently fields mostly with peppers and cereals, among the fields, for example, a spiny liquorice *Glycirhizza echinata* was recorded, lots of *Prunus* sp. and *Crataegus* sp., behind them stands mostly with *Populus nigra* or *Fraxinus angustifolia*. A grass Sorghum halapense was spotted at a dump place near the village Vrbjani, which is famous for it's large population of white storks (Velevski et al., 2010).

South of the site, along a path to another Neolithic tell-site by a village Borotino (lined by *Echium italicum*, *Cichorium intybus* and *Elymus farctus*), a mosaic of mesic fields, meadows and pastures is still maintained by an agricultural rotation system, where on the laid-off field species like wild carrot (*Daucus carota*) and lettuce (*Lactuca serriola*) grow 2 m high; many weeds were collected in the area such as *Nigella arvensis*, *Anagallis arvensis*, *Marrubium peregrinum*, of others *Stachys* cf. *byzantina*, *Althea* cf. *setosa* and at least two species of *Salvia* sp.; of grasses *Bromus* (*B. secalinus, B. scoparius, B. hordeaceous, B. japonicus, B. tectorum*), *Dasypyrum villosum, Aegilops cylindrica* and *Cynodon dactylon*. Species of *Setaria* sp. and *Hordeum* sp. were spotted as well.

East of the tell, mostly tobacco fields spread on a large area, alternating with cattle-herd pastures, incl. some *Trifolium* meadows. A grass dominating some places at Lokvi Natural Monument about a 1km N-E from the site turned to be (after detailed microscopical examination) *Festuca valesiaca*, making it a patchy short-grass steppe. However, technically, the habitat can be also considered as a seasonal/intermittent saline/brackish/alkaline marshes/pools wetland (according to a Ramsar definition). A genesis of such geomorphology and habitats on salty soils (which are predominant around Vrbjanska Čuka, according to Macedonian Soil Atlas) is of palaeoecological interest because, for example, in Pannonia, the salt-affected soils developed mostly 7000-5000 yrs ago, while in the Balkans they are natural, but expand as a consequence of an anthropic influence in some cases (Eliáš et al., 2013).

Through species such as *Eryngium campestre*, *Centaurea* (probably mostly *C. stoebe* subsp. *australis*, referred in a Matevski et al.(2015)), *Botriochloa ischaemum* or *Achillea* sp., the vegetation on the plain is connected to a vast area of grasslands, herbs, shrubs and scattered trees on south and west-facing slopes, grazed around Prilep mostly by goats and horses; many typical steppe species were collected on those hills, such as *Stipa capillata*, *Chrysopogon gryllus*, *Allium flavum*, *Astragalus* sp., *Hyssopus officinalis*, *Euphorbia cyparissias, E. myrsinites, Poa bubosa, Scabiosa* sp., *Sedum album*; others, such as *Verbascum phoeniceum*, *Paeonia officinalis*, *Linum flavum, Linum hirsutum* or *Stipa pulcherrima* (syn. *Stipa grafiana*), were collected here by a Czech-Bulgarian botany student Jan/Ivan Mrkvička during the 1st world war (Velenovský, 1922). Quite similar vegetation, sharing many species, can be noted about 80 km south on slopes by Vegoritida lake and phytocenologically, both those alliances harbor in *Astragalo-Potentilletalia*, *Festuco-*

Brometa (Matevski et al., 2015, Pirini et al., 2014). Surroundings of Prilep-Pletvar was also recognized as an Important Plant Area in 2008 and sorted to Scardo-Pindic province of a sub-Middle-European-Balkan subregion of a Middle-European biogeographical region; habitats of grasses/herbs were assesed here as 6210 (*) Semi-natural dry grasslands and scrubland facies on calcareous substrates (Festuco-Brometalia) and 34.3 Dense perrenial grasslands & middle European steppes (Plantlife, IPA database). The place is also known as a centre of endemism of the country's lowland belt (Čarni & Matevski, 2015) Species collected from around VČ and Prilep in 2017-19, which were determined to some level are listed in an Appendix.

To conclude on the environmental settings of the agricultural settlement, evidence of riparian vegetation, deciduous forests, grasslands, ruderal and weed flora from the Neolithic layers on site was already brought (Beneš et. 2018, Mazzuco et al. 2022), as well as faunal remains belonging to both wet and dry lands (Beneš et al., 2018;, Stojanovski et al., 2020), and the same habitats can be seen here until today.

2. Materials and methods

Soil samples for the analysis were collected during field campaigns in 2016-2018, when the Archaeobotanical summer school organized by LAPE (Laboratory of Archaeobotany and Palaeoecology at Faculty of Science, University of South Bohemia) took place in Prilep and Vrbjanska Čuka in cooperation with Prilep Museum, Institute for old Slavonic culture (Prilep) and Centre for Prehistoric research ((Skopje). Aerial photo of the trench with analyzed samples scheme is presented on Fig. 2 and a list of samples with basic description is given in Table I.

Table I: A description of the analysed samples with colours assessed according to a Munsell color chart.

Sample	Munsell	Description of the context
U137_06	10YR 5/2	bottom of the Neolithic pit in quadrant 29
Q30_U71	10YR 5/2	around the stone deposition in quadrant 30
U11	10YR 5/2	context full of river mussels and pottery
U7 D169	7.5YR 6/4	daub sample
U287 D7	10YR 7/4	daub sample
Q22U315(2)	10YR 5/1	black infill of intact daub layers
U47	10YR 6/2	bottom of infill of structure 47
P17/10	10YR 6/1	Bottom of the profile P17(Q17); 68-75 cm
P17/05	10YR 3/1	black daub in the middle of the profile P17; 40-45 cm
P17/05	10YR 6/1	layer directly above the daub in the P17; 30-40 cm

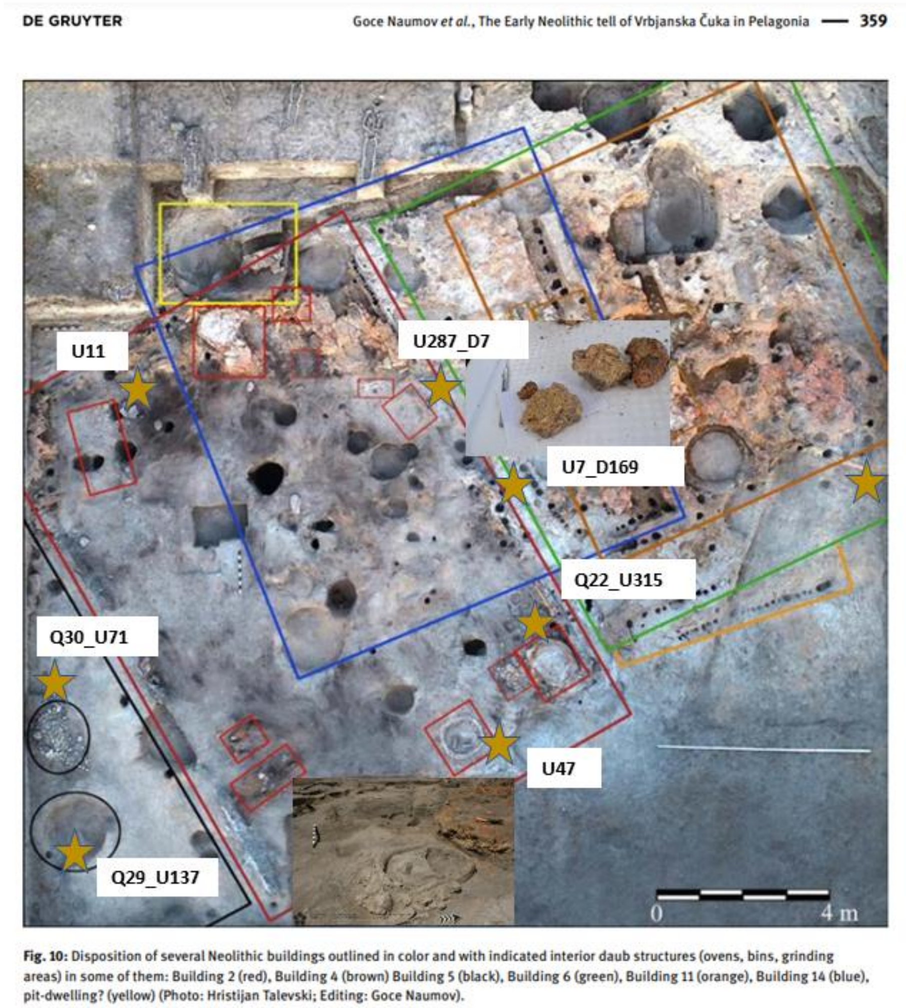


Fig. 2: A spatial scheme of the samples in southern part of the trench.
Photo: Hristijan Talevski, reprinted from Naumov et al., 2021.

Focusing on the Neolithic layers, sediment from bottom of an only large pit (Quadrant 29 Unit 137) dated to this period was sampled in 2017, marked 137_06; a whole profile of this pit was taken, but there was no stratigraphy of the infill recorded by archaeologists (and the upper samples, when analysed preliminarily, appeared quite the same), so only the bottom sample got fully quantified.

Close to the pit, by southern wall of the trench, a deposition of fragmented grinding stones (Fig. 3) was freshly unearthed in 2017; samples were taken from a posthole in an immediate vicinity west of the accumulation (Q30U_71).

Fig. 3: The deposition of grinding stones Photo: LAPE.

At a western part of the trench, Unit 11 was sampled and described as ashy layer behind Building 2, containing lots of river mussels and the fine red pottery.

Daub samples were obtained by crushing some of the pieces of the architecture (on the left on Fig. 4) analysed by Jana Anvari in 2018 and their position in the scheme is now approximate, most probably they did originated in the large daub structures in the middle of the area excavated in 2017-18.

Fig. 4: analysed pieces of daub (left) and the intact daub structure (right) of which an infill was taken. Photo: left – author, right – LAPE.

By the northern part of the trench (in 2017), a 70 cm stratigraphy of the Neolithic layers was preserved, from which the bottommost layer was analysed (P17_10, which contained large bones and pottery), together with layer of black daub sealing the lower part (P17_5d) and the layer above the daub (P17_5)(Fig. 5, below).

Fig 5: The profile in quadrant 17 (75 cm). Photo: author.

The very inside of structure interpreted as an oven in the last publication (U47, Naumov et al., 2021) was sampled (Fig. 6) and further some intact situations in an area surrounding the large working space in a north-east corner of the Building 2.(Fig 4), Q22U315(2).During the excavation, more documented context were taken, but turned out not to be suitable for the analysis because of an expected contamination from younger horizons or recent soil (especially in case of Building 2 ,,floors").

Fig. 6: The working space (left) and surroundings of U47 (right). Photo: left-author, right – (LAPE).

For a plant reference collection, Poaceae specimens from Pelagonia were chosen, representing either wild relatives of cereals (*Secale montanum, Aegilops cylindrica, Taeniatherum caput-medusae, Dasypyrum villosum*) or species indicative for different environments, such as wetland species *Glyceria maxima*, collected in České Budějovice in 2018 (reported from Pelagonia marsh in Country Study for Biodiversity, 2003), *Beckmannia eruciformis* which is an obligate halophyte, dryland *Festuca valesiaca* or the savannoid C4 grass C*hrysopogon gryllus*). A recent domesticated einkorn wheat (*Triticum monococcum*) grown in Vitín near České Budějovice, Czech Republic, was also included, to explore variations in its shapes.

Sample preparation followed a protocol elaborated by Albert & Weiner (2001), optimized for equipment available in the LAPE laboratory. Samples were slightly crushed, placed in glass Petri dishes, and dried at 50°C in a laboratory drier. After sieving on 0,4 mm mesh, a certain amount was weighed and put into 50 ml centrifuge tubes. Calcium carbonates were dissolved by adding 15 ml of 15% hydrochloric acid (HCl), and their amount was estimated on a scale of 0-5, according to a level of fizzing. Sediment was left in HCl for several hours until reactions with all the carbonates were over (with tips open, except when shaking it by hand a couple of times during the process). After that, samples were slowly fulfilled with distilled water and centrifuged at 2500 rpm; this step was repeated twice in order to prevent the mixing of acid with the following reagents. Clays were extracted by repeatedly adding a solution of sodium hexametaphosphate 50g/1l and centrifugation on 1000 rpm. After another neutralisation by distilled water and centrifugation, a concentrated nitric acid (20ml of 30% HNO_3) was added to eliminate organic compounds, mixing with samples by a laboratory vortex and leaving them for two hours (with tips still open, except the vortexing), then lowering the concentration by adding distilled water to a top of the tubes and the other day, centrifugation again on 2500 rpm and getting off the supernatant. Obtained mineral fractions were dried at 50°C in smaller Petri dishes, then scraped with glass sticks, carefully washed in ethanol, and kept under an aluminium foil to prevent their contamination from the air during manipulation with the dried samples, which were weighted again and kept in aluminium sheets, until the next steps were prepared. A sediment in this stage of the protocol is called Acid Insoluble Fraction.

At a final stage of the separation, 3 ml of sodium polytungstate (SPT, $Na_6H_2W_{12}O_{40}$) calibrated on 2,35 g/cm3 density was put to 15 ml polypropylene tubes (of which those with rounded bottoms attested better) and the samples carefully transported in. After mixing by vortex until any possible lumps got dispersed, the tubes were placed in a centrifuge and run on 800 rpm for 10 min. The heavy liquid did successfully separated the phytolith fraction fig which was transported back to the 50 ml tubes (meanwhile flushed just with a tap water) by plastic pipettes (new for each sample).

Sometimes, due to a large amount of phytoliths, this has not been much effective, and a better way appeared in carefully just spilling off the supernatant creating a kind of well-separated plug made of the SPT bonded phytoliths. When it was uncertain whether all the phytolith fraction got moved (usually when the pipette took all the liquid, but the upper fraction obviously remained), the step was repeated with fresh SPT and another centrifugation. Phytoliths in tubes were then supplemented by distilled water to maximum, vortexed, and centrifuged again at 2500 rpm or more if the pellet was loosening during spilling off the supernatant. In the end, phytoliths were removed to clean small Petri dishes (carefully washed and dried upside down) by adding about 15 ml of ethanol (C_2H_6O); quick vortexing and leaking everything to the dish, flushing the rest by a pipette filled with ethanol, immediately covered and transported to a drier. After drying and scratching off the clean phytoliths of the dishes with glass sticks again, the dust gained was weighted on analytical weights, and a certain amount was transported to a microscopic slide (weighted again) with a few drops of Euparal epoxide, covered with a large, microscopic glass and the rest is kept in small plastic tubes or aluminium foil sealed in plastic bags.

Manufacturing of permanent slides was evaluated as an advantage in phytolith quantification when counting can be eventually stopped and renewed at any time, centred on another part of the glass when needed, or when going back to single particles hard to describe; however, it disables a further rotation of the phytoliths, so every each sample was observed first and mostly photographed only in distilled water, which allowed to the learn the 3D shapes of the phytoliths necessary for further description of the morphotypes during the quantification.

Microscopic slides were prepared by precisely weighting a small amount of phytoliths on a slide and a concentration of phytoliths was quantified as a number of phytoliths per 1g of AIF (Albert & Weiner, 2001). Phytoliths were observed by a Leica (DM2500P) polarising microscope on 500x or 200x magnification and described after an International Code for Phytolith Nomenclature 2.0 (Neumann et al., 2019) and around 250 particles were counted for a sample, which was attested as a minimum limit for a representative quantification (Zurro, 2018). Skeletons, i. e., aggregates of more than one phytolith, were counted as a single particle.

The plant parts for the reference collection were mounted in 30 ml of 65% HNO_3 for several hours and later, the concentration of the acid was lowered by distilled water (slowly and carefully because a reaction may appear) and centrifuged at 2500 rpm; this step was repeated 3x, and after that, the samples were dried by the same method as samples above.

Graphs of phytolith morphotypes were elaborated in Tillia programme (Grimm, 1992). Phytolith morphotypes in all the samples were processed by a simple unimodal correspondence analysis using a Canoco5 programme (Šmilauer & Lepš, 2014).

3. Results

Phytoliths were successfully extracted from all the soil samples and a table is provided containing the levels of fizzing, weights of the original sieved material, AIF, clean phytolith fraction and counted phytolith concentrations (Tab. II).

The content of calcium carbonates was very high in most of the samples, when a zero value appeared only in case of U7_D169 and lower than maximum in the two other daub samples. A highest concentration of phytoliths was counted for U11, while the lowest in sample P137_06; the concentration ranges from 11,741,739 (U11) phytoliths per 1 gram of the Acid Insoluble Fraction to 50,337 (P137_06).

Tab II: Samples with recorded level of fizzing, original weight of samples, weight of AIF, pure phytolith fractions and counted phytolith concentrations.

Sample	Fizzing (0-5)	weight (g)	weight of AIF (g)	phytolith fraction (g)	phytoliths per gram AIF
P 137/06	5	1,226	1,002	0,235	50,377
30/71	5	1,15	0,853	0,273	872,372
JB U11	5	1,24	0,67	0,394	11,741,739
U7 D169	0	0,612	0,534	0,107	479,387
U287 D7	3	0,58	0,329	0,055	631,099
Q22U315(2)	5	0,868	0,607	0,15	3,350,033
Q22U314U47	5	0,76	0,357	0,142	8,899,018
P17/05	5	1,086	0,731	0,157	1,915,291
P17/05d	4	1,135	0,883	0,223	5,744,514
P17/10	5	1,37	1,081	0,427	3,959,878

Described phytolith morphotypes and their amount in the samples are presented on two graphs; the first graph bears non-stratigraphical samples where phytolith counts are represented in bars (ordering is only a technical one, Fig. 7), while the second graph belongs to the profile in the quadrant 17 and a silhouette expression was chosen to highlight the trends (Fig. 8). The second graph is, therefore, stratigraphical and shows the phytolith morphotypes in a "developmental" sequence. The morphotypes were sorted into categories according to their determinable taxonomy (Poaceae chaff, Poaceae SC (short cells), leaves/stems (Poaceae) and dicotyledonous plants; the rest of morphotypes is out of categories, since they cannot be determined.

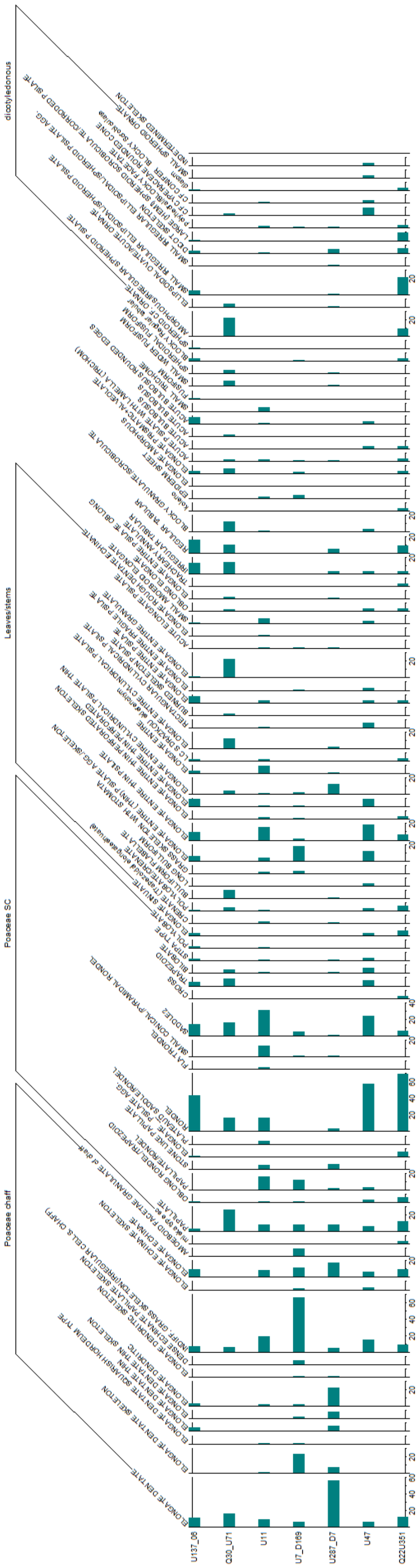


Fig. 7: Diagram of the non-stratigraphical sampes.

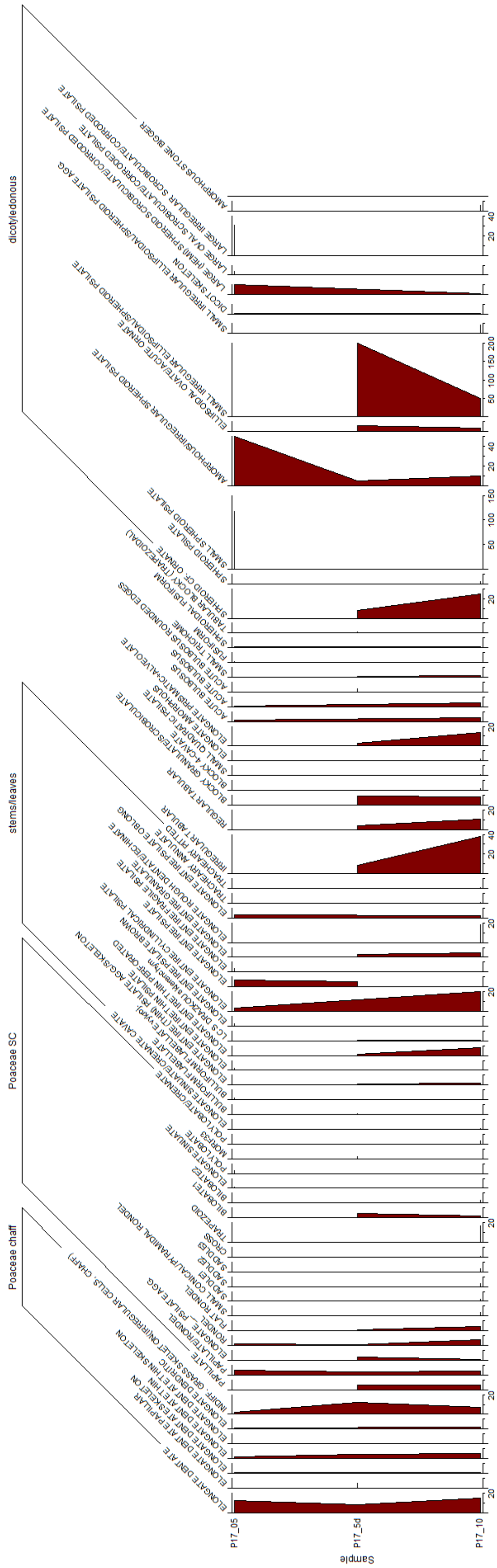


Fig. 8: Diagram bearing the samples from P17.

Regarding the non-stratigraphical samples, the results reveal a highest abundance of morphotypes in Poaceae chaff category (in samples U7 D169 and U287 D7, while the rest of the samples were rich in the Poaceae SC category, where RONDEL was the best represented.

In case of the second graph, dicotyledonous phytoliths yield the highest abundances, while the chaff appears continuously in a low amount. A shift in morphotypes is visible between P17_5d and P17_5.

Results of the correspondence analysis with all the samples included and 20 the best explained morphotypes are visualised below on Fig. 9. All four axes together explained 64% of a variation.

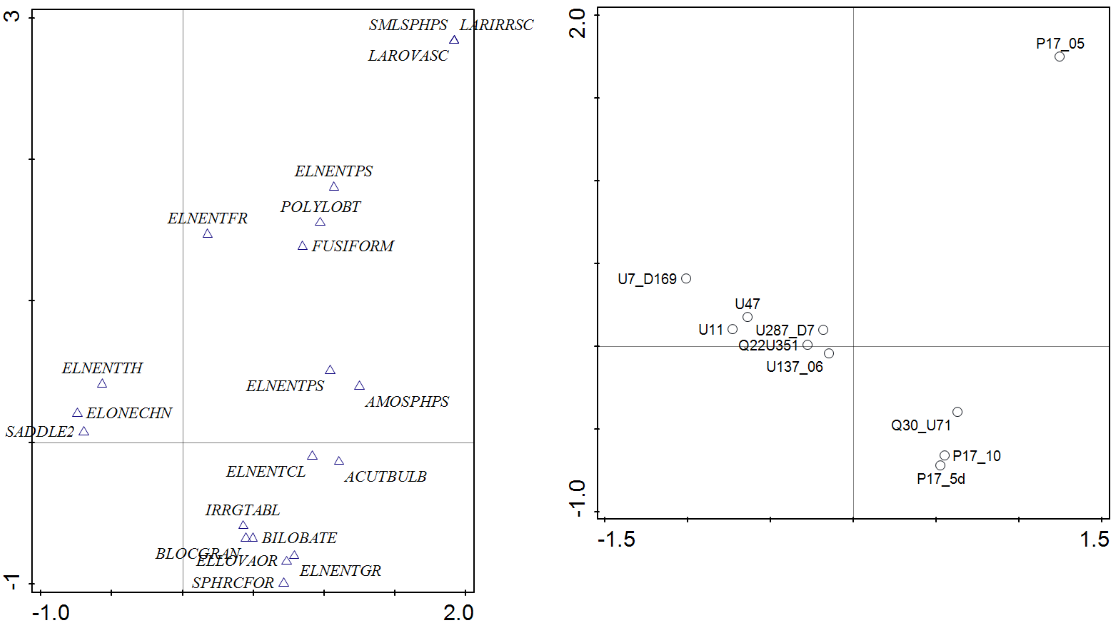
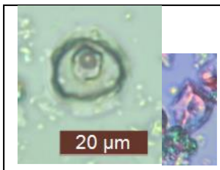
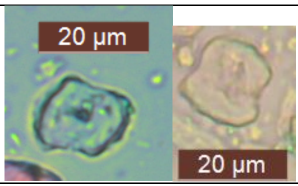
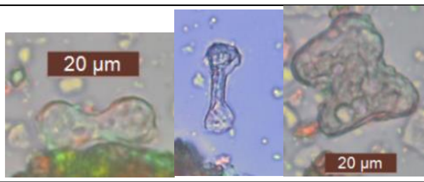
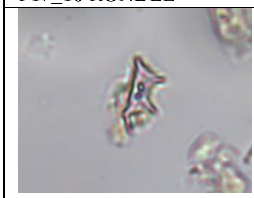
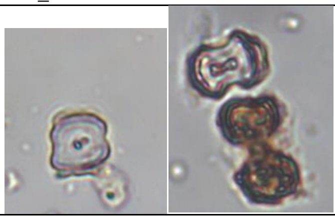
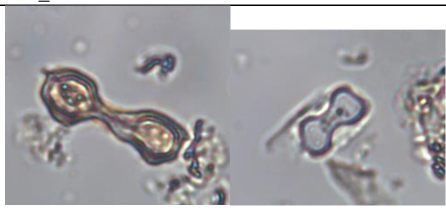
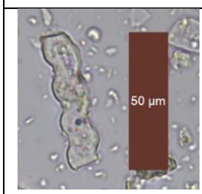
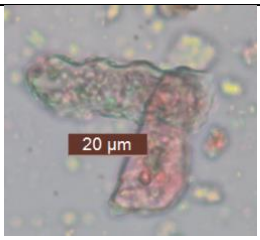
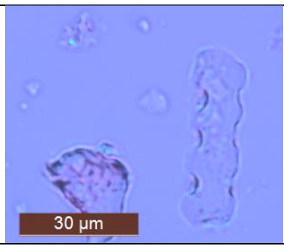
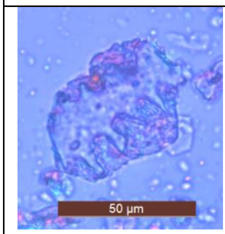
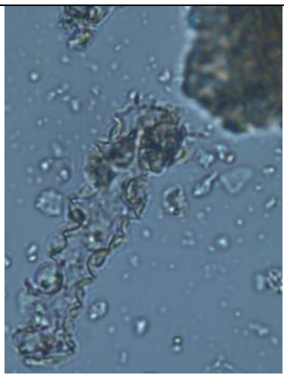
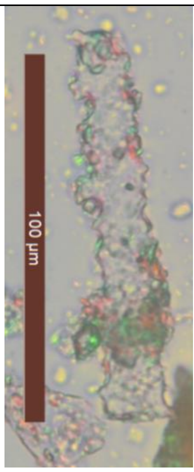
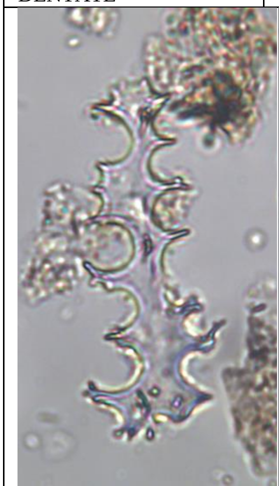
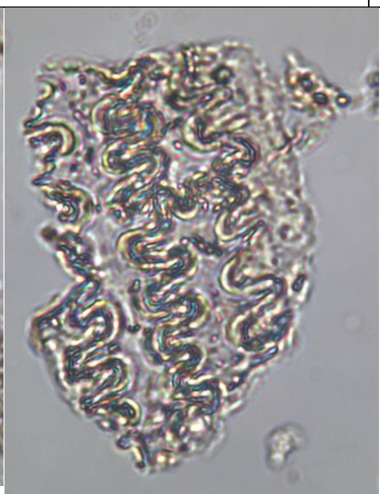
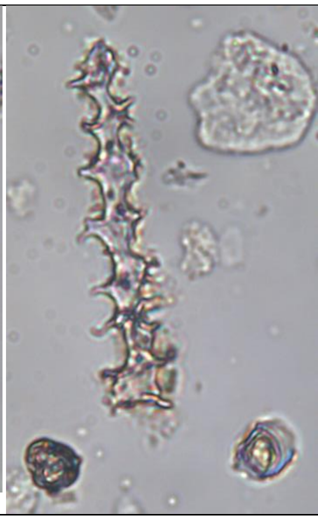


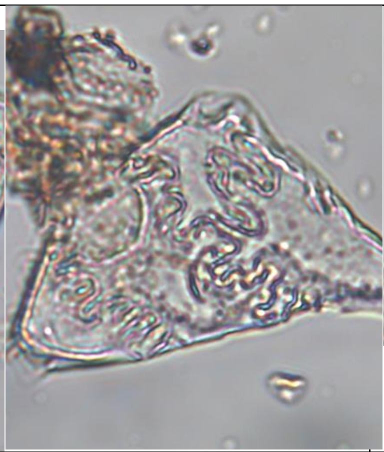
Fig. 9: Results of the correspondence analysis with 10 samples and 20 morphotypes included.

The diagram is showing a cluster of samples from the southern part of the trench (U47, U11, Q22, U351, U137_06) together with the daub sample U287D7, while the sample from around the stone accumulation groups with the lower layers from P17. The upper part (P17_05) demonstrates a different phytolith composition, as well as the daub U7_D169. From a single morphotype point of view (on the left), the SMALL SPHEROID PSILATE, LARGE OVAL AND IRREGULAR SCROBICULATE are grouping together towards P17_5, while on the other end of the y axis, morphotypes such as ELONGATE ENTIRE GRANULATE, SPHEROID cf. ORNATE or BILOBATE can be found. .Selected morphotypes are presented in Table III below. Phytoliths were documented with the same magnification and resolution, hence the figures without a scale are comparable to the scale where present.

Tab III. Selected phytolith morphotypes.

		
P17_10 RONDEL	P17_10 SADDLE	P17_10 BILOBATE
		
U11 RONDEL	U11 SADDLE+BILOBATE	U11 BILOBATE
		
P17_10 POLYLOBATE	P17_10 ELONGATE SINUATE	P17_10 POLYLOBATE

		
U11 INDETERMINED	U11 POLYLOBATE	U11 ELONGATE SINUATE 2x
		
P17_10 ELONGATE DENTATE	P17_10 ELONGATE DENTATE	P17_10 ROUGH ELONGATE DENTATE
		
U11 ELONGATE DENTATE/DENDRITIC	U11 ELONGATE DENTATE SKELETON	U11 ELONGATE DENTATE/DENDRITIC

		
U11 INDETERMINED GRASS SKELETON	U11 INDETERMINED GRASS SKELETON	U11 INDETERMINED GRASS SKELETON

Regarding the reference collection, phytoliths were successfully documented in all the processed plant parts and examples are presented on several pages in the Appendix, together with examples of the phytoliths from the daub.

4. Discussion

The analysis of plant phytoliths from the Neolithic site yielded a number of new findings, which are discussed and commented in the following paragraphs. First comment is given on the taphonomy and distribution of phytoliths in the sediment, noting the potential for capturing human activity at the site. Then the potential of phytolith analysis for reconstructing the Neolithic tell environs is discussed.

Concerning the taphonomy, phytoliths at the bottom of P17 appear to be a bit „melted", which may be due to post-depositional factors; it is known that the amorphous silica is better soluble in alkalic, rather than acid environment (Cabanes et. al, 2011) and hence the taphonomy perhaps could be caused by a fluctuating water level rich in calcium, in some periods after the initial phase of the settlement. Given the fact that the sediments on Vrbjanska Čuka are strongly calcareous, which was proven by testing the soil for carbonates (and also obvious from basically testing soil textures), the conditions for such a taphonomic processes are met).

Another issue of taphonomy is burning on phytoliths and its traces, which were noted already in Beneš et al. (2018); even though some of the contexts were apparently burned, containing microcharcoal and carbonized seeds (e.g., U11), a quantification of it was abandoned because it would create too many phytoliths categories and also the problem is rather complex, since brown to black colour of phytoliths can be caused by other factors as well (Devos et al., 2020; Parr, 2006). In the case of the chaff remains, a potential was recognized in states of their preservation; skeletons with rounded edges were proposed to be digested by ruminants (Madella, 2007), those with specifically cut sharp edges might indicate specific ways of processing (Portillo et al., 2017; Cummings, 2007) and finally, a number of phytoliths articulated in single husk skeletons may indicate a water regime during the growth (Shillito, 2011); all such a taphonomical states were observed in the samples; however, quantification of it was not performed, because of the environmental conditions which could influence the„melting" and because the size of the skeletons may be affected by the centrifugation (Shillito, 2011) (and again, it would create too many categories to handle).

Discussing the spatial distribution of phytolith morphotypes which could reveal the activities on site, according to the results it is not possible to determine precisely where did the cereal processing took place, since most of the indicative morphotypes such as ELONGATE DENTATE, ELONGATE DENDRITIC and its SKELETONs were present in all the expected processing-related contexts in moderate rates, while most of it was recovered from the daub samples.

The scarcity of the cereal residues around the stone accumulation may be explained by heir secondary deposition, and in the case of pit 137_06 by its different function - in the CA graph, this sample appears one of the most neutral. In the case of Q22U315(2), the cereal residues may have not been recovered in higher abundances due to sampling since the working surface of the large structure was not sampled directly and samples were taken rather from under the attached intact daub structures.

The CA diagram also indicates grouping of the lower samples of P17 with a sample(s) around the stone deposition, which may help confirm a simultaneous occupation with related activities.

The richest context in grass morphotypes generally turned out to be U11, which could be perhaps considered as an accumulation of fuel mixed with other household waste, given its origin in the deposition of river mussels and pottery and due to a presence of similar straw phytoliths referred in the literature as particles of animal dung (Delhon et al., 2008), here the best represented by ELONGATE ENTIRE THIN skeleton, and the overall „burned'' character of the material; such an interpretation might be in congruence with the far highest phytolith concentration.

The high abundance of a SMALL IRREGULAR/SPHEROID PSILATE, indicating probably a dicotyledonous leaf mesophyll (although they do appear as well for example in some fruits, see Neumann et al., 2019) could be possibly explained by storage of twigs as fodder or they might have come from a simple deposition of leaves from a tree growing near. Some twigs and branches may have been indeed incorporated in the architecture, as the layers are considered (as far as I know) to be burned and unburned destructions of houses; roof constructions made of such a material and straw are known in the area ethnographically (Friesem et al., 2014) and a presence of a rounded BULLIFORM FLABELLATE in the layers, indicating a usage of a rather wide-leave grasses, might support such an interpretation, together with the CA diagram, which clearly show a different composition of P17_5. The branches may have been also collected for fuel and disposed here as an ash (after Tsartsidou & Kotsakis, 2020). Given a wisible shift in a shape of those small phytoliths, a combination of the options can be considered as well. The daub layer in P17 is well different from the composition of daub in the central area, where mostly Poaceae remains were found. That reveals interesting variations in the architectural techniques, together with preliminary results of morphological analysis of the daub, where impressions of straw were mostly observed in general (Naumov et al, 2021). A recovery of chaff in the daub samples is not very surprising since it was observed macroscopically and hence chosen for the analysis (the results thus are not generally representative), it is, however, interesting that the material was quite homogenous, not mixed with many other plant resources, nor any clays (it consisted of a calcium-binded silt instead). A pattern of cereal phytoliths embedded mostly in a daub was also found at a Trypilian site Maidentske in Ukraine (Dal Corso et al., 2018) and imprints of *Triticum monococcum* were observed also on a site Balgarčevo (Marinova, 2017). In this analysis, it can be further noted that while one unit of the daub contained mostly single ELONGATE DENTATE phytoliths, the other kept them in the form of SKELETONs, suggesting a different decomposition level of the original material.

Discussing directly the chaff residues, after a comparison with available literature (Ball et al., 1999; Ryan, 2011; Madella et al., 2014) and own reference collection, some phytolith skeletons with distinct morphologies could be attributed to *Triticum* (probably *T. monococcum*, which is the most represented in macroremains (Vychronová, 2022; Antolín et al., 2020)); their shapes, however, appear somehow unconsolidated, not very regular and straight, compared to the reference. Skeletons of the processed wild cereal-related grasses, compared to a reference given in Rosen (1992), did not show the expected patterns, as *Aegilops cylindrica* expressed a pattern similar to an oat grass, while the rye *Secale montanum* would quite well correspond to a pattern of wheat. *Dasypyrum villosum* creates husk structures also resembling wheat but with a rather specific shape of the ELONGATE DENTATE, which was also recovered in the sediments, thus the presence of this grass in the Neolithic cannot be excluded. *Taeniatherum caput-medusae* creates quite dense structures with many trichomes, which are well distinctive on their own and were not observed in the samples. Speaking of the non-cereal Poaceae chaff types, Especially in U11, some of the skeletons probably belongs to wild grasses and generally, many grass skeletons were recovered, whose taxonomy was not possible to determine. Elongate dentate type prevailing in P17_10 (middle of a second row in a Table III p.22, which is not obvious from the graphs but was differentiated when counting) and present in other samples as well cannot be assesed as a cereal residue, because it was observed that it had a different design of the tissue than the Triticeae have.

Having in mind the above-mentioned results of the pottery shred analysis suggesting usage of leaves of Poaceae, some of the BULLIFORM FLABELLATE and as well the SADDLE morphotype do visually match with a grass *Cynodon dactylon* (reference in Chauhan et al., 2011).

LARGE HEMI-SPHEROID PSILATE/ SCROBICULATE phytolith (often brown) deserves attention in a matter of potential dicotyledonous food resources, as it was found also in dental calculus of an individual at Mesolithic/Neolithic Lepenski Vir, where it is reported as a phytolith of „bark" and discussed as a possible source of famine food (Jovanović et al., 2021).

A higher presence of the diagnostic grass short cells (SC) such as RONDEL, SADDLE, and to a lesser extent BILOBATE in the southern part of the trench is useful for the hypothetical reconstruction of the environment, despite it is hard to judge how much of the material was brought by the people and how much of the material could have been accumulated on the tell for example in the form of dust, because it is known that phytoliths can be transported by wind, especially in dry and sparsely wooded environments (Madella & Lancelotti, 2012) and that might be even more relevant in case of those small short cells; on the other hand, it might be potentially used in the archaeological research when differentiating between open spaces and structures with a roof.

A problem here might be an underrepresentation of potentially exploited wetland vegetation since one of the best candidates *Typha* sp. probably does not produce leaf phytoliths (Pearce & Ball, 2020), and only weak structures with single phytoliths were observed in *Glyceria maxima*. Of the possible wetland resources, skeletons are probably of *Juncus* sp. were sporadically recovered (ELONGATE ALVEOLATE + skeleton) (after Tsartsidou et al., 2007)

A comparison with the reference collection of the recent grasses did not brought any apparent results, although a certain potential was recognized, since the grasses as *Festuca valesiaca* and *Chrysopogon gryllus* did contain the same morphotypes, which were observed in the samples; however, a confirmation of their identities would must have been necessary to test by advanced methods, such as phytolith morphometrics.

When comparing the gain phytolith spectra to other Neolithic sites in south-east Europe, generally, sites from Romania (Danu et al., 2020; Malaxa et al., 2020) seem to share higher abundances of RONDELs, while BILOBATEs are represented on Vrbjanska Čuka in lower frequencies and SADDLEs in a higher; a large proportion of Pooid phytoliths was recovered also in the samples from Palimbela Kolindros, where the results are given in phytolith categories (Tsartsidou & Kotsakis,2020).

According to findings of phytolith analysis in palaeoecology, high rates of RONDELs in soils are usually associated with xerothermic grasslands of Pooid species (Golyeva, 2007; Sylantieva et al., 2018; Pető, 2013), while „chloridoid" SADDLE phytoliths generally belong to C4 grasses, well-adapted to arid environments and the BILOBATEs were both of a Panicoid type (C_4) and the Stipa-type (C_3)(Twiss et al., 1969; Piperno, 2006, Solomonova et al., 2017).

Hence, together with the results of the malacological and zoological analysis performed at Vrbjanska Čuka, (Beneš et al., 2018, Stojanovski et al., 2020), the phytolith spectra further argues that open and dry environments must have been present already in the Neolithic, at least to some extent. Such a finding may be somehow important for enlightening the process of a neolithization of the European continent, since it is not fully understood, how did the first farmers (especially in case of Pelagonia) advanced so rapidly through the fertile, but initially forested flood plains (Krauß et al., 2018). A same question could be adressed here as in central Europe, where it was debated for a long time, whether the steppe islands were already existing at the time of arrival of the first farmers or to what an extent they were created by them, bringing the anthropogenic and steppe vegetation within (Hejcman et al. 2013).

In the Republic of North Macedonia, a debate on a natural appearance of steppe grasslands has accelerated only recently, when *Stipa* plants (*S. ukrainica* and *S. crassiculmis* subsp. *picentina*) from Vardar region were published and proposed to be disjunct relicts of a Pleistocene vegetation (Kabaš et al., 2019a; Kabaš et al., 2019b); however, a prevailing opinion sustains, that all the Macedonian steppic flora is of secondary origin, established after a detoriation of mid-Holocene forests (Matevski et al., 2015; Matevski et al., 2008). Quite elsewhere, grasslands with the savannoid species *Chrysopogon gryllus* are considered to be native, natural or semi-natural vegetation - e.g. in Republic of Moldova, they are thought to appear for the first time in a Middle Miocene (Lazu et al., 2019). In the review elaborated by Lazu et al. (2019), discussing in detail Eurasian phytocenoses with *Chrysopogon gryllus*, the Pelagonian communities in North Macedonia were only mentioned, while in the following review, discussing associations both with *C. gryllus* and *Botriochloa ischaemum* (Lazu et al. 2021), they were not included for a no apparent reason, which only highlights a need for further research.

5. Conclusion

The analysis of phytoliths from Vrbjanska Čuka did further confirmed usefulness of an application of the analytical discipline in the archaeological praxis, bringing some first quantified observations for the Neolithic period in North Macedonia. A new light was shed on a genesis of the archaeological deposits and activities around building 2, complementing the archaeobotanical and archaeological data, despite the analysis has failed to identify the ultimate spot of the cereal processing, since most of the phytoliths were embedded in the daub and in the other contexts, they were present with moderate rates. Apart from the cereal cultivation, exploitation of other food resources, such as wild grasses and a „bark" can be proposed as a hypothesis and tested in further research.

Regarding the environmental conditions, the phytolith analysis is in congruence with the already published results of plant macroremain and faunal analysis, bringing evidence of the presence of grasslands in the critical period of the site occupation, probably alternating with spring seasonal shallow water pans, surrounded by a river, smaller streams, discrete permanent ponds or a lake. However, it still needs to be made clear to what extent the landscape may have been already open at the time of the first agriculturalists' arrival.

A methodological suggestion arising of the thesis is that wild grasses, especially the close relatives of wheat, should be studied more in detail to avoid biases when interpretating the phytolith data concerning the early agriculture.

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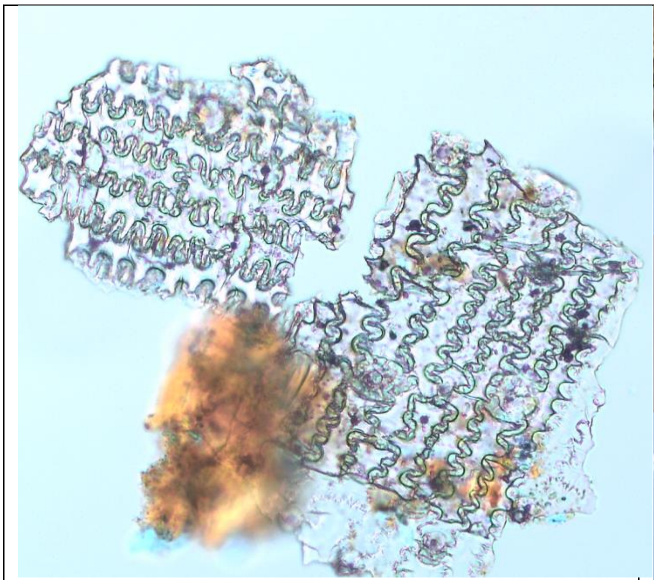
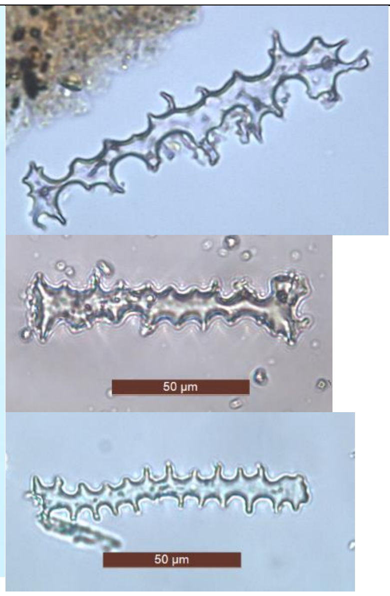
Appendices

Appendix 1. The list of plants collected near Vrbjanska Čuka

The following list contains plants which were collected by the author in 2017-18 mostly around Vrbjanska Čuka, further on Markovi Kuli hill above Prilep and in a lesser extent, at the Natural monument Lokvi-Golemo Konjari about 1 km N-E from the tell-site.

Chrysopogon gryllus , *Koeleria* sp., *Secale montanum* , *Stipa capillata, Botriochloa ischaemum* , *Taeniatherum caput-medusae, Poa bulbosa* , *Avena* sp., *Anthyllis vulneraria, Astragalus* sp.,*Scleranthus perrenis, Oxytropis pilosa, Allium flavum, Dianthus* sp., *Euphorbia cyparissias, Eryngium campestre, Marrubium peregrinum, Teucrium polium, Scabiosa* sp., *Orlaya* sp., *Thymus* sp., *Bromus hordeaceous, B. secalinus, B. scoparius, B. tectorum,B. japonicus, B. arvensis, Cynosurus echinatus, Dasypyrum villosum, Aegilops cylindrica, Cynodon dactylon, Trisetum* sp., *Antoxanthum* sp., *Hordeum* sp., *Festuca valesiaca, Spergularia rubra, Carex muricata* agg., *Carex* cf. *stenophylla, Carex* cf. *melanostachya, Achillea millefolium* agg., *Melilotus albus, Trifolium campestre, T. arvense, T. dubium, T. incarnatum,, Potentilla argentea* agg., *Cynoglossum* sp., *Galium verum, Matricaria recutita, Cichorium intybus, Onopordum acantheum, Centaurea idaea, Centaurea stoebe, Tanacetum vulgare, Calendula officinalis, Anthemis tinctoria, Inula* sp., *Artemisia campestris, A. vulgaris, Erigeron canadensis, Consolida regalis, Nigella arvensis, Ranunculus arvensis, Anagallis arvensis, Delphinium* sp., *Linaria* sp. *Althaea* cf. *setosa, Pseudolysimachion spicatum, Mentha* sp., *Ajuga chamaepytis, Medicago* cf., *falcata, Lotus corniculatus, Hypericum* sp., *Daucus carota, Lactuca serriola, Echium italicum, Verbascum blattaria, Clinopodium vulgare, Stachys* sp., *Salvia* sp., *Glycyrhizza echinata, Elymus farctus, Beckmania eruciformis, Polypogon monspeliensis, Bulboschoenus maritimus* agg., *Cyperus longus, Sorghum halapense*

Appendix 2: Examples of phytoliths recovered from Vrbjanska Čuka (following pages)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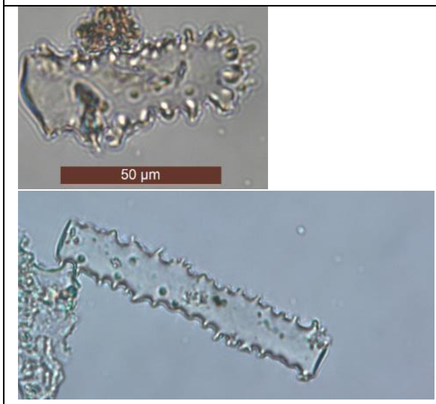	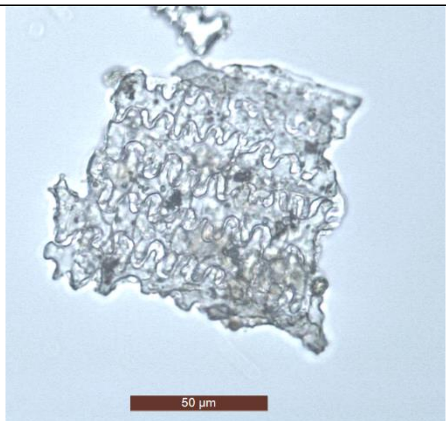
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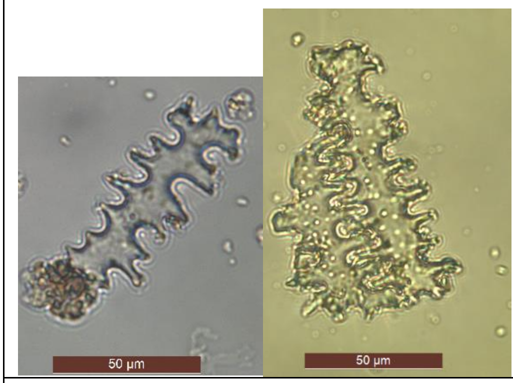
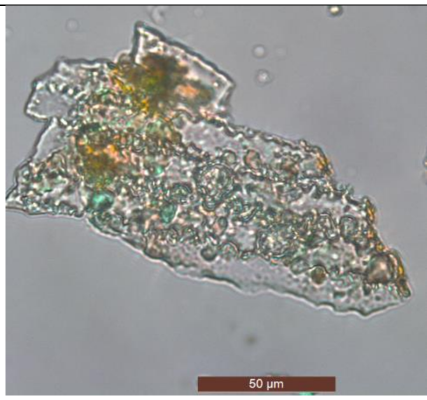
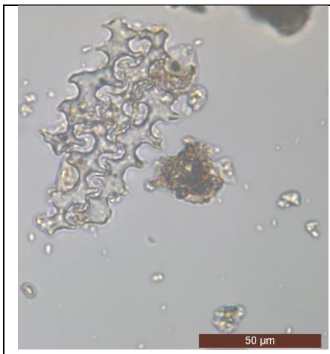
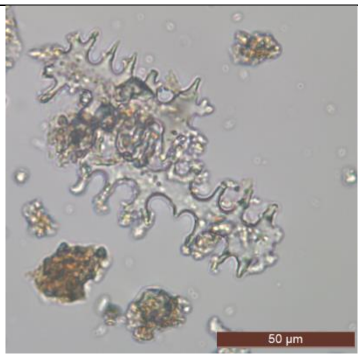
	
U7_D169 ELONGATE DENTATE+SKELETON 500x	U7_D169 INDETERM. GRASS SKELETON, 500x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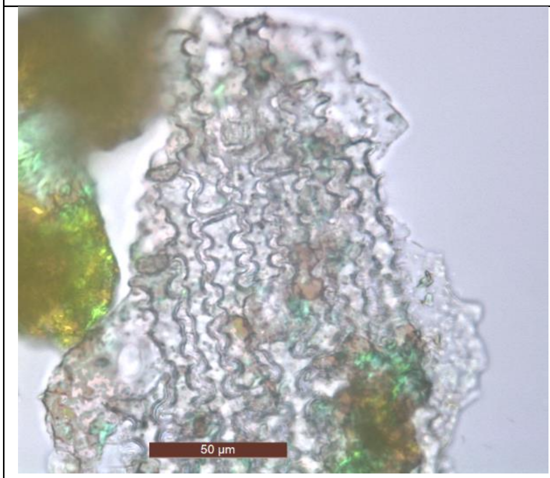
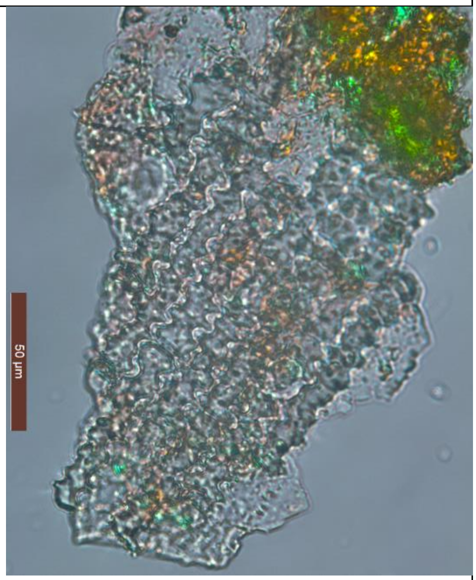
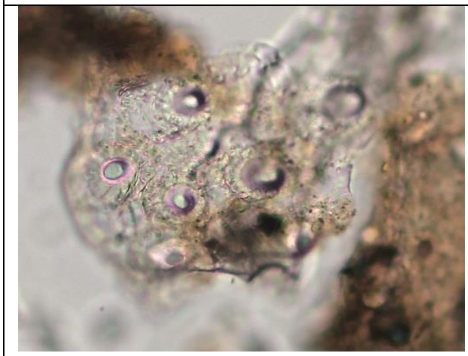
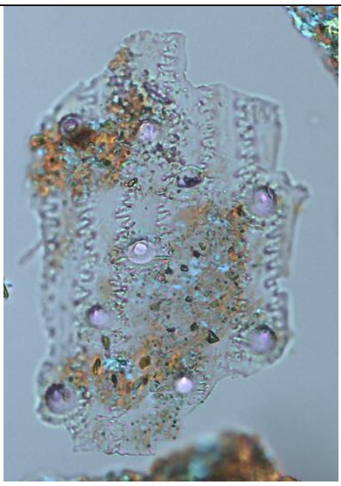
	
U7_D169 ELONGATE DENTATE SKELETON , cf. *Triticum* 500x	U7_D169 ELONGATE DENTATE SKELETON , cf. *Triticum* 500x

	
U7_D169 ELONGATE DENTATE SKELETON , 500x	U7_D169 ELONGATE DENTATE SKELETON , cf. *Triticum* 500x
	
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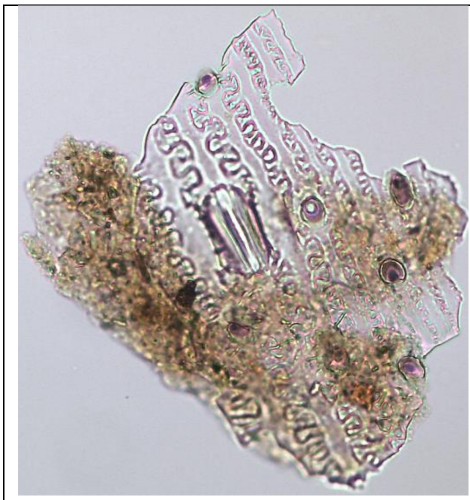
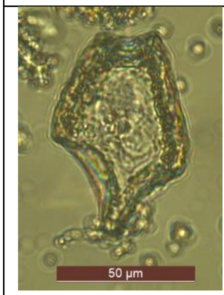
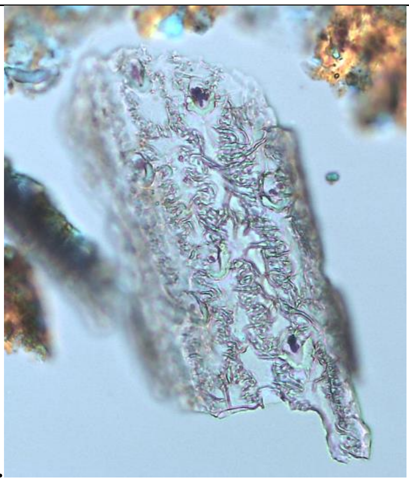
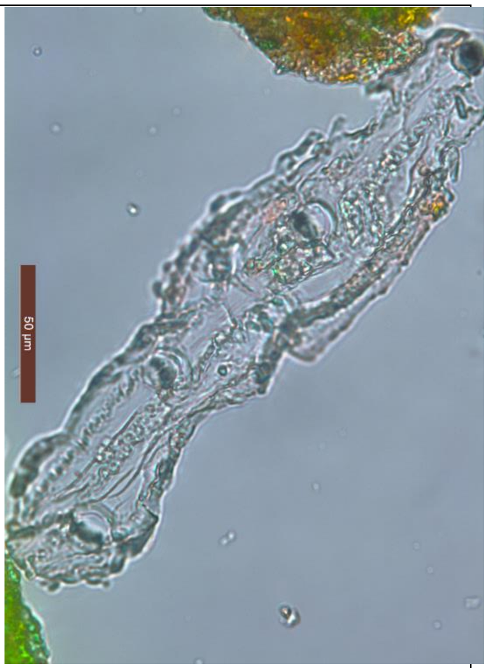
	
U7_D169 INDETERM. chaff (Poaceae) 500x	U7_D169 INDETERM. chaff (Poaceae) with BILOBATEs, 500x
	
U287_D7 BULLIFORM FLABELLATE 500x	U7_D169 ELONGATE ALVEOLATE 500x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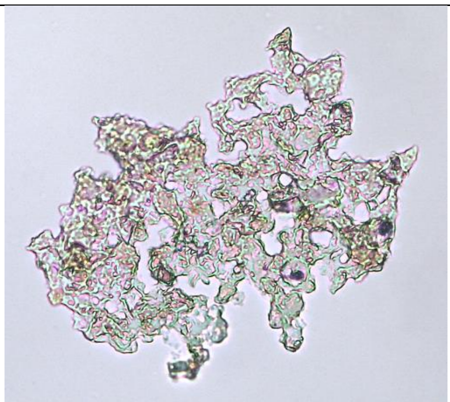
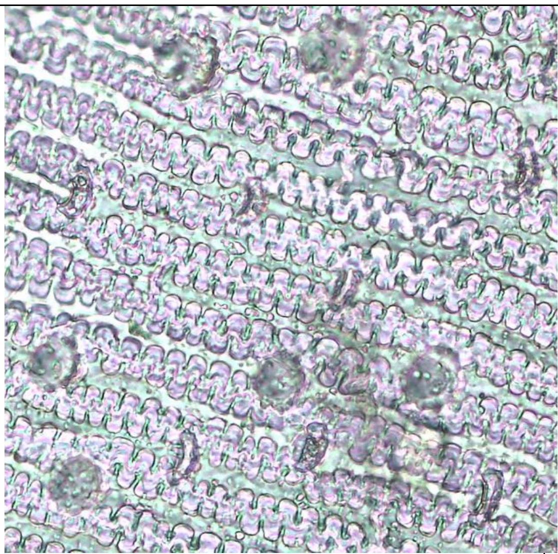
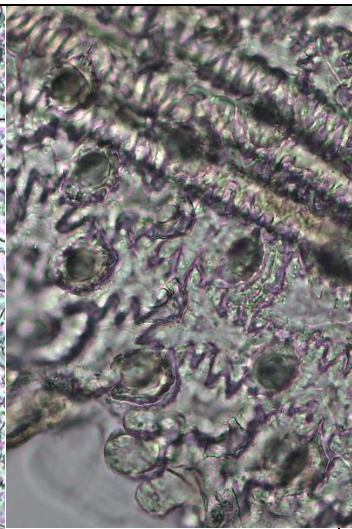
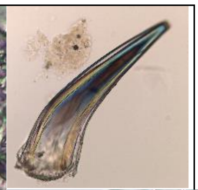
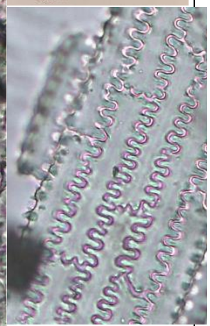
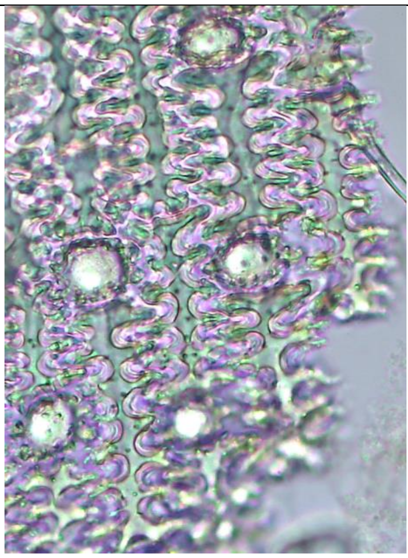
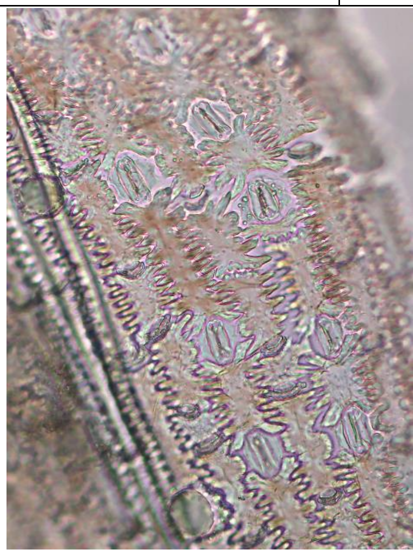
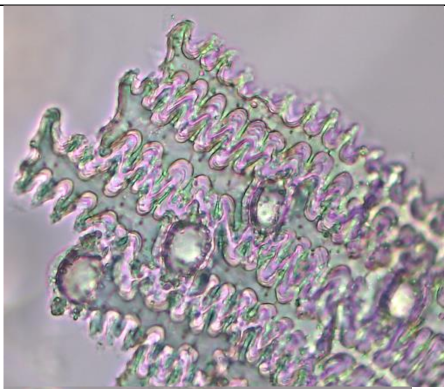
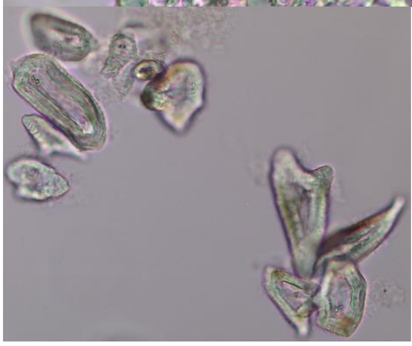
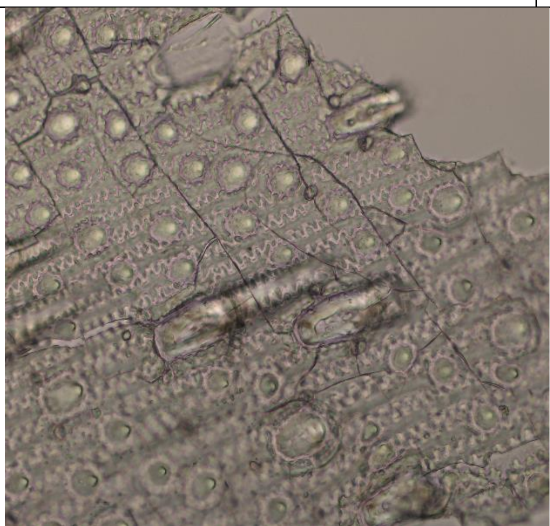
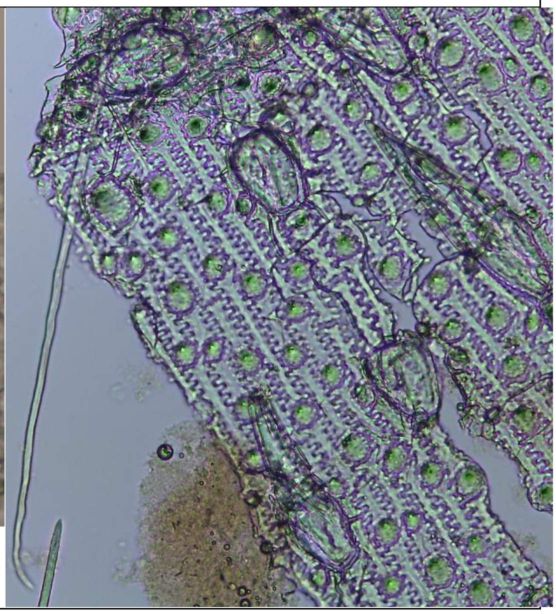
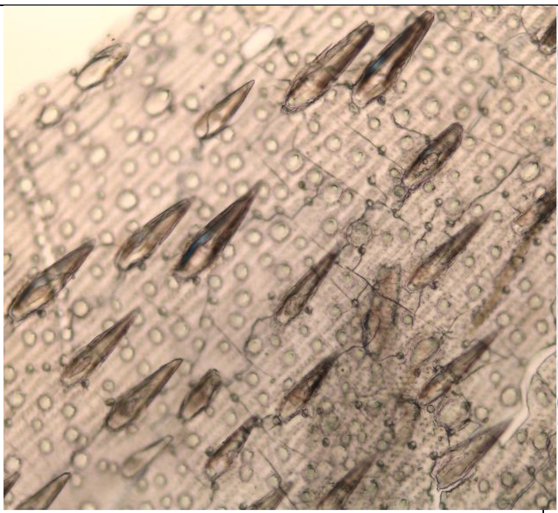
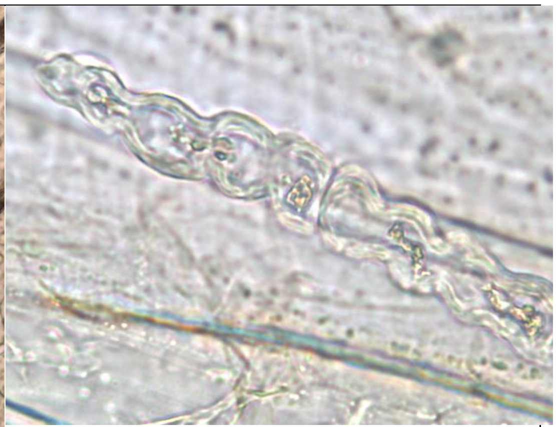
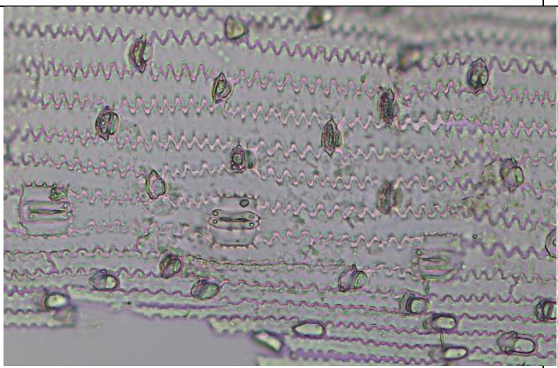
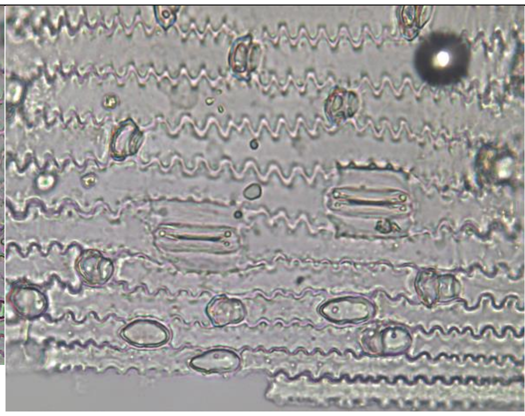
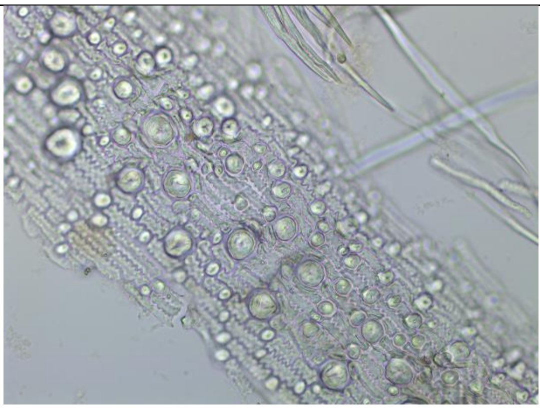
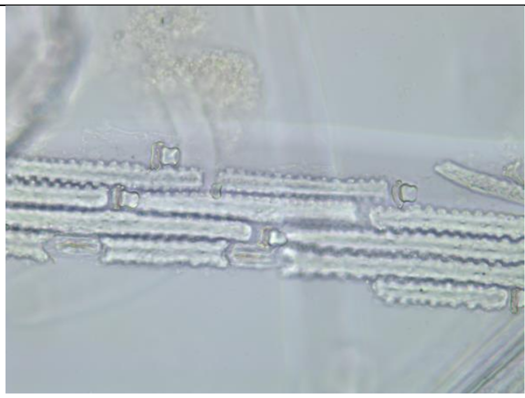
	
U7_D169 INDETERM. SKELETON 500x	U7_D169 INDETERM. SKELETON 500x

Appendix 3: Examples of recent plant phytoliths from the reference collection. (No scale).

Secale montanum chaff		  
Aegilops cylindrica chaff		

	 	
Taeniatherum caput-medusae *chaff*		

		
Festuca valesiaca *leaf*		
Chryso-pogon gryllus *chaff*		

Beckman-nia erucifor-mis *chaff*