PNAS
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Supplementary Information for

Archaeobotanical evidence reveals the origins of bread 14,400 years ago in northeastern Jordan

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This PDF file includes:

www.pnas.org/cgi/doi/10.1073/pnas.1801071115

Supplementary Information Text
Identification of plant tissues in the food remains from Shubayqa 1

Following previously tested identification criteria (*1-3*), for this study we considered these main plant tissues: layers present in the cereal kernels (pericarp and seed coat), husks (epidermis of paleas and lemmas), other parenchyma tissues (pulses and tubers), vascular tissues (tubers), and starch granules were considered. From these, the following were found in the food remains from Shubayqa 1:

Tissue layers present in cereal kernels including:

-Pericarp layers (bran)

Pericarp layers include elements such as the epicarp, the mesocarp and the transverse cell layers (*4*). From these, the latter two were identified in Shubayqa 1.

The mesocarp consists of two or three layers of cells which are known as longitudinal cell layers. These layers of cells are commonly identified in the composition of archaeological cereal foods, including those analyzed in this study.

Transverse cells layers are also known as cross-cells and tube cells in cereal grains. They are found beneath the mesocarp and these are the most often identified layers in archaeological food remains such as those from Shubayqa 1. The transverse layers consist of rows of elongated cells, like those seen for the epicarp layers, but arranged side to side instead of end to end.

It has been possible to identify several fragments of longitudinal and transverse cell layers of the cereal kernel in the food remains from Shubayqa 1 (**Fig. S2**, **S3**). In wild cereal species, such as those present in the macrobotanical assemblage from Shubayqa 1, longitudinal and transverse cells tend to be narrower and longer in comparison to their domesticated successors.

-Endosperm

Aleurone layer/aleurone cells: The aleurone layer encloses the outer surface of the starchy endosperm in cereal grains. It consists of a single layer of cells in most cereals such as wheats and oats, whereas the aleurone layer is multicellular in barley species. Aleurone cells vary in size depending on the cereal species however their shape is uniform. When seen in cross-section they appear cuboid in shape, while when seen from their top view they seem more rounded polygonal. The aleurone cells' cytoplasm is densely packed with aleurone grains (considered as protein bodies, storage protein) and lipid droplets (spherosomes). The patches of aleurone layer found in the food remains from Shubayqa 1 were in their majority characterized as single-celled aleurone layers and therefore most likely derived from cereals such as wheat or oat species both present in the macrobotanical assemblage (*5*). In some cases, the remains of a possible second aleurone layer have been observed, however, due to the poor preservation of the cells structure and shape, it is inconclusive whether these indicate barley (**Fig. S4**).

Starch parenchyma: These cells form most of the seed and they are large, isodiametric and thin-walled cells, which contain starch grains. They vary greatly in shape and size depending on the species of cereal although their measures are commonly under 20 μm in diameter. Patches of parenchyma cells from cereal grains have been identified in several food fragments from Shubayqa 1, normally immediately beneath the visible aleurone layer (e.g. sample no. 17).

Other parenchymatic tissue

Parenchyma cells are commonly thin-walled, and their main function is to give mechanical support to the plant, and serve for storage and packing (*6*). The main diagnostic characteristics of the parenchyma cells are their shape and size. The other parenchyma cells observed in the food remains from Shubayqa 1 were broadly isodiametric and 20-40 μm in diameter, and included a characteristic pitting (**Fig. S5**). The size of the cells does not coincide with storage parenchyma of the endosperm and cotyledon of seeds, which is commonly less than 20 μm in diameter (*6*). The

parenchyma cells found in the food remains were similar in terms of size to those found in vegetative storage organs (see **Fig. S6**, *5*). Small and not well preserved starch grains were only visible in some of the parenchyma cell walls (see **Fig. S5B**). The characteristics of the annulus and hilum were not observable, reducing the chances of identification. No raphides, druses or other crystals were observed in the parenchyma cells.

Vascular tissue

In the food remains from Shubayqa 1, vascular tissue was identified both in transverse section (**Fig. S5A**) and in longitudinal section (e.g. **Fig. S7A**). However, it should be stated that no attempts were done to identify these vascular elements taxonomically as the areas preserved were too small for this purpose.

In some of the samples (e.g. sample no. 2) vascular bundles were identified in transverse section. Vascular bundles comprise two main types of conducting tissues: a) thick-walled cells, which are in charge of water and solute transportation, and are known as xylem; and b) thin-walled cells in charge of sugar and nutrient transportation, and which are referred to as phloem. The xylem often survives well to charring, as a result of its thick and non-living lignified walls (*6*). Diagnostic characters are limited to its shape in transverse section and its diameter.

As Hather points out (*6*), sometimes vascular tissues of the bundle are deteriorated leaving a cavity, but the vascular bundle can still be identified by the sheath fibers (sclerenchyma) that surround the bundle and the position of the long axes of elongated parenchyma cells radiating away from the bundle (e.g. **Fig. S5A**).

However, most common in the food remains from Shubayqa 1 was the presence of xylem vessels in longitudinal section (**Fig. S7A**). These are recognized by the very characteristic lignin rings or spirals (**Fig. S7B**). They are long thick-walled hollow tubes that allow water to easily move up through the plant.

Analysis and identification of starch grains in the food remains from Shubayqa 1

A small subsample was taken from each 'food' sample, leaving the larger portion behind for future analyses. The subsamples were lightly ground in a mortar and pestle and then mounted directly in Entellan. The slides were scanned using a Zeiss Imager.M2m microscope in their entirety at 20x for large or notable starches. One (if the number of starches were quite low) or two rows were selected and scanned at 40x from all of the slides to identify smaller starches. This method of counting provided an efficient presence/absence analysis (*7, 8*). The starches were classified according to their size, shape, lamellae, hilum location (centric, eccentric), hilum shape (closed or open i.e. 'pit') and extinction cross (*7, 9*) and described according to terms defined in the International Code For Starch Grain Nomenclature (*10*).

Four starch types were identified within the charred food remains (see **Table S3**). These types might, with further comparative collection work, be subject to finer identifications. In this study conservative identification to general taxa parts (i.e. root-type) or genera-types (i.e. *Avena*-type) have been employed. Notably, type I, the largest proportion of the starch recovered could, with further comparative work be a good candidate for a more precise identification. Several examples of this type have been recovered 'bound in tissue' or 'in situ' (**Figure S9**), providing a clear population of starches to help aid identification. In addition to the 'types' there is a fifth category of 'indet'. These starches were too degraded to classify beyond being a possible starch grain, generally missing key features such as birefringence or extinction cross (**Figure S9**):

Type I (**Figure S9**): Centric hilum, some appear to have closed hilum, but many of the starches appear to have a 'pit' (may be a central vacuole or open hilum). Faint lamellae were visible in some granules. Granules appear simple or semi-compound (in groups of two and three). They are circular, while the shape of the extinction cross arms suggests a discoid profile. Extinction cross features straight arms except where the starches are semi-compound. They often feature one or

two sides with pressure facets. Where they are pressed up against one another the arms of the extinction cross appear to bend. They range in size between 7-16 μm. Not similar to Piperno's AHT group (*Aegilops*, *Hordeum* and *Triticum*). Does not have A, B pairings (all similar size range). Generally much smaller than the AHT size range (i.e. 8-52 with av. between 17-25)(*11*). The shape, central hilum and size range tentatively suggest Type I is most likely from an aerial storage organ (seed or grain)(*12*). However, *Bolboschoenus* sp. (see **Figure S10**) tuber appears to compare favorably (shape, closed and open hila and faint lamellae). While the modern starch on average (7.4 μm, based on 50 grain measurements) falls within the size range of the archaeological starch, type I includes larger starches (i.e. 10-16 μm). As such, a more secure identification is not possible. However, Cyperaceae tuber generally cannot be ruled out without further comparative work.

Type II (root-type) (**Figure S11**): Eccentric hilum, closed. Clearly defined 'heavy' lamellae. Granules appear simple. They are oval to tear-drop in shape. They range in size between 15-38 μm.

Type III (**Figure S12**): Centric hilum, with 'pit' (may be a central vacuole). Faint lamellae. Granule is simple. Disc shaped with each edge featuring a pressure facet (7 clear facets around the periphery). 18.68 μm.

Type IV (*Avena*-type) (**Figure S13**): Centric hilum. Granule is simple, or compound. Faceted on two sides with semi-circular base (*13*). Quite angular. 5-9 μm.

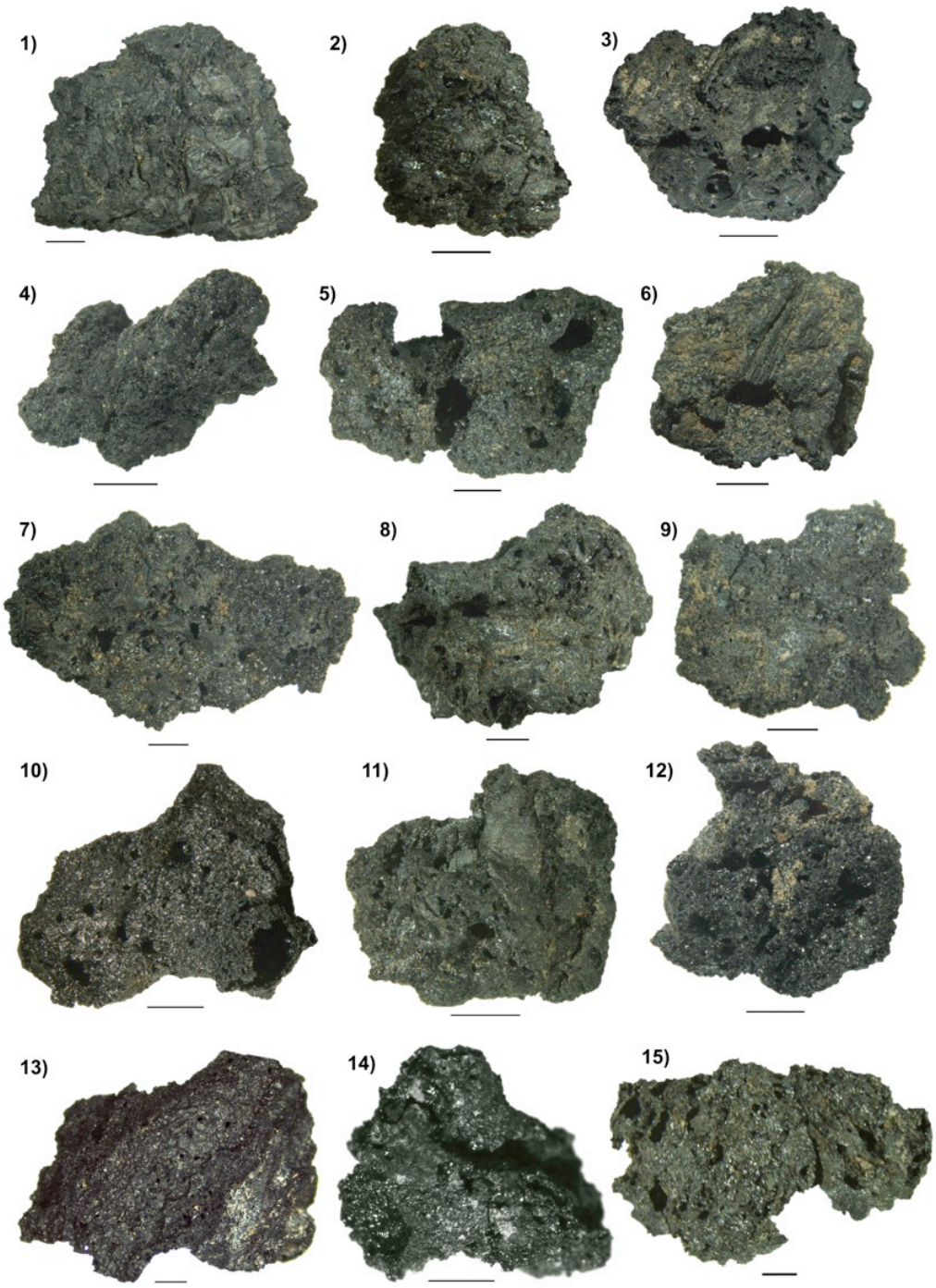

Fig. S1. Bread-like remains from Shubayqa 1 ordered according to their sample number (1-24). Scale is 1 mm.

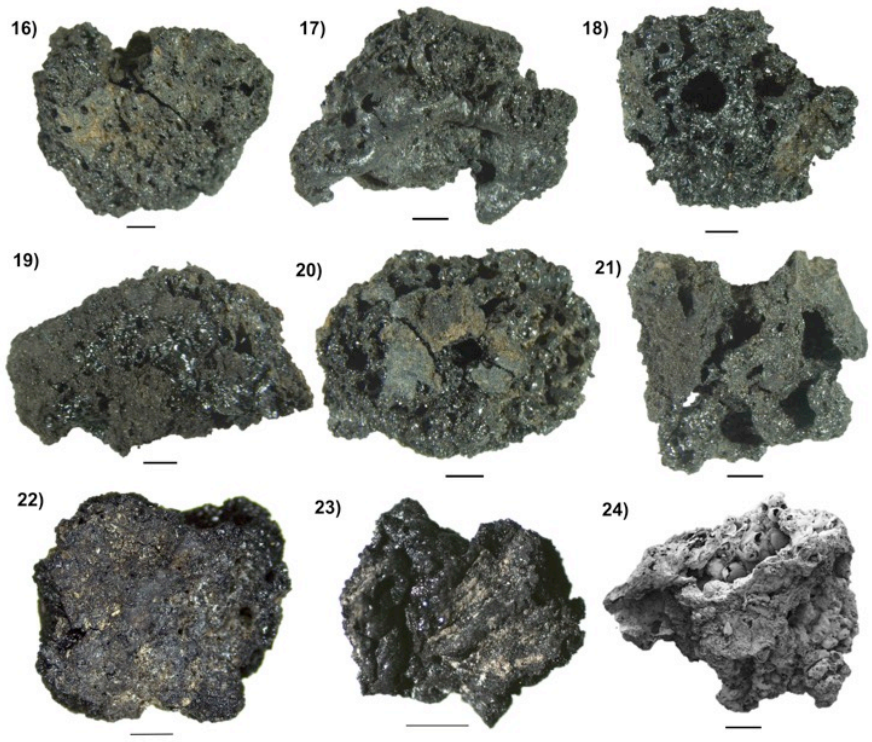

Fig. S1. (continue).

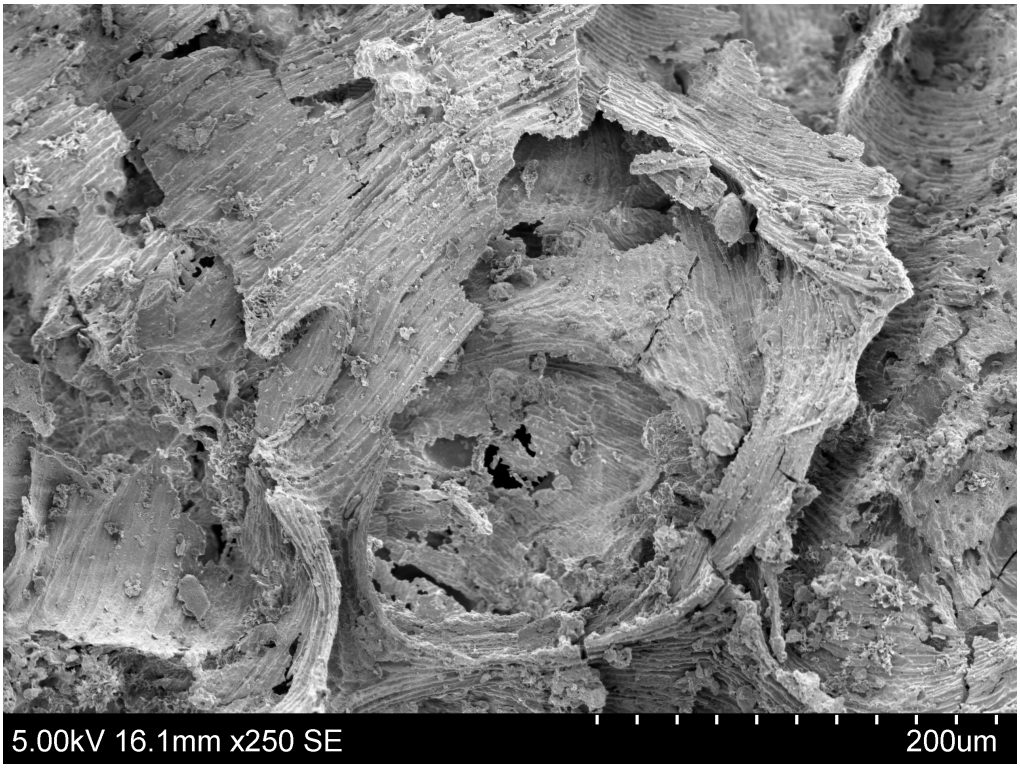

Fig. S2. Detail of longitudinal and transverse cell layers observed in the matrix of a food fragment from Shubayqa 1, sample no. 1.

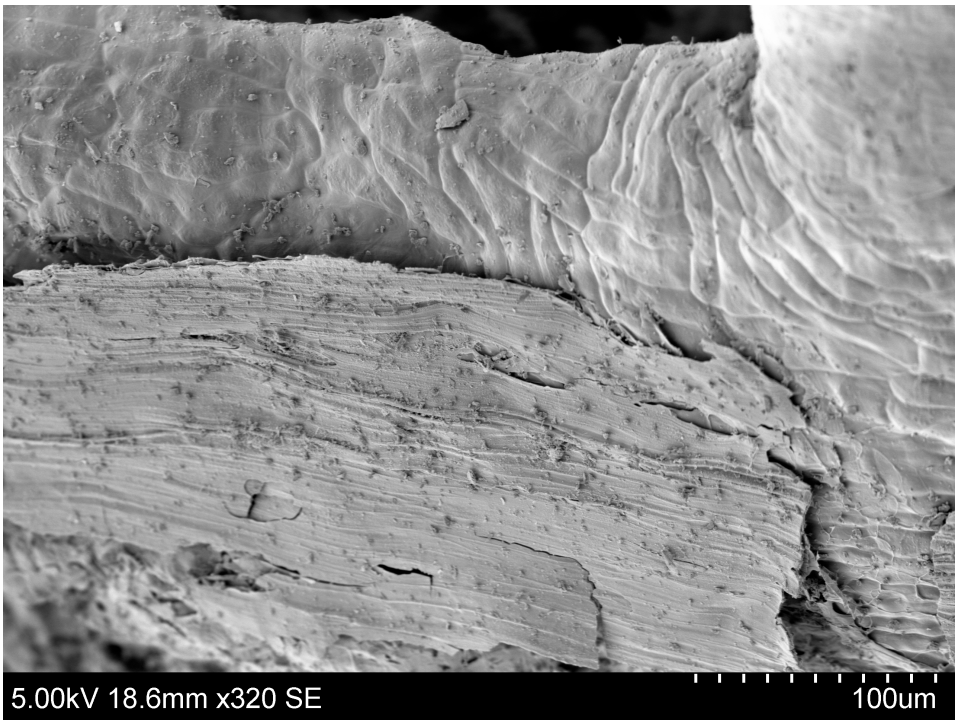

Fig. S3. Transverse and longitudinal cells from modern *Secale montanum* (wild rye).

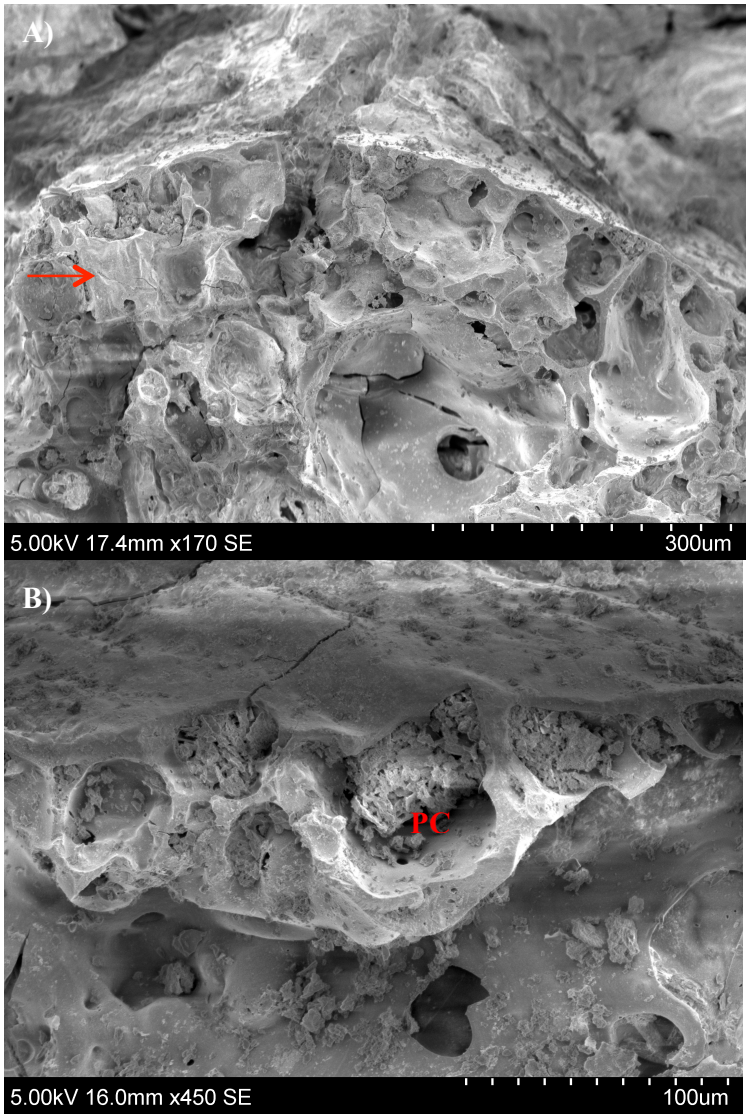

Fig. S4. Detail of cross-sections of cereal grain observed in a food fragment from Shubayqa 1. A) sample no. 17 showing at least single-cell aleurone layer (the red arrow marks a possible second layer) and damaged starch parenchyma cells (enlarged image of the specimen shown in the main text, Fig. 3B); B) detail of the aleurone layer with protein cells (PC) from sample no. 17.

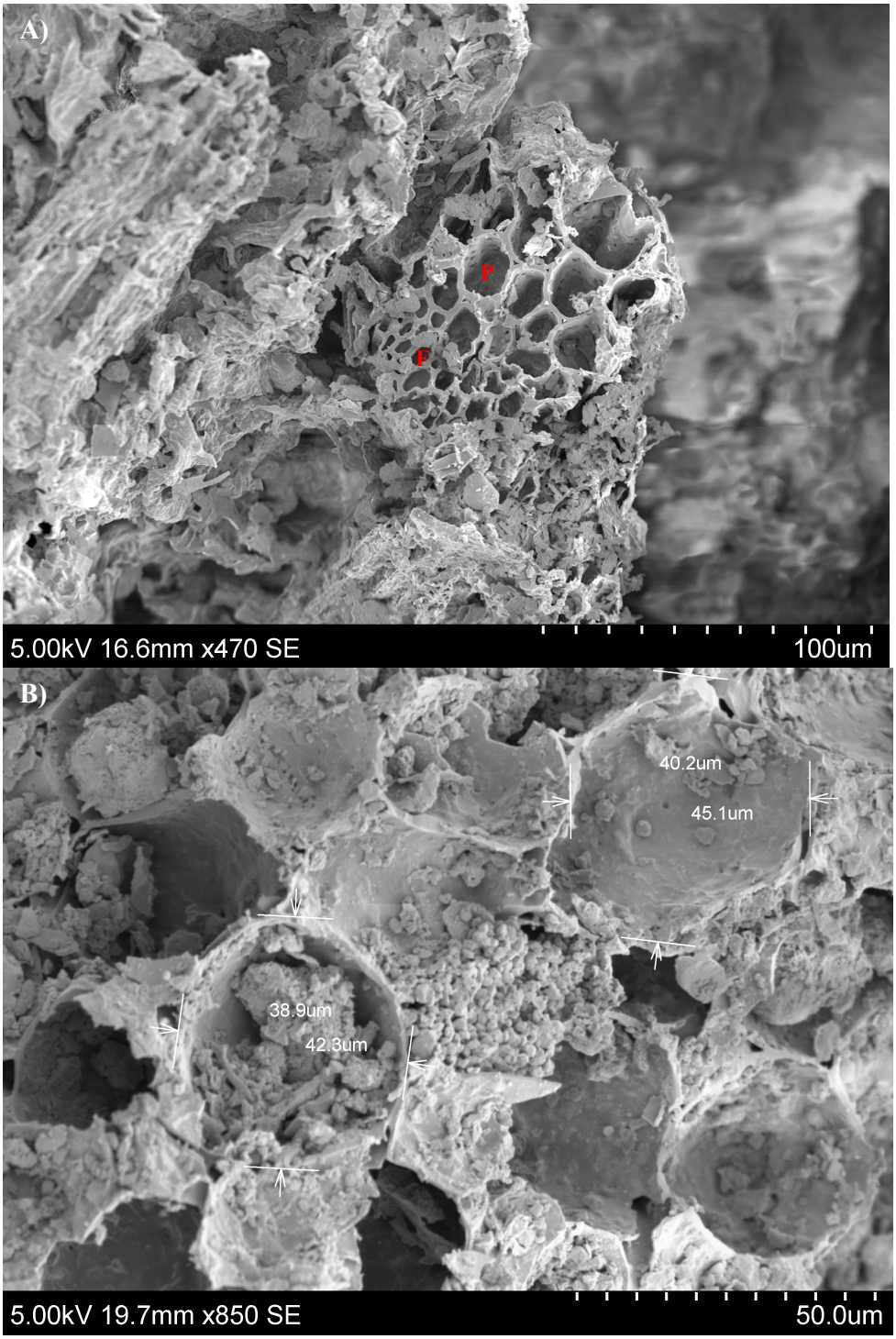

Fig. S5. Example of parenchyma and vascular tissues in transverse section observed in the food remains from Shubayqa 1. A) sample no. 2, parenchyma area (P) surrounding a possible vascular bundle with preserved fiber sheets (F). B) Food sample no. 12 showing the size of the parenchyma cells preserved and starch granules.

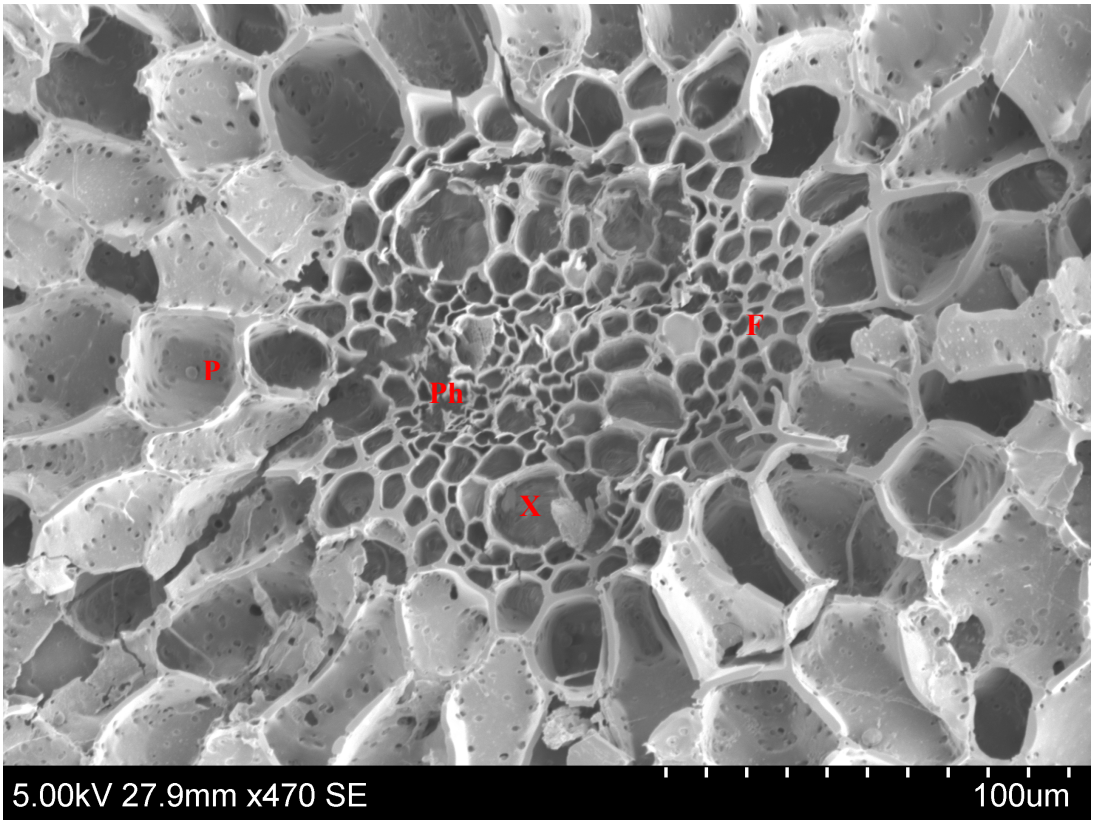

Fig. S6. For comparative purposes, this image shows a club-rush tuber (*Bolboschoenus* sp.) from Shubayqa 1 with parenchyma cells (P) surrounding a vascular bundle in transverse section that includes fiber sheets (F), xylem (X) and partially deteriorated phloem (Ph). Note that the size and the pitting of the parenchyma cells are very similar to that observed in Fig. S4.

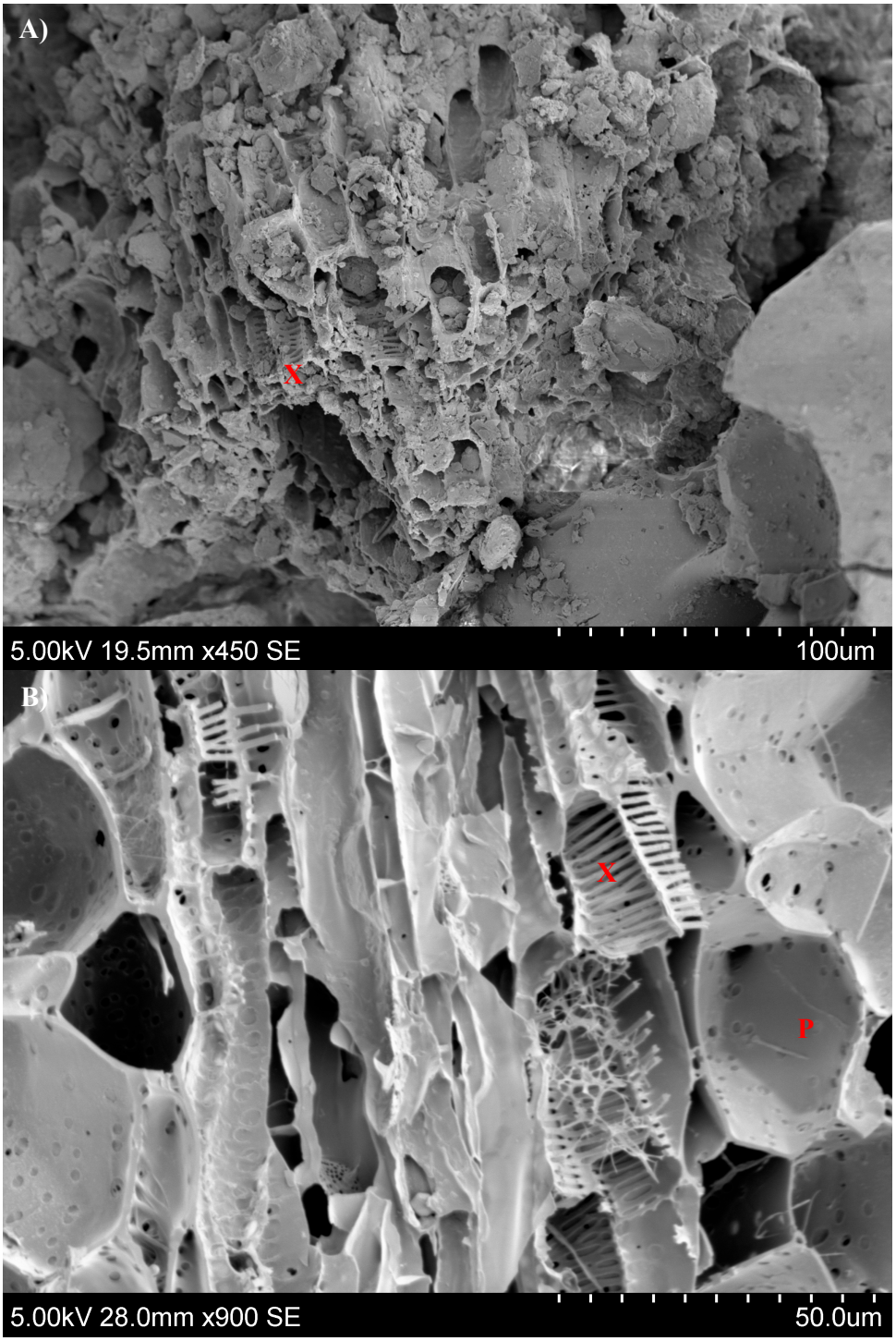

Fig. S7. Comparison of vascular tissue in longitudinal section: A) Food sample no. 12 showing a vascular tissue in longitudinal section with xylem vessels preserved (X) (enlarged image of the specimen shown in the main text, Fig. 3C). B) A club-rush (*Bolboschoenus* sp.) tuber from Shubayqa 1 showing a similar vascular tissue in longitudinal section (X, xylem) surrounded by parenchyma cells (P).

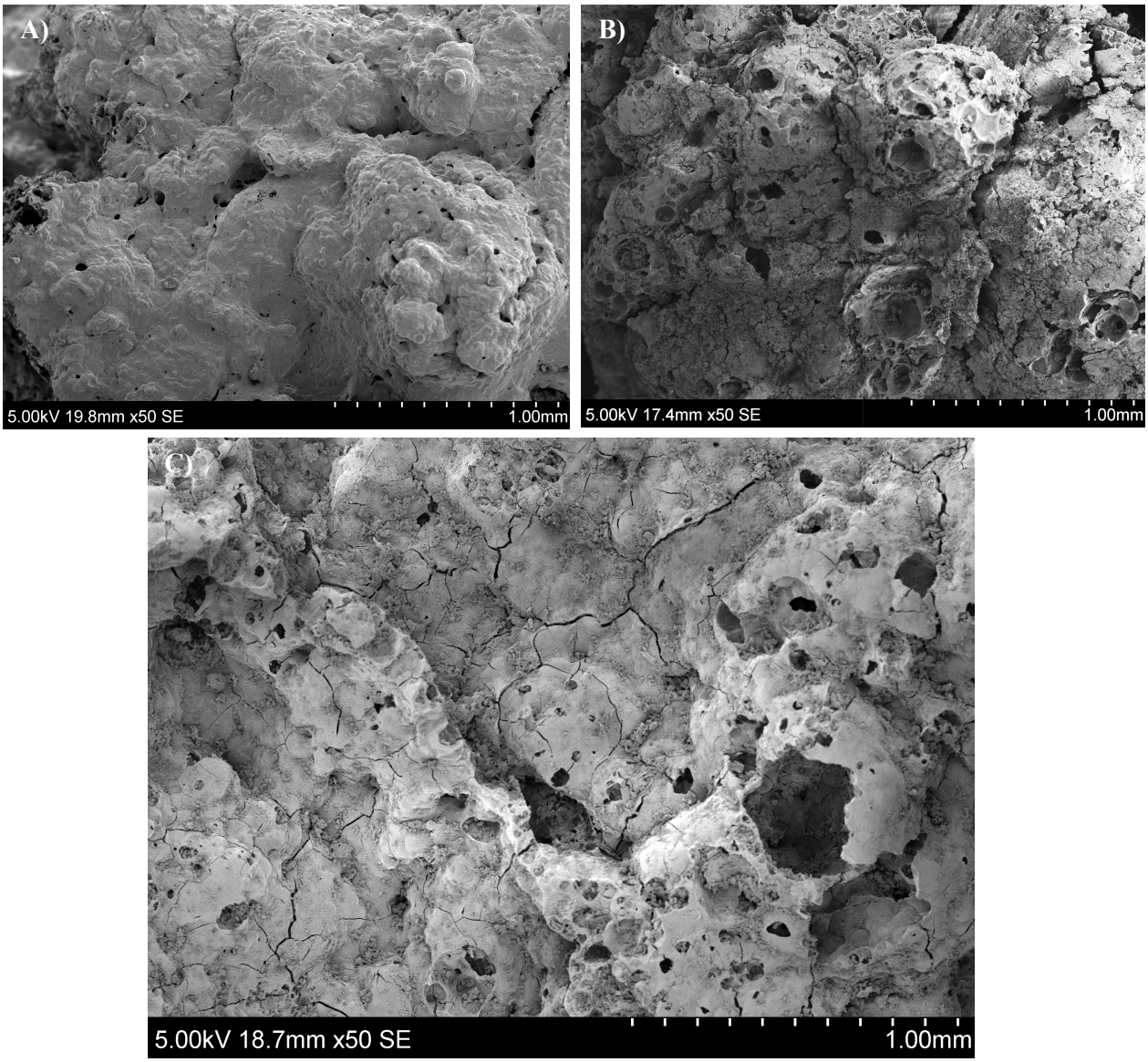

Fig. S8. Comparison of microstructures of different bread-like products showing low density of micropores and small closed voids: A) experimental bread made of barley (*Hordeum vulgare*) (*14*); B) flat bread-like remains from Pre-Pottery Neolithic B Çatalhöyük (Turkey) showing matrix type 3 (*14*); B); C) sample no. 7 from Shubayqa 1.

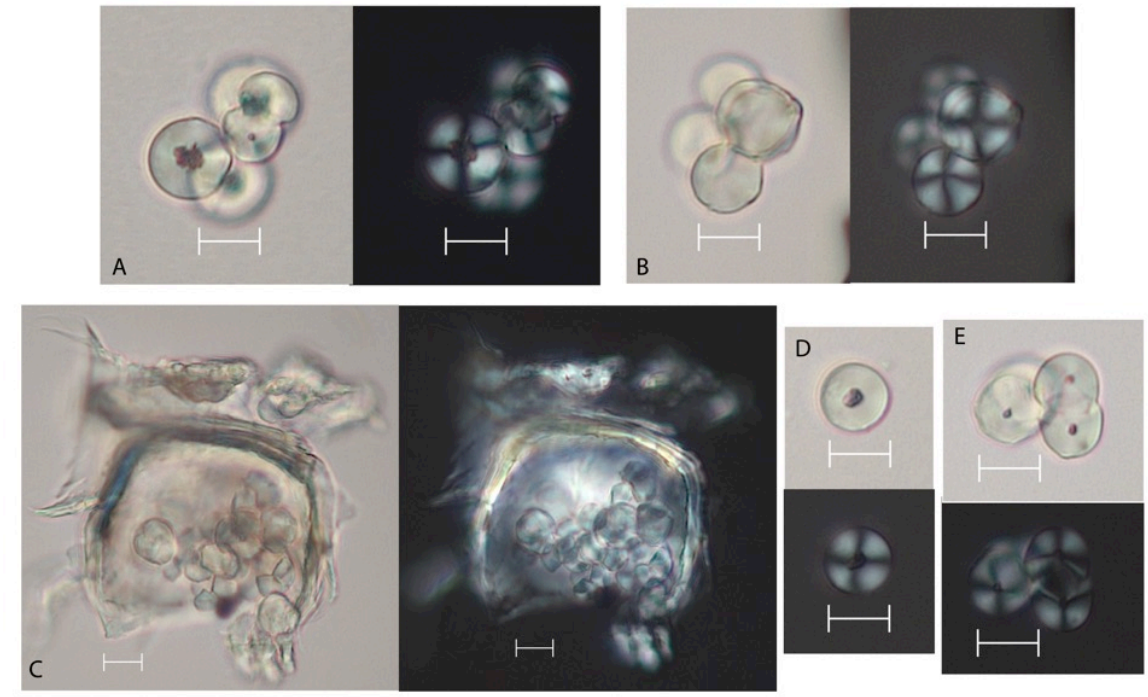

Fig. S9. Type I Starch. A) open hilum 'pit' may represent 'enlargement' of the hilum from processing damage, and closed hilum, semi-compound; B) closed hilum, semi-compound; C) starches in plant tissue; D) open hilum 'pit', simple; E) smaller open hilum 'pit', semi-compound (scale 10 μm).

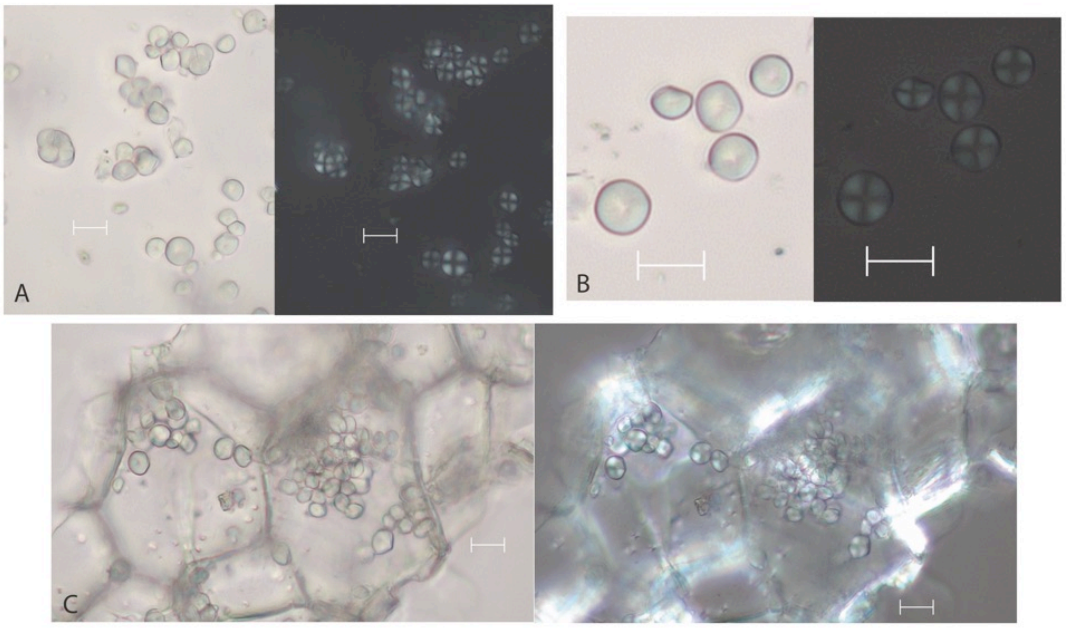

Fig. S10. *Bolboshoenus* sp. starch (tuber). A) simple and semi-compound forms, with centric hilum; B) note, central depression (may be susceptible to development of 'pit' in archaeological assemblage); C) starches in plant tissue (scale 10 μm).

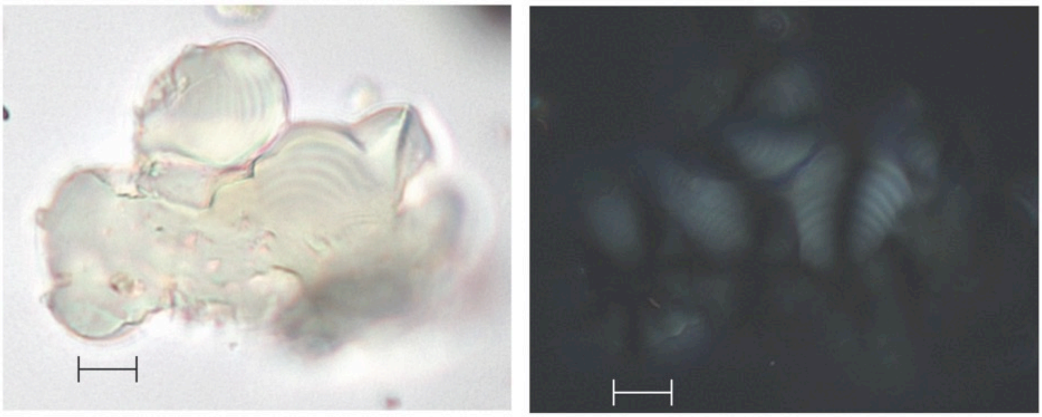

Fig. S11. Type II Starch (scale 10 μm). Starches are partially gelatinized.

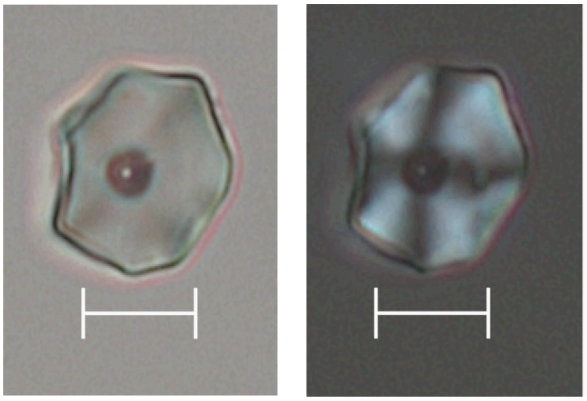

Fig. S12. Type III Starch (scale 10 μm).

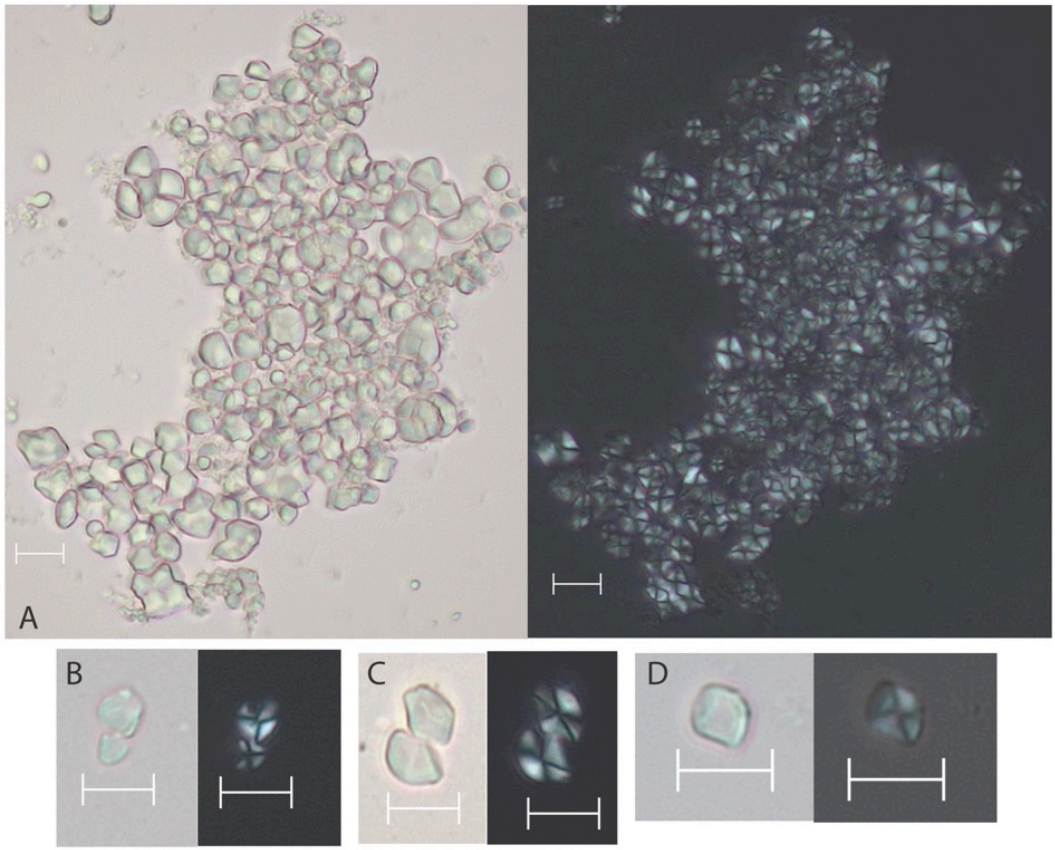

Fig. S13. Modern *Avena sativa* Starch (A) and Type IV Starch (*Avena*-type) (B-D) (scale 10 µm).

Table S1. General description of the bread-like remains from Shubayqa 1 under the low-magnification microscope.

Nº	Context	Width (mm)	Thickness (mm)	Length (mm)	Transverse section	Texture	Particles
1	86	5.4	3.1	6.9	rectangular, flat, regular	One side with smooth surface. The other side shows a mixture of compressed plant tissue, mushy texture	Ground grain fragment (hilum visible) in one of the sides
2	86	3.4	2.5	3.8	rectangular, flat, regular	Heterogeneous mixture of compressed plant tissue in both sides	Vascular material
3	86	4.3	2.8	4	square, irregular	Voids in both sides (mainly small <0.2 mm), slightly vitrified	
4	83	3.4	2.9	4.2	triangle, irregular	Voids in both sides (primarily small <0.1 mm), slightly vitrified	Small fragment of a ground grain
5	86	4.2	3	6.1	square, irregular	Voids in both sides (primarily small <0.1 mm), slightly vitrified	Small fragments of grains
6	83	3.8	2	4.2	square, regular	One side with voids (mainly small <0.1 mm), vitrified area. The other flat surface, with small melted-like patterns	Stem-like plant impression in the smooth and flat side
7	42	5	3.5	8.3	rectangular, flat, regular	One side slightly vitrified, porous (primarily small voids 0.1 mm). The other side with compacted semi-rounded structures that protrude	
8	86	3.2	2.8	4.8	rectangular, irregular	One side slightly vitrified, with voids (c. 0.1 mm). The other side smooth surface (no voids)	
9	49	4.2	1.5	4.9	rectangular, flat, regular	One side slightly vitrified, voids (primarily small <0.1 mm). The other is smooth, low number of voids	

Nº	Context	Width (mm)	Thickness (mm)	Length (mm)	Transverse section	Texture	Particles
10	86	4.5	1.3	5.1	upper part rounded with compressed edges, bottom part flat	One side smooth and very vitrified (like melted), with stalk/stem impression similar to Cyperaceae. The other side slightly vitrified, with small (<0.1 mm) and some larger voids (up to 0.5 mm)	Stalk/stem impression in the smooth side
11	86	3.4	2.2	3.9	square, irregular	One side slightly vitrified and porous (small voids 0.1 mm). The other side has a mixture of compressed plant tissue, mushy texture	
12	128	4.1	2	4.5	rounded, irregular	One side slightly vitrified with many small voids (<0.1 mm). The other side has a plant impression similar to Cyperaceae stalk. This side is melted, has very small voids	Stalk/stem impression in the smooth side
13	86	3.3	2.6	4.1	triangular, irregular, flat	One side smooth, with 0.1-0.2mm sized voids, and the other side with many medium sized voids (0.8 mm) and vitrified	
14	86	4.2	2.1	4.2	triangular, irregular, flat	Both sides characterised by smooth surfaces. In one of them small (<0.1 mm) pores cover half of the surface	
15	86	6	2	10	rectangular, flat, regular	Both sides slightly vitrified and with small (<0.1 mm) and some larger voids (up to 1 mm).	
16	86	7.5	4.1	8	rectangular, flat, regular	The outer surface in both sides is smooth, almost with no voids, whilst the interior looks very porous (small <0.1 mm and larger 0.4 mm voids)	

Nº	Context	Width (mm)	Thickness (mm)	Length (mm)	Transverse section	Texture	Particles
17	86	6.2	4	8	rounded, irregular	One side is vitrified and contains ground cereal remains, and a smooth area with many voids (possibly endosperm). The other side has a smooth surface and the layer below comprises large number of small voids (small voids <0.1 mm)	Small fragments of ground grain
18	86	6.5	4	7.5	rounded, rectangular	One side very vitrified, with small voids (<0.1 mm) and one large void (1.5 mm diameter). The other side has a smooth surface and comparatively lower proportion of small voids	
19	86	4	3	8.5	rectangular, flat, regular	Both sides with small vitrified voids (max. 0.5 mm) covered by a smooth layer (no voids)	
20	86	6	c. 3.5	7.5	rectangular, flat, regular	Both sides with small very vitrified voids (mainly small >0.1 mm, max. 1mm) covered by a smooth layer (no voids)	
21	86	6	3	6	square, irregular	One side has smooth surface and some scattered voids. The other has many voids (1-2 mm diameter) and it is vitrified	
22	86	0.5	0.3	0.6	square, regular	Both sides with soft surfaces, and the layer below characterised by small voids (mainly <0.1 mm)	
23	86	2.3	1.1	3.1	square, irregular	Both sides with smooth surface and some small voids (0.1 mm)	Monocot cells
24	86	4.4	1.8	4.6	square, irregular	Both sides very vitrified and with a large number of medium size voids (0.4 mm)	

Table S2. Tissue-based analyses of the bread-like remains from Shubayqa 1 under the SEM. Sample 13 and 15 were only studied under low-magnification microscope and subsequently sent for starch analyses. This was carried our with the purpose of comparing the presence of starch granules in samples that underwent SEM analyses (i.e. gold-coating etc.) and those that did not. However, the results were inconclusive at this regard.

Nº	Average void size (mm)	Percentage of voids	Type of voids	Average particle size (mm)	Average number of particles	Type of Particles
1	0.2	15%	Cracks and channel voids	1.4	2	Cereal grain fragments with transverse and longitudinal cells (bran) and aleurone layer
2	0.15	10%	Cracks and channel voids	0.5	>10	Parenchyma area (20-40 μm), vascular bundles and transverse and longitudinal cells (bran)
3	0.15	20%	Closed voids small	0.05	1	Longitudinal and transverse cells (bran)
4	0.1	20%	Micropores and small closed voids	1.2	2	Ground cereal grain fragments with longitudinal and transverse cells (bran) and kernel layers
5	0.1	15%	Micropores and small closed voids	0.9	1	Cereal grain fragments with visible kernel layers
6	0.15	20%	Small closed voids	0.05	1	Longitudinal and transverse cells (bran), small area
7	0.15	15%	Micropores and small closed voids	0.9	1	Parenchyma area (20-40 μm)
8	0.15	20%	Closed voids	0.1	1	Longitudinal and transverse cells (bran)
9	0.05	20%	Micropores	-	-	None
10	0.2	10%	Closed voids and micropores	0.1	2	Small area showing longitudinal and transverse cells (bran) located in the middle of the matrix, aleurone layer
11	0.05	10%	Cracks and channel voids	1.3	3	Ground cereal grain epidermis layers. Big areas of longitudinal and transverse cells (bran) all over the matrix
12	0.2	25%	Closed voids	0.1	1	Parenchyma area (20-40 μm) and vascular tissue
14	0.05	7%	Micropores	1.1	1	Aleurone layer
16	0.1	25%	Closed voids	-	-	None
17	0.2	10%	Closed voids	2	1	Ground cereal grains, at least single-celled aleurone layer in cross section, cereal parenchyma (endosperm <20 μm)
18	0.15	10%	Closed voids	-	-	None
19	0.25	20%	Closed voids	-	-	None

20	0.125	30%	Micropores and small closed voids	-	-	None
21	0.25	20%	Closed voids	0.3	1	Single-celled aleurone layer in cross section, cereal parenchyma (endosperm <20 μm), vascular material
22	0.075	10%	Micropores	0.2	1	Longitudinal and transverse cells (bran)
23	0.075	5%	Closed voids	0.15	1	Longitudinal and transverse cells (bran)
24	0.4	10%	Closed voids	0.3	1	Longitudinal and transverse cells (bran)

Table S3. Results of the starch analyses in the bread-like remains from Shubayqa 1.

Sample Nº	Starch Type				
	I	II	III	IV	Indeterminate
1	X				X
4					X
13	X				
14	X		X	X	X
15	X	X			X
24	X				X

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