

# Dental calculus reveals Mesolithic foragers in the Balkans consumed domesticated plant foods

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Researchers agree that domesticated plants were introduced into southeast Europe from southwest Asia as a part of a Neolithic “package,” which included domesticated animals and artifacts typical of farming communities. It is commonly believed that this package reached inland areas of the Balkans by ~6200 calibrated (cal.) BC or later. Our analysis of the starch record entrapped in dental calculus of Mesolithic human teeth at the site of Vlasac in the Danube Gorges of the central Balkans provides direct evidence that already by ~6600 cal. BC, if not earlier, Late Mesolithic foragers of this region consumed domestic cereals, such as *Triticum monococcum*, *Triticum dicoccum*, and *Hordeum distichon*, which were also the main crops found among Early Neolithic communities of southeast Europe. We infer that “exotic” Neolithic domesticated plants were introduced to southern Europe independently almost half a millennium earlier than previously thought, through networks that enabled exchanges between inland Mesolithic foragers and early farming groups found along the Aegean coast of Turkey.

Mesolithic foragers | starch analysis | domesticated cereals | forager/farmer interaction | human dental calculus

It is commonly assumed that the spread into Europe of domesticated plants, cereals, and pulses (1, 2) was part of a Neolithic “package,” which included domesticated animals and artifacts typical of farming communities (e.g., ceramics, polished stone axes). Researchers agree that such a package was introduced into southeast Europe from southwest Asia and reached inland areas of the Balkans by ~6200 calibrated (cal.) BC or later. Genetic (3) and isotope data (4) have revealed that demographic movements brought these Neolithic novelties from the Near East into Europe through the process by which scarce Mesolithic foragers were either quickly replaced or assimilated into Neolithic lifeways. Isotope analyses have also suggested that Mesolithic diets were largely based on terrestrial, marine, or riverine protein-rich resources (5–7) with scanty evidence for the consumption of plants (8). Here we show that Late Mesolithic foragers of the central Balkans consumed domesticated specimens of wheat and barley (namely grass species of Triticeae tribe) at least from ~6600 cal. BC, almost half a millennium earlier than previously thought. We found that starch granules entrapped in dental calculus of Mesolithic (~6600–5900 cal. BC) human teeth at the site of Vlasac (the Danube Gorges) correspond to those found in the teeth of Neolithic (~5900–5700 cal. BC) individuals at the neighboring site of Lepenski Vir. Our results show that these starch granules originate from the consumption of main domestic crops found among Early Neolithic communities of southeast Europe (9)—that is, *Triticum monococcum* (einkorn wheat), *Triticum dicoccum* (emmer wheat), and/or *Hordeum distichon* (barley). As genetic and botanical studies have ruled out European ancestry for domestic wheat/barley and confirmed the Near East as the source of these crops (2, 10, 11), our assay provides the earliest evidence that domesticated plants were transmitted independently from other components of the Neolithic package through existing networks that enabled exchanges between inland Mesolithic foragers and early farming groups from ~6600 cal. BC onwards.

## The Sites

The Danube Gorges area is split by the River Danube between the territories of present-day Romania and Serbia (Fig. 1). In this region, more than 20 sites with Mesolithic deposits were discovered in the mid-1960s and yielded unprecedented data for other areas with known Mesolithic presence in Europe (12–15).

The site of Vlasac is situated in the Lady Whirlpool’s Gorge of the Danube on the Serbian side of the river (Fig. 1). The first excavations at the site were carried out in 1970–1971 as part of a rescue project (16). New excavations at Vlasac began in 2006, and the investigations of the site are ongoing (17). The resumed work at Vlasac has covered an area of 326 m<sup>2</sup> (Fig. S1) and takes place upslope from the excavation area investigated in 1970–1971. Radiocarbon dates from both old and new excavations suggest that the site was more or less continuously occupied from the Early Mesolithic, from ~9500 cal. BC, but the intensity of occupation increases from the mid-eighth millennium BC (18, 19). The Late Mesolithic occupation/use of the site covers the period between ~7400 and ~6200 cal. BC. New research at Vlasac has indicated that the site was continuously used throughout the period of the Mesolithic–Neolithic transition—that is, ~6200–5900 cal. BC. Finally, there is also evidence for the use of this site in the course of the regional Early/Middle Neolithic (~6000/5950–5500 cal. BC). During the Early Neolithic phase, the first chaff-tempered ceramic finds appeared at Vlasac (17, 18). Late Mesolithic domestic features, such as trapezoidal dwellings and numerous and overlapping rectangular stone-lined hearths, testify to the fact that this was a relatively large and likely sedentary complex forager settlement (16, 18). The total number of formal burials at Vlasac excavated in

## Significance

The starch record entrapped in dental calculus of Mesolithic human teeth from the site of Vlasac in the central Balkans provides direct evidence that complex Late Mesolithic foragers of this region consumed domesticated cereal grains. Our results challenge the established view of the Neolithization in Europe that domestic cereals were introduced to the Balkans around ~6200 calibrated (cal.) BC as a part of a “package” that also included domesticated animals and artifacts, which accompanied the arrival of Neolithic communities. We infer that Neolithic domesticated plants were transmitted independently from the rest of Neolithic novelties from ~6600 cal. BC onwards, reaching inland foragers deep in the Balkan hinterland through established social networks that linked forager and farmer groups.

Author contributions: E.C. designed research; E.C. and A.R. performed research; M.E. performed anthropological analysis of dental remains from the sites presented in the paper; D.B. excavated the site of Vlasac from 2006–2009 and provided chronological framework; E.C. and A.R. analyzed data; and E.C., A.R., M.E., and D.B. wrote the paper.

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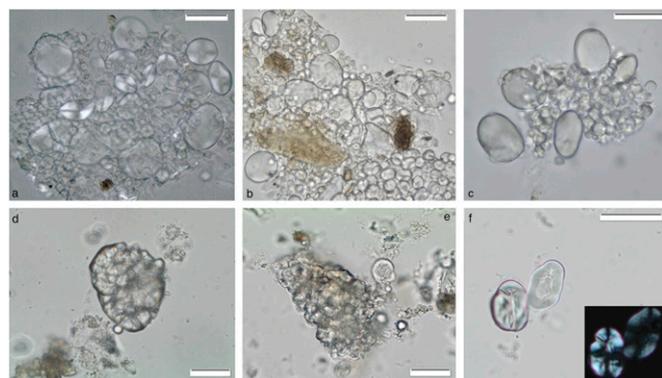


**Table 1. Context, provenance, chronology, sex, and age of individuals analyzed for dental calculus from Vlasac and Lepenski Vir and summary data on the quantity of starch granules, descriptions of their features, and proposed identifications**

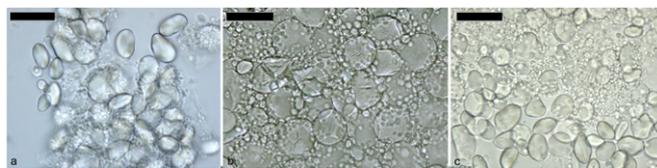
Individual/burial no. (dating)	Sex/age	Tooth no.	Quantity	2D/3D morphology	Hilum	Lamellae	Proposed identification
<b>Vlasac</b>							
U 115 (LM: ~6800–6500 cal. BC)	?/adult	28	2	Irregular triangular of oval	Central, slightly sunken	Not visible	Unidentified starch granules (see <a href="#">Supporting Information</a> )
H232 (LM: TPQ OxA-20702: 6636–6476 cal. BC)	f/~25 y	28	<5	Round to oval in 2D view/distinctive lenticular shape when rotated	Central, with distinctive extinction cross	Present, concentrated around the hilum	Triticeae
H317 (LM: ~6500 cal. BC)	f/~30 y	14	No starch				
U 326 (LM: ~6500 cal. BC)	m/~50 y	1, 2	>50	Round to oval in 2D view/distinctive lenticular shape when rotated	Central, with distinctive extinction cross	Present, concentrated around the hilum	Triticeae
U 64.x11/H81 (LM: OxA-20762: 6639–6440 cal. BC)	m/~40 y	20, 26, 27, 29, 30, 31	>100	Round to oval in 2D view/distinctive lenticular shape when rotated	Central with distinctive extinction cross	Present, concentrated around the hilum	Triticeae
			<5	3D polyhedral shape	Central with fissures radiating from it	Not visible	Paniceae
U 222.x18 (LM: bottom of the sequence, ~6700 cal. BC)	?/adult	3	2	Oval in 2D view/ kidney 3D morphology	Central, slightly sunken	Present, barely visible	Fabeae
U 24.x30 (LM-M/N transition)	?/adult	32	No starch				
U 44 (LM-Meso/Neo transition: ~6700–5900 cal. BC)	?/adult	27	<5	Oval in 2D view/ kidney 3D morphology	Central, slightly sunken	Present, barely visible	Fabeae
			<5	Round to oval in 2D view/distinctive lenticular shape when rotated	Central, with distinctive extinction cross	Present, concentrated around the hilum	Triticeae
H53 (M/N transition: OxA-16544: 6006–5838 cal. BC)	f/~50 y	3, 28, 29	>5	Round to oval in 2D view/distinctive lenticular shape when rotated	Central with distinctive extinction cross	Present, concentrated around the hilum	Triticeae
			> 200	Large suboval lumps of starches	Central	Not visible	Avena
<b>Lepenski Vir</b>							
8 (EN: AA-58319: 5710–5520 cal. BC; OxA-25207: 5982–5760 cal. BC)	f/~40 y	44	>5	Round to oval in 2D view/distinctive lenticular shape when rotated	Central, with distinctive extinction cross	Present, concentrated around the hilum	Triticeae
			<5	Oval in 2D view/ kidney 3D morphology	Central, slightly sunken	Present, barely visible	Fabeae
20 (EN: ~6000–5700 cal. BC)	f/~40 y	48	<5	Round to oval in 2D view/distinctive lenticular shape when rotated	Central, with distinctive extinction cross	Present, concentrated around the hilum	Triticeae
			1	Irregular triangular of oval	Central, slightly sunken	Not visible	Unidentified starch granules (see <a href="#">Supporting Information</a> )
32a (EN: OxA-5828: 6066–5727 cal. BC)	f/~50–60 y	42, 43, 36	> 200	3D polyhedral shape	Central with fissures radiating from it	Not visible	Paniceae

high number of B-type grains, and granules are also attached to the calculus matrix (Fig. 3 *A* and *B*). Bimodal distribution is known for Triticeae tribe (e.g., *Triticum* spp., *Hordeum* spp.) (Fig. 4 and Fig. S7), but none of these species had wild progenitors in the Balkans (9, 10). Thanks to the modality of preservation, it was possible to exclude wild species of the Triticeae and Bromideae tribes (known to have bimodal starch granules) that could have been eaten at the time in the region (31, 32) on the basis of their morphology (Figs. S7 *A–H* and S8*A*) as well as most recent literature on phylogenetic evaluation of Poaceae species (33). It was also noticed that recovering good quality seeds and starch granules from some of the mentioned wild wheat species was very difficult. Furthermore, *Aegilops* spp. are absent from assemblages with analyzed macrobotanical remains found at Mesolithic and Early Neolithic sites in the central Balkans (10). Finally, none of the species of the genus *Bromus* growing in the region are bimodal (SI Results). Starch granules of the Triticeae tribe with morphologies remarkably similar to those found on Late Mesolithic and Mesolithic–Neolithic phase individuals from Vlasac were found in Early Neolithic individuals from Lepenski Vir (Fig. 3*C*). A further confirmation that archaeological starch granules belong to domesticated species of the Triticeae tribe is provided by the pollen record, as very large grains of Cerealia grasses were recovered in human coprolites from two Late Mesolithic sites in the Danube Gorges (Vlasac and Icoana) and were interpreted as coming from domesticated species (29, 34). At Vlasac, Gramineae pollens of Cerealia type with a diameter no larger than 38.5  $\mu\text{m}$  are dominant in coprolites from lower Mesolithic horizons (1.6%). This diameter is considered the limit between spontaneous and domesticated Cerealia grasses. The quantity of small Cerealia pollens decreases in coprolites from upper Mesolithic horizons, where large Cerealia-type grains with diameters larger than 50  $\mu\text{m}$  (sometimes up to 53  $\mu\text{m}$ ) appear (3.5%). Interestingly, in upper Mesolithic horizons, pollen grains of legumes have also been found (29).

**Type II.** Type II of starch granules was found in the form of intact large suboval aggregates of small compound granules (Fig. 3*D*). Compared with our reference collection and literature (30), both compound granules and the aggregate are consistent in size and shape with those of the species from the Aveneae tribe (oats tribe), both wild (e.g., *Avena sterilis*, *Phalaris minor*) (Fig. S8 *B*



**Fig. 3.** Starch granules entrapped in archaeological dental calculus: (A) starch compound in Late Mesolithic calculus (Vlasac H232); (B) bimodal distribution of lenticular starch grains in calculus dated to the Late Mesolithic (Vlasac U64,  $\times 11$ ); (C) starch compound in Early Neolithic calculus from Lepenski Vir (Lepenski Vir 20); (D) large starch compound found in calculus (Vlasac H53) consistent with Aveneae tribe; (E) cluster of polyhedral starch grains with central hilum and fissures associated with the Paniceae tribe (Vlasac U64); (F) starch grain entrapped in Mesolithic–Neolithic calculus (Vlasac U44). Note the oval shape, kidney-like shape 3D morphology, and sunken central hilum, which are consistent with genus *Vicia* (Vlasac U44). (Scale bar, 20  $\mu\text{m}$ .)



**Fig. 4.** Experimental starches: (A) *Hordeum distichon*; (B) *Triticum monococcum*; (C) *Triticum dicoccum*. (Scale bar, 20  $\mu\text{m}$ .)

and *C*) and domesticated (e.g., *Avena sativa*). Starch granules of the Aveneae tribe were found in the Late Mesolithic samples only, and they could have been either deliberately harvested, exchanged, or ingested as weeds of crops.

**Type III.** A third group of starch granules also retrieved in dense clusters, in one case of over 200 granules, is consistent with those of the Paniceae tribe. This type of granule has a 3D polyhedral shape, with central hilum and fissures radiating from it (Fig. 3*E*, Table 1, and Fig. S8 *F–J*). Although an overlap in size and shape exists among the smaller starch granules of the tribe (35), the large number of our starches and their overall morphologies suggest species of the genus *Setaria* spp. as potential candidates (Fig. S8 *F* and *G*). Although several species of the genus *Setaria* are diffused in the central Balkans (e.g., *Setaria viridis*, *Setaria verticillata*, etc.), a secure identification of archaeological starches needs to be taken with caution, whereas identification to tribe level is rather secure. Starch granules of the Paniceae tribe were retrieved from our samples dated to the Mesolithic, Mesolithic–Neolithic transition, and Early Neolithic phases.

**Type IV.** The fourth type of starch granules is consistent with the Fabaeae tribe (peas and beans) and is remarkably consistent with those found in the genus *Vicia* (vetches) for its small oval 2D shape and its kidney-shape 3D morphology and hilum sunken in a central cleft fissure (Fig. 3*F*, Table 1, and Fig. S8*J*). These were lower in number. Species of vetches can grow as weeds of crops, and sometimes are eaten raw.

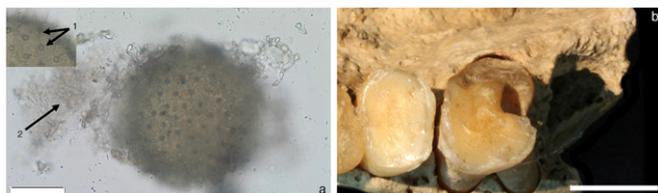
Single starch granules were found in a number of samples, but these were low in number and often damaged, and it was difficult to reach a secure identification for them. These starches were considered undiagnostic, as they could belong to groups that could not be identified because they were damaged by processing and chewing.

Finally, a pollen grain, characterized by a spheroidal and pantaporate morphology, ca. 50  $\mu\text{m}$  in diameter, pores between 3 and 5  $\mu\text{m}$ , and spines protruding from the surface, was found embedded in the calculus matrix (Fig. 5*A*) and identified as *Malva* sp. (36). The presence of such pollen in Late Mesolithic calculus could be suggestive of the consumption of honey or its use as herbal medicine. In addition, nondietary evidence in dental calculus is consistent with a variety of animal (e.g., feather barbules) and plant remains (wood debris and plant fibers) (Figs. S9 *C* and *D* and S10), likely to be the result of exposure to dust generated by crafts. Such remains being below 20  $\mu\text{m}$  are interpreted as potential respiratory irritants (37).

The analysis of dentoalveolar pathologies has shown the absence of caries and periapical lesions in examined individuals from Vlasac. However, antemortem loss of two teeth (38 and 48) has been recorded in one case (U64,  $\times 11$ ). Root exposure caries were found in Neolithic individual 32a from Lepenski Vir (teeth 16, 25, and 26) (Fig. 5*B*). Individuals 32a (tooth 17) and 20 (teeth 45 and 46) exhibit antemortem tooth loss. Lastly, three periapical lesions were identified in individual 8 (teeth 12, 21, and 23) from Lepenski Vir.

## Discussion and Conclusions

Our evidence suggests that starch granules found in large numbers in dental calculus of both Late Mesolithic and Mesolithic–Neolithic individuals from Vlasac are consistent with tribes of



**Fig. 5.** (A) Large spheroidal and pantaporate pollen of *Malva* sp. with spines protruding from the surface (1, spines and pores; 2, remains of ancient calculus (Vlasac U64)); (B) caries found in Neolithic individual 32a from Lepenski Vir. (Scale bar: A, 20  $\mu$ m; B, 1 cm.)

plants that became key staple domestic foods with the start of the Neolithic in this region. The status of preservation of entire “groups” of starch granules suggests that their presence in the calculus matrix could be the result of inhalation or ingestion during processing rather than ingestion after cooking, a pathway of inclusion of starch granules rarely taken into consideration in other studies (see [Supporting Information](#)). The data indicate that the earliest domesticated seeds would have reached the Danube Gorges area already by the mid-seventh millennium BC, if not earlier—that is, at least four centuries before what is commonly believed. In Greece, at the site of Franchthi Cave (Fig. 1), three carbonized seeds of *T. dicocum* from mixed Final Mesolithic/Initial Neolithic aceramic units were directly AMS-dated to between 6750 and 6430 cal. BC (95% confidence), suggesting the presence of domesticated emmer wheat in southern Greece likely before ~6500 cal. BC (38), which is contemporaneous with the analyzed Mesolithic individuals in the Danube Gorges. Although the dated *T. dicocum* seeds from Franchthi were tentatively attributed to the Neolithic occupation (38), in the light of our results one may speculate that they relate to forager rather than first farming occupation of the site. These dates are also broadly contemporaneous with the Early Neolithic occupation at Ulucak (level VI) on the Turkish side of the Aegean Sea (Fig. 1) (39). A recent series of dates from the Early Neolithic sites of Dikili Tash, Mavropigi, and Paliambela in Macedonia (Fig. 1) suggest that Neolithic communities settled in the region already by ~6500 cal. BC (40). In the central Balkans, the earliest date for an Early Neolithic occupation comes from the site of Blagotin (Serbia) (Fig. 1), where OxA-8608 (7480  $\pm$  55) dated a red deer antler to 6440–6230 cal. BC (95% confidence) (41).

It remains difficult to evaluate the importance of carbohydrate foods in the overall subsistence of Late Mesolithic foragers at Vlasac (25), as it is likely that fish was an important staple resource (5). However, antemortem tooth loss documented in some individuals could indicate a deteriorated dental health status due to specific dietary habits (e.g., glucose/fructose consumption derived from plants and honey). The absence of caries during the Mesolithic at Vlasac does not contradict the evidence about the consumption of plants rich in fermentable carbohydrates and could directly be related to high calculus rates documented among Mesolithic individuals at Vlasac, as calculus forms during the process of mineralization whereas caries develops through the process of tooth demineralization (42). The similarities between starch grains present in the calculus of Mesolithic individuals from Vlasac and Early Neolithic individuals from Lepenski Vir provide evidence that Mesolithic foragers of the Danube Gorges must have had acquired domestic grains before farming practices became rooted in the Balkans more permanently ~6200/6100 cal. BC. This could be similar to

the process that led to the spread of domesticated maize through trade networks among forager communities of North America before evidence of its cultivation (43, 44). It has also been argued that domesticated wheat reached Mesolithic Britain already by 6000 cal. BC—that is, 2,000 y before full-scale agricultural practices were adopted (45). In the central Balkans, foragers’ familiarity with Cerealia grasses from ~6500 cal. BC, if not earlier—that is, at least 400 y before their full cultivation in this regional context—might have eased the later quick adoption of agricultural practices. The existence in this region of large sedentary settlements of complex foragers along with numerous grinding stone tools (Fig. S2) is in agreement with our results that processing of plant foods, including domesticated species, might have been common already in the Late Mesolithic. This significantly challenges our hitherto understanding of pre-Neolithic adaptations in southeast Europe and suggests that elements of the “Neolithic package” were transmitted piecemeal and at different times using established networks of interaction between foragers and farmers.

## Methods

Calculus sampling took place on aluminum foil with starch-free gloves worn at all times. The average weight for calculus samples was 0.983 g. Once removed, samples were sealed into sterile Eppendorf tubes. In the laboratory, samples underwent specifically developed decontamination procedures (46) ([SI Methods](#)). Extraction and mounting of the microfossils entombed in the calculus matrix involved a weak solution 0.06 N of HCl ([SI Methods](#)). A solution of 50:50 glycerol and ultrapure water was used to allow for the rotation of microfossils. Examination of microfossils was carried out using Zeiss and Olympus compound polarized microscopes (100–630 $\times$ ) at the University of York and a Leica DM2500 polarized microscope (100–1,000 $\times$ ) at the University of Cambridge. Starch granules were identified on the basis of their 3D morphology, presence, and shapes of features (lamellae, hilum, bumps, and depressions of their surface), characteristics of the extinction cross under polarized light microscopy. A large collection of microremains from modern plants native to the central Balkans and the Mediterranean region collected at the Botanical Garden of Belgrade and stored at the University of Cambridge was used as an experimental reference. A collection of plants of north European, Mediterranean, and north African origin as well as nondietary items (e.g., plants used for crafts and occupational dust such as wood and pottery) hosted by the University of York was also consulted and successfully used before in other studies (35, 46). Fresh and dry botanical samples were ground in agate mortar using distilled water. Different modalities of grinding were applied to record level of damage and/or change in the size of granules. Ground plant material has also been left in water to understand the swelling process. Our reference collection also comprised experimentally processed seeds (e.g., boiled, ground, chewed) ([SI Materials](#)). Criteria of identification that are current standards in the field of modern and ancient starch granules research (47) were followed.

Caries and periapical lesions have been recorded macroscopically and using a magnifying lens. Visibility location and size of caries were described following the methodology proposed by Hillson (42). Both caries and periapical lesions were recorded at the individual tooth level (42, 48) and the Fédération Dentaire Internationale system was used for tooth numbering (49).

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- Asouti E, Fuller D (2013) A contextual approach to the emergence of agriculture in southwest Asia: Reconstructing Early Neolithic plant-food production. *Curr Anthropol* 54:299–345.
- Zeder MA (2008) Domestication and early agriculture in the Mediterranean Basin: Origins, diffusion, and impact. *Proc Natl Acad Sci USA* 105(33):11597–11604.
- Mathieson I, et al. (2015) Eight thousand years of natural selection in Europe. *Nature*, 10.1101/016477.

- Borić D, Price TD (2013) Strontium isotopes document greater human mobility at the start of the Balkan Neolithic. *Proc Natl Acad Sci USA* 110(9):3298–3303.
- Bonsall C, et al. (1997) Mesolithic and Early Neolithic in the Iron Gates: A palaeodietary perspective. *J Eur Archaeol* 5:50–92.
- Mannino MA, Thomas KD (2009) *Mesolithic Horizons*. Paper presented at the Seventh International Conference on the Mesolithic in Europe, eds McCartan S, Woodman P, Schulting R, Warren G (Oxbow Books, Oxford), pp 140–145.

7. Shulging R (2015) *The 150th Anniversary of the Discovery of Mesolithic Shellmiddens*—Vol. 2, eds Bicho N, Detry C, Price TD, Cunha E (Cambridge Scholars Publishing, Cambridge, UK), pp 153–172.
8. Hardy K (2007) . Food for thought: Starch in Mesolithic diet. *Mesol Miscell* 18(2):2–11.
9. Filipović D (2013) Southwest Asian founder- and other crops at Neolithic sites in Serbia. *Eur J Archaeol* 4:195–215.
10. Weiss E (2012) *Domestication of Plants in the Old World*, eds Zohary D, Hopf M, Weiss E (Oxford Univ Press, Oxford), 4th Ed, pp 1–8.
11. Harris DR (1996) *The Origin and Spread of Agriculture and Pastoralism in Eurasia* (UCL Press, Oxford).
12. Bonsall C (2008) *Mesolithic Europe*, eds Bailey G, Spikins P (Cambridge Univ Press, Cambridge, UK), pp 238–279.
13. Borić D (2011) *Beginnings – New Research in the Appearance of the Neolithic Between Northwest Anatolia and the Carpathian Basin*, ed Krauß R (Verlag Marie Leidorf GmbH, Rahden, Germany), pp 157–203.
14. Radovanović I (1996) *The Iron Gates Mesolithic* (International Monographs in Prehistory, Ann Arbor, MI).
15. Srejović D (1972) *Europe's First Monumental Sculpture: New Discoveries at Lepenski Vir* (Thames and Hudson, London).
16. Srejović D, Letica Z (1978) *Vlasac: Mezolitsko naselje u Djerdapu (I Arheologija)* (Srpska akademija nauka i umetnosti, Belgrade).
17. Borić D, et al. (2014) Late Mesolithic lifeways and deathways at Vlasac (Serbia). *J Field Archaeol* 39:4–31.
18. Borić D, French C, Dimitrijević V (2008) Vlasac revisited: Formation processes, stratigraphy and dating. *Doc Praehist* 35:261–287.
19. Borić D, Griffiths S (2015) The living and the dead, memory and transition: Bayesian modelling of Mesolithic and Neolithic deposits from Vlasac, the Danube Gorges. *Oxf J Archaeol* 34(4):343–364.
20. Roksandić M (2000) Between foragers and farmers in the Iron Gates Gorge: Physical anthropology perspective. Djerdap population in transition from Mesolithic to Neolithic. *Doc Praehist* 27:1–100.
21. Bonsall C, et al. (2015) New AMS <sup>14</sup>C dates for human remains from Stone Age sites in the Iron Gates reach of the Danube, southeast Europe. *Radiocarbon* 57(1):33–46.
22. Borić D, Dimitrijević V (2007) When did the 'Neolithic package' reach Lepenski Vir? Radiocarbon and faunal evidence. *Doc Praehist* 35:53–72.
23. Borić D, Dimitrijević V (2009) Apsolutna hronologija i stratigrafija Lepenskog Vira. *Starinar* 57/2007:9–55.
24. Mason S, Boroneanț V, Bonsall C (1996) Plant remains from Schela Cladovei, Romania. *Mesolithic Miscellany* 17:11–14.
25. Marinova E, et al. (2013) Wild plant resources and land use in Mesolithic and Early Neolithic south-east Europe: Archaeobotanical evidence from the Danube catchment of Bulgaria and Serbia. *Offa* 69/70:467–478.
26. Willis KJ (1994) The vegetation history of the Balkans. *Quat Sci Rev* 13:769–788.
27. Magyari G, et al. (2010) Braun, rapid vegetation response to Late Glacial and Early Holocene climatic fluctuation in the South Carpathian Mountains (Romania). *Quat Sci Rev* 35:116–130.
28. Gigov A (1972) Pollen analysis. *Europe's First Monumental Sculpture: New Discoveries at Lepenski Vir*, ed Srejović D (Thames and Hudson, London), pp 185–186.
29. Cărciumaru M (1978) *Vlasac. A Mesolithic settlement in the Iron Gates. 2 Geology–Biology–Anthropology*, eds Srejović D, Letica Z (Srpska Akad. Nauka Umetnosti, Belgrade), pp 31–34.
30. Piperno DR, Weiss E, Holst I, Nadel D (2004) Processing of wild cereal grains in the Upper Palaeolithic revealed by starch grain analysis. *Nature* 430(7000):670–673.
31. Kuzmanović N, Vukojičić S, Barina Z, Lakušić D (2013) *Sesleria serbica* (Poaceae), a neglected species of the Balkan Peninsula. *Botanica Serbica* 37:113–120.
32. Kilian B, et al. (2011) *Aegilops. Wild crop relatives: Genomic and breeding resources*. *Cereals*, ed Kole C (Springer, Heidelberg), pp 1–76.
33. Matsushima R, Yamashita J, Kariyama S, Enomoto T, Sakamoto W (2013) A phylogenetic re-evaluation of morphological variations of starch grains among Poaceae species. *J Appl Glycosci* 60:37–44.
34. Cărciumaru M (1973) Analiza poliniča a coprolitelor din stătuinea arheologică de la Icoana (Defileul Dunării). *Studii și Cercetări de Istoria Veche* 24:5–13.
35. Lucarini G, Radini A, Barton H, Barker G (2016) The exploitation of wild plants in Neolithic North Africa. Use-wear and residue analysis on non-knapped stone tools from the Haua Fteah cave, Cyrenaica, Libya. *Quat Int* 410:77–92.
36. Sawyer R (2006) *Pollen Identification for Beekeepers* (CLE Print Ltd, Cardiff, United Kingdom).
37. Pope CA, 3rd, Dockery DW (2006) Health effects of fine particulate air pollution: Lines that connect. *J Air Waste Manag Assoc* 56(6):709–742.
38. Perlès C, Quiles A, Valladas H (2013) Early seventh-millennium AMS dates from domestic seeds in the Initial Neolithic at Franchthi Cave (Argolid, Greece). *Antiquity* 87: 1001–1015.
39. Çilingiroğlu C, Çakırlar C (2013) Towards configuring the Neolithisation of Aegean Turkey. *Doc Praehist* 40:21–29.
40. Karamitrou-Mentessidi G, et al. (2013) New evidence on the beginning of farming in Greece: The Early Neolithic settlement of Mavropigi in western Macedonia (Greece). *Antiquity Project Gallery* 87. Available at antiquity.ac.uk/projgall/mentessidi336/. Accessed August 17, 2016.
41. Whittle A, et al. (2002) In the beginning: New radiocarbon dates for the Early Neolithic in northern Serbia and south-east Hungary. *Antaeus* 25:63–117.
42. Hillson S (2001) Recording dental caries in archaeological human remains. *Int J Osteoarchaeol* 11:249–289.
43. Dickau R, Ranere AJ, Cooke RG (2007) Starch grain evidence for the preceramic dispersals of maize and root crops into tropical dry and humid forests of Panama. *Proc Natl Acad Sci USA* 104(9):3651–3656.
44. Barton H, Torrence R (2015) Cooking up recipes for ancient starch: Assessing current methodologies and looking to the future. *J Arch Sci* 56:194–201.
45. Smith O, et al. (2015) Archaeology. Sedimentary DNA from a submerged site reveals wheat in the British Isles 8000 years ago. *Science* 347(6225):998–1001.
46. Hardy K, et al. (2015) Dental calculus reveals potential respiratory irritants and ingestion of essential plant-based nutrients at Lower Palaeolithic Qesem Cave Israel. *Quat Int* 398:129–135.
47. Torrance R, Barton H (2007) *Ancient Starch Research* (Left Coast Press, San Francisco).
48. Buikstra JE, Ubelaker DH (1994) *Standards for Data Collection from Human Skeletal Remains* (Arkansas Archeological Survey, Fayetteville, AR).
49. Fédération Dentaire Internationale (FDI) (1971) Two-digit system of designating teeth. *Int Dent J* 21:104–106.
50. Cook G, et al. (2002) Problems of dating human bones from the Iron Gates. *Antiquity* 76:77–85.
51. Warinner C, et al. (2014) Pathogens and host immunity in the ancient human oral cavity. *Nat Genet* 46(4):336–344.
52. Svoboda C (1970) Grasses (Poaceae) from the Balkan Peninsula. *Studii și cercetări, Biologie* 17:19–45.
53. Zaharieva M, Prosperi JM, Monneveux P (2004) Ecological distribution and species diversity of Aegilops L. genus in Bulgaria. *Biodivers Conserv* 13:2319–2337.
54. Howard T, et al. (2011) Identification of a major QTL controlling the content of B-type starch granules in Aegilops. *J Exp Bot* 62(6):2217–2228.
55. Yang X, Perry L (2013) Identification of ancient starch grains from the tribe Triticeae in the North China Plain. *J Arch Sc* 40:3170–3177.
56. Henry AG, Hudson HF, Piperno DR (2009) Changes in starch grain morphologies from cooking. *J Arch Sc* 36:915–922.
57. Stoddard FL, Sarker R (2000) Characterization of starch in Aegilops species. *Cereal Chem* 77:445–447.
58. Radini A, et al. (2016) Neanderthals, trees and dental calculus: New evidence from El Sidrón. *Antiquity* 90:290–301.
59. Flodin U, Ziegler J, Jönsson P, Axelsson O (1996) Bronchial asthma and air pollution at workplaces. *Scand J Work Environ Health* 22(6):451–456.
60. Oladele AK, Aina JO (2007) Chemical composition and functional properties of flour produced from two varieties of tigernut (*Cyperus esculentus*). *Afr J Biotechnol* 6(21): 2473–2476.
61. Antonović D (2006) *Stone Tools from Lepenski Vir* (Arheološki Institute, Belgrade).
62. Madella M, Lancelotti C, García-Granero J (2013) Millet microremains—An alternative approach to understand cultivation and use of critical crops in Prehistory. *J Arch Anthropol Sci* 8:17–28.
63. Bergfjord C, et al. (2010) Comment on “30,000-year-old wild flax fibers”. *Science* 328(5986):1634–1634, author reply 1634.
64. Blatt SH, et al. (2011) Dirty teeth and ancient trade: Evidence of cotton fibres in human dental calculus from Late Woodland, Ohio. *Int J Osteoarchaeol* 21:669–678.
65. Buckley S, Usai D, Jakob T, Radini A, Hardy K (2014) Dental calculus reveals unique insights into food items, cooking and plant processing in prehistoric central Sudan. *PLoS One* 9(7):e100808.
66. Dove CJ, Agreda A (2007) Differences in plumulaceous feather characters of dabbling and diving ducks. *Condor* 109:192–199.
67. Dove CJ (1998) Feather evidence helps clarify locality of anthropological artifacts in the Museum of Mankind. *Pac Stud* 21:73–85.
68. Harwood HP (2011) Identification and description of feathers in Te Papa's Maori cloaks. *Tuhinga* 22:125–147.
69. Dove CJ, Koch S (2011) Microscopy of feathers: A practical guide for forensic feather identification. *Microscope* 59:51–71.
70. Borić D, et al. (2014) Late Mesolithic lifeways and deathways at Vlasac (Serbia). *J Field Archaeol* 39(1):4–31.

# Supporting Information

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## SI Materials

**Vlasac.** Radiocarbon dates from the most recent work at Vlasac are given in Table S1. Bayesian statistical modeling of the dates is available for the vertical sequence of burials in trench 3/2006 (Fig. S4), and these results are cited in italics to separate them from individually calibrated dates (19). Radiocarbon ages of human burials have been corrected for the aquatic reservoir effect due to the consumption of fish based on the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values obtained as part of the AMS radiocarbon dating process. Age offsets are calculated using method 1, as outlined by Cook et al. in 2002 (50). The dates from the most recent excavation phase at Vlasac suggest that the excavated zone of the site was occupied from the end of the eighth millennium BC until the beginning of the sixth millennium BC. This time span is representative of the duration of Late Mesolithic mortuary activities in trench 3/2006 with a concentration of burials with available dental material, which was analyzed for starch preservation. More detailed chronological, stratigraphic, and contextual information is provided below for each of the samples on which dental calculus yielded results:

- i) Unit 115 (Fig. S4) represents a cremation zone found in the vertical sequence of Mesolithic burials in trench 3/2006. This zone is located below the level of primary disturbed burial H81, which is directly dated by OxA-20762 to 6600–6400 cal. BC (95% probability), and it cuts primary disturbed burial H136 dated by OxA-18865 to 6630–6480 cal. BC (95% probability). The analyzed disarticulated dental remains are likely from the individual H136.
- ii) Primary female adult burial H232 (Fig. S4) is indirectly dated by OxA-20702 to 6620–6480 cal. BC (95% probability), which dates a carbonized Cornelian cherry *Cornus mas* pit found in a cremation pit directly beneath burial H232 (Fig. S4) and acts as the *terminus post quem* for the burial. However, the archaeological context suggests that the cremation event and subsequent inhumation burial must have taken place as part of the same burial event and are thus contemporaneous (17, 19).
- iii) Primary adult male burial H326 is found in trench 3/2008 as an undisturbed single burial in the vicinity of several other burials (Fig. S1) and has not been directly AMS-dated. On the basis of the dating of a bone tool in the nearby context 320 by OxA-24769– of 6640–6490 cal. BC (95% confidence) (Table S1) as well as on the basis of the burial's extended supine inhumation body posture and orientation being parallel with the Danube with the head pointing in the downstream direction, this burial is dated to the Late Mesolithic phase of the site's use, around the mid-seventh millennium BC.
- iv) The disarticulated mandible marked as U64. × 11 (Fig. S4) was found in the fill of primary disturbed adult female burial H63, AMS-dated by OxA-16542 to 6240–6010 cal. BC (95% probability). This mandible (Fig. S5 A and C) comes from the individual found in primary disturbed, chronologically older adult male burial H81 dated by OxA-20762 to 6600–6400 cal. BC (95% probability). There were several other skeletal elements from this disturbed burial found in the fill of primary disturbed burial H63 (Fig. S5B).
- v) Dental remains in unit 222. × 18 are found in the layer of paleosol at the bottom of the stratigraphic sequence in trench 3/2006. There is no radiocarbon date for this layer, but based on the stratigraphic sequence in trench 3/2006, the remains in this layer are contemporaneous with or earlier in age than the dated remains in layer unit 282 in the same trench, which

gave two dates on two different bone tools: OxA-24809 and OxA-24810 gave ranges of 6990–6650 and 7000–6600 cal. BC (95% probability), respectively.

- vi) Primary female adult burial H53 (Fig. S4) is indirectly dated with OxA-16544 to 6010–5840 cal. BC (95% probability), which dates a red deer skull deposited directly over the stone slab that covered the pelvic area of the deceased woman found in burial H53. Hence the date represents a *terminus ante quem* for the individual in burial H53. The *terminus post quem* for burial H53 is OxA-16542 that directly dates burial H63, found in a stratigraphic superposition directly underneath burial H53, to 6240–6020 cal. BC (95% probability). However, it is very likely that the date obtained for the red deer skull and the chronological age of burial H53 are by and large contemporaneous.
- vii) Unit 44 relates to the layer that was found on top of stone plaques and slabs directly covering burial H53 (Fig. S4). This layer contained numerous finds of disarticulated human remains, including the dental remains analyzed here. These remains come from an adult individual and most likely can be associated with the primary disturbed adult female individual in burial H63, the skull of which was missing and which was dated by OxA-16542 to 6240–6020 cal. BC (95% probability), but it cannot be excluded at this stage that these remains belong to an older adult disturbed burial found in this particular burial location.

**Lepenski Vir.** More detailed chronological, stratigraphic, and contextual information is provided below for each of the samples from Lepenski Vir on which dental calculus yielded results:

- i) Primary adult female burial 8 lay in the fill of trapezoidal building 24, some 20 cm above the limestone floor of the building and must have been interred in this space after the abandonment and complete or partial backfilling of the semisubterranean space of this building structure. It was found on the lateral right side in a crouched position and was symmetrically facing another crouched burial in the same building space (burial 9) (Fig. S6A). These two interments are very likely broadly contemporaneous or took place within the existing memory of the first burial event. OxA-25207 and AA-58319 directly date these burials (13, 21) (see Table 1).
- ii) Primary adult female burial 20 is found in quad. b/10 as a single inhumation placed on the lateral left side in a crouched position within a stone construction (Fig. S6B). There is no direct AMS date for this individual, and it is dated to the Lepenski Vir phase III based on its stratigraphic position and its body posture.
- iii) Primary adult female burial 32a (Fig. S6C) is found on the lateral right side in a crouched posture at the bottom of the multiple burial place in quad. d/1. There was another crouched burial marked as 32b placed directly on top of the individual marked as burial 32a. The burial is directly dated by OxA-5828 (see Table 1).

## SI Methods

**Calculus Decontamination Procedure.** The fleck of calculus was first observed under incident light microscopy, and the contaminant soil adhering to the surface was removed following established protocols (35, 51). The protocol consisted of the following steps: A fine acupuncture needle was wet in HCl 0.06 M. A very small

area of dirt adhering to the surface was gently dislocated and scraped off. The calculus was then washed in ultrapure water and left to dry. It was then examined again, and the procedure was repeated until the fleck was clean. Once the surface was satisfactory clean, the calculus samples were removed using a sterile blade and then with a fine sterile acupuncture needle. Further, they were slid into sterile Eppendorf tubes. Ultrapure water was added, and the calculus was washed up to three times to remove any trace settled on it. Once washed, the calculus samples were placed into new sterile Eppendorf tubes and degraded in a weak solution 0.06 N of HCl, enough to cover the entire calculus. The contaminant soil removed was also placed on a slide and examined, as an extra safety measure, and no starch was recovered from the contaminant soil. A portion of the dissolving calculus was siphoned out with its natant and mounted on clean slides. A solution of 50:50 glycerol and ultrapure water was added to the drop on the slide to allow for the rotation of microfossils, a step necessary during the identification. It has been documented how such protocols allow the retrieval of all debris undamaged and often calculus fragments still dissolving can be observed under the microscope, providing further evidence of the integrity of the analysis (51).

**Experimental Collection.** Reference collection of modern starch granules included plants native to the central Balkan region as well as many others from the wider Mediterranean region stored at the George Pitt-Rivers laboratory at the University of Cambridge. Furthermore, a reference collection hosted at the Department of Archaeology at the University of York that contains plants of North European, Mediterranean, and Northern African origin, including nondietary items, as well as processed and chewed food specimens (e.g., plants used for cordage and containers and occupational dust such as wood and pottery) was also consulted. Such reference collection has been successfully used before in other studies (35, 51). The reference collection was prepared in a clean laboratory, with handlers wearing dust-free gloves. Starch granules were extracted by gently grinding/squashing seeds in a clean jade mortar/pestle, first with and then without a drop of ultrapure water and as gentle as possible to try to obtain starch granules still in situ in the amyloplast. Starch granules were mounted using the same mounting media used for the calculus samples to secure a comparison as close as possible to the remains observed in ancient samples.

**Details on the Identification of the Tribe Triticeae and Aveneae.** The identification of starch granules with bimodal distribution consisting of A- and B-type starch granules as domesticated species of the Triticeae tribe was made based on morphological and archaeological considerations mentioned in the main text of this article as well as distributions of wild species in the region (32). Following Svoboda (52), we considered the presence of wild taxa of the Triticeae tribe, and the following species are well-known: *Aegilops cylindrica* (A-type: min., 10.3; max., 28.5; mean, 22.5), *Aegilops geniculata* (A-type: min., 10.04; max., 31.12; mean, 22.34), *Aegilops neglecta* (A-type: min., 10.2; max., 19.28; mean, 12), and *Aegilops triuncialis* subsp. *Triuncialis* (A-type: min., 10.23; max., 34.2; mean, 21.5) (53). In our reference collection, we also have *Aegilops speltoides* (A-type: min., 10.2; max., 34.6; mean, 19.4) and *Aegilops comosa* (A-type: min., 10.3; max., 38.5; mean, 23) (see Fig. S7). *Aegilops peregrina* and *Aegilops crassa* are not bimodal (54). The possibility that archaeological starch granules were consistent with one of such *Aegilops* species was excluded through experimental comparison as well as on the basis of recent literature. First of all, published data show that very often starch granules of the *Aegilops* are large and round, reaching often 40  $\mu\text{m}$  in size, whereas species of domesticated Triticeae are often below this size (55). However, in our reference collection of plant specimens from the area, this is not often the case (as shown earlier in this paragraph).

Second, although the majority of the archaeological starch granules assigned to the Triticeae tribe and proposed as domesticates are still lodged together and therefore difficult to fully measure, many are clearly within the range of domesticated species published by others (56). This being said, some of the starch granules can reach up to 40  $\mu\text{m}$ , and in our reference collection, granules of domesticates can easily reach that size too. Such differences with published data can be explained by different growing conditions, as a number of environmental factors can affect the size of starch granules. Using specimens from the Balkan region was therefore very helpful. Due to the fact that archaeological starch granules are still lodged together, in our comparison we also focused on their overall morphology, the presence/absence/shape/location of lamellae, as well as the way the starch granules appear as a “cluster.”

Third, in the archaeological samples, a large number of starch granules were very clearly still lodged together as they would be in the amyloplast. This aspect allowed us to establish their strong and remarkable bimodal distribution where the large lenticular granules, A-type, are seen emerging from the mass of a high number of B-type starch granules (Fig. 3A and B). In the light of such observations, we compared starch granules in their amyloplast in wild species of Triticeae, using both reference material and published data as detailed below. In wild species of wheat (*Aegilops*) as well as in genera of the Triticeae tribe present in the Balkans, such as *Elymus* and *Agropyrum*, in our reference collection, the large A-type granules are predominant in number, an aspect that becomes even clearer when these are viewed in situ (57). A-type starch granules of the *Aegilops* species have clearly a round 2D shape, sometimes irregular and with lumps, and a large number of lamellae across the entire granules, denser in the central area of the granules near the hilum, have been observed (see examples of *A. cylindrica* and *A. speltoides* in our reference collection; Fig. S7A and B), and this has also been pointed out in earlier published work (30). Our archaeological samples are clearly not as round but more oval in 2D shape and they lack the number of lamellae across the entire granule as seen in the *Aegilops* species. Also, the distribution of lamellae in archaeological granules is different from various species of *Aegilops* we have analyzed and we exclude this aspect as being related to cooking on the basis of experimental comparison.

Our observations are supported by a number of studies on modern starch granules. A recent phylogenetic reevaluation of morphological variations of starch grains among Poaceae species has stressed that clear bimodal simple grain types characterize species belonging to the *Triticum* and *Hordeum* genera but is lacking in a number of wild wheat species of *Aegilops*, including some of the species known to be present in the Balkans (32, 33). It has also been known for some time now that species of *Aegilops* with bimodal distribution have low B-type starch (57), confirming observations from our reference collection. Traits on B-type starch are thought to be under genetic control (31). Thanks to the extraordinary modality of preservation of our archaeological starch granules and to the morphological and genetic aspects described above, we could exclude species of *Aegilops*. Within the Triticeae tribe, we also considered *Hordeum murinum*, as this wild species is documented in the region. However, we excluded this species on morphological grounds, as large A-type granules were consistently smaller than ours (Fig. S7C).

Fourth, we examined species of *Elymus* spp. (e.g., *Elymus caninus*, A-type: min., 10.3; max., 29.03; mean, 18.34) (Fig. S7G) and *Agropyrum* spp. (e.g., *Agropyrum doricum*, A-type: min., 10.5; max., 19.35; median, 12.4; e.g., *Agropyrum farctus*, A-type: min., 10.25; max., 31.5; median, 16.2) (Fig. S7H), which also look more elongated growing in the Balkans, and excluded them on morphological grounds and appearance of starch granules in the amyloplasts. In our reference collection, the starch granules have clearly different overall morphology, and the A-type granules are

more oval and smaller than ours (see Fig. S7 for an example of such starch granules in their amyloplast). We believe our results to be reliable, as we evaluated the morphology of starch granules of interest in this study by using plant material from the region under study as well as other regions. We would also like to stress that the extraction of starch granules from species of *Elymus* and *Agropyrum* required several attempts to be successful despite the fact that the plants were mature, suggesting a lower production of starch granules of these species compared with other species of the Triticeae tribe.

Lastly, although the bimodal distribution is known for at least one species in the Bromideae tribe, *Bromus catharticus* (57), the latter is not native to the Eurasian region. However, we also excluded other species of the genus *Bromus* (e.g., *Bromus secalinus*, *Bromus alopecuroides*, *Bromus hordeaceus*, *Bromus arvensis*) on morphological grounds derived from both our reference collection and previously published data (Fig. S8A) (57). Such species have starch granules that do not appear bimodal, and starch granules are around or below 11  $\mu\text{m}$ . As we excluded wild species of the Triticeae and Bromideae tribes to be the origin of our starch granules, this leaves us with the domesticated species of the Triticeae tribe available at the time. Finally, the characteristics used in our comparison/identification related to 3D morphology and lamellae have already been used as criteria for separation among *Aegilops* spp., *Triticum* spp., and *Hordeum* spp. when a large number of well-preserved starch granules as well as archaeobotanical data were available (30).

The identification of the tribe Aveneae was achieved by comparison with published data as well as through the evaluation of experimental starch granules in our reference collection, as mentioned in the main text of the article (Fig. S8B and C). Compound granules are known in different species of plants including the Poaceae (33). In our reference collection, we noticed morphological similarities of Aveneae with compound granules of *Sesleria caerulea*, a species growing wild in many areas of Europe (Fig. S8D). Additionally, some species of such genus are still harvested today as wild food in the central Balkans (e.g., *Sesleria serbica*) (31). However, in *S. caerulea*, the single starch granules forming the compound lump and the lump itself were smaller than the archaeological ones. A number of processes can cause starch granules to swell, including water, cooking, and even grinding. To exclude that the larger starch granules observed in our ancient samples were those of *Sesleria* species enlarged by the processes listed above, we carried out experimental work to see if starch granules of these species could overlap with those of the Aveneae species. We were able to exclude this possibility as our experiments show how starches of the genus *Sesleria* lose their morphology when ground or boiled for even just a minute (Fig. S8E). On the other hand, variations caused by cooking/processing in species of the Triticeae tribe were found to be consistent with those of other published studies (54).

**Considerations on the Conditions of Preservation of Starch Granules in Our Samples and Potential Pathways of Inclusion.** We have found that our starch granules (found in large numbers and still lodged together in the amyloplast) exhibit a good overall state of preservation. Alterations of starch granules due to diagenetic processes, processing, and cooking have been the subject of a number of studies and remain an important area of research in the field of ancient starch research (44). Although it is assumed that starch granules in the mouth are by and large the result of deliberate consumption of processed and cooked plants, and therefore morphological changes are to be expected, an increasing number of papers show that a variety of pathways to the inclusion of such remains are equally possible, including accidental inhalation (58). The presence of well-preserved starch granules in our ancient samples, including those still apparently lodged in their amyloplast, could be the results of ingestion during the processing of the seeds before cooking. Plant processing for fibers as well as for

starchy food generates a high amount of debris, especially when carried out for prolonged periods of time. This is a well-studied aspect of “occupational” health for the consequences that such activities have on the respiratory system when inhaled (59). Although technology and scale of processing changed over time, the processing of plant material is inevitably a “dusty craft” that results in large quantities of particles to be released in the process. Such particles can settle on hands, cloths, and hair as well as become airborne and enter the human mouth by transfer from hand to mouth or by inhalation. These processes increase the chances of uncooked and low-processed remains to become incorporated into the calculus matrix. Such a pathway of inclusion is very likely and can easily explain the good state of preservation in our remains and should always be taken into account. We also stress that many species of starchy food are consumed raw, even bitter tubers such as tigernut (*Cyperus esculentus*) (60). Legumes are consumed raw even today in many countries, including some European countries, such as Italy, and could result in well-preserved starch granules. Although we cannot be sure that no processing occurred in our remains, prolonged cooking and high processing result in the release of starch granules out of their amyloplast, something we can exclude in this unique case. Some of our starch granules have cracks that are very likely the result of grinding, as documented also by stone tools found at both of the examined sites (15–17, 61) (Fig. S2), and this could clearly generate the release in the air of starch masses that would therefore not be grinded for prolonged periods of time or cooked, hence their still being lodged in the amyloplast in great conditions of preservation. This could also explain some of the largest starch granules, as grinding can enlarge the starch granules (56).

## SI Results

Supplementary information about dietary remains identified in the Lepenski Vir dental calculus as well as nondietary remains identified in dental calculus from Vlasac and Lepenski Vir are provided below.

**Dietary Remains.** The analysis of dental calculus obtained from individuals 8, 20, and 32a from Lepenski Vir yielded starch grains associated with diet. In particular, three different typologies of starch granules were identified and assigned to two tribes of the Family Poaceae and to the Family Fabaceae.

The first type of starch granules show a bimodal distribution characterized by the presence of both small round granules with a central hilum, rarely larger than 10  $\mu\text{m}$ , and large oval/lenticular morphology, with central hilum and lamellae (Fig. 3C). On the basis of the experimental comparison (Fig. S7D–F), these starches were interpreted as belonging to the Triticeae tribes (barley and wheat tribe) of the family Poaceae and were attributed to the domesticated species according to the consideration made in *Considerations on the Conditions of Preservation of Starch Granules in Our Samples and Potential Pathways of Inclusion*.

The second group of starch granules was retrieved in dense clusters and is consistent with the grains of the tribe Paniceae. This type of starch granule shows a faceted shape, a central hilum, and fissures often radiate from it (Fig. S9A and B). The large number of our starch granules (sometime more than 200 per cluster) and the morphology of the single grains suggest an attribution to the genus *Setaria* spp. grains (see Fig. S8F–I for experimental comparison). The presence of such starch granules is also remarkable. Species of the tribe Paniceae are in fact part of an important group of food plants known as small millets with lower archaeological visibility compared with others. Further details on this topic were published by Madella et al. (62).

Finally, a small number of simple starch granules were found in samples that had lost characteristics useful for the identification, often had no extinction cross visible, and appeared somewhat

gelatinized. Due to the low number of these starch-type granules and the evident loss of their original morphology, it is not possible to put forth a positive identification. Such starch granules were described as unidentified.

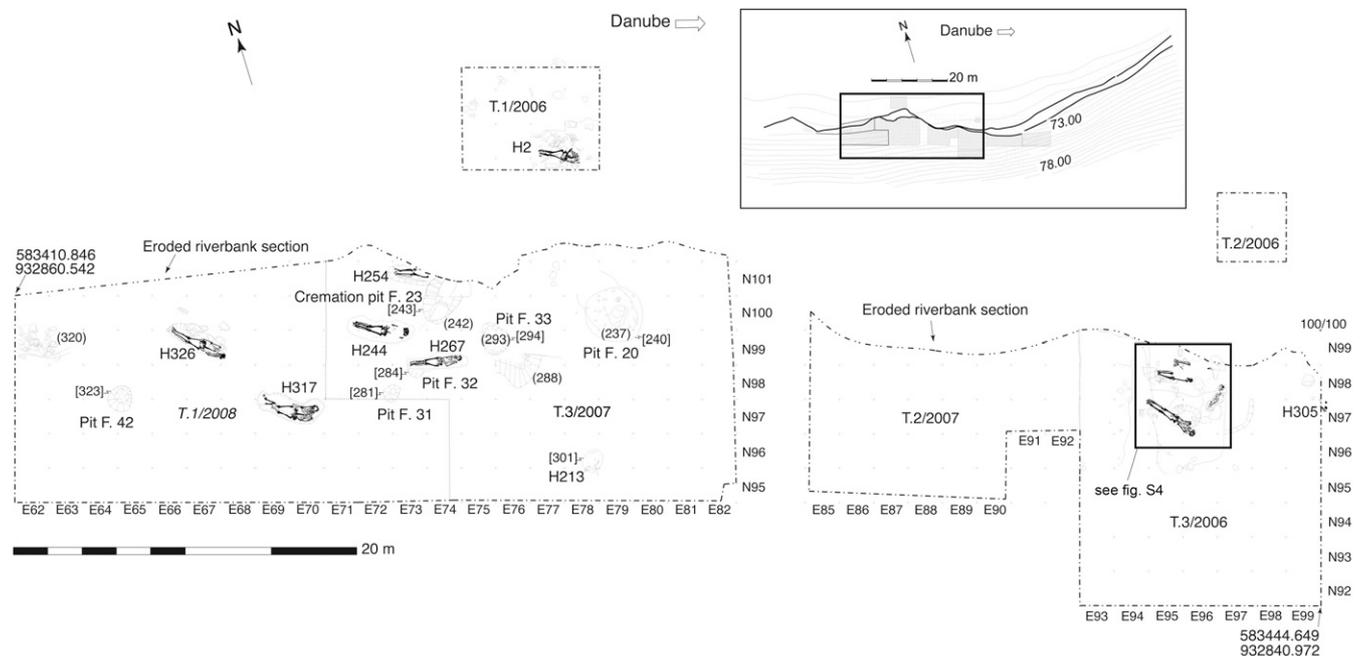
**Nondietary Remains.** All of the nondietary remains of the debris found in analyzed dental calculus were consistent with respirable fractions of particulate matter, which being below 20  $\mu\text{m}$  would also be naturally airborne, and all of them are therefore potentially respiratory irritants (37). Our finds therefore document the potential of human dental calculus to entrap environmental debris potentially harmful to people, as suggested by an increasing number of studies (46).

**Vlasac.** Nondietary evidence in dental calculus includes a variety of animal and plant remains. Small fragments of conifer wood (with some of its characteristic tracheid fibers with bordered pits clearly visible) and a large amount of plant fibers (22 in total) of varied diagnostic and nondiagnostic nature were found in a number of individuals and were particularly abundant in Mesolithic individual U64.  $\times$  11 from Vlasac. Potential bast fibers of plant origin were often observed still in situ (Fig. S10A), in different forms of preservation; some of them (seven in total) showed a narrow lumen and dislocation bands when polarizer and analyzer were oriented at 90 degrees to one another, typical of plants used for fibers in cordage and textiles such as nettle, flax, and hemp (63). Considering the region and period, the most likely candidate for our finds is nettles (*Urtica* spp.). Bast fibers potentially resulting from the processing of plants for cordage or textile were found in dental calculus in other studies (53, 64, 65). Among other remains were two unidentified fungal spores (for problems with the identification of spores, see ref. 46), microcharcoal and nondiagnostic

plant remains showing no diagnostic feature to allow for a positive identification.

Animal remains were consistent with fragments of feather barbules, which have been identified upon the shape and features at their nodes (Fig. S10 C and E) and Lepidoptera scales (butterfly and moth), the latter identified for their distinctive morphology of blade and pedicel (Fig. S10 B and D). Lepidoptera scales have been recently found also in a dental calculus sample of *Homo erectus* from the Qesem cave in Israel (46), showing that such evidence can survive considerable periods of time. Pathways of inclusion of such remains vary (46). Fragments of bird feathers identified in the dental calculus of some Late Mesolithic individuals from Vlasac show shapes and features at their nodes that would be consistent with various species of ducks of the Anatidae family. These identifications are supported by the presence of identified bird remains, such as mallard *Anas platyrhynchos* and teal *Anas crecca*, in the Late Mesolithic levels at Vlasac (17). Bird feather barbules could be the result of exposure to dust generated when plucking birds, whereas Lepidoptera scales are present in the airborne dust in the natural environment. Such remains therefore could represent inhaled material or material accidentally ingested as settled dust on food.

**Lepenski Vir.** Nondietary evidence in the Lepenski Vir dental calculus was consistent with animal debris and particularly with feather barbules. Two fragments from Neolithic calculus show triangular-shaped nodes, which are typically located on the downy barbules of Anseriform birds inhabiting aquatic environments (Fig. S9C), and pronged nodes suggestive of the Anatidae family (Fig. S9D) (66–69).



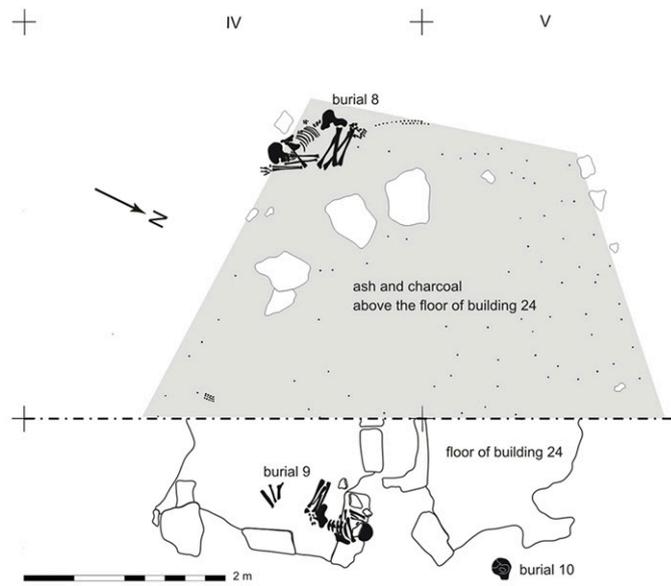
**Fig. S1.** Site plan of Vlasac, reprinted from ref. 70. Late Mesolithic pits and burials (indicated with "F." and "H." prefixes, respectively) in the central part of the area excavated in 2006–2009 in the southernmost part of the site with trenches 1–3/2006, 2–3/2007, and 1/2008 (indicated with "T." prefixes). Context numbers are in brackets.



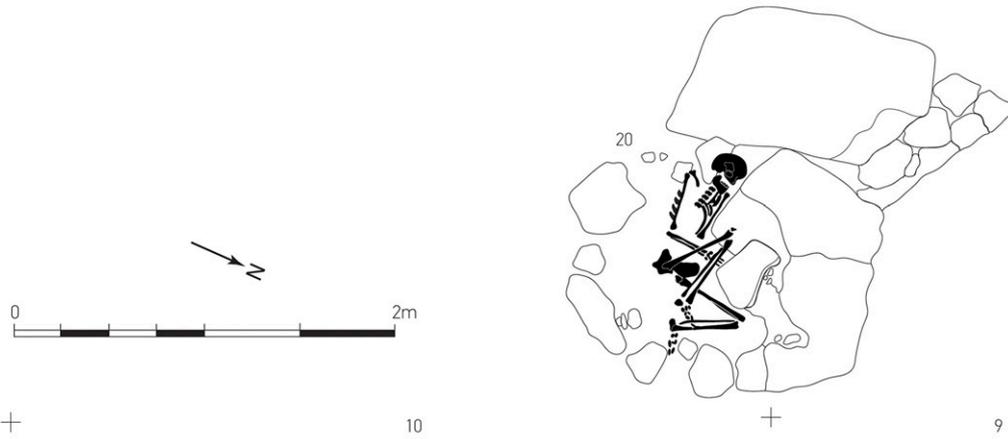




**Fig. S5.** Human remains from Vlasac. Adult female burial H63 (A) with disarticulated (B and C) cranial and postcranial bones of adult male H81 that was several centuries older.



a

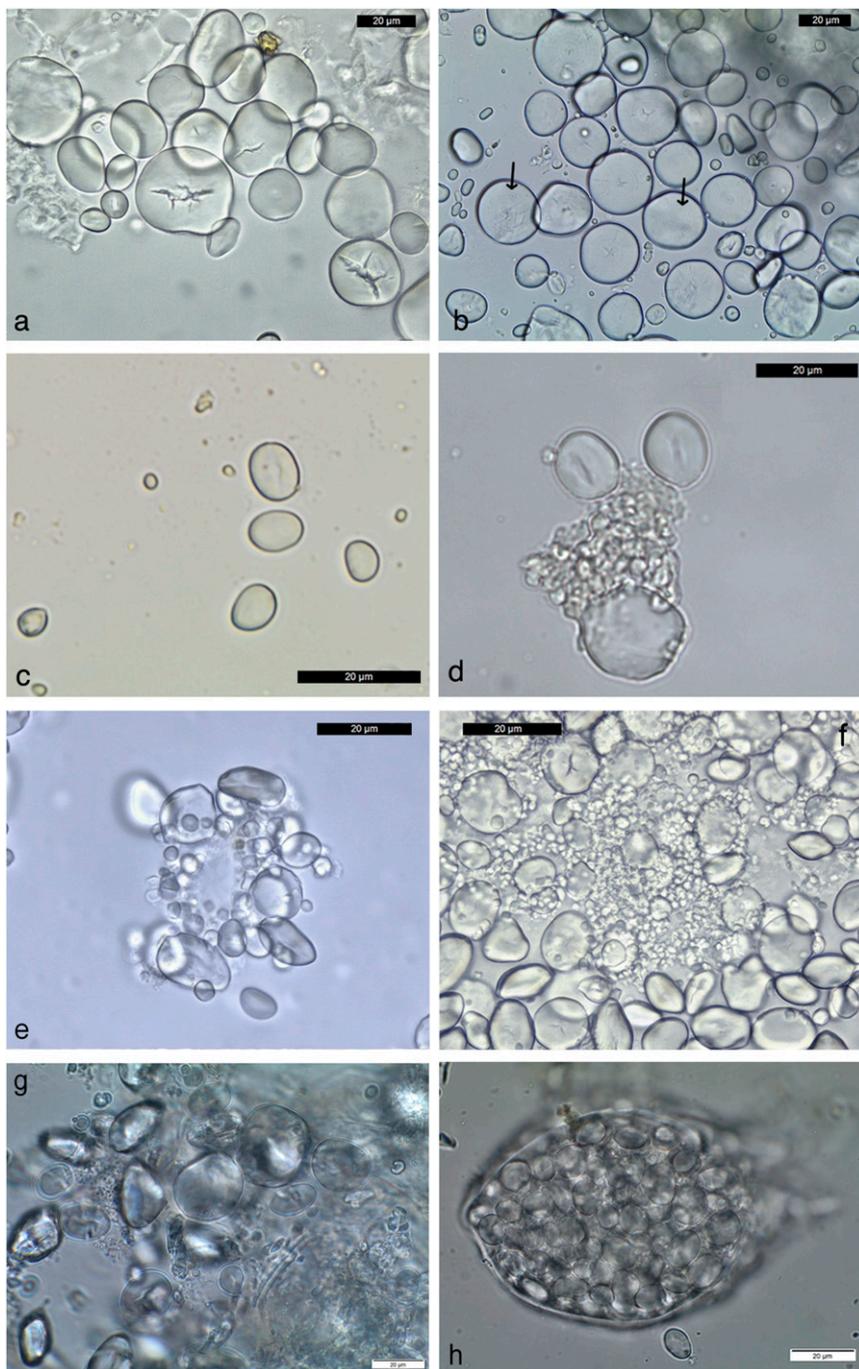


b

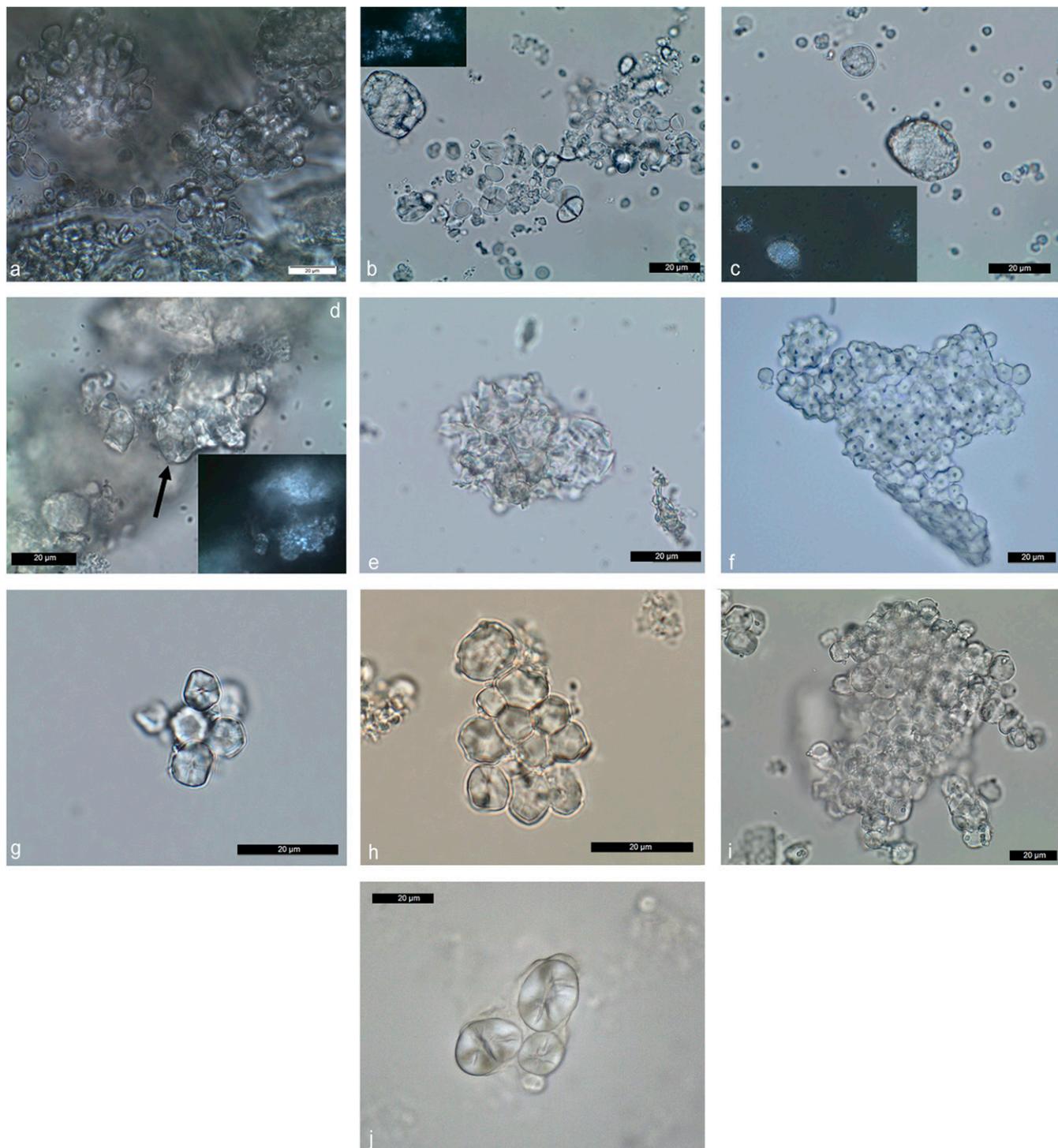


c

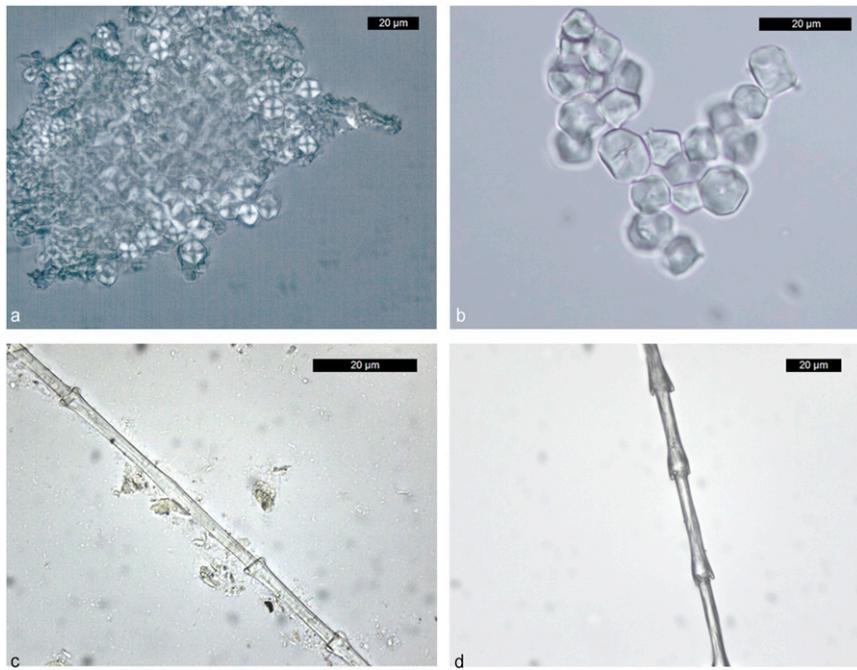
**Fig. S6.** Early Neolithic burials at Lepenski Vir analyzed for dental calculus in this paper: (A) LV8; (B) LV20; (C) LV32a.



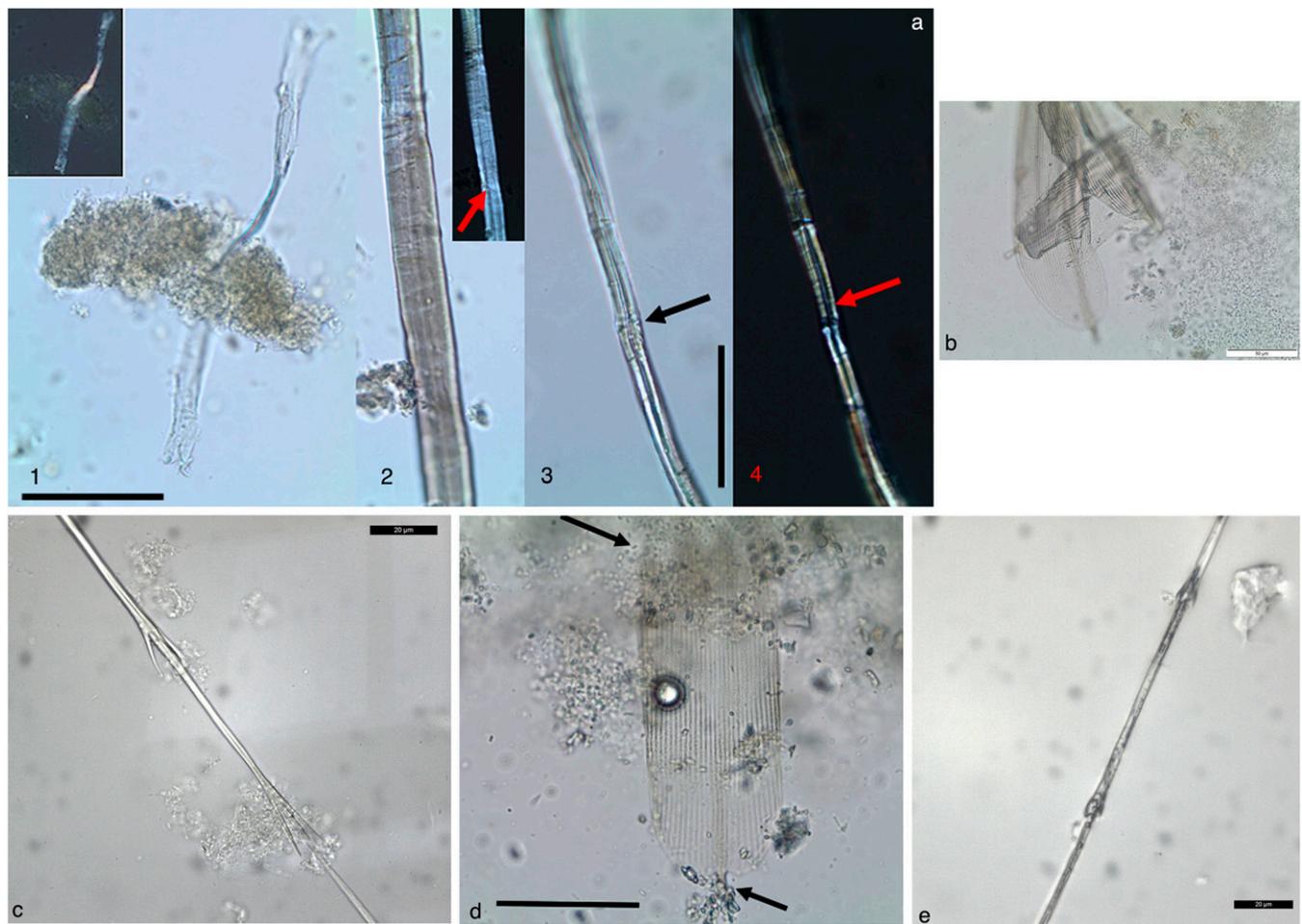
**Fig. S7.** Experimental starches. Characteristic starch grains from modern seeds of Triticeae tribe: (A) *Aegilops comosa* and (B) *Aegilops speltoides*. The arrows indicate the lamellae: (C) *Hordeum murinum*; (D) *Hordeum distichon*; (E) *Triticum dicoccum*; (F) *Triticum monococcum*; (G) *Elymus caninus*; (H) *Agropyrum doricum*.



**Fig. S8.** Experimental starch grains. Characteristic starch grains from modern seeds of Bromidae, Aveneae, Paniceae, and Fabaeae tribes: (A) *Bromus arvensis*; (B) *Avena sterilis*; (C) *Phalaris minor*; (D) *Sesleria caerulea*; (E) *Sesleria caerulea* boiled for 1 min; (F) *Setaria viridis*; (G) *Setaria glauca*; (H) *Penisetum elatum*; (I) *Panicum (Echinochloa) crusgalli*; (J) *Vicia selvatika*.



**Fig. S9.** Evidence of dietary and nondietary remains from Early Neolithic individuals from Lepenski Vir: (A) compound of starch grains with faceted morphology and perpendicular extinction cross embedded in dental calculus (32a); (B) compound of starch grains with faceted morphology and evident central fissure (32a); (C and D) fragments of feather downy barbules with triangular-shaped nodes typical of Anseriformes (C) and asymmetric short pronged nodes typical of Anatidae (D) (32a).



**Fig. S10.** Evidence of nondietary remains from individuals from Vlasac: (A) bast fibers embedded in calculus (U64); (B) lepidoptera scale (U44); (C) feather barbule with long pronged nodes attributed to *Anatidae* (H53); (D) lepidoptera scale (H53); (E) feather barbule with short pronged nodes attributed to *Anatidae* (H232). (Scale bar: A and D, 20  $\mu\text{m}$ .)

**Table S1. AMS dates from new excavations at Vlasac (2006–2009), calibrated using OxCal v. 4.0**

Site/lab no.	Context and material	<sup>14</sup> C age, B.P.	δ <sup>15</sup> N, ‰	δ <sup>13</sup> C, ‰	68.2% confidence, cal. BC	95.4% confidence, cal. BC
						95.4% probability, cal. BC
OxA-16544	Context 19, red deer skull (VL50) over burial H53, trench 3/2006 (22/04/2006)	7035 ± 40	6.8	−21.3	5990–5900	6010–5840 6010–5840
OxA-16542	Burial H63, human rib (08/07/2006)	7701 ± 39* Corrected: 7261 ± 60 <sup>†</sup>	17.0	−17.7	6220–6070	6240–6020 6240–6010
OxA-16539	Context 40, x.8, trench 3/2006, square 95/96 (20), large mammal bone (VL18) (30/05/2006)	7425 ± 39	6.8	−21.7	6370–6250	6400–6230
OxA-20762	Burial H81, human femur – proximal diaphysis, trench 3/2006	8125 ± 45* Corrected: 7685 ± 64	14.0	−19.3	6590–6470	6640–6440 6600–6440
OxA-20702	Burial H232, charred pit of Cornelian cherry in context 249 beneath the skeleton	7725 ± 40	—	−22.8	6600–6510	6640–6480 6620–6480
OxA-24769	Context 320, x.17 (tool on red deer antler with traces of green pigment)	7738 ± 35	6.6	−22.0		6640–6490
OxA-16540	Context 118, x.1, bone projectile point (VL21), above the floor context 149 of feature 12, trench 3/2006 (18/07/2006)	7764 ± 38	7.7	−22.1	6650–6540	6660–6490 6690–6560
OxA-16541	Burial H2, human rib (VL42), trench 1/2006 (10/04/2006)	8228 ± 40* Corrected: 7788 ± 60 <sup>†</sup>	16.3	−18.2	6690–6530	6780–6470
OxA-18865	Burial H136, human right tibia (VL1/2008), trench 3/2006 (20/07/2006), trench 1/2007	8231 ± 36* Corrected: 7791 ± 58 <sup>†</sup>	16.2	−18.5	6690–6530	6780–6480 6630–6480
OxA-24809	Context 282, x.7 (tool on large mammal bone), trench 3/2006	7943 ± 40	6.2	−22.1	7030–6710	7040–6700 6990–6650
OxA-24810	Context 282, x.6 (tool on large mammal bone), trench 3/2006	7952 ± 38	5.2	−21.2	7030–6770	7040–6700 7000–6600
OxA-24811	Context 314, x.54, spit 2 (tool on large mammal bone), trench 1/2009	7905 ± 39	7.4	−22.6	6900–6660	7030–6650
OxA-21962	Context 314, x.23, spit 1, roe deer skull (S3/2009), trench 1/2007	8050 ± 40	5.0	−23.0	7080–6840	7140–6830

The δ<sup>15</sup>N values are used to estimate percentage of aquatic diet. The last column shows 95% ranges both of individually calibrated dates (confidence) and Bayesian modeling (*probability*) where available.

\*Values are corrected for ages affected by the aquatic reservoir effect (δ<sup>15</sup>N values > +10‰) using method 1 as suggested by Cook et al. in 2002 (50).

<sup>†</sup>100% reservoir correction applied (440 ± 45 y).