nature human behaviour

ARTICLES

https://doi.org/10.1038/s41562-021-01083-y

Evidence for early dispersal of domestic sheep into Central Asia

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The development and dispersal of agropastoralism transformed the cultural and ecological landscapes of the Old World, but little is known about when or how this process first impacted Central Asia. Here, we present archaeological and biomolecular evidence from Obishir V in southern Kyrgyzstan, establishing the presence of domesticated sheep by ca. 6,000 BCE. Zooarchaeological and collagen peptide mass fingerprinting show exploitation of *Ovis* and *Capra*, while cementum analysis of intact teeth implicates possible pastoral slaughter during the fall season. Most significantly, ancient DNA reveals these directly dated specimens as the domestic *O. aries*, within the genetic diversity of domesticated sheep lineages. Together, these results provide the earliest evidence for the use of livestock in the mountains of the Ferghana Valley, predating previous evidence by 3,000 years and suggesting that domestic animal economies reached the mountains of interior Central Asia far earlier than previously recognized.

The early Holocene domestication of crops and livestock in the Fertile Crescent is among the earliest in the world, with the first traits of domestication appearing in plants and animals by 7,500 BCE[1–6]. The food-producing economy of this region, based on sheep, goats, cows, cereals and legumes, launched humanity's first agricultural demographic transition[6,7], which would eventually reshape human populations, both genetically and culturally, across the ancient world[8]. Recent human genomic studies have clearly illustrated that the Neolithization of western Eurasia involved a demic wave of expansion as peoples of southwest Asian ancestry expanded and admixed with local populations in Europe and West Asia[9–11]. Understanding the dynamics of early Neolithic dispersals informs the processes that shaped the trajectory of human societies in Eurasia.

Archaeozoological evidence suggests that the first progenitors of domesticated goats and sheep came under sustained, multigenerational human control by ca. 11,000–9,000 years ago in a region stretching from eastern Anatolia to the Zagros Mountains of Iran and Iraq[12–14]. Following their initial domestication, animal livestock (including sheep and goats, as well as cattle) and plant crops from this region dispersed across Eurasia and Africa in one of the most important globalization processes in human prehistory[15]. While these three livestock species sometimes moved together into new regions as part of a 'Neolithic package', domesticated sheep and goats became particularly widespread in the ancient world, reaching areas across Europe and the Mediterranean by ca. 6,000 BCE and North Africa by 5,000 BCE[15].

Although the impact of Western Asian livestock dispersals on the ancient cultural landscape of western Eurasia and Africa has been substantial, the spread of animal domesticates to the Eurasian interior is poorly understood[16,17]. Domesticated sheep and goat are not found in the archaeological record of the eastern steppe region of Inner Asia until the arrival of the pastoralist Afanasievo culture ca. 3,000 BCE[18,19], and agropastoralism has only been traced to the early third millennium BCE in Central Asia[20–22]. However, Soviet archaeologists have long hypothesized a much earlier arrival of agropastoralism from southwest Asia between the seventh and fourth millennia BCE associated with the Kelteminar culture (Fig. 1)[23–27]. This poorly understood culture, concentrated in the Khoresm region beyond the southern edge of the Aral Sea, is characterized by their microlith technology, distinctive arrowheads with a prominent notch cut out at their base, handmade coarseware pottery with pointed bases and geometric incisions and a mixed pastoralist–hunting–fishing economy.[26,28–31] Cattle and ovicaprid (sheep/goat) remains dating to at least the fifth millennium BCE have been reported from Kelteminar sites, but they have not been subjected to detailed zooarchaeological analysis[27,32], and thus it is not clear whether they represent wild or domesticated populations.

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Fig. 1 | Modeled dispersal of domestic animals into Central Asia. Proposed centre of sheep and goat domestication (dark red) and early Holocene dispersals out of western Eurasia (information from Vigne[16] and Murphy[90]) alongside relevant archaeological cultures and newly hypothesized dispersal event(s) (red dashed arrow). Inset map of Obishir V and the Ferghana Valley situated within the mountainous zone of Central Asia.

More recently, extensive research at the Djeitun site in southern Turkmenistan (Fig. 1) has revealed a complex agropastoral system, relying on harvesting tools, grinding stones, irrigation and a mixed crop assemblage, including glume wheats and barley, dated to ca. 6500 BCE. Based on the predominance of *Ovis* and *Capra* remains at Djeitun, along with their small size and some distinct morphological traits, domesticated sheep and goat are also thought to have been an important part of the subsistence economy at Djeitun[33–36].

Soviet scholars have also hypothesized the presence of another Neolithic culture known as the Hissar in the Hissaro-Alay Mountains of Tajikistan (Fig. 1). In its classic formulation, the Hissar culture is dated to between the sixth and second millennia BCE and is characterized by a pebble and microblade lithic technology, which includes geometric microliths[37]. Importantly, scholars have also argued that the Hissar economy included goat and sheep pastoralism[37]. However, as at Djeitun, the inference of domestic animals at Hissar sites has been based largely on the high frequency of *Ovis* and *Capra* remains and indirect lines of evidence such as site location, rather than direct evidence of human management.

Taken together, these archaeological finds provide evidence for the presence of Neolithic material culture and domestic plants, along with tentative evidence for domestic animals, along the margins of Central Asia during the seventh millennium BCE. However, most of the relevant scholarly research on these cultures was performed prior to the widespread availability of scientific methods, including radiocarbon dating via accelerator mass spectrometry. Moreover, no direct archaeobotanical or zooarchaeological evidence for food production has been found east of the Kopet Dag piedmont of Turkmenistan prior to ca. 3,500 BCE[22]. With the advent of powerful new methods in archaeological science over the past decade, it is now possible to revisit these Soviet-era cultural designations and Neolithic dispersal models and to test these hypotheses in a robust scientific framework.

Here we investigate animal husbandry at the early Holocene site of Obishir V in southern Kyrgyzstan. Located in the heart of Central Asia within the Inner Asian Mountain Corridor, Obishir V is situated along the southern margin of the Ferghana Valley, a historically significant crossroads for the exchange of people and animals across eastern and western Eurasia[21]. Through new excavations, we identified stone tools and faunal remains suggestive of grain processing and livestock pastoralism, dating to the late seventh millennium BCE. Zooarchaeological analysis and collagen fingerprinting reveal a faunal assemblage consisting primarily of *Ovis* and *Capra*, which based on cementum annulation appear to have been killed during the autumn season. Based on DNA sequences from five well-preserved specimens, we further identify at least four of these animals as *O. aries* within the diversity of domestic sheep, and showing genetic affinity to modern Anatolian and South Asian breeds. The presence of these domestic sheep at Obishir V ca. 6,000 BCE suggests that pastoral livestock species reached the foothills of the Pamiro-Alay mountains millennia earlier than previously recognized. These findings warrant a re-evaluation of the timing and routes of the earliest eastward agropastoral expansions and the role of domestic plants and animals in the development of social complexity in this crossroads region.

Results

Site excavation. The site of Obishir V (39° 57′ N, 71° 16′ E) is located near the Town of Aidarkyen, in Batken Prefecture of Osh Province, along the northern edge of the Pamiro-Alay range in the southern Ferghana Basin in Kyrgyzstan (Fig. 1). The area has an arid, montane climate with cold winters and hot summers, situated at an elevation of roughly 1,700 m above sea level. The site itself consists of a small rockshelter, originally identified by U.I. Islamov in 1965 and excavated throughout the 1960s and 1970s[38]. These initial excavations explored an area of 141 m^2. Renewed excavations began at the site in 2015, revealing a stratigraphic sequence more than 4 m in depth, with five primary layers (Fig. 2). The sedimentary sequence was formed largely through colluvial rockfall and scree accumulation from Palaeozoic limestone and shale, which form the steep hillside. The lowermost layer, layer 5, consists of an aeolian loess-like deposit,

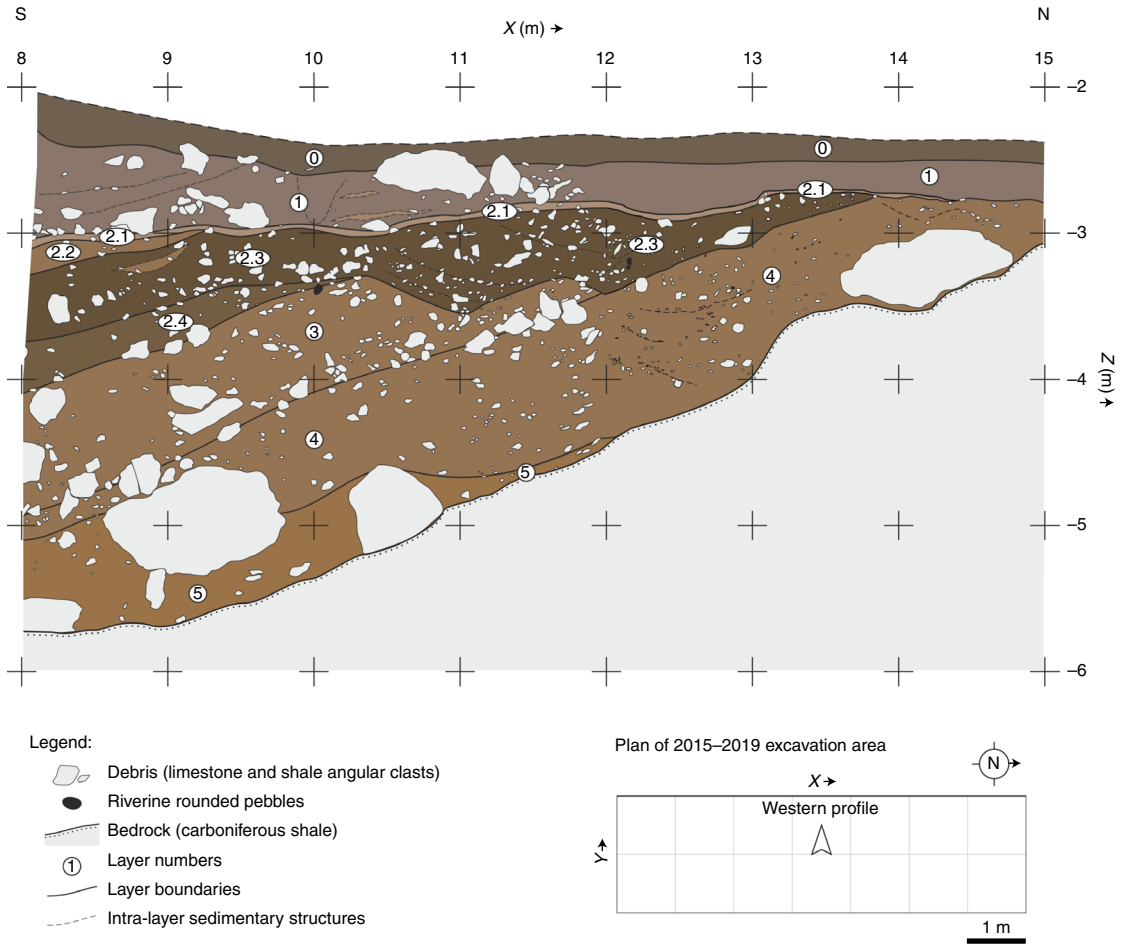

Fig. 2 | Stratigraphic profile of Obishir V. The key layers are layer 0 (modern topsoil), layer 1 (Bronze through Middle Ages), layer 2 (early Holocene strata, including occupation surfaces and palaeosol), layers 3 and 4 (underlying colluvium containing contemporaneous and earlier cultural material) and layer 5 (a sterile final late Pleistocene/initial early Holocene loess deposit).

Table 1 | Bayesian radiocarbon model (uniform prior) for Obishir V, by stratigraphic series, produced in OxCal using the INTCAL20 calibration curve[65]

Boundary	Modelled date (1σ)	Modelled date (2σ)
Layer 1 end	447–1372 cal CE	424 cal CE–present
Layer 1 start	1,783–939 cal BCE	2,499–902 cal BCE
Layer 2–3 end	2,897–2,342 cal BCE	2,914–1,624 cal BCE
Layer 2–3 start	7,349–6,876 cal BCE	7,781–6,817 cal BCE
Layer 4 end	8,129–7,555 cal BCE	8,160–7,161 cal BCE
Layer 4 start	8,608–7,875 cal BCE	9,827–7,819 cal BCE
Layer 5 end	10,124–8,276 cal BCE	11,581–7,993 cal BCE
Layer 5 start	11,958–8,905 cal BCE	14,200–8,300 cal BCE

For radiocarbon dates and model distributions, see Supplementary Information.

which passes upward into layer 4 with a weakly developed palaeosol. Layer 5 was dated to approximately 10,000 BCE using thermoluminescence (Table 1 and Supplementary Information). Layers 2, 3 and 4 contain cultural deposits formed through complex sedimentary processes, involving some downslope relocation. One radiocarbon date on unidentified animal bone suggests possible cultural activity in layer 4 beginning as early as ca. 8,198–7,820 cal BCE. Layer 2 is a dark organic palaeosol and occupation surface with cultural activity clustering around ca. 6,000 cal BCE, underlain by several metres of colluvium that contain roughly contemporaneous cultural material, including from layer 3 (Supplementary Information). Dates from animal bones and charcoal recovered from layer 1 suggest that this layer formed much later and dates from the late Bronze Age through the Early Middle Ages (Supplementary Information).

Comparing the lithic assemblage of Obishir V with earlier materials from Central Asia reveals an important economic transition characterized by a decline in the use of projectiles and a concomitant increased use of microblades for carcass processing, as well as an increased emphasis on grinding stones for food production. This lithic transition is reflected across much of mid-Holocene Central Asia[39]. Stone tools from layers 2–4 reveal a lithic industry based on pressure knapping of microblades, with important similarities to the Hissar culture, including a technological emphasis on retouched bladelets, trapezoids, end-scrapers and grinding stones, and evidence of processing of wild or domestic grain[33]. In addition, as seen in the Kelteminar culture[23], the Obishirian industry also utilized pressure knapping from prismatic and *cis*-prismatic cores, but only yielded a handful of bullet cores and lacked other diagnostic features of the Kelteminar stone tool assemblage, such as scalene triangles, sickle blades and 'Kelteminar points'. Finally, excavations at Obishir V recovered ornamental stone pendants and grinding stones, perhaps associated with food production, that have close parallels in both Hissar and Kelteminar cultures[31,40].

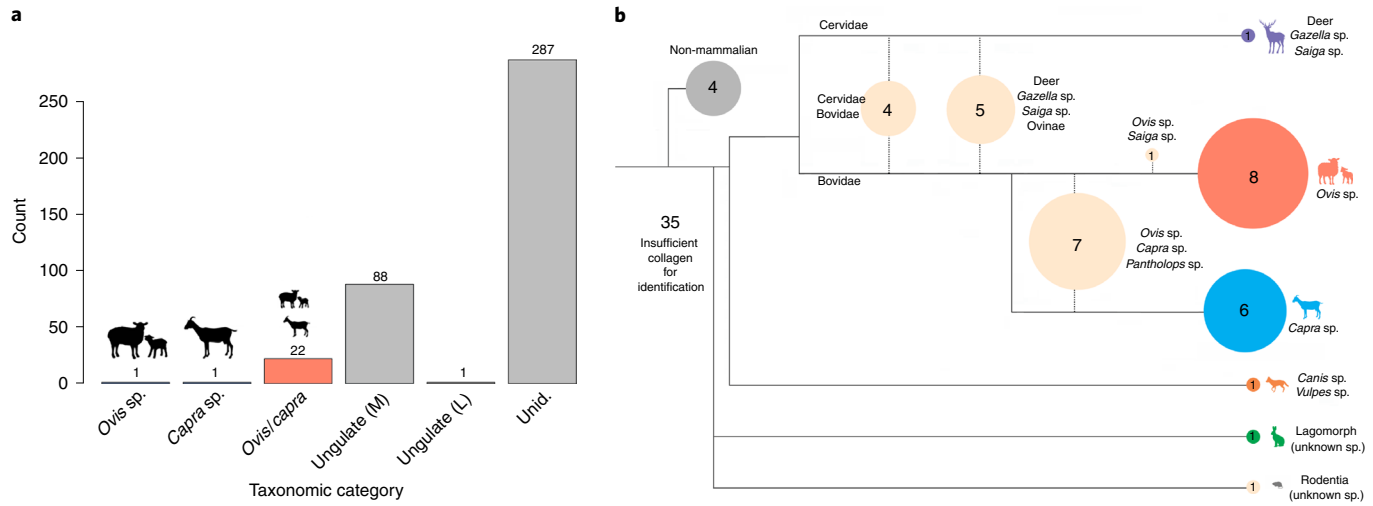

Fig. 3 | Identification of animal remains at Obishir V by ZooMS. a, Morphology-based identifications of animal remains from layers 2–4 at Obishir V, by number of identified specimens (NISP) and taxonomic category. **b**, Collagen peptide-based identifications for layers 2–4, square R8 at Obishir V, grouped by general phylogenetic relationships. Each colored circle represents a different identified taxon. Specimens with a grey circle are missing necessary peptide markers for more detailed identification, and silhouettes depict a range of possible taxa from which specimens in that category may derive. Icons created or modified by Hans Sell. Unid., unidentified.

In addition to lithics, Obishir V also yielded a small archaeofaunal assemblage, which has allowed an investigation of subsistence changes underlying the technological shift towards microblades. Although the faunal material (Supplementary Data A) is highly fragmented, layers 2–4 contained a total of 400 animal bone fragments. Of these, 24 could be morphologically identified as *Ovis* ($n = 1$), *Capra* ($n = 1$) or *Ovis/Capra* ($n = 22$). An additional 89 were identified as ungulate (88 medium-sized ungulates and 1 large ungulate), and 287 fragments were not identifiable to a taxonomic class (Fig. 3a). While the assemblage displayed many specimens with burn marks ($n = 36$), some with cut marks ($n = 5$) and others with spiral fracture ($n = 3$), only a small number of these anthropogenically modified specimens were taxonomically identifiable (*Ovis*, $n = 1$; ovicaprid, $n = 1$). A single human tooth was identified in the assemblage (a deciduous incisor shed from a child of approximately 6 years of age); however, this specimen was not recovered in stratigraphic context but was found during screening of sediments at the contact between layer 1 and 2. Radiocarbon date estimates for this tooth suggest that it belongs to the late Holocene cultural level 1 (Supplementary Information). Despite the small sample size and heavy fragmentation of the skeletal assemblage, the archaeofaunal material suggests that early Holocene animal economies at Obishir incorporated meaningful exploitation of sheep and goat. We next applied biomolecular methods to test whether these animals represent wild or domestic individuals, and to assess their antiquity directly.

Archaeofaunal remains. Zooarchaeology by mass spectrometry (ZooMS) analysis[41] of 74 archaeofaunal skeletal fragments from square R8, layers 2–4 (Supplementary Data B) confirms the presence of *Ovis* and *Capra* in the early Holocene layers at Obishir V, and among identifiable specimens suggests a nearly complete economic emphasis on these two taxa. Although nearly half of the assemblage from square R8 no longer had identifiable collagen ($n = 35$), the largest number of identifiable specimens were assigned as sheep ($n = 8$) or goat ($n = 6$), or ovicaprid lacking the necessary peptide marker to distinguish between sheep and goat ($n = 7$; Fig. 3b). We also identified one deciduous tooth belonging to a canine (*Canis*, likely a domestic dog or fox), one rodent bone, one lagomorph bone and one bone broadly assigned to deer/*Saiga*/gazelle. On the basis of observed peptide markers, four specimens appeared non-mammalian, four were assigned only to Cervidae/Bovidae and six additional specimens were consistent with either sheep or deer/*Saiga*/gazelle. Comparing the faunal assemblages of the Neolithic layers 2–4 at Obishir V with an Early Bronze Age assemblage from the nearby Alay Valley[21] shows that an emphasis on *Ovis*, augmented by *Capra*, is characteristic of local Neolithic/Bronze Age pastoral subsistence strategies. Later, during the first millennium BCE and first millennium CE, the Obishir V fauna also includes additional domesticated taxa, such as *Bos* sp. and *Equus* sp. (identified through DNA analysis as a male domesticated donkey, *E. asinus*; Supplementary Information). Dietary focus on domestic sheep, supplemented by goat and other taxa, characterizes modern herding lifeways in the region[42], and may reflect cultural preferences or herd compositions optimized to the dry montane environment.

Cementum analysis and dental eruption/wear. Cementum analysis of intact tooth specimens can provide insights into the age structure and management of animal herds, including the season during which animals are culled. Cementum is a connective calcified tissue that is deposited on the outer surface of the dental root, linking it with the fibres of the periodontal ligament. Consisting of a collagen matrix within which hydroxyapatite crystals form, its growth is continuous throughout an animal's life[43–45] and follows an annual cycle that typically produces light-coloured bands of low-mineral-density growth during the late spring to fall followed by dark banding of high mineral density during the non-growth, winter season[46–48]. On the basis of these patterns, the season of death for the animal can be established by identifying the growth status of the final mineral deposit[44,49–51].

Four faunal teeth (OB20-01, OB20-06, OB20-07 and OB20-09) were sufficiently intact to allow cementum analysis, and it was possible to identify at least one region of interest (ROI) per specimen based on annulation patterns (Supplementary Information). The four teeth likely represent four distinct individuals: three *Ovis* (as indicated by ZooMS and ancient DNA (aDNA)), and one *Ovis/Capra* (identified only as sheep/cervid by ZooMS from poor collagen preservation, but *Ovis/Capra* based on morphology). Across these four specimens, the cementum was globally well preserved. Limited recrystallizations were identified, but not concerning the

Table 2 | Osteological information and wear, age (cementum) and sex (DNA) estimates for teeth recovered from early Holocene cultural layers at Obishir V, along with taxonomic identifications from peptide fingerprinting

Layer	ID number	Species ID (ZooMS)	DNA sex/ species	Element	Side	Wear stage (Payne 1973)	Age (cementum)	Estimated season of death (cementum)	Notes	Direct radiocarbon date
1–2 contact	DA-OBI-1518-20.4	*Ovis*	*O.aries*, male	Lower incisor (permanent)	R	—				4,260 ± 36
2	DA-OBI-1518-20.7	*Ovis*	—	Lower M1 (permanent)	R	C-I	5 years	Late cementogenenesis (late fall?)		
2	DA-OBI-1518-20.6	*Ovis*	*O.aries*, female	Lower I3 (deciduous)	L	—	2 years	Late cementogenenesis (late fall?)		7,012 ± 45
2	DA-OBI-1518-20.5	*Ovis*	—	Lower P4 (deciduous)	L	B-C			Deciduous (<18 months)	
2.1	DA-OBI-1518-20.9	Deer/*Saiga*/ sheep/gazelle	—	Upper M1 (permanent)	R	B	1 year	Late cementogenenesis (late fall?)		
2.2	DA-OBI-1518-20.1	*Ovis*/*Capra*	*O.aries*, female	Upper M3 (permanent)	L	G-I	5 years	Late cementogenenesis (late fall?)	Hyper-cementosis advanced wear	6,989 ± 45
2.2	DA-OBI-1518-20.2	Deer/*Saiga*/ sheep/gazelle	—	Upper P2 (permanent)	R	—				
2.3	DA-OBI-1518-20.8	*Capra*	—	Upper P3 (permanent)	R	—				

Grey shading indicates DNA-based identification as domestic *O. aries*.

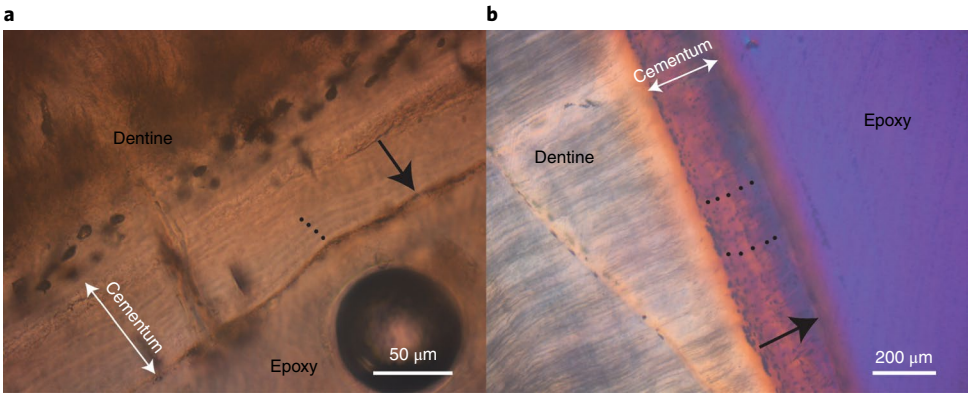

Fig. 4 | Cementum analysis of sheep and goat remains from Obishir V. a, OB20-01. A thick deposit of mixed cementum is followed by incremental acellular cementum with extrinsic fibrils. Within it, four pairs of growth zones + annuli can be seen. The last increment is a complete growth zone, marking the end of the seasonal record. The cementum is well preserved and is not affected by post mortem modification in the ROI. **b**, OB20-07. Annuli are underlain by a line of cementocytes, showing five complete growth zones (the last one constituting the outermost increment). Observations were conducted in cellular cementum because no extrinsic fibrils of acellular cementum were present on the tooth. For both teeth, observations were conducted under polarized light with the insertion of a lambda plate under high magnification (500×). Dots show the location of annuli (winter bands); black arrow indicates the direction of cementum growth.

outermost deposits, and no microbial damage was identified. Localized weathering influenced the cementum exterior on two specimens, but in both cases at least one tooth root was sufficiently preserved to allow reliable observation. Two teeth exhibited evidence of an older age-at-death (4 and 5 years, respectively), while the remaining two belonged to juvenile animals (1 and 2 years; Table 2). For all four teeth, the final increment of growth was nearly equivalent in width to the previous increment, suggesting the animals were at the end of their growth phase (fall or early winter) at the time of death (Fig. 4). Late-fall livestock culling patterns are a typical feature of pastoralist herd management[52].

Animal DNA. Five tooth fragments from Obishir V, square R8, layer 2 were selected for genetic analysis, on the basis of preservation and identification as *Ovis* ($n = 4$) or *Capra* ($n = 1$) through ZooMS (Table 3). Ancient DNA was successfully extracted from all five fragments, built into Illumina libraries and sequenced using a shotgun strategy. We observed high variability in endogenous DNA preservation across the fragments, ranging from 0.2% to 8.3%, and all five specimens exhibited patterns of post mortem DNA damage consistent with ancient DNA (Table 3 and Supplementary Information). We next compared genome-wide sequences of the Obishir V specimens with published genomes of modern

Table 3 | Whole mitogenome DNA results for specimens analysed in this study

Specimen ID	Species	Reads sequenced	Endogenous DNA	No. of genome-wide SNPs	Damage					Sex	No. of mt reads	mt genome coverage	mt haplogroup
					First base 3′	Second base 3′	First base 5′	Second base 5′	Avg frag. length				
OB20-01	*O. aries*	18,506,454	8.32%	494,366	0.1242	0.0372	0.1628	0.0309	59.7	XX	718	1.9×	A
OB20-06	*O. aries*	17,858,515	6.25%	316,536	0.0797	0.0291	0.0787	0.0157	55.86	XX	6,846	13.6×	A
OB21-06	*O. aries*	25,946,522	0.23%	16,784	0.0758	0.0372	0.0688	0.0165	54.49	XX	399	0.69×	n.d.
OB20-04	*O. aries*	23,830,365	7.61%	473,283	0.1621	0.0352	0.1683	0.0212	48.97	XY	1589	3.6×	A
OB21-04	*Capra sp.*	27,141,767	0.27%	19,313	0.0975	0.0419	0.1155	0.0316	59.35	XY	37	0.3×	n.d.

Notes n.d., not determined Genome-wide SNPs are those from the ISGC SNP chip. HVRI and MT-CYTB numbering for sheep is relative to the sheep reference genome NC001941; numbering for goat is relative to the goat reference genome KR059154.

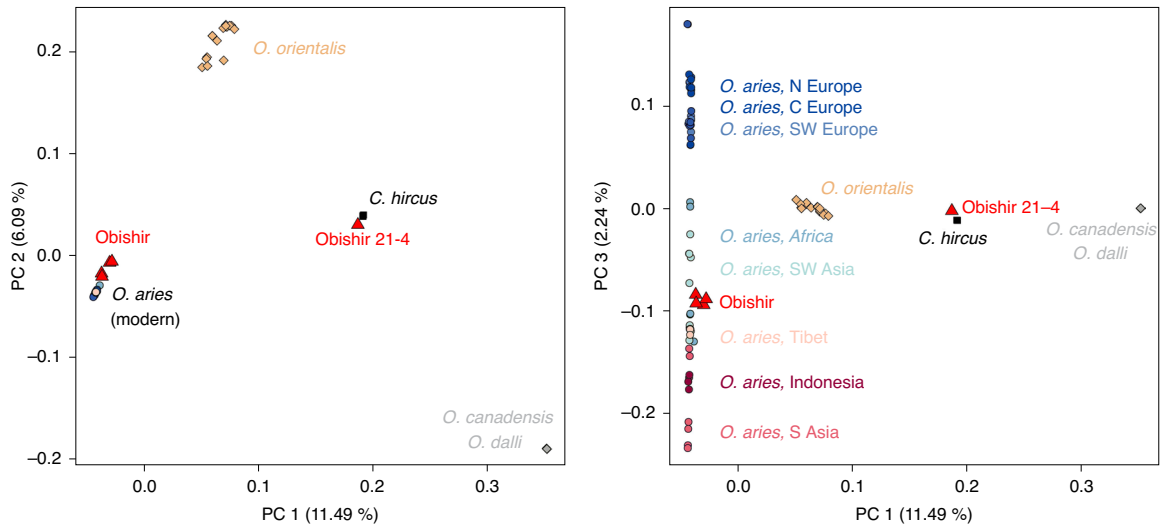

Fig. 5 | PCA results of Obishir V sheep and goat samples. a,**b** The results for principal component (PC) 1 versus PC 2 (**a**) and PC 3 (**b**), projected onto a reference database of wild sheep taxa as well as domestic sheep and goat specimens from across Eurasia and Africa.

domestic sheep and goats from Eurasia and Africa, as well as wild Asiatic mouflon using principal component analysis (PCA). Four of the Obishir V specimens (OB20-01, OB20-06, OB21-06 and OB20-04) clustered together with present-day domesticated sheep breeds (*O. aries*) while being clearly separated from wild sheep species (Fig. 5). Based on the autosomal-to-sex chromosome ratio in the samples, three sheep (OB20-01, OB20-06 and OB21-06) were identified as female (Table 3 and Supplementary Information) and one sheep (OB20-04) was identified as male. Specimens OB20-01 and OB20-06 were directly dated through ultrafiltration to 6,989 ± 45 and 7,012 ± 45 ^{14}C years BP, or between 5,983 and 5,751, and 5,991 and 5,771 cal BCE, respectively (2σ calibrated range). Both specimens showed good measures of quality control (fraction of modern carbon 0.4189 ± 0.00231 (1σ) for OB20-1 and 0.4178 ± 0.0023 (1σ) for OB20-06). No material remained for direct dating of the third *Ovis* specimen with poor coverage (OB21-06), while specimen OB20-04 was dated to the Early Bronze Age (ca. 3,003–2,701 cal BCE, 2σ). The single *Capra* tooth (OB21-04), recovered from the contact between layers 1 and 2, was revealed to be Late Bronze Age in origin (Supplementary Information).

In PCA space, the Obishir V sheep genomes fall close to modern southwest and Central Asian sheep breeds (Fig. 5). In addition, the Obishir V sheep carry hypervariable region I (HVRI) polymorphisms that are shared with domestic sheep breeds but that are not found in modern wild sheep (Supplementary Information). For three of the four Obishir V sheep (OB20-01, OB20-04 and OB20-06), we obtained sufficient mitogenome coverage (1.9–13.6x) to perform phylogenetic analysis comparing the Obishir V samples with 160 modern mitogenomes (Supplementary Data C) of *O. aries* (domestic sheep), *O. ammon* (wild argali), *O. vignei* (wild urial), *O. orientalis ophion* (wild Cyprus mouflon) and *O. aries musimon* (wild European mouflon). We found that Neolithic Obishir V sheep, as well as the single Early Bronze Age specimen, all clustered within *O. aries* haplogroup A, a major mitochondrial DNA subclade of domestic sheep (Fig. 6 and Table 3).

Discussion

Our identification of *O. aries* belonging to mitochondrial haplogroup A at Obishir V ca. 6,000 BCE has critical implications for our understanding of transcontinental connections and domestic animal dispersals. Although our assemblage is small and badly fragmented, ZooMS analysis enabled us to confirm inferences based on morphological comparisons that the Obishir V economy included both sheep and goat (*Ovis* and *Capra* sp.). Wild *Ovis* and *Capra* in this region include the large argali sheep (*O. ammon*) and Siberian ibex (*C. sibirica*), but a ratio of roughly 3:1 between *Ovis* and *Capra* has characterized pastoralist archaeological assemblages in Central and Inner Asia since the Bronze Age[22,52], and is also typical of other early western Eurasian pastoral assemblages[53]. Based on the ZooMS results, *Ovis* and *Capra* appear to account for the majority of the unidentifiable fragments in the layer 2 faunal assemblage.

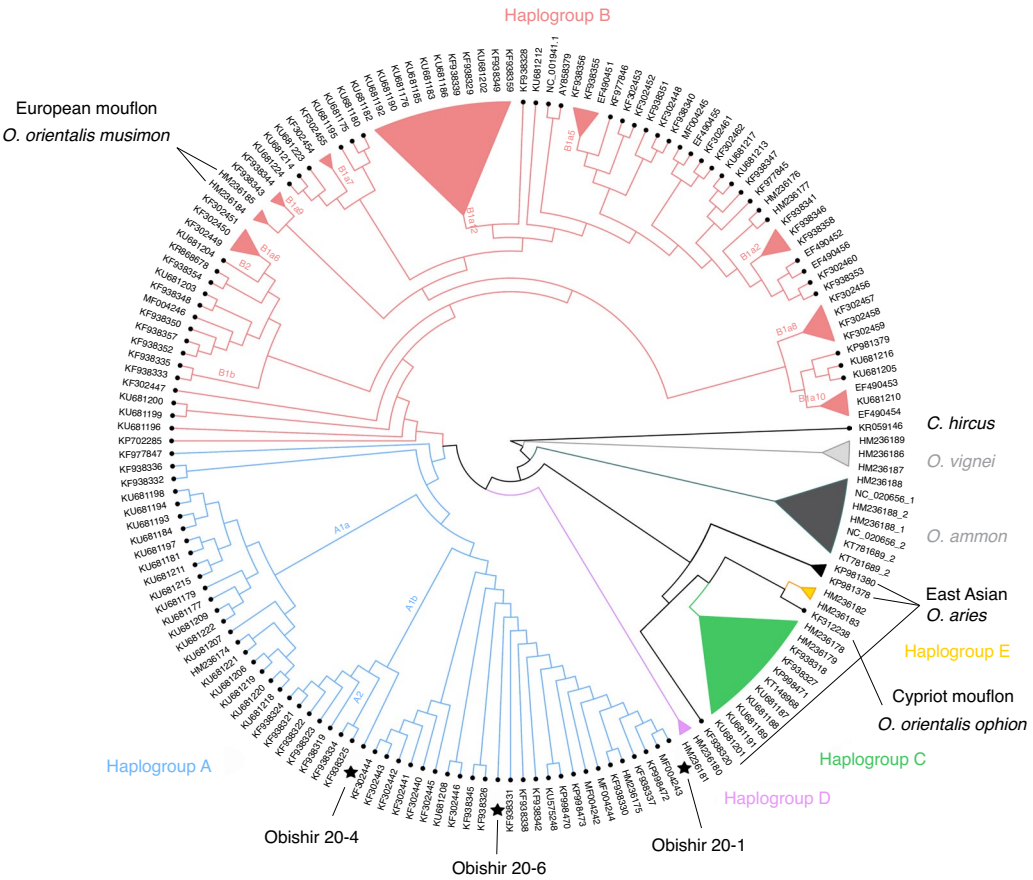

Fig. 6 | Phylogenetic tree produced using maximum parsimony. The tress shows the whole mitochondrial genome relationships of the Obishir V specimens (stars) versus modern reference genomes of the domestic sheep *O. aries* (haplogroups A–E), the European mouflon (*O. aries musimon*), the Cypriot mouflon (*O. orientalis ophion*), the wild urial (*O. vignei*) and the wild argali (*O. ammon*), with *C. hircus* as an outgroup. Major haplogroups A–E are labelled following previous published studies[91]. For full details on tree generation, see Supplementary Information.

Further genetic analysis suggests that the Obishir V includes domestic animals. Mitochondrial DNA data recovered from sheep teeth confirms that these specimens fall within the present-day genetic diversity of domestic *O. aries*, and at least three (two Neolithic and one Early Bronze Age specimen) are members of haplogroup A. This haplogroup has been hypothesized to be linked with the earliest domestication of sheep that widely dispersed throughout Eurasia, as it is the only haplogroup found among the local sheep populations of North Africa, Britain and Northern Europe[54,55]. Significantly, haplogroup A is also found at high frequency in early domestic sheep populations in East Asia[56]. Information available for domesticated sheep suggests that domesticated sheep are monophyletic with five distinct haplogroups (haplogroups A–E)[57,58], with the sole exception of the Cypriot mouflon (*O. orientalis ophion*) and the European mouflon (*O. aries musimon*). These subspecies are often considered to be relict populations of early domesticated European sheep. The identification of Obishir V specimens as members of haplogroup A does not rule out the possibility that this haplogroup was present in a previously undocumented wild species of *Ovis* exploited by ancient Central Asian hunters. However, the fact that all Obishir V specimens with complete mitochondrial genomes are nested *within* the range of established diversity in domesticated sheep but do not form a basal lineage or cluster with other wild sheep taxa (Fig. 6) casts doubt on this possibility. Moreover, our PCA comparison of Obishir V sheep places them between modern South Asian, East Asian and European populations (Fig. 5).

Based on their co-occurence with *O. aries*, the *Capra* sp. specimens recovered at Obishir V may also represent domestic animals. However, no haplogroup assignment could be made for the *Capra* specimen due to the poor preservation of the recovered DNA, and the only directly dated *Capra* specimen, recovered from the contact between layers 1 and 2, was dated to the early first millennium BCE. As a result, future research is necessary to assess whether the *Capra* specimens at Obishir V are indeed reliably associated with the early occupation layer and how they relate to known ancient populations of wild and domestic animals.

Careful excavation and direct radiocarbon dating of analysed specimens (including two teeth identified through aDNA as *O. aries*: specimens OB20-01 and OB20-06) confirms the deep antiquity of the Obishir V sheep specimens. Radiocarbon dating of faunal remains at Obishir V provides a robust chronology, dating the initial occupation of layers 2 and 3 to before 6,000 BCE. Bayesian model estimates for the onset of occupation in this layer range between 7,800 and 6,800 cal BCE at the 2σ range (Table 1). The earliest radiocarbon date in the early Holocene cultural layer comes from unidentified wood charcoal (Obishir V, R-11, depth 352, layer 2), which may be influenced by old wood effect and may not reliably date cultural activity at the site. During the early formation of this layer, geogenic material and archaeological material may have been re-deposited and mixed together over a period of two or three millennia. Thus, while human activity at the site might have begun as early as 9,800–7,800 cal BCE with unidentified faunal materials in layer 4, specimens identified as *Ovis* and *Capra* (as well as those of

similar size class but identified only to lower-resolution taxonomic categories) produced radiocarbon dates clustering around ca. 6,000 BCE (Supplementary Information). Sample sizes are too small to demonstrate clear patterns of management by humans, but age and seasonality data from the Obishir V teeth provide important context supporting the genetic findings, and strengthen the interpretation of Obishir V sheep as domestic animals. Incremental cementum banding on four intact teeth from Obishir V shows that two animals died between 1 and 2 years of age, a pattern consistent with early pastoral culling practices across southwest Asia after 7,500 BCE and probably representing a focus on meat or dairy rather than wool[59]. The slaughter of mature females (older than 2 years) that have failed to reproduce or are small in body size is another hallmark of pastoral management of a breeding herd[12,59,60]. Based on cementum analysis, the female *O. aries* specimens identified through DNA sequencing were 2+ years of age and 5+ years of age (Table 2). Another deciduous premolar (<18 monoths) and several unfused metapodial shafts reinforce the impression of the regular slaughter of juvenile individuals at Obishir V. Analysis of banding patterns indicates seasonal slaughter of Obishir V sheep and goat in the fall or early winter in all analysed teeth. Among contemporary pastoral sheep and goat dietary economies, it is common to cull animals that are unlikely to survive the winter in late autumn, so as to improve winter herd survival and store meat more easily over winter months[61]. The consistency in the apparent seasonality of death among all four teeth inspected for cementum analysis implies either: (A) seasonal occupation of Obishir V during the fall months paired with hunting of juveniles and mature females or (B) regular late-season slaughter of domestic animals.

Although our previous research[21] demonstrated that pastoral economies had been present in the region since the early Bronze Age, until now, no direct evidence had been found for domestic animals in montane Central Asia during the early Holocene. Our findings indicate that the subsistence behaviours that underpinned these nascent interaction networks could date from at least three millennia earlier than previously demonstrated. Assuming a single centre of domestication in the Near East, the evidence from Obishir V would appear to directly demonstrate the movement of domestic animals deep into the Eurasian interior, either through human movements or cultural exchange with western Eurasia. The timing of probable domesticated animal use at Obishir V chronologically aligns with the first dispersal of domesticated bovids into Mediterranean North Africa[15] and the arrival of domestic sheep and goat in the Kopet Dag of southern Turkmenistan[34]. Therefore, Obishir V may form an extended outward branch of animals from southwest Asia by before ca. 6,000 BCE (Fig. 1). In comparison with the Iranian Plateau and the Levant, the relatively more challenging ecological setting of montane Central Asia (with its high altitude, hard frosts and cold winters) implies that such conditions were not an impermeable ecological barrier for the dispersal of domestic animals during the mid-Holocene, and raise the possibility that similar assemblages may exist across other areas of Central Asia.

The presence of probable domestic sheep in the Ferghana Basin by the seventh millennium BCE may warrant reconsideration of the timing of other components of the 'Neolithic package', including goat, cattle and crops such as wheat and barley, in the Inner Asian Mountain Corridor. Our identification of *O. aries* dated to the early Bronze Age (OB20-04, dating to ca. 3,003–2,701 cal BCE at the 2σ level) from layer 2, along with the faunal remains from layer 1 (Supplementary Data A, B) point to continuous use of domestic animals in the Ferghana region from their Neolithic introduction throughout the Middle Ages. In later history and prehistory, the southern Ferghana region became linked to northwest China through networks of pastoral mobility that drove exchange across the interior[62]. Our research shows that such networks could date from at least three millennia earlier than previously demonstrated.

Based on the material culture links between Obishir V and other important Neolithic horizons (such as Hissar and Kelteminar), as well as the paucity of scientific study on highly fragmented early faunal assemblages from the Pamiro-Alay and Tian Shan region, we suggest that Obishir V may form part of a much broader phenomenon linked with early pastoralist or agropastoralist economies (Fig. 1). If so, the region may have been a key node in an early dispersal system for southwest Asian domesticated bovids and, possibly, plants into other areas of East and South Asia. No archaeobotanical data are yet available for Obishir V. Future investigation of this and other mid-Holocene sites in Central Asia should assess the possibility that domestic plants were also dispersed into the region during the mid-Holocene, and clarify whether this newly identified Neolithic expansion was demic (accompanied by human dispersals, as in much of Eastern Europe) or took place through transfer of organisms and technologies, as in much of non-Mediterranean Africa[63] to better understand the cultural and ecological dynamics of the Neolithic transition across the ancient world. Until now, archaeological data have pointed to a relatively late arrival of the agropastoral economy to interior Central Asia, around 3,500 BCE. Results from Obishir V suggest that the chronology of Neolithization in this region is in need of revision. Archaeological investigation of the early Holocene deposits at the rockshelter of Obishir V have revealed major changes in material culture, along with fragmented faunal material potentially linked to an early pastoral economy. Using a combination of collagen fingerprinting, ancient DNA and thin-section cementum analysis, we propose an early dispersal of domestic sheep into the Ferghana Valley by at least 6,000 BCE, concurrent with other out-migrations from Western Asia into the Old World. These results support Soviet scholarship hypothesizing a Neolithic dispersal of food-producing economies into Central Asia through connections with Western Asia, and raise the possibility that domesticated sheep, perhaps in association with other early domesticated plants and animals, could have dispersed into the continental interior millennia earlier than previously recognized.

Methods

Excavation. Excavations at the Obishir V site were carried out between 2015 and 2019 by a joint Russian–Kyrgyzstani archaeological expedition. In 2015–2016, researchers working at the site excavated an area of 8 m^2 adjoining the 1968–1969 excavation area (Supplementary Information). Each artefact (lithic and faunal remains) greater than 1 cm in size was individually piece-plotted with 1 mm accuracy using a Leica Total Station TS02 plus in tandem with the Trimble software EDM Mobile, and documented along with contextual information (orientation, dip, inclination, find category, stratigraphic level, etc.). Fragments smaller than this were collected in bulk for each 0.25 m^2 area. Features (natural or anthropogenic) were recorded with the total station using outlines, surface points and breaklines. Large surfaces, such as walls of old excavation pits, sections and archaeological horizons with numerous artefacts, were also documented using photogrammetry and three-dimensional (3D) scanning. Digital records were supplemented by field drawings and notes. All sediment obtained during the excavation was wet-screened with mesh of size of 2.0 mm, and finds were documented according to excavated square, layer and depth. Stratigraphic units were defined during the excavation based on grain size composition, consistency, colour, presence of erosional surfaces and accumulation of limestone clasts.

Radiocarbon dating. Nine radiocarbon samples of identified and unidentified animal bone and tooth ($n = 7$) and charcoal ($n = 2$) were selected from various depths across the stratigraphic unit of layers 2–4 and submitted for radiocarbon dating at the Oxford Radiocarbon Accelerator Laboratory, the Center for Isotope Research at the University of Groningen, the Golden Valley Laboratory at Novosibirsk and the University of Arizona AMS Laboratory. Portions of domestic sheep teeth OB20-01, OB20-06 and OB20-04 from layer 2 used in genomic analysis were submitted to the University of Arizona Accelerator Mass Spectrometry Laboratory. Collagen was obtained from dentin using acid–base–acid (ABA) pretreatment, gelatinization, 0.45 micron filtration and ultrafiltration. The quality control parameters, collagen yield of 7.1%, 6.4% and 7.2%, carbon yield of 42.9%, 36% and 36% and $d^{13}C$ values of −19.7, −19.3 and −19.6 ± 0.1 per mil, indicated good preservation[64]. The atomic C:N ratio for OB20-01 (3.2) and OB20-04 (3.2) also fell within ranges indicating good preservation, although no material remained from tooth OB20-06 for C:N measurement after ^{14}C analysis. All dates were calibrated in OxCal using the INTCAL20 calibration curve[65].

We performed Bayesian stratigraphic phase modelling (using ordered phases and combining layers 2 and 3 due to their apparent mixing) in the software OxCal with a uniform prior using all available radiocarbon dates from Obishir V, and two thermoluminescence dates from layer 5, following methods outlined by Ramsey[66]. Because charcoal dates, particularly those from layer 2, suggest an old wood effect that produced unsuccessful models in OxCal, only radiocarbon dates from bone and teeth were included in the final chronology. The models provided good agreement, and repeating the analysis using a general outlier model did not significantly alter estimated parameters. The OxCal code used in the analysis, along with the human tooth dating methods, are outlined in Supplementary Information.

Thermoluminescence dating. During the 2017 field season, two sediment samples were collected from the southern portion of the western excavation profile of layer 5 at a depth of 295 and 335 cm below the surface. We measured the deposit moisture in each sample and, after drying, determined the dose rate (DR) using a MAZAR gamma spectrometer. Concentrations of ^{226}Ra, ^{228}Th and ^{40}K in each sample were obtained from 20 measurements lasting 2,000 s each. We established equivalent dose (ED) on the 63–80 mm polymineral fraction, after 10% HCl and 30% H_2O_2 washing and ultraviolet optical bleaching. The samples were irradiated with 20, 30, 40, 50 and 100 Gy doses from ^{60}Co gamma source. Before measurement, we heated the samples at 140 °C for 3 h. A sample pre-treated in this way was used to determine the ED by the thermoluminescence multiple-aliquot regenerative technique[67], according to the description published by Fedorowicz et al.[68]. Curve registration was performed on a RA'94 (Mikrolab) thermoluminescence reader coupled with an EMI 9789 QA photomultiplier. Finally, we calculated thermoluminescence age according to Frechen[69]. A detailed description of the preparation and the equipment used in the study is provided in Fedorowicz et al.[68].

Zooarchaeology and ZooMS. For each specimen, we conducted taphonomic analysis and morphology-based species identification using comparative faunal collections held by the Russian Academy of Sciences-Siberia in their field station at Aidarkyen, Kyrgyzstan, and attempted refits for all specimens to control for issues of fragmentation. We recorded taphonomic indicators such as rodent and carnivore damage, root etching, and weathering[70], along with evidence for anthropogenic modification such as spiral fracturing, cut marks and burning via visual inspection (Supplementary Data A). In addition, we used collagen fingerprinting (ZooMS) to taxonomically identify a subset of this material, all derived from 2017 excavations. We analysed all bone specimens from square R8, layers 2.2, 3 and 4, as well as all bone specimens from square R8, layer 1.3 using ZooMS. To do so, we demineralized 10 mg of bone using 50 mM ammonium bicarbonate buffer (Sigma-Aldrich) produced in UHQ water and pH adjusted to 8.0 using ammonium hydroxide (1 M), following the protocol outlined by van Doorn et al.[71]. The extracted collagen was digested into component peptides using trypsin (Pierce), and was purified using Pierce C18 tips (Thermo Scientific), eluting in 50% acetonitrile (Sigma-Aldrich) and 0.1% trifluoroacetic acid (Sigma-Aldrich). Samples were then spotted on Bruker AnchorChip with Bruker Peptide Calibration Standard in calibration spots directly neighbouring the samples. Mass spectrometric analysis was conducted using a Bruker Autoflex Speed LRF matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) device in the dedicated ZooMS Laboratory at the Max Planck Institute for the Science of Human History, Jena, Germany. The acquisition used the following parameters: 4,000 laser shots at 50–60% intensity (50 shots per spot), mass range 600–3,500 Da and reflector mode. Identifications were made using published reference spectra from the Eurasian mammals database[72] and are reported according to the level of taxonomic specificity. In some cases (for example, *Ovis* versus muskox and chamois), species that were not necessarily separable on the basis of observed peptide markers were inferred on the basis of known habitat distribution. All peptide marker data are provided in Supplementary Data B.

Cementum analysis. First, all analysed teeth were 3D scanned using a MicroCT scanner, and washed in 90% ethanol solution. A cast of the occlusal surface was retained for later micro-wear analyses. Thin sections were prepared following recommendations by Rendu[73]. Teeth were embedded in epoxy within a vacuum chamber (at 0.2 bar pressure). They were then cut at thickness of 250 µm using an Isomet5000 automatic saw, and ground to thickness of 100 µm with a PressiCube grinding machine. For each tooth, four independent thin sections were prepared and analysed independently. Observations were conducted under polarized light both with and without the insertion of a lambda plate[74], using a Leica 2500P microscope equipped with a Leica DC 120 camera. Post-depositional modifications were analysed systematically following Geusa et al.[75] and Stutz[74]. Measurements of the increments were conducted using ImageJ software following methods outlined by Lieberman et al.[76].

DNA analysis. *Extraction.* DNA was extracted from six teeth using silica-based purification in a dedicated laboratory for aDNA at PACEA (UMR 5199, University of Bordeaux). We incubated 100–250 mg of bone powder in 1 mL extraction buffer (0.5 M EDTA, 0.25 M Na_2HPO_4, 50 µg mL^{-1} proteinase K) for 48–72 h at 37 °C. DNA was purified from the collected supernatant using a method adapted from the QIAquick MinElute purification kit protocol (Qiagen).

PCR amplification. Purified DNA was amplified by quantitative polymerase chain reaction (qPCR) using a LightCycler 96 Instrument (Roche Life Science). Primer pairs were designed to amplify two regions of the mitochondrial DNA, a 130 bp region from cytB and 170 bp from the hypervariable region (see Supplementary Information for a list of the primers used and product sizes). We obtained each PCR product at least twice, conducting standard negative control to ensure the authenticity of the results. We used 1 µL of extract in 10 µL reaction with 1× FastStart Essential DNA Green Master and 1 µM of each primer. Products were sequenced by capillary electrophoresis at Genewiz (Leipzig, Germany). Sequences were visually inspected and manually curated, assembled and aligned using Geneious software suite v9.1.8 (https://www.geneious.com).

Library construction and sequencing. We used DNA extracts to build double-stranded Illumina libraries following the protocol described by Gorge et al.[77]. We sequenced whole-genome shotgun libraries for ~500,000 reads on an Illumina NextSeq 500 at the Institut de Recherche Biomédicale des Armées in Brétigny-sur-Orge, France, to assess DNA preservation. DNA extracts of five samples produced in Bordeaux were sent to the dedicated cleanroom facilities of the Max Planck Institute for the Science of Human History. There, 20 µL of the extract was used to build double-stranded libraries with partial treatment of the uracil-DNA glycosylase enzyme (UDG-half)[78]. The resulting genetic libraries were double indexed and further amplified[79]. Finally, all five samples were shotgun sequenced for ~20 M reads on an Illumina HiSeq run with a 75-cycle single-end configuration. Sequence authenticity was estimated on libraries subjected to either partial or no USER treatment using MapDamage[80].

We trimmed adapter sequences from generated sequences, merging overlapping paired-end reads and filtering out reads shorter than 30 bases using Clip and Merge. We then mapped our filtered reads to both the *O. aries* genome assembly (International Sheep Genome Consortium build Oarv3.1) and the *C. hircus* genome assembly (ARS1) using BWA[81], and against the full mitochondrial genome of *O. aries* (NC_001941.1) with a duplication of its first 500 bases at the end to ensure mapping of the reads overlapping the junction resulting from the virtual linearization of the circular mitogenome.

PCA of genome-wide sequences. Genotypes were called for the 38.5M single-nucleotide polymorphisms (SNPs) from the International Sheep Genomics Consortium (ISGC) SNP project from 60 domesticated sheep along with three specimens of North American bighorn sheep (*O. canadensis*) and two Dall sheep (*O. dalli*). We drew a single allele at random for each position (minimum mapping and base quality of 30) using PileupCaller (https://github.com/stschiff/sequenceTools), rendering the individuals from the dataset homozygous for each locus. The two files were then merged using Plink v1.9[82]. To augment the ISGC SNP discovery panel, we added published genome data of 16 Asiatic mouflons (*O. orientalis*)[83]. For this, we downloaded FastQ files for the 16 individuals from the National Center for Biotechnology Information Sequence Read Archive under the accession number PRJNA24020 and aligned reads to the oviAri4 reference genome using BWA-mem v0.7.17[81]. We removed PCR duplicates using the MarkDuplicates module of the picardtools program v2.20.0 (https://broadinstitute.github.io/picard/). We then retained properly paired reads with mapping quality score 30 or higher using SAMtools v1.9[84]. For the SNPs from the ISGC SNP project, we calculated genotype likelihoods using GATK UnifiedGenotyper (v3.8.1.0) with '–genotype_likelihoods_model SNP–output_mode EMIT_ALL_SITES –allSitePLs' options[85]. Then, we used an in-house python script to calculate posterior genotype probability with a non-default prior [0.4995, 0.0010, 0.4995] to reduce reference bias, following the approach taken in the Simons Genome Diversity Project[86]. We took genotypes with posterior probability 0.9 or higher and kept the remaining ones as missing. Finally, we calculated sequencing coverage with Qualimap v2.2.1, assigning biological sex to each individual based on the X to autosome coverage ratio (males around 0.5, females around 1.0). Modern wild and domestic sheep samples used from the ISGC, along with 16 Asiatic mouflons, and the three goat genomes are indicated in Supplementary Data D. We then conducted PCA using smartPCA implemented in EIGENSOFT, projecting ancient sequences from Obishir onto the first three components of the PCA defined by the full dataset (including goat, wild sheep and domestic sheep reference specimens) to visualize diversity among sheep species.

Mitochondrial genome analysis. We produced a multiple genome alignment of our newly reconstructed sequences (excluding OB21-04 and OB21-06 because of low coverage) along with 160 present-day sequences from various breeds of modern *O. aries*, *O. ammon*, *O. vignei*, *O. orientalis ophion* and *O. aries musimon* (Supplementary Data C), using a modern *C. hircus* as outgroup, with Geneious software suite v9.1.8. Gaps were removed from the alignment. Phylogenetic trees were inferred using both the maximum-parsimony (MP) method (Fig. 6) and maximum likelihood (ML) with Tamura–Nei model (Supplementary Information) in MEGA version X[87–89]. We visualized and manipulated phylogenetic trees in FIGTREE v1.4.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Shotgun sequencing raw files are available at the European Nucleotide Archive (ENA) database under accession number PRJEB41594.

Received: 7 September 2020; Accepted: 17 February 2021;
Published online: 08 April 2021

References

1. Asouti, E. & Fuller, D. Q. A contextual approach to the emergence of agriculture in southwest Asia: reconstructing early Neolithic plant-food production. *Curr. Anthropol.* **54**, 299–345 (2013).
2. Conolly, J. et al. Meta-analysis of zooarchaeological data from SW Asia and SE Europe provides insight into the origins and spread of animal husbandry. *J. Archaeol. Sci.* **38**, 538–545 (2011).
3. Larson, G. et al. Current perspectives and the future of domestication studies. *Proc. Natl Acad. Sci. U. S. A.* **111**, 6139–6146 (2014).
4. Willcox, G. Measuring grain size and identifying Near Eastern cereal domestication: evidence from the Euphrates Valley. *J. Archaeol. Sci.* **31**, 145–150 (2004).
5. Willcox, G. & Stordeur, D. Large-scale cereal processing before domestication during the tenth millennium cal BC in northern Syria. *Antiquity* **86**, 99–114 (2012).
6. Larson, G. & Fuller, D. Q. The evolution of animal domestication. *Annu. Rev. Ecol. Evol. Syst.* **45**, 115–136 (2014).
7. Bocquet-Appel, J. Paleoanthropological traces of a Neolithic demographic transition. *Curr. Anthropol.* **43**, 637–650 (2002).
8. Bellwood, P. *First Farmers: the Origins of Agricultural Societies* (Wiley, 2004).
9. Omrak, A. et al. Genomic evidence establishes Anatolia as the source of the European Neolithic gene pool. *Curr. Biol.* **26**, 270–275 (2016).
10. Olalde, I. et al. The genomic history of the Iberian Peninsula over the past 8000 years. *Science* **363**, 1230–1234 (2019).
11. Brace, S. et al. Ancient genomes indicate population replacement in early Neolithic Britain. *Nat. Ecol. Evol.* **3**, 765–771 (2019).
12. Zeder, M. A. & Hesse, B. The initial domestication of goats (*Capra hircus*) in the Zagros mountains 10,000 years ago. *Science* **287**, 2254–2257 (2000).
13. Daly, K. G. et al. Ancient goat genomes reveal mosaic domestication in the Fertile Crescent. *Science* **361**, 85–88 (2018).
14. Alberto, F. J. et al. Convergent genomic signatures of domestication in sheep and goats. *Nat. Commun.* **9**, 813 (2018).
15. Zeder, M. A. in *Human Dispersal and Species Movement: from Prehistory to the Present* (eds Petraglia, M.D., Crassard, R. & Boivin, N.) p. 261 (Cambridge Univ. Press, 2017).
16. Vigne, J.-D. Early domestication and farming: what should we know or do for a better understanding? *Anthropozoologica* **50**, 123–151 (2015).
17. Pereira, F. & Amorim, A. *Encyclopedia of Life Sciences* https://doi.org/10.1002/9780470015902.a0022864 (2010).
18. Hermes, T. R. et al. Mitochondrial DNA of domesticated sheep confirms pastoralist component of Afanasievo subsistence economy in the Altai Mountains (3300–2900 cal BC). *Archaeol. Res. Asia* **24**, 100232 (2020).
19. Wilkin, S. et al. Dairy pastoralism sustained eastern Eurasian steppe populations for 5,000 years. *Nat. Ecol. Evol.* **4**, 346–355 (2020).
20. Hermes, T. R. et al. Early integration of pastoralism and millet cultivation in Bronze Age Eurasia. *Proc. Biol. Sci.* **286**, 20191273 (2019).
21. Taylor, W. et al. Early pastoral economies along the Ancient Silk Road: biomolecular evidence from the Alay Valley, Kyrgyzstan. *PLoS ONE* **13**, e0205646 (2018).
22. Spengler, R. N. & Willcox, G. Archaeobotanical results from Sarazm, Tajikistan, an early Bronze Age settlement on the edge: agriculture and exchange. *Environ. Archaeol.* **18**, 211–221 (2013).
23. Korobkova, G. F. *Tools and Economy of the Neolithic Populations in Central Asia* (Nauka, 1969).
24. Masson, V. M. *The Jeitun Settlement: the Emergence of a Productive Economy* (Nauka, 1971).
25. Itina, M.A. *History of Steppe Tribes of Southern Aral Sea Region (2–1 Ka BP)* (Nauka, 1977).
26. Lamberg-Karlovsky, C. C. The Bronze Age khanates of Central Asia. *Antiquity* **68**, 398–405 (1994).
27. Brunet, F. Pour une nouvelle étude de la culture néolithique de Kel'teminar. *Ouzbékistan. Paléorient* **31**, 87–105 (2005).
28. Masson, V. M. The Bronze Age in Khorasan. *Hist. Civiliz. Cent. Asia* **1**, 225 (1999).
29. Ranov, V. A. Hissar culture - Neolithic mountain regions of Central Asia. in *Stone Age of Northern, Middle and Eastern Asia* 27–28 (Nauka, 1985).
30. Yablonsky, L. T. Kelteminar craniology. Intra-group analysis. *Sov. Ethnogr., Mosc., USSR Acad. Sci.* **2**, 127–140 (1985).
31. Vinogradov, A. V. *Drevnie okhotniki i rybolovy Sredneaziatskogo Mezhdurechija (Former hunters and fishermen of Central Asian Mesopotamia)* (Nauka (Science), 1981a).
32. Vinogradov, A. V. in *Kul 'tura i iskusstvo drevnego Khorezma (Culture and Art of Ancient Khoresam)* (eds Itina, M. A., Raporort, J. A. et al.) pp. 88–98 (1981b).
33. Harris, D. R., *Origins of Agriculture in Western Central Asia* https://doi.org/10.9783/9781934536513 (Univ. of Pennsylvania, 2010).
34. Dolukhanov, P. M. The ecological prerequisites for early farming in southern Turkmenia. *Sov. Anthropol. Archeol.* **19**, 359–385 (1981).
35. Lisitsina, G. N. in *The Bronze Age Civilization of Central Asia: Recent Soviet Discoveries* (ed. Kohl, P. L.) pp. 350–358 (M. E. Sharpe, 1981).
36. Larkum, M. in *Origins of Agriculture in Western Central Asia: An Environmental–Archaeological Study* (ed. Harris, D.) pp. 142–149 (Univ. of Pennsylvania Museum of Archaeology and Anthropology, 2010).
37. Ranov, V. A. & Korobkova, G. F., Tutkaul – multilayered settlement site of the Gissar culture in southern Tajikistan. *Sov. Archaeol.* 133–147 (1971).
38. Islamov, U. I., *Obishirian Culture* (FAN, 1980).
39. Fedorchenko, A. Y. et al. Personal ornament production technology in the early Holocene complexes of western Central Asia: insights from Obishir-5. *Archaeol., Ethnol. Anthropol. Eurasia* **46**, 3–15 (2018).
40. Szymczak, K. & Khudzhanazarov, M., Exploring the Neolithic of the Kyzyl-Kums. Ayakagytma "The Site" and other collections. Swiatowit Suppl. Ser. P: Prehistory and Middle Ages 11. *Central Asia–Prehist. Stud* (2006).
41. Buckley, M., Collins, M., Thomas-Oates, J. & Wilson, J. C. Species identification by analysis of bone collagen using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* **23**, 3843–3854 (2009).
42. Kerven, C., Steimann, B., Ashley, L., Dear, C. & ur Rahim, I. *Pastoralism and Farming in Central Asia's Mountains: a Research Review* (Univ. of Central Asia, 2011).
43. Lieberman, D. E. Life history variables preserved in dental cementum microstructure. *Science* **261**, 1162–1164 (1993).
44. Klevezal, G. A. *Recording Structures of Mammals: Determination of Age and Reconstruction of Life History* (A. A. Balkema, 1996).
45. Diekwisch, T. G. The developmental biology of cementum. *Int. J. Dev. Biol.* **45**, 695–706 (2001).
46. Klevezal, G. & Kleinenberg, S. Age determination of mammals from annual layers in teeth and bones of mammals. *Trans. from Russian by the Israel Program for Scientific Translations, Jerusalem* (1969).
47. Grue, H. & Jensen, B., Review of the formation of incremental lines in tooth cementum of terrestrial mammals [age determination, game animal, variation, sex, reproductive cycle, climate, region, condition of the animal]. *Dan. Rev. Game Biol.* **11** (1979).
48. Stock, S. R. et al. Cementum structure in Beluga whale teeth. *Acta Biomater.* **48**, 289–299 (2017).
49. Gordon, B. C. *Of Men and Reindeer Herds in French Magdalenian Prehistory* https://doi.org/10.30861/9780860545040 (BAR Publishing, 1988).
50. Pike-Tay, A. *Red Deer Hunting in the Upper Paleolithic of South-West France: a Study in Seasonality* (BAR Oxford, 1991).
51. Burke, A. & Castanet, J. Histological observations of cementum growth in horse teeth and their application to archaeology. *J. Archaeol. Sci.* **22**, 479–493 (1995).
52. Frachetti, M. D. Multiregional emergence of mobile pastoralism and nonuniform institutional complexity across Eurasia. *Curr. Anthropol.* **53**, 2–38 (2012).
53. Stiner, M. C. et al. A forager–herder trade-off, from broad-spectrum hunting to sheep management at Aşıklı Höyük, Turkey. *Proc. Natl Acad. Sci. U. S. A.* **111**, 8404–8409 (2014).
54. Demirci, S. et al. Mitochondrial DNA diversity of modern, ancient and wild sheep (*Ovis gmelinii anatolica*) from Turkey: new insights on the evolutionary history of sheep. *PLoS ONE* **8**, e81952 (2013).
55. Meadows, J. R. S., Hiendleder, S. & Kijas, J. W. Haplogroup relationships between domestic and wild sheep resolved using a mitogenome panel. *Heredity* **106**, 700–706 (2011).
56. Cai, D. et al. Early history of Chinese domestic sheep indicated by ancient DNA analysis of Bronze Age individuals. *J. Archaeol. Sci.* **38**, 896–902 (2011).
57. Meadows, J. R. S., Cemal, I., Karaca, O., Gootwine, E. & Kijas, J. W. Five ovine mitochondrial lineages identified from sheep breeds of the Near East. *Genetics* **175**, 1371–1379 (2007).
58. Bruford, M. W. & Townsend, S. J., in *Documenting Domestication: New Genetic and Archaeological Paradigms* (eds Zeder, M. A., Bradley, D. G. & Emshwiller, E. A.) pp 306–316 (Univ. of California Press, 2006).
59. Arbuckle, B. S. & Atici, L. Initial diversity in sheep and goat management in Neolithic south-western. *Asia. Levant.* **45**, 219–235 (2013).
60. Hesse, B. Slaughter patterns and domestication: the beginnings of pastoralism in western Iran. *Man* **17**, 403–417 (1982).
61. Fijn, N., *Living with Herds: Human–Animal Coexistence in Mongolia* (Cambridge Univ. Press, 2011).

62. Frachetti, M. D., Smith, C. E., Traub, C. M. & Williams, T. Nomadic ecology shaped the highland geography of Asia's Silk Roads. *Nature* **543**, 193 (2017).
63. Jerardino, A., Fort, J., Isern, N. & Rondelli, B. Cultural diffusion was the main driving mechanism of the Neolithic transition in Southern Africa. *PLoS ONE* **9**, e113672 (2014).
64. van Klinken, G. J. Bone collagen quality indicators for palaeodietary and radiocarbon measurements. *J. Archaeol. Sci.* **26**, 687–695 (1999).
65. Reimer, P. J. et al. The IntCal20 Northern Hemisphere radiocarbon age calibration curve (0–55 cal kBP). *Radiocarbon* **62**, 725–757 (2020).
66. Ramsey, C. B. Bayesian analysis of radiocarbon dates. *Radiocarbon* **51**, 337–360 (2009).
67. Wintle, A. G. & Prószyńska, H. TL dating of loess in Germany and Poland. *PACT* **9**, 547–554 (1983).
68. Fedorowicz, S. et al. Loess–paleosol sequence at Korshiv (Ukraine): chronology based on complementary and parallel dating (TL, OSL), and litho-pedosedimentary analyses. *Quat. Int.* **296**, 117–130 (2013).
69. Frechen, M. Systematic thermoluminescence dating of two loess profiles from the Middle Rhine Area (FRG). *Quat. Sci. Rev.* **11**, 93–101 (1992).
70. Behrensmeyer, A. K. Taphonomic and ecologic information from bone weathering. *Paleobiology* **4**, 150–162 (1978).
71. van Doorn, N. L., Hollund, H. & Collins, M. J. A novel and non-destructive approach for ZooMS analysis: ammonium bicarbonate buffer extraction. *Archaeol. Anthropol. Sci.* **3**, 281 (2011).
72. Welker, F. et al. Palaeoproteomic evidence identifies archaic hominins associated with the Châtelperronian at the Grotte du Renne. *Proc. Natl Acad. Sci. U. S. A.* **113**, 11162–11167 (2016).
73. Rendu, W. Hunting behavior and Neanderthal adaptability in the Late Pleistocene site of Pech-de-l'Azé I. *J. Archaeol. Sci.* **37**, 1798–1810 (2010).
74. Stutz, A. J. Polarizing microscopy identification of chemical diagenesis in archaeological cementum. *J. Archaeol. Sci.* **29**, 1327–1347 (2002).
75. Geusa, G. et al. in *Osteodental Biology of the People of Portus Romae (Necropolis of Isola Sacra, 2nd-3rd Cent. AD)* (eds Bondioli, L. & Macchiarelli, R.) (Roma, 1999).
76. Lieberman, D. E., Deacon, T. W. & Meadow, R. H. Computer image enhancement and analysis of cementum increments as applied to teeth of *Gazella gazella*. *J. Archaeol. Sci.* **17**, 519–533 (1990).
77. Gorgé, O. et al. Analysis of ancient DNA in microbial ecology. *Methods Mol. Biol.* **1399**, 289–315 (2016).
78. Rohland, N., Harney, E., Mallick, S., Nordenfelt, S. & Reich, D. Partial uracil-DNA-glycosylase treatment for screening of ancient DNA. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **370**, 20130624 (2015).
79. Kircher, M., Sawyer, S. & Meyer, M. Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. *Nucleic Acids Res.* **40**, e3 (2012).
80. Ginolhac, A., Rasmussen, M., Gilbert, M. T. P., Willerslev, E. & Orlando, L. mapDamage: testing for damage patterns in ancient DNA sequences. *Bioinformatics* **27**, 2153–2155 (2011).
81. Li, H. & Durbin, R. Fast and accurate long-read alignment with Burrows–Wheeler transform. *Bioinformatics* **26**, 589–595 (2010).
82. Purcell, S. et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).
83. Li, X. et al. Whole-genome resequencing of wild and domestic sheep identifies genes associated with morphological and agronomic traits. *Nat. Commun.* **11**, 2815 (2020).
84. Li, H. et al. The sequence alignment/map format and SAMtools. *Bioinformatics* **25**, 2078–2079 (2009).
85. DePristo, M. A. et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat. Genet.* **43**, 491 (2011).
86. Mallick, S. et al. The Simons Genome Diversity Project: 300 genomes from 142 diverse populations. *Nature* **538**, 201–206 (2016).
87. Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **35**, 1547–1549 (2018).
88. Stecher, G., Tamura, K. & Kumar, S. Molecular evolutionary genetics analysis (MEGA) for macOS. *Mol. Biol. Evol.* **37**, 1237–1239 (2020).
89. Tamura, K. & Nei, M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* **10**, 512–526 (1993).
90. Murphy, D. J. *People, Plants and Genes: The Story of Crops and Humanity* (Oxford Univ. Press, 2007).
91. Lv, F.-H. et al. Mitogenomic meta-analysis identifies two phases of migration in the history of eastern Eurasian sheep. *Mol. Biol. Evol.* **32**, 2515–2533 (2015).

Acknowledgements

The authors thank D. Paul and S. Palstra for performing radiocarbon dating of tooth enamel, and E. Rannamäe for assistance with manuscript preparation. Cementum analyses were funded through the CemeNTAA project, via the French National Agency for Research (ANR-14-CE31-0011). Geological investigations were supported by the National Science Center, Poland (grant no. 2018/29/B/ST10/00906). Sampling for ZooMS, DNA and radiocarbon analysis (Golden Valley Laboratory) and lithic analysis of Obishir V were supported by RSF project no. 19-78-10053, 'The emergence of food-producing economies in the high mountains of interior Central Asia'. Ancient DNA analyses were conducted with the support of the palaeogenomic platform from the UMR5199 PACEA Universite de Bordeaux and the European Research Council under the European Union's Horizon 2020 research and innovation programme under grant agreement no. 804884-DAIRYCULTURES. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Author contributions

W.T.T.T. and S.Sh. designed the research, collected data, conducted analysis and wrote the manuscript. M.P., C.P., A.A., W.R., C.J., T.H., and C.W. collected data, conducted analysis and helped to write the manuscript. M.T.K., G.B., S.Sc., G.H., R.Sp., R.St., J.M., A.S., S.F., L.O., K.D. and A.K. collected data, conducted analysis and assisted in data interpretation. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41562-021-01083-y.

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Peer review information *Nature Human Behaviour* thanks Suzanne Birch, Laurent Frantz and Eve Rannamae for their contribution to the peer review of this work.

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Last updated by author(s): Sep 11, 2020

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Software and code

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Data collection	No software used
Data analysis	Radiocarbon dates Bayesian analysis of radiocarbon dates was performed in OxCal v. 4.4 (https://c14.arch.ox.ac.uk/oxcal.html) ZooMS Mass spectrometry identifications were performed using the open-source software MMass (http://www.mmass.org/) Cementum analysis Measurements of the increments were conducted using ImageJ (https://imagej.nih.gov/ij). PCR amplification Sequences were visually inspected and manually curated, assembled and aligned using Geneious software suite v9.1.8 (https://www.geneious.com). Library construction and sequencing Sequence authenticity was estimated on libraries subjected to either a partial or no USER treatment using MapDamage. We drew alleles at random using PileupCaller (https://github.com/stschiff/sequenceTools), rendering the individuals from the dataset homozygous for each locus. The two files were then merged using Plink v1.9. We then conducted principal component analysis using smartPCA implemented in EIGENSOFT. We mapped our filtered reads to both the Ovis aries genome assembly (international Sheep Genome consortium build Oarv3.1) and the Capra hircus genome assembly (ARS1) using BWA. We produced a multiple genome alignment of our newly reconstructed sequences with Geneious software suite v9.1.8. Gaps were removed from the alignment and a Maximum Parsimony tree was built using MEGA version X.Phylogenetic trees were visualized and manipulated in FIGTREE v1.4.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Graphs
All graphs were produced and edited in R (https://cran.r-project.org/) and Adobe Photoshop

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Behavioural & social sciences study design

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Study description	This study is a mixed-methods, interdisciplinary analysis of archaeological materials from the site of Obishir V, in Kyrgyzstan. All available archaeological materials (as of 2017) were selected for the analysis, of which the best preserved specimens (n=6) were selected for genomic sequencing.
Research sample	All available skeletal remains from 2017 and early excavations were chosen for study. For ZooMS analysis, all materials from one square of the excavation (R8) were selected for study.
Sampling strategy	All available materials from the archaeological assemblage were chosen for study.
Data collection	Researchers were blind to any hypothesis during data collection, which did not involve human participants. Excavation Excavations at Obishir V site carried out between 2015-2019 by a joint Russian-Kyrgyzstan archaeological expedition. In 2015–2016, researchers working at the site excavated an area of 8 m2 adjoining the 1968–1969 excavation area (Supplementary Appendix A). Each artifact (lithic and faunal remains) greater than 1 cm in size was individually piece-plotted with 1 mm accuracy using a Leica Total Station TS02 plus in tandem with the Trimble software EDM Mobile, and documented along with contextual information (orientation, dip, inclination, find category, stratigraphic level, etc.). Fragments smaller than this were collected in bulk for each 0.25 m2 area. Features (natural or anthropogenic) were recorded with the total station using outlines, surface points and breaklines. Large surfaces, such as walls of old excavation pits, sections and archaeological horizons with numerous artefacts were also documented using photogrammetry and 3D-scanning. Digital records were supplemented by field drawings and notes. All sediment obtained during the excavation was wet-screened with mesh of a size of 2.0 mm, and finds were documented according to excavated square, layer and depth. Stratigraphic units were defined during the excavation based on grain size composition, consistency, color, presence of erosional surfaces and accumulations of limestone clasts. Thermoluminescence dating During the 2017 field season, two sediment samples were collected from the southern portion of the western excavation profile of layer 5 at a depth of 295 and 335 cm below the surface. We measured deposit moisture in each sample, and after drying determined dose rate (DR) using a MAZAR gamma spectrometer. Concentrations of 226Ra, 228Th, 40K in each sample were obtained from twenty measurements lasting 2000 s each. We established equivalent dose (ED) on the 63-80 mm polymineral fraction, after 10% HCl and 30% H2O2 washing and UV optical bleaching. The samples were irradiated with 20 Gy, 30 Gy, 40 Gy, 50 Gy and 100 Gy, doses from 60Co gamma source. Before measurement, we heated the samples at 140 degrees for 3 hours. A sample pre-treated in this way was used to determine the equivalent dose (ED) by the TL multiple-aliquot regenerative technique (67), according to the description published by Fedorowicz et al. (68). Curve registration was performed on RA'94 (Mikrolab) thermoluminescence reader, coupled with EMI 9789 QA photomultiplier. Finally, we calculated TL age according to Frechen (69). Zooarchaeology and Zooarchaeology by Mass Spectrometry For each specimen, we conducted taphonomic analysis and morphology-based species identifications using comparative faunal collections held by the Russian Academy of Sciences-Siberia in their field station in Aidarkyen, Kyrgyzstan, and attempted refits for all specimens to control for issues of fragmentation. We recorded taphonomic indicators such as rodent and carnivore damage, root etching, and weathering (70), along with evidence for anthropogenic modification such as spiral fracturing, cut marks, and burning via visual inspection (Supplementary Appendix C). In addition, we used collagen fingerprinting (or Zooarchaeology by Mass Spectrometry, also known as ZooMS) to taxonomically identify a subset of this material, all derived from 2017 excavations. We

analyzed all bone specimens from Square R8, Layers 2.2, 3, and 4, as well as all bone specimens from Square R8, Layer 1.3 using ZooMS. To do so, we demineralized 10 mg of bone using 50mM ammonium bicarbonate buffer (Sigma-Aldrich) produced in UHQ water and pH adjusted to 8.0 using Ammonium Hydroxide (1M), following the protocol outlined by (71). The extracted collagen was digested into component peptides using trypsin (Pierce), and was purified using Pierce C18 tips (Thermo Scientific), eluting in 50% acetonitrile (Sigma-Aldrich) and 0.1% TFA (Sigma-Aldrich). Samples were then spotted on Bruker AnchorChip with Bruker Peptide Calibration Standard in calibration spots directly neighbouring the samples. Mass spectrometric analysis was conducted using a Bruker Autoflex Speed LRF MALDI-TOF in the dedicated ZooMS Laboratory at the Max Planck Institute for the Science of Human History in Jena, Germany.

Cementum analysis
First, all analyzed teeth were 3D scanned using a MicroCT scanner, and washed in a 90% ethanol solution. A cast of the occlusal surface was retained for later micro-wear analyses. Thin sections were prepared following recommendations by Rendu (73). Teeth were embedded in epoxy within a vacuum chamber (at 0.2 bars pressure). They were then cut at a thickness of 250µm using an Isomet5000 automatic saw, and ground to a thickness of 100µm with a PressiCube grinding machine. For each tooth, four independent thin sections were prepared and analyzed independently. Observations were conducted under polarized light and polarized/analyzed light both with and without the insertion of a lambda plate (74), using a Leica 2500P microscope equipped with a Leica DC 120 camera. P

DNA Extraction
DNA was extracted from 6 teeth using silica-based purification in a dedicated laboratory for ancient DNA at PACEA (UMR 5199, University of Bordeaux). We incubated between 100-250 mg of bone powder in a 1 mL extraction buffer (0.5 M EDTA, 0.25 M Na2HPO4, 50 µg/mL proteinase K) for 48-72 h at 37°C. DNA was purified from the collected supernatant using a method adapted from the QIAquick MinElute purification kit protocol (Qiagen).

PCR amplification
Purified DNA was amplified by qPCR using a LightCycler® 96 Instrument (Roche Life Science). Primer pairs were designed to amplify 2 regions of the mitochondrial DNA, a 130 bp region from cytB and 170bp from the hypervariable region (Supplementary Appendix F: list of primers used and product sizes). We obtained each PCR product at least twice, conducting standard negative control to ensure authenticity of the results. We used 1 µL of extract in 10 µL reaction with 1X FastStart Essential DNA Green Master and 1µM of each primer. Products were sequenced by capillary electrophoresis at Genewiz (Leipzig, Germany).

Library construction and sequencing
We used DNA extracts to build double-stranded Illumina libraries following the protocol described in Gorge et al (77). We sequenced whole genome shotgun libraries for ~ 500 000 reads on an Illumina NextSeq 500 at the Institut de Recherche Biomédicale des Armées in Brétigny-sur-Orge, France, to assess DNA preservation. DNA extracts of five samples produced in Bordeaux were sent to the dedicated clean room facilities of the Max Planck Institute for the Science of Human History. There, 20 ul of the extract were used to build double stranded UDG-half libraries (78). The resulting genetic libraries were double indexed and further amplified (79) Finally, all five samples were shotgun sequenced for ~20M reads on an Illumina HiSeq run with a 75-cycles single-end configuration.

Timing	Archaeological research was conducted in the summer of 2017, with ZooMS conducted in Fall 2017, and radiocarbon dating and DNA sequencing from early 2018 through spring of 2020.
Data exclusions	No data were excluded from the analyses.
Non-participation	No human participants were used in the study
Randomization	No human participants were used in the study, and no data were allocated into groups

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Methods

n/a	Involved in the study
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Palaeontology and Archaeology

Specimen provenance	Specimens all come from the archaeological site of Obishir V in Aidaryken, Kyrgyzstan, and research was conducted under Permit #0040/02-05 issued by the The Field Committee of the Institute of History and Cultural Heritage of the National Academy of Sciences, Kyrgyz Republic on 19 April, 2017. Specific provenance for each object is provided in the supplementary material.

Specimen deposition	All specimens are stored in the collections at the Institute for Archaeology and Ethnography, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russian Federation
Dating methods	Nine radiocarbon samples on identified and unidentified animal bone and tooth samples (n = 7) and charcoal (n = 2) were selected from various depths across the stratigraphic unit of layers 2-4, and submitted for radiocarbon dating at the Oxford Radiocarbon Accelerator Laboratory, the Center for Isotope Research at the University of Groningen, and the Golden Valley Laboratory at Novosibirsk. A portion of tooth 20-1 was submitted to the University of Arizona Accelerator Mass Spectrometry Laboratory. Collagen was obtained from 20-1 dentin using Acid-Base-Acid (ABA) pretreatment, gelatinization, 0.45 micron filtration, and ultrafiltration. Quality control parameters: collagen yield (7.1%); carbon yield (42.9%); CN ratio (3.2); and d13C (19.7 +/- 0.1 per mil), indicated good preservation (64). All dates were calibrated in OxCal using the INTCAL13 calibration curve (65). We performed Bayesian stratigraphic phase modeling (using ordered phases and combining Layers 2-4) in the software OxCal with a uniform prior using all available radiocarbon dates from Obishir V, and two thermoluminescence dates from Layer 5 (using OxCal's C_date function), following methods outlined by Ramsey (66). We also modeled the cultural occupation of Layer 2 separately, using only bone and tooth samples using a uniform prior. The models provided good agreement, and repeating the analysis using a general outlier model did not significantly alter estimated parameters. OxCal code used in the analysis, along with human tooth dating methods are outlined in Supplementary Appendix B.

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Ethics oversight	All research was conducted in concurrence with ethical guidelines of the Max Planck Institute for the Science of Human History, Jena, Germany.

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