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## **Animal fibre use in the Keriya valley (Xinjiang, China) during the Bronze and Iron Ages: A proteomic approach**

Clara Azemard Azemard, Antoine Zazzo, Arul Marie, Sébastien Lepetz,  
Corinne Debaine-Francfort, Abduressul Idriss, Séverine Zirah

### ► To cite this version:

Clara Azemard Azemard, Antoine Zazzo, Arul Marie, Sébastien Lepetz, Corinne Debaine-Francfort, et al.. Animal fibre use in the Keriya valley (Xinjiang, China) during the Bronze and Iron Ages: A proteomic approach. *Journal of Archaeological Science*, 2019, 110, pp.104996. 10.1016/j.jas.2019.104996 . hal-02348818

**HAL Id: hal-02348818**

**<https://hal.science/hal-02348818v1>**

Submitted on 5 Nov 2019

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1 **Animal fibre use in the Keriya valley (Xinjiang, China) during the Bronze and Iron Ages:**  
2 **A proteomic approach**

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5 Clara Azémard<sup>1,2</sup>, Antoine Zazzo<sup>2</sup>, Arul Marie<sup>1</sup>, Sébastien Lepetz<sup>2</sup>, Corinne Debaine-Francfort<sup>3</sup>,  
6 Abduressul Idriss<sup>4</sup>, Séverine Zirah<sup>1</sup>

7 1 – Unité Molécules de Communication et Adaptations des Microorganismes (MCAM), Muséum  
8 national d’Histoire naturelle, CNRS, CP 54, 63 rue Buffon, 75005 Paris, France.

9 2 – Unité Archéozoologie, Archéobotanique: Sociétés, pratiques et environnements (AASPE),  
10 Muséum national d’Histoire naturelle, CNRS, CP 56, 55 rue Buffon, 75005 Paris, France.

11 3 – Archéologies et Sciences de l’Antiquité (ArScAn / Archéologie de l’Asie centrale.  
12 Peuplements, milieux, techniques, UMR 7041), CNRS, Maison de l’Archéologie et de  
13 l’Ethnologie René Ginouvès, 21 allée de l’Université, F-92023 Nanterre cedex, France.

14 4 – Institut d’Archéologie et du Patrimoine du Xinjiang, Beijing Nanlu, Urumqi 830011, Xinjiang,  
15 R.P. China.

16  
17  
18 **Abstract**

19 Textile technology strongly advanced with sedentism and pastoralism. During prehistory, many  
20 populations settled in central Eurasia, a place of extensive exchange and cultural contact. In the  
21 Taklamakan desert, the dry climate enabled good preservation of ancient textiles. The study presented  
22 here aimed to identify animal fibres from Bronze Age and Iron Age sites in the Keriya valley  
23 (Xinjiang, China) using proteomics. A large corpus of 109 keratin extracts obtained from raw fibres or  
24 textiles was analysed, enabling us to establish a corrected and improved list of peptide markers for the  
25 identification of species, not only among the family Bovidae, but also for camels and humans. In total,  
26 we were able to identify 97% of the sampled objects to the taxonomic level of tribe and 85% of  
27 caprines to the level of genus. The assemblage was dominated by hair of sheep (57.8%) followed by  
28 goat (16.5%), cattle (8.3%), camel (0.9%), human (0.9%) and non-differentiated Caprinae (sheep or  
29 goat) (12.8%). The study showed a continuity between the two sites in this respect. It revealed a  
30 choice of raw material linked to the function of the textile, with most woven textiles being made from  
31 sheep’s wool and most pelt being obtained from goat. Comparison with the bone assemblage of one of  
32 the sites provided insight into the herd management strategies. The results confirm the heuristic  
33 potential of the proteomic approach for the determination of archaeological fibres and for textiles  
34 studies in general. Data are available via ProteomeXchange with identifier PXD012189.

35 **Keywords:** Proteomics, keratin, textiles, animal fibres, Xinjiang, Iron Age, Bronze Age

36  
37 **1. Introduction**

38  
39 Textile technology played a central role in the development of past societies (Good, 2001; Hardy,  
40 2007). The first archaeological evidence for pelts dates back to 90,000–80,000 years ago, whereas the  
41 first evidence for twisted fibres appears as clay impressions 27,000 years ago (Hardy, 2007). The  
42 invention of looping and weaving opened up many possibilities for the creation of textile products,  
43 from nets to clothing. Starting in the Holocene, domestication and agriculture enabled the increase of  
44 textile production by supplying animal or vegetal fibres (Bender Jørgensen et al., 2018; Rast-Eicher,  
45 2005). Therefore, the study of textiles can provide much information about resources, manufacturing  
46 techniques, trade, and culture through time. In contrast to other materials, such as ceramics, bone, or  
47 metal, textiles are very sensitive to degradation and are rarely preserved in archaeological contexts.  
48 Preservation occurs in specific environments, such as deserts, bogs, or permafrost (Gleba, 2011; Good,

49 2001; Hardy, 2007; Strand et al., 2010).

50 The preserved fibres are generally studied from the point of view of technology (of weaving, dyes,  
51 etc.), but their specific origin (vegetal or animal) is rarely studied in depth. A taxonomic determination  
52 of the animal or plant species at the origin of the textile is often made, but the rarity of the remains  
53 does not allow questions related to either the production methods of these fibres (organisation of  
54 livestock or crops) or the cultural choices underlying the activities to be addressed. Such questions are  
55 addressed using information from animal bones and teeth (often more numerous) or plant remains  
56 (generally rarer) found in archaeological sites, but it is difficult to use these proxies to define in detail  
57 the types of textiles produced and their use. When there are many textiles on a site, we can increase the  
58 number of taxonomic identifications and directly acquire information on the animals, plants, herds,  
59 and fields that are at their origin.

60 Generally, fibre identification is undertaken by microscopy. The morphological observation of animal  
61 fibres by optical and scanning electron microscopy normally permits specialists to clearly separate  
62 animal families. To achieve this, they focus on different parameters, such as the colour and diameter of  
63 the fibre, the morphology of the scales, and the structure of the medulla (Houck, 2010; Thomas et al.,  
64 2012; Rast-Eicher, 2016). However, alteration of the fibre due to diagenesis can make these techniques  
65 inapplicable to archaeological remains. In addition, these techniques cannot always differentiate  
66 between certain related species, such as sheep and goat. More importantly, wool characteristics have  
67 changed through time, so the general morphology of ancient fibre may not correspond to that of  
68 modern wool from the same species (Rast-Eicher, 2016). For any one of these reason, the  
69 differentiation of archaeological fibres can be very challenging.

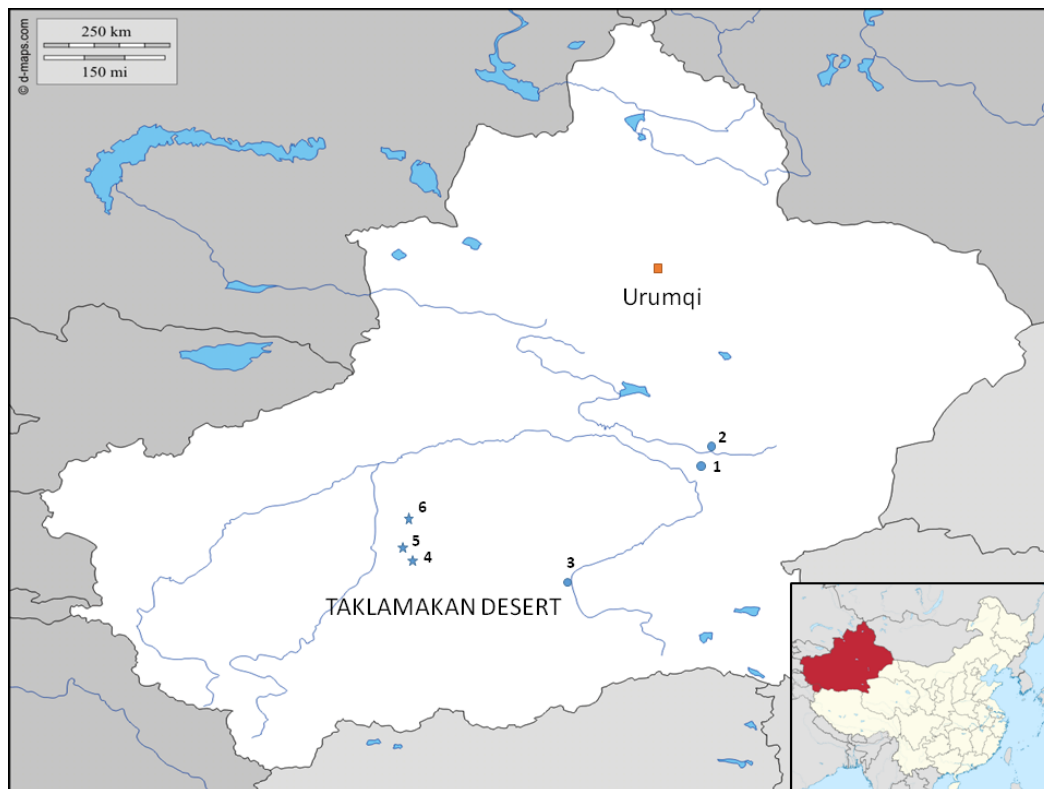
70 Palaeoproteomics has recently emerged as a powerful method to characterise the fraction of proteins  
71 preserved over time, providing a molecular signature related to the nature and taxonomy of  
72 archaeological samples (Cappellini et al., 2014; Hendy et al., 2018). This approach involves the  
73 hydrolysis of protein extracts, followed by analysis by mass spectrometry. It can generate diagnostic  
74 peptides that help discriminate among animal species, through their  $m/z$  value and fragmentation  
75 pattern related to their amino acid sequence (Cleland and Schroeter, 2018). The main proteins present  
76 in hair and fur are keratins. They constitute a large family of proteins forming heteropolymeric  
77 filaments (Plowman, 2007; Schweizer et al., 2006) incrustated into a matrix of keratin-associated  
78 proteins (KAPs) that stabilise the filament structure through extensive disulphide bonding (Gong et al.,  
79 2012; Marshall et al., 1991). Keratins are classified into two families: type I keratins (40–55 kDa),  
80 which are acidic, and type II keratins (55–70 kDa), which are basic or neutral. The revised  
81 nomenclature of mammalian keratins names type I keratins from K31 to K40 and type II keratins from  
82 K81 to K87 (Schweizer et al., 2006). Keratin K33 generally exists as two isoforms, named K33a and  
83 K33b. However, the annotation of keratins in databases is often heterogeneous or related to previous  
84 classifications. Thus, wool microfibrillar keratins are often named based on the old nomenclature,  
85 which relies on electrophoresis separation: components 8C-1, 8C-2, 8A and 8B for type I and  
86 components 5, 7A, 7B, and 7C for type II (Plowman, 2003; Miao et al., 2018). A correspondence  
87 between the nomenclatures is provided in the Supplementary material Table S1. Proteomic analysis of  
88 hair, fur, and textiles was first conducted on modern samples to assess the quality of textile products  
89 (Hollemeier et al., 2002; Hollemeier and Heinzle, 2007; Clerens et al., 2010; Plowman et al., 2012;  
90 Paoletta et al., 2013; Vineis et al., 2014; Li et al., 2016; Plowman et al., 2018). Analysis of ancient  
91 samples in order to characterise remains of Neolithic human hair and clothes (Hollemeier et al., 2008,  
92 2012; Fresnais et al., 2017) and for other, archaeozoological applications (Solazzo, 2017; Solazzo et  
93 al., 2017, 2014, 2013, 2011) has permitted researchers to refine taxonomic attributions, in some cases  
94 increasing knowledge of the fabrication of ancient textiles (Fresnais et al., 2017; Solazzo et al., 2011).  
95 The high sequence similarity between keratins of the same type and between proteins of closely  
96 related species makes keratin identification difficult (Plowman, 2007). Nevertheless, diagnostic

97 peptides have been proposed to differentiate between related animal species, such as a fragment of  
98 K33, which differs by only one amino acid between goat and sheep (Solazzo et al., 2014, 2013). These  
99 markers are not always detected in proteomic studies of archaeological fibres, which leaves many  
100 unidentified samples. New markers and identification keys are essential for the improvement of  
101 archaeological fibre identification.

102 Here we present the results of a proteomic study aimed at identifying ancient fibres from the Xinjiang  
103 region at the species level. Xinjiang is an arid region of northwestern China particularly rich in well-  
104 preserved organic remains, including textiles and furs (Wang, B., 1999; Good, 1998; Keller et al.,  
105 2001; Liu et al., 2011; Mallory and Mair, 2000; Zhao, 2004, 2002, 2001, 1999).

106 Located in the eastern part of Central Asia, Xinjiang was a nodal point within the large Eurasian  
107 sphere of contact and interaction known as the Silk Roads, and within their forerunners. Many sites  
108 from the Bronze and Iron Age have been discovered so far (Chen and Hiebert, 1995; Jia et al., 2007,  
109 2010), the oldest dated around 2500-2000 BCE. Isotopic analyses on vegetal remains made it possible  
110 to enlighten us on human diet, subsistence strategies, and cross-cultural contacts from the Late Bronze  
111 Age to early historic times, both in northern and in southern Xinjiang (Wang, W. et al., 2017;. Wang,  
112 T. et al., 2017). However, the textile remains found to date come mainly from eastern Xinjiang (the  
113 Wupu cemetery, near Hami); from the Lopnor region (including the Xiaohe and Gumugou cemeteries)  
114 (Li, 2007; Liang et al., 2012; Qiu et al., 2014; Qu et al., 2017; Wang, 2014; Yang et al., 2014); and  
115 from the Tarim Basin, in both the Turfan region (including the Yanghai and Subeixi cemeteries) and  
116 the southern part of the Taklamakan desert (notably the Keriya Valley and the Chärchan area) (Wang,  
117 B., 1999; Good, 1998; Wang et al., 2016).

118 We focused on two sites in the Tarim basin (southern Xinjiang), where large corpuses of textiles and  
119 furs have been unearthed: Djoumboulak Koum (also called Yuansha gucheng), which is an Iron Age  
120 fortified settlement, and the Northern Cemetery, which dates from the Bronze Age (Figure 1)  
121 (Debaine-Francfort and Idriss, 2001; Debaine-Francfort, 2013). Discovered by the Sino-French  
122 Archaeological Mission in Xinjiang, both are located in the now-dry protohistoric delta of the Keriya  
123 River, which crosses the Taklamakan desert from south to north. Both have yielded large corpuses of  
124 textiles and furs that are being studied in a multidisciplinary way. Our research not only aims to  
125 discriminate between native and exogenous fauna, it also focusses on the methods used to process the  
126 fibres and initiates wider research on the evolution of fleeces and textiles from the Bronze Age to the  
127 Iron Age and a comparison with contemporaneous fleeces and textiles found in Europe. This  
128 information is cross-referenced with that provided by archaeozoology to supplement our knowledge  
129 on the history of breeding, transhumance practices, and hunting. This work, which has already  
130 revealed the complexity and diversity of weaving techniques and dyeing practices, suggests that the  
131 textile industry had a major importance in the ancient societies of the Keriya valley (and beyond)  
132 (Cardon et al., 2013; Debaine-Francfort and Idriss, 2001). The often poor state of preservation of the  
133 fibres, which is sometimes insufficient to enable their taxonomic determination using microscopy, has  
134 led us to adopt non-zooarchaeological analytical methods to overcome this taphonomic handicap and  
135 to thus address unanswered questions about the raw materials used in the manufacture of these textiles.  
136



137  
 138 **Figure 1.** Map showing some of the archaeological sites in the Xinjiang Uyghur autonomous region –1: Xiaohe, 2:  
 139 Gumugou, 3: Chärchan, 4: Karadong, 5: Djouboulak Koum (Yuanshagucheng), 6: Northern Cemetery.

140

141 In this article, we report on how a proteomics approach was used on raw fibres and textiles in order to  
 142 identify the origin of animal fibres from these two archaeological sites. The taxonomic specificity of  
 143 keratins was evaluated on a large corpus of samples, with the aim to define new peptide markers for  
 144 species differentiation. The fibre identification was used to figure out the relation between domestic  
 145 animal species and type of textile produced and to document the diachronic development of textile use  
 146 in Xinjiang from the Bronze Age to the Iron Age.

147

## 148 2. Material & Methods

149

### 150 2.1. Samples

151

152 The samples come from Djouboulak Koum and the Northern Cemetery site. The Sino-French  
 153 archaeological mission in Xinjiang found both sites, located in the protohistoric delta of the Keriya  
 154 River, in the Taklamakan desert.

155 Djouboulak Koum is an Iron Age settlement and the Northern Cemetery is a cemetery dating from  
 156 the Bronze Age. Djouboulak Koum (mid-1st millennium BCE), was found in 1994. The area enclosed  
 157 by the walls covers 10 hectares and is surrounded by various cemeteries. The sedentary population  
 158 practiced agriculture, producing millet, wheat, and barley thanks to an elaborate system of irrigation  
 159 (Debaine-Francfort et al., 2010; Debaine-Francfort and Idriss, 2001). The Northern Cemetery dates  
 160 from c. 1950–c. 1400 BCE (Debaine-Francfort, 2013, 2019; Cardon et al., 2013; Mair, 2014; Aoudia  
 161 et al., 2016; Qu et al., 2017). Discovered in 2008, and unfortunately severely looted, it shows  
 162 astonishing similarities with the famous and much better preserved cemetery of Xiaohe, located 600  
 163 km to the east (Bergman, 1939; Xinjiang wenwu kaogu yanjiusuo, 2003, 2007; Li, W., 2007; Mair,  
 164 2006). These two contemporary funerary sites belong to the same culture, but since the Northern

165 Cemetery is connected with remains of dwellings that are without equivalent, it is more than just the  
166 “twin” of Xiaohe: these remains (the earliest of which date back to about 2500 cal. BC) represent the  
167 earliest known human settlement in the Tarim basin.

168 The Northern Cemetery initially contained many graves divided into at least two levels, similar to  
169 those of Xiaohe, which has yielded 167 tombs in five levels. The structure of the tombs is regular. The  
170 deceased, wrapped in a shroud, were placed in a coffin with a lid covered with the pelts of sheep or  
171 cattle that had been freshly slaughtered. Naked, they wore only felt caps of various types, leather  
172 shoes, and a loincloth (for women) or a fringed belt (for men), a costume very different from the pants,  
173 skirts, and tunics worn by the Iron Age populations buried at Djoumboulak Koum.

174 A set of 109 sampled objects from the two Keriya valley sites was studied (Figure 2, Supplementary  
175 Material Table S2). The samples were grouped into the following categories: The category textile  
176 refers to all fibres transformed by weaving, twisting, and similar techniques (e.g., felt, clothing,  
177 shrouds). Within this category, we separated out the shoes. The category raw fibres refers to all furs,  
178 pelts, and other unprocessed raw fibres. A total of 93 sampled objects come from the Northern  
179 Cemetery (35 raw fibres, 56 textiles, 2 shoes) and 16 from Djoumboulak Koum (10 raw fibres, 5  
180 textiles, 1 shoe).

181 In addition, we collected modern reference fibres in 2012, in the Arkhangai aimag, near Oziit,  
182 Mongolia, namely, one sample from goat and one from sheep.



183  
184 **Figure 2.** Sampled objects from the Bronze Age site of the Northern Cemetery. Clockwise from top left: a  
185 dark cord, a woven textile, a pelt, and a shoe.  
186

## 187 2.2. Sample preparation

188

189 First, the samples were washed with milliQ water + 2% Decon™ Decon 90 under stirring, until clear.  
190 Then they were washed 2 × 15 min in a MeOH/CHCl<sub>3</sub> solution (1:2) under stirring and 15 min in  
191 milliQ water under sonication. During this protocol, textiles tend to disintegrate, contrary to  
192 unprocessed hair.

193 The sample preparation protocol was adapted from Paolletta et al. (2013). Three mL of 25 mM Tris-  
194 HCl, 2.4 M thiourea, 5 M urea (pH 8.5), and dithiothreitol (DTT, 100 mM) were added to 20 mg of  
195 washed sample. The solution was left three days under stirring at 50°C, then filtered on a Buchner

196 funnel. A volume of 100  $\mu\text{L}$  of protein extract was reduced and alkylated: (1) Reduction was  
197 performed by addition of 100  $\mu\text{L}$  of  $\text{NH}_4\text{HCO}_3$  (100 mM) and 5  $\mu\text{L}$  of DTT (200 mM in the  $\text{NH}_4\text{HCO}_3$   
198 solution) followed by 1 h incubation at room temperature; (2) alkylation was performed by addition of  
199 4  $\mu\text{L}$  of a 1 M solution of iodoacetamide, followed by 1 h of incubation in the dark at room  
200 temperature. To this new solution, we added 20  $\mu\text{L}$  of the 200 mM DTT solution, after which it was  
201 incubated for 1 h. Finally, 1  $\mu\text{L}$  of trypsin gold® (1 g/L in 50 mM acetic acid) and 770  $\mu\text{L}$  of milliQ  
202 water were added and the 1 mL solution was incubated at 37°C for 16 h. This step was followed by  
203 solid phase extraction on a C8 cartridge (Sep-Pak C8 Plus, 400 mg sorbent, 37–55  $\mu\text{m}$  particle size,  
204 Waters). Conditioning of the cartridge was done with 8 mL HPLC-grade acetonitrile (AcN) and 4 mL  
205 milliQ-water + 0.1% formic acid (FA). After loading 1 mL of sample, the cartridge was washed with 1  
206 mL  $\text{H}_2\text{O}$  + 0.1% FA and eluted with 2 mL  $\text{H}_2\text{O}$  + 0.1% FA / AcN 20:80 (v/v). The samples were  
207 dried under vacuum, after which they were resuspended in 1.5 mL  $\text{H}_2\text{O}$  + 0.1% FA / AcN 70:30 (v/v).  
208

### 209 2.3. Analysis of the trypsin digests by mass spectrometry

210  
211 The trypsin digests were analysed by ultra-high-performance liquid chromatography–mass  
212 spectrometry (UHPLC-MS) on an Ultimate 3000-RSLC system (Thermo Scientific) connected to an  
213 electrospray ionization–quadrupole–time of flight (ESI Q-TOF) instrument (Maxis II ETD, Bruker  
214 Daltonics). The separation was achieved using a RSLC Polar Advantage II Acclaim column (2.2  $\mu\text{m}$ ,  
215 120 Å, 2.1  $\times$  100 mm, Thermo Scientific) with the following gradient of mobile phase A (milliQ water  
216 + FA 0.1%) and B (LC-MS grade AcN + FA 0.08%), at 300  $\mu\text{L}/\text{min}$ : linear increase from 5% B to  
217 60% B for 12 min, linear increase to 100% B for 0.5 min, plateau at 100% B for 0.5 min, decrease to  
218 5% B for 0.5 min, equilibration for 4 min (total run time 17.5 min). The MS detection was carried out  
219 in positive mode in the range  $m/z$  100–2000. The source parameters were as follows: nebulizer gas 35  
220 psi, dry gas 8 L/min, capillary voltage 3500 V, end plate offset 500 V, temperature 200 °C. Analyses  
221 were performed using collision-induced dissociation in data dependent auto-MS/MS mode, using the  
222 following set-up: preferred charge states: 1–4, unknown charge states excluded, cycle time 3 s, MS  
223 spectra rate: 2 Hz, MS/MS spectra rate: 2 Hz at 16.000 counts increasing to 6 Hz at 160.000 counts or  
224 above. MS/MS active exclusion was set after one spectrum unless intensity increased fivefold.  
225 Collision energy was automatically calculated from  $m/z$  and charge states. The MS instrument was  
226 calibrated at each run start using a sodium formate solution consisting of 10 mM sodium hydroxide in  
227 isopropanol / 0.2% formic acid (1:1 v/v). Four samples (P189, P226, P227, and P239) were analysed  
228 on our previous LC-MS instrumentation, a U3000 micro-HPLC system connected to a QStar Pulsar  
229 ESI-Q-TOF-MS instrument, using the conditions described by Cersoy et al. (2019).  
230

### 231 2.4. Data analysis

232  
233 The LC-MS/MS data were converted to mgf files using DataAnalysis software (Bruker Daltonics). A  
234 database search was carried out against the SwissProt and NCBI databases simultaneously with  
235 Mascot (MatrixScience.com, using the online version or an in-house licence). We restricted the search  
236 to Mammalia (mammals) and allowed 2 missed cleavages. We considered carbamidomethylation of  
237 cysteine as a fixed modification and deamidation of glutamine and asparagine as well as oxidation of  
238 methionine as variable modifications. Charge states 1+, 2+, and 3+ were considered with 10 ppm  
239 tolerance for precursor ions and 0.05 Da tolerance for MS/MS fragments (25 ppm for precursor ions  
240 and 0.5 Da tolerance for MS/MS fragments for the 4 samples run on the QStar MS instrument). The  
241 statistic decoy was applied to minimise false-positive match. In addition, the samples with highest

242 scores were treated using the error-tolerant mode in order to identify potential additional  
243 modifications. A new search was then performed on the samples with ambiguous or failed  
244 identification, considering lysine carbamylation as variable modification or error-tolerant mode,  
245 against a restrained database constituted of the keratin sequences present in NCBI (50,422 sequences).  
246 Mascot results were inspected manually in the “protein family summary” mode in order to determine  
247 the most confident identification, paying attention to the section “subsets and intersections,” which  
248 sometimes contained the best protein hits. The mass spectrometry proteomics data and Mascot results  
249 have been deposited in the ProteomeXchange Consortium via the PRIDE (Vizcaíno et al., 2016)  
250 partner repository with the dataset identifiers PXD012189 and 10.6019/PXD012189.  
251 To be able to distinguish among animal species, a list of peptide markers was constituted based on the  
252 literature (Izuchi et al., 2013; Paoletta et al., 2013; Solazzo et al., 2014, 2013, 2011) and the Mascot  
253 searches against the NCBI and Swissprot databases. In particular, unique peptides assigned by Mascot  
254 were assessed for their taxonomic potential. The specificity of the markers was checked by protein  
255 alignments generated with Muscle (<https://www.ebi.ac.uk/Tools/msa/muscle>) and Blast  
256 (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).  
257 The correspondence analysis was performed on the freely available R environment version 3.4.3  
258 ([www.r-project.org](http://www.r-project.org)), using the package ade4 (Dray and Dufour, 2007).

### 259 3. Results

260  
261 Protein inference is the process used to assemble identified peptides into a list of proteins that are  
262 believed to be present in a sample (He et al., 2016). This process is challenging in the case of keratins,  
263 given their high intra- and inter-specific sequence similarities. First, a systematic analysis of the best  
264 protein hits obtained for each sample was carried out (Supplementary material Table S3). Although a  
265 specific peptide can scarcely be assigned unambiguously to a specific keratin protein, this step  
266 permitted us to delineate the most confident taxonomic identifications as well as the most represented  
267 keratins. Type I keratins provided by far the highest scores, followed by type II keratins and,  
268 sometimes, KAPs. This step proposed sheep, goat, cattle, and, to a much lesser extent, camel and  
269 human as the best identifications. While identification of camel (1 sample) was straightforward,  
270 identification of Bovidae was sometimes ambiguous, due to the difficulty of distinguishing among the  
271 closely related sequences of goat, sheep, and cattle and to their high similarity with the sequences of  
272 certain Cervidae (deer family). Identification of human (2 samples) was also to some extent  
273 ambiguous, because it could indicate sample contamination and because there are high sequence  
274 similarities within primate keratins. The best protein matches were keratins K33b and K31 for sheep  
275 and cattle and K33a and K31 for goat (Supplementary material Table S4).

276 Then we searched for peptide markers permitting distinction among closely related species. These  
277 markers are listed in Table 1. Their distribution in the 109 archaeological and 2 reference samples is  
278 reported in Supplementary material Table S5, and their specificity is shown in Supplementary material  
279 Table S6. Alignment of representative sequences of type I keratins (Supplementary material Figure  
280 S1) showed that the C-terminal region is the most informative regarding species identification. The  
281 peptide at position 307-329 (given for type I K33B from *Ovis aries*, NP\_001185999.1), already  
282 reported in the literature (Clerens et al., 2010; Solazzo et al., 2013), appears to be a good marker,  
283 presenting sequences specific to sheep (YSCQLSQVQSLIVNVESQLAEIR), goat  
284 (YSCQLNQVQSLIVNVESQLAEIR), undetermined caprine (YSCQLNQVQSLISNVESQLAEIR),  
285 human (primate, YSSQLSQVQRLITNVESQLAEIR), and camel  
286 (YGSQLSQVQGLITNVEHQLAEIR). In addition, we observed another informative peptide from  
287 keratin K31 or K33, with sequences specific to goat (CGPCSSYVR), non-goat Bovidae (sheep, cattle,  
288 deer), caprine, cattle, camel, and primate. Only the peptides specific to goat, non-goat Bovidae, and



289 camel were detected in our samples. The corresponding MS/MS spectra are shown in Figure 3.  
 290 Although short (9 amino acids) and located at the C-terminus of the keratin proteins, these peptides  
 291 were detected in many samples (15 out of 17 goat samples), sometimes providing the only specific  
 292 clue to a goat taxonomic identification. A new non-cattle marker from keratin K31,  
 293 GDLERQNQEYQVLLDVR, was also identified (Supplementary material Figure S2A). This sequence  
 294 is present in various mammals but not in cattle species. It is derived from peptide  
 295 QNQEYQVLLDVR, reported in wool keratin (Cardamone et al., 2009), but this latter peptide was not  
 296 validated in our study as an informative marker since it was detected in caprine as well as cattle  
 297 samples. Type I keratins were revealed to be ambiguous for identifying cattle because the identified  
 298 peptide markers were generally shared with *Cervus elaphus hippelaphus* (Central European red deer)  
 299 and *Odocoileus virginianus texanus* (Texas white-tailed deer). A new marker,  
 300 ILERSQQQEPLLCPNYQSYFR, was identified from keratin 31 (Supplementary material Figure  
 301 S2B), but it, too, is shared with deer. Type II keratins were revealed to be more informative in that  
 302 respect. Alignment of representative sequences of type II keratins (Supplementary material Figure S3)  
 303 permitted us to identify new cattle peptide markers in the N-terminal and central region of the proteins,  
 304 such as AGYCSR, GLNMDNIVAEIK, and GLNMDNIVAEIKAQYDDIASR from keratin K83 and  
 305 ALPAFSCVSACGPRPGR from keratin K86 (Supplementary material Figure S4). In addition, the C-  
 306 terminal region constituted an informative region for differentiating between the other species. In  
 307 particular, region 448-487 (position relative to keratin K85 from *Ovis aries*, K2M3\_SHEEP:  
 308 QIASGPVATGG SITVLAPDSCQPR) contains markers specific to caprine, goat, sheep, camelid, and  
 309 primate. While type I keratin markers identified for humans were common in certain non-human  
 310 primates, type II keratins provided one marker (VSSVPSNSNVVVGTTNACAPSAR) specific to  
 311 *Homo sapiens*. Finally, KAPs, although detected in the best-preserved samples only, provided  
 312 additional peptide markers. Most of the samples displayed non-discriminant KAPs peptides, such as  
 313 (R)FWPFALY, detected in 72 samples identified as caprine or cattle. However, we identified markers  
 314 from KAPs from cattle (SSCCQPCCLPIR), non-cattle Bovidae (SLCGSGYGYGSR), and camel  
 315 (TSTLSRPCQTTYSGSLGFGSR). These markers are shown on the alignment of representative KAPs  
 316 in Figure S5.

317 **Table 1.** List of peptide markers used to distinguish among species.

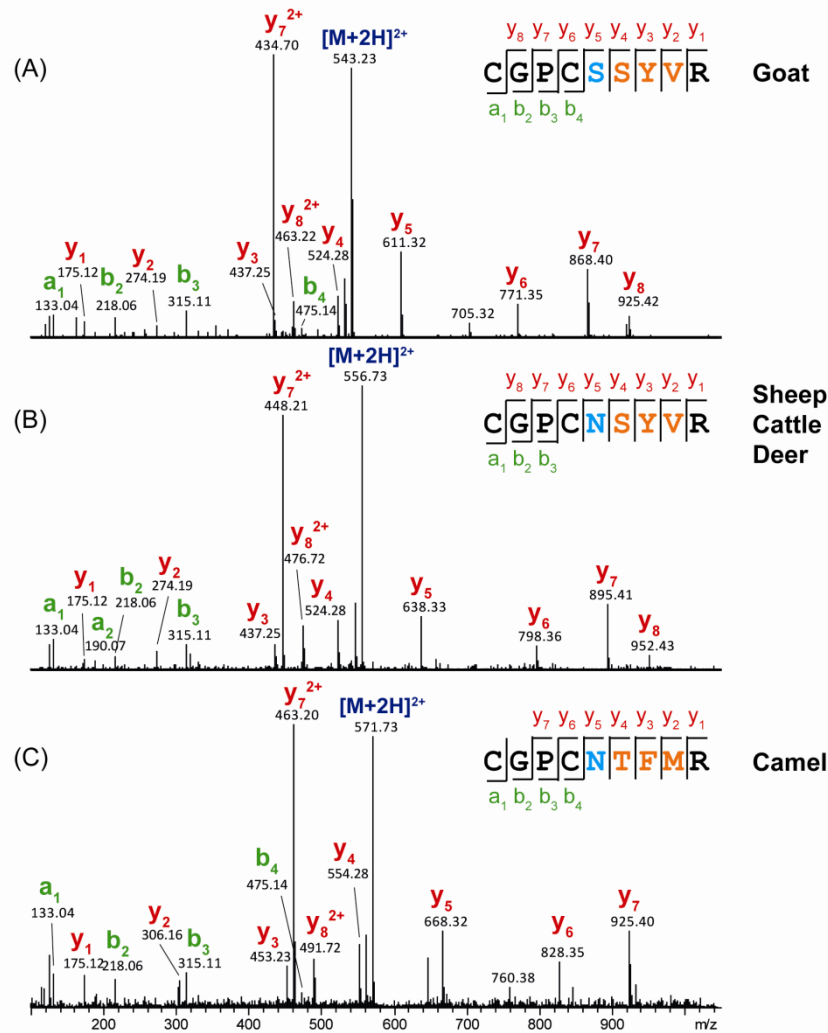
Attribution	Sequence	Best match keratin	Number of observations	Mean score $\pm$ sd	Reference
<b>Type I keratin markers</b>					
sheep	YSCQLSQVQSLIVNVESQLAEIR	K33b	62	92.0 $\pm$ 14.6	(Clerens et al., 2010; Solazzo et al., 2013)
goat	YSCQLNQVQSLIVNVESQLAEIR	K31,	13	99.7 $\pm$ 13.8	(Clerens et al., 2010; Solazzo et al., 2013)
		:			
		:			
non-caprine (cattle, deer)	YSCQLAQVQGLIGNVESQLAEIR	K31,	9	127.7 $\pm$ 12.8	(Clerens et al., 2010; Solazzo et al., 2013)
		:			
		:			
caprine (sheep, goat)	YSCQLNQVQSLISNVESQLAEIR	K31	45	78.1 $\pm$ 24.1	(Clerens et al., 2010; Solazzo et al., 2013)
cattle, deer	ILERSQQQEPLLCPNYQSYFR	K31	4	28.5 $\pm$ 4.2	This study
non-cattle (various mammals)	GDLERQNQEYQVLLDVR	K31	81	33.2 $\pm$ 11.0	This study
non-cattle (various mammals)	ARLESEINTYR	K31, K33	90	39.3 $\pm$ 13.5	(Clerens et al., 2010; Solazzo et al., 2013)

non-cattle (various mammals)	LESEINTYR	K31, K33	97	70.9 ± 10.6	(Clerens et al., 2010; Solazzo et al., 2013)
goat	CGPCSSYVR	K31	15	41.2 ± 11.9	This study
non-goat (cattle, sheep, deer)	CGPCNSYVR	K31	33	31.8 ± 15.5	(Clerens et al., 2010; Solazzo et al., 2013)
camel	YGSQLSQVQGLITNVEHQLAEIR	K33	1	81	(Solazzo et al., 2013)
camel	CGPCNTFMR	K33a	1	32	This study
camelid	QTEELNKQVVSSEQLQSNQAEIHELRR	K33a	1	37	This study
primate	YSSQLSQVQRLITNVESQLAEIR	K33a	2	99.5 ± 3.5	This study
primate	YSSQLSQVQR	K31	2	75.0 ± 11.3	This study
primate	LITNVESQLAEIR	K31	2	95.5 ± 9.2	This study
primate	DNAELENLIR	K31, K33a	2	60.0 ± 2.8	This study
<b>Type II keratin markers</b>					
non-cattle (various mammals)	DLNMDCIVAEIKAQYDDIASR	K83	22	13.7 ± 10.0	(Clerens et al., 2010)
caprine (sheep, goat)	GGVACGGLTYSSSTAGR	K85	64	60.6 ± 17.5	(Clerens et al., 2010; Solazzo et al., 2013)
non-caprine (cattle, deer)	GGVTCGGLTYSTTAGR	K85	9	78.0 ± 30.9	(Solazzo et al., 2013)
goat	(K)SDLEANAEALIQETDFLR(R)	K81	10	23.8 ± 12.5	This study
cattle and primate	(K)SDLEANVEALIQEIDFLR	K81	4	18.8 ± 6.2	(Solazzo et al., 2013)
cattle	AGYCSR	K83	2	16.0 ± 14.1	This study
cattle	GLNMDNIVAEIKAQYDDIASR	K83	3	45.3 ± 23.6	This study
cattle	GLNMDNIVAEIK	K83	4	86.3 ± 6.2	(Solazzo et al., 2013)
cattle	ALPAFSCVVSACGPRPGR	K86	5	32.2 ± 12.7	(Solazzo et al., 2013)
goat, horse, deer	AFSCVVSACGPRPSR	K81	17	15.7 ± 6.8	This study
sheep	QIASGPVATGGSITVLAPDSCQPR	K85	26	15.6 ± 14.2	(Clerens et al., 2010; Solazzo et al., 2013)
non-sheep	QIASGPVATGGSITVLAPDSCVPCQPR	K85	6	22.2 ± 16.0	(Solazzo et al., 2013)
camelid	DLNLDIVAEIKEQYDDIAR	K81	1	54	This study
primate	GGVVCGDLCASTTAPVVSTR	K86	1	54	This study
human	VSSVPSNSNVVVGTTNACAPSAR	K86	1	39	This study
<b>KAPs markers</b>					
caprine (sheep, goat) and cattle	(R)FWPFALY	-	72	15.1 ± 1.8	(Clerens et al., 2010)
cattle	SSCCQPCLPIR	-	1	26	This study
non-cattle (caprine, deer, antelope)	SLCGSGYGYGSR	-	3	59.3 ± 11.2	(Clerens et al., 2010; Solazzo et al., 2013)
camel	TSTLSRPCQTTYSGSLGFGSR	-	1	39	This study

318

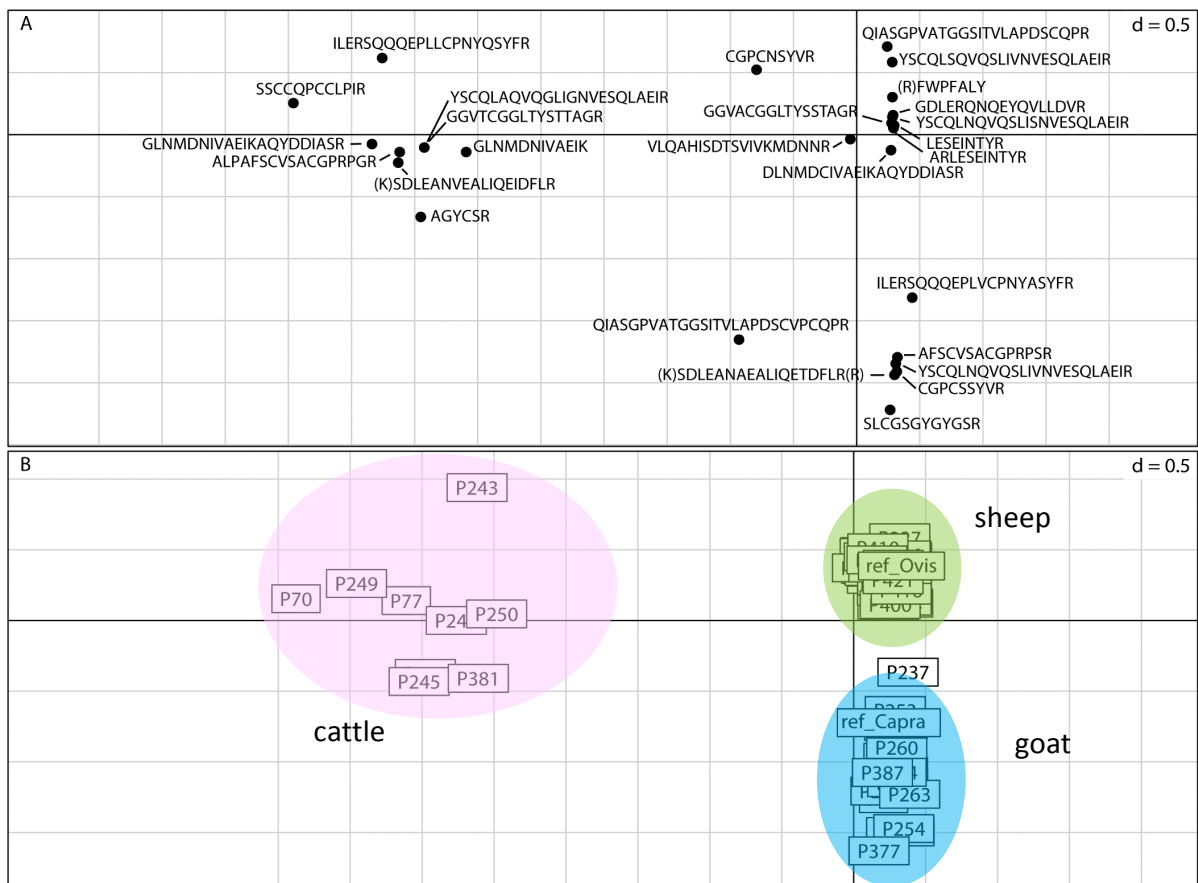
319 One important step to validate the peptide markers was to assess their presence or absence in each  
320 sample (Supplementary material Table S5). Indeed, database incompleteness and intra-specific  
321 sequence variability may alter their specificity. For example, sequence VLQAHISDTSVIVKMDNNR  
322 appeared to be specific to the species *Bubalus bubalis* (water buffalo) based on our database search.  
323 However, this peptide was detected not only in samples assigned to cattle, as one might expect, but  
324 also in those assigned to goat and sheep. In addition, this peptide was generally proposed with a low  
325 score (< 10) and high expectation value (~ 1000), indicative of a high chance of it being a random  
326 match. This sequence was therefore discarded from the list of peptide markers. Another example of a  
327 discarded sequence is LLEGQEQR, specific to sheep based on our database search but detected in our  
328 study in samples identified as goat, cattle, camel, and human. The specificity of Bovidae markers was

329 also revised. For example, sequence DLNMDCIVAEIKAQYDDIASR, assigned to diverse mammals  
 330 including sheep, but not to cattle or goat, in the existing databases, was detected in samples identified  
 331 in our study as both sheep and goat, and thus was proposed as a “non-cattle” marker. The high number  
 332 of samples used in this study proved to be very efficient to test and establish a list of validated specific  
 333 markers (Table 1). Note that certain of the marker peptides contain missed cleavages, as already  
 334 reported (Solazzo, 2017). This may relate to protein modifications that compromise enzymatic  
 335 digestion.



336  
 337 **Figure 3.** MS/MS spectra of the C-terminal type I keratin markers. (A) [M+2H]<sup>2+</sup> species of peptide CGPCSSYVR, specific  
 338 to goat (*m/z* 543.23, retention time 3.2 min, collision energy 22.6 eV), (B) [M+2H]<sup>2+</sup> species of peptide CGPCNSYVR,  
 339 specific to sheep, cattle, and deer (*m/z* 556.73, retention time 3.1 min, collision energy 23.4 eV), (C) [M+2H]<sup>2+</sup> species of  
 340 peptide CGPCNTFMR (*m/z* 571.73, retention time 3.9 min, collision energy 24.3 eV). Cysteine residues are  
 341 carbamidomethylated.

342 These peptide markers permitted us to clearly distinguish among goat, sheep, and cattle samples, as  
 343 illustrated in a correspondence analysis (Figure 4). Out of the 109 samples, 64 were identified as  
 344 sheep, 17 as goat, 14 as undetermined caprine, and 9 as cattle based on Mascot and peptide marker  
 345 searches (Table 2). In addition, one Djoumboulak Koum sample was identified as camel and a dark  
 346 twisted cord from the Northern Cemetery (Figure 2) was characterised as human hair. Note that  
 347 caprine samples P237 and P263 revealed a similar profile. Only these two samples showed peptide  
 348 ILERSQQQEPLVCPNYASYFR assigned to the ambiguous sequence  
 349 ILERSQQQEPLVCPNYXSYFR, which is specific to goat based on our database search. They were  
 350 designated as caprine due to their ambiguous marker profile.

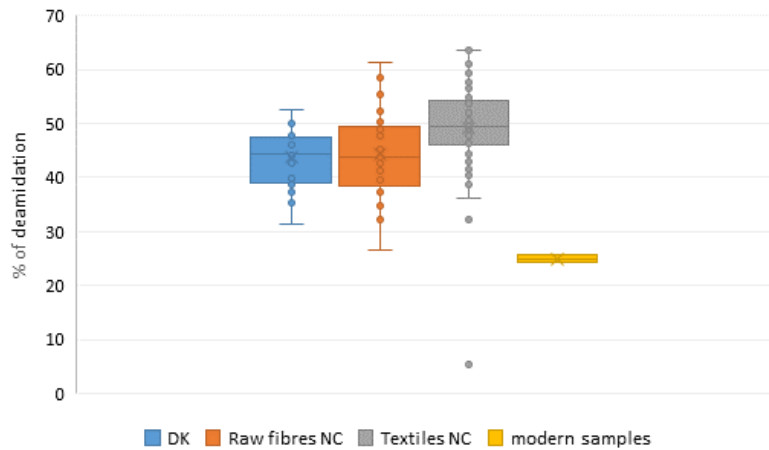


352 **Figure 4.** Correspondence analysis of Bovidae peptide markers–based identification, based on two axes. Scatter plots  
 353 showing the peptide markers (A) and samples (B).  
 354  
 355

	Djouboulak Koum	Northern Cemetery
Sheep	10	53
Goat	2	16
Caprine	2	12
Cattle	1	8
Camel	1	0
Unknown	0	3
Human	0	1
total	16	93

356 **Table 2.** Proteomic-based taxonomic identifications for the two sites.  
 357

358 The database search revealed important deamidation of asparagine and glutamine amino acids, as  
 359 already reported (Solazzo et al., 2014) (Figure 5). Although the average percentage of deamidation  
 360 sites of the archaeological sample (around 46.8%) was nearly double that of modern fibre (24.3% and  
 361 25.6% for goat and sheep, respectively), the maximum protein sequence coverage was generally  
 362 similar. Indeed, the maximum percentage ranged between 14% and 62%, with a median of 47%, for  
 363 the archaeological sampled objects and between 49% and 38% for the modern references used in this  
 364 study.



365  
 366 **Figure 5.** Percentage of deamidation sites for the modern and archaeological samples. DK = Djoumboulak Koum. NC =  
 367 Northern Cemetery.  
 368

369 Finally, a database search in the error-tolerant mode revealed lysine carbamylation as a frequent  
 370 modification, as already reported (Clerens et al., 2010; Solazzo et al., 2013). This modification may  
 371 arise from sample treatment, which includes a long incubation under the presence of urea (Sun et al.,  
 372 2014). However, if it increased the complexity of digests analysed, it did not affect protein/peptide  
 373 identification since the peptides were generally present in both modified and unmodified forms.

374

#### 375 4. Discussion

376

##### 377 4.1. Keratins identification, variation and degradation

378

379 A high percentage of archaeozoological identifications was obtained due to good sample preservation,  
 380 owing to the dry environment at the two sites. In total, 97% of the samples were identified to the tribe  
 381 level or lower, and 85% of caprines were identified to the genus level. This result confirms that  
 382 proteomics, when allied with detection of appropriate markers, is a powerful technique to differentiate  
 383 ancient animal fibres. One limitation of this technique is that it requires complete and reliable  
 384 sequence databases. Thus, a key challenge to extend the potential of palaeoproteomics is to enrich  
 385 those protein sequence databases, particularly with ancient species and with amino acid variations  
 386 resulting from single nucleotide polymorphism.

387 The best-hit proteins were generally the type I keratins K33 and K31. The representation of type II  
 388 keratins and KAPs strongly differed depending on sample preservation. For keratin K33, sheep and  
 389 cattle exhibited K33b as the most represented isoform, while goat exhibited K33a as the most  
 390 represented isoform. In goat, keratin K33a is highly expressed in winter and constitutes a major  
 391 component of cashmere (Seki et al., 2011), which explains its high representation in goat samples. In  
 392 addition, single nucleotide polymorphisms have been reported in keratin K33a gene sequences among  
 393 domestic and wild goats, resulting in amino acid differences among the individual goats (Seki et al.,  
 394 2011) at positions 24 (S or P), 104 (Q or H), 202 (R or Q), and 271 (E or D). Position 104 corresponds  
 395 to the peptide marker ILERSQQQEPLVCPNYXSYFR, where X denotes glutamine or histidine at  
 396 position 104. In our study, we found two samples (P237 and P263) with an alanine at position 104,  
 397 which suggests a new polymorphism in K33a keratin. Since these two samples did not exhibit any  
 398 specific goat peptide markers, they were categorised as undetermined caprine. However, the presence  
 399 of the peptide AFSCVSACGPRPSR in P263, which was detected in 16 out of 17 goat samples and  
 400 could correspond to goat, horse, or deer based on the databases, strongly supports that they are goat

401 samples with a particular polymorphism in the gene encoding K33a. If the discrimination between  
402 goat and sheep was feasible in most of the cases, distinction among breeds appears challenging in the  
403 case of archaeological samples, since their distinction relies on different levels of KAPs or minor  
404 proteins (Li et al., 2018; Plowman et al., 2018).

405 The difference in the deamidation ratio between archaeological and modern samples was remarkably  
406 low (Figure 5). The hair of Andean mummies, also preserved under a dry climate, showed more than  
407 85% deamidated sites according to Fresnais et al. (2017). A comparison of Djoumboulak Koum and  
408 the Northern Cemetery raw fibres and textiles shows that the difference in deamidation rates between  
409 those two categories is minor. The textiles seem slightly more degraded compared with the raw fibres  
410 – and they appeared more fragile during the wet pre-treatment steps – most probably due to alterations  
411 related to manufacturing and dyeing processes.

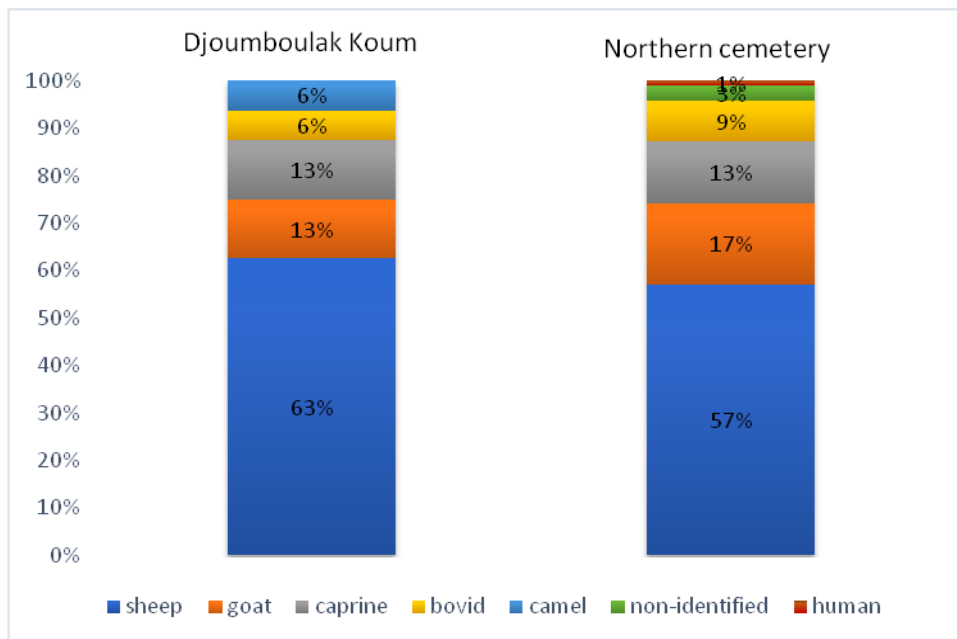
412  
413 4.2. The use of animal  
414 fibres in textile manufacture in Xinjiang

415  
416 Looking at the fibre identifications, it seems that not all animals present in this region were used for  
417 their fibres. A previous archaeozoological study showed that several bones of horse and dog were  
418 recovered at Djoumboulak Koum (Lepetz, 2001, Figure 8), but no fibre from these species was  
419 identified by our proteomics study. The number or choice of sampled objects may have influenced this  
420 result. We note that few horsehair hairnets were identified by the archaeologists, and it seems that  
421 horsehair was reserved for this specific use. An independence chi-square test was used to detect  
422 association between species distribution and collection site. It revealed that the two sites are  
423 independent and thus that their distribution cannot be considered identical (chi-square = 6.85, p-value  
424 = 0.33). However, both sites showed a high proportion of sheep (above 50%), and lower proportions  
425 of goat and cattle (Figure 6).

426 For Bronze Age cemeteries of the so-called Xiaohe culture, osteological data (Debaine-Francfort,  
427 2019) show the frequent presence of bovine heads placed in the graves and attached to poles marking  
428 the presence of the graves. In addition, cattle hides were placed on the coffins. At Xiaohe, where the  
429 tombs are best preserved, painted bovine skulls with massive horns were also placed on the shroud  
430 (Debaine-Francfort, 2019). Cattle also appear in other forms, notably as cut ears and tendons, but also  
431 as cow dung, dairy products, cosmetic sticks, and glue (Qiu, Z. et al, 2014; Liang, Y. et al., 2012;  
432 Yang, Y., 2014; Rao et al., 2015). DNA study of the cattle indicates that they are *Bos taurus*,  
433 originally of West Asian origin (Cai, D. et al., 2014). This reveals the importance of this animal in  
434 Bronze Age cultures. But because these are very particular choices related to the ritual character of the  
435 site, it is not possible to use these osteological data to deduce in detail the use of animals in the  
436 economy of these societies and therefore to state, for example, that cattle were more numerous than  
437 sheep in the herds. Moreover, our study shows a clear preference for sheep wool in textile  
438 manufacturing at both sites. It can therefore be deduced that (1) there was a choice in the animals used  
439 in funeral practices; (2) sheep had an important place in artisanal activities; and (3) care must be taken  
440 to consider all these elements when addressing issues of breeding strategies and, more broadly, of the  
441 food economy.

442

443



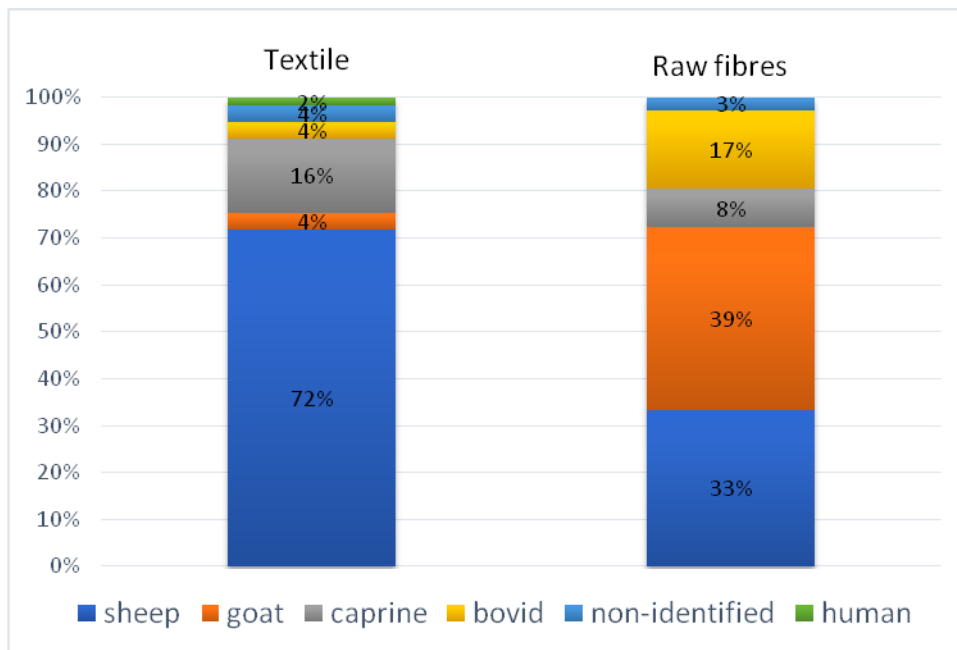
444 **Figure 6.** Taxonomic distribution of fibre origin at Djoumboulak Koum and the Northern Cemetery.  
 445

446 A human hair string was identified from the Northern Cemetery. Archaeologists have previously  
 447 described braided or twisted human hair postiches from Djoumboulak Koum. These artefacts are often  
 448 present in steppe traditions, and various examples have been found in Xinjiang and Altai graves  
 449 (Debaine-Francfort and Idriss, 2001). Thus, this cord from the Northern Cemetery may be a postiche  
 450 element that belongs to this tradition.

451  
 452 One hair sample collected in the F4 settlement unit at Djoumboulak Koum was identified as camel,  
 453 whereas no camel fibre was identified in the 93 sampled objects from the Northern Cemetery. This  
 454 difference in camel hair occurrence could result from the difference in social function between the two  
 455 sites. But it comes from a refuse pit containing animal remains.

456  
 457 The large number of objects sampled at the Northern Cemetery (35 raw fibres and 58 textiles)  
 458 permitted us to assess the preferential uses of each species (Figure 7). The species distribution  
 459 revealed a strong difference between raw fibres and textiles. Indeed, 72% of the textile fibres were  
 460 made from sheep's wool, whereas only 4% were made of goat hair. On the contrary, most of the goat  
 461 fibres were found as raw material, mostly furs or pelts. The softness of sheep fibre compared with that  
 462 of goat may explain this difference. Cattle fibres, like goat fibres, were mostly present as furs, but  
 463 these were also used in the confection of shoes. This use most probably relied on their coarse,  
 464 resistant, and thick skin. Shoelaces were made either from undetermined caprine or from sheep,  
 465 revealing objects made from a mix of fibres. However, the number of shoe samples is too small to  
 466 generalise about the use of cattle hides. The results from an ongoing study on a larger corpus from the  
 467 Northern Cemetery, Djoumboulak Koum, and Xiaohe provide better insight into shoe manufacture,  
 468 revealing that attention was paid to the choice of the raw fibre in the fabrication of textiles and the use  
 469 of furs from the Keriya population.

470

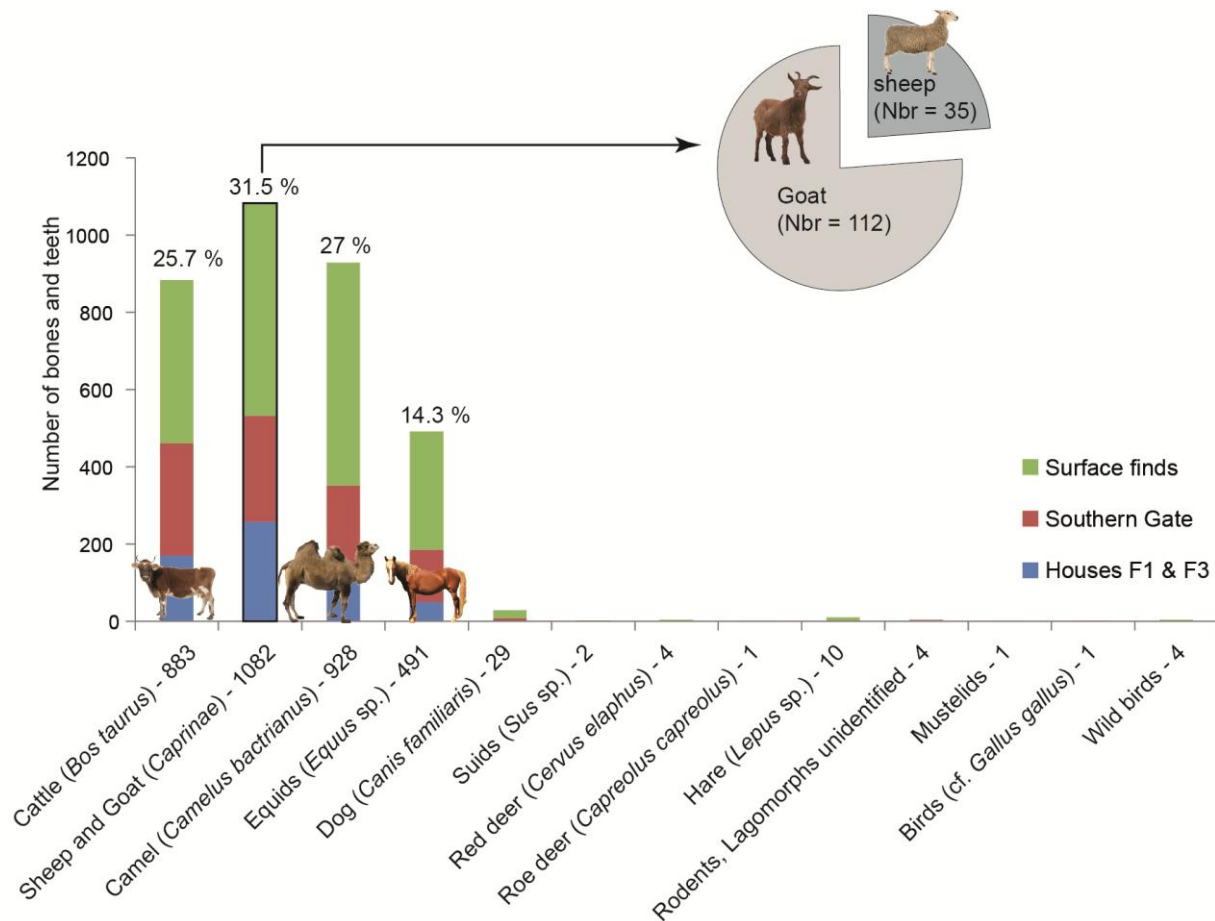


471  
 472 **Figure 7.** Species distribution by type of product found at the Northern Cemetery.  
 473

474 At Djoumboulak Koum, animal bones and teeth are mostly (99%) from domestic species (goat, sheep,  
 475 dog, cattle, camel, horse). Only 1% was attributed to wild animals (Lepetz, 2001) (Figure 8), which is  
 476 consistent with the fibre assemblage. However, the importance of each of these domestic species  
 477 differs within the two sub-assemblages. Caprines represent only 31.5% of the bone remains, whereas  
 478 they dominate (87.5%) the fibre assemblage. Cattle and camel are well represented (>25%) in the  
 479 remains (NISP), while we identified only 1 item representing camel and 1 representing cattle from our  
 480 16 fibre samples. Finally, 14.3% of the bones come from equids, while no horse was found in our  
 481 proteomics study. Regarding caprines, among the bones, the majority of those identified below the  
 482 subfamily level are goat rather than sheep (76% vs. 24%), while we observe the opposite trend for the  
 483 fibres (12.5% vs. 62.5%). These differences could be explained by the small number of fibre samples  
 484 collected from Djoumboulak Koum (n=16), which may not be representative of the site as a whole.  
 485 Alternatively, it might also indicate that animal fibre use was species-dependent and reflected the  
 486 needs of the population. Sheep may well have represented a small minority of the farmed animals and  
 487 were largely used for textile manufacture. The sheep herd would have been managed to provide a  
 488 sufficient quantity of wool from live sheep. In contrast, obtaining goat pelts would have required the  
 489 animals to be slaughtered.

490





491  
 492 **Figure 8.** Animal taxa identified at Djoumboulak Koum. Nbr = Number. The remains of the South Gate and in houses F1 and  
 493 F2 were found during the excavations. Manual collect is a collection from the surface of the ground during surveys (not  
 494 excavation).  
 495

496 Many textiles remains have been found in Eurasia, in the form of fibres as well as ceramic impressions  
 497 or carbonised fragments, for instance at Begash (Kazakhstan) (Doumani Dupuy et al., 2018; Doumani  
 498 and Frachetti, 2012). Unfortunately, despite the numerous textiles found, their identification mostly  
 499 remains at a very basic taxonomic level (i.e., animal vs. vegetal). When the issue of textiles is  
 500 addressed, it is often from a technological point of view, through the analysis of weaving techniques or  
 501 the identification of dyes (Keller et al., 2001; Kramell et al., 2014; Liu et al., 2011; Zhang et al., 2008).  
 502 These studies only touch upon the link with pastoralism. It would be interesting to perform proteomics  
 503 on the textiles from Chärchän (1000 BCE to 3rd century CE) and Karadong (3rd to early 4th century  
 504 CE), where possible mohair goat wool textiles were described (Desrosiers and Debaine-Francfort,  
 505 2016; Good, 1998), to validate these results. If goat fibres were used, it would reveal an interesting  
 506 difference in fibre use between different periods in the Taklamakan desert. The origin of mohair goat  
 507 is still not fully understood (Ryder, 1993), and its presence at these sites could be evidence in favour  
 508 of a central Asia origin. If we focus on the Keriya valley, the Karadong site revealed not only woollen  
 509 but also silk and cotton textiles, as proof of the increase in exchange and the transfer of goods linked  
 510 to the appearance of the Silk Road (Desrosiers and Debaine-Francfort, 2016). This change in textile  
 511 fabrication habits certainly would have had an influence on herd management strategies.

512  
 513  
 514  
 515

516 5. Conclusion

517

518 The dry climate of the Tarim Basin and the Taklamakan desert is highly favourable to the preservation  
519 of animal fibre. The results of this study confirm the heuristic potential of the proteomic approach for  
520 the determination of archaeological fibres and for textiles studies in general, especially when the fibres  
521 are degraded and their distinctive features are no longer observable or interpretable by other methods.  
522 In combination with other analytical approaches, such as microscopy, as well as archaeozoological and  
523 palaeoenvironmental studies, it also holds more global potential for archaeology that goes far beyond a  
524 simple case study.

525 The good state of preservation and the use of proteomics enabled us to identify 97% of the remains to  
526 the tribe level or lower and 85% of the caprines to the genus level. This study permitted us to extend  
527 the list of peptide markers useful for species identification within Bovidae, but also for camel and  
528 human. The comparison between the two Keriya valley sites, Djoumboulak Koum and the Northern  
529 Cemetery, showed similarities in the fabrication and use of textiles, with a predominance of sheep and  
530 goat fibres, followed by cattle. Camel and human fibres were used only occasionally. During the  
531 Bronze Age, sheep were largely used for manufactured woollen textiles, whereas goats provided  
532 mostly furs and pelts. The few shoes analysed are made from cattle hide, while the shoelaces are made  
533 from sheep or undetermined caprine fibres. A comparison of the taxonomic composition of the fibre  
534 assemblage at Djoumboulak Koum and at the Northern Cemetery with the respective bone assemblage  
535 suggests the occurrence of specific herd management strategies according to the animal species, in  
536 order to provide enough raw material. Our analysis underlines the need to base argumentation on the  
537 widest possible range of data types if the question of the economics of these societies, and of their  
538 rites, is to be effectively addressed. It would be highly desirable to extend this approach to other sites  
539 that provided textiles, in order to further explore regional differences in the use of the animal fibres  
540 and the changes caused by the spread of cotton and silk in this region.

541

542

543 Acknowledgments

544

545 We thank the members of the Sino-French archaeological Mission in Xinjiang who provided the  
546 samples. All proteomics experiments were conducted in the bioorganic mass spectrometry platform at  
547 the National Natural History Museum in Paris. We are also thankful for the critical comments of the  
548 three reviewers that greatly helped to improve this paper as well as S. Needs for English editing.

549 Funding: This work was supported by a grant from Agence Nationale de la Recherche under the  
550 LabEx ANR-10-LABX-0003-BCDiv, in the program “Investissements d’avenir” n\u0001 ANR-11-  
551 IDEX-0004-02.

552

553 Declarations of interest: none

554

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