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1 **Looking for constraints: exploring proteins characterisation in arid**  
2 **environments to unravel past human adaptations to climate change**

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21

22 **Keywords**

23 Prehistoric archaeology; climate change; subsistence strategies; ancient proteins; arid environments

## 24 **Résumé**

25 La pression environnementale est l'un des facteurs ayant probablement affecté les populations  
26 humaines passées et leur développement cognitif. L'adoption progressive d'animaux et de plantes  
27 domestiques représente un tournant dans la Préhistoire humaine. Or, retracer l'adoption de ces  
28 pratiques et leur potentiel lien avec des événements climatiques majeurs peut s'avérer un véritable  
29 défi pour l'archéologie préhistorique. En effet, les dernières décennies ont montré que les périodes  
30 de refroidissement ou d'assèchement environnemental ont pu jouer un rôle plus important qu'on  
31 ne le pensait dans le développement de la cognition humaine et, par conséquent, dans l'adoption  
32 de certaines pratiques de subsistance. L'avènement des méthodes biomoléculaires, qui permettent  
33 de caractériser le matériel génétique conservé dans les écofacts et les artefacts archéologiques, a  
34 permis d'apporter de nouvelles données concernant notre compréhension des populations passées.  
35 Avec de meilleures chances de survie dans les contextes archéologiques, les protéines anciennes  
36 ont d'ores et déjà prouvé leur potentiel pour aider à la compréhension de nombreuses  
37 problématiques archéologiques. Afin d'apporter de nouvelles données concernant les réponses  
38 humaines aux pressions climatiques dans l'adoption de pratiques d'élevage, cet article présente une  
39 étude paléoprotéomique préliminaire axée sur les restes de Bovidae provenant de sites  
40 archéologiques de contextes secs à arides en Jordanie, au Soudan et en Namibie. Les spécimens  
41 ont été sélectionnés pour couvrir une période couvrant la fin du *Younger Dryas* en Jordanie au *Little*  
42 *Ice Age* en Namibie.

43

## 44 **Abstract**

45 Environmental pressure is one of the factors that probably affected past human populations and  
46 their cognitive behaviour. One spinning point in human prehistory is the progressive adoption of  
47 domestic animals and plants. However, tracing the adoption of these practices and their relation  
48 to major climatic events could be a real challenge in prehistoric archaeology. In fact, these past

49 decades have proven that the cooling or dryer environmental periods might have played a greater  
50 role than assumed in the development of human cognition, and consequently, for the adoption of  
51 certain subsistence practices. The advent of biomolecular methods, allowing for the  
52 characterisation of genetic material preserved in archaeological ecofacts and artefacts have  
53 increasingly extended our knowledge of past populations. With higher chances of survival within  
54 archaeological contexts, ancient proteins have revealed their potential in helping to shed light on  
55 numerous archaeological questions. For the purpose of trying to infer human responses to climatic  
56 pressure in the adoption of herding practices, this paper presents a preliminary palaeoproteomics  
57 study focusing on remains of Bovidae from dry to arid archaeological sites from Jordan and  
58 Namibia. Specimens were selected so that they cover a period from hunting and gathering practices  
59 to established pastoralism and covering environmental conditions from the end of the Younger  
60 Dryas in Jordan to the Little Ice Age in Namibia.

61

62 **Introduction**

63 *Cognition and influence of climate on adoption of various subsistence strategies*

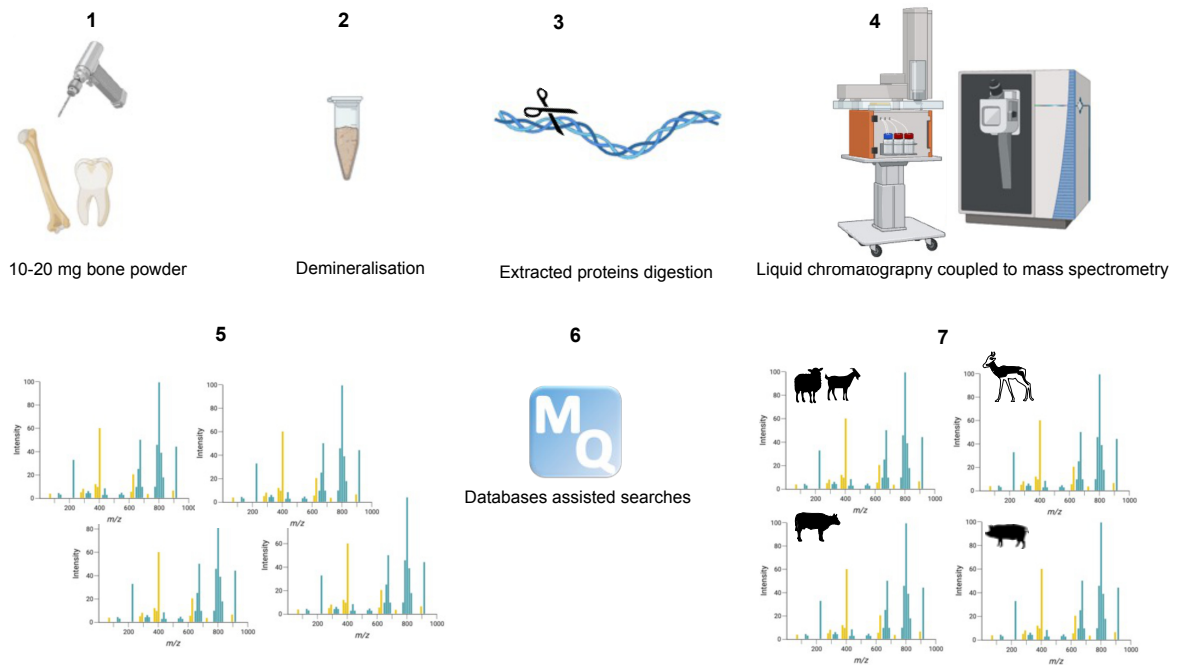
64 The relationship existing between human cognition and culture (in a very broad acceptance) has  
65 already been discussed extensively, especially in the frame of thinking and material culture  
66 production. This entire discipline called cognitive archaeology, has greatly improved our  
67 understanding of the links between cognitive development and production (whether it be material,  
68 spiritual or social)<sup>1</sup>. However, assessing these links is a real archaeological challenge when  
69 concerning changes in subsistence strategies. There is, in fact, no *production* per se when focusing  
70 on subsistence in Prehistory. One way of characterising subsistence in archaeology is through the  
71 study of faunal remains. Multiple lines of evidence can be used to do so, from comparative  
72 anatomy to traceology or molecular identifications.

73 *The Levant and Africa as case studies: arid environments and the adoption of herding practices*

74 One of the major shifts human populations had to experienced lies in the advent of  
75 domestication, also referred to as the “Neolithic revolution”. Not only did it lead to major  
76 biological and societal changes (both for animals/plants and humans), but it also re-shaped the  
77 environment. Rather seen as a complex process than an abrupt shift, the domestication of animals  
78 happened in the Zagros mountain in current Middle East<sup>2,3</sup>. Domestication is a relatively long  
79 process occurring over generations that implies the replacement of one wild population by a  
80 domestic one. Nowadays, sheep, *Ovis aries* and goats, *Capra hircus*, lie at the centre of many  
81 economies across the globe and are thus of interest as to where and how they were domesticated  
82 in the first place. The two species thus result from the management of their wild ancestors, the  
83 Asiatic mouflon, *Ovis orientalis* and the wild bezoar, *Capra aegagrus*, initiated after the end of the  
84 Younger Dryas, around 9,600 before common era (BCE)<sup>4</sup>. The initial management of these species  
85 lead to their domestic forms by 7000 BCE<sup>5</sup>. Introduced in Africa later in comparison, around the  
86 seventh millenium BCE in the northeastern part of the continent, they are documented around  
87 the turn of the era in the southernmost tip<sup>6</sup>.

88 *State of the art on studies of ancient proteins in the Levant and Africa*

89 Molecular methods employed in archaeology are numerous and one could think about  
90 stable isotopes, dating methods or ancient DNA (aDNA) analyses. These methods can be  
91 employed to recover information about past diet, environments, chronologies or past populations  
92 gene pools. One molecule of choice is thus aDNA, allowing to infer



93  
94 *Figure 1: General description of the palaeoproteomics workflow when looking for species identification of archaeological remains.*

95  
96 *Hypothesis and question(s) driving the study*

97 This project revolves around the question of the influence of climatic events on the adoption  
98 of herding practices in challenging environments. The archaeological material was selected for the  
99 triple purpose of: covering a large chronology covering the advent of herding practices as long as  
100 various climatic events that might have influenced the adoption of such practices; securely  
101 identifying the species of the specimens (*i.e.* either from domestic sheep or goat, wild gazelles or  
102 wild caprines in the case of the Jordanian sites); and finally, confirming the effectiveness of ancient  
103 proteins characterisation in the dry and arid environments of the Near East and Africa from  
104 various time-scales. In order to gain insights at these questions, we selected three archaeological

105 sites located in Jordan and covering a large chronology from the Natufian to the end of the PPNA:  
106 Shubayqa 1 and 6 and Shkarāt Msaied (see extensive description in the Material and Methods  
107 section following). We also included two African sites corresponding to different environmental  
108 conditions: SJE 270 in Sudan and Leopard Cave in Namibia, representing the current climatic  
109 conditions and the Little Ice Age, respectively. We selected samples along the stratigraphy to  
110 identify potential shifts in subsistence strategies, although our sample size remains sparse due to  
111 unknown protein preservation. We compared two ancient proteins extraction protocols to identify  
112 a potential ideal extraction strategy for the sampled remains and analysed them using liquid  
113 chromatography coupled to tandem mass spectrometry. Finally, we used a database partially  
114 developed during the study, to identify the archaeological specimens as specifically as possible.

115

## 116 **Material and method**

117 *About having the proper references – establishment of bovids COL1 sequences*

118 Since (palaeo)proteomics rely on the comparison of the obtained data to available protein  
119 references, many bioarchaeologists already faced the issue of the lack of reference in previous  
120 studies<sup>7,8</sup>. A substantial amount of time of this work was thus dedicated to the re-analysis of COL1  
121 sequences data to allow for the species identification of arid environments Bovidae (for an detailed  
122 description, see ref.<sup>9</sup>). The establishment of this accurate referential to work with is however critical  
123 to go beyond “classical” comparative anatomy and morphology-based methods and thus, enhance  
124 our comprehension of species distribution, ecology and subsequent human exploitation.

125 Then, we recently published a new COL1 reference database accessible to anyone willing  
126 to use it<sup>9</sup> and including nineteen species of wild Bovidae falling in the “size 2/3 bovids” category  
127 (classically employed by zooarchaeologists when preservation of the archaeological material does  
128 not allow to identify the remains more specifically). Mostly composed of wild gazelles that can be  
129 confused with sheep and goat, the database also includes two species of wild caprines, the Barbary  
130 sheep, *Ammotragus lervia*, and the Nubian ibex, *Capra nubiana*. Even though the database used to

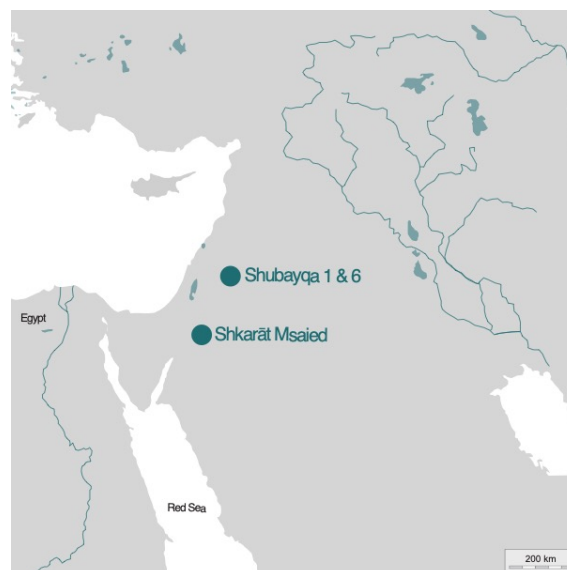
131 assign the studied archaeological specimens is lacking endemic species from Jordan, it presents  
132 African species of the same genera. Indeed, given the consistency of COL1 sequences within the  
133 genera of family Bovidae (we did not observe any amino acid substitution from genus *Gazella*, and  
134 very few in the different Antilopinae species for instance), we did not expect any mis assignment  
135 that might have been due to a database bias.

136 *Archaeological context*

137 Shkarāt Msaied and Shubayqa 1 & 6

138 Located in current Jordan, the archaeological sites of Shkarāt Msaied and Shubayqa 1 and  
139 6 are covering a large chronology from early Natufian to the pre-pottery neolithic B (PPNB), with  
140 remains of domesticated sheep and goats morphologically identified at both sites.

141 Shubayqa 1 and 6 are located less than a kilometre apart, on the edge of the Qa' Shubayqa  
142 basin in the Harrat ash-Sham desert in north-eastern Jordan (Fig. 2,<sup>10,11</sup>). The stratigraphy indicates  
143 sporadic occupations from 12400 BCE to 9650 BCE at Shubayqa 1<sup>10</sup>, when Shubayqa 6 was  
144 occupied continuously from 10420 to 8640 BCE<sup>12</sup>. Both sites thus cover a time-range spanning  
145 from the Natufian to the PPNA, covering environmental conditions including the Bølling-Allerød,  
146 the Younger Dryas and the return to milder conditions during the Pre-Boreal oscillation<sup>5</sup>.



147  
148 *Figure 2: Locations of the Levantine archaeological sites included in the project.*

149 Shkarāt Msaied is almost at the opposite side of the country, north of Petra in south-  
150 western Jordan, close to the Israeli border (Fig. 2)<sup>13</sup>. The stratigraphy indicates human occupation  
151 starting during the middle PPNB (MPPNB) around 8000 BCE<sup>14,15</sup>. Circular stone buildings and  
152 material culture related to daily life (cooking areas) have been excavated, associated with what was  
153 described as a “small village”<sup>16,17</sup>. Thus, occupations at Shkarāt Msaied correspond to the Holocene  
154 climatic optimum, environmental conditions resembling the ones currently observed in the region.

#### 155 SJE 170, Sudan

156 Between 1960-1964, extensive rescue excavation campaigns were conducted by a team of  
157 Nordic prehistorian in Nubian Sudan. The sites discovered and documented by the Scandinavian  
158 Joint Expedition in Sudanese Nubia (SJE) are curated in different places of Scandinavia, including  
159 Denmark, at the Zoological Museum (Univ. of Copenhagen)<sup>18</sup>. We accessed domesticated caprines  
160 material from site number 270, a funerary context in which the caprines were originally associated  
161 with human burials<sup>19</sup> (Fig. 3). The 270 site has previously been dated from 1950 to 1750 BCE,  
162 period associated with C-group culture, with large buildings and wealthy burials. Resembling  
163 Bronze Age cultures in other parts of the world<sup>20</sup>, and contemporary of the Middle Kerma phase  
164 for site 270, C-group Nubian populations were close to the river Nile and experienced  
165 environmental conditions resembling the ones occurring nowadays in south Egypt and north  
166 Sudan, consecutive to the mid-third millennium BCE aridification<sup>21</sup>.



Figure 3: Location of the African sites included in the project.

### Leopard Cave, Namibia

Leopard Cave is located in the Erongo mountains, north of the capital of Namibia and has been discovered in 2007<sup>22</sup> (Fig. 3). The rock shelter presents a chronologically long sequence, from the end of the Middle Stone Age (MSA) to the Later Stone Age (LSA)<sup>23</sup>. Recently, our team has revised the morphological assignation to sheep for some of the faunal remains of the sites, pushing back the arrival of the domesticates at the site to *ca* 1000 CE<sup>7</sup>. We indeed confirmed the presence of sheep at the site during the Little Ice Age, but not earlier.

#### *Proteins extraction and characterisation*

A total of 47 archaeological remains morphologically identified as sheep/goat, antelopes/gazelles or ‘unidentified Bovidae’ were selected. An additional set of eight samples from Shkarāt Msaied from which shotgun proteomics data were already available were included to our analyses. All processed sampled are described in Table 1.

Table 1: Archaeological specimens and corresponding dating, culture and morphological identification selected for the study. Specimens marked with \* stand for the data re-analysis performed by the first author for the Jensen et al. 2023 paper. All other samples were prepared and analysed following the arid environment protocol.

Archaeological site	Sample code	Dating (years BCE)	Culture	Skeletal element	Morphological ID	Reference
Shubayqa 1	SH_1	12300-12000	Natufian	L. humerus	<i>Gazella</i> sp.	Richter <i>et al.</i> 2017
	SH_2	12300	Natufian	R. tibia	<i>Gazella</i> sp.	Richter <i>et al.</i> 2016
	SH_3	12300	Natufian	L. tibia	<i>Gazella</i> sp.	Richter <i>et al.</i> 2016
	SH_4	12300-12000	Natufian	L. tibia	<i>Gazella</i> sp.	Richter <i>et al.</i> 2016
	SH_5	11200-10000	Natufian	L. humerus	<i>Gazella</i> sp.	Richter <i>et al.</i> 2016
	SH_6	10000-9800	Natufian	Tibia	<i>Gazella</i> sp.	Richter <i>et al.</i> 2016
Shubayqa 6	SH_7	10400-10000	Natufian	L. humerus	<i>Gazella</i> sp.	Richter <i>et al.</i> 2016
	SH_8	9000	PPNA	Humerus	<i>Capra/Oris</i>	
	SH_9	test pit	N/A	Tibia	<i>Capra/Oris</i>	
Shkarāt Msaied	SM_20	7000	LPPNB	Femur	<i>Capra/Oris</i>	Bangsgaard 2008
	SM_21	7000	LPPNB	Humerus	<i>Capra/Oris</i>	Bangsgaard 2009
	SM_22	7000	LPPNB	Humerus	<i>Capra</i> sp.	Bangsgaard 2010
	SM_23	7000	LPPNB	Humerus	<i>Capra/Oris</i>	Bangsgaard 2011
	SM_24	7000	LPPNB	Tibia	<i>Capra/Oris</i>	Bangsgaard 2012
	SM_25	7000	LPPNB	Mandible	<i>Capra/Oris</i>	Bangsgaard 2013
	SM_26	7000	LPPNB	Tibia	<i>Capra/Oris</i>	Bangsgaard 2014
	SM_27	7000	LPPNB	Humerus	<i>Capra/Oris</i>	Bangsgaard 2015
	SM_28	7000	LPPNB	Humerus	<i>Capra/Oris</i>	Bangsgaard 2016
	SM_29	7000	LPPNB	Mandible	<i>Capra/Oris</i>	Bangsgaard 2017
	SM_30	7000	LPPNB	Mandible	<i>Capra/Oris</i>	Bangsgaard 2018
	SM_31	7000	LPPNB	Tibia	<i>Capra</i> sp.	Bangsgaard 2019
	SM_32	7000	LPPNB	Tibia	<i>Capra/Oris</i>	Bangsgaard 2020
	SM_33	7000	LPPNB	Mandible	<i>Capra/Oris</i>	Bangsgaard 2021
	SM_34	7000	LPPNB	Humerus	<i>Capra</i> sp.	Bangsgaard 2022
	SM_35	7000	LPPNB	Humerus	<i>Capra</i> sp.	Bangsgaard 2023
	SM_36	7000	LPPNB	Tibia	<i>Capra/Oris</i>	Bangsgaard 2024
	SM_37	7000	LPPNB	Humerus	<i>Capra</i> sp.	Bangsgaard 2025
	SM_38	7000	LPPNB	Humerus	<i>Capra</i> sp.	Bangsgaard 2026
	SM4*	8000	MPPNB	Petrous	Caprinae	Jensen <i>et al.</i> 2023
	SM5*	8000	MPPNB	Petrous	Caprinae	Jensen <i>et al.</i> 2023
	SM8*	8000	MPPNB	Petrous	Caprinae	Jensen <i>et al.</i> 2023
SM10*	8000	MPPNB	Petrous	Caprinae	Jensen <i>et al.</i> 2023	
SM12*	8000	MPPNB	Petrous	Caprinae	Jensen <i>et al.</i> 2023	
SM18*	8000	MPPNB	Petrous	Caprinae	Jensen <i>et al.</i> 2023	
SM23*	8000	MPPNB	Petrous	Caprinae	Jensen <i>et al.</i> 2023	
SM26*	8000	MPPNB	Petrous	Caprinae	Jensen <i>et al.</i> 2023	
SJE 270	SJE_10	2500-2050	C-group	Tibia	<i>Capra/Oris</i>	Johansson 2014
	SJE_11	2500-2050	C-group	Tibia	<i>Capra/Oris</i>	Johansson 2014

	SJE_12	2500-2050	C-group	Cranium	<i>Ovis aries</i>	Johansson 2014
	SJE_13	2500-2050	C-group	Tibia	<i>Capra/Ovis</i>	Johansson 2014
	SJE_14	2500-2050	C-group	Mandible	<i>Ovis aries</i>	Johansson 2014
	SJE_15	2500-2050	C-group	Mandible	<i>Ovis aries</i>	Johansson 2014
	SJE_16	2500-2050	C-group	Mandible	<i>Capra/Ovis</i>	Johansson 2014
	SJE_17	2500-2050	C-group	Vertebra	<i>Capra/Ovis</i>	Johansson 2014
	SJE_18	2500-2050	C-group	Mandible	<i>Ovis aries</i>	Johansson 2014
	SJE_19	2500-2050	C-group	Tibia	<i>Capra/Ovis</i>	Johansson 2014
Leopard Cave	LC_166	1st c.	LSA	Unidentified	Size 2/3 bovids	Pleurdeau et al. 2012
	LC_167	1st c.	LSA	Unidentified	Size 2/3 bovids	Pleurdeau et al. 2012
	LC_168	1st c.	LSA	Unidentified	Size 2/3 bovids	Pleurdeau et al. 2012
	LC_169	1st c.	LSA	Unidentified	Size 2/3 bovids	Pleurdeau et al. 2012
	LC_170	1st c.	LSA	Unidentified	Size 2/3 bovids	Pleurdeau et al. 2012
	LC_171	1st c.	LSA	Metatarsal	Size 2/3 bovids	Pleurdeau et al. 2012
	LC_172	1st c.	LSA	Vertebra	Size 2/3 bovids	Pleurdeau et al. 2012
	LC_173	1st c.	LSA	Unidentified	Size 2/3 bovids	Pleurdeau et al. 2012
	LC_174	1st c.	LSA	Unidentified	Size 2/3 bovids	Pleurdeau et al. 2012

184            Approximately 20 mg of bone powder was sampled for each of the 47 archaeological  
185 specimens using a diamond drill or disc, cleaned thoroughly in between samples with 96% ethanol  
186 to avoid cross-contamination. Proteins extractions were carried out as follow: the 47 specimens  
187 and one experimental blank (hereafter, first batch) were treated using the ‘arid environment  
188 protocol’ that was optimised<sup>24,25</sup>; while the other eight (hereafter, second batch) were treated using  
189 the new ‘Tryps-IN’ simplified protocol that our team published recently<sup>15</sup>. All samples underwent  
190 enzymatic hydrolysis using trypsin, allowing to break down the proteins into smaller pieces, or  
191 peptides.

192            All 56 samples were analysed by liquid chromatography coupled to tandem mass  
193 spectrometry (LC-MS/MS) at the Center for Protein Research (CPR, Novo Nordisk Foundation,  
194 Univ. of Copenhagen). First, the peptides contained in each sample were separated regarding their  
195 affinity with the liquid chromatography column internal phase. Consequently, molecules were  
196 ionised (addition of a positive charge), separated based on their mass over charge ratio ( $m/z$ ) in  
197 the mass spectrometer, and detected. The resulting data for each sample consist in a set of spectra,

198 each corresponding to a specific peptide, allowing the bioinformatic reconstruction of the initial  
199 protein.

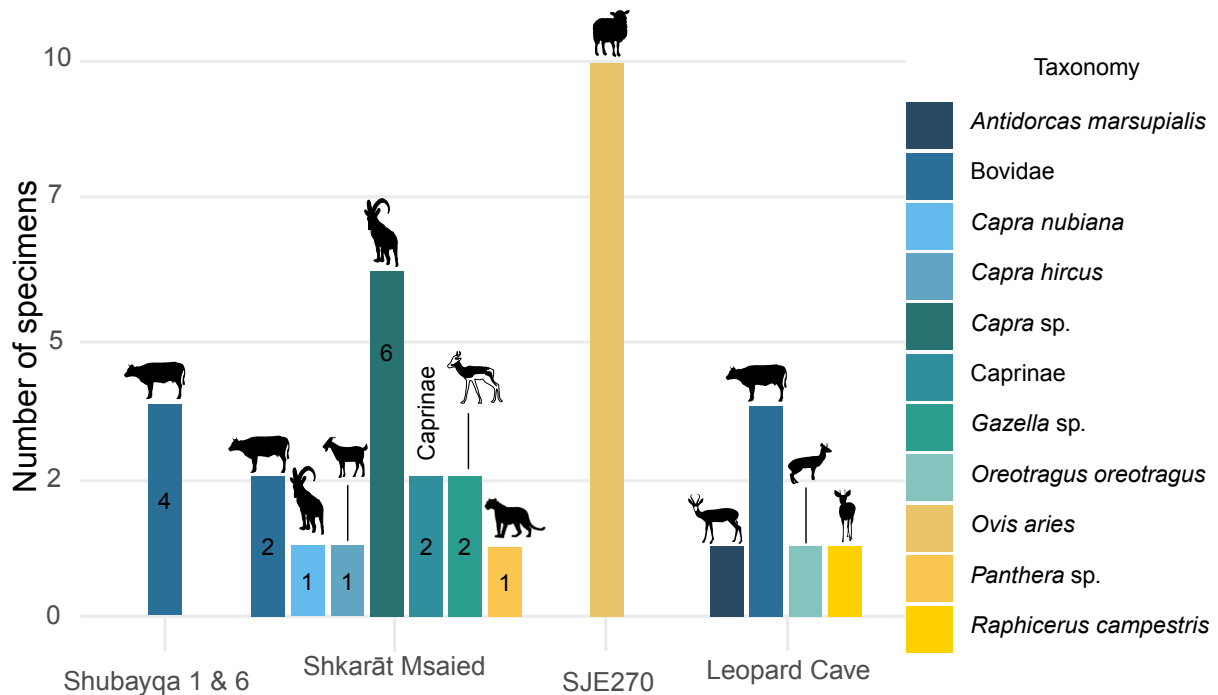
200 All raw data were search against the reference Bovidae COL1 database described in a  
201 previous section and available in ref.<sup>9</sup> using the MaxQuant software (v. 2.3.0,<sup>26</sup>). Parameters for  
202 the different searches were set as follow: we allowed 2 tryptic missed-cleavages, post-translational  
203 modifications included oxidation (M), hydroxyproline, deamidation (N, Q) and false discovery rate  
204 (FDR) was set to 1%. All other parameters were set as default. Identifications were considered  
205 confident when at least 2 peptide spectral matches (PSMs) for one species were observed. A more  
206 descriptive methodology is available in refs.<sup>25,9</sup> and<sup>15</sup>.

207

## 208 **Results and discussion**

### 209 *Molecular identifications of remains from arid environments*

210 Overall, data quality was uneven in between the different archaeological sites. Only three  
211 samples from Shubayqa (1&6) allowed for a very limited number of characterised peptides,  
212 preventing more specific identification than to family level. Over the initial 18 samples from  
213 Shkarāt Msaied, seven allowed for identification from family to species level. However, it is worth  
214 noticing that the samples prepared using the “Tryps-IN” protocol yielded at least genus level  
215 identifications, even though they were almost two centuries older than the previous set. Samples  
216 from site 270 of the SJE were all assigned to sheep, when six out of nine samples from Leopard  
217 Cave present preserved peptides allowing assignation from family to species level (Fig. 4).



218

219 *Figure 4: Molecular taxonomic identifications for all the studied sites of the project.*

220 *Towards an ideal protocol for ancient protein extraction from Holocene arid environments?*

221 Two preparation protocols were used here. Although our initial intention was not to  
 222 compare their efficiency, the analysed specimens originated from the same site, allowing for  
 223 comparison. We observe enhanced identification rates for the specimens analysed using the Tryps-  
 224 IN protocol compared to the ones using the “arid environment” protocol. Not only the number  
 225 of “identifiable” samples was higher with Tryps-IN (100% against 39% for the other protocol),  
 226 but identification were more specific. Given these conclusions, we have confidence that if the  
 227 Tryps-IN protocol was going to be used again on all other samples analysed in this paper, it will  
 228 result in enhance number of identified specimens.

229 *Morphology vs molecular identifications*

230 The analyses presented here allowed to refine the morphological identifications in some  
 231 cases. Proteins did not seem to be preserved enough at Shubayqa 1 and 6 to allow any  
 232 identification, except for one sample for which the preserved peptides did not allow enough  
 233 sequence coverage to confidently identify below family level.

Table 2: Comparison of morphological and molecular identifications obtained for all the analysed samples.

Archaeological site	Sample code	Morphological ID	Molecular ID
Shubayqa 1	SH_1	<i>Gazella</i> sp.	
	SH_2	<i>Gazella</i> sp.	
	SH_3	<i>Gazella</i> sp.	
	SH_4	<i>Gazella</i> sp.	
	SH_5	<i>Gazella</i> sp.	Bovidae?
	SH_6	<i>Gazella</i> sp.	
Shubayqa 6	SH_7	<i>Gazella</i> sp.	
	SH_8	<i>Capra/Ovis</i>	Bovidae?
	SH_9	<i>Capra/Ovis</i>	Bovidae?
Shkarāt Msaied	SM_20	<i>Capra/Ovis</i>	<i>Capra hircus</i>
	SM_21	<i>Capra/Ovis</i>	Caprinae
	SM_22	<i>Capra</i> sp.	
	SM_23	<i>Capra/Ovis</i>	<i>Gazella</i> sp.
	SM_24	<i>Capra/Ovis</i>	
	SM_25	<i>Capra/Ovis</i>	
	SM_26	<i>Capra/Ovis</i>	
	SM_27	<i>Capra/Ovis</i>	
	SM_28	<i>Capra/Ovis</i>	
	SM_29	<i>Capra/Ovis</i>	
	SM_30	<i>Capra/Ovis</i>	
	SM_31	<i>Capra</i> sp.	Bovidae?
	SM_32	<i>Capra/Ovis</i>	
	SM_33	<i>Capra/Ovis</i>	
	SM_34	<i>Capra</i> sp.	<i>Capra nubiana</i>
	SM_35	<i>Capra</i> sp.	Caprinae
	SM_36	<i>Capra/Ovis</i>	Bovidae?
	SM_37	<i>Capra</i> sp.	
	SM_38	<i>Capra</i> sp.	
	SM4	Caprinae	<i>Capra</i> sp.
SM5	Caprinae	<i>Capra</i> sp.	
SM8	Caprinae	<i>Panthera</i> sp.	
SM10	Caprinae	<i>Capra</i> sp.	
SM12	Caprinae	<i>Gazella</i> sp.	
SM18	Caprinae	<i>Capra</i> sp./Antilopinae	

	SM23	Caprinae	<i>Capra</i> sp.
	SM26	Caprinae	<i>Capra</i> sp.
SJE 270	SJE_10	<i>Capra/Ovis</i>	<i>Ovis aries</i>
	SJE_11	<i>Capra/Ovis</i>	<i>Ovis aries</i>
	SJE_12	<i>Ovis aries</i>	<i>Ovis aries</i>
	SJE_13	<i>Capra/Ovis</i>	<i>Ovis aries</i>
	SJE_14	<i>Ovis aries</i>	<i>Ovis aries</i>
	SJE_15	<i>Ovis aries</i>	<i>Ovis aries</i>
	SJE_16	<i>Capra/Ovis</i>	<i>Ovis aries</i>
	SJE_17	<i>Capra/Ovis</i>	<i>Ovis aries</i>
	SJE_18	<i>Ovis aries</i>	<i>Ovis aries</i>
	SJE_19	<i>Capra/Ovis</i>	<i>Ovis aries</i>
Leopard Cave	LC_166	Size 2/3 bovids	<i>Raphicerus campestris</i>
	LC_167	Size 2/3 bovids	
	LC_168	Size 2/3 bovids	<i>Antidorcas marsupialis</i>
	LC_169	Size 2/3 bovids	Bovidae?
	LC_170	Size 2/3 bovids	Bovidae?
	LC_171	Size 2/3 bovids	Bovidae?
	LC_172	Size 2/3 bovids	
	LC_173	Size 2/3 bovids	<i>Oreotragus oreotragus</i>
	LC_174	Size 2/3 bovids	

235 One domestic goat, one gazelle (genus *Gazella*) and a wild goat were identified, along with  
236 two Caprinae and two Bovidae. The domestic and wild goat were discriminated using the single  
237 peptide variant described in ref. <sup>9</sup>, located on the alpha 1 chain of COL1. Since our database  
238 contains only two species of genus *Capra*, the domestic goat *Capra hircus* and the wild Nubian ibex,  
239 *Capra nubiana*, we can confidently argue that the sample identified as *C. hircus* indeed belong to the  
240 latter species. However, the case of the wild *Capra* is different. Since *C. nubiana* is the only reference  
241 in our database, and even though the species is indeed ecologically distributed in the vicinity of  
242 Shkarāt Msaied, we cannot rule out the possibility of the specimen belonging to another species  
243 of ibex, such as *C. aegagrus*. We therefore need to expand our reference sequences of the genus in  
244 order to assign the archaeological specimen to one species or the other.

245 All specimens sampled from the 270 site of the SJE were identified as domestic sheep.  
246 Samples all presented the alpha 1 chain of COL1 peptide that differs from all other species of  
247 either Caprinae or Antilopinae observed in both Barbary (*Ammotragus lervia*) and domestic sheep.  
248 Considering the provenance and dating of the samples, we can confidently rule out the possibility  
249 of them belonging to another species (or wild sheep for instance). Indeed, domestic sheep arrived  
250 in Sudan around the V<sup>th</sup> millennium BCE and thus, the eventuality of the studied samples to belong  
251 to the wild species *A. lervia*, can be excluded.

252 Out of nine samples from Leopard Cave, six presented preserved peptides. For three of  
253 them, the number of peptides was too low to go further than Family level identification. The other  
254 three were assigned to species level, all three to different wild antelopes species. One present the  
255 protein signature of a steenbok, *Raphicerus campestris*; another of a springbok *Antidorcas marsupialis*  
256 and finally, the last sample present peptides of a klipspringer, *Oreotragus oreotragus*. Springbok and  
257 klipspringer have specific peptides that allow to distinguish them from all other Antilopinae, when  
258 steenbok present a combination of COL1 peptides that makes the species rather easily  
259 distinguishable from the other antelopes. The identified samples present either the corresponding  
260 species-specific peptides or combination, allowing to be confident in the subsequent species  
261 assignation. Moreover, these three species are easily observable nowadays in southern Africa, and  
262 especially in Namibia where the site is located<sup>27</sup>. Considering that the current environmental  
263 conditions were established after the end of the AHP, the probability for finding these species in  
264 a first century BCE archaeological site is more than probable.

265 *Climatic influence over subsistence strategies in past Levantine and African populations*

266

## 267 **Conclusion**

268 Palaeoproteomics is rather new field in the landscape of archaeological sciences<sup>28</sup> and it might be  
269 tempting to see it as a more “efficient” way than comparative anatomy or morphometrics for  
270 identifying osteological remains. However, it might be necessary to remind several critical points

271 when working with the molecular method: first, even if the method falls into the “-omics”, it  
272 should be seen as part of archaeometry, and therefore as a methodology in the service of  
273 archaeology and its -many- questions; second, without the initial morphological identification, we  
274 would be completely blind in our analyses and even though it is technically feasible, it is also time  
275 and resources consuming.

276

#### 277 **Data availability**

278 Data will be made available upon publication.

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301

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