



Unmatched DNA preservation prove arctic hare and sheep wool in Norse Greenlandic textile from “The Farm Beneath the Sand”



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ABSTRACT

GUS (*Gården under sandet- The Farm Beneath the Sand*) is a Greenlandic Norse settlement site 80 km from Nuuk in the former Norse western settlement occupied between 1000 and 1400 CE. Renowned for its excellent preservation caused by its interment under large quantities of sand and permafrost after its abandonment, GUS is unique in Norse Greenlandic contexts as perishable materials and artefacts are extremely well preserved. Some aspects of fibre admixtures used in Norse Greenlandic clothing are unknown to us, but of great relevance to understanding the history of the colony and its subsistence practices. Here we present the results of shotgun genomic data from 11 samples originating from ten archaeological textiles from a variety of different Norse Greenlandic sites. The obtained sequences were mapped to mitochondrial genomes of 15 diverse mammals and only samples from GUS had any endogenous DNA (4,5 and 3,5%), resulting in a 70x mt-genome of arctic hare (*Lepus arcticus*) and a 20x mt-genome of domestic sheep (*Ovis aries*). The evidence of arctic hare in Greenlandic textile is one of the few examples confirming the use of exotic textiles and mixtures of wool types in Norse clothing. Furthermore, the study overall finds the biological material from GUS to have unmatched DNA preservation, indicating potential for genetic investigations in the future from this locality.

1. Introduction

Textiles were an important commodity in the life of the Norse settlements of the North Atlantic, where they were used in garment construction, as tents, sails, and household commodities such as blankets, pillows, sacks etc. (Hayeur Smith, 2015, 2014a, 2014b). Moreover, textiles themselves as elements of material culture can also be used to provide intriguing insights into the social and environmental surroundings of the people using them. During the Little Ice Age, the Greenlandic Norse settlers faced harsh climatic deterioration and were, subsequently, dependant upon warmer clothing. Unlike the Inuit, the Norse used fibers differently and continued to wear European style garments made of coarse woollen homespun that they themselves wove in Greenland during the 10th–15th century (Hayeur Smith, 2015, 2014a). Neither archaeological nor documentary evidence indicate that the Norse used furs in the same manner as the Inuit and did not possess the same range of sewing techniques or the integration of multiple skins in individual garments to make best use of each pelt's aesthetic and/or functional properties. Zooarchaeology of the North Atlantic has also suggested that the use of goat (*Capra hircus*) was more prevalent in

Greenland than in other Norse settlements of the North Atlantic (Mainland and Halstead, 2005). Recent research into ratios of European domesticates also revealed that overall Norse Greenland cattle (*Bos taurus*) declined through time, while caprines did not (Smiarowski and McGovern, 2012). Comparing zooarchaeological data from both Iceland and Greenland (McGovern et al., 2014), it was found that, by the 13th century, goat numbers in Iceland had decreased among caprines while in Greenland the ratios between goat and sheep remained relatively even (McGovern et al., 2014). Furthermore, the presence of goat is evident in a substantial amount of Greenlandic archaeological textiles (Østergård, 2004), supporting heavy utilization of the species.

Nonetheless, it should be noted that goat was not the only addition to sheep's wool the Norse used and it is strongly considered that additional arctic species may have been utilised (Østergård, 2004). From GUS it has been suggested that 12 raw fibre (including pelts) samples were from animals other than sheep or goat, specifically cattle, arctic hare, muskox (*Ovibos moschatus*), caribou (*Rangifer tarandus*), wolf (*Canis lupus*), arctic fox (*Vulpes lagopus*), bison (*Bison bison*), black bear (*Ursus americanus*) and/or brown bear (*Ursus arctos*) (Walton Rogers, 1998). The bison and pigmented bear furs (thus excluding the polar

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Table 1
Samples analysed in this study.

Sample number	Museum collection	Site	Textile type
D51992-9	NNMA	Narsaq Ø17a	Tabby, z/z (14/6, 10/5)
D51992-17	NNMA	Narsaq Ø17a	Pile weave, z/s (7/4)
D5 1992-23	NNMA	Narsaq Ø17a	2/2 twill, z/s (7/4)
A-3	NMD	Brattahlid Ø29a	2/2 twill, z/s (9/18)
x1702	NMD	Qorlortup Itinna Ø34	2/2 twill, z/s, (11/15)
D24 1991 55b	NMD	Abels farm Ø167	2/2 twill, z/s (8/7)
KNK 1950x776a	NNMA	GUS	2/2 twill, z/s (8/10)
KNK 1950x776b	NNMA	GUS	2/2 twill, z/s (8/10)
D11920-c	NMD	Sandnes V51	2/2 twill, z/s (9/10)
x1024	NMD	Niaquusat V48	2/2 twill, z/s (12/7)
D12557 b	NMD	Ujarassuit (Anavik) V7	2/2 twill, z/s (8/8)

Abbreviations for collections above are NNMA for Greenland's National Museum and Archives and NMD National Museum of Denmark.

bear; *Ursus maritimus*), suggest trade and contact with North America possibly via the Inuit (Walton Rogers, 1998). The bison, muskox and bears have since been reanalysed using ancient DNA (aDNA) (Sinding et al., 2015) and have been demonstrated to be horse (*Equus caballus*), and goat. While not a part of that study, arctic hare was also identified visually on two textiles from GUS. One of these samples (KNK 1950x776) is a 2/2 twill woven with two white bands, both of which had been analysed and interpreted by Østergård (2004) and Walton Rogers (1998). It was confirmed to be a white band of arctic hare woven into black goat hair (Walton Rogers, 1998; Østergård, 2004). However, aDNA analysis was not performed on this material for those publications. aDNA could provide an elegant alternative method of confirmation of the presence of arctic hare, adding further to the use of local and exotic materials in Greenlandic Norse textiles.

In this paper shotgun sequences were obtained from 11 yarn samples from 8 Norse Greenlandic sites, such as Narsaq (Ø17a), Brattahlid (Ø29a), Abels Farm (Ø167), Qorlortup Itinna (Ø34), GUS, Sandnes (V51), Niaquusat (V48) and Ujarassuit (Anavik) (V7) (see Table 1 for details). The material from GUS includes artefact no. KNK 1950x776 from which we tested both the white – presumed arctic hare material and the dark wool presumed to be goat (Walton Rogers, 1998; Østergård, 2004). The purpose of the sequencing was to identify the species of origin in the yarn at various sites across Norse Greenland and to investigate the possible presence or absence of arctic hare fur and/or other fibre mixtures in KNK 1950x776.

2. Methods and materials

All textiles are stored and belong to collections from both Greenland's National Museum and Archives as well as in the National Museum of Denmark, details of each sample is given in Table 1.

2.1. DNA extraction

All handling and preparation of subsamples, extracts and DNA libraries was conducted in specialised facilities designed to it, following standard procedures for ancient DNA analyses (Cappellini et al., 2011; Orlando et al., 2011; Sinding et al., 2012). Ca. 50–150 mg of textile was cleaned in 1% bleach solution and subsequently 70% ethanol. Extraction was preformed using a digestion buffer equal to Sinding et al. (2015), incubating for 12 h at 56 °C in a 2 ml DNA-lobind (Eppendorf). Digestion was hereafter centrifuged at 3.000 × g for 1 min, the supernatant was taken and mixed with 1 ml Phenol, rotated for 5 min, then centrifuged at 3.000 × g for 3 min, the aqueous layer mixed with 1 ml chloroform, and then centrifuged at 3.000 × g for 3 min. The aqueous layer was then mixed with a binding buffer optimized for ancient and/or highly fragmented DNA (Allentoft et al., 2015) in a binding

apparatus (Dabney et al., 2013) at a ratio 1:10. The binding apparatus was centrifuged at 90° at 500 × g for 30 min, the MinElute column (Qiagen) in the apparatus was hereafter placed in a 2 ml collection tube and filters were washed in 700 µl of PE buffer (Qiagen), centrifuged at 10.000 × g for 1 min and dry spun after removal of flow-through at 13.000 × g for 2 min. The columns was then place in 1,5 ml DNA-lobind (Eppendorf) tubes and eluted by two steps of 25 µl EB buffer (Qiagen), each incubating at 37 °C for 15 min and centrifuged at 10.000 × g for 1 min.

2.2. Sequencing

A mitochondrial genetic 16S marker was targeted by PCR as detailed in Sinding et al. (2015). However, due to low success the protocol was switched to a next generation sequencing protocol. Using NEBNext DNA Sample Prep Master Mix Set 2 (E6070S - New England Biolabs Inc., Beverly, MA, USA) and Illumina-specific adapters (Meyer and Kircher, 2010), a NGS blunt-end library was built on 42,5 µl of extract (re-extracted as described above) for every sample. Library building followed manufactures instructions with a few modifications: given the fragmented structure of ancient DNA the initial nebulization step was avoided. End-repair, adapter ligation and adaptor fill-in was performed as in Schroeder et al. (2015), however with 42,5 µl DNA template and the buffer detailed in Allentoft et al. (2015) as binding buffer in the end-repair step. Of the final 50 µl library 2 PCR amplifications and indexing reactions were made containing 32 µl H₂O, 5 µl 10X PCR buffer, 2 µl BSA (20 mg/ml), 1 µl dNTPs (25 mM), 1,5 µl of each of Illumina's Multiplexing PCR primer (1,5 µM of inPE1.0 5'-AATGATACGGCGAC-CACCGAGATCTACACTCTTCCCTACCGACGCTTCCGATCT and a custom-designed index primer with a six nucleotide index 5'-CAAGC-AGAAGACGGCATCGAGATNNNNNNGTGACTGGAGTTC), and 2 µl PfuTurbo Cx Hotstart DNA Polymerase (Agilent Technologies). Thermocycling was done for 2 min at 95 °C, followed by 12–18 cycles of 30 s at 95 °C, 30 s at 60 °C and 40 s at 68 °C, and 7 min elongation at 68 °C. The two amplifications was purified together over one MinElute (Qiagen) column, following manufactures guidelines, eluted in 35 µl EB (Qiagen). DNA concentration in purified amplified libraries was measured using a Agilent 2100 bioanalyzer, pooled in equimolar amounts and 'shot-gun' sequenced on a Illumina HiSeq 2500 platform and 100 bp single read chemistry.

2.3. Analyses

Post-sequencing read processing was preformed using the PALEOMIX pipeline (Schubert et al., 2014). Adaptor removal and trimming of low quality bases (BaseQ < 5 or Ns) was done in AdapterRemoval v2.0.0 (Schubert et al., 2016), removing reads shorter than

Table 2
Mapping and blasting results of NGS reads.

Sample	Read yield	% Reads mapped	Most reads mapped	Coverage	Genbank blast max score	Genbank blast query cover
Ø17a D51992-9	16,604,165	0,002%	Human	1,33	1147	73%
Ø17a D51992-17	34,565,886	0,000%	–	–	–	–
Ø17a D5 1992-23	45,501,285	0,000%	–	–	–	–
Ø29a A-3	10,413,829	0,001%	Human	0,24	329	17%
Ø34 x1702	43,816,295	0,000%	–	–	–	–
Ø167 D24 1991 55b	19,960,089	0,000%	–	–	–	–
KNK 1950x776(a)	18,958,171	0,035%	Sheep	20,16	29024	99%
KNK 1950x776(b)	39,486,496	0,045%	Hare	70,07	29593	99%
V51 D11920-c	10,215,116	0,000%	–	–	–	–
V48 x 1024	8,717,619	0,000%	–	–	–	–
V7 D12557 b	15,138,847	0,000%	–	–	–	–

25 bp or with over 50 bp missing data. Read damage patterns were assessed using mapDamage v2.0.5 (Jónsson et al., 2013). All retained reads were mapped to 28 diverse reference mammalian mitochondrial genomes (see Supplementary table a for details) with BWA-MEM v0.7.5a (Li, 2013), using a minimum read mapping quality of 15. PCR duplicates were identified and filtered by the 5'-end mapping coordinate using Picard v1.140 (<http://broadinstitute.github.io/picard/>). For each sample, and based on which reference mitochondrial genomes had most reads mapped to, we obtained the consensus sequence. To investigate taxonomic makeup of sequence, each consensus was blasted against Genbank (NCBI) (see Supplementary table b for details) with BLASTN v2.3.1. (Altschul et al., 1997). For each of the consensus sequences, we aligned them together with other closely-related sequences downloaded from Genbank (NCBI). For sample KNK 1950x776(a), we used 95 modern *Ovis* mitochondria, represented by domestic sheep (*O. aries*) and wild subspecies (*O. canadensis*, *O. ammon*, *O. vignei*, *O. musimon*, *O. orientalis*; see Supplementary table c for details). For sample

KNK 1950x776(b), 11 lagomorphs, specifically arctic hare (*L. arcticus*), domestic rabbit (*Oryctolagus cuniculus*) and wild hare species (*L. timidus*, *L. corsicanus*, *L. othus*, *L. townsendii*, *L. granatensis*, *L. capensis*, *L. americanus* and *L. californicus* (see Supplementary table d for details). These sequences were aligned with MAFFT v7.305 (Katoh and Standley, 2013) and maximum likelihood phylogenetic analyses performed with RAxML v8.2.9 (Stamatakis, 2014).

3. Results

3.1. DNA sequence generation

Standard PCR, targeting the 16S marker, was only successful for sample KNK 1950x776(b) and only after three repeated attempts. Given the lack of success in all other samples these products were not sequenced. All samples successfully amplified as libraries and sequencing yielded a total of 263,377,798 reads. Table 2 gives details on samples

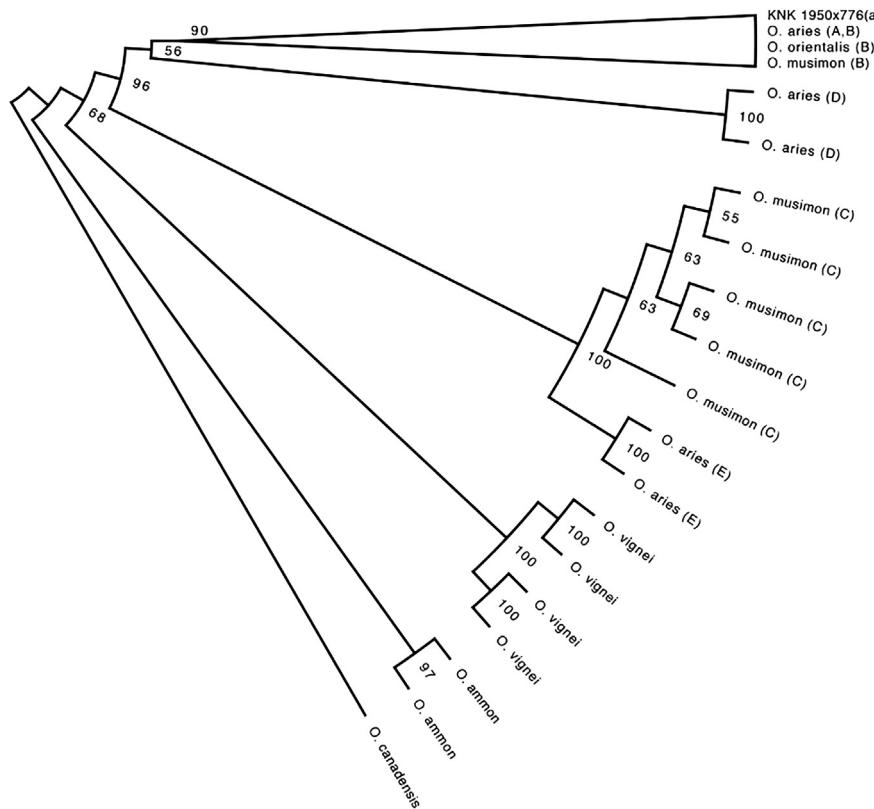


Fig. 1. Sheep phylogeny for KNK 1950x776(a).

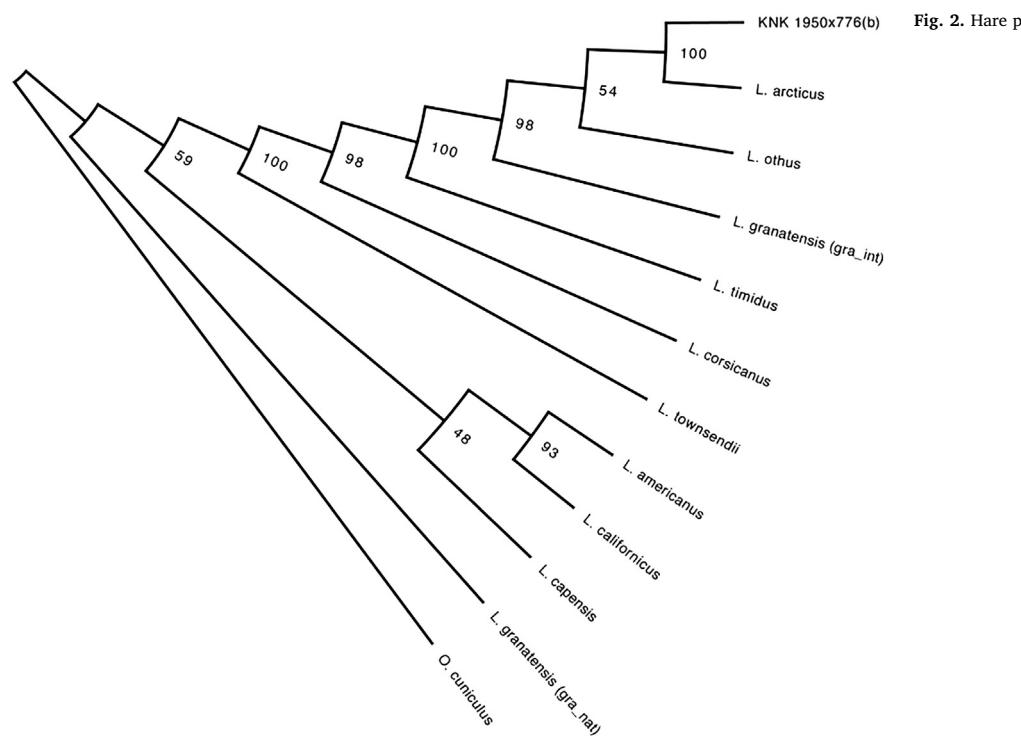


Fig. 2. Hare phylogeny for KNK 1950x776(b).

and which of them successfully yielded enough mitochondrial genomic data to determine a species of origin, assuming a minimum of 0,001% reads mapping to a mammalian mitochondrial reference (see Supplementary for statistics on mapping (table a) and blasting (table b)). Consensus sequences of KNK 1950x776(a) and KNK 1950x776(b) have been submitted on Genbank under the accession numbers KY786037–KY786038.

Sample is the name of the specific sample. Read yield is the total number of reads from the specific library after filtering. % Reads mapped is the percentage of reads mapping to the specific reference, which had the highest amount of reads mapping. Most reads mapped is the common name of the reference sequence where most reads for the specific library mapped. Coverage is the coverage of the specific consensus sequence. Genbank blast max score is the highest alignment score from that database sequence in Genbank. Genbank blast query cover is the percentage of query covered by alignment to the database sequence in Genbank in percentage.

3.2. Phylogeny of sheep mitochondrial lineages

The sheep consensus sequence of KNK 1950x776(a) was aligned together with 96 reference sheep sequences downloaded from GenBank (Supplementary table c) and their phylogenetic relationships reconstructed (See Fig 1). Our consensus sequence was placed within a monophyletic clade with 90% bootstraps containing the vast majority of domestic sheep haplogroup A and B representing 69 individuals, as well as two *O. musimon* and one *O. orientalis* (see Supplementary Fig. S1 for full phylogeny). The presence of wild subspecies likely indicates hybridisation or haplotypes shared from the ancestors of domestic sheep. The specific placement of the KNK 1950x776(a) mitochondria is from among domestic sheep B haplotypes, however it is not possible to associate KNK 1950x776(a) with a specific breed or geographic location among modern sheep, due to the lack of structure among B haplotypes.

Phylogenetic arrangement of KNK 1950x776(a) with 95 reference sequences rooted to bighorn sheep (*O. Canadensis*). Node labels represent bootstrap support for clade, tip labels represent subspecies affiliation of branch, “A, B, C, D, E” represent haplogroups of

mitochondria in domestic sheep. The upper branch represents a collapsed clade of closely related sequences (see Supplementary Fig. S1 for details).

3.3. Phylogeny of hare mitochondrial lineages

The hare consensus sequence of KNK 1950x776(b) was aligned together with 11 reference mitochondrial genome sequences downloaded from Genbank (Supplementary table d) and their phylogenetic relationships reconstructed (See Fig 2). KNK 1950x776(b) clustered together with the sequence of a Canadian arctic hare with a bootstrap support of 100%, supporting the same species affiliation. There are two sequences for Granada hare that are distantly related according to the tree. This is a well known phenomena attributed to ancient hybridisation between Granada hare and European hare (*L. europaeus*), introducing paraphyletic mitochondrial lineages in Granada hare (Melo-Ferreira et al., 2014).

Phylogenetic relationships of KNK 1950x776(b) with 11 reference sequences rooted to domestic rabbit (*O. cuniculus*). Node labels represent bootstrap supported for each clade, tip labels represent species affiliation of branch.

4. Discussion

4.1. aDNA analysis

Even with an extreme low cut-off of 0,001% reads mapping to a mammalian mitochondrial reference, only four libraries passed the cut off presented in Table 2. In fact, most samples had < 0,001% reads mapping to a mammalian reference. We considered these levels of endogenous DNA content too low, therefore failing to present any reliable interpretation of species origin (for details see Supplementary table A). Generally, for the failed samples, extremely low levels of reads mapped to either human, sheep or hare most likely represent various forms of contamination either pre, during or post excavation, handling prior to lab work, cross-contamination in lab, or errors and index contamination in library amplification and subsequent sequencing. From the four

samples that passed the threshold, two of them (Ø17a D51992-9 and Ø29a A-3) had 0,002% and 0,001% reads mapping to the human reference, respectively. We interpret this as human DNA contamination rather than endogenous DNA in the samples; the source of contamination is hard to pinpoint but likely related to unsterile handling of the sample in excavation and curating. The two samples from GUS, KNK 1950x776(a) and (b) had 0,035% and 0,045% reads mapping to a reference mitochondrial genome, giving a 20,16x sheep and 70,07x hare mitochondrial genome, respectively. These levels of coverage are a good indication of the samples' species of origin but, to fully validate the result, we performed further analysis of the two sequences.

4.2. Phylogenetic reconstruction of GUS material

The mitochondrial genome of KNK 1950x776(a) is firmly placed with the domestic sheep, specifically the B haplogroup. This clade is the largest group of domestic sheep mitochondrial lineages, with the highest frequency in modern European breeds, but also well represented in numerous breeds globally (Lv et al., 2015), precluding the association of the KNK 1950x776(a) haplotype to any specific breed but supporting a sheep origin of the textile sample. We were not able to fully reconstruct the phylogeny presented in Lv et al., 2015, as we consistently obtained the A2 sub haplogroup falling as sister clade to the B clade rather than A clade. However, since KNK 1950x776 is tightly within the main B diversity and given that solving sheep mitochondrial phylogeny is not a focus of this study, we will not address the issue further in this paper (Supplementary Fig. S1). The phylogenetic analysis of the hare KNK 1950x776(b) consensus sequence, mainly the firm clustering with the mitochondrial genome of a Canadian arctic hare, greatly supports an arctic hare origin probably from local Greenlandic individual.

4.3. DNA preservation in GUS

This study attempted to include samples from a wide range of Greenlandic Norse sites, from both Eastern and Western settlements. Unfortunately, apart from the site of GUS, which presents excellent preservation conditions, the endogenous DNA content from the other sites was too low to allow for species identification. The only reliable species identification possible was from the two KNK 1950x776 samples (GUS), that proved to be a piece of homespun cloth of sheep's wool (previously identified as goat; Østergård, 2004; Walton Rogers, 1998) with a strip of arctic hare woven through the middle creating the effect of two white strips in the textile. At the time of writing, this is the first piece of cloth from GUS genetically proven to include arctic hare and thereby evidence of a mixing of European domesticates and wild arctic species in Norse textiles. The extremely poor level of DNA preservation in the other sites (apart from GUS) suggests that future attempts to test these samples should be performed with different methods, with the use of proteomics that is known to have a greater resilience than DNA (Brandt et al., 2014). Genetic testing of material from GUS has been performed before (Hebsgaard et al., 2009; Sinding et al., 2015) but this is the first study assessing DNA quality and quantity in GUS in comparison to other archaeological sites in Greenland.

These results from GUS have opened future opportunities and furthered our understanding of the Greenlandic Norse, their social and economic use of secondary animal products as well as their wider range of socioeconomic relations during that time period. The textile findings demonstrated here illustrate the potential for further genetic and even genomic investigations of material from GUS, adding more perspectives to the many ongoing multidisciplinary investigations at GUS.

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