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Characterization of an archaeological decorated bark cloth from Agakauitai Island, Gambier archipelago, French Polynesia

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ABSTRACT

Bark cloth ('tapa/kapa') is a fabric made from beaten plant fibres. In the Pacific tapa made of paper mulberry has been of great cultural importance and its use is associated with both utilitarian and ceremonial contexts. In the 19th century, traditional bark cloth was largely replaced by Western cloth. On some islands, tapa making was banished with the arrival of missionaries and Christianization. This is the case for the Gambier Islands in French Polynesia. Only a few tapa pieces from this island group survive and are held in Museum collections.

In this work, we present results of the analysis of a bark cloth bundle discovered at the Te Ana o te Tetea cave on Agakauitai in the Gambier Archipelago. The bundle was made up of large and small strips of thin tapa, with some watermarks left by the beaters. Associated with the tapa, were a piece of wood and cordage. A few of the bark cloth samples showed symmetrical black lines along some of the folds. This paper presents the results of a number of analyses performed on the bark cloth bundle from this island with the aim of determining its age, if the decorations were man-made and the plant species used for its manufacture. Samples were dated by Accelerator Mass Spectrometry (AMS) and the designs were analyzed by portable X-ray fluorescence (XRF) and Scanning Electron Microscopy - Energy Dispersive X-ray spectroscopy (SEM-EDX) for elemental characterization. Raman spectroscopy was also performed in order to assess the chemical nature of pigments. These analyses allow us to conclude that the finds date to the pre-European contact period for this island group and that these lines can be attributed to man-made designs. In addition, genetic analysis of the ribosomal region were performed to identify the species used in its manufacture, which indicate that the plant used to make the tapa cloth was *Broussonetia papyrifera* or paper mulberry. The availability of new genetic sequencing techniques allow for new and very sensitive analyses of archaeological material that require careful handling from the beginning in order to avoid sample contamination.

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1. Introduction

In 2007 a decomposing tapa bundle reported to belong to a funerary context was found by a fisherman in the Te Ana o te Tetea cave (site 190-02-AGA) on Agakauitai Island, located to the south-east of Taravai Island in the Gambier island group (Fig. 1). The bundle was retrieved from the cave and held in a private home for a number of years. On October 18, 2012, the remains were placed in custody at the Musée de Tahiti et des Îles, located at Punauia, Tahiti. Shortly after this, one of us (AS) was made aware of the existence of the tapa bundle. Little is presently known in terms of the context of this find, as no information was recorded at the time. Nothing of the original configuration is now known, except that it was opened and unwrapped for the first time on a family kitchen table. A number of the small remnants showed some black lines and markings that resemble ink drawings along the folds. It is not known where these lines were placed in the original configuration.

The island of Agakauitai is closely associated with oral traditions of Mangareva, the main island of the Gambier group, particular with the history of two chiefly brothers. Buck (1938) reports that for chiefly funerals the bodies were wrapped in cloth and placed on wooden tables near the sea and final disposal was in caves or in the sea. Large quantities of bark cloth were brought by mourners and stacked beside the corpse when it was placed in a cave. The royal cave Te Ana-o te Tetea was reported in 1934 to have contained "a great deal of cloth … and samples of the material were still present … though quantities had been previously removed by visitors" (Buck, 1938: 254). Emory (1939) reported that the cave was the burial place of these two brothers mentioned in the oral tradition, and was told that the British archaeological expedition to Easter Island, Pitcairn and Mangareva led by Katherine Routledge, visited the site in 1921.

The first European to visit the archipelago was the British Captain James Wilson in 1797. Later Captain Beechey of the British Expedition to the Pacific and Bering Strait, (1825–1828) visited it again in 1826 (Buck, 1953). In 1827, a schooner from Chile arrived for pearl fishing, and in 1832, a second Chilean pearl fishing expedition with a crew from the Austral islands with an English pastor visited the Gambier archipelago. Neither stayed for long. The first French missionaries arrived in August 1834 and settled on the biggest island, Mangareva (Delaire, 2008). Although the French Catholic missionaries were the first permanent European settlers, there may have been occasional visitors before them – attracted by the pearlfishing. In the Tuamotus, the closest island group, the first recorded visit of a missionary is 1821, however no record exists that these visited Mangareva.

The plant commonly used for making tapa in Mangareva was paper mulberry (*Broussonetia papyrifera* (L.) L'Herit. Ex Vent), which disappeared from the Gambier group in the late 20th century. Few people still know and recognize this plant today. Extensive plantations of paper mulberry were described by the first missionaries, particularly on the island of Akamaru (Laval, 1938). Father Honoré Laval[1] recounts that on Mangareva there were two varieties, one cultivated and one feral with different names (*puri* and *ute*). The materials and methods once used in the tapa making process are now only known through historic descriptions by these missionaries and from some explorers' logs.

Tapa cloth was still used and made in 1838, as we can read from some of the missionary letters. For a religious procession organized by the church the "… *women have been over a month beating the paper mulberry to make toga, of rereki and tupunu. In other letters I have mentioned what toga and rereki are. The tupunu is a large piece of toga (native cloth) which is folded when it is still wet*" (Laval, 1879a:95)[2] However soon after, the missionaries started to introduce cotton and a spinning/weaving enterprise on the island. In 1840 father Honoré Laval writes "… *in addition there are a number who spin cotton habitually. There are now eight workshops, each with thirty people who have recently spun in the space of ten weeks 851 lb of yarn*" (Laval, 1879a:123)[3].

On arrival to the islands, father H. Laval relates how the dead bodies were "*wrapped in a kind of fabric of the country not unlike paper which they called tapa. These bodies were not interred in the ground like in Europe but they leave them exposed in a box, or lay them on a tripod; in the latter case they built a little roof with leaves to protect them from the rain; in this way they are set out to dry out … -We cannot take a step without finding these here and there; sometimes they are small children, that died without baptism, […] sometimes they are adults …*" (Laval, 1879b: 36)[4]. By 1840 the Catholic cemetery was already in use.

The texts give only brief or vague accounts of tapa manufacturing techniques, particularly in what concerns the preparation of colorants, dyes or pigments. Few tapa pieces from Mangareva have survived until modern times, as most were destroyed by the zeal of these Catholic missionaries that settled on the island. A few pieces are held in museum collections: two large pieces are in the ethnographic collections of the British Museum and two smaller pieces in the Musée de Tahiti et des Îles in Tahiti, French Polynesia. The two pieces in the British Museum were collected by Captain Beechey in 1828; one measures 2.0 by 1.20 m, while the other measures 1.20 × 0.92 m and both are painted with large black triangles arranged in a symmetrical pattern. One of the pieces at the Musée de Tahiti et des Îles is a small rectangle of very white tapa that was used for making a marriage register by the missionaries in 1835. The other is a bundle of tapa, very similar in appearance to the one found by the fisherman on Agakauitai Island. No information is available for this second bundle, but its similarity suggests that it might well come from the same context as the one we are describing here.

To date, there has been very limited scientific investigations on the paints or dyes used on Pacific tapa (Smith and Te Kanawa, 2008). To our knowledge only one study has been undertaken at the Bishop Museum using a multi-analytical approach to determine the colourants used in ethnographic Hawaiian kapa (Bisulca et al., 2014). The authors were able to document the presence of

[1] Father Honoré Laval was the principal missionary together with father Rouchouze, responsible for the conversion to Christianity of the people on Gambier Islands, often referred to as Mangareva, the name of the main island of the group. He lived there between 1835 and 1850. His role in the history of these islands is quite ambiguous. He is famous for the rigor in which he proceeded in the task of conversion destroying all signs of heathen activities, to which also all material culture produced before the advent of Christianity was victim. At the same time his descriptions of pre-contact life on the island are full of ethnographic details and key works in the study of Mangarevan history.

[2] Our translation of: Les femmes avaient employé plus d'un mois à battre le mûrier à papier pour se faire des toga, des rereki et des tupunu. J'ai déjà dit dans d'autres lettres ce que c'est que toga et rereki. Le tupunu est une grande pièce de toga (étoffe du pays) que l'on plisse lorsqu'elle est encore mouillée.

[3] Our translation of: De plus il y en a un certain nombre qui filent habituellement le coton. Elles composent à présent huit ateliers, chacun de trente personnes, qui ont produit dernièrement, dans l'espace de dix semaines 851 livres de fil.

[4] Our translation of: *Je viens de vous dire que dans leurs fêtes ils introduisent des corps morts; ce n'est pas qu'ils vont les déterrer; ils ont ici la coutume de les bien envelopper dans une espèce d'étoffe du pays qui ressemble assez à du gros papier et qu'ils appellent tapa. Ces corps ainsi disposés ne sont point mis dans la terre comme en Europe: ou bien ils les laissent exposés dans une case, ou bien ils les mettent sur une espèce de trépied; dans ce dernier cas on leur fait un petit toit avec des feuilles pour les défendre de la pluie; on les met ainsi afin qu'ils se dessèchent d'eux-mêmes; mais je ne sais pas qu'elle en est la fin. On ne peut pas faire un pas sans en rencontrer çà et là; tantôt ce sont de petits enfants, …tantôt ce sont des adultes …*

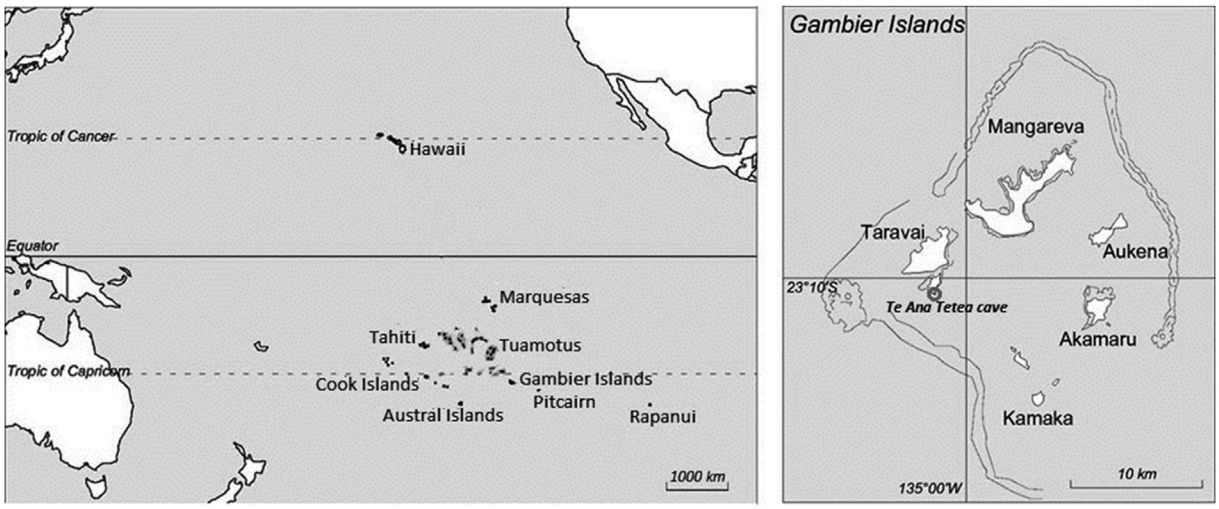

Fig. 1. Map of the Pacific with location of the Gambier Archipelago. Right: Grey circle indicates de location of Te Ana o te Tetea cave, in relation to the main islands of the Gambier island group.

traditional pigments and dyes, such as iron oxide, carbon black, turmeric among others and the incorporation of imported Western materials in the 19th century. No information is available in this respect for Mangarevan tapa or from other Pacific islands.

1.1. Genetic analysis of bark cloth

Our group has previously undertaken the genetic analysis of contemporary bark cloth (Moncada et al., 2013). This molecular study of bark cloth adds a new source of materials of plant origin to the characterization of other old plant materials such as wood (Speirs et al., 2009) or papyrus (Marota et al., 2002). We have shown that it is possible to extract and amplify DNA from bark-cloth using specific molecular markers. The use of the ribosomal DNA region (rDNA) for the analysis of ethnographic material is particularly suitable for species identification (Martin and Alvaro, 2001; Zhao et al., 2005). Here, we analyzed the most variable region within the rDNA region, which corresponds to the internal non-coding transcribed spacer (ITS) sequences, in order to identify the species used in the manufacture of this archaeological tapa. The genetic analysis of these archaeological samples of pre-European bark cloth using ancient DNA techniques provides an exciting and unique opportunity to deepen our understanding of past tapa culture from one of the most remote islands in the Pacific.

1.2. Aims

The aims of this research were to determine the age of a bark cloth bundle from the island of Agakauitai in the Gambier archipelago, to characterize its line drawings by analyzing the composition of the putative pigments present on this textile and to attempt a genetic analysis of the DNA extracted from the bark cloth fibres and associated cordage. In order to determine the plant species employed for its manufacture, a specific molecular marker

Fig. 2. The collection of tapa pieces, cordage and the piece of wood that comprise the find. On the bottom left: cardboard with tapa fragments that have the line drawings. Right: Detail of the fibre cordage remains that tied the bundle together, and detail of a fine tapa cloth showing the watermarks left by the grooves of the wooden beater on the wet cloth during the manufacturing process.

was used. This work represents the first analysis of bark cloth from an archaeological context from the Gambier archipelago and, to the best of our knowledge, from Remote Oceania.

2. Materials and experimental methods

2.1. Materials

In the Musée de Tahiti et des Îles we found one box with several layers of small tapa pieces, and two bags with bundled-up tapa (which we labelled "Bag A" and "Bag B"), one bag of cordage and a piece of wood that apparently was part of the same find (Fig. 2). Some of the tapa pieces had black markings on them, such as fine lines and a number of these suggest symmetrical designs (Fig. 3).

As controls we used three samples from contemporary *B. papyrifera* leaves, one from the island of Ua Pou in the Marquesas archipelago (BQUCH0041), one from Savaii in Samoa (BQUCH0082) and one from Fiji (BQUCH0246), from the genomic DNA bank of our collection of contemporary *B. papyrifera* samples from the Pacific. Additionally we used two contemporary tapa samples from Easter Island.

2.2. Analytical procedures

The first steps undertaken at the Museum in Tahiti were to unwrap the bundles and unfold the tapa pieces in order to document and clean the samples manually. Manual cleaning involved lightly brushing of samples with a clean brush in order to remove dirt, and insect remains. After cleaning, the fragments with the black lines were each photographed and the lines were traced on Mylar film. Finally, the collection was re-bagged for storage in acid free neutral bags made of Tyvek® (Du Pont).

Samples for analytical procedures were then taken from both the painted and the unpainted tapa. Analyses performed included radiocarbon dating at the Waikato Radiocarbon Facility in New Zealand, Scanning Electron Microscopy (SEM) at the facilities of the Faculty of Chemical and Pharmaceutical Sciences of the University of Chile in Santiago, Chile and pigment analysis at the Laboratorio de Análisis e Investigaciones Arqueométricas (LAIA), Universidad de Tarapacá, Chile. The genetic analyses were performed at the ancient DNA laboratory of the Faculty of Medicine and at the Faculty of Chemical and Pharmaceutical Sciences at the University of Chile and the ancient DNA laboratory belonging to the Anatomy Department at the University of Otago, New Zealand.

2.2.1. Dating

A cloth sample for radiocarbon dating was taken from the bark cloth bundle from bag A, and prepared following standard accelerator mass spectrometry (AMS) protocols, whereby the bark cloth was washed in dilute HCl to remove surface contamination, and then were treated with a series of dilute HCl, multiple NaOH and HCl washes (UCI, 2011a) prior to CO_2 collection and conversion to graphite (UCI, 2011b). AMS graphite targets were measured at the Keck Radiocarbon Laboratory, University of California, Irvine. The $\delta^{13}C$ value was measured on prepared graphite using the AMS spectrometer. The radiocarbon date has therefore been corrected for isotopic fractionation as per convention (Stuiver and Polach, 1977). However, the AMS-measured $\delta^{13}C$ value can differ from the $\delta^{13}C$ of the original material and it is therefore not reported.

2.2.2. Image analysis

Samples were observed initially with a FEI Inspect F50, High Resolution Scanning electron Microscope, held at the Faculty of Chemical and Pharmaceutical Sciences, University of Chile. Small samples from two different pieces were placed directly on the sample holder and observed under very low voltage (1 kV) in order to avoid damage to the samples. Pieces from where samples were taken are shown in Figs. 4 and 5, and correspond to a 3 × 3 mm

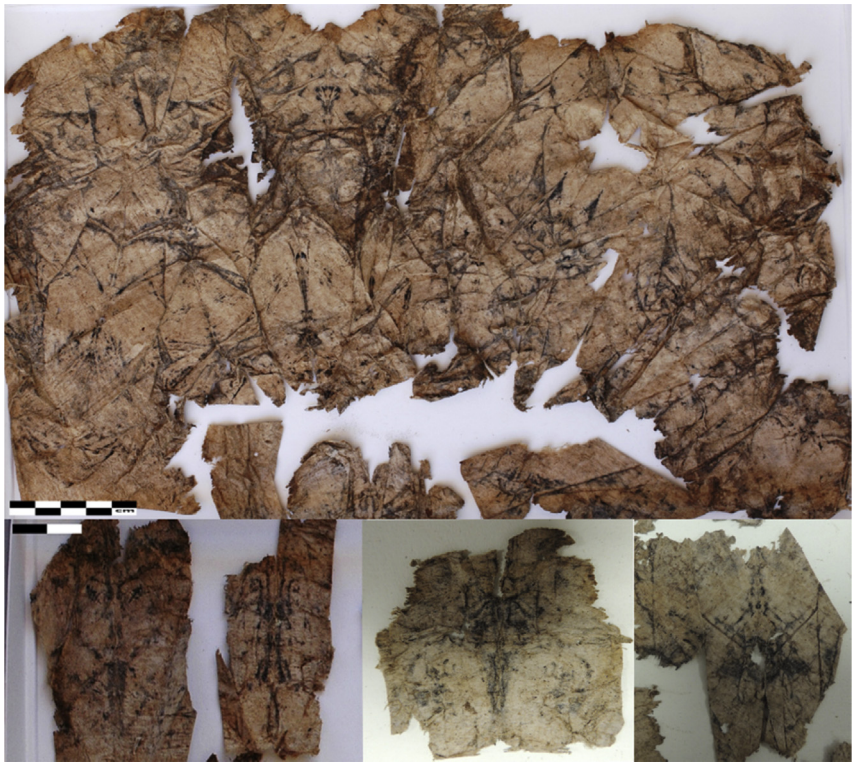

Fig. 3. Examples of the tapa fragments with black line drawings. Note that most of markings are located along folds of the cloth and several seem to have a symmetrical pattern.

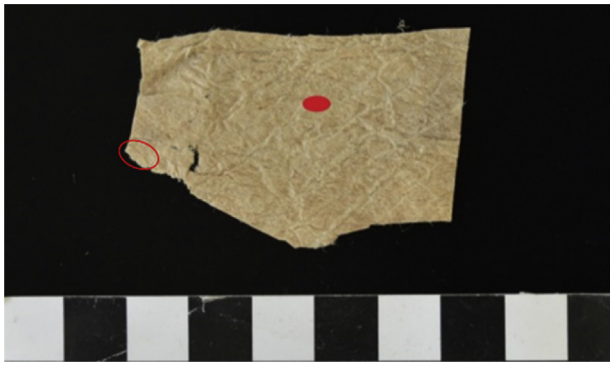

Fig. 4. Plain tapa sample. The red dot indicates the location of UV light, X-ray fluorescence (XRF) and Raman spectroscopy. The red circle indicates subsample taken for SEM imaging. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fragment of plain tapa and a 0.5 mm length fragment with black stain. Images under different magnifications were taken from several sections of each sample.

Samples were subjected to the following analyses at the analytical laboratory from the University of Tarapacá: conventional microscope observation with different lights and magnification in order to assess the presence of possible colouring, as well as the existence of erased paint traces. SEM images were also taken of the coloured fragment at a 70° angle in order to observe the bark cloth in cross section. In addition, a small sample was mounted in resin for stratigraphic observation and to determine the position of the pigments in the fibre structure. This was performed with a scanning electron microscope fitted with a Jeol JCM-6000 Energy Dispersive X-ray (EDX) detection system. Parameters were 15 kV with a magnitude of 200×. Experimental conditions and spectral resolution were adjusted for optimal data recording.

2.2.3. Elemental analyses

X-ray fluorescence (XRF) and Raman spectroscopy were performed to determine the elemental and mineral composition of the putative pigments. X-Ray Fluorescence (XRF) analysis was performed with an XRF Bruker Tracer III-SD portable device with a detector fitted 10 mm2 XFlash® SDD, Peltier cooled and equipped with an X-ray tube Rh target; max voltage 40 kV. The specifications for this particular study were high voltage 40 kV of energy, 37.8 μA of intensity and an acquisition time of 180 s. Data were collected and plotted using the Tracer software S1PXRF 3.8.3. All results obtained were semi-quantitative. For comparison, results were standardized as areas for each element. Standardization was performed through the following formula: $S = A/\Sigma A$, where S= Net Standardized, A = Net area and ΣA = Sum of Net area.

Raman spectra were taken on small samples on the dark markings (Figs. 4 and 5) and recorded on a Raman Renishaw InVia Reflex system, equipped with a c 785 nm excitation source, a Leica microscope and an electrically cooled charge-coupled device detector. Micro-Raman measurements were calibrated using a Si wafer and a 50× objective. Spectra were obtained with 1–5 scans of 10–50 s. The 785 laser beam was adjusted to between 0.0001 y 1%. Spectra were recorded in the 1800-200 cm^{-1} region to observe the Raman spectra. Data were collected and plotted using the programs WIRE 3.4 and GRAMS/AI 8.

2.2.4. Genetic analyses

According to the recommendations for work with ancient DNA (Poinar and Cooper, 2000), all samples were extracted at least in duplicate and the extraction procedure repeated in two different facilities (the ancient DNA facilities of the University of Otago and the ancient DNA facility of the Faculty of Medicine, University of Chile), as summarized in Tables 2 and 3. Independent amplifications for each sample were performed by two operators in two countries at three different institutions: The ancient DNA facilities of the University of Otago, the ancient DNA facility of the Faculty of Medicine, University of Chile, and the Dept. of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, University of Chile.

2.2.4.1. DNA extraction. Fragments ranging from approximately 3 mg–62 mg (of approximately 1 cm), were taken from each archaeological specimen, including the bark cloth, the fibres, and a small fragment of bark from the wood that was part of the bundle. For each sample set, negative controls were included, which were processed simultaneously. Each sample was torn and cut lengthwise with a scalpel on a clean surface before adding reagents for DNA extraction. DNA extractions were performed according to the protocol designed and described in Moncada et al. (2013) based on the protocol described by Lodhi et al. (1994) with some additional modifications. This protocol consisted of a homogenization of the

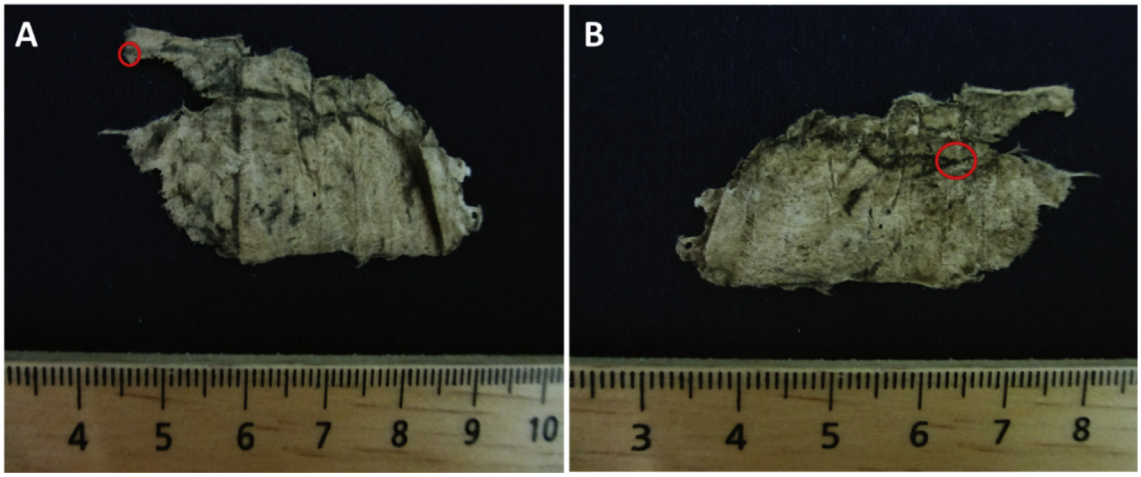

Fig. 5. Tapa sample with black lines. A. The red circle indicates were the subsample for SEM imaging was taken. B. Reverse side of the same sample. The red circle indicates were XRF and RAMAN spectra were taken. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

tissue in an extraction buffer containing 2% CTAB, incubation at 60 °C, extraction of proteins and impurities with organic solvents (chloroform and isoamylic alcohol) and precipitation of nucleic acids using cold absolute ethanol in the presence of salt. The optimized protocol consisted of 3–10 mg of the bark cloth samples that were incubated at 60 °C for 8 h on a rotary shaker with the extraction buffer. Finally, obtained DNA was resuspended in 100 μL of distilled water. Negative controls, that contained extraction buffer but lacked the study sample, were treated exactly as all samples for the rest of the extraction protocol. As positive controls, two contemporary leaf samples of paper mulberry and three paper mulberry bark-cloth samples (from Hawaii and Samoa) as described above were included and processed in a separate laboratory. DNA concentration and quality (Absorbance ratio 260 nm/280 nm) were measured using a NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and by fluorescence analysis using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies) according to manufacturer's instructions.

2.2.4.2. Polymerase chain reaction (PCR) amplification of the ITS1 region and sequence analyses. Starting material for all amplifications was DNA obtained from extractions described in Table 2. PCR amplifications of the region corresponding to the Internal Transcribed Sequence (ITS1) of the nuclear ribosomal DNA were performed. The ITS1 region was amplified using primers ITS-A (5'-GGAAGGAGAAGTCGTAACAAGG-3') and ITS-C (5'-GCAATTCA-CACCAAGTATCGC-3') (Blattner, 1999), obtaining an amplification product of approximately 300bp. In each case, PCR reactions were carried out using 2 μL of undiluted DNA extracted from the bark cloth, fibre and bark samples. PCR reactions included a negative (blank) control, adding the appropriate amounts of sterile distilled water to the mix in all experiments. Amplifications of DNA extracted from a contemporary *B. papyrifera* leaf sample were performed in separate laboratories as positive reaction controls.

A) Analyses performed at the facilities of the University of Chile (Faculty of Medicine and Faculty of Chemical and Pharmaceutical Sciences): PCR reaction mixtures consisted of 2 μL of genomic DNA, 2.5 mM $MgCl_2$, 0.625 mM dNTPs, 0.25 μM of each primer, 1 mg/mL of BSA and 0.2 U/μl of GoTaq® G2 Flexi DNA Polymerase or GoTaq® G2 Hot Start Polymerase (Promega, Madison, WI, USA) in a final volume of 20 μl. The PCR program consisted of an initial denaturation step at 94 °C for 5 min, followed by 40 cycles with a denaturation step at 94 °C for 1 min, an annealing stage at 60 °C for 1 min, an extension at 72 °C for 1 min with a final extension at 72 °C for 7 min. Amplicons were separated by electrophoresis on 1.5% agarose gels in 0.5 × TBE buffer, dyed with GelRed™ Nucleic Acid Gel Stain (Biotium, Inc.) and visualized under UV light. Samples were sequenced at Macrogen Inc. (Seoul, South Korea). For bioinformatic analysis, polymorphisms from all sequences were visualized and checked on electropherograms using Bio Edit 7.2.3 software. ITS sequences were aligned using the Clustal W method included in Mega 6 (Tamura et al., 2013). Species determination was performed comparing sequences of interest with the BLAST database.

B) Analyses performed at the facility of the University of Otago: The PCR mixture consisted of 2 μL of genomic DNA extracted from the bark cloth samples, 0.5 mM MgCl2, 0.625 mM of dNTPs, 0.25 μM of each primer, 1U/rx of KAPA HiFi HotStart DNA Polymerase (Kapa Biosystems, Wilmington, MS, USA) and Molecular Biology grade water to complete a final volume of 20 μL. The PCR program used was that recommended by the manufacturer, which consisted of an initial denaturation at 95 °C for 3 min, 40 cycles of 98 °C for 20 s, 60 °C for 15 s and 72 °C for 20 s, followed by a final extension step at 72 °C for 1 min. The amplicons were separated by electrophoresis on 1.5% agarose gels in 1% TAE buffer using Gel Red dye (Biotium Inc.) for nucleic acids. Samples were sequenced at the Genetic Analysis Service of the University of Otago (Dunedin, New Zealand). For bioinformatic analysis, polymorphisms were visualized and checked on electropherograms from all sequences using Bio Edit 7.1.3.0 software. ITS sequences were aligned with the CLC sequence viewer 7.6.1 software, using the Clustal W method. Species determination was performed comparing sequences of interest with the BLAST database.

3. Results

3.1. Description of the textile

The tapa fragments varied in thickness and texture. The general colouring of the tapa was light brown to light tan. Some fragments had clear line impressions from the use of incised or grooved beaters. These fragments were very thin and almost translucent against the light. Other fragments from the assortment were coarser and had much denser fibres, as if the beating process had been shorter. Fragment size varied from 4 cm to 37 cm in length. Some of the edges of the painted fragments indicate that the pieces were cut to size with either scissors or a sharp knife. Most of the fragments with the black markings were very thin and flexible, however only some had the impressions from the beaters (See Figs. 2 and 3).

3.2. Dating

The radiocarbon dates taken on the bark cloth sample were calibrated using OxCal v4.2 (Bronk-Ramsey, 2013) with the IntCal13 (Reimer et al., 2013) calibration curves. The 68.2% probability distributions for these calculations are shown in Table 1, with most dates clearly displaying a wide calibrated age range with two peaks, the most recent corresponding to AD 1800 (Fig. 6A). To refine the dating of the tapa cloth sample we used the *terminus ante quem* ("before" constraint in OxCal) which sets a date before which the calibrated age range must occur (Fig. 6B). This *prior* historical information is incorporated in a Bayesian statistical model, resulting in more precise calendar results (Bronk Ramsey, 2009).

Table 1
Radiocarbon determinations on organic materials from Agakauitai Island, Gambier Islands.

Laboratory code	Material	^{14}C Age (years BP) plus error	Calibrated age BC/AD (68.2% probability) Unmodelled	Calibrated age BC/AD (68.2% probability) Modelled
Wk-40170	Tapa cloth	214 ± 20	1650–1670 AD 1780–1800 AD 1940-0[a] AD	1650–1670AD 1780–1800 AD

[a] Date may extend out of range (i.e. modern).

Table 2
Summary of extractions: DNA concentration of archaeological and contemporary barkcloth and paper mulberry leaf samples, 260nm/280 nm ratio, and yields of the DNA extractions performed in three different facilities.

	Extractions at the aDNA Lab, Faculty of Medicine University of Chile			Extractions at the aDNA Lab, University of Otago								
	Extraction 1			Extraction 2			Extraction 3			Extraction 4		
Samples	[DNA] ng/µL PG	[DNA] ng/µL Abs	$A_{260/280}$	[DNA] ng/µL Abs	$A_{260/280}$	Yield (ng DNA/mg)	[DNA] ng/µL Abs	$A_{260/280}$	Yield (ng DNA/mg sample)	[DNA] ng/µL Abs	$A_{260/280}$	Yield (ng DNA/mg sample)
TM BA 1 *tapa*	4.6	469.0	1.25	226.9	1.17	1445	114	1.38	1216	204	1.35	1076
TM BA 2 *tapa*	2.2	72.2	1.29	8.4	1.38	95	19,8	1.52	228	16.4	1.49	180
TM BB 1 *tapa*	2.6	504.6	1.3	15.3	1.35	128	32,5	1.34	325	27.7	1.29	170
TM BB 2 *tapa*	4.9	1257.3	1.36	193.6	1.37	1713	225	1.36	1974	259	1.49	1715
Bark 1	2.9	3120.3	1.35	1167	1.36	4541	198	1.34	1771	53.2	1.32	512
Bark 2	3.2	4310.3	1.38	-	-	-	-	-	-	-	-	-
Fibre 1	2.1	1539.3	1.35	-	-	-	-	-	-	-	-	-
Fibre 2	3.3	2012.7	1.36	-	-	-	-	-	-	-	-	-

coloured extract - : not assayed
PG: Picogreen; Abs: Absorbance

Table 3
Summary of amplifications performed in three different laboratories using two DNA polymerases.

Amplification enzyme	Amplification with GoTaq® G2 Hot Start Polymerase			Amplification with KAPA HiFi HotStart DNA Polymerase			Amplification with GoTaq® G2 Flexi DNA Polymerase			
Amplification facility	Faculty of Medicine, University of Chile			Anatomy Department, University of Otago			Faculty of Chemical and Pharmaceutical Sciences, University of Chile			
Extraction number	Extraction 1			Extraction 2		Extraction 3	Extraction 2	Extraction 3	Extraction 4	
Amplification	Ampl. 1	Ampl. 2	Ampl. 3	Ampl. 1	Ampl. 2	Ampl. 1	Ampl. 1	Ampl. 1	Ampl. 1	Ampl. 2
TM BA 1	x	x	–	x	✓	x	✓	✓	✓	x
TM BA 2	✓	✓	–	x	✓	x	x	x	x	✓
TM BB 1	x	✓	✓	x	✓	x	✓	✓	✓	✓
TM BB 2	x	✓	✓	✓	*x*	x	x	x	x	✓
Bark 1	x	x	✓	x	x	x	x	x	x	x
Bark 2	x	x	x	–	–	–	–	–	–	–
Fibre 1	x	✓	✓	–	–	–	–	–	–	–
Fibre 2	x	✓	✓	–	–	–	–	–	–	–

✓: successful amplification; x: no amplification; –: not assayed.

3.3. Microscopic observation and UV light

Arrangement of fibres and their structure was observed under SEM. Both pigmented and non-pigmented fragments of bark cloth samples were chosen for observation. Non-pigmented samples display the individual fibres arranged in parallel bundles in different orientations (Fig. 7).

Light microscopy observation of one of the samples with different magnification shows the presence of black coloring traces. Under fluorescent light, the black lines do not fluoresce (data not shown). Therefore, the black colouring may not be a dye from an organic fluorescent compound, or from an inorganic mineral, that could also fluoresce (Guibault, 1990). The absence of fluorescence also suggests that no organic compound was used as a binder. All pigmented samples taken from a piece with black lines observed with SEM, showed a coating that covers and obscures the individual fibres. When zooming in on this coating we observed that it penetrates between the fibres. (Fig. 8). A small sub-sample was

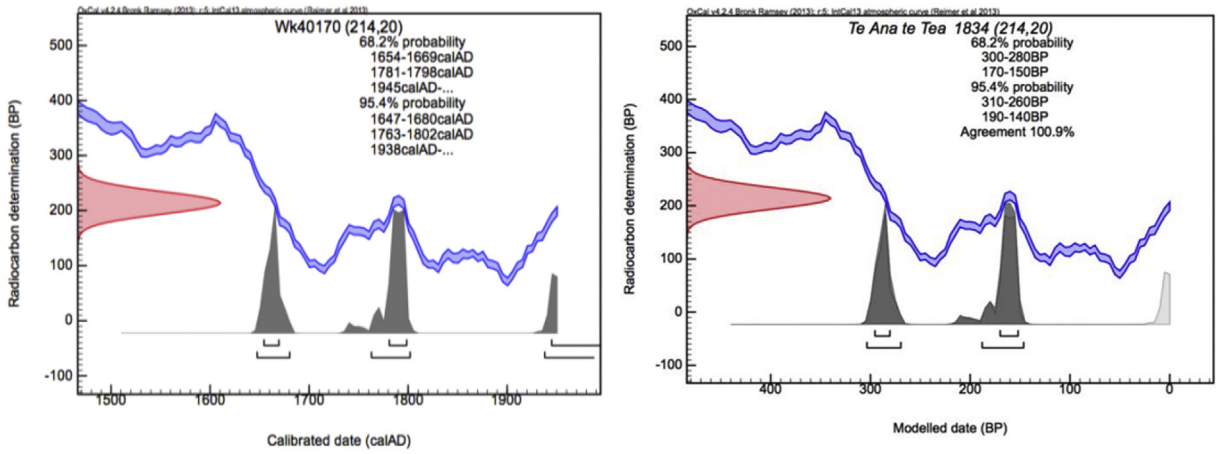

Fig. 6. Calibration curves for the radiocarbon dates of the tapa samples. Panel A. Calibrated radiocarbon age for Agakauitai tapa cloth sample. Panel B. Calibrated radiocarbon age for Agakauitai tapa cloth sample, assuming a *terminus ante quem* of AD 1834±5yrs.

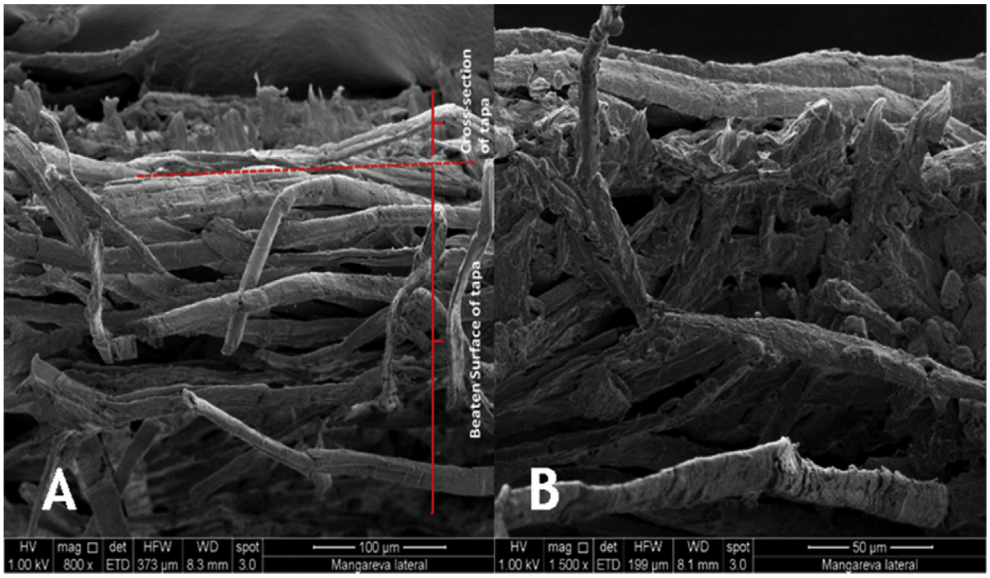

Fig. 7. SEM image of unpainted beaten tapa. A. Surface and cross section of sample. B. Close up of the cross section of the sample.

mounted in resin in order to perform a stratigraphic scan (see Fig. 9A). We conducted separate analyses of different spots in order to observe the presence of the carbon in the upper layers of the sample. We obtained an EDX spectrum for one of the spots (see Fig. 9, Panel B) detecting the presence of carbon in the paint layer, as well as other minor elements such as aluminum, silica and sodium. The presence of gold is due to the sample preparation. Finally when plotting the distribution of the lines on the image we were able to show that the distribution of the carbon coincides with the spectrum obtained by EDX. (Fig. 9B).

3.4. XRF and Raman spectroscopy

The XRF spectra did not allow a precise elemental characterization of the black lines. Due to the detection limits of the equipment, carbon could not be identified. A Raman spectrum of the black parts of the sample was obtained. The spectral profile shows a region with high relative intensity, between 1300 and 1600 cm^{-1}. The bands in the upper region (of 1391 and 1577 cm^{-1}) allow to infer the presence of carbon. The small peak (500 and 1000 cm^{-1}) may be indicative of the presence of salt, however the chemical nature of the salt could not be identified. Measurements were also taken on the surface on areas that did not show the black colouring, however no spectrum could be obtained due to high fluorescence (Fig. 10).

3.5. DNA analyses from bark cloth, fibres and bark

DNA extractions from the bark cloth, fibre and bark samples yielded concentrations ranging between 2.1 and 4.9 ng/µL of DNA as measured by the Quant-iT™ PicoGreen® dsDNA Assay Kit (Table 2). The same samples extracted at the U. of Otago yielded negative concentrations using this quantification method. Nonetheless, absorbance spectra of samples showed a small peak at 260 nm suggesting the presence of DNA. A representative profile of each kind of sample is presented in Fig. 11. The 260 nm/280 nm ratios of DNA extractions performed both at the facilities of the U. of Chile and at the U. of Otago, lie outside the optimal 1.8–2.0 range, since extraction solutions were of a light brown colour. Table 2 shows the concentration, 260 nm/280 nm ratio and yields

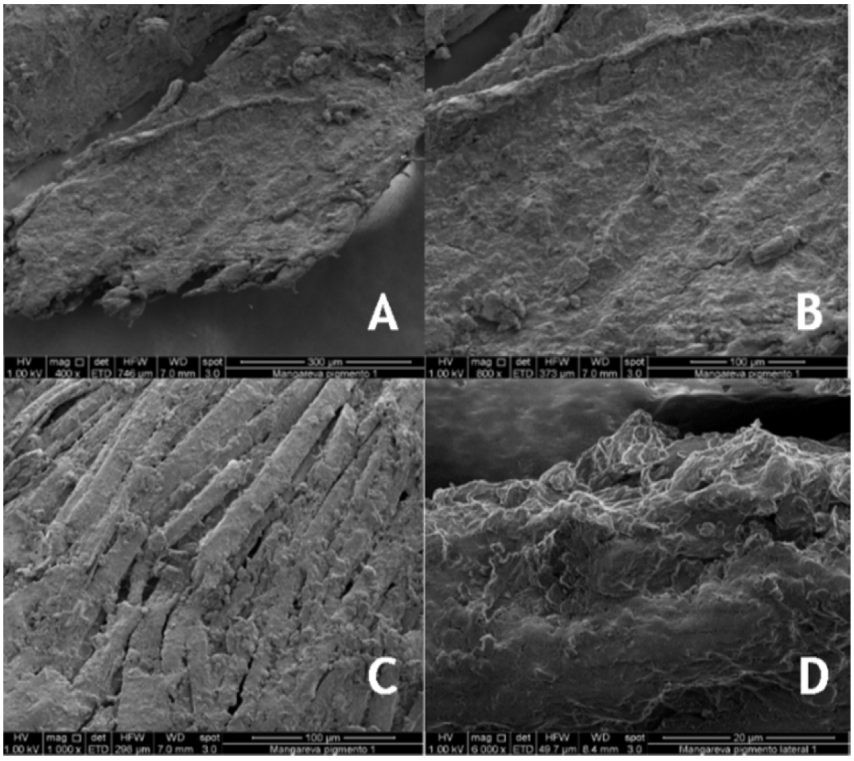

Fig. 8. SEM image of the surface of the painted tapa sample. A and B: Notice the layer that hides the individual plant fibres. Close-up of the same sample. C: Fibres covered in some type of substance that sticks and penetrates the fibre surface. D: Close-up of the sample in cross section. Notice that the individual fibres are not recognizable.

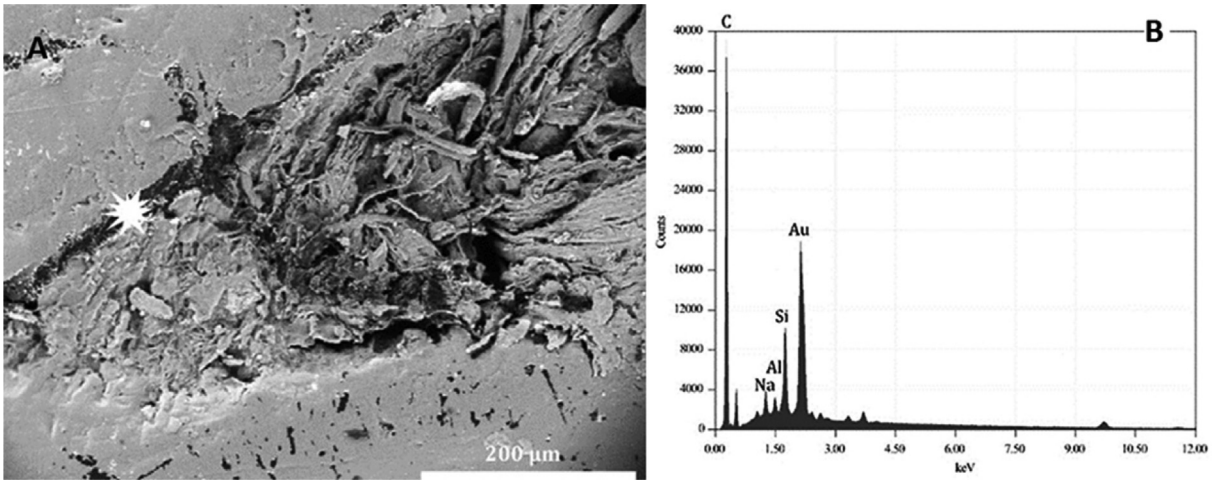

Fig. 9. SEM image of stratigraphic analyses. A) Image by SEM of stratigraphic preparation of fibre sample. The star indicates the presence of carbon coating. B) EDX spectrum of coated painting on plant fibre showing the peaks for carbon in the paint layer, as well as other minor elements such as aluminum, silica and sodium.

obtained from extractions of the archaeological samples and of contemporary controls performed at the different facilities. Analysis of the integrity of the DNA obtained from the different samples was analyzed by gel electrophoresis on 0.8% agarose gels (Fig. 12). DNA from all archaeological samples and contemporary bark cloth was highly degraded, in contrast to high molecular DNA obtained from a leaf sample (Fig. 12). Extraction controls do not display any bands.

Although the DNA extracted from the samples was highly degraded, it was possible to amplify the ITS1 region of all seven archaeological samples. Amplification of samples was obtained in different PCR assays, as shown in Fig. 13. DNA from the contemporary leaf and bark cloth samples amplified consistently, as previously described for contemporary bark cloth (Moncada et al., 2013). Sequence analysis of obtained amplification products was possible in all samples. Sequences from three archaeological bark cloth samples were identified as *Broussonetia papyrifera* (TM BA1, TM BA2 and TM BB1). Sample TM BA1 was identified by the presence of the characteristic DNA motif present in samples of *B. papyrifera* from Remote Oceania, although the rest of the sequence was of very low quality and not readable (Fig. 14A). Samples TM BA2 (Fig. 14B) and TM BB1 (Fig. 14C) present the polymorphism that identifies the Oceanian haplotype of this species, in contrast to the Asian haplotype reported by our group

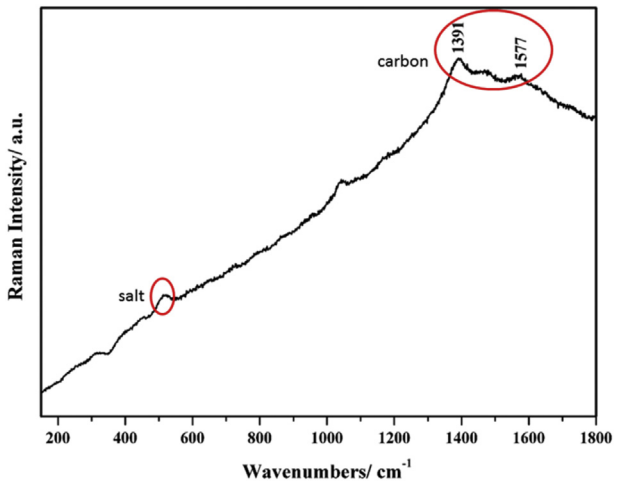

Fig. 10. Raman spectrum of the coating on the fibre surface. The peaks in the upper region of 1391 and 1577 cm^{-1} allow to infer the presence of carbon. The small peaks (500 cm^{-1} and 1000 cm^{-1}) may be indicative of the presence of salt.

(Seelenfreund et al., 2011). Bacterial cloning and sequencing of amplicons from some samples from TM BA2 that yielded unreadable sequences were performed in order to separate these mixed sequences. The analysis of ten clones yielded sequences of contaminant fungal and plant species (SITable1). The DNA sequence retrieved from the bark cloth sample TM BB2 matched only with tomato (*Solanum lycopersicum*) and the DNA sequence from fibre sample 2 matched with *Brassica oleracea and Solanum lycopersicum*. The sequences from amplified bark samples were of low quality and did not match with any woody species. In addition, sequences matching with an assortment of species were retrieved from all samples, as shown in Table 4.

4. Discussion and conclusions

In this study, we examined archaeological samples of bark cloth

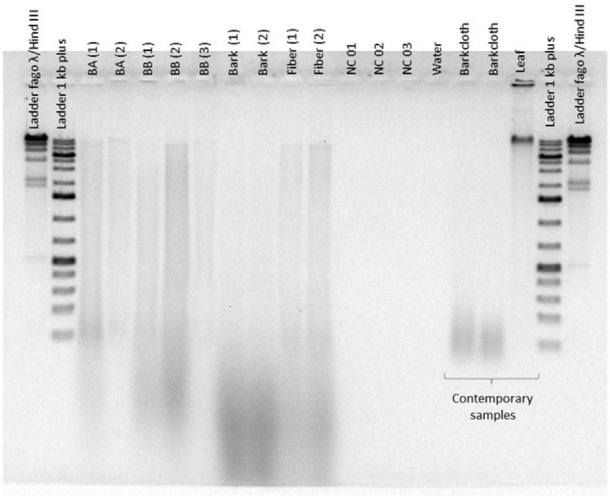

Fig. 12. Genomic DNA extracted from bark cloth, fibre and bark from archaeological samples. BA, BB: Bark cloth samples from bags A and B, respectively. NC: negative extraction controls. Leaf: contemporary *B. papyrifera* leaf sample (Fiji, BQUCH0246). The contemporary bark cloth samples are from Easter Island. Electrophoresis on 0.8% agarose gel. Numbers in parenthesis represent number of replicate extractions.

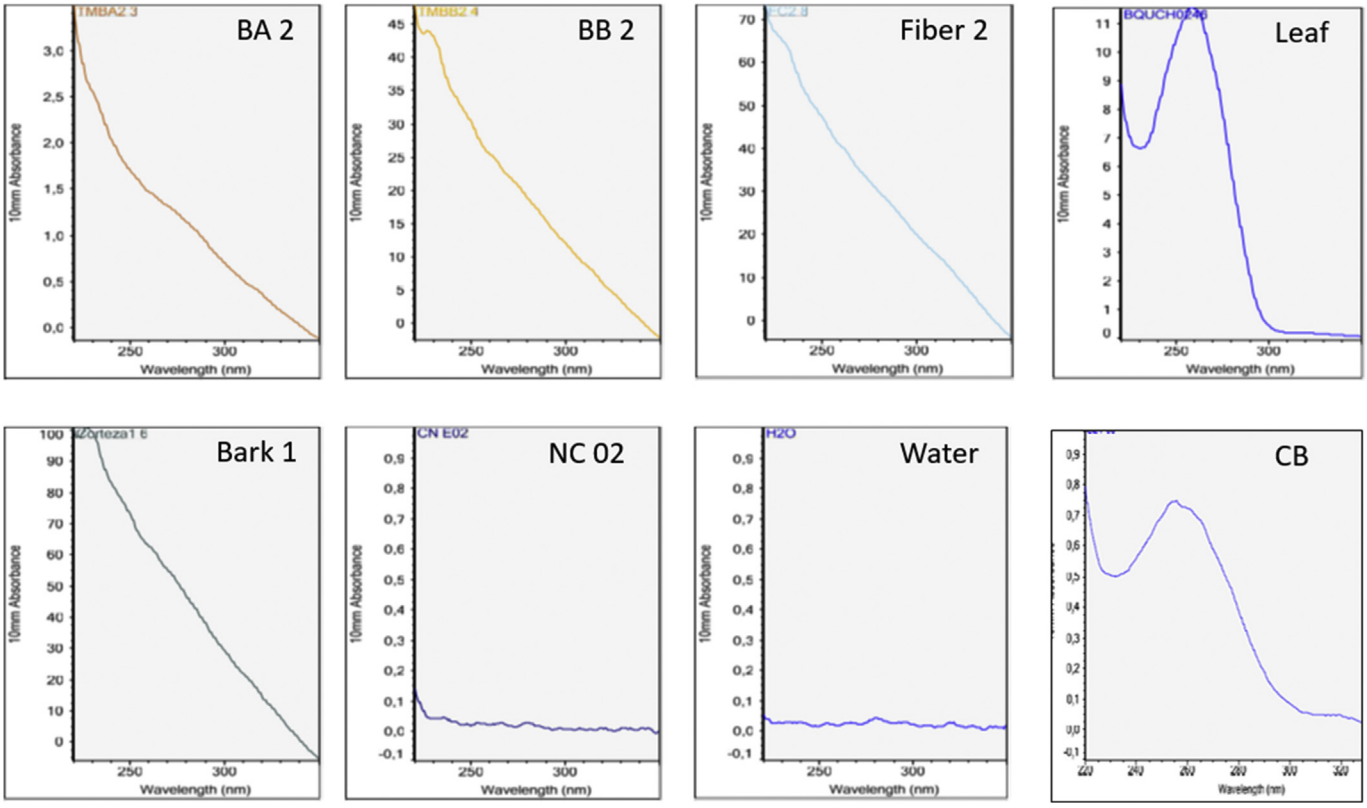

Fig. 11. Absorption profiles of DNA extracted from bark cloth, fibre and bark samples. BA, BB: bark cloth samples from bags A and B, respectively. NC: negative extraction control. CB: Contemporary bark cloth. Leaf: BQUCH0246 from Fiji.

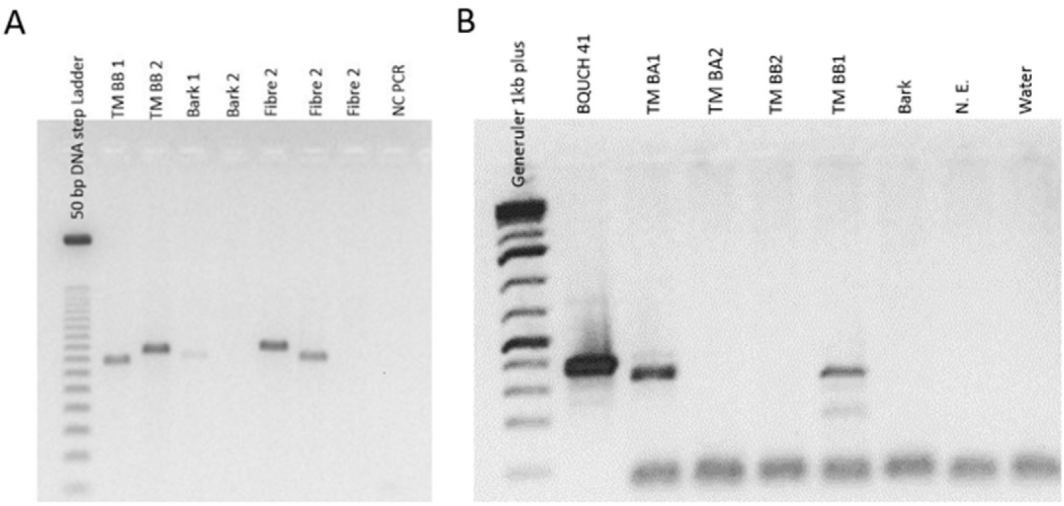

Fig. 13. Amplifications of the ITS-1 region from DNA samples obtained from the bark cloth samples. Amplicons of 300–350 bp are shown. A: amplification with GoTaq® G2 Hot Start Polymerase (Faculty of Medicine, University of Chile). B: amplification with Go Taq® G2 Flexi DNA Polymerase (Faculty of Chemical and Pharmaceutical Sciences, University of Chile). TM BA1, TM BA2, TM BB1 and TM BB2 and the bark sample correspond to DNA from the archaeological sample. Sample BQUCH41 corresponds to contemporary DNA of *B. papyrifera* leaves (positive control). Electrophoresis on 1.5% agarose gels. N.E = Negative Extraction control. Water = negative PCR control (water).

and associated fibres recovered from a funeral cave on the Gambier Islands, a region from which very few objects of material culture have survived over time. We used multiple experimental approaches in order to obtain as much information as possible from this valuable and unique find.

Our first question was to establish an approximate date for the manufacture of the bark cloth. The modeled calibrated age ranges (95.4% probability) shown in Fig. 6A indicate a probable date of manufacture in the mid 17th century AD to the end of the 18th century. To refine the dating of the tapa cloth sample we used the *terminus ante quem* ("before" constraint in OxCal) which sets a date before which the calibrated age range must occur. A date of 1834 coincides with the arrival of the first missionaries on the island and we have therefore chosen this date as a conservative estimate of the minimum age of the cloth (Fig. 6B). The inner bark of tapa in modern ethnographic context may occasionally be dried for storage (personal observation by AS), however it is usually used within a year of harvesting. Therefore, we suggest that this finding likely corresponds to tapa manufactured before the arrival of Europeans settlers to the island.

The next step consisted of a detailed analysis of the fibre structure and the character of the black markings found on the bark cloth. The scanning electron microscopy images indicated the presence of various layers of intertwined fibres. In addition, analysis of the dark regions of the tapa cloth showed a thick layer covering the fibres that resembled a dense paste, consistent with the application of pigments or paint. Results obtained by Raman spectroscopy indicate that several of the observed signals correspond to vibration modes associated to carbon. These results are confirmed by SEM-EDX elemental analysis and are consistent with ethnographic information regarding the use of charcoal as an important element in manufacture of black paint (Brigham, 1911; Kooijman, 1972). It is possible that inorganic compounds could also have been used as pigments, because other elements such as aluminum, silica and sodium can be detected by EDX. In addition, an undetermined salt was detected by RAMAN analysis.

Most information of the composition of paints, pigments or dyes used in Polynesian bark cloth derive from ethnographic sources (Kooijman, 1972), providing data on the main plants for dyes and paints. Red or rust brown red was usually obtained from the juice of the tree bishop wood (*Bischofia javanica*) often mixed with red ochre. On Tahiti red colour was produced from the steeped juice of the stem of the dye fig (*Ficus tinctoria)*. A yellow colour is obtained from the roots of the turmeric (*Curcuma longa)* or the roots of noni (*Morinda citrifolia)* (Neich and Pendergrast, 1997). In Samoa red ground ochre is spread over the whole surface of the textile and then rubbed into the fibre with a pad dipped in *Bischofia javanica* extract. The bark of the candlenut tree (*Aleurites moluccana*) produces a brown colour. Charcoal blacks are made by charring plant tissues such as wood or nuts. The soot of burnt candlenuts is commonly used as black dye. In the Austral Islands, the soot of burnt bark of the ironwood tree (*Casuarina equisetifolia*) was also used (Kooijman, 1972). In New Guinea, black and browns are also obtained from certain muds (Neich and Pendergrast, 1997; Hill, 2001). Similarly, on Easter Island reds and blacks were commonly obtained from minerals. Charcoal was also commonly used for black paints (Kooijman, 1972; Métraux, 1971; Englert, 1980). Media commonly used with charcoal are either oils such as candlenut or coconut oil, or plant saps containing resins.

Our interpretation of these results is that the markings indeed seem to correspond to intentional applications, and are most probably not the result of fungal degradation. We have not undertaken a systematic analysis of these line drawings, since this is beyond the scope of this study. This is a difficult task, since these small fragments were found out of the original context in no particular pattern, and therefore can be observed from all possible angles and are hence open to multiple interpretations.

We have also attempted a genetic analysis of these samples using a molecular marker that is useful for species identification. To the best of our knowledge, this is the first time that the extraction of DNA from bark cloth over 150 years old is achieved. As indicated, we have previously reported the successful extraction of DNA from contemporary bark cloth samples (Moncada et al., 2013). Starting from two bark cloth, two fibre and one bark sample, we were able to obtain amplifiable DNA from the bark cloth and one of the fibre samples. It is important to note that each textile and therefore each sample in question may have been manufactured from various individual plants of the same or from different fibre producing species. For making tapa, bark from several plants is often used and sometimes bark from different species can be mixed (Buck, 1964). For the Gambier archipelago only the use of paper mulberry and breadfruit are mentioned (Buck, 1938), with no evidence of the

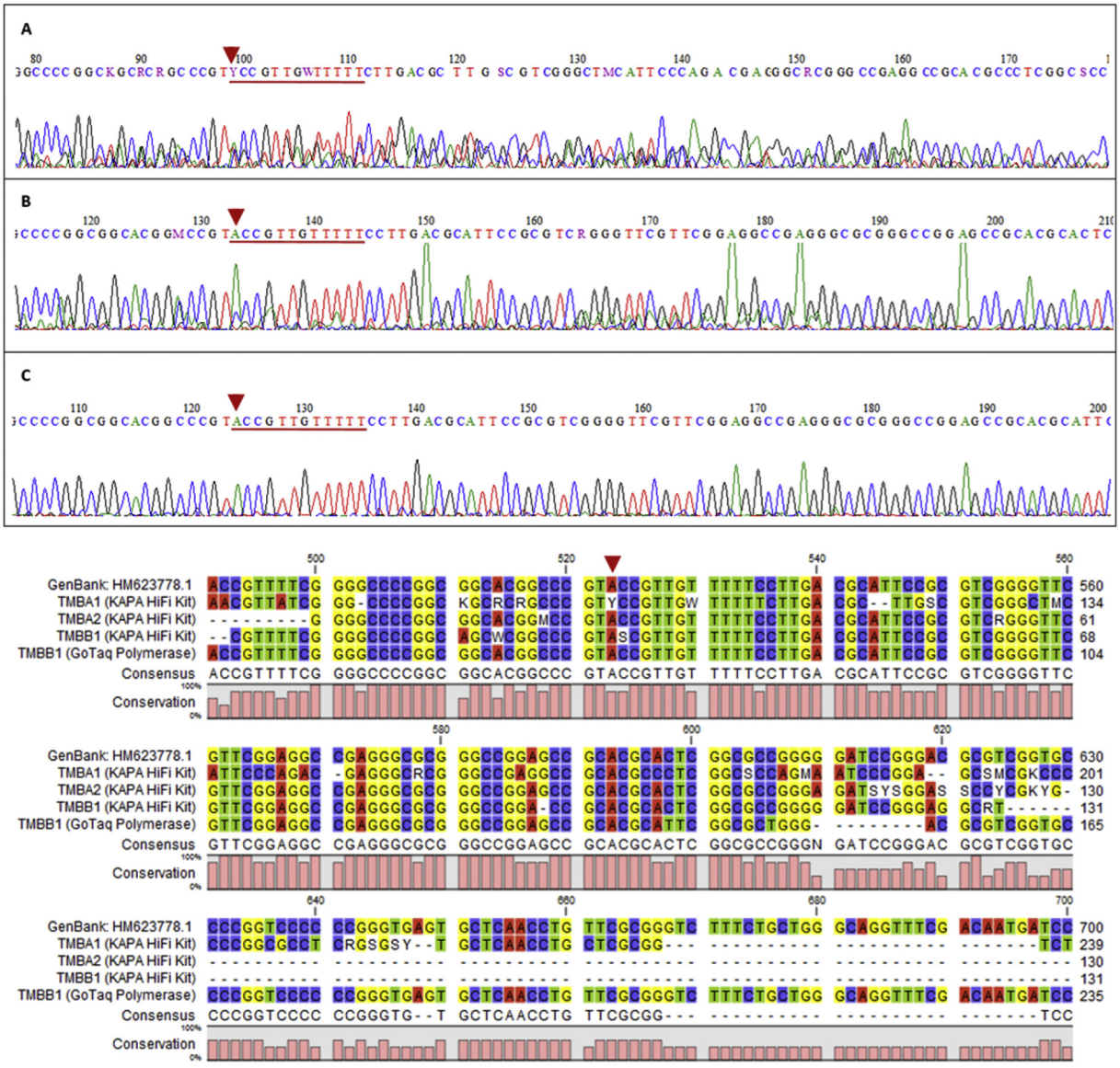

Fig. 14. Paper mulberry ITS1 sequences identified in archaeological bark cloth samples. Upper panel: Red line and red arrowheads indicate the conserved *B. papyrifera* motif within the ITS1 sequence (Seelenfreund et al., 2011). The reverse sequence obtained with the ITS-C primer is shown in all electropherograms. A: ITS1 sequence from TM BA1 sample amplified with the KAPA HiFi Hot Start DNA Polymerase. B: ITS1 sequence from the TM BA2 sample amplified with the KAPA HiFi Hot Start DNA Polymerase. C: ITS1 sequence from the TM BB1 sample amplified with the GoTaq® G2 Flexi DNA Polymerase. Lower Panel: Red arrowhead indicates the polymorphic position that is characteristic for Remote Oceania. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mixing of the two plant fibres. As expected, not all of our samples yielded DNA useful for PCR amplification. However, in those samples that yielded readable sequences after PCR amplification, we identified a range of species, but only one of these, *B. papyrifera*, is a species commonly used as a fibre source for making bark cloth in the Pacific. As Buck (1938) mentions, this was the preferred plant for making cloth. The identification of paper mulberry as the main fibre source in the manufacture of these textiles was achieved analyzing DNA at two different facilities and using two different DNA polymerases for amplification (see Table 3). In addition, the presence of the characteristic *B. papyrifera* Oceanian polymorphism (Seelenfreund et al., 2011; González-Lorca et al., 2015) in these samples suggests a continuity of the use of this species. It also suggests the continuous use of the same plants since the early colonization of this island group, because paper mulberry in the Pacific is reproduced asexually. The results reported in this work also strongly suggest that only *B. papyrifera* was used to make this textile.

In addition to identifying *B. papyrifera* sequences, the presence of a range of species was found in all samples. One of these corresponded to DNA from a fungus (*Aspergillus fumigatus*), which is commonly found in decaying organic matter, and could be an expected host on a decomposing textile or associated corpse. In addition to fungi, we identified several plant species such as *Robinia pseudoacacia*, a tree native to the USA and introduced to many places in the world, including some Pacific islands, tomato (*Solanum lycopersicum*), broccoli (*Brassica oleracea*), dandelion (*Leontodon* sp.), wheat (*Triticum* sp.) and other grasses. The presence of sequences from all these species likely reflect contemporary contamination of the samples under study. As indicated in the introduction, the find was initially handled by lay people and first kept in a plastic food container and then spread open on a kitchen table. The findings likely reflect the range of foods placed either in the container or on this table or tablecloth. The complications

Table 4
Species identified by ITS1 sequences retrieved from the bark cloth and fibre samples.

Sample	Amplification with GoTaq® G2 Hot Start Polymerase		Amplification with KAPA HiFi HotStart DNA Polymerase		Amplification with GoTaq® G2 Flexi DNA Polymerase					
	Faculty of Medicine, University of Chile		Anatomy Department, University of Otago		Faculty of Chemical and Pharmaceutical Sciences, University of Chile					
	Extraction 1		Extraction 2		Extraction 2		Extraction 3		Extraction 4	
	ITS-A	ITS-C	ITS-A	ITS-C	ITS-A	ITS-C	ITS-A	ITS-C	ITS-A	ITS-C
TM BA 1	X	X	Unreadable	No match, *Broussonetia papyrifera* motive	*Lagenaria siceraria*	*Lagenaria siceraria*	*Leontodon* sp.	*Leontodon* sp.	Unreadable	Unreadable
TM BA 2	*Robinia pseudoacacia*	*Robinia pseudoacacia*	Unreadable	*Broussonetia papyrifera*	X	X	X	X	Unreadable	Unreadable
TM BB 1	*Cenchrus hohenackeri*	*Uncultured Eukaryote clone CMH056 Triticum aestivum Cenchrus hohenackeri*	Unreadable	*Broussonetia papyrifera*	*Aspergillus fumigatus*	*Aspergillus fumigatus*	*Broussonetia papyrifera*	*Broussonetia papyrifera*	*Solanum lycopersicum Prunus avium*	*Solanum lycopersicum Prunus avium*
TM BB 2	*Solanum lycopersicum Prunus avium*	*Solanum lycopersicum Prunus avium*	*Solanum lycopersicum Prunus avium*	No match	X	X	X	X	Unreadable	Unreadable
Bark 1	*Demodex brevis Triticum petropavlovskyi Uncultured eukaryote clone CMH056 Triticum aevestium*	*Salacia chinensis Uncultured eukaryote clone CMH056*	X	X	X	X	X	X	X	X
Bark 2	X	X	–	–	–	–	–	–	–	–
Fibre 1	*Triticum petropavlovskyi Uncultured eukaryote clone CMH056 Triticeae Triticum aevestium*	*Uncultured eukaryote clone CMH056*	–	–	–	–	–	–	–	-
Fibre 2	*Brassica oleracea Solanum lycopersicum*	*Brassica oleracea Solanum lycopersicum*	–	–	–	–	–	–	–	-

X: no amplification; –: not assayed.

encountered in the genetic analysis are due, at least in part, to inexperienced handling of the find in its initial stages. No precautions were taken to avoid any type of contamination with present day plant or animal materials. Only once the find was transferred to the Museum of Tahiti, were the use of gloves was included into the handling protocol for the textiles. This may explain the unexpected presence of *Solanum lycopersicum* and other species in several amplification reactions. We need to stress the importance of careful handling of archaeological finds, starting at the site location, especially in view of the increasingly sensitive techniques made available by genetic and other analytical tools. The contemporary contamination can easily obliterate the limited and endogenous DNA remaining in the sample. We wish to stress that even minute amounts of modern biological materials that contain intact DNA that amplifies easily, in contrast to the degraded DNA remains from the archaeological materials may spoil the possibility of its successful genetic analysis. In spite of the lack of initial professional handling, the following of stringent protocols at the ancient DNA laboratory, finally permitted successful characterization of this valuable and unique find.

The multidisciplinary approach for the study of the remains of the archaeological decorated bark cloth from Agakauitai Island, Gambier Archipelago has yielded a wealth of unexpected and interesting information on the bark cloth tradition from pre-European culture from the Gambier Islands. There are no written or material records that bark cloth from this island group was decorated with line drawings. The only surviving record is of tapa painted with large black triangles arranged in a symmetrical pattern. Line drawings such as these are not common in the Pacific as bark cloth decorations, and therefore further study of these patterns is called for. Finally, the genetic analysis of this find confirms that *B. papyrifera* was present on the Gambier archipelago in the pre-contact period and was used for tapa making. This work is the first study that successfully extracts DNA and analyses genetic information from a bark cloth textile found in an archaeological context in Oceania.

Authors' contributions

AS conceived the idea, procured permits and samples. AS and MS secured funding. AMR performed textile conservation and textile characterization. SG, JC and MS performed elemental analysis. XM, CP, BP and OK performed genetic analyses. DS supervised genetic analyses. FP carried out the dating analysis. LM-S and MM provided access to ancient DNA lab space and reagents. AS, DS, MS, FP wrote the manuscript. All authors read, commented and

approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jas.2016.10.008.

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