



## Megafaunal split ends: microscopical characterisation of hair structure and function in extinct woolly mammoth and woolly rhino



Silvana R. Tridico <sup>a,b,\*</sup>, Paul Rigby <sup>c</sup>, K. Paul Kirkbride <sup>d</sup>, James Haile <sup>a,b</sup>, Michael Bunce <sup>a,b</sup>

<sup>a</sup> Ancient DNA Laboratory, School of Veterinary and Life Sciences, Murdoch University, Perth, Western Australia 6150, Australia

<sup>b</sup> Trace and Environmental DNA Laboratory, Department of Environment and Agriculture, Curtin University, Perth, Western Australia 6845, Australia

<sup>c</sup> CMCA, The University of Western Australia, Perth, Western Australia 6009, Australia

<sup>d</sup> School of Chemical and Physical Sciences, Flinders University, GPO Box 2100, Adelaide, South Australia 5001, Australia

### ARTICLE INFO

#### Article history:

Received 2 August 2013

Received in revised form

28 October 2013

Accepted 28 October 2013

Available online 25 November 2013

#### Keywords:

Woolly mammoth

Woolly rhinoceros

Hair

Microscopy

Taphonomy

Permafrost

### ABSTRACT

The large extinct megafaunal species of the Late Pleistocene, *Mammuthus primigenius* (woolly mammoth) and *Coelodonta antiquitatis* (woolly rhino) are renowned for their pelage. Despite this, very little research has been conducted on the form and function of hair from these iconic species. Using permafrost preserved hair samples from seven extinct megafaunal remains, this study presents an in-depth microscopical characterisation of preservation, taphonomy, microbial damage, pigmentation and morphological features of more than 420 hairs. The presence of unique structural features in hairs, from two extinct megafauna species, such as multiple medullae and unparalleled stiffness suggests evolution of traits that may have been critical for their survival in the harsh arctic environment. Lastly, despite popular depictions of red-haired and/or uniformly coloured mammoths, a closer examination of pigmentation reveals that mammoth coats may have exhibited a mottled/variegated appearance and that their 'true' colours were not the vivid red/orange colour often depicted in reconstructions. Insights gained from microscopical examination of hundreds of extinct megafauna hairs demonstrate the value of extracting as much morphological data as possible from ancient hairs prior to destructive sampling for molecular analyses.

© 2013 Elsevier Ltd. All rights reserved.

### 1. Introduction

Mammalian hair predominantly consists of the protein keratin, which due to its chemical structure is highly durable. This resilience is responsible for the survival and preservation of hair for millennia in remains that have been exposed to diverse and extreme conditions such as freezing, burial and mummification. Hair preserved in archaeological and palaeontological contexts is now sought after as a source of "pure" preserved ancient DNA (Gilbert et al., 2008; Rasmussen et al., 2011); however there is much to be gained from the morphological analysis of hair before it is destructively sampled.

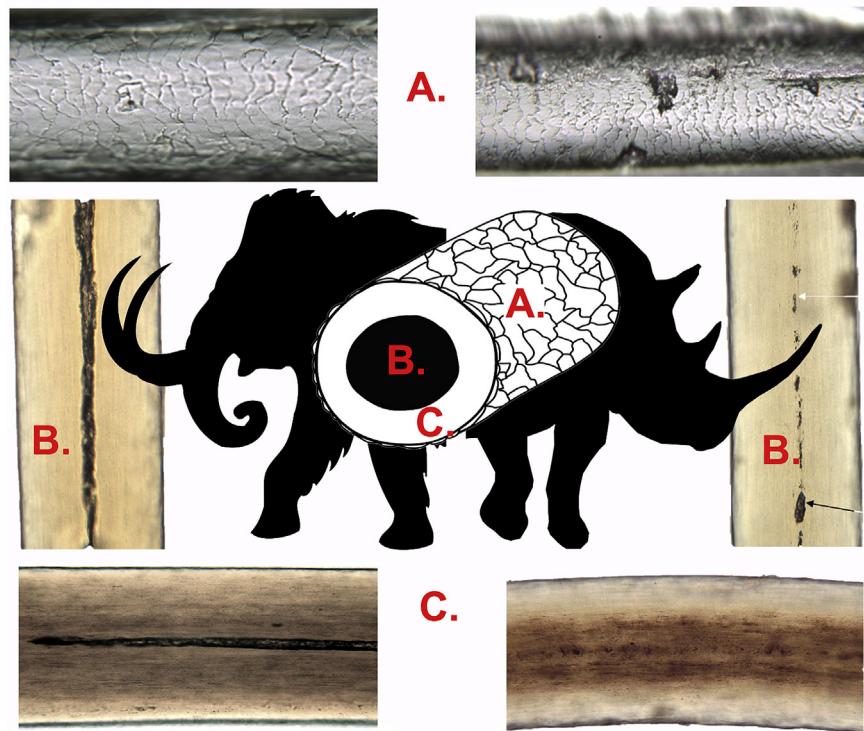
Mammalian hair is essentially composed of three layers consisting of the outermost cuticle, an inner cortex and a central core or medulla (Fig. 1). Close inspection of animal pelts reveals the presence of three distinct types of hair: overhairs, guard hairs and

underhairs. Overhairs are the most prominent and coarsest of hairs on the pelage (coat) and are commonly circular in cross-sectional shape. Guard hairs are less coarse and shorter than overhairs; guard hairs exhibit an array of medullae morphologies, scale patterns and cross-sectional shapes that are usually diagnostic for a particular taxon (Teerink, 1991). The underhairs are shorter and much finer; they range from being wavy, lightly curled to tightly curled and commonly show circular cross-sections. In most mammalian hairs there is a gradation from one hair 'type' to another. This gradation is not abrupt as shown by the presence of 'transitional' hair types, which bear 'hybrid' features.

All mammalian hair shares similar chemical and physical composition and structure. Cross-sectional shapes, medullae morphologies and scale pattern not only differentiate human hair from animal but may also assist in differentiating animal hairs that originate from different taxa. Furthermore, mammalian hairs exhibit intra- and interspecies variance in profile and morphological characteristics depending on the somatic origin (body area that hair originates from) (Brunner and Coman, 1974; Teerink, 1991). While many extant taxa have been studied with regards to hair form and function, for obvious reasons, extinct species have received much less attention.

\* Corresponding author. Trace and Environmental DNA Laboratory, Department of Environment and Agriculture, Curtin University, Perth, Western Australia 6845, Australia. Tel.: +61 8 82785731.

E-mail address: [silvanatridico@yahoo.com](mailto:silvanatridico@yahoo.com) (S.R. Tridico).



**Fig. 1.** Schematic diagram of a generic mammalian hair (centre) that consists of three major components. (A) The outermost cuticle, (B) the central core or medulla which may be continuous (left) or interrupted (right) and (C) cortex that contains pigment granules (melanins) which may be uniformly distributed across the hair shaft (left) or medially distributed (right).

The woolly mammoth (*Mammuthus primigenius*) is probably the most iconic and charismatic of all the extinct northern megafauna and is renowned for its size and hairy coat. Vast numbers of these animals roamed Eurasia and North America in the Pleistocene before becoming extinct on the mainland some 10,000 years ago. The species clung to existence until the last known individuals, comprising a dwarf island population on Wrangel Island, vanished some 4000 years ago (Vartanyan et al., 1993). The causes underlying the extinction of woolly mammoth still remain elusive – a complex interplay of climate and anthropogenic influences is currently proposed (Lorenzen et al., 2011). Despite becoming extinct a few thousand years ago a great deal is known about the woolly mammoth, and it is arguably one of the best-understood representatives of the extinct megafauna. Their relative abundance and wide geographic range increased the probability of discovering their remains; their demise and subsequent entombment in a natural freezer ensured exceptional preservation.

In contrast, the woolly rhinoceros (*Coelodonta antiquitatis*) is less well understood. This is probably due to the paucity of mummified remains (compared to woolly mammoth) that have been discovered, which may reflect the more restricted geographic distribution of this species (it was absent from large areas of the high Arctic, for example) and possibly lower population density, relative to that of the woolly mammoth.

The morphology of woolly mammoth and woolly rhinoceros bones, teeth and carcasses have been extensively studied and documented contributing a wealth of knowledge with regards to their natural history and adaptations to surviving cold temperatures (Boeskorov, 2004). Woolly mammoth were also among the first species to be investigated using PCR of ancient mitochondrial (Paabo et al., 1989) and nuclear DNA (Greenwood et al., 1999). The advent of next generation sequencing enabled researchers to

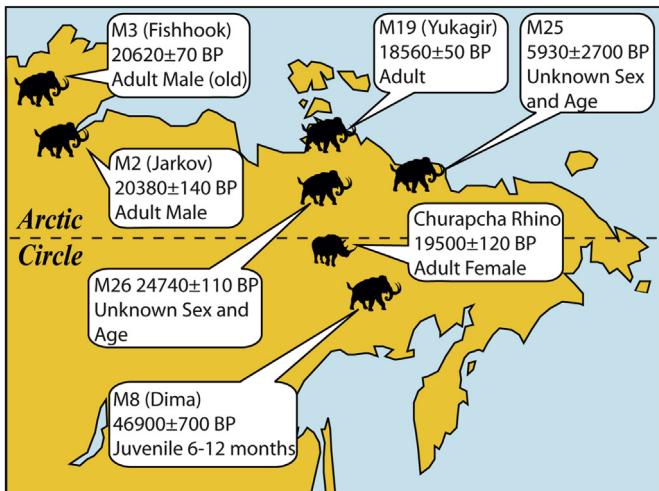
sequence short, fragmented strands of mammoth DNA using the elephant genome as a scaffold (Miller et al., 2008). Significantly, the substrate used for this genome was mammoth hair due to the high levels (relative to contaminating environmental sequences) of endogenous mammoth DNA compared to bone (Gilbert et al., 2008) (Gilbert et al., 2007). The survival of woolly mammoth hair entombed in permafrost for millennia is testament to the resilience of the biopolymer keratin to withstand harsh environmental conditions and insults. In contrast to the woolly mammoth's genome and skeletal morphology, hairs comprising the thick woolly coat, for which this species and (woolly rhino) are famously known, have received little detailed morphological examinations. The objective of the current study is to conduct detailed and comprehensive microscopical examination of hairs from these extinct megafauna in order to investigate possible relationships between hair structure and the environment these animals inhabited and study the effects of taphonomy.

## 2. Materials and methods

### 2.1. Materials

A total of six woolly mammoth (Jarkov, Yukagir, Dima, Fishhook, M25 and M26) and one woolly rhinoceros (Churapcha) hair samples were examined. The original geographic locations in which the remains of these megafauna were found and specimen details are presented in Fig. 2 and in more detail in other publications (Gilbert et al., 2007, 2008).

Adult African elephant (*Loxodonta africana*) hairs were obtained from the United States Fisheries and Wildlife Forensic Laboratory and Aalborg Zoo, Denmark. Adult Asian elephant (*Elephas maximus*) hairs were obtained from Copenhagen Zoo, Denmark. Somatic



**Fig. 2.** Sites of recovery of woolly mammoth hair and woolly rhino hair which were used in the present study, detailing identification details, radio carbon dated ages, sex and age for each hair sample used in the present study.

origins of *Loxodonta* hairs were flank and lower leg/top of foot area, and head, flank, dorsum and lower leg/foot area of the *Elephas* individual. All extant animal hair samples were obtained in accordance with the relevant legislation for the importation of samples from animal species listed in Appendix 1 of CITES. Megafauna samples used in this current study may not have contained representatives of all hairs types present on the living animal.

## 2.2. Methods

Preliminary examinations of each hair sample were conducted macroscopically (naked eye) and at low magnification (6–40 $\times$ ) using a stereomicroscope. Hair types were assigned in accordance with Brunner and Coman classification (Brunner and Coman, 1974). Representative hair types from each sample were subsequently selected for detailed examinations and microscopical analyses at higher magnifications using transmitted light microscopy (100–400 $\times$  magnification), scanning electron microscopy and confocal microscopy. A total of approximately 420–450 hairs were examined in both macro- and microscopical detail.

### 2.2.1. Scale cast pattern and cross-sections

Scale cast patterns and cross-sections were produced in accordance with the methodology of Brunner and Coman (1974). Briefly, a cover slip was coated with clear nail polish and the hair was placed on the wet polish; once hardened the hair was removed leaving a scale impression. Cross-sections were obtained by placing hairs in acetate fibres vertically in holes drilled into a stainless steel plate. A razor blade was used to cut the protruding hair and acetate bundle. Accurate shaft diameters were obtained from whole mounts and cross-sections. Scale bars are not included for scale cast images as the entire hair shaft may not be in contact with the medium.

### 2.2.2. Transmitted light microscopy (TLM)

Hairs were permanently mounted using Safe-T-Mounting permanent mounting medium (FRIONINE Pty Ltd., refractive index ~1.52); all were mounted between conventional glass microscope slides and cover slips (0.17 mm thick). Microscopy was performed on an Olympus compound transmitted light microscope equipped with UPLFL20x Semi apochromatic, UPLAN40x Apochromatic objectives. Images were acquired with an Olympus DP 70 camera and associated software.

### 2.2.3. Confocal microscopy

Confocal microscope images were collected using a modification of published methodology (Kirkbride and Tridico, 2010). A Nikon A1RMP equipped with a Nikon PlanApo VC 60 $\times$  oil immersion NA 1.40 objective was used for all imaging. Multiphoton imaging was used employing 800 nm laser excitation and detection through 450/50 nm, 525/50 nm, 595/50 nm and 704/32 nm bandpass filters. Z stacks were collected through the entire hair thickness typically using z steps of 1  $\mu$ m. Image data sets were processed using Nikon NIS Elements and Nikon NIS Viewer.

### 2.2.4. Scanning electron microscopy (SEM)

Each hair sample was affixed to double sided adhesive tape attached to a 12.6 mm diameter aluminium stub then coated with a 90 nm layer of gold in a Balzers Union Ltd. Sputter coater (Liechtenstein) before being examined and photographed in a Philips XL20 Scanning Electron Microscope (the software for image capture is part of the microscope operating software).

## 3. Results and discussion

### 3.1. Morphological features of permafrost preserved hair

Like most mammals, woolly mammoth and woolly rhino coats comprised multiple hair types each of which were different in regards to structure, colour and microscopical characteristics. Hairs from each megafauna species were categorised on the basis of their macroscopic appearance into overhairs, guard hairs and underhairs in accordance with Brunner and Coman (1974). Macroscopically, overhairs and guard hairs exhibited a variety of colours, ranging from colourless, to dingy yellow, bright red/orange and brown. In contrast, underhairs were either colourless or dingy yellow. Microscopical examination of each hair type revealed unique structures and a variety of post-mortem/taphonomic artifacts.

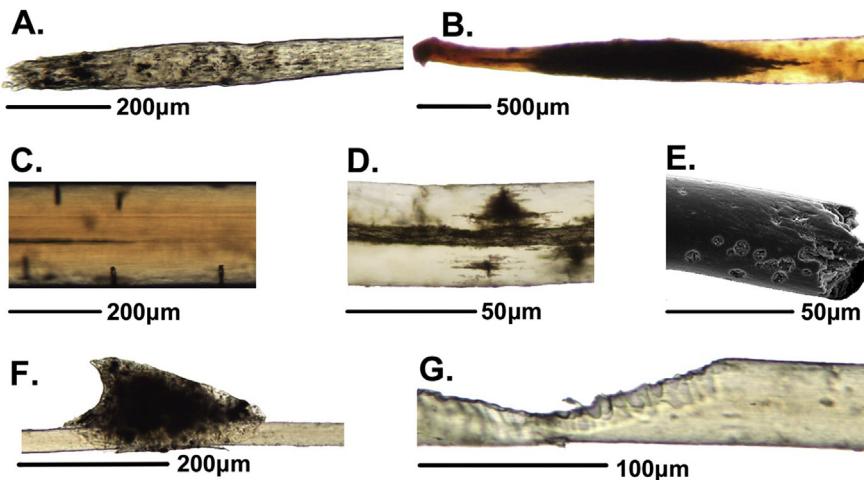
### 3.2. Preservation and damage

Although hair is remarkably resilient it is not immune to post-mortem degradation processes – the hairs reported upon here were no exception despite being predominantly frozen since death. Notably, Jarkov, Dima and M26 woolly mammoth hairs exhibited a phenomenon known as post-mortem banding (or putrid root) (Fig. 3). Post-mortem banding has been studied extensively in human hairs and it solely occurs at the proximal (root) end of hairs that are attached to decomposing bodies; this process is thought to occur from bacterial action and appears to be accelerated in warm and humid conditions and retarded in colder ones (Koch et al., 2013).

The presence of post-mortem banding reveals that the bodies of Dima, Jarkov and M26 mammoths underwent some degree of putrefaction before being frozen. To the best of the authors' knowledge the presence of this post-mortem artifact in animal hairs and ancient animal hairs has not been previously published and as such represents a novel and significant finding.

Evidence of insect activity was found on woolly rhino hairs in the form of cuspatate markings (or "bite marks") (Fig. 3) but whether this artifact occurred as a result of 'ancient' taphonomy or 'modern' taphonomy (e.g. during storage) is unknown. Evidence of antemortem insect activity is also apparent as nit (hair lice) sacs were observed on woolly rhino hair (Fig. 3); lice lay eggs on hair shafts close to the skin, as body heat is required in order for the eggs to hatch.

Hairs buried in soil are susceptible to degradation by keratinophilic fungi that live in soil. They obtain nutrients from digesting keratin containing biological matter such as hooves, horns and hair.



**Fig. 3.** Examples of ante-mortem and taphonomic (post-mortem) artifacts present on extinct mega fauna hair shafts. (A) Jarkov (woolly mammoth) underhair bearing normal root. (B) Jarkov underhair with the centrally placed, dark post-mortem banding in the shaft at the proximal (root) end. (C) Perpendicular needle-like fissures caused by keratinophilic fungal invasion of Jarkov (woolly mammoth) overhair. (D) Conical fissures caused by keratinophilic fungi invasion of M26 (woolly mammoth) guard hair. (E) SEM image showing circular surface degradation and/or points of entry by keratinophilic fungi in Jarkov (woolly mammoth) overhair (F) Woolly rhino under-hairs with the ante-mortem deposition of a hair louse egg case. (G) Cuspatate, insect bite-marks on woolly rhino guard hair shaft.

Fungal digestion of hairs is well studied and reported in the literature (Blyskal, 2009). Evidence of fungal damage was variable in the permafrost preserved hair with widespread fungal growth in some hairs (e.g. M25) and negligible growth in others (e.g. Dima); this may reflect the environment in which the animal carcass was interred i.e. keratinophilic fungi are strictly aerobic and would not survive in an anaerobic environments. Examples of fungal invasion of hairs are illustrated in Fig. 3 and S1–S3.

In woolly mammoth and woolly rhino hairs that did not show evidence of keratinophilic fungal activity the multiple medullae-like structures retained their fine, narrow parallel 'track-like' appearance. This contrasted with the situation in hairs that were infected by fungi, where the medullae-like structures were enlarged and dark (Fig. S3). It would appear that fungal hyphae find it easier to digest areas such medullary canals once they have entered the shaft, as illustrated in Fig. S1B; in essence these keratinophilic fungi digest the hair from the inside out, starting with the medulla. This was also noted by English (1963) 'As soon as the fungus reaches the medulla hyphae begin to grow along it. Growth is much more rapid than through the cortex'.

The degree of bacterial, fungal and insect activity on a hair sample may be a valuable indication of its 'purity' for future genetic and isotopic studies that are complicated by post-mortem contamination by microorganisms.

### 3.3. Roots

Although most of the hairs studied were fragments (i.e. root absent), a significant number of hairs bore intact roots. The majority of hairs with roots were underhairs with the remaining roots being present on coarser guard hairs (additional information and images provided in Fig. S4).

The large number of hairs indicated that these hairs most likely became detached from the body as a result 'skin slippage,' a phenomenon that commonly occurs in the early stages of decomposition, rather than becoming detached from mummified or frozen remains. Mummified skin is leathery and the removal of intact hairs (i.e. bearing roots) would be almost impossible to achieve without breaking the shaft. The premise that some of the bodies were decomposing is further supported by the presence of post-mortem banding in some of the hairs as illustrated in Fig. 3B.

### 3.4. Surface features and scale patterns

Woolly mammoth and woolly rhino guard hairs exhibited comparable surface scale patterns (Fig. S5) which alternated from irregular wave/mosaic pattern and broad petal (nomenclature according to Brunner and Coman (1974)). The overall appearance of the cuticles, which were not prominent, was that the cuticle edges were broadly curved or straight. By analogy with extant mammals that have similar scale patterns, this indicates that individual hairs would not easily interlock, but may freely 'slide' over each other, ensuring these hairs remained separate. This may represent an adaptation to discourage matting or tangling of these hairs (see further discussion below).

The scale arrangements in the finer underhairs were broad petal, with rounded, non-prominent edges. This arrangement, like the overhairs and guard hairs, would have discouraged the hairs from becoming matted, but would have encouraged the hairs to become loosely intertwined, thereby facilitating the formation of insulating thermal air-pockets.

### 3.5. Internal structures-medullae

The medulla, when present in modern mammalian hairs, is almost exclusively single and placed centrally in the hair shaft. Notable exceptions occur in human coarse and stiff beard-, sideburn- and moustache hairs, which may exhibit a double medulla. Our present study revealed two additional mammalian species that exhibit multiple medullae in some of their hairs; *L. africana* (lower leg/foot hairs) and *Elephas maxima* (dorsal and head hairs) as illustrated in Fig. S6.

The most significant characteristic of all woolly mammoth and woolly rhino overhairs was the presence of multiple medullae-like structures, which were often present in greater numbers than seen in samples from extant mammals previously discussed. These structures were manifested as numerous parallel lines that occurred at many radial positions throughout the axis of the shaft (Fig. 4). The greatest number of these structures occurred, without exception, in the coarsest overhairs. In regards to the guard hairs however, an apparent correlation exists between shaft diameter and number of 'medullae' present. Only single medullae were

found in the finer guard hairs. Multiple medulla-like structures were not seen in the fine underhairs (Fig. S7).

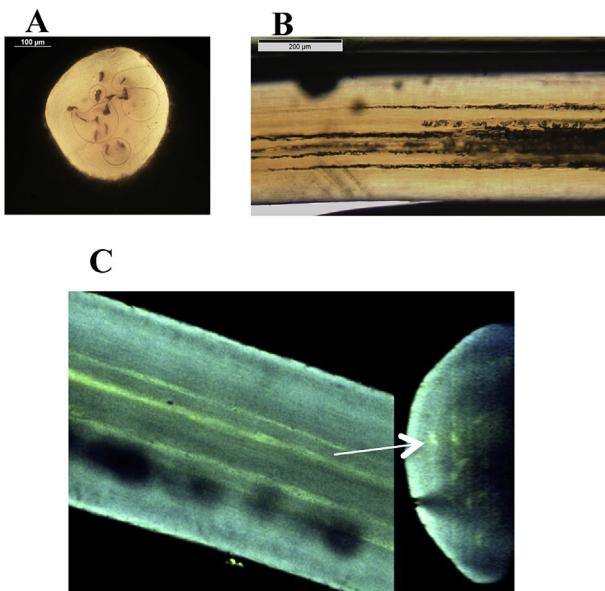
In comparison to woolly mammoth and woolly rhino hairs, and *Loxodonta* hairs, the majority of *Elephas* hairs microscopically were opaque due to the heavy concentration of pigment granules within the cortex (Fig. S6). Therefore, it is possible that dense pigment granules may mask multiple medullae-like structures, if present. In addition, compared to their hirsute elephantid progenitors, extant elephants possess a very sparse pelage and their hairs are mostly coarse and bristle-like.

Gilbert et al. (2007) and Lister and Bahn (2007) depict transverse cross-sections of woolly mammoth hair with multiple dark structures in the cortex. Although these structures are reported as nuclear remnants (Gilbert et al., 2007) or pigmentation (Lister and Bahn, 2007) they are so similar to the structures we observed in the current study (Fig. 4A) that we suspect they are neither pigment nor nuclear remnants. Our findings demonstrate that longitudinal views of these features show them to be elongated parallel lines running along the length of the shaft (Fig. 4B and C) – this observation does not support premises of these structures being nuclear remnants or pigmentation. Nuclear remnants are significantly smaller than the structures depicted and pigmentation is granular and scattered throughout the shaft.

We hypothesize that these medullae-like structures are a cold adaptation that assists their survival in Arctic conditions. Support for this hypothesis is explored in the following section.

### 3.6. Form and function

Through the course of the Pleistocene, megafauna had to adapt and change in order to survive harsh environmental conditions; Campbell et al. (2010) describe an adaptive physiochemical adaptation of woolly mammoth haemoglobin that aided in its survival in cold conditions. We suggest that multiple medullae-like structures in hairs from two extinct megafauna species may result from



**Fig. 4.** Example of multiple medullae-like structures prevalent in extinct megafauna hairs. (A) Transverse cross-section of Jarkov (woolly mammoth) overhair showing dark multiple medullae-like structures throughout the shaft. (B) Longitudinal TLM image of cross-sectioned hair (A) showing multiple medullae-like structures. (C) Confocal virtual cross-section of woolly rhino overhair (approximately 210  $\mu\text{m}$  diameter) showing multiple medullae-like structures throughout the shaft. These structures are parallel in the longitudinal view (left image) and as small spots in the virtual transverse cross-section (arrow). Scale bars (A) 100  $\mu\text{m}$ , (B) 200  $\mu\text{m}$ .

convergent evolution of structures that, in combination with the density of their coats, may have been critical for their survival. Like 'rods' of reinforcing metal in concrete, multiple medullae may have strengthened the hairs in order to maintain shape and orientation and resist distortion. It was noted that woolly mammoth and woolly rhino overhairs were very strongly resistant to being bent and manipulated, and were noticeably 'springy' and very smooth, almost slippery, to the touch. These attributes probably prevented the long overhairs and coarsest guard hairs becoming intertwined and/or matted. Matted hair is likely to be less efficient at channelling moisture/water and snow away from the body, which would have proved fatal in the depths of an arctic winter. The 'springiness' of overhairs might also be attributed to a different type of keratin in these hairs, which is currently being investigated.

The discovery of sebaceous glands in mummified woolly mammoth remains by Repin et al. was significant as '...sebaceous glands are a sign of cold adaptation' (Repin et al., 2004). These glands secrete an oily/waxy substance (sebum), which lubricates the skin and hair surface and acts as natural water repellent. Given the similarity in morphology and texture of woolly rhino and woolly mammoth hairs it is not unreasonable to assume woolly rhino skin also contained sebaceous glands that served the same purpose as those found in the woolly mammoths. The waxy/slippery feel to the overhairs may have arisen by the presence of sebum. This too is currently under investigation.

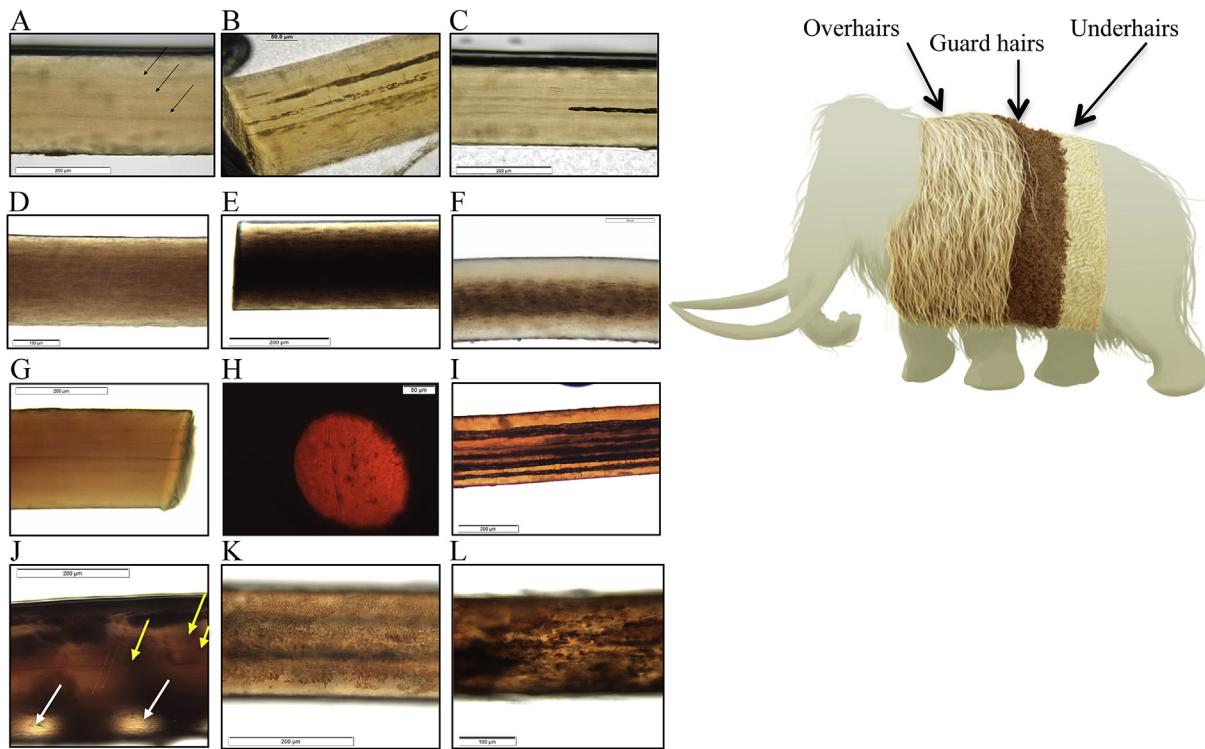
Mammalian underhair (or underfur) acts as an insulating layer that assists thermoregulation by forming insulating air pockets between the intertwined hairs. Woolly mammoth and woolly rhino underhairs were comparable to modern, extant mammal underhairs.

Woolly mammoth underhairs exhibited uniform shaft diameters (which measured approximately 20–100  $\mu\text{m}$ ); all were wavy but in addition numerous hairs were tightly coiled and difficult to separate. Woolly rhino underhairs, whilst exhibiting wavy and lightly curled hairs similar to those found on the woolly mammoth, did not exhibit the tightly coiled underhairs and as consequence were easier to separate. Woolly rhino underhairs measured approximately 20–100  $\mu\text{m}$  in diameter. The profiles of the thickest underhairs differed to those from woolly mammoth in that they were 'buckled' along the length of the shaft (Fig. S8). It is reasonable to assume that like coarse human beard hairs or pubic hairs, these 'buckled' shafts would not have lain flat but may have afforded the animal a 'puffier' or bulkier appearance than the woolly mammoth whose hairs were not buckled.

Each of the above proposed structural adaptations to woolly mammoth and woolly rhino pelage may have increased the effectiveness of their woolly coats, 'Effective pelage can extend a little further the meager calories in winter food.... Woolliness can mean the difference between life and death.' (Guthrie, 1990).

### 3.7. Colour and pigmentation

Mammalian hair colouration is one of the most conspicuous phenotypes; in some animals it plays diverse and significant roles such as sexual attraction, sexual dimorphism and camouflage. However, on the basis of the results of this study, there is no indication that any of these functions applied to woolly mammoth and woolly rhino. Hair colour, length and type appeared to be equally represented in each of the samples, irrespective of the age and sex of the specimen they were taken. Macroscopically and microscopically, woolly mammoth and woolly rhino overhairs, guard hairs and underhairs varied in colour from colourless, to dingy yellow, red/orange and brown (which ranged from pale brown to dark brown, almost black). The majority of overhairs and thicker guard hairs from the woolly mammoths and woolly rhino



**Fig. 5.** Examples of natural and 'acquired' colouration in overhairs and guard hairs from two extinct megafauna species. Images A–C represent natural colouration of overhairs that are devoid of pigmentation. Images D–F show the distribution of pigment in guard hairs that were either uniformly pigmented (D, E) or medial (F). The image (right) shows a 'deconvoluted' view of the distribution of these hair types comprising woolly mammoth (and woolly rhino) pelages. Images G–I show 'acquired' colouration present on the inside of hair shafts devoid of pigmentation. The homogeneous red-orange colouration throughout the hair is evident in transverse cross-section (H). Images J–L reveal red/orange colouration due to 'debris' on the outer surface of hair shafts. The woolly rhino overhair (J) shows breaches in the surface debris reveal three underlying colourless areas hair shafts (thick arrows) and faint multiple medullae are apparent (fine arrows) Scale bars: A, C, E, G, H, J, L: 200  $\mu$ m; B, I: 50  $\mu$ m; E: 200  $\mu$ m and K: 100  $\mu$ m.

were vivid red/orange colour or 'fox red' as described by Krefft (1969). Close examination of woolly mammoth and woolly rhino hairs revealed that their colours could be attributed to either natural pigmentation or 'acquired' colouration (discussed below).

### 3.8. Natural pigmentation

The diversity of mammalian hair colour is attributed to the quality, quantity and ratio of two melanins (pigment types), eumelanin (predominant in dark brown/black hairs) and pheomelanin (predominant in red and blonde hairs) (Ito and Wakamatsu, 2003; Lister and Bahn, 2007). Pigmentation in hairs is usually found as granules in the cortex of the hair shaft; its distribution may be uniformly or medially distributed (around the central axis of the shaft). In hairs from some animals (but not humans) a unique feature is one in which the hair shaft shows natural, abrupt colour changes (commonly known as banding). These hairs may be bi- or tri-coloured along the length of the shaft. If present in sufficient quantities these hairs may give the pelage a mottled or speckled appearance.

Microscopical examination of woolly mammoth and woolly rhino hairs revealed visible pigment in many guard hairs and underhairs, but absent in overhairs (Fig. 5A–C). Where present, pigment distribution was either uniformly distributed or medially distributed as illustrated in Fig. 5D–F; however, medial pigmentation was the most prevalent distribution in hairs from both extinct megafauna species, as is also the case in extant elephantids. Like extant elephantids, Yukagir, Jarkov, M25 and M26 woolly mammoths also exhibited bi-coloured hairs (Fig. S9); bi-coloured mammoth hairs are also noted by Lister and Bahn (2007). These hairs were coarse and bristle-like, similar to both species of extant

elephantids. No bi-coloured hairs were evident in the woolly rhino sample.

Underhair from woolly mammoth and woolly rhino were comparable exhibiting colourless, pale yellow or pale brown hairs. Pigment granules in coarser underhairs were sparse and uniformly distributed within the shaft. Guard hairs from Yukagir woolly mammoth were notably darker and more heavily pigmented compared with the samples from other woolly mammoths and woolly rhino. This may be due to the pelage of this animal being significantly darker than the hairs of other megafauna studied or the hairs originated from a different somatic origin (body area).

### 3.9. 'Acquired' colouration

Current literature attributes red/orange colour of extinct megafauna overhairs and guard hairs to the oxidation of melanin pigment granules as a result of interment over millennia (Lister and Bahn, 2007). It is generally accepted that eumelanin and pheomelanin pigment granules are susceptible to photo degradation via UV in sunlight (Krefft, 1969; Lee, 2010). However, although this chemical reaction undoubtedly accounts for some of the red/orange colouration seen in these megafauna hairs, it cannot be the sole cause because hair totally lacking pigment granules also showed this colour that was more vivid than seen in pigmented hairs.

Krefft concluded that multiple processes were acting upon hairs each resulting in colour changes. He acknowledged the effects of photo oxidation of pigments and found that the red/orange ('fox-red') colouration not only occurred in pigmented hairs, but also in hairs totally lacking pigment granules; he concluded that this could be attributed to the breakdown of tyrosine residues in keratin. This process resulted in colouration that was homogenously distributed

throughout the entire hair shaft (Krefft, 1969). We observed a number of homogenously coloured 'fox red' hairs from both extinct megafauna species, predominantly in overhairs and coarsest guard hairs as illustrated in Fig. 5G–I. On the basis of the work conducted by Krefft it is likely that this colouration may be attributed to the chemical breakdown of keratin.

However, many overhairs bore red/orange debris or a 'sheath' encasing the shaft (Fig. 5J–L). This may be due to a fungal deposit. The present study supports the premise that the natural coat colour of an individual animal was probably not uniform and certainly not red/orange in colour. Instead, the results of this current study strongly indicate that woolly mammoth and woolly rhino pelages may have exhibited a variety of colours comprising hairs of different colours from different somatic origins and/or hair type. A modern day example of just such a pelage is present on the musk oxen (*Ovibos moschatus*), whose pelage is likened to that of the woolly mammoth, which has white hair on its muzzle, top of head, forelocks and saddle. This is in stark contrast to the remainder of the body on which the hairs are rich red/brown in colour.

Workman et al. (2011) assert that 'light coloured woolly mammoths probably were very rare, or even non-existent.' The current study of woolly mammoth and woolly rhino hairs does not support this premise as we found an abundance of colourless hairs in all samples and on both species. It does, however, suggest that woolly mammoths and woolly rhino pelages comprised light and dark coloured hairs with lighter hairs predominating, especially amongst underhairs. On the basis of the mixture of pigmented, non-pigmented and bi-coloured hairs found in each sample examined, woolly mammoth and woolly rhino coats were likely to have exhibited heterogeneity in colour rather than homogeneity. The arrangement of hair types comprising the pelages would be colourless, long overhairs covering a mixture of pigmented and non-pigmented guard hairs, all of which covered predominantly colourless underhairs for both species of extinct megafauna (Fig. 5). Furthermore, it is possible that woolly mammoths and woolly rhinos may have shown a mottled ('salt and pepper') appearance to their coats if bi-coloured hairs occurred *en masse*.

Perhaps further genetic studies on hairs, for which the phenotype is self-evident, may further elucidate extinct megafauna pelage colouration.

#### 4. Conclusion

The results of the present study demonstrate new insights into woolly mammoth and woolly rhino hairs and their preservation in permafrost. In particular, regarding the structure and colour of woolly mammoth and woolly rhino pelages, detailed microscopical examinations enable development of a more accurate picture of pelage appearance, form, function and colour than currently exists. This study challenges the current view that pelages of these two species were uniform in colour; the findings indicate that they were likely to exhibit a variegated colouration with long colourless overhairs covering a mixture of bi-coloured, uniformly coloured brown or red/brown and colourless guard hairs, and innumerable colourless underhairs. The presence of multiple medullae-like features in two extinct megafauna species is suggestive of convergent evolution of traits that, together with their woolly coats, may have helped them to survive the thermally, and in winter nutritionally, challenging environments of the Pleistocene glaciations. Future morphological examinations of woolly mammoth and woolly rhino hairs taken from known areas of the body would undoubtedly shed further light on the colouration and distribution of hair types on their pelages. The present study demonstrates the importance of familiarity and expertise in the microscopical and morphological examination of hairs to reveal aspects of megafauna

hairs that might have remained hidden. We advocate that there is much to be gained from morphological and microscopic examination of hair prior to any destructive sampling for molecular analyses. A multi-disciplinary approach to the examination of extinct megafauna remains can only continue to enhance our knowledge of these iconic species.

#### Acknowledgements

It is with gratitude and appreciation that we thank Professors Tom Gilbert, Eske Willerslev and their collaborators including Andrei Sher for providing the ancient megafauna hair samples. Professor Adrian Lister for reviewing the manuscript, Gordon Thompson for his patience and SEM skills, Copenhagen Zoo, Aalborg Zoo (Dk), Natural History Museum of Denmark and United States Fisheries and Wildlife Laboratory for provision of extant elephantid hairs. We also thank Dr. Eline Lorenzen for the use of her cartoons and Riana Sellek for her patience and skill in producing the woolly mammoth artwork in Fig. 5. The authors acknowledge the CMCA at The University of Western Australia, a facility funded by the University, State and Commonwealth Governments. MB funded by an Australian Research Council Future Fellowship (FT0991741).

#### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.quascirev.2013.10.032>.

#### References

- Blyskal, B., 2009. Fungi utilizing keratinous substrates. *Int. Biodeterior. Biodegrad.* 63, 631–653.
- Boeskorov, G.M., 2004. Quaternary mammal collections in the museums of Yakutsk (Eastern Siberia, Yakutia, Russia). *Cranium* 21, 14.
- Brunner, H., Coman, B., 1974. The Identification of Mammalian Hairs. Inkata Press, Melbourne, Australia.
- Campbell, K.L., Roberts, J.E.E., Watson, L.N., Stetefeld, J., Sloan, A.M., Signore, A.V., Howatt, J.W., Tame, J.R.H., Rohland, N., Shen, T.-J., Austin, J.J., Hofreiter, M., Ho, C., Weber, R.E., Cooper, A., 2010. Substitutions in woolly mammoth hemoglobin confer biochemical properties adaptive for cold tolerance. (LETTERS) (–Report). *Nat. Genet.* 42, 536–537.
- English, M.P., 1963. The saprophytic growth of keratinophilic fungi on keratin. *Mikol. Iekarska* 2.
- Gilbert, M.T., Drautz, D.I., Lesk, A.M., Ho, S.Y., Qi, J., Ratan, A., Hsu, C.H., Sher, A., Dalen, L., Gotherstrom, A., Tomsho, L.P., Rendulic, S., Packard, M., Campos, P.F., Kuznetsova, T.V., Shidlovskiy, F., Tikhonov, A., Willerslev, E., Iacumin, P., Buigues, B., Ericson, P.G., Germonpre, M., Kosintsev, P., Nikolaev, V., Nowak-Kemp, M., Knight, J.R., Irzyk, G.P., Perbost, C.S., Fredrikson, K.M., Harkins, T.T., Sheridan, S., Miller, W., Schuster, S.C., 2008. Intraspecific phylogenetic analysis of Siberian woolly mammoths using complete mitochondrial genomes. *Proc. Natl. Acad. Sci. U. S. A.* 105, 8327–8332.
- Gilbert, M.T., Tomsho, L.P., Rendulic, S., Packard, M., Drautz, D.I., Sher, A., Tikhonov, A., Dalen, L., Kuznetsova, T., Kosintsev, P., Campos, P.F., Higham, T., Collins, M.J., Wilson, A.S., Shidlovskiy, F., Buigues, B., Ericson, P.G., Germonpre, M., Gotherstrom, A., Iacumin, P., Nikolaev, V., Nowak-Kemp, M., Willerslev, E., Knight, J.R., Irzyk, G.P., Perbost, C.S., Fredrikson, K.M., Harkins, T.T., Sheridan, S., Miller, W., Schuster, S.C., 2007. Whole-genome shotgun sequencing of mitochondria from ancient hair shafts. *Science* 317, 1927–1930.
- Greenwood, A.D., Capelli, C., Possnert, G., Pääbo, S., 1999. Nuclear DNA sequences from late Pleistocene megafauna. *Mol. Biol. Evol.* 16, 1466–1473.
- Guthrie, R.D., 1990. Frozen Fauna of the Mammoth Steppe The Story of Blue Babe. The University of Chicago.
- Ito, S., Wakamatsu, K., 2003. Quantitative analysis of eumelanin and pheomelanin in humans, mice, and other animals: a comparative review. *Pigment Cell Res.* 16, 523–531.
- Kirkbride, K.P., Tridico, S.R., 2010. The application of laser scanning confocal microscopy to the examination of hairs and textile fibers: an initial investigation. *Forens. Sci. Int.* 195, 28–35.
- Koch, S.L., Michaud, A.L., Mikell, C.E., 2013. Taphonomy of hair – a study of post-mortem root banding. *J. Forens. Sci.* 58 (Suppl. 1), S52–S59.
- Krefft, M.D.S., 1969. About postmortem changes in the structure and color of hairs and other keratin-containing dermal substances. *Arch. Fuer Kriminol.* 143.
- Lee, W.-S., 2010. Hair photoaging. In: Trueb, R.M., Tobin, D.J. (Eds.), *Aging Hair*. Springer-verlag.

Lister, A., Bahn, P., 2007. *Mammoths Giants of the Ice Age*. University of California Press, Los Angeles, USA.

Lorenzen, E.D., Nogués-Bravo, D., Orlando, L., Weinstock, J., Binladen, J., Marske, K.A., Ugan, A., Borregaard, M.K., Gilbert, M.T.P., Nielsen, R., Ho, S.Y.W., Goebel, T., Graf, K.E., Byers, D., Stenderup, J.T., Rasmussen, M., Campos, P.F., Leonard, J.A., Koepfli, K.-P., Froese, D., Zazula, G., Stafford, T.W., Aaris-Sorensen, K., Batra, P., Haywood, A.M., Singarayer, J.S., Valdes, P.J., Boeskorov, G., Burns, J.A., Davydov, S.P., Haile, J., Jenkins, D.L., Kosintsev, P., Kuznetsova, T., Lai, X., Martin, L.D., McDonald, H.G., Mol, D., Meldgaard, M., Munch, K., Stephan, E., Sablin, M., Sommer, R.S., Sipko, T., Scott, E., Suchard, M.A., Tikhonov, A., Willerslev, R., Wayne, R.K., Cooper, A., Hoffreiter, M., Sher, A., Shapiro, B., Rahbek, C., Willerslev, E., 2011. Species-specific responses of Late Quaternary megafauna to climate and humans. *Nature* 479, 359–364.

Miller, W., Drautz, D.I., Ratan, A., Pusey, B., Qi, J., Lesk, A.M., Tomsho, L.P., Packard, M.D., Zhao, F., Sher, A., Tikhonov, A., Raney, B., Patterson, N., Lindblad-Toh, K., Lander, E.S., Knight, J.R., Irzyk, G.P., Fredrikson, K.M., Harkins, T.T., Sheridan, S., Pringle, T., Schuster, S.C., 2008. Sequencing the nuclear genome of the extinct woolly mammoth. *Nature* 456, 387–390.

Paabo, S., Higuchi, R.G., Wilson, A.C., 1989. Ancient DNA and the polymerase chain reaction. The emerging field of molecular archaeology. *J. Biol. Chem.* 264, 9709–9712.

Rasmussen, M., Guo, X., Wang, Y., Lohmueller, K.E., Rasmussen, S., Albrechtsen, A., Skotte, L., Lindgreen, S., Metspalu, M., Jombart, T., Kivisild, T., Zhai, W., Eriksson, A., Manica, A., Orlando, L., De La Vega, F.M., Tridico, S., Metspalu, E., Nielsen, K., Avila-Arcos, M.C., Moreno-Mayar, J.V., Muller, C., Dorch, J., Gilbert, M.T., Lund, O., Wesolowska, A., Karmin, M., Weinert, L.A., Wang, B., Li, J., Tai, S., Xiao, F., Hanihara, T., van Driem, G., Jha, A.R., Ricaut, F.X., de Knijff, P., Migliano, A.B., Gallego Romero, I., Kristiansen, K., Lambert, D.M., Brunak, S., Forster, P., Brinkmann, B., Nehlich, O., Bunce, M., Richards, M., Gupta, R., Bustamante, C.D., Krogh, A., Foley, R.A., Lahr, M.M., Balloux, F., Sicheritz-Ponten, T., Villem, R., Nielsen, R., Wang, J., Willerslev, E., 2011. An aboriginal Australian genome reveals separate human dispersals into Asia. *Science* 334, 94–98.

Repin, V.E., Taranov, O.S., Ryabchikova, Tikhonov, A.N., Pugachev, V.G., 2004. Sebaceous glands of the woolly mammoth, *Mammuthus primigenius* Blum: histological evidence. *Dokl. Biol. Sci. Proc. Acad. Sci. USSR, Biol. Sci. Sect./Transl. Russ.* 398, 382–384.

Teerink, B.J., 1991. *Hair of West European Mammals: Atlas and Identification Key*. Cambridge University Press, New York.

Vartanyan, S.L., Garutt, V.E., Sher, A.V., 1993. Holocene dwarf mammoths from Wrangel Island in the Siberian Arctic. *Nature* 362, 337–340.

Workman, C., Dalen, L., Vartanyan, S., Shapiro, B., Kosintsev, P., Sher, A., Gotherstrom, A., Barnes, I., 2011. Population-level genotyping of coat colour polymorphism in woolly mammoth (*Mammuthus primigenius*). *Quat. Sci. Rev.* 30, 2304–2308.