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1 **Megafaunal split ends: Microscopical characterisation of hair structure and**
2 **function in extinct woolly mammoth and woolly rhino.**

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13

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17

18 **ABSTRACT**

19 The large extinct megafaunal species of the Late Pleistocene, *Mammuthus*
20 *primigenius* (woolly mammoth) and *Coelodonta antiquitatis* (woolly rhino) are
21 renowned for their pelage. Despite this, very little research has been conducted on the
22 form and function of hair from these iconic species. Using permafrost preserved hair
23 samples from seven extinct megafaunal remains, this study presents an in-depth
24 microscopical characterisation of preservation, taphonomy, microbial damage,
25 pigmentation and morphological features of more than 420 hairs. The presence of
26 unique structural features in hairs, from two extinct megafauna species, such as
27 multiple medullae and unparalleled stiffness suggests evolution of traits that may have
28 been critical for their survival in the harsh arctic environment. Lastly, despite popular
29 depictions of red-haired and/or uniformly coloured mammoths, a closer examination
30 of pigmentation reveals that mammoth coats may have exhibited a mottled/variegated
31 appearance and that their ‘true’ colors were not the vivid red/orange color often
32 depicted in reconstructions. Insights gained from microscopical examination of

33 hundreds of extinct megafauna hairs demonstrate the value of extracting as much
34 morphological data as possible from ancient hairs prior to destructive sampling for
35 molecular analyses.

36 **1. INTRODUCTION**

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

45

46 Mammalian hair is essentially composed of three layers consisting of the outermost
47 cuticle, an inner cortex and a central core or medulla (Fig.1). Close inspection of
48 animal pelts reveals the presence of three distinct types of hair: overhairs, guard hairs
49 and underhairs. Overhairs are the most prominent and coarsest of hairs on the pelage
50 (coat) and are commonly circular in cross-sectional shape. Guard hairs are coarser and
51 larger than underhairs; guard hairs exhibit an array of medullae morphologies, scale
52 patterns and cross-sectional shapes that may be diagnostic for a particular taxon
53 ([Teerink, 1991](#)). The underhairs are shorter and much finer; they range from being
54 wavy, lightly curled to tightly curled and commonly show circular cross-sections. In
55 most mammalian hairs there is a gradation from one hair ‘type’ to another. This
56 gradation is not abrupt as shown by the presence of ‘transitional’ hair types, which
57 bear ‘hybrid’ features.

58 All mammalian hair shares similar chemical and physical composition and structure.
59 Cross-sectional shapes, medullae morphologies and scale pattern not only
60 differentiate human hair from animal but may also assist in differentiating animal
61 hairs that originate from different taxa. Furthermore, mammalian hairs exhibit intra-
62 and interspecies variance in profile and morphological characteristics depending on
63 the somatic origin (body area that hair originates from) ([Brunner, 1974](#); [Teerink,](#)
64 [1991](#)). While many extant taxa have been studied with regards to hair form and
65 function, for obvious reasons, extinct species have received much less attention.

66

67 The woolly mammoth (*Mammuthus primigenius*) is probably the most iconic and
68 charismatic of all the extinct northern megafauna and is renowned for its size and
69 hairy coat. Vast numbers of these animals roamed Eurasia and North America in the
70 Pleistocene becoming extinct on the mainland some 10,000 years ago. The species
71 clung to existence until the last known individuals, comprising a dwarf island
72 population on Wrangel Island, vanished some 4,000 years ago ([Vartanyan et al.,](#)
73 [1993](#)). The causes underlying the extinction of woolly mammoth still remain elusive
74 – a complex interplay of climate and anthropogenic influences is currently proposed
75 ([Lorenzen et al., 2011](#)). Despite becoming extinct a few thousand years ago a great
76 deal is known about the woolly mammoth, and it is arguably one of the best-
77 understood representatives of the extinct megafauna. Their relative abundance and
78 wide geographic range increased the probability of discovering their remains; their
79 demise and subsequent entombment in a natural freezer ensured exceptional
80 preservation.

81

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

88

89 The morphology of woolly mammoth and woolly rhinoceros bones, teeth and
90 carcasses have been extensively studied and documented contributing a wealth of
91 knowledge with regards to their natural history and adaptations to surviving cold
92 temperatures ([Boeskorov, 2004](#)). Woolly mammoth were also among the first species
93 to be investigated using PCR of ancient mitochondrial ([Paabo et al., 1989](#)) and
94 nuclear DNA ([Greenwood et al., 1999](#)). The advent of next generation sequencing
95 enabled researchers to sequence short, fragmented strands of mammoth DNA using
96 the elephant genome as a scaffold ([Miller et al., 2008](#)). Significantly, the substrate
97 used for this genome was mammoth hair due to the high levels (relative to
98 contaminating environmental sequences) of endogenous mammoth DNA compared to
99 bone ([Gilbert et al., 2008](#)) ([Gilbert et al., 2007](#)). The survival of woolly mammoth

100 hair entombed in permafrost for millennia is testament to the resilience of the
101 biopolymer keratin to withstand harsh environmental conditions and insults. In
102 contrast to the woolly mammoth's genome and skeletal morphology, hairs comprising
103 the thick woolly coat, for which this species and (woolly rhino) are famously known,
104 have received little detailed morphological examinations. The objective of the current
105 study is to conduct detailed and comprehensive microscopical examination of hairs
106 from these extinct megafauna in order to investigate possible relationships between
107 hair structure and the environment these animals inhabited and study the effects of
108 taphonomy.

109

110 **2. Materials and Methods**

111 *2.1. Materials*

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

117

118 Adult African elephant (*Loxodonta africana*) hairs were obtained from the United
119 States Fisheries and Wildlife Forensic Laboratory and Aalborg Zoo, Denmark. Adult
120 Asian elephant (*Elephas maximus*) hairs were obtained from Copenhagen Zoo,
121 Denmark. Somatic origins of *Loxodonta* hairs were flank and lower leg/top of foot
122 area, and head, flank, dorsum and lower leg/foot area of the *Elephas* individual. All
123 extant animal hair samples were obtained in accordance with the relevant legislation
124 for the importation of samples from animal species listed in Appendix I of CITES.
125 Megafauna samples used in this current study may not have contained representatives
126 of all hairs types present on the living animal.

127

128 *2.2. Methods*

129 Preliminary examinations of each hair sample were conducted macroscopically
130 (naked eye) and at low magnification (6-40x) using a stereomicroscope. Hair types
131 were assigned in accordance with Brunner and Coman classification ([Brunner, 1974](#)).
132 Representative hair types from each sample were subsequently selected for detailed
133 examinations and microscopic analyses at higher magnifications using transmitted

134 light microscopy (100-400x magnification), scanning electron microscopy and
135 confocal microscopy. A total of approximately 420-450 hairs were examined in both
136 macro- and microscopic detail.

137

138 *2.2.1. Scale cast pattern and cross-sections*

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

147

148 *2.2.2. Transmitted Light Microscopy (TLM)*

149 Hairs were permanently mounted using Safe-T-Mounting permanent mounting
150 medium (FRIONINE Pty Ltd, refractive index ~1.52); all were mounted between
151 conventional glass microscope slides and cover slips (0.17mm thick). Microscopy
152 was performed on an Olympus compound transmitted light microscope equipped with
153 UPLFL20x Semi apochromatic, UPLANO40x Apochromatic objectives. Images were
154 acquired with an Olympus DP 70 camera and associated software.

155

156 *2.2.3. Confocal Microscopy*

157 Confocal microscope images were collected using a modification of published
158 methodology ([Kirkbride, 2010](#)). A Nikon A1RMP equipped with a Nikon PlanApo
159 VC 60x oil immersion NA 1.40 objective was used for all imaging. Multiphoton
160 imaging was used employing 800nm laser excitation and detection through 450/50nm,
161 525/50nm, 595/50nm and 704/32nm bandpass filters. Z stacks were collected through
162 the entire hair thickness typically using z steps of 1µm. Image data sets were
163 processed using Nikon NIS Elements and Nikon NIS Viewer.

164

165 *2.2.4. Scanning Electron Microscopy (SEM)*

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

171 **3. RESULTS AND DISCUSSION**

172 *3.1. Morphological features of permafrost preserved hair*

173 Like most mammals, woolly mammoth and woolly rhino coats comprised multiple
174 hair types each of which were different in regards to structure, color and microscopic
175 characteristics. Hairs from each megafauna species were categorised on the basis of
176 their macroscopic appearance into overhairs, guard hairs and underhairs in accordance
177 with Brunner and Coman ([Brunner, 1974](#)). Macroscopically, overhairs and guard
178 hairs exhibited a variety of colors, ranging from colorless, to dingy yellow, bright
179 red/orange and brown. In contrast, underhairs were either colorless or dingy yellow.
180 Microscopic examination of each hair type revealed unique structures and a variety of
181 post-mortem/taphonomic artifacts.

182

183 *3.2 Preservation and Damage*

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

192

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

198

199 Evidence of insect activity was found on woolly rhino hairs in the form of cusped
200 markings (or “bite marks”) (Fig. 3) but whether this artifact occurred as a result of
201 ‘ancient’ taphonomy or ‘modern’ taphonomy (e.g. during storage) is unknown.
202 Evidence of *ante-mortem* insect activity is also apparent as nit (hair lice) sacs were
203 observed on woolly rhino hair (Fig. 3); lice lay eggs on hair shafts close to the skin, as
204 body heat is required in order for the eggs to hatch.

205

206 Hairs buried in soil are susceptible to degradation by keratinophilic fungi that live in
207 soil. They obtain nutrients from digesting keratin containing biological matter such as
208 hooves, horns and hair. Fungal digestion of hairs is well studied and reported in the
209 literature ([Blyskal, 2009](#)). Evidence of fungal damage was variable in the permafrost
210 preserved hair with widespread fungal growth in some hairs (e.g. M25) and negligible
211 growth in others (e.g. Dima); this may reflect the environment in which the animal
212 carcass was interred i.e. keratinophilic fungi are strictly aerobic and would not survive
213 in an anaerobic environments. Examples of fungal invasion of hairs are illustrated in
214 Fig. 3 and S1-S3.

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

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

228

229 3.3. Roots

230 Although most of the hairs studied were fragments (i.e. root absent), a significant
231 number of hairs bore intact roots. The majority of hairs with roots were underhairs

232 with the remaining roots being present on coarser guard hairs (additional information
233 and images provided in Fig. S4).

234

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

244

245 *3.4. Surface features and Scale patterns*

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

255

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

261

262 *3.5. Internal structures-Medullae*

263 The medulla, when present in modern mammalian hairs is, almost exclusively single
264 and placed centrally in the hair shaft. Notable exceptions occur in human coarse and
265 stiff beard-, sideburn- and moustache hairs, which may exhibit a double medulla. Our

266 present study revealed two additional mammalian species that exhibit multiple
267 medullae in some of their hairs; *Loxodonta africana* (lower leg/foot hairs) and
268 *Elephas maxima* (dorsal and head hairs) as illustrated in Fig. S6.

269 The most significant characteristic of all woolly mammoth and woolly rhino overhairs
270 was the presence of multiple medullae-like structures, which were often present in
271 greater numbers than that seen in samples from extant mammals previously discussed.
272 These structures were manifested as numerous parallel lines that occurred at many
273 radial positions throughout the axis of the shaft (Fig. 4). The greatest number of these
274 structures occurred, without exception, in the coarsest overhairs. In regards to the
275 guard hairs however, an apparent correlation exists between shaft diameter and
276 number of ‘medullae’ present. Only single medullae were found in the finer guard
277 hairs. Multiple medulla-like structures were not seen in the fine underhairs (Fig. S7).

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

284 Gilbert *et al* ([Gilbert et al., 2007](#)) and Lister and Bahn ([Lister, 2007](#)) depict transverse
285 cross-sections of woolly mammoth hair with multiple dark structures in the cortex.
286 Although these structures are reported as nuclear remnants ([Gilbert et al., 2007](#)) or
287 pigmentation ([Lister, 2007](#)) they are so similar to the structures we observed in the
288 current study (Fig. 4A), that we suspect they are neither pigment nor nuclear
289 remnants. Our findings demonstrate that longitudinal views of these features show
290 them to be elongated parallel lines running along the length of the shaft (Fig 4B and
291 4C) this observation does not support premises of these structures being nuclear
292 remnants or pigmentation. Nuclear remnants are significantly smaller than the
293 structures depicted and pigmentation is granular and scattered throughout the shaft.

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

297

298 3.6. Form and function

299 Through the course of the Pleistocene, megafauna had to adapt and change in order to

300 survive harsh environmental conditions; Campbell *et al.* ([Campbell et al., 2010](#))
301 describe an adaptive physiochemical adaptation of woolly mammoth haemoglobin
302 that aided in its survival in cold conditions. We suggest that multiple medullae-like
303 structures in hairs from two extinct megafauna species may result from convergent
304 evolution of structures that, in combination with the density of their coats, may have
305 been critical for their survival. Like ‘rods’ of reinforcing metal in concrete, multiple
306 medullae may have strengthened the hairs in order to maintain shape and orientation
307 and resist distortion. It was noted that woolly mammoth and woolly rhino overhairs
308 were very strongly resistant to being bent and manipulated, and were noticeably
309 ‘springy’ and very smooth, almost slippery, to the touch. These attributes probably
310 prevented the long overhairs and coarsest guard hairs becoming intertwined and/or
311 matted. Matted hair is likely to be less efficient at channeling moisture/water and
312 snow away from the body, which would have proved fatal in the depths of an arctic
313 winter. The ‘springiness’ of overhairs, might also be attributed to a different type of
314 keratin in these hairs, which is currently being investigated.

315

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

325

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

330 Woolly mammoth underhairs exhibited uniform shaft diameters (which measured
331 approximately 20-100µm); all were wavy but in addition the numerous hairs were
332 tightly coiled and difficult to separate. Woolly rhino underhairs whilst exhibiting

333 wavy and lightly curled hairs similar to those found on the woolly mammoth, did not
334 exhibit the tightly coiled underhairs and as consequence were easier to separate.
335 Woolly rhino underhairs measured approximately 20-100µm in diameter. The profiles
336 of the thickest underhairs differed to those from woolly mammoth in that they were
337 ‘buckled’ along the length of the shaft (Fig. S9). It is reasonable to assume that like
338 coarse human beard hairs, or pubic hairs, these ‘buckled’ shafts would not have lain
339 flat but may have afforded the animal a ‘puffier’ or bulkier appearance than the
340 woolly mammoth whose hairs were not buckled.

341

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

346

347 *3.7. Colour and Pigmentation*

348 Mammalian hair colouration is one of the most conspicuous phenotypes; in some
349 animals it plays diverse and significant roles such as sexual attraction, sexual
350 dimorphism and camouflage. However, on the basis of the results of this study, there
351 is no indication that any of these functions applied to woolly mammoth and woolly
352 rhino. Hair colour, length and type appeared to be equally represented in each of the
353 samples, irrespective of the age and sex of the specimen they were taken.
354 Macroscopically and microscopically, woolly mammoth and woolly rhino overhairs,
355 guard hairs and underhairs varied in colour from colourless, to dingy yellow,
356 red/orange and brown (which ranged from pale brown to dark brown, almost black).
357 The majority of overhairs and thicker guard hairs from the woolly mammoths and
358 woolly rhino were vivid red/orange colour or ‘fox red’ as described by Krefft ([Krefft,
359 1969](#)). Close examination of woolly mammoth and woolly rhino hairs revealed that
360 their colours could be attributed to either natural pigmentation, or ‘acquired’
361 colouration (discussed below).

362

363 *3.8. Natural Pigmentation*

364 The diversity of mammalian hair colour is attributed to the quality, quantity and ratio
365 of two melanins (pigment types), eumelanin (predominant in dark brown/black hairs)

366 and phaeomelanin (predominant in red and blonde hairs) ([Ito and Wakamatsu, 2003](#);
367 [Lister, 2007](#)). Pigmentation in hairs is usually found as granules in the cortex of the
368 hair shaft; its distribution may be uniformly or medially distributed (around the
369 central axis of the shaft). In hairs from some animals (but not humans) a unique
370 feature is one in which the hair shaft shows natural, abrupt colour changes (commonly
371 known as banding). These hairs may be bi-or tri-coloured along the length of the
372 shaft. If present in sufficient quantities these hairs may give the pelage a mottled or
373 speckled appearance.

374 Microscopic examination of woolly mammoth and woolly rhino hairs revealed visible
375 pigment in many guard hairs and underhairs, but absent in overhairs (Fig. 5A-C).
376 Where present, pigment distribution was either uniformly distributed or medially
377 distributed as illustrated in Fig. 5D-F; however, medial pigmentation was the most
378 prevalent distribution in hairs from both extinct megafauna species, as is also the case
379 in extant elephantids. Like extant elephantids, Yukagir, Jarkov, M25 and M26 woolly
380 mammoths also exhibited bi-coloured hairs (Fig. S8); bi-coloured mammoth hairs are
381 also noted by Lister and Bahn ([Lister, 2007](#)). These hairs were coarse and bristle-like,
382 similar to both species of extant elephantids. No bi-coloured hairs were evident in the
383 woolly rhino sample.

384

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

392

393 *3.9. 'Acquired' Colouration*

394 Current literature attributes red/orange colour of extinct megafauna overhairs and
395 guard hairs to the oxidation of melanin pigment granules as a result of interment over
396 millennia ([Lister, 2007](#)). It is generally accepted that eumelanin and phaeomelanin
397 pigment granules are susceptible to photo degradation via UV in sunlight ([Krefft,](#)
398 [1969](#); [Lee, 2010](#)). However, although this chemical reaction undoubtedly accounts for
399 some of the red/orange colouration seen in these megafauna hairs, it cannot be the

400 sole cause because hair totally lacking pigment granules also showed this colour that
401 was more vivid than seen in pigmented hairs.

402

403 Kreffft concluded that multiple processes were acting upon hairs each resulting in
404 colour changes. He acknowledged the effects of photo oxidation of pigments and
405 found that the red/orange ('fox-red') colouration not only occurred in pigmented
406 hairs, but also in hairs totally lacking pigmentation; he concluded that this could be
407 attributed to the breakdown of tyrosine residues in keratin. This process resulted in
408 colouration that was homogenously distributed throughout the entire hair shaft
409 ([Kreffft, 1969](#)). We observed a number of homogenously coloured 'fox red' hairs from
410 both extinct megafauna species, predominantly in overhairs and coarsest guard hairs
411 as illustrated in Fig. 5 G-I. On the basis of the work conducted by Kreffft it is likely
412 that this colouration may be attributed to the chemical breakdown of keratin.

413 However, many overhairs bore red/orange debris or a 'sheath' encasing the shaft (Fig.
414 5 J-L). This may be due to a fungal deposit. The present study supports the premise
415 that the natural coat colour of an individual animal was probably not uniform and
416 certainly not red/orange in colour. Instead, the results of this current study strongly
417 indicate that woolly mammoth and woolly rhino pelages may have exhibited a variety
418 of colours comprising hairs of different colours from different somatic origins and/or
419 hair type. A modern day example of just such a pelage is present on the musk oxen
420 (*Ovibos moschatus*), whose pelage is likened to that of the woolly mammoth, which
421 has white hair on its muzzle, top of head, forelocks and saddle. This is in stark
422 contrast to the remainder of the body on which the hairs are rich red/brown in colour.

423

424 Workman et al ([Workman et al., 2011](#)) assert that 'light coloured woolly mammoths
425 probably were very rare, or even non-existent.' The current study of woolly mammoth
426 and woolly rhino hairs does not support this premise as we found an abundance of
427 colourless hairs being in all samples and on both species. It does, however, suggest
428 that woolly mammoths and woolly rhino pelages comprised light *and* dark coloured
429 hairs with lighter hairs predominating, especially amongst underhairs. On the basis of
430 the mixture of pigmented, non-pigmented and bi-coloured hairs found in each sample
431 examined woolly mammoth and woolly rhino coats were likely to have exhibited
432 heterogeneity in colour rather than homogeneity. The arrangement of hair types
433 comprising the pelages would be colourless, long overhairs covering a mixture of

434 pigmented and non-pigmented guard hairs all of which covered predominantly
435 colorless underhairs, for both species of extinct megafauna (figure 5). Furthermore, it
436 is possible that woolly mammoths and woolly rhinos may have shown a mottled ('salt
437 and pepper') appearance to their coats if bi-colored hairs occurred *en masse*,
438 Perhaps further genetic studies on hairs, for which the phenotype is self-evident, may
439 further elucidate extinct megafauna pelage colouration.

440

441 **4. Conclusion**

442 The results of the present study demonstrate new insights into woolly mammoth and
443 woolly rhino hairs and their preservation in permafrost. In particular, regarding the
444 structure and colour of woolly mammoth and woolly rhino pelages, detailed
445 microscopical examinations enable development of a more accurate picture of pelage
446 appearance, form, function and colour than currently exists. This study challenges the
447 current view that pelages of these two species were uniform in colour; the findings
448 indicate that they were likely to exhibit a variegated coloration with long colorless
449 overhairs covering a mixture of bi-coloured, uniformly coloured brown or red/brown
450 and colourless guard hairs, and innumerable colourless underhairs. The presence of
451 multiple medullae-like features in two extinct megafauna species is suggestive of
452 convergent evolution of traits that, together with their woolly coats, may have helped
453 them to survive the thermally, and in winter nutritionally, challenging environments of
454 the Pleistocene glaciations. Future morphological examinations of woolly mammoth
455 and woolly rhino hairs taken from known areas of the body would undoubtedly shed
456 further light on the colouration and distribution of hair types on their pelages. The
457 present study demonstrates the importance of familiarity and expertise in the
458 microscopical, morphological examination of hairs to reveal aspects of megafauna
459 hairs that might have remained hidden. We advocate that there is much to be gained
460 from morphological and microscopic examination of hair prior to any destructive
461 sampling for molecular analyses. A multi-disciplinary approach to the examination of
462 extinct megafauna remains can only continue to enhance our knowledge of these
463 iconic species.

464

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477

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569

570

571 **MAIN FIGURES: LEGENDS**

572 Figure 1. Schematic diagram of a generic mammalian hair (centre) that consists of
573 three major components. (A) The outermost cuticle, (B) the central core or medulla
574 which may be continuous (left) or interrupted (right) and (C) cortex that contains
575 pigment granules (melanins) which may be uniformly distributed across the hair shaft
576 (left) or medially distributed (right).
577

578 Figure 2. Sites of recovery of woolly mammoth hair and woolly rhino hair which
579 were used in the present study, detailing identification details, radio carbon dated
580 ages, sex and age for each hair sample used in the present study.
581

582 Figure 3. Examples of ante-mortem and taphonomic (*post-mortem*) artifacts present
583 on extinct megafauna hair shafts.

584 (A) Jarkov (woolly mammoth) underhair bearing normal root.

585 (B) Jarkov underhair with the centrally placed, dark post-mortem banding in the shaft
586 at the proximal (root) end.

587 (C) Perpendicular needle-like fissures caused by keratinophilic fungal invasion of
588 Jarkov (woolly mammoth) overhair.
589 (D) Conical fissures caused by keratinophilic fungi invasion of M26 (woolly
590 mammoth) guard hair.
591 (E) SEM image showing circular surface degradation and/or points of entry by
592 keratinophilic fungi in Jarkov (woolly mammoth) overhair
593 (F) Woolly rhino under-hairs with the *ante-mortem* deposition of a hair louse egg
594 case.
595 (G) Cusped, insect bite-marks on woolly rhino guard hair shaft.
596

597 Figure 4. Example of multiple medullae-like structures prevalent in extinct megafauna
598 hairs.

599 (A) Transverse cross-section of Jarkov (woolly mammoth) overhair showing dark
600 multiple medullae-like structures throughout the shaft. (B) Longitudinal TLM image
601 of cross-sectioned hair (A) showing multiple medullae-like structures.
602 (C) Confocal virtual cross-section of woolly rhino overhair (approximately 210µm
603 diameter) showing multiple medullae-like structures throughout the shaft. These
604 structures are parallel in the longitudinal view (left image) and as small spots in the
605 virtual transverse cross-section (arrow).
606 Scale bars (A) 100µm, (B) 200µm
607

608 Figure 5. Examples of natural and ‘acquired’ coloration in overhairs and guard hairs
609 from two extinct megafauna species. Images A-C represent natural coloration of
610 overhairs which are devoid of pigmentation. Images D-F show the distribution of
611 pigment in guard hairs which were either uniformly pigmented (D, E) or medial (F).
612 The image (right) shows a ‘deconvoluted’ view of the distribution of these hair types
613 comprising woolly mammoth (and woolly rhino) pelages.

614 Images G-I show ‘acquired’ coloration present on the *inside* of hair shafts devoid of
615 pigmentation. The homogeneous red-orange colouration throughout the hair is evident
616 in transverse cross-section (H).

617 Images J-L reveal red/orange colouration due to ‘debris’ on the *outer* surface of hair
618 shafts. The woolly rhino overhair (J) shows breaches in the surface debris reveal three
619 underlying colourless areas hair shafts (thick arrows) and feint multiple medullae are
620 apparent (fine arrows)

621 Scale bars: A, C, E, G, H, J, L: 200µm; B, I: 50µm; E: 200µm and K: 100µm
622

623

624

Supplemental Information: Figure Legends

625 Figure S1. Further examples of keratinophilic activity on extinct megafauna hair.

626 (A) Large fungal ‘blooms’ on the surface of a woolly rhino guard hair devoid of
627 visible pigmentation but with a feint single medulla in the centre of the shaft.

628 (B) Fishhook (woolly mammoth) overhair exhibiting fine keratinophilic fungi hyphae
629 ‘targeting’ medullae (arrow).

630 (C) Severe keratinophilic fungal destruction of the integrity of M25 (woolly
631 mammoth) guard hair shaft resulting in the exposure of the underlying cortex (arrow).

632 (D) Scale cast pattern of M25 hair, which reveals imprints of a severely damaged
633 shaft, stripped of cuticle and two areas of exposed cortex (arrows)

634 Scale Bars: (A) 200µm, (B) 100µm, (C) 200µm

635

636 Figure S2. (Top panel) The extent of the destructive nature of surface feeding
637 keratinophilic fungi is evident in the TLM image of Fishhook (woolly mammoth)
638 overhair with extensive surface keratinophilic fungal damage masking internal
639 features (left). The extent of the surface damage is evident in the irregular outline of
640 the transverse cross-section in which dark multiple medullae-like structures are
641 visible (right).

642 (Bottom panel) Woolly rhino overhair that has not suffered from keratinophilic fungal
643 attack. The TLM image (left) shows feint multiple medullae-like structures (arrows).
644 The corresponding cross-section (right) bears a smooth outline and discrete black
645 medullae-like structures throughout the cortex. Hairs from both species were devoid
646 of pigmentation.

647 All scale bars 100µm (except lower left, bar 200µm)

648

649 Figure S3. The effect of keratinophilic invasion on extinct megafauna medullae.

650 (Top left) Woolly rhino overhair unaffected by keratinophilic fungal activity exhibits
651 medullae-like structures as feint parallel lines in the cortex (black arrows). The
652 corresponding transverse cross section presented at top right in which central
653 medullae-like structures appear diffuse.

654 (Bottom left) Jarkov (woolly mammoth) overhair in which medullae-like structures
655 have been invaded by keratinophilic fungi, resulting in their enlargement and dark
656 colouration. The corresponding transverse cross-section is presented at bottom right
657 in which the black multiple medullae-like structures are markedly darker and more
658 obvious in comparison to the top right image. Both hairs colourless (devoid of
659 pigmentation)

660 Scale bars: Top left 100µm, top right 200µm; bottom left 200µm, bottom right 100µm

661

662

663 Figure S4. Examples of roots from woolly mammoth underhairs (top panel) and guard
664 hairs (lower panel). Underhairs exhibited elongated roots, whilst guard hair roots were
665 shorter and wider.

666

667 Figure S5. Examples of scale patterns commonly found on woolly mammoth and
668 woolly rhino guard hairs. (Top) Irregular wave-like pattern at the proximal to mid-
669 shaft region of the hair.

670 (Centre) Irregular mosaic-like pattern at mid-shaft region of the hair.

671 (Bottom) Irregular wave-like pattern at distal shaft of the hair

672 (Note: smooth 'cylindrical' features are excess casting material).

673

674 Figure S6. Multiple medullae-like structures evident in (A) *Loxodonta* foot and lower
675 leg hairs and (B) guard hair of Fishhook (woolly mammoth) hair.

676 (C) Example showing the opaque nature of many *Elephas* hairs due to heavy
677 pigmentation (bar = 200µm).

678 (D) Single central medulla visible in the lighter part of an *Elephas* bi-colored
679 (banded) head hair (bar = 100µm)

680 (E) Possible multiple medullae-like structures in an *Elephas* bi-colored (banded)
681 dorsal hair (over-exposed in order to visualise these structures) (bar = 100µm)

682

683 Figure S7. Examples of decreasing medullae like structures related to decreasing shaft
684 diameter.

685 (A) Fishhook (woolly mammoth) overhair showing numerous medullae-like
686 structures in cross-section in the left panel (scale bar 100 μ m) and longitudinal TLM
687 image of the hair (scale bar 200 μ m).

688 (B) Jarkov (woolly mammoth) guard hair few medullae-like structures in cross-
689 section in the left panel (bar 100 μ m) and longitudinal TLM image of the hair (scale
690 bar 200 μ m).

691 (C) Virtual cross-section of Dima (woolly mammoth) finer guard hair showing a
692 single medulla (left panel) and the longitudinal image of the hair showing a single,
693 central medulla (arrows) (bar 100 μ m).

694 (D and E) Transverse, physical cross-sections of woolly rhino (D) and Yukagir
695 (woolly mammoth) fine underhairs that, like the majority of mammalian underhairs,
696 are circular and devoid of medullae.

697

698 Figure S8. Examples of bi-coloured (banded) guard hairs. (A) TLM image showing a
699 darker pigmented proximal half (root end) of a Yukagir (woolly mammoth) hair shaft.
700 The image on the right is the lighter portion of the mid-distal hair shaft.

701 (B) M25 (woolly mammoth) bicoloured hair showing heavy pigmentation in the mid-
702 shaft area and the right image showing medial pigmentation of the distal part of the
703 shaft. (Scale bars: 200 μ m).

704

705 Figure S9. Profiles of underhairs from woolly mammoth (left panel, and woolly rhino
706 (right panel). Both megafauna species exhibited comparable underhairs in size and
707 appearance with the exception of the majority of coarsest woolly rhino underhairs
708 consistently showing uneven shaft diameters caused by 'buckling' as illustrated in the
709 four images on the right panel. (Lowest image on RHS panel: scale bar 50 μ m, top
710 three image scale bars 100 μ m)