= GENERAL BIOLOGY ====

Hair Microstructure of the First Calf of Woolly Rhinoceros *Coelodonta antiquitatis* Found

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Abstract—Hair microstructure of the first calf of the woolly rhinoceros *Coelodonta antiquitatis* found in Sakha in 2014 (the neck and hind leg hair) was examined by the light and electron scanning microscopy. The calf hair features were compared with those of two adults studied earlier. The calf coat color was much lighter than in adults, from pale ashy to blond. The extent of hair differentiation, dimensional and pigmentation indices were lower in the calf than in adult rhinoceroses. There was no medulla in the calf hairs, while in those of adults it was occasionally found. The cortical and cuticular layer microstructure was similar in all the animals compared. In both calf and adult hairs, there were traces of mechanical and fungal damage.

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Adult woolly rhinoceroses (Coelodonta antiquitatis Blumenbach, 1799) are known to have a multideck, thick, and long woolly coat with a well-developed underfur and perfect heat-insulating properties to provide survival under severe conditions of the Ice Age [1–4]. However, the hair coat of this species is still poorly studied, because the findings are extremely rare. In particular, there is no data on its age-related changes. The first finding of the frozen mummy of a calf of the woolly rhinoceros (WR) named Sasha provided an opportunity to compare the calf hairs with those of adults studied earlier [3]. The calf was found in the defrosted coastal outcrop of the unnamed Semilyakh River tributary (outskirts of the Abyi village, Republic of Sakha). As judged from the body size (172 cm from nostrils to the tail base) and the state of dental system, the suckling was 1.0- to 1.5-year-old [5]. Radiocarbon dating of the animal bones (GrA6329)

and a skin sample (GrA63289) yielded the age estimations of 42 and 34.7 ka BP, respectively [5].

The objective of this study was to examine the juvenile hair coat of the little rhinoceros and evaluate the age-related distinctions and the mode of adaptation to environmental conditions.

The hairs were grouped according to the categories and dimensional orders depending of the length, thickness, and shaft configuration. There were 20 groups of differently sized hairs (in total, 80 hairs) taken from the neck side and dorsal foot surface (Fig. 1a). Hair preparation for the light microscopy using a Leica DMLS and a Leica DFC 320 microscopes (Leica, Germany) and for scanning electron microscopy using a JSM 840 A (Jeol, Japan) and a VEGA SEM (Tescan, Czech) microscopes has been made by the standard technique [6]. The longitudinal and transverse sections and the shaft surface were gold-coated by ion sputtering using an Edwards Sf150 A device (Edwards High Vacuum International, United Kingdom). The preparations were examined and photographed at an acceleration voltage of 15 kV. In comparative morphological analysis, we used our previous data r [3, 5] on the hind limb hair of an adult WR female found in lower reach of the Kolyma River near the Chersky village on the Malaya Filippovka River (collection number 79328, Sakha Academy of Sciences, Yakutia, dated 39 140 years BP).

The following features of the WR calf hair coat were observed. The calf wool was thick, unicolored, light (up to blond), and of relatively small length (up to 14–15 cm). It was evenly distributed over the entire body including neck and hind leg (Fig. 1 a). In adults, the wool hairs were of more saturated ash-beige color

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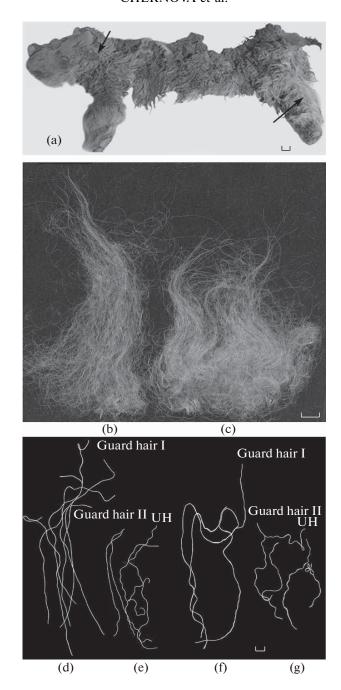


Fig. 1. The sites of (a) wool sampling (indicated with arrows) and wool differentiating on (b, d, e) the side neck surface and (c, f, h) hind leg of the woolly rhino calf *Coelodonta antiquitatis* from the Semilyakh River. (a) Photo; (b—h) microphotography. Scale, 1 cm. Here and in Fig. 2: UH, underhairs.

with an admixture of dark-brown or almost black thick and long overhairs. No overhairs were found in WR calf. The calf wool consisted of the guard hairs of two dimensional orders and a few underfur (Figs. 1b, 1c). In contrast to the calf, in adults, the body wool was more differentiated: apart from overhairs, they had guard hairs, and their underfur consisted of three dimensional orders (Figs. 2a, 2). In the calf, the wool was similarly differentiated (in hair length and thickness) on different body areas, namely, the neck and

hind leg (Figs. 2a, 2b). The hind leg of an adult female was covered with two-layer wool: the upper layer was formed by long overhairs and guard hairs; the lower layer, by short underfur; there was no intermediate layer (Fig. 2a). Thickness variation of different hair categories was similar in different body areas of both the calf and adults. On the calf neck and leg, as well as on the adult female leg, hair thickness was sharply reduced from the upper to the lower layer, but on the female body, the transition was more gradual [3]

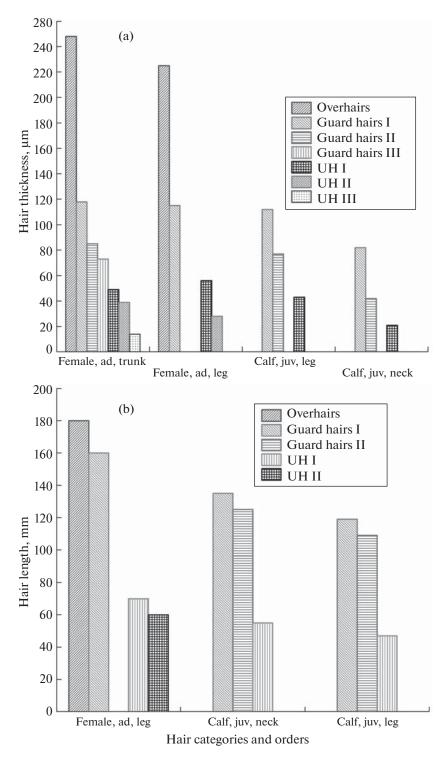


Fig. 2. The hair coat differentiation by (a) length and (b) thickness of hair fragments from the calf and adult females of woolly rhino *Coelodonta antiquitatis*.

(Fig. 2b). Thus, topographical distinctions in the wool differentiation were more pronounced in adults than in the calf. The wool length and differentiation in the calf suggests that it did not play as an important role in thermal protection as in adult WR. However, the WR calf wool was still longer than, or as long as, that of adult caribou or moose in winter [7].

In the calf, the thick and long guard hairs of the first order (guard hair I, Figs. 1d, 1f) were lightly curled, while the thinner guard hairs II (Figs. 1e, 1h) were tightly curled, twisted, and turned mostly in the upper part of the shaft. Guard hairs I and II have a fusiform shaft configuration with a slight expansion in the shaft upper quarter. Accordingly, the cross-sec-

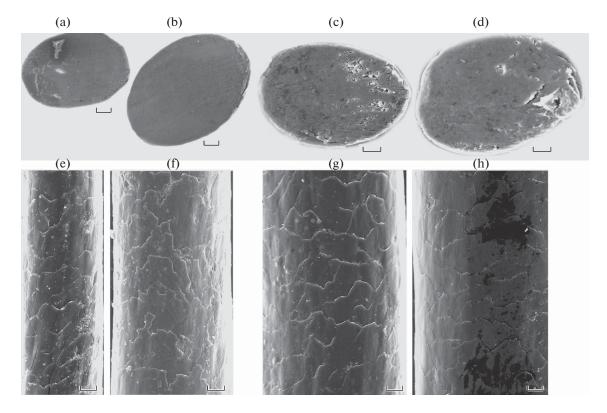


Fig. 3. Hair architectonics on the (a, b, e, f) neck and (c, d, h, i) hind leg of the woolly rhino calf *Coelodonta antiquitatis*. (a-d), the shaft cross-sections, (e, h), the shaft basis cuticle; (f, i), the middle-of-shaft cuticle. Here and in Fig. 4e, data of scanning electron microscopy with a scale of $10 \mu m$.

tions along the entire hair length have a regular round shape or they are slightly flattened (Figs. 3a–3d). In guard hair I, the apexes were usually broken and polished, while in guard hair II, they were thin. The calf underhairs were long, thick and tightly curled. Unlike differentiation of the adult underhairs, where up to three dimensional orders can be distinguished, differentiation of the calf underhairs was insignificant (Figs. 2a, 2b). At the moment of the calf death, many guard hairs and underhairs were growing, because of which the root and basal parts of the hair shaft were swollen, twisted and contained discrete pigment granules or their accumulations, which is typical of the anagen growth phase.

In the compared specimens, microstructure of the main hair layers (cuticle, medulla, and core) was generally similar, with exception of the fact that medulla was not found in the calf hairs, while in some large hairs of adults it was occasionally observed [1–3]. In the calf, the ribbon-like cuticle ornament and the scale height varied insignificantly along the shaft (Figs. 3e–3i). Near the shaft base, cuticle was ringed (the shaft was completely wrapped by a scale) and consisted of low scales (14–18 µm) with wavy and smoothed apical edges. Upward the shaft, cuticle is semi-ringed, the scales were larger (19–20 µm) with triangular apexes and heavily indented apical edges. In general, the calf cuticle ornament of different hair categories was

almost the same. Both cuticle ornament and scale dimensions were quite similar to those of adults.

As in adults, the cortical layer of the calf hair was loose, with numerous longitudinal cracks. The calf hair diffuse pigmentation was poorly expressed, while in adults, it was clearly distinguishable, and pigment was sometimes assembled in a longitudinal band located in the shaft central part.

The hairs of all specimens studied, including the calf, were heavily damaged and had traces of mechanical impacts and fungal infection. A large portion of the calf hairs had numerous local and parallel scratches around the shaft at an angle to its longitudinal axis (Figs. 4a–4d); the shafts themselves devoid of cuticle were crumpled, bent, and even twisted (Fig. 4 e). These orderly arranged damages on the shafts suggest that the calf hairs were exposed to a uniform strong mechanical impact, probably, chewing.

Our study has shown that the wool of juvenile and mature WRs had both similar features and differences.

The calf coloration was much lighter than that of adult WRs.

The calf wool was relatively short and evenly distributed over the entire body, while the adult wool was much longer and varying over the body.

Unlike the adults, the WR calf had hair which was poorly differentiated and deprived of overhairs, rough

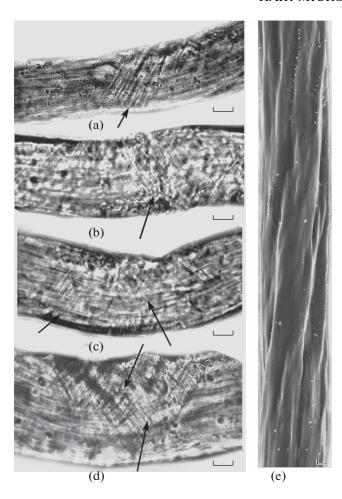


Fig. 4. The hair shaft deformation in the calf of woolly rhino *Coelodonta antiquitatis*. (a–d) The crossing strokes-cracks (indicated with arrows) on the shaft bends; (e) destroyed cuticle and longitudinal cortical depression; (a–d) micrographs.

pile, and the multi-deck layer of guard hairs and underfur.

The hair shaft configuration, cuticle and cortical microstructure of the corresponding hair categories

were quite similar in the compared specimens, though specific medulla was occasionally found only in adult WRs.

The calf died at the stage of intense hair growth (probably, during molting), apparently, in the summer—autumn period.

The similar hair thickness in adults and the calf suggests that the relatively short but thick guard hairs provided a defense against mechanical damage.

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