

Identification of Hairs of the Woolly Mammoth *Mammuthus primigenius* and Woolly Rhinoceros *Coelodonta antiquitatis* Using Scanning Electron Microscopy

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Hair of the woolly mammoth *Mammuthus primigenius* Blumenbach, 1799 has been studied for many years. Judging from the ancient rock paintings, figurines, and preserved hair fragments, as well as from the data of numerous paleontological researches, the mammoth hair was long (up to 1 m or even longer), thick, multi-tiered, and with topographical differences. The hair coat of the woolly rhinoceros (WR) *Coelodonta antiquitatis* Blumenbach, 1799 was also thick and long. The wool of both species was three-tiered: the most numerous downy hairs, which formed a thick underhair, the long and sparse intermediate (or guard) hairs, and the sparsest overhair giving fluffiness to the wool. The hairs grew in the skin singly and had sebaceous glands [1, 2].

Tens of the dead mammoth bodies were found in permafrost, and the mammoth hair microstructure was the subject of numerous studies [3–6]. Only a few overhairs have the fragmented medulla consisting of meshes irregular in shape and with jagged edges; they are loosely arranged along the hair axis. The mammoth hair cuticle, which is not annular because the scales do not fully encircle the shaft, is heavily damaged as a rule, and the free edges of the scales are broken.

The WR remnants with soft tissues are much more rarely found in the permafrost than the mammoth remnants; therefore, the WR woolly coat has been much less studied. Until now, no fundamental differ-

ences have been found in the hair structure between recent rhinoceros species and WR: as in the mammoth, rhinoceros hairs are deprived of medulla, have a mosaic cuticle pattern and the true cylindrical shaft. All of the main hair structures are nonspecific; therefore, it is impossible to determine even generic affiliation of wool [7]. The lack of hair coat and subcutaneous fatty tissue in recent rhinoceros, as well as the presence of numerous large and actively secreting apocrine glands are believed to be related to specific thermoregulation in these inhabitants of tropics [8]. Under the conditions of the Ice Age, the heavy hair coat of WR contributed to their survival.

In the latest report [6], the authors did not differentiate between the mammoth and WR hairs in regard to morphometry (unfortunately, the actual data are not presented, although 420 hairs have been examined), color, shaft configuration, and other parameters. A general description was given for these two species. The authors have confirmed the tiered woolly-coat structure of both mammoth and WR and disproved the notion that the mammoth coat was dark brown or black. They have emphasized the common features of the hair architectonics in both species, i.e., “multiple medulla,” stiffness, and heterogeneity; these features are believed to be related to severe environmental conditions of the habitat.

Thus, the structural characteristics of the mammoth and WR hairs remain incomplete and obscure, and the hair identification on the basis of microstructure have not yet been developed. Nevertheless, identification of representatives of the mammoth fauna, which are sympatric in many areas of northern Eurasia, still remains an urgent problem having not only theoretical but also practical importance for paleontological studies. In this study, we aimed at determining the species specificity and adaptive microstructure of hairs in these two species.

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The starting point of our study was tangled hairs of a fossil animal (the collection number F-2362 of the Ice Age Museum, X-sample) found on the middle Bolshaya Chukochya River (Yakutia), but it was impossible to determine visually whether these hairs belonged to mammoth or WR.

Other wool fragments found in Yakutia in different years that were morphologically studied, were from two WR (Churapchinsky, collection number 2114, Diamond and Precious Metal Geology Institute, Siberian Branch, Russian Academy of Sciences, Yakutia, and Chersky, no. 7938, Ya, Academy of Sciences of the Republic of Sakha (Yakutiya)) and six mammoths (Bolshoi Lyakhovskii Island, without a collection number, Ust'-Lenskii Reserve Museum; Kulara, no. 6832, Diamond and Precious Metal Geology Institute, Siberian Branch, Russian Academy of Sciences, Yakutsk; Ol'chanskii, no. 7911, Ya, Academy of Sciences of Sakha Republic (Yakutiya), Yakutsk; Yukagirskii, no. 7863, Ya, Academy of Sciences of the Republic of Sakha (Yakutiya), Yakutsk; Oiogosskii Yar, no. 1, OYu (Yukagir community), Yakutsk; Malyi Lyakhovskii Island, without a number, Mammoth Museum, Yakutsk). The hair types were determined with a binocular magnifier to divide them into categories and orders, and the thickness of hair shaft and medulla (if present) were measured under a light microscope. The largest overhairs and guard hairs of the first order (guard I) were studied using JSM 840A (JEOL, Japan) and VEGA SEM (TESCAN, Czech) scanning electronic microscopes. Scanning of the intact hair surface and of transverse and longitudinal shaft sections was conducted. The morphometric data were analyzed using the Statistica 6.0 software.

According to the data of the electron-microscopic hair examination, the hair coats of both mammoth and WR were equally well differentiated; therefore, analysis of the hair categories and orders provides no opportunity to distinguish these species. The wool length of the two species is impossible to compare because completely preserved hairs were found in only a few cases; the heavily broken hairs and hair fragments were found as a rule. The hair thickness was a parameter the most accessible for studying (Fig. 1a). The maximum thickness of the mammoth overhairs and guard I exceeded significantly the same parameter in WR ($p < 0.003$ according to Student's test in both cases), which may help to distinguish the hairs of the two species. However, in the X-sample, hair thickness was similar to that in WR and significantly less than in mammoth ($p < 0.01$). When comparing the thickness of hairs taken from different skin areas of the Yuribey mammoth [9], the authors suggested that the X-sample hairs could be from the mammoth belly. However, discriminant analysis of the morphometric data (the hair thickness and cuticle index for hairs of all three

categories) suggest that the X-sample hair could belong to either mammoth or WR (Fig. 1b).

The shaft configuration of the slightly curved overhairs and guard hairs was usually cylindrical in shape in both species and in the X-sample, but there were a few slightly flattened and deformed hairs with oval, flattened, triangular, and ovoid cross-sections and even with a narrow longitudinal groove in the bottom half of the shaft, which agrees with the published data [1]. Shaft deformation was probably a result of strong mechanical compression within the permafrost soil. Since hair cuticle is heavily damaged, as a rule, the large hairs look like an elastic fishing line. The underhairs are long, unusually thick along the entire shaft length, and crimped to different extent. Hair crimpiness is believed to give compactness to the hair coat [9]. However, the high density of wool when the hair medulla is underdeveloped is the cause of a smaller air space within the hair coat, which reduces the heat-proofing capacity of the coat. Hence, during the lifetime, the mammoth and WR wool was thick rather than compacted.

The cuticle of most X-sample hairs is heavily damaged and preserved only on individual areas of the shaft. The cuticle is either half-annular or annular, flattened to different extent in overhairs and guard hairs, the scale free edges are strongly indented, and many scales are extremely high (20–35 μm). Nevertheless, the large overhairs from the body of WR no. 1 have an extremely low cuticle (up to 8–10 μm) lying at an angle of 30° to the shaft transverse axis, although the guard hair cuticle is high like in mammoth. In the black rhinoceros *Diceros bicornis* L., 1758, the cuticle is only of 5–8 μm in height [10]. Probably, the large scales of cuticle appear in fossil species because of destruction of the cuticle outer layers and uncovering the deeper layers as a result of the long-term maceration. However, the intact cuticles of the guard hairs and underhairs are also high: 20–25 and 15–20 μm in mammoth and up to 35 μm in WR. The free cuticle edges are wavy and slightly indented. The cuticle index (the ratio of the maximum scale height to the shaft diameter) is significantly different in mammoth and WR ($p < 0.001$ in both cases) especially in underhairs. However, in the X-sample, the cuticle index is indistinguishable from that in either species ($p = 0.1$); therefore, the species affiliation cannot be determined on the basis of this trait.

The medulla was only in some large hairs of the mammoth, WR, and X-sample. Medulla is difficult to distinguish under a light microscope, because, when the damaged coat hair is embedded into a liquid medium (water, glycerol, xylene, etc.), the solution penetrates the unprotected and pigment-deprived medulla so rapidly that medulla becomes indiscernible within several seconds.

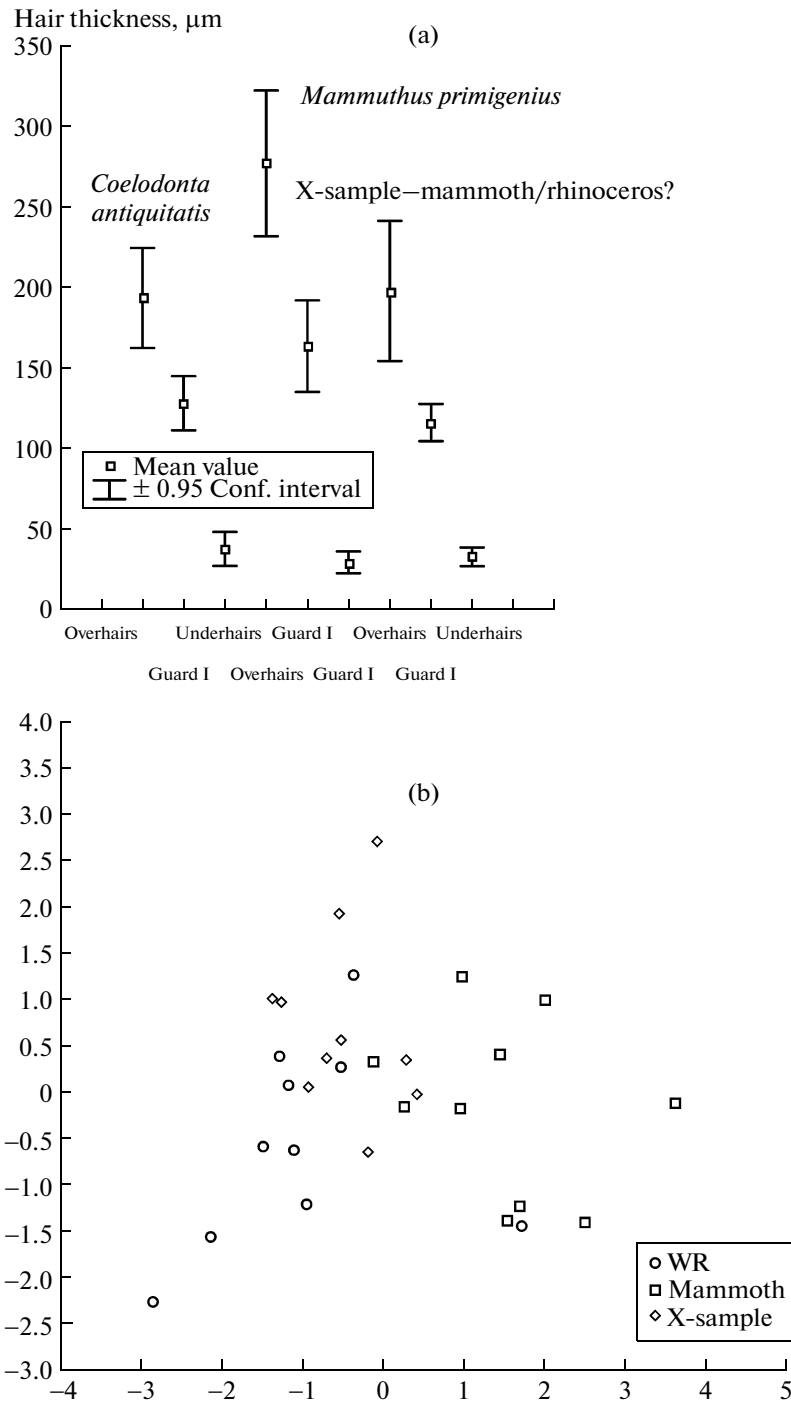


Fig. 1. Morphometry of the overhairs and guard hairs of *Mammuthus primigenius*, *Coelodonta antiquitatis*, and the unknown X-sample (mammoth/rhinoceros?). (a) The maximum hair thickness ($n = 90$); (b) discriminant analysis of morphometric data ($n = 120$).

In some hairs of WR and the X-sample, the fragmented single-row medulla is well developed (if present), and sometimes it occupies as much as half of the shaft thickness (Figs. 2b, 2c). The medullar cavity is filled with large cylindrical discs lying in a row and separated by narrow air layers (Figs. 2g, 2h). This kind

of structure we have described only in vibrissa of weasel *Mustela nivalis* L., 1758 (Fig. 2i) [13]. The maximum cylinder size in rhinoceros is $140 \times 60 \mu\text{m}$ at a guard hair thickness of $140 \mu\text{m}$ (the medulla occupies 43% of the shaft thickness), while in weasel, the respective parameters are $20 \times 25; 80 \mu\text{m}$, and 40%.

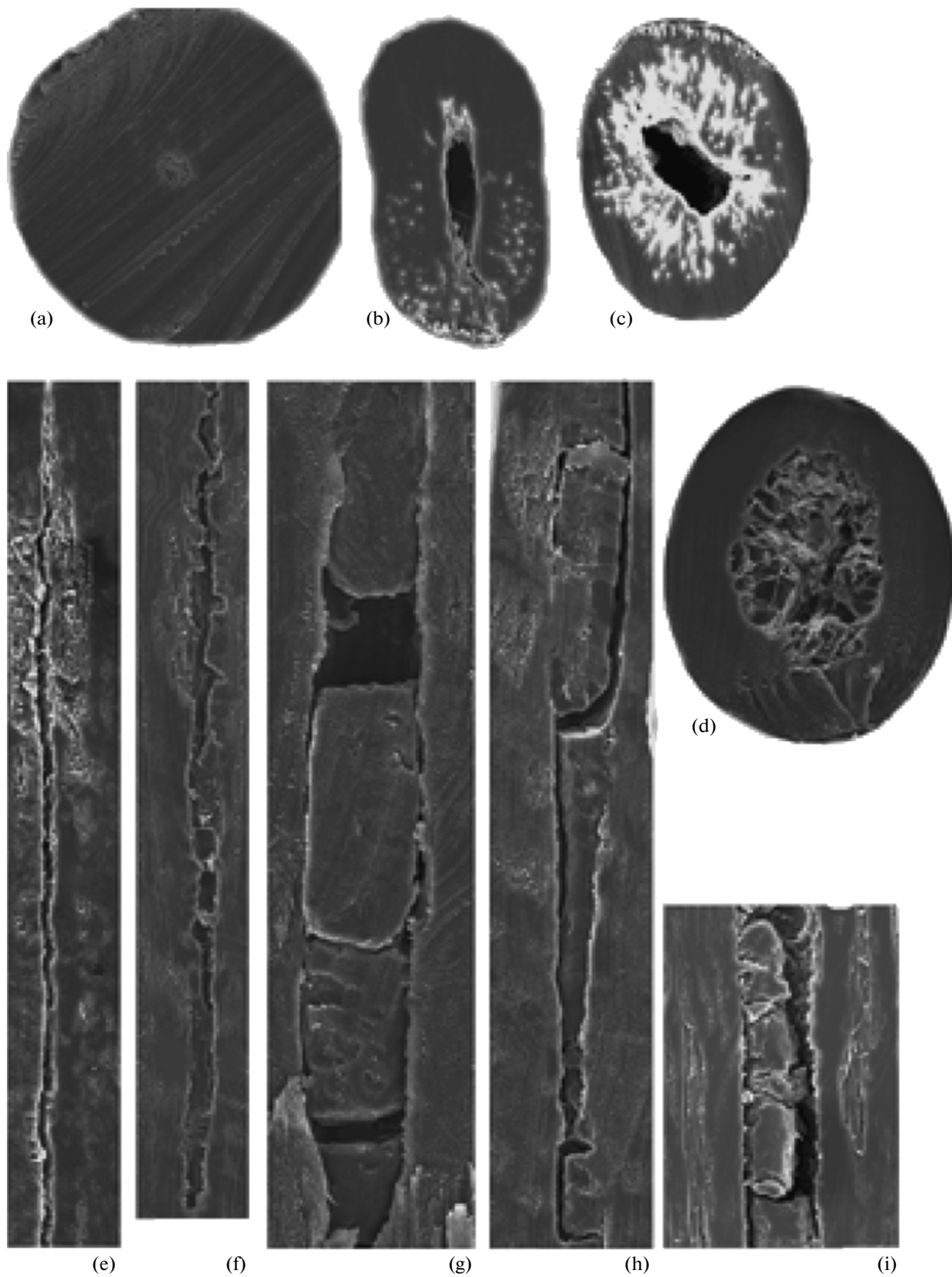


Fig. 2. Architectonics of the overhairs of (a, e, f) *Mammuthus primigenius*, (b, g) *Coelodonta antiquitatis*, (c, h) unknown X-sample (mammoth/rhinoceros?), (d) *Bison* spp., and (i) vibrissa of *Mustela nivalis* on the (a–d) transversal and (e–i) sagittal sections. Scanning electron microscopy (SEM). Scale: (a–d) 20 μ m; (e–i) 10 μ m.

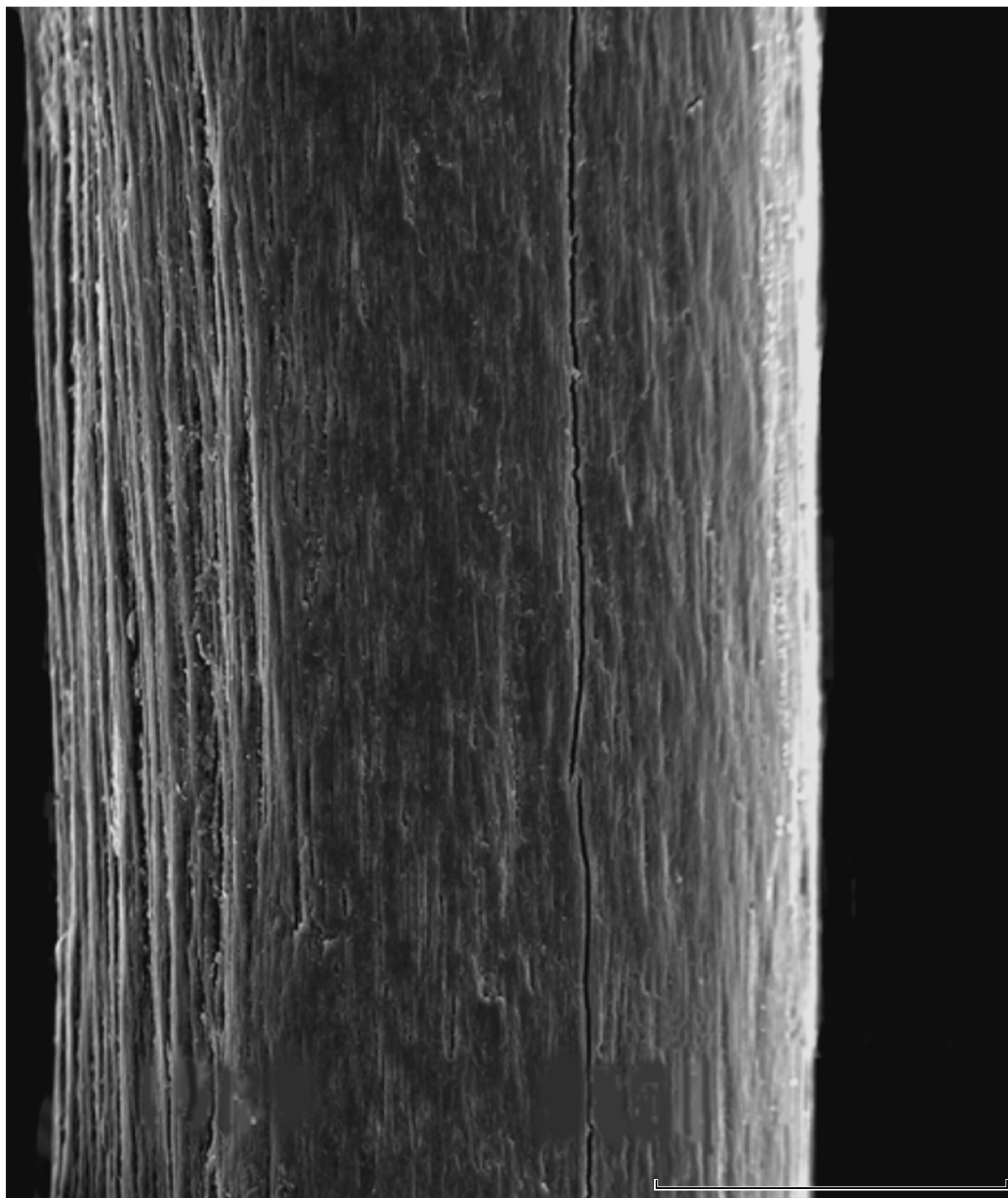


Fig. 3. Longitudinal fissures in the damaged core of a *Coelodonta antiquitatis* guard hair shaft basis with a completely disrupted cuticle. They are erroneously believed to be the “multiple medulla.” SEM data. Scale, 100 μm .

The one-row medulla consists of the alternating dense horny cylindrical bodies and narrow air intervals. This structure undoubtedly improves the shaft stiffness and protects it effectively from the mechanical impacts, such as a touch of substrate (in the case of weasel vibrissa) or rough vegetation (WR wool).

Nothing of that kind was observed in the mammoth hairs. The hair medulla (if any) is degenerated and looks like an uneven, sometimes beads-like, narrow cord, either continuous or fragmented, which is stretched through the middle of the shaft and occupies from 5 to 15% of the hair thickness (Figs. 2a, 2e, 2f).

The internal medullar structure is disrupted, as a rule, and the details of its architectonics are indiscernible except for the fine septa.

In the X-sample, the hairs of another species were found, and comparative analysis indicated that these were hairs of the ancient bison *Bison* sp. (Fig. 2d) [11]. The hair medulla of bison is of medium development, and it has a particular openwork structure.

The hair core of rhinoceros and mammoth is rather dense, but with numerous longitudinal gaps (Fig. 3) [3]. The gaps or “fusi” are present during the natural process of the hair shaft keratinization to ensure certain resilience of the hair core and a possibility of movement of tissue substances and gases. Another type of gaps, “fractile fusi,” appears when the core cells are moving apart in response to a mechanical compression within the permafrost ground [3]. They probably have no species specificity and often detectable in hairs of another species, including humans [12]. Gaps are well discernible on the longitudinal and transverse sections of the shaft; they are more numerous, bigger, and elongated (along the shaft) at the base of a hair and in the thicker hairs. Like the clusters of pigment granules, the gaps look dark under a light microscope, and special techniques have been developed to differentiate them [3]. However, some researchers erroneously believe that these fissures are the medullary cords, and they describe so-called multi-cord “multiple medulla” in the mammoth and WR hairs [6] or the medulla in the form of thin layers between the core cells [13].

Thus, the main adaptation of the mammoth and WR to severe environmental conditions was the composite hair coat differentiation, extreme length and thickness of hairs of every category, and thick, high underwool of wavy underhairs. The maximum thickness of the overhairs and guard I hairs in mammoth exceeds significantly these parameters in WR, and the two species can be differentiated on this basis. Hair color of various hair categories is similar in both species except for the presence of the red-brown hairs in certain areas of the mammoth body, which, however, depends on the degree of preservation of the material. Both species are rather similar in configuration of hairs of different categories. The microstructure of hair cuticle and core has no pronounced species specificity. In general, most hairs of mammoth and WR either have no medulla or contain degenerated medulla, which is of no value for the hair heat-proofing properties, while the hair strength is ensured by the core layer. Nevertheless, the architectonics of the medulla may serve as the main key for diagnosing these species. Unlike the mammoth hairs, some hairs of WR have a medulla similar to that of vibrissae in some mammalian species, and the medullar structure of

these hairs improves hair strength at the expense of their heat-proofing properties.

According to all these traits, the X-sample contains numerous mammoth hairs and a few hairs of WR and ancient bison. The origin of tangles formed by hairs of various species can be only assumed. This could be either a simultaneous death of various animals in a natural trap or accumulation of dead bodies because of action of a natural agent (e.g., water). Probably, further findings will provide answers to these questions.

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