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The morphological basis of the armor-like folded skin of the greater Indian rhinoceros as a thermoregulator

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Abstract. The armor-like folded skin of the Indian rhinoceros (*Rhinoceros unicornis*) was examined by CT image analyses and microscopic observation. The three-dimensional reconstructed images demonstrate that the folded skin has the subcutaneous tissues including cutaneous muscles and connective tissues inserted to the deepest holes and grooves of 2–3 mm thickness in each fold. The cutaneous muscles are well-developed in subcutaneous tissues, in which many small blood vessels are found. We conclude that the folded skin acts as a thermoregulator, since the thin blood vessels and capillaries and cutaneous muscles in the subcutaneous tissues transmit the thermal energy from the core region of the body to the skin folds. We suggest that the greater Indian rhinoceros, *Rhinoceros unicornis*, has evolved the extraordinary thermoregulation mechanism in the folded skin adapted to high temperature in the tropical and subtropical regions.

Key words: CT, folded skin, *Rhinoceros unicornis*, thermoregulation, three-dimensional image analysis.

The greater Indian rhinoceros has appearance of wearing armor. The skin of the species is equipped with deep folds in neck, shoulder and hip regions and with convex tubercles in proximal and lateral areas of the four limbs. The hairless skin is thick and hard in these areas, and these specialized structures of the skin represents the most extraordinary silhouette in all mammalian species. The function of folds of the skin structure has remained unclear. However, the greater Indian rhinoceros frequently wallows and bathes in water (Nowak 1999), and it suggests that the skin folds may contribute to the heat radiation to regulate the body temperature in the high air temperature condition. Since the non-destructive examination by the CT scanner could be usefully undertaken on the soft part structure including skeletal muscles in the newborn of the greater Indian rhinoceros (Endo et al. 2009), we applied the CT scan and the related image analysis to continuously observe the internal structure of the large areas of the skin and to morphologically confirm the thermoregulating function of the folds in this study. The histological examinations of the subcutaneous tissues were also carried out to detail the morphological bases of the thermoregulating function of the skin folds.

Materials and methods

The dead body of an greater Indian rhinoceros (*Rhinoceros unicornis*) of 36 years old male was donated to The University Museum, The University of

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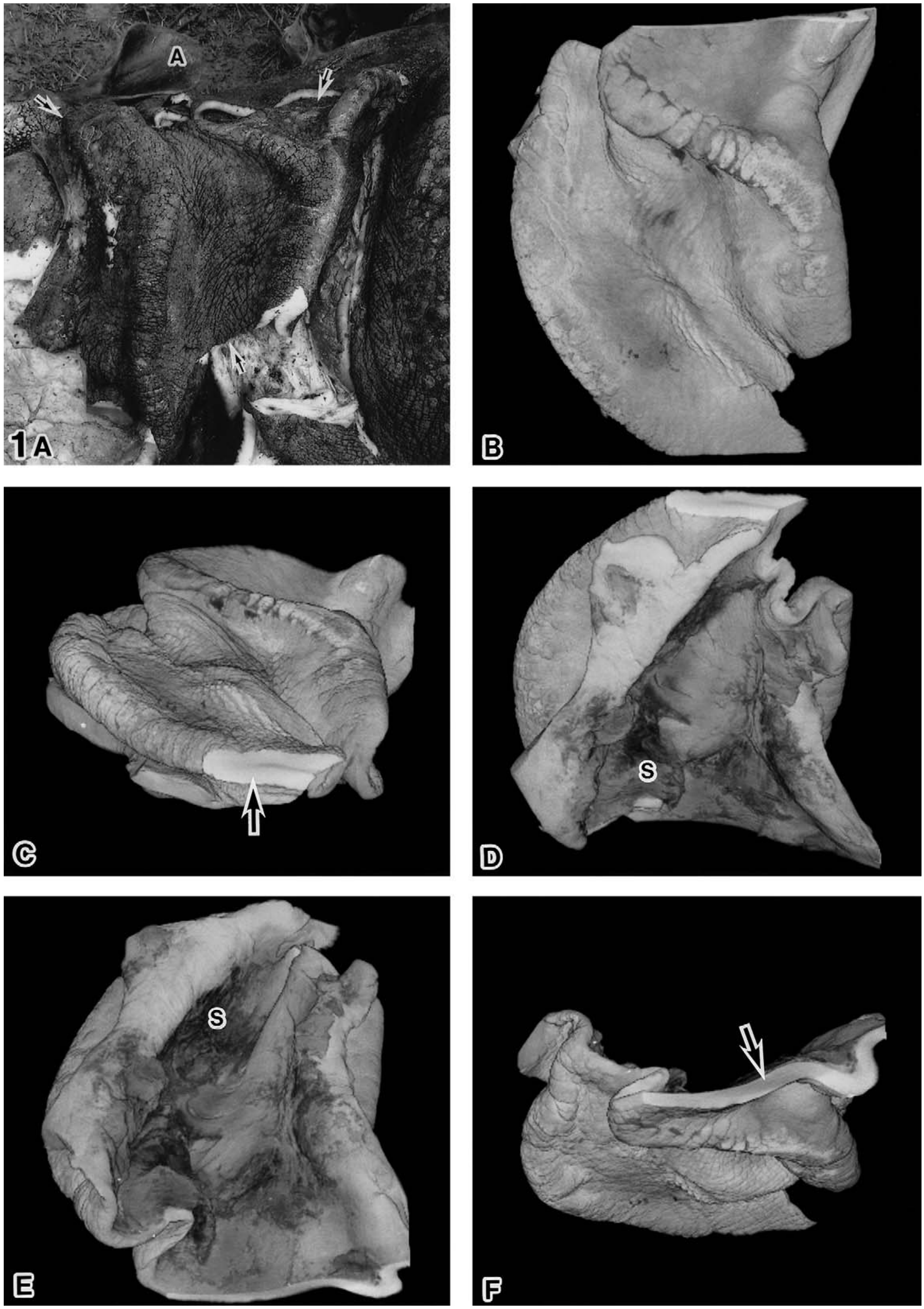

Fig. 1. A) The excised skin (small arrows) including the deep folds and convex tubercles. The caudo-lateral area of the head and the cranial area of the shoulder region. Dorsal direction at the top and rostral at the left. A, auricle of the left side. B)–F) The three-dimensional reconstructed images of the excised skin from the CT data are rotated. Figure 1B is shown from the same aspect of 1A. The sections (large arrows) of the skin are seen and the subcutaneous regions (S) can be observed from the reverse side of the skin.

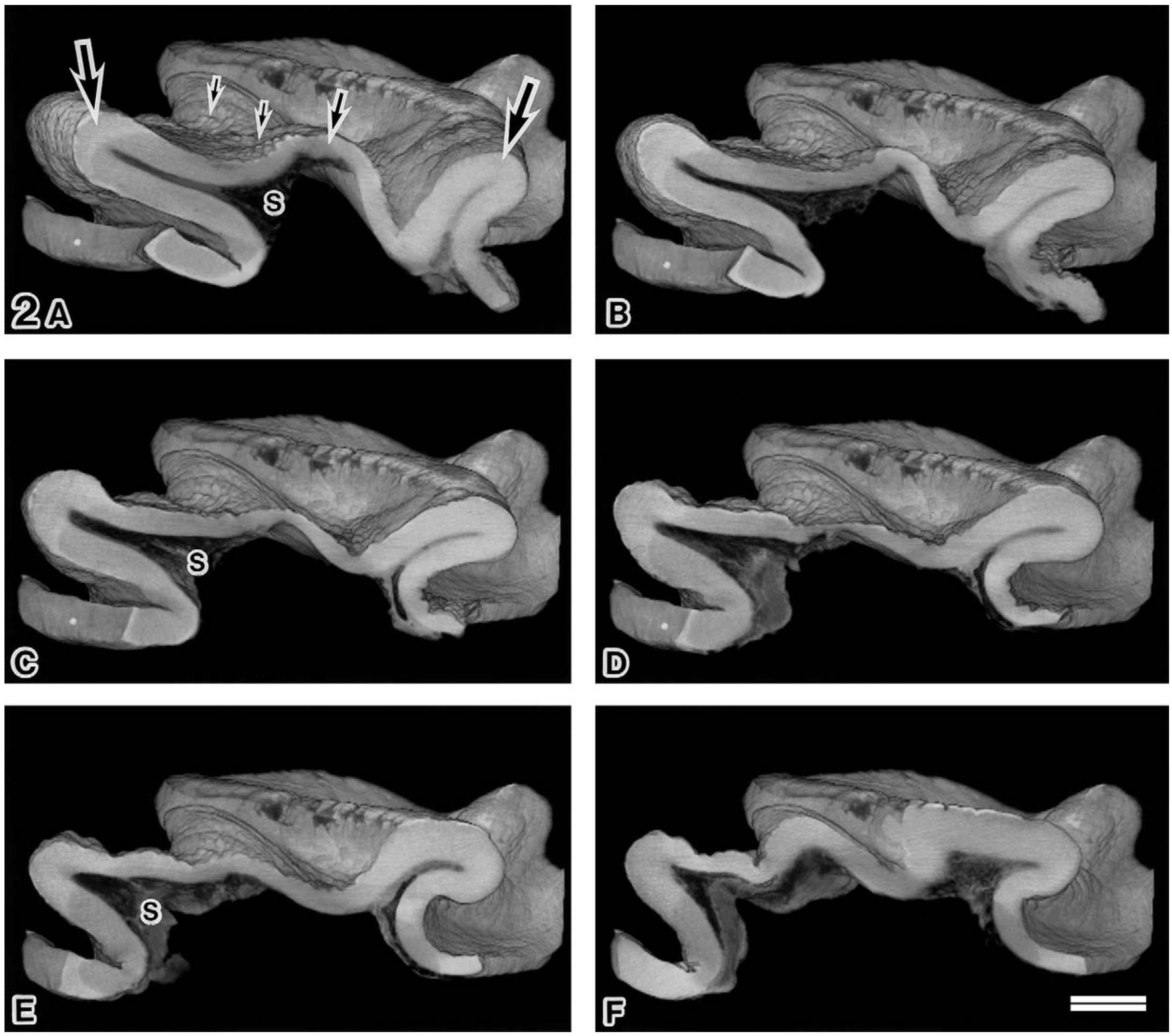

Fig. 2. The three-dimensional reconstructed images of the skin folds. The folds are horizontally sectioned from A (ventral) to F (dorsal) at an interval of 30 mm. The epidermis and dermis are thicker in the fold area (large arrows) than in the flat region (intermediate arrow). Subcutaneous connective tissues including the thick cutaneous muscles are seen (S). The convex tubercles of the skin surface (small arrows) are not morphologically related to the subcutaneous tissues. Scale bar = 50 mm.

Tokyo, by Higashiyama Zoo and Botanical Gardens. After the pathological checks the folded skin of about 1400 square centimeter was excised from the neck and scapula areas of the dead body and sectioned in various directions by CT (Computed tomography) (Toshiba Aquilion 16: Toshiba Medical Systems, Japan) scanner at 0.5 mm thickness without gap. The three-dimensional images were reconstructed from the DICOM-formatted files by the Voxel Transmission (Volume Rendering) techniques through an image analyzing system (AZE Virtual Place: AZE Corporation, Tokyo, Japan). Adequate thresholds were selected and the sections were axially and horizontally obtained from the three-dimensional images to effectively visualize the internal structure of the folded skin. In addition to the macroscopic CT examinations, we chose up the part of the subcutaneous tissues and muscles of the folded skin for light microscopy, and tissue blocks were excised and fixed in 10% formalin solution. After 24 hours of fixation, the blocks were dehydrated in ethanol, treated with xylene and embedded in paraffin. The blocks were sectioned at 4 μm in thickness, and the sections were stained with haematoxylin and eosin, and examined by light microscopy.

Results

The folds of the skin and the convex tubercles are deeply and highly developed in the caudo-lateral area of the head and the cranial area of the shoulder region (Fig. 1A). This area of about 1400 square centimeter

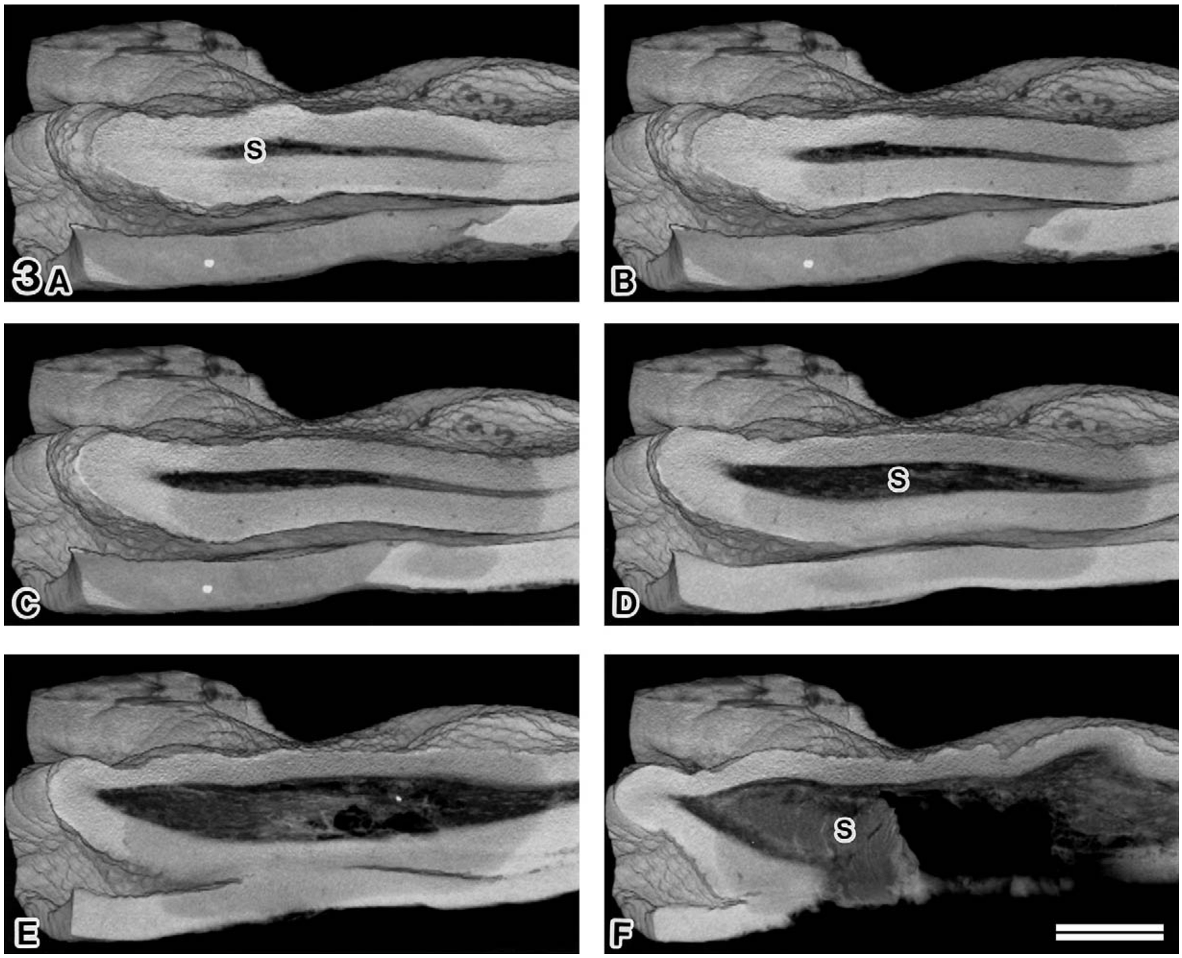

Fig. 3. The vertical (transversal) sections of the reconstructed images from the same data of the Figure 2. The folds are sectioned from A (cranial) to F (caudal) at an interval of 30 mm. The subcutaneous tissues (S) are also distributed even in the thin space of the folds. Scale bar = 50 mm.

was used for the CT examination (Figs. 1B–F). The thick epidermis and dermis wall is entirely developed in all areas of the excised skin. The shoulder regions with deep folds possess the thicker cutaneous structure of about 20–30 mm thickness (Figs. 2A–F). The subcutaneous tissues are extended under the skin. It is inserted even into the deepest holes and grooves and the subcutaneous structure is 2–3 mm in thickness in these parts of the fold (Figs. 2B, C, D). The folds of the skin consist of the relatively thicker epidermis and dermis and extended subcutaneous tissues. In the other aspect of the CT reconstructed images (Figs. 3A–F), the thin subcutaneous tissues are inserted into all areas of the holes and grooves of the folded skin. The holes and grooves become wider in the basal part of the skin fold (Figs. 3D, E), in which the subcutaneous tissues possess larger volume. The convex tubercles and protrusions consist of the keratinized surface of the epidermis (Figs. 2A, C, D, E) and are not morphologically related to the development of the subcutaneous structure. The cutaneous muscles are also inserted into the deepest part of the holes and grooves (Figs. 2A, 2C and 3A). The cutaneous muscles seem light and dense, whereas the collagen fibers appear dark and diffused in the CT sections.

From the microscopic observations, the musculatures of the neck cutaneous muscle are well-developed within subcutaneous tissues (Fig. 4). The many smaller blood vessels are distributed in the tissues (Fig. 4A), while the relatively larger veins are encountered (Fig. 4B). The specialized structure consisting of the network of the capillaries is not seen in the subcutaneous regions. We did not try to observe the distribution of the sweat glands in the skin.

Discussion

Both greater Indian and Sumatran rhinoceroses are equipped with the armor-like deeply-folded skin in the

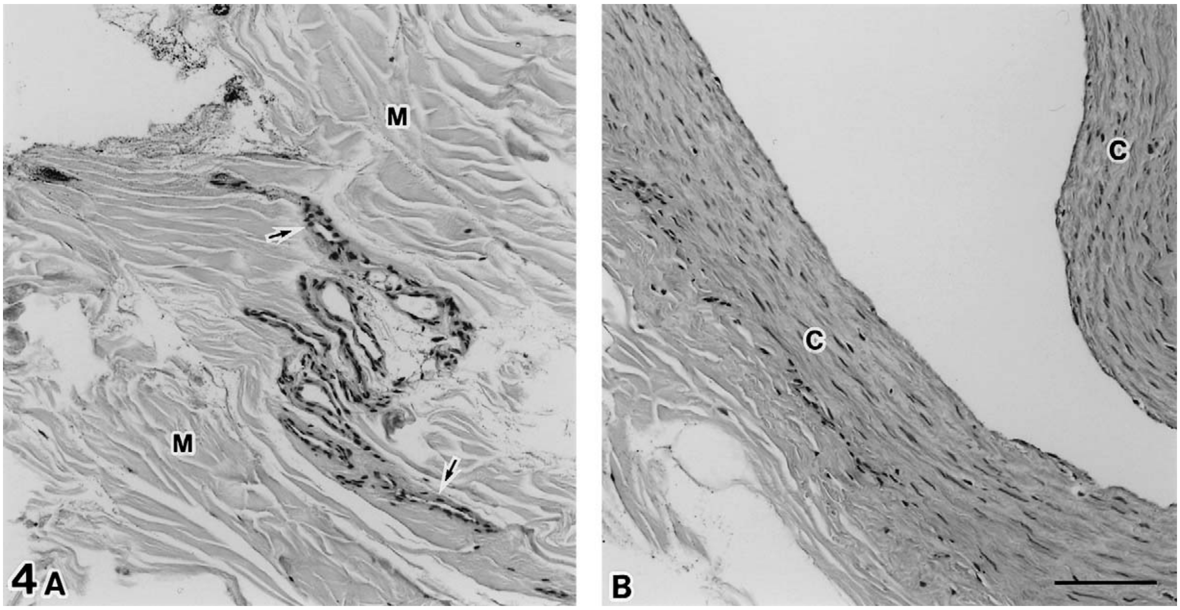

Fig. 4. Sections of the subcutaneous tissue. Stained with haematoxylin and eosin. Scale bar = 100 µm. A) The thick musculature (M) occupies the tissues. Many small blood vessels (arrows) are encountered among the musculatures. B) The vein with collagen fibers in wall (C) can be observed in the tissues.

neck, shoulder and hip regions. The function of the skin folds has remained unclear. However, since the greater Indian rhinoceros bathes in lakes, rivers and pools and this behavior is frequent during hot season (Nowak 1999), it has been suggested that the folded skin may contribute to the thermoregulation and protection against flies in their body.

Although the living rhinoceroses have hairless skin, the wooly rhinoceroses such as *Coelodonta*, the extinct group distributed in northern districts of Eurasian continent in the late Pleistocene interestingly possessed the long hair in their body (Kahlke and Lacombat 2008). The variability of the skin appearances in the rhinoceroses has attracted the mammologists, especially the functional morphologists and the clinical veterinarians, since the specialization of the skin is morphologically related to the thermoregulation of the body and the protection against the flies. The thick folded skin also may act as an effective barrier against various external parasites. We think that the woolly rhinoceroses might survive the cold temperature depending on the skin with the long hair. In contrast, for the rhinoceros species distributing in the lower latitude and warmer districts, we suggest that the armor-like folded skin may function as a thermoregulation system of the body. As the animal stays in the river and swamp, the folded skin with larger surfaces can play a role of a heat radiator to resist the high body temperature. Since the folded skin holds much water after leaving swamp, the heat-radiation effect maybe continues also on the ground.

However, the fold should contain the subcutaneous tissues and the cutaneous muscles with the blood vessels or capillaries to act as an effective radiator. The present CT examinations demonstrate that the folded skin has the subcutaneous tissues extended and inserted into the deepest holes and grooves of the skin at least in the shoulder region. Neither specialized network of the capillaries nor counter-current blood vessels contributing to the heat exchange between the deep and superficial regions of the body, for example as shown in flippers of dolphin or legs of birds and sloth (Scholander 1955; Scholander and Schevill 1955; Scholander and Krog 1957), are encountered in the subcutaneous structure, but many thin arteries and the thick musculatures can be histologically found. We point out that the subcutaneous tissues in the fold acts as a thermoregulator, since the small blood vessels and capillaries transmit the thermo energy from the core region of the body to the skin folds. We suggest that the enlarged cutaneous musculatures can also easily release the heat from the body rather than the deep skeletal muscles. The distribution of the capillaries should be quantitatively examined in the future to speculate the function of the heat-radiation.

The greater Indian rhinoceros has the largest body in all living rhinoceroses. Since the extraordinarily large body disturbs the species to regulate the body temperature under the high temperature condition, we suggest that only this genus is necessarily equipped with the effective armor-like thermoregulators. The ancient DNA analysis revealed that the genus *Rhinoceros* is closely related to the *Coelodonta* including wooly rhinoceroses (Orland et al. 2003). It is suggested that the Family Rhinocerotidae could be easily adapted to various natural temperature conditions by the drastically morphological changes of the skin, since the living *Rhinoceros* species have poorly hairs and pores in their skin surface. We conclude that the two endangered species, *Rhinoceros unicornis* and *R. sumatrensis*, are the extreme examples in which the large-sized species have evolved the extraordinary thermoregulation mechanism in the folded skin adapted to the high air temperature in the tropical and subtropical regions among Rhinocerotidea species.

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