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Characteristics of the microscopic hair structure of domestic mammals from *Equidae* family

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Abstract

Hair is an indispensable component of the animal body. Having structural features of the structure, it allows you likely to identify the type and age of animals, conditions of keeping animals, feeding and even sex. Paleontologist's findings prove, the hairline stores the undisputed information on its “owner” for thousands of years. According to the results of the conducted research it is established, that the hair coat of the studied animal species – *Equinus asinus* and *Equus caballus* – has significant differences in the structure of the brain substance and superficial drawing of the cuticle. Microscopic examination of discolored samples of animal hair well-recognizes the structure of the brain substance, which makes it possible to differentiate the species of animal. The brain substance in the donkey mane hair occupies most of the hair, is represented by densely grouped cells, sometimes interrupted, whereas in the horse mane hair, it has the appearance of grouped rounded cells with small intervals between sections of 6–10 cells. The brain substance of the donkey covering hair is represented by cells of different size and shape, which disappear from the middle of the hair to the peripheral end. This tendency is also typical for the brain substance of the horse covering hair, but unlike donkey hair – cells of the same size, begin with a continuous cord at a distance of 1–1.5 mm from the root of the hair, towards the peripheral end of the hair the gaps between them increase to the complete disappearance of cells. Ultramicroscopic examination of the cuticle superficial drawing of hair samples allowed to establish the peculiarities of two species of the same animal genus. The donkey and horse mane hair had almost the same thickness, the number of scales (waves) per 100 μm of hair length and the size of the scales (wavelength), however, the overall drawing was significantly different. Superficial drawing of hair cuticle from horse mane represented by irregular waves with sharp pointed edges of scales, instead, the donkey has fringed edges of scales. The horse's covering hair was thicker than the donkey's hair and had differences in the location and shape of the scales. Superficial drawing of covering hair cuticle of donkey represented by a regular wave of scales with clear and even edges, while the scales on the surface of the covering hair of the horse have indistinct torn edges and collected in intermittent (irregular) waves.

Key words: hair, *Equinus asinus, Equus caballus*, light microscopy, scanning electron microscopy, morphometric parameters.

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1. Introduction

Notoriously, hair analysis based on morphological features like cuticle scale patterns, medulla and pigment or molecular analysis of DNA and protein, trace elements, chemicals etc. can help in pursuing studies of extinct organisms, dietary habits, evolution, ancient gene functions, forensic toxicology studies, etc. (Teerink, 1991; Wolinsky, 2010). Morphological analysis of mammalian hairs may help in species identification, understanding evolutionary effects including adaptive alterations due to climate changes in many species or origin of hairs (Mansilla et al., 2011; Chernova et al., 2016a; Pikhtirova & Ivchenko, 2019).

Osthaus et al. (2018) established that donkeys' hair coats do not significantly differ across the seasons. All three measurements of the insulation properties of the hair samples indicate that donkeys do not grow a winter coat and that their hair coat was significantly lighter, shorter and thinner than that of horses and mules in winter. In contrast, the hair coats of horses changed significantly between seasons, growing thicker in winter.

Hair keratin can stand vagaries of nature and may be well preserved in fossilized hairs found in ice, amber, mummies, scats (or coprolites) of carnivores, bird pellets etc. Ancient hairs are also found in archeological investigations in form of artifacts made by animal hairs like paint brushes, apparel, cordages etc. Analysis of ancient hairs can be useful for study of cuticle patterns, medulla, pigments, isotope ratios etc. (Gharu & Trevedi, 2016).

In some hairs, scale patterns are not distinct enough to discriminate between mammalian species though are helpful in discriminating filament from that of mammalian hair

(Vullo et al., 2010). SEM studies shows similarity of hair cuticle, cortical structure in hairs from matted wool ("wads"), the woolly rhinoceros (rhino) *Coelodonta antiquitatis* and woolly mammoth *Mammuthus primigenius*. However, medulla structure is specific for species. Further, similarity of rhino hair structure with vibrissae of predatory small mammals is reported (Chernova et al., 2015a; 2015b). Extant and fossil dog hairs of Chukotka (Russia) analysis helped in identification of species (Chernova et al., 2016b).

Comparative study on hairs from ancient frozen bison and extant species shows change in cuticle thickness and hair medulla for better insulation but the morphology of hairs has not significantly changed during the course of evolution (Chernova & Kirillova, 2013; Kirillova et al., 2015). Similar findings are also reported in study on fossilized hairs from *Equus* species (*E. lenensis*, *E.* conf. *lenensis*, and *E. caballus*) and modern horses (*E. caballus*) (Spasskaya et al., 2012). Further, these studies also indicate close relationship between ancient and modern respective species (Kirillova et al., 2015); specifying that possibly order or family specific features are retained despite some modifications due to environmental changes. On the other hand, no significant differences are found in fossilized hairs (found in amber from the Font-de-Benon quarry at Archingeay-Les Nouillers in Charente-Maritime (southwestern France) and the hairs from extant species. Similarly, cave lion *Panthera spelaea* Goldfuss, 1810 fossil hairs and *Panthera leo* hairs do not differ in morphological features (Kirillova et al., 2015).

Moreover, morphological similarity may exist due to similar habitats and climatic conditions but there may be genetic differences as seen in comparative morphological and protein analysis on hairs from Holocene "Yukagir Horse" (*Equus spp*.), Lena and modern Yakutian horses (Chernova et al., 2015c). Thus, alterations or lack of modifications in hair morphology for adaptations for insulation are species specific.

Some researchers suggest a light microscopy method for hair identification (Kotsiumbas et al., 2010). Bonnie et al. (2010) used cross section analysis and light microscopy to analyze the tail hair morphology of African elephants, Asian elephants, and giraffes and found that cross-sectional shape, pigment placement, and pigment density are useful morphological features for distinguishing the three species.

Also, Vullo et al. (2010) found that the morphology of hair cuticula may have remained unchanged throughout most of mammalian evolution. The association of these hairs with a possible fly puparium provides paleoecological information and indicates peculiar taphonomic conditions.

Clement et al. (1981) approves that the classification is based on the fine morphology and the ultrastructure of the medulla and the differences encountered in the main mammalian orders. Furthermore the chronology of these characteristics follows that of mammalian phylogenesis. The ultrastructural characteristics of the medullar of human hair are totally different from those of the hair of all other mammals (Feughelman, 1997).

The arrangement of the cortical cells of human hairs was observed to be fairly irregular with conspicuous interdigitations of cell boundaries as compared with that of animal hairs. The medulla of human and animal hairs were filled with cellular remnants of destroyed medullary cells showing fibrous structures. The lamellar structures of cuticular cells became very clearly visible. The number and the overall thickness of cuticular cell layers in human and animal hairs investigated varied considerably between scalp and pubic hairs and from animal to animal (Sato et al., 1982).

One of the most modern and accurate laboratory methods is scanning electron microscopy, which allows differentiation of biological objects at the microscopic level (Rogers, 1959; Kunytskyi & Kupyna, 1998; Salyha & Snitynskyi, 1999; Pikhtirova & Ivchenko, 2018).

Van den Broeck et al. (2001) and Popescu et al. (2010) stated a fact that the scanning electron microscopic observation of hair samples is a fast and valuable method for identifying hair types with useful applications in different disciplines such as mammalian biology, the textile industry and forensic medicine.

Hair coat properties of donkeys, mules and horses in a temperate climate was carried out by Osthaus et al. (2018) but ultramicrostructural comparison was not carried out.

The aim of the study was to investigate the microstructure of donkey's (*Equinus asinus*) and horse's (*Equus caballus)* hair samples and to identify the characteristic features inherent in the investigated species of animals.

2. Materials and methods

The studies were carried out in the Department of Anatomy, Normal and Pathological Physiology and Laboratory of Electron Microscopy of Sumy National Agrarian University.

Animals

For this purpose, samples of hair from mane (15 hairs per animal) and spine area (15 hairs per animal) from 7 donkeys (*Equinus asinus*) and 7 horses (*Equus caballus)* were selected.

The hair color and samples length were identified before microscopic examination.

Light microscopy

Investigation of the medulla structure was performed using microscope "XS 2610 (MICROmed, Poltava, Ukraine)" in the magnification range from 250 to 400 times and microphotograph Micro Capture Ver 6.9.3. Sample preparation included: selection of biological material; washing; discoloration (30 % H_2O_2 at room temperature for 30–60 min.); fixation on glass slide and placement on the microscope object table.

The following parameters were determined on the photo: the structure of the brain substance (medulla) and its relation to the total thickness of the hair.

Electron microscopy

The microstructure of the hair surface was examined using a scanning electron microscope REM-106I (Selmi, Sumy, Ukraine) in the magnification range from 500 to 1500 times. The preparation of samples for study on the scanning electron microscope (SEM) included the following steps (Rogers, 1959): selection of biological material; washing; degreasing (dipping in 96 % alcohol); placing the samples on the objective table; silver dusting by VUP and placement in the chamber of scanning electron microscope.

The morphometric characteristics were described by photo and SEM-images using a digital image analysis program Digimiser 4.0 (Med Calc Softwar). The following parameters were determined on the SEM-image (GHEP-ISFG, 2015): hair thickness, frequency of scales (as the average number of scales along the line at 100 μm in hair length), the transverse size of the widest part of the scales, the angular characteristics of the scales "teeth" (in the presence of a characteristic feature in some species of animals). The program used allowed the statistical calculation of the average minimum and maximum values of the studied parameters (Figure 1).

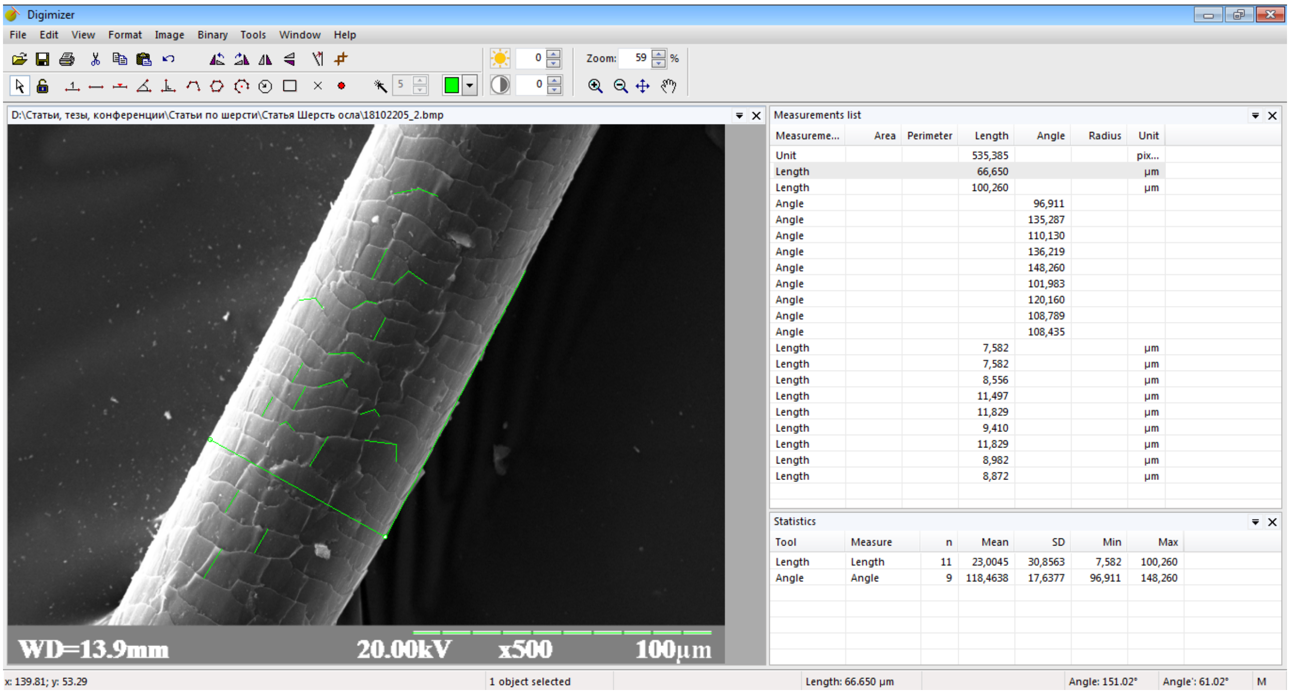


Fig. 1. Investigation of the morphometric parameters of *Equinus asinus* hair by digital SEM-image in programs Digimizer 4.0

Statistical data processing

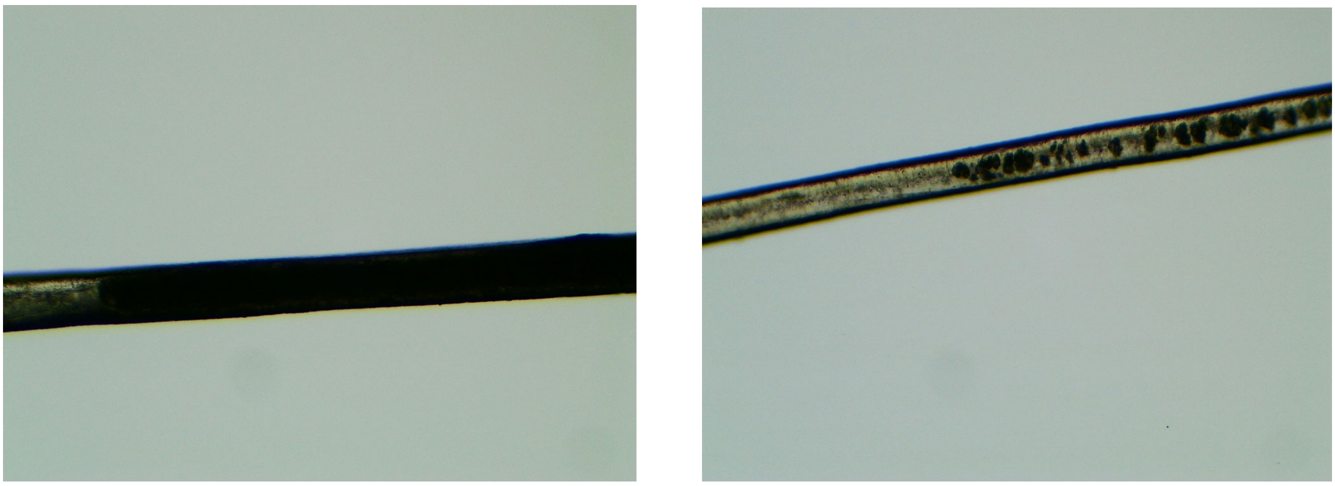
The results of the study were elaborated statistically using one-factor analysis of variance ANOVA in Statistica 10.0 software. Significance was declared at $P < 0.05$, $P < 0.01$ and differences between means with $0.05 < P < 0.10$ were accepted as representing tendencies.

3. Results and discussion

The horse hair specimens were different from the donkey hair in length and color. The investigated donkey hair obtained from the mane area was black, length 7.5–13 cm, and from the spine section – gray, some samples – with black and white strokes, length 1.5–2.6 cm. The horse mane's hair was from 8 to 14 cm long and brown color, and from the spine section – 0.7–1.7 cm and light brown, almost red, color.

Light microscopy

It is known that the nature of the brain substance – the location and shape of the medullary cells – is important for identifying the species of animals (Teerink, 1991; Kotsiumbas et al., 2010; Wortmann, 2014). According to the results of microscopic studies, it was found that the enlightened hair of a mane and cover hair donkey (Figure 2) had significant differences. Thus the brain substance of mane hair (Figure 2 a) is represented by a wide and continuous weight of cells from the root to the peripheral end of the hair, sometimes with cell-free sections. The ratio of brain matter to the total thickness of the hair is 1 : 0.58.

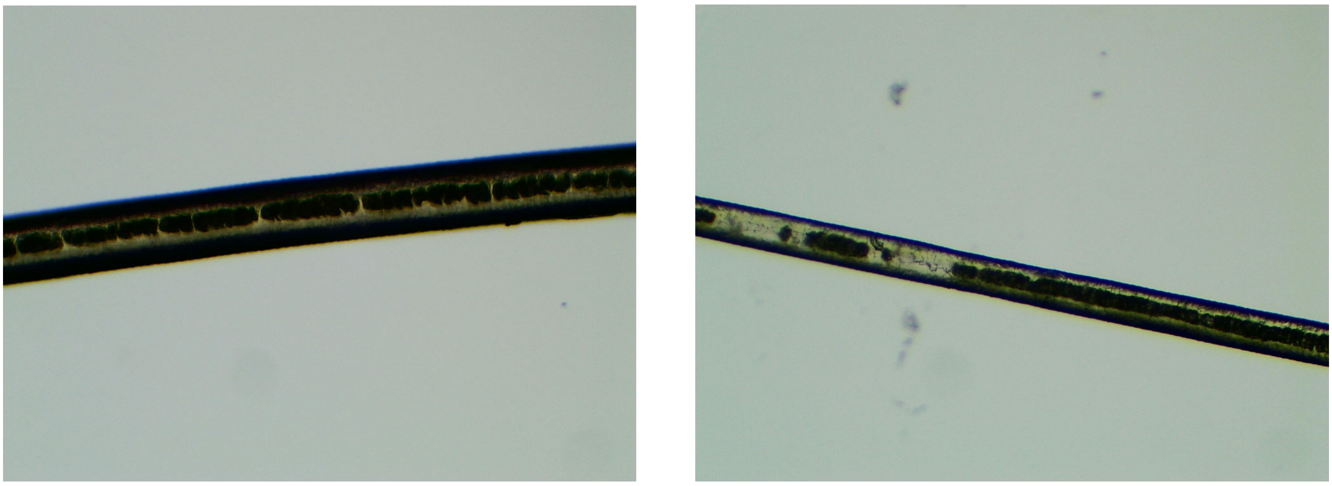


a б

Figure 2. Donkey hair (*Equinus asinus*): a – mane; b – covering hair; x 400.

The brain substance of the donkey covering hair (Figure 2 b) is represented by separate chaotically placed cells of different size and shape, which completely disappear from the root to the peripheral end of the hair. The ratio of brain matter to the total thickness of the hair is 1 : 0.56.

The character of the brain substance in the enlightened horse mane hair (Figure 3 a) is represented by groups of cells of a round shape, the number and size of which do not change from the root to the peripheral end of the hair. The ratio of brain matter to the total thickness of the hair is 1 : 0.28.



а б

Figure 3. Horse hair (*Equus caballus*): a – male; b – covering hair; x 400.

The brain substance at the beginning of the horse covering hair (Figure 3 b) is represented by a continuous cord of the round shape cells, which begins at a distance of 1–1.5 mm from the root of the hair. In the direction of the peripheral end of the hair cells of the brain substance are placed in section, the gaps between the cells increase until they disappear completely. The ratio of brain matter to the total thickness of the hair is 1 : 0.71 – 1 : 0.41.

Electron microscopy

Many studies have confirmed the fact that the surface pattern of the cuticle in different species of animals is different (20, 21). According to the results of the researches it can be noted that the thickness of the investigated samples of hair obtained from animals of the species *Equinus asinus* and *Equus caballus* has the following differences (Table 1). The size and placement of scales on the hair surface (cuticle pattern) are close to the animals' hair types within the same animal species.

Table 1

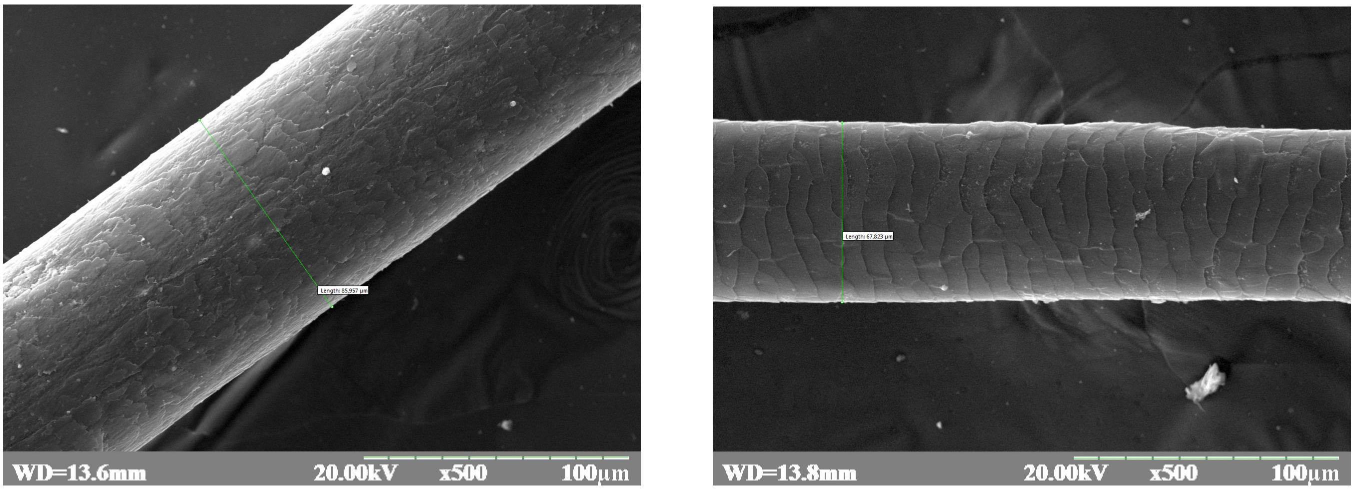
Morphometric characteristics of *Equinus asinus* and *Equus caballus* hair (n = 420)

№	Investigated samples		Hair thickness, µm	The number of scales (waves) on 100 µm hair length	The size of the scales (wavelength), µm		
					Mean	Min	Max
1	Donkey hair (*Equinus asinus*)	mane	105.46 ± 0.3	11–12	11.48	5.50	17.21
		covering hair	67.82 ± 0.2	11–12	9.64	6.31	14.83
2	Horse hair (*Equus caballus)*	mane	109.62 ± 0.3	10–11	10.03	4.28	12.56
		covering hair	75.45 ± 0.2	11–12	9.34	6.11	14.44

Hair from a donkey mane has cylindrical form (Figure 4 a). Scales on the hair surface are different in shape and size, don't have clear edges, longitudinal direction and closely adjacent to the surface. The superficial picture of the cuticle looks like an irregular wave formed from individual medium-sized petals with fringed (torn off) edges. The number of waves on the hair surface per 100 µm of hair length – 11–12. The size of the scales (wavelength) is 11.48 µm in average. The outer edge of the scales forms angles of different meanings, 110° in average.

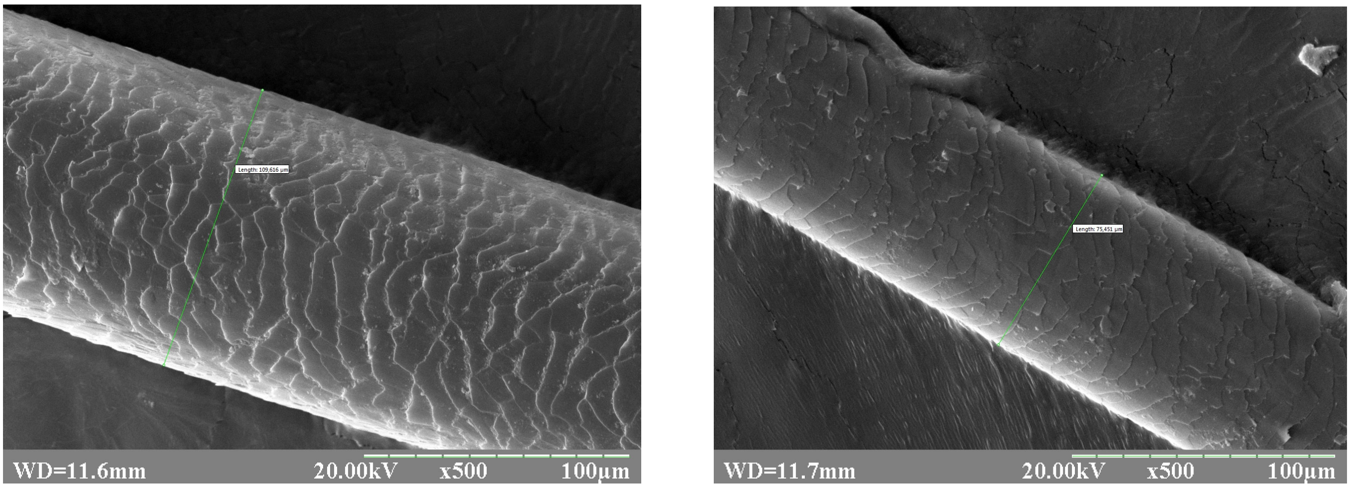
Donkey covering hair are also cylindrical in shape (Figure 4 b), but by 1.55 times smaller than mane hair. Scales on the hair surface are same in shape and size, have clear edges, longitudinal direction and closely adjacent to the hair surface. Cuticle picture looks like a regular wave formed by continuous petals with smooth edges. The number of waves on the hair surface per 100 µm of hair length – 11. The size of the scale (wavelength) is 9.64 µm in averages. The outer edge of the scales forms angles of different meanings, 127° in average.

Hair from a horse mane has cylindrical form (Figure 5 a). Scales on the hair surface are different in shape and size, have clear edges, longitudinal direction and closely adjacent to the hair surface. Cuticle picture looks like a irregular wave formed by the average size of individual petals with smooth sharp edges. The number of waves on the hair surface per 100 µm of hair length – 10–11. The size of the scale (wavelength) is 10.03 µm in averages. The outer edge of the scales forms angles from 77.84° to 128.82°.



a b

Figure 4. Donkey hair (*Equinus asinus*): a – mane; b – covering hair



a b

Figure 5. Horse hair (*Equus caballus*): a – male; b – covering hair

Horse's covering hair by 1.45 time less, than the hair from mane and has a cylindrical shape (Figure 5 b). Scales on the hair surface are different in shape and size, have not clear edges (torn off), longitudinal direction, loosely adjacent to the hair surface. Cuticle picture looks like a regular wave formed by separate petals with fringed edges. The number of waves on the hair surface per 100 µm of hair length – 11–12. The size of the scale (wavelength) is 9.44 µm in averages. The outer edge of the scales forms angles of different meanings, 112° in average.

4. Conclusions

Determination of morphological features of animal hair structure has important differential value not only from a scientific but also a practical point of view. As a result of our research, we have established the morphological and morphometric features of the hair structure *Equus asinus* (donkey) and *Equus caballus* (horse). It is proved that an important differential value have:

1. the shape, size and arrangement of cells in the brain substance of the hair and the ratio of the brain substance to the total thickness of the hair.

2. superficial drawing of hair cuticle – the shape of the scales and the nature of their edges, the waves regularity.

Donkey (*Equus asinus*): Mane: brain substance – tightly placed cells in the form of a continuous strand along the entire length of the hair, sometimes with cell-free gaps; superficial drawing of the cuticle – irregular wave formed from scales with fringed edges. Covering hair: brain substance – different cell in sizes and shapes that completely disappear from the middle to the peripheral end of the hair; superficial drawing of the cuticle – regular wave formed from scales with clear even edges.

Horse (*Equus caballus*): Mane: brain substance – sectioned cells of round shape along the entire length of the hair; superficial drawing of the cuticle – irregular wave formed by scales with sharp pointed edges. Covering hair: brain substance – continuous cord of the round shape cells, which begins at a distance of 1–1.5 mm from the root of the hair, towards the peripheral part, the gaps between cells increase until they disappear completely; superficial drawing of the cuticle – irregular wave formed from scales with fringed edges.

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Conflict of interest

The authors declared that there is no conflict of interest.

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