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Typical microscopic hair structure of the family Equidae, which includes horses and donkeys.

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Abstract

Animals just cannot survive without hair. The building's design elements make it possible to determine things like animal species, age, housing circumstances, diet, and even sexual orientation. According to paleontologists, the hairline remembers certain details about its "owner" even after thousands of years have passed. Research shows that there are notable variations in the hair coats of the two species studied (Equus caballus and Equus asinus) in terms of the structure of the brain content and the superficial drawing of the cuticle. Animal species may be identified by a microscopic analysis of hair that has been bleached, since the structure of the brain matter is easily recognized. Donkey mane hair's brain material appears as tightly clustered, occasionally interrupted cells, whereas horse mane hair's brain substance appears as clustered, spherical cells with short spaces between sections of 6-10 cells. Donkey hair represents the brain material, which is made up of cells of varying sizes and shapes that gradually diminish from the hair's surface as one moves from its center to its tip. Like donkey hair, horse hair has a similar pattern in its brain substance: cells of the same size begin with a continuous cord at a distance of 1-1.5 mm from the root of the hair, but the gaps between them increase to the complete disappearance of cells as you move toward the hair's peripheral end. Hair samples were examined under an ultramicroscope to determine the differences between two species of the same animal genus. The thickness, number of scales (waves), and size of the scales (wavelength) in donkey and horse mane hair were almost same, but the overall drawing was quite different. Horse mane cuticles are shown as sharply pointed waves on the surface design, whereas donkey mane scales are drawn with fringed edges. The covering hair of the horse was thicker than that of the donkey, and the scales were located and shaped differently. The donkey's outside cuticle is shown as a smooth, continuous wave of scales, whereas the horse's outer cuticle is depicted as a collection of scales with blurry, ripped edges that form uneven waves.

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

1. Introduction

Studies of extinct organisms, diet, evolution, ancient gene functions, forensic toxicology, etc. can benefit from hair analysis based on morphological features such as cuticle scale patterns, medulla and pigment, or molecular analysis of DNA and protein, trace elements, chemicals, etc. (Teerink, 1991; Wolinsky, 2010). Hairs on mammals may be studied morphologically to learn more about the species, their evolution (Mansilla et al., 2011However, fossilized hairs (such as those discovered in amber from the Font-de-Benon quarry near Arch-ingeay-Les Nouillers in Charente-Maritime (southwestern France)) show no discernible variations from modern-day hairs. Similar to Pan- thera leo hairs, fossil hairs from the cave lion Panthera spelaea Goldfuss, 1810 show no morphological differences from Pan- thera leo hairs (Kirillova et al., 2015).Comparative morphological and protein research on hairs from Holocene "Yukagir Horse" (Equus spp.), Lena, and current Yakutian horses reveals genetic differences despite superficial similarities owing to shared habitats and climatic circumstances (Chernova et al., 2015c).

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Consequently, species-specific modifications in hair morphology for insulation are rare.A light microscopy technique has been proposed for hair identification by several researchers (Kotsiumbas et al., 2010). Researchers Bonnie et al. (2010) found that the cross-sectional shape, pigment placement, and pigment density of the tail hairs of African elephants, Asian elephants, and giraffes are useful morpho- logical features for distinguishing the three species.The form of hair cuticula may have stayed mostly unaltered during much of mammalian history, as discovered by Vullo et al. (2010). Paleoecological information and unusual taphonomic circumstances are provided by the likely presence of a fly puparium near these hairs.Clement et al. (1981) agree that the categorization is justified because of the similarities and contrasts between the fine morphology and ultrastructure of the medulla in the major mammalian orders. Furthermore the timing of these charac- teristics mirrors that of mammalian phylogenesis. The medullar of human hair has ultra-structural properties that are unlike those of the hair of any other animal (Feughelman, 1997).When compared to animal hairs, the organization of human hairs' cortical cells was found to be quite irregular, with noticeable interdigita- tions of cell borders. Fibrous cellular remains of dead medullary cells were abundant in the medulla of both human and animal hairs. Cuticular cells' lamellar structures became strikingly apparent. Cuticular cell layers differed greatly in quantity and overall thickness between scalp and pubic hairs and across animals (Sato et al., 1982).Scanning electron microscopy is a cutting-edge technique that enables for precise microscopic differentiation of biological things (Rogers, 1959; Kunytskyi & Kupyna, 1998; Salyha & Snitynskyi, 1999; Pikhtirova & Ivchenko, 2018).SEM observation of hair samples is a rapid and valuable method for identifying hair types, with applications in fields as diverse as mammalian biology, the textile industry, and forensic medicine, as stated by Van den Broeck et al. (2001) and Popescu et al. (2010).

Osthaus et al. (2018) compared the qualities of the hair coats of donkeys, mules, and horses living in a temperate environment, however they did not compare the hair's ultramicrostructure.

The purpose of this research was to compare the microstructure of hair samples taken from donkeys (Equinus asinus) and horses (Equus ca- ballus) in order to determine distinguishing features between the two species.

Materials and methods

The studies were carried out in the Department of Anat- omy, Normal and Pathological Physiology and Laboratory of Electron Microscopy of Sumy National Agrarian Univer- sity.

Animals

For this purpose, samples of hair from mane (15 hairsper animal) and spine area (15 hairs per animal) from7 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 us- ing microscope "XS 2610 (MICROmed, Poltava, Ukraine)" in the magnification range from 250 to 400 times and mi- crophotograph Micro Capture Ver 6.9.3. Sample preparation included: selection of biological material; washing; discol- oration (30 % H_2O_2 at room temperature for 30–60 min.); fixation on glass slide and placement on the microscopeobject table.

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

Electron microscopy

The microstructure of the hair surface was examined us- ing a scanning electron microscope REM-106I (Selmi, Su- my, 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.

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The morphometric characteristics were described by photo and SEM-images using a digital image analysis pro- gram 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 pres- ence 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 pa- rameters (Figure 1).

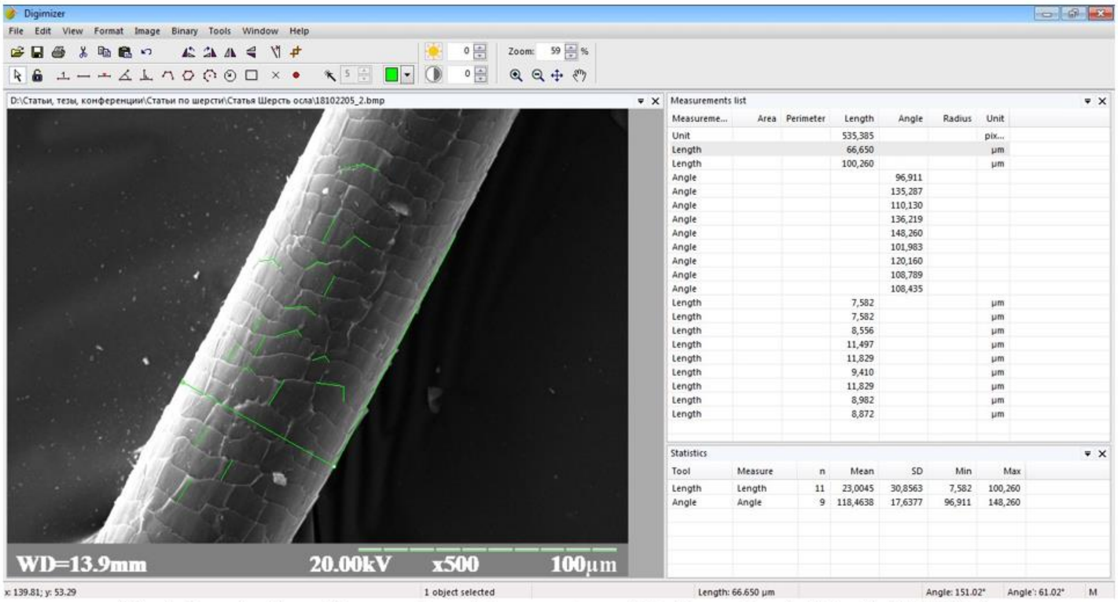

Fig. 1. Investigation of the morphometric parameters of *Equinus asinus* hair by digital SEM-image in programs Digimizer 4.0

Statistical data processing

The results of the study were elaborated statistically us- ing 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. Results and discussion The horse hair specimens were different from the donkey hair in length and color. The investigated donkey hair ob- tained from the mane area was black, length 7.5–13 cm, and from the spine section – gray, some samples – with blackand 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 enlight- ened hair of a mane and cover hair donkey (Figure 2) had significant differences. Thus the brain substance of manehair (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.

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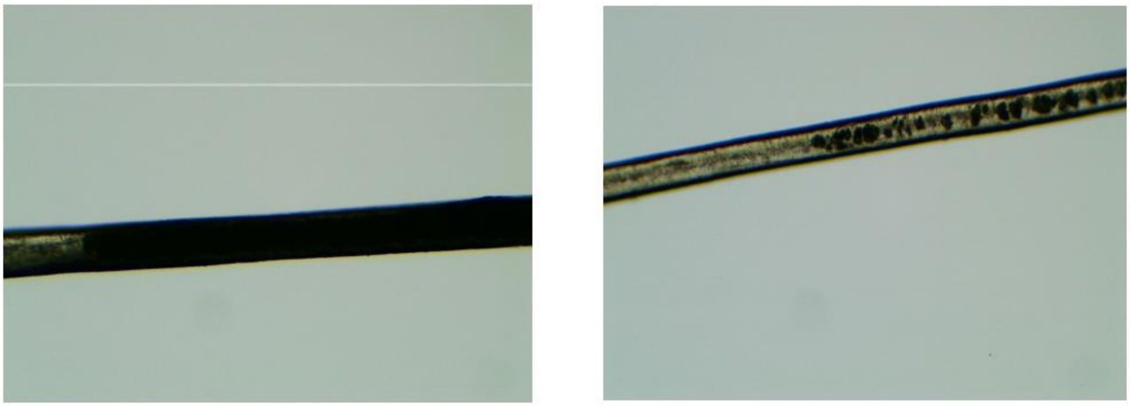
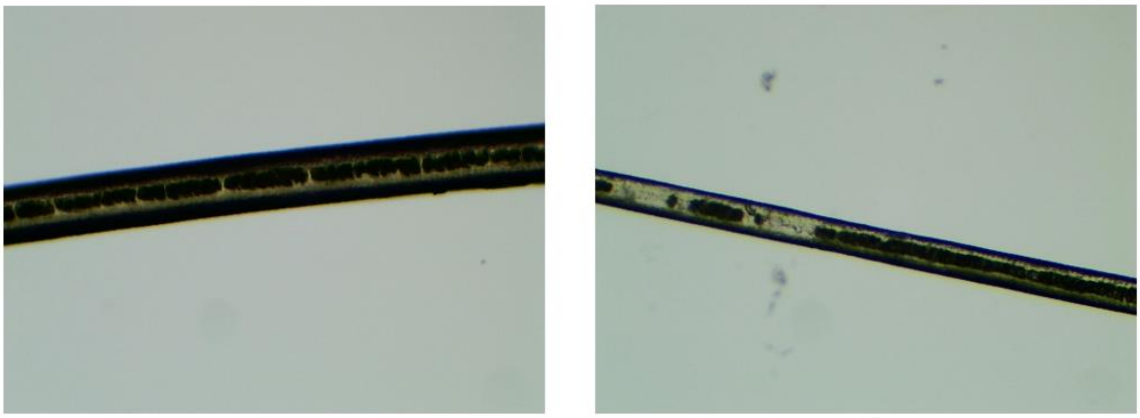


а б
Figure 2. Donkey hair (*Equinus asinus*): a – mane; b – covering hair; x 400.

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 ofcells 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 is1 : 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 cover- ing 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 dif- ferent (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.

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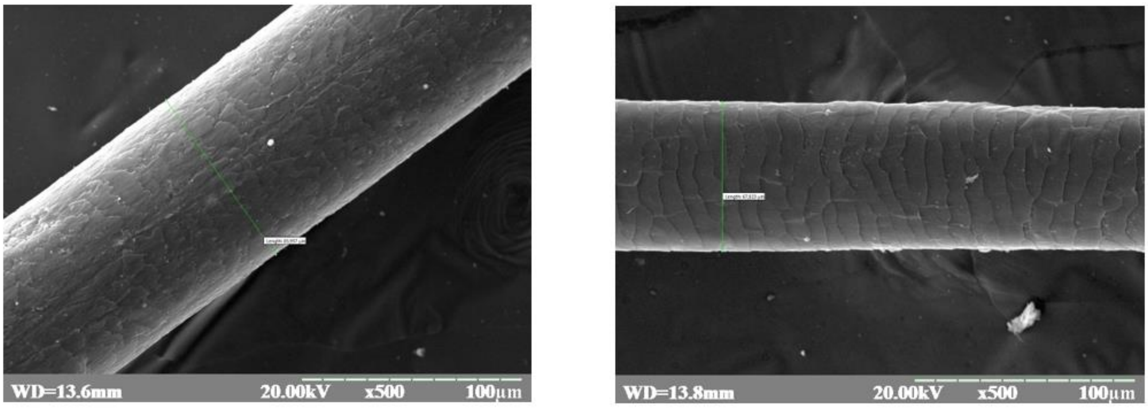
Table 1
Morphometric characteristics of *Equinus asinus* and *Equus caballus* hair (n = 420)

№	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 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 num- ber of waves on the hair surface per 100 µm of hair length – The size of the scale (wavelength) is 9.64 µm in averag- es. 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 closelyadjacent to the hair surface. Cuticle picture looks like airregular wave formed by the average size of individualpetals with smooth sharp edges. The number of waves onthe 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

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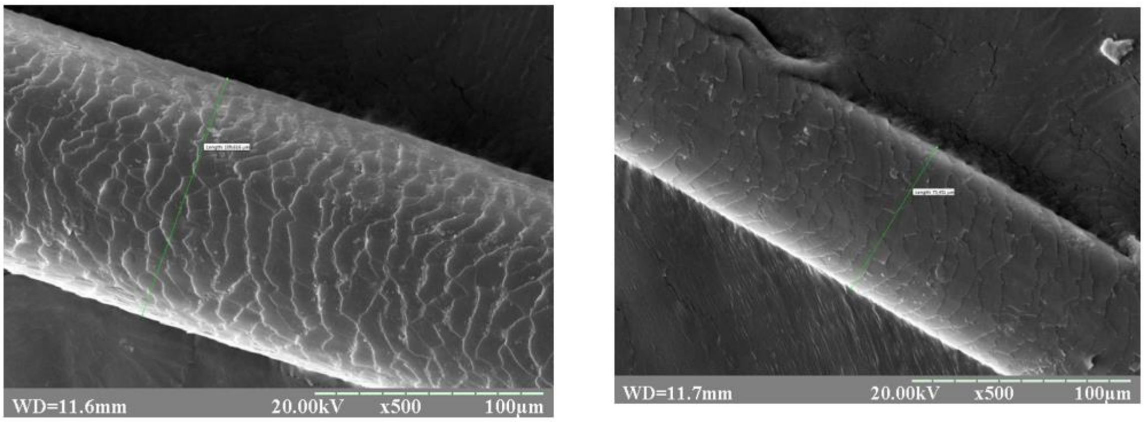

a b

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 adja- cent to the hair surface. Cuticle picture looks like a regular wave formed by separate petals with fringed edges. Thenumber 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.

Conclusions

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 sub- stance – 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 – sec- tioned cells of round shape along the entire length of thehair; 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 draw- ing of the cuticle – irregular wave formed from scales with fringed edges.

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