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Morphological study of the hairs of prey mammals from the fauna of Bulgaria

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Abstract

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The morphological specifics of the fur can be used for identification in ecological and zoological studies, in forensic and veterinary medical examinations. The scale pattern of the hair is one of the important identifying features and can be used as a "fingerprint". In this study, the morphological characteristics of the guard hairs of eleven species of prey mammals, present in the fauna of Bulgaria were studied. The values for the hair length, the total hair thickness, the medulla thickness and medullary index were found in the studied animals. The combined use of morphometric values and the "scale model" of the hair – the medullary index (MI) are indexes that are applied for greater data reliability.

Keywords: prey mammals; hairs; histology

Introduction

The fur of the animals forms an additional protective layer on the epidermis of the skin. It protects the body surface from both mechanical damage and excessive heat radiation (Sarma et al., 2021; Mihaylov & Kirilov, 2022).

The hairs (*pilli*) that compose the fur are epidermal formations of the skin. They are flexible, elastic, filamentous structures that are composed of hair follicle, root (*radix pilli*) located in the dermis of the skin and an external and visible part located above the epidermis – a shaft (*scapus pilli*) (Lee et al., 2014; Sarma et al., 2021).

The study of fur of the animals has fundamental morphological importance (Sapundzhiev et al., 2006). One of the reasons for the investigation of the fur is the identification of animals (Knecht, 2012; Sari & Arpacik, 2018; Sarma et al., 2021; Mihaylov & Kirilov, 2022). That is necessary to make difference between animals of similar species.

The scale pattern of the hair is one of the important features for identification and can be used as a "fingerprint" (Partin, 2004). Another reason that hairs may be studied is the distribution of the animals – the areas are investigated to

determine if the species is presented in that area (Tremori et al., 2018; Sarma et al., 2021). The third reason to study the hair is because of the fact that it is fun, inspiring, and educational (Partin, 2004). The investigation of the hairs in faecal samples and their identification answers the question "Who eats what?" That is essential for the relationships between predator and prey in the healthy ecosystems (Symondson, 2002; Sheppard & Harwood, 2005).

The hairs are highly resistant to atmosphere conditions and decomposition. These properties make them an almost ideal type of physical proof (Lungu et al., 2007; Sarma et al., 2021).

The structure and the characteristics of the hair shaft of the farm animals has been studied by light microscopy (Moskov & Madrov, 1963) and by electron microscopy (Blažej et al., 1989; Stanley & Magney, 1992).

The studies of the hair shaft of the wild animals (Sapunjiev et al., 2006; Partin, 2004; Lungu et al., 2007; Tremori et al., 2018; Sarma et al., 2021; Mihaylov & Kirilov, 2022) collect a database that can be used for identification in ecological and zoological studies, in forensic and veterinary expert reports. These data can be used successfully to track predators and to identify their victims and in the illegal hunting of wild animals. In an ecological aspect, they clarify the cases of conflict between the wild animals and the humans (Mihaylov & Kirilov, 2022). In this aspect, the topic of the study is to investigate the morphological features of the guard hairs from mammalian prey animals, some of which are characteristic of the fauna of Bulgaria.

Material and Methods

Guard hairs from nutria (*Myocastor coypus*), hare (*Lepus europaeus*), roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*) and squirrel (*Sciurus vulgaris*) were collected in our field investigations and by hunters.

Guard hairs from red deer (*Cervus elaphus*), fallow deer (*Dama dama*), mouflon (*Ovis musimon*), American bison (*Bison bison*), and yak (*Bos grunniens*) were obtained from animals at Stara Zagora Zoo with the courtesy of its management. Hairs from wild goat (*Rupicarpa rupicarpa*) were delivered by employees of State Forestry "Izvor".

The hair samples were obtained from the dorsolateral part of the body of each species (Wallis, 1993) during autumn/winter season of 2022 year and were placed separately in labeled polyethylene bags.

The length of the hairs was determined using a digital caliper (Neiko 01407A Stanless Steel 6-Inch Digital Caliper with Extra-Large LCD Screen and Instant SAE-Metric Conversion) with an accuracy of 0.1 cm (Mihaylov et al., 2014). In order to avoid errors due to the distortion or curling of the hairs, they were stretched on glass slides and taped. Their length was determined with an accuracy of 0.1 cm (Mihaylov & Kirilov, 2022).

To prepare permanent histological preparations, hair samples were washed in soapy water to remove contaminants and rinsed in distilled water 2–3 times. They were then placed in ether/alcohol (50:50) for 2–3 min to remove fat (Kshirsagar et al., 2009). The dried guard hairs were placed on glass slides and embedded in Canadian balsam (Sapundzhiev et al., 2006). Peroxide bleaching was carried out on hypermelanotic hairs (Lungu et al., 2007). The preparations were observed on a Galen III microscope following the sublimation of Canada balsam. The measurements of analogous sections from the middle of the guard hairs of the different animal species was carried out by an ocular micrometer. The average number of the samples was seven, at a magnification of 100x and 400x.

The diameter of the hair shaft and pith were measured. Imaging of identically selected sections of the integumentary hairs was conducted with a Digital Camera MDCE-5C. The medullary index – MI was calculated, as the ratio of medulla thickness to the total hair thickness. Medullary Index (Kirk, 1953; Lungu et al., 2007; Sarma et al., 2021). The processing of the received data was carried out using Statistica 7.

Results and Discussion

The three morphological layers of the hairs (Deedrick & Koch 2004; Debelica & Thies, 2009; Knecht, 2012) – medulla (central layer), cortex (intermediate layer) and cuticle (outer layer) are easily detectable (Negi et al., 2017) by microscopic study. The different thickness of the medulla and the cortex in the different animal species forms a kind of scale pattern (Partin, 2004), which is specific and can be used in their identification. In some animals the total thickness of the hair is greater but the medulla is thinner or vice versa. For more accuracy the medullary index can be used (Kirk, 1953; Lungu et al., 2007; Sarma et al., 2021; Mihaylov & Kirilov, 2022) (Table 1).

The study of the guard hairs of the roe deer (*Capreolus capreolus*) – shows that their medulla occupies 75% of the thickness of the hair – D – 112.28 ± 2.45 μm. It is composed of polygonal, dorsoventrally flattened cells, with strong pigmentation and air spaces between them. The cortical layer is clearly visible and well distinguishable from the medulla (Figure 1). Sari & Arpacik (2018) classify the cortical layer as narrow. The cells of the cortex are elongated, parallel to the longitudinal axis of the hair, and less pigmented than those of the medulla. MI was calculated – 0.75. Our results

Table 1. Values of the length, total hair thickness, medulla thickness and medullary index in the studied animals

No	Species	Hair length, cm, Mean ± SE	Diameter of hair shaft (D) (μm) (Mean ± SE)	Medulla Diameter (MD) (μm) (Mean ± SE)	Medullary Index (MI): Medulla Diameter/ Diameter of hair shaft
1	Roe deer (*Capreolus capreolus*)	6.2 ± 0.12	112.28 ± 2.45	84.00 ± 1.21	0.75
2	Fallow deer (*Dama dama*)	4.0 ± 0.06	220.43 ± 2.43	189. 74 ± 1.19	0.86
3	Red deer (*Cervus elaphus*)	5.6 ± 0.50	227.53 ± 5.28	192.59 ± 1.42	0.85
4	Chamois (*Rup. rupicarpa*)	5.7 ± 0.42	203.17± 4.12	198.24± 1.67	0.98
5	Muflon (*Ovis aries*)	6.2 ± 0.57	157.48 ± 3.12	155.12± 1.75	0.98
6	Yak (*Bos grunniens*)	9.6 ± 1.89	59.12 ± 1.34	17.52 ± 0.46	0.29
7	Bison (*Bison bison*)	6.4±1.72	108.57 ± 2.44	52.53 ± 0.61	0.48
8	Hare (*Lepus europaeus*)	4.2 ± 0.26	73.43 ± 5.29	59.62 ± 3.14	0.81
9	Nutria (*Myocastor coypus*)	5.5 ± 0.07	171.43 ± 2.55	94.00 ± 0.97	0.55
10	Common squirrel (*Sciurus vulgaris*)	2.7±0.37	87.58 ± 2.71	70.54 ± 3.42	0.80
11	Wild boar (*Sus scrofa*)	8.9 ± 0.23	254.00 ± 1.23	240.71 ± 3.31	0.95

support the research of some authors (Kulak & Wajdzik, 2006; Lungu et al., 2007) about the hairs of the roe deer.

The observation of the guard hairs of fallow deer (*Dama dama*) –found that the diameter of their hair shaft D – 220.43 ± 2.43 μm was twice greater than that of the doe – D – 112.28 ± 2.45 μm. The cells of the medulla are oval-spherical, closely packed together, highly pigmented, and definitely demonstrate a "medullary lattice pattern" (Figure 2).

At first look, due to the strong pigmentation, the medulla fills almost the entire oval of the shaft – Figure 2, but after lightening the hair with peroxide (Lungu et al., 2007) ((Figure 3), the cortex becomes clearly noticeable. The cells of the cortex, as in the doe, are elongated and oriented parallel to the longitudinal axis of the hairs. We calculated MI – 0.86.

The thickness of the hair shaft of the red deer (*Cervus elaphus*) (Figure 4), is calculated D – 227.53 ± 5.28 μm. The cells of the medulla are arranged in a "medullary lat-

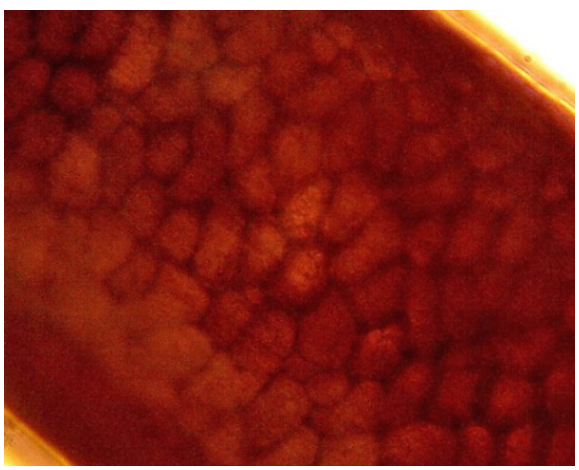

Fig. 2. Fallow deer (*Dama dama*) × 400

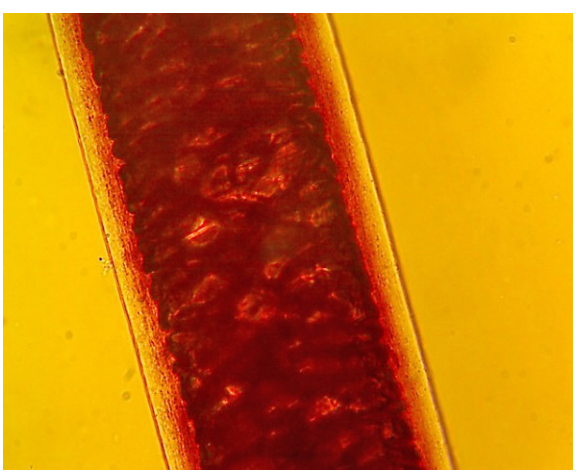

Fig. 1. Roe deer (*Capreolus capreolus*) × 400

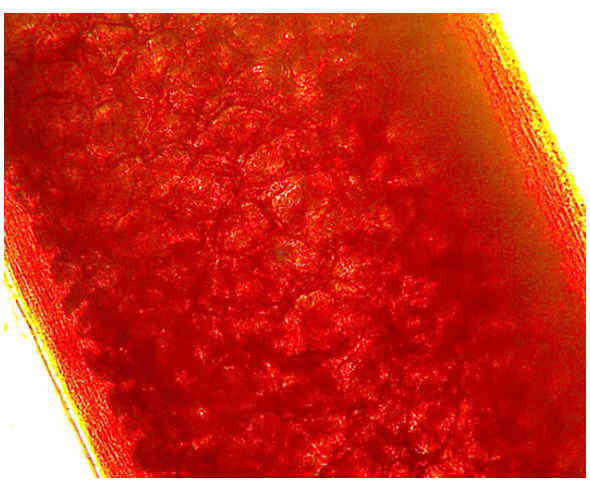

Fig. 3. Fallow deer (*Dama dama*) × 400

tice pattern" and they are highly pigmented. After lightening the hairs with peroxide, it is possible to observe the fine cortical layer, composed by less pigmented cells, which are elongated and oriented along the longitudinal axis of the hair. We found that MI is 0.85. Some authors (Deedrick & Koch, 2004; Debelica & Thies, 2009) report that the hairs of representatives of the Cervidae family are indistinguishable, because of their gross appearance and microscopic features, and the cortex is not observed. However, our results for red deer and fallow deer hairs support the studies (Lungu et al., 2007; Sari & Arpacik, 2018; Dehury et al., 2019), that in these species, the cortex is observed. We found that MI of the hairs of red deer is – 0.85 and that of the hairs of the fallow deer is 0.86.

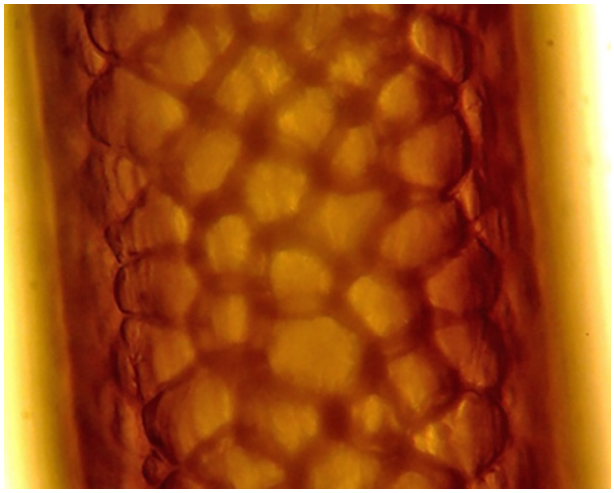

Fig. 4. Red deer (*Cervus elaphus*) × 400

The guard hairs of the wild goat (*Rupicapra rupicapra*) (Figure 5) is with thickness D – 203.17 ± 4.12 μm. Their medullary structure at x 100 magnification strongly resembles that of the red deer and fallow deer (Figure 5). A mistake can be made in their identification. At higher magnification (Figure 6) and in an lightened section, the fine cortex between the cuticle and the medulla of the hair is noticeable. The medulla of the hair has a lattice pattern and occupies a larger proportion of the diameter of the hair, compared to that of the fallow deer and the red deer. It is reasonable, and MI – 0.98 in the wild goat is greater compared to that of the fallow deer – MI – 0.86 and the red deer MI – 0.85. Our results on hair shaft thickness and MI in the wild goat confirm the results of some authors (Lungu et al., 2007) for this animal species.

The results of the morphological study of mouflon hairs (*Ovis aries*) (Figure 7) show that the thickness of the hair shaft is: D – 157.48 ± 3.12 μm. Cuticle cells are clearly visible and tile-like. The cells of the medulla are arranged in a lattice pattern, but unlike fallow deer, red deer, and wild goat, their shape is not oval-spherical. They are dorsoventrally flattened, as in roe deer. In lightened sections of the hairs, cells from the thin cortical layer can be seen. They are elongated and oriented along the longitudinal axis of the hair. For mouflon's hair we calculated MI – 0.98, same as for the wild goat. A similar description for the peculiarities of the mouflon hair's construction is presented by De Marins & Aspera (2006), who report that "the medulla fills the entire width of the hair and the cortex is very narrow or not noticeable". Unfortunately, the same authors did not report D and MI values for mouflon's hair because the accent of their study was on the hair changes during the domestication of the species.

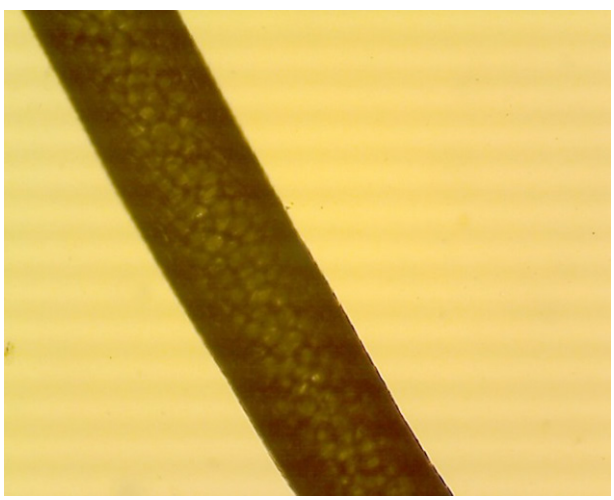

Fig. 5. Wild goat (*Rupicarpa rupicarpa*) × 100

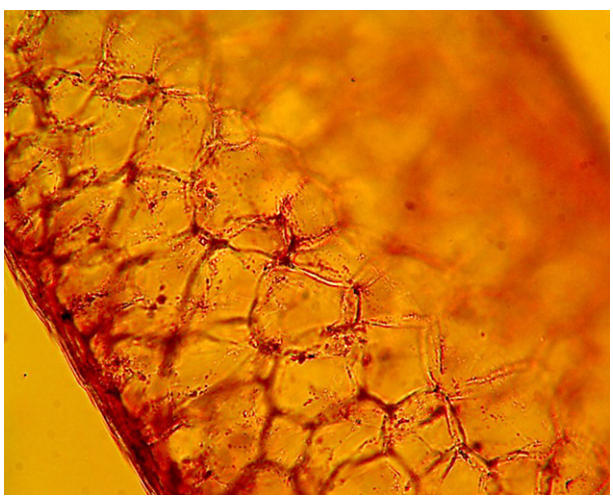

Fig. 6. Wild goat (*Rupicarpa rupicarpa*) × 400

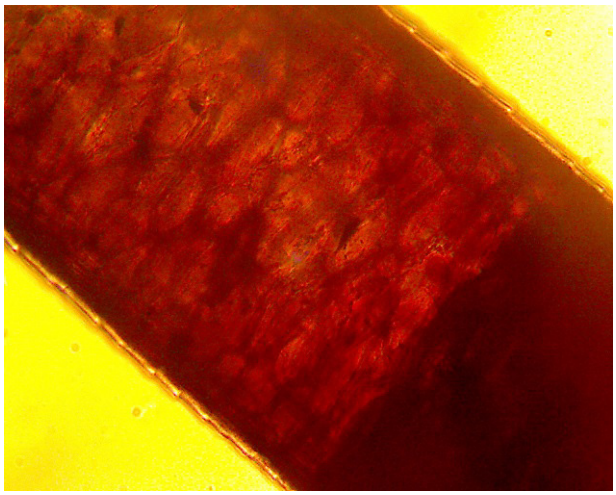

Fig. 7. Mouflon (*Ovis aries*) × 400

The study of the yak hairs (*Bos grunniens*) (Figure 8), shows that they are thinner than all studied, presented above. We found the thickness of the hair shaft in the yak – D – 59.12 ± 1.34 μm. The medulla of the hairs is amorphous, with strong pigmentation of the cells. The well-formed cortex has less pigmentation of the cells and is clearly distinguishable. The highly developed cortex affects the MI value – 0.29, which we calculated. In their study some authors (Sarma et al., 2021) presented values close to ours for hair shaft thickness in the yak – D – 66.08 ± 3.67 μm, but we cannot accept their presented values for MI – 0.895.

In the microscopic study of bison hairs (*Bison bison*) (Figure 9), the thickness of the hair shaft is determined – D – 108.57 ± 2.44 μm. Similar values for the thickness of the hair shaft, but in adult European bison (*European bison*) are presented (Sztych & Olech, 2016). The highly developed cortical layer is found. It is composed of elongated cells oriented parallel to the length of the hair shaft. The pigmentation of the cells from the cortex is not uniform – the cells that are close to the medulla are more darkly pigmented than those that are closer to the cuticle. A decrease in the pigmentation of the cells from the cortex in the direction from the medulla to the cuticle is observed. The well-developed cortex in bison hairs affects the MI value – 0.48.

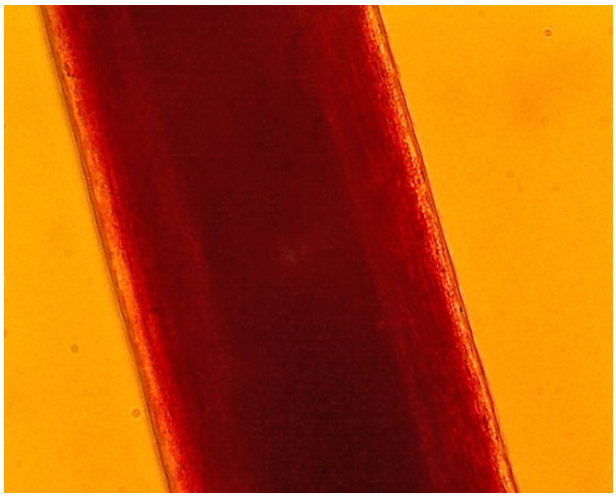

Fig. 9. Bison (*Bison bison*) × 400

The thickness of the hair shaft in the hare (*Laepus europeus*) (Figure 10) is D – 73.43 ± 5.29 μm. The medulla is very well developed – M – 59.62 ± 3.14 μm. Its cells are pigmented, and their arrangement resembles that of "corn kernels in a corncob". Air bubbles are observed in many of the cells of the medulla, giving it a mosaic-like appearance. In a

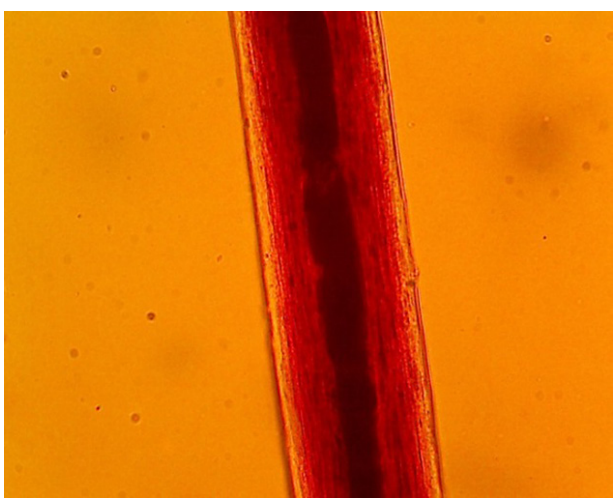

Fig. 8. Yak (*Bos grunniens*) × 400

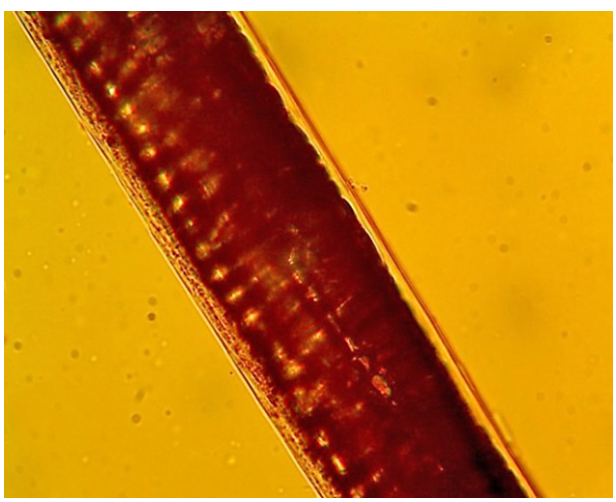

Fig. 10. Hare (*Lepus europaeus*) × 400

Fig. 11. Hare (*Lepus europaeus*) × 400

study for hair's structure, but in the domestic rabbit (Zheng et al., 2011; Guo et al., 2012) reported that medulla's cells form columns along the hair. According to them, the number of the columns of the medullary cells changes depending on the thickness of the hair shaft. Even within a single rabbit hair, the number of the columns of the medullary cells varies from the root to the tip, which we do not dispute.

The cortical layer in the covering hairs of the hare (Figure 11), is clearly distinguishable but poorly developed. The cells from the cortex are elongated and oriented along the longitudinal axis of the hair. They are less pigmented than the cells of the medulla. The thin cortical layer of the hairs of the wild rabbit affects the medullary index, which we determined – MI – 0.81.

In different sections along the length of the guard hairs from a hare, areas with weaker pigmentation of medullary cells can be observed in the medullary layer (Figure 11). According to us that is related to the formation of the disguise coloration of the hare's fur.

The guard hairs of the nutria (*Myocastor coypus*) (Figure 12), are rough and the thickness of the hair shaft is D – 171.43 ± 2.55 µm. The medulla is clearly distinguishable and occupies more than half of the hair's diameter, M – 94.00 ± 0.97 µm. The cells of the medulla are pigmented and air bubbles are observed between them. The medullary cells (Figure 12), are dorsoventrally flattened, oriented transversely along the length of the hair shaft and form three columns – two peripheral and one central. Debelica & Thies (2009) describe the cells in the medulla of the nutria's hair as "flattened cells with some appearance of rows". The cortical layer of the nutria hairs is well formed, its cells are less pigmented than those of the medulla and oriented parallel to the length of the hair. The well-developed cortical layer affects the MI value of 0.55 that we calculated.

In the common squirrel (*Sciurus vulgaris*) (Figure 13), the thickness of the hair shaft is D – 87.58 ± 2.71 µm. The medulla is well formed and occupies most of the hair's diameter – M – 70.54 ± 3.42 µm. The cells of the medulla are pigmented and air bubbles are visible in places, giving them a mosaic appearance, similar to that of the wild rabbit's hairs. We assume that the cells of the medulla in the squirrel's hairs are arranged in four to five columns, oriented along the longitudinal axis of the hair, similar to the wild rabbit's hairs, although they are not so clearly defined. Debelica & Thies (2009) describe the medulla in another squirrel species – Eastern Gray Squirrel (*Sciurus caroliensis*) as "unordered, disturbed, flattened with cortical intrusions on the edges".

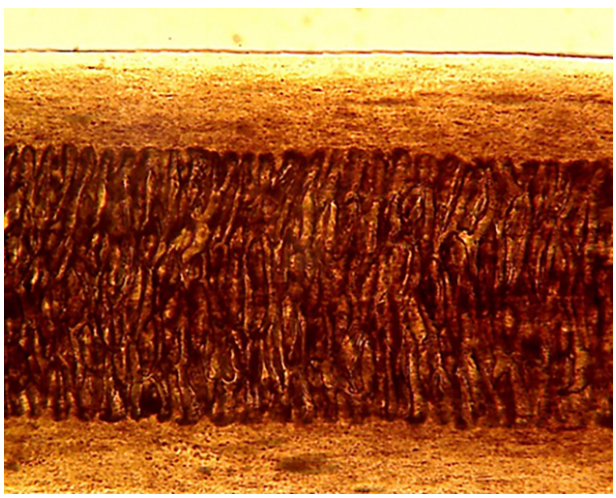

Fig. 12. Nutria (*Myocastor coypus*) × 400

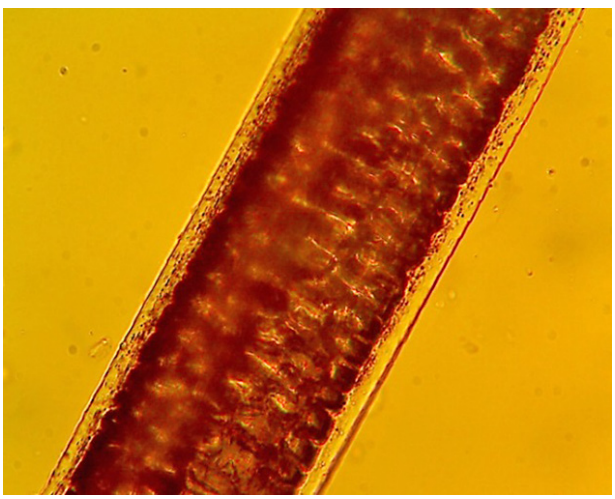

Fig. 13. Common squirrel (*Sciurus vulgaris*) × 400

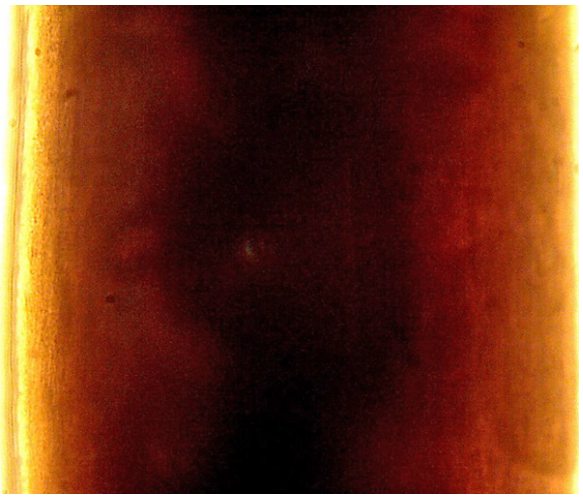

Fig. 14. Wild boar (*Sus scrofa*) × 400

Similar information to our description for the structure of the medulla in the common squirrel (*Sciurus vulgaris*) is presented by Lee et al. (2014), who describe the medulla as "zipper-shaped". The cortical layer of the squirrel's hair is distinct but thin. Its cells are less pigmented than those of the medulla and oriented parallel to the longitudinal axis of the hair. Due to the less developed cortical layer MI is – 0.80.

The hairs of the wild boar (*Sus scrofa*) (Figure 14), are easily recognizable macroscopically because they are thick and have split tips, which is also reported by Lee et al. (2014). The thickness of the hair shaft in the wild boar is D – 254.00 ± 1.23 μm. The medulla is continuous, amorphous and homogeneous, but according to Debelica & Thies (2009), it occupies the entire shaft. However, in their study, Sari & Arpacik (2018) report a broad cortex in the wild boar. After treating the hairs with peroxide, we found a clearly distinguishable and thin cortex in the wild boar's hairs (Figure 14). The cells of the cortex are less pigmented than those of the medulla and we found for the wild boar's hairs MI – 0.95.

Conclusion

Based on the obtained results of our study, we found that the thickest guard hairs are those of the wild boar (*Sus scrofa*) – D – 254 ± 1.23 μm. We observe a thin cortex and the ends of the hairs are split.

In the representatives of the family Cervidae – red deer (*Cervus elaphus*) and fallow deer (*Dama dama*), the hairs are difficult to be distinguished from each other because their microscopic characteristics are similar. A cortex is found.

In the representatives of the family Bovidae – wild goat (*Rupicarpa rupicarpa*) and muflon (*Ovis aries*), the microscopic characteristics of the guard hairs are similar – MI – 0.98, the cells of the medulla are arranged in a "medullary lattice pattern", but in the muflon, they are dorsoventrally flattened. In the roe deer (*Carpeolus carpeolus*), in the wild goat (*Rupicarpa rupicarpa*), in the red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) the cells of the medulla of the hairs are oval. In the yak (*Bos grunniens*) and bison (*Bison bison*), which are representatives of the family *Bovidae*, a definite differentiation can be made on the basis of the microscopic characteristics of the guard hairs. In the yak, MI of the guard hairs is 0.29, and in bison, the MI is 0.48.

The guard hairs of the nutria (*Myocastor coypus*) are clearly distinguished from those of the wild rabbit (*Laepus europeus*) and the common squirrel (*Sciurus vulgaris*). In the nutria the cells of the medulla of the guard hairs are dorsoventrally flattened and the cortex is wide – MI – 0.55, while in the guard hairs of the wild rabbit and the common squirrel, the medulla is wider than the cortex – MI is 0.81 in the wild rabbit and MI – 0.80 in the common squirrel. Due to the similar values of MI in the wild rabbit and the common squirrel, and the similar appearance of their medullary cells, for differentiation, it is necessary to search for non-pigmented areas in the medulla, which is specific for the guard hairs of the wild rabbit.

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