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The dorsal guard hair identification key of Korean small mammals (Rodentia and Lagomorpha)

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Abstract: We analyzed the hair microstructure of Korean small terrestrial mammals, such as Rodentia and Lagomorpha, to classify the characteristics of hair morphology. *Micromys minutus* showed a unicellular irregular type of medulla structures; on the other hand, the other mammals showed multicellular structures. Regarding the cuticular scale structures, the Rodentia species exhibited a narrow and broad diamond petal type, while the Lagomorpha species exhibited an elongated petal type. Interestingly, the hair cross-sections showed quite unique characteristics. We constructed hair identification keys to distinguish species with a single hair. The dichotomous key of Rodentia and Lagomorpha can be used for their behavioral ecology and dietary analysis of upper predators to serve as the basis for ecological research.

Keywords: hair microstructure, identification key, Lagomorpha, Rodentia, small mammal

INTRODUCTION

Mammal hair plays an important role in body shape maintenance, thermoregulation, protection from pollution and waterproofing. There have been many studies that use the microstructure of hair, especially for the establishment of an identification key for various species. The microstructure of hair can be distinguished by the medulla (the pith of a hair), the cuticular (the outermost layer) and the cortex (the layer between the cuticular and medulla). Teerink (1991) observed the hair of mammals distributed in Western Europe. In that research, Teerink analyzed hair of the Carnivora, Artiodactyla, Insectivora, and Chiroptera, and reported that the hair cross-section of Rodentia and Lagomorpha is very distinctive, and that feature can be used as an index for species identification.

There are several reasons why microstructure research of small mammal hair should be conducted. First, Korean mammal fauna is characteristic in that it is composed mainly of small mammals (Order Rodentia and Lagomorpha) rather than medium- and large-sized wild mammals. Species belonging to the two orders have similar hairy morphological appearances (color and length, etc.), so it is not easy to distinguish only through macroscopic observation. Second, Lee *et al.* (2014) only classified the Rodentia and Lagomorpha orders by medulla structures and cuticular scales. In addition to the medulla structures and cuticular scales, the feature of hair cross-section could also be an important hair identification key (Teerink 1991). Thus, more detailed and informative microstructures were used to establish a standard method for the identification of the Rodentia and Lagomorpha. Lastly, the research of the microstructure of small mammal hair can be applied to various parts of real life. In the past, incidents related to food sanitation often occurred in Korea, and most of the cases were related to species belonging to order Rodentia, and the hairs or carcasses of rodents were mixed into food. Furthermore, since rodent hair has been detected in the feces of small predators in Korea, i.e., the leopard cat (*Prionailurus bengalensis*) and yellow-throated marten (*Martes flavigula*), during ecological surveys, the identification key for Rodentia and Lagomorpha using the microstructure of hair could be beneficial for the ecological research and dietary analysis of predators. In addition to the above, research for Rodentia and Lagomorpha can be applied in various fields, including forensic science (Sato *et al.* 2010), taxonomy (Sessions *et al.* 2009) and archaeology (Spangenberg *et al.* 2010; Vullo *et al.* 2010).

The main purpose of this research is to characterize the microstructure of dorsal guard hair of Korean small mammals belonging to order Rodentia and Lagomorpha, and to establish a criterion for species identification using these characteristics.

MATERIALS AND METHODS

We collected hair samples from stuffed specimens deposited in the National Institute of Biological Resources (NIBR) in Korea. In detail, the samples we included the samples for research consist of eight species from the order Rodentia (*Micromys minutus*, Eurasian harvest mouse, $n=3$; *Eothenomys regulus*, Red-backed vole, $n=11$; *Apodemus agrarius*, Striped field mouse, $n=19$; *A. peninsulae*, Korean wood mouse, $n=5$; *Rattus norvegicus*, Common rat, $n=8$; *Sciurus vulgaris*, Eurasian red squirrel, $n=5$; *Pteromys volans*, Flying squirrel, $n=4$; *Tamias sibiricus*, Siberian chipmunk, $n=8$) and one species from the order Lagomorpha (*Lepus coreanus*, Korean hare, $n=4$) (Table 1). We utilized dorsal guard hair from mature animals because it is known that guard hair is informative in species identification, as it exhibits diagnostically reliable features (De Marinis and Asprea 2006; Lynch *et al.* 2012). To examine the microstructure of hair, we treated the hair samples following a previously used method by Lee *et al.* (2014) and Lee *et al.* (2015).

We collected five dorsal guard hairs from each individual to observe the medulla structure of the hair shield using a light microscope (CX31; Olympus, Tokyo, Japan) equipped with a 100× lens. We also observed the hair shield region to analyze the cross-section of hairs because

Table 1. Microstructure features of hair of Rodentia and Lagomorpha

Order	Family	Genus	Species	Medulla structures	Cuticular scales	Hair cross-sections
Rodentia	Muridae	*Micromys*	*M. minutus* ($n=3$)	Unicellalur irregular	Narrow diamond-shaped petal	Concavo-convex type
		Eothenomys	*E. regulus* ($n=11$)	Multicellular with isolated	Broad diamond-shaped petal	Quadriconcave type
		Apodemus	*A. agrarius* ($n=19$)	Multicellular with striped margin	Narrow diamond-shaped petal	Concavo-convex type
			A. peninsulae ($n=5$)	Multicellular with striped margin	Broad diamond-shaped petal	Concavo-convex type
		Rattus	*R. norvegicus* ($n=8$)	Multicellular with striped margin	Narrow diamond-shaped petal	Oval type
	Sciuridae	*Sciurus*	*S. vulgaris* ($n=5$)	Multicellular with net shaped	Narrow diamond-shaped petal	Concavo-convex type
		Pteromys	*P. volans* ($n=4$)	Multicellular with numerous squared cells	Broad diamond-shaped petal	Biconcave type
		Tamias	*T. sibiricus* ($n=8$)	Multicellular with isolated	Broad diamond-shaped petal	Oval type
Lagomorpha	Leporidae	*Lepus*	*L. coreanus* ($n=4$)	Multicellular with striped margin	Elongated-petal	Dumb-bell type

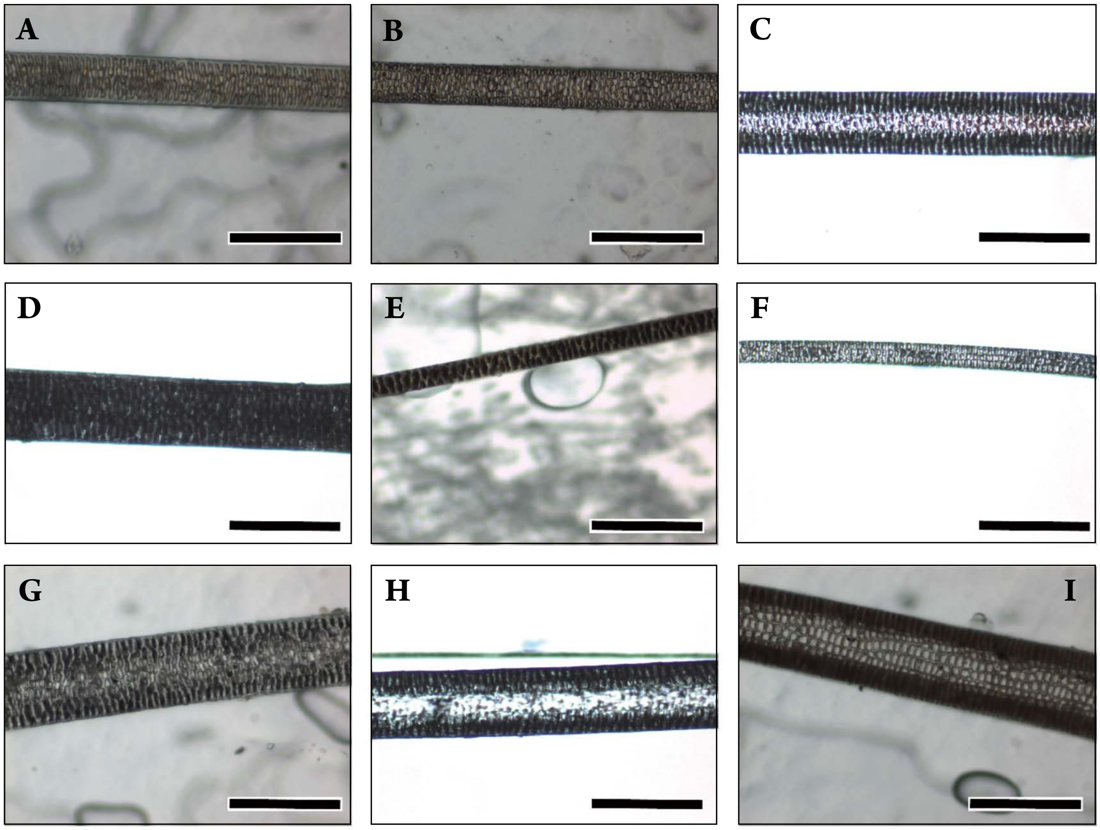

Fig. 1. Medulla structures of the hair shield of nine species of Rodentia and Lagomorpha. Most of the nine species show unique multicellular structures. (A) *Tamias sibiricus*, (B) *Eothenomys regulus*, (C) *Apodemus agrarius*, (D) *Sciurus vulgaris*, (E) *Micromys minutus*, (F) *Pteromys volans*, (G) *Rattus norvegicus*, (H) *Apodemus peninsulae*, and (I) *Lepus coreanus*. All scale bars are 150 μm.

Rodentia and Lagomorpha hair is characterized by a thick shield region (De Marinis and Paolo 1993). Generally, the cuticular scales of the hair shield show an irregular wave pattern in all species, while those in hair shafts have a variety of features (Smith 1933; Teerink 1991). Thus, we used scanning electron microscopy (SEM TM-1000; Hitachi, Tokyo, Japan) to observe hair cross-sections in hair shield and cuticular scales in hair shaft. We measured the guard hair width with ImageJ (available for download at https://imagej.nih.gov/ij/), and the mean value of each species is presented in Figure 4, respectively.

RESULTS

Descriptions of the medulla structures, cuticular scales, and cross-section type of each species are summarized in Table 1. We classified the medulla structures, cuticular structures, and cross-section type according to Teerink (1991). The medulla structures of the Rodentia and Lagomorpha orders showed distinct features, such as multicellular, unicellular, and squared cellular features. *M. minutus* showed unicellular irregular structures in the hair shield region (Fig. 1E). *S. vulgaris* exhibited a multicellular structure with a net shape in its hair (Fig. 1D), similar to that of carnivores (Lee *et al.* 2014). *L. coreanus* (Fig. 1I), *R. norvegicus* (Fig. 1G), *A. agrarius* (Fig. 1C), and *A. peninsulae* (Fig. 1H) have multicellular structures with striped margins. *P. volans* had numerous squared cells (Fig. 1F), while *T. sibiricus* (Fig. 1A) and *E. regulus* (Fig. 1B) were multicellular with isolated structures (Table 1).

All cuticular scales of the hair shaft regions of Rodentia showed a petal-shaped pattern; *T. sibiricus*, *E. regulus*, *P. volans*, and *A. peninsulae* showed a broad diamond-shaped petal (Fig. 2A, B, F, and H); *A. agrarius*, *S. vulgaris*, *M. minutus*, and *R. norvegicus* exhibited a narrow diamond-shaped petal (Fig. 2C, D, E, and G). However, those of *L. coreanus* showed an elongated petal pattern (Fig. 2I).

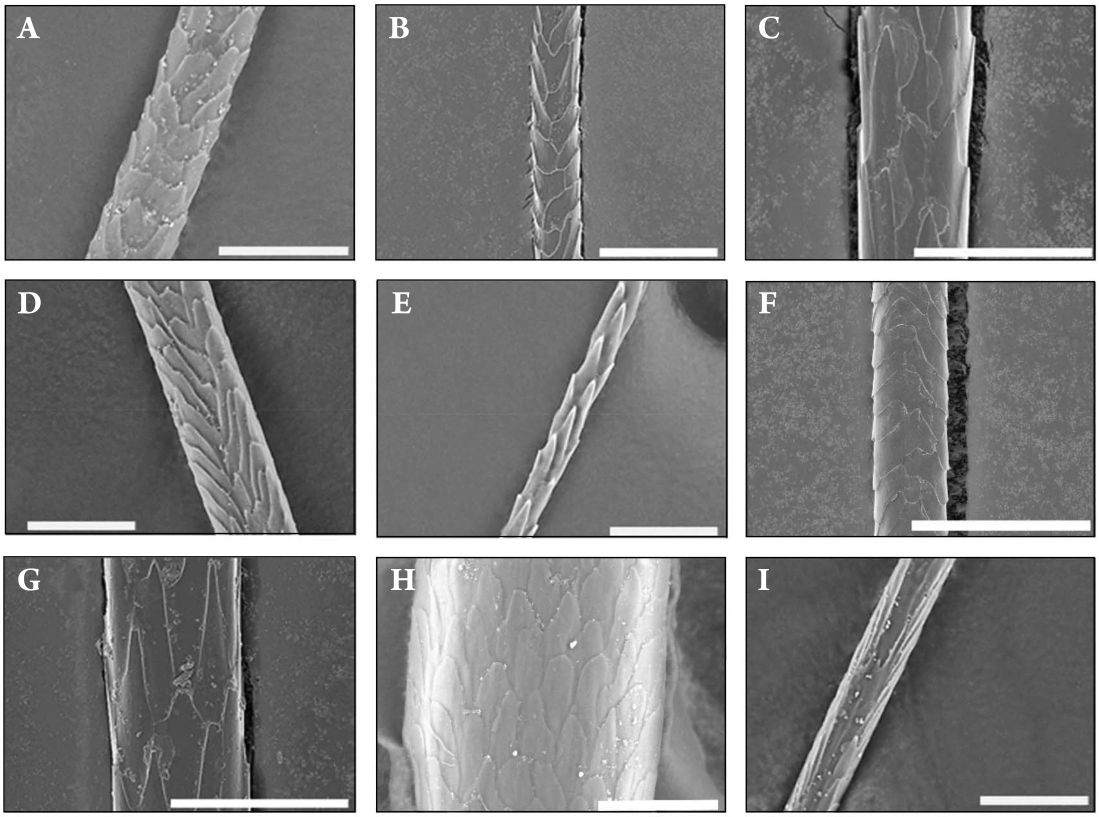

Fig. 2. Cuticular scales of the hair shaft of nine species of Rodentia and Lagomorpha. All species show diamond petal patterns in their cuticular scales. (A) *Tamias sibiricus*, (B) *Eothenomys regulus*, (C) *Apodemus agrarius*, (D) *Sciurus vulgaris*, (E) *Micromys minutus*, (F) *Pteromys volans*, (G) *Rattus norvegicus*, (H) *Apodemus peninsulae*, and (I) *Lepus coreanus*. All scale bars are 50 μm.

Regarding the cross-sections of the hair shield region, Rodentia and Lagomorpha exhibited quite unique features; *T. sibiricus* showed an oval type (Fig. 3A); *A. agrarius*, *S. vulgaris*, *M. minutus*, *R. norvegicus*, and *A. peninsulae* showed a concavo-convex type characteristic (Fig. 3C, D, E, G and H); *P. volans* exhibited a biconcave type (Fig. 3F). Interestingly, *E. regulus* and *L. coreanus* were very distinct types, quadriconcave and dumb-bell, respectively (Fig. 3B and I).

The guard hair widths and statistical boxplots of the width values are shown in Figure 4. The hair widths of Rodentia and Lagomorpha were distributed between 25 μm and 175 μm. Using the microstructure of three parts (the medulla structure, cuticular scales, and hair cross-sections), we could construct the dichotomous key for identification (Table 2). We determined that the starting identification key was *M. minutus* because it was easy to recognize the medulla of *M. minutus*, as it had unicellular structures, unlike the others.

DISCUSSION

In this study, we analyzed the medulla structures, cuticular scales, cross-section type, and hair width of nine species from the Rodentia and Lagomorpha orders of the Korean Peninsula and observed the differences among them. Then, we established the hair identification key to identify species using the morphological differences in hair among species. The hair microstructure (medulla, cuticular scales and cross-sections) that we analyzed did not show high heterogeneity among the nine species, but it allowed identification when used in combination.

Additionally, we propose a relatively easy method for species identification by combining a quantitative hair width analysis method and microstructural observation. Through various field research activities, such as collecting feces or securing stomach contents from dead bodies, researchers can often secure undigested hair samples of prey. In this case, researchers can use a general stereomicroscope

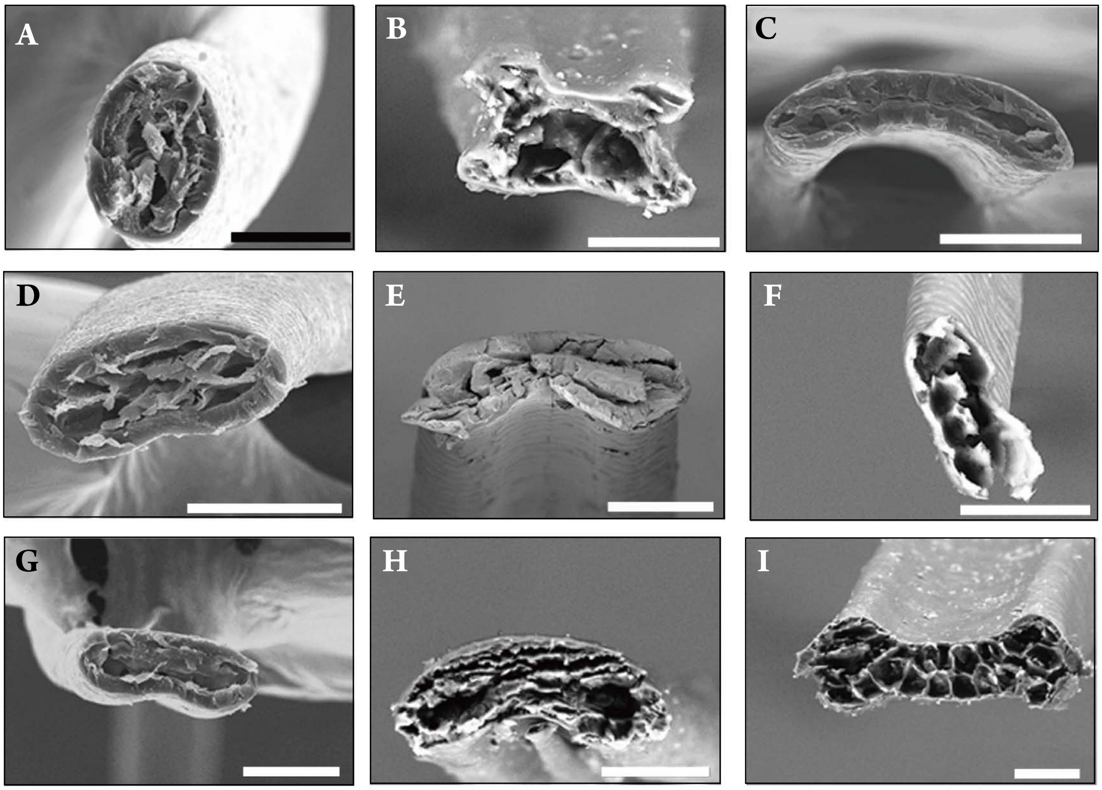

Fig. 3. Hair cross-sections of the hair shield of nine species of Rodentia and Lagomorpha. Nine species exhibit quite unique features in hair cross-sections. (A) *Tamias sibiricus*, (B) *Eothenomys regulus*, (C) *Apodemus agrarius*, (D) *Sciurus vulgaris*, (E) *Micromys minutus*, (F) *Pteromys volans*, (G) *Rattus norvegicus*, (H) Apodemus peninsulae, and (I) *Lepus coreanus*. Scale bars are 25 μm (A); 30 μm (B, E, and F); 50 μm (D, G, H, and I); and 100 μm (C).

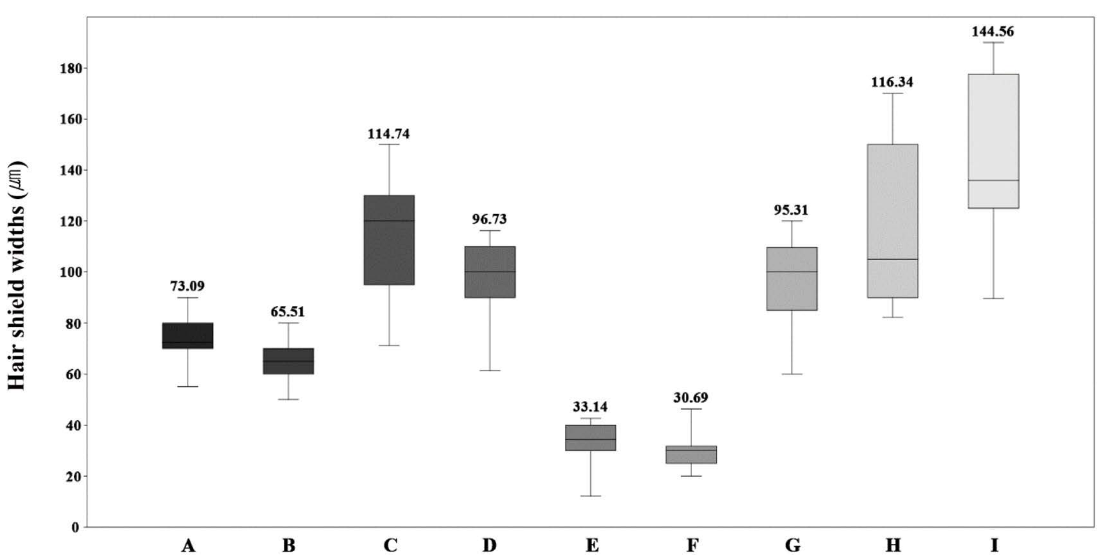

Fig. 4. Boxplot of the hair widths of nine species of Rodentia and Lagomorpha. (A) *Tamias sibiricus*, (B) *Eothenomys regulus*, (C) *Apodemus agrarius*, (D) *Sciurus vulgaris*, (E) *Micromys minutus*, (F) *Pteromys volans*, (G) *Rattus norvegicus*, (H) *Apodemus peninsulae*, and (I) *Lepus coreanus*. The mean value of each species is shown above the box plot of each species.

Table 2. Hair identification key of Rodentia and Lagomorpha

1.	Unicellular irregular in medulla	*Micromys minutus*	
	Medulla structures other than above		2
2.	Multicellular with net shaped in medulla	*Sciurus vulgaris*	
	Medulla structures other than above		3
3.	Multicellular with striped margin in medulla		4
	Multicellular in medulla		5
4.	Tiny-squared cells in medulla	*Lepus coreanus*	
	Medulla structures other than above		7
5.	Broad diamond shaped petal in cuticular scales		6
6.	Oval shape in cross-section	*Tamias sibiricus*	
	Quadricconcave shape in cross-section	*Eothenomys regulus*	
	Biconcave shape in cross-section	*Pteromys volans*	
7.	Broad diamond shaped petal in cuticular scales	*Apodemus peninsulae*	
	Narrow diamond shaped petal in cuticular scales		8
8.	Concavo-convex shape in cross-section	*Apodemus agrarius*	
	Oval shape in cross-section	*Rattus norvegicus*	

installed in the laboratory to take pictures of these samples and then use free software (for example, ImageJ software, Image Processing and Analysis in Java https://imagej,nih.gov/ij/) to obtain the width value of the hair. Additionally, this software can be used to compare the resulting values with the data values obtained from this study.

As shown in this study, *L. coreanus* showed distinctive microstructural characteristics among the species used in this research. Previous studies have shown that the guard hair of *L. coreanus* has a similar feature in outer appearance to *P. bengalensis* and *T. sibisicus* (*L. coreanus* was thin, beige and dark brown alternately, and *P. bengalensis* and *T. sibiricus* was thin, beige and black alternately; Lee *et al.* 2014). On the other hand, comprehensive results from this and previous studies show that the microstructures of the three species were quite different, and these results may provide a clear basis for identification.

Species that belong to the families Rodentia and Lagomorpha are the main prey of a variety of carnivores. The identification of the gut and feces contents of mammalian predators showed a major problem in that the prey's obvious characteristics, i.e., size, color and shape, are lost during the process of mastication and digestion (Day 1966). Since feline species, a major predator group, control the population size of prey and maintain balance (Schonewald-Cox *et al.* 1991), studies on their ecology are important, and the results of our study (hair microstructure of small mammals) are likely to be useful in such investigations (identification of prey).

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