Species Identification Key of Korean Mammal Hair

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ABSTRACT. The hair microstructures of Korean terrestrial mammals from 23 species (22 wild and one domestic) were analyzed using light and scanning electron microscopy (SEM) to construct a hair identification key. The hairs were examined using the medulla structures and cuticular scales of guard hairs from the dorsal regions of mature adult animals. All cuticular scale structures in the hair of Rodentia, Lagomorpha, Carnivora, and Insectivora showed unicellular patterns, respectively. Rodentia, Lagomorpha, and Carnivora showed the petal pattern, and those of Artiodactyla and Chiroptera showed the wave pattern and coronal pattern, respectively. Carnivora showed the petal pattern, and those of Artiodactyla and Chiroptera showed the wave pattern and coronal pattern, respectively. Rodentia, Lagomorpha, and Carnivora showed the unicellular pattern, and those of Artiodactyla and Chiroptera showed the wave pattern and coronal pattern, respectively. Carnivora did not show the medulla structures in their hair. We found that it is possible to distinguish between species and order based on general appearance, medulla structures, and cuticular scales. Thus, we constructed a hair identification key with morphological characteristics from each species. This study suggests that hair identification keys could be useful in fields, such as forensic science, food safety, and foraging ecology.

KEYWORDS: cuticular scale, hair identification key, mammalian hair microstructure, medulla structures.

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Mammal hair plays an important role in thermoregulation, body shape maintenance, waterproofing and protection from pollution. There are 2 types of mammal hair: guard hairs that are generally thick and bristly and fine hairs that are curled and thin [4, 16, 17]. A single hair can be separated into three cross-sectional regions: cuticular scale, medulla, and cortex [17].

The morphological characteristics of the cuticular scales and medulla structures have been used to distinguish among mammalian species. Since identification of mammal hair was first performed by Hausman [6], further research has been conducted on various hair characteristics. Mathiak [8] established a systematic identification key of southern Michigan mammals using light microscopy.

To identify mammal hair, most researchers have generally used light and electron microscopy. Light microscopy is the traditional tool used for identification of hair, while scanning electron microscopy (SEM) has recently become more common. The SEM has superior ability over light microscopy for hair studies [13, 18], as it is able to view details of cuticular scales. Moyo et al. [10] also used computer pattern recognition techniques with SEM microphotographs for mammal hair identification. DNA analysis of hair is also used for species identification; however, time and knowledge of DNA techniques are required for this procedure. Furthermore, it is difficult to extract DNA from hair, unless some root material is present. Thus, light microscopy and SEM are quite useful ways to identify mammal hair. Results from hair identification keys based on light microscopy and SEM observations have been applied to scientific research in fields, such as taxonomy [12], ecology [2, 3, 5, 7, 21], forensic science [11] and archeology [15, 19]. For example, mammal hair found in the field could be useful for habitat studies of endangered species, as well as the study of feeding habits using scat analysis. Moreover, a hair can be a clue to control poaching of wildlife using a hair identification key. Thus, hair identification keys will be useful to Korean mammal research, and their use represents a first step to securing Korean biological resources.

In this study, hair samples from 23 species of South Korean terrestrial mammals were studied using light microscopy and SEM. The aims of this study are 1) to classify mammal hair based on morphological characteristics and 2) to construct identification keys based on mammal hair characteristics.

MATERIALS AND METHODS

The hair samples were obtained from stuffed specimens in the National Institute of Environmental Research (NIER) and from road kill species collected country-wide by the Conservation Genome Resource Bank for Korean Wildlife (CGRB) at Seoul National University in Korea. We studied the hair structure of 23 species representing 6 orders, 13 families and 21 genera of wild (N=22: Eothenomys...
regulus, Apodemus agrarius, Apodemus peninsulae, Rattus norvegicus, Sciurus vulgaris, Pteromys volans, Tamias sibiricus, Lepus coreanus, Nyctereutes procyonoides, Mustela sibirica, Lutra lutra, Martes flavigula, Prionailurus bengalensis, Hydropotes inermis, Capreolus pygargus, Moschus moschiferus, Nemorhaedus caudatus, Sus scrofa, Crocidura lasiura, Crocidura saucovolens, Mogera robusta and Pipistrellus abramus) and domestic (N=1: Bos taurus coreanae) terrestrial mammals, all found in South Korea (Table 1). Six of 22 wild species collected are classified as endangered: Pteromys volans, Lutra lutra, Martes flavigula, Prionailurus bengalensis, Moschus moschiferus and Nemorhaedus caudatus.

All of the samples were dorsal guard hair from mature adult animals. Guard hairs are important in species identification as they exhibit diagnostically reliable features [4, 17]. Hair samples were washed in water containing a detergent rinsed sequentially in water, distilled water and stored in 70% alcohol, according to Teerink [17].

Dorsal guard hairs (five hairs from each individual) were

### Table 1. Macroscopic characters of Korean mammal hair

| Order      | Family           | Genus            | Species                  | Profile      | General appearance                          | Length (cm; mean ± SD) |
|------------|------------------|------------------|--------------------------|--------------|---------------------------------------------|------------------------|
| Rodentia   | Muridae          | Eothenomys       | 1. Eothenomys regulus (N=11) | Straight     | Thin, weak beige and dark gray tip          | 1.10 ± 0.17            |
|            |                  | Apodemus         | 2. Apodemus agrarius (N=19) | Straight     | Thin, beige and dark gray tip               | 0.88 ± 0.11            |
|            |                  |                  | 3. Apodemus peninsulae (N=5) | Straight     | Thick, dark gray and light gray tip         | 0.95 ± 0.10            |
|            |                  | Rattus           | 4. Rattus norvegicus (N=8) | Straight     | Thin and gray                              | 1.35 ± 0.19            |
|            | Sciuridae        | Sciurus          | 5. Sciurus vulgaris (N=5)  | Straight     | Thin and dark gray                         | 1.33 ± 0.18            |
|            |                  | Pteromys         | 6. Pteromys volans (N=3)   | Straight     | Thin and gray                              | 1.60 ± 0.32            |
|            |                  | Tamias           | 7. Tamias sibiricus (N=8)  | Straight     | Thin, beige and black alternately          | 1.27 ± 0.21            |
| Lagomorpha | Leporidae        | Lepus            | 8. Lepus coreanus (N=3)    | Straight     | Thin, beige and dark brown alternately     | 3.22 ± 0.68            |
|            | Canidae          | Nyctereutes      | 9. Nyctereutes procyonoides (N=10) | Undulated | Thick, light beige and black tip          | 7.64 ± 0.89            |
|            | Mustelidae       | Mustela          | 10. Mustela sibirica (N=9) | Straight     | Thin, long hair and light brown            | 2.10 ± 0.43            |
|            |                  | Lutra             | 11. Lutra lutra (N=5)      | Straight     | Thin, white and dark gray                  | 2.32 ± 0.32            |
|            | Carnivora        | Martes           | 12. Martes flavigula (N=5) | Straight     | Thin, weak beige and dark gray or black tip | 3.02 ± 0.51            |
|            | Felidae          | Prionailurus     | 13. Prionailurus bengalensis (N=9) | Straight     | Thin, beige and black alternately          | 3.97 ± 0.45            |
|            | Cervidae         | Hydropotes       | 14. Hydropotes inermis (N=9) | Wavy         | Thick, white and dark beige                | 4.76 ± 1.56            |
|            |                  | Capreolus        | 15. Capreolus pygargus (N=9) | Wavy         | Thick, white and dark beige                | 4.0 ± 0.33             |
|            | Moschidae        | Moschus          | 16. Moschus moschiferus (N=4) | Wavy         | Thick, beige and dark gray tip             | 5.42 ± 0.56            |
|            | Bovidae          | Nemorhaedus       | 17. Nemorhaedus caudatus (N=5) | Undulated   | Thin, light gray and black tip             | 8.58 ± 1.07            |
|            |                  | Bos              | 18. Bos taurus coreanae (N=7) | Straight     | Thin, shiny and brown                      | 3.79 ± 0.52            |
|            | Suidae           | Sus              | 19. Sus scrofa (N=10)      | Straight     | Thick, bristly, dark brown and split tip   | 7.01 ± 2.55            |
|            | Insectivora      | Soricidae        | 20. Crocidura lasiura (N=3) | Zigzagged    | Thin, dark gray and weak orange tip        | 0.62 ± 0.11            |
|            |                  | Tadpoleida       | 21. Crocidura shantungensis (N=7) | Zigzagged  | Thin and dark gray                         | 0.38 ± 0.08            |
|            | Chiroptera       | Pipistrellus     | 22. Mogera robusta (N=5)   | Zigzagged    | Thin, long hair and dark gray              | 0.87 ± 0.10            |
|            | Vespertilionidae | Pipistrellus abramus (N=4) | Undulated   | Thin and dark brown                        | 0.55 ± 0.07            |
mounted on glass slides with double-sided sticky tape and measured at the hair shaft and shield using a light microscope (CX31, OLYMPUS, Tokyo, Japan) equipped with 10× and 4× lenses for medulla structures. To analyze the cuticular scales (hair shaft region), which can have many variations [14, 17], we used the SEM (TM-1000, HITACH, Tokyo, Japan).

Medulla structures and cuticular scales were classified according to terminology in Teerink [17]. Net-shape, zipper-shape, columnar and mesh are new terms that we present to describe the morphological characteristics of the hair medulla structures in this study.

RESULTS

The macroscopic view for hair profiles: The macroscopic observation of the guard hair is the first step for hair identification [4]. Most hair profiles were straight and undulated types. The hair of the *Sus scrofa* can be easily identified by general appearance and was quite thick and split ended (Table 1). The general appearance of Cervidae and Moschidae hair was uniquely wavy, and that of Insectivora was zigzagged (Table 1). The hair of Cervidae, Moschidae and Insectivora was classified to family and order levels by general appearance; however, we were not able to identify the hair to species level. Due to the dull hair color of 23 samples, it was difficult to identify by color. Thus, microscopic inspection was needed for hair identification.

The microscopic view for cuticular scales: In the cuticular scale structure, the morphological characteristics of the hair shaft can be used in the differentiation of species [14, 17]. Thus, we examined the cuticular scale structures in the shaft region of the hair samples in this study. Hair cuticular scales are summarized in Table 2.

| Order    | Family      | Genus         | Species                  | Shaft                        |
|----------|-------------|---------------|--------------------------|------------------------------|
| Rodentia | Muridae     | Eothenomys    | *Eothenomys regulus*     | Broad diamond-shaped petal   |
|          |             | Apodemus      | *Apodemus agrarius*      | Narrow diamond-shaped petal  |
|          |             | *Apodemus peninsulæ* |              | Broad diamond-shaped petal   |
|          | Sciuridae   | Rattus        | *Rattus norvegicus*      | Narrow diamond-shaped petal  |
|          |              | Sciurus       | *Sciurus vulgaris*       | Narrow diamond-shaped petal  |
|          |              | Pteromys      | *Pteromys volans*        | Broad diamond-shaped petal   |
|          |              | Tamias        | *Tamias sibiricus*       | Broad diamond-shaped petal   |
| Lagomorpha | Leporidae    | Lepus         | *Lepus coreanus*         | Elongated petal              |
| Carnivora | Canidae     | Nyctereutes   | *Nyctereutes procyonoides* | Broad diamond-shaped petal   |
|          | Mustelidae  | Mustela       | *Mustela sibirica*       | Narrow diamond-shaped petal  |
|          |              | Lutra         | *Lutra lutra*            | Narrow diamond-shaped petal  |
|          |              | Martes        | *Martes flavigula*       | Broad diamond-shaped petal   |
| Felidae  | Prionailurus |              |                          | Narrow diamond-shaped petal  |
| Artiodactyla | Cervidae    | Hydropotes    | *Hydropotes inermis*     | Regular wave                 |
|          |              | Capreolus     | *Capreolus pygargus*     | Regular wave (Pineal wave)   |
|          | Moschidae   | Moschus       | *Moschus moschiferus*    | Regular wave (Frilled wave)  |
|          | Bovidae     | Nemorhaedus   | *Nemorhaedus caudatus*   | Regular wave                 |
|          |              | Bos           | *Bos taurus coreanae*   | Regular wave                 |
| Suidae   | Sus          | *Sus scrofa*  |                          | Irregular wave               |
| Insectivora | Soricidae  | Crocidura     | *Crocidura lastiura*     | Narrow diamond-shaped petal  |
|          |              | Crocidura sauveolens |              | Narrow diamond-shaped petal  |
| Talpidae | Mogera       | *Mogera robusta* |              | Narrow diamond-shaped petal  |
| Chiroptera | Vespertilionidae | Pipistrellus | *Pipistrellus abramus*  | Simple coronal               |

Table 2. Cuticular scale structures of Korean mammal hair
wave pattern, while *Sus scrofa* showed an irregular wave pattern (Fig. 3). The external morphology of the hair of three deer species (*Hydropotes inermis*, *Capreolus pygargus* and *Moschus moschiferus*) in the family Artiodactyla was quite similar according to the naked eye, showing a regular wave in the hair cuticular scale structure. *Capreolus pygargus* showed a regular wave with a pineal shape, while *Moschus moschiferus* showed a frilled regular wave (Fig. 3A, 3B and 3C). To observe the cuticular scale structures of three species of Insectivora, we examined the node region. *Crocidura lasiura*, *Crocidura shantungensis* and *Mogera robusta* showed a narrow diamond-shaped petal. (Fig. 4A, 4B and 4C). *Pipistrellus abramus* showed cuticular scales that were simple coronal (Fig. 4D). The simple coronal pattern was represented throughout the hair in this species.

The microscopic view for medulla structures: The medulla structures were observed in the shield and shaft regions of the hairs. However, four orders have no characteristic medulla structure (Carnivora, Artiodactyla, Insectivora and Chiroptera) in their hair shaft; thus, hair shaft regions are included as figures (Figs. 7, 8 and 9). Hair medulla structures are summarized in Table 3.

Rodentia and Lagomorpha showed a multicellular in rows structure in the hair shield region. Additionally, *Eothenomys regulus*, *Apodemus agrarius*, *Rattus norvegicus*, *Sciurus vulgaris*, *Pteromys volans*, *Tamias sibiricus* and *Lepus coreanus* showed a multicellular in rows structure in the hair shield region. *Apodemus peninsulae* showed simple composition of numerous cells in the hair shield region (Fig. 5). However, the medulla structure was separated into three types in the hair shaft region. *Eothenomys regulus* showed a ladder-like unicellular structure, while *Apodemus agrarius* and *Rattus norvegicus* showed a multicellular in rows structure. *Apodemus peninsulae* also showed multicellular structures, and *Sciurus vulgaris*, *Pteromys volans*, *Tamias sibiricus* and *Lepus coreanus* showed a zipper-shaped multicellular structure (Fig. 6). A net-shaped multicellular structure with scallops was observed in the hair shields of all Carnivora species, with the exception of *Nyctereutes procyonoides*. In the net-shaped medulla structure, that of *Mustela sibirica* was a cellular imbricated structure (Fig. 7B). *Lutra lutra* and *Prionailurus bengalensis* showed a thick cortex layer.
and vacuoles, respectively (Fig. 7C and 7E). The hair shaft of *Mustela sibirica*, *Martes flavigula*, *Lutra lutra* and *Prionailurus bengalensis* showed an irregular unicellular structure, while *Nyctereutes procyonoides* showed a net-shaped multicellular structure throughout the hair. Artiodactyla showed the same medulla structure from the shaft to the shield, contrary to other orders, which showed different structures in the shaft and shield. *Hydropotes inermis*, *Capreolus pygargus* and *Moschus moschiferus* showed a mesh structure in the medulla (Fig. 8A, 8B and 8C); *Nemorhaedus caudatus* showed a net-shaped multicellular structure with a cortex layer (Fig. 8D); *Sus scrofa* and *Bos taurus coreanae* showed a columnar structure (Fig. 8E and 8F). *Crocidura lasiura* and *Crocidura shantungensis* showed a ladder-like unicellular regular structure in the shield and shaft regions (Fig. 9A and 9B). However, *Mogera robusta* showed a unicellular irregular structure in the shield (Fig. 9C). *Pipistrellus*...
**DISCUSSION**

In this study, we observed the difference in cuticular scale structures and medulla structures in the hair samples of 23 species. Although the morphologies of hairs were similar among Korean terrestrial mammals, cuticular scales and medulla structures are sufficient to distinguish between species and orders and can be used to construct an identification key.

In the examined hair samples, the medulla hair shaft structure was generally unicellular [17]. However, the medullas of Rodentia and Lagomorpha showed a multicellular structure in the hair shaft and shield, while the hair shaft of Carnivora showed a net-shaped multicellular structure with scallops. These differences are the basis for distinguishing among these orders. Moreover, due to fact that the tiny square-shaped structure of Leporidae is also found in other species in Lagomorpha [1], we argue that it is characteristic of Lagomorpha. The identifying characteristic of Artiodactyla is that the medulla structure is consistent throughout the entire hair [4]. Interestingly, the medulla structure of *Nemorhaedus caudatus* is similar to that of Carnivora. The hair of *Nemorhaedus caudatus* has been confused with that of *Nyctereutes procyonoides* due to the similar external morphology of the hair. To distinguish between these two species, the cuticular scales must be analyzed.

Because the medulla structures of all three species in Insectivora showed the same unicellular structure, it is difficult to distinguish among *Crocidura lasiura*, *Crocidura shantungensis* and *Mogera robusta*. Thus, this key is not appropriate for distinguishing among these three species; however, hair length and hair color can be used to easily distinguish among these species (Table 4). *Crocidura lasiura*, *Crocidura shantungensis* and *Mogera robusta* exhibit nodes and inter-nodes in the hairs, which are morphological characteristics of these species [14]. Mammals’ fine hairs generally have nodes and inter-nodes [8], and it appears that their body hairs are fine as an adaption for digging. *Crocidura lasiura* and *Crocidura shantungensis* had the same medulla and cuticular structures.
as North American Soricidae and Sri Lankan shrews. Therefore, we argue that these traits are characteristic of Soricidae [9, 14].

In Artiodactyla, the macroscopic characteristics and medulla structures of *Hydropotes inermis*, *Capreolus pygargus* and *Moschus moschiferus* are the same. In this case, the medulla structure cannot be used as an identifying feature among these three species. However, we found that the cuticular scales were different among the three species (Fig. 3A, 3B and 3C). These different cuticular scales can be used as an identification key to distinguish among these species (Table 4).

Upon inspection, the whole hair type of Rodentia, Lagomorpha and Carnivora is characterized by a thick shield

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### Table 4. Hair identification key of Korean mammals

| No. | Description                                                                 | Species                                    |
|-----|----------------------------------------------------------------------------|--------------------------------------------|
| 1.  | Thick, bristly, and split tip of hairs.                                    | *Sus scrofa*                               |
| 2.  | Cuticular scale pattern simple coronal throughout the hairs (Fig. 4D).     | *Pipistrellus abramus*                     |
| 3.  | Cuticular scale pattern regular wave throughout the hairs (Fig. 3A, 3B, 3C, 3D and 3F). | *Hydropotes inermis*                      |
|     | Cuticular scale pattern petal shaped at the shaft on the hairs (Figs. 1, 2 and 4). | *Capreolus pygargus*                      |
| 4.  | Cuticular scale pattern wave throughout the hairs (Fig. 3A).               | *Moschus moschiferus*                      |
|     | Medulla structure net-shaped in the shaft of the hairs (Fig. 1E).           | *Lepus coreanus*                           |
|     | Hairs other than above.                                                     |                                            |
| 5.  | Medulla structure net-shaped multicellular (Figs. 3D and 8D).               | *Nemorhaedus caudatus*                     |
|     | Medulla structure columnar (Figs. 3F and 8F).                               | *Bos taurus coreanae*                     |
|     | Cuticular scale pattern net-shaped multicellular-in-rows in the shield and ladder-like in the shaft of the hairs (Figs. 1A, 1B, 1C, 1F, 2B, 2C, 2E, 4A, 4B and 4C). | *Moschus moschiferus*                     |
|     | Cuticular scale pattern wave throughout the hairs (Fig. 3A).               | *Lepus coreanus*                           |
|     | Medulla structure net-shaped and medulla margins with scalloped only in the shield of the hairs (Figs. 2B, 2C, 2E, 7B, 7C and 7E). | *Eothenomys regulus*                      |
|     | Hairs other than above.                                                     |                                            |
| 6.  | Medulla structure columnar (Figs. 3F and 8F).                               | *Nyctereutes procyonoides*                 |
|     | Cuticular scale pattern net-shaped throughout of the hairs (Figs. 2A and 7A). | *Eothenomys regulus*                      |
| 7.  | Medulla structure net-shaped and scale margins scalloped in the shield of the hairs (Figs. 2D and 7D). | *Martes flavigula*                        |
|     | Medulla structure net-shaped multicellular-in-rows throughout of the hairs (Figs. 1D, 1E, 2D, 5G). | *Apodemus agrarius*                       |
|     | Medulla structure multicellular-in-rows in the shield and zipper-shaped in the shaft of the hairs (Figs. 1G, 1H, 2E, 6G, 6H). | *Apodemus agrarius*                       |
|     | Hairs other than above.                                                     |                                            |
| 8.  | Zigzagged, thin and with dark gray and weak orange tip of the hair; mean length 6.2 mm (Figs. 4A and 9A). | *Crocidura lasiura*                       |
|     | Zigzagged, thin and with dark gray and weak orange tip of the hair; mean length 3.8 mm (Figs. 4B and 9B). | *Crocidura shantungensis*                 |
|     | Medulla structure unicellular irregular in the shield and zigzagged, thin and dark gray hair; mean length 8.6 mm (Figs. 4C and 9C). | *Mogera robusta*                          |
| 9.  | Thin and straight hair with beige and dark gray tip (Figs. 1B and 5C).      | *Sciurus vulgaris*                         |
| 10. | Thin and straight hair with gray (Figs. 1C and 5E).                        | *Rattus norvegicus*                       |
| 11. | Thin and straight hair with light brown (Figs. 2B and 7B).                  | *Mustela sibirica*                        |
| 12. | Thin and straight hair with beige and black alternately (Figs. 2E and 7E).  | *Lutra lutra*                              |
|    | Thin and straight hair with gray (Figs. 1G, 6E and 6F).                    | *Pteromys volans*                         |
|    | Thin and straight hair with a beige and black alternately (Figs. 1H, 6G and 6H). | *Tamias sibiricus*                        |
The whole hairs of Artiodactyla, Insectivora and Chiroptera were line type, node type [14] and coronal type, respectively (Fig. 10). Although it is not known whether hair type is related to diet type, we were able to use cuticular scales to determine feeding ecology at the order level. The cuticular scales of carnivores and omnivores were an irregular wave, petal shaped pattern and a coronal shaped pattern. Herbivores exhibited a regular wave pattern. We simply suggest that the petal shaped pattern can only be used to differentiate between carnivores, omnivores and herbivores.

The hair of *Sus scrofa* can be easily classified by the naked eye and is quite thick, bristly and split at the top (Table 1). Because the hair of *Sus scrofa* can be easily distinguished from that of other animals, our identification key chart was started with the hair of *Sus scrofa* (Table 4), as shown in De Marinis and Aspera [4]. Because the hair of some animal cannot be classified using cuticular scales and medulla structures, the hair of those animals should be classified by macroscopic view for hair profile and hair general appearance.

Many studies have constructed identification keys of mammal hair, but the present study is first conducted in Korea. The identification key presented in this study provides a foundation for ecological field research, forensic science and the food safety in Korea. Furthermore, we suggest adding cross-sections of hair, body regions, ages and hair width to create a more complete identification key for Korean mammal hair.

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Fig. 9. Light micrographs of medulla structures from Insectivora hair shafts and shields. (A) Crocidura lasiura, (B) Crocidura shantungensis, (C) Mogera robusta, (D) Pipistrellus abramus. All scale bars indicate 150 μm.

Fig. 10. The drawings show characteristics of the whole hair morphology. (A) Arviodaectyla, (B) Rodentia, Lagomorpha and Carnivora, (C) Insectivora, (D) Chiroptera. a: node, b: inter-node. Size and diameter of hairs are disregarded.

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