What determines the success of the species identification? The identification of 10 deer (Cervidae) species in China based on multiple parameters of hair morphology

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What determines the success of the species identification? The identification of 10 deer (Cervidae) species in China based on multiple parameters of hair morphology

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Hair morphological structure is widely utilized for species identification based on the differentiation of scales and medullar pattern of mammal hairs. To determine what may influence the accuracy of identification using hair morphology, we measured and calculated 11 parameters of hair morphometry of 10 deer species in China. Our results suggested that the morphological parameters of deer hairs have extensive overlap within Cervidae species and we obtained a correct discriminant rate of 90.1% for 10 deer species. For the five sympatric deer species in the northeastern forests of China, 94.2% of hairs can be identified correctly, with a correct discriminant rate of 89.7% and 83.9% when the hair tip or root was absent, respectively. When both hair tip and root were absent, we obtained a correct discriminant rate of 73.6%. In addition, we obtained a correct discriminant rate of 97.9% for five sympatric deer species using a blind test approach to remove observer bias. Hair morphological characteristics are similar within the family or genus because of their close genetic relationships. Furthermore, species with similar living habitat conditions may have similar hair morphological structure. These factors influence discriminant capacity, and we evidently cannot identify them more accurately when using only one morphological parameter of hair. While understanding the above, our quantitative multi-parameter morphometric analyses successfully identified the hairs of deer, and likely have significant applications concerning further mammal species.

Keywords: Cervidae, hair, identification, morphometry

Microscopic examination of prey hair microstructures retained in scat or feces has been the primary method for analyzing the diet of carnivores (Hausman 1924, Day 1966, Odden et al. 2010). Because of its powerful identification capability, it is also an effective method for exploring resource utilization and studying the interspecific relationships among sympatric predators (Gómez-Ortiz and Monroy-Vilchis 2013, Gómez-Ortiz et al. 2015). Microscopic examination was the only tool available until the emergence of modern identification techniques based on molecular biology in the 1980s. However, DNA technology has been limited in its use for the analysis of carnivore diets because it has strict requirements for sample quality (Gu et al. 2018). Often, samples of scat were collected in the field a few days after they were excreted by animals, and the scat was degraded by microorganisms shortly thereafter. As a result, PCR amplification of the samples could not be conducted, leading to a failure to identify the species (Gosselin et al. 2017, Xiong et al. 2017). Therefore, diet analysis techniques based on hair morphology are still effective methods for species identification and have been widely utilized (De Marinis and Asprea 2006, Chetri et al. 2017, Preez et al. 2017).

Cervidae are a primarily prey group for large carnivores, and we have often found their hairs in the scat of carnivores, which we identified based on their unique morphological features. Deer pelage is mainly composed of guard hairs and has consistent hair profiles and microstructures (Jin et al. 2005). The medulla is formed of four to six angular cells arranged irregularly to fill the entire hair. The cortex is paper thin and seems to be absent (Teerink 1991). The cuticular scales overlap, giving them a diamond shape, and the adjacent scales have straight margins (mosaic type) (Sheng et al. 1993), but the cuticolar scale pattern changes along the hair and associates with species (Sun et al. 2003, Hou et al. 2008).

The hair micromorphological characteristics of different species are not significantly differentiated, and may even

overlap; therefore, species are hard to classify if they have a close genetic relationship (i.e. within a family or genus), which may lead to ambiguous or incorrect identifications. For example, there are five sympatric deer species in the northeastern forests of China whose hairs are frequently found in the scat of the Amur tiger *Panthera tigris altaica* and leopard *Panthera pardus orientalis* (Kerley et al. 2015, Gu et al. 2018). It is difficult to clearly identify Cervidae species because of the similarity in their hair profiles and medullar and cuticular scale patterns. However, multiple parameters of cuticular patterns have been applied to subgroup differentiation in the Cervidae (Meyer et al. 2001). Sato et al. (2006) used multiple regression analysis to identify the hairs of dogs and cats based on the numerical characterization of the morphology of hairs. They obtained high ratios of correct discrimination even though the hairs were potentially damaged.

In this study, we hypothesized that deer species have similar numerical features of hair (length, width, medulla and cuticle) and that the five sympatric deer in northeastern China have a close genetic relationship (within a family or genus), which may lead to ambiguous or incorrect identification. Furthermore, we hypothesized that individuals of the five species, which live in similar environmental conditions, have similar hair morphologies. We will verify our hypothesis by measuring and comparing their multiple parameters of hair morphology. Finally, we analyzed the features that lead to mistakes in species identification using hair morphological structures.

Material and methods

Hair samples

In this study, samples collected from the Fur Specimen Museum of Northeast Forestry University. We selected Cervidae guard hairs,and all from individuals adults. Winter guard hairs were collected from 10 deer species within 2 subfamilies (Cervinae and Odocoileinae) in China: red deer, *Cervus elaphus* , n = 8; white-lipped deer, *Przewalskium albirostris*, n = 5; sika deer, *Cervus nippon* , n = 5; Mi-deer, *Elaphurus davidianus*, n = 16; sambar, *Rusa unicolor*, n = 4; Eld's deer *Cervus eldii*, n = 5; hog deer, *Axis porcinus*, n = 2; Siberian roe deer, *Capreolus pygargus*, n = 7; moose, *Alces alces*, n = 5; and reindeer, *Rangifer tarandus*, n = 3.

Eld's deer, sambar and hog deer are only distributed in tropical and subtropical regions, sika deer are widespread, and *Cervus nippon hortulorum* is distributed in the northeastern forest region discussed in this study. The white-lipped deer is a unique Cervidae that is only distributed on the Tibetan Plateau above an elevation of 3000–5000 m. The Mi-deer is a reintroduced species that has been distributed in the Central region of China since the 1990s. Sika deer *C. nippon hortulorum*, red deer, Siberian roe deer, moose and reindeer are sympatric species, occurring in the temperate and subfrigid climatic zones, and they are widely distributed in the northeastern forests of China. They are prey items for big cats and other medium-large carnivores, such as wolves *Canis lupus* and wolverines *Gulo gulo*, in that area.

Based on our experiences in the field, large carnivores ignore the distal end of prey (e.g. the head and limbs) (Gu et al. 2018); therefore, hair from the trunk was used in the present study. Twenty hairs were randomly taken from anatomical regions, such as the dorsum, abdomen or hip.

Numerical morphology

The hairs were washed in a supersonic cleaning machine, and the cuticular and medullar slides were prepared according to the methods of Teerink (1991). Eleven hair parameters were measured using an optical microscope and TCapture 5.1 software (Motic Co., Ltd, China) and are illustrated in Fig. 1, 2. The hair parameters were determined as follows:

(1) The length (CHs, mm) was measured with a ruler from the tip to the root of the hair.
(2) The maximum width (MaxWid, μm) and maximum medulla width (Max MeWid, μm) were measured at the point of the maximum width along the hair shaft using a microscope equipped with a micrometer.
(3) The non-medulla length at the distal end of the hair (disNon-MedLen, μm) and non-medulla length at the proximal end of the hair (proNon-MedLen, μm) were measured at the tip and root of the hair, respectively, using a microscope equipped with a micrometer.

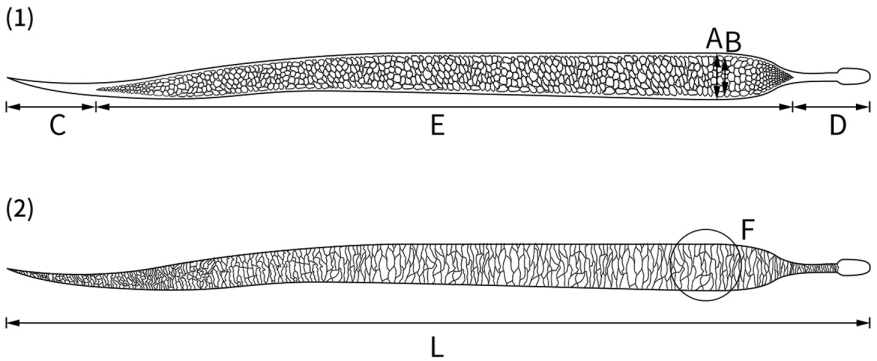

Figure 1. Numerical morphology of hair. A: maximum width, MaxWid (μm); B: maximum medulla width, Max MeWid (μm); C: non-medulla length at distal hair, disNon-MedLen (μm); D: non-medulla length at proximal hair, proNon-MedLen (μm); E: the length of medulla; F: the scale counts per 200 μm length, scCnt; L: the length, CHs (mm); MI: medulla index = B/A × 100; Non-MI: non-medulla index = (C + D)/L × 100.

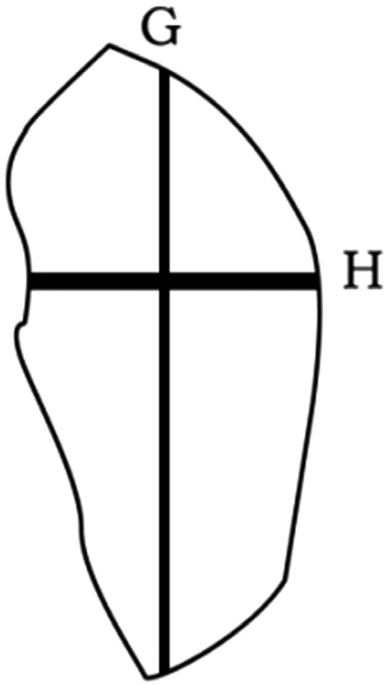

Figure 2. Numerical morphology of scale G: the scale width, scWid (μm); H: the scale height, scHei (μm); Y-/X: Y-/X- Feret = H/G.

(4) The scale counts (scCnt) were the number of scale free edges along the maximum width of the hair shaft per unit length (200 μm).
(5) The scale width (scWid, μm) and scale height (scHei, μm) were measured along the maximum width of the hair shaft using a microscope equipped with a micrometer, and 10 scales per hair were measured.

Moreover, three indexes were calculated using the formula shown below: the medulla index (MI), non-medulla index (Non-MI) and Y-/X-Feret (Y-/X).

$$\text{Medulla index (MI)} = \frac{\text{The maxmum medulla width along the hair shaft}}{\text{The maxmum width along the hair shaft}} \times 100\% \quad (6)$$

$$\text{Non-medulla index (Non-MI)} = \frac{\text{The non-medulla total length at distal and proximal end of the hair}}{\text{The length of the hair}} \times 100\% \quad (7)$$

$$\text{Y}-/\text{X}-\text{Feret (Y}-/\text{X)} = \frac{\text{The scale height}}{\text{The scale width}} \quad (8)$$

Statistical analysis

Statistical analysis of the numerical morphology of the hair

Using species as the dependent variable and numerical morphology as the independent variable, a database was established using SPSS Statistics 17.0 software (Sato et al. 2006). A one-way ANOVA was conducted on the mean values of the numerical indexes of hair morphology. Differences between the species were determined using subsequent post hoc tests.

Discriminant analysis

We carried out bivariate correlation analysis on the independent variables in the database. After the bivariate correlation analysis, variables were selected according to the analysis results. The discriminant analysis function were determined by stepwise method using the selected variables in SPSS Statistics 17.0 software (Aljandali 2017).

Blind test of assignment correction

Based on the discriminant functions, we blind tested 20% of the deer hairs of the five sympatric deer species in the northeastern forests of China, all from the same sample used for the database (Park et al. 2019). The membership probability of the blind test sample was obtained.

Cluster analysis

We used hierarchical cluster analysis to identify clusters of the 10 deer species based on 11 hair parameters using the Ward minimum variance method (Ward 1963). This method uses Euclidian distances as measures of similarity and produces clusters according to a criterion that minimizes variance within clusters. We plotted the taxonomic clusters of the 10 deer species by referring to Smith and Xie (2010) and used hierarchical cluster analysis to identify clusters of the 10 deer species based on 11 hair parameters. Then, we compared the overlap between the two clusters and analyzed the relationship between them.

Results

Quantitative overlapping characteristics of guard hairs from 10 deer species

Table 1 shows that CHs in the Cervidae ranged between 18 and 139 mm (Table 1). *Rusa unicolor* had the shortest CHs, and *Alces alces* had the longest CHs.There were significant differences in their CHs (ANOVA, $F = 258.416$, $p < 0.001$,

Table 1. Hair length (CHs) of 10 deer species in the Cervidae.

Species	Mean (mm) ± SD	Min.–Max. (mm)	CV
Rusa unicolor	18.43 ± 4.40	12.00–28.00	0.24
Cervus eldii	30.35 ± 3.49	24.00–40.00	0.11
Axis porcinus	35.70 ± 4.13	28.00–40.00	0.12
Elaphurus davidianus	51.07 ± 8.70	31.00–70.50	0.17
Przewalskium albirostris	62.25 ± 10.27	42.50–89.00	0.17
*Rangifer tarandus**	31.92 ± 6.12	24.50–43.00	0.19
*Capreolus pygargus**	42.95 ± 3.84	25.00–49.20	0.09
*Cervus elaphus**	43.98 ± 8.42	27.50–64.30	0.19
*Cervus nippon**	49.67 ± 14.90	20.50–68.70	0.30
*Alces alces**	139.05 ± 15.22	105.00–164.00	0.11

* Sympatric Cervidae on northeast forest of China.

n = 63). Based on a multiple comparison, the CHs of the five sympatric species (*Rangifer tarandus*, *Capreolus pygargus*, *Cervus elaphus*, *Cervus nippon* and *Alces alces*) differed significantly (ANOVA, $F = 390.337$, $p < 0.001$, $n = 28$) for every species, except for those of *Capreolus pygargus* and *Cervus elaphus*.

Table 2 shows that the MaxWid of the Cervidae hairs ranged between 154 mm and 794 mm. The MaxWid and Max MeWid differed significantly ($p < 0.001$, $n = 63$). The MI of the Cervidae hairs ranged from 92% to 98%, except for that of *Rusa unicolor*, *Cervus eldii* and *Axis porcinus*, in which MI was below 90%, and the three deer species had the thinnest MaxWid and Max MeWid. No significant differences were observed in the MI between the species.

Compared the MaxWid and Max MeWid of the five sympatric deer species, the result showed that there were significant differences for each of the five sympatric deer species ($p < 0.001$, $n = 28$). But no significant differences were observed in the MI between the sympatric Cervidae species.

Table 3 shows that the disNon-MedLen in the Cervidae ranged between 66 μm and 2105 μm, and the proNon-MedLen ranged from 313 μm to 1295 μm. They both had a wide range. These values differed significantly for every species ($p < 0.001$, $n = 63$). The Non-MI in the Cervidae ranged between 1.14% and 10.77%; the species living in tropic and subtropic regions (*Rusa unicolor*, *Elaphurus davidianus*, *Cervus eldii* and *Rusa unicolor*) had Non-MI values greater than 5%. *Rusa unicolor* had the highest Non-MI value. In contrast, the species living in the moderate and subfrigid regions had Non-MI values less than 4%; *Alces alces* had a Non-MI value of 1.14%, which was the lowest of all the species.

Based on a multiple comparison test, the disNon-MedLen differed significantly (ANOVA, $F = 51.214$, $p < 0.001$, $n = 28$) for all of the five sympatric deer species except for *Cervus nippon* and *Rangifer tarandus*. No significant differences were observed in proNon-MedLen between the sympatric Cervidae species. And the non-MI of the five sympatric deer species differed significantly (ANOVA, $F = 76.816$, $p < 0.001$, $n = 28$) for every species except for *Cervus elaphus* and *Rangifer tarandus*.

Table 4 shows that the scWid of the Cervidae cuticular scales ranged between 35 μm and 67 μm. There were significant differences in the scWid between species (ANOVA, $F = 115.780$, $p < 0.001$, $n = 63$). The scHei of the cuticular scales ranged from 8 μm to 27 μm. *Rusa unicolor* had the narrowest scHei and *Cervus elaphus* had the widest scHei (ANOVA, $F = 46.093$, $p < 0.001$, $n = 63$). Y-/X was the ratio of scale height to width. *Cervus nippon*, *Cervus eldii* and *Axis porcinus* had the minimum Y-/X values; however, *Cervus eldii* and *Axis porcinus* also had the maximum scale counts, with more than 16 scales per 200 μm length at the maximum width of the hair shaft.

The scHid of the five sympatric deer species differed significantly (ANOVA, $F = 98.989$, $p < 0.001$, $n = 28$) for every species except for *Cervus elaphus* and *Alces alces*, *Rangifer tarandus* respectively; *Cervus elaphus* and *Rangifer tarandus* based on multiple comparison. No significant differences were observed in the scWid between the sympatric Cervidae species. The Y-/X values of the five sympatric deer species differed significantly (ANOVA, $F = 49.400$, $p < 0.001$, $n = 28$) for every species except for *Cervus elaphus* and *Alces alces*, *Rangifer tarandus* respectively; *Cervus elaphus* and *Rangifer tarandus* based on multiple comparison. No significant differences were observed in the scCnt between the sympatric Cervidae species.

Discriminant analysis results of the hairs from 10 deer species

Eight numerical features were selected based on the correlation coefficient and t-test conducted before the discriminant analysis: CHs, MaxWid, Max MeWid, disNon-MedLen, proNon-MedLen, scCnt, scWid and scHei.

Table 5 indicates that, with the exception of *Rusa unicolor* and *Alces alces*, there were a few incorrectly discriminated in all other species. A discriminant function established using eight numerical features, the accuracy of the correct discrimination was 90.1% for the 10 deer (Cervidae) species in China.

Discriminant analysis results of five sympatric Cervidae in the northeastern forests of China

Cervidae are the most important prey group for big cats and other carnivores in the northeastern forests of China. Table 6 indicates that there were a few incorrectly discriminated Cervidae samples, with the exception of samples *Capreolus pygargus* and *Alces alces*. Using eight numerical features, we established a discriminant function, and the accuracy of the discrimination was 94.2% for the five sympatric Cervidae in the northeastern forest of China.

We supposed that the guard deer hair found in the scat of carnivores were incomplete fragments. We obtained the

Table 2. Hair medulla parameters of 10 deer species in the Cervidae.

Species	MaxWid (μm)	Max MeWid (μm)	MI (%)
Rusa unicolor	221.75 ± 13.89	171.25 ± 12.13	77.81 ± 5.82
Cervus eldii	154.00 ± 20.88	120.75 ± 15.67	78.79 ± 7.92
Axis porcinus	179.50 ± 13.01	152.50 ± 14.77	84.86 ± 3.53
Elaphurus davidianus	451.75 ± 65.93	436.61 ± 63.45	96.51 ± 1.34
Przewalskium albirostris	794.57 ± 124.33	766.58 ± 114.66	96.58 ± 1.78
*Rangifer tarandus**	370.19 ± 49.31	364.04 ± 48.91	98.32 ± 0.56
*Capreolus pygargus**	374.65 ± 37.47	360.65 ± 37.35	96.21 ± 1.98
*Cervus elaphus**	344.66 ± 59.81	337.75 ± 50.67	96.89 ± 1.42
*Cervus nippon**	276.62 ± 63.77	266.72 ± 63.08	96.30 ± 2.11
*Alces alces**	598.57 ± 116.85	552.38 ± 102.95	92.59 ± 4.80

* Sympatric Cervidae on northeast forest of China.

Table 3. Hair non-medulla parameters of 10 deer species in the Cervidae.

Species	disNon-MedLen (μm)	proNon-MedLen (μm)	Non-MI (%)
Rusa unicolor	1510.00 ± 576.73	313.98 ± 172.27	10.77 ± 5.05
Cervus eldii	808.35 ± 252.38	722.25 ± 324.28	5.13 ± 1.50
Axis porcinus	932.50 ± 234.41	977.00 ± 390.76	5.50 ± 2.07
Elaphurus davidianus	2105.49 ± 1000.30	1295.88 ± 351.28	6.70 ± 2.39
Przewalskium albirostris	389.19 ± 156.50	940.06 ± 98.18	2.21 ± 0.59
*Rangifer tarandus**	402.08 ± 219.67	645.77 ± 93.67	3.36 ± 0.77
*Capreolus pygargus**	66.86 ± 38.30	725.47 ± 72.91	1.86 ± 0.24
*Cervus elaphus**	564.03 ± 136.54	1072.50 ± 164.25	3.81 ± 0.89
*Cervus nippon**	440.29 ± 263.23	969.86 ± 457.35	2.75 ± 0.98
*Alces alces**	800.60 ± 466.36	748.76 ± 409.23	1.14 ± 0.48

* Sympatric Cervidae on northeast forest of China.

following discriminant classification after removing the independent variables related to the hair tip and root.

(1) For the samples lacking a hair tip, we obtained a discrimination accuracy of 89.7% with six indexes: MaxWid, Max MeWid, proNon-MedLen, scCnt, scWid and scHei.
(2) For the samples lacking a hair root, we obtained an accuracy of 83.9% with six indexes: MaxWid, Max MeWid, disNon-MedLen, scCnt, scWid and scHei.
(3) For the samples lacking both a hair tip and root, we obtained a discrimination accuracy of 73.6% with five indexes: MaxWid, Max MeWid, scCnt, scWid and scHei.

Blind test for five sympatric deer species

The results showed that, in the blind test, all the hair samples from the deer species were correctly identified except those of *Cervus elaphus*, which had 6.2% incorrect assignment. In total, the blind test accuracy of the five sympatric deer species was 97.9%. We also conducted a blind test of the discriminant function when the hair tip or root were absent. The results showed that the accuracy of the blind test was 89.6% and 83.3%, respectively,. The accuracy of the blind test was 75.0% when both the hair root and tip were absent (Table 7).

Cluster analysis of both hair and taxon characteristics of Cervidae

Figure 3 shows that the hair characteristics of the 10 Cervidae deer species can be divided into three groups. The first group includes *Cervus eldii*, *Axis porcinus* and *Rusa unicolor*, which are all distributed in the southern tropical and subtropical regions of China. The second group includes *Rangifer tarandus*, *Capreolus pygargus*, *Cervus elaphus* and *Cervus nippon*, which are mainly distributed in the northern temperate zone of China. Although *Cervus nippon* is widely distributed in China, only *Cervus nippon hortulorum* was discussed in this study. The last group includes *Przewalskium albirostris*, *Alces alces* and *Elaphurus davidianus*, which are mainly distributed in the alpine and high-altitude areas of China. *Elaphurus davidianus* is a reintroduced species, but northeastern China is their historical distribution area.

Discussion

The unique profile characteristics of the deer hairs

The hairs of Cervidae are mainly composed of guard hairs and a few of fluff, the guard hairs have more Cervidae-specific characteristics than the fluff hairs (Jin et al. 2005, Hou et al. 2008), so we try to use guard hairs for species identification if they are found in carnivore scat. The guard hairs of Cervidae have some unique profile characteristics, which usually makes it easy to distinguish them from hairs of other species. 1) The color is relatively simple, although some species (such as *Cervus nippon*) have spots covering their pelage. However, it is difficult to distinguish them at the species level based on the hair color in the carnivore feces, their color can be similar to those of Bovidae. Although pigmentation is variable characters and its usefulness in species identification is correspondingly limited (De Marinis and Asprea 2006), it can be used as the first step in species identification. 2) The shape

Table 4. Hair cuticles scales parameters of 10 deer species in the Cervidae.

Species	scWid (μm)	scHei (μm)	Y-/X	scCnt
Rusa unicolor	35.25 ± 12.30	8.63 ± 2.22	0.28 ± 0.13	24.60 ± 3.17
Cervus eldii	48.50 ± 5.87	12.50 ± 2.57	0.26 ± 0.05	17.95 ± 1.88
Axis porcinus	47.50 ± 4.86	11.00 ± 2.11	0.23 ± 0.05	16.80 ± 1.55
Elaphurus davidianus	67.60 ± 7.00	19.86 ± 3.33	0.29 ± 0.06	6.83 ± 4.07
Przewalskium albirostris	63.49 ± 6.36	27.50 ± 3.94	0.44 ± 0.08	5.44 ± 2.37
*Rangifer tarandus**	58.46 ± 8.58	27.12 ± 2.89	0.48 ± 0.10	7.30 ± 0.83
*Capreolus pygargus**	55.90 ± 9.11	22.38 ± 5.52	0.40 ± 0.08	4.33 ± 1.91
*Cervus elaphus**	56.32 ± 7.88	27.76 ± 5.40	0.51 ± 0.14	6.73 ± 3.32
*Cervus nippon**	53.26 ± 8.64	13.74 ± 3.28	0.27 ± 0.10	6.46 ± 4.61
*Alces alces**	57.38 ± 8.46	27.35 ± 5.39	0.48 ± 0.07	7.71 ± 0.85

* Sympatric Cervidae on northeast forest of China.

Table 5. Classification results of 10 deer species in the Cervidae.

Species	1	2	3	4	5	6	7	8	9	10
1. *Rusa unicolor*	**100**									
2. *Cervus eldii*		**80.0**	20.0							
3. *Axis porcinus*		10.0	**90.0**							
4. *Elaphurus davidianus*				**84.5**			5.2	9.3	1.0	
5. *Przewalskium albirostris*					**97.0**		1.5	1.5		
6. *Rangifer tarandus**						**96.2**			3.8	
7. *Capreolus pygargus**						4.4	**82.3**		13.3	
8. *Cervus elaphus**			1.3	1.3		6.2	1.3	**86.2**	3.7	
9. *Cervus nippon**		1.4	1.4				1.4		**95.8**	
10. *Alces alces**										**100**

* Sympatric Cervidae on northeast forest of China; The numbers on the first line are corresponding with the species on left row.

of the guard hairs of Cervidae is different from the typical fusiform shape of the carnivore's hairs, they are also different than hairs of other mammalian orders. The width of the hair shaft is not significantly change from the tip to the root in Cervidae (Meyer et al. 2001). However, the guard hairs suddenly thin at the point where the hair approaches the root, which is a unique characteristic that is only observed in Cervidae and Moschidae. 3) The guard hairs are fragile, and can be broken easily. The guard hairs have well-developed medullary structures. The medulla index is more than 90%, except for that of *Rusa unicolor*, *Cervus eldii* and *Axis porcinus*, for which the medulla index is more than 75%. In contrast, the cortex is paper thin and seems to be absent, making deer hairs fragile. For this reason, we found that the deer hairs were always fragmentary in the carnivore feces (Shores et al. 2015). In other words, we can initially distinguish the deer hair or not according to profile characteristics. Then try to identify the species of Cervidae using multiple parameters of hair morphology.

The identification characteristics in Cervidae

Cervidae are distributed worldwide, from tropical areas to the arctic, occurring in the various temperature zones of China. *Rusa unicolor*, *Cervus eldii* and *Axis porcinus* live in the tropical and subtropical regions of China. Their CHs is less than 40 mm, their MI is below 85% and their Non-MI is more than 5% (more than 10% in *Rusa unicolor*). However, the Y-/X in these three species are less than 0.3 and scCnt is more than 16, suggesting that their hair scales are arranged in a dense, flattened pattern. The cuticular scCnt is related to the mechanical resistance of the hair. Usually, the scale at the distal end of the hair is dense and flat (Hausman 1920).

In contrast, the five sympatric species were distributed in the mid-temperate and cold temperate zones and have a hair length of more than 40 mm (up to 139 mm in moose), except for that of *Rangifer tarandus*. The CHs is directly related to heat insulation. The MI of these species is more than 92%, and the Non-MI is less than 4%. The Y-/X is more than 0.4 except those of *Cervus nippon*, and all the scCnt is less than 8, indicating that the scales are arranged in a loose, mosaic pattern. The hair of deer has extremely well-developed medullary structures, which is an important strategy for adapting to cold environments for species that lack the fluff hairs. The guard hairs allow the species to withstand more cold air by increasing the volume of the medullary cavity of the hair and increasing the effect of heat insulation.

Table 6. Classification results of five sympatric Cervidae on northeast forest of China.

Species	1	2	3	4	5
1. *Rangifer tarandus*	**92.3**		3.8	3.8	
2. *Capreolus pygargus*		**100**			
3. *Cervus elaphus*	1.3	2.5	**88.8**		7.5
4. *Cervus nippon*	1.7	1.4	1.4	**95.7**	
5. *Alces alces*					**100**

The hair characteristics have species specificity, which is an important basis for zoological taxonomy. In this study, we found that the deer species have similar hair characteristics, which may be related to their close genetic relationships. This means that some of the deer species, such as *Cervus nippon* and *Cervus elaphus*, have similar phylogenetic characteristics. However, the hair characteristics of Cervidae are strongly affected by the climatic habitat in which the species are found. *Rusa unicolor* and *Przewalskium albirostris* are phylogenetically related, but the two species are distributed in different habitats and have completely different hair microstructures. *Przewalskium albirostris* is a unique deer species of the Qinghai-Tibet Plateau. To survive in the alpine environment of the plateau, it has long hair, well-developed medullae, and loosely arranged scales and its pelage features are similar to those of deer species in cold areas (Chen and Wang 1991). *Rusa unicolor* are distributed in subtropical forests, and the relatively low level of medullary development and densely arranged scales are the characteristic of wool fiber protection. Therefore, the hair of Cervidae shows significant characteristics of zonal adaptability, not only in macro characteristics but also in some micro-structures, such as medullary and scale patterns.

Application of hair quantitative morphological characteristics

Since humans began to study the microscopic morphology of mammalian hair, researchers have been committed to utilizing hair morphology for species identification and zoological taxonomy. The profile, cross-sectional morphology, cuticular scale and medullar pattern of the different mammalian groups have been applied to the dietary analysis of carnivores (Betsch 2014, Sugimoto et al. 2016). However, the type and shape of the scales at different locations along one

Table 7. The blind test accuracy of five sympatric Cervidae on northeast forest of China.

Species	The sample size of blind test	The hair completed; 8 numerical features	The hair tip absented; 6 numerical features	The hair root absented; 6 numerical features	The hair absented both at the hair tip and root; 5 numerical features
1. *Rangifer tarandus*	5	100.0%	100.0%	80.0%	80.0%
2. *Capreolus pygargus*	9	100.0%	66.7%	88.9%	66.7%
3. *Cervus elaphus*	16	93.8%	87.5%	62.5%	50.0%
4. *Cervus nippon*	14	100.0%	100.0%	100.0%	100.0%
5. *Alces alces*	4	100.0%	100.0%	100.0%	100.0%
Total	48	97.9%	89.6%	83.3%	75.0%

hair are different, and the similarity in the medullar pattern between sibling species is very high (Meyer et al. 2001, Kuhn and Meyer 2010). Monroy-Vilchis et al. (2005) focused on the intraspecific individual variation of mammals hair and found just the medulla morphology was only constant factor. Within a family or genus, it is difficult to successfully discriminate species by means of only a single medullar or cuticular scale pattern. Wildlife forensic medicine has been working on discriminating animal hair using multivariate morphological function to improve discriminant accuracy (Oli 1993, Rowe 2001, Anwar et al. 2011, Kerley et al. 2015). Sato et al. (2006) obtained a discriminant rate of 96% and 99% for cat hair and dog hair, respectively, with 13 hair parameters. The three subfamilies of deer species were classified by Meyer et al. (2001) using the ratio of the height to the width of scales, the scale index and the diameter of the hair. Hall-Aspland and Rogers (2006) distinguished potential prey of four leopard seals *Hydrurga leptonyx* using a six-dimensional cross-sectional quantitative feature of hair. We obtained a high discriminant accuracy of 90.1% for 10 species of Cervidae using eight quantitative parameters. Furthermore, we obtained a discriminant accuracy of 94.2% for the five sympatric deer species in the northeastern forests of China, where they are potential prey for medium-large carnivores, such as Amur tiger and leopard.

Due to the fragile nature of the deer's hair, the hairs remaining in the carnivore's feces are usually fragmentary. According to our experience, generally less than 20% of the hairs are complete. Even so, we still obtained a discrimination rate of 83% after removing some of the variables for incomplete hair roots or tips. However, if both the hair root and tip were missing, only a 73% discriminant rate was obtained using our discrimination functions. Clearly, in the process of discriminant analysis, as the number of quantitative parameters decreases, the discrimination accuracy will also decrease. Therefore, we strongly recommend selecting complete hair samples as often as possible. While doing this work, the taxon and the environmental driving factors should also be understood to avoid mistakes in species identification.

In our study, when using hair morphology to identify species of the same genus or family, we suggest the following: 1) the potential identified species should be reduced as far as possible so that getting higher discriminant accuracy. As shown by our results, we obtained a higher discriminant accuracy for the five sympatric deer species than for the

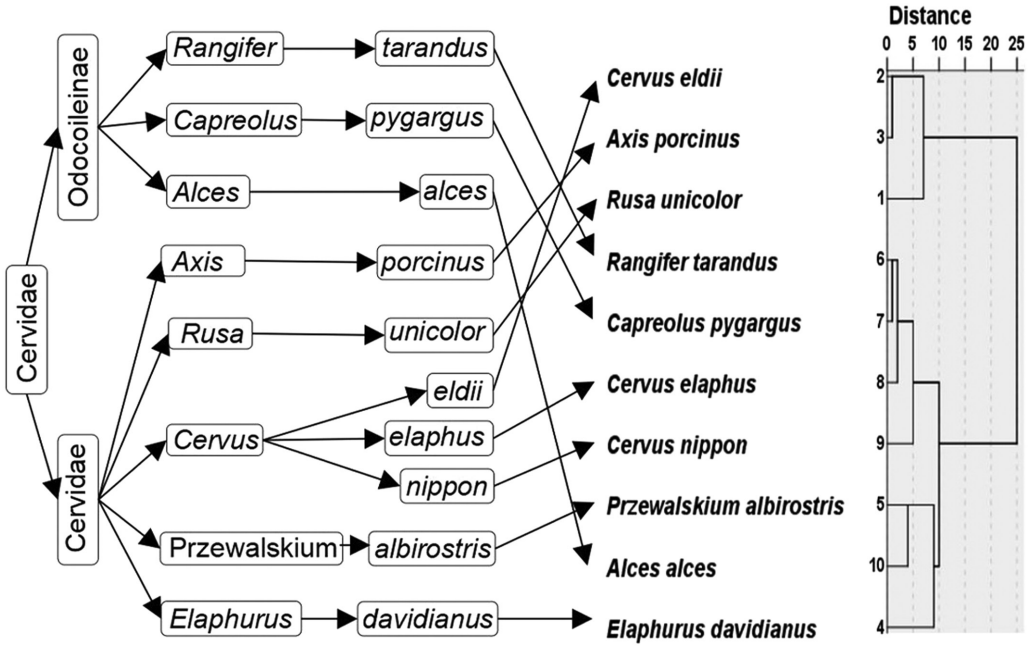

Figure 3. Relationship between hierarchical cluster analysis and taxonomy of the 10 deer species.

group of 10 species. 2) Complete hairs with typical characteristics of a given species should be selected. For example, researchers should try to select the guard hairs instead of the fluff hairs in Cervidae. 3) Some quantitative characteristics should be removedif they have no significantly differences to reduce confusion for species identification.

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