

On the hairs and hair cuticle structure of douc langurs (*Pygathrix* sp.) and the Tonkin snub nosed monkey (*Rhinopithecus acunculus*)

Clara Stefen¹ and Franziska Szokoli²

¹ Senckenberg Naturhistorische Sammlungen Dresden, Museum für Tierkunde Dresden, Königsbrücker Landstrasse 159, 01109 Dresden, Germany. Corresponding author <clara.stefen@senckenberg.de>

² Technical University Dresden, Teplitzer Straße 51, 01219 Dresden, Germany

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Summary

The study describes and compares the hair cuticle structure of the three species of douc langurs, *Pygathrix cinerea*, *P. nemaeus* and *P. nigripes* in comparison to *Rhinopithecus avunculus*. In the latter the hair diameter and cuticle height is smaller than in the three species of *Pygathrix*. The cuticles in general have smooth to crenate edges and are variable in all regions of the hair. A clear differentiation of the species on the basis of hair cuticle structure is not possible. Only the discriminant analysis shows some separation between the species.

Về các loại lông và cấu trúc biểu bì lông của các loài chà vá (*Pygathrix* sp.) và voọc mũi hếch (*Rhinopithecus acunculus*)

Tóm tắt

Nghiên cứu này mô tả và so sánh cấu trúc biểu bì lông của ba loài chà vá, *Pygathrix cinerea*, *P. nemaeus* và *P. nigripes* với sự so sánh với loài voọc mũi hếch *Rhinopithecus avunculus*. Về sau cùng, đường kính lông và chiều cao biểu bì là nhỏ hơn trong ba loài của giống *Pygathrix*. Nhìn chung, các biểu bì có cạnh trơn đến hình tai bèo và thay đổi trong tất cả các vùng lông. Sự khác biệt rõ ràng của các loài về gốc của cấu trúc biểu bì lông là không thể. Chỉ có phân tích biệt số cho thấy một vài phân cách giữa các loài.

Introduction

Currently three species of douc langurs (*Pygathrix*) are recognized in Vietnam. All three species are relatively poorly known. In outer morphology the coloration of the thigh pelage is the most obvious difference between the three Vietnamese species: being red in *P. nemaeus*, black in *P. nigripes* and grey in *P. cinerea*. The grey-shanked douc langur (*P. cinerea*) has only recently been described due to differences in coloration (Nadler, 1997). The species status was supported by molecular genetic data (Roos, 2004). The grey-shanked douc langur is listed as “Critically Endangered”, and is one of the world’s 25 most endangered primates (Mittermeier et al. 2009). The red-shanked douc langur and the black-shanked douc langur (*P. nigripes*) are listed as “Endangered” (IUCN, 2009). The Tonkin snub-nosed monkey (*Rhinopithecus avunculus*) is endemic to Vietnam, listed as “Critically Endangered” and also belongs to the world’s 25 most endangered primates.

Douc langurs belong to the subfamily Colobinae within the family Cercopithecidae. The Colobinae

include the genera *Presbytis*, *Nasalis*, *Rhinopithecus*, *Semnopithecus*, *Trachypithecus* and *Pygathrix* (Brandon-Jones et al., 2004). Molecular genetic data support a monophyletic clade consisting of *Nasalis*, *Pygathrix* and *Rhinopithecus*, with *Rhinopithecus* and *Pygathrix* probably being sister taxa (Sternier et al., 2006).

The hair cuticle structure has been studied for several taxonomic groups and in different regions and is used in comparative determination of wild animals, such as in the analysis of scats to determine food items. Some comparative studies, particularly of the hair cuticle structure of European mammals, are given by Debrot et al. (1982), Teerink (1991) and Meyer et al. (2002). The hair cuticle structure of primates has rarely been studied aside from in *Homo sapiens*.

In this paper the hairs and the hair cuticle structure of the three species of douc langurs is described in order to see if it offers the possibility to differentiate the species. Also, comparisons are made to *Rhinopithecus avunculus*.

Material and Methods

The fur of mammals consists of two types of hair: primary or guard hairs, also called overhair, and secondary hairs, which are usually curled and form the insulating wool hair coat and are therefore also called underhair. To study the cuticle structure, guard hairs were used. The shaft (the region of the hair that extends beyond the skin surface) has three regions: the apical region or tip that often has a spindle-shaped or flattened thickening called the shield, underneath which the medium region is followed by the basal region that thins towards the base of the hair. The cuticle structure varies in the regions, particularly in the apical and basal. (Meyer et al. 2002; Teerink 1991) The hairs of the studied species do not show a shield and thus the middle portion of the hair was considered the medium part.

The study is based on the hairs of 10 skins of *Pygathrix*: 2 of *P. cinerea* (B24006, B 24817), 6 of *P. nemaeus* (B13913, B19400, B19854, B15777, B19853, B19855), 2 of *P. nigripes* (B19857, B19856) and 1 of *Rhinopithecus avunculus* (B18785) from the Senckenberg Naturhistorische Sammlungen Dresden, Museum für Tierkunde (MTD). For each species, 15 primary hairs from the back and from the thigh were cut from the skins at their very base, cleaned in warm tepid water and fixed on cardboard. Here the lengths of the hairs were measured and coloration was noted. Short pieces of the tips, medium region, and bases of hair from the specimen's backs were taken and mounted on aluminum stubs, then coated with platinum for 120 seconds for use with the scanning electron microscope (SEM). Pictures of the hairs were taken in the SEM at different resolutions. Hair diameter, height of cuticle, cuticle area and number of cuticles per mm² were recorded from the medium part of the hairs. Measurements of the cuticle structures were done on the SEM photos using the program "Image J" on a PC.

Statistical analyses of the measurements include students' t-tests for statistically significant differences between the species and discriminant analyses of all species. The DA was done using Wilk's lambda statistic, and all variables were entered simultaneously, with equal prior probabilities of group membership based on the pooled within-group covariance matrix.

All statistical analyses were performed using SPSS 16.

Results

Outer morphology and coloration

The hairs of the thigh and back of the *Pygathrix* species differ in coloration. The main characteristic of the three species is the coloration of the thigh and thus it is not surprising that these hairs are black in *P. nigripes*, grey in *P. cinerea* and red in *P. nemaeus*. The hairs of the thigh are uniform in color and are

shorter than the hairs of the back in all three species. The hairs of the back are clearly banded with creamy white and dark brown or black bands; the dark bands are usually slightly longer (about 4 mm) compared to the light ones (about 3 mm). The base is usually creamy and the tip is always dark in the three species of *Pygathrix*.

In the studied specimen of *R. avunculus* the hairs of the back and thigh are of a similar brownish grey color.

A thickened shield cannot be observed in any of the hairs of the studied species.

Hair length and diameter

The length of back hairs varied greatly between the individuals of *Pygathrix* studied, while the thigh hairs appeared more uniform in size. In Table 1 the descriptive statistics of hair lengths are given. The hairs of the back are longest in *P. cinerea*. This is supported by statistically significant differences in the back hair length between *P. cinerea* and *P. nemaeus*, *P. cinerea* and *P. nigripes* and *P. cinerea* and *R. avunculus*. Also the thigh hairs are significantly longer in *P. cinerea* than in *P. nemaeus* and *P. nigripes* (Fig. 1). The back hairs are significantly longer in *P. nigripes* than in *P. nemaeus*.

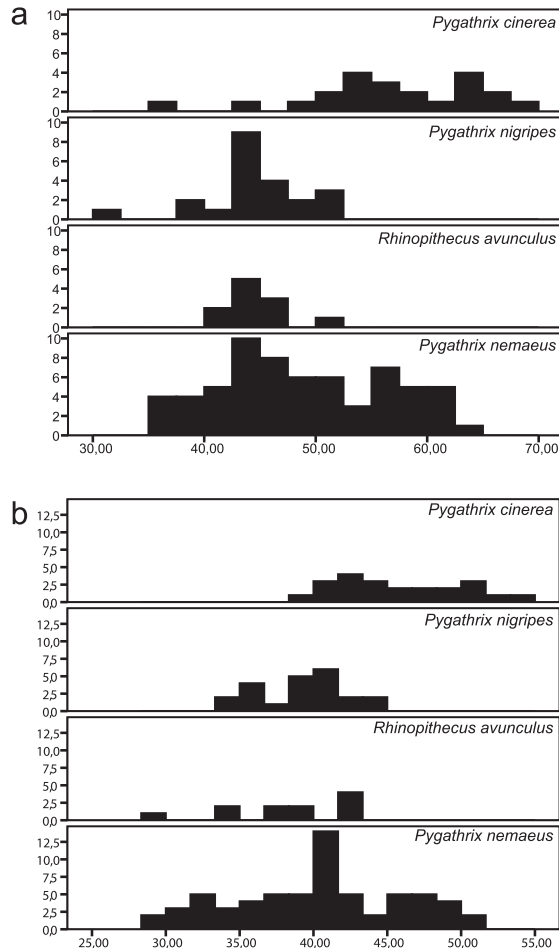


Fig. 1. Frequency of hair length of back (a) and thigh (b) of the three species of *Pygathrix* and *Rhinopithecus avunculus*.

The hairs of *R. avunculus* are clearly thinner than the hairs of *Pygathrix*. *P. cinerea* has the largest hair diameters (Table 2).

Hair cuticle structure of the species

The gross morphology of the hair cuticle structure of the hairs on the back of the three species of *Pygathrix* is fairly similar (Fig. 2 and 3a-c). Despite the cleaning of hairs prior to analysis the base often showed more dirt particles than the rest of the hair and the scale edges were often not as clear as in other parts of the hair.

In *P. nemaeus* the base of the scales are usually crenate to slightly crenate and even fairly smooth (Fig. 2c). The scales differ more in height in areas with crenate scales than in areas with smooth scales. Generally the scales are long and form a flat mosaic (see Meyer et al., 2002). The scales are of intermediate height and are usually at a right angle to the long axis of the hair, though deviations of about 25° occur. In the medium part the scales are smoothly crenate, have an intermediate height and are usually at a right angle to the long axis of the hair, although deviations of ~20° occur. At the tip, the scales are smoothly crenate, and in some areas the scales are fairly high.

In *P. cinerea* the cuticle structure is very similar. At the base, the scales are crenate to nearly smooth; in the middle part more crenate, and crenate and higher at the tip (Fig. 2d-f). At the base and in the medium part the scales are usually rectangular to the long axis of the hair. Also, strongly bent scales can be seen at the tip, forming the single chevron pattern of Meyer et al. (2002).

The pattern in *P. nigripes* (Fig. 3a-c) is again very similar to *P. nemaeus*. Overall scale edges appear more smooth than in *P. nemaeus* and in the medium part scales are in some areas arranged in the single chevron pattern which is not visible at the base or tip.

In *Rhinopithecus avunculus* (Fig. 3d-f) the scales at the base are smooth to slightly crenate and the middle part is usually also crenate at the tip. Overall the scales are at a right angle to the long axis to the hair, although slight deviations occur.

There is noticeable variation in crenation of the edges of the scales in all regions of the hair in all studied species. In general the scales at the base are smoother and higher than in the middle part. The tip in particular shows fairly irregular scale edges and higher scales. Therefore, hair cuticle structure in the apical part appears the most irregular. However, even in the middle part, sometimes scales are more crenate and irregular.

The variability in cuticle height is reflected by the large range and high standard deviation in all species (Table 2). In *R. avunculus* the cuticle height and number of cuticles per mm² is similar to that in the *Pygathrix* species, but the cuticle area and hair diameter are markedly smaller (Table 2).

Discussion

Overall there is variability in hair cuticle structure in all parts of the hairs. Variability is minimal in the medium part of the hair below the shield and therefore this area is regarded most suitable for the study and measurement of cuticles (Meyer et al., 2002). Nevertheless, even in this region of the hair there is a high variability in all measured parameters (Table 2).

The study of the cuticle structure in all parts of the hairs did not indicate clear differences useful for differentiating the species although that could have been expected as hairs are sometimes used to determine species (e.g. in stomach contents or feces). However, Meyer et al. (1997) noted that the identification of mammals with the hair cuticle structure is sometimes difficult due to the variability along

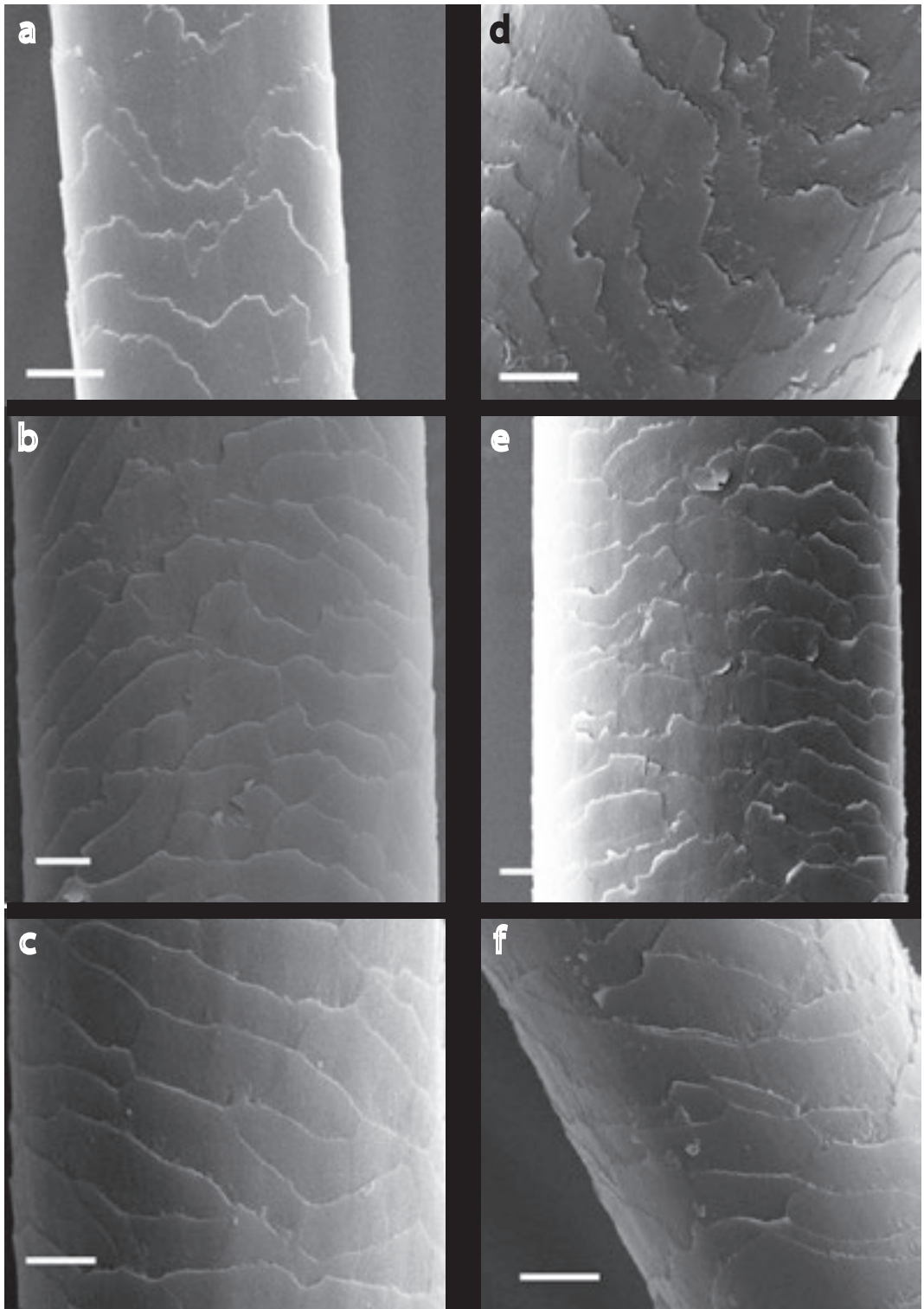


Fig.2. Hair cuticle structure of hairs of the back of *Pygathrix nemaeus* (a-c: a MTD B 19400, b, c MTD B 19855) and *Pygathrix cinerea* (d-f: d, e MTD B24817, f MTD B 24006). Apical part (a, d), medium part (b, e) and basal part (c, f) of the hair. Scale bar = 10 μ m.

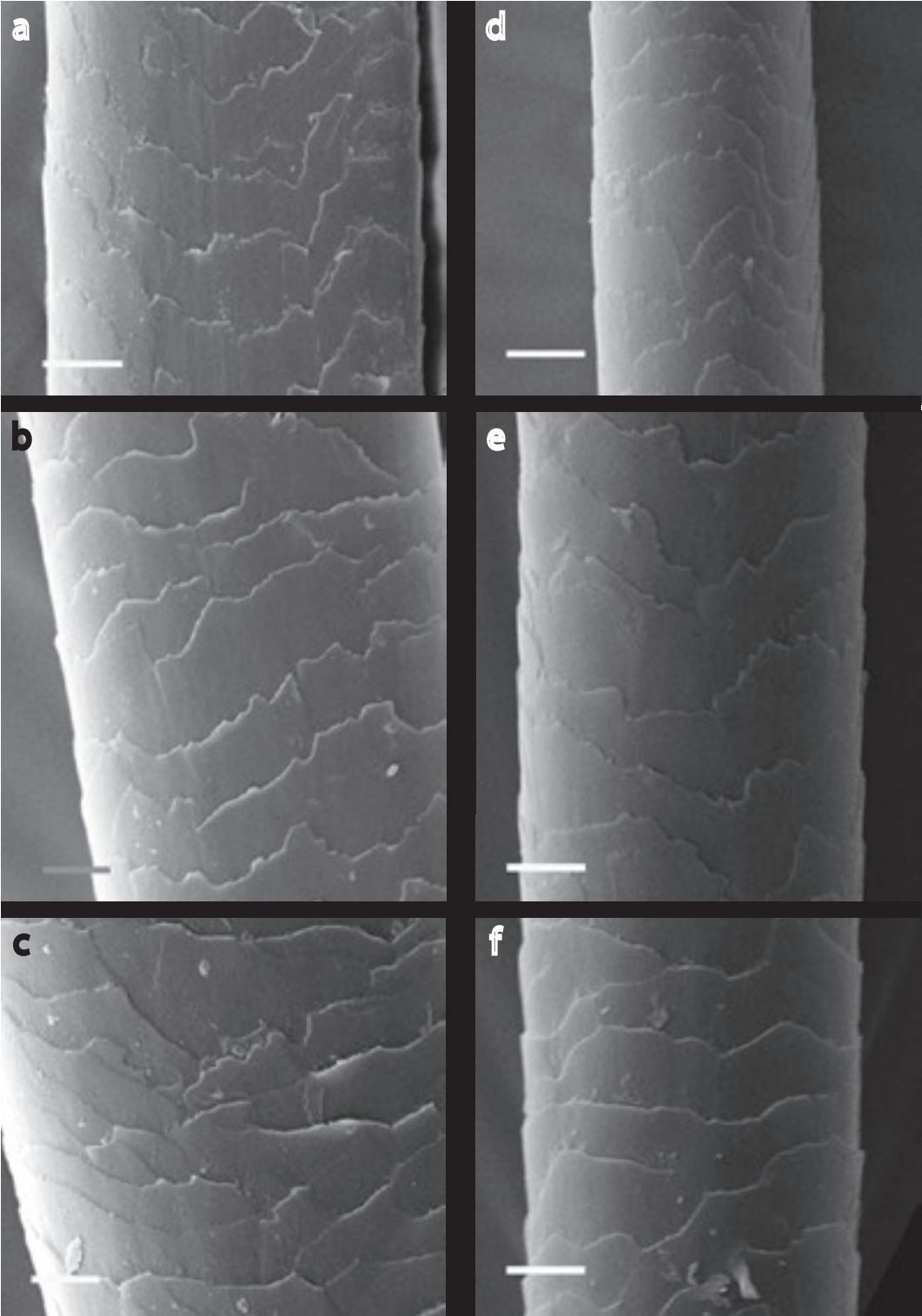
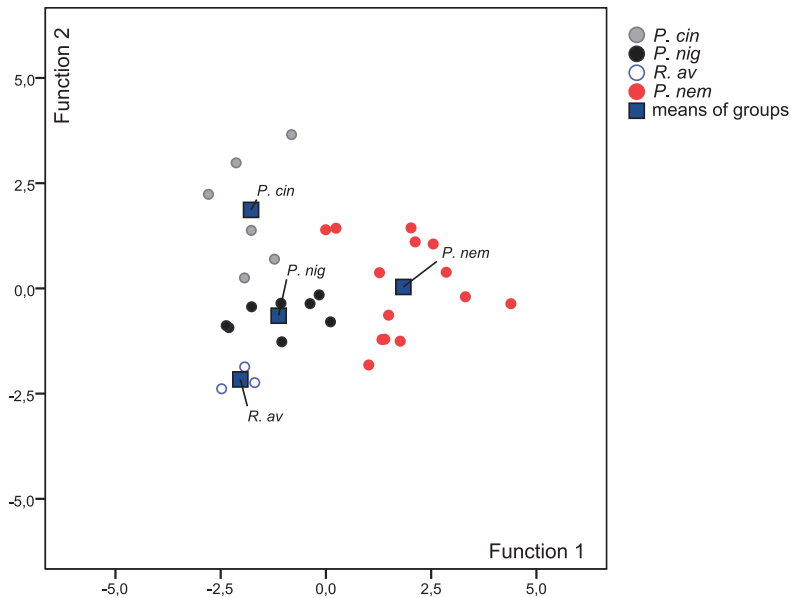


Fig.3. Hair cuticle structure of hairs of the back of *Pygathrix nigripes* (a-c, MTD B19857) and *Rinopithecus avunculus* (d-f, MTD B18785). Apical part (a, d), medium part (b, e) and basal part (c, f) of the hair. Scale bar = 10 μ m.

the hair and between different types of hair. In this study only hairs of the same region of the back were used thus reducing the difficulty to distinguish between cuticle patterns between hair types. The three species of Vietnamese douc langurs are distinguished on the color of their thigh but also on the basis of molecular genetic data (Roos, 2004).

The hair diameter and the cuticle area are smaller in *Rhinopithecus avunculus* than in the three species of *Pygathrix*. These two parameters mainly influence the functions of the discriminant analysis (DA, Fig. 4). The DA shows some separation of the samples: *Rhinopithecus avunculus* is separated from the others by function 1, which is mainly influenced by hair diameter and the three species of *Pygathrix* are separated by function 1 and 2, the latter of which is mainly influenced by cuticle area. The sample presented here might be regarded small for statistical analysis, but this is due to the availability of skins of douc langurs. Even though the same number of specimens could not be studied for each species, differences that might be useful to differentiate between the species should have been detected.



Structure Matrix

	Function		
	1	2	3
cuticle area	.708*	.133	-.683
hair diameter	-.055	.882*	-.411
cuticles/mm ²	.079	.031	.918*
cuticle heigth	-.016	.009	-.467*

Common correlation within the groups between discriminant variables and canonical discriminant functions.

Variables are ordered according to their absolute correlations within the functions

* greatest absolute correlation between each variable and a discriminant function

Fig.4. Discriminant analysis (DA) based on several hair and cuticle parameters of the middle part of hairs of the back of *Pygathrix cinerea* (*P. cin*), *P. nigripes* (*P. nig*), *P. nemaeus* (*P. nem*) and *Rhinopithecus avunculus* (*R. av*).

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