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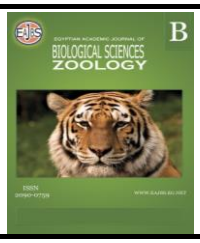

Morphological Studies on the Guard Hair of the Mona Monkey (*Cercopithecus mona*) in Omo-Shasha-Oluwa Forest Reserves of Southwest Nigeria

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ABSTRACT

Guard hairs of mammals are useful in identifying species. Morphological characteristics of the guard hairs were used to determine differences in the pelage of mona monkeys (*Cercopithecus mona*) in Omo-Shasha-Oluwa Forest Reserves in South western Nigeria. Using standard procedures, five strands of guard hair from each location were observed under a light microscope attached with a digital camera for the determination of histological structures. Qualitative and quantitative characteristics of the hair were examined and data obtained directly from the microscope. Data on the cuticle, cortex and medullar dimensions (μm) of the hair were analysed descriptively and inferentially using SPSS Version 20 respectively. Variance analysis was used to compare the means of the quantitative parameters of the hair. Statistically significant means ($P \leq 0.05$) were separated using LSD post hoc test. A continuous medulla pattern was common in all locations. Amorphous medulla was found in only Omo and Shasha monkeys. The longest scale length and width of 218.76 ± 60.29μm and 725.76 ± 155.91μm respectively were recorded in Omo Forest Reserve. Medullary diametre, index, and a fraction of monkeys in Omo and Shasha were significantly different ($P \leq 0.05$) from that of Oluwa. Based on the qualitative and quantitative characteristics of the guard hair, the mona monkeys in Omo and Shasha had features that were similar. The findings can be useful in explaining the close proximities and interactions of this species in the three Reserves. The information can be used as a guide in conservation, forensic and other scientific researches.

INTRODUCTION

Hair is one of the features mammals possess. It is an epidermal exoskeleton that consists of cells that occur in three concentric layers, viz: cuticle, cortex, and medulla. The configuration of hair varies considerably from species to species. This variation can be used to identify a particular species through various methods of hair analysis (Bhat *et al.*, 2014). Hair has been known to play a vital role for identifying mammals. The presence of mammalian hair in an area can be used in establishing and monitoring the presence of a mammal species in that area (Yasser *et al.*, 2018). Based on morphological features of hair, some animal species can be differentiated easily. This makes morphological identification of hairs vital in mammalian wildlife identification (Farag, 2015).

There is likelihood that two hair samples may be similar and differentiating them could be difficult if the variation range overlap. The medulla is an important characteristic in

identifying hair morphology. The presence, absence, continuous, discontinuous uniserial, amorphous medulla in hair shafts is used in describing medulla distribution and consequently in associating it with a species (Verma and Joshi, 2012).

Non-invasive techniques were developed to survey and monitor wild animals undisturbed in their natural environment. The technique involves observing animals directly or indirectly through the use of tracks, footprint tunnels, scats, hair snares, hair snags, and sensor cameras. These have been used for surveying wild mammals, especially rare or elusive species (Cornally and Lawton, 2016). Non-invasive techniques have their advantages and disadvantages. The collection of hair samples may seem easy, but it requires training and experience to use them for species identification.

Non-invasive methods using hair morphological analyses are simple, low-cost and have been used for breed identification for animal production (Felix *et al*., 2019). Mammalian hairs, except those of humans, are taxon-specific in terms of the medullae characteristics, scale patterns and cross-sectional shapes (Tridico, 2015). Where morphological distinctive features are not too clear, a combination of morphological (which is time and cost-effective) and molecular (use of DNA testing) analyses would provide valid taxon-specific results (Mariacher *et al*., 2019).

There are numerous studies on the hair morphology of different wildlife (buffalo, deer, hare, howler monkey, hyena, leopard, lion, tiger, and wild goat) and domestic (cow, goat, sheep) mammals from different parts of the world. Hair from different parts of the animals' bodies was studied and shown to be valuable for forensic and zoological purposes, and dietary ecology of predators (Davis, 2010; Verma and Joshi, 2012; Bhat *et al*., 2014; Farag, 2015; Cornally and Lawton, 2016; Sari and Arpacik, 2018; Tremori *et al*., 2018; Desai *et al*., 2019). Few of these studies involved non-human primates. Some of those studies showed cannibalism and infanticide in chimpanzees (Walker *et al*., 2018). Hair morphology characteristics could provide information on the identity of animals (Tridico *et al*., 2014), non-human primates inclusive.

Lack of information on the morphological histology of the Nigerian mona monkey hair, vis-à-vis differences in the same ecological area, information that would be relevant to park rangers, forensic and scientific research purposes. This study aims at using a light microscope to obtain the morphological characteristics of guard hair samples of mona monkeys (*Cercopithecus mona*) in three different but contiguous Omo-Shasha-Oluwa Forest Reserves complex and show the phenotypic similarities or otherwise of this species. This study determined and contrasted the morphological characteristics of guard hair of mona monkeys in Omo, Shasha, Oluwa Forest Reserves located respectively in Ogun, Osun, and Ondo States in South western Nigeria. Since there is a dearth of information on this aspect of the species, the results of this study will not only serve as guidelines on hair morphological protocol, but as a data base in wildlife forensics in Nigeria.

MATERIALS AND METHODS

Study Areas:

The guard hairs of mona monkeys were collected from Omo, Shasha and Oluwa Forest Reserves (OSOLFR) located in the southwestern part of Nigeria, on Latitude 4˚10' to 4˚57'N and Longitude 6˚30' to 7˚21'E (Fig. 1). The area is made up of a 2462km^2 contiguous forests in Ogun, Osun and Ondo States of Nigeria. Omo Forest Reserve (OFR) is 1325 km^2, Shasha Forest Reserve (SFR) covers about 310km^2, while Oluwa Forest Reserve (OlFR) covers about 827 km^2. Rainfall and temperature within the reserves averaged 2050 mm and 27˚C respectively (Adedeji and Adeofun, 2014).

Vegetatively, the study area is a lowland tropical rainforest that has been degraded to

secondary forests, thickets, and farmlands of annual and perennial crops, with the exception of some parts of the Forest Reserves (Adedeji and Adeofun, 2014). Apart from the Strict Nature Reserve (SNR) situated at the north-western part of OFR, the forest is largely converted to mono plantations, mainly *Gmelina arborea* (Okoli and Ola-Adams, 1987).

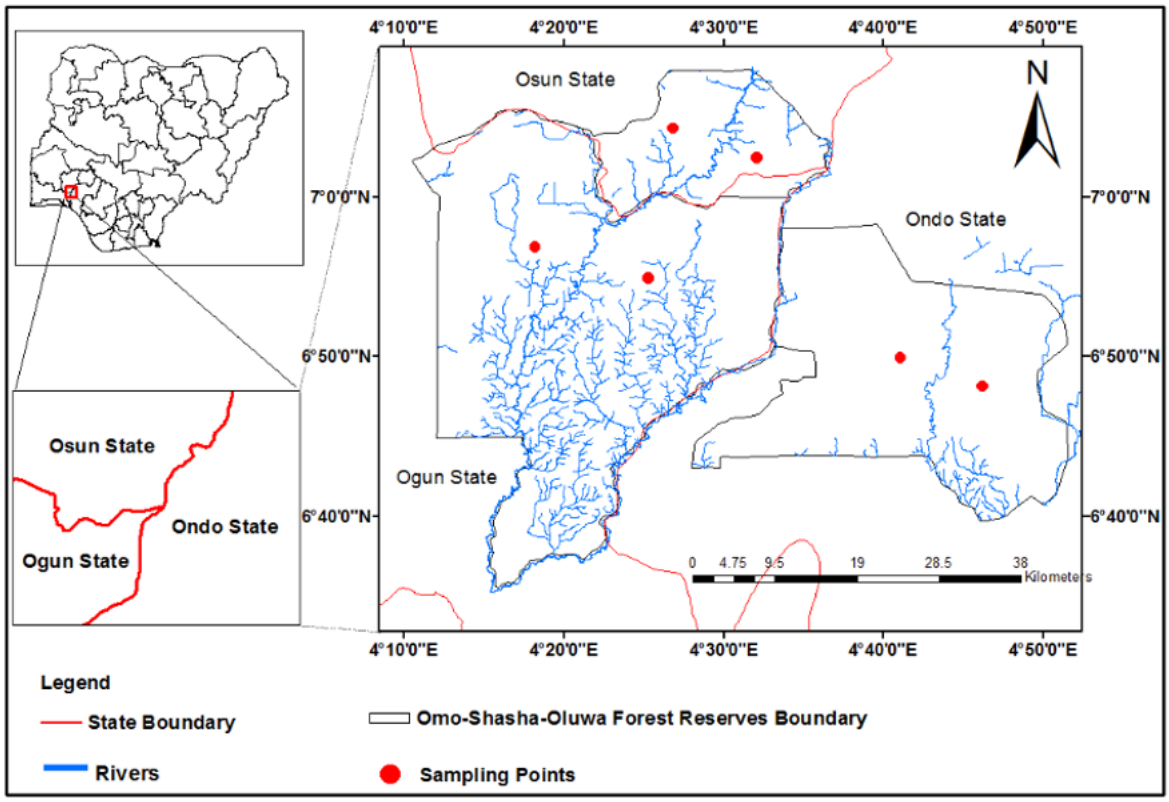

Fig 1: Map of Omo-Shasha-Oluwa Forest Reserves in Ogun, Osun and Ondo States respectively

Hair Sample Collection:

Guard hairs of moan monkeys were obtained from hunters who operate around two selected areas within a forest reserve that is separated by a river (Fig. 1). This was done with the assistance of field staff that knows these hunters. At least 10 hair shafts were placed in labelled acid free paper envelopes immediately after collection and placed in zip-lock bags with silica gel and stored at room temperature until morphological analysis was carried out (Garcia-Alaniz *et al.*, 2010).

Hair Sample Preparation For Morphological Examination:

Hair slides were prepared using the method described in Deedrick and Koch (2004a). Guard hair samples were cleaned by immersion in 70 % ethanol for 5 minutes, and allowed to dry. For each location, five hair strands were placed on five separate slides that were initially smeared with glycerine, and then covered with a cover slip. These were examined at x10 magnifications for medullae pattern under a light microscope that had a digital camera attached, and photomicrographs were taken.

The same hairs were placed again in ethanol for cleaning. They were removed, air dried, and used as scale cast to obtain scale impressions for cuticle scale pattern determination. This was to check the difficulty associated with direct observation of scale patterns (Zafarina and Panneerchelvam, 2009). Scale casts were investigated through the use of clear nail polish. This was improvised to replace gelatine used by Cornally and Lawton (2016) and Yasser *et al*. (2018). Clear nail polish was placed on another slide, allowed to set for two minutes. Hair was placed on the set polish with fine-pointed forceps, placed in a dust-

free area, and allowed to dry for five minutes after which the hair was gently removed leaving scale patterns on the slide. The cuticle scale patterns were observed under a light microscope at x40 magnification.

The qualitative parameters studied were shaft diameter, cuticle scale pattern, margin type and distance, and medulla pattern and structure. The quantitative characteristics studied were medulla diametre (μm), hair diametre (μm), medullary index, and fraction. Medullary and hair diametres were measured using a calibrated micrometer in the eyepiece. Medullary index was calculated as medulla diametre/hair diametre. Meduallary fraction was calculated as (medulla diametre/hair diametre) x 100 (Kitpipit and Thanakiatkrai, 2013).

For the scale cast, scale length and width were determined in μm by using a calibrated micrometer in the eyepiece. These were determined from randomly selected cuticle scales (Kitpipit and Thanakiatkrai, 2013).

Morphological Evaluation:

All morphological examinations were conducted in the instrument room, Department of Zoology, University. Cuticle scale patterns and medullae characteristics were determined by using available animal hair keys in literature as guide. These included Deedrick and Koch (2004b), Knecht (2012), Cornally and Lawton (2016), and Yasser *et al*. (2018).

Statistical Analysis:

Means, standard deviation, and analysis of variance were conducted using SPSS (Version 25) to determine the differences between the quantitative characteristics. A P-value of less than 0.05 was considered significant. A post-hoc test using the least significant difference was carried out where means between locations were significantly different.

RESULTS

Qualitative Characteristics of Mona Monkey in Omo-Shasha-Oluwa Forest Reserves:

The qualitative characteristics (scales of the cuticle and medulla) of mona monkeys in OSOLFR are shown in Table 1. These characteristics were different for the three Reserves. The cuticle scale margin of mona monkey hairs from OFR and OlFR were ‘crenate’, but ‘smooth’ in SFR. The cuticle scale margin distance was ‘distant’ in OFR, but ‘intermediate’ in both SFR and OlFR. The medulla pattern was ‘continuous’ in the three locations. An ‘amorphous’ medulla structure was common in mona monkeys in Oluwa and Shasha, but ‘uniserial’ in mona monkeys in Oluwa Forest Reserve. The photographs of the cuticle scale and medulla patterns of mona monkeys in OSOLFR are shown in Fig. 2. The cuticle scale patterns were ‘imbricate’ in OFR and OlFR, but ‘coronal’ in SFR; the medulla pattern was ‘continuous’ in the three locations.

Table 1: Qualitative characteristics of guard hairs of mona monkeys in OSOLFR

Qualitative traits	Cuticle scale characteristics			Medulla characteristics	
Location	Margin Type	Pattern	Margin Distance	Pattern	Structure
Omo	Crenate	Imbricate	Distant	Continuous	Amorphous
Shasha	Smooth	Coronal	Intermediate	Continuous	Amorphous
Oluwa	Crenate	Imbricate	Intermediate	Continuous	Uniserial

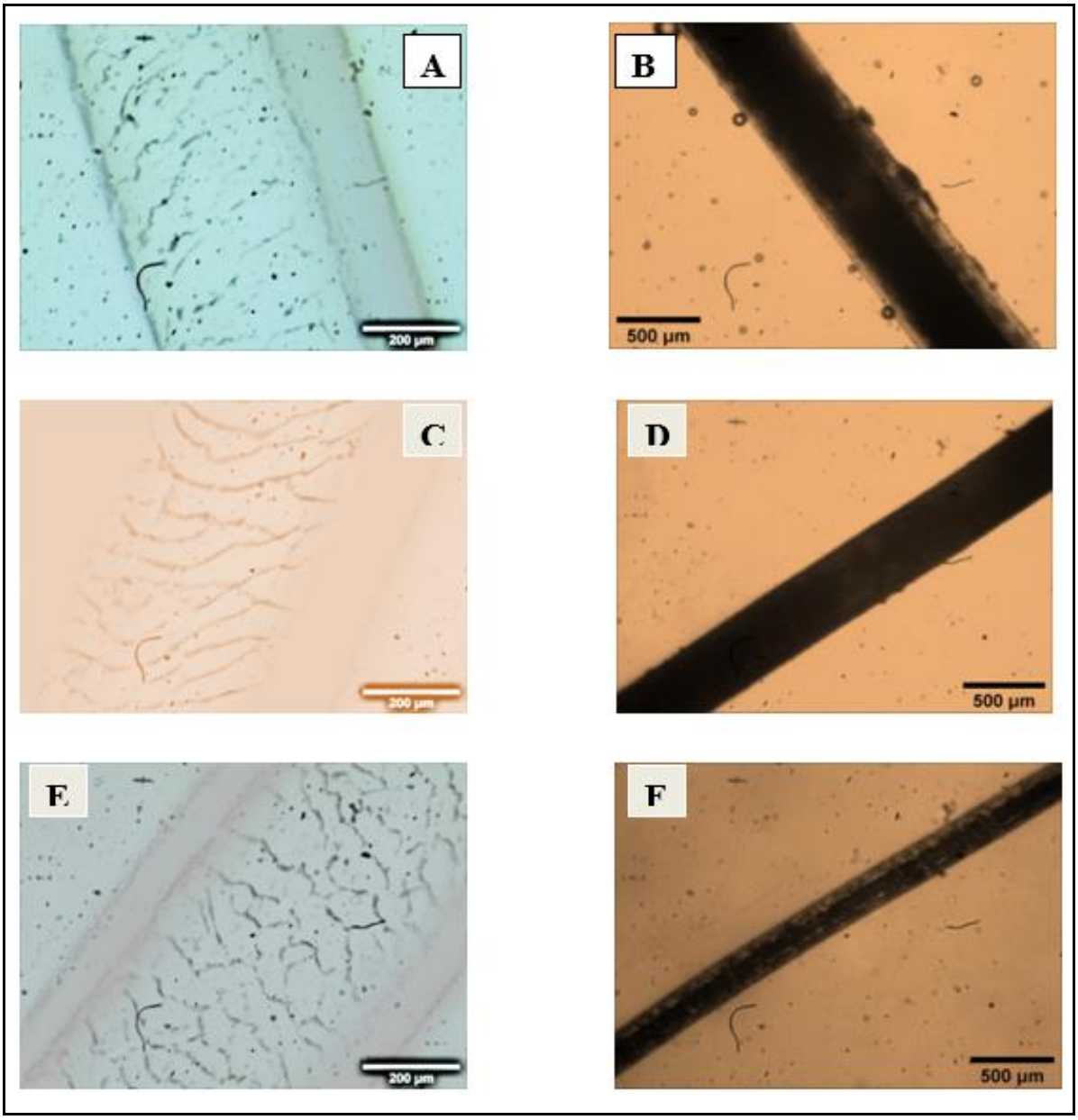

Fig. 2: Hair scale pattern and medulla of mona monkey in OSOLFR
A = Cuticle scale pattern, and B = Medulla of mona monkey hair in Omo Forest Reserve.
C = Cuticle scale pattern, and D = Medulla of mona monkey hair in Shasha Forest Reserve.
E = Cuticle scale pattern, and F = Medulla of mona monkey hair in in Oluwa Forest Reserve.

Quantitative Values of Hair Morphology of Mona Monkeys in Omo-Shasha-Oluwa Forest Reserves:

Mona monkeys in OFR had the highest values in medullary diametre (226.00±59.73), shaft diametre (387.00±75.38), scale length (218.76±60.29), and scale width (725.76±155.91) than monkeys from the other Reserves (Table 2). Apart from scale width, the mean values of all the other quantitative characteristics were greater in mona monkeys in OFR and SFR than those in OlFR. These values were significant at $P < 0.05$ (Table 3). Which location was significant from another was shown in Table 4. Medullary diametre, index, and fraction of monkey hairs in OFR and SFR were significantly different ($P \leq 0.001$) from that of OlFR. The scale length was significantly different ($P \leq 0.05$) between the monkeys in OFR and SFR.

Table 2: Mean and Standard Deviation of morphological characteristics of mona monkeys' hairs in OSOLFR

Location	N	Medullary Diametre (µm)	Shaft Diametre (µm)	Medullary Index	Medullary Fraction	Scale length (µm)	Scale Width (µm)
Omo	5	226.00±59.73	387.00±75.38	0.58±0.13	58.4±12.60	218.76±60.29	725.76±155.91
Shasha	5	198.00±24.90	311.00±97.04	0.66±0.11	66.20±11.03	180.15±46.01	468.78±114.18
Oluwa	5	33.00±5.70	230.00±49.63	0.15±0.04	14.60±3.51	165.29±16.61	547.15±133.63

Table 3: Analysis of variance of the morphological characteristics mona monkey in OSOLFR

Morphological characteristics	df	Mean Square	F	Significance
Medullary Diametre	2	54381.667	38.660	0.000**
Shaft Diametre	2	30821.667	5.265	0.023*
Medullary Index	2	0.387	39.633	0.000**
Medullary Fraction	2	3868.200	39.633	0.000**
Scale Length	2	86734.642	4.714	0.031*
Scale Width	2	3809.817	1.896	0.192

Note: ** = P ≤ 0.001, *=P ≤ 0.05

Table 4: Post-hoc of mona monkey quantitative characteristics between OSOLFR

Dependent Variable	(I) Location	(J) Location	Mean Difference (I-J)	SE	Significance
Medullary diameter	Omo	Oluwa	193.00000*	23.72060	0.000***
	Shasha	Oluwa	165.00000*	23.72060	0.000***
Shaft diameter	Omo	Oluwa	157.00000*	48.39077	0.007**
Medullary index	Omo	Oluwa	0.43800*	0.06248	0.000***
	Shasha	Oluwa	0.51600*	0.06248	0.000***
Medullary fraction	Omo	Oluwa	43.80000*	6.24820	0.000***
	Shasha	Oluwa	51.60000*	6.24820	0.000***
Scale length	Omo	Shasha	256.97800*	85.79193	0.011*

*** = P ≤ 0.001, ** = P ≤ 0.01, *=P ≤ 0.05

DISCUSSION

The qualitative characteristics of coronal, crenate, imbricate, and smooth cuticle scales have been reported for different wildlife (Deedrick and Koch, 2004b; Kencht, 2012; Bhat, 2014: and Yasser *et al*., 2018). In comparing the qualitative characteristics of *C. mona* across the three forest reserves within the same ecological zone, it was interesting to observe unexpected variations in the cuticle scale margin type, and distance, and patterns. This finding varied with Tridico (2015) where medullae characteristics and scale patterns were regarded as taxon-specific. This implies that the morphological features of the mona monkey in all the three forest reserves should be the same. The observed variations in this study could be attributed to individual differences (intra-species diversity) and the immediate local environmental conditions. Those with similar cuticle and medulla characteristics could be members that perhaps lived together as near kins, but got separated with the presence of Reserve boundaries that operate only as human delineations. The golden standard of

molecular studies would be useful in establishing this relationship between individuals from the three Reserves (Mariacher *et al*., 2019). The cost, time, and technical expertise demanded for a molecular test is a great hindrance within the terrain where quick action and decision need to be made.

The 'continuous' medullary pattern observed for the three locations was similar to that observed in brown howler monkey (*Alouatta guariba*) in Brazil (Tremori *et al*., 2018). The 'coronal' cuticle scale pattern of monkeys in SFR was simply making them resemble the 'imbricate' pattern for those in OFR and OlFR.

Having quantitative characteristics that were significantly different across OSOlFR not only explains intra-species differences but established it. The variations in the parametres were between OFR-SFR and OlFR. The hair morphologies of mona monkeys in OFR and SFR were more similar than those in OlFR. Based on the qualitative and quantitative characteristics of the guard hair, the mona monkeys in Omo and Shasha had features that were similar. They could be closely related. In the absence of local data to compare this work, the hair morphology characteristics established from this study could be useful as a key for mona monkey identification and baseline guide for future studies of this species.

CONCLUSION

In this study, an attempt was made in providing a fast way of analyzing guard hair morphologies of mona monekeys using a light microscope to determine their differences in the contiguous OSOlFR in Southwestern Nigeria. There were dissimilarities in the qualitative features and significant differences in the quantitative morphological characteristics of the guard hair of mona monkeys from the three forest reserves. The differences were less between monkeys in Omo and Shasha Forest Reserves. Molecular studies could be needed to confirm the relationships between individuals in these two forest reserves. With limited literature on this type of study in the locale, the information from this simple, time and cost-effective technique could be quite vital for future comparison with other similar works. It could be handy for wildlife conservationists, zoological and forensic researches.

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