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Guard hair micro-morphology of four non-human primates in Shasha Forest Reserve, Osun State, Nigeria

Fatsuma Olaleru[*1, 2], Temitope R. Olugbebi[1] and Michelle I. Fasona[1]

[1] *Department of Zoology, Faculty of Science, University of Lagos, Nigeria*
[2] *TETFund Centre of Excellence for Biodiversity Conservation and Ecosystem Management, University of Lagos, Nigeria*

*Correspondence: *folaleru@unilag.edu.ng* ORCID: https://orcid.org/0000-0002-3362-8668

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Abstract Mammalian guard hairs have been used for their identification and have been proved useful in wildlife population surveys and trafficking. The qualitative and quantitative features of the dorsal guard hairs of four non-human primates (NHPs) from Shasha Forest Reserve were studied using standard procedures to determine their differences. The NHPs were mona (MM), putty-nosed (PNM), and white-throated (WTM) monkeys, and red-capped mangabey (RCM). The qualitative features determined were medulla pattern, and structure, scale margin distance and type, and scale pattern. The quantitative values studied were scale length and width, shaft diameter (µm), medulla diameter, and medulla index and fraction. Continuous medulla pattern was the only qualitative feature common to all the species. The MM hair had the highest recorded values for all morphological characteristics except shaft diameter and scale width in which PNM had the highest value of 323.00 ± 58.37 and 630.55 ± 213.95µm respectively. The medullary diameter, index and fraction, and shaft diameter were highly significant ($P<0.001$) among all the species. Post-hoc comparison showed that the medullary diameter, index, and fraction of hairs of the MM was significantly different ($P\leq0.001$) from the other three NPHs. The shaft diameter of the MM was also significantly different from that of the PNM ($P\leq0.001$), and WTM ($P\leq0.004$). The scale width of RCM differed from PNM ($P\leq0.01$), and WTM ($P\leq0.05$). These empirically established morphological differences in guard hairs of the four NHPs in SFR would be useful in verifying their habitat occupancy and forensic evidence in case of illegal trafficking.

Keywords. Dorsal hair, medulla hair pattern, mona monkey, non-invasive studies, qualitative hair features.

1 Introduction

The proper management and conservation of wild animals require population monitoring through regular surveys. Such surveys provide a greater understanding of the distribution, extent, and status of populations, thereby facilitating the protection of threatened or rare species (Rylands *et al*. 2008, Plumptre *et al.* 2013). Population surveys of terrestrial mammals have been conducted by direct or indirect techniques.

Direct techniques could be through live trapping or observation and counting of the target species. Live trapping has been used for enumerating small mammals, but it requires adequate knowledge of the species, training, and in many instances a license (Bertolino *et al.* 2009). Even though live trapping could provide information on abundance, species, and sex of individuals captured, it could lead to capture of non-target species, implication of stress to the trapped animals, and interferes with their activities (Chiron *et al*. 2018). Large mammals have been enumerated through observation and sighting of target species.

Population surveys of wild mammals especially arboreal species could be demanding in terms of the efforts in sighting, and the time to enumerate them correctly. Nocturnal arboreals have been enumerated through the use of camera traps (Bowler 2017). Direct observation through line transects, and total/sweep count, have been used for the enumeration of non-human primates (NHPs) in forest habitats (Plumptre *et al*. 2013). Direct counts of individuals in a troop or numbers of troops per distance covered, their dispersion and time used for such surveys are some of the parameters considered. In rainforests, such exercises could be quite difficult given the poor visibility of individuals, especially elusive species such as the putty-nosed monkey. Indirect signs such as nest counts, scats/dung, and group calls have been used to survey fleeing or hideous species (Plumptre 2000, Plumptre *et al*. 2013). Motion sensor cameras and hair tubes/lures have been used to study dietary composition, population genetics, and habitat occupancy of mammals (Menike *et al*. 2012, Cornally and Lawton 2016, Bowler 2017). These methods are non-invasive, and require no direct contact with the target animals. They are labour-efficient, less costly, reduce or have no interference with the animals' activities, and do not expose researchers and the animals to zoonotic diseases (De Bondi *et al*. 2010). Some of the indirect methods could be limiting especially if the study species have uneven distribution or a low population density (Ruell *et al*. 2009).

The use of hair has proved to be quite reliable in non-invasive studies of the presence of mammals, especially those that have low population density in a location or cryptic ones (Paez *et al*. 2021). It has been used to study the diet of carnivores and omnivores, and identify road kills, habitat occupancy and population genetics (Menike *et al*. 2012). The presence and morphological analyses of chimpanzee hair found in the faeces of an adult chimpanzee in Gombe National Park, Tanzania were used to confirm cannibalism/infanticide within this taxon (Walker *et al*. 2017). Silver hair found in night nests has been used to indicate the presence of adult male gorillas (Gray *et al*. 2009). Hair tubes/ lures of different designs have been used for hair sample collection from different terrestrial mammals (Cornally and Lawton 2016). The hair tubes/ lures must be located in different parts of the study location in order to obtain representative hair samples. Hair samples are obtained when they get stuck to the strong adhesive glue applied to a rubber band stretched across the entrance to the tube (McAney 2011). For arboreal mammals, hair samples could be obtained at nest sites (Gray *et al*. 2009), water sites, or where they are known to feed and/or groom. Hair samples could also be obtained from dead animals or museum specimens.

Hair is strong and does not decompose fast, thereby making it easy to preserve. Its durability and resilience are due to the presence of keratin, a sulphur-containing protein in its cortex. Keratin makes hair to be less soluble and resistant to many chemical agents that could degrade it (Velasco *et al.* 2009). The long chain nature of keratin makes the hair have a regular structure that is flexible (de Sá Dias 2004). This feature makes the hair retain its elasticity for a long time, thereby enhancing its durability.

Each mammalian species has a unique hair morphology that has been used to identify them. This is because the hairs vary in colour and have different morphological arrangements (Farag *et al.* 2015). Each mammalian species has a different hair configuration in the way the concentric layers of the cuticle, cortex and medulla are arranged. Scale patterns and cross-sectional shapes are some of the basic information required in documenting the hair morphology of mammals (Taru and Backwell 2014). Analysing these configurations can be used to identify a species (Bhat *et al*. 2014). The micro-morphological characteristics of mammalian hair have been studied in forensic medicine (Meyer *et al*. 2000), wildlife biology (Sahajpal *et al*. 2009) and other disciplines.

Olaleru *et al.* (2020) provided a comparison of the mona monkey hair morphologies from Omo, Shasha, and Oluwa Forest Reserves in southwestern Nigeria. Olugbebi *et al*. (2021) compared the morphological features of guard hair of some non-human primates in Omo Forest Reserve and reported significant differences among the species. There is limited information on the morphological features of hairs of some members of the Family Cercopithecidae (cheech-pouched monkeys) that are found in Shasha Forest Reserve (SFR). In this study, we compared the morphological characteristics of guard hair of four species of the Cercopithecidae Family: mona monkey (*Cercopithecus mona*), putty-nosed monkey (*Cercopithecus nictitans*), white-throated monkey (*Cercopithecus erythrogaster*), and red-capped mangabey (*Cercocebus torquatus*) in SFR in Southwestern Nigeria. The findings would serve as a database in contrasting ecological studies, such as their habitat occupancy and predators. It could be useful in identifying members of this family in the event it is the only evidence found on poachers or wildlife traffickers.

2 Material and Methods

2.1 Study area

The Shasha Forest Reserve is located in Ife South Local Government Area of Osun State (Figure 1). It lies between Latitudes 6º 50’ and 7º 50’ N and Longitudes 4º 15’ and 4º58’ E. It is part of the contiguous Omo-Oluwa-Shasha Forest Reserves complex in southwestern Nigeria (Oludare and Clement 2014). It is the smallest of the three Reserves, covering about 310km^2 (Alo *et al*. 2020). It has a mean annual rainfall of about 2050 mm and mean monthly temperature of about 27˚C. Shasha Forest Reserve is a lowland tropical rainforest. It has been reduced, due to anthropogenic activities, to

a secondary forest, thickets and farmlands of annual and perennial crops (Adedeji and Adeofun 2014, Chenge and Osho 2016).

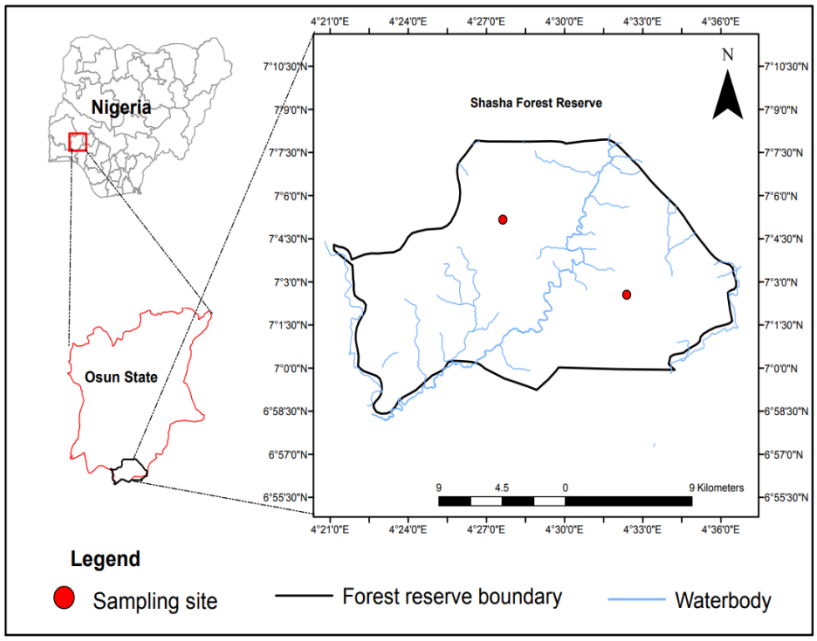

Fig 1: Map of Shasha Forest Reserve in Osun State, Nigeria

2.2 Hair sample collection and preservation

Guard hair strands from the dorsal regions of the studied non-human primates (NHPs), namely, mona monkey (MM), putty-nosed monkey (PNM), white-throated monkey (WTM), and red capped mangabey (RCM) were collected from Shasha Forest Reserve with the help of hunters and field guides. These were stored in labelled acid free paper envelopes and placed in zip lock bags that had silica gel, and kept at room temperature until they were used for the studies (Garcia-Alaniz *et al.* 2010). Guard hairs were used because they produce pelage (coat) colour, are useful in differentiating species (Tridico 2005, Knecht 2012), and possess the features for which microscopic identification could be made (Tridico 2015).

2.3 Preparation of hair samples for morphological examination

For each species, five strands of hair were randomly picked and prepared for viewing under the microscope as described by Deedrick and Koch (2004a). To remove dirt, the hair strands were immersed for five minutes in 70% ethanol, removed and air dried at room temperature (Verma and Joshi 2012). Each hair strand for each species was placed on glycerin smeared slide, and then covered with a cover slip. These were used

for medullae pattern studies under light microscope that was attached to a digital camera for photomicrograph production at a magnification of x10.

For scale cast, the same hair was cleaned in ethanol, removed and dried. This was used for scale impressions and cuticle pattern determination. Clear nail polish was used to replace gelatin used by Yasser *et al*. (2018) to produce cuticular scale pattern imprint. The polish was placed on another slide, allowed to set for two minutes, cleaned hair strand picked with fine-tipped forceps was placed on it and allowed to dry for five minutes. Gentle removal of the hair leaves the scale pattern which was viewed under light microscope at a magnification of x40.

2.4 Hair morphological evaluation and determination

The qualitative parameters studied were medulla pattern and structure, cuticle scale distance, margin type, and distance. The quantitative characteristics studied were medulla diameter (μm), hair diameter (μm), medullary index, and fraction. Hair and medullary diameters were measured at random points using a calibrated micrometer in the eyepiece. Medullary index was calculated as medulla diameter/hair diameter. Medullary fraction was calculated as (medulla diameter/hair diameter) x 100 (Kitpipit and Thanakiatkrai 2013). Using the scale cast, the entire 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).

All morphological examinations were conducted in the instrument room, Department of Zoology, University of Lagos. To ensure non mix-up of samples, the hair samples from each species were prepared and observed separately one after the other. Cuticle scale patterns and medullae characteristics were determined by using available animal hair keys in literature as a guide. These included Deedrick and Koch (2004b), Knecht (2012), Cornally and Lawton (2016), and Yasser *et al*. (2018).

2.5 Data analyses

The data were analysed descriptively using Microsoft excel and inferentially using SPSS (Version 25). Analysis of variance was used for the quantitative characteristics to determine differences in guard hair within and between species. A post-hoc test using least significant difference was used to separate means that were significant at $P \leq 0.05$.

3 Results

3.1 Qualitative characteristics of selected monkey species in Shasha Forest Reserve

Table 1 showed the qualitative characteristics of guard hairs from four non-human primate species in SFR. None of them had the same features, even though all had

'Continuous' medulla pattern. All species except MM had 'Uniserial' medulla structure, and 'Distant' scale margin.

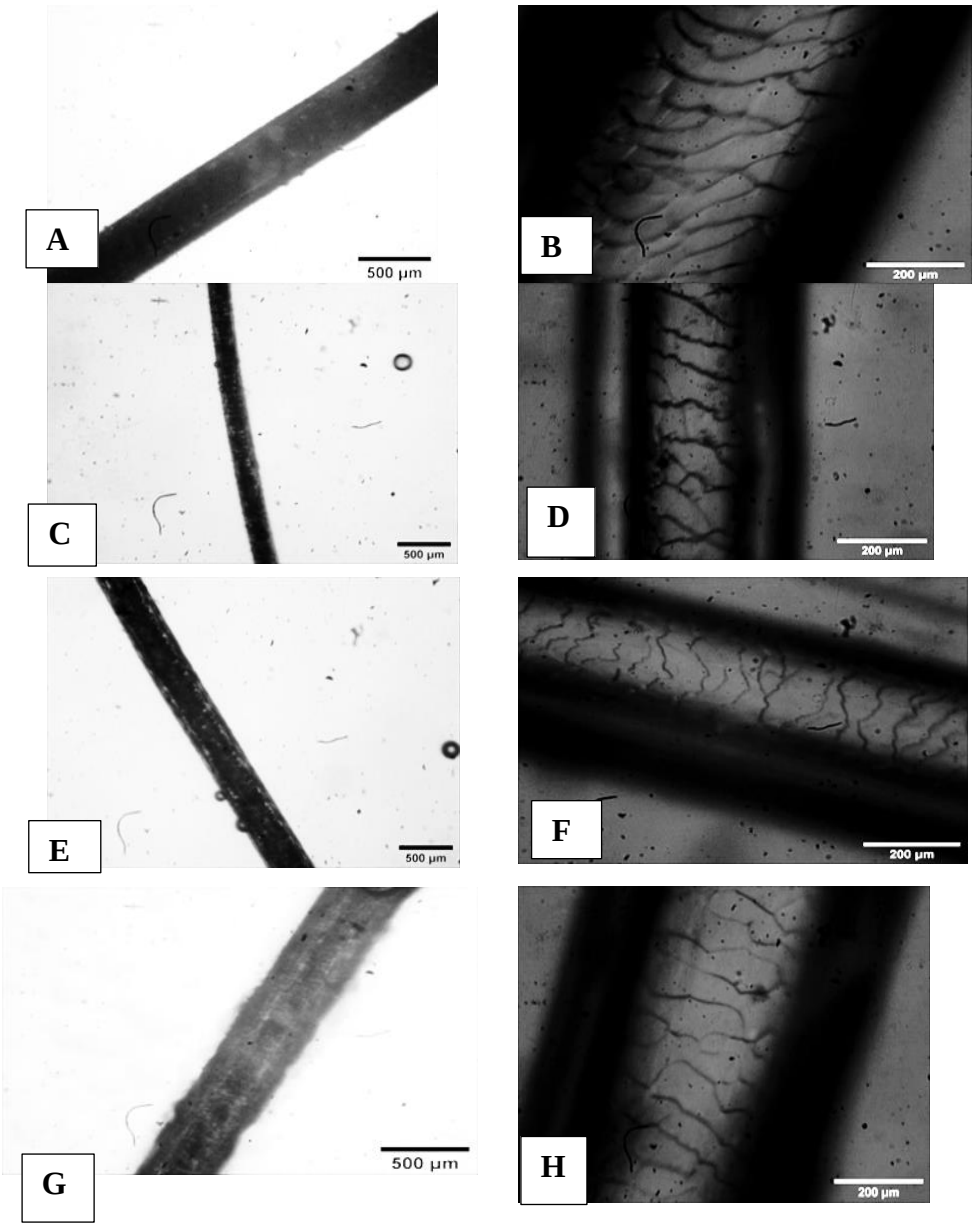

Plate 1: Photomicrographs of medulla and scale pattern of the guard hair of four studied NHPs in Shasha Forest Reserve

(A and B = Respective medulla and scale pattern of the hair of mona monkey; C and D = Respective medulla and scale pattern of the hair of putty-nosed monkey; E and F = Respective medulla and scale pattern of the hair of white-throated monkey; G and H = Respective medulla and scale pattern of the hair of red-capped mangabey)

Plate 1 shows the photomicrographs of guard hairs of the four monkey species studied. The scale patterns for mona and putty-nosed monkeys were coronal (A and C), while those of white throated monkey and red-capped mangabey were imbricate (E and G). The MM and RCM had 'Smooth' scale margin type, while MM and PNM had 'Coronal' scale pattern. Apart from scale margin type which were 'Smooth' and 'Crenate' respectively in RCM and WTM all the other qualitative features were the same for these two species. The hairs of PNM and WTM differed only in the scale pattern which were 'Coronal' and 'Imbricate' respectively.

Table 1: Qualitative characteristics of guard hair from mona, putty-nosed, red-capped mangabey and white-throated monkeys in Shasha Forest Reserve.

Hair features	Medulla Features		Scale Features		
Sample	Pattern	Structure	Margin distance	Margin type	Pattern
Mona monkey's hair,	Continuous	Amorphous	Intermediate	Smooth	Coronal
Putty-nosed monkey's hair	Continuous	Uniserial	Distant	Crenate	Coronal
White throated monkey's hair	Continuous	Uniserial	Distant	Crenate	Imbricate
Red-capped mangabey's hair	Continuous	Uniserial	Distant	Smooth	Imbricate

3.2 Quantitative morphological characteristics of guard hairs of four species of non-human primates in Shasha Forest Reserve

Table 2 shows the mean and standard deviation in the quantitative characteristics of the guard hair of four non-human primates in SFR. The mona monkey hair had the highest recorded values for all morphological characteristics except shaft diameter and scale width in which putty-nosed monkey had the highest value of 323.00±58.37 and 630.55 ±213.95µm respectively. Putty-nosed monkey was recorded to have the lowest value of medullary index which was 0.10±0.03µm.

Table 2: Mean and standard deviation of morphological characteristics of hair from four NHPs in Shasha Forest Reserve**.**

Features Sample	N	Medullary diameter (µm)	Medullary index	Medullary fraction	Shaft diameter (µm)	Scale length (µm)	Scale Width (µm)
MMH	5	198.00±24.90	0.66±0.11	66.20±11.03	311.00±97.04	180.15±46.01	468.78±114.18
PNMH	5	32.00±8.37	0.10±0.03	10.20±3.03	323.00±58.37	175.00±19.95	630.55±213.95
WTMH	5	28.00±5.70	0.20±0.06	19.60±6.31	149.00±34.89	179.19±47.88	394.10±101.65
RCMH	5	32.00±6.71	0.18±0.04	17.80±4.09	183.00±26.36	175.49±44.78	448.15±54.02

MMH= Mona monkey's hair, PNMH= Putty-nosed monkey's hair, WTMH= White-throated monkey's hair, RCMH= Red-capped mangabey's hair

3.3 Differences in the morphological characteristics of guard hairs between the four studied non-human primate species in Shasha Forest Reserve

Table 3 shows the ANOVA of the quantitative features of guard hairs of four studied NHPs in SFR. There were no significant differences ($P \leq 0.05$) between species for scale

length and scale width, while other characteristics such as medullary diameter, index and fraction, and shaft diameter were highly significant (P<0.001).

Table 3: Analysis of variance in the morphological characteristics of guard hairs of the four non-human primate species in Shasha Forest Reserve.

Morphological Characteristics	Samples	Df	Mean Square	F	Significance
Medullary diameter	Between Groups	3	35018.333	182.506	<0.001*
	Within Groups	16	191.875		
	Total	19			
Shaft diameter	Between Groups	3	39085.000	10.608	<0.001*
	Within Groups	16	3684.375		
	Total	19			
Medullary index	Between Groups	3	0.325	69.366	<0.001*
	Within Groups	16	0.005		
	Total	19			
Medullary fraction	Between Groups	3	3249.783	69.366	<0.001*
	Within Groups	16	46.850		
	Total	19			
Scale length	Between Groups	3	33.568	0.020	0.996
	Within Groups	16	1703.124		
	Total	19			
Scale width	Between Groups	3	51777.954	2.874	0.069
	Within Groups	16	18016.031		
	Total	19			

Note: * = P ≤ 0.001

3.4 Significant difference in the morphological characteristics between the guard hairs of the four studied non-human primates in Shasha Forest Reserve

Table 4 showed the post hoc separation of means. The medullary diameter, index, and fraction of guard hairs of the MM were significantly different at P ≤ 0.001 from the guard hairs of the other three NPHs. The shaft diameter of the MM was also significantly different from that of the PNM (P ≤ 0.001), and WTM (P ≤ 0.004). The shaft diameters of the WTM and PNM were both different from that in RCM. The PNM had medullary index and fraction that both differed from that of RCM. The scale width of RCM differed from that of PNM (P ≤ 0.01), and with that of WTM (P ≤ 0.05).

Table 4: The post hoc on morphological hair characteristics between the four studied non-human primate species in Shasha Forest Reserve.

Dependent Variable	(I) Species	(J) Species	Mean Difference (I-J)	Std. Error	Sig.
Medullary diam.		PNM	170.000	8.761	<0.001***
	MM	WTM	166.000	8.761	<0.001***
		RCM	166.000	8.761	<0.001***
Shaft diameter	MM	PNM	162.000	38.389	0.001***
		WTM	128.000	38.389	0.004**
	WTM	RCM	-140.000	38.389	0.002**
	PNM	RCM	-174.000	38.389	<0.001***
Medullary index		PNM	0.466	0.043	<0.001***
	MM	WTM	0.484	0.043	<0.001***
		RCM	0.560	0.043	<0.001***
	PNM	RCM	0.094	0.043	0.045*
Medullary fraction		PNM	46.600	4.329	<0.001***
	MM	WTM	48.400	4.329	<0.001***
		RCM	56.000	4.329	<0.001***
	PNM	RCM	9.400	4.329	0.045*
Scale width	RCM	PNM	236.448	84.891	0.013**
		WTM	182.392	84.891	0.047*

MM = Mona monkey, PNM = Putty-nosed monkey, WTM = White-throated monkey, RCM = Red-capped mangabey; *** = $P \leq 0.001$, ** = $P \leq 0.01$, *=$P \leq 0.05$

4 Discussion

The objective of this study was to compare the morphological characteristics of the guard hair of the four members of the Cercopithecidae family in Shasha Forest Reserve. Our findings showed that none of the NHPs had the same qualitative hair features. These confirmed their unique specificity. They had some similarities in some features that could make it difficult not to mistake one for the other, if other features are not used. For instance, they all had the same medullary pattern that was 'Continuous', a pattern that was reported brown howler monkey (*Alouatta guariba*) hair from Brazil (Tremori *et al.* 2018), and in domesticated animals such as camel, cow, horse, sheep, cat and dog in Egypt (Yasser *et al*. 2018). Using this feature alone is not advisable.

All the quantitative parameters were different too, but with some being similar between two species. The medullary diameters of PNM and RCM in this study site were similar. These were also similar to that for blue Nile monkey (*Cercopithecus mitis*) reported Farag *et al*. (2015). The medulla diameter of WTM though different from those of the rest in the same location, was similar to that obtained by Tremori *et al.* (2018) for brown howler monkeys.

The similarity in cuticular scale pattern in hairs of MM and PNM, and that of WTM and RCM could infer relatedness. Cornally and Lawton (2016) explained that many closely related species share similar cuticular scale patterns. The 'Distant' scale margin

of hairs recorded for all the species apart from MM implies that the scales were less dense or relatively sparse. Scale density is shown in how they are packed and could be described as 'Close' when dense (Cornally and Lawton 2016). The similarities between species in some qualitative features at the microscopy level has limited inference (Tridico 2005).

Similarities of species in qualitative hair features did not translate to similarities in quantitative characteristics. For instance, there was a unique similarity in the qualitative hair features between red-capped mangabey and white throated monkey, and between putty-nosed and white throated monkeys. Quantitatively, the medullary and shaft diameters, and the medullary index and fraction of the species were significantly different. These differences in the morphology of the hair confirms the samples were from different species. The results obtained could be used at least at the preliminary stages of evidence of habitat occupancy, dietary component of predators, or exhibits on poachers. Although cheaper when compared to standard DNA profiling, hair morphology screening can be time consuming. With more detailed study techniques, especially at the molecular level, more differences could be established in the hairs of these species. Molecular studies could perhaps show if these similarities could be due to genetic relatedness or adaptation to the environment.

5 Conclusions

The use of mammalian hair for micro-morphological studies is relatively cheap and easy to assess, The identity of these four species of NHPs have been buttressed further by the empirical data provided through the micro-morphological study of their guard hairs. The differences in the hair features verified the presence of these species in SFR. With the present results as a database, hair samples collected at feeding, sleeping or watering sites could be analysed and the outcome compared to verify which species of NHPs the hair belongs to. This result could also be useful database for comparing other poached members in the future. For ecological and conservation uses, environmental DNA studies will be needed to map out the spatial distribution of these species in the Reserve.

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