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Cranial and External Morphology of Male and Female Orange Headed Tree Squirrels (*Funisciurus leucogenys*) in Selected Locations of Savannah Forest in Nigeria

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Authors' contributions

This work was carried out in collaboration between the authors. Author AIA designed the study and wrote the protocol. Author AOB carried out the field work and performed the statistical analysis, manage the literature and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

Article Information

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Original Research Article

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ABSTRACT

This study examined the differences in cranial and external morphology of male and female tree squirrel species (*Funisciurus leucogenys*) from three different locations in Savannah vegetation zone of Nigeria. The tree squirrels were collected from contracted local hunters which identify the tree squirrel. After collection and identification to the generic level, they were transferred to the laboratory in absolute ethanol. About 183 skulls were prepared (58 male and 125 female), the skull and other body parameters were measured using digital venire calliper. The results showed that the body parameters (HBL, TL, TBL, EL, HFL, and BW) of the female specimen measured were different from male and the body parameters of the specimen from one location were slightly different from another. Also, the cranial measure showed some similarities and slight differences between male and female specimen among the locations (Asejire, Ilorin, and Ogbomosho). In conclusion, the cranial and body morphology parameters of male and female tree squirrels from the



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three locations (Asejire, Ilorin, and Ogbomosho) showed that despite that the tree squirrels were from different locations, they were of the same genus (*F. leucogeny*), which indicated that locations might not have an effect on the specimen.

Keywords: Rodents; crania parameters; measurements; specimen; abundance.

1. INTRODUCTION

The mammalian fauna of the woodlands of West Africa, which is around 320 species, comprise about a fourth of mammalian assorted variety in Africa [1]. Rodents (Order: Rodentia) are the world's most different well-evolved creatures, establishing 42% of worldwide warm-blooded animal assorted variety [2]. Among individuals from the Order Rodentia, squirrels of the family Sciuridae is one of the biggest. Squirrels (family: Sciuridae) consisting of approximately 279 species and 51 genera, are one of the most diverse and variable families of living mammals (Thorington and Hoffman, 2005). The family Sciuridae includes tree squirrels, ground squirrels and flying squirrels. Tree squirrels have long, hairy tails, sharp paws, and expansive ears, with some having very much created ear tufts [3]. Tree squirrels have been used to measure the shape and richness of forest because they rely on the mature forest for a nest site, food, cowl and their daily activities [4]. Disturbance of such wooded area condition in any way constantly create a hassle for tree squirrels, which occasionally lead to everlasting habitat loss.

The Orange-headed tree squirrel (*Funisciurus leucogenys*) which is very distinctive and easily identified has head and cheeks which are orange-red, with black speckling on the crown of the head, and black on and behind ears [5]. Dorsal pelage in the species is dark grey-brown to brown-black, not speckled, and yellowish on shoulders. The pale side stripe on each flank in the species is broken into short lines anteriorly and spots posteriorly, while the ventral pelage is pale orange-red [6].

The tail in the orange-headed tree squirrel is similar in colour to the back and is usually held (like all squirrels in this genus) curled forward over the back with the tip pointing upwards [6]. The reproductive habits in the species which are very different from those of the typical members of the genus could be an example of extreme niche separation, eliminating competition with other arboreal squirrels, but obviously, no conclusion can be reached until further accurate observations are made [7].

The Nigerian subspecies of Orange-headed tree squirrel is *F. leucogeny olivae* while the other subspecies, *F. leucogenysauriculatus*, and *F. leucogenysboydi*, occur in the Cameroon highland close to the Nigerian border. Both species have been reported to have blacker on the head than in the *F. lolivae* [6].

The field of morphometric is involved with techniques for the description and statistical evaluation of form variation inside and amongst samples of organisms and of the evaluation of form alternate because of growth, experimental treatment or evolution. Morphometric methods are used each time one needs to describe and to compare shapes of organisms or of unique organs. The samples may symbolize geographic localities, developmental stages, genetic results and environmental effects [8]. Cranium measurement of the equal species or exclusive species from the identical vicinity or one-of-a-kind location can additionally be used to differentiate between species [9].

The skull is an odd vicinity of phylogenetic research for assessing mammalian relationships and it serves as the foremost skeletal component from which morphological measurements are taken [9]. According to Zelditch et al. [10], refined differences can be shown cranial via measurements and quantitative comparisons of the skull amongst rodents. Corti et al. [11] pronounced that considering that the skull consists of the predominant sensory organs, the genius, and the feeding apparatus, it also includes a lot of information on the phylogeny. ontogeny and adaptation of rodents. It is as a consequence no longer surprising that skull characters have been the major supply of study in rodent morphology.

Happold [12] was able to discover specific species of tree squirrels in Nigeria after which there is little or no documentation on tree squirrels in Nigeria. Due to deforestation urbanization and the use of wild animals as means of income in Nigeria, there is need to carry out the study on tree squirrels mainly Orange-headed tree squirrel in some phase of savannah vegetation sector in Nigeria. Hence, this study was aimed to evaluating the body and cranial morphology of tree squirrel (*F. leucogenys*) in some sections of savannah vegetation quarter in Nigeria to establish the effects of urbanization and deforestation on tree squirrels.

2. MATERIALS AND METHODS

2.1 Study Area and Sampling Location

Squirrel samples were collected within the savannah vegetation zones of Nigeria (Fig.1). The squirrel samples were collected from Asejire, llorin, and Ogbomosho. The specimens were collected from local hunters for 19 months period (November 2011 – May 2013) to cover both rainy and dry seasons. The tree squirrels were bought from local hunters after the specific type has been shown to them. The bought specimens (Orange headed tree squirrel) after identification were transferred to the laboratory in jars containing 96% ethanol solution. Specimens that could not be examined immediately were preserved in jars containing 96% ethanol solution [13].

2.2 Specimen Identification

Specimens were preliminarily identified to the generic level, using an identification key prepared by Happold [12] and the sexes were determined by visual inspection of the external genitalia.

2.3 Skeletal Preparation

The skull of the sacrificed specimen was severed from the neck, skinned and the surrounding musculature was removed with the aid of knife and scissors. Skeletal preparation was carried out using the Long Island Natural History Museum guide [14] on how to prepare skeletal material.

The skull was soaked in water, inside a plastic container, for two weeks to ensure that the remaining attached flesh was degraded through bacterial maceration. The skull was then cleared with a fine-tooth brush; the skull, which was completely devoid of flesh, was then degreased by placing it in a solvent (kerosene) contained in a sealed glass container for two weeks. The skull was then bleached by soaking in 20% hydrogen peroxide for two days, removed and allowed to dry in a Petri dish at room temperature, over a period of five days.

2.4 Cranial and External Measurements

27 cranial and 6 external measurements were taken on each of the specimens according to the method of Rasmussen and Thorington [15], and Song et al. [16] using digital caliper (RUPAC, Italy), and digital weighing balance for the body weight with values allowed to be in 2 decimal places. The cranial measured parts included: Length of Nasals (NL), Breadth of Nasals (NB), Interorbital Breadth (IOB), Zygomatic Breadth (ZB), Breadth behind Postorbital Process (POB), Breadth of Braincase (BCB), Greatest Length of skull (GLS), Depth of Incisor (ID), Depth of Braincase (BCD), Occipitonasal Length (ONL), Length of Auditory Bulla (BL), Rostrum Breadth (ROB), Rostrum Length (ROL), Length of Diastema (DL), Length of Bony Plate (BPL), Breadth of Bony Plate (BPB), Postpalatal Length(PPL), Interseptal Breadth (ISB), Mastoid



Fig. 1. Maps showing the study areas, (a) Asejire and Ogbomosho, (b) Ilorin Sources; [17,18] modified Keys: (1) is Asejire, (2) Ogbomosho

Length (MTL), Breadth across Occipital Condyle (OCB), Length of Incisive Foramen (IFL), Breadth across the Incisive Foramina (IFB), Breadth of First Upper Molar (M1B), Length of Maxillary Toothrow (TRL), Mandibular Toothrow Length (LMT), Mandible Length (ML), Height of Mandible (THM) (Fig. 2) while the Body weight and external measurements included:

Head-body length (HBL), Tail length (TL), Total body length (TBL), Ear length (EL), Hind Foot length (HFL) as shown in Fig. 2.



LEGEND Α. NL = Length of Nasals NB = Breadth of Nasals **IOB = Interorbital Breadth** ZB = Zygomatic Breadth POB = Breadth behind Postorbital Process BCB = Breadth of Braincase G LS = Greatest Length of skull B. ID = Depth of Incisor BCD = Depth of Braincase ONL = Occipitonasal Length BL = Length of Auditory Bulla C. **ROB = Rostrum Breadth ROL = Rostrum Length** DL = Length of Diastema **BPL = Length of Bony Plate** BPB = Breadth of Bony Plate PPL = Postpalatal Length ISB = Interseptal Breadth MTL = Mastoid Length OCB = Breadth across Occipital Condyle C.1 IFL = Length of Incisive Foramen IFB = Breadth across the Incisive Foramina C.2 M1B = Breadth of First Upper Molar TRL = Length of Maxillary Toothrow D LMT = Mandibular Toothrow Length ML = Mandible Length THM = Height of Mandible

Fig. 2. Localization of the 27-craniodental measurements recorded in this study. Abbreviations for variables are defined in material and methods. Diagrammatic representation of measurements taken from a generalized tree squirrel skull and mandible; (A) Skull, dorsal view (B) Skull lateral view (C) Skull ventral view (C1) Incisive foramina (C2) Maxillary check-teeth and (D) Left mandible, lateral view. Adapted from 15 &16



Fig. 3. Diagrammatic representation of the external measurement of tree squirrel (Lateral view)

Source: 12, modifies Keys: 1. Head Body Length (HBL), 2. Tail length (TL), 3. Ear Length (EL), 4. Hind foot Length (HFL), 5. Total body Length (TBL= 1+2)

2.4.1 Sampling locations

The study was carried out in two different locations in South Western Nigeria.

- a. Asejire: 07°21′N 07°22′N and 004°08′E -004°10′E
- b. Ilorin: 08°29′N- 08°31′N and 004°21′E-004°22′E
- c. Ogbomosho: 08°08′N- 08°10′N and 004°13′E- 004°14′E

2.5 Statistical Analysis

Data generated from this work were subjected to descriptive statistics such as mean, and standard deviation. One-way analysis of variance (ANOVA) was used to determine the significant differences between the means while the significant means were separated at P < 0.05, using Duncan multiple Range Test from System Analysis Software (SAS) [19]. The inferential statistics employed were correlation and Principal Components Analysis (PCA) computed using Paleontological Statistics (PAST) version 1.75.

3. RESULTS

The *Funisciurus leucogenys* caught in all the locations were brown-black with strips. Some of the specimens had speckled rufous on the head and speckled rufous absent in some individuals. The tail had back pelage while the ventral pelage was creamy-white.

3.1 Composition and Abundance

One hundred and eighty-three (183) of Funisciurus leucogenys were collected across the studied locations (Asejire, Ilorin, and Ogbomosho) (Table 1). In Asejire location, the highest number of the specimen was collected which make about 37.02% of the total of sample collected. In Ogbomosho location, 60 specimens were collected, and this makes about 33.15% of the specimen. In Ilorin location, the least specimen was collected and it was about 29.83% of the total collection. The amount collected for this duration of the study was lower than what was expected and higher than *Heliosciurus rufobrachium* collected from the same location [7].

In all locations, higher numbers of females of *F. leucogenys* were collected (Table 1). The sex ratios (M: F) in the various locations were: Asejire 1:2, Ilorin 1:3.5, and Ogbomosho 1:1.5. The overall comparison showed that the sex ratio in the savannah zone was 1:2. Higher numbers of males of *F. leucogeny* specimens were collected from two locations (Asejire and Ogbomosho) when compared to the number of male sample in llorin.

The measured morphometric parameters of *F. leucogeny* sample in three locations (Asejire, llorin, and Ogbomosho) are shown in Table 2a & b. Table 2a shows measured the morphometric parameters of male *F. leucogenys* from different locations. The Head-body length (HBL) of the specimens sampled from different locations differs accordingly. Specimens from llorin had the highest HBL mean value (209.80 mm) and ranged between 179 – 265 mm. Specimens from Asejire followed specimens from llorin with the mean value of HBL of 198.30 mm and ranged from 121 to 243 mm. Specimen from Ogbomoso had the least mean value of HBL of

Locations	Male	Female	Total	Percentage
Asejire	21	48	67	37.02
llorin	12	42	54	29.83
Ogbomoso	25	35	60	33.15
Total	58	125	183	100

 Table 1. Composition and abundance of Funisciurus leucogenys caught during the period of study

194.20 mm and ranged between 141 to 221 mm. The Head-body length (HBL) of female *F. leucogenys* specimens caught from the same locations have a similar trend (Table 2b). The HBL of the specimen from llorin was the highest (229.8 mm), followed by Asejire (200.6 mm) and lastly Ogbomoso (199.2 mm).

The Tail length (TL) values of the specimen from llorin (male) was the highest (210.6 mm), followed by TL of the specimen from Asejire (196.3 mm) and Ogbomoso having the least value of TL (181.5 mm). The TL of the female specimen from llorin was the longest (227.1 mm), followed by Asejire (199.5 mm) and the least was from Ogbomoso (194.5 mm). The mean range of the TL for the male specimen from all the locations was between 96 – 285 mm. The mean range for TL for the female specimen was 142mm for the specimen from Ogbomoso and 296 mm for the specimen from Asejire (Table 2 a & b).

The Ear length (EL) value of specimen from Ogbomoso(male) was the highest (15.7 mm), followed by specimen from Asejire (14.8 mm) and 14.6 mm from Ilorin (Table 2 a). The EL for the female specimen (Table 2 b) was 15.6 mm for the specimen from Ogbomoso, 15.1 mm for the specimen from Asejire and 14.9 mm for the specimen from Ilorin. The total body length (TBL) for the male specimen from all the locations ranged between 384. 1-421.9 mm. Specimen from Ilorin has the highest TBL (421.9 mm), followed by the specimen from Ogbomoso (392.2 mm) and 384.1 mm for the specimen from Asejire.

The Hind-foot length (HFL) for the male specimen from all the location showed that specimen from Ogbomoso has the highest value (45.9 mm) while specimens from Asejire and llorin have 44.5 and 43.1 mm, respectively. The HFL for female specimen were 45.9 mm (Ogbomoso) and 43.8, 43.3 mm for specimens from Asejire and llorin, respectively. The Body weight (BW) of the male specimen from all the location was smaller when compared with the

female (Table 2 a & b). The male specimen body weights were 182.2, 180.9, and 150.4 g for the specimen from Ilorin, Asejire, and Ogbomoso. The body weight for the female specimens was 205.9, 199.2 and 192.4 g (Asejire, Ilorin, and Ogbomoso) respectively.

3.2 Variations in the Measured Cranial Parameters across Locations

The measurement of cranial parameters across the three sampled locations (Asejire, Ilorin, and Ogbomosho) are shown in Fig. 3 a & b. NL (Length of Nasal), BCB (Breadth of Braincase), ISB (Inter-septal breadth), IFL (Length of incisive foramen) and LMT (Mandibular toothrow length) were similar in the specimen from all the location between male and female. While NB (Breadth of Nasal), IOB (Interorbital Breadth), GLS (Greatest length of skull),ROL (Rostrum length),BPB (Breadth of Bony Plate), POB (Breadth behind postorbital process),ID (Depth of Incisor),BL (Length of Auditory Bulla), ROB (Rostrum Breadth), PPL (Postpalatal length), IFB (Breadth across the incisive foramina), DL (Length of Diastema) and MIB (Breadth of First Upper Molar) differ between male and female specimen from the three locations (Asejire, Ilorin and Ogbomoso).

The male specimen from Ilorin location differ in cranial parameters, ZB (Zygomatic Breadth), ID (Depth of Incisor),ONL (Occupational Length), POB (Breadth behind postorbital process), BPL (Length of Bony Plate), PPL (Postpalatal length, TRL (Length of maxillary toothrow), ROB (Rostrum Breadth) and OCB (Breadth across occipital condyle) when compared to male specimens from Asejire and Ogbomoso. The female specimen from Ilorin differs in cranial parameter (NB, ZB, GLS, ONL, ML, and THM) when compared with female specimens from Asejire and Ogbomoso.

The male specimen from Asejire and Ilorin differ from male specimen from Ogbomoso in some cranial parameters (GLS, BCD, BL, IFB and ML), while the female specimen from the two locations

а												
Locations	Locations HBL		TL		TBL		EL		HFL		BW (g)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Asejire	198.3 ^a ±2.8	121-243	196.3 ^a ±2.4	96-285	390.5 ^ª ±2.9	217-425	14.8 ^a ±0.2	11-18	44.5 ^a ±0.5	40-52	180.9 ^a ±3.3	53-300
llorin	209.8 ^a ±3.1	179-265	210.6 ^b ±2.7	182-307	419.9 ^{ab} ±7.2	377-568	14.6 ^a ±0.3	13-16	43.1 ^{ab} ±0.3	41-54	182.2 ^b ±5.2	115-325
Ogbomoso	194.2 ^ª ±2.1	141-221	181.5 ^ª ±2.1	142-208	376.2 ^ª ±3.3	283-429	15.7 ^ª ±0.3	13-18	45.9 ^a ±0.4	42-49	150.4 [°] ±5.1	60-198

Table 2. Comparison of the measured morphometric parameter of *Funisciurus leucogenys* caught in four different locations during the period of study (a) male, (b) Female (mm)

b

Locations	Н	BL	TI	-	TI	BL	E	Ľ	HF	=L	BW	(g)
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Asejire	200.6 ^a ±2.8	161-237	199.5 ^ª ±5.4	152-296	384.1 ^a ±7.5	352-435	15.1 ^ª ±0.1	12-17	43.8 ^ª ±0.3	41-56	205.9 ^a ±3.7	158-274
llorin	229.8 ^a ±4.1	179-275	227.1 ^b ±4.7	171-345	421.9 ^{ab} ±7.2	352-585	14.9 ^ª ±0.3	13-17	43.3 ^{ab} ±0.3	39-47	199.2 [⊳] ±5.5	148-260
Ogbomoso	199.2 ^a ±2.1	182-294	194.5 ^ª ±5.1	142-212	396.2 ^ª ±8.3	270-444	15.6 ^ª ±0.3	14-18	45.9 ^a ±0.4	40-49	192.4 [°] ±5.1	155-251

*Column means with the same superscript are not significantly different (P > 0.05) from each other. HBL – Head Body Length; TL – Tail Length; TBL – Total Body Length; EL – Ear Length; HFL – Hind Foot Length; BW – Bodyweight

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Fig. 4. Histogram showing the cranial measurements of tree squirrel (*Funisciurus leucogenys*) specimen from three locations (Asejire, Ilorin & Ogbomoso). (a) Male (b) Female

(Asejire and Ilorin) differ from female specimen from Ogbomoso in cranial parameters such as ROL, BPB, MLT, OCB, and TRL, respectively.

Ilorin and Ogbomoso specimen are similar in cranial parameters MLT and IOB in the male specimen. They (Ilorin and Ogbomoso) are also similar in BCD in the female specimen. The specimens (both male and female) from the two locations differed in MLT, IOB, and BCD when compared to the specimen from Asejire.

Cranial parameters measured were subjected to a Principal Component Analysis (PCA). PCA showed correlations and variance among male and female specimens collected from the three locations in this study. The Principal Component Analysis divided the cranial parameter measured between male and female into three [3] namely the PC [1,2,3] representing each location (Asejire, Ilorin and Ogbomosho) respectively. The eigenvalue and percentage variance for male and female specimen from the locations are shown in Table 3a. From this Table, only PC1 (Asejire) of Eigenvalue 3312.14 and percentage variance of 99.87 were most significant being higher than the Joliffe cut- off 82.75.

In Table 3b, it is observed that PC1 (male and female specimen from Asejire) had the highest eigenvalue of 2.89142 and percentage correlation of 99.87 compared to all other specimens. Here in correlation, the Joliffe cut-off is 0.7. The loading plot in Fig. 4 showed the variance that occurred in the cranial parameters measured among male and female specimen from the study locations.

A scatter plot diagram in Fig. 5 showed a cluster eclipse of all the cranial parameters of male and female from all the locations (Asejire, Ilorin, and Ogbomosho). The plotting showed that all the cranial parameters measured from male and female specimens in all the locations belong to the same homogeneous population, though there might be a very little variation which is statistically insignificant.

Table 3. The eigenvalue and the percentage (a) variance (Joliffe cut-off of 82.75) and percentage (b) Correlation (Joliffe cut-off of 0.7) of male and female of *F. leucogenys* from three different locations

PC		а	b			
	Eigenvalue	% Variance	Eigenvalue	% Correlation		
1	3312.14	99.872	2.98112	99.872		
2	0.62142	0.11420	0.00482	0.11382		
3	0.14516	0.01380	0.00126	0.01418		



Fig. 5. PCA scatter plot for *Funisciurus leucogenys* specimens showing relationship between sexes (M = male (red), F = female (blue))

4. DISCUSSION

The composition and the abundance of tree squirrel from the three locations (Asejire, Ilorin, and Ogbomoso) showed that female F. leucogenys were more than the male. Although, the abundance of female than male may be attributed to the search of food and mating period which make the female fall victim of the hunters during the study period. The territorial defence that is carried out by the male may also be responsible for the decrease in the number of male tree squirrel in this study. Palombit [20] reported that female squirrel is more dominant in various locations due to reproduction and nursing of their young ones. Urbanization can also contribute negatively to the abundance of tree squirrel in Nigeria. Increase in population which led to the development of rural area poses a big threat to tree squirrel since most of their drey nest will be destroyed during deforestation. Urban and rural developments have been reported to lead to an overall loss of native biodiversity [21]. Bamidele and Akinpelu [7] also reported an increase in the amount of female Red-legged Sun Squirrel (H. rufobrachium) caught in some locations in Nigeria (Asejire, Ilorin, and Ogbomoso).

parameters Variations cranial and in external body measurements of tree squirrels from different locations (Asejire, Ilorin, and Ogbomoso) may be attributed to so many factors among which is the sex, metabolism rate, climate factors and mating success [22]. Sex (male and female) difference may be responsible for a variation on external body measurement of the tree squirrels. Female species are expected to have high body weight and some other parts of the body than the male due to reproduction and nursing of the young ones. Heldslab et al. [23] reported that female mammal's ability to bear the energy cost of pregnancy and lactation may be affected by the amount of the body fat she can store. The stored body fat may be responsible for the increase in body weight experienced in this study. Although the body weight and other parts of the female specimen slightly different from the male, there was a positive correlate on (0.72)among the external body parameters measured among the specimen from the three locations. The correlation is similar to our previous study on Red-legged Sun Squirrel from the same locations [7].

The abundance of food in a location such as Asejire and llorin may also be responsible for the

high body weight recorded among the specimens from such location. Heldslab et al. [23] showed that in the natural animal population, higher food abundance leads to higher birth rate and body weight, while food restriction leads to stunted growth, delay sexual maturation and inhibit mating. Age of the specimen may also be responsible for the difference in body weight and size. Younger specimen (tree squirrel) is expected to have lower body weight compared to older specimen, but age was not considered for this study.

The cranial parameters measured between male and female specimen showed a lot of similarity and differences among the specimen from different locations. Although, cranial parameters have been used to differentiate among species [10]. Our findings showed that despite the differences in the cranial parameters measured, the tree squirrels from the three locations are of the same genius (F. leucogenys). The scatter plot showed a lot of overlap in the cranial parameters measured between the male and female specimen and the eigenvalue also confirmed it. The similarities in cranial parameters may be due to the zone from which the specimens were collected (Savannah). This finding was the same about Red legged trees squirrel from Savannah zone in Nigeria [7].

The differences in the cranial parameter measured may be associated with the climate factors, metabolism rate, competition and mating success among the specimens from the different locations [22]. Although, size variation is generally considered to be related to environmental gradient than shape [24], the difference in the cranial parameters measured may also be associated with climate change.

5. CONCLUSION

The cranial and external body measurement between male and female tree squirrel (*F. leucogenys*) in this study shows that the specimen is of the same genus. The male and female specimens from all the locations have little difference in external and cranial parameters measured but no distinctive different to categorize them to another genus of tree squirrel. From this study, there is no new species of Orange headed tree squirrel (*F. leucogenys*) from the locations of study.

DISCLAIMER

This paper is based on preliminary dataset. Authors declare that "We will like to state

categorically here that dealing with local hunters for the collection of the specimen was private matter because the specimen was usually hunted by them to make money without collecting ethical clearance or permission in their various location."

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ETHICAL APPROVAL

As per international standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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