

Comparison of cranial and body morphology of tree squirrels (*Helioscarius rufobrachium*) in selected locations of rainforest in Nigeria

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Abstract

This study examined the differences in cranial morphology of tree squirrel species (*Heliosciuru srufobrachium*) from four different locations in rainforest part of Nigeria. Locally fabricated live traps made of wire-mesh and steel were used to capture the squirrels. Trapped tree squirrels were immediately transferred to the laboratory in cages, where they were euthanized by placement in a bell-jar containing chloroform-soaked cotton wool. Individuals from two locations (33 from Ile Ife and 49 from Emure Ekiti) were then preliminarily identified to the generic level, using an identification key. Eighty-two (82) skulls were prepared from them, using Long Island Natural History Museum Guide. The sexes of the specimens were determined by visual inspection of the external genitalia. Twenty-seven (27) cranial and six (6) external body parameters were measured using digital Vernier caliper. One-way analysis of variance at $p < 0.05$ was used to determine the difference in measured body parameters and Principal Component Analysis was used to differentiate the measured skull parameters. The results showed that the body parameters; head body length, tail length, tail body length, ear length, hind foot length and body weight measured were slightly different from one location to another. Also, the cranial measurements showed some similarities and differences between the two locations (Ile Ife and Emure Ekiti). In conclusion, the cranial measurements of the tree squirrels from the two locations (Ile Ife and Emure Ekiti) showed slight differences despite the fact that the tree squirrels are from the same genus (*H. rufobrachium*).

Keywords: *Heliosciurus rufobrachium*; rainforest; tree squirrel; cranial; morphometric.

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Introduction

Conservation of biodiversity has been described as one of the principal environmental challenges for humanity (Wilson, 1992). Although considerable scientific efforts are devoted to conservation issues, however, a gap exists between theory and practices in conservation activities. Considerable attention and funds are directed toward a few species of high economic or emotional interest (Amori and Gippoliti, 2000). Tree squirrels across the globe are considered at risk (Koprowski and Steele, 1998). Awareness of the conservation status of squirrel when viewed in the context of the ecological significance of taxa takes on great importance. The major threats to squirrels' persistence are habitat loss and fragmentation, over-exploitation, persecution, and invasive species (Steele and Koprowski, 2001).

Tree squirrels are excellent indicators of forest health and structure (Carey, 2000; Steele and Koprowski, 2001) due to their dependence on mature forests for seeds, nest sites, cover, and microclimates for food storage (Steele and Koprowski, 2001). The presence, demographics, and

habitat use of tree squirrels could indicate the status of forested ecosystems. The geographic range distribution of squirrels is of precarious conservation status with 88% of nine *Sciurus*, 67% of three *Tamiasciurus*, and 100% of two *Glaucomys* receiving legal protection due to concerns about persistence in a portion of the species' range. Squirrels rely on mature forests that produce quantities of seed, shaded microclimates for fungal growth and seed storage, and nest cavities (Steele and Koprowski, 2001). Disturbance of such conditions is not conducive to short-term persistence of populations in a local area. Management schemes that do not promote return of forests to such conditions after disturbance will therefore impede re-establishment and persistence of squirrels (Koprowski, 2005).

Heliosciurus rufobrachium (Red-legged Sun-squirrels) live in rainforest habitats as well as in relic forests in the savanna where there are tall trees. Rosevear (1969) reported that the distribution patterns of *H. rufobrachium* do not overlap except along the forest-savanna boundary. The Red-legged Sun squirrels were also



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reported to be diurnal, nest in hollows high up in trees and feed on a variety of fruits and nuts, and sometimes may eat insects. *H. rufobranthium* has been recorded in Ibadan, Ilase, Benin, Calabar, Lagos, Osu and Upper Ogun Ranch of Nigeria (Happold, 1987).

Morphometric analysis is a tool used in extracting information about biological material and biological processes. It is defined as the quantitative characterization, analysis, and comparison of biological forms (Roth and Mercer, 2000). The field of morphometry is concerned with methods for the description and statistical analysis of shape variation within and among samples of organisms and of the analysis of shape change as a result of growth, experimental treatment or evolution.

Morphometric methods are needed whenever one needs to describe and to compare shapes of organisms or of particular organs. The samples may represent geographic localities, developmental stages, genetic effects and environmental effects (Rohlf and Marcus, 1993).

Since 1987, there is little or no report on the tree squirrel (*H. rufobranthium*) in rainforest part of Nigeria. Hence, this study aims to compare the cranial morphology of tree squirrel (*H. rufobranthium*) in some parts of rainforest in Nigeria to establish effect of urbanization, deforestation and location on tree squirrel.

Materials and methods

Study area

Squirrel samples were collected within the rainforest zones of Nigeria. The squirrel samples were collected from Ile Ife (07° 21' N-07° 22' N and 004° 08' E-004° 10' E), Osun State, Nigeria, and Emure Ekiti (07° 25' N-07° 27' N and 005° 25' E-00° 27' E), Ekiti State, Nigeria.

Sample collection

The specimens (82 in total, 33 from Ile Ife and 49 from Emure Ekiti) were collected using locally fabricated live traps made of wire-mesh and steel of 18 x 18 x 45 cm (Plate 1) for a 19 month's period (November 2011-May 2013). The traps were baited with palm kernel, fresh corn, and groundnuts and were checked every morning and evening for trapped specimens. The trapped specimens were immediately transferred to the laboratory in cages, where they were euthanized by placement in a bell-jar containing chloroform-soaked cotton wool. Specimens that could not be examined immediately were preserved by immersion in jars containing 96% ethanol solution (Olayemi, 2006).

Specimen identification

Specimens were preliminarily identified to the generic level, using an identification key prepared by Happold (1987) and the sexes were determined by visual inspection of the external genitalia.

Skeletal preparation

The skull of each of the sacrificed specimens 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* (2000) 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 bacteria maceration. The skull was then washed 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.

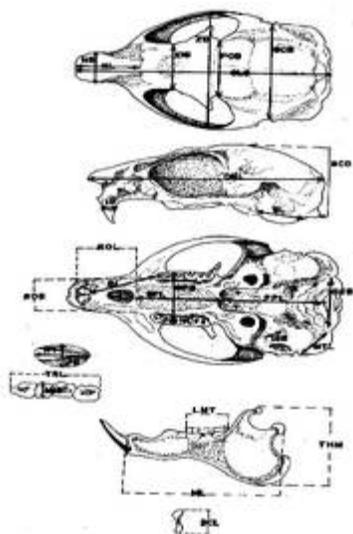
Cranial and external measurements

Twenty-seven (27) cranial and six (6) external measurements were taken on each specimen according to the method of Rosevear (1969), Happold (1987), Rasmussen and Thorington (2008), and Song *et al* (2012) using digital caliper (RUPAC, Italy). The Body weight (BW) was taken using digital weighing balance (Ohaus Scout Pro SPX422. NJ) The cranial measured parts include: 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 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) (Figure 1) while the external measurements include: Head body length (HBL), Tail length (TL), Total body length (TBL), Ear length (EL), Hind Foot length (HFL) as shown in Figure 2.

Figure 3 showed the map of Nigeria with the two States (Osun and Ekiti) as a whole and Figure 3b showed the map of the specific towns (Ile Ife and Emure Ekiti) where the specimens for the study were collected.

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine the significant difference between the means, while the significant mean was separated at $p < 0.05$ using Least Significant Difference (LSD) test from System Analysis Software (SAS Institute, 1997). Principal Component Analysis (PCA) was carried out with PAST version.



Legend

A.

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

Figure 1: Localization of the 27-craniodental measurements recorded in this study. Abbreviations for variables are defined in materials 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 Rasmussen and Thorington, 2008; Li *et al* 2012.

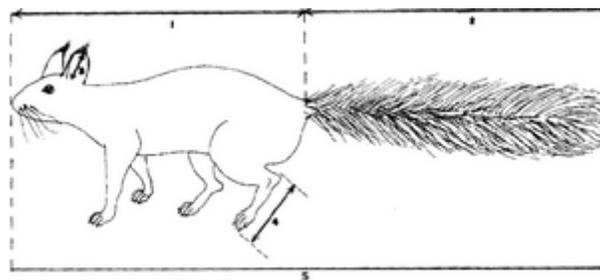


Figure 2: Diagrammatic representation of external measurements that were taken from a generalized tree squirrel (lateral view).

Source: Happold, 1987 modified.

Keys: 1. Head Body Length (HBL), 2. Tail Length (TL), 3. Ear Length (EL), 4. Hind foot Length (HFL), 5. Total body length "5"

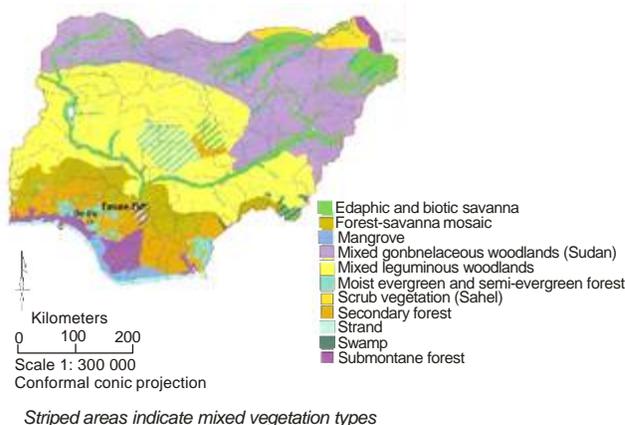


Figure 3: Map of Nigeria showing the sampling locations with respect to the vegetation zones modified.

Source: Macmillan Nigerian Publishers (2006).

Results and discussions

In both locations, higher numbers of males of *H. Rufobranthium* were collected compared to female (Table 1). The numbers of male to female in the various locations were: 18 to 15 (Ile Ife), and 31 to 18 (Emure Ekiti) respectively.

The variation in the number of specimens caught in location in Rainforest zone may be attributed to so many factors such as, type of trap used, the location of collection, types of foods in the location, predators (Dwiyahreni, 2003) and habitat fragmentation (Jaseenet *al* 2017). Although, *H. rufobranthium* specimens were collected during this study, but the quantity collected was small compared to the period of collection. This is an indication that tree squirrels in rainforest part of Nigeria are at risk due to urbanization and deforestation. These findings have been supported by Koprowski and Steele (1998), who reported that population of tree squirrels across the globe are considered at risk due to changes in food abundance, increasing inter- and intra-species competition, urbanization and predation by humans.

Table 1: Composition of *H. rufobranthium* caught during the period of study.

Locations	Male	Female	Coordinates
Ile Ife	18	15	07° 21' N- 07°22' N and 004° 08' E-004° 10' E
Emure Ekiti	31	18	07° 25' N-07° 27' N and 005° 25' E-005° 27' E.
Total	49	33	

The measured morphometric parameters of *H. Rufobranthium* caught in the two locations are shown in the Table 2. The Head Body Length (HBL) of the specimens caught in the two locations have significant difference ($p < 0.05$), with different range of 210-260 for Ile Ife and 220-250 for Emure Ekiti. The Tail Length (TL) also showed significant difference at $p < 0.05$, for specimens from the specimens collected from the two locations.

The Total Body Length (TBL), Ear length (EL), Hind foot length (HFL) and Body weight (BW) followed the same pattern like that of HBL and TL. There was significant difference between specimens from Ile Ife and Emure Ekiti at $p < 0.05$. Also, the range measure of the specimens was wide apart from the two locations. In most cases, the least measure part in specimen from Emure Ekiti was always higher than that of specimen from Ile Ife.

Body measurement has been found useful in differentiating between types of sifakas (*Propithecus*). Lehman *et al* (2005) used the mean body size to correlate the climate data, annual rainfall and seasonality among different types of sifakas in Madagascar. They reported that the mean body size was positively correlated with annual rainfall and negatively correlated with seasonality, with body size difference attributed to variations in habitat productivity and resource seasonality.

The significant difference at $p < 0.05$ for all the parameters (HBL, TL, TBL, EL, HFL and BW) measured for the specimens collected in Ile Ife and Emure Ekiti locations may be attributed different factors such as age and sex of the specimens. Abundance of food in all the locations was not considered but it may also contribute to higher value of the body measurement of the specimens from Emure Ekiti. The report of Selonen *et al* (2016) showed the effect of food abundance on reproduction of

two arboreal squirrel species. Abundance of food lead to high reproduction rate which can also be said to lead to high growth rate. Selonen *et al* (2016) reported higher body measurements in female flying squirrel than male. They (Selonen *et al*, 2016) also reported that age difference affected the body parts of flying squirrels, which may also affect the tree squirrels.

Variations in the measured cranial parameters across locations

Analyses of measured cranial parameters across the two sampled locations (Ile Ife and Emure Ekiti) are shown in Table 3. NL (Length of Nasal), NB (Breadth of Nasal), IOB (Interorbital Breadth), ZB (Zygomatic Breadth), POB (Breadth behind postorbital process), BCB (Breadth of Brain case), ONL (Occupational Length), ROB (Rostrum Breadth), OCB (Breadth across occipital condyle), IFL (Length of Incisive foramen), IFB (Length across the incisive foramina), LMT (Mandibular tooththrow length), and THM (Height of mandible) were found to be significantly different ($p < 0.05$) in the populations of *H. rufobranthium* across the two locations of study.

The remaining cranial parameters measured, GLS (Greatest Length of Skull), ID (Depth of Incisor), BCD (Depth of Braincase), BL (Length of Auditory Bulla), ROL (Rostrum length), DL (Length of Diastema), BPL (Length of Bony Plate), BPB (Breadth of Bony Plate), PPL (Post palatal Length), ISB (Inter-septal breadth), MTL (Mastoid Length), MIB (Breadth of First Upper Molar), TRL (Lengths of Maxillary Tooththrow), and ML (Mandible Length), were not significant different ($p > 0.05$) in populations of *H. rufobranthium* across the two locations of study.

The cranium is a peculiar area of phylogenetic research for assessing mammalian relationships and it serves as the major skeletal component from which morphological measurements are taken (Voss and Jansa, 2009). According to Zelditch *et al* (2012), subtle cranial differences can be shown by measurements and quantitative comparisons of the skull among rodents. Corti *et al* (2000) reported that since the skull contains the major sensory organs, the brain and the feeding apparatus, it also contains a lot of information on the phylogeny, ontogeny and adaptation of rodents. It is thus not surprising that skull characters have been the primary source of study in rodent morphology.

Table 2: Comparison of measured morphometric parameters of *H. rufobranthium* caught in four different locations during the period of study (mm).

Locations	HBL		TL		TBL		EL		HFL		BW	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
IleIfe	236.8 ^a ±	210-	225.5 ^a ±	201-	441.9 ^a ±	401-	15.0 ^a ±	13-18	47.5 ^a ±0	41-55	282.2 ^a ±	192-
	1.8	260	2.4	295	2.5	555	0.5		.3		3.3	427
Emure Ekiti	241.5 ^b ±	220-	282.5 ^b ±	258-	524.8 ^b ±	508-	16.8 ^b ±	13-17	54.3 ^b ±0	44-55	319.6 ^b ±	244-
	2.5	250	3.1	284	3.4	535	0.2		.3		4.2	359

*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 – Body Weight.

Table 3: Comparison of cranial parameter of *H. rufobranthium* across the two locations of the study.

Parameters	Ile Ife	Emure Ekiti
NL	14.7 ^a ± 0.2	15.6 ^b ± 0.2
NB	7.9 ^a ± 0.2	9.4 ^b ± 0.2
IOB	13.2 ^a ± 0.2	16.2 ^b ± 0.2
ZB	27.2 ^a ± 0.5	29.0 ^b ± 0.5
POB	15.2 ^a ± 0.1	16.8 ^b ± 0.1
BCB	20.2 ^a ± 0.3	22.8 ^b ± 0.3
GLS	51.1 ^a ± 0.9	51.7 ^a ± 0.9
ID	2.8 ^a ± 0.1	2.9 ^a ± 0.1
BCD	17.0 ^a ± 0.1	17.3 ^a ± 0.1
ONL	49.2 ^a ± 0.5	50.2 ^b ± 0.5
BL	9.7 ^a ± 0.1	9.3 ^a ± 0.1
ROB	7.1 ^b ± 0.3	7.4 ^a ± 0.3
ROL	10.2 ^a ± 0.3	10.4 ^a ± 0.3
DL	12.1 ^a ± 0.3	12.5 ^a ± 0.3
BPL	14.3 ^a ± 0.2	14.4 ^a ± 0.2
BPB	12.2 ^a ± 0.2	12.7 ^a ± 0.2
PPL	19.0 ^a ± 0.5	18.9 ^a ± 0.3
ISB	5.8 ^a ± 0.1	5.4 ^a ± 0.3
MTL	9.2 ^a ± 0.3	9.7 ^a ± 0.3
OCB	12.4 ^b ± 0.3	11.6 ^a ± 0.2
IFL	5.7 ^b ± 0.3	4.8 ^a ± 0.3
IFB	3.1 ^b ± 0.1	2.4 ^a ± 0.3
MIB	2.7 ^a ± 0.1	2.7 ^a ± 0.3
TRL	9.9 ^a ± 0.3	9.8 ^a ± 0.3
LMT	9.3 ^b ± 0.1	8.9 ^a ± 0.3
ML	29.2 ^a ± 0.4	29.4 ^a ± 0.5
THM	17.2 ^a ± 0.3	18.9 ^b ± 0.3

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 Length (MTL), Breadth across Occipital Condyle (OCB), Length of Incisive Foramen (IFL), Breadth across the Incisive Foramina (IFB), Breadth of First Upper Molar (MIB), Length of Maxillary Tooththrow (TRL), Mandibular Tooththrow Length (LMT), Mandible Length (ML), Height of Mandible (THM). (Length of Incisive Foramen), IFB (Breadth across Incisive Foramina), LMT (Mandibular Tooththrow Length) and ML (Mandible Length).

Although the tree squirrels' populations were of the same genus (*Helioscirus*) and from the same rainforest in Nigeria, the cranial measurements showed some similarities and difference in the cranial parts measured. In most of the cranial parameters (skull part) measured, specimen from Emure Ekiti have higher value when compared with the Ile Ife locations.

This is similar to the report of Li *et al* (2012), who reported craniometrics differences among the four species of giant flying squirrels (*Petauristayunanensis*, *P. philippensis*, *P.hainana*, *P.petaurista*) in China. There is a lot of overlap in the cranial parts measured among the different locations. This is in line with the report of Canady *et al* (2015) who reported overlap in some cranial parts measured among the Eurasian Red Squirrels (*Sciurus vulgaris*) from Slovakia. Also, Song *et al* (2012) measured

26 cranial variables in 60 adult specimens of *Petauris tayunanensis*, *P. philippensis*, *P. hainana*, and *P. petaurista* to establish significant difference in craniometrics states among the four species. Their results revealed no sexual dimorphism in any of the four species but confirmed significant craniometrics differences among the four species in both the principal components analysis (PCA) and discriminant function analysis (DFA), with the greatest distinction observed between *P. petaurista* and other *Petaurista* species.

The overlap recorded was associated with sex and age of the squirrels. For further confirmation of the differences, *H. rufobranthium* specimens collected from the two locations were pooled and subjected to PCA to determine whether the recorded variations were significant. To the best of our knowledge, this is the first study on *H. rufobranthium* in rainforest that deal with cranial measurements.

Table 4: Principal components of cranial measurements of *H. rufobranthium* compared based on location.

PC	Eigen value	% Variance
1	13.1768	77.359
2	2.58643	15.184
3	1.27018	7.4570
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	0	0
16	0	0
17	0	0
18	0	0
19	0	0
20	0	0
21	0	0
22	0	0
23	0	0
24	0	0
25	0	0
26	0	0
27	0	0

The Eigen values on the PCA loadings (Table 4) showed that PC1 contributed 67.432% of the variation while PC2 contributed 25.378% of the variance. The PCA loadings (Table 4) showed that nineteen (19) cranial characters were discriminatory between the populations from the various locations. The discriminatory characters were: NL (length of Nasals), NB (Breadth of Nasals), IOB (Interorbital Breadth), ZB (Zygomatic Breadth), POB (Breadth behind Postorbital Process), BCB (Breadth of Braincase), GLS (Greatest Length of Skull), ID (Depth of Incisor), BCD, (Depth of Braincase), ONL (Occipitonasal Length), BL (Length of Auditory Bulla), DL (Length of Diastema), BPL (Length of Bony Plate), BPB (Breath of Bony Plate), ISB (Interseptal Breath), MIB (Breadth of First Upper Molar), LMT (Mandibular Toothrow Length), ML (Mandible Length) and THM (Height of Mandible).

All these cranial characters showed the discrimination among the locations. According to the report of Li *et al* (2012) on the cranial measurement of giant flying squirrels in China and Canady *et al* (2015) on cranial measurement of Red Squirrel, they both reported some discrimination on the skulls measured. They both attributed the differences to sex of the squirrels and the age. The same reason may be responsible for what we observed since sex and age were not taken into consideration for this study.

Table 5: PCA Loadings showing the discriminatory characters in *H. rufobranhium* populations across the two locations.

Factors	PC 1	PC 2
NL	-0.7011	
NB	-0.7754	
IOB	-0.7900	
ZB	-0.7837	
POB	-0.5280	
BCB	-0.7895	
GLS		0.7082
ID	-0.7091	
BCD		0.6070
ONL	-0.7582	
BL		0.7352
DL	-0.7607	
BPL		0.5842
BPB		0.5019
ISB		0.7053
MIB		0.7640
LMT		0.6358
ML		0.6766
THM	-0.6692	

The discriminatory cranial characters between the sexes were: IOB (Interorbital Breadth), ZB (Zygomatic Breadth), GLS (Greatest Length of Skull), ID (Depth of Incisor), BCD (Depth of Braincase), ONL (Occipitonasal Length), ROB (Rostrum Breadth), ROL (Rostrum Length), DL (Length of Diastema), BPB (Breadth of Bony Plate), PPL (Post Palatal Length), MTL (Mastoid Length), OCB (Breadth across Occipital Condyle), IFL (Length of Incisive Foramen), IFB (Breadth across Incisive Foramina), LMT (Mandibular Toothrow Length), ML (Mandible Length) and THM (Height of Mandible)

Figure 4 shows the PCA scatter plots for *H. rufobranhium* specimens showing the relationships between the means of cranial features of populations from the two locations. The scatter diagram showed that populations from Ile Ife were slightly different from those from Emure Ekiti location.

The differences observed maybe attributed to availability of food, urbanization and predators which influence the type of life style tree squirrels where living. Also, the age of the specimens maybe another factor responsible for the difference since age, food urbanization and predation has been factors reported to influence reproduction and body mass of squirrels (Dwiyahreni, 2003).

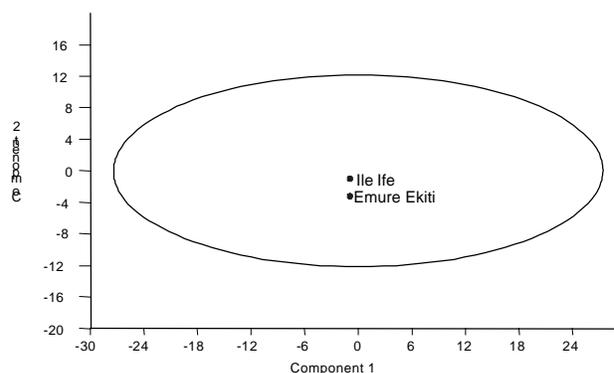


Figure 4: PCA scatter plots for *H. rufobranhium* specimens showing the relationship among the populations across the two locations.

Conclusion

H. rufobranhium in Ile Ife and Emure Ekiti, Nigeria, have similar body and cranial measurements despite different locations of collection. Cranial measurements can serve as a good tool to differentiate between populations of tree squirrels, but molecular techniques (using DNA, PCR and sequencing) are required to confirm the influence of some factors (climate change and deforestation) that may contribute to changes in the populations of tree squirrels.

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