Int. J. Morphol.,
37(3):1003-1007, 2019.

A Gross Morphometric Study of Olfactory Brain Components in the Rufous Sengi (*Elephantulus rufescens*)

Estudio Morfométrico de los Componentes Olfativo del Cerebro en el Rufo Sengi (*Elephantulus rufescens*)

Boniface Mwanzia Kavoi[1] & Mosiany Letura Kisipan[2]

KAVOI, B. M. & KISIPAN, M. L. A gross morphometric study of olfactory brain components in the rufous sengi (*Elephantulus rufescens*). *Int. J. Morphol., 37(3)*:1003-1007, 2019.

SUMMARY: The gross morphometric features of mammalian olfactory system components show variations that may be attributed to dietary and ecological factors. We analyzed volumes and linear dimensions of olfactory brain components (OBC) namely, olfactory bulb (OB), olfactory tract (OT) and olfactory stria (OS) in an Afrotherian insectivore, the rufous sengi. These findings were then compared with those obtained previously in dogs (carnivore), goats (herbivore) and humans (omnivore). Volumes, lengths and breadths of the OBC were compared with those of the cerebral hemisphere (CH) and the whole brain (WB) by working out their ratios (%). In the sengi, the volume of OBC: WB was 1.03 %, length of OBC: CH = 58.08 % and breadth of OB: CH = 28.97 %. In an earlier report by Kavoi & Jameela respective values for the above parameters were 0.03 %, 21.47 % & 8.94 % in humans, 0.77 %, 51.87 % & 29.73 % in goats and 1.95 %, 72.30 % & 42.91 % in dogs. These observations suggest that the anatomical design of OBC happens in a manner that mimics an animal's level of reliance on the sense of smell vis-à-vis feeding lifestyles, habitat and dynamics of evolution.

KEY WORDS: Macroscopic; Morphometry; Olfactory brain; Sengi.

INTRODUCTION

The small family of elephant shrews or sengis (order Macroscelidea) represents a monophyletic radiation endemic to Africa and which was long placed within the polyphyletic group 'Insectivora' (Rathbun & Rathbun, 2006). Later, taxonomic repositioning saw the shifting of the sengi from 'Insectivora' to superorder Afrotheria, a group of placental mammals whose ancestral lineage can be traced to ~11 million years ago (Smit *et al*., 2011). Being Afrotherians, sengis share similar evolutionary origin with elephants, hyraxes, dugongs, sea cows, aardvarks, golden moles and tenrecs (Dengler-Crish *et al*., 2006). The tenrec brain is reputed for its resemblance to that of ancestral mammals owing to its relative small size with little neocortex (Künzle, 2003). Elephants, on the other hand, present an extreme situation with respect to body and brain size and social complexity (Hakeem *et al*., 2005). Given this diversity, Afrotherians seem to represent an untapped resource of information about sensory systems and brain organization in mammals.

Kaufman *et al*. (2013) demonstrated that sengis have relatively large brains when compared with similarly-sized terrestrial mammals that share a similar diet. Indeed, the brain of the sengi has a remarkably prominent olfactory center and a hippocampus (part involved in spatial memory and navigation), which is over three times larger than that of basal insectivores (Stephan & Andy, 1964), larger than that of most primates, and equal in relative size to that of humans (Stephan, 1983). In their habitat, sengis use their long trunk-like noses to probe through crevices in search of prey, particularly ants and termites (Rathbun, 2005). In regard to locomotor behavior, sengis differ quite remarkably when compared with most other insectivore-grade mammals in that their movement is notably agile (Rathbun, 2005). Worthwhile to note also is that sengis create and maintain a complex olfaction-dependent trail system that enables them to escape predators and to exhibit social monogamy, a unique characteristic among mammals which is necessitated by factors like resource and female

[1] Department of Veterinary Anatomy and Physiology, Faculty of Veterinary Medicine, University of Nairobi, Riverside Drive, Nairobi, Kenya.
[2] Department of Veterinary Anatomy and Physiology, Egerton University, P.O. BOX 536-20115, Egerton, Kenya.

dispersion, indirect paternal investment and male mate guarding (Rathbun, 2005; Rathbun & Rathbun).

In the literature, it is evident that earlier authors paid little attention in understanding how evolutionary dynamics affect olfactory system architecture and how this influences neural mechanisms and behavior in Afrotheria. It has been shown in several studies (Barton *et al*., 1995; Finlay & Darlington, 1995; Meisami & Bhatnagar, 1998; Barton, 1999) that ecological and behavioral factors impact, to a great degree, on the size and morphology of specific nervous structures in mammals. In a recent study (Kavoi, 2018), nasal olfactory structures in sengis were compared with those of other placentals with the observation that the anatomy of such structures was influenced by feeding lifestyles and habitat. To explore this further and to generate new information, we analyzed volumes and linear dimensions of the sengi's OB, OT & OS and the ratios of these parameters with respect to the cerebrum and the entire brain. These data were then compared with those reported for other eutherian mammals in Kavoi & Jameela (2011).

MATERIAL AND METHOD

Experimental animals. Seven male (body weight: 40-56 grams) rufous sengis (*Elephantulus rufescens*) were captured in Sherman life traps from the outskirts of Voi town, located 350 km South-East of Nairobi. These animals, which provided brains for this study and organ from other body systems for investigations by my colleagues, were trapped under permit (no. 4004) from the Kenya Wildlife Service. All procedures related to the use of these animals were approved by the Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nairobi, and strictly adhered to the guidelines provided in the Animals (Scientific Procedures) Act, 1986.

Fixation and harvesting of the brains. Immediately after capture, animals were sacrificed by injecting them with lethal doses of pentobarbital sodium (140 mg/kg) through the intraperitoneal route. The thoracic cavity of the freshly euthanized animals was opened up and brains were fixed by perfusion through the heart using 10 % formaldehyde. The head of the carcass was then chopped off and harvesting of the brain from the cranial cavity was performed as highlighted in Onyono *et al*. (2017). In brief, bones of the skull were broken using a dissecting knife and a pair of thumb forceps to expose the brain, which was then removed (with the bulbs intact) and allowed to fix further by immersing it overnight in 10 % formaldehyde.

Measurement of linear dimensions. Linear measurements of the cerebrum were carried out (on the brain in situ) as shown in Figure 1. Separation of the OBC namely, OB, OT and OS was performed as outlined in Figure 2. After separating the OBC, linear dimensions were then determined as shown in Figure 3. Vernier calipers, thread and meter rule were used to measure the greatest lengths and breadths of the above mentioned structures. Two trained technicians performed the measurements with intra and inter-observer errors of between 2 to 3 %.

Determination of volume of the brain and its olfactory structures. The volume of the entire brain and that of the OBC (dissected out from the brains as illustrated in Figure 2) were determined using the method of Scherle (1970). To achieve this, a container filled with physiological saline was placed on an electronic analytical balance and the structure of interest (initially suspended with a fine thread from a clamp attached onto a stand) was fully immersed in the saline. The change in weight reading (in grams) equals to the volume of the structure (in cubic centimeters).

Analysis of data. Measurement of volumes and linear dimensions on the brain and its components were repeated three times and value means were recorded together with their standard deviations (SDs). Comparisons of the measurement values of OBC with those of the cerebrum and the entire brain were expressed as ratios (%).

RESULTS

Table I provides data on volumes of the sengi's OB and OBC and the proportions of the volumes of these structures to those of the cerebrum and the entire brain. Additionally in this table, the sengi's volumes and volume ratios for the above structures are compared with those already recorded in Kavoi & Jameela for dogs (carnivores), sheep (herbivores) and humans (omnivores). In the sengi, the volume (in mm^3) was 0.03 ± 0.01 for the OB alone, 0.07 ± 0.03 for the OBC and $6.78.3 \pm 1.49$ for the entire brain (Table I). Volume proportion of the OB to the brain was 0.44 % in the sengi and this value exceeded that reported for the dog, goat and humans by 0.13 %, 0.26 % and 0.43 %, respectively. The volume ratio of the sengi's OBC to the brain, which was estimated at 1.03 %, was greater than that of the goat and humans by 0.26 % and 1.00 %, respectively, but was 0.92 % less than that of the dog (Table I).

Linear dimensions (greatest lengths and breadths) of OBC and the cerebrum are presented in Table II. The length of sengi's OBC and the cerebrum were 11.5 ±1.04 and 19.8

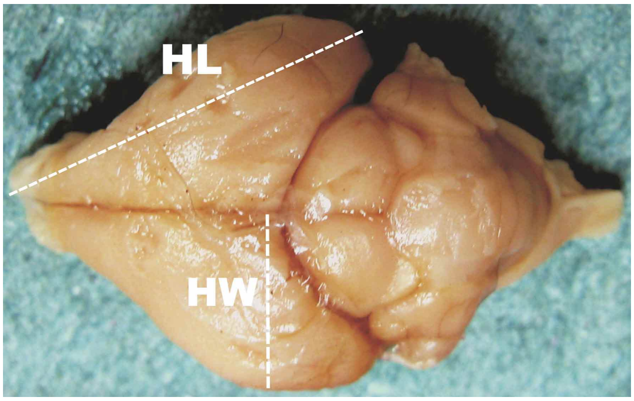

Fig. 1. A macrograph showing how linear measurements were carried out on the sengi's brain. HL: greatest length of the cerebral hemisphere is measured from the dorsal aspect of the brain. HW: hemisphere width or breadth is taken at its widest points from the dorsal aspects of the brain.

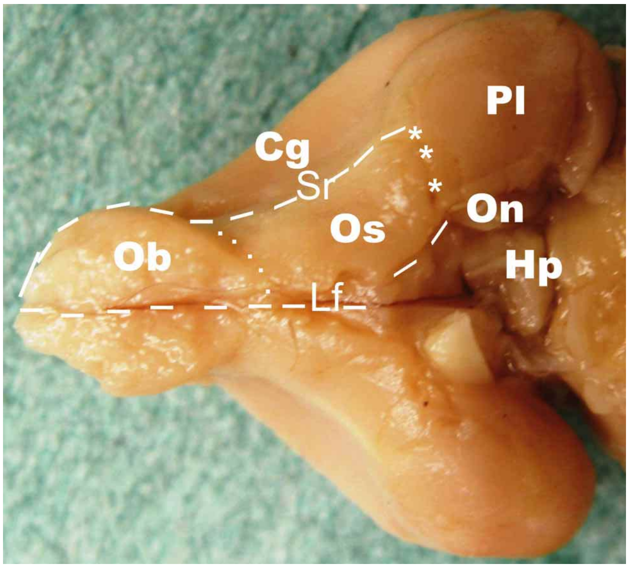

Fig. 2. A ventral view of the cerebrum illustrating how olfactory brain components were marked out for separation. The olfactory bulb (Ob), olfactory stria (Os) and olfactory tract (on which the bulb sits) are marked out for dissection along the sulcus rhinalis (Sr) and the longitudinal fissure (Lf) and extending posteriorly to the lateral fossa (*). Abbreviations Pl, Cg, Hp and On stands for piriform lobe, lateral cerebral gyrus, hypothalamus and optic nerve respectively.

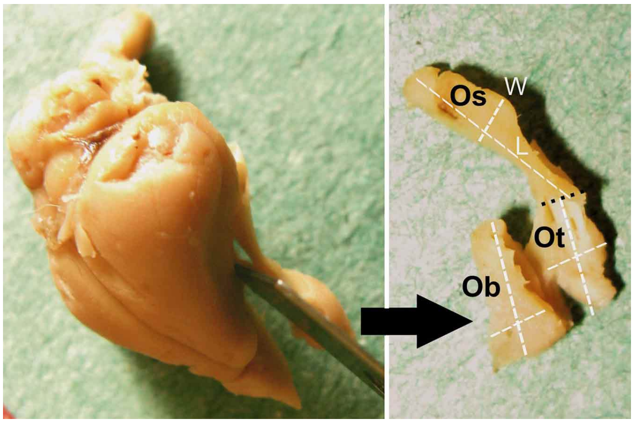

Fig. 3. Macrographs demonstrating how olfactory brain components were separated and linear dimensions measured. A blind dissection is done to remove the components, en bloc, after which the olfactory bulb (Ob) is detached from the underlying olfactory tract (Ot) to allow measurements of the greatest length (L) and width (W) of the bulb, tract and stria (Os).

± 2.10 mm, respectively, while breadths for the OB and the hemisphere measured 2.10 ±0.58 and 7.25 ± 0.64 mm respectively. In the sengi, length proportion of the OBC to that of the hemisphere was 58.08 %, a value that was less than that noted in dogs by 14.30 % but greater than that obtained in goats and humans by 6.21 % and 36.61 % respectively (Table II). OB to hemisphere breadth ratio in sengis (28.97 %) fell below that observed in dog and the goat by 13.94 and 0.76 %, respectively, but was greater than that of humans by 20.03 % (Table II).

DISCUSSION

This study aims at understanding how dietary, ecological and evolutionary factors shape the anatomical design of the nose-to-brain olfactory pathway in mammals. The data presented here serve to complement that in Kavoi in which the microanatomy of nasal olfactory structures was compared in animals leading different dietary lifestyles. Here, we analyze the gross morphometry of three OBC: (1)

Table I. Comparison of volumes of the sengi's OB, OBC and WB and their ratios (%) with those reported previously in Kavoi & Jameela (2011) in the dog, goat and human. OB, OBC and WB stand for olfactory bulb, olfactory brain components and whole brain respectively.

SPECIES	VOLUMES (mm^3)			RATIOS	
	OB	OBC	WB	OB: WB	OBC: Brain
Sengi	0.03 ± 0.01	0.07 ±0.03	6.78.3 ± 1.49	0.44	1.03
Dog	0.18 ±0.02	1.15 ±0.04	58.98 ±3.01	0.31	1.95
Goat	0.17 ±0.01	0.75 ±0.05	97.33 ± 8.79	0.18	0.77
Human	0.06 ±0.01	0.31 ±0.02	1175 ±52.44	0.01	0.03

Values are means (mm^3) ±SD.

Table II. Comparison of lengths and widths of the sengi's OBC and CH with those recorded earlier in Kavoi & Jameela (2011) in the dog, goat and human. OBS and CH denote olfactory brain components and cerebral hemispheres respectively.

SPECIES	LENGTH (mm)			WIDTH (mm)		
	OBC	CH	Ratio (%)	OB	CH	Ratio (%)
Sengi	11.5 ±1.04	19.8 ± 2.10	58.08	2.10 ±0.58	7.25 ± 0.64	28.97
Dog	48.20 ±1.92	66.67 ±1.53	72.30	10.8 ±1.64	25.17 ±0.76	42.91
Goat	34.50 ±1.30	66.50 ± 2.12	51.87	8.25 ±0.96	27.75 ±1.77	29.73
Human	36.25 ±1.70	168.86± 10.53	21.47	5.50 ±0.71	61.50 ±2.02	8.94

Values are means (mm) ±SD.

OB, a part of the forebrain that receives neural input about odors detected by primary olfactory neurons in the nasal cavity, (2) OT, a bundle of nerve fibers that connect the OB with the olfactory cortex and (3) OS, the ridges formed, laterally and medially, when fiber bundles of the OT split on reaching the olfactory areas of the cortex (Price, 2004). In the paper by Kaufman *et al*., a notably high encephalization (ratio between actual brain mass and predicted brain mass for an animal of a given size) is reported in sengis and this has been interpreted to mean that brain-body allometry in the sengi matches that of larger-brained non insectivorous groups rather than smaller-brained insectivores.

The sengi is an Afrotheria (a base group in mammalian radiation) that leads a strictly insectivorous lifestyle (Rathbun, 2005) and is reputed to exhibit a wide range of olfaction-dependent behaviors including social monogamy, lack of nest use, absentee maternal care of neonates and production of small precocial litters (Rathbun, 2005, 2009). Undoubtedly therefore, sengis require an olfactory system that is refined to a level that meets the functional demands of the aforementioned behaviors. The OB is a structure of reputable evolutionarily significance that antedates the appearance of the six-layered mammalian cerebral cortex and whose mass has been shown through scaling studies to be a function of its neuron number (Ribeiro *et al*., 2014). Data obtained in this study show the volume and linear measurement proportions of the OB and its projection structures (OT & OS) to that of the cerebrum and the whole brain to exceeds that recorded previously in Kavoi & Jameela for the goat, a browser herbivore whose olfactory cue is comparatively less important (Gelez & Fabre-Nys, 2004) and human, a primate that puts more reliance to stereoscopic vision at the expense of smell (Ross, 1995). However, values for the parameters mentioned above are less in the sengi than in the dog, a relatively generalized carnivore that relies heavily on the olfactory cue (Gittleman, 1989). Scaling of OBs with brain size across animal orders has been applied to determine likely ancestral states and to test for correlations between OB sizes and habitat, ecology and behavior (Corfield *et al*., 2015). Besides, magnetic resonance imaging work by Haehner *et al*. (2008) demonstrated a direct relationship between OB volume and odor detection ability.

In conclusion, data generated from this work show that the proportionate sizes and volumes of OBC (compared to cerebrum and the entire brain) vary according to olfactory function demand levels vis-à-vis dietary behaviors. Morphometrically, OBC were more advanced in the Afrotherian insectivore (the sengi) than in the primate (human) and the herbivore (goat) but less so compared to the carnivore (dog). Based on these observations, we assert that different selective pressures, which undoubtedly have a bearing on evolutionary dynamics, have acted upon the olfactory system of these animals to produce the observed outcomes. Future work aimed at shedding more light on the inferences made here concerning the sengi and other mammalian groups should incorporate more representative species and should analyze the OBC for variations in microscopic morphometry.

ACKNOWLWDGEMENT

We thank the Kenya Wildlife Service for granting us the permission to trap the animals. Mr. Ben Agwanda of the National Museums of Kenya assisted in the trapping of the animals. Mr. Francis Okumu helped in harvesting of the brains. This study was supported by a grant from the Deans Committee of the University of Nairobi.

KAVOI, B. M. & KISIPAN, M. L. Estudio morfométrico de los componentes olfativos del cerebro en el rufo Sengi (*Elephantulus rufescens*). *Int. J. Morphol., 37(3)*:1003-1007, 2019.

RESUMEN: Las características morfométricas de los componentes del sistema olfativo de los mamíferos muestran variaciones que pueden atribuirse a factores dietéticos y ecológicos. Analizamos los volúmenes y las dimensiones lineales de los componentes cerebrales olfativos (CCO), es decir, la médula oblonga (MO), el tracto olfatorio (TO) y la estría olfatoria (SO) en un insectívoro de Afrotherian, el sengi rufo. Estos hallazgos fueron comparados con los obtenidos previamente en perros (carnívoros), cabras (herbívoros) y humanos (omnívoros). Los volúmenes, longitudes y anchuras de los CCO se compararon con los del hemisferio cerebral (HC) y el cerebro completo (CC) mediante el cálculo de sus proporciones (%). En el sengi, el volumen de los CCO: CC fue de 1,03 %, la longitud de CCO: HC = 58,08 % y la amplitud de MO: HC = 28,97 %. En un informe anterior de Kavoi & Jameela, los valores respectivos para los parámetros anteriores fueron 0,03 %, 21,47 % y 8,94 % en humanos, 0,77 %, 51,87 % y 29,73 % en cabras y 1,95 %, 72,30 % y 42,91 % en perros. Estas observaciones sugieren que el diseño anatómico de la CCO se realiza de una manera que imita el nivel de confianza de un animal en el sentido del olfato en relación con los estilos de vida, el hábitat y la dinámica de la evolución.

PALABRAS CLAVE: Macroscópico; Morfometría; Cerebro olfativo; Sengi.

REFERENCES

Barton, R. A. *The evolutionary Ecology of the Primate Brain*. In: Lee, P. (Ed.). Comparative Primate Socioecology. Cambridge, Cambridge University Press, 1999. pp.167-203.

Barton, R. A.; Purvis, A. & Harvey, P. H. Evolutionary radiation of visual and olfactory brain systems in primates, bats and insectivores. *Philos. Trans. R. Soc. Lond. B Biol. Sci., 348(1326)*:381-92, 1995.

Corfield, J. R.; Price, K.; Iwaniuk, A. N.; Gutierrez-Ibañez, C.; Birkhead, T. & Wylie, D. R. Diversity in olfactory bulb size in birds reflects allometry, ecology, and phylogeny. *Front. Neuroanat., 9*:102, 2015.

Dengler-Crish, C. M.; Crish, S. D.; O'Riain, M. J. & Catania, K. C. Organization of the somatosensory cortex in elephant shrews (*E. edwardii*). *Anat. Rec. A Discov. Mol. Cell Evol. Biol., 288(8)*:859-66, 2006.

Finlay, B. L. & Darlington, R. B. Linked regularities in the development and evolution of mammalian brains. *Science, 268(5217)*:1578-84, 1995.

Gelez, H. & Fabre-Nys, C. The "male effect" in sheep and goats: a review of the respective roles of the two olfactory systems. *Horm. Behav., 46(3)*:257-71, 2004.

Gittleman, T. L. *Carnivore Behaviour, Ecology and Evolution.* London, Chapman & Hall Ltd., 1989.

Haehner, A.; Rodewald, A.; Gerber, J. C. & Hummel, T. Correlation of olfactory function with changes in the volume of the human olfactory bulb. *Arch. Otolaryngol. Head Neck Surg., 134(6)*:621-4, 2008.

Hakeem, A. Y.; Hof, P. R.; Sherwood, C. C.; Switzer, R. C. 3rd.; Rasmussen, L. E. & Allman, J. M. Brain of the African elephant (Loxodonta africana): neuroanatomy from magnetic resonance images. *Anat. Rec. A Discov. Mol. Cell Evol. Biol., 287(1)*:1117-27, 2005.

Kaufman, J. A.; Turner, G. H.; Holroyd, P. A.; Rovero, F. & Grossman, A. Brain volume of the newly-discovered species Rhynchocyon udzungwensis (Mammalia: Afrotheria: Macroscelidea): implications for encephalization in sengis. *PLoS One, 8(3)*:e58667, 2013.

Kavoi, B. M. & Jameela, H. Comparative morphometry of the olfactory bulb, tract and stria in the human, dog and goat. *Int. J. Morphol., 29(3)*:939-46, 2011.

Kavoi, B. M. Light and scanning electron microscopy of the olfactory mucosa in the rufous sengi (*Elephantulus rufescens*). *Anat. Histol. Embryol., 47(2)*:167-73, 2018.

Künzle, H. Neocortical connections with perihippocampal and periamygdalar regions in the hedgehog tenrec. *Anat. Embryol. (Berl.), 207(4-5)*:389-407, 2003.

Meisami, E. & Bhatnagar, K. P. Structure and diversity in mammalian accessory olfactory bulb. *Microsc. Res. Tech., 43(6)*:476-99, 1998.

Onyono, P. N.; Kavoi, B. M.; Kiama, S. G. & Makanya, A. N. Functional Morphology of the Olfactory Mucosa and Olfactory Bulb in Fossorial Rodents: The East African Root Rat (*Tachyoryctes splendens*) and the Naked Mole Rat (*Heterocephalus glaber*). *Tissue Cell, 49(5)*:612-21, 2017.

Price, J. L. *Olfaction*. In: Paxinos, G. & Mai, J. M. (Eds.). The Human Nervous System. 2nd ed. San Diego, Elsevier, 2004. pp.1198-212.

Rathbun, G. B. & Rathbun, C. D. Social structure of the bushveld sengi (*Elephantulus intufi*) in Namibia and the evolution of monogamy in the Macroscelidea. *J. Zool., 269*:391-9, 2006.

Rathbun, G. B. *Order macroscelidea*. In: Skinner, J. D. & Chimimba, C. T. (Eds.). The Mammals of the Southern African Subregion. 3rd ed. Cape Town, Cambridge University Press, 2005. pp.22-34.

Rathbun, G. B. Why is there discordant diversity in sengi (Mammalia: Afrotheria: Macroscelidea) taxonomy and ecology? *Afr. J. Ecol., 47(1)*:1-13, 2009.

Ribeiro, P. F.; Manger, P. R.; Catania, K. C.; Kaas, J. H. & Herculano-Houzel, S. Greater addition of neurons to the olfactory bulb than to the cerebral cortex of eulipotyphlans but not rodents, afrotherians or primates. *Front. Neuroanat., 8*:23, 2014.

Ross, C. F. Allometric and functional influences on primate orbit orientation and the origins of the Anthropoidea. *J. Hum. Evol., 29(3)*:201-27, 1995.

Scherle, W. A simple method for volumetry of organs in quantitative stereology. *Mikroskopie, 26(1)*:57-60, 1970.

Smit, H. A.; Jansen Van Vuuren, B.; O'Brien, P. C. M.; Ferguson-Smith, M.; Yang, F. & Robinson, T. J. Phylogenetic relationships of elephant-shrews (Afrotheria, Macroscelididae). *J. Zool., 284(2)*:133-43, 2011.

Stephan, H. & Andy, O. J. Quantitative comparisons of brain structures from insectivores to primates. *Am. Zool., 4*:59-74, 1964.

Stephan, H. Evolutionary trends in limbic structures. *Neurosci. Biobehav. Rev., 7(3)*:367-74, 1983.

Corresponding author:
Dr. Boniface M. Kavoi
Department of Veterinary Anatomy and Physiology
Faculty of Veterinary Medicine
University of Nairobi
Riverside Drive
P.O. Box 30197- 00100
Nairobi
KENYA

E-mail: drkanvo@yahoo.com

Received: 18-12-2018
Accepted: 07-02-2019