Original Article

Sahel Journal of Veterinary Sciences

Sahel J. Vet. Sci. Vol. 19, No. 3, Pp 20-27 (2022)

Article History
Received: 28-02-2022
Revised: 08-08-2022
Accepted: 27-08-2022
Published: 30-09-2022

Morphology and Morphometry of the Brain of African side necked turtle *(Pelusios castaneus)*: A Preliminary Investigation

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ABSTRACT

The brain and spinal cord make up the central nervous system and studying its morphology and morphometry in African side necked turtle (*Pelusios castaneus*) enhance understanding the neurobiology of this reptile. The current study is aimed to document the normal features on gross morphology and morphometry of the brain of *Pelusios castaneus,* and discuss the structure-function paradigm. The study was conducted on six (6) brains of African side necked turtle without sex differences. The main morphologic features observed were: 1- a pear-shaped olfactory brain, sand-wished between the two eyes and grossly divided into i) olfactory bulb, ii) olfactory tract and iii) olfactory lobe. 2- smooth cerebrum lacking gyri and sulci, and narrowed rostrally but broad caudally, 3- bi-lobed optic structure separating the cerebrum from cerebellum, 4- developed cerebellum with large corpus cerebelli and small flocculus. 5-pons and medulla oblongata were developed, and made up the myelencephalon. The mean body, head and brain weights were 111.69±21.04g, 4.58±0.60g and0.35±0.04g respectively. While brain weight accounts for 0.34% of total body weight and 8% of head weight, brain somatic index was 0.0034±0.0004 and head brain index was 7.60±0.70 respectively. The study provided baseline data on the gross morphology and morphometry of the brain of African side necked turtle (*Pelusios castaneus*); which is of great benefit in understanding the neurobiology of this reptile.

Key words: African Side Necked Turtle; Brain; Smooth cerebrum; Morphology; Morphometric

INTRODUCTION

The brain, a component of the central nervous system, is one of the most important organs in the body and controls many important functions (Shoshani *et al.* 2006). Despite these important functions, little information is available regarding the African side necked turtle (*Pelusios castaneus*). The reptilian nervous system could appear simple anatomically, but there is great functional diversity in behaviours which are specie-specific, and in the pattern of adaption to the diverse niches (Wyneken, 2007). Superimposed upon morpho-functional similarities of the reptilian nervous system is the brain and other several variations. These reflects the differences in gross body structure and evolutionary history among turtle (Wyneken, 2007) of which the African side necked turtle is not an exception. African side necked turtle (*Pelusios castaneus*) is known for their characteristic inability to fully withdraw their heads into the shells (Olukole *et al.* 2014), but instead, draws it to the side and folds it beneath the upper edge of their shell (Broadley and Boycott, 2009; Olukole *et al.* 2014). The African sideneck turtle (*Pelusios castaneus*) is a freshwater turtle belonging to the family *Pelomedusidae*, and widely distributed in the continent of West Africa, spreading from the Guinea savannah region and Senegal down to the Northwestern Angola region (Kirkpatrick, 1995; Olukole *et al.* 2014). *Pelusios castaneus* is a small to medium size turtle presenting extensive plastron that may have a hinge present between the pectoral and abdominal scutes (Olukole *et al.* 2010; 2014). Anatomical studies on this specie of reptile are scarce. Little studies available are mainly on reproduction (Olukole *et al.* 2014; Olukole and Oke 2020), and on the morphology and function of its feeding apparatus (Lemell *et al.* 2000). Concerning the nervous system, available literature is on the brain of the snapping turtle (*Chelydra serpentina*) (Garofeanu *et al.* 2004). There is however, lack of information on the functional morphology and morphometrics of the brain of the African sideneck turtle (*Pelusios castaneus*). Therefore, the aim of this research was to study the gross morphology and morphometrics of the brain of the African sideneck turtle (*Pelusios castaneus*), to explore the physical features of this turtle specie for identification and propose some of its morpho-functional paradigms.

MATERIALS AND METHODS

This study was conducted in the Gross Veterinary Anatomy Laboratory, Faculty of Veterinary Medicine, University of Abuja, Gwagwalada Abuja Nigeria. Six (6) apparently healthy African side necked turtle (*Pelusios castaneus*) without sex differences were used for this study. The turtles were purchased from a local hunter at the back of abattoir, Gwagwalada Area Council Abuja and were transported by road to the Neuroscience Unit, Department of Veterinary Anatomy, University of Abuja, Nigeria. Each of the African side necked turtle (*Pelusios castaneus*) was weighed on a Lark electronic balance (LP 502A, China) with sensitivity of 0.1 to 5kg with a sensitivity of 500g/0.01g (Oyelowo *et al.,* 2017a) and the weights were recorded. The turtles were then anaesthetized with intraperitoneal injection of ketamine/xylazine according to the protocol described by Usende *et al.* (2016). The turtles were then immediately decapitated at the occipito-atlantal junction and the weight of the head was taken before a perfusion fixation was slowly done on the brain tissue using 1ml of 10% solution of neutral buffered formalin (NBF) via both carotid arteries. After perfusion, heads were descalped by removing skin and muscle around the skulls. Brains were maintained inside the cranium to preserve the natural shape, and were then immersion-fixed in 10% NBF solution for 48 hours. In order to facilitate penetration of the fixative into the brain tissue (for optimal fixation of the brains), skulls were carefully cracked open. After 48 hours of fixation, the fixed brain tissues were carefully removed from the skull with their thin dural covering still intact. Brains were then exposed by the removal of the dura coverings. All brains were then used for gross morphological descriptions and morphometrical analysis.

After gross morphological descriptions, brain measurements were carried out using digital Vernier calliper, protractors, set square, treads and divider following the method described by Usende *et al.* (2020a). Briefly, the following parameters (Figure.1) were measured on the brains used for this study:

i. Weight of Animal (WOA): live animal weight was taken with a table top sensitive Lark electronic balance (LP 502A, China)
ii. Weight of Animal Head (WOAH): Weight of the animal head was taken with a table top sensitive Lark electronic balance (LP 502A, China)
iii. Head Somatic Index (HIS): This was calculated as the weight of the head as a proportion of the total body weight as follows:
HSI =Head weight/Body weight X 100
iv. Weight of Brain (WOB): Animal brain weight was taken with Lark electronic balance
v. Brain Somatic index (BSI): This was calculated as the weight of the whole brain as a proportion of the total body weight as follows:
BSI =Brain weight/Body weight X 100
vi. Brain Head Index (BHI): This was calculated as the weight of the whole brain as a proportion of the head weight as follows:
BSI =Brain weight/Head weight X 100
vii. Length of Brain (LOB): maximum length of the brain; taken from the tip of the olfactory bulb to the most caudal portion of the medulla oblongata
viii. Width of Brain (WOB): maximum width of the brain; taken as the distance from the two most lateral aspects of the brain
ix. Olfactory Brain Length (OBL): length of the olfactory brain; taken as the distance from the tip of the right and left olfactory bulb through the olfactory tract to its rhinal sulcus divided by two and represented mathematically as: ROBL+LOBL/2 where ROBL is the Right olfactory brain length and LOBL is the left olfactory brain length respectively.
x. Olfactory Lobe Width (OLW): width of the olfactory brain; taken as the distance from the most medial to the most lateral aspect of the right and left olfactory lobes divided by two and represented mathematically as: ROLW+LOLW/2, where ROLW is the right olfactory lobe wide and LOLW is the left olfactory lobe width respectively.
xi. Cerebrum Length (CL): maximum length taken from the tip of the frontal lobe to the most caudal portion of the occipital lobe of the cerebrum
xii. Cerebrum Width (CW): maximum length across the most lateral portions of the parietal lobes of the cerebrum
xiii. Cerebellum Length (CbL): maximum length taken from the most rostral portion to the most caudal part of the cerebellum
xiv. Cerebellum Width (CbW): maximum length across the most lateral portion of the cerebellum
xv. Optic Lobe Length (OLL): maximum length taken from the most rostral portion of the optic lobe (caudal to the pineal gland) to the caudal portion of the optic lobe (cranial portion of the cerebellum).
xvi. Optic Lobe Width (OpLW): taken as the distance from the most medial to the most lateral aspect of the right and left optic lobes divided by two and represented mathematically as: ROpLW+LOpLW/2, where ROpLW is the right optic lobe width and LOpLW is the left optic lobe width respectively.

Statistical Analysis

All numeric data obtain were expressed as Mean ± Standard Error of Mean (Mean ± SEM), using graph pad prism 5 and Microsoft Excel 2007. All pictures were reconstructed with the use of CorelDRAW version 12 software.

RESULTS

After the dissection of the turtle head and successfully exhuming the brain, the following observations were recorded;

Physical Features of African Side Necked Turtle

All the African side necked turtle used in the project has a dark coloured carapace on its dorsal surface with lighter brown- white coloured plastron on its ventral surface. The white portion, when present is more centrally placed. The carapace and plastron are joined at the latero- ventral edges of the shell. All the African side necked turtle used has a short tail which projects below the caudal portion of the carapace. The carapace had 37 scutes and the plastron had 13 scutes. Among the scutes of the carapace 13 were centrally placed and lined around by 24 marginal scutes.

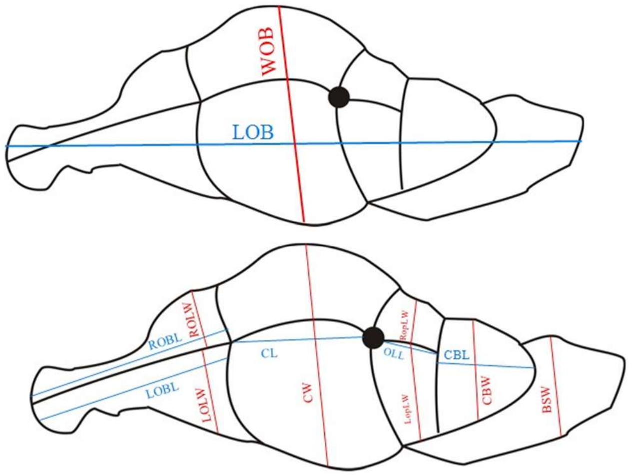

Figure. 1: Schematic representation of the measurements of major subdivisions of the brain of the African side necked turtle (*Pelusios castaneus*) (dorsal views). LOB: Length of brain, WOB: Width of brain, CL: length of cerebrum, CW: Width of cerebrum, CBL: length of cerebellum, CBW: width of cerebellum; ROBL: length of right olfactory brain; ROBW: width of right olfactory brain; LOBL: length of left olfactory bulb; LOBW: width of left olfactory bulb; CL: length of cerebrum; CW: width of cerebrum, OLL: length of optic lobe, RopLW: Right optic lobe width, LopLW: Light optic lobe width

Gross Morphology of the Brain

Figures 2 - 5 showed different anatomical regions of the brains of the African side necked turtle studied. The brain was entirely located in the cranium covered by thin scaly skin layer (Figure. 2) and appeared as a long tract- like organ with various structural convolutions (Figure. 2 and 3). The brain was entirely covered by meninges which appeared greyish in color. Findings in this study showed that the brain of African side necked turtle is grossly divided into the forebrain (prosencephalon), the mid-brain (mesencephalon) and the hindbrain (rhombencephalon). A flexure was seen between the midbrain and hindbrain and on a dorsal view while the most rostral portion of the forebrain was the olfactory brain system and the paired cerebral hemispheres. A paired optic lobe (mesencephalon) followed next before the unpaired cerebellum resting on the pons and medulla oblongata (Figure 3). To enable easy description, the brain of the African side necked turtle used for this study was divided regionally into olfactory brain, the cerebrum, the optic lobe, the cerebellum and the pons and medulla oblongata rostro-caudally (Figure 3). These various anatomical regions have their own peculiarities.

The Olfactory Brain

The olfactory brain of the African side necked turtle was pear shaped and grossly divided into 1) the olfactory bulb, 2) the olfactory tract and 3) the olfactory lobe (Figure 3). These structures were paired and projected from the two hemispheres of the cerebrum. The olfactory bulb and olfactory lobe were two bulges connected together by the olfactory tract. The olfactory lobe was the greater bulge, caudally placed and in contact with the cerebrum. The olfactory bulb was the more rostral free end and smaller than the olfactory lobe. The olfactory brain of the African side necked turtle appeared dark yellow in color, and this color distinct it from other parts of the brain grossly (Figure 3). A rhinal fissure (which is a small depression) separates the olfactory brain from cerebral hemisphere. *In-situ*, the olfactory brain was sand-wished between the two eyes and the olfactory bulb portion extended most rostrally at the apex of the eye. The olfactory brain continued with the cerebrum caudally (Figure 3).

The Cerebrum

The cerebrum was the second organ following the olfactory brain caudally. The African side necked turtle cerebrum was divided into two anatomically symmetrical hemispheres by a longitudinal fissure. African side necked turtle cerebrum appeared somewhat oval in shape, whitish in colour, and was larger than the olfactory brain in size. The two hemispheres were joined medially by a falx cerebri, a fold of dura mater. The cerebrum of African side necked turtle cerebrum appeared smooth lacking gyri and sulci and can be said to be lissencephalic. The cerebrum rest on underlying structures of the brain stem and partly visible from the ventral view. The cerebral hemisphere was narrow rostrally and broad caudally (Figure. 3). Three major fissures were observed on the cerebral hemisphere: 1) the rostral rhinal fissure which separate the cerebral hemisphere from the olfactory bulb, 2) the longitudinal fissure which separate both left and right cerebral hemispheres, and 3) the transverse fissure which separate the cerebral hemisphere from the caudally placed cerebellar hemisphere.

The Optic Lobe

In the African side necked turtle, the optic lobe also known as the tectum was well developed and situated caudal to the cerebrum. The optic lobe was seen dorsally as a raised bi-lobed structure separating the cerebrum from the cerebellum (Figure 3). It was shaped like a pair of oval balls.

The Cerebellum

The cerebellum was seen as a prominent structure on the brain stem, located at the dorsal portion of the brain (Figure 3). It is caudal to the optic lobe and appear smooth (lissencephalic). It was separated from the optic lobe by a cranial fissure and shaped like a hemisphere. Typically, all the African side neck turtle studied had large corpus cerebelli and small flocculus.

The Pons and Medulla Oblongata

The pons and medulla oblongata were developed, and made up the myelencephalon of the African side necked turtle. Rostral was the pons, and the medulla oblongata was caudal (Figure 4). The caudal portion of the medulla oblongata appeared thinner than the rostral portion and connect the brain to the spinal cord. Although not as distinct as what is seen in the mammals, the myelencephalon (pons and medulla oblongata) had a groove on both its dorsal and ventral aspect. The grooves were the dorsal and ventral medial sulcus, respectively and divided the myelencephalon into two equal halves (Figure 3).

Brain Weight and Morphometrics

Figure 1 represents landmarks for the morphometric parameters taken and the results of the morphometric measurements were presented in Table 1 and graphically represented in Figure 4. The mean body weight, mean head weight and mean brain weight of the African side necked turtle used for this study were 111.69±21.04g, 4.58±0.60g and 0.35±0.04g respectively (Table 1). The mean brain weight therefore accounts for about 0.34% of the total body weight and 8% of the head weight (Figure 4). Whereas the head somatic index was 0.043±0.003, the brain somatic index was 0.0034±0.0004 and the head brain index was 7.60±0.70. The head somatic and brain head index percentages are represented in Figure 4 and were 4% and 8% respectively. The length and width of the whole brain of the African side necked turtle used were 2.05±0.13cm and 0.8±0.03cm respectively (Table 1).

The mean olfactory brain length and width were 0.45±0.08cm and 0.50±0.06cm, and the mean optic lobe length and width were 0.3±0.02 and 0.43±0.01 respectively. The mean cerebrum length and width were 0.8±0.63 and 0.8±0.03, and the mean cerebellum length and width were 0.4±0.4 and 0.36±0.03 respectively (Table 1).

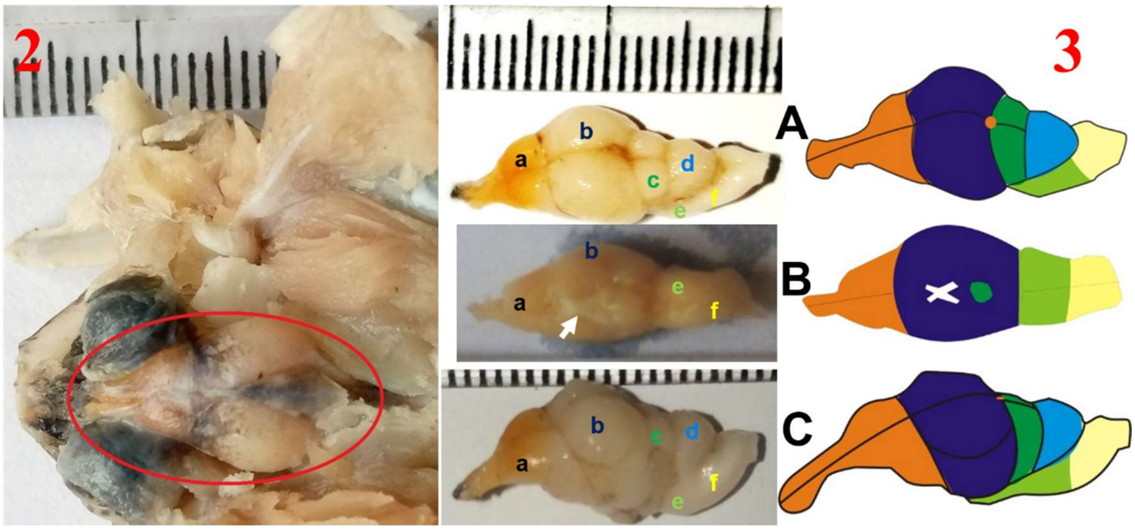

Figure. 2: Brain of African side necked turtle (*Pelusios castaneus*) *in situ* showing the brain (in red circle) covered with meninges. Note the position of the olfactory brain sand wish between the two eyes. Scale bar =0.3cm. **Figure 3:** Brain of African side necked turtle (*Pelusios castaneus*) in dorsal (A), ventral (B) and lateral (C) views and their reconstructions showing different anatomical features. a: olfactory brain; b: cerebrum; c: optic lobe; d: cerebellum; e: pons; f: medulla oblongata; white arrow: optic chiasm. Scale bar =0.3cm

DISCUSSION

The brain, due to its function has been said to be the most important organ in an animal's body, and makes up part of the central nervous system, together with the spinal cord (Skagges, 2021). However, there exist some differences and similarities in the brain and olfactory brain morphology of different animal species including the African side necked turtle (*Pelusios castaneus*). First, we explore the physical features of this turtle specie for identification. The present findings on the physical features of the African side necked turtle (including its dark-coloured carapace on its dorsal surface with lighter brown- white coloured plastron on its ventral surface, short tail which projects below the caudal portion of the carapace and retractable head which lies sideways in the shell) were in accordance to earlier reports by Olukole *et al,* (2010).

However, some variations were seen in the external morphological feature and morphometric data generated herein and are discussed below. This present study showed that the brain of the African side necked turtle consists of forebrain (prosencephalon), mid-brain (mesencephalon) and the hindbrain (rhombencephalon) and this conforms to the basic pattern of brain morphology in reptiles (Wyneken, 2007; Naumann *et al,* 2015). The developed forebrain seen in the African side neck turtle might be a reason for a good sense of smell, taste rhythms and sensory-motor integration and mediation (Wyneken, 2007) for this specie of reptile.

Interestingly, we showed that olfactory brain in this species of reptile is elaborate, divided into an initial olfactory bulb, a middle olfactory tract and a caudal olfactory lobe. It is worth stating that social life, animal survivability and sexual behaviour are strongly influenced mainly by the olfactory system (Shipley *et al.*2004; Amir and John, 2006). Morphologically, the olfactory brain system of all the African side necked turtle (*Pelusios castaneus*) studied herein were well developed, conspicuously and completely seen in lateral, ventral and dorsal views, typical of reptiles (Wyneken, 2007). The present finding is also similar to findings reported in rodents such as the grasscutter (Byanet *et al.* 2009) and the African giant rats (Nzalak *et al.* 2008; Ibe *et al.* 2014; Musa, 2015). In elephant and man however, the olfactory bulb is completely inconspicuous and not visible from a dorsal view (Shoshani *et al.* 2006). Studies have also shown a complete absence of olfactory bulb in whales (Marino *et al.* 2003). We hypothesize that the elaborate olfactory brain system seen in this present study may facilitate the use of this reptile for experimental procedures involving smell and some neurodegenerative diseases like the Parkinson disease. Moreover, it has been reported that the size of the olfactory brain constitutes a good indication of acuity of olfaction (Rombaux *et al.* 2006; Ibe *et al.* 2014, Nakamuta, *et al.* 2016). The present report of elaborate olfactory brain system could be reason why this reptile may be able to use the sense of smell in locating food, sexual partner and identifying predators. Similar findings have been reported in the African striped ground squirrel (Joanna *et al.* 2005), and suggested as an ideal model for radio-tracking data collection (Linn and Key, 1996), African giant rats, in landmines sniffing (Ibe *et al.* 2014; Usende *et al.* 2017; 2018; 2020b). However, a detailed histological examination of the olfactory brain system of the African side necked turtle (*Pelusios castaneus*) is required.

The present study revealed that the cerebrum is separated from the optic lobe by a transverse fissure and both hemispheres joined medially by a falx cerebri. Similar findings have been reported by (Wyneken, 2007) for the reptilian species. We also reported herein that the cerebrum of the African side necked turtle appeared smooth lacking gyri and sulci and are said to be lissencephalic. This report is typical of the reptilian species and in sea turtles (Wyneken, 2007). Similar lissencephalic brain has reported in hystricomorphs (Dozo *et al.* 2004) and rodents including the African giant rats (Nzalak *et al.*2008; Ibe *et al.* 2014; Musa, 2015). In the mammals, and associated with increasing brain functions, the cerebrum presents significant variations across species, ranging from the small and smooth (lissencephalics) cerebral cortex of mice, to the large and profoundly folded (gyrencephalic) cerebral cortex typical of humans (Rakic, 1995).

The optic lobe also known as the tectum of the African side necked turtle are reported herein to be well developed bi-lobed structure, oval ball shaped and caudal to the cerebellum. These findings are typical of the reptilian optic lobe (Wyneken, 2007). Reports have shown that the optic lobe receives both auditory and visual inputs (Wyneken, 2007) and that the size of the optic lobe correlate with important visual stimulus in animals ((Kardong-Edgren *et al.* 2005; Wyneken, 2007).

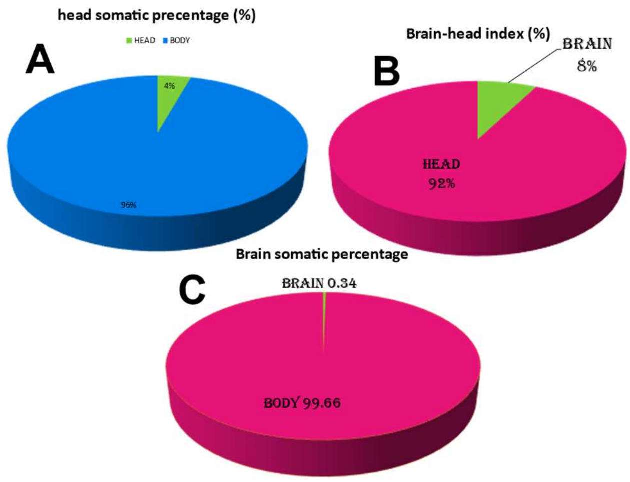

Figure 4: Pie chart representation of the head somatic percentage (A), Brain head index (B) and Brain somatic percentage of the African side necked turtle (*Pelusios castaneus*)

It has also been reported that together with the cerebellum, the optic lobes are responsible for the elaborate feeding behavior (Abrahao and Shibatta, 2015). Specifically, the optic lobe, through the optic nerve receives sensory information from the retina, generating signals that provide coordinating motor responses of the cerebellum (Meek and Nieuwenhugs, 1998). In addition, the optic lobes also receive auditory and somatosensory information creating retinotopic maps of the environment (Butler and Hodos, 2005). We hypothesized that the combination of motor coordination of the optic lobes, cerebellum and eyes of the African sided necked turtle may be responsible for stimulus directing the reptile to capture prey, exhibit its sexual behaviour and locate and survive its environment.

Table 1: Mean ± SD values of measured parameters of the brain of the African side necked turtle *(Pelusios castaneus)*

Parameters	Mean ± SD
Body weight (g)	111.69±21.04
Head weight (g)	4.58±0.58
Brain weight (g)	0.35±0.04
Brain length (cm)	2.05±0.13
Brain width (cm)	0.80±0.03
Olfactory brain length (cm)	0.45±0.08
Olfactory brain width (cm)	0.53±0.06
Cerebrum length (cm)	0.63±0.06
Cerebrum width (cm)	0.80±0.03
Optic lobe length (cm)	0.22±0.02
Optic lobe width (cm)	0.43±0.02
Cerebellum length (cm)	0.30±0.04
Cerebellum width (cm)	0.36±0.03

We also showed herein that the cerebellum of the African side necked turtle was seen to be a prominent structure on the brain stem. Reports have shown that the size of the cerebellum suggests, to a great extent the engagement of the brain in animals' lifestyle (Usende *et al.* 2020a). The cerebellum is part of the hind brain and integrates proprioception, hearing, touch, vision and motor inputs playing vital role in maintaining postural equilibrium in almost all vertebrates (Wyneken, 2007). However, cerebellar size varies greatly among species, especially with locomotory behaviour; being larger in aquatic and climbing species and smaller in ground dwelling species (Wyneken, 2007). We reported herein, that the cerebellum of the African side neck turtle has large corpus cerebelli and small flocculus. This finding is typical of the reptilian cerebellum (Kardong-Edgren *et al.* 2005; Wyneken, 2007).

This present study also reported that the pons and medulla oblongata were developed, and made up the myelencephalon of the African side necked turtle. In reptiles, while information on the pons is scarce, the medulla oblongata is fairly conservative but functions as in other vertebrates (Wyneken, 2007) housing the auditory, proprioceptive visceral and respiratory centers as well as regulation heart rate and gastrointestinal secretions and mobility (Kardong-Edgren *et al.* 2005; Wyneken, 2007).

Morphometric data are known for their usefulness for theoretical importance of functional morphology, either as absolute or relative size of particular organ of interest (Saber and Gummow 2014; Oyelowo *et al.* 2017b), and we present for the first time, data on the morphometrics of the brain of African side necked turtle (*Pelusios castaneus*). In our report, mean body weight, mean head weight and mean brain weight of the African side necked turtle were 111.69±21.04g, 4.58±0.60g and0.35±0.04g respectively and we showed that while brain weight accounts for 0.34% of the total body weight and 8% of the head weight, brain somatic index was 0.0034±0.0004 and the head brain index was 7.60±0.70 respectively. Earlier, reports have shown that a great deal of relationship of brain weight to body weight exist, both within and between most classes of vertebrates (Quay, 1972). In their report (Quay, 1972) and using the turtle, *Pseudemys scripta (Schoepff)* showed that analysis of weight of various regions of the brain could predict a good basis for external measurement of the shell. This is open for exploration in the African side necked turtle (*Pelusios castaneus*) especially as little or nothing is known about the development and growth of the brain of this species as well as other reptilian specie (Quay, 1972; Wyneken, 2007). The length and width of olfactory brain, optic lobe, cerebrum and cerebellum presented herein may have contributed a great deal to the overall brain weight of the African side necked turtle. This is although, a major generalizations and mathematical formulations and therefore may require major attention. Similar argument has been made by Quay (1972) concerning generalizations and mathematical formulations. It has been documented that growth continues in some brain regions of the adult turtle *Pseudemys scripta* (Quay, 1972) and this may be the situation with the African side necked turtle (*Pelusios castaneus*). However, this requires investigations and the morphometric herein presented may serve as a guide.

In conclusion, this study presents preliminary data on the morphology and morphometry of the brain of the African side necked turtle *(Pelusios castaneus*) with discussion on structure function paradigm, and form part of the continuing effort to document various aspects of the anatomy of this specie.

Conflict of Interest

The authors declare that they have no conflict of interest

Authors' Contribution

UIL, OFO, ARO, TAS and MSA conceptualized, designed and planned the study. UIL, ARO, OFO, SI, RAA and TAS performed the experiment. UIL, ARO, OFO and SI financed the research. ARO, UIL OFO and SI analysed the data. UIL and OFO supervised the work. All authors have read and approved the final manuscript.

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