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ORIGINAL ARTICLE

Anatomical Studies of the Gastrointestinal Tract of the Striped Sand Snake (*Psammophis Sibilans*)

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SUMMARY

A study was carried out on the gross anatomical, morphometric features and histology of the gastrointestinal tract of the Striped Sand Snake (*Psammophis sibilans*). Ten snakes (five males and five females) were euthanized and dissected for the study. The gastrointestinal tract appeared as a straight tubular organ from oral cavity to cloaca, owning to the absence of a cecum. The stomach could be distinguished as a spindle-shaped bulge along the length of the gastrointestinal tract, longitudinal folds (rugae) were observed for the inner wall of the stomach. The small intestine in this species is thrown into rings; the cranial portion is the duodenum and posterior portion the ileum. The ileum widens up into the colon, it also loses its rings at this point. The cranial portion of the large intestine is the colon, and the caudal portion is the rectum. The mean snout-vent length (cm) in males was recorded as 94.02 ± 1.1 while in females it was recorded as 48.28 ± 1.4. The stomach was the shortest segment while the esophagus was the constituting almost half of the total length of the snout-vent length. The wall of the esophagus, stomach, small intestine and large intestine was built up of the following layers from outside inwards; serosa, muscularis, submucosa and mucosa, while the stomach consists of a new layer, subserosa which follows the serosa. The entire length of the gastrointestinal tract was lined by simple columnar epithelium (ciliated in the esophagus) and contains goblet cells except in the stomach and rectum where these cells are absent. The esophageal mucosa contained extensive folds, and the lining epithelium consisted of ciliated columnar epithelium, lamina propria, muscularis mucosae and goblet cells. Areas of lymphatic aggregation were also found in the mucosal layer. The stomach mucosa is thrown into folds and was lined through its length with columnar epithelium that showed numerous invaginations, gastric pits, which led to glandular structures, the gastric glands. In the small intestine (duodenum and ileum) the mucosal lining of the intestinal villi is uniform throughout. It is composed of three types of cells; simple columnar cells, goblet cells and lymphatic nodules. The large intestine consists of the colon and rectum. With their villi relatively short compared to those in the small intestine.

Key words: Sand snake, *Psammophis sibilans*, gastrointestinal tract, gross, morphometry, histology.

INTRODUCTION

Reptiles have been suggested to be useful models for the study of the physiological regulation of the gastrointestinal system because they respond well to feeding even more than other commonly used experimental mammals such as mice, rats, rabbit and pigs (Secor and Diamond, 1998).

The snake digestive system has several specialized regions, the esophagus, stomach, small intestine (duodenum, ileum and jejunum) and large intestine (caecum, colon and rectum), rate of digestion is dependent of body temperature because they are cold-blooded animals (Goin 1962, Spellerberg, 1982). Their movement and secretary activities results in optional digestive and presentation to the absorptive surface of an appropriate substrate. These processes are governed largely by; rate at which ingesta pass along the tract and their sequential exposure to the digestive enzymes. Rates of gastric digestion and gastric secretion increase with increasing Tb in N. natrix, the grass snake (Skoczylas 1970a, 1970b)

Psammophis sibilans is a snake of the genus Psammophis, a huge snake that can have a maximum length of about 180cm, but the range for adults is 90-110cm while juveniles have a range length of 19-28cm. (Boulenger, 1890)

All snakes are strictly carnivorous, eating small animals including lizards, other snakes, small mammals, birds, eggs, fish, snails or insects (Mehrtens, 1987). Because snakes cannot bite or tear their food to pieces, they must swallow prey whole. The body size of a snake has a major influence on its eating habits. Smaller snakes eat smaller prey. (Behler, 1979)

During times of fasting the small intestine becomes less active and atrophies (Cossins and Roberts, 1996).

The alimentary tract of reptiles is similar to higher vertebrates with some exceptions. The esophagus shows adaptive modifications from group to group. In turtles, the esophagus has heavily keratinized papillae that protect the mucosa from abrasive diet such as speculated sponges and jellyfish, and also may act as filtering devices. In lizards, it is formed of folds lined by ciliated columnar epithelium with goblet cells. Some snakes have mucous glands along their submucosa (Elliott, 2007). The muscularis mucosa of the oesophagus is absent in many species of reptiles but may be found in some species of turtles (Elliott, 2007). Oppel (1896, 1897& 1900) described the alimentary canal of reptiles; Greschik (1917) studied the anatomy and histology of the alimentary canal of both *Ableparus pannonicus* and *Anguis fragilis*. Langley (1881) gave an account on the histology and physiology of pepsin-forming glands in some reptiles. Beguin (1904 a & b) studied the esophageal glands of reptiles. Staley (1925) gave a brief account on the structure of gastric glands of *Alligator mississippiensis*. Beattie (1926) described the ileo-caecal region of *Tupinambis teguexin*.

A study already conducted on *Psammophis sibilans* tongue, indicated that it is divided into three separate portions; anterior free bifurcated tongue tips followed by a portion which protrudes out of the mouth during tongue flicking, and a posterior portion of the tongue that remained almost entirely within the mouth during protrusion. The tongue is suspended in the floor of the mouth by a sheet that is dorsally stiffened by the larynx and trachea (El-sayyad *et al.*, 2011).

A review of anatomical studies on animals in Nigeria has showed that most of these studies were dealt with members of the class Mammalia and Aves. On the other hand, only little attention was paid to the members of the class Reptilia. This indicated that the present subject did not receive the necessary attention. This stimulated the authors to carry out some work in this field on a

Plate 1: Showing the male *Psammophis sibilans* before dissection

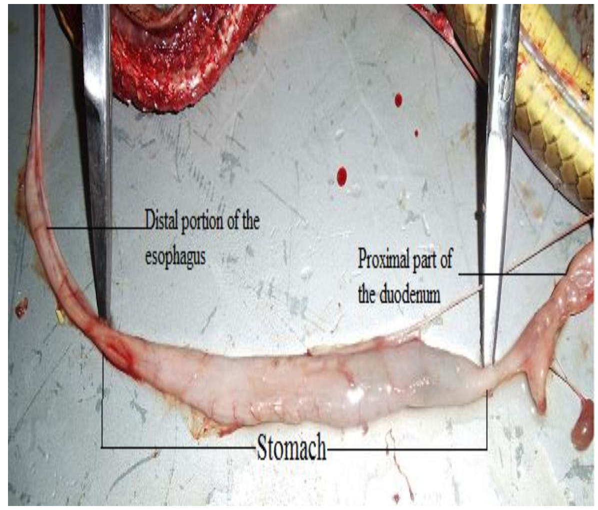

Plate 2: Showing transition from esophagus to stomach with evident widening of the esophagus (female)

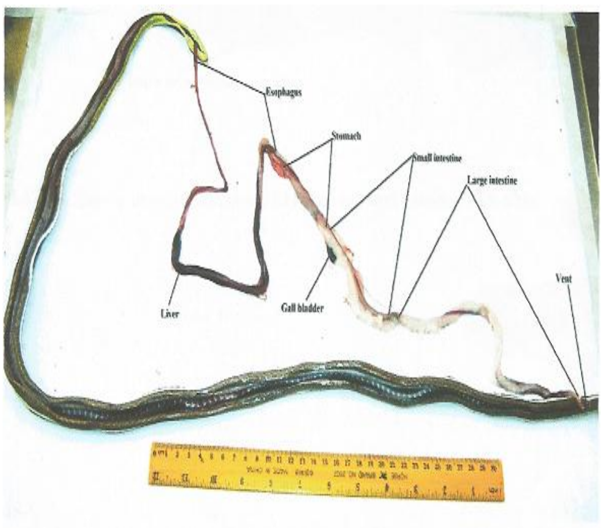

Plate 3: Photograph of a fresh isolated alimentary canal of a male *Psammophis sibilans* still attached showing (the esophagus, stomach, small and large intestine)

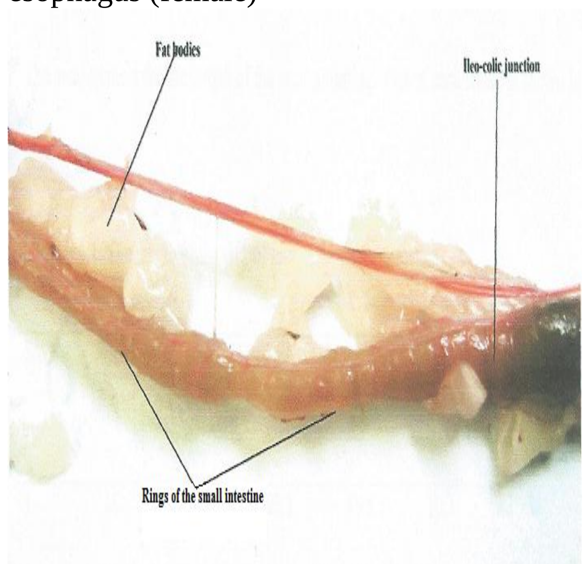

Plate 4: Photograph of the ileo-colic junction showing the short transverse rings of the small intestine (male)

member of the class Reptilia.

MATERIALS AND METHODS

Ten specimens of the snake (*Psammophis sibilans*) were caught by hand from different localities of Zaria, Kaduna State, Nigeria. (Plate 1)

The reptilian species were sacrificed according to the local experimental animal ethics committee of the Faculty of Veterinary, University of Ilorin.

The specimens were euthanized using chloroform. Snakes were complete specimens, in good body condition with no signs of infection or disease.The snout-vent length (SVL) of the snake was measured using thread then placed on a ruler. Following the measurements of the snout-vent length (SVL), a ventral midline incision was made to expose the internal organs. After dissecting fat and connective tissue, the length of the coelomic spaces occupied by the oesophagus, lung, liver, stomach, small intestine and large intestine was measured (Plate 3). With the snake on its back, the internal organs were measured in situ from proximal to distal. Weights of snake or GIT were not taken.

After dissection the entire gastrointestinal tract starting from the esophagus to the cloaca was removed and processed for investigation. Photographs of the entire GIT in-situ were taken using a digital camera (Sony Cybershot 14.1 megapixels DSC-W370). The GIT specimens were identified, removed, photographed and described.

Samples of different segments of the GIT (esophagus, stomach, small intestine and large intestine) were taken and kept inside a labeled container with 10% buffered neutral Formaldehyde for one week.

The tissues were cleared in xylene before infiltration with molten paraffin wax. Transverse sections of five microns (5µm) thick each of the esophagus, stomach, small intestine and large intestine were cut from the embedded tissues using a rotatory microtome (model 42339) and mounted on a clean glass slide in the presence of egg albumin, dried at room temperature and stained with Haematoxylin and Eosin(Fischer, Jacobson and Seller 2008)

Photomicrographs of the prepared slides were taken using a digital eye piece (Hoe Science Tools microscope digital camera 5MP) attached to a Leitz (HM-Lux) binocular light microscope and a computer and then studied at x 40 and x 100 magnifications.

RESULTS

Gross

Examination of the internal organs in-situ shows that there was no demarcation between the thoracic and abdominal cavities; therefore snakes have a single body chamber (thoraco-abdominal) and do not have separation of abdomen and thorax as in mammals (separated by the diaphragm). Both abdominal and thoracic organs run side by side longitudinally (i.e. esophagus, stomach, heart and lungs). They are attached to each other by sheets and strands of connective tissue mesh that allow for some limited movement of these organs (Plate 3).

Esophagus: The esophagus courses alongside the trachea and extends from the pharyngeal region to the stomach (Plate 2). It appeared more like an elastic band in close contact with the trachea. It also has longitudinal folds on the inner surface.

Stomach: The stomach appears as a wider tube compared to the esophagus. There is no line of demarcation between the esophagus and the stomach, just a funnel-shaped widening of the esophagus around the distal portion of the liver and caudal to the heart (Plate 3). The stomach then narrows again as it enters the duodenum which gives it a spindle-shaped appearance (Plate 2). Upon incision, longitudinal folds (rugae) were observed for the inside wall of the stomach.

Small intestine: The small intestine in this species is thrown into rings but relatively straight (Plate 3). The first loop after the stomach, receiving the pancreatic ducts is considered as the duodenum, the rest is the ileum. Therefore the cranial portion is the duodenum and posterior portion the ileum. There is no external or internal indication of the transition between duodenum and ileum as they pass indistinguishably into one another. There was no segment called jejunum.

Large intestine**:** This species lack a caecum. The ileum widens up into the colon, it also loses its rings at this point (Plate 3 & 4). Next to the colon is the short rectum. The rectum opens into the cloaca which is of a

Table 1: Gross anatomy measurements of total body length and visceral organs of the snake (*Psammophis sibilans*). All measurements were in centimetre from proximal to distal

Structure	Male Mean ± SD	Relative lengths (OL/SVL)	Female Mean ± SD	Relative lengths (OL/SVL)
Snake length	153.20 ± 1.4		70.8 ± 2.2	
Snout-vent Length	94.02 ± 1.1		48.28 ± 1.4	
Esophagus	48.92 ± 0.6	52	24.50 ± 0.7	50.7
Stomach	10.40 ± 0.2	11	6.56 ± 0.3	13.6
Small intestine	16.20 ± 0.2	17.2	9.44 ± 0.3	19.5
Large intestine	18.78 ± 0.3	19.9	7.78 ± 0.3	16.1

OL, Organ length; SVL, Snout-vent length; SD, Standard deviation of mean

smaller diameter. Its cranial portion is the colon, and the caudal portion is the rectum. There is no structural demarcation between the colo-rectum as there are no distinguishing morphological features.

Morphometrics: The male is longer than the female having a mean length of 153.20 ± 1.4 compared to 70.8 ± 2.2 in females. Similarly all parameters were longer in the male than the female. (Table I). The measurement for the length of the gastrointestinal tract of each snake is shown in table 1.

The average snout-vent length was 94.02 ± 1.1cm for males (Table 1) and 48.28 ± 1.4 for females (Table 1). The stomach and small intestine is relatively longer in females than males while the males have a relatively longer esophagus and large intestine (Table 1). The relative length of GIT segment (SVL) to the snake length was relatively longer in females with 68.2 and relatively shorter in males with 61.4.

Histology

Esophagus: The alimentary tract of the Psammophis sibilans revealed the typical layers seen in higher vertebrates; mucosa, submucosa, tunica muscularis and serosa. The esophageal mucosa contained extensive folds, and the lining epithelium consisted of ciliated columnar epithelium, lamina propria, muscularis mucosae and goblet cells (Plate 5).

The cilia are found arranged along the outer border of the mucosal epithelium. Each columnar cell has an oval nucleus which rests near its base.

Areas of lymphatic aggregation were found in the mucosal layer.

Stomach: The stomach is composed of the following layers; serosa, sub-serosa, sub-mucosa and mucosa. The mucosa is thrown into folds and was lined through-out its length with columnar epithelium that showed numerous invaginations, gastric pits, which led to glandular structures, the gastric glands. The mucosa also contained the muscularis mucosae. The submucosa was connective tissue layer with blood vessels (Plate 6).

Small intestine: Photomicrograph of the presented in plate 7 and 8. The lumen of the small intestine is relatively narrow due to the presence of extremely long and coiled villi. The outer-most layer is the serosa and consists of simple squamous epithelial cells. The sub-mucosa is vascular and enters in the formation of intestinal villi while the mucosa is devoid of muscularis mucosae.

In the duodenum and ileum, the intestinal mucosal villi is uniform throughout and the mucosa cells are of three types; simple columnar cells, goblet cells and lymphatic

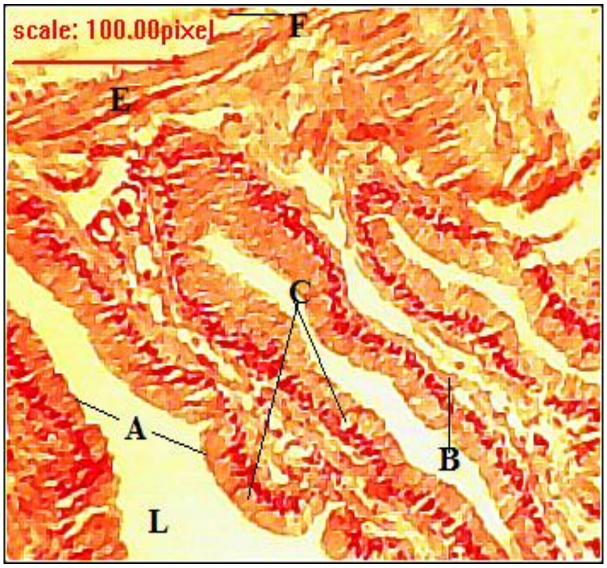

Plate 5: Photomicrograph of the transverse section of the esophagus showing; Ciliated Columnar Epithelium (A), muscularis mucosae (B), goblet cells (C), submucosa (D), tunica muscularis (E), serosa (F) and Lumen (L). (H&E × 100)

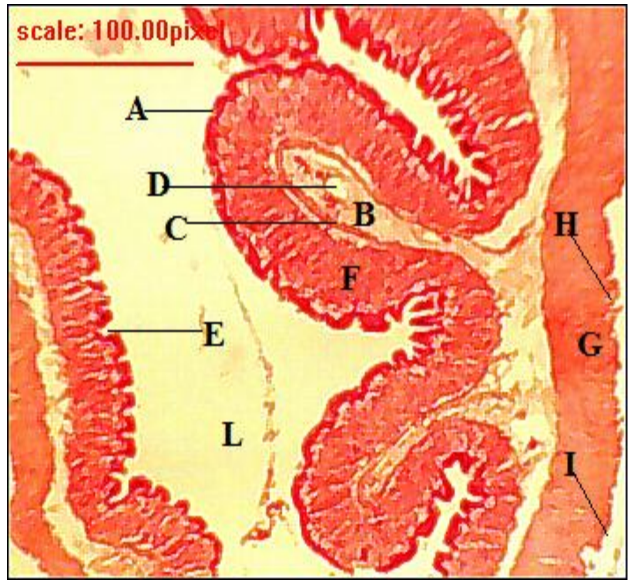

Plate 6: Photomicrograph of the transverse section of the stomach showing; simple columnar epithelium (A), submucosa (B), muscularis mucosae (C), blood vessel in the submucosa (D), gastric glands (F), muscularis layer (G), subserosa (H), serosa (I) and lumen (L). (H&E × 100)

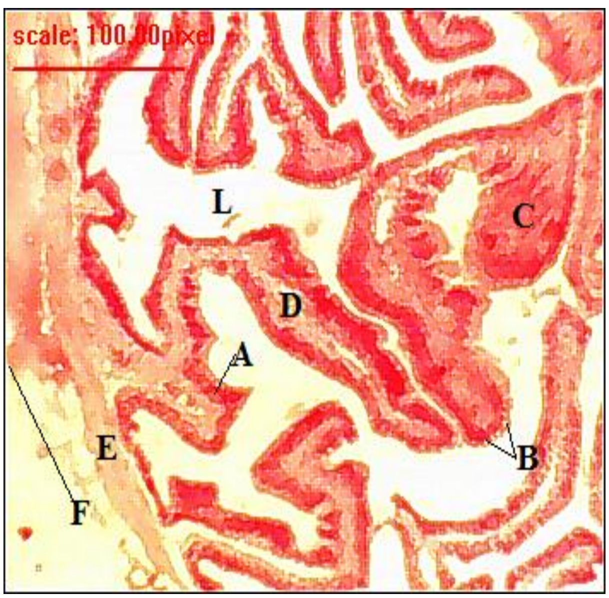

Plate 7: Photomicrograph of the transverse section of the duodenum showing; the mucosa lined by simple columnar epithelium (A), goblet cells (B), lymphatic nodules (C), submucosa (D), tunica muscularis (E), serosal layer (F) and lumen (L). (H&E × 40)

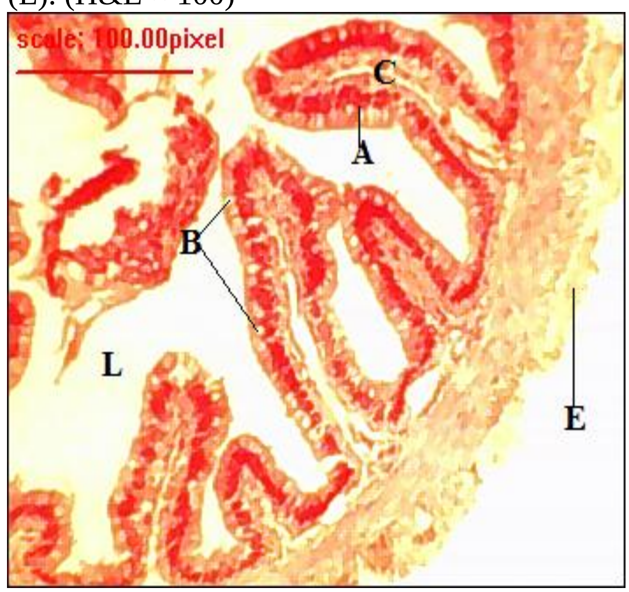

Plate 8: Photomicrograph of the transverse section of the ileum showing; the mucosa lined by simple columnar epithelium (A), goblet cells (B), submucosa (C), tunica muscularis (D), serosa (E) and lumen (L). (H&E × 100)

nodules. The lamina propria is rich in lymphocytic infiltrations. No intestinal glands and crypts were found in the submucosa of the duodenum and ileum.

Large intestine: The photomicrograph is presented in plates 9 and 10. The large intestine presented two parts; the colon and rectum. The villi are relatively short compared to those in the small intestine. The mucosa of the colon consist of simple columnar epithelium and few goblet cells, while that of the rectum contains only simple columnar cells with no goblet cells. The mucosa muscularis layer thins out from the esophagus down to the rectum. This means that the large intestine of this species of snake lack muscularis mucosae.

DISCUSSION

Results of current study show that the digestive system of the snake is structurally and functionally very similar to other reptiles. In reptiles the stomach varies in shape (Elliott, 2007). The stomach of turtles has greater and lesser curvatures, crocodiles have a saccular stomach, and lizards have an ovoid one, while the stomach of snakes is elongated in shape (Madrid *et al*., 1989).
The stomach may closely resemble in diameter to that of the oesophagus and be indistinguishable from it when it is empty. However, if fully distended with a prey, the stomach may occupy much larger portion of the coelomic cavity (Greene, 1997). Contrary to gopher snakes where a palpable thickening was noted between the oesophagus and the stomach (Khamas and Reeves, 2011), there was no such structure in the *P. sibilans*. On the contrary, Gans (1977) stated in general term that in many snakes, the border between the oesophagus and stomach is indicated by the nature of the mucus membrane and that is the only way one can differentiate the two from each other. Even with the absence of feed in the sand snake the stomach was distinguishable giving out a spindle-shaped appearance.

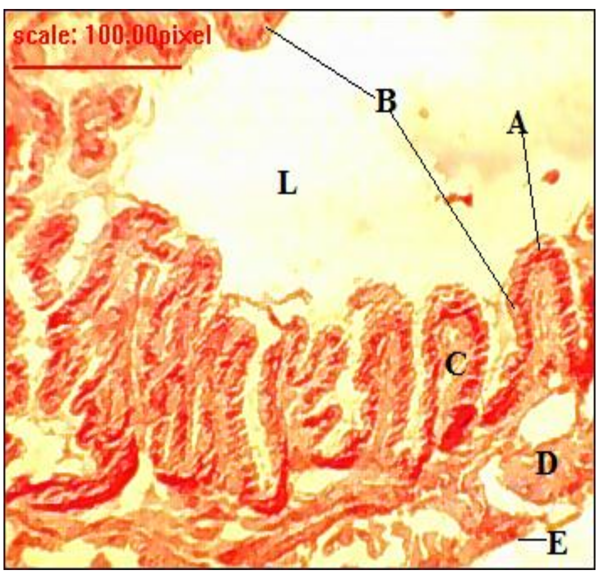

Plate 9: Photomicrograph of the transverse section of the colon showing; the mucosa lined by simple columnar epithelium (A), goblet cells (B), submucosa (C), tunica muscularis (D), serosa (E) and lumen (L). (H&E × 40)

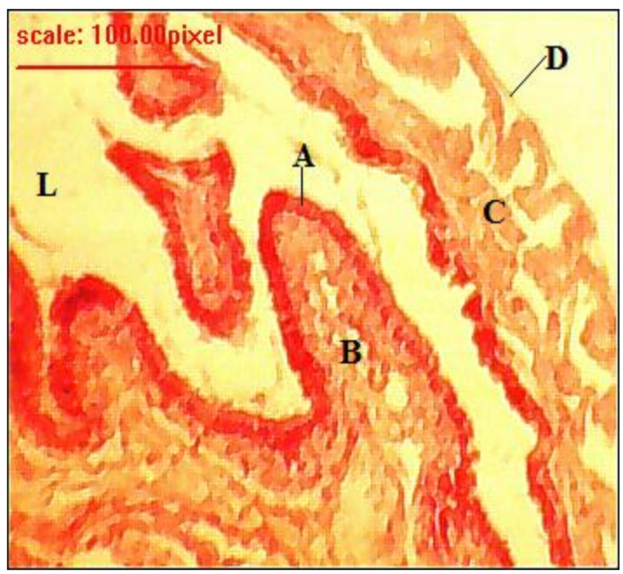

Plate 10: Photomicrograph of the transverse section of the rectum showing; the mucosa lined by simple columnar epithelium (A), submucosa (B), tunica muscularis (C), serosa (D) and lumen (L). (H&E × 40)

The small intestine is in short rings and straight (not looped), in contrast to the highly convoluted small intestine of turtles (Parsons and Cameron, 1977). This could be because the digestive system of these snakes is well adapted to their intermittent feeding behavior or rather their posture.

This species lack a caecum. The ileum widens up into the colon, it also loses its folds at this point. It is not known why some snakes have a caecum and others do not, but the appendage is generally found in herbivorous animals but not in carnivores (Douglas Mader, 1995).

This study shows that the body of the snake is made up of a single chamber (no separation between the thoracic and abdominal cavity) i.e. they lack a diaphragm as described by (Douglas Mader, 1995).

The gastrointestinal tract is larger and longer in males compared to female and may be due to the fact that the males are larger than the females but the gastrointestinal tract is relatively shorter in males than in females.

Although, the lining epithelium of the esophagus of some reptiles is similar to that of mammals; stratified squamous epithelium but it is also known that some species are different and lined with columnar epithelium (Elliott, 2007) and that is in agreement with the result reported here. However in *Ablephorus pannonicus* (Greschik, 1917), *Chamaeleon vulgaris* (Bishai, 1960), *Uromastyxphilbyi* (Farag, 1982), *Chalcides levitoni* (El-Taib eta!., 1982), *Mauremyes caspica* (El-Taib and Jarrar, 1983) and *Acanthodactylus boskianus* (Dehlawi and Zaher, 1985a) where the mucosal membrane of the anterior region of the esophagus declares the present configuration, while it is composed of non-ciliated simple columnar and goblet cells in its posterior region. The present study reveals that the esophageal mucosa is thrown into several longitudinal folds. This feature is common for all the described reptiles e.g. *Uromastyx philibyi* (Farag, 1982) and *Pristurus rupestris* (Dehlawi and Zaher, 1985b).

The stomach of the examined species is characterized by the presence of a relatively thick gastric muscularis layer which is obviously a good adaptation for breaking up food to small pieces through strong muscular contractions. The gastric mucosa of *Psammophis sibilans* is characterized by gastric glands.

Microscopic examination of the intestinal mucosa indicated the presence of extremely long and coiled villi to compensate the shortness of the small intestine. Such a histological feature may allow efficient absorption of the digested food.

Examination of the mucosal epithelium of the small intestine, (duodenum and ileum) of the studied species revealed the absence of the intestinal glands (Crypts of Lieberkuhn). Such a condition was recorded in the intestinal mucosa of *Mabuya quinquetaeniata*, Chalcides ocellatus (Anwar and Mahmoud, 1975), Agama stelilo (Amer and Ismail, 1976), and *Pristurus rupestris* (Dehlawi and Zaher, 1985b). On the contrary, the presence of the intestinal glands was recorded in the intestinal mucosa by (Toro, 1930) and (Farag, 1982) in crocodilians, and *Uromastyx philipyi*, respectively.

The present study revealed the complete absence of glandular crypts in the mucosa of the rectum of the studied species. Such a condition is concordant to what were recorded by (Farag 1982) and (Dehlawi and Zaher, 1985b) in *Uromastyx philipyi* and *Pristurus rupestris*, respectively; But contained few goblet cells in the mucosa of the colon, a similar finding by (Dehlawi and Zaher, 1989) in *Coluber florulentus.*

CONCLUSION

The relative length of GIT segment (SVL) to the snake length was relatively longer in females with 68.2 and relatively shorter in males with 61.4. Other anatomical structures studied both gross and histologic were highly insightful and significant compared to the normal structures studied in other animal species, which is a reflection of the external morphology and mode of life of the animal. This to the authors' knowledge is the first time the gastrointestinal tract of a Nigerian indigenous snake is being studied. Further physiological and histochemical studies will be required to reveal certain points of significance.

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