Morphometric analysis of tongue and dentition in hedgehogs and pangolins

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SUMMARY

The gross anatomy, morphometric and histology of the tongue and dentition were studied in African hedgehogs and pangolins. In this study, eight hedgehogs and six pangolins were used. The average body weight and body length of hedgehogs and pangolins were 210.00 ± 11.03 g, 15.80 ± 0.09 cm and 1449.42 ± 220.15 g, 115.75 ± 6.75 cm, respectively. The average tongue weight and length of hedgehogs and pangolins were 1.32 ± 0.09 g, 3.10 ± 0.51 cm, and 19.75 ± 0.85 g, 66.38 ± 11.92 cm respectively, while the relative tongue weight (RTW) and relative tongue length (RTL) were 6.27%, 21.62% and 1.10%, 1.92% respectively. The findings of this work provide baseline data that could be relevant for understanding the anatomical adaptation for their diet, tongue, and dentition.

Key words: Morphometry – Tongue – Hedgehog – Pangolin – Adaptation – Diet

INTRODUCTION

These two mammals were chosen because of the inaccessibility and dearth of documented data on their relation, which correlates

their tongue and dentition. The hedgehog Atelerix albiventris and Pangolin, Manis tricus*pis*, are insectivorous terrestrial mammals (Adeniyi et al., 2010; Stevens and Lowe, 2005; Hildebrand and Goslow, 2001). However, much work has been done on the comparative anatomy of tongue of the hedgehog, bat and rat (Chivers and Hladic, 1980; Ofusori et al., 2008; Adenivi et al., 2010). There are three types of pangolins in Africa the giant pangolin, the tree pangolin and (the most widespread) the ground pangolin. Pangolins have small heads and long, broad tails. They are toothless and have no external ears, although their hearing is good. Their sense of scent is well-developed, but their sight is poor. The weight of the protective keratinous scales and skin make up about 20% of the pangolin's weight. The animal preens itself by scratching with its hind legs, lifting its scales so the claws can reach the skin. It also uses its tongue to remove insects from under the scales (African Wildlife Foundation, 2011). The tongue is a highly muscular organ covered in a squamous epithelium for deglutition, taste, and for making communicative sounds (Standring et al., 2005; Stevens and Lowe, 2005). It is partly oral and partly pharyngeal in position, and is attached by its muscles to the hyoid bone, mandible, styloid

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processes, soft palate and the pharyngeal wall in most mammals (Standring et al., 2005). However, in pangolins the larger part lie in the thoracic and abdominal parts; while the teeth, though lacking in pangolins, also form part of the structures that are found in the oral cavity, their shape, size and arrangement depend on dietary intake (Taylor et al., 1998). There are three basic tooth forms in dentition: incisiform, caniniform, and molariform. Incisiform teeth (incisors) are cutting teeth; caniniform teeth (canines) are piercing or tearing teeth, and molariform teeth (molars and premolars) are grinding teeth and have a number of cusps on an otherwise flattened biting surface (Standring et al., 2005; Stevens and Lowe, 2005). Since pangolins have no teeth, the gizzardlike stomach is specially adapted for grinding food. The process is helped along by the small stones and sand pangolins consume (African Wildlife Foundation, 2011).

To the best of my knowledge no precise work has been done to relate all these differences with the micro-and macro-anatomical adaptation that the tongue and teeth of these two insectivorous mammals have adopted. The soft internal parts of invertebrates, along with their flesh, do contain protein and fat, which provide the nutrient for the hedgehog, pangolins and other ant-eaters (Redford and Dorea, 1983). The sand and detritus adsorbed together with termites have been reported to add bulk to the digestive load of these insectivorous animals and hence reduce the caloric proportion of their digestive content (Redford and Dorea, 1983; Adeniyi et al., 2010).

Here I report a comparative study of the morphology, macro-and micro-structural organization of the tongue, and dentition of the two insectivorous mammals developed to successfully manipulate their different diets and compare the morphological differences.

MATERIALS AND METHODS

Care of the animals

Eight hedgehogs weighing on average 210 g and four Pangolins weighing on average 1449.42 g of both sexes were used. The hedgehogs were procured from local sellers in Ilorin, Nigeria, following ethical clearance, and maintained in the animal holding facilities of the same Department. The Pangolins were also procured from the *Asejire* Community of Oyo State, Nigeria. They were fed with insects and had free access to water. The handling and care of the animals conformed to the animal rights Committee of the University of Ilorin, Nigeria, and the rules guiding Good Laboratory Practice were also adhered to.

Excision of tongues

After the animals had been sacrificed by chloroform inhalation, the tongues were excised from the animals, blotted using filter paper and their wet weight was recorded, using a Gallenkamp electric balance (Model FA2104A). The antero-posterior lengths (APL) of the tongues were also recorded using a transparent ruler. The tissues were rapidly transferred into a bottle containing 10% formol saline for 48 hours. The teeth were carefully observed, counted and recorded.

Histological procedures

The oral part of the tongues were carefully excised and processed routinely for paraffin embedding. Serial sections (transverse and longitudinal) were obtained at 5 μ m-thickness with a rotary microtome and subjected to Haematoxylin and Eosin (H&E) staining (Adeniyi et al., 2010; Bancroft and Stevens, 1999). The sections were mounted and examined under a light microscope and a photomicrograph of each slide was taken for further analysis.

Statistical analyses

The data were expressed as means \pm Standard Error of the Mean (SEM). The data were evaluated statistically with SPSS software, version 14.0. One-way analysis of variance (ANOVA) at a level of significance of 2 = 0.05 was used. The relative tongue weight (RTW) and relative tongue length (RTL) of each animal were calculated using the following formulae:

$$RTW = \frac{Tongue weight}{Body weight} \ge 100\%$$

$$RTL = \frac{100 \text{gut Length}}{\text{Body Length}} \ge 100\%$$

(Note: Body Length = nose to tail length of the animal).

RESULTS

Morphological analyses

Gross anatomy. The Pangolin's tongue is bigger and longer than that of the hedgehog.

The pangolin's tongue is divided into three parts: anterior/ oral part; middle/ thoracic part, and posterior/ abdominal part (see Fig. 2). The abdominal part of the pangolin's tongue is girded on the two lateral sides (right and left sides) by a Y-shape cartilage (Y-cartilage) and the cartilage ends by attaching to the peritoneum which is inserted in to the right posterior abdominal wall. The Y-cartilage is attached anteriorly to the thoracic cage (see Fig. 2).

Tongue weight. There were significant differences (p < 0.05) in tongue weight between the three mammals. The Pangolins had the highest tongue weight (19.75 ± 0.85 g) in comparison with the hedgehogs (1.32 ± 0.09 g) and this was correlated with their body weight (Table 1).

Table 1. Comparative morphometric values of means \pm SE of the tongue weight (g), length (cm) and volume (ml) of hedgehogs and pangolins. APL: Antero-posterior length; RTW: Relative Tongue Weight; RTL: Relative Tongue Length; * statistically significant difference.

Weight	Hedgehogs $(n = 8)$	Pangolins (n = 6)
Body Weight (g)	210.00 ± 11.03	1449.42* ± 220.15
Body length (cm)	15.80 ± 0.09	115.75* ± 6.75
Tongue weight (g)	1.32 ± 0.09	19.75* ± 0.85
Tongue APL (cm)	3.10 ± 0.51	66.38* ± 11.92
Tongue Volume (ml)	0.92 ± 0.12	$17.00^* \pm 1.00$
RTW (%)	6.27* ± 0.21	1.10 ± 0.17
RTL (%)	21.62* ± 0.61	1.92 ± 0.05

Relative tongue weight (RTW). Using Students' t test (p<0.05), a significant difference was seen in the RTW among the two mammals. The hedgehog RTW (6.27 x 10^{-2}) was significantly higher than that of the pangolin (1.10 x 10^{-2}) (Table 1).

Relative tongue length (RTL). The Hedgehog RTL (21.62 x 10^{-2}) was significantly greater than that of the pangolin (1.92 x 10^{-2}) (Table 1).

Table 2. Comparative morphometric values of the mean ± SEM of the tongue weight (g), length (cm) and volume (ml) of hedgehogs and pangolins. I: incisors; C: Canine; PM: premolar; M: molar.

Animals	Dental formula	Total teeth
Hedgehog	2[I ³ ₂ C ¹ ₁ PM ³ ₂ M ³ ₃]	36
Pangolins	$2[I_0^0 C_0^0 PM_0^0 M_0^0]$	Nil

Histological observations

The microscopy observations made from histological preparations with Haematoxylin (H) -Eosin (E) revealed the following (see Fig. 1):

Epithelial lining: the epithelial lining in the hedgehog tongue is thicker than that of the pangolin; it is almost twice the thickness and





Fig. 1. Photomicrographs of the Tongue in the two mammalian species (x 320). All sections are longitudinal. E: epithelium; CM: Circular muscle; LM: Longitudinal muscle; LP: lamina propia; G: Gland; BV: Blood vessel.

the pangolin tongue lacks the convolutions seen in the hedgehog.

Muscle: The patterns of arrangement of the circular and longitudinal muscles in the two mammals are histologically similar, although that of the pangolin were more prominent.

Lamina propria and papilla: The LP was more distinctly outlined, in the hedgehog



Fig. 2. Photograph of the Pangolin tongue.

tongue, unlike that seen in the pangolin which has hardly any; the fungiform and filiform papillae were well outlined in the hedgehog, unlike those in pangolin.

Glands and Blood Vessels: in the hedgehog the serous glands were more prominent in their tongues compared with those of pangolin; the pangolin tongue was better vascularized than that of the hedgehog.

DISCUSSION

In mammals, taste receptors on the tongue can detect five primary flavours: sweet, bitter, sour, salty and umami - a savoury or meaty taste. The sense of taste plays an important role in mammals' decisions about what to eat and what to avoid, and scientists have identified many of the underlying genes and their functions (DUHS, 2011). In this study, the average tongue weight, length and volume of the hedgehog and pangolin were found to be 1.32 g, 3.10 cm, 0.92 ml and 19.75 g, 66.38 cm, 17.00 ml; those of pangolin being statistically higher (p<0.05). Although the histological outlines of the epithelial lining of these two insectivorous mammals were different from those of the pangolin lacking convolutions and thicker than that of the hedgehog, the muscular pattern was not significantly different and this could be a result of the same role of the tongue in the two mammals, as reported by Adeniyi et al. (2010), Young et al. (2006) and Standring et al. (2005). The papillae, filiform, fungiform and circumvallate, foliate, although not well outlined in the two mammals, were more rudimentary in the pangolin as compared to those seen in the hedgehog (Young et al., 2006). The upper blood vessels in the histological outline of the pangolin tongue is an indication of an abundant blood supply; this may be an adaptive mechanism of the pangolin (since it lacks teeth for food manipulation), for a high work-load involved in food manipulation. The findings in this study are also in agreement with the report of Taylor et al. (1998). The dentition patterns in the two species were different: pangolins lack a set of teeth while the hedgehog has a set of 36 teeth for food manipulation before it is swallowed. The lack of a set of teeth in pangolin might account for its very strong and long tongue, and needed for special support for tongue, provided by the Y – cartilage and the thoraco-abdominal wall (see Fig. 2). Thus it may be concluded that histomorphological analyses of the tongue and the dentition in the two insectivorous mammals studied must be involved in their respective dietary intake and habits.

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