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HISTOLOGICAL STUDY OF TONGUE IN *ROUSETTUS AEGYPTIACUS* IN THE SOUTHWEST OF IRAN (JAHROM)

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ABSTRACT

Bats make up a large proportion of mammalian diversity in Iran. Highest ranking sites for *Rousettus aegyptiacus* presence are located in Jahrom in southwest of Iran. Dominant vegetation in this region is Palm, Jujube, Figure, and Citrus. Ten specimens were captured using mist-net (4 cm mesh). Species were identified according to their morphological characters, and then autopsied and their digestive systems were observed for determination of diet types. Tongues were fixed in 10% formaldehyde, samples were taken from tip, middle and hind tongue and placed in tissue processor and dehydrated in graded series of ethanol (70%-100%), then impregnated with paraffin and serial Sections (4µ thickness) were prepared. sections were subjected to Haematoxylin and Eosin (H&E) stains and mounted on binocular microscope and micrographs were taken with digital camera.

Presence of seeds, pieces of leaf and fruit in their gut and feces showed their frugivory diet type. Elongated and movable tongue and tine keratinized epithelium especially on ventral surface of tongue, numerous and different types (4 types) of papilla on the dorsal surface of tongue in different locations and orientation were seen in this species. Hyaline cartilage was observed in tip of tongue.

KEYWORDS: Diet Type, Hyaline Cartilage, Lingual Papillae, Pollination

INTRODUCTION

Bats with nearly 1250 species are the second largest group of mammals and play ecological and economic role in our community (Kunz & Fenton, 2006). Frugivorous bats disperse seeds and pollinate flowers by feeding on fruit, pollen and nectar, on the other hand, insectivore bats play an important role in biological defense by feeding pests (Kunz & Fenton, 2006).

Tongue as taste organ have important role in digestion of food in all vertebrates (Pastor et al., 2008). Tongue's structure has been studied in many mammals as rats (Iwasaki et al., 1999), mice (Iwasaki et al., 1996), guinea pigs (Kobayashi, 1990), flying squirrels (Emura et al., 1999) and rabbits (Nonaka et al., 2008). Variation in the distribution and types of lingual papillae were indicated (small, nipple-shaped projection) related to manner of food uptake (Iwasaki, 2002).

Microscopic observation of the tongue of bats have been reported in insectivorous families such as Molossidae (Gregorin, 2003; Azzali et al., 1991), Hipposideridae, Rhinolophidae and frout eating bats such as *Pteropus vampyrus* (Emura et al., 2002), Cynopterus brachyotis, *Pteropus giganteus* (Kobayashi et al., 2004) and in fruit eater bats such as *Rossetus aegyptiacus* (Jackowiak et al, 2009).

There are various types of lingual papillae on the dorsal surface of tongue. These papillae are divided into mechanical (Filiform and Conical) and gustatory papillae (fungiform and vallate). Shape, position and distribution of these lingual papillae play important role in eating habits (Hanna et al,. 2009).

MATERIALS AND METHODS

The Egyptian fruit bat (*Rousettus aegyptiacus*: Figure 1) belongs to the Pteropodidae family. Large populations of this species are present in the southwest of Iran (Jahrom). Presence of large caves, ponds and rich flora in this region are the main reasons for the presence of this species in the region. This species feed on fruit pulp, juice, nectar, pollen and leaf, and play a very important role as a seed disperser and pollinator species of many important flowers and trees like the Jahrom's vegetation.

Jahrom district with 5498 km^2 area is located in the southwest of Iran between 53º, 30' to 53º, 34' E & 28º to 28º, 29' N, and between 985 to 1120m above M.S.L. (Figure 2).

Numerous caves, richness of flora and warm weather are reasons for presence of large populations and high diversity of bats.

Ten specimens were captured using mist-net (with different sizes and 4 cm mesh), autopsied in lab and their digestive systems were observed for determination of their diet types. Their tongues were cut from the root and their length and width were measured. Samples were taken from tip, middle and hind of tongue, placed in Tissue processor for 15 hours and prepared for histological study as below:

The samples were fixed in 10%, formaldehyde, dehydration in graded series of ethanol (70%, 80%, 99% and 100%), clearing with transfer trough xylene and impregnation with hot liquid paraffin for stabilization. Then Embedding and sectioning of tissues using a microtome and preparation of serial sections (transverse and longitudinal) with 4µ thickness. The sections were stined using a combination of Haematoxylene and Eosin (H&E). Sections were mounted on binocular light microscope and photomicrograph of each slide was taken for further analysis.

RESULTS

Dental formula: In upper jaw was $I_4\ C_2\ PM\ _6M_4$ and in lower jaw was $I_4\ C_2\ PM\ _6\ M_6$, incisors were very small, canines tall and sharp and molar were large. Seeds, pieces of leaf and fruit were observed in the gut and the feces of specimen.

Tongue was long, muscular and movable. It was divided into three areas: apex, body and root (Figure 3). Mean tongue weight was 3.20g ±0.50, mean total tongue length was 4.20 cm ±0.90, mean tongue width was 0.58 cm ±0.56 at the tip, 0.98 cm ±0.32 in the middle and 1.1cm ±0.40 at the root. The anterior free part of the tongue was 1.6cm±0.35. Numerous projection or papilla with tiny lamina propria were found on the dorsal surface of tongue (Figure 3), however no papillae were found on the ventral surface. Serous and mucous glands were recognized in the thick connective tissue or the lamina propria under the non-keratinized squamous epithelium. Longitudinal and transverse bundles of muscle fibers were surrounded by collagenous fibers below the connective tissue (Figure 4).

Four kinds of lingual papillae (filiform, conical, fungiform and vallate) as following, were found on the dorsal surface of tongue:

- Filiform papillae were distributed on the whole dorsal surface of the tongue. They were divided to 3 types with different size and position:
    - **Small Filiform Papillae:** Hair–shape with needlelike scale projections, were on the anterior part of the tongue (Figure 5).

- Giant filiform papillae with smooth surface, thick connective tissue in body and trifid posterior process that overlap each other (Figure 6). Directions of giant papillae were toward the mid zone of tongue and pharynx. Small papillae were in the front of giant papilla and their directions were toward the posterior of tongue. Type 1 was fewer than type 2, but it had sharper projections. Although types 1 & 2 overlap in sides of tongue, they are recognized from each other by their size and direction.
- **Bifid Filiform Papillae:** This type is similar to giant type but had bifid ends and oriented to the lateroposterior of tongue (Figure 6).

- **Fungiform Papillae:** Large, mushroom form with many taste buds (As shown in Figure 7). These papillae were observed on all of surface especially at the tip and lateral margins of tongue between filiform papillae.
- Conical papillae were distributed on the lateroposterior dorsal surface of the tongue and have have thick connective tissue with keratinized stratified squmosal epithelium (Figure 7).
- Vallate papillae were three in number and large, flattened and triangle in form (Figure 8). They were situated on the behind of mid region of tongue and surrounded by circle grooves which contain many taste buds (Figure 9). In one of the specimens these papilla were situated on a striaght line. These papillae and the associated mucosal and serous glands were not prominent.

Many taste buds were found on the posterior and sides of tongue. Serose and mucus glands and lingual tonsils which were collection of lymphocyte cell were founded on the root zone and related with fungiform and vallate papillae (Figure 4, 10).

Histological observation showed a thick cartilage situated under the connective tissue in the anterior part of tongue (Figure 10).

DISCUSSIONS

Dental and papilla types and presence of seeds, pieces of leaf and fruit in the gut and the feces of specimens, showed their frugivory habit.

Some degree of structural variation especially in shape and number of papilla on the dorsal surface of the tongue was identified in mammals (Taiwo et al., 2009). Three types of papilla were identified in insectivorous bats (Pastor et al., 1993; Jackowiak et al,. 2009), whereas there are four types of papillae (filiform, fungiform, conical and vallate) with different distribution on the dorsal surface of tongue in fruit eater bats (Emura et al., 2009). In this spices, papilla distribution was limited to the dorsum of tongue whereas in some of mammals they were also seen on the anterior and ventral surfaces part (Khaksary et al., 2010). In fruit eater bats, considerable length of the anterior free part of the tongue facilitates the movement of the tongue (Greenbaum & Phillips, 1974) and different types of filiform papillae according to their location in aid of eating fruit, seed and leaf (Emura et al., 2001).

Papilla's situation are different and related to their function (Jackowiak, 2009; Rex et al., 2010). Brush-like filiform papillae (type 1) with rough tips attach to the food particles and enables them to scoop up nectar (Gonzalez-Terrazas1 et al., 2012), pollen and semi liquid food in great quantities. Filiform papillae (Type 2) with smooth surface and trifid tip may help in more efficient uptake of semi liquid food and transportation of food to the pharynx (Jackowiak et al, 2009). This type that occupies more sites, increases absorption surface. Third kind of these papillae with sharp bifid tip which waere located in latroposterior of tongue make a large surface for leading food to the pharynx.

All filiform papillae have processes with scale keratinized squmosal epithelium that attach to particles and are useful for uptake of semiliquid food (Toprak, 2006). Fungiform papillae in some of mammals such as buffalo (Khaksary et al., 2010) hasn't taste buds but in this species, numerous fungiform papillae with many taste buds were observed. Fungiform and vallate papillae (gustatory papillae) were covered by a very thin keratinized layer (Emura et al., 2009) because they have taste buds (Hwang & Lee, 2007) and cause the food particles to be exposed to the taste buds and facilitate absorption of food. Number of vallate papillae in mammals is different and depends on food habit (More et al., 2001). Most fruit eater bats (Emura et al., 2002) have three vallate papillae and insectivore bats have two or noting (Son & Lee, 2000). Collagenous fibers were found around the muscles in the anterior of tongue, these fibers help to the extension of tongue.

As it was reported in the previous studies (Emura et al., 2001) in this species, unlike insectivorous bats, number of fungiform papillae is high and probably like the fruit eater bats such as fox flying (Emura et al., 2002) and even omnivores mammals such as human and other primates (Taiwo et al., 2009) might be the result of the food habit or large tongue size and aid to gustatory sensitivity. In present study it was seen that like in humans, fungiform papillae were located on the tip, root and sides of the tongue (Kullaa et al., 1987) that enable them to take a wide range of foods (Emura et al., 2001).

Shape and distribution of the lingual papillae by making a rough surface in the front of tongue are effective in touching and taking of the food particles (park & lee, 2009) but posterior papilla, participate in transportation of food (Pastor et al., 1993). Specific shape of the vallate papilla and their contact with taste buds play important role in tasting.

Number of circumvallate papillae in bats is different and depends on food habit. Insectivore bats have two vallate papillae (Son et al., 2000) but not only frugivores bats, also most of the fruit eater mammals have three vallate papillae. In addition, number of large fungiform papillae located in the center of posterior region of tongue were different with insectivor bats (Azzali et al., 1991; Emura et al., 2001).

Lamina propia in dorsal surface of tongue was thinner than that of ventral surface. This layer is thinner than that in the most mammals (Taiwo et al., 2009). Serous glands were not prominent (Toprak, 2006) because they feed on soft materials such as pollen and nectar , which do not require high salivary secretion for swallowing, unlike the rodents and carnivores. Conical papillae were located in the posterior region of the tongue where mucous glands with tine keratinized squmosal epithelium and many taste buds were found. These findings show that gustatory function is developed and adapted to food uptake.

Presence of cartilage in the front part of the tongue of this spices which was reported for the first time, is important for movement of the tongue. Muscles, cartilage and collagenous fibers beside this antilogous tissue facilitate movement of the tongue.

CONCLUSIONS

The present study showed that the shape and the distribution of lingual papillae depend on the type of diet and location of these papilla on the tongue. Structural characteristics of the papilla are appropriate to their function. Presence of cartilage in the tip of tongue, facilitate its movement.

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APPENDICES

Figure 1: *Rossetus agyptiacus*

Figure 2: Map of Jahrom (Iran)

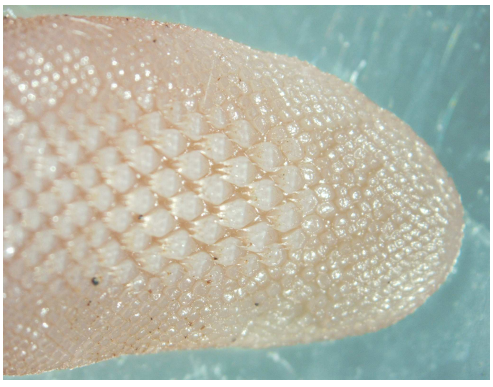

Figure 3: Dorsal Surface of Tongue

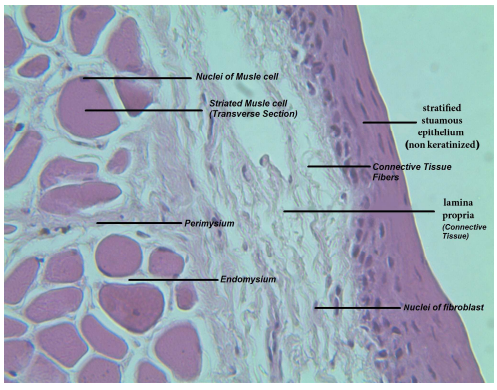

Figure 4: Ventral Surface of Tongue

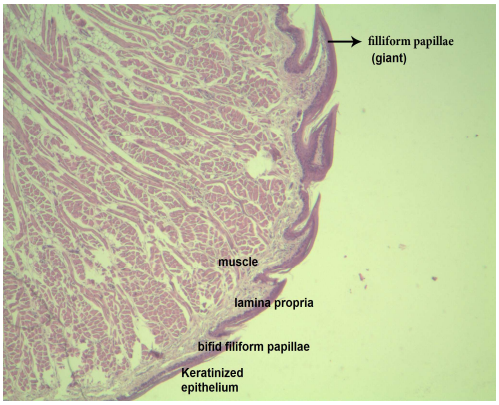

Figure 5: Filliform Papillae (Type I)

Figure 6: Filliform Papillae(Type II, III)

Figure 7: Fungiform and Conical Papillae

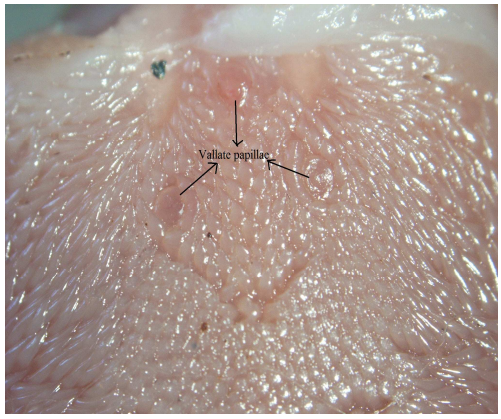

Figure 8: Triangels form Vallate Papilla

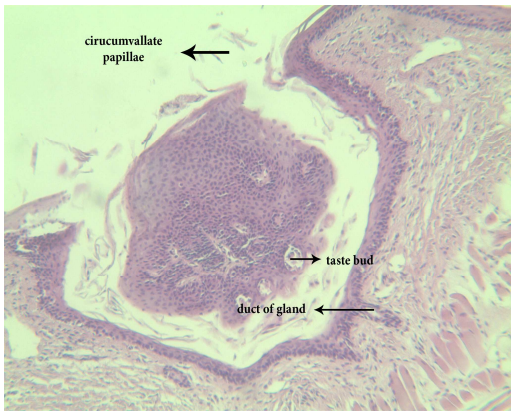

Figure 9: Vallate Papillae and Test Bud

Figure 10: Cartilage in the Tip of Tongue