

Comparative Histological Studies on the Tongue of Three Species of Egyptian Bats

Fatma M.A. Taki-El-Deen, Samia M.I. Sakr and Mohamed.A.Shahin

Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University – Egypt
Dr.fatma.bio@gmail.com

Abstract: The present study aims to describe the histological structure of the tongues of three species of bats which having different diets and live in different habitates in Egypt. This work was done on three species of bats; the frugivorous bat *Rousettus aegyptiacus*, the insectivorous bat *Rhinopoma hardwickie* and the tomb-inhabiting bat *Taphozous perforatus*. These animals were caught from their natural habitats in Egypt; dissected and their tongues were fixed in the appropriate fixatives for light microscopical investigations. The histological results revealed that the dorsal surface of the tongue of the three bats is formed of three consecutive layers; mucosa, submucosa and muscularis. The mucosal layer is similar in the three bats. It consists of stratified squamous epithelium and contains three types of papillae but this layer is very thick in both insectivorous and tomb-inhabiting bats. The submucosa also appears thicker in the frugivorous bat than that in both the insectivorous and tomb-inhabiting bats. It is built up of loose connective tissue, small blood vessels and lymphatics. The muscularis is formed of an outer thin layer of circular muscle fibers and an inner thick layer of longitudinal muscle fibers in both the frugivorous and tomb-inhabiting bats but this layer is constructed of circular muscle fibers arranged in different directions in the insectivorous bat. The histochemical results showed differences in the stainability and distribution of neutral and acid mucopolysaccharides in the lingual glands of the three species of Egyptian bats.

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1. Introduction

The study of the histological and the histochemical aspects of the tongues of the species of different classes of vertebrates, and specifically bats, had attracted the attention of certain investigators. Earlier anatomical and microscopic observations of bat tongues have primarily focused on insectivorous species from families of Vespertilionidae and Rhinolophidae (Azzali *et al.*, 1991; Pastor *et al.*, 1993), Molossidae (Gregorin, 2003), Hipposideridae (Sharma *et al.*, 1999) and Noctilionidae (Elizalde-Arellano *et al.*, 2004).

With regard to fruit- and nectar- eating bats, the microstructure of the tongue has been investigated in short- nosed fruit bat (*Cynopterus brachyotis*) (Emura *et al.*, 2001), the large flying fox (*Pteropus vampyrus*) (Emura *et al.*, 2002) and the species belonging to phyllostomidae (Winter and Von Helversen, 2003). The findings of these authors showed the occurrence of two types of mechanical papillae (i.e. filiform and conical papillae) for food manipulation and two types of gustatory papillae (i.e. fungiform and vallate papillae) for taste perception.

Mahmoud *et al.* (2002) investigated the morphology of the donkey tongue and its papillae. The authors found that the filiform papillae were distributed mainly on the dorsum of the tongue while the fungiform papillae appeared scattered mainly on the lateral surfaces.

Iwasaki (2002) examined the fundamental importance of morphology in the evolution of the

vertebrate tongue, focusing on the origin of the tongue and on the relationship between morphology and environmental conditions. The author examined the tongues of various extant vertebrates, including those of amphibians, reptiles, birds and mammals. The study revealed a relationship between changes in the structure of the tongue and changes in habitat from freshwater to land or sea water.

The structure of the tongue of the marsupial feathertail glider (*Acrobates pygmeus*) was studied by Jackowiak and Godynicki (2007). On the dorsal surface of the tongue they distinguished three types of lingual papillae; mechanical filiform papillae and gustatory fungiform and vallate papillae. The fungiform papillae have a single taste bud and are uniformly scattered between filiform papillae only on the anterior half of the tongue. On the smooth root of the tongue, three oval vallate papillae are arranged in the form of a triangle.

Rather recently, Jackowiak and his team (2009) in their study on the microstructure of lingual papillae in the Egyptian fruit bat (*Rousettus aegyptiacus*), demonstrated that the small and giant filiform papillae were present in anterior part of the tongue and tilted to the back of the tongue. The filiform papillae with elongated processes were arranged on each side of the tongue and oriented perpendicularly to the median line of the tongue.

Benetti *et al.* (2009) studied the tongue of *Bradypus torquatus*. They noted that the rostral part of the tongue presents a round apex and covered by filiform and fungiform lingual papillae and a

ventral smooth surface. The vallate papillae presented numerous taste buds in the wall of epithelial cells.

Ebru *et al.* (2010) in their investigation of the histological features of lingual papillae on the dorsal surface of the Zavot cattle tongue, observed the presence of five types of papillae on the dorsal surface of the tongue, namely filiform, fungiform, circumvallate, lenticular and conical papillae.

Recently, Sakr and Ramadan (2010) in their study on the histological studies on the stomach of some Egyptian bats reported that there were differences in the histological structure of the mucosa between frugivorous, insectivorous and tomb-inhabiting bats; this may be correlated with the differences in their habitats or the nature of their feeding habits.

The available literature indicated that few studies have been carried out on the histology and histochemistry of the tongue of these Egyptian bats. So, the present study was performed to investigate the histological structure and the histochemical characteristics of the lingual papillae of the mucosa of three species of bats, and discuss the structural features in relation to feeding habits of these animals.

2. Material and Methods

The collected Experimental animals:

In the present work, specimens of three species of Egyptian bats living in different habitats: the frugivorous bat *Rousettus aegyptiacus*, the insectivorous bat *Rhinopoma hardwickie* and the tomb-inhabiting bat *Taphozous perforates* were used. The frugivorous bats were collected from the vegetative and garden areas at El-Fayom Governorate. The insectivorous bats were caught from Abou-Rawash Constituency, whereas the tomb-inhabiting bats were gathered from certain uninhabited and deserted areas near El-Suez Governorate.

A- Histological preparations:

Each bat was dissected; tissue samples of the tongues were immediately excised, cut into small pieces, fixed rapidly in 10% neutral formalin solution, and then dehydrated in ascending series of ethyl alcohol, cleared in terpineol and embedded in paraffin wax. Sections of 4-6 μ m thick were stained with haematoxylin and eosin (Bancroft and Gamble, 2002), microscopically examined and photomicrographs were made as required.

B- Histochemical preparations:

For the demonstration of mucopolysaccharides, muscles and connective tissue small pieces of the tongues were fixed in 10% neutral formalin, the following staining methods were used:

1. Alcian PAS for the demonstration of both acid and neutral mucopolysaccharides (Hotchkiss, 1948).

2. Masson's trichrome stain to demonstrate muscles and connective tissue (Roy Mahoney, 1973).

3. Results

Histological results:

1- The tongue of the frugivorous bat, *Rousettus aegyptiacus*:

Examination of the histological sections obtained from the tongue of the frugivorous bat, *Rousettus aegyptiacus*, showed that it consists of three consecutive layers; mucosa, submucosa and muscularis (Fig.1). The first layer consists of stratified squamous epithelium and contains three types of papillae: filiform, fungiform and circumvallate papillae as illustrated in Figures 1, 2, 7 and 8. The filiform papillae are the most abundant and scattered all over the dorsal surface of the tongue. These papillae are subdivided into small and giant one (Figs. 1 & 7). They are characterized by a curved shape and a stratified epithelium bordered by a basal generative layer. The filiform papillae of the frugivorous bat exhibit weak keratinization (Figs. 2, 4 & 6). The fungiform papillae are scattered among the filiform papillae. The tops of these papillae have dome-shaped upper surface (Fig. 8). The circumvallate papillae are large flattened structures, project above the surface of the tongue and are surrounded by deep invaginations of the surface epithelium (Figs. 2&8). The second layer is submucosa appears as a wide and thick layer and consists of dense connective tissue which takes green colour with Masson's trichrome stain (Figs.7&8). The third layer (muscularis) is constructed of an outer thin layer of circular muscle fibres and an inner thick layer of longitudinal muscle fibres (Figs.1, 2&8). The longitudinal component is much distinct in this frugivorous bat as compared to the other two studied species (Figs.1-12).

2- The tongue of the insectivorous bat, *Rhinopoma hardwickie*:

The histological section of the tongue of the insectivorous bat, *Rhinopoma hardwickie* showed that, it is formed likewise of the three distinct layers; mucosa, submucosa and muscularis (Fig.3). The mucosa consists of very thick stratified squamous epithelium and contains the three types of papillae: filiform, fungiform and circumvallate papillae (Figs.4, 9 & 10). The filiform papilla is a conical mass (process) of stratified squamous epithelial cells. It is a slender sharp pointed structure (Figs.4&10). The fungiform papilla is a mushroom-shaped and has a core of connective tissue. The fungiform papilla is round in shape and its top is covered by thin keratinized epithelium (Fig. 9). The circumvallate papillae are flattened structures, exhibit a thin stratum corneum (Figs. 4& 9). The submucosa is built up of connective tissue rich with blood vessels and takes green colour with

masson's trichrome stain. The third layer (muscularis) is striated muscle bundles that arranged in different directions as illustrated in Figures 3, 9 & 10.

3- The tongue of the tomb-inhabiting bat, *Taphozous perforatus*:

The examination of histological section of the tongue of this bat revealed that it consists of three consecutive layers; the mucosa, submucosa and muscularis (Fig. 5). The mucosa is formed of very thick layer of Stratified squamous epithelium and contains three types of papillae: filiform, fungiform and circumvallate papillae (Figs.6&12). The filiform papillae are characterized by a curved shape with acuminate ends, and weakly keratinized stratified epithelium (Figs. 6&12). The fungiform papilla possesses dome-like shape, and its outermost stratified squamous epithelium is covered by a thin corneum (Figs. 6&12). The circumvallate papillae appear as disc- or rod-like structures on the surface of the tongue (Fig. 5). The submucosa consists of connective tissue which takes green colour with Masson's stain (Figs.11&12) and blood vessels; it is much thinner as compared with those of *Rousettus aegyptiacus* (Fig.8) or *Rhinopoma hardwichi* (Fig.10). The muscularis is constructed of an outer thin layer of circular muscle fibers and an inner thick layer of longitudinal muscle fibers (Figs.5&7).

Histochemical observations:

1-The tongue of the frugivorous bat, *Rousettus aegyptiacus*:

Examination of stained sections of the tongue of the frugivorous bat *Rousettus aegyptiacus*, revealed that the lingual glands of the tongue can be divided into two types; serous and mucous. The serous gland cells are pyramidal in shape and have single, round, centrally located nuclei. It gives red colour reaction with Alcian PAS stain, denoting the neutral mucopolysaccharide secretions. The mucous glandular cells are irregular in shape with basely flattened nuclei. It gives blue colour with Alcian PAS, denoting the neutral and acidic nature (Fig.13).

2- The tongue of the insectivorous bat, *Rhinopoma hardwiche*:

The lingual glands of the tongue of this bat are stained with red and blue colours with Alcian blue PAS method indicating the presence of neutral mucopolysaccharides at nearly same amount. The lingual glands showed the neutral and acid mucopolysaccharide secretions (Fig.14).

3-The tongue of the tomb-inhabiting bat, *Taphozous perforatus*:

In the tongue of the tomb- inhabiting bat, *Taphozous perforatus*, the lingual glands are mucous. The application of Alcian blue-PAS method showed the presence of acid mucopolysaccharides (Fig.15).

4.Discussion

On the surface of the tongue of mammals, there are various kinds of lingual papillae including filiform, fungiform, circumvallate and foliate papillae, each having different morphological structure and shape. Distribution of these lingual papillae has been considered to be related to species eating habits (Fawcett, 1986).

The diversity and differences of food habits in bats resulted in adaptations and modifications in the morphology of the tongue and teeth (Iwasaki, 2002). Various investigations have been reported regarding the morphology of tongue in long-Nosed bat (Greenbaum & Philips, 1974), Japanese long-fingered bat (Kobayashi & Shimamura, 1982), European common bat (Pastor *et al.*, 1993), lesser dog- faced fruit bat (Emura *et al.*,2001), large flying fox (Emura *et al.*,2001) and the Japanese pipistrelle (Emura *et al.*,2009).

Histological observation of the tongue of the investigation three species of bats; the frugivorous bat *Rousettus aegyptiacus*, the insectivorous bat *Rhinopoma hardwiche* and the tomb-inhabiting bat *Taphozous perforatus* showed that it is composed of three layers; mucosa, submucosa and muscularis. The dorsal surface of the tongue is rough due to the presence of three types of papillae; filiform, fungiform and circumvallates papillae. These observations provided similar results to those of Kobayashi & Shimamura (1982) and Pastor *et al.* (1993) in the insectivorous bats (*Miniopterus schreibersi* and *Pipistrellus pipistrellus*; respectively), Ismail (2000) in *Poecilictis libyca*, as well as with those of Emura *et al.* (2001; 2002 and 2012) in the fruit eating bats (*Pteropus vampyros* and *Cynopterus brachyotis*). These authors reported three types of lingual papillae on the dorsal surface of their examined species of bats; filiform, fungiform and vallate papillae).

The anatomy and histology of the tongue of frugivorous bat, *Certurio senex* were reviewed by Elizalde- Arellano *et al.*, (2004). The authors postulated that fungiform and circumvallate papillae were absent and numerous short single-pointed papillae and large papillae were observed on the dorsal surface of the tongue. This finding is contradicting that reported in the present investigation in the tongue of the frugivorous bat, *Rousettus aegyptiacus*, in which filiform, fungiform and circumvallate papillae are observed.

The number of vallate papillae in different species of bats was reported by several authors. There were two vallate papillae in the Japanese long-fringed bat (Kobayashi & Shimamura, 1982) and four vallate papillae in the long-nosed bat (Greenbaum & Philips, 1974). The present histological and histochemical observations could not detect or arrived to the exact number of vallate papillae in the three examined species of bats.

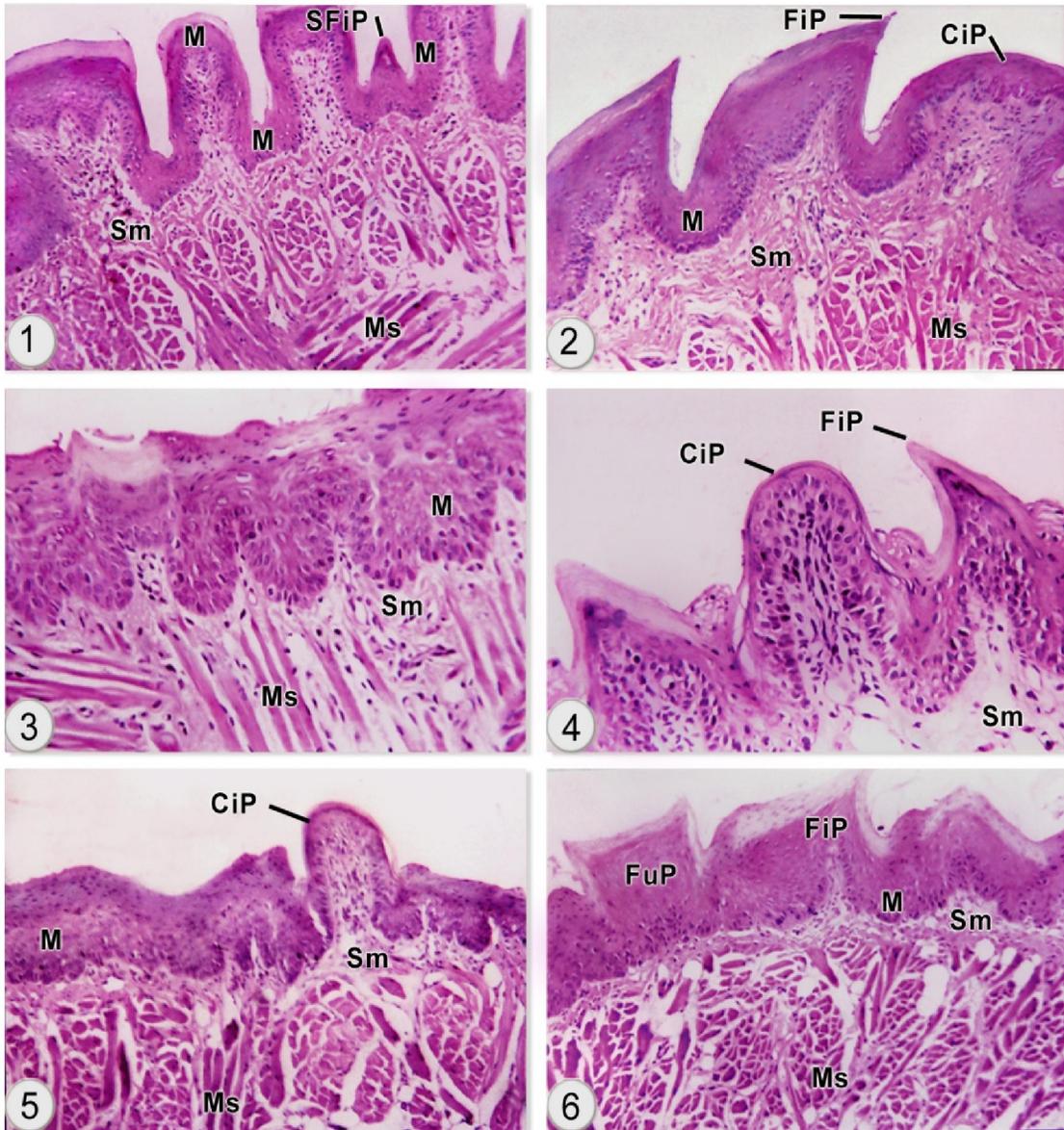


Figure (1): Photomicrograph of transverse section of the tongue of the Egyptian frugivorous bat stained with H&E showing the mucosa (M), submucosa (Sm), muscularis (Ms), and small filiform papilla (SFiP). X100

Figure (2): Photomicrograph of transverse section of the tongue of the Egyptian frugivorous bat stained with H&E showing the mucosa (M), submucosa (Sm), muscularis (Ms), filiform papilla (FiP) and circumvallate papilla (CiP). X200

Figure (3): Photomicrograph of transverse section of the tongue of the insectivorous bat stained with H&E showing the mucosa (M), submucosa (Sm) and muscularis (Ms). X200

Figure (4): Photomicrograph of transverse section of the tongue of the insectivorous bat stained with H&E showing the submucosa (Sm), filiform papilla (FiP) and circumvallate papilla (CiP). X200

Figure (5): Photomicrograph of transverse section of the tongue of the tomb - bat stained with H&E showing the mucosa (M), submucosa (Sm), muscularis (Ms) and circumvallate papilla (CiP). X100

Figure (6): Photomicrograph of transverse section of the tongue of the tomb - bat stained with H&E showing the mucosa (M), submucosa (Sm), muscularis (Ms), filiform papilla (FiP) and fungiform papilla (FuP) X200

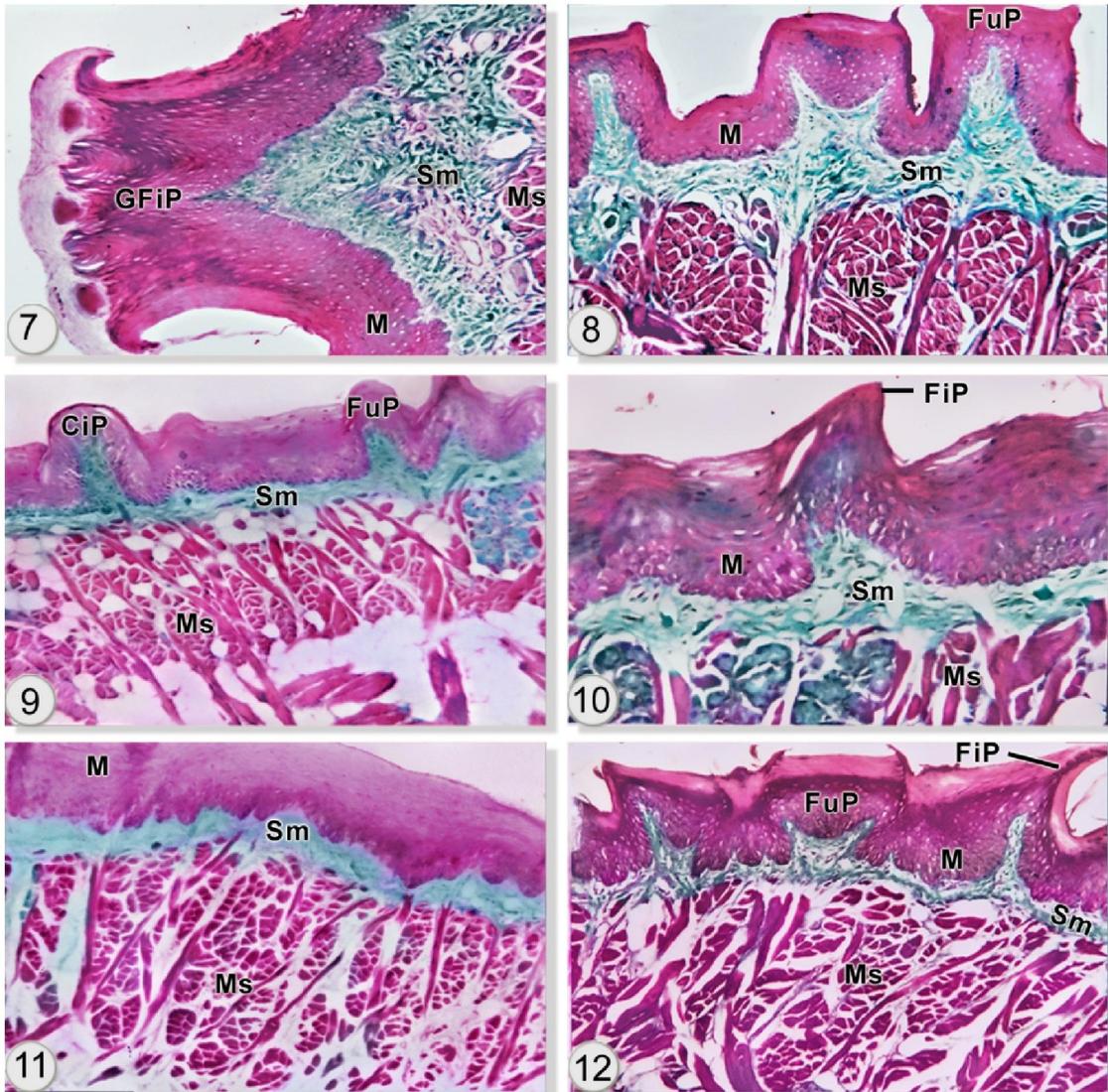


Figure (7): Photomicrograph of transverse section of the tongue of the Egyptian frugivorous bat stained with Masson's trichrome stain showing the mucosa (M), submucosa (Sm), muscularis (Ms) and giant filiform papilla (GFIP).
X100

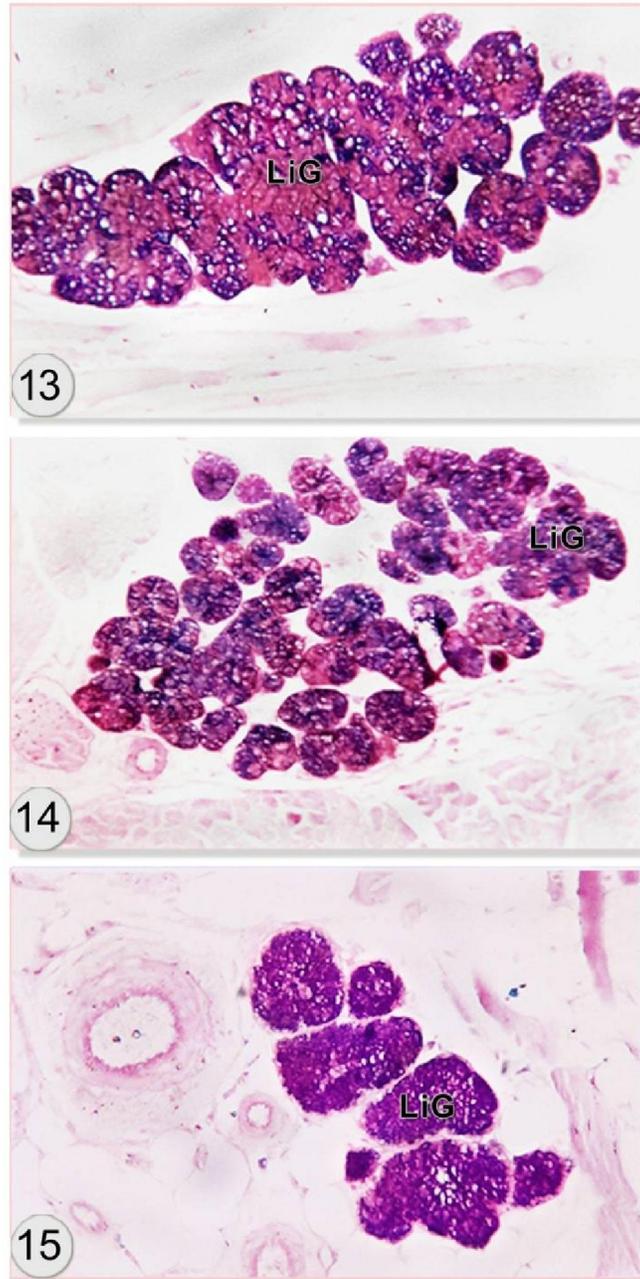
Figure (8): Photomicrograph of transverse section of the tongue of the Egyptian frugivorous bat stained with Masson's trichrome stain showing the mucosa (M), submucosa (Sm), muscularis (Ms) and fungiform papilla (FuP).
X100

Figure (9): Photomicrograph of transverse section of the tongue of the insectivorous bat stained with Masson's trichrome stain showing the submucosa (Sm), muscularis (Ms), fungiform papilla (FuP) and circumvallate papilla (CiP).
X100

Figure (10): Photomicrograph of transverse section of the tongue of the insectivorous bat stained with Masson's trichrome stain showing the mucosa (M), submucosa (Sm), muscularis (Ms) and filiform papilla (FiP).
X200

Figure (11): Photomicrograph of transverse section of the tongue of the tomb - bat stained with Masson's trichrome stain showing the mucosa (M), submucosa (Sm) and muscularis (Ms).
X100

Figure (12): Photomicrograph of transverse section of the tongue of the tomb - bat stained with Masson's trichrome stain showing the mucosa (M), submucosa (Sm), muscularis (Ms), filiform papilla (FiP) and fungiform papilla (FuP).
X200



Figures (13): Photomicrograph of transverse section of the tongue of the Egyptian frugivorous bat stained with Alcian PAS stain showing the lingual glands (LiG). X200

Figure (14): Photomicrograph of transverse section of the tongue of the insectivorous bat stained with Alcian PAS stain showing the lingual glands (LiG). X200

Figure (15): Photomicrograph of transverse section of the tongue of the tomb - bat stained with Alcian PAS stain showing the lingual glands (LiG). X200

Such information will be performed through scanning electron microscopy in forthcoming studies. On the other hand, the flattened shape of circumvallate papillae observed in the present investigation in the tongues of *Rousettus aegyptiacus* and *Rhinopoma hardwickie*, resembled those described in the tongues of other species of

bats, *Pteropus vampyrus* (Emura *et al.*, 2002) and *Myotis macrodactylus* (Hwang & Lee 2007).

Different types of papillae are observed in the dorsal surface of chiropteran tongues (Greenbaum & Philips, 1974; Kobayashi & Shimamura, 1982; Pastor *et al.*, 1993; Emura *et al.*, 2001, 2002). In insectivorous bats, the Japanese long-fringed bat (*Miniopterus schreibesi*) and the European common

bat (*Pipistrellus pipistrellus*), the filiform papillae are classified into five types (Kobayashi & Shimamura, 1982) and three types (Pastor *et al.*, 1993) respectively.

On the other hand, in the fruit eating bats (the large flying fox and the lesser dog-faced fruit bat) the filiform papillae are classified into six types (Emura *et al.*, 2002) and five types (Emura *et al.*, 2010), respectively. The present observations of the fruit-eating bat (the Egyptian rousette bat) showed that the filiform papillae classified into small and giant papillae. These papillae appear to be the most abundant and scattered all over the dorsal surface of the tongue. This observation confirms the findings of Jackowiak *et al.* (2009) who reported the presence of small and giant filiform papillae on the anterior part of the tongue of the Egyptian fruit bat. This arrangement of filiform papillae in the Egyptian fruit bat is considered to be useful for the efficient uptake of semi liquid food as it can be collected toward the median line of the tongue.

In the present investigation, the fungiform papillae are spread among the filiform papillae. The circumvallate papillae are raised slightly above the general surface level of the tongue and are surrounded by deep invaginations of the surface epithelium. The types of gustatory papillae (i.e. fungiform and vallate papillae) in the three species of bats under investigation are generally similar to those observed by Greenbaum and Phillips, 1979 in long-nosed bats. The variety of the histological characteristics of the lingual papillae of the three bats related to be kind of diet and feeding habits. This assumption confirms the finding of Ebru *et al.* (2010) in Zavot cattle.

The nature of the lingual papillae of the three studied bats has mucopolysaccharide secretions. This observation agrees with that of Taki-El-Deen (2003) in her study on *Taphozous nudiventris*.

In conclusion, marked differences were noticed in the histological structure of the tongue of the three Egyptian bat species, in addition to the histochemical reactivities of neutral and acid mucopolysaccharides in the lingual glands.

Such dissimilarities are probably due to environmental factors, or may be correlated to the mode of occurrence of certain kinds of food and deprivation of other kinds in their habitats.

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