Histological study of tongue in insectivore bat (*Rhinopoma hardwickii*)

1Sara Mohebinia and Frangis Ghassemi

1Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom, Iran.
2Department of Biology, Jahrom branch, Islamic Azad University, Jahrom, Iran.

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**ABSTRACT**

Insectivore bats play an important role in biological defense by feeding on pests. In this survey, the characteristics of tongue as the main organ for getting food were studied by light microscope. The present study was conducted on five tongues of insectivore bat (*Rhinopoma hardwickii*). The tongues were dissected from root and measured weight, anterioposterior length and diameter in apex, body and root of them. Then they were fixed in formaldehyde (10%) and were prepared for light microscopic study by tissue processor. The samples were dehydrated in graded series of ethanol (70%-100%), impregnated with paraffin and serial sections with 4μ thickness were obtained. The sections were subjected to H&E stains, mounted on light microscope and were prepared photomicrographs. The tongue in this bat was muscular with 1.34 cm long and weighed about 1.20 g. The histological observation indicated that keratinized stratified squamous epithelium and keratinization with variable degrees were on both surface of tongue. Three types of papilla (filiform, fungiform and circumvallate) with different distribution and density were observed on the dorsal surface. Two types of filiform papilla with different size and shape were on the whole of dorsum of tongue. A few fungiform papilla were on midlateral and single circumvallate papilla were situated in root of tongue. Mucous and serous glands, mass of muscles and collagenous fibers were observed especially on ventral surface. In conclusion, morphology of tongue can be related to diet type.

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**INTRODUCTION**

Insectivorous bats involve in the biological control by feed on insect pests, so they are very important in ecosystem. The histology of the tongue was studied in many vertebrates [25,14,11,21,27]. The anatomical study of tongue specially lingual papilla also were done on fruit eater bat [3,4,9,6] and insectivorous species belong to families such as Molossidae [7], Phyllostomidae [8], Rhinolophidae [21] and Vespertilionidae [23,10].

There are various types of lingual papillae including filiform, fungiform, circumvallate, lentiform and foliate papillae on the surface of the tongue of mammals [13]. They have different morphological structure and role [14,2]. These papillae are gustatory or mechanical.

In present study, histological structure of the tongues in relation to diet was studied in *Rhinopoma hardwickii* as insectivorous bat.

**MATERIALS AND METHODS**

Sampling was carried out with hand and using mist-net (in different sizes and 4 cm mesh) from Tadvan cave in the southwest of Iran (28˚50'45"N, 53˚19'46"W) with 200m length and 20-65m altitude (Fig. 1).

*Rhinopoma hardwickii* or less mouse-tailed bats belong to Rhinopomatidae that are insectivore. Their diet is less diverse than other bats and beetles comprising up to 50%.

Tongues of five bats were dissected from the root, measured their length and width. The 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 stained using combination of Haematoxyline and Eosin (H & E). Sections were mounted on binocular light microscope and photomicrograph of each slide was taken for further analysis.

**Results:**

The muscular tongue weighed about 1.20 g and elongated 1.34 cm. Three areas as apex, body and root that were respectively 0.1, 0.15, 0.35cm width distinguished in the dorsal surface of the tongue. The apex of tongue was narrow and rough with thickly keratinized papillae (Fig. 2). It is composed of mucosa, submucosa and muscularis layer. The epithelium was stratified squamous with keratin spatially on dorsum (Fig. 3, 5). Three types of lingual papillae include filiform, P, fungiform, P and vallate, P (Table. 1), thin connective tissue with blood vessel and glands was observed on dorsal surface (Fig. 4).

Epithelium and keratin in ventral surface were thinner than dorsal surface. Many glands (serous and mucous gland), thick lamina propria and muscularis layer (circular and longitudinal) and collagenous fibres was observed in this surface (Fig. 3).

Needlelike filiform papillae with smal size and high density were known as Type I placed in apex. Giant and bifid filiform papillae with smooth surfac and trifid process as Type II were suited mid and posterior of tongue (Fig. 3).

One vallate papillae with concave shape and numerous taste bud that surrounded by cleft were between posterior of lingual body and root. Many glands were around this papillae and connected to its cleft (Fig. 5).

Fungiform papillae were Large, mushroom form with thick lamina propria in core placed between filiform papilla espitialy on midlateral of tongue (Fig. 3).

<table>
<thead>
<tr>
<th>Papillae type</th>
<th>lengh(cm)</th>
<th>width(cm)</th>
<th>Distribution</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filiform</td>
<td>0.12, 0.12, 014</td>
<td>0.11, 0.10, 0.10</td>
<td>Whole of dorsal surface</td>
<td>High</td>
</tr>
<tr>
<td>Fungiform</td>
<td>0.18, 0.16</td>
<td>0.12, 0.10</td>
<td>Mid lateral</td>
<td></td>
</tr>
<tr>
<td>Few</td>
<td>MP</td>
<td>M P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vallate (one)</td>
<td>0.22</td>
<td>0.22</td>
<td>Posterior of body</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1: Map of research station (of Iran)
Fig. 2: Dorsal surface of tongue in *Rhinopoma hardwickii*

![Dorsal surface of tongue](image)

**Fig. 3: Photomicrograph of lingual papillae** (*Rhinopoma hardwickii*) Stain: H&E, 40x

**Discussion:**

Variations of food and habits in bats resulted in adaptations and modifications in the morphology of the tongue and teeth. Although there are six types of papillae in mammals [13] only three types of them were observed in this species (*hardwickii*) as insectivore bat (Fig. 3, 5). Distribution and types of these lingual papillae are related to species eating habits [4].

Lingual papillae especially filiform P by making rough surface in the front of tongue are effective in touching and taking of the food particles (Fig. 4) and facilitate the transfer of food [21]. According to previous findings, small and giant filiform papillae with orientation forward to posterior aid to swallowingon [15].

Fungiform papillae have a few taste bud in some mammals [14] but there was one taste bud was seen in this research (Fig. 5). The fungiform and vallate papillae were placed on midlateral and posterior of tongue due to causes the food particles to be exposed to the taste buds. The number of circumvallate papillae in bats is different and depends on food habit [1]. Noting to four was reported in insectivore bats [24,14].

The variation in the distribution and types of lingual papillae were related to manner of food uptake [13].

Most of serous and mucosa glands were observed on dorsal surface. This finding showed that these glands were near the gustatory papillae as fungiform and vallate [26]. Because food were digested and exposed to taste buds. As for the relation between dietary intake and oral glands, may be diet type is effective. Probably food particle were ingested fast and most of digestion were occurred after passing the mouth.

Lack of keratin on the ventral surface epithelium cause to contact food and gland's secretion. Presence of serous glands aid to digestion of protein and the insect body is composed of a high amount of protein (Fig. 6). Thick muscularis and hyalin cartilage from apex to root, also aid to movement of tongue [15] that is necessary for predatory behavior in bat (Fig. 7).
Fig. 4: Photomicrograph of lingual apex (*Rhinopoma hardwickii*) Stain: H&E, 10x

![Image of lingual apex](image1.png)

Fig. 5: Photomicrograph of lingual papillae (*Rhinopoma hardwickii*) Stain: H&E, 40x

![Image of lingual papillae](image2.png)

Fig. 6: Photomicrograph of lingual gland (*Rhinopoma hardwickii*) Stain: H&E, 10x

![Image of lingual gland](image3.png)

Fig. 7: Photomicrograph of lingual cartilage (*Rhinopoma hardwickii*) Stain: H&E, 40x

![Image of lingual cartilage](image4.png)
Conclusion:
The present study showed that the type and the distribution of lingual papillae probably due to diet habit.

ACKNOWLEDGMENTS

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REFERENCES


