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Study on lingual morphology of tongue of Insectivorous Bat, *Hipposideros speoris*

Dr.Asha Vilas Ramteke

Assistant Professor

Department of Zoology, Seth Kesarimal Porwal College, Kamptee, Distt. Nagpur.

E- mail : asharamteke 08@ gmail.com.

ABSTRACT:

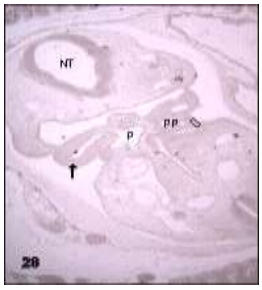
Elongated and movable tongue of *Hipposideros speoris* with highly keratinized epithelium as well as numerous and different types of papilla on the dorsal surface of tongue shows its insectivorous habit. Variation in the distribution and types of lingual papillae related to which types of food uptake. A conspicuous feature of the diets of insectivorous, feeds on a variety of insects. Insectivorous bats consume diversity of insects such as Lepidoptera, Diptera, Coleoptera and Hymenoptera. This diversity of feeding habits directly reflects on the tongue morphology. For their insectivorous feeding habits their lingual morphology of tongue are very appropriate. Present research paper was based on the histological structure of different types of mechanical and gustatory lingual papillae of tongue of *H. speoris* at different developmental stages of tongue i.e. Prenatal, Postnatal and Adult.

KEY WORDS: Bat**,** *Hipposideros speoris*; Insectivorous Microchiroptera; Tongue.

INTRODUCTION:

Tongue of mammals plays important role in feeding such as collecting solutions, taste of food and swallowing it. The tongue is a muscular organ and consists of heavy network of interlacing bundles with greater mobility and rigidity. In vertebrates, the foundation of the visceral portion developed from branchial arches. At the early stage of embryonic development, the primitive gut appears as a cavity without any opening, latter the oral region is established by the ectodermal depressions i.e. stomodaeum. The mammalian lingual organ developed from the palatal shelves. The fusion of palatal shelves separated the nasal and oral region. The tongue arises from first arch tissue and the root of tongue clothed by endoderm originated from second and third branchial arch (Pattern 1968). The sensory innervations of this part done by trigeminal nerve which were originates from the first pharyngeal arch. (Moore 1982, Spielmal *et. al*., Barlow et. al., 1995).

Fig. 1.



▪ ***Fig. 1.*** *T. S. of H. speoris embryo (at early limb bud stage with C. R. length 2.5mm) showing the cephalic and visceral portion of head. The appearance of the developing notochord is a thickened ectodermal area at anterior of the primitive streak. Neural plate formed from the notochord, which immediately become folded into neural tube. Visceral portion of head has stomodeal depression, paired maxillary process i.e. hyoid arch (arrow head), mandibular arches (long arrow) and branchial arches (small arrow) are well identified at that stage. Pharynx is formed from forgut, which had a four pharyngeal pouches are arranged opposite to the branchial groove. Neural tube- NT. Buccal cavity- BC. Maxillary Process- MP. Pharyngeal pouches- PP. Pharynx- P. x 35.*

The mammalian animals have different species with morphological diversity of tongue depends on the dietary habits and their living environment. Mammalians tongue at the dorsal surface characteristically covered by different types of papilla which have different specialized distributional location. Nearly all mammalian animals have the same type of papillae, filiform, fungiform and foliate or circumvallate papillae but there is major difference on the subtype of filiform ones. In mammals, the taste buds have receptor cells located on certain papillae. The tongue and their lingual papillae of the mammalian animals show morphological variations which depends on the evolutions, taxonomy of mammalian species and environment in which the mammals live. The morphology and function of tongue in different species of bats were different.

Sonntag (1920), studied the comparative anatomy of the mammalian tongues and observed the taste buds were associated with fungiform and circumvallate papillae of the tongue in Chiropterans.

Kobayashi and Shimamura (1982) studied the anatomical and histological structure of Japanese long-fingered bats, *Miniopterus scheribersi fuliginosus* by light microscope and scanning electron microscope.

Uieda (1986), studied the comparative morphology tongue in three species of Vampire bats, *Desmodus Rotundus*, *Diaemus*, and *Diphylla.*

Pastor e*t. al*., (1993), noticed in *Pipestrellus pipestrellus* that, the filiform papillae have pores and microridges add for the production and distribution of mucus over the papillary surface.

Our aim of this research paper was to study the histological structure of lingual papillae of Insectivorous species, i.e. *Hipposideros speoris*. *H. speoris* belong to Class "Mammalia",

Order is “Chiroptera”, Suborder is “Microchiroptera” and Family is “Rhinolophidae”. This insectivorous species generally habitat in hot humid caves, tunnels, underground dilapidated rooms. Common name of *H. speoris* is “Indian Horse-Shoe Nose Bat". **Fig. 2 &3.**

Fig 2

Fig 3

- **Fig.2.** *Photograph of female H. speoris to show external morphology.*
- **Fig.3.** *Photograph of huge colony of H. speoris. The colony comprises hundreds of individuals in the underground dilapidated dark rooms of old fort at Ballarshah, District Chandrapur.*

MATERIAL AND METHODS

Preserved material was used for this work. For histological study, the tongue was fixed in different fixative. Alcoholic Bouins, Aqueous Bouins and 10% formalin for 24 hours, then washed overnight in running tap water and dehydrates by passing through different grades of ethyl alcohol, cleared in xylene and embedded in Paraffin.The sections were cut at 5-7 um with the help of rotary microtome. For routine histological observations, were stained with Haemato-Xyline-Eosin method.

RESULT : Anterior tip of insectivorous bat, *H. speoris* was triangular and at the ventral side possessing a prominent central groove in-between the two lateral sides **(Fig 4 & 5.**

Fig 4

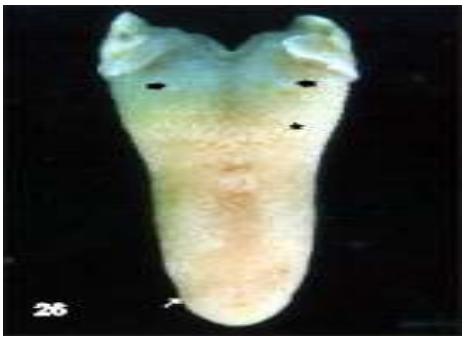


Fig 5

Fig 5

Figs.4 and 5. *Photographs of the tongue of H. speoris to show dorsal (Fig.4) and ventral (Fig.5) surface. The insectivorous bat had a small anterior blunt tip with a cluster*

of filiform papillae on the dorsal part of the tongue. The filiform papillae are smaller (small arrow) at the anterior portion and larger (star) towards the posterior region. Fungiform papillae are embedded between the filiform papillae. Two circumvallate papillae (thick arrows) are present at the posterior region (Fig.4). One-third part of anterior tongue was free at the ventral side (Fig.5); possessing a prominent central groove (arrow head) in-between the two lateral ridges(arrow)x8

Tongue of *H.S.* at prenatal stage covered large area of the dorsal surface of tongue with papilla. Beneath the papillary region, the lingual mass composed of lingual muscles, lingual glands and vasculature embedded in the connective tissue. **(Fig 6).** Major salivary gland lie deeply among the muscle bundles, connective tissue and blood vessels. The gland had small ducts for secretion. **(Fig.8).** Dorsal surface of tongue covered with round shaped fungiform papillae surrounded by scale-like filiform papillae. **(Fig.7)**.

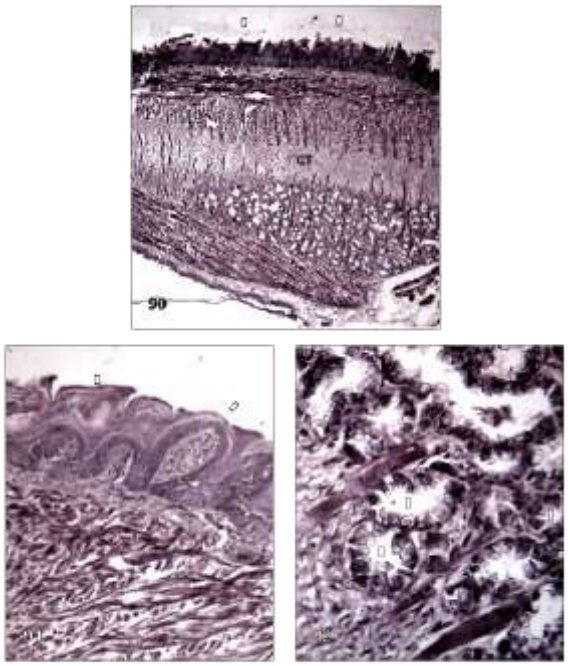


Fig.6, 7 & 8

Tongue of *H.S.* at the postnatal stage with body weight 19 gms. The length, breadth and weight of tongue are 10mm, 4mm and 0.05gms.The anterior blunt tip of tongue covered by scale-like, hook like filiform papillae. **(Fig.9,10).** The posterior region gives bumpy appearance to the tongue. The tongue was increase in size along with the size of papilla and its related taste buds as compared to their late prenatal stage**. Table I& II shows e measurement of different papilla and taste buds**. (Ramteke A.V. 2011). In

the lingual mass the network of muscle bundles and blood vasculature was more complex. In circumvallate and in some fungiform papillae, the taste buds were increase in size and numbers. (Farbman 1965, Mistretta *et. al*., 1988, Yamaguchi *et. al*., 2000). The different types of mechano and chemosensitive papillae at early postnatal stages were performing certain functions but their functional maturity reached by the age of three weeks when animals attends from liquid suckling to digestion of solid food (Lyubimova and Esakov, 1979, Trzeielinska-Lorych *et. al*., 2009).

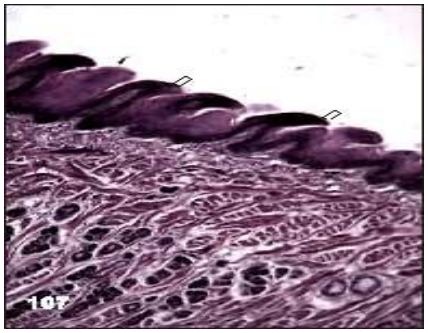
TABLE I & II: Shows Measurement of Different Type of Papilla and Taste Buds

TABLE I.

Sr. No.	Species	Stage	Filiform Papillae						Fungiform papilla		Taste bud of Fungiform papilla		Taste bud cell of fungiform papilla	
			Scale like		Horney		Tricuspid							
			H	B	H	B	H	B	H	B	H	B	H	B
1.	*H. speoris*	Prenatal	12. 8	9. 6	11. 2	12. 8	8. 0	20. 8	12. 8	9. 6	-	-	-	-
		Postnatal	19. 2	14. 4	20. 8	12. 8	9. 6	-	19. 2	16. 0	-	-	-	-
		Adult	12. 8	9. 6	16. 0	17. 6	12. 8	17. 6	22. 4	6. 4	17. 6	9. 6	8. 0	3. 2

TABLE II.

Sr. No.	Species	Stage	Circumvallate Papilla		Taste bud of circumvallate papilla		Taste bud cell of circumvallate papilla		Cells of salivary gland		Salivary gland (diameter)	
			H	B	H	B	H	B	H	B	L	B
1.	*H. speoris*	Prenatal	20. 8	17. 6	14. 4	6. 4	12. 8	3. 2	12. 8	3. 2	4. 8	1. 6
		Postnatal	12. 8	14. 4	16. 0	6. 4	12. 8	3. 2	12. 8	3. 2	4. 8	1. 6
		Adult	32. 0	17. 6	19. 2	11. 2	16. 0	4. 8	6. 4	3. 2	4. 8	1. 6



- *Fig. 9. Section of tongue of Hipposideros speoris at the postnatal stage to show the anterior blunt tip of tongue covered by scally filiform papillae (arrow heads) embedded in squamous stratified epithelium. x 100.*
- *Fig. 10. Section showing the hook like filiform papillae (arrow heads) at the posterior region; gives the bumpy appearance to the tongue. x 100.*

The tongue of bat at adult stage is a highly specialized muscular organ covered by different types of lingual papillae. The small insectivorous bat *H.S.* shows the smallest tongue. The dorsal surface of the tongue was covered mainly by mixture of filifrom papillae i.e. Scale like, tricuspid and few pointed hook like papillae in between laying the fungiform papillae. Anterior blunt tip of the tongue possess few tricuspid papillae **(Fig. 11).** The filiform papillae are smaller at the anterior portion and larger towards the posterior region. A highly keratinized parakeratotic layer comprises the entire surface of these papillae. Anterior two-thirds of the dorsal surface was roughened by many fine projections i.e. the papillary area give a brush like appearance **(Fig. 12)**.Beneath the surface epithelium in the mucous membrane, the striated muscle bundles are observed running in different planes i.e. Vertical, longitudinal and transverse. The connective tissue, blood vessels, lingual glands and nerve cells are present around the muscle bundles. The groups of major salivary glands **(Fig 15**) with numerous small ducts for secretion were observed towards the base of the tongue. In the posterior region, the circumvallate papillae are formed by twelve or thirteen comparatively large circular projections, each of which was surrounded by narrow groove. These papillae lie in a "V" shaped line, with the apex towards the back of the tongue **(Fig. 13)**. The elongated taste buds occupied the lateral wall of the vellum in the cavity of stratified epithelium. The base of the taste buds rests upon the corium of the mucous membrane **(Fig.14)**. The large lingual glands with numerous small ducts are observed in the posterior region of the tongue amongst the muscle bundles, connective tissue and blood vessels as well as occurred at various portions of the dorsal surface.

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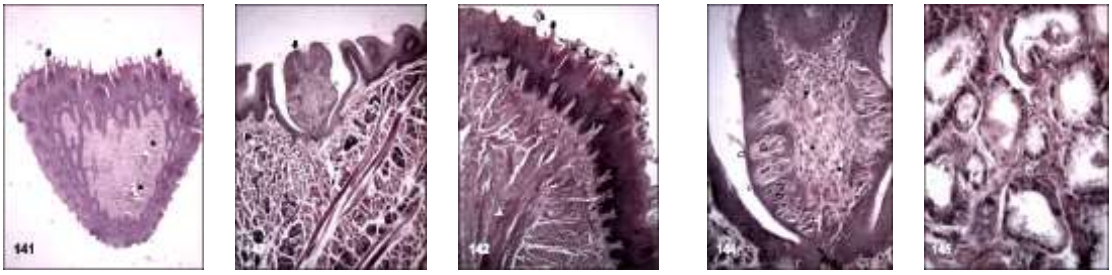

- *Fig 11. T. S. of tongue of adult Hipposideros speoris showing the anterior region of the tongue. Anterior triangular blunt tip had a cluster of tricuspid papillae (thick arrows) surrounded by scaly filiform papillae, which are more keratinized. Beneath the surface epithelium, the mucous membrane consists of the striated muscles (stars). x 80.*
- *Fig 12. Part of the section to show the anterior two thirds of the upper surface was roughened by many fine projections (thick arrows) i.e. the papillary area gives plushlike appearance to the dorsal surface. Under the surface epithelium the striated muscles running in a 3 different planes i.e. vertical, longitudinal and transverse (arrow heads). x 100.*
- *Fig. 13. Section to show the circumvallate papilla (thick arrow) at the posterior region. The papillae lie in a V shaped line and consists the taste buds in the lateral wall of stratified epithelium. x 40.*
- *Fig. 14. Part of Fig. 143 magnified showing the circumvallate papillae formed by large circular projections surrounded by narrow groove. The taste buds (arrow heads) are elongated and lie in the cavity of stratified epithelium. The base of the taste buds rests upon the corium of the mucous membrane (stars) and consists the pale stained of spindle shape of cells of taste bud (small arrows). x 250.*
- *Fig. 15. Section showing the serous glands (arrow heads) with numerous small ducts. These types of lingual glands were the small tubulo-acinose mucous type and occurred at the various portions of the dorsal surface of the tongue, being especially abundant in the lymphoid area of tongue. x 300.*

DISCUSSION:

Insectivorous bats exhibit high species diversity (Simmons 2005). Some of morphological difference of tongue between different species of insectivorous bat is due to different passive prey selection, variation in diet composition and habitat that maintain high insect diversity. Coexisting bat species frequently differ in resources use in at least 1 niche dimension and thus avoid competition (Saunders & Barclay 1992). Differences in ecomorphological traits such as wing shape, body size and sensory cues are associated with differences in the access of bat species to food (Adridge & Rautenbach 1987). Dietary composition of insectivorous bat species are mostly contains 4 insects orders (like Coleoptera, Diptera, Lepidoptera and Hymenoptera). A conspicuous feature of the diets of insectivorous bats feeds on a variety of insects for example, beetles, orthopterans and moths. Insectivorous bats, feeds mainly on flying insects and does not take small terrestrial vertebrates. Lepidoptera (moths) or Coleoptera (beetles) or both dominate in the diet of Hipposiderid bats. According to previous studies, almost all Hipposiderid bats consume mainly Coleoptera (larger species of insects), (Vaughan 1977, Aldridge & Rautenbach 1987). They can consume mainly nocturnal and actively flying insects. For insectivorous species, food abundance, echolocation and the ability to detect prey may be the most necessary determinants of habitat use. In general, areas that favor movement which have high food availability are visited more frequently (Hagen and Sabo 2011). Higher concentration of insects and ease of travel and prey capture may result in a higher activity of aerial insectivorous bats. Ma *et. al* (2008), discussed that the highly specialized ability of Insectivorous bats that can pick up the wing beats of their prey in echo-cluttered environment. Prey-specific modulations in the constant frequency component of the returning echo, which are induced by the alternating wing movement relative to the bats. So, the diet of insectivorous bats generally seems to be influenced by echolocation characteristic. They use high frequency echolocation and rely on hearing as their major locational sense.

Different variety of insect in the diet of Insectivorous bats is affected by wing morphology of bats flight in open and cluttered space, echolocation call and body size of bats. Due to species-specific differences in traits consume different resources and shows greater impact on their tongue morphology. Hipposideridae has been predicted to use both open and cluttered space for feeding (Norberg & Rayner 1987). Hipposideridae generally can forage on larger and harder prey such as beetles. Because of the variation in prey hardness, the bite performance influences the diet composition of bats (Aquirre *et. al*. 2003). Due to consumption of hard prey, they have a need of more secretion for making the food more lubricants, so large size of lingual gland with numerous small ducts are observed in the posterior region of the tongue. Secretion of glands are added from the major salivary glands of the mouth.

The tongue of insectivorous bats is extremely complex and the epithelium of the anterior half of the tongue is extremely keratinized. Stratification and keratinization is a common feature of mammalian lingual epithelium. The keratinization of lingual epithelium were changed from moist or wet to dry environment.

The dorsal surface of insectivorous bats is covered with filiform, fungiform and circumvallate papillae. Dorsal anterior portion of the tongue has a numerous filiform papillae those are distributed all over and consists of a slender vascular core of connective tissue which covered by cornified stratified squamous epithelium. The numbers of fungiform papillae are few and are interspersed among filiform. Fungiform are rounded, non-cornified and broader than base. Generally in insectivorous bats, three types of conical papillae were observed at the anterior part of tongue according to their Shape, Size, Distributive pattern and Form. Small conical papillae (I and II) present at the apex, which mainly involved in touch and attachment to the food, while small conical papillae (II and III) present at the anterior

part of tongue and their function is the taking food from both peripheral sides to median sulcus or central portion and at last transport it into oral cavity and also involved in mastification process.

CONCLUSION:

Elongated and movable tongue of *H.S.* covered by highly keratinized epithelium. Numerous and different types of papilla on the dorsal surface of tongue shows it's insectivorous feeding habit. Variation in the distribution and types of lingual papillae related with, which type of food uptake by different families of insectivorous bats. The structure of tongue changed according to the habits and environment condition, so the major relationship between morphology of tongue with surrounding climate. The absence or presence of keratinization on the lingual epithelium related to the habitat of tongue and environmental condition.

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