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ISSN 2249-3352 (P) 2278-0505 (E)
Cosmos Impact Factor-5.86

DEVELOPMENTAL BIOLOGY OF TONGUE OF *ROUSETTUS LESCHENAULTI*

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Abstract: There are 4,800 species of mammals currently living on Earth. These mammals are divided into three subclasses i.e. Prototheria, Metatheria and Eutheria. These three subclasses are further divided into 26 or more orders. These are all species of mammals that exhibit tremendous morphological, physiological and developmental diversity. Yet embryonic development has been studied in only a few mammalian species. Among mammals, bats are second only to rodents with regards to species number and habitat range and are the most abundant mammals in undisturbed tropical regions. Bats, the second largest order of mammals and characterized as the only mammals to have evolved true flight are divided into two suborders, the Megachiroptera and Microchiroptera. In mammals the tongue can be classified as intra-oral; Marsupialia (Dasyurus, Sarcophilus, Trichosurus): Rodentia (Rattus): Carnivora (Felis canis): Artiodactyla (Camelus, Bovis, Ovis): Perissodanivora (Equus): Primates (Homocallicebus) and extraoral : Monotremata (Tachyglossus): Marsupialia (Tarsiper): and Pholidota (manis). The intra-oral tongue are mainly spatulate-shaped and oval in cross section provided with bilateral lingual arteries, veins and nerves, while the extra-oral tongue are fusiform in shape and either triangular or circular in cross section with single lingual artery veins and nerves. In the present study, it is examined the staged development of tongue of *Rousettus leschenaultia* at the early limb bud stage, late limb bud stage, paddle stage, phalange stage, late prenatal stage, postnatal stage and adult. The tongue of embryo of *R.L.* shows well developed tongue, dorsal surface covered with lingual papillae; below the epithelium a well - developed network of lingual muscles and blood vessels. Mucous and salivary glands were well developed and embedded in the connective tissue at the posterior region. At fetal stage, the epithelium layer of tongue appeared to be periderm. Fungiform papillae were developed earlier than filiform papillae. Just after the birth, the periderm disappeared and few filiform papillae were developed.

Key words:

Introduction: The tongue of *Rousettus leschenaultia* is a highly muscular organ attached to floor of the oral cavity surrounded by different types of foliate papillae on the dorsal, while the ventral region was devoid of papillae. *Rousettus leschenaulti* is a medium-sized fruit bat with a short tail. Ear pinnae are hairless and separated by philtrum. The globe of the eyes are prominent, large and dark brown in colour. The pelage is dull grey brown throughout the body. They are generally habitat in roosts in caves, tunnels; disused buildings and rarely in trees. This bat species is found in a variety of habitats ranging from tropical forests to urban environments. It roosts in caves, old abandoned buildings and tunnels and other such structures. The frugivorous bat *Rousettus leschenaultia* have a wide habitat tolerance but their day roosts were invariably humid. *Rousettus leschenaultia* preferred to roost in deserted buildings, historical monuments. A colony of this animal can contain upto several thousands individuals. It feeds on fruits, nectar and flowers. *Rousettus leschenaultia* belonging the Phylum –Chordata, Subphylum is Vertebrata, Class is Mammalia, Order is Chiroptera, Family is Pteropodidae, Subfamily is Pteropodinae, Genus is *Rousettus* and Species is *leschenaultia.*

A literature survey reveals that meager information is available on the detailed structure of tongue of bat in general, (Suthers, 1970; Glass 1970, Winkelmann 1971, Greenbaum and Phillips 1974, Wille 1954, Griffiths 1978,1981,1982, Uieda 1986, Griffiths and Criley 1987 and on Indian bats in particular (Agarwal and gupta 1982; Singh and Bhatti 1993.

Mistretta et. al. (1988), observed the morphology and total numbers of fungiform papillae in receptive fields of single Chorda tympani fibers during the different stages of development in sheeps viz, Fetal, Perinatal and Postnatal. They observed that decreased the number of taste buds and numbers of fungiform papillae at the time of development. The increasing or decreasing level of taste bud dependent on the increasing size of fungiform papillae because the number of papillae on the tongue were always remain constant but the size of papillae increases with increasing the number of taste bud. In Perinatal stage, the taste receptive fields were more static and due to the hyperinnervation of nerve fibres increasing the number of taste bud as well as the size of papillae because the significant relation between fungiform papillae size and the numbers of taste buds as per papillae. At the postnatal stages, the number of taste buds decreases because of the elimination of some innervation. In perinatal stage, taste buds and chorda tympani nerve fibres were more in number, while less in postnatal stage because of some changes occurred in morphology and in neural organizations.

Yamaguchi *et.al.* (2000) studied on age related alteration of taste bud distribution in the common marmosets. The number of taste buds of soft palate foliate as well as the circumvallate papilla were different at the different postnatal stages. The number of filiform taste buds at one day of birth was 334, only 20% of all taste buds at the time of birth possessed a taste pore. 39% of soft palate beared 174 taste buds at one day and possessed a taste pore. The number of taste buds with taste pore at one day was small,l at the center of circumvallate papilla had 19 to 59 taste buds. He observed that the total number of taste buds increased with age and reached a maximum at 2 months of age and decreased there after. The decreased in the number of taste buds with increasing age, may change taste sensitivity.

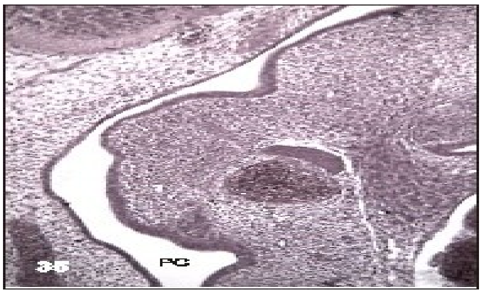
Uppal *et. al.*(2006) studied the histogenesis of tongue from early prenatal stages of murrah buffalo and noticed that, at the earlier stages, the epithelium was either simple, cuboidal or simple columnar, but become stratified at the 79 days of age. Undifferentiated taste buds and muscle fibers were formed at 95 days of fetal age. Some glandular acini and muscle striations also observed at 137 days of fetal age.

Dmitrieva (1985) studied the development of taste buds of fungiform and vallate papillae in three stages of rat embryo. In the first stage at 16-17th day of gestation, the presence of nerve fibres in connective tissue of papilla. In second stage at 19th days of gestation, nerve entering the epithelium and forming afferent synaptic contact between differentiating epithelial cells and nerve fibres. In third stage 21-22nd days of gestation, the cluster of differentiating epithelial cell attained a form, which was similar to mature taste buds.

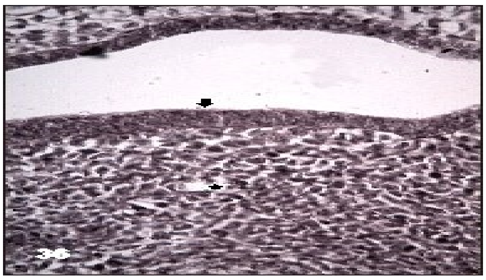
Boshell *et. al.* (1980), observed the development of different types of papilla at different age group of human tongue. At fetal stage, the epithelium layer of tongue appeared in the form of periderm. Fungiform papillae were developed earlier than filiform papillae. Just after the birth, the periderm had disappeared and few filiform were observed.

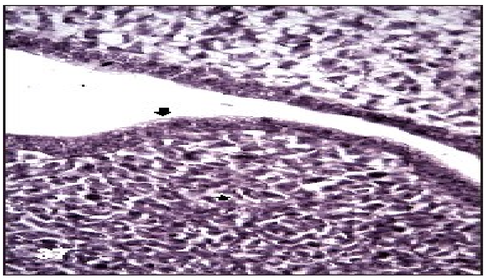
In present research paper, it is studied the stage development of tongue of *Rousettus leschenaultia*. The tongue of *R.L.* is highly muscular organ attached to floor of the oral cavity, surrounded by different types of foliate papillae on the dorsal side. In vertebrates, the foundation of the visceral 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. the stomadaeum. The development of oral cavity consists; (1) The origin of mandibular arch from paired primordial, (2) Lower and upper jaw established from the maxillary process. (3) The main part of the palate was derived from the upper jaw, which arises from the maxillary process.

The embryo of *Rousettusn leschenaultia* with C.R. length 5 mm at limb bud stage also showed developing mandibular and hyoid arch in the pharyngeal pouch **(Fig. 1,2,3&4)**. At this stage of development, the lateral mesoderm became split into somatic and splanchnic mesoderm covering the coelom. The lines of demarcation between the splanchnic and somatic layers determine by intermediate mesoderm. Next developmental stage of tongue is lingual papillae. When the lingual papillae take shape the primordial of taste buds recognized in the lingual epithelium which indicated their appearance of small cell clusters. Further development of tongue shows these pale-staining cell groups sharper with definite pore at the surface.



- Fig.1. Section of the embryo of *R. leschenaulti* (with C.R. length 5mm) shows the development of mandibular and hyoid arches. Heart (H) in pericardial coelom- (PC) .
- Fig.2. Magnified part of Fig. 1 to showing the heart is nearly a straight tube which started with elongation and bending with clearly recognized beginning of primary regional divisions as sinus venosus, the atrium and the ventricles. Pericardial coelom- (PC)





- Figs.3 and 4 . Magnified view of Fig. 1 to show the differentiating lingual muscles (star) in the connective tissue lined with compactly arranged cells in epithelial layer (thick arrow).

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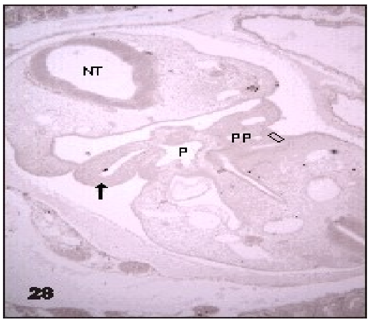
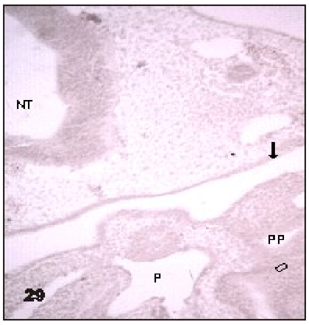
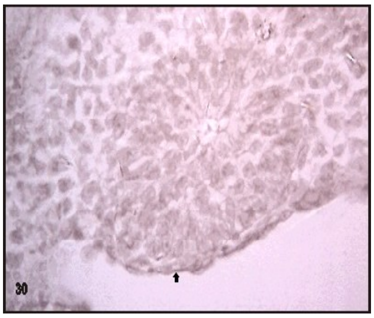
Result: The embryo of R. leschenaultia with C.R. Length 5mm at limb bud stage also showed the primitive streak and the notochord. Notochord is a thickened ectodermal area at anterior end of primitive streak. Neural plate formed from the notochord, which are immediately become folded into neural tube. Visceral portion of head are stomodeal depression, paired maxillary process i.e. hyoid arch, mandibular arches and branchial arches are well identified at that stage **(Fig. 5).** Pharynx is formed from forgut with four pharyngeal pouches arranged opposite to the branchial groove. At, this stage of development in the visceral of head **(Fig.6)**, the developing lingual musles (mandibular arch and hyoid arch) in the buccal cavity were observed. The caudal boundary of the oral cavity is less complex and being constituted by the mandibular arch alone. The origin of mandibular arch from paired primordial involved in the formation of lower jaw. Next behind the mandibular arch lies the hyoid arch while the branchial arch lies behind the hyoid arch. The origin of mandibular arch from the paired primordial arches appears first on either sode of the midline of buccal cavity are marked by the local thickening and these local thickening due to the rapid proliferation of mesenchymal tissue beneath the overlying epithelium are known as lateral swelling **(Fig. 7)**, beneath them a small median elevation known as tuberculum impar was observed. Behind the tuberculum impar is another median elevation known as copula. On either side of the copula, the rapid growing adjacent tissue of second, third and fourth branchial arches were present.At this stage of development, the lateral mesoderm became split into somatic and splanchnic mesoderm covering the coelom. The somatic mesoderm covers the outer wall of heart and the splanchnic mesoderm forms the supporting membrane of heart suspended in pericardial coelom. The lines of demarcation between the splanchnic and somatic layers determine by intermediate mesoderm.



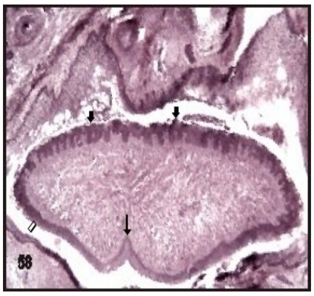


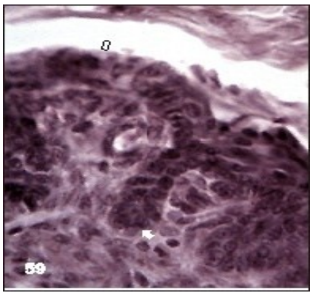


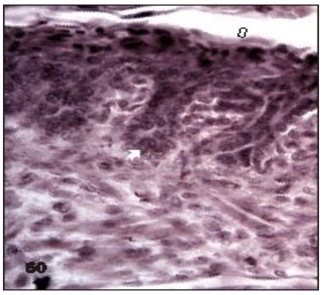
- Fig. 5. T. S. of *H. speoris* embryo (at early limb bud stage with C. R. length 2.5mm) showing the cephalic and visceral portion of head. The appearance of the developing notochord is a thickened ectodermal area at anterior of the primitive streak. Neural plate formed from the notochord, which immediately 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.
- Fig. 6. Enlarged part of Fig. 5 to show the visceral portion of head with developing lingual muscle: mandibular arch (long arrow) and hyoid arch (arrow head). The origin of mandibular arch from paired primordial, involved in the formation of lower jaw. Behind the mandibular arch lie the hyoid arch and the branchial (small arrow) lie behind the hyoid arch. Buccal cavity- BC. Pharynx- P.
- Fig.7. Part magnified from Fig.5 showing the buccal cavity marked by the local thickening due to the rapid proliferation of mesenchymal tissue beneath the overlying epithelium, known as lateral swelling; beneath this is a

small median elevation of tuberculum impar. Behind the tuberculum impar is another median elevation known as copula (thick arrow).

The embryo of *Rousettus leschenaultia* with C.R. length 9mm at phalange stage shows well developed papillary region on the dorsal and lateral sides. Central papillary region is more prominent than the lateral margins. At this stage the median lingual septum seperates the striated muscles into two lateral halves. The lingual septum extends from the base to the tip of tongue **(Fig. 8).** The mucous membrane of the tongue is lined by a striatified epithelium, which gives it a rough appearance to the dorsal surface. Some parts of papillary containing end-bulbs, project beneath stratified epithelium **(Fig. 9 and 10).**

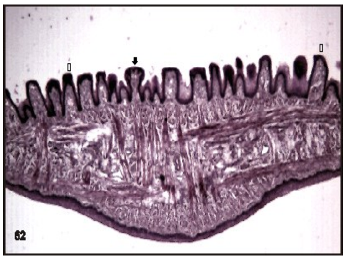


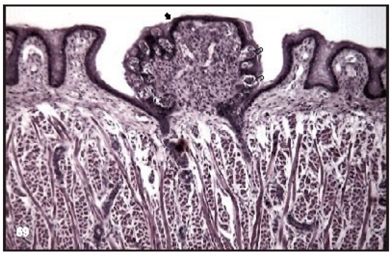




- Fig.8. Section of the embryo of *Rousettus leschenaulti* (with C.R. length 9mm at phalange stage) showing well differentiated papillary surface. Central papillary region (thick arrows) is more prominent than the lateral margins (arrow heads). The lingual septum separates the fibers of striated muscle into two lateral halves (small arrow).
- Fig.9 and 10. Magnified parts of the Fig. 58 to show the differentiating papillae lined by a stratified epithelium (arrow heads), which gives rough appearance to the dorsal surface. Some parts of papillae containing end-bulbs (thick arrow) beneath the epithelium, the papillae contain the connective tissue and blood capillaries.

The prenatal male embryo of Rousettus leschenaultia collected on 23.3.2008 with body weight 120 gms; shows the tongue weighing 0.64 gms, possessing the length 28mm and bredth 9mm. The dorsal surface shows different types of papilla, viz filiform, fundiform and circumvallate papilla. On the anterior side, round shaped fungiform papillae were observed in-between the differentiating tricuspid and scaly filiform papillae. The outer surface of papillae was covered with keratinized cell layer, while the blood capillaries embedded in connective tissue were present inside the papilla. The developing network of musculature, blood vessels and mucous glands were observed surrounded by connective tissue under the papillary surface **(Fig. 11)**. In the fungiform papillae developing taste buds are observed towards outer surface, covered by a smooth stratified squamous epithelium.The differentiating filiform papillae were also surrounded by the stratified squamous epithelium. At the anterior-lateral region, the scaly filiform papillae was arranged serially in diagonal rows directed posteriorly against the surface of the tongue. The ventral surface of the tongue was also covered with stratified squamous epithelium. The cluster of large sized developing mechanical tricuspid papillae were more keratinized in nature and surrounded by the stratified squamous epithelium. These papillae covered a large area of the middle part of the dorsum. Inside the papillae, blood capillaries are noticed in the connective tissue.The posterior surface of tongue covered dorso-laterally by numerous posteriorly directed horney filiform papillae. The circumvallate papillae **(Fig. 12)** are large circular projection present on the posterior region of the dorsal surface of tongue and are bounded by a deep circular furrow. The three circumvallate papillae i.e. one central-median and two lateral forms a triangle on the posterior end of the tongue. Taste buds composed of cells with large nucleus are generally covered the side walls but not the top of papilla. Bunches of mucous and salivary glands are present in the mucous membrane of tongue beneath the epithelium amongst the muscle bundles. The glands open upon its epithelial surface and pour their secretions into the trench at the base of tongue confined to the region of circumvallate papillae.**(Pg. no. 59 Last para)**





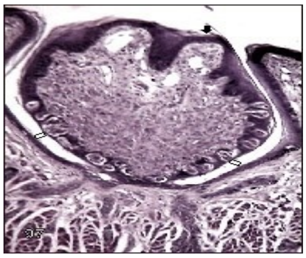


Fig. 11 T. S. of anterior part of tongue at prenatal stage of *Rousettus leschenaulti* to show the differentiating papilla, viz. filiform (arrow heads), fungiform (thick arrow). The differentiating filiform papillae covered the major part of tongue, in between fungiform papillae were observed.

Fig. 12. Section through the posterior part of tongue to show the circumvallate papilla (thick arrow). Taste buds (arrow heads) are located in the lateral-dorsal epithelium of the papilla.

Fig. 13. Photograph to show well developed circumvallate papillae (thick arrow) at the posterior region with clearly identified taste buds (arrow heads) in the circular epithelial surface, mainly in the lateral wall of circular furrow. These papillae are much broader than the other form of papillae. These papillae just like as an inverted conical excavation, lined by an invagination of the stratified epithelium; which thus forms a deep trench about the base of the papillae.

The tongue of postnatal suckling of *Rousettus leschenaultia* were increased in size as compared to their late prenatal stages. In the lingual mass the network of muscle bundles and blood musculature was more complex, increased in numbers as well as in size. The different types of filiform papillae were bigger in size and more keratinized on the dorsal surface. The fungiform and circumvallate papillae were bigger in size and well differentiated as compared to the prenatal stages. In circumvallate and some fungiform papillae, the taste buds were increase in size and in numbers **(Fig.13).** It is observed that the number and height of receptor cells with well developed nucleus in the taste buds was also increased and supplied with nerve fibres. The large part of the posterior half of lingual mas was occupied by mucous and salivary glands beneath the connective tissue. The lingual glands open outside by means their ducts and supplied with dense network of blood capillaries.

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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.	*R. leschenaulti*	Prenatal	24. 0	30. 4	16. 0	4. 8	6. 4	3. 2	11. 2	8. 0	6. 4	1. 6
		Postnatal	19. 2	22. 4	19. 2	4. 8	6. 4	3. 2	6. 4	6. 4	9. 6	1. 6
		Adult	40. 0	36. 8	20. 8	8. 0	20. 8	3. 2	16. 0	12. 8	6. 4	1. 6

TABLE I. The ratio of a) weight of tongue with body mass and b) length of free anterior surface with total length of tongue of adult *R. leschenaultia.*

TABLE II . Measurements of different types of papillae and taste buds in adult R. leschenaultia.

TABLE III. Measurements of different types of papillae and taste buds in adult *R. leschenaulti.*

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.	*R. leschenaulti*	Prenatal	24. 0	11. 2	28. 8	17. 6	11. 2	14. 4	22. 4	16. 0	-	-	-	-
		Postnatal	16. 0	14. 4	20. 8	16. 0	12. 8	-	25. 6	22. 4	-	-	-	-
		Adult	28. 8	20. 8	28. 8	22. 4	20. 8	12. 8	44. 8	36. 8	17. 6	9. 6	6. 4	4. 8

Discussion: The mammalial lingual organ develops from the palatal shelves. The development of the tongue begins in the fourth week of gestation. It is derived from pharyngeal arches 1-4 (forms the mucosa of the tongue) and the occipital somites (forms the musculature of the tongue). The tongue arises from first arch tissue and root of tongue clothed by endoderm originated from second and third branchial arches. In the first stage of development, lingual and median swelling appear. Lateral lingual swelling and Tuberculum impar (Medial swelling) derived from the 1st pharyngeal arch paired lateral primordial of mandibular arch called lateral lingual swelling. Between them is a small median elevation known as tuberculum impar. Behind the tuberculum is another median elevation called Copula. Copula (Hypobranchial eminence) derived from the 2nd, 3rd and 4th pharyngeal arches and are forms the mucosa of the posterior one third of the tongue. The small median pit called Foramen caecum formed between the tuberculum impar and copula, separating the body and root of tongue. Epiglottal swelling derived from the 4th pharyngeal arch and forms the Epiglottis. During the fourth week, the lateral lingual swelling overgrow the tuberculum impar and merge together forming the mucosa of the anterior two third of the tongue. Their line of fusion is marked by the Median sulcus of the tongue. Within the copula, the third pharyngeal arch component overgrows the second arch and forms the mucosa of the posterior one third of the tongue. The anterior two/third and posterior one/third fuse forming a V-shaped groove known as the terminal sulcus. At the centre of this groove is the foramen caecum, a pit which represents the place of origin of the thyroid gland. The intrinsic and extrinsic muscles of the tongue are derived from occipital somites, which are segments of mesoderm in the region of the upper neck. The somites migrated from the neck anteriorly to give rise to the muscles of the tongue (Yamane A. 2005). Since the mucosa overlying their area of the tongue has its origin from the first pharyngeal arch, it receives its sensory innervation from the mandibular branch of the “V” cranial nerve (trigeminal nerve).

In the early stages of development in mammals, lingual papillae are developed through special and complex epithelial-mesenchymal interactions. At embryonic stage; the lingual epithelium covering the dorsal surface of the tongue, which is thought to occur as filiform papillae as striated cuboidal epithelium cells. Microvilli were observed on the surface of cuboidal epithelium cells and showed charactristics before development began. Under the lingual epithelium, a single layer of mesenchymal cells was observed. At the next developmental stage, the microvilli disappeared and the elongated and thickened lingual epithelium was developed on the dorsal surface of the tongue, confirming that the development of the filiform papillae was initiated and showed a typical striated squamous epithelium composed of cuboidal cells in the basal layer and squamous cells in the apical cell layers. At the next developmental stage, the lingual epithelium was become more compact. The morphological changes of the surface and the development of filiform papillae with pointed and rounded ends began. Arch like structures that migrated upward of the condensed mesenchymal cells located below the epithelium. At Neonatal development stage, a clearly distinguished striated squamous epithelium of filiform papillae with connective tissue. Filiform papillae with a keratinized stratified squamous epithelium and irregular connective tissue thought to be mature structure. The development of the filiform papillae initiated at embryonic developmental stage and completed before birth. The development of the filiform papillae initiated late and completed quickly in comparison with the fungiform papillae and is completed through embryonic and neonatal development (Kim J. Y. *et. al*. 2003). The first sign of development of taste bud on the lingual epithelium occurs at the 8th week of gestation. Between the nineth an eleventh week of gestation, many taste bud primordia develop. In mammals, lingual taste buds can form only within papillae, which indicates that taste bud-component cells exist before taste buds differentiate and are restricted to gustatory papillae. Taste neuroepithelium consists of a host of taste buds widely distributed over tongue, pharynx, palate and epiglottis. Taste is a contact chemical sense innervated by sensory components of the VIIth, IXth and Xth cranial nerves. Taste buds are aggregated in relation to three kinds of papillae, Fungiform, Foliate and Circumvallate. Fungiform papillae are widely distributed on the top and sides of the tongue. Foliate papillae are found on the posterior and lateral surfaces of the tongue and are innervated by both the Chorda tympani (VIIth) and glossopharyngeal (IXth) cranial nerves. Circumvallate papillae innervated by the glossopharyngeal (IXth) nerve, (Frank, 1991). Circumvallate papillae are much larger and contain thousands of taste buds. Each taste buds in any these papillae consists of 40-50 modified epithelial cells, grouped in four distinct types of cells; basal cells, dark cells, light cells and intermediate cells. The latter three cell types are also known as type II and III taste cells. We do know that, in mammals, lingual taste buds can form only within papillae, which indicates that taste bud competent cells exist before taste bud differentiate and are restricted to gustatory papillae, (Mistretta and Hill 1995). It is possible that these cells become specific as gustatory papillae initially develop (Barlow L.A. 2000). Taste buds receive rich innervation, about 50 nerve fibers innervate in one bud, but each nerve fiber gets input from at least five taste buds. Taste buds on the larynx and epiglottis are abundant at birth but begin to disappear during infancy. Finally, the tongue is left as the major organ of taste, with separate regional areas of innervation. The number of taste buds on the anterior two-third of the tongue is not great and they tend to disappear progressively with age.

The present research paper embodies detailed observation on the histology of the prenatal, postnatal and adult tongue of *Rousettus leschenaulti.* At the phalange stage of *Rousettus leschenaulti* with C.R. length 9mm, shows the tongue as a sac of mucous membrane filled with mass of lingual muscle, which were covered by superficial lingual epithelium with well identified papillary surface. In the prenatal embryo of *Rousettus leschenaulti,* the dorsal surface of the well-developed tongue covered by well differentiated different types of papilla. A well-developed network of lingual muscles and vasculature was observed. In the posterior region, the salivary and mucous glands embedded in the mucous membrane. The postnatal sucklings shows further development of lingual organ. The tongue of adult *Rousettus leschenaulti,* at the anterior-dorsal side shows a cluster of large sized mechanical tricuspid filiform papillae directed towards the posterior surface. The pointed hook like horny and bifid filiform papillae covered the anterior dorsal surface while large scaly, horny and three circumvallate papillae were present in the posterior region of tongue.

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