BIODIVERSITAS
Volume 24, Number 4, April 2023
Pages: 2302-2314

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240444

Morphological identification of the squirrel (*Callosciurus notatus*) tongue through Scanning Electron Microscopy (SEM) and histochemistry

EMILIA IKA MEGAWATI, STANISLAUS IVAN DAVIN PRADIPTA, ULFAH DAMIA, ULAYATUL KUSTIATI, HEVI WIHADMADYATAMI, DWI LILIEK KUSINDARTA♥

Department of Anatomy, Faculty of Veterinary Medicine, Universitas Gadjah Mada. Jl. Fauna 2, Sleman 55281, Yogyakarta, Indonesia.
Tel./fax.: +62-274-560861, ♥email: indarta@ugm.ac.id

Manuscript received: 14 January 2023. Revision accepted: 27 April 2023.

Abstract. *Megawati EI, Pradipta SID, Damia U, Kustiati U, Wihadmadyatami H, Kusindarta DL. 2023. Morphological identification of the squirrel (*Callosciurus notatus*) tongue through Scanning Electron Microscopy (SEM) and histochemistry. Biodiversitas 24: 2302-2314.* Squirrel (*Callosciurus notatus*) plays an important role in the agricultural ecosystem. Their urine and feces kill coconut tree pests and help preserve coconut farming productivity. This research focuses on the morphological identification of the tongue and lingual papillae distribution of the Callosciurus notatus's tongue. The tongue morphology and lingual papillae distribution of *Callosciurus notatus* were investigated through two different methods: using scanning electron microscopy (SEM) and histology. This paper provides macroscopic and histological tongue morphology and lingual papillae distribution of *Callosciurus notatus.* Macroscopically, the tongue is divided into the apex, corpus, and radix. The dorsal surface of the apex the groove and lateral prominence were occur. SEM and light microscopy observation showed that the apex bears three types of papillae: leaf-like filiform, bifid filiform, and fungiform. The corpus includes wide leaf-like filiform papillae, saw-like filiform papillae, bifid filiform papillae, and fungiform papillae. On the radix, there are conical like filiform papillae, fungiform papillae, conical and vallatae papillae. The lateral part of radix showed Weber's glands that react strongly positively by Alcian Blue (AB) pH 2.5 and periodic acid Schiff (PAS) staining; Weber's glands consist of neutral and acidic mucin. Von Ebner's glands were at the textus muscularis striatus syncytialis near the vallatae papillae, which react weakly positively to PAS stain and did not react to AB pH 2.5 stain; von Ebner's glands consist of neutral mucin. Masson Trichrome stain revealed collagen structure on lamina propria mucosae and textus muscularis striatus syncytialis of apex; moderately on lamina propria mucosae and lesser on textus muscularis striatus syncytialis of the corpus and radix.

Keywords: Conical filiform, fungiform, lingual glands, lingual papillae, vallatae

INTRODUCTION

Squirrels are omnivorous mammals that live on almost all continents. There are about 278 species of squirrels, with its diversity of morphology, one of which is *Callosciurus notatus* that comes from the Rodentia and family *Sciuridae* (Baker 2016). *Callosciurus notatus* has a brownish-red or orange color underside, brown on the dorsal side, and white and black on the lateral line. They have good eyesight, sharp claws, and long tails (Thorington et al. 2012) Their diets consist of whole grains, fruits, leaf shoots, flowers, insects and anthropods, tree bark, and resins. Plantain squirrels have been observed to be particularly fond of figs and will often seek out fig trees for sustenance. They are also known to consume flowers and nectar from various plant species. Interestingly enough, they have been spotted raiding bird nests for eggs or chicks as well. Their feeding behavior shows them actively searching for food in trees and shrubs during the day while spending nights in tree holes or nests made up of leaves. These squirrels have strong jaws which allow them to crack open hard-shelled nuts with ease. In urban environments where they are commonly found scavenging trash cans or stealing snacks from humans in parks or gardens; this dietary habit can pose problems if it becomes too habitual since they may become dependent upon human-provided nutrition rather than finding natural sources themselves. Though their varied diets make *Callosciurus notatus* adaptable creatures able to survive in a range of environments by seeking out different types of nourishment available to them at any given location within their habitat range (Thorington et al. 2012). The presence of the *Callosciurus notatus* plays an important role in the ecosystem, especially in coconut plantations. Urine and feces from *Callosciurus notatus* can be a natural pesticide to kill pests on coconut leaves that cause tree unproductivity (Andalisa et al. 2018).

The process of obtaining energy and nutrients in the digestive tract through chemical digestion may involve a variety of procedures. During a meal, in which food is taken in, food is moved from outside the oral cavity into the esophagus and then the stomach by a series of precise and coordinated movements of several structures, including the tongue, lips, cheeks, jaw, soft palate, and hyoid. The events that occur during feeding—including the acquisition and transport of food, physical and mechanical breakdown of food, bolus aggregation, and swallowing—are distinct and complex in their own right but closely related both

physiologically and functionally. The tongue is an important organ in the process of digestion of food. Tongue is a muscular organ which provides multiple functions to the species. The ability of the tongue will influence the diversity of tongue morphology between species such as processing food (e.g., catching the food, transporting, swallowing the food, dringking-lapping and or sucking), and non-feeding activities (e.g., grooming or vocal modulation) (König and Liebich 2020; Abumandour 2014; Williams 2019; Haggag et al. 2020). In addition, the environment and or habitats also take part in the tongue morphology evolution (Iwasaki et al. 2019; Williams 2019). Furthermore, morphology, distribution, amount and the type of vertebrate lingual papillae are mainly influenced by diet (Pastor et al. 2011; Iwasaki 2019; Damia et al. 2021).

Several studies for the morphology of the tongue in Rodentia order have been carried out on rats (*Rattus norvegicus*) (Iwasaki et al. 1987; Goździewska-Harłajczuk et al. 2018), guinea pigs (*Cavia porcellus*) (Ciena et al. 2017; Sakr et al. 2017), hazel dormouse (*Muscardinus avvelanarius*) (Wolczuk 2014), vole banks (*Clethrinomys glareolus*) (Jackowiak and Godynicki 2005), capybara (*Hydrochaeris hydrochaeris*) (Watanabe et al. 2013), degu (*Octodon degus*) (Cizek et al. 2017), hedgehog (*Atelerix albiventris*) (Emura 2019), Persian squirrel (*Sciurus anomalus*) (Sadeghinezhad et al. 2018), Pallas's squirrel (*Callosciurus erythraeus*) (Yoshimura et al. 2018), and flying squirrels (*Pteromys momonga* and *Petaurista leucogenys*) (Emura et al. 1999; Emura 2019). From the studies there is a variation on the type of the lingual papillae and also the presence of the taste buds. The taste buds appear not only on the fungiform papillae but also on the lateral wall of the foliate papillae. The variation could be caused due to the wide range of feeding habit between species. In addition, keratinization on the surface of the tongue has already reported. There were significant differences in the epithelial layers in both the degree and time of keratinization. The thickest layer of keratin covers the mechanical papillae, which form a protective layer on the surface of the tongue. The tongue is also composed of muscle tissue with various arrangements of skeletal muscle fibers (longitudinal, transverse, and vertical muscle fibers). The lingual glands located within the tongue muscle tissue, there are Blandin-Nuhn glands, von Ebner's glands and Weber's glands (Sadeghinezhad et al. 2018).

Furthermore, the data of anatomical studies of the squirrel (*Callosciurus notatus*) have been carried out on the oral cavity, which deals with the teeth of red squirrel (*Sciurus vulgaris*), ligaments, bones and the pulmo of ground squirrel (*Geosciurus inauris*). There are no recent data that describe the lingual tongue morphology of *Callosciurus notatus* in detail. In Indonesia solely the distribution of squirrels is widely from Sumatra to Java Island. Since there is very lack of data on the animal biodiversity mainly for the squirrel (*Callosciurus notatus*) in Indonesia, this study aims to analyze the morphology of the tongue and lingual papillae of squirrel (*Callosciurus notatus*) using scanning electron microscopy (SEM) and histochemical stain including Hematoxylin-Eosin (HE), Alcian Blue (AB pH 2.5), Periodic Acid Schiff (PAS), and Masson Trichrome (MT). We hope with this study will fill the gap in the basic knowledge variety of biodiversity and provide new insight into the knowledge mainly of the influence of the diet, living region on the morphology and type of the species lingual papillae.

MATERIALS AND METHODS

Animal specimens

Six adults *Callosciurus notatus,* 4 females and two males, weighing 190-250 g. Squirrels were captured by using small mammal traps with the help of experts that were placed on the tree branches around the farming area in Yogyakarta, Indonesia during the day, coconut and peanut butter, were used as baits. The Ethical Committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia, approved the capture process and specimens processing (approval number 0141EC-FKH/int./2019). The animal specimens' morphological characteristics were identified in the Laboratory of Animal Systematics, Faculty of Biology, Universitas Gadjah Mada. The squirrel was anesthetized, perfused, and euthanized in the Laboratory of Anatomy, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Conservation status

Callosciurus notatus used in this study is not on any conservation list in Indonesia. According to the International Union for Conservation of Nature Red List of Threatened Species, *Callosciurus notatus* is categorized as Least Concern.

Gross macroscopy analysis

Six *Callosciurus notatus* samples were anesthetized using a combination of ketamine 10 mg/kg body weight (Kepro, Maagdenburgstraat, The Netherlands) and xylazine 2 mg/kg body weight (Interchemie, Metaalweg, The Netherlands). Animals were placed in dorsal position and perfused through intracardiac using physiological saline (NacalaiTesque, Kyoto, Japan) and formaldehyde 4% (Nacalai Tesque, Kyoto, Japan) for sample fixation. Animals were euthanized, and the blood flowing out was replaced by physiological saline. Tongue samples were obtained by opening the oral cavity and separating the mandibular with the basal tongue. The sample was washed using physiological saline. The tongue samples were divided into two groups for scanning electron microscopy (SEM) and histochemical staining. Three samples for SEM were stored in SEM fixative (0.5% glutaraldehyde (Chem Cruz, Dallas, Texas, USA), formaldehyde 1.5% (Nacalai Tesque, Kyoto, Japan), and 100 g hepes (Chem Cruz, Dallas, Texas, USA) for a minimum 6-8 hours. Meanwhile, sample for histochemical staining was stored in paraformaldehyde 4%. Macroscopic observations were made using a camera (EOS 7000D, Canon, Tokyo, Japan).

Scanning electron microscope

The fixed tongue samples were washed with physiological saline for 5 minutes 5 times. Tongues were dehydrated using a graded series of ethanol (KgaA, Darmstadt, Germany) and trimmed in the border of corpus and radix before fixing it on conductive metal plates. The samples were dried using a vacuum system (25°C, 4 Pa; Buehler 1000 Vacuum System, Stuttgart, Germany), and they were coated with platinum ion coater. Then, tongues were observed using SEM with 15kV voltage and magnifications of 30x, 100x, 200x, 250x, 300x, and 350x.

Histochemistry (HE, AB pH 2.5, PAS and Masson's Trichrome)

Tongues were trimmed on the apex, corpus, and radix, then gauzed and washed with running water. Samples were dehydrated using a graded series of ethanol and cleared using xylene. For reinforcing consistency, the samples were soaked in paraffin solution and placed in an incubator. The samples were embedded and blocked in paraffin (Leica Biosystems, Wetzlar, Germany). The sections were cut to 8 µm thick using a rotary microtome (Yamato RV 240, Tokyo, Japan), transferred to a water bath, placed on a coated slide (Leica, Wetzlar, Germany), and then placed slides on a slider warmer overnight at 40°C. Then, sample slides were deparaffinized in xylene and rehydrated using a graded series of ethanol. These samples were stained according to the procedure of HE (Biooptica, San Faustino-Milano, Italy), AB pH 2.5 (Bio Optica Improving Pathology, Milano, Italy), PAS Hotchiss-Mc Manus kit (Bio Optica Improving Pathology) and MT stainings (Bio Optica Improving Pathology) separately. The samples were observed using light microscopy (BX51, Olympus, Tokyo, Japan), and the sample's photomicrographs were captured using Optilab (Optilab, Yogyakarta, Indonesia).

RESULTS AND DISCUSSION

Macroscopic observation

Macroscopic observation and SEM showed *Callosciurus notatus*'s tongues can be divided into three regions: apex linguae, corpus linguae, and radix linguae. The length of each tongue region is the same for apex, corpus, and radix is 100 ±1 mm. The width of the tongues is 90±10 mm with an apex width of 70±40 mm, and it widens to the radix (Figure 1A). The dorsal surface of the apex is divided into anterior, lateral, and medial parts. A median groove was observed on the anterior part (Figure 1D). The corpus's dorsal surface is divided into lateral part and medial part. The lateral part of the corpus can be determined from the lateral prominence, and fossa linguae appeared in the median part in each lateral section (Figure 1C). Macroscopically, fungiform papillae and three vallatae papillae appear on the dorsal surface (Figure 1B). A frenulum lingua on the ventral tongue connects the tongue to the mandible.

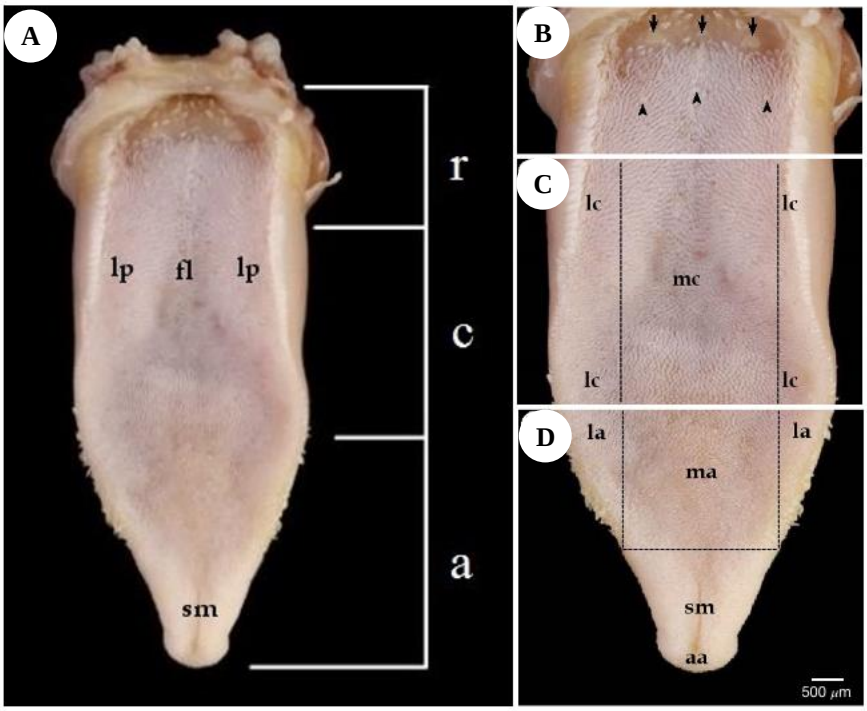

Figure 1. Gross macroscopy images of *Callosciurus notatus* tongue. A. *Callosciurus notatus* tongue is divided into apex, corpus and radix regions. B. Macrosopic dorsal view of the radix region. C. Macroscopic dorsal view of the corpus region. D. Macroscopic dorsal view of the apex region. a: apex, c: corpus, r: radix, lp: lateral prominentia on lateral corpus, fl: fossa linguae on medial corpus, sm: sulcus medianus, arrow: vallatae papillae, arrowhead: fungiform papillae, lc: lateral corpus, mc: medial corpus, la: lateral apex, ma: median apex, aa: anterior apex

SEM

SEM observation showed the distribution and variation of the lingual papillae morphology on the dorsal surface of the tongue. There were two types of lingual papillae: mechanic papillae (filiform and conical) and gustatory papillae (fungiform and vallatae). Filiform papillae were found on the dorsal tongue and divided into five subtypes: leaf-like filiform, wide leaf-like filiform, saw-like filiform, conical like filiform papillae and bifid filiform papillae. Fungiform papillae spread among filiform papillae. Three vallatae papillae were found on the posterior radix, forming a "V" formation pointing caudally (Figure 2, Table 1).

Apex

The dorsal surface of *Callosciurus notatus* tongue apex contains leaf-like filiform papillae, bifid filiform papillae, and fungiform papillae (Figure 2, 3A, Table 1). Leaf-like filiform papillae dominate the dorsal surface of the apex; it has a long shape with a pointy end (Figure 3). On the anterior part, these papillae are directed toward the medial along the medial groove (Figure 2). On the lateral posterior part, these papillae were directed toward the caudomedial, and on the medial posterior part, these papillae are directed toward the caudal position of the lingua (Figure 2, 3B, 3C, 3E). Bifid filiform papillae were found on the median groove area with similar traits as leaf-like filiform papillae but had two processes pointed to caudodorsal (Figure D). Fungiform papillae resembling shape of fungus were found, (Figure 3F).

Corpus

There are four subtypes of papillae on the corpus dorsal surface: wide leaf-like filiform, bifid biliform, saw-like filiform, and fungiform (Figure 4A). Wide-leaf-like filiform papillae were found on lateral and medial parts between apex and corpus. These papillae are shaped like leaf-like filiform papillae with a pointed tip and a short and wide base. On the lateral part, these papillae were directed toward the medial and shifted caudomedially toward the medial part of the tongue (Figure 4B and 4E). Bifid filiform papillae in the corpus were wider in size than those in the apex (Figure 4C). Saw-like filiform papillae with more than two processes dominate the medial corpus and appear on each lateral side. These papillae lead to the caudal on the medial part, while on the lateral part, these papillae were directed toward the caudomedial (Figure 4D). Fungiform papillae were found distributed among filiform papillae and were larger in size than those in the apex (Figure 4F).

Radix

The dorsal surface of the radix is dominated by conical filiform papillae on the lateral and medial parts (Figure 5A), resembling a cone with pointed end (Figure 5D). The lateral section papillae were projected caudomedially with the medial part pointed to the caudal part of the tongue (Figure 5B). Fungiform papillae were found among the filiform papillae (Figure 5C). Three vallate papillae, surrounded by a groove and thick outer wall, were found on the posterior radix (Figure 5E). In addition, conical papillae were present with a long and oval-shaped blunt end (Figure 5F)

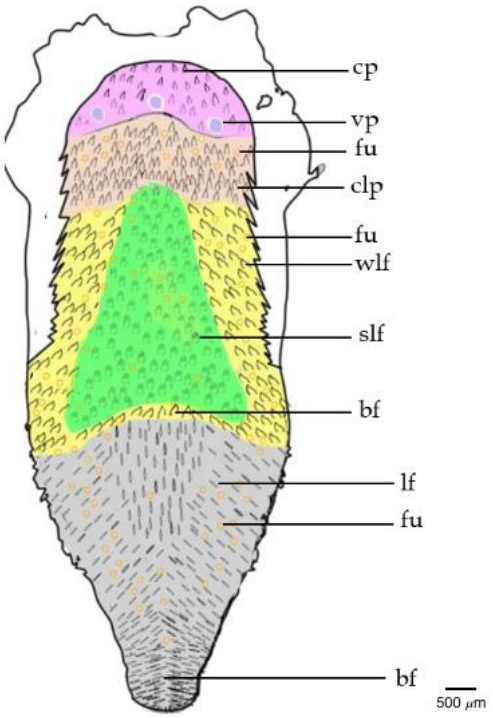

Figure 2. Schematic illustration of the *Callosciurus notatus* tongue with the distribution of the papillae. bf: bifid filiform papillae, fu: fungiformes papillae, lf: leaf like filiform papillae, slf: saw like filiform, wlf: wide leaf like filiform, clp: conical like papillae, cp: conicae papillae, vp: vallate papillae

Table 1. Distribution and sizes of the lingual papillae on the tongue *Callosciurus notatus*, + represent for found and - represents for absent

Type of papillae	*Apex*	*Corpus*	*Radix*	Length (μm)	Width (μm)
Leaf like filiform papillae	+	-	-	150± 13	30 ± 12
Wide leaf like filiform papillae	-	+	-	106 ± 33	49 ± 14
Saw like filiform papillae	-	+	-	224 ± 27	117 ± 8
Bifid filiform papillae	+	+	-	171 ± 17	74 ± 11
Conical like filiform papillae	-	-	+	240 ± 47	95 ± 11
Vallatae papillae	-	-	+	367 ± 93	367 ± 93
Conicae papillae	-	-	+	352±69	133±29
Fungiformes papillae	+	+	+	A: 96±43	A: 96±43
				C: 181±47	C: 181±47
				R: 242±24	R: 242±24

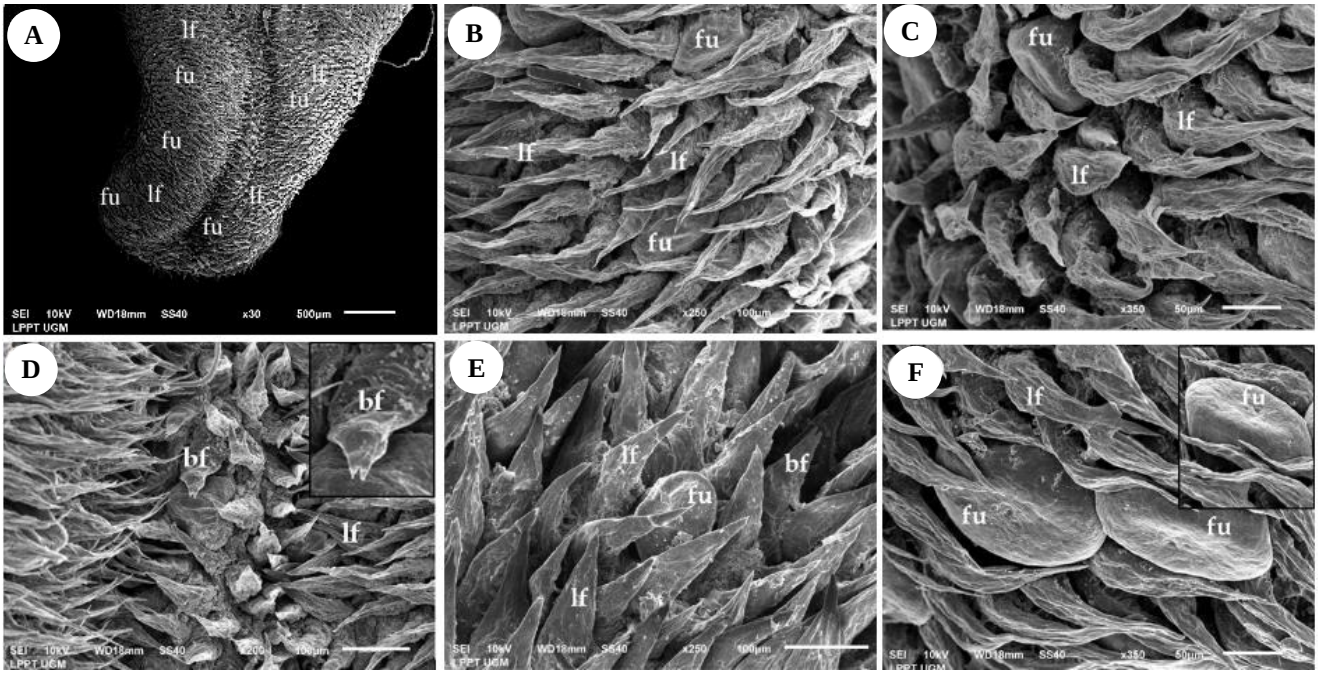

Figure 3. SEM images of the apex region of *Callosciurus notatus* tongue. A. Distribution of papillae on apex region. B. High magnification of lateral sinister apex. C. High magnification of lateral dexter apex. D. Papillae of the sulcus medianus region at anterior apex. E. High magnification of leaf-like filiform papillae. F. High magnification of funiform papillae. lf: leaf-like filiform papillae, fu: fungiform papillae, bf: bifid filiform papillae

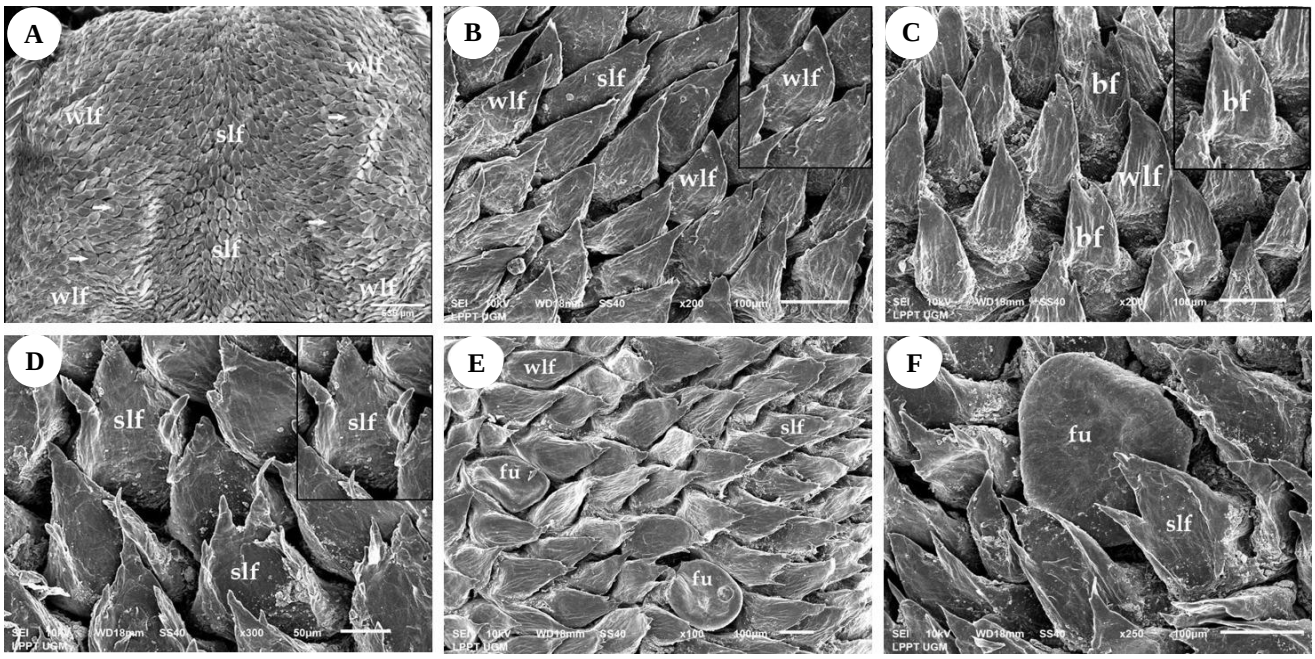

Figure 4. SEM images of the corpus region of *Callosciurus notatus* tongue. A. Distribution of papillae on corpus region. B. Lateral margin of corpus. C. Anterior margin of corpus. D. High magnificationof saw-like filiform papillae on medial corpus. E. Lateral margin of corpus. F. High magnification of fungiform papillae. slf: saw-like filiform papillae, wlf: wide leaf-like filiform papillae, fu (arrow in A): fungiform papillae, bf: bifid filiform

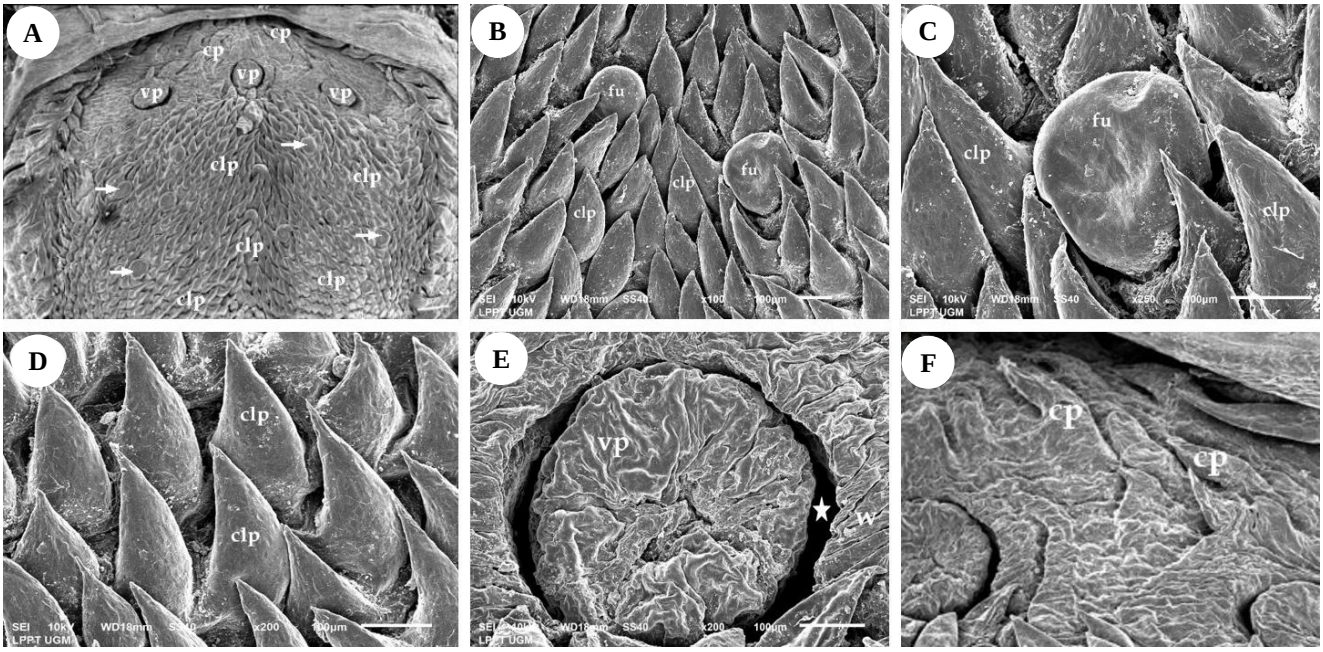

Figure 5. SEM images of the radix region of *Callosciurus notatus* tongue. A. Distribution of papillae on radix region. B. Medial part of radix. C. High magnification of fungiform papillae. D. High magnification of vallatae papillae. E. High magnification of the vallate papillae with the thick wall (w) and the deep groove surrounding the papillae. F. High magnification of conicae papillae. clp: conical like filiform papillae, vp: vallatae papillae, fu (arrow in A): fungiform papillae, w: wall, star: deep groove

Histological observation

HE staining revealed that the *Callosciurus notatus* tongue has two layers, the tunica mucosae and the textus muscularis striatus syncytialis. The tunica mucosae were divided into lamina epithelialis mucosae, arranged from epithelium stratificatum squamosum and lamina propria mucosae (Figure 6A, 6C, 9A, 9D, 12A). The textus muscularis striatus syncytialis exhibits several fibers that are transversal and longitudinal. In line with the SEM observations, the histological findings also showed several types of papillae: filiform, fungiform, conical, and vallate.

AB Ph 2.5 and PAS shows the distribution and type of lingual gland secretion. Weber's glands are on the lateral posterior radix and von Ebner's glands are near the vallatae papillae. Both glands are mainly found in the textus muscularis striatus syncitialis layer. AB pH 2.5 stains acid muco substances and acetic mucins, while PAS stains neutral muco substances. The magenta stain of PAS indicates neutral carbohydrate mucous, while blue stains of AB pH 2.5 indicates acid secretes.

MT shows the structure of connective tissue, especially collagen fibers in a green stain (Fig 8, 11, 14). Strongly staining at the lamina propria mucosae and the textus muscularis striatus syncyialis of the apex, moderate staining at the region of the corpus and radix at the lamina propria mucosae and weak staining at the textus muscularis striatus syncytialis were observed.

Apex

Histological observation of HE stained sections shows that filiform papillae on the apex consisted of the epithelium stratificatum squamosum cornificatum with thick keratin. Leaf-like filiform papillae have slender and pointed tips, while bifid filiform papillae have tips of lamina epithelial mucosae that split in two (Figure 6D). Fungiform papillae were less observed than filiform papillae on the surface of the apex. These papillae were composed of epithelium stratificatum squamosum cornificatum with thin keratin. The dorsal surface contained a taste bud, enabling the papillae to perform taste recognition (Figure 6B).

AB pH 2.5 and PAS stain were negative, indicating no lingual gland in all layers of the apex (Figure 7B and 7C). This finding is consistent with the result of HE, which did not show lingual glands on the apex (Figure 7A). MT staining showed a highly green stain of collagen connective tissue in lamina propria mucosae and between the muscles in the textus muscularis striatus syncytialis (Figure 8).

Corpus

The results revealed that lingual papillae on the corpus were dominated by highly keratinized epithelium (Figure 9A and 9D). Saw-like filiform papillae have a wide shape with lamina propria mucosae and lamina epithelialis mucosae divided into more than two processes with a thick keratinized (Figure 9B). Bifid filiform papillae on the corpus have a structure resembling the letter "U" with the more obvious loop hole in lamina propria mucosae followed by the cleaving of lamina epithelialis mucosae (Figure 9C). Wide leaf-like filiform papillae have a wide figure with a thick epithelium stratificatum cornificatum (Figure 9E). Fungiform papillae were covered by thin keratin with a taste bud on the dorsal surface (Figure 9F).

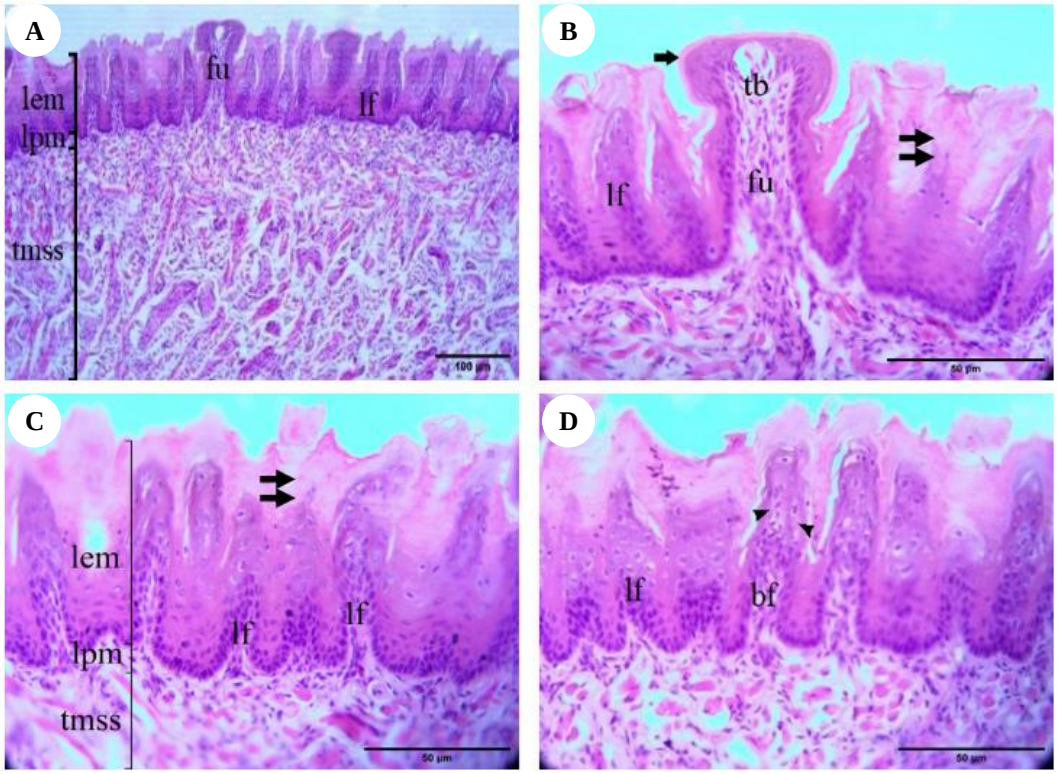

Figure 6. Photomicrographs of the apex region of *Callosciurus notatus* tongue with HE staining. A. Three layers, lamina epithelialis mucosae, lamina propria mucosae and textus muscularis striatus syncytialis are observed in the apex region, containing leaf-like filiform papillae and fungiform papillae. B. Fungiform papillae are characterized by thin keratinization (double black arrow) with a taste bud found between leaf-like filiform papillae. C. Leaf-like filiform papillae characterized by thick keratinization (double black arrow) are observed. D. Bifid filiform among leaf-like filiform papillae characterized by split lamina epithelialis (arrowhead) are observed. lem: lamina epithelialis mucosae, lpm: lamina propria mucosae, tmss: textus muscularis striatus syncytialis, lf: leaf-like filiform, fu: fungiform papillae, tb: taste bud, bf: bifid filiform papillae

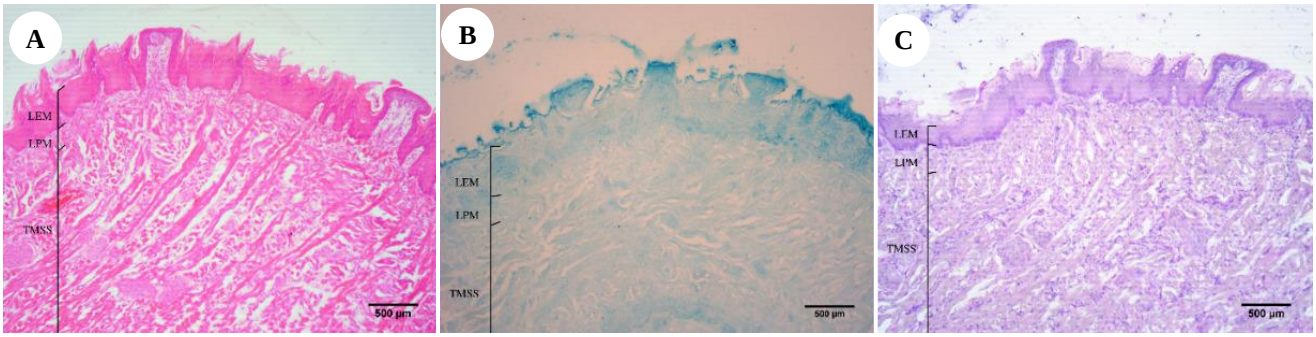

Figure 7. Photomicrographs of the apex region of *Callosciurus notatus* tongue. A. HE staining shows the absence of lingual glands. B. AB pH 2.5 staining shows no positive reaction to lingual glands. C. PAS staining shows no positive reaction to lingual glands. LEM: lamina epithelialis mucosae, LPM: lamina propria mucosae, TMSS: textus muscularis striatus syncytialis

AB pH 2.5 and PAS stainings also showed negative results, indicating the absence of lingual glands of the corpus (Figure 10B and 10C). This is consistent with the HE staining result that showed no lingual glands in three layers of the corpus (Figure 10A). MT staining showed that collagen connective tissue had a moderate green stain in lamina propria mucosae on the corpus and less green stain in the textus muscularis striatus syncytialis (Figure 11).

Radix

The radix exhibited four types of papillae: fungiform, conical filiform, vallatae, and conicae (Figure 12A). Fungiform papillae are larger and dome-like, with taste buds on the dorsal surface (Figure 12B). Conical filiform papillae are shaped like wide leaf-like filiform papillae with thicker lamina epithelialis mucosae and the lamina propria mucosae are more pointed, resembling a horn (Figure 12C). Vallate papillae have a rounded shape and

are surrounded by a canal and outer wall formed by invagination in the surface of the epithelium around these papillae with several taste buds present on its lateral wall (Figure 12E). Von Ebner glands can be observed in the textus muscularis striatus synctialis of the vallatae papillae (Figure 12D). Conicae papillae were blunt shaped with a thick keratinized epithelium (Figure 12F).

AB pH 2.5 and PAS stain demonstrated two types of lingual glands; von Ebner's glands at the textus muscularis striatus syncytialis near the vallatae papillae (Figure 13B); and Weber's glands at the lateral posterior radix (Figure 13A and 13C). Weber's glands revealed a strong positive reaction (+++) to AB pH 2.5 (blue-stained) (Figure 13D and 13F). The PAS stain (magenta-stained) (Figure 13G and 13I) indicates that Weber's glands, secreted the mucous, secrete neutral and acidic mucin. Von Ebner's glands revealed no reaction to AB pH 2.5 (Figure 13E) and produce purple stain on PAS stain, indicating that von Ebner's glands are secreting the moderate positive of serous and neutral (++) (Figure 13H).

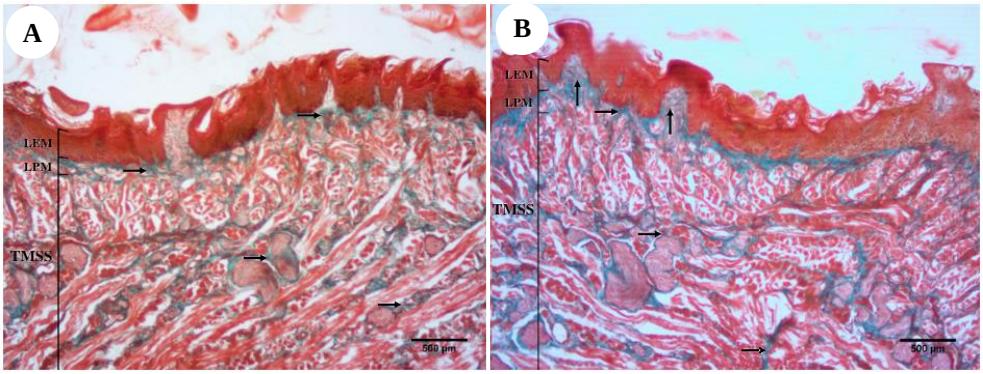

Figure 8. Photomicrographs of the apex region of *Callosciurus notatus* tongue with MT staining. Collagen structures in lamina propria mucosae and between muscle fibers in textus musclaris striatus syncytialis are stained in green (black arrow). LEM: lamina epithelialis mucosae. LPM: lamina propria mucosae, TMSS: textus musclaris striatus syncytialis

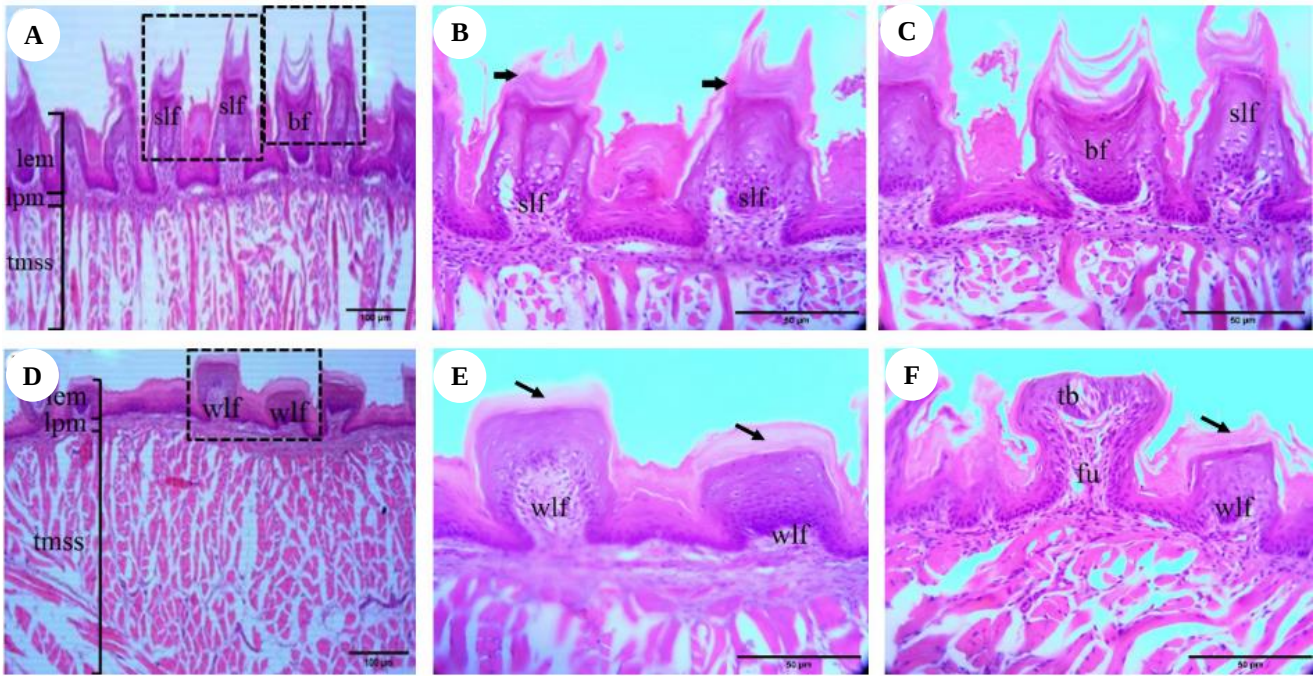

Figure 9. Photomicrographs of the corpus region of *Callosciurus notatus* tongue with HE staining. A. Three layers, lamina epithelialis mucosae, lamina propria mucosae, textus muscularis striatus syncytialis, are observed in the corpus region. Mucosae lingual shows saw-like filiform and bifid filiform papillae (dotted square). B. Saw-like filiform papillae characterized by two or more processes in lamina epithelialis mucosae and lamina propria mucosae, with thick keratinization (black arrow) are observed. C. Bifid filiform papillae characterized by split lamina epithelialis mucosae and lamina propria mucosa is observed. D. Lateral margin of the corpus shows wide leaf-like filiform papillae in mucosae lingual (dotted square). E. Wide leaf-like filiform papillae characterized by thick ephitelium stratificatum cornificatum are observed. F. Fungiform papillae (fu) is characterized by thin keratinization (black arrow) with a taste bud. lem: lamina epithelialis mucosae, lpm: lamina propria mucosae, tmss: textus muscularis striatus syncytialis, slf: saw-like filiform papillae, bf: bifid filiform papillae, wlf: wide leaf-like filiform papillae, fu: fungiform papillae, tb: taste bud

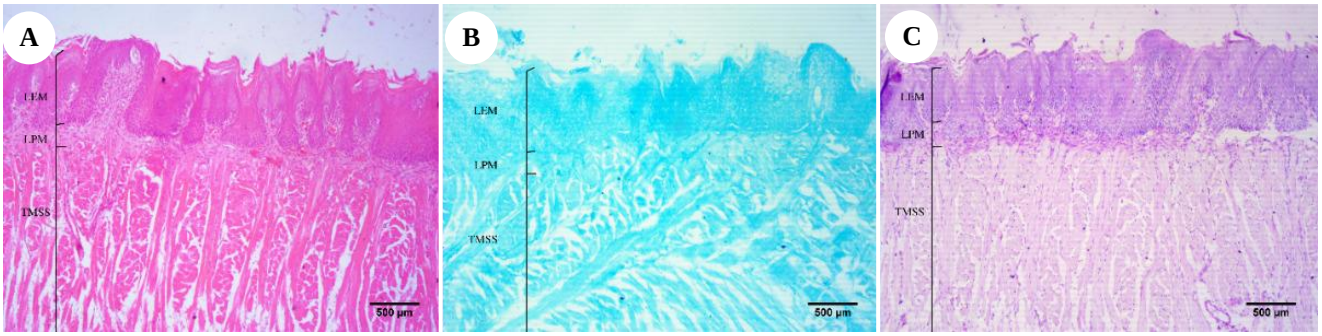

Figure 10. Photomicrographs of the corpus region of *Callosciurus notatus* tongue. A. HE staining shows the absence of lingual glands. B. AB pH 2.5 shows no positive reaction to lingual glands. C. PAS staining shows no positive reaction to lingual glands. lem: lamina epithelialis mucosae, lpm: lamina propria mucosae, tmss: textus muscularis striatus syncytialis

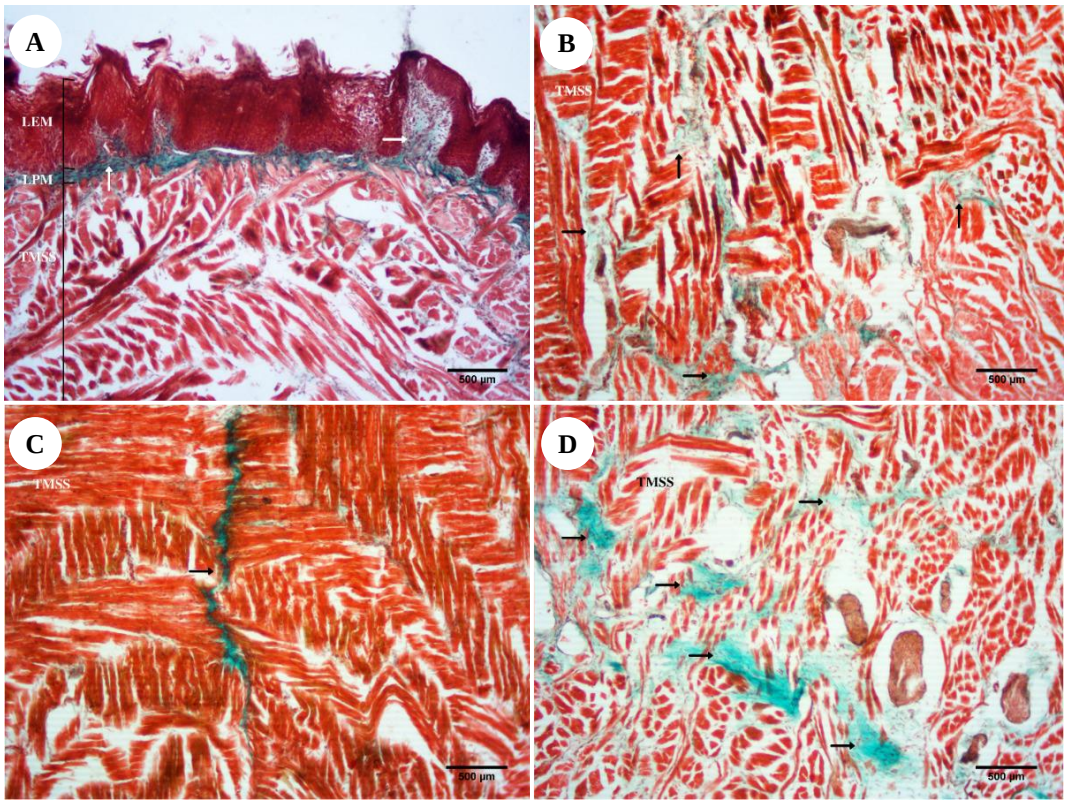

Figure 11. Photomicrographs of the corpus region of *Callosciurus notatus* tongue with MT staining. A. Collagen structure shown in green on lamina propria mucosae. B,C,D. Collagen structures are less between muscle fibers. lem: lamina epithelialis mucosae, lpm: lamina propria mucosae, tmss: textus muscularis striatus syncytialis

Discussion

Our observation revealed that macroscopically, the tongue of *Callosciurus notatus* is divided into the apex, corpus, and radix, meanwhile SEM and light microscopy observation showed that the apex bears three types of papillae: leaf-like filiform, bifid filiform, and fungiform. The corpus includes wide leaf-like filiform papillae, saw-like filiform papillae, bifid filiform papillae, and fungiform papillae. On the radix, there are conical like filiform papillae, fungiform papillae, conical and vallatae papillae. In addition on Weber's glands and Von Ebner's glands presence at the textus muscularis striatus syncytialis of the tongue.

From the macroscopic observation, the tongue of *Callosciurus notatus* as divided into three parts: apex, corpus, and radix. This division of tongue is similar to the pig (*Sus domesticus*), cow (*Bos taurus*), and horse (*Equus caballus*) (König and Liebich 2020), and in some others, rodents such as hazel dormouse *(Muscardinus avellanarius*) (Wolczuk 2014), guinea pig (*Cavia porcellus)* (Ciena et al. 2017), white mouse (*Ratus norvegicus*) (Davydova et al. 2017), Persian squirrel (*Sciurus anomalus*) (Sadeghinezhad et al. 2018), Pallas's squirrel (*Callosciurus erythraeus thai)* (Yoshimura et al. 2018), and flying squirrel (*Pteromys momonga)* (Emura 2019). The sulcus medianus linguae was found at the anterior part of apex from the tongue of *Callosciurus notatus* similar to those in Hazel Dormouse (*Muscardinus avellanarius)* (Wolczuk 2014), Capybara (*Hydrochaeris hydrochaeris)* (Watanabe et al. 2013), white

mouse (*Ratus norvegicus*) (Davydova et al. 2017), and Persian squirrel (*Sciurus anomalus)* (Sadeghinezhad et al. 2018). Lateral prominentia are present on the dorsal lateral surface of the tongue. This structure is a conformation of the fossa linguae that narrowed down toward the radix. In the white mouse (*Ratus norvegicus*), Hazel Dormuse (*Muscardinus avellanarius)*, flying squirrels (P*teromys momonga)*, and Japanese lesser flying squirrels (*Petaurista leucogenys*) have a wide prominentia at the posterior of corpus without the formation of fossa linguae (Ciena et al. 2017; Davydova et al. 2017; Yoshimura et al. 2018; Emura 2019). According to Sadeghinezhad et al. (2018), the prominentia, characterized by enriched muscle, helps in the food grinding process by smashing the food between the tongue and palatine.

The observation through SEM and histological staining found that lingual papillae of *Callosciurus notatus* tongue are based on the function, divided into 2 types; mechanica (filiform and conicae) and gustatory papillae (fungiform and vallatae). This arrangement of papillae is similar in another ordo of Rodentia (Wolczuk 2014; Ciena et al. 2017; Davydova et al. 2017; Goździewska-Harłajczuk et al. 2018; Sadeghinezhad et al. 2018; Yoshimura et al. 2018; Emura 2019).

Filiform papillae of *Callosciurus notatus* tongue spread widely at the dorsal surface of tongue similar to degu (*Octodon degus*) (Cizek et al. 2017), Persian squirrels (*Sciurus anomalus)* (Sadeghinezhad et al. 2018), and Pallas's Squirrel (*Callosciurus erythraeus thai)* (Yoshimura et al. 2018). Filiform papillae on *Callosciurus notatus* are grouped into 5 subtypes based on the difference of shape and characteristic (leaf-like filiform, bifid filiform, wide leaf-like filiform, saw-like filiform, and conical like filiform papillae). According to Saragih et al. (2020), filiform papillae are divided into type 1 and type 2. Papillae type 1 usually can be discovered at the anterior part of the tongue and is characterized by a hair-like structure (long and slim) along with a brush-like texture. These papillae help with gripping and binding food. Filiform papillae type 2 is mostly distributed along the lingual prominentia. These papillae are triangle-shaped with a smooth surface and a thick body to hold the food until the food is swallowed. Based on these, those filiform papillae from *Callosciurus notatus* contain papillae type 1 (papillae leaf-like filiform and bifid filiform) and type 2 (papillae saw-like filiform, conical like filiform, and wide leaf-like filiform). Filiform papillae in Hazel Dormouse (*Muscardinus avellanarius)* (Wolczuk 2014) are divided into 2 subtypes, Pallas's Squirrel (*Callosciurus erythraeus thai*) (Yoshimura et al. 2018) 1 type, guinea pig (*Cavia porcellus)* (Ciena et al. 2017) 4 subtypes and fruit bat (*Rosettus amplexicaudatus)* (Gunawan et al. 2019) 5 subtype. Histologically, filiform papillae of *Callosciurus notatus* are coated with a thick layer of keratinized. In contrast to Persian squirrels (*Sciurus anomalous)*, the filiform papillae are coated with a thin layer of keratin (Sadeghinezhad et al. 2018).

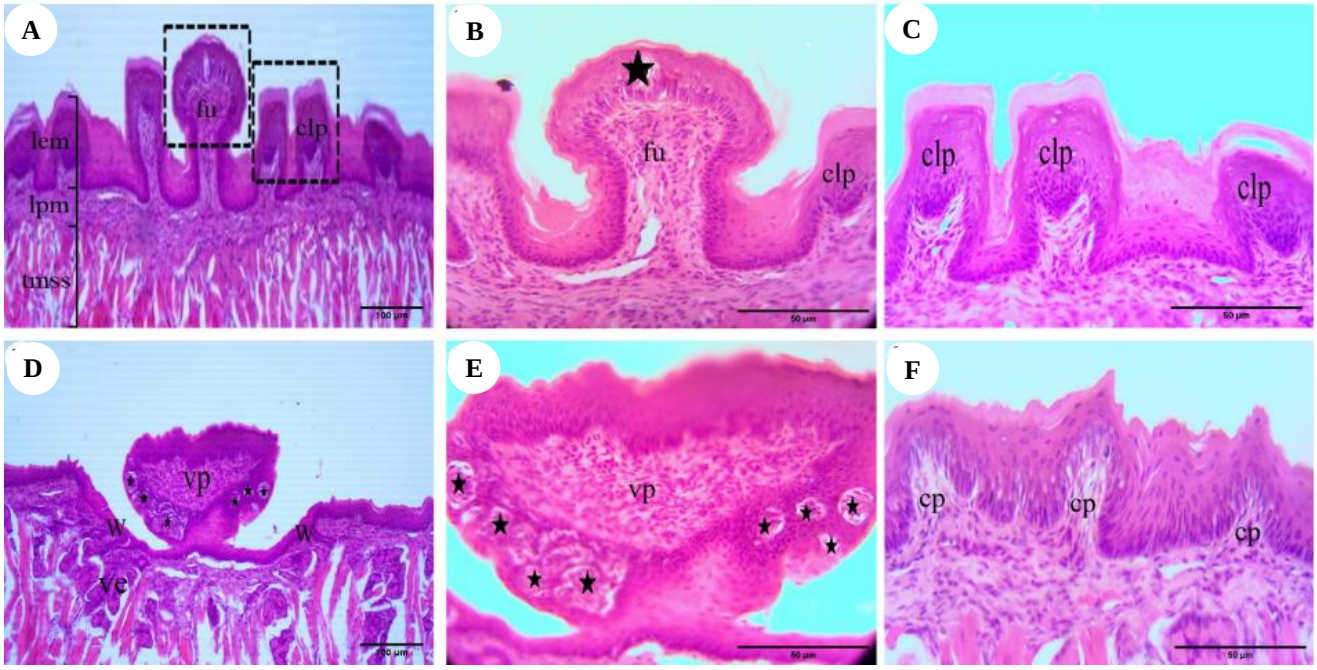

Figure 12. Photomicrograph of the radix region of *Callosciurus notatus* tongue with HE staining. A. Three layers, lamina epithelialis mucosae, lamina propria mucosae and textus muscularis striatus syncytialis, are observed. Mucosae lingual shows conical like filiform and fungiform papillae. B. Fungiform papillae characterized by a taste bud on dorsal surface. C. Conical like filiform papillae are observed. D. Vallatae papillae equipped with several taste buds, and surrounded by a canal and outer wall. E. High magnification of vallatae papillae. F. Conicae papillae with blunt shaped thick keratinization are observed. lem: lamina epithelialis mucosae, lpm: lamina propria mucosae, tmss: textus muscularis striatus syncytialis, clp: conical like filiform papillae, fu: fungiform papillae, star: taste bud, vp: vallatae papillae, W: wall, cp: conicae papillae

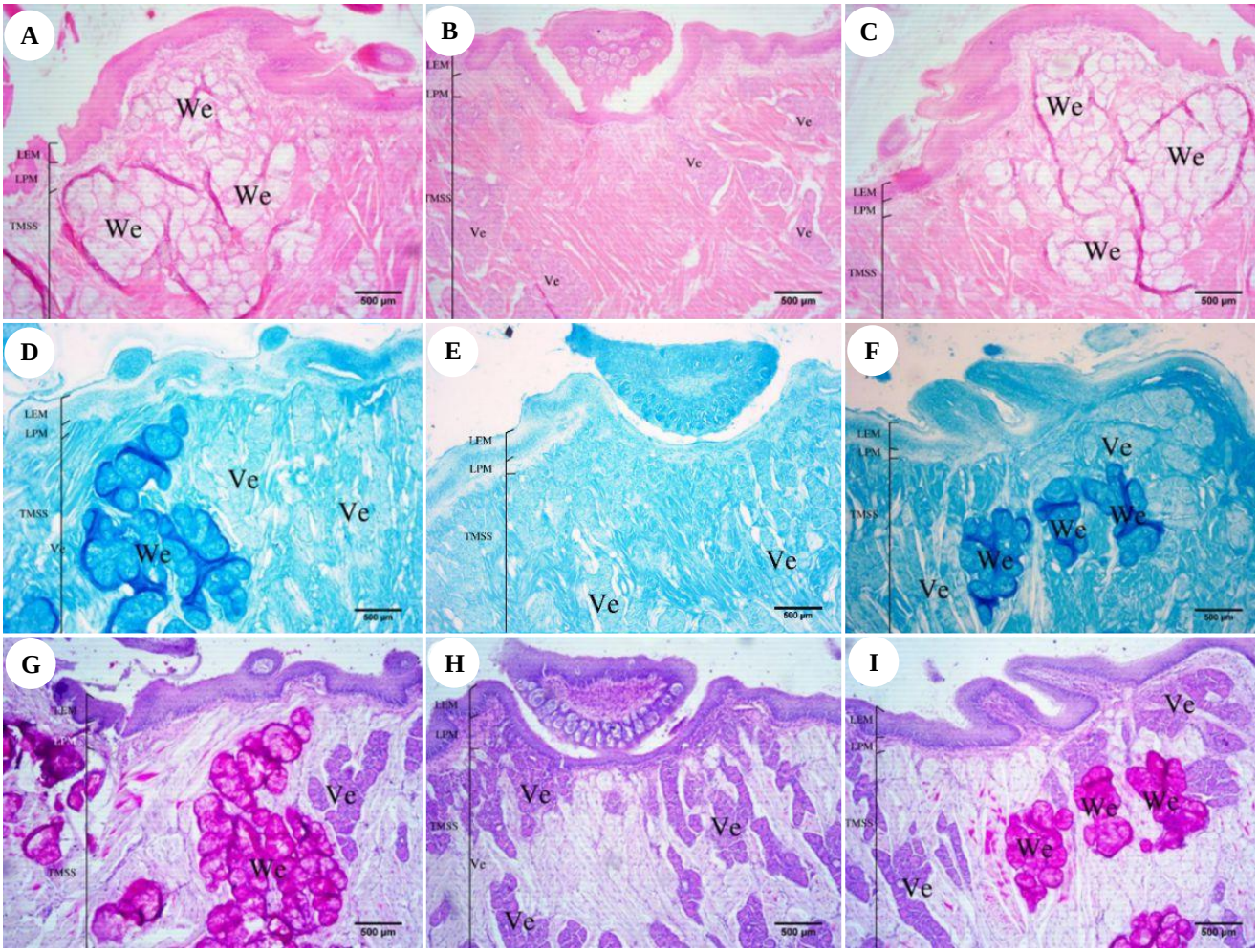

Figure 13. Photomicrographs of the radix region *Callosciurus notatus* tongue. A, B, C. HE staining shows the lingual glands including Weber's glands and von Ebner's glans. D, E, F. AB pH 2.5 staining shows strong positive reaction to Weber's glands and no positive reaction to von Ebner's glands (G, H, I) PAS staining shows strong positive reaction to Weber gland's and weak reaction to von Ebner's glands. lem: lamina epithelialis mucosae, lpm: lamina propria mucosae, tmss: textus muscularis striatus syncytialis. We: Weber's glands, Ve: von Ebner's glans

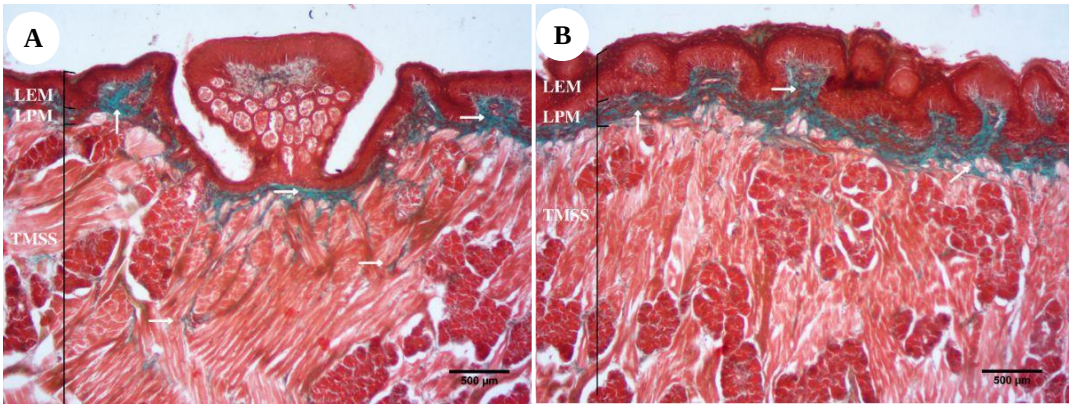

Figure 14. Photomicrographs of the corpus region of *Callosciurus notatus* tongue with MT staining. Collagen structure stained in green is observed moderately on lamina propria mucosae and less or absent on tunica muscularis striatus syncytialis. lem: lamina epithelialis mucosae, lpm: lamina propria mucosae, tmss: textus muscularis striatus syncytialis

Based on the SEM and histology observation, conicae papillae can be found on the radix of *Callosciurus notatus* tongue similar to Pallas's Squirrel (*Callosciurus erythraeus thai)* (Yoshimura et al. 2018), degu (*Octodon degus)* (Cizek et al. 2017), and Hazel Dormouse (*Muscardinus avellanarius)* (Wolczuk 2014). Histologically, the conicae papillae of *Callosciurus notatus* are coated with thick keratin. Conicae papillae are a modification form of filiform papillae, which protect the mucous membrane of the mouth when chewing and prevent retraction when the food is being swallowed (Saragih et al. 2020). Therefore, the keratinized filiform papillae and conicae papillae can relate to the function of papillae in terms of holding food, in addition this may be linked to the type of diet.

Based on SEM and histology observation, fungiform papillae spread between filiform papillae. These findings are in line with the research on some species such as Pallas's Squirrel (*Callosciurus erythraeus thai)* (Yoshimura et al. 2018), flying squirrel (*Pteromys momonga)* (Emura 2019), *Sciurus anomalus* (Sadeghinezhad et al. 2018), and *Hydrochaeris hydrochaeris* (Watanabe et al. 2013). From the histological observation, fungiform papillae have taste buds on the dorsal surface; however, it differs from Persian squirrels (*Sciurus anomalus*) (Sadeghinezhad et al. 2018), which stated that have 1-4 taste bud on the dorsal surface. The difference in the amount of taste bud on fungiform papillae depends on the type of food that is consumed. Omnivorous animals have more fungiform papillae than herbivores (Wolczuk 2014; Saragih et al. 2020). Vallatae papillae were found on the radix through observation from SEM and histology. Three vallatae papillae with a V-shape formation that the tip faced the caudal. Those papillae were also found in other studies, such as Persian squirrels (*Sciurus anomalus)* (Sadeghinezhad et al. 2018), Pallas's Squirrel (*Callosciurus erythraeus thai*) (Yoshimura et al. 2018), and flying squirrels (*Pteromys momonga)* with the same amount and formation (Emura 2019). On other rodents, such as white mouse (*Rattus norvegicus*) (Reginato et al. 2014), a single vallate papillae on the medial radix is observed; guinea pig (*Cavia porcellus)* (Ciena et al. 2017) exhibit two vallate papillae. Vallate papillae numbers differ according to their feeding habits and habitats. Increasing numbers of vallatae papillae correspond to the sensitivity of gustatory function (Wolczuk 2014; Sadeghinezhad et al. 2018). Based on observation of SEM and histology, papillae vallatae are surrounding by a groove. The canal that surrounds vallatae papillae helps increase the accessibility of taste buds toward food. Histologically, vallatae papillae are structured with epithelium stratificatum squamosum cornificatum with a thin layer of keratin and many taste buds in the lateral papillae. Similar traits were also found in the studies of guinea pig (*Cavia porcellus)* (Ciena et al. 2017) and Persian squirrels (*Sciurus anomalus)* (Sadeghinezhad et al. 2018). Histochemical analysis in this research by AB pH 2.5 and Periodic Acid Shiff (PAS) staining helps identify sialomucin or sulfomucin, which is acidic mucin and glycan, glycoconjugate, glycoprotein and is neutral or weak acidic (Nikumbh et al. 2012; Sheehen and Hrapchak 1980). Weber's glands and von Ebner's glands are identified at the radix region of *Callosciurus notatus* tongue. In mammals, there are 3 types of saliva glands: Blandin-Nuhn glands located at the tip of tongue, von Ebner glands located at gustatoria papillae, and Weber glands located at the radix of the tongue. Weber's glands are secretory mucous glands, and von Ebner's glands are secretory serous glands (Eurell and Frappier 2013; Singh and Sandhya 2018). Von Ebner's and Weber's gland can be found in both Persian squirrels (*Sciurus anomalus)* (Sadeghinezhad et al. 2018) and rat (*Rattus norvegicus)* (Goździewska-Harłajczuk et al. 2018). In agouti (*Dasyprocta aguti Linnaeus)* (Ciena et al. 2013) Weber's gland is absent. AB pH 2.5 and PAS staining reacted strongly positive (+++) at the Weber's glands on lateral posterior radix of *Callosciurus notatus*. This result was also found in the study of Persian squirrels (*Sciurus anomalus)* (Sadeghinezhad et al. 2018). The positive reaction toward the AB pH 2.5 and PAS staining indicates that the secretion of these glands is a neutral carbohydrate (glycan, glycoconjugate, glycoprotein) and acidic mucin (sialomucin or sulfomucin). The secretion of Weber's glands located at the posterior of the tongue is predicted to facilitate the tongue's movement for swallowing dry food, considering that the food of *Callosciurus notatus* is solid foods that tend to be dry. Von Ebner's glands identified near the vallatae papillae with no reaction to AB pH 2.5 and produce in purple (react weakly positive) on PAS stain indicates that von Ebner's glands are moderate serous glands secreting neutral serous (++). AB staining did not react to neutral mucin; meanwhile, PAS staining was used to check the presence of neutral mucin, which showed in strong magenta (Nikumb et al. 2012; Suvarna et al. 2019).

In addition, Masson Trichrome's staining was used to show collagen connective tissue to differentiate it from muscle tissue. Collagen connective tissue showed in green and was visible at the lamina propria mucosae and the textus muscularis striatus sycytialis of the apex; moderately at the lamina propria mucosae corpus and radix; lesser at the textus muscularis striatus syncytialis. Iwasaki et al. (2012) studied using confocal laser scanning microscopy and found collagen connective tissue type 3 at the lamina propria mucosae and the textus muscularis striatus syncytialis. They also found connective tissue consisting of collagen connective tissue, elastic connective tissue, and reticular connective tissue. Connective collagen tissue was stained in green; meanwhile, elastic connective tissue did not react to Masson Trichrome's staining (Eroschenko 2017; Suvarna et al. 2019). Collagen connective tissue can be seen in an abundant amount in the apex and could be due to the apex is not connected to the mandible through frenulum lingual, and the apex can move freely. Therefore, it requires more connective tissue than the corpus and radix that are connected to the mandible.

In conclusion, this research provides detailed morphological information on the tongue and lingual papillae of *Callosciurus notatus* from Yogyakarta, Java, Indonesia, based on observations using scanning electron microscopes and light microscopes. The lingual papillae's shape, structure, and distribution are related to the type of food and its function. The results obtained in this study are expected to be able to fill the information gaps related to

adaptation knowledge of individuals on the type of food and shelter; it is hoped that in the future, it can become a source of biodiversity diversity databases, especially in Indonesia.

ACKNOWLEDGEMENTS

The authors would like to thank the Integrated Laboratory for Research and Testing, Universitas Gadjah Mada, Yogyakarta, Indonesia for the use of scanning electron microscopy. The authors also wish to thank Yusuf Umardani for the excellent technical assistance.

REFERENCES

Abumandour MMA. 2014. Morphological comparison of the filiform papillae of New Zealand white rabbits (*Oryctolagus cuniculus*) as domestic mammals and egyptian fruit bat (*Rousettus aegyptiacus*) as wild mammals using scanning electron microscopic specimens. Intl J Morphol 32 (4): 1407-1417. DOI: 10.4067/S0717-95022014000400045.

Andalisa L, Rizaldi R, Nurdin J. 2018. Estimation of kapapa bajing population (*Callosciurus notatus* Boddaert, 1785) family Sciuridae in Nagari Koto Dalam, Padang Sago, Sumatera Barat. Metamorfosa J Biol Sci 213: 210-213. DOI: 10.24843/metamorfosa.2018.v05.i02.p11.

Bacha WJ, Bacha LM. 2000. Color Atlas of Veterinary Histology Second Edition. Lippincott Williams and Wilkins, Pennsylvania.

Baker N. 2016. Plantain Squirrel - *Callosciurus notatus*. Ecology Asia 8235. https://www.ecologyasia.com/verts/mammals/plantain_squirrel.htm.

Ciena AP, Bolina CDS, Almeida SRYD, Rici REG, Oliveira MFD, Silva MCPD, Miglino MA, Watanabe I. 2013. Structural and ultrastructural features of the agouti tongue (*Dasyprocta aguti* Linnaeus 1766). J Anat 223 (2): 152-158. DOI: 10.1111/joa.12065.

Ciena AP, Santos ACD, Vasconcelos BG, Rici REG, Neto ACDA, Almeida SRYD, Miglino MA, Watanabe I. 2017. Morphological characteristics of the papillae and lingual epithelium of guinea pig (*Cavia porcellus*). Acta Zool 100: 53-60. DOI: 10.1111/azo.12230.

Damia U, Anjani A, Wihadmadyatami H, Kusindarta DL. 2021. Identification of the lingual papillae in the sugar glider (*Petaurus breviceps*) by scanning electron microscopy and light microscopy. Anat Histol Embryol 50 (6): 918-930. DOI: 10.1111/ahe.12734.

Davydova L, Tkach G, Tymoshenko A, Moskalenko A, Sikora V, Kyptenko L, Lyndin M, Muravskyi D, Maksymova O, Suchonos O. 2017. Anatomical and morphological aspects of papillae epithelium muscles and glands of rats' tongue: Light scanning and transmission electron microscopic study. Interv Med Appl Sci 9 (3): 168-177. DOI: 10.1556/1646.9.2017.21.

Emura S, Tamada A, Hayakawa D, Chen H, Jamali M, Taguchi H, Shoumura S. 1999. SEM study on the dorsal lingual surface of the flying squirrel *Petaurista leucogenys*. Ann Anat 181 (5): 495-498. DOI: 10.2535/ofaj.79.113.

Emura S. 2019. Morphology of the lingual papillae of the Japanese lesser flying squirrel and four-toed hedgehog. Okajimas Folia Anat Jpn 96 (1): 23-26. DOI: 10.2535/ofaj.96.23.

Eroschenko VP. 2017. diFiore's Atlas of Histology With Functional Correlations 13ed. Lippincott Williams and Wilkins.

Eurell JA, Frappier BL. 2013. Dellman's Textbook of Veterinary Histology. Jhon Wiley & Sons, New Jersey.

Goździewska-Harłajczuk K, Nawrot JK, Barszcz K Marycz K, Nawara T, Modlińska K, Stryjek R. 2018. Biological aspects of the tongue morphology of wild-captive WWCPS rats: A histological histochemical and ultrastructural study. Anat Sci Intl 93 (4): 514-532. DOI: 10.1007/s12565-018-0445-y.

Gunawan G, Saragih G.R, Umardani Y, Karnati S, Wihadmadyatami H, Kusindarta DL. 2019. Morphological study of the lingual papillae in the fruit bat (*Rousettus amplexicaudatus*) by scanning electron microscopy and light microscopy. Anat Histol Embryol 49 (2): 173-183. DOI: 10.1111/ahe.12509.

Iwasaki SI, Miyata K, Kobayashi K. 1987. Comparative studies of the dorsal surface of the tongue in three mammalian species by scanning electron microscopy. Cells Tissues Organs 128 (2): 140-146. DOI: 10.1159/000146330.

Iwasaki SI, Yoshizawa H, Aoyagi H. 2012. Localization of type III collagen in the lingual mucosa of rats during the morphogenesis of circumvallate papillae. Odontology 100: 10-21. DOI: 10.1007/s10266-011-0020-7.

Iwasaki SI, Erdoğan S, Asami T. 2019. Evolutionary Specialization of the Tongue in Vertebrates: Structure and Function. In: Bels V, Whishaw I. (eds.) Feeding in Vertebrates. Fascinating Life Sciences. Springer, Cham. DOI: 10.1007/978-3-030-13739-7_10.

Jackowiak H, Godynicki S. 2005. The distribution and structure of the lingual papillae on the tongue of the bank vole *Clethrionomys glareolus*. Folia Morphol 64 (4): 326-333.

König HE, Liebich HG. 2020. Veterinary Anatomy of Domestic Animals: Textbook and Colour Atlas, 7th Edition. Thieme, New York.

Nikumbh RD, Nikumbh DB, Umarji BN. 2012. Mucin histochemical study of the colon in normal and malignant lesions. Intl J Health Sci Res 2 (7): 20-32.

Pastor JF, Barbosa MFJ, Paz DM, Garcia M, Ferrero E. 2011. Functional and comparative study of lingual papillae in four species of bear (Ursidae) by scanning electron microscopy. Microsc Res Technol 74 (10): 910-919. DOI: 10.1002/jemt.20975.

Reginato GDS, Bolina CDS, Watanabe I, Clena AP. 2014. Three-dimensional aspects of the lingual papillae and their connective tissue cores in the tongue of rats: A scanning electron microscope study. Sci World J 2014: 841879. DOI: 10.1155/2014/841879.

Sadeghinezhad J, Tootian, Javadi F. 2018. Anatomical and histological structure of the tongue and histochemical characteristics of the lingual salivary glands in the Persian squirrel (*Sciurus anomalus*). Anat Sci Intl 93 (1): 58-68. DOI: 10.1007/s12565-016-0367-5.

Sakr SMI, Taki-El-Deen FMA, Aboelwafa HR. 2013. Comparative light and scanning electron microscopic study of the lingual papillae in three different mammalian animals, *Hemiechinus auritus* (Erinaceomorpha: Erinaceidae) *Cavia porcellus* (Rodentia: Caviidae) and *Mustela nivalis vulgaris* (Carnivora: Mustel). Life Sci J 10 (4): 3082-3093.

Saragih G, Gunawan G, Umardani Y, Karnati S, Kusindarta DL, Wihadmadyatami H. 2020. Morphological and scanning electron microscopic study of the lingual papillae in the Javan Pipistrelle (*Pipistrellus javanicus*). Anat Histol Embryol 49 (6): 718-727. DOI: 10.1111/ahe.12566.

Sheehan DC, Hrapchak BB. 1980. Theory and Practice of Histotechnology, 2nd Edition. Battelle Press, Ohio.

Singh KKP, Sandhya K. 2018. Comparative histological study on lingual glands of some mammals. J Dent Med Sci 17 (8): 22-28.

Suvarna SK, Layton C, Bancroft JD. 2019. Bancroft's Theory and Practice of Histological Techniques. Churcill Livingstone, London. DOI: 10.1016/C2015-0-00143-5.

Thorington RW, Koprowski JJL, Steele MA, Whatton JF. 2012. Squirrels of The World. The Johns Hopkins University Press, Bartimole.

Watanabe I, Santos Haemmerle CA, Dias FJ, Cury DP, Silva MCP, Sosthines MCK, Santos TC, Guimaraes JP, Miglino MA. 2013. Structural characterization of the capybara (*Hydrochaeris hydrochaeris*) tongue by light, scanning, and transmission electron microscopy. Microsc Res Technol 76: 141-155.

Williams SH. 2019. Feeding in Mammals: Comparative, Experimental, and Evolutionary Insights on Form and Function. In: Bels V, Whishaw I. (eds.) Feeding in Vertebrates. Fascinating Life Sciences. Springer, Cham. DOI: 10.1007/978-3-030-13739-7_18.

Wolczuk K. 2014. Dorsal surface of the tongue of the hazel dormouse *Muscardinus avellanarius*. Scanning Electron Light Microsc Stud 59 (1-4): 35-47. DOI: 10.2478/zoop-2014-0004.