The Structure of the Lingual Papillary of the Asian Palm Civet's (Paradoxurus hermaphorditus): The Analysis of Feeding Habit Adaption using Gross-Morphometric, Scanning Electron Microscope, Light Microscope (Histochemistry Staining)

Dwi Kusindarta[1], Felix Chaya Eka Saputra[1], Anni Nurliani[2], Ignasius Gracia[1], and Hevi Wihadmadyatami[1]

[1]Universitas Gadjah Mada Fakultas Kedokteran Hewan
[2]Universitas Lambung Mangkurat Fakultas Matematika dan Ilmu Pengetahuan Alam

January 27, 2023

Abstract

The Asian palm civet (*Paradoxurus hermaphroditus*) is a wild mammal which is living in nature in Indonesia forest. Recently due to the highest demand of civet coffee, push the changes of the living environment of the Asian palm civet as the pet or captive breed. Changes in the environment from the wild to human intervention cause shift in their behaviour and feeding patterns. This is thought possibly to affect it's anatomical structure and histology digestive organ and mainly the lingual papillae as an entrance of the food. This study aims to analyse the anatomical structure of the lingual papillae and the secretions of the lingual glands in *Paradoxurus hermaphroditus* due to the changes of the food behaviour by gross macroscopy, scanning electron microscopy (SEM) and light microscopy (LM) in the form of histochemical staining (hematoxyline eosin, Alcian Blue-Periodic Acid Schiff, and Masson's Trichrome). The SEM results revealed that several type lingual papillae are found on the tongue of the *Paradoxurus hermaphroditus* they are: fungiform, arrowhead filiform, giant arrowhead filiform, scale-like filiform, conical, and circumvallate papillae. The histological results showed that on the radix of tongue of the civet are found Weber's glands which produce the secretion of neutral and acid mucin. Collagen fibers were found scattered in all of the lamina propria and some of the tunica muscularis with higher collagen intensity at the apex. In conclusion, these results provide the new insight of the adaptation form of the lingual papillae of the Asian palm civet due to their changes of feeding behaviour based on the type, number, distribution of the papillae, as well as the type of lingual gland secret and the presence of the collagen fibre.

The Structure of the Lingual Papillary of the Asian Palm Civet's (*Paradoxurus hermaphorditus*): The Analysis of Feeding Habit Adaption using Gross-Morphometric, Scanning Electron Microscope, Light Microscope (Histochemistry Staining)

Felix Chaya Eka Saputra[1#]., Anni Nurliani[2,3#], Ignasius Gracia P.D.W[1].,

Hevi Wihadmadyatami[1], Dwi Liliek Kusindarta[1*]

[1]Department of Anatomy, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia, 55281

[2]Post Doctoral Fellow, Department of Anatomy, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia, 55281

[3]Department of Mathematics and Natural Sciences, Lambung Mangkurat University, South Kalimantan, Indonesia, 70714

#Both authors contribute equally

*Corresponding author:

Dwi Liliek Kusindarta

Email: indarta@ugm.ac.id

Phone: +6282137067422

ABSTRACT

The Asian palm civet (*Paradoxurus hermaphroditus*) is a wild mammal which is living in nature in Indonesia forest. Recently due to the highest demand of civet coffee, push the changes of the living environment of the Asian palm civet as the pet or captive breed. Changes in the environment from the wild to human intervention cause shift in their behaviour and feeding patterns. This is thought possibly to affect it's anatomical structure and histology digestive organ and mainly the lingual papillae as an entrance of the food. This study aims to analyse the anatomical structure of the lingual papillae and the secretions of the lingual glands in *Paradoxurus hermaphroditus* due to the changes of the food behaviour by gross macroscopy, scanning electron microscopy (SEM) and light microscopy (LM) in the form of histochemical staining (hematoxyline eosin, Alcian Blue-Periodic Acid Schiff, and Masson's Trichrome). The SEM results revealed that several type lingual papillae are found on the tongue of the *Paradoxurus hermaphroditus* they are: fungiform, arrowhead filiform, giant arrowhead filiform, scale-like filiform, conical, and circumvallate papillae. The histological results showed that on the radix of tongue of the civet are found Weber's glands which produce the secretion of neutral and acid mucin. Collagen fibers were found scattered in all of the lamina propria and some of the tunica muscularis with higher collagen intensity at the apex. In conclusion, these results provide the new insight of the adaptation form of the lingual papillae of the Asian palm civet due to their changes of feeding behaviour based on the type, number, distribution of the papillae, as well as the type of lingual gland secret and the presence of the collagen fibre.

Keywords: *Paradoxurus hermaphroditus,* lingual papillae, lingual glands, collagen

Highlights

- This study provides the first detailed information about the structure and type of secretion of the lingual tongue papillae and glands of the*Paradoxurus hermaphorditus*
- Scanning electron microscopy and light microscopy in the histochemistry staining were done to determine the type of papillae, type of the secret of the lingual glands and histological structure of the tongue
- Six types of papillae presents on the tongue of *Paradoxurus hermaphorditus, there are:* fungiform, arrowhead filiform, giant arrowhead filiform, scale-like filiform, conical, and circumvallate papillae

INTRODUCTION

Paradoxurus hermaphorditus (P. hermaphorditus) or the Asian palm civet is a mammal belonging to the family Vevirridae (Baker et al., 2008). *P. hermaphorditus* or Asian palm civet is one of Indonesian wild animal which is quite well known throughout the world as an effect of the famous civet coffee. The civet will only smell and eat the best coffee beans, then digest and ferment the coffee beans, the seeds remain intact with the faeces and are collected for further processing to become civet coffee (Marcone, 2004). The current level of civet coffee popularity has resulted in a decrease in the number of *P. hermaphorditus* populations in nature, but the number of captive breeds has increased. This phenomenon to keep them as captive breeds actually causes many changes in the behaviour of the Asian palm civet and a decrease in productivity on the producing of civet coffee. The change in their habitat causes changes in the diet pattern of the Asian palm civet when it is still in the wild compared to when living intervention by humans.

In nature, Asian palm civet often eat fruit such as mangoes, bananas, rambutan and papaya. In addition to fruits, Asian palm covet are very fond of the sap from the flowers of the palm tree, besides that they also eat mice, birds, insects, worms, seeds, eggs, reptiles, slugs, and scorpions (Burton, 1968; Grzimek et al., 2004; Jumhawan et al., 2016). Meanwhile in captivity, food intake is served by humans, and the type of food is different from their natural habitat. It will cause the change and adaptation of organs involved in the digestion process. One of the organs that may be affected by this habit change is the tongue as the first organ in the digestive tract. The tongue plays an important role in food intake in vertebrates. It serves the mechanical function of manipulating food and sensory function related to taste (Klinic et. al., 2010; Reginato et. al., 2014). Therefore, morphological variations in vertebrate tongues directly show the result of adaptation to the type of natural food in their habitat.

Many studies on the dorsal surface of animal tongues have been carried out such as the fruit bat *Rousettus ampelxicaudatus* (Gunawan et al., 2019), the insect bat *Pipistrellus javanicus* (Saragih et al., 2020), the flying squirrel *Hylopetes platyurus*(Wihadmadyatami et al., 2020), the flying squirrel *Tupaia javanica* (Gartiwa et al., 2021), sugar glider *Petaurus breviceps*(Damia et al., 2021), and javan mongoose *Herpestes Javanicus*(Kusuma et al., 2022). These studies provide evidence of variation in papillary morphology and distribution. However, morphological studies on the tongue of the Asian palm civet (*P. hermaphorditus*) form Yogyakarta, Indonesia as one of the source of civet coffee have been not conducted yet. Therefore, this study will focus to describe complete morphology and distribution of lingual papillae and glands as well as the distribution of the collagen fiber of the Asian palm civet's tongue in the form of gross macroscopy, as well as the scanning electron microscope and histological observation (hematoxylin-eosin, AB-PAS, Masson Trichome Staining) to provide a clear visualisation of each papilla and to observe the characteristic of the secretory glands for better understanding to the lingual papillae adaptation of function related the type of food and place of living.

MATERIAL AND METHODS

Animal Samples

Eight asian palm civets (*Paradoxurus hermaphorditus*) were obtained from breeding in the Special Region of Yogyakarta regardless of age and sex. Samples were anesthetized and euthanized using lethal doses of 10 mg/kg BW of ketamine (Interchemie, Metalweeg, Holand) and Xylazine 2 mg/kg BW (Intercemie, Metaalweg, Holand) and directly continued for perfusion. To identify the morphological characteristics, the analysis was carried out in the Laboratory of Animal Systems, Faculty of Biology, Universitas Gadjah Mada

Conservation Status

The asian palm civet (*P. hermaphorditu* s) used in this study is not on any conservation lists in Indonesia and is not protected by the national laws of the Republic of Indonesia, in addition based on the IUCN the *P. hermaphorditu* s categorized as the least concern species.

Gross Macroscopy Analysis

The oral cavity was separated between the base of the tongue and the mandible. The hyoid bone is excised and the tongue is retracted, then cut caudally to the larynx. Then the sample was rinsed using 0.9% Physiological NaCl for five minutes, five times. Macroscopic observations were made on all samples using a Canon EOS 7000D digital camera (Canon, Tokyo, Japan). Then the samples were prepared for SEM and LM.

Scanning Electron Microscopy (SEM)

Samples were stored in SEM fixative solution (0.5% glutaraldehyde, 1.5% paraformaldehyde, Hepes, and Phosphate Buffer Saline (PBS) working for a minimum of 6 – 24 hours. The fixed samples were then washed with 0.9% NaCl solution for 5 times. Then the dehydration process was carried out using a graded ethanol solution. Then the sample was trimmed at the apex, corpus, and radix borders and fixed on a carbon plate. Then the sample was dried using a vacuum system (BUEHLER, Castable Vacuum System). Then the sample was coated using platinum coater (JEOL auto fine coater JEC-3000FC) (4.5 PA). Finally, the sample was inserted into the electron microscope (JEOL JSM-6510LA) and observed at voltage of 15 kV and magnifications of 27 times, 30 times, 50 times, 70 times, 80 times, 100 times, and 190 times.

Morphometric Analysis

To do the morphometric analysis of the lingual papillae the ImageJ software was applied (NIH, Maryland, US). The SEM images was opened in the software; then, the measurement scale was equalized to the scale bar. Subsequently, a sample of papillae was selected, to measure the length a straight line was drawn from the tip to the base of the papilla. In addition, to measure the width and diameter of the papilla, a straight line was drawn from end to end on the widest side of the papilla. The same steps were done for all type of papillae and the measurement were done in triplicate for each type of papillae, then the mean and deviation standard of each result was calculated by GraphPad Prism7 (La Jolla, CA, USA).

Preparation for the LM

The tongue that had been stored in a 4% paraformaldehyde fixative solution for 24 hours was then trimmed in a predetermined region. The sample was then washed with running water after which it was dehydrated with a graded ethanol solution. After that, the clearing process was carried out with xylol solution. The next step was paraffin infiltration in the incubator. After that, embedding was carried out in paraffin blocks and blocking by allowing paraffin to harden at room temperature. Then, the blocks were cut using a rotary microtome with thickness of 8 mm. Sample that had been cut was placed on a water bath and then transferred to the surface of a coated slide. The slides were then placed on a slide warmer for 24 hours at temperature of 40°C and were labeled with the preparation code. The slides then stained with hematoxylin eosin (HE), others were stained with periodic acid-schidd (PAS) and Masson's Trichrome.

Light Microscopy (LM)

Hematoxylin Eosin

The slides were immersed in Xylol (KgaA, Darmstadt, Germany)solutions for 5 minutes each for the deparaffinization process. The slides were then rehydrated by immersing the slides in absolute ethanol (KgaA, Darmstadt, Germany) solutions then gradually decreasing (95%, 90%, 80% ethanol and 70% ethanol) respectively for 3 minutes each. Furthermore, the staining stage was carried out using Hematoxylin and Eosin (Leica, Wetzlar, Germany), which was started by immersing the sample in the hematoxylin solution for 1 minute followed by immersing the sample in the Eosin solution for 1 minute. Then the sample dehydration process was carried out to remove excess liquid using graded alcohol, for 1 minute each in 70%, 80%, and 90% ethanol. Furthermore, the clarification process was carried out by immersing the slides in Xylol (KgaA, Darmstadt, Germany) solutions for 5 minutes each. The last process in HE staining is mounting the sample using Canadian balsam (Leica. Wetzlar, Germany). HE staining of the sample then could be observed using a light microscope.

Alcian Blue Staining

The staining process was initiated by deparaffinization and then stained using the AB staining kit (Bio-Optica, Milan, Italy) which was initiated by rinsing the slides with running water. Then the slide was dripped with AB reagent pH 2.5 as much as 2 drops and then allowed to stand for 30 minutes. The slide was then immersed again in water and then dripped with 2 drops of sodium tetraborate reagent and allowed to stand for 10 minutes. Then the slide is dripped with 2 drops of carmalum solution and left for 5 minutes, then rinsed in running water for 5 minutes and dehydrated with graded alcohol and clarified using xylol. The final step is mounting using Balsam Canada. Samples that have been stained and can be observed using a light microscope.

Periodic Acid shiff Staining

The next stage was PAS staining using the PAS Kit (Bio-Optica, Milan, Italy) which was started by soaking the slide in water, then adding 2 drops of Periodic Acid solution and letting it stand for 10 minutes. The slide was rinsed with water after that it was given 2 drops of Schiff's reagent and left for 15 minutes. Then the slide was rinsed again with water and given 2 drops of potassium meta-bisulphite solution and waited for 2 minutes. Then the slide was dried and added 10 drops of fixative solution, allowed to stand for 2 minutes and then immersed in water. The slide was then given 10 drops of Mayer's Hemalum solution, left for 3 minutes, then rinsed in running water for 5 minutes. After that, the slides were washed in running water and dehydrated with graded alcohol and clarified using xylol. The final step is mounting using Balsam Canada. Samples that have been stained with the AB PAS kit can then be observed using a light microscope.

Masson's Trichrome Staining

The staining process begins with the deparaffinization process using xylol solution and the rehydration process with absolute ethanol solution, 90%, 80% and 70%. Masson Trichrome staining using the Masson Trichrome kit (Bio-Optica, Milan, Italy). The staining stage begins by dripping Weigert's iron hematoxylin A solution and then adding 2 drops of Weigert's iron hematoxylin B solution each, then let stand for 10 minutes. Then the slide was cleaned without water rinsing and given a solution of 3 drops of picric acid alcoholic stable and left in the open air for 4 minutes. The slides were rinsed quickly in distilled water, then 3 drops of Ponceau acid fuchsin solution were added and left for 4 minutes.The slide was immersed in distilled water and added 3 drops of Phosphomolybdic acid solution and then let stand for 10 minutes. Furthermore, the slide was cleaned without water rinsing and added 3 drops of light green solution as the final colouring, then waited for 5 minutes.

The final stage is that the slides are immersed in distilled water and the dehydration process is carried out quickly with graded alcohol, then proceed with the clearing process using xylol solution and ending with the mounting process using Balsam Canada. Slides that have been stained can be seen when observed using a light microscope.

RESULTS

Gross Morphology of the Tongue

The results of macroscopic observations showed that the tongue of*Paradoxurus hermaphroditus* was divided into three regions, namely the apex which is the most anterior part, the corpus which is the middle part, and the radix which is the most posterior part and is adjacent to the larynx (Figure 1A). The boundary between the apex, corpus, and radix can be seen from the changes in the size and type of papilla. On the ventral side, the tongue is connected to the mandible by the frenulum of the tongue which is located at the junction of the apex and body. Macroscopically, the dorsal surface of the apex can be divided into two regions based on the distribution of the papillae, namely the medial apex and the lateral apex (Fig. 1B). The region of the body is divided into two regions according to the distribution of the papillae, namely the anterior body and the posterior body (Fig. 1C). The radix region is divided into two regions according to the distribution of the papillae, namely the anterior radix region and the posterior radix region (Fig. 1D). In the posterior radix, there is a circumvallate papilla formation which is in the central forming a V formation and pointing to the anterior of the tongue.

Scanning Electron Microscope

Apex

The apex of the tongue is divided into two regions, lateral and medial. In the medial part of the apex, there are arrowhead papillae. These papillae are directed caudally and are characterized by their pointed tip that has up to 6-8 lateral processes (Fig. 2B). In the lateral part of the apex, there are long, oblong conical papillae that point to the caudomedial of the tongue (Fig. 2D). These papillae are oval-shaped with blunt ends and have no processes (Fig. 2E). On the medial apex, fungiform papillae were scattered which are relatively large in size. The features of these papillae are polygonal and dome-like with their medial areas becoming concave (Fig. 2C). There were 35-40 fungiform papillae in total in the apex region (Fig. 2A).

Corpus

The corpus is divided into two regions, anterior and posterior corpus. In the anterior part of the corpus there are two types of filiform papillae, namely giant arrowhead and scale like filiform. Giant arrowhead papillae are characterized with a sharp tip shape accompanied by up to 8-10 processus originating from the lateral side (Figure 3B). Giant arrowhead papillae on the anterior of the corpus lead to caudomedial. The difference between the arrowhead papilla at the apex and the giant arrowhead papilla at the corpus lies in the direction and the size of the papilla. Among the giant arrowhead papillae are also scattered fungiform papillae. These papillae look similar to those at the apex (Fig. 3C). In the posterior part of the corpus there are filiform scale-like papillae as the border between the corpus and the radix. The filiform scale-like papillae are characterized by an oblong shape with a pointed tip like scales. These papillae have processes on the lateral to caudal sides of about 4-6 pieces (Fig. 3E). In the medial region of the posterior corpus there are foliate papillae with small and short features with a pointed tip (Fig. 3D).

Radix

The radix is divided into three regions, namely anterior, medial, and posterior. In the anterior radix, there are blunt conical papillae which is oval like a cone but much smaller in size and have a blunt tip (Fig. 4A). In the posterior radix there are conical papillae that point caudomedially toward the larynx (Fig. 4E). The characteristics of these papillae are tapered oval in shape without the processes. In the medial radix there are circumvallate papillae which have taste buds with large and round shapes and are surrounded by an invagination of thick wall. These papillae form in a V formation that points anteriorly to the tongue. The circumvallate papilla is the papilla with the largest size but the least number of papillae (Fig. 4D). Fungiform papillae are scattered all over the radix with diameter of 397.7 $\pm$ 17 µm. The fungiform papillae present on the radix are flat in the center (Fig. 4B).

Light Microscopy

Hematoxylin Eosin (HE) Staining

The apex area is histologically divided into three layers, namely the lamina epithelial mucosal, the lamina propria mucosal, and the tunica muscularis striatus syncytialis. In the apex area, arrowhead filiform papillae and fungiform papillae were found. The arrowhead filiform papillae are thickly keratinized with a shape resembling an arrowhead. These papillae were evenly distributed at the apex of the tongue (Fig. 5A). Fungiform papillae have a thinner keratinization with small base then widen on the surface. The fungiform papillae are located between the arrowhead filiform papillae (Fig. 5B).

The corpus of the tongue is also divided into same three layers as in the apex. In the corpus area, fungiform papillae, giant arrowhead papillae filiform, and scale-like filiform papillae were observed (Fig. 6A). The fungiform papillae are dome-shaped with thin keratinization and have scattered taste buds (Fig. 6C). The giant arrowhead filiform papillae have a wider and a blunter tip than the arrowhead filiform papillae present at the apex (Fig. 6B). The scale-like filiform papillae are wider than other papillae in this region.

The radix of the tongue is also divided into same three layers as in the apex and corpus (Fig. 7A). In the radix area there are circumvallate papillae, conical papillae, and fungiform papillae. The circumvallate papillae have a large circular shape with a canal invaginating the surrounding epithelium. The circumvallate papillae have taste buds scattered on their surface (Fig. 7B). The conical papillae have an elongated oval shape with thin keratinization. Conical papillae were seen in the lateral area of the radix (Fig. 7C). The fungiform papillae have the same shape as the fungiform present at the apex and corpus. The fungiform papillae are dome-shaped with scattered taste buds (Fig. 7D). In the radix, weber's glands were observed under the papillae.

Histochemistry Staining

Alcian Blue (AB) Staining

The results of Alcian Blue (AB) staining on the tongue of Asian palm civet shows that the tongue is histologically divided into the same three layers as in the HE staining, namely the lamina epithelial mucosal, the lamina propria mucosal, and the tunica muscularis striatus syncytialis. In the apex and corpus areas, there was no visible tissue part that reacted positively to AB (Fig. 8A, B, C, D). In the radix area there are Weber's glands whose secretions react positively to AB (Fig. 8E). A positive AB result is indicated by the presence of a dark blue colour on the tongue tissue in the radix area. The dark blue colour indicates that Weber's glands produce an acidic mucopolysaccharide secretion (Fig. 8F).

Periodic Acid shiff (PAS) Staining

From the results of Periodic Acid shiff (PAS) staining of the tongue of*Paradoxurus hermaphroditus* , the formation of the tongue layer in all regions consists of the same three layers, namely, the lamina epithelial mucosal, the lamina propria mucosal, and the tunica muscularis striatus syncytialis (Figure 9A). Based on histochemical analysis, it was known that the tongue of *Paradoxurus hermaphroditus* has Weber's glands (Fig. 9B). Weber's glands are located below the circumvallate papillae on the radix and in the posterior region of the radix. In the posterior radix region, there was a positive reaction to the PAS stain, which is shown in magenta red colour (Fig. 9E, F). This magenta colour indicates that the von Ebner glands secrete mucous carbohydrates with neutral properties.

Masson's Trichrome Staining

Masson's Trichrome staining on the tongue of the Asian palm civet showed that the tongue in all regions was divided into the same three layers, namely the lamina epithelial mucosal, the lamina propria mucosal, and the tunica muscularis striatus syncytialis. In the lamina propria in all regions there are collagen fibers marked with a greenish blue colour. The apex region has fewer collagen fibers (Figs. 10A, B). Collagen fibers are also seen in some areas of the tunica muscularis in corpus and radix (Figs. 10C, D, E, F). The muscle fiber is stained bright red and the epithelium is stained darker red. In the radix area there are Weber's glands which are not stained by Masson's Trichrome staining.

DISCUSSION

The results of observations of the tongue of *Paradoxurus hermaphroditus* showed that the tongue was divided into three regions; apex, corpus, and radix. The apex is the most anterior part of the tongue that can move freely. The corpus is the middle part that is attached to the frenulum. The radix is the most posterior part that connects to the hyoid and mandible (Frandson et al., 2009). The tongue of *Paradoxurus hermaphroditus* has a median sulcus located from the apex to the posterior corpus and three circumvallate papillae that form the letter "V" towards the posterior. This circumvallate papilla structure is also found in several other carnivores, such as the Egyptian domestic cat (*Felix catus*) (El-Bably and Tolba, 2015), the Egyptian mongoose (*Herpestes ichneumon*) (Selim and Samir, 2018), and the Raccoon dog (*Nyctereutes procyonoides*) (Emura et al., 2006) with different number of circumvallate papillae.

Papillae on the tongue of *Paradoxurus hermaphorditus* are divided into two types; gustatory papilla as a sensory organ and mechanical papilla to facilitate the process of taking and processing food in the mouth. Gustatory papillae are grouped into fungiform papillae and circumvallate papillae, whereas mechanical papillae are grouped into filiform papillae and conical papillae. This grouping of papillae was also found in other animal species such as Markoz goat (Goodarzi and Hoseini, 2015), wild cats (Haddad et al., 2019), and laboratory rats (Daydova et al., 2017).

As a mechanical papillae, filiform papillae function to facilitate individuals to take food. Filiform papillae have many subtypes depending on the species with different types of feed and habitat (El-bably and Tolba, 2015). In *Paradoxurus hermaphroditus* , filiform papillae are scattered over the apex and corpus. In the apex region, arrowhead filiform papillae were found. In the corpus region, giant arrowhead filiform papillae and scale-like filiform papillae were found. From the observation, arrowhead filiform and giant arrowheads filiform papillae have a similar shape, with a triangular base of the lamina propria. The scale-like filiform papillae are flat and short, resembling reptile scales (Daydova et al., 2017). A similar division of filiform papilla is also found in *Rattus norwegicus* (Iwasaki, 2002).

The conical papillae of *Paradoxurus hermaphroditus* were found only in the lateral region of the radix. The conical papilla is characterized by a raised oval shape with a blunt tip and thick keratinization that serves to facilitate swallowing of food (Park and Lee, 2009). Conical papillae are also only found in the radix area of some animals such as *Herpestes ichneumon* (Salim and Samir, 2018),*Felis domesticus* (Iwasaki, 2002), and *Rousettus amplexicaudatus* (Gunawan et al., 2019).

Fungiform papillae of *Paradoxurus hermaphroditus* were found throughout the whole tongue region. According to Iwasaki (2002), fungiform papillae were evenly distributed from the apex to the radix, with the highest number found in the corpus area. Other animal studies such as *Herpestes ichneumon* (Selim and Samir, 2018) and*Nyctereutes procyonoides* (Emura et al., 2006) have shown that fungiform papillae are scattered in the apex and corpus areas. Fungiform papillae have a dome-like shape with thin keratinization and a concave tip with taste buds scattered on the surface (Haddad et al., 2019).

The circumvallate papillae are the largest papillae found on the tongue. Three fungiform papillae of *Paradoxurus hermaphroditus* were found in the radix area. The circumvallate papilla has the largest diameter characterized by round shape and the presence of an invagination of the surrounding epithelium with numerous taste buds in the lateral surface (Haddad et al., 2019, Gunawan et al., 2019). Papilla circumvallate has the main function as a sensory system on the tongue to recognize whether the food is edible or poisonous. The number and size of circumvallate papillae vary for carnivores with an average amount of three to six (Haddad et al., 2019).

Alcian Blue and Periodic Acid Schiff histochemical staining techniques on *Paradoxurus hermaphroditus* in the apex and corpus of the tongue do not show any part of the tissue that reacted positively to AB or PAS staining. The tongue on the radix of the Asian palm civet with AB staining is found to have Weber's glands whose secretions reacted positively, marked by a dark blue colour. The dark blue colour indicates that the mucopolysaccharide secretion is acidic. Weber's glands also show a positive PAS reaction marked by a magenta red colour indicating a neutral substance. This is in accordance with the explanation from Avery (2002) that the Weber's glands produce secretions that are useful for moistening food so that it helps the task of taste receptors on the papillae of the tongue and makes it easier to swallow food. Weber's glands which showed an acid secretion in AB and neutral secretion on PAS staining indicated that the weber glands of *Paradoxurus hermaphroditus* secreted two different types of mucosal secretions, as research by Haddad and Yassear (2018) showed that rabbit's weber glands produce acid secretions on AB and neutral on PAS.

Masson's Trichome staining on the tongue of *Paradoxurus hermaphroditus* showed the presence of collagen fibers marked by blue colour in all the lamina propria and some part of the tunica muscularis. The epithelium of the papillae is stained dark red and the tunica muscularis is stained lighter red. The red-coloured epithelium is more concentrated because the epithelium is on the surface area and has many gaps and pores so that it absorbs more color (El-deen and Shahin, 2013). Collagen fibers scattered on the tongue function as connective tissue that helps maintain the structural integrity of the tongue and helps the elasticity of the tongue in processing food (Goździewska-Harłajczuk et al., 2018).

CONCLUSION

Overall, this study provides the first detailed information on the tongue papillae of the Asian palm civet in Yogyakarta, Indonesia based on macroscopic and microscopic examination. The shape and distribution of papillae on the tongue depends on the type of food and the structural characteristics of each papillae are adapted to their function.

CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflicts of interest concerning the research, authorship, or publication of this article.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Post Doctoral Fellowship Universitas Gadjah Mada for funding this work . The authors thank to Golda Rani Saragih, D.V.M., Yusuf Umardani, S.T., M.Eng. for his excellent technical assistance and also Integrated Laboratory for Research and Testing for the use of Scanning Electron Microscope.

DATA AVAILABILITY STATEMENT

The author elects to not share data

REFERENCES

Avery J K. 2002. Essential of oral histology and embryology. 2nd ed. 182-193 Mosby.

Baker, N., Lim, K. K. P., & Nature Society (Singapore). (2008). *Wild animals of Singapore : a photographic guide to mammals, reptiles, amphibians and freshwater fishes* . Draco Pub. and Distribution.

Burton, M. 1968. *University Dictionary of Mammals of the World* . New York,NY : Crowell

Damia, U., Anjani, A.K., Wihadmadyatami, H., Kusindarta, D.L. 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 https://doi.org/10.1111/ahe.12734

Davydova, L., Tkach, G., Tymoshenko, A., Moskalenko, A., Sikora, V., Kyptenko, L., Lyndin, M., Muravskyi, D., Maksymova, O., and Suchonos, O. 2017. Anatomical and morphological aspects of papillae, epithelium, muscles, and glands of rats' tongue: Light, scanning, and transmission electron microscopic study. *Interventional Medicine and Applied Science* , *9* (3), 168–177. https://doi.org/10.1556/1646.9.2017.21

El-Bably, S.H., Tolba, A.R. 2015. Morph-metrical study of the tongue (*Lingua*) of the adult Egyptian domestic cat (*Felis domesticus*). *Inter J Vet Sci, 4(2) : 69-74*

El-Deen., T., Shahin, M. 2013. Comparative histological studies on three species of Egyptian bats. *Life Science Journal 2013 (10)2*

Emura, S., Okumura, T., Chen, H., Shoumura, S. 2006. Morphology of the Lingual Papillae in the Raccoon Dog and Fox. *Okajimas folia anatomica Japonica* . 83. 73-6. 10.2535/ofaj.83.73.

Frandson, R. D., Wilke, W. L., & Fails, A. D. (2019). Anatomy and Physiology of Farm Animals seventh Edition. *The Canadian Veterinary Journal* .

Gartiwa, G., Damia, U., Megawati, E.I., Pradipta, S.I.D., Gunawan, G., Karnati, S., Wihadmadyatami, H., Kusindarta, D.L. 2021. Morphological characterization of Horsfield's treeshrew Tupaia javanica lingual papillae: light microscopy and scanning electron microscopy studies.*Anatomia, Histologia, Embryologia*https://doi.org/10.1111/ahe.12724

Grzimek, B., N. Schlager, D. Olendorf. 2004. *Grzimek's Animal Life Encyclopedia* . Detroit: Gale

Goździewska-Harłajczuk, K., Klećkowska-Nawrot, J., Barszcz, K., Marycz, K., Nawara, T., Modlińska, K., and Stryjek, R. 2018. Biological aspects of the tongue morphology of wild-captive WWCPS rats: a histological, histochemical and ultrastructural study. *Anatomical Science International* , *93* (4), 514–532. https://doi.org/10.1007/s12565-018-0445-y

Goodarzi, N., Hoseini, T.H. 2015. Fine Structure of Lingual Papillae in the Markhoz Goat (Iranian Angora): A Scanning Electron Microscopic Study. *International Journal of Zoological Research, 11: 160-168.*

Gunawan, G., Saragih, G, R., Umardani, Y., Karnati, S., Wihadmadyatami, H., Kusindarta, D, L. 2019. 'Morphological study of the lingual papilae in the fruit bat (Rousettus amplexicaudatus) by scanning electron microscopy and light microscopy', Journal of Veterinary Medicine Series C: Anatomia Histologia Embryologia, (June), pp. 1–11. doi: 10.1111/ahe.12509

Haddad, K. M., Yasear, A. Y. 2018. Weber's salivary glands of rabbit: Histological and histochemical studies. *Biochemical and Cellular Archives* , *18* (1), 557–560.

Iwasaki, S. I. 2002. 'Evolution of the structure and function of the vertebrate tongue', Journal of Anatomy, 201(1), pp. 1–13

Kilinc, M., Erdogan, S., Ketani, S., Ketani, M. A. 2010. Morphological study by scanning electron microscopy of the lingual papillae in the middle east blind mole rat (*Spalax ehrenbergi* , Nehring, 1898).*Anatomia Histologia Embryologia* . 39(6), 509–515.

Kusuma, I.F., Damia, U., Megawati, E.I., Saputra, F.C.E., Karnati, S., Kusindarta, D.L., Wihadmadyatami, H. 2022. Morphology of lingual papillae in the Javan mongoose (Herpestes javanicus) by scanning electron microscopy and light microscopy. Anat Histol Embryol. 2022*https://doi.org/10.1111/ahe.12848*

Jumhawan, U., Putri, S. P., Yusianto, Bamba, T., & Fukusaki, E. 2016. Quantification of coffee blends for authentication of Asian palm civet coffee (Kopi Luwak) via metabolomics: A proof of concept. *Journal of Bioscience and Bioengineering* . https://doi.org/10.1016/j.jbiosc.2015.12.008

Marcone, M. F. (2004). Composition and properties of Indonesian palm civet coffee (Kopi Luwak) and Ethiopian civet coffee. *Food Research International* , *37* (9), 901–912. https://doi.org/10.1016/j.foodres.2004.05.008

Park, J.W., and Lee, J.-H. .2009. Comparative Morphology of the Tongue of *Miniopterus schreibersi fuliginosus* and *Pipistrellussavii* . *Applied Microscopy, 39(3), pp. 267–276.*

Reginato, G. S., Bolina, C. S., Watanabe, I., Ciena, A. P. 2014. Three-Dimensional Aspects of the Lingual Papillae and Their Connective Tissue Cores in the Tongue of Rats: A Scanning Electron Microscope Study. *The Scientific World Journal* , 841879.

Saragih GR, Gunawan G, Umardani Y, Karnati S, Kusindarta DL, Wihadmadyatami H. Morphological and scanning electron microscopic study of the lingual papillae in the Javan Pipistrelle (Pipistrellus javanicus). Anat Histol Embryol. 2020;00:1–10. https://doi.org/10.1111/ ahe.12566

Selim, A., and Samir, R. 2018. Light and Scanning Electron Microscope Studies of the Tongue of the Egyptian Mongoose (Herpestes ichneumon).*Journal of Cytology & Histology* , *09* (01), 1–6. https://doi.org/10.4172/2157-7099.1000499

Wihadmadyatami, H., Saragih, G.R., Gunawan, G., Mataram, M.B.A., Kustiati, U., Kusindarta, D.L. Morphological study of the lingual papillae of Jentink's flying squirrel (*Hylopetes platyurus*).*Thai Journal of Veterinary Medicine* 2020 Vol.50 No.2 pp.239-249

FIGURE LEGEND

Figure 1. The dorsal surface of the tongue of *Paradoxurus hermaphroditus* macroscopically. (A) The tongue is divided into 3 regions, namely apex (A), corpus (C) and radix (R); the medial groove or median sulcus (mg) and 3 circumvalate papillae (cv) in the radix area were observed. (B) The apex of the tongue is divided into medial apex (ma) and lateral apex (la). (C) Part of the corpus of the tongue is divided into anterior and posterior bodies; the anterior body is further subdivided into the medial anterior corpus (mac) and the lateral anterior corpus (lac); The posterior corpus is divided into the medial posterior corpus (mpc) and the lateral posterior corpus (lpc). (D) Radix region of the tongue is divided into medial radix (mr) and lateral radix (lr) with circumvallate papillae (cv) clearly visible in the medial radix area.

Figure 2. Scanning Electrone Microscopy (SEM) image of the apex region of tongue of *Paradoxurus hermaphroditus* . (A) Distributed papillae at the apex of the tongue. Arrowhead filiform papillae (af);

fungiform papillae (fu); conical papillae (cp) (B) arrowhead filiform papilla (af) at medial apex 190x magnification. (C) fungirform papilla (fu) at medial apex 190x magnification. (D) conical papilla (cp) at lateral apex 90x magnification. (E) conical papilla (cp) at lateral apex 190x magnification.

Figure 3 Scanning Electrone Microscopy (SEM) of the corpus of tongue of*Paradoxurus hermaphroditus* . (A) Distribution of papillae on the corpus of the tongue. giant arrowhead filiform papillae (gaf); scale like filiform papilla (sc); fungiform papillae (fu); foliate papilla (f) (B) giant arrowhead filiform papilla (gaf) on the posterior lateral of the corpus, 30x magnification. (C) giant arrowhead filiform papilla (gaf) and fungiform papilla (fu) on the lateral posterior corpus, 190x magnification. (D) foliate papilla (f) and scale like filiform papilla (sc) on medial posterior corpus, 190x magnification. (E) scale like filiform papilla (sc) on medial posterior corpus, 190x magnification.

Figure 4. Scanning Electron Microscopy (SEM) image of the radix region of tongue of *Paradoxurus hermaphroditus* . (A) Distribution of papillae on the radix of the tongue. Conical papillae (cp); circumvallate papillae (v); fungiform papillae (fu); blunt conical papillae (bc). (B) fungiform papillae (fu) and taste buds (tb) on the anterior radix, 100x magnification. (C) fungiform papillae (fu) and blunt conical papillae (bc) on the anterior radix, 50x magnification. (D) circumvallate papillae (v); wall (w); groove (g) on the anterior radix, 80x magnification. (E) conical papilla (cp) on the posterior radix, 50x magnification.

Figure 5. Histology of the apex of tongue of *Paradoxurus hermaphroditus* using the hematoxylin-eosin (HE) staining technique. (A) Apex of the tongue which is divided into lamina epithelial mucosa (lem), lamina propria mucosa (lpm), and tunica muscularis striatus syncytialis (tmss); distribution of fungiform papillae (fu) and arrowheads filiform papillae (ah) at the apex, 4x magnification. (B) Structure of fungiform papillae and arrowheads filiform papillae, 10x magnification.

Figure 6. Histology of the corpus of the tongue of *Paradoxurus hermaphroditus* with hematoxylin-eosin (HE) staining technique (A). The corpus area is divided into 3 layers; lamina epithelial mucosa (lem), lamina propria mucosa (lpm), and tunica muscularis striatus syncytialis (tmss); fungiform papillae (fu) and scale-like filiform papillae (sc), 4x magnification. (B) Giant arrowhead filiform papilla (gaf) with obtuse triangular cross section, 10x magnification. (C) Fungiform papilla (fu) with taste buds (arrow sign) observed, 10x magnification.

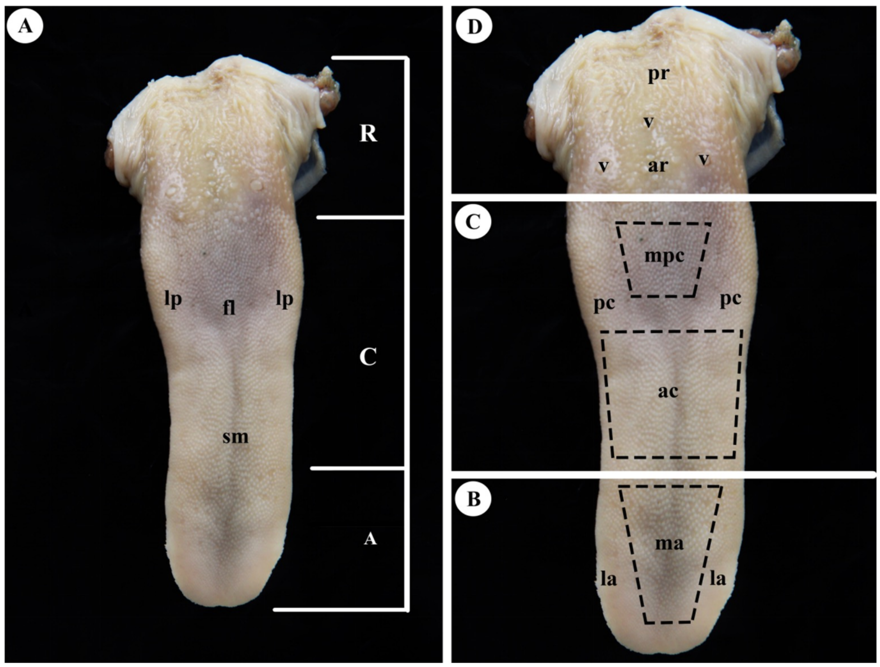
Figure 7. Histology of the radix of tongue of *Paradoxurus hermaphroditus* using the hematoxylin-eosin (HE) staining technique. (A) The radix is divided into three layers; lamina epithelial mucosa (lem), lamina propria mucosa (lpm), tunica muscularis striatus syncytialis (tmss) and circumvallate papillae (cv) with invagination of the surrounding epithelium, 4x magnification. (B) Taste buds (tb) that appear to be scattered on the surface of the circumvallate papillae were observed with 10x magnification. (C) Conical papilla (cp) which is oval in shape with thick keratinization, Weber's glands (wg) are seen which are spread evenly on the underside of the papilla in the radix area. (D) Fungiform papillae (fu) with thin keratinization observed at 10x magnification showed taste buds (arrow sign) spreading over the papillary surface area.

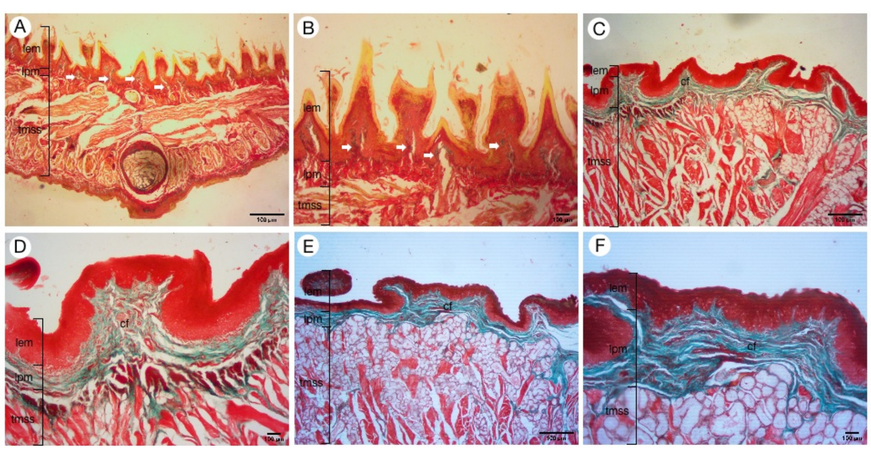
Figure 8. Histology of the tongue of *Paradoxurus hermaphroditus*with Alcian Blue (AB) staining. (A) At the apex of the tongue there is no tissue that reacted positively to AB staining at 4x magnification. The tongue is divided into three layers; lamina epithelial mucosa (lem), lamina propria mucosa (lpm), and tunica muscularis striatus syncytialis (tmss). (B) Apex of tongue with AB staining at 10x magnification. (C) At the corpus of the tongue there is no tissue that reacted positively to AB staining at 4x magnification. (D) Corpus of tongue with AB staining at 10x magnification. (E) In the radix area of the tongue, it was observed that Weber's glands (wg) reacted positively to AB, which was indicated by a dark blue color in the tissue. (F) 10x magnification on Weber's glands (wg) that react positively to AB staining.

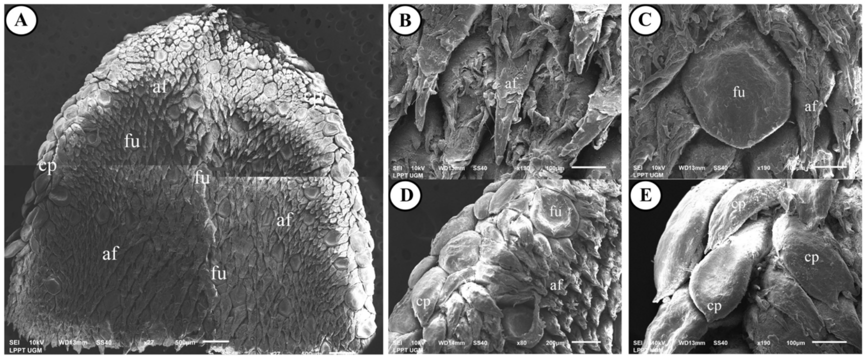
Figure 9. Histology of the tongue of *Paradoxurus hermaphroditus*with Periodic Acid Schiff (PAS) staining. (A) At the apex of the tongue there is no tissue that reacted positively to PAS staining at 4x magnification. The tongue is divided into three layers; lamina epithelial mucosa (lem), lamina propria mucosa (lpm), and tunica muscularis striatus syncytialis (tmss). (B) Apex of the tongue with PAS staining at 10x magnification. (C) At the corpus of the tongue there is no tissue that reacted positively to PAS staining at 4x magnification.

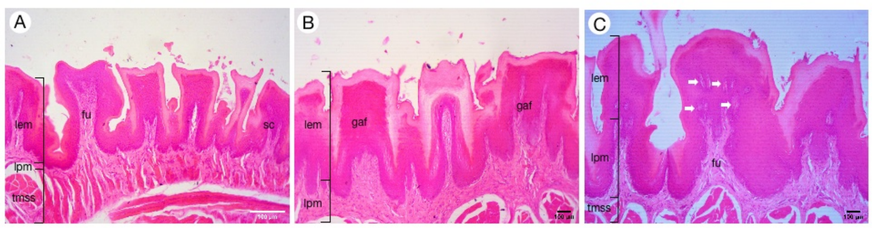
(D) Corpus of the tongue with PAS staining at 10x magnification. (E) In the radix area of the tongue, it was observed that Weber's glands (wg) reacted positively to AB indicated by a magenta red color in the tissue. (F) 10x magnification on Weber's glands (wg) that reacted positively to PAS staining.

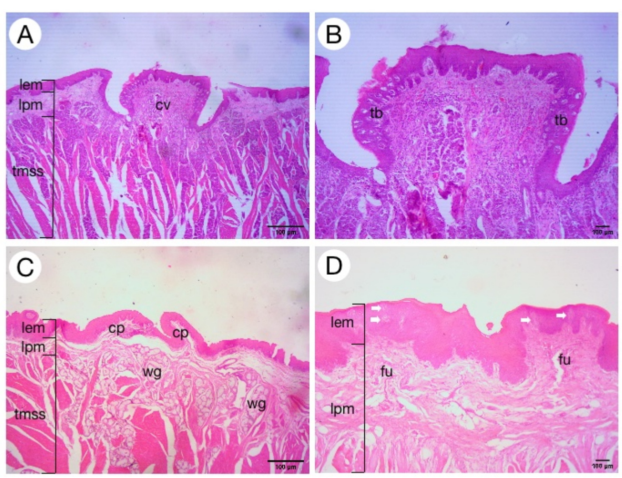
Figure 10. Histology of the tongue of *Paradoxurus hermaphroditus* with Masson's Trichrome staining. (A) At the apex of the tongue, there are few collagen fibers (arrow sign) that are stained bluish in the lamina propria mucosa (lpm). (B) Collagen fibers (arrow sign) in the lamina propria mucosa of the apex were observed at 10x magnification. (C) Collagen fibers (cf) are clearly visible in the lamina propria mucosa of the corpus of the tongue and part of the tunica muscularis striatus syncytialis (tmss). (D) Collagen fibers (cf) in the area of the corpus of the tongue are observed at 10x magnification. (E) Collagen fibers (cf) in the radix area are clearly visible in the lamina propria and part of the tunica muscularis. (F) Collagen fibers (cf) in the lamina propria of radix were observed with magnification of 10x.

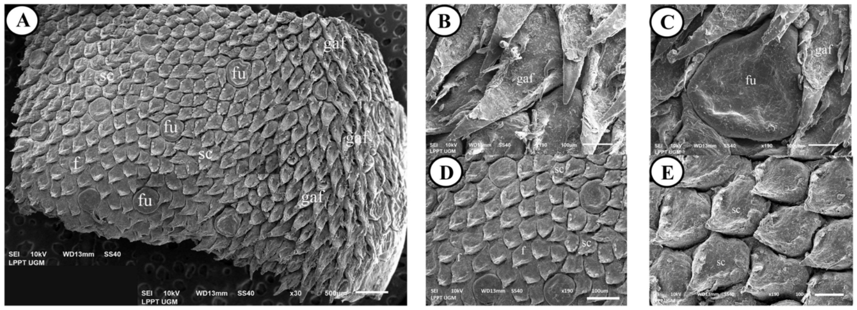


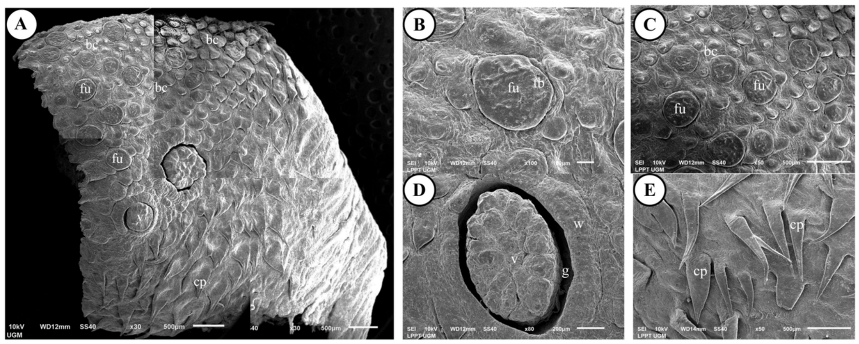


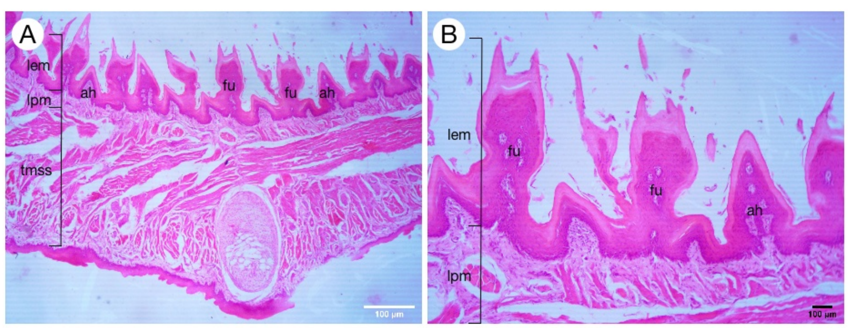

A
af
fu
cp
fu
af
af
fu
B
af
C
fu
af
D
fu
af
cp
E
cp
cp
cp

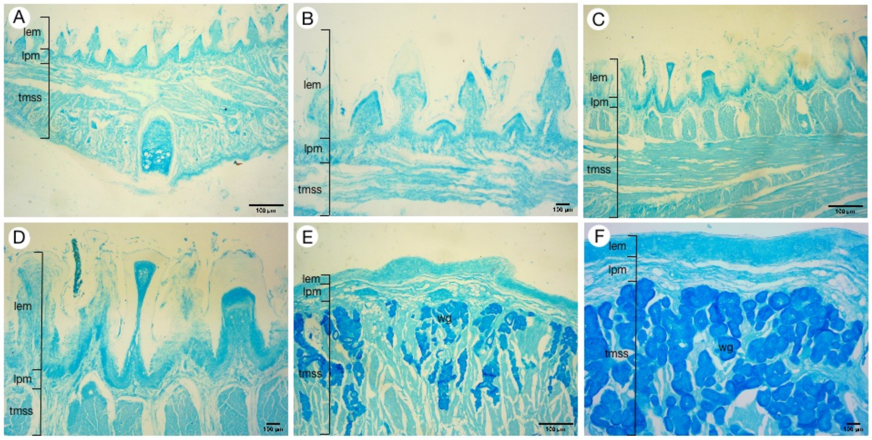

A
lem
fu
sc
lpm
tmss
B
lem
gaf
gaf
lpm
C
lem
lpm
fu
tmss

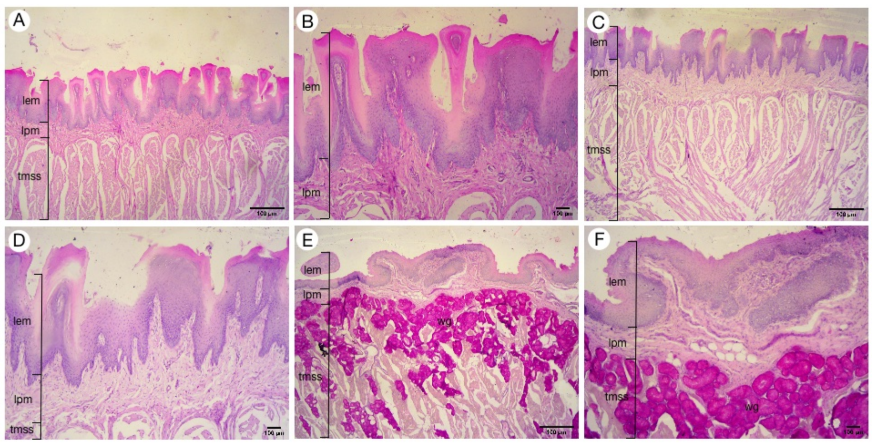

A
lem
lpm
cv
tmss
B
tb
tb
C
lem
cp
cp
lpm
wg
wg
tmss
D
lem
fu
fu
lpm


A
gaf
sc
fu
fu
sc
f
fu
gaf
SEI 10kV WD13mm SS40
LPPT UGM
x30 500μm
B
gaf
C
fu
gaf
D
sc
f
sc
E
sc


A
bc
bc
fu
bc
fu
v
cp
10kV WD12mm SS40
UGM
x30 500μm
B
fu
fb
C
bc
fu
fu
D
w
v
g
E
cp
cp


A
lem
lpm
ah
fu
fu
ah
tmss
100 μm
B
lem
fu
fu
ah
lpm
100 μm


A
lem
lpm
tmss
B
lem
lpm
tmss
C
lem
lpm
tmss
D
lem
lpm
tmss
E
lem
lpm
wg
tmss
F
lem
lpm
wg
tmss


A
lem
lpm
tmss
B
lem
lpm
C
lem
lpm
tmss
D
lem
lpm
tmss
E
lem
lpm
wg
tmss
F
lem
lpm
tmss
wg