BIODIVERSITAS
Volume 24, Number 6, June 2023
Pages: 3512-3518

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240649

The tongue morphology of *Pteropus vampyrus* from Timor Island, Indonesia: New insights from scanning electron and light microscopic studies

YULFIA NELYMALIK SELAN[1], HEVI WIHADMADYATAMI[2], ARIS HARYANTO[3], DWI LILIEK KUSINDARTA[2,♥]

[1]Department of Anatomy, Physiology, Pharmacology, and Biochemistry, Universitas Nusa Cendana. Jl. Adisucipto Penfui, Kupang 85001, East Nusa Tenggara, Indonesia
[2]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
[3]Department of Biochemistry, Faculty of Veterinary Medicine, Universitas Gadjah Mada. Jl. Fauna 2, Sleman 55281,Yogyakarta, Indonesia

Manuscript received: 24 February 2023. Revision accepted: 26 June 2023.

Abstract. *Selan YN, Wihadmadyatami H, Haryanto A, Kusindarta DL. 2023. The tongue morphology of* Pteropus vampyrus *from Timor Island, Indonesia: New insights from scanning electron and light microscopic studies. Biodiversitas 24: 3512-3518.* The large flying fox (*Pteropus vampyrus* Linnaeus, 1758) is a Southeast Asian megabat species includes with frugivorous bats. The tongue plays a pivotal role in taking, chewing, and swallowing food. The structure of the bat tongue hampers considerable variation, mainly in the papilla. Variations occur owing to the feeding habits, environment, and adaptation of bats to their environments. The aim of this study was to clarify the morphological structure of the tongue of *P. vampyrus* obtained from the island of Timor, East Nusa Tenggara, Indonesia, by using Scanning Electron Microscope (SEM) and Light Microscopy (LM). This study included six adult bats regardless of sex. Macroscopically, the tongue of *P. vampyrus* consists of three parts: the apex, corpus, and radix. SEM and LM confirmed that the apex presents filiform papillae of several subtypes, including scale-like filiform, giant trifid, and small crown-like papillae. In addition, the apex features fungiform and transitional papillae between the giant trifid and small crown-like papillae. Furthermore, the corpus consists of filiform papillae (leaf-like filiform and large crown-like papillae) and fungiform papillae. The radix consists of filiform papillae (long conical, leaf-like filiform, and short conical papillae), fungiform papillae, and three V-shaped circumvallate papillae pointing to the larynx.

Keywords: Light microscopy, lingual papilla, *Pteropus vampyrus*, Scanning Electron Microscopy, tongue

INTRODUCTION

The large flying fox (*Pteropus vampyrus* Linnaeus, 1758) is a megabat native to tropical and subtropical forests in Asia, including India, China, Indonesia, Malaysia, and the Philippines. *Pteropus vampyrus* can grow up to 3 feet in length and have wingspans reaching up to 6 feet. One unique feature of the large flying fox is its striking appearance. Its fur ranges from dark brown to reddish brown in color and has a slight golden sheen under certain lighting conditions. The bat also has distinctive facial features, including a long snout and large eyes. Large flying foxes are social animals that typically shelter in colonies ranging from a few individuals to more than 10,000 bats (Hengjan et al. 2017). *Pteropus vampyrus* is frugivorous and feed primarily on fruits such as bananas, figs, mangoes, and guavas. They have a sharp sense and echolocation system, which helps them locate ripe fruit from great distances. Apart from fruits, some flying fox species also consume nectar and pollen from flowering trees, which they collect by hovering over flowers with their wings flapping rapidly (Aboelnour et al. 2020; Pulscher et al. 2021).

Studies have suggested that large flying foxes adapt to their environment by consuming leaves when food sources are scarce. However, it is uncommon for them to eat leaves regularly because leaves have low nutritional value compared with fruits. While they do not eat insects or other animals directly, researchers have discovered that a small amount of their diet consists of insects because they often consume whole fruits, which may contain insects inside them. The large flying fox has developed an efficient way of finding food within its habitat through adaptations such as specialized diets and heightened senses (Pulscher et al. 2021). Large flying foxes are magnificent creatures that play an important role in the ecosystem. Their unique adaptations and diet help maintain balance in nature. However, their populations are declining rapidly because of habitat loss and hunting.

The tongue plays a pivotal role in taking, chewing, and swallowing food, the initial process of digestion, which also includes water absorption, voice modulation, and breastfeeding (Abumandour 2014; Williams 2019; König and Liebich 2020). The tongue morphology can vary according to diet, habitat, taxonomy, and animal behavior (Iwasaki et al. 2019; Damia et al. 2021). Differences in tongue morphology among various animals are related to the type of food and adaptation to various environments (Gunawan et al. 2019). In animals, the structure of the tongue papilla is an essential factor in processing food in the oral cavity (Abumandour and El-Bakary 2013; Igbokwe et al. 2021).

Several studies have used SEM to examine the tongues of bats, including large flying fox (*Pteropus vampyrus*) from Japan (Emura et al. 2002), *Myotis macrodactylus* Temminck, 1840 (Hwang and Lee 2007), *Pipistrellus savii* Bonaparte, 1837 (Park and Lee 2009), *Rousettus aegyptiacus* E.Geoffroy, 1810 (Jackowiak et al. 2009; Abumandour and El-Bakary 2013; Massoud and Abumandour 2020), *Rousettus amplexicaudatus* E.Geoffroy, 1810 (Gunawan et al. 2019), *Pipistrellus javanicus* Gray, 1838 (Saragih et al. 2020), and *Pipistrellus kuhlii* Kuhl, 1817 (Massoud and Abumandour 2020). Research results showed papillary variations among these species. In frugivorous bats such as *P. vampyrus*, *R. aegyptiacus*, and *R. amplexicaudatus*, the type of papilla, especially sensory papillae such as filiform and fungiform papillae, greatly varies. By contrast, in *M. macrodactylus*, *P. javanicus*, and *P. kuhlii*, the variation of filiform papillae is less and more dominated by mechanical papillae. Until now, no specific study has discussed in detail the morphological structure of the tongue papilla in *P. vampyrus* from Timor Island, Indonesia. Therefore, this study focused on determining the macroscopic and microscopic structures of the lingual papillae of *P. vampyrus* from Timor Island using SEM and LM.

MATERIALS AND METHODS

Animal samples

Six fruit bats (*P. vampyrus*) from Oeekam Village, Noebeba District, Soe, and East Nusa Tenggara, Indonesia, were examined. The bats were caught at night using nets and transported in cloth bags to the laboratory. They were sexually mature but not pregnant or lactating. The bats' morphological characteristics were identified at the Animal Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Ethical clearance

The Ethics Commission of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia, approved the study (approval number: `3/EC-FKH/Int./2021).

Conservation status

The *P. vampyrus* used in this study is listed in the International Union for Conservation of Nature in the Nearly Threatened category.

Gross macroscopic analysis

Six samples of *P. vampyrus* were used for macroscopic analysis. All samples were anesthetized with combination of high dose combination of ketamine (10 mg/kg of body weight [b.w.]; Kepro, Maagdenburgstraat, Holland) and xylazine (2 mg/kg of [b.w.]; Kepro), during anesthetized condition the intracardial perfusion were run. After the perfusion, the animals then were laid down in the dorsal position, and then the oral cavity was opened to separate the maxillary and mandibular parts. The basal part of the tongue was separated from the mandible, the hyoid bone was cut, the tongue was removed, and the caudal part of the larynx was cut. The tongues were washed with 0.9% NaCl (Nacalai Tesque, Kyoto, Japan) for five repetitions for 5 minutes each. Each tongue was macroscopically observed using EOS 7000D camera (Canon, Tokyo, Japan). The tongues were divided into two groups for SEM and LM examinations (hematoxylin-eosin staining).

Scanning electron microscopy

Six tongues of *P. vampyrus* were used in this study. The tongues were fixed with 0.5% glutaraldehyde (Chem Cruz, Santa Cruz Biotechnology, Dallas, Texas, USA), 1.5% formaldehyde (Nacalai Tesque), and 100 g HEPES (Chem Cruz, Santa Cruz Biotechnology) in 100 mL phosphate-buffered saline. Furthermore, the tongues were dehydrated using graded ethanol. The samples were then vacuum dried (Buehler 1000 Vacuum System, Stuttgart, Germany), coated with platinum in sputter coating (Jeol JEC-3000FC, Tokyo, Japan), and observed under an electron microscope (Jeol-JSM6510LA, Tokyo, Japan) at 15 kV.

Hematoxylin-eosin staining

Six tongues of *P. vampyrus* were fixed with 10% formaldehyde (Nacalai Tesque) in phosphate-buffered saline, dehydrated with graded ethanol (Interchemie, Metaalweg, Holland), purified with xylol (Interchemie), and embedded in liquid paraffin (Leica, Weztlar, Germany). The paraffin blocks were cut into 5-µm-thick sections and stained with hematoxylin and eosin (Leica). The samples were observed on LM (Olympus, Tokyo, Japan). The images were then documented with the Optilab software (Optilab, Yogyakarta, Indonesia), and ×40 magnification was applied for all the samples.

RESULTS AND DISCUSSION

Gross macroscopy of the tongue

On macroscopic examination, the tongue of *P. vampyrus* was elongated, flat, and round on the base adjacent to the larynx. The tongue can move freely in the oral cavity. A lingual frenulum on the ventral surface joined the tongue to the mandible. The tongue consisted of three parts: the apex, corpus, and radix (Figure 1A). From the six samples of the gross macroscopy measurement of the length of the tongue is almost uniform with the average total length are 55 mm, including the apex (400 mm), corpus (100 mm), and radix (50 mm). The apex was longer than the corpus and radix, occupying half of the tongue. The apex consisted of the anterior, medial, and lateral parts (Figure 1B). The corpus consisted of the medial and lateral parts and had lingual prominence (Figure 1C). The radix consisted of medial and lateral parts and had three circumvallate papillae near the larynx (Figure 1D).

Scanning electron microscopy

Apex

At the apex, several types of filiform papillae, including scale-like, giant trifid, and small crown-like papillae, were observed. The scale-like papillae occupied the anterior apex (Figure 2A). These papillae had 4 or 5 anterior

processes (Figure 2B). The lateral apex was occupied by a small crown-like papillae (Figure 2C) until they were adjacent to the lateral corpus. The small crown-like papillae had 10-12 keratinized anterior and lateral processes (Figure 2D, E). The transitional papillae were located between the small crown-like and giant trifid filiform papillae (Figure 2C). Fungiform papillae were scattered among the small crown-like papillae (Figure 2D). The median apex was covered by a large giant trifid filiform papilla (Figure 2C), which had three posterior processes (Figure 2F).

Corpus

The corpus of the tongue had two main parts: the medial and lateral areas. The medial area had large crown-like and fungiform papillae (Figure 3A). The large crown-like papillae had 10-12 processes on both the anterior and lateral sides (Figure 3B, C). Both lateral sides of the corpus were occupied by leaf-like papillae, where the fungiform papillae were located (Figure 3D).

Radix

The radix of the tongue was divided into lateral, medial, and posterior areas. The medial area had large crown-like and short conical papillae (Figure 4A, C, F). The lateral area had long conical and rosette-shaped filiform papillae (Figure 4D). In the posterior part of the radix, three circumvallate papillae formed a "V" shape bordering the larynx. Each circumvallate papilla had a groove and wall (Figure 4B, E).

In addition, based on the data of the scanning electron microscope on the apex, radix, and corpus, we describe in detail the distribution as well the size of each type of the lingual papillae (Table 1).

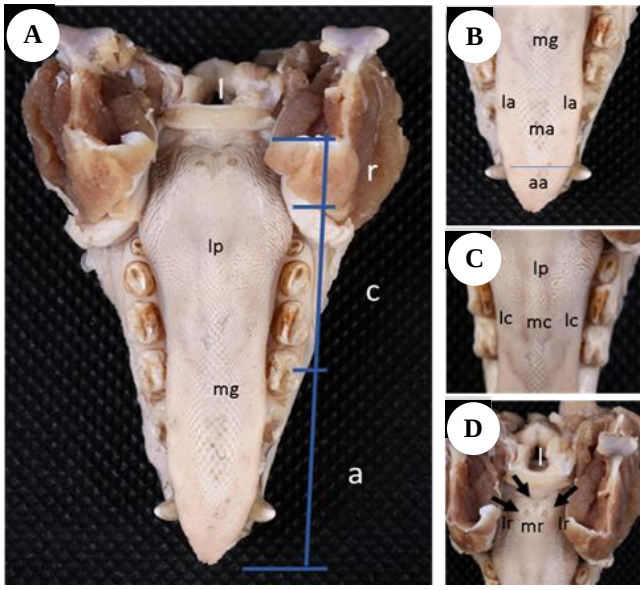

Figure 1. Gross macroscopy of the tongue of *Pteropus vampyrus*. A. Macroscopic depiction of the tongue: the apex (a), corpus (c), radix (r), larynx (l), median groove (mg), and lingual prominence (lp), B. Macroscopic dorsal view of the apex of the tongue: anterior apex (aa), lateral apex (la), medial apex (ma), and median groove (mg), C. Macroscopic dorsal view of the corpus of the tongue: lateral corpus (lc), medial corpus (mc), and lingual prominence (lp), D. Macroscopic dorsal view of the corpus of the tongue: lateral radix (lr), medial radix (mr), larynx (l), and papilla circumvallate (black arrow)

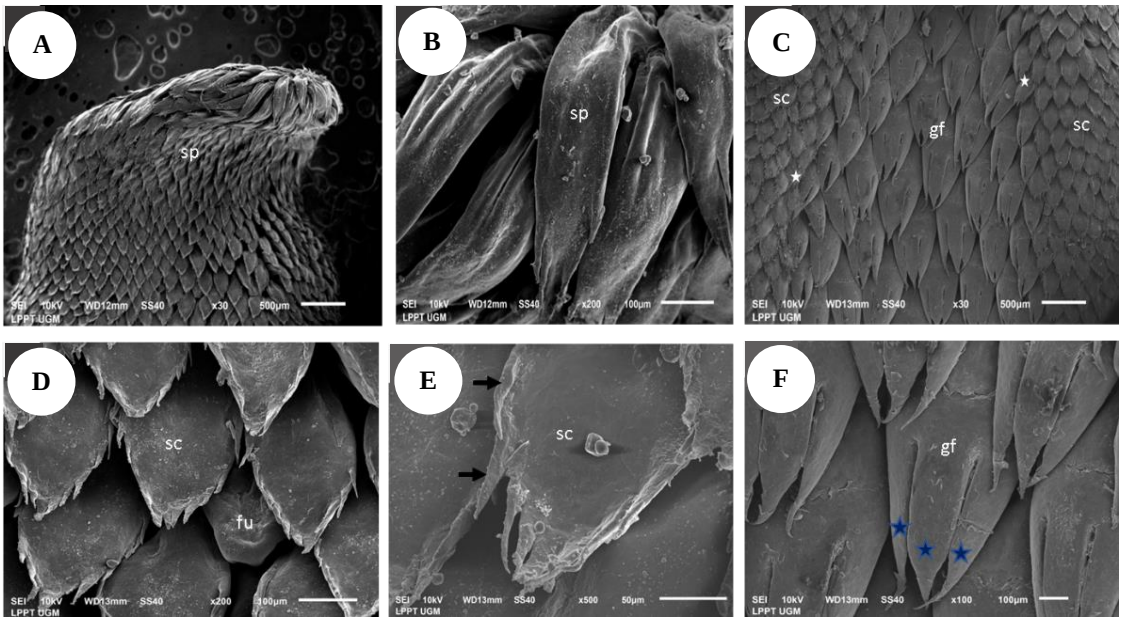

Figure 2. Scanning Electron Microscopy (SEM) images of the apex region of the tongue of *Pteropus vampyrus*. A. Anterior apex and scale-like papilla (sp), B. Scale-like papilla (sp) on the anterior apex at high magnification (×200), C. Posterior apex, giant trifid filiform papilla (gf), small crown-like papilla (sc), and transitional papilla (white star), D. Small crown-like papilla (SC) on the lateral apex at high magnification (×200) and fungiform papilla (fu), E. Small crown-like papilla (sc) with anterior processes (black arrow), F. Giant trifid filiform papilla (gf) with posterior processes (blue star)

Light microscopy (Hematoxylin-eosin staining)

Apex

The histological examination revealed that the apex consisted of filiform and fungiform papillae. The three subtypes of filiform papilla, namely, scale-like, giant trifid, and small crown-like papillae, were composed of a keratinized squamous complex epithelium (Figure 5A-E). Fungiform papillae were present among the filiform papillae, and each fungiform papilla contained 1-2 taste buds (Figure 5C, D).

Corpus

The histological structure of the corpus was the same as that of the apex. The filiform papillae were composed of keratinized squamous complex epithelia. The corpus had two subtypes of filiform papillae: large crown-like and leaf-like papillae (Figure 6A-C). Large crown-like papillae were found on the medial side of the corpus, and leaf-like papillae were found on the lateral side.

Radix

The histological examination revealed that the radix consisted of conical and circumvallate papillae (Figure 7). Conical papillae were found on the lateral side of the radix (Figure 7A). The lingual glands were found in the muscular layer (Figure 7B). On the lateral side of the radix, circular taste buds were found (Figure 7B). Circumvallate papillae were spherical and surrounded by walls with deep grooves (Figure 7C). There were 20-22 taste buds observed on the circumvallate papillae and their walls (Figure 7D).

Discussion

In bats, adaptations can be in the form of the morphology and structure of the tongue papillae, which is related to the diet of bats, including insectivores, frugivores, nectavores, and carnivores. This study used the tongue of *P. vampyrus* from the island of Timor, East Nusa Tenggara, Indonesia, to examine the structure and morphology of its tongue papillary adaptation according to ecological conditions. Our macroscopic observation revealed that the tongue of *P. vampyrus* consists of three parts: the apex, corpus, and radix. SEM and LM confirmed that the apex presents filiform papillae of several subtypes such as the scale-like filiform, giant trifid, and small crown-like papillae. In addition, on the apex, fungiform and transitional papillae were found between the giant trifid and small crown-like papillae. Furthermore, the corpus consists of filiform papillae (leaf-like filiform and large crown-like papillae) and fungiform papillae. The radix consists of filiform papillae (long conical, leaf-like filiform, and short conical papillae), fungiform papillae, and three V-shaped circumvallate papillae pointing to the larynx.

Table 1. Distribution and sizes of the lingual papillae on the tongue *Pteropus vampyrus* (+: found; -: absent)

Type of papillae	Apex	Corpus	Radix	Length (µm)	Wide (µm)
Scale-like papilla	+	-	-	493±52	122±27
Small crown-like papilla	+	-	-	339±52	191±17
Giant trifid papilla	+	-	-	1.026±88	442±34
Large crown-like papilla	-	+	-	412±46	201±28
Fungiform papilla	+	+	-	202±50	136±40
Leaf-like papilla	-	+	-	278±30	242±12
Long conical papilla	-	-	+	1.358±107	274±35
Rosette shape filiform papillae	-	-	+	156±23	146±19
Short conical papilla	-	-	+	646±111	259±60
Circumvallate papilla	-	-	+	316±60	457±24

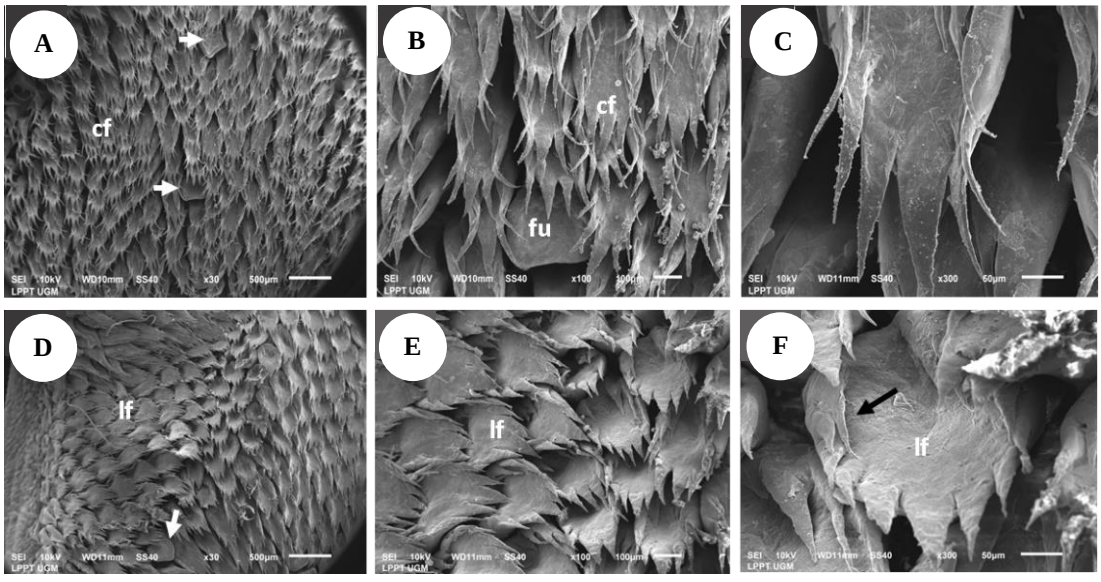

Figure 3. Scanning Electron Microscopy (SEM) images of the corpus region of the tongue of *Pteropus vampyrus*. A. The central area of the corpus, large crown-like papilla (cf), and fungiform papilla (white arrow), B. Large crown-like papilla (cf) and fungiform papilla (fu) at high magnification (×100), C. Large crown-like papilla at high magnification (×300), D. Lateral corpus area, leaf-like filiform papilla (lf), and fungiform papilla (white arrow), E. Leaf-like papilla (lf) at high magnification (×100), F. Leaf-like papilla with anterior processes (black arrow) at high magnification (×300)

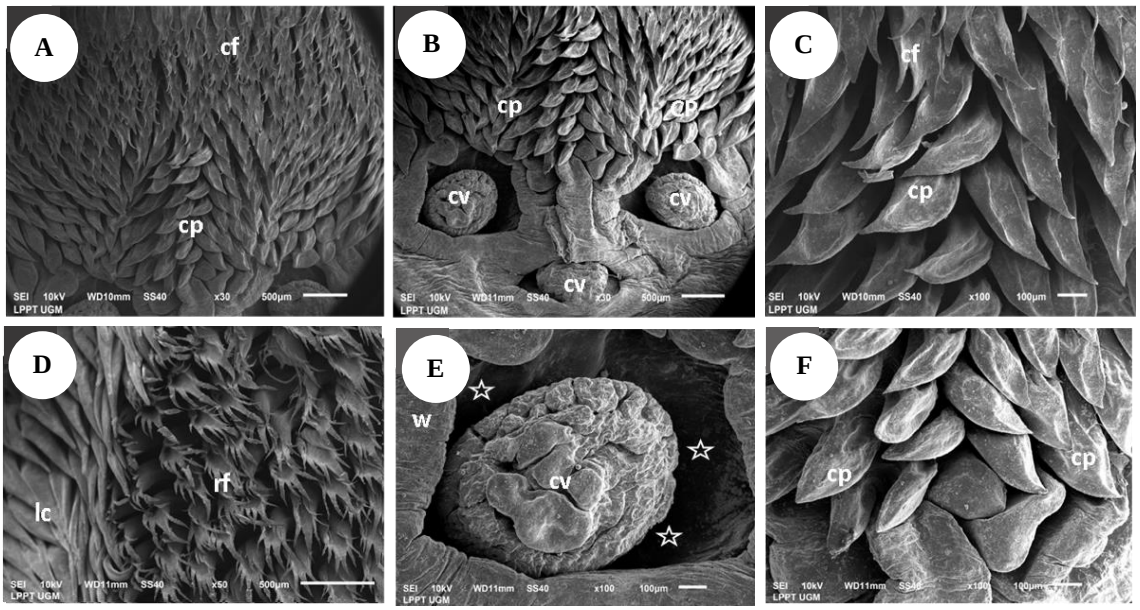

Figure 4. Scanning Electron Microscopy (SEM) images of the radix region of the tongue of *Pteropus vampyrus*. A. Large crown-like papilla (cf) and short conical papilla (cp), B. Short conical papilla (cp) and circumvallata papilla (cv), C. Large crown-like papilla (cf) and short conical papilla (cp) at high magnification (×100) on the medial side, D. Long conical papilla (lc) and rosette-shaped filiform papillae (rf) on the lateral side, E. Circumvallata papilla (cv) with grooves (*) and outer walls (w), F. High-magnification image of the short conical papilla (cp)

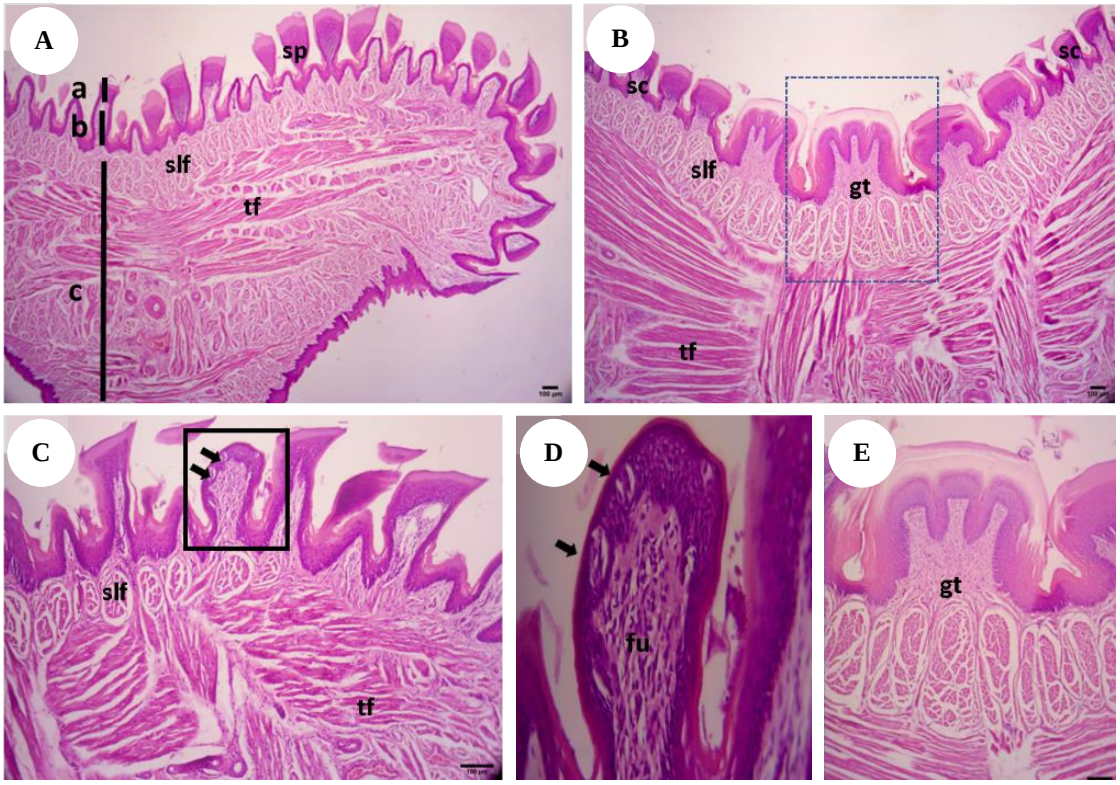

Figure 5. Photomicrographs of the apex of the *Pteropus vampyrus* tongue with hematoxylin-eosin staining. A. The apex is histologically divided into three layers: the lingual mucosa (a), lingual aponeurosis (b), and lingual muscle (c). In addition, the lingual mucosa of the anterior apex shows a scale-like papilla (sp). Meanwhile, the muscular layer consists of superficial longitudinal fibers (slf) and transverse fibers (tf), B. At the median region of the apex, giant trifid papilla (gt) and small crown-like papilla (sc) were found, C. High magnification of the fungiform papilla with taste buds (black arrow), superficial longitudinal fibers (slf), and transverse fibers (tf), D. Taste buds can be observed with high magnification of the fungiform papilla (black arrow), E. High magnification of the giant trifid papilla on the medial region of the apex

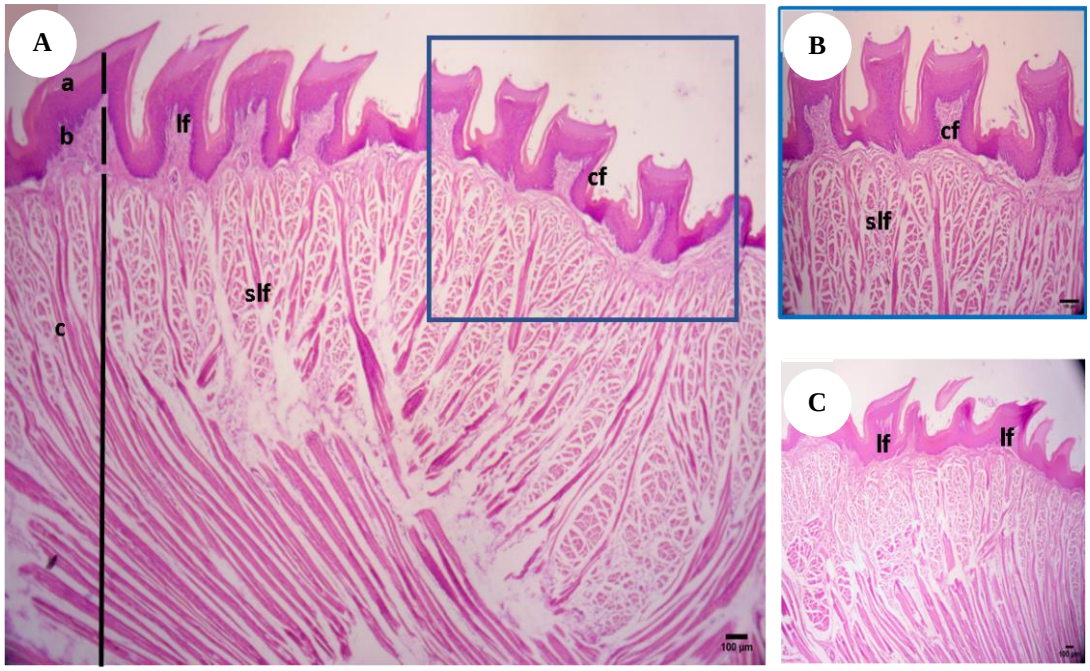

Figure 6. Photomicrographs of the corpus of the *Pteropus vampyrus* tongue with hematoxylin-eosin staining. A. Like the apex, the corpus is histologically divided into three layers: the lingual mucosa (a), lingual aponeurosis (b), and lingual muscle (c). In addition, the lingual muscle consists of superficial longitudinal fibers (slf). The lingual mucosa has a leaf-like filiform papilla (lf) and a large crown-like papilla (cf), B. High magnification of the large crown-like papilla (cf) on the median region of the corpus, C. Leaf-like papilla (lf) of the lateral corpus

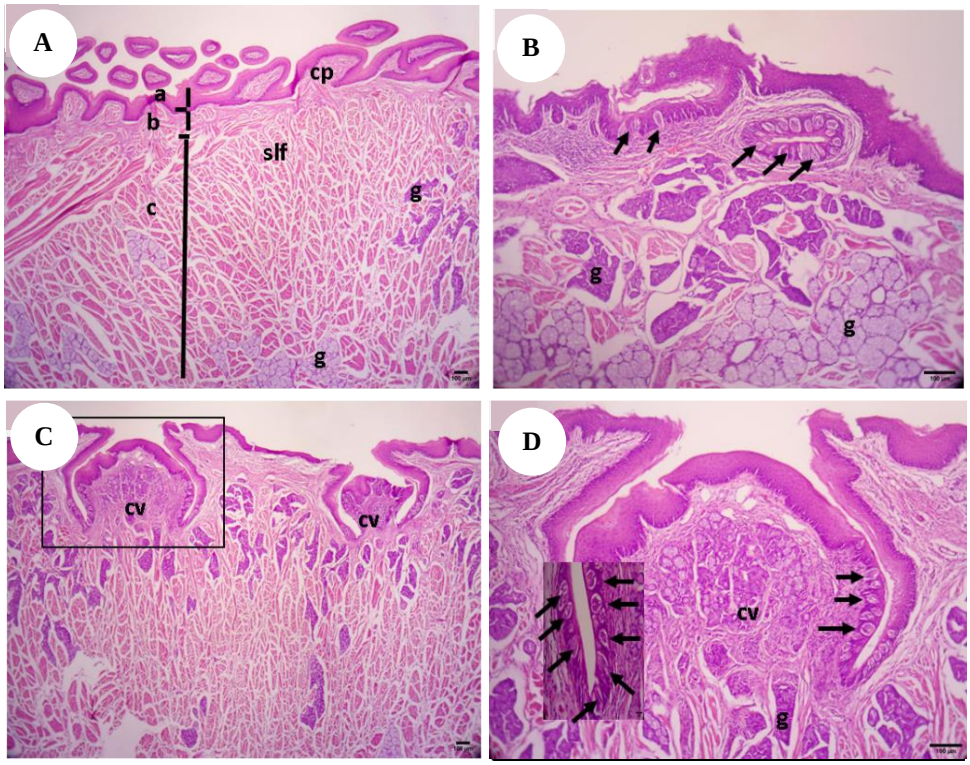

Figure 7. Photomicrographs of the radix of the tongue of *Pteropus vampyrus* with hematoxylin and eosin staining. A. The radix region histologically consists of three layers (as do the apex and corpus): the lingual mucosa (a), lingual aponeurosis (b), and lingual muscle (c). In addition, the lingual muscle consists of superficial longitudinal fibers (slf) and some glandular fibers (g). On the mucosa, conical filiform papillae (cp) are present, B. High magnification of the mucosa of the lateral radix with taste buds (black arrow) and glandular fibers (g), C. In the mucosa of the circumvallate papilla (cv), abundant taste buds can be found (black arrow), D. High magnification of the circumvallate papilla (cv), with abundant taste buds (black arrow) on its mucosa

Gross macroscopy of the *P. vampyrus* tongue showed that the tongue structure has three parts: the apex, corpus, and radix. This structure is the same with mammals in general (Al-bazii et al. 2020), fruit bats (Emura et al. 2002; Gunawan et al. 2019; Massoud and Abumandour 2020; Igbokwe et al. 2021), and some insectivorous bats (Massoud and Abumandour 2020; Saragih et al. 2020). The *P. vampyrus* tongue is long and has an anterior part that can move freely in the oral cavity. By contrast, in the posterior part, a lingual frenulum on its ventral part connects the tongue with the mandible. These results are similar to those of studies on other fruit bats (Abumandour and El-Bakary 2013; Gunawan et al. 2019). Several studies have reported no median sulcus in the tongue of some bats (Emura et al. 2001; Jackowiak et al. 2009; Abumandour and El-Bakary 2013). Previous studies (Gunawan et al. 2019; Massoud and Abumandour 2020) have also found a sulcus protruding toward the profundus at the median sulcus of the tongue, a characteristic of some mammals. The posterior center of the *P. vampyrus* tongue features a protrusion called lingual prominence. These findings are in line with those of previous studies that observed the same prominence (Gunawan et al. 2019; Kandyel et al. 2022). In some animals, protrusion is considered a characteristic of herbivores such as rodents (Wannaprasert 2017). The tongue protrusion in herbivores allows for the tongue and palate to grind and crush food in the oral cavity. Meanwhile, the prominence and shape of the tongue, which is elongated, pointed, and can be extended in fruit-eating bats (frugivorous), make it easier for bats to eat fruit and nectar (Gonzalez-Terrazas et al. 2016; Gunawan et al. 2019).

The SEM and histology results of the *P. vampyrus* tongues in this study showed a mechanical papilla (filiform papilla) and two gustatory papillae (fungiform and circumvallate papillae). The filiform papilla has eight subtypes, starting from the apex, corpus, and radix. The apex has three filiform papillary subtypes: scale-like filiform, giant trifid, and small crown-like papillae). The corpus has two filiform papillary subtypes (leaf-like and large crown-like papillae). The radix has three filiform papillary subtypes (long conical, rosette-shaped filiform, and short conical papillae). The filiform papilla subtype at the apex is in accordance with the finding of a study on *P. vampyrus* (Emura et al. 2002). However, the filiform papillary subtypes in the corpus and radix have variations. Several studies have found that the proportions of the filiform papillary subtypes vary among other fruit-eating bats as follows: *Pteropus vampyrus*, six (Emura et al. 2002); *Rousettus amplexicaudatus*, six (Gunawan et al. 2019); *Rousettus aegyptiacus*, five (Emura et al. 2012) or six (Abumandour and El-Bakary 2013); and *Eidolon helvum* (Kerr 1972), five (Igbokwe et al. 2021).

Giant trifid papillae are commonly found in fruit- and nectar-eating bats, including *C. brachyotis* (Emura et al. 2001), *R. aegyptiacus* (Abumandour and El-Bakary 2013), *R. amplexicaudatus* (Gunawan et al. 2019), and *E. helvum* (Igbokwe et al. 2021). The giant trifid papilla expands the surface of the tongue and collects nectar (Gunawan et al. 2019). In addition, it can catch and puncture the skin of ripe fruit and flowers by pressing it using both the surface of the tongue and the rough palate to get the juice (Igbokwe et al. 2021). The anterior and posterior processes of the giant trifid papilla help direct the food caught with the canines. The filiform papilla on the anterior part of the tongue compensates for the absence of the upper and lower incisors, forming a space between the canines. This space allows the tongue of fruit- and nectar-eating bats to extend and move when feeding (Abumandour and El-Bakary 2013; Mqokeli and Downs 2013).

Fungiform papillae were scattered among the filiform papillae, except for giant trifid and long conical papillae. These results align with those of previous studies by Emura et al. (2002). Fungiform papillae have many taste buds and function as taste sensors (Park and Lee 2009). The number of fungiform papillae is proportional to the food a species consumes (Hwang and Lee 2007). The presence of fungiform papillae with taste buds on the anterior part of the tongue explains the greater involvement of taste sensation for the types of food (fruit or flower) in the surrounding area, which may be related to the response to food selection (Igbokwe et al. 2021).

Three circumvallate papillae were found in the tongue of *P. vampyrus*. The finding on the circumvallate papillae is in line with some studies on fruit-eating bats (Emura et al. 2002; Jackowiak et al. 2009; Emura et al. 2012; Gunawan et al. 2019), although some insectivore bats have two circumvallate papillae, such as *Miniopterus schreibersi fuliginosus*, *P. savii* (Park and Lee 2009), *P. javanicus* (Saragih et al. 2020), and *M. macrodactylus* (Hwang and Lee 2007). The number of circumvallate papillae in various animals is related to the type of food consumed.

In addition, the histological observation revealed three tongue layers: the lingual mucosa, lingual aponeurosis, and lingual muscle. The lingual mucosa is composed of the epithelial squamous complex and papillae of the tongue (filiform, fungiform, and circumvallate). Filiform papillae were scattered throughout the (dorsal) surface of the tongue. They were keratinized and could be categorized into several types. Fungiform papillae were dome-like and scattered among the filiform papillae at the apex and corpus. Circumvallate papillae were larger than the other papillae and had an epithelial surface. Lingual aponeurosis is a layer composed of connective tissue, blood vessels, lymph nodes, and nerves. Lingual muscles were composed of transverse and longitudinal fibers. These findings are in concordance with observations for *Rousettus aegypticus* E.Geoffroy, 1810, *R. amplexicaudatus*, *P. javanicus*, and *Rhinopoma hardwickii* (Taki-El-Deen et al. 2013; Abumandour 2014; Gunawan et al. 2019; Saragih et al. 2020; Abumandour et al. 2022).

Pteropus vampyrus is a fruit- and nectar-eating bat. The presence of fungiform and three circumvallate papillae compensates for the absence of foliate papillae in this bat. The number of circumvallate papillae is related to the level of taste sensitivity, while the distribution of fungiform papillae is specialized to improve taste perception before the food is swallowed. The variations of filiform, fungiform, and circumvallate papillae in *P. vampyrus* from Timor Island reflect the adaptation of these fruit- and

nectar-eating bats to the limited and seasonal food availability in the area around the rainforest on the island of Timor.

In conclusion, this study provides detailed information about the papillae of the tongue of *P. vampyrus* from Timor Island, Indonesia, on the basis of SEM and histological observations. The shape, structure, and distribution of filiform papillae are associated with functional roles in the food-consuming process. By contrast, fungiform and circumvallate papillae are associated with taste sensation.

REFERENCES

Aboelnour A, Noreldin AE, Massoud D, Abumandour MMA. 2020. Retinal characterization in the eyes of two bats endemic in the Egyptian fauna, the Egyptian fruit bat (*Rousettus aegyptiacus*) and insectivorous bat (*Pipistrellus kuhlii*), using the light microscope and transmission electron microscope. Microsc Res Tech 83 (11): 1391-1400. DOI: 10.1002/jemt.23530.

Abumandour MMA, El-Bakary RMA. 2013. Morphological and scanning electron microscopic studies of the tongue of the Egyptian fruit bat (*Rousettus aegyptiacus*) and their lingual adaptation for its feeding habits. Vet Res Commun 37 (3): 29-38. DOI: 10.1007/s11259-013-9567-9.

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.

Abumandour MMA, Madkour N, Morsy K, Haddad S, Abourashed NM, E1-Basyouny HA, Kandyel RM. 2022. Lingual papillary system of the Egyptian mouse-tailed bat (*Rhinopoma hardwickii*): New insights to its feeding strategies using scanning electron microscope, and immunohistochemical analysis of keratin. Microsc Res Technol 85 (6): 2192-2205. DOI: 10.1002/jemt.24076.

Al-bazii SJ, Obeid AK, Hameed RM. 2020. Comparative morpho-histological studies of tongue papillae in the pre-pubertal and adult stages of Awassi sheep (*Ovis ovis*). Intl J Pharm Res 12 (4): 4218-4224. DOI: 10.31838/ijpr/2020.12.04.580.

Damia U, Anjani AK, 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.

Emura S, Hayakawa D, Chen H, Shoumura S, Atoji Y, Agungpriyono S. 2001. SEM study on the dorsal lingual surface of the lesser dog-faced fruit bat, *Cynopterus brachyotis*. Okajimas Folia Anat Jpn 78 (4): 123-128. DOI: 10.2535/ofaj1936.78.4_123.

Emura S, Hayakawa D, Chen H, Shoumura S, Atoji Y, Wijayanto H. 2002. SEM study on the dorsal lingual surface of the large flying fox, *Pteropus vampyrus*. Okajimas Folia Anat Jpn 79 (4): 113-119. DOI: 10.2535/ofaj.79.113.

Emura S, Okumura T, Chen H. 2012. Morphology of the lingual papillae in the Egyptian rousette bat (*Rousettus aegyptiacus*). Okajimas Folia Anat Jpn 89 (3): 61-66. DOI: 10.2535/ofaj.89.61.

Gonzalez-Terrazas TP, Koblitz JC, Fleming TH, Medellín RA, Kalko EKV, Schnitzler H-U, Tschapka M. 2016. How nectar-feeding bats localize their food: Echolocation behavior of *Leptonycteris yerbabuenae* approaching cactus flowers. PLoS ONE 11 (9): e0163492. DOI: 10.1371/journal.pone.0163492.

Gunawan G, Saragih GR, 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.

Hengjan Y, Pramono D, Takemae H, Kobayashi R, Iida K, Ando T, Kasmono S, Basri C, Fitriana YS, Arifin EMZ, Ohmori Y, Maeda K, Agungpriyono S, Hondo E. 2017. Daytime behavior of *Pteropus vampyrus* in a natural habitat: The driver of viral transmission. J Vet Med Sci 79 (6): 1125-1133. DOI: 10.1292/jvms.16-0643.

Hwang H-S, Lee J-H. 2007. Morphological study on the dorsal lingual papillae of *Myotis macrodactylus*. Korean J Electron Microsc 37 (3): 147-156.

Igbokwe CO, Bello UM, Mbajiorgu FE. 2021. Anatomical and surface ultrastructural investigation of the tongue in the straw-coloured fruit bat (*Eidolon helvum*, Kerr 1972). Anat Histol Embryol 50 (3): 448-458. DOI: 10.1111/ahe.12648.

Iwasaki S-I, 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, Trzcielińska-Lorych J, Godynicki S. 2009. The microstructure of lingual papillae in the Egyptian fruit bat (*Rousettus aegyptiacus*) as observed by light microscopy and scanning electron microscopy. Arch Histol Cytol 72 (1): 13-21. DOI: 10.1679/aohc.72.13.

Kandyel RM, Abumandour MMA, Mahmoud SF, Shukry M, Madkour N, El-Mansi A, Farrag FA. 2022. Structural and functional adaptation of the lingual papillae of the Egyptian fruit bat (*Rousettus aegyptiacus*): Specific adaptive feeding strategies. Folia Morphol (Warsz) 81 (2): 400-411. DOI: 10.5603/FM.a2021.0042.

König HE, Liebich HG. 2020. Veterinary Anatomy of Domestic Animals. 7th eds. Scatthauer, Germany.

Massoud D, Abumandour MMA. 2020. Anatomical features of the tongue of two chiropterans endemic in the Egyptian fauna: The Egyptian fruit bat (R*ousettus aegyptiacus*) and insectivorous bat (*Pipistrellus kuhlii*). Acta Histochem 122 (2): 151503. DOI: 10.1016/j.acthis.2020.151503.

Mqokeli BR, Downs CT. 2013. Palatal and lingual adaptations for frugivory and nectarivory in the Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*). Zoomorphology 132 (1): 111-119. DOI: 10.1007/s00435-012-0170-3.

Park JW, Lee JH. 2009. Comparative morphology of the tongue of *Miniopterus schreibersi fuliginosus* and *Pipistrellus savii*. Appl Microsc 3 9 (3): 267-276.

Pulscher LA, Dierenfeld ES, Welbergen JA, Rose KA, Phalen DN. 2021. A comparison of nutritional value of native and alien food plants for a critically endangered island flying-fox. PLoS ONE 16 (5): e0250857. DOI: 10.1371/journal.pone.0250857.

Saragih GR, 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 Hist Embryol 49 (6): 718-727. DOI: 10.1111/ahe.12566.

Taki-El-Deen FMA, Sakr SMI, Shanin MA. 2013. Comparative histological studies on the tongue of three species of Egyptian bats. Life Sci J 10 (2): 633-640.

Wannaprasert T. 2017. Morphological characteristics of the tongue and lingual papillae of the large bamboo rat (*Rhizomys sumatrensis*). Anat Sci Intl 93 (3): 323-331. DOI: 10.1007/s12565-017-0414-x.

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.