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Morphology features and microanatomy of the tongue papillae of the *Eonycteris spelaea*: Scanning electron microscopy and light microscopy

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Abstract

Background: *Eonycteris spelaea* (*E. spelaea*) is a sizable nectar-feeding bat that falls within the taxonomic classification of order Chiroptera and family Pteropododae. The form and structure of the tongue play a crucial role for bats in the intake and digestion of food in their mouth. Each papilla's morphology, dimensions, spatial arrangement, and physiological role exhibit variations among different animal species, contingent upon their respective lifestyles.

Aim: This research attempts to examine the morphology and microstructure of the *E. spelaea* tongue papillae, collected from Timor Island, East Nusa Tenggara, Indonesia.

Methods: This study aimed to achieve a scanning electron microscope and a light microscope in the presence of hematoxylin-eosin staining and employed a sample of 6 sexually indiscriminate adult *E. spelaea* bats.

Results: The tongue of *E. spelaea* is separated into three distinct parts: the apex, corpus, and radix. The structure's apex contains filiform papillae, which come in many varieties, such as scale-like filiform papilla, enormous trifid papilla, and little crown-like papilla. Additionally, there is a cluster of fungiform papillae on the outside edge of the highest point and transitional papillae connecting the large trifid papillae with the smaller crown-like papillae. The corpus section comprises two papilla types: filiform papilla (leaf-shaped filiform papilla and big crown-like papilla) and fungiform papilla. The radix comprises the elongated conical papilla, rosette-shaped filiform papilla, short conical papilla, transitional papilla, and three circumvallate papillae at the back of the tongue.

Conclusion: The tongue papillae of *E. spelaea* comprise a wide variety of mechanic papillae and also sensory papillae which have specific dietary regimens in their living habitat.

Keywords: *E. spelaea*, Light microscopy, Tongue papillae, Scanning electron microscopy.

Introduction

Eonycteris spelaea (*E. spelaea*), also known as the Dobson's Long-tongued Fruit Bat (1781), Lesser Dawn Bat, dawn bat, or Cave Fruit Bat, is a species that mostly feed on fruit and nectar (Bumrungsri *et al*., 2013; Peng *et al*., 2017; Waldien *et al*., 2020) and classified under the taxonomic order Chiroptera and the family Pteropododae (Waldien *et al*., 2020). The *E. spelaea* is found in South Asia and Southeast Asia and has a flight range of 30 km, during which it searches for food from its perch (Bumrungsri *et al*., 2013; Acharya *et al*., 2015). *E. spelaea*, as a nectar-feeding bat, regularly visits flowering trees for a minimum of four months, specifically during the pollination period (Acharya *et al*., 2015). The presence of pollen from fruit trees in the surrounding ecosystem will be crucial for the long-term sustenance of bat nectar. *E. spelaea* can be observed alone or in small clusters during foraging. Their visits to flowers to extract nectar typically endure for 2–3 seconds (Acharya *et al*., 2015). *Eonycteris spelaea* is highly proficient at pollinating mangroves, primary forests, and fruit crops such as durian and beans (Bumrungsri *et al*., 2013; Mohamed *et al*., 2016). The reproductive activity of *E. spelaea* is continuous throughout the year. Females give birth to a single offspring per year, correlated with the flowering of specific plants serving food (Bumrungsri *et al*., 2013).

The tongue is a crucial component of the oral cavity that performs vital functions in the first stages of digestion,

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such as grasping, chewing, swallowing, breastfeeding, and even modulating the voice (Saragih *et al*., 2020). Tongue shape in different species can be influenced by factors such as the type of food, habitat, or environment (Okada and Schraufnagel, 2005; Gunawan *et al*., 2019). The morphology and composition of the tongue play a role in the ingestion and digestion of food within the oral cavity of bats (Abumandour and El-Bakary 2013; Igbokwe, Bello, and Mbajiorgu 2021). Each tongue papilla's morphology, dimensions, dispersion, and physiological role vary among animal species, influenced by their lifestyles (Damia *et al*., 2021; Kusuma *et al*., 2022; Megawati *et al*., 2023; Anjani *et al*., 2023).

Several studies have utilized scanning electron microscopy (SEM) to examine the papillae of the tongue in various bat species. These include Pteropus vampyrus (Emura *et al*., 2002), Myotis macrodactylus (Hwang and Lee 2007), Pipistrellus savii (Park and Lee 2009), Rousettus aegyptiacus (Abumandour and El-Bakary 2013; Massoud and Abumandour 2020), Rousettus amplexicaudatus (Gunawan *et al*., 2019), Pipistrellus javanicus (Saragih *et al*., 2020), and Pipistrellus kuhli (Massoud and Abumandour 2020). This research attempts to analyze the tongue papillae's composition and arrangement in the fruit-eating bat *E. spelaea*'s tongue, which is native to East Nusa Tenggara, Indonesia. The study will primarily utilize light microscope and scanning electron microscope (SEM) observations to analyze the tongue papillae's distribution and structure.

Material and Methods

Specimens of animals

The specimens included in this study consisted of six *E. spelaea* bats. The bat species was extracted from Oeekam Village, East Nusa Tenggara, Indonesia. Nocturnal Bats were brought to the laboratory after being caught with nets. The trapped bats are in a state of sexual maturity and are not expecting nor producing milk. The morphological traits were determined at the Animal Systematics Laboratory in the Faculty of Biology at Universitas Gadjah Mada.

Conservation status

The *E. spelaea* is registered as least concern (LC) in the International Union for Conservation of Nature (IUCN).

Animal preparatory procedures

The six animals were rendered unconscious using a dosage of 10 mg/kg B.W. of ketamine (Kepro, Maagdenburgstraat, The Netherland) and 2 mg/kg B.W. of xylazine (Interchemie, Metaalweg, The Netherland), prior termination with lethal ketamine and xylazine, perfusion was conducted by injecting the animal with physiological NaCl (313-20-05, Nacalai Tesque, Kyoto, Japan) and 10% formaldehyde (16222-65, Nacalai Tesque, Kyoto, Japan) through the heart to maintain their preservation. Ketamine and xylazine were administered at high dosages following the perfusion. The tongue samples were acquired through the dissection of the cavum oris in order to isolate the maxillary and mandibular sections. The mandibular section is then detached from the lower jaw, the hyoid bone is incised, subsequently, the tongue is extracted, and the posterior portion of the tongue is isolated from the larynx. The tongue organ was rinsed with a 0.9% physiological sodium chloride solution five times, each lasting five minutes.

SEM

Three tongues were extracted from the fixative and washed using a 0.9% NaCl solution (313-20-05, Nacalai Tesque, Kyoto, Japan). The samples underwent dehydration using a variety of ethanol solutions (64-17-5, Merck-KgaA, Darmstadt, Germany). The tongue was put onto a metal plate. The samples underwent vacuum drying at a temperature of 25°C and a pressure of 4 Pa using the Buehler 1000 Vacuum System (Buehler 1000, Stuttgart, Germany). They were subsequently coated with platinum using sputter coating (JEC-3000FC, JEOL, Tokyo, Japan). The specimens were analyzed using a SEM (SM6510LA, JEOL, Tokyo, Japan). The SEM operated at an acceleration current of 15 kV.

Hematoxylin–Eosin staining

The samples were placed in a tissue cassette and subjected to a washing process. Subsequently, the samples underwent dehydration and cleansing utilizing Xylene (108297, Merck-KgaA, Darmstadt, Germany), followed by immersion and embedding in paraffin (39601006, Leica Biosystems, Wetzlar, Germany). Finally, they were incubated at a temperature of 58°C–60°C for a duration of 24 hours. 8-micrometer thick samples were obtained by sectioning with a rotary microtome (RV 240, Yamato Asaka, Japan) and then mounted onto slides coated with gelatine (9000-70-8, KgaA, Darmstadt, Germany). The samples were subsequently stained using a standard histological technique including the application of hematoxylin and eosin (HE05-M06002; Bio-Optica, Milan, Spain). Inspections were conducted using a light microscope (BX51, Olympus, Tokyo, Japan). Materials' photographic micrographs were captured utilizing Optilab (Optilab Observer and Viewer, Miconos, Yogyakarta, Indonesia).

Ethical approval

The capture procedure received approval from the Ethics Commission of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia (EC number: 00133/EC-FKH/Int./2021).

Results

Macroscopic analysis

Eonycteris spelaea's tongue is elongated and flat and moves freely in the oral cavity, and the base of the tongue is rounded, bordering the larynx. The tongue consists of the apex, corpus, and radix (Fig. 1A). The length of the tongue approximately reaches 6.0

± 0.2cm, the length of the apex is 4.5 ± 0.5 cm, the length of the corpus is 1cm, and the radix is 0.5 ± 0.1 cm. The apex of the tongue comprises anterior, medial, and lateral (Fig. 1B), and the tongue's corpus comprises medial and lateral with a tongue protrusion (Fig. 1C). In contrast, the root of the tongue consists of medial, lateral, and posterior, with three circumvallate papillae bordering the larynx (Fig. 1D). The ventral part contains the tongue frenulum, which unites the tongue with the mandible.

SEM

Apex

The apex of the tongue has different kinds of filiform papilla, namely scale-like papilla, giant trifid papilla, and small crown-like papilla (Table 1). The scale-like papilla is present at the anterior apex (Fig. 2A). This papilla has 4–5 anterior processes (Fig. 2B). A small crown-like papilla (Fig. 2C) is lateral to the apex and borders the lateral corpus. Small crown-like papilla has keratin on the anterior and lateral processes of 10–14 (Fig. 2D and E). The giant trifid filiform papilla and the small crown-like papilla are separated by the transient papilla (Fig. 2C). Amidst the small crown-like papillae, fungiform papillae were discernible (Fig. 2D). The median part of the apex is filled with quite large filiform papilla, namely giant trifid filiform papilla (Fig. 2E). On the lateral part of the apex, there is an accumulation of fungiform papillae (Fig. 2F).

Corpus

The tongue corpus of *E. spelaea* is divided into two primary sections: the medial and lateral segments. The central portion of the structure consists of the

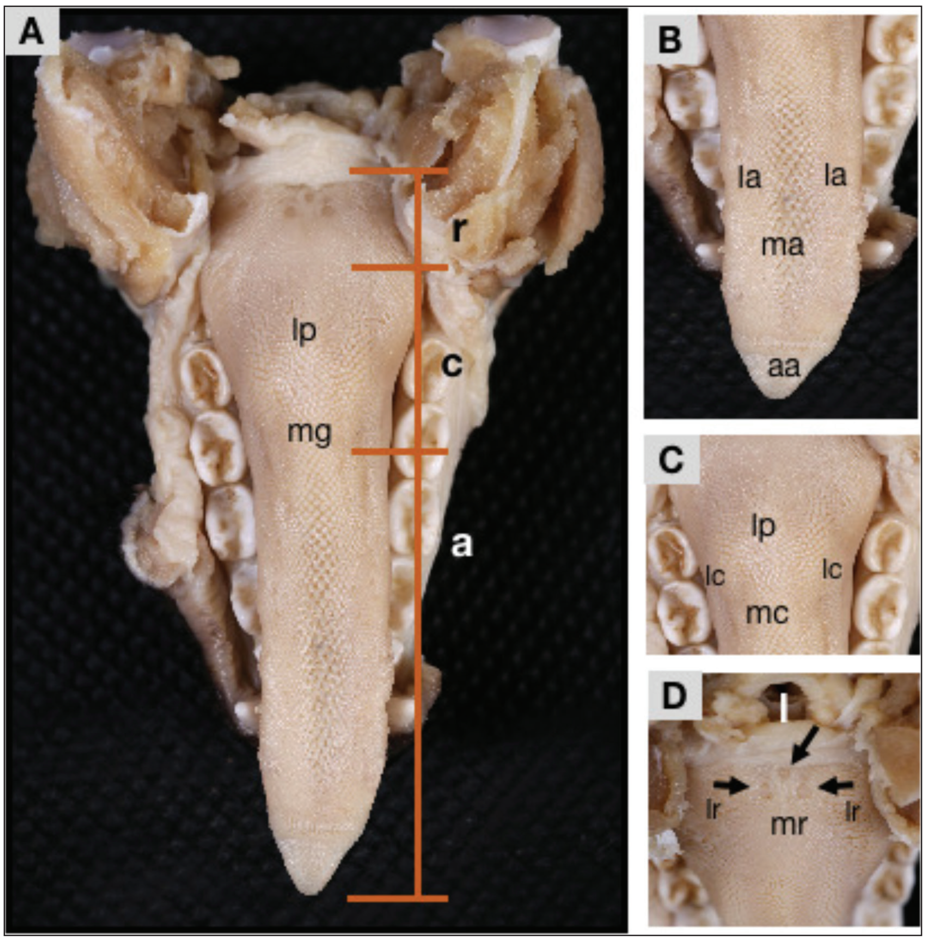

Fig. 1. Gross macroscopy of the lingual of *E. spelaea*. (A) Macroscopic depiction of the tongue of *E. spelae*; apex (a); corpus (c); radix (r); median groove (mg); lingual prominence (lp). (B) Macroscopic dorsal view of the apex of the tongue of *E. spelaea*. Anterior apex (aa); lateral apex (la); medial apex (ma). (C) Macroscopic dorsal view of the corpus of tongue of *E. spelaea*. Lateral corpus (lc); medial corpus (mc); lingual prominence (lp). (D) Macroscopic dorsal view of the corpus of the tongue of *E. spelaea*. Lateral radix (lr); medial radix (mr); larynx (l); papilla circumvallate (black arrow).

Table 1. Types of papillae and their distribution on the tongue of *E. spelaea.*

Papilla type	Apex	Corpus	Radix	Length (µm)	Width (µm)
Scale-like papilla	+	−	−	592 ± 69	308 ± 28
Small crown-like papilla	+	−	−	287 ± 20	231 ± 13
Giant trifid filiform papilla	+	−	−	1,255 ± 25	348 ± 18
Fungiform papilla	+	+	−	313 ± 69	200 ± 45
Leaf-like papilla	−	+	−	337 ± 27	173 ± 23
Long conical papilla	−	−	+	796 ± 76	346 ± 78
Rosette shape filiform papilla	−	+	+	324 ± 56	139 ± 14
Short conical papilla	−	−	+	179 ± 10	87 ± 6
circumvallate papilla	−	−	+	456 ± 118	333 ± 13

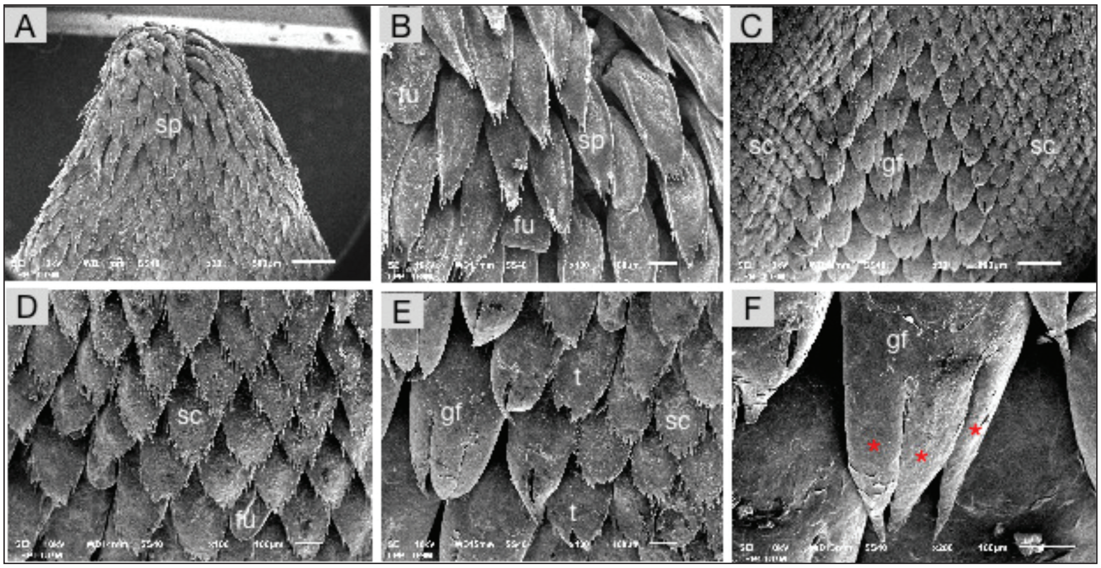

Fig. 2. SEM images of the apex region of the tongue of *E. spelaea*. (A) Anterior apex; Scale-like papilla (sp). (B) Scale-like papilla on the anterior apex at high magnification (100x). (C) Medial of apex; Giant trifid filiform papilla (gf); Small crown-like papilla (sc); transitional papilla (*) between small crown-like papilla and giant trifid filiform papilla. (D) Small crown-like papilla (sc) on the lateral apex at high magnification (100x). (E) Giant trifid filiform papilla (gf) with posterior prosessus (blue star) at high magnification (100x). (F) Small crown-like papilla (sc); Fungiform papilla (white arrow) between small crown papilla and accumulation fungiform papilla (fu) at the lateral region of the posterior apex.

leaf-shaped filiform papilla and fungiform papilla (shown by the white arrow) (Fig. 3A). The leaf-like papilla has 8–10 processes in the anterior and lateral regions (Fig. 3A and B). Large crown-like papillae are present on both lateral sides of the corpus, with fungiform papillae situated between the leaf-like papillae (Fig. 3C). Fig. 3D shows fungiform papillae on the side adjacent to the huge crown-like papillae.

Radix

Eonycteris spelaea possesses lateral, medial, and posterior regions in its radix or root area. Figure 4A and B shows the presence of a rosette-shaped filiform papilla and a short conical papilla on the medial side. Additionally, there is a transitional papilla located between the rosette-shaped filiform papilla and the short conical papilla. Horizontally, there are elongated conical papillae and filiform papillae arranged in a rosette arrangement (Fig. 4D–F). Located at the posterior section of the root are three circumvallate papillae arranged in a "V" shape, adjacent to the larynx. Figure 4B and C shows that each circumvallate papilla is accompanied by a groove and a substantial surrounding wall.

Microanatomy analysis

Apex

The histological examination of the tongue with hematoxylin-eosin staining reveals three distinct layers: the mucosa layer, the aponeurosis layer, and

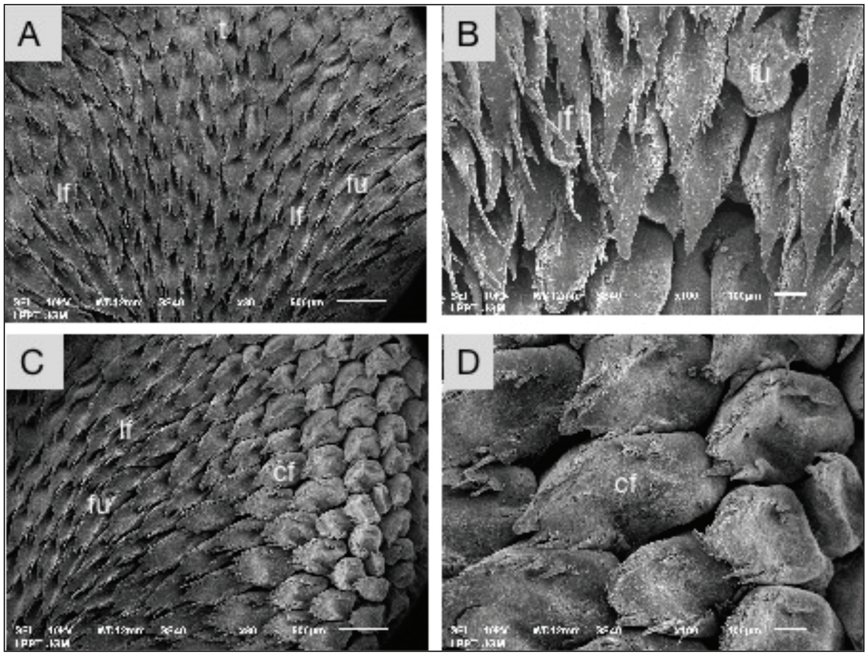

Fig. 3. SEM images of the corpus region of the tongue of *E. spelaea.* (A) Leaf-like filiform papilla (lf); Fungiform papilla (white arrow). (B) High magnification (100x) image of the Leaf-like filiform papilla (lf). (C) Large crown-like papilla (cf); Leaf-like filiform papilla (lf); Fungiform papilla (fu). (D) Large crown-like papilla (cf); accumulation of Fungiform papilla (fu).

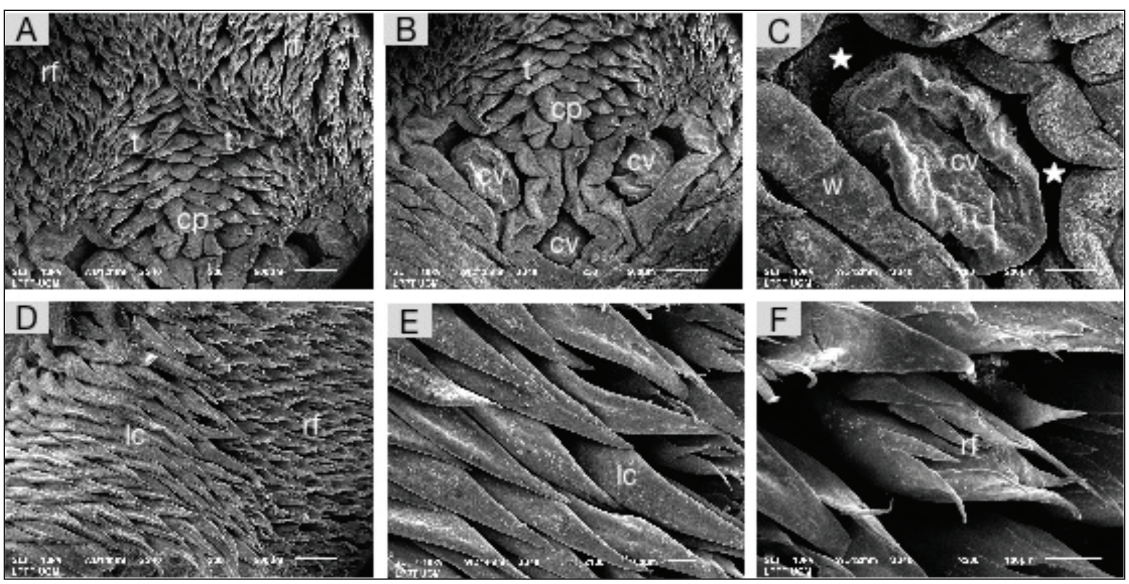

Fig. 4. SEM images of the Radix region of the tongue of *E. spelaea*. (A) on the radix, the rosette shape filiform papillae (rf), Short Conical papilla (cp), and transitional papilla (t) are distributed. (B) short conical papilla (cp); Circumvallata papilla (cv); transitional papilla (t) between rosette shape filiform papillae and short conical papilla. (C) Circumvallate papilla (cv), which has grooves (white star) and outer walls (w). (D) Long conical papilla (lc)on the lateral side; rosette shape filiform (rf). (E) High magnification image of the long conical papilla (lc). (F) High magnification image of the rosette shape filiform papillae (rf).

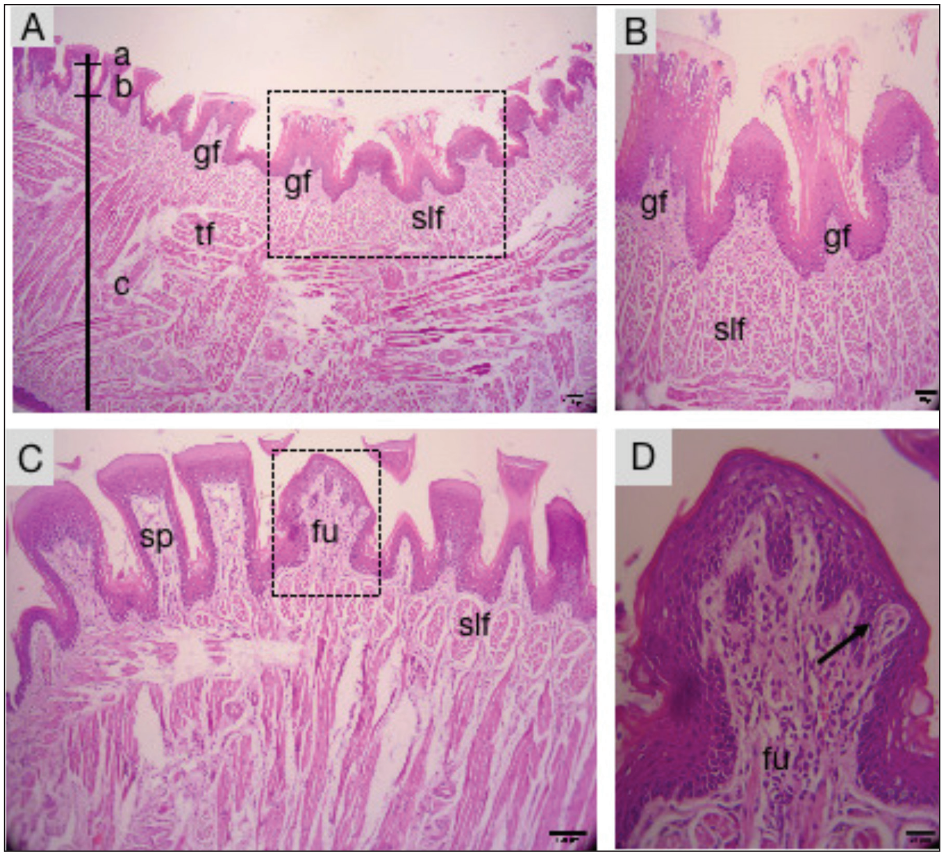

Fig. 5. Photomicrograph of the papilla on the lingual apex of the *E. spelaea*. with hematoxylin-eosin staining. (A) the apex is histologically divided into three layers: lingual mucosa (a), lingual aponeurosis (b), and lingual muscle (c). In addition, on the lingual mucosa of the anterior apex, find the scale-like papilla (sp); meanwhile, the muscularis layer can be seen in the superficial longitudinal fibres (slf) and transverse fibres (tf). (B) At the median region of the apex, find the giant trifid papilla (gt) and superficial longitudinal fibres (slf) at high magnification. (C) High magnification of the fungiform papilla (fu), scale-like papilla (sp), and superficial longitudinal fibres (slf) at the anterior apex. (D) High magnification of the fungiform papilla a taste buds can be observed (black arrow).

the muscularis layer (Fig. 5 A–E). The tongue mucosa consists of a stratified squamous epithelium and tongue papillae, including filiform, fungiform, and circumvallate papillae. Filiform papillae are comprised of many subtypes that are distributed across the whole surface of the tongue. The fungiform papilla possesses a convex shape and is situated amidst the filiform papilla at the tip and corpus. The circumvallate papilla is characterized by its substantial size and the presence of epithelial tissue on its surface. The tongue aponeurosis is a stratum consisting of connective tissue, lymphatic vessels, blood vessels, and nerves. The tongue muscle consists of both longitudinal and transverse muscle strands. The apex comprises the filiform papilla and the fungiform papilla. There are three distinct varieties of filiform papilla: gigantic trifid papilla, scale-like papilla, and small crown-like papilla. These papillae are made up of keratinized squamous complex epithelium (Fig. 5A–E). The fungiform papillae are located in between the filiform papillae, and each individual fungiform papilla contains either one taste bud (Fig. 5C and D).

Corpus

The histology of the tongue corpus is identical to apex, specifically consisting of filiform papillae formed of keratinized complex squamous epithelium. The corpus contains two types of filiform papillae: leaf-like papillae and enormous crown-like papillae (Fig. 6A and B). The prominent crown-like papilla is situated on the inner portion of the corpus, while the leaf-like papilla is found on the outside portion of the corpus. The fungiform papilla is positioned amidst the larger crown-like papilla and contains one taste bud (Fig. 6B).

Radix

The histology of the radix exhibits conical papilla and circumvallate papilla (Fig. 7). There are conical papillae on the root surface's lateral aspect (Fig. 7A, B, and E). Tongue glands are present in the muscle layer (Fig. 7A–

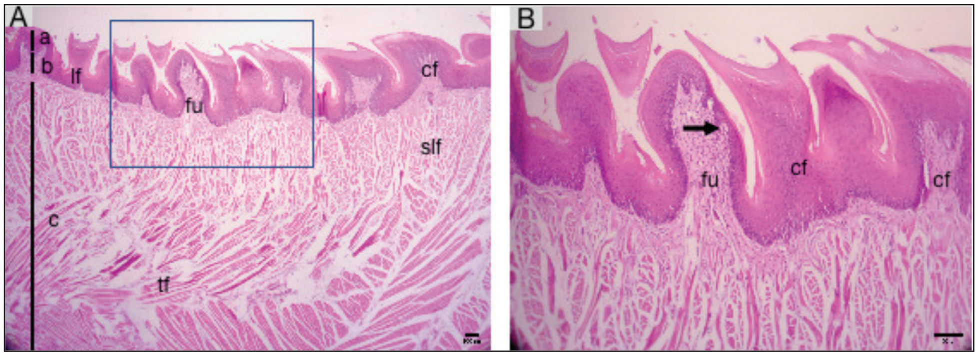

Fig. 6. Photomicrograph of the corpus of the *E. spelaea* tongue with hematoxylin-eosin staining. (A) like the apex, the tongue corpus is histologically devided into three layers: lingual mucosa (a), lingual aponeurosis (b); and lingual muscle (c); the superficial longitudinal fibers (slf) and transverse fibers (tf); fungiform papilla, leaf-like filiform papilla (lf) and large crown-like papilla (cf). (B) High magnification of the fungiform papilla (fu), a taste bud can be observed (black arrow) and Large crown-like papilla (cf).

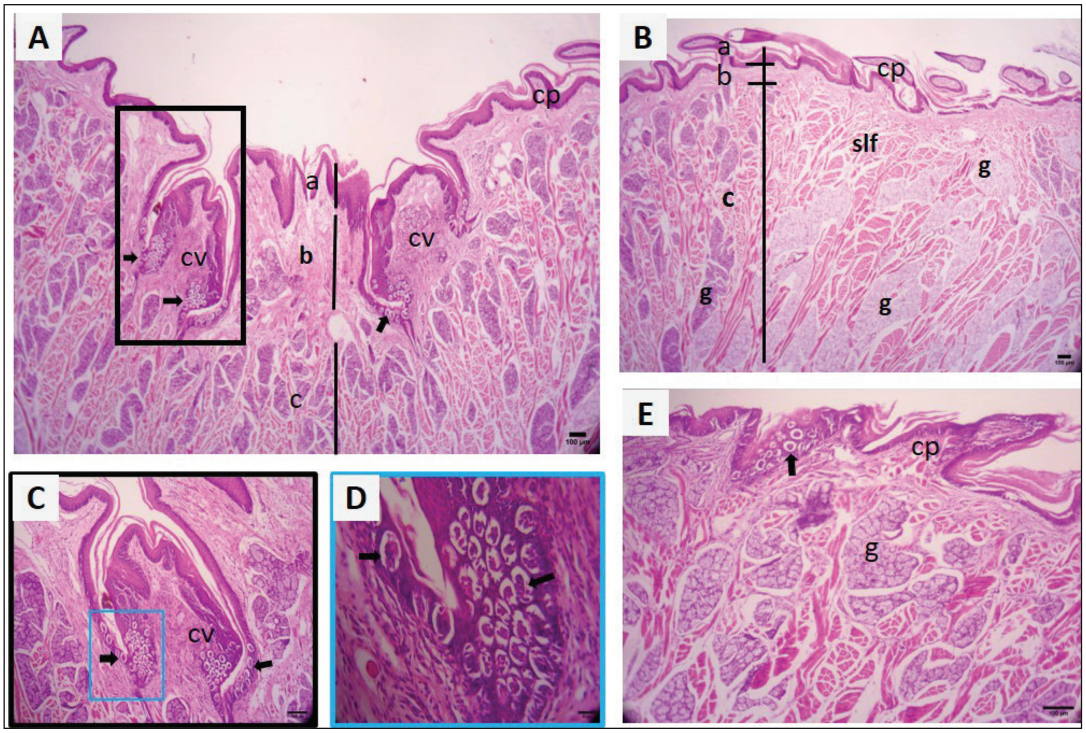

Fig. 7. Photomicrograph of the radix of the tongue of *E. spelaea*, with hematoxylin and eosin staining. (A) The radix region histologically consists of three layers (as do the apex and corpus): lingual mucosae (a), lingual aponeurosis (b), and lingual muscle (c). The circumvallate papilla (cv) abundant taste buds can be found (arrow) and conical papilla (cp) on the surface of the mucosa. (B) The layers of the tongue are, consist of lingual mucosae (a), lingual aponeurosis (b), and lingual muscle (c). The muscle layer also shows superficial longitudinal fibers (self) and some glandular (g). (C) High magnification of circumvallate papilla (cv), abundant taste buds can be found (arrow). (D) High magnification of the circumvallate papilla's taste buds (arrow). (E) Conical papilla (cp) and taste buds (arrow) of the lateral radix view.

E). Taste buds were observed on the surface of the tongue, including the lateral root (Fig. 7E). The circumvallate papilla is round in shape and is encircled by a wall that contains deep grooves (Fig. 7A and C). There are over 30 taste buds located on both the circumvallate papilla and its protective wall (Fig. 7A, C, and D).

Discussion

Bats are classified according to their diet, specifically as frugivores, nectivores, insectivores, or carnivores. Notably, the composition of food and the immediate surroundings can impact the morphology and configuration of the tongue, which serves as a digestive organ responsible for receiving and processing food within the oral cavity. This adaptation enables animals to adjust to the availability of food to fulfill their nutritional requirements. The papilla is the section of the tongue that possesses the ability to adjust to different types of food. In this instance, it pertains to the morphology, dimensions, arrangement, and role of each papilla (Gartiwa *et al*., 2021; Anjani *et al*., 2023).

The tongue of *E. spelaea*, a frugivore bat, is divided into three distinct parts: the apex, corpus, and radix. The corpus contains a medial sulcus extending in the profundus' direction. This feature is present in various bat species, including Rousettus amplexicaudatus (Gunawan *et al*., 2019), Rousettus aegyptiacus (Abumandour and El-Bakary, 2013), and Pipistrellus kuhlii (Massoud and Abumandour, 2020). The extension of the tongue is a distinctive feature that enables the palate and tongue to pulverize and masticate food within the mouth. In certain frugivore bats, the elongated and pointed shape of the tongue allows for extension, facilitating the consumption of fruit and nectar (Gonzalez-Terrazas *et al*., 2016). The tongue shape of *E. spelaea* allows for efficient movement during the process of piercing and extracting fruit. This includes adaptations of the palate and teeth that facilitate the extraction of fruit juice and nectar. The tongue frenulum, located on the underside of the tongue, is a structure that connects the tongue to the mandible.

Multiple SEM studies have revealed differences in filiform papillae among different bat tongues. For instance, Eidolon helvum (Kerr, 1972) exhibits five distinct sub-types of filiform papillae (Igbokwe *et al*., 2021). R. aegyptiacus, R. amplexicaudatus, and Pteropus vampyrus each possess six distinct sub-types of filiform papillae (Abumandour and El-Bakary, 2013; Gunawan *et al*., 2019; Selan *et al*., 2023). Additionally, *E. spelaea* (Dobson, 1871) exhibits seven distinct sub-types of filiform papillae: scale-like filiform papillae, enormous trifid papillae, small crown-like papillae, leaf-like papillae, huge crown-like papillae, conical papillae, and rosette-shaped filiform papillae. The filiform papillae are distributed across the apex, corpus, and radix. Filiform papillae have a mechanical function in aiding food ingestion (Gunawan *et al*., 2019). The characteristics of filiform papillae vary across species and are adapted to specific feeding behaviors. In nectar-feeding bats, the large, thread-like papillae on their tongues increase the surface area for nectar collection and have a coarse texture aiding in penetrating the skin of soft fruits, allowing the bats to extract fluids by rubbing the fruit against their tongue and the ridged hard palate Gunawan *et al*. (2019). These filiform papillae are mechanical structures assisting in the ingestion process and can vary in subtypes based on the contact frequency with food and individual eating patterns (Gartiwa *et al*., 2021). Furthermore, filiform papillae play a crucial role in increasing the gripping of food on the tongue surface, facilitating the efficient movement of food towards the pharynx (Mqokeli and Downs, 2012).

The fungiform papillae found in *E. spelaea* are distributed sporadically amid the filiform papillae. This aligns with research conducted on multiple species of frugivorous and nectarivorous bats (Emura *et al*., 2002; Gunawan *et al*., 2019). Furthermore, the development of fungiform papillae was observed on the lateral surface of the apex and corpus of the tongue of *E. spelae*. The taste buds possess a heightened taste sensor that aids in the acquisition of food for nutritional purposes. The taste buds are located within the fungiform papillae and serve the purpose of discerning the flavors of food (Park and Lee, 2009). The front part of the tongue, specifically the apex and corpus, contains fungiform papillae with the taste buds. These taste buds have a role in determining the sort of food that is preferred for ingestion (Hwang and Lee, 2007). The ability to sort the food can be attributed to the influence of taste perceptions derived from the variety of fruits and flowers. It is believed that the taste buds are connected to the decision-making process for food selection (Igbokwe *et al*., 2021). Similar to Pipistrellus javanicus (Saragih *et al*., 2020) and *R. amplexicaudatus* (Gunawan *et al*., 2019), fungiform papillae in *E. spelae* serve a gustatory function.

The presence of circumvallate papillae in bats is contingent upon their dietary preferences. For instance, hematophagous bats, as observed by Masuko *et al*. (2007), lack circumvallate papillae. Several insectivores, including Miniopterus schreibersi fuliginosus, Pipistrellus savii, Myotis macrodactylus, and *P. javanicus*, possess two circumvallate papillae (Hwang and Lee, 2007; Park and Lee, 2009; Saragih *et al*., 2020). The study reveals that *E. spelaea* has three circumvallate papillae on the tongue's posterior part (radix) adjacent to the larynx. This finding is consistent with the number of circumvallate papillae observed in fruit and nectar bats (Emura *et al*., 2002; Jackowiak and Godynicki, 2009; Gunawan *et al*., 2019; Selan *et al*., 2023). Lowland tapirs (Tapirus terrestris), similarly frugivorous, possess nine circumvallate and foliate papillae (Goździewska-Harłajczuk *et al*., 2020). This distinction is believed to be associated with their adaptation to the mastication of food within the mouth cavity. The circumvallate papillae in *E. spelaea* contain around 30 taste buds, enabling the interpretation of the flavor of consumed food, such as nectar, pollen, and fruit. This feature compensates for this species' lack of foliate papillae.

The tongue of *E. spelaea* exhibits a microanatomical structure consisting of three distinct layers: the mucosa layer, aponeurosis layer, and muscularis layer. The mucosal layer contains epithelial tissue that exhibits distinctive keratinization. Moreover, the tongue is a flexible organ, aiding bats in manipulating their food. Histology studies of muscle fiber have revealed the precise positioning of muscle fiber bundles within the muscle layer, which is near the mucosa layer of the bat tongue. Tongue motions are facilitated by the existence of muscle fibers, enabling a wide range of motion. The findings of this study are consistent with the results obtained from previous research (Gunawan *et al*., 2019; Selan *et al*., 2023). Tongue glands are present in the muscularis layer, particularly in the corpus and root regions. Similar to other mammals, like Aonyx cinereus, the tongue's intermuscularis layer contains serous and mucus glands (Anjani *et al*., 2023).

The shape of the tongue is crucial for efficient movement, particularly in activities like mastication and speech production. The intrinsic muscle fibers of the tongue significantly contribute to its shape and movement (Sato *et al*., 1990). Tongue movement is closely related to changes in shape, as the tongue is a constant volume structure that deforms to produce movement (Liu *et al*., 2009). Research has shown that tongue movements directly impact masticatory efficiency, with electromyographic analysis reflecting the influence of tongue movements on suprahyoid muscles (Oguchi *et al*., 2016). Moreover, the strength and tonus of the tongue muscles are linked to the execution of lingual movements, underscoring the importance of tongue mobility in various tasks (Amaral *et al*., 2020).

Conclusion

This study presents the initial comprehensive data on the papillae of *E. spelaea* tongue from Oeekam Village, Noebeba District, Soe, East Nusa Tenggara, Indonesia with a SEM and light microscope. This study discovered that bats possess two primary categories of papillae: mechanical and sensory. Each type exhibits diverse forms of specialization, which can be attributed to variations in the species' diet and habitat.

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Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contributions

Conceptualization, H.W. and D.L.K.; Methodology, H.W. and D.L.K.; Software, Y.N.S and G.R.S.; Validation, H.W. and Y.N.S.; Formal analysis, D.L.K., H.W., and Y.N.S.; Investigation, Y.N.S and U.K.; Resources, H.W. and D.L.K.; Data curation, H.W. and Y.N.S.; Writing—original draft preparation, Y.N.S and H.W.; Writing—review and editing, G.R.S.; Visualization, U.K and A.H.; Supervision, D.L.K., H.W., and A.H.; Project administration, H.W. and D.L.K.; Funding acquisition, D.L.K. and H.W. All authors have read and agreed to the published version of the manuscript.

Data availability

All data supporting the findings of this study are available within the manuscript.

References

Abumandour, M.M.A. and El-Bakary, R.M.A. 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), 229–238.

Acharya, P.R., Racey, P.A., Sotthibandhu, S. and Bumrungsri, S. 2015. Feeding behaviour of the dawn bat (*Eonycteris spelaea*) promotes cross pollination of economically important plants in Southeast Asia. J. Pollinat. Ecol. 15(0), 44–50.

Amaral, M.S., Furlan, R.M.M.M., de Las Casas, E.B. and Motta, A.R. 2020. The influence of tongue mobility on children's performance in computer games that depend on lingual movements. .J Oral. Rehabil. 47(10), 1233–1241.

Anjani, A.K., Saragih, G.R., Wihadmadyatami, H. and Kusindarta, D.L. 2023. Lingual morphology of domesticated Asian small-clawed otters in Yogyakarta, Indonesia. Vet Med (Praha). 68(3), 91–105.

Bumrungsri, S., Lang, D., Harrower, C., Sripaoraya, E., Kitpipit, T. and Racey, P. 2013. The dawn bat, *Eonycteris spelaea* dobson (Chiroptera: Pteropodidae) feeds mainly on pollen of economically important food plants in Thailand. Acta Chirop. 15, 95–104.

Damia, U., Anjani, A.K., Wihadmadyatami, H. and 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.

Emura, S., Hayakawa, D., Chen, H., Shoumura, S., Atoji, Y. and 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.

Gartiwa, G., Damia, U., Megawati, E.I., Pradipta, S.I.D., Gunawan, G., Karnati, S., Wihadmadyatami, H. and Kusindarta, D.L. 2021. Morphological characterization of Horsfield's treeshrew Tupaia javanica lingual papillae: light microscopy and scanning electron microscopy studies. Anat. Histol. Embryol. 50(5), 801–811.

Gonzalez-Terrazas, T.P., Koblitz, J.C., Fleming, T.H., Medellín, R.A., Kalko, E.K. V, Schnitzler, H.-U. and Tschapka, M. 2016. How nectar-feeding bats localize their food: echolocation behavior of leptonycteris yerbabuenae approaching cactus flowers. PLoS One. 11(9), e0163492.

Goździewska-Harłajczuk, K., Hamouzova, P., Klećkowska-Nawrot, J., Barszcz, K. and Cizek, P. 2020. Microstructure of the surface of the tongue and histochemical study of the lingual glands of the lowland tapir (Tapirus terrestris Linnaeus, 1758) (Perissodactyla: Tapiridae). Animals. 10(12), 2297.

Gunawan, G., Saragih, G.R., Umardani, Y., Karnati, S., Wihadmadyatami, H., and Kusindarta, D.L. 2020. 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.

Hwang, H.S. and Lee, J.H. 2007. Morphological study on the dorsal lingual papillae *of Myotis macrodactylus*. Appl. Microsc. 37(3), 147–156.

Igbokwe, C.O., Bello, U.M. and Mbajiorgu, F.E. 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.

Jackowiak, H., Trzcielinska-Lorych, J. and 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.

Kusuma, I.F., Damia, U., Megawati, E.I., Saputra, F.C.E., Karnati, S., Kusindarta, D.L. and Wihadmadyatami, H. 2022. Morphology of lingual papillae in the Javan mongoose (*Herpestes javanicus*) by scanning electron microscopy and light microscopy. Anat. Histol. Embryol. 51(6), 756–768.

Liu, Z.J., Shcherbatyy, V., Kayalioglu, M. and Seifi, A. 2009. Internal kinematics of the tongue in relation to muscle activity and jaw movement in the pig. J. Oral. Rehabil. 36(9), 660–674.

Massoud, D. and Abumandour, M.M.A. 2020. Anatomical features of the tongue of two chiropterans endemic in the Egyptian fauna; the Egyptian fruit bat (*Rousettus aegyptiacus*) and insectivorous bat (*Pipistrellus kuhlii*). Acta. Histochem. 122(2), 151503.

Masuko, T., Boaro, S., König-Junior, B., Cabral, R. and Paulo, O. 2007. Comparative Scanning Electron Microscopic Study of the Lingual Papillae in Three Species of Bats (Carollia perspicillata, Glossophaga soricina and Desmodus rotundus). Microscopy Microanal. 13(S02), 280–281.

Megawati, E.I., Pradipta, S.I.D., Damia, U., Kustiati, U., Wihadmadyatami, H. and Kusindarta, D.L. 2023. Morphological identification of the squirrel (*Callosciurus notatus*) tongue through scanning electron microscopy (SEM) and histochemistry. Biodiversitas. 24(4), 2302–2314.

Mohamed, N.Z., Anuar, M., and Jones, G. 2016. The potential significance of nectar-feeding bats as pollinators in mangrove habitats of Peninsular Malaysia. Biotropica. 4 (48), 425–428.

Mqokeli, B. and Downs, C. 2012. Palatal and lingual adaptations for frugivory and nectarivory in the Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*). Zoomorphology. 132(1), 111–119.

Oguchi, H., Watanabe, T., Nakamura, N. and Watanabe, S. 2016. Influence of tongue movements on masticatory efficiency. Dent. Oral. Craniofac. Res. 2(6):1–6.

Okada, S. and Schraufnagel, D.E. 2005. Scanning electron microscopic structure of the lingual papillae of the common opossum (*Didelphis marsupialis*). Microsc. Microanal. 11(4), 319–332.

Park, J. and Lee, J.H. 2009. Comparative morphology of the tongue of *Miniopterus schreibersi* fuliginosus and *Pipistrellus savii*. Appl. Microsc. 39(3), 267–276.

Peng, X., He, X., Liu, Q., Sun, Y., Liu, H., Zhang, Q., Liang, J., Peng, Z., Liu, Z. and Zhang, L. 2017. Flight is the key to postprandial blood glucose balance in the fruit bats *Eonycteris spelaea* and Cynopterus sphinx. Ecol. Evol. 7(21), 8804–8811.

Saragih, G.R., Gunawan, G., Umardani, Y., Karnati, S., Kusindarta, D.L. and 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.

Sato, I., Suzuki, M., Sato, M., Sato, T. and Inokuchi, S. 1990. A histochemical study of lingual muscle fibers in rat. Okajimas. Folia. Anat. Jpn. 66(6), 405–415.

Selan, Y.N., Wihadmadyatami, H., Haryanto, A. and Kusindarta, D.L. 2023. The tongue morphology of *Pteropus vampyrus* from Timor Island, Indonesia: New insights from scanning electron and light microscopic studies. Biodiversitas. 24(6), 3512–3518.

Waldien, D.L., Adleson, S. and Wilson, Z. 2020. *Eonycteris spelaea*. IUCN Red List Threatened Species. 2020, e.T7787A22128326.