Journal of Tropical Biodiversity and Biotechnology
Volume 09, Issue 04 (2024): jtbb90995
DOI: 10.22146/jtbb.90995

Research Article

Morphological Structure of the Tongue of *Gekko gecko* in Yogyakarta, Indonesia

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Keywords:
Gekko gecko
Histology
Morphology
Papilla
Tounge
Submitted:
23 November 2023
Accepted:
18 June 2024
Published:
06 December 2024
Editor:
Ardaning Nuriliani

ABSTRACT
Gekko gecko is a member of the order Squamata from the family Gekkonidae and mainly feeds on small insects. This study aims to determine the morphology of the tongue of *Gekko gecko* through Scanning Electron Microscopy (SEM), hematoxylin–eosin (HE) staining, and immunohistochemistry (IHC). Six adult *Gekko gecko* were obtained from the Special Region of Yogyakarta, and *Gekko gecko* tongue samples were stored in SEM fixative solution and then observed with SEM. For histochemical and IHC staining, tongue samples were processed into paraffin blocks and cut into 8 μm-thick sections. The SEM revealed three types of papillae: dome-shaped papillae at the apex, fan-shaped papillae at the corpus, and scale–like papillae at the radix. Histological observations showed that the tongue of the *Gekko gecko* was composed of tunica mucosa and tunica muscularis, and goblet cells were present in the lamina of the epithelial mucosa. Meanwhile, no taste buds were found. Immunoreactivity against PGP 9.5 was observed on the tunica muscularis of the apex, corpus, and radix. Taken together, this study provides new insight into the tongue morphology of *Gekko gecko* and is dominated by mechanical papillae on the tongue surface.

INTRODUCTION
The Gekko gecko or house gecko is under the class Reptilia, order Squamata, suborder Sauria (Lacertilian), and family Gekkonidae (Hildyard 2001). *Gekko gecko* in Southeast Asia are naturally found in Indonesia, Malaysia, Singapore, the Philippines, Thailand, Vietnam, and Cambodia and have spread to Northeast India, south China, Hong Kong, and Nepal (Rocha et al. 2015). *Gekko gecko* in Indonesia are found in Java, Sumatra, Kalimantan, Sulawesi, Lesser Sunda (Bali), and Maluku. *Gekko gecko* mainly inhabits areas with many trees, such as forests, but are also often found in the building (Carranza & Arnold 2006; Cahyani et al. 2023). It is nocturnal and mainly feed on small insects (Partasasmita et al. 2016).

The tongue is one of the most important parts of the digestive tract. In most reptiles, amphibians, and mammals, the tongue is used to hold food. The tongues of lepidosaurians are divided into three parts: apex, corpus, and radix (Jamniczky et al. 2009). The tongues of most iguanas and geckos are wide and short with a wide and rounded tip (Schwenk 2000). *Gekko gecko* also uses its tongue to clean it eyes because it has no eyelids (Kusrini 2019).

The tongues of geckos in the Gekkonidae family have three types of papillae. For example, *Gekko japonicus* have dome-shaped, fan-shaped, and scale–like papillae (Iwasaki 1990), and *Tarentola annularis* have dome-shaped, fan-shaped, and broad scale–like papillae (Bayoumi et al. 2011). In *Gekko japonicus,* the epithelia of dome-shaped papillae comprise stratified columnar epithelial cells, whereas the epithelia of fan-shaped papillae comprises stratified squamous epithelial cells (Iwasaki 1990). Moreover, the epithelia of the dome-shaped papillae of *Tarentola annularis* are composed of non-keratinised stratified squamous epithelial cells, whereas the epithelia of the fan-shaped and broad scale–like papillae are composed of keratinised layered stratified squamous epithelial cells (Bayoumi et al. 2011). These studies revealed variations in the morphology and distribution of the papillae on the dorsal tongue surfaces in geckos and lizards, but bibliographical data on the structure of tongue papillae of *Gekko gecko* are limited.

MATERIALS AND METHODS

Ethical approval

The experimental procedures were approved by the ethics committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia (approval number: 0101/EC-FKH/Int./2021).

Animal Sample

Gekko gecko were identified at the Animal Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada. The tongue samples of *Gekko gecko* were obtained from collectors. A high-dose anesthetic combination of ketamine (Kepro, Maagdenburgstraat, Holand) and xylazine (Interchemie, Metaalweg, Holand) was administered: 20 mg/kg body weight for the ketamine and 3 mg/kg body weight for xylazine. *Gekko gecko* tongue samples were prepared by separating the maxilla and mandible. Then, dirt was removed by brushing the tongues of the *Gekko gecko* with a fine brush. The tongue was removed by cutting the base and separating it from the mandible.

Conservation status

According to the International Union for Conservation of Nature Red List of Threatened Species, *Gekko gecko* is categorised as Least Concern. Furthermore, according to Convention on International Trade in Endangered Species of Wild Fauna and Flora, *Gekko gecko* is included in CITES Appendix 2.

Sample Preparation

Three tongue samples were immersed in 4% paraformaldehyde (KgaA, Darmstadt, Germany) for HE (HE; Bio-Optica, Milan, Spain) staining, and three other samples were immersed in SEM fixative solution (0.5% glutaraldehyde (Chem Cruz, Texas, US), 1.5% paraformaldehyde (Nacalai Tesque, Tokyo, Japan), Hepes (Chemcruz), and PBS (Nacalai Tesque, Tokyo, Japan) for at least 6–8 h.

The length of the tongue was measured using calipers from the tip to the base and each sample was measured in triplicate. The samples were then observed with a camera (Canon EOS 7000D, Tokyo, Japan). After macroscopic observation, the samples were prepared for Scanning Electron Microscopy (SEM) and light microscopy.

Scanning Electron Microscopy

Tongue samples were fixed for at least 24 h, divided into apex, corpus, and radix parts, and washed with PBS (Nacalai Tesque, Tokyo, Japan). Then, the samples were dehydrated in an ethanol solution (KgaA, Darmstadt, Germany) for 5–10 min twice and fixed on a conductive metal plate with carbon tape. The samples were dried using a vacuum system (BUEHLER- Castable Vacuum System, Stuttgart, Germany) for 60 min and then coated with a coater (JEOL-auto fine coater JEC-3000FC, Tokyo, Japan) using platinum for 120 s. Finally, each sample was inserted into an electron microscope (JEOL JSM-6510LA, Tokyo, Japan) and observed at a voltage of 10 kV and magnifications of 30×, 100×, and 300×.

Histological Staining

Tongues fixed in 4% paraformaldehyde (KgaA, Darmstadt, Germany) fixative solution for at least 24 h were cut into three parts: apex, corpus, and radix. The samples were then washed with running water, dehydrated with a graded ethanol solution, cleared with xylol (Leica, Wetzler, Germany), placed in paraffin (Leica Biosystems, Wetzlar, Germany), and embedded in paraffin blocks. The samples were cut transversely and the resulting 8 µm-thick sections were stained with hematoxylin and eosin (HE; Bio-Optica, Milan, Spain).

Immunohistochemistry for PGP 9.5

The samples were fixed in 4% paraformaldehyde (KgaA, Darmstadt, Germany) for 24 h and processed for routine paraffin embedding. The blocks were cut into 8 µm-thick cross-sectional sections, mounted on gelatin-coated microscope slides, and processed for immunohistochemistry. Serial sections were deparaffinized and rehydrated, rinsed several times with PBS, and blocked with 10% normal goat serum for 1 h. Polyclonal rabbit anti-PGP 9.5 (Invitrogen, Waltham, MA, USA) was diluted in a permeabilising solution (PBS, 0.2% Triton X 100, 0.1% sodium azide) according to optimal dilutions and placed on the slides overnight at room temperature. The sections were rinsed with PBS and incubated with secondary antibody poly-HRP goat anti-rabbit IgG (Fine test, Wuhan, China) for 60 min. After DAB was added (Fine test, Wuhan, China), the sections were observed with a light microscope (Nikon, Tokyo, Japan).

RESULTS

Morphology of the Tongue

Gross Morphology

Macroscopic observations of the *Gekko gecko* tongue show four surfaces: a dorsal surface, a ventral surface, and two lateral surfaces. The tongue is triangular, flat, short, and pale pink, with a wide tip and rounded shape, widening and thickening posteriorly. It is attached to the base of the buccal cavity along the ventral median line and has a total length of approximately 2 cm. It is divided into three parts: the apex which is the most anterior part of the tongue, the corpus, and the radix which is the most posterior near the larynx and blackish in color. The difference can be seen through the dorsal view. Papillae lingualis are found on the entire dorsal to lateral surface. The ventral sulcus is found on the ventral surface along the apex region to the radix region of the tongue (Figure 1).

The apex region of the *Gekko gecko* tongue starts from the anterior part to the curve that separates the apex and corpus regions. The *Gekko gecko* tongue in the apex region has a rounded and flattened tip shape anteriorly and its size thickens further posteriorly. Similar to other types of reptiles, *Gekko gecko* has bifurcated or branching tongue. However, the bifurcation on the *Gekko gecko* tongue is extremely small and does not split the apex of the tongue deeply, and thus the branching or bifurcation can only be seen from an extremely close distance. Papillae lingualis, which has a smooth and flat surface, is found on the dorsal surface of the tongue's apex.

The corpus region is between the apex region on the anterior part of the tongue, and the radix region is on the posterior part. The border of the corpus region with the apex region can be observed from the curve that separates the apex and corpus regions (Figure 1). It can also be located according to changes in the shapes of the scattered papillae (Figures 2 and 3). The tongue papillae scattered on the *Gekko gecko* tongue in the apex region have flat and smooth surfaces. By contrast, the tongue papillae scattered in the corpus region have rougher surfaces than those in the apex region. The border of the corpus and radix regions shows "V"-shaped branching, where the corpus region ends at the beginning of the branching and continues to the radix region.

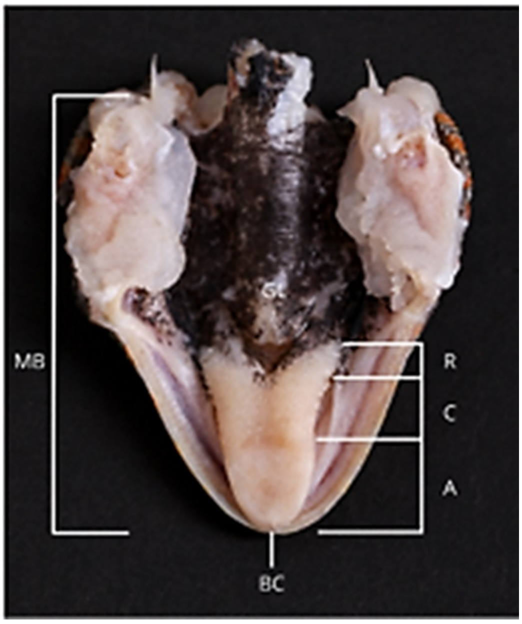

Figure 1. Macroscopic view of *Gekko gecko* tongue in the dorsal section (A = apex region, C = corpus region, R = radix region, BC = bifurcation, = GL = glottis, MB = mandible).

The radix region of the *Gekko gecko*'s tongue is the most posterior, borders the glottis, and is characterised by "V"-shaped branching that splits the region. The glottis is found between the two radix regions. The presence of blackish patches on the posterior part of the tongue radix characterise the radix region.

Scanning Electron Microscopy

Apex

The dorsal surface of the *Gekko gecko*'s tongue at the apex was observed using a scanning electron microscope at 30× magnification. Tongue papillae was found at the apex, and the apex has a smooth texture and flat surfaces and are arranged tightly (100× magnification). Bifurcation or branching on the anterior part of the apex at 100× magnification is fairly small and does not split the apex of the tongue as deeply as that in other types of reptiles. At 300× magnification, the apex contains dome-shaped papillae, which are polygonal and have different shapes. Dome-shaped papillae have short and uniform size, rendering the dorsal surface of the apex smooth and flat (Figure 2).

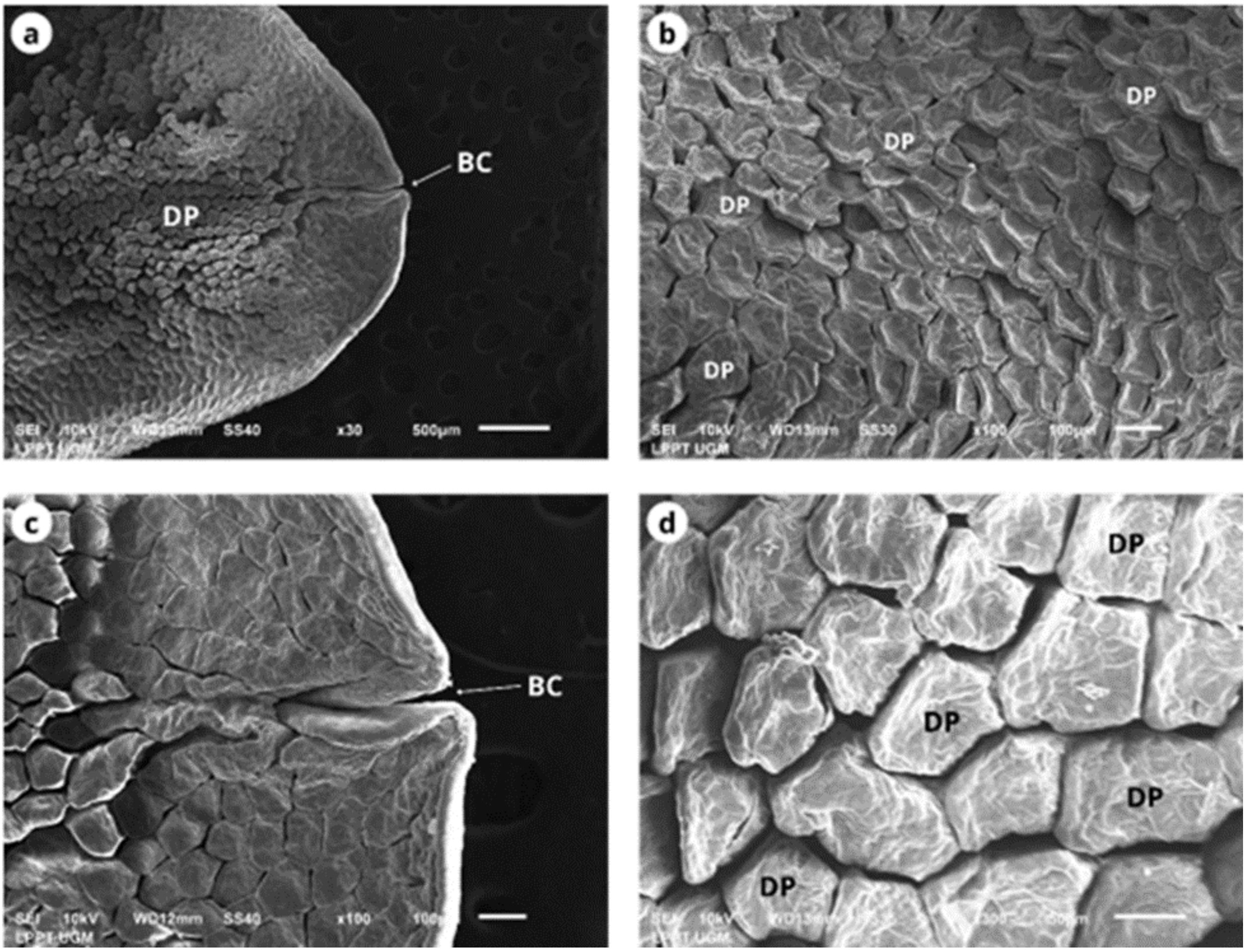

Figure 2. Scanning Electron Microscopy (SEM) image of the tongue of *Gekko gecko* at the apex. (a) *Gekko gecko*'s tongue apex section at 30× magnification; (b) apex at 100× magnification shows visible papillae with smooth and flat surfaces; (c) anterior part of the apex at 100× magnification shows visible bifurcation (BC); (d) apex at 300× magnification shows visible dome-shaped papillae (DP).

Corpus

The dorsal surface of the *Gekko gecko*'s tongue at the corpus was observed with a Scanning Electron Microscopy at 30× magnification. The dorsal surface has uneven and smooth texture, similar to the apex. At 100× magnification, the dorsal surface in the corpus section shows long and slender fan-shaped tongue papillae arranged loosely, thus showing an uneven and smooth surface similar to the dorsal surface of the tongue at the apex. At 300× magnification, the surfaces of the fan-shaped papillae have a small polygonal shapes with tapered ends (Figure 3).

Radix

The dorsal surface of the radix of *Gekko gecko*'s tongue was observed with a Scanning Electron Microscope at 30× magnification. The dorsolateral side has tongue papillae, and the dorsal surface of the radix part has an area without papillae (NP) on the middle dorsal side and can be observed at 30× magnification. At 100× magnification, the dorsolateral side of the radix part of the tongue show tongue papillae with folds and scale–like shapes. At 300× magnification, the papillae have wide triangular shapes and tips that point caudally (Figure 4).

Histological Structure of the Tongue

The histological structure of the *Gekko gecko*'s tongue was observed using the HE-stained cross-sections of the tongue apex, corpus, and radix, and the morphology of each part was further observed. *Gekko gecko*'s tongue is

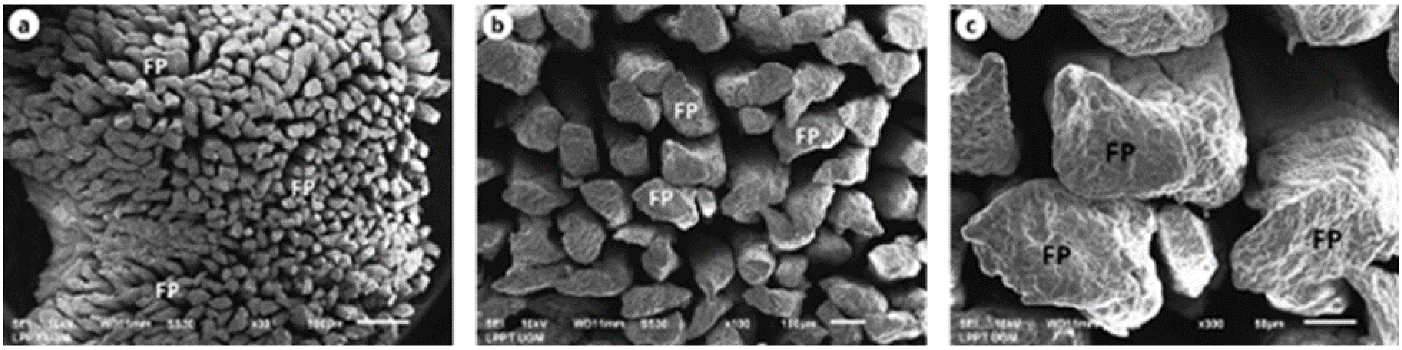

Figure 3. Scanning Electron Microscopy (SEM) image of the corpus of *Gekko gecko*'s tongue: (a) corpus section at 30× magnification; (b) corpus at 100× magnification shows papillae with loose surfaces; (c) corpus with 300× magnification shows the formation of fan-shaped papillae (FP).

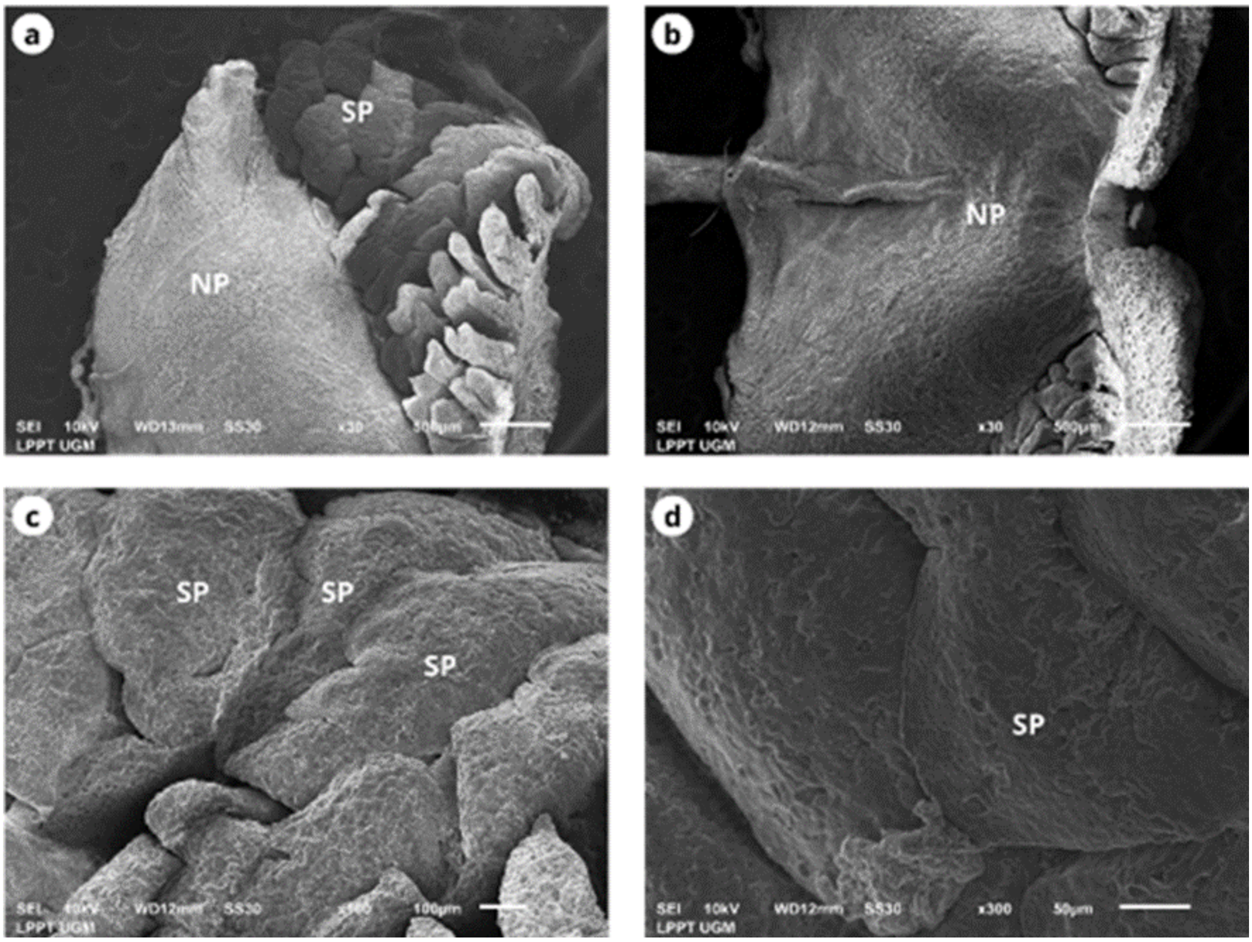

Figure 4. Scanning Electron Microscopy (SEM) image of the radix section. (a) Radix section at 30× magnification; (b) radix at 30× magnification showing the tongue area without papillae; (c) radix at 100× magnification showing scale–like papillae (SP); (d) scale–like papillae at 300× magnification.

microscopically composed of two tunicas: tunica mucosa (lamina epithelial mucosa and lamina propria mucosa) and tunica muscularis composed of several muscles. The histological structures of the *Gekko gecko*'s tongue have similar arrangements in the apex, corpus, and radix, especially in the corpus and radix

Apex

The histological structure of the HE-stained *Gekko gecko*'s tongue at the apex is flat and wide. The apex is histologically composed of two tunica, namely, tunica mucosa and tunica muscularis. The tunica mucosa is composed of lamina epithelial mucosa and lamina propria mucosa. The mucosal lining of the tunica mucosa is lined by the stratified squamous epithelium. The tunica muscularis is composed of several types of muscles: dorsal longitudinal muscle, which is in the dorsal part of the tunica muscularis, transverse muscle, and several hyoglossus muscles in the ventral part. The papillae lingualis is found on the dorsal and lateral parts of the tongue. The tongue papillae found on the tongue of *Gekko gecko* at the apex are dome-shaped and appear like short stalks with flat and wide surfaces (Figure 5).

Corpus

The histological structure of the *Gekko gecko*'s tongue was observed using HE-stained corpus section. The structure shows a wide and flat shape but is thicker than that observed in the apex. The corpus is composed of two tunica, namely tunica mucosa and tunica muscularis. Tunica mucosa comprises lamina epithelial mucosa and lamina propria mucosa. The LEM is composed of stratified squamous epithelium. Tunica muscularis is composed of several types of muscles different from those observed in the apex. The muscularis tunica in the corpus is composed of genioglossus muscles on the left and right regions that forms the corpus's lateral border. The entoglossal processus of the hyoid bone is found at the ventral center between the hyoglossus muscles. The tongue of papillae are found on the dorsal to lateral part of the tongue. The tongue papillae found on the corpus are slender and long fan-shaped papillae (Figure 6).

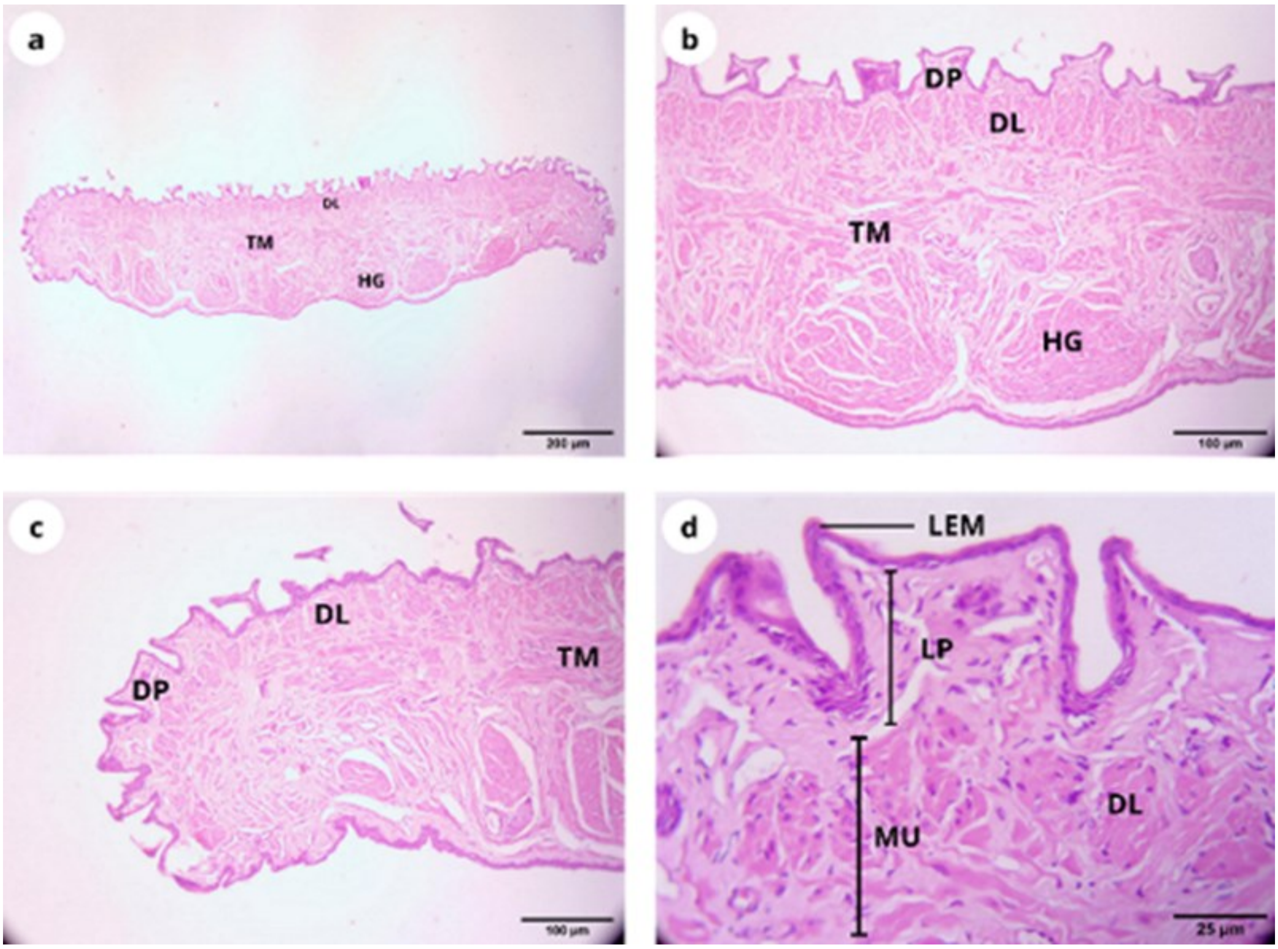

Figure 5. Histological structure of the tongue of a *Gekko gecko* in hematoxylin–eosin-stained apex. (a) The overall shape of the histological structure of the *Gekko gecko*'s tongue at the apex at 4 × 10 magnification; (b) apex at 10 × 10 magnification shows the dorsal longitudinal muscle (DL), transverse muscle (TM), and hyoglossus muscle (HG); (c) apex at 10 × 10 magnification shows the formation of dome-shaped papillae (DP); (d) dome-shaped papillae with a 40 × 10 magnification shows the lamina epithelial mucosa (LEM), lamina propria mucosa (LP), lamina muscularis (MU).

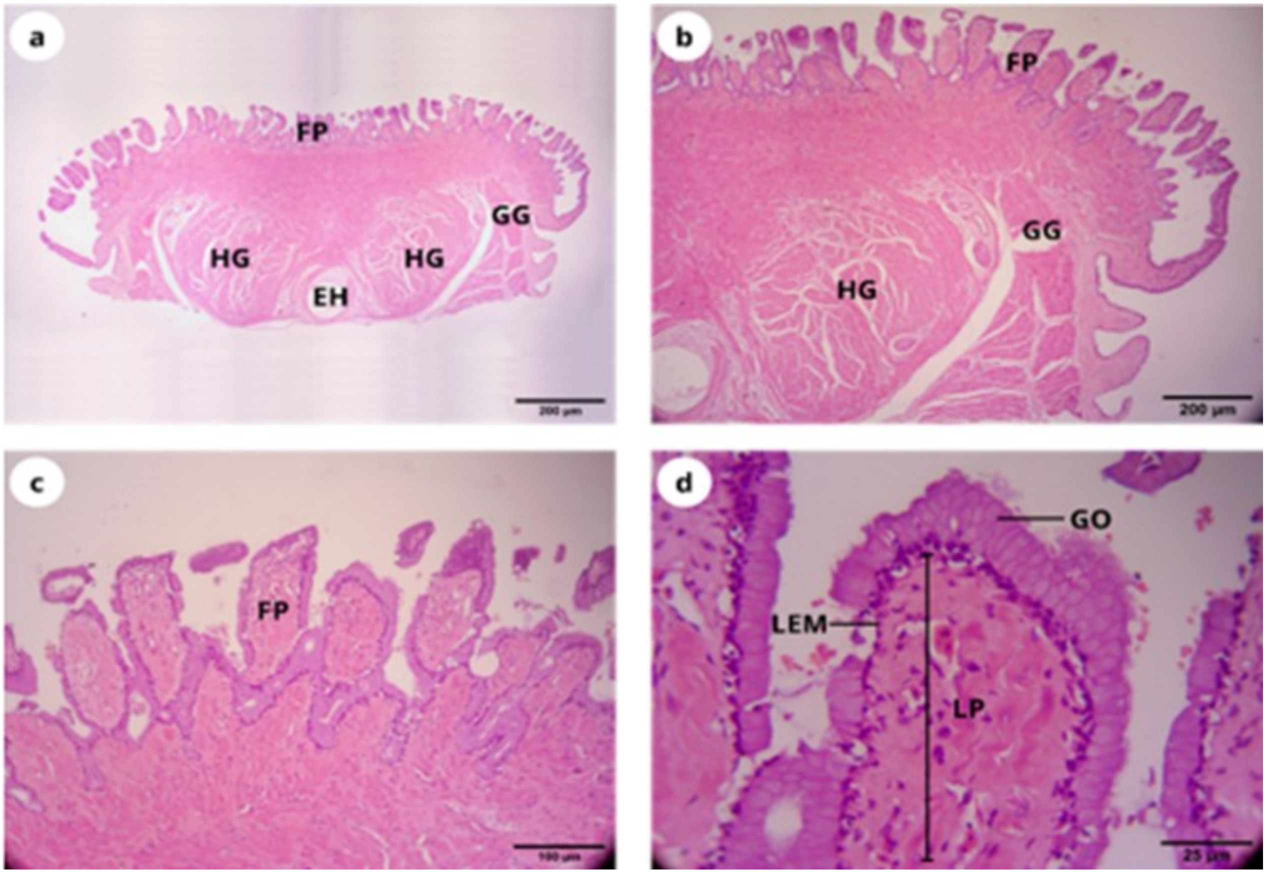

Figure 6. Histological structure of the tongue hematoxylin–eosin-stained corpus. (a) The overall shape of the histological structure of the *Gekko gecko* tongue in the corpus section with a 4 × 10 magnification; (b) corpus with a 10 × 10 magnification showing fan-shaped papillae (FP), genioglossus muscle (GG), and hyoglossus muscle (HG); (c) corpus with 10 × 10 magnification showing fan-shaped papillae (DP); (d) fan-shaped papillae with 40 × 10 magnification showing lamina epithelial mucosa (LEM), lamina propria mucosa (LP), goblet cluster (GO), and hyoid bone (EH).

Radix

The histological structure of the *Gekko gecko*'s tongue in HE-stained radix section was observed. The structure in this section has a wide and flat shape and is thicker than that in the apex but thinner than that in the corpus. The radix and corpus have a similar histological structure arrangement. The radix is composed of two tunics, namely, tunica mucosa and tunica muscularis. Tunica mucosa comprises LEM and lamina propria mucosa. The lamina epithelial mucosa is composed of stratified squamous epithelium. Tunica muscularis in the radix has a similar arrangement to that in the corpus; the genioglossus muscle on the left and right sections of the tongue formed the lateral border of the radix. The entoglossal processus of the hyoid bone on the tongue radix is at the ventral center between the two hyoglossus muscles. The tongue papillae is on the dorsolateral part of the tongue, and the dorsal center has no tongue papillae. The tongue papillae found on the radix part of the *Gekko gecko*'s tongue are scale–like papillae with shaped-like folds resembling scales (Figure 7).

Immunoreactivity of PGP 9.5 on *Gekko gecko*'s tongue

Cells positive for PGP 9.5 protein were observed in the sensory cells of the muscle fiber of the tunica muscularis. Immunoreactive cells are dominant in the genioglossus muscles in the corpus and radix and scattered in the tunica muscularis on the apex (Figures 8A, 8B, 8C).

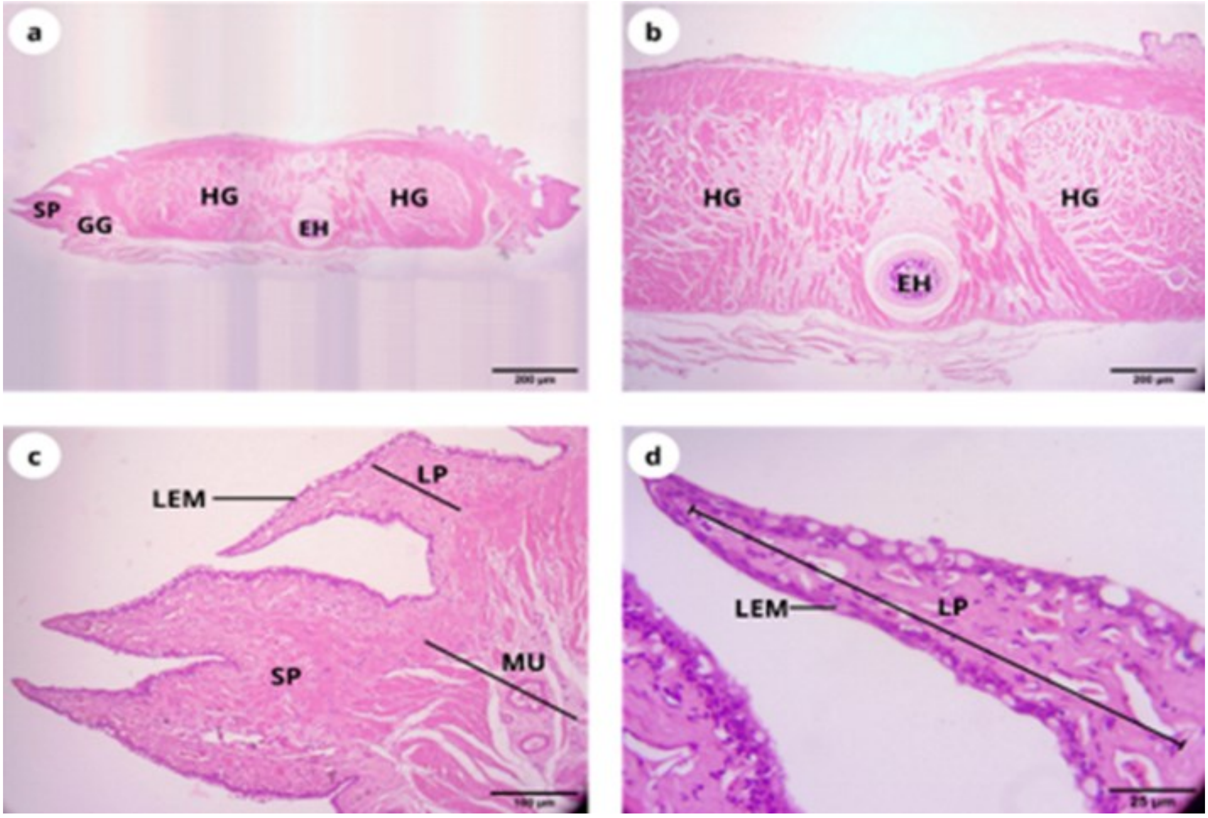

Figure 7. Histological structure of the tongue of a *Gekko gecko* in hematoxylin–eosin-stained radix section. (a) The overall shape of the histological structure of the *Gekko gecko*'s tongue in the radix section at 4 × 10 magnification; (b) radix with a 10 × 10 magnification showing scale–like papillae (SP), genioglossus muscle (GG), and hyoglossus muscle (HG); (c) radix at 10 × 10 magnification showing the formation of scale–like papillae (SP); (d) scale–like papillae with 40 × 10 magnification showing Lamina Epithelial Mucosa (LEM) and Lamina Propria Mucosa (LP); Hyoid Bone (EH), Lamina Muscularis (MU).

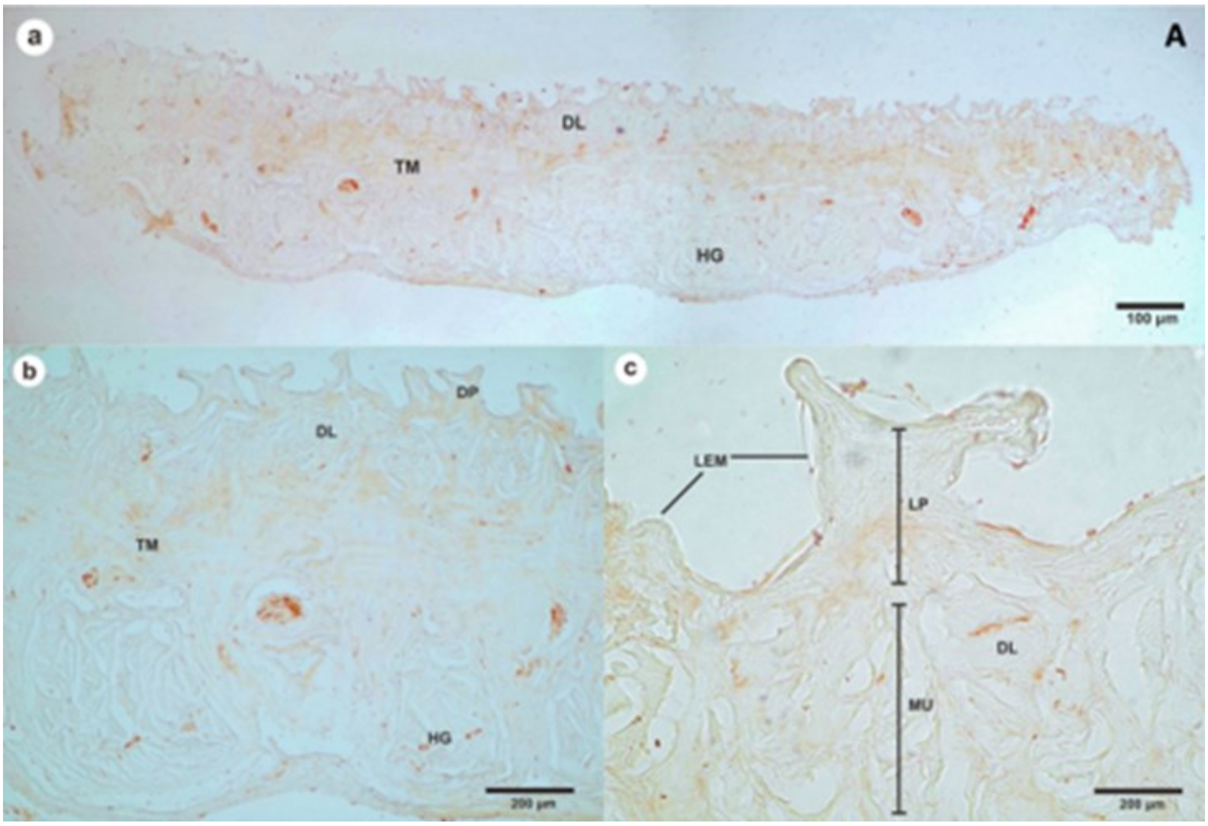

Figure 8A. Micrographs of cells immunoreactive to PGP 9.5 in the apex. The sensory cells immunoreactive for PGP-9.5 protein are scattered in the tunica muscularis, (a) Scale bar: 100 µm; (b and c) Scale bar: 200 µm; dorsal longitudinal muscle (DL); tunica muscularis, transverse muscle (TM); hyoglossus muscle (HG), lamina epithelial mucosa (LEM); Lamina muscularis (LM).

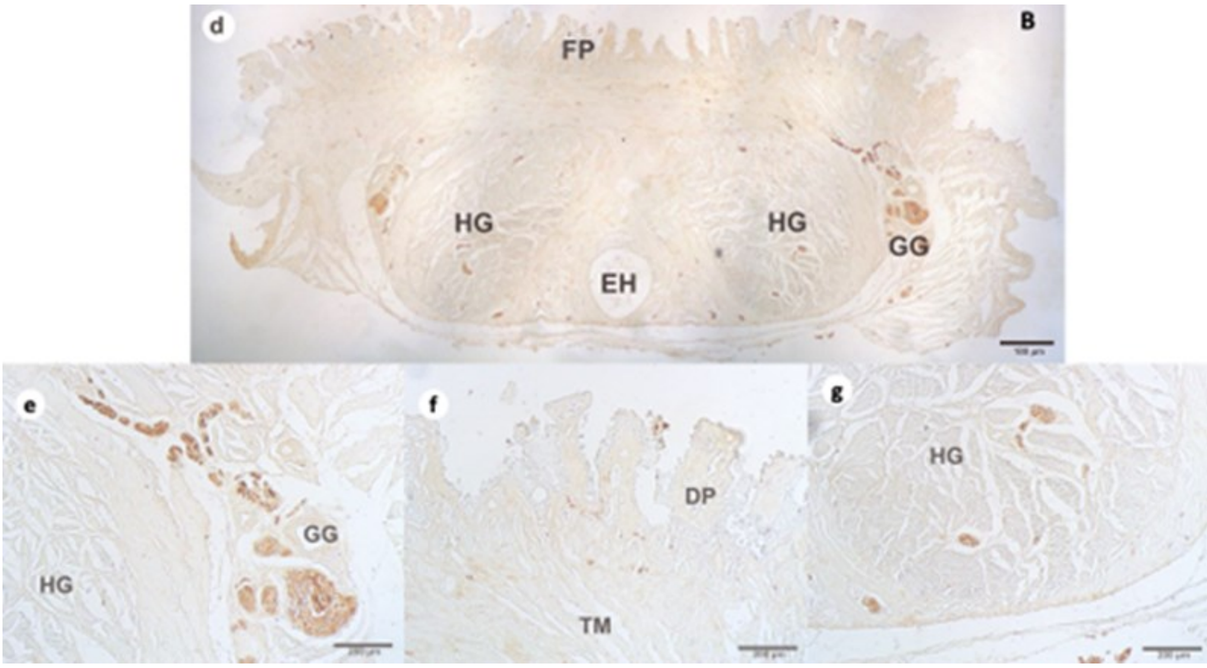

Figure 8B. Micrographs of cells immunoreactive to PGP 9.5 in the corpus. Sensory cells immunoreactive for PGP-9.5 protein are scattered in the tunica muscularis mainly on the genioglossus muscle (GG), (d) Scale bar: 100 µm; (e, f, g) Scale bar: 200 µm; Fan-shaped Papillae (FP); Dome-shaped Papillae (DP); Transverse Muscle (TM); Hyoglossus Muscle (HG), Lamina Epithelial Mucosa (LEM); Lamina muscularis (LM); Hyoglossus Muscle (HG).

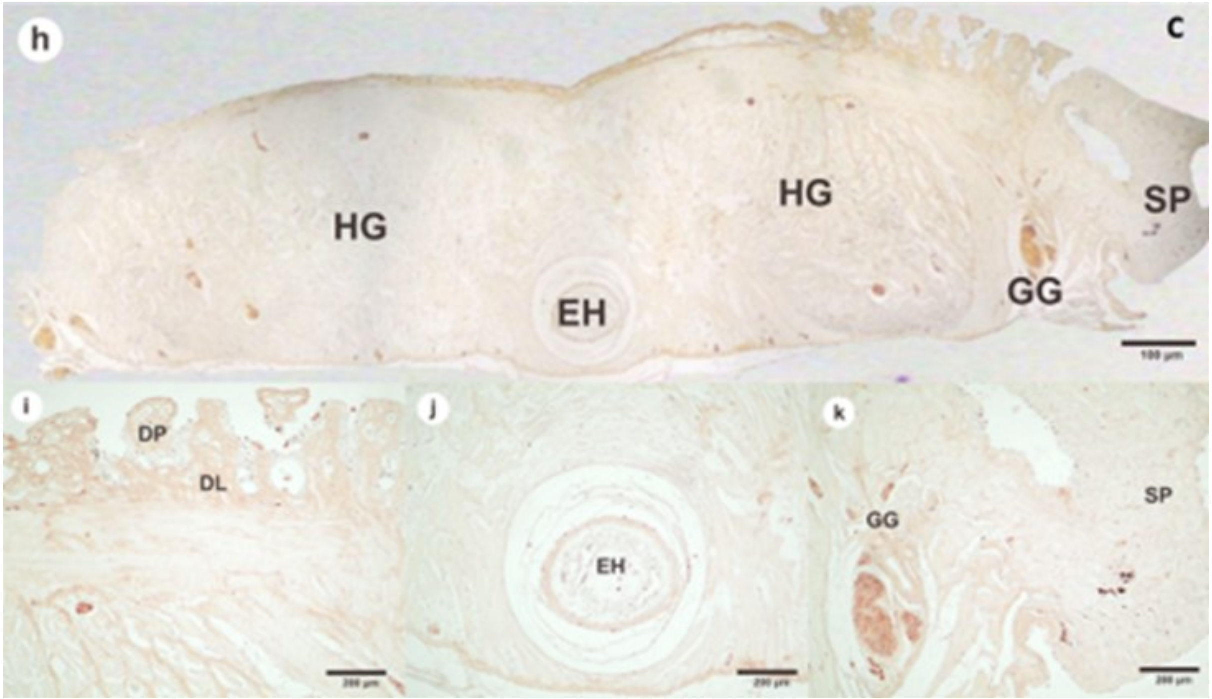

Figure 8C. Micrographs of cell immunoreactive to PGP 9.5 in the radix. The sensory cells immunoreactive to PGP-9.5 protein are present scattered in the tunica muscularis mainly on the genioglossus muscle (GG), (h) Scale bar: 100 µm; (i,j, and k) Scale bar: 200 µm; Scale–like Papillae (SP); Hyoglossus Muscle (HG); Hyoid Bone (EH).

DISCUSSION

Macroscopic observation showed that the *Gekko gecko*'s tongue has a similar shape to the tongues of other lizard species, such as *Gekko japonicus* (Iwasaki 1990), *Eublepharis macularius* (Jamniczky et al. 2009), *Uromastyx aegypticus* (Bayoumi et al. 2011), *Tarentola annularis* (Bayoumi et al. 2011), and *Hemidactylus flaviviridis* (Al-Fartwsy et al. 2016), which have triangular and dorsoventrally flattened tongues. Bifurcation is a special formation found on the apex of a reptile's tongue, dividing the tip of the apex. *Gekko gecko* shows bifurcation at the apex, but the bifurcation is extremely small and does not split the apex deeply in contrast to the bifurcation found in other reptiles, such *Elaphe climacophora* (Iwasaki et al. 1996), *Psammophis sibilans* (El-Mansi et al. 2020), *Varanus niloticus* (Sheren et al. 2018), and *Varanus bengalensis* (Pathak et al. 2015), which exhibit deep branching. Small and shallow bifurcation is also found in other lizards, such as *Gekko japonicus* (Iwasaki 1990), *Eublepharis macularius* (Jamniczky et al. 2009), *Tarentola annularis* (Bayoumi et al. 2011), and *Hemidactylus flaviviridis* (Al-Fartwsy et al. 2016). The tongue the house gecko is similar to that tongue of a plant-eating lizard. *Gekko gecko* and *Uromastyx aegypticus* have different bifurcation shapes (Bayoumi et al. 2011). According to Bayoumi et al. (2011), bifurcation in reptiles is phylogenetic in nature and thus has no significant effect on diet. However, diet can affect the morphological structure of a bifurcation. Wide and deep bifurcation in *Uromastyx aegypticus*, which is a herbivorous lizard, is suitable for cutting various types of plants, whereas a narrow and shallow bifurcation found in the insectivorous *Tarentola annularis* and *Gekko gecko* is suitable for swallowing insects (Bayoumi et al. 2011). In snakes, the tongue does not function as an organ and help in food ingestion but is used for olfaction in cooperation with the vomeronasal organ (Bayoumi et al. 2011). Difference in bifurcation shape between snakes and monitor lizards has deep ramifications, and the small and shallow bifurcation in the tongues of house geckos may be due to difference in prey search process (Schwenk 1994). Reptiles with deep bifurcations (snakes, amphisbaenians, teiids, varanids, and helodermatids) can follow prey trails through odor or scent, whereas iguanas and gekkonids showing bifurcation with small indentations do not search for prey by following scent trails (Schwenk 1994).

SEM results showed that the tongue papillae on the dorsal surface of *Gekko gecko*'s tongue were similar to those found on the tongues of other lizards, including *Takydromus tachydromoides* (Iwasaki & Miyata 1985), *Gekko japonicus* (Iwasaki 1990), *Eublepharis macularius* (Jamniczky et al. 2009), *Uromastyx aegypticus* (Bayoumi et al. 2011), *Hemidactylus flaviviridus* (Al-Fartwsy et al. 2016; Bayoumi et al. 2011), *Tarentola annularis* (Bayoumi et al. 2011), and *Uromastyx aegypticus* (Bayoumi et al. 2011). The dorsal surface of the apex of *Gekko gecko*'s tongue was smooth and flat and composed of polygonal dome-shaped papillae, which were short, uniform, and tightly arranged. The DP on the tongue of the house gecko are similar to those found on the dorsal surface of the apex in the tongues of *Gekko japonicus* (Iwasaki 1990) and *Tarentola annularis* (Bayoumi et al. 2011). According to Schwenk (2000), papillae on the apex of a gecko's tongue are smooth and flat and can be used to clean the eyes. Long and slender FP are found in the corpus. The papillae are loosely arranged, and thus the dorsal surface has an uneven and smooth surface. The FP on the corpus are similar to those found in *Gekko japonicus* (Iwasaki 1990) and *Tarentola annularis* (Bayoumi et al. 2011). Meanwhile, scale–like papillae on the dorsolateral side in the radix have tight folds resembling wide triangular scale shapes and tips leading to the caudal portion. The scale–like papillae on the radix of the house gecko's tongue are similar to those found on the radices of the tongues of *Gekko japonicus* (Iwasaki 1990) and *Tarentola annularis* (Bayoumi et al. 2011).

The histological structure was then observed using 8 μm-thick samples. The outermost layer of the *Gekko gecko*'s tongue is a LEM composed of stratified squamous epithelial cells. Clusters of goblet cells were found in the LEM in the FP located in the corpus. The mucosal LP is beneath the LEM which is composed of connective tissues. Tunica muscularis is composed of several types of muscles that are in the ventral part of the LP mucosa. The apex of the *Gekko gecko*'s tongue is histologically composed of two tunica, namely, tunica mucosa and tunica muscularis. Tunica mucosa is composed of LEM and LP mucosa. The LEM of the *Gekko gecko*'s tongue at the apex is composed of stratified squamous epithelial cells, similar to that found in the tongues of *Eublepharis macularius* (Jamniczky et al. 2009) and *Tarentola annularis* (Bayoumi et al. 2011). The tunica muscularis is composed of several types of muscles, including the dorsal longitudinal muscle in the most dorsal part of the tunica muscularis, transverse muscle, and several bundles of hyoglossus muscles in the ventral part. The muscle structure found at the apex of the *Gekko gecko*'s tongue is similar to that of *Eublepharis macularius* (Jamniczky et al. 2009). When used in cleaning the eyes, the apex of *Gekko gecko*'s tongue is assisted by several bundles of hyoglossus muscles arranged on the ventral part of the apex (Schwenk 2000). The structure of the muscles that constitutes the apex of the *Gekko gecko*'s tongue is similar to that of the *Gerrhonotus multicarinatus* lizard's tongue (Smith & Mackay 1990), where four bundles of hyoglossus muscles are found, each surrounded by a circular muscle, a transverse muscle on the dorsal part of the hyoglossus muscles, and a DL muscle (Smith & Mackay 1990). The tongue papillae at the apex of the *Gekko gecko*'s tongue—the dome-shaped papillae—appear like short stalks with flat and wide surfaces. Taste buds were not found in the apex of the *Gekko gecko*'s tongue. According to Jamniczky et al. (2009), most geckos may not have taste buds. This feature could have been influenced by the form of adaptation. Interestingly, immunohistochemical staining with PGP 9.5 showed immunoreactivity in the apex, corpus, and radix portions containing tunica muscularis, especially in the genioglossus muscles. These findings demonstrated that the gecko's tongue represented one of the most specialised compound sensory systems among vertebrates. This system may play an important role in chemical and mechanical information processing for preys. Moreover, a correlation may be hypothesised with the vomeronasal system stimulated by tongue flicking. Food odor revealed by the nasal olfaction triggers the mechanism of tongue flicking, and the vomeronasal system provides information on the volatile components of the odor (Filoramo & Schwenk 2009). Furthermore, the tongue papillae found at the apex of the *Gekko gecko*'s tongue are similar to those found at the apex of the tongue of *Eublepharis macularius* (Jamniczky et al. 2009), which are DP called cuboid papillae and at the apex of the tongue of *Tarentola annularis* (Bayoumi et al. 2011), which are DP shaped like short stalks.

The corpus and radix part of the *Gekko gecko*'s tongue are largely the same but differ in the papillae they contain. The corpus and radix of *Gekko gecko*'s tongue are histologically composed of tunica mucosa and tunica muscularis. The lining of lamina epithelialis mucosa is composed of stratified squamous epithelial cells, similar to that in the tongue of Tarentola annularis (Bayoumi et al. 2011). Tunica muscularis in the corpus and radix have similar arrangements, namely, a genioglossus muscles that forms the lateral border of the radix of the tongue and two bundles of hyoglossus muscles on the left and right portions. The entoglossal process of the hyoid bone is found on the tongue radix in the center between two hyoglossus muscles. The muscle structure that composes *Gekko gecko*'s tongue in the corpus and radix is similar to that in the tongue of *Eublepharis macularius* (Jamniczky et al. 2009); that is, two bundles of hyoglossus muscles on the right and left are separated by a connective tissue, and between the two muscles, a processus entoglossal of the hyoid bone is located in the ventral center of the tongue. The muscle structure in *Gekko gecko*'s in the corpus and radix has a similar arrangement to that in the tongue of the *Gerrhonotus multicarinatus* (Smith & Mackay 1990). Two bundles of hyoglossus and genioglossus muscles form the lateral boundary of the tongue. The histological structures of the corpus and radix of *Gekko gecko*'s tongue differ in the type of papillae. The corpus contains long and slender FP on the dorsal and lateral parts, whereas the radix contains scale–like papillae with folds resembling scales found on the dorsolateral tongue. Papillae found on the corpus and radix are similar to those found on the tongue of *Eublepharis macularius* (Jamniczky et al. 2009). FP are called leaf-like papillae on the corpus, and scale–like papillae are called fungiform papillae on the radix. The papillae on the corpus of the *Gekko gecko*'s tongue are also similar to those found on the corpus of the tongue of *Tarentola annularis* (Bayoumi et al. 2011), which also show FP. Clusters composed of goblet cells are found in the FP in the corpus and produce mucus in the oral cavity (Jamniczky et al. 2009).

The differences in the type of tongue papillae between the *Gekko gecko* and other lizards can be attributed to species-specific variations in tongue morphology. Different reptilian species exhibit distinct types of tongue papillae (Gewily et al. 2021). These variations are observed across different reptilian species and among mammals (Emura & El-Bakary 2014).

Furthermore, the presence of specific tongue papillae types, such as filiform, fungiform, foliate, and vallate papillae, are common in mammals, including rodents (Čížek et al. 2016; Wannaprasert et al. 2019). The diversity, distribution, and morphology of tongue papillae have been studied extensively in various mammals (Erdoğan et al. 2014; Toprak 2023). Additionally, the tongue papillae of different mammalian species exhibit unique characteristics, such as the presence of gustatory and nongustatory papillae, which are distributed in a species-specific manner on the tongue surface (Pastor et al. 2017). In the *Gekko gecko*, the gustatory papillae are not present.

The differences in the type of tongue papillae between *Gekko gecko* and other lizard species can be attributed to species-specific adaptations in tongue morphology. These variations reflect the diverse evolutionary adaptations that have shaped the tongue structures of reptiles and mammals, highlighting the importance of tongue papillae in various functions, such as taste perception, and mechanical manipulation of food (Jackowiak & Godynicki 2007; Manley & Kraus 2010). Additionally, the morphology of tongue papillae can vary regionally within the tongue and is influenced by factors, such as feeding habits, climate conditions, and type of food particles. Moreover, the complexity of tongue structures, including the papillae, can be linked to specific modes of adaptations of reptiles, such as geckos, which are known for their exceptional high-high frequency vision and hearing (Abbate et al. 2019).

CONCLUSION

This work offers the first description of the anatomy of the *Gekko* gecko's tongue in Yogyakarta, Indonesia, also examining the distribution of tongue papillae. This information may offer insight into *Gekko gecko*'s feeding strategies and ecological roles.

AUTHORS CONTRIBUTION

Conceptualisation, H.W.; Methodology, H.W.; Software, K.A., T.W.P., and V.B.; Validation, K.A., A., and T.B.; formal analysis, K.A., G.R.S., HyW., V.B.; Investigation, K.A. and U.K.; Resources, K.A., T.W.P., and HW.; Data Curation, K.A., A.; Writing—Original Draft Preparation, U.K., H.W., and G.R.S.; Writing—Review and Editing, H.W. and U.K.; Visualization, K.A.; Supervision, H.W.; Project Administration, U.K; Funding Acquisition, H.W. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

The authors would like to thank the Integrated Laboratory for Research and Testing Universitas Gadjah Mada for allowing us to use the scanning electron microscope and Yusuf Umardani, S.T., M.Eng. Virginia Fahrizza Amalya, S.Si. for excellent technical assistance.

CONFLICT OF INTEREST

None of the authors have any competing interests to disclose.

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