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Original Research Article

Anatomical and Histological Features of Lingual Papillae on Tongue in Squirrel (*Sciurus vulgaris*)

Key words

light microscopy;
lingual papillae;
rodent;
squirrel;
taste bud

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Abstract: This study examined the anatomical and histological features of lingual papillae in squirrels. Two adult male squirrel tongues were used as the material in the study. Three parts were detected in the tongue: apex, corpus, and radix. There was a median sulcus on the apex of the tongue and an intermolar prominence on the body of the tongue. Five types of papillae such as filiform, conical, fungiform, vallate, and foliate were observed on the tongues of the squirrels. Filiform papillae were located from the apex of the tongue to the root as the dominant papilla. Conical papillae were observed on the intermolar prominence, on the sides of the tongue root, and between the vallate papillae. The direction of this papillae was oriented caudomedially. Fungiform papillae were randomly distributed among the filiform papillae. These papillae were mushroom shaped and had slits that separated them from filiform papillae. Three vallata papillae, arranged in a triangle with the apex pointing backward, were found on the root of the tongue. These papillae were surrounded by a trench. Foliate papillae were observed like mucosal folds in the caudolateral part of the tongue. On light microscopic examination, lingual papillae were covered with stratified squamous epithelium and had connective tissues. There were varying degrees of keratinization on the epithelial surfaces of the papillae. Although taste buds were seen in the epithelial layer of the fungiform and vallate papillae, they were not observed in the epithelium of the grooves of the foliate papillae. The findings obtained in the study were compared with those obtained from the lingual papillae of other rodents, and similarities and differences were revealed.

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Introduction

Rodents are an order that includes about half of the known mammals in the world. Of the 166-167 mammals recorded in Türkiye, 65 belong to the rodent order. One of the best known rodent species is the squirrel (*Sciurus vulgaris*), which is very common in the Anatolian part of Türkiye (1).

The tongue plays an important role in nutrition, along with the other organs located in the oral cavity. The morphological and histological features of the tongue in mammals are a reflection of the differences between the diets of living things (2). In the tongue mucosa, there are two types of papillae, mechanical and gustatory, according to their functions. Mechanical papillae are keratinized. In the gustatory papillae, the epithelial layer contains taste buds sensitive to taste (3)

To date, macroscopic, light microscopic, and scanning electron microscopic studies have been carried out on the distribution, types, and structures of lingual papillae in many mammalian species. Macroscopic and light microscopic properties of both mechanical and gustatory papillae found on tongues of many species belonging to the rodent order were investigated. Accordingly, the macroscopic, light microscopic and scanning electron microscopic structures of the papillae of the mouse (4-7), rat (8-11), guinea pig (12, 13), porcupine (14, 15), hamster (16), Japanese lesser flying squirrel (17), Jentink's flying squirrel (18), greater cane rat (19), American beaver (20), capybara (21), agouti (22), hazel dormouse (23), large bamboo rat (24) and paca (25) were examined.

In the detailed literature review, it was determined that the macroscopic and light microscopic structures of the fungiform and vallate papillae, which are gustatory papillae in squirrels, were examined (26, 27). However, no study has been found on mechanical papillae and foliate papillae on the tongue. This study was conducted to reveal the anatomical and histological features of the papillae on the tongue mucosa of squirrels and to determine the similarities and differences between the data obtained from this study and the data obtained from other rodent studies.

Materials and methods

Ethical Approval

This study was conducted with permission from the Turkish Ministry of Agriculture and Forestry Management (permission number: E-21264211-288.04-7695477). Ethical approval was obtained from the Research Ethics Committee of the Veterinary Control Central Research Institute on October 26, 2022 (decision number: 2022/29).

Animal

As the study specimens, two male squirrels (*Sciurus vulgaris*) sent to Etlik Veterinary Control Central Research Institute Pathology Laboratory were used. The oral cavities of the squirrels were carefully opened and tongue samples were removed from the oral cavity. Fresh tongue specimens were washed with physiological saline. Measurements were made with the help of a digital caliper from these tongues and macroscopic photographs (Canon IXUS75 7.1 megapixel) were viewed with a digital camera.

Light microscopy

Histological tissue samples were fixed in 10% formaldehyde solution for 24 h. Samples were taken from the apex, corpus and radix parts of the dorsal part of the fixed tongues and from the ventral part of the apex of the tongue. For light microscopic evaluation; sample washing, dehydration, paraffin saturation and embedding were performed with alcohol and xylol series on an automatic tissue tracking device (Leica ASP 300S). Sections of 5 µm thickness were obtained from the prepared paraffin blocks with a microtome (Shandon/finesse) and placed on slides. These slides were kept in an oven at 60°C for 40 min. Then, it was stained with the Hematoxylin-Eosin stain method with a fully automatic (Shandon/varistain Gemini) device. The obtained preparations were examined under a light microscope with attachment (Leica DM2500 LED). Necessary assessments were made and microscopic pictures of the sections were taken.

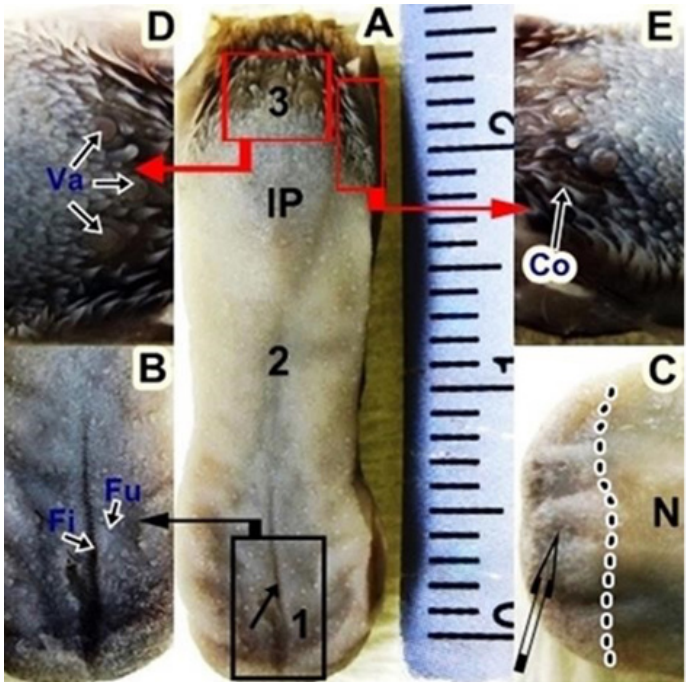

Figure 1: A: General macroscopic view of the tongue. 1: apex of tongue, 2: body of tongue, 3: root of tongue, arrow: median sulcus, IP: intermolar prominence. B: General macroscopic view from the dorsal part of the apex of the tongue. Fu: fungiform papillae, Fi: filiform papillae. C: General macroscopic view from the ventral part of the apex of the tongue. white arrow: filiform and fungiform papillae, N: nonpapillary part. D: General macroscopic view from the root of the tongue. Va: vallate papillae. E: Macroscopic view from the lateral part of the root of the tongue. Co: large conical papillae

Results

Macroscopic evaluation of the tongue

The squirrel's tongue was rectangular with an oval apex. A median sulcus was present at the apex of the tongue. A slightly prominent intermolar prominence was observed between the body and the root of the tongue. The length of the tongue was 28 mm on average, its width was 8 mm at the apex, 6 mm in the body and 9 mm at the root (Figure 1A). Five types of papillae on the tongue of squirrels were observed: filiform papillae (Figure 1B, C), conical papillae (Figure 1E), fungiform papillae (Figure 1B, C), vallate papillae (Figure 1D) and foliate papillae.

Filiform papillae

Filiform papillae were distributed on the dorsal surface of the tongue from the apex to the vallate papillae. Filiform papillae were observed as the predominant papilla type on the dorsal surface of the tongue. The tips of these papillae were caudally inclined (Figure 2A). These were also observed in the ventrolateral region of the apex of the tongue (Figure 1C). On light microscopic examination, it was determined that the filiform papillae were covered with stratified squamous epithelium. There was a varying degree of keratinized layer in the epithelial layer of these papillae (Figure 2B, C).

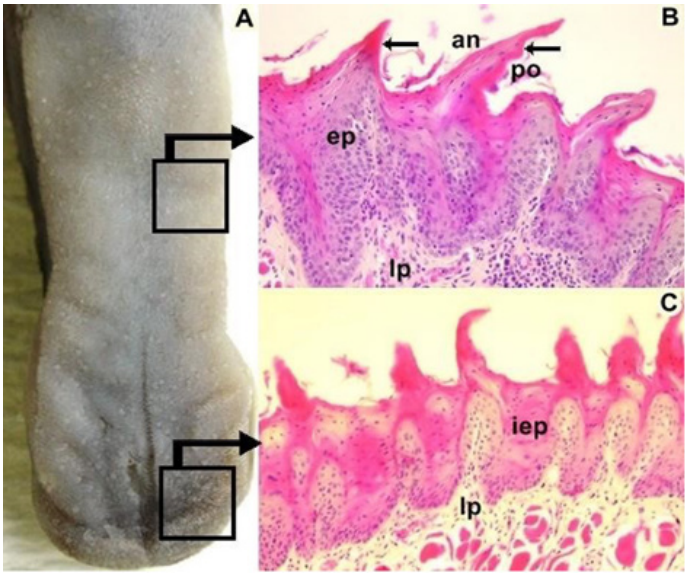

Figure 2: A: Macroscopic view of filiform papillae. B: Light microscopic view of filiform papillae on the body of the tongue. ep: epithelial layer, lp: connective tissue, an: anterior part of the filiform papilla, po: posterior part of the filiform papilla, arrows: keratin layer, H&E, 100 µm. C: Light microscopic view of the filiform papillae at the apex of the tongue. iep: interpapillary epithelial layer, lp: connective tissue, H&E, 200 µm

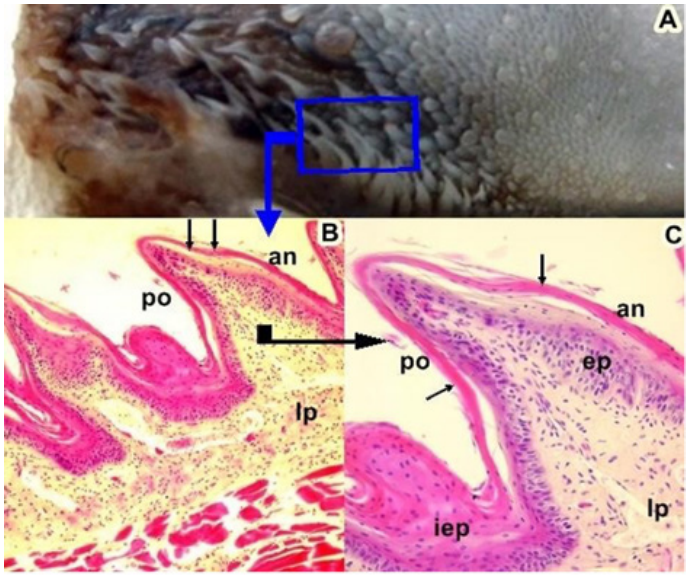

Figure 3: A: Macroscopic view of conical papillae. B: Light microscopic view of conical papillae. lp: connective tissue, an: anterior part of conical papilla, po: posterior part of conical papilla, arrows: keratin layer, H&E, 200 µm. C: Light microscopic view of a conical papilla. ep: epithelial layer, iep: interpapillary epithelial layer, lp: connective tissue, an: anterior part of conical papilla, po: posterior part of conical papilla, arrows: keratin layer H&E, 100 µm

Conical papillae

Conical papillae were detected on the intermolar prominence in the body of the tongue, on the side of the root part of the tongue and in the root part of the tongue. The conical papillae, which were on the side and behind the vallate papillae, were greater and their directions were also oriented caudomedially (Figure 3A). The conical papillae located in front of the vallate papillae and on the intermolar prominence were smaller and were directed caudally. On the microscopic examination, it was found that the conical papillae started with a thick root from the tongue mucosa and ended bluntly. The anterior and posterior parts of the conical papillae had the same thick keratin layer. The epithelial layer among the papillae was found to be thicker than the papillary epithelial layer (Figure 3B, C).

Fungiform papillae

Fungiform papillae were scattered among the filiform and conical papillae from the apex of the tongue to the vallate papillae. These papillae, round and resembling mushrooms were separated from the filiform papillae by a slit. Fungiform papillae were specially observed in the apex and body of the tongue. These papillae were not observed in the median sulcus in the middle of the apex of the tongue (Figure 4A). Fungiform papillae were also detected in the ventrolateral part of the apex of the tongue (Figure 1C). The average diameter of these papillae was around 0.34 mm. A weak keratinized layer was observed on the epithelial surface of the fungiform papillae on light microscopic examination (Figure 4B, C). Intraepithelial taste buds were observed in the dorsal epithelial layer of these papillae and their number was few. These taste buds were opened into the oral cavity through the taste pores. Dark and light-stained cells were observed in the taste buds (Figure 4D).

Vallate papillae

Three vallate papillae, arranged in a triangle with the apex pointing backward were found on the root of the tongue. They are surrounded by a circular trench that separates them from the rest of the tongue's surface. Conical papillae were present between and around these papillae. The average length of the vallate papillae was 0.96 mm, and the width was around 0.74 mm. (Figure 5A). On light microscopic examination, the taste buds of the vallate papillae were located intraepithelially in the trench-facing epithelial layer of the papillae. These taste buds were arranged perpendicular to the length of the epithelial layer and were observed in the epithelial layer up to half the trench (Figure 5B, C). Taste buds in the vallate papillae were observed to open into the papilla trenches through the taste pores. (Figure 5B). Dark and light-stained cells were detected in these taste buds (Figure 5D). While there was no keratinized layer on the epithelial surface of the vallate papillae, there was very weak keratinization on the epithelial surfaces facing the trench (Figure 5C).

Foliate papillae

Mucosal folds similar to those of foliate papillae were detected on the caudolateral sides of the root of the tongue. The number of these mucosal folds ranged from 10 to 14 (Figure 6A). On light microscopic examination, papillary protrusions and papillary clefts were quite evident. Taste

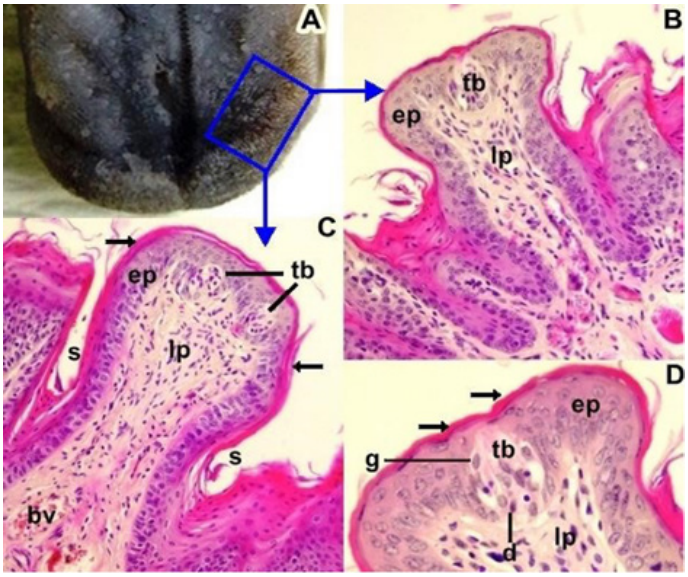

Figure 4: A: Macroscopic view of fungiform papillae. B: Light microscopic view of a fungiform papilla with one taste bud. ep: epithelial layer, lp: connective tissue, tb: taste bud, H&E, 100 µm. C: Light microscopic view of fungiform papilla with two taste buds. ep: epithelial layer, lp: connective tissue, s: papilla slits, bv: blood vessel, tb: taste buds, arrows: keratinized layer, H&E, 100 µm. D: Light microscopic view of a taste bud. ep: epithelial layer, lp: connective tissue, tb: taste bud, g: light-stained cell, d: dark-stained cell, arrows: keratinized layer, H&E, 50 µm

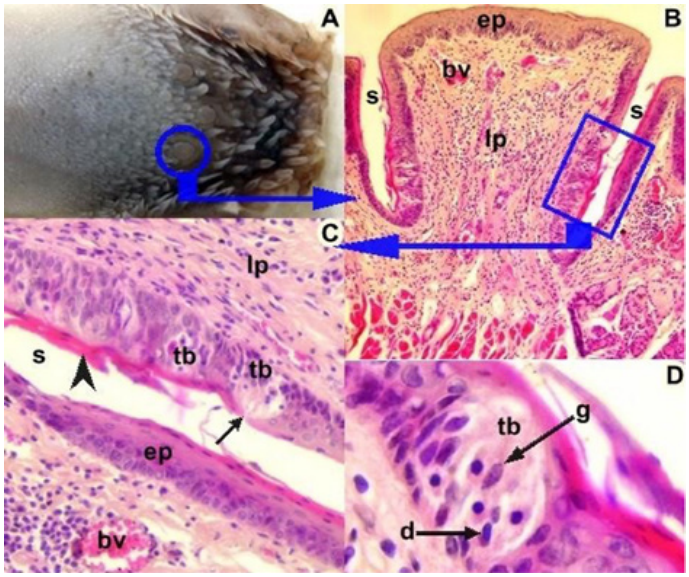

Figure 5: A: Macroscopic view of vallate papillae. B: Light microscopic view of a vallate papilla. ep: epithelial layer, lp: connective tissue, bv: blood vessel, s: trench of papilla, H&E, 200 µm. C: Light microscopic view of taste buds in the epithelial layer facing the trench in the vallate papilla. ep: epithelial layer, lp: connective tissue, s: trench of papilla, bv: blood vessel, tb: taste buds, arrow: taste pore, arrowhead: keratinized layer, H&E, 100 µm. D: Light microscopic view of a taste bud. tb: taste bud, g: light-stained cell, d: dark-stained cell, H&E, 50 µm

buds were absent in the epithelial layer facing the clefts and in the dorsal epithelium of the foliate papillae. A prominent keratinized layer was detected in the surface epithelial layer and in the epithelial layer facing the clefts of these papillae (Figure 6B, C).

Discussion

The median sulcus was detected on the apex part of the tongue in the squirrel, similar to what has been reported in the tongues of rats (8), WWCPS rat (11), porcupines (14, 15), Jentink's flying squirrel (18), agouti (22), hazel dormouse (23) and large bamboo rat (24). However, it has been reported that the median sulcus is absent in rodents such as guinea pigs (12), capybaras (21) and rock cavy (28). In this study, five types of tongue papillae filiform, conical, fungiform, vallate and foliate were detected on the tongue of the squirrel. The shape and distribution of these papillae were similar to the papillae found on the tongue of rats (8), Japanese lesser flying squirrel (17), Jentink's flying squirrel (18), hazel dormouse (23) and paca (25).

Filiform papillae were a common and abundant type of papillae on the tongue, extending from the apex to the root. The tips of these papillae were generally oriented in the caudal direction. The distribution of filiform papillae on the tongue in squirrels was similar to the distribution of filiform papillae found in the tongues of Wistar rat (10), guinea pig (12), Jentink's flying squirrel (18) and agouti (22). A weak keratinized layer was present on the epithelial surface of the filiform papillae on the tongue in squirrels, similar to reports of the filiform papillae in rats (8) and Jentink's flying squirrels (18).

Conical papillae on the tongue in squirrels were detected on the intermolar prominence located on the body of the tongue, on the sides of the root part of the tongue and on the root part of the tongue. The conical papillae located on the sides of the root part of the tongue were quite large and oriented caudomedially. Previos studies have reported that conical papillae are located in the middle of the root of the tongue in hazel dormouse (23) and on the intermolar prominence of the root of the tongue in rock cavy (28) and degu (29). The distribution of conical papillae on the tongues of Japanese lesser flying squirrel (17), Jentink's flying squirrel (18) and paca (25) were similar to our study findings. The presence of a thick keratinized layer detected on the epithelial surface of the conical papillae in this study was consistent with the expression of a keratinized layer on the surface of the conical papilla epithelium of tongues in the Jentink's flying squirrel (18) and rock cavy (28). It is considered that the large conical papillae on the tongue in squirrels are effective in sending the nutrients taken into the oral cavity to the pharynx.

Fungiform papillae were randomly distributed on the dorsal surface from the apex to the root of the tongue. These papillae were also detected in the ventrolateral part of the apex of the tongue. Some researchers reported that fungiform papillae were located on the apex and the body parts of the tongue in the rat (9), WWCPS rat (11), agouti (22), hazel dormouse (23), large bamboo rat (24), paca (25) and rock cavy (28). It has been reported that these papillae are

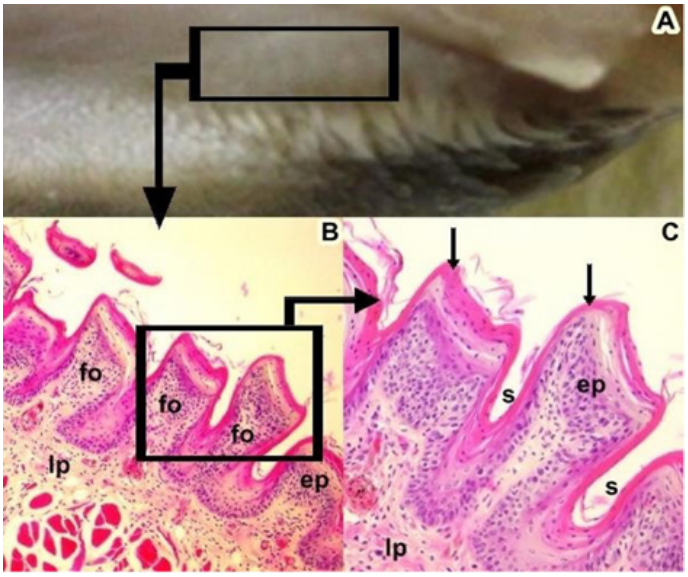

Figure 6: A: Macroscopic view of foliate papillae. B: Light microscopic view of foliate papillae. fo: foliate papillae folds, ep: epithelial layer, lp: connective tissue, H&E, 200 µm. C: Light microscopic view of two folds of foliate papillae. ep: epithelial layer, lp: connective tissue, s: grooves of papillae, arrows: keratinized layer, H&E, 100 µm

not found at the apex of the tongue in porcupines (14), they are located in the body and posterior third of the tongue and at the tip of the tongue and on the intermolar prominence in porcupines (15). It has been reported that there are no fungiform papillae on the tongue in degu (29). Contrary to the findings of this study, it has been reported that there are no fungiform papillae in the ventrolateral part of apex of the tongue in the hamster (16) and rock cavy (28). In this study, a few taste buds located intraepithelially in the dorsal region of the epithelial layer of the fungiform papilla were detected. One taste bud was observed in the fungiform papillae epithelial layer in mice (4), rat (8), Jentink's flying squirrel (18) and hazel dormouse (23). A few taste buds were detected in the epithelial layer of the fungiform papillae in guinea pig (12) and American beaver (20) and between 1 and 4 in large bamboo rats (24), and generally, 4-5 in porcupines (14). Dedection of several taste buds in the epithelial layer of the fungiform papillae in this study was similar to those reported in guinea pigs (12) and American beavers (20). The weak keratin layer detected on the fungiform papillae epithelial surface was consistent with findings in other rodent species (11, 12, 18, 22, 24, 26).

The number of vallate papillae on the root part of the tongue varies among rodent species. Accordingly, one vallate papilla was detected on the root part of the tongue in mice (7) and rats (8, 11). Two vallate papillae were observed on the root part of the tongue in the guinea pig (12), capybara (21), large bamboo rat (24), paca (25), rock cavy (28) and degu (29). Three vallate papillae were seen on the root part of the tongue in the Japanese lesser flying squirrel (17), Jentink's flying squirrel (18), American beaver (20), hazel dormouse (23) and squirrel (27). In agouti (22), there were four vallate papillae on the root of the tongue. In this study, three vallate papillae were detected on the root of the tongue, similar to those reported in the literature (17, 18, 20, 23, 27). In the squirrel, the taste buds of the vallate papillae were detected in the epithelial layer of the papillae facing the trench. These taste buds were opened to trench via taste pores. Taste buds of vallate papillae were detected in the epithelial layer on both walls of the trench in mice (7), WWCPS rats (11), guinea pigs (12, 13) and agouti (22). Taste buds of vallate papillae were observed in the epithelial layer on the interior wall of the trench in American beaver (20), Jentink's flying squirrel (18), hazel dormouse (23), large bamboo rat (24), squirrel (27) and degu (29). A weak keratinized layer was detected on the epithelium surface of vallate papillae in WWCPS rat (11), guinea pig (12), Jentink's flying squirrel (18), agouti (22) and large bamboo rat (24). A thick keratinized layer was observed on the epithelium surface of vallate papillae in guinea pigs (13), American beaver (20) and hazel dormouse (23). In this study, it was found that there was no keratinized layer on the surface of the epithelium of vallate papillae.

In the squirrel, mucosal folds similar to foliata papillae were detected in the caudolateral part of the tongue. The number of these mucosal folds ranged from 10 to 14. The number of foliate papillae mucosal folds were three in hazel dormouse (23) and four to six in mice (6). These mucosal folds were observed as five pairs of epithelial folds in the rat (9), WWCPS rat (11) and guinea pig (12). In the American beaver (20), 22-25 foliate mucosal folds were determined. Contrary to the reports that it is found caudolaterally between the body and root of the tongue in other rodent species, 170-200 foliate papillae were detected on the intermolar prominence in the dorsal part of the tongue in rats (8). In Jentink's flying squirrels (18), they were seen as mucosal folds in the dorsal part of the apex of the tongue. The absence of foliate papillae on the tongue of the large bamboo rat has been reported (24). In this study, contrary to the literature (6, 11, 12, 20, 23, 25, 28), taste buds were not observed in the epithelial layer facing the clefts of the foliate papillae mucosal folds. A prominent keratinized layer detected on the surface epithelium of foliate papillae in the squirrel was consistent with what has been reported in other rodent species (12, 23).

Conclusions

As a result, it was found that there were five types of lingual papillae, mechanical and gustatory, on the tongue of the squirrels. According to the comparison with other rodent species with lingual papillae, differences were detected, especially in conical and foliate papilla types, while other papilla types were found to have more similarities. It has been concluded that these similarities and differences can be due to the diet and nutrient selection of squirrels. It is considered that the findings of this study will contribute to the knowledge in the field of rodent anatomy and histology.

Acknowledgements

This study was conducted with permission from the Turkish Ministry of Agriculture and Forestry Management (permission number: E-21264211-288.04-7695477). Ethical Approval was obtained from the Research Ethics Committee of the Veterinary Control Central Research Institute on October 26, 2022 (decision number: 2022/29).

The availability of data and materials. Data sets analyzed during the current study are available from the corresponding author (BT) upon reasonable request.

Competing interests. There was no conflict of interest with regard to the authors reporting their findings.

Author contributions. This article was written by evaluating the anatomical and histological data planned by (BT). (BK) performed histological studies.

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Anatomske in histološke značilnosti jezičnih papil na jeziku pri veverici (*Sciurus vulgaris*)

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Izvleček: V tej študiji smo preučevali anatomske in histološke značilnosti jezičnih papil pri vevericah. V študiji smo uporabili dva jezika odraslih samcev veveric. Določili smo tri dele jezika: vrh, telo in koren. Na vrhnjem delu jezika je bila sredinska brazda, na telesu jezika pa medmolarna izboklina. Na jezikih veveric smo opazili pet vrst papil: nitaste, konične, gobaste, otočkaste in listaste. Nitaste papile so bile prevladujoče, prisotne od vrha do korena jezika. Konične papile so bile prisotne na intermolarnem izrastku, na straneh korena jezika in med otočkastimi papilami. Usmerjene so bile kavdomedialno. Gobaste papile so bile naključno razporejene med nitastimi papilami. Te papile so bile v obliki gobe z režami, ki so jih ločevale od filiformnih papil. Na korenu jezika so bile tri otočkaste papile razporejene v trikotnik z nazaj obrnjenim vrhom. Obdane so bile z jarkom. Listaste papile so bile v obliki gube sluznice v kavdolateralnem delu jezika. Pod svetlobnim mikroskopom so bile jezične papile pokrite z večskladnim ploščatim epitelijem, imele so vezivno tkivo. Na epitelijskih površinah papil so bile vidne različne stopnje poroženevanja. Čeprav so bile v epitelijski plasti gobastih in otočkastih papil vidne brbončice, jih v epiteliju žlebov listastih papil ni bilo opaziti. Ugotovitve, pridobljene v tej študiji, smo primerjali z ugotovitvami študij jezičnih papil drugih glodavcev ter prikazali podobnosti in razlike med njimi.

Ključne besede: svetlobna mikroskopija; jezične papile; glodavec; veverica; brbončica