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DOI: 10.5603/FM.a2021.0023

Article type: Original article

Submitted: 2020-11-26

Accepted: 2021-01-27

Published online: 2021-03-01

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Morphological structure of the tongue of the European badger (*Meles meles*)
A. Haligur et al., Tongue morphology in badger

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ABSTRACT
This study aimed to reveal the morphological aspects of the tongue and investigate the histologic and scanning electron microscopic (SEM) findings of the papillae on the tongue of the European badger. Two adult European badgers were used as material. The tongues of the European badgers were removed via the dissection method, and morphometric measurements were taken. The tongues were fixed in 10% buffered formalin solution for histological examination, and the SEM procedure was applied to the tongues. Three types of papillae were observed on the tongues: filiform, fungiform, vallate papillae. The different shapes (hook or conical structures) of filiform papillae were observed on the whole tongue. Fungiform papillae were found scattered in filiform papillae. Six vallate papillae were found on the dorsal face of the middle and root of the tongues. In the root of the tongue, the lingual tonsil was observed in the lentiform shape. It is thought that this research will provide contributions to morphological knowledge about badger tongue that species poor known.

Key words: badger, morphology, papillae, SEM, wild animal
INTRODUCTION

The European badger (*Meles meles*) belongs to the order Carnivora of the Mustelidea subfamily and is found all around the world [1, 2]. The badger population in Turkey is endangered, and therefore these animals are protected through an international agreement with BERN and CITES [3].

The diet of the badger consists of species of larvae, pupae, and adults of different species of insects, small animal species, such as worms and snails, and animals, including frogs, reptiles, mice, and small rabbits. It consumes more herbal nutrients than other carnivore species. The badger uses its tongue similar to Carnivora [4].

Many studies have been conducted to investigate the tongue of different animals using scanning electron microscopic (SEM), histological, morphological or functional analyses. Among the animals investigated are the civet cat [5], silver fox [6], porcupine [7, 8], mice [9], Asian black bear and panther [10], bush dog [11], Japanese badger [12] and mongoose [13, 14]. The aim of this study was to determine the possible SEM, histological and morphological changes in the papillae and tongue of the European badger. These results were compared with other studies in the literature to emphasize the morphologic and microscopic diversities among species. This study is also hoped to contribute to improving our current knowledge related to the badger.

MATERIALS AND METHODS

This study was performed with permission from the General Directorate of Nature Conservation and National Parks of the Ministry of Forestry and Water Affairs (Permission number: 38002405-445.05-177733). Also this study was accepted by the animal experiments local ethics committee of the Cukurova University (decision number: 2017/6-5).

In this study, two dead adult European badgers (*Meles meles*) [2 female (9.21-11.72 kg)] were used as materials. This animals died by traffic accident in Ceyhan region. Classic dissection method was made and removed the tongue from the head. Morphometric measurements were taken from the tongue by using a digital caliper. The methods used in tissue processing are described below.

Histological procedure
After the tongue of European badgers had been cleaned with physiological saline solution and examined anatomically, the appropriate sized parts of the tongue were fixed in 10% formaldehyde for 24 hours in order to preserve the tissue structure in the nearest living state and facilitate to get thin sections by increasing hardness. Tissue samples were washed for removing of formaldehyde and passed through a series of low to high alcohol grades to remove water. After paraffin embedding, the blocks were cut transversely into 5 µm sections and stained with haematoxylin and eosin (H&E) for general histological examination. After staining, it was dehydrated by passing through alcohol series, cleared in xylene and mounted with entellan. The slides were examined histologically using by light microscope (Lyca) and taken its photographs with Lyca digital camera.

**SEM procedure**

First of all, trimmed specimens (0.5 ×0.5 cm) were washed twice with 0.1 M (pH 7.4) phosphate buffer solution and then were kept in 2.5% gluteraldehyde solution for two days at 4°C. After that period, the tissues were kept in 1% osmiumtetroxide (OsO₄) for 1 hour. The tissues were passed through sequential acetone series and dried with critical point drying (CPD). They were coated with gold by using Polaron SC7620 Sputter Coater and finally the images were taken with LEICA LEO 440 trade scanning electron microscopy in various magnifications.

**RESULTS**

**Morphological findings**

The tongue of the badger is elongated with an oval flat apex (Figure 1). In this study, the tongue was examined in three parts: the tip, middle and root. The length of the tongue from the tip to the root was measured as 8.4 cm and 8.7 cm. The thickness was 0.128-0.172 cm at the tip, 1.326-1.393 cm at the center, and 1.667 -1.701 cm at the root. The width of the tongue was 2.315-2.394 cm at the tip and 2.669-2.704 cm at the root. Accordingly, a significant expansion of the tongue was observed.

There were six vallate papillae on the tongue. The vallate papilla was seen as irregular; so, it was not in a V shape. It was determined that the cranial first two of these papillae were prominent, and the last papilla was less pronounced. Especially in the
middle part of the midline near the corpus, fungiform papillae were visualized in the external aspect. Filiform papillae were found along with the entire tongue. On both lateral sides of the tongue, it was seen that the shape of the filiform papillae changed in the caudal 1/3 plane. In the caudal part of the tongue, filiform papillae were present but the number and shape were more pronounced and less in number than in the apex. Foliate papillae were not observed in any part of the tongue.

**Histological findings**

Filiform papillae were composed of stratified squamous epithelium (Figure 2/A). The dorsal surface of the papillae was observed to have prominent keratinization structure. Fungiform papillae were observed among the filiform papilla (Figure 2/B). Conical and hook-shaped papillae were found in sections taken from the root of the tongue (Figure 2/C). These were localized between the filiformis papillae. There were several taste buds covered with squamous epithelium (Figure 2/D). The top of the papillae vallate was seen to slightly collapse inwards, forming a wide encircling groove. The vallate papillae were found to be surrounded by several papillary projections.

**SEM findings**

In the SEM analyses of the tongues, the filiform (Figures 3/1, 2-f), fungiform (Figures 3/1, 2-fu), vallate papillae (Figures 3/3, 4-v) were identified. In addition to these main papillae, hook (Figure 3/3-h) and conical papillae (Figure 3/3-c) were seen on the dorsal surface of the European badger tongue.

The examination of the SEM images of both European badgers revealed that the dorsal surface of the filiform papillae of the middle was flattened. These papillae were observed to approach the caudal of the tongue, and looked like an arrow. The cranial part of the papillae was flat on the dorsal surface of the tongue. The number of filiform papillae was decreased toward the back of the tongue. However, these papillae were seen less than in the remaining parts of the tongue. In the same area, some of the filiform papillae were observed to have gland ducts.

Fungiform papillae were found scattered. Especially in the middle tongue, fungiform papillae were higher in number than filiform papillae, and they had an
irregular ovoid shape. In addition, fungiform papillae were extensively located on the 1/3 middle part of the tongue bilateral but they were not seen at the end of the tongue.

The vallate papillae were mostly found on the dorsal face of the middle and end of the tongue. A total of six vallata papillae were seen and four of these structures differed from the others. These four vallata papillae were faintly observed while the remaining two were marked. The top of the vallata papillae collapsed inwards, and their edges were fragmented. Papillae foliate were not observed, but the lateral edges of the tongue were differentiated in terms of filiform papillae, which did not contain a ductal structure. At the root of the tongue were lentiform-shaped structures of lingual tonsillae. These structures were located under the epiglottis and arranged in a sequential “U” shape.

**DISCUSSION**

The position of the papillae, structure, shape and length of the tongue vary among animal species. The length of the tongue was reported to be approximately 9 cm for young lions [15], 7-8 cm for the silver fox [6], raccoon dog and fox [16], 20 cm for tigers [17], 7-7.5 cm for the Japanese badger [12], 12.3 cm for pumas [18] and in 6-7 cm for porcupines [7, 8]. In this study, the length of the European badger tongue was measured as 8.4-8.7 cm. It was also found that the length of the tongue differed from the other species, which was considered to be due to the habitat.

In the silver fox [6], puma [18], tiger [17], young lion [15] and Carnivora [11, 19], the filiform papillae are found from the apex linguæ to the radix linguæ on the dorsal surface of the tongue. In addition, these papillae are seen on the ventral surface of the tongue, especially lateral edges in the tiger [17], in puma [18], young lion [15] and silver fox [6]. These papillae are located on the median channel of the dorsal surface of the tongue and have a similar to the hook of a meerkat [20]. These papillae have different types (bifurcation, papule-like, cylindrical, conical and blunt) in pumas [18]. In porcupines [8] they have two different shapes (one flat and the other cylindrical). On the margin of the tiger tongue, filiform papillae are divided into hemispherical, horny-shaped, giant club-shaped, conical, hoe-shaped or bifid types in the different parts of the tongue [17]. The current study was carried out with the European badger and revealed the presence of filiform papillae on the whole surface of the tongue (mostly at the tip
and middle tongue). These papillae were similar both in terms of type and location to the meerkat [20], raccoon dog and fox tongue [16]. In the present study, another finding related to the filiform papillae was the presence of keratinized areas in these papillae, which is consistent with the finding reported by Selim and Samir [14]. In addition, unlike the Japanese badger [12], these papillae were detected in a small number in the root of the tongue (in hook, conic and filiform shapes). The epithelial structures of the filiform papillae in the European badger were similar to those of young lions [15].

In contrast to the literature [14, 17], in this study, the fungiform papillae were located in the posterior (tip) part of the tongue. Moreover, these papillae were generally observed on both sides of the middle part of the tongue. The shape of the fungiform papillae is reported to be flat at the upper surface in the silver fox [6], and smooth and dome-like in the Japanese badger [12]. These papillae are located on the apex and lateral region of the tongue and have as mushroom-like shape in squirrels [21] and a hemispherical or horn shape on the margin of the tongue in the tiger [17]. In this study, different from the literature [6, 21, 22, 23] the fungiform papillae were found to have an irregular ovoid shape, and extensively observed after the 1/3 middle part of the European badger tongue bilaterally. A few taste buds were found at the top of the fungiform papillae in the Japanese badger [12]. In this study, some of the buds were seen on both the upper and ventrolateral surfaces of the fungiform papillae as different from the Japanese badger [12].

The number of vallate papillae varies according to species; e.g., four for the silver fox [6] and puma [18], six for the civet cat [5] and Japanese badger [12], 7-8 for the Asian black bear and 5-9 for lion [24], 4 for tigers [17] and 28 for porcupines [7]. In the present study, the number of papillae was six as in the civet cat [5] and Japanese badger [12]. The vallate papillae are in a “V” shape in other species of animals; however, in the European badger, they were not regularly shaped, similar to the case observed in the raccoon dog and fox [16]. While the location of the papillae was similar to that of other animals, they had a different distribution. In addition, while the cranial first two filiform papillae were quite prominent, the others were not as pronounced in the European badger. This is another finding that differentiates the European badger tongue from the other animal species.
In the literature, it is that the civet cat [5] has two foliate papillae and occur from 4-5 laminas in silver fox [6]. Foliate papillae are not present in young lions [15], pumas [18] and tigers [17]. In this study, foliate papillae were not seen. The bilateral edges of the root tongue were similar to those of the Japanese badger [12] reported by Yoshimura.

CONCLUSIONS

In conclusion, this study shows that the number and position of papillae in the tongue of wild animals differ between wild species. It is considered that the anatomic, histological and SEM findings of the data obtained from the present study will contribute to the literature.

Acknowledgement

This study was supported by Cukurova University Scientific Research Projects (Number: TSA-2017-8852).

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**Figure 1.** The general appearance of the dorsal surface of European Badger tongue. D: Different form of papillae. Fu: Fungiform papillae, V: Vallate papillae, D: Differentiated filiform papillae.

**Figure 2.** Histological sections of different regions of the tongue. A: Filiform papillae in the apex tongue, HxE, Bar=50 μm. B: Fungiform and filiform papillae in the middle of the tongue (F: Filiform papillae-arrows, Fu: Fungiform papillae-arrows), HxE, Bar=50μm. C: Appearance of the different papillae on the root of the tongue, (Co: Conic papillae-arrow, F: Filiform papillae-arrow and H: Hook shaped-arrow) HxE, Bar=50 μm. D: Fungiform papillae on the middle tongue, (Tb: Taste buds-arrows) HxE, Bar=50 μm.

**Figure 3.** The tip of tongue and general appearance of the dorsal surface papillae. 1. Localization of the papillae fungiformes and scattered within the filiform papillae (fu: Fungiform papillae, f: Filiform papillae), SEM magnification 65X, Bar=200 µm. 2. General aspect of the middle of the tongue (fu: Fungiform papillae, f: Filiform papillae), SEM magnification 100X, Bar=200 µm. 3. Markedly vallata papillae and surrounding structure (c: conic papillae, h: hook shape papillae, v: vallate papillae), SEM magnification 51X, Bar=200 µm. 4. Longitudinal section of the vallate papillae (v: vallate papillae), SEM magnification 150X, Bar=200 µm.

**Figure 4.** General appearance of the dorsal surface of papillae and lingual tonsillae on root of tongue (C: conic papillae, L: lentiform structure of lingual papillae), SEM magnification 58X, Bar=200 µm.
