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MORPHOLOGICAL STUDY OF THE BRANDT'S HEDGEHOG, *PARAECHINUS HYPOMELAS* (EULIPOTYPHLA, ERINACEIDAE), TONGUE

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Morphological Study of the Brandt's Hedgehog, *Paraechinus hypomelas* (Eulipotyphla, Erinaceidae), Tongue. Goodarzi, N., Azarhoosh, M. — The morphology and histological structure of two adult Brandt's hedgehog, *Paraechinus hypomelas*, (Brandt, 1836) tongue were examined by light and scanning electron microscopy. On the dorsal surface of the tongue, three types of papillae were observed: filiform, fungiform and vallate papillae. Apex and corpus of the tongue as well as the lateral surface of the corpus were covered with numerous filiform papillae with bifurcated tip, while the epithelium lining the ventral lingual surface was free from papillae. Discoid shape fungiform papillae were scattered over the entire surface of the lingual apex, corpus and lateral surface uniformly between the filiform ones without regional variation in number and size. Three elliptical or oval vallate papillae in an inverted triangle form were found on the root of the tongue. Each papilla had a lobulated and very irregular dorsal surface. Both fungiform and vallate papillae contain taste buds. The foliate papillae was absent. Overall, the present findings reveal that despite some similarities, the lingual papillae of the Brandt's hedgehog as an omnivore animal has spices-specific characteristics compare to the *Erinaceous auritus* as an insectivore species. This finding provides a set of basic data about the morphology of tongue and its lingual papillae in Brandt's hedgehog.

Key words: Brandt's hedgehog, lingual papillae, morphology, tongue, scanning electron microscopy.

Introduction

Animal feeding mechanism is an important factor that helps to adaptation to environment and survival (Darwish, 2012). In this mechanism, the tongue as a principal organ, plays a pivotal role. Further, it has been established that there are a close correlations between anatomical structure of tongue and its functional roles such as food transport and manipulation from one side, and habitat in which animal lives on the other (McClung, Goldberg, 2000; Schwenk, 2000; Iwasaki, 2002; Darwish, 2012).

Many studies in various mammalian species indicate that there is a wide variability in morphological features of lingual papillae and a close correlation between lingual papillae structure, feeding habits and taxonomy (Jackowiak, Godinicki, 2005). Although, this variability is remarkable between high systemic units, such as orders and families, interspecies differences are also seen frequently (Iwasaki, 2002; Kobayashi et al., 2005; Emura et al., 2006; Jackowiak et al., 2004).

Structure and distribution of the lingual papillae in different mammalian species include rodents (Kobayashi et al., 1989; Wolczuk, 2014; Nonaka et al., 2008; Goodarzi, 2014; Ciena et al., 2013; Shindo et al., 2006; Watanabe et al., 1997; Kilinc et al., 2010), carnivores (Emura et al., 2013; Emura et al., 2002; Kobayashi et al., 1988; Iwasaki, Miyata, 1989), ruminants (Quayyum et al., 1988; Kumar et al., 1998; Scala et al., 1993; Eerdunchaolu et al., 2001; Goodarzi, Shah-hoseini, 2015; Kurtul, Atalgin, 2008). were the subject of studies.

Brandt's hedgehog (*Paraechinus hypomelas*) is a member of the Erinaceidae family which lives in arid desert and mountainous areas of the Middle East and Middle Asia. This species has an average weight about 500–1,000 g and average body length about 25 cm (Hutterer et al., 2005).

The aim of the present study was to investigate the microscopic structure of the tongue as well as the morphological characteristics of its papillae in the Brandt's hedgehog (*Paraechinus hypomelas*) using light and scanning electron microscopy.

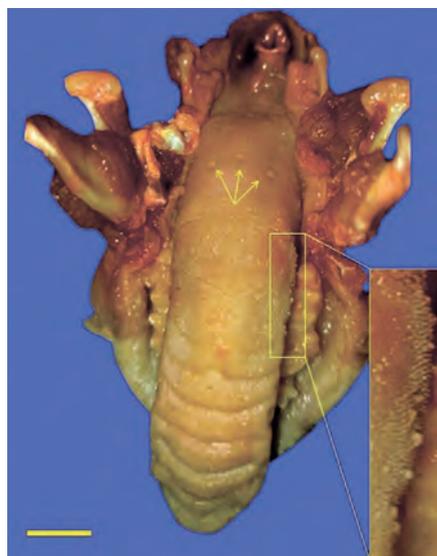


Fig. 1. Macroscopic appearance of the dorsal surface of the tongue of Brandt's hedgehog: arrows point to vallate papillae, right lower rectangle magnifies the filiform and fungiform papillae on the lateral surface of the tongue. Scale bar 5 mm.

Material and methods

Animals. Two male animals with an average body weight 1150 ± 250 g and poor physical conditions were found from the rural region around the city of Kermanshah, west of Iran and used for this study. The subjects were euthanized with chloroform inhalation.

Light microscopy. Following immediate dissection, the tongues were fixed in 10 % neutral buffer formaldehyde for two weeks. After this time, the specimens were cut into three parts; apex, body and root and were processed and embedded in paraffin for light microscopic observations. The paraffin embedded blocks were sectioned at $5\mu\text{m}$ thickness and stained with hematoxylin and eosin and Masson's trichrome.

Scanning electron microscopy. For electron microscopy, the tongues were fixed in 2.5 % glutaraldehyde for 48 h. The samples were then post-fixed with 1 % osmium tetroxide solution. After dehydration in ascending concentration of ethanol series, the specimens were dried at critical point drier, mounted on the aluminum Stubs and coated with gold. The specimens were examined at various angles under a scanning electron microscope (VEGA, TESCAN, Brno, Czech Republic) at accelerating voltages of 15 kV.

Results

In macroscopic observation, no lingual prominence and median sulcus were observed on the tongue. Numerous filiform papillae were visible on the dorsal surface of the tongue with fungiform papillae scattered among them. The lateral surface of the tongue contains taller filiform papillae and a few fungiform papillae (fig. 1). Three oval vallate papillae were seen posteriorly on the root of the tongue. The arrangement of vallate papillae was so that they were located on the corners of a triangle. One was directed towards the pharynx and two another — towards the apex of the tongue (fig. 1).

Light microscopic examinations showed that the tongue consisted of three layers; the mucosa, submucosa and muscular layer (fig. 2). The mucosa has cornfield stratified squamous epithelium and shows tree type of the lingual papillae; filiform, fungiform

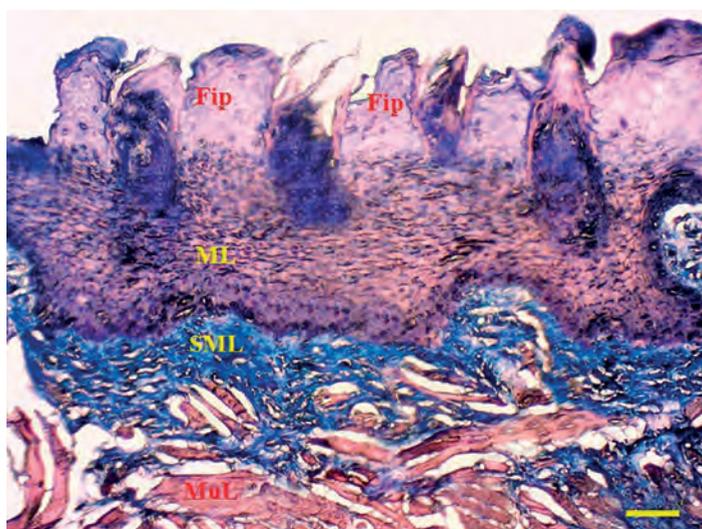


Fig. 2. Light microscopic micrograph of a cross section of the lingual apex showing: ML mucosal layer, SML submucosal layer, MuL muscular layer, Fip filiform papillae. Masson's trichrome staining. Scale bar $100\mu\text{m}$.

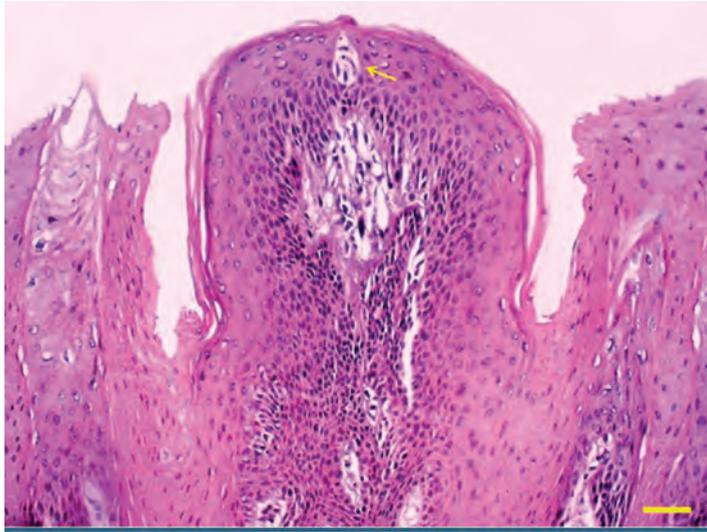


Fig. 3. Cross section of the lingual radix showing a vallate papillae. Arrow points to the taste bud. Hematoxylin and eosin (H&E) staining. Scale bar 50 μm .

and vallate papillae, while the epithelium of the ventral lingual surface has no papillae. The submucosa was seen as a dense connective tissue with Masson's trichrome staining. The muscular layer consisted of muscle fibers with longitudinal, transverse and vertical orientation. At cross section of the vallate and fungiform papillae the taste bud was observed opening onto the lingual surface (fig. 3).

In scanning electron microscopic examination, the dorsal surface of the anterior two-third of the tongue bore numerous long, slender filiform papillae with bifurcated sharp tip which tended to bend caudally. A bulb-like structure formed the basal portion of these

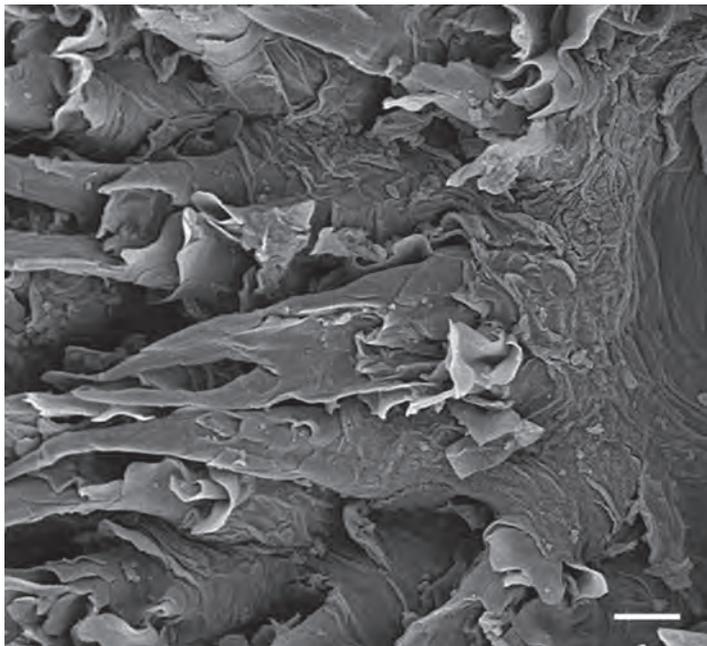


Fig. 4. SEM micrograph of the lingual corpus showing the filiform papillae with bifurcated tip which tend to bend caudally. Scale bar 25 μm .

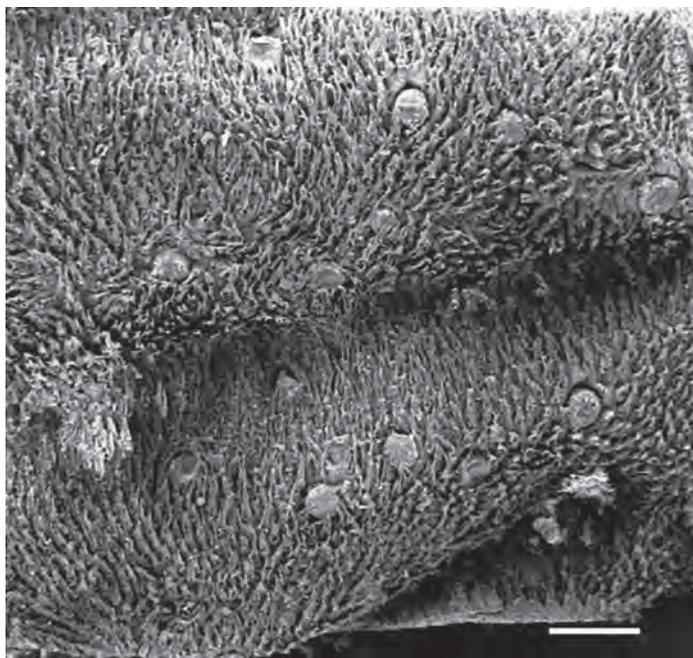


Fig. 5. SEM micrograph of the lingual corpus with filiform papillae which directed toward the midline of the tongue. Scale bar 500 μm .

papillae (fig. 4). The filiform papillae of the lingual apex were oriented caudally, whereas, some of the papillae of the lingual corpus were directed towards the midline of the tongue (fig. 5). Furthermore, the first ones were more slender than later ones and have a single tip

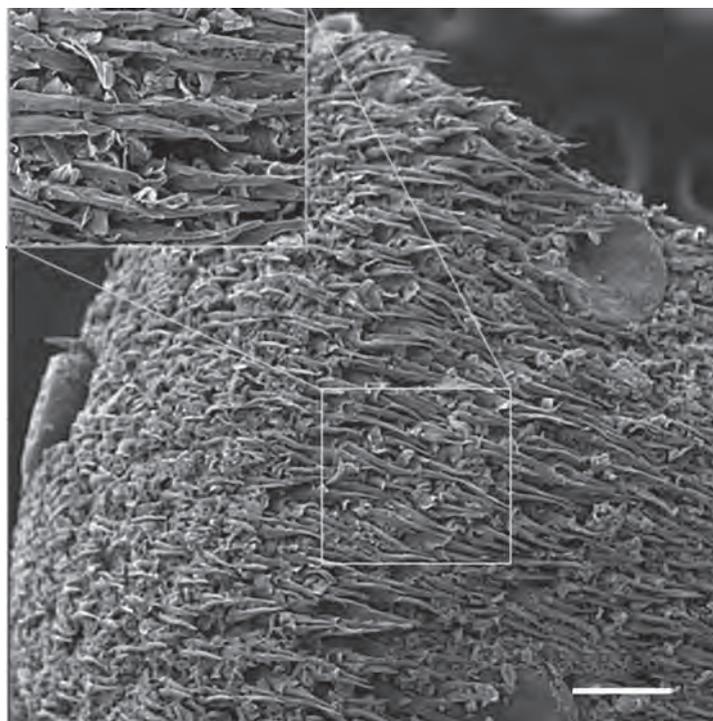


Fig. 6. SEM micrograph of the lingual apex showing slender and caudally directed filiform papillae with single tip. Scale bar 200 μm .

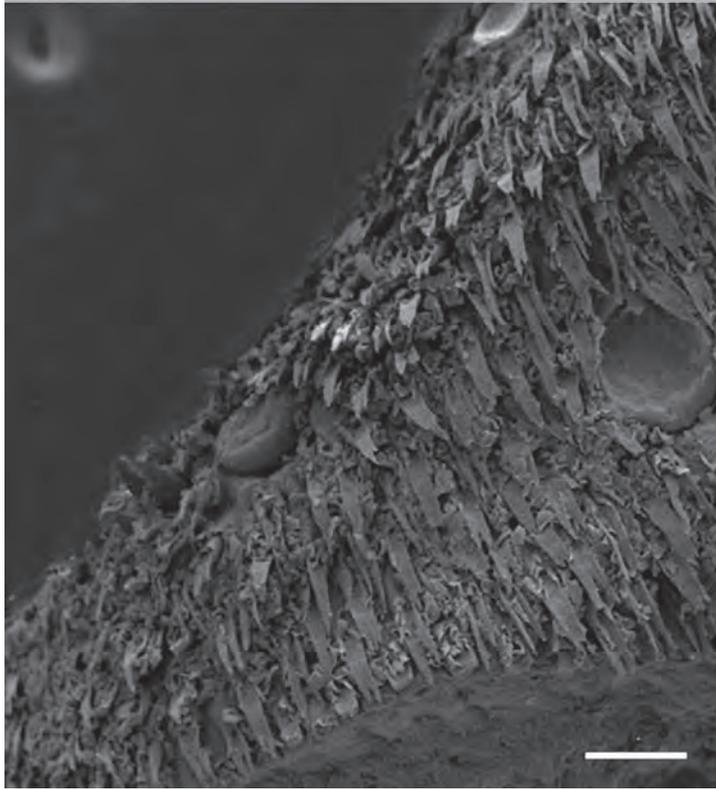


Fig. 7. SEM micrograph of the lateral surface of the tongue which contains the ventrally directed filiform papillae and discoide fungiform papillae. Scale bar 200 μm .

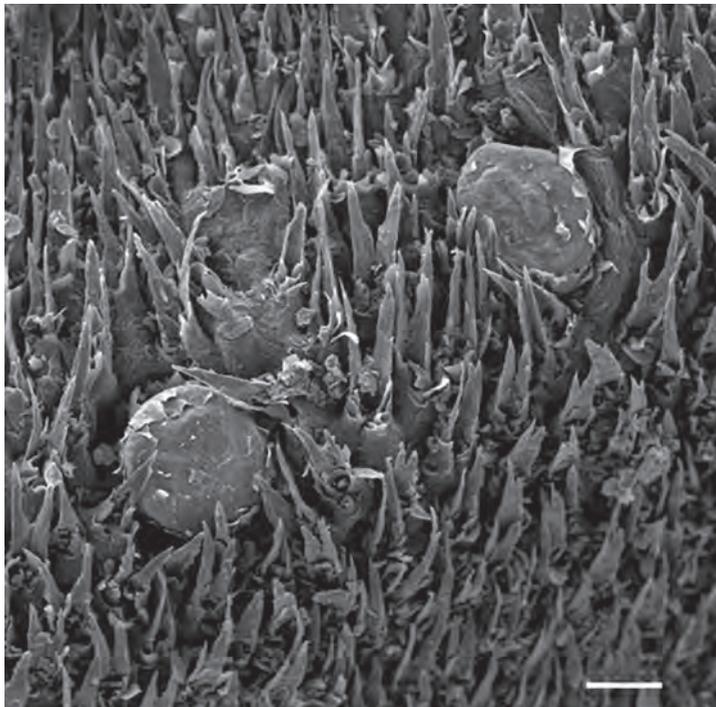


Fig. 8. SEM micrograph of the lingual corpus showing discoide fungiform papillae among the filiform papillae. Scale bar 100 μm .

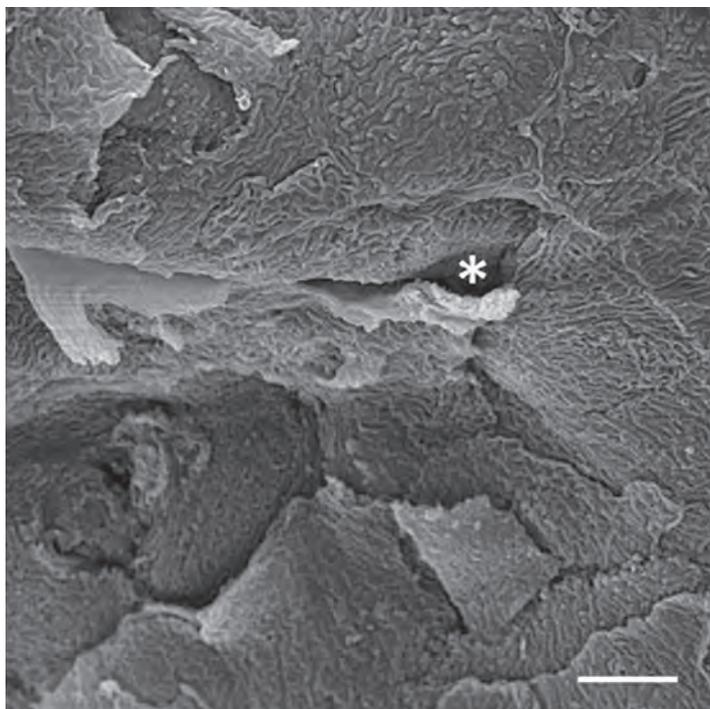


Fig. 9. SEM micrograph of the dorsal surface of the fungiform papillae at higher magnification. Asterisk indicates a taste pore. Scale bar 5 μm .

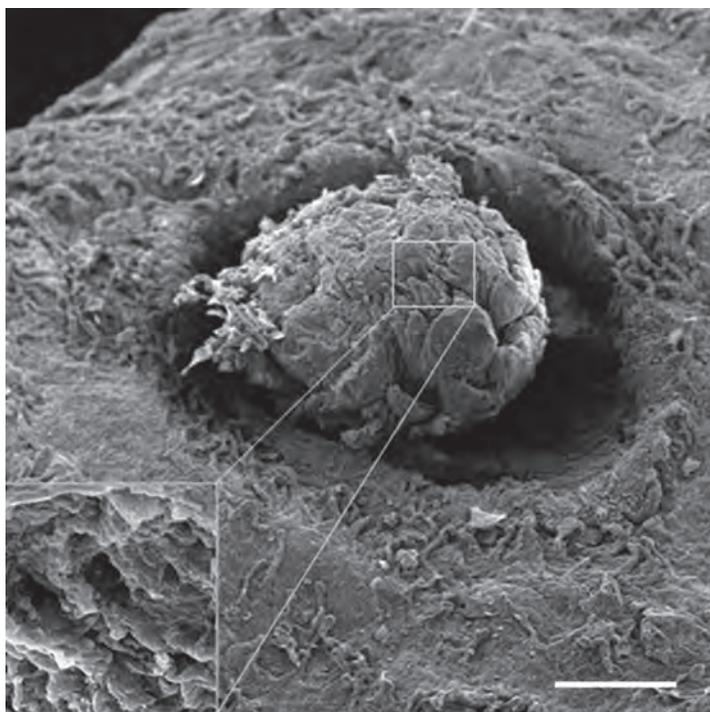


Fig. 10. SEM micrograph of the lingual radix showing vallate papillae. Note to the lobulated and irregular surface of the papillae and the shallow gustatory groove surrounded it. Left lower rectangle shows a taste pore at higher magnification. Scale bar 200 μm .

(fig. 6). No filiform papillae were seen on the lingual radix. The lateral surface of the tongue was covered with ventrally-oriented filiform papillae (fig. 7). The discoid fungiform papillae were scattered among the filiform papillae in the anterior two-third of the dorsal lingual surface. Each fungiform has a concave dorsal surface and was encircled by a narrow papillary groove (fig. 8). A few fungiform papillae were also seen on the lateral lingual surface (fig. 7). At higher magnification, taste pores could be seen between the shingle-like flattened cells of a stratified squamous epithelium covered the surface (fig. 9). On the lingual radix, the body of the vallate papillae was encircled by a shallow continuous gustatory groove and a thin annular pad or vallium of the lingual mucosa. The dorsal surface of the vallate papillae was highly irregular which appears to be lobulated and epithelial lining revealed irregular microplacae (fig. 10). No foliate papillae were seen.

Discussion

The lingual prominence presents in many species such as rodents (Ciena et al., 2013; Shindo et al., 2006; Kilinc et al., 2010) and ruminants (Zheng, Kobayashi, 2006), while it was not seen in carnivores and Pigs (Emura et al., 2006; Kumar, Bate, 2004). In general, lingual prominence is a characteristic of herbivore species which helps them to grind food (Shindo et al., 2006). The tongue of the Brandt's hedgehog (*Paraechinus hypomelas*) has no lingual prominence or median sulcus. The Brandt's hedgehog considered as omnivorous species feeding on insects, snails, frogs and toads, snakes, bird eggs, carrion, mushrooms, grass roots, berries, melons and watermelons. The median sulcus is reported on the apex of the tongue of many rodents including rat, mouse, bank vole and American beaver as a specific feature (Kobayashi et al., 1989; Iwasaki et al., 1997; Jackowiak, Godynicki, 2005; Shindo et al., 2006), with varying length and width. The median sulcus was not seen in the guinea pig (Kobayashi, 1990). Sabry et al. (2015) reported a distinct median sulcus in the tongue of *Erinaceous auritus* which is in contrast with our finding.

Lingual papillae are different with respect to their shape, size, number, orientation and distribution between various mammalian species (Iwasaki, 1992; Iwasaki et al., 1996; Abumandour, El-Bakary, 2013). This may be due to the different diet, feeding habits and handling of food in mouth (Emura et al., 2002).

In the present study three types of lingual papillae were found on the lingual mucosa of the Brandt's hedgehog. One type of mechanical papillae as filiform papillae, and two types of gustatory papillae namely fungiform and vallate papillae.

The filiform papillae have a wide variety of morphological structures among mammalian species. These variations in filiform structure could be due to masticatory methods and dietary habits (Yoshimura et al., 2009). Iwasaki et al. (1987 a, b) reported that in rats and mice three distinct types of filiform papillae are found in three different parts of the tongue, in particular: simple cylindrical in the anterior; large, conical on the intermolar eminence, and small conical in the posterior. Furthermore, it seems that the variation in the filiform papillae morphology is related to the geographical region where species is distributed. In this regard, Abumandour, El-Bakary (2013) described six types of filiform papillae in Egyptian fruit bat captured from Egypt, while Jackowiak et al. (2009) found three types of these papillae in Egyptian fruit bat captured from Poland.

The filiform papillae found in the present study were similar to those reported in *Dasyopus hybridus* (Ciuccio et al., 2010), mice (Toprak, 2006) and the Persian squirrel (Goodarzi, 2014) due to its bifurcated tip. Four different types of filiform papillae (thread-like, single, bifurcated and short conical) on different region of the tongue were described by Sabry et al., (2015) in *Erinaceous auritus*. On the contrary, two types of filiform papillae, single and bifurcated were found in the Brandt's hedgehog and no other regional variations were seen. Moreover, it has been reported that filiform papillae in *Erinaceous auritus* has two to four accessory process in different region of the tongue (Nasr, 2012). It should be noted that this species considered as an insectivore animal. Therefore, due to the omnivorous feeding

habits of the Brandt's hedgehog, the difference in the appearance of the filiform papillae may be explained by different feeding habits of these species.

Functionally, the fungiform papillae could be classified into three types; gustatory papillae, which have taste bud as it was seen in Middle East blind mole rat (Kilinc et al., 2010), cattle, horse (Chamorro et al., 1986), mechanical papillae which have no taste bud like those seen in goat (Kurtul, Atalgin, 2008) and mixed type, means that some papillae have taste bud and some have not any taste bud as it was described in Australian Megachiroptera (Birt et al., 1997). The shape of the fungiform papillae have a wide variety of rectangle in Egyptian fruit bat (Abumandour, El-Bakary, 2013), Dome-shape in dog and fox (Emura et al., 2006), Mushroom-shape in rat (Nasr et al., 2012; Kurtul, Atalgin, 2008) and elliptical or circular in *Sorex caecutiens* (Park, Lee, 2009). The fungiform papillae of the present study have a discoid shape and the presence of taste pore suggests that they have gustatory function as revealed in other species (Kilinc et al., 2010; Chamorro et al., 1986; Goodarzi, 2014) and in *Erinaceous auritus* (Nasr, 2012).

The fungiform papillae in guinea pig (Kobayashi, 1990), goat (Kurtul, Atalgin, 2008), Persian squirrel (Goodarzi, 2014) and maned sloth (Benetti et al., 2009) are reported to be concentrated on the lingual apex and also on both lateral borders. However, fungiform papillae in the common shrew are restricted to the lingual corpus (Jackowiak et al., 2004). Nasr (2012) reported that fungiform papillae of *Erinaceous auritus* are populated on the apex of the tongue and form clusters of two or three papillae. But our observations show that fungiform papillae are distributed uniformly on the entire dorsal lingual surface in the Brandt's hedgehog.

It is well established that the number of vallate papillae differs among the animal species from absent in Cape hyrax (Emura et al., 2008) and hematophagous bats (Masuko et al., 2007), one in mouse, rat and hamster. (Kobayashi et al., 1989; Iwasaki et al., 1997). Two in rabbit, guinea pig and opossum (Kulawik, Godynicki, 2007; Kobayashi, 1990; Krause, Cutts, 1982), tree in common tree shrew, flying squirrel, Persian squirrel, American beaver and Egyptian fruit bat (Chunhabundit et al., 1992; Emura et al., 1999; Goodarzi, 2014; Shindo et al., 2006; Abumandour, El-Bakary, 2013), 3–6 in dog and cat (Boshell et al., 1982; Iwasaki, Miyata, 1989) and 5–26 in ruminants (Tadjalli, Pazhoomand, 2004; Kurtul, Atalgin, 2008). Further, many species-specific characteristics in morphology of the vallate papillae have been shown. For instance, small conical and filiform projection on the dorsal surface of the papillae in bush dog donkey (Emura et al., 2000) and multiple secondary papillae in giant panda (Pastor et al., 2008).

The vallate papillae of the Brandt's hedgehog in number and shape (three elliptical or oval papillae) were similar to those reported in *Erinaceous auritus* (Nasr, 2012) and arranged in a triangular pattern on the lingual radix similar to those described in the Persian squirrel (Goodarzi, 2014), Egyptian fruit bat (Abumandour, El-Bakary, 2013) and *Erinaceous auritus* (Nasr, 2012). But, in comparison with *Erinaceous auritus*, the dorsal surface of the vallate papillae in the Brandt's hedgehog was more irregular and has lobulated appearance. Unlike *Erinaceous auritus* (Nasr, 2012; Sabry et al., 2015) where a pair of foliate papillae in the lateroposterior part of the lingual radix was described, these papillae were not seen in the Brandt's hedgehog.

Overall, the present findings reveal that despite some similarities, the lingual papillae of the Brandt's hedgehog has species-specific characteristics compare to the *Erinaceous auritus* which indicate its different feeding habits. This is the first description of the lingual papillae morphology in Brandt's hedgehog providing a set of basic data and can be useful for the better understanding of the nutrition and feeding habits in this species.

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