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RESEARCH

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Anatomical and histological study on the tongue of two different species *Ptychadena mascareniensis* and *Hemidactylus turcicus* from the Egyptian environment

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Abstract

Background The tongue plays definite and important role in simplifying food intake and also, exhibits significant morphological distinctions to acclimatize the environment. This study aimed to investigate the variations in gross and microscopic anatomy of the tongue in two tetrapod classes *Ptychadena mascareniensis* from class: Amphibia and *Hemidactylus turcicus* from class: Reptilia to correlate the structure and functions of the tongue in relations to the habitat and feeding mechanisms.

Results All the modifications perceived in the tongues of the premeditated animals explained the relationship between adaptation of each animal to its feeding habit and habitat. Amphibians which live in and nearby freshwater showed no keratinization in their lingual epithelium. However, reptiles, which live in terrestrial habitats, have shown keratinization and ideal forms of lingual papillae which considered the most remarkable feature of the histological structures of the reptilian tongues and reflect their adaptations to the dry habitat in which they live.

Conclusion There were some variations reported in histological structure, gross anatomy and ultrastructure morphology of the tongue from the two tetrapod classes, the Amphibia (*Ptychadena mascareniensis*) and Reptilia (*Hemidactylus turcicus*). The present study is the first one in Egypt that deals with the tongue structures and functions in one of the amphibians Ptychadena mascareniensis. Concerning the lizard Hemidactylus turcicus.

Keywords Gross anatomy, Amphibia, Reptilia, Habitat, Feeding mechanisms

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Background

The morphology and structures of the tongue differ significantly among animal species according to various functions of each respective tongue (Santos et al., 2011). The tongue has a wide diversity of functions such as prey capture, drinking and swallowing (Bels et al., 1994; Darwish, 2012; Schwenk & Throckmorton, 1989). There are many correlations between the tongue anatomy; functions and the environmental conditions of the animal (Herrel et al., 2005a, 2005b; S. Iwasaki, 2002; Ross McClung & Goldberg, 2000). Anatomically, the tongue consists of apex, body and root (Alberts, 2015; Jackowiak & Godynicki, 2005).

Histologically, the tongue consists of three layers; mucosa, submucosa and muscularis. The mucosa is a stratified squamous epithelium; the submucosa is a thin layer of connective tissue containing blood vessels. The muscularis consists of bundles of skeletal muscle fibers arranged in many directions, (Alberts, 2015).

The lingual mucosa of dorsal, lateral border, and some extent to ventral surface of apex had lingual papillae (filiform, fungiform), while the lingual mucosa of body especially at torus linguae had conical papillae, but circumvallate papillae observed at the caudal part of body and root (Farrag et al., 2022). All papillae are covered by stratified squamous epithelium that differs by the thickness and keratinization only (Iwasaki & Miyata, 1985; Wassif, 2001). The distribution and random orientation of individual skeletal muscle fibers in the tongue allows for increased movement during chewing and swallowing (Eroschenko, 2017).

Study of different classes of vertebrates revealed a relationship between changes in the appearance of the tongue and changes of habitats from a freshwater environment to a terrestrial environment. In addition, there are a relationship between the extent of keratinization of the lingual epithelium and the transition from a moist or wet environment to a dry environment (Iwasaki & Wanichanon, 1993).

Reptiles may live in fresh water, seawater and on the land. On land, however, habitats vary significantly although temperatures are usually relatively high. Some reptiles live in habitats with extremely high temperatures and high humidity, others live at very low humidity with extreme variations in temperature, and others still live at moderate temperatures and humidity (Winokur, 1988). The most interesting features of the histological structures of the reptilian tongues that reflect adaptations to a dry or wet habitat are stratification and keratinization of the lingual epithelium (Elsheikh et al., 2013).

The reptilian tongues are characterized by morphological and functional variations among species (Cooper, 1995, 2003; El-Sayyad et al., 2011). Lizards are especially interesting group to investigate the tongue function and morphology, as the tongue is specialized for different functions in different clades (EI-Bakary et al., 2008), (Ozelmas et al., 2005; Cooper, 1995; Herrel, Canbek, Schwenk, 1995). Among reptilian animals, there are structural variations especially in size and shape of the dorsal papillae (Abbate et al., 2008). Apparently, the differences between the tongue surfaces of various reptiles depend on dissimilarities in diet, feeding habits and handling of the food in the mouth (Darwish, 2012). From the developmental point of view, it has been reported that in most mammals, keratinization of the epithelium begins with the appearance of the filiform papillae, just before birth (Iwasaki et al., 1998).

To our knowledge, the present study is the first one in Egypt that deals with the tongue structures and functions in one of the amphibians *Ptychadena mascareniensis* and reptilian *Hemidactylus turcicus* emphasizing onto the habitats and the mechanisms of feeding.

Methods

Ten specimens of the tongue from both classes: class Amphibia (*Ptychadena mascareniensis*) and class Reptilia (*Hemidactylus turcicus*), were collected from the following localities in Egypt to be used in this study.

Experimental design

Experimental animals

Ptychadena mascareniensis (Common name: Mascarene frog) ($n=5$) is recorded in Egypt in Nile Valley and Delta. It lives in freshwater in swamps, marshes and pans (Harper et al., 2010). It was collected from Abou Rawash, Giza, Egypt.

Mascarene frog has a medium-sized body with long legs. There are two light lines along its back, giving this frog its name. There are several dark squarish markings on its dorsum. The snout is long and pointed, and the nostrils are nearer to the snout than to the eyes (Harper et al., 2010). It is carnivorous and feeds on terrestrial beetles, bugs, spiders, earth worms and snails (Measey et al., 2009).

Hemidactylus turcicus (Common name: Mediterranean Gecko) ($n=5$). This species is very common in Egypt. It is terrestrial and mostly associated with human dwellings. It resides commonly on old buildings, rock walls, and burial vaults. The body and limbs are of moderate size. The head has rounded snout, and the tail is long, cylindrical and tapering. It is carnivorous and feeds on insects and spiders (Vogrin & Miklic, 2005). The specimens for this study were collected from Baltim, Kafr El Sheikh; Northern of Egypt.

Animals were collected alive and transferred to the Histology Laboratory, Department of Zoology and Entomology, Faculty of Science, Helwan University. Animals were killed by inhalation of chloroform. Later, the lower jaws were dissected, and the tongues were dissected out and processed for investigations.

Gross anatomy: All tongues were dissected out from the mandible, and then, their lengths were measured and photographed for gross anatomy description.

Light microscopy preparations: Fresh tongue specimens were fixed in aqueous Bouin's solution, dehydrated in ascending grades of ethyl alcohol (70, 80, 90 and 100%), cleared in xylene and embedded in paraffin wax. The thickness of longitudinal and serial transverse sections was 4 µm. Later on, they were stained with Haematoxylin and Eosin, Mallory's Triple and Periodic Acid Schiff's reagent (PAS) according to the applied protocol. The sections were then examined and photographed using the *Leica* optic photomicroscope.

Histological and histochemical methods

Hematoxylin and eosin stain for histological examination of tissues

Slides were de-paraffinized in xylene, hydrated in descending grades of ethyl alcohols (100, 90, 80, and 70%), and stained with Haematoxylin. Afterward, they were rinsed in water, stained with Eosin, cleared in xylene, mounted in DPX, and then, covered with coverslips (Suvarna et al., 2018).

Mallory's triple stain for collagen content

Slides were de-paraffinized in xylene, hydrated in descending grades of ethyl alcohols (100, 90, 80, and 70%), rinsed in running tap water, placed in acid fuchsine stain, and then, placed in phosphomolybdic acid solution. Subsequently, they were placed in aniline blue/orange G stain, rinsed in 95% ethyl alcohol, dehydrated in absolute ethyl alcohol, cleared in xylene, mounted in DPX, and then, they covered with coverslips (Suvarna et al., 2018).

Periodic acid Schiff's reaction (PAS) for demonstration of general carbohydrate content

Slides were de-paraffinized in xylene, hydrated in descending grades of alcohols (100, 90, 80, and 70%), and rinsed in distilled water. Later on, they were placed in 0.5% Periodic acid stain, rinsed in distilled water, placed in Schiff's reagent, washed under tap water, dehydrated through descending grades of ethyl alcohol, cleared in xylene, mounted in DPX, and then, they were covered with coverslips. Serial transverse and longitudinal sections were processed and kept for microscopic examination and photography (Suvarna et al., 2018).

Preparation of specimens for scanning electron microscopic investigation (SEM)

Tongue specimens were fixed in 2.5% buffered glutaraldehyde for 48 h, followed by dehydration in ascending grades of ethyl alcohol. Afterward, they were infiltrated with amyl acetate and then, dried by the critical point during using liquid carbon dioxide. The samples were mounted on metal stubs, coated with gold, and observed at accelerating voltages of 15 kV with A Joel scanning electron microscope (SEM) et al. Azhar University, Cairo, Egypt.

Results

Gross morphology of the tongue

The tongue of Ptychadena mascareniensis

The tongue of the mascarene frog, *Ptychadena mascareniensis* was triangular in shape and large in size. The mean length was 1.4 cm. The anterior region of the tongue (the apex) was narrow and attached to the bones of the lower jaw. The middle region of the tongue (the body) was broad in comparison with the anterior region. The posterior region of the tongue (the root), which protrudes out of the mouth during the tongue flicking, was broad, blunt and free. The buccal cavity was filled with mucous secretions (Fig. 1A).

The tongue of Hemidactylus turcicus

The tongue of the Mediterranean gecko, *Hemidactylus turcicus* was dorsoventrally flattened, slightly triangular and moderate in size. The mean length was 1.1 cm. The anterior region of the tongue (the apex) was rounded, narrow and free. The middle region (the body) was broader than the anterior region. The posterior region (the root) was broader caudally toward the base and fixed to the lower jaw. Examination of the buccal cavity showed the presence of mucous secretions (Fig. 1B).

Histological; histochemical and scanning electron microscope (SEM) examination

Light microscopy examination of the histological sections revealed that the tongue basically consists of three consecutive regions: anterior (apex), middle (body) and posterior (root). Each part composes of three layers, mucosa, submucosa and muscularis. The mucosa consists of stratified squamous epithelium, and the underlying submucosa consists of dense connective tissue, while the muscularis that consists of skeletal muscle fibers was oriented as circular and longitudinal bundles.

The tongue of Ptychadena mascareniensis

Examination of the histological sections revealed that the mucosa of the anterior region consists of non-keratinized

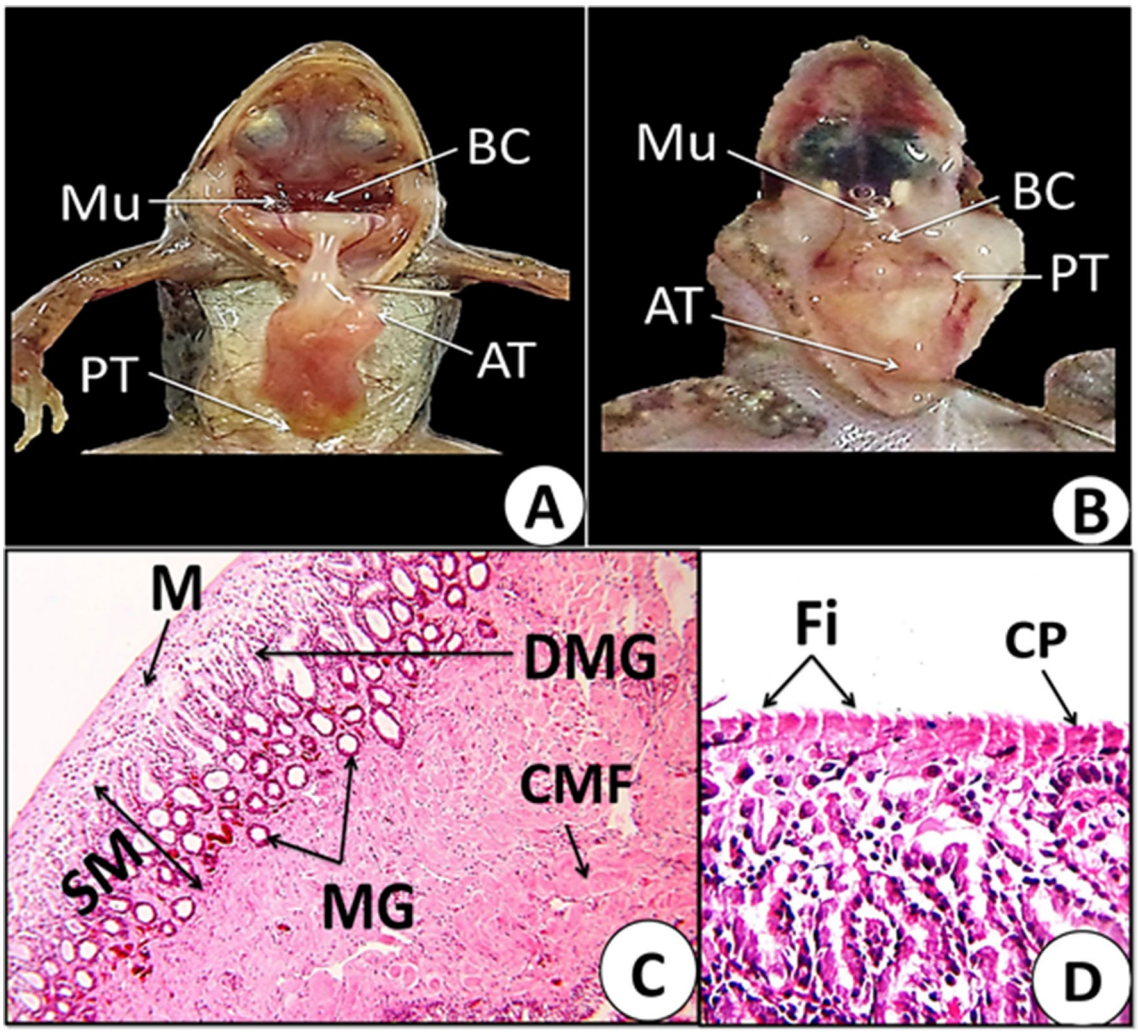

Fig. 1 **A** A photomicrograph to show the anterior and posterior parts of the tongue (AT & PT); buccal cavity (BC) of *Ptychadena mascareniensis* filled with mucus (Mu), **B** The buccal cavity (BC) of *Hemidactylus turcicus* filled with mucoid secretions (Mu) and showing the anterior and posterior parts of the tongue (AT & PT); **C** a photomicrograph of a longitudinal section of the middle region of the tongue of *P. mascareniensis* stained with H & E showing mucosa (M), submucosa (SM), mucous glands (MG) and their ducts (DMG) and circular muscle bundles (CMB), X 100; **D**, a photomicrograph of a longitudinal section of the posterior region of the tongue of *P. mascareniensis* stained with H & E showing keratinized filiform papillae (Fi) and cuboid (CP) papillae on the mucosa (M), X 400

stratified squamous epithelium, while the mucosa of the middle and posterior regions is composed of keratinized stratified squamous epithelium. The middle and posterior regions were rich in mucous gland lined with cuboidal epithelium (Fig. 1C). Mucous secretion appears as pink color in the sections stained with PAS. The mucosa of the posterior region showed the presence of keratinized filiform and cuboid papillae as well as the taste disks which were also, confirmed by the SEM examination (Figs. 1D, 2A and B). The muscularis of the anterior region consists of circular bundles of skeletal muscle fibers and few longitudinal bundles in between. Small amount of collagen was detected between the muscle fibers as shown in the sections stained with Mallory's triple stain. The muscularis of the middle region consists of bundles of circular muscle fibers only (Fig. 1C), while that of the posterior region consists of bundles of circular and longitudinal muscle fibers. The two regions are characterized by a relatively high amount of collagen between muscle fibers (Fig. 2C). PAS reaction showed considerable carbohydrate content along all parts of the tongue.

The tongue of Hemidactylus turcicus

Although gross examination to the tongue of *Hemidactylus turcicus* did not show any bifurcation at the tip of the tongue. However, microscopic examination disclosed

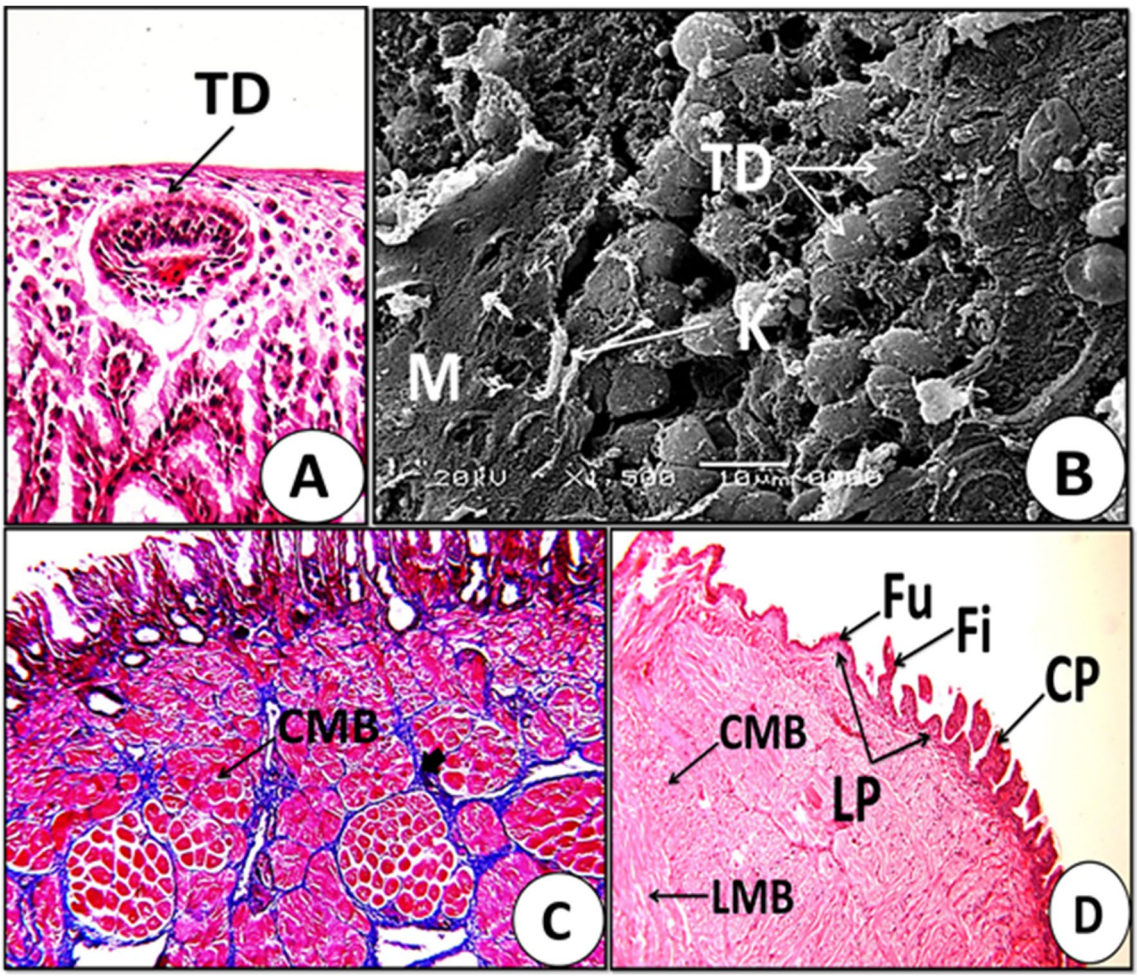

Fig. 2 **A** A photomicrograph of a longitudinal section of the posterior region of the tongue of *Ptychadena mascareniensis* stained with H & E showing taste disk (TD), X 400; **B** scanning electron micrograph of the dorsal surface of the posterior region of the tongue of *P. mascareniensis* showing mucosa (M), keratinization (K) and taste disks (TD), Scale bar 10 µm, X 1.500; **C** a photomicrograph of a longitudinal section of the middle region of the tongue of Ptychadena mascareniensis stained with Mallory's triple stain showing the collagen content presented in dark blue color (arrow) surrounds the circular muscle bundles (CMB), X 100; **D** a photomicrograph of a longitudinal section of the anterior region of the tongue of *Hemidactylus turcicus* stained with H & E showing fungiform (Fu), filiform (Fi), cuboid papillae (CP), lamina propria (LP), circular muscle bundles (CMB) and longitudinal muscle bundles (LMB), X 40

the presence of a bifurcation. At the anterior region of the tongue, the mucosa layer comprised of keratinized stratified squamous epithelium. The bifurcated part was keratinized but not providing with papillae. The mucosa of the rest of the anterior region was provided with cuboid papillae and few numbers of filiform and fungiform papillae. The papillae were overlapped and posteriorly oriented. Each papilla enclosed a connective tissue core rich in blood vessels and continuous with the lamina propria (Figs. 2D and 3A). Some taste buds were detected within the epithelium which covering the cuboid papillae. SEM examination indicated the presence of taste buds at this region of the tongue (Fig. 3B). Numerous mucous glands form crypt-like invaginations between the bases of the papillae. PAS reaction showed dark pink color mucoid secretions inside the glands. The mucosa of the middle region consists of keratinized stratified squamous epithelium but less thick than that of the anterior region also, provided with filiform and few fungiform papillae. At the posterior region, the mucosa was folded and lacks any papillae. Many taste buds were detected within the mucosa and contain sensory and support cells (Fig. 3C). In addition, the SEM examination showed the presence of micropores. In this posterior region, the mucosa composed of pseudo-stratified columnar epithelium provided with goblet cells and the posterior extremity was provided with dense microvilli. In addition, many mucous glands were detected in association with the mucosa.

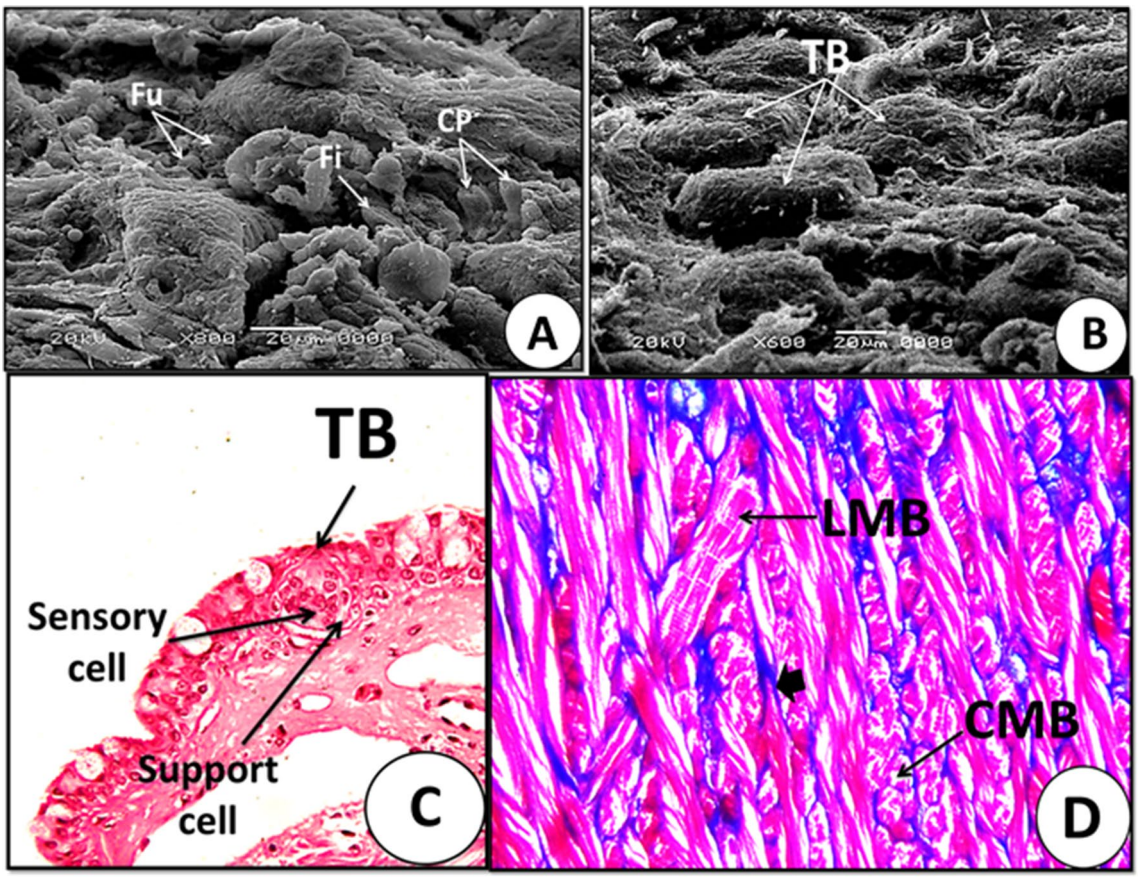

Fig. 3 **A** A scanning electron micrograph (SEM) of the dorsal surface of the anterior region of the tongue of *Hemidactylus turcicus* showing fungiform (Fu), filiform (Fi) and cuboid papillae (CP). Scale bar 20 µm, X 8.00; **B** scanning electron micrograph of the dorsal surface of the anterior region of the tongue of *H. turcicus* showing taste buds (TB), Scale bar 20 µm, X 6.00; **C** a photomicrograph of a longitudinal section of the posterior region of the tongue of *Hemidactylus turcicus* stained with H & E showing taste bud (TB) provided with sensory and support cells, X 400; **D** a photomicrograph of a longitudinal section of the anterior region of the tongue of *Hemidactylus turcicus* stained with Mallory's triple stain showing collagen content appeared in dark blue color (arrow) and surrounds the circular (CMB) and longitudinal muscle bundles (LMB), X 400

PAS reaction showed the presence of the mucoid secretions inside the goblet cells and the mucous glands.

Regarding the muscularis, all regions of the tongue consist of parallel longitudinal and circular bundles of muscle fibers with relatively high percentage of longitudinal fibers in the anterior region. An imperative content of collagen fibers between the muscle fibers and bundles was noted using Mallory's triple stain (Fig. 3D). PAS reaction showed the presence of high carbohydrate content throughout the muscularis layer.

Discussion

A comparison of the gross and microscopic anatomy of the tongue of tetrapod species of the mascarene frog (*Ptychadena mascareniensis*) revealed that the anterior extremity was attached to the mandible, while its posterior end was free and protruded during flicking. Similar results were reported by Elsheikh et al., 2013. The fixed anterior end and the protrusion of the posterior one allow the frog to catch the prey. Also, the movements of the tongue during feeding in different groups of frogs were studied and reported by (Savitzky, 2002).

In this study, histological and SEM examinations to the tongue of the frog showed that the lingual mucosa consists of keratinized stratified squamous epithelium in the middle and in the posterior regions while, no keratinization was observed in the anterior region. Our observations were contradicted with the previous studies on amphibians where they reported the absence of keratinization in the lingual mucosa of the tongues regarding the middle and posterior regions (Elsheikh et al., 2013; Graziadei & DeHan, 1971; Iwasaki & Kobayashi, 1988, 1989; Zylberberg, 1977;).

The presence of thin keratinization in the lingual mucosa of mascarene frog may be related to the nature of feeding habit which was usually a preyer characterized

by having tough tongue surfaces. In addition, the results showed the presence of taste disks in the mucosa of the posterior region of the tongue of the mascarene frog. Osculati & Sbarbati, 1995 described the taste disks in the tongue of frogs as a prototype of the vertebrate taste buds. The presence of taste disks seems to be a common characteristic feature in the tongue of frogs. Moreover, authors reported that the taste disks occur in metamorphosed anuran and urodelian individuals (Graziadei & DeHan, 1971; Jasiński, 1979; Osculati & Sbarbati, 1995). Recently, Budzik et al. suggested that *Incilius alvarius* possesses a pool of taste organs, which does not change with age, while the area of taste disks sensory zones increases (Budzik et al., 2018).

Elsheikh et al., 2013 and XIE et al., 2014 reported that in *Bufo regularis*, *Salamandra salamandra*, *Hynobius dunni* and *Cynops orientalis*, the taste disks used to make the animal feels the different flavors of food stimulation. In the present study, the filiform and cuboid papillae were present in the mucosa of the posterior region of the tongue of the mascarene frog. In addition, the results revealed that the mucosa of the latter regions was rich in mucous glands. The interaction between the filiform papillae and the mucous secretion together with the surface of the prey helps the feeding habit.

This suggestion is in agreement with the findings of Kleinteich and Gorb (Kleinteich & Gorb, 2016). The presence of filiform and fungiform papillae has been reported by many authors in previous studies that dealt with different amphibian species (Elsheikh et al., 2013; Guiraldelli et al., 2011; Helff & Mellicker, 1941; Iwasaki & Kobayashi, 1989; Iwasaki & Wanichanon, 1991, 1993; Iwasaki et al., 1998; Jaeger & Hillman, 1976; Ojima et al., 1997). Our data were supported by the presence of an abundant amount of collagen which, surrounds the muscle bundles in middle and posterior region to increase the strength and flexibility of the muscle fibers during feeding mechanism.

The current study revealed the presence of a bifurcation at the anterior extremity concerning the tongue of *Hemidactylus turcicus*. However, Darwish reported that feeding habit in bifurcated insectivores lizards reflects that the insect may be swallowed, and bifurcation could facilitate this process (Darwish, 2012).

The same results were proven by Elsheikh et al. considering *Chalcides ocellatus* (Elsheikh et al., 2013); Abo-Eleneen and El-Bakry (2017).

Iwasaki showed that the tongue tip of *Takydromus tachydromoides* was bifurcated at the anterior end, while that of the *Gekko japonicus* tongue was slightly bifurcated (Iwasaki, 1992).

However, this phenomenon of bifurcation was present in all squamates except the Dibamidae (Greer, 1985), the agamid *Laudakia stellio* (Koca et al., 2007). The results also showed that the mucosa of the anterior and middle regions consists of keratinized stratified squamous epithelium and provided with filiform, fungiform and cuboid papillae. Taste buds were also detected in addition to numerous mucous glands. The mucosa of the posterior region consists of pseudostratified columnar epithelium provided with goblet cells containing secretory granules and dense microvilli. Jamniczky et al. described the presence of glandular goblet clusters on the exposed stalks of papillae in the middle and posterior tongue regions in *Eublepharis macularius* (Jamniczky et al., 2009).

Conclusion

In this comparative study, the morphology and the structure of the tongue of the two tetrapod classes (Amphibia and Reptilia) shed the light on the vital roles of adaptation in relation to the structure and functions of their tongues. Such adaptations were studied throughout the movement between fresh water and terrestrial area. In *Ptychadena,* keratinization of the lingual epithelium was noted as a new foundation for the progress in ingestion of the tough preys. The bifurcation in the tongue tip of *Hemidactylus* helps in facilitating the swallowing of insects. However, the presence of micropores helps in holding the mucus for lubrication.

Abbreviations

AT	Anterior part of tongue
BC	Buccal cavity
C	Cuboid papilla
CMB	Circular muscle bundle
DMG	Duct of mucous gland
Fi	Filariform papilla
FT	Forked part of tongue
Fu	Fungi form papilla
G	Goblet cell

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Author contributions

Experimental design was contributed by EY, OH. Data interpretation was contributed by OH, ZSH, BH, EY. Histological examination was contributed by ZSH, BH. Writing and revision were contributed by OH, ZSH, EY, MM. All authors read and approved the final manuscript.

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Availability of data and materials

Data available on request.

Declarations

Ethics approval and consent to participate

The study was approved by the ethical committee at the Faculty of Science, Helwan University (approval number HU2020/ZASG0220/10), and according

to the National Institutes of Health guide for the care and use of laboratory animals (NIH publications No. 8023, received 1978).

Consent for publication
Not applicable.

Competing interests
The authors declare that there is no competing interests, including financial and non-financial.

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