Comparative Histological and Ultrastructural Study of the Tongue in *Ptyodactylus guttatus* and *Stenodactylus petrii* (Lacertilia, Gekkonidae)

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Abstract: The present study deals with examined the gross structure and lingual surface at light and ultrastructural level of two Gekkonidae species ; *Ptyodactylus guttatus* and *Stenodactylus petrii* collected from different regions of Sinai. Bifurcation is more detected in *Stenodactylus petrii*.. The distribution pattern of the mechanical filiform papillae varied between both species, being more abundant in *Ptyodactylus guttatus* in the lingual apex and similar in the lingual body. The examined lingual papillae are of flattened and conical filiform types. The distal margin of the root possessed serrated lingual surface in *Stenodactylus petrii* and pattern of scutate semi-like papillae in *Ptyodactylus guttatus*. In addition, the distal margin showed abundant glandular distribution associated with dense distribution of microvilli and microridges on lingual papillae facilitated for feeding habits.
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1. Introduction

The feeding mechanism is an important factor that determines the success of adaptation of vertebrates to their environment and of their persistence through procreation (Roth & Wake, 1989). The tongue is considered a key innovation in the evolution of a terrestrial lifestyle as it allows animals to transport food items through the oral cavity. In lizards, the tongue is specialized for different functions including prey capture, as in iguanian lizards, (Schwenk & Throckmorton, 1989; Herrel *et al.,* 1995), prey transport and swallowing in most lizards (Delheusy & Bels, 1992; Herrel *et al.,* 1996, 1997). Secondarily, the tongue and hyobranchial system has been coopted for a wide diversity of functions such as drinking (Sherbrooke *et al.,* 2007; Jamniczky *et al.,* 2009). Also, the tongue is specialized for another functions such as breathing (Bels *et al*., 1994; Schwenk, 1995); defensive display in some scincids (e.g., shingleback lizards, Gans *et al.,* 1985) and spectacle cleaning in geckoes (Simon, 1983). In snakes, the tongue appears specialized for chemoreceptive purposes from the external environment by means of tongue flicks or tongue touches (Graves & Halpern,1989;Mason,1992; Schwenk, 2000).

Moreover, there are fairly strong correlations between tongue anatomy, its functional roles as food transport and manipulation (McClung & Goldberg, 2000; Schwenk, 2000), and the environmental conditions in which animals use their tongues or the hyobranchial system (i.e., water vs. air; Iwasaki, 2002).

Among reptilian individuals, there are structural variations especially in size and shape of the dorsal papillae (Wassif & El-Hawary 1998; 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 (Pianka, 1986; Mohammed, 1987 & 1992).

The present study aims to clarify the relationship between the histological and ultrastructural features of the dorsal lingual epithelium of two Gekkonidae lizards; *Ptyodactylus guttatus* and *Stenodactylus petrii* by light and scanning electron microscopy.

2. Materials and Methods

Fourteen adult geckos, *Ptyodactylus guttatus* and *Stenodactylus petrii* were collected from different regions of Sinai and sacrificed by ether anaesthesia. They were decapitation and their lower jaws, including the tongues, were removed. Macroscopic structure of the tongue structure were examined and photographed.

Light microscopy:

After sacrificing the specimens, the tongues were separated and immediately fixed in bouin's solutions for 24 hours. These were followed by dehydration in ascending series of ethanol alcohol, cleared in xylene and mounted in molten paraplast 58-62 C. Serial 5–7 µm thick histological sections at both transverse and longitudinal line were made and stained with haematoxylin and eosin and investigated under bright field light microscopy and photographed.

Scanning Electron Microscopy (SEM) :

Extra fresh tongue specimens were removed and immediately fixed in 2.5 % glutaraldhyde in phosphate buffer, pH 7.4 followed by washing in phosphate buffer and dehydrated in ascending grades of ethyl alcohol and critically drying in carbon dioxide apparatus. The specimens were coated with gold in sputter coater and their dorsal lingual mucosa were viewed using a Joel scanning electron microscope.

3. Results
Gross morphology:

The tongue is relatively of moderate size, slightly triangular in shape with a rounded apex and broader caudally toward the base. It is dorso-ventrally flattened and consists of three parts; lingual apex, body and root. The tongue of *Ptyodactylus guttatus* is markedly larger comparing with that of *Stenodactylus petrii*. It occupied the greater part of the floor of the oral cavity. Macroscopic observation showed no existence of bifurcation. There is structural variations of the distal region adjacent to tongue root in both species. The periphery of the tongue of the root is idented like comb teeth in *S. petrii* and folded forming semi-like scutate papillae in *P. guttatus*.   The tongue of *Stenodactylus petrii* attains 8 mm length and 5 mm width at its base. However the tongue of *Ptyodactylus guttatus* reached to approximately to 11 mm length and 6 mm width at its base (Fig.1 A&B).

Scanning Electron Microscopy:

SEM shows that the overall morphology of both tongues is dorsally flattened with a blunt apex (Figs.2-5,A). Bifurcation of the tongue is only detected in *S. petrii* and missing in *P. guttatus*. The lateral margin of the bifurcated tongue is keratinized (Fig.2,C).There is no detected gustatory papillae in the dorsal lingual surface of both examined species. Filiform papillae are widely distributed all over the dorsal surface of the tongue in both examined geckos. In *S. petrii*, flattened filiform papillae (dome-shaped) are compactly distributed all over the apical lingual surface on both sides of the bifurcation tip as well as in the proximal lingual body. The diameter of each flattened papilla is 30 μm width (Fig. 2B). The whole surface of each papilla showed abundant distribution of both microridges and microvilli (Fig.2 D-E). On the other hand, *P. guttatus* possessed two kinds of filiform papillae ; flattened (dome-shaped) filiform papillae (25 μm width) and conical filiform papillae (20 μm width).

In the lingual body of *S. petrii* , two types of lingual papillae are distinguished; flattened and conical filiform papillae. The distal end of the lingual body adjacent to the triangular glandular region, the lingual mucosa become lobulated with detected microridges covering its surface. Although there is a close similarity of arrangement of flattened and conical filiform papillae on the lingual surface of *P. guttatus*, the distal end of lingual body just adjacent to the peripheral margin of the triangular gland region revealed the presence of characteristic structure semi-like scutate papillae (Fig.5D).

The hind triangular part of the tongue in both species is limited by the opening of the pharynx outlined by regular marginal folds arranged parallel to each other. The lingual mucosa adjacent to the pharynx opening showed abundant glandular opening (Figs.3,C,D & 5 C).

Histological structure:

In both species, the lingual mucosa is covered with different pattern of lingual papillae; which are widely distributed all over the dorsal surface. Each papilla have a dense connective tissue core rich in blood vessels and penetrates deeply into the center of each papilla. The lamina propria is continuous with the connective tissue core. The lingual epithelium of each papilla is mainly stratified in its apical surface and composed of single columnar epithelial cells in the lateral areas (Figs.6, 7).

The lingual apex, is covered by stratified squamous epithelium relatively thick and keratinized in *P. guttatus* compared with that of *S. petrii*. (Figs. 6B,7C). Both flattened and conical filiform papillae have dense connective tissue core distributed mainly in its central region (6A,B;7E,G).

On the other hand, the lingual mucosa of median tongue region possessed similar pattern structure of epithelial stratification, however no keratinzation was detected. The distal tongue region exhibit tubular glands traversed the lingual mucosa. Glandular goblet cells are detected (Figs.6 C&D ; 7D). The papillae of the lingual body are relatively taller comparing with the other kinds. The intrinsic musculature of the tongue is less prevalent in the lingual body more than that of the lingual apex.

4. Discussion

The present studies showed morphological variations of the two Gekkonidae species; *Ptyodactylus guttatus* and *Stenodactylus petrii*. The *Stenodactylus petrii* possessed bifurcation of lingual apex which completely missing in *Ptyodactylus guttatus*. Forked tongues may provide more surface available for sensory function in lizards.Forked tongue apex is detected in different reptilian species such as *Takydromus takydromoides* (Iwasaki & Miyata, 1985) and *Gecko japonicas* (Iwasaki ,1990). There is no reflect of feeding habit on the bifurcation of tongue but   these structural pattern may be of

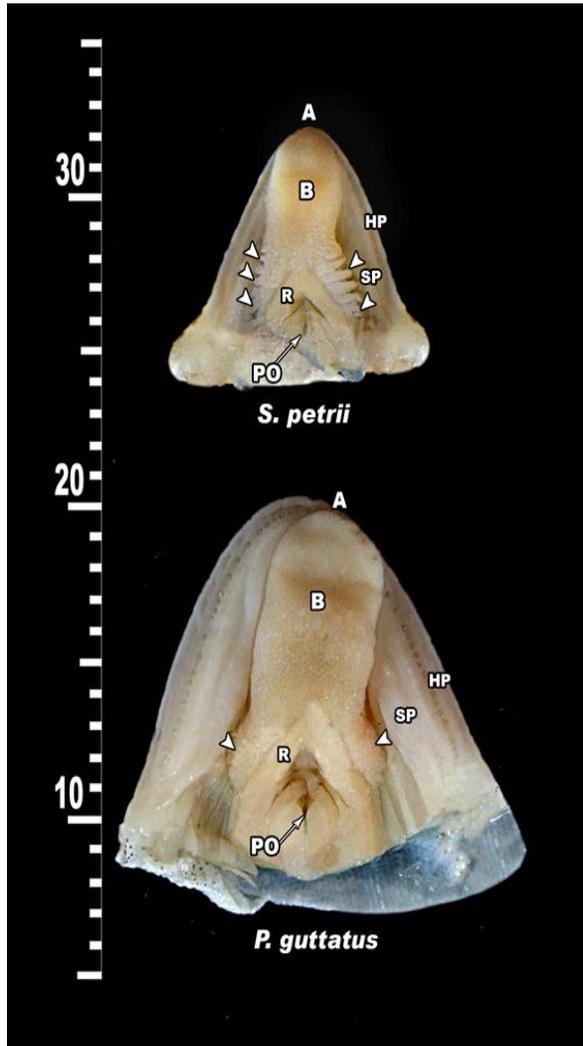phylogenetic importance in reptilian species according to Schwenk (1988) .

Fig. 1: Photomacrographs of dorsal tongue of *S. petrii* and *P. guttatus* showing triangular structure with marked enlarged size in *P. guttatus*. The interphase between the proximal and distal margin become serrated in *S. petrii* and lobulated in *P. guttatus*. Arrow heads indicate the indentation of the lingual surface in close associations with the triangular pharynx opening.
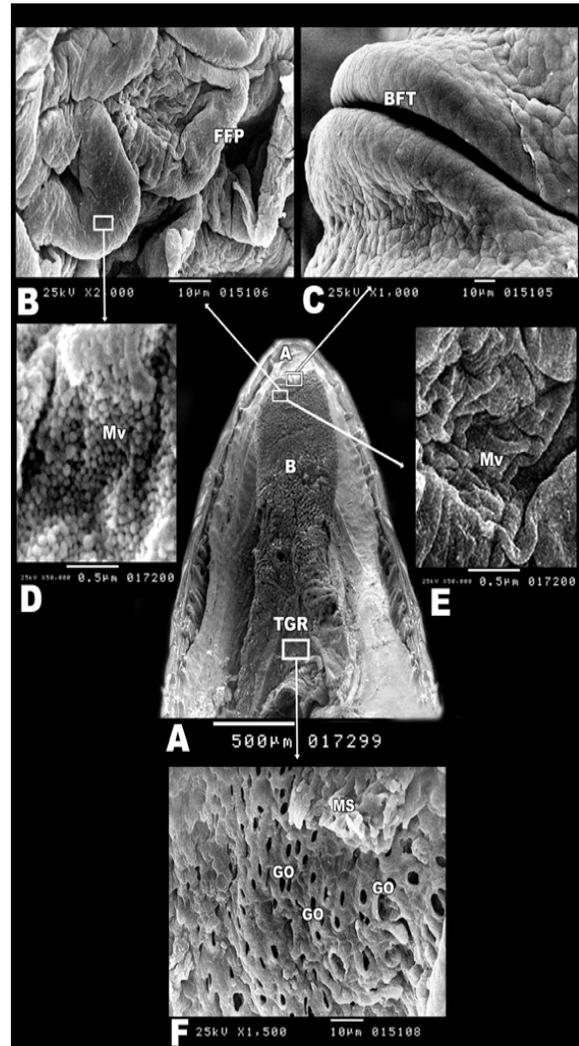**(Abbreviations; Apex, A; Body, B; Hard palate, HP; Pharynx opening, PO; Root, R, SP, Serrated papillae).**

Fig. 2 (A-F). Scanning electron micrographs of dorsum tongue of S. petrii showing bifurcated tongue lined by conical filiform papillae having microridges. The distal parts show numerous glandular opening.
Abbreviations; Apex, A; Body, B; Bifurcated tip, BFT; Conical filiform papilla, CFP; Microvilli , Mv; GO, Glandular opening; Tall tubular filiform papillae, TFP; Triangular glandular region ,TGR.
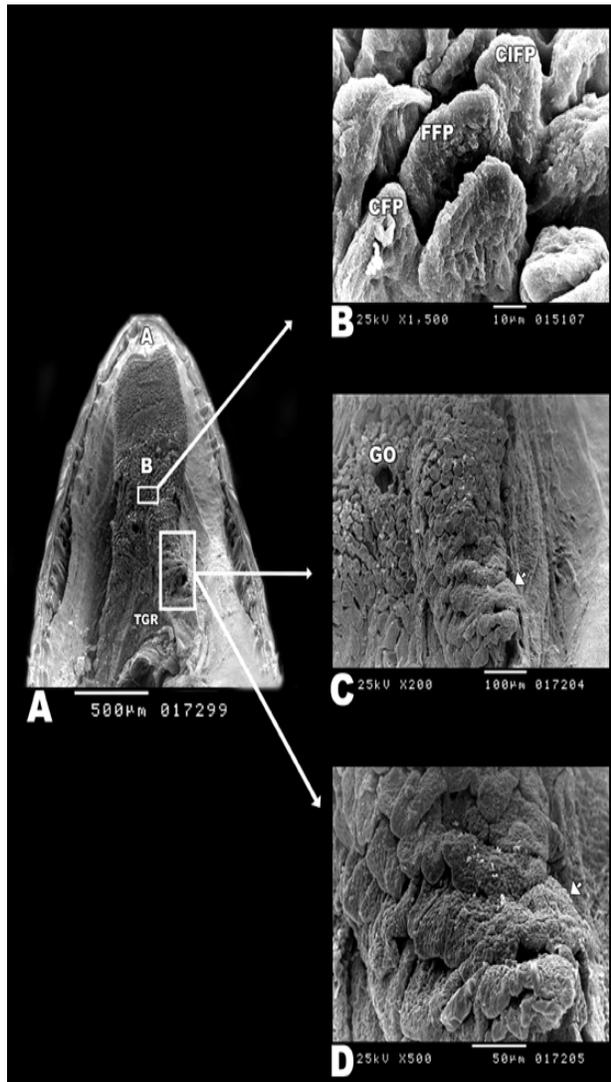
Fig. 3 (A-F). Scanning electron micrographs of dorsum tongue of *S. petrii* showing medium tongue having lingual mucosa formed mainly of conical filiform papillae. The distal end near the triangular glandular region showing scutate- like papillae as mentioned by arrow heads.
Abbreviations; Apex, A; Body, B; Bifurcated tip, BFT; Conical filiform papilla, CFP; Cylindrical filiform papillae, CIFP; Microvilli , Mv; GO, Glandular opening; Tall tubular filiform papillae, TFP; Triangular glandular region ,TGR.
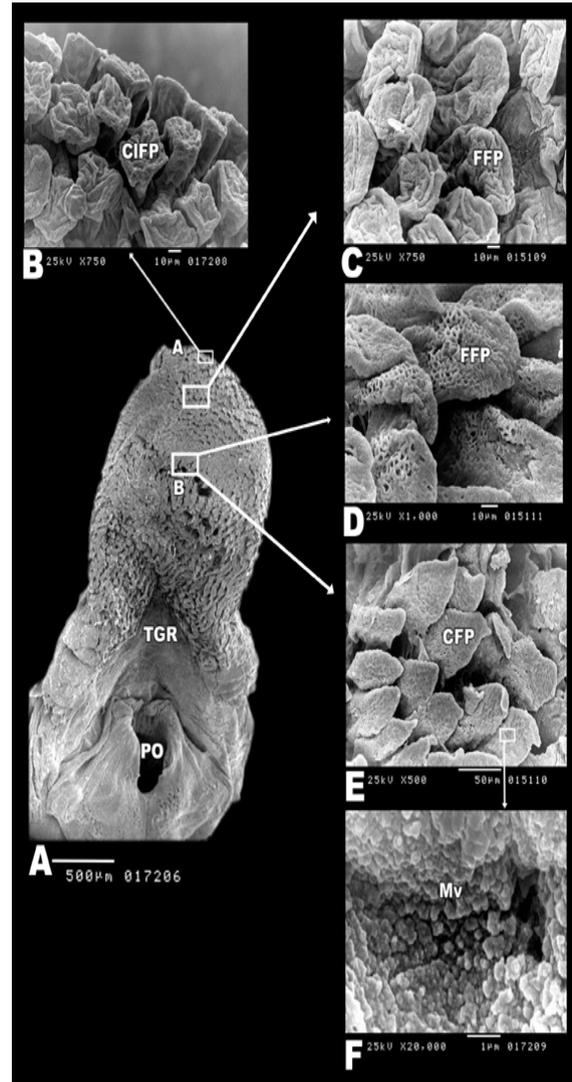

Fig. 4(A-F). Scanning electron micrographs of dorsum tongue of *P. guttatus* showing tongue lined with pattern of conical & filiform papillae having microridges. The distal parts show numerous glandular openings.
Abbreviations; Apex, A; Body, B; Bifurcated tip, BFT; Conical filiform papilla, CFP; Cylindrical filiform papillae, CIFP; Microvilli , Mv; GO, Glandular opening; Flattened filiform papillae: FFP ; MV, Microvilli; Tall tubular filiform papillae, TFP; Triangular glandular region ,TGR.
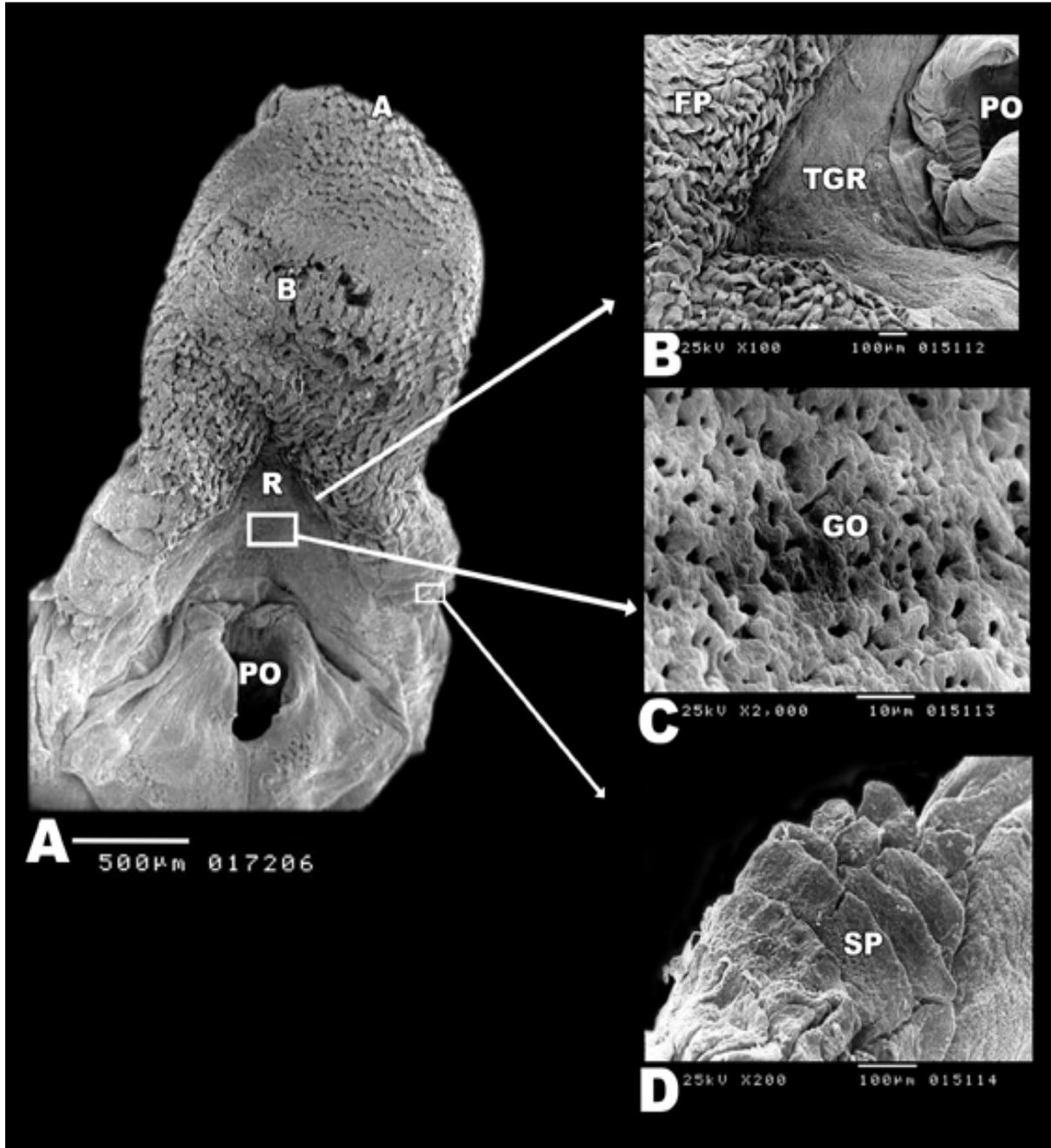
Fig. 5(A-D). Scanning electron micrographs of dorsum tongue of *P. guttatus* showing distal tongue region with abundant glandular opening. The distal periphery show numerous scutate- like papillae. Abbreviations; Apex, A; Body, B; GO, Glandular opening; Filiform papillae: FP ; Pharynx opening, PO;R, Root; Scutate like papillae, SP; Triangular glandular region ,TGR.
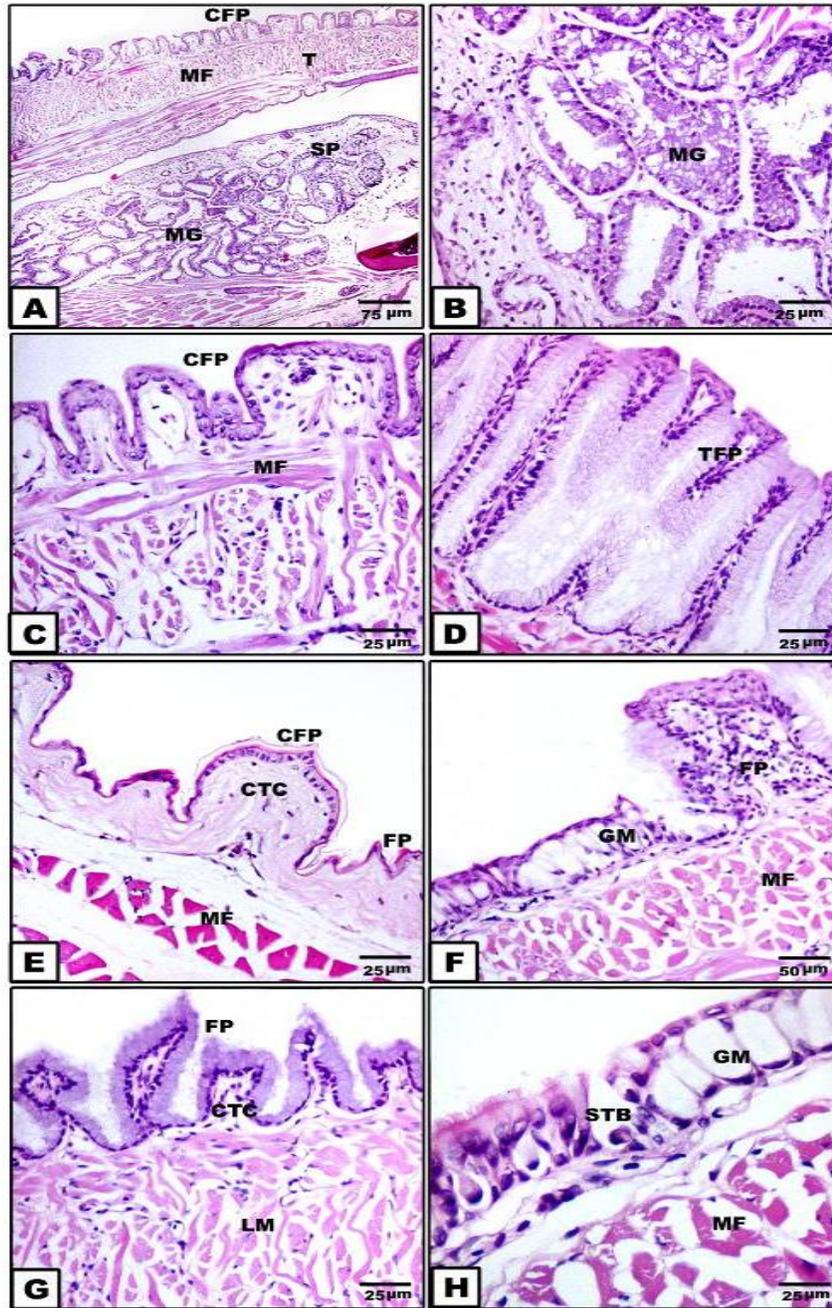
Fig. 6(A-H). photomicrographs of histological sections of dorsum tongue of *S. Petrii.*. A. Medium tongue showing conical filiform papillae. B. Showing hind part with lingual mucosa formed of glandular cells and underlying several branches of mucous glands. C,E&F. Showing conical filiform papillae. D. Medium tongue showing lingual mucosa with abundant distribution of tall filiform papillae. E-G. Medium showing abundant conical and filiform papillae. H. Showing hind part with lingual mucosa formed of glandular cells
Abbreviations; Apex, A; Body, B; Bifurcated tip, BFT; Conical filiform papilla, CFP; Cylindrical filiform papillae, CFP; Connective tissue core, CTC; Glandular mucosa, GM; Microvilli , Mv;, Glandular opening, GO; Flattened filiform papillae: FFP ; Filiform papillae: FP ; Longitudinal muscle, LM; Muscle fiber, MF; Mucous gland, MG; Microvilli, MV,; Tall tubular filiform papillae, TFP.
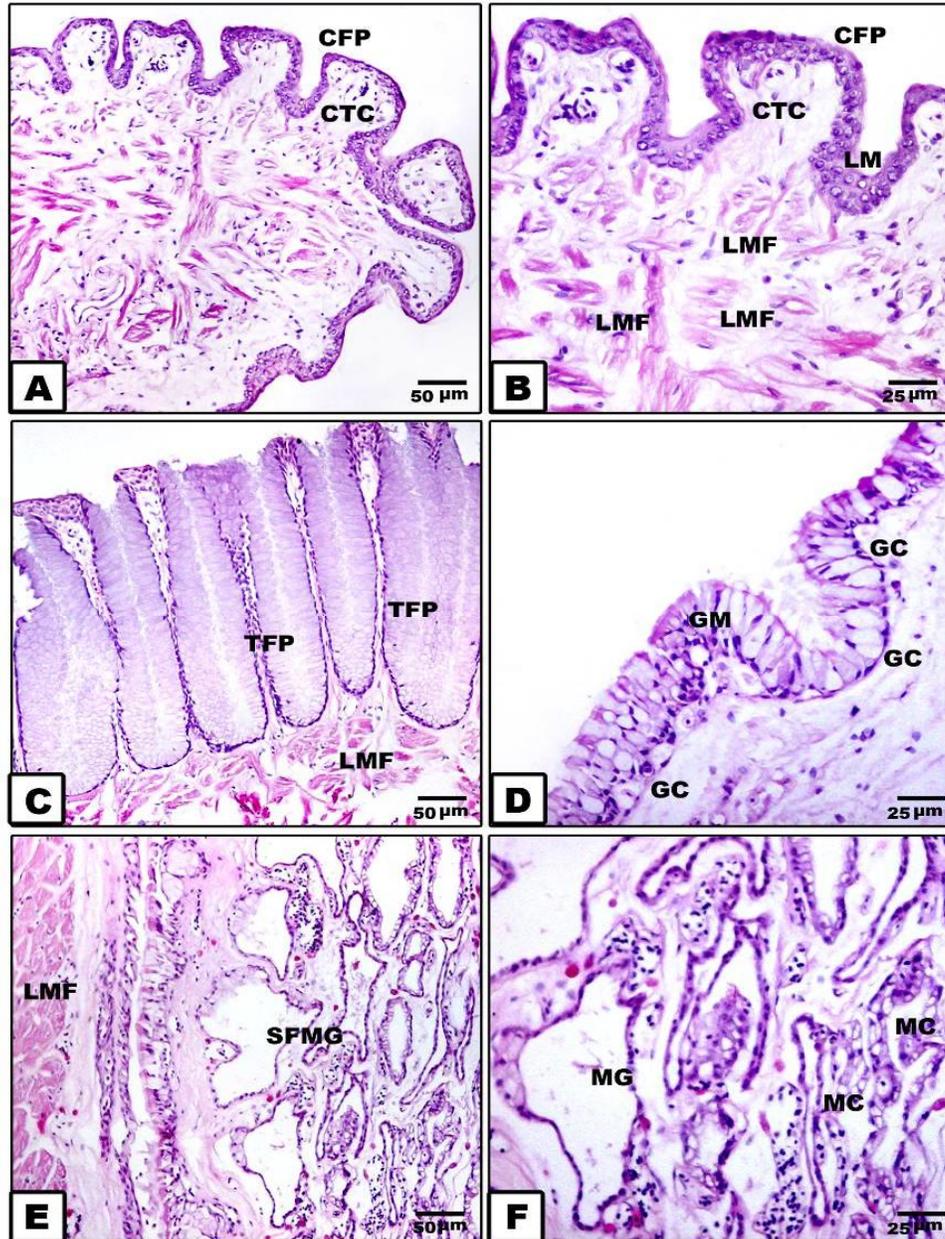
Fig. 7 (A-F). photomicrographs of histological sections of dorsum tongue of _P. guttatus_. A&B. Proximal tongue showing conical filiform papillae. C. Medium tongue showing lingual mucosa with abundant distribution of tall filiform papillae. B-F. Showing hind part with lingual mucosa formed of glandular cells and underlying several branches of mucous glands.
Abbreviations; Apex, A; Body, B; Bifurcated tip, BFT; Conical filiform papilla, CFP; Cylindrical filiform papillae, CFP; Connective tissue core, CTC; Glandular cell, GC; Flattened filiform papillae: FFP ; Filiform papillae: FP ;Longitudinal muscle, LM; Muscle fiber, MF; Mucous gland, MG; Microvilli, MV; Tall tubular filiform papillae, TFP.

Feeding habit in bifurcated insectivores lizards reflect that the insect may be swallowed and bifurcation may facilitated this process. The tongue has two principal functions in squamates: feeding and chemoreception. From a mechanical point of view, these functions impose conflicting demands on tongue structure that have been resolved historically in clade-specific patterns (Schwenk, 1993, 2000). At the same time, however, the similarities in ultrastructural features may reflect phylogenetic relationships between reptiles, as is the case also at the macroscopic and light- microscopic level (Iwasaki &Kumakura,1994; Iwasaki *et al.*, 1996).

The tongue potentially serves several different functions during feeding in lizards (Schwenk, 2000): (1) as a prehensile organ to capture food; (2) to manipulate food in the oral cavity after capture, either by positioning it between upper and lower tooth rows for gnawing, or to transport it towards the throat for swallowing; and/or (3) during swallowing, either to pack food into the pharynx or, along with the hyobranchial apparatus, to compress the pharynx in order to squeeze food into the oesophagus where peristalsis takes over transport of the bolus through the gut. The bifurcation of the tongue in lizard is unclear, although its presence is correlated with a direct connection between the vomeronasal chemosensory organs and the oral cavity through apertures in the anterior palate. Thus, the notch is assumed to be functionally related to chemoreception (Schwenk, 1993, 1994).

In the examined lizard species, there are differences among different regions of the tongue in the structure of the lingual epithelium which become keratinized at the lingual apex, non-keratinized in the lingual radix and those in the intermediate region between the lingual apex and the radix exhibit a transition, in terms of the keratinization of the epithelial cells, from one form to the other. Similar findings were reported by Iwasaki & Miyata (1985) and Iwasaki (1990).

The morphological structure of reptile's tongue is extremely interesting. Until now many researchers have focused on the ultrastructure (Filoramo & Shwenk, 2000; Sherbrooke *et al*., 2007; Abbate *et al.*, 2008; Jamniczky *et al*., 2009; Marycz *et al*., 2009) on the tongue of reptiles living in normal environmental conditions at relatively constant temperature and unchanged lifestyle.

Both earlier results (Iwasaki & Miyata,1985; Iwasaki,1990) and the present study demonstrate that the dorsal lingual surface of the studied lizard species showed different pattern of mechanical papillae ranged from flattened filiform papillae in its apical margin in *S. petrii* to both flattened and conical filiform papillae which may of great importance of

facilitated carrying of food items to the oral region. The lingual body supported by more abundant both kinds of lingual papillae besides the presence of characteristic organized structure at the peripheral region of the triangular root varied markedly between both species , being idented like comb teeth in *S.petrii* and folded forming semi-like scutate papillae and these structures have certain roles in feeding habits and swallowing of food materials.

Similar structural pattern of lingual papillae were reported in lizard species *Takydromus tachydromides, Gekko japonicus* (Iwasaki,1990; Iwasaki & Kobayashi,1992). The conical, cylindrical and flattenened papillae are closely similar in both species.

Microridges are widely observed on the dorsal lingual surface, especially on the interpapillar surface of mammals (Iwasaki *et al.*, 1987 a,b). Also, the present results indicate that microvilli are predominant on the dorsal lingual surface of *S. petrii* and *P.guttatus* and that the development of microridges is not (less) pronounced. Similar results were observed for *G. japonicus* (Iwasaki,1990) while the microridges are indicated but were less pronounced in *G. japonicus* than in *T. tachydromides* (Iwasaki & Miyata, 1985).

According to Sperry &Wassersug (1976), the observed microridges of filiform papillae may play a role in the retention and spreading of mucus on the epithelial cell surface. Microvilli on the surface of the oral epithelial cells are also thought to have a function similar to that of the microridges.

The differences of microvilli and distribution of glandular region on the distal tongue region may reflect the mucoid pattern of dorsal lingual epithelium of *S. petrii* and *P. guttatus* in swallowed food materials. Similar findings were reported by (Iwasaki,1990; Iwasaki & Kumakura,1988).

In mammals, the tongue functions mainly as an organ for the uptake of food and for the sense of taste (Graziadei,1969), and it is not very important for the secretion of mucus or serous granules, since the salivary glands mainly perform this function. Several secretory glands are also located around the oral cavity in reptiles, for example, palatine, sublingual, supralabial,and infralabial glands (Kochva,1978).

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