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Functional morphology of the tongue of lizard *Salvator merianae* (Reptilia: Squamata)

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ABSTRACT
The reptilian tongue has morphological and functional variations according to the feeding mechanisms and habitat of the species. This study aimed to understand the morphofunctional characteristics of the tongue of the lizard *Salvator merianae*, through anatomical, histological and scanning electron microscopy analyses. This study involves five adult animals, which were collected in the municipality of Viçosa, Minas Gerais, from Brasil. The tongue of *S. merianae* was protractile and bifid, important to drive molecules from the environment to the vomeronasal organ and thus identify odors and locate prey, besides being strongly muscular and with keratinized and pigmented stratified epithelium for protection. Mucous secretion was present as an important tool for lubrication and protection in view of the protrability of the tongue and the great presence of bacteria. Morphological specialization of the *S. merianae* tongue reflect its direct participation in food assimilation, with squamiform papillae for the mechanical function of maintaining prey in the oral cavity and contributing to swallowing. Taste buds were not found, which excludes the tongue participation in the taste, although it has olfactory sensory function in association with the vomeronasal organ.

Key Words: Histology; Digestive System; Mouth; Lacertilia.

Salvator merianae (Duméril and Bibron, 1839) is a species of lizard belonging to the Teiidae family, found in Argentina, Uruguay, Paraguay and all regions of Brazil (except the Amazon Forest) (Vanzolini *et al*., 1980; Péres Júnior, 2003). This species occurs in open areas of the Cerrado, edges of gallery forests and within more open forests. It has daytime habits, is heliophile and active throughout the day, spending most of the time looking for prey, which is found with the help of its long, bifid and protractile tongue (Vitt, 1995). It has an omnivorous feeding habit, which includes vertebrates, vegetables, mollusks, arthropods and even carrion in their diet (Ávila-Pires, 1995).

The main role of the tongue is facilitating feeding, whether for catching prey, transporting food during swallowing and, or, sensory function (Schwenk, 2000). Reptiles can live in fresh water, sea water and on land, with very variable temperature and humidity levels. The reptilian tongue presents morphological and functional variations between species, reflecting differences in habitat, feeding habit and behavior, that is important for the survival of reptiles in different environments (Iwasaki, 2002; Koca *et al*., 2007).

Thus, this study aimed to understand the morphofunctional characteristics of the tongue of *S. merianae*, through macro and microscopic analysis. Five adult (four females and one male) animals of this species were used. The animals were collected in the municipality of Viçosa, in the Zona da Mata Mineira region, from Brasil (IBAMA license: 10504-1). The experiment was conducted in accordance with the "Ethical Principles for the Use of Laboratory Animals" (Brazilian College of Animal Experimentation - COBEA, 1991) andit was approved by the Ethics Committee for the Use of Animals of the Universidade Federal de Viçosa (protocol 27/2016). The animals were euthanized with an intraperitoneally injected overdose of pentobarbital. After euthanasia, the tongue fragments were collected, washed in saline solution and fixed in Carson's formalin (Carson *et al*., 1973) for 24 hours. The fragments were analyzed for anatomical description, with the use of a stereoscopic microscope (Olympus SZ40). The tongue was divided into three regions for histological analysis: anterior (apical), middle and posterior (basal) thirds. Then, fragments of these regions were

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submitted to histological processing (Tolosa *et al*., 2003): dehydrated in a growing ethyl series, included in glycol-methacrylate resin and sectioned by a rotating microtome (RM2055, Leica), which provided 3 μm thick semi-serial cross-sections. The sections were stained with toluidine blue in 1% sodium borate, for histological description, and subjected to the following histochemical methods: Alcian blue (AB) pH 2.5 and pH 0.5, aiming to detect acid mucins and sulfated acid mucins, respectively (Bancroft and Stevens, 1996); periodic acid of Schiff (PAS) for neutral mucins (Pearse, 1968); Sudam black for lipids (McManus and Mowry, 1960) and Ponceau's xylidine (PX) (Kiernan, 1990) for the identification of general proteins. The observation and photographic record of the sections were performed in a light microscope coupled with a digital camera (Olympus BX53).Fragments from the three regions were also used for scanning electron microscope analysis. They were dehydrated in an ethanol series, critical point driedby the carbon evaporator (Quorum Q150 T), placed in stubs and then metallized with gold (Balzers Union FDU 010 Modular Metallizer). Next, the material was analyzed using a scanning electron microscope (Leo, 1430VP).

As anatomical results, we verified that the tongue of the lizard *S. merianae* is elongated, protractile, with a tapered and bifurcated apex, gradually becoming wider and thicker after the apex. The apex has a lateral fold along its bifurcations, and its texture is almost smooth (Fig. 1A). The lingual dorsum protrudes laterally to connect to the venter, which is slightly narrower, cylindrical and bipartite, with a deep central groove and folds arranged obliquely. The middle region is pigmented and irregular on the dorsal surface, with a scaly texture that extends to the posterior region (Fig. 1B).

The tongue protractile and bifurcated allows animals to capture chemical substances from both the environment and the potential prey and lead these substances to the vomeronasal organ, a small tubular structure located in the postero-inferior portion of the cartilaginous nasal septum, close to the union with the vomer, which has an auxiliary olfactorial function (Burghardt, 1970). Some studies indicate the occurrence of a co-evolutionary process between the tongue and the vomeronasal organ in the irradiation of squamates,which is correlated to the shape, elongation and bifurcation of the tongue (Cooper, 1994; Cooper, 1995a; Cooper, 1997a; Cooper, 1997b; Filoramo and Schwenk, 2009).

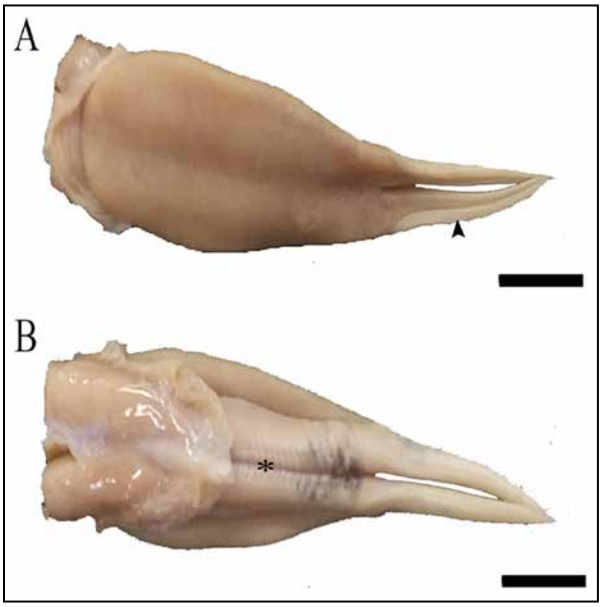

Figure 1. Macroscopic images of *Salvator merianae* tongue. A) Dorsal face of the tongue, showing the bifurcated apex with lateral folding (arrowhead). B) Ventral face of the tongue, with deep central groove (*). Bar: = 1cm

As histological results, we observed that the tongue of *S. merianae* is a muscular organ highly innervated and vascularized. The epithelium is stratified squamous, with two thick bands of keratin in the tongue apex, where a large amount of loose connective tissue and little skeletal muscle tissue are also observed (Fig. 2A). The epithelial type of the lizard's tongue, with layers of keratin and pigmentation, provides important protection in view of the protractile ability of this organ. In lizards, differences in relation to the histological structure of the tongue epithelium have been reported between species in different habitats (Schwenk, 1985; Schwenk, 1986; Smith, 1988; Schwenk, 1989; Iwasaki, 1990; Toubeau *et al*., 1994; Iwasaki, 2002). One of them refers to the level of epithelial keratinization, which may vary according to the humidity levels of the environment, with greater keratinization in the tongue of animals from dry and hot environments (Iwasaki, 1990; Iwasaki 2002). The *S.merianae* lizard inhabit dry environments with high temperatures (Winokur, 1988), thus, the keratinization is extremely important to protect the protractile tongue of these animals. In some groups of lizards, such as *Gekko japonicus* and *Takydromus tachydromoides*, differences were observed in the keratinization of the epithelium between the regions of the tongue. The epithelial cells in the tongue apex are keratinized; those of the posterior of the tongue are not keratinized; and those in the

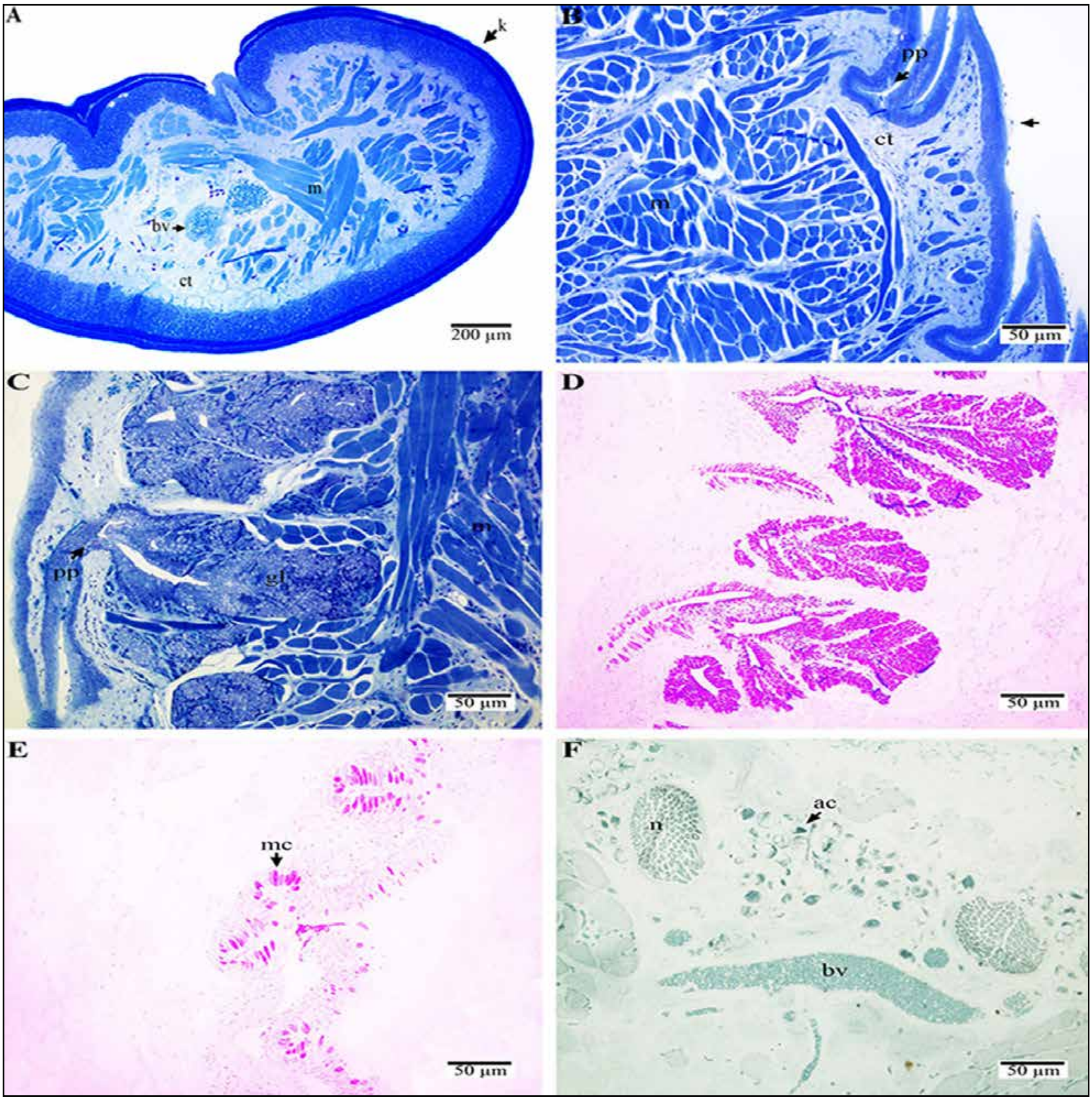

Figure 2. Light microscopy images of *Salvator merianae* tongue. A) Apex of the tongue with stratified squamous epithelium and two keratin bands (k). Blue toluidine staining. B) Middle region of the tongue, with well-developed musculature (m) and squamiform papillae (pp) of stratified squamous epithelium, with bacteria (arrow) on the surface. Blue toluidine Staining. C) Posterior region of the tongue, with well-developed musculature (m) and squamiform papillae (pp), among which there are branched tubular mucous glands (gl). Blue toluidine staining. D) Posterior region of the tongue, with PAS-positive branched tubular mucous glands. Schiff periodic acid Staining (PAS). E) Posterior end of the tongue, of prismatic epithelium with PAS-positive mucous cells (mc). Schiff periodic acid staining (PAS). F) Posterior region of the tongue, with adipose cells (ac) and nerve bundles (n). Sudam Black coloring. bv: blood vessel; ct: connective tissue.

intermediate region show some transition (Iwasaki and Miyata, 1990; Iwasaki, 1990), which partly co-rroborates the results found in *S. merianae*, in view of the presence of keratin in the apical region (and noton basal region) of its tongue, which is more exposed to the environment.

In the middle and posterior regions of the *S. merianae* tongue, there are squamiform papillae on the dorsal surface, where bacteria are frequently observed (Fig. 2B and C). Tongue papillae of lizards are described as filiform, with the ends having varied shapes: rounded, conical, cylindrical, fan-shaped or squamiform (Iwasaki, 1990; Darwish, 2012), depending on the species and region of the tongue.

These differences are due to the feeding habit and the way the species handles food (Pianka, 1986). Squamiform filiform papillae, such as those found in *S. merianae*, assist in the mechanical function of maintaining prey in the oral cavity, also contributing to swallowing (Schwenk, 2000; Silva, 2015). The same papillary shape has been described for the *Anthrosaura* genus, which also belongs to the Teiidae family (Hoogmoed and Avila-Pires, 1992), which may have a phylogenetic correlation. Since no taste buds were observed in the tongue papillae of *S. merianae*, these papillae have a purely mechanical function, without involvement with the sense of taste. The absence of taste buds may reflect the generalist feeding habit of this species and the greater importance of the olfaction for food selection, with the participation of the tongue transferring molecules from the environment to the vomeronasal organ.

In the middle and posterior regions of the *S. merianae* tongue, the muscle tissue is predominant, arranged in different directions and in a spaced way (Fig. 2B and C), which allows for wide-ranging movements forthe capture of odorous molecules, handling and swallowing of prey (Silva, 2015). In the posterior region of *S. merianae* tongue, there are branched tubular mucous glands that flow between the papillae, with PAS-positive and AB-PX-negative secretion (Fig. 2D). Further posteriorly, at the pharyngeal end, the epithelium becomes ciliated prismatic, with PAS-positive and AB-PX-negative mucous cells (Fig. 2E). Mucous secretion is important due to its lubricating action, prevention of damage to the epithelium, aid in swallowing and defense against harmful bacterial colonization (Arellano *et al*., 1999; Radaelli *et al*., 2000; Domeneghini *et al*., 1998). Neutral mucus (PAS staining) is denser and thicker than acidic mucus (AB staining) and therefore seems to have a great capacity to form protective barriers (Beamish *et al*., 1972). Therefore, neutral mucus (PAS-positive) observed in *S. merianae* is especially important in view the great exposure of its protractile tongue, the great amount of bacteria in its mouth and the generalist diet of this species. On the other hand, no serous cells or granules were observed in the *S. merianae* tongue, considering the PX-negative reaction. Therefore, there is no secretion of enzymes or other proteins, which differs from the observed in some species of reptiles, such as the *American chameleon* and *Anolis carolinensis*, whose tongue epithelium presents cells with mucous and serous granules (Rabinowitz and Tandler, 1986).

Tongue base of *S. merianae* presents many adipocyte cells and nerve bundles intermingling muscular tissue, as evidenced by Sudan black staining (Fig. 2F). The rich vascularization, innervation and adiposity detected in the tongue of this species are consistent with the high activity of this organ. The adipose tissue found in the *S. merianae* tongue may be an energy reserve to be used during the hibernation period in the coldest months (Derickson, 1976; Abe, 1983). In the Southeastern Brazil, teiú lizards remain active during the hot and humid season (August to April), and retract in shelters during the cold and dry months (May to July) (Abe, 1983).

Scanning electron microscopy analysis corroborate the observations made in the anatomical and histological studies. There are small undulations at the apex of the tongue that may be involved with the adhesion of molecular odoriferous, and the scaly papillae starting right after the apical bifurcation (Fig. 3A). The surface of the tongue has flattened polygonal cells, making it difficult to see its limits, especially at the apex where there are layers of keratin, which leave the surface looking like rough plates (Fig. 3B). The papillae extend along the tongue dorsum and its surface is flattened (Fig. 3C). The lateroventral portion of the tongue has no papillae, but there are folds arranged obliquely, with a flattened surface similar to that of the back (Fig. 3E). The posterior end of the tongue also has no papillae. Its surface has a velvety appearance, due to the presence of cells with numerous apical projections, which certainly represents the transition to the ciliated epithelium of the pharynx (Fig. 3F).

This study described the morphology of the tongue of the lizard *Salvator merianae* and the functional implications in the face of the feeding habit and behavior. The tongue of *S. merianae* is protractile and bifid, with great exposure to the environment, requiring protection mechanisms, such as keratinized pigmented stratified epithelium and mucus secretion. The great presence of bacteria reflects the generalist feeding habit of this species. The *S. merianae* tongue plays a direct role in the acquisition of food, presenting itself as a robust structure, extremely muscular and full of squamiform lingual papillae on the dorsal surface, which are involved in the apprehension and swallowing of prey. The absence of taste buds on the tongue of this species reveals that this organ plays no role in tasting, but is crucial for olfaction in cooperation with

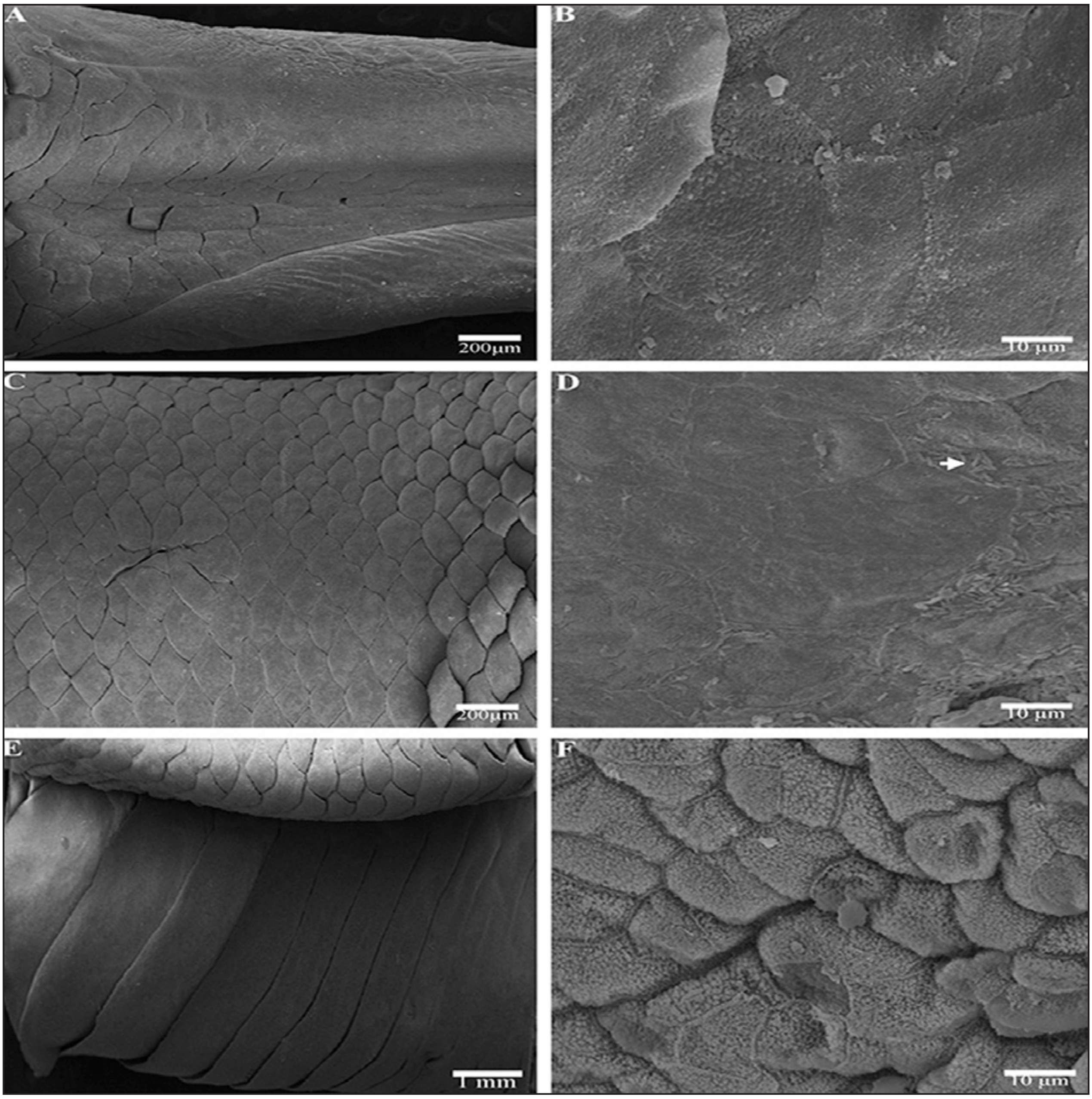

Figure 2. Scanning microscopy images of *Salvator merianae* tongue. A) Dorsal surface of the apical region. B) Detail of the epithelium of the apical region, with a flattened aspect. C) Dorsal surface of the middle region, with squamiform papillae. D) Detail of the papillae lining epithelium, with a flattened aspect and presence of bacteria (arrow). E) Lateroventral surface of the tongue, with oblique folds. F) Posterior end of the tongue, with a velvety surface due to the presence of cells with numerous apical projections.

the vomeronasal organ, which favors their foraging behavior for locating prey.

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