Nota

Cuad. herpetol. 37 (1): 65-72 (*2023)*

Functional morphology of the tongue of snake *Bothrops jararaca* (Reptilia: Squamata)

Maria Luiza Assis Rodrigues[1], Sirlene Souza Rodrigues Sartori[1]

[1] Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil

Recibida: 05 Enero 2022
Revisada: 04 Marzo 2022
Aceptada: 04 Octubre 2022
Editora Asociada: S. Quinzio

doi: 10.31017/CdH.2023.(2022-001)

ABSTRACT
The tongue has a series of functions that can be related to feeding, such as transporting food to the back of the mouth, sensory function and capturing prey. The tongue of the reptiles has great morphological and functional variations between orders. This study aimed to described the morphological characteristics of the tongue of the snake *Bothrops jararaca*, relating them to the habitat and eating habits of this species. This work used five adult animals which were collected in the municipality of Viçosa, in the Zona da Mata Mineira region. The animals were euthanized, with anesthetic overdose, for the removal of the tongue, which was used for histological processing and scanning electron microscopy. The tongue had stratified epithelium with an extensive degree of keratinization and pigmentation. It was shown to be strongly muscular, innervated and vascularized. Keratinization and pigmentation is an important protection mechanism, due to the protrability of this organ, which is exposed to the environment to capture odors and locate prey. Mucous secretion was present as an important tool for lubrication and protection of the lingual lining. The tongue of the *B. jararaca* has no papillae and taste buds, being its function strictly olfactory, in association with the vomeronasal organ.

Key Words: Histology; Microscopy; Buccal cavity; Viperidae.

Bothrops jararaca (Wied-Neuwied, 1824), popularly known as jararaca, is a species of snake of the family Viperidae found in Brazil, Paraguay and northern Argentina (Warrell, 2004). It inhabits forests but can also be found in degraded areas (Campbell and Lamar, 2004). It is a poisonous species with mainly nocturnal habits (Sazima, 1992; Warrell, 2004) and ambush behavior, which can also use the active search strategy, depending on the size of the prey (Greene, 1997). It feeds mainly on small vertebrates, but adult and young individuals have different feeding habits. The young animals prey mainly on frog amphibians, while the adults, prey on rodents (Sazima, 1992).

Generally, the main function of the tongue is to contribute to feeding, participating in the capture of prey, ingestion and swallowing of food, tasting and, in some reptiles, it can play a role in olfaction in association with the vomeronasal organ (Schwenk, 2000; Iwasaki, 2002). In snakes, there is a growing evidence that the vomeronasal organ and, indirectly, tongue flicking, is involved in many activities including tailing, recognizing, striking, and swallowing prey, predator recognition, courtship, maternal care, aggregation, exploration, and habitat selection (Burghardt, 1980; Teshera and Clark, 2021).

The tongue of reptiles has morphological and functional variations between orders, reflecting differences in feeding, habitat and habits (Iwasaki, 2002; Koca *et al*., 2007). Thus, this study aimed to describ the morphological characteristics of the tongue of *Bothrops jararaca*, through macro and microscopic analysis.

For this work, five adult animals of *Bothrops jararaca* species were used. The animals were collected in the municipality of Viçosa, in the Zona da Mata Mineira region (IBAMA license: 10504-1). The entire procedure was conducted in accordance with the "Ethical Principles for the Use of Laboratory Animals" (Brazilian College of Animal Experimentation - COBEA, 1991), which was approved by the Ethics Committee for the Use of Animals (CEUA) of Federal University of Viçosa (protocol 27/2016). The animals were euthanized with an intraperitoneally

Author for correspondence: sirlene.rodrigues@ufv.br

injected overdose of pentobarbital. After euthanasia, the animals were measured and weighed. Tongue fragments were collected 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 (apex), middle (body) and posterior (root) thirds. Then, fragments of these regions were 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 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 acidic mucins and mucin sulfated acids, respectively (Bancroft and Stevens, 1996); periodic acid of Schiff (PAS) for neutral mucins (Pearse, 1968); Sudan 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 placed in stubs, taken to the carbon evaporator (Quorum Q150 T) and then metallized with gold by the Balzers Union FDU 010 Modular Equipment, composed of Metallizer ("Sputter coating attachment") SCA 010. Next, the material was analyzed using a scanning electron microscope (Leo, 1430VP).

Among the results of this work, it was observed that the *Bothrops jararaca*' tongue is surrounded by the buccal floor, which is a tubular structure housing the tongue (Fig. 1A). The tongue of this species is long, elongated and narrow, tapering at the apical end, which is bifurcated (Fig. 1A). Pigmentation is observed throughout the tongue (Fig. 1B), especially on the dorsum, whose texture is almost smooth, with small transverse grooves arranged dorsolaterally (Fig. 1C). In the venter, a deep central groove is observed, starting from the bifurcation and arranged throughout the tongue (Fig. 1B).

The protractile and bifurcated tongue of the *Bothrops jararaca* allows the capture of chemical substances from the environment and potential prey, taking these substances to the vomeronasal organ. Also known as the Jacobson organ, it is a small tubular structure with auxiliary olfactorial function, located in the posterior-inferior portion of the cartilaginous nasal septum, close to the union with the vomer (Burghardt, 1970). Some studies indicate the occurrence of a co-evolutionary process between the tongue and the vomeronasal organ in the irradiation of squamates (Cooper, 1994; Cooper, 1997a; Filoramo and Schwenk, 2009). This process is related to the shape and elongation of the tongue and presence of bifurcation (Cooper, 1994; Cooper, 1995; Cooper, 1997b). Parker *et al.* (2008) suggested that the lingual bifurcation in snakes serves to increase area of chemosensory detection.

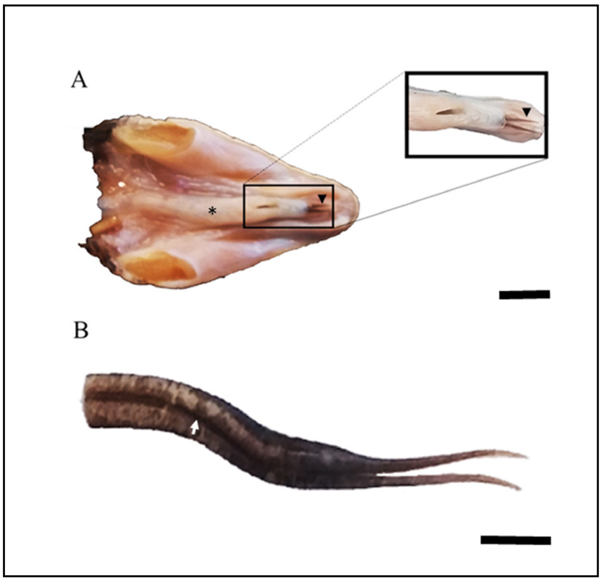

Figure 1. Anatomy of the tongue of *Bothrops jararaca*. A) Buccal floor forming a tubular structure (asterisk) that surrounds the tongue, whose apex is bifurcated (insert, arrow head). B) Ventral face of the tongue with deep central groove (arrow). Scale: 1cm

Histologically, the tongue of the snake *B. jararaca* has a stratified squamous epithelium widely keratinized and pigmented throughout its length (Fig. 2A). This characteristic may be related to the "flicking" pattern (when the tongue protrudes out of the mouth) of the tongue of this species, due to external stimuli, such as the presence of prey or predators, also involving behavioral and seasonal aspects (Gove, 1979; Graves and Halpern, 1990; Bels *et al.*, 1994). The "flicking" of a snake's tongue is a form of adsorption of odorant in the air (Halpern *et al.*, 1986). In other words, it has a sensory function, capturing odorous molecules for the vomeronasal organ. Therefore, the tongue is strongly exposed to environmental aggressions, while keratin and pigment protect and provide resistance against da-

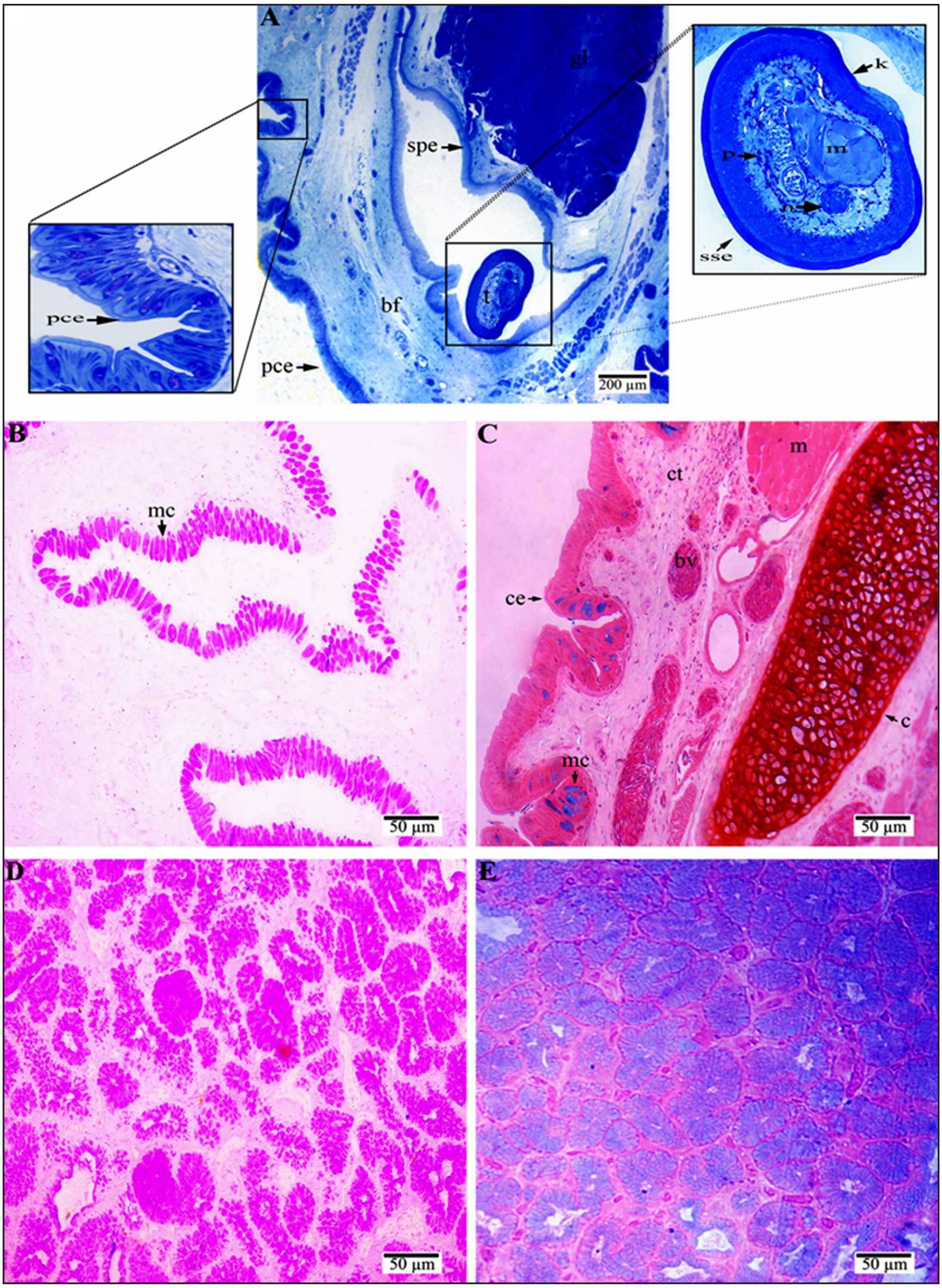

Figure 2. Light microscopy photos of the tongue and buccal floor of *Bothrops jararaca*. A) Buccal floor (bf) of pseudostratified ciliated epithelium (pce) on the external surface and stratified polyhedral epithelium (spe) on the internal surface, facing the cavity where the tongue (t) is located, which has a squamous stratified epithelium (sse) with keratin (k) and pigmentation (p). Anterior region; toluidine blue staining. B) Buccal floor, showing the pseudostratified ciliated epithelium and PAS-positive mucous cells (mc). Anterior region; periodic acid Schiff (PAS) staining. C) Buccal floor, showing the pseudostratified ciliated epithelium with AB-positive mucous cells (mc). Posterior region; Alcian blue (AB) and safranin staining. D) Branched tubular mucous gland of the buccal floor, with PAS-positive secretion. Middle region; periodic acid Schiff (PAS) staining. E) Branched tubular mucous gland with AB-positive secretion. Middle region; Alcian blue (AB) and safranin staining. bv: blood vessel; c: cartilage; ct: connective tissue; gl: gland; m: muscle (skeletal striated); n: nerve.

mage (Stern, 1980; Avery, 1987). *Bothrops jararaca* inhabits dry environments with high temperatures, thus, keratinization is extremely important to protect the protractile tongue of these animals.

In *Bothrops. jararaca,* the tongue is highly muscular, filled with skeletal striated muscle, which is arranged in a unidirectional and compacted manner, permeated by blood vessels and nerve bundles; and showed no lingual glands and no adipose tissue (Fig. 2A). This disposition of the muscle tissue, unidirectional and juxtaposed, may imply more targeted movements of the tongue, since the tongue of this species is not related to the admission of food, but to the capture of odorous molecules. The muscular tissue allows the sliding and stretching of the tongue, in the "tongue-flicking" behavior (El-sayyad, 2011). According to Smith *et al.* (1990), the tongue of snakes is composed of a few muscle groups, which are responsible for its flicking movement, and, despite general similarities, distinct patterns of intrinsic tongue musculature characterize each infraorder of snakes.

Through histochemical methods, it was observed that the oral floor is lined with pseudostratified ciliated epithelium (Fig. 2A) with PAS-AB-positive mucous cells (Fig. 2B and C), and filled with loose connective tissue with blood vessels, muscle bundles and a well-developed compound gland, whose duct opens in the cavity where the tongue is located (Fig. 2A). This cavity is lined with stratified polyhedral epithelium. The compound gland can be classified as branched tubular mucosa, with PAS-AB-positive and PX-negative secretion (Fig. 2 D and E), characteristic of sublingual salivary gland. Mucous secretion is important due to its lubricating action, prevention of damage to the epithelium, aid in swallowing and defense against harmful bacterial colonizations (Arellano *et al.*, 1999; Radaelli *et al.*, 2000; Domeneghini *et al.*, 2005). Neutral mucus is denser than acidic mucus and therefore has a greater capacity to form protective barriers (Beamish *et al.*, 1972). The protection and lubrication provided by mucous secretion are crucial in view of the protractability of the tongue, especially in the snake *B. jararaca*, whose tongue moves within the tubular structure of the oral floor. No serous cells were observed in the glands of the studied specie. 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 *Anolis carolinensis*, whose lingual epithelium presents cells with mucous and serous granules (Rabinowitz and Tandler, 1986).

According to scanning microscopy, on the dorsum of the tongue of *B. jararaca*, small longitudinal grooves are observed, in addition to transverse grooves on the dorsolateral edges (Fig. 3A). There are porous undulations along the dorsum and venter of the tongue (Fig. 3B), and the epithelial surface has a flattened but irregular appearance (Fig. 3C). The snake *B. jararaca* showed no lingual papillae and the tongue is almost smooth, which is certainly related to the fact that the tongue of this species, as well as for other snakes, are not important for the direct admission of food, but exclusively used in cooperative olfaction, together with the vomeronasal organ (McDowell, 1972; Gillingham and Clark, 1981). In snakes, the vomeronasal organ facilitates prey identification, courtship, and aggregation (Teshera and Clark, 2021). According to the review made by these authors, all viperids that hunt large prey inject venom to kill it, and this reliance on venom is associated with a suite of related physiological and behavioral traits that has facilitated the evolutionary success of this group. One of these traits is the process termed strike-induced chemosensory searching. This specialized form of chemosensory searching is a critical aspect of the overall phenotype that allows viperids to kill and ingest prey items that are not only well defended with teeth and claws, but also can be larger than the snake itself. The vomeronasal organ and the tongue comprise the accessory olfactory system, which is one of three major chemosensory systems possessed by squamates, the other two being gustation and the main olfactory system. The prioritization of the accessory olfactory system over the main olfactory system occurs in snakes and some lizards.

Corroborating the histological findings, in scanning electron microscopy the buccal floor had a velvety surface on its external face (facing the oral cavity) (Fig. 3D), certainly due to the presence of ciliated cells. On the internal face (facing the cavity where the tongue is housed), its surface was fragmented (Fig. 3E), due to the presence of polyhedral cells.

This study aimed to describe the morphological structure of the tongue of *B. jararaca*. The tongue of this species is protractile and bifid, with great exposure to the environment for the identification of odors and the location of prey, requiring protection mechanisms, such as stratified pigmented keratinized epithelium and mucus secretion. This structure

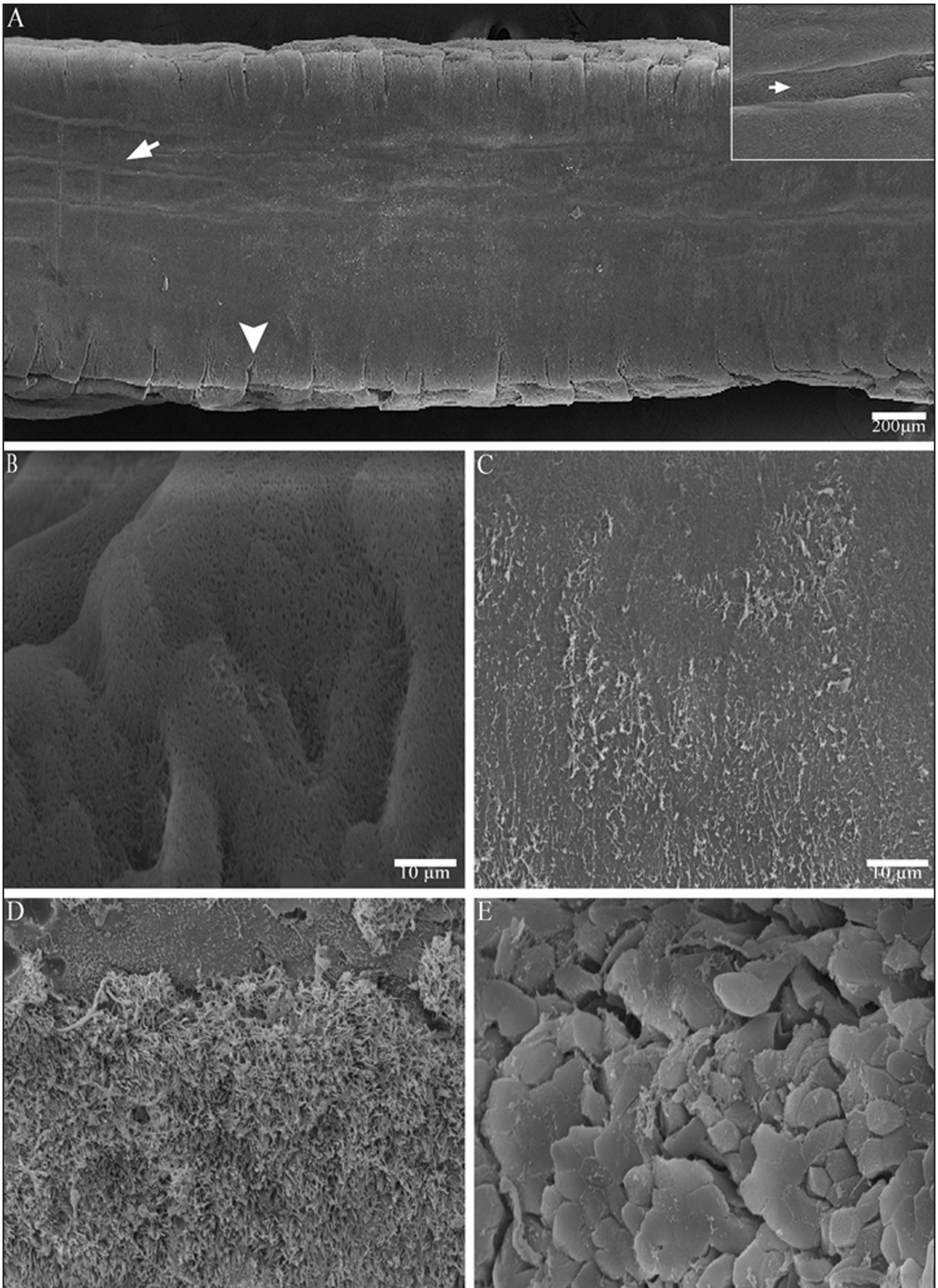

Figure 3. Scanning microscopy photos of *Bothrops jararaca* tongue. A) Dorsum of the tongue showing the small longitudinal grooves (arrow) and transverse grooves (arrow head). B) Undulations with a porous aspect on the lingual surface. C) Flattened epithelium of the lingual surface. D) Surface with velvety appearance, due to the presence of ciliated cells. E) Surface with a fragmented aspect, due to the presence of polyhedral cells.

has a strictly sensory function, with participation in the sense of smell, presenting a smooth surface devoid of papillae. The absence of taste buds on the tongue of this species reveals that this organ has no role in tasting. Thus, there is a clear relationship between the morphology of the tongue and the feeding behavior of this species of reptiles, and in the case of the *Bothrops jararaca* with an important olfactory sensory function for capturing prey.

Acknowledgments

We would like to thank the Department of Animal Biology and the Microscopy and Microanalysis Nucleus of the Federal University of Viçosa, Minas Gerais, Brazil.

Literature cited

Arellano, J.M.; Dinis, M.T. & Sarasquete, C. 1999. Histomorphological and histochemical characteristics of the intestine of Senegal Sole, Solea senegalensis. *European Journal of Histochemistry* 43: 121-133.

Avery, J.K. 1987. Developmental prenatal craniofacial skeleton: 42-53. In: Avery, J.K. (ed.), Oral Development and Histology. Baltimore.

Beamish, R.J. 1972. Lethal pH for white sucker *Catostomus comersoni* (Lacépedè). *Transactions of the American Fisheries Society* 101: 335-358.

Bels, V.L.; Chardon, M. & Kardong, K.V. 1994. Biomechanics of the hyolingual system in Squamata. *Advanced in Comparative and Environmental Physiology* 18:197-240.

Burghardt, G.M. 1970. Communication by Chemical signals: 241-308. In: Johnston, J. W.; Moulton, D.G. & Turk, A. (ed/s.), Chemical perception in reptiles. Appleton-Century-Crofts. 1970, New York.

Burghardt, G.M. 1980. Behavioral and stimulus correlates of vomeronasal functioning in reptiles: feeding, grouping, sex, and tongue use: 275-301. In: Miiller-Schwarze, D. & Silverstein, R.M. (ed/s), Chemical Signals in Vertebrates. Plenum. 1980, New York.

Campbell, J.A.; Lamar, W. W. 2004. Venomous Reptiles of the Western Hemisphere. *The Canadian Field Naturalist* 119: 148-149.

Cooper, W.E. 1994. Chemical discrimination by tongue-flicking in lizards: a review with hypoteses on its origin and its ecological and phylogenetic relationships. *Journal of Chemical Ecology* 20: 439-486.

Cooper, W.E. 1995. Foraging mode, prey chemical driscrimination, and phylogeny in lizards. *Animal Behavior* 50: 973-985.

Cooper, W.E. 1997a. Independent evolution of squamate olfaction and vomerolfaction and correlated evolution of vomerolfaction and lingual structure. *Amphibia-Reptilia* 18: 85-105.

Cooper, W.E. 1997b. Correlated evolution of prey chemical discrimination with foraging, lingual morphology and vomeronasal chemoreceptor abundance in lizards. *Behavior Ecology and Sociobiology* 41: 257-265.

Domeneghini, C.; Panneli, R.S.; Veggetti, A. 1998. Gut glycoconjugates in *Sparus aurata* L. (Pisces, Teleostei). A comparative histochemical study in larval and adult ages. *Histology and Histopathology* 13: 359-372.

El-Sayyad, H.I.H.; Sabry, D.A.; Khalifa, S.A.; Abou-El-Naga, A.M.; Foda, Y.A. 2011. Studies on tongue of reptilian species *Psammophis Sibilans*, *Tarentola Annularis* and *Crocodylus Niloticus*. *International Journal of Morphology* 29: 1139-1147.

Filoramo, N.I. & Schwenk, K. 2009. The mechanism of chemical delivery to the vomeronasal organs in squamates reptiles: A comparative morphological approach. *Journal of Experimental Zoology* 311(1): 20-34.

Gillingham, J.C.; Clark, D.L. 1981. Snake tongue-flicking: Transfer mechanics to Jacobson's organ. *Canadian Journal of Zoology* 59: 1651-1657.

Gove, D.A. 1979. Comparative study of snake and lizard tongue-flicking, with an evolutionary hypothesis. *Zeitschrift für Tierpsychologie* 51: 58-76.

Graves, B.M. & Halpern, M. 1990. Roles of vomeronasal organ chemoreception in tongue flicking, exploratory and feeding behavior of the lizard, *Chalcides ocellatus*. *Animal Behavior* 39: 692-698.

Greene, H.W. 1977. Snakes. The Evolution of Mystery in Nature. University of California Press. Los Angeles.

Halpern, M.; Schulman, N.; Kirschenbaum, D.M. 1986. Characteristics of earthworm washings detected by the vomeronasal system of snakes: 63-77. In: Duvall, D.; Müller-Schwartz, D.; Silverstein, R.M (ed/s.), Chemical Signals in Vertebrates. Plenum Press, New York.

Iwasaki, S. 2002. Evolution of the structure and function of the vertebrate tongue. *Journal of Anatomy* 201: 1-13.

Koca, Y. B.; Oguz, E.O. & Osanç, E. 2007. Morphology, and muscle and papillae volume ratios, of the tongue of *Laudakia stellio* (Agamidae, Squamata): A Histological and Stereological Study. *Zoological Science* 24: 899-905.

Mcdowell, S.B. 1972. The evolution of the tongue of snakes, and its bearing on snake origins: 191-273. In: Dobzhansky, T., Hecht, M.K. & Steere, W.G (ed/s.), Evolutionary Biology. Meredith Corp, New York.

Parker, M.R.; Young, B.A. & Kardong K.V. 2008. The forked tongue and edge detection in snakes (Crotalus oreganus). An experimental test. *Journal of Comparative Psychology* 122: 35-40.

Rabinowitz, T. & Tandler, B. 1986. Papillary morphology of the tongue of the American chameleon: *Anolis carolinensis*. *The Anatomical Record* 216: 483-489.

Radaelli, G.C.; Domeneghini, A.; Arrighi, M. & Mascarello, F. 2000. Ultrastructural features of the gut in the while sturgeon, *Acipenser transmontanus*. *Histology and Histopathology* 15: 429-439.

Sazima, I. 1992. Natural history of the jararaca pit viper, *Bothrops jararaca*, in southeastern Brazil: 199-216. In: Campbell, J.A. & Brodie, E.D (ed/s.), Biology of the pitvipers. Selva, Texas.

Schwenk, K. 2000. Feeding in the lepidosaurus: 175-291. In: Schwenk, K (ed), Feeding: form, function, and evolution in tetrapod vertebrates. Academic Press, San Diego.

Smith, K.K. & Mackay K.A. 1990. The morphology of the intrinsic tongue musculature in snakes (Reptilia, ophidia): Functional and phylogenetic implications. *Journal of Morphology* 205: 307-324.

Stern, I.B. 1980. Oral Mucous Membrane: 261-335. In: Bhaskar, S. N (ed), Orban's Oral Histology And Embryology. Mosby Co, St. Louis.

Theshera, M.S. & Clark, R.W. 2021. Strike-Induced Chemosensory Searching in Reptiles: A Review. *Herpetological Monographs* 35: 28-52.

Warrell, D.A. 2004. Snakebites in central and south america: epidemiology, clinical features, and clinical management: 709-761. In: Campbell, J.A. & Lamar, W.W (ed/s.), The venomous reptiles of the western hemisphere. Comstock Publishing Associates, Ithaca and London.