

## Morphological and ultrastructural characteristics of the tongue of wild boar

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### ABSTRACT

The present study aimed to describe the structural and ultrastructural morphological characteristics of the lingual epithelium and the connective tissue cores (CTCs) of wild boar (*Sus scrofa*). The tongues were processed for light microscopy, scanning electron microscopy, and transmission electron microscopy. In this study, we revealed the filiform, fungiform, foliate, and vallate papillae. The filiform papilla is elongated with a conical shape and its CTC has a conical shape; the fungiform papilla is rounded with a dome-shape and its CTC is flower bud; the foliate papilla is formed by four pairs of epithelial folds and irregular grooves, and its CTC is thin with adjacent conjunctive projections, and taste buds and serous glands in the epithelial layer have been evidenced; and the vallate papilla is oval surrounded by a groove with increases of epithelium surface, and the CTC is formed by numerous connective projections lined by a deep groove. Also noted were serous gland and taste buds on the medial wall of the vallate papilla. The epithelium has the keratinized, granular, spinous, basal, and lamina propria layers. In conclusion, we found new descriptions and shapes of the CTCs of the lingual papillae. In addition, we demonstrated the epithelium structural characteristics, the nuclear distribution between the epithelial layers, and the ultrastructural aspects of the dorsal epithelium of the tongue.

**Key words:** Tongue; lingual papillae; epithelium; morphology; wild boar.

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## Introduction

The tongue is an important organ in the oral cavity for the functions of apprehension and transportation of food, swallowing,<sup>1</sup> and cleaning.<sup>2</sup> Also, it assists in social functions such as grooming and vocalization.<sup>3</sup> The development and evolution of the tongue and the lingual papillae can be influenced by their habitat and their functional aspects.<sup>2,4</sup> The tongue and lingual papillae demonstrate wide structural and ultrastructural characteristics among mammals, such as those described for rodents,<sup>5-10</sup> equines and ruminants,<sup>11-13</sup> canines,<sup>14</sup> sloth,<sup>15</sup> Carnivora order,<sup>1,2,16,17</sup> Artioctyla order,<sup>18</sup> lesser hedgehog tenrec,<sup>19</sup> rabbit,<sup>20</sup> and bats.<sup>21</sup> The lingual mucosa has a highly differentiated system of papillae, with mechanical and gustatory functions, and those mucosa changes in epithelial morphology between the species are related to the type of food and adaptation of the animal to environmental conditions.<sup>22,23</sup> Filiform papillae (mechanical function) and fungiform, vallate and foliate papillae (gustative function) are found in domestic mammals, however, in animals with herbivore diet, foliate papillae are commonly absent.<sup>13,24</sup> The structure of connective tissue cores (CTC) is influenced by the diet due to the need for chewing according to the food,<sup>25</sup> and the characteristics of CTC can be used to denominate the morphologies present in the tongue.<sup>5</sup> In this study, we propose to analyze the morphology and ultrastructure characteristics of the dorsal epithelium and the CTC of the tongue of the wild boar, *Sus scrofa* (Order: Artiodactyla; Family: Suidae) This species has an omnivorous diet that involves the consumption of plant material (roots and seeds), invertebrates (earthworms), and vertebrates.<sup>26</sup> We reveal the differences observed between the lingual papillae that can contribute to future analyses and comparative descriptions among various mammalian species with similar habitat and eating habits.

## Materials and Methods

The tongues of 13 male wild boars were analyzed. These samples were donated by Fleury Brothers Avarenga LLC., located at Araçariçuama, São Paulo, Brazil. All procedures adopted in this study follow the ethical principles of the Ethics Committee on Animal Experimentation (CEUA process n° 7891310114), at the Faculty of Veterinary Medicine, University of São Paulo, São Paulo, Brazil.

### Light microscopy

The samples of the tongue (n=5) were fixed in 10% formalin solution, dehydrated in increasing series of alcohol, and embedded in paraffin blocks in histological sections of 6-10 µm thickness. The sections were stained in hematoxylin-eosin (HE) for evidence of the structural characteristics of the tongue.<sup>27</sup> The slides were examined by light microscopy (LM) (Carl Zeiss Microimaging, Axiokop 40, Göttingen, Germany) at the Faculty of Veterinary Medicine, University of São Paulo, São Paulo, Brazil.

### Scanning electron microscopy

The samples of the tongue (n=2) were immersed in a modified Karnovsky solution, washed in buffer, and divided for conventional and macerated scanning electron microscopy (SEM) techniques. For the extraction of the dorsal epithelium and analysis of the CTCs, the sample was immersed in a 10% NaOH solution for five days at room temperature and washed in distilled water with frequent changes for three days at 4°C. After this stage, all the samples were dehydrated in series of ethanol (60% to absolute), dried

in critical point apparatus (Balzers CPD-030) utilizing CO<sub>2</sub> liquid, mounted in metallic basis, and covered with gold ion (Balzers-SDC-040).<sup>5,28</sup> The samples were examined by a scanning electron microscopic LEO 435 VP at the Department of Surgery, Faculty of Veterinary Medicine, University of São Paulo, São Paulo, Brazil.

### Transmission electron microscopy

For transmission electron microscopy (TEM) analysis, the samples (n=2) were fixed in modified Karnovsky solution. The tissues were rinsed in 0.1 M sodium phosphate buffer (pH 7.4), post fixed in 1% osmium tetroxide aqueous solution for 2 h at 4°C, dehydrated in an increasing series of ethanol followed by propylene oxide, and embedded in Spurr<sup>TM</sup> resin. The sections were obtained using a Reichert Ultracut-E (C. Reichert AG, Vienna, Austria), stained with 4% uranyl acetate and 0.4% lead citrate for 3 min.<sup>29</sup> The sections were examined by transmission electron microscope JEOL 1010 (Jeol Co., Tokyo, Japan) at the Institute of Biomedical Sciences, University of São Paulo, Brazil.

## Results

The tongue of the Wild Boar measured about on average 8.5 cm and the presence of four types of papillae on the dorsal surface of the tongue was noted: filiform, fungiform, foliate, and vallate. The filiform and fungiform papillae were distributed in the total dorsal epithelium, and fungiform papillae and foliate papillae were observed in the lateral-caudal margin of the tongue. Additionally, two vallate papillae were observed in the caudal region of the dorsal surface.

### Light microscopy

The filiform papillae demonstrated elongated and conical shape with a thick keratin layer and the lamina propria. Highlighted is the observation that the epithelial layer had many cell nuclei from the basal layer to the stratum corneum (Figure 1a). The fungiform papillae were rounded with a dome shape with a thin keratinized layer, and taste buds on the epithelium surface (Figure 1b). The foliate papilla demonstrated a thin keratin layer, with taste buds in the epithelial layer of the groove, and the serous gland was noted (Figure 1c). The vallate papilla was surrounded by a groove with a thin keratinized epithelial layer, the taste buds were found in the medial wall of the groove, and the serous gland was observed (Figure 1d).

### Scanning electron microscopy

The filiform papilla revealed a conical shape with wide base, and squamous epithelium in the interpapillary zone (Figure 2a). After removing the dorsal epithelium, the CTC proved also to be of conical shape in proximity to other filiform papillae (Figure 2b).

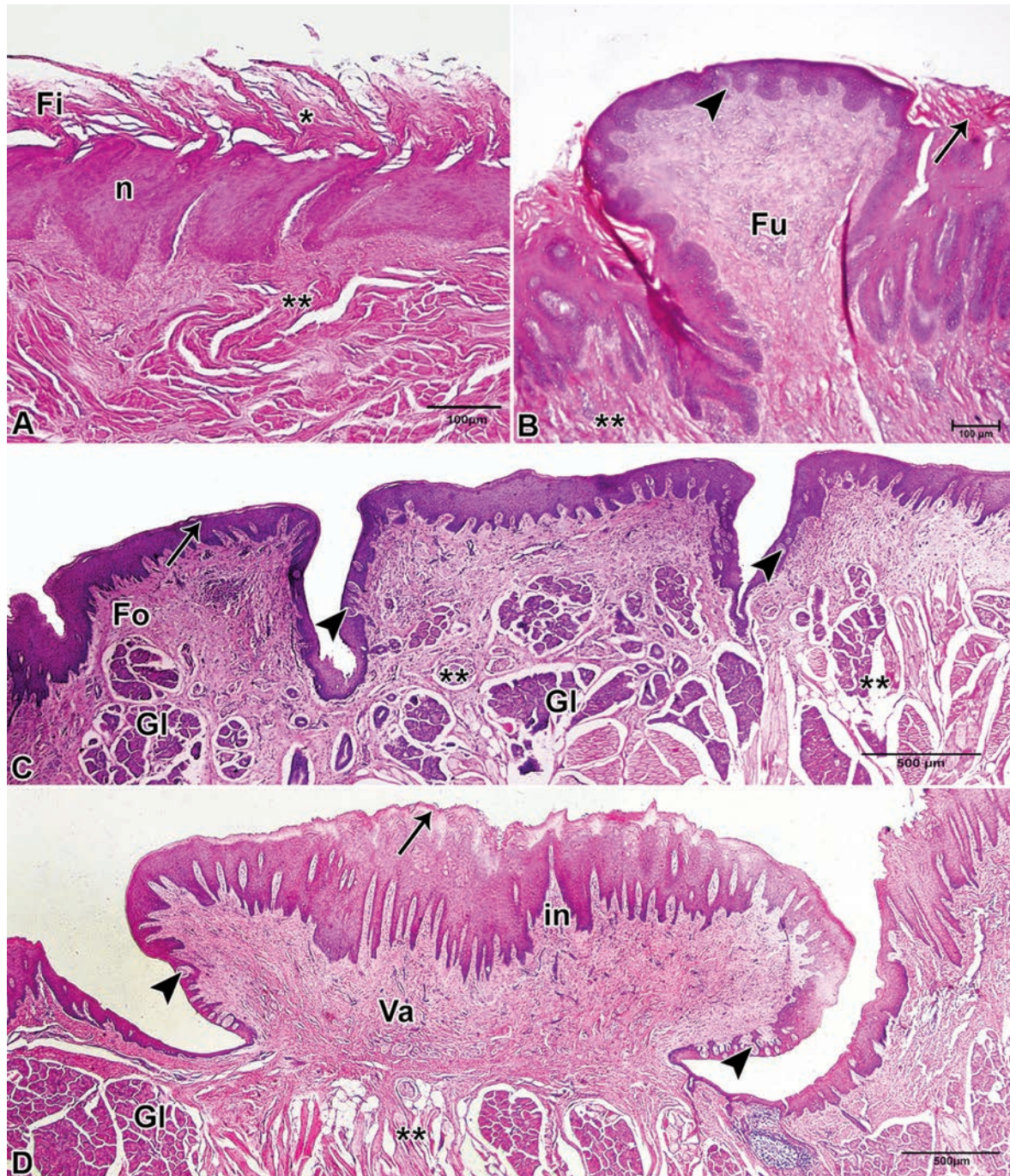
The fungiform papillae demonstrated rounded morphology with a dome-shape (Figure 2c). In the CTC, we observed the flower-bud shape with lateral linear projections from base to apex, and the support of the taste buds could be observed (Figure 2d). The foliate papillae had four pairs of epithelial folds separated by irregular grooves (Figure 2e). After removal of the epithelial layer, irregular grooves delimited by thin CTC were observed, as well as adjacent conjunctive projections (Figure 2f).

The vallate papillae had an oval shape, and were observed surrounded by a full groove forming the trench. Epithelium projections at the center of the papilla were noted (Figure 2g). The CTC demonstrated numerous connective projections lined by a deep groove. Also noted was the opening of ducts (Figure 2h).

### Transmission electron microscopy

Through the ultrastructural analysis, the cell layers were observed: keratinized; granular; spinous; basal; and lamina propria. The keratinized layer showed irregular shapes and other cell layers were superimposed in parallel (Figure 3a), interconnected by numerous desmosomes surrounded by amorphous substances, and the presence of some cell nuclei was noted

(Figure 3c). At high magnification, bundles of intermediate filaments (tonofilaments) were observed (Figure 3d). The granular layer had various keratohyalin granules, and the cell nucleus (Figure 3b). In the basal layer, several hemidesmosomes in the limit area that separates the basal and the lamina propria layers were observed, as also was evidenced a dense layer of collagen (Figure 3 e,f).



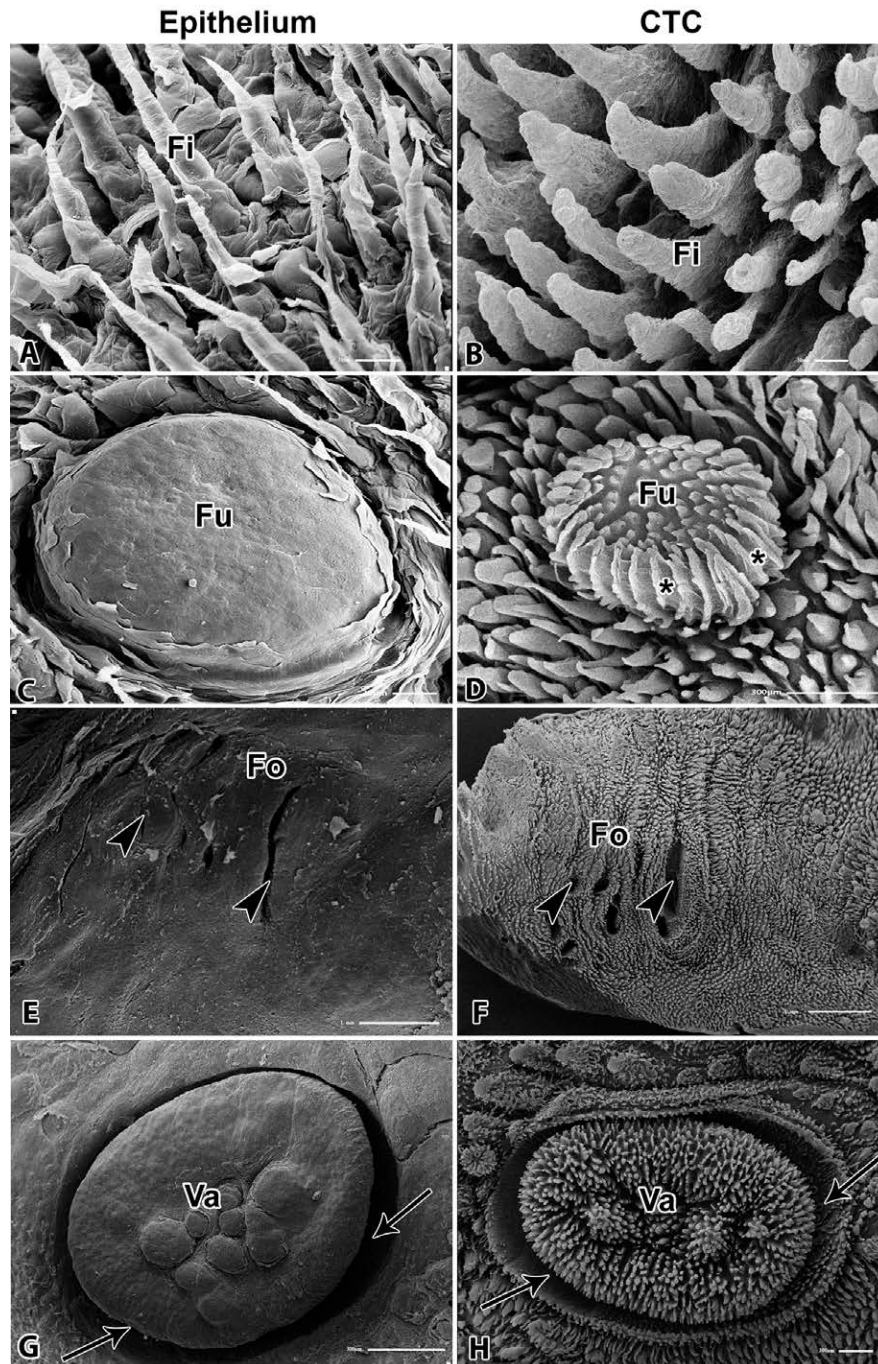
**Figure 1.** Light microscopy of the tongue of the wild boar. A) The filiform papillae (Fi) are elongated, had a thick epithelial layer (\*), cell nuclei in the epithelial layer (n), and lamina propria (\*\*) can be observed. B) The fungiform papillae (Fu) has a thin keratinized layer (arrow); the lamina propria (\*\*), and the taste bud (arrowhead) are noted. C) The foliate papilla (Fo) demonstrates the keratin layer (arrow), the taste buds (arrowhead), and also reveals small invaginations in the papillary-interface and serous glands (Gl) in the lamina propria (\*\*). D) Vallate papilla (Va) has a keratin layer on the surface (arrow), the taste buds (arrowhead), and the connective tissue invaginations (in) in the epithelial-tissue interface are observed. Additionally, the serous glands (Gl) in the lamina propria (\*\*) are observed.

## Discussion

We demonstrate the macroscopic, microscopic, and ultramicroscopic characteristics of the filiform, fungiform, foliate, and vallate papillae. The dorsal epithelium was composed of the keratinized, granular, spinous, basal, and lamina basal layers. Also

characterized were the dispositions of the taste buds, cell nuclei, and the distribution of the serous glands.

The histological results demonstrated that the epithelial mucosa layer was composed of squamous epithelium with various degrees of keratinization. The filiform papillae had a thick epithelial layer with numerous cell nuclei and were similar to bharal,<sup>18</sup> goat,<sup>24</sup> tayra,<sup>2</sup> roan antelope,<sup>30</sup> and capybara.<sup>31</sup>

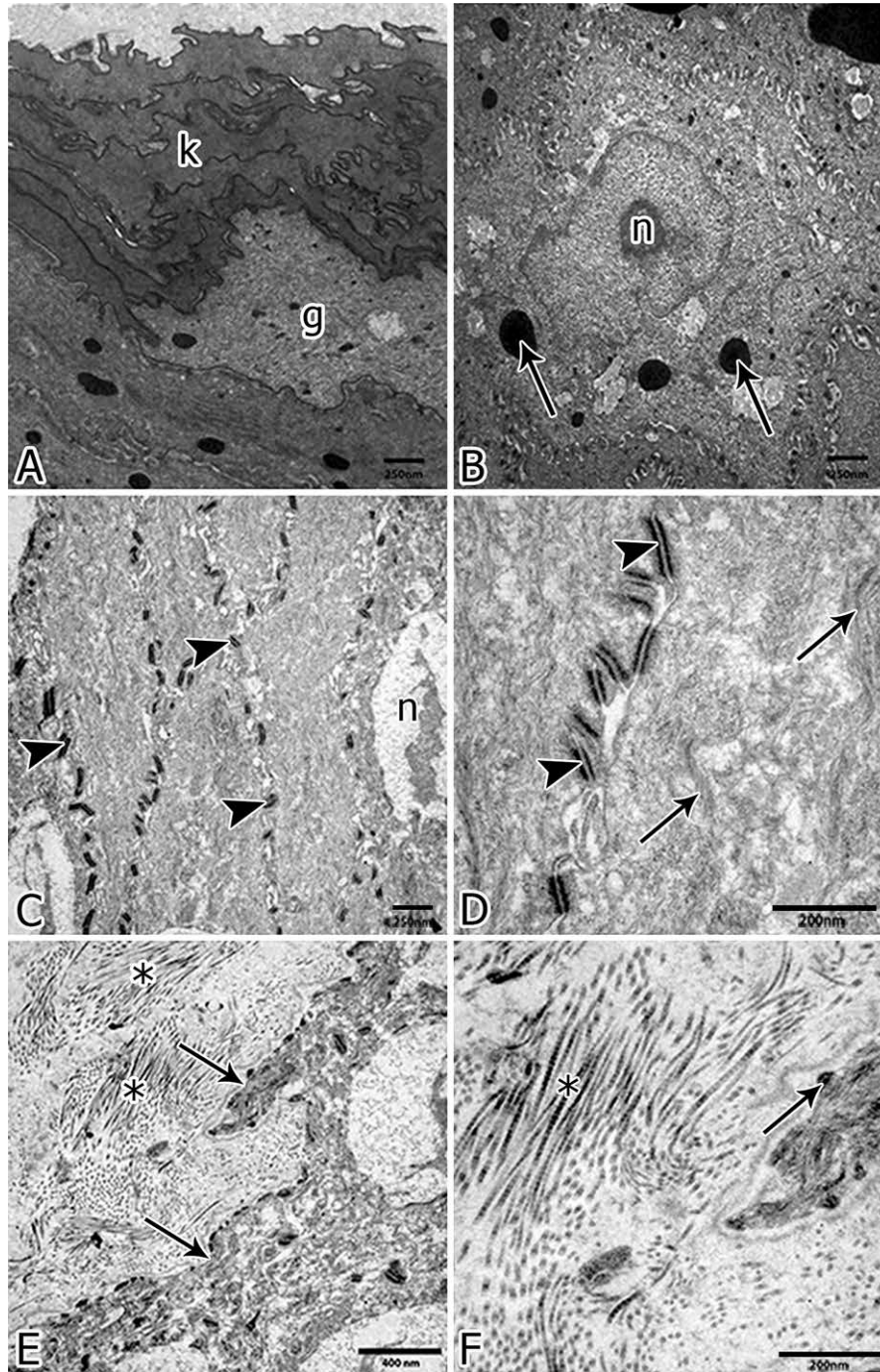


**Figure 2.** Scanning electron microscopy of the dorsal epithelium of the tongue of the wild boar. A) The filiform papillae (Fi) are shown elongated and pointed. B) The connective tissue cores (CTC) of the filiform papillae (Fi) with conical shapes. C) The fungiform papillae with dome shape have the keratinized epithelial cells (Fu). D) The CTC of the fungiform papilla (Fu) reveals a bud-flower shape and at the lateral portion, linear projections (\*) extending from base to apex. E) The foliate papillae (Fo) shows epithelial folds separated by irregular ridges (arrowhead). F) The CTC of the foliate papillae (\*\*) showed grooves delimited by irregular slender projections (arrowhead). G) Vallate papilla (Va) delimited by a full groove (arrows), has an oval shape. H) The CTC of the vallate papilla (Va) reveals many thin connective projections bordered by a deep groove (arrows). Scale bars: A,B) 1 mm; C,D,H) 300  $\mu$ m; E,E,G) 30  $\mu$ m.

The three-dimensional analysis revealed that the filiform papillae were elongated and conical in shape, located in the rostral and middle regions of the tongue, and CTCs had conical shapes. The shape of the filiform papilla differed from the trifurcated forms,<sup>2</sup> and from studies that demonstrate two distinct morphologies.<sup>1,32,33</sup> The morphologies of the filiform papillae can be subclassified into the following subtypes: two (insectivorous bat);<sup>23</sup> three (fruit

bat);<sup>21,23</sup> four (New Zealand white rabbits);<sup>34</sup> or six (fruit bats).<sup>34</sup>

The CTC of filiform papillae of the Wild Boar resembles a conical shape described for the leopard,<sup>16</sup> however, it differs from the U-shape,<sup>35</sup> projections that are finger-like and triangular shape,<sup>36</sup> from CTC formed by main and secondary processes and by multiple processes,<sup>18</sup> and from the CTCs divergent according to the region of the tongue.<sup>25</sup>



**Figure 3.** Transmission electron microscopy of the dorsal epithelium of the tongue of the wild boar. A) The epithelial cells of the keratinized layer with irregular shapes (k) and cells (g) of the granular layer containing few granules superimposed in parallel. B) Keratohyalin granules (arrows) and the cell nucleus (n) are observed. C) Desmosomes uniting epithelial cells (arrowhead), presence of cell nuclei (n). D) Observed desmosomes (arrowhead), and intermediate filaments bundles (arrows). E) Limit area between the lamina propria and the basal layer (arrows), showing bundles of collagen fibers (\*). F) Shows the hemidesmosomes, electrondense basal lamina (arrowheads) and lamina propria, as well as the presence of collagen fibers (\*).

The filiform papillae are the most abundant in the mammalian tongue, distributed over the dorsal surface and decreasing in the direction of the caudal region of the tongue.<sup>3</sup> These results are similar to those observed in the lesser hedgehog,<sup>19</sup> giraffe,<sup>37</sup> capybara,<sup>31</sup> and roan antelope,<sup>30</sup> however, it differs from the filiform papilla subtype in the rostral direction,<sup>34</sup> and in the medial and medioposterior directions.<sup>21</sup>

The fungiform papillae revealed a thin keratinized epithelial layer and the taste buds on the surface. Similar morphology was found in agouti<sup>38</sup> and capybara,<sup>31</sup> nevertheless, there are no taste buds in the fungiform papillae of coatis.<sup>39</sup> In the pig there are conical-like fungiform papillae in the proximity of the vallate papillae, and mushroom-shape fungiform papillae, although the number of taste buds varies between the subtypes.<sup>33</sup> The fungiform papillae demonstrate taste buds located apically in bat species<sup>10,23</sup> and in the domestic pig.<sup>33</sup> The fungiform papillae were distributed in the middle and lateral-caudal regions of the dorsal surface, whose characteristics are similar to those described in the pig,<sup>32</sup> goat,<sup>24</sup> dog,<sup>14</sup> and paca.<sup>40</sup>

The three-dimensional analysis demonstrated that the fungiform papilla was rounded and resembles a fungiform papilla type described for the Egyptian fruit bat,<sup>21</sup> but differs from the mushroom shape,<sup>17,33</sup> dome-like shape with varied sizes,<sup>10</sup> and the rectangular fungiform papillae.<sup>21</sup> After epithelium removal, the CTCs have a bud-flower shape and this differs from the volcano shape,<sup>28</sup> the rose-bud shape,<sup>37</sup> the handle shape,<sup>41</sup> the dome shape,<sup>42</sup> and the columnar shape.<sup>36</sup>

We can emphasize that the foliate papilla demonstrated a large number of taste buds in the epithelium of the medial wall of the groove, and also the serous glands were observed. The presence of taste buds in the lower region of the wall of the groove has been described for the pig.<sup>32</sup> The foliate papilla does not present taste buds in suckling and adult feral cat individuals,<sup>17</sup> and the foliate papillae are not very developed in the Artiodactyla order.<sup>30</sup> The foliate papillae presented three to four pairs of epithelial folds, separated by irregular parallel grooves, located in the lateral-caudal margin of the tongue. This morphology differs from that of the interdigitation pattern with shallow sulcus<sup>17</sup> and the cone-like projections found on the papilla surface.<sup>36</sup> This description is similar to that found for the wild boar and differs from the pig due to the irregularity of the number of leaves, the arrangement of the sulcus, and the presence of pseudo papillae.<sup>33</sup>

The location of these papillae is similar to those reported in rodent,<sup>43</sup> rats,<sup>28</sup> agouti,<sup>38</sup> and sloth.<sup>15</sup> Moreover, the number of epithelial folds varies with the species; bears have five to six pairs of foliate papillae separated by deep grooves in the posterior third of the lateral margin of the tongue.<sup>44</sup> Already the arctic foxes have between two and three pairs of foliate papillae of the tongue in the caudal region.<sup>45</sup> Their CTC presented irregular ridges and delimitations of laminar projections and differed from long cone-like conjunctive projections,<sup>36</sup> from small projections surrounded with laminar projections,<sup>5</sup> from thin parallel laminae with projections on the part superior,<sup>38</sup> and from the CTC with parallel arrangement.<sup>31</sup>

Regarding the structure of the vallate papilla, we reveal a thick epithelial layer and numerous taste buds in the inferior region of the medial wall of the groove, also described for ocelot<sup>1</sup> and tayra;<sup>2</sup> however, this differs from the central duct opening in papilla described for the muntjac deer.<sup>25</sup> The presence of the serous gland was similar to those described for the dromedary,<sup>46</sup> agouti,<sup>38</sup> sloth,<sup>15</sup> llamas,<sup>11</sup> and rats.<sup>6</sup> We observed two papillae vallate in the caudal region with oval shape delimited by a groove with irregular projections at the center of papillae, and the CTC have different shapes and the constitution of thin filaments bordered by a deep groove. The three-dimensional morphology of the vallate papillae is similar to that of the feral cat,<sup>17</sup> but differs from the pig in the absence

of the annular projection.<sup>32</sup> Also, the morphology of the vallate papilla can be rounded,<sup>2</sup> and rounded with an annular pad.<sup>13</sup> The irregular projections found on the surface of the vallate papilla are similar to the pseudo papillae described for wild boar and pigs. In addition, epithelial micro-folds have been described and it has been demonstrated that filiform papillae cover the annular projection in the vallate papillae of pigs.<sup>33</sup>

The CTC of vallate papilla of the wild boar differs from the descriptions of CTC formed by numerous spines,<sup>18,3</sup> surfaces with ditches,<sup>16</sup> and concave surface surrounded by a conjunctive wall-like projection.<sup>36</sup> Among the species, there is a variation in the number of pairs of vallate papillae in three or four pairs for the alpaca,<sup>11</sup> two pairs in the agouti,<sup>38</sup> and five to six pairs in the dromedary.<sup>46</sup> The keratinized layer was formed by cells with irregular shapes and superimposed in parallel. This arrangement of cells is similar to that described for the guinea pig<sup>5</sup>, however, in agouti, the cells present as a saw-like pattern.<sup>38</sup> The granular layer revealed several keratohyalin granules and in a study with WWCP rats, these granules were located mainly in the anterior part of the filiform papillae.<sup>8</sup> The basal layer presented hemidesmosomes and a layer of dense collagen at the interface with the lamina propria. The interface between the basal layer and the lamina propria has an area of subepithelial connective tissue,<sup>38</sup> where fibroblasts are present.<sup>5,31</sup> Through the LM, SEM, and TEM analysis, it can be stated that the morphological characteristics of the lingual papillae of animals of all species, including the wild boar, depend on their diet and habitat, and this was corroborated by several authors.<sup>6,7,19,47</sup>

The wild boar is a terrestrial species with an omnivorous diet,<sup>26</sup> and the presence of foliate papillae is expected because it is only absent in herbivorous animals.<sup>13,24</sup> This species consumes plant material, invertebrates, and vertebrates,<sup>26</sup> and the diet influences the lingual papillae morphology,<sup>22,23</sup> and the CTC morphology.<sup>25</sup> In addition, the keratinization of the tongue and its papillae are characteristic of terrestrial animals due to the evolution of these species.<sup>4</sup>

In conclusion, we have described the new descriptions and shapes of the CTCs of the lingual papillae of the wild boar. Additionally, we have demonstrated the epithelium structural characteristics and the ultrastructural aspects of the dorsal epithelium of the tongue.

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