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Macroscopic and Microscopic Analysis of the Tongue of the Common Opossum (*Didelphis marsupialis*)

CELINA A.F. MANÇANARES,[1] AMILTON C. SANTOS,[2] MARIA V. PIEMONTE,[2] BRUNO G. VASCONCELOS,[2] ANA F. CARVALHO,[3] MARIA A. MIGLINO,[2] CARLOS E. AMBRÓSIO,[1] AND ANTÔNIO C. ASSIS NETO[1]*

[1]*Department of Basic Sciences of the School of Animal Science and Food Engineering, University of São Paulo, São Paulo, Brazil*
[2]*Department of Surgery, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil*
[3]*University Center of the Octavio Bastos Educational Foundation, São João da Boa Vista, Brazil*

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ABSTRACT We performed a macroscopic and microscopic study of the tongues of common opossums, *Didelphis marsupialis,* from South America. We studied two males and two females. We collected morphometric data on the tongue with precision calipers. For the light microscopy and scanning electron microscopy analyses, we fixed tissue fragments in 10% formaldehyde and 2.5% glutaraldehyde, respectively. The opossum tongues averaged 5.87 ± 0.20 cm in length, 3.27 ± 0.15 cm in width at the lingual body, and 3.82 ± 0.15 cm in width at the root. The mean thickness of the lingual body was 1.8 ± 0.1 cm, and the thickness of the root was 3.82 ± 0.15 cm. Sharp filiform papillae were scattered across the entire tongue; conical filiform papillae occurred on the lingual body and tongue tip; fungiform papillae were scattered among the filiform papillae on the lingual body and tongue tip; and there were three vallate papillae at the root of the tongue. We found two strands of papillary projections in the tongue root. Despite the low variability observed in the lingual papillae, the morphological data obtained in this study may be related to the opossum's diverse food habits and the extensive geographic distribution of the species throughout America. *Microsc. Res. Tech. 75:1329–1333, 2012.*

INTRODUCTION

The common opossum is a mammal from the subclass Theria; infraclass Metatheria; order Marsupialia; family Didelphidae; subfamily Didelphinae, genus *Didelphis* (Orr, 1986; Zeller, 1999). Two species are found in South America: *Didelphis marsupialis* and *Didelphis albiventris*.

D. marsupialis is found in humid regions of Brazil such as the Amazon Forest and the southeast. *D. albiventris* is found in northeastern and central Brazil (Cáceres et al., 2008; Cerqueira, 1985). Although these marsupial species are common in South America, few studies have addressed their biology, ecology, and food habits (Cáceres, 2000; Cerqueira, 1985; Lessa and Geise, 2010).

Species of the genus *Didelphis* are omnivorous and largely generalist feeders. Their diet varies seasonally and is primarily composed of insects, birds, eggs, small mammals, fruits, seeds, leaves, and, less frequently, reptiles, amphibians, and mollusks (Aguiar et al., 2004; Aragona and Marinho-Filho, 2009).

The mammalian tongue exhibits different morphological adaptations in different species, which serve highly specialized feeding functions (Dyce et al., 2010; Okada and Schraufnagel, 2005) such as food capture, water uptake, the movement of food in the mouth, swallowing, suckling (Guimarães et al., 2011), and taste (Du Toit, 2003). Additionally, the functional stimuli of the tongue play a role in facial growth and development (Bezerril et al., 2005).

The dietary habits of species are directly related to tongue texture, which is determined by an assemblage of different types of papillae (Abreu et al., 2006; Branco et al., 2012; Ciuccio et al., 2008). There are several functional types of lingual papillae, such as the mechanical papillae (filiform, conical, and marginal) and the gustatory papillae (vallate, fungiform, and foliated) (Branco et al., 2011; Okada and Schraufnagel, 2005). Our aim in this study was to describe the macroscopic and microscopic aspects of the tongue of the common opossum (*D. marsupialis*). Our results present information that is relevant to comparative animal anatomy.

MATERIAL AND METHODS

Animals

We studied two males and two females of *Didelphis marsupialis.* The animals were acquired from the Department of Animal Morphology of the University Center of the Octavio Bastos Educational Foundation (Centro Universitário da Fundação de Ensino Octávio Bastos – UniFEOB), São João da Boa Vista, Brazil, and from the Department of Anatomy of Domestic and Wild Animals, School of Veterinary Medicine (Faculdade de Medicina Veterinária e Zootecnia – FMVZ), University of São Paulo (USP), São Paulo, Brazil. The study was approved by the Bioethics Committee of FMVZ/USP and registered under protocol number 493/2004.

Laboratories

We worked in the Laboratory of Animal Morphology of UniFEOB, São João da Boa Vista, São Paulo, and

*Correspondence to: Antônio Chaves de Assis Neto, Faculdade de Medicina Veterinária e Zootecnia, Programa de Pós-graduação em Anatomia dos Animais Domésticos e Silvestres, Universidade de São Paulo – USP. Av. Prof. Dr. Orlando Marques de Paiva, CEP 05508-270, São Paulo – SP, Brazil. E-mail: antonioassis@usp.br

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TABLE 1. The tongue opossums (Didelphis marsupialis) length, width, and thickness measures evidencing the average and standard deviations

Sample	Length (cm)	Root width (cm)	Body width (cm)	Root thickness (cm)	Body thickness (cm)
an. 1	6	4.4	5.3	4.0	1.8
an. 2	6	4.5	5.3	3.9	1.9
an. 3	5.8	4.1	5.0	3.7	1.8
an. 4	5.7	4.1	5.0	3.7	1.7
Average	5.875	4.275	5.15	3.825	1.8
Deviation	±0.15	±0.20	±0.17	±0.15	±0.08

the Laboratory of Histology and Electronic Microscopy of FMVZ/USP, São Paulo, Brazil.

Morphometric Analysis

We measured the opossum tongues with precision calipers. The means and standard deviations of the morphometric data are presented in Table 1.

Light Microscopy

Samples were dehydrated in a graded ethanol series (60%–100%), cleared in xylene, and embedded in Histosec (Merck; Tolosa et al., 2003). Sections of 5 μm were obtained on a microtome (Leica RM 2155) and stained with Hematoxylin-eosin. Microscopy slides were mounted with Entellan (Historesin Merck). We utilized a microscope (Leica DM 2000) coupled with an image capture system to study cell morphology.

Scanning Electron Microscopy

Tissue fragments were fixed in glutaraldehyde (Propylene oxide EM Grade - Polysciences, Inc., USA), washed in 0.1 M phosphate buffer at a pH of 7.4, and post-fixed in 1% osmium tetroxide (Spurr's Kit - Electron Microscopy Sciences Co., USA). Then, tissue fragments were dehydrated in a graded ethanol series (50%, 70%, 90%, and 100%) and dried in a critical point dryer (Balzers PCD 020). Tissues fragments were fixed in metal supports (stubs) and then sputter coated with gold (Emitech K550). The analysis was performed on an electron microscope, model Leo 435 VP.

Nomenclature

We used the nomenclature established by the International Committee on Veterinary Histological Nomenclature (1994) and the International Committee on Veterinary Gross Anatomical Nomenclature (2005).

RESULTS

Macroscopic Analysis

The opossum tongue extends from the oropharynx and is located within the oral cavity. It is divided into three parts: the posterior fixed root, the anterior free tip, and the fixed lingual body, located between the two other portions. Morphometric data are summarized in Table 1. The ventral surface of the tongue is smooth, whereas the dorsal surface is irregular due to tongue papillae. Filiform, fungiform, and vallate papillae were found on the dorsal surface (Figs. 1 and 2).

The macroscopic and microscopic analyses indicated the presence of two types of filiform papillae: sharp filiform papillae and tall conical filiform papillae. Sharp filiform papillae were the most common and were scattered throughout the dorsal surface of the tongue, whereas conical filiform papillae were less numerous and located only on the tongue tip and the lingual body. There were no filiform papillae at the root of the tongue (Figs. 1A, 1B, and 1E).

Fungiform papillae were less common and occurred among the filiform papillae on the tongue tip and lingual body. Additionally, there were marginal fungiform papillae surrounding the tongue tip and the two posterolateral folds near the tip (Fig. 2A).

We found three isolated vallate papillae at the tongue root in the studied specimens, which were not surrounded by any other type of papillae (Figs. 1F, 1I, and 1J). We did not find foliated, conical, or lentiform papillae. Two strands of papillary projections were observed at the tongue root projecting toward the oropharynx (Fig. 1F).

Light and Scanning Electron Microscopy

Light microscopy revealed that the lamina propria of the tongue is formed by two layers: (1) a surface layer of stratified squamous epithelium, in which the lingual papillae are found (Figs. 1C, 1D, 1G, and 1H); and (2) a basal layer (submucosa) of dense irregular connective tissue consisting of vessels, nerves, and serous glands (Fig. 2E). Light microscopy also revealed morphological differences between the sharp filiform papillae and the tall conical filiform papillae (Fig. 1E). The sharp filiform papillae contained fewer vesicles than the tall conical filiform papillae (Fig. 2C). Prominent marginal fungiform papillae were observed throughout the lateral folds and the tongue tip (Fig. 2C).

Taste buds were closely related to the epithelium of the vallate papillae. These taste buds were cylindrical and surrounded by clusters of epithelial cells (Figs. 1G and 1H). There were also deep sulci around the isolated vallate papillae (Figs. 1I and 1J). The intrinsic tongue muscles of the submucosal layer were arranged in longitudinal and transverse bundles (Fig. 2D). Furthermore, there was unilocular fat tissue in the adventitia layer of the lingual body (Fig. 2F). The ventral surface of the tongue lacks papillae and is covered by the squamous epithelium in the mucosa and by dense irregular connective tissue in the submucosa (Fig. 2G).

DISCUSSION

The topography of the tongue in the oral cavity of *D. marsupialis* is similar to the descriptions by Dyce et al. (2010), Ellenport (1986), and Schaller et al. (1999) in domestic mammals.

The lingual papillae of *D. marsupialis* are irregularly distributed on the dorsal surface and absent from the ventral surface. This is similar to the morphologies of other small mammals (Abreu et al., 2006; Branco et al., 2011, 2012; Chamorro et al., 1987; Ciuccio et al., 2008, 2010; Martinez et al., 1998; Watanabe et al., 1988, 2009) (Table 2).

The types of lingual papillae and their distribution, morphology, and abundance are important factors to consider when analyzing the feeding habits of a species. The structural details of the lingual papillae in marsupials are related to their diet and ecology (Okada and Schraufnagel, 2005).

The types of papillae that we observed in the studied specimens of the common opossum from Brazil are similar to those found in the same species from North America (Okada and Schraufnagel, 2005).

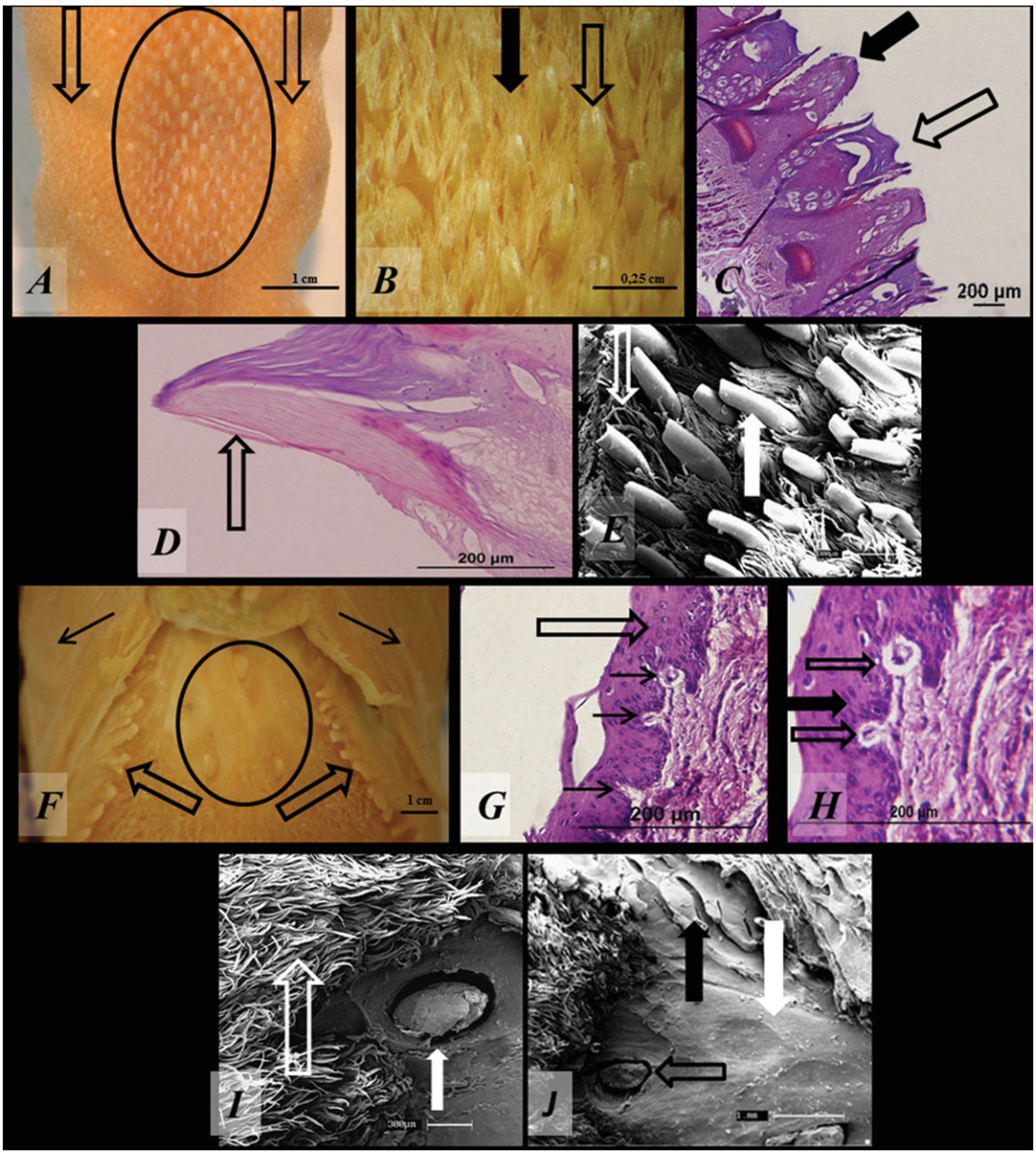

Fig. 1. Tongue opossum in macroscopic view, light microscopy (LM) and scanning electron microscopy (SEM). **A**: Macroscopic view of the body of the tongue with sharp and tall conical filiform papillae (arrows) and fungiform papillae (circle). Bar: 1 cm. **B**: macroscopic view of the body of the tongue showing sharp filiform papillae (filled arrow) and tall conical filiform papillae (arrowhead). Bar: 0.25 cm. **C**: LM (HE) of the sharp filiform papillae (filled arrow) and tall conical filiform papillae (arrowhead). Bar: 200 µm. **D**: LM (HE) in sharp filiform papillae (arrow). Bar: 200 µm. **E**: SEM of the body of the tongue showing sharp filiform papillae (arrowhead) and tall conical filiform papillae (filled head) Bar: 300 µm. **F**: macroscopic view of the root of the tongue with three vallate papillae (circle), papillary cords projections (arrows), muscular revetment of the root of the tongue (thin arrow). Bar: 1 cm. **G**: LM (HE) vallate papillae in nonkeratinized-stratified squamous epithelium (large arrow) and taste buds (thin arrow). Bar: 200 µm. **H**: LM (HE) vallate papillae in nonkeratinized-stratified squamous epithelium (filled arrow) and taste buds empty arrows). Bar: 200 µm. **I**: SEM of the root of the tongue showing sharp filiform papillae (arrowhead) and vallate papillae (filled arrow). Bar: 300 µm. **J**: SEM of the root of the tongue showing vallate papillae (arrowhead), papillary projections (black arrow) and deprived papillae region (white arrow). Bar: 1 mm.

However, Okada and Schraufnagel emphasized the angioarchitecture of the tongue. Similar papillae have also been found in *D. virginiana* and *D. albiventris* (Krause and Cutts, 1982; Martinez et al., 1998) (Table 2). This similarity in the lingual papillae may be due to a shared omnivorous feeding habit and does not appear to be affected by differences in habitat or food. The diet of a widely distributed species may vary between different biomes or habitats (Lessa and Geise, 2010).

The distributions of the fungiform and filiform papillae observed in this study are similar to those described

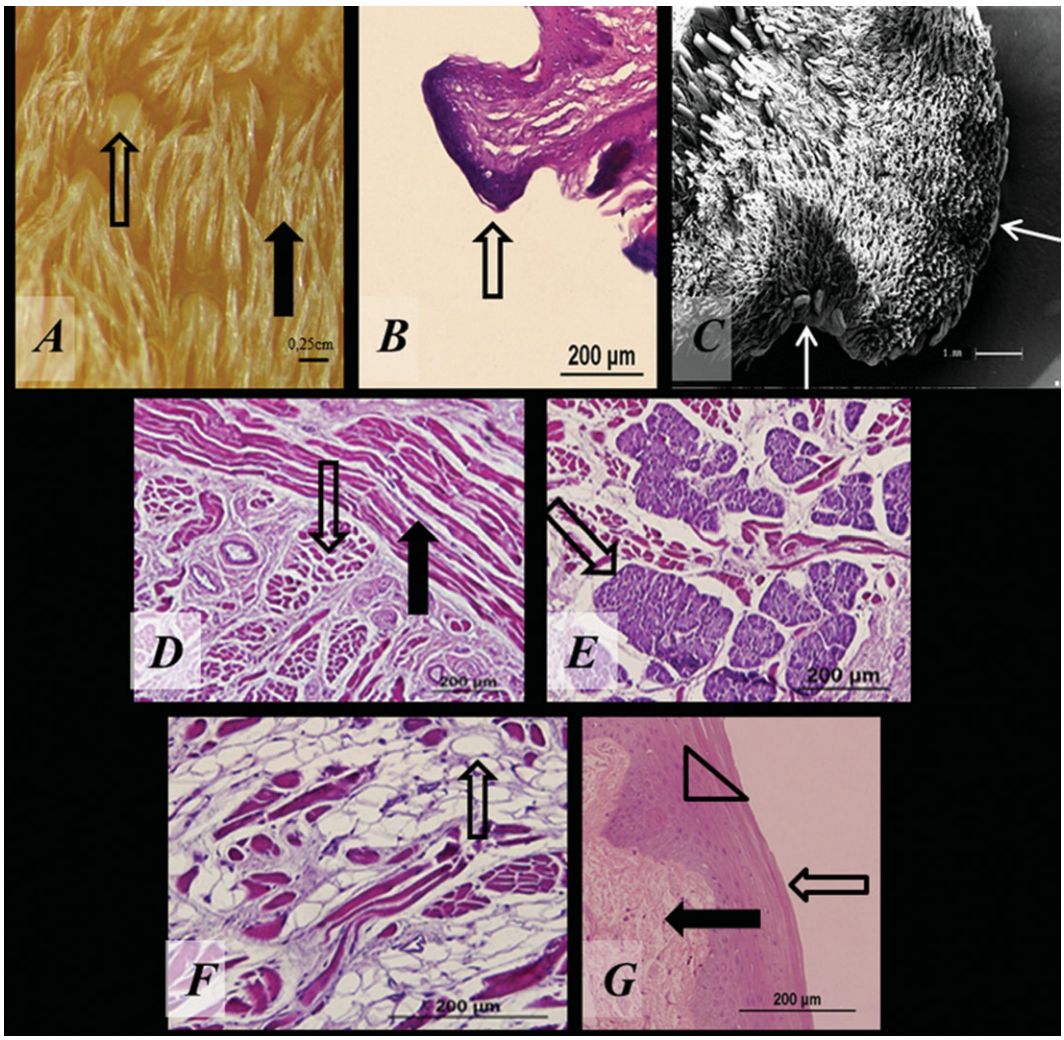

Fig. 2. Tongue opossum in macroscopic view, light microscopy (LM) and scanning electron microscopy (SEM). **A**: macroscopic view of the apex tongue with filiform papillae (filled arrow) and fungiform papillae (arrowhead). Bar: 0.25 cm; **B**: LM of fungiform papillae. Bar: 200 µm; **C**: SEM of the apex of the tongue showing the fungiform papillae (arrows). Bar: 1 mm; **D**: LM (HE) of the body of the tongue showing transverse muscle layer (filled arrow) and longitudinal muscle layer (arrowhead). Bar: 200 µm; **E**: LM of the root of the tongue showing serous acini (arrow). Bar: 200 µm; **F**: LM of the root of the tongue showing unilocular adipose tissue (arrow). Bar: 200 µm; **G**: LM of the ventral region of the tongue showing keratinized stratified squamous epithelium (arrowhead), not modeled dense connective tissue (filled arrow) and keratin layer (arrowhead). Bar: 200 µm.

TABLE 2. Statement of presence of tongue lingual papillae available in marsupial and others mammalian

	Papillae (present or absent)						
	Filiform						
Species (author)	Sharp	Tall conical	Conical	Fungiform	Vallate number	Foliated	Lentiform
Marsupials							
Didelphis marsupialis (current research)	+	+	−	+	3	−	−
Didelphis virginiana (Martinez et al., 1998)	+	+	−	+	3	−	−
Didelphis albiventris (Martinez et al., 1998)	+	+	−	+	2	−	−
Didelphis marsupialis (Okada and Schraufnagel, 2005)	+	+	−	+	3	−	−
Other mammalian							
Oryctolagus cuniculus (Chamorro et al., 1987)	+	−	+	+	2	+	−
Felis catus (Chamorro et al., 1987)	+	−	+	+	6	+	−
Zaedyus pichiy (Ciuccio et al., 2008)	+	−	−	+	2	−	−
Tayassu pecari (Watanabe et al., 2009)	+	−	−	+	2	−	−
Dasypus hybridus (Ciuccio et al., 2010)	+	−	−	+	2	−	−
Sotalia guianensis (Guimarães et al., 2011)	+	−	+	−	−	−	−
Saimiri sciureus (Branco et al., 2011)	+	−	−	+	+	+	−
Callithrix penicillata (Branco et al., 2012)	+	−	−	+	3	−	−

+, present; −, absent.

in species with different feeding habits, such as rabbits, cats, horses, llamas, rats, armadillos, peccaries, and primates (Abreu et al., 2006; Banks, 1991; Branco et al., 2011, 2012; Chamorro et al., 1987; Ciuccio et al., 2008, 2010; Martinez et al., 1998; Watanabe et al., 1988, 2009) (Table 2). The marginal fungiform papillae surrounding the posterolateral folds and tongue tip are unique to opossums and have not been described in other land mammals. Aside from opossums, marginal papillae have only been described for Guiana dolphins (*Sotalia guianensis)* (Guimarães et al., 2011).

The *D. marsupialis* tongues examined in this study also exhibited some unique features that have not been described for other opossum species (Krause and Cutts, 1982; Martinez et al., 1998). These features include the strands of papillary projections on the tongue root, which have also been described in *D. marsupialis* specimens from North America (Okada and Schraufnagel, 2005). These papillary projections on the tongue root seem to be used to direct food into the oropharynx. However, further studies regarding the masticatory and swallowing mechanisms in this species are required.

The tall, conical, thick filiform papillae, and the sharp filiform papillae were present in both the *D. marsupialis* specimens examined in this study and those from a study on North American specimens (Okada and Schraufnagel, 2005). Similar results were also found for *D. virginiana* and *D. albiventris* (Krause and Cutts, 1982; Martinez et al., 1998) (Table 2).

The two types of filiform papillae found in *D. marsupialis* may reflect the constantly changing diet resulting from its characteristic omnivorous habit, but the papillae do not appear to be influenced by habitat (Aguiar et al., 2004; Okada and Schraufnagel, 2005).

The number and distribution of vallate papillae in these Brazilian specimens of *D. marsupialis* are similar to the findings for North American specimens of *D. marsupialis* (Okada and Schraufnagel (2005) and for *D. virginiana* (Chamorro et al., 1987), but differ from the findings for *D. albiventris* (Martinez et al., 1998) (Table 2). These differences may be related to the different habitat and feeding habits of *D. albiventris*.

The distribution of lingual papillae is very similar across the three *Didelphis* species, even though the species are found in different regions of the Americas. This fact reflects their common omnivorous habits. Krause and Cutts (1982) argue that the marsupial tongue has features that serve as the basis for structures in other mammals, although some of these structures were lost during evolution. The results of this study contribute to the knowledge of the lingual morphology and biology of *D. marsupialis,* which is widely distributed in the Americas. Our data could be used in studies of taxonomy, natural selection, and the anatomical and physiological adaptations of mammals in general.

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REFERENCES

Abreu MAMM, Weckx LLM, Hirata CHW. 2006. Aspectos histológicos e ultraestruturais da língua em ratos desnutridos. Rev Bras Otorrinolaringol 72:523–527.

Aguiar MS, Ferigolo J, Rossi JL, Jr, Gioso MA. 2004. Atrição dental em *Didelphis albiventris* e *D. marsupialis* (Marsupialia, Didelphimorphia, Didelphidae) do Sul do Brasil. Cienc Rural 34:1127–1132.

Aragona M, Marinho-Filho J. 2009. História natural e biologia reprodutiva de marsupiais no Pantanal, Mato Grosso, Brasil. Zoologia 26:220–230.

Banks WJ. 1991. Histologia Veterinária Aplicada, 2nd ed. São Paulo, Brazil: Manole. 629 p.

Bezerril DD, Barreto J, Macari S, Felício CM. 2005. A língua: Características morfológicas normais e alterações. Rev. Ibero-am. Odontopediatr. Odontol Bebê 8:264–270.

Branco E, Guimarães JP, Miglino MA, Lacreta ACC, Jr, Ishizaki MN, Gomes BD, Muniz JAPC, Imbeloni A, Fioretto ET, Lima AR. 2011. Ultrastructural aspects of Lingual Papillae in squirrel monkey (*Saimiri sciureus*). Microsc Res Tech 74:484–487.

Branco E, Pereira WL, De Lima AR, Franciolli AL, Rici RE, Miglino MA, Muniz JA, Imbeloni A. 2012. Ultrastructural aspects of *Callithrix penicillata* lingual papillae. Microsc Res Tech 75:282–284.

Cáceres NC. 2000. Population ecology and reproduction of the white-eared opossum *Didelphis albiventris* (Mammalia, Marsupialia) in an urban environment of Brazil. Ciencia e Cultura, Sao Paulo, Brazil 52:171–174.

Cáceres NC, Carmignotto AP, Fischer E, Santos CF. Mammals from Mato Grosso do Sul, Brazil. 2008. Check List 4:321–335.

Cerqueira R. 1985. The distribution of *Didelphis* in South America (Polyprotodontia, Didelphidae). J Biogeog 12:135–145.

Chamorro CA, Sandoval J, Fernandez JG, Fernandez M, De Paz P. 1987. Estudio comparado de las papilas linguales del Gato (*Felis catus*) y del Conejo (*Oryctolagus cuniculus*) mediante el microscopio electronico de barrido. Anat Histol Embriol 16:37–47.

Ciuccio M, Estecondo S, Casanave EB. 2008. Scanning electron microscopy study of the dorsal surface of the tongue in *Zaedyus pichiy* (Mammalia, Xenarthra, Dasypodidae). Int J Morphol 26:13–18.

Ciuccio M, Estecondo S, Casanave EB. 2010. Scanning electron microscopy study of the dorsal surface of the tongue of *Dasypus hybridus* (Mammalia, Xenarthra, Dasypodidae). Int J Morphol 28:379–384.

Du Toit DF. 2003. The tongue structure and function relevant to disease and oral health. South African Assoc Dent J 58:375–383.

Dyce KM, Sack WO, Wensing CJG. 2010. Tratado de anatomia veterinária, 4th ed. Rio de Janeiro: Guanabara Koogan. 813 p.

Ellenport CR. 1986. Sistema Digestivo do Carnívoro. In: Getty R, editor. Anatomia dos Animais Domésticos, version 2, 5th ed. Rio de Janeiro: Interamericana. 1445 p.

Guimarães JP, Mari RB, Marigo J, Rosas FCW, Watanabe I. 2011. Light and scanning electron microscopic study of the tongue in the Estuarine Dolphin (*Sotalia guianensis* van Bénéden, 1864). Zool Sci 28:617–622.

International Committee on Veterinary Histological Nomenclatura. 1994. Nomina Histological, 2nd ed. Rev Zurich. 137 p.

International Committee on Veterinary Gross Anatomical Nomenclature. 2005. Nomina Anatomica Veterinaria, 5th ed. Hannover: Columbia, Gent, Sapporo. 165 p.

Krause WJ, Cutts H. 1982. Morphological observations on the papillae of the Opossum tongue. Acta Anat 113:159–168.

Lessa GL, Geise L. 2010. Hábitos alimentares de marsupiais didelfídeos brasileiros: Análise do estado de conhecimento atual. Oecol Austral 14:901–910.

Martinez M, Martinez FE, Pinheiro PFF, Almeida CCD, Guida HL, Watanabe I. 1998. Light and scanning electron microscopic study of the vallate papillae of the Opossum (*Didelphis albiventris*). Rev Chil Anat 16:67–73.

Okada S, Schraufnagel DE. 2005. Scanning electron microscopic structure of the lingual papillae of the common opossum (*Didelphis marsupialis*). Microsc Microanal 11:319–332.

Orr RT. 1986. Biologia dos vertebrados, 5th ed. São Paulo: Roca. 674 p.

Schaller O. 1999. Nomenclatura anatômica veterinária ilustrada. São Paulo: Manole. 614 p.

Tolosa EMC, Rodrigues CJ, Behmer AO, Freitas Neto AG. 2003. Manual de técnicas para histologia normal e patológica, 2nd ed. Manole: Barueri. 331 p.

Watanabe I, Ogawa K, Yamada E. 1988. Taste buds of rabbits foliate papillae. A scanning electron microscopy study. Ciênc Cult 40: 787–790.

Watanabe I, Guimarães JP, Boleta AS, Almeida SRY, Righeti MMT, Santos TC, Miglino MA, Kfoury Júnior JR. 2009. Nerve endings of filiform, fungiform and vallate papillae of dorsal tongue mucosa of white-lipped peccary (*Tayassu pecari*). Neurohistological observations. Pesq Vet Bras 29:281–285.

Zeller U. 1999. Mammalian reproduction: Origin and evolutionary transformations. Zool Anzeiger. 238:117–130