

## MORPHOLOGICAL AND ULTRASTRUCTURAL ANALYSIS OF THE TONGUE OF *Gracilinanus microtarsus* (WAGNER, 1842)

### ANÁLISE MORFOLÓGICA E ULTRAESTRUTURAL DA LÍNGUA DA *Gracilinanus microtarsus* (WAGNER, 1842)

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**ABSTRACT:** The *G. microtarsus* is a small marsupial belonging to the Didelphidae family and may be found mainly in the Atlantic Rainforest of the Brazilian Southeastern region. *G. microtarsus* has important ecological importance in the dispersion of seeds of several plant species. Current research characterizes morphologically six tongues of adult male and female specimens, which are fixed in 10% formaldehyde. The tongues were analyzed, measured and photographed "in situ" and "ex situ". Anatomy, light microscopy and ultrastructural analysis were performed. Macroscopically the tongue has root, a fixed body and a free apex. The tongue has fungiform papillae and high conical and acute filiform papillae across the dorsal surface. It also featured three vallate papillae located at the root and marginal fungiform papillae at the apex. Microscopically, the tongue is composed of longitudinal and transversal muscle fibers. Blood vessels, nerves, serous, mucous and mixed acini are found in non-modeled connective tissue. Ventrally, the tongue is lined by a keratinized stratified pavement epithelium. The morphology of the tongue of *G. microtarsus* resembles that described in carnivores and marsupials Didelphidae regarding its microscopic structure, differing only in the distribution and number of papillae.

**KEYWORDS:** Didelphidae. Digestory apparatus. Lingual papillae. Marsupials.

### INTRODUCTION

Marsupials, such as *Gracilinanus microtarsus* are included among the commonly called opossums, *cuícas* and *catitas*, all of which belong to the family Didelphidae (ORR, 1986) and is a very important family among marsupials due to its high number of species (GONÇALVES et al., 2009). *Gracilinanus microtarsus* is one of the smallest marsupials on Earth, measuring between 20 and 25 cm long, with the tail making more than a half of its length (LIMA et al., 2013). Specimens of the species have body mass average between 10 and 44 grams and exhibit nocturnal and arboreal habits (EISENBERG; REDFORD, 1999; CÁCERES et al., 2008; LOBO et al., 2014). They have frugivorous-omnivorous feeding habits with a very varied diet, consisting of insects, fruits and small vertebrates. Since it feeds on small fruits, *G. microtarsus* is a species of considerable importance in dispersing seeds of trees (LOBO et al., 2014).

It is known that animals need raw material and energy for growth, maintenance and reproduction process. The processing of raw material derived from food requires several organs

which constitute the digestory apparatus (LOBO et al., 2014). The tongue is an organ of the digestory apparatus and is responsible for moving food in the mouth in mastication and swallowing. It is also related to taste due to the taste buds (MANÇANARES et al., 2012). The tongue is also associated with speech in humans. Moreover, the tongue also participates in the functional stimuli that act on the growth and development of the face (BEZERRIL et al., 2005).

Members of research group have made morphological comparative studies related with digestory organs in omnivorous wild mammals as *Nasua nasua* (SANTOS et al., 2010; 2012; SOUZA et al., 2012), *Procyon cancrivorus* (SANTOS et al., 2012; 2013), *Didelphis marsupialis* (MANÇANARES et al., 2012), *Callithrix jacchus*, *C. penicillata* and *C. geoffroyi* (BERTASSOLI et al., 2013) in order to establish possible morphological pattern between the species.

Current study describes anatomically, histologically and ultrastructurally the tongue of *G. microtarsus* for a deeper knowledge of the biology of this species which is widely distributed in Brazil. In fact, the species is highly interesting for studies

related to natural selection, anatomical and physiological adaptations and ecological studies of mammals in general.

## MATERIAL AND METHODS

### Animals

For this study, six adult male and female specimens of *G. microtarsus* were used and fixed in 10% formaldehyde solution. The specimens were provided by the Research Laboratory of Morphological Sciences of the University Center of Education Foundation Octavio Bastos, authorized by the Brazilian Institute of the Environment 02001.007176/03-69.

### Macroscopic analysis and measurements

For the macroscopic analysis of the tongue, the jaws of the animals were moved for better *in situ* measurement, description and photographic documentation. The macroscopic photo documentation was performed with a Sony Mavica digital camera 3.2 MP. Measurements of total length, width of root, apex, body and thickness were taken.

### Light Microscopy (LM)

For microscopic analysis, the tongue was sectioned into root, body and apex, and the fragments were fixed in 10% formaldehyde solution. Samples were then dehydrated in an ethanol series at increasing concentrations (from 70 to 100%) and diaphanized in xylene, followed by paraffin embedding.

Samples were cut at 5µm thickness by microtome LEICA 2165 and the sections were stained with hematoxylin and eosin (HE), Picrosirius and Masson's trichrome. After staining, slides were mounted with cover slips and glue. The material was analyzed, mapped and photographed with a light microscope Nikon Eclipse E-400.

### Scanning Electron Microscopy (MEV)

For the analysis in scanning electron microscopy, fragments of tongues were fixed in glutaraldehyde 1M (EM Grade Propylene oxide, Polysciences Inc., USA), followed by washing of samples with 0.1 M phosphate buffer at pH 7.4 and post-fixed in osmium tetroxide 1% (Spurr Spurr's kit, Electron Microscopy Sciences, USA). Tissue fragments were fixed in metal supports (stubs) and coated with gold ("sputtering" Emitech 550 K). The analysis was performed with Electron Microscope Leo 435 VP.

## RESULTS

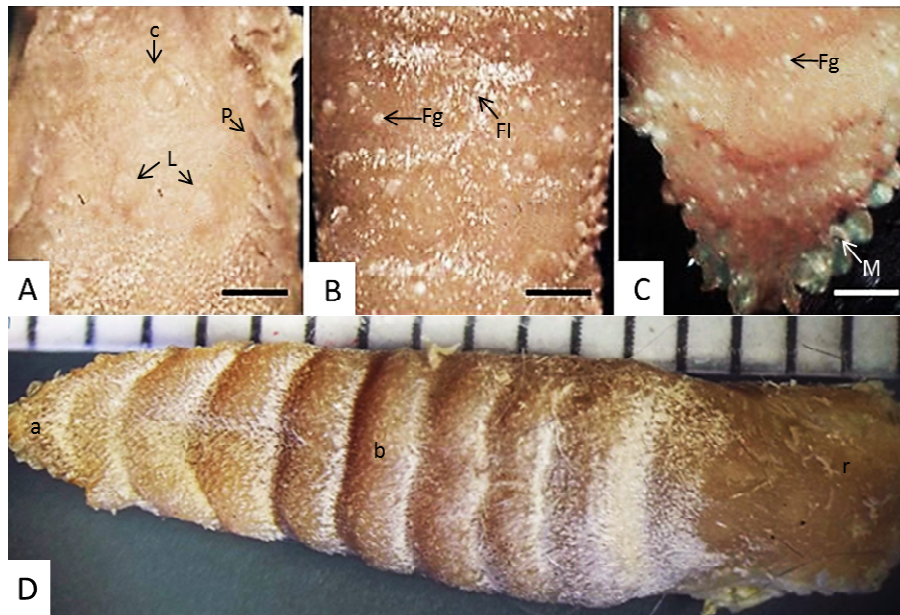
### Macroscopic analysis

The tongue of *G. microtarsus* extends from the oropharynx and occupies most of the oral cavity. It is laterally limited by the teeth, caudally by the pharyngeal arch and dorsally by the hard and soft palates. The tongue has a root, a fixed body and a free apex, trapped to the floor just by the lingual frenulum. The tongue measures  $1.85 \pm 0.06$  cm in length; it is  $0.45 \pm 0.06$  cm wide at the base;  $0.48 \pm 0.05$  cm wide in the body;  $0.38 \pm 0.05$  cm wide at the apex; it is  $0.3 \pm 0$  cm thick at the root;  $0 \pm 0.4$  cm thick on the body and  $0 \pm 0.1$  cm thick at the apex. The tongue has high conical and acute filiform papillae and fungiform papillae across the dorsal surface; marginal fungiform papillae at the margin of the apex; three vallate papillae, one central and two lateral at the root; and papillary projections at the lateral region of the root (Figure 1).

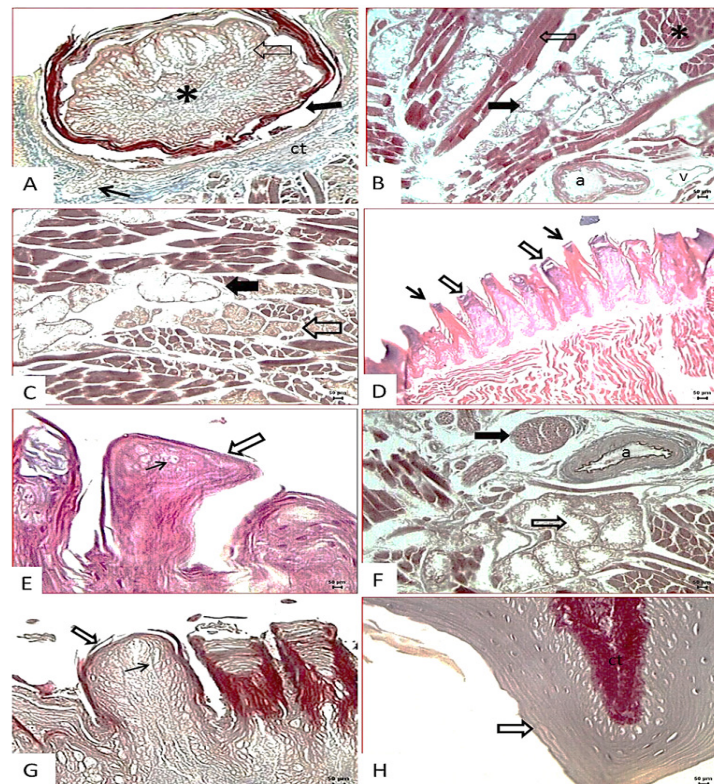
### Light and scanning electron microscopy

The dorsal surface of the root revealed three vallate papillae, a central one and two lateral, surrounded by a groove, with numerous taste buds and serous salivary glands. The root surface also presented fungiform papillae and acute and high conical filiform papillae. The ventral surface was lined by non-keratinized stratified squamous epithelium. The tongue had striated transversal and longitudinal muscle, serous, mucous and mixed acini. In the lamina of the lingual papillae, there was a layer of stratified squamous epithelium and a basal layer of non-modeled dense connective tissue with vessels and nerves. Unilocular adipose tissue was also reported in this region (Figure 2).

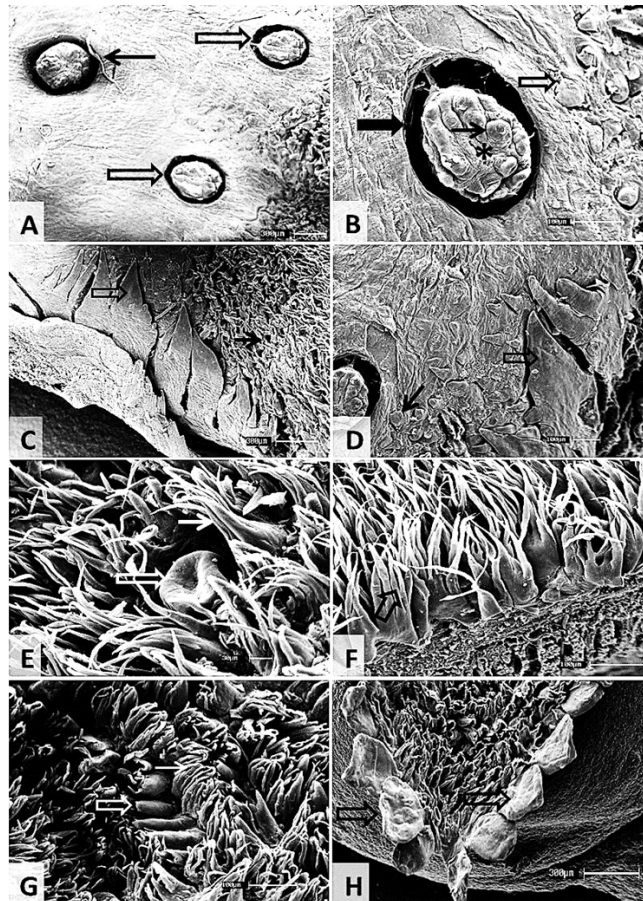
Images of scanning electron microscopy showed the different types of lingual papillae: high conical and acute filiform papillae and fungiform papillae across the dorsal surface; marginal fungiform papillae located at the margin of the apex and three vallate papillae at the root. Papillary projections were also reported (Figure 3).



**Figure 1.** Tongue of *G. microtarsus*. A: central vallate papillae (c), lateral vallate papillae (L) and papillary projections (P). B: fungiform papillae (Fg) and filiform papillae (Fl). C: fungiform papillae (Fg) and marginal fungiform papillae (M). D: apex (a), body (b) and root (r) of the tongue. Bars: 1 mm.



**Figure 2.** Photomicrography of the tongue of *G. microtarsus*. A: vallate papillae (\*) taste buds (empty arrow), groove (full arrow), connective tissue (ct) and Von Ebner gland (thin arrow). Masson's trichrome. B: transversal muscle (\*), longitudinal muscle (empty arrow), mixed acini (full arrow), artery (a) and vein (v). HE. C: mucous (full arrow) and serous acini (empty arrow). Picrossirius. D: high conical (empty arrows) and acute conical filiform papillae (thin arrows). HE. E: marginal fungiform papillae (empty arrow) and taste buds (thin arrow). HE. F: nerve bundle (full arrow), artery (a) and mixed acini (empty arrow). Picrossirius. G: fungiform papillae (empty arrow) and taste buds (thin arrow). Picrossirius. H: keratinized stratified squamous epithelium (arrow) and connective tissue (ct). Picrossirius. Bars: 50 $\mu$ m.



**Figure 3.** Electromicrography of the tongue of *G. microtarsus*. A: central vallate papillae (thin arrow) and lateral papillae (empty arrows). Bar: 100 $\mu$ m. B: lateral vallate papillae (\*), surrounded by a groove (full arrow) and taste buds (thin arrow) and fungiform papillae (empty arrow). Bar: 300 $\mu$ m. C: papillary projections (empty arrow) and acute filiform papillae (thin arrow). Bar: 300 $\mu$ m. D: papillary projections (arrow empty) and fungiform papillae (thin arrow). Bar: 100 $\mu$ m. E: fungiform papillae (empty arrow) between the acute filiform papillae (thin arrow). Bar: 30 $\mu$ m. F: acute filiform papillae (empty arrow). Bar: 100 $\mu$ m. G: acute filiform papillae (thin arrow) and high conical filiform papillae (empty arrow). Bar: 100 $\mu$ m. H: marginal fungiform papillae (empty arrows). Bar: 300 $\mu$ m.

## DISCUSSION

The tongue of mammal is divided into apex, body and root regions. As a rule, the dorsum of the tongue mucosa presents several types of papillae which may be mechanical-type as filiform and conical and gustatory-type as fungiform, foliate and vallate. These papillae become highly differentiated in shape, size, number, distribution and function among the various animal species (BUTENDIECK; VARGAS, 1998; MANÇANARES et al., 2012).

The dorsum of the tongue of *G. microtarsus* is divided into apex, body and root and presents acute and high conical filiform, fungiform and vallate papillae. Marginal papillae are present only at the apex, similar to those in *Didelphis virginiana*, *D. albiventris* (MARTINEZ et al., 1998) and *D. marsupialis* (MANÇANARES et al., 2012).

The types of lingual papillae found in *G. microtarsus* differ from those other mammalian, such as *Nasua nasua* (SOUZA et al., 2012), *Oryctolagus cuniculus* (NONAKA et al. 2008), *Felis catus* (CHAMORRO et al., 1987) and *Callithrix penicillata* (BRANCO et al. 2012) with only acute filiform papillae. In the case of *F. catus*, and *Kerodon rupestris* (SANTOS et al., 2015) foliate papillae have also been described (CHAMORRO et al. 1987). Mammal species and lingual papillae distribution are described below (Table 1).

**Table 1.** Distribution of lingual papillae in mammalian species of different taxa.

Specie (author)	Filiform		Fungiform	Vallate (number)	Conical	Foliated
	High conical	Acute conical				
<i>Gracilinanus microtarsus</i> (This research)	+	+	+	3	-	-
<i>Didelphis virginiana</i> (MARTINEZ et al., 1998)	+	+	+	3	-	-
<i>Didelphis albiventris</i> (MARTINEZ et al., 1998)	+	+	+	2	-	-
<i>Didelphis marsupialis</i> (MANÇANARES et al. 2012)	+	+	+	3	-	-
<i>Kerodon rupestris</i> (SANTOS et al., 2015)	-	+	+	2	+	+
<i>Oryctolagus cuniculus</i> (NONAKA et al. 2008)	-	+	+	2	-	+
<i>Nasua nasua</i> (SOUZA et al. 2012)	-	+	+	9	+	-
<i>Felis catus</i> (CHAMORRO et al. 1987)	-	+	+	6	+	+
<i>Callithrix penicillata</i> (BRANCO et al. 2012)	-	+	+	3	-	-

The dorsum of *G. microtarsus*'s tongue is lined with keratinized stratified squamous epithelium whereas in its interior non-modeled dense connective tissue and transversal and longitudinal striated muscles are found. Mucous and serous acini, lymphoid nodules, adipose tissue and nerves are present, as described in *D. marsupialis* (MANÇANARES et al., 2012), *Nasua Nasua* (SOUZA et al., 2012) and domestic animals such as cats and dogs (BUTENDIECK; VARGAS, 1998).

Since the *G. microtarsus* presents three vallate papillae, as reported in *D. virginiana* (MARTINEZ et al., 1998) and *D. marsupialis* (MANÇANARES et al., 2012), this characteristic is due to the fact that these animals belong to the

same family Didelphidae (ORR, 1986). On the other hand, *D. albiventris* (MARTINEZ et al., 1998) has only two vallate papillae.

The morphology of the tongue of *G. microtarsus* resembles that described in carnivores and marsupials Didelphidae with regard to its microscopic structure and merely differs in the distribution and number of papillae.

#### ACKNOWLEDGEMENTS

The authors would like to thank Dr. Rose Ely Grassi Ricci for technical support with scanning electron microscopy. Thanks are also due to FAPESP for its financial support.

**RESUMO:** O *G. microtarsus* é um pequeno marsupial pertencente à família Didelphidae, que pode ser encontrado principalmente na Mata Atlântica da região sudeste brasileira. O *G. microtarsus* possui importância ecológica na dispersão de sementes de diversas espécies de plantas. A pesquisa atual caracteriza morfológicamente as línguas de seis animais adultos, fixados em formol a 10%. As línguas foram analisadas, mensuradas e fotografadas. Foram realizadas análises anatômicas, microscópicas de luz e ultraestruturais. Macroscopicamente a língua possui raiz, um corpo fixo e um ápice livre. A língua possui papilas fungiformes, filiformes cônicas altas e agudas em toda a superfície dorsal. Também possui três papilas valadas localizadas na raiz e papilas fungiformes marginais no ápice da língua. Microscopicamente, a língua é composta por fibras musculares longitudinais e transversais. Os vasos sanguíneos, nervos e ácinos serosos, mucosos e mistos são encontrados em tecido conjuntivo não modelado. Ventralmente, a língua é revestida por um epitélio pavimentoso estratificado queratinizado. A morfologia da língua de *G. microtarsus* se assemelha ao descrito em carnívoros e marsupiais Didelphidae quanto à sua estrutura microscópica, diferindo apenas na distribuição e número de papilas.

**PALAVRAS-CHAVE:** Aparelho digestório. Didelphidae. Marsupiais. Papilas linguais.

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