

# Scanning Electron Microscopy Study of the Dorsal Surface of the Tongue in the Slow Loris (*Nycticebus coucang*) and Pygma Slow Loris (*N. pygmaeus*)

Estudio de Microscopía Electrónica de Barrido de la Superficie Dorsal de La Lengua en Lori Perezoso (*Nycticebus coucang*) y Lori Perezoso Pigmeo (*N. pygmaeus*)

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**SUMMARY:** The dorsal surface of the tongues of the Slow Loris and the Pygma Slow Loris were examined by employing scanning electron microscopy techniques. Three types of the papillae are present on their dorsal surface of the tongue: filiform, fungiform and vallate. The filiform papillae are located the apex and the body of the tongue, and we observed that each of them has a gustatory pore. The fungiform papillae, scattered singly among the filiform papillae, distribute mainly on the apex of the tongue. The vallate papillae are located along the diversing arms of the V-shaped boundary between the anterior and posterior regions of the tongue. In addition, no foliate papillae were observed. The morphological characteristics of the dorsal surface of the tongues in Slow Loris and Pygma Slow Loris are similar to each other.

**KEY WORDS:** *Nycticebus*; Lingual Papillae; Primate; SEM; Tongue.

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## INTRODUCTION

The Slow Loris (*Nycticebus coucang*) and the Pygma Slow Loris (*Nycticebus pygmaeus*), two distinct species of *Nycticebus*, one genus of Lorisidae, belong to the Strepsirrhini, an ancient group of primates. They are the only nocturnal animals among all the primates that have been found in China so far (Nekaris & Nijman, 2007). Tropical and subtropical evergreen and semi-evergreen rainforests with continuous dense canopies and forest edges are their preferred habitats. The Slow Loris mainly lives in tropical and subtropical evergreen and semi-evergreen rainforests, and the Pygma Slow Loris lives in semi-evergreen and secondary forests (Srivastava & Mohnot, 2001). Their main distribution area in China is tropical and subtropical rainforest in Yunnan and southern Guangxi, China. They feed on a mixed diet, with the majority of the food items consisting of fruits, vegetables, insects, honey, fresh leaves and berries.

Numerous studies on the structure of the dorsal surface of the tongue in vertebrates have been reported: in reptile (Carmignani & Zacccone, 1975); in amphibians (Zuwaa & Jakubowski, 2001; Zuwaa & Jakubowski, 2007); in birds (Emura *et al.*, 2009; Emura *et al.*, 2008), and in mammals (Levin & Pfeiffer, 2002; Yoshimura, 2002). Many studies have investigated the structure of the dorsal surface

of the tongue in primates (Machida *et al.*, 1967; Arvidson, 1976) and, in monkeys, *Macaca fuscata* and *Cercopithecus aethiopus* (Emura *et al.*, 2002), *Macaca irus* (Iwasaki, 1992) and *Macaca fuscata* (Iwasaki *et al.*, 1992a). Unfortunately, there is no report on the morphological characteristic of the lingual papillae under SEM in Slow Loris and Pygma Slow Loris. The aim of this study was to examine the dorsal surface of the lingual papillae of Slow Loris and Pygma Slow Loris, and to distinguish the differences between the tongues of them.

## MATERIAL AND METHOD

Seven adult Slow Loris and 4 adult Pygma Slow Loris were rescued from wildlife traffickers by the Wildlife Rescue and Rehabilitation Center of Henan province in October 2010 in Xinxiang, Henan province, China. The average weights of them were 963.8 g and 391.7 g. Adult Slow Loris and 4 adult Pygma Slow Loris that died eventually due to the lack of rescue experience and guidance were used in our study. And the other 6 were taken to Xishuangbanna Nature Reserve for releasing. There was no animal sacrificed in this study.

For scanning electron microscopic (SEM) observation, tongue samples were fixed at 4 °C in 2.5 % glutaraldehyde with phosphate buffer (pH 7.2). After washing in fresh buffer, the tissues were additionally fixed for 1 h in 1 % buffered aqueous OsO<sub>4</sub> solution. They were dehydrated in a series of acetone concentration, starting with a 50 % solution, and then dried with critical-point-dryer, coated with gold and observed at various different angles under the scanning electron microscope (SEM) FEL QUANTA 450 at 30KV.

## RESULTS

The tongue of the adult slow Loris measured about 24 mm in length and about 18 mm in width, and that of the adult Pygma slow Loris was about 17 mm and 6 mm, respectively. Three types of the papillae are present on the dorsal surface of the tongue of the Slow Loris and the Pygma Slow Loris: filiform, fungiform and vallate (Figs. 1, 2 and 3).

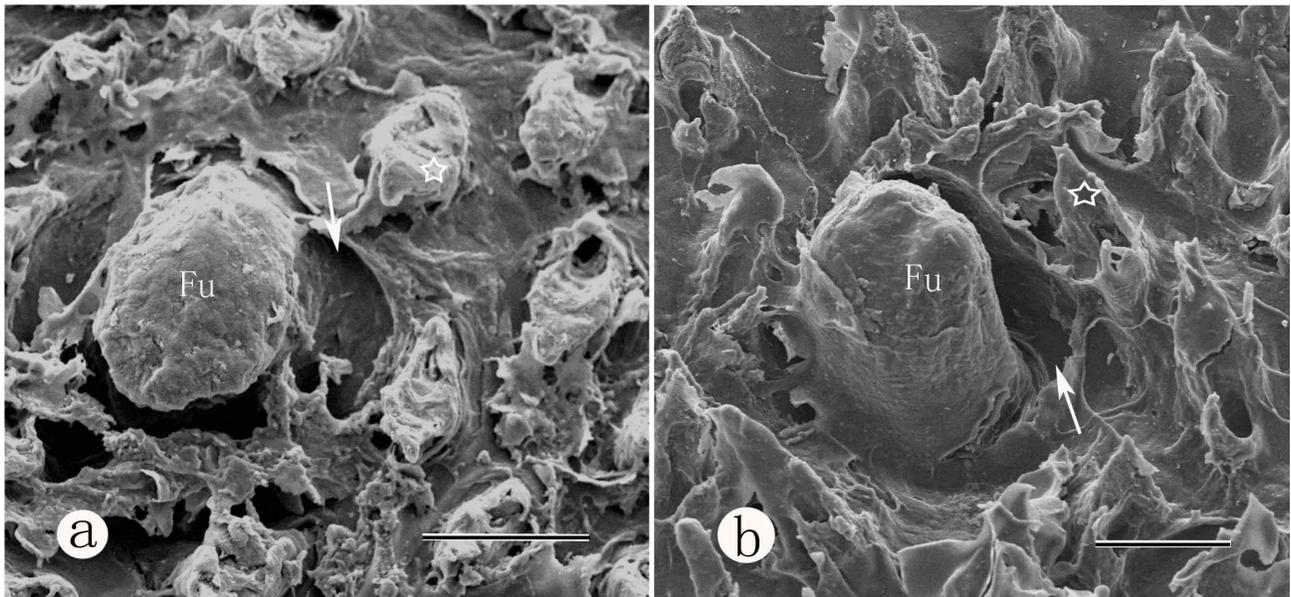


Fig. 1. A set of scanning electron micrographs of Fungiform papillae. (a)Slow Loris. (b) Pygmy Loris. Fu, fungiform papillae; Arrows, openings of the lingual glands; Star showed filiform papillae. Scale bar =100 mm.

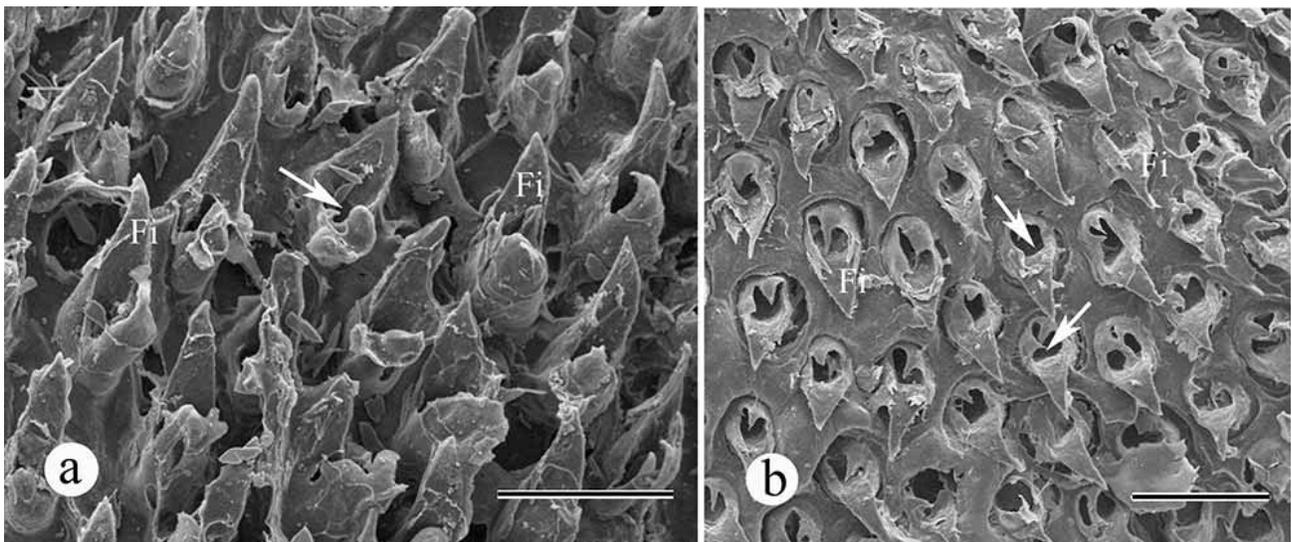


Fig. 2. A set of scanning electron micrographs of filiform papillae. (a)Slow Loris. (b) Pygmy Loris. Fi, filiform papillae; Arrows, openings of the lingual glands. Scale bar = 250 mm.

The filiform papillae, which are conical or branched, consisting of one main thick process and several slender accessory processes, can be found on the apex and body of the tongue, pointing principally towards the pharynx (Fig. 2a, 2b). Filiform papillae are arranged in distinct rows, decreasingly in number and length throughout the posterior third. Furthermore, the filiform papillae are abundant at the tip of the tongue. Each filiform papilla is approximately 100  $\mu$ m in diameter in Slow Loris (Fig. 2a) and 105  $\mu$ m in Pygma (Fig. 2b). Each filiform papilla possesses a gustatory pore which is approximately 80  $\mu$ m in diameter. There is a circular concavity in each pore. The filiform show conical shaped in Slow loris, and the filiform papillae showed flat-conical shaped in Pygma slow loris.

The fungiform papillae, distributed mainly on the apex of the dorsal surface, present a round or dome in shape. And they are scattered singly among the filiform papillae. They are of larger size to the filiform ones; each fungiform papillae are approximately 126  $\mu$ m in diameter in Slow Loris (Fig. 1a) and 153  $\mu$ m in Pygma (Fig. 1b).

The vallate papillae, have the round or oval shape, located along the diversing arms of the V-shaped boundary between the anterior and posterior regions of the tongue. And they are sunk into the surface of the mucous membrane and each is surrounded by a deep, circular, furrow (Fig. 3). The vallate papillae is approximately 316  $\mu$ m in diameter in Slow loris (Fig. 3A ) and 264  $\mu$ m in Pygma (Fig. 3B).

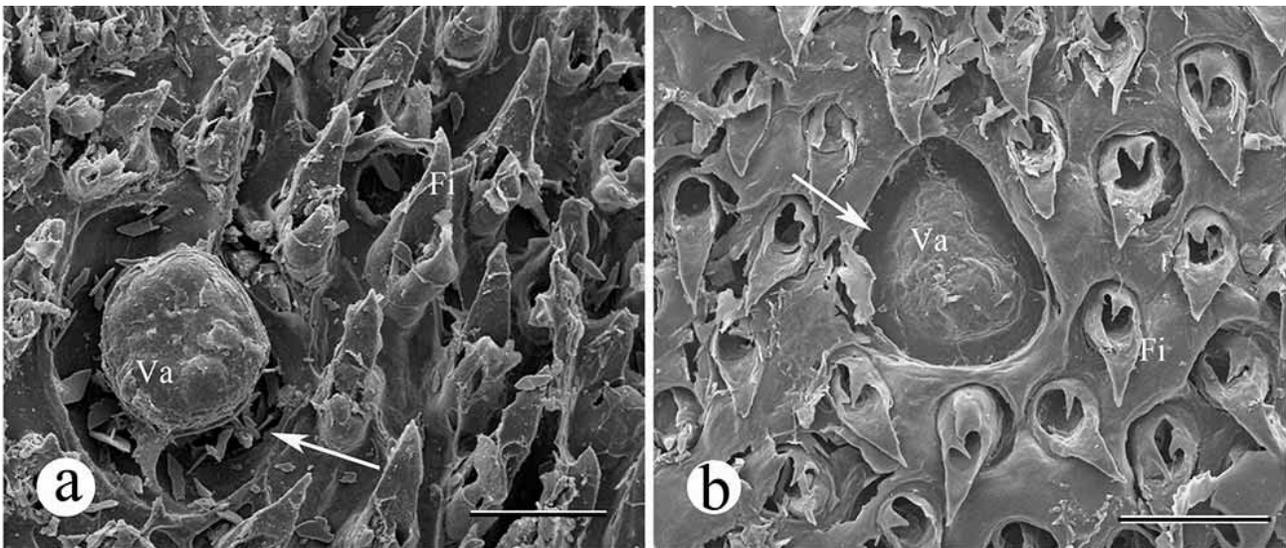


Fig. 3. A set of scanning electron micrographs of vallate papillae. (A)Slow Loris. (B) Pygmy Loris. Va, vallate papillae. Fi, filiform papillae. Arrows, circular furrow can be seen. Scale bar =250  $\mu$ m.

## DISCUSSION

This study has demonstrated three types of papillae, filiform, fungiform and vallate, in Slow Loris and Pygma Slow Loris, employing scanning electron microscopic technique. Overall, the morphological patterns of the tongues were not different from other species in primates (Arvidson; Ferguson, 1980; Iwasaki, 1992; Iwasaki *et al.*, 1992a; Emura *et al.*, 2002).

Study on the Slow loris interspecific at the genetic level by Chen *et al.* (2004) suggested that there were significant differences between Slow Loris and Pygma Slow Loris, which showed that they were two efficient species. Additionally, our previous work has also mentioned (Xie *et al.*, 2013).

Previous research in the dorsal surface of the tongue in mammals has emphasized the connective tissue cores and the different types of filiform papillae (Yoshimura; Emura *et al.*, 2004). In this paper, we discussed the pattern of the filiform papillae already. And numerous filiform papillae cover the surface of the lingual dorsum, seems to be similar in the tongue of *Bradypus torquatus* (Benetti *et al.*, 2009), *Myocastor coypus* tongue (Ünsal *et al.*, 2003) and *Macaca fuscata* and *Cercopithecus aethiopus* tongues (Emura *et al.*, 2002). According to Iwasaki (2002), the characteristic of the lingual papillae, especially filiform papillae, are similar within the same genus, which is consistent with our observations in Slow Loris and Pygma Slow Loris. The appearance of the keratinization of the epithelium in the

filiform papillae, which is similar in most mammals, can sustain strong physical force (Iwasaki, 2002). It is noted that the filiform papillae are easily bent in the direction of the radix but not in the opposite direction, being probably connected the need for moving the food taken into the mouth (Iwasaki, 2002). At a high degree of magnification, we observed a gustatory pore in the central of the each filiform papillae, probably related to the types of food and the feeding habits in Slow Loris and Pygma Slow Loris. Moreover, further studies are needed to evaluate such assumption.

Regarding the fungiform papillae, several microridges can be identified on the dorsal surface of the fungiform papillae. The pattern and the shape have been established in the literature, referring to the reports in primates (Kobayashi *et al.*, 2004). The fungiform papillae in Slow Loris and Pygma Slow Loris, furnished with taste buds, perform gustatory functions in monkey (Arvidson).

The distribution of vallate papillae in Slow Loris and Pygma Slow Loris, with three papillae arranged in an inverted pattern of V-shape, is also found in primates (Kubota & Hayama, 1964; Emura *et al.*, 2002). The shape of the posterior central papillae differs from the lateral ones as is mentioned in Marmosets (Kubota & Hayama) and Saimiri (Iwasaki *et al.*, 1988; Matsukawa & Okada, 1994). In addition, the number of the vallate papillae is various, such as Japanese monkey (Iwasaki *et al.*, 1992b). At a high degree

of magnification, circular furrows are evident. The circular furrows, surrounding the vallate papillae, separates the vallate papillae from the mucosa covered with filiform papilla.

According to Kubota & Hayama, the tongues of pigmy and common marmosets have the foliate papillae with a few clefts, while, Matsukawa & Okada stated that no foliate papillae were observed in the squirrel monkey and marmoset. And in our study, we could not find any foliate papillae in Slow Loris and Pygma Slow Loris.

The morphological characteristics of the tongues in Slow Loris and Pygma Slow Loris are similar to each other. However, the feature of the filiform papillae is different. The morphological variations of the tongues, especially the filiform papillae in Slow Loris and Pygma Slow Loris may be the reason of being dependent on the evolutionary taxonomy of primate species.

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ZHAOHUI, X. & ZHANG, R. Estudio de microscopía electrónica de barrido de la superficie dorsal de la lengua en lori perezoso (*Nycticebus coucang*) y lori perezoso pigmeo (*N. pygmaeus*). *Int. J. Morphol.*, 35(2):520-524, 2017.

**RESUMEN:** La superficie dorsal de la lengua en lori perezoso y lori perezoso pigmeo fue examinada utilizando técnicas de microscopía electrónica de barrido. Se observaron tres tipos de papilas presentes en la superficie dorsal de la lengua: filiforme, fungiforme y valada. Las papilas filiformes se localizaban en el ápice y en el cuerpo de la lengua, y observamos que cada una tenía un poro gustativo. Las papilas fungiformes están distribuidas individualmente entre las papilas filiformes, principalmente en el ápice de la lengua. Entre las regiones anterior y posterior de la lengua se observan las papilas valadas a lo largo de las extensiones del margen en forma de 'V'. Además, no se observaron papilas foliadas. Las características morfológicas de la superficie dorsal de las lenguas en lori perezoso y lori perezoso pigmeo son similares entre sí.

**PALABRAS CLAVE:** *Nycticebus*; Papilas linguales; Primate; SEM; Lengua.

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