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Characteristics of the Gustatory Organs on the Tongue of the Chinese Fire-bellied Newt (*Cynops orientalis*): Light and Scanning Electron Microscopy Study

Características de los Órganos Gustativos en la Lengua del Tritón Vientre de Fuego Chino (*Cynops orientalis*): Estudio por Microscopía de Luz y Electrónica de Barrido

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SUMMARY The dorsal surface of the tongue gustatory organs of Chinese fire-bellied newt (*Cynops orientalis*) was observed by employing the light and scanning electron microscopy (SEM) techniques. The results revealed that the rostral and median part of the tongue presents a round apex and covered by taste disks (TDs). They are usually roundish or ellipsoidal in shape and are 20-35 mm in diameter. The many openings of the lingual glands are 4-8 mm in diameter exist in the lateral border or median part of lingual body. The gustatory organs on the tongue did not differed form those presented in other species in Caudates. These may indicate the functions of gustatory organs on the tongue related to their life habit.

KEY WORDS: Tongue; Lingual epithelium; Histology; Taste disks; Caudates.

INTRODUCTION

Many studies have demonstrated that the gustatory organs on the tongue of vertebrates in the form of taste buds (TBs) (Iwasaki, 2002; Mistretta & Liu, 2006; Abbate *et al*., 2010). However, previous morphological descriptions of gustatory organs in developing Anurans showed that there are two successive generations of taste organs: premetamorphic TBs in larval forms and taste disks (TDs) in postmetamorphic animals (Graziadei & DeHan, 1971; Zylberberg, 1977; Iwasaki *et al*. 1989a, 1989b; Witt, 1993; Osculati & Sbarbati, 1995; Zuwala, 2002; Iwasaki & Wanichanon, 2005). In adult specimens of Caudata such as *Salamandra salamandra* (Zuwala & Jakubowski, 2001), *Hynobius dunn*i (Zuwalla *et al*. 2002) and *Triturus alpestris* (Zuwala & Jakubowski, 2007) have been investigated that taste disks structure as gustatory organs, similar to adult Anura. In the oral cavity of mature specimens gustatory organs only classified as TB-type were reported in axolotl *Ambystoma mexican* (Northcutt *et al*., 2000), *Triturus pyrrhogaster* (Toyoshima & Shimamura, 1987) and *Necturus maculosus* (Cumnings *et al*., 1987; Delay & Roper, 1988).

The purpose of this study was to investigate, with light and scanning electron microscopy, the dorsal epithelium of the gustatory organs type of the adult Chinese fire-bellied newt *Cynops orientalis* (David, 1873) and compare the results of the observations with those previous reported for those that of other Caudata. In order to clarify the relationship between the structure features of the gustatory organs and the lifestyles of newts that live in different environment.

MATERIAL AND METHOD

Twelve adult newt of both sexes, length approximately 40 mm and weighing 1.9-2.3 g, which were captured from countryside near Guangshui, Hubei province, China, were used in the present study. After anesthetization with a lethal dose of sodium pentobarbital, the animals were killed by decapitation. The livers were immediately taken for later analysis. For light microscopy, the samples were fixed in 10% neutral buffered formaldehyde and Bouin's solution without acetic acid (3:1 mixture of saturated solution of picric acid in water and formalin) for 48 h. Samples were then dehydrated through a series of graded alcohols, cleared in xylene, infiltrated, and embedded into paraffin. Tongue

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paraffin wax blocks were cut into 7 μm thick sections, stained with hematoxylin and eosin, and then they were examined under Nikon TE2000-U microscope.

For scanning electron microscopic (SEM) observation, tongue samples were fixed at 4 ºC 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 O_SO_4 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) KYKY-EM3200 at 5-15kV.

RESULTS

The tongue of Chinese fire-bellied presents around apex, measuring about 4.2 mm in length (except lingual root), 3.2 mm of width and 1.6 mm of thickness. Three parts are distinguished in the dorsal surface of the tongue: the apex, the body and the root. On the apex dorsal surface of the tongue, a large number of TDs are distributed by SEM observation. They are usually roundish or ellipsoidal in shape and are 20-35 μm in diameter (Fig. 1). The sensory epithelium of the TDs consist large mucous cells are relatively large irregular encircled by narrow apical sheet of 3-4 wing cells can be seen (Fig. 2). The strips of wing cells separate the mucous cells, where a tuft protruding apical microvillar processes are discernible. The surface of posterior part of tongue body distribute round knob-like structure about 5-10 μm in diameter can be seen (Fig. 3). Those processes presumed sensory cell. In some cases, a single opening is found at the lateral of the TD, outer

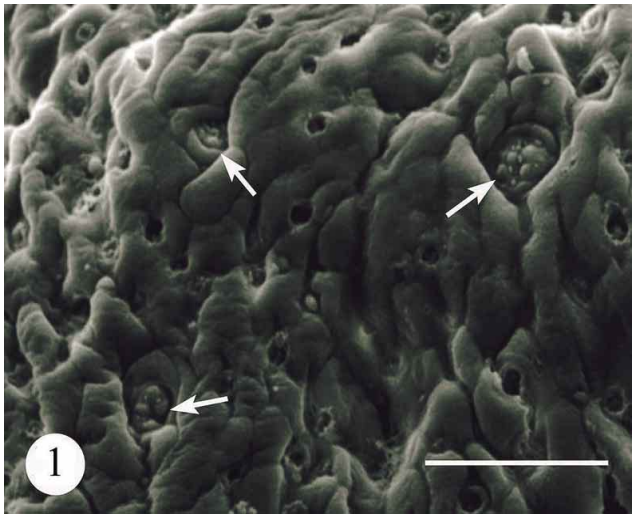

Fig. 1. Dorsal surface of the tongue of *C. orientalis* with developed taste discs (TDs, arrows). Scale bar = 50 μm.

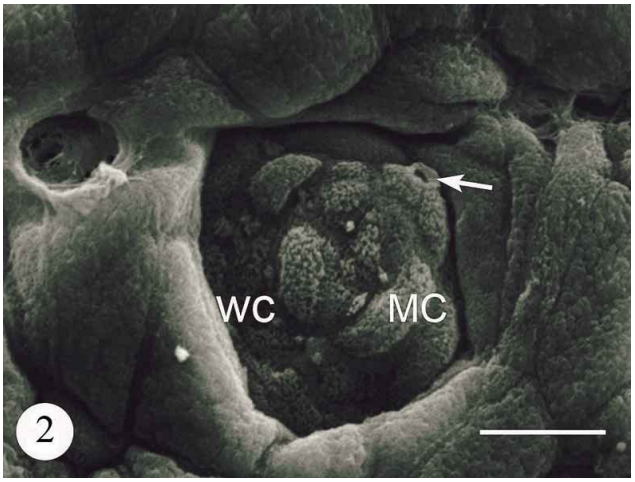

Fig. 2. Sensory area of lingual taste discs of *C. orientalis*, a single opening is found at the lateral of the TD (arrow). MC, mucous cell; WC, wing cell. Scale bar = 10 μm.

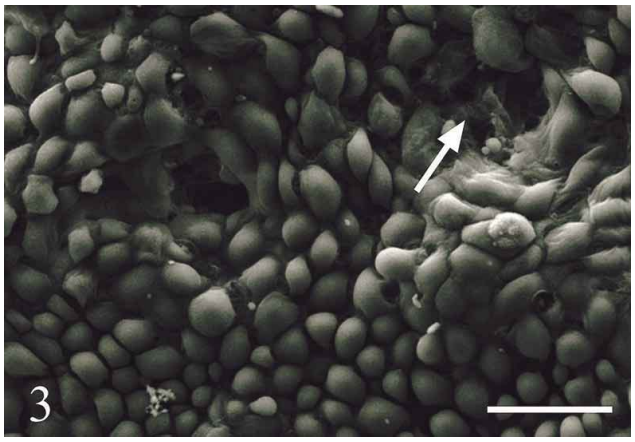

Fig. 3. The many openings of the lingual glands exist in the dorso-lateral surface of lingual apex of *C. orientalis*. Scale bar =10 μm.

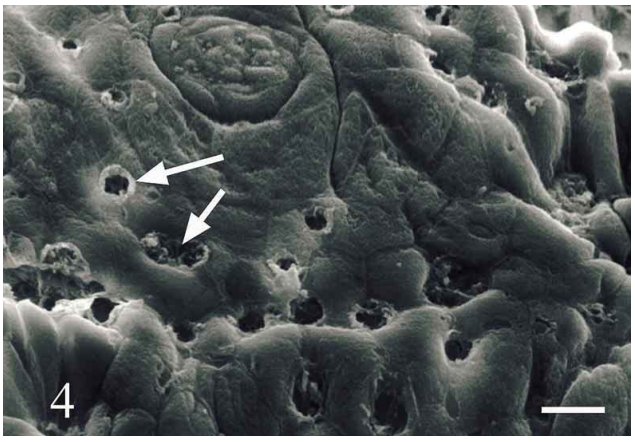

Fig. 4. The openings of the lingual glands in the lingual body of *C. orientalis*. Arrow, varying size pores integrated. Scale bar = 40 μm.

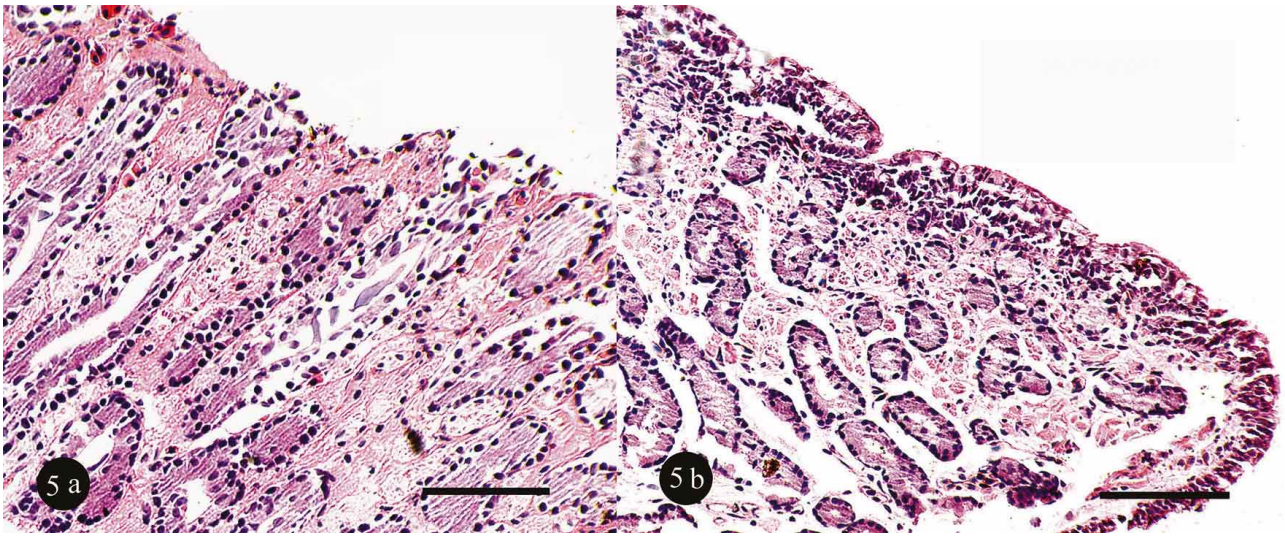

Fig. 5. The light microscopy examine the apex of the tongue of *C. orientalis*, the epithelial mucosal cells are arranged multi-layered. a. sagittal section; b, longitudinal section. Scale bar= 100 μm.

opening where semitransparent gelatinous substance attached to the orifice of the opening, a majority of TD have no opening. However, the many openings of the lingual glands, diameter in 4-8 μm, exist in the lateral border or median part of lingual body (Fig. 4).

By the light microscopy examinations of longitudinally sectioned show that on the apex of the tongue, the epithelial mucosal cells are arranged multi-layered, the cell number up to the highest at the bottom of the layers at the side. It consists of interlacing bundles of striated muscle that cross one another. The dense lamina propria is continuous with the interstitial connective tissue of the muscle. In vertical sections, the number of glandular ducts can be seen aligned in a rather amorphous substance (Fig. 5a and 5b). It is presumed to be glands of mucosa.

DISCUSSION

The present study demonstrated the characteristics of epithelial cell layers of Chinese fire-bellied tongue. Results indicated that the structure of the tongue in the Chinese fire-bellied does not differ from that present in other species in Caudates, such as *Salamandra salamandra* (Zuwala & Jakubowski, 2001) and *Hynobius dunni* (Zuwala *et al*.), alpine newt *Triturus alpestris* (Zuwala & Jakubowski, 2007) and a few Anuran families (Zuwala; Paulson *et al*., 1995).

Chinese fire-bellied belongs to the tailed amphibians in vertebrates, and amphibians usually live in and around freshwater, so the surface of the oral cavity around the tongue is wet, even on land, amphibians are not generally exposed to extremely dry conditions, and, consistently, no keratinization is found in the amphibian lingual epithelium (Iwasaki). In accord with our observation by SEM, that they were no keratinization of filiform papillae and circumvallate papillae.

The morphological and histological features of mammalian tongues reflect the differences among the life-styles of mammals and relate to the animal habitat (Iwasaki). This animal´s living habitat is widely distributed in the hilly plains of Central and Southeastern China from 30 to 1500 m above sea level (the provinces of Henan, Southern Anhui, Jiangsu, Zhejiang, Guangxi, Fujian, Southern Hubei, and Hunan). Their habitats are suitable water bodies at various altitudes, mountain ponds, small brooks and flooded fields in mountain valleys (Zhao & Hu, 1988), as well as for many species of water-living animals, They use suction feeding for intraoral food transport, where water is drawn into the oral cavity by suddenly lowering the floor of the mouth, so that water movement is the main mechanism of intraoral food transport. Besides, the tongue occupies space within the oral cavity that could otherwise be utilized for volumetric expansion and suction (Beisser *et al*., 1998).

There are taste disks distributed on the dorsal surface of the tongue both the apex and body, at the same time, a large number of tongue gland openings distributed around the TDs nearly, and the secretions can continuously flushing the ring groove of gustatory cells, the clearance of bacteria impurities and food residue, it is good for the

TDs to feel the different flavors of food stimulation, which reveals that the living environment of Chinese fire-bellied is more stringent. Chinese fire-bellied mainly prey on aquatic insects, insect eggs and other small aquatic animals, the living environment of the waters clear, with the aquatic plant growing, so the high requirements of food fresh and living environment leads to the development of sensitive taste receptor. As for the distribution of a large number of TDs and taste glands in the tongue body, probably for its smooth swallowing and plays an important role in the completion on eating food, and is also helpful for the Chinese fire-bellied to swallow food associated with fixed food by mucus. Nomura *et al*. (1979), studied the structure of the oral cavity lining in tadpoles of *Rana japonica*, and described a group of innervated cells in the apex of each premetamorphic, suggesting that they may have a chemoreceptive function.

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RESUMEN: La superficie dorsal de la lengua de los órganos gustativos del tritón de vientre de fuego chino (*Cynops orientalis*) se observó mediante microscopía de luz y electrónica de barrido (SEM). Los resultados revelaron que la parte rostral y mediana de la lengua presenta un ápice redondo y cubierto por discos sensoriales. Estos por lo general tienen una forma redondeada o elipsoidal con un diámetro de 20-35 mm. Las numerosas aperturas de las glándulas linguales tienen un diámetro de 4-8 mm en el margen lateral o en la parte mediana de cuerpo lingual. No se observaron diferencias en los órganos gustativos linguales al comparar estos con otras especies de caudados. Estos pueden indicar funciones de los órganos gustativos de la lengua relacionadas con su hábito de vida.

PALABRAS CLAVE: Lengua; Epitelio lingual; Histología; Discos sensoriales; Caudados.

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