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Comparative study on the tongue of *Bufo regularis* and *Chalcides ocellatus* in relation to their habitats

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Abstract The present study investigated the structure of the tongue of the toad, *Bufo regularis* and the lizard, *Chalcides ocellatus*. They have different feeding habits and live in different habitats. The tongue of the toad contains two types of lingual papillae; fungiform papillae and filiform papillae. The fungiform papillae are usually scattered among the filiform papillae and are believed to function in gustation and in the secretion of salivary fluid. Scanning electron microscopical studies revealed that no ciliated cells were observed on the surface of the filiform papillae or in the surrounding area of the sensory disc. In *C. ocellatus* the tip of the tongue is bifurcated and keratinized. The dorsal surface of the tongue is covered with several types of papillae; irregular, scale and ridge-shaped. Taste buds were present in the epithelium of the tongue. The lingual glands consist of mucous cells that form crypt-like invaginations between papillae. The present study revealed that there is a marked correlation between the structure of the tongue of both *B. regularis* and *C. ocellatus* and habitats and feeding mechanism of the two species.

Introduction

The feeding mechanism is clearly an important factor that determines the success of adaptation of vertebrates to their environment and of their persistence through procreation (Roth and Wake, 1989). The tongue has a characteristic form and plays a principal role in feeding, together with other organs within and near the oral cavity, in particular in tetrapods. Most adult amphibians have a tongue (Helff, 1929), as do all known reptiles. Thus it is likely that the tongue appeared with the establishment of tetrapods and this structure seems to be related, to some extent, to the terrestrial lifestyle (Helff, 1929). We can infer that the main role of the tongue is to facilitate eating on land, in co-operation with other organs within and near the oral cavity. Amphibians usually live in and around fresh water, and 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 (Graziadei and Dehan, 1971; Iwasaki and Kobayashi, 1989; Iwasaki, 2002).

The morphology of the gustatory organs present in mature anuran amphibians has been extensively studied (Zuwala, 2002; Zuwala and Jakubowski, 2000). It has been shown that anurans are not the only group of vertebrates equipped with gustatory organs of the taste disc type. In mature caudate spec-

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imens such as *Salamandra salamandra* (Zuwala and Jakubowski, 2001) and *Hynobius dunni* (Zuwala et al., 2002), taste discs function as gustatory organs, similar to mature anura. Also, in alpine newt *Triturus alpestris* there are well developed taste discs that differ from taste buds mainly by their structurally diversified set of associated cells; mucous, wing and glial cells (Zuwala and Jakubowski, 2007).

In some vertebrates, the tongue plays additional roles; these are especially significant in reptiles. The tongue of lizards is used for diverse functions such as lingual prey prehension in iguanian lizards (Herrel et al., 1995), prey transport and swallowing in most lizards (Delheusy and Bels, 1992; Herrel et al., 1996, 1997, 1998). Also in *Ptyodactylus guttatus* and *Stenodactylus petrii* (lacertilian, Gekkonidae) the dorsal surface of the tongue showed abundant glandular distribution associated with dense distribution of microvilli and microridges on lingual papillae which facilitated feeding habits (Darwish, 2012). Tongue extrusions, such as flicks or touches, can be used in the detection of specific species (Cooper and Vitt, 1984; Simon, 1985), kin recognition (Werner et al., 1987), sex recognition and courtship (Cooper and Vitt, 1984; Cooper et al., 1986).

The lingual papillae entirely cover the dorsal surface of the tongue. They differ in size, form, number and function and are species-variable. The lingual papillae consist of a dermal core covered with stratified squamous epithelium (Iwasaki et al., 1989; Chunhabundit et al., 1992; Emura et al., 2000a,b, 2001). Many studies have demonstrated that the dorsal surface of the reptilian tongue is rich in lingual papillae (Jamniczky et al., 2009; Cizek et al., 2011). On the other hand, Iwasaki and Miyata (1985) reported that no lingual papillae are located on the surface of the anterior bifurcated area of the adult Japanese lizard tongue.

In terrestrial reptiles, the salivary glands are separate from the tongue (Kent, 1978), and the relative number of cells with secretory granules is relatively low in the lingual epithelium (Iwasaki and Miyata, 1985; Iwasaki and Kumakura, 1994). The lingual glands consist of numerous mucocytes that form a simple mucous epithelium that covers most of the dorsal and lateral surfaces of the tongue in iguanid lizards like *Oplurus cuvieri* (Delheusy et al., 1994). These numerous mucous glands are observed in Sphenodon and terrestrial tortoises (Schwenk, 1986; Winokur, 1988). Keratinization has been observed among various reptilian species on evolutionary trend, in conjugation with adaptation to dry land (Iwasaki and Kobayashi, 1992; Iwasaki and Kumakura, 1994).

The purpose of this investigation was to find out the relation between the adaptive structures of the tongue and the type of food in the toad, *Bufo regularis* and the lizard, *Chalcides ocellatus*.

Materials and methods

The observations were conducted on seven roofs and tongues of each adult toad *B. regularis* and tongues of lizard *C. ocellatus*. The tongues were dissected from the mandible, fixed in 10% neutral formaldehyde, and routinely prepared for observation using scanning electron microscopy .The samples of the tongue were dehydrated in a series of ethanol (70–99.8%) and acetone, and subsequently dried with the critical-point drier Tousimis Audosamdri-815. The dried material was coated by a gold sputter coater (SPI-Module). The material was stored over silica gel, and observed with a scanning electron microscope JEOL (JSM-5400 LV) in Regional Center of Mycology, Al-Azhar University, Cairo, Egypt.

Results

The tongue of ***B. regularis***

A toad usually lives in and around freshwater, and the surface of the oral cavity around the tongue is wet. Even on land, a toad is not generally exposed to extremely dry conditions, and consistently, no keratinization is found in the toad's lingual epithelium. Toads are remarkable in having a tongue that is propelled from the mouth, to impact on, adhere to, and pull prey into the buccal cavity. The gustatory organs of the toad seem to differentiate into a specialized structure called, the taste disc (Fig. 1).

In the present investigation, the oral cavity of the toad contains two different types of taste organs: taste buds and taste discs. The peripheral gustatory apparatus is composed of papillary and non-papillary sense organs. The papillary sense organs are taste discs (TDs) located on the dorsal surface of the tongue at the apical surface of fungiform papillae (Fig. 1). The non-papillary sense organs are situated both on the floor and roof epithelia of the mouth. The taste buds located on the palate have a slightly convex surface with deep-set, irregular margins and are slightly smaller (Fig. 2).

Tongue of the toad can be recognized into proximal, middle, and apical portions. The proximal portion is attached to the jaw and at the end of the apical portion two horns are present (Fig. 1). The epithelium covering the dorsal surface of the tongue forms predominantly numerous branched filiform and scattered fungiform papillae (Fig. 3). Micropits or mucous glands are present among the papillae (Fig. 4).

The filiform papillae are characterized by the fork-shaped processes. The number of processes in fork-shaped filiform papillae are varied (Fig. 5) .No ciliated cells were observed on the surface of the filiform papillae or in the surrounding area of the sensory disc.

The fungiform papillae have the form of an inverted truncated cone with a convex base constituting the chemoreceptor

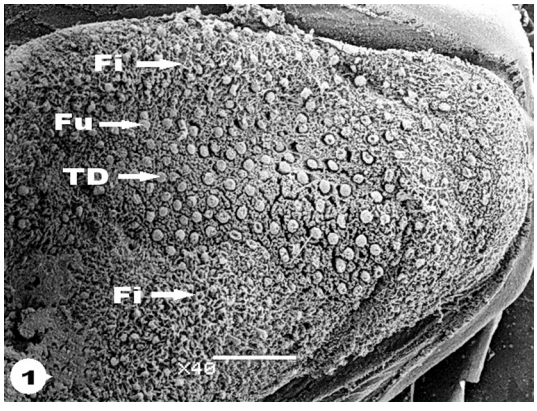

Figure 1 Scanning electron micrograph of the tongue of *Bufo regularis* showing the tongue, filiform papillae (Fi) fungiform papilla (Fu) and taste disc (TD).