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Research Article

Morphological, histological and ultrastructural studies on the tongue of *Chamaeleo chamaeleon*

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Abstract

Chamaeleon belongs to the most primitive lizard clade (Iguania), members of which have characteristically long tongue mainly used to capture the prey. Scanning electron microscopic investigation of lingual mucosa of the anterior part of chamaeleon tongue revealed that the lingual surface is completely covered with three forms of filliform papillae; conical leaflet structure with pointed curved end, flattened surface and longitudinal papillae. Numerous mucous gland openings are spread in lingual mucosa. The free border of the dorsal surface is formed of longitudinal lingual strands separated by median grooves connected internally by building muscle structures. At light microscopic level, the lingual mucosa is covered by columnar epithelium and maintained internally forming tubular glands enclosed in between mucous secretion. Numerous goblet cells are distributed in-between the columnar cells. The free edge of the lingual mucosa was less keratinized. Transmission electron microscopic observations showed numerous desmosomes in-between electron-dense keratinocytes of the stratum granulosum. Few numbers of keratohyaline granules were detected manifesting the reduction of keratinization.

Keywords: Chamaeleon; lingual papillae; filliform papillae; serrated filaments

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Introduction

Chameleon belongs to the most primitive lizard clade (Iguania), members of which characteristically use their tongue to capture prey items (Schwenk and Throckmorton, 1989; Schwenk, 2000). Chameleons diverge from the primitive prey capture mode by projecting their tongue ballistically up to twice their body length to capture prey (Wainwright et al., 1991).

Tongue was the predominant prey capture tool in all members of the most primitive lizard clade, the Iguania (i.e. Iguanidae, Agamidae and Chamaeleontidae). The mechanism by which the prey adhered to the tongue of iguanid lizards during capture was thought to be based on adhesive bonding and/or interlocking (Bramble and Wake, 1985). Since the chameleon tongue pad contains a large number of epithelial glands and possesses numerous papillae that can lock into surface irregularities on the prey (Schwenk, 1983), both wet adhesion and interlocking presumably played an important role during prey capture. Although the chameleon tongue is generally considered to be an example of an adhesive bonding system (Bramble and Wake, 1985), suction was suggested as a possible mechanism that would enable chameleons to capture large prey (Schwenk, 1983). Indeed, because the strength of the adhesive bond was limited by the surface area of the tongue contacting the

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prey (Emerson and Diehl, 1980), this system places severe limitations on the maximal prey size that can effectively be transported by the tongue. The most interesting histological feature of reptilian tongues reflects adaptations to a dry habitat but stratification and keratinization of the lingual epithelium were the most common features (Iwasaki & Kumakura, 1994). By contrast, the American chameleon's tongue is intimately involved in feeding. A large part of the lingual epithelium consists of cells with secretary granules, many of which are mucous and some are serous (Rabinowitz & Tandler, 1986). Thus the shape and structure of the tongue differ significantly among reptiles, reflecting the various functions of each respective group.

Little studies have been conducted on the tongue of of chameleon but scarce information is available about the lingual structure component. The present study focused mainly on the gross macroscopic structure beside the analysis of the lingual papillae structure under light and scanning electron microscopes.

Materials and Methods

Chamaeleo chamaeleon, Linnaeus, 1758: Class reptilia ▶ Order Squamata ▶ Suborder, Iguania ▶ Family, Chamaeleontidae ▶ Subfamily, Chamaeleoninae ▶ Genus, Chamaeleo Laurenti, 1768 were collected from Giza Governorate, Abou-Rawash zone and used in the present work. Classification was carried out according to Berre (2009). Five individuals were used during the present work. They were sacrificed, dissected and tongue were removed and processed for the following investigations.

Morphological studies

The tongue specimens were removed photographed and described.

Histological studies

Fresh tongue specimens were immediately fixed in 10% formal saline, dehydrated in ascending grades of ethyl alcohol, cleared in zylol and mounted in molten paraplast 58-62ºC. Five µm thick histological sections were cut and stained with haematoxylin and eosin. Samples were investigated with bright field light microscopy.

Scanning electron microscopic study

For topographic studies, extra fresh tongue specimens were fixed immediately in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) followed by washing in 5% sucrose in 0.1 M cacodylate buffer. The specimens were dehydrated using a graded acetone series (25%, 50%, 75%, 95%, and 100% acetone) and critically dried in a carbon dioxide apparatus. The specimens were coated with gold and viewed using a Joel 5300JSM microscope (Musashino 3 Chome, Akishima, Tokyo 196-8558, Japan).

Transmission electron microscopic study

Extra fresh tongue specimens were immediately fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). After washing the specimens in the buffer, they were post-fixed in a buffered solution of 1% osmium tetra oxide at 4°C for 1.5 h, dehydrated in an ascending ethanol series, and embedded in epoxy resin. Ultrathin sections were cut with a diamond knife on an LKB Ultratome IV (LKB Instruments, Bromma, Sweden), mounted on grids, stained with uranyl acetate and lead citrate, and examined under a Joel 100CX transmission electron microscope.

Results

Morphological observation

Macroscopically the tongue is composed of three differentiated successive zones. The proximal part is a textured mass markedly flattened, thickened and sticky. The median part is elongated tube-like structure and appeared fleshy being formed of muscular tissue. The posterior part of the tongue was attached to the hyoid region. The anterior part was carefully investigated in the present study. The anterior part showed free conical shaped border. The median sulcus was ill-differentiated from the dorsum anterior lingual region (Fig. 1 A1).

Histological observation

At the light microscopic level, the lingual mucosa was lined by columnar epithelium and maintained internally forming tubular glands enclosed in between mucous secretion. Numerous goblet cells were distributed in-between the columnar cells. The lingual papillae were regularly arranged with their curved pointed edge directed posteriorly to the tongue root. The core of the papillae was filled with fine connective tissue core containing numerous blood vessels (Fig. 1A1-A3).

Scanning electron microscopy

The lingual mucosa was composed of parallel lingual strands separated by median grooves connected internally by muscular structures (Fig. 2, A-D). Each lingual strand surfaces carried two different forms of filiform papillae; one took the conical form and the other acquired the slender-pattern. Numerous mucous gland openings were detected throughout the lingual surface. Each was completely ensheathed by clusters of conical-shaped papillae (Fig. 3 A-B).

Transmission electron microscopy

Transmission electron microscopic observations possessed numerous desmosomes in between electron-

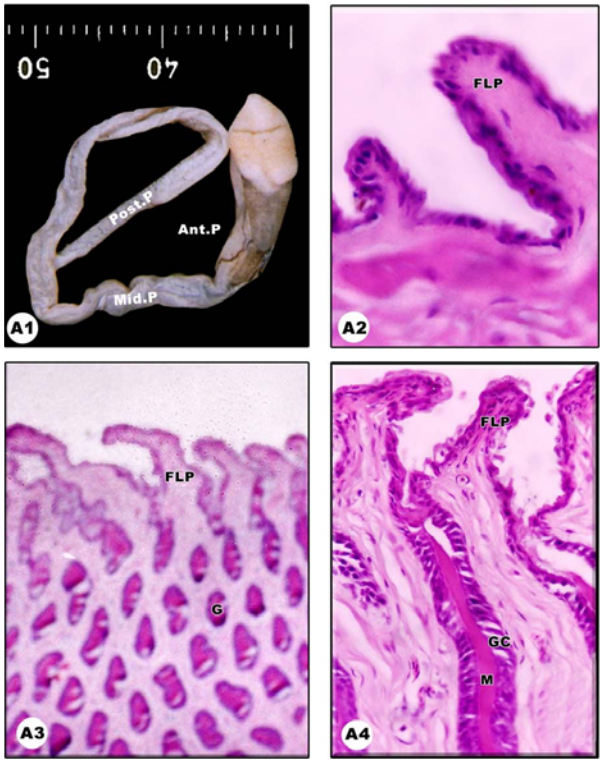

Fig.1 (A1-A4): Chameleon Tongue A1: Photomacrographs of gross dorsal tongue structure. A2-A4: Photomicrographs of longitudinal transverse histological section of dorsum lingual mucosa showing conical filliform papillae radially arranged and enclosed mucous gland containing hyaline secretion. HE (A3X160-A2-A4 250)

Fig. 2 (A-D): SEM of dorsal view of *Chameleon chameleon* lingual mucosa. A&C. Showing densely compacted conical filiform papillae with broad base and pointed curved end. B&D. Showing mucous gland opening

dense keratinocytes of the stratum granulosum. Few numbers of keratohyaline granules were detected (Fig. 4A-B).

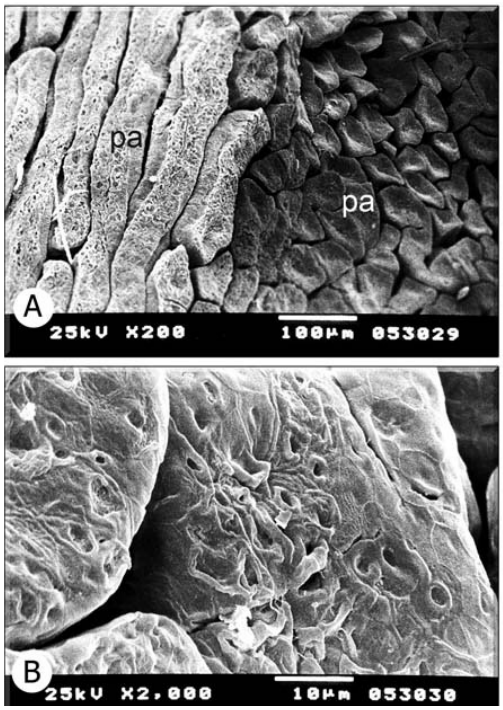

Fig. 3 (A-B): SEM of dorsal view of *Chameleon chameleon* lingual mucosa showing longitudinal and flattened lingual papillae

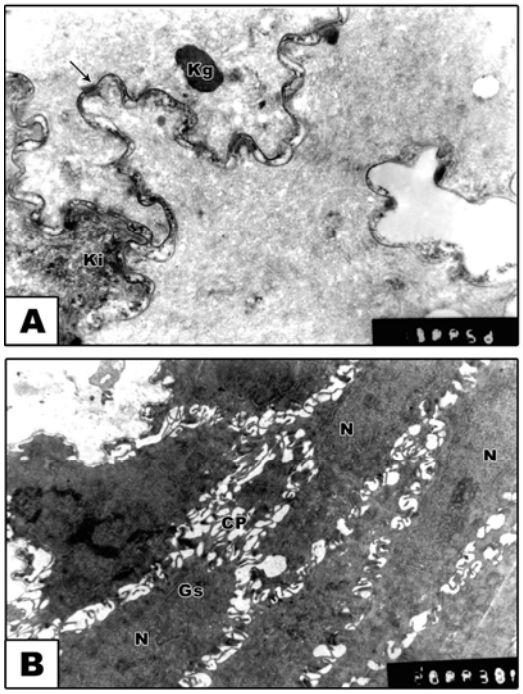

Fig. 4 (A-B): Transmission electron micrographs of lingual mucosa showing electron-dense keratinocytes separated by desmosomes (arrow head). Keratohyalin granules (Kg) are abundant in cytoplasm of keratinocytes. Lead citrate and Uranyl acetate X 7500

Discussion

The characteristic proximal lingual body of *Chamaeleo chamaeleon* possessed the lingual mucosa containing lingual papillae, mucous glands and other ordinary structures facilitate the animals for prehension and feeding. Filliform of different structures formed the mechanical parts and infiltrated by tubular glands containing mucoid secreting cells facilitated preying behaviour.

According to Bramble and Wake (1985) the prehensile tongue was the predominant prey capture mode in all members of the most primitive lizard clade, the Iguania (i.e. Iguanidae, Agamidae and Chamaeleonidae). The mechanism by which the prey adheres to the tongue of iguanid lizards during capture was thought to be based on adhesive bonding and/or interlocking. Since the chameleon tongue pad contained a large number of epithelial glands and possesses numerous papillae that can lock into surface irregularities on the prey (Schwenk, 1983), both wet adhesion and interlocking presumably played an important role during prey capture.

It is generally thought that chamaeleons, like other iguanians, rely on serous and mucous secretions and on interlocking to hold the prey on the tongue after capture (Bramble and Wake, 1985; Bell, 1989; Bels et al., 1994). On the basis of morphological and photographic data, Schwenk (1983) suggested that during prey capture, the tongue hits the prey and is splayed, resulting in the forcible discharge of mucous. Interlocking by free-standing cells on the tongue surface and suction were also put forward as possible adhesive mechanisms (Schwenk, 1983). Schwenk (1983) revealed that suction played an important role in the mechanics of chameleon tongue prehension. More than two-thirds of the total force generated by the tongue in chameleons was due to suction, thus enabling the animals to capture much larger prey (up to 15% of their body mass) than would be possible using surface phenomena alone. The maximum size of prey effectively transported with the tongue in a generalized agamid lizard, *Plocederma stellio* (Schwenk, 2000). There was no indication of the presence of gustatory papillae. Carnivorous feeding of the animals reflected on the presence of slight cornification of the lingual surface at light microscopic level. Reduction of keratinization is also confirmed by a decrease of keratohyaline granules in the keratinocytes of the stratum granulosum layer. These cells are the source of the stratum corneum.

It was concluded that the proximal lingual body is composed mainly of lingual mucosa and internal muscle for prehension.

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