

Scanning Electron Microscopy of the Lingual Dorsal Surface of the Japanese Lizard, *Takydromus tachydromoides*

By

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Summary: Scanning electron microscopy was employed to investigate the ultrastructure of lingual dorsal epithelial cells of the lizard, *Takydromus tachydromoides*. The specimens were prepared by a method involving osmium post-fixation and acid treatment to remove extracellular attached material.

The tongue of this species is long and slender. The anterior one third is bifurcate. The part comprising the posterior two thirds is wider than the anterior one. The dorsal surface of the anterior one third was found to be composed of an almost smooth surface, and the dorsal surface of the middle one third was entirely covered with scale-like papillae. The dorsal surface of the posterior one third had transverse ridges.

At higher magnifications, the epithelial cell surface of the anterior bifurcate portion revealed widely distributed microridges and cell marginal thickening. The epithelial surface of the scale-like papillae showed ring pattern microridges and cell marginal thickening. The frontal surface of the posterior ridges had abundant pores, on the surface of which globular substance was sometimes attached. Around the pores, a fine network pattern was recognized.

There have been many scanning electron microscopic studies on the lingual dorsal epithelial surface of mammals (Graziadei, 1969; Švejda und Škach, 1971; Yoshioka and Muto, 1976; Kessel and Kardon, 1979; Shimizu et al., 1979; Kobayashi and Shimamura, 1982; Steflik et al., 1983; Iwasaki et al., 1984a, b) and amphibia (Graziadei, 1969; Graziadei and DeHan, 1971; Düring and Andres, 1976). However, few scanning electron microscopic observations have been reported for the lingual surface of reptiles. The purpose of the present study was to observe the intact real surface of the lingual dorsal epithelium of the Japanese lizard,

Takydromus tachydromoides. For this purpose, the mucus covering the lingual epithelium was removed by acid hydrolysis. Employing the same method as in previous studies (Iwasaki et al., 1984a, b).

Materials and Methods

Tongues from three male and three female adult Japanese lizards, *Takydromus tachydromoides*, were used. The animals were perfused from the heart with Karnovsky fixative containing glutaraldehyde and paraformaldehyde under ether anaesthesia.

The tongues were then removed and refixed with the same fixative. After rinsing in 0.1 M cacodylate buffer, the materials were post-fixed in phosphate-buffered 1% osmium tetroxide solution at 37°C for 2 hours, and treated with 8 N hydrochloric acid at 60°C for 30 minutes to remove extracellular attached substance by acid hydrolysis. This was followed by dehydration, critical point drying and gold-ion sputtering in that order. Finally, the specimens were observed by scanning electron microscopy.

Results

The tongue of this species is long and slender with a bifurcation in the anterior one third. The posterior two thirds are wider than the anterior part. A transverse depression, making a distinction between the bifurcation and the posterior area, was found to be located in the marginal region between these two areas (Fig. 1). The surface of the bifurcation was flat from the tip (Fig. 2) to the base (Fig. 3). Abundant scale-like papillae were closely arranged on the middle dorsal surface. The interpapillar epithelium was deeply sunk. The papillae near the median line revealed a fan-shape, and those in the lateral dorsal surface formed transverse long protuberances in rows. Both types of papillae were inclined towards the back (Fig. 4). The fan-shaped papillae usually had a cusp. A few circular swellings and abundant pits were scattered on the surface of the papillae. The basal width of the papilla was about 200 μm , and its height was 200 to 300 μm (Fig. 5). The long protruding papillae in the lateral dorsal surface had many notches on the apical edge. The height of these papillae was 150 to 250 μm . A few circular swellings and abundant pits were also distributed on their surface (Fig. 6).

At higher magnifications, well developed microridges were found to be widely distributed on the surface of the epithelium in the anterior bifurcation. Cell marginal thicken-

ing was clearly observed (Fig. 7).

Ring pattern microridges were widely distributed on the surface of the scale-like papillae of the middle one third area. Cell marginal thickening was clearly observed. The diameter of the rings was about 0.5 μm (Fig. 8). The dorsal surface of the posterior one third formed transverse ridges. The part of each ridge on the median line was located ahead forming an arrow-head pattern. Many pores were scattered on the frontal and dorsal surface of these ridges (Fig. 9). Such pores were very sparse on the rear surface of the ridges (Fig. 10). Some of these pores did not have any attached substance on their surface, and contained compact materials inside (Fig. 11). Globular mucous substances were attached to other pores and often linked together (Fig. 12). The surface around the pores with and without globular substances showed a fine network pattern (Fig. 13).

Discussion

Usually, the oral epithelial surface of vertebrates covered with mucous substance in the living state. This is one reason for the difficulty in observing the real epithelial surface of the oral cavity by scanning electron microscopy in vertebrates. Especially on the lingual dorsal surface, abundant mucous substance tends to pile up on the interpapillar concave surface. Recently, it has become possible in mammals by employing osmium post-fixation and hydrolysis to remove the mucous substance from the dorsal surface including the interpapillar surface without damage to the epithelial cells themselves (Iwasaki et al., 1984a, b). In the present study, therefore, the same method as used in mammals was applied to the tongue of a reptile, the Japanese lizard, *Takydromus tachydromoides*. The results of this study show that the mucus could be removed almost completely from the lingual epithelial surface and the damage to the cell surface itself was not so distinct. Based on

these findings, it is concluded that acid hydrolysis after osmium post-fixation at a higher temperature than that of the ordinary method may be effective for the removal of mucous substance from the lingual surface of the Japanese lizard.

A general view of the tongue of some reptiles was presented by Goin and Goin (1962). However, few studies have been made on the tongue of reptiles by scanning electron microscopy. The present results demonstrate that the lingual dorsal surface of the lizard abounds with lingual papillae as it does in mammal (Švejda und Škach, 1971; Yoshioka and Muto, 1976; Kessel and Kardon, 1979; Shimizu et al., 1979; Kobayashi and Shimamura, 1982; Steflik et al., 1983; Iwasaki et al., 1984a, b) and amphibia (Graziadei and DeHan, 1971; Graziadei, 1975; Düring and Andres, 1976). However, the situation must be examined in more species of reptiles.

Microridges are widely observed on the lingual dorsal surface, especially the inter-papillary surface, of mammals (Iwasaki et al., 1984a,b). The present results indicate that microridges are also widely distributed on the lingual dorsal surface of the Japanese lizard, and that uncommon ring pattern microridges are widely spread on the surface of the scale-like papillae. Fahrenbach and Knutson (1975) considered that microridges represented an adaptational structure of the epithelium to friction, while Sperry and Wassersug (1976) suggested that microridges might play a role in holding mucus and appeared to facilitate the spread of mucus. In the tongue of the Japanese lizard, microridges might also be suitable structures for the holding and spreading of mucus on the epithelium.

References

- 1) Düring, M. v. and Andres, K.H. : The ultrastructure of taste and touch receptors of the frog's taste organ. *Cell Tiss. Res.*, **165** : 183-198, 1976.
- 2) Fahrenbach, W. H. and Knutson, D. D. : Surface adaptations of the vertebrate epidermis to friction. *J. Invest. Dermat.*, **65** : 39-44, 1975.
- 3) Goin, C. J. and Goin, O.B. : Introduction to herpetology. W. H. Freeman & Co., San Francisco, 1962.
- 4) Graziadei, P. P. C. : The ultrastructure of vertebrate taste buds. Ed. C. Pfaffmann, "Olfaction and taste". Rockefeller University Press, New York, 315-330, 1969.
- 5) Graziadei, P. P. C. and DeHan, R. S. : The ultrastructure of frogs' taste organs. *Acta Anat.*, **80** : 563-603, 1971.
- 6) Iwasaki, S., Sakata, K., Miyata, K., Mori, H. and Kobayashi, K. : Fine structure of the lingual dorsal epithelium of mongolian gerbil. *Jpn. J. Oral Biol.*, **26** : 292-296, 1984a.
- 7) Iwasaki, S. and Kobayashi, K. : Comparative studies on the lingual dorsal surface in three mammalian species by scanning electron microscopy. Proceedings of the 3rd Asia-Pacific Conference on Electron Microscopy: 277-278, 1984b.
- 8) Kessel, R. G. and Kardon, R. H. : Tissue and organ; a text-atlas of scanning electron microscopy. W.H. Freeman & Co., San Francisco, 1979.
- 9) Kobayashi, S. and Shimamura, A. Comparative anatomical observations of the tongue of the Japanese long-fingered bats, *Miniopterus schreibersi fuliginosus*. *Okajimas Fol. Anat. Jpn.*, **58** : 923-932, 1982.
- 10) Shimizu, T., Kusano, T. and Wada, M.: The fine structure of the papillary epithelium on the tongue of albino rats and albino mice. *Zool. Mag.*, **88** : 188-192, 1979.
- 11) Sperry, D.G. and Wassersug, R. J. : A proposed function for microridges on epithelial cells. *Anat. Rec.*, **185** : 253-258, 1976.
- 12) Steflik, D. E., Singh, B. B., Mckinney, R.V. Jr. and Boshell, J.L.: Correlated TEM, SEM, and histological observations of filiform papillae of the cow tongue. *Acta Anat.* **117** : 21-30, 1983.
- 13) Švejda, J. und Škach, M. : Die Zunge der Ratte im Raster Elektronenmikroskop (Stereo-scan). *Z. mikrosk.-anat. Forsch.*, **84** : 101-116, 1971.
- 14) Yoshioka, I. and Muto, H. : Surface structure of the tongue, palate and buccal mucosa of the rat (Scanning electron microscopic studies on the oral mucosa, 3). *Okajimas Fol. Anat. Jpn.*, **52** : 597-312, 1976.

Explanation of Figures

Plate I

- Fig. 1. Total view of the tongue of a Japanese lizard by scanning electron microscopy. The tongue is long and slender with a bifurcation in the anterior one third. The posterior two thirds are wider than the anterior part. A transverse depression (arrows), a distinction between the bifurcation and the posterior area, is recognized in the marginal region between these two areas. X 18.
- Fig. 2. Apex of the bifurcation. The surface is flat without papillae. X 1300.
- Fig. 3. Basal surface of the bifurcation. The sulcus (arrow) is a crevice of bifurcation. The epithelial cell surface is flat without papillae. Right in this photo is the apical direction. X 430.

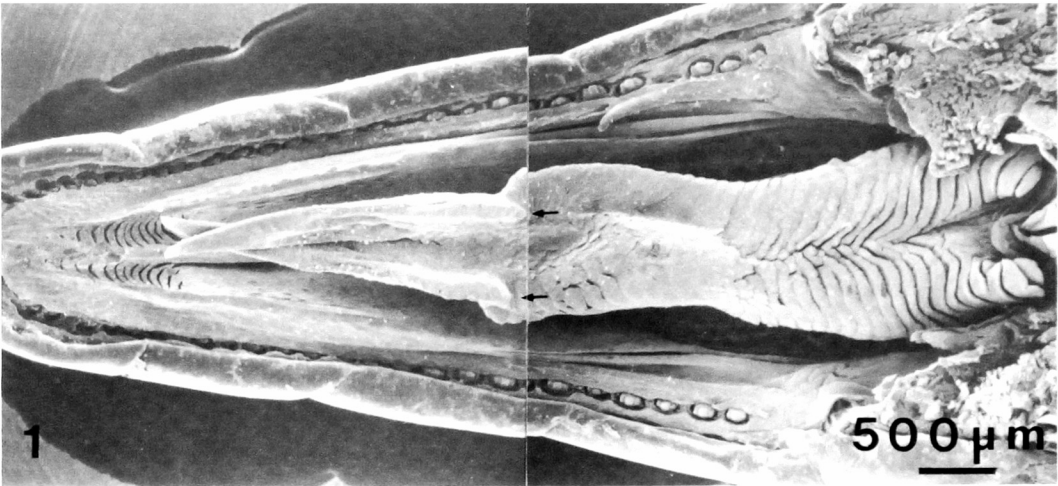


Plate II

- Fig. 4. Abundant scale-like papillae on the dorsal surface of the middle one third. The interpapillar epithelium is deeply sunk. The papillae near the median line show a flat fan-shape (arrows), and the papillae in the lateral dorsal surface form transverse protuberances in rows (curved arrows). Both types of papillae are inclined towards the back. Right in this photo is the apical direction. X 130.
- Fig. 5. Fan-shaped scale-like papillae near the median line. This type of papilla has a cusp (thick arrows). A few circular swellings (curved arrows) and abundant pits (thin arrows) are scattered on the surface of the papillae. The basal width of each papilla is 200 to 300 μm . X 320.
- Fig. 6. Long scale-like papillae on the lateral dorsal surface. Many notches are arranged on the apical edge of the papillae (thick arrows). The height of these papillae is about 200 μm . A few circular swellings and abundant pits (thin arrows) are observed on their surfaces. X 540.
- Fig. 7. Higher magnification of the epithelial cell surface in the anterior bifurcation. Well developed microridges are widely distributed on the cell surface. Cell marginal thickening is clearly seen. X 8000.

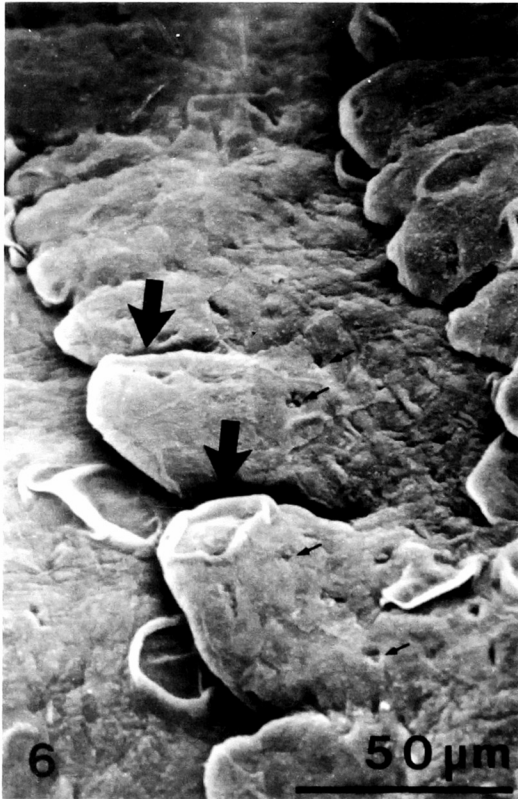
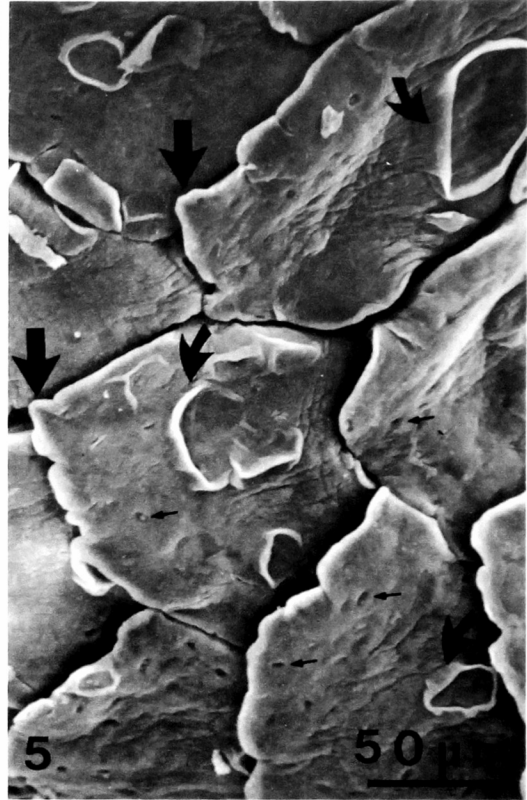
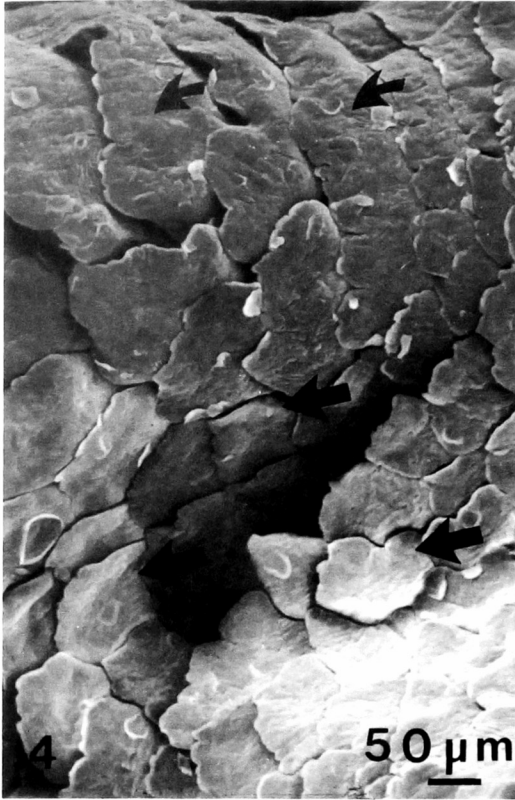


Plate III

- Fig. 8. Higher magnification of the epithelial cell surface of a fan-shaped scale-like papilla. Ring pattern microridges are widely distributed on the cell surface. The diameter of the ring is about $0.5 \mu\text{m}$. X 10000.
- Fig. 9. Dorsal surface of the posterior one third. Transverse ridges are arranged in rows. The part of each ridge on the median line is located ahead forming an arrow-head. Many pores are scattered on the frontal and dorsal surfaces of these ridges. Upwards in this photo is the apical direction. X 530.
- Fig. 10. One of the ridges on the dorsal surface of the posterior one third. Many pores are present on the frontal and dorsal surfaces of the ridges. Such pores are very few on the rear surface of the ridges. Right in this photo is the apical direction. X 520.
- Fig. 11. Higher magnification of the dorsal epithelial surface of a ridge. Each pore without attached substances are attached to the pores and often linked together. X 2700.

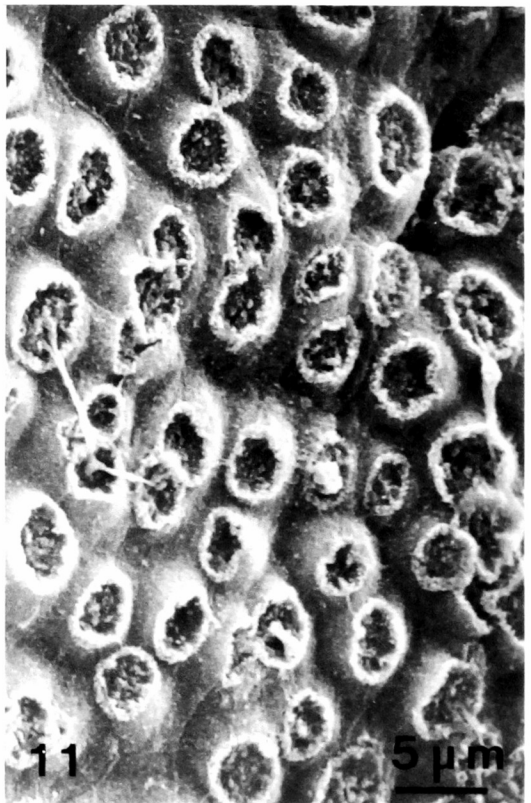
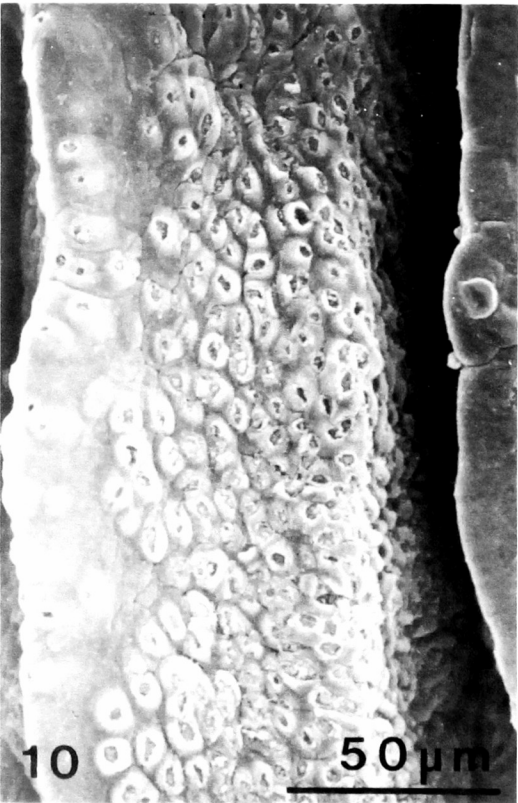
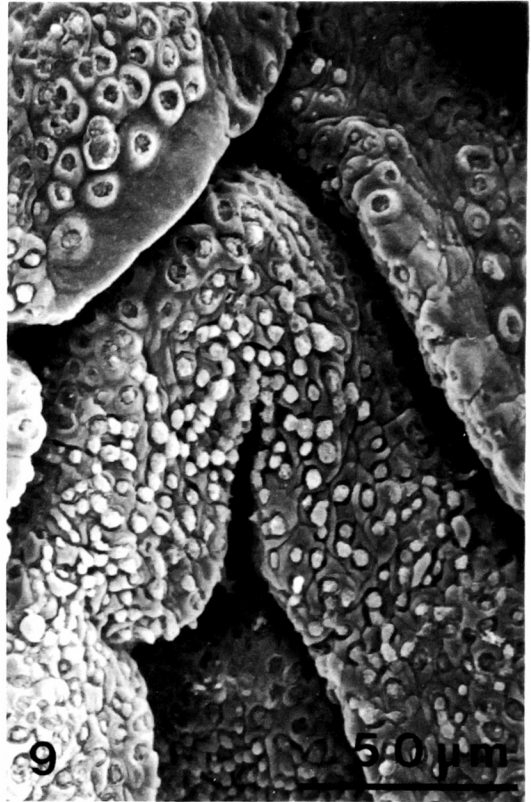
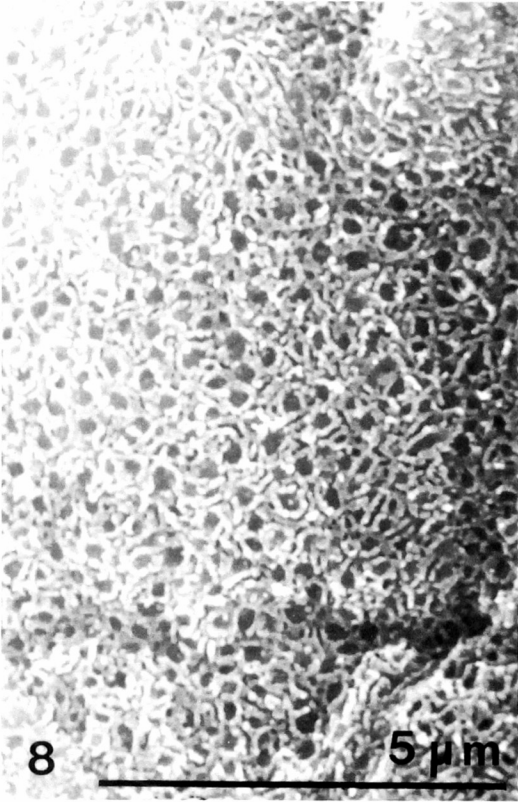


Plate IV

Fig. 12. Higher magnification of the dorsal epithelial surface of a ridge. Globular mucous substances are attached to the pores and often linked together. X 2700.

Fig. 13. Higher magnification of the dorsal surface of a ridge. The epithelial cell surface around the pores shows a fine network pattern. X 14000.

