



CHAPTER 5

HISTOLOGICAL FEATURES AND SURFACE MORPHOLOGY OF THE TONGUE



5.1 INTRODUCTION

The basic histological features of the avian tongue, especially in domestic birds, have been described in numerous species (see Calhoun, 1954 and McLelland, 1979 for a review of the earlier literature; Warner *et al.*, 1967; Koch, 1973; Hodges, 1974; McLelland, 1975; Nickel *et al.*, 1977; Homberger and Meyers, 1989; Gargiulo *et al.*, 1991; Porchescu, 2007). Echoing the suggestion by Gardner (1926, 1927) that microscopic data would enhance the understanding of macroscopic features, recent studies have generally combined light and scanning electron microscopy with the basic gross morphological features (Kobayashi *et al.*, 1998; Jackowiak and Godynicki, 2005; Jackowiak and Ludwig, 2008; Tivane, 2008). More specialized studies include those on the structure and secretions of salivary glands (Samar *et al.*, 1999; Liman *et al.*, 2001; Al-Mansour and Jarrar, 2004) and sensory structures of the tongue including taste buds (Botezat, 1910; Moore and Elliott, 1946; Lindenmaier and Kare, 1959; Gentle, 1971a, b; Berkhoudt, 1985) and Herbst corpuscles (Berkhoudt, 1979).

In contrast to the numerous gross morphological descriptions (see Chapter 4) available on the ratite tongue, there is very little information available on the histology of this region in ratites. The only histological study of the emu tongue is that of Crole and Soley (2008), which briefly outlines the main features observed by light microscopy. Other studies documenting the histology of ratite tongues are those of Feder (1972) for the greater rhea and Porchescu (2007), Jackowiak and Ludwig (2008) and Tivane (2008) for the ostrich. Scanning electron microscopy has only been employed for the ostrich tongue (Jackowiak and Ludwig, 2008; Tivane, 2008).

This chapter presents the first definitive histological and SEM description of the emu tongue and reviews, consolidates and compares the limited information on the histological features of the ratite tongue available in the literature.



5.2 MATERIALS AND METHODS

The heads of 23 sub-adult (14-15 months) emus of either sex were obtained from a local abattoir (Oryx Abattoir, Krugersdorp, Gauteng Province, South Africa) immediately after slaughter of the birds. The heads were rinsed in running tap water to remove traces of blood and then immersed in plastic buckets containing 10% buffered formalin. The heads were allowed to fix for approximately four hours while being transported to the laboratory, after which they were immersed in fresh fixative for a minimum period of 48 hours. Care was taken to exclude air from the oropharynx by wedging a small block of wood in the beak.

For light microscopy, five tongues were removed and cut into appropriate longitudinal and transverse sections to represent the body and root of the tongue, and the frenulum. The samples were dehydrated through 70, 80, 96, and 2X 100% ethanol and further processed through 50:50 ethanol:xylol, 2X 100% xylol and 2X paraffin wax (60-120 minutes per step) using a Shandon Excelsior Automatic Tissue Processor (Shandon, Pittsburgh, PA, USA). Tissue samples were then imbedded manually into paraffin wax in plastic moulds. Sections were cut at 4-6 μm , stained with Haematoxylin and Eosin (H&E) and Periodic Acid Schiff stain (PAS) (McManus, 1946) and viewed and micrographed using an Olympus BX50 equipped with the analySIS CC12 Soft Imaging System (Olympus, Japan).

An additional three heads were collected from birds (5, 15 months & 5 year-old birds) specifically for scanning electron microscopy. The heads were fixed in 10% buffered formalin overnight. Samples of the caudo-dorsal tongue body, tongue root and tongue body ventrum were removed and rinsed in distilled water to remove all traces of phosphate buffer. The samples were dehydrated through an ascending ethanol series (50, 70, 80, 90, 96 and 3X 100%). Due to the size of the tissue blocks, each dehydration step took 60 minutes. The blocks were then critical point dried from 100% ethanol through liquid carbon dioxide in a Polaron E300 Critical Point Drier (Polaron, Watford, England). After critical point drying the samples were mounted on round or rectangular (depending on sample size) aluminium viewing stubs with a conductive paste, Silver Dag (Dag 580 in alcohol), and sputter coated with a thin layer of palladium using a Polaron SEM E5100 coating unit. Areas of interest were viewed using a Philips XL 20 SEM operated at 8kV. Images were digitally captured using analySIS[®] 3.1 software (Soft Imaging



System GmbH) and described. The terminology used in this study is that of *Nomina Anatomica Avium* (Baumel *et al.*, 1993).

5.3. RESULTS

5.3.1 Light microscopic observations

5.3.1.1 Tongue body

The tongue body consisted essentially of an epithelial lining, a wide connective tissue layer (the lingual submucosa) containing glands, lymphoid tissue, Herbst corpuscles, blood vessels and nerves, and a core formed by the lingual skeleton and associated striated muscle (Figs. 5.1, 5.2, 5.6). Both the dorsal and ventral surfaces of the tongue were invested by a non-keratinised stratified squamous epithelium (*Epithelium stratificatum squamosum*) (Fig. 5.7). The dorsal epithelium was marginally thicker than the ventral epithelium (Fig. 5.9), displayed a lower frequency of connective tissue papillae and contained melanocytes.

The *stratum basale* of the *dorsum linguae* consisted of a single, compact layer of low columnar cells with vertically oriented nuclei. Interspersed between the epithelial cells were numerous melanocytes from which pigment-containing dendritic processes projected into the overlying *stratum spinosum* (Fig. 5.7). In the lateral lingual papillae, the melanocytes were situated at the tips in the *stratum basale* and underlying connective tissue. The *stratum spinosum* was composed of a variable number of layers of polygonal cells. These cells typically contained a large, round, centrally positioned nucleus and were separated from neighbouring cells by a relatively wide intercellular space spanned by numerous inter-connected cytoplasmic processes. Nucleoli were particularly prominent in the cells of the *stratum spinosum* (Fig. 5.7). The more superficial cells of this layer were observed to flatten and assume a horizontal orientation. The nuclei were similarly flattened, pale in appearance and displayed a prominent mass of heterochromatin which was generally associated with the nuclear membrane. These cells constituted the origin of the *stratum corneum* which was composed of a variable number of nucleated cell layers stretching to the epithelial surface (Fig. 5.7). The cells of this layer were compactly arranged and displayed a substantial degree of surface sloughing (see SEM). The dorsal epithelium was interrupted at regular intervals by the ducts of large, simple branched tubular mucus-secreting glands (Fig. 5.8) (see below) situated in the underlying connective tissue.



The epithelium of the *ventrum linguae* was similar in composition to that of the dorsum except for the obvious absence of melanocytes (Figs. 5.10, 5.12). The *stratum corneum* was poorly developed in some areas with rounded cells more typical of the *stratum spinosum* stretching to the epithelial surface. Isolated patches of ciliated columnar cells were confined to this aspect of the tongue and when observed on the epithelial surface, were often associated with aggregations of lymphoid tissue (Fig. 5.15) and/or gland openings. The mucosa at the junction between the tongue ventrum and frenulum exhibited folds (Fig. 5.5). In some instances the ventral epithelium was obliterated by large aggregations of lymphoid tissue emanating from the underlying connective tissue layer (Fig. 5.16). In contrast to the tongue body dorsum, the epithelium of the ventrum was interrupted by the ducts of both large simple branched tubular mucus-secreting glands and small simple tubular mucus-secreting glands (Figs. 5.5, 5.12).

Underlying the epithelium on all aspects of the tongue surface was a dense, irregular fibrous connective tissue layer, the lingual submucosa (*Tela submucosa linguae*) that stretched from the base of the epithelium to the lingual skeleton and associated striated muscle. It was thickest at the centre of the dorsal tongue body and tapered towards the margins (Fig. 5.9). This tissue penetrated the epithelial layer in the form of connective tissue papillae richly supplied with capillaries (Figs. 5.7, 5.8, 5.10). Melanocytes were heavily concentrated around these capillaries. The papillae on the tongue body dorsum were often irregular in number, orientation and length, with some penetrating close to the epithelial surface; with those on the ventrum being more regularly arranged and variable in depth of penetration.

The lingual submucosa was dominated by the presence of large, simple branched tubular mucus-secreting glands (*Glandulae linguales*) that occupied the full width of the layer, being absent only from the lateral lingual papillae (Figs. 5.9, 5.10), excepting the most caudal ones, and ending abruptly where the tongue body merged with the frenulum. These structures presented oblong, round, oval or pear-shaped profiles (Figs. 5.1, 5.8, 5.11). The glands accounted for the bulk of the tongue parenchyma (Figs. 5.1, 5.2, 5.4-5.6) and varied in size with the largest and most branched being found near the midline where the connective tissue layer was the thickest. Each gland was surrounded by a condensed layer of connective tissue resulting in the formation of distinct glandular units. Numerous fine septa radiated from the containing fibrous layer to separate the individual tubular (sometimes tubulo-alveolar) secretory acini. The septa were richly supplied with capillaries. The secretory acini emptied into a large central lumen which in some



glands was clearly lined by a pseudostratified ciliated columnar or simple ciliated columnar epithelium (Fig. 5.14). The lumen narrowed as it passed through the epithelium, forming the secretory duct. This duct was lined by a single layer of vertically oriented squamous cells continuous with the surface layer of the epithelium (see SEM) although in some instances a ciliated columnar epithelium was observed along part of the duct.

The acini displayed varying degrees of secretory activity. Active acini were lined by typical mucus-secreting cells with basally-positioned round vesicular, or dark, flattened nuclei (Fig. 5.13). The ample apical cytoplasm was filled with a granular, lightly basophilic material that demonstrated a positive PAS reaction (Figs. 5.6, 5.9). Inactive acini were composed of a simple cuboidal epithelium with relatively less and darker staining cytoplasm with a round central nucleus. The released mucus was visible in the lumen of some acini and in the central lumen as wispy, stringy accumulations of blue-purple material. The glandular units represented the doughnut-shaped structures seen macroscopically (see Chapter 4), with the secretory acini forming the pale ring and the central lumen/duct forming the dark central spot.

In addition to the large branched glands described above, the tongue ventrum also displayed numerous small, simple tubular mucus-secreting glands (Fig. 5.5, 5.12, 5.15). These glands were partly intra-epithelial in location, extending only a short distance into the underlying connective tissue and were composed of cells with similar features to those lining the active acini in the larger branched glands. The gland lumen was narrower than that of the larger glands and the portion traversing the epithelium was lined by mucus-secreting cells. Simple tubular glands, in addition to the large simple branched tubular glands, were also absent from the lateral lingual papillae.

Specialised sensory nerve endings in the form of Herbst corpuscles (*Corpusculum lamellosum avium*) (Figs. 5.5, 5.17, 5.18) were also a common feature of the connective tissue layer. These large, pale lamellated bodies occurred singly, were randomly distributed and were closely associated with the large branched glands, although always separated from them by an intervening layer of connective tissue. The distribution of the corpuscles varied with some being positioned just beneath the epithelium (superficial) and others abutting the lingual skeleton (deep) (Fig. 5.17). They exhibited round or oval profiles, although irregular forms were also observed, and they displayed morphological features typical of Pacinian (Herbst) corpuscles (Figs. 5.17, 5.18). The neural component (nerve terminal/axon) of the corpuscle was centrally



situated and surrounded by a series of closely apposed lamellae forming a distinct zone, the inner core. This zone was also characterised by the presence of a number of Schwann cell nuclei. Surrounding the inner core was a series of loosely arranged, concentric lamellae (fibrocytic lamellae) separated by obvious spaces. This region (the outer core) formed the bulk of the tissue surrounding the neuronal component and displayed relatively few nuclei. The entire corpuscle was closely invested by a capsule formed by a thin, fibrous connective tissue layer displaying numerous fibroblast nuclei (Fig. 5.18). The Herbst corpuscles were similar to those observed elsewhere in the oropharynx (see Chapter 3 - Fig. 3.28).

Lymphoid tissue in the tongue body was confined to the ventrum where it generally occurred as large diffuse accumulations situated immediately beneath the epithelium (Fig. 5.5, 5.15, 5.16). The larger aggregations were associated with the glandular tissue (which in some instances invaded the glandular tissue particularly near the lumen) whereas smaller isolated patches (Fig. 5.15) occurred throughout the connective tissue layer and also in the tips of the lateral lingual papillae (Fig. 5.10). The large aggregations were sometimes confined to the connective tissue but were also observed to penetrate the epithelium, obliterating the normal structure of this layer (Fig. 5.16). Nodular lymphatic tissue in the form of lymphoid follicles was present within some of the diffuse accumulations. The follicles were always positioned toward the deeper aspect of the aggregations (Fig. 5.16).

The deeper region of the lingual submucosa was compressed into a narrow conspicuous layer between the base of the glands and the perichondrium of the lingual skeleton or the perimysium of the associated skeletal muscle bundles. This layer displayed large blood vessels (Fig. 5.8) and nerves from which smaller subdivisions radiated between the glandular tissues. Melanocytes were concentrated around the large blood vessels on the dorsum of the tongue body.

The core of the tongue body was formed by the **lingual skeleton** which comprised the rostral projection of the *basihyale* and the *paraglossum* (Fig. 5.6). The rostral projection of the *basihyale* was situated ventral to the *paraglossum*. It was round in cross-section, composed of hyaline cartilage and invested by a thin perichondrium flanked by adipose tissue (Fig. 5.6). The caudal aspect showed signs of ossification. The *paraglossum* was dorso-ventrally flattened (Figs. 5.1, 5.2) and thinned where it lay above the rostral projection of the *basihyale*, giving it a butterfly appearance in cross-section (Fig. 5.6). It was also composed of hyaline cartilage and surrounded by a delicate perichondrium.



Skeletal muscle fibres (*Musculi linguae*) were observed ventral to the *paraglossum* (Fig. 5.2, 5.5). The fibres were grouped into fascicles which in turn formed muscle bundles (which would represent the intrinsic hyolingual muscles described by Bonga Tomlinson (2000)) that ran rostrally from the base of the *paraglossum* on either side of the rostral projection of the *basihyale* to end rostral to the mid-ventral aspect of the *paraglossum*. The muscle bundles were attached along their length to the ventral aspect of the *paraglossum* through merging of the respective perimysium and perichondrium. The muscle bundles also tapered in a caudo-rostral direction and could be seen macroscopically as the crura on the ventrum of the tongue body (see Chapter 4 - Fig. 4.6).

5.3.1.2 Tongue root (Figs. 5.3, 5.4)

The epithelium covering the tongue root displayed similar features to that of the ventrum of the tongue body, except that the islands of ciliated columnar epithelium observed on the body were not seen on the tongue root. The underlying connective tissue was similar to that of the tongue body, but was slightly less densely packed. Both types of glands were present and similar to those of the tongue body. The large glands were concentrated mainly in the midline of the tongue root and were more loosely spaced than those of the tongue body. These glands formed the faint doughnut-shaped structures seen macroscopically in this region (see Chapter 4). The small simple tubular mucus-secreting glands were scattered over the rest of the area and concentrated on the caudally pointed tongue root tip. Melanocytes were present only in those specimens that had a pigmented tongue root. The melanocytes, when present, were restricted to the caudal tongue root tip. Occasional small diffuse lymphoid aggregations were present in the underlying connective tissue. Herbst corpuscles were present in very low numbers and associated with the larger glands. There was no core formed by the lingual skeleton and muscular tissue was only present below the connective tissue on the lateral edges (Fig. 5.3).

In one specimen an epithelial modification with features similar to those of a taste bud (*Caliculus gustatorius*) was found on the tongue root close to the glottis. It was an isolated structure clearly demarcated from the surrounding epithelial tissue, oval in shape and contained a group of elongated, vertically oriented cells apparently opening into a central pore (Fig. 5.19). It was not possible with any certainty to identify supporting cells from sensory cells within the structure although supporting elements appeared to surround the sensory cells. (Fig. 5.19).



5.3.1.3 Frenulum

The epithelial covering of the frenulum showed similar characteristics to that of the ventrum of the tongue body with which it was continuous and typically did not reveal melanocytes. Only simple tubular mucus-secreting glands were present. The frenulum revealed a core of loose irregular connective tissue containing large blood vessels and non-medullated nerves. Large aggregations of lymphoid tissue similar to those observed on the tongue ventrum were consistently present in the folded tissue at the junction of the ventrum of the tongue body and the frenulum (Figs. 5.5, 5.16).

5.3.2 Scanning electron microscopic observations (Figs. 5.20-5.28)

On low magnification the dorsum of the tongue body appeared 'flaky', due to the desquamation of individual surface cells of the *stratum corneum* (Fig. 5.20, 5.26). All the surface cells were flattened and polygonal-shaped (Fig. 5.20). On higher magnification the surface cells revealed a complex pattern of microplicae and the cell boundaries were clearly demarcated. The only other notable feature of this region was the presence of large openings of the underlying mucus-secreting glands (see histology). Most of the openings were obscured by glandular secretions and cell debris (Fig. 5.20). All the gland openings on this surface were of similar size.

The rostral part of the tongue body ventrum displayed similar features to that of the dorsum. The caudo-lateral aspect of the ventrum was also similar to the dorsum; however, small openings were apparent and were randomly and unevenly distributed amongst the larger openings (Fig. 5.21). (This observation confirmed the presence of both the simple tubular and large simple branched tubular mucus-secreting glands seen histologically). There was also less desquamation of the surface cells (Fig. 5.21). The cells immediately surrounding the small gland openings displayed a velvety pattern on low magnification. Higher magnification revealed that this pattern was due to the surface of these cells displaying densely packed microvilli (Fig. 5.22). Microvilli also adorned the surface of the cells forming the duct opening. The ring of microvilli-adorned cells around the duct openings made an abrupt transition to the surrounding surface cells demonstrating microplicae (Figs. 5.22, 5.23).



That part of the tongue body ventrum bordered by the above areas (essentially the surface overlying the rostral projection of the *basihyale* and the area adjacent to both it and the frenulum) displayed different features to the rest of the tongue. The typical desquamating cell surface was replaced by an undulating, uneven lumpy surface (Fig. 5.24). This surface was characterised by cells which were not clearly demarcated from each other due to a dense covering of microvilli. These microvilli were interspersed with patches of cilia, which had an uneven distribution (Figs. 5.24, 5.25). Gland openings were present in this region and ranged from very large, to large (the same size as on the dorsum) and small. Smaller openings were often located in groups or rows and were dispersed amongst the larger openings. Some of the larger openings appeared to be split into 2-3 openings by a septum.

The central region of the tongue root (Fig. 5.26) appeared similar to the dorsum of the tongue body, displaying both individual desquamating surface cells and large gland openings (Fig. 5.28). The lateral edges and caudal projection of the root displayed areas of markedly less surface cell desquamation. On the lateral edges, both small and large gland openings were observed (Figs. 5.26, 5.27). Mucus secretion often obscured or plugged the openings. On the caudal projection, only small gland openings were obvious.

The basic surface features were similar in all the age groups studied, although a greater degree of desquamation was noted in the older birds.

5.4 DISCUSSION

5.4.1 Light microscopical features

5.4.1.1 General features of the tongue body

Although the dorsal and ventral surfaces of the emu tongue appear similar macroscopically (see Chapter 4), it is possible to distinguish the two surfaces histologically. The dorsum contains melanocytes, has only large simple branched, mucus-secreting glands penetrating the epithelium, and lymphoid tissue is absent. The tongue ventrum is free of melanocytes, has aggregations of diffuse and nodular lymphoid tissue, patches of ciliated columnar epithelium and openings of both large and small simple mucus-secreting glands. It is also a noteworthy observation that histologically the entire tongue ventrum lacks melanocytes, yet macroscopically the ventral surface appears lightly pigmented. No such differentiation was noted for the dorsum and



ventrum of the tongue body in the greater rhea (Feder, 1972) or ostrich (Jackowiak and Ludwig, 2008; Tivane, 2008).

The connective tissue papillae penetrating the dorsal epithelium in the emu were often irregular in frequency, orientation and length, with some penetrating close to the epithelial surface. Those of the tongue ventrum were more regularly arranged than in the dorsum and similar in appearance to those described in the ostrich (Tivane, 2008). Feder (1972) reported intraepithelial capillaries looping up to half the distance of the epithelium of the greater rhea tongue, a feature not noted in the emu.

5.4.1.1.1 Epithelium

The stratified squamous epithelium covering all aspects of the emu tongue was non-keratinised, confirming the finding of Crole and Soley (2008). Faraggiana (1933) also noted, macroscopically, that the emu tongue mucosa showed no signs of cornification. The stratified squamous epithelium of the greater rhea (Feder, 1972) and ostrich (Porchescu, 2007; Jackowiak and Ludwig, 2008; Tivane, 2008) tongues is also reported to be non-keratinised. This contrasts with the general statement that the tongue of most birds displays a keratinised epithelium (Iwasaki, 2002) as illustrated, for example, in the penguin, white bulbul and various domestic species (Koch, 1973; Hodges, 1974; McLelland, 1975; Kobayashi *et al.*, 1998; Al-Mansour and Jarrar, 2004). It has also been reported that in some birds (Warner *et al.*, 1967; Jackowiak and Godynicki, 2005) the tongue ventrum is keratinised while the dorsum is non-keratinised.

In the emu the dorsal epithelium was observed to be thicker than that of the tongue ventrum, a feature also noted in the ostrich (Jackowiak and Ludwig, 2008). However, the dorsal epithelium of the emu tongue is unusually thin when compared to the thickness of the dorsal epithelium found, for example, in the chicken (Hodges, 1974) and quail tongues (Warner *et al.*, 1967). A reason for this phenomenon may be found in the feeding method of palaeognaths (Bonga Tomlinson, 2000; Gussekloo and Bout, 2005) where the tongue is not involved in food manipulation and the surface therefore requires less mechanical protection.

An interesting finding on the ventrum of the tongue was the abrupt transition from a stratified squamous epithelium to isolated patches of simple columnar epithelium with or without cilia. This type of epithelium most often occurred in the vicinity of underlying lymphoid tissue. Feder



(1972) encountered a similar phenomenon of epithelial transition in a hatchling female greater rhea. The author noted that the caudal palate, oral floor, tongue base and tongue ventrum showed large islands of cylindrical (columnar) epithelium with kinocilia. These islands apparently increased in density aborally. The functional importance of this type of epithelium is not clear (except for the obvious possibility of mucous clearance) and further studies will be required for a more definitive explanation.

5.4.1.1.2 Glands

The glands in the emu tongue are ubiquitous and occur in the connective tissue of the tongue body, root and frenulum, but not in the lateral lingual papillae, excepting the most caudal ones. Tucker (1958) notes that the size and number of glands present in the oropharynx of vertebrates are influenced by the environment and condition of the animal and it appears plausible that the emu displays a high gland density in the tongue (and oropharynx, see Chapter 3) due to its relatively dry diet. The glands in the greater rhea (Feder, 1972; personal observation) and ostrich (Porchescu, 2007; Jackowiak and Ludwig, 2008; Tivane, 2008) tongue are also found throughout the parenchyma and are located within the connective tissue, a feature apparently typical for ratites. There is a greater concentration of glands in the emu tongue than in the oropharynx (see chapter 3), a similar situation to that noted in the penguin (Samar *et al.*, 1999).

The naming of avian salivary glands has in the past been found to be inconsistent and confusing (Ziswiler and Farner, 1972), with most descriptions being based on human directional terminology (Anthony, 1919; Ziswiler and Farner, 1972; Hodges, 1974; Nickel *et al.*, 1977; Jackowiak and Godynicki, 2005) which is used to describe the location of the glands. According to Anthony (1919) the sparrow, robin, swallow and pigeon have the following groups of lingual glands: inferior, superior, anterior superior and posterior superior lingual glands. Ziswiler and Farner (1972) divide the salivary glands into superior and inferior groups. The glands in the chicken (McLelland, 1975) occur as the paired rostral lingual glands and the unpaired median caudal lingual gland, or as the anterior (tongue body?) and posterior (tongue root?) lingual glands (Hodges, 1974; Nickel *et al.*, 1977). The tongue of the white eagle shows anterior and posterior glands (Jackowiak and Godynicki, 2005) while those of the quail are classified as lingual, pre-glottal and laryngeal (Liman *et al.*, 2001). Tucker (1958) notes that lingual salivary glands of vertebrates can be grouped into anterior, posterior, inferior and superior glands, with frenular and basal glands only occurring in mammals. In some birds, the glands may be restricted



to certain areas of the tongue (Kobayashi *et al.*, 1998; Al-Mansour and Jarrar, 2004) which makes naming of the glands more precise.

Despite the occurrence of glands throughout the emu tongue, they can be grouped according to their location into dorsal, rostro-ventral, caudo-ventral, frenular (previously not said to occur in birds (Tucker, 1958) and radical (tongue root). Jackowiak and Ludwig (2008) identified dorsal, ventral and tongue-root lingual glands in the ostrich. Although Tivane (2008) describes and illustrates lingual glands in the ostrich, no specific groupings were identified. The naming of the emu (present study) and ostrich (Jackowiak and Ludwig, 2008) lingual glands thus differs from the earlier works where human anatomical terminology was used (see above). Although noting the presence of mucus-secreting cells, Bonga Tomlinson (2000) states that there are no salivary glands in the tongue of the greater rhea. However in the study by Feder (1972) in the same species it is clearly stated and illustrated that the tongue body is filled with glands. The description of the pre-glottal salivary glands in the quail (Liman *et al.*, 2001) fits the location (between the caudal lingual papillae and glottis) of the tongue root. This group of glands was named the radical glands in the emu (present study) and tongue-root glands in the ostrich (Jackowiak and Ludwig, 2008). The grouping of glands is complicated by the fact, as noted by Tucker (1958), that the areas of the salivary glands tend to merge with one another, particularly in birds.

The lingual salivary glands of the emu are of two types, namely, mucus-secreting (PAS positive) simple tubular glands and large simple branched, tubular glands. The large glands are seen macroscopically as doughnut-shaped structures with their openings to the surface appearing as a small central spot or depression. The lingual glands of the ostrich were classified as simple tubular and large simple branched tubular glands by Tivane (2008) whereas Jackowiak and Ludwig (2008) classified them as simple tubular and complex alveolar glands. The lingual glands of the greater rhea (Feder, 1972) are numerous and are described as tubulo-alveolar with no further mention being made of their size or more detailed structure. The two types of glands in the emu differed in distribution, a feature also noted in the ostrich (Jackowiak and Ludwig, 2008; Tivane, 2008). In the emu the dorsal and rostro-ventral glands are of the large simple branched tubular type, the frenular glands are exclusively of the simple tubular type and the caudo-ventral and radical lingual glands are composed of both types. Despite obvious structural differences between the emu and ostrich tongues (see Chapter 4) a similar distribution of the two types of glands is apparent in the ostrich (Jackowiak and Ludwig, 2008; Tivane, 2008). In the



ratite species studied (emu, ostrich and greater rhea) all the glands were exclusively mucus-secreting. The salivary glands in birds are generally tubular in nature with serous elements normally being absent (Ziswiler and Farner, 1972), a feature also apparent in the ratites. The lingual glands of the emu were similar to those depicted in other bird species, although the structural classification differed (Samar *et al.*, 1999; Bacha and Bacha, 2000; Liman *et al.*, 2001; Al-Mansour and Jarrar, 2004; Jackowiak and Godynicki, 2005).

The lumen of some of the large simple branched glands in the emu displayed a ciliated columnar epithelium, presumably to assist in mucus transport as there was no obvious evidence (with the staining techniques used) of smooth muscle elements around the glands. The mucus-secretions accumulate in the large lumen below the epithelium and move through short ducts to the surface. Thus extrusion of the viscid secretion and its transport to the epithelial surface may be effected by cilia, where present, as well as by pressure built up by the accumulated secretion. Hodges (1974) notes that the presence of smooth muscle fibres around salivary glands is disputed in birds. The large glands in the emu are surrounded by a conspicuous connective tissue capsule, a feature also noted in the ostrich (Jackowiak and Ludwig, 2008), and which distributes a rich capillary plexus between the acini.

Both the emu and greater rhea have pigmented tongue bodies although in the emu the pigmentation is restricted to the dorsum. In the emu, melanocytes are distributed in the *Str. basale* and underlying connective tissue and also concentrated around the blood vessels. When viewed macroscopically, pigmentation is uniform across the whole surface. However, the melanocytes in the greater rhea tongue (Feder, 1972) are concentrated around the base of the glands encasing them like a basket. This phenomenon causes the pigmentation to appear dotted across the surface. Thus every dark spot in the greater rhea tongue represents a gland (personal observation) whereas in the emu tongue the glands are seen as pale doughnut-shaped structures below the pigmented surface.

The main function of the lingual salivary glands in birds is to provide moisture and lubrication to food boli (Nickel *et al.*, 1977; King and McLelland, 1984; Gargiulo *et al.*, 1991; Liman *et al.*, 2001; Al-Mansour and Jarrar, 2004). Jackowiak and Ludwig (2008) proposed that due to the high concentration of mucous glands located in the shortened tongue body of the ostrich, the main function would be to produce copious amounts of mucus which would lubricate the oropharynx and assist in rolling or sliding the food over the smooth tongue surface towards the



oesophagus. Whereas it is true that mucus production by the tongue would assist in the transport of food in this fashion, these authors failed to review any of the existing literature on the feeding method of palaeognaths which indicate that the emu and other ratites employ a ‘catch and throw’ (Gusseklou and Bout, 2005) or cranioinertial (Bonga Tomlinson, 2000) feeding method whereby the food bolus travels from the bill tip to the oesophageal entrance (Gusseklou and Bout, 2005). As the tongue is depressed during this movement it plays a limited role in transport of food through the oropharynx. Therefore the proposed function of the lingual salivary glands of the ostrich by Jackowiak and Ludwig (2008) is questionable. Thus it would be reasonable to assume that food boli in the emu would be moistened and lubricated by salivary glands of the pharyngeal region and not of the tongue directly (the food is thrown caudal to the tongue).

The lingual glands of birds are also responsible for providing a moist environment in the oropharynx, a hydrophilic surface on the tongue as well as protection from micro-organisms (Gargiulo *et al.*, 1991). Similar functions could also be attributed to the emu lingual glands. Tabak *et al.* (1982) note further that the mucins have the effect of protecting the tongue surface against coarse material and desiccation, and modulate microbial flora.

5.4.1.1.3 Herbst corpuscles

The Herbst corpuscles in the emu tongue body occur both superficially (below the epithelium) and deep (overlying the *paraglossum*) and are mostly associated with the large glands. They are found in smaller numbers in the tongue root, also associated with the large glands. No sensory corpuscles were found in the greater rhea tongue (Feder, 1972) although the author notes that the possibility of their presence could not be excluded. Herbst corpuscles were also absent from the tongue of the ostrich (Tivane, 2008) and their presence was not noted in the same species by Porchescu (2007) or Jackowiak and Ludwig (2008). The presence of Herbst corpuscles in the avian tongue has been confirmed by Ziswiler and Farner (1972) and Berkhoudt (1979) in the duck tongue.

The Herbst corpuscles in the tongue of the emu displayed similar characteristics to those observed in the emu oropharynx (see Chapter 3) and to those found in the ostrich (Tivane, 2008). In the emu Herbst corpuscles, a capsule, an outer zone (subcapsular space), an inner core with a lamellated appearance (formed by specialised Schwann cells) and a central axon could be identified. The avian Herbst corpuscle capsule is continuous with the perineurium of the nerve



fibre and the lamellae consist of delicate connective tissue (Nickel *et al.*, 1977). Gottschaldt (1985) provides a review of the earlier literature as well as a description of Herbst corpuscles; from this it is apparent that the emu Herbst corpuscle, at the light microscopic level, appears similar to other avian Herbst corpuscles. A more detailed comparative study will be needed to ascertain the similarity between the Herbst corpuscles in the ratite tongue and avian Herbst corpuscles of the oropharyngeal cavity.

Herbst corpuscles are comparable to Pacinian corpuscles found in mammals and are lamellated sensory receptors sensitive to pressure and vibration, being the most widely distributed receptors in the skin of birds (see Gottschaldt, 1985 for review of earlier literature; Nickel *et al.*, 1977). Harrison (1964) classified the tongue of birds according to function noting that in some birds the tongue functions as an organ of touch. The tongue of the emu, as well as that of other ratites, is short in comparison to the bill and is unable to protrude (see Chapter 4). Bonga Tomlinson (2000) and Gussekloo and Bout (2005) studied eating and drinking in palaeognaths and concluded that the tongue plays no role in manipulating or contacting food. Therefore, the fact that the emu possesses a tongue apparently equipped as an organ of touch, in contrast to the situation in the greater rhea (Feder, 1972) and ostrich (Tivane, 2008), is unusual. It is possible that the emu may use its tongue in a way not previously described in other ratites during eating or investigatory behaviour. Further studies will be needed to determine this possibility. The tongue may also, by virtue of the Herbst corpuscles, play a role in food selection by determining the texture of ingested food, a possibility also considered by Crole and Soley (2008).

5.4.1.1.4 Lymphoid tissue

Lymphoid tissue is present as aggregations on the ventrum, frenulum, lateral papillae tips and root of the emu tongue. The aggregations are mostly associated with glands or are positioned just beneath the epithelium. Hodges (1974) noted that lymphoid tissue is frequently found in the connective tissue surrounding salivary glands in adult birds. The only other mention of lymphoid tissue in a ratite tongue is that of Tivane (2008) in the ostrich. According to Rose (1981) a notable amount of lymphoid tissue is contained within the walls of the digestive tract in birds and constitutes part of the secondary lymphoid tissue. Furthermore, lymphoid tissue is abundant in the oropharynx of birds (Rose, 1981) although no specific mention is made to its presence in the tongue. Thus a comparison can not be drawn between the lymphoid tissue in the emu tongue and that of other avian tongues (where present).



Diffuse lymphoid tissue was the most common type observed in the emu tongue. When present, within the diffuse lymphoid tissue, nodular lymphoid tissue was most commonly encountered at the junction of the frenulum with the tongue body. The ostrich tongue contained small amounts of diffuse lymphoid tissue mainly associated with the glands (Tivane, 2008). In the emu, in areas where the epithelium was invaded by underlying lymphoid tissue, the epithelium would often display a change to a columnar ciliated epithelium (see above). This was especially prominent in the frenular folds. The significance of this phenomenon remains undetermined.

Lymphocytes constitute the main component of lymphoid tissue, with the T-lymphocytes being responsible for cell mediated immune responses and the B-lymphocytes, which synthesize and secrete antibodies after transforming to plasma cells, providing humoral immunity (Rose, 1981). The tongue of the emu, by virtue of the notable amounts of lymphoid tissue, would therefore also appear to play an important immunological function.

5.4.1.1.5 Lingual skeleton

The *paraglossum* in the emu tongue body is situated centrally in the parenchyma and consists entirely of hyaline cartilage (Crole and Soley, 2008; present study). The positioning of the *paraglossum* (*Os entoglossum*) within the tongue body of the greater rhea (Feder, 1972) is similar to that of the emu although no mention is made of its histological structure. In contrast, the ostrich has paired paraglossals which are also composed of hyaline cartilage (Tivane, 2008). In ratites the *paraglossum* remains cartilaginous and does not ossify in older birds (Bonga Tomlinson, 2000), a situation also apparent in the emu.

The rostral projection of the *basihyale* in the emu lies ventral to the *paraglossum*, is round in cross section and composed of hyaline cartilage showing areas of ossification near its centre (Crole and Soley, 2008; present study). A similar structure is present in the ostrich (Tivane, 2008), and, as in the emu, was surrounded by a distinct perichondrium, skeletal muscle, loose connective tissue, blood vessels, nerves and fat cells. Feder (1972) made no mention of the rostral projection of the *basihyale* or its histological structure in the greater rhea tongue. The rostral projection of the *basihyale* in the ostrich is a flattened rectangle, cartilaginous in younger birds and showing signs of ossification in older birds (Tivane, 2008). Jackowiak and Ludwig (2008) seem to have mistaken the rostral projection of the *basihyale* in the ostrich for the



paraglossum. The authors reported the '*paraglossum*' as spatula-shaped and cartilaginous. This description is more befitting of the rostral projection of the *basihyale*. Porchescu (2007) also depicts the rostral projection of the *basihyale* in the ostrich as cartilaginous. Thus it would seem this structure in both the emu and ostrich is largely cartilaginous with some signs of ossification. This may very well be an age related phenomenon, which, however, was not confirmed in the present study.

5.4.1.1.6 Lingual musculature

The only musculature in the emu tongue is skeletal muscle fibres which attach to the ventral aspect of the *paraglossum*. This is a similar finding to that in the greater rhea (Feder, 1972). Intrinsic musculature is absent from the tongue in birds, excepting parrots (Ziswiler and Farner, 1972; Koch, 1973; Nickel *et al.*, 1977; McLelland, 1990), with the rostral third of the tongue being completely free of musculature (Nickel *et al.*, 1977). In the emu, the rostral aspect of the tongue is also free of musculature (Crole and Soley, 2008; present study).

The only muscles that move the tongue of birds are those of the hyobranchial apparatus (Harrison, 1964; Koch, 1973) which form the extrinsic musculature of the emu tongue. The movement of the tongue during eating and drinking of palaeognaths as described by Bonga Tomlinson (2000) and Gussekloo and Bout (2005) would seem to indicate that the tongue is not an active participant in swallowing. During swallowing the hyobranchial apparatus is retracted and causes tongue retraction through the attachment of the striated muscle to the ventral aspect of the *paraglossum* and by virtue of the rostral portion of the *basihyale* being imbedded in the tongue body. In the emu, the function of the muscle attaching to the ventral aspect of the *paraglossum* would similarly be to effect the retraction of the tongue.

5.4.1.2 Tongue root - Taste buds

A structure resembling a taste bud was located in the epithelium on the tongue root. This is the first report of a taste bud in a ratite tongue. No taste buds were observed in the tongue of the greater rhea, although their existence could not be ruled out (Feder, 1972). Similarly, taste buds have not been reported in the ostrich tongue (Jackowiak and Ludwig, 2008; Tivane, 2008). Although only a single taste bud was identified in the emu tongue these structures were observed more frequently on the caudal oropharyngeal floor and proximal oesophagus (see Chapter 3).



Some confusion exists in the literature regarding the naming of the caudal extremity of the tongue body (the tongue base) and the tongue root (Moore and Elliott, 1946) with both terms being used interchangeably (McLelland, 1975). The lack of consensus regarding which parts constitute the tongue has led to disagreement in the literature as to whether taste buds occur on the tongue of birds or not (Moore and Elliott, 1946). Based on the work of Lillie (1908) and Bradley (1915) it is generally accepted that the border between the tongue body and root is the row of caudal lingual papillae (Moore and Elliott, 1946; Gentle, 1971b; Nickel *et al.*, 1977; Bailey *et al.*, 1997). The importance of clarity in correctly identifying and naming the various components of the tongue has been pointed out by Moore and Elliott (1946), particularly in regard to the location of taste buds. Failure to recognize the caudal aspect of the tongue (the tongue root) as part of the tongue could lead to invalid conclusions about the presence of taste buds in this organ, as they are reportedly concentrated in this region (Moore and Elliott, 1946; Gentle, 1971b; Nickel *et al.*, 1977; Bacha and Bacha, 2000; Al-Mansour and Jarrar, 2004). Due to the confusion in correctly identifying the tongue root in ratites, it is possible that taste buds were not located in the tongue during previous studies (Feder, 1972; Tivane, 2008) if the root was not identified, sectioned and examined. The number of taste buds in the chicken are reported to increase with age (Lindenmaier and Kare, 1959). If this phenomenon applies to ratites it may be another reason why Feder (1972) did not find taste buds in the greater rhea tongue, due to the young age of the birds examined. Thus it would seem that future investigation of the tongue root of ratites is warranted to definitively determine whether these structures are present or not.

Birds display a very low number of taste buds in comparison to other vertebrates (Berkhoudt, 1985). The paucity of taste buds in the avian tongue is due to the fact that unlike mammals, birds do not break down their food orally (Gentle, 1971a); therefore the food is not in contact with the tongue for long. Thus the emu, which swallows its food whole and uses the 'catch and throw' (Gussekkloo and Bout, 2005) or cranioinertial feeding method (Bonga Tomlinson, 2000) in which the food lands near or into the oesophageal entrance before being swallowed, would have limited need for taste on the tongue. It would therefore seem appropriate that if any receptors were found in the emu tongue, they would be extremely sparse and located on the most caudal extremity thereof (the root).

A reason for the difficulty in locating taste buds, as noted by Moore and Elliott (1946), is the fact that they are obscured by the connective tissue papillae and by the ducts of glands traversing the



epithelium. Due to the many deep connective tissue papillae and many gland openings in the emu tongue these factors would certainly complicate and mask the identification of taste buds. Taste buds are most often associated with glands or occur free in the mucosa (Botezat, 1910; Gentle, 1971b; Nickel *et al.*, 1977; Berkhoudt, 1985; Bacha and Bacha, 2000). The structure found on the emu tongue root was not associated with a gland opening and was isolated in the epithelium.

The structure resembling a taste bud found on the emu tongue root was similar to the isolated receptors depicted by Botezat (1910) for birds and was an entity discernable from the surrounding epithelium. The putative taste bud revealed what appeared to be a taste pore at the epithelial surface and was composed of elongated cells typical of those described in birds (Berkhoudt, 1985). However it was not possible to distinguish clearly between supporting and sensory cells. The taste bud on the tongue root of the emu appeared similar in shape to that described and depicted for birds in general (Botezat, 1910; Moore and Elliott, 1946; Gentle, 1971b; Nickel *et al.*, 1977; Lindenmaier and Kare, 1959; Warner *et al.*, 1967). Taste buds in birds also appear similar to those found in other vertebrates (Moore and Elliott, 1946; Gentle, 1971b). A more detailed comparative study will be needed to ascertain whether the taste buds on the ratite tongue are comparable to those found on other avian tongues.

The most obvious function of taste buds on the tongue of the emu would be the discrimination of food. Again, because of the reduced, non-protrusible tongue of the emu which does not contact food during the cranioinertial method of feeding (Bonga Tomlinson, 2000), the role of the tongue as a sense organ is debatable. There seems little opportunity for food to contact the tongue root to be tasted. However, Bonga Tomlinson (2000) describes the tongue as scraping the palate during retraction and swallowing. It may therefore be possible that only after food ingestion can the emu taste the ingesta. The tongue scrapes off food that may have stuck (due to the abundant mucus secretion, see Chapter 3) to the oropharyngeal roof while travelling from the bill tips to the oesophageal entrance. The sense of taste is an important motivator for feeding as well as initial food selection in birds (Gentle, 1971a). Initial food selection may thus not be an important function of taste in the emu. In birds food selection is also based on size, shape, colour and texture as well as taste and olfaction (Berkhoudt, 1985). It would seem plausible that all these factors would also influence the food intake in the emu. It is also suggested (Huchzermeyer, personal communication) that the sparse taste buds in the emu may be involved in the selection of potable drinking water, particularly in their natural arid environment.



5.4.2 Scanning electron microscopy (SEM) features

The description of the surface morphology was based mainly on observations of the 5 month-old specimen, although the basic features observed were consistent with those of the older birds.

The SEM findings revealed that the various surfaces of the tongue displayed features similar to those found in the oropharynx and proximal oesophagus (see Chapter 3). The tongue body dorsum displayed similar features (large gland openings and desquamating surface cells) to those described for the ostrich tongue (Jackowiak and Ludwig, 2008; Tivane, 2008). The large openings on the tongue body (dorsum and ventrum) of the emu also appeared similar to those depicted in the white eagle tongue (Jackowiak and Godynicki, 2005). SEM confirmed the distribution of glands in the emu tongue noted by light microscopy (see above). The large openings represented the underlying large simple branched tubular mucus-secreting glands and the smaller openings represented the small simple tubular mucus-secreting glands. Isolated patches of ciliated cells on the tongue ventrum, as seen by light microscopy, were also confirmed by SEM. Microridges described on the surface of keratinised cells in the tongue of the white eagle (Jackowiak and Godynicki, 2005) appear similar to the microplacae observed on the non-keratinised cells found on all surfaces of the emu tongue.

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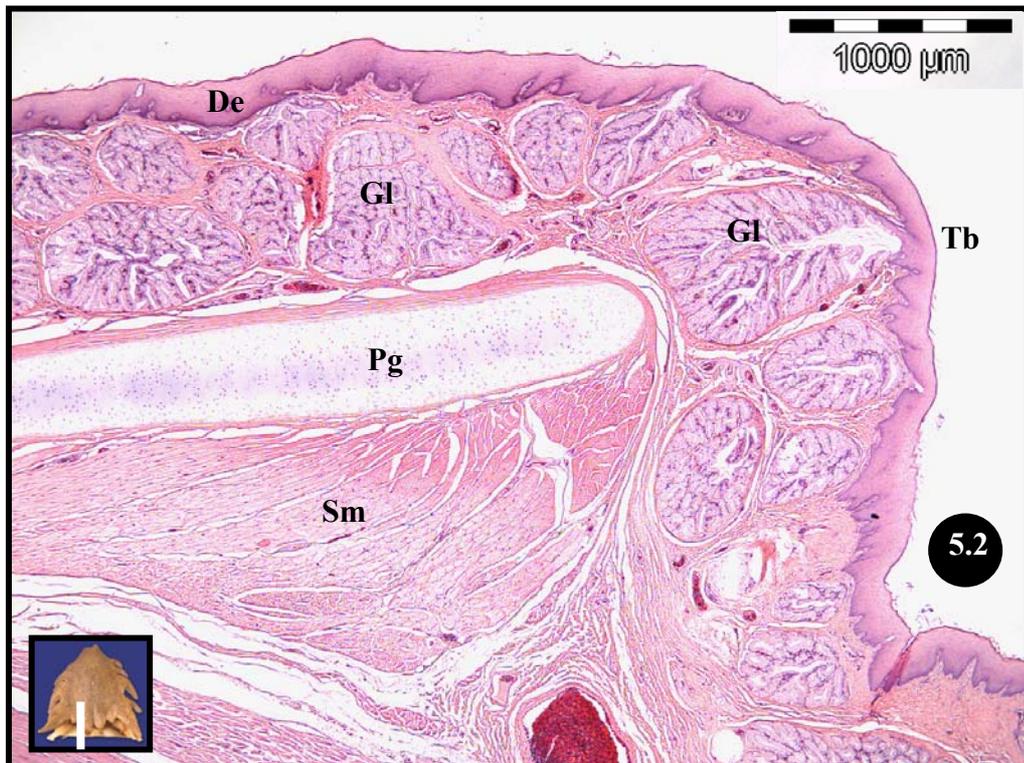
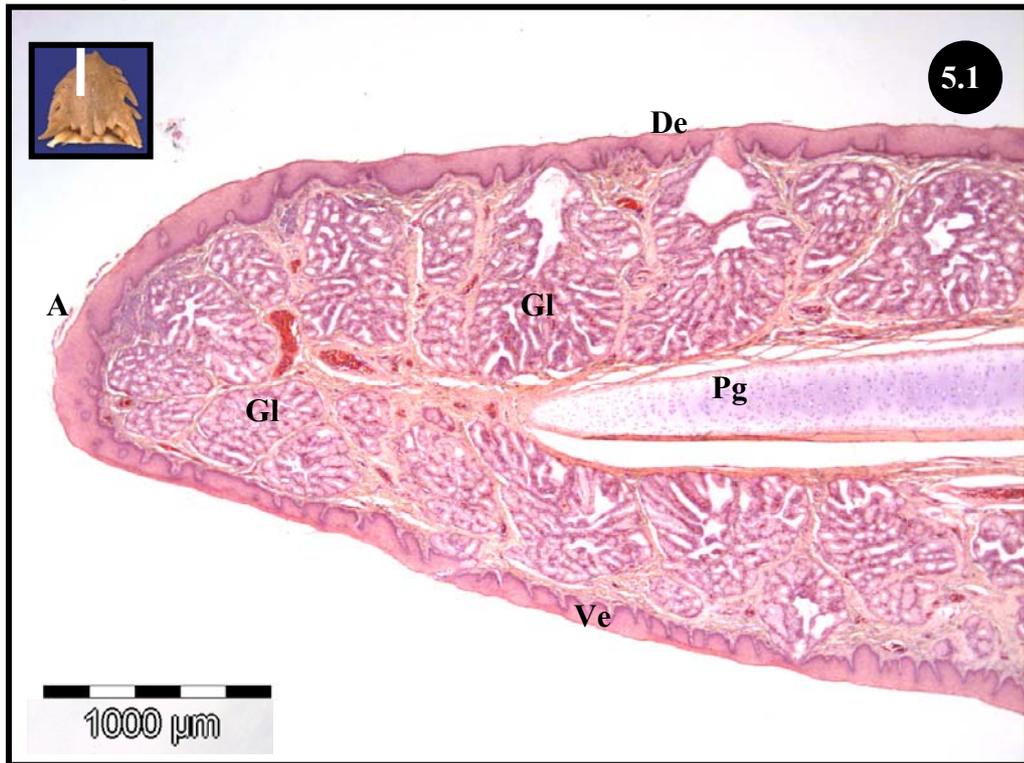
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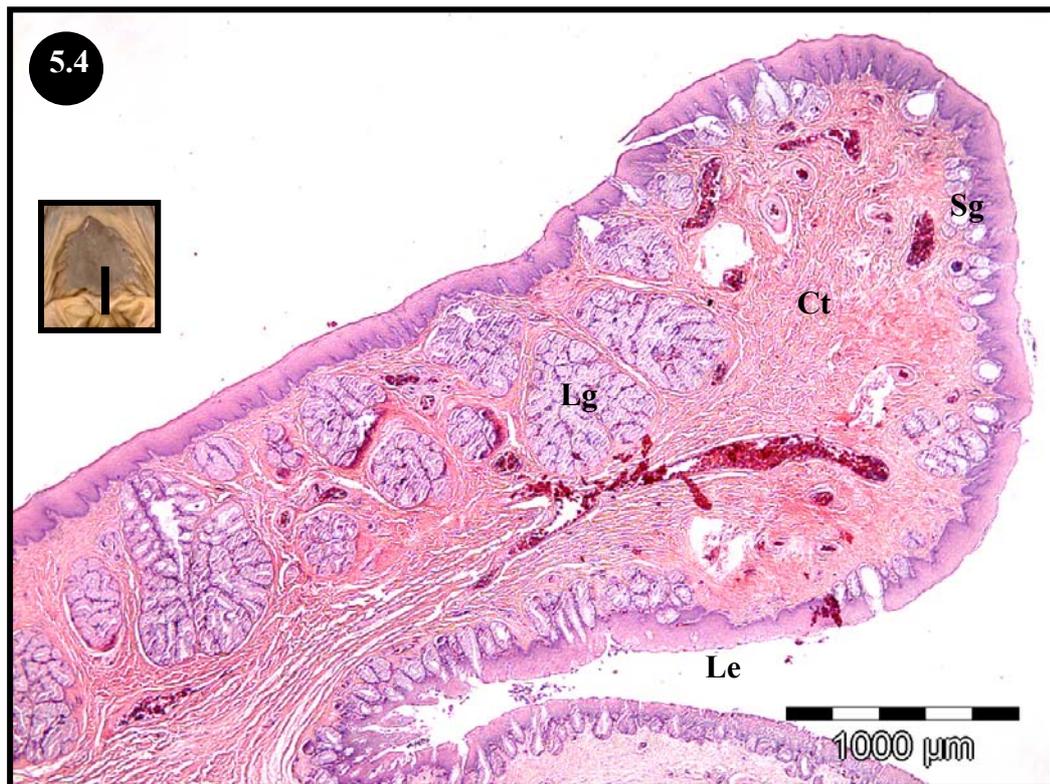
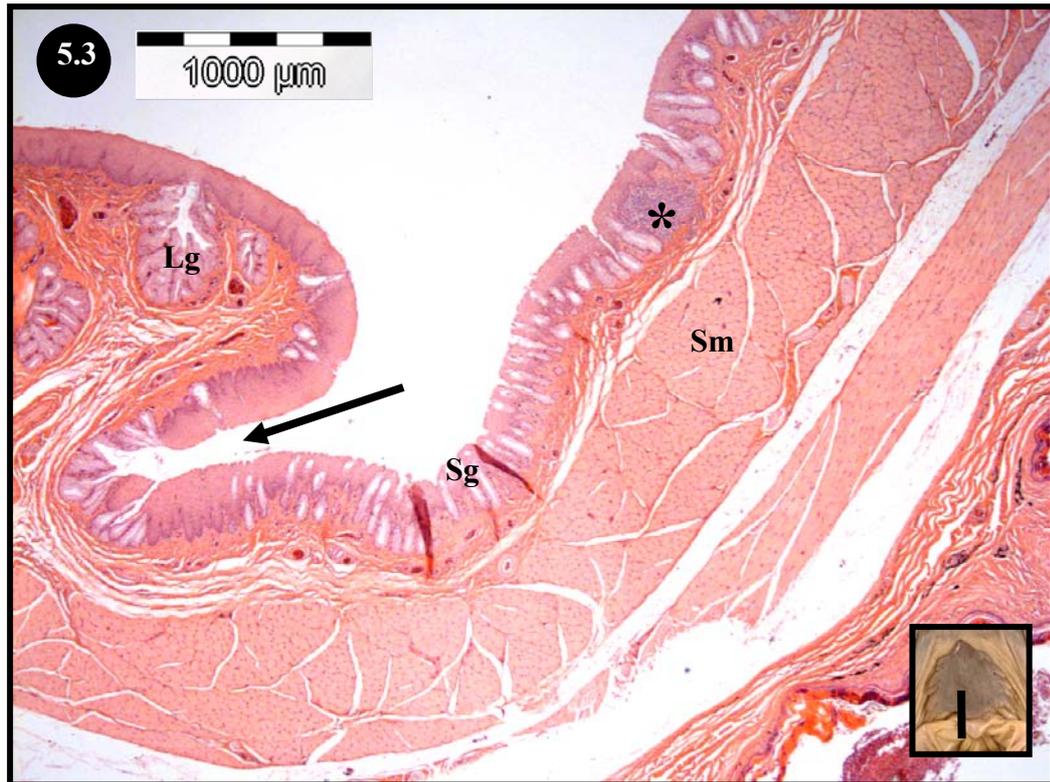
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5.6 FIGURES



Figures 5.1 and 5.2: Longitudinal sections of the tongue body representing the rostral (Fig. 5.1) and caudal (Fig. 5.2) regions. The *paraglossum* (Pg) forms the core between the connective tissue layer (lingual submucosa) filled with large, simple branched glands (Gl). Note the large amount of skeletal muscle (Sm) attaching at the base of the *paraglossum*. Apex (A), tongue base (Tb), dorsal epithelium (De), ventral epithelium (Ve).



Figures 5.3 and 5.4: Paramedian (Fig. 5.3) and median longitudinal (Fig. 5.4) sections of the tongue root depicting simple tubular glands (Sg), lymphoid tissue (*) and skeletal muscle (Sm) in the paramedian section. Large simple branched tubular glands (Lg) are a feature of the median section. Connective tissue (Ct), shallow retrolingual recess (arrow), laryngeal entrance (Le).

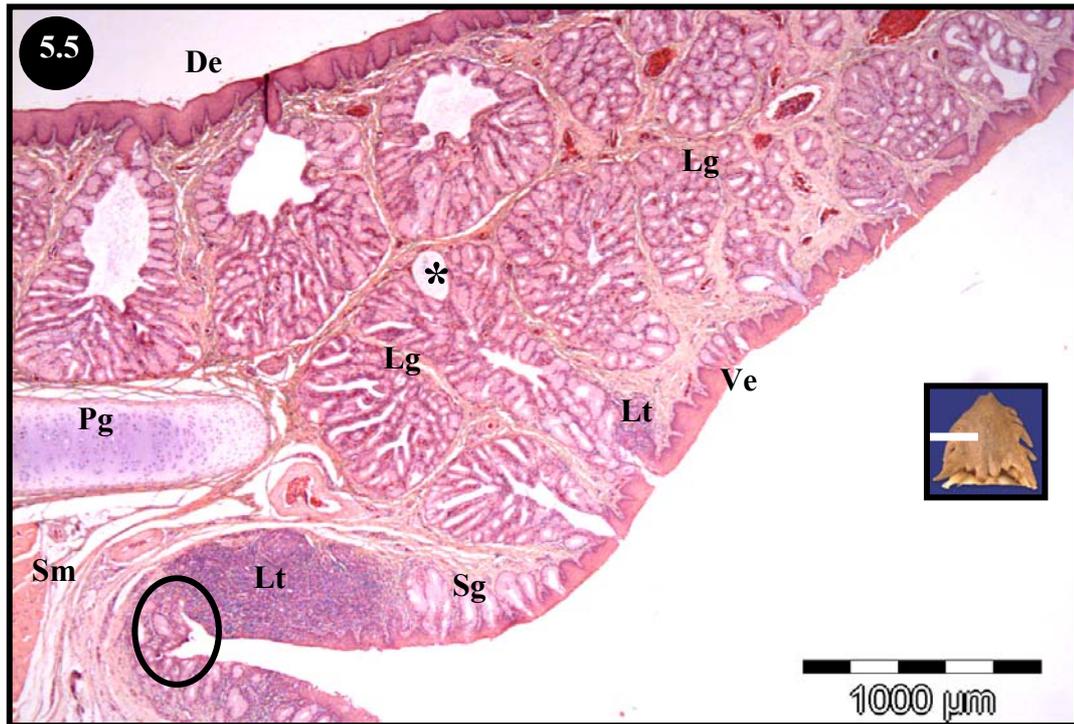


Figure 5.5: Cross section of the lateral tongue body and papillae base demonstrating large simple branched tubular glands (Lg) and associated Herbst corpuscle (*). Note the simple tubular glands (Sg) and lymphoid tissue (Lt) exclusively present on the ventrum. *Paraglossum* (Pg), skeletal muscle (Sm), dorsal epithelium (De), ventral epithelium (Ve), mucosal folds of ventrum at frenular junction (encircled).

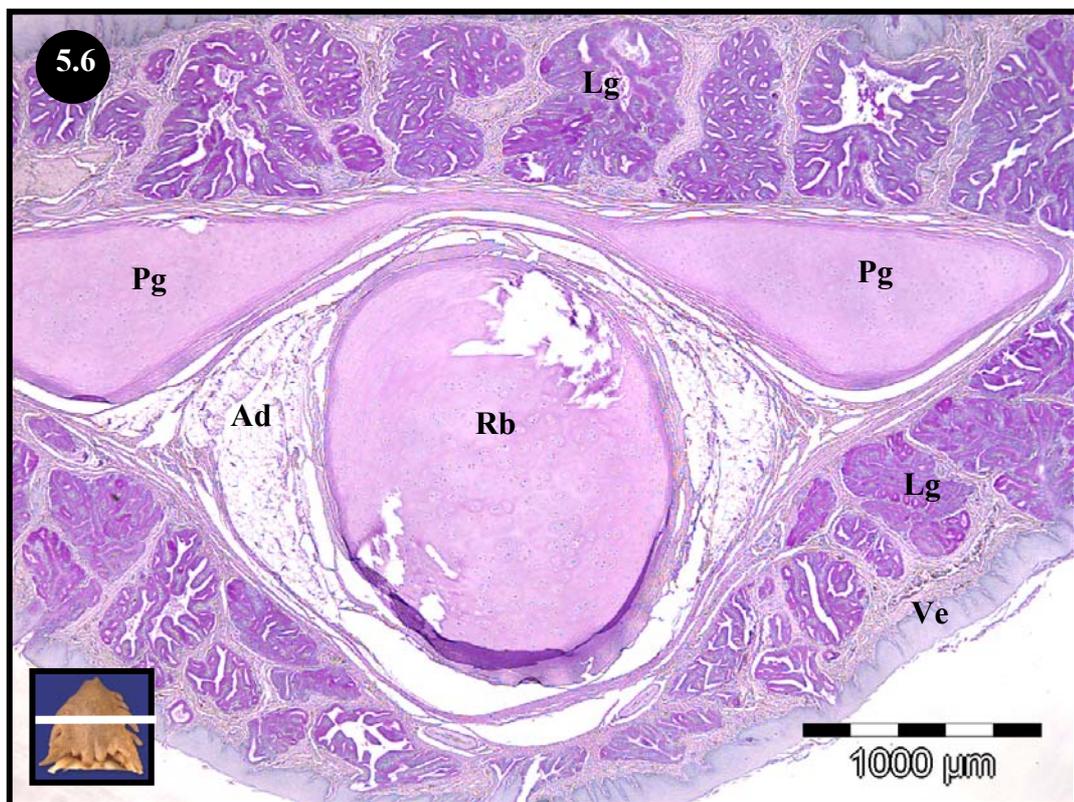


Figure 5.6: Cross section of the middle of the tongue body showing the topography of the lingual skeleton within the parenchyma. The *paraglossum* (Pg) lies dorsal to the rostral projection of the *basihyale* (Rb) which is flanked by adipose tissue (Ad). Large simple branched tubular glands (Lg), ventral epithelium (Ve). PAS stain.

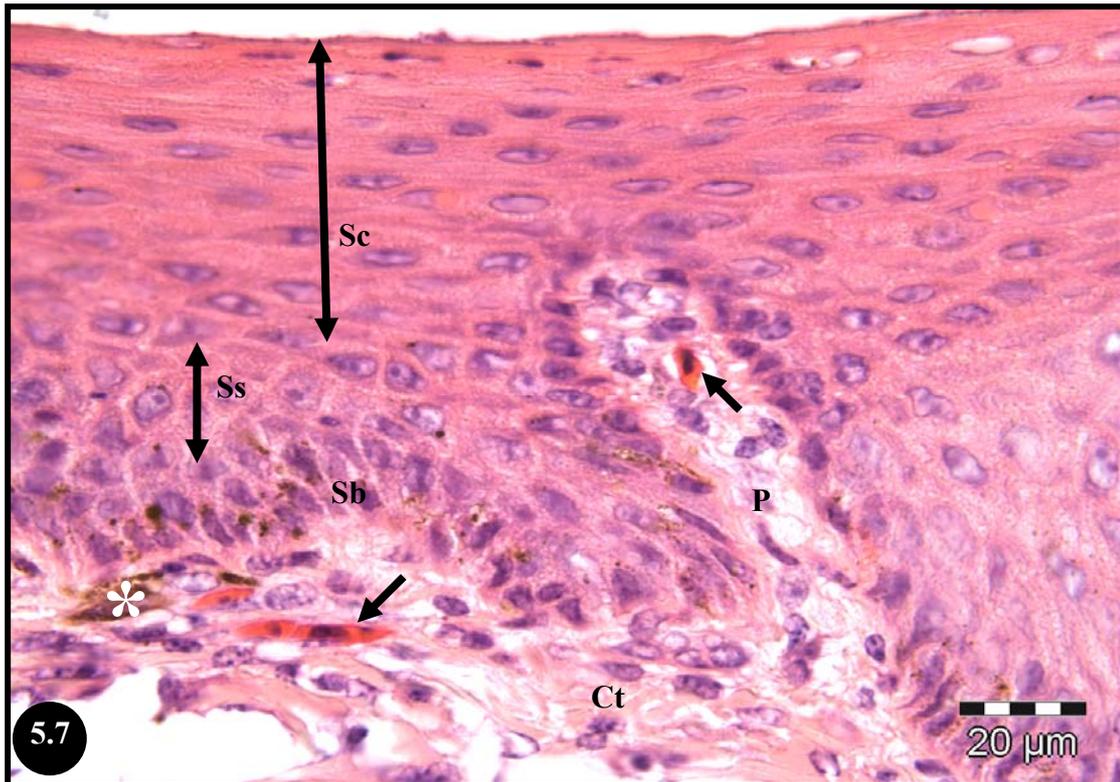


Figure 5.7: The non-keratinised stratified squamous epithelium of the tongue dorsum displaying the *Str. basale* (Sb) with melanocytes (*) some of which lie in the connective tissue beneath the *Str. basale*, *Str. spinosum* (Ss) and *Str. corneum* (Sc). Connective tissue (Ct), connective tissue papilla (P), capillary (arrows).

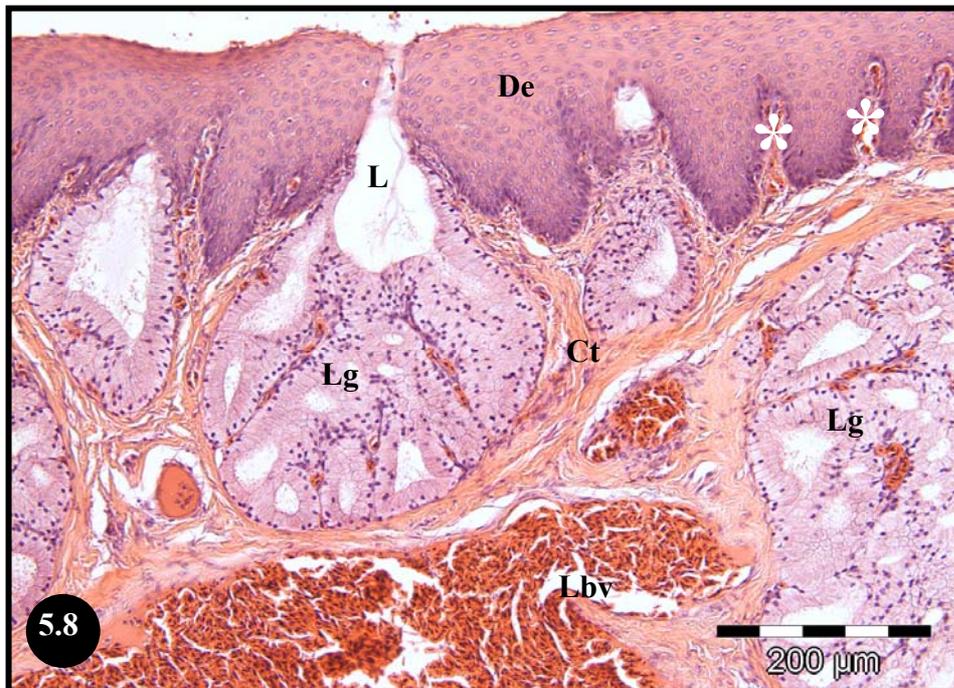


Figure 5.8: Low magnification of the tongue dorsum showing the duct of a large simple branched tubular gland (Lg) passing through the epithelium (De). Lumen (L), connective tissue (Ct), connective tissue papillae (*), large blood vessel (Lbv).

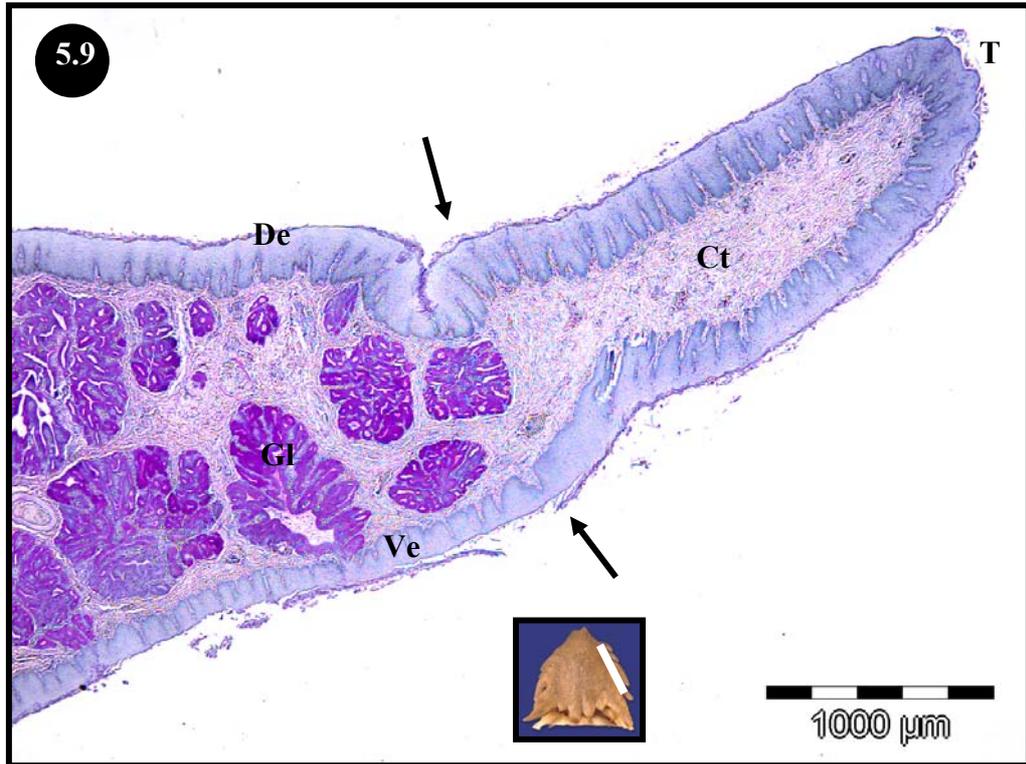


Figure 5.9: Lateral lingual papilla in longitudinal section with the glandular tissue showing a positive PAS reaction. Note the abrupt termination (arrows) of the glands (Gl) leaving only connective tissue (Ct) filling the space between the dorsal (De) and ventral epithelium (Ve). Papilla tip (T).

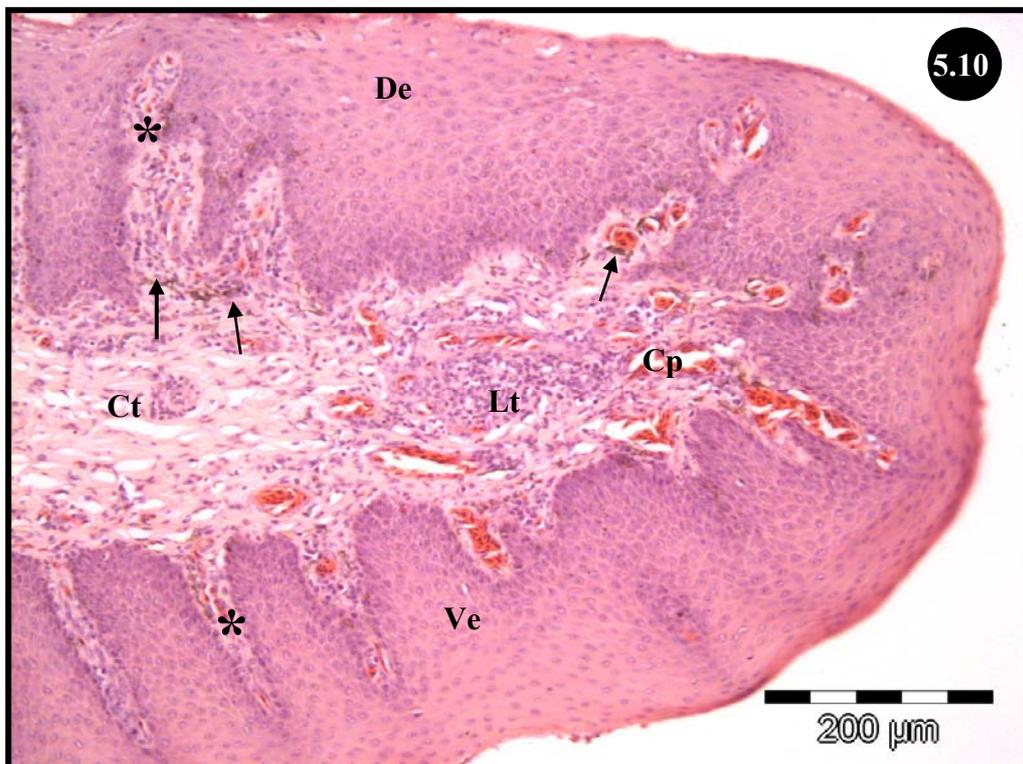


Figure 5.10: Longitudinal section of a lateral lingual papilla tip. Note the presence of a rich capillary plexus (Cp) and an aggregation of diffuse lymphoid tissue (Lt) within the supporting connective tissue (Ct). Deep connective tissue papillae carrying capillaries (*) penetrate the epithelium. Melanocytes (arrows), dorsal (De) and ventral epithelium (Ve).

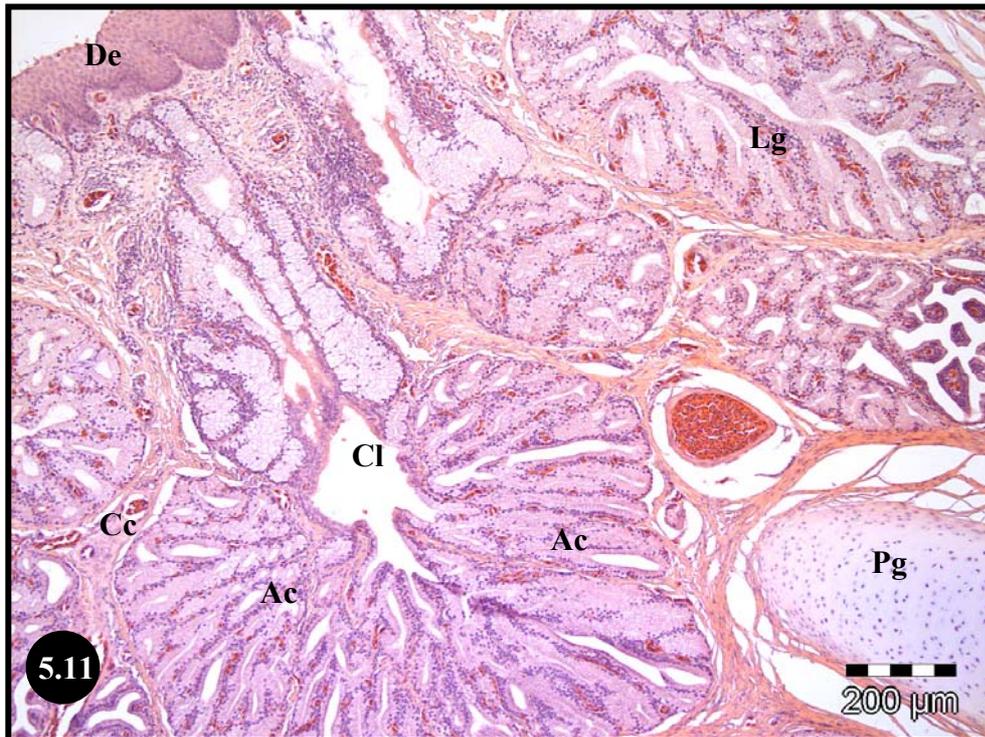


Figure 5.11: The typical structure of the large simple branched tubular mucus-secreting glands (Lg) in longitudinal section illustrating the numerous acini (Ac) which open into the central lumen (Cl). A connective tissue capsule (Cc) surrounds each gland. *Paraglossum* (Pg), dorsal epithelium (De).

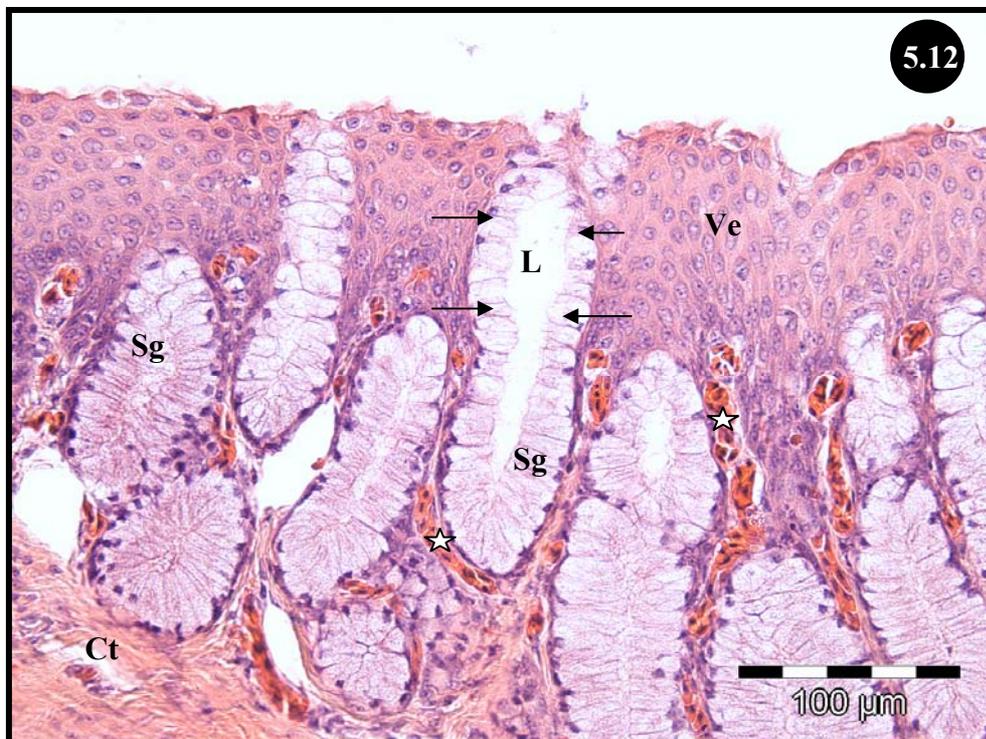


Figure 5.12: Tongue ventrum illustrating the small simple tubular mucus-secreting glands (Sg) opening onto this surface. The glands are seen in longitudinal section with much of their length restricted to the epithelial layer. The lumen (L) is lined by secretory cells (arrows). Capillaries (stars), connective tissue (Ct), ventral epithelium (Ve).

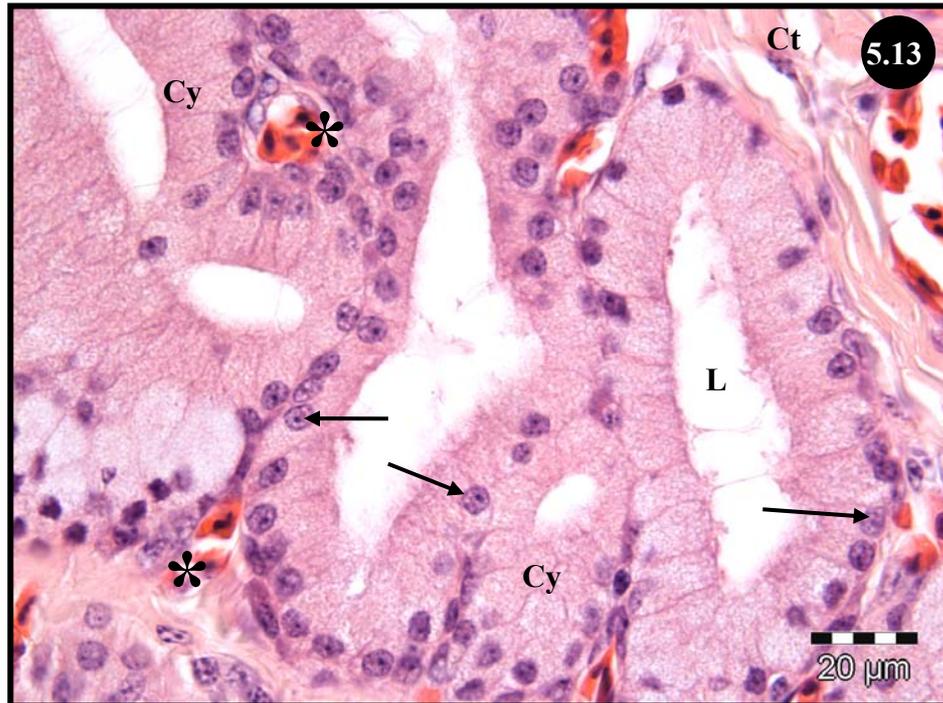


Figure 5.13: High magnification showing details of the acini of the large simple branched tubular mucus-secreting glands. The acini show typical properties of mucus-secreting cells, with a basal nucleus (arrows) and basophilic foamy cytoplasm (Cy). Lumen of acinus (L), capillaries (*), connective tissue (Ct).

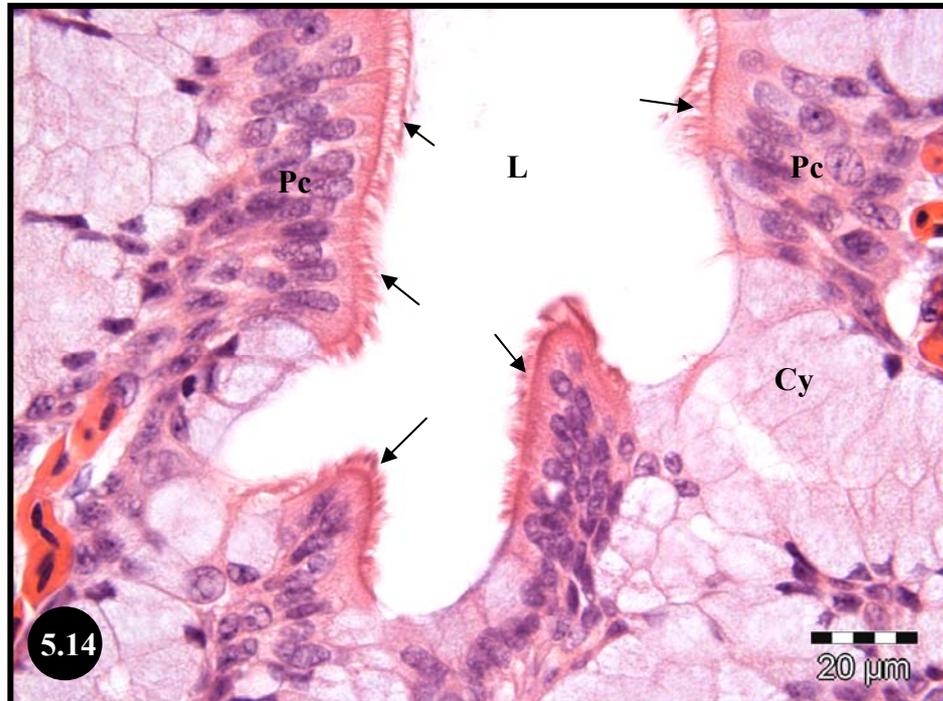


Figure 5.14: Pseudostratified ciliated columnar epithelium (Pc) lining part of the lumen (L) of a large simple branched tubular gland. Basophilic cytoplasm (Cy) of the adjacent mucus-secreting cells. Cilia (arrows).

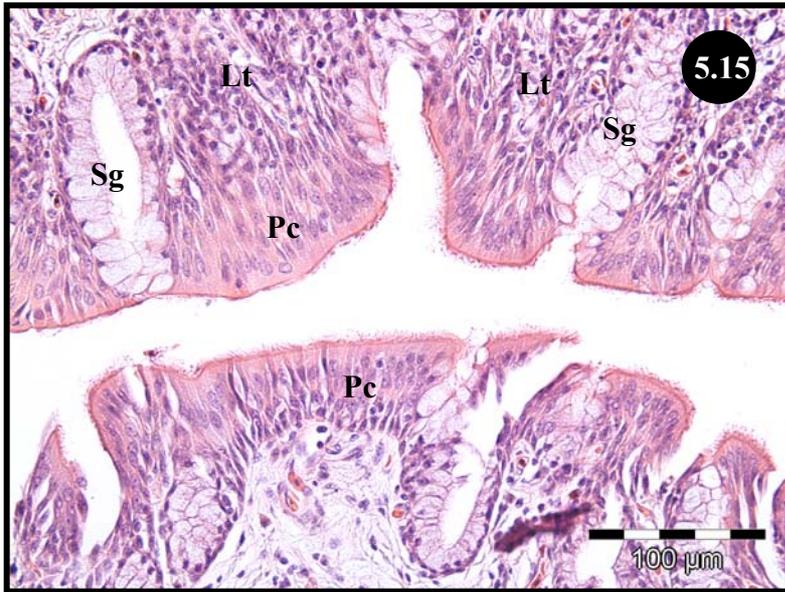


Figure 5.15: The folded ventrum of the tongue close to the frenulum. Note the ciliated pseudostratified columnar epithelium (Pc) and areas of diffuse lymphoid tissue (Lt). Simple tubular glands (Sg) are found in this region.

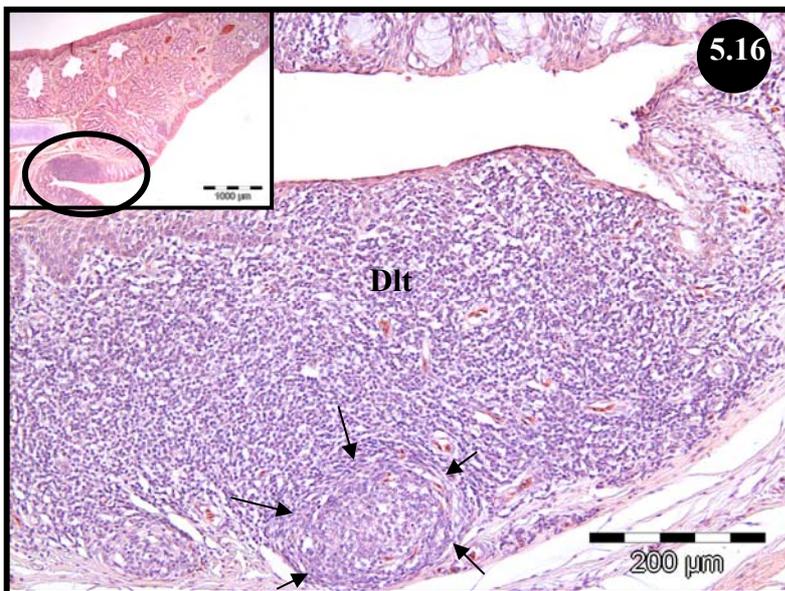


Figure 5.16: Junction of the tongue ventrum with the frenulum (inset) showing the large patch of diffuse lymphoid tissue (Dlt) consistently found in this region. Note the obliteration of the epithelial tissue by the lymphocytes and the nodular lymphoid tissue (arrows) situated at the base of the diffuse lymphoid tissue aggregation.

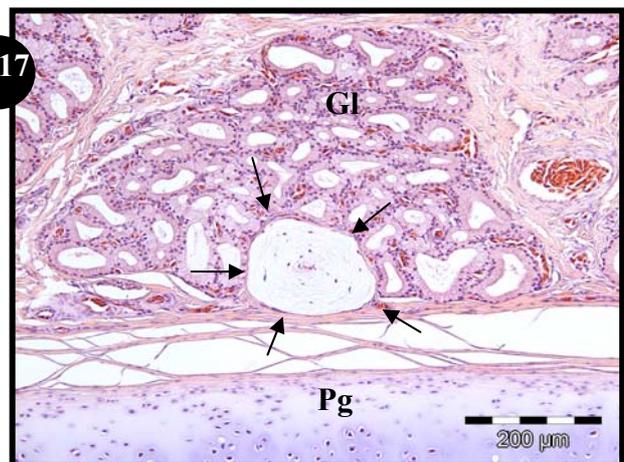
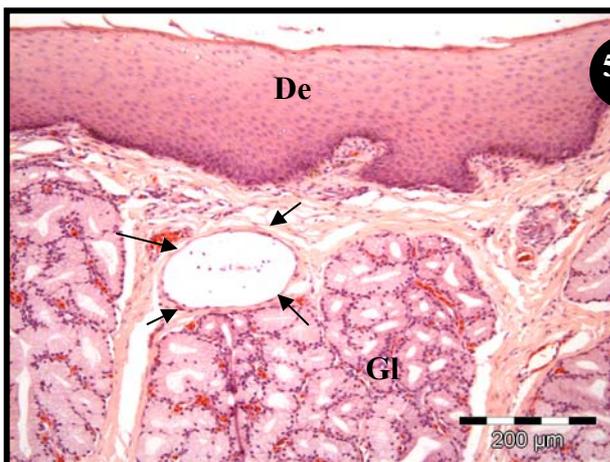


Figure 5.17: Dorsum of the tongue showing Herbst corpuscles (arrows) associated with the large simple branched tubular glands (Gl), one situated superficially just beneath below the dorsal epithelium (De) and one deeply positioned adjacent to the *paraglossum* (Pg).

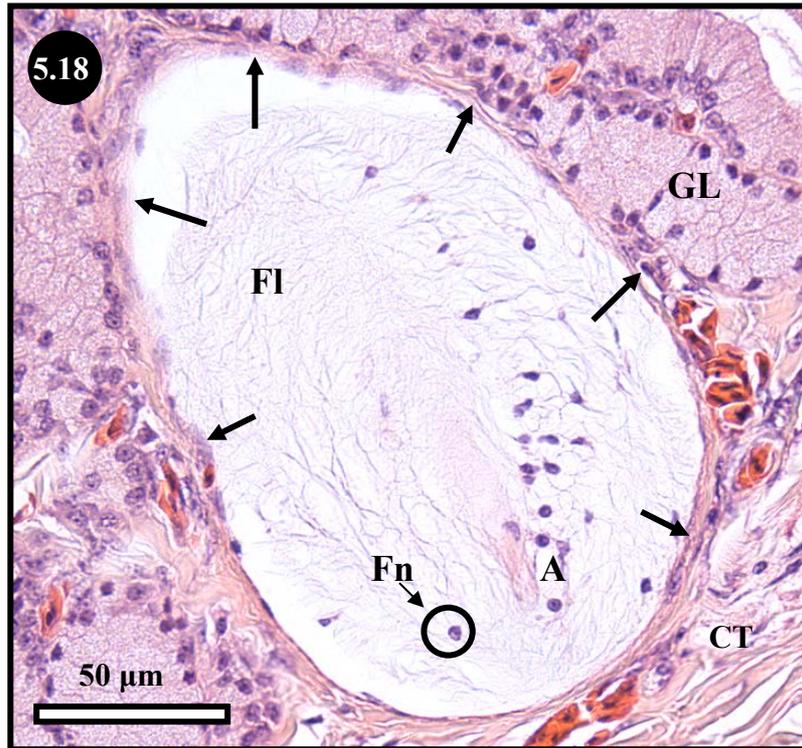


Figure 5.18: High magnification of a Herbst corpuscle showing the fibrous capsule (arrows) surrounding the outer core of fibrocytic lamellae (Fl) containing sparse fibrocytic nuclei (Fn). Central pink axon (A), glandular tissue (Gl), connective tissue (Ct).

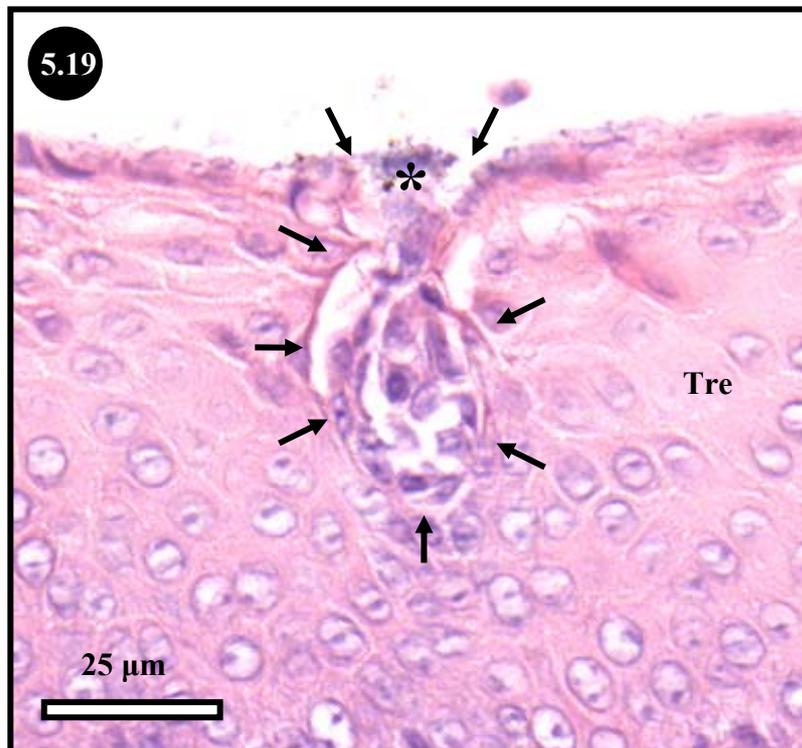


Figure 5.19: A structure resembling a taste bud observed on the tongue root close to the glottis. This structure is clearly demarcated (arrows) from the tongue root epithelium (Tre). Putative taste pore (*).

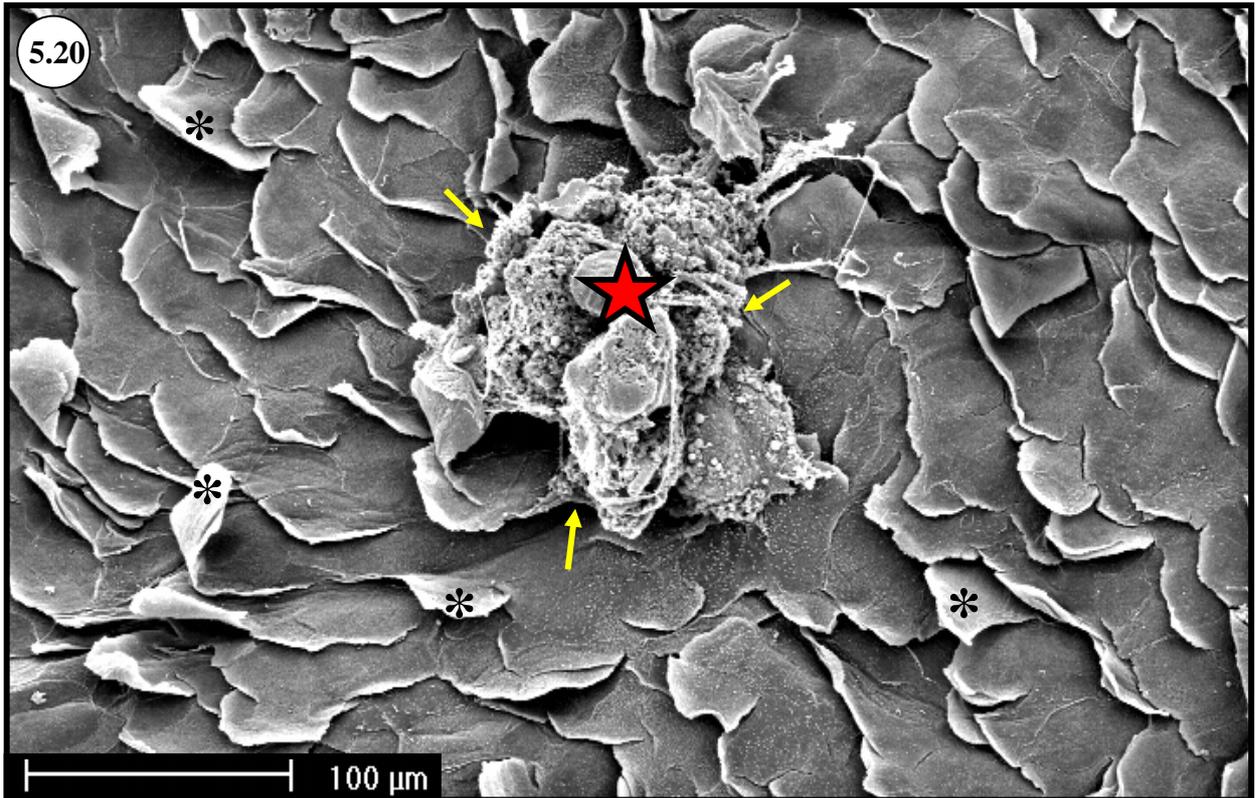


Figure 5.20: Dorsal tongue body demonstrating a large gland opening (yellow arrows) obscured by the mucus-secretion (red star) of the underlying gland. Note the individual desquamating surface cells (*) characteristic for this surface. x260.

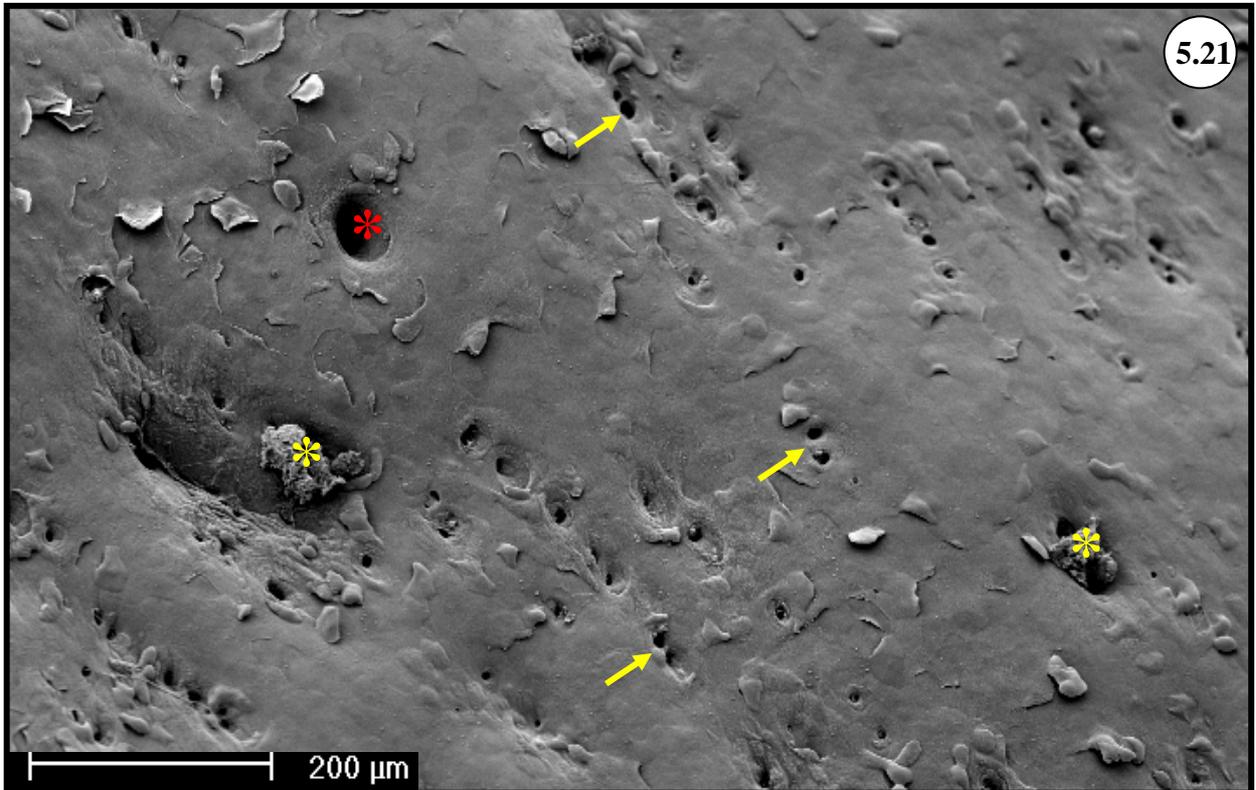


Figure 5.21: The caudo-lateral aspect of the ventral tongue body showing both large (red *) and small (arrows) openings. Mucus secretion (yellow *) is visible in some of the larger openings. Note the low frequency of desquamating surface cells. x120.

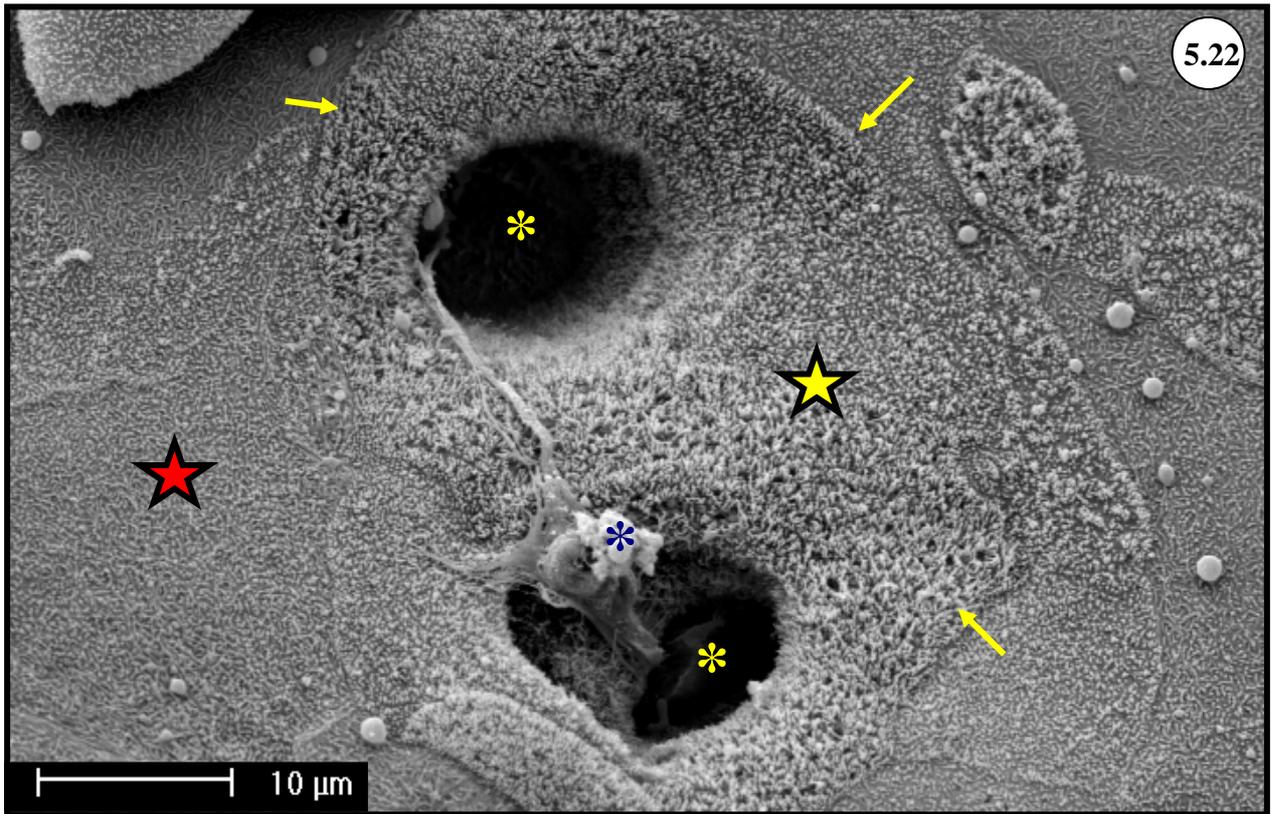


Figure 5.22: Caudo-lateral aspect of the ventral tongue body. Note that the cells around the small gland openings (yellow *) display dense microvilli (yellow star) on their surface. The transition between the ring of cells displaying microvilli and the surrounding cells with microplacae (red star) is abrupt (yellow arrows). Secreted mucus (blue *). x1925.

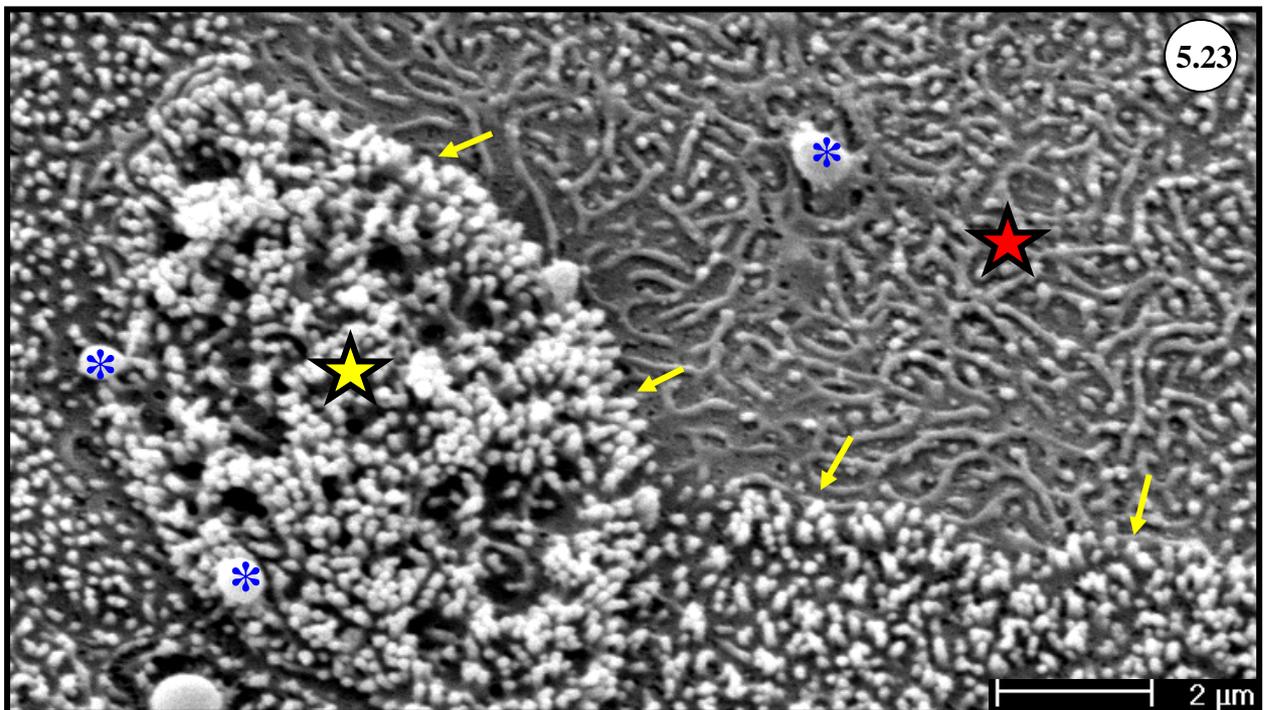


Figure 5.23: High magnification of the transition from microvilli (yellow star) to microplacae (red star) on the caudo-lateral aspect of the ventral tongue body. Note the abrupt transition (yellow arrows) as well as the presence of small globular structures (blue *) on the surface of both cell types. x7700.

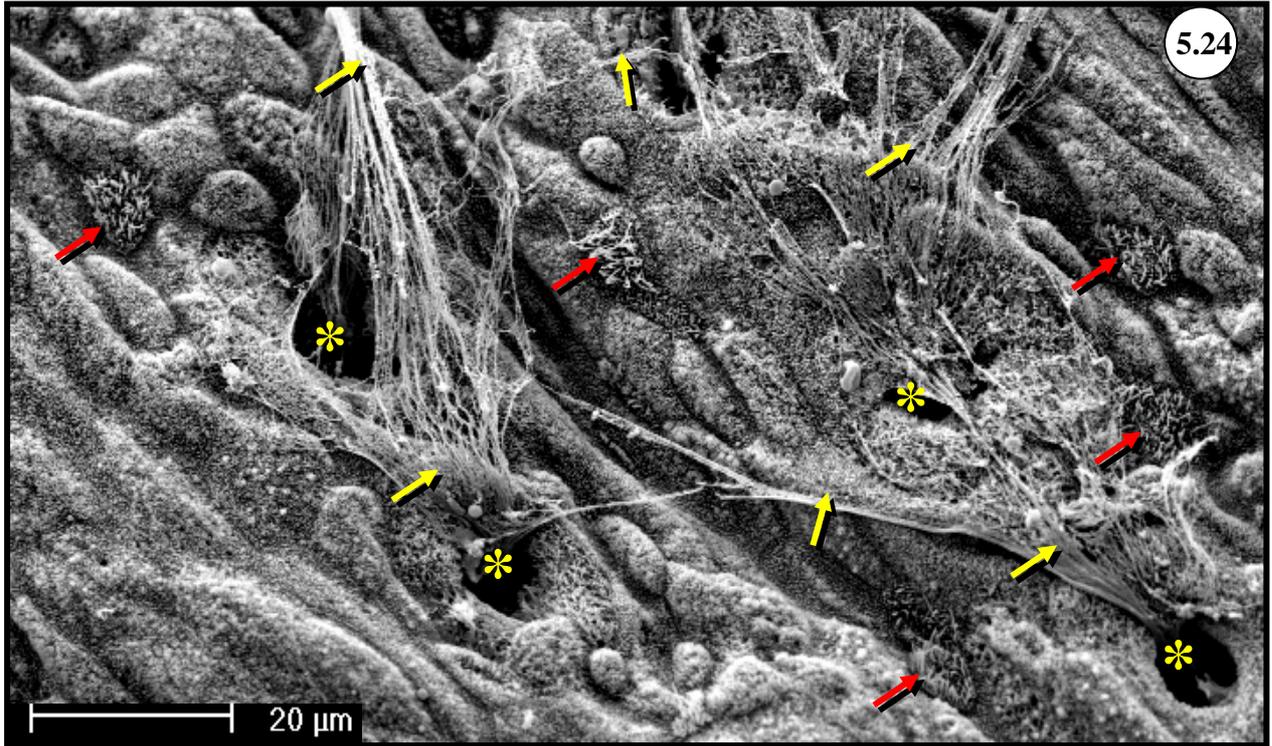


Figure 5.24: Mid tongue body ventrum. Numerous small openings (yellow *) showing strands of mucus secretion (yellow arrows) from the underlying glands are visible. All the surface cells of this region displayed densely-packed microvilli. Occasional ciliated cells (red arrows) also occurred in this region. x990.

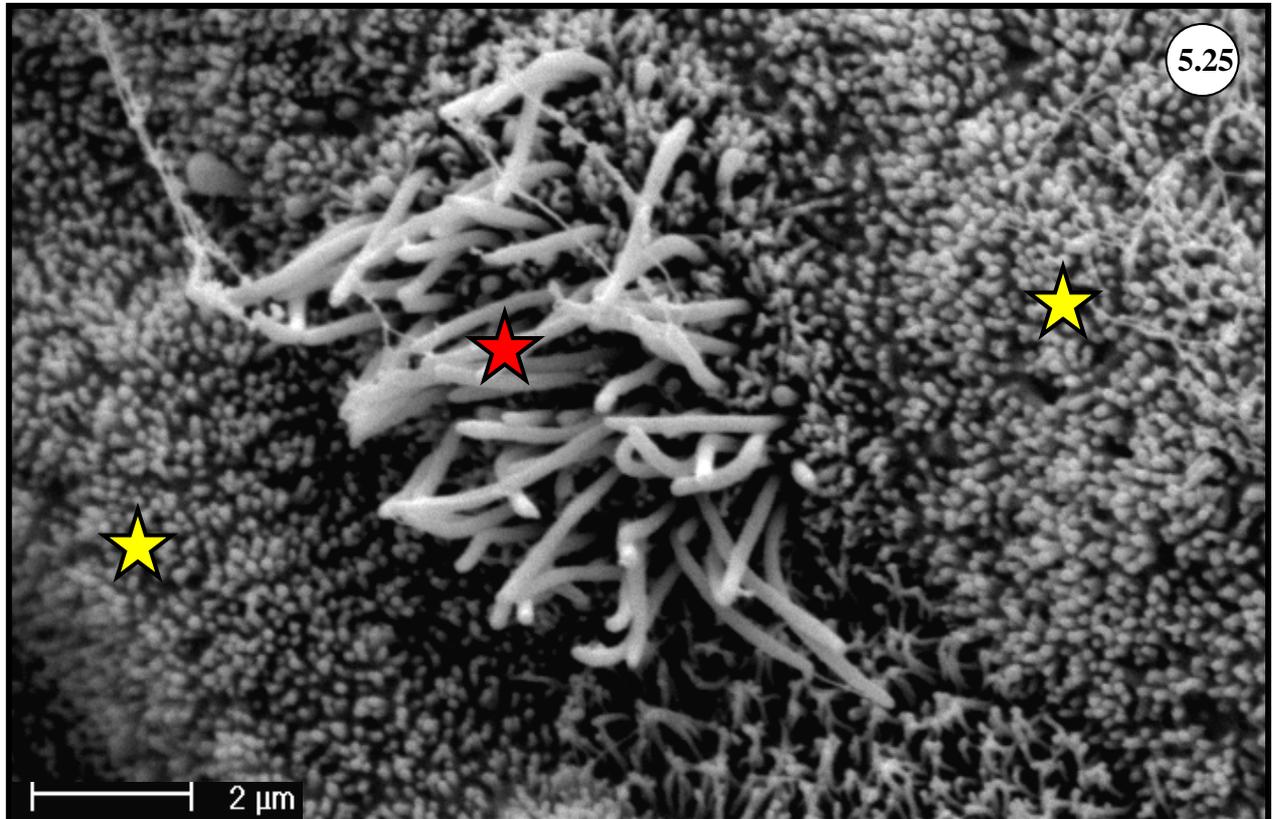


Figure 5.25: High magnification of a ciliated cell (red star) interposed between the cells displaying microvilli (yellow stars) on the ventrum of the mid tongue body. x7910.

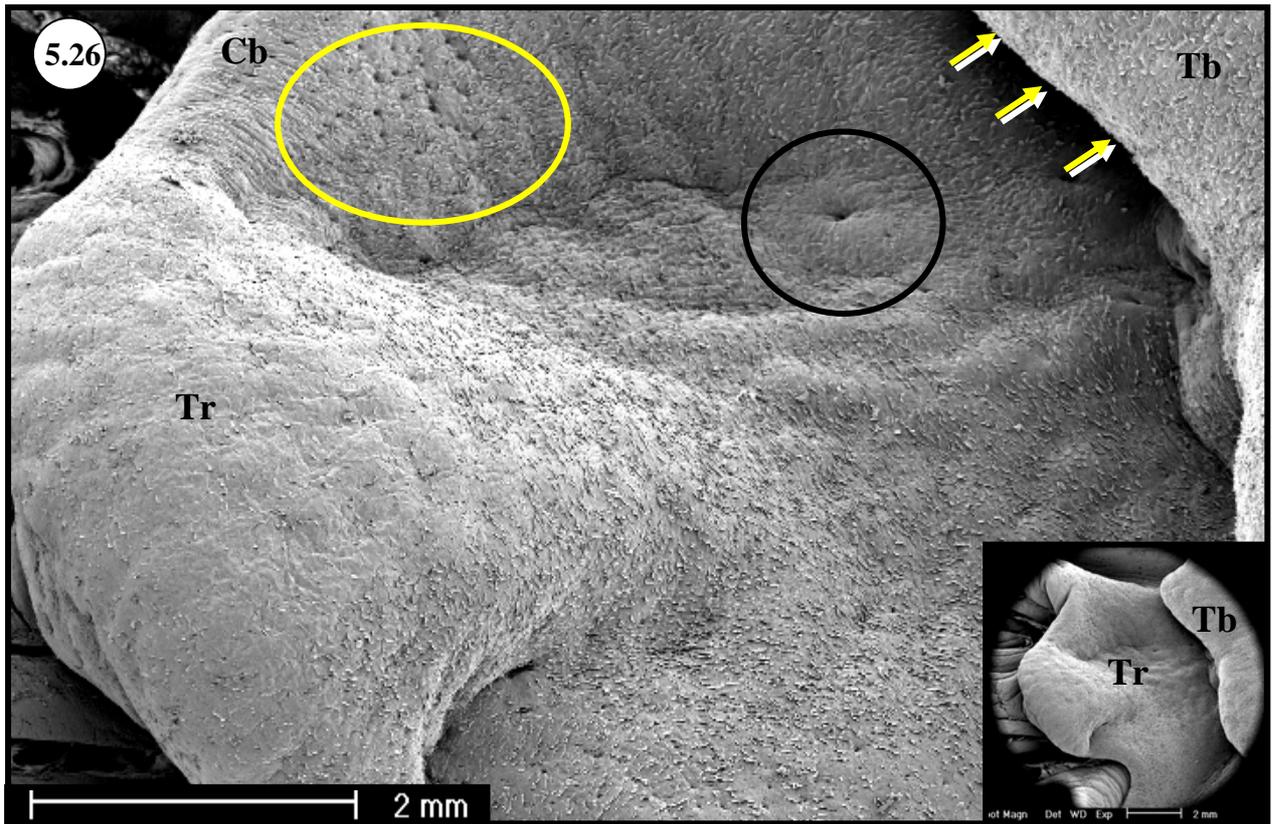


Figure 5.26: Low magnification of the dorsal tongue body (Tb) and tongue root (Tr). Note the flaky appearance of both surfaces due to the desquamation of individual surface cells and the large gland opening (black circle) in the mid tongue root and small gland openings (yellow circle) on the lateral edges and mucosa covering the underlying *ceratobranchiale* (Cb). Small retrolingual recess (yellow arrows). x16; inset x8.

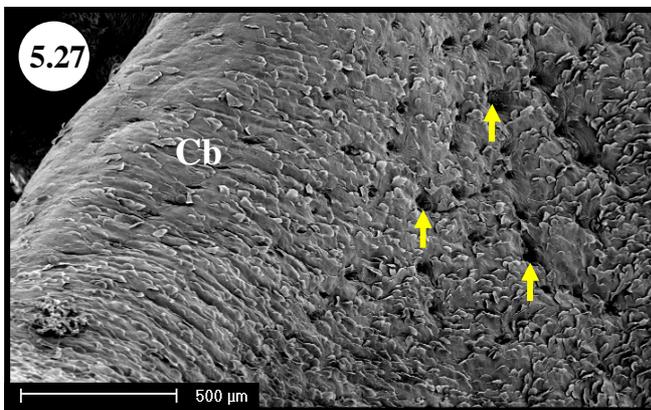


Figure 5.27: Enlargement of the yellow encircled area in Fig. 5.26 showing the numerous small gland openings (yellow arrows) on the lateral edge of the tongue root and mucosa covering the underlying *ceratobranchiale* (Cb). Note also the flaky appearance due to the desquamating surface cells. x60.

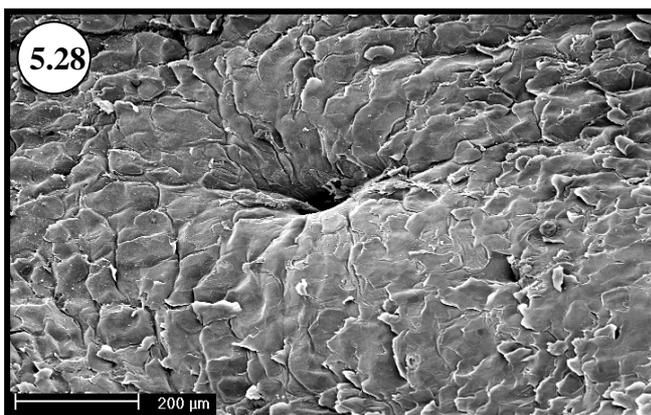


Figure 5.28: Enlargement of the black encircled area in Fig. 5.26 showing a large gland opening in the mid region of the tongue root. Note the raised edges around the opening and the vertical orientation of the cells forming the duct opening. x120.