



Morphology of the tongue of the emu (*Dromaius novaehollandiae*). II. Histological features

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ABSTRACT

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Although a number of brief, fragmented descriptions have been provided on the gross morphology of the ratite tongue, very few studies have documented the histological structure of this organ. This paper presents the first definitive histological description of the emu tongue and reviews, consolidates and compares the scattered information on the histology of the ratite tongue available in the literature. Five tongues were removed from heads obtained from birds at slaughter and fixed in 10 % neutral buffered formalin. Appropriate longitudinal and transverse segments were removed, routinely processed for light microscopy, and sections examined after staining with H & E and PAS. The entire tongue (body and root) is invested by a non-keratinized stratified squamous epithelium. The supporting connective tissue of the tongue dorsum displays only large, simple branched tubular mucus-secreting glands, whereas the caudal tongue body ventrum and tongue root, in addition to these glands, also exhibits small, simple tubular mucus-secreting glands. Herbst corpuscles are associated with the large, simple branched glands. Lymphoid tissue is restricted to the tongue ventrum and is particularly obvious at the junction of the ventral tongue body and frenulum where a large aggregation of diffuse lymphoid tissue, with nodular tissue proximally, was consistently observed. A structure resembling a taste bud was located in the epithelium on the caudal extremity of the tongue root of one bird. This is the first reported observation of taste buds in ratites. Forming the core of the tongue body is the cartilaginous *paraglossum* lying dorsal to the partially ossified rostral projection of the *basihyale*. The histological features of the emu tongue are generally similar to those described for the greater rhea and ostrich, except that taste buds were not identified in these species. The results would suggest that the emu tongue functions as a sensory organ, both for taste and touch (by virtue of taste receptors and Herbst corpuscles, respectively), as well as fulfilling an immunological function.

Keywords: *Dromaius novaehollandiae*, emu, histology, ratite, tongue

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, McFarland & Wilson 1967; Koch 1973; Hodges

1974; McLelland 1975; Nickel, Schummer & Seiferle 1977; Homberger & Meyers 1989; Gargiulo, Lorvik, Ceccarelli & Pedini 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 a description of the basic gross morphological features (Kobayashi, Kumakura, Yoshimura, Inatomi & Asami 1998; Jackowiak & Godynicki 2005; Jackowiak & Ludwig 2008; Tivane 2008). More special-

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ized studies on the tongue include those on the structure and secretions of salivary glands (Samar, Avila, De Fabro, Porfirio, Esteban, Pedrosa & Peinado 1999; Liman, Bayram & Koçak 2001; Al-Mansour & Jarrar 2004), and sensory structures, including taste buds (Botezat 1910; Moore & Elliott 1946; Lindenmaier & Kare 1959; Gentle 1971a, b; Berkhoudt 1985) and Herbst corpuscles (Berkhoudt 1979).

In contrast to the numerous gross morphological descriptions of the ratite tongue (Meckel 1829; Cuvier 1836; MacAlister 1864; Gadow 1879; Owen 1879; Pycraft 1900; Göppert 1903; Duerden 1912; Faraggiana 1933; Roach 1952; Feder 1972; McCann 1973; Cho, Brown & Anderson 1984; Fowler 1991; Bonga Tomlinson 2000; Gussekloo & Bout 2005; Porchescu 2007; Crole & Soley 2008, 2009; Jackowiak & Ludwig 2008; Tivane 2008), very little information is currently available on the histology of this organ in ratites. The only studies documenting the histology of ratite tongues are those of Feder (1972) for the greater rhea (*Rhea americana*), Porchescu (2007), Jackowiak & Ludwig (2008) and Tivane (2008) for the ostrich (*Struthio camelus*), whereas Crole & Soley (2008) briefly outlined the salient features of the emu (*Dromaius novaehollandiae*) tongue observed by light microscopy.

This study presents the first definitive histological 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.

MATERIALS AND METHODS

The heads of five 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 % neutral buffered formalin. They were allowed to fix for approximately 4 h while being transported to the laboratory, after which they were immersed in fresh fixative for a minimum period of 48 h. Care was taken to exclude air from the oropharynx by wedging a small block of wood in the beak.

Each tongue (body and root) was removed from the head by incising the tissues peripheral to it, including the frenulum, and cutting through the ceratobranchials, to free it from the oropharyngeal floor. The tongue was cut into appropriate longitudinal

and transverse sections to represent its body and root, 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 min 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 (PAS) stain (McManus 1946), and viewed and micrographed using an Olympus BX50 equipped with the analySIS CC12 Soft Imaging System (Olympus, Japan).

The terminology used in this study is that of Nomina Anatomica Avium (Baumel, King, Breazile, Evans & Vanden Berge 1993).

RESULTS

Tongue body

Macroscopically, the tongue consists of a pigmented, triangular, dorsoventrally flattened body, bearing lateral and caudal lingual papillae as well as a variably pigmented, triangular root with its caudal extremity projecting into the laryngeal entrance (glottis) (Crole & Soley 2009).

Histologically, the tongue body consists of an epithelial lining, a wide connective tissue layer (lingual submucosa) which contains glands, lymphoid tissue, Herbst corpuscles, blood vessels and nerves, and a core which is formed by the lingual skeleton (Fig. 1, 10 and 11) and associated striated muscle (Fig. 1 and 11). Both the dorsal and ventral surfaces of the tongue are invested by a non-keratinized stratified squamous epithelium (*Epithelium stratificatum squamosum*) (Fig. 3, 4 and 6). The dorsal epithelium is marginally thicker than the ventral epithelium (Fig. 3), displays a lower frequency of connective tissue papillae and contains melanocytes.

The *Stratum basale* of the tongue body dorsum consists of a single, compact layer of low columnar cells with vertically oriented nuclei. Interspersed between the epithelial cells are numerous melanocytes from which pigment-containing dendritic processes project into the overlying *Stratum spinosum*. In the lateral lingual papillae the melanocytes are situated at the tips in the *Stratum basale* and underlying connective tissue of both the dorsal and ventral surfaces. The *Stratum spinosum* is composed of a variable number of layers of polygonal cells. These cells typically contain a large, round, centrally posi-

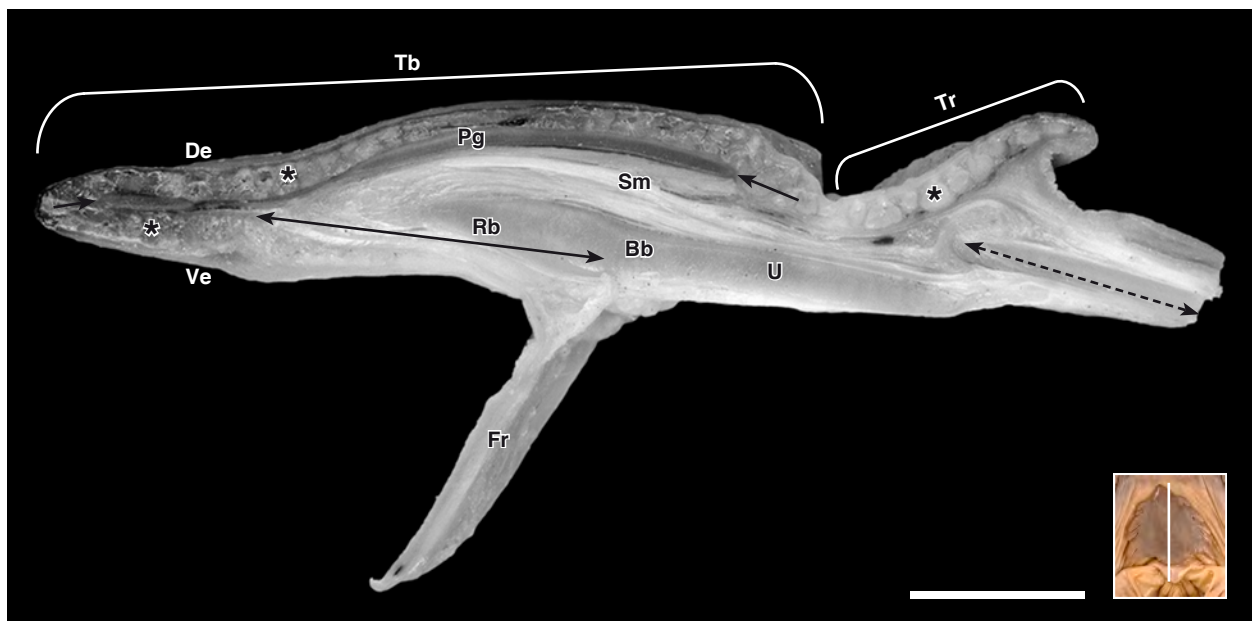


FIG. 1 Mid-sagittal section through the emu tongue illustrating the long rostral body (Tb), caudal root (Tr) and ventrally positioned frenulum (Fr). The core of the rostral tongue body is formed by the *paraglossum* (Pg, extremities indicated by the arrows) and the rostral projection of the *basihyale* (Rb). (The solid double-headed arrow indicates the actual extent of the Rb). Note the continuity between the rostral projection of the *basihyale*, the body of the *basihyale* (Bb) and the *urohyale* (U) in the caudal tongue body. Large, simple branched tubular mucus-secreting glands (*) are visible macroscopically as pear-shaped or round structures in the tongue body and root. Skeletal muscle fibres (Sm) attach to the ventral aspect of the *paraglossum*. Dorsal epithelium (De), ventral epithelium (Ve), cricoid cartilage (dotted double-headed arrow). Bar = 5 mm

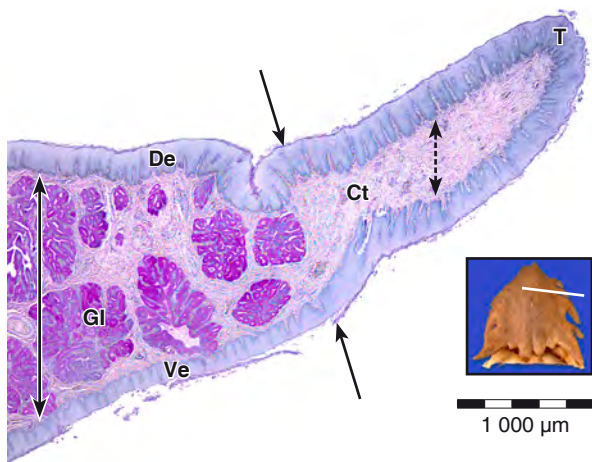


FIG. 2 Lateral lingual papilla in longitudinal section with the glandular tissue showing a positive PAS reaction. Note the abrupt termination (arrows) of the glands (Gl) leaving only connective tissue (Ct) filling the space between the dorsal (De) and ventral epithelium (Ve). The thinning of the connective tissue layer from medial (solid double-headed arrow) to lateral (dotted double-headed arrow) is also apparent. Papilla tip (T)

tioned nucleus and are separated from neighbouring cells by a relatively wide intercellular space spanned by numerous interconnected cytoplasmic processes. Nucleoli are particularly prominent in the cells of the *Stratum spinosum*. The more superficial cells of this layer are observed to flatten and assume a horizontal orientation. The nuclei are similarly flattened, pale in appearance and display a prominent mass of heterochromatin which is generally associated with the nuclear membrane. These cells constitute the origin of the *Stratum corneum* which is composed of a var-

iable number of nucleated cell layers which stretch to the epithelial surface. The cells of this layer are compactly arranged and display a substantial degree of surface sloughing. The dorsal epithelium is interrupted at regular intervals by the ducts of large, simple branched tubular mucus-secreting glands (Fig. 4) (see below) situated in the underlying connective tissue.

The epithelium of the tongue ventrum (Fig. 6) is similar in composition to that of the dorsum except for

the obvious absence of melanocytes. The *Stratum corneum* is poorly developed in some areas, displaying rounded cells more typical of the *Stratum spinosum* stretching to the epithelial surface. Isolated patches of ciliated columnar cells are confined to this aspect of the tongue and, when observed on the epithelial surface, are often associated with aggregations of lymphoid tissue and/or gland openings. The mucosa at the junction between the tongue ventrum and frenulum exhibits folds (Fig. 9). In some instances the ventral epithelium is obliterated by large aggregations of lymphoid tissue emanating from the underlying connective tissue layer. In contrast to the dorsum, the epithelium of the tongue ventrum is interrupted by the ducts of both large, simple branched tubular mucus-secreting glands (Fig. 9) and small, simple tubular mucus-secreting glands (Fig. 6). The latter are limited to the caudal region of the tongue ventrum and appear to be concentrated at the junction with the frenulum.

Underlying the epithelium on all aspects of the tongue surface is a dense, irregular fibrous connective tissue layer, the lingual submucosa (*Tela submucosa linguae*) that stretches from the base of the epithelium to the lingual skeleton and associated striated muscle. It is thickest at the centre of the dorsal tongue body and tapers towards the margins (Fig. 2). This tissue penetrates the epithelial layer in the form of connective tissue papillae which are richly supplied with capillaries (Fig. 3 and 4). Melanocytes are heavily concentrated around these capillaries.

The lingual submucosa is dominated by the presence of large, simple branched tubular mucus-secreting glands (*Glandulae linguales*) (Fig. 1, 2, 4, 7, 9, 10 and 11) that occupy the full width of the layer, being absent only from the tips of the lateral lingual papillae (Fig. 2), except for the most caudal ones, and end abruptly where the tongue body merges with the frenulum. These structures present oblong, round, oval or pear-shaped profiles (Fig. 1, 2, 4, 7, 9, 10 and 11). The glands account for the bulk of the tongue parenchyma (Fig. 1, 9, 10 and 11) and vary in size with the largest and most branched being found near the midline where the connective tissue layer is the thickest. Each gland is surrounded by a condensed layer of connective tissue resulting in the formation of distinct glandular units. Numerous fine septa radiate from the containing fibrous layer to separate the individual tubular secretory acini. The septa are richly supplied with capillaries. The secretory acini empty into a large central lumen which in some glands is clearly lined by a pseudostratified ciliated columnar (Fig. 5) or simple ciliated columnar

epithelium. The lumen narrows as it passes through the epithelium (Fig. 4), forming the secretory duct. This duct is lined by a single layer of vertically oriented squamous cells continuous with the surface layer of the epithelium, although in some instances a ciliated columnar epithelium is observed along part of the duct.

The glandular acini display varying degrees of secretory activity. Active acini are lined by typical mucus-secreting cells with basally-positioned round vesicular, or dark, flattened nuclei (Fig. 5). The ample apical cytoplasm is filled with a granular, lightly basophilic material that demonstrates a positive PAS reaction (Fig. 2 and 10). Inactive acini are composed of cuboidal cells displaying a smaller amount of darker staining cytoplasm surrounding a round, centrally positioned nucleus. The released mucus is visible in the lumen of some acini and in the central lumen as wispy, stringy accumulations of blue-purple material. The glandular units represent the doughnut-shaped structures seen macroscopically (Crole & Soley 2009), 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 caudal aspect of the tongue ventrum also displays numerous small, simple tubular mucus-secreting glands (Fig. 6 and 9). These glands are partly intra-epithelial in location, extend for only a short distance into the underlying connective tissue and are composed of cells with similar features to those lining the active acini in the larger branched glands. The gland lumen is narrower than that of the larger glands and the portion traversing the epithelium is lined by mucus-secreting cells (Fig. 6). Simple tubular glands, in addition to the large, simple branched tubular glands, are also absent from the tips of the lateral lingual papillae.

Specialized sensory nerve endings in the form of Herbst corpuscles (*Corpusculum lamellosum avium*) (Fig. 7 and 8) are also a common feature of the connective tissue layer. These large, pale lamellated bodies occur singly, are randomly distributed and are closely associated with the large, simple branched tubular glands, although always separated from them by an intervening layer of connective tissue. The distribution of the corpuscles varies, with some being positioned just beneath the epithelium (superficial) (Fig. 8) and others abutting the lingual skeleton (deep) (Fig. 7). They exhibit round or oval profiles, although irregular forms are also observed, and display morphological features typical of Pacinian and Herbst corpuscles. The neural component (nerve

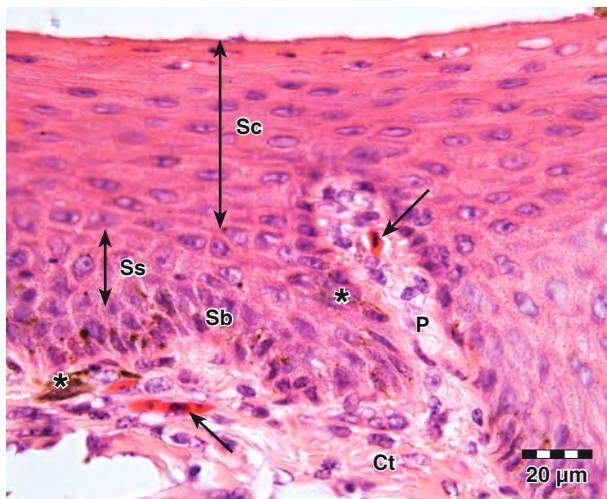


FIG. 3 The non-keratinized stratified squamous epithelium of the tongue dorsum displaying the *Str. basale* (Sb) with melanocytes (*) some of which lie in the connective tissue beneath the *Str. basale*, *Str. spinosum* (Ss) and *Str. corneum* (Sc). Connective tissue (Ct), connective tissue papilla (P), capillary (arrows)

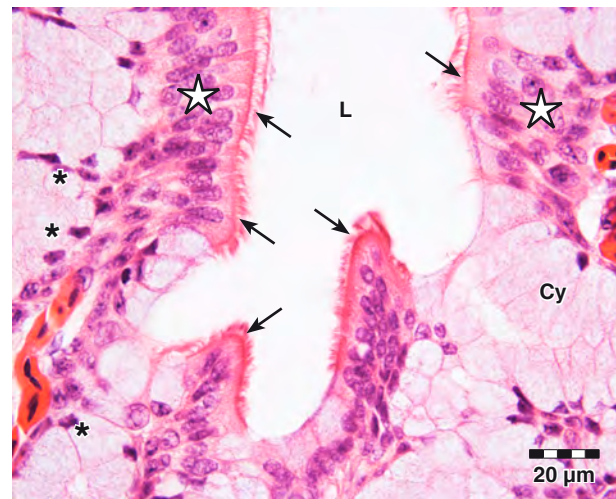


FIG. 5 Pseudostratified ciliated columnar epithelium (white stars) lining part of the lumen (L) of a large, simple branched tubular gland. Note the basophilic cytoplasm (Cy) of the adjacent mucus-secreting cells and their basally-positioned, dark flattened nuclei (*). Cilia (arrows)



FIG. 4 Low magnification of the tongue dorsum showing the duct of a large, simple branched tubular gland (Lg) passing through the epithelium (De). Lumen (L) narrowing through the epithelium, connective tissue (Ct), connective tissue papillae (*), large blood vessel (white star)

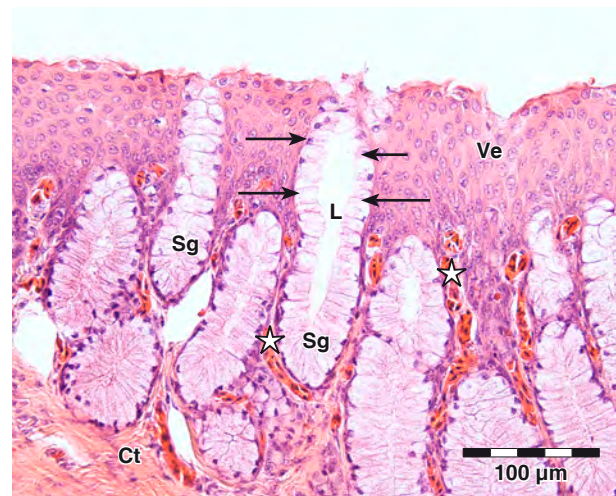


FIG. 6 Caudal aspect of the tongue ventrum illustrating the small, simple tubular mucus-secreting glands (Sg) opening onto this surface. The glands are seen in longitudinal section with much of their length restricted to the epithelial layer. The lumen (L) is lined by secretory cells (arrows). Capillaries (stars), connective tissue (Ct), ventral epithelium (Ve)

terminal/axon) of the corpuscle is centrally situated (Fig. 8) and surrounded by a series of closely apposed lamellae forming a distinct zone, the inner core. This zone is also characterized by the presence of a number of Schwann cell nuclei. Surrounding the inner core is a series of loosely arranged, concentric lamellae (fibrocytic lamellae) separated by obvious spaces (Fig. 8). This region (the outer core) forms the bulk of the tissue surrounding the neuronal component and displays relatively few nu-

clei (Fig. 8). The entire corpuscle is closely invested by a capsule (Fig. 8) formed by a thin, fibrous connective tissue layer displaying numerous fibroblast nuclei.

Lymphoid tissue in the tongue body is confined to the ventrum where it generally occurs as large diffuse accumulations situated immediately beneath the epithelium (Fig. 9). The larger aggregations are associated with the glandular tissue whereas small-

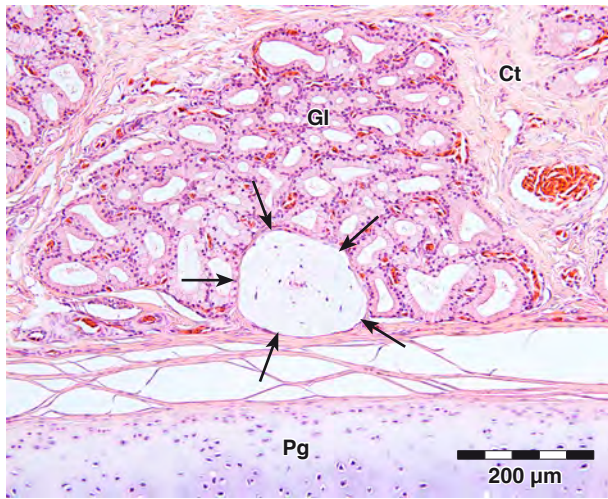


FIG. 7 Dorsum of the tongue showing a Herbst corpuscle (arrows) deeply positioned adjacent to the *paraglossum* (Pg). The corpuscle appears in cross-section and is closely associated with a large, simple branched tubular gland (Gl). Connective tissue (Ct)

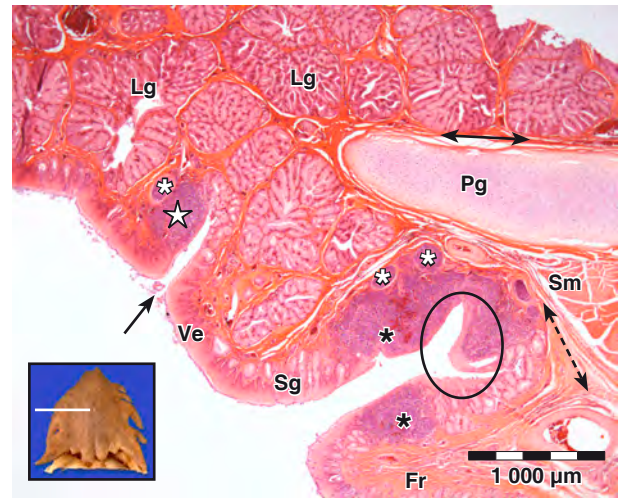


FIG. 9 Cross-section of the lateral tongue body and papillae base demonstrating large, simple branched tubular glands (Lg). Note the simple tubular glands (Sg) and diffuse (black *) and nodular (white *) lymphoid tissue exclusively present on the tongue ventrum. *Paraglossum* (Pg), skeletal muscle (Sm), ventral epithelium (Ve), mucosal folds of ventrum at frenular junction (en-circled), compressed submucosa surrounding the *paraglossum* (double-headed arrow) and skeletal muscle (dotted double-headed arrow), frenulum (Fr), large gland opening (arrow) with lymphoid tissue invading the glandular tissue (white star)

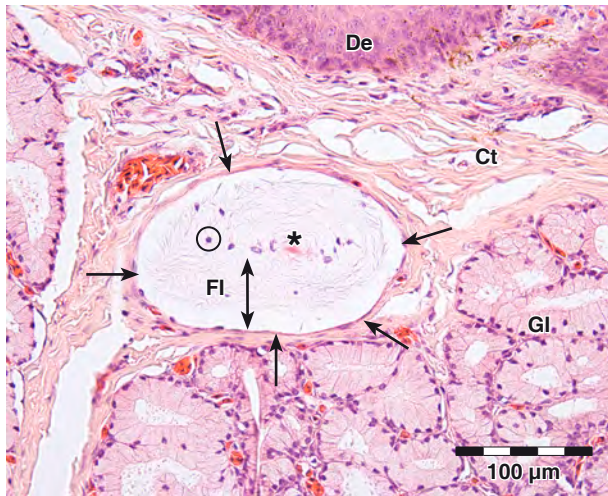


FIG. 8 Dorsum of the tongue showing a Herbst corpuscle (arrows) situated superficially just beneath the dorsal epithelium (De). The corpuscle appears in cross-section and is associated with a large, simple branched tubular gland (Gl). The fibrous capsule (arrows) surrounding the outer core of fibrocytic lamellae (Fl) contains sparse fibrocytic nuclei (encircled). Central axon (*), connective tissue (Ct)

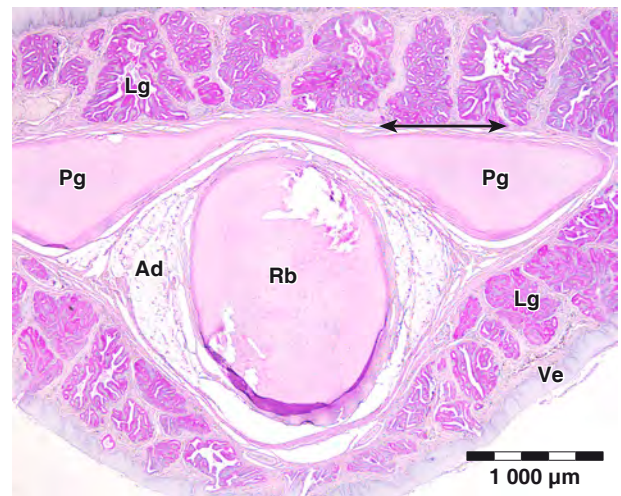


FIG. 10 Cross section of the middle of the tongue body showing the topography of the lingual skeleton within the parenchyma. The *paraglossum* (Pg) lies dorsal to the rostral projection of the *basihyale* (Rb) which is flanked by adipose tissue (Ad). Large, simple branched tubular glands (Lg), ventral epithelium (Ve), compressed submucosa (double-headed arrow). PAS stain

er isolated patches occur throughout the connective tissue layer and also in the tips of the lateral lingual papillae. The large aggregations are sometimes confined to the connective tissue but are also ob-

served to penetrate the epithelium, obliterating the normal structure of this layer. Infiltration of the glandular tissue, particularly in the vicinity of the lumen and secretory duct, is also obvious (Fig. 9). Nodular

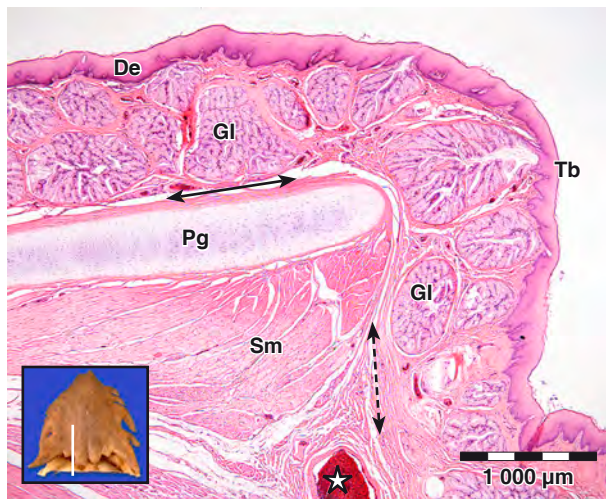


FIG. 11 Longitudinal section of the caudal tongue body. The *paraglossum* (Pg) forms the core between the connective tissue layer (lingual submucosa) filled with large, simple branched tubular glands (Gl). Note the large amount of skeletal muscle (Sm) attaching at the base of the *paraglossum*. Tongue base (Tb), dorsal epithelium (De), compressed submucosa dorsal to the *paraglossum* (double-headed arrow) and surrounding the skeletal muscle (dotted double-headed arrow), large blood vessel (white star)

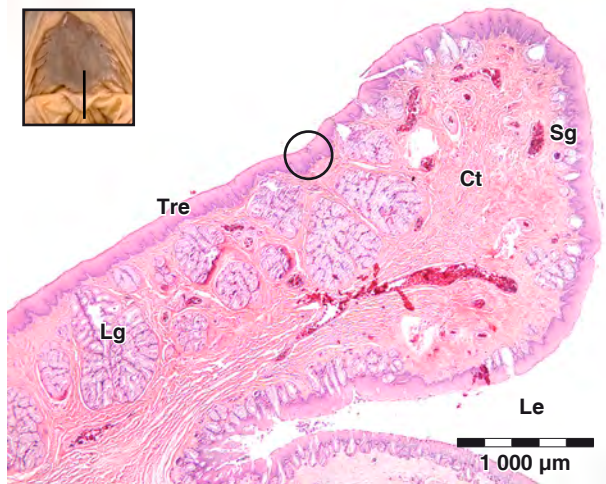


FIG. 12 Median longitudinal section of the tongue root depicting small, simple tubular glands (Sg), and large, simple branched tubular glands (Lg). Connective tissue (Ct), laryngeal entrance (Le), tongue root epithelium (Tre), encircled area is enlarged in Fig. 13

lymphatic tissue in the form of lymphoid follicles is present within some of the diffuse accumulations (Fig. 9). The follicles are always positioned toward the deeper aspect of the aggregations.

The deeper region of the lingual submucosa is compressed into a narrow conspicuous layer between the base of the large, simple branched tubular glands and the perichondrium of the lingual skele-



FIG. 13 Enlargement of the area encircled in Fig. 12 showing 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) and is comprised of vertically oriented cells. Putative taste pore (star). Bar = 25 µm

ton or the perimysium of the associated skeletal muscle bundles (Fig. 9, 10 and 11). This layer displays large blood vessels (Fig. 4 and 11) and nerves from which smaller subdivisions radiate between the glandular tissues. Melanocytes are concentrated around the large blood vessels on the dorsum of the tongue body.

The core of the tongue body is formed by the lingual skeleton which comprises the rostral projection and body of the *basihyale*, the rostral aspect of the *urohyale* (Fig. 1 and 10) and the *paraglossum* (Fig. 1, 9, 10 and 11). The rostral projection of the *basihyale* is situated ventral to the *paraglossum* (Fig. 10). It is round in cross-section, composed of hyaline cartilage and invested by a thin perichondrium flanked by adipose tissue (Fig. 10). The caudal aspect shows signs of ossification. The *paraglossum* is dorsoventrally flattened (Fig. 1, 9, 10 and 11) and thins where it lies above the rostral projection of the *basihyale*, giving it a butterfly appearance in cross-section (Fig. 10). It is also composed of hyaline cartilage and surrounded by a delicate perichondrium.

Skeletal muscle fibres (*Musculi linguae*) are observed ventral to the *paraglossum* (Fig. 1 and 11). The fibres are grouped into fascicles which in turn form muscle bundles (which would represent the intrinsic hyolingual muscles described by Bonga Tomlinson (2000)) that run rostrally from the base of the *paraglossum* (Fig. 11) on either side of the rostral projection of the *basihyale* to end rostral to the mid-ventral aspect of the *paraglossum*. The muscle

bundles are attached along their length to the ventral aspect of the *paraglossum* through merging of the respective perimysium and perichondrium, and taper in a caudo-rostral direction; they can be seen macroscopically (Fig. 1).

Tongue root

The epithelium covering the tongue root displays similar features to that of the ventrum of the tongue body. The underlying connective tissue is similar to that of the tongue body, but is slightly less densely packed. Both types of glands are present and similar in structure to those of the tongue body. The large, simple branched tubular mucus-secreting glands are concentrated mainly in the midline of the tongue root (Fig. 1 and 12) and are more loosely spaced than those of the tongue body. These glands form the faint doughnut-shaped structures seen macroscopically in this region (Crole & Soley 2009). The small, simple tubular mucus-secreting glands are scattered over the rest of the area and concentrated on the caudally pointing tongue root tip (Fig. 12) and on the lateral edges of the tongue root. Melanocytes are present only in those specimens that display a pigmented tongue root, and when observed, are restricted to the caudal tongue root tip. Occasional small diffuse lymphoid aggregations are present in the underlying connective tissue. Herbst corpuscles occur in very low numbers and are associated with the larger glands in similar fashion to that noted in the tongue body. There is no core formed by the lingual skeleton, and muscular tissue is only present below the connective tissue on the lateral edges.

In one specimen an epithelial modification with features similar to those of a taste bud (*Caliculus gustatorius*) was observed on the tongue root close to the glottis (Fig. 12). It was an isolated structure, clearly demarcated from the surrounding epithelial tissue, oval in shape, and consisted of a group of elongated, vertically oriented cells apparently opening into a central pore (Fig. 13). 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. 13).

Frenulum

The epithelial covering of the frenulum shows similar characteristics to those of the ventrum of the tongue body with which it is continuous and typically does not reveal melanocytes. Only simple tubular mucus-secreting glands are present. The frenulum

reveals a core of loosely arranged dense irregular connective tissue containing large blood vessels and non-medullated nerves. Large aggregations of diffuse lymphoid tissue similar to those observed on the tongue ventrum are consistently present in the folded tissue at the junction of the ventrum of the tongue body and the frenulum (Fig. 9).

DISCUSSION

General features of the tongue body

Although the dorsal and ventral surfaces of the emu tongue appear similar macroscopically, it is possible to distinguish the two surfaces histologically. The dorsum contains melanocytes and has only large, simple branched tubular mucus-secreting glands penetrating the epithelium. 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, simple branched tubular and small, simple tubular mucus-secreting glands. A noteworthy observation is that, histologically, the entire tongue ventrum lacks melanocytes, yet macroscopically it appears lightly pigmented. No such histological differentiation was noted for the dorsum and ventrum of the tongue body in the greater rhea (Feder 1972) or ostrich (Jackowiak & Ludwig 2008; Tivane 2008).

The connective tissue papillae penetrating the dorsal and ventral epithelium in the emu tongue typically carried capillaries deep within the epithelium and were structurally similar to those described in the ostrich (Tivane 2008). Feder (1972) reported capillaries looping up to half the distance of the epithelium of the greater rhea tongue, a feature also noted in the emu (present study).

Epithelium

As previously noted (Crole & Soley 2008), the stratified squamous epithelium covering all aspects of the emu tongue was non-keratinized, a feature also reported in the greater rhea (Feder 1972) and ostrich (Porchescu 2007; Jackowiak & Ludwig 2008; Tivane 2008). This contrasts with the general statement that the tongue of most birds displays a keratinized epithelium (Iwasaki 2002) as illustrated, for example, in penguins (*Spheniscus demersus*, *Spheniscus humboldti*, *Pygoscelis papua*, *Eudyptes chrysolophus*) (Kobayashi *et al.* 1998), white-cheeked bulbul (*Pycnonotus leucogenys*) (Al-Mansour & Jarrar 2004) and various domestic species (Koch 1973;

Hodges 1974; McLelland 1975). It has also been reported that in some birds (Warner *et al.* 1967; Jackowiak & Godynicki 2005) the tongue ventrum is keratinized while the dorsum is non-keratinized.

In the emu the dorsal epithelium was observed to be thicker than that of the ventral epithelium, a feature also noted in the ostrich (Jackowiak & 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 (*Coturnix coturnix japonica*) (Warner *et al.* 1967). A reason for this phenomenon may be found in the feeding method of palaeognaths (Bonga Tomlinson 2000; Gussekloo & Bout 2005) where the tongue is not involved in food manipulation and the surface would therefore require less mechanical protection.

An interesting finding on the tongue ventrum 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 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 in the emu tongue is not clear (except for the obvious possibility of mucous clearance) and further studies will be required before a more definitive explanation can be advanced.

Glands

The glands in the emu tongue are ubiquitous and occur within the connective tissue (lingual submucosa) of the tongue body, root and frenulum, but not in the tips of the lateral lingual papillae, except for 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 due to its relatively dry diet. The glands in the greater rhea (Feder 1972) and ostrich (Porchescu 2007; Jackowiak & Ludwig 2008; Tivane 2008) tongues are also found throughout the parenchyma, a feature apparently typical for ratites, and are located within the connective tissue.

The naming of avian salivary glands has in the past been found to be inconsistent and confusing (Zis-

wiler & Farner 1972), with most descriptions of their location being based on human directional terminology (Antony 1920; Ziswiler & Farner 1972; Hodges 1974; Nickel *et al.* 1977; Jackowiak & Godynicki 2005). According to Antony (1920) the sparrow (*Passer domesticus*), robin (*Erithaeus rubecula*), swallow (*Chelidon urbica*) and pigeon (*Columbia livia*) have the following groups of lingual glands: inferior, superior, anterior superior and posterior superior lingual glands. Ziswiler & Farner (1972) divide the salivary glands into superior and inferior groups. The glands in the chicken tongue (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 & Godynicki 2005) while those of the quail are classified as lingual, preglottal 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 & Jarrar 2004) which makes their naming more precise.

Despite the occurrence and merging of glandular fields throughout the emu tongue, the lingual glands can be classified according to their location into dorsal, rostroventral, caudoventral, frenular (previously not said to occur in birds [Tucker 1958]) and radical (tongue root) groups. Jackowiak & Ludwig (2008) identified dorsal, ventral and tongue-root lingual glands in the ostrich tongue. 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 & Ludwig 2008) lingual glands thus differs from the earlier works in which 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) of the same species it is clearly stated and illustrated that the tongue body is filled with such glands. The description of the preglottal salivary glands in the quail (Liman *et al.* 2001) fits the location (between the caudal lingual papillae and glottis) of the tongue root glands. This group of glands was named the radical glands in the emu (present study) and tongue-root glands in the ostrich (Jackowiak & 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 (Crole & Soley 2009). The lingual glands of the ostrich were classified as simple tubular and large, simple branched tubular glands by Tivane (2008), whereas Jackowiak & 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 being 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 & Ludwig 2008; Tivane 2008). In the emu the dorsal and rostroventral glands were of the large simple branched tubular type, the frenular glands were exclusively of the simple tubular type, and the caudoventral and radical lingual glands were composed of both types. A similar distribution of the two types of glands is apparent in the ostrich (Jackowiak & 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 usually being absent (Ziswiler & 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 & Bacha 2000; Liman *et al.* 2001; Al-Mansour & Jarrar 2004; Jackowiak & 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 beneath 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 & Ludwig 2008), which distributes a rich capillary plexus between the acini.

Both the emu (Crole & Soley 2008; present study) and greater rhea (Feder 1972) 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 appears uniform across the whole surface (Crole & Soley 2008). 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 the pale doughnut-shaped structures just below the pigmented surface (see above).

The main function of the lingual salivary glands in birds is to provide moisture and lubrication to food boli (Nickel *et al.* 1977; King & McLelland 1984; Gargiulo *et al.* 1991; Liman *et al.* 2001; Al-Mansour & Jarrar 2004). Jackowiak & Ludwig (2008) proposed that due to the high concentration of mucus-secreting 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 indicates that the emu and other ratites employ a 'catch and throw' (Gusseklou & Bout 2005) or cranioinertial (Bonga Tomlinson 2000) feeding method whereby the food bolus travels from the bill tip to the oesophageal entrance (Gusseklou & 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 & 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 (personal observation) 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 protec-

tion from micro-organisms (Gargiulo *et al.* 1991). Similar functions could also be attributed to the emu lingual glands. Tabak, Levine, Mandel & Ellison (1982) note further that the mucins produced have the effect of protecting the tongue surface against coarse material and desiccation, and modulate microbial flora.

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, simple branched tubular glands as previously reported (Crole & Soley 2008). 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 & Ludwig (2008). The presence of Herbst corpuscles in the avian tongue has been confirmed by Ziswiler & Farner (1972) and Berkhoudt (1979) in the duck tongue.

The Herbst corpuscles in the tongue of the emu (present study) displayed similar characteristics to those observed in the ostrich oropharynx (Tivane 2008). In the emu Herbst corpuscles, a capsule, an outer zone (subcapsular space), an inner core with a lamellated appearance (formed by specialized 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 a 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 length of the bill and is unable to protrude. Bonga Tomlinson (2000) and Gussekloo & 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 & Soley (2008).

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 (where they are situated adjacent to the lumen) 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, although nodular tissue also occurred, particularly 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 ciliated columnar 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.

Lingual skeleton

The *paraglossum* in the emu tongue body is situated centrally in the parenchyma and consists entirely of hyaline cartilage (Crole & 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*, and is round in cross section and composed of hyaline cartilage showing areas of ossification near its centre (Crole & Soley 2008; present study). A similar structure is present in the ostrich (Tivane 2008), and, as in the emu, is 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 & Ludwig (2008) possibly mistook the rostral projection of the *basihyale* in the ostrich for the *paraglossum*, as they reported the '*paraglossum*' to be 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 tongue as cartilaginous. Thus it would seem that this structure in both the emu and ostrich is largely cartilaginous, showing some signs of ossification.

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 & 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 & 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 tongue. The movement of the tongue during eating and drinking in palaeognaths, as described by Bonga Tomlinson (2000) and Gussekloo & Bout (2005), would seem to indicate that the tongue is not an active participant in swallowing. During swallowing the hyobranchial apparatus is retracted, causing retraction of the tongue. This is achieved through the attachment of striated muscle fibres from the hyobranchial apparatus 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 fibres attaching to the ventral aspect of the *paraglossum* would similarly be to effect the retraction of the tongue.

Tongue root – taste buds

A structure resembling a taste bud was located in the epithelium of the tongue root in the emu. 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 & Ludwig 2008; Tivane 2008).

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 & Elliott 1946) as both of these terms are 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 & 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 & Elliott 1946; Gentle 1971b; Nickel *et al.* 1977; Bailey, Mensah-Brown, Samour, Naldo, Lawrence & Garner 1997). This border coincides with the boundary between the oral and pharyngeal cavities as described for *Anas* spp. by Zweers *et al.* (1977, cited by McLelland 1993). The importance of clarity in correctly identifying and naming the various components of the

tongue has been pointed out by Moore & 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 & Elliott 1946; Gentle 1971b; Nickel *et al.* 1977; Bacha & Bacha 2000; Al-Mansour & 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; Crole & Soley 2008; Tivane 2008) simply because the root was not identified, sectioned and examined. The number of taste buds in the chicken are reported to increase with age (Lindenmaier & 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). Their paucity 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 & 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 its most caudal extremity (the root). Typical taste buds have also been noted in the caudal oropharyngeal floor and proximal oesophagus in this species (personal observation).

A reason for the difficulty in locating taste buds, as noted by Moore & 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, which in birds are most often associated with glands or occur free in the mucosa (Botezat 1910; Gentle 1971b; Nickel *et al.* 1977; Berkhoudt 1985; Bacha & Bacha 2000). The structure resembling a taste bud found on the emu tongue root was not associated with a gland open-

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

The most obvious function of taste buds on the emu tongue is for the discrimination of food, but, because of the tongue's reduced and non-protrusible nature it has limited contact with food during the cranioinertial method of feeding described by Bonga Tomlinson (2000). The role of the tongue as a sense organ is therefore debatable as there seems little opportunity for food to contact its root to be tasted. However, it has been reported (Bonga Tomlinson 2000) that the tongue scrapes the palate during the process of retraction and swallowing, thus removing food that may have become lodged on the oropharyngeal roof while being moved from the bill tip to the oesophageal entrance. As the emu employs a similar feeding strategy, it seems possible that ingesta is tasted only after it has been swallowed. The sense of taste is an important motivator for feeding as well as initial food selection in birds (Gentle 1971a), although, for the reasons outlined above, this may not be so important for food selection 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 (F.W. Huchzermeyer, personal communication 2009) that the sparse taste buds in the emu may be involved in the selection of potable drinking water, particularly in their natural arid environment.

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