THE MORPHOLOGY OF THE ORAL CAVITY, PHARYNX AND OESOPHAGUS OF THE OSTRICH (*STRUTHIO CAMELUS*)

by

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DECLARATION

I declare that the dissertation which I hereby submit for the degree Master of Science at the University of Pretoria is my own work and has not been submitted by me for a degree at another university.

DEDICATION

I dedicate this work to my late father Dinis Constantino Tivane, for enlightening my life with his love, guidance and support…I MISS YOU PAI!

SPECIAL THANKS

I would like to thank:

Firstly, Almighty Lord, our Saviour, for the blessings He always sends to me.

My parents, Dinis Constantino Tivane and Florinda Manuel Cossa, for giving me the chance to study and for their love and support throughout these years of study.

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SUMMARY

THE MORPHOLOGY OF THE ORAL CAVITY, PHARYNX AND OESOPHAGUS OF THE OSTRICH (*STRUTHIO CAMELUS*)

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Most descriptions of the ostrich oropharynx and oesophagus are superficial and supply little meaningful morphological data. It was therefore the aim of this study to address this deficiency by means of a macroscopic and histological study of this region. The results were supplemented by data obtained by scanning electron microscopy.

Macroscopic observations confirmed that in the ostrich the oral and pharyngeal cavities formed a single structure and could not be separated using visual criteria. The most obvious components observed in the roof of the oropharynx were the palate, the choana, the infundibular cleft and the pharyngeal folds, and on the floor, the interramal region, the tongue and the laryngeal mound. The prominent median longitudinal fold running along the palate and the numerous folds in the interrammal region of the floor contained a concentration of Herbst (Pacinian) corpuscles.

The ramphotheca forming the rim of the oral cavity carried a sharp tomium along the rostral aspect of the mouth. which would assist the ostrich in tearing off plant material. It was further observed that both the roof and floor of the oropharynx could be macroscopically divided into two regions based on colour differences in the mucosa. The pale rostral regions were lined by a keratinized stratified squamous epithelium whereas the darker, more caudally positioned regions demonstrated a thicker non-keratinised epithelium and, in the case of the roof, a glandular layer.

None of the regions of the upper digestive tract sampled revealed structures resembling taste buds and it would appear as if taste plays no role in the selection of food in the ostrich. The presence of large numbers of Herbst corpuscles in the palate may indicate the importance of texture in the selection of food in this species.

In addition to confirming the folded nature of the ostrich tongue, this study revealed that the deep pouch formed by the dorsal tongue fold is further subdivided by a smaller secondary fold into dorsal and ventral recesses. The function of this structural adaptation is unclear but the large increase in surface area produced by the folds, and by virtue of the numerous mucous producing glands found in the mucosa, would presumably enhance mucous production and secretion required for ingesting often dry and difficult to swallow plant material. In addition to the tongue, the entire caudal aspect of the oropharynx was well-equipped with glandular tissue. Other adaptations for swallowing food included the presence of a highly folded mucosa in the interramal region which would indicate that the floor of the oral cavity in the ostrich is capable of a certain degree of distension to accommodate the accumulation of food in the oral cavity prior to swallowing. In similar fashion the longitudinal mucosal folds present throughout the oesophagus, as in other avian species, would also allow for distension of this organ when swallowing bulky food items.

The pharyngeal folds that lie caudal to and around the opening of the Eustachian tubes in ratites are often referred to as the "tonsils" although no histological information has been presented to support this observation. This study revealed that the pharyngeal folds are filled with masses of diffuse and nodular lymphatic tissue and that epithelial folds emanating from the infundibular cleft and retropharyngeal recess formed tonsillar crypts surrounded by the lymphatic tissue.

It has been well documented that in most species of birds papillae are found throughout the oropharynx. Papillae have also been described in ratites, mainly on the tongue and at the caudal aspect of the larynx. Whether the projections observed on the laryngeal mound of the ostrich in this study can be viewed as pharyngeal papillae remains debatable. Likewise, the lingual papillae seen in the ostrich were poorly developed and rudimentary. Compared to other birds, therefore, it is clear that the oropharynx of the ostrich is poorly equipped with papillae.

This study confirmed that the hyobranchial apparatus consists of both central and paired caudo-lateral components, the former represented by the paraglossum and fused basihyale and urohyale, and the latter by the ceratobranchiale and the epibranchiale. The most important finding was that the paraglossum of the ostrich consisted of paired caudo-laterally directed cartilages that were connected rostrally to each other by fibrous connective tissue, and which supported the ventro-lateral aspect of the tongue. This information on the paraglossum has not previously been reported. The horns of the hyobranchial apparatus did not pass close to the skull as previously reported but in fact curved downwards away from the skull. The larynx consisted of the cricoid, procricoid and two arytenoid cartilages as is found in birds in general

It can be concluded that the present study, in addition to confirming the basic features of the oropharynx previously described for the ostrich, clarified the contradictory information presented in the literature and also provided new, unreported morphological data, some of which may be important when studying nutrition in these birds.

CHAPTER 1

GENERAL INTRODUCTION

1.1. HISTORICAL BACKGROUND

The ostrich (*Struthio camelus*) belongs to the Ratidae, a family of birds that includes the emu (*Dromaius novaehollandiae*), cassowary (*Casuarius casuarius*), kiwi (*Apteryx australis*) and rhea (*Rhea americana*). A number of these birds, namely the ostrich, rhea and emu have become commercially important and are farmed in various parts of the world for their meat, skins and feathers.

The ostrich has been utilized as a source of meat since historical times and was apparently considered a delicacy by the Romans. The Roman Emperor, Heliogabalus reportedly had six hundred ostrich brains prepared for one of his feasts and Apicius left the recipe of a special sauce used for dressing ostrich meat (Anon., 1952). The Arabs hunted this bird for its meat, skin, feathers and fat, the latter being much sought after for its nutritional and medicinal properties (Anon., 1952). The San also hunted ostriches for their meat, and used the eggshells for storing water (Anon., 1952).

The domestication and breeding of ostriches in South Africa began around 1850 as a direct response to the dramatic decline in the numbers of wild ostriches which had been hunted for their feathers (Holtzhausen & Kotzé, 1990), and also because wild ostriches were unmanageable (Osterhoff, 1979). Organised ostrich farming, however, was only established from about 1863, mainly in the Karoo and Eastern Cape following the introduction of wire fencing and Lucerne farming (Smit, 1963; Holtzhausen & Kotzé, 1990).

In South Africa the ostrich feather industry collapsed in 1914 as a result of the negative effects of the First World War, and the number of birds decreased from 250,000 in 1913 to 32,000 in 1930 (Smit, 1963). In subsequent years the industry was rebuilt based on the utilization of the entire bird, with the feathers, fresh meat, dried meat (biltong), and in

particular the skin, assuming new-found economic importance. Ostrich farming has now been established world-wide, with farming operations in Europe mainly being stocked with birds imported from Africa and Israel (Drenowatz *et al*., 1995; Deeming & Angel, 1996).

1.2. OSTRICH NUTRITION

The ostrich is the largest living bird, measuring up to 2.75 m and weighting up to 150 kg (Deeming, 1999). In Southern Africa the ostrich can be found in a variety of habitats such as desert grassland, semi-arid savannas, Karoo shrub land and coastal fynbos. "Coastal fynbos" is unique to South Africa and is characterized by a preponderance of small shrub bushes of the *Protea* and *Erica* families (Dean *et al.*, 1994).

Adult ostriches are almost exclusively vegetarian, although they do swallow dry bones (Williams *et al*., 1993; Dean *et al*., 1994; Milton *et al.,* 1994). They select foods by visual means and use the beak for pulling at vegetation and stripping leaves from shrubs and woody plants (Dean *et al*., 1994). The diet of the ostrich varies in accordance with the available plant life in their habitat. Ostriches prefer green annual grasses and forbs, but they also eat leaves, flowers and fruit (Williams *et al*., 1993; Dean *et al*., 1994). Milton *et al*., (1994) reported the preference of the ostriches for forbs low in phenolics and high in fibre. In the ostrich industry, farmers and feed companies usually prepare what are considered appropriate formulations for each stage of development, using ingredients selected to provide adequate nourishment and also additives to improve the bird's immunity to disease (Tully & Shane, 1996).

Despite the availability of commercial ostrich rations, it has been noted that little information in the field of nutrient requirements for the ostrich diet is actually available (Hicks, 1990; Beavers, 1992; Scheideler & Angel 1994; Westendorf & Altizin, 2003). Some researchers suggest turkey as the best avian model to predict approximate nutrient needs for the ostrich (Ullrey & Allen, 1996). However, rations for ostriches are also formulated using the nutritional requirements which have been investigated in other avian (mainly domestic) species (Beavers, 1992).

1.3. ECONOMIC IMPORTANCE OF THE OSTRICH (*Struthio camelus*)

In South Africa the ostrich industry forms a small but important component of the farming sector and the thousands of birds slaughtered annually are an important earner of foreign exchange. Until recently, the skin [which, after tanning, is favoured because of its durability compared to other types of leather (Minnaar, 1998; Dzoma & Gerry, 1998)] formed the most valuable part of the bird, earning 75 – 80% of the potential income from each animal. This was followed by the meat (20%) and feathers (less than 5%) (Van Zyl, 1997). More recent figures, however, indicate that meat is now the most valuable part of the bird (50% of potential income), followed by the skin (45%) and the feathers (5%) (see Brand & Gous, 2006). This trend is apparent in various countries around the world where the ostrich is utilized more as a meat animal, with birds usually being slaughtered at 9 months of age due to the decline in feed efficiency after this age (Deeming, 1999). The increasing popularity of ostrich meat is due to its reputation as a high protein food with very low cholesterol levels. Ostrich meat has therefore become an important product and ostrich farming possesses enormous growth potential. However, the industry in South Africa is beset by numerous production problems, the cumulative effect of which results in fewer birds reaching slaughter age with resultant loss of earnings for the producers. The current efficiency rate of world ostrich production is stated to be five to six birds slaughtered per breeding hen per year (see Brand & Gous, 2006).

1.4. SOURCE, COLLECTION AND PREPARATION OF MATERIAL USED IN THIS STUDY

The heads of fifteen 12 – 14 month-old ostriches of either sex were obtained from a local ostrich abattoir (Ostriches Galore, Krugersdorp, Gauteng, South Africa) where the birds were slaughtered for their skin and meat. After the heads had been removed from the carcasses they were immediately immersed in plastic buckets containing 10% buffered formalin and allowed to fix for approximately 4 hours while being transported to the laboratory. At the laboratory the heads were immersed in fresh fixative (10% buffered formalin) for a minimum period of 48 hours. Care was taken to exclude air from the oropharynx and proximal oesophagus by wedging a small block of wood in the beak. Ten formalin-preserved heads were utilised for a

description of the gross anatomical features and topographical relationships of the structures in the oropharyngeal cavity.

Samples of the mucosa from various parts of the oropharynx were removed from five heads and processed routinely for light microscopy (LM) and scanning electron microscopy (SEM) (See Chapter 3). In addition, the heads of five one-day-old and five three month-old ostrich chicks of either sex were obtained from birds sacrificed during a nutritional trial at the Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, and immersion fixed in 10% buffered formalin in similar fashion to those of the 12 – 14 month-old ostriches. These specimens were used to determine the possible presence of any age-related gross morphological changes.

1.5. AIM OF THE STUDY

Despite fluctuations in the fortunes of the ostrich industry, the ostrich remains an extremely valuable farmed animal in South Africa as well as in other parts of the world. Any information that leads to improved production of this bird will enhance the economic viability of ostrich farming. Although the formulation of commercial diets for ostriches suitable for South African conditions is currently taking place, little attention has been paid to the possible effects such rations may have on the digestive tract of the birds. This situation is partly due to the paucity of meaningful morphological information that is currently available on the structure of the digestive tract, particularly of the oropharynx and oesophagus.

This study aims to address the lack of meaningful morphological information on the oropharynx and oesophagus of the ostrich by presenting the macroscopic and light-microscopic features of the oral cavity (including the tongue), pharyngeal cavity and oesophagus, and also to clarify the often contradictory information presented in the literature. The light microscopic (LM) study will concentrate on a description of the various tissue layers found in the above-mentioned regions of the upper digestive tract and will be supplemented by a detailed scanning electron microscopy (SEM) study of the appropriate surface features. Scanning Electron Microscopy provides a convenient overall view of surface features and is valuable in correlating data obtained by other microscopic techniques. The results will be compared with published information on ratites and other birds.

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CHAPTER 2

MORPHOLOGY OF THE OROPHARYNGEAL CAVITY: GROSS ANATOMICAL FEATURES

2.1 INTRODUCTION

The gross anatomical features of the oropharynx of birds have been described in a number of wild and domestic species. One of the most comprehensive papers is that of Göppert (1903) which compared the oropharynx of numerous species and served as the basis for later descriptions of this region (McLelland, 1979). The structure of the oropharynx of domestic species such as the fowl, duck and goose (Göppert, 1903; Ellenberger and Baum, 1943; Koch, 1973; McLelland, 1975; Nickel *et al*., 1977) have also received wide attention (for additional papers detailing the oropharynx of the duck and goose see McLelland, 1975) and the anatomy of this region in the turkey is reported to be similar to that of the chicken (McLelland, 1975). Various other studies have concentrated on the description of specific morphological features of the region such as taste buds, Herbst corpuscles and salivary glands (See Chapter 3).

Specific attention has been given to the tongue and larynx of birds, both structures forming much of the floor of the oropharynx. The morphological features of the avian tongue have been described in numerous species (see McLelland, 1979 for a review of the earlier literature) and the structural adaptations of this organ linked to diet and mode of feeding (Lucas, 1896, 1897; Gardner 1926, 1927). Many of these studies, particularly the earlier works, presented comparative information on the macroscopic features of the tongue with a view to providing taxonomic data (see eg. Lucas, 1896, 1897; Gardner 1926, 1927). Recent studies have generally supplied more comprehensive information on tongue structure by utilising both light and electron microscopy in addition to macroscopic descriptions (Kobayashi *et al*., 1998; Jackowiak and Godynicki, 2005). The structure of the avian larynx has been detailed in a number of birds (Faraggiana, 1933) including domestic species (Ziswiler & Farner, 1972; White, 1975; Nickel *et al*., 1977).

Although Göppert (1903) provides a very accurate description and sketch of the oropharynx of the ostrich, most descriptions of this region in ratites are superficial and supply little meaningful morphological data. Bezuidenhout (1999) briefly describes some basic features of the oropharynx in the ostrich whereas Duerden (1912) simply mentions that the tongue "is very short, blunt and non-protrusable" and that in the "middle hinder part of the floor is a circular opening, the glottis" which opens into the trachea. The basic morphological features of the oropharynx of the rhea have also been illustrated (Gussekloo & Bout, 2005). The tongue and laryngeal mound of the ostrich, rhea and emu have been described and illustrated in some detail by Faraggiana (1933) and brief descriptions (generally defining the shape) of the ostrich, rhea, emu and cassowary tongue have been published (Gadow, 1879; Feder, 1972; Cho *et al*., 1984; Fowler, 1991).

In view of the lack of information regarding the gross anatomical features of the upper digestive tract of the ostrich, this chapter provides a detailed description of the macroscopic features of the oral cavity (including the tongue) and pharynx (including the laryngeal mound) and compares the results with published information on ratites and, where appropriate, with birds in general. The terminology used is that of *Nomina Anatomica Avium* (Baumel, King, Breazile, Evans & Vanden Berge, 1993).

2.2 MATERIALS AND METHODS

2.2.1 Experimental Animals

The heads of fifteen 12 – 14 month-old ostriches of either sex were obtained from a local ostrich abattoir (Ostriches Galore, Krugersdorp, Gauteng, South Africa) where the birds were slaughtered for their skin and meat. After the heads had been removed from the carcasses they were immediately immersed in plastic buckets containing 10% buffered formalin and allowed to fix for approximately 4 hours while being transported to the laboratory. At the laboratory the heads were immersed in fresh fixative (10% buffered formalin) for a minimum period of 48 hours. Care was taken to exclude air from the oropharynx and proximal oesophagus by wedging a small block of wood in the beak. Samples of the mucosa from various parts of the oropharynx were removed from five heads and processed routinely for light microscopy (LM) and scanning electron microscopy (SEM) (See Chapter 3). In addition, the heads of five one-

day-old and five three month-old ostrich chicks of either sex were obtained from birds sacrificed during a nutritional trial at the Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, and immersion fixed in 10% buffered formalin in similar fashion to those of the 12 – 14 month-old ostriches. These specimens were used to determine the possible presence of any age-related gross morphological changes.

2.2.2 Preparation of specimens for Gross Anatomy and Topography

Ten formalin-preserved heads were utilised for a description of the gross anatomical features and topographical relationships of the structures in the oropharyngeal cavity. The heads were removed from the fixative, rinsed in running tap water to remove excess formalin and incised along one commisure of the mouth to expose the oral cavity, pharynx and proximal oesophagus. The macroscopic features were described and photographically recorded using a Nikon Coolpix 995 digital camera (Nikon, Tokyo, Japan). Dried skulls, as well as dried whole specimens of the tongue, laryngeal mound, trachea and hyoid apparatus, from two 12-14 month-old birds, were used to provide supporting evidence for the anatomical description.

2.1 RESULTS

2.3.1 Rostral boundaries of the oropharynx

The oropharyngeal cavity was bell-shaped (Fig. 2.1) and dorso-ventrally flattened, resulting in limited space within the cavity when the beak was closed. No obvious morphological distinction could be made between the oral cavity and the pharynx and both cavities formed a common chamber.

The rostral part of the oropharynx (essentially representing the oral cavity) was bounded ventrally by the mandibular ramphotheca and dorsally by the maxillary ramphotheca. The mandibular ramphotheca was a pale, creamy colour and was clearly demarcated from the rest of the floor of the oral cavity with which it was continuous. It extended bilaterally as a zone of cornified epithelium from the angulus oris. The ramphotheca was of equal width where it lay over the ramus of the mandible but widened rostrally to form a plate-like region above the mandibular rostrum (*rostrum mandibulae*) (Fig. 2.1). A variable number of fine, rostro-

laterally oriented folds continuous with the mucosal folds on the inter-ramal region were observed on the mandibular ramphotheca, and a raised, sharp edge, the tomium (*tomium mandibulare*) was apparent along the lateral border of the rostral half. At the tip of the beak the tomium was interrupted by the rostral termination of the gonys, a midventral, thickened, plate-like component of the external ramphotheca which extended from the tip of the mandible to the skin forming the rostral limit of the inter-ramal region. The gonys was supported by a broad thickening of the bone on the ventral surface of the mandibular rostrum in the vicinity of the mandibular symphysis (*symphysis mandibularis*). The termination of the gonys effectively formed the unguis or nail which was continuous with the tomium, of similar height and varied in width between 12 – 15 mm. A short region of the mandibular tomium just caudal to the tip of the beak was finely serrated. The remaining lateral surface of the mandibular ramphotheca caudal to the tomium was smooth and rounded (Figs. 2.1 and 2.2).

The maxillary ramphotheca was not as clearly demarcated from the surrounding tissue when compared to the mandibular ramphotheca. As in the latter, the tomium was only apparent along the rostral half of the maxillary ramphotheca. The lateral margin of the caudal maxillary ramphotheca formed a rounded, cord-like structure which extended to the corners of the mouth (Figs. 2.1 and 2.2). At the tip of the beak the tomium was interrupted by the rostral termination of the culmen (a mid-dorsal plate of the external ramphotheca which extended from the tip of the upper beak to the skin of the forehead) (Fig. 2.1) in similar fashion to that observed on the mandible. This structure (effectively the maxillary unguis or nail) was also continuous with the tomium, again of similar height and varied in width between 12 – 13 mm. The rostral part of the maxillary tomium and the maxillary unguis exhibited a higher profile than the equivalent structures on the mandible. The rostral part of the culmen was supported by a thickening of the dorsal tip of the premaxillary bone providing further support that the termination of the culmen formed a nail (*unguis*).

2.3.2 Roof of the oropharynx

The roof of the cavity demonstrated two regions (of approximately equal area) based on differences in the colour of the mucosa. The demarcation of the two regions was abrupt (Figs. 2.1 and 2.2). The mucosa of the rostral and rostro-lateral region was pale and tightly stretched, and divided into two halves by a prominent raised longitudinal mucosal ridge or fold, the median palatine ridge (*Ruga palatina mediana*) that extended rostrally from the apex of the

choana to the tip of the beak, ending against the unguis. The tip of the ridge narrowed into a sharp point and flattened, effectively subdividing the ramphotheca above the maxillary rostrum into two halves (Fig. 2.1). At a point approximately half way along the median ridge the mucosa in this region displayed fine, oblique mucosal folds which radiated rostro-laterally towards the rampotheca where they became progressively larger. LM revealed that the mucosa in this region was non-glandular (see below)

The mucosa of the caudal and caudo-medial region was darker in colour, had a sponge-like texture and surrounded the choana and opening of the Eustachian tubes. When viewed from beneath, the choana formed a bell- or inverted V-shaped depression subdivided along the midline by a prominent mucosal ridge. The ridge was deeper rostrally where it inserted arrowhead-like into the apex of the choana. It became shallower caudally where it expanded just beneath the caudal border of the choana to form a shallow crescent that demarcated the rostral boundary of the infundibular cleft. At the lateral borders of the choana lay the openings of the internal nares, each demarcated dorso-medially by a low mucosal ridge. This ridge formed the lateral border of a relatively deep, blind-ending mucosal recess (Figs. 2.1 – 2.3).

Caudal to the choana was the median infundibular cleft (*Rima infundibuli*) which represented the opening to the pharynx of the infundibulum into which, in turn, the left and right Eustachian tubes opened. The infundibular cleft extended from a crater-like depression lying caudal to the crescent-shaped ridge described above and continued caudally to subdivide the caudal portion of the pharyngeal mucosa into two overlapping mucosal (pharyngeal) folds. The free borders of the folds were rounded and each contributed to the formation of a deep retro-pharyngeal recess which was continuous with the longitudinally folded mucosa of the oesophagus (Figs. 2.1 and 2.3). The thickened nature of the mucosa in this region was due to the presence of numerous, well-developed mucous-producing glands as revealed by LM (see Chapter 3). The openings of these glands could be seen as small pit-like depressions throughout the surface of this region, particularly in the vicinity of the pharyngeal folds.

2.3.3 Floor of the oropharynx

The floor of the oropharynx was formed by the interramal region (*Regio interramalis*), the tongue and the laryngeal mound (Fig. 2.1). The triangular interramal region was accommodated between the rami of the mandible and formed the floor of the oral cavity

rostral to the tongue. It extended bilaterally around both the tongue and the laryngeal mound, eventually merging with the oesophageal mucosa. The mucosa of this region displayed two components based on differences in colour. The largest component was a pale colour (shown by LM to be aglandular – see Chapter 3) and occupied the rostral and rostro-lateral aspects of the oral cavity. (Figs. 2.1 and 2.2). The smaller creamy-pink component (darker) (also non-glandular but with a thicker epithelial component as shown by LM – see below) occupied a limited area in the immediate vicinity of the tongue, both rostrally and laterally (Figs. 2.1 and 2.2), curved around the laryngeal mound, and represented that part of the interramal region that merged with the oesophageal mucosa.

The entire interramal region was characterised by a series of mucosal folds, the most obvious of which was a median longitudinal fold running from beneath the tongue to the tip of the mandibular ramphotheca. This fold originated in the darker region of the interramal mucosa as two moderately sized latero-medial folds that converged on the midline where they met, at the junction between the darker and lighter mucosa, with two smaller medial folds to form a pair of larger median folds. These folds in turn merged approximately half-way along the interramal region to form a single fold which enlarged appreciably within the relatively smooth rostral termination of the interramal region. In similar fashion to that of the median palatine ridge, this fold continued rostrally, effectively dividing the ramphotheca covering the mandibular rostrum into two halves before narrowing to terminate against the mandibular unguis. Unlike the median palatine ridge, the greater part of the median fold and its components were fleshy in nature and displayed a zig-zag pattern. The rostral part of the fold, however, was more rigid and showed no folding (Figs. 2.1 and 2.2).

Two additional sets of folds were observed to originate from the floor of the pharynx on either side of the laryngeal mound. Each set consisted of approximately four longitudinal folds, the largest of which was laterally positioned and emanated from the rostro-lateral border of the overlying pharyngeal fold. This tall, fleshy fold ran along the lateral aspect of the interramal region but was separated from the ramphotheca of the mandibular ramus by a deep, narrow cleft. The fold terminated at approximately the level of the tip of the tongue in the form of a number of small radiating folds traversing the aglandular mucosa. In addition, small obliquely oriented folds branched from the main fold along much of its length, those extending from the lateral aspect of the main fold running onto the mandibular ramphotheca. The most medially positioned of the folds was also large and fleshy in nature and extended from the caudal and

caudo-lateral aspects of the body of the tongue, coursing around the laryngeal mound to merge with the criss-crossing folds of the widened portion of the proximal oesophagus. This fold formed a deep recess between itself and the laryngeal mound (Figs. 2.1, 2.4 and 2.5).

Positioned between the large medial and lateral folds were two or more smaller folds that were continuous with the proximal oesophagus and which branched rostrally onto the mucosa of the interramal region (Figs. 2.1, 2.2, 2.4 and 2.5). Although most of the smaller folds in this region were longitudinally oriented, numerous oblique and transverse folds were observed to link the three major sets of longitudinal folds.

The ostrich tongue was a small, stubby, U-shaped structure that lay approximately in the middle of the floor of the oropharynx. It was a pale, creamy pink colour in formalin-fixed specimens and had a firm texture. From the blunt apex the body widened caudally forming two short arms, each of which terminated in an elongated, relatively slender, rounded tip reminiscent of a papilla (Figs. 2.1, 2.2, 2.4 and 2.5). The base of the tongue lay relatively far from the entrance of the larynx (the glottis) and the stretch of mucosa lying between the two structures demonstrated a number of longitudinal folds (Figs. 2.4 and 2.5). Whether this region represented the root of the tongue was morphologically difficult to determine. With the beak closed the tongue fitted snugly against the glandular mucosa around the rostral aspect of the choanae. The stubby, thickened nature of the tongue resulted in the formation of dorsal, ventral and lateral surfaces, each of which displayed a subtle, cobblestone appearance. (Fig. 2.2). Each rounded unit (cobblestone) contained a centrally positioned opening which represented the duct of large mucus-producing glandular units situated in the mucosa (see Chapter 3).

The dorsal surface of the tongue was folded back on itself resulting in the formation of a deep, blind-ending pocket. Median sections of the tongue revealed that the "pocket" was divided into dorsal and ventral recesses by a relatively large secondary mucosal fold (Figs, 2.4 and 2.6). There was no communication between the two recesses and both opened caudally opposite the entrance to the glottis (Fig. 2.6). The ventral aspect of the tongue was attached to the floor of the oropharynx by the frenulum (*frenulum linguae*) which was triangular in shape. The broad base of the frenulum was caudally positioned with the apex directed rostrally. A shallow recess lay at the junction between the frenulum and the ventrum of the tongue and a shallow median sulcus was apparent towards the tip of the ventrum. The smooth mucosa of

the frenulum commonly displayed numerous folds with oblique and longitudinal folds being particularly obvious along the lateral edges (Fig.2.2). It was also clear from median sections that the body of the tongue was supported by the paired cartilaginous paraglossals and the cartilaginous rostral process of the basihyale, whereas the root was supported by the ossified body of the basihyale and rostral process of the cricoid cartilage of the larynx (Fig. 2.6) (see Chapter 4).

A short distance from the base of the tongue was the laryngeal mound, a raised, oval or shield-shaped structure which occupied the caudal third of the floor of the oropharyngeal cavity. It was related rostrally to the base of the tongue and caudally to the oesophagus. The more rostral star-shaped portion of the mound housed the glottis which in fixed specimens was opened wide in a V-shaped configuration, with the arms of the V directed rostro-laterally (Figs. 2.1, 2.4 and 2.5). The rim of the glottis was demarcated by two prominent mucosal ridges or lips which were elevated above the surface of the mound. The ridges did not meet at the base of the glottis, leaving a small gap between them (Figs. 2.4 and 2.5). When the beak was closed, the glottis was perfectly aligned with the common openings of the choana.

The star-shaped portion of the mound housing the glottis was inclined in a dorso-rostral direction giving it a horizontal orientation in respect of the rostral portion of the oro-pharyngeal cavity and was characterized by the presence of three pairs of prominent projections that gave it a star-shaped appearance. The rostral pair was laterally directed whereas the caudal and caudo-medial pairs were caudally directed (Figs. 2.1, 2.4 and 2.5). The caudo-medial pair were closely apposed and in some specimens appeared to form a single structure, although a subtle medial groove could be discerned (Fig. 2.5). The projections varied in structure, most appearing flattened and of simple design (Figs. 2.1 and 2.5), while others were more bulbous in nature with intervening grooves giving them the appearance of papillae (Fig. 2.4). The paired rostral and caudal projections were supported by extensions of the arytenoid cartilages (see Chapter 4) whereas it appeared as if the caudo-medial projections existed simply as mucosal extensions. The more caudal aspect of the laryngeal mound was structurally uncomplicated and represented that portion of the larynx supported by the cricoid cartilage (see Chapter 4).The mucosa covering the mound was relatively smooth in appearance and was continuous caudally with the prominently folded mucosa of the oesophagus.

2.4 DISCUSSION

2.4.1 General features

The boundaries and components of the oral and pharyngeal cavities of the ostrich agree with the general avian pattern as previously described (Göppert, 1903; Farner & King, 1972; McLelland, 1979, 1993) and with the brief description of Bezuidenhout (1999). As noted above, no obvious morphological distinction could be made between the oral cavity and the pharynx and both cavities formed a common chamber. This situation is apparent in most avian species due to the absence of a soft palate and oropharyngeal isthmus (McLelland, 1979, 1993). However, Zweers *et al*. (1977) (cited by McLelland, 1993), place the boundary between both cavities in the duck at the level of the caudal lingual papillae. Employing embryological data, it has been suggested (Lucas & Stettenheim, 1972 – cited by McLelland, 1993) that the dorsal transverse boundary lies between the choana and the rima infundibuli, stretching laterally to the angles of the jaws, and that the ventral transverse boundary lies between the paraglossal and basihyal bones (see Chapter 4).

The ramphotheca forming the rim of the oral cavity in ratites reportedly "shows very little adaptation and the rims are relatively round and blunted" (Gussekloo, 2006). This is certainly true for the caudal aspect of the ramphotheca in the ostrich. However, the more rostral component of both the mandibular and maxillary ramphotheca possess a raised, sharp edge, the tomium located along the lateral border. The existence of this structural adaptation would lend support to the observation that ostriches use their large beaks to tear off plant material (Brand & Gous, 2006). The distinct difference in width observed between the mandibular and maxillary ramphotheca in the ostrich has also been illustrated in the rhea (Gussekloo & Bout, 2005).

2.4.2 Roof of the oropharynx

The roof of the oropharynx exhibited the basic features previously described and illustrated in the ostrich by Göppert (1903). In contrast to various other avian species, where the mucosa of the palate frequently forms lateral, median and intermediate ridges (McLelland, 1979), the mucosa of the palate in the ostrich formed a single median longitudinal ridge (*ruga palatina mediana*) which extended the length of the palate rostral to the tip of the choana. A similar,

single median ridge is also illustrated in the palate of the rhea (Gussekloo & Bout, 2005; Gussekloo, 2006). Histology indicated that this structure in the ostrich may play an important mechano-sensory function (see Chapter 3). As illustrated by Göppert (1903) and confirmed in the present study, the choana in the ostrich is very short and restricted to the caudal aspect of the palate, a feature shared with a few other species such as herons and ducks (McLelland, 1979). The illustration of the oropharynx of the rhea provided by Gussekloo & Bout (2005) also indicates a similar situation in this species. Likewise, as determined in the present study, the caudally directed papillae that reportedly form a typical feature of the palate and roof of the pharynx in many avian species (Göppert,1903; McLelland, 1979) are totally absent in the ostrich and seemingly also in the rhea (Gussekloo & Bout, 2005). An interesting finding from the present study was the division of the palate into two distinct regions based on differences in the colour of the mucosa. The illustration of the ostrich oropharynx by Göppert (1903) also appears to indicate this regional differentiation. Histology revealed that the distinction was due to the presence of mucus-secreting glands in the darker region surrounding the choana and extending onto the pharyngeal folds (see Chapter 3).

The most distinct features of the pharynx observed in this study and also by Göppert (1903) were the presence of large pharyngeal folds and a long pharyngeal cleft (*Rima infundibuli*). The pharyngeal cleft is described as a median longitudinal fissure which connects the infundibulum (into which the Eustachian tubes [*Tubae auditivae*] open) to the pharynx (McLelland 1979, 1993). The observation that the auditory tubes open independently into the infundibulum in ratites, as opposed to opening via a common tube as in the chicken and dove) (McLelland, 1993) was not confirmed in the present study. The massive pharyngeal folds in the ostrich were seen to overlap on the midline as also noted by McLelland (1979). These structures contained mucus-secreting glands and masses of lymphoid tissue (see Chapter 3), thus constituting pharyngeal tonsils. McLelland (1979) states that these structures make "a sharp boundary with the oesophagus". Although this appears to be the case when viewed macroscopically, the statement is misleading as this study demonstrated that the pharyngeal folds form a deep recess before merging with the tissue of the proximal oesophagus.

2.4.3 Floor of the oropharynx

This study demonstrated that the floor of the oropharynx of the ostrich consisted of three components, the interramal region (*regio interramalis*), the tongue and the laryngeal mound (*mons laryngealis*).

The triangular interramal region has previously been mentioned or illustrated in the ostrich (Göppert, 1903; Bezuidenhout, 1999). The present study identified this region as being heavily folded, with the most prominent feature being a double/single large median mucosal fold that effectively divided the interramal region into two halves. As in the median palatine ridge, this fold was richly supplied with Herbst corpuscles (see Chapter 3) which would suggest a mechano-sensory function for this structure. A similar, but in this instance single median fold, is also illustrated in the interramal region of the rhea (Gussekloo & Bout, 2005; Gussekloo, 2006). It has been noted that in birds equipped with oral sacs for carrying food, the inner wall of the sacs is greatly folded in the empty state to allow for expansion when filled (Bock *et al*., 1973). The presence of a highly folded mucosa would indicate that the interramal region in the ostrich is therefore capable of a degree of distension to accommodate the accumulation of food in the oral cavity prior to swallowing. The scooping of water into the beak during drinking would also require temporary storage in the interramal region (distension therefore important) prior to the water moving to the proximal oesophagus where it is accumulated until the head is raised (F. W. Huchzermeyer – personal communication). The deep gullies formed between the mucosal folds that run around the tongue and laryngeal mound would also act to channel the water to the proximal oesophagus.

This study confirmed the basic observation by previous authors (Gadow, 1879; Göppert, 1903; Duerden, 1912; Cho *et al*., 1984; Bezuidenhout, 1999; that the ostrich tongue is small, triangular or U-shaped, has a smooth appearance and is non-pigmented, and also that the dorsal surface folds back on itself to form a pouch or pocket (Göppert, 1903; Faraggiana, 1933; Fowler, 1991). Although smooth, the surface of the ostrich tongue has a cobble-stone appearance due to the presence of large mucus-producing glands located just beneath the surface. Faraggiana (1933) made a similar observation, noting that the tongue surface is composed of many tightly-packed semi-circular papillae. This feature has not been noted in any other previous investigations.

The present work also confirmed the observations of Göppert (1903) and Faraggiana (1933) that the caudal aspect of the body of the tongue tapered bilaterally to form papillae-like extensions. It is well documented that in many avian species, including domestic birds, the tongue is adorned with lingual papillae (see, for example, Göppert 1903; Gardner, 1926, 1927; McLelland, 1979; Kobayashi *et al.*, 1998, Nickel *et al.*, 1977). However, the situation in ratites is less clear and conflicting information has been presented regarding the presence of lingual papillae. Gussekloo (2006) notes that in ratites "only the papillae behind the larynx (papillae pharyngis caudoventrales) are clearly recognizable", whereas Tomlinson (2000), commenting on the role of the ratite tongue in pushing a food bolus from the pharynx to the oesophagus, states that "The tongue is therefore very short and relatively broad without clear adaptations other than the papillae linguae caudales that stabilize the food bolus during the final transport into the oesophagus." The structures seen in some specimens of the ostrich tongue certainly resemble papillae (caudal lingual papillae) and it would appear as if a similar structural adaptation occurs in the rhea (*Rhea americana*) tongue (sketch by Gussekloo & Bout, 2005, personal observations – M.R. Crole & J.T.Soley). The paired papillae-like extensions at the base of the ostrich tongue are poorly developed in comparison to those observed in the rhea and whether they represent true lingual papillae is not clear. Lingual papillae are generally accepted to assist in swallowing food, but in the ratites where the small tongue is viewed as a rudimentary organ adapted for the rapid swallowing of bulky food items (McLelland, 1979), the assistance of the weakly developed papillae in the ostrich may be of limited value. In the emu the serrated edge described by Cho *et al.* (1984) certainly represents lingual papillae. Additional observations in the emu have revealed that both lateral and caudal sets of papillae are indeed present and well-developed in this species (M.R. Crole – personal observations). Gadow (1879) notes that the lateral edges of the cassowary tongue carry small, caudally directed points which would also appear to represent lingual papillae.

The folded nature of the ostrich tongue has previously been described (Göppert, 1903; Faraggiana, 1933; Fowler, 1991). In addition to confirming this observation, the present study revealed that the deep pouch formed by the dorsal tongue fold is further subdivided by a smaller, but substantial secondary fold, into dorsal and ventral recesses. The function of this structural adaptation is unclear but the large increase in surface area produced by the folds, and by virtue of the numerous mucus producing glands found in the mucosa (see Chapter 4), would presumably enhance mucus production and secretion required for ingesting often dry and difficult to swallow plant material. As noted by Gussekloo (2006) "The products of the

salivary glands [in birds] have functions in the intraoral transport (e.g. in slide-and-glue transport), but mainly in the lubrication of food."

The laryngeal mound (*Mons laryngealis*) in avian species is a raised structure lying immediately caudal to the tongue and which carries on its rostral aspect the glottis (McLelland, 1979). Similar to most bird species, the laryngeal mound in the ostrich was situated close to the base of the tongue with only a small stretch of intervening tissue lying between the two structures (McLelland, 1979). In the ostrich the part of the mound carrying the glottis (supported by the arytenoid cartilages – see Chapter 4) was a star- or shield-shaped structure, characteristic features that were also illustrated by Göppert (1903) and Faraggiana (1933). The glottis typically lay directly opposite the caudal aspect of the choana as described for most avian species (McLelland. 1979). The observation in the ostrich that the lips of the glottis did not meet at the base was also illustrated by Faraggiana (1933). It has been well documented that in most species of birds papillae are found in the oropharynx at the edges of the choana, the base of the tongue, and caudal to the larynx and infundibular cleft (Göppert, 1903; McLelland, 1979; Gussekloo, 2006). However, in ratites, according to Gussekloo (2006), the only papillae which can be readily recognized are those situated at the base of the larynx (*papillae pharyngis caudoventrales*). These papillae have been described in the rhea (Gussekloo, 2006). Whether the projections observed on the laryngeal mound of the ostrich can be viewed as pharyngeal papillae remains debatable, although the caudo-medial pair, which are unsupported by the arytenoid cartilages, do resemble papillae. In some ostrich specimens the various paired projections were seen to be subdivided (see Fig. 2.4) creating the impression of a caudal row of papillae very similar in appearance to those illustrated for the rhea (Gussekloo, 2006). Compared to other birds, however, it is clear that the oropharynx of the ostrich (and ratites in general) is poorly equipped with papillae.

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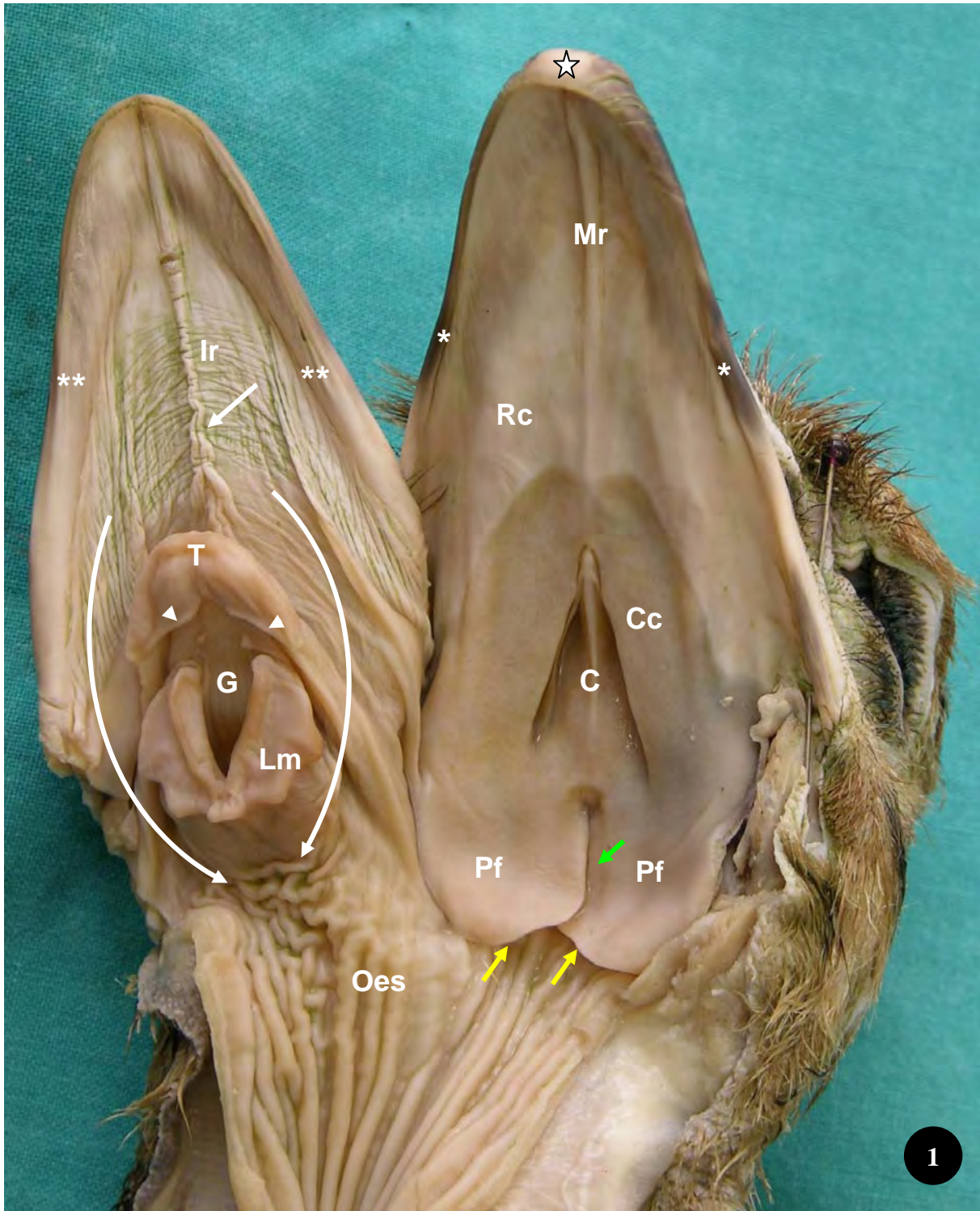
Figure 2.1: Ostrich head and proximal oesophagus opened along the left side to reveal the macroscopic features of the oropharynx. The maxillary ramphotheca (asterisks) are substantially narrower than the mandibular ramphotheca (double asterisks). Note the contribution of the culmen (star) to the tomium of the upper beak. The roof of the oropharynx displays a median palatine ridge (Mr), a pale rostral component (Rc) and darker caudal component (Cc), the bell-shaped choana (C) and the infundibular cleft (green arrow). Note the overlapping pharyngeal folds (Pf) on either side of the cleft and the retro-pharyngeal recess (yellow arrows) where the folds become continuous with the proximal oesophagus (Oes). The floor of the oropharynx reveals the highly folded interramal region (Ir) dominated by a large median fold (white arrow). The folds proceed caudally around the tongue (T) and laryngeal mound (Lm) to the proximal oesophagus as indicated by the curved arrows. Arrowheads indicate the point of reflection of the dorsal surface of the tongue to form a deep pocket. The laryngeal mound displays three pairs of projections and the V-shaped glottis (G).
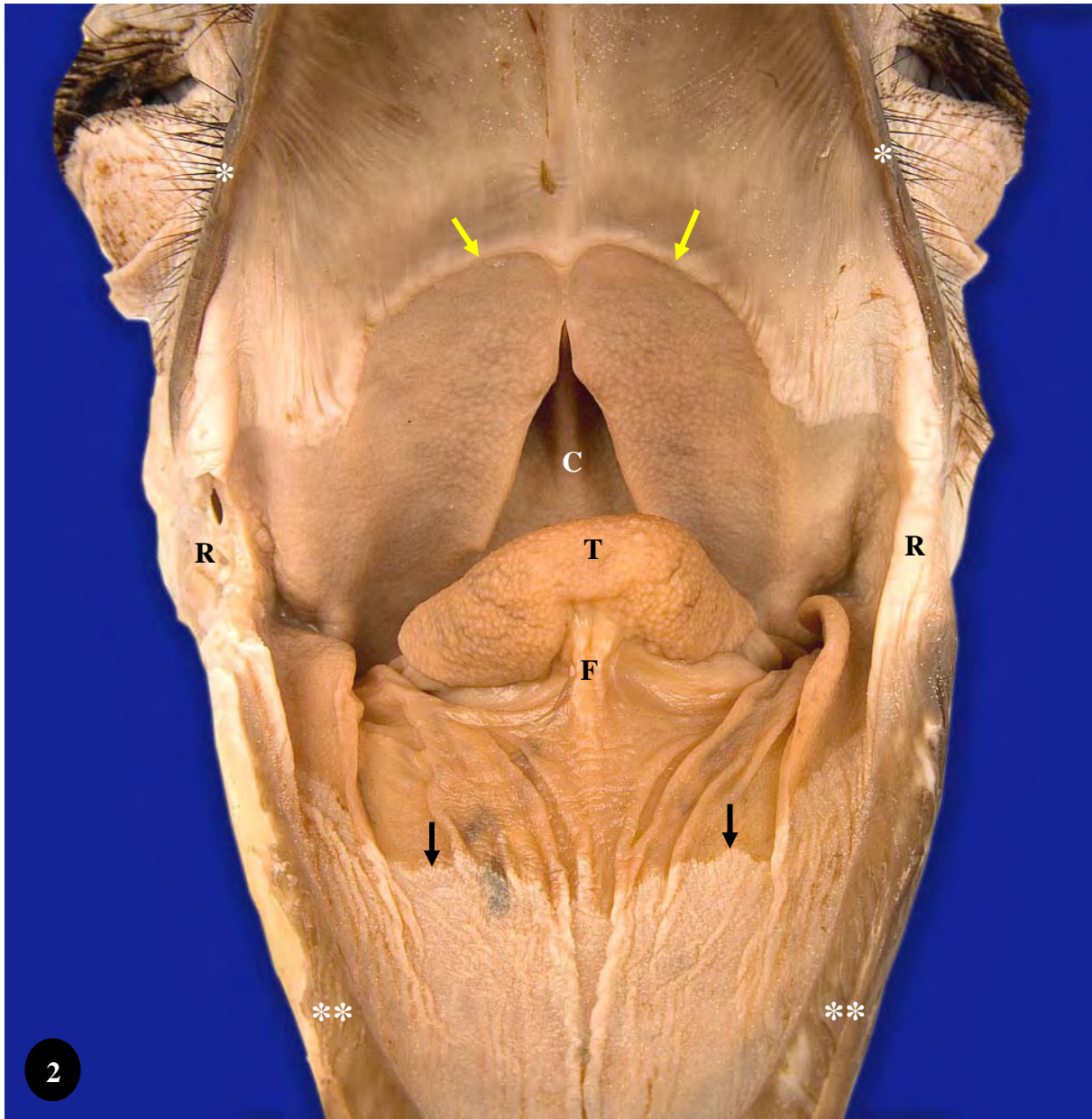
Figure 2.2: Maximum gape of the beak revealing the caudal aspect of the oropharynx in the ostrich. The tongue has been retracted from the floor of the oropharynx to expose the thick, non-glandular mucosa lying beneath and lateral to the tongue. The abrupt transition from a thin, keratinised stratified squamous epithelium (pale colour) to a thick, non-keratinised stratified squamous epithelium (light brown colour) is clear (arrows). A similar colour transition is seen on the roof of the oropharynx (yellow arrows), in this instance indicating the demarcation of the glandular (pink) and non-glandular (pale colour) regions. The glands in the region around the choanae (C) and in the tongue (T) are seen as small, rounded structures beneath the surface. Note the numerous folds on the floor of the oropharynx (interramal region) and the frenulum (F). Both sets of ramphotheca (asterisks) are rounded at their caudal aspect and terminate at the rictus (R). The mandibular ramphotheca (double asterisks) are visibly wider than the maxillary ramphotheca.
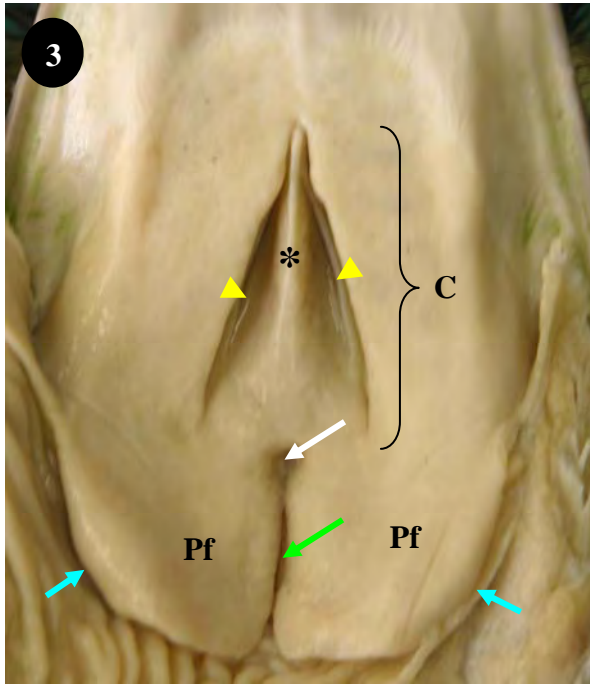
Figure 2.3: Enlargement of the caudal region of the roof of the oropharynx illustrating the choana (C) which forms a bell- or inverted V-shaped depression subdivided along the midline by a prominent mucosal ridge (asterisk). The openings of the internal nares are demarcated dorso-medially by low mucosal ridges (yellow arrowheads). The infundibular cleft (green arrow) extends from a crater-like depression (white arrow) to subdivide the caudal portion of the pharyngeal mucosa into two overlapping mucosal (pharyngeal) folds (Pf). The free borders of the folds are rounded and form a deep retro-pharyngeal recess (turquoise arrows) before becoming continuous with the mucosa of the proximal oesophagus.
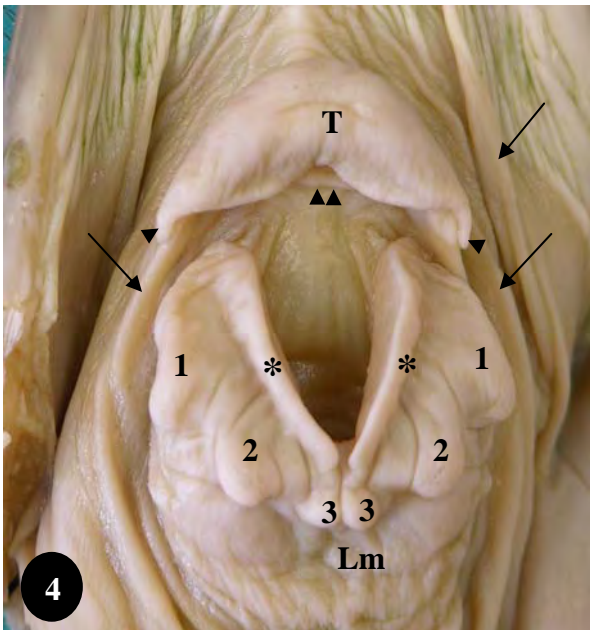
Figure 2.4: Caudal region of the floor of the oropharynx showing details of the tongue (T) and laryngeal mound (Lm). Note the two small caudally directed papillae at the base of the tongue (arrowheads) and the secondary tongue fold (double arrowhead). The elevated lips of the glottis (asterisks) are prominent and the three pairs of projections (1,2 and 3) on that part of the mound formed by the arytenoid cartilages appear in the form of papillae. The caudo-medial projections (3) are clearly separated in this specimen. The large mucosal folds (arrows) emanating from the base of the tongue and the interramal region are seen passing around the laryngeal mound.
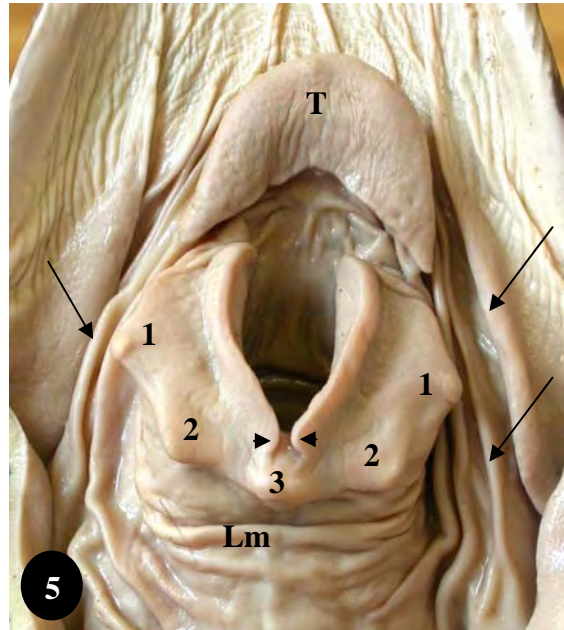

Figure 2.5: The tongue (T) and laryngeal mound (Lm) of another specimen illustrating the more typical appearance of the star-shaped region of the laryngeal mound. The three pairs of projections (1, 2 and 3) are simple in design with the caudo-medial pair (3) appearing almost fused. The lips of the glottis do not meet at the base resulting in a small gap between them (arrowheads). The papillae-like extensions at the caudo-lateral aspect of the tongue are not obvious in this specimen. Mucosal folds (arrows).
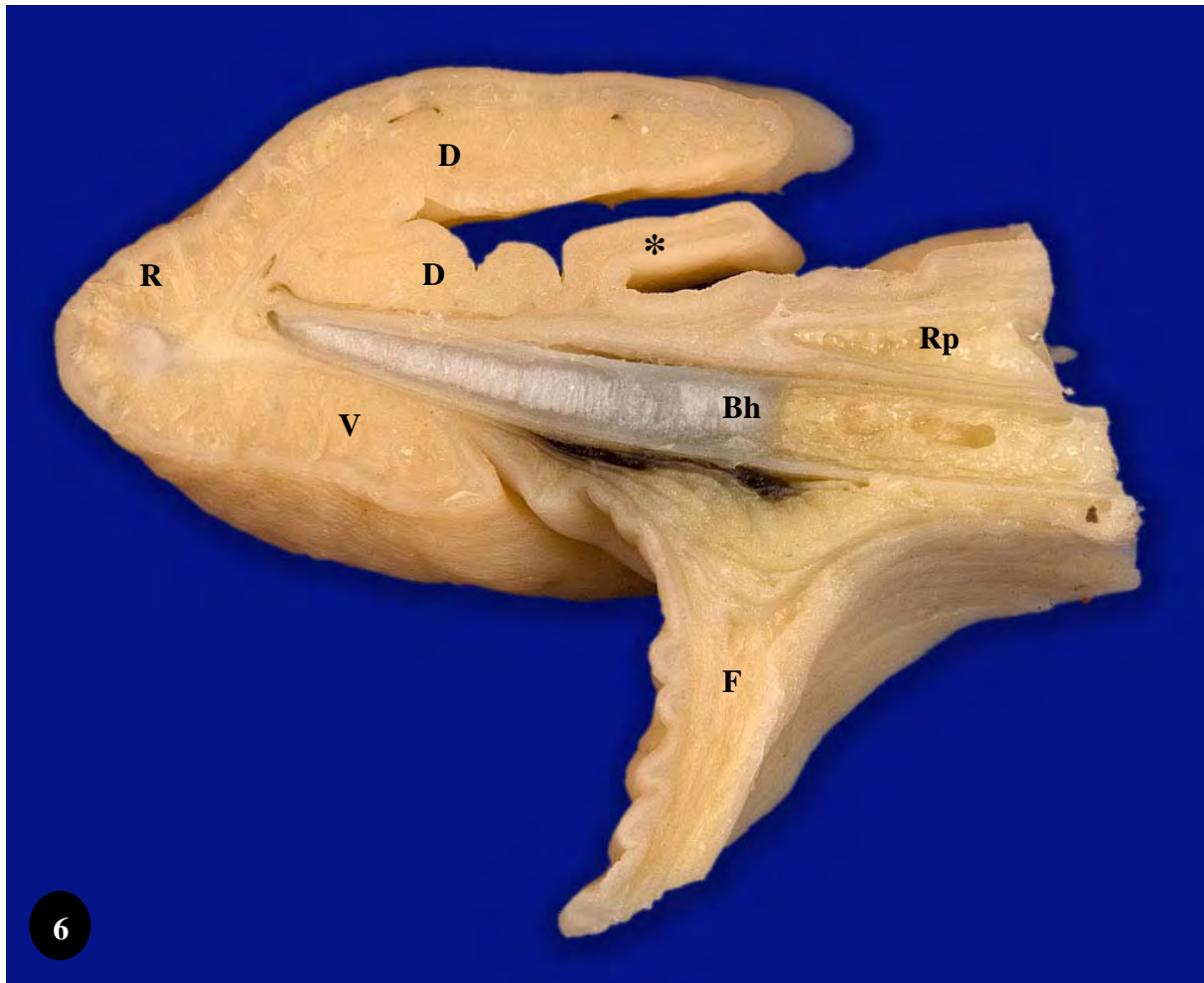
Figure 2.6: A mid-saggital section through the tongue showing the blunt rostral aspect (R), the dorsal surface (D) folded back on itself, forming a pocket divided into dorsal and ventral recesses by a secondary mucosal fold (asterisk), the ventral surface (V) and the frenulum (F). The basihyale (Bh) and more dorsally positioned rostral process (Rp) of the cricoid cartilage form supporting structures for the tongue (for details of the elements of the hyobranchial apparatus supporting the tongue see Figure 4.7 in Chapter 4).

CHAPTER 3

MORPHOLOGY OF THE OROPHARYNGEAL CAVITY: HISTOLOGICAL FEATURES AND SURFACE MORPHOLOGY

3.1 INTRODUCTION

Although some anatomical information regarding the oropharynx of ostriches and other ratites has been presented, very little information is available on the morphological and histological features of the upper digestive tract of these birds.

The studies of the morphology of the upper digestive tract of domestic and wild birds date back to the earlier 1900's (Botezat, 1904, 1906, 1910; Bath, 1906; Greschik, 1917 a, b). Most of these studies concentrated on the description of specific morphological features of this region such as taste buds and salivary glands (Lindenmaier & Kare, 1959; Gentle, 1971; Ziswiler & Farner, 1972; Wissman, 2002; Iwasaki, 2002) (see http://people.eku.edu/ritchisong/ birddigestion.html). In later studies a number of authors described the histochemistry and morphology of the cutaneous sensory end-organs known as Vater-Pacinian corpuscles and which have been referred to in various body regions of birds as Herbst corpuscles, Leydig's body and Rauber's end-organ (Winkelman & Myers, 1961; Warner *et al*., 1967; Anderson & Nafstad, 1968; Wight *et al*., 1970; Gottschaldt & Lausmann, 1974).

The basic macroscopic features of the oropharynx of the ostrich and rhea have been recorded (Göppert, 1903; Duerden, 1912; Bezuidenhout, 1999; Gussekloo & Bout, 2005) and the tongue and laryngeal mound of the ostrich, rhea and emu have been described and illustrated in some detail by Faraggiana (1933). Brief descriptions (generally defining the shape) of the ostrich, rhea, emu and cassowary tongue have also been published (Gadow, 1879; Feder, 1972; Cho *et al*., 1984; Fowler, 1991). However, with the exception of the study by Feder (1972) on the digestive tract of the rhea, no detailed histological information has been presented on the oropharynx of ratites.

In view of the almost complete lack of information regarding the histological features of the oropharynx of the ostrich and ratites in general, this chapter presents the morphology of selected

regions of the oropharynx as observed by light microscopy. Scanning electron microscopy (SEM) was employed to provide complementary data on the surface features of these regions.

3.1 MATERIALS AND METHODS

3.2.1. Experimental animals

The heads of five 12 – 14 month-old ostriches of either sex were obtained from a local ostrich abattoir (Ostriches Galore, Krugersdorp, Gauteng, South Africa) where the birds were slaughtered for their skin and meat. After the heads had been removed from the carcasses they were immediately immersed in plastic buckets containing 10% buffered formalin and allowed to fix for approximately 4 hours while being transported to the laboratory. At the laboratory the heads were immersed in fresh fixative (10% buffered formalin) for a minimum period of 48 hours. Care was taken to exclude air from the oropharynx and proximal oesophagus by wedging a small block of wood in the beak. Tissues were sampled and prepared for light microscopy (LM) and scanning electron microscopy (SEM) as outlined below.

3.2.2. Light microscopy (LM)

Samples from the roof and floor of the oropharynx were collected from various regions as indicated in Figure 3.1. Samples of the roof were taken from the palate (including the median palatine ridge) [Site 1], the transition zone between the pale and dark mucosa of the palate [Site 2], and from the pharyngeal folds. The latter were sampled at two sites; one to include the wall of the infundibular cleft in the vicinity of the opening of the Eustachian tubes [Site 3], and the other to include the retro-pharyngeal recess at the caudal aspect of the pharyngeal fold [Site 4]. Samples of the floor were collected from the interramal region (*regio interramalis*) [Site 5], the transition between the light and dark mucosa of the interramal region [Site 6], the tongue [Site 7] and the laryngeal mound (*mons laryngealis*) [Site 8]. Mid-sagittal sections of the tongue were used to view the dorsal, ventral and rostral surfaces. The laryngeal mound was sampled to include the lips of the glottis and the supporting arytenoid cartilage.

Samples for LM were dehydrated through 70, 80, 96 and 2X100% 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 embedded manually into paraffin wax in plastic moulds. Sections were cut at

4-6 µm, stained with Haematoxylin and Eosin (H & E), and viewed and micrographed using an Olympus BX50 equipped with the analySIS CC12 Soft Imaging System (Olympus, Japan).

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Scanning Electron Microscopy (SEM)

Samples for SEM were collected from sites adjacent to those sampled for light microscopy and included the palate, pharyngeal fold (at the infundibular cleft), interramal region and the tongue. Tissue blocks were rinsed in water for several hours to remove any traces of phosphate buffer and routinely dehydrated through an ascending ethanol series (50, 70, 90, 95 and 3X100%). Due to the size of the tissue blocks, each dehydration step took 60 minutes. Thereafter the blocks were critical point dried from 100% ethanol through liquid CO_2 in a Polaron E3000 Critical Point Drier (Polaron, Watford, England), attached to rectangular alluminium supports with Silver Dag to expose the epithelial surface and sputter coated with a thin layer of palladium in a Polaron SEM E5100 coating unit. The samples were viewed in a Philips XL 20 scanning electron microscope operated at 7kV.

3.2 RESULTS

3.3.1. Roof of the oropharynx

As observed macroscopically, the roof of the oropharynx displayed two distinct regions based on the colour of the mucosa; a pale rostral component and a darker staining caudal component concentrated around the choana and the infundibular cleft (see Chapter 2).

3.3.1.1 Pale rostral (non-glandular) component

This region was composed of a relatively thin yet heavily keratinised stratified squamous epithelium supported by an irregular dense connective tissue layer (lamina propria) (Figs. 3.2 and 3.3). The stratum basale consisted of a single layer of tightly packed columnar cells with dense, vertically oriented, elongated nuclei. The basal lamina was indistinct in H & E-stained sections. The stratum spinosum consisted of a number of cell layers. The layers immediately above the stratum basale displayed polyhedral cells containing a round, centrally-positioned nucleus. In the more superficial layers the cells progressively became more flattened to form the horizontally oriented cells of the stratum corneum. This transformation was accompanied by the accumulation of kerato-hyalin granules in the cell cytoplasm which was obvious in the superficial layers of the stratum spinosum and in the initial layers of the stratum corneum (Fig.

3.2). A clearly defined stratum granulosum was not apparent. The stratum corneum formed half the thickness of the epithelium and consisted of numerous layers of densely packed, flattened cells. The superficial layers were devoid of nuclei and a stratum disjunctum formed by layers of desquamating cells could be observed (Figs. 3.2 and 3.3).

The underlying lamina propria was thicker than the epithelium and consisted of variably oriented bundles of collagen fibres and interspersed blood vessels and nerves. No connective tissue papillae were observed to penetrate the epithelial layer and the interface between the lamina propria and epithelium was even except for occasional short projections carrying capillaries (Fig. 3.2). Glandular tissue was absent and there were no obvious signs of lymphatic tissue. The most conspicuous feature of this layer was the presence of randomly distributed, large, pale lamellated bodies lying within the deeper aspect of the lamina propria (Figs. 3.3 and 3.4). These structures exhibited round, ovoid or elongated profiles and displayed morphological features typical of Pacinian (Herbst) corpuscles (Fig. 3.2 – inset and Fig 3.6). The neural component (nerve terminal) 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 separated by obvious spaces. This region (the outer core) formed the bulk of the capsule surrounding the neuronal component and displayed relatively few nuclei. The entire corpuscle was closely invested by a thin, fibrous connective tissue layer displaying numerous fibroblast nuclei (Fig. 3.2 – inset). Myelinated nerves were sometimes observed in close asociation with the wall of the corpuscles. Immediately beneath the lamina propria was a layer of loose connective tissue characterised by the presence of large vascular and nerve plexuses and concentrations of adipose tissue.

A prominent feature of the rostral region was the presence of a median longitudinal ridge (*ruga palatina mediana*) running from from the choana to the tip of the beak (see Chapter 2). Transverse sections of the ridge revealed a mass of irregular dense connective tissue invested by the same keratinised stratified squamous epithelium described above. The ridge formed deep lateral recesses at its point of origin from the palate. Concentrated within the ridge was a U-shaped collection of between 10 to 15 large Pacinian corpuscles (100-250 µm in diameter) located around a core of loose connective tissue that was continuous with the same tissue lying beneath the base of the ridge (Fig. 3.3). A large artery typically lay in this tissue immediately beneath the ridge and was surrounded by numerous veins and nerves. Branches of these vessels and nerves proceeded into the core of the ridge where they surrounded the Herbst corpuscles.

At the point of transition between the pale rostral component of the palate and the darker appearing region, the keratinised stratified squamous epithelium displayed obvious morphological changes. The epithelium was observed to progressively thicken due to increased development of the stratum germinativum (mainly the stratum spinosum) whereas the stratum corneum reduced in thickness until it eventually disappeared (Fig. 3.4). This signalled the transformation of the keratinised epithelium into a non-keratinised type which displayed nucleated cells in the superficial layers of the stratum corneum. Despite becoming non-keratinised, desquamation of individual surface cells was still apparent. Accompanying the changes in the epithelium was the emergence of connective tissue papillae penetrating the epithelium and carrying capillaries. These structures were initially short and obliquely oriented but became longer and vertically inclined where the epithelium thickened and became non-keratinised (Fig. 3.4). The underlying connective tissue layers remained unchanged except that scattered lymphocytic infiltrations became more common, often associated with the ubiquitous Herbst corpuscles. At the point of transition, however, the lamina propria became filled with large, branched, tubular, mucus-producing glands (Fig. 3.5) which marked the junction between the pale, non-glandular rostral component of the roof of the oro-pharynx and the glandular, more caudal component.

In the glandular region the stratum basale presented as a layer of cuboidal, sometimes spherical cells. The stratum spinosum was the dominant layer with individual cells becoming progressively flattened to form the nucleated cells of the stratum corneum (Fig. 3.6). The lamina propria became completely filled with large, circular glandular units. Each unit consisted of numerous tubular secretory endpieces that radiated towards a central duct which opened onto the surface of the epithelium. Some of the endpieces showed evidence of coiling while others adopted a more flask-shaped appearance. The glands were classified as simple branched tubular, mucus-secreting glands. The secretory cells were typically columnar in nature and pale staining with basally compressed nuclei. Stringy accumulations of mucus were present in the lumen of the secretory endpieces and in the secretory duct. The glands were separated from each other by connective tissue septa carrying numerous blood vessels and nerves (Fig. 3.5). As a result of the accumulation of glandular tissue the vascular and nerve plexuses, as well as the decreasing number of Herbst corpuscles, were compressed into a narrow region of the lamina propria between the necks of the glands and the base of the epithelium (Figs. 3.5 and 3.6). Aggregations

of lymphatic tissue were also observed in this zone and were also associated with the capsule of the glands.

3.3.1.2 Darker caudal (Glandular) component

The darker mucosa of the roof of the oropharynx represented the glandular tissue of this region and exhibited the basic morphological features outlined above. However, in that part of the glandular region represented by the pharyngeal folds a dramatic increase in both the size of the glandular units and the accumulation of masses of lymphatic tissue was observed.

Transverse sections through the pharyngeal fold and the infundibular cleft revealed the following features. The surface epithelium was of the non-keratinised, stratified squamous type previously described and was characterized by the presence of numerous deep, regularly spaced connective tissue papillae. The lamina propria was filled with large, elongated, mucus-secreting glandular units that opened onto the surface via a single secretory duct for each unit. Each unit was enclosed in a capsule formed by the connective tissue of the lamina propria. Thinner strands from the capsule sub-divided the gland into smaller segments. The various connective tissue elements associated with the glands carried blood vessels and nerves emanating from the loose connective tissue beneath the lamina propria (Fig. 3.7). Dense masses of lymphatic tissue were present within many of the glands, often obliterating the normal structure of the gland (Fig. 3.10). The lymphatic tissue displayed both diffuse and nodular accumulations. The epithelium was generally not affected by invading lymphatic tissue although localized areas of infiltration were observed, mainly associated with the main secretory ducts of the glands. Herbst corpuscles were seldom observed in the glandular region of the roof of the oropharynx.

At the rim of the infundibular cleft the epithelium thinned markedly and the large glandular units were replaced abruptly by numerous, closely packed, simple tubular, mucus-secreting glands (Fig. 3.8). Throughout most of the length of the medial wall of the cleft the simple tubular glands and overlying epithelium were almost totally obliterated by dense masses of lymphatic tissue that contained numerous round to oval nodular aggregations (Figs. 3.8 and 3.11). In places the overlying epithelium invaginated to form deep, sub-divided tonsillar crypts, each surrounded by lymphatic tissue with many nodules. The lining of the crypts was reduced, in places, to a single layer of columnar, ciliated cells, although in most areas it appeared as a thin yet stratified layer with the superficial layer of cells again appearing ciliated. The epithelial invaginations also displayed occasional small groups of simple tubular glands.

At the caudal aspect of the pharyngeal folds where they formed a deep retro-pharyngeal recess a similar situation was apparent to that described for the infundibular cleft. The thick epithelium on the surface of the pharyngeal fold was again seen to thin appreciably where the mucosa folded to form the pharyngeal recess and the large glandular units were replaced by a row of simple tubular glands (Figs. 3.9 and 3.10). Obliteration of the epithelium and glands by lymphatic tissue was again obvious and the formation of tonsillar crypts surrounded by masses of nodular lymphatic tissue similar to that present in the inner wall of the infundibular cleft was evident (Figs. 3.10 and 3.12). Towards the base of the retro-pharyngeal recess the epithelium assumed its normal appearance (non-keratinised stratified squamous) and featured rows of closely packed simple tubular mucus-secreting glands with only a few patches of diffuse lymphatic tissue being present. It was clear from these observations that each pharyngeal fold represented a well-developed and immunologically active tonsil.

3.3.2. Floor of the oropharynx

The floor of the oropharynx was composed of the interramal region, the tongue and the laryngeal mound.

3.3.2.1 Interramal region

The extensive interramal region forming much of the floor of the oropharynx was also divided into two components based on colour differences (see Chapter 2). The pale component which made up most of this region was remarkably similar in histological structure to that of the rostral non-glandular component of the roof described above, but differed in the following respects. The surface was more undulating which was indicative of the numerous folds seen macroscopically. The stratum corneum of the keratinized stratified squamous epithelium was not as well-developed and constituted approximately a third to a quarter of the total thickness of the epithelium. Small, bulbous connective tissue papillae penetrated a short distance into the epithelium (Figs. 3.13 – 3.15). The features of the lamina propria and the underlying loose connective tissue, and the location of blood vessels and nerves, were also similar to that observed in the non-glandular region of the roof of the oropharynx (Fig. 3.15). Numbers of Herbst corpuscles were positioned relatively deep within the lamina propria and were concentrated in large numbers in the mucosal folds (Figs. 3.13 and 3.14). This was particularly obvious in the large median fold that divided the floor of the interramal region in two, and which demonstrated

as many as 18 corpuscles in some profiles. This fold was of similar structural design to that of the median palatine ridge described above, the most notable features being the concentration of Herbst corpuscles and the large artery situated at the base of the fold (Fig. 3.13).

At the point of transition between the pale rostral mucosa of the interramal region and the darker appearing zone around the tongue, the keratinized stratified squamous epithelium described above changed into a non-keratinised stratified squamous epithelium which showed a four-fold increase in thickness (Fig. 3.16). The connective tissue papillae became more regularly shaped, deeper and more evenly spaced than those in the keratinized part of the epithelium. Herbst corpuscles were still evident in the lamina propria but were situated far closer to the epithelium than before (Fig. 3.16), probably due to the thickening of the epithelial layer. Both the irregular dense and loose connective tissue elements revealed morphological features similar to those previously described.

3.3.2.2 The tongue

Macroscopically the blunt nature of the tongue presented three surfaces; dorsal, ventral and rostral. The epithelium covering the dorsal surface of the lingual fold was typically non-keratinised stratified squamous in nature (Fig. 3.17). The cells of the single layered stratum basale were round to cuboidal in shape and covered by a thick stratum spinosum displaying the typical polyhedral cells and intercellular bridges characteristic of this layer. These cells were observed to flatten, giving rise to the six to eight layers of flattened cells with horizontally elongated nuclei that constituted the stratum corneum. Numerous individual cells were seen to desquamate from the surface of the epithelium, a phenomenon graphically illustrated in SEM preparations (see below). The connective tissue papillae penetrating the epithelium in this region were relatively short and often obliquely oriented. The underlying layer of irregular dense connective tissue (lamina propria) contained a single layer of small, evenly spaced, simple branched tubular mucus-producing glands surrounded by blood vessels and nerves (Fig. 3.17).

The ventral surface of the lingual fold revealed similar properties to that of the dorsal surface except that the lamina propria housed a row of massive rectangular glandular units (simple branched tubular mucus-producing glands) that opened into the lumen of the lingual pocket (Fig. 3.17). The lamina propria within the dorsal lingual fold was effectively divided into two parts by a thin layer of loose connective tissue emanating from the base of the fold, one half housing the smaller glands and the other the larger units (Fig. 3.17). At the point where the surface

epithelium of the tongue, having formed the dorsal lingual fold, was again reflected caudally, the large glandular units were abruptly replaced by a sheet of simple tubular mucus-producing glands located just beneath the epithelium. This arrangement was obvious along the rest of the lingual surface, including the secondary lingual fold, becoming continuous with the epithelial lining of the proximal oesophagus,

The epithelium covering the rostral and ventral aspects of the tongue was similar to that described on the dorsal surface except that the connective tissue papillae were more numerous, deeper, and vertically oriented. At the transition between the dorsal and rostral surfaces the layer of small glands was gradually replaced by larger units (Fig. 3.18). These glands continued along the ventral surface of the tongue and ended abruptly where the epithelium was reflected onto the frenulum. The secretory cells of all the glandular tissue were columnar elements with a pale, almost granular cytoplasm and basally compressed nuclei. Occasional aggregations of diffuse lymphatic tissue were observed, generally within the glandular tissue, but which formed a small part of the tongue parenchyma. The rostral projection of the basihyale supporting the tongue parenchyma (Fig. 3.18) was composed of hyaline cartilage and was surrounded by a distinct perichondrium, skeletal muscle elements (concentrated on the ventral surface) and loose connective tissue containing numerous blood and lymphatic vessels as well as fat cells. In some sections profiles of the paired paraglossals (also composed of hyaline cartilage) were observed.

3.3.2.3 The laryngeal mound

The laryngeal mound was lined by a non-keratinised stratified squamous epithelium similar to that investing the tongue. The supporting lamina propria was, as in the rest of the oropharynx, composed of irregular dense connective tissue and well-supplied with blood vessels and nerves. The lips of the glottis were filled with large, simple, branched tubular mucus-secreting glands similar to those previously described (Fig. 3.19). At the point where the epithelium of the glottis formed the lining of the larynx (at the level of the arytenoid cartilage), the large glands abruptly ended and were replaced by a layer of simple tubular mucus-secreting glands. This was accompanied by a marked thinning of the epithelium. The large glands also petered out a short distance beyond the base of the glottis where it joined the laryngeal mound. Here the connective tissue papillae appeared less well organised and developed than in the rest of the glottis (Fig. 3.19). The mound displayed no glandular tissue and was lined only by the epithelium and supporting lamina propria. Diffuse and nodular lymphatic tissue was apparent in the lips of the glottis (often associated with the glands) and in the laryngeal mound at the base of the glottis.

3.3.3. Scanning electron microscopy

3.3.3.1. Roof of the oropharynx

All regions of the palate were characterised by obvious and dense desquamation of the superficial cells of the epithelium. The rostral aglandular region demonstrated what appeared to be a smooth surface at low magnification and which revealed large plates of desquamating cells (Figs. 3.20 and 3.21). The transition to the glandular region was abrupt, the latter region being distinguished by the presence of round to slit-like openings which were shown by LM to represent the openings of the secretory ducts of the underlying glands (Fig. 3.21 and 3.22). The openings were frequently filled with cell debris and glandular secretions (Fig. 3.24 and 3.26). The opening itself was surrounded by a concentric arrangements of surface cells, many of which were in the process of desquamating (Fig. 3.23). Higher magnification of the glandular area showed the desquamation of numerous individual cells and tracts of mucus being released from the gland openings. The mucus sometimes accumulated in the form of extensive sheets on the epithelial surface. The glandular region in the vicinity of the infundibular cleft revealed similar surface morphology and was in places characterised by mucus from neighbouring duct openings merging to form geometric patterns (Fig. 3.25).

3.3.3.2. Floor of the oropharynx

Low magnification SEM of the interramal region revealed that a complex system of smaller mucosal folds were situated on the floor of the mouth and which emanated from the deeper aspects of the longitudinal folds described macroscopically (see Chapter 2). The smaller folds emerged at an angle from the primary folds and branched and anastomosed, forming an intricate network that enclosed numerous small openings. The larger longitudinal folds displayed a series of transverse grooves and ridges (Fig. 3.27) and all surfaces were covered by a layer of desquamating, clearly defined, plate-like cells (Fig. 3.28). All surfaces of the tongue and the glottis revealed features similar to those seen in the glandular region on the roof of the oropharynx (Figs. 3.29 – 3.31 and Fig. 3.33). The duct openings of the underlying lingual glands were again the most obvious feature and were seen to contain plugs of mucus and trapped, desquamated epithelial cells (Figs 3.30 – 3.31 and 3.33). Cell sloughing was also obvious on all surfaces and involved single cells or small groups of cells (Figs. 3.29 – 3.31 and Fig. 3.33). The aglandular surface of the laryngeal mound beyond the lips of the glottis displayed only desquamating epithelial cells (Fig. 3.32).

3.4. DISCUSSION

3.4.1 Microscopical features

Epithelium

The results of the present study suggest that, although presenting little variation in morphology, the epithelium of the oropharyngeal cavity of the ostrich showed some similarities to that of other avian species (Ziswiler & Farner, 1972; McLelland, 1979). As in other birds, the epithelium in the ostrich was of the stratified squamous type which in some regions was keratinized. In the current study the keratinization was seen to be restricted to the rostral palate and to the rostral part of the interramal region, representing the pale rostral components of the mucosa found in both the roof and floor of the oropharynx. This appears to also be the situation in the rhea where the keratinised epithelium is reportedly confined to the oral cavity (Feder, 1972). However, towards the choana in the roof of the pharynx, dorsally, and the region beneath the tongue and laryngeal mound, ventrally (representing the darker caudal component in both parts of the oropharynx), there was a sharp transition from the strongly keratinised stratified squamous epithelium to a non-keratinised stratified squamous epithelium. This transition of epithelial types was in agreement with what has been reported by Ziswiler and Farner (1972) in other avian species. Ziswiler and Farner (1972) and Hodges (1974) affirmed that in avian species the occurrence of keratinisation is restricted to the roof of the orpharyngeal cavity and to the surface of the tongue. In contrast to the situation in most birds where the surface of the tongue is reported to be keratinised, the ostrich tongue reveals a non-keratinised epithelium. A similar report in the rhea (Feder, 1972) would seem to indicate that ratites possibly differ from other avian families in this respect.

Herbst Corpuscles

In the underlying irregular connective tissue beneath the epithelium of the palate and the interramal region of the ostrich was the notable presence of Herbst corpuscles. Although individual corpuscles were sometimes observed in the glandular regions of the oropharynx, they were mostly restricted to the keratinised regions of the oral cavity. Herbst corpuscles with a similar distribution have also been described in the rhea (Feder, 1972). This observation correlates well with the research findings on Herbst corpuscles previously reported in the domestic fowl (Winkelman & Myers, 1961; Andersen & Nafstad, 1968; Wight *et al*., 1970; Ziswiler & Trunka, 1972; Calhoun, 1974), Japanese Quail (Warner *et al.,* 1967), geese (Gottschaldt & Lausmann, 1974) and ducks (Berkhoudt, 1976). This study also revealed that the Herbst corpuscles observed

in the ostrich oropharynx were structurally similar to those described in other birds (Winkelmann & Myers, 1961; Andersen & Nafstad, 1968; Wight *et al*., 1970; Ziswiler & Trnka, 1972). The results of the present study indicated that Herbst corpuscles are concentrated in the median longitudinal ridge (*ruga palatina mediana*) of the palate and also in the median fold (as well as in smaller folds) located in the interramal region. The concentration of large numbers of these bodies in the median palatine ridge appears to be a unique feature, particularly when compared to the lateral location of Herbst corpuscles reported beneath the hard palate of the domestic fowl (Wight *et al.*, 1970). It is suggested, based on the observation of Ross and Pawlina (2006) that the Herbst corpuscles concentrated in the above mentioned areas should be considered to function as "deep pressure receptors for mechanical and vibratory pressure." The presence of Herbst corpuscles in the ostrich oropharynx, particularly in the apparent absence of taste buds, may very well indicate that these structures play a role in determining the texture of ingested material.

Glands

The glandular tissue of the oropharynx was located in the underlying irregular dense connective tissue beneath the epithelium and consisted exclusively of mucus-producing units. Two types of mucus glands were described. The most abundant type formed large, branched, tubular glands and were distributed in the roof of pharynx (caudal region of the palate and the pharyngeal folds) and in the tongue. The second type comprised smaller simple tubular glands that were distributed in the transition zones between the caudal limits of the oropharynx and the proximal oesophagus, both dorsally and ventrally. In fowl the occurrence of glands in the roof, floor, body of the tongue and in the pharyngeal walls is a common feature (Fahrenholz, 1937; Ziswiler and Farner, 1972). The characteristics of the glands in the domestic fowl (McCallion & Aitken, 1953) showed similarities with the observations in the present study, where it was noted that each mass of glands was composed of a number of units, each of which consisted of a number of lobules composed of many secretory endpieces opening into a common cavity. The common cavity drained the secretion into a duct which opened onto the surface of the epithelium. Statements concerning the glands and the presence of taste buds in avian species has been described by Bath, (1906); Botezat, (1904, 1906, 1910), Greschik, 1971a, b; Warner *et al*., 1967; McLelland, 1979. According to these authors, the presence and distribution of taste buds in avian species appears to be more abundant in the soft part of the palate, around the glottis, beneath the tongue and scattered in areas near the salivary glands where it is associated with the ducts of these glands. However, in the present study no taste buds were present in the oropharynx of the ostrich. Therefore, it is suggest that further studies be carried out to finally determine the presence or not of taste buds in the oropharynx of the ostrich.

Lymphatic Tissue

The results of the current study indicate that the irregular dense connective tissue layer beneath the epithelium was often infiltrated by lymphatic tissue accumulations either diffuse or nodular. These lymphocytic aggregations were frequently associated with the glands, except in underlying connective tissue of the palate and the interramal region where they were associated with the Herbst corpuscles. Additionally, it was noted in the present study that lymphatic tissue either diffuse or in aggregations, was closely associated with the glandular tissue, particularly with the connective tissue septa between the glands. A similar observation was made by Hodges (1974) in avian species. He described lymphoid tissue as frequently found in the connective tissue septa of the glands of adult birds. The lymphatic tissue also formed massive diffuse and nodular aggregations in the pharyngeal folds which suggested the presence of "tonsils" in this region. Supporting these findings, McLelland (1979) reported the presence of the lymphatic nodules (*lymphonoduli pharyngeales*) in the roof of the pharynx, in the region of the pharyngeal cleft and in the pharyngotympanic infundibulum in birds. This work represents the first histological evidence of pharyngeal tonsils in the ostrich.

3.4.2. Scanning electron microscopy (SEM)

The SEM findings confirmed the LM observations related to the occurrence and distribution of glandular tissue in the oropharynx and also revealed the transition between the keratinized (non-glandular) and non-keratinised (glandular) regions. The most obvious features identified were: (a) the distinct desquamation of surface cells from both the keratinised and non-keratinised regions of the epithelium, (b) the concentric arrangement of the lining cells of the glandular duct openings and (c) the strands of mucus associated with the duct openings. No specialized surface features were observed and due to a lack of similar information in other bird species no specific conclusions can be drawn.

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http://people.eku.edu/ritchisong/birddigestion.html
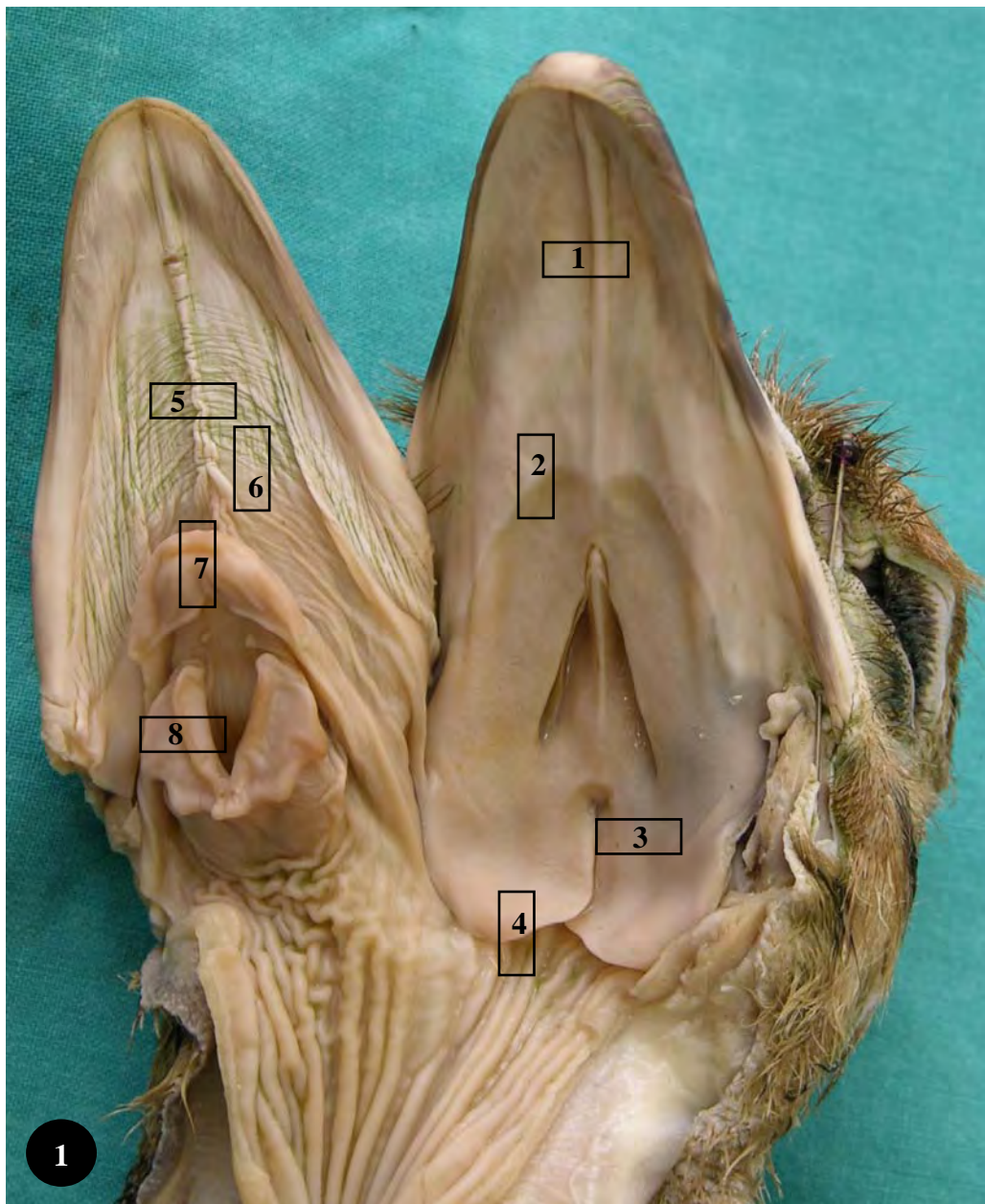
Figure 3.1: View of the roof and floor of the oropharynx indicating the various sampling sites for light microscopy and scanning electron microscopy. Site 1 – palate (including the median palatine ridge); Site 2 – transition zone between the pale and dark mucosa of the palate; Site 3 – pharyngeal fold including the wall of the infundibular cleft in the vicinity of the opening of the Eustachian tubes; Site 4 – pharyngeal fold including the retro-pharyngeal recess; Site 5 – the interramal region; Site 6 – the transition between the light and dark mucosa of the interramal region; Site 7 – mid-sagittal sections of the tongue; Site 8 – the laryngeal mound including the lips of the glottis. Sections for scanning electron microscopy were taken from adjacent sites.
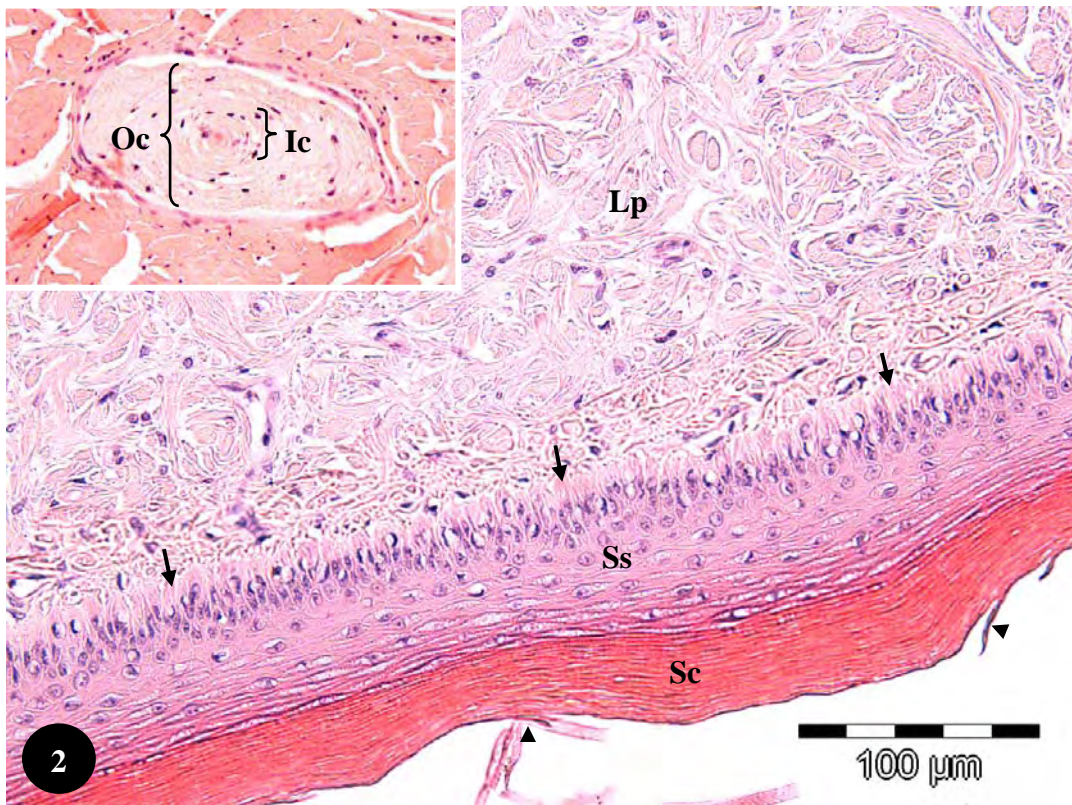
Figure 3.2. High power view of the aglandular region of the palate. Note the thickness of the stratum corneum (Sc) in relation to the stratum spinosum (Ss) and stratum basale (arrows). Sloughing of the surface cells is obvious (arrowheads) The lamina propria (Lp) consists of irregular dense connective tissue. Inset: A Herbst corpuscle displaying a characteristic lamellated appearance. An inner core (Ic) contains the axon and in turn is surrounded by the outer core (Oc).
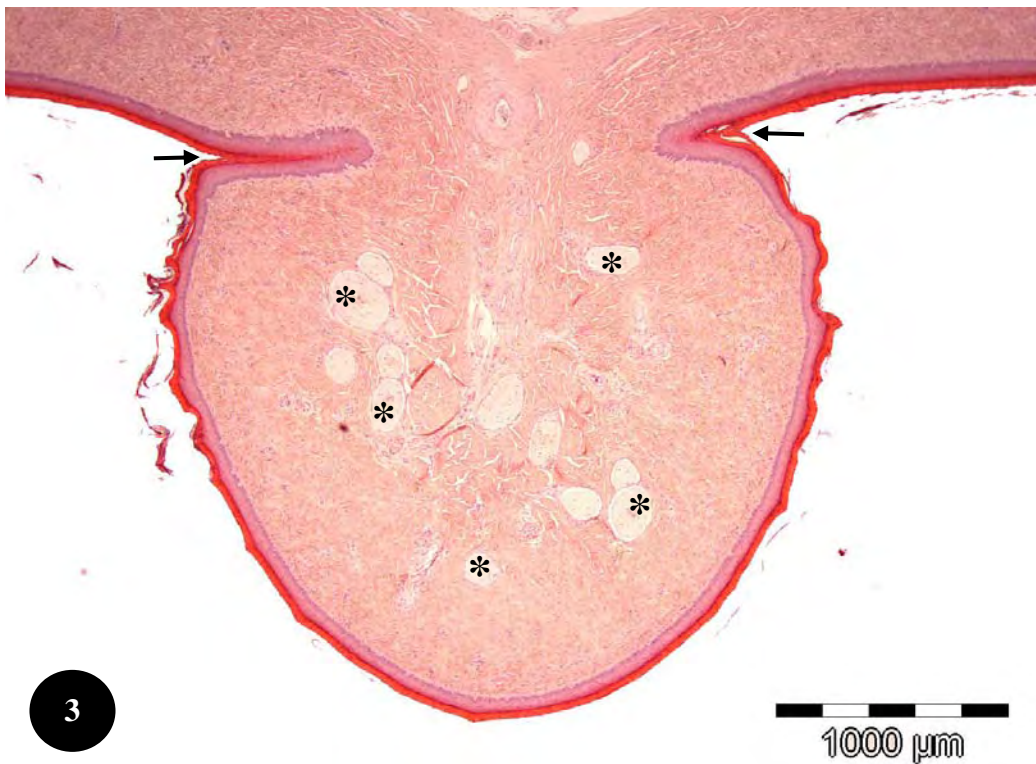
Figure 3.3: A transverse section of the medial longitudinal ridge within the non-glandular part of the palate. Note the lateral recesses formed where the ridge extends from the surface of the palate (arrows) and the U-shaped collection of Herbst corpuscles (asterisks) concentrated around the vascular core of the ridge. The epithelial features are similar to those seen in Figure 1 above.
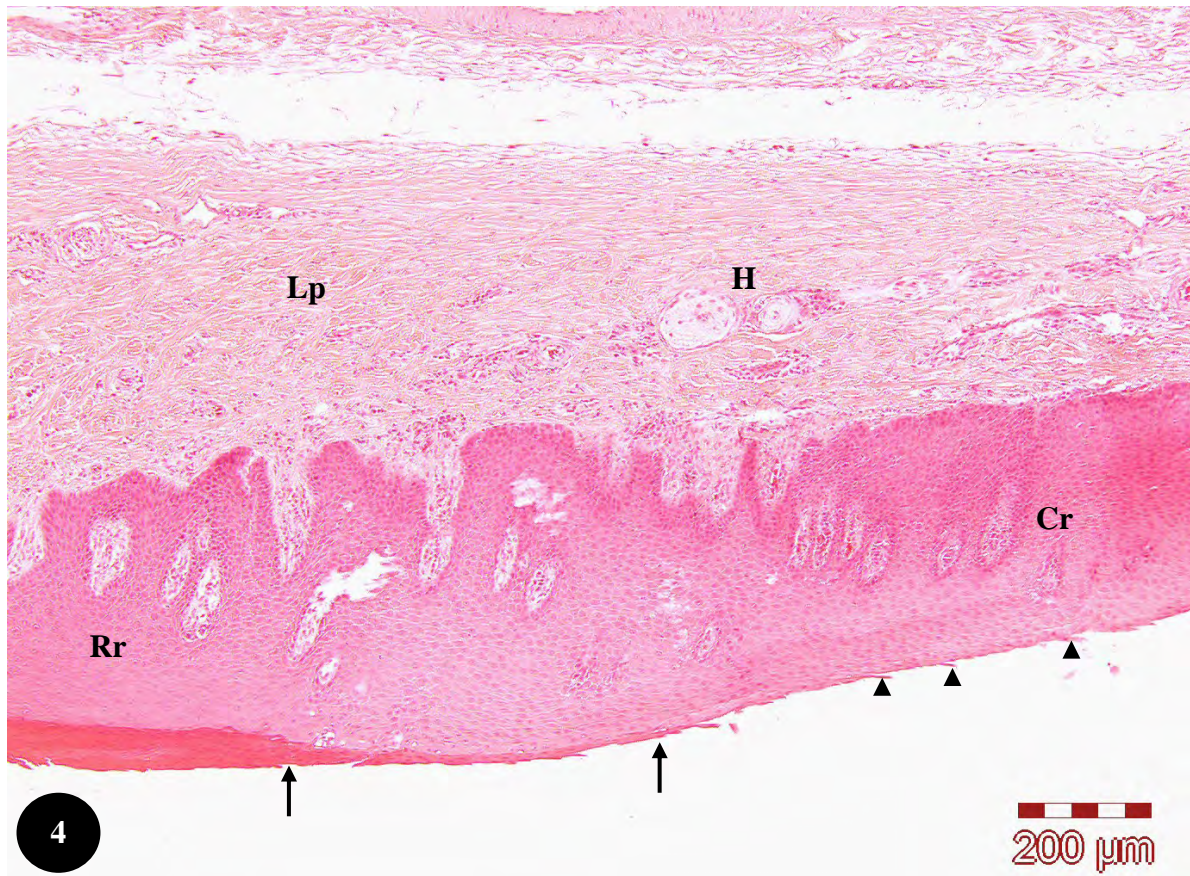
Figure 3.4: A section of the palate showing the transition between the keratinised stratified squamous epithelium of the non-glandular rostral region (Rr) and the thicker non-keratinised stratified squamous epithelium of the caudal glandular region (Cr) just prior to the appearance of the glandular tissue. Note the gradual loss of the keratinised layer (between arrows). Two Herbst corpuscles (H) appear in the lamina propria (Lp). Desquamation of surface cells of the non-keratinised epithelium is evident (arrowheads).
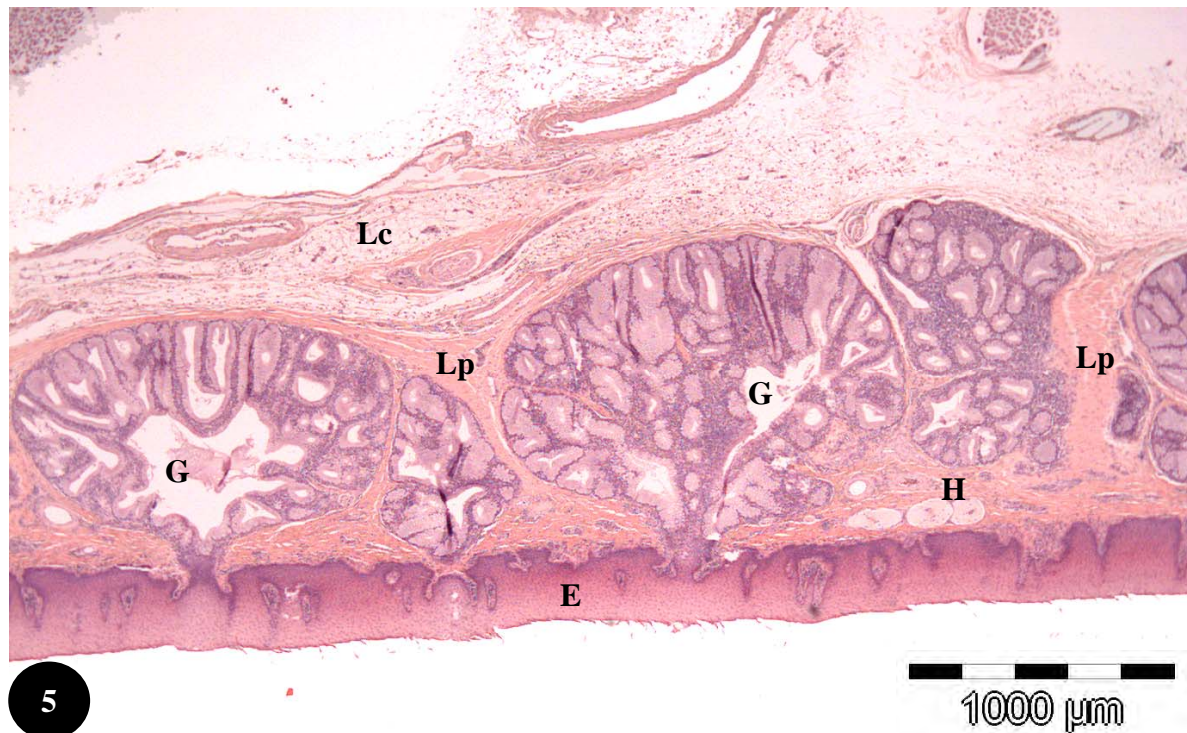
Figure 3.5: Initial segment of the glandular region just caudal to the area depicted in Figure 3 above. The non-keratinised epithelium (E) displays numerous connective tissue papillae and the lamina propria (Lp) is filled with large, round glandular units (G). Three Herbst corpuscles (H) lie close to the epithelium and large blood vessels and nerves lie in the deeper loose connective tissue (Lc).
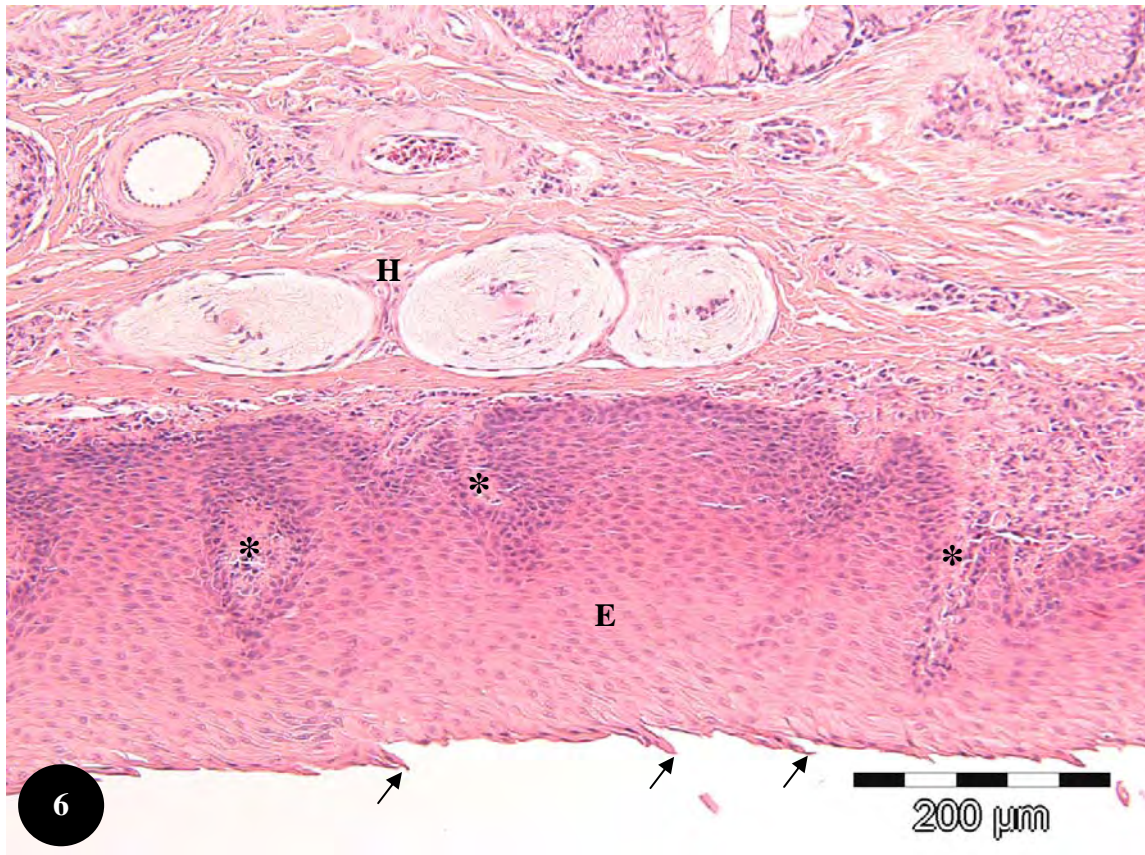
Figure 3.6: A higher magnification of the three Herbst corpuscles (H) illustrated in Figure 3.5 above. Note their lamellated appearance and close proximity to the epithelium (E). Connective tissue papillae (asterisks) are observed to penetrate deep within the epithelium. Note the sloughing of the superficial cells of the stratum corneum (arrows).
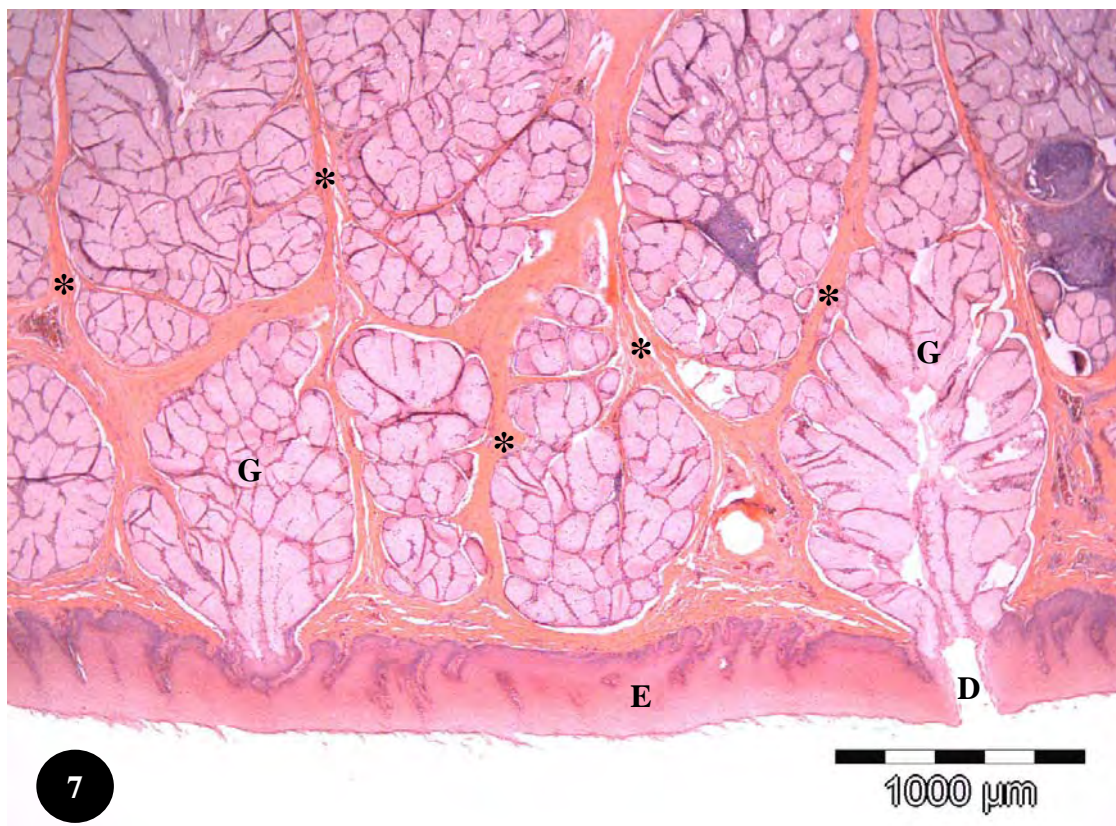
Figure 3.7: Section of the pharyngeal fold showing the closely packed, rectangular glands (G) found in this region. The branched tubular secretory endpieces empty into a single, large secretory duct (D) that opens onto the surface of the epithelium (E). Note the connective tissue strands from the lamina propria encapsulating the glands (asterisks).

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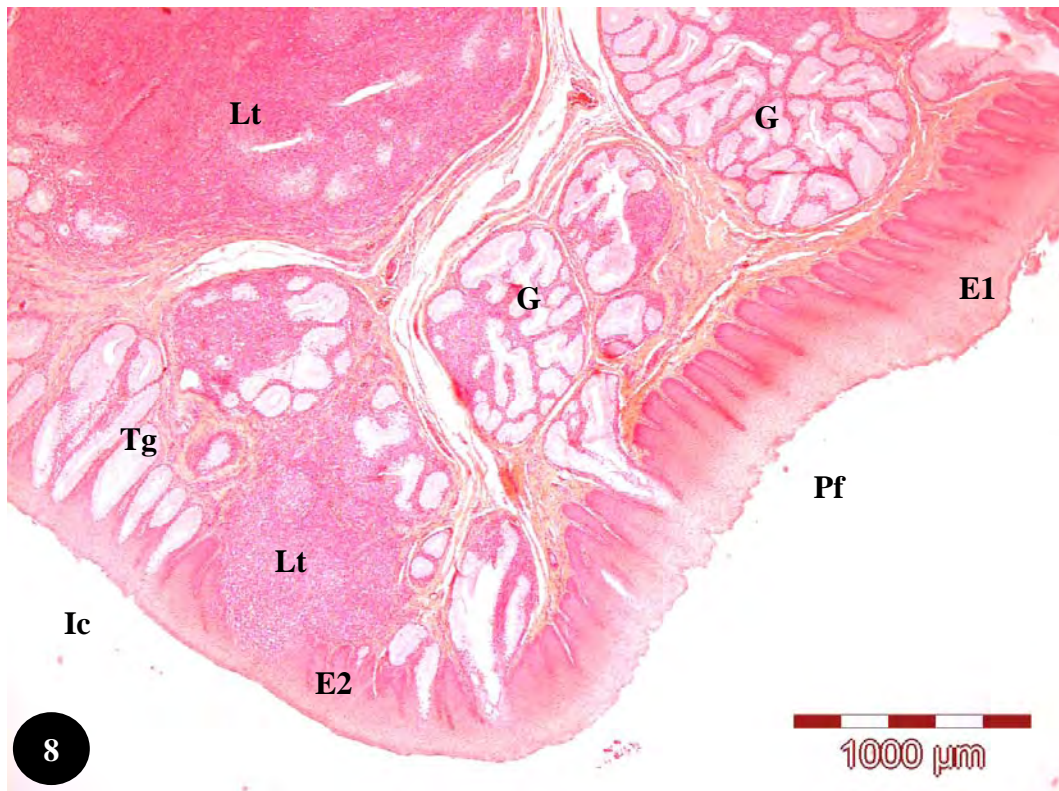
Figure 3.8: Rim of the infundibular cleft showing the transition from the surface of the pharyngeal fold (Pf) to the wall of the infundibular cleft (Ic). The pharyngeal aspect of the fold displays a thick non-keratinised epithelium (E1) with numerous connective tissue papillae and large underlying glands (G). In contrast, the wall of the infundibular cleft reveals a thinner epithelium (E2), infiltrations of lymphatic tissue (Lt) and a row of simple tubular, mucous-secreting glands (Tg).
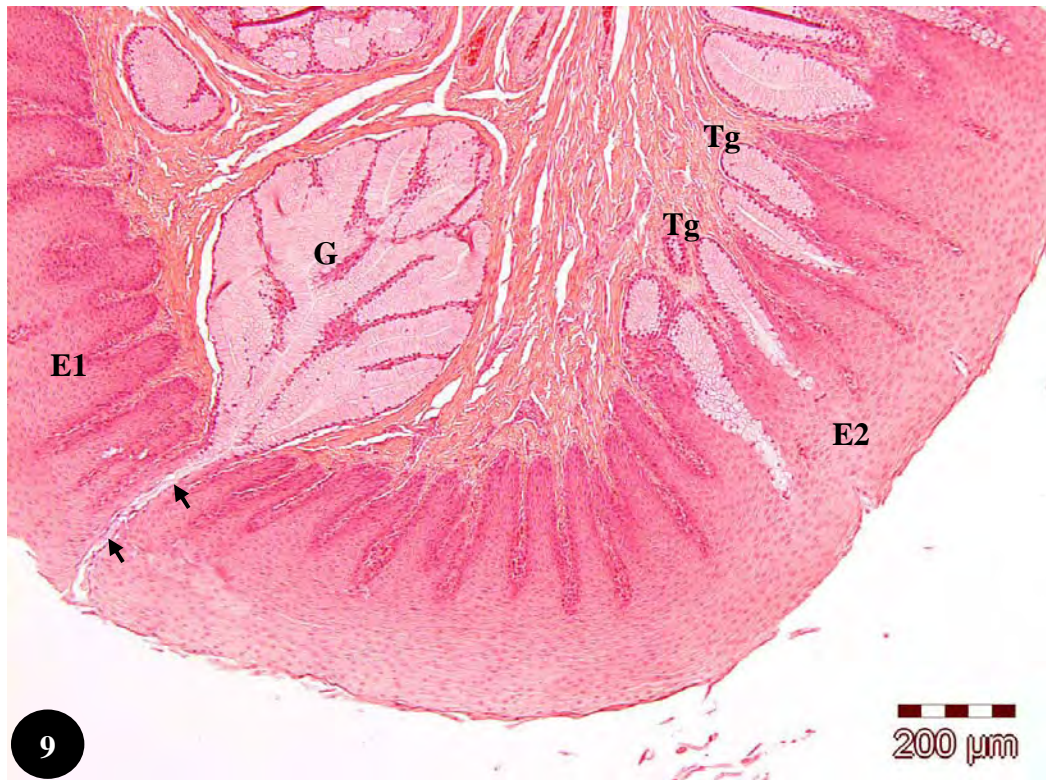
Figure 3.9: A similar transitional region to that shown in Figure 3.8 but in this instance illustrating the caudal aspect of the pharyngeal fold where it is reflected to form the retropharyngeal recess. The sequence of morphological changes is identical to that described in Figure 3.8 above. Large, branched tubular, mucous-producing glands (G), epithelium lining the surface of the pharyngeal fold (E1), epithelium lining the retropharyngeal recess (E2), simple tubular glands (Tg). Note the long duct (arrows) linking the gland (G) to the surface of the epithelium.
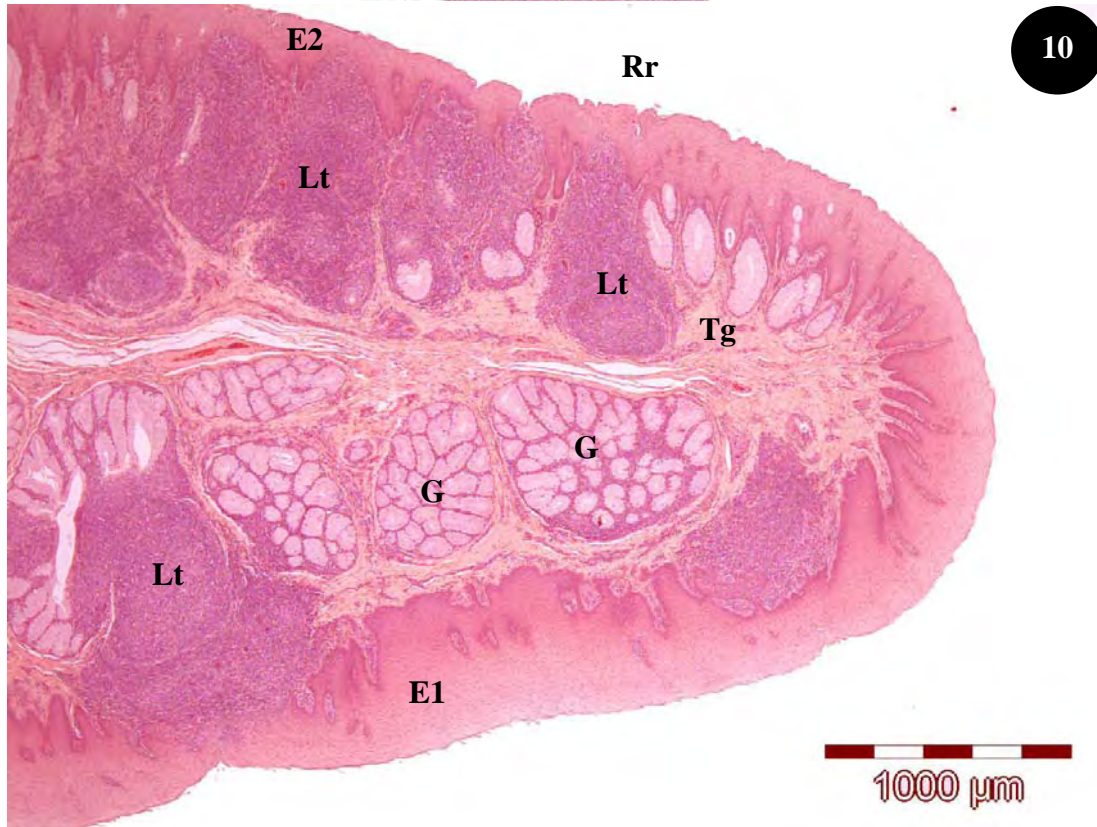
Figure 3.10: A lower magnification micrograph of the caudal aspect of the pharyngeal fold showing the complete transition in this region from the thick non-keratinised epithelium (E1) and large underlying glands (G) to the thinner epithelium (E2), infiltrations of lymphatic tissue (Lt) and row of simple tubular, mucous-secreting glands (Tg) typical of the lining of the retropharyngeal recess (Rr). Most of the simple tubular glands have been obliterated by the lymphatic tissue.
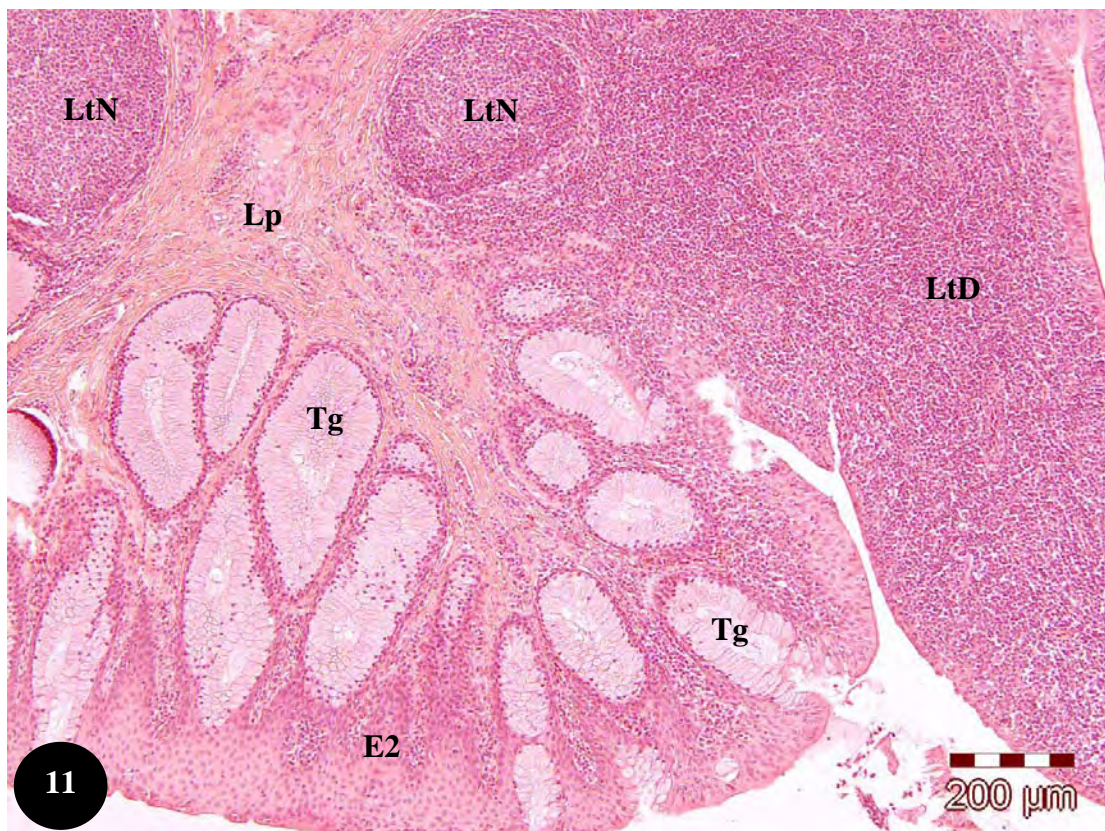
Figure 3.11: Section from the wall of the infundibular cleft showing the obliteration of the simple tubular glands (Tg) by diffuse (LtD) and nodular (LtN) lymphatic tissue. Epithelium (E2), Lamina propria (Lp).
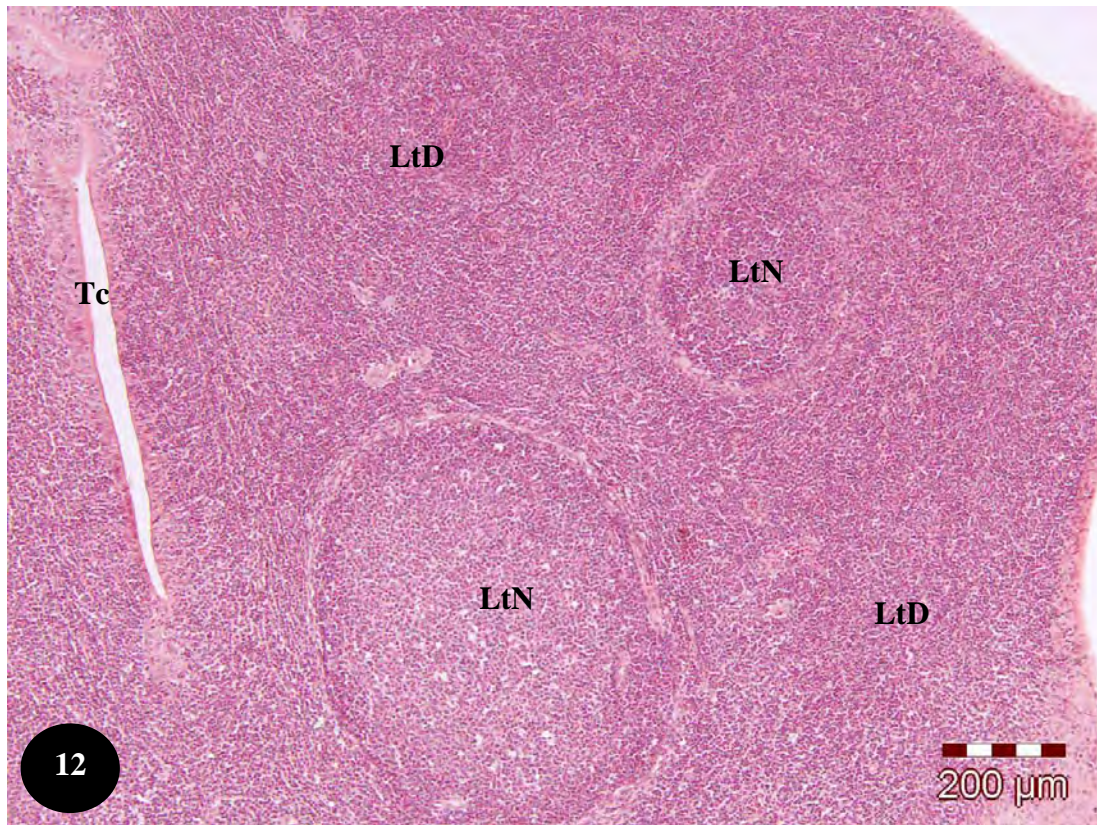
Figure 3.12: The tonsillar tissue of the pharyngeal fold composed of masses of diffuse (LtD) and nodular (LtN) lymphatic tissue. A small section of one of the tonsillar crypts (Tc) is evident.
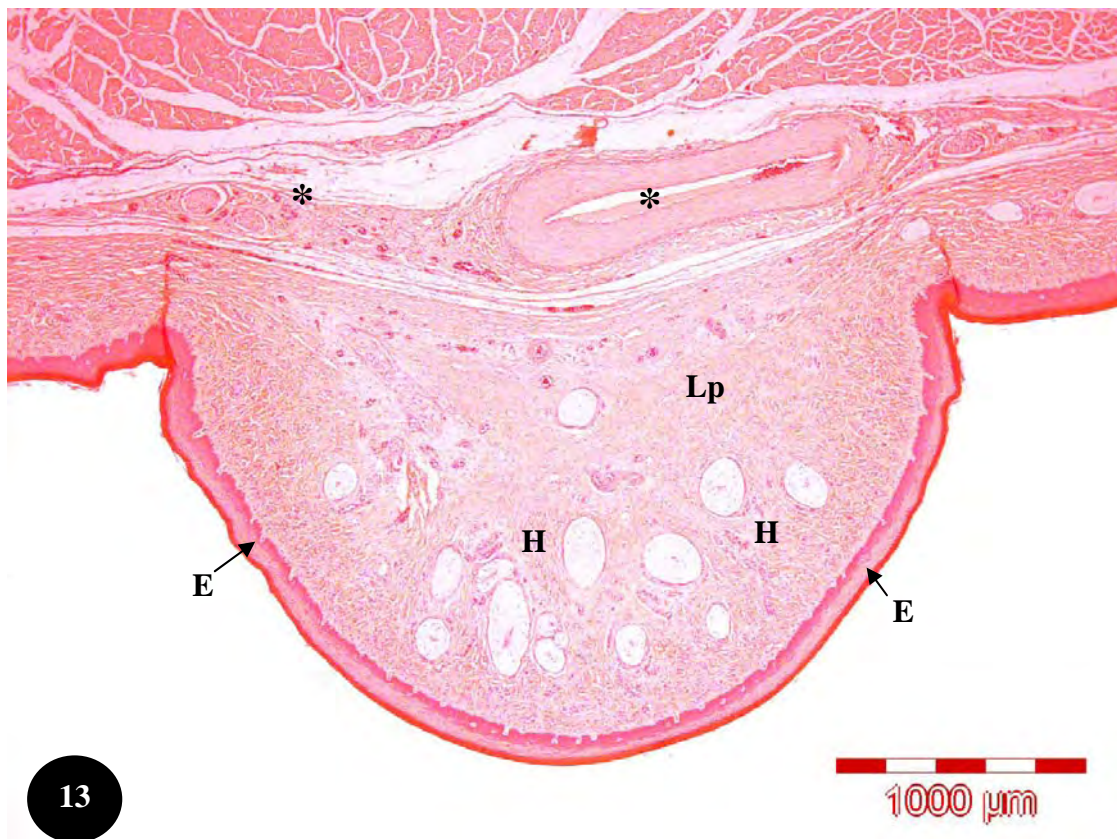
Figure 3.13: A transverse section through the median fold on the floor of the interramal region of the oropharynx. Note the similarity between this fold and the median palatine ridge found on the palate in respect of the concentration of Herbst corpuscles (H) and the large blood vessels and nerves (asterisks) within loose connective tissue situated at the base of the fold. Lamina propria (Lp), epithelium (E).

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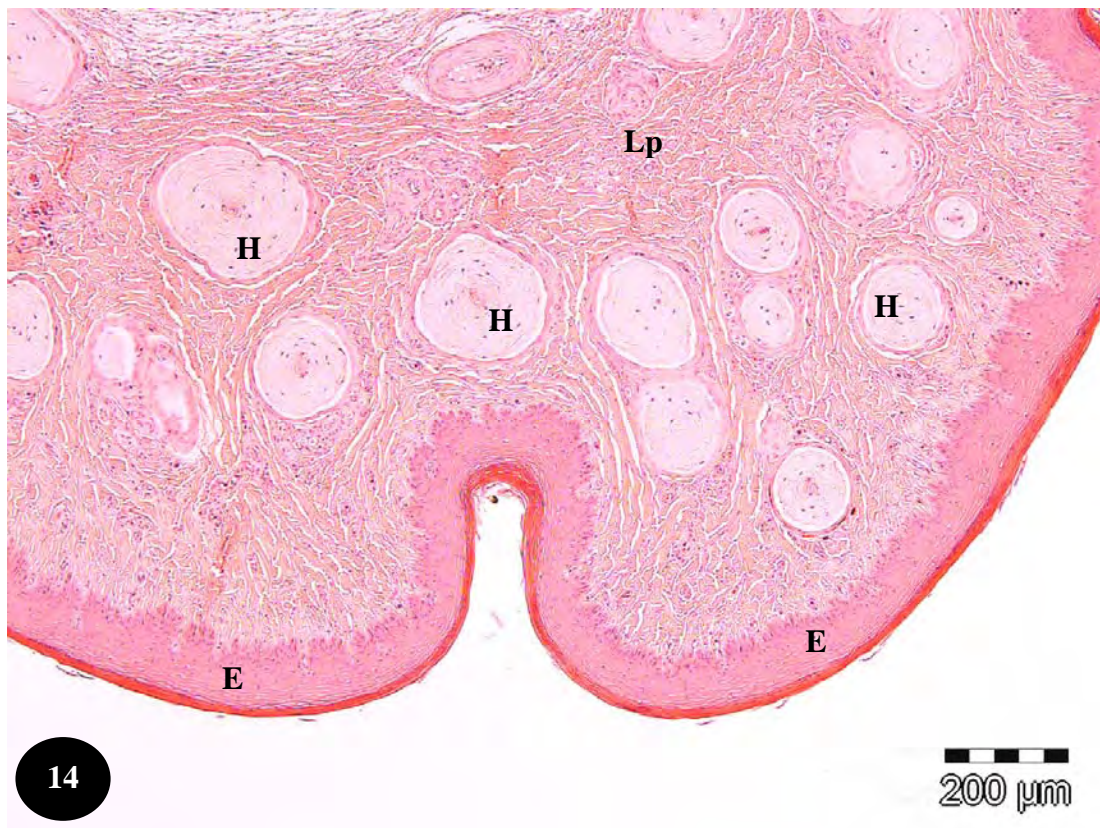
Figure 3.14: Higher magnification of a smaller, double fold from the interramal region. The Herbst corpuscles (H) are again conspicuous within the lamina propria (Lp). Epithelium (E).
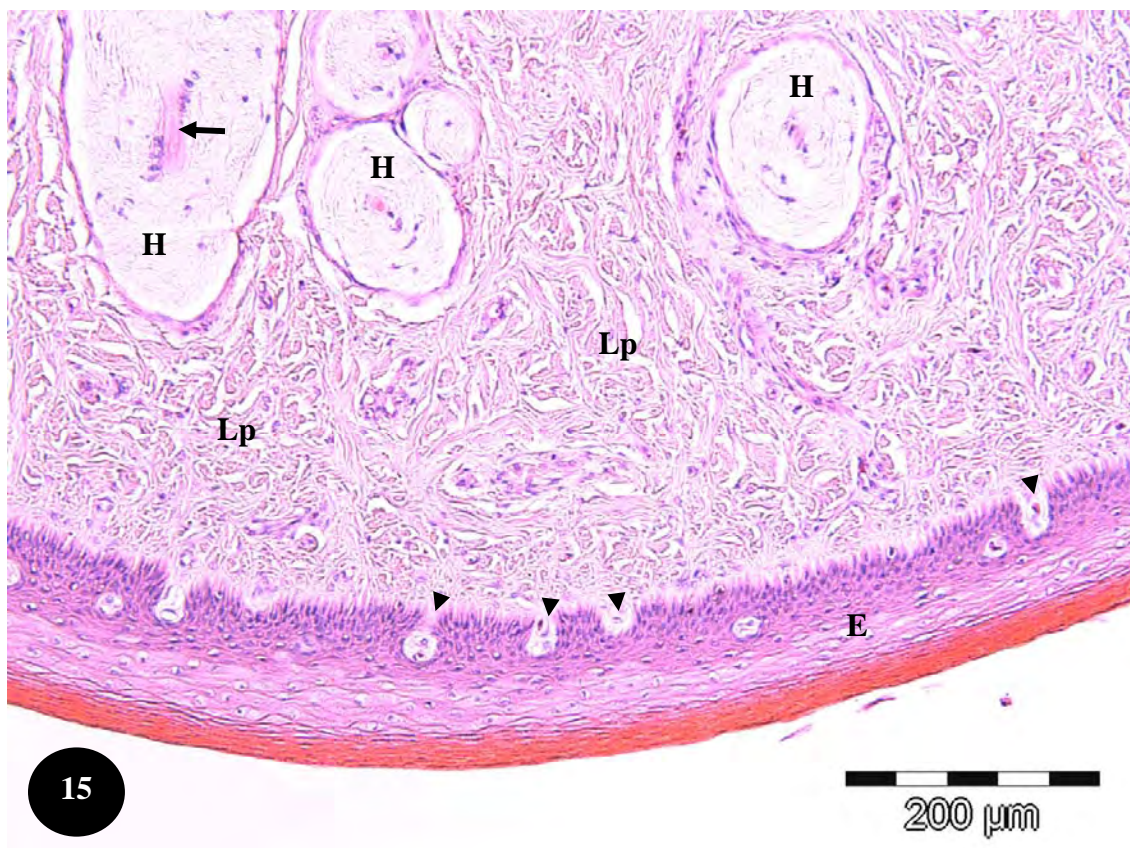
Figure 3.15: Higher magnification of one of the folds in the interramal region illustrating the lamellated appearance of the Herbst corpuscles (H), one of which demonstrates the nerve terminal in longitudinal section (arrow). Note the irregular dense connective tissue of the lamina propria (Lp), the small, bulbous connective tissue papillae (arrowheads) and the various layers of the keratinised stratified squamous epithelium (E).

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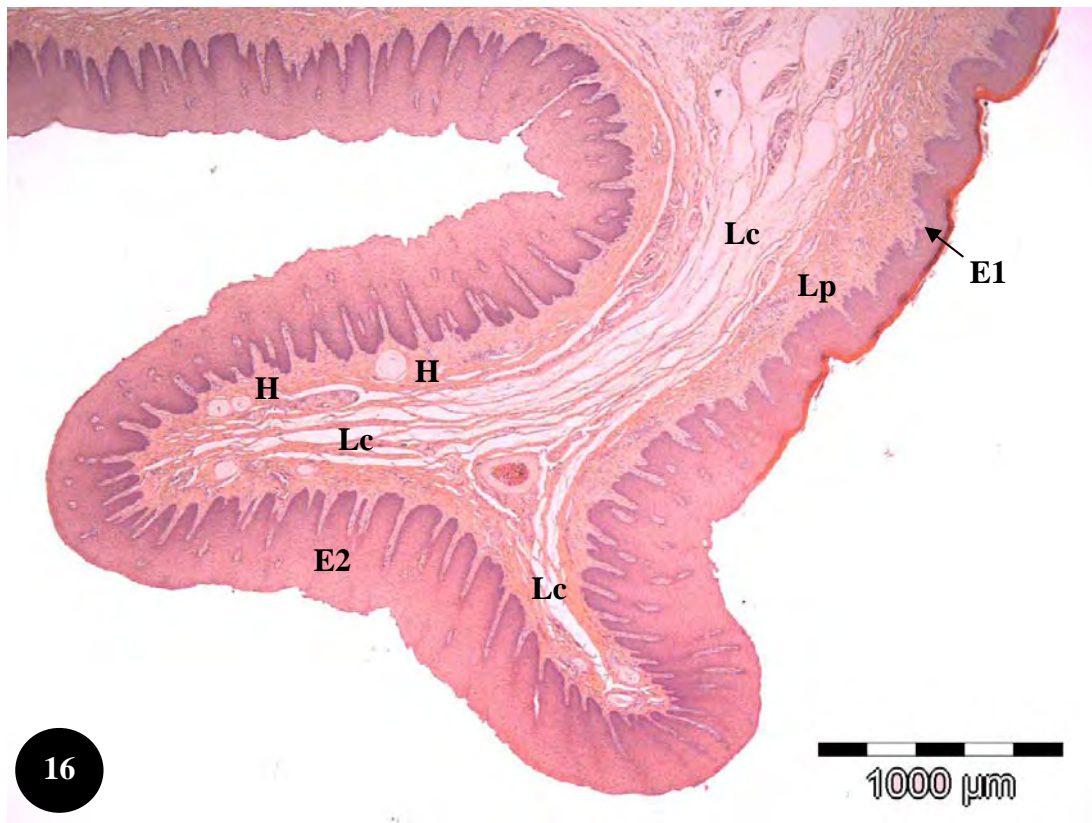
Figure 3.16: A tissue fold from the interramal region demonstrating the transition from a thin keratinised stratified squamous epithelium (the pale rostral component) (E1) to a thick, non-keratinised stratified squamous epithelium (the darker region in the vicinity of the tongue) (E2). Note the well-developed connective tissue papillae in the thicker epithelium, some Herbst corpuscles (H) in the lamina propria (Lp) and the core of loose connective tissue (Lc) within the folds.
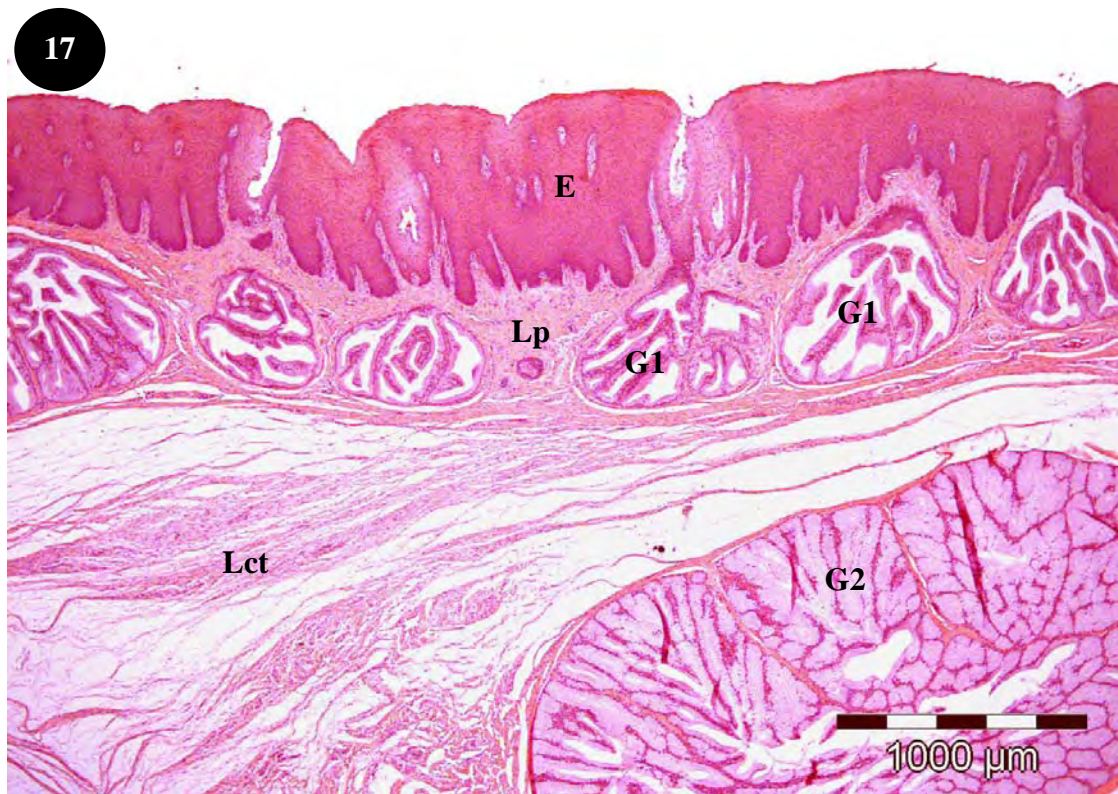
Figure 3.17: The dorsal surface of the dorsal lingual fold showing the thick, non-keratinised stratified squamous epithelium (E), the row of small, branched tubular, mucous-producing glands (G1) in the lamina propria (Lp) and the large, branched tubular, mucous-producing glands (G2) which open on the ventral surface of the lingual fold (not shown). Loose connective tissue (Lct).
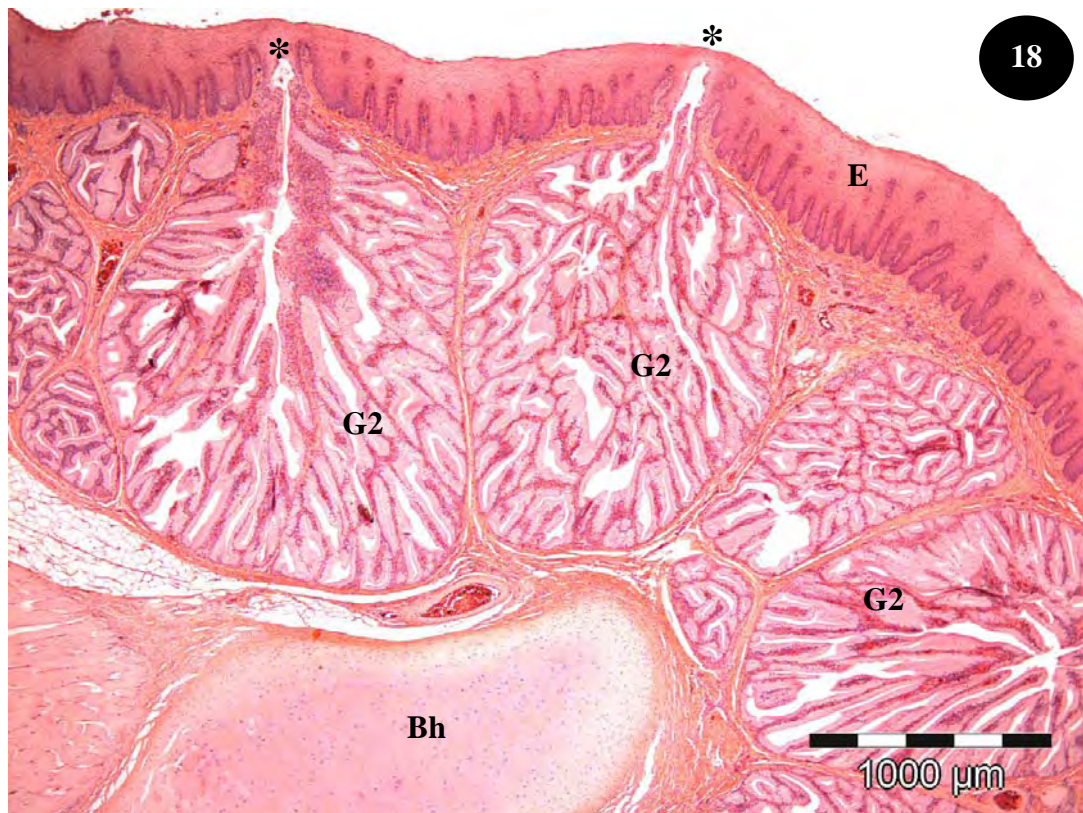
Figure 3.18: The rostro-ventral surface of the tongue showing the large, branched tubular, mucous-producing glands (G2) found in this region. The ducts of two glands (asterisks) are seen opening onto the ventral surface of the tongue. The epithelium (E) displays numerous well-developed connective tissue papillae and the tip of the cartilaginous rostral process of the basihyale (Bh) is visible.
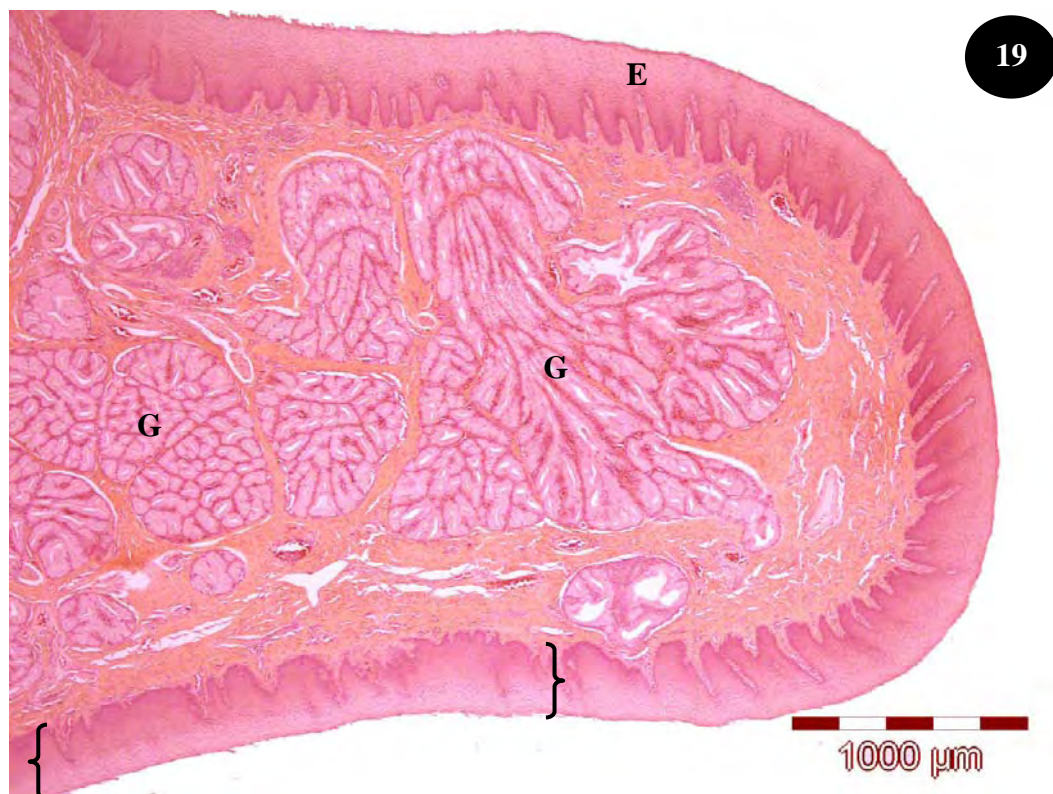

Figure 3.19: The rim of the glottis revealing the concentration of large, branched tubular, mucous-producing glands (G) situated within the irregular dense connective tissue core. The non-keratinised stratified squamous epithelial lining (E) appears similar throughout, although the connective tissue papillae in that part of the epithelium continuous with the laryngeal mound (not shown) appear less well-organised and developed (between brackets).
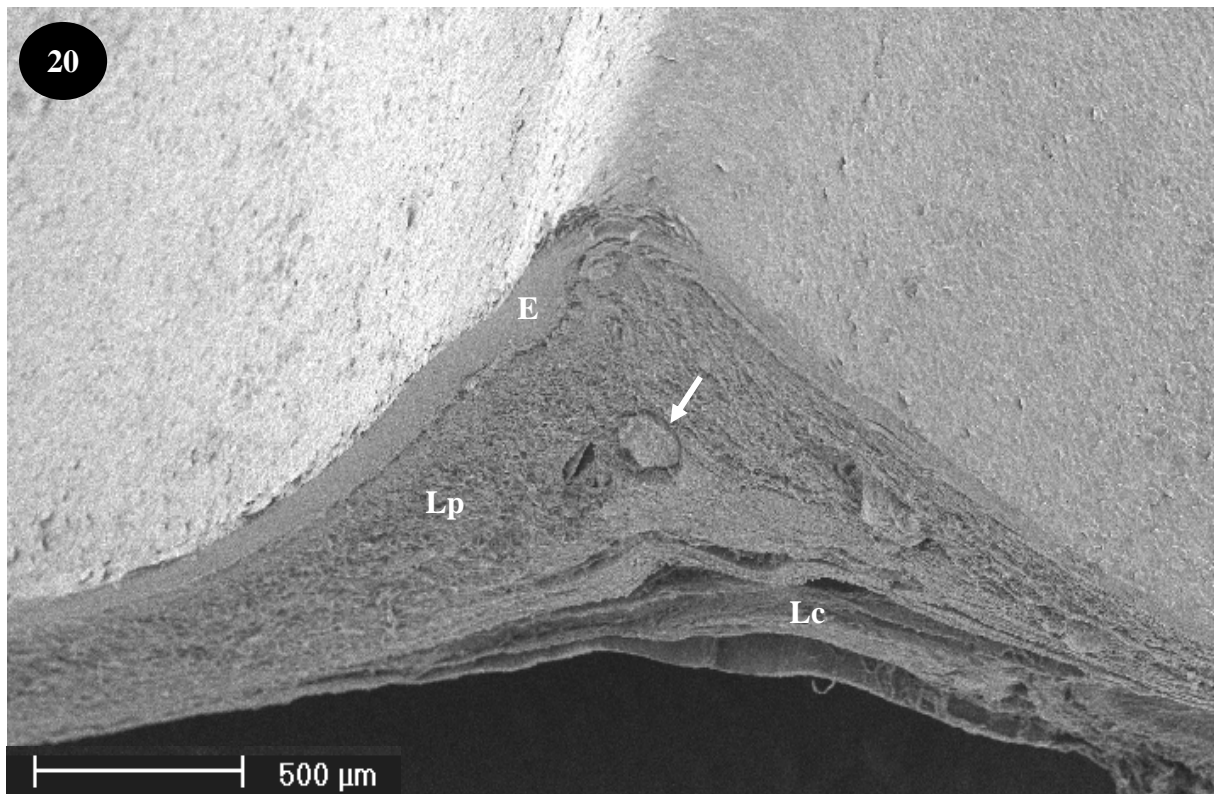
Figure 3.20: Transverse section through the median palatine ridge on the roof of the oropharynx showing the thin, keratinised stratified squamous epithelium (E), the lamina propria of irregular dense connective tissue (Lp) and the underlying loose connective tissue layer (Lc). The large round round structure (arrow) represents the large artery situated at the base of the fold.
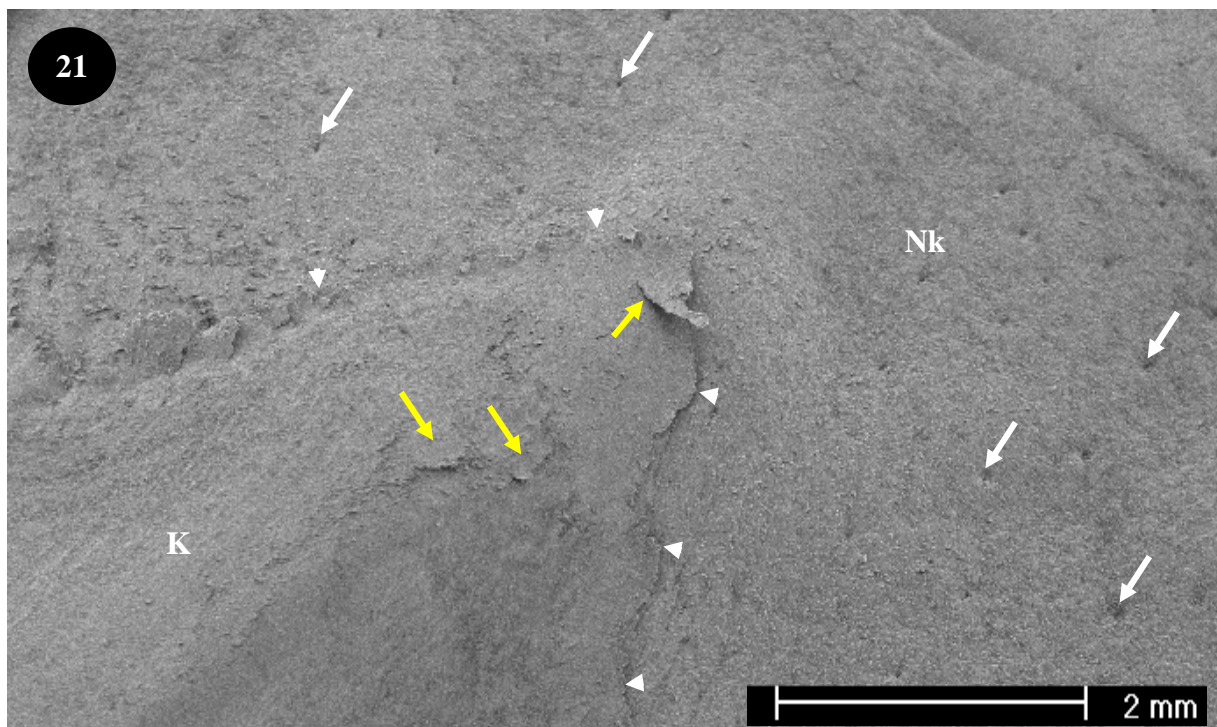

Figure 3.21: The transitional region between the aglandular (keratinised) (K) and glandular (non-keratinised) (Nk) epithelium of the palate (arrowheads). The glandular epithelium displays numerous gland openings (white arrows) whereas the aglandular epithelium shows sloughing of sheets of cells (yellow arrows).
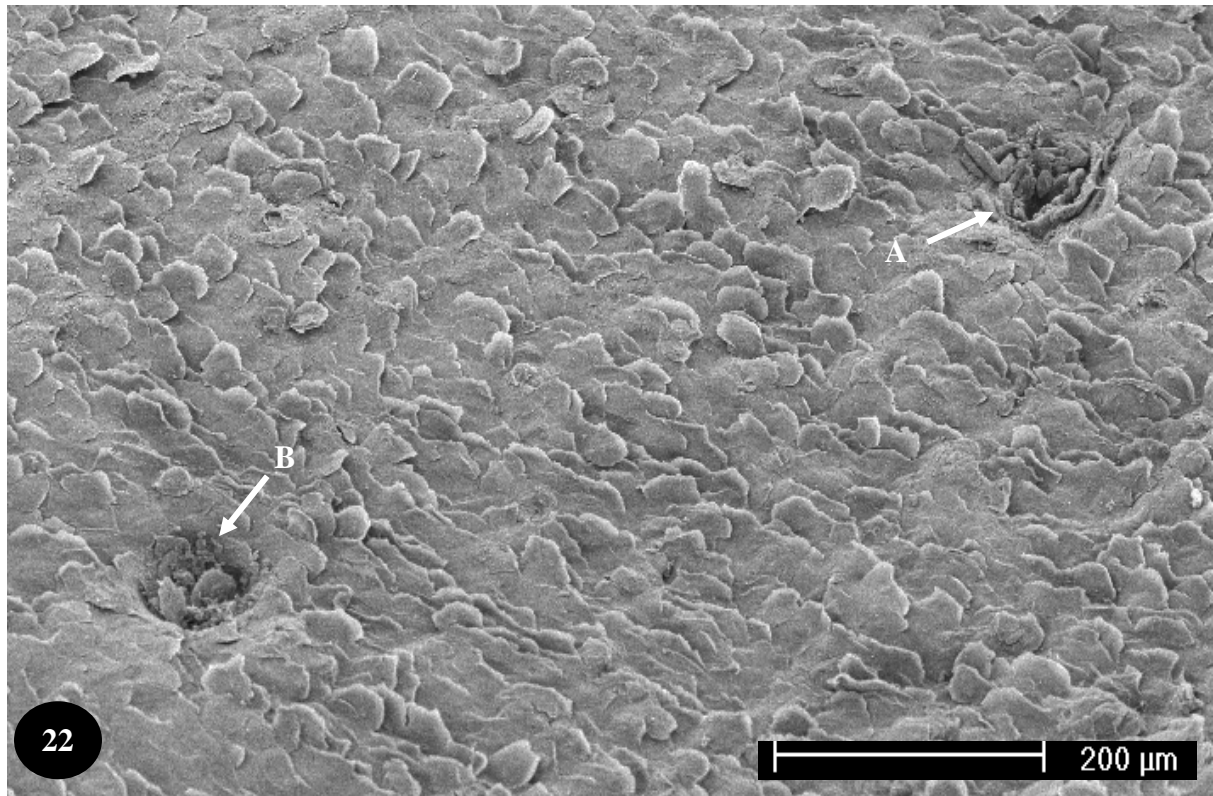
Figure 3.22: A higher magnification of the glandular region of the palate. Two gland openings (A and B) are shown (enlarged in Figures 3.23 and 3.24 below). Note the loose nature of the surface cells of the non-keratinised epithelium.
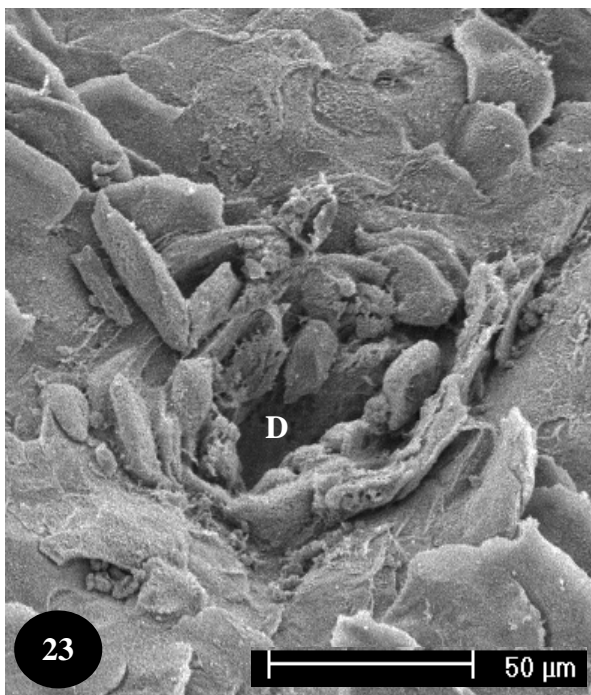

Figure 3.23: Enlargement of gland opening A. Note the concentric arrangement of the cells forming the lining of the duct (D).
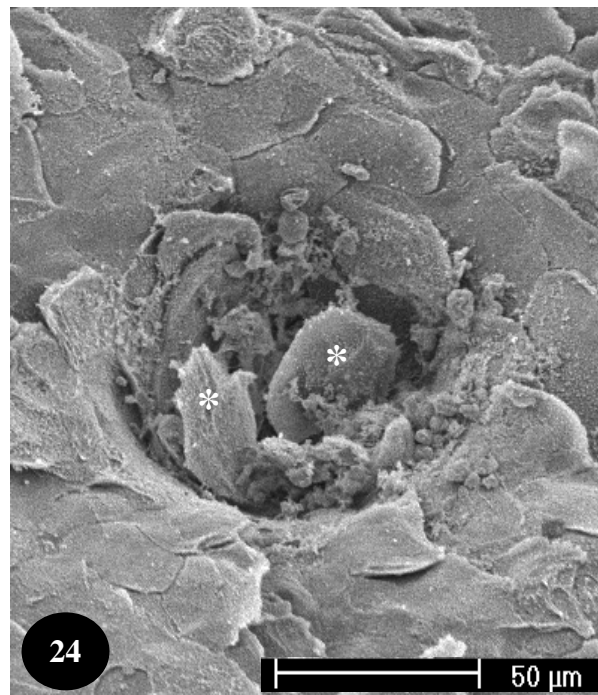

Figure 3.24: Enlargement of gland opening B. The lumen of the duct is filled with mucus and entrapped desquamated epithelial cells (asterisks)
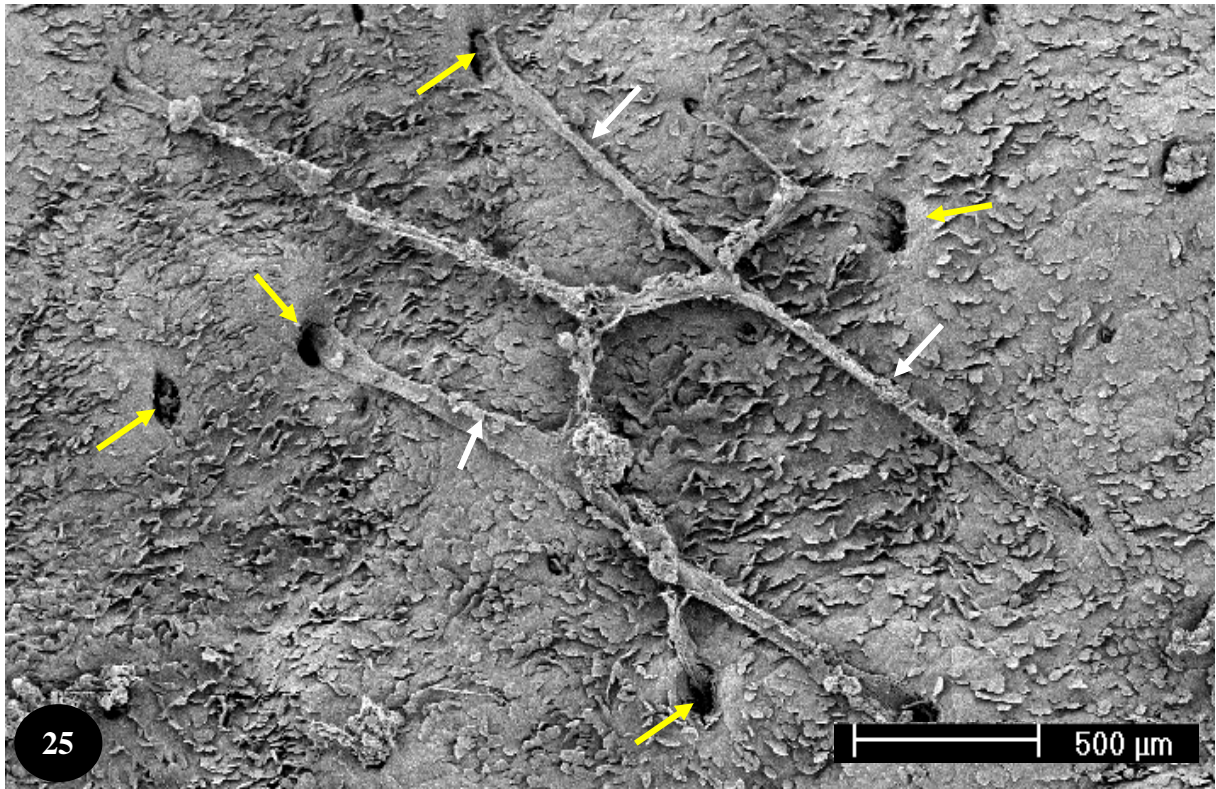
Figure 3.25: Pharyngeal fold displaying numerous evenly spaced gland openings (yellow arrows), many of which are seen expelling streams of mucus which have become interconnected (white arrows).
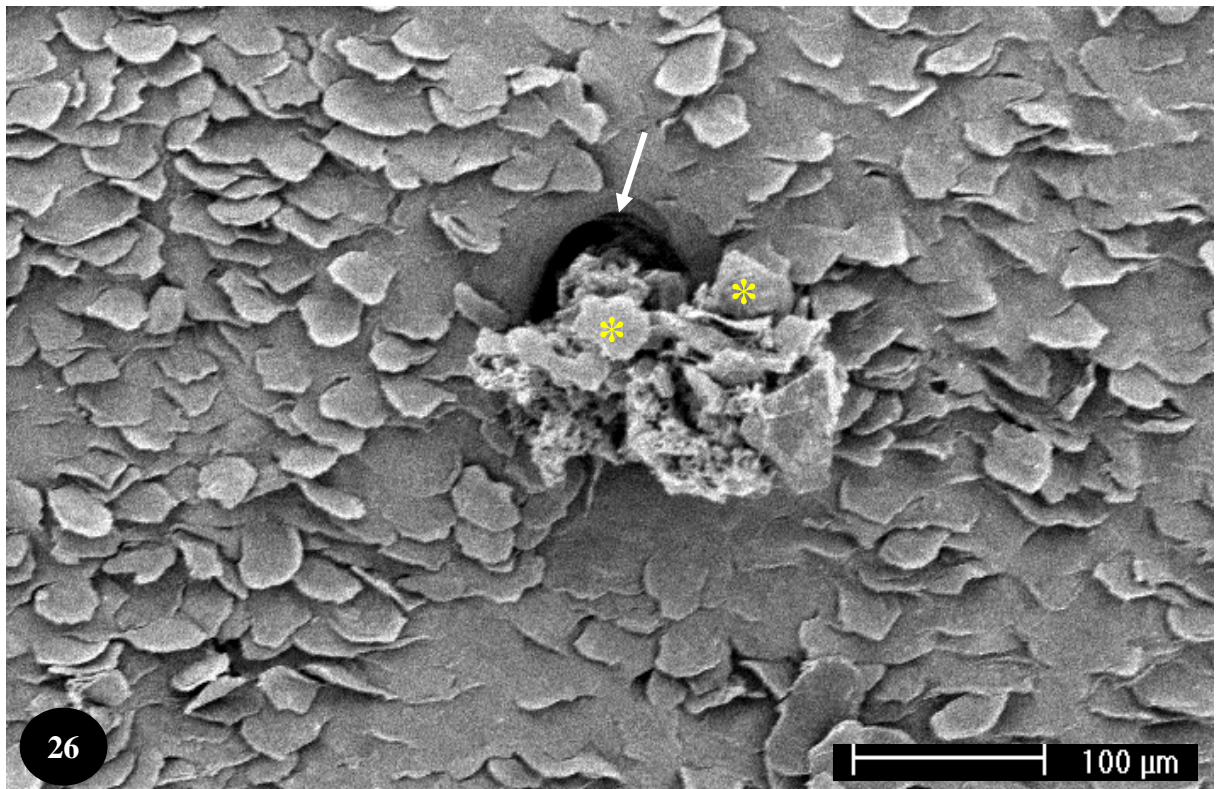

Figure 3.26: Enlargement of a gland opening (arrow) from the same region depicted in Figure 3.25. A mass of mucous containing trapped epithelial cells (asterisks) is seen emerging from the gland opening. Note also the loose nature of the surface epithelial cells.
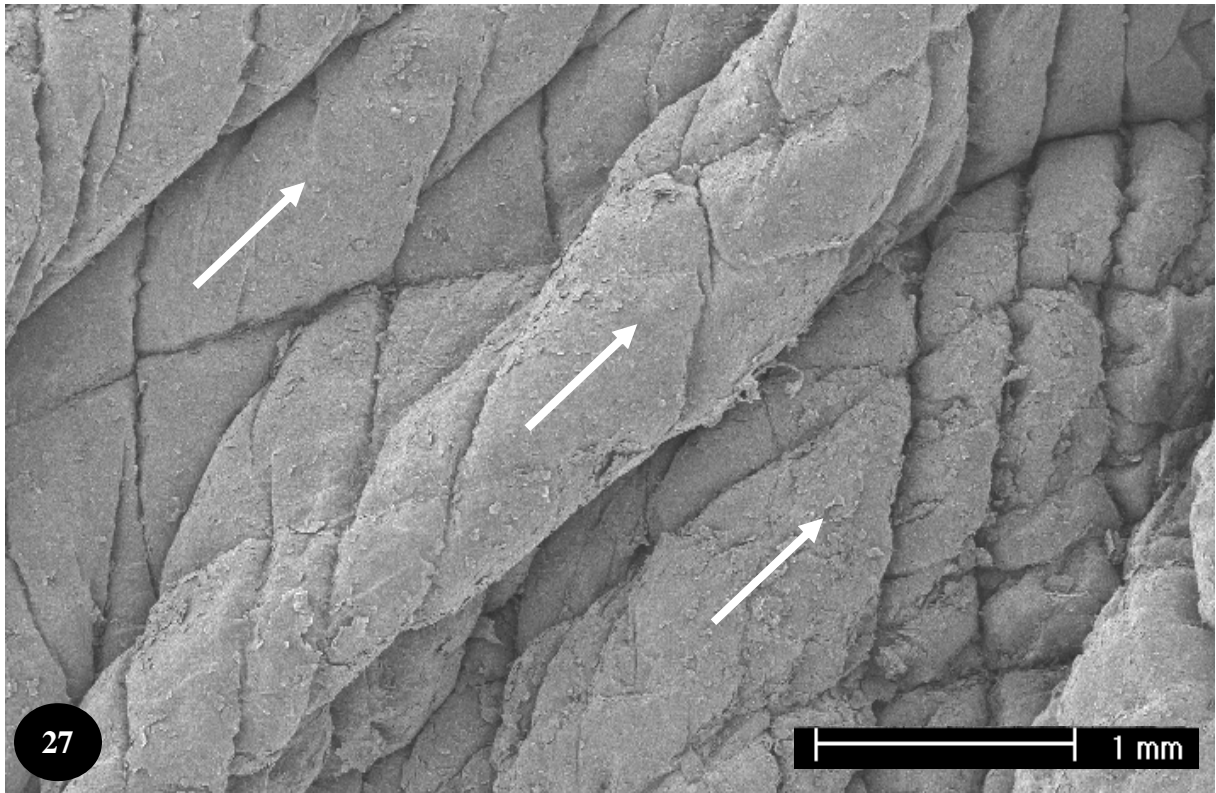
Figure 3.27: Aglandular interramal region on the floor of the oropharynx. The longitudinal mucosal folds (arrows) display alternating transverse grooves and ridges.
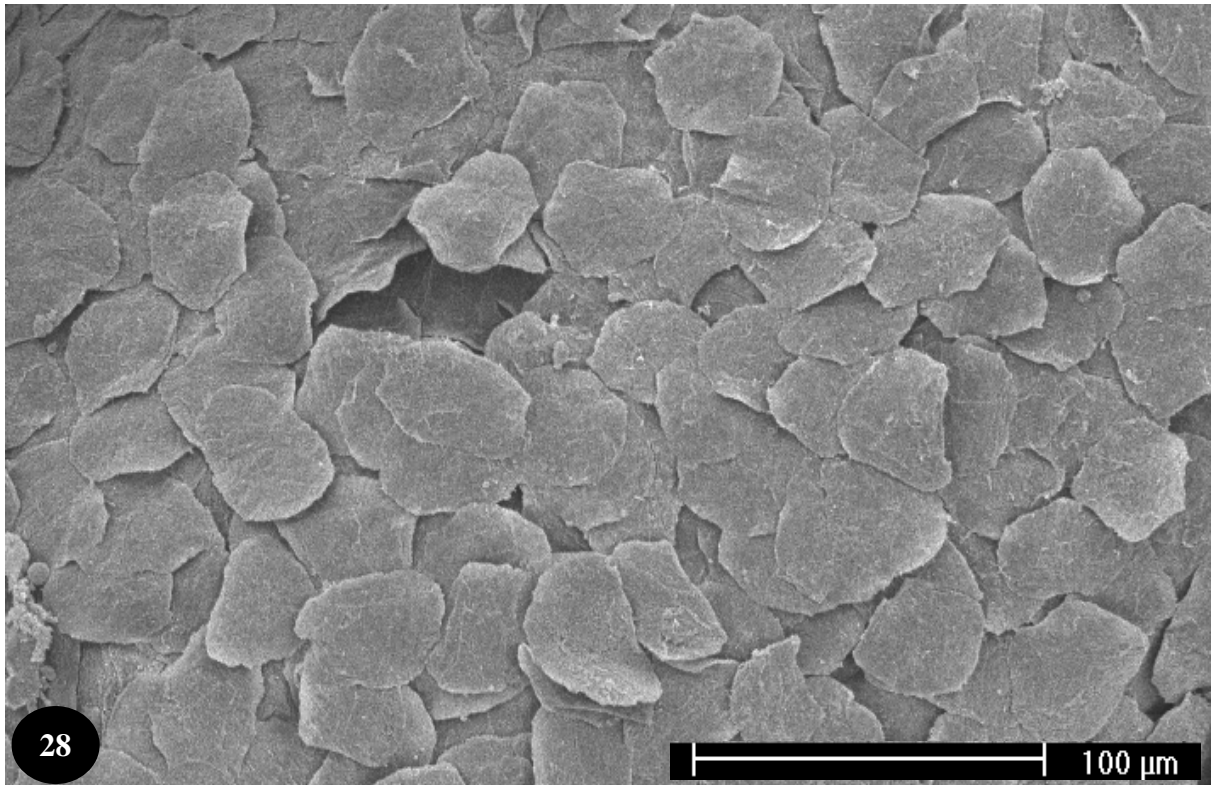
Figure 3.28: Desquamating superficial cells of the keratinised stratified squamous epithelium covering the aglandular interramal region.

Figure 3.29: Dorsal surface of the tongue showing two gland openings (arrows) and desquamating surface cells.
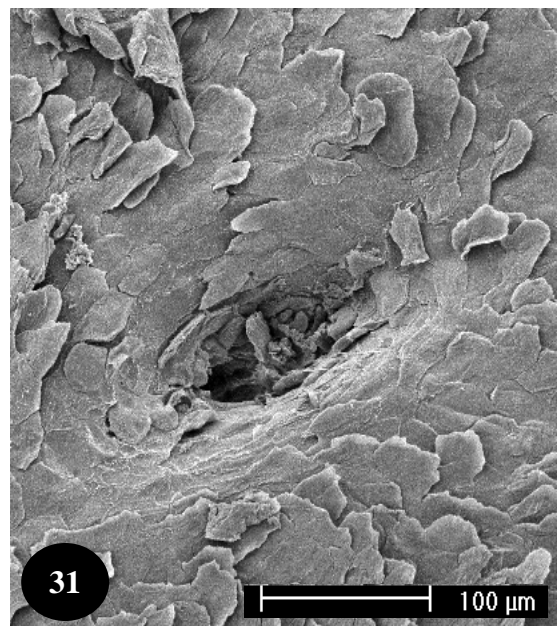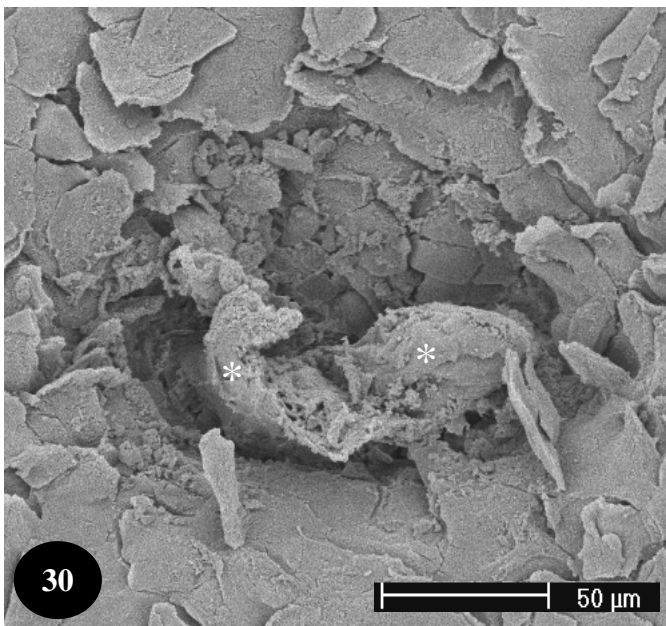
Figures 3.30 and 3.31: Higher magnification of gland openings on the dorsal surface of the tongue showing (Figure 3.30) a plug of mucus (asterisks) filling the opening and (Figure 3.31) the concentric arrangement of cells around the gland opening.
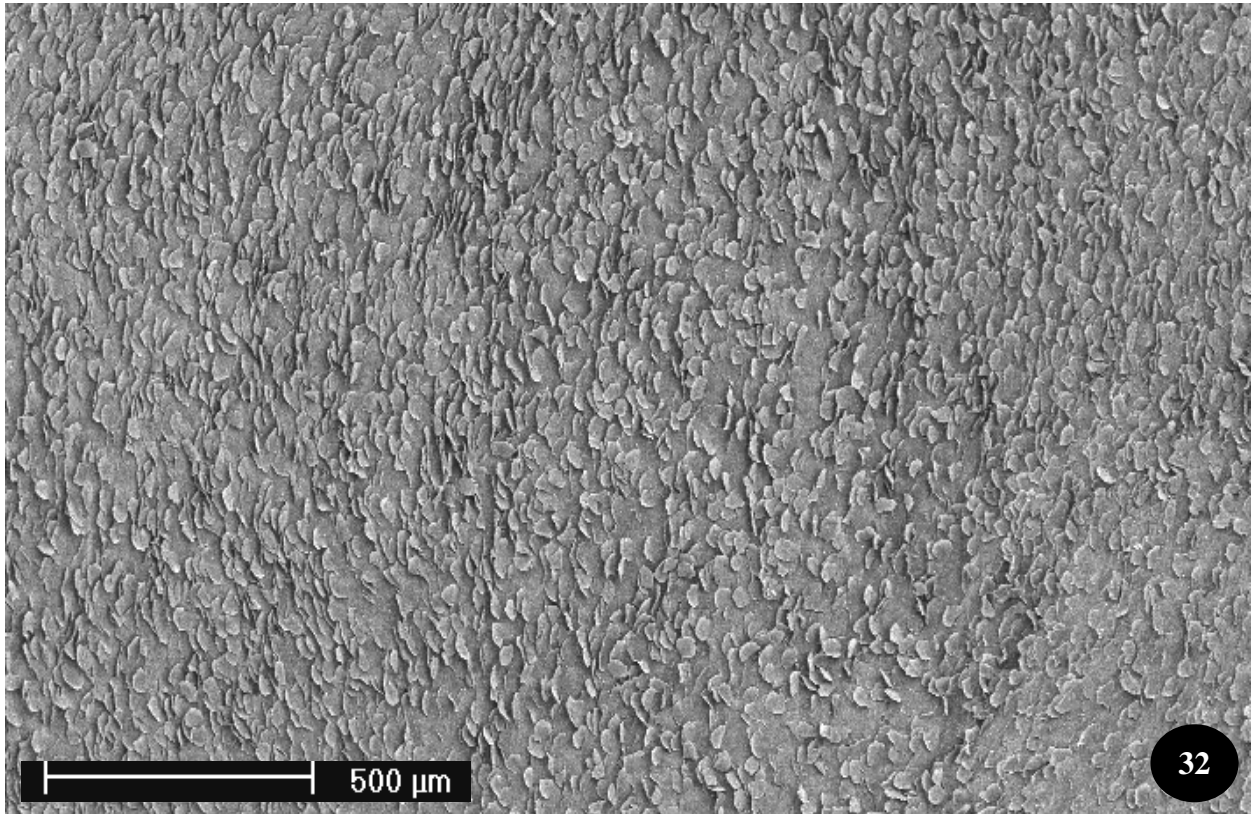
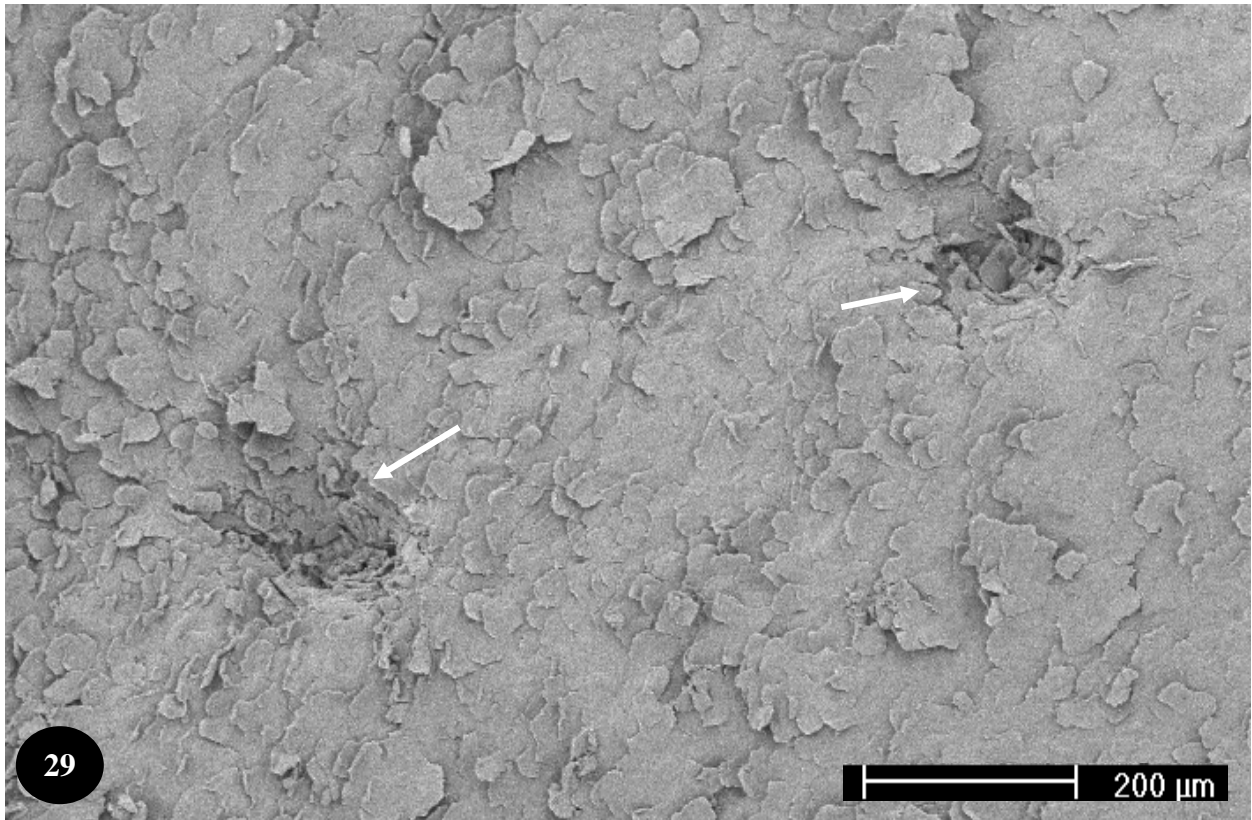Figure 3.32: Typical features of the surface of the laryngeal mound beyond the lips of the glottis. Only desquamating surface epithelial cells are visible in this aglandular region.

Figure 3.33: High magnification of a gland opening on the lips of the glottis. Strands of mucus are visible (arrows) as well as desquamating surface epithelial cells.

GROSS MORPHOLOGY AND TOPOGRAPHICAL RELATIONSHIPS OF THE HYOBRANCHIAL APPARATUS AND LARYNGEAL CARTILAGES

4.1 INTRODUCTION

In a comprehensive study of the lingual apparatus of the domestic chicken, Homberger & Meyers (1989) note that in birds the lingual apparatus "cooperates with the jaw apparatus and the larynx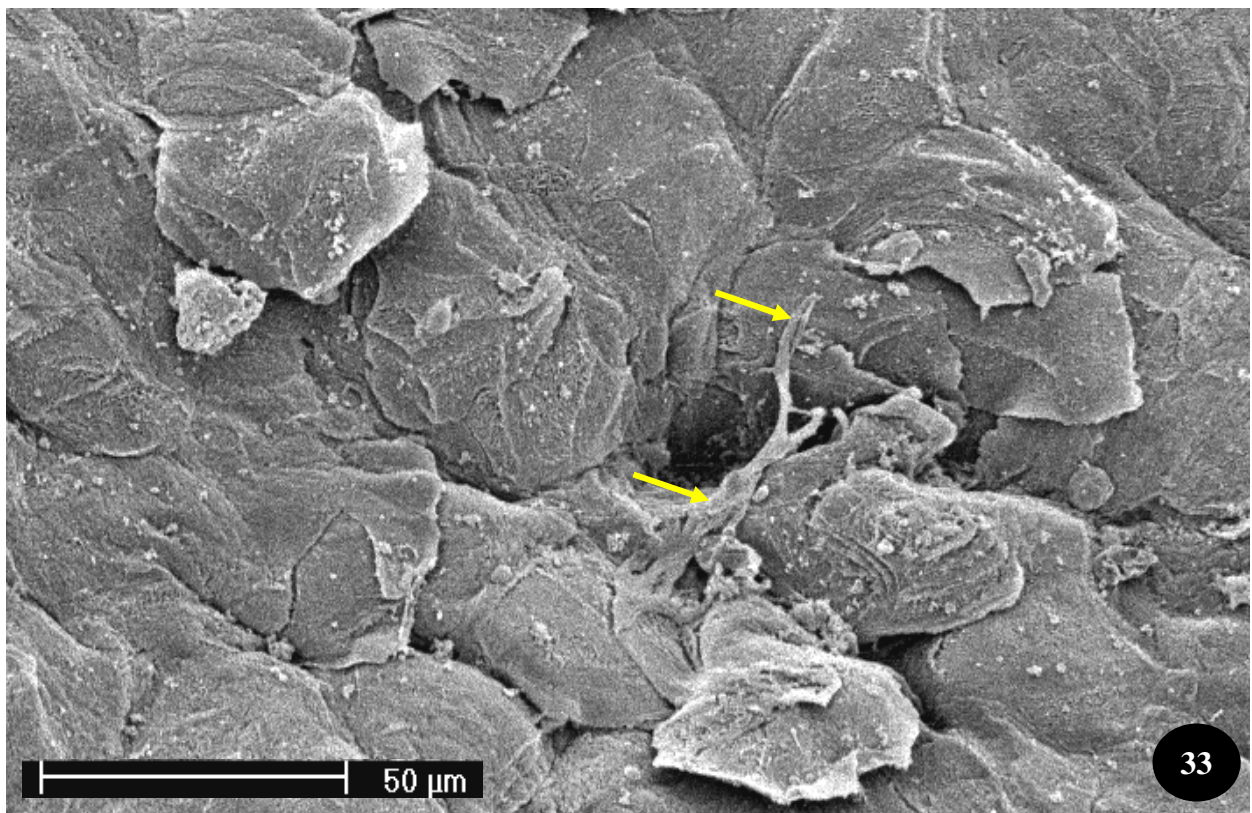 in generating carefully coordinated movements during various behaviors, such as feeding and drinking." As an important component of the lingual apparatus, the hyobranchial apparatus, by virtue of its role in providing an attachment for lingual muscles and fasciae and anchoring certain salivary glands (Homberger & Meyers, 1989) forms an essential structural element of the upper digestive tract. Likewise, the larynx which constitutes a considerable part of the floor of the oropharynx is reported to play "an important part in several functions, including respiration and feeding" (McLelland, 1989).

The hyobranchial apparatus of birds has been described in a number of species, particularly in galliform birds (see review by Homberger & Meyers (1989)) and its structure has been clearly defined. The various components of the apparatus originate principally from elements of the hyoid arch and the more caudally positioned branchial arches (Baumel & Witmer, 1993). Although species-based structural differences occur, and taking differences in anatomical nomenclature into account, the basic components of the avian hyobranchial apparatus are the paraglossum, basihyale, urohyale and *cornu branchiale* composed of the ceratobranchiale and epibranchiale (King & McLelland, 1984; Homberger & Meyers, 1989; Baumel & Witmer, 1993).

The structure of the avian larynx has also been reported in a number of studies (see review by McLelland, 1989) and the laryngeal cartilages have been described in a variety of species (Boccius, 1858; White, 1975; Bock, 1978; King, 1993; Zweers *et al.*, 1981; Hogg, 1982; McLelland, 1989). From these studies it was concluded that birds have four laryngeal cartilages, namely cricoid, procricoid, and left and right arytenoid cartilages.

Information on the hyobranchial apparatus and laryngeal cartilages of the ostrich (and ratites in general) is sketchy, incomplete and in some instances inaccurate. Fowler (1991) notes that "The larynx of the ratites is well developed. There are no vocal folds and no epiglottal cartilage, but the arytenoids, cricoid, and thyroid cartilages can be identified." In contrast, Bezuidenhout (1999) reports that the laryngeal skeleton of the ostrich "consists of two cricoid and two arytenoid cartilages" whereas McLelland (1989) identifies, based on a sketch by S.S.White, arytenoid, cricoid and procricoid cartilages in the larynx of the emu. A sketch of the laryngeal muscles of the ostrich in the same paper (McLelland, 1989) indicates a similar arrangement of cartilages in this species.

The morphology of the hyobranchial apparatus of the ostrich has been briefly described by Bezuidenhout (1999) who notes that it consists of three central components (entoglossum, basibranchiale rostrale and basibranchiale caudale), of which the first two support the tongue, and four caudo-lateral components (paired ceratobranchial and paired cartilaginous parts) which "suspend the apparatus from the ventral surface of the cranium". Duerden (1912) simply notes that the tongue "is supported upon the hyoid or tongue bone". The most comprehensive account of the ratite hyobranchial apparatus is that of Webb (1957) who described its structure in the ostrich embryo and noted that it consisted of a basihyale, urohyale, *os entoglossum*, ceratobranchial and epibranchial. An *os entoglossum* has been described to run the length of the tongue in the rhea (Feder, 1972). The lack of meaningful morphological descriptions of both the hyobranchial apparatus and the larynx of ratites is further complicated by the use of variable terminology in naming anatomical structures, a situation also apparent during a study of the galliform hyoid skeleton (Homberger & Meyers, 1989).

In view of the paucity of published information on the hyobranchial apparatus and laryngeal cartilages of ratites, and considering the need for a better understanding of the basic anatomy of these important supportive components of the upper digestive tract, this chapter provides a description of the gross morphology and topographical relationships of the hyobranchial apparatus and laryngeal cartilages in the ostrich. The terminology used is that of *Nomina Anatomica Avium* (Baumel, King, Breazile, Evans & Berge, 1993).

4.2 MATERIALS AND METHODS

The heads of nine semi-adult (12 to 14 month-old) ostriches were obtained from a commercial abattoir (Oryx Abattoir, Krugersdorp, Gauteng Province, South Africa) immediately after the

birds had been slaughtered. The heads were rinsed in running tap water to remove traces of blood and immersion-fixed in 10% buffered formalin for at least 48 hours. The fixed specimens were rinsed in running water for 2 days after which they were carefully dissected as follows to expose the relevant anatomical features. The skin and subcutaneous tissue was removed from the ventral aspect of the mandible and adjacent neck region of three heads to expose the *in situ* positioning of the hyoid apparatus and its relationship to the larynx (Figs. 4.1 – 4.3). The hyobranchial apparatus, together with the tongue, larynx and trachea, was removed as a unit from the remaining six heads. Three of these specimens were air-dried to demonstrate the topographical relationship of the various components, while the remaining three were freed of all mucous membranes and connective tissue to expose the underlying cartilaginous/bony structures (Figs. 4.4 – 4.6 and 4.8). Median longitudinal sections of the tongue were also prepared to determine the relationship between elements of the hyobranchial apparatus and larynx, and the tongue parenchyma (Fig. 4.7). In order to confirm the findings in semi-adult birds, and to determine any age-related changes, the heads of two one day-old ostrich chicks and two 3 month-old birds sacrificed as part of a nutritional trial at the Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, were also examined. The morphology and topographical relationships of the hyoid apparatus and laryngeal cartilages were described and digitally recorded using a Nikon 4500 Coolpix 995 (Nikon, Tokyo, Japan) digital camera.

4.3 RESULTS

4.3.1 The hyobranchial apparatus (*Apparatus hyobranchialis*)

The hyobranchial apparatus of the ostrich consisted of two centrally positioned elements (components) (paired paraglossals and a fused basihyale and urohyale) and paired caudo-lateral elements, the horns (*cornu*) each consisting of the ceratobranchiale and epibranchiale (Figs. 4.4 – 4.6).

The paraglossals formed the most rostral component of the hyobranchial apparatus and consisted of paired, relatively narrow, flat, caudo-laterally directed cartilages that were connected rostrally to each other (and along the rostral third of their length to the tip of the underlying rostral extension of the fused basihyale and urohyale) by a sheet of loosely arranged fibrous connective tissue. (Figs. 4.5 and 4.6). Each cartilage curved caudo-laterally to form the cartilaginous support of the ventro-lateral aspect of the tongue, was broader rostrally where they were attached to each other and tapered caudally to end in a pointed tip. (Figs. 4.4 – 4.7).

The remaining central component, which represented the basihyale and urohyale described in birds (Baumel & Witmer, 1993), consisted of a single, dorso-ventrally flattened, cartilaginous unit composed of an octagonal-shaped body (*corpus*) from which extended rostral and caudal projections (Figs. 4.4 and 4.5). In most of the 12-14-month-old birds the corpus was ossified (Figs. 4.4, 4.5 and 4.7) but in some specimens from this group, and in three-month-old and one-day old birds, remained cartilaginous. The fused nature of this part of the hyobranchial apparatus was obvious even in the one-day-old birds examined. For convention and ease of comparison the corpus and its rostral projection are referred to as the basihyale (*Os basibranchiale rostrale*) and the caudal projection as the urohyale (*Os basibranchiale caudale*). The rostral projection of the basihyale extended from the rostral facet of the corpus in the form of a flattened rectangle. A broad band of fibrous connective tissue attached the dorsal surface of the rostral projection and body of the basihyale to the ventral surface of the rostral process of the cricoid cartilage of the larynx (Figs 4.4 and 4.6). The ventral aspect of the tip of the rostral projection was related to the rostral aspect of the paraglossals as described above. The lateral facets of the corpus of the basihyale (which adopted a concave shape) formed the site of articulation for the proximal aspect of the rami of the ceratobranchiale (see below) (Figs. 4.4 and 4.5). The urohyale projected from the caudal facet of the corpus of the basihyale. It was as wide as the facet at its point of origin but narrowed progressively to end in a sharp point in the region of the sixth tracheal ring (Fig. 4.5). The wider rostral part of the urohyale was attached along its dorsal surface to the ventral aspect of the body of the cricoid cartilage of the larynx by fascia and muscular tissue, whereas the tapered distal part was similarly attached to the proximal trachea.

The most proximal part of the paired hyobranchial horns was the ceratobranchiale. This was the longest component of the hyobranchial apparatus, measuring approximately 71mm (n = 10) in semi-adult birds. The ceratobranchiale was ossified in all the age groups studied and consisted of a cylindrical shaft with bulbous proximal and distal extremities. The latter fitted snugly with the concave articular facets of the basihyale corpus and proximal end of the epibranchiale, respectively (Figs. 4.4 and 4.5). The distal part of the horns was represented by the epibranchiale. This was the second longest component of the hyobranchial apparatus, measuring approximately 42mm (n = 8) in semi-adult birds. It was a cartilaginous, cylindrical structure of similar diameter proximally to that of the bulbous termination of the ceratobranchiale, but tapered distally to form a small, sometimes gently curved, point (Figs 4.1, 4.2 and 4.4). There was no difference in length between the left and right ceratobranchiale or epibranchiale.

4.3.2 Laryngeal cartilages (*Cartilagines larynges*)

The laryngeal skeleton of the ostrich was composed of cricoid, procricoid and paired arytenoid cartilages. The cricoid cartilage, which formed the bulk of the skeleton, was ring-shaped and consisted of the body (*corpus*) ventrally, and from which emanated the wings (*alae*) which extended caudo-laterally and dorsally to complete the ring (Figs 4.3, 4.5, 4.6 and 4.8). The wings articulated with one another dorsally and also with the ventro-lateral aspect of the procricoid (Fig. 4.8). In semi-adult birds the body displayed a central, plate-like, ossified region (Figs 4.4 and 4.5) from which extended a blunt-ending, ossified, rostral projection (Fig. 4.6). This projection was loosely attached along its ventral surface to the dorsal aspect of the body of the basihyale by a layer of connective tissue. The tip of the projection was connected to the tip of the rostral projection of the basihyale by a strong band of fibrous connective tissue. This band of tissue was firmly attached to the ventral surface of the tip of the cricoid projection and to the dorsal aspect of the tip of the projection of the basihyale. For most of its length the band was loosely attached to the dorsal surface of the projection of the basihyale (Figs. 4.4 and 4.6). In younger birds the body of the cricoid cartilage remained cartilaginous. The caudal margin of the cricoid cartilage was attached by mucous membrane to the proximal cartilage rings of the trachea. The point of contact between the two structures was inclined in a ventro-rostral direction.

The small, centrally positioned procricoid cartilage was situated just caudal to the caudal extremity of the glottis and effectively linked the caudal aspect of the paired arytenoids with the tips of the wings of the cricoid cartilage. The procricoid was oriented at almost 90 degrees to the other elements of the laryngeal skeleton and formed a single, shield-shaped structure which articulated with the caudo-medial aspect of the paired arytenoids and with the rostro-medial aspect of the wings of the cricoid (Fig. 4.8). The articulation of the procricoid with the wings of the cricoid, and the articulation of the wings with each other, created a Y-shaped point of contact between the two cartilages.

The two arytenoid cartilages lay dorsally and formed the margins of the glottis. Each of the arytenoids was dorso-ventrally flattened with a straight medial border on which the lips of the glottis were accommodated (Figs 4.6 and 4.8). Despite the relatively high profile of the lips, they were not supported internally by extensions from the medial border of the arytenoids. The lateral borders had a scalloped appearance due to the presence of two large lateral projections. The larger rostral projections were caudo-laterally directed, hook-like structures, whereas the smaller

caudal projections were blunt and more caudally directed (Fig 4.6). These projections formed the support of the mucosal elaborations seen externally on the laryngeal mound.

The positioning of the arytenoid cartilages relative to the wings of the cricoid cartilage resulted in the formation of two large, cat's-eye-shaped gaps between them. These were covered by the mucous membrane lining the laryngeal cavity and which was attached to the ventral surface of the arytenoids. (Figs 4.6 and 4.8).

4.3.3 Topographical relationships

The hyobranchial apparatus was attached rostrally to the parenchyma of the tongue via the cartilaginous rostral projection and part of the body of the basihyale which lay dorsal to the frenulum. The rostral process of the cricoid cartilage of the larynx was also observed to support the root of the tongue (Fig. 4.7). The remaining elements of the central components of the hyobranchial apparatus (body of basihyale and the urohyale) firmly anchored the tongue to the larynx (via the body of the cricoid cartilage) and proximal trachea (via the first six tracheal rings) (Fig. 4.3). The ossified ceratobranchiale extended latero-caudally from the body of the basihyale and continued caudally over the cricoid cartilage of the larynx and the lateral musculature of the neck, a short distance beneath, but medial to, the ramus of the mandible. In the vicinity of the *pars caudalis* of the ramus of the mandible, the ceratobranchiale articulated with the cartilaginous epibranchiale which curved sharply caudo-ventrally to attach to the dorso-lateral cervical musculature. From its point of origin on the body of the basihyale, the horns of the hyobranchial apparatus (ceratobranchiale and epibranchiale) were firmly attached to all underlying structures by a double-layered sheath of fascia, the *fascia vaginalis* (Figs. 4.1 – 4.3).

4.4. DISCUSSION

4.4.1. Hyobranchial apparatus

The results of this study broadly confirmed the findings of Webb (1957) and Bezuidenhout (1999) that the hyobranchial apparatus of the ostrich consists of both central and paired caudo-lateral components. In agreement with Webb (1957) the paired caudo-lateral components (horns or cornu) of the apparatus consisted of the ceratobranchiale (which articulated with the body of the basihyale) and the epibranchiale (which articulated with the ceratobranchiale), both components being rod-like structures which appeared circular in cross-section. Bezuidenhout

(1999) provided no structural detail of the cornu, noting simply that they consisted of paired ceratobranchial and cartilaginous parts, the latter presumably representing the epibranchiale described in the present study and by Webb (1957).

In defining the central component, Bezuidenhout (1999) briefly mentioned that it consisted of the entoglossum, basibranchiale rostrale and basibranchiale caudale, again without providing any structural detail. The present study revealed that the paraglossum (entoglossum) of the ostrich consisted of paired caudo-laterally directed cartilages that were connected rostrally to each other by fibrous connective tissue, and which supported the ventro-lateral aspect of the tongue. This description of the paraglossum differs markedly from that of Webb (1957) who describes the os entoglossum as a single structure represented by the widened, disc-like anterior portion of the basihyale, noting further that it "is situated in the tongue and therefore corresponds to the "os entoglossum"". Based on the sketch of the "hyoid apparatus" of a 37-day old ostrich embryo provided by Webb (1957) it would appear that the os entoglossum described by the author actually represents the rostral projection of the basihyale described in the present study and that the forming paraglossals were not observed by Webb (1957). Faraggiana (1933) also describes an os entoglossum as revealed in a median section of the ostrich tongue and notes that it consists of spongy bone in the root which continues towards the apex as a cartilaginous appendix. This description typically reflects the bony and cartilaginous components of the basihyale identified in the present study and not the paraglossum. The sketch provided by Faraggiana (1933) also illustrates the tip of the rostral process of the cricoid cartilage of the larynx and its dorsal orientation in respect of the basihyale as observed in the present study. Feder (1972) notes that the *os entoglossum* (paraglossum) of the rhea tongue extends from just beyond the base of the tongue to near the tip. The paraglossals were clearly discernable in both the three-month-old and one-day-old chicks examined in the present study. Paired paraglossals have also been described in Psittaciform birds (Mivart, 1895; Beddard, 1898; Homberger, 1986), the separate elements being bound rostrally by a cartilaginous or bony isthmus (Mivart, 1895; Beddard, 1898) and some reports on the domestic fowl describe the *os entoglossum* as being double (Nickel *et al*., 1977). Unlike in the domestic fowl and duck where the paraglossum consists of bony (caudal) and cartilaginous (rostral) parts (Ellenberger & Baum, 1943; McLelland, 1975; Homberger & Meyers, 1989), the paraglossals in the ostrich are cartilaginous, even in the 12 – 14-month-old birds.

In the ostrich, the remaining central component of the hyobranchial apparatus consisted of a single fused structure representing the basihyale and urohyale described in other birds. The fused

structure demonstrated an octagonal-shaped body (*corpus*) from which extended rostral and caudal projections. Webb (1957) also described a fused structure composed of a dorso-ventrally flattened median basal plate and which anteriorly formed the "os entoglossum" and posteriorly the tapered urohyale. Webb (1957) further notes that the median basal plate (fused basihyale and urohyale) "is in no way sub-divided either by ossification or by being laid down as separate areas of chondrification", even in 27 and 30 day-old embryos. The fused nature of the basihyale and urohyale in the ostrich, even during embryonal stages, contrasts sharply with the situation in other avian species where the two components are separate in young birds, although fused to one another in adults (Baumel & Witmer, 1993). Despite being fused, the central component of the hyobranchial apparatus in the ostrich consists essentially of three parts, namely, a central body (*corpus* or *basihyoideum*) with which the paired ceratobranchiale articulate laterally, and rostral and caudal projections representing the rostral (lingual) process and urohyale, respectively. In common with the duck and goose the corpus in the ostrich is a flattened structure unlike the rod-like form reported in the fowl and pigeon (Nickel *et al*., 1977).

Webb (1957) states that in both ostrich embryos and adult birds the only ossified components of the hyobranchial apparatus is the ceratobranchiale, while all the other parts remain cartilaginous. The results of the present study disagree with this statement as it was demonstrated that in most of the 12 – 14 month-old birds studied, the body of the fused basihyale and urohyale was clearly ossified in addition to the ossification of the paired ceratobranchiale. As the oldest birds studied in the present work were 12 – 14 months-old, it is impossible to speculate on whether additional parts of the ostrich hyobranchial apparatus become ossified over time as is the case in the domestic fowl where parts of the paraglossum, urohyale and epibranchiale are ossified in addition to the paired ceratobranchiale (Homberger & Meyers, 1989)

4.4.2. Laryngeal cartilages

Some confusion exists in the literature regarding the identification and number of cartilages forming the larynx in ratites. Fowler (1991) describes arytenoids, cricoid and thyroid cartilages in ratites, whereas Bezuidenhout (1999) describes two cricoid and two arytenoids cartilages in the ostrich. In accordance with the information on birds in general (McLelland, 1989), this study demonstrated that the larynx of the ostrich consisted of the criocoid, procricoid and two arytenoid cartilages, and confirmed the absence of thyroid and epiglottic cartilages (Nickel *et al*., 1977; King and McLelland, 1984).

The large cricoid cartilage was composed of a body and two wings that articulated caudally with each other. A similar situation, based on the work of S.S. White, is illustrated for the emu and the ostrich (McLelland, 1989). Whereas the tip of the cricoid is reportedly cartilaginous in the domestic fowl (White, 1975; McLelland, 1989) and crows (Zweers & Berkhoudt, 1987), this part of the ostrich cricoid cartilage, as well as part of the body, is ossified. Fusion of tracheal cartilages with the caudal aspect of the body of the cricoid as described in some bird species (Boccius, 1858; Zweers *et al*., 1981) was not observed in the ostrich. The small procricoid cartilage of the ostrich was rhomboidal in outline and articulated both with the arms of the cricoid cartilage and the caudo-medial aspect of the paired arytenoid cartilages as reported in other birds, including the emu (McLelland, 1989; King, 1993).

The paired arytenoid cartilages were flat and showed no elaborations except for the presence of two large lateral projections which distinguish these cartilages from those of the emu (McLelland, 1989 – sketch by S.S. White). The rostral and caudal processes typical of other avian species such as the domestic fowl (White, 1975; McLeland, 1989) were not observed in the ostrich, the arytenoid cartilages in this species appearing to consist only of a body (*corpus*). Ossification of the laryngeal cartilages in the domestic fowl is reported to occur in the body and wings of the cricoid and the body of the procricoid and arytenoid cartilages (Hogg, 1982), whereas in the pigeon the wings of the cricoid and tail of the procricoid remain cartilaginous while the body of the arytenoids is only partially ossified (Zweers *et a*l., 1981). In the 12 – 14-month-old ostriches studied only part of the body and the rostral process of the cricoid cartilage were ossified. This supports he observation that in birds the "cricoid cartilage tends to early ossification" (Nickel *et al*., 1977). Whether ossification eventually occurs with age in other parts of the laryngeal skeleton of the ostrich remains unknown.

It is concluded that, although the ostrich larynx displays the typical combination of cartilages described for birds in general, it differs markedly in respect of the relatively simple design of the paired arytenoid cartilages.

4.4.3 Topography

Bezuidenhout (1999) reported that the horns of the hyobranchial apparatus in the ostrich "suspend the apparatus from the ventral surface of the cranium," while Webb (1957) noted that the cartilaginous epibranchiale "extends to the posterior end of the skull." In the various age groups of birds examined in the present study it was observed that the paired cornu, while indeed

suspending the hyobranchial apparatus, at no point passed close to the skull, but in fact curved downwards (caudo-ventrally) away from the skull. This contrasts with the situation in the domestic fowl where the horns of the hyobranchial apparatus curve upwards to attach to the occipital region of the skull (Homberger & Meyers, 1989). In both the ostrich and fowl (Homberger & Meyers, 1989) the attachment of the ceratobranchiale and epibranchiale to the underlying structures was effected by a sheath-like fascia, the *fascia vaginalis*.

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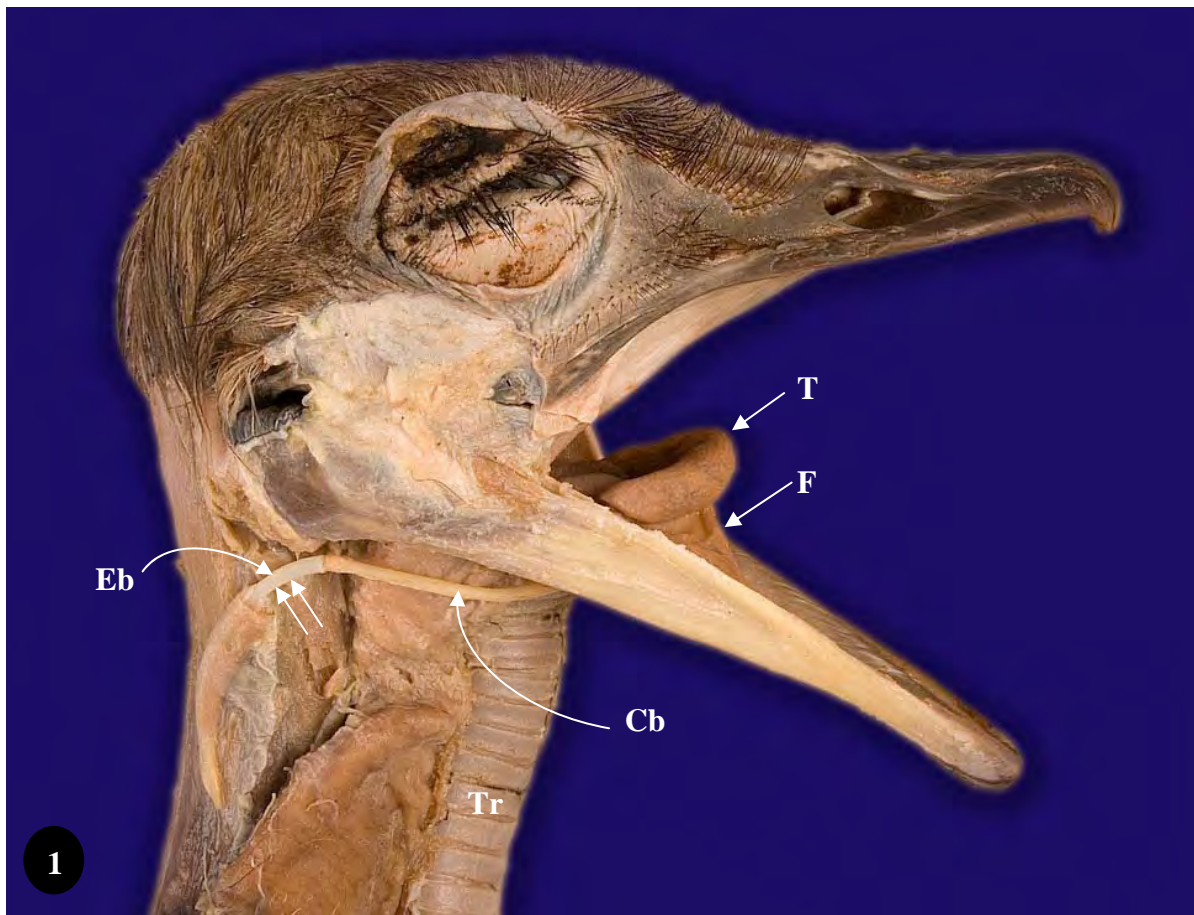
Figure 4.1: Right lateral view of the head and proximal neck region of the ostrich dissected to reveal the *in situ* positioning of the hyobranchial apparatus. The right horn of the apparatus is represented by the ceratobranchiale (Cb) and epibranchiale (Eb). Note that the fascia vaginalis that houses the horns has been removed except for that portion covering the distal part of the epibranchiale (double arrows). Tongue (T), frenulum (F), trachea (Tr).
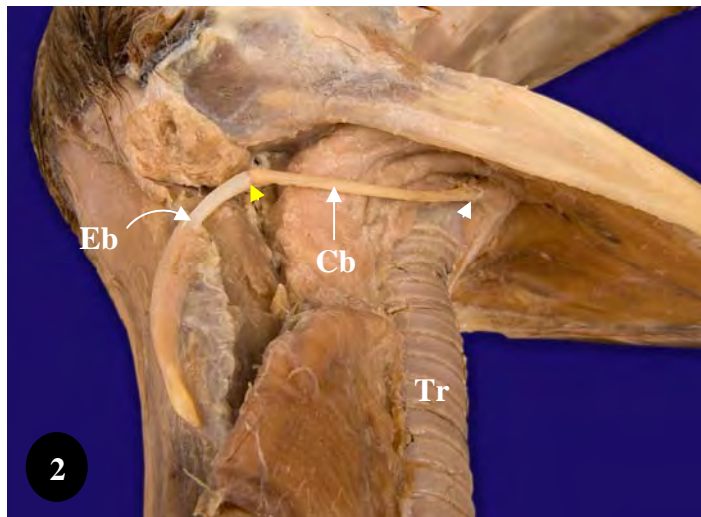

Figure 4.2: Ventro-lateral view of the head illustrated in Fig. 4.1 showing the connection of the ceratobranchiale (Cb) to the body of the basihyale (arrowhead). Note the colour difference between the ossified ceratobranchiale and the cartilaginous epibranchiale (Eb) and the articulation (yellow arrowhead) between the two components. Trachea (Tr).
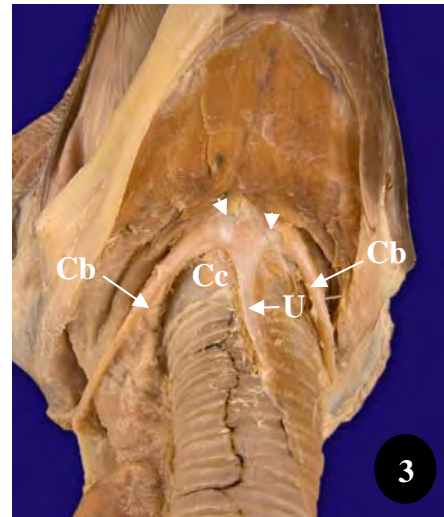

Figure 4.3: Ventral view of the head demonstrating the connection of both the left and right ceratobranchiale (Cb) to the body of the basihyale (arrowhead). The urohyale (U) is firmly attached to the body of the cricoid cartilage (Cc) of the larynx and to the first number of tracheal rings.
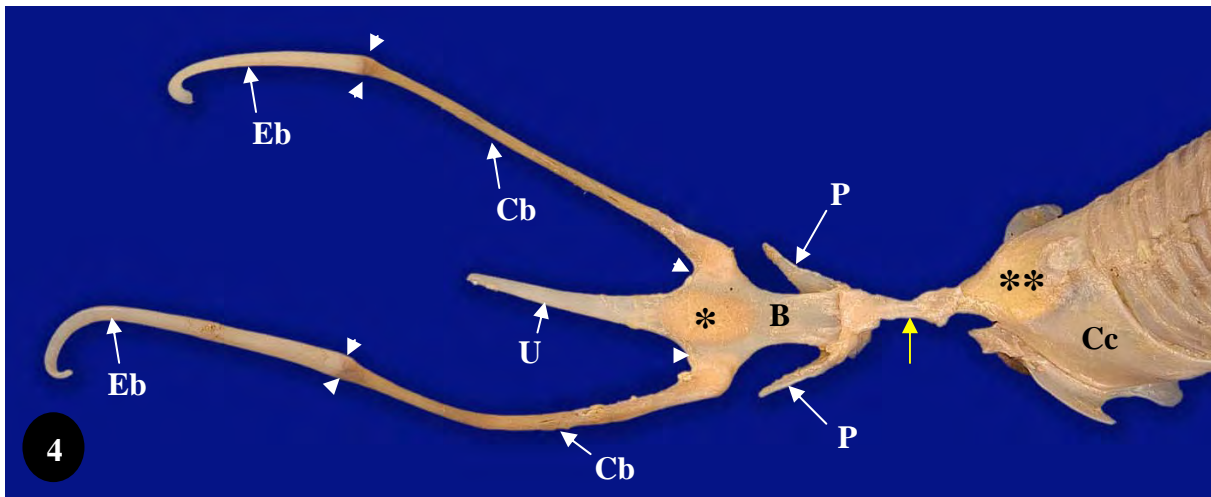
Figure 4.4: Dorsal view of the hyobranchial apparatus showing the paired paraglossals (P), the rostral projection and body of the basihyale (B), the urohyale (U), ceratobranchiale (Cb) and epibranchiale (Eb). The reflected band of fibrous connective tissue (yellow arrow) attaches the rostral projection and body of the basihyale to the rostral projection of the cricoid cartilage (Cc). The points of articulation between the ceratobranchiale and body of the basihyale (single arrowheads) and between the ceratobranchiale and epibranchiale (double arrowheads) are shown. The regions of ossification of the body of the basihyale (asterisk) and the body of the cricoid cartilage (double asterisk) are clearly visible.
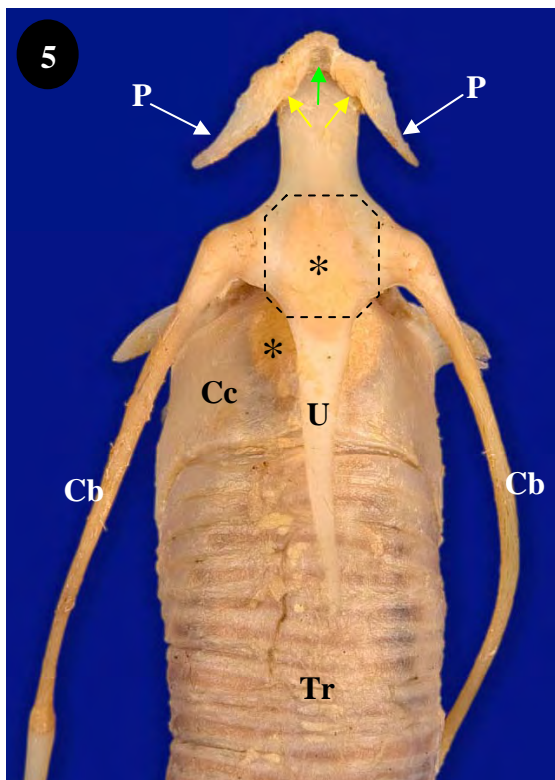

Figure 4.5: Enlarged ventral view of the hyo-branchial apparatus and laryngeal cartilages. The paired paraglossals (P) are attached to each other (green arrow) and to the rostral process of the basihyale (yellow arrows) by fibrous connective tissue. Note the octagonal shape of the body of the basihyale (broken line), and the ossification of the body of both the basihyale and cricoid (Cc) cartilage (asterisks). The narrow tail of the urohyale (U) extends along approximately six tracheal rings. Ceratobranchiale (Cb), trachea (Tr).
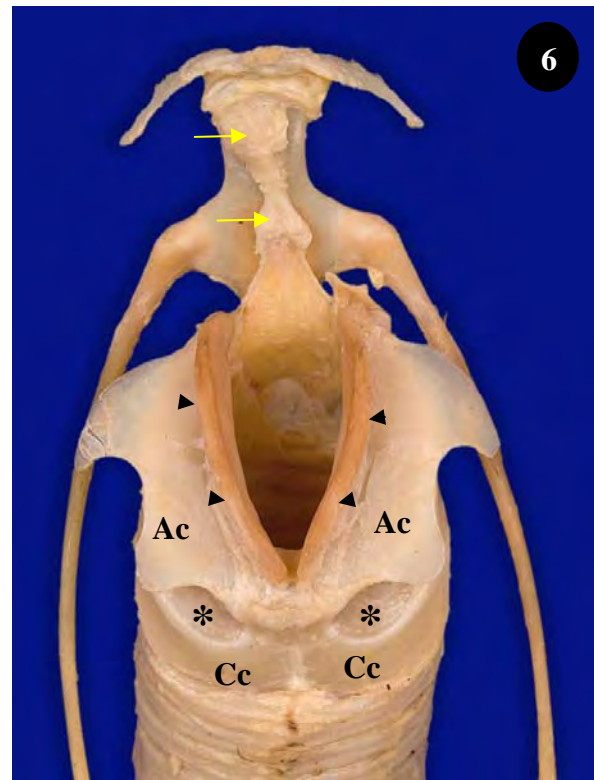

Figure 4.6: Enlarged dorsal view of the hyobranchial apparatus and laryngeal cartilages. The fibrous attachment between the rostral process of the cricoid cartilage and the basihyale (arrows) is obvious. The arytenoid cartilages (Ac) with their distinctive lateral projections are shown. Note the mucosa-lined apertures (asterisks) between the arytenoid cartilages and the wings of the cricoid cartilage (Cc). The raised rim of the glottis (arrowheads) represents well-developed mucosal folds not removed during dissection of the larynx.
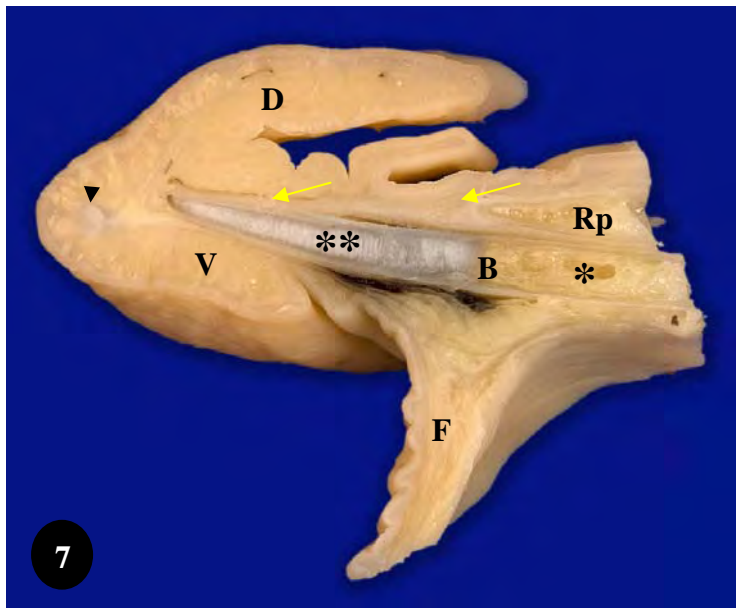
Figure 4.7: A mid-saggital section through the tongue showing the relationship between the basihyale (B) and the more dorsally positioned rostral process (Rp) of the cricoid, which in this specimen is ossified. The body of the basihyale is ossified (asterisk) whereas the rostral process remains cartilaginous (double asterisk). Only a small portion of the paraglossum (arrowhead) is visible on the photograph. Note the band of dense connective tissue running between the rostral process of the basihyale and the rostral process of the cricoid cartilage (yellow arrows). Dorsal surface (D), Ventral surface (V), Frenulum (F).
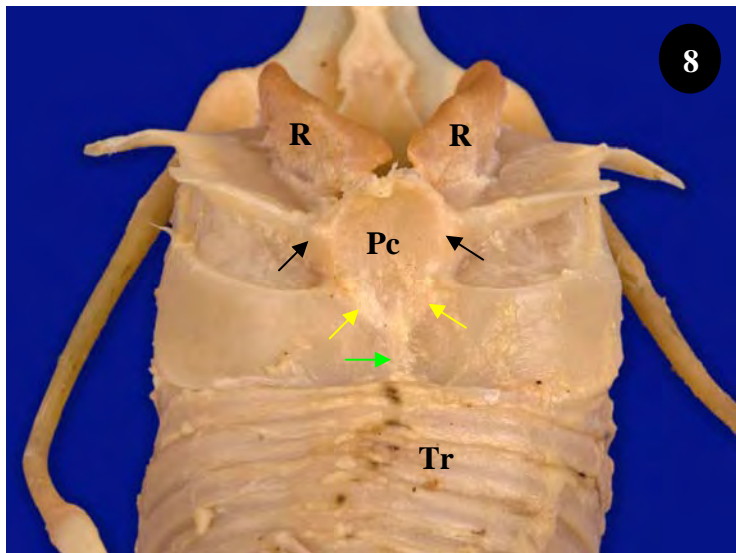
Figure 4.8: A caudo-rostral view of the dorsal aspect of the laryngeal skeleton illustrating the union of the procricoid cartilage (Pc) with the wings of the cricoid cartilage (yellow arrows) and the caudo-medial aspect of the two arytenoid cartilages (black arrows). The wings of the cricoid cartilage also link with each other below the procricoid cartilage (green arrow). The elevated mucosal ridges (R) running along the rim of the glottis are obvious. Trachea (Tr)

CHAPTER 5

MORPHOLOGY OF THE OESOPHAGUS

5.1   INTRODUCTION

The morphology and microanatomy of the avian oesophagus and crop has been the subject of numerous investigations (Ziswiler & Farner, 1972). Most of these studies concentrated on the appearance of the mucosal folds and on histological descriptions of the oesophagus in domestic birds such as the chicken (*Gallus gallus*) and turkey (*Meleagris galloparo*). Descriptive studies were also carried out in various exotic birds such as herons, hawks, owls, penguins, auks, pelicans, ducks and geese (Barthels, 1895; Kaden, 1936; Niethamer, 1933; Ivey & Edgar, 1952; Calhoun, 1954; Malewitz & Calhoun, 1958; Allenspach, 1964; Ziswiler, 1967a,b).

The macroscopic structure of the avian oesophagus has been studied in a variety of species. Structurally, the mucosal surface of the oesophagus is strongly folded longitudinally (*plicae Oesophageales*). It was reported that the size of the oesophageal folds was dependent on the size of the food the birds swallowed. In fact, the authors demonstrated that the oesophagus was widest in the species that swallowed large pieces of food and also in those that stored food along the whole length of the organ (Barthels, 1895; Malewitz & Calhoun, 1958; Hodges, 1974).

The internal lining of the oesophagus in birds has in general the same morphology as in other vertebrates (Ivey & Edgar, 1952; Ziswiler & Farner, 1972). However, contradictory reports have been presented concerning the number of tissue layers in the oesophagus. Some authors such as Barthels, (1895), Kaden, (1936), Niethammer, (1933), Ziswiler, (1967a,b), Feder, (1972a) and McLelland (1979), described four tissue layers (mucous membrane, submucosa, muscular tunic and adventitia), while Wheather *et al*., (1987), Warner *et al*., (1967), Malewitz and Calhoun, (1958), Ivey and Edgar, (1952), Ziswiler & Farner, (1972) and Hodges, (1974) described seven tissue layers, namely stratified squamous epithelium, lamina propria, *muscularis mucosae*, submucosa, tunica muscularis consisting of two layers (inner circular and outer longitudinal) and adventitia.

From the luminal side the oesophagus in general consists of a thick stratified squamous epithelium. This layer is underlaid by a lamina propria, where the oesophageal mucous glands occur. Wheather *et al*., (1987) and Hodges (1974) reported the presence of nodules of lymphatic tissue (*lymphonoduli oesophageales*), frequently associated with the glands. Beneath the lamina propria, the muscularis mucosae is found, underlaid by a thin layer of loose connective tissue, the submucosa, followed by the tunica muscularis, which consists of a thick inner circular layer and a thinner outer longitudinal layer. Connective tissue fibres and myenteric plexuses commonly separate these two layers of the tunica muscularis. Externally the oesophagus has a tunica adventitia, a layer of loose connective tissue consisting of elastic fibres and which contain blood vessels and nerves.

MacAlister, (1864) and Bezuidenhout, (1986; 1999) described the topography of the oesophagus in the ostrich. The oesophageal mucosal lining has been studied by Feder (1972a) in the rhea *(Rhea americana)* and by Herd (1983) in the emu (*Dromaius novaehollandiae*). Hanke (1957) described the histology of the oesophagus of some *Tinamidae* species, which are closely related to the ratites.

According to Feder (1972a) the oesophageal epithelium of the rhea consisted of a stratum basale and a stratum spinosum. The lamina propria contained simple tubular mucous glands that stained intensely red with mucicarmine. The mucus in the glands is composed of neutral mucopolysaccharides (Feder, 1972a). Herd (1983) stated that "the epithelial lining of the oesophageal mucosa of the emu is a stratified squamous epithelium followed by a lamina propria consisting of loose connective tissue. The *muscularis mucosae* was separated from the double layer of tunica muscularis by a thin submucosa consisting of loose connective tissue".

Although information on the morphology of the oesophagus of some ratites has been provided by the above-mentioned authors, a comprehensive histological study of this region of the upper digestive tract in the ostrich is not available. For this reason, this chapter presents a detailed description of the macroscopic, microscopic and scanning electron microscopic features of the oesophagus of the ostrich (S*truthio camelus)*, and compares the results with published information on this species, with other ratites and with birds in general.

5.2. MATERIALS AND METHODS

5.2.1. Experimental animals

Entire oesophagi were obtained from five, 12 – 14-month-old clinically healthy *Struthio camelus* specimens, of either sex, which had been raised on a breeding farm and were slaughtered commercially for their skin and meat. To prevent rapid degenerative changes after death, samples were collected on site at the abattoir. Animals were immobilized by mechanical or electrical means before slaughter (see details in chapter 2). Although death was immediate, carcasses were left until all obvious signs of post mortem tremor had ceased before commencing the skinning process. All oesophagi were removed from carcasses after the skinning process had been completed, rinsed in tap water and immersion-fixed in 10% buffered formalin. During the evisceration, the oesophagus and trachea were detached at the cranial cervical region and at the proximal part of the proventriculus. The oesophagi were isolated from the trachea and trimmed of excess fascia and further processed for light microscopy (LM), and scanning electron microscopy (SEM) as described below.

5.2.2. Topography and macroscopical features

Two ostriches from the collection held at the Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, were carefully dissected to provide a description of the anatomical features and topographical relationship of the oesophagus and associated structures in the cervical and thoracic regions. The oesophagi were then incised longitudinally to expose the macroscopical features of the oesophageal lumen.

5.2.3. Light microscopy (LM)

Five oesophagi were collected from the 12 – 14-month-old ostriches for light microscopy. The samples were immersion-fixed in 10% buffered formalin and irrigated with the fixative from both cut ends using a 20 ml syringe. They were then re-immersed in the fixative bath for a minimum period of 48 hours. Following fixation, transverse segments were collected from just behind the laryngeal mound and from the proximal, middle and distal regions. The samples were dehydrated through 70, 80, 96 and 2X100% ethanol and further processed through 50:50 ethanol:xylol, 2X 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 embedded manually into paraffin wax in plastic moulds. Sections were cut at 4-6 µm, stained with Haematoxylin and Eosin (H&E) and viewed and micrographed using an Olympus BX50 microscope equipped with the analySIS CC12 Soft Imaging System (Olympus, Japan).

5.2.4. Scanning electron microscopy (SEM)

Samples for SEM from three birds were collected from sites adjacent to those sampled for light microscopy. Tissue blocks were rinsed in water for several hours to remove any traces of phosphate buffer and routinely dehydrated through an ascending ethanol series (50, 70, 90, 95 and 3X100%). Due to the size of the tissue blocks, each dehydration step took 60 minutes. Thereafter the blocks were critical point dried from 100% ethanol through liquid CO_2 in a Polaron E3000 Critical Point Drier (Polaron, Watford, England), attached to rectangular alluminium supports with Silver Dag to expose the epithelial surface and sputter coated with a thin layer of palladium in a Polaron SEM E5100 coating unit. The samples were viewed in a Philips XL 20 scanning electron microscope operated at 7kV.

5.3. RESULTS

5.3.1. Macroscopic features

The oesophagus was a tubular organ that originated from the caudal aspect of the oropharynx and could be divided into cervical and thoracic components. In the cranial part of the neck the cervical oesophagus lay in the midline and dorsal to the trachea to which it was attached along its ventrum by connective tissue. The position of the cervical oesophagus changed more caudally where it was situated to the right of the midline between the jugular vein dorsally, and the trachea laterally (Fig. 5.1). The thoracic oesophagus ran dorsal to the trachea and syrinx and between the bifurcation of the trachea, and was closely related to the cervical, clavicular and cranial thoracic air sacs. The oesophagus opened into the proventriculus on the left side of the body cavity (Fig. 5.1).

The internal surface of the oesophagus was longitudinally folded. Four regional sub-divisions could be distinguished (the region just behind the laryngeal mound, the proximal oesophagus, the middle oesophagus and the distal oesophagus). The divisions were described based on the number and shape of the folds in each region. The most proximal region just behind the laryngeal mound consisted of numerous fine anastomosing and branching folds (Fig. 5.2). The

proximal oesophagus presented well demarcated longitudinal folds, approximately 12 in number (Fig. 5.3). In the middle region the oesophageal folds were consolidated into approximately 8-9 longitudinal folds with larger spaces between the folds (Fig. 5.4).

In the distal region, the folds were sparse, with large spaces between them. No cardiac sphincter was observed at the gastro-oesophageal junction which was clearly defined by the abrupt change in the structure and colour of the mucosa (Fig. 5.5).

The transition between the oesophagus and the proventriculus was abrupt, as mentioned above, and was morphologically indicated by a marked thinning of the epithelial lining representing the ridged gastro-oesophageal junction. The cream coloured oesophagus with regular folds was different in appearance compared to the yellowish-brown and relatively irregular appearance of the folds of the proventriculus. The openings of the proventricular glands were also obvious. (Fig. 5.5).

5.3.2. Light microscopy (LM)

Based on the macroscopic features outlined above and on preliminary histological observations, the segments of the oesophagus for LM and SEM could be grouped into four regions, namely: the oesophagus just behind the laryngeal mound, proximal oesophagus, middle oesophagus, and distal oesophagus.

The wall of the oesophagus was composed of a mucosa (which consisted of a thick stratified squamous epithelium, a lamina propria and a *muscularis mucosae*), submucosa, tunica muscularis (consisting of inner circular and outer longitudinal layers) and adventitia. In cross sections of the proximal, middle and distal oesophagus, the mucosa displayed a number of longitudinal folds which projected into the lumen. The number of folds tended to diminish towards the distal region. The detailed histological description of the four regions of the oesophagus which follows was based on transverse sections.

5.3.2.1. Oesophagus just behind the laryngeal mound

The epithelium was non-keratinized stratified squamous (Fig. 5.6). The lamina propria consisted of bundles of collagen fibres, blood vessels, nerve fibres, and numerous simple tubular mucus-

secreting glands. Diffuse or nodular lymphocytic aggregations, covered by connective tissue septa were distributed in the lamina propria and associated with the glands.

The *muscularis mucosae* was well developed although in some regions it was interrupted by connective tissue fibres and vascular and nerve plexuses. The *muscularis mucosae* did not accompany the lamina propria into the smaller mucosal folds, whereas in the larger folds it accompanied the lamina propria into the folds. The folds in this region appeared to be lower than those of the proximal and middle oesophagus (Fig. 5.6).

The thin submucosa consisted of loose connective tissue and contained large arteries, small veins and nerve fibres (Fig.s. 5.6 & 5.7). The tunica muscularis consisted only of a single circular layer (Fig. 5.6). The adventitia consisted of loose connective tissue that contained arteries and nerves.

5.3.2.2. Proximal oesophagus

The epithelial lining of the proximal oesophagus consisted of a thick non-keratinized stratified squamous epithelium with numerous openings for the ducts of the glands. The epithelium consisted of three cell layers. The basal layer (*stratum basale*) consisted of relatively high epithelial cells whereas the most superficial layer (*stratum corneum*) consisted of flattened squamous epithelial cells showing elongated nuclei. Between these two layers there was a layer of spherical epithelial cells, the *stratum spinosum* (Fig. 5.8).

The lamina propria consisted of a thick layer of dense connective tissue containing many blood vessels and nerve fibres. Some loose connective tissue and thin bands of collagen fibres were evident in the sample. The lamina propria followed the contours of the epithelium into the oesophageal folds (Fig. 5.10). Immediately beneath the epithelium was a capillary plexus, which was linked to a network of blood vessels located in the dense connective tissue. Prominent simple tubular mucous-secreting glands were present in the lamina propria. The epithelium forming the duct lining of the glands became progressively thinner towards the oesophageal lumen (Fig. 5.8). The glands were frequently associated with lymphoid tissue aggregations, either diffuse or nodular (Figs.5.9 & 5.10)

The thick muscularis mucosa followed the contours of the lamina propria into the folds. Connective tissue strands separated the muscle bundles of the *muscularis mucosae* (Figs.5.9 & 5.10).

The submucosa was poorly developed and consisted of loose connective tissue sandwiched between the *muscularis mucosae* and the tunica muscularis (Figs.5.9 & 5.10). It contained arteries, veins and nerve fibres. The tunica muscularis consisted of an inner circular layer (well developed), and an outer longitudinal layer (poorly developed) (Fig. 5.9). Loose connective tissue, myenteric plexuses and blood vessels were found between the two layers.

5.3.2.3. Middle oesophagus

Microscopically, the morphology of the middle oesophagus resembled that of the proximal oesophagus. In the samples examined it was evident that there were fewer folds than in the proximal oesophagus.

5.3.2.4. Distal oesophagus

The basic morphology of the distal oesophagus was much the same as the proximal and middle oesophagus. There seemed to be a little more diffuse and nodular lymphoid tissue infiltrations in this region compared to the other regions and it was evident that there were fewer folds. The folds were also not as well developed as in the above described regions.

5.3.3. Scanning Electron Microscopy (SEM)

Cross-sections of the oesophagus revealed similar features to those observed by LM. The folded mucosa could be clearly seen (Fig. 5.11). The various connective tissue components of the oesophageal wall could also be clearly observed (Fig. 5.12). The proximal region displayed a series of closely positioned longitudinal folds. High magnification of the longitudinal folds revealed numerous fine transverse grooves where the openings of the glands could be observed (Fig. 5.13). The stratified squamous epithelium showed signs of desquamation. Desquamated cells surrounded the openings of the glands (Fig. 5.15). These cells were sometimes covered by mucus released by the glands (Fig. 5.15). Sheets of mucus were seen on parts of the longitudinal folds (Fig. 5.14). No ciliated cells were seen in any of the regions studied.

5.4. DISCUSSION

5.4.1. Macroscopic features

MacAlister (1864), Deeming (1996) and Bezuidenhout (1986, 1999) provided a relatively detailed description of the topographical relationship of the oesophagus in the ostrich. These authors noted that in the cervical region the oesophagus lies on the right side of the neck, dorsally to the trachea and the external jugular vein. In the cranial thoracic region, it passed dorsally to the two bronchi and was situated between the heart and the lungs. At the level of the sixth cervical rib the oesophagus expands and opens into the proventriculus (Huchzermeyer, 1998; Bezuidenhout, 1999).

Much of the description of the topography of the oesophagus was supported by the work of Duerden (1912) who also noted that the initial portion of the oesophagus was pouch-like. He postulated that the food was stored there when the bird was eating, prior to being passed into the narrower distal part of the organ when the bird raised its head.
.
The results of the present study confirmed the findings of the above-mentioned authors, namely, that the oesophagus in the ostrich arose dorsally to the tracheal opening on the caudal aspect of the oropharynx. It then continued on the right side of the neck, attached to the trachea by connective tissue fibres.

The occurrence of longitudinal folds running throughout the oesophagus of the ostrich is typical of birds in general and had also been described in other ratites (Barthels, 1895; Duerden, 1912; Feder, 1972b; Herd, 1983; Fowler, 1991). However, a distinction was made between the longitudinal folds of the ostrich and those of other birds. According to Duerden (1912) and Fowler (1991), the internal lining of the oesophagus of the ratites is very distensible and when contracted displays numerous longitudinal folds. Herd (1983) confirmed the above-mentioned authors' findings, emphasizing the fact that the mucous membrane is arranged in longitudinal folds to allow the distension of the organ when the animal swallows bulky food. In addition, the author suggests that the stretching nature of the oesophagus facilitates the storage of the food in the absence of a crop.

The observations of the above-mentioned authors on the typical pattern of the internal lining of the oesophagus in ratites were clearly confirmed in the present study. The most remarkable

feature observed macroscopically in this study was the occurrence of the mucosal folds throughout the oesophagus. The region just behind the laryngeal mound revealed branching and anastomosing folds.

Although investigators frequently note the occurrence of longitudinal folds in birds, none of the earlier studies divided the avian oesophagus into specific regions or zones. However, specific differences in respect of the oesophageal folds in different regions were made by Puterill (2002) in the Nile crocodile.

The present investigation revealed that the oesophagus of the ostrich could be divided into three regions (proximal, middle and distal) based on the appearance and number of mucosal folds. The longitudinal oesophageal folds were present throughout the organ, but showed a decrease in number from about 12 in the proximal oesophagus to about 7 in the distal oesophagus. The folds also became lower towards the distal oesophagus.

5.4.2. Histological features

Epithelium

Little histological information on the oesophagus of the ostrich is available. McLelland (1979) and Fowler (1991) reported that the surface of the organ presented a cornified appearance and Duerden (1912) stated that the "internal mucous lining of the oesophagus of the ostrich was richly supplied with mucus glands" which help facilitate the passage of food.

Barthels (1895) described that the internal morphology of the oesophagus in *Rhea americana* consisted of only a few longitudinal folds. He noted that the typical histological pattern of the oesophagus was the same as in other birds and that the mucosa consisted of the usual layers described in other birds. He also noted the presence of very small and round cells on the surface of the oesophageal mucosa.

Hodges (1974), did not discuss the number of epithelial cell layers, but described the oesophageal epithelium as a stratified squamous epithelium with a characteristic basal germinal layer, with the cells becoming flattened towards the lumen. Kudo (1970) and Herd (1983) described a typical stratified squamous epithelium composed of numerous epithelial cell layers with the cells becoming flattened towards the lumen.

In this study no signs of keratinisation were observed and the findings therefore differ from the findings of McLelland (1979) and Fowler (1991) who note that a degree of keratinisation is present in birds including ratites. However, the epithelial cellular components described by Kudo (1970) and Herd (1983) were confirmed in the present study, namely flattened cells represented in the uppermost layer, relatively high cells represented in the basal layer with an additional layer between them. The outer epithelial cells had a tendency to desquamate.

Lamina propria

Hodges (1974) noted that lymphoid tissue was associated with the oesophageal glands and penetrated either individually or in aggregates into the glands, whereas Malewitz and Calhoun (1958) reported a reduced presence of lymphoid tissue in the lamina propria. In this investigation, although the lymphoid tissue was frequently associated with the glands, it never penetrated inside the glands. The number of lymphoid aggregates increased towards the distal part of the oesophagus. Barthels (1895) described the region beneath the epithelium as an irregular dense connective tissue. The author also described the oesophageal glands, located in the connective tissue, as very small, with a long body. The ducts were clearly situated in the mucosa and the surface of the glands was covered by thin connective tissue. The results of this study supported these findings.

Muscularis mucosae

Calhoun (1954), Warner *et al.,* (1967), Ziswiler & Farner (1972) and Hodges (1974) described the *muscularis mucosae* in birds as a band of longitudinal muscle with the same thickness as the external longitudinal layer of the tunica muscularis. These findings were confirmed in the ostrich in this study. However, in the region immediately caudal to the laryngeal mound, the *muscularis mucosae*) had the same thickness as the circular layer of the tunica muscularis (the only layer present. Connective tissue strands, nerve fibres and arteries interrupted the *muscularis mucosae*. This layer extended into the folds of the mucosa, which confirms the findings of Malewitz and Calhoun (1958).

Submucosa

Calhoun (1954) and McLelland (1979) both describe the submucosa in birds as a thin layer of loose connective tissue located between the *muscularis mucosae* and the inner circular layer of the tunica muscularis. This study confirmed the typical appearance of the avian submucosa.

Tunica muscularis

Barthels (1895) stated that the muscle layer (tunica muscularis) of the rhea was very well developed and was subdivided into three layers, namely an outer longitudinal, middle circular and inner longitudinal layers. This is contradictory to the usual two layers described in this study and in the majority of the birds including the ratites described above. Between the inner longitudinal and middle circular layers, Barthels (1895) observed a connective tissue layer, 100µm thick, and also that the outer longitudinal layer was covered by connective tissue rich in blood supply.

It was clear from the present study that in the ostrich the tunica muscularis consisted of an outer longitudinal and an inner circular layer, the latter being better developed that the former, and that the two layers were separated by connective tissue bands containing the myenteric plexuses and blood vessels. This study also showed that the tunica muscularis immediately caudal to the laryngeal mound consisted only of a single layer

5.4.3. Scanning electron microscopy (SEM)

The SEM findings of the present study supported the folded appearance of the oesophageal mucosa seen macroscopically. In the material studied, the proximal region showed a highly folded surface and desquamation of surface epithelial cells.

Although Kudo (1970) studied in detail the basal epithelial cells of the stratified squamous epithelium in the oesophagus of the chicken and pigeon using transmission electron microscopy, his results are not helpful for the comparison of the scanning electron microscopic observations of the present work. No SEM studies have previously been done on the ostrich oesophagus and it is therefore not possible to make comparative comments regarding the results obtained in this investigation. However, the results obtained by SEM confirmed most of the LM findings specifically regarding the surface morphology of the oesophagus. The lack of ciliation and the superficial cellular desquamation in the proximal oesophagus revealed by SEM was similar to the LM findings. SEM also revealed the branching and anastomosing of the mucosal folds in the

proximal region, which confirms the macroscopic findings observed in this investigation. The openings of the glands (most of them revealing mucus secretions), which were observed frequently in LM sections of the proximal region of the oesophagi examined, were confirmed in the SEM study.

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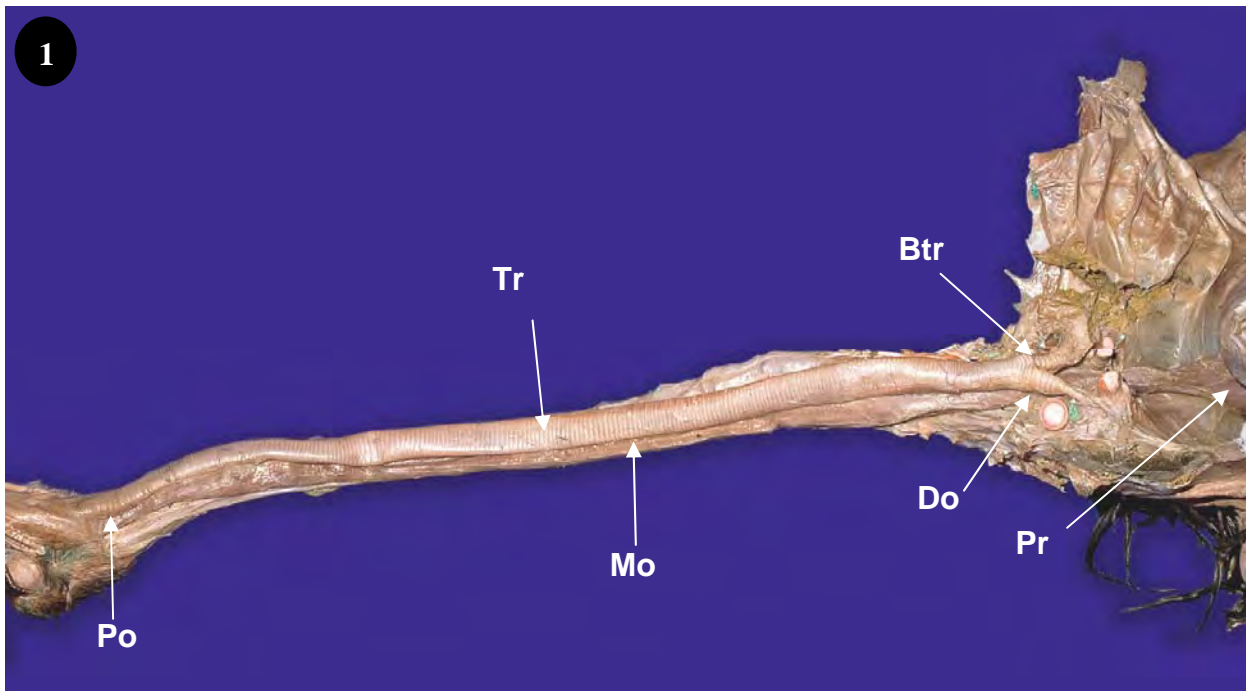
Figure 5.1: Ventro- lateral view showing the course of the oesophagus. The proximal oesophagus (Po) lies in the midline, dorsal to the trachea (Tr). The middle oesophagus (Mo) is located in the right side of the trachea, while the distal oesophagus (Do) runs dorsal to the trachea and between the bifurcation of the trachea (Btr) to join the proventriculus (Pr).
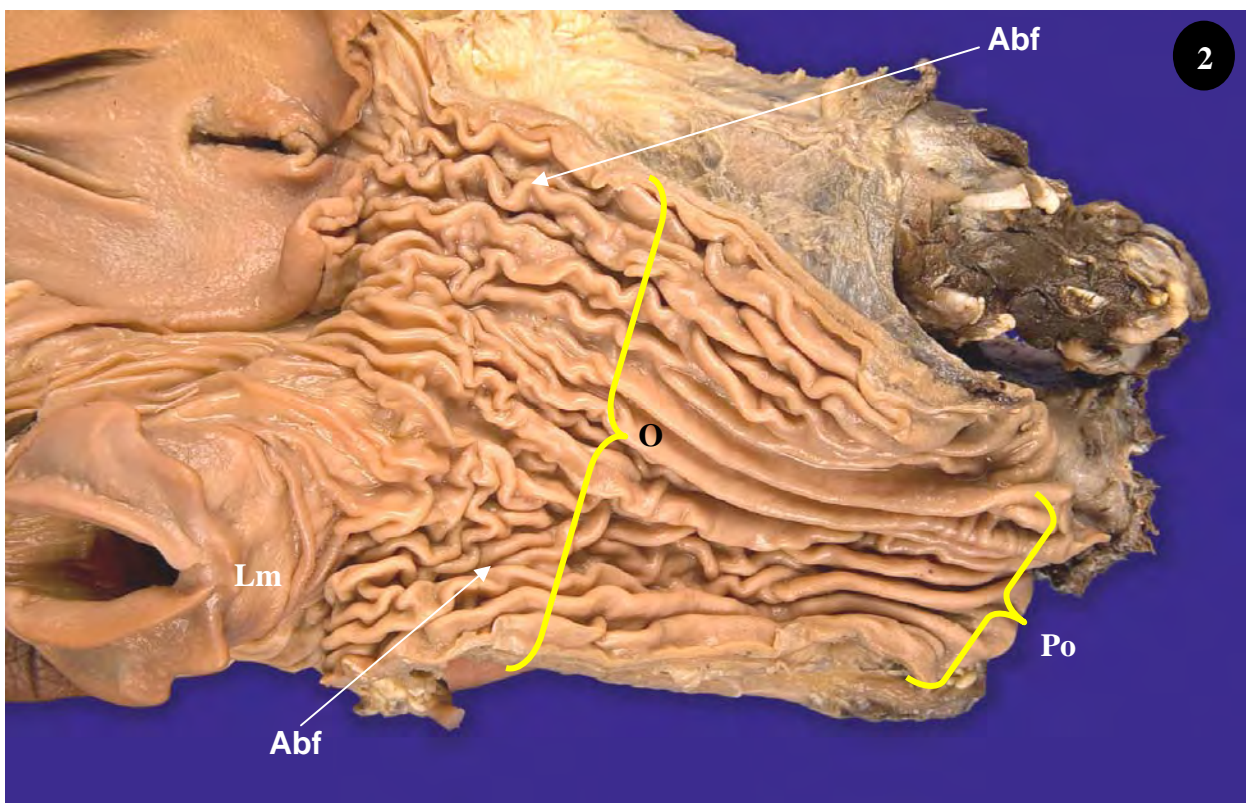

Figure 5.2: Oesophagus just behind the laryngeal mound. Anastomosing and branching folds (Abf) of the oesophagus (O) are present just behind the laryngeal mound (Lm). Note that the folds become longitudinally oriented in the proximal oesophagus (Po).

Figure 5.3. Proximal oesophagus showing the well-demarcated longitudinal folds. Approximately 12 folds are present.

Figure 5.4. Middle region of the oesophagus showing approximately 8 longitudinal folds with larger spaces occurring between them.
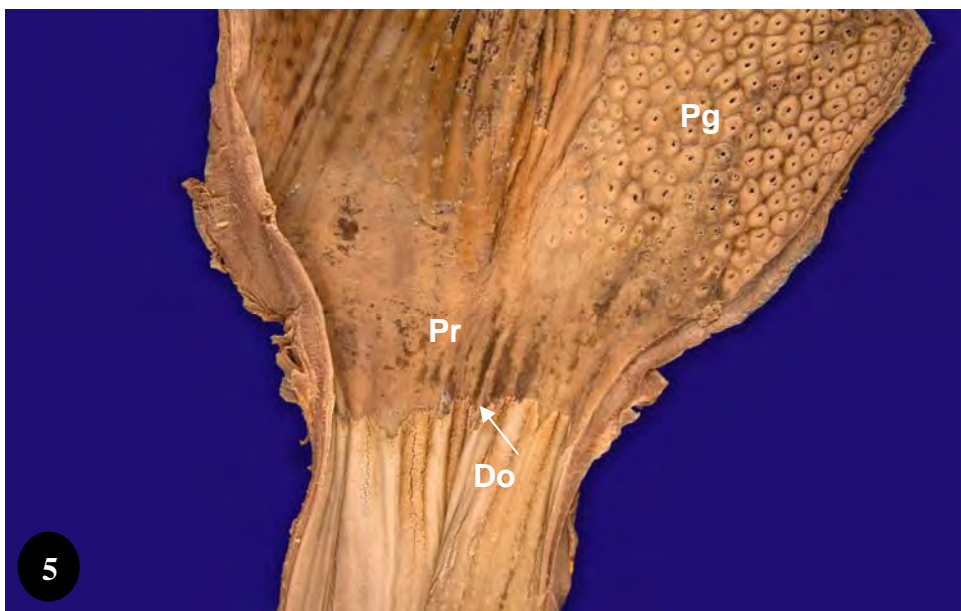
Figure 5.5. Transition between the distal oesophagus (Do) and the proventriculus (Pr). Note the small number of folds in the distal oesophagus and the gastro-oesophageal junction (arrow). The cream colour of the oesophagus and the yellowish-brown colour of the proventriculus can also be seen. Note the openings of the proventricular glands (Pg)
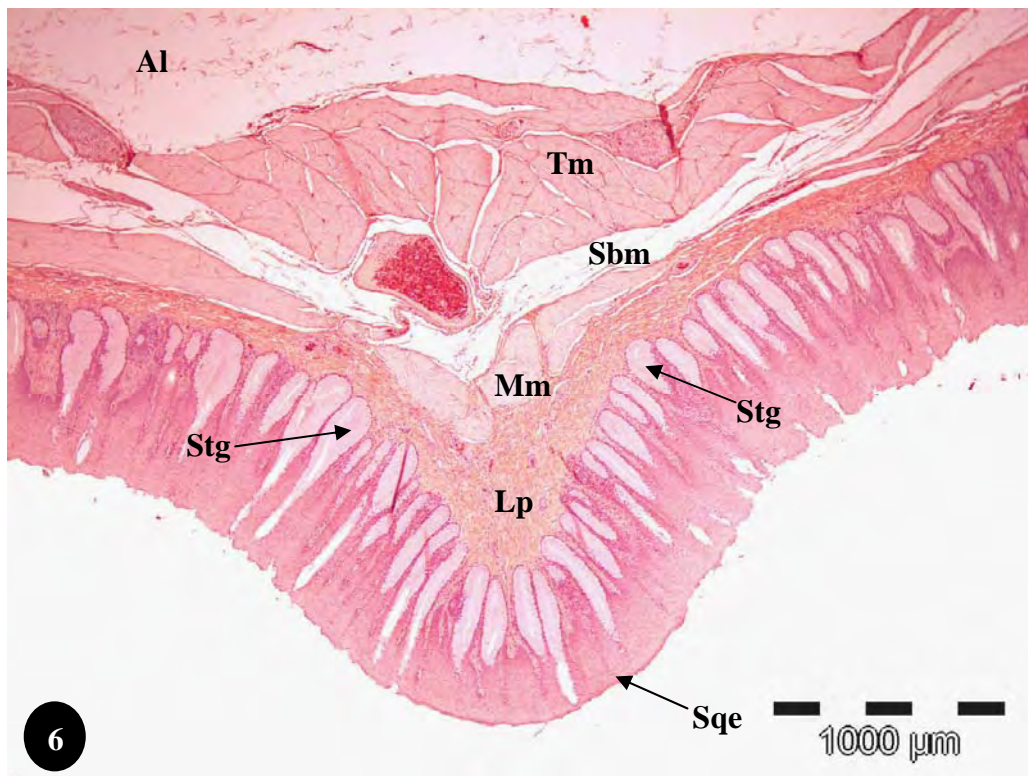
Figure 5.6: Oesophagus just behind the laryngeal mound demonstrating a shallow fold. Note the tissue layers: stratified squamous epithelium (Sqe), the thick lamina propria (Lp) which contains simple tubular glands (Stg). The muscularis mucosae (Mm) does not follow the contours of the fold. The submucosa (Sbm) consists of loose connective tissue and is situated between the muscularis mucosa (Mm) and the tunica muscularis (Tm), which is composed of a single layer of smooth muscle. Note the adventitia layer (Al).
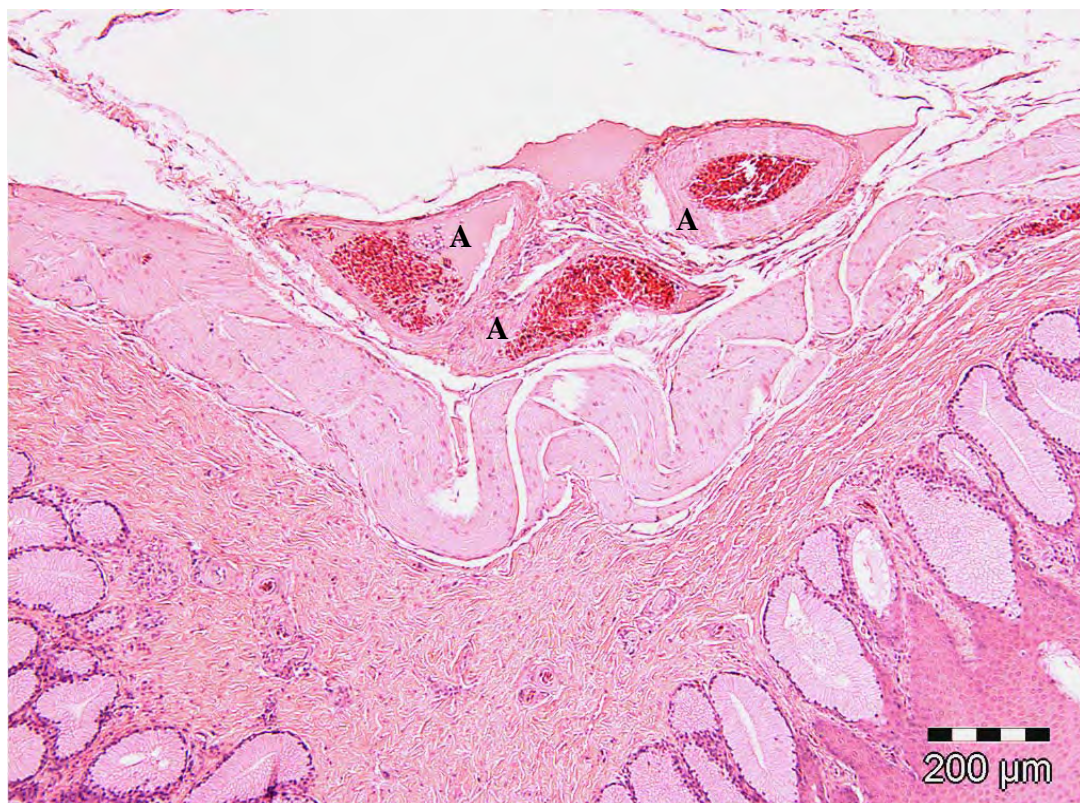
Figure 5.7: Higher magnification of the oesophagus just behind the laryngeal mound showing the large arteries (A) in the submucosa.
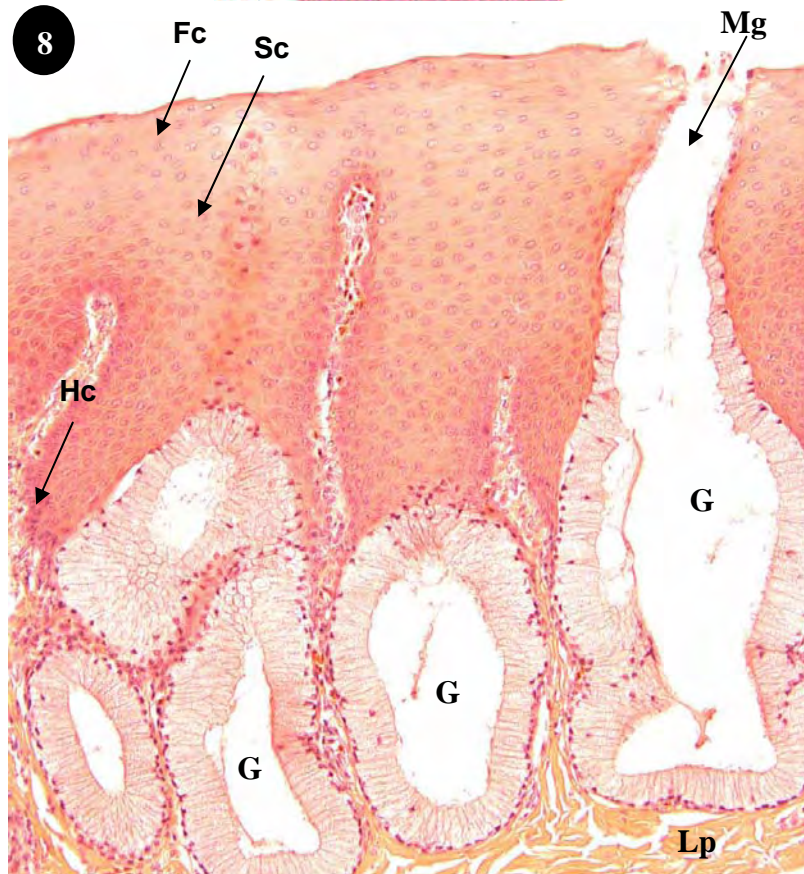
Figure 5.8: Proximal oesophagus. The basal layer consisted of relatively high epithelial cells (Hc) whereas the most superficial layer consisted of flattened squamous epithelial cells (Fc) showing elongated nuclei. Between these two layers there was a layer of spherical epithelial cells (Sc). Lamina propria (Lp). Simple tubular mucus-secreting glands (G).
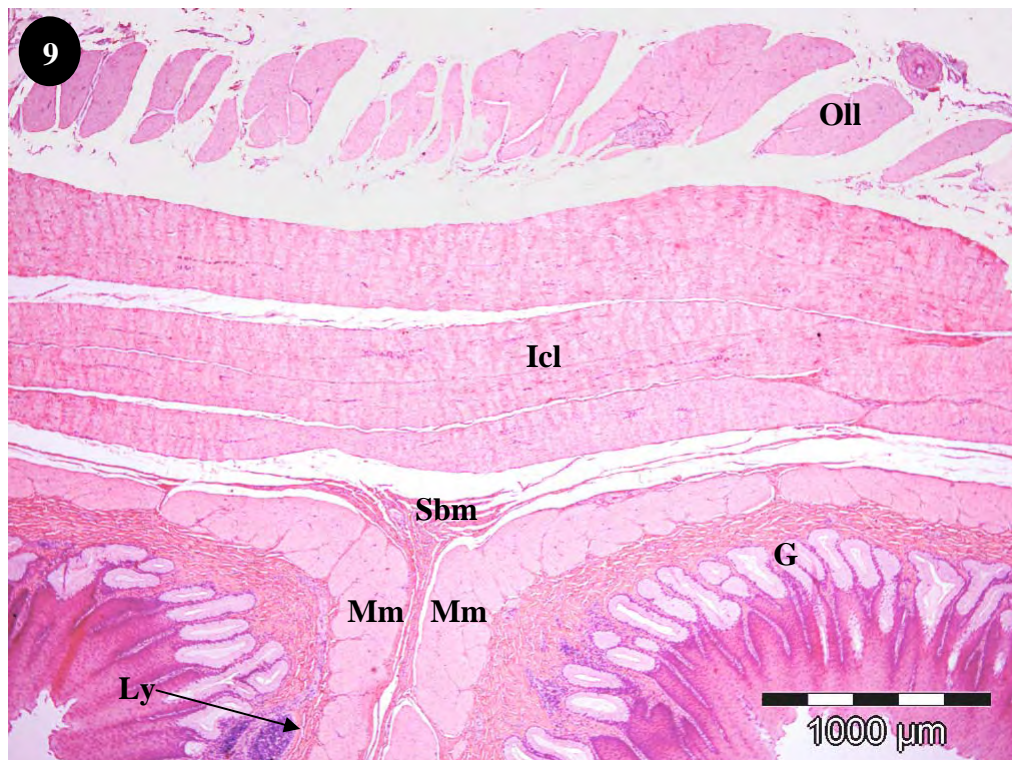

Figure 5.9: Proximal oesophagus. Note the thick inner circular layer of the tunica muscularis (Icl) compared to the outer longitudinal layer (Oll). See also the submucosa (Sbm) sandwiched between the *muscularis mucosae* (Mm) and the inner circular layer (Icl) of the tunica muscularis. Small lymphocytic infiltrations (Ly) were scattered in the lamina propria associated with the glands (G)
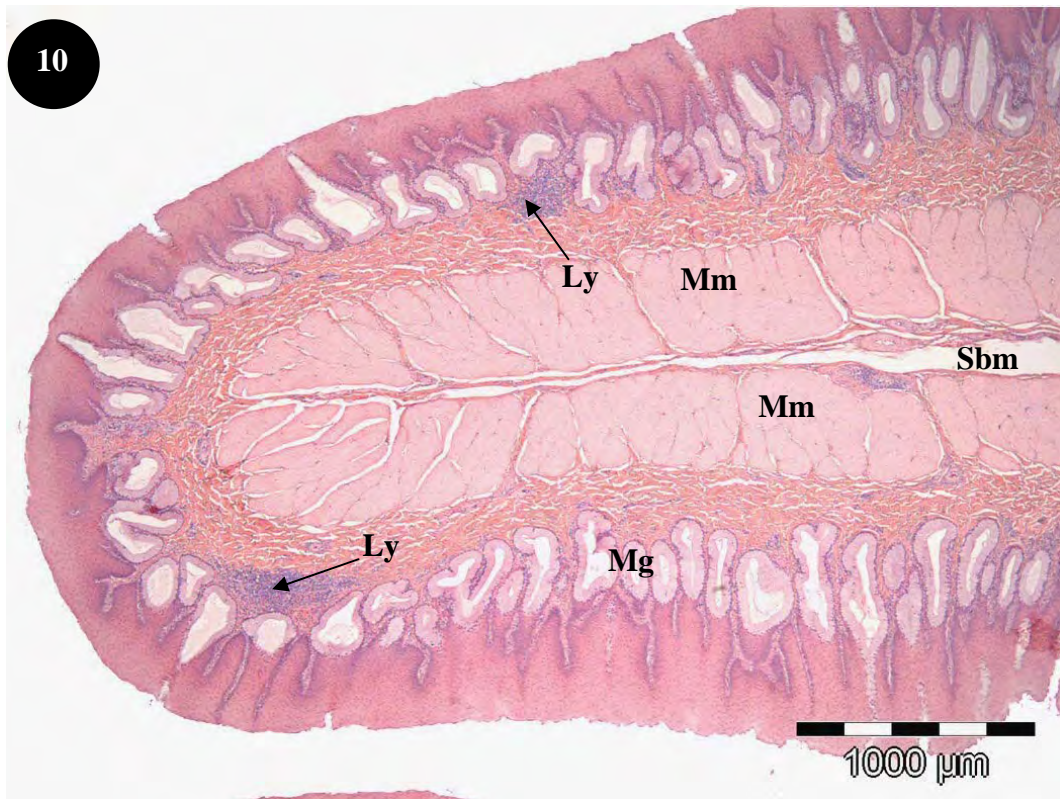
Figure 5.10: Mucusal fold of the proximal oesophagus. Note the submucosa (sbm) situated between the layers of the *muscularis mucosae* (mm). Lymphocytic aggregations (Ly) are associated with the mucous glands (mg). The thick *muscularis mucosae* follows the contours of the lamina propria into the fold. Connective tissue strands separate the muscle bundles of the *muscularis mucosa*e.
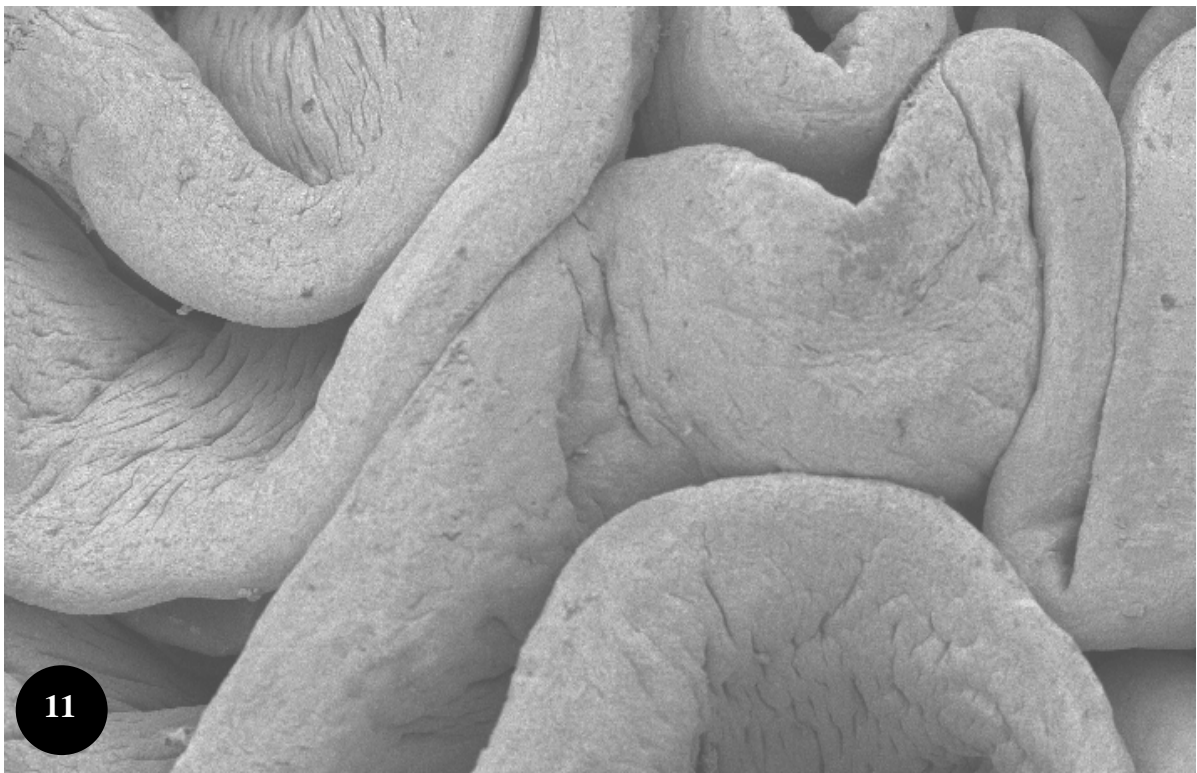

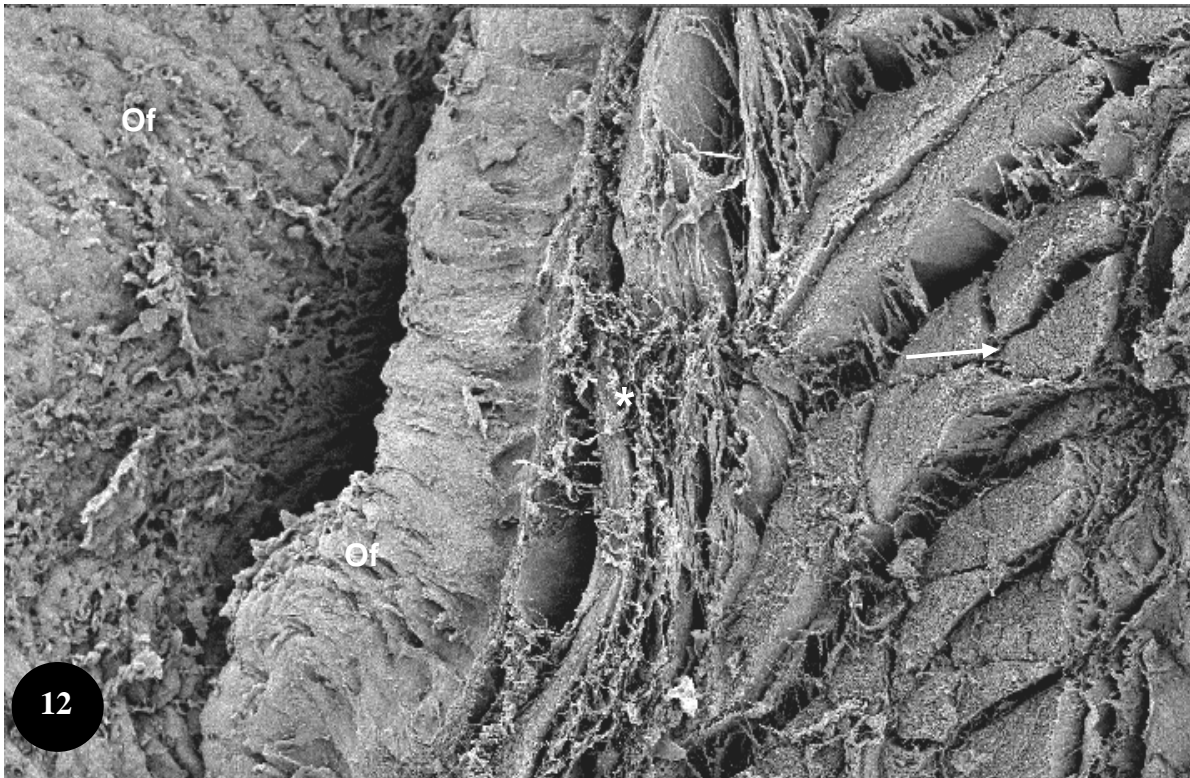Figure 5.11: Typical mucosal folds of the proximal oesophagus as seen by SEM.

Figure 5.12: Connective tissue components of the oesophageal wall viewed by SEM. Note elastic fibres (*) and fibrous dense connective tissue (arrow). Note the oesophageal folds (Of)
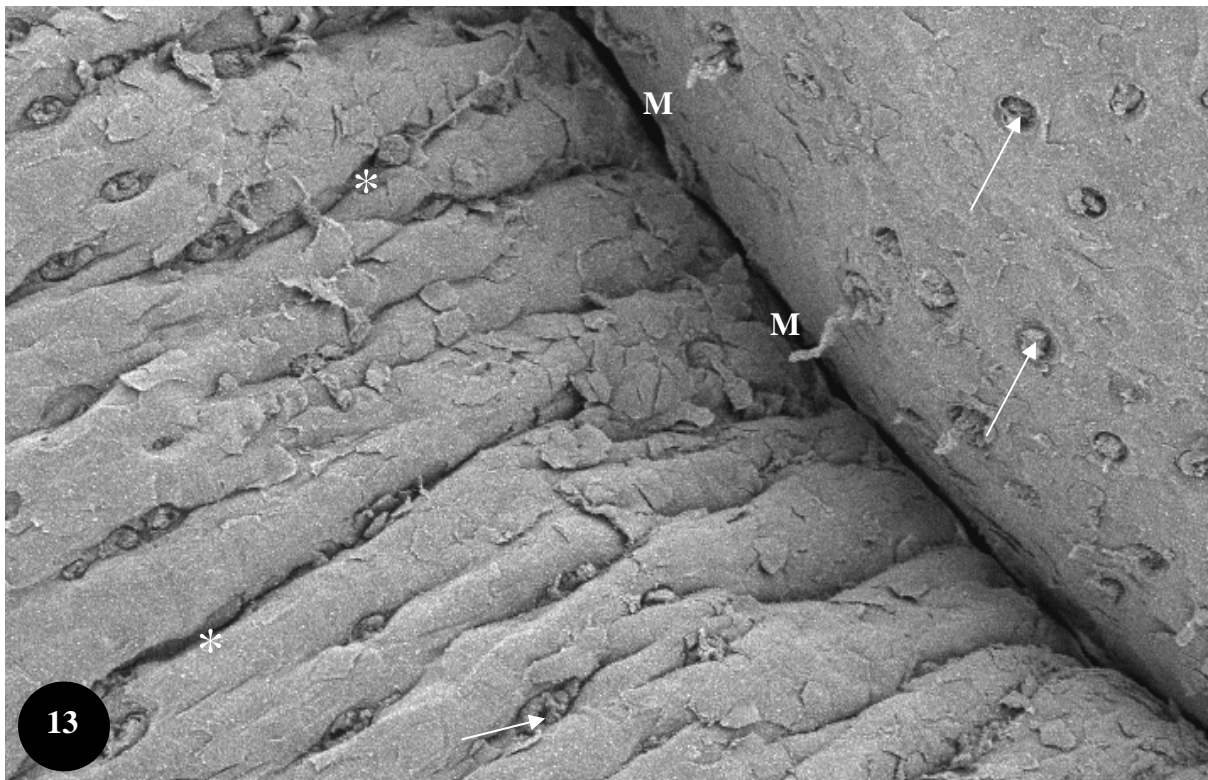

Figure 5.13: High magnification of the longitudinal folds showing transverse grooves (*) with the openings of the glands (arrows). Mucus can be seen in the openings of some glands (M)
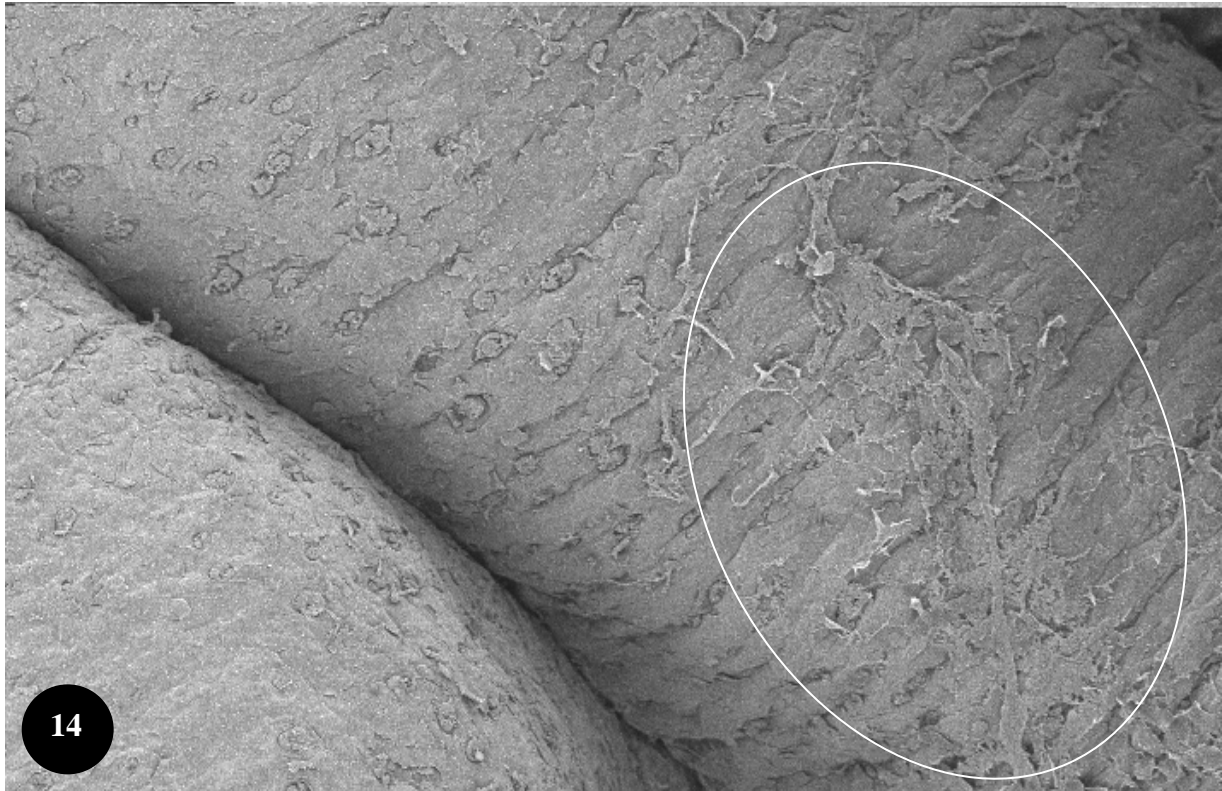
Figure 5.14: A lower magnification of the oesophageal folds. Note mucus (encircled) released by the glands.
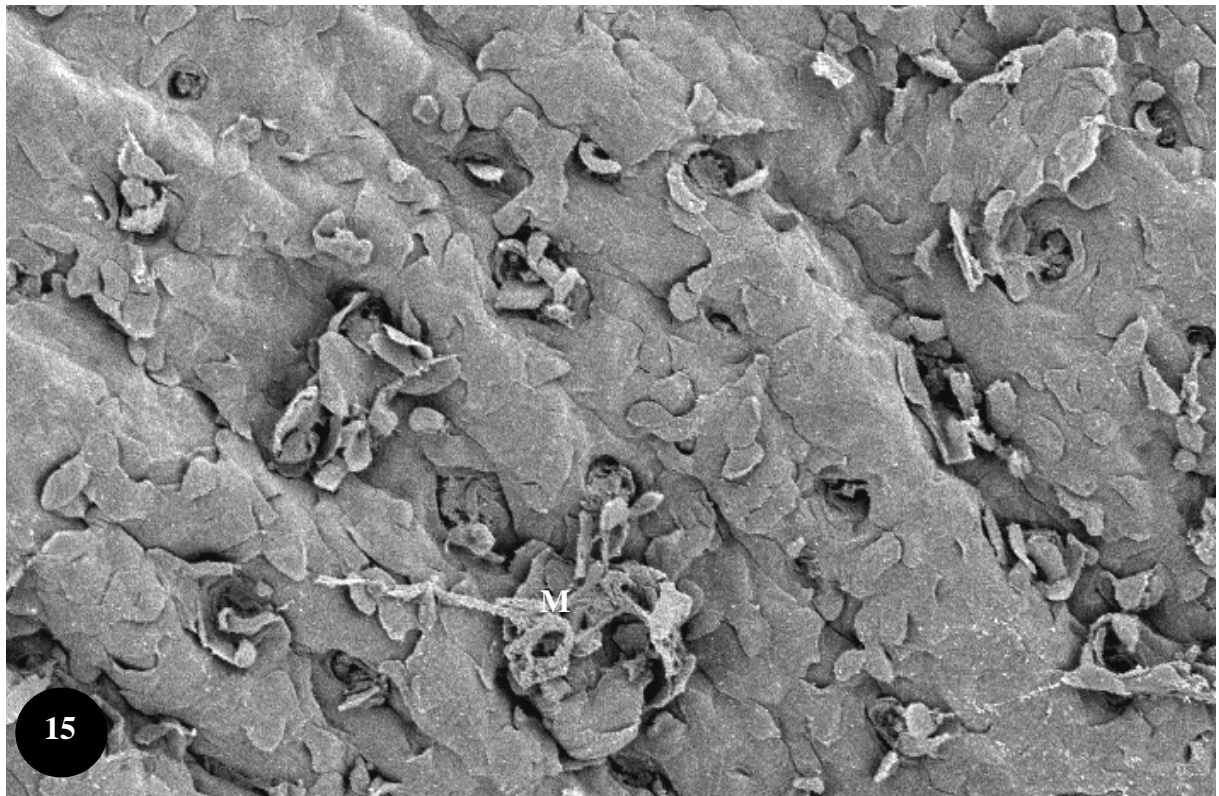

Figure 5.15: Higher magnification of the surface of the longitudinal folds clearly showing the desquamation of superficial cells. Mucus (M) released by the glands can also be seen.

CHAPTER 6

GENERAL CONCLUSIONS

Although a number of earlier studies provided information (in some instances illustrated by sketches) on the upper digestive tract (oropharynx and oesophagus) of ratites, including the ostrich, most descriptions of this region are superficial and supply little meaningful morphological data. It was therefore the aim of this study to complement the available knowledge on this part of the digestive tract with a more detailed account of the morphological features of the oropharynx and oesophagus in the ostrich, and also to clarify the often contradictory information presented in the literature.

Macroscopic observations confirmed that in the ostrich the oral and pharyngeal cavities formed a single structure and could not be separated using visual criteria. The most obvious components observed in the roof of the oropharynx were the palate, the choana, the infundibular cleft and the pharyngeal folds, and on the floor, the interramal region, the tongue and the laryngeal mound. These components have previously been briefly described. Additional information provided by the current study point to the existence of previously unreported structural modifications in the oropharynx that may influence the selection and manipulation of food by the ostrich.

An important observation was that the previously illustrated prominent median longitudinal fold running along the palate contained a concentration of Herbst (Pacinian) corpuscles. These structures were commonly found throughout the non-glandular regions of the palate and interramal region of the floor where they were also seen to concentrate at the tips of the numerous mucosal folds present in this region. Herbst corpuscles are believed to act as deep pressure receptors for mechanical and vibratory pressure and although described in other avian species have not previously been noted in the oropharynx of ratites. To what extent Herbst corpuscles may play a role in determining the texture of ingested material remains to be determined.

This study also revealed that the ramphotheca forming the rim of the oral cavity carried a sharp tomium along the rostral aspect of the mouth. The existence of this structural adaptation would lend support to the observation that ostriches use their large beaks to tear off plant material. It was further observed in the ostrich that both the roof and floor of the oropharynx could be macroscopically divided into two regions based on colour differences in the mucosa. In the roof and floor the pale rostral region was shown histologically to be lined by a keratinized stratified squamous epithelium. The darker mucosa lining the caudal aspect of the roof was composed of a non-keratinised stratified squamous epithelium overlying a well-developed glandular layer. In contrast, the darker mucosa on the caudal aspect of the floor, while also lined by a non-keratinised stratified squamous epithelium, was free of glandular tissue. These features have not previously been reported.

None of the regions of the upper digestive tract sampled revealed structures resembling taste buds and it would appear as if taste plays no role in the selection of food in the ostrich. This observation is supported by the suggestion that the colour and surface texture of feed is more important than any other qualities in the acceptance or refusal by ostriches of newly introduced feed. The presence of large numbers of Herbst corpuscles, particularly in the rostral palate and interramal region, may further indicate the importance of texture in the selection of food in this species.

This study also confirmed the rudimentary nature of the ostrich tongue which is said to be adapted, as in all ratites, for the rapid swallowing of large food items. Evidence has been presented that the caudal lingual papillae that lie at the base of the tongue in ratites such as the rhea assist in stabilizing the food bolus during its final transportation to the oesophagus. In the ostrich, however, the caudal lingual papillae are poorly developed and their role in stabilizing food would appear to be of limited value. In addition to confirming the folded nature of the ostrich tongue, the present study revealed that the deep pouch formed by the dorsal tongue fold is further subdivided by a smaller, but substantial secondary fold, into dorsal and ventral recesses. The function of this structural adaptation is unclear but the large increase in surface area produced by the folds, and by virtue of the numerous mucus producing glands found in the mucosa, would presumably enhance mucus production and secretion required for ingesting often dry and difficult to swallow plant material. In addition to the tongue, the entire caudal aspect of the oropharynx was well-equipped with glandular tissue. Massive branched tubular mucus-producing glands were situated around the choana and in the pharyngeal folds

and glottis. Tightly packed sheets of simple tubular mucus-producing glands extended from the retropharyngeal recess and base of the laryngeal mound and were present throughout the oesophagus. The additional capacity of these regions to produce large amounts of mucus would further assist in conveying food to the stomach.

Other adaptations for swallowing food were also identified in the study. The presence of a highly folded mucosa in the interramal region would seem to indicate that the floor of the oral cavity in the ostrich is capable of a certain degree of distension to accommodate the accumulation of food in the oral cavity prior to swallowing. In similar fashion the longitudinal mucosal folds present throughout the oesophagus, as in other avian species, would also allow for distension of this organ when swallowing bulky food items.

The pharyngeal folds that lie caudal to and around the opening of the Eustachian tubes in ratites are often referred to as the "tonsils" although no histological information has been presented to support this observation. This study revealed that the pharyngeal folds are filled with masses of diffuse and nodular lymphatic tissue and that epithelial folds emanating from the infundibular cleft and retropharyngeal recess formed tonsillar crypts surrounded by the lymphatic tissue. It was also observed that diffuse lymphatic aggregations occurred throughout most regions of the oropharynx although none of these areas matched the concentration of tonsillar tissue seen in the pharyngeal folds. The oropharynx therefore also serves an important immune function.

It has been well documented that in most species of birds papillae are found in the oropharynx at the edges of the choana, the base of the tongue, and caudal to the larynx and infundibular cleft. Papillae have also been described in ratites, mainly on the tongue and at the caudal aspect of the larynx. Whether the projections observed on the laryngeal mound of the ostrich in this study can be viewed as pharyngeal papillae remains debatable, although the caudo-medial pair, which were unsupported by the arytenoid cartilages, do resemble papillae. Likewise, the lingual papillae seen in the ostrich were poorly developed and rudimentary. Compared to other birds, therefore, it is clear that the oropharynx of the ostrich is poorly equipped with papillae.

Although the hyobranchial apparatus of the ostrich has been described in a number of studies, differences in nomenclature and in the identification of certain components have made a definitive description difficult. This study confirmed that the hyobranchial apparatus consists

of both central and paired caudo-lateral components, the former represented by the paraglossum and fused basihyale and urohyale, and the latter by the ceratobranchiale (which articulated with the body of the basihyale) and the epibranchiale (which articulated with the ceratobranchiale). The most important finding was that the paraglossum of the ostrich consisted of paired caudo-laterally directed cartilages that were connected rostrally to each other by fibrous connective tissue, and which supported the ventro-lateral aspect of the tongue. This information on the paraglossum has not previously been reported. The statements in the literature that the horns of the ostrich hyobranchial apparatus pass close to the skull (as is the case in the domestic fowl) were shown in this study to be inaccurate. It was observed that the paired cornu, while indeed suspending the hyobranchial apparatus, at no point passed close to the skull, but in fact curved downwards (caudo-ventrally) away from the skull.

Some confusion also exists in the literature regarding the identification and number of cartilages forming the larynx in ratites. This study demonstrated that the larynx of the ostrich consisted of the cricoid, procricoid and two arytenoid cartilages, and confirmed the absence of thyroid and epiglottic cartilages. Although the ostrich larynx displays the typical combination of cartilages described for birds in general, it differs markedly in respect of the relatively simple design of the paired arytenoid cartilages.

It can be concluded that the present study, in addition to confirming the basic features of the oropharynx previously described for the ostrich, clarified the contradictory information presented in the literature and also provided new, unreported morphological data. The most important findings were:

- the occurrence of transitional zones in the mucosa of the palate and interramal region.
- the occurrence of concentrations of Herbst corpuscles in the mucosal ridges of the palate and interramal region.
- the distribution of glandular tissue at the caudal aspect of the oropharynx.
- the sub-division of the lingual pocket into dorsal and ventral recesses.
- the existence of paired paraglossals supporting the tongue parenchyma in conjunction with other elements of the hyobranchial apparatus.
- the existence of a deep retropharyngeal recess at the junction of the pharyngeal folds and the proximal oesophagus.
- the existence of well-developed pharyngeal tonsils with crypts.