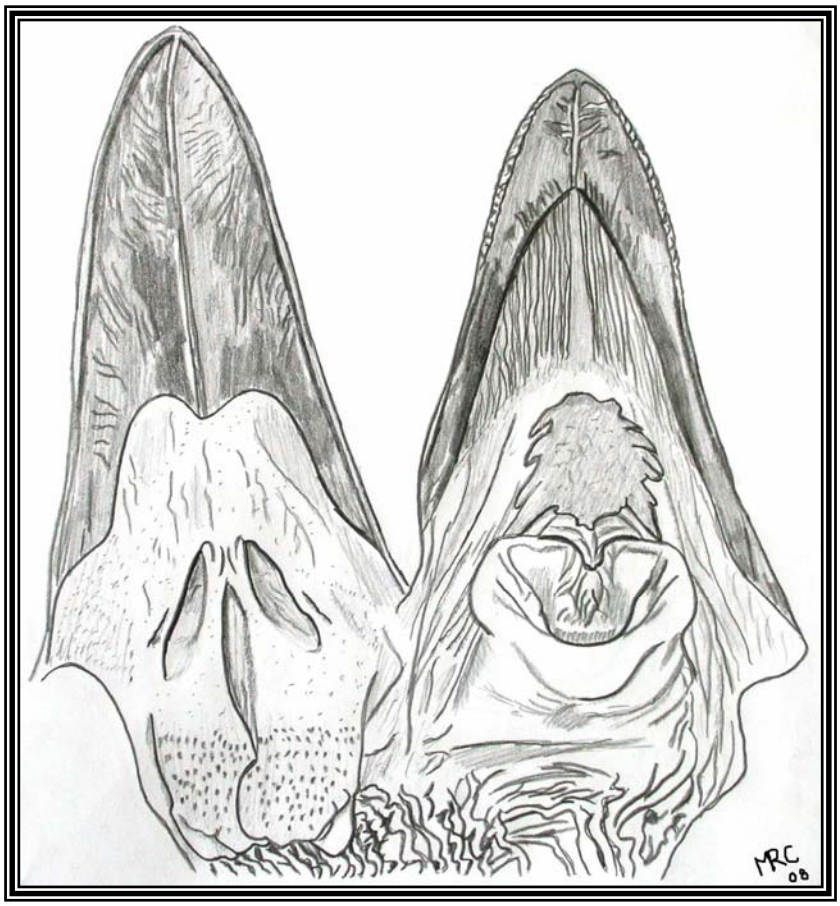
A Gross Anatomical and Histological Study of the Oropharynx and Proximal Oesophagus of the Emu (*Dromaius novaehollandiae*)

by

MARTINA RACHEL CROLE



Submitted in partial fulfilment of the requirements for the degree MSc

DEPARTMENT OF ANATOMY AND PHYSIOLOGY

FACULTY OF VETERINARY SCIENCE

UNIVERSITY OF PRETORIA

PRETORIA

2009

Supervisor:

Professor Doctor John Thomson Soley

Department of Anatomy and Physiology

Faculty of Veterinary Science

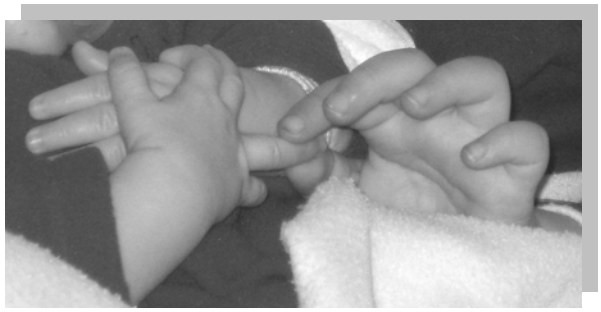
University of Pretoria

Pretoria

DECLARATION

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

Dedication



To Damién and Jayden van den Berg:

The most endearing brothers I have ever known.

Dear Baby Jay

…It was but a breath of time,
And your smiling, precious soul was amongst us.
…It was but another, brief,
Breath of time,
….and you had left us. Departed…..……
Yet, for all the time,
…For every breath that will still fill our lungs,
All who cared so deeply for you,
…Will never forget you, never stop loving you.
And our hearts will be joyously filled;
…Overflowed,
With the memory of you, Jayden,
…Of every second you were with us.
You live on, here, in us, who love you,
……..Forever.

To my dearest little friend

Damién

You have stolen my heart,
Right from the start;
From your intelligence and questions,
To your incontrovertible perceptions.
Your lucid imagination, leaves no room,
for our adult stagnation.
The freedom with which you love
And the freedom, with which
you live, is exceptionally
enlightening and truly inspiring.

CONTENTS

ACKNOWLEDGEMENTS

My promoter, Prof. John Soley, who guided and taught me so patiently, thoroughly and with all his heart. Thank you for your dedication, you have given me a solid foundation.

The Head of the Department of Anatomy and Physiology, Faculty of Veterinary Science, Prof. Herman Groenewald. Thank you for the trust and belief you show in me and my work. I value your inputs and the freedom with which you allow me to work.

Mr. Peter Duncan for providing the emu heads and for his willingness to secure samples for future work. Joan and Adrian Perry for providing an emu head for SEM. Dr. Catarina Tivane for the initial specimen collection.

Erna Van Wilpe and Lizette du Plessis of the Electron Microscopy Unit, Department of Anatomy and Physiology, for their kind assistance in sample processing, viewing and use of printing facilities. My colleagues and support staff from the Department of Anatomy and Physiology including Martè Smit for technical assistance and preparation of articles and Leon De Villiers and Adam Flink for specimen storage and handling.

Alan Hall, Andre Botha and Chris van der Merwe from the Laboratory for Microscopy and Microanalysis, University of Pretoria, for kindly allowing me use of the SEM's.

Joey Breedt from the Section of Pathology, Faculty of Veterinary Science, for the preparation of histological sections; Charmaine Vermeulen for photography of the gross anatomy specimens; Dr. Kerstin Junker from the Department of Veterinary Tropical Diseases for her assistance in photographing histology slides. Drs. Fritz and Hildegard Huchzermeyer for article translations.

Carole Long, Secretary of the Otanewainuku Kiwi Trust, Avi Holzapfel (leader of the Kiwi Recovery Group) and Susan Cunningham from New Zealand for their assistance in acquiring literature on the kiwi; Peter Johnston from the Liver Transplant Unit and Department of Anatomy, University of Auckland, New Zealand for his information on the cassowary and assistance in the acquisition of literature.

The University of Pretoria for financial support of this project.

SUMMARY

A Gross Anatomical and Histological Study of the Oropharynx and Proximal Oesophagus of the Emu (*Dromaius novaehollandiae*)

by

MARTINA RACHEL CROLE

SUPERVISOR: Professor John T. Soley

DEPARTMENT: Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, Republic of South Africa.

DEGREE: MSc (Veterinary Sciences)

This study describes the gross anatomical, histological and surface morphological features of the oropharynx and proximal oesophagus of the emu in order to address the scarcity of information on this region in this commercially important bird. Heads obtained from birds at slaughter (and a younger and older bird from emergency farm slaughter) were used for this study and described using basic gross anatomical and histological techniques, supplemented by scanning electron microscopy. The findings of the study were compared with the relevant literature.

The oral and pharyngeal cavities could not be morphologically separated and formed a single cavity. This cavity was dorso-ventrally flattened and clearly divided, both on the floor and the roof, into rostral pigmented and caudal non-pigmented parts. The non-pigmented floor housed the tongue and laryngeal mound which had a wide glottis and no papillae. The choana was triangular-shaped, with a small caudo-lateral fold on either side, and was situated in the non-pigmented part of the roof. Caudal to the choana were two rounded pharyngeal folds with a

pitted ventral surface. A small bilateral projection from the caudo-lateral edge consisted mainly of diffuse lymphoid tissue. The pharyngeal folds contained numerous large simple branched tubular mucus-secreting glands as well as large accumulations of lymphoid tissue.

The pigmented regions of the roof and floor were aglandular and lined by a keratinised stratified squamous epithelium which, particularly in the roof, contained numerous Herbst corpuscles in the underlying connective tissue. SEM revealed the surface to be composed of sheets of desquamating flattened polygonal cells. The non-pigmented regions were glandular and lined by a non-keratinised stratified squamous epithelium. Surface cells displayed a pattern of microplicae or microvilli while individual surface cells were seen to desquamate. The connective tissue housed small, simple tubular and large, simple branched tubular mucus-secreting glands, Herbst corpuscles (only absent from the pharyngeal folds and proximal oesophagus), lymphoid tissue, blood vessels and nerves. The glands of the upper digestive tract were polystomatic and named as follows according to their location: Caudal intermandibular, lingual, crico-arytenoid, oral angular, caudal palatine, pharyngeal and oesophageal. The openings of the glands to the surface were seen on SEM as variably sized holes on the surface, some being obscured by mucus secretions from the underlying glands. Taste receptors were sparse and present only in the caudal non-pigmented oropharyngeal floor, tongue root and proximal oesophagus. Accumulations of lymphoid tissue were identified at the junction between the two regions of the roof, and in the non-pigmented roof, the non-pigmented floor, tongue ventrum, root and frenulum, proximal oesophagus and pharyngeal folds. The consistent dense accumulation of lymphoid tissue in the pharyngeal folds constituted pharyngeal tonsils (*Lymphonoduli pharyngeales*). The lymphoid tissue of the non-pigmented floor was visible macroscopically as round raised nodules. Specific, unnamed larger lymphoid tissue aggregations were located at the junction of the tongue ventrum and frenulum and in the small folds lateral to the choana. Surface morphology, as seen by SEM, revealed a pattern of microridges on the surface cells of the keratinised areas, whereas the surface cells of the non-keratinised areas displayed microplicae, microvilli and cilia. Microvilli and cilia were associated with the gland openings and ducts.

The proximal oesophagus was a cylindrical tube with a longitudinally folded mucosa and displayed the typical tissue layers described in birds. The mucosa was formed by a non-keratinised stratified epithelium which on SEM showed minimal surface desquamation. The *lamina propria* contained numerous simple tubular mucus-secreting glands which sometimes branched and occasional diffuse lymphoid tissue aggregations. The gland openings to the surface

were seen on SEM as small and large dark holes. The *muscularis mucosae* was very prominent and was a longitudinal smooth muscle layer separating the mucosa from the submucosa. The *tunica muscularis* was composed of a thicker inner circular and a thinner outer longitudinal smooth muscle layer surrounded by the outer loose connective tissue forming the *tunica adventitia*.

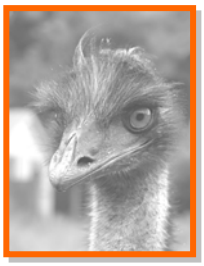
The emu tongue was divided into a body and a root. The body was triangular, dorso-ventrally flattened, pigmented and displayed caudally directed lingual papillae on both the lateral and caudal margins. The root, a more conspicuous structure in comparison to other ratites, was triangular, with a raised bulbous component folding over the rostral part of the laryngeal fissure. The lingual skeleton was formed by the triangular-shaped *paraglossum* (hyaline cartilage), forming the core of the tongue body, and the rostral projection of the *basihyale*, ventral to the *paraglossum*. Following the general trend in ratites, the emu tongue was greatly reduced in comparison to the bill length and specifically adapted for swallowing during the cranioinertial method of feeding employed by palaeognaths.

The tongue was invested by a non-keratinised stratified squamous epithelium. The glands in the connective tissue formed the bulk of the parenchyma and were composed of both small simple tubular and large simple branched tubular mucus-secreting glands similar to those seen in the oropharynx. The lingual glands were grouped as follows: dorsal and rostro-ventral (large glands), caudo-ventral and radical (large and small glands) and frenular (small glands). The large glands were visible macroscopically as doughnut-shaped structures. Melanocytes were absent from the tongue ventrum and occasionally from the tongue root. Lymphoid tissue was absent from the tongue dorsum. Herbst corpuscles were present in the tongue body and root and generally closely associated with the large mucus-secreting glands. The surface morphology varied in the different regions of the tongue. The dorsal and rostro-ventral tongue body showed individual desquamating cells and large gland openings only, the caudo-lateral ventrum showed less desquamation and both large and small openings. The mid-ventral aspect had an undulating uneven appearance with round raised cells on the surface which were densely packed with microvilli. Very large, large and small openings were present in this region and ciliated cells occurred in the vicinity of gland openings.

This study presented various unique findings regarding the morphology of the emu oropharynx compared to other ratites. Although the sense of taste has been confirmed in many avian species,

this study presented the first evidence of taste in the emu and ratites in general and suggests the possibility of taste being previously overlooked in the other birds studied (ostrich and greater rhea). The tongue root of the emu was clearly defined and is unique in structure and possible function amongst the ratites and other birds. Previously unmentioned functions of the emu tongue revealed by this study include: touch (Herbst corpuscles), taste (taste bud), lubrication and mechanical protection (mucus-secreting glands), immunological (lymphoid tissue) and digestive (swallowing). It was also noted that the various structures and organs of the oropharynx revealed important and often interesting differences between the emu and the other ratites documented. The prominent serrations of the rostral mandibular tomia of the emu also appear to be unique amongst ratites. The presence and wide distribution of Herbst corpuscles within the emu oropharynx and tongue show these areas to be highly sensitive to touch. The caudo-lateral projections of the pharyngeal folds effectively formed pharyngeal tonsils, a feature not apparent in other ratites. Despite the differences noted between the emu and other ratites it was possible to discern a common pattern of structures and features, with their modifications, both within and forming the oropharynx in this group of birds.

CHAPTER 1



GENERAL INTRODUCTION

Members of the *ratidae* (flightless birds with no keel on the sternum) have assumed an ever increasing commercial importance and the ostrich, rhea and emu are farmed extensively throughout the world for their skins, meat, feathers and fat (Gillespie and Schupp, 1998; Sales, 2007). Emu farming in South Africa is a relatively new enterprise and efforts to place this emerging industry on a sound financial basis are hamstrung by a lack of basic knowledge on the biology of this bird. Although a number of studies have been carried out on the digestive tract of ratites, these have concentrated mainly on the gastro-intestinal tract (Owen, 1841, 1879; Gadow, 1879; Pycraft, 1900; Mitchell, 1901; Cho *et al*., 1984; Herd, 1985; Bezuidenhout, 1999; Potter *et al*., 2006), with little detailed information being provided on the structure of the upper digestive tract (oropharynx and oesophagus). This region is of considerable importance considering that it is the first area for food selection and intake which is vital to the nutrition and growth of the animal and therefore its commercial viability.

The gross morphology of the upper digestive tract of many species of birds has been extensively studied (for a review of the earlier literature see McLelland, 1979). More recent studies on this region have concentrated on relating structure to function and in providing more detailed morphological descriptions using a wider variety of techniques including immuno-cytochemistry and scanning and transmission electron microscopy (Gargiulo *et al*., 1991; Kobayashi *et al*., 1998; Samar *et al*., 1999; Liman *et al*., 2001; Jackowiak and Godynicki, 2005). However, most of this work has focused on specific areas or structures of the upper digestive tract, such as the tongue (Lucas, 1896; 1897; Gardner, 1926, 1927; Kobayashi *et al*., 1998; Jackowiak and Godynicki, 2005; Rossi *et al*., 2005). This organ has been studied in respect of its function (McLelland, 1979; Bonga Tomlinson, 2000; Gussekloo and Bout, 2005) and classification (Lucas, 1896, 1897; Gardner, 1926, 1927; Harrison, 1964; Iwasaki, 2002), whereas the structure and secretion of the lingual salivary glands (Samar *et al*., 1999; Liman *et al*., 2001; Al-Mansour and Jarrar, 2004) have also been investigated.

Other studies have concentrated on the distribution and classification of the glands within the oropharynx (Tucker, 1958; Warner *et al*., 1967; Bailey *et al*., 1997; Samar *et al*., 1999; Liman *et al*., 2001) as well as of the taste end-organs of birds (Bath, 1906; Botezat, 1910; Moore and Elliott, 1946; Lindenmaier and Kare, 1959; Gentle, 1971a, b). The avian oesophagus has also been described for many species, generally as part of studies dealing with the digestive tract as a whole (Calhoun, 1954; Ziswiler and Farner, 1972; Hodges, 1974; Nickel *et al*., 1977; McLelland, 1979; Bailey *et al*., 1997; Bacha and Bacha, 2000; Gussekloo, 2006).

In contrast to the wealth of information available on this region in birds in general, studies on the upper digestive tract of ratites are superficial, brief, fragmented and often difficult to interpret (Sales, 2006). This situation is further compounded by the fact that only single specimens were sometimes described, particularly in the earlier studies (see Faraggiana, 1933).

Much of the available information has centred on gross morphological descriptions of the ratite tongue, the most extensive report being that of Faraggiana (1933) who compared the tongue and laryngeal mound of the ostrich, rhea and emu. Descriptions of the ratite tongue have appeared in numerous publications over the years (Meckel, 1829; Cuvier, 1836; MacAlister, 1864; Gadow, 1879; Owen, 1879; Pycraft, 1900; Göppert, 1903; Duerden, 1912; Faraggiana, 1933; Roach, 1952; Feder, 1972; McCann, 1973; Cho *et al*., 1984; Fowler, 1991; Bonga Tomlinson, 2000; Gussekloo and Bout, 2005; Porchescu, 2007; Crole and Soley, 2008; Jackowiak and Ludwig, 2008; Tivane, 2008), the majority of which, however, are brief and superficial.

The shape of the tonsils, as with the tongue, is also reported to vary between the ratites. A brief comparison is provided by Cho *et al*. (1984), which is vague and open to interpretation, giving little information on the specific location or structure of the tonsils. The authors simply note that "The ostrich tonsils and tongue are smooth, blunt and U-shaped. In the Darwin's rhea both tongue and tonsils have simple, pointed V-shaped tips. The tonsils in the emu are similar to the rhea but have a small flap laterally" (Cho *et al*., 1984).

Brief descriptions, as well as illustrations, of the ratite oropharynx or parts thereof have been supplied for the ostrich (Göppert, 1903; Faraggiana, 1933; Bonga Tomlinson, 2000), greater rhea (Pycraft, 1900; Faraggiana, 1933; Bonga Tomlinson, 2000; Gussekloo and Bout, 2005), kiwi (Owen, 1879; McCann, 1973) and emu (Faraggiana, 1933; Bonga Tomlinson, 2000). More recent studies incorporating gross morphological descriptions, light microscopy (Porchescu,

2007; Jackowiak and Ludwig, 2008; Tivane, 2008) and scanning electron microscopy (Jackowiak and Ludwig, 2008; Tivane, 2008) have supplied more comprehensive data of this region in the ostrich. Functional studies on the eating behaviour of ratites, involving structures of the upper digestive tract, have been documented using the ostrich, emu and greater rhea (Bonga Tomlinson, 2000) or greater rhea only (Gussekloo and Bout, 2005) as models.

Histological studies of the upper digestive tract of ratites include those of Feder (1972) on the tongue and oesophagus of the greater rhea, Herd (1985) on the oesophagus of the emu, Crole and Soley (2008) on the tongue of the emu, Jackowiak and Ludwig (2008) on the tongue of the ostrich, and Porchescu (2007) and Tivane (2008) on the oropharynx and oesophagus of the ostrich.

In respect of the emu, the tongue, and a description of its margins, surfaces and papillae have been reported, based on a single specimen (Faraggiana, 1933). Cho *et al*. (1984) describe the tongue as having a serrated edge and Bonga Tomlinson (2000) illustrates the tongue's outline in relation to surrounding structures and notes the presence of papillae. A brief histological description of this organ is supplied by Crole and Soley (2008). As part of a study on the anatomy and histology of the gut of the emu, Herd (1985) measured and briefly described the histology of the oesophagus based on two specimens.

As is evident from the above review, very little information is currently available on the morphology of the upper digestive tract of the emu, with only the tongue and oesophagus briefly being described. In view of the lack of any detailed information on the morphology and topographical relationships of the structures forming the upper digestive tract of the emu, this study aims to provide essential baseline data on a previously neglected segment of the digestive tract of this commercially important bird. The work will also provide additional data of academic significance enabling more accurate comparisons to be made between members of this important avian family.

The aims of the study are the following:

- To provide a comprehensive gross morphological description of the upper digestive tract (oropharynx and proximal oesophagus) of the emu,
- To describe the histological and surface morphological features of selected areas of the oropharynx and proximal oesophagus,
- To link microscopic findings to the gross morphology and formulate postulations for function,
- To critically appraise the existing literature on the topic and
- To gather base-line data for future studies.

The envisaged benefits arising from this study are the following:

- As morphology is so intimately linked to function, accurate, detailed morphological descriptions of the areas studied will lead to postulation of function.
- A sound knowledge of normal gross anatomical and histological features, including possible individual variations, will greatly assist in recognising pathology thus providing more accurate diagnostics and will aid in accurate tissue sampling.
- The collection of base-line data on the emu will provide a greater platform for an improved understanding of comparative ratite biology, will add to the data base of avian biology in general, may lead to the discovery of novel structures and will be of taxonomic value.
- A more accurate appreciation of the structure of the upper digestive tract will provide a greater insight into food selection and feeding behaviour of this bird and may possibly impact on feed formulation.

REFERENCES

AL-MANSOUR, M.I. & JARRAR, B.M. 2004. Structure and secretions of the lingual salivary glands of the white-cheeked bulbul, *Pycnonotus leucogenys* (*Pycnontidae). Saudi Journal of Biological Sciences,* 11:119-126.

BACHA, W.J. & BACHA, L.M. 2000. Digestive system, in *Color Atlas of Veterinary Histology,* edited by D. Balado. Philadelphia: Lippincott Williams & Wilkins: 121-157.

BAILEY, T.A., MENSAH-BROWN, E.P., SAMOUR, J.H., NALDO, J., LAWRENCE, P. & GARNER, A. 1997. Comparative morphology of the alimentary tract and its glandular derivatives of captive bustards. *Journal of Anatomy,* 191:387-398.

BATH, W. 1906. *Die Geschmacksorgane der Vögel und Krokodile*. Berlin: In Kommission bei R. Friedländer & Sohn.

BEZUIDENHOUT, A.J. 1999. Anatomy, in *The Ostrich, Biology, Production and Health*, edited by D. C. Deeming. Wallingford, UK: CABI Publishing: 13-49.

BONGA TOMLINSON, C.A. 2000. Feeding in paleognathous birds, in *Feeding: Form, Function, and Evolution in Tetrapod Vertebrates,* edited by K. Schwenk. San Diego: Academic Press: 359-394.

BOTEZAT, E. 1910. Morphologie, Physiologie und phylogenetische Bedeutung der Geschmacksorgane der Vögel. *Anatomischer Anzeiger,* 36:428-461.

CALHOUN, M.L. 1954. *Microscopic Anatomy of the Digestive System of the Chicken*. Ames, Iowa: Iowa State College Press.

CHO, P., BROWN, B. & ANDERSON, M. 1984. Comparative gross anatomy of ratites. *Zoo Biology,* 3:133-144.

CROLE, M.R. & SOLEY, J.T. 2008. Histological structure of the tongue of the emu (*Dromaius novaehollandiae*). *Proceedings of the Microscopy Society of Southern Africa,* 38:63.

CUVIER, G. 1836. *Leçons d'anatomie comparée*, Third edition. Volumes 1 & 2, edited by M. Duméril. Bruxelles: Dumont.

DUERDEN, J.E. 1912. Experiments with ostriches XVIII. The anatomy and physiology of the ostrich. A. The external characters. *Agricultural Journal of the Union of South Africa*, 3:1-27.

FARAGGIANA, R. 1933. Sulla morfologia della lingua e del rialzo laringeo di alcune specie di uccelli Ratiti e Carenati non comuni. *Bollettino dei Musei di Zoologia e Anatomia comparata*, 43:313-323.

FEDER, F-H. 1972. Zur mikroskopischen Anatomie des Verdauungsapparates beim Nandu (*Rhea americana*). *Anatomischer Anzeiger*, 132:250-265.

FOWLER, M.E. 1991. Comparative clinical anatomy of ratites. *Journal of Zoo and Wildlife Medicine*, 22:204-227.

GADOW, H. 1879. Versuch einer vergleichenden Anatomie des Verdauungssystemes der Vögel. *Jenaische Zeitschrift für Medizin und Naturwissenschaft*, 13:92-171.

GARDNER, L.L. 1926. The adaptive modifications and the taxonomic value of the tongue in birds. *Proceedings of the United States National Museum*, 67:Article 19.

GARDNER, L.L. 1927. On the tongue in birds. *The Ibis*, 3:185-196.

GARGIULO, A.M., LORVIK, S., CECCARELLI, P. & PEDINI, V. 1991. Histological and histochemical studies on the chicken lingual glands. *British Poultry Science*, 32:693-702.

GENTLE, M.J. 1971a. Taste and its importance to the domestic chicken. *British Poultry Science*, 12:77-86.

GENTLE, M.J. 1971b. The lingual taste buds of *Gallus domesticus*. *British Poultry Science*, 12:245-248.

GILLESPIE, J.M. & SCHUPP, A.R. 1998. Ratite production as an agricultural enterprise. *The Veterinary Clinics of North America. Food Animal Practice*, 14:373-386.

GÖPPERT, E. 1903. Die Bedeutung der Zunge für den sekundären Gaumen und den Ductus nasopharyngeus. *Morphologisches Jahrbuch*, 31:311-359.

GUSSEKLOO, S.W.S. 2006. Feeding structures in birds, in *Feeding in Domestic Vertebrates: From Structure to Behaviour*, edited by V. Bels. Wallingford, UK: CABI Publishing: 14-19.

GUSSEKLOO, S.W.S. & BOUT, G.R. 2005. The kinematics of feeding and drinking in palaeognathous birds in relation to cranial morphology. *Journal of Experimental Biology*, 208:3395-3407.

HARRISON, J.G. 1964. Tongue, in *A New Dictionary of Birds*, edited by A.L. Thomson. London: Nelson: 825-827.

HERD, R.M. 1985. Anatomy and histology of the gut of the emu *Dromaius novaehollandiae*. *Emu*, 85:43-46.

HODGES, R.D. 1974. The digestive system, in *The Histology of the Fowl*. London: Academic Press: 35-47.

IWASAKI, S. 2002. Evolution of the structure and function of the vertebrate tongue. *Journal of Anatomy*, 201:1-13.

JACKOWIAK, H. & GODYNICKI, S. 2005. Light and scanning electron microscopic study of the tongue in the white tailed eagle (*Haliaeetus albicilla, Accipitiridae, Aves*). *Annals of Anatomy*, 187:251-259.

JACKOWIAK, H. & LUDWIG, M. 2008. Light and scanning electron microscopic study of the structure of the ostrich (*Strutio camelus*) tongue. *Zoological Science*, 25:188-194.

KOBAYASHI, K., KUMAKURA, M., YOSHIMURA, K., INATOMI, M. & ASAMI, T. 1998. Fine structure of the tongue and lingual papillae of the penguin. *Archivum Histologicum Cytologicum*, 61:37-46.

LIMAN, N., BAYRAM, G. & KOÇAK, M. 2001. Histological and histochemical studies on the lingual, preglottal and laryngeal salivary glands of the Japanese quail (*Coturnix coturnix japonica*) at the post-hatching period. *Anatomia*, 30:367-373.

LINDENMAIER, P. & KARE, M.R. 1959. The taste end-organs of the chicken. *Poultry Science*, 38:545-549.

LUCAS, F.A. 1896. The taxonomic value of the tongue in birds. *Auk*, 13:109-115.

LUCAS, F.A. 1897. The tongues of birds. *Report of the United States National Museum,* 1895:1003-1020.

MACALISTER, A. 1864. On the anatomy of the ostrich (*Struthio camelus*). *Proceedings of the Royal Irish Academy,* 9:1-24.

MCCANN, C. 1973. The tongues of kiwis. *Notornis,* 20:123-127.

MCLELLAND, J. 1979. Digestive system, in *Form and Function in Birds,* edited by A.S. King & J. McLelland. San Diego, California: Academic Press: 69-92.

MECKEL, J.F. 1829. *System der vergleichenden Anatomie*. Halle: Der Rehgerschen Buchhandlung.

MITCHELL, P.C. 1901. On the intestinal tract of birds; with remarks on the valuation and nomenclature of zoological characters. *Transactions of the Linnean Society of London. Zoology,* 8:173-275.

MOORE, D.A. & ELLIOTT, R. 1946. Numerical and regional distribution of taste buds on the tongue of the bird. *Journal of Comparative Neurology,* 84:119-131.

NICKEL, R., SCHUMMER, A. & SEIFERLE, E. 1977. Digestive system, in *Anatomy of the Domestic Birds.* Berlin: Verlag Paul Parey: 40-50.

OWEN, R. 1841. On the anatomy of the southern apteryx (*Apteryx australis,* Shaw). *Transactions of the Zoological Society of London,* 2:257-301.

OWEN, R. 1879. *Memoirs on the extinct and wingless birds of New Zealand; with an appendix of those of England, Australia, Newfoundland, Mauritius and Rodriguez.* Volume 1. London: John van Voorst.

PORCHESCU, G. 2007. Comparative morphology of the digestive tract of the Black African ostrich, hen and turkey. PhD thesis (in Russian), Agrarian State University of Moldova.

POTTER, M.A., LENTLE, R.G., MINSON, C.J., BIRTLES, M.J., THOMAS, D. & HENDRIKS, W.H. 2006. Gastrointestinal tract of the brown kiwi (*Apteryx mantelli*). *Journal of Zoology,* 270:429-436.

PYCRAFT, W.P. 1900. On the morphology and phylogeny of the palaeognathae (*Ratitae and Crypturi*) and neognathae (*Carinatae*). *Transactions of the Zoological Society of London,* 15:149-290.

ROACH, R.W. 1952. Notes on the New Zealand kiwis (1). *The New Zealand Veterinary Journal,* 1:38-39.

ROSSI, J.G., BARALDI-ARTONI, S.M., OLIVEIRA, D., FRANZO, C.V.S. & SAGULA, A. 2005. Morfologia do bico e da língua de perdizes *Rhynchotus rufescens. Ciência Rural,* 35:1098-1102.

SALES, J. 2006. Digestive physiology and nutrition of ratites. *Avian and Poultry Biology Reviews,* 17:41-55.

SALES, J. 2007. The emu (*Dromaius novaehollandiae*): A review of its biology and commercial products. *Avian and Poultry Biology Reviews,* 18:1-20.

SAMAR, M.E., AVILA, R.E., DE FABRO, S.P., PORFIRIO, V., ESTEBAN, F.J., PEDROSA, J.A. & PEINADO, M.A. 1999. Histochemical study of Magellanic penguin (*Spheniscus magellanicus*) minor salivary glands during postnatal growth. *Anatomical Record,* 254:298-306.

TIVANE, C. 2008. A Morphological Study of the Oropharynx and Oesophagus of the Ostrich (*Struthio camelus*). MSc dissertation, University of Pretoria, South Africa.

TUCKER, R. 1958. Taxonomy of the salivary glands of vertebrates. *Systematic Zoology,* 7:74-83.

WARNER, R.L., MCFARLAND, L.Z. & WILSON, W.O. 1967. Microanatomy of the upper digestive tract of the Japanese quail. *American Journal of Veterinary Research,* 28:1537-1548.

ZISWILER, V. & FARNER, D.S. 1972. Digestion and the digestive system, in *Avian Biology,* edited by D.S. Farner, J.R. King & K.C. Parkes. New York: Academic Press: 344-354.

CHAPTER 2

GROSS MORPHOLOGY OF THE OROPHARYNGEAL CAVITY AND PROXIMAL OESOPHAGUS

2.1 INTRODUCTION

Despite numerous studies investigating the intestinal tract of ratites (Owen, 1841; Gadow, 1879; Pycraft, 1900; Mitchell, 1901; Herd, 1985; Bezuidenhoudt, 1999; Potter *et al*., 2006; Porchescu, 2007) there is very little comprehensive information available on the structure of the upper digestive tract (oral cavity, tongue, pharynx and oesophagus) of these birds. In contrast, the upper digestive tract of many other species of birds has been described in some detail (for a review of the earlier literature see Calhoun, 1954; Warner *et al*., 1967; McLelland, 1979).

The most comprehensively studied ratite in respect of the upper digestive tract is the ostrich and this region, or parts thereof, have been illustrated and described in a number of publications (Göppert, 1903; Faraggiana, 1933; Porchescu, 2007; Jackowiak and Ludwig, 2008; Tivane, 2008) with the most comprehensive work being that of Tivane (2008) who combined gross morphological descriptions with histology and scanning electron microscopy of the oropharynx and oesophagus. Descriptions, as well as illustrations of the ratite oropharynx or parts thereof have also been supplied for the greater rhea (Gadow, 1879; Pycraft, 1900; Faraggiana, 1933; Gussekloo & Bout, 2005), kiwi (Owen, 1879) and emu (Faraggiana, 1933, Bonga Tomlinson, 2000). No complete description of the emu oropharynx is currently available and the existing information, which records the structure of the tongue and laryngeal mound, is, in part, inaccurate or misleading (see Chapter 4).

The most complete comparative work on the ratite oropharynx is that by Cho *et al*. (1984) who noted that the shape of the tonsils, as with the tongue, varies between the ratites. The description is vague and open to interpretation, giving little information on the specific location or structure of the tonsils. The authors simply note that “The ostrich tonsils and tongue are smooth, blunt and U-shaped. In the Darwin’s rhea both tongue and tonsils have simple, pointed V-shaped tips. The tonsils in the emu are similar to the rhea but have a small flap laterally” (Cho *et al*., 1984). It is

clear from the existing literature on the topic that a comprehensive description of the upper digestive tract of ratites is sorely lacking, particularly in respect of the emu.

Emu farming in South Africa is a relatively new enterprise and efforts to place this emerging industry on a sound financial basis are hamstrung by a lack of basic knowledge on the biology of this bird. The upper digestive tract is of considerable importance considering that it is the first area for food selection and intake which is vital to the nutrition and growth of the animal and therefore its commercial viability. This chapter presents the first definitive macroscopic description of the oropharynx of the emu and reviews, consolidates and compares scattered information on the gross morphology of the ratite oropharynx available in the literature.

2.2 MATERIALS AND METHODS

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

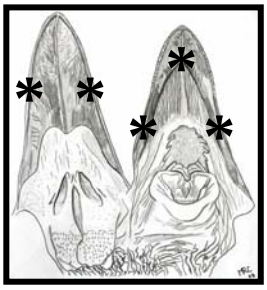
The specimens were rinsed in running tap water and each preserved head was used to provide information on the gross anatomical features of the oropharyngeal cavity. This was achieved by incising the right commisure of the beak, disarticulating the quadratomandibular joint and reflecting the mandible laterally to openly display the roof and floor of the oropharynx (Fig. 2.2). Relevant features were described and recorded using a Canon 5D digital camera with a 28-135 mm lens and a Canon Macro 100mm lens for higher magnification photographs.

The terminology used in this study was that of Nomina Anatomica Avium (Baumel *et al*., 1993).

2.3 RESULTS

The oropharyngeal cavity consisted of the oral (*Cavum oralis*) and pharyngeal (*Cavum pharyngis*) cavities (Figs. 2.1, 2.2), which could not be morphologically distinguished from each other. The oropharyngeal cavity was bounded laterally and rostrally by the tomia of the *rhamphotheca*, dorsally by the oropharyngeal roof, choana and pharyngeal folds, ventrally by the mandibular rhamphotheca and soft interramal region and caudally by the proximal oesophagus. The oropharyngeal cavity was dorso-ventrally flattened in the closed gape and housed the tongue and laryngeal mound. The oropharyngeal floor was triangular (Figs. 2.2, 2.7) and the oropharyngeal roof was pear-shaped (Figs. 2.2, 2.10).

2.3.1 Rhamphotheca



The mandibular rhamphotheca (Figs. 2.1, 2.2, 2.3, 2.7) was a dark brown/black colour in formalin fixed specimens and had a rubbery/leathery texture. Viewed from dorsally, it consisted of two long thin arms originating caudally from the fleshy angle of the mouth (mandibular rictus) which followed the contours of the mandibular rami and converged rostrally to meet and form a flattened plate overlying the mandibular rostrum (Figs. 2.1, 2.2, 2.3, 2.7). The rostral plate displayed a clear median sulcus which overlay the mandibular symphysis (Fig. 2.3). The sulcus was bordered on either side by a slight ridge and extended from the caudal edge of the mandibular nail (*Unguis mandibularis*) to the caudal edge of the rostral plate (Figs. 2.3). The rostral plate bore a series of transverse grooves extending the full width of the *rhamphotheca* (Figs. 2.3, 2.7). These varied in number and depth between the specimens.

The mandibular tomia (*Tomium mandibulare*) (the cutting edge of the *rhamphotheca*), were relatively wide caudally and presented a smooth and rounded surface forming a blunt cutting edge (Figs. 2.1, 2.2, 2.4, 2.7). The rostral third of the mandibular tomia bore serrations (*Lamellae rostri*) with rostrally pointing tips forming a sharp cutting edge (Figs. 2.3, 2.4). The right side (range: 18-27) almost always displayed a higher number of serrations than the left side (range: 19-26). The average total number of rostral lamellae for each bird was 44.6 (range: 38-52). The serrations were fairly uniform in profile for each specimen (Figs. 2.1, 2.3, 2.4, 2.7), but varied

between the specimens, being prominent in some and less distinct in others. The serrations abutted the most rostral tip of the mandible, the mandibular nail, which was represented by a smooth, pointed, lightly pigmented thickening which formed a raised tip (Fig. 2.3). The mandibular nail was the most rostral extremity of the *gonys*, a thickened component of the external mandibular rhamphotheca (Fig. 2.4).

The left and right maxillary rhamphotheca extended from the rostral border of each maxillary rictus to the maxillary nail (*Unguis maxillaris*) where they merged to form a broad shelf (maxillary rostrum) similar to, but larger, than the rostral plate of the mandible (Fig. 2.10). It was similar in colour and texture to the mandibular rhamphotheca. The maxillary rostrum was concave and was indiscernible from the pigmented region of the roof. The maxillary tomia (*Tomium maxillare*) (Figs. 2.1, 2.2, 2.5, 2.6, 2.10) were smooth (non-serrated) and narrower than the mandibular tomia and formed a sharper cutting edge. The tip of the maxillary rostrum displayed a prominent maxillary nail (*Unguis maxillaris*) (Figs. 2.5, 2.6) which represented the most rostral tip of the *culmen*, a structure comparable to the *gonys*, but occurring on the maxilla (Fig. 2.5). The rostral tip of the unguis was lightly pigmented in most specimens (Fig. 2.5). In the closed gape the maxillary unguis projected rostral to and overlapped the mandibular unguis.

The *Rima oris* was formed by the maxillary and mandibular tomia. Caudally, in the closed position, the maxillary and mandibular tomia directly opposed each other. Rostrally, in the region where the serrations originated, the mandibular tomia lay medial to the maxillary tomia and the mandibular nail lay ventral and caudal to the maxillary nail. In lateral profile, the serrated part of the mandible had a slight ventral inclination from the origin of the serrations to the tip of the bill.

2.3.2 The floor of the oropharynx

The oropharyngeal floor was divided into the interramal region, consisting of a rostral pigmented and a caudal non-pigmented part, tongue (see Chapter 4) and laryngeal mound (Fig. 2.7).

2.3.2.1 Interramal region - Rostral pigmented part (Figs. 2.1, 2.2, 2.7)

This region was situated rostral to the tongue and was bordered laterally and rostrally by the mandibular rhamphotheca. It represented the intra-oral tissue overlying the *mentum*. This region was triangular in outline with a rounded apex pointing rostrally and was dark ash-grey in colour. The base was clearly demarcated from the caudal non-pigmented region and had a scalloped outline. The median sulcus in the *rhamphotheca*, overlying the mandibular symphysis, continued caudally through this region as a smooth well defined light-grey line. The mucosa on either side of this line was divided into two columns composed of fine longitudinal folds (Fig. 2.2). The two medial columns were divided by and situated on either side of the obvious median smooth line, while the two lateral columns bordered the medial side of the *rhamphotheca*. The demarcation between the lateral and medial columns was not always well-defined, but was generally indicated by a thin light grey line. The lateral boundaries of the lateral columns tapered caudally onto the medial border of the *rhamphotheca*, ending by merging imperceptibly with the non-pigmented medial part of the mandibular rictus.

2.3.2.2 Interramal region - Caudal non-pigmented part (Figs. 2.1, 2.2, 2.7)

This region lay rostral and ventral to the body of the tongue and extended laterally around the tongue and laryngeal mound. The part situated in the midline and ventral to the tongue, was smooth and continuous caudally with the frenulum of the tongue. On either side of the smooth area, the tissue was thrown into longitudinal folds scattered with small raised nodules (Fig. 2.1). The folds followed the contours of the lateral sides of the laryngeal mound (medially) and the medial edge of the caudal mandibular rami (laterally), diverging from the smooth area ventral to the tongue, around the laryngeal mound, and converging caudal to the mound as they joined the origin of the oesophageal folds (Fig. 2.7). Two definite larger flat folds were identifiable, one on either side of the laryngeal mound, running medial to the rhamphotheca. They originated at the rostral border of the non-pigmented region and ended at the angle of the mouth. The folds lay flat on the floor with their free edge facing medially and enclosing a medially opening recess. These paired folds were also defined by a difference in colour, appearing slightly darker than the rest of the non-pigmented floor.

2.3.2.3 The tongue (see Chapter 4)

2.3.2.4 The laryngeal mound (*Mons laryngealis*) (Figs. 2.1, 2.2, 2.7, 2.8, 2.9)

The laryngeal mound projected dorsally from the floor of the oropharynx and was situated caudal to the tongue and rostral to the oesophagus. The lateral edges did not contact the mandibular rami. The laryngeal mound was supported by the circular cricoid cartilage, the paired dorsal arytenoid cartilages and the procricoid cartilage which connected the arytenoids caudally (Figs. 2.8, 2.9). The laryngeal fissure (glottis) (viewed dorsally) was wide rostrally and narrowed caudally. This was due to the lateral divergence of the arytenoid cartilages as they proceeded rostrally. The caudal protuberance of the tongue root (see Chapter 4) overlapped the rostro-medial part of the laryngeal fissure. Caudal to the tongue root and lying on the rostro-ventral floor of the larynx were 3-5 raised prominent, longitudinally plicated mucosal folds (Figs. 2.7, 2.8, 2.9). The middle fold was always the largest and longest. The mucosa supported by the arytenoid cartilages displayed a double fold separated by an intervening groove. The medial fold had a raised, sharp edge which terminated caudally as a bulbous protuberance. The medial folds formed the lateral edges of the glottis (*Rima glottis*) (Figs. 2.8, 2.9). The larger lateral folds presented gently rounded contours and merged caudally with the medial folds to form a single structure linked by the underlying procricoid cartilage. The mucosa covering the laryngeal mound was smooth and non-pigmented. Caudally, the mucosa merged with that of the oesophagus and became longitudinally folded.

2.3.3 The roof of the oropharynx

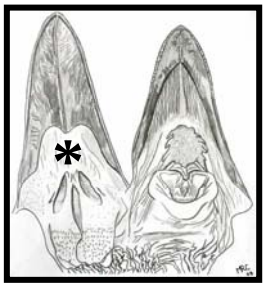
The oropharyngeal roof consisted of a rostral pigmented region clearly demarcated from a caudal non-pigmented region which housed the *choana*, and two pharyngeal folds which extended caudally from the non-pigmented region (Fig. 2.10).

2.3.3.1 Pigmented region (Figs. 2.1, 2.2, 2.10)

The colour and texture of the pigmented region of the roof was similar to that of the *rhamphotheca* and it was difficult to clearly distinguish the two

components (Fig. 2.10). It occupied approximately the rostral two thirds of the roof. Its shape was that of an arrow-head, with the tip pointing rostrally and the two elongated caudal arms extending to the rostral edge of the maxillary rictus. A prominent median palatine ridge (*Ruga palatina mediana*), bordered bilaterally by shallow sulci, extended from the maxillary unguis to the border of the pigmented and non-pigmented regions of the roof. The median sulcus of the rostral mandibular plate corresponded to the median palatine ridge of the maxilla, and the two ridges on either side of the mandibular sulcus corresponded to the sulci bordering the median palatine ridge.

2.3.3.2 Non-pigmented region (Figs. 2.1, 2.2, 2.10)



The outline of the non-pigmented region of the oropharyngeal roof (excluding the pharyngeal folds) was bell-shaped, with the base facing caudally. The rounded rostral border was indented caudally by the abrupt termination of the median palatine ridge at the junction of the pigmented and non-pigmented regions. The lateral borders extended to the maxillary rictus and ran parallel to the slits forming the choana (see below). The caudal border ended approximately level with the base of the choana, merging imperceptibly with the non-pitted surface of the pharyngeal folds. The maxillary rictus formed the most caudo-lateral extent of this region. The tissue had a lumpy uneven appearance and closer inspection revealed that the underlying tissue contained light-coloured doughnut-shaped structures, each with a dark, central spot (Fig. 2.11). Light microscopy confirmed each of the doughnut-shaped structures to be a glandular unit (see Chapter 3).

2.3.4 Choana (Figs. 2.1, 2.2, 2.10, 2.12, 2.13)

The choana was formed by paired, slit-like, oblique, oblong openings (the internal nares), resulting in a triangular-shaped choana. The paired slits originated rostro-medially and proceeded caudo-laterally, their line of direction being parallel to the border between the pigmented and non-pigmented regions of the roof. The two slits were separated by a wide raised ridge with a groove running down its midline and continuing to the infundibular cleft (*Rima infundibuli*). The infundibular cleft, housing the individual openings of the Eustachian tubes (McLelland, 1993), continued caudally as the separation between the two

pharyngeal folds. In the most rostro-medial area between the two slits of the choana (the intervening ridge) were a few raised nodules which in the closed gape contacted the caudal point of the tongue root. On either side of the choana on the most caudo-lateral edge was a small fold of tissue (mucosal fold), concealing a small blind-ending pouch or recess, with its opening facing the choana.

2.3.5 Pharyngeal folds (*Plica pharyngis*) (Figs. 2.2, 2.10, 2.14, 2.15, 2.16, 2.17, 2.18, 2.19)

The pharyngeal folds were paired, U-shaped structures with the rounded free base facing caudally. They were divided into a smooth, attached rostral part and a pitted, free caudal part. The folds overlapped each other medially. The two pharyngeal folds formed the most caudal extent of the oropharyngeal roof and were connected laterally to the maxillary rictus. They originated caudal to the base of the choana and were separated rostrally by the infundibular cleft. The point where the pharyngeal folds were unattached was marked by a pitted horizontal line. Caudal to this line, the ventral surface of the folds displayed a deeply pitted surface in contrast to the dorsal surface that was smooth and free of large pits. Attached to the dorsal aspect of the caudo-lateral edge of each fold was a smooth rounded structure (caudo-lateral projection) that protruded beyond the margins of the fold. A blind-ending pouch or recess was formed between the ventrum of the protrusion and the dorsum of the pharyngeal fold (Fig. 2.14).

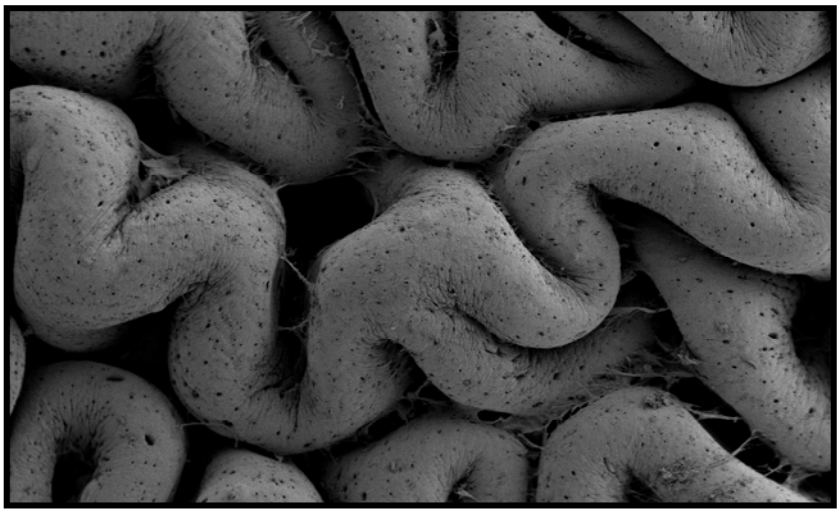
2.3.6 Proximal cervical oesophagus (*Oesophagus pars cervicalis*) (Figs. 2.2, 2.15, 2.19, 2.20)

The proximal oesophagus originated dorsal to the trachea and proceeded from the caudal end of the laryngeal mound caudally down the neck. It soon occupied a position lateral to the trachea and to its right. The oesophageal mucosa was non-pigmented and displayed a smooth surface thrown into prominent longitudinal folds. These folds proceeded from the oesophageal origin up to the end of the specimens studied. The proximal oesophagus of the emu was flaccid and wide in its natural state but appeared collapsed on itself in the preserved oesophagi which varied in cross-sectional shape from triangular to oval to circular.

The transition from oropharynx to oesophagus was not clearly demarcated on the oropharyngeal floor. The longitudinal folds on either side of the laryngeal mound converged caudal to the mound and merged with the longitudinal folds of the oesophagus. There was a raised transverse ridge caudal to the laryngeal mound, over which the longitudinal folds ran. This was not always as obvious in all specimens.

The transition from oropharyngeal roof to oesophagus was much more abrupt and clearly demarcated. The pharyngeal folds obscured the oesophageal origin. Their dorsal surface lay in contact with the oesophagus and formed a retropharyngeal recess, lined ventrally by the dorsal surface of the pharyngeal folds and dorsally by the longitudinally folded mucosa representing the origin of the oesophagus (Fig. 2.15).

In the fresh state, the longitudinally folded nature of the mucosa was not always apparent. However, following fixation the pattern of mucosal folds was prominent. The folds were raised off the floor, had rounded contours and were convoluted. Branching and anastomosing of the folds were also characteristic for this region (Figs. 2.15, 2.20). There were an average number of 16 folds in the proximal oesophagus (n=10) with a range of 14 – 26. The mucosa had a smooth appearance and was non-pigmented.



2.4 DISCUSSION

2.4.1 Oropharynx

In the emu the oral and pharyngeal cavities could not be morphologically distinguished from one another and therefore formed one combined cavity, namely, the oropharynx, a feature also noted in the ostrich (Tivane, 2008). As birds lack a soft palate (McLeod, 1939; Nickel *et al*., 1977; McLelland, 1975, 1979, 1990, 1993) and pharyngeal isthmus (McLelland, 1975, 1979, 1990, 1993) the occurrence of a combined oropharynx is typical of avian species (McLeod, 1939; Koch, 1973; Hodges, 1974; Nickel *et al*., 1977; King and McLelland, 1984; McLelland, 1975, 1979, 1990). The precise point where the oral and pharyngeal cavities join one another is impossible to determine (McLelland, 1975). However, some authors have named certain landmarks which they use to divide the oral and pharyngeal cavities, namely the last row of caudal pointing papillae on the palate (Koch, 1973; Hodges, 1974; McLelland, 1975) or the space between the choana and infundibular cleft (Hamilton, 1952; Nickel *et al*., 1977; King and McLelland, 1984). Lucas and Stettenheim, 1972 (cited by McLelland, 1993) using embryological evidence, note that the dorsal transverse boundary of the roof lies between the choana and infundibular cleft, stretching to the lateral angle of the jaws, while the ventral transverse boundary lies between the paraglossal and basihyal bones.

2.4.2 Rhamphotheca

The term *rhamphotheca* denotes the *Stratum corneum* of the epidermis covering the bill (Hodges, 1974; Clark, 1993). The *rhamphotheca* forming the most lateral limits of the oropharynx shows some special modifications in the emu. The most rostral extremity of both upper and lower bills display a distinct hook-like or nail-like structure, the mandibular and maxillary nail (*unguis*), a structure also evident in the ostrich (Tivane, 2008) and greater rhea (personal observation), but not in the kiwi (Roach, 1952). The mandibular and maxillary nails have been reported in procellariform, most pelecaniform (Clark, 1993) and anseriform birds (Berkhoudt, 1975; Nickel *et al*., 1977; Clark, 1993; Gussekloo, 2006).

The upper and lower beak function as prehensile organs (McLeod, 1939; Calhoun, 1954; Nickel *et al*., 1977); therefore these two structures would assist in the incomplete breaking down of food

(Nickel *et al*., 1977) as well as in its procurement and handling. Due to the absence of teeth in birds (McLeod, 1939; McLelland, 1975, 1979; Nickel *et al*., 1977; King and McLelland, 1984), these structures are replaced by the tomia (McLelland, 1975, 1979; Nickel *et al*., 1977; King and McLelland, 1984). The rostral mandibular tomia in the emu bear serrations (*Lamellae rostri*) and the maxillary tomia are narrow, strong and sharp. The rostral mandibular tomia of the ostrich revealed fine serrations (Tivane, 2008) whereas those of the greater rhea are entirely smooth (personal observation). The finding in the emu and ostrich contrasts with the statement by Gussekloo and Bout (2005) that the bill in ratites is relatively less adapted and non-specialised due to its sole function of holding food and that the tomia are blunt and rounded. Davies (1978) notes that the bill of the emu requires little strength due to their diet and that these birds only require the ability to ingest large objects. However, the nails of the bill together with the sharp and serrated tomia, present a formidable combination of tearing and pecking power.

2.4.3 Oropharyngeal floor

This study revealed the floor of the oropharynx of the emu to consist of four clearly discernable parts and structures, the interramal region, divided into rostral pigmented and caudal non-pigmented regions, the tongue (see chapter 4) and the laryngeal mound.

2.4.3.1. Oropharyngeal floor - Interramal region

Although the interramal region of the emu showed few remarkable features, in comparison to that of the ostrich (Göppert, 1903; Faraggiana, 1933; Porchescu, 2007; Jackowiak and Ludwig, 2008; Tivane, 2008) and greater rhea (Gussekloo and Bout, 2005; personal observation), the emu shows a more distinct demarcation between the rostral and caudal interramal regions. In the ostrich the entire interramal region is similar in colour (Porchescu, 2007; Jackowiak and Ludwig, 2008; Tivane, 2008) whereas in the emu the rostral region is pigmented in contrast to the non-pigmented caudal region. In the greater rhea, the lateral portions of the caudal interramal region display a pigmented surface in the form of small dark dots (personal observation). In the emu the surface of the rostral component displays a different pattern of folds (columns of fine longitudinal folds) to those of the comparable region in the ostrich. This area in the ostrich is characterised by irregular longitudinal folds, with a single or double larger fold, extending from the bill tip to the frenulum (Tivane, 2008). Although Tivane (2008), quoting Gussekloo and Bout

(2005) refers to folds in the interramal region in the greater rhea, this area is entirely smooth and displays no folds (personal observation).

The membranous floor of the oropharyngeal cavity is highly distensible in some groups of birds (Ziswiler and Farner, 1972), a similar feature also noted in the emu. The non-pigmented interramal area displayed a series of longitudinal folds which diverged around the laryngeal mound. The most lateral of those folds was large and conspicuous, a feature also illustrated in the ostrich (Göppert, 1903; Faraggiana, 1933; Porchescu, 2007; Jackowiak and Ludwig, 2008; Tivane, 2008) but not in the greater rhea (personal observation).

Two reasons can be advanced for the presence of folds in the caudal interramal region in the emu. In the 'catch and throw' feeding method employed by ratites (Gussekloo and Bout, 2005) the gape needs to be enlarged to allow the accelerated food particle/s to travel beyond the tongue and laryngeal mound into the proximal oesophagus. Yet, in the closed gape, the oropharyngeal cavity presents as a dorso-ventrally flattened structure. Thus enlargement of the cavity is necessary during eating. Gussekloo and Bout (2005) attribute the enlargement of the gape to depression of the tongue only. In the folded interramal region, depression of the tongue would allow for a greater enlargement of the gape than would a non-folded region. Tivane (2008) suggests that the folded nature of the ostrich oropharyngeal floor would allow food to be accumulated prior to swallowing, yet as seen from the feeding method described above ratites do not house food in the oral cavity prior to swallowing. Therefore this function of the distensible floor in the ostrich is questionable.

The second reason advanced for the presence of the folds in the interramal region would be for the process of fluid ingestion. During drinking in ratites (Gussekloo and Bout, 2005), the lower bill is inserted into the water and the head moved forward, using the lower bill as a scoop. Again, the folded nature of the oropharyngeal floor would allow the distensibility required to hold sufficient quantities of water to swallow as well as for the channelling of fluids around the laryngeal mound.

2.4.3.2. Laryngeal mound

The laryngeal mound of the emu is a prominent feature in the oropharynx and forms the most caudal structure of the oropharyngeal floor. This is in agreement with the general pattern in

avians (Nickel *et al*., 1977; King and McLelland, 1984). In most birds the glottis, which is situated on the dorsal surface of the laryngeal mound, usually lies directly ventral to the caudal part of the choana (McLelland, 1979; Bailey *et al*., 1997). However, in the emu, which has an undivided choana (see discussion below), the glottis underlies the entire choana. This arrangement was also noted in the ostrich (Tivane, 2008) and greater rhea (personal observation) and appears to be the general pattern in ratites. The caudal margin of the laryngeal mound is sloped and the pharyngeal folds overlie this sloped area (Nickel *et al*., 1977), a feature also noted in the emu. This arrangement allows for closure of the oesophagus during respiration (Nickel *et al*., 1977). The illustrations of Porchescu (2007) and Tivane (2008) seem to confirm a similar situation in the ostrich.

The glottis in palaeognaths is relatively wider than in neognaths (Pycraft, 1900). The laryngeal fissure (glottis) in the emu is rhomboid-shaped (Faraggiana, 1933) and is wider rostrally than caudally. The extension of the tongue root into the rostral aspect of the laryngeal entrance (Faraggiana, 1933; present study) represented an interesting modification not observed or illustrated in other ratites (ostrich and greater rhea) (Göppert, 1903; Faraggiana, 1933; Gussekloo and Bout, 2005; Jackowiak and Ludwig, 2008; Porchescu, 2007; Tivane, 2008). It is of importance that the glottis is closed during swallowing (Kaupp, 1918; Nickel, *et al*., 1977; McLelland, 1990) to prevent the inhalation of anything except air. The respiratory route, during swallowing, is occluded by closure of the laryngeal fissure by the *M. constrictor glottides* (King, 1993). The positioning of the tongue root would appear to assist in sealing the rostral aspect of the larynx during closure of the glottis, almost assuming the role of an epiglottis. An epiglottis, however, is not present in birds (MacAlister, 1864; Kaupp, 1918; Calhoun, 1954; King and McLelland, 1984; Nickel *et al*., 1977). This argument regarding the role of the tongue root functioning as an epiglottis in the emu has been proposed by Gadow (1879) but disputed by Faraggiana (1933). Koch (1973) considers folds opposite the tongue base (i.e. tongue root) to be a form of rudimentary epiglottis. Indeed, it seems plausible that in birds with such a wide glottis (emu and ostrich) a structure would be necessary to assist in closure of the glottis. Owen (1879) describes a fold in the base of the kiwi tongue which can be retracted to cover the glottis. A fold or pocket has also been described at the base of the tongue body in the ostrich (see Chapter 4, Table 4.1). However, the only function attributed to this fold is the production of mucus (Tivane, 2008). Further studies will be required to determine whether the lingual pocket of the ostrich may perform a similar function to that of the kiwi (Owen, 1879).

A unique feature of the emu larynx is the presence of 3-5 raised folds situated immediately caudal to the tongue root. The function of these folds is unknown and their presence was not depicted in the illustration of the emu laryngeal entrance by Faraggiana (1933). The shape of the glottis of the emu observed in the present study differs from that depicted by Faraggiana (1933) and Bonga Tomlinson (2000). Whereas Faraggiana (1933) depicts the glottis with a constriction in the midline, Bonga Tomlinson (2000) shows the glottis as oblong and more similar to that of the ostrich (Bonga Tomlinson, 2000). None of these features were noted in the specimens studied. From the present observations the emu glottis is defined as being narrow caudally and widening rostrally as the arytenoid cartilages diverged. Reports in the literature indicate that the shape of the laryngeal mound and glottis differs between the ratites. These observations are compared with the results of the present study in Table 2.1.

Many bird species display papillae on the laryngeal mound caudal to the glottis (King and McLelland, 1984; Bailey *et al*., 1997; McLelland, 1989). The laryngeal mound of ratites, however, is described as being smooth (McLelland, 1989), a feature also noted in the emu. Yet, as can be seen in the table below (Table 2.1), some of the ratites, namely the greater rhea and kiwis, possess papillae, even if ill-defined. Whether the lateral projections of the arytenoid cartilages in the ostrich (Tivane, 2008) can be considered as papillae remains debatable. The laryngeal mound is supported by the cricoid, two arytenoid (Kaupp, 1918; McLelland, 1989) and procricoid cartilages (totalling four) and their associated muscles, connective tissue and covering mucosa (McLelland, 1989). A similar situation is apparent in the emu (present study) and also in the ostrich (Tivane, 2008).

Though mainly associated and studied with the respiratory tract, the laryngeal mound of the emu fulfils both a respiratory and digestive function. In respect of its respiratory function, the laryngeal mound brings the glottis into contact with the choana allowing an open passage of air-flow directly from the external nares to the trachea and air sacs. The proximal oesophagus of the emu appears to lack an upper sphincter, in contrast to the situation in mammals, thus it is important that the oesophagus remains closed during respiration to prevent the movement of air into the digestive tract. The pharyngeal folds which overlie the caudal laryngeal mound (Nickel *et al*., 1977) are reported to close off the oesophagus in birds during respiration. The substantial pharyngeal folds observed in the emu and also illustrated in the ostrich (Göppert, 1903; Porchescu, 2007; Tivane, 2008) would seemingly also fulfil this function.

Table 2.1 Comparative morphological features of the ratite laryngeal mound

Species	Shape of laryngeal mound	Shape of Glottis	Papillae on the caudal margin	Projections from the laryngeal cartilages
Emu (*Dromaius novaehollandiae*)	Raised, triangular with a flat rostral aspect[8]	Rhomboid-shaped[2] Wider rostrally and narrowing caudally[8]	No papillae on caudal edge[8]	Two small projections off the caudal arytenoid lips[8]
Ostrich (*Struthio camelus*)	Raised, oval, shield-shaped[6]	Wide, triangular[2], V-shaped[6]	Ill-defined papillae[2]	Arytenoids: Polygonal contours[2], three paired projections around the glottis[6]
Greater Rhea (*Rhea americana*)	Slopes caudally[2]	Thinner & longer than ostrich, triangular[2]	Three thick lobes on either side[2], Variable number[9, #]	Rounded, smooth contours, no projections[9]
Cassowary (*Casuarius casuarius*)	Raised, oval-shaped[7]	Short and narrow[7]	None[7]	Rounded contours, no projections[7]
Kiwi (*Apteryx australis mantelli*) [1,3]	Similar in outline to a Porcupine-fish swim-bladder[3]	Narrow[3]	Two elongate, square, smooth, thick, and apparently glandular folds or processes, the obtuse free margins face caudally[1]	-
(*Apteryx haasti*) [3]	Not as well-defined as above[3]	Large, with two 'glands' rostrally[3]	Two large, deeply divided, ovoid lobes, pits rostral to these structures[3]	-
(*Apteryx oweni*) [3]	Less defined than both above[3]	Partially obscured by caudal part of tongue[3]	Two fleshy, divided, oblong lobes with pitted surface[3]	-

(Underlined names indicate a sketch is supplied, bold indicates photographs.) [#]Extrapolated from 4, 5.

[1]Owen (1879), [2]Faraggiana (1933), [3]McCann (1973), [4]Bonga Tomlinson (2000), [5]Gussekloo and Bout (2005), [6]**Tivane** (2008), [7]Johnston (Personal communication), [8]**Present study**, [9]Personal observation.

In ratites the laryngeal mound also plays an important role in swallowing (digestive function) as it retracts, together with the tongue, during this process (Bonga Tomlinson, 2000; Gussekloo, 2006), a function which can also be attributed to the emu laryngeal mound. Furthermore, the tongue root and lips of the closed glottis fit neatly into the groove down the midline of the choana in the emu. During swallowing, when the tongue and laryngeal mound are retracted, these structures would be able to scrape food particles from the concavity of the choana and infundibular cleft thus cleaning this region and preventing the build-up of food particles which could possibly be inhaled or even occlude the internal nares.

2.4.4 Oropharyngeal roof

The oropharyngeal roof of the emu is divided into rostral pigmented and caudal non-pigmented regions, and two pharyngeal folds. The choana is situated in the non-pigmented region.

2.4.4.1 Pigmented and non-pigmented regions of the roof

The roof of the oropharynx in the emu is clearly divided into rostral pigmented and caudal non-pigmented regions. The caudal non-pigmented component housed the choana and infundibular cleft. Two distinct regions were also visible in the ostrich; however, in this species the entire roof was non-pigmented (Tivane, 2008). The transition between the two parts of the roof was abrupt in the emu (present study) and ostrich (Tivane, 2008). In the emu, a well-defined median palatine ridge ran the full length of the pigmented region, ending abruptly at the transition to the non-pigmented part. A median palatine ridge was also present in the ostrich (Tivane, 2008), represented a far more prominent structure than that of the emu, and ended abruptly between the two regions of the roof, as in the emu.

The rostral pigmented region of the roof of the emu was shown histologically to be aglandular (see Chapter 3), a similar finding to that in the comparable region in the ostrich (Tivane, 2008). The caudal non-pigmented region of the roof of the emu represented the glandular portion (see Chapter 3), which was again similar to the situation in the ostrich (Tivane, 2008). The caudal part of the roof of the greater rhea is also reported to be glandular (Feder, 1972).

The entire oropharyngeal roof in the emu was smooth and, with the exception of the median palatine ridge, showed no evidence of papillae or rugae. There were also no papillae or rugae present on the oropharyngeal roof of the ostrich (Tivane, 2008), greater rhea (Gussekloo and Bout, 2005) and kiwi (Owen, 1879). This is contrary to the situation in most birds were papillae and rugae are commonly present (see for example, Owen, 1879; Barge, 1937; Calhoun, 1954; McLelland, 1975, 1979, 1990; Bailey *et al*., 1997).

2.4.4.2 Choana

The choana of the emu was a triangular-shaped structure situated in the caudal non-pigmented region of the roof. In ratites, including the emu (present study) and ostrich (Göppert, 1903; Porchescu, 2007; Tivane, 2008), and in herons and ducks (Barge, 1937; McLelland, 1979) the choana is restricted to the caudal part of the roof and is short. In most other birds the choana is a longer structure consisting of a rostral slit and a wider caudal part (Barge, 1937; McLelland, 1975, 1979; Nickel *et al*., 1977; Bailey *et al*., 1997). The rostral slit is often closed off by the dorsum of the tongue (McLelland, 1975; Nickel *et al*., 1977; Bailey *et al*., 1997) whereas the caudal part overlies the glottis during respiration (Nickel *et al*., 1977).

The shape of the choana differs between the ratites and is compared in Table 2.2. The choana of palaeognaths is reported to be wide and triangular or cordiform while that of neognathous birds is slit-like (Pycraft, 1900). In the duck and goose however, the choana is a short wide oval (McLeod, 1939; Koch, 1973). Although the choana of ratites is divided by a septum (Pycraft, 1900) it appears that the grooved septum observed in the emu is unique.

The choana of the emu formed the communication between the nasal and oropharyngeal cavities as reported in other birds (Pycraft, 1900; Barge, 1937; Koch, 1973; King and McLelland, 1984; Bailey *et al*., 1997).

Caudal to the choana in the emu (as in other ratites), a cleft was formed between the pharyngeal folds, the infundibular cleft. This cleft was less obvious in its origin than that of the ostrich, although its origin in the greater rhea is also difficult to determine (see Table 2.2). In birds the infundibular cleft houses the common opening of the paired Eustachian tubes (Pycraft, 1900; McLeod, 1939; Ziswiler and Farner, 1972; McLelland, 1975, 1979; King and McLelland, 1984; Tivane, 2008) although in ratites each Eustachian tube is reported to open independently into the infundibulum (McLelland, 1993). This was not confirmed in the present study.

Table 2.2

Comparative features of the ratite choana, infundibular cleft and pharyngeal folds

Species	Choana	Infundibular cleft	Pharyngeal folds
Emu[5, 8] (*Dromaius novaehollandiae*)	Triangular – Two oblong slits following the lateral triangle edge, divided by a ridge with a median groove.[8]	Deep, grooved with no clear distinction from the groove in the midline of the choana.[8]	Two large overlapping U-shaped folds with rounded caudal edges and pitted ventral surfaces. Small projection on the caudo-lateral edge forming a pocket with the pharyngeal fold.[8] Similar to Darwin's rhea with small flaps laterally.[5]
Ostrich[3, 5, 6, 7] (*Struthio camelus*)	Bell/inverted V-shaped depression with prominent mucosal ridge in the midline[7, +]	Clear point of origin caudal to the choana.[+] Crater-like depression caudal to the crescent-shaped ridge of the choana.[7]	Two large folds with rounded caudal edges, pitted ventral surface.[+, 7] Blunt and U-shaped.[5]
Greater Rhea[2, 4, 9] (*Rhea americana*) Darwin's rhea[5] (*Pterocnemia pennata*)	Elliptical to teardrop-shaped with the median septum extending about half the length.[*, 9] -	Very wide, essentially forming the caudal half of the choana.[9] -	Rudimentary, very small, firmly attached and no free caudal edge. Caudo-lateral edge has a small indentation.[9] Pointed V-shaped tips[5]
Kiwi[1] (*Apteryx australis*)	Two linear slits, close together, parallel to the beak axis[1]	Straight, short and clearly defined.[#]	Two rectangular folds, with an undulating caudal free end.[#]

(Underlined names indicate a sketch is supplied, bold indicates photographs.) [#]Extrapolated from 1. [*]Extrapolated from 2, 4. [+]Extrapolated from 3, 6.

[1]Owen (1879), [2]Pycraft (1900), [3]Göppert (1903), [4]Gussekloo and Bout (2005), [5]Cho *et al.* (1984), [6]**Porchescu** (2007), [7]**Tivane** (2008), [8]**Present study**, [9]Personal observation.

2.4.4.3 Pharyngeal folds

The pharyngeal folds represented the most caudal structures of the oropharyngeal cavity in the emu. The comparative structure of the pharyngeal folds of ratites is described in Table 2.2. With the exception of the small ventro-lateral projection (see below), the pharyngeal folds of the emu most closely resemble those of the ostrich.

Cho *et al*. (1984) refer to the pharyngeal folds as tonsils and note that the shape of the tonsils differs between the ratites (see Table 2.2). The caudal edge of the emu pharyngeal folds is rounded yet Cho *et al*., (1984) describe the pharyngeal folds of Darwin's rhea as pointed and similar to that of the emu, yet no pointed tips were observed in any of the emu specimens studied. The emu pharyngeal folds seem unique amongst the ratites in that they possess an extra feature in the form of a small ventro-lateral projection which forms a pocket between its ventral surface and the dorsal surface of the pharyngeal fold.

2.4.5 Proximal cervical oesophagus

The proximal cervical oesophagus of the emu, after its origin dorsal to the trachea, soon occupied a position to the right of the trachea. This is similar to the finding in other ratites (Fowler, 1991), namely the ostrich (Bezuidenhout, 1999; Tivane, 2008), kiwi (Owen, 1879) and for birds in general (Pernkopf and Lehner, 1937; McLeod, 1939; Koch, 1973; McLelland, 1975, 1979; Nickel *et al*., 1977; King and McLelland, 1984; Bailey *et al*., 1997).

The avian oesophagus is a long distensible tube (Calhoun, 1954; Ziswiler and Farner, 1972; Koch, 1973; Hodges, 1974; Nickel *et al*., 1977; McLelland, 1979; King and McLelland, 1984; Bailey *et al*., 1997; Gussekloo, 2006) demonstrating a longitudinally folded mucosa (Pernkopf and Lehner, 1937; Warner *et al*., 1967; Ziswiler and Farner, 1972; Nickel *et al*., 1977; McLelland, 1979; King and McLelland, 1984; Bailey *et al*., 1997; Gussekloo, 2006). It is also apparent that longitudinal folds of the oesophageal mucosa are a feature of the ratite oesophagus and which is therefore also highly distensible (Gadow, 1879; Pernkopf and Lehner, 1937; Tivane, 2008 (ostrich); Gadow, 1879; Feder, 1972 (greater rhea); Owen, 1879; Pernkopf and Lehner, 1937 (kiwi); Meckel, 1829; Gadow, 1879 (cassowary)). As previously noted by Herd (1985), the lumen of the proximal oesophagus of the emu, exhibits a series of well-developed

longitudinal folds. An average number of 16 folds were present in the emu oesophagus in comparison to 10-12 in the greater rhea (Feder, 1972) and 12 in the ostrich (Tivane, 2008).

The oesophagus transports food from the oropharynx to the stomach (Hodges, 1974; Davies, 1978) and performs an important storage function (Ziswiler and Farner, 1972). The avian oesophagus is generally greater in diameter (Ziswiler and Farner, 1972; McLelland, 1979; King and McLelland, 1984; Gussekloo, 2006) than that of mammals (McLelland, 1979; King and McLelland, 1984; Gussekloo, 2006). This is due to the limited ability of birds to break down their food orally (Gussekloo, 2006). The distensibilty of the oesophagus is particularly important in birds which swallow bulky food (Ziswiler and Farner, 1972; Gussekloo, 2006). A distensible oesophagus would be of great importance in the emu which employs the cranioinertial feeding method, as described by Bonga Tomlinson (2000). That the emu possess a distensible oesophagus is evident from the prominent folded mucosa it displays (see above) and also by virtue of the relatively large diameter of the proximal region. In the cranioinertial feeding method food is passed directly from the bill tips to the oesophageal entrance resulting in the oesophagus receiving completely unaltered food items and even stones in the case of the ostrich (Huchzermeyer, 1998) The proximal oesophagus is more distensible and folded than the distal parts in the ostrich (Tivane, 2008) and kiwi (Owen, 1879), possibly to accommodate the feeding method mentioned above. Another important adaptation of the oesophagus for swallowing large food items is that of lubrication (Ziswiler and Farner, 1972; Hodges, 1974). This is made possible in the emu by the ubiquitous presence of mucus-secreting glands in the lamina propria (Herd, 1985; Chapter 3). Thus the proximal oesophagus of the emu displays three main adaptations allowing it to receive and handle large, orally unaltered, food items: 1.) the diameter is relatively large, 2.) the mucosa is longitudinally folded allowing great distensibility and 3.) the numerous mucus-secreting glands provide copious amounts of mucus to lubricate the lumen and food for ease of transport.

2.5 REFERENCES

BAILEY, T.A., MENSAH-BROWN, E.P., SAMOUR, J.H., NALDO, J., LAWRENCE, P. & GARNER, A. 1997. Comparative morphology of the alimentary tract and its glandular derivatives of captive bustards. *Journal of Anatomy,* 191:387-398.

BARGE, J.A.J. 1937. Kopfdarm. A. Mundhöhle und ihre Organe. 1. Mundhöhlendach und Gaumen, in *Handbuch der vergleichenden Anatomie der Wirbeltiere,* edited by L. Bolk, E. Göppert, E. Kallius & W. Lubosch. Berlin: Urban and Schwarzenberg: 29-48.

BAUMEL, J.J., KING, A.S., BREAZILE, J.E., EVANS, H.E. & VANDEN BERGE, J.C. 1993. *Handbook of Avian Anatomy: Nomina Anatomica Avium*. Second edition. Cambridge, Massachusetts: Nuttall Ornithological Club.

BERKHOUDT, H. 1975. The epidermal structure of the bill tip organ in ducks. *Netherlands Journal of Zoology*, 26:561-566.

BEZUIDENHOUDT, A.J. 1999. Anatomy, in *The Ostrich: Biology, Production and Health,* edited by D.C. Deeming. Wallingford, UK: CABI Publishing: 13-49.

BONGA TOMLINSON, C.A. 2000. Feeding in paleognathous birds, in *Feeding: Form, Function, and Evolution in Tetrapod Vertebrates,* edited by K. Schwenk. San Diego: Academic Press: 359-394.

CALHOUN, M.L. 1954. *Microscopic Anatomy of the Digestive System of the Chicken*. Ames, Iowa: Iowa State College Press.

CHO, P., BROWN, B. & ANDERSON, M. 1984. Comparative gross anatomy of ratites. *Zoo Biology,* 3:133-144.

CLARK, G.A. 1993. Anatomia topographica externa, in *Handbook of Avian Anatomy: Nomina Anatomica Avium.* Second Edition, edited by J.J. Baumel, A.S. King, J.E. Breazile, H.E. Evans & J.C. Vanden Berge. Cambridge, Massachusetts: Nuttall Ornithological Club: 7-16.

DAVIES, S.J.J.F. 1978. The food of emus. *Australian Journal of Ecology,* 3:411-422.

FARAGGIANA, R. 1933. Sulla morfologia della lingua e del rialzo laringeo di alcune specie di uccelli Ratiti e Carenati non comuni. *Bollettino dei Musei di Zoologia e Anatomia comparata,* 43:313-323.

FEDER, F-H. 1972. Zur mikroskopischen Anatomie des Verdauungsapparates beim Nandu (*Rhea americana*). *Anatomischer Anzeiger,* 132:250-265.

FOWLER, M.E. 1991. Comparative clinical anatomy of ratites. *Journal of Zoo and Wildlife Medicine,* 22:204-227.

GADOW, H. 1879. Versuch einer vergleichenden Anatomie des Verdauungssystemes der Vögel. *Jenaische Zeitschrift für Medizin und Naturwissenschaft,* 13:92-171.

GÖPPERT, E. 1903. Die Bedeutung der Zunge für den sekundären Gaumen und den Ductus nasopharyngeus. *Morphologisches Jahrbuch,* 31:311-359.

GUSSEKLOO, S.W.S. 2006. Feeding structures in birds, in *Feeding in Domestic Vertebrates: From Structure to Behaviour,* edited by V. Bels. Wallingford, UK: CABI Publishing: 14-19.

GUSSEKLOO, S.W.S. & BOUT, G.R. 2005. The kinematics of feeding and drinking in palaeognathous birds in relation to cranial morphology. *Journal of Experimental Biology,* 208:3395-3407.

HAMILTON, H.L. 1952. *Lillie's Development of the Chick.* Third Edition. New York: Henry Holt.

HERD, R.M. 1985. Anatomy and histology of the gut of the emu *Dromaius novaehollandiae. Emu,* 85:43-46.

HODGES, R.D. 1974. The digestive system, in *The Histology of the Fowl.* London: Academic Press: 35-47.

HUCHZERMEYER, F.W. 1998. Diseases of ostriches and other ratites. Pretoria, South Africa: Agricultural Research Council.

JACKOWIAK, H. & LUDWIG, M. 2008. Light and scanning electron microscopic study of the structure of the ostrich (*Strutio camelus*) tongue. *Zoological Science,* 25:188-194.

KAUPP, M.S. 1918. *The Anatomy of the Domestic Fowl*. Philadelphia: W.B. Saunders Company.

KING, A.S. 1993. Apparatus respiratorius [Systema respiratorium], in *Handbook of Avian Anatomy: Nomina Anatomica Avium.* Second Edition, edited by J.J. Baumel, A.S. King, J.E. Breazile, H.E. Evans & J.C. Vanden Berge. Cambridge, Massachusetts: Nuttall Ornithological Club: 257-299.

KING, A.S. & MCLELLAND, J. 1984. Digestive system, in *Birds - Their Structure and Function.* Second Edition. London: Bailliere Tindall: 86-87.

KOCH, T. 1973. Splanchnology, in *Anatomy of the Chicken and Domestic Birds,* edited by B.H. Skold & L. DeVries. Ames, Iowa: The Iowa State University Press: 68-69.

MACALISTER, A. 1864. On the anatomy of the ostrich (*Struthio camelus*). *Proceedings of the Royal Irish Academy,* 9:1-24.

MCCANN, C. 1973. The tongues of kiwis. *Notornis,* 20:123-127.

MCLELLAND, J. 1975. Aves digestive system, in *Sisson and Grossman's The Anatomy of the Domestic Animals,* edited by C.E. Rosenbaum, N.G. Ghoshal & D. Hillmann. Philadelphia: W.B. Saunders Company: 1857-1867.

MCLELLAND, J. 1979. Digestive system, in *Form and Function in Birds.* Volume 1, edited by A.S. King & J. McLelland. San Diego, California: Academic Press: 69-92.

MCLELLAND, J. 1989. Larynx and trachea, in *Form and Function in Birds.* Volume 4, edited by A.S. King & J. McLelland. San Diego, California: Academic Press: 69-104.

MCLELLAND, J. 1990. Digestive system, in *A Colour Atlas of Avian Anatomy,* edited by J. McLelland. Aylesbury, England: Wolfe Publishing Ltd.: 47-49.

MCLELLAND, J. 1993. Apparatus digestorius [Systema alimentarium], in *Handbook of Avian Anatomy: Nomina Anatomica Avium.* Second Edition, edited by J.J. Baumel, A.S. King, J.E. Breazile, H.E. Evans & J.C. Vanden Berge. Cambridge, Massachusetts: Nuttall Ornithological Club: 301-328.

MCLEOD, W.M. 1939. Anatomy of the digestive tract of the domestic fowl. *Veterinary Medicine*, 34:722-727.

MECKEL, J.F. 1829. *System der vergleichenden Anatomie*. Halle: Der Rehgerschen Buchhandlung.

MITCHELL, P.C. 1901. On the intestinal tract of birds; with remarks on the valuation and nomenclature of zoological characters. *Transactions of the Linnean Society of London. Zoology*, 8:173-275.

NICKEL, R., SCHUMMER, A. & SEIFERLE, E. 1977. Digestive system, in *Anatomy of the Domestic Birds.* Berlin: Verlag Paul Parey: 40-50.

OWEN, R. 1841. On the anatomy of the southern apteryx (*Apteryx australis*, Shaw). *Transactions of the Zoological Society of London*, 2:257-301.

OWEN, R. 1879. Memoirs on the extinct and wingless birds of New Zealand; with an appendix of those of England, Australia, Newfoundland, Mauritius and Rodriguez. Volume 1. London: John van Voorst.

PERNKOPF, E. & LEHNER, J. 1937. Vorderdarm. A. Vergleichende Beschreibung des Vorderdarmes bei den einzelnen Klassen der Kranoten, in *Handbuch der vergleichenden Anatomie der Wirbeltiere*, edited by L. Bolk, E. Göppert, E. Kallius & W. Lubosch. Berlin: Urban and Schwarzenberg: 349-559.

PORCHESCU, G. 2007. Comparative morphology of the digestive tract of the Black African ostrich, hen and turkey. PhD thesis, Agrarian State University of Moldova.

POTTER, M.A., LENTLE, R.G., MINSON, C.J., BIRTLES, M.J., THOMAS, D. & HENDRIKS, W.H. 2006. Gastrointestinal tract of the brown kiwi (*Apteryx mantelli*). *Journal of Zoology*, 270:429-436.

PYCRAFT, W.P. 1900. On the morphology and phylogeny of the palaeognathae (*Ratitae and Crypturi*) and neognathae (*Carinatae*). *Transactions of the Zoological Society of London*, 15:149-290.

ROACH, R.W. 1952. Notes on the New Zealand kiwis (1). *New Zealand Veterinary Journal*, 1:38-39.

TIVANE, C. 2008. A Morphological Study of the Oropharynx and Oesophagus of the Ostrich (*Struthio camelus*). MSc dissertation, University of Pretoria, South Africa.

WARNER, R.L., MCFARLAND, L.Z. & WILSON, W.O. 1967. Microanatomy of the upper digestive tract of the Japanese quail. *American Journal of Veterinary Research*, 28:1537-1548.

ZISWILER, V. & FARNER, D.S. 1972. Digestion and the digestive system, in *Avian Biology*, edited by D.S. Farner, J.R. King & K.C. Parkes. New York: Academic Press: 344-354.

2.6 FIGURES

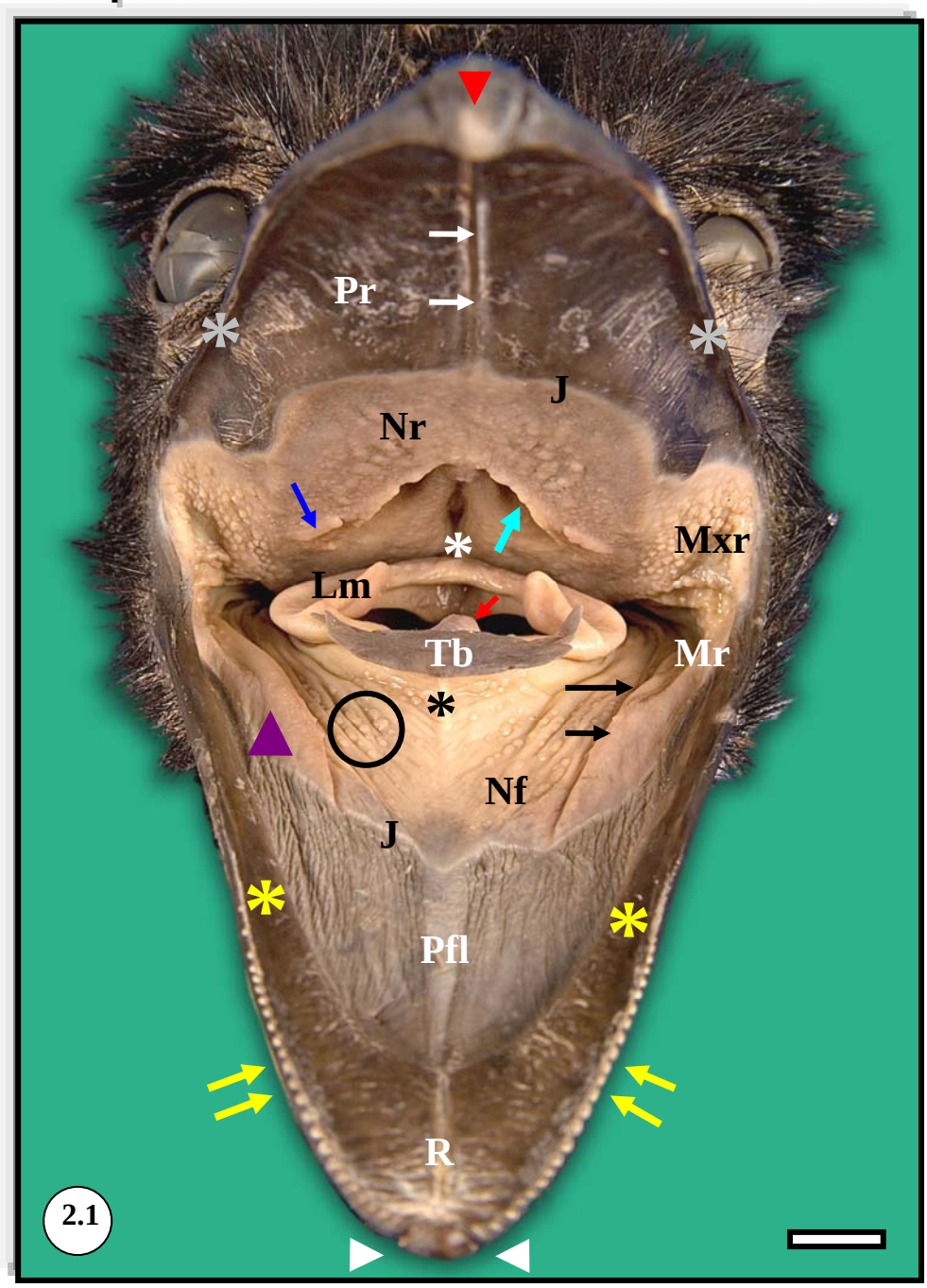


Figure 2.1: Rostral view of the full gape of the emu illustrating the major gross anatomical features visible. The oropharynx is divided into a rostral pigmented floor (Pfl) and roof (Pr) and caudal non-pigmented floor (Nf) and roof (Nr), bordered by the maxillary (grey *) and mandibular (yellow *) rhamphotheca. The serrations on the mandibular tomium are clearly visible (double yellow arrows) as are the junctions (J) between the pigmented and non-pigmented regions. Other noticeable features are the maxillary (red arrowhead) and mandibular (white arrowheads) nails, mandibular rostrum (R), large lateral mucosal fold (purple arrowhead) with associated medial facing groove or recess (black arrows), the tongue frenulum (*), body (Tb) and root (red arrow), nodules on the non-pigmented floor (encircled), laryngeal mound (Lm), mandibular (Mr) and maxillary (Mxr) rictus, median palatine ridge (white arrows), choana (turquoise arrow), small mucosal fold lateral to the choana (blue arrow) and infundibular cleft (white *). Bar = 5mm.

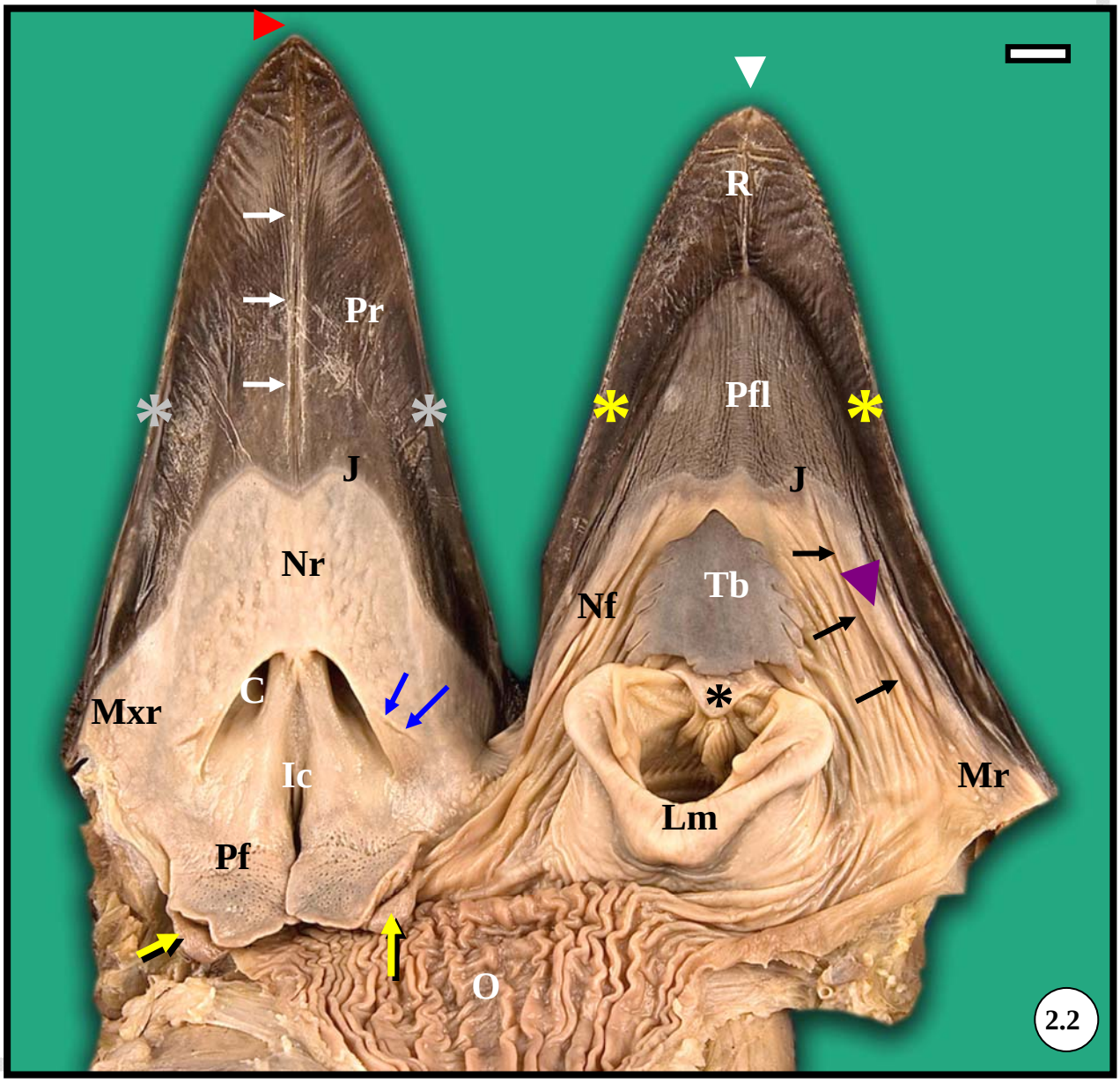


Figure 2.2: Gross anatomical features of the floor and roof of the emu oropharynx. The right commisure has been incised and the two components reflected. The oropharynx is divided into a rostral pigmented floor (Pfl) and roof (Pr) and a caudal non-pigmented floor (Nf) and roof (Nr), bordered by the maxillary (grey *) and mandibular (yellow *) rhamphotheca. Note the smooth rostral and pitted caudal components of the pharyngeal folds (Pf) with the caudo-lateral tissue projection (yellow arrows), and the convoluted longitudinal folds of the proximal oesophagus (O). Other noticeable features are the maxillary (red arrowhead) and mandibular (white arrowhead) nails, mandibular rostrum (R), junctions between pigmented and non-pigmented regions (J), large lateral mucosal fold (purple arrowhead) with associated medial facing groove or recess (black arrows), the tongue body (Tb) and root (black *), laryngeal mound (Lm), mandibular (Mr) and maxillary (Mxr) rictus, median palatine ridge (white arrows), choana (C), small mucosal fold lateral to the choana (blue arrows) and infundibular cleft (Ic). Bar = 5mm.

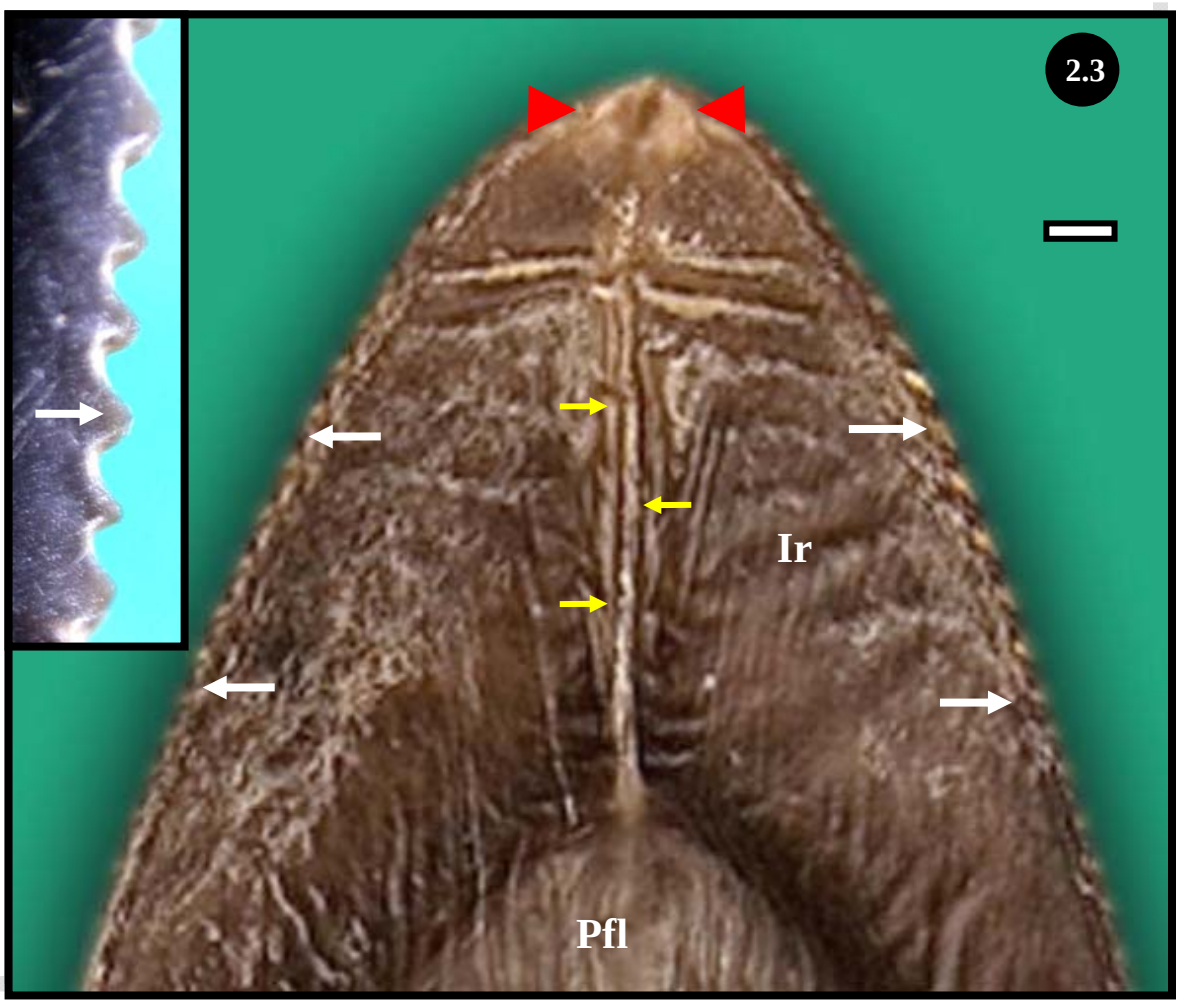


Figure 2.3: The flattened rostral plate formed by the internal rhamphotheca (Ir) overlying the mandibular rostrum. Note the median sulcus (yellow arrows) extending from the mandibular nail (red arrowheads) to the pigmented interramal floor (Pfl). Rostral lamellae (white arrows). Inset: High magnification of the rostral lamellae (white arrow) present on the mandibular tomium. Bar = 1mm.

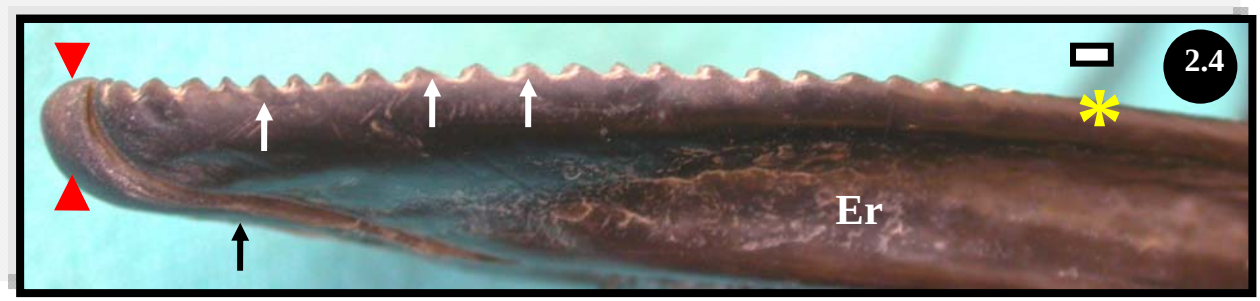
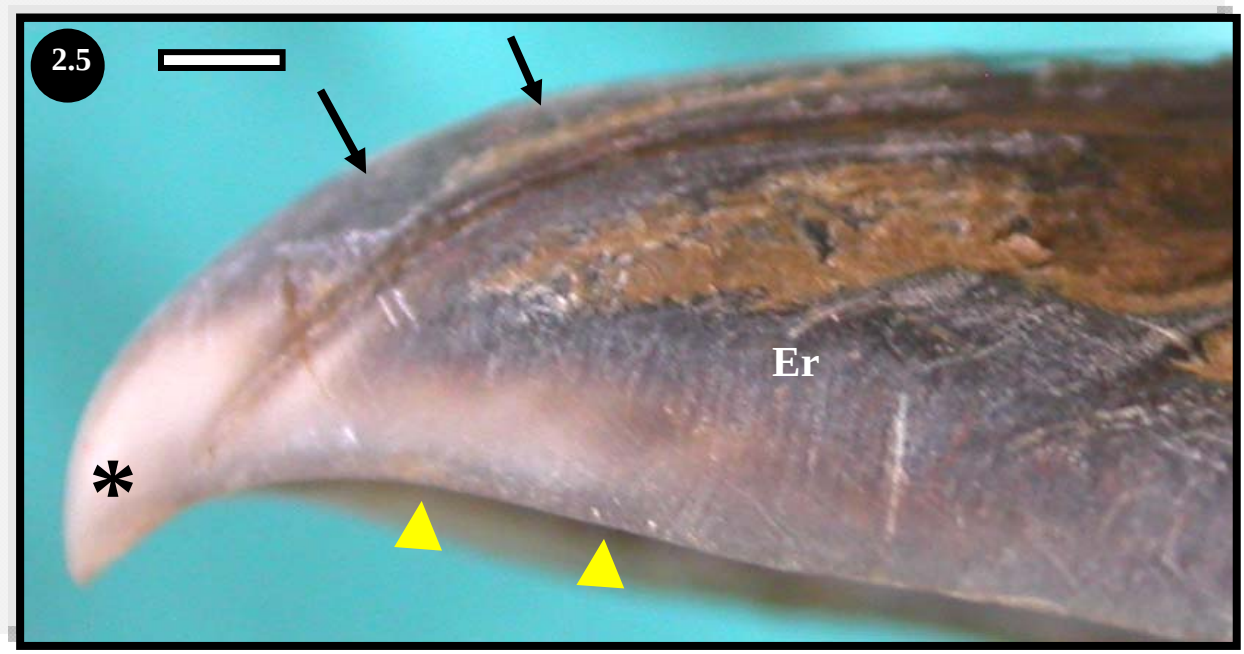


Figure 2.4: Lateral profile of the external mandibular rhamphotheca (Er) showing the smooth mandibular tomium (yellow *) proceeding rostrally to the serrated cutting edge (white arrows). Note how the *gonys* (black arrow) ends rostrally as the mandibular nail (red arrowheads). Bar = 1mm.



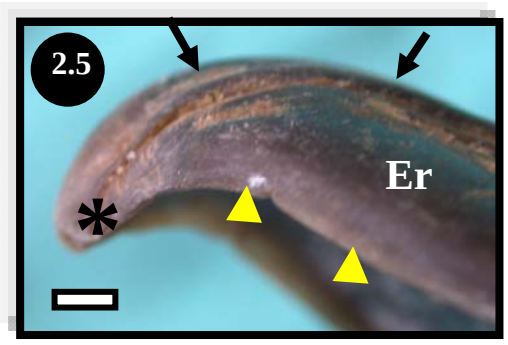


Figure 2.5: The external maxillary rostrum displaying the maxillary nail (*), the *culmen* (black arrows) on the dorsal surface of the beak and the sharp maxillary tomium (yellow arrowheads). External rhamphotheca (Er). Bar = 2mm.

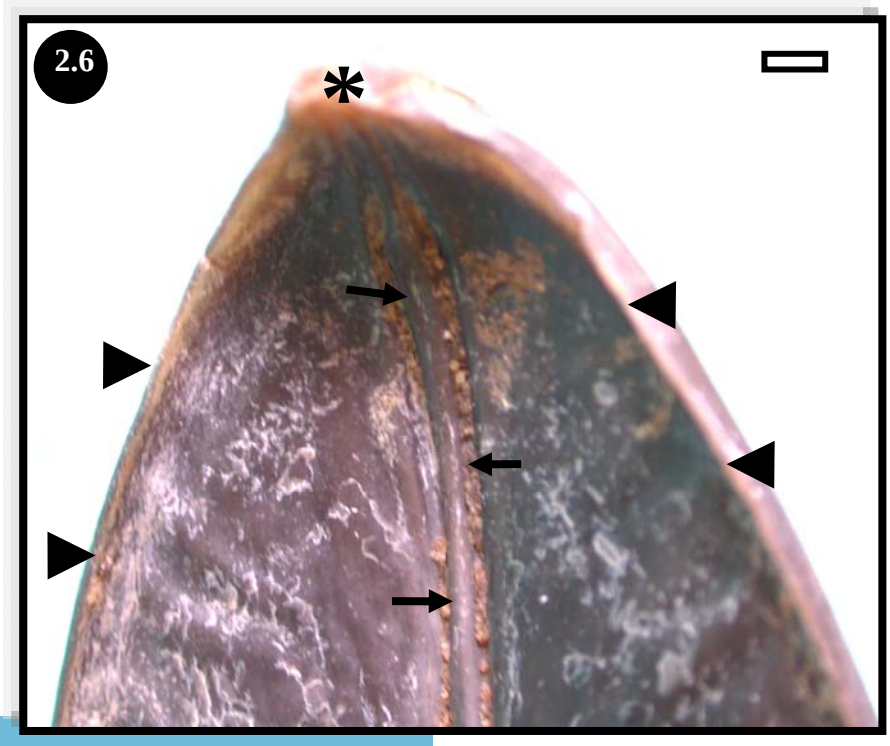


Figure 2.6: Maxillary rostrum, intra-oral view. The maxillary nail (*) can be seen projecting below the concavity (area between arrowheads) of the maxillary rostrum. Tomia (arrowheads) and median palatine ridge (arrows). Bar = 1mm.

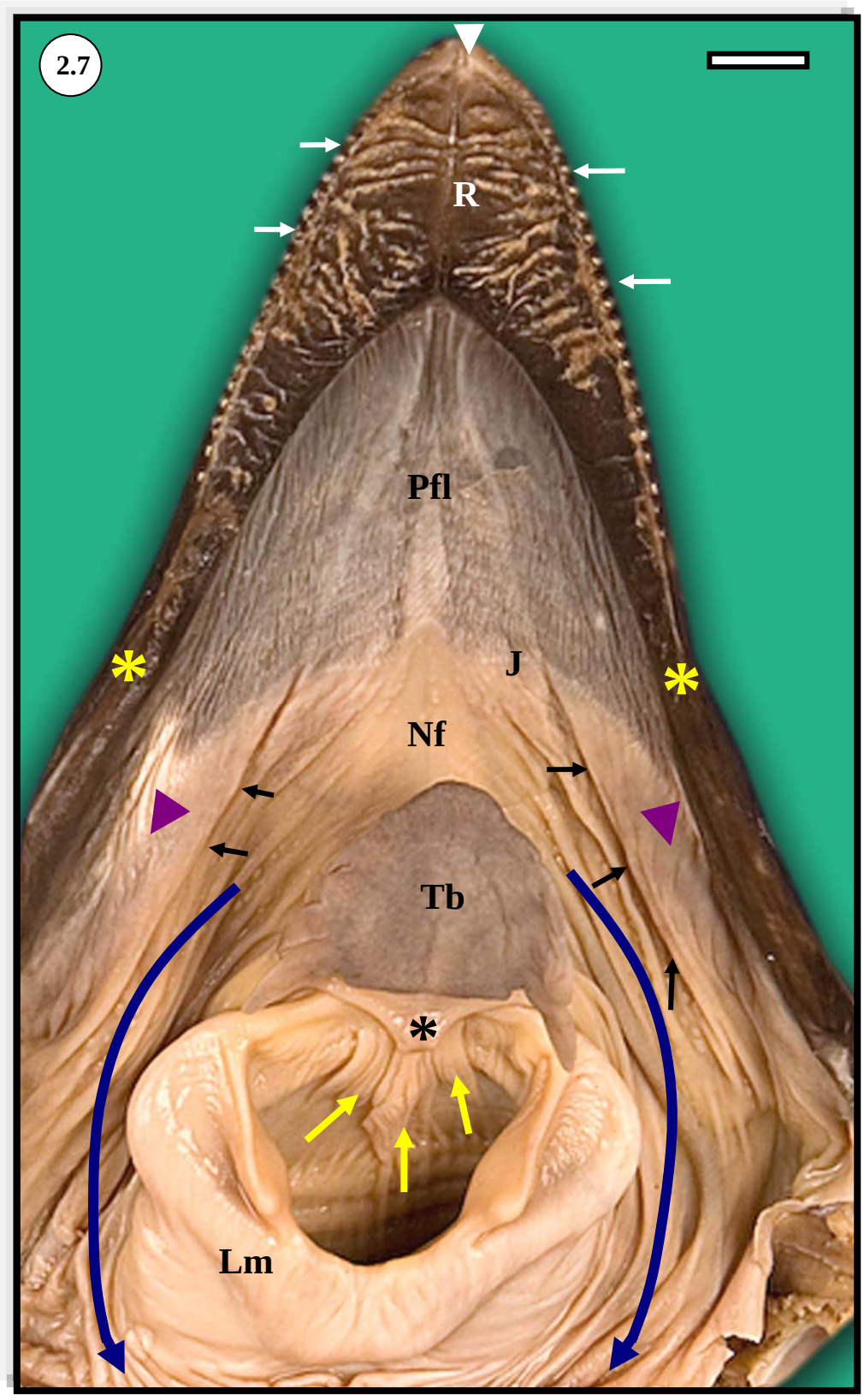
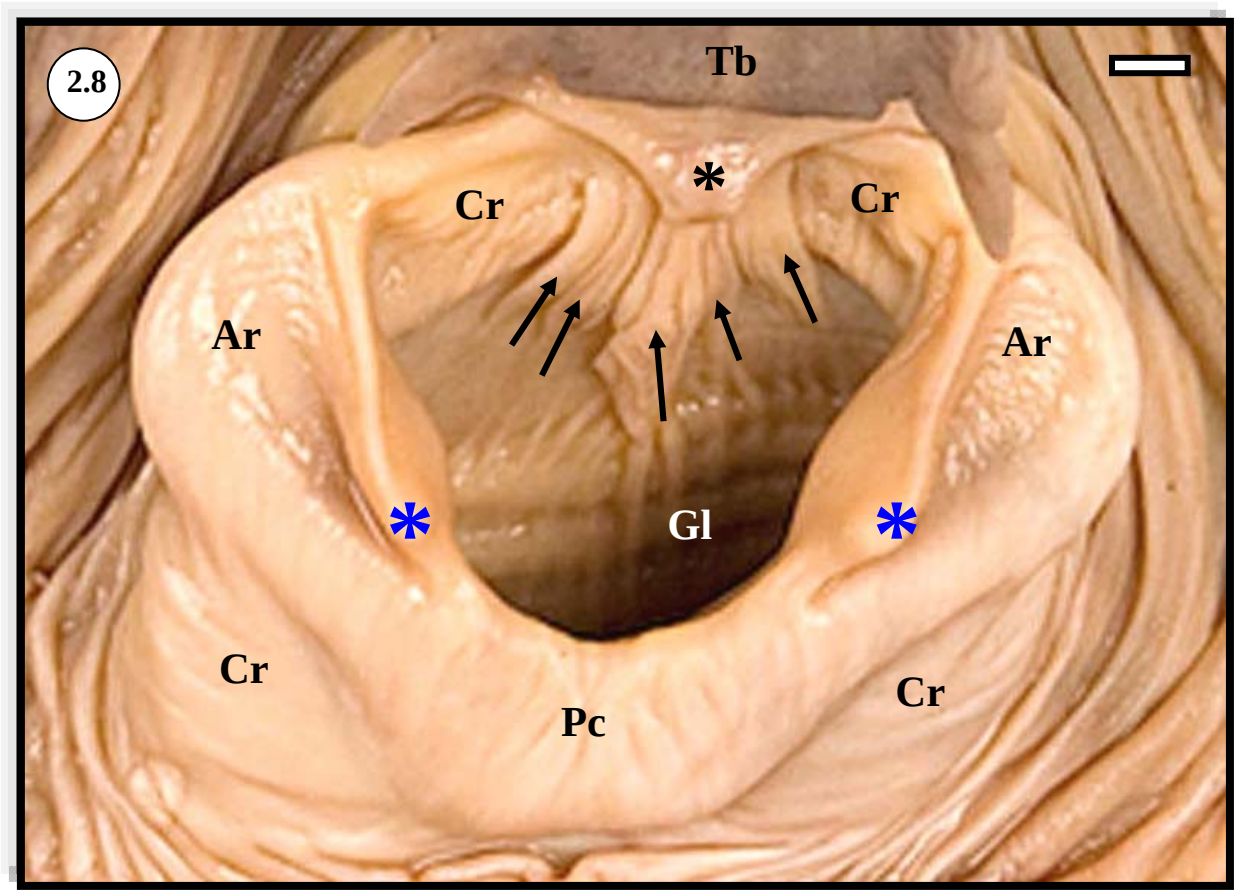


Figure 2.7: Gross anatomical features of the floor of the oropharynx. The interramal region is divided into a rostral pigmented (Pfl) and a caudal non-pigmented (Nf) part with a clear junction (J) marking the transition. The caudal region contains the tongue body (Tb) and root (*) and laryngeal mound (LM). The large lateral folds of the caudal floor are indicated (purple arrowheads) together with their associated medially opening groove or recess (black arrows). The smaller folds (blue arrows) follow the contours of the laryngeal mound. Mandibular rostrum with transverse ridges (R), mandibular nail (white arrowhead), rostral lamellae (white arrows) and smooth tomia (yellow *), mucosal folds at laryngeal entrance (yellow arrows). Bar = 5mm.



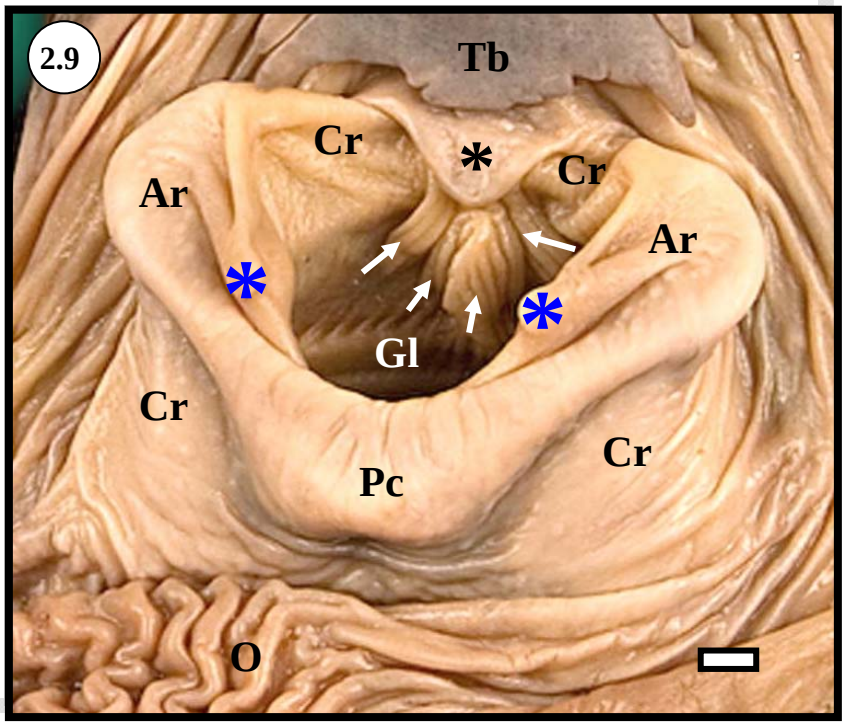


Figure 2.8 and 2.9: Dorsal view of the laryngeal mound of the emu showing the covering of smooth mucosa and the wide glottis (Gl). The circular cricoid (Cr), two dorsal arytenoid (Ar) and procricoid (Pc) cartilages support the larynx. Note the tongue root (black *) overlapping the glottis, the prominent mucosal folds (arrows) caudal to the root and the protuberances (blue *) projecting off the medial lips of the arytenoid cartilages. Tongue body (Tb), proximal oesophagus (O). Bar = 2mm.

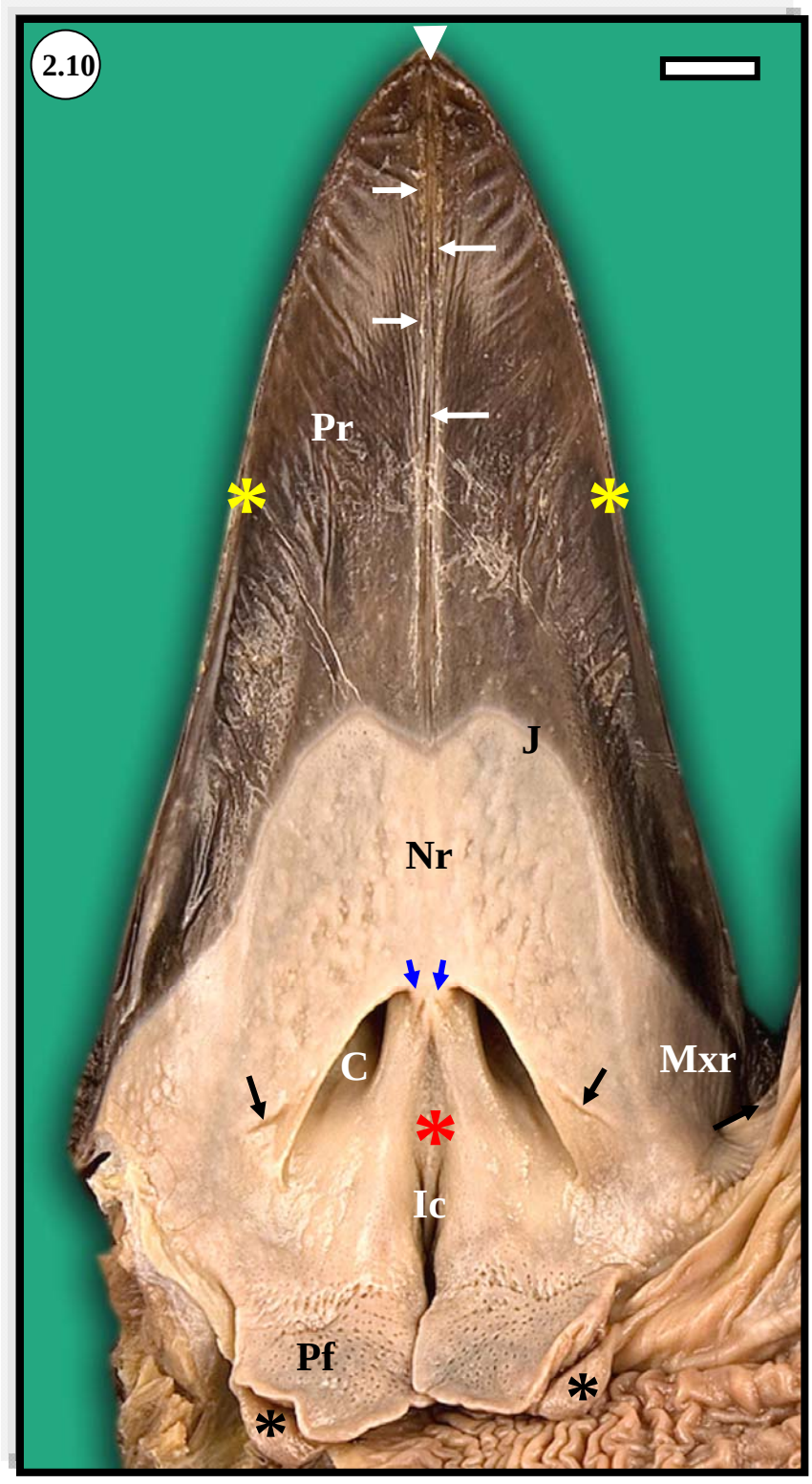


Figure 2.10: Gross anatomical features of the roof of the oropharynx of the emu. The junction (J) between the pigmented (Pr) and non-pigmented regions of the roof (Nr) is sharply demarcated. The pigmented roof is similar in colour to the maxillary rhamphotheca (yellow *) and displays a median palatine ridge (white arrows) down its midline. The division between the rhamphotheca and pigmented region is obscure. The choana (C) flanked by two small folds laterally (black arrows) and small raised nodules rostrally (blue arrows) is situated in the caudal non-pigmented roof. The pharyngeal folds (Pf) and their lateral projections (black *) are seen to form the most caudal extent of the oropharyngeal roof. Maxillary nail (white arrowhead), maxillary rictus (Mxr), median grooved septum (red *), infundibular cleft (Ic). Bar = 5mm.

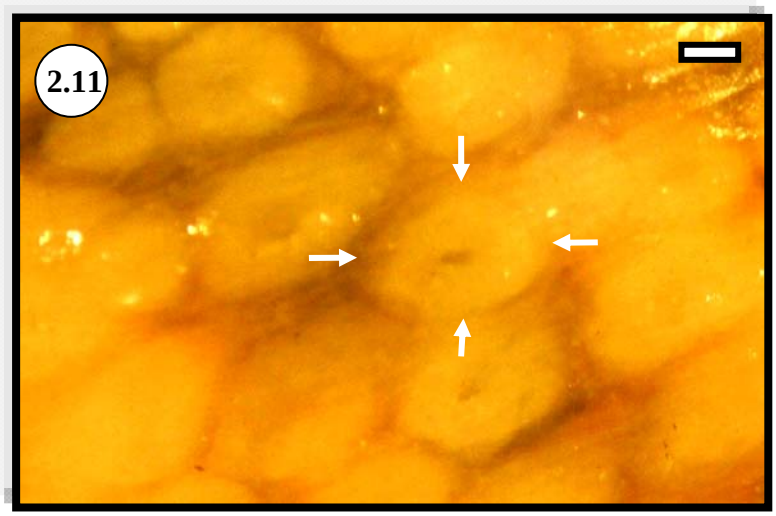


Fig. 2.11: High magnification of the doughnut-shaped structures lying beneath the mucosa of the non-pigmented roof. The outline of a single doughnut is shown by the white arrows and represents a glandular unit with the dark central spot indicating the gland opening. Bar = 200µm.

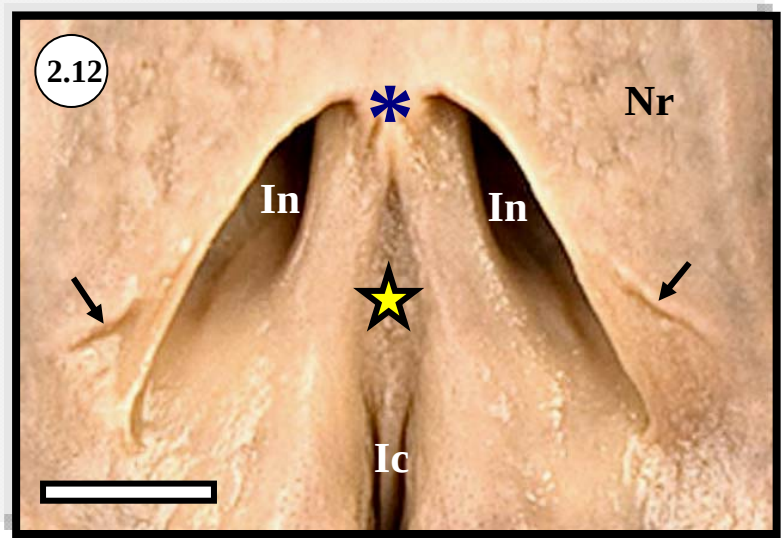


Fig. 2.12: The triangular choana of the emu with the two internal nares (In) separated by a median grooved septum (yellow star). The small nodules (blue *) are seen at the rostral choanal extremity. Non-pigmented roof (Nr) infundibular cleft (Ic), caudo-lateral mucosal folds (arrows). Bar = 5mm.

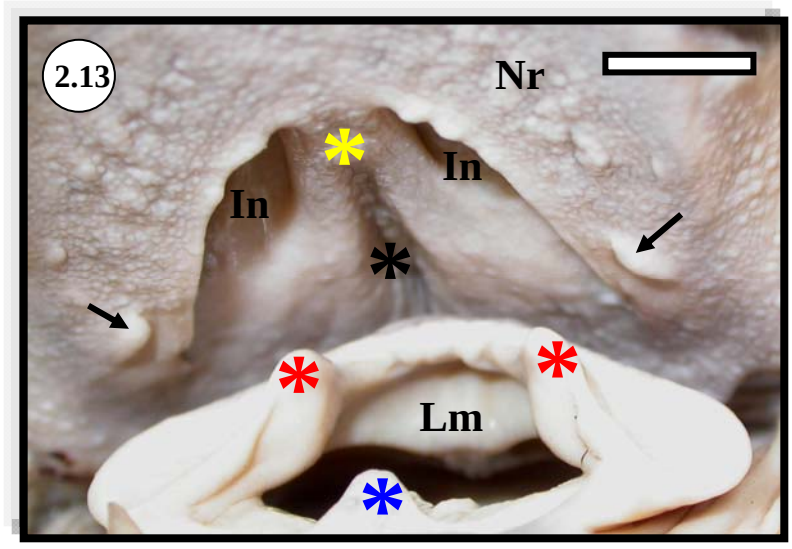


Fig. 2.13: Caudal view of the choana and laryngeal mound illustrating the functional relationship of the two structures. When the glottis is closed, the medial lips of the arytenoid cartilages (red *) and tongue root tip (blue *) align to move through the median grooved septum (black *) of the choana when the laryngeal mound (Lm) and the tongue (not shown) are retracted. Note the small mucosal folds (arrows) near the caudo-lateral edges of the choana. Non-pigmented roof (Nr), internal nares (In). Bar = 5mm.

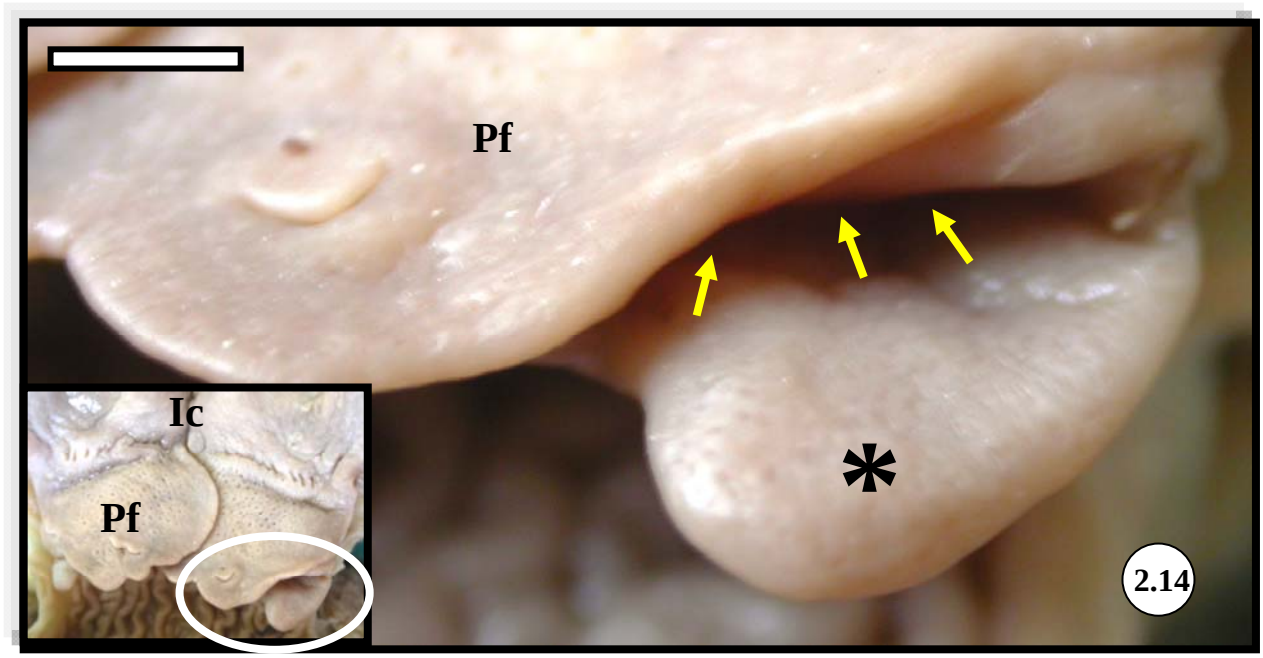


Figure 2.14: High magnification of the caudal pharyngeal fold (encircled area in inset). The caudo-lateral projection (*) forms a pocket or recess (yellow arrows) with the dorsal aspect of the pharyngeal fold (Pf). Note the medial overlapping of the free caudal aspect of the pharyngeal folds in the inset. Infundibular cleft (Ic). Bar = 1mm.

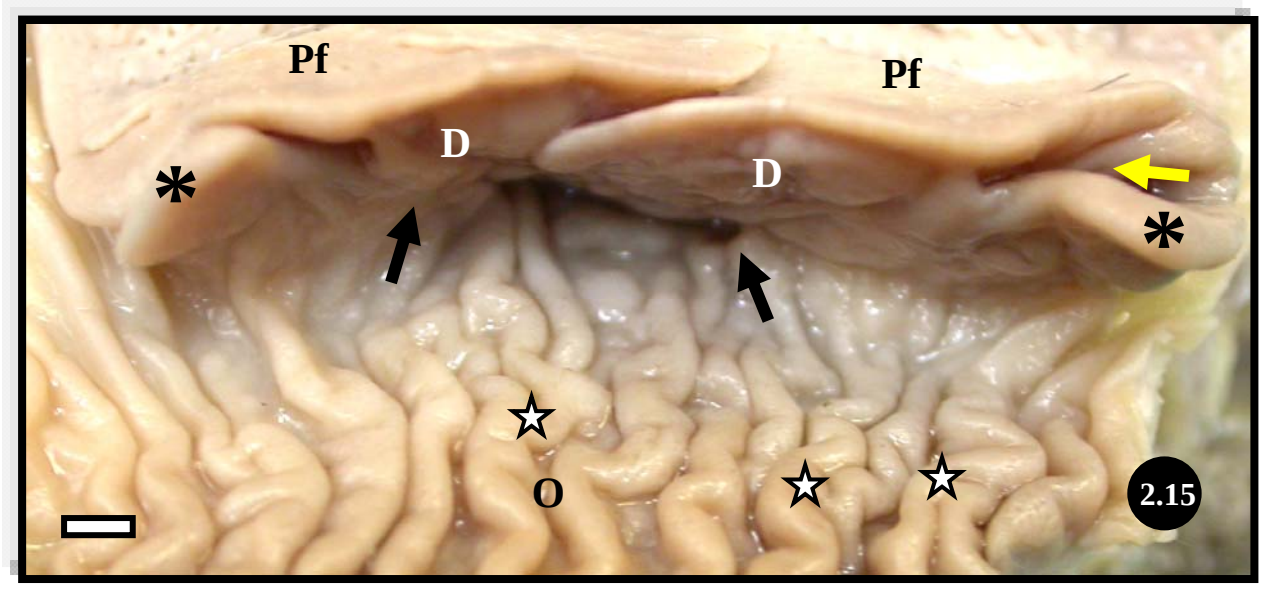


Figure 2.15: Caudal limit of the oropharynx showing the dorsal aspect (D) of the pharyngeal folds (Pf) forming a retropharyngeal recess (black arrows) where the mucosa of the folds is reflected and continued caudally as the proximal oesophagus (O). Note the wavy appearance of the oesophageal folds which branch and anastomose (starts). Lateral tissue projection (*), pocket or recess (yellow arrow). Bar = 1mm.

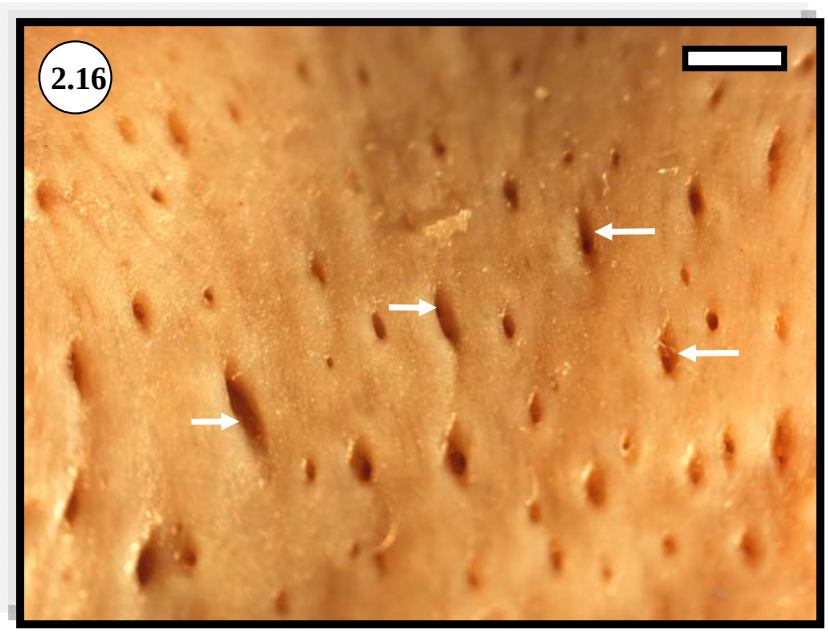


Fig. 2.16: The ventral surface of the caudal free part of the pharyngeal fold. The deeply pitted surface is made up of numerous large openings (white arrows) of underlying glands. Bar = 2mm.

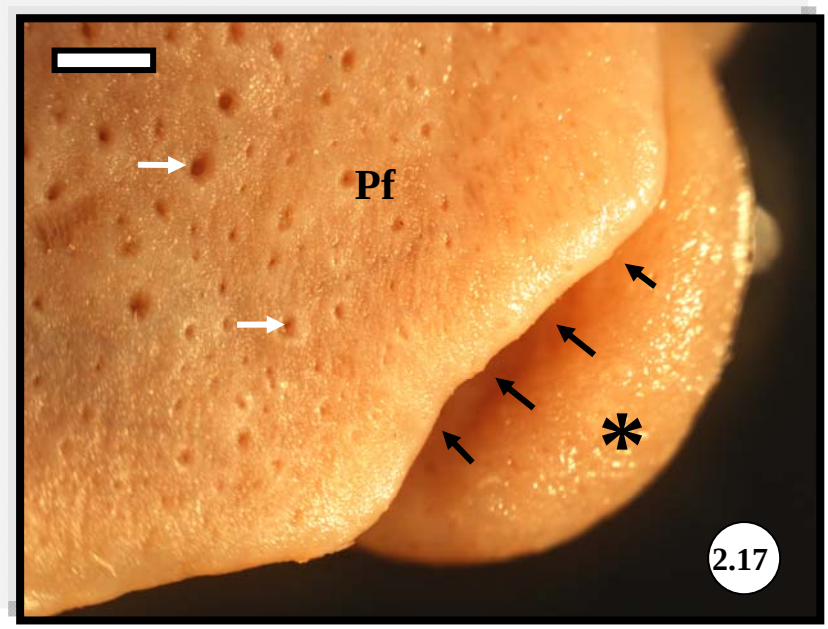


Fig. 2.17: Ventral view of the lateral projection (*) of the caudal part of the pharyngeal fold (Pf). A pocket or recess (black arrows) is formed between the fold and the projection. Gland openings (white arrows). Bar = 1mm

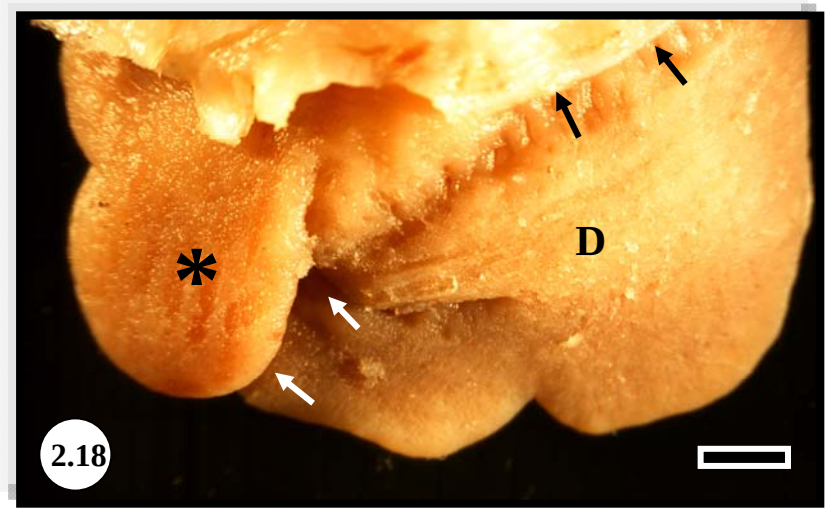


Fig. 2.18: Dorsal view (D) of the caudal part of the pharyngeal fold and projection (*). The pocket or recess is indicated by the white arrows and the reflection of the mucosa to form the retropharyngeal recess is indicated by the black arrows. Bar = 2mm.

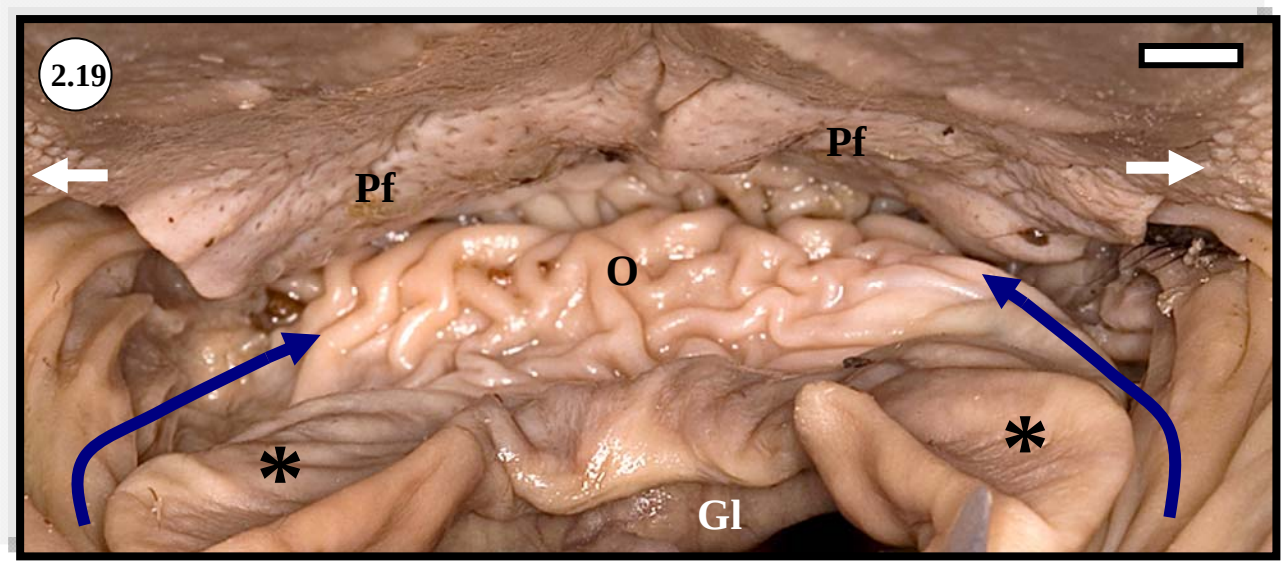


Figure 2.19: The entrance to the proximal oesophagus (O) seen from the gape of the emu (laryngeal mound depressed). The mucosal folds of the caudal oropharyngeal floor are indicated by the curved blue arrows. Pharyngeal folds (Pf), maxillary rictus with nodules (white arrows), arytenoid cartilages (*), glottis (Gl). Bar = 2mm.

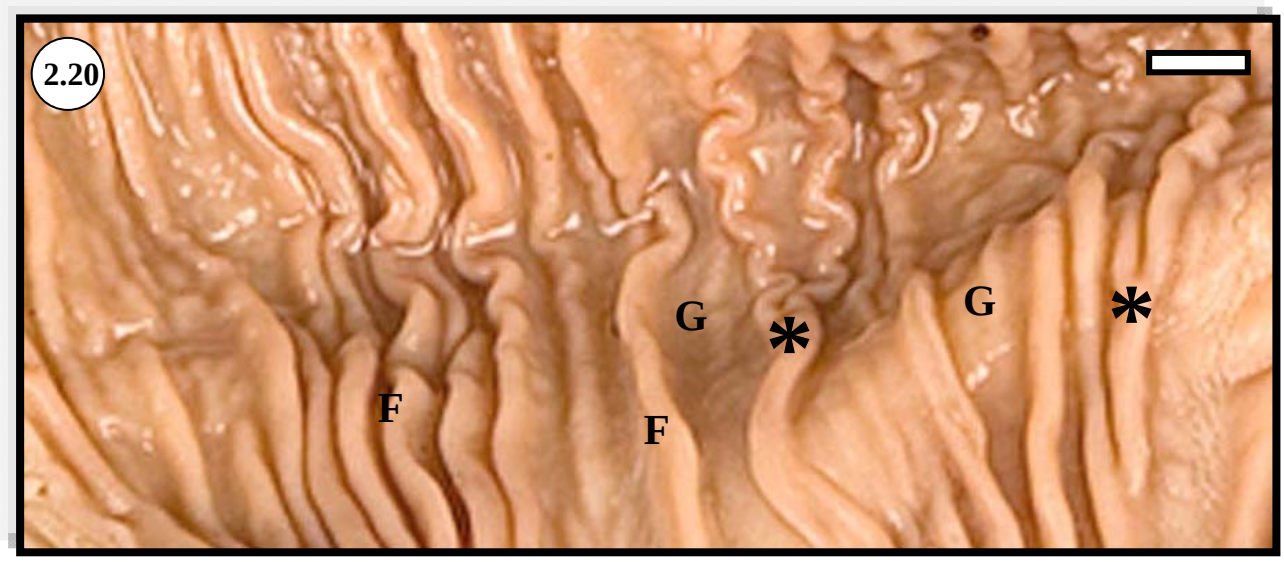
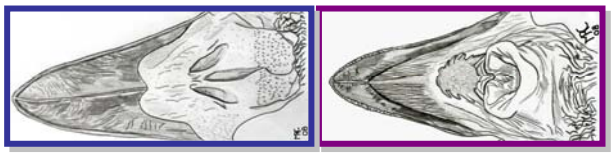


Figure 2.20: The proximal oesophagus showing the highly longitudinally folded nature of this region. Note the wavy appearance of the folds (F) and occasional branching and anastomosing (*). Intervening grooves (G). Bar = 2mm.

CHAPTER 3



HISTOLOGICAL FEATURES & SURFACE MORPHOLOGY OF THE OROPHARYNGEAL CAVITY & PROXIMAL OESOPHAGUS

3.1 INTRODUCTION

Although the histological features of the oropharyngeal cavity of many avian species have been described, often only certain structures or aspects of the oropharynx were studied, for example the tongue (see chapter 5). Owing to their commercial importance, domestic poultry and more specifically the fowl, have received the most attention (Calhoun, 1954; Koch, 1973; Hodges, 1974; McLelland, 1975, 1979, 1990; King and McLelland, 1984; Banks, 1993; Bacha and Bacha, 2000). Although the macroscopic features of the oropharynx or parts thereof have been described in ratites (Meckel, 1829; Cuvier, 1836; Gadow, 1879; Owen, 1879; Pycraft, 1900; Duerden, 1912; Faraggiana, 1933; McCann, 1973; Cho *et al*, 1984; Bonga Tomlinson, 2000) and other birds (see McLelland, 1979 for a review of earlier literature), the omission of histological data has severely restricted the value of these reports. Gardner (1926) has emphasised the importance of providing histological data, together with macroscopic descriptions, for a more in-depth understanding of structures and their function.

There have been very few histological studies of the ratite oropharynx and none detailing the histological or scanning electron microscopical features of the emu oropharynx. Histological studies of this region in ratites have been limited to the greater rhea (Feder, 1972) and ostrich (Porchescu, 2007; Tivane, 2008), whereas the surface morphology of the entire oropharynx has only been described in the ostrich (Tivane, 2008).

The histological structure of the avian oesophagus displays a remarkable uniformity (Pernkopf and Lehner, 1937; Calhoun, 1954; Warner *et al*., 1967; Ziswiler and Farner, 1972; Koch, 1973; Hodges, 1974; McLelland, 1975, 1979, 1990; Nickel *et al*., 1977; King and McLelland, 1984; Banks, 1993; Bacha and Bacha, 2000; Gussekloo, 2006) with the greatest variation appearing to be the presence of either a keratinised or non-keratinised stratified squamous epithelium lining

the organ. An additional variation (at the gross anatomical level) is the absence or presence of a crop.

Histological features of the ratite oesophagus have been documented for the greater rhea (Feder, 1972), ostrich (Porchescu, 2007; Tivane, 2008) and emu (Herd, 1985). These studies all show a similarity between the histology of the ratite oesophagus and that of birds in general, namely a non-keratinised stratified squamous epithelium, oesophageal glands in the distal portion of the *lamina propria* and the presence of a well-developed *muscularis mucosae*. The only histological study of the oesophagus of the emu is that of Herd (1985) in which only two specimens were used. The surface morphology of the ratite oesophagus has only been described in the ostrich (Tivane, 2008).

The emu is a commercially important bird and its nutrition and health are paramount to the success of any commercial operation. The emu enjoys a varied diet (Davies, 1978); however, nothing is known of the microstructure of the oropharynx which could affect food selection and intake. For example, it is not known if the emu has a sense of taste. It is therefore necessary to investigate the microstructures of the emu oropharynx to identify structural features that could influence nutrition, food intake and subsequent ingestion, as well as providing a foundation for the recognition of pathology in this region. This chapter will also provide comparative information for future studies of the ratite oropharynx.

3.2 MATERIALS AND METHODS

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

After rinsing five of the heads in running tap water, the right commisure of the beak was incised and the mandible reflected laterally by disarticulating the quadratomandibular joint to openly

display the roof and floor of the oropharynx and the proximal oesophagus (Fig. 3.1). Appropriate longitudinal and transverse sections representing areas of interest were excised from the oropharynx and proximal oesophagus (Fig. 3.1). The samples were dehydrated through 70, 80, 96, and 2X 100% ethanol and further processed through 50:50 ethanol: xylol, 2X xylol and 2X paraffin wax (60-120 minutes per step) using a Shandon model 2LE Automatic Tissue Processor (Shandon, Pittsburgh, PA, USA). Tissue samples were then imbedded manually into paraffin wax in plastic moulds. Sections were cut at 4-6 μm, stained with Haematoxylin and Eosin (H&E) and Peroidic Acid Schift Stain (PAS) (McManus, 1946) and viewed and micrographed using an Olympus BX50 equipped with the analySIS CC12 Soft Imaging System (Olympus, Japan).

An additional three heads were collected from birds (5, 15 months & 5 year-old birds) specifically for scanning electron microscopy. The heads were fixed in 10% buffered formalin overnight. Appropriate samples of the oropharynx were removed (Fig. 3.47) after the heads had been rinsed in running water for several hours to remove all traces of phosphate buffer. The samples were dehydrated through an ascending ethanol series (50, 70, 80, 90, 96 and 3X 100%). Due to the size of the tissue blocks, each dehydration step took 60 minutes. The blocks were then critical point dried from 100% ethanol through liquid carbon dioxide in a Polaron E300 Critical Point Drier (Polaron, Watford, England). After critical point drying the samples were mounted on round or rectangular (depending on sample size) aluminium viewing stubs with a conductive paste, Silver Dag (Dag 580 in alcohol), and sputter coated with a thin layer of palladium using a Polaron SEM E5100 coating unit. Areas of interest were viewed using a Jeol NeoScope JCM-5000 SEM operated at 10kV and a Jeol JSM-840 SEM operated at 5kV. Images were digitally captured using Start JCM-5000 and Orion 6.60.4 software, respectively, and described.

The terminology used in this study was that of Nomina Anatomica Avium (Baumel *et al*., 1993).

3.3 RESULTS

3.3.1 Light microscopic observations

3.3.1.1 Intra-oral mandibular bill skin (Figs. 3.2 – 3.4)

The mandibular bill skin (seen macroscopically as the mandibular rhamphotheca) consisted of a heavily keratinized, pigmented, stratified squamous epithelium, overlying a layer of dense irregular connective tissue (corium). The epithelium formed up to half the thickness of the bill skin. The *Str. basale* consisted of a tightly packed layer of columnar cells that were interspersed with melanocytes, which were also found in the connective tissue immediately beneath the *Str. basale*. This layer was followed by a thin *Str. spinosum*, with the rest of the epithelium composed of an extensive *Str. corneum* (*rhamphotheca*). The *Str. spinosum* appeared in places to be absent and in other areas it was two-three cell layers thick. There was no obvious *Str. granulosum*. The *Str. corneum*, which was by far the greatest component of the epithelium, consisted of a narrower, deeper, darker area and a wider, more superficial, lighter area. The *Str. basale* rested on a fine layer of connective tissue which merged with the dense irregular connective tissue forming the corium. The corium displayed localised areas of loose connective tissue resting on the periosteum of the mandible, and which contained numerous blood vessels, nerves and Herbst corpuscles. The Herbst corpuscles were of varying sizes with the majority being large in size. They were found singly or stacked, grouped or in longitudinal chains and were evenly distributed throughout the corium.

3.3.1.2 Oropharyngeal floor

Based on macroscopic observations (see Chapter 2) the oropharyngeal floor could be divided into the interramal region (*Regio interramalis*) composed of a rostral pigmented part and a caudal non-pigmented part, and the tongue (see Chapter 5) and laryngeal mound which were situated within the caudal interramal region.

3.3.1.2.1 Interramal region – Rostral pigmented part (Figs. 3.5 – 3.7)

The pigmented area directly caudal to the mandibular rostrum consisted of a keratinized stratified squamous epithelium overlying loosely arranged dense irregular connective tissue, up to six times the width of the epithelium. The intra-oral tissues were separated from the underlying integument by a layer of skeletal muscle fibres. The epithelium was undulating, representing the longitudinal mucosal folds and alternating grooves seen macroscopically. Melanocytes were concentrated in the *Str. basale* of the folds, with some cells extending into the *Str. spinosum*. In contrast, the density of melanocytes was greatly reduced in the grooves and where present these cells were scattered in the underlying connective tissue immediately below the *Str. basale* (Figs. 3.5, 3.6). The connective tissue housed numerous capillaries, small nerves and Herbst corpuscles. Connective tissue papillae were absent in this region. The Herbst corpuscles were mainly situated in the connective tissue beneath the mucosal folds (Fig. 3.7). Large blood vessels and nerves were located directly above the skeletal muscle layer.

3.3.1.2.2 The area of transition (Fig. 3.8)

The transitional region was characterised by a loss of mucosal folds and a marked thickening of the epithelium. Melanocytes gradually decreased in density, initially disappearing from the connective tissue and then also from the *Str. basale*. In addition to thickening, the epithelium changed to a non-keratinised stratified squamous epithelium, up to three times the thickness of the rostral keratinized epithelium. A short distance after the loss of the keratinised layer large, simple branched tubular glands (in the large lateral folds) or simple tubular glands (in the region medial to the large lateral folds) appeared in the underlying connective tissue. The epithelium was penetrated by connective tissue papillae carrying capillaries at their tips.

3.3.1.2.3 Interramal region – Caudal non-pigmented part (Figs. 3.9, 3.10)

This region consisted of a non-keratinised stratified squamous epithelium which overlay a gland-rich connective tissue layer. The epithelium was obliterated in certain areas by large

accumulations of diffuse lymphoid tissue, and which formed round masses emanating from the underlying connective tissue. Between the gland ducts traversing the epithelium were connective tissue papillae which carried capillaries at their tips. Structures resembling taste buds (Fig. 3.17, 3.19) occurred in the epithelium of this region and were very sparse and not associated with gland openings. They were clearly demarcated from the surrounding epithelium which encapsulated them and were composed of elongated elements which could not be clearly distinguished as sensory or supporting cells.

The dense irregular connective tissue beneath the epithelium contained mainly simple tubular mucus-secreting glands (PAS positive). These glands were confined to the more superficial zone of the connective tissue and were densely packed (Fig. 3.10). Large simple branched tubular mucus-secreting glands occurred on the dorsal aspect of the large lateral fold of tissue running parallel to the mandibular rami (Fig. 3.9). However, the blind ending groove or recess enclosed by the large fold and the area medial to it, contained only simple tubular mucus-secreting glands. A rich capillary plexus surrounded the larger glands and the ducts penetrated the full length of the epithelium, opening into the oropharyngeal cavity. The glands present in this area were structurally similar to those described for the tongue (see Chapter 5). The connective tissue beneath the mucosal folds in this region formed a thick core that supported each fold. Situated at the base of the fold was a large artery while nerve and vascular plexuses were situated near the base of the glands (Figs. 3.9, 3.10). Due to the thinning of the connective tissue in the grooves between the folds, the base of the simple tubular glands lay in close proximity to the underlying layer of skeletal muscle which demarcated the intra-oral tissue and integument.

3.3.1.2.4 Mandibular rictus (Figs. 3.11 – 3.13)

The intra-oral mandibular portion of the angle of the mouth (mandibular rictus) was a non-pigmented longitudinally folded tract of tissue. The mandibular rhamphotheca formed its lateral border and it was continuous with the caudal non-pigmented interramal space medially. At the point where the rhamphotheca merged with the non-pigmented tissue, the epithelium was seen to change from a lightly keratinized stratified squamous type with melanocytes to a non-

keratinised stratified squamous type devoid of melanocytes. The epithelium rested on a layer of dense irregular connective tissue, rich in nerves and blood vessels. This connective tissue also housed numerous simple tubular mucus-secreting glands (Fig. 3.12) which opened onto the surface via a duct lined by similar cells to those composing the secretory part of the gland. These glands were situated superficially, directly below the *stratum basale* of the epithelium and appeared partly intraepithelial in location. Larger simple branched tubular mucus-secreting glands (Figs. 3.11, 3.13) were situated deeper within the connective tissue than the smaller glands. The ducts of the larger glands which opened through the epithelium were lined by invaginated squamous cells from the epithelium which were vertically oriented. The tissue in this area was folded and in some of the folds large aggregations of lymphoid tissue were observed. Some of the aggregations contained a well circumscribed lymphatic nodule, surrounded by a thin layer of connective tissue (Fig. 3.12). The connective tissue papillae penetrated deep into the epithelium and carried capillaries in their tips (Fig. 3.11). Herbst corpuscles occurred in the connective tissue and were mostly associated with the capsule of the large glands (Fig. 3.13), although isolated corpuscles also appeared in the connective tissue (Fig. 3.12). Below the layer of dense irregular connective tissue was a layer of more loosely arranged dense irregular connective tissue, housing nerves and larger blood vessels (Fig. 3.11). This connective tissue rested on skeletal muscle.

3.3.1.3 Laryngeal mound (*Mons laryngealis*) (Figs. 3.14 – 3.15)

The tissue covering the laryngeal mound was smooth and non-pigmented and consisted of a non-keratinised stratified squamous epithelium which was thinner in the glandular region and thicker in the aglandular region (see below). Beneath the epithelium was a dense irregular connective tissue layer which formed widely spaced papillae that extended a short distance into the epithelium. The connective tissue layer housed mucus-secreting glands, Herbst corpuscles, lymphoid tissue, blood vessels and nerves and rested on skeletal muscle. The laryngeal mound displayed both glandular and aglandular regions. Simple branched tubular mucus-secreting glands (similar to those found elsewhere in the oropharynx) were situated on the dorso-lateral surface of the arytenoid cartilages (Figs. 3.15, 3.38) while the rest of the laryngeal mound was free of glands (Figs. 3.14, 3.38). At intervals, there were small aggregations of diffuse lymphoid tissue, which partially invaded the epithelium. The lymphoid aggregations consisted of scattered lymphocytes separated by connective tissue strands. Herbst corpuscles were present in low

numbers, and were either associated with the glands (Fig. 3.15) or lay isolated in the connective tissue (Fig. 3.14).

3.3.1.4 Laryngo-oesophageal junction (Fig. 3.16)

Macroscopically, the smooth non-pigmented caudal aspect of the laryngeal mound, changed abruptly to the longitudinally folded mucosa of the proximal oesophagus. The histological structure of the caudal aspect of the laryngeal mound was similar to that of the aglandular region of the mound (Fig. 3.14). At the point where the underlying skeletal muscle dissipated, simple tubular glands appeared in the *lamina propria,* marking the transition to the proximal oesophagus, the structure of which is described below. A structure resembling a taste bud was located in the epithelium of this area (Fig. 3.18). It consisted of a small group of vertically oriented cells lying within a depression in the epithelium and from which small cilia-like structures projected to the surface.

3.3.1.5 Oropharyngeal roof

The oropharyngeal roof was divided into the areas identified macroscopically (see Chapter 2), namely, the rostral pigmented region, the caudal non-pigmented region (housing the choana) and the two pharyngeal folds (Fig. 3.1).

3.3.1.5.1 Pigmented region (Figs. 3.20, 3.21)

The surface lining consisted of a keratinized, pigmented, stratified squamous epithelium (Fig. 3.20), overlying a dense irregular connective tissue layer. The *Str. corneum* formed up to half the thickness of the epithelium. The melanocytes (Fig. 3.20) were mainly confined to the *Str. basale*, but extruded pigment granules were also observed in the more superficial layers of the epithelium, particularly the *Str. spinosum*. The connective tissue abutted the periosteum of the underlying bone and was in places up to four times the thickness of the epithelium. It housed an extensive collection of large nerves and blood vessels as well as numerous Herbst corpuscles (Fig. 3.21). The Herbst corpuscles ranged in position from immediately below the epithelium, to just above the periosteum, although most of these structures were situated centrally in the connective tissue. They varied in size, occurred both

singly, in groups or in chains, and were more or less evenly distributed throughout the tissue. They were comparable in structure, and similarly arranged, to those in the mandibular bill skin (Figs. 3.2, 3.4). In the region forming the median palatine ridge, the connective tissue greatly increased in thickness (Fig. 3.21). At the base of the ridge was a large artery which was a consistent feature in all the specimens studied. In places, the connective tissue formed small, regular papillae which penetrated the epithelium. However, the epithelium and connective tissue generally showed a smooth interface.

3.3.1.5.2 Transitional area (Figs. 3.22, 3.23)

The transition from the pigmented oropharyngeal region to the non-pigmented region was marked by a gradual disappearance of melanocytes from the *stratum basale* of the epithelium; a gradual increase in thickness of the epithelium as it became non-keratinised; the appearance of connective tissue papillae; and the presence of a small aggregation of diffuse lymphoid tissue within the underlying connective tissue (Fig. 3.23). The deep zone of the connective tissue merged with the supporting connective tissue of the respiratory epithelium lining the nasal cavity (Fig. 3.23).

3.3.1.5.3 Non-pigmented region (Figs. 3.24, 3.25)

The surface was covered by a non-keratinised, non-pigmented stratified squamous epithelium supported by an underlying layer of dense irregular connective tissue. Connective tissue papillae carrying a rich capillary plexus penetrated the epithelium, up to half its depth, at regular intervals. The connective tissue contained large simple branched tubular mucus-secreting glands PAS positive (Figs. 3.24, 3.25) which changed in shape (in a rostral to caudal direction) from dorso-ventrally flattened to more dorso-ventrally elongated. Herbst corpuscles, located in the connective tissue, were most often associated with the glands, as seen elsewhere in the oropharynx, or occurred isolated in the connective tissue (Fig. 3.24). Each gland was surrounded by a capsule of dense irregular connective tissue and was similar in structure to those described in the tongue (see Chapter 5). Rostrally, the tissues of the non-pigmented region were separated from the deeper lying respiratory tissue by an abrupt transition from dense to loose irregular connective tissue. At this junction, large blood vessels and nerves

were observed. Caudally, a skeletal muscle layer originated which separated the respiratory and oral components from each other. Aggregations of diffuse lymphoid tissue (Fig. 3.25) associated with the ducts of the glands, were occasionally observed. Caudally, occasional simple tubular mucus-secreting glands were interspersed between the larger, simple branched tubular glands.

3.3.1.5.3.1 Maxillary rictus (Figs. 3.26-3.29)

The tissue in this region was similar to that described above for the non-pigmented region. It differed slightly in that the dense connective tissue layer was relatively wider with a greater amount of loose connective tissue, carrying blood vessels and nerves, present below the dense connective tissue layer (Fig. 3.26). A higher frequency of diffuse lymphoid tissue aggregations (Fig. 3.27), associated with both the glands (predominantly large, simple branched tubular) and isolated in the connective tissue, as well as a higher frequency of deep penetrating connective tissue papillae, (Fig. 3.26) were observed. Herbst corpuscles were present and were mostly arranged in groups and not associated with the glands (Fig. 3.28 – inset). The corpuscles were structurally similar to those found elsewhere in the oropharynx (see Chapter 5). The fibrocytic lamellae forming the outer core of the corpuscles demonstrated a faint PAS-positive reaction (Fig. 3.29).

3.3.1.5.3.2 Non-pigmented region – fold caudo-lateral to the choana (Figs. 3.30, 3.31)

The ventral aspect of the small fold of tissue observed at the caudo-lateral edge of the choana displayed similar features to that of the non-pigmented region (Fig. 3.30) with which it was continuous. The epithelium lining the dorsum of the fold (effectively forming the ventrum of the pocket) changed from a stratified squamous type to a ciliated columnar epithelium, which only occurred within the confines of the pocket. This transition was characterised by the appearance of large aggregations of diffuse and nodular lymphoid tissue which occupied the connective tissue enclosed within the blind-ending pocket (Figs. 3.30, 3.31). The presence of lymphoid tissue in this region was a consistent feature in all the specimens examined. The supporting connective tissue housed glands, lymphoid tissue, Herbst corpuscles, blood vessels and nerves. In the connective tissue adjacent to the blind-ending pocket was a large muscular artery (Figs. 3.30, 3.31). The glands within the pocket were simple tubular mucus-secreting

glands (PAS-positive, Fig. 3.31), although the lateral aspects of the pocket contained both the above mentioned glands as well as large, simple branched tubular mucus-secreting glands (Fig. 3.31).

3.3.1.6 Pharyngeal folds (Figs. 3.32 – 3.37)

The rostral fixed part of the pharyngeal folds was continuous with the oropharyngeal roof caudal to the choana. The epithelium and connective tissue elements were similar to those described for the non-pigmented region of the roof. There was a low frequency of simple tubular mucus-secreting glands scattered amongst the evenly spaced large simple branched tubular mucus-secreting glands (PAS positive, Fig. 3.33), which formed the bulk of the glandular tissue. The lumen of some of the glands was lined by a pseudostratified ciliated columnar epithelium (Fig. 3.35). Most of the glands were associated with variably sized aggregations of diffuse lymphoid tissue. Caudally (seen macroscopically as large pitted openings), the glands increased in size as did the associated aggregations of lymphoid tissue. Randomly distributed units of nodular lymphoid tissue occurred within the diffuse lymphoid aggregations. More caudally the epithelium was penetrated by a lower frequency and regularity of connective tissue papillae. Below the dense irregular connective tissue was a thin layer of loose irregular connective tissue containing blood vessels and nerves as well as adipose tissue. Below this layer was a layer of skeletal muscle.

The free part of the pharyngeal folds displayed the largest glands and the greatest amount of associated lymphoid tissue. At the most caudal extremity, the pharyngeal fold was separated by a crypt that was only present in the free part of the fold (Fig. 3.34). It varied in depth between the specimens. The tissue flap forming the ventral boundary of the crypt (continuous with the ventral surface of the pharyngeal fold) was generally free of lymphoid tissue, the connective tissue layer was thinner, and large, simple branched tubular glands (more typical of the rest of the oropharynx) opened into the oropharynx. The glands on the opposite side of the fold and which opened into the crypt were both large, simple branched tubular and, more rostrally, simple branched tubular glands (Fig. 3.34). The part of the pharyngeal fold forming the dorsal boundary of the crypt contained a few simple branched tubular glands and a large mass of diffuse lymphoid tissue. Dorsal to the crypt, a pocket or recess was formed between the dorsum

of the pharyngeal fold and a caudo-lateral projection of tissue (see Chapter 2). The part of the tissue projection protruding beyond the pharyngeal fold contained both types of glands and scant lymphoid tissue (Fig. 3.34). However, the pocket or recess was lined almost entirely by a dense mass of diffuse lymphoid tissue, with only occasional simple tubular glands being seen (Figs. 3.34, 3.37). Variable amounts of nodular lymphoid tissue were also present in the tissue lining the recess (Figs. 3.36, 3.37). The dorsal surface of the pharyngeal fold consistently displayed a small nodule of lymphoid tissue which protruded into the pocket or recess, suspended by a stalk of connective tissue (Fig. 3. 36). The dorsal aspect of the projection merged rostrally with the dorsal aspect of the pharyngeal fold, which rostrally reflected on itself and marked the beginning of the proximal oesophagus, forming the depth of the retropharyngeal recess (Fig. 3.37). This portion of the pharyngeal fold showed fewer and smaller amounts of lymphoid tissue and contained simple tubular glands only. The oesophagus in the retropharyngeal recess displayed features similar to the rest of the proximal oesophagus (see below).

3.3.1.7 Proximal cervical oesophagus (*Oesophagus Pars cervicalis*) (Figs. 3. 39 – 3.46)

The oesophagus was composed from within outwards of four main layers, namely, the mucosa, submucosa, muscular layer (*Tunica muscularis*) and adventitia (Figs. 3.39, 3.40).

The mucosa was formed by a non-keratinised stratified squamous epithelium, a dense irregular connective tissue (*lamina propria*) and a thick longitudinally oriented smooth muscle layer, the *muscularis mucosae.* The epithelium was penetrated by the long necks of glands emanating from the *lamina propria,* and contained sparse taste buds. The taste buds were elongated structures, which were clearly discernable from the surrounding epithelium and displayed a definite taste pore (Fig. 3.46). They were not directly associated with the glandular tissue. The taste buds were composed of vertically oriented cellular elements that displayed both round vesicular nuclei and denser, more elongated nuclei. However, it was not possible with the staining technique used to discern sensory and supporting cells from one another or the presence of nerve processes. The *lamina propria* housed numerous mucus-secreting glands (PAS positive), which were of the simple tubular type, some of which were branched. In the mucosal folds the glands did not appeared to penetrate far into the *lamina propria* (in the mucosal folds the *lamina propria* was thick due to the absence of the *muscularis mucosae*) (Figs. 3.40-3.43). In contrast, in the mucosal grooves, the glands appear to occupy the

full width of the *lamina propria* (in the mucosal grooves the *lamina propria* was thin due to the presence of the *muscularis mucosae*) (Figs. 3.40, 3.43). The glands displayed features typical of the mucus-secreting glands (Figs. 3.44, 3.45) described in the oropharynx. Unlike in the oropharynx, no large, simple branched tubular glands were observed. Diffuse lymphoid tissue was also present in the *lamina propria* and was situated between the numerous glands which were often excluded (Fig. 3.43). The *muscularis mucosae* was the most prominent layer in the oesophagus but did not extend into the folds of the mucosa (Figs. 3.39-41, 3.43). It was composed of longitudinally arranged smooth muscle cells and was similar in thickness to the *tunica muscularis.* The longitudinal folds of the proximal oesophagus were formed by the epithelium and *lamina propria* only.

The submucosa was a very thin connective tissue layer which in places was hardly discernable (Figs. 3.39, 3.40). It carried large blood vessels and nerves as well as the submucosal plexus.

The *tunica muscularis* (Figs. 3.39, 3.40, 3.43) was composed of a thicker inner circular and thinner outer longitudinal layer of smooth muscle. Between the two layers was a nerve plexus and associated neurons, the myenteric plexus.

The *tunica adventitia* (Fig. 3.39), composed of loose connective tissue, formed the outermost layer of this region and contained large blood vessels, nerves and adipose tissue.

3.3.2 Scanning electron microscopic observations

Samples for SEM (Fig. 3.47) were taken from the interramal region (including the rostral pigmented and caudal non-pigmented parts, and large lateral fold), the pigmented and non-pigmented parts of the roof (including the median palatine ridge), the ventrum of the pharyngeal fold and tissue projection, and the proximal oesophagus.

3.3.2.1 Oropharyngeal floor

At low magnification two distinct parts were visible, a region displaying many crevices and folds (representing the rostral longitudinally folded keratinised oropharyngeal floor) (Figs. 3.48, 3.49) and a smoother region consisting of a few larger, well-defined folds (representing the caudal

non-keratinised glandular oropharyngeal floor) (Fig. 3.51). High magnification of the longitudinal folds of the rostral region revealed numerous oblique, transverse and longitudinal fissures on the surface (Figs. 3.49, 3.50). Few individual desquamating cells (Fig. 3.50) were visible on the surface. The transition between the keratinised and non-keratinised regions (Fig. 3.48) was broad and bordered rostrally by the abrupt ending of the longitudinal folds and caudally by the flaky appearance of the non-keratinised region. The transitional zone was composed of a broad sheet of desquamating cells (Fig. 3.48), similar to those observed in the keratinised region of the oropharyngeal roof (see below). The large lateral fold of the non-keratinised region displayed individually desquamating cells, giving it a flaky appearance, and large, evenly dispersed openings, often obscured by mucus-secretion from the underlying glands (Fig. 3.56). In the younger bird, the fold medial to the large lateral fold displayed a similar surface but with fewer openings (Figs. 3.51, 3.52). The surface of the more medial folds (Fig. 3.55) was uneven and undulating. High magnification of these folds revealed surface cells covered with dense microvilli (Fig. 3.57) which were compacted at the cell boundaries, thus clearly demarcating the individual cells (Figs. 3.55, 3.57). Numerous small openings were also present on this surface and were also surrounded by cells densely packed with microvilli (Figs. 3.55, 3.57). In the older birds, the region medial to the large lateral fold displayed numerous evenly spaced small openings. In all the specimens studied, the grooves between the folds displayed a lumpy, uneven surface with large and small openings (Figs. 3.51, 3.52). This surface was covered by cells densely packed with microvilli, and which were concentrically arranged around the gland openings (Figs. 3.54, 3.57). The large openings were situated in the walls of the grooves (Figs. 3.51, 3.52) whereas the smaller openings occupied the depths of the grooves. The large openings were lined by a concentric pattern of cells giving them a ridged appearance (Figs. 3.52, 3.53).

3.3.2.2 Oropharyngeal roof

Two different regions of the roof were apparent at low magnification, a smooth rostral area representing the keratinised region and a ‘flaky’ caudal area representing the non-keratinised region (Fig. 3.60). The transition between the two regions was abrupt (Fig. 3.60). The smooth area typically displayed sheets of desquamating cells (Fig. 3.58). Individual cells were polygonal in shape and displayed microridges on their free surface (Fig. 3.59). The non-keratinised region of the oropharyngeal roof displayed individual desquamating cells or rows of cells giving it a more flaky appearance (Fig. 3.60, 3.61). The surface cells in this region were also polygonal

shaped. Large gland openings (Figs. 3.61, 3.62) as well as numerous small gland openings (Figs. 3.63, 3.64) were visible in this region. The smaller gland openings (found in the more caudal aspect of the non-keratinised oropharyngeal roof) were surrounded by concentrically arranged cells covered by a dense mass of microvilli (Fig. 3.64).

3.3.2.3 Pharyngeal folds

At low magnification the surface of the pharyngeal folds displayed similar features to that of the non-keratinised roof, exhibiting a flaky appearance due to the desquamation of individual cells (Fig. 3.65, 3.69). Higher magnification of the surface cells revealed a complex pattern of branching and anastomosing microplicae (Fig. 3.66). The complexity of this pattern varied between individual cells. However, in the immediate vicinity of gland openings the surface cells displayed dense masses of microvilli (Figs. 3.67, 3.68). The gland openings in this region (Figs. 3.65, 3.67, 3.69) were more numerous and larger than those of the oropharyngeal roof. Both large and small gland openings were present, with the former (presumably representing the openings of the underlying large, simple branched tubular glands) being more numerous and apparent. The cells forming the ducts of the large glands were vertically aligned, in some instances appearing to form folds (Fig. 3.67). The duct lining cells also displayed masses of microvilli (Fig. 3.67) and were continuous with the zone of similarly adorned cells surrounding the duct openings. The gland openings were often filled with a plug of mucous (Fig. 3.67) and patches of cilia were apparent (Figs. 3.67, 3.68). Small, randomly distributed globular structures were also present (Fig. 3.68).

In the younger bird the caudo-lateral tissue projection of the pharyngeal fold displayed a more irregular surface than the pharyngeal fold (Fig. 3.69, 3.70). The large gland openings (Figs. 3.69, 3.70) were bigger than those of the pharyngeal fold and appeared raised or crater-like (Figs. 3.69, 3.70). Small gland openings surrounded by circumferentially oriented cells (Fig. 3.71) were also present. The surface cells adopted a variety of shapes, their cells boundaries were not clearly defined and they were covered with masses of densely-packed microvilli (Figs. 3.71, 3.72). Occasional ciliated cells were interspersed between the microvilli-rich cells (Fig. 3.72). Numerous small raised nodules (presumably rounded cells) (Figs. 3.70, 3.72) were situated on the surface of this tissue and displayed a pattern of microplicae (Fig. 3.71, 3.72). Numerous cell projections were apparent in this region and occurred in the form of long slender rods or club-shaped structures (Figs. 3.71, 3.72). Numerous globular structures lay scattered on or between

the surface cells (Figs. 3.71, 3.72). This region in the older birds, however, was characterised by an increase in individual surface cell desquamation and large gland openings.

3.3.2.4 Proximal oesophagus

On low magnification, the mucosal folds of the proximal oesophagus appeared as smooth, gently rounded, longitudinally oriented structures exhibiting a convoluted or wavy pattern. A degree of branching and anastomosing was observed while strands of mucus were visible between and adhering to the folds (Fig. 3.73). The surface of the folds was pitted by the openings of underlying glands (see light microscopy). Both large and small openings were apparent, with the small openings being more numerous and generally scattered around the larger openings (Figs. 3.73-3.75). Strands of mucus representing the secretions from the underlying glands were visible in most of the openings and occasionally on the cell surfaces (Figs. 3.75, 3.76, 3.78). In the young bird, desquamating surface cells, unlike the rest of the oropharynx, were not a feature of this region. The surface cells were polygonal and characterised by clearly demarcated cell boundaries accentuated by an accumulation of microvilli (Figs. 3.75, 3.77, 3.78). All cell surfaces in the proximal oesophagus, including the cells lining the gland duct openings, displayed densely arranged microvilli (Figs. 3.76-3.78). Scattered, raised nodules (Fig. 3.74) lay on the surface between the gland openings. In the older birds, gland openings were more crater-like in appearance and the surface cells with microvilli were restricted to the regions immediately surrounding and lining the gland openings. Thus the predominant cell surface pattern in the older birds for this region was microplicae.

3.4 DISCUSSION

3.4.1 The oropharynx

3.4.1.1 Epithelium

The entire oropharyngeal cavity of the emu was lined by a stratified squamous epithelium. This is the same finding for the ostrich (Tivane, 2008) and for other birds in general (Fahrenholz, 1937; Calhoun, 1954; Warner *et al*., 1967; McLelland, 1975, 1979; Nickel *et al*., 1977; King and McLelland, 1984). The *stratum corneum* of the epithelium covering the bill is termed the *rhamphotheca* (Hodges, 1974). The *stratum granulosum* is not very apparent in the emu, a

feature also noted in the chicken (Hodges, 1974). The epithelium of the rostral oropharynx in the emu contains melanocytes and is keratinised, and manifests macroscopically as the rostral pigmented parts of the floor and roof (see Chapter 2). Although the greater rhea (Feder, 1972) and ostrich (Tivane, 2008) do not have a rostral pigmented oropharynx, the epithelium of the rostral part of the roof is also keratinised in both species, as well as the rostral floor in the ostrich (Tivane, 2008). Feder (1972) makes no mention of the histology of the oropharyngeal floor in the greater rhea. The transition between the rostral keratinised stratified squamous epithelium and the caudal non-keratinised stratified squamous epithelium in the emu is abrupt, a feature also noted in the ostrich (Tivane, 2008). Thus the epithelia lining the emu, greater rhea and ostrich oropharyngeal cavities are similar. Keratinisation of the oropharyngeal epithelium also occurs in other birds to varying degrees (Fahrenholz, 1937; Nickel *et al*., 1977; McLelland, 1979; King and McLelland, 1984).

In areas subject to abrasion the epithelium is keratinised (McLelland, 1979; King and McLelland, 1984) and the degree of keratinisation varies according to the amount of mechanical stress involved (Nickel *et al*., 1977). In the emu, the rostral pigmented parts of the floor and roof of the oropharynx are keratinised. In the cranioinertial feeding method employed by ratites (Bonga Tomlinson, 2000; Gussekloo and Bout, 2005), the food is handled by the bill tips only and held in the most rostral portions of the oropharynx prior to being transported to the proximal oesophagus. Therefore the keratinisation of these areas in the emu as well as in other ratites such as the ostrich (Tivane, 2008) that employ the same feeding strategy, protects the parts of the rostral oropharynx involved in the handling of food and thus subject to the most abrasion.

3.4.1.1.2 Taste buds (*Caliculi gustatorii*)

In birds, the presence or absence of taste buds in the oropharynx has been heavily debated due to the different eating habits and diet of birds (Moore and Elliott, 1946). In the emu, structures resembling taste buds are located in the oropharyngeal epithelium in the caudal interramal region, the tongue root (see Chapter 5) and at the laryngo-oesophageal junction. This is the first report of taste buds in the oropharynx of a ratite. No taste buds were identified in the greater rhea (Feder, 1972) or ostrich (Tivane, 2008), although, the former author notes that the possibility of their existence could not be ruled out.

Birds possess a very low number of taste buds in comparison to other vertebrates (Berkhoudt, 1985). This would be true for the emu as putative taste buds were very sparse and only observed in a few sections. As the emu swallows its food whole, employing the 'catch and throw' (Gussekloo and Bout, 2005) or cranioinertial feeding method (Bonga Tomlinson, 2000), in which the food lands near or into the oesophageal entrance before swallowing, there would be a limited need or opportunity for taste during the intra-oral transport of food. It would thus seem appropriate that any taste receptors found in the emu oropharynx would be sparse and located in the most caudal regions.

A reason for the difficulty in locating taste buds, as noted by Moore and Elliott (1946), is the fact that they are obscured by the connective tissue papillae and the ducts of glands traversing the epithelium. Submucosal papillae and salivary ducts can also easily be mistaken for taste buds, depending on the plane of sectioning (Lindenmaier and Kare, 1959). Moreover, taste buds are most often associated with glands (Gentle, 1971b; Bacha and Bacha, 2000). The presence of many deep connective tissue papillae and gland openings in the emu oropharynx would certainly complicate and mask the identification of taste buds in this species. Taste buds can either occur free in the mucosa or be associated with salivary glands (Botezat, 1910; Nickel *et al*., 1977; Berkhoudt 1985). The structures found in the emu oropharynx were not associated with gland openings and were distinct entities within the epithelium. A definite taste pore as well as vertically oriented elongated cells were identified, although it was not possible to discern supporting from sensory cells as described by Berkhoudt (1985).

The structures resembling taste buds found in the emu oropharynx were similar to the isolated receptors depicted by Botezat (1910) and appeared similar in shape to those described and depicted for birds in general (Botezat, 1910; Moore and Elliott, 1946; Gentle, 1971b; Nickel *et al*., 1977; Lindenmaier and Kare, 1959; Warner *et al*., 1967). Taste buds in birds also appear similar to those found in other vertebrates (Moore and Elliott, 1946; Gentle, 1971b). However, a more detailed comparative study will be needed to ascertain whether the taste buds in the ratite oropharynx are comparable to those found in other birds. Further studies will also be needed, employing alternative staining techniques, to fully describe the structure of the emu taste buds.

The most obvious function of taste buds in the emu would be the discrimination of food. The sense of taste is an important motivator for feeding as well as for initial food selection in birds (Gentle, 1971a). Taste encourages nutrient intake as well as helping to discriminate against

possible harmful foods (Kare and Rogers, 1976) by screening the intake of food and water (Berkhoudt, 1985). Initial food selection, however, may not be an important function of taste in the emu, as noted above, due to the particular feeding method of this bird where food is most likely only tasted after ingestion. In birds, food selection is also based on size, shape, colour and texture as well as taste and olfaction (Berkhoudt, 1985). It would seem plausible that all these factors would also influence food intake in the emu.

3.4.1.3 Connective tissue

The layer of connective tissue supporting the epithelium of the oropharynx in the emu could not be clearly divided into a *lamina propria* and a submucosa, a feature also noted in the greater rhea (Feder, 1972) and chicken (Calhoun, 1954). This is due to the absence of a *muscularis mucosae* (Calhoun, 1954). Thus for the purposes of this study this tissue was termed the underlying connective tissue. The connective tissue in the emu formed capsules around the glands, and housed blood vessels, nerves, Herbst corpuscles, melanocytes, lymphoid tissue and glandular tissue, in similar fashion (except for the melanocytes) to that described in the ostrich (Tivane, 2008).

3.4.1.3.1 Glands (*Glandulae oris, Glandulae pharyngis*)

Glandular tissue was a major feature of the non-pigmented regions of the emu oropharynx and was located in the connective tissue of the non-pigmented floor, tongue (see Chapter 5), lips of the glottis, the non-pigmented roof, rictus and pharyngeal folds. The environment and condition of the animal is reported to influence both the size and number of glands present in the oral and pharyngeal cavities (Tucker, 1958) and glands are best developed in birds with a dry diet, such as seed or insect eaters (King and McLelland, 1984). The emu has a varied di*et al*so consuming seeds and insects (Davies, 1978), thus there is a high gland density in the emu oropharynx. The glands in the greater rhea (Feder, 1972) and ostrich (Porchescu, 2007; Tivane, 2008) oropharynx are also abundant in comparable regions to those in the emu (see Chapter 2).

The nomenclature used to describe the grouping of avian salivary glands has been found in the past to be both inconsistent and confusing (Ziswiler and Farner, 1972). This is partly due to the fact that in birds the regions of glandular tissue tend to merge with one another (Tucker, 1958). Fahrenholz (1937) grouped the oropharyngeal glands of birds into: mandibular, lingual and

crico-arytenoid glands in the floor, and palatine and sphenopterygoid glands in the roof. Tucker (1958), alternatively, distinguishes the oral angular glands (often rudimentary), the palatine group consisting of palatine, median, lateral, anterior, posterior, internal and external glands, the intermandibular group consisting of anterior, posterior, external posterior and inferior posterior glands and the pharyngo-oesophageal group consisting of pterygoidal palatine, crico-arytenoidal and oesophageal glands. Thus the glandular regions in the emu oropharynx were grouped and named according to their location (Fig. 3.38). The following groups were recognised, namely, caudal intermandibular, lingual (see Chapter 5), crico-arytenoid, oral angular (buccal), caudal palatine and pharyngeal glands. The groups of glands identified in the greater rhea and ostrich were not named (Feder, 1972; Tivane, 2008). The two types of glands (large, simple branched tubular and small, simple tubular glands, see below) observed in the emu oropharynx differed in distribution. The caudal intermandibular glands were formed by both types of glands. The crico-arytenoid glands were composed of the large simple branched tubular type and the oral angular glands consisted of both types. The caudal palatine and pharyngeal glands consisted predominantly of the large simple branched tubular units with only a few simple tubular glands being present.

Two types of salivary glands were evident in the emu, namely, small simple tubular mucus-secreting glands (single and branched) and large simple branched tubular mucus-secreting glands, similar to those noted in the ostrich (Tivane, 2008). The glands in the greater rhea (Feder, 1972) were described as being tubulo-alveolar with typical mucus-secretory features. No further mention of size or details of their structure were provided (Feder, 1972). Hodges (1974) and McLelland (1979) state that the salivary glands of the oral and pharyngeal cavity in birds are compound tubular structures. Although large, the branched tubular glands seen in the emu did not reveal a complex duct system and were therefore not compound in nature. Tubular glands are the most common type found in birds with the alveolar type being the exception (Fahrenholz, 1937). The large glands manifested as the doughnut-shaped structures observed macroscopically with their openings to the surface the small central spot or depression (also noted by Gardner (1927) in other birds studied). The openings of the salivary glands of the chicken (King and McLelland, 1984) and ostrich (Tivane, 2008) are also seen as small openings macroscopically.

In birds, definitive salivary glands do not occur; instead they are replaced by collections of large numbers of simple and branched tubular mucus-secreting glands lined by large mucus cells (Banks, 1993). Thus the salivary glands of birds are mostly a collection of individual glands

combined in a glandular field forming a polystomatic gland (Fahrenholz, 1937). This situation is evident in the emu as well as in the greater rhea (Feder, 1972) and ostrich (Tivane, 2008). Monostomatic salivary glands are, however, found in poultry (Saito, 1965). Collections of glands with a single opening form a continuous layer in the connective tissue of the fowl (McLelland, 1975, 1979). In all the ratite species studied (emu, ostrich and greater rhea) all the glands were mucus-secreting only. The salivary glands in birds are most often tubular with the serous elements normally absent (Ziswiler and Farner, 1972), a feature also apparent in the ratites. The glands of the emu oropharynx compare to the similar simple branched tubular, tubulo-alveolar and alveolar mucus-secreting glands found in many birds (Calhoun, 1954; Warner *et al*., 1967; Hodges, 1974; McLelland, 1975, 1979; Samar *et al*., 1999).

The lumen of some of the large, simple branched glands of the pharyngeal folds in the emu displayed a pseudostratified ciliated columnar epithelium, presumably to assist in extrusion of mucus from the glands. The mucus secretions of the oropharyngeal glands apparently accumulate in the large lumen below the epithelium and moves to the surface through short ducts. Thus extrusion of viscid secretion may be due to the action of cilia, where present, as well as through pressure build-up of accumulated secretions. The large openings would offer little resistance to the passage of the secretions. Hodges (1974), notes that the presence of smooth muscle fibres around glands is disputed in birds. The large glands in the emu are surrounded by a clear connective tissue capsule with no evidence of smooth muscle (with the staining techniques used), a finding similar to that in the ostrich (Tivane, 2008). Connective tissue capsules around glands in other birds have also been noted (Warner *et al*., 1967; Hodges, 1974). However, in the quail (Warner *et al*., 1967) smooth muscle fibres were identified surrounding the glands in the oropharynx.

The main function of the salivary glands in birds is mucogenesis to form saliva (Ziswiler and Farner, 1972) which provides moisture and lubrication for food boli (Ziswiler and Farner, 1972; Nickel *et al*., 1977; King and McLelland, 1984; Gargiulo *et al*., 1991; Liman *et al*., 2001). Mucins are visco-elastic organic components of mucus formed by high molecular weight glycoproteins and coat all mucosal surfaces (Tabak *et al*., 1982). They provide protection from desiccation and mechanical damage, help maintain cellular water balance, provide lubrication and are antimicrobial in action (Tabak *et al*., 1982). Sticky saliva also assists in the backward propulsion of food and prevents regurgitation (McLelland, 1990). All these functions would be fulfilled by the mucus-secreting glands in the emu oropharynx.

3.4.1.3.2 Herbst corpuscles

Herbst corpuscles were located throughout the oropharynx of the emu, except for the pharyngeal folds, and in the glandular regions were mostly associated with the large glands. Sensory corpuscles occur in the roof of the greater rhea (Feder, 1972) and the oropharynx (excepting the tongue) of the ostrich (Tivane, 2008). Herbst corpuscles occur in the beak of ducks and geese, the oral cavity, tongue, subcutaneous connective tissue, muscles and adjacent to joints. In the deep dermis they are found in the legs, beak and feathered skin (Gottschaldt, 1985). Their presence in the oropharynx of many birds has also been confirmed (Wight *et al*, 1970; Ziswiler and Farner, 1972; Hodges, 1974; Berkhoudt, 1979).

The dermis (corium) of the bill skin in the emu was aglandular and contained numerous Herbst corpuscles. Herbst corpuscles have also been found in the bill skin of domestic poultry (Calhoun, 1954; Warner *et al*., 1967; Berkhoudt, 1979) and the bill of the kiwi (Cunningham *et al*., 2007). In the keratinised, aglandular regions of the emu oropharynx, the corpuscles were situated near the base of the connective tissue layer and were mostly single but sometimes occurred in groups or chains. In comparison to the rest of the structures and regions of the emu oropharynx, the corpuscles were mainly concentrated in the pigmented roof. In the ostrich (Tivane, 2008) the median palatine ridge was a very pronounced structure in comparison to that in the emu (see Chapter 2). Herbst corpuscles were concentrated in this ridge, as well as in the mucosal ridges on the floor of the oropharynx (Tivane, 2008). However, no such concentration of corpuscles was noted in the emu median palatine ridge. In contrast, they were evenly distributed throughout the pigmented roof, and the median ridge/s on the oropharyngeal floor (present in the ostrich [Tivane, 2008]), were absent in the emu. The corpuscles decreased in number in the non-keratinised (non-pigmented glandular) regions of the oropharynx, a finding similar to that in the greater rhea (Feder, 1972) and the ostrich (Tivane, 2008).

The connective tissue encapsulating the avian Herbst corpuscle is reported to be continuous with the perineurium of the nerve fibre supplying it and the lamellae consist of delicate connective tissue (Nickel *et al*., 1977). The continuity between the Herbst corpuscle capsule and the perineurium of the associated nerve could not be demonstrated in the emu material studied. The structure of the Herbst corpuscles in the oropharynx of the emu was similar to those identified in the tongue (Crole and Soley, 2008; Chapter 5) and in the ostrich oropharynx (Tivane, 2008). The

emu Herbst corpuscle is also similar to those described in the chicken (Cobb and Bennet, 1970; Wight, 1970; Hodges, 1974; Dimitrov, 2003). Gottschaldt (1985) provides a review of the earlier literature, as well as a description of Herbst corpuscles; from this it is apparent that the emu Herbst corpuscle, at the light microsopic level, appears similar to other avian Herbst corpuscles. A more detailed comparative study of these structures, however, will be needed to clarify this situation.

3.4.1.3.3 Lymphoid tissue

In the emu oropharynx, lymphoid tissue was located in the connective tissue of the caudal non-pigmented glandular interramal region, the tongue (see Chapter 5), the rictus, the junction of the pigmented and non-pigmented roof, the mucosal folds lateral to the choana, the infundibular cleft and pharyngeal folds and was mainly associated with the glands present in these regions. The association of lymphoid tissue with glands has been noted in the ostrich (Tivane, 2008) and in other birds (Calhoun, 1954; Warner *et al*., 1967; Hodges, 1974). Lymphoid tissue is abundant in the oropharynx of birds (Rose, 1981) and is especially concentrated in the pharyngeal region (Barge, 1937; Nickel *et al*., 1977; McLelland, 1979) where it has been termed the *lymphonoduli pharyngeales* (Rose, 1981; Rautenfeld, 1993). The pharyngeal folds in the emu represented the *lymphonoduli pharyngeales* (pharyngeal tonsils).

Lymphoid tissue occurred in the emu oropharynx as numerous areas or patches of diffuse lymphoid tissue, some of which featured nodular concentrations. The occurrence of both diffuse and nodular lymphoid tissue was noted in the ostrich (Tivane, 2008) as well as in other birds (Ziswiler and Farner, 1972; Nickel *et al*., 1977). Nodular lymphoid tissue was mainly seen in the rictus, the mucosal folds lateral to the choana and the pharyngeal folds. Each pharyngeal fold in the emu demonstrated a small protrusion of tissue on its caudo-lateral edge. This tissue was almost entirely lymphoid in nature (composed of both diffuse and nodular tissue). This feature of the emu pharyngeal fold is unique amongst the ratites.

Lymphocytes constitute the main component of lymphoid tissue, with the T-lymphocytes being responsible for cell mediated immune responses and the B-lymphocytes, which synthesize and secrete antibodies after transforming to plasma cells, providing humoral immunity (Rose, 1981).

3.4.2 Proximal cervical oesophagus

As previously described (Herd, 1985, and confirmed in the present study), the oesophagus of the emu is composed, in sequence, of a stratified squamous epithelium overlying a loose connective tissue *lamina propria* containing glands, a longitudinal *muscularis mucosae*, a thin submucosa and a broad inner circular and thin outer longitudinal external muscle layer. Additional to the description of Herd (1985), it was noted in the present study that the epithelium is non-keratinised, the glandular tissue is composed of tubular and simple branched tubular mucus-secreting glands (PAS-positive staining), lymphoid tissue is present in the *lamina propria*, the muscle layers are composed of smooth muscle and the outermost layer is the *tunica adventitia*. It is unclear from the study of Feder (1972) and Herd (1985) which part of the oesophagus was sampled in the greater rhea and emu respectively, however, the results from this study show the proximal oesophagus of the emu to be similar to the results of Herd (1985).

The oesophagus of the greater rhea (Feder, 1972) and ostrich (Tivane, 2008) is also lined by a non-keratinised stratified squamous epithelium, as in the emu (present study). This is a feature common to most birds (Pernkopf and Lehner, 1937; Calhoun, 1954; Warner *et al*., 1967; Hodges, 1974; McLelland, 1975; Bacha and Bacha, 2000). However, in some birds this epithelium may be partially or completely keratinised (Koch, 1973; King and McLelland, 1984; McLelland, 1990), a feature not seen in the emu (present study). Fowler (1991) states that the ratite oesophagus appears cornified, but as indicated above, the epithelium in the emu remains uncornified. In the hatchling greater rhea, sheets of ciliated columnar epithelium, in the process of sloughing, were observed on the stratified squamous epithelium (Feder, 1972). Although ciliated cells were seen elsewhere in the oropharynx, ciliation was never observed in the emu oesophagus.

Taste buds were found in the proximal oesophagus of the emu. This is the first report of such structures in the ratite oesophagus. They had the typical appearance of those described for birds (Botezat, 1910; Moore and Elliott, 1946; Gentle, 1971b; Nickel *et al*., 1977; Lindenmaier and Kare, 1959; Warner *et al*., 1967) and were similar to those identified in the emu oropharynx (see above). The presence of taste buds in this segment of the emu upper digestive tract is probably not unusual as in the eating method employed by this bird (Bonga Tomlinson, 2000; Gussekloo and Bout, 2005) the oesophagus is one of the first areas to receive ingesta. Thus food selection by taste in the emu may most likely occur after swallowing (see above).

Despite the occurrence of large amounts of lymphoid tissue in the avian oesophagus (Pernkopf and Lehner, 1937) it is only mentioned in a few studies (Warner *et al*., 1967; Banks, 1993). Lymphoid tissue in the oesophagus is termed *lymphonoduli oesophageales* (Rose, 1981), however, its actual existence in birds is questioned by Rautenfeld (1993). Although specific aggregations of lymphoid tissue are formed in the oesophagus of the emu, they do not constitute oesophageal tonsils. Both the ostrich (Tivane, 2008) and the emu display lymphoid tissue in the oesophagus. The lymphoid tissue of the emu was mainly composed of diffuse lymphoid tissue and was situated in the *lamina propria* in association with the glands. This tissue would obviously imply an immunological function for the oesophagus, as in the oropharynx.

A prominent feature of the avian oesophagus is the presence of numerous simple tubular mucus-secreting glands, also noted in the ostrich (Tivane, 2008) and greater rhea (Feder, 1972). In the emu, the oesophageal glands are situated in the *lamina propria* (Herd, 1985) (although much of their length is enclosed in the epithelial lining) (present study) into which they extend for only a short distance, a feature similar to that in the ostrich (Porchescu, 2007; Tivane, 2008) and greater rhea (Feder, 1972). This is in contrast to mammals where glands are situated in the submucosa (Ross *et al*., 2003). In birds the oesophageal glands are noted to lie in the *tunica mucosae* (Ziswiler and Farner, 1972) or more specifically, the *lamina propria* (McLelland, 1975). In the emu the glands are simple tubular, sometimes branched, mucus-secreting (PAS-positive) glands. Oesophageal glands of other birds have been reported to range from tubular to alveolar (Ziswiler and Farner, 1972), mainly alveolar with some branching (Warner *et al*., 1967) or branched (Koch, 1973). Hodges (1974) notes that in the chicken, the same type of glands found in the oropharynx occur in the oesophagus. The simple tubular glands also occurred in the oropharynx (see above) and tongue (see Chapter 5) in the emu.

The *muscularis mucosae* in the emu represented the thickest layer of the oesophagus and consisted of longitudinally oriented (Herd, 1985; present study) smooth muscle fibres, a feature noted in the greater rhea (Feder, 1972) and ostrich (Tivane, 2008). This appears to be a general feature of the avian oesophagus (Calhoun, 1954; Warner *et al*., 1967; Ziswiler and Farner, 1972; Hodges, 1974; Gussekloo, 2006). In the greater rhea (Feder, 1972) and ostrich (Tivane, 2008) oesophagus the *muscularis mucosae* was present in the longitudinal folds of the mucosa, a feature not noted in the emu. However, Tivane (2008) reported that the folds of the proximal oesophagus in the ostrich were lower than those situated more distally and that the *muscularis*

mucosae was only present in the larger folds. Thus the presence of the *muscularis mucosae* in the larger folds of the distal oesophagus of the emu cannot be ruled out.

The submucosa in the emu oesophagus was weakly developed (Herd, 1985; present study) and was situated between the *muscularis mucosae* and *tunica muscularis*. It was composed of a loosely arranged irregular dense connective tissue and carried blood vessels and nerves (submucosal plexus). This finding is similar to that in the greater rhea (Feder, 1972) and ostrich (Tivane, 2008) as well as in other birds (Calhoun, 1954; Warner *et al*., 1967; Ziswiler and Farner, 1972; Hodges, 1974; Gussekloo, 2006).

In the emu, the *tunica muscularis* was composed of a thicker inner circular and thinner outer longitudinal (Herd, 1985; present study) smooth muscle layer and was surrounded by the loose irregular connective tissue of the adventitia. Both of these layers were similar to those described for the greater rhea (Feder, 1972) and ostrich (Tivane, 2008). The features of the *tunica muscularis* and adventitia of the emu were also typical for those described in other birds (Pernkopf and Lehner, 1937; Calhoun, 1954; Warner *et al*., 1967; Ziswiler and Farner, 1972; Hodges, 1974; Banks, 1993; Gussekloo, 2006). Although Owen (1879) reported that the oesophagus of the kiwi contained an outer circular and inner longitudinal layer, the uniformity of the layers of the muscular tunic described in ratites and other birds (see above) would make it seem unlikely that this arrangement would differ in the kiwi.

3.4.3 Scanning electron microscopy

The description of surface features was based mainly on observations of the 5 month-old specimen, although the basic features observed were consistent with those of the older birds. The main difference appeared to be an increase in cell sloughing in the older birds and the replacement of large areas of cell surfaces displaying microvilli (young bird) by surfaces displaying microplicae (older birds).

The SEM findings for the oropharyngeal floor of the emu revealed a difference in appearance of the keratinised and non-keratinised surfaces noted histologically. The keratinised region displayed sheets of desquamating cells whereas the non-keratinised region displayed individual desquamating cells. Individual desquamating cells were also a feature noted in the oropharynx and oesophagus of the ostrich (Tivane, 2008).

In the emu, higher magnification of the surfaces studied in the oropharynx and proximal oesophagus, revealed 4 different cell surface features, namely: microridges, microplicae, microvilli and cilia. Microridges were present on the surface cells of the keratinised areas only. In the non-keratinised regions, microplicae were present on cells free of microvilli and cilia as well as on the raised round cells of the pharyngeal folds. Microvilli were present on cells lining all small gland openings and large gland openings or parts there of. Microvilli also adorned cell surfaces in areas surrounding the gland openings and the luminal surface of the proximal oesophagus. Cilia were present in isolated patches in the ducts of gland openings and in the vicinity of the openings. No specialised features were noted for the ostrich (Tivane, 2008).

The openings seen in all regions of the emu oropharynx represented the underlying glands. Large openings represented those of the large simple branched tubular mucus-secreting glands whereas the small openings represented the simple tubular mucus-secreting glands. Both large and small openings were often filled with cellular debris and mucus-secretions from the underlying glands. In the ostrich (Tivane, 2008) only one type of opening was described (which also represented underlying glands) and showed similar features to that of the large gland openings observed in the emu.

Although only simple tubular (and sometimes branched) glands were identified histologically in the proximal oesophagus, both large and small openings were observed on the luminal surface using SEM. The small openings were more numerous and represented the simple tubular glands seen histologically, a feature also noted in the ostrich (Tivane, 2008). However, it was not possible to ascertain what type of underlying glands the large openings represented. Following the pattern seen in the oropharynx, it may be possible that the large openings represent large, simple branched tubular glands, such as those commonly seen in the oropharynx, or are merely enlarged openings of the simple tubular glands. However, the large, simple branched tubular glands were not observed histologically.

Although taste buds were identified histologically, structures typically representing taste buds were not resolved by SEM. However, due to the relatively small areas sampled for SEM and the scarcity of taste buds in the emu oropharynx, this study does not rule out the possibility of these structures being identified by SEM. Another possibility could be that the taste buds may be difficult to visualise due to their size and morphological characteristics, and may possibly even

be masked by mucus-secretions or desquamating cells. Further studies, incorporating larger tissue samples will be needed to positively identify these structures in the oropharynx and proximal oesophagus of the emu using this technique.

3.5 REFERENCES

BACHA, W.J. & BACHA, L.M. 2000. Digestive System, in *Color Atlas of Veterinary Histology,* edited by D. Balado. Philadelphia: Lippincott Williams & Wilkins: 121-157.

BANKS, W.J. 1993. Comparative organology, in *Applied Veterinary Histology,* edited by R.W. Reinhardt. St. Louis: Mosby-Year Book, Inc.: 356-360.

BARGE, J.A.J. 1937. Mundhöhlendach und Gaumen, in *Handbuch der vergleichenden Anatomie der Wirbeltiere,* edited by L. Bolk, E. Göppert, E. Kallius & W. Lubosch. Berlin: Urban and Schwarzenberg: 29-48.

BAUMEL, J.J., KING, A.S., BREAZILE, J.E., EVANS, H.E. & VANDEN BERGE, J.C. 1993. *Handbook of Avian Anatomy: Nomina Anatomica Avium*. Second Edition. Cambridge, Massachusetts: Nuttall Ornithological Club.

BERKHOUDT, H. 1979. The morphology and distribution of cutaneous mechanoreceptors (Herbst and Grandry corpuscles) in bill and tongue of the mallard (*Anas Platyrhynchos* L.). *Netherlands Journal of Zoology,* 30:1-34.

BERKHOUDT, H. 1985. Structure and function of avian taste buds, in *Form and Function in Birds.* Volume 3, edited by A.S. King & J. McLelland. London: Academic Press: 463-491.

BONGA TOMLINSON, C.A. 2000. Feeding in paleognathus birds, in *Feeding: Form, Function, and Evolution in Tetrapod Vertebrates,* edited by K. Schwenk. San Diego: Academic Press: 359-394.

BOTEZAT, E. 1910. Morphologie, Physiologie und phylogenetische Bedeutung der Geschmacksorgane der Vögel. *Anatomischer Anzeiger,* 36:428-461.

CALHOUN, M.L. 1954. *Microscopic Anatomy of the Digestive System of the Chicken*. Ames, Iowa: Iowa State College Press.

CHO, P., BROWN, B. & ANDERSON, M. 1984. Comparative gross anatomy of ratites. *Zoo Biology,* 3:133-144.

COBB, J.L.S. & BENNET, T. 1970. Herbst corpuscles in the smooth muscles in the wings of chicks. *Experientia*, 26:768-769.

CROLE, M.R. & SOLEY, J.T. 2008. Histological structure of the tongue of the emu (*Dromaius novaehollandiae*). *Proceedings of the Microscopy Society of Southern Africa,* 38:63.

CUNNINGHAM, S., CASTRO, I. & ALLEY, M. 2007. A new prey-detection mechanism for kiwi (*Apteryx spp.*) suggests convergent evolution between paleognathous and neognathous birds. *Journal of Anatomy,* 211:493-502.

CUVIER, G. 1836. *Leçons d'anatomie comparée.* Third Edition. Volumes 1 & 2, edited by M. Duméril. Bruxelles: Dumont.

DAVIES, S.J.J.F. 1978. The food of emus. *Australian Journal of Ecology,* 3:411-422.

DIMITROV, D. 2003. Encapsulated Nerve endings in the lachrymal glands of broiler chickens – A light microscopic study. *Trakia Journal of Sciences*, 1:38-41.

DUERDEN, J.E. 1912. Experiments with ostriches XVIII. The anatomy and physiology of the ostrich. A. The external characters. *Agricultural Journal of the Union of South Africa,* 3:1-27.

FAHRENHOLZ, C. 1937. Drüsen der Mundhöle. In: *Handbuch der vergleichenden Anatomie der Wirbeltiere*, edited by L. Bolk, E. Göppert, E. Kallius & W. Lubosch. Berlin: Urban and Schwarzenberg: 115-206.

FARAGGIANA, R. 1933. Sulla morfologia della lingua e del rialzo laringeo di alcune specie di uccelli Ratiti e Carenati non comuni. *Bollettino dei Musei di Zoologia e Anatomia comparata,* 43:313-323.

FEDER, F-H. 1972. Zur mikroskopischen Anatomie des Verdauungsapparates beim Nandu (*Rhea americana*). *Anatomischer Anzeiger,* 132:250-265.

FOWLER, M.E. 1991. Comparative clinical anatomy of ratites. *Journal of Zoo and Wildlife Medicine,* 22:204-227.

GADOW, H. 1879. Versuch einer vergleichenden Anatomie des Verdauungssystemes der Vögel. *Jenaische Zeitschrift für Medizin und Naturwissenschaft,* 13:92-171.

GARDNER, L.L. 1926. The adaptive modifications and the taxonomic value of the tongue in birds. *Proceedings of the United States National Museum,* 67:Article 19.

GARDNER, L.L. 1927. On the tongue in birds. *The Ibis,* 3:185-196.

GARGIULO, A.M., LORVIK, S., CECCARELLI, P. & PEDINI, V. 1991. Histological and histochemical studies on the chicken lingual glands. *British Poultry Science,* 32:693-702.

GENTLE, M.J. 1971a. Taste and its importance to the domestic chicken. *British Poultry Science,* 12:77-86.

GENTLE, M.J. 1971b. The lingual taste buds of *Gallus domesticus*. *British Poultry Science,* 12:245-248.

GOTTSCHALDT, K.-M. 1985. Structure and function of avian somatosensory receptors, in *Form and Function in Birds.* Volume 3, edited by A.S. King & J. McLelland. London: Academic Press: 375-462.

GUSSEKLOO, S.W.S. 2006. Feeding structures in birds, in *Feeding in Domestic Vertebrates: From Structure to Behaviour*, edited by V. Bels. Wallingford, UK: CABI Publishing: 14-19.

GUSSEKLOO, S.W.S. & BOUT, G.R. 2005. The kinematics of feeding and drinking in palaeognathous birds in relation to cranial morphology. *Journal of Experimental Biology,* 208:3395-3407.

HERD, R.M. 1985. Anatomy and histology of the gut of the emu *Dromaius novaehollandiae*. *Emu,* 85:43-46.

HODGES, R.D. 1974. The digestive system, in *The Histology of the Fowl*. London: Academic Press: 35-47.

KARE, M.R. & ROGERS, J.G. 1976. Sense organs. Taste, in *Avian Physiology*, edited by P.D. Sturkie. Berlin: Springer-Verlag.

KING, A.S. & MCLELLAND, J. 1984. Digestive system, in *Birds – Their Structure and Function.* Second edition. London: Bailliere Tindall: 86-87.

KOCH, T. 1973. Splanchnology, in *Anatomy of the Chicken and Domestic Birds*, edited by B.H. Skold & L. DeVries. Ames, Iowa: The Iowa State University Press: 68-69.

LIMAN, N., BAYRAM, G. & KOÇAK, M. 2001. Histological and histochemical studies on the lingual, preglottal and laryngeal salivary glands of the Japanese quail (*Coturnix coturnix japonica*) at the post-hatching period. *Anatomia*, 30:367-373.

LINDENMAIER, P. & KARE, M.R. 1959. The taste end-organs of the chicken. *Poultry Science*, 38:545-549.

MCCANN, C. 1973. The tongues of kiwis. *Notornis*, 20:123-127.

MCLELLAND, J. 1975. Aves digestive system, in *Sisson and Grossman's The Anatomy of the Domestic Animals*, edited by C.E. Rosenbaum, N.G. Ghoshal & D. Hillmann. Philadelphia: W.B. Saunders Company: 1857-1867.

MCLELLAND, J. 1979. Digestive system, in *Form and Function in Birds.* Volume 1, edited by A.S. King & J. McLelland. San Diego, California: Academic Press: 69-92.

MCLELLAND, J. 1990. Digestive system, in *A Colour Atlas of Avian Anatomy*, edited by J. McLelland. Aylesbury, England: Wolfe Publishing Ltd.: 47-49.

MCMANUS, J.F.A. 1946. Histological demonstration of mucin after periodic acid. *Nature (London)*, 158:202.

MECKEL, J.F. 1829. *System der vergleichenden Anatomie*. Halle: Der Rehgerschen Buchhandlung.

MOORE, D.A. & ELLIOTT, R. 1946. Numerical and regional distribution of taste buds on the tongue of the bird. *Journal of Comparative Neurology*, 84:119-131.

NICKEL, R., SCHUMMER, A. & SEIFERLE, E. 1977. Digestive system, in *Anatomy of the Domestic birds*. Berlin: Verlag Paul Parey: 40-50.

OWEN, R. 1879. *Memoirs on the extinct and wingless birds of New Zealand; with an appendix of those of England, Australia, Newfoundland, Mauritius and Rodriguez.* Volume 1. London: John van Voorst.

PERNKOPF, E. & LEHNER, J. 1937. Vorderdarm. A. Vergleichende Beschreibung des Vorderdarmes bei den einzelnen Klassen der Kranoten. In: *Handbuch der vergleichenden Anatomie der Wirbeltiere*. edited by L. Bolk, E. Göppert, E. Kallius & W. Lubosch. Berlin: Urban and Schwarzenberg: 349-559.

PORCHESCU, G. 2007. Comparative morphology of the digestive tract of the black African ostrich, hen and turkey. PhD thesis, Agrarian State University of Moldova.

PYCRAFT, W.P. 1900. On the morphology and phylogeny of the palaeognathae (*Ratitae and Crypturi*) and neognathae (*Carinatae*). *Transactions of the Zoological Society of London,* 15:149-290.

RAUTENFELD, D.B.V. 1993. Systema lymphaticum et splen [Lien], in *Handbook of Avian Anatomy: Nomina Anatomica Avium.* Second Edition, edited by J.J. Baumel, A.S. King, J.E. Breazile, H.E. Evans & J.C. Vanden Berge. Cambridge, Massachusetts: Nuttall Ornithological Club: 477-492.

ROSE, M.E. 1981. Lymphatic system, in *Form and Function in Birds.* Volume 2, edited by A.S. King & J. McLelland. London: Academic Press: 341-372.

ROSS, M.H., KAYE, G.I. & PAWLINA, W. 2003. Digestive System II: Esophagus and Gastrointestinal Tract, in *Histology. A Text and Atlas.* Fourth Edition. Philadelphia: Lippincott Williams & Wilkins: 474-531.

SAITO, I. 1965. Comparative anatomical studies of the oral organs of the poultry. IV. Macroscopical observation of the salivary glands. *Bulletin of the Faculty of Agriculture, Miyazaki University*, 12:110-120.

SAMAR, M.E., AVILA, R.E., DE FABRO, S.P., PORFIRIO, V., ESTEBAN, F.J., PEDROSA, J.A. & PEINADO, M.A. 1999. Histochemical study of Magellanic penguin (*Spheniscus*

magellanicus) minor salivary glands during postnatal growth. *Anatomical Record*, 254:298-306.

TABAK, L., LEVINE, M., MANDEL, I. & ELLISON, S. 1982. Role of salivary mucins in the protection of the oral cavity. *Journal of Oral Pathology*, 11:1-17.

TIVANE, C. 2008. A Morphological Study of the Oropharynx and Oesophagus of the Ostrich (*Struthio camelus*). MSc dissertation, University of Pretoria, South Africa.

TUCKER, R. 1958. Taxonomy of the salivary glands of vertebrates. *Systematic Zoology*, 7:74-83.

WARNER, R.L., MCFARLAND, L.Z. & WILSON, W.O. 1967. Microanatomy of the upper digestive tract of the Japanese quail. *American Journal of Veterinary Research*, 28:1537-1548.

WIGHT, P.A.L., SILLER, W.G. & MACKENZIE, G.M. 1970. The distribution of Herbst corpuscles in the beak of the domestic fowl. *British Poultry Science*, 11:165-170.

ZISWILER, V. & FARNER, D.S. 1972. Digestion and the digestive system, in *Avian Biology*, edited by D.S. Farner, J.R. King & K.C. Parkes. New York: Academic Press: 344-354.

3.6 FIGURES

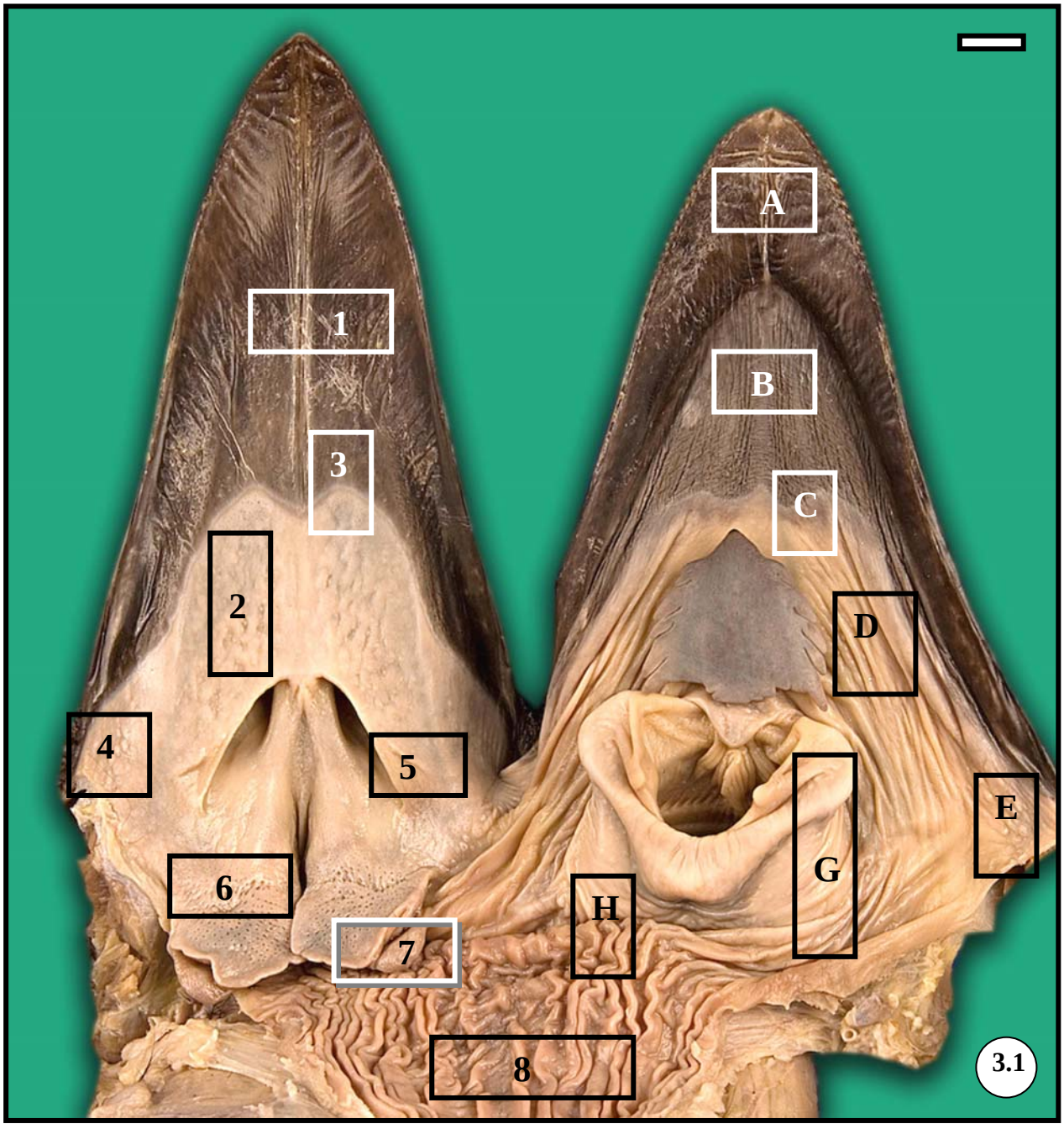


Figure 3.1: Emu head opened and the two halves reflected to show the areas sampled for light microscopy.

Floor of the oropharynx: The pigmented mandibular rhamphotheca (A), the pigmented rostral interramal region (B), the area of transition (C), the caudal non-pigmented interramal region (D), the mandibular rictus (E), the laryngeal mound (G) and the transition to the oesophagus (H).

Roof of the oropharynx: The pigmented roof (1), the non-pigmented roof (2), the transitional area (3), the maxillary rictus (4), the mucosal flap lateral to the caudal choana (5), rostral attached pharyngeal fold (6), caudal free pharyngeal fold and the caudo-lateral projection (7), proximal oesophagus (8). Bar = 5mm.

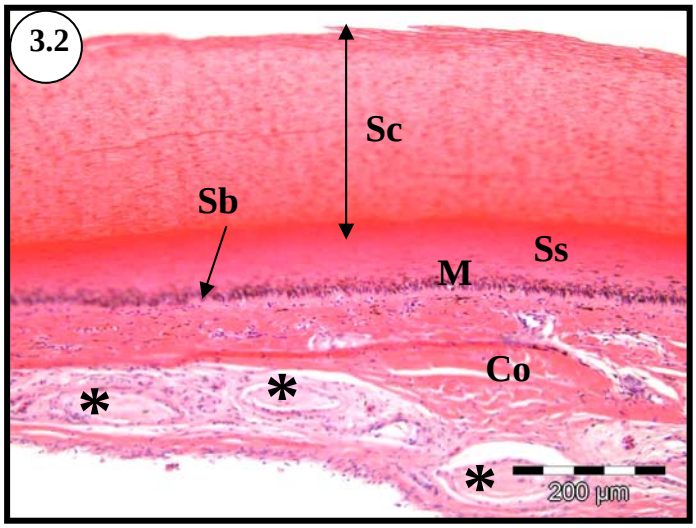


Figure 3.2: The mandibular bill skin showing the S*tratum corneum* (Sc) (*rhamphotheca*) overlying the *str. spinosum* (Ss) and *Str. basale* (Sb). The corium (Co) houses Herbst corpuscles (*). Melanocytes (M) are concentrated in the *Str. basale*.

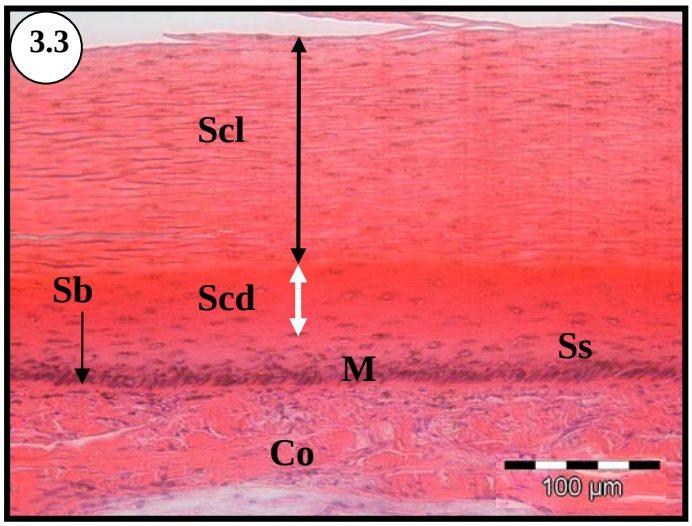


Figure 3.3: The *rhamphotheca* of the mandibular bill skin formed by the dark (Scd) and light regions of the *Str. corneum* (Scl). S*tr. spinosum* (Ss), *Str. basale* (Sb), melanocytes (M) and corium (Co).

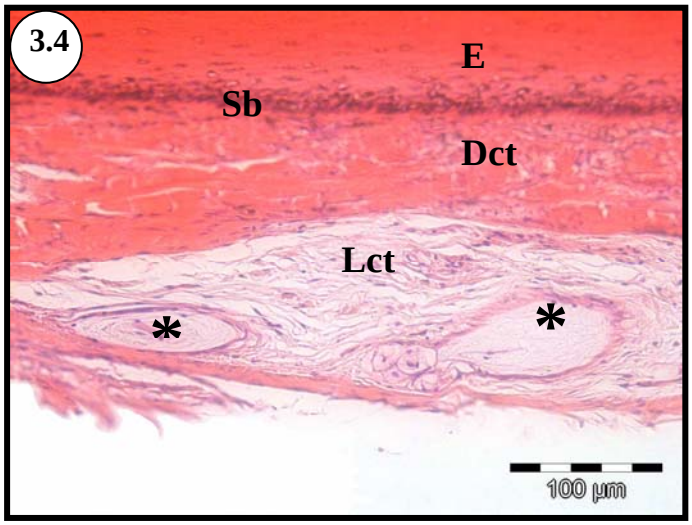


Figure 3.4: The corium of the mandibular bill skin displaying the dense connective tissue (Dct) typical of this layer and an area of loose connective tissue (Lct) housing Herbst corpuscles (*). Epithelium (E), *Str. basale* (Sb) with melanocytes (dark line).

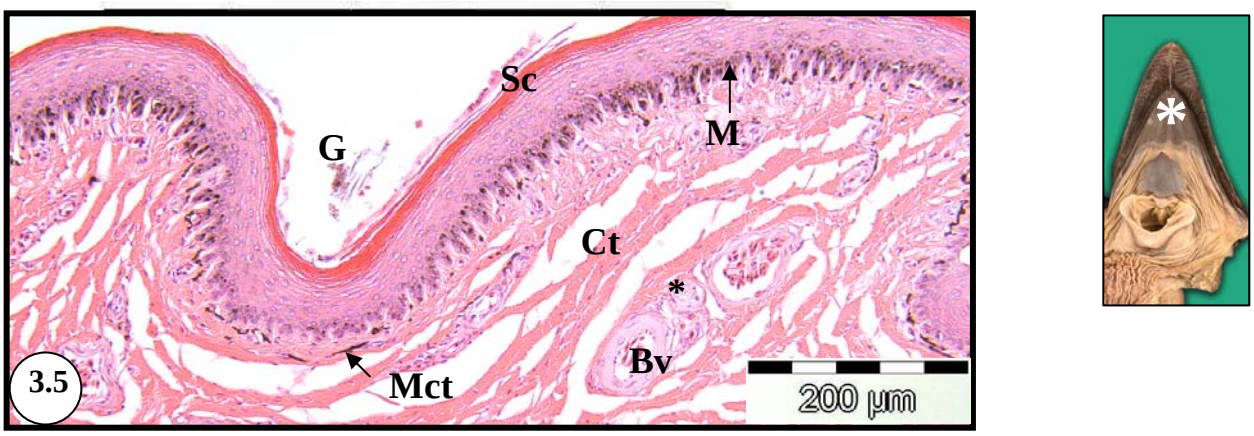


Figure 3.5: Two folds and an intervening groove (G) in the rostral pigmented interramal region. *Str. corneum* (Sc), melanocytes (M) in the *Str. basale*, melanocytes in connective tissue (Mct), connective tissue (Ct), blood vessel (Bv) and nerve (*).

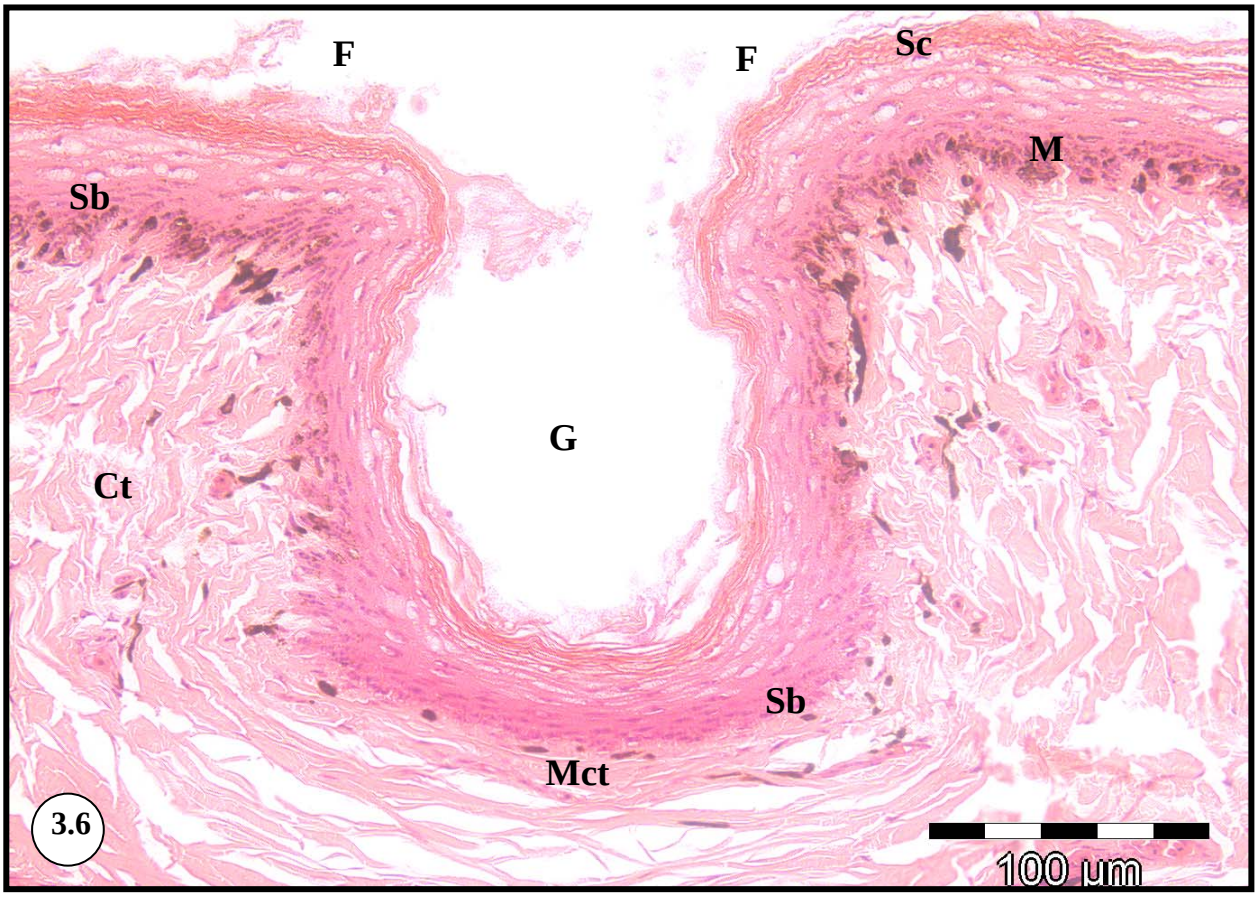
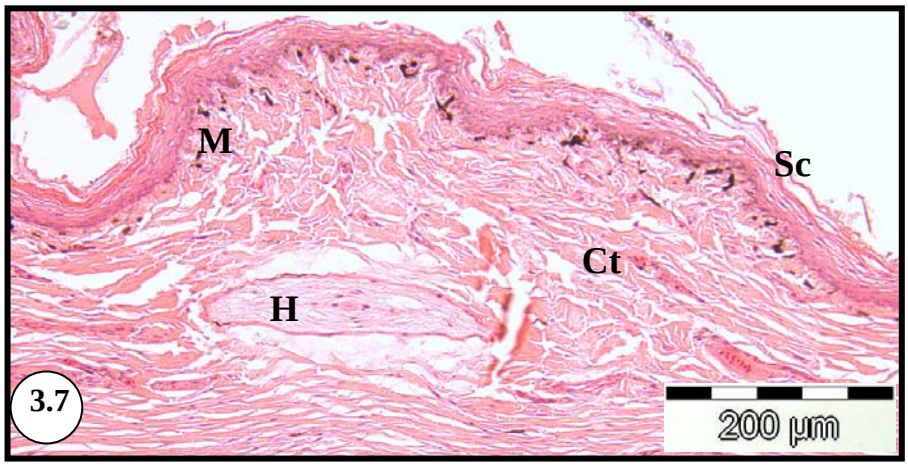


Figure 3.6: Two folds and an intervening groove (G) in the rostral pigmented interramal region. Note the concentration of melanocytes (M) in the *Str. basale* (Sb) of the fold (F), their disappearance from this layer in the groove and their presence (Mct) restricted to the underlying connective tissue (Ct). *Str. corneum* (Sc).



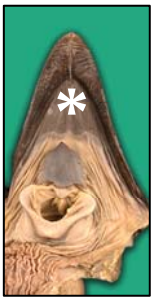
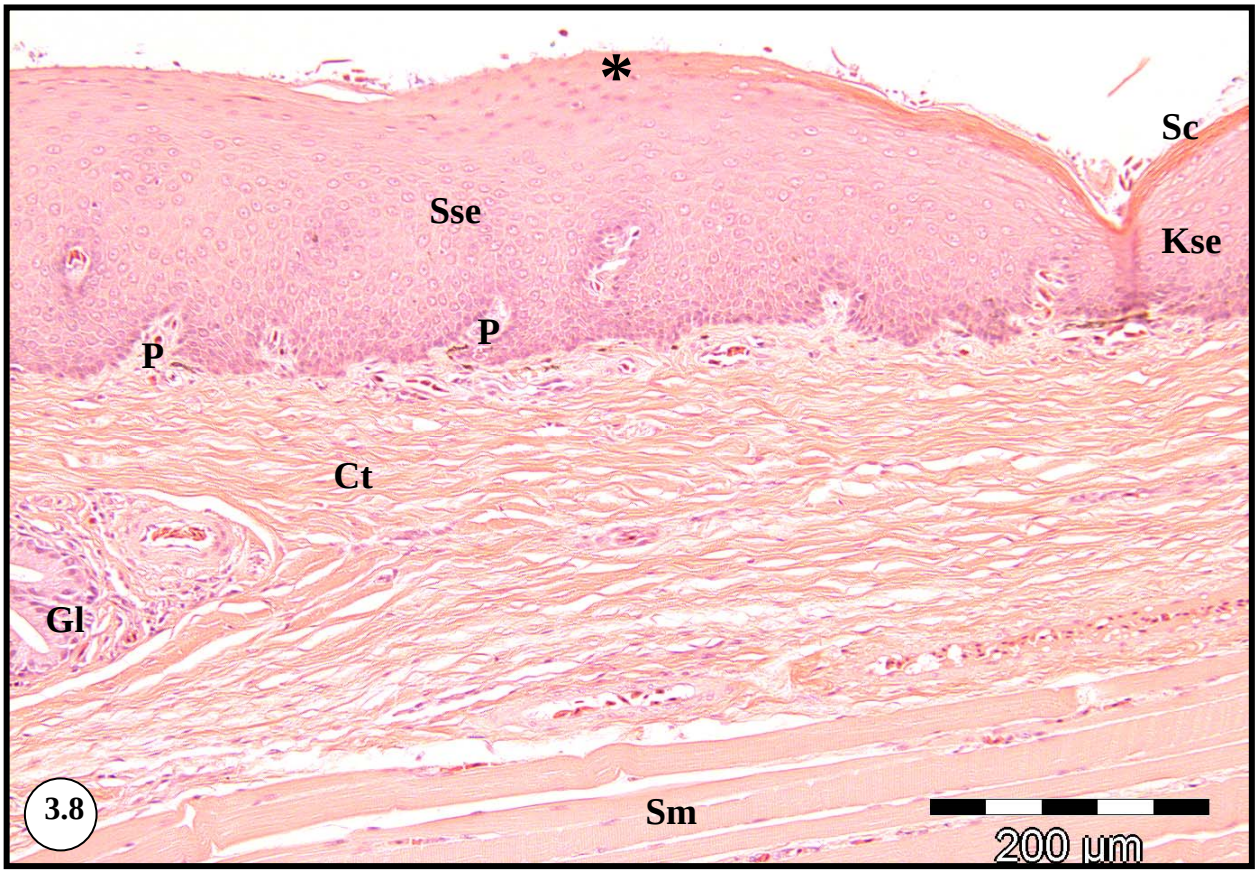


Figure 3.7: A Herbst corpuscle (H) in the connective tissue (Ct) of the rostral pigmented interramal region. Note the desquamation of the *Str. corneum* (Sc). Melanocytes (M).



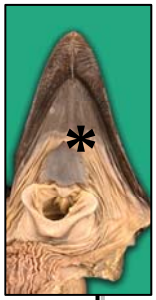


Figure 3.8: Floor of the oropharynx showing the zone of transition from the keratinised stratified squamous epithelium (Kse) to the thicker non-keratinised stratified squamous epithelium (Sse). Note the attenuation of the keratinised *Str. corneum* (Sc) and its eventual disappearance (*) as well as the appearance of connective tissue papillae (P) and glands (Gl) in the non-keratinised region. Connective tissue (Ct), skeletal muscle (Sm).

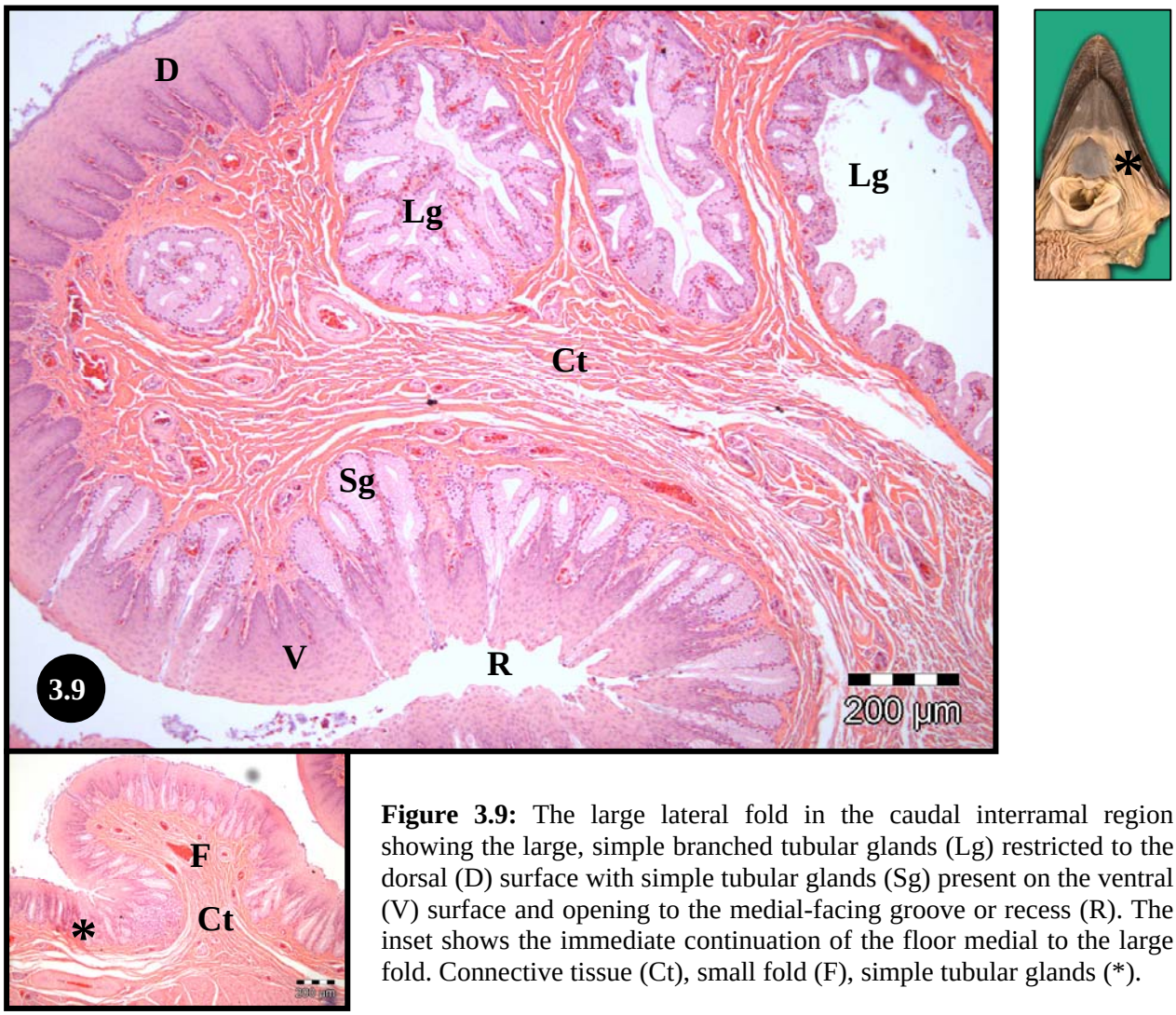


Figure 3.9: The large lateral fold in the caudal interramal region showing the large, simple branched tubular glands (Lg) restricted to the dorsal (D) surface with simple tubular glands (Sg) present on the ventral (V) surface and opening to the medial-facing groove or recess (R). The inset shows the immediate continuation of the floor medial to the large fold. Connective tissue (Ct), small fold (F), simple tubular glands (*).

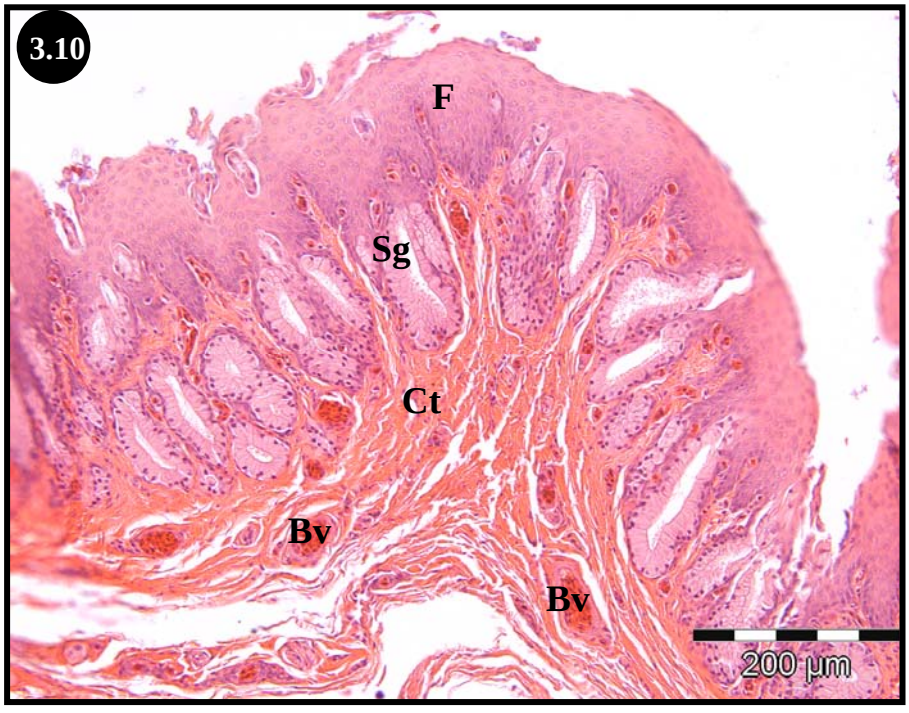


Figure 3.10: Enlargement of a similar area to that shown in the inset in Fig. 3.9. The fold (F) of the caudal interramal region displays simple tubular mucus-secreting glands (Sg) only. Note the numerous blood vessels (Bv) within the underlying connective tissue (Ct).

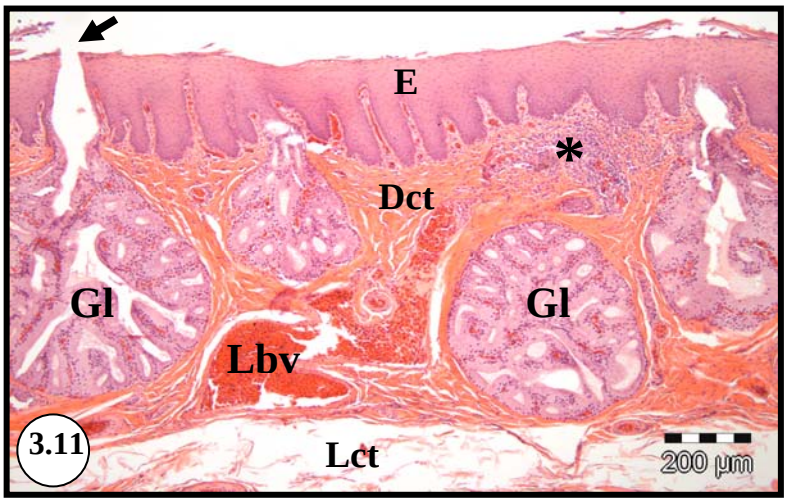
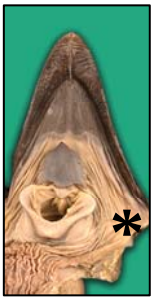


Figure 3.11: Mandibular rictus displaying large, simple branched tubular glands (Gl), diffuse lymphoid tissue (*) and large blood vessels (Lbv) in the underlying dense connective tissue (Dct). Note the gland opening (arrow) coursing through the epithelium (E) and the regular, deep connective tissue papillae. Loose connective tissue (Lct).



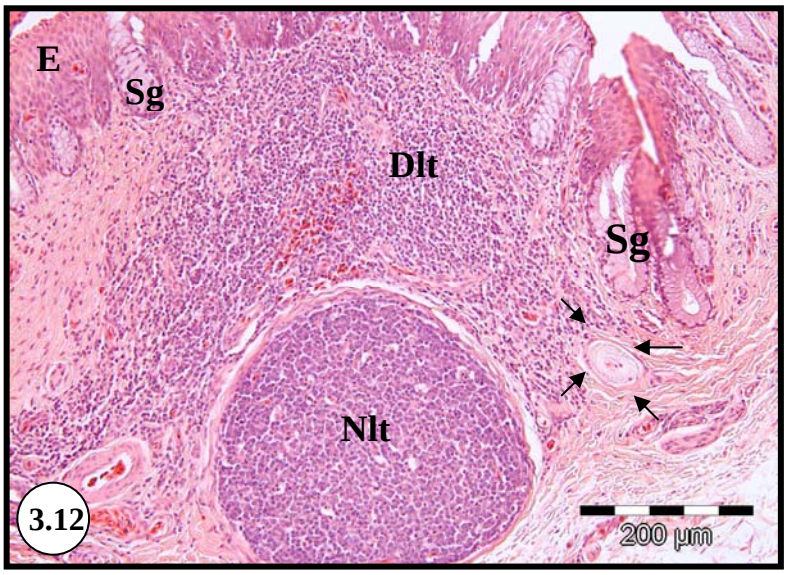


Figure 3.12: A collection of diffuse lymphoid tissue (Dlt) and nodular lymphoid tissue (Nlt), simple tubular glands (Sg) and a Herbst corpuscle (arrows) in the mandibular rictus. Epithelium (E).

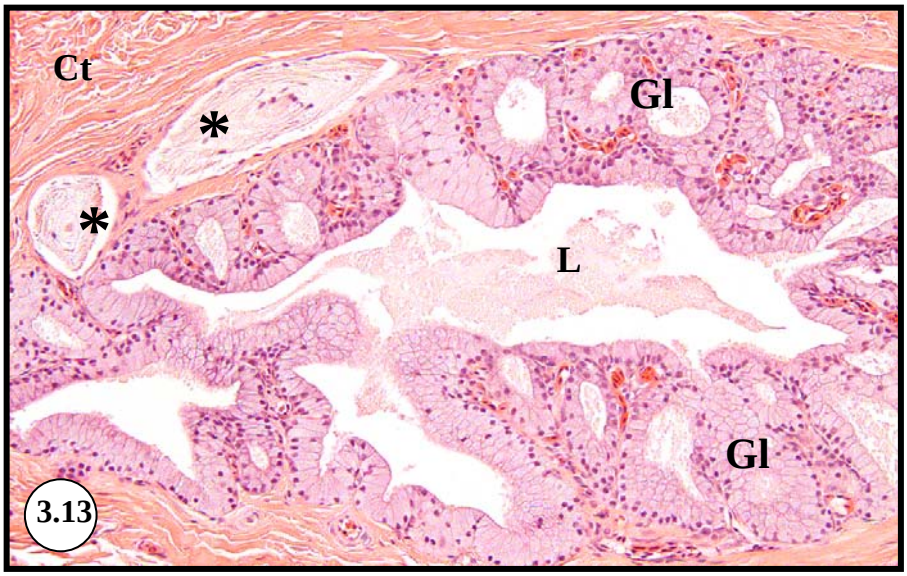


Figure 3.13: A large simple branched tubular gland (Gl) with associated Herbst corpuscles (*) in the connective tissue (Ct) of the mandibular rictus. Note the large lumen (L) filled with pale basophilic material (mucus).

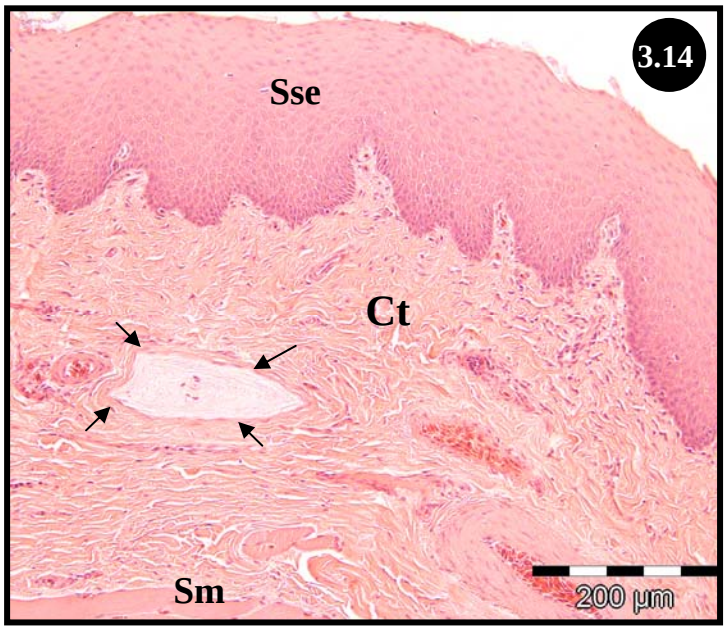
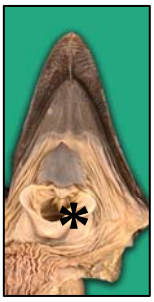


Figure 3.14: Aglandular region of the laryngeal mound displaying a thick stratified squamous epithelium (Sse) and dense irregular connective tissue (Ct) resting on skeletal muscle fibres (Sm). A single Herbst corpuscle is outlined by the arrows.



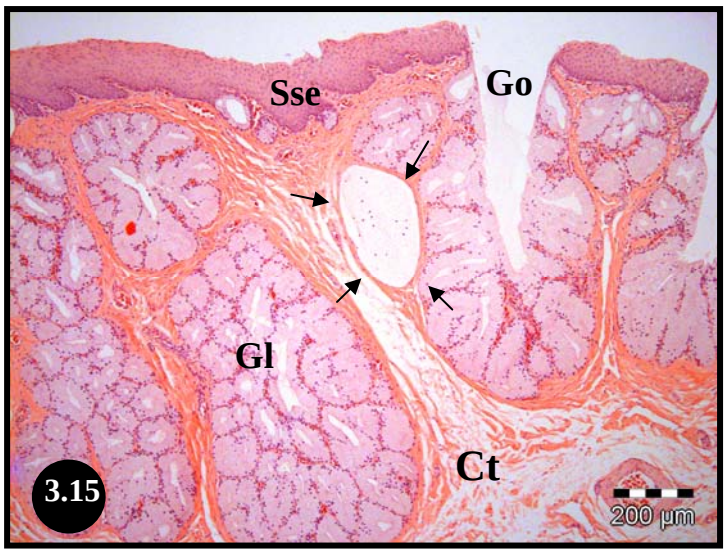


Figure 3.15: Glandular region of the laryngeal mound displaying a thinner stratified squamous epithelium (Sse) than the aglandular region. The underlying connective tissue (Ct) contains large simple branched tubular glands (Gl). Note the Herbst corpuscle (arrows) associated with the glands. Gland opening (Go).

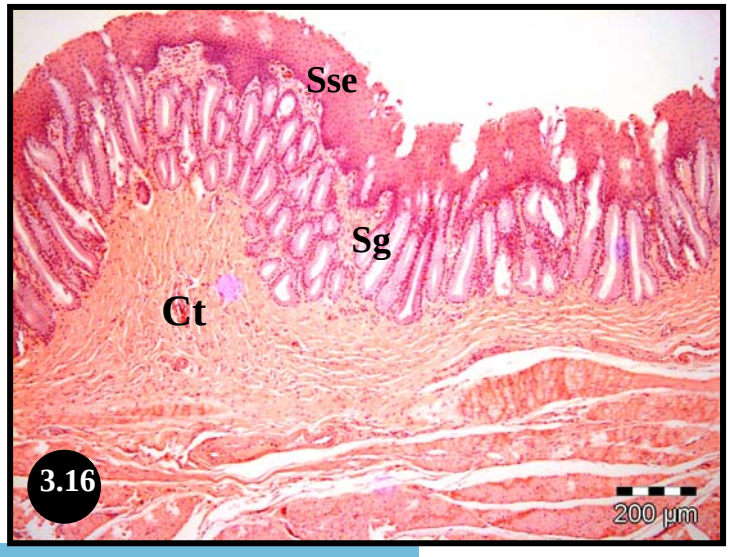


Figure 3.16: The laryngo-oesophageal junction marked by the appearance of simple tubular glands (Sg). Stratified squamous epithelium (Sse), connective tissue (Ct).

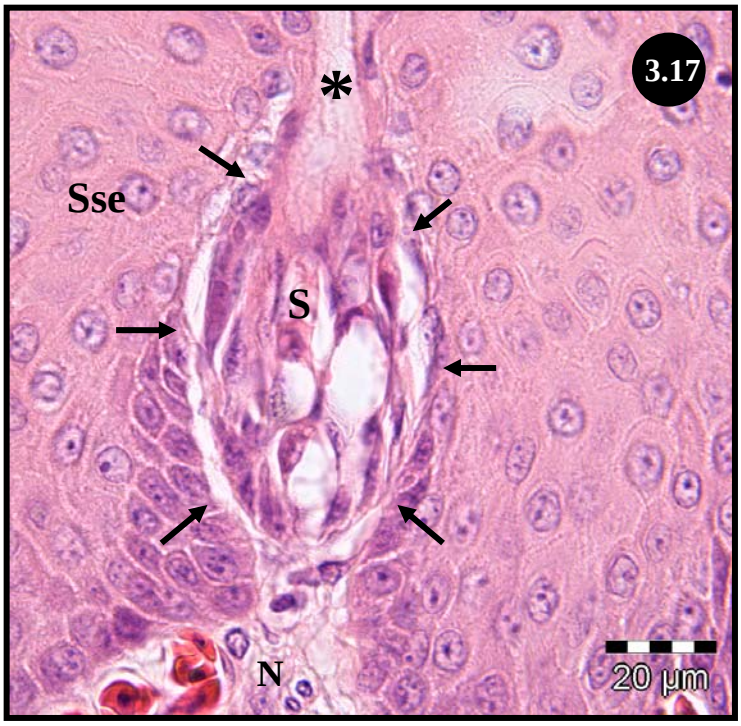


Figure 3.17: A taste bud in the non-pigmented floor, adjacent to the tongue body. The taste bud is demarcated (arrows) from the surrounding stratified squamous epithelium (Sse). Sensory and supporting cells (S) are not clearly defined. Opening to the surface (*). Nerve (N).

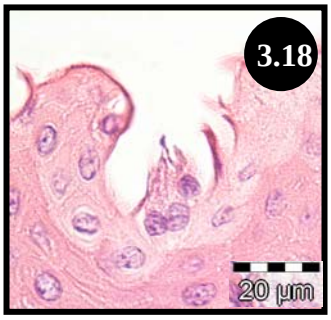


Figure 3.18: Putative taste bud at the laryngo-oesophageal junction.

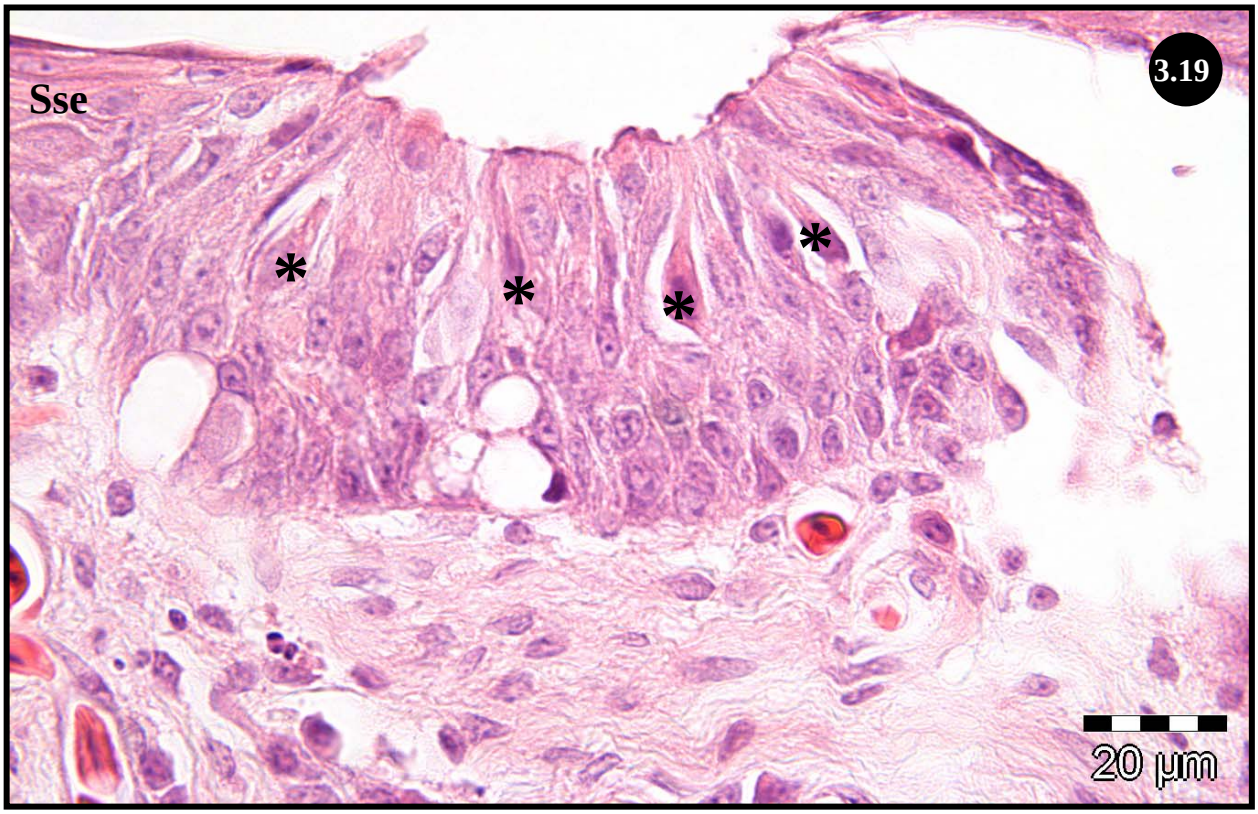


Figure 3.19: A circumscribed area in the stratified squamous epithelium (Sse) of the non-pigmented floor showing a collection of vertically oriented cells (*) with features typical of a taste bud.

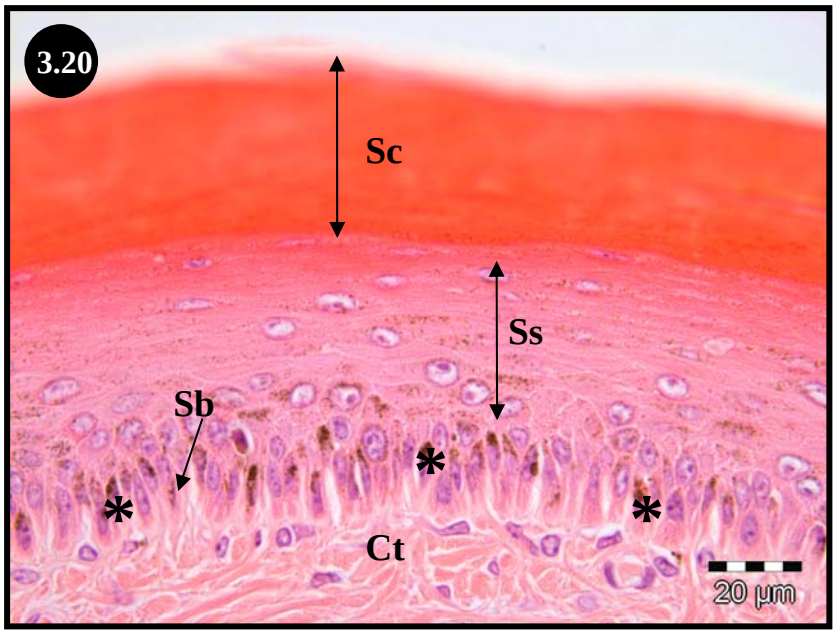


Figure 3.20: Epithelial lining of the pigmented region of the oropharyngeal roof. Note the columnar cells of the *stratum basale* (Sb) and the interspersed melanocytes (*) which are also obvious in the *stratum spinosum* (Ss). The thickness of the *stratum corneum* (Sc) places it out of the plane of focus. Connective tissue (Ct).

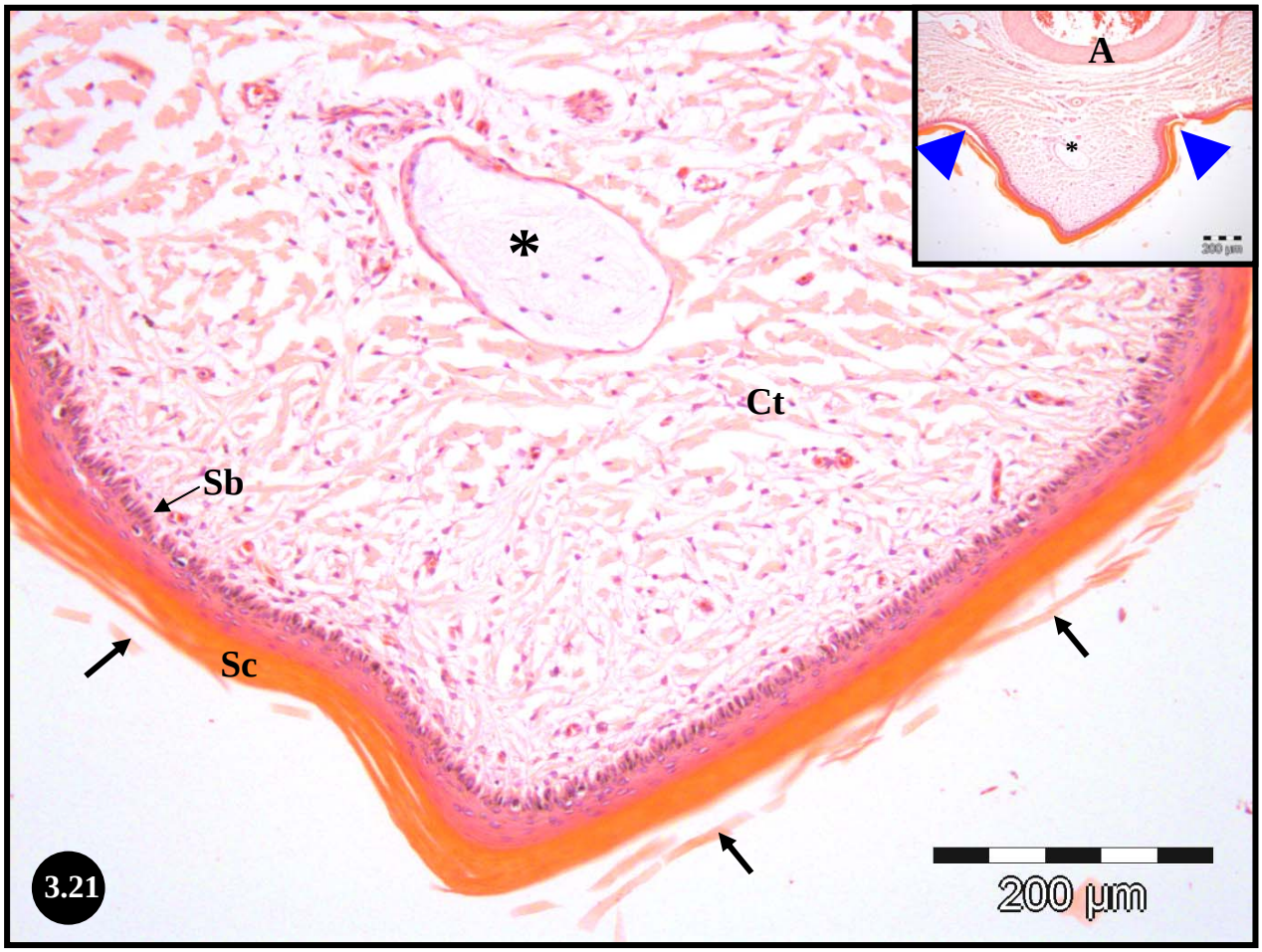


Figure 3.21: Transverse section through the median palatine ridge. The low magnification inset demonstrates the boundaries of the ridge (blue arrowheads) and the large artery (A) typically situated at its base. At higher magnification a single Herbst corpuscle (*) is seen in the less compacted region of the underlying connective tissue (Ct). Desquamation (arrows) of the surface cells of the *str. corneum* (Sc) is obvious. *Str. basale* (Sb).

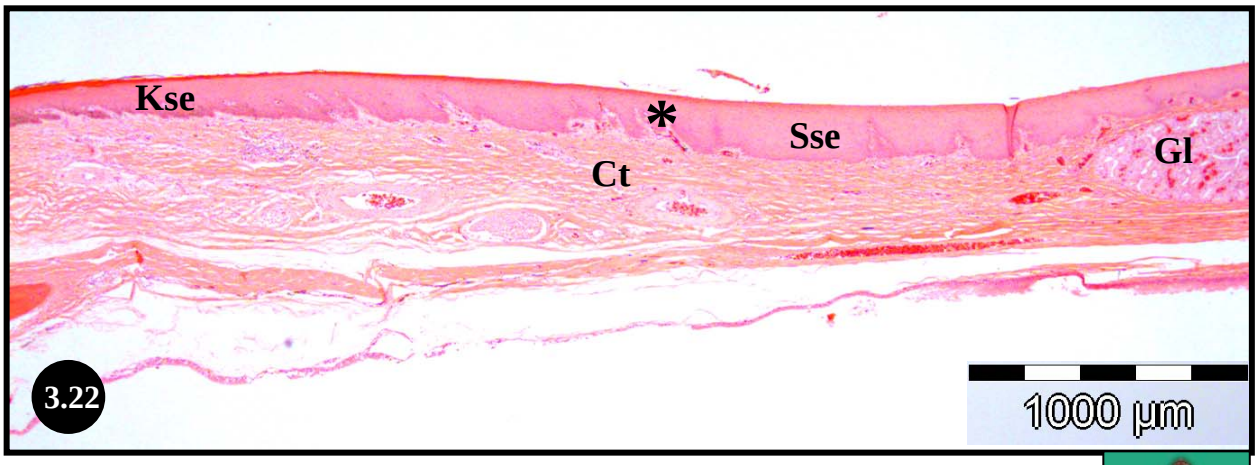


Figure 3.22: Transition between the pigmented and non-pigmented regions of the oropharyngeal roof. The keratinized stratified squamous epithelium (Kse) of the aglandular pigmented region gradually widens (*) as it changes to the non-keratinised stratified squamous epithelium (Sse) of the glandular non-pigmented region. Gland (Gl), connective tissue (Ct).

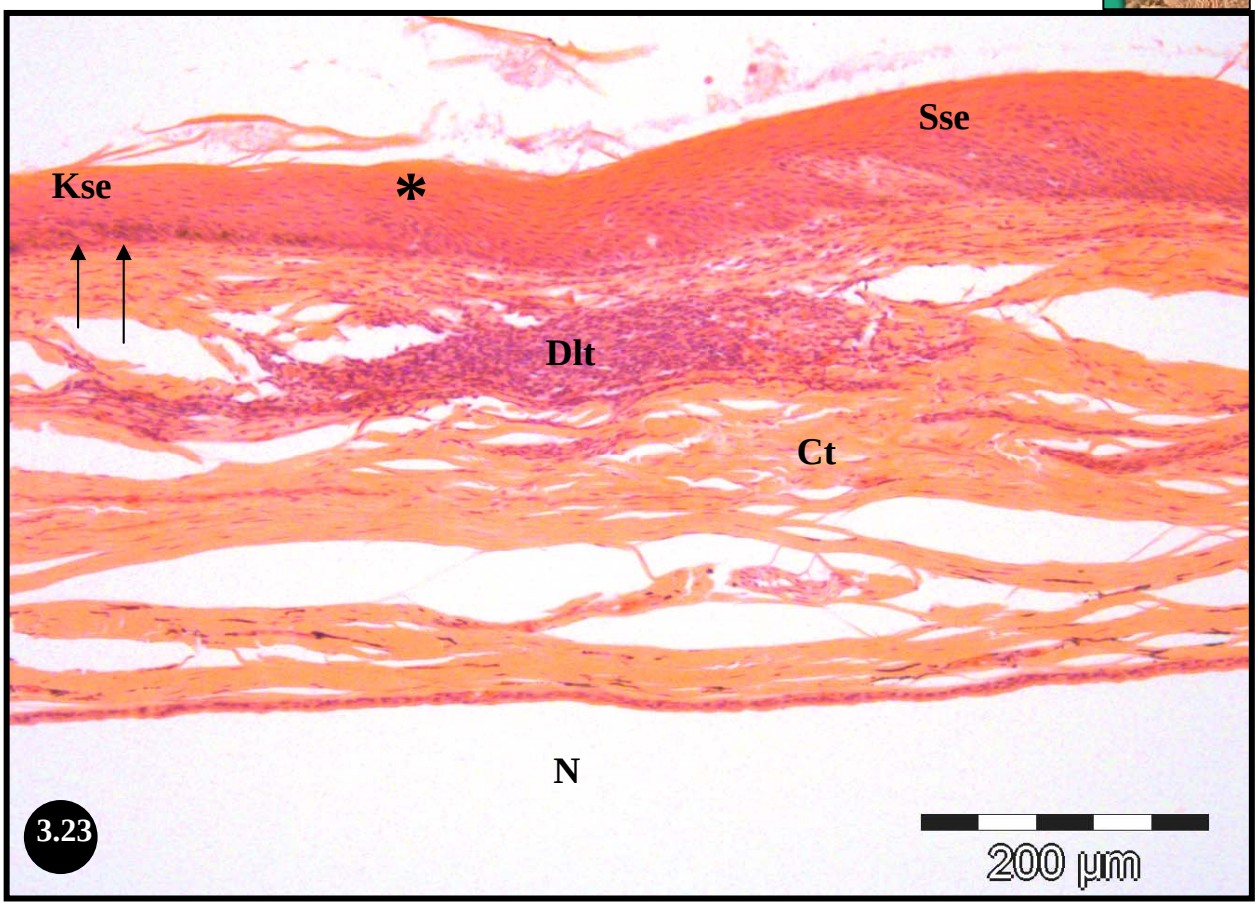


Figure 3.23: An area similar to that shown in Fig. 3.22 but demonstrating an aggregation of diffuse lymphoid tissue (Dlt) below the transition from a keratinized stratified squamous epithelium (Kse) to a thicker non-keratinised stratified squamous epithelium (Sse). Melanocytes (arrows), transition area (*), connective tissue (Ct) shared between the oral and nasal (N) portions of the roof.

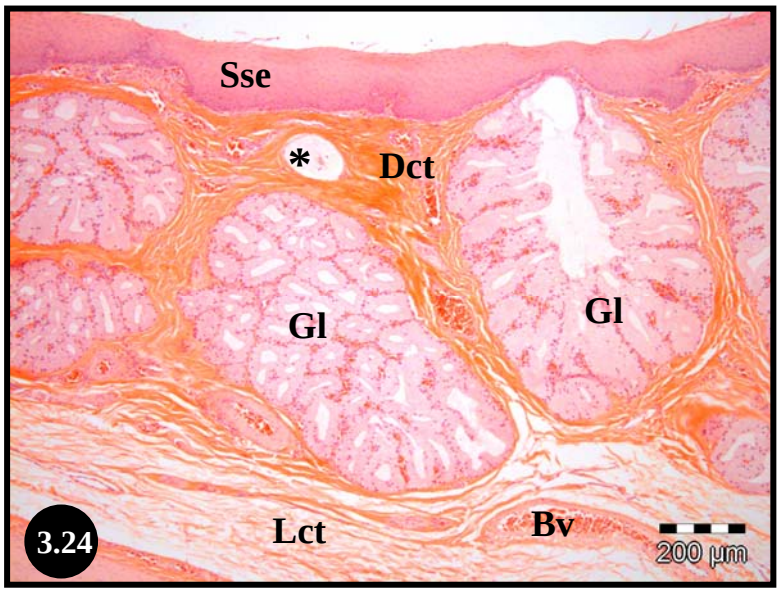


Figure 3.24: The non-pigmented, glandular oropharyngeal roof lined by a stratified squamous epithelium (Sse). Note how the dense connective tissue (Dct) houses the glands (Gl) and a Herbst corpuscle (*) whereas the deeper, more loosely arranged connective tissue (Lct) contains large blood vessels (Bv).

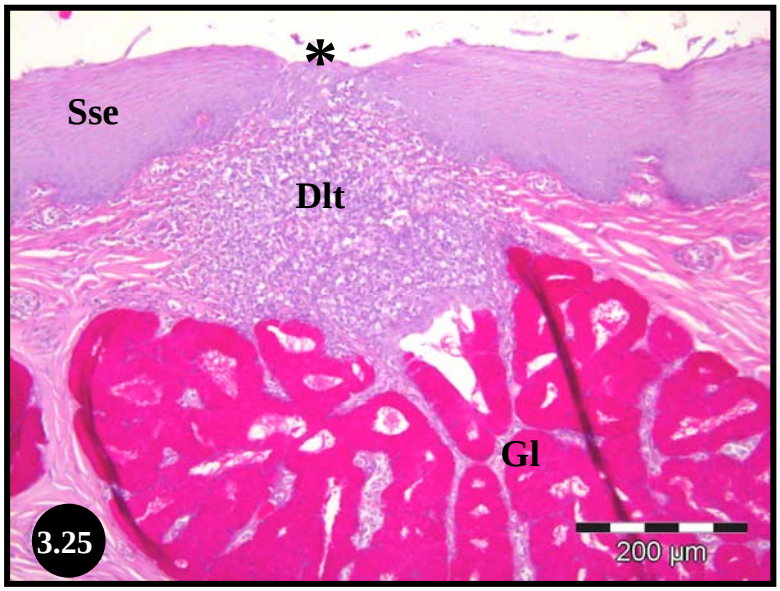
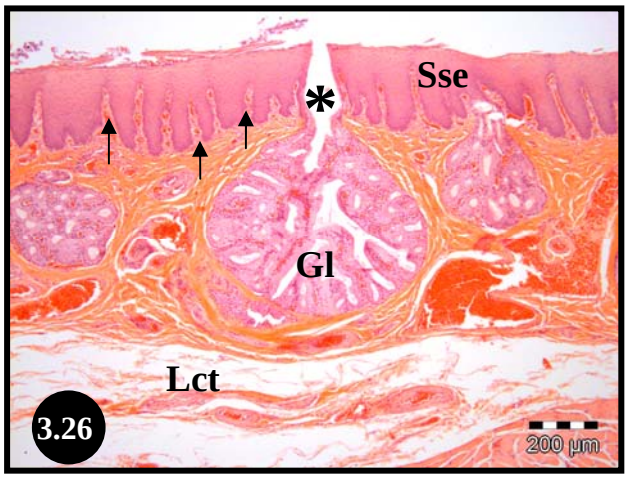


Figure 3.25: Glandular region of the oropharyngeal roof showing the PAS-positive staining reaction of the large, simple branched tubular glands (Gl). The diffuse lymphoid tissue (Dlt) obliterates part of the overlying stratified squamous epithelium (Sse), appearing to breach the surface (*).



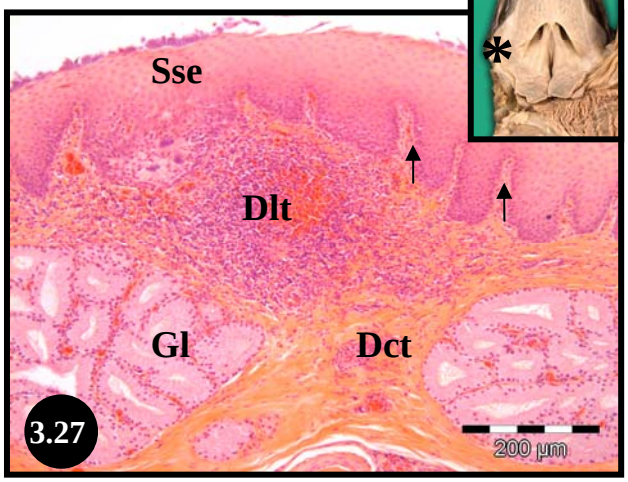


Figure 3.26 & 3.27: Sections through the mucosa of the maxillary rictus indicating the regularity and depth of the connective tissue papillae (arrows) in the maxillary rictus. Large, simple branched tubular glands (Gl) and aggregations of diffuse lymphoid tissue (Dlt) are present in the dense connective tissue (Dct). Loose connective tissue (Lct), gland opening (*), stratified squamous epithelium (Sse).

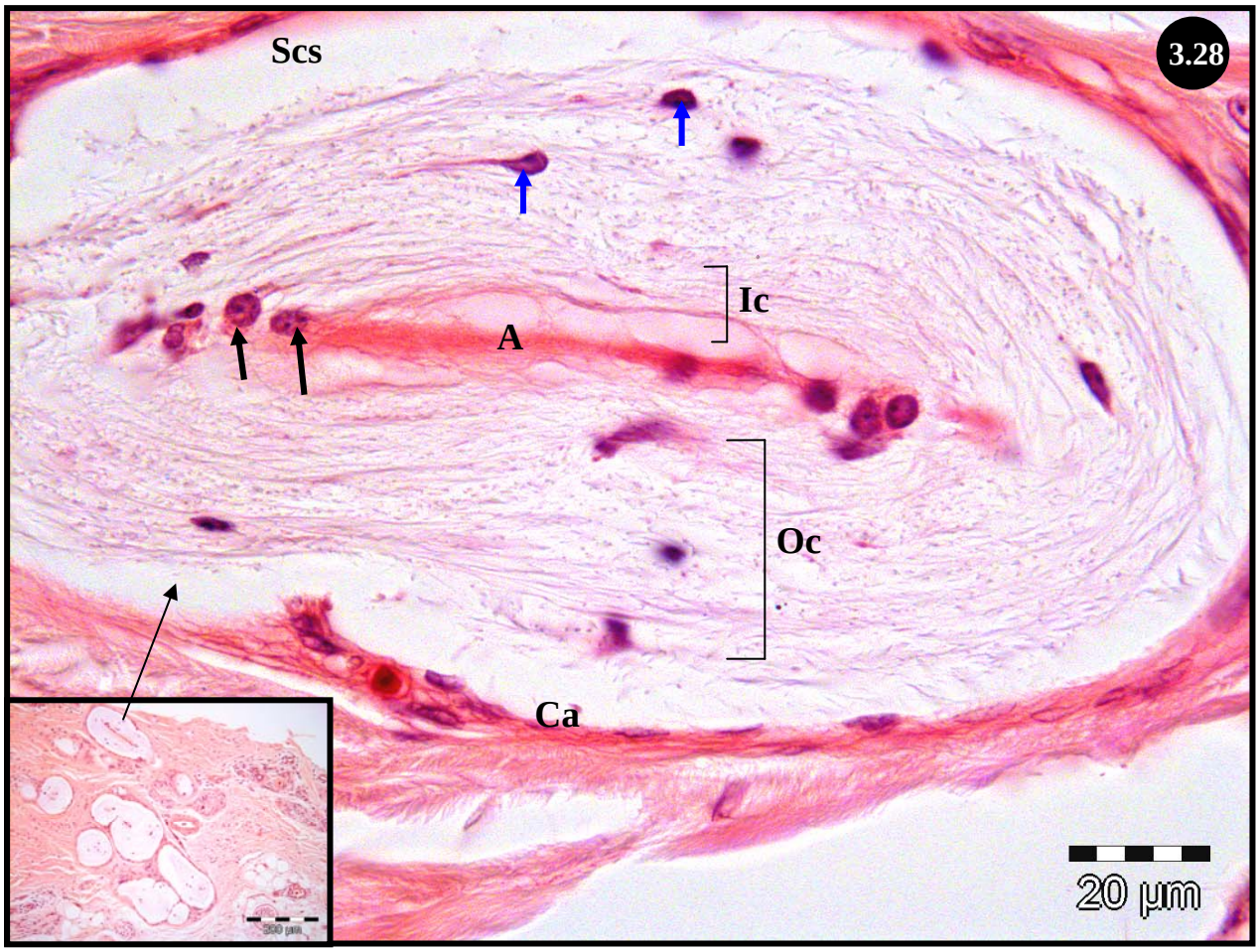


Figure 3.28: A group of Herbst corpuscles at the maxillary rictus (inset). Higher magnification of one of the corpuscles in longitudinal section details the central pink axon (A) surrounded by the inner core (Ic) with Schwann cell nuclei (black arrows), the outer core (Oc) with fibroblast nuclei (blue arrows) and the subcapsular space (Scs) below the fibrous outer capsule (Ca).

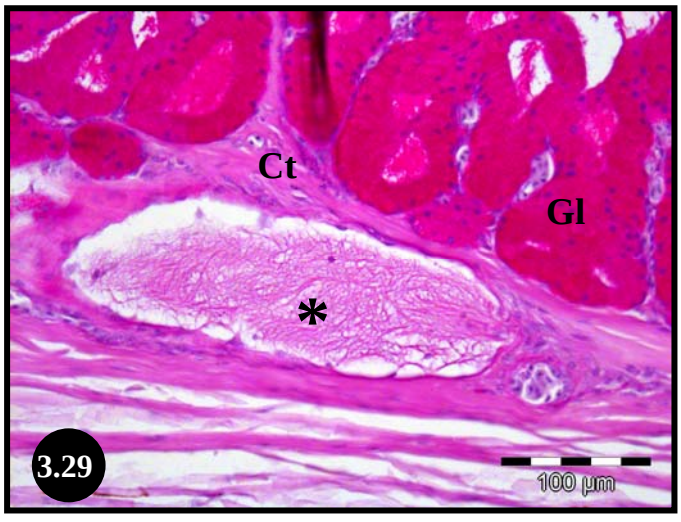


Figure 3.29: The PAS-positive staining reaction shown by the simple branched tubular glands (Gl) of the maxillary rictus. The fibrocytic lamellae of a Herbst corpuscle (*) also demonstrate a faint PAS-positive reaction. Connective tissue (Ct).

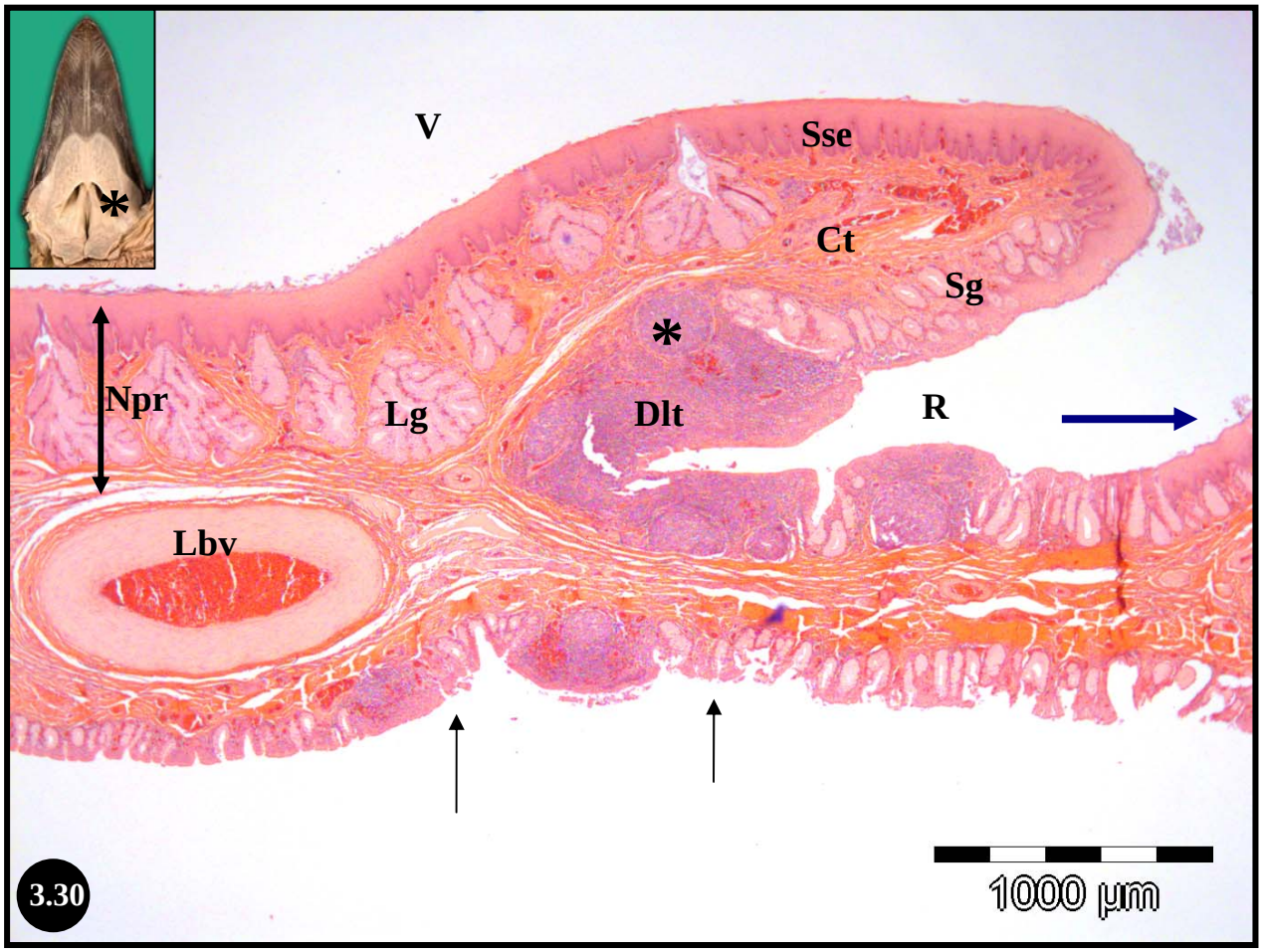


Figure 3.30: Transverse section of the small fold on the glandular region of the oropharyngeal roof lateral to the choana. Note the large, simple branched tubular glands (Lg) on the ventral surface (V) and the simple tubular glands (Sg) lining the recess (R) beneath the fold. Similar glands occur in the deeper lying respiratory mucosa (arrows). The large blood vessel (Lbv) and diffuse (Dlt) and nodular (*) lymphoid tissue situated at the angle of the recess, were consistently present. Stratified squamous epithelium (Sse), tissue of non-pigmented roof (Npr), direction of the choana (blue arrow), connective tissue (Ct).

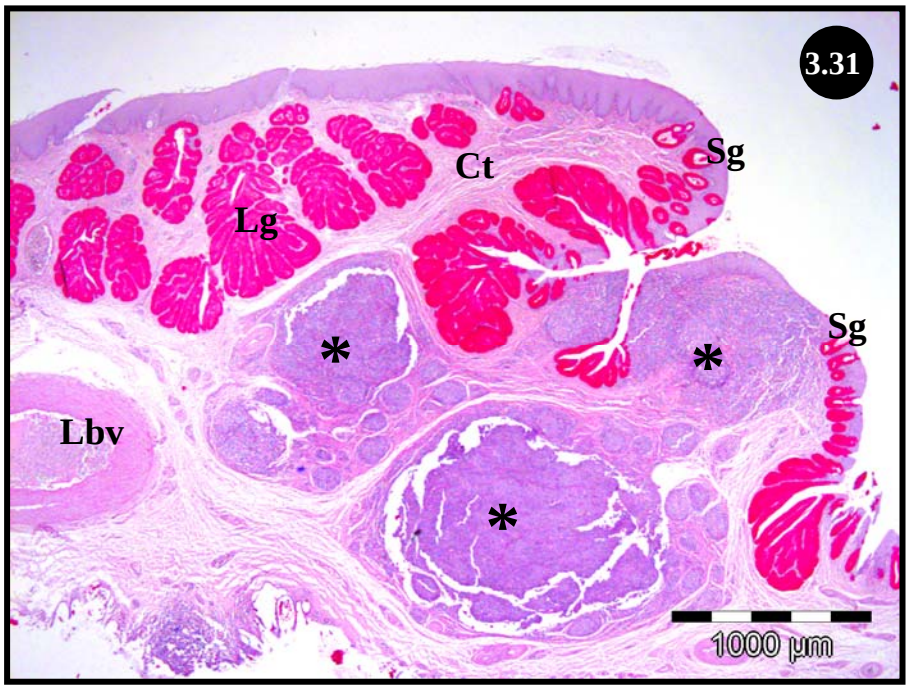


Figure 3.31: A similar view of the fold to that depicted in Fig. 3.30 but sectioned closer to its edge. The large, simple branched tubular glands (Lg) are confined mainly to the ventral surface of the pharyngeal fold and the lateral edges of the recess. These glands and the simple tubular glands (Sg) display a PAS-positive staining reaction. Large blood vessel (Lbv), connective tissue (Ct), diffuse lymphoid tissue (*).

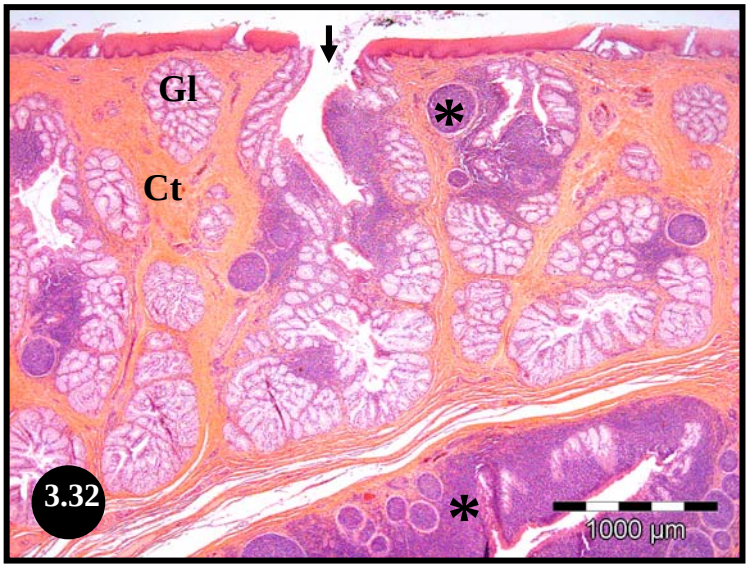


Figure 3.32: Ventral surface of the pharyngeal fold displaying numerous, large, simple branched tubular glands (Gl) and associated lymphoid tissue (*) in the connective tissue (Ct). The lymphoid tissue consists of diffuse and nodular accumulations. Note the large opening to the surface of a gland (arrow).

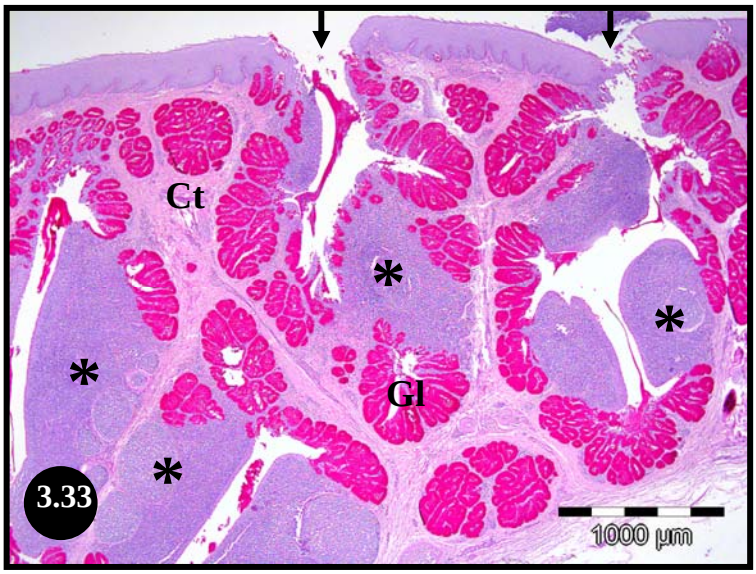


Figure 3.33: Similar region to that shown in Fig. 3.32 illustrating the PAS-positive staining reaction of the glandular tissue (Gl). Note the large accumulations of lymphoid tissue (*) associated with the glands. Connective tissue (Ct), large gland openings (arrows).

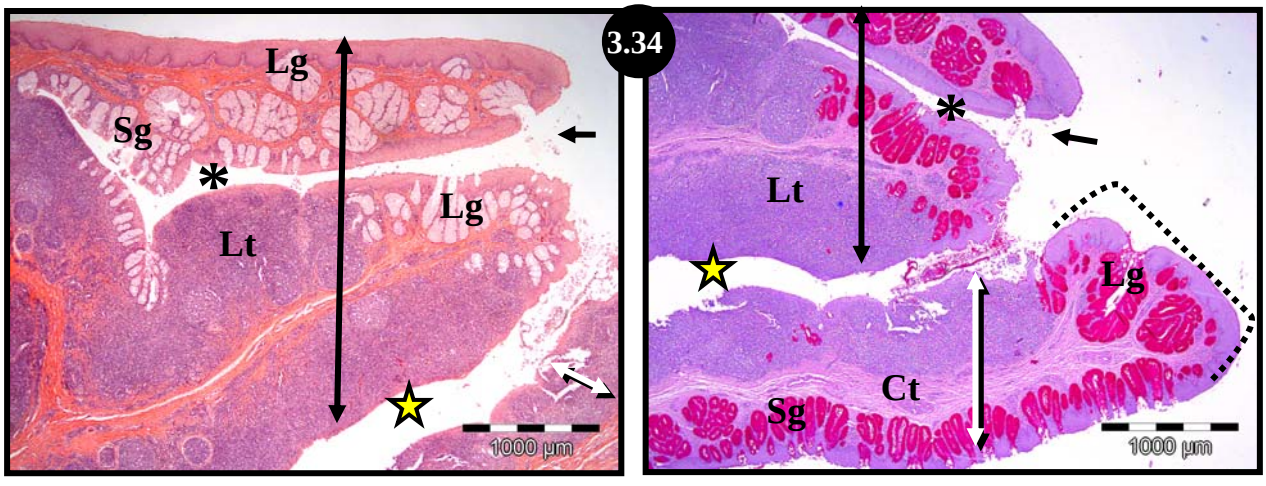


Figure 3.34: Caudo-lateral aspect of the pharyngeal fold (black double-headed arrows) depicting the large opening (arrows) to the tonsilar crypt (*). Note the PAS-positive staining reaction of the figure on the right, showing the mucus-secreting properties of the glands. Connective tissue (Ct), lymphoid tissue (Lt), large, simple branched tubular glands (Lg), simple tubular glands (Sg), pocket or recess (yellow star) between the pharyngeal fold and the caudo-lateral tissue projection (white double-headed arrows). Protruding surface of the caudo-lateral projection (dotted bracket) (see Chapter 2 - Fig. 2.17).

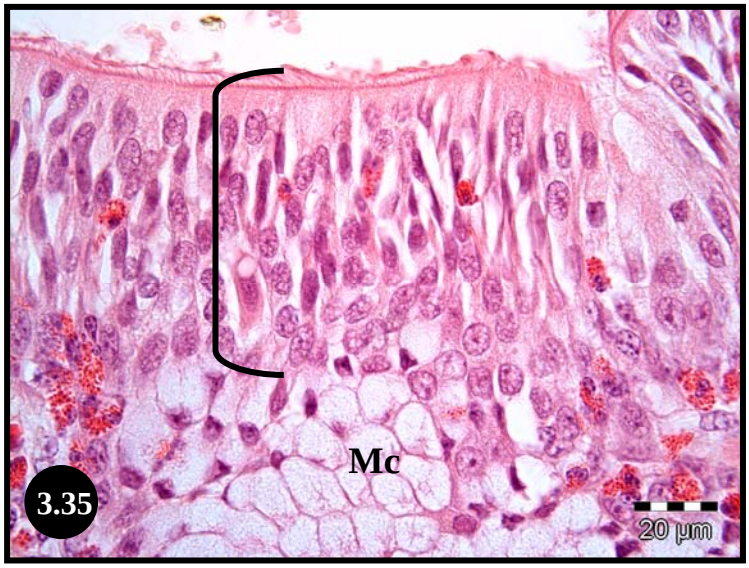


Figure 3.35: Psuedostratified ciliated columnar epithelium (bracket) lining the lumen of a large, simple branched mucus-secreting gland in the pharyngeal fold. Mucous cells (Mc).

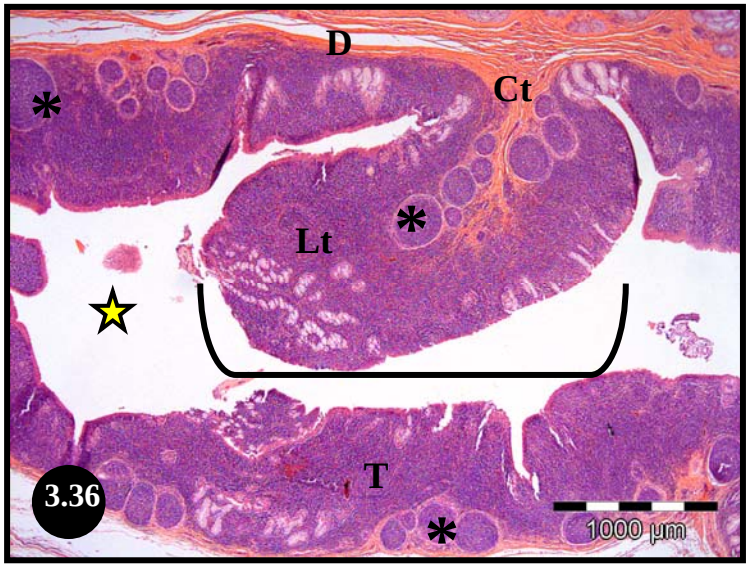


Figure 3.36: Pocket or recess (yellow star) between the dorsal surface of the pharyngeal fold (D) and the ventrum of the caudo-lateral tissue projection (T). Note the large nodule (bracket) of lymphoid tissue (Lt) projecting dorsally into the recess from the pharyngeal fold, held by a connective tissue (Ct) stalk. Nodular lymphoid tissue (*).

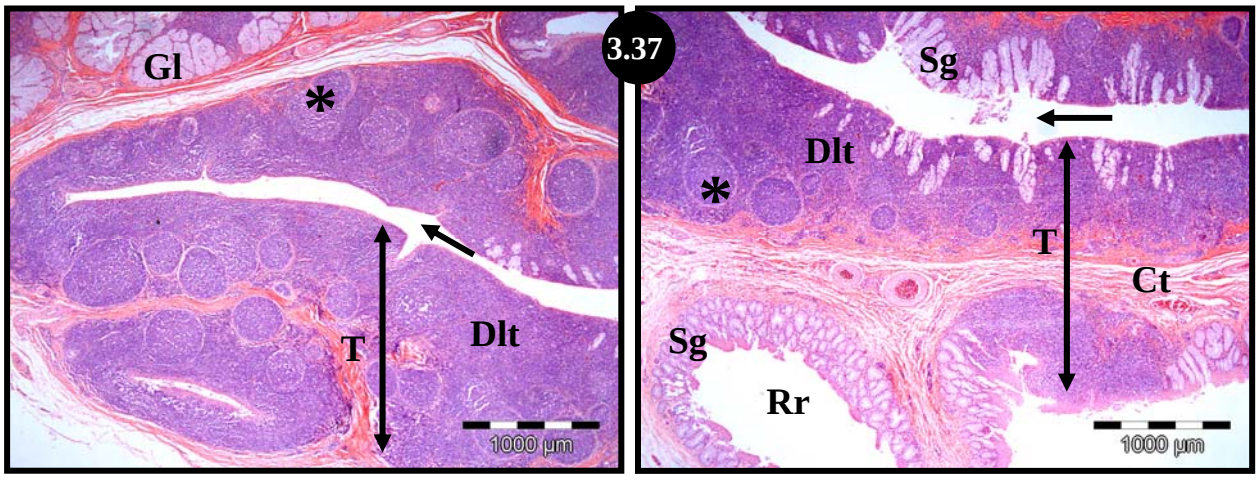


Figure 3.37: Rostral extent of the pocket or recess (arrows) illustrated in Fig. 3.36. The figure on the right also shows the rostral extent of the retropharyngeal recess (Rr). The caudo-lateral tissue projection (T) formed the ventral border of the retropharyngeal recess. Connective tissue (Ct), diffuse (Dlt) and nodular (*) lymphoid tissue, large simple branched tubular gland (Gl), simple tubular glands (Sg).

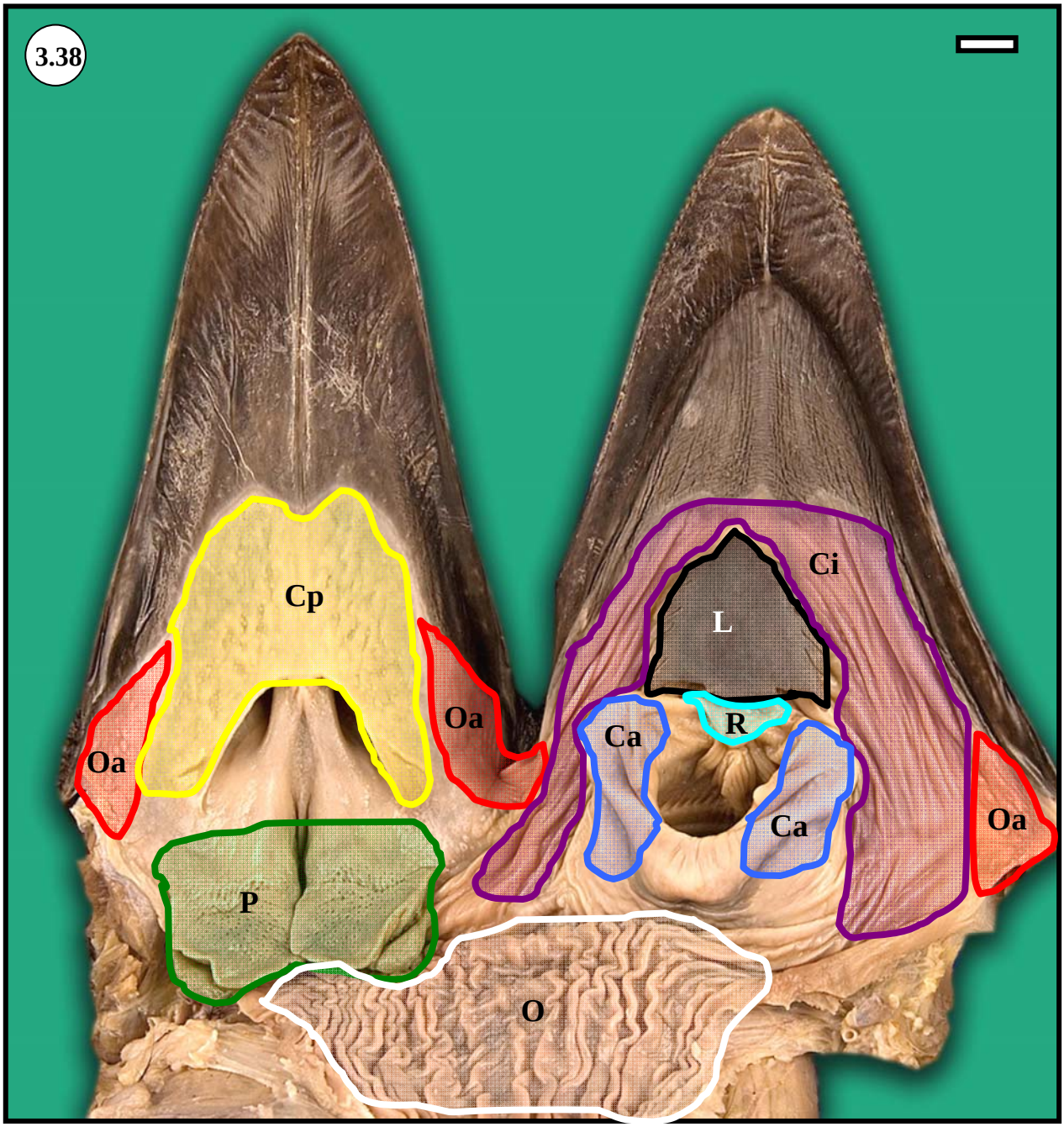


Figure 3.38: Schematic representation of the mucus-secreting glandular fields identified in the oropharynx and proximal oesophagus of the emu: Caudal intermandibular (Ci, purple), lingual (L, black), radical (R, turquoise), crico-arytenoid (Ca, blue), oral angular (Oa, red), caudal palatine (Cp, yellow), pharyngeal (P, green), oesophageal (O, white) glands. Bar = 5mm.

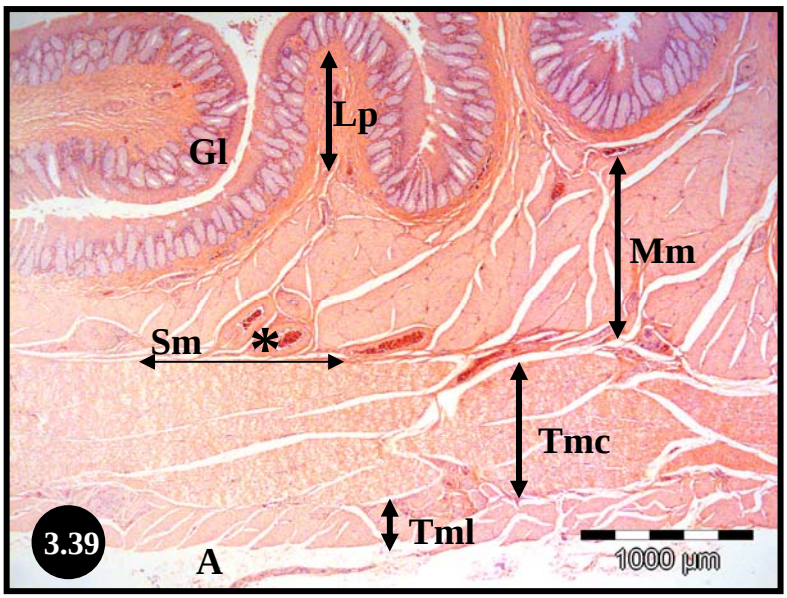


Figure 3.39: Transverse section of the proximal oesophagus depicting the *lamina propria* (Lp) containing simple tubular glands (Gl), the thick longitudinal *muscularis mucosae* (Mm), the very thin submucosa (Sm) with blood vessels (*), the inner circular (Tmc) and outer longitudinal (Tml) layers of the *tunica muscularis* and the adventitia (A).

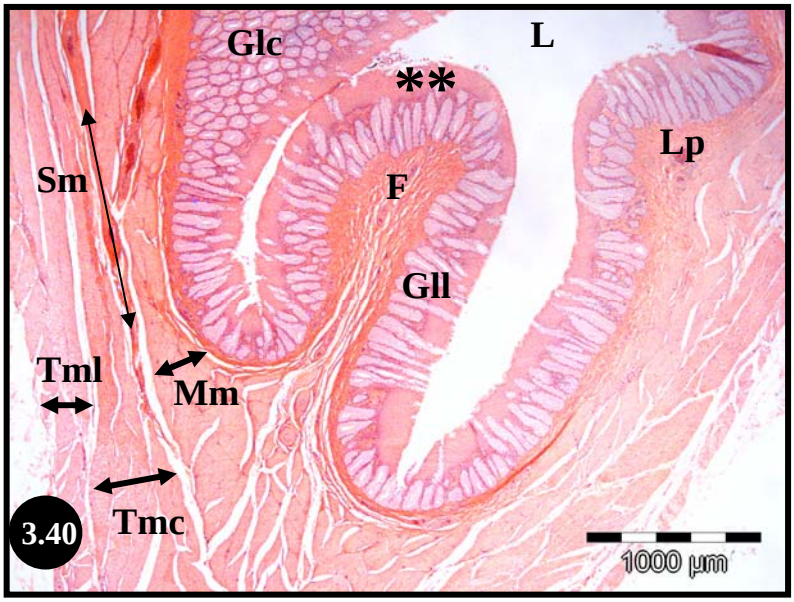


Figure 3.40: Transverse section of the proximal oesophagus depicting the epithelium (**), *lamina propria* (Lp) containing simple branched glands seen in longitudinal (Gll) and cross section (Glc), the thick longitudinal *muscularis mucosae* (Mm), the very thin submucosa (Sm), and the inner circular (Tmc) and outer longitudinal (Tml) layers of the *tunica muscularis*. Note how the mucosa is thrown into folds (F) which fill the lumen (L).

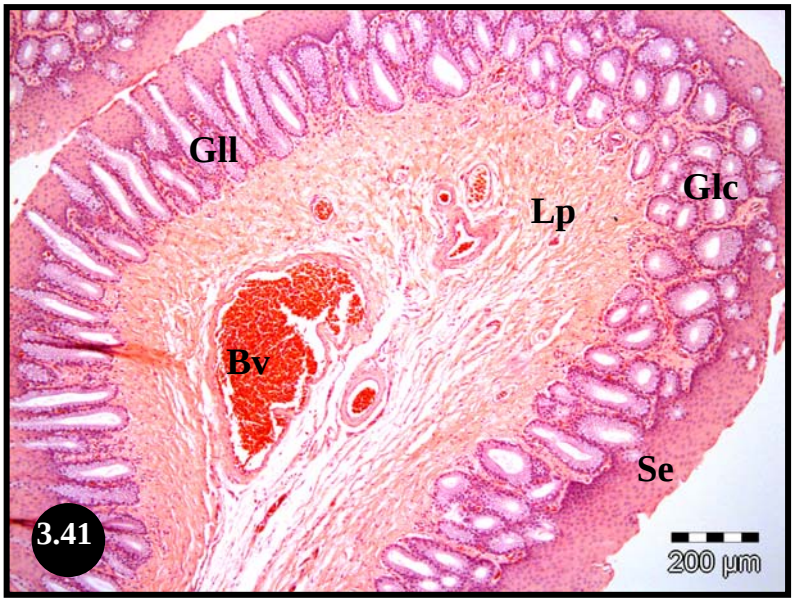


Figure 3.41: A mucosal fold in the proximal oesophagus consisting of a core of connective tissue (*lamina propria*) (Lp), containing simple tubular glands in longitudinal (Gll) and cross section (Glc). Note the large blood vessel (Bv) carried in the centre of the fold as well as the absence of the *muscularis mucosae*. Stratified squamous epithelium (Se).

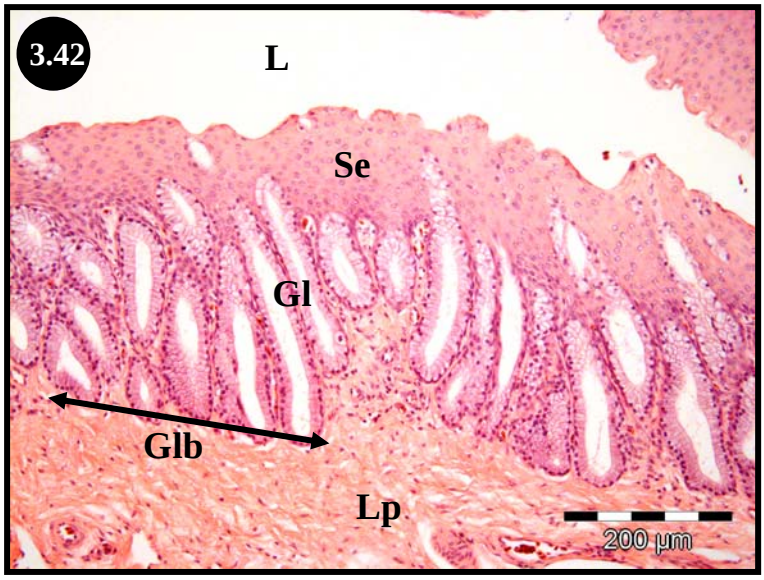


Figure 3.42: High magnification of the simple tubular glands (Gl) situated in the *lamina propria* (Lp) of the proximal oesophagus. Note that the base of the glands (Glb) extend only a short distance into the *lamina propria.* Non-keratinised stratified squamous epithelium (Se). Lumen (L).

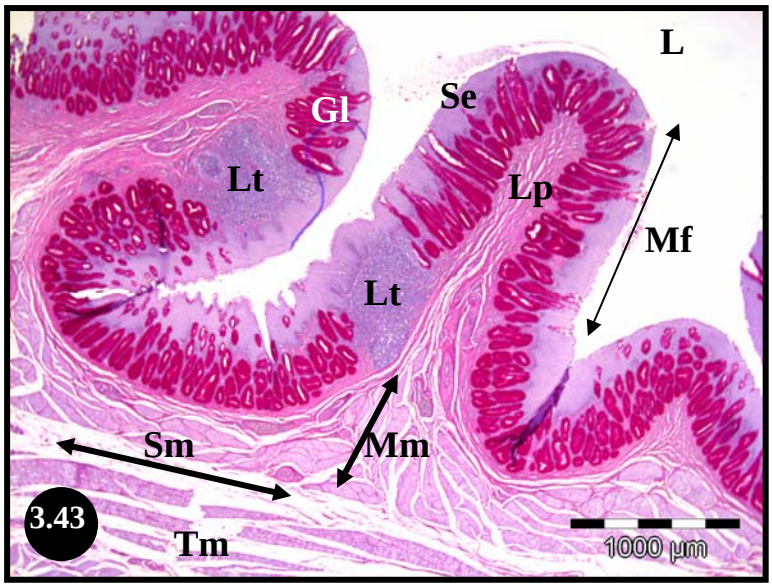
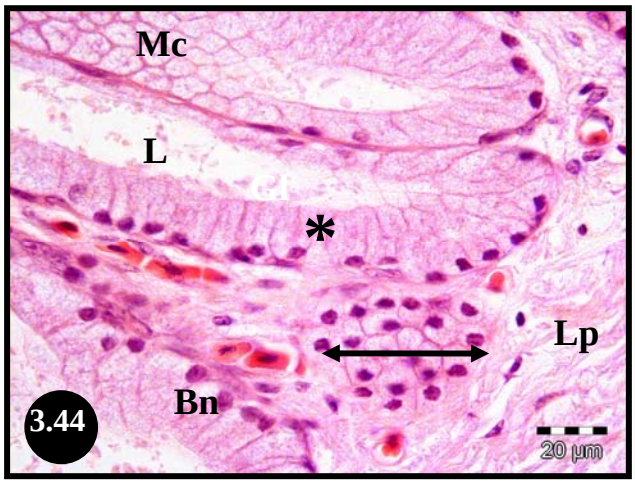


Figure 3.43: PAS-positive staining reaction of the simple tubular mucus-secreting glands (Gl) in the proximal oesophagus. Aggregations of lymphoid tissue (Lt) lie between the glands. Lumen (L), mucosal fold (Mf), *muscularis mucosae* (Mm), submucosa (Sm), *tunica muscularis* (Tm), stratified squamous epithelium (Se).



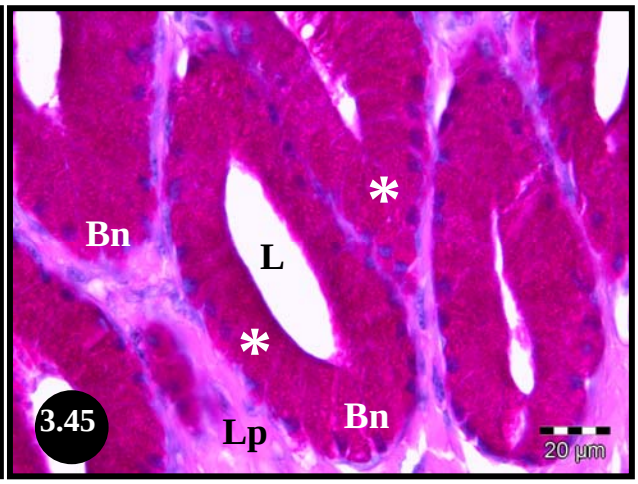


Figure 3.44 & 3.45: High magnification of the mucus-secreting cells (Mc) which form the simple tubular oesophageal glands (PAS-positive stain reaction, Fig. 3.45). Note the typical features, basal nuclei (Bn) and basophilic foamy cytoplasm (*), of the mucus-secreting cells. Lumen (L), *lamina propria* (Lp), cross section of the basal part of the cells (double-headed arrow).

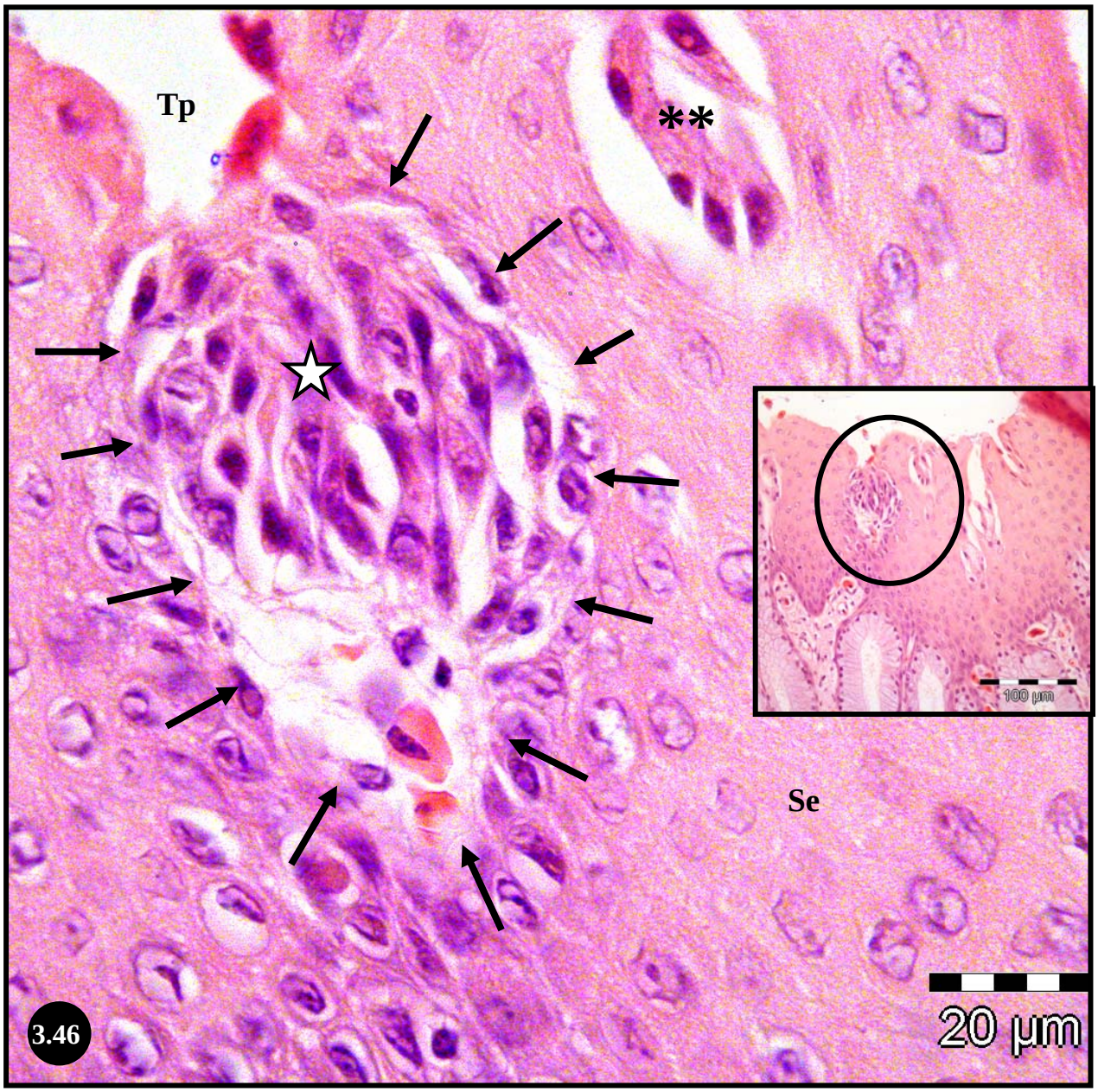


Figure 3.46: Enlargement of a taste bud (circled in inset) located in the non-keratinised stratified squamous epithelium (Se) of the proximal oesophagus. Structures identifiable were the taste pore (Tp), encapsulating epithelium (arrows) and vertically oriented, elongated cells (star). ** indicates another possible taste bud sectioned superficially.

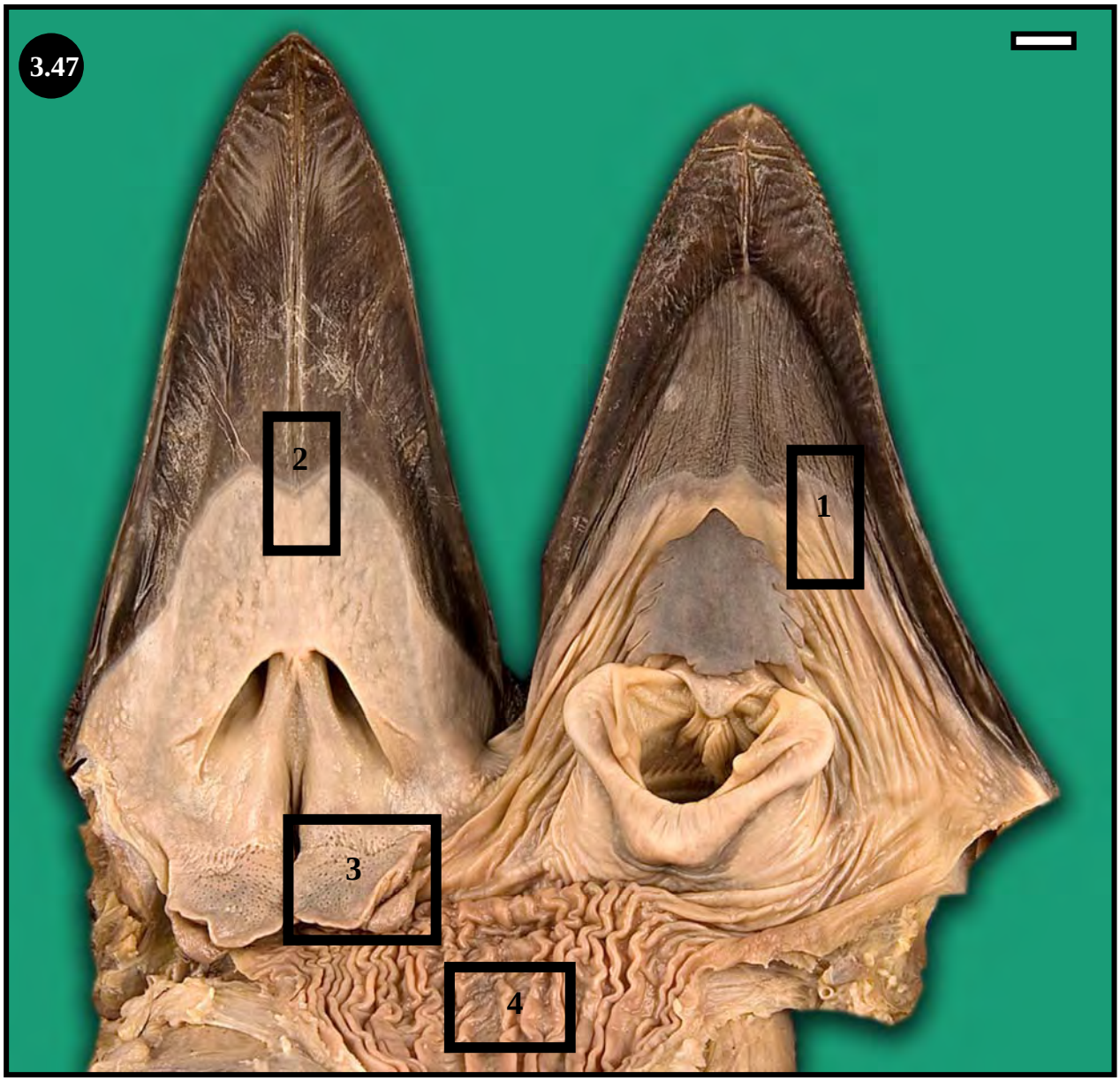


Figure 3.47: Sample areas selected for scanning electron microscopy of the emu oropharynx: Rostral pigmented and caudal non-pigmented floor, including the large lateral fold and smaller folds (1), pigmented and non-pigmented roof, including the median palatine ridge (2), ventral surface of the pharyngeal fold including the caudo-lateral protrusion (3), proximal oesophagus (4). Bar = 5mm.

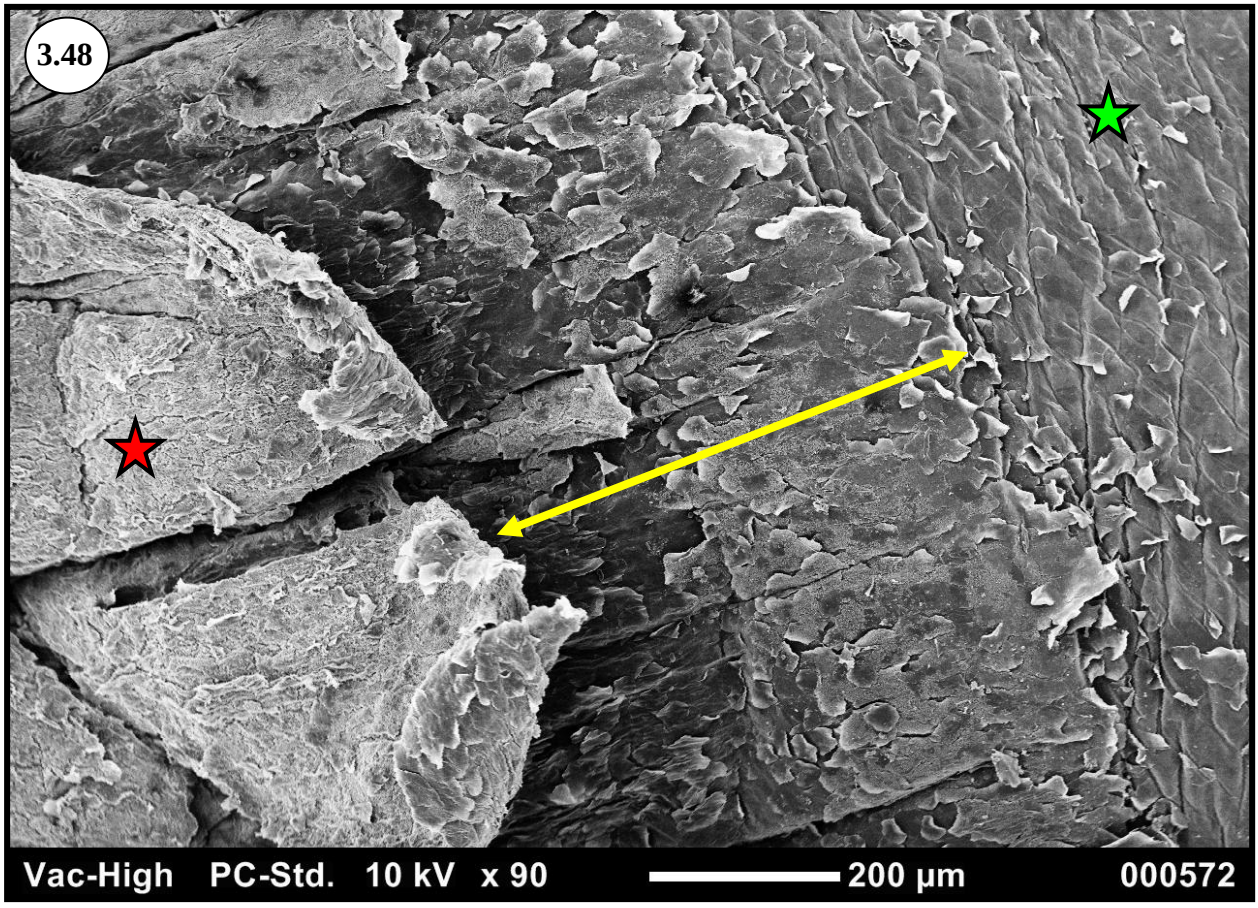


Figure 3.48: Low magnification of the oropharyngeal floor showing the transition (yellow arrow) from the rostral, longitudinally folded, keratinised region (red star) to the caudal non-keratinised region (green star). Note the individual desquamating surface cells in the non-keratinised region and the sheets of desquamating cells in the transitional zone.

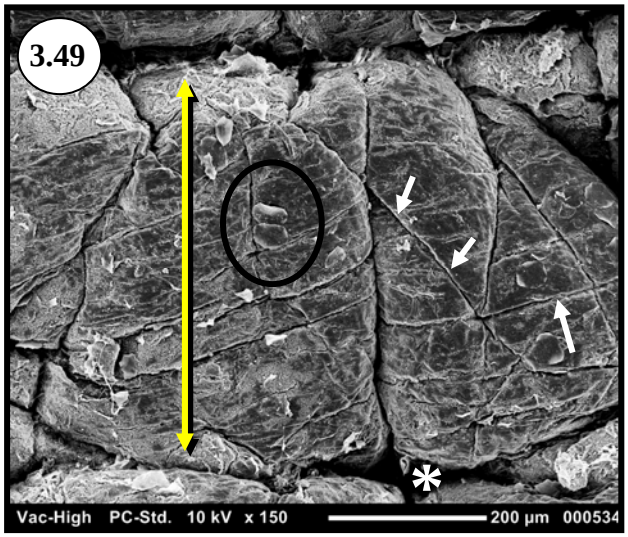


Figure 3.49: Higher magnification of a fine longitudinal fold (yellow arrow) of the keratinised region of the oropharyngeal floor displaying numerous smaller transverse, oblique and longitudinal fissures (white arrows). Groove between the folds (*).

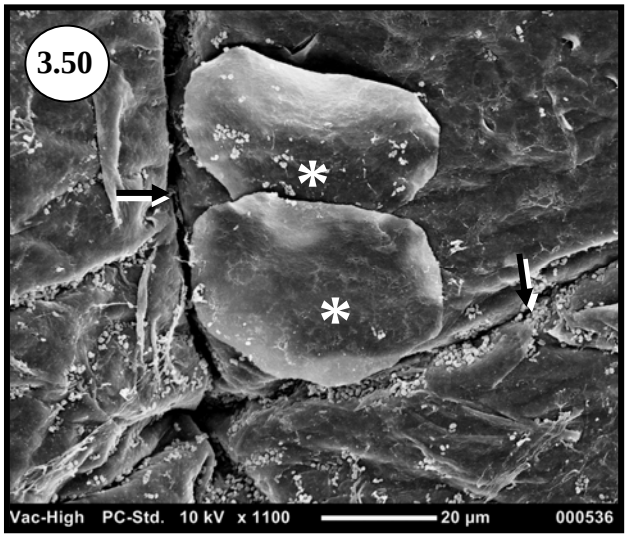


Figure 3.50: Higher magnification of the area encircled in Fig. 3.49. The surface is mainly smooth with only a few individual desquamating cells (*). Fine longitudinal and transverse fissures (arrows).

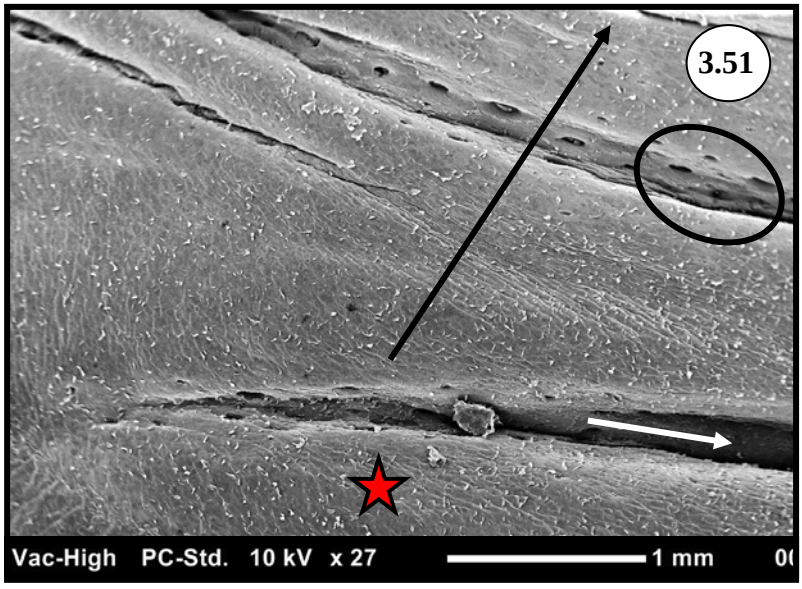


Figure 3.51: Low magnification of the non-keratinised oropharyngeal floor showing the origin of the large lateral mucosal fold (red star), the corresponding recess it encloses (white arrow) and the smaller folds (black arrow) towards the medial aspect of the floor. Note the numerous large gland openings in the grooves (encircled).

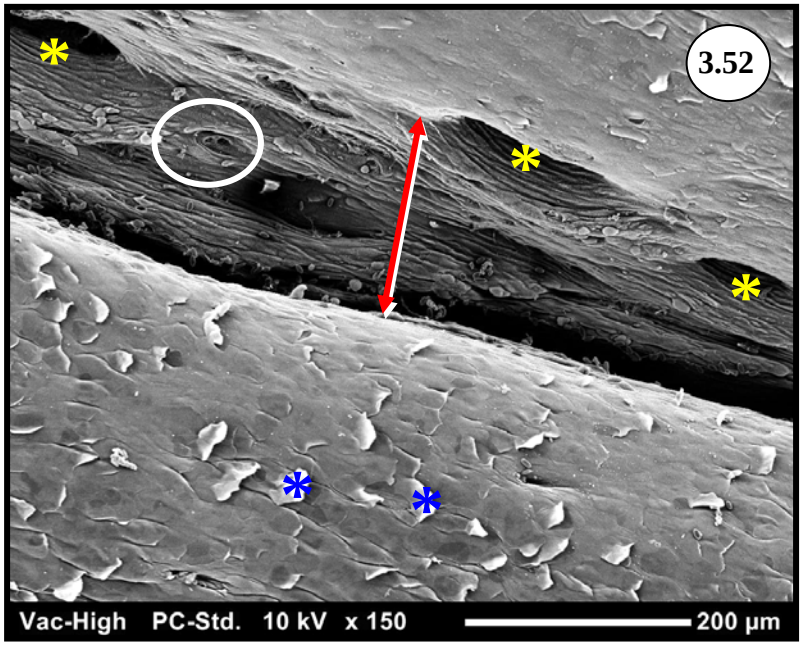
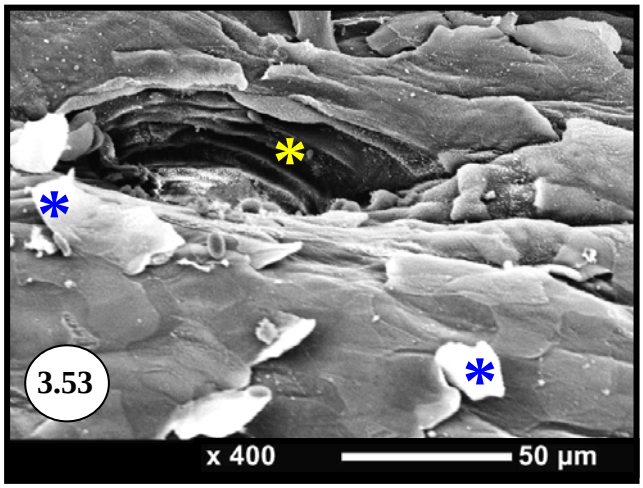


Figure 3.52: Higher magnification of the area encircled in Fig. 3.51. Note the difference in surface pattern from the desquamating cells (blue *) on the folds to a more undulating pattern in the groove (red arrow). Numerous large openings (yellow *) and smaller openings (encircled) are present in the groove.

Figure 3.54: Enlargement of the area encircled in Fig. 3.52 showing two smaller gland openings. The surface of the cells in this region are covered by a dense mass of microvilli. Strands of mucus lie between the two openings (yellow arrow).



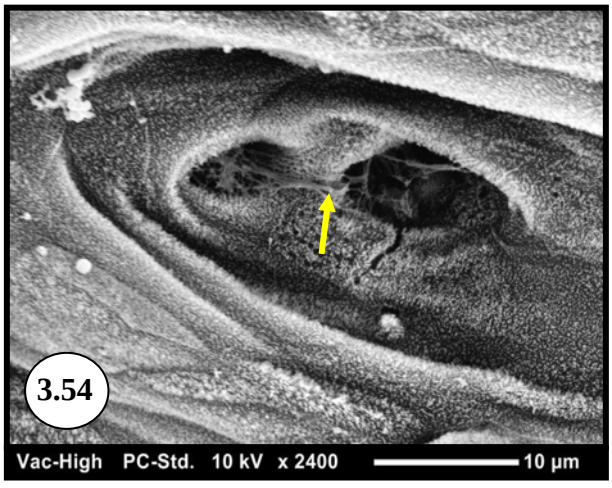


Figure 3.53: Higher magnification of a large gland opening in the groove shown in Fig. 3.52. Note the concentric arrangement of the cells lining the large opening (yellow *). Desquamating surface cells (blue *).

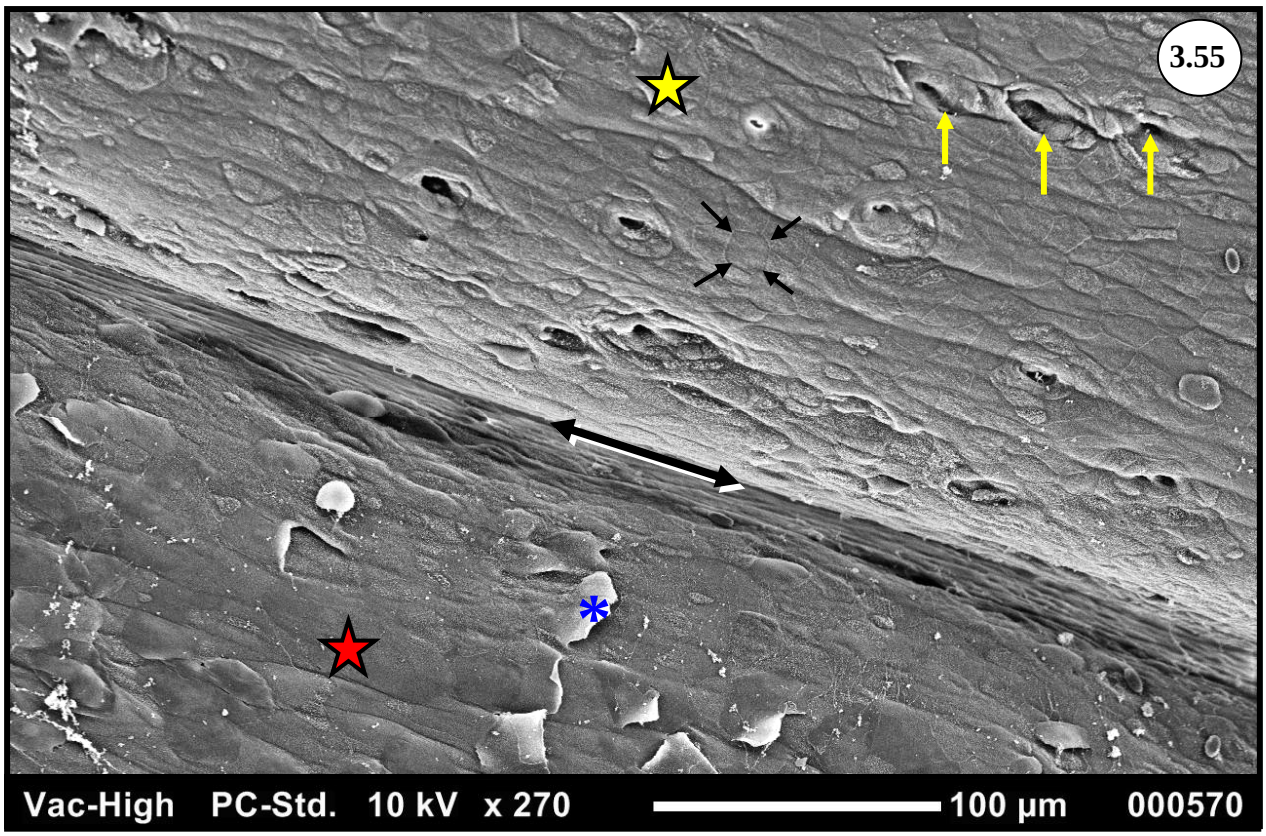


Figure 3.55: Different surface patterns of the smaller folds of the non-keratinised oropharyngeal floor medial to the large lateral fold. One fold (red star) displays a flaky surface due to individual cell desquamation (blue *). A second more medially situated fold (yellow star) shows an uneven surface with clearly demarcated cell boundaries (black arrows) and numerous small openings (yellow arrows). Groove (double-headed black arrow).

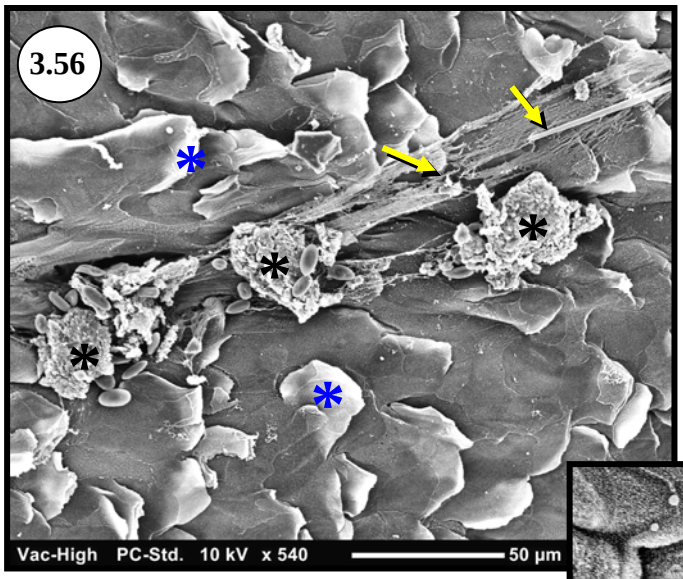


Figure 3.56: Higher magnification of the large lateral fold of the oropharyngeal floor. Note the desquamating surface cells (blue *) and large openings obscured by mucus-secretion (black *) from the underlying glands. Strands of mucus (yellow arrows).

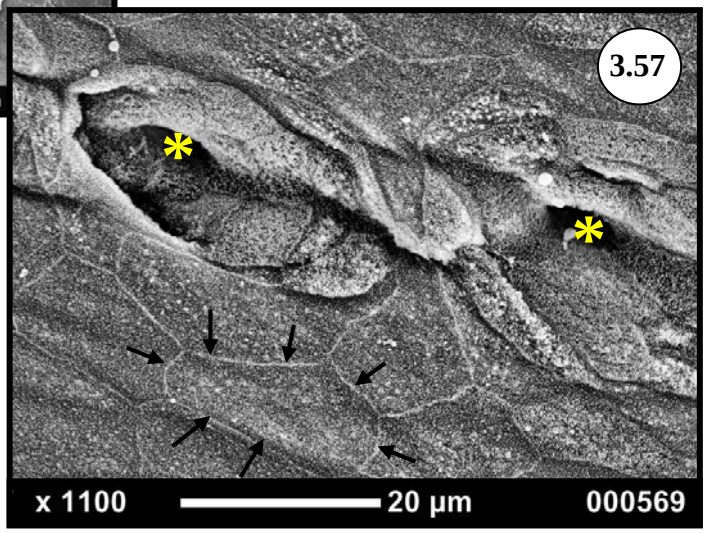


Figure 3.57: Higher magnification of the area depicted by the middle yellow arrow in Fig. 3.55. All the cell surfaces are covered by microvilli which compact to form well demarcated cell boundaries (black arrows). Small gland openings (yellow *).

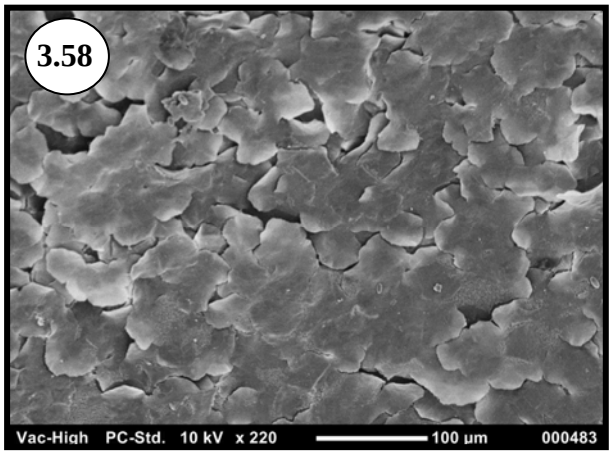


Figure 3.58: The rostral keratinised region of the oropharyngeal roof displaying sheets of desquamating cells.

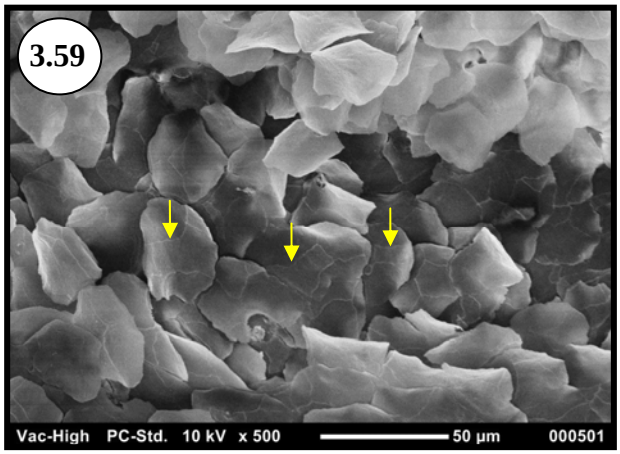


Figure 3.59: Higher magnification of Fig. 3.58 showing the microridges (arrows) on some of the cells.

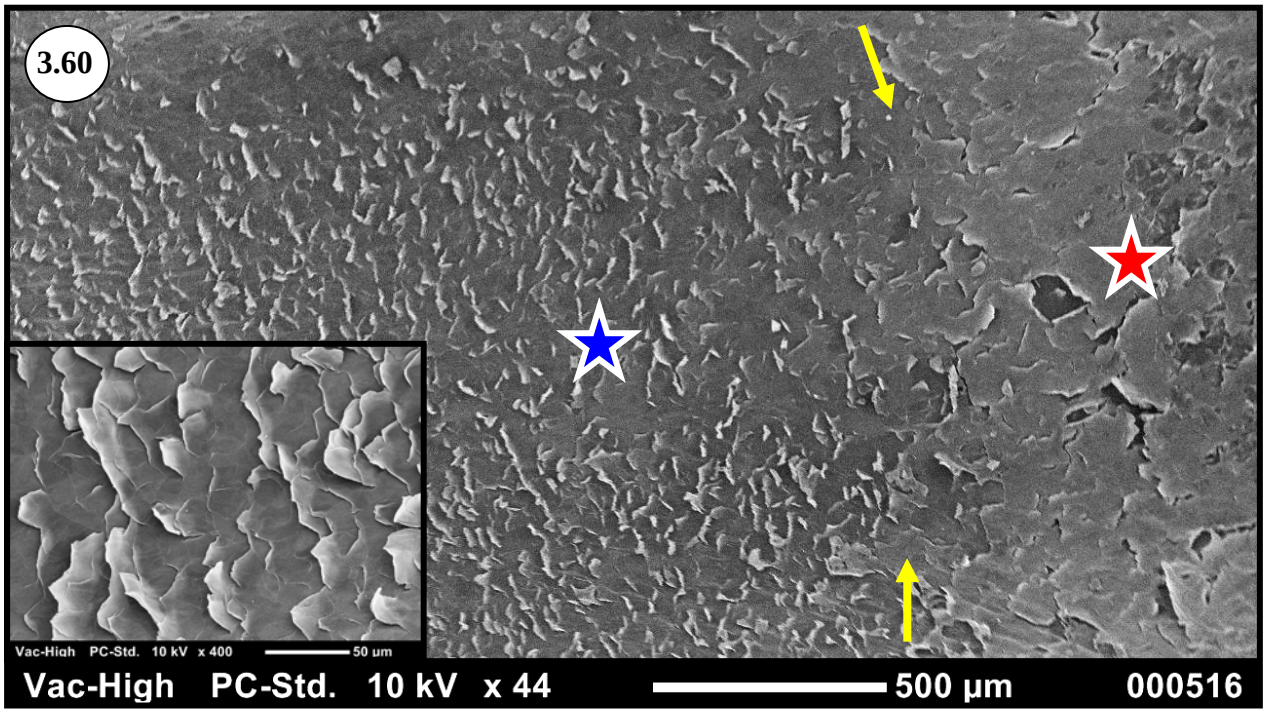


Figure 3.60: Roof of the oropharynx showing the abrupt transition (arrows) from the smooth keratinised region with sheets of desquamating surface cells (red star) to the flaky non-keratinised region with its individual desquamating surface cells (blue star and inset). The inset shows the rows of desquamating cells in the non-keratinised region at higher magnification.

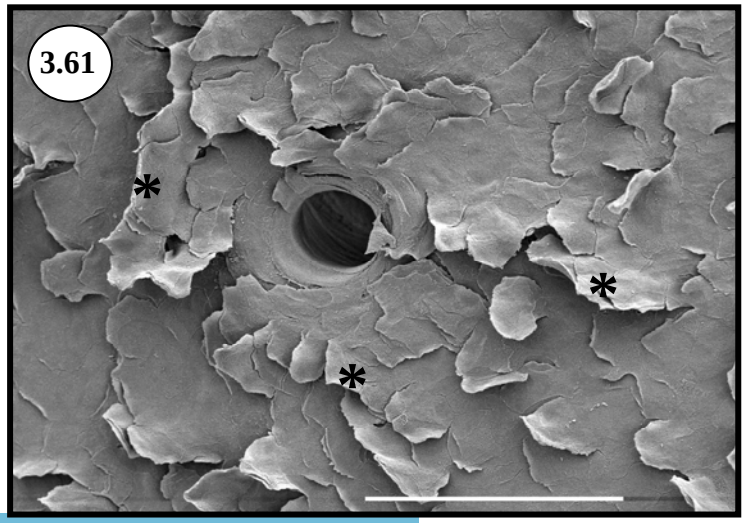


Figure 3.61: Large gland opening between the desquamating cells (*) of the non-keratinised oropharyngeal roof. Note the concentric arrangement of the cells lining the duct. x370; Bar = 100 µm.

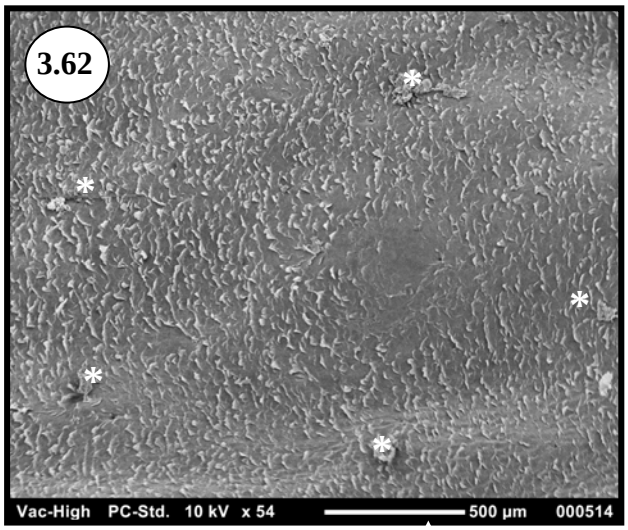


Figure 3.62: The non-keratinised roof of the oropharynx illustrating the wide, evenly distributed large gland openings (*) observed in this region.

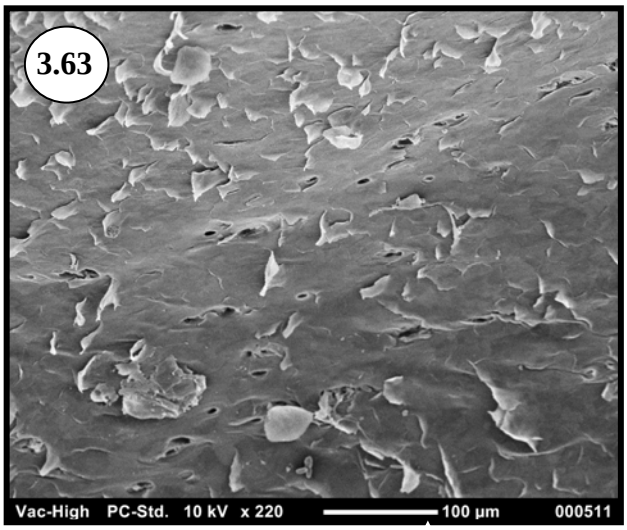


Figure 3.63: The close distribution of small gland openings (small black holes) on the more caudal aspect of the non-keratinised oropharyngeal roof.

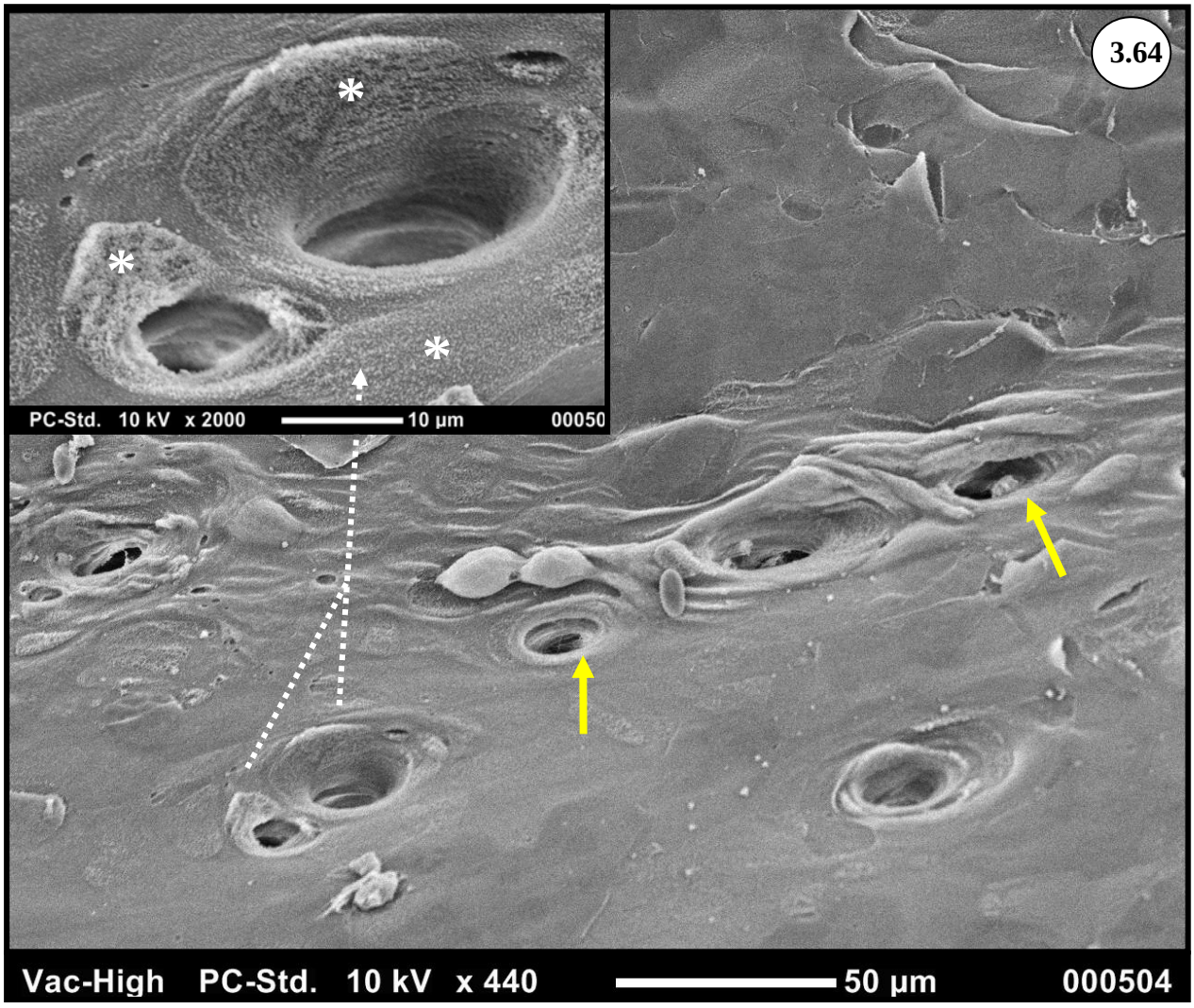


Figure 3.64: The non-keratinised oropharyngeal roof. Note the numerous small gland openings (arrows) and the microvilli (*) on the concentrically arranged cells surrounding the openings. Cells with similar features line the gland ducts (inset).

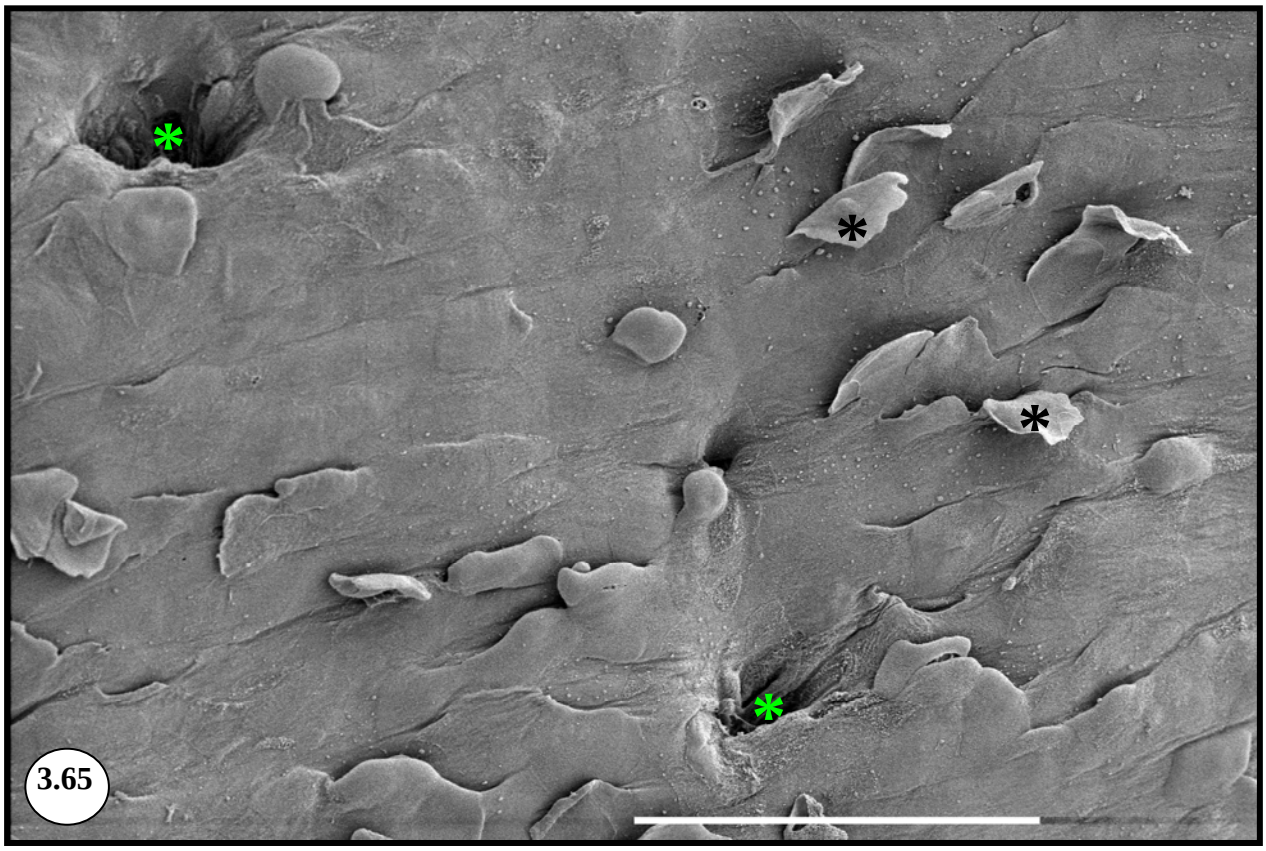


Figure 3.65: Ventral surface of the pharyngeal fold showing individual desquamating surface cells (black *) and large gland openings (green *). The surface appears smooth at this magnification. x350; Bar = 100 µm.

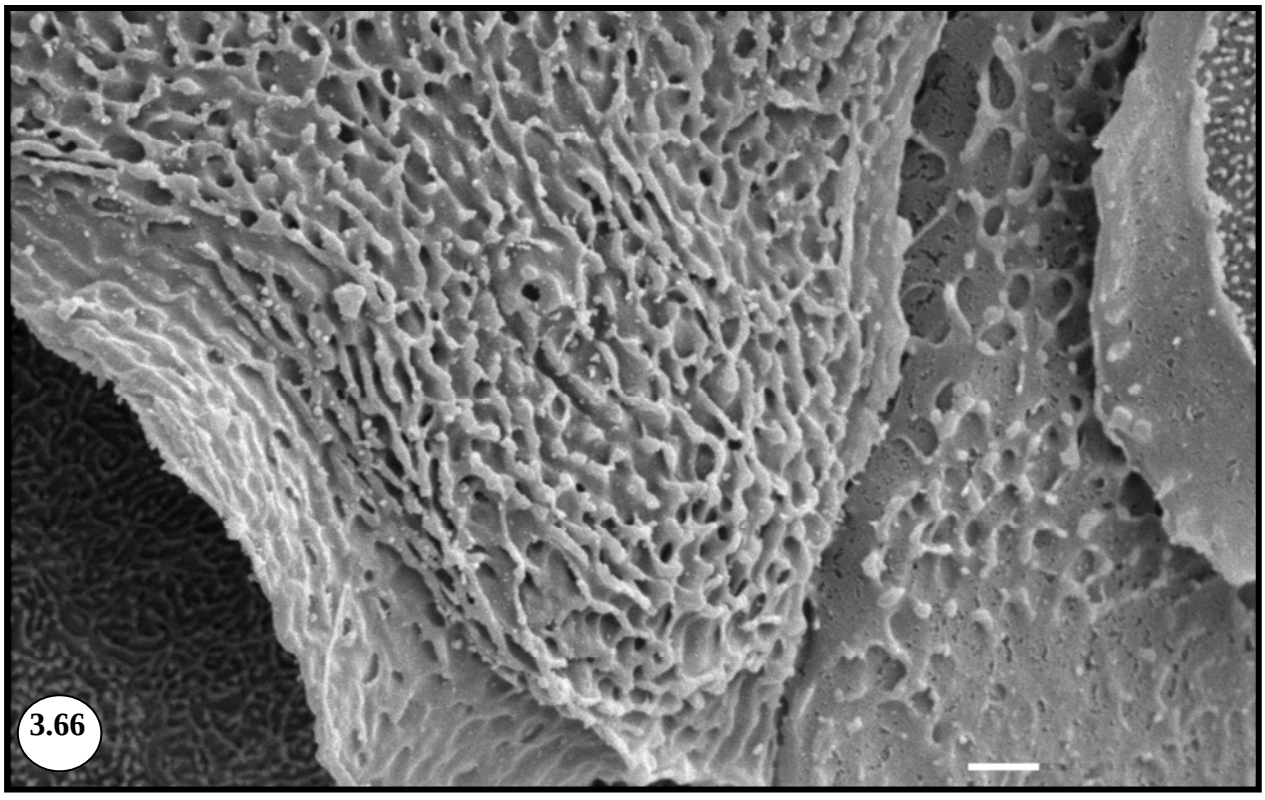


Figure 3.66: Detail of the pattern of microplicae evident on the surface cells of the ventral aspect of the pharyngeal fold. x6000; Bar = 1 µm.

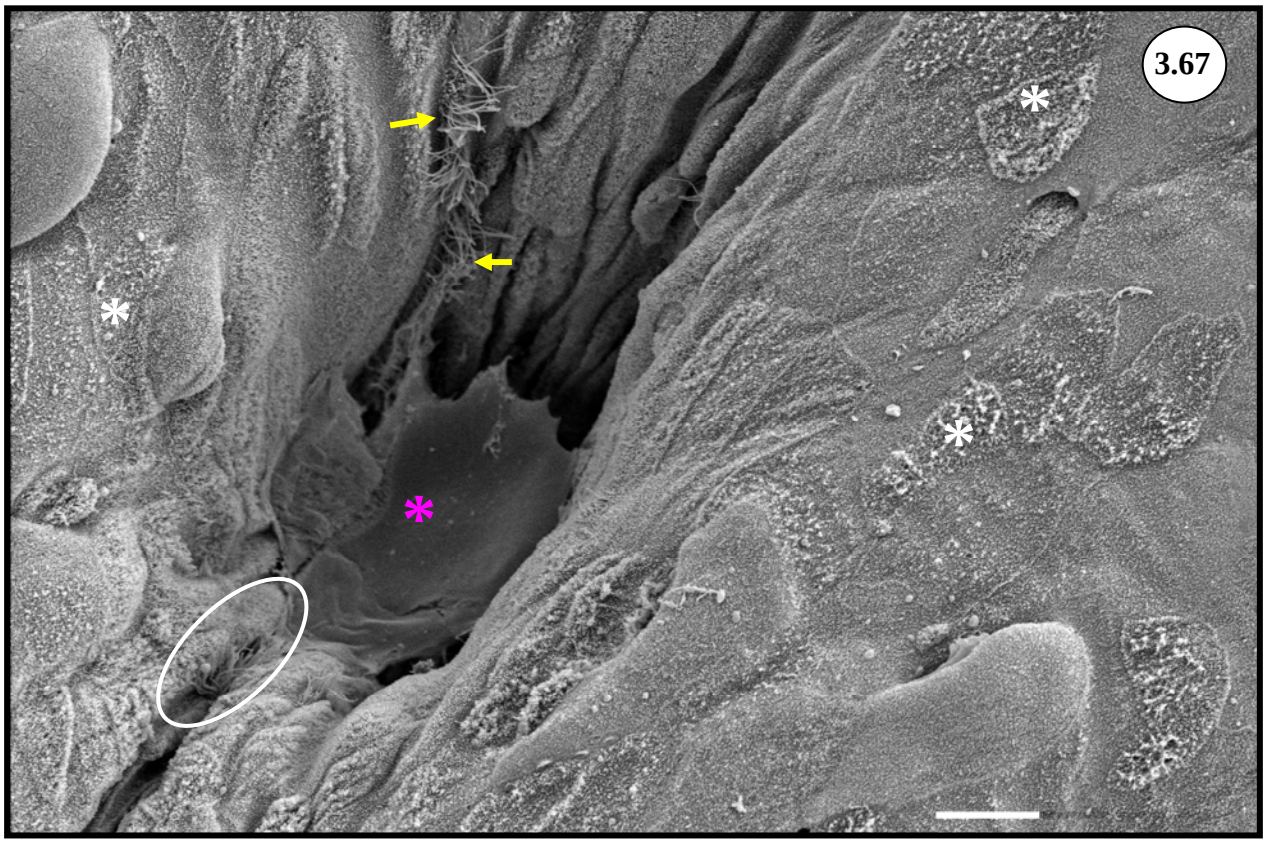


Figure 3.67: Large gland opening on the ventral surface of the pharyngeal fold revealing a mucus plug (pink *) filling the opening. Note the vertically aligned cells and ciliated cells (arrows and circled) associated with the duct opening. The cell surfaces in the vicinity of the opening display masses of microvilli (white *). x900; Bar = 10 µm.

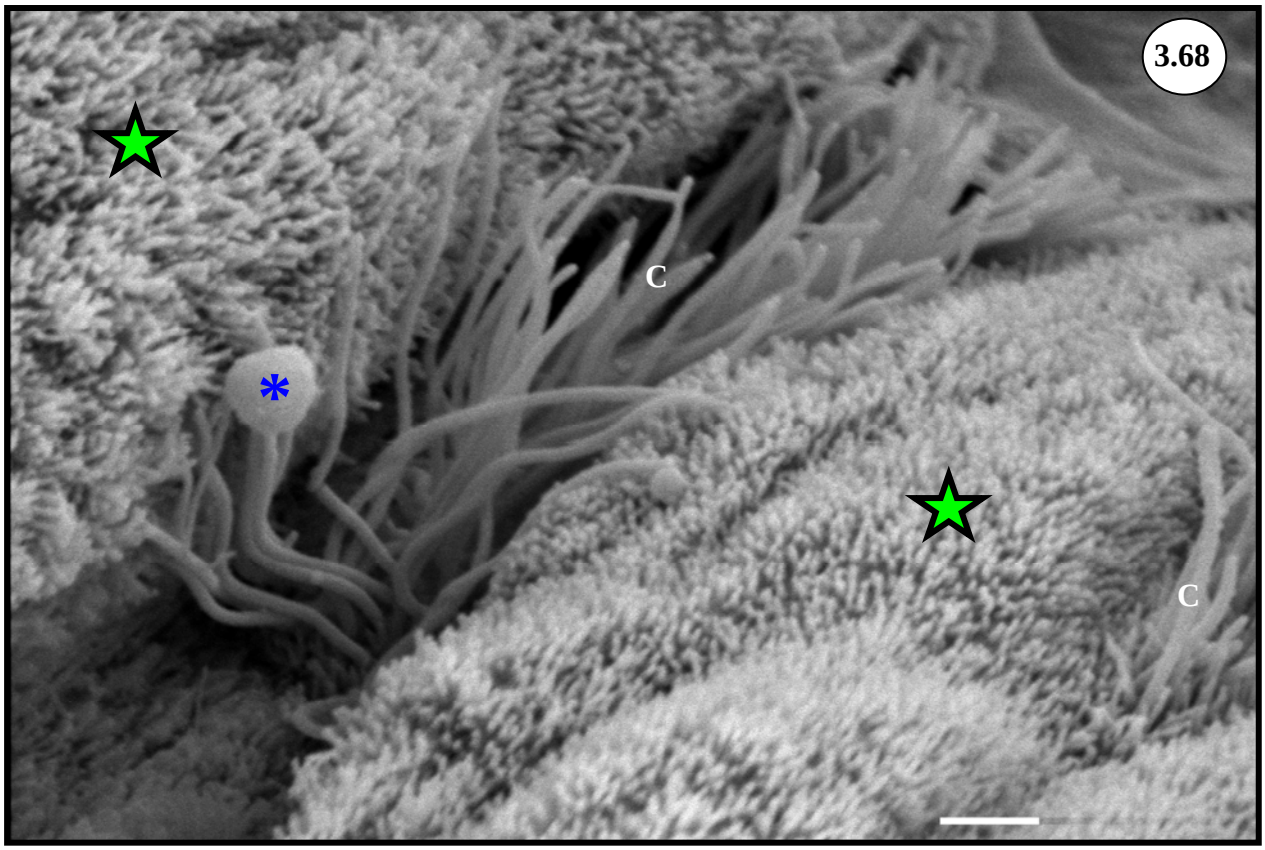


Figure 3.68: Enlargement of the encircled area in figure 3.67. The cell surfaces display masses of microvilli (green stars) and numbers of cilia (C). A globule (blue *) appears trapped by the cilia. x8500; Bar = 1 µm.

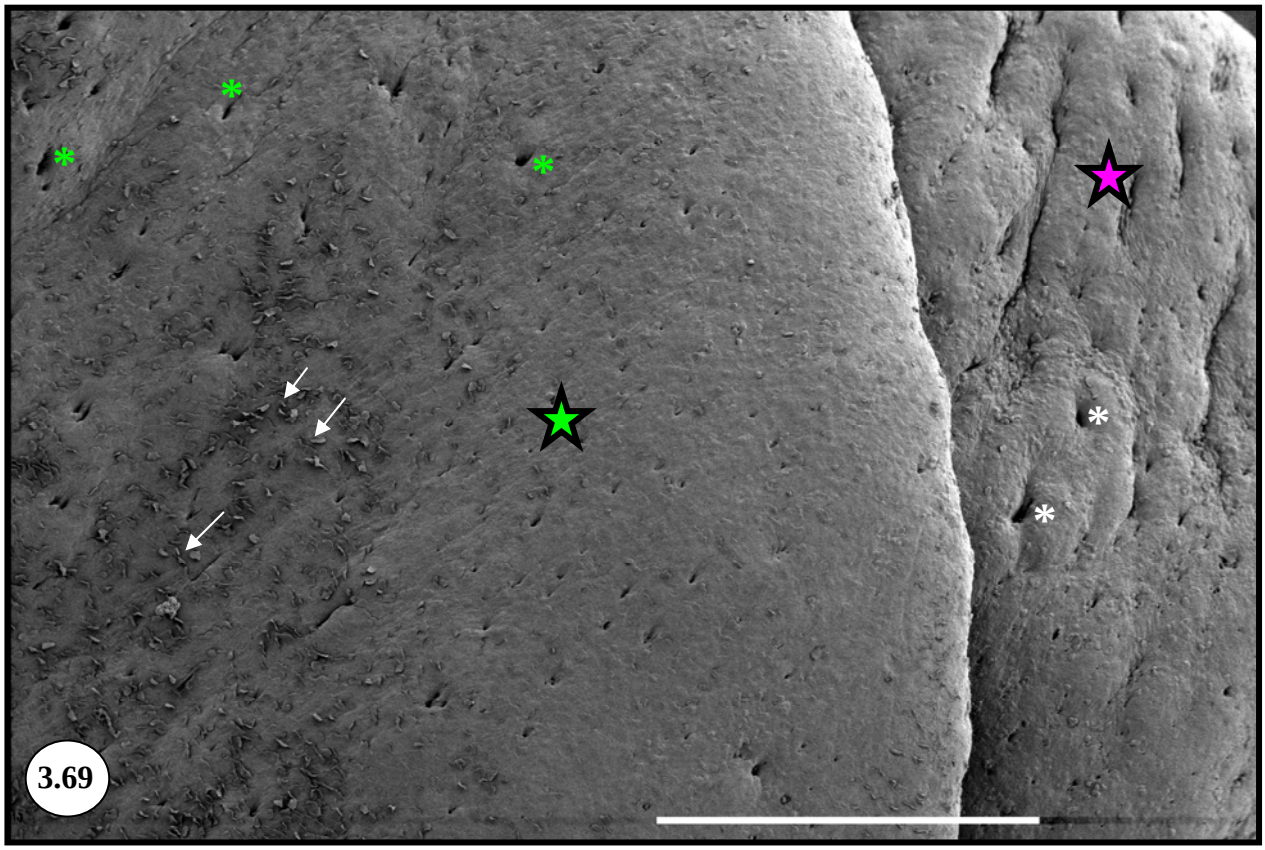


Figure 3.69: Ventral surfaces of the pharyngeal fold (green star) and caudo-lateral tissue projection (pink star). The most notable features are the large gland openings (green *) and desquamating cells (arrows). Note the crater-like features of the large gland openings (white *) on the caudo-lateral tissue projection. x33; Bar = 1 mm.

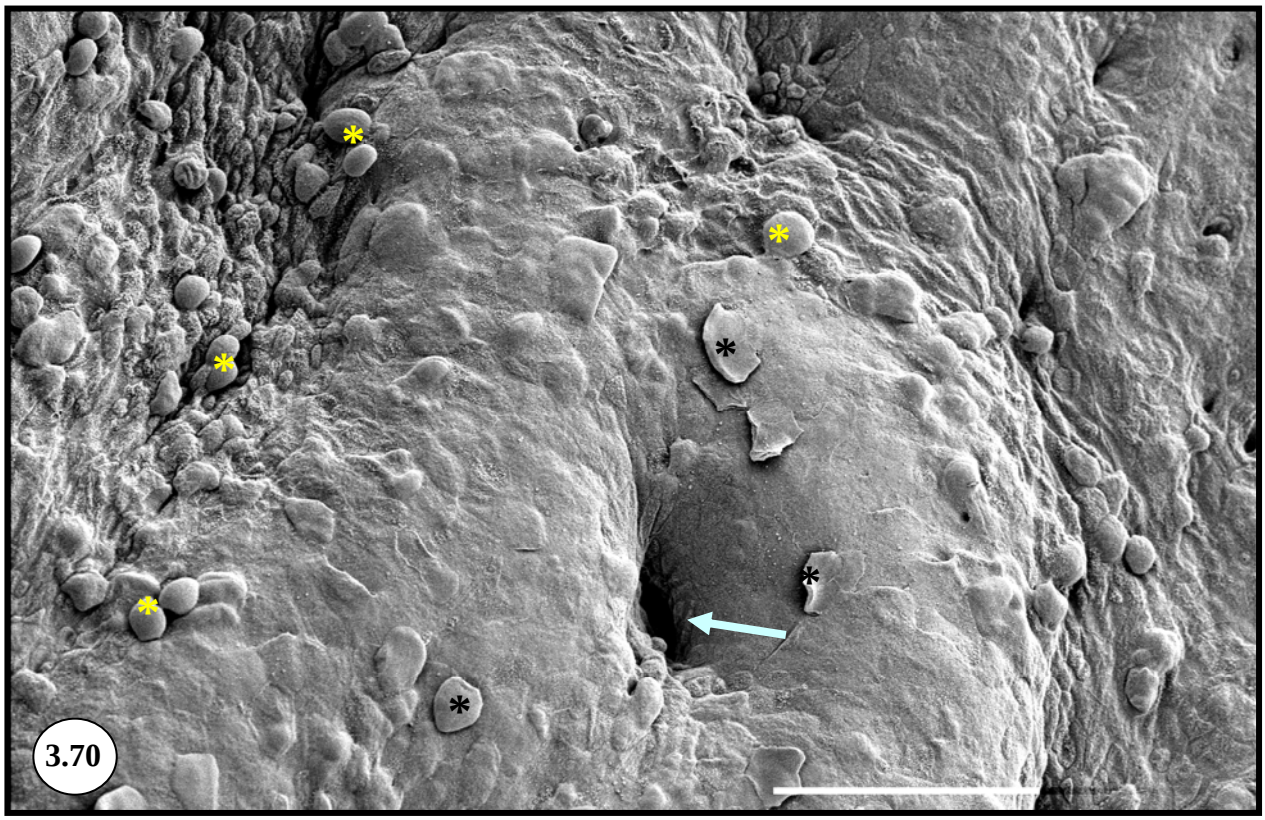


Figure 3.70: Large gland opening (arrow) on the caudo-lateral tissue projection of the pharyngeal fold. Note the raised nodules (yellow *) projecting off the surface, isolated desquamating cells (black *) and the raised rim of the gland opening. x230; Bar = 100 µm.

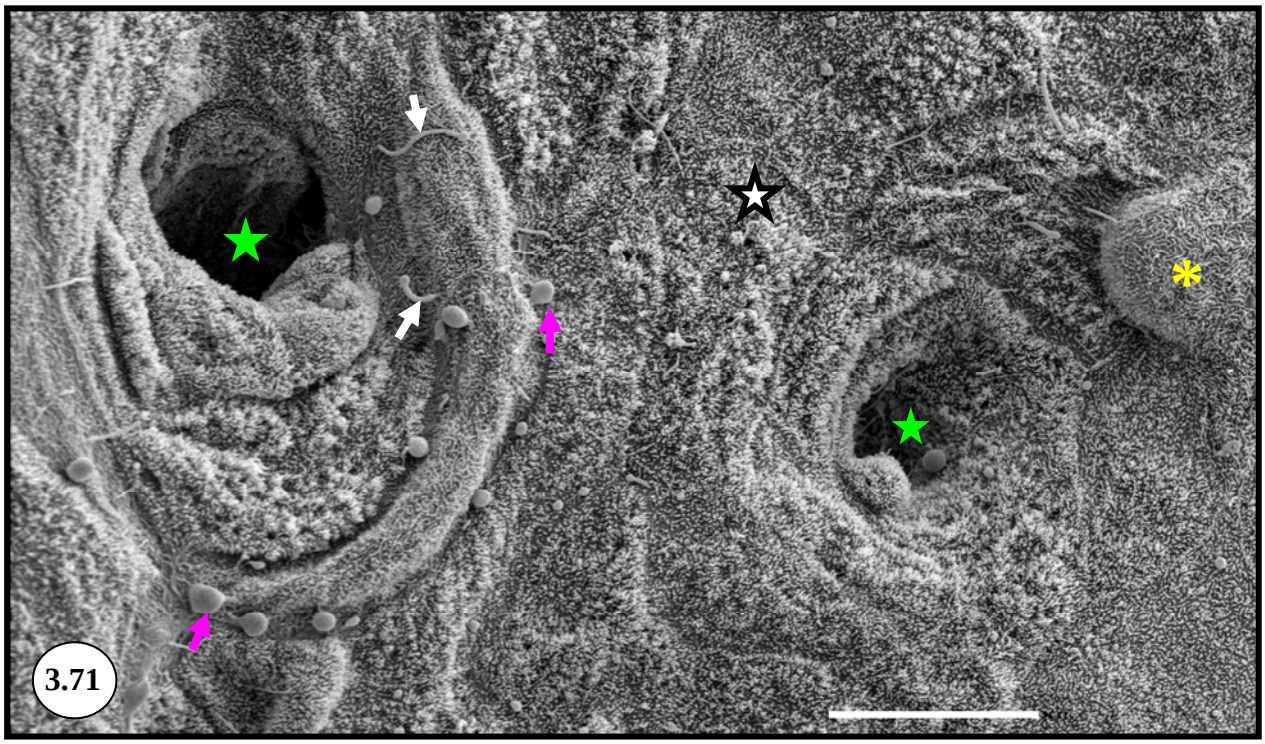


Figure 3.71: Small gland openings (green stars) of the caudo-lateral tissue projection of the pharyngeal fold. Note the microplicae of the nodule (yellow *) in contrast to the dense microvilli (white star) of the surface cells. Note also the circumferential arrangement of cells around the gland openings. Rod-like and club-shaped (white arrows) cell projections and globules (pink arrows). x1800; Bar = 10 µm.

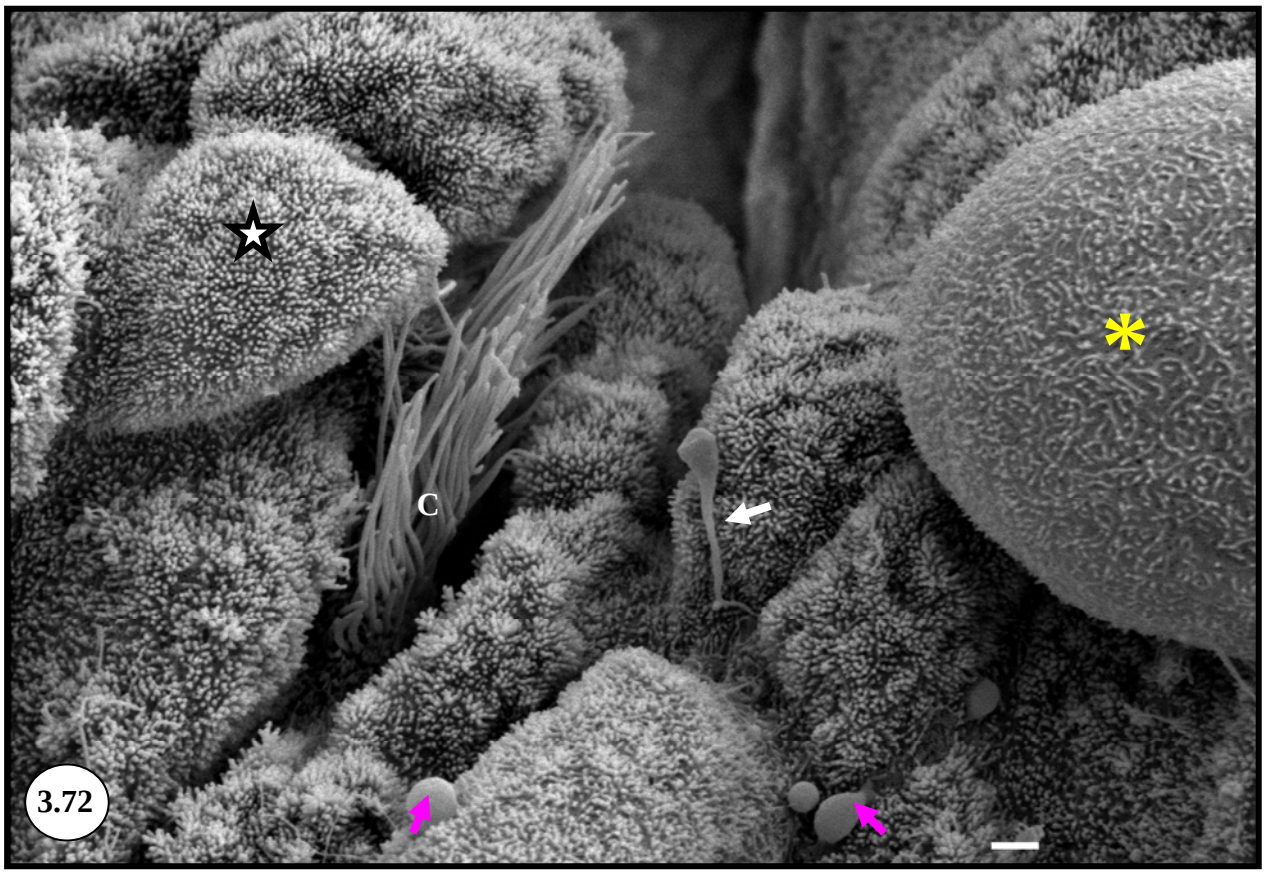


Figure 3.72: Detail of features of the caudo-lateral tissue projection of the pharyngeal fold illustrating the dense microvilli (star), a nodule (yellow *) with microplicae, cilia (C), club-shaped (white arrow) cell projection. Small globular structures are also visible (pink arrows). x4000; Bar = 1 µm.

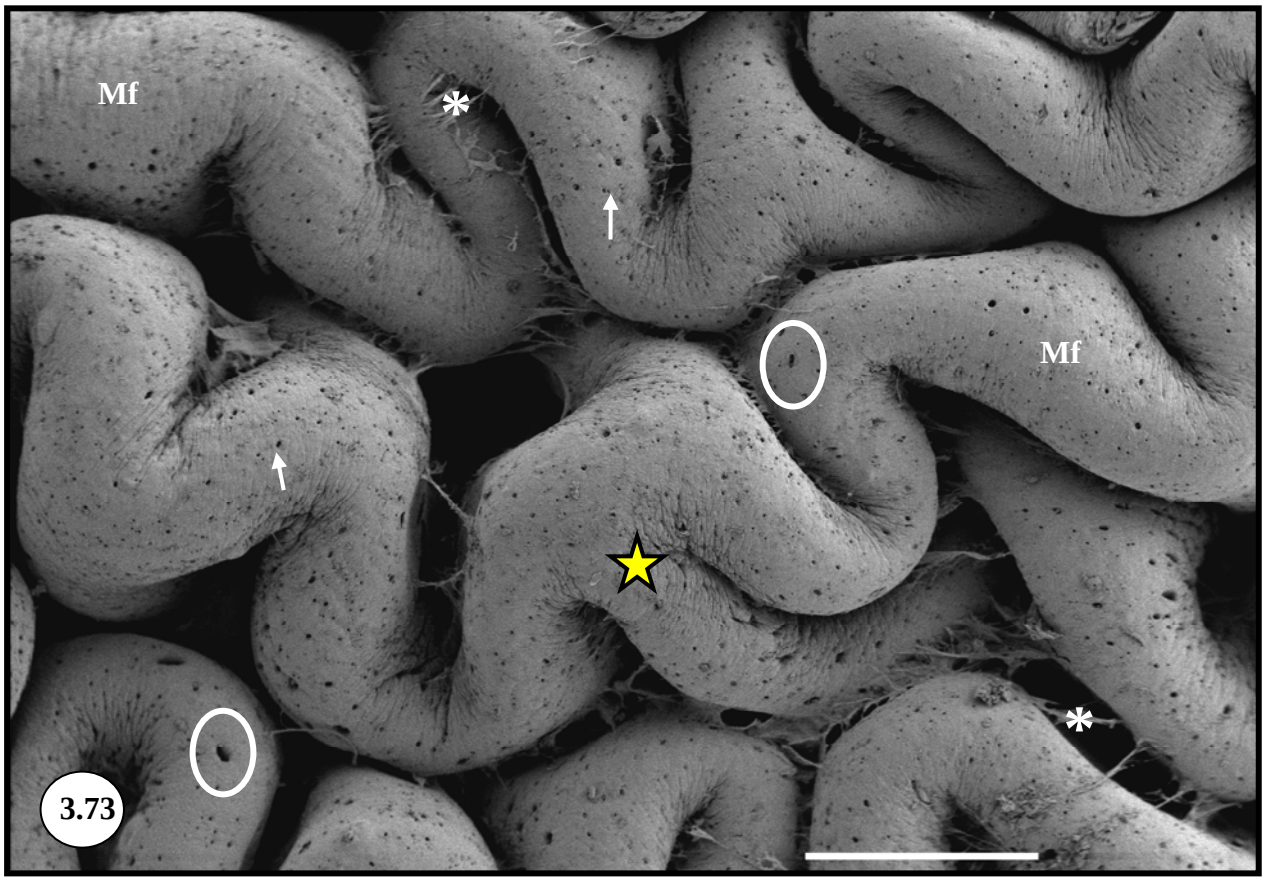


Figure 3.73: Low magnification of the longitudinal mucosal folds (Mf) of the proximal oesophagus. Note the wavy, convoluted appearance of the folds, a degree of branching (star) and the interconnecting strands of mucus (*). Numerous large (encircled) and small (arrows) gland openings occur throughout the folds. x20; Bar = 1 mm.

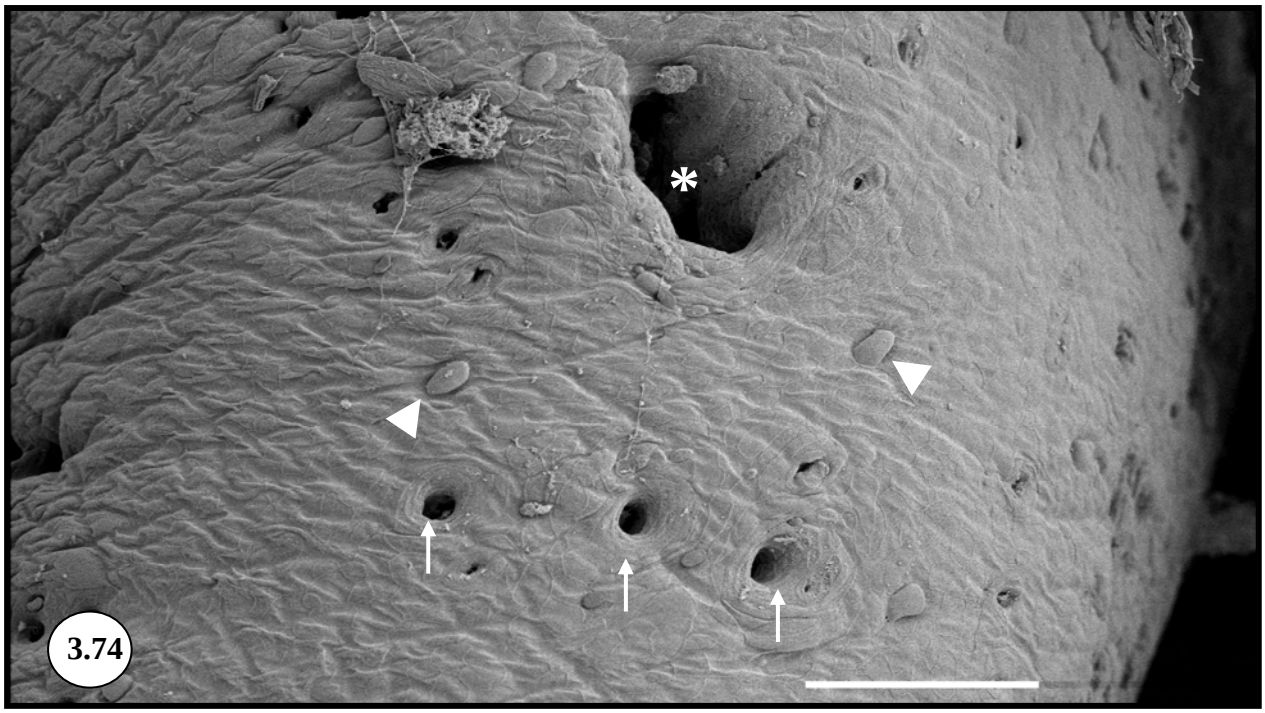


Figure 3.74: Higher magnification showing large (*) and small (arrows) gland openings, as well as raised nodules (arrow heads) on a mucosal fold of the proximal oesophagus. Note the relatively smooth surface devoid of obvious cell sloughing and the concentric arrangement of surface cells around the gland openings. x200; Bar = 100 µm.

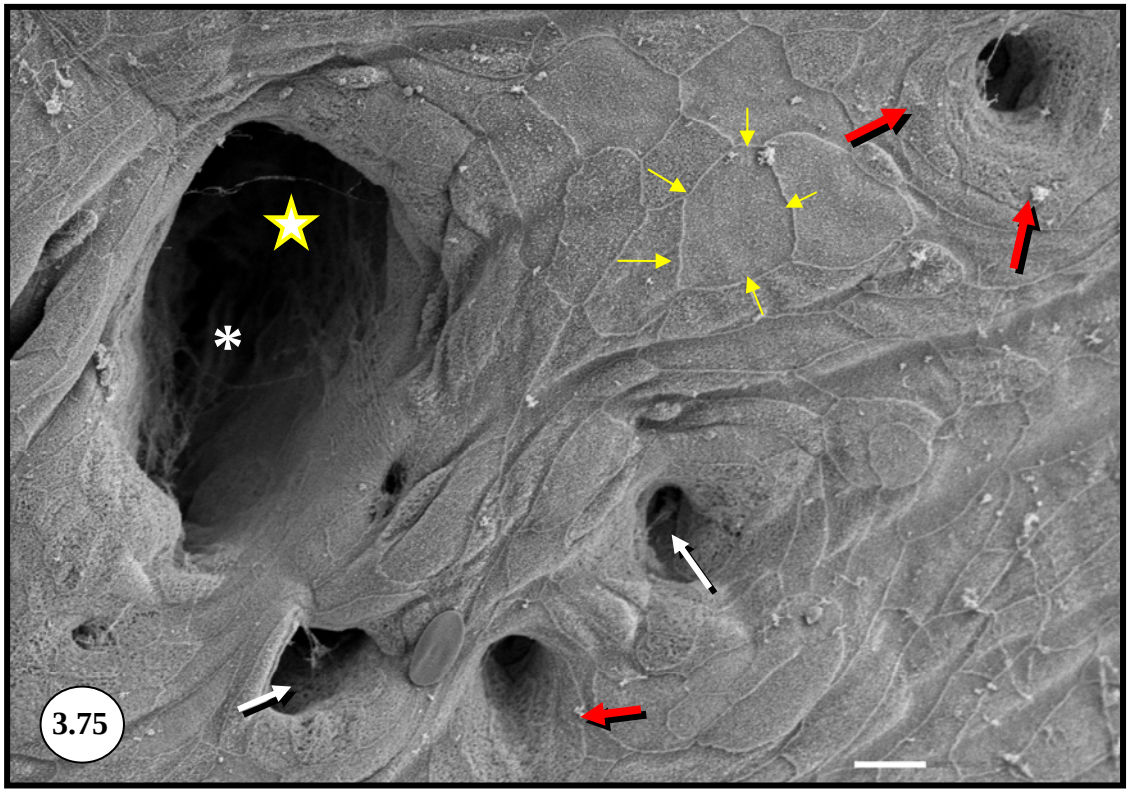


Figure 3.75: A large opening (star) surrounded by a cluster of small openings (white arrows) in the proximal oesophagus. Note the clear demarcation (yellow arrows) of the surface cell boundaries and the concentric arrangement of cells around the openings (red arrows). Mucus-secretion (*). x700; Bar = 10 µm.

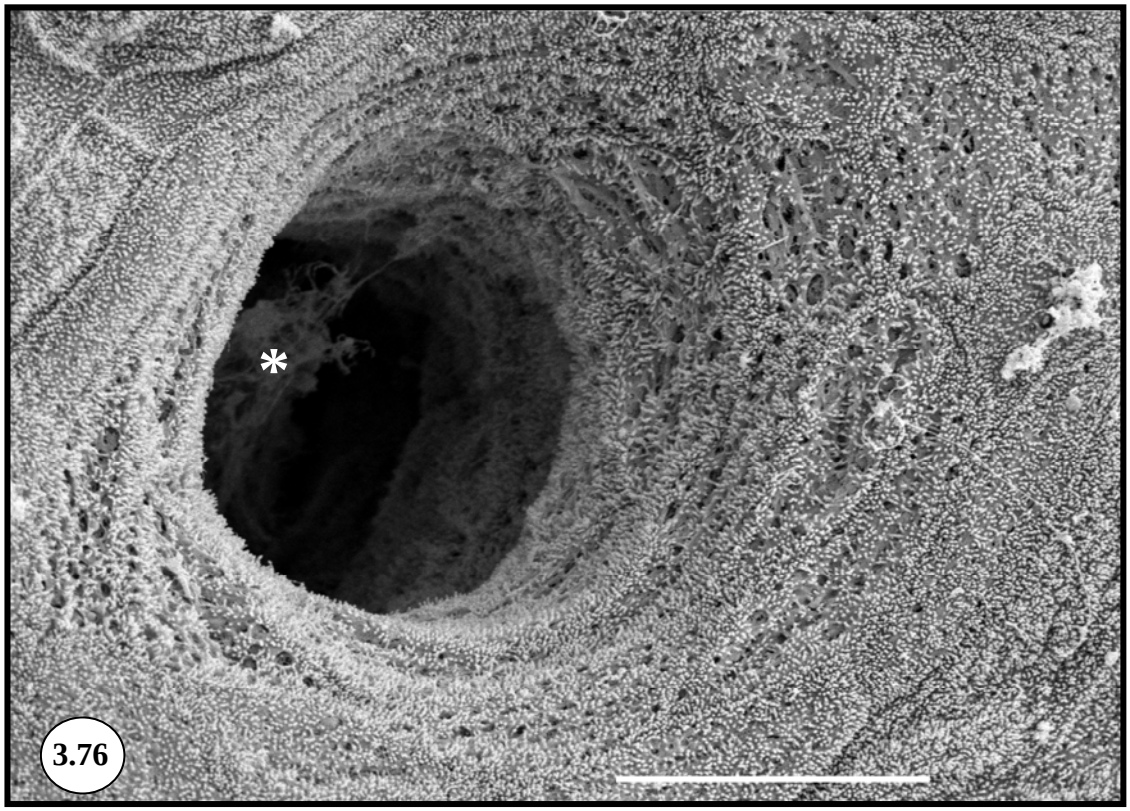


Figure 3.76: Mucus-secretion (*) partially protruding from a small gland opening of the proximal oesophagus. Note that the entire surface is covered by a dense mass of microvilli extending into the gland opening. x3000; Bar = 10 µm.

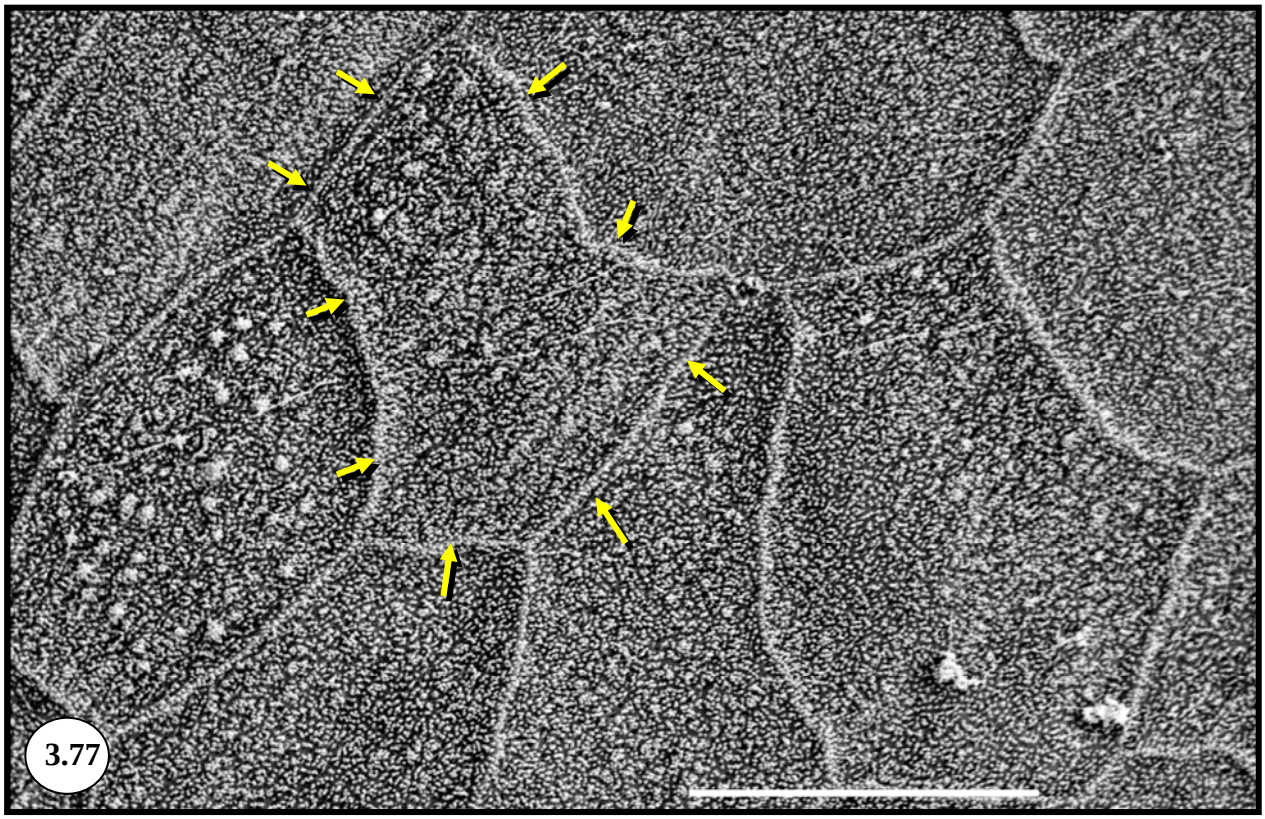


Figure 3.77: High magnification of the surface cells of the proximal oesophagus displaying clearly demarcated cell boundaries (arrows) and densely packed microvilli. Note the polygonal shape of the surface cells. x3000; Bar = 10 µm.

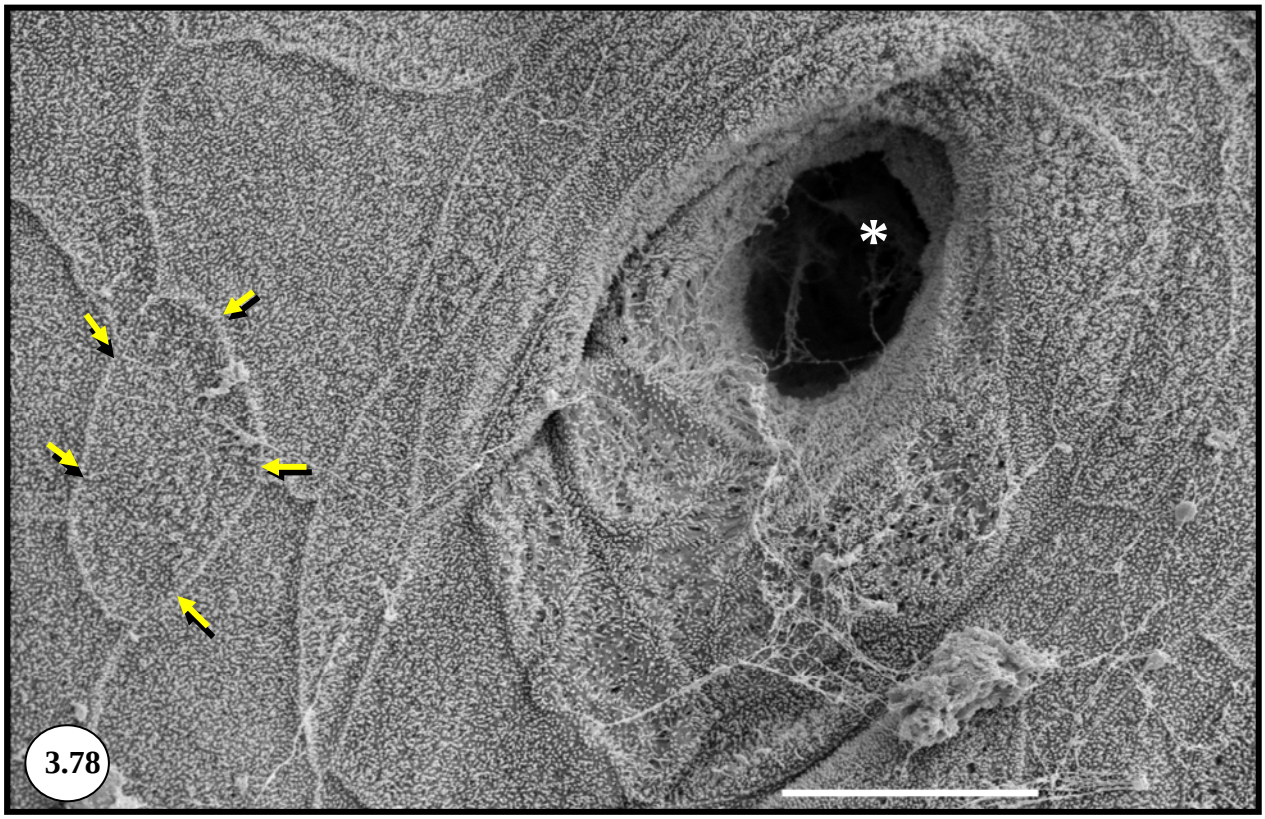


Figure 3.78: A small gland opening surrounded by concentrically arranged surface cells. Thread-like strands of mucus (*) lie in the duct of the gland and on the surface cells. Note the well-defined cell boundaries (arrows) and densely packed microvilli. x2200; Bar = 10 µm.

CHAPTER 4

GROSS MORPHOLOGY OF THE TONGUE

4.1 INTRODUCTION

The gross 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 (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 (Lucas, 1896, 1897; Gardner, 1926, 1927; Harrison, 1964). This information was subsequently utilised to classify the tongue of birds into various categories. Gardner (1926, 1927) for example, recognised eight categories based on the function and adaptations of this organ. Harrison (1964), on the other hand, proposed the classification of avian tongues into five functional groups, namely, tongues specialised for collecting food, eating, swallowing, taste and touch, and nest building.

Due to their commercial importance, the tongue and associated hyobranchial apparatus of domestic poultry have been described in detail (Hodges, 1974; McLelland, 1975; Gargiulo *et al*., 1991; Nickel *et al*., 1977; Homberger and Meyers, 1989; see Calhoun, 1954 for a review of the earlier literature).

During the past 180 years numerous publications on the ratite tongue have appeared in the form of sketches, descriptions and comparisons (Meckel, 1829; Cuvier, 1836; MacAlister, 1864; Gadow, 1879; Owen, 1879; Pycraft, 1900; Göppert, 1903; Duerden, 1912; Faraggiana, 1933; Roach, 1952; Feder, 1972; McCann, 1973; Cho *et al*., 1984; Fowler, 1991; Bonga Tomlinson, 2000; Gussekloo and Bout, 2005; Porchescu, 2007; Crole and Soley, 2008; Jackowiak and Ludwig, 2008; Tivane, 2008). Many of these studies, however, provide incomplete and sometimes misleading information on the macroscopic features of this organ. This situation is exacerbated by the fact that some descriptions are based on limited numbers of specimens ranging from embryos to fully mature birds, resulting in conflicting information that is difficult to interpret. The most comprehensive studies of a ratite tongue are those of Jackowiak and

Ludwig (2008) and Tivane (2008) on the ostrich, although the former authors neglected to reference any of the earlier literature on this topic.

To date there have only been four reports on the gross morphology of the emu tongue. The most complete description is that of Faraggiana (1933) who studied a single excised specimen of the tongue and laryngeal mound. Crole and Soley (2008) described the basic features of the emu tongue. In a study of feeding in palaeognathous birds, Bonga Tomlinson (2000), depicts the outline of the emu tongue in relation to the hyobranchial apparatus and surrounding mandibular rami, and briefly describes the presence of lingual papillae. Cho *et al*. (1984) simply note that "the emu tongue has a serrated edge".

This chapter presents the first definitive morphological description of the emu tongue and reviews, consolidates and compares the scattered information on the morphological features of the ratite tongue available in the literature. This study not only contributes to a better understanding of the upper digestive tract of the emu but also provides data that can be utilised for more meaningful future comparative studies of the ratite tongue.

4.2 MATERIALS AND METHODS

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

The specimens were rinsed in running tap water and each preserved head was used to provide information on the gross anatomical features of the tongue and its topographical relationships within the oropharyngeal cavity. This was achieved by incising the right commisure of the beak, disarticulating the quadratomandibular joint and reflecting the mandible laterally to openly display the roof and floor of the oropharynx (Fig. 4.1). The length (from the apex to the caudal edge of the caudal papillae) and width (between the tips of the last lateral papillae) (Fig. 4.2) of 16 tongues were measured and the lateral and caudal lingual papillae counted. The bill length

was measured on the mandibular rhamphotheca from the commisure to the rostral bill tip. Relevant anatomical features were described and recorded using a Canon 5D digital camera with a 28-135mm lens and a Canon Macro 100mm lens for higher magnification photographs.

Three tongues were removed from the heads by lifting the organ from the floor of the oropharynx and cutting through the frenulum as well as the paired *ceratobranchiale* and *urohyale* of the hyobranchial apparatus. The mucosa was stripped from the tongues to expose the intraglossal elements (Figs. 4.7, 4.8) of the hyobranchial apparatus.

The terminology used is that of Nomina Anatomica Avium (Baumel *et al*., 1993).

4.3. RESULTS

4.3.1 Topography

The tongue of the emu consisted of a rostral pigmented body and a caudal, variably pigmented root, both of which lay within the confines of the non-pigmented regions of the roof and floor of the oropharynx (Fig. 4.1). The tongue body occupied the middle third of the floor of the oropharynx and was a triangular structure with the apex pointing rostrally. The tongue root (Figs. 4.1, 4.4) extended from the caudal lingual papillae to the glottis and was flanked by, but did not extend to, the paired *ceratobranchiale* of the hyobranchial apparatus. In the closed gape, the caudal margin of the tongue body lay beneath and in contact with the rostral border of the choana, whereas the triangular tongue root fitted snugly into the rostral aspect of the choana. In some tongues the apex was observed, in the closed gape, to make contact with the base of the median palatine ridge which originated at the border of the pigmented and non-pigmented regions of the oropharyngeal roof.

4.3.2 Tongue body (*Corpus linguae*)

The tongue body was dorso-ventrally flattened (Fig. 4.5) with the dorsum slightly raised in the centre and sloping towards the margins. The body varied in length between 21-27 mm (average of 23.6 mm), and in width between 20-29 mm (average of 25.9 mm) (Fig. 4.2). The apex (*Apex linguae*) was rudimentary and varied in shape from a sharp point (Fig. 4.1), to a blunt or rounded tip. In some instances the apex was invaginated by a shallow groove forming two smaller points (Fig. 4.2). The dorsal surface (*Dorsum linguae*) was pigmented giving it an ash-grey/brown colour in formalin-fixed specimens (Figs. 4.1, 4.2). However, in the specimens used for scanning electron microscopy, the tongues were of variable pigmentation, ranging from pigmented papillae only, to pigment mainly associated with the dorsal blood vessels, to no pigmentation at all. The ventral surface (*Ventrum linguae*) (Fig. 4.6) was lighter in colour than the dorsal surface with the epithelium appearing glass-like (transparent). The rostro-medial region of the tongue ventrum was slightly concave. A conspicuous, light-coloured, finger-like line extended along the midline from the tip of the frenulum to end bluntly caudal to the apex (Fig. 4.6). This line represented the rostral projection of the *basihyale* (see below) (Fig. 4.8). From the rostro-lateral surfaces of the frenulum two raised bands (crura) (Fig. 4.6), were directed and tapered towards the apex. Numerous pale doughnut-shaped structures with a darker centre were clearly visible beneath both the dorsal and ventral surfaces of the tongue body (Figs. 4.2, 4.3, 4.6). Light microscopy confirmed that each of these structures constituted a glandular unit with a central lumen/duct opening onto the lingual surface (Crole and Soley, 2008; see Chapter 5). In some tongues, these structures were obscured due to a darker colouration of the dorsum and only the openings, resembling pits, were visible (Fig. 4.4).

4.3.3 Margins (*Margo linguae*)

The three margins of the tongue body displayed two sets of lingual papillae (Figs. 4.1, 4.2), the left and right lateral lingual papillae (*Papillae linguae laterales*) and the caudal lingual papillae (*Papillae linguae caudales*).

The first lateral papillae originated on either side of and just caudal to the apex. These were the smallest of the lateral papillae and were directed laterally or caudo-laterally. The rest of the papillae progressively pointed more caudo-laterally and became longer and more

slender. The last papillae were the longest and most caudally directed, and in some specimens exhibited a pale tip. In some instances individual papillae emanated from the base of adjacent papillae (Fig. 4.2) and not directly from the lingual margin. The number of papillae present on the lateral lingual margins was variable and not necessarily equal on both sides. Although the left and right lateral margins demonstrated a similar range of papillae (3-8 on the left side and 5-8 on the right side), there appeared to be a consistently higher number of papillae on the right margin than compared to the left. The average number of lateral papillae on the tongues studied totalled 11.2. The doughnut-shaped structures seen below the surface (Fig. 4.3) ended abruptly just beyond the root of the lingual papillae, although in the last lateral and caudal papillae they extended to the papillae tips.

The caudal lingual papillae (Figs. 4.1, 4.2, 4.4) were rudimentary and poorly defined compared to the lateral papillae and demarcated the caudal boundary of the tongue body. In some instances (n=4) the caudal papillae appeared as a fused, centrally positioned structure with variable incisures and small projections (Fig. 4.4). In other specimens (n=4) the fused component was flanked on either side by a single, more typical papilla. In a number of tongues (n=8) the fused component displayed a shallow median groove resulting in the formation of two median papillae which were accompanied by a variable number (0-2) of adjacent papillae (Fig. 4.2). The caudal papillae varied in number between 1-4 (average 2.5). In one specimen, a structure similar in appearance to a lingual papilla was observed to project dorsally from the mucosa covering the left *ceratohyale*, just caudal to the last lateral papilla.

4.3.4 Tongue root (*Radix linguae*)

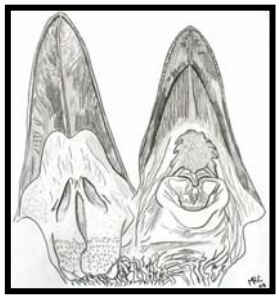
The tongue root (Figs. 4.1, 4.4) was a fleshy triangular structure, which in most specimens was non-pigmented. The caudal extremity of the root ended as a rounded raised bulbous structure (pigmented in some specimens) that extended into the rostral aspect of the laryngeal fissure (glottis). The mucosa of the tongue root was continuous with the rest of the mucosa covering the oropharyngeal floor and formed a shallow groove where it abutted the paired *ceratobranchiale* and the raised margins of the laryngeal fissure (Fig. 4.4). The surface of the root displayed the same doughnut-shaped structures seen on the tongue body, particularly in the midline. A shallow retrolingual recess existed between the ventral aspect of the caudal lingual papillae and the tongue root.

4.3.5 Frenulum (*Frenulum linguae*)

The frenulum (Figs. 4.5, 4.6) was a fleshy non-pigmented structure attaching the caudal half of the tongue body to the oropharyngeal floor. It was triangular in shape, with the rostral attachment to the ventrum of the tongue forming the point of the triangle. The mucosa along the lateral edges was thrown into longitudinal folds. These folds were obliterated when the tongue body was lifted dorsally from the oropharyngeal floor (Fig. 4.5). The rostral point of the frenulum housed the body of the *basihyale* while the two lateral edges enclosed the rostral parts of the paired *ceratobranchiale* which merged rostrally with the body of the *basihyale* (Fig. 4.6). Extending caudally from the body of the *basihyale*, along the midline, was the *urohyale*, also housed within the frenulum (Fig. 4.6) (see also Fig. 4.8).

4.3.6 Lingual skeleton

The lingual skeleton consisted of the *paraglossum* and the rostral projection of the *basihyale*, both of which were imbedded within the tongue body (Figs. 4.7, 4.8). The *paraglossum* was a broad, thin, teardrop-shaped cartilaginous plate imbedded within the lingual parenchyma. The rostral tip was pointed while the base varied from gently rounded, to scalloped. The *paraglossum* was situated dorsal to the rostral projection of the *basihyale*, to which it was attached by loose connective tissue. The *basihyale* ran almost the full length of the *paraglossum*, ending near its rostral tip. The edges of the *paraglossum* did not extend to the apex or lingual margins or into any of the lingual papillae.



4.4 DISCUSSION

4.4.1 Topography

There is no definitive information in the literature on the topography of the emu tongue within the oropharynx. The sketch by Faraggiana (1933) shows the tongue in relation only to the laryngeal mound whereas Bonga Tomlinson (2000) simply depicts the outline of the emu tongue body in relation to the hyobranchial apparatus and mandibular rami. From the specimens examined in the current study it was observed that the apex of the tongue did not extend further than half the distance from the commisure to the rostral bill tip. This contrasts with the positioning of the tongue body indicated by Bonga Tomlinson (2000), which shows it to occupy a far more rostral position relative to the surrounding structures. However, despite differences in the appearance of the various ratite tongues, the topographical relationships of this organ in the emu are generally similar to those illustrated in the ostrich (Göppert, 1903; Faraggiana, 1933; Bonga Tomlinson, 2000; Porchescu, 2007; Jackowiak and Ludwig, 2008; Tivane, 2008), greater rhea (Gadow, 1979; Pycraft, 1900; Faraggiana, 1933; Gussekloo and Bout, 2005), cassowary (P. Johnston, personal communication) and kiwi (Owen, 1879; McCann, 1973).

The general shape of the tongue in birds usually mimics that of the bill (Bradley, 1915; McLeod, 1939; Harrison, 1964; Koch, 1973; Hodges, 1974; Nickel *et al*., 1977) or the palate (McLelland, 1979). However, in comparison to other bird families, the ratite tongue is greatly reduced in length relative to the bill (Faraggiana, 1933; Ziswiler and Farner, 1972; McLelland, 1979; Bailey *et al*., 1997; Bonga Tomlinson, 2000; Gussekloo and Bout, 2005; Jackowiak and Godynicki, 2005; Jackowiak and Ludwig, 2008), a feature also noted in the emu (see Table 4.1). Tongue structure in birds is highly variable and closely related to feeding (McLelland, 1979), with the ratite tongue being described as a rudimentary or vestigial organ adapted for rapid swallowing of large food items (Gadow, 1879; Pycraft, 1900; McLelland, 1979; Bonga Tomlinson, 2000). Two specific adaptations of the avian tongue for swallowing have been recognised, namely, the occurrence of caudally directed lingual papillae (Harrison, 1964; McLelland, 1979; King and McLelland, 1984) and/or a reduction in tongue size (McLelland, 1979). The emu tongue body displays both of the above mentioned adaptations, as does that of the cassowary (P. Johnston, personal communication). Two reasons for tongue reduction in ratites can be advanced. In birds that swallow food whole (Harrison, 1964; McLelland, 1979) the tongue is unnecessary and

therefore rudimentary (Harrison, 1964; King and McLelland, 1984) as well as non-protrusable (King and McLelland, 1984). It is also suggested that because of the cranioinertial feeding method employed by ratites, a longer tongue extending to the bill tip would be injured due to the rapid bill closure involved in this feeding method (Bonga Tomlinson, 2000).

4.4.2 Shape

There are surprisingly few accounts documenting the general appearance of the emu tongue, with both Fowler (1991) and Sales (2006, 2007) simply quoting the observation of Cho *et al*. (1984) that "the tongue of the emu has a serrated edge". The fringed appearance of the emu tongue body is also illustrated by Bonga Tomlinson (2000). The most comprehensive description of the general shape of the emu tongue is that of Faraggiana (1933) who described the basic features noted in this study. However, as this author was limited to a single specimen, some differences were apparent. In addition to the rounded apex described by Faraggiana (1933), pointed or split apices were observed whereas the tongue body appeared broader than that depicted in the earlier study.

It is clear from previous studies that the shape of the tongue body differs between ratites (Cho *et al*., 1984). These differences in tongue shape are compared in Table 4.1 and indicate that the tongues of the emu and cassowary (P. Johnston, personal communication) share similar gross morphological features. It should be noted, however, that it is not only tongue shape that differs between ratites. The appearance of the tongue body margins, tongue root, the prevalence of pigmentation, tongue size relative to the length of the bill, the occurrence of special features (for example, the lingual pocket in the ostrich), and the shape and composition of the *paraglossum* all define differences in ratite tongue structure and appearance (see Table 4.1).

It is also noteworthy that in birds with an omnivorous diet the tongue conforms to a generalised pattern described as triangular with a pointed apex, with the chief adaptive feature being that of caudally pointing spines (papillae) on the caudal margin (Gardner, 1927). This statement would certainly be true for the emu, which also enjoys a varied diet (Davies, 1978).

Table 4.1 Comparative features of the ratite tongue

Species	Body shape	Root shape	Pigmentation	Body margins	[+]Tongue length compared to lower bill length (%)
Emu (*Dromaius novaehollandiae*)	Triangular[15, 20]	Triangular[15, 20]	Body: Yes[15, 20], variable[21] Root: Variable[20]	Serrated [9, 13, 14, 15, 20] Lateral[9, 14, 15, 20] and caudal papillae[9, 15, 20]	20.8[#] – 23.8[#]
Ostrich (*Struthio camelus*)	Triangular or ∩-shaped [4, 6, 13, 14, 17, 18] Short and or blunt[3, 4, 6, 8, 13, 14, 17, 18], caudal "lingual pocket"[1, 2, 9, 14, 16, 17, 18]	Flat[17, 18, 21]	Body: No[18] Root: No[18, 21]	Smooth [18] Two caudolateral projections (Lingual horns) [1, 2, 7, 9, 17, 18]	20[9] - 21.4[#] 25[17]
Greater Rhea (*Rhea americana*)	Triangular with rounded apex[9, 21]	Flat[21]	Body: Yes,[9, 11] the lingual horns not[9, 21] Root: No[21]	Smooth [9, 14] Two globose, bilateral caudolateral papillae[14], Two caudal lingual horns/projections [9, 21]	19[#] - 20.9[#]
Darwin's rhea (*Pterocnemia pennata*)	V-shaped with pointed apex [13]	-	-	Smooth[13]	-
Cassowary (*Casuarius casuarius*)	Triangular, longer than wide [4] Rostral rounded apex free of papillae, no caudal papillae[19]	Flat[19]	Body: No[19] Root: No[19]	Backward pointing tips[4], Denticulate [9] Similar to the emu but a different pattern[19]	13[19]
Kiwi (*Apteryx australis mantelli*) (*Apteryx haasti*) (*Apteryx oweni*)	Triangular Long-pyriform; tip obtuse, retuse or truncate.[12] Oblong, constriction below transverse midline; apex truncate or retuse.[12] Similar to *A. haasti*, with larger constriction.[12]	(Depicted, but not labelled [12])	No[5, 12] No[12] No[12]	Smooth[5, 12] Blunt[12] Folded[12]	9.5* – 14.2*

[+] These are approximate measurements. * Extrapolated from the measurements in Roach (1952) (Species not mentioned); [#]Own measurements; (Underlined names indicate a sketch is supplied, bold indicates photographs.)

[1] Meckel (1829) [2] Cuvier (1836), [3]MacAlister (1864), [4]Gadow (1879), [5]Owen (1879), [6]Pycraft (1900), [7]Göppert (1903), [8]Duerden (1912), [9]Faraggiana (1933), [10]Roach (1952), [11]**Feder** (1972), [12]McCann (1973), [13]Cho *et al.* (1984), [14]Bonga Tomlinson (2000), [15]Crole & Soley (2008), [16]**Porchescu** (2007), [17]**Jackowiak & Ludwig** (2008), [18]**Tivane** (2008), [19]P. Johnston (Personal communication), [20]**Present study**, [21]Personal observation.

4.4.3 Lingual papillae

Lingual papillae (dorsal, lateral and caudal) are a common feature of the avian tongue and have been described in numerous species (Gardner, 1926, 1927; McLelland, 1979; King and McLelland, 1984; Bailey *et al*., 1997; Kobayashi *et al*., 1998; McLelland, 1990) including domestic poultry (Calhoun, 1954; Ziswiler and Farner, 1972; McLelland, 1975; Nickel *et al*., 1977; King and McLelland, 1984; McLelland, 1990). However, it would appear that lingual papillae are not a common or well-developed feature in ratites (Table 4.1), a characteristic also noted by Bonga Tomlinson (2000). Apart from the lateral papillae of the emu and cassowary (Gadow, 1879; Pycraft, 1900) the rest of the ratites documented display smooth lateral tongue margins. In the little spotted kiwi (McCann, 1973) the lateral tongue margins are narrowly infolded, but show no papillae.

The lateral lingual papillae of the emu tongue show a lack of bilateral symmetry which involves differences in both number and shape, with a greater number of papillae usually being observed on the right margin. Faraggiana (1933) also noted that the number of papillae were not the same on each side of the tongue body whereas Bonga Tomlinson (2000) provides a definitive number of five lingual papillae on the lateral margins. In contrast, as noted in this study, the numbers of papillae display a normal variation between specimens of 3-8 on the left and 5-8 on the right margins.

The caudal lingual papillae of the emu tongue are rudimentary compared to other bird species and even though identifiable, are often not well-developed. The sketch by Bonga Tomlinson (2000) neglects to depict the caudal lingual papillae in this species. In comparison to the other ratites, the emu appears to be the only member which possesses structures recognisable as caudal lingual papillae (Table 4.1). However, in the ostrich and greater rhea (Table 4.1) the caudo-lateral aspect of the tongue body displays papillae-like extensions. Whether these structures represent true caudal lingual papillae remains undetermined.

The function of the lingual papillae is reportedly to assist in the aboral transport of food (McLelland, 1979; King and McLelland, 1984). In the emu the lingual papillae may be instrumental in removing smaller food particles from the roof of the oropharynx in a similar fashion to that proposed by Bonga Tomlinson (2000) for palaeognathous birds (see below).

4.4.4 Tongue root

Some confusion exists in the literature regarding the naming of the caudal extremity of the tongue body (the tongue base) and the tongue root (Moore and Elliott, 1946) with both terms being used interchangeably (McLelland, 1975). In domestic poultry the tongue is clearly defined into a free rostral tip (apex), a body and a caudal root (McLelland, 1993). Descriptions of the tongue using this terminology exist for a number of species (see, for example, Faraggiana, 1933; Bailey *et al*., 1997; Jackowiak and Godynicki, 2005; Jackowiak and Ludwig, 2008). Based on the work of Lillie (1908) and Bradley (1915) it is generally accepted that the border between the tongue body and root is the row of caudal lingual papillae (Moore and Elliott, 1946; Gentle, 1971; Nickel *et al*., 1977; Bailey *et al*., 1997). Some authors appear to use the term 'tongue base' synonymously with 'tongue root' (Nickel *et al*., 1977; Gussekloo and Bout, 2005). In some studies the caudal aspect of the tongue body has been termed the tongue base (Warner *et al*., 1967; McLelland, 1975; Bhattacharyya, 1980; Bonga Tomlinson, 2000) or even the tongue root (Koch, 1973; McLelland, 1979; McLelland, 1990; Kobayashi *et al*., 1998) whereas in other publications the term tongue base is used but not defined (Bacha and Bacha, 2000; Calhoun, 1954). Alternative terminology used for the tongue root includes the posterior part of the tongue (Gentle, 1971), the sensory area (Bhattacharyya, 1980) and the preglottal part of the tongue (Homberger and Meyers, 1989; Liman *et al*., 2001).

The importance of clarity in correctly identifying and naming the various components of the tongue has been pointed out by Moore and Elliott (1946), particularly in regard to the location of taste buds. Failure to recognise the caudal aspect of the tongue (the tongue root) as part of the tongue could lead to invalid conclusions about the presence of taste buds in this organ, as they are reportedly concentrated in this region (Moore and Elliott, 1946; Gentle, 1971; Nickel *et al*., 1977; Bacha and Bacha, 2000; Al-Mansour and Jarrar, 2004).

A clearly defined triangular structure represents the tongue root in the emu and is positioned between the caudal margin of the tongue body and the laryngeal entrance. This structure seems to be unique to the emu as in other ratites the tongue root is represented by a featureless stretch of mucosa (Table 4.1). The structure of the tongue root in kiwi species (McCann, 1973) is unclear. The extension of the tongue root into the rostral aspect of the laryngeal entrance (Faraggiana, 1933; present study) represented an interesting modification not observed or illustrated in other ratites (ostrich and greater rhea) (Göppert, 1903; Faraggiana, 1933; Gussekloo

and Bout, 2005; Porchescu, 2007; Jackowiak and Ludwig, 2008; Tivane, 2008). The positioning of the tongue root would also appear to assist in sealing the rostral part of the larynx when the glottis is closed, almost assuming the role of an epiglottis, which is not present in birds (Kaupp, 1918; Calhoun, 1954; King and McLelland, 1984; Nickel *et al*., 1977). This argument regarding the role of the tongue root functioning as an epiglottis in the emu has been proposed by Gadow (1879) but disputed by Faraggiana (1933). The tongue root of the emu also appears to play a special role in assisting to close off of the rostral aspect of the choana in the closed gape. The choana of most birds is divided into a rostral slit-like part (*pars rostralis*) and a caudal triangular part (*pars caudalis*) (King, 1993) with the tongue commonly closing off the rostral part of the choana (McLelland, 1975, 1979). In the emu, the triangular choana (Fig. 4.1) is not divided into rostral and caudal parts and therefore the tongue body plays no part in closing off the choana in the closed gape. Instead, the tongue root partially closes off the rostral aspect of the choana in this species.

4.4.5 Frenulum

Little mention is made in the literature of the frenulum in birds. A possible reason for this may be its general lack of remarkable features, serving simply to attach the tongue to the oropharyngeal floor (McLelland, 1979). In the emu, the frenulum is a relatively large structure which houses part of the hyobranchial apparatus. The lateral margins are longitudinally folded which would seem to indicate that the tongue is capable of a certain degree of movement. This observation lends further support to the role played by the tongue of palaeognaths in cranioinertial feeding and in drinking. During swallowing in palaeognaths the tongue is lifted and contacts the palate before moving caudally, thereby scraping any food caudal to the tongue into the proximal oesophagus (Bonga Tomlinson, 2000). Palaeognaths transport food from their bill tips to the oesophageal entrance via the cranioinertial feeding method (Bonga Tomlinson, 2000), also described as the 'catch and throw' method by Gussekloo and Bout (2005). The transport of food into or close to the oesophageal entrance is facilitated by a large gape and marked depression of the tongue. Tongue depression enlarges the 'buccal cavity' (oropharyngeal cavity), which assists in moving food to the caudal oropharynx, while retraction of the tongue assists in the final transport of fluid to the oesophagus during drinking (Gussekloo and Bout, 2005). Therefore, despite the emu tongue showing such relatively reduced dimensions and rigidity, it possess a surprisingly large range of movements in both the rostro-caudal (though

unable to protrude) and dorso-ventral planes by virtue of the relatively large, folded frenulum and the association of the hyobranchial apparatus with the tongue body and frenulum.

4.4.6 Lingual skeleton

The lingual skeleton of the emu is formed by the median, unpaired *paraglossum* and the rostral projection of the *basihyale* of the hyobranchial apparatus. The *paraglossum* is related dorsally to the rostral projection of the *basihyale* as also described by Bonga Tomlinson (2000) in the emu and the greater rhea. However, the findings of this study contrasted with those of Bonga Tomlinson (2000) in that the rostral projection of the *basihyale* extended further rostrally, ventral to the *paraglossum,* than that depicted by the above author.

The *paraglossum* of the emu was teardrop-shaped with a pointed rostral tip and a rounded base, while, it is depicted by Parker (1866), in *Dromaius irroratus*, as inverted heart-shaped and by Bonga Tomlinson (2000), in *Dromaius novaehollandiae*, as arrowhead-shaped. In ratites the *paraglossum* remains cartilaginous and does not ossify in older birds (Bonga Tomlinson, 2000), a situation also apparent in the emu (see Chapter 5). The shape of the *paraglossum* differs between the ratites. The *paraglossum* of the emu (*Dromaius irroratus* and *novaehollandiae*), rhea (*Rhea americana*) and cassowary (*Casuarius bennetii*) are all basically arrowhead shaped, although individual differences are apparent, particularly regarding the form of the base (Parker, 1866; Bonga Tomlinson, 2000; present study). The *paraglossum* of the kiwi (*Apteryx australis*) (Parker, 1891) is also a single structure but is much narrower than that of the emu, rhea and cassowary and has a split, elongated base. The ostrich *paraglossum* is divided into two narrow *paraglossalia* which flank the rostral projection of the *basihyale* and are located ventro-lateral to it (Bonga Tomlinson, 2000; Tivane, 2008). This arrangement differs radically from that of the emu and the other ratites, where the rostral projection of the *basihyale* lies ventral to the *paraglossum*, and has lead to some authors not recognising or misinterpreting the narrow, paired structure (Meckel, 1829; Parker, 1866; Webb, 1957; Jackowiak and Ludwig, 2008).

The tongue of birds is a rigid organ due to the presence of the *paraglossum* (Koch, 1973) and, except in parrots, the absence of intrinsic musculature (Ziswiler and Farner, 1972; Koch, 1973; Nickel *et al.,* 1977; McLelland, 1990). The rigidity afforded by the *paraglossum* in palaeognathous birds is needed for the swallowing phase in order to push the food into the oesophagus. The rostral projection and body of the *basihyale*, situated ventrally in the tongue

body, connects the hyobranchial apparatus with the tongue, and due to its close association, retracts the tongue during swallowing. The great mobility of the hyobranchial apparatus in birds, attributed to the fact that it does not articulate with the skull (McLeod, 1939), is the main contributor to the movement of the tongue (King and McLelland, 1984; Bonga Tomlinson, 2000).

4.5 REFERENCES

AL-MANSOUR, M.I. & JARRAR, B.M. 2004. Structure and secretions of the lingual salivary glands of the white-cheeked bulbul, *Pycnonotus leucogenys* (*Pycnontidae). Saudi Journal of Biological Sciences,* 11:119-126.

BACHA, W.J. & BACHA, L.M. 2000. Digestive system, in *Color Atlas of Veterinary Histology,* edited by D. Balado. Philadelphia: Lippincott Williams & Wilkins: 121-157.

BAILEY, T.A., MENSAH-BROWN, E.P., SAMOUR, J.H., NALDO, J., LAWRENCE, P. & GARNER, A. 1997. Comparative morphology of the alimentary tract and its glandular derivatives of captive bustards. *Journal of Anatomy,* 191:387-398.

BAUMEL, J.J., KING, A.S., BREAZILE, J.E., EVANS, H.E. & VANDEN BERGE, J.C. 1993. *Handbook of Avian Anatomy: Nomina Anatomica Avium*. Second Edition. Cambridge, Massachusetts: Nuttall Ornithological Club.

BHATTACHARYYA, B.N. 1980. The morphology of the jaw and tongue musculature of the common pigeon, *Columba livia*, in relation to its feeding habit. *Proceedings of the Zoological Society, Calcutta,* 31:95-127.

BONGA TOMLINSON, C.A. 2000. Feeding in paleognathus birds, in *Feeding: Form, Function, and Evolution in Tetrapod Vertebrates,* edited by K. Schwenk. San Diego: Academic Press: 359-394.

BRADLEY, O.C. 1915. *The Structure of the Fowl*. London: A. and C. Black, Ltd.

CALHOUN, M.L. 1954. *Microscopic Anatomy of the Digestive System of the Chicken*. Ames, Iowa: Iowa State College Press.

CHO, P., BROWN, B. & ANDERSON, M. 1984. Comparative gross anatomy of ratites. *Zoo Biology,* 3:133-144.

CROLE, M.R. & SOLEY, J.T. 2008. Histological structure of the tongue of the emu (*Dromaius novaehollandiae*). *Proceedings of the Microscopy Society of Southern Africa,* 38:63.

CUVIER, G. 1836. *Leçons d'anatomie comparée*, Third Edition. Volumes 1 & 2, edited by M. Duméril. Bruxelles: Dumont.

DAVIES, S.J.J.F. 1978. The food of emus. *Australian Journal of Ecology,* 3:411-422.

DUERDEN, J.E. 1912. Experiments with ostriches XVIII. The anatomy and physiology of the ostrich. A. The external characters. *Agricultural Journal of the Union of South Africa,* 3:1-27.

FARAGGIANA, R. 1933. Sulla morfologia della lingua e del rialzo laringeo di alcune specie di uccelli Ratiti e Carenati non comuni. *Bollettino dei Musei di Zoologia e Anatomia comparata,* 43:313-323.

FEDER, F-H. 1972. Zur mikroskopischen Anatomie des Verdauungsapparates beim Nandu (*Rhea americana*). *Anatomischer Anzeiger,* 132:250-265.

FOWLER, M.E. 1991. Comparative clinical anatomy of ratites. *Journal of Zoo and Wildlife Medicine,* 22:204-227.

GADOW, H. 1879. Versuch einer vergleichenden Anatomie des Verdauungssystemes der Vögel. *Jenaische Zeitschrift für Medizin und Naturwissenschaft,* 13:92-171.

GARDNER, L.L. 1926. The adaptive modifications and the taxonomic value of the tongue in birds. *Proceedings of the United States National Museum,* 67:Article 19.

GARDNER, L.L. 1927. On the tongue in birds. *The Ibis,* 3:185-196.

GARGIULO, A.M., LORVIK, S., CECCARELLI, P. & PEDINI, V. 1991. Histological and histochemical studies on the chicken lingual glands. *British Poultry Science,* 32:693-702.

GENTLE, M.J. 1971. The lingual taste buds of *Gallus domesticus*. *British Poultry Science,* 12:245-248.

GÖPPERT, E. 1903. Die Bedeutung der Zunge für den sekundären Gaumen und den Ductus nasopharyngeus. *Morphologisches Jahrbuch,* 31:311-359.

GUSSEKLOO, S.W.S. & BOUT, G.R. 2005. The kinematics of feeding and drinking in palaeognathous birds in relation to cranial morphology. *The Journal of Experimental Biology,* 208:3395-3407.

HARRISON, J.G. 1964. Tongue, in *A New Dictionary of Birds,* edited by A.L. Thomson. London: Nelson: 825-827.

HODGES, R.D. 1974. The digestive system, in *The Histology of the Fowl*. London: Academic Press: 35-47.

HOMBERGER, D.G. & MEYERS, R. 1989. Morphology of the lingual apparatus of the domestic chicken *Gallus gallus,* with special attention to the structure of the fasciae. *American Journal of Anatomy,* 186:217-257.

JACKOWIAK, H. & GODYNICKI, S. 2005. Light and scanning electron microscopic study of the tongue in the white tailed eagle (*Haliaeetus albicilla, Accipitiridae, Aves*). *Annals of Anatomy,* 187:251-259.

JACKOWIAK, H. & LUDWIG, M. 2008. Light and scanning electron microscopic study of the structure of the ostrich (*Strutio camelus*) tongue. *Zoological Science,* 25:188-194.

KAUPP, M.S. 1918. *The Anatomy of the Domestic Fowl*. Philadelphia: W.B. Saunders Company.

KING, A.S. 1993. Apparatus respiratorius [Systema respiratorium], in *Handbook of Avian Anatomy: Nomina Anatomica* Avium *Second Edition,* edited by J.J. Baumel, A.S. King, J.E. Breazile, H.E. Evans & J.C. Vanden Berge. Cambridge, Massachusetts: Nuttall Ornithological Club: 257-299.

KING, A.S. & MCLELLAND, J. 1984. Digestive system, in *Birds - Their Structure and Function.* Second Edition. London: Bailliere Tindall: 86-87.

KOBAYASHI, K., KUMAKURA, M., YOSHIMURA, K., INATOMI, M. & ASAMI, T. 1998. Fine structure of the tongue and lingual papillae of the penguin. *Archivum Histologicum Cytologicum,* 61:37-46.

KOCH, T. 1973. Splanchnology, in *Anatomy of the Chicken and Domestic Birds,* edited by B.H. Skold & L. DeVries. Ames, Iowa: The Iowa State University Press: 68-69.

LILLIE, F.R. 1908. *The Development of the Chick*. New York: Henry Holt and Co.

LIMAN, N., BAYRAM, G. & KOÇAK, M. 2001. Histological and histochemical studies on the lingual, preglottal and laryngeal salivary glands of the Japanese quail (*Coturnix coturnix japonica*) at the post-hatching period. *Anatomia,* 30:367-373.

LUCAS, F.A. 1896. The taxonomic value of the tongue in birds. *Auk,* 13:109-115.

LUCAS, F.A. 1897. The tongues of birds. *Reports of the United States National Museum,* 1895:1003-1020.

MACALISTER, A. 1864. On the anatomy of the ostrich (*Struthio camelus*). *Proceedings of the Royal Irish Academy,* 9:1-24.

MCCANN, C. 1973. The tongues of kiwis. *Notornis,* 20:123-127.

MCLELLAND, J. 1975. Aves digestive system, in *Sisson and Grossman's The Anatomy of the Domestic Animals,* edited by C.E. Rosenbaum, N.G. Ghoshal & D. Hillmann. Philadelphia: W.B. Saunders Company: 1857-1867.

MCLELLAND, J. 1979. Digestive system, in *Form and Function in Birds.* Volume 1, edited by A.S. King & J. McLelland. San Diego, California: Academic Press: 69-92.

MCLELLAND, J. 1990. Digestive system, in *A Colour Atlas of Avian Anatomy,* edited by J. McLelland. Aylesbury, England: Wolfe Publishing Ltd: 47-49.

MCLELLAND, J. 1993. Apparatus digestorius [Systema alimentarium], in *Handbook of Avian Anatomy: Nomina Anatomica Avium.* Second Edition, edited by J.J. Baumel, A.S. King, J.E. Breazile, H.E. Evans & J.C. Vanden Berge. Cambridge, Massachusetts: Nuttall Ornithological Club: 301-328.

MCLEOD, W.M. 1939. Anatomy of the digestive tract of the domestic fowl. *Veterinary Medicine,* 34:722-727.

MECKEL, J.F. 1829. *System der vergleichenden Anatomie*. Halle: Der Rehgerschen Buchhandlung.

MOORE, D.A. & ELLIOTT, R. 1946. Numerical and regional distribution of taste buds on the tongue of the bird. *Journal of Comparative Neurology*, 84:119-131.

NICKEL, R., SCHUMMER, A. & SEIFERLE, E. 1977. Digestive system, in *Anatomy of the Domestic Birds*. Berlin: Verlag Paul Parey: 40-50.

OWEN, R. 1879. *Memoirs on the extinct and wingless birds of New Zealand; with an appendix of those of England, Australia, Newfoundland, Mauritius and Rodriguez*. Volume 1. London: John van Voorst.

PARKER, T.J. 1891. Observations on the anatomy and development of Apteryx. *Philosophical Transactions of the Royal Society of London, B.,* 182:25-134.

PARKER, W.K. 1866. On the structure and development of the skull in the ostrich tribe. *Philosophical Transactions of the Royal Society of London,* 156:113-183.

PORCHESCU, G. 2007. Comparative morphology of the digestive tract of the black African ostrich, hen and turkey. PhD thesis (in Russian), Agrarian State University of Moldova.

PYCRAFT, W.P. 1900. On the morphology and phylogeny of the palaeognathae (*Ratitae and Crypturi*) and neognathae (*Carinatae*). *Transactions of the Zoological Society of London,* 15:149-290.

ROACH, R.W. 1952. Notes on the New Zealand kiwis (1). *The New Zealand Veterinary Journal,* 1:38-39.

SALES, J. 2006. Digestive physiology and nutrition of ratites. *Avian and Poultry Biology Reviews,* 17:41-55.

SALES, J. 2007. The emu (*Dromaius novaehollandiae*): A review of its biology and commercial products. *Avian and Poultry Biology Reviews,* 18:1-20.

TIVANE, C. 2008. A Morphological Study of the Oropharynx and Oesophagus of the Ostrich (*Struthio camelus*). MSc dissertation, University of Pretoria, South Africa.

WARNER, R.L., MCFARLAND, L.Z. & WILSON, W.O. 1967. Microanatomy of the upper digestive tract of the Japanese quail. *American Journal of Veterinary Research,* 28:1537-1548.

WEBB, M. 1957. The ontogeny of the cranial bones, cranial peripheral and cranial parasympathetic nerves, together with a study of the visceral muscles of *Struthio*. *Acta Zoologica,* 38:81-202.

ZISWILER, V. & FARNER, D.S. 1972. Digestion and the digestive system, in *Avian Biology,* edited by D.S. Farner, J.R. King & K.C. Parkes. New York: Academic Press: 344-354.

4.6 FIGURES

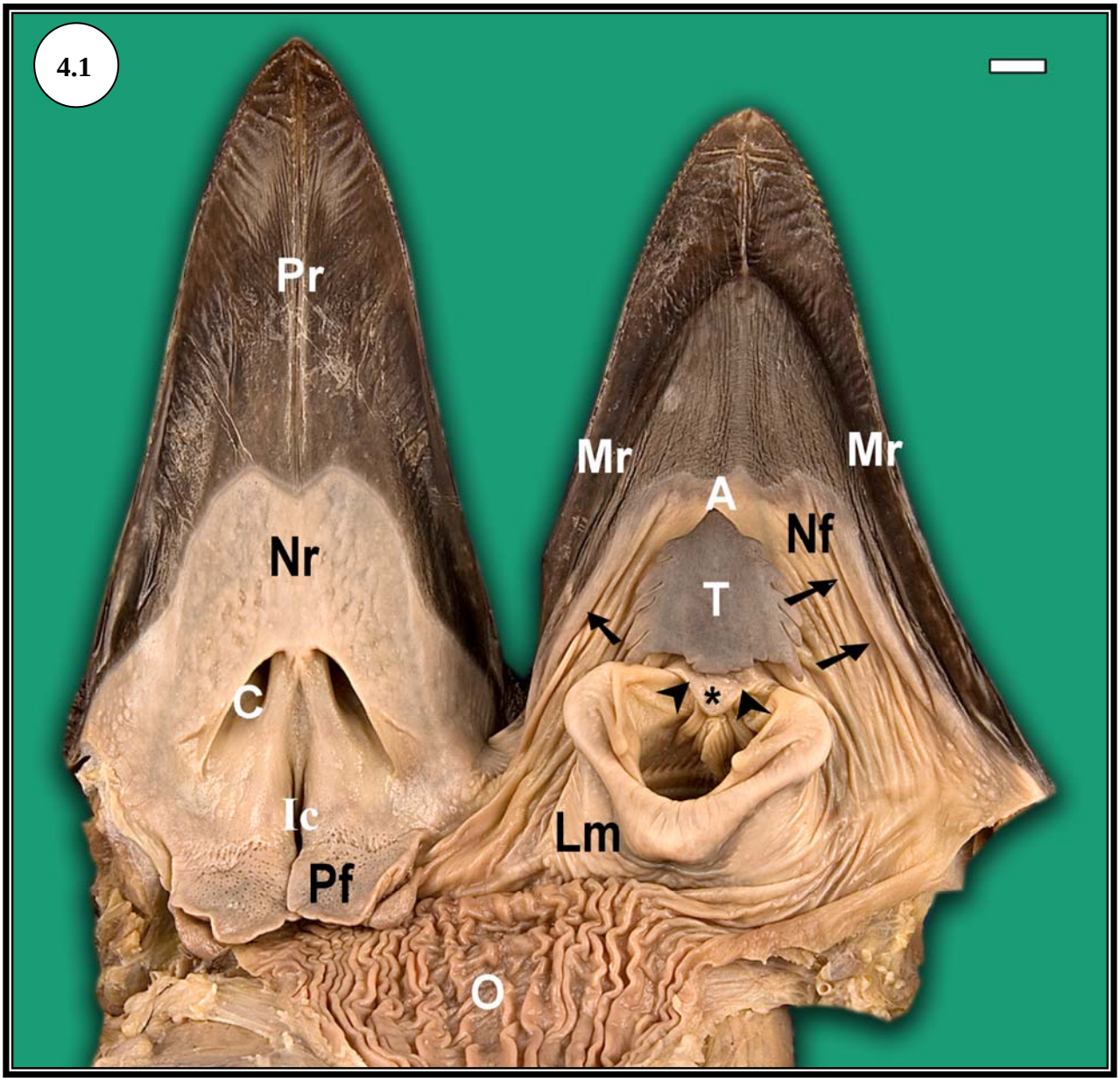


Figure 4.1: Emu head opened along the right commisure to reveal the positioning of the tongue within the oropharynx. The body of the tongue (T) lies within the non-pigmented region of both the roof (Nr) and floor (Nf) of the oropharynx, and the small tongue root (*) extends from the base of the tongue body to the rostral tip of the glottis (arrowheads). The apex (A) of the tongue lies close to the border of the pigmented and non-pigmented regions. Other noticeable features of the oropharynx include the broad mandibular rhamphotheca (Mr), the interramal region of the non-pigmented floor with its numerous folds (arrows), the laryngeal mound (Lm), the median palatine ridge (Pr), the choana (C), infundibular cleft (Ic), pharyngeal folds (Pf) and proximal oesophagus (O). Bar = 5mm.

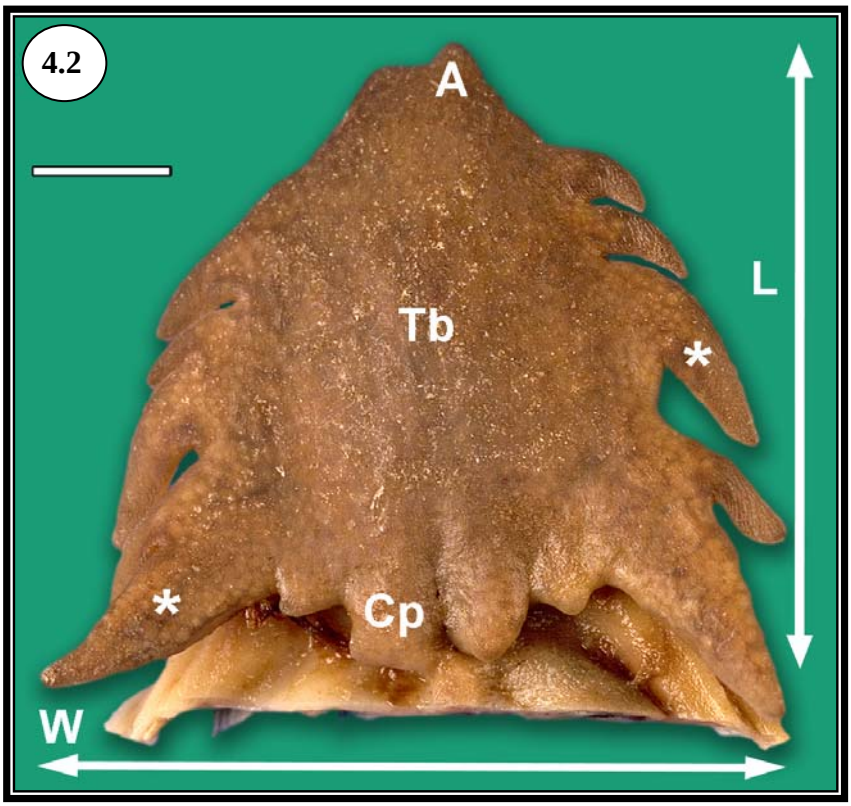


Figure 4.2: Dorsal view of the tongue body (Tb) showing the apex (A), lateral lingual papillae (*) and caudal lingual papillae (Cp). Tongue body length (L) was measured from the apex to the caudal papillae. The width (W) was measured between the tips of the last lateral papillae. Bar = 5mm.

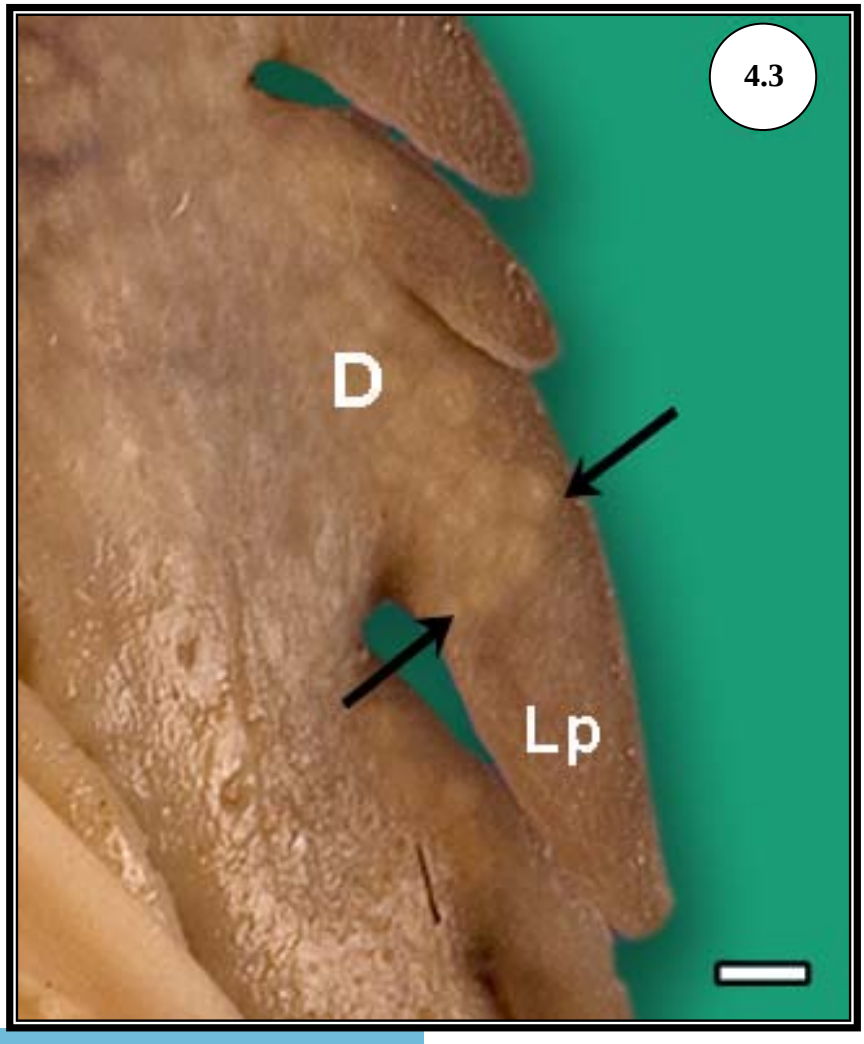


Figure 4.3: Ventral view of the lateral lingual papillae showing the abrupt transition (arrows) between the presence of doughnut-shaped structures (D) and the unelaborated surface of the papillae (Lp). Bar = 1mm.

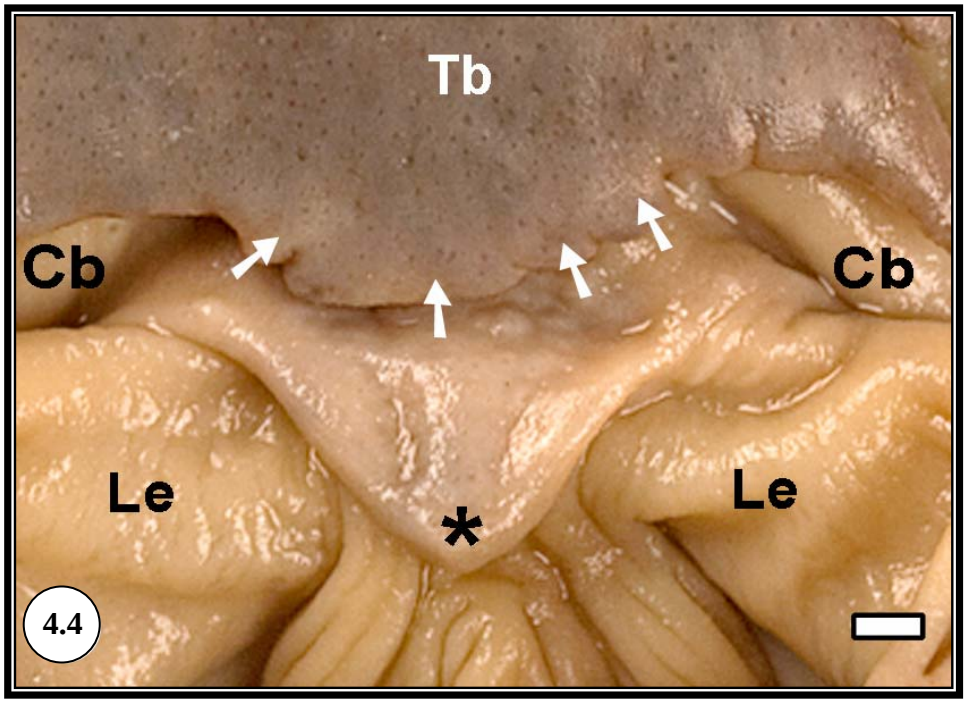


Figure 4.4: Dorsal view of the triangular tongue root, showing the tongue root tip (*) folding over the laryngeal entrance (Le). In this specimen, the caudal lingual papillae (arrows) of the tongue body (Tb) appear fused with variable incisures and small projections being apparent. The rostral parts of the paired *ceratobranchiale* (Cb) are seen bordering the tongue root. Note the pitted surface of the tongue body, representing the openings of the large underlying glands.
Bar = 1mm.

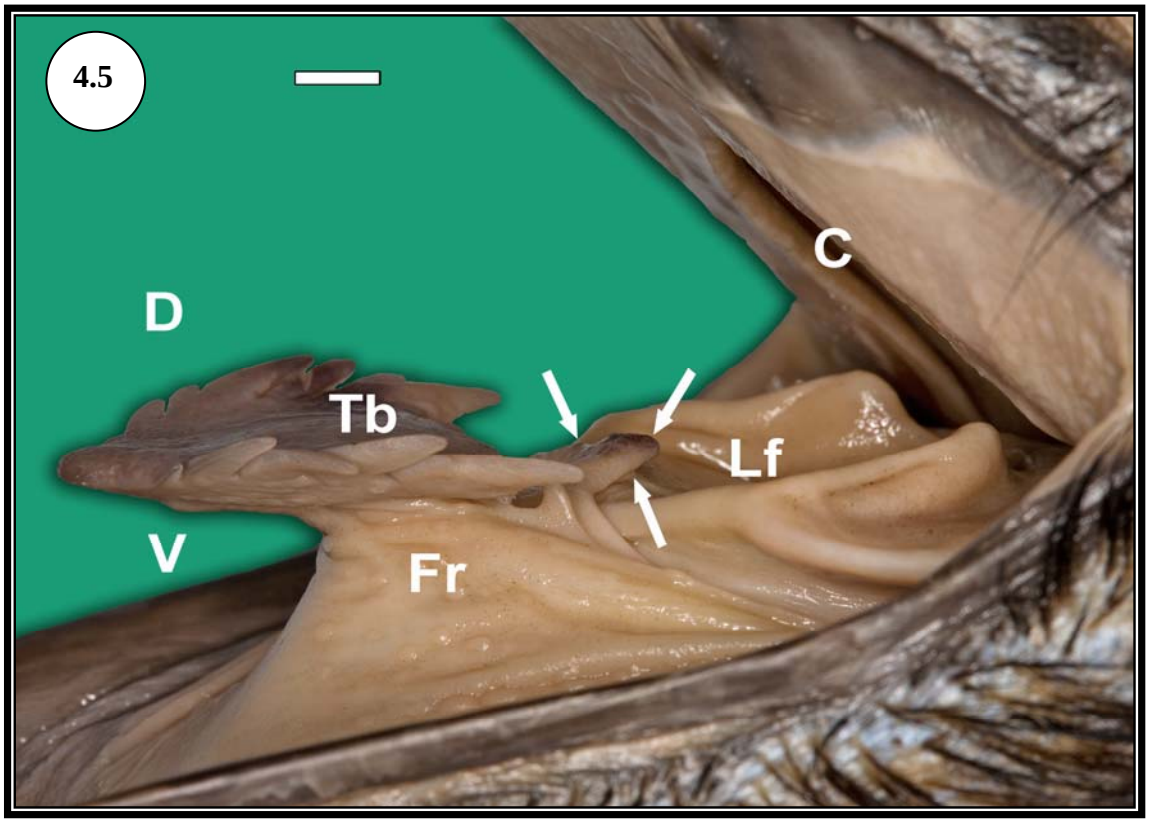


Figure 4.5: The dorso-ventrally flattened tongue body (Tb) shown in lateral profile. The folds of the frenulum (Fr) are not visible as the tongue body is in the raised position**.** Dorsum (D), ventrum (V), tongue root tip (arrows), Laryngeal fissure (Lf), choana (C). Bar = 5mm.

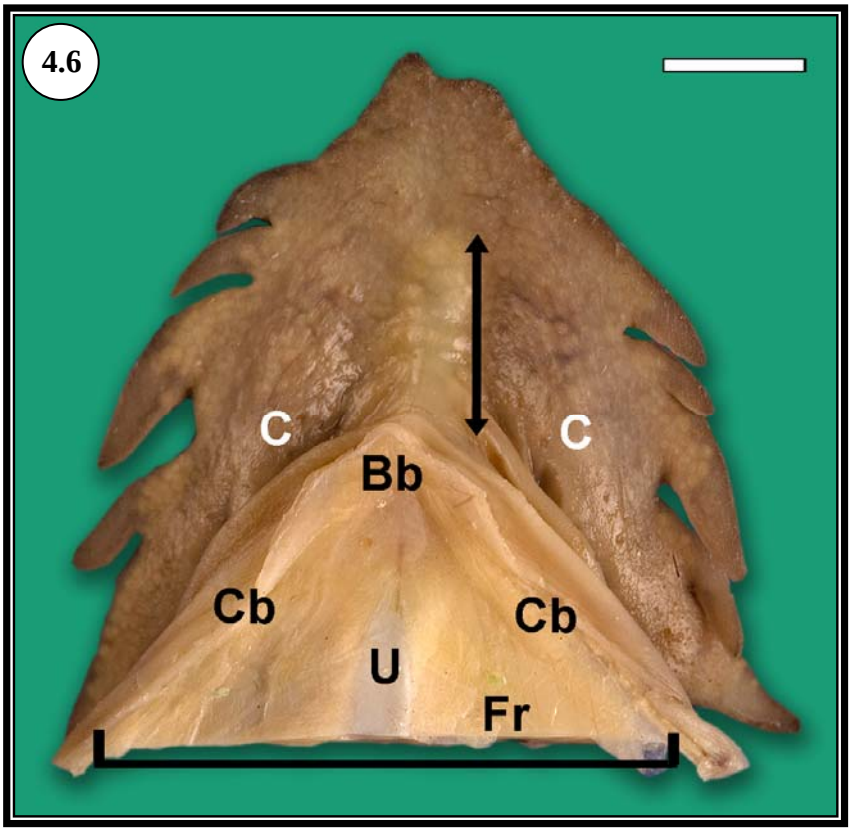
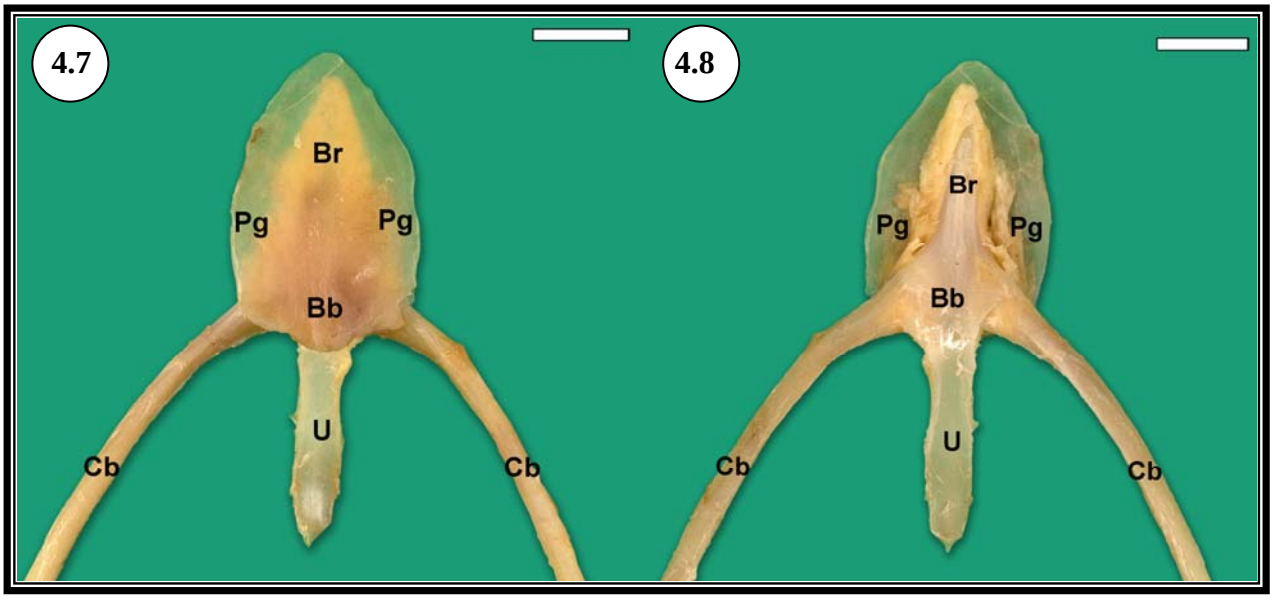


Figure 4.6: The tongue body and frenulum in ventral view. Note the extent of the rostral projection of the *basihyale* (double-headed arrow). The position of the body of the *basihyale* (Bb), rostral parts of the paired *ceratobranchiale* (Cb) and the *urohyale* (U) are indicated and occur in triangular formation running within the frenulum (Fr). The doughnut-shaped structures can be clearly seen below the surface. Crura (C). Bar = 5mm.



Figures 4.7 and 4.8: The lingual skeleton shown in dorsal (4.7) and ventral (4.8) view. The broad *paraglossum* (Pg) lies dorsal to the rostral projection of the *basihyale* (Br) within the tongue body. The body of the *basihyale* (Bb), the rostral parts of the paired *ceratobranchiale* (Cb) and the *urohyale* (U) are all imbedded within the frenulum (See Fig. 4.6). Bar = 5mm.

CHAPTER 5

HISTOLOGICAL FEATURES AND SURFACE MORPHOLOGY OF THE TONGUE

5.1 INTRODUCTION

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

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

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

5.2 MATERIALS AND METHODS

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

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

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

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

5.3. RESULTS

5.3.1 Light microscopic observations

5.3.1.1 Tongue body

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5.3.1.2 Tongue root (Figs. 5.3, 5.4)

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

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

5.3.1.3 Frenulum

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

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

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

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

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

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

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

5.4 DISCUSSION

5.4.1 Light microscopical features

5.4.1.1 General features of the tongue body

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

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

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

5.4.1.1.1 Epithelium

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

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

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

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

5.4.1.1.2 Glands

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

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

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

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

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

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

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

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

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

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

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

5.4.1.1.3 Herbst corpuscles

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

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

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

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

5.4.1.1.4 Lymphoid tissue

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

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

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

5.4.1.1.5 Lingual skeleton

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

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

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

5.4.1.1.6 Lingual musculature

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

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

5.4.1.2 Tongue root - Taste buds

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

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

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

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

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

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

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

5.4.2 Scanning electron microscopy (SEM) features

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

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

5.5 REFERENCES

AL-MANSOUR, M.I. & JARRAR, B.M. 2004. Structure and secretions of the lingual salivary glands of the white-cheeked bulbul, *Pycnonotus leucogenys* (*Pycnontidae). Saudi Journal of Biological Sciences,* 11:119-126.

ANTHONY, M. 1919. Über die Speicheldrüsen der Vögel. *Zoologische Jahrbücher. Abteilung für Anatomie,* 41:547.

BACHA, W.J. & BACHA, L.M. 2000. Digestive system, in *Color Atlas of Veterinary Histology,* edited by D. Balado. Philadelphia: Lippincott Williams & Wilkins: 121-157.

BAILEY, T.A., MENSAH-BROWN, E.P., SAMOUR, J.H., NALDO, J., LAWRENCE, P. & GARNER, A. 1997. Comparative morphology of the alimentary tract and its glandular derivatives of captive bustards. *Journal of Anatomy,* 191:387-398.

BAUMEL, J.J., KING, A.S., BREAZILE, J.E., EVANS, H.E. & VANDEN BERGE, J.C. 1993. *Handbook of Avian Anatomy: Nomina Anatomica Avium*. Second Edition. Cambridge, Massachusetts: Nuttall Ornithological Club.

BERKHOUDT, H. 1979. The morphology and distribution of cutaneous mechanoreceptors (Herbst and Grandry corpuscles) in bill and tongue of the mallard (*Anas platyrhynchos* L.). *Netherlands Journal of Zoology,* 30:1-34.

BERKHOUDT, H. 1985. Structure and function of avian taste buds, in *Form and Function in Birds.* Volume 3, edited by A.S. King & J. McLelland. London: Academic Press: 463-491.

BONGA TOMLINSON, C.A. 2000. Feeding in paleognathous birds, in *Feeding: Form, Function, and Evolution in Tetrapod Vertebrates,* edited by K. Schwenk. San Diego: Academic Press: 359-394.

BOTEZAT, E. 1910. Morphologie, Physiologie und phylogenetische Bedeutung der Geschmacksorgane der Vögel. *Anatomischer Anzeiger,* 36:428-461.

BRADLEY, O.C. 1915. *The Structure of the Fowl*. London: A. and C. Black, Ltd.

CALHOUN, M.L. 1954. *Microscopic Anatomy of the Digestive System of the Chicken*. Ames, Iowa: Iowa State College Press.

CROLE, M.R. & SOLEY, J.T. 2008. Histological structure of the tongue of the emu (*Dromaius novaehollandiae*). *Proceedings of the Microscopy Society of Southern Africa,* 38:63.

FARAGGIANA, R. 1933. Sulla morfologia della lingua e del rialzo laringeo di alcune specie di uccelli Ratiti e Carenati non comuni. *Bollettino dei Musei di Zoologia e Anatomia comparata,* 43:313-323.

FEDER, F-H. 1972. Zur mikroskopischen Anatomie des Verdauungsapparates beim Nandu (*Rhea americana*). *Anatomischer Anzeiger,* 132:250-265.

GARDNER, L.L. 1926. The adaptive modifications and the taxonomic value of the tongue in birds. *Proceedings of the United States National Museum,* 67:Article 19.

GARDNER, L.L. 1927. On the tongue in birds. *The Ibis,* 3:185-196.

GARGIULO, A.M., LORVIK, S., CECCARELLI, P. & PEDINI, V. 1991. Histological and histochemical studies on the chicken lingual glands. *British Poultry Science,* 32:693-702.

GENTLE, M.J. 1971a. Taste and its importance to the domestic chicken. *British Poultry Science,* 12:77-86.

GENTLE, M.J. 1971b. The lingual taste buds of *Gallus domesticus*. *British Poultry Science,* 12:245-248.

GOTTSCHALDT, K.-M. 1985. Structure and function of avian somatosensory receptors, in *Form and Function in Birds.* Volume 3, edited by A.S. King & J. McLelland. London: Academic Press: 375-462.

GUSSEKLOO, S.W.S. & BOUT, G.R. 2005. The kinematics of feeding and drinking in palaeognathous birds in relation to cranial morphology. *Journal of Experimental Biology,* 208:3395-3407.

HARRISON, J.G. 1964. Tongue, in *A New Dictionary of Birds,* edited by A.L. Thomson. London: Nelson: 825-827.

HODGES, R.D. 1974. The digestive system, in *The Histology of the Fowl*. London: Academic Press: 35-47.

HOMBERGER, D.G. & MEYERS, R. 1989. Morphology of the lingual apparatus of the domestic chicken *Gallus gallus,* with special attention to the structure of the fasciae. *American Journal of Anatomy,* 186:217-257.

IWASAKI, S. 2002. Evolution of the structure and function of the vertebrate tongue. *Journal of Anatomy,* 201:1-13.

JACKOWIAK, H. & GODYNICKI, S. 2005. Light and scanning electron microscopic study of the tongue in the white tailed eagle (*Haliaeetus albicilla, Accipitiridae, Aves*). *Annals of Anatomy,* 187:251-259.

JACKOWIAK, H. & LUDWIG, M. 2008. Light and scanning electron microscopic study of the structure of the ostrich (*Strutio camelus*) tongue. *Zoological Science,* 25:188-194.

KING, A.S. & MCLELLAND, J. 1984. Digestive system, in *Birds - Their Structure and Function.* Second Edition. London: Bailliere Tindall: 86-87.

KOBAYASHI, K., KUMAKURA, M., YOSHIMURA, K., INATOMI, M. & ASAMI, T. 1998. Fine structure of the tongue and lingual papillae of the penguin. *Archivum Histologicum Cytologicum,* 61:37-46.

KOCH, T. 1973. Splanchnology, in *Anatomy of the Chicken and Domestic Birds,* edited by B.H. Skold & L. DeVries. Ames, Iowa: The Iowa State University Press: 68-69.

LILLIE, F.R. 1908. *The Development of the Chick*. New York: Henry Holt and Co.

LIMAN, N., BAYRAM, G. & KOÇAK, M. 2001. Histological and histochemical studies on the lingual, preglottal and laryngeal salivary glands of the Japanese quail (*Coturnix coturnix japonica*) at the post-hatching period. *Anatomia,* 30:367-373.

LINDENMAIER, P. & KARE, M.R. 1959. The taste end-organs of the chicken. *Poultry Science,* 38:545-549.

MCLELLAND, J. 1975. Aves digestive system, in *Sisson and Grossman's The Anatomy of the Domestic Animals,* edited by C.E. Rosenbaum, N.G. Ghoshal & D. Hillmann. Philadelphia: W.B. Saunders Company: 1857-1867.

MCLELLAND, J. 1979. Digestive System, in *Form and Function in Birds.* Volume 1, edited by A.S. King & J. McLelland. San Diego, California: Academic Press: 69-92.

MCLELLAND, J. 1990. Digestive system, in *A Colour Atlas of Avian Anatomy,* edited by J. McLelland. Aylesbury, England: Wolfe Publishing Ltd.: 47-49.

MCMANUS, J.F.A. 1946. Histological demonstration of mucin after periodic acid. *Nature (London)*, 158:202.

MOORE, D.A. & ELLIOTT, R. 1946. Numerical and regional distribution of taste buds on the tongue of the bird. *Journal of Comparative Neurology,* 84:119-131.

NICKEL, R., SCHUMMER, A. & SEIFERLE, E. 1977. Digestive system, in *Anatomy of the Domestic Birds.* Berlin: Verlag Paul Parey: 40-50.

PORCHESCU, G. 2007. Comparative morphology of the digestive tract of the black African ostrich, hen and turkey. PhD thesis (in Russian), Agrarian State University of Moldova.

ROSE, M.E. 1981. Lymphatic system, in *Form and Function in Birds.* Volume 2, edited by A.S. King & J. McLelland. London: Academic Press: 341-372.

SAMAR, M.E., AVILA, R.E., DE FABRO, S.P., PORFIRIO, V., ESTEBAN, F.J., PEDROSA, J.A. & PEINADO, M.A. 1999. Histochemical study of Magellanic penguin (*Spheniscus magellanicus*) minor salivary glands during postnatal growth. *Anatomical Record,* 254:298-306.

TABAK, L., LEVINE, M., MANDEL, I. & ELLISON, S. 1982. Role of salivary mucins in the protection of the oral cavity. *Journal of Oral Pathology*, 11:1-17.

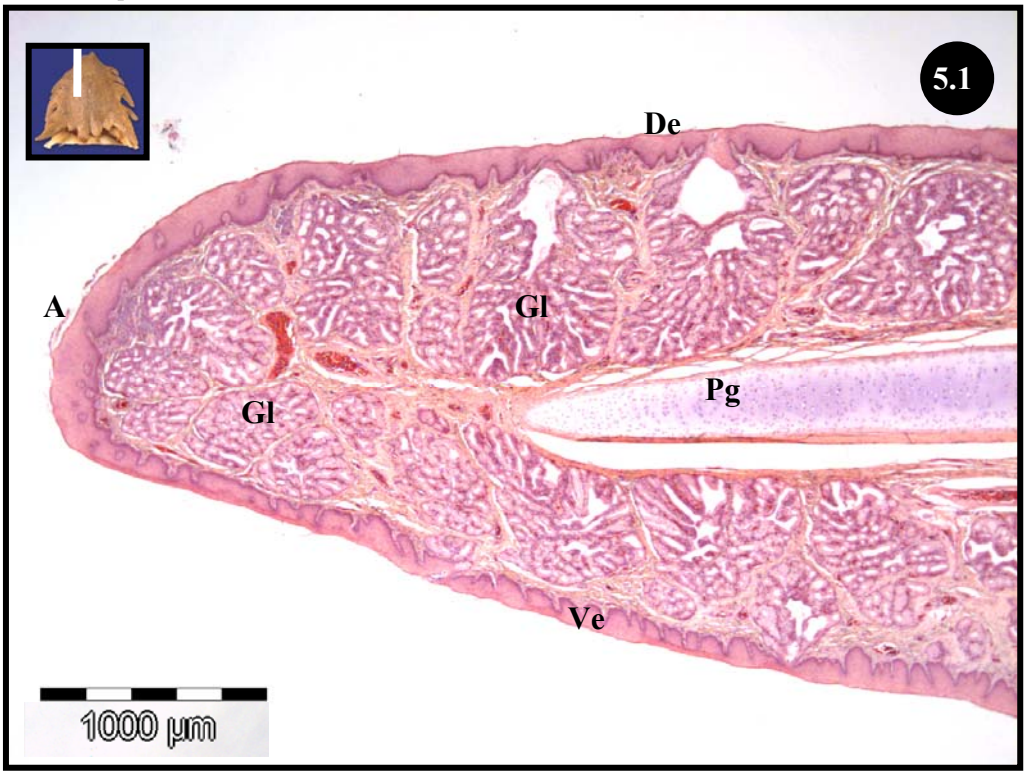
TIVANE, C. 2008. A Morphological Study of the Oropharynx and Oesophagus of the Ostrich (*Struthio camelus*). MSc dissertation, University of Pretoria, South Africa.

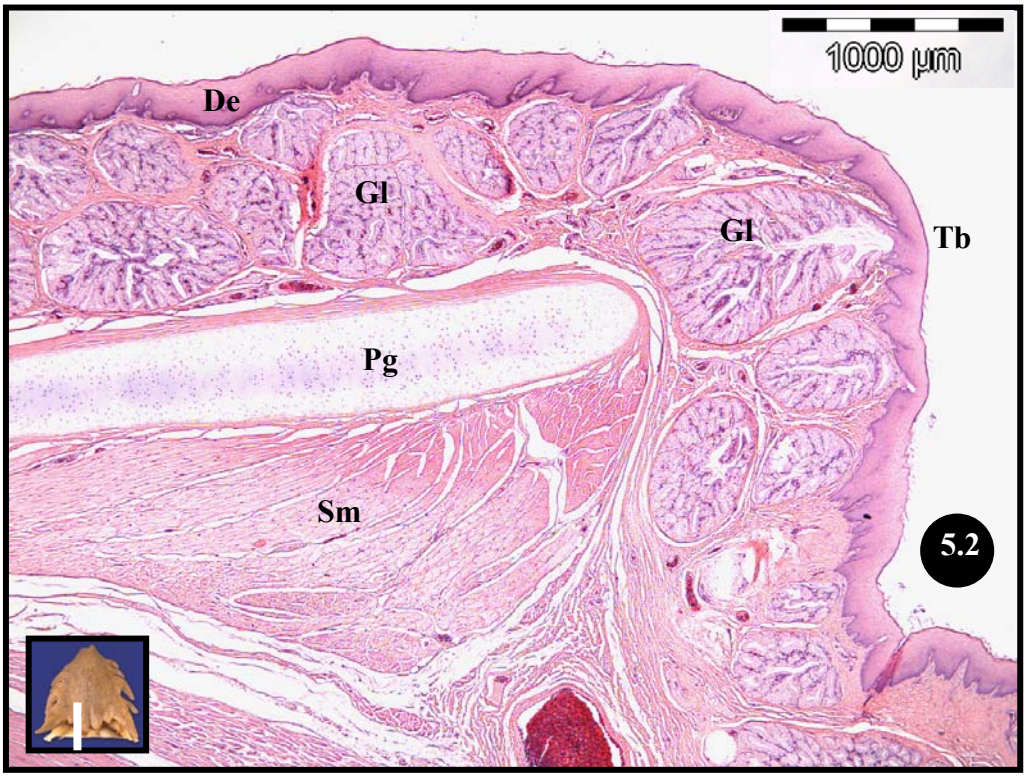
TUCKER, R. 1958. Taxonomy of the salivary glands of vertebrates. *Systematic Zoology,* 7:74-83.

WARNER, R.L., MCFARLAND, L.Z. & WILSON, W.O. 1967. Microanatomy of the upper digestive tract of the Japanese quail. *American Journal of Veterinary Research,* 28:1537-1548.

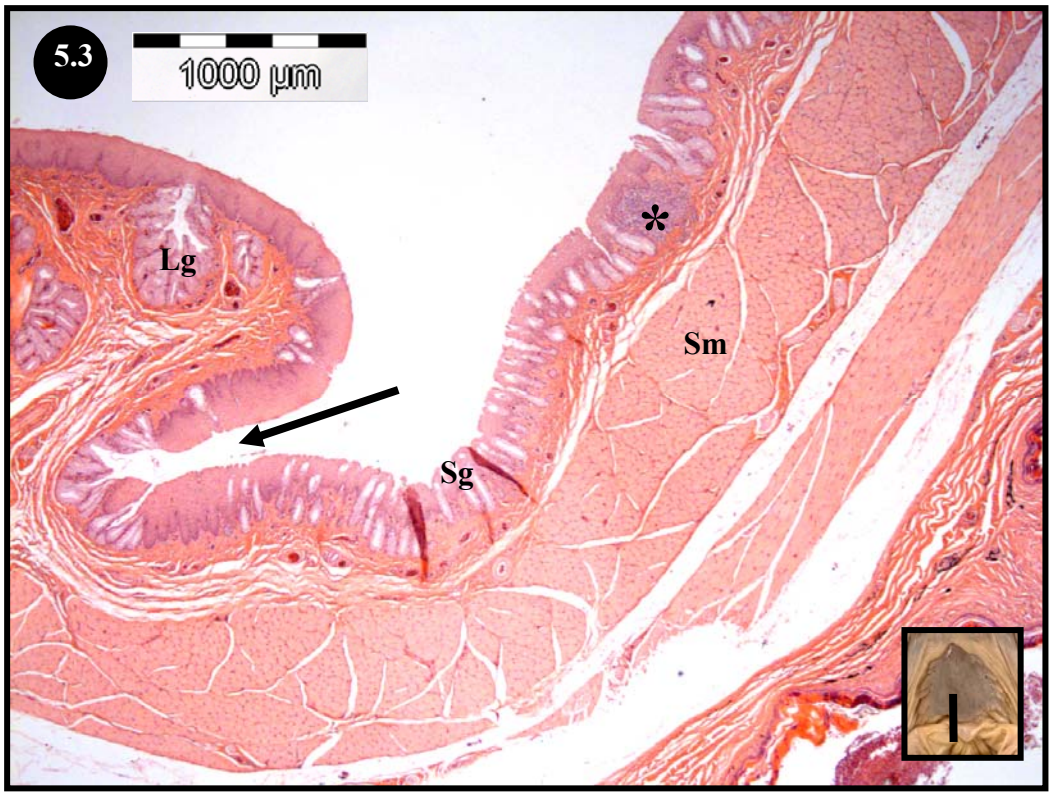
ZISWILER, V. & FARNER, D.S. 1972. Digestion and the Digestive System, in *Avian Biology,* edited by D.S. Farner, J.R. King & K.C. Parkes. New York: Academic Press: 344-354.

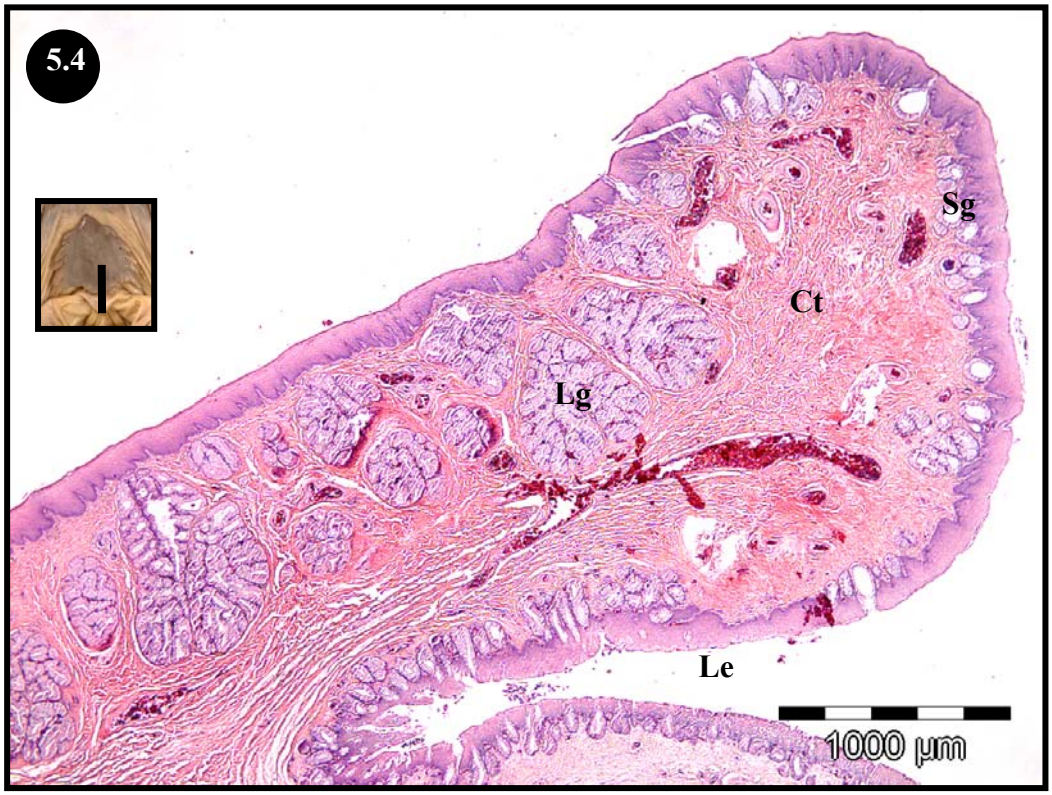
5.6 FIGURES





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





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

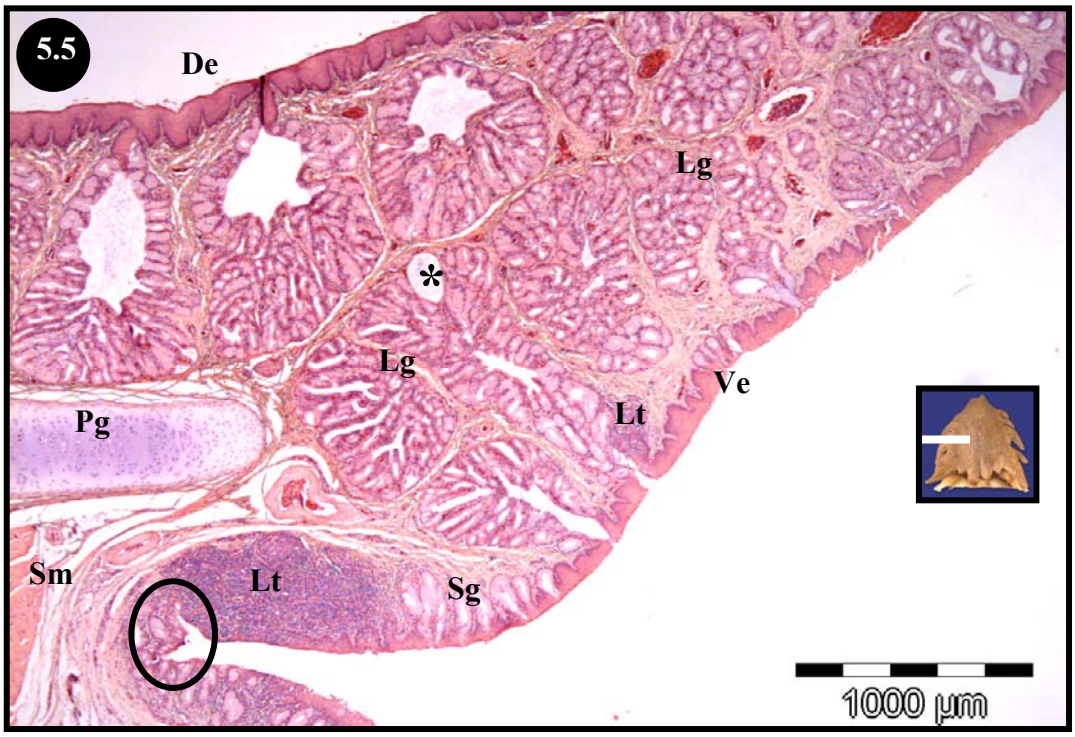


Figure 5.5: Cross section of the lateral tongue body and papillae base demonstrating large simple branched tubular glands (Lg) and associated Herbst corpuscle (*). Note the simple tubular glands (Sg) and lymphoid tissue (Lt) exclusively present on the ventrum. *Paraglossum* (Pg), skeletal muscle (Sm),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

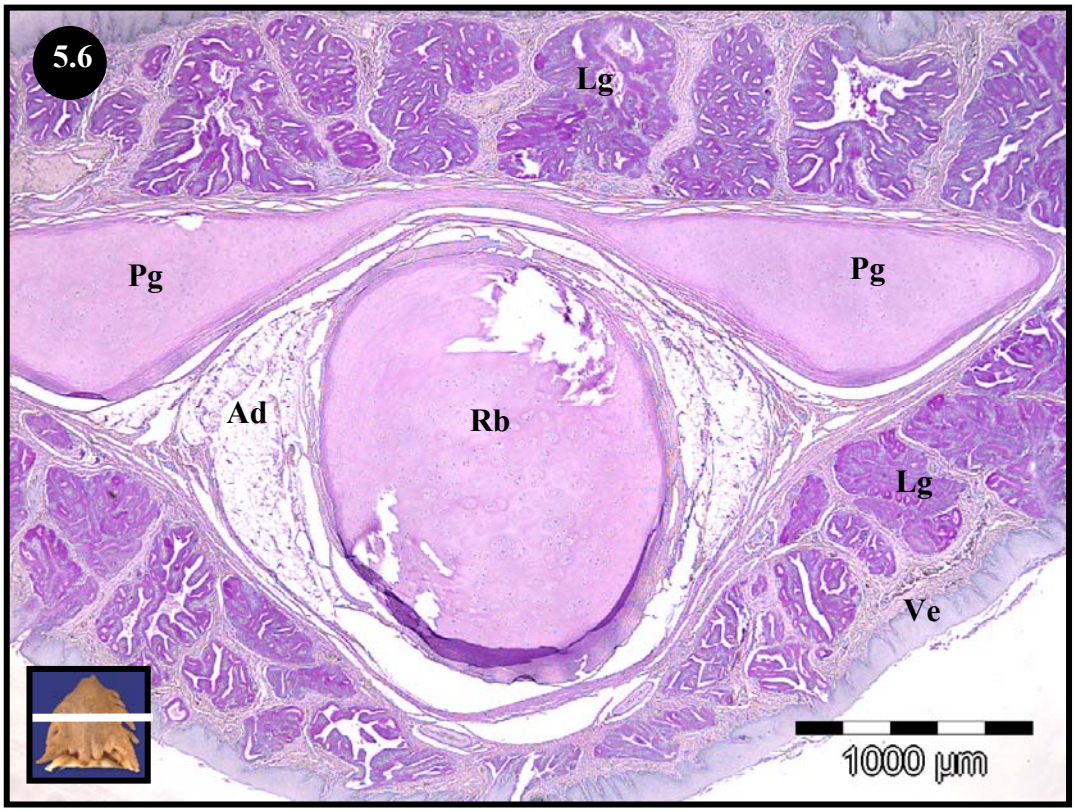


Figure 5.6: Cross section of the middle of the tongue body showing the topography of the lingual skeleton within the parenchyma. The *paraglossum* (Pg) lies dorsal to the rostral projection of the *basihyale* (Rb) which is flanked by adipose tissue (Ad). Large simple branched tubular glands (Lg), ventral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

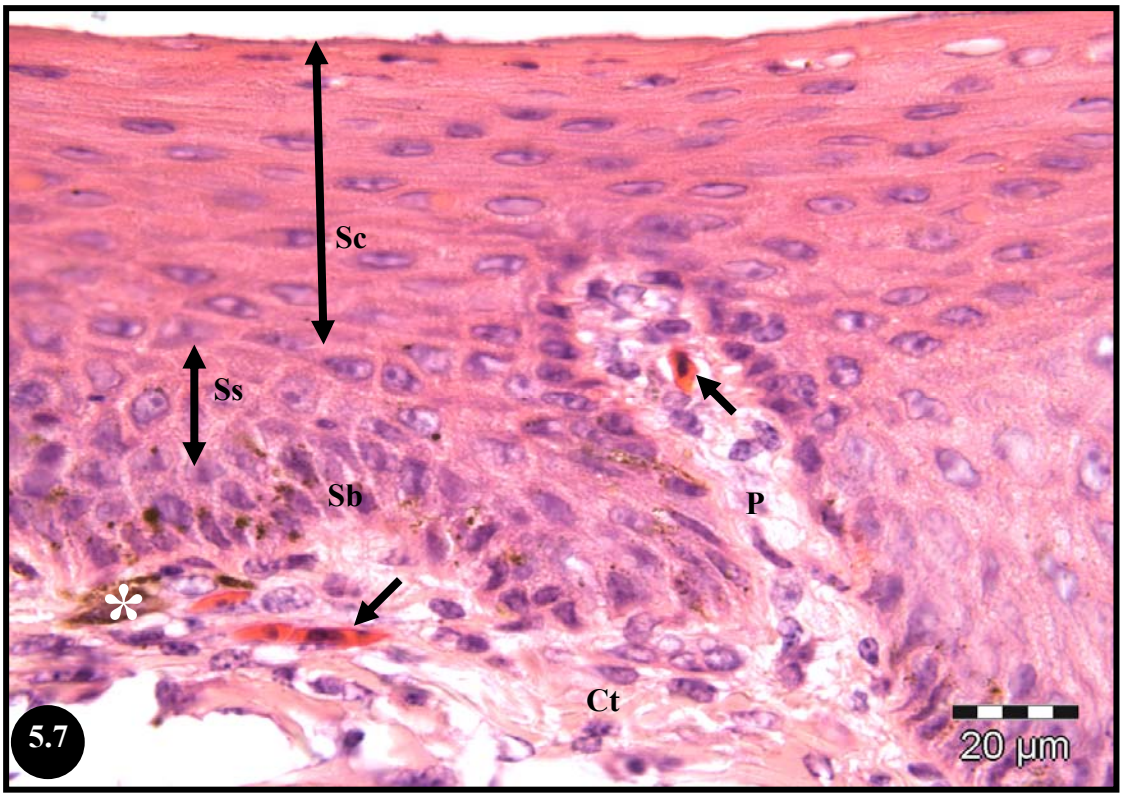


Figure 5.7: The non-keratinised stratified squamous epithelium of the tongue dorsum displaying the S*tr. basale* (Sb) with melanocytes (*) some of which lie in the connective tissue beneath the S*tr. basale*, S*tr. spinosum* (Ss) and S*tr. corneum* (Sc). Connective tissue (Ct), connective tissue papilla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

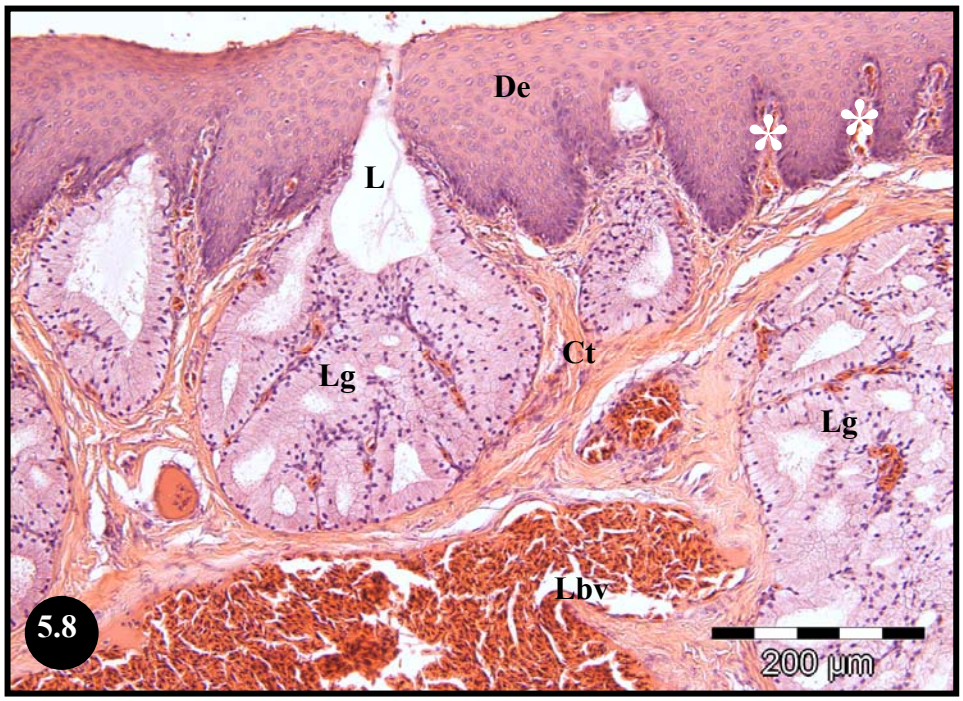


Figure 5.8: Low magnification of the tongue dorsum showing the duct of a large simple branched tubular gland (Lg) passing through the epithelium (De). Lumen (L), connective tissue (Ct), connective tissue papillae (*), large blood vessel (Lbv).

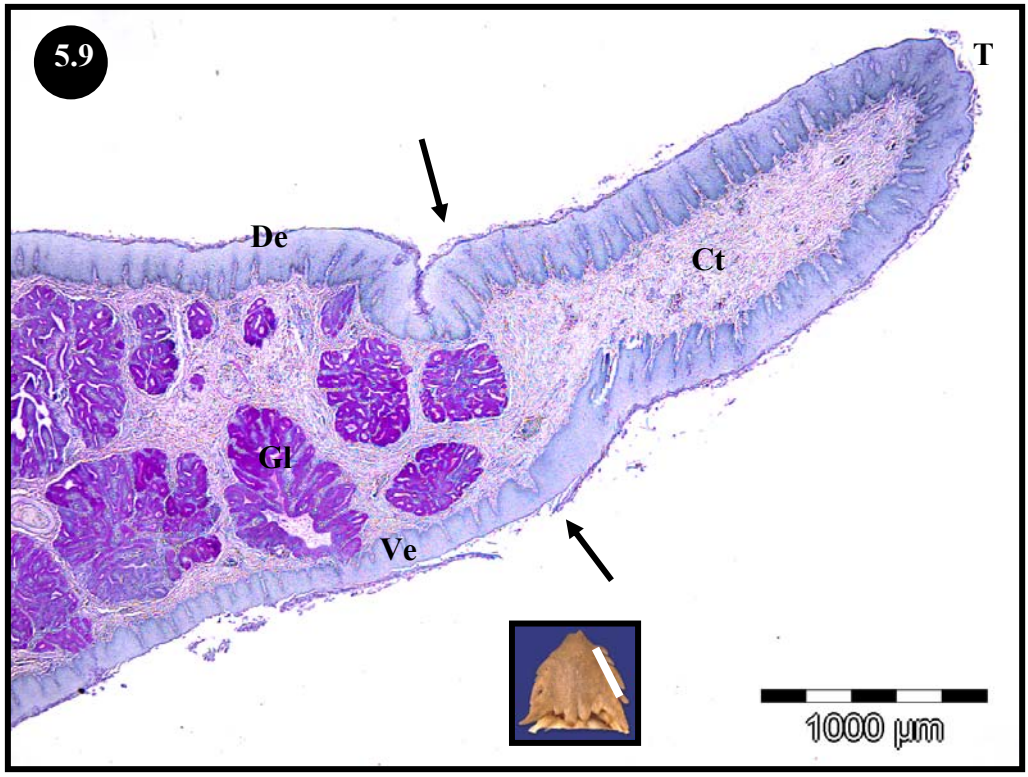


Figure 5.9: Lateral lingual papilla in longitudinal section with the glandular tissue showing a positive PAS reaction. Note the abrupt termination (arrows) of the glands (Gl) leaving only connective tissue (Ct) filling the space between the dorsal (De) and ventral epithelium (Ve). Papilla tip (T).

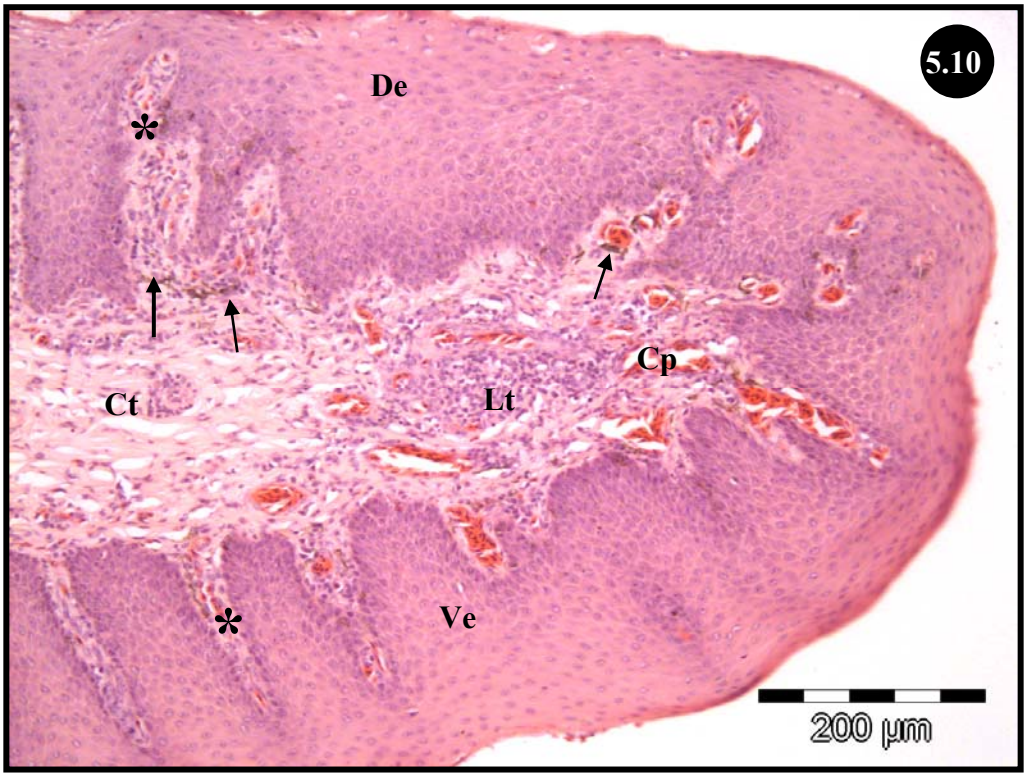


Figure 5.10: Longitudinal section of a lateral lingual papilla tip. Note the presence of a rich capillary plexus (Cp) and an aggregation of diffuse lymphoid tissue (Lt) within the supporting connective tissue (Ct). Deep connective tissue papillae carrying capillaries (*) penetrate the epithelium. Melanocytes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

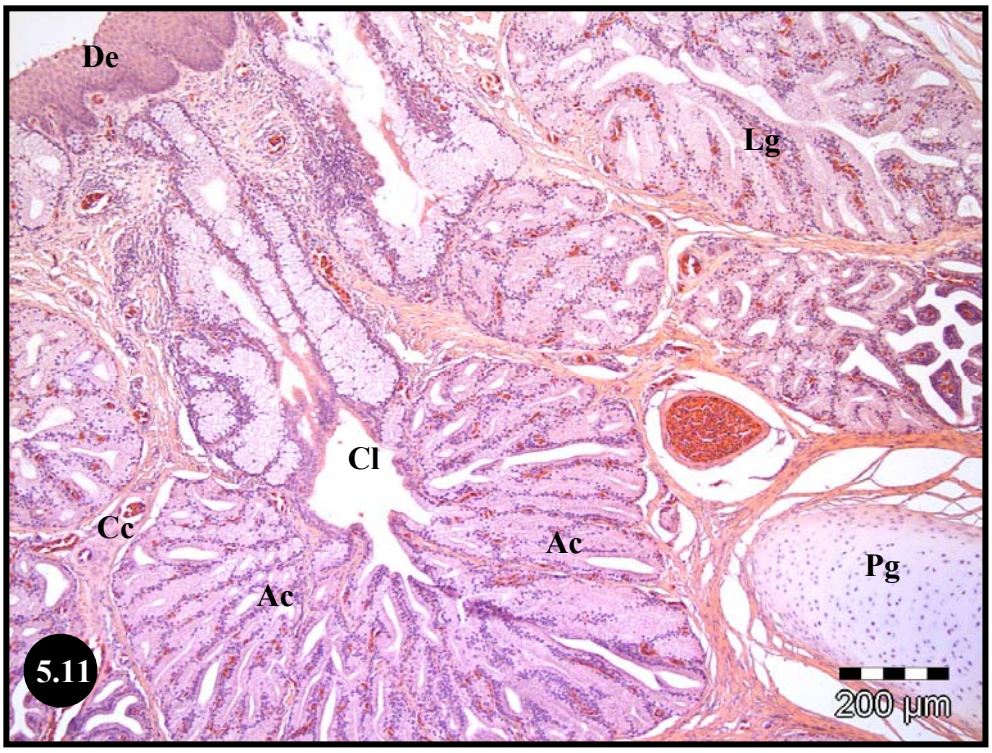


Figure 5.11: The typical structure of the large simple branched tubular mucus-secreting glands (Lg) in longitudinal section illustrating the numerous acini (Ac) which open into the central lumen (Cl). A connective tissue capsule (Cc) surrounds each gland. *Paraglossum* (Pg), dorsal epithelium (De).

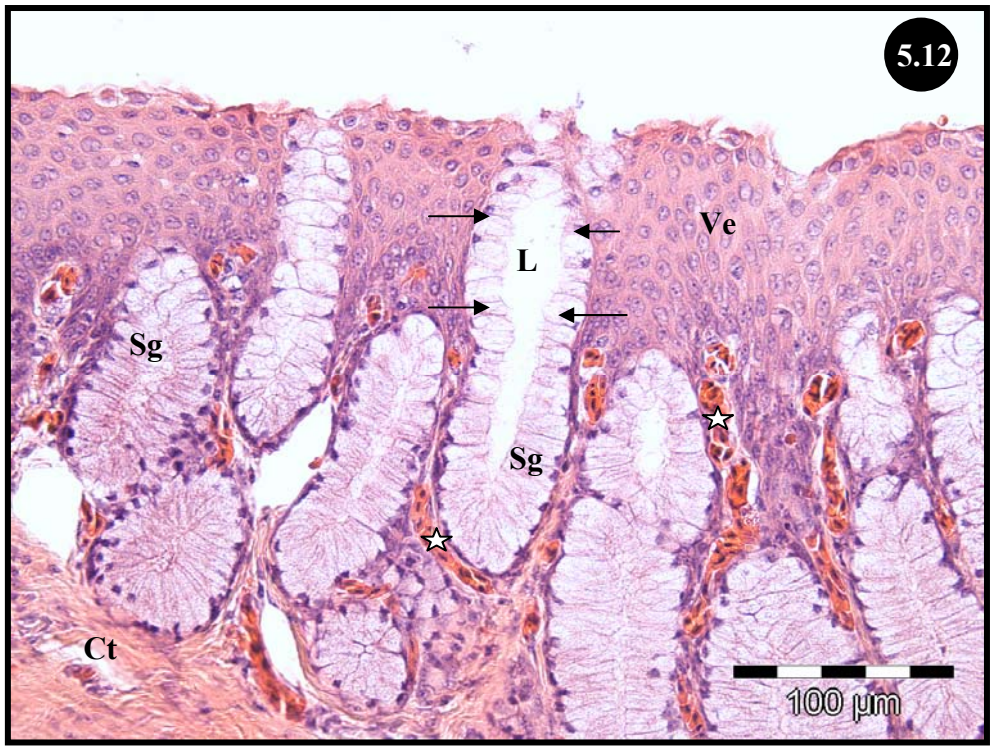


Figure 5.12: Tongue ventrum illustrating the small simple tubular mucus-secreting glands (Sg) opening onto this surface. The glands are seen in longitudinal section with much of their length restricted to the epithelial layer. The lumen (L) is lined by secretory cells (arrows). Capillaries (stars), connective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

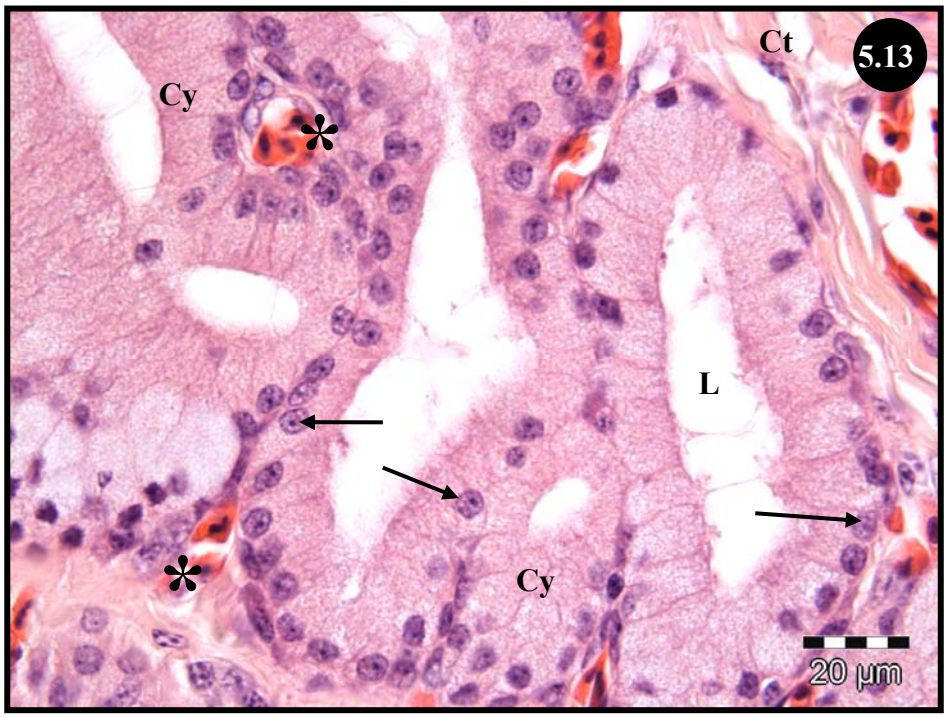


Figure 5.13: High magnification showing details of the acini of the large simple branched tubular mucus-secreting glands. The acini show typical properties of mucus-secreting cells, with a basal nucleus (arrows) and basophilic foamy cytoplasm (Cy). Lumen of acinus (L), capillaries (*), connective tissue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

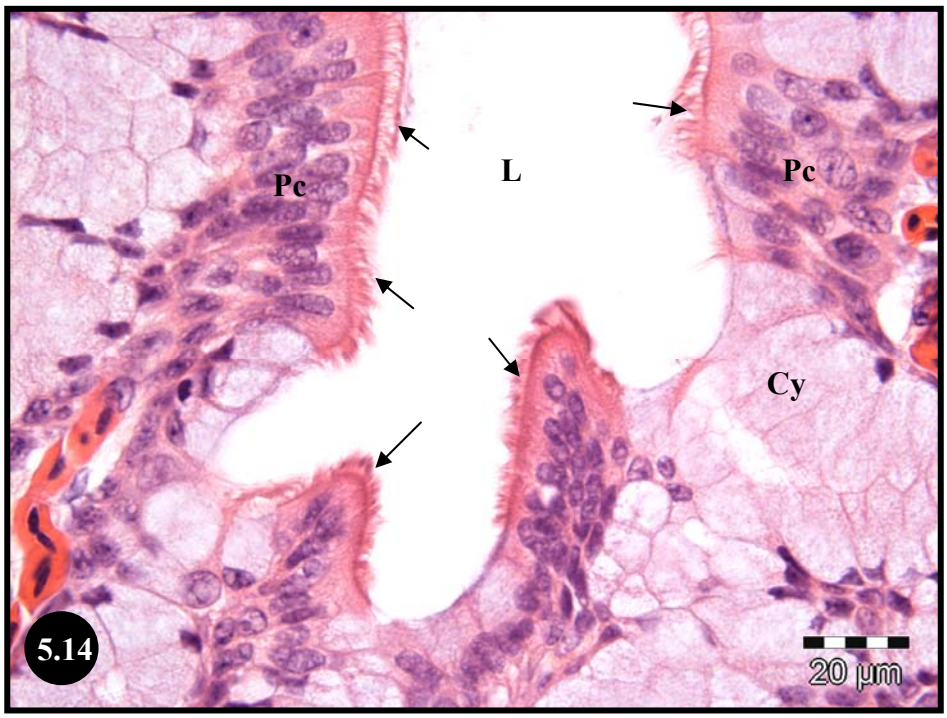


Figure 5.14: Pseudostratified ciliated columnar epithelium (Pc) lining part of the lumen (L) of a large simple branched tubular gland. Basophilic cytoplasm (Cy) of the adjacent mucus-secreting cells. Cilia (arrows).

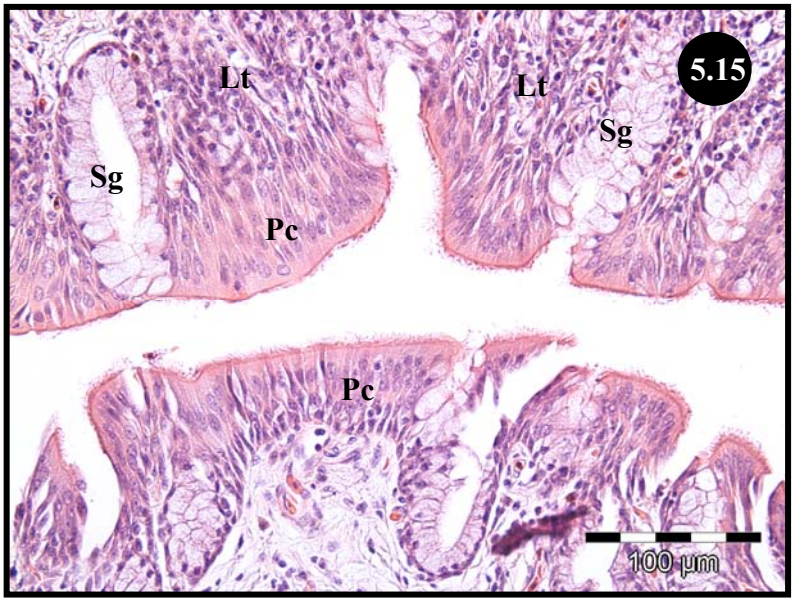


Figure 5.15: The folded ventrum of the tongue close to the frenulum. Note the ciliated pseudostratified columnar epithelium (Pc) and areas of diffuse lymphoid tissue (Lt). Simple tubular glands (Sg) are found in this region.

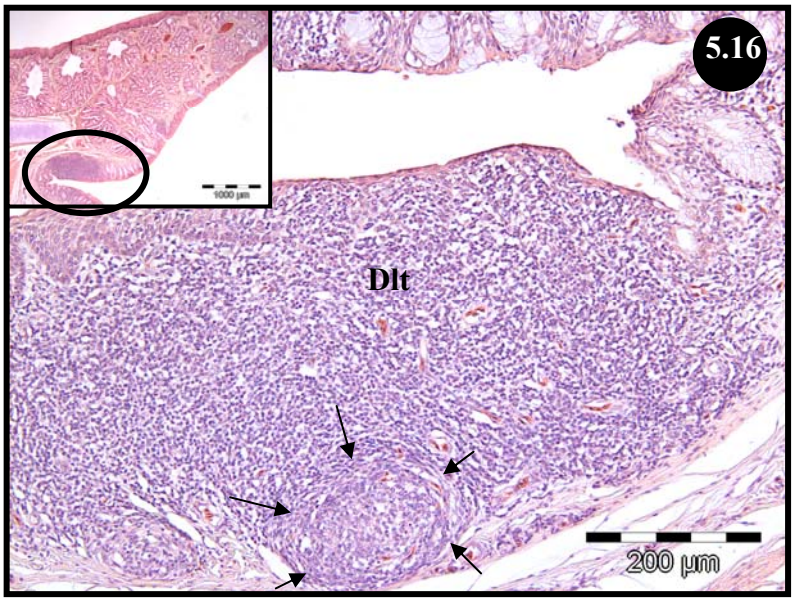


Figure 5.16: Junction of the tongue ventrum with the frenulum (inset) showing the large patch of diffuse lymphoid tissue (Dlt) consistently found in this region. Note the obliteration of the epithelial tissue by the lymphocytes and the nodular lymphoid tissue (arrows) situated at the base of the diffuse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

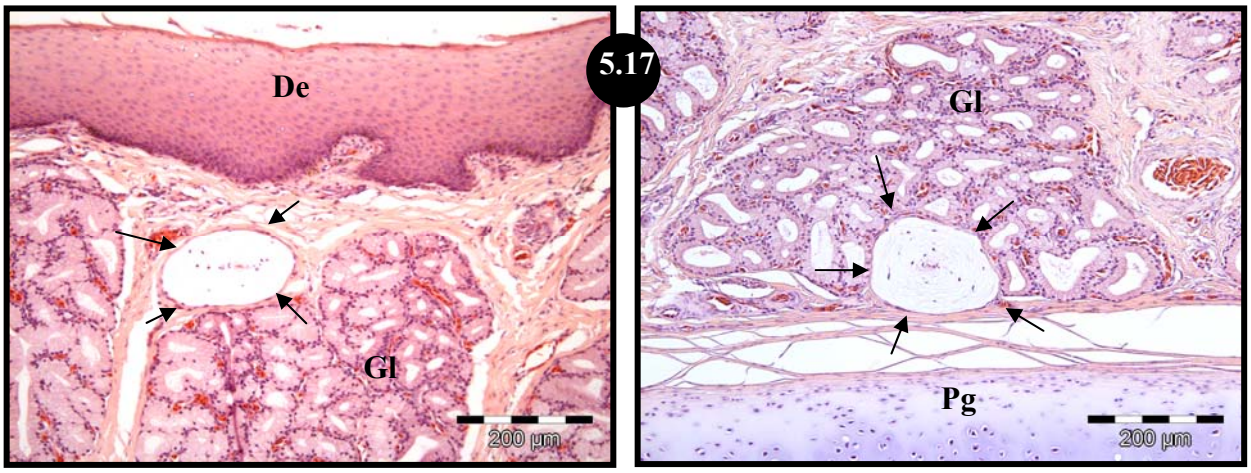


Figure 5.17: Dorsum of the tongue showing Herbst corpuscles (arrows) associated with the large simple branched tubular glands (Gl), one situated superficially just beneath below the dorsal epithelium (De) and one deeply positioned adjacent to the *paraglossum* (Pg).

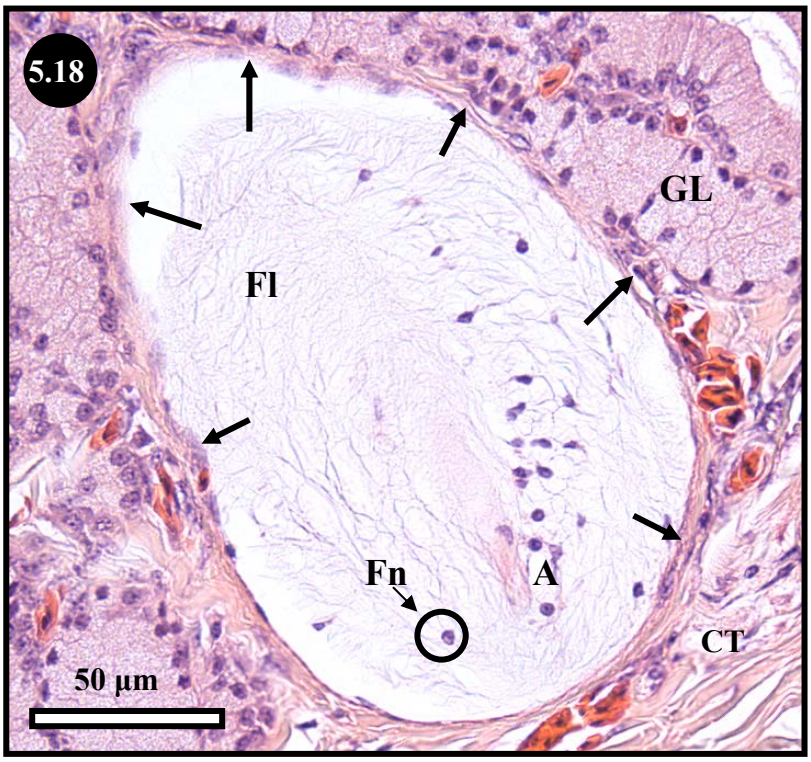


Figure 5.18: High magnification of a Herbst corpuscle showing the fibrous capsule (arrows) surrounding the outer core of fibrocytic lamellae (Fl) containing sparse fibrocytic nuclei (Fn). Central pink axon (A), glandular tissue (Gl), connective tissue (Ct).

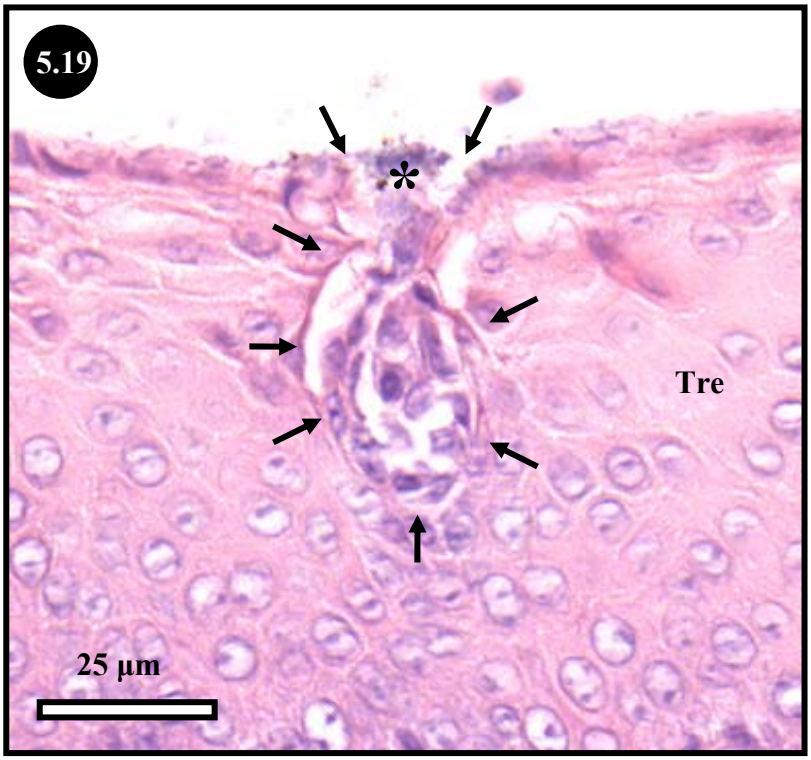


Figure 5.19: A structure resembling a taste bud observed on the tongue root close to the glottis. This structure is clearly demarcated (arrows) from the tongue root epithelium (Tre). Putative taste pore (*).

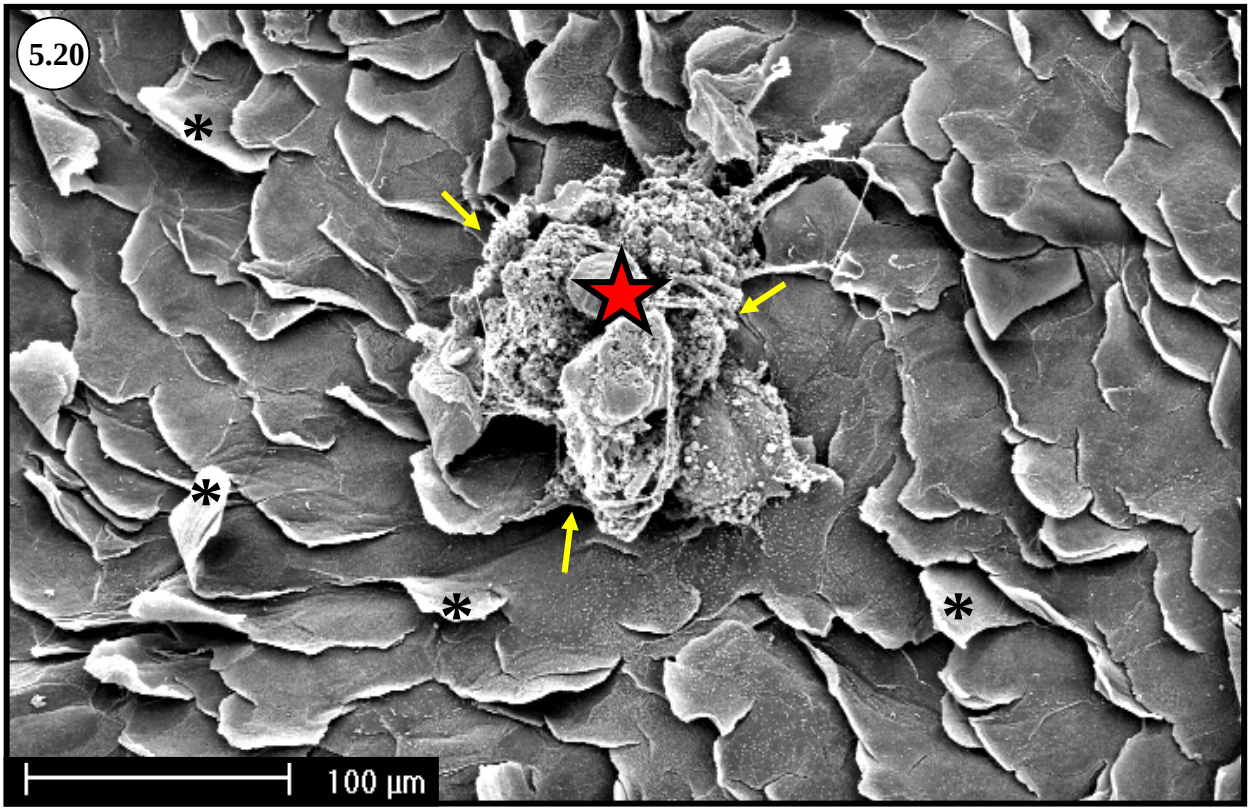


Figure 5.20: Dorsal tongue body demonstrating a large gland opening (yellow arrows) obscured by the mucus-secretion (red star) of the underlying gland. Note the individual desquamating surface cells (*) characteristic for this surface. x260.

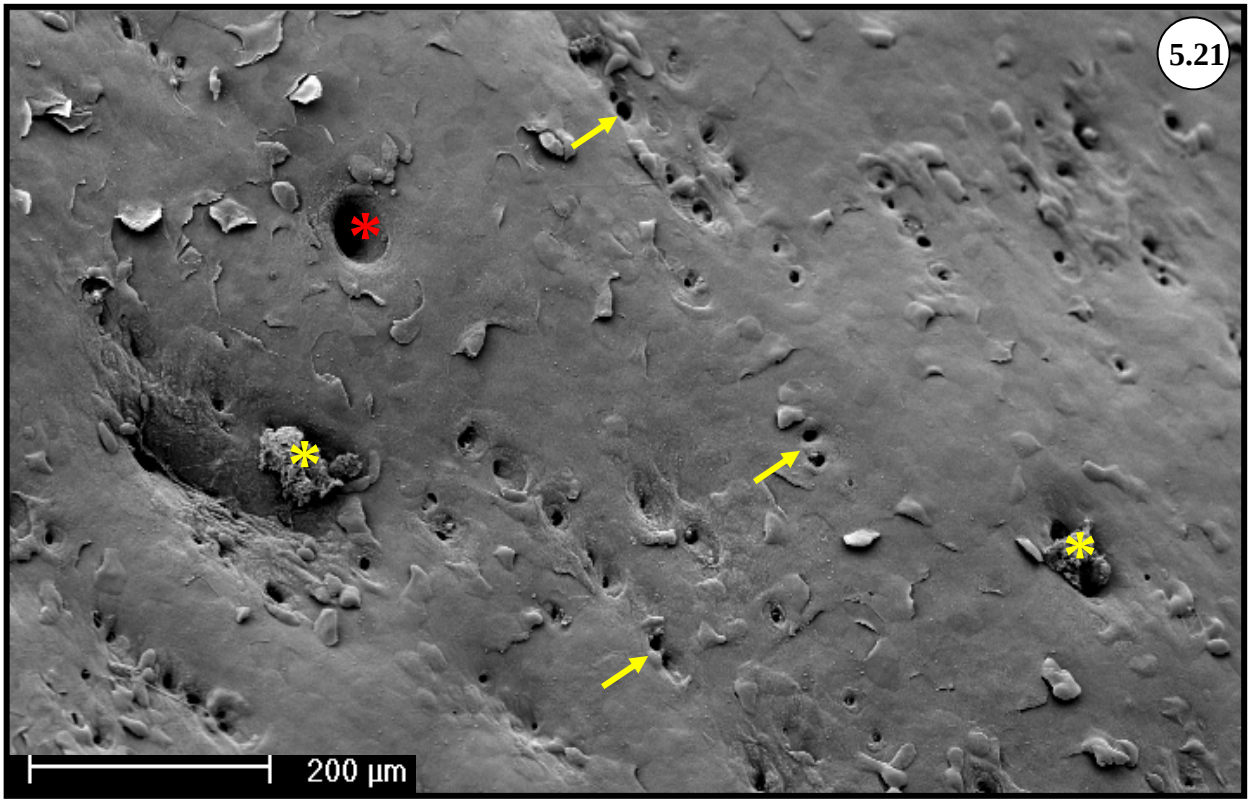


Figure 5.21: The caudo-lateral aspect of the ventral tongue body showing both large (red *) and small (arrows) openings. Mucus secretion (yellow *) is visible in some of the larger openings. Note the low frequency of desquamating surface cells. x120.

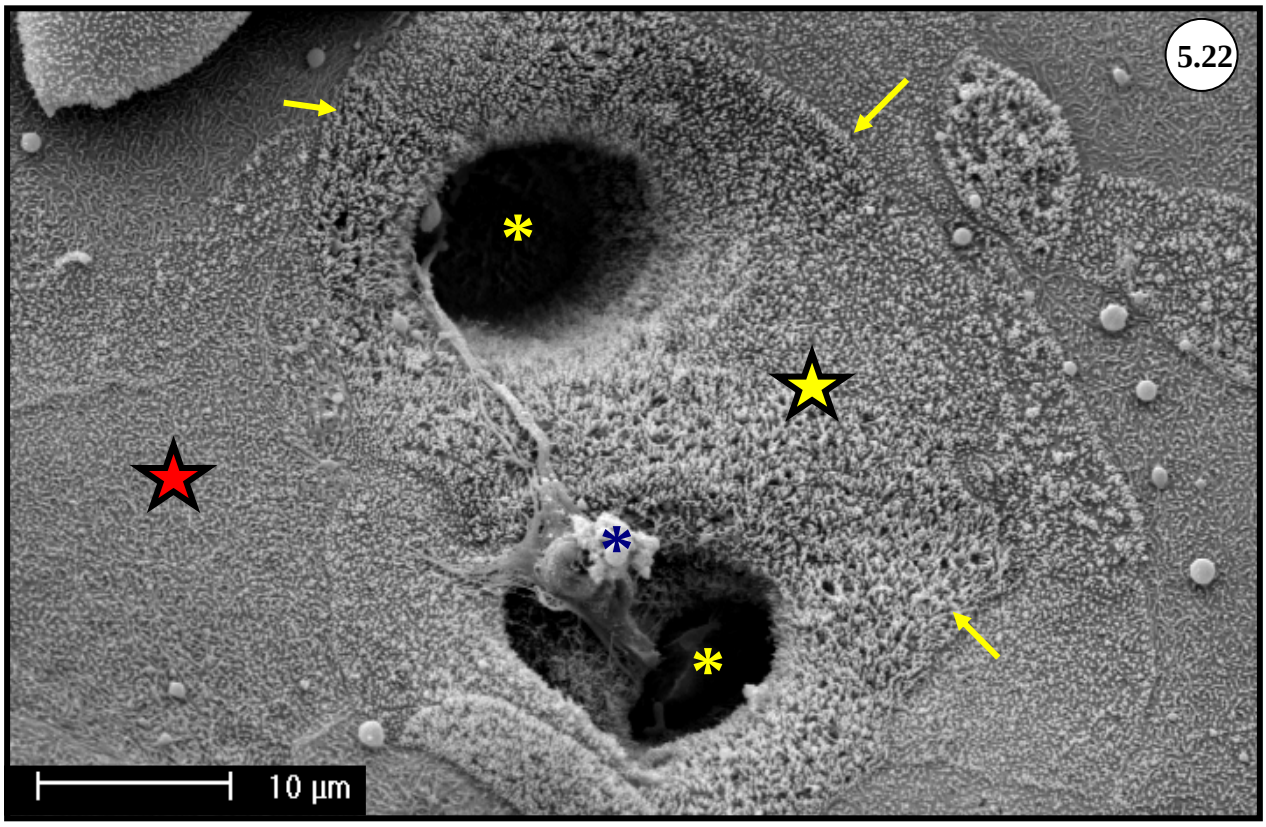


Figure 5.22: Caudo-lateral aspect of the ventral tongue body. Note that the cells around the small gland openings (yellow *) display dense microvilli (yellow star) on their surface. The transition between the ring of cells displaying microvilli and the surrounding cells with microplicae (red star) is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

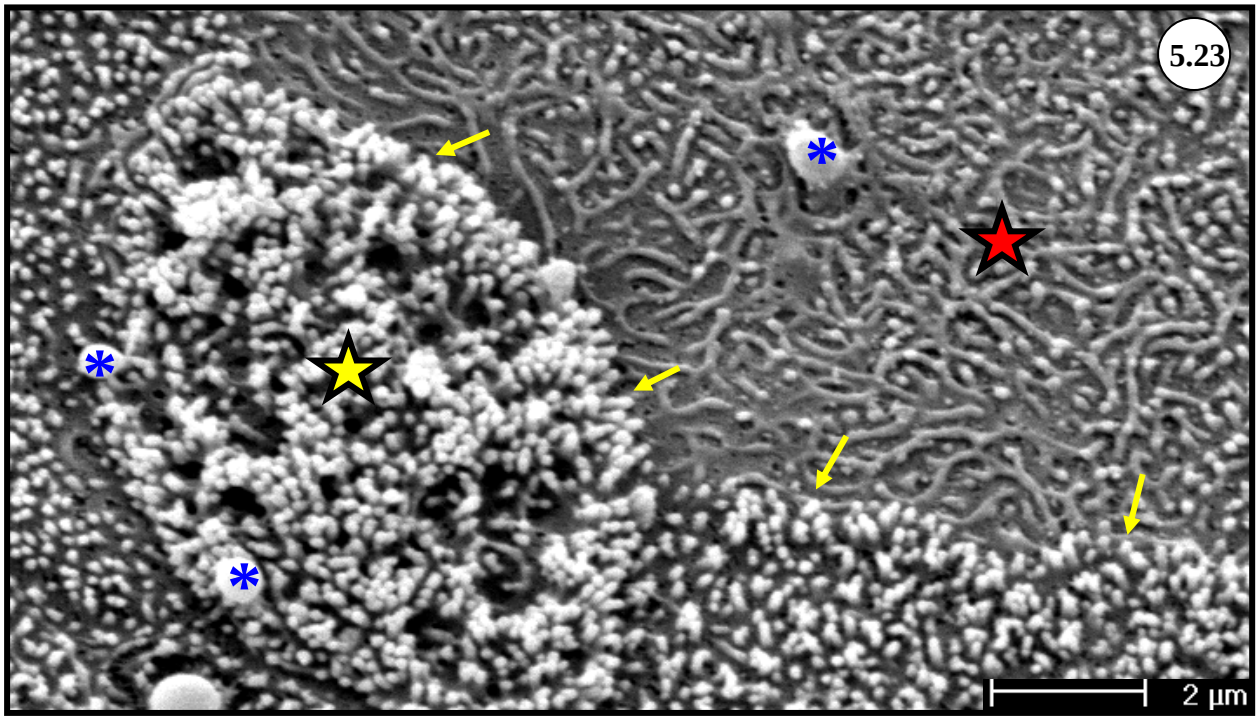


Figure 5.23: High magnification of the transition from microvilli (yellow star) to microplicae (red star) on the caudo-lateral aspect of the ventral tongue body. Note the abrupt transition (yellow arrows) as well as the presence of small globular structures (blue *) on the surface of both cell types.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

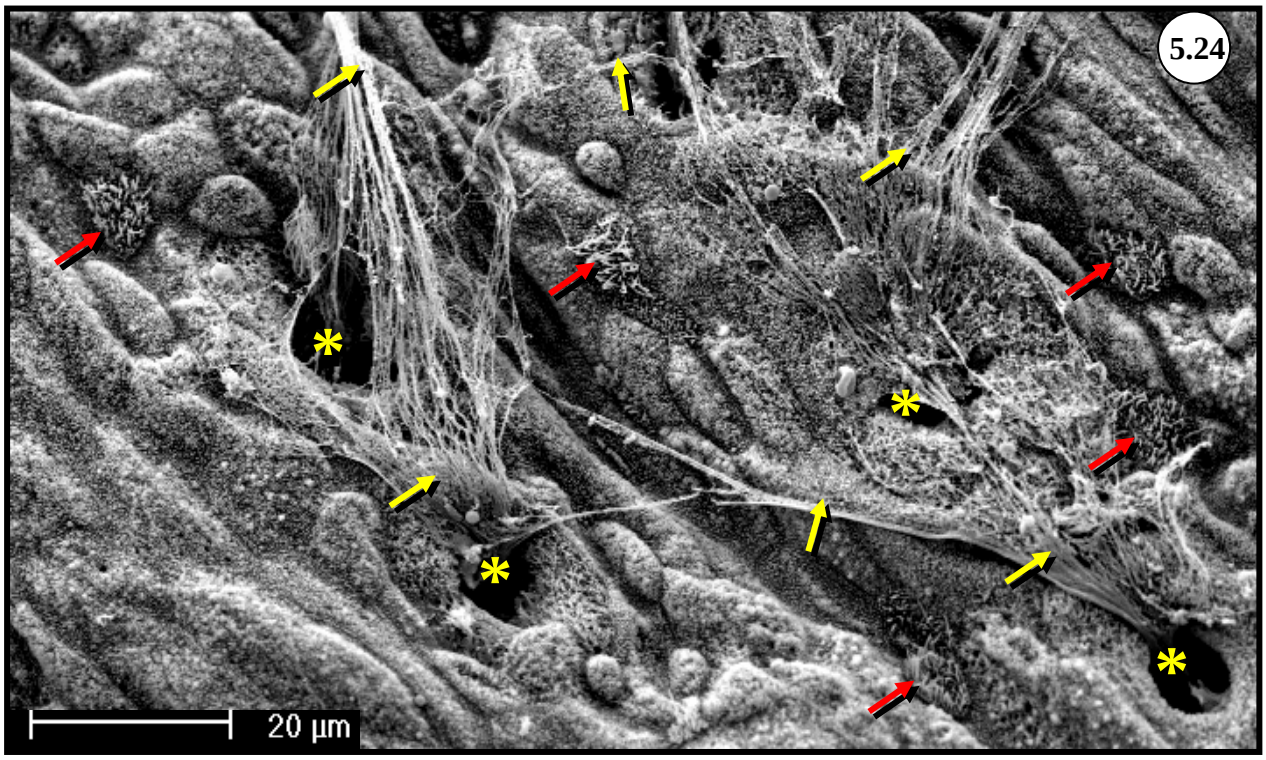


Figure 5.24: Mid tongue body ventrum. Numerous small openings (yellow *) showing strands of mucus secretion (yellow arrows) from the underlying glands are visible. All the surface cells of this region displayed densely-packed microvilli. Occasional ciliated cells (red arrows) also occurred in this region.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

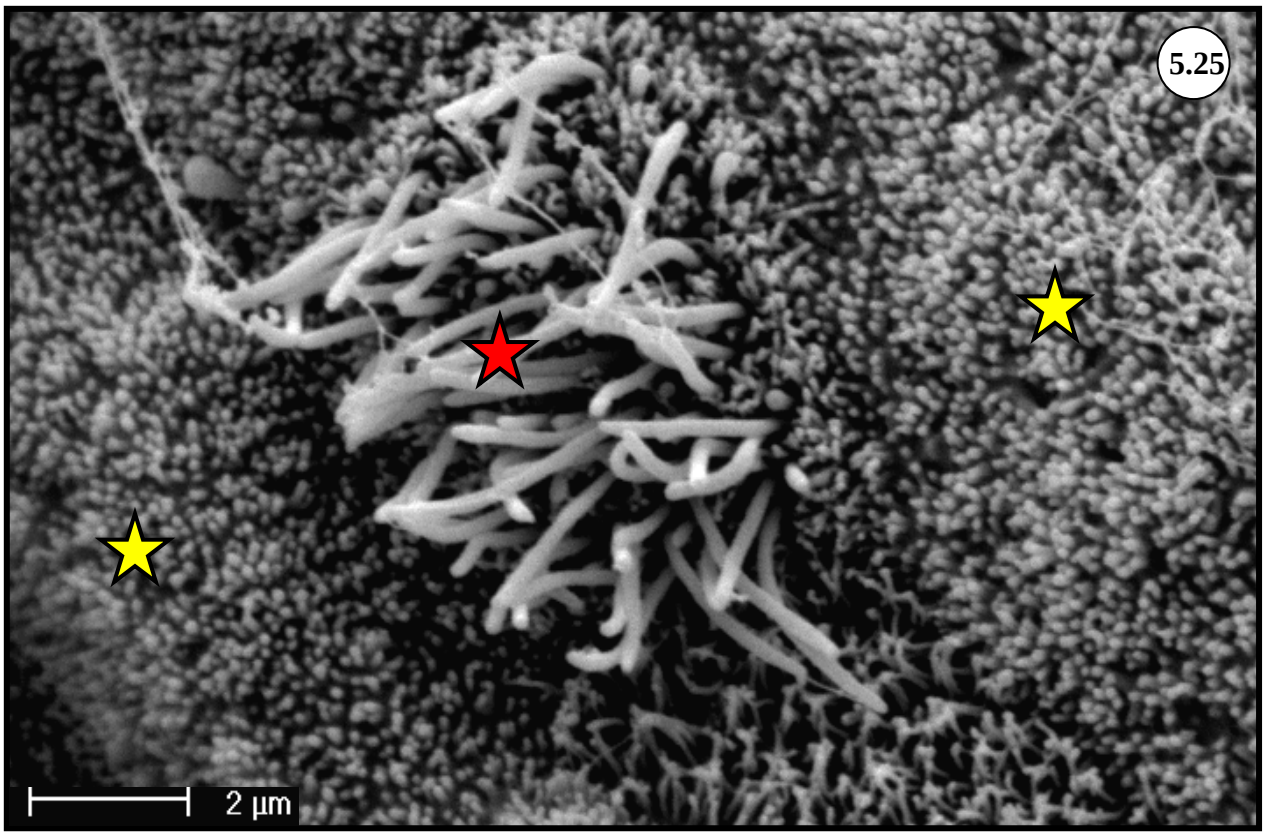


Figure 5.25: High magnification of a ciliated cell (red star) interposed between the cells displaying microvilli (yellow stars) on the ventrum of the mid tongue body. x7910.

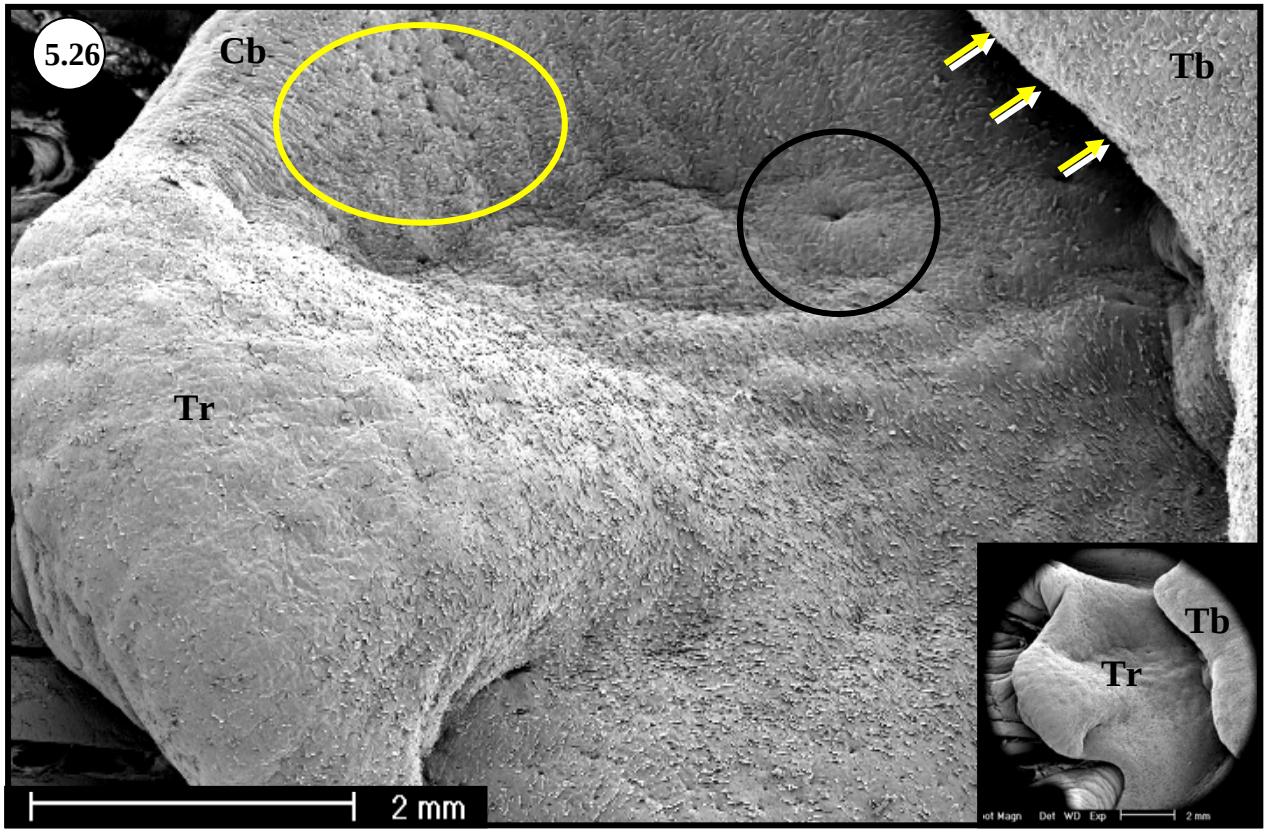


Figure 5.26: Low magnification of the dorsal tongue body (Tb) and tongue root (Tr). Note the flaky appearance of both surfaces due to the desquamation of individual surface cells and the large gland opening (black circle) in the mid tongue root and small gland openings (yellow circle) on the lateral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

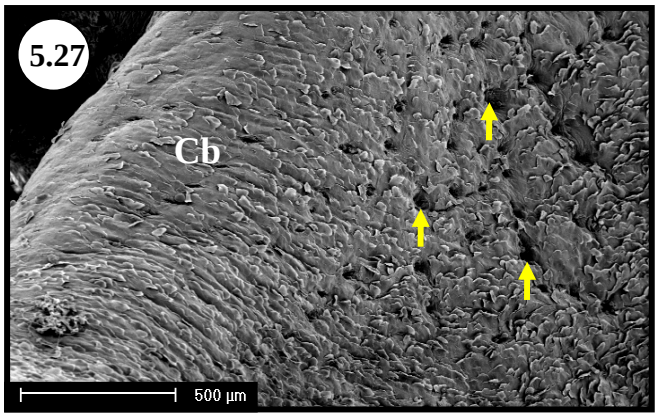


Figure 5.27: Enlargement of the yellow encircled area in Fig. 3.26 showing the numerous small gland openings (yellow arrows) on the lateral edge of the tongue root and mucosa covering the underlying *ceratobranchiale* (Cb). Note also the flaky appearance due to the desquamating surface cells. x60.

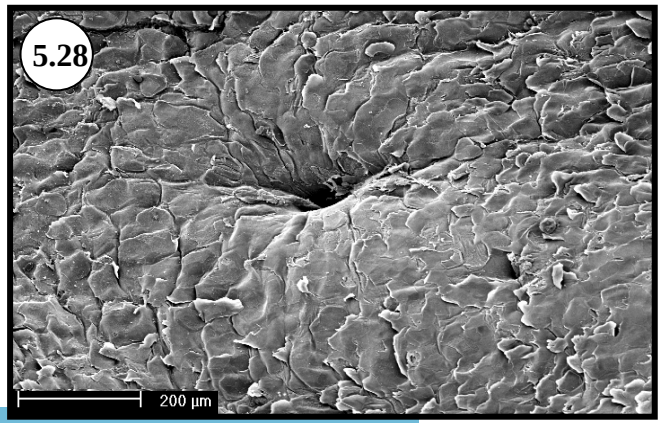
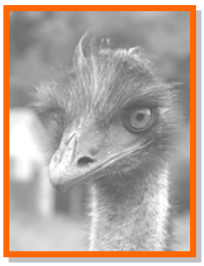


Figure 5.28: Enlargement of the black encircled area in Fig. 5.26 showing a large gland opening in the mid region of the tongue root. Note the raised edges around the opening and the vertical orientation of the cells forming the duct opening. x120.

CHAPTER 6



GENERAL CONCLUSIONS

The upper digestive tract of the emu has received little attention in the past, with only the tongue and laryngeal mound being briefly described and the oesophagus documented in two specimens. The emu is deemed a commercially important bird and thus a sound knowledge of the basic biology of this bird is imperative. This study described the detailed gross anatomy and histology of the oropharyngeal cavity and the structures and features therein as well as the proximal oesophagus. The morphology of the surface features was described using scanning electron microscopy.

The oral and pharyngeal cavities of the emu, as in other birds, could not be distinguished from one another using recognisable morphological features and thus formed one cavity, the **oropharynx**. This cavity was dorso-ventrally flattened in the closed gape and bounded laterally by the tomia. Both the floor and roof of the cavity were divided into rostral aglandular pigmented regions, lined by a keratinised stratified squamous epithelium, and caudal non-pigmented glandular regions, lined by a non-keratinised stratified squamous epithelium. The non-pigmented floor housed the tongue and laryngeal mound. The non-pigmented roof housed the choana and merged with the two pharyngeal folds, separated at their origin by the infundibular cleft. Numerous Herbst corpuscles were located in the connective tissue in the pigmented regions. Thus these areas would have a high sensitivity to touch and texture. This may be an important function considering the investigative nature of this bird as well as being important for food selection. Herbst corpuscles are a common feature in the ratite oropharynx, and are described in the greater rhea, ostrich and kiwi. The oropharynx of the emu therefore reflects the general pattern of the ratites with a few modifications and differences.

The emu has prominent mandibular and maxillary **nails**, features only previously identified in pelicans, gulls and ducks. The ostrich also has such structures, thus the ratites can be included amongst the birds with nails on the bill tips. The **serrated tomia** of the mandible were a unique feature of the emu. Such structures are also present in the ostrich but are very rudimentary. It

has been previously stated that the emu has no need for a strong bill due to its diet. However, the nails and serrations provide a strong gripping and tearing instrument. The numerous Herbst corpuscles also provide a high degree of sensitivity.

The non-pigmented floor displayed many small **folds** and two larger, flat glandular folds. Numerous nodules were also seen, representing lymphoid tissue aggregations. The ingestion method of the emu has been previously described, where the food travels from the bill tips to the oesophageal entrance, thus bypassing structures in the oropharynx. To allow the passage of ingesta through the dorso-ventrally flattened oropharynx, the tongue is used to depress the oropharyngeal floor, thus enlarging the cavity. The folded nature of the floor would allow for such enlargement. During fluid ingestion, the folded floor would be distensible, allowing for the accumulation of fluid in the oropharynx before lifting the head to transport fluid to the oesophagus.

Following the general trend in ratites, the emu **tongue** is greatly reduced in comparison to the bill length and is specifically adapted for swallowing during the cranioinertial method of feeding employed by palaeognaths. It was not only the shape of the tongue that differed between ratites, as previously reported, but also the colour of the tongue, the appearance of the tongue margins and root, the length of the tongue in comparison to the bill, and the shape of the *paraglossum*. Previously, the only function attributed to the emu tongue was that of retraction during swallowing. However, it was seen from this study that the tongue has at least four main functions, namely: 1.) digestive (role in swallowing), 2.) sensory (taste and touch), 3.) immunological and 4.) mechanical protection (by virtue of mucus-secretion).

Although the **laryngeal mound** of the emu has been previously described, important differences were noted in this study. The laryngeal mound has been depicted as being similar to that of the ostrich, although it clearly differs. The glottis is wide rostrally and narrows caudally. There are no papillae on the laryngeal mound. The three to five longitudinal folds lying ventrally in the laryngeal entrance have not been previously noted. Although the function of these folds was not determined in this study they seem to be a unique feature of the emu compared to the other ratites. The glottis of ratites is relatively larger in comparison to that of other bird families. Birds do not posses an epiglottis; however, due to the wide glottis present in the emu and ostrich, it appears possible that the tongue possesses special modifications to assist in closing the glottis during ingestion. The shape and location of the emu tongue root would indicate that it may fulfil

such a function. The laryngeal mound of the emu performs both a respiratory and digestive (swallowing) function. The crico-arytenoid glands are located on the emu laryngeal mound. Their mucus-secretion would assist in the digestive function of the mound and contribute to lubrication of the oropharynx. Herbst corpuscles, attributing a sense of touch to the laryngeal mound, are also present. The laryngeal mound differs between the ratites with regard to shape, glottis and papillae.

In the emu the **choana** is triangular with a wide, median grooved ridge separating the two oblong internal nares. The shape of the choana differs between the ratites, with that of the emu appearing unique. The median groove of the ridge continues caudal to the choana as the **infundibular cleft**. The infundibular cleft in the emu was less defined than that of the ostrich.

The two large **pharyngeal folds** of the emu were similar to those of the ostrich and displayed a high density of glandular and lymphoid tissue. The emu had, additionally, a small tissue projection on the caudo-lateral edge of the folds, composed almost entirely of lymphoid tissue, which together with the pharyngeal folds, effectively formed pharyngeal tonsils (*lymphonoduli pharyngeales*). The shape and size of the pharyngeal folds differ between the ratites. The pharyngeal folds of the emu fulfil a mechanical function of closing off the oesophageal entrance during respiration, an immunological function and a protective function (attributed to mucins supplied by the numerous mucus-secreting glands located in the folds).

The observations of the **proximal oesophagus** confirmed the features previously described for the emu oesophagus as well as for other ratites and birds in general. Additionally, the identification of taste buds within the epithelium was a previously unreported observation. This study is the first report of taste buds in a ratite oesophagus. As food is transported to the oesophageal entrance and largely bypasses the structures in the oropharynx, the location of taste buds in the proximal oesophagus seems a logical finding as the emu may discriminate the food while swallowing and thus be able to decide whether more of that particular food should be ingested. The oesophagus of the emu shows three main adaptations for the ingestion of large food particles: 1.) the diameter is relatively large, 2.) the mucosa is longitudinally folded allowing great distensibility and 3.) the numerous mucous glands secrete copious amounts of mucus to lubricate the lumen and food for ease of transport.

The following groups of **salivary glands** were identified: caudal intermandibular, lingual (dorsal, rostro-ventral, caudo-ventral, frenular and radical), crico-arytenoid, oral angular, caudal palatine, pharyngeal and oesophageal. The mucous glands were small, simple tubular and large, simple branched tubular in the oropharynx, and simple tubular (occasionally branched) in the oesophagus. The main function of the glands is mucus production which contains mucins. Mucins provide protection from desiccation and mechanical damage, help maintain cellular water balance, provide lubrication and are antimicrobial in action. Sticky saliva also assists in the backward propulsion of food and prevents regurgitation.

Herbst corpuscles in the emu were most numerous in the dermis of the bill skin. They varied in size and grouping, with most occurring singly and others arranged in longitudinal chains. They occurred in the connective tissue underlying the pigmented oropharyngeal roof and floor. In the non-pigmented glandular regions they were associated mainly with the larger glands. Their numbers diminished in a caudal direction. They were absent from the pharyngeal folds and proximal oesophagus only. Herbst corpuscles also occur in the ostrich, greater rhea and kiwi oropharynx. Their ubiquitousness in the emu oropharynx indicates that the upper digestive tract is highly sensitive to touch and thus may play an important role in food selection by virtue of texture.

The **lymphoid tissue** in the emu oropharynx and proximal oesophagus occurs mainly as accumulations of diffuse lymphoid tissue. This tissue was located in the connective tissue at the junction between the pigmented and non-pigmented roof; ventrum, frenulum and root of the tongue; the non-pigmented oropharyngeal floor; the rictus; oesophagus; and particularly in the pharyngeal folds. In the glandular areas, the diffuse lymphoid tissue was mostly associated with the ducts of the large glands. The epithelium overlying the lymphoid tissue often showed a change from a stratified squamous epithelium to a psuedostratified ciliated columnar epithelium. Only *Lymphonoduli pharyngeales* (pharyngeal tonsils) were identified in the emu.

Taste buds in the emu were isolated structures found in the epithelia of the non-pigmented oropharyngeal floor, tongue root and proximal oesophagus. They were clearly demarcated from the surrounding epithelium, displayed a taste pore and contained vertically oriented elongated cells. These presumably represented the sensory and supporting cells which could not be distinguished from one another by the staining techniques used in this study. This is the first report of taste buds in the emu and ratites in general.

SEM confirmed a number of features noted histologically and provided corroboratory evidence regarding the distribution of the different types of glands. The keratinised regions of the rostral parts of the oropharynx displayed sheets of desquamating cells which revealed a pattern of microridges on their surface. The non-keratinised regions of the oropharynx revealed both individual desquamating surface cells, which displayed a complex surface pattern of microplicae, or regions of clearly demarcated cells, which displayed a surface adorned with microvilli. Cilia were present in the ducts of some of the large glands, as well as on the tongue ventrum near the opening of glands. Openings in the surface were round to oval and were generally lined or bordered by concentrically arranged cells. Large openings representing the ducts of the underlying large, simple branched tubular glands, often displayed cilia and emerging mucus-secretions. Small openings, lined and surrounded by dense microvilli, represented the openings of the underlying small simple tubular (sometimes branched) glands. Larger openings were generally evenly distributed, whereas the smaller openings mostly occurred in groups, or near the large openings. No meaningful comparisons can be made to other ratites regarding surface morphology of the oropharynx and proximal oesophagus due to the absence of detailed information in previously published works.