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Comparative anatomy of the larynx of the minke whale, *Balaenoptera acutorostrata* and the pygmy right whale, *Caperea marginata*.

by

Desray Reeb

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Supervisor: Dr P. B. Best
Mammal Research Institute
Department of Zoology
University of Pretoria
Pretoria

Abstract

The laryngeal apparatus of *C. marginata* is described for the first time and proves to be entirely different from that of any mysticete so far described, especially in the position of the laryngeal sac which is separate from the tracheal rings and lies to the right of the animal. Massive ontogenetic development of the sac in adult males is demonstrated, far greater than that seen in a series of two juvenile and two adult *B. acutorostrata* also examined. Histological analysis of the laryngeal sacs of both *C. marginata* and *B. acutorostrata* shows the walls to be highly muscular, well innervated and vascularised, indicating an active organ. Coiled blood vessels and nerves found in *C. marginata* support the theory that in this species the sac undergoes extensive expansion and contraction, and a possible association with the unique thoracic structure of the species is suggested. The roles of the laryngeal sac and arytenoid cartilages in mysticete sound production are discussed.

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CHAPTER 1: INTRODUCTION

The "ghostly, eerie" sounds, described by many whalers and fishermen on their various expeditions, were man's first encounters with whale vocality (Payne & McVay 1971). These reports and other statements made by observers in the field were discouraged by the prevailing scientific opinion that whales were mute, presumably because they were said to lack vocal cords. Sound is poorly transmitted between water and air, and because an immersed man's hearing is quite dull, it was not until World War II, when research in sonar and anti-submarine warfare provided the facilities for listening underwater, that it became generally known that many species of whales are vocal. It was at this time that the first recordings of whale vocalisations were made (Schevill 1964).

Sound is perhaps the major sense used by cetaceans. The physical properties of water allow sound to be propagated much more effectively than it can be in air. This advantage has been put to good use by cetaceans, who use sound in two main ways - for echolocation and for communication. Mysticetes (baleen whales) are not known to echolocate and, although short pulses resembling clicks have been heard in the presence of certain species, they are certainly not typically heard from such species (Evans 1987), and have not been demonstrated experimentally to have any echolocatory function.

In general the sounds made by odontocetes (toothed whales) can be classified into three general categories: tonal whistles, pulsed sounds of very short duration used in echolocation, and less distinct pulsed sounds such as cries, grunts and barks (Richardson, Greene Jr., Malme & Thomson 1995) Most odontocete whistles are narrow-band sounds - sometimes tones, having most of their energy below 20 kHz (Richardson *et. al.* 1995). However, besides broad-band clicks, which can contain any frequency, marine odontocetes are not known to produce sustained frequencies much below 500 Hz, and most of their vocal activity is at frequencies above 20 kHz (Payne & Webb 1971). Pulsed calls are very complex with energy at 500 Hz to 24 kHz and pulse repetition rates up to 5000 per sec (Schevill & Watkins 1966; Ford & Fisher 1982).

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Scientists are divided in their opinions about the site(s) of odontocete sound production. There are two schools of thought; those who predict its genesis from somewhere in the larynx (Lawrence & Schevill 1965), and those who predict its genesis from somewhere in the soft anatomy of the forehead (Evans & Prescott 1962; Norris 1964), especially from the region of the nasal plugs.

All the theories to date concerning the mechanism of delphinid sound production have implicated the larynx (arytenoepiglottic tube), the complicated diverticuli associated with the blowhole mechanism, the large muscular plugs that seal off the internal nares, or various combinations of these. The driving mechanism has been thought to be pneumatic, mechanical or both. Various combinations of internal sound transmission paths have been considered: air-muscle/fat-water; air-bone-water; tissue-water (Norris 1969; Evans 1973).

A group of mainly European advocates (Purves 1967; Purves & Pilleri 1983) claim that the sound source is located within the larynx and a second theory, favoured by mainly North American researchers places the sounds source in the nasal plug area just below the blowhole (Evans & Prescott 1962; Norris 1964; Norris 1969; Norris & Harvey 1973; Evans 1973; Evans & Maderson 1973; Amundin 1991). More recent studies have shown the upper nasal pathways to be the source of sound generation. Diercks *et. al.* (1971, in Amundin 1991) used a multiple array of contact hydrophones, which was placed on the rostrum and the melon of dolphins to show that the location of the click sound source was in the nasal area. Cineradiographic evidence from live, phonating dolphins obtained by Norris *et. al.* (1971, in Amundin 1991) and Dormer (1979) showed sound generation and air recycling taking place in the upper nasal pathways and diverticula. There has not, as yet, been any experimental demonstration that the larynx may be involved in sound production in odontocetes.

Mysticete sounds are varied and complex, consisting for the most part of lower and longer sounds than have yet been recorded from odontocetes. Clark (1990) describes mysticetes as producing vocal and non-vocal sounds. Non-vocal sounds include blow, slap and miscellaneous (rubbing against objects, flatulence or baleen rattle) sounds, while vocal sounds include calls and songs.

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A broad classification of vocal mysticete sounds would consist of the following (Fish, Sumich & Lingle 1974; Winn & Perkins 1976; Thompson, Winn & Perkins 1979; Watkins 1981; Cummings, Thompson & Ha 1986):

1. Tonal pulses and typical low frequency moans, between 0.4 and 36 secs in duration and of 12 to 500 Hz frequency, but usually between 20 and 200 Hz. Moans may either contain strong harmonic structure or be pure tone, such as the 20 Hz signals recorded from fin whales (Schevill, Watkins & Backus 1964; Thompson, Findley & Vidal 1992). Clark (1990) describes the principal energy for these "simple" calls as being below 1000 Hz. All but the sei (*Balaenoptera borealis*) whale are known to produce these sounds.

2. Grunt-like thumps and knocks of shorter average duration than moans (50 to 500 msec), and between 40 and 200 Hz. Clark (1990) extended the average duration of these sounds to between 50 and 100 msec being in the 100-1000 Hz range. The humpback (*Megaptera novaeangliae*), southern (*Eubalaena australis*) and northern (*E. glacialis*) right, bowhead (*Balaena mysticetus*), gray (*Eschrichtius robustus*), fin (*Balaenoptera physalus*) and minke (*B. acutorostrata*) whales are all known to produce these sounds.

3. Chirps, cries and whistles, with frequencies above 1 kHz. Chirps are generally pulses producing short (50-100 msec) discrete tones which change frequency rapidly and are not harmonically related, whereas cries and whistles are pure tonal with or without harmonics.

4. Click-like sounds or pulses of a fraction of a second in duration (0.5 to 5 msec) with frequencies from 3 to as high as 30 kHz, recorded in the blue whale (*Balaenoptera musculus*) (Beamish & Mitchell 1971; Cummings & Thompson 1971). Click-like sounds of either pure frequency or broad band sounds are reported from *B. acutorostrata*, *E. robustus*, *M. novaeangliae*, *B. borealis*, *Balaenoptera edeni*, and *B. physalus*.

To this list, Clark (1990) adds what he refers to as complex calls. Complex calls are broadband, pulsive signals which consist of variable mixtures of amplitude, modulation of noise and/or a frequency-modulated fundamental. Typical bandwidths for complex pulsive signals are in the

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500-5000 Hz range. Complex calls are often described as sounding like screams, roars and growls. Complex calls have been recorded from *B. mysticetus, M. novaeangliae, E. australis* and *E. glacialis*.

Humpback whales produce a series of ordered themes containing a number of repetitive phrases in the form of a song, which may last 8-30 minutes (Payne & McVay 1971) and which may be repeated more or less exactly for several hours at a time, with no breaks longer than one minute (Payne & Webb 1971). Humpbacks are not the only baleen whales producing repetitive or monotonous vocalizations. According to Cummings and Philippi (1970) there is some evidence that northern right whales (*E. glacialis*) do so as well. But according to Clark (1990) southern right whales have been recorded extensively during their mating seasons but no sounds resembling song have been recorded and it is therefore assumed that this species does not sing. Songs have been reported for bowhead (Ljungblad, Thompson & Moore 1982; Würsig & Clark 1993) and fin whales (Watkins, Tyack, Moore & Bird 1987). The most precisely repeating sounds yet ascribed to a mysticete are probably the 20 Hz signals produced by fin whales (Schevill *et. al.* 1964; Watkins *et. al.* 1987; Thompson *et. al.* 1992).

Many speculations have been made, but as yet, the mechanism by which mysticetes generate these sounds is unknown. Schulte (1916) mentions a "subcircular diverticulum from the dorsal wall of the respiratory passage" and a 'spritzsack' along the anterior wall, however, Howell (1970) did not find any true diverticula, only a slight folding and wrinkling of the rostral end of the mucosa.

Initial anatomical work on the laryngeal area of mysticetes was recorded from the late eighteenth to the late nineteenth centuries. In 1787, Hunter discovered the presence of a laryngeal sac on the ventral surface of the larynx of *Balaenoptera rostrata* (*B. acutorostrata*). The laryngeal sac was later recorded by Eschricht & Reinhardt (1866), Carte & Macalister (1868), Turner (1872), Watson & Young (1879), Beauregard & Boulart (1882), Dubois (1886), Benham (1901), Schulte (1916), Hosokawa (1950), Yablokov, Bel'kovich & Borisov (1974), Quayle (1991) and Haldiman & Tarpley (1993) for a variety of species including *B. mysticetus, B. acutorostrata, M. novaeangliae, Eubalaena australis, B. borealis, Delphinapterus leucas* and *Physeter catodon.*

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The muscular, "sac-like" structure connected to the ventral wall of the mysticete larynx has been referred to, by many authors, in a variety of ways i.e., "laryngeal pouch" (Turner 1872), "air bag/laryngeal sac" (Murie 1870, in Watson & Young 1879), "sub-laryngeal pouch" (Benham 1901), "ventral air-sac" (Watson & Young 1879), "saccus laryngis ventralis" (Hosokawa 1950) and "epiglottic cavity" (Yablokov *et al.* 1974). For the purposes of this study, this structure will be referred to as the "laryngeal sac".

The cetacean laryngeal apparatus is constructed, as in other mammals, of a cartilaginous framework and several muscles connecting the cartilages. These form as a whole a tubular organ with the laryngeal cavity in it. The inner surface is covered with a mucous membrane continuous with that of the pharynx upwards and of the trachea downwards (Hosokawa 1950).

The laryngeal cartilages are five in number, three of which (thyroid, cricoid and epiglottic) are unpaired and the other two (arytenoid) are paired (Carte & Macalister 1868; Turner 1872; Hosokawa 1950).

As in other mammals, the laryngeal muscles in mysticetes are classified into two groups, extrinsic and intrinsic. The extrinsic muscles are those connecting the larynx with neighbouring structures and the intrinsic muscles are those which begin and end within the larynx itself (Hosokawa 1950). Up to 17 different muscles (Carte & Macalister 1868; Benham 1901) have been described in the larynx of baleen whales (Yablokov *et al.* 1974).

Eschricht & Reinhardt (1866) pointed out that the most essential peculiarity of the larynx of mysticetes, as compared with that of the odontocetes, is in its allowing the mucous membrane of the respiratory canals, by means of an opening on the ventral surface, to appear in the form of a sac with an exterior covering of a strong layer of muscles. A similar sac is found in the respiratory canals of many terrestrial mammals, as is very well known. In most cases the sac appears between the hyoid bone and the thyroid cartilage, though in others, especially in some of the apes, it appears between the thyroid and cricoid cartilages, or between the latter and the first ring of the trachea; the last-mentioned case most resembles that of the mysticetes (Eschricht & Reinhardt 1866).

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In their studies of a juvenile and neonate female respectively, Carte & Macalister (1868) and Benham (1901) found no vestiges of vocal chords or of a ventricle, or a lateral laryngeal sacculus in *B. acutorostrata*. However, situated in front of the cavity of the larynx, and opening by a wide orifice immediately at the root of the epiglottis, was a remarkable large musculo-membranous mesial sac or laryngeal pouch, which extended downwards and backwards in front of the trachea. Its walls, which were thick, were almost entirely composed of circular muscle fibres. The interior of the sac communicated directly with the central portion of the laryngeal cavity by a wide orifice and was lined by a continuation of the mucous membrane of the laryngeal cavity.

Likewise, in a 6 meter foetus of the blue whale, *Balaenoptera musculus*, Turner (1872) found that there were no true vocal cords passing from the thyroid to the arytenoid cartilages, or laryngeal ventricles. However, on each side within the aperture of the glottis, a short distance below the free edge of the aryteno-epiglottidean folds, a slight fold of the mucous membrane extended obliquely in the antero-posterior direction. These folds might be regarded as rudimentary false vocal cords. An interesting structure that was noticed by Turner (1872) was a "great laryngeal pouch or cul-de-sac" that was connected with the larynx. He describes the laryngeal sac as being 25.5 cm in length. In the same species, Beauregard & Boulart (1882) reported a female foetus of 3.6 m as having a laryngeal sac 21 cm in length (measured from anterior portion to laryngeal cavity).

Hosokawa (1950) conducted a detailed study of the laryngeal apparatus of two female sei whales (*B. borealis*), 12.7 and 13.9 meters in length. In both specimens he found a sac attached to the ventral wall of the larynx, elongated downwards through the whole length of the trachea. The cavity within the sac was elongated longitudinally and communicated with the proper cavum laryngis through a slit along nearly the whole length of the arytenoid cartilage. The inner surface of the sac had many folds and grooves and many granular prominences were visible on the mucous membrane. Judging from this structure, Hosokawa (1950) concluded that it seemed certain that this sac was capable of extension and contraction in the living whale.

Why this structure occurs in mysticetes, as well as the anatomical nature of the laryngeal sac, have been very differently answered by anatomists. Hosokawa (1950) proposed that a key for settling

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the problem may lie in the fact that the laryngeal sac in the right and humpback whales is not as large as those of the rorquals, showing an intermediate form between the laryngeal recess found in odontocetes (Dubois 1886) and the laryngeal sac in the mysticetes (Eschricht & Reinhardt 1866; Carte & Macalister 1868; Benham 1901; Hosokawa 1950). Evaluation of Hosokawa's hypothesis is complicated by the fact that the only references he cites refer to studies on juvenile animals.

The laryngeal sac, according to Dubois (1886) and Benham (1901), is derived from the downward "sagging" of the thyro-arytenoid muscle, so as to project between thyroid and cricoid cartilage which has led to the oblique and nearly vertical position of the glottis.

When contemplating the origin of the laryngeal sac one is tempted to see some interrelation between the laryngeal sac and the aryteno-epiglottideal tube of odontocetes - to assume in some way that the function of the sac of the mysticetes is taken on by the glottideal tube and the elaborate "spiracular sacs" of the odontocetes. This leads us to look for any homologue in the odontocete of the laryngeal sac of the mysticete (Benham 1901).

Eschricht & Reinhardt (1866) stated that a laryngeal sac was present only in mysticetes, but Watson & Young (1879) point out that although the laryngeal sac is consistently recorded in mysticetes, in which it attains its greatest development, this structure cannot be regarded as a specific character of the mysticetes. An analogous sac has been described in *Grampus griseus*, which, according to Murie (1870, in Watson & Young 1879) "fills in great part the angle of junction between the enlarged epiglottis and the thyroid cartilage, but does not reach the posterior border of the latter". The arrangement in *D. leucas* is similar to that described for *G. griseus* and this fact corroborates Murie's assertion that the above apparent distinction between the mysticetes and odontocetes is "one rather of degree than of kind" (1870, in Watson & Young 1879).

As previously stated, the laryngeal sac is a median, ventral evagination of the muscular wall of the larynx, between the thyroid and cricoid cartilages which is post-thyroideal in position. In the odontocetes, no such structures occur in the same relative position. A small median sac has been described by various authors (Murie 1870, in Watson & Young 1879) in various odontocetes, but

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this sac has glandular walls and occurs between the base of the epiglottis and the anterior border of the thyroid cartilage in a pre-thyroideal position (Benham 1901). Murie (1870, in Watson & Young 1879), Turner (1872) and Watson & Young (1879) regard this sac as homologous with that of the mysticetes, and Dubois (1886) supports their opinion. The latter author further includes in the homology, small lateral, glandular outgrowths (also known as Morgagni's ventricles) at the sides of the base of the epiglottis and projecting, more or less, over the upper margin of the thyroid; these ventricles are known in a great variety of mammals.

In all these cases, the sac or sacs lie above the thyroid cartilage. Of the mammals, it is only the mysticetes that possess a post-thyroideal pouch.

Benham (1901) states that it is unacceptable to assume that the median post-thyroideal laryngeal sac of the mysticetes is truly and genetically homologous with that of the odontocetes and other mammals - whether median or lateral - which is pre-thyroideal. Yablokov *et al.* (1974) concur with Benham (1901) and state that in referring to the comparative anatomic aspect, "this cavity cannot be homologised either with the small dilatations on the floor of the larynx of toothed whales as previously assumed by Murie (1870, in Watson & Young 1879) and Turner (1872) or to the known structures in terrestrial mammals".

As yet, the function or purpose of the laryngeal sac is uncertain. Several hypotheses have been put forward, of which three seem the most plausible.

Rawitz (1900 in Hosokawa 1950), suggested that the laryngeal sac acts as **an apparatus for preventing the entrance of water and food into the respiratory canal**. The contraction of the massive muscles of the sac would make the laryngeal sac, as well as the larynx itself, firm and solid, so as to avoid accidentally swallowing large quantities of food with water into the larynx and trachea. Alternatively, the blast of air produced by contraction of the laryngeal sac would prevent the entrance of water and food into the respiratory canal. Based on the laryngeal sac's position in *B. mysticetus* as an integral part of the ventral tracheal wall that bulges dorsally into the tracheal lumen, Haldiman & Tarpley (1993) found that enlargement of the sac should close off the trachea.

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A second hypothesis proposes that **the sac is concerned with complete utilization of oxygen in the inspired air**. Schulte (1916) speculated that by the contraction and relaxation of the laryngeal sac during submergence, a circulation of air in the wide trachea and bronchi might be set up, which would favour the absorption of oxygen by bringing the air in these passages more rapidly into contact with the respiratory membrane than could be done by the usual diffusion currents. Alternatively, Negus (1962) suggested the "rebreathing of air", whereby the sac might act as an air-reservoir so that when the animal is submerged for a prolonged period of time, the used-up air which has been in contact with the pulmonary epithelium would mix with the relatively unused air which has lain in the sac. This mixed air, when blown back into the lungs, would provide a fresh supply of oxygen and take up CO_2 so that submergence times could be prolonged.

In the third hypothesis, **the sac is related to phonation**. According to Turner (1872) the mechanism of phonation in Balaenidae (right whales) was such that the elongated caudal processes of the arytenoid cartilages would be drawn near to each other and vibrate from a strong expiration. If such a mechanism of phonation be true, we must bear in mind the possible utility of the laryngeal sac.

In 1950, Hosokawa reviewed the hypotheses that mysticete whales may recycle inspired air using the laryngeal sacs and that these sacs may possibly be involved in sound production. Apparently, he was unaware of the characteristics of mysticete sounds, which were not generally known at the time.

In studying the humpback whale, Quayle (1991) postulated that air forced from the laryngeal sac between the arytenoids causes the air column in the sac and perhaps the nasopharynx to vibrate. The resulting pressure fluctuations are transmitted through the soft tissues of the whale into the surrounding water. While the sac was compressed, the trachea would be occluded and gas exchange could presumably continue uninterrupted in the lungs. Conceivably the sac could be refilled from the thoracic air while the whale remained submerged.

The acquisition of laryngeal material from four pygmy right whales, *Caperea marginata*, (and photographs of the visceral anatomy of another - Plates 1 and 2) has revealed a marked

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development of the laryngeal sac in adults compared to juveniles. The larynx of this species has not been described previously, nor has the striking ontogenetic development of the laryngeal sac in any baleen whale, although most dissections have been of foetal or juvenile material. To investigate the ontogeny of the laryngeal apparatus in another mysticete, frozen material from four minke whales, *B. acutorostrata* (an adult male and female and a juvenile male and female) has been obtained.

Caperea marginata (Gray 1846) is the smallest mysticete, adult females reaching 6.5 m in length, and is usually placed in a family of its own, the *Neobalaenidae*: it exhibits characteristics of both balaenopterids and right whales.

Balaenoptera acutorostrata (Lacépède 1804) is the smallest of the balaenopterids, seldom exceeding a length of 10.1 m.

Minke whale vocalisations have been described as a series of low-frequency grunts, thumps and ratchets. Most are trains of sound at 100-200 Hz which seem to make the call of each individual unique (Stewart & Leatherwood 1985). They also produce pure frequency pulsed sounds at 4-8 kHz involving series of clicks for 6-8 seconds at a time, possibly used for echolocation. Beamish & Mitchell (1973) recorded short pulse length audio frequency sounds in the presence of a minke whale. In another study conducted by Winn & Perkins (1976) grunts and thump-like sounds were recorded. The grunt-like sounds had low, restricted frequencies with the greatest energy somewhat variable between 80 and 140 Hz. The thump-like sounds had broadband energy from below 100 Hz to at least 800 Hz with maximum energy between 100 and 200 Hz. Ratchet-like pulses had an energy peak centred at about 850 Hz with harmonics extending up to at least 6 kHz and single pulse durations between one and 6 msec. A few sounds were pulse-like and varied in frequency from 3.8 to 12 kHz. Durations varied from one to 5 msec for the highest frequency classes but were 16 to 20 msec long for the lower frequency class. A large number of high frequency zip-like clicks were recorded with principal energy in the 5 to 6 kHz region but with significant energy near 14 kHz and some energy beyond 20 kHz. These clicks were 0.5 to one msec in duration.

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Pygmy right whale sounds have only been recorded once, in Nov 1986-Feb 1987 when a juvenile spent more than 10 weeks in the harbour at Portland, Victoria (Dawbin & Cato 1992). The sounds recorded were intense, thump-like pulses, which occurred in pairs and in one sample a trio was recorded. The duration of single pulses varied from 140 to 225 msec and the number of cycles from 11 to 19. The frequency at the start of a burst varied from about 90 Hz to in excess of 135 Hz, but the final value was always about 60 Hz. The time from the start of the first pulse to the start of the second pulse in the pairs varied from 430 to 510 msec.

The sounds reported for the pygmy right have most similarity to those of a minke whale recorded in the Antarctic. In both cases the sounds show a downsweep in frequency over much the same range for about the same duration, but the minke whale sounds occurred individually rather than in pairs or trios (Schevill & Watkins 1972; Dawbin & Cato 1992). This would seem to indicate a common method of sound production and similar acoustic structures (Watkins 1981; Dawbin & Cato 1992).

In this project, the morphology and anatomy of the laryngeal apparatus of *C. marginata* will be described, and compared with that of *B. acutorostrata*, with special emphasis on the ontogeny of the laryngeal sac in both species and its possible significance in phonation. This study adds to the previous descriptions of the laryngeal apparatus of minke whales by including both adult and juvenile specimens of both sexes, and by adding descriptions of the fine anatomy.

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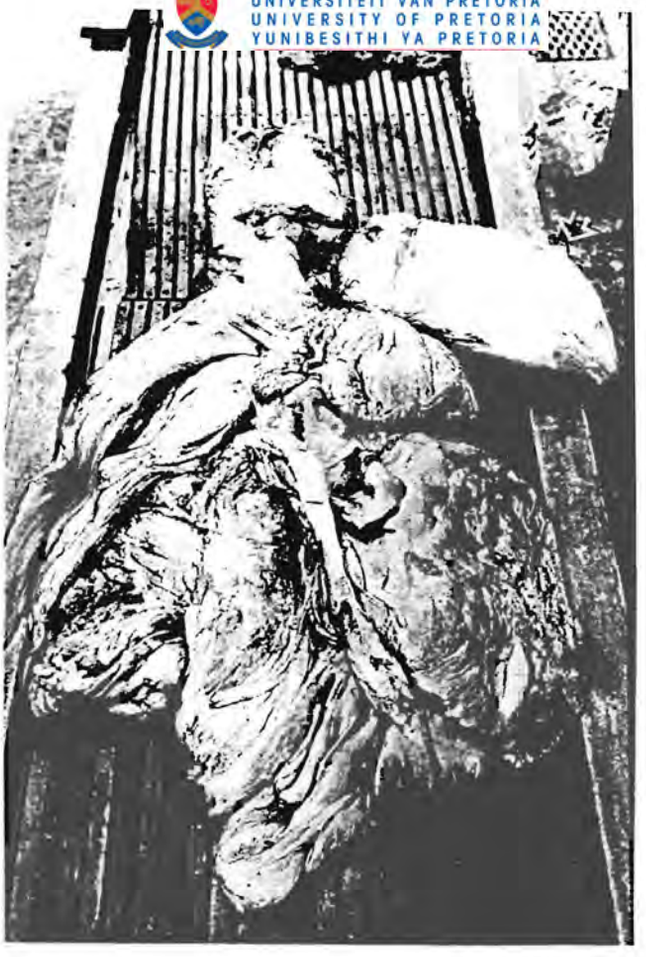

Plate 1: Ventral view of the viscera and laryngeal apparatus of an adult male *C. marginata*, stranded at Bordjies Drif, Cape Point Nature Reserve on 25/05/82 - (# 82/11). Photographs taken by PBB. Note laryngeal sac lying above the heart (arrow).

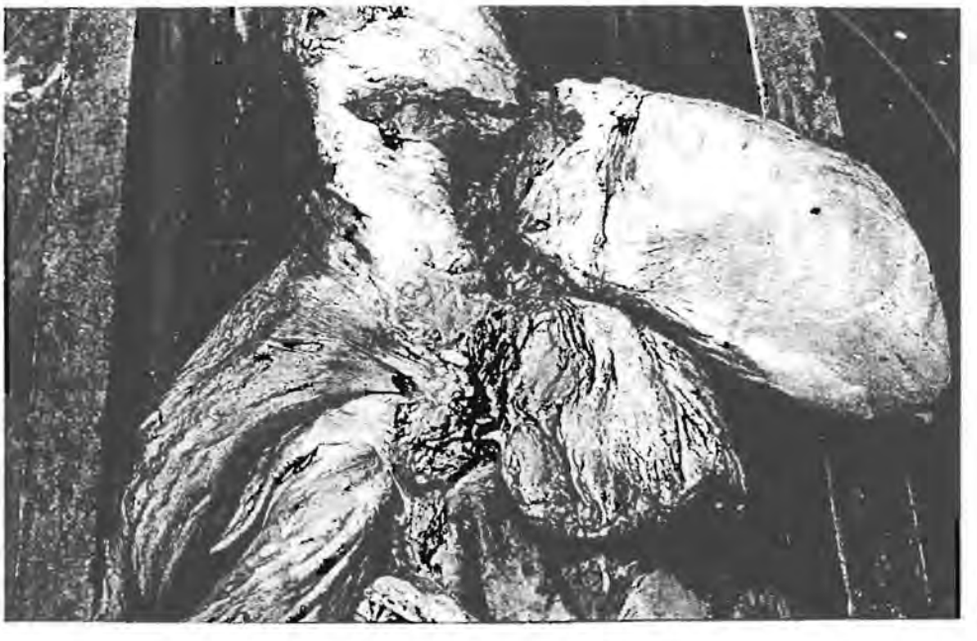

Plate 2: Ventral view of the laryngeal sac of an adult male *C. marginata*, details as above.

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1.1 PROBLEMS WITH NOMENCLATURE

Because most of the anatomical papers referred to in this study were written over a century ago, the nomenclature used to describe various species of cetaceans sometimes differs from that in current usage and it is not always clear to which species they refer. To this end, the table below summarises the nomenclature used by various authors to describe their specimens, together with the presently accepted taxonomy and revised identifications where necessary (together with the justification therefor).

Table 1: List of nomenclature used by various authors with current interpretation of the species involved and justifications therefor.

Authors and associated nomenclature	Present identifications and justifications
Carte & Macalister (1868) 1. *B. rostrata*	***B. acutorostrata*** -♀ - 5.6 m - synonymised by author.
Turner (1872) 1. *B. sibbaldii*	***B. musculus*** - ♀ approx. 23.9 m (measured along the mid-line of the back from the tip of the lower jaw to the end of the tail) - dark steel grey / almost black in colour, with a generally black ventral surface being mottled with white silvery grey patches. Black baleen.
Beauregard & Boulart (1882) 1. *B. sibbaldii* 2. *B. musculus* 3. *B. antipodum*	***B. musculus*** - ♂ foetus, 3.60 m long - D Robineau (pers. comm.). Authors state that this animal is the same species as Turner's specimen. ***B. physalus*** -♀ , 12 m long - D Robineau (pers. comm.) ***E. australis*** -♀ foetus, 55 cm long -according to Hershkovitz (1966).
Dubois 1886 1. *B. sibbaldii*	**B. musculus** - ♀ foetus, 2.27 cm long - Author states that this animal is the same species as Turner's specimen.

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Largely on morphological grounds, four "forms" of minke whales have been recognised, one in the North Atlantic and one in the North Pacific (referred to *B. a. acutorostrata* and *B. a.davidsoni* respectively by Omura 1975), and two forms in the Southern Hemisphere. One of the latter was recognised as *B. a. bonaerensis* by Omura (1975) but the second, a smaller "dwarf" form was only described in 1985 (Best 1985; Arnold, Marsh & Heinsohn 1987), and has not yet been given a scientific name. On genetic grounds, the *bonaerensis* form could be considered specifically distinct from that in the North Pacific (Wada & Numachi 1991; Hoelzel & Dover 1991).

If this is accepted, the laryngeal material examined in this study should be regarded as referring to *Balaenoptera bonaerensis* Burmeister, 1867, making this study the first description of the laryngeal anatomy of this species. But, due to the continuing confusion surrounding the nomenclature of the minke whale, the specimens are referred to as *B. acutorostrata* in this text.

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CHAPTER 2: MATERIALS AND METHODS

Laryngeal material from a juvenile female, juvenile male, an adult female and an adult male (specimen numbers 44, 159, 15 and 13, respectively) *B. acutorostrata* was obtained from the 1993/1994 Japanese Whale Research Programme under special permit in the Antarctic (JARPA) (Table 2), and was frozen immediately after collection.

Table 2: Collection details of *B. acutorostrata* specimens during the 1993/94 season.

Details (specimen #)	Juvenile ♂ (13)	Adult ♂ (159)	Juvenile ♀ (44)	Adult ♀ (15)
Total length of animal (m)	5.3	8.5	5.7	8.9
Time of capture	16:21, Dec 8	11:28, Jan 18	15:19, Dec 14	10:22, Dec 9
Time of treatment	17:05, Dec 8	12:30, Jan 18	16:20, Dec 14	12:30, Dec 9
Post-mortem times (hr-min)	0-45	1-00	1-00	2-10
Age (years)	2	31	2	-*
Locality	60-69 S 70-130 E	60-69 S 70-130 E	60-69 S 70-130 E	60-69 S 70-130 E

* Age undetermined due to broken ear plug

Laryngeal material from two juvenile females, one juvenile male and one adult male (specimen nos 89/3, 90/12, 21/27 and 93/07, respectively) *C. marginata* was available (Table 3). All were strandings on the coast of Southern Africa. All of the specimens, except one juvenile female (specimen no 89/3) were preserved in 10% formalin. Specimen 89/3 was frozen.

Radiographs of the whole laryngeal apparatus of the adult male *B. acutorostrata*, as well as of the juvenile male and female *C. marginata* were taken using a Shimodzu Medical X-ray Unit at the South African Museum. The KV's ranged between 52-70 using Trimax mammography film. Manual processing was undertaken using Polycon developer, with a development time of 4 minutes, and Perfix fixer, with a fixing time of 2 minutes.

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Table 3: Collection details of *C. marginata* specimens.

Details (specimen #)	Juvenile ♀ (89/3)	Juvenile ♀ (90/12)	Juvenile ♂ (91/27)	Adult ♂ (93/07)
Total length of animal (m)	3.3	3.7	3.2	5.9
Date of stranding	17/02/89	04/03/90	30/03/91	15/05/93
Post-mortem times	12-24 hours	24-48 hours	24-48 hours	several days
Locality	Walvis Bay, Namibia	Murdock Valley, Simonstown	Salt works, Walvis Bay, Namibia	Buffels Bay, Cape Point Nature Reserve

All frozen material was stored at -18 °C. Before dissection, each specimen was placed in a cold room (2 °C) where it was left to defrost. The juvenile material took approximately 3 days to defrost while the adult material required approximately 4 days to defrost. Once defrosted, the material was moved to a wet laboratory for dissection. After each session the material was returned to the cold room.

All formalin-fixed material was soaked in fresh water before dissections were undertaken. The adult *C. marginata* material was soaked in fresh water for 24 hours, while that from the juveniles was soaked overnight.

Each step of the dissections was recorded on film, using a Nikon F-601 Quartz Date camera with Fuji colour slide, 400 ASA film. Observations were also recorded as physical notes and diagrams.

The dissections of the minke whale material were carried out according to the techniques and diagrams of Benham (1901).

The frozen juvenile female specimen of *C. marginata* consisted not only of the laryngeal apparatus, but the viscera as well. This provided an opportunity to test whether the introduction of water or air into the trachea would cause any reaction in the laryngeal sac.

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A 1 m airhose attached to a pump was placed in the pharynx, held at the *aditus laryngis* (Benham 1901) and then air was introduced into the laryngeal area. Observations taken before air was introduced, during the inflow and after the airflow was cut-off, were recorded on film, using a Nikon F-601 Quartz Date camera with Fuji colour slide, 400 ASA film.

Tissue samples from the various muscles, mucosas and epithelia of the laryngeal apparatus of both species were taken using scalpels and were preserved in 10% buffered formalin solution in 125 ml sample bottles.

Each sample was dehydrated and embedded in wax, following standard histological procedure. Sections between 2 µm and 5 µm thick were cut on a rotary microtome, mounted onto slides and stained. Three slides were prepared of each sample; the first was stained with haemotoxylin and eosin, using standard histological procedure (Drury & Wallington 1967; Bancroft & Stevens 1982), the second with Masson's trichrome (procedure described below) and the third with Victoria Blue (procedure described below).

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PROCEDURE FOR MASSON'S TRICHROME STAIN (Drury & Wallington 1967; Bancroft & Stevens 1982)

1. Bring sections to distilled water
2. Stain in Weigert's Iron haematoxylin for 20-30 minutes
3. Wash in water
4. Differentiate in 1% acid-alcohol until only the nuclei are stained
5. Wash in water until sections are blued - 5 minutes
6. Stain in 1% Ponceau 2R in 1% Acetic acid - 5 minutes
7. Rinse rapidly in distilled water
8. Mordant and diffuse in 1% aq phosphomolybdic acid until collagen is decolourized, muscle, red blood cells and fibrin remaining red - 3 minutes
9. Drain slide and counterstain with 2% Light green in 2% acetic acid - 2 minutes
10. Rinse, dehydrate, clear and mount

RESULTS:

Nuclei -	blue-black
Cytoplasm, muscle, Acidophil granules -	red
Collagen, cartilage, mucin, Basophil granules -	green

PROCEDURE FOR AN ELASTIN STAIN WITH VICTORIA BLUE (Lustgarten 1886, in Brontë Gatenby & Beams 1950); Drury & Wallington 1967; Bancroft & Stevens 1982)

Victoria Blue 4R	1 gm
New fuchsin	1 gm
Crystal violet	1 gm

Dissolve in 200 ml of hot distilled water then add in the following order -

Resorcin	4 gm
Dextrin	1 gm
30% ferric chloride	50 ml (freshly prepared)

Boil for 5 minutes then filter when hot. Transfer precipitate plus filter paper to original beaker

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and redissolve in 200 ml of 95% alcohol. Boil on a hot plate or in a water bath for 15-20 minutes. Filter and make up to 200 ml with 95% alcohol. Finally add 2 ml of concentrated HCl.

1. Take sections to water.
2. Stain in 0.5 % potassium permanganate - 5 minutes
3. Rinse well in distilled water then 0.5 % Oxalic acid
4. Rinse in water - 95 % alcohol - 2 minutes
5. Use freshly filtered stain in Coplin jar for 1-3 hours or overnight in 0.5 stain and 0.5 96 % alcohol
6. Rinse in several changes of 96 % alcohol
7. Wash in Distilled water
8. Counterstain 2-3 minutes with von Gieson
9. Take through absolute alcohol and xylol - mount

For Australian Antigen stain overnight.

RESULTS:

Elastin - Black

Australian Antigen - Grey-black

Each stain was used in order to highlight specific characteristics of the various tissue samples i.e. Masson's trichome stain for muscle and connective tissue; Victoria Blue for elastic fibres and mucous cells; Haemotoxylin and Eosin for general histology. Once prepared, the slides were examined using a Zeiss light microscope (magnifications are indicated on the various plates).

Slides were photographed using a Zeiss Axiophot photomicroscope, loaded with 35mm 100 ASA colour slide film which was processed by and at The University of Cape Town's Medical School.

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CHAPTER 3: RESULTS

As defined here, the laryngeal apparatus of *B. acutorostrata* and *C. marginata* begins anteriorly with the nasopharynx (Quayle 1991) and ends posteriorly with the trachea supported by tracheal rings (Plate 3). The larynx in mysticetes is hereby defined as a muscular sphincter which separates the oral from the nasal passageways and which includes the epiglottis, glottis and aryteno-epiglottic folds.

3.1 Gross anatomy of *Balaenoptera acutorostrata*

Lengths of the laryngeal apparatus' (measured from nasopharynx to end of tracheal rings) of *B. acutorostrata* ranged from 57.7 cm in the juvenile female (Plate 4), 57.8 cm in the juvenile male (Plate 5) to 85.5 cm and 1.1 m in the adult male (Plate 6) and female (Plate 3), respectively. These measurements are approximate bearing in mind that most of the nasopharynges of the various specimens were lost during their initial removal.

a) Bones

From a ventral perspective, the most outstanding features on the surface of the apparatus of *B. acutorostrata* are the hyoid and sternum bones (Plate 7), the latter covering more or less the posterior third of the apparatus and the former lying in close association with the tracheal area. These bones provide points of attachment for various muscles and upon removal of the styloid bones and os hyoides, the muscular character of the ventral surface of the laryngeal apparatus is evident.

The hyoid bones described in this study include the tympanohyal, stylohyal-epihyal, basihyal and thyrohyals referred to by Mead (1997, pers. comm.), with the stylohyal-epihyals being synonymous with the styloid bones and the basihyal and thyrohyals being synonymous with the os hyoides.

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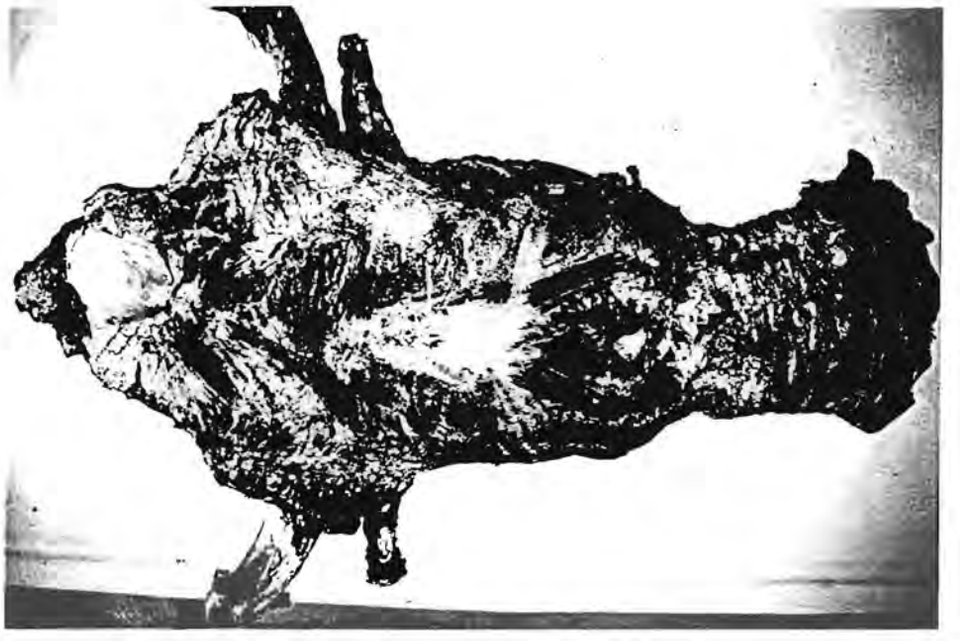

Plate 3: Dorsal view of the laryngeal apparatus of an adult female (# 15) *B. acutorostrata*.

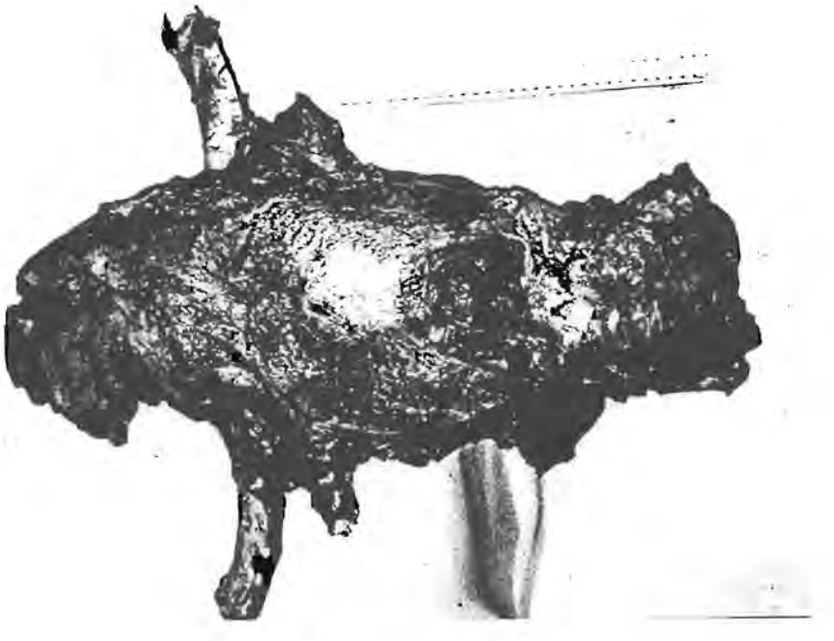

Plate 4: Dorsal view of the laryngeal apparatus of a juvenile female (# 44) *B. acutorostrata*.

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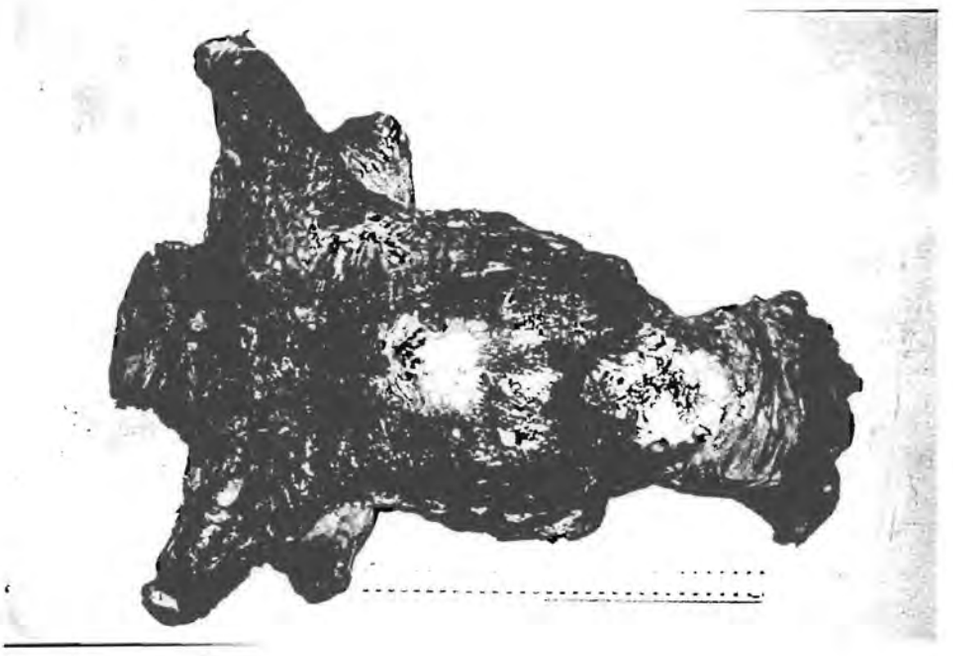

Plate 5: Dorsal view of the laryngeal apparatus of a juvenile male (# 13) *B. acutorostrata*.

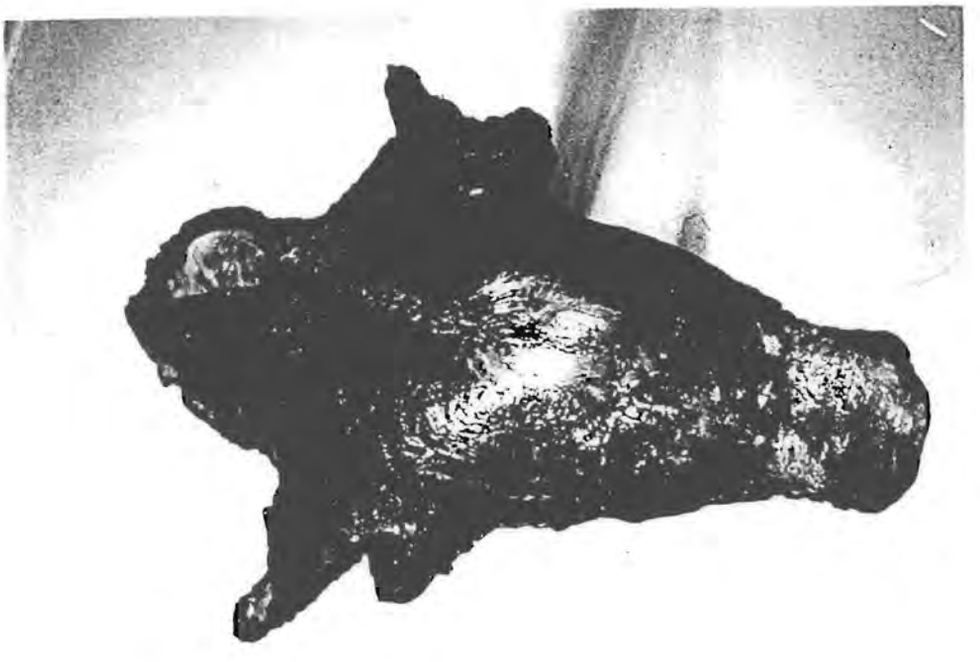

Plate 6: Dorsal view of the laryngeal apparatus of an adult male (# 159) *B. acutorostrata*.

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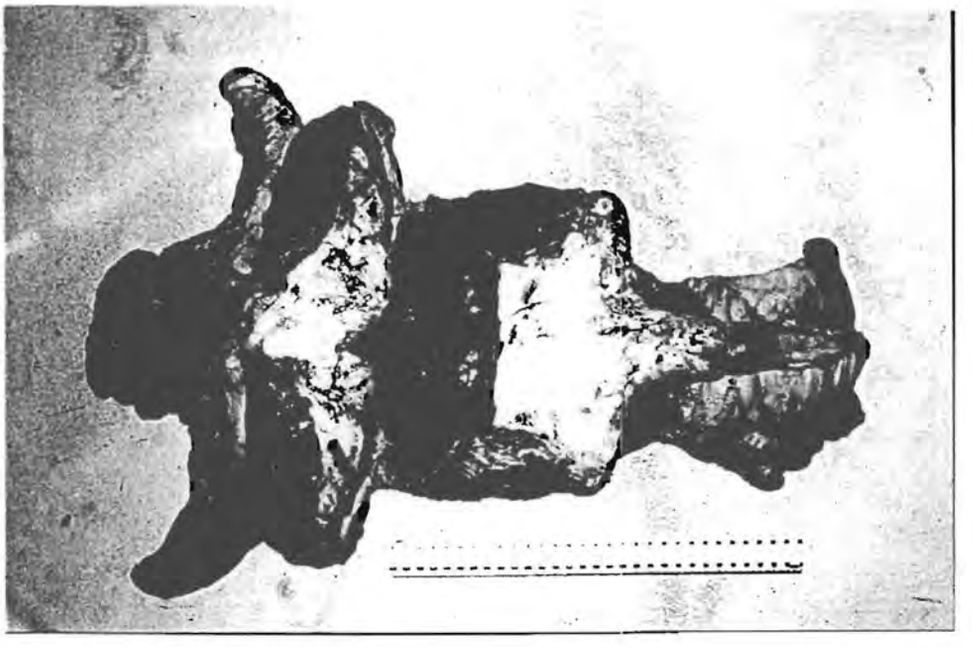

Plate 7: Ventral view of the laryngeal apparatus of a juvenile male *B. acutorostrata* showing the anteriorly situated hyoid bones and the posteriorly occurring sternum bone.

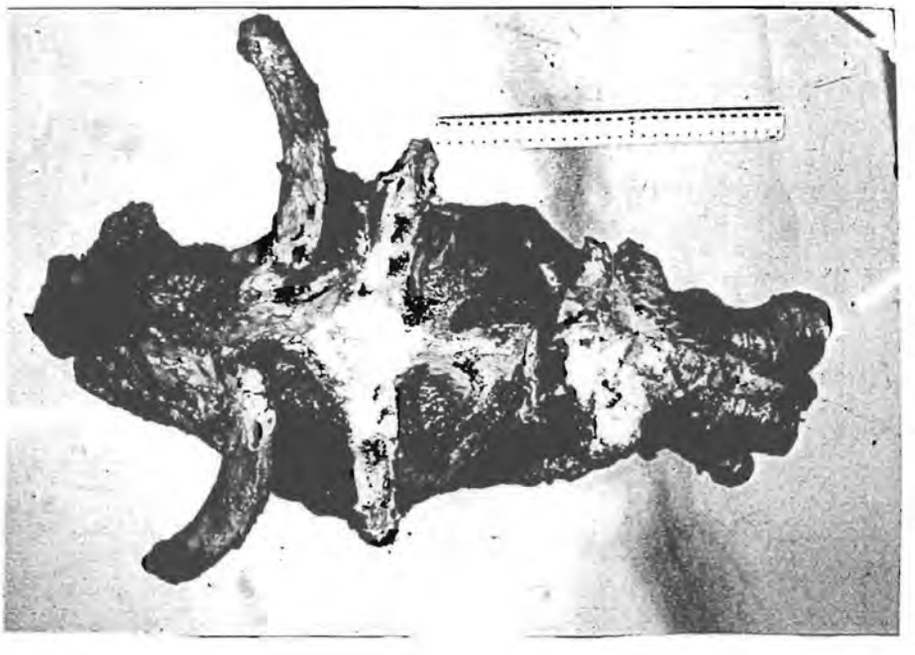

Plate 8: Ventral view of the hyoid bones attached to the laryngeal apparatus of a juvenile female *B. acutorostrata*.

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i) Hyoid bones

The hyoid, as in all other cetaceans, is composed of three separate bones - the os hyoides and the two styloid bones (Plate 8). The os hyoides can be distinguished as the middle bone, lying anterior to the thyroid cartilage (Plate 9). It extends laterally as two cornua, while its anterior margin bears two anterior projections, separated from each other by a notch in the mesial line. Although the bones and the anterior projections naturally increase in size from the juveniles to the adults, there is much individual variation. In the juvenile male, the posterior margin of the body of the os hyoides is rounded in a posterior direction (Plate 9), whereas in the juvenile female, the posterior margin possesses a slight indentation (Plate 10). The slight notch or indentation seen in the juvenile female is also present in the adult male but is absent in the adult female. Instead, the adult female possesses an even, concave, posterior margin (Plate 11). The os hyoides in all the specimens is curved laterally.

The two styloid bones are found anterior to the cornua of the os hyoides, lying on the ventral surface of the nasopharynx (Plate 12). The bones are attached around their margins, by muscle, to the wall of the nasopharynx. Each bone is of uniform thickness and is essentially convex in shape, though (in the case of the adult male) the posterior margins are not entirely smooth. In the adult female and the juvenile male, the notch on the anterior margin of the os hyoides lies in between the styloid bones whereas in the adult male and juvenile female, the styloid bones are closer together, with the anterior notch of the os hyoides lying beneath the mid-ventral meeting of these bones.

Measurements of the hyoid bones are given in Table 4.

ii) Sternum bones

Sternum bones were only available for the juvenile specimens. The sternum is situated on the ventral wall of the trachea and consists of two lateral cornua which join together mesially to form the body. In the juvenile female (Plate 12), the body continues anteriorly in a triangular formation whereas in the male it forms a smooth rectangular plate (Plate 13). The anterior margin of the

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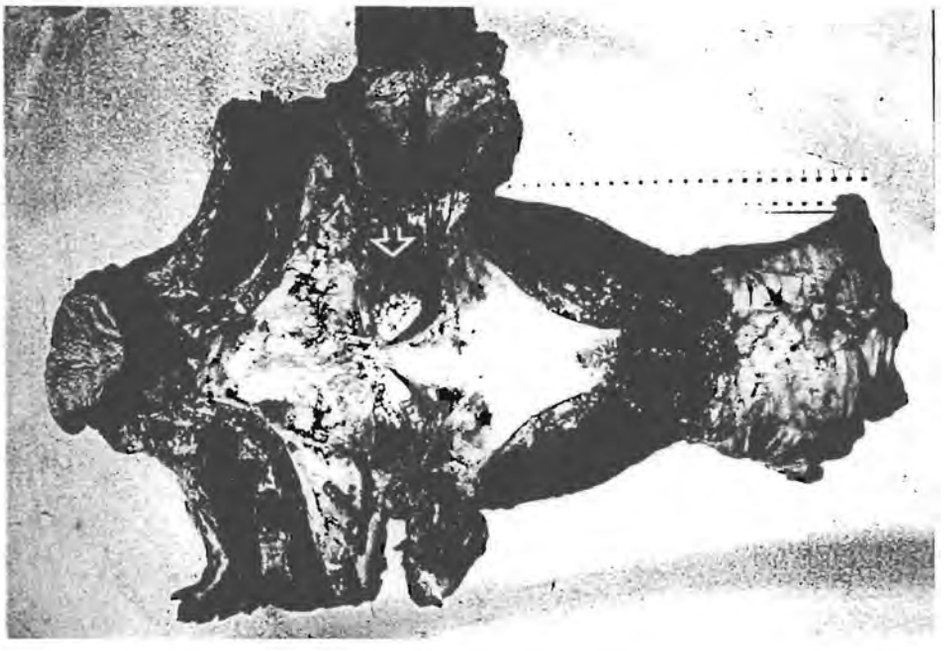

Plate 9 Ventral view of the laryngeal apparatus of a juvenile male *B. acutorostrata* showing the os hyoides (with a rounded posterior margin) lying anterior to the thyroid cartilage. Note the thyro-hyoid muscles anterior to the thyroid cartilage and attached to the posterior margin of the os hyoides (arrow).

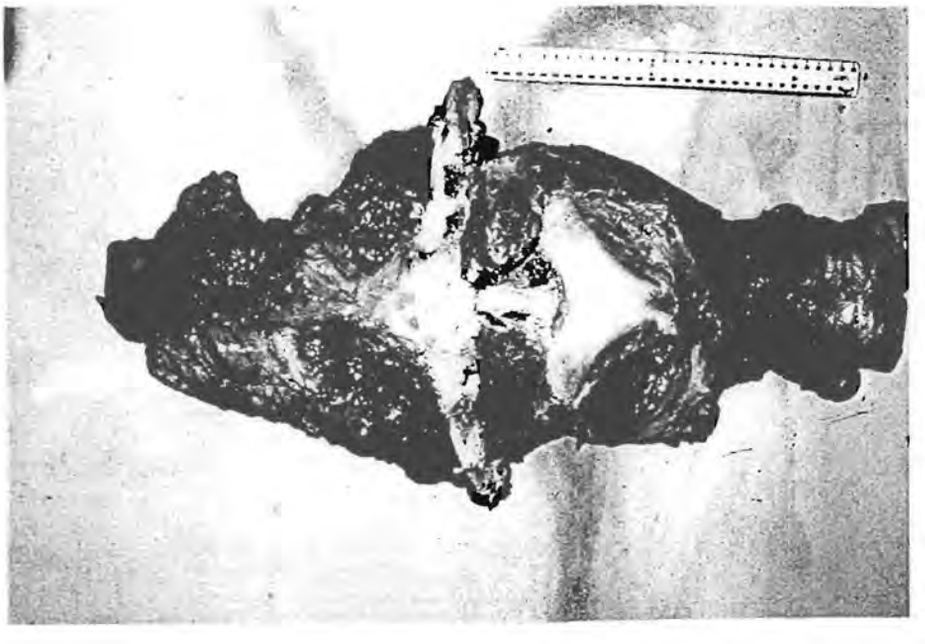

Plate 10: Ventral view of the laryngeal apparatus of a juvenile female *B. acutorostrata* showing the slight indentation on the posterior margin of the os hyoides, lying anterior to the thyroid cartilage.

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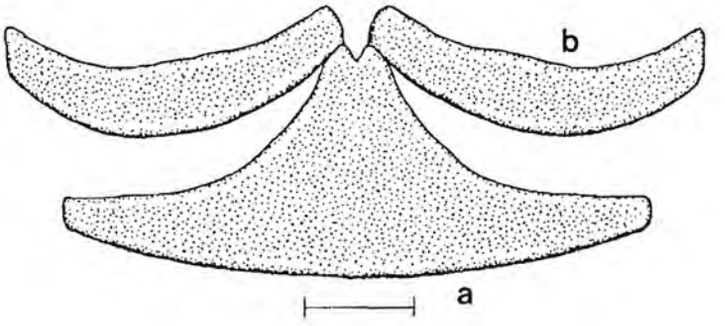

Plate 11: Ventral view of the os hyoides (a) and styloid bones (b) of an adult male *B. acutorostrata* (# 159) showing an even concave posterior margin. Note scale bar = 10 cm.

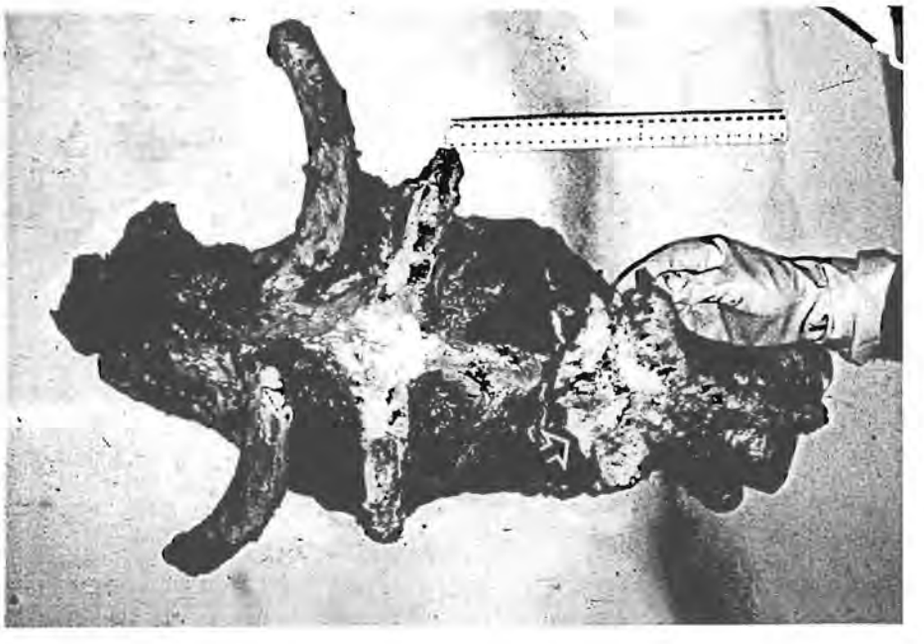

Plate 12: Ventral view of the styloid and sternum bones of a juvenile female *B. acutorostrata*. The styloid bones are found anterior to the cornua of the os hyoides. The anterior margin of the sternum bone is triangular in shape. Note the muscular attachment on the anterior margin of the sternum (possible sterno-thyroid muscle) (arrow).

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Table 4 Measurements associated with the laryngeal apparatus of *B. acutorostrata*.

Cartilage/Bone (specimen #)	Measurements (cm)	Juvenile ♂ (13)	Adult ♂ (159)	Juvenile ♀ (44)	Adult ♀ (15)
Entire apparatus	Total length (m)	57.8	85.5	57.7	110.0
Thyroid cartilage	Anterior margin	16.8	22.5	17.3	26.2
	Body width	21.3	29.0	22.5	30.0
	Total length	19.2	28.5	14.3	31.2
Cricoid cartilage	Length	13.6	22.0	14.0	21.2
	Width	13.2	21.5	12.0	27.7
	Length ventral LHS cornu	3.7	2.5	6.0	10.5
	Length dorsal LHS cornu	3.0	2.0	5.0	6.0
Epiglottic cartilage	Total length	15.6	28.5	14.3	24.9
Arytenoid cartilages	Depth of notch (between lips)	0.5	1.8	0.8	0.6
	Ave width of lips (widest area)	7.5	12.0	6.4	10.6
	Height of lips (LHS) - tip of lip to cartilage - mid-dorsal measurement	1.6	4.6	2.6	2.2
	Height of lips (RHS) - tip of lip to cartilage - mid-dorsal measurement	2.0	3.8	2.4	2.2
	Ave total length	19.2	28.5	14.3	31.2
Os hyoides	Length cornua LHS	19.5	23.5	17.5	31.0
	Length cornua RHS	19.5	24.2	17.7	31.0
	Width of body - measured from bottom of "v" notch	9.4	13.1	8.4	16.7
Styloid bones	Total length (LHS)	21.7	34.3	25.1	39.1
	Total length (RHS)	21.0	35.2	24.3	39.1
	Average width - at thickest region	4.3	7.5	4.7	9.0

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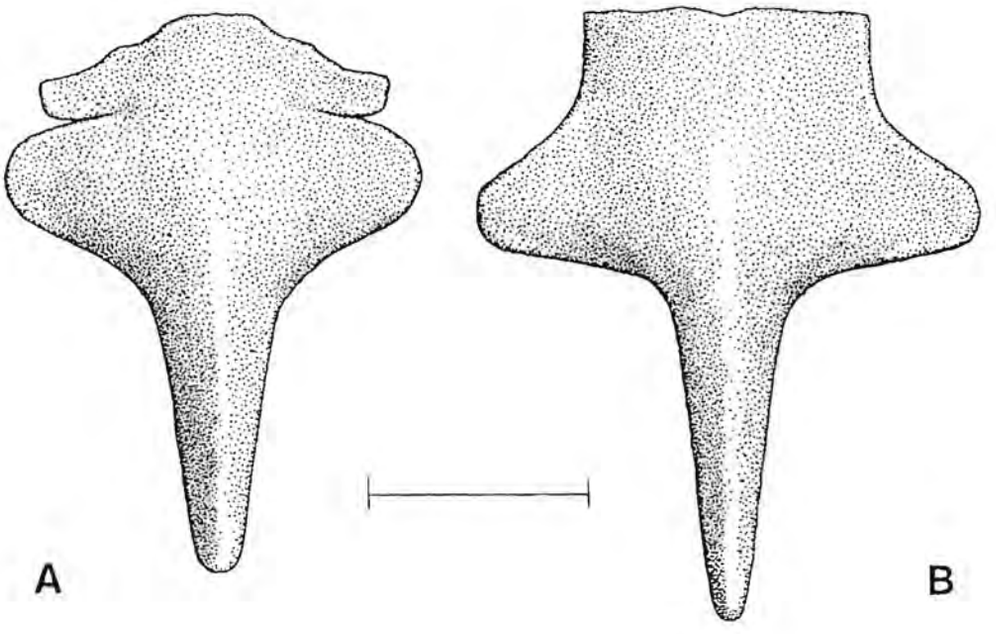

Plate 13: Ventral view of the sternum bone of (A) a juvenile female (# 44) and (B) a juvenile male (# 13) *B. acutorostrata*. Note scale bar = 10 cm.

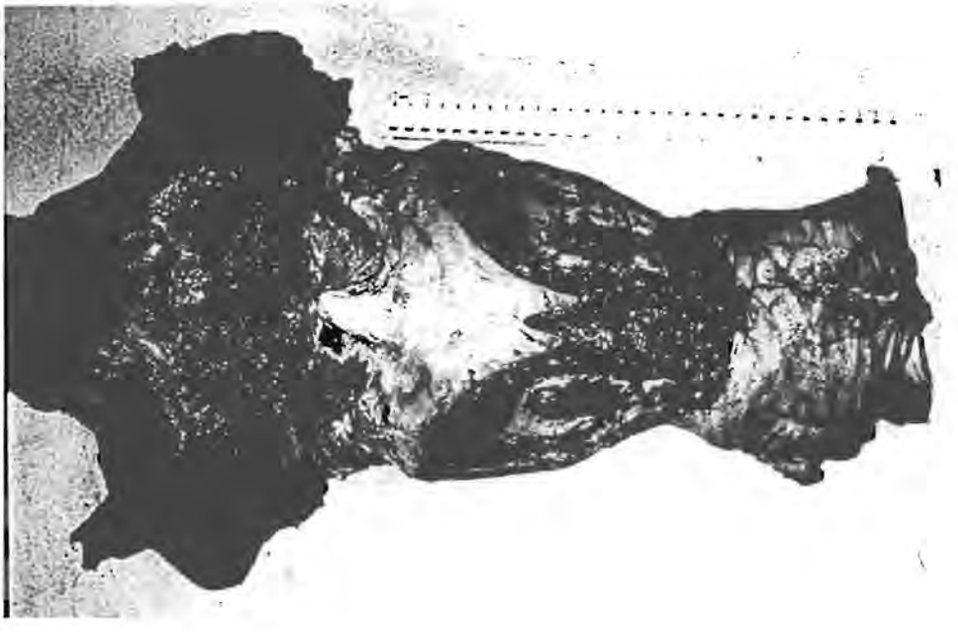

Plate 14: Ventral view of the laryngeal apparatus of a juvenile male *B. acutorostrata* showing the mid-ventral situation of the thyroid cartilage.

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sternum is flat and provides a surface for muscle attachment. Posteriorly, the body forms a dagger-like extension narrowing along its anterior-posterior axis, and ending in a pointed tip, which extends as far as the anterior margin of the fifth tracheal ring.

b) Cartilages

The laryngeal cartilages in each specimen are five in number, the single thyroid, cricoid and epiglottic cartilages and the paired arytenoids. The outstanding characteristic of these cartilages is their general flexibility and "softness" which is characteristic of both the juveniles and the adults.

Naturally, the various structures, bones and cartilages increase in size from the juveniles to the adults, but much individual variation exists. Measurements of all the cartilages are given in Table 4.

i) Thyroid cartilage

The thyroid cartilage occurs in the mid-ventral region of the laryngeal apparatus (Plate 14). The thyroid cartilage is made up of a horizontal body which gives rise on either side to posterior, vertical cornua. The body of this cartilage is flat with a reduced anterior-posterior diameter (Plate 15). From a posterior aspect, the anterior margin of the body is convex and the posterior margin is concave, the latter possessing a median v-shaped notch (Plate 15). In the adult female the body of the thyroid cartilage possesses a slightly raised ridge along the mid-ventral line. Posterior cornua occur at the left and right extremities of the body and it is at these points that the anterior margin of the body is thickest, forming tubercles (Plate 15). On the outer margins of the tubercles both cornua curve backwards, forming prominent concave arches, resting on either side of the larynx. As each cornu arches concavely, its original band-like character changes to a more rod-like one, showing slight thickening at the posterior tip (Plate 15). It is at these tips that each cornu is articulated with the cricoid cartilage (Plate 16).

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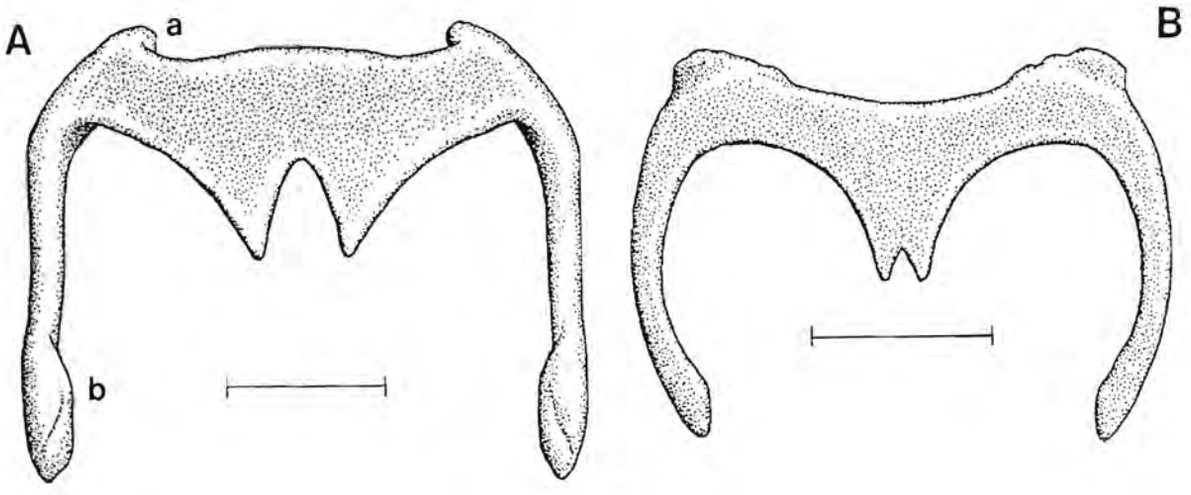

Plate 15: Ventral view of the thyroid cartilage of (A) an adult female (# 15) and (B) a juvenile female *B. acutorostrata*. Note (a) anterior tubercles (well-developed in the adult), notch on posterior margin of the thyroid body and (b) the posterior thickening of the cornua (much thicker in the adult). Note scale bar = 10 cm.

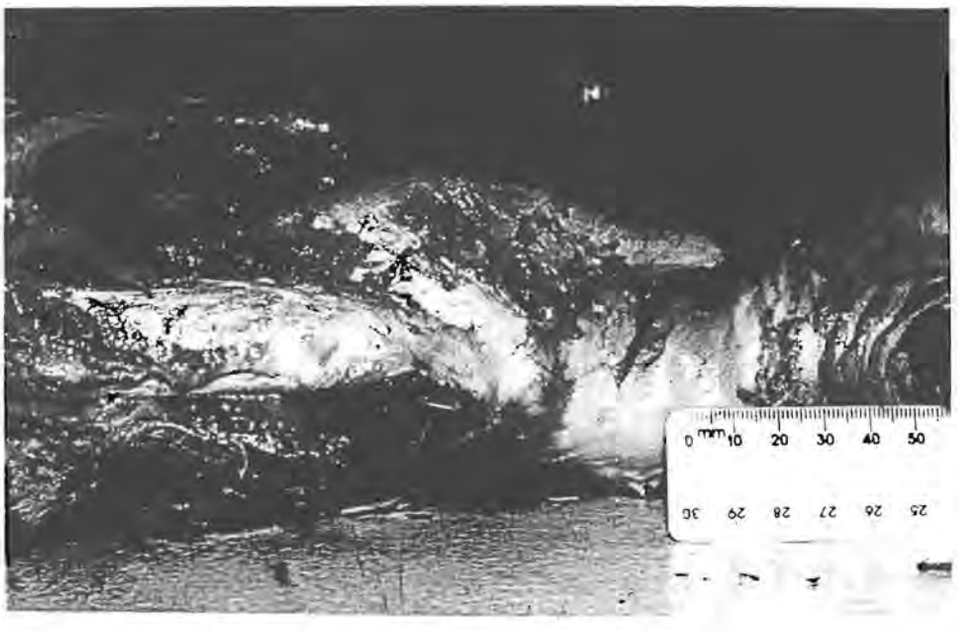

Plate 16: Lateral view of the right hand side of the laryngeal apparatus of a juvenile female *B. acutorostrata* showing the articulation between the thyroid and cricoid cartilages.

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ii) Cricoid cartilage

The cricoid cartilage, is incomplete ventrally with a dorsally positioned body which continues laterally and ventrally to form two cornua (Plate 17). The body is a large, almost square plate of cartilage which provides facets of articulation with both the arytenoid and the thyroid cartilages, the joints of which have fibrous capsular membranes. The arytenoidal articular facets occur on either side of the anterior margin of the cricoid body. Each facet forms a convex depression lying at an oblique angle to the longitudinal axis of the body of the animal. In between the arytenoid articular facets, the body of the cricoid cartilage is convex in shape. The posterior margin of the body of the cricoid forms a rounded prominence which is continuous with the upper tracheal rings (Plates 18 and 19) - the number of which seems to vary with the size of the animal. The cricoid prominence is continuous with the first tracheal ring in the juvenile female, it borders on the anterior margin of the first tracheal ring in the juvenile male, is continuous between the first and second tracheal ring in the adult male and is continuous with the first three tracheal rings in the adult female. The middle of the lateral margins of the body of the cricoid tend to move inward towards each other, causing their posterior ends to point away from the body. It is at these ends that the facets for articulation with the thyroid cornua are found (Plate 17). Posterior to each facet, the body of the cricoid proceeds ventrally to form its cornua. The part of the cartilage which joins the body of the cricoid to its cornua is more scallop-shaped in the males and the cornua are shorter compared to those of the females (Table 4). The cornua extend to the first tracheal ring in the juvenile male and are in line with the second tracheal ring in the adult male. In the females, however, the cornua lie in line with the fourth and third tracheal rings in the juvenile and adult, respectively. From the dorsal perspective (Plate 19), the cricoid body is slightly concave in the middle. Along the mid-dorsal line of the body, small indentations occur. These indentations range from 0.5 mm in diameter to approximately 1 cm in diameter in the juvenile female and the adults and are possibly neuro-vascular foramina (R Alexander, S A Museum 1997, pers. comm.). However, these indentations were not detected in the juvenile male.

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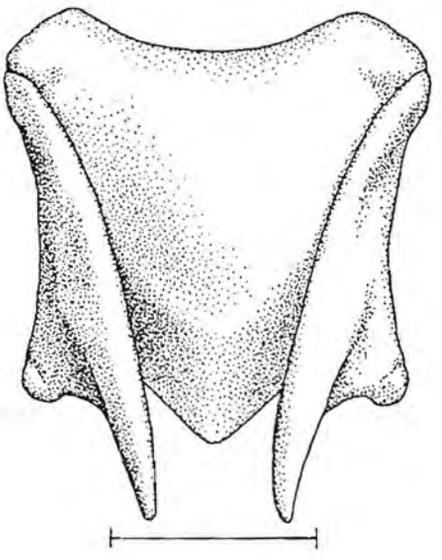

Plate 17: Ventral view of the cricoid cartilage of a juvenile female *B. acutorostrata* (# 44). Note that the cartilage is incomplete ventrally and the facets for articulation occur on the four "corners" of the cartilage. Note scale bar = 10 cm.

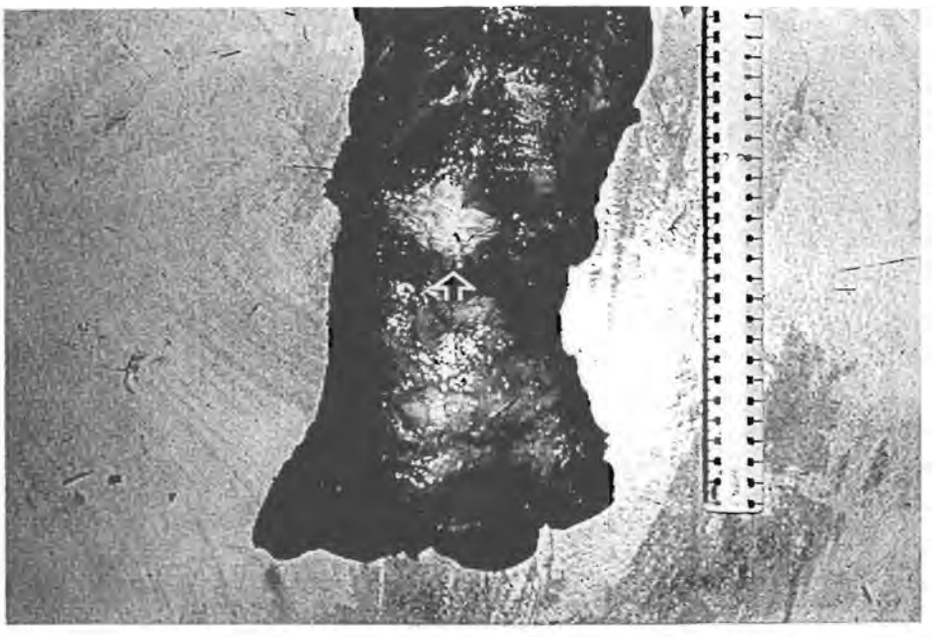

Plate 18: Dorsal view of the rounded prominence of the posterior margin of the body of the cricoid cartilage (arrow), of a juvenile female *B. acutorostrata*, which is continuous with the tracheal rings.

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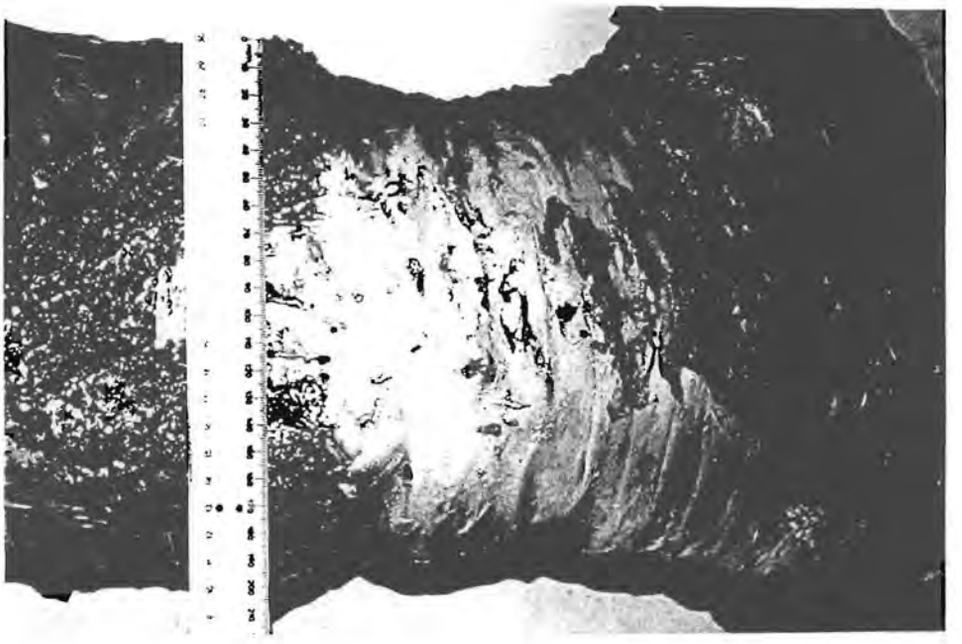

Plate 19: Dorsal view of the body of the cricoid cartilage of an adult female *B. acutorostrata*, being slightly concave along the mid-line. Note foramina.

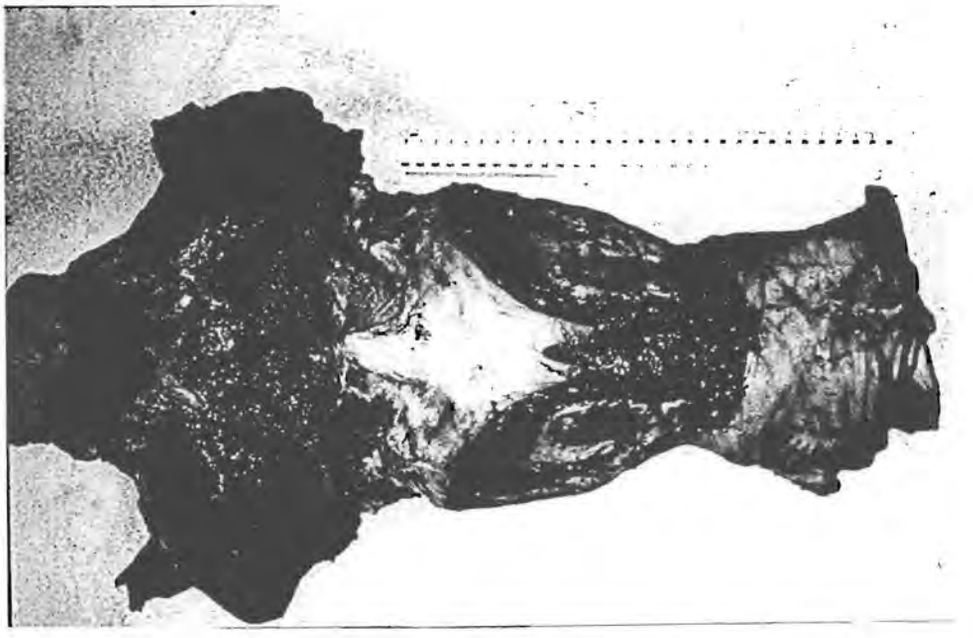

Plate 20a: Ventral view of the laryngeal apparatus of a juvenile male *B. acutorostrata* showing the epiglottic projection occurring anterior to the thyroid cartilage.

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iii) Epiglottic cartilage

The first indication of the epiglottic cartilage in this species is a small region that projects through the ventral muscular surface, anterior to the thyroid cartilage (Plates 20a and 20b). This region is continuous with the bulk of the cartilage as it appears ventrally above the arytenoid bodies (Plate 21). This cartilage, together with the epiglottic folds extends over the tips of the arytenoid bodies to form a hood-like arrangement (Plate 22). Once removed, the shape of the cartilage is that of a shoe-horn, consisting of a very thin anterior plate surrounding a wide shallow groove, with a thickened extension at its posterior end (Plates 23a and 23b). Table 4 shows the measurements of the epiglottic cartilage in each specimen.

iv) Arytenoid cartilages

The arytenoid cartilages consist of antero-ventrally situated, crescentic "wing-like" plates which terminate anteriorly as horn-like processes (arytenoid bodies). Dorsally these bodies continue to form the processi musculares and, posterior to this, they become thinner and taper to form the processi vocales (Plate 24a). The "wing-like" plates are connected to the other two processes by thick basal regions (Plate 24b). The left and right wings approach one another ventrally, at their extremities, so that the arytenoid bodies point dorsally towards the nasopharynx. The arytenoid bodies project beneath the root of the epiglottis forming the ventral margin of the entrance of the larynx (Plate 25). In the juveniles, the arytenoid bodies project an average of 3,6 cm from the floor of the pharynx, and in the adults an average of 6,5 cm. The processi musculares are somewhat "bell-shaped", with the base of the "bells" being incorporated into the processi vocales (Plate 24a). The anterior margins of the processi musculares are slightly rounded so as to accommodate the convexity of the articular facets on the cricoid body (Plate 26). The processi vocales are conical in shape, with their long axes being directed backwards, and lie parallel to each other along the ventral wall of the larynx, forming the dorsal wall of the laryngeal sac (Plate 26). The distal ends are connected by connective tissue. A wide aperture occurs between the processi vocales through which the sac communicates with the trachea (Plate 26). The arytenoid bodies are connected by connective tissue which is continuous with the laryngeal mucosa and which

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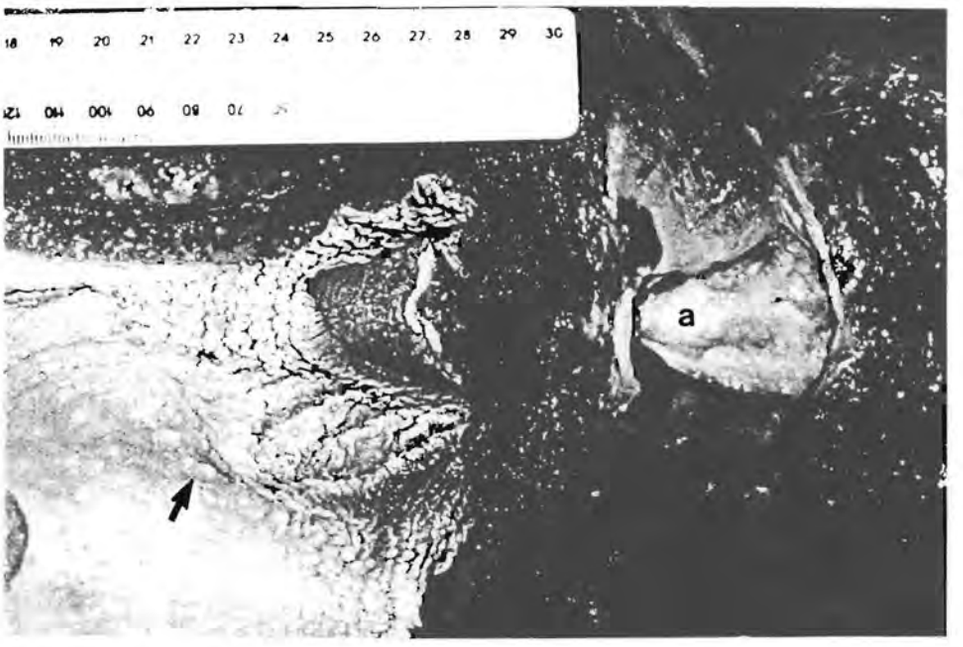

Plate 20b: Ventral view of (a) the epiglottic protrusion, occurring posterior to the nasopharynx. Note knob-like structures which occur randomly between the pits and below the anterior end of the nasopharynx (arrow).

Plate 21: Dorsal view of the epiglottis (lifted) showing (a) the epiglottic ridge of a juvenile male *B. acutorostrata*, which lies over the arytenoid cartilages (ventral view). Note arytenoid lips (arrow).

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Plate 22: Lateral view of the hood-like arrangement of the epiglottic cartilage of an adult female *B. acutorostrata*. Epiglottic folds occur on either side of the "hood", supported by the hyo-epiglottic muscles.

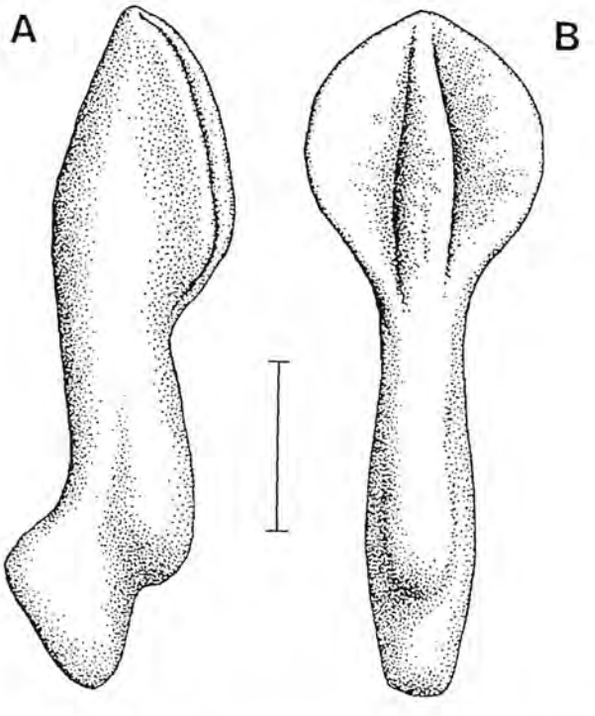

Plate 23: (A) Latero-dorsal view of the epiglottic cartilage of an adult male *B. acutorostrata* (# 159) showing its shoe-horn shape. The posterior region forms the epiglottic protrusion. (B) Dorsal view of the epiglottic cartilage of an adult male *B. acutorostrata* (# 159). The anterior "lips" of the cartilage are parted to reveal the epiglottic ridge. Note scale bar = 5 cm.

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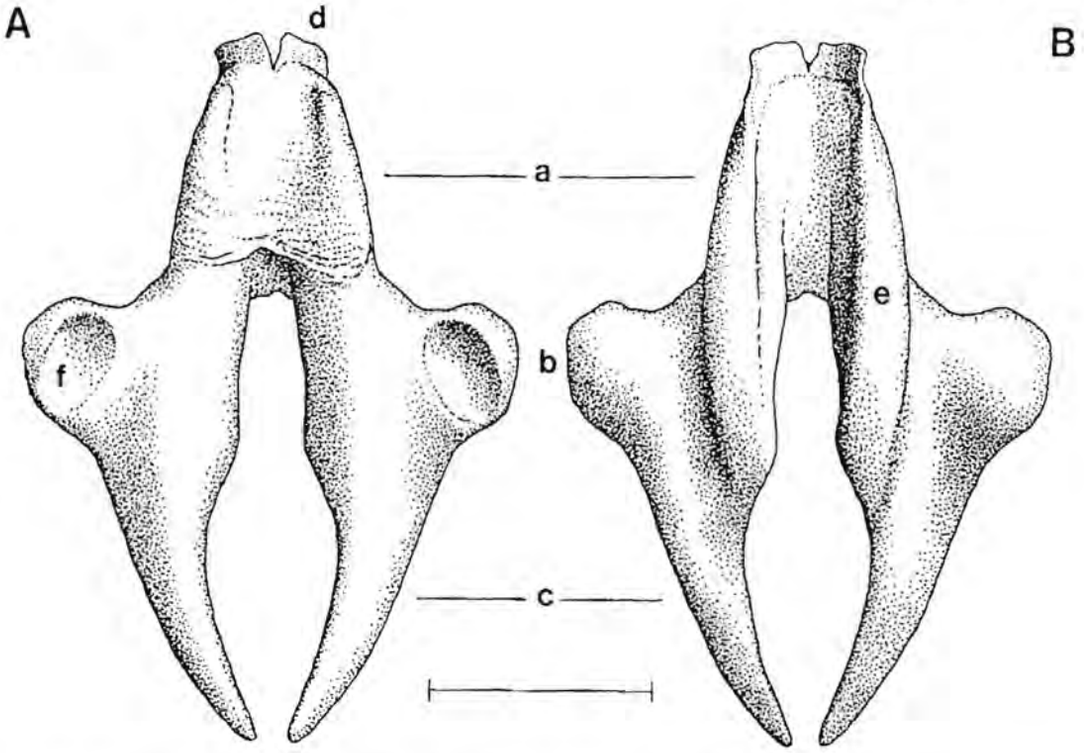

Plate 24: (A) Dorsal view and (B) ventral view of the arytenoid cartilages of an adult male *B. acutorostrata* (# 159). Labels are as follows: a = arytenoid bodies; b = processi musculares; c = processi vocales; d = arytenoid lips; e = ridges of the arytenoid bodies; f = crico-arytenoid facets. Note scale bar = 10 cm.

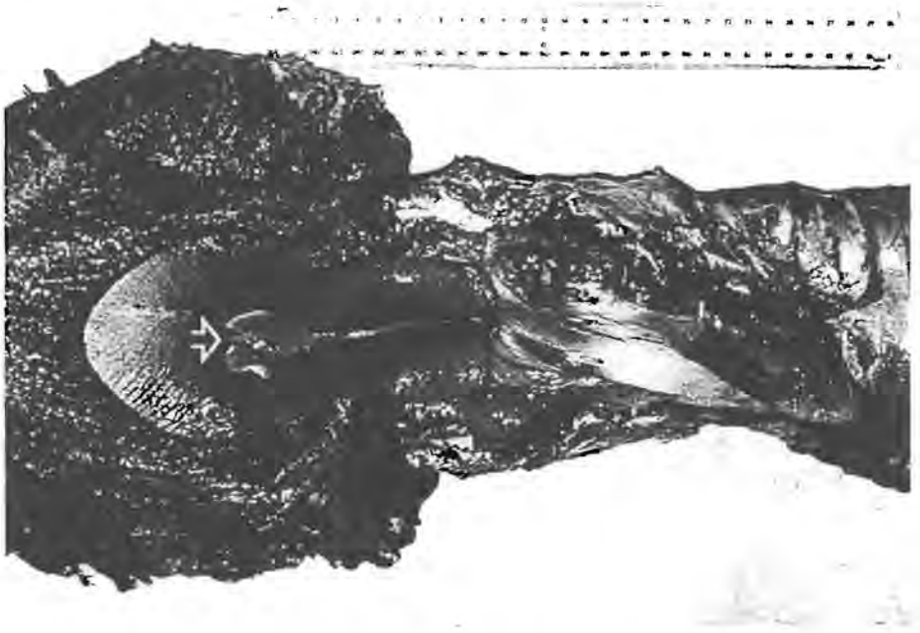

Plate 25: Ventral view showing the arytenoid bodies projecting beneath the root of the epiglottis (pulled back) of a juvenile female *B. acutorostrata*, forming the ventral margin of the entrance into the larynx. Note the arytenoid lips (arrow).

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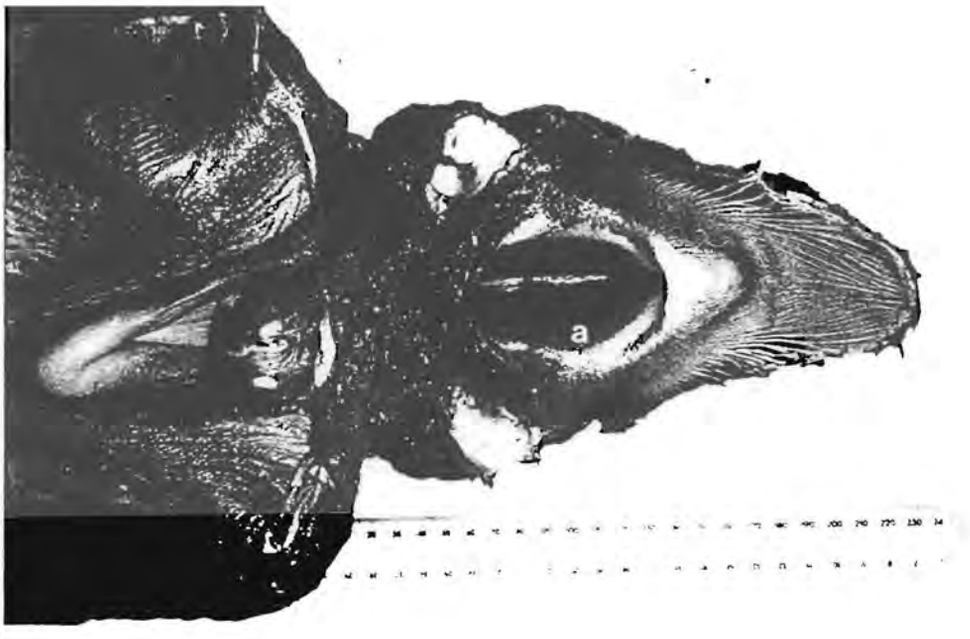

Plate 26: Dorsal view of (a) the arytenoid cartilages and the glottis forming the dorsal wall of the laryngeal sac of a juvenile male *B. acutorostrata*. Note rounded nature of the processi musculares and the folds of the laryngeal sac mucosa.

extends past the bodies forming rounded "lips" (Plates 24a, 24b and 25). These "lips" join together, forming the shape of the letter "m".

c) Myology of the laryngeal apparatus

The musculature of the laryngeal apparatus of *B. acutorostrata* was similar to that described by Benham (1901), so the terminology used by this author for the major muscles of the laryngeal apparatus is applied to this description (Plates 27a and 27b). The various extrinsic and intrinsic muscles of the laryngeal apparatus are composed of highly vascularised, skeletal muscle tissue.

i) Extrinsic muscles

On the posterior margins of the styloid bones, bordered by the cornua of the os hyoides, lies a long rectangular muscle, which could possibly be the **sterno-hyoid** (Plate 28). The raised anterior margin of the os hyoides seems to separate this muscle into 2 sections. In the juvenile male, this muscle takes its posterior origin from the anterior, flat region of the sternum, and passes upwards, being inserted into the posterior margin of the os hyoides. This muscle forms two quadrilateral masses, rather than one long one. This muscle was not observed in the other specimens, presumably being removed during initial dissection.

Arising from the anterior border of the thyroid cartilage, the triangular **thyro-hyoid** muscles stretch forward and attach to the posterior margins of the cornua of the os hyoides (Plate 9). The **sterno-thyroid** muscle was not clearly discernible, if at all present, but the juvenile female specimen did display some muscular attachment on the anterior margin of the sternum, which seemed to stretch anteriorly and cover the ventral face of the thyroid cartilage (Plate 12).

ii) Intrinsic muscles

Upon the removal of the sternum bones, posterior to the thyro-hyoid muscles and anterior to the tracheal rings, the **thyro-cricoid** muscle can be seen lying in line with the mid-line of the thyroid cartilage. Anteriorly this muscle reaches the epiglottic cartilage (Plate 29) while posteriorly it

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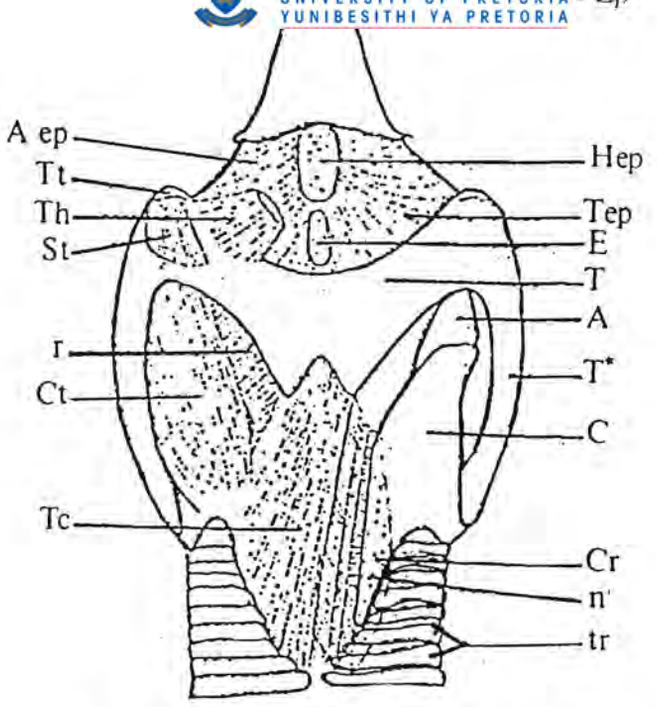

Plate 27a: Ventral view of entire larynx of *B. acutorostrata*, as seen after the removal of the hyoid bones and the wall of the pharynx. Some of the muscles have been removed from the right side of the figure, so as to show the shape and relations of the cartilages. The right longitudinal muscle (thyro-cricoid) of the sublaryngeal pouch has been cut away so as to exhibit the deeper circular muscles. Letters have the following significance: A: arytenoid cartilage; A.ep: aryteno-epiglottid muscle; C: cricoid cartilage; C.c: Ventro-posterior cornu of cricoid cartilage; C.t: Crico-thyroid muscle; E: epiglottid cartilage; Ep: epiglottis; H.ep: Hyo-epiglottid muscle; n: Transverse and circular muscles of the sublaryngeal pouch; r: accessory crico-thyroid muscle; S.t: sterno-thyroid muscle; T: thyroid cartilage; T*: posterior cornu of thyroid cartilage; T.c: thyro-cricoid muscle; t.ep: thyro-epiglottid muscle; T.h: thyro-hyoid muscle; tr: tracheal rings; T.t: tubercle or ridge on the anterior margin of the thyroid cartilage. (Taken from Benham 1901)

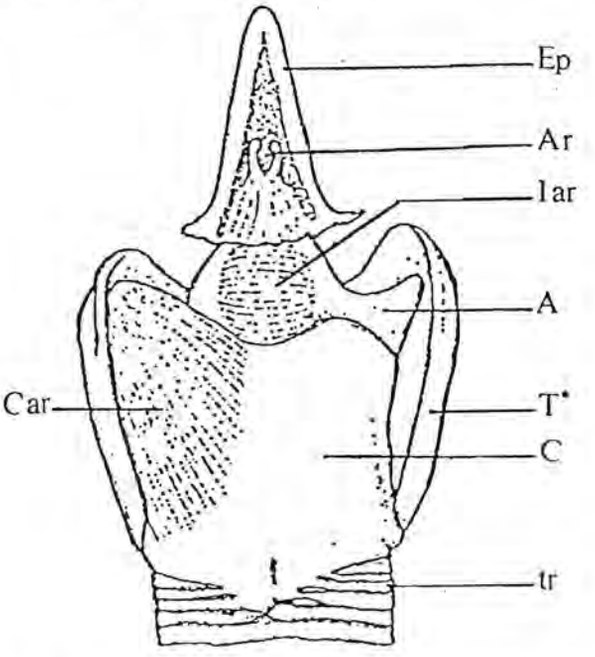

Plate 27b: Dorsal view of the larynx of *B. acutorostrata*. Some of the muscles have been removed from the right side. Note continuity of the upper tracheal rings with the cricoid cartilage. Labelling as above except for I.ar: inter-arytenoid muscle; C.ar: (posterior) crico-arytenoid muscle. (Taken from Benham 1901).

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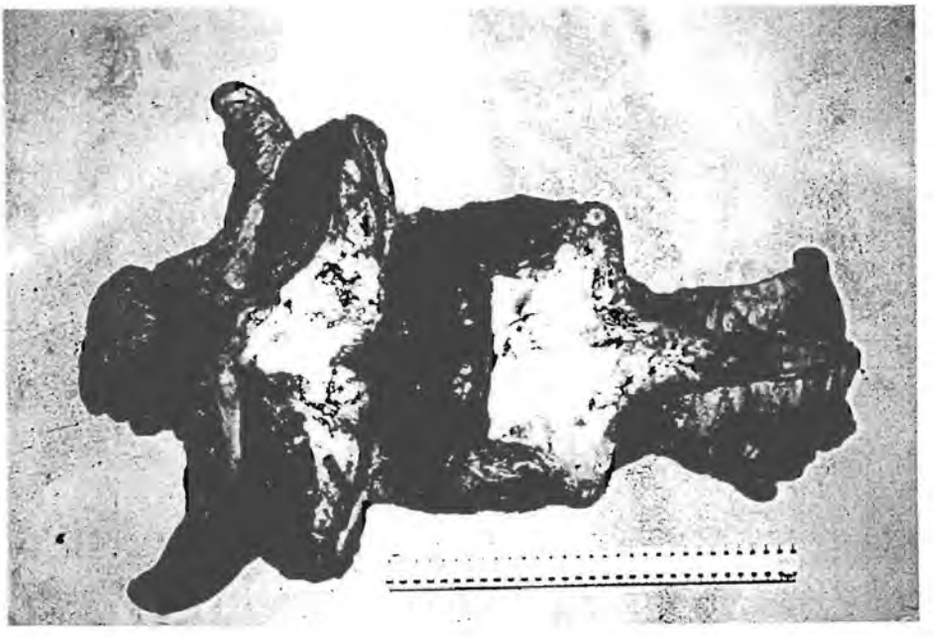

Plate 28: Ventral view of the laryngeal apparatus of a juvenile male *B. acutorostrata* showing the hyoid and sternum bones. Note the association of the rectangular sterno-hyoid muscle with the anterior margin of the sternum bone.

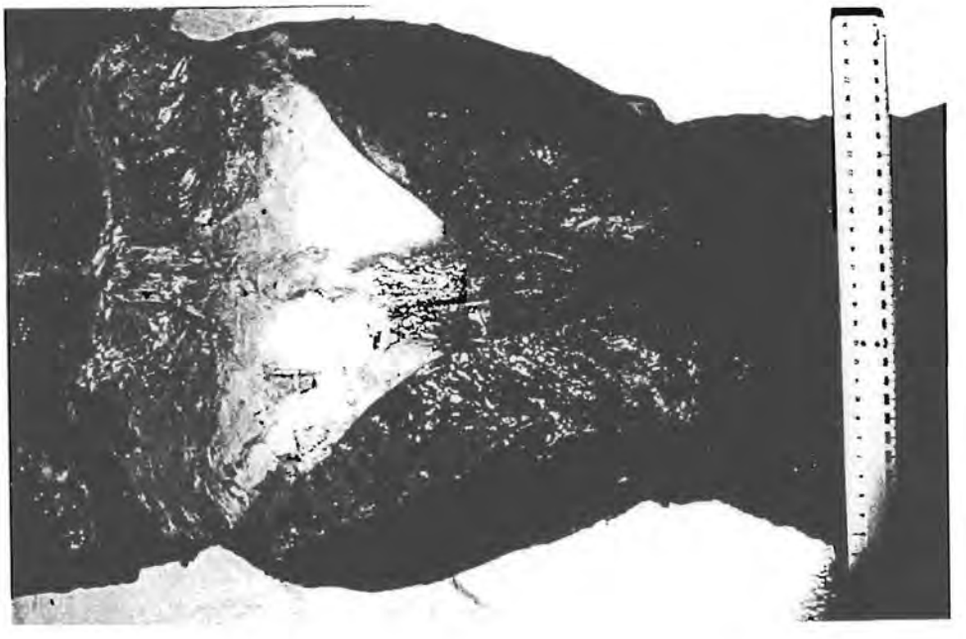

Plate 29: Ventral view of the thyro-cricoid muscle (in line with the mid-line of the thyroid cartilage) of an adult female *B. acutorostrata*.

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stretches as far as the tracheal rings, forming part of the outer wall of a blind-ending sac which terminates at the anterior margin of the tracheal rings (Plate 30).

The epiglottis and thyroid cartilages lie posterior to the os hyoides. Only a small part of the epiglottic cartilage protrudes through the **thyro-epiglottic, aryteno-epiglottic** and **hyo-epiglottic** muscles and is situated in line with the middle of the convex anterior margin of the thyroid cartilage (Plate 31). The thyro-epiglottic muscle begins on the inner, lateral side of the body of the thyroid cartilage and passes anteriorly to join the aryteno- and hyo-epiglottic muscles which, together with the epiglottic cartilage, form the epiglottis. The outer margin of the wing and the posterior processus vocales of the arytenoid cartilage provide attachment for the aryteno-epiglottic muscle. This muscle extends to the epiglottic cartilage and penetrates the thyro- and hyo-epiglottic muscles in which it is embedded. The hyo-epiglottic muscle is inserted in the antero-ventral region of the epiglottic cartilage and passes along the sides of the epiglottis (Plate 22). The **thyro-arytenoid** muscle is not very conspicuous and lies across the aryteno-epiglottic muscle. The origin of the thyro-arytenoid muscle is not clearly defined, but it seems to arise beneath the crico-arytenoid muscle from the thyroid cartilage region and continues towards the bodies of the arytenoid cartilages.

Within the arches of the body of the thyroid cartilage, originating from the latero-ventral face of the cricoid body, the **crico-thyroid** muscle fibres extend to the inner surface of the posterior cornu of the thyroid cartilage (Plate 32). Once removed, the **accessory crico-thyroids** are visible, while the lateral part of the cricoid body is also exposed (Plate 33).

The dorsal surface of the laryngeal apparatus is also very muscular in character (Plate 34), although here, only two muscles are distinguishable, namely, the crico-arytenoid and the inter-arytenoid muscles. The **crico-arytenoid** muscle covers most of the dorsal body and lateral surfaces of the cricoid cartilage, with the lateral portion extending beneath the crico-thyroid muscle. The lateral and dorsal portions of the crico-arytenoid cannot be distinguished from one another and together form one muscle mass (Plate 34). This muscle mass extends to the processus muscularis of the arytenoid cartilage. The second muscle found on the dorsal surface of the laryngeal apparatus is the **inter-arytenoid**, and its position is (as its name suggests)

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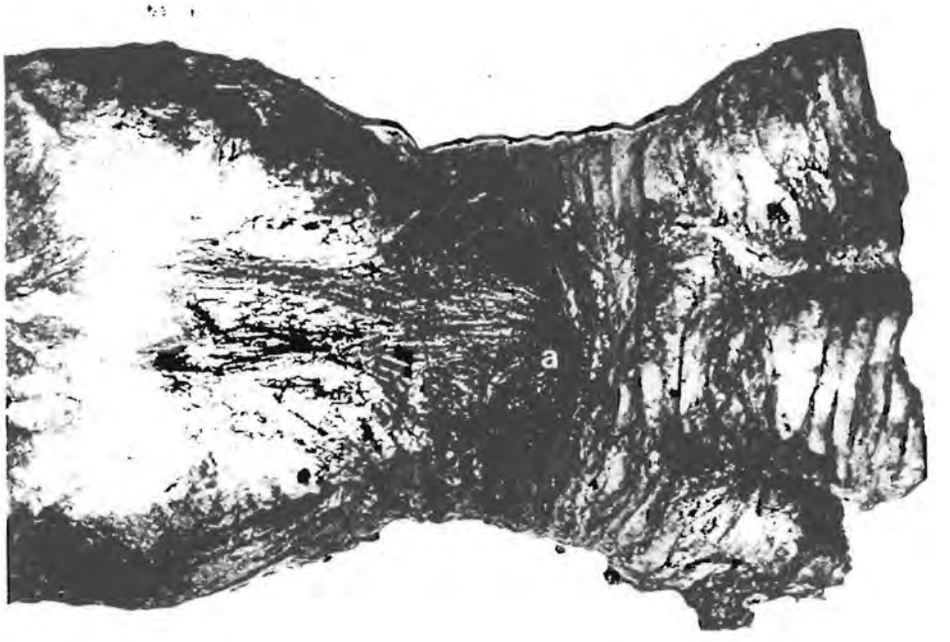

Plate 30: Ventral view of (a) the posterior margin of the thyro-cricoid muscle of an adult female *B. acutorostrata* forming part of the outer wall of the laryngeal sac.

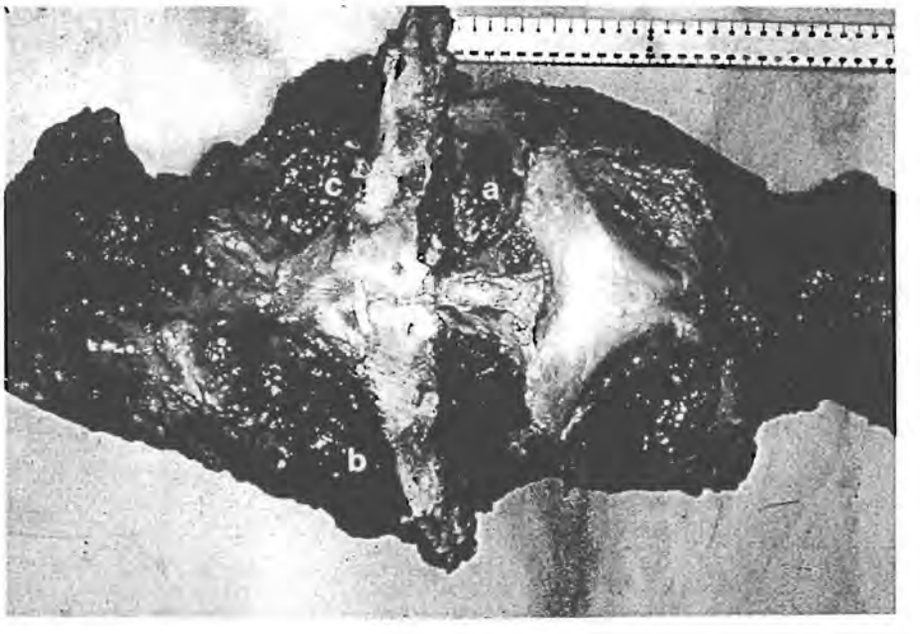

Plate 31: Ventral view of the (a) thyro-epiglottic, (b) aryteno-epiglottic and (c) hyo-epiglottic muscles of a juvenile female *B. acutorostrata*

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Plate 32: Lateral view of the right portion of the crico-thyroid muscle and the thyroid cartilage of an adult female *B. acutorostrata*.

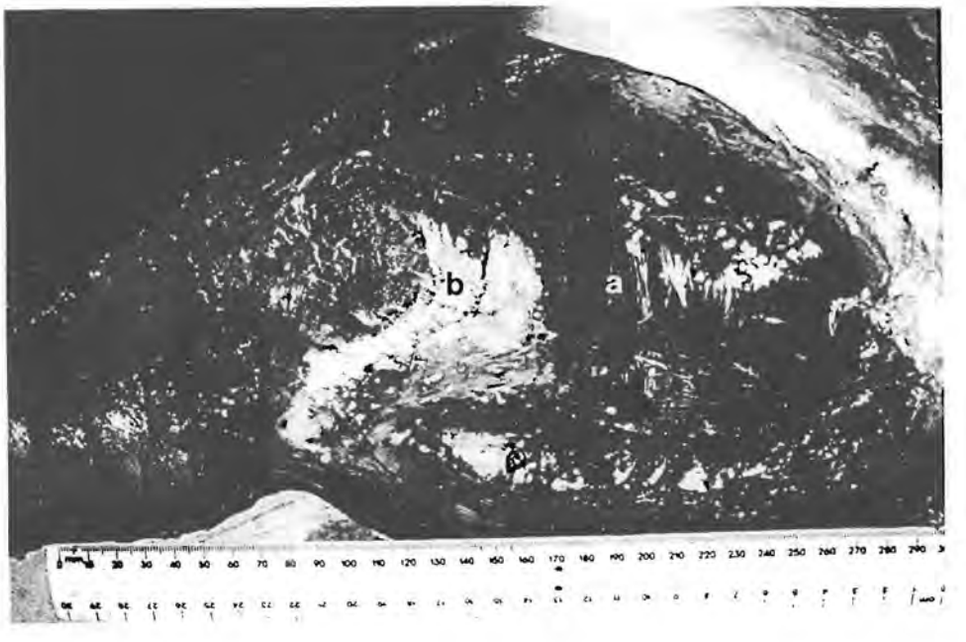

Plate 33: Lateral view of the left portion of the (a) accessory crico-thyroid muscle of an adult female *B. acutorostrata*, with (b) the lateral part of the cricoid cartilage exposed.

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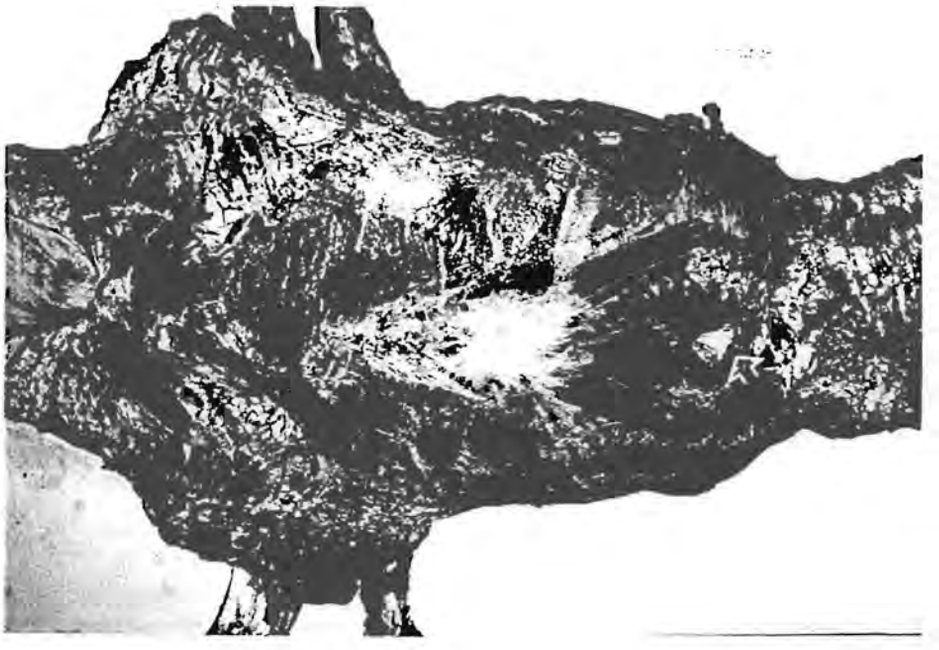

Plate 34: Dorsal view of the muscular nature of the laryngeal apparatus of an adult female *B. acutorostrata*. Note the oesophageal opening (arrow) just above the tracheal rings.

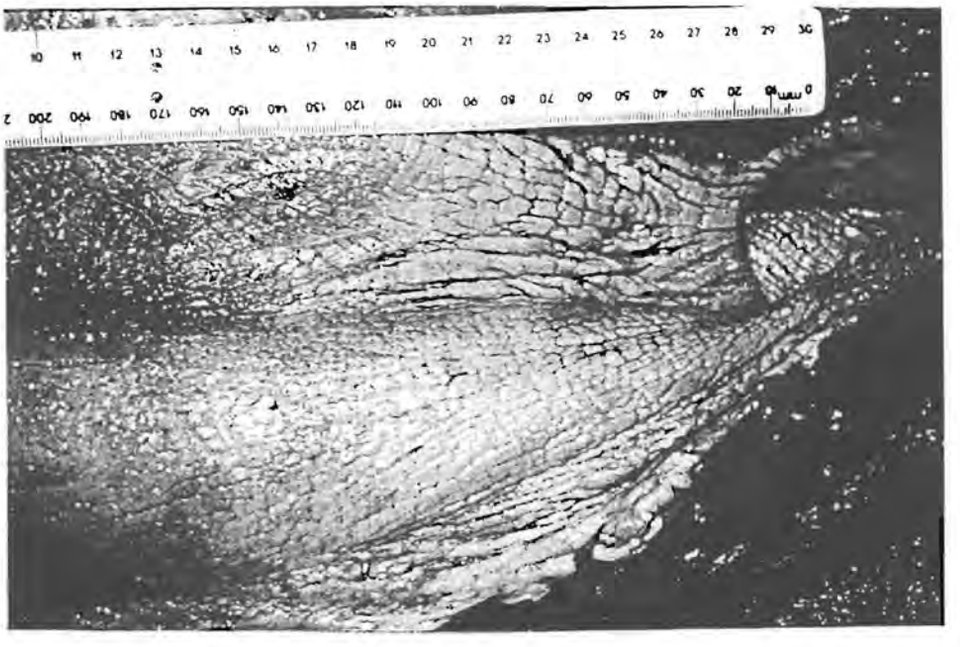

Plate 35: Ventral view of the nasopharynx of an adult female *B. acutorostrata* showing cuboidal and rectangularly shaped tissue on the surface of the mucosa.

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between the paired arytenoid cartilages, arising from the arytenoid "wings". The posterior opening of the oesophagus lies between the arytenoid "wings" and in the midline of the cricoid body on the dorsal surface (Plate 34).

3.2 Histology of the laryngeal apparatus of *B. acutorostrata*

Beginning at the anterior end of the apparatus and proceeding in a posterior direction, the nasopharynx, lying on the ventral side of the animal, is lined by a mucosal layer which on inspection consists of cuboidal and rectangularly-shaped units (being cuboidal close to the epiglottis and becoming rectangular anteriorly) (Plate 35). Histologically, the mucosa consists of a thick layer of stratified squamous epithelium which covers unusually dense concentrations of connective (collagenous) tissue. Muco-serous glands and ducts lie loosely in the connective tissue, which also divides them into distinct lobules. A finger-like dermis reaches into the epidermis (Plate 36). The "fingers" are made up of connective tissue and arise from the lamina propria. Besides these tissue formations, the surface of the mucosal layer is pitted. Tiny pin-holes also penetrate the mucosa throughout the length of the nasopharynx. The mucosa originating in the nasopharynx is dark grey but becomes light pink in colour in the pharynx, with infusions of grey throughout. The grey mucosal tissue is more pronounced in the females particularly in the juvenile, where most of the nasopharynx is dark grey (Plate 37). This grey coloration is due to the presence of melanocytes in the epidermal layer (Plate 38). In the juvenile male, a few small knob-like structures are found to occur randomly between the pits and below the anterior end of the nasopharynx (Plate 20b). In the adults, the muscular tissue around the nasopharynx seems to be penetrated by a network of thin, white-coloured deposits, forming a pattern much like that of the cells of a bee-hive. Upon visual inspection, this network seemed to be made up of adipose tissue, indicating the possible presence of smooth muscle. But histological analysis revealed only small amounts of adipose tissue interspersed between the muscle tissue, which was identified as skeletal muscle. The nasopharynx ends at the posterior narial opening.

The mucosal layer of the nasopharynx is continuous with the narial opening, but adopts a distinctive "elasticated" or "gathered" appearance at the edges of the opening (Plates 20b and 25). The epithelium in this region is not as thick as that found in the nasopharynx (Plate 39). Many

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Plate 36: Transverse section of the nasopharyngeal mucosa of an adult male *B. acutorostrata*. Note the finger-like dermis reaching into the epidermis (arrow) as well as large amounts of adipocytes. (Mag. 50x, Masson's Trichrome (MT) stain).

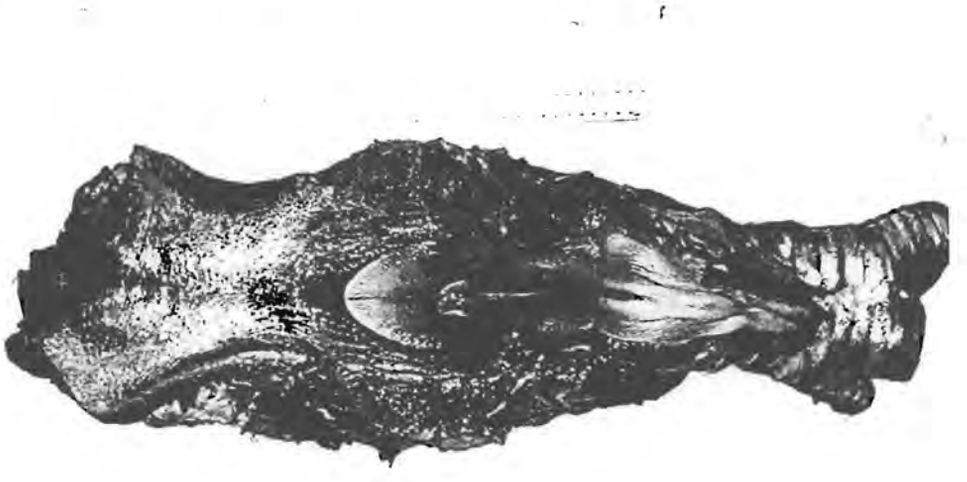

Plate 37: Ventral view of the laryngeal apparatus of a juvenile female *B. acutorostrata* showing the grey mucosal tissue (epiglottis is pulled back).

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Plate 38: Transverse section of the nasopharyngeal mucosa of a juvenile female *B. acutorostrata* indicating collections of melanocytes at the base of the epidermal layer (arrow). (Mag. 100x, Haemotoxylin & Eosin (H/E) stain).

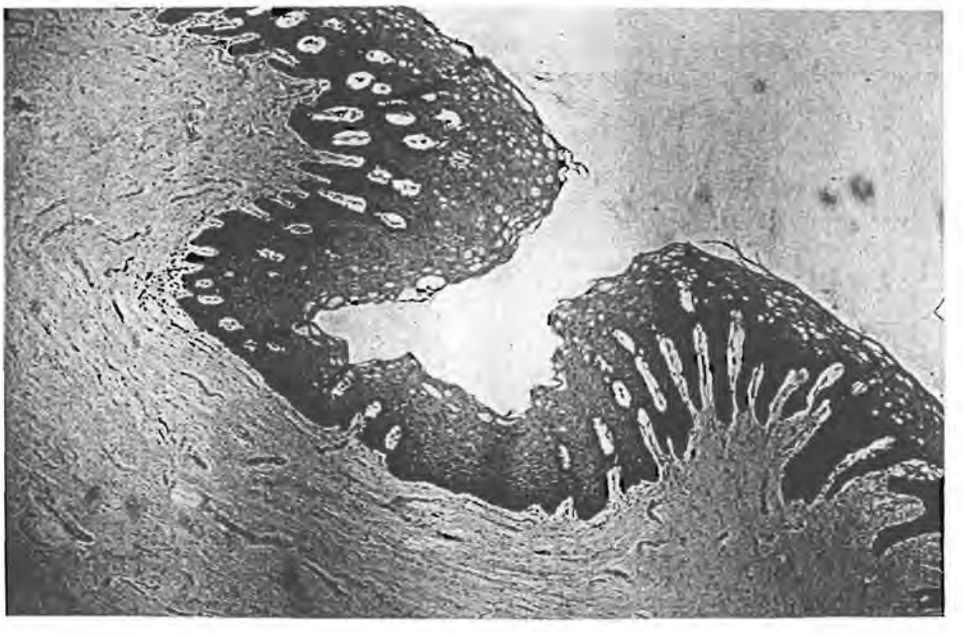

Plate 39: Transverse section of the mucosa of the narial opening of an adult male *B. acutorostrata* indicating undulations of the mucosa. (Mag. 50x, Victoria Blue (VB) stain).

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muco-serous glands are present, with most of the glands being mucosal. Skeletal muscle as well as a small amount of connective tissue occurs between the epithelium and the glands. Huge ducts are present along with many blood vessels and capillaries. Lymphocytes are also present in this region and, as in the nasopharynx, collagenous "fingers" protrude into the epidermal layers.

The epiglottis originates from the floor of the pharynx. It is conical in shape, narrowing anteriorly, ending in a somewhat rounded tip, providing a seemingly hood-like appearance (Plate 40). From the dorsal perspective, the margins of the epiglottis are rounded and eventually pass into the aryteno-epiglottic folds. The dorsal surface is grooved, possessing a distinct ridge down the middle (Plate 41). A simple squamosal epithelium lines the epiglottic ridge, which consists entirely of adipose tissue (Plate 42). The ridge is thickest at its middle point and extends downwards into the laryngeal sac. The anterior portion of the epiglottis lies in a furrow on the posterior portion of the nasopharynx; this is accomplished by its free end being inserted into the posterior narial opening (Plate 43).

Simple squamosal epithelial cells characterise the epidermal layer of the pharynx, and due, once again, to the presence of melanin in the basal layer of the epidermis, the anterior region of the pharynx in the juveniles is distinctly grey in colour (Plate 43). In the adults, however, the grey coloration is not as marked, as is the case for the nasopharynx (Plate 44). A longitudinal incision along the sagittal plane of the pharynx reveals the epiglottis, and two clear groups of crypts on the left and right sides of the epiglottis (Plate 45). The crypts along the pharynx begin just below the anterior opening and continue until approximately 5 cm from the posterior tip of the epiglottis (Plate 45). Each group of crypts has, on its side closest to the epiglottis, a "crypt passage/pocket", i.e. the lining of the pharynx "invaginates" (Plate 45). These "invaginations" are also continuous with crypts (Plate 46). Thereafter, there are a few smaller crypts leading into the laryngeal area. The crypts gradually diminish in size until they disappear entirely towards the middle of the pharynx, where the mucosa becomes smooth and continues in this manner until it becomes continuous with the oesophageal mucosa.

The oesophageal mucosa is folded and, once again, distinct left and right groups of crypts occur on each side (Plate 47). The oesophagus is lined with a notably thick stratified-squamous

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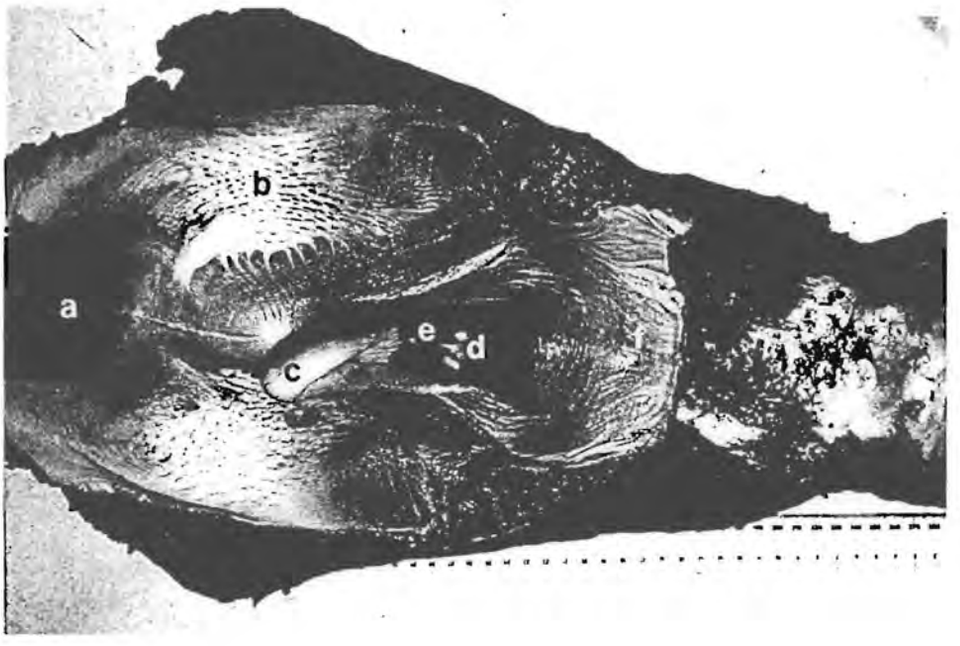

Plate 40: Sagittal section along the dorsal side of the laryngeal apparatus of a juvenile male *B. acutorostrata* exposing the (a) nasopharynx, (b) pharynx, (c) epiglottis, (d) arytenoid bodies, (e) arytenoid lips and (f) the oesophagus. Note the shape and origin of the epiglottis.

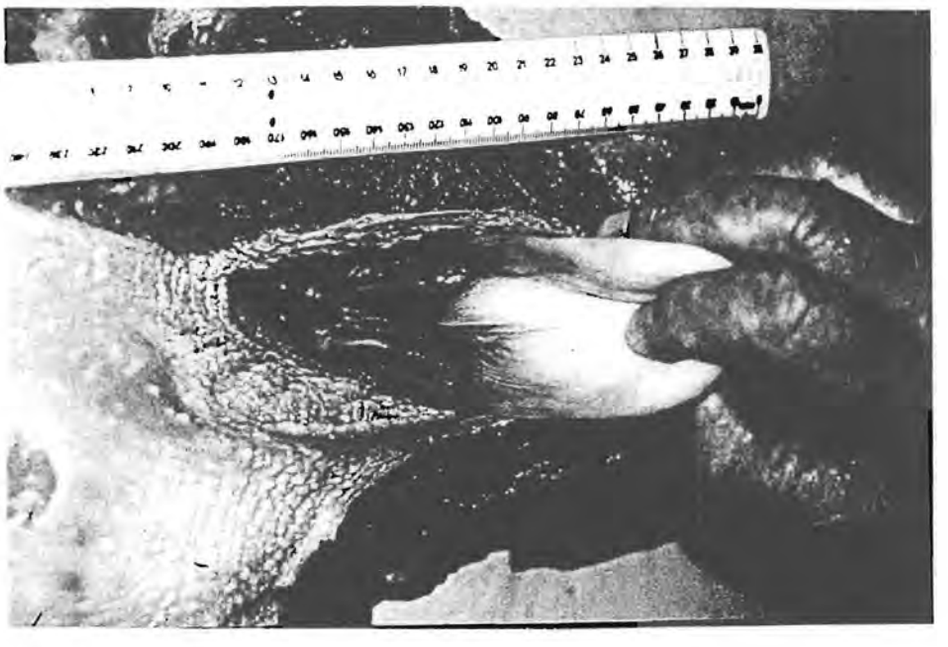

Plate 41: Ventral view of the epiglottis (pulled back) exposing the epiglottic ridge of a juvenile male *B. acutorostrata*.

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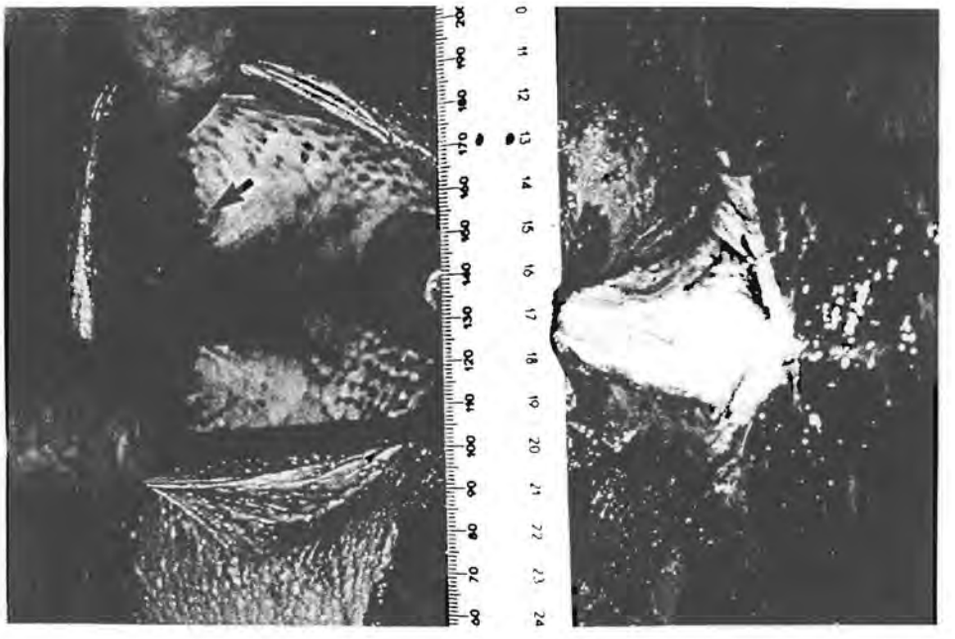

Plate 46: Ventral view of the epiglottic protrusion of a juvenile male *B. acutorostrata* (posterior nare pulled back). Note the presence of crypts in the crypt passages (arrow).

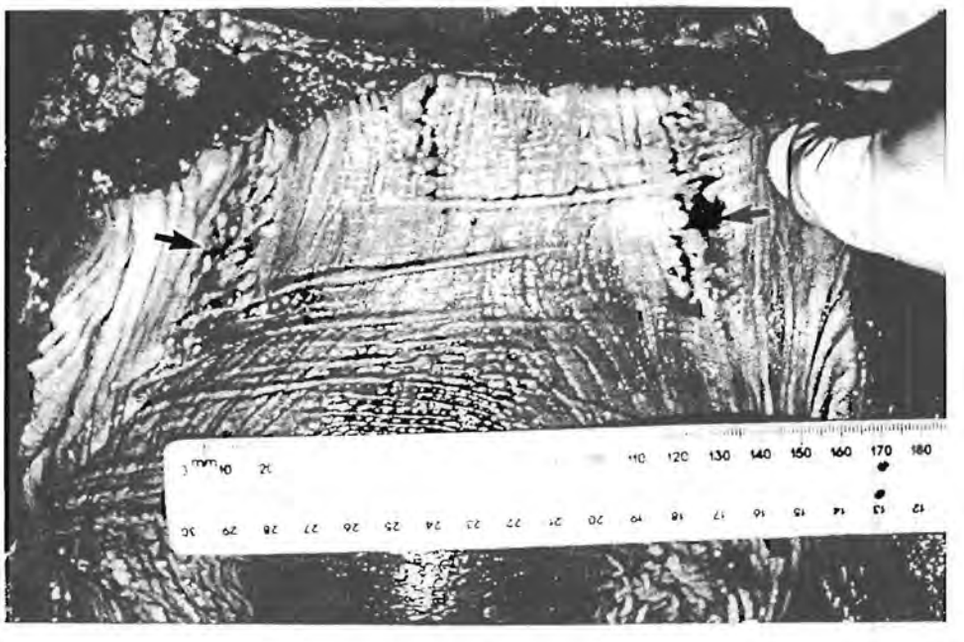

Plate 47: Dorsal view of the oesophagus of an adult female *B. acutorostrata* (exposed by a sagittal incision) showing distinct groups of crypts (arrows).

epithelial layer and mucous glands are present in great numbers (Plate 48). The base of each crypt has a duct opening into it and they are surrounded by lymphocytes. Enormous lymph nodes (resembling tonsils) are present amongst the many glands (Plate 49).

As previously mentioned, the arytenoid bodies project beneath the root of the epiglottis. The lips which join the anterior tips of the arytenoid bodies possess a stratified squamous epithelial layer which covers mainly connective tissue intermingled with collagen fibres (Plate 50). This region forms the posterior margin of the entrance into the laryngeal area, leading into the trachea. The tracheal mucosa lines the ventral surface of the body of the cricoid cartilage and the dorsal wall of the laryngeal sac (Plate 51). The lining of the trachea is brown in colour and is characterised (on the dorsal wall of the laryngeal sac) by tasselated folds (Plates 26 and 52) which are covered by a thick stratified columnar epithelium (Plate 53). The epithelium is thicker in the depressions compared to the edges of the protrusions (Plate 54). This epithelium is not typical stratified columnar epithelium as the top-most cells are not squamous but columnar in nature. On inspection, the cells merge from thick stratified squamous cells to less thick cells, to stratified columnar cells which merge with very thin pseudo-stratified columnar cells. Small aggregations of lymphocytes are present as well as goblet cells. Mucous glands are present, but are restricted to regions of the epidermis (Plate 55).

The trachea continues posteriorly where it is surrounded and supported by cartilagenous (tracheal) rings and eventually splits to form the bronchi (Plates 18 and 56). The tracheal rings are incomplete ventrally and terminate with rounded ends. Dorsally, preceeding rings are fused with succeeding rings making the definition of individual rings quite difficult.

The upper tracheal rings are continuous dorsally with the cricoid cartilage (Plates 19 and 57) and ventrally they provide attachment for the laryngeal sac (Plate 58).

The laryngeal sac lies on the ventral aspect of the larynx, below the thyroid cartilage and between the cricoid cornua (Plate 58). The ventral wall of the sac is formed by a thick skeletal muscular layer (Plate 59). The mucosa of the laryngeal sac is smooth with two distinct rows of crypts on either side of the sac which diminish in size at the posterior end of the sac and meet up to form a group of smaller crypts around the rounded end of the sac (Plate 60). Pseudo-stratified

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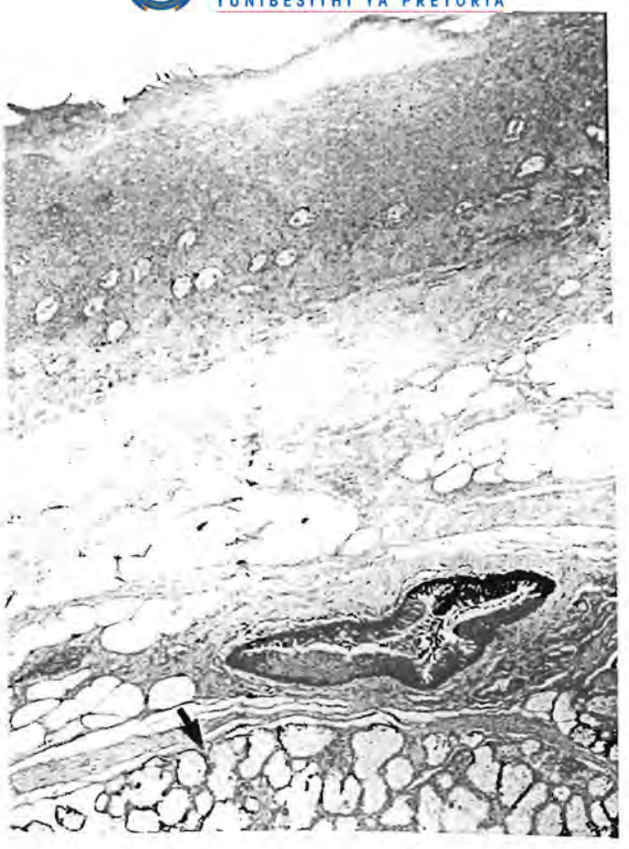

Plate 48: Transverse section of the oesophageal mucosa of an adult male *B. acutorostrata* showing the thick stratified squamous epithelial layer and large mucous glands (arrow). (Mag. 50x, H/E).

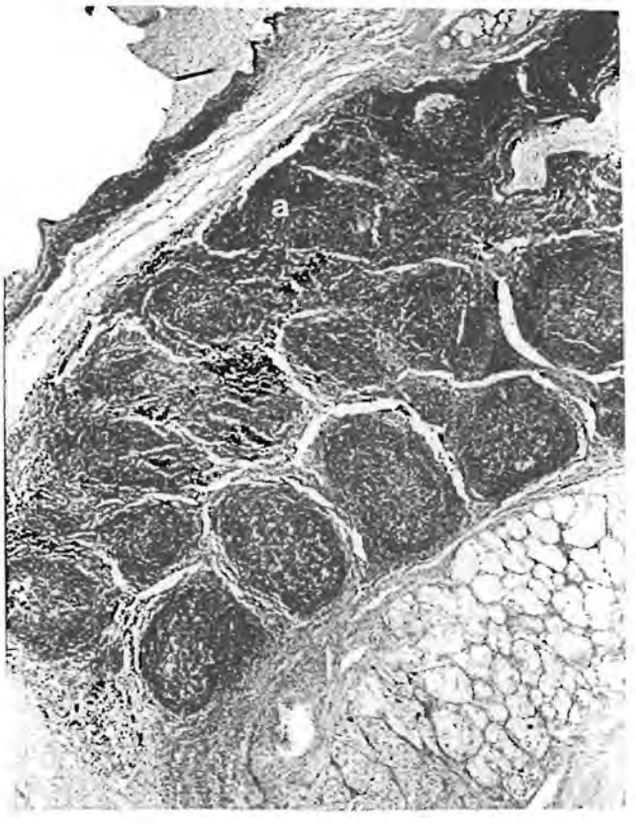

Plate 49: Transverse section of the oesophageal mucosa of an adult female *B. acutorostrata* showing (a) enormous lymph nodes occurring below the epidermis and above large glands. (Mag. 50x, H/E).

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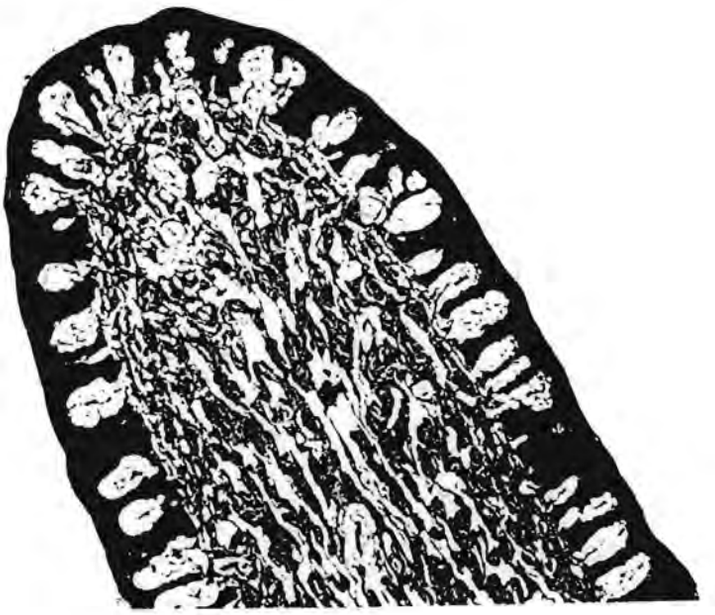

Plate 50: Transverse section of the right hand side arytenoid lip of an adult female *B. acutorostrata* . Note the large amounts of connective tissue (blue/green). (Mag. 50x, VB).

Plate 51: Lateral view of (a) the laryngeal sac and the tracheal rings of an adult female *B. acutorostrata* showing that the tracheal mucosa is continuous with the ventral surface of the body of the cricoid cartilage and the the dorsal wall of the laryngeal sac.

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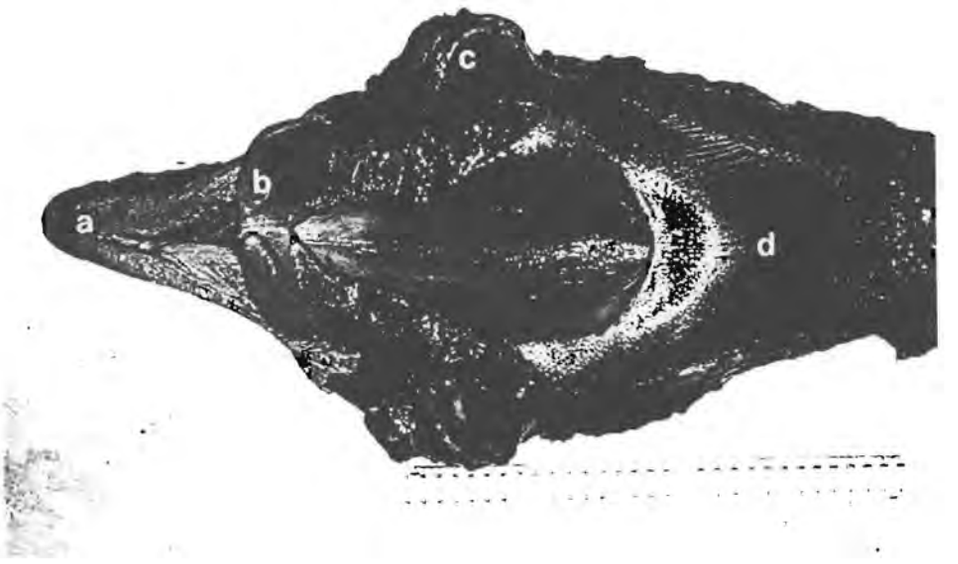

Plate 52: Dorsal view of the (a) epiglottis, (b) tips of the arytenoid bodies, (c) processi musculares and the (d) laryngeal sac of an adult female *B. acutorostrata*. Note the folded nature of the tracheal/external laryngeal sac mucosa.

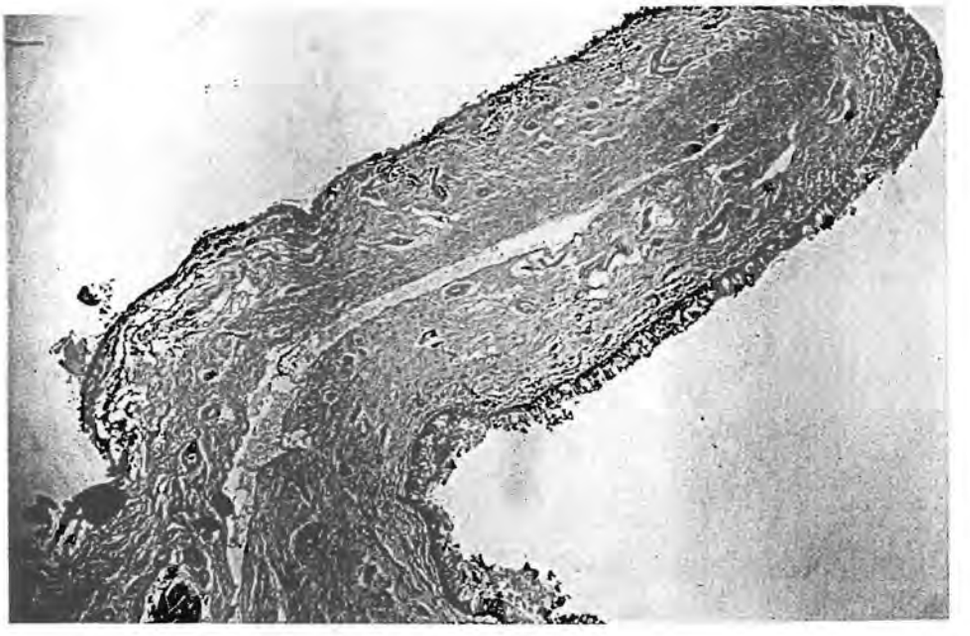

Plate 53: Transverse section of the tracheal mucosa of an adult male *B. acutorostrata* showing the folded nature of the mucosa. (Mag. 50x, H/E).

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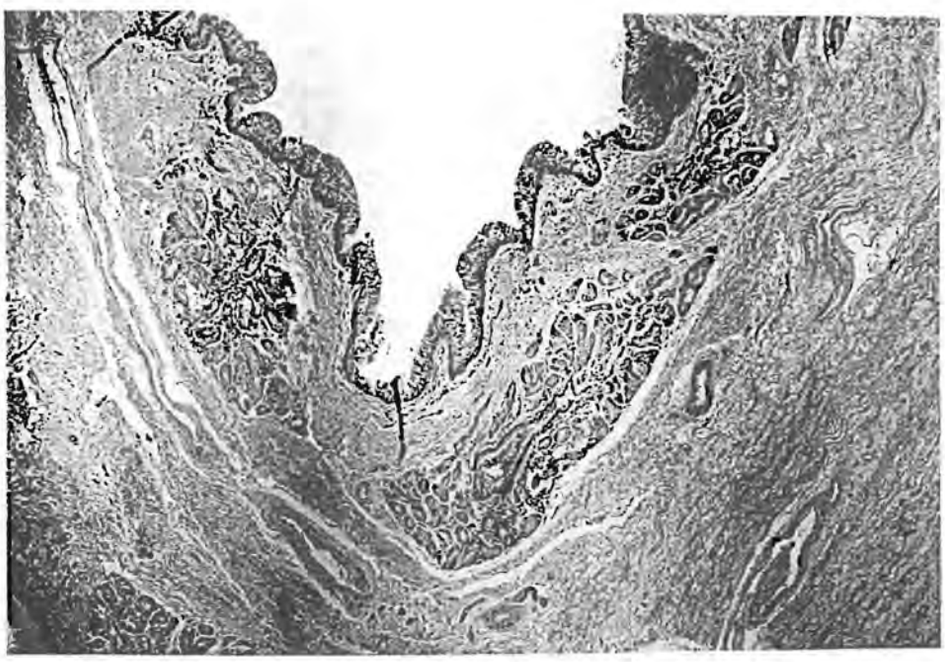

Plate 54: Transverse section of the tracheal mucosa of an adult male *B. acutorostrata* showing the thicker epithelium in the depressions of the mucosa. (Mag. 50x, H/E).

Plate 55: Transverse section of the tracheal mucosa of an adult male *B. acutorostrata* showing the restriction of mucous glands to the epidermal region. Note the atypical epithelial structure and ducts which pierce the epithelium. (Mag. 50x, H/E).

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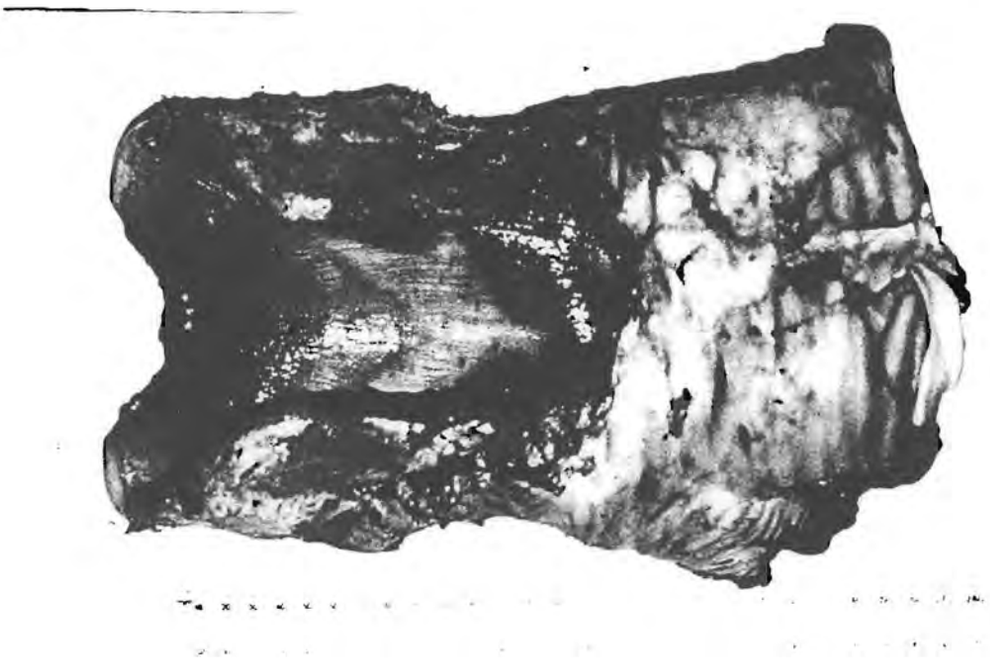

Plate 56: Ventral view of the cricoid cartilage continuous with the tracheal rings of a juvenile male *B. acutorostrata*, which split to form the bronchi.

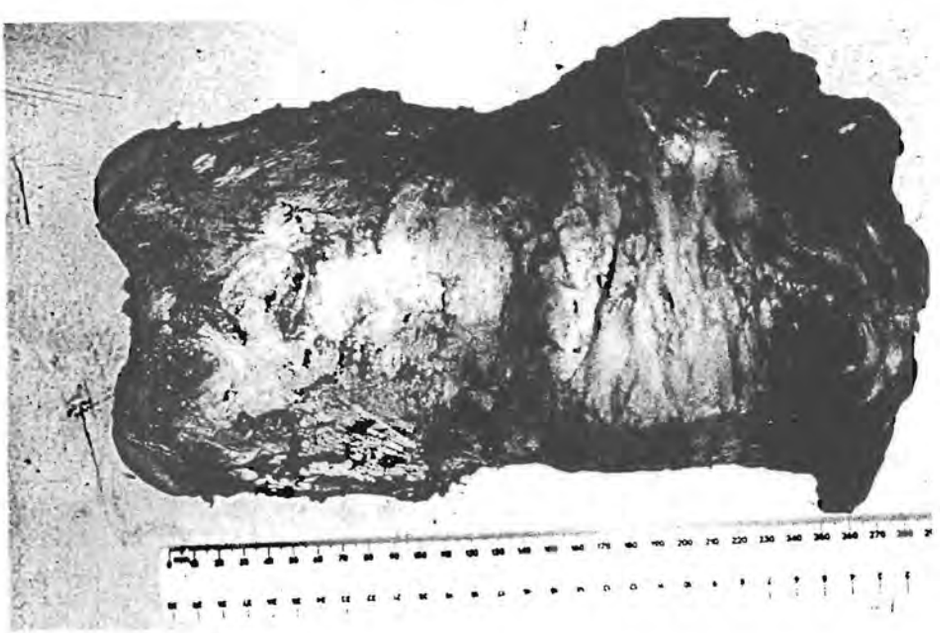

Plate 57: Dorsal view of the body of the cricoid cartilage of a juvenile male *B. acutorostrata* which is continuous with the tracheal rings.

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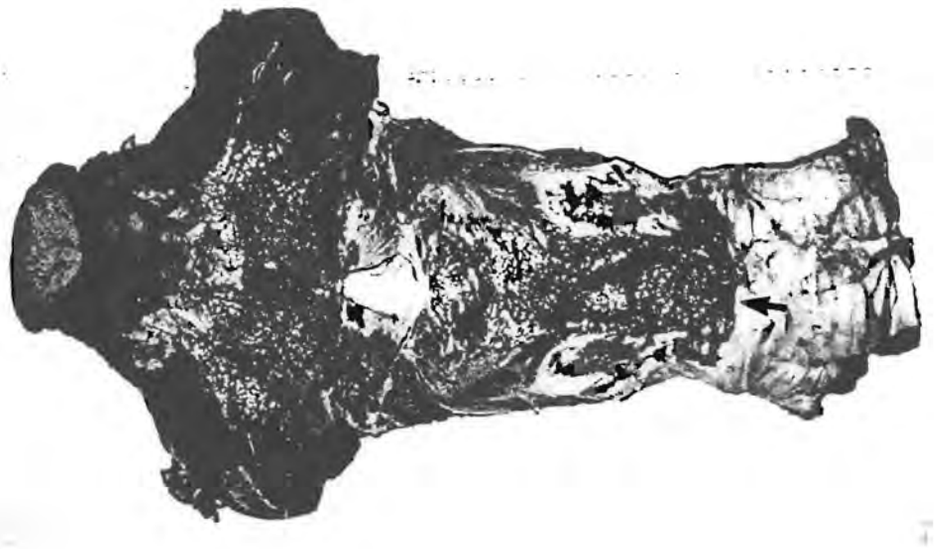

Plate 58: Ventral view of the laryngeal apparatus of a juvenile male *B. acutorostrata*. Note the posterior attachment of the laryngeal sac with the tracheal rings (arrow).

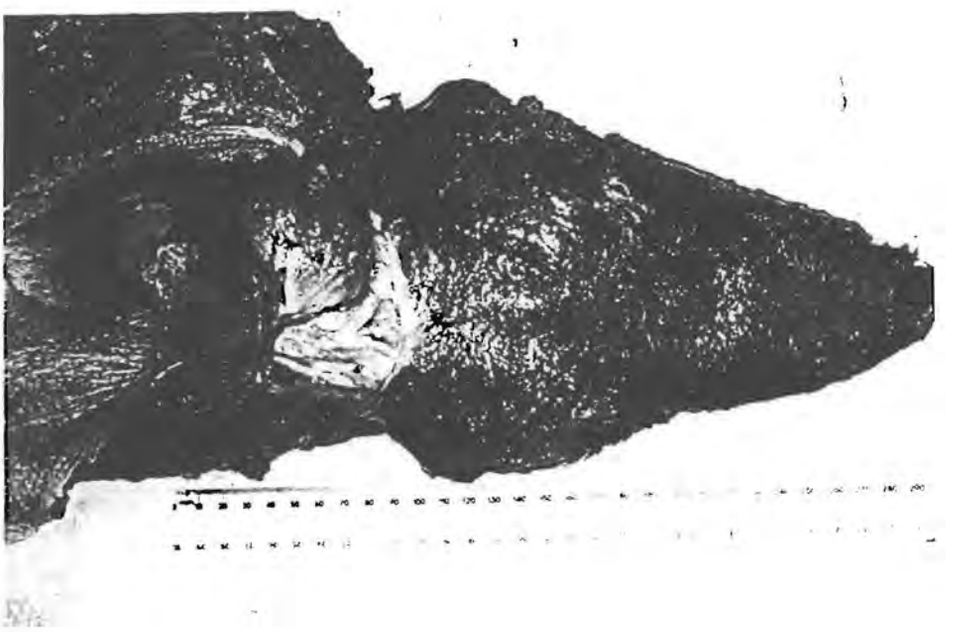

Plate 59: Ventral view of the muscular wall of the laryngeal sac of a juvenile male *B. acutorostrata*.

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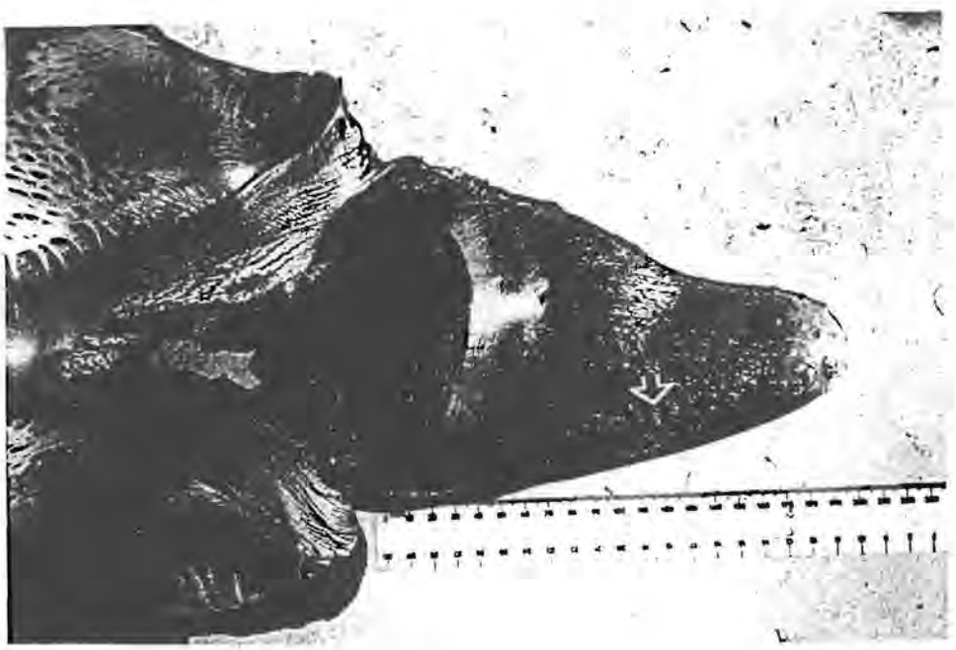

Plate 60: Dorsal view of the mucosa of the laryngeal sac of a juvenile male *B. acutorostrata*. Note organisation of crypts (arrow) (sac turned inside out).

Plate 61: Transverse section of the mucosa of the laryngeal sac of an adult male *B. acutorostrata*. Note lymphoid tissue in depression. (Mag. 100x, VB).

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columnar epithelium lines the inside of the laryngeal sac (Plate 61). Beneath the epidermis there are three layers of dense connective tissue with extremely large scattered serous glands, adipose tissue interspersed with connective tissue, and skeletal muscle, respectively (Plate 62).

Skeletal muscle is present in all the layers, but increases in abundance towards the exterior of the sac. This whole region is infiltrated with lymphocytes and lymphoid tissue is found in the depressions occurring along the epidermis (Plate 61). Deep ducts leading from the many glands are lined with pseudo-stratified cuboidal epithelial cells, which are unusually elongated (Plate 63). These ducts appear to pierce the epithelium of the laryngeal sac (Plate 63). The whole structure is innervated and very well vascularised, having adipose tissue, elastin and collagen fibres dispersed throughout (Plate 64). Measurements taken from the bottom of the notch of the thyroid cartilage to the end of the sac reveal its length to be 11 cm in the juvenile male (Plate 60), 11.3 cm in the juvenile female (Plate 65), 22.5 cm in the adult male (Plate 66) and 28.8 cm in the adult female (Plate 67). The width of the sac decreases posteriorly to give the sac a triangular shape (Plates 59 and 65-67). Measured at their widest points, in the same order as above, the sacs measured, 8.3 cm, 12.2 cm, 15.4 cm and 20.9 cm, respectively.

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Plate 62: Transverse section of the dorsal mucosa of the laryngeal sac of an adult male *B. acutorostrata*. Note muscle interspersed between glands, adipose tissue and blood vessels. (Mag. 100x, VB).

Plate 63: Transverse section of the ventral mucosa of the laryngeal sac of an adult female *B. acutorostrata* showing ducts leading from glands which are lined with elongated, cuboidal epithelial cells and lymphatic tissue below the epidermis (arrow). (Mag. 100x, H/E).

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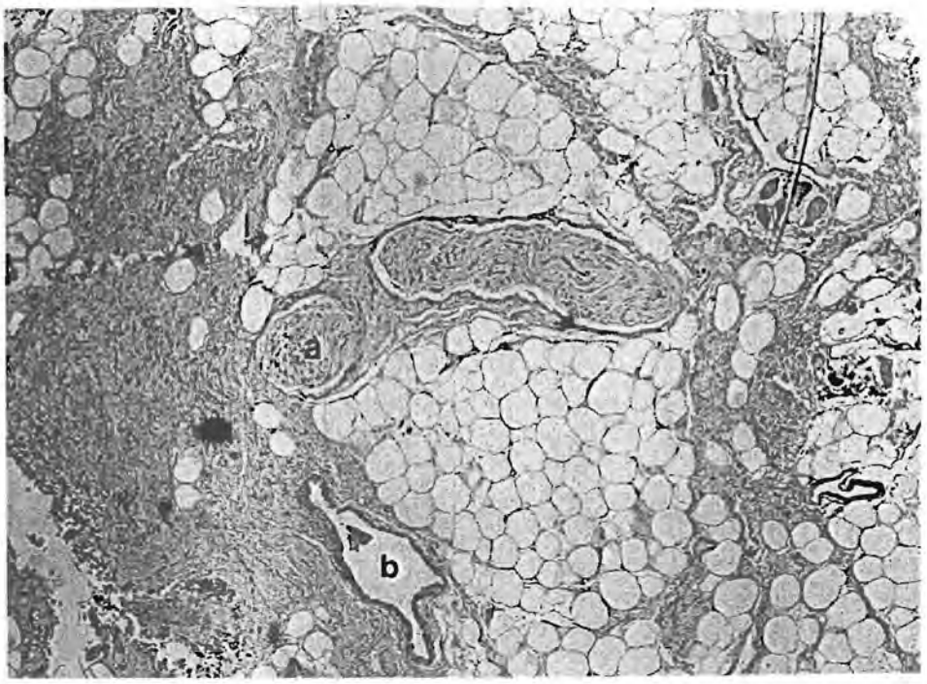

Plate 64: Transverse section of the ventral wall of the laryngeal sac of an adult male *B. acutorostrata* showing (a) large nerves and (b) blood vessels. (Mag. 100x, H/E).

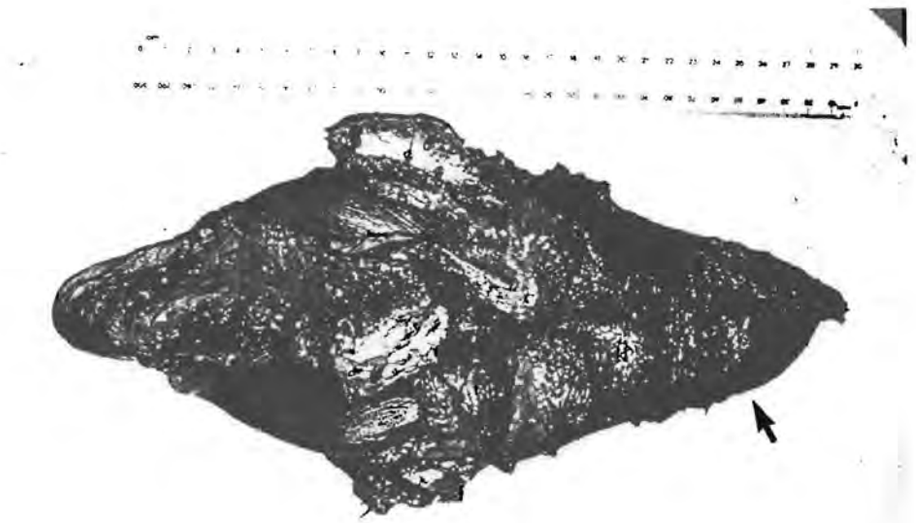

Plate 65: Ventral view of the laryngeal sac (arrow) of a juvenile female *B. acutorostrata*.

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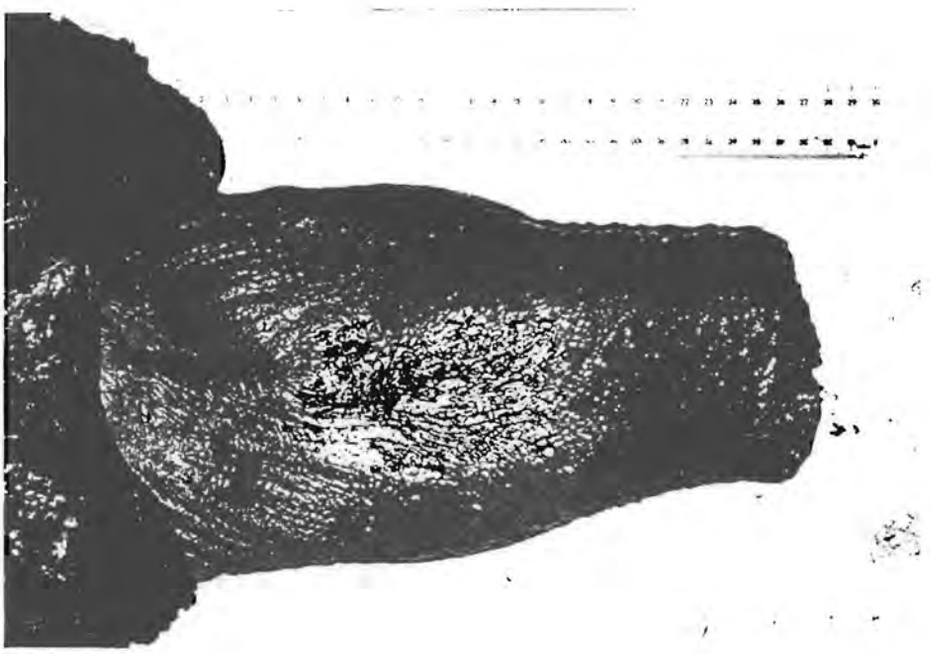

Plate 66: Ventral view of the laryngeal sac of an adult male *B. acutorostrata* (sac turned inside out).

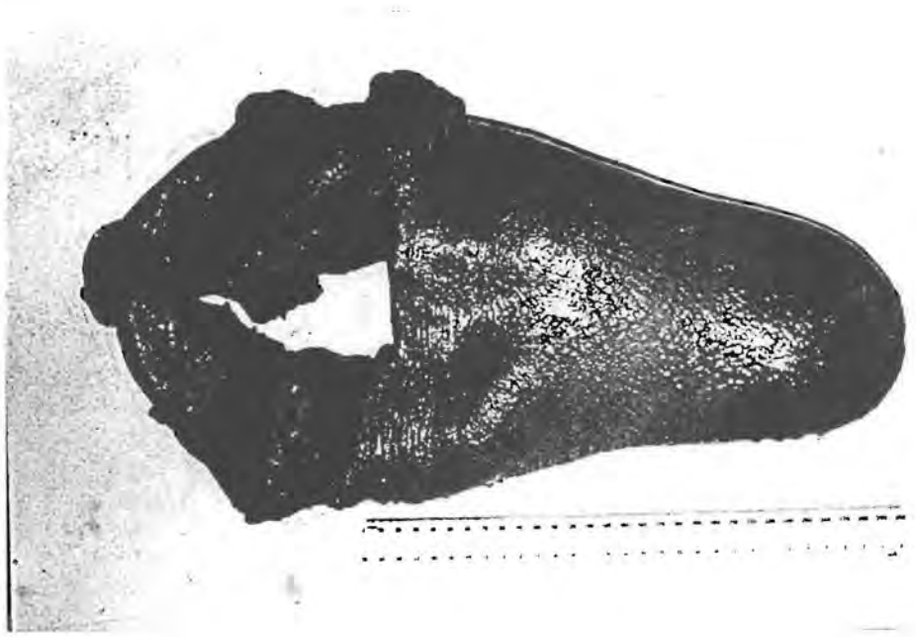

Plate 67: Dorsal view of the laryngeal sac of an adult female *B. acutorostrata* (sac turned inside out).

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3.3 Gross anatomy of *Caperea marginata*

The overall organisation of the laryngeal apparatus of *C. marginata* conforms with that of *B. acutorostrata*, but structural differences do occur, and these are discussed below.

Lengths of the laryngeal apparatus' were measured from the nasopharynx to end of tracheal rings (when present), and ranged from 23.4 cm in the juvenile male (91/27) (Plate 68), 29.2 (Plate 69) to 37.2 cm (Plate 70) in the juvenile females (90/12 and 89/3, respectively) and 60.9 cm in the adult male (93/07) (Plate 71). These measurements are as accurate as possible, bearing in mind that the nasopharynges and tracheal rings were not complete in most of the specimens. However, the complete viscera of the last-mentioned juvenile female (89/3) was available, so that all the tracheal rings remained intact (Plate 70).

The most outstanding character, evident on the ventral wall of the laryngeal apparatus of the juvenile female (90/12), was the presence of part of the aorta (which moves from the left hand to the right hand side of the apparatus) and the ducti arteriosus and ligamentum (Plate 73).

The only adult specimen was procured after the process of autolysis had already begun and unfortunately, when preserved, the apparatus underwent considerable distortion during subsequent fixation in a large drum. Consequently, even though some assumptions could be made with reference to the juvenile specimens, the gross morphological character of the adult larynx of this species was difficult to determine from this specimen.

a) Bones

Unfortunately none of the specimens of *C. marginata* possessed sternum bones, and hyoid bones were only preserved in one of the juvenile females (89/3) (Plate 72).

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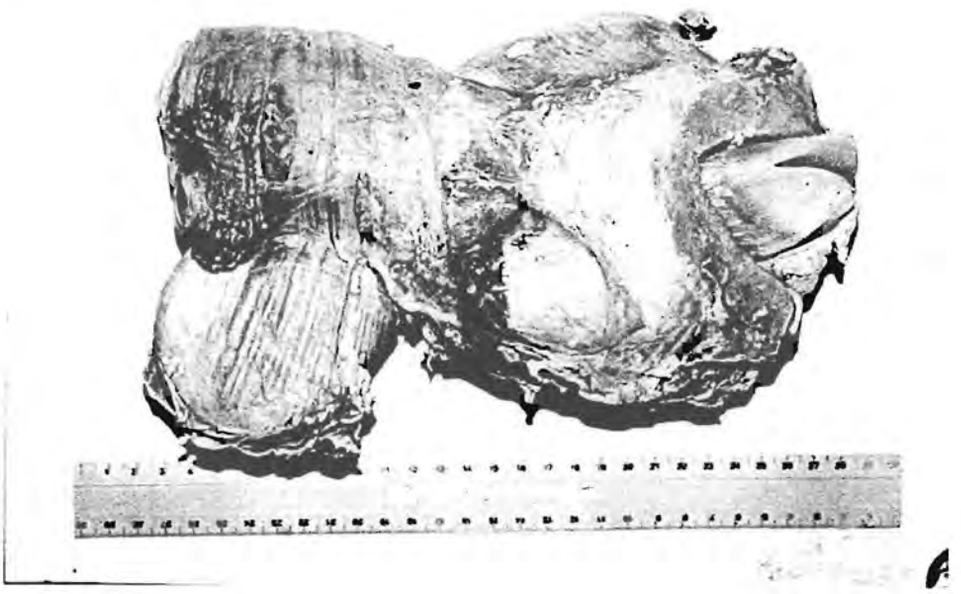

Plate 68: Dorsal view of the laryngeal apparatus of a juvenile male *C. marginata*.

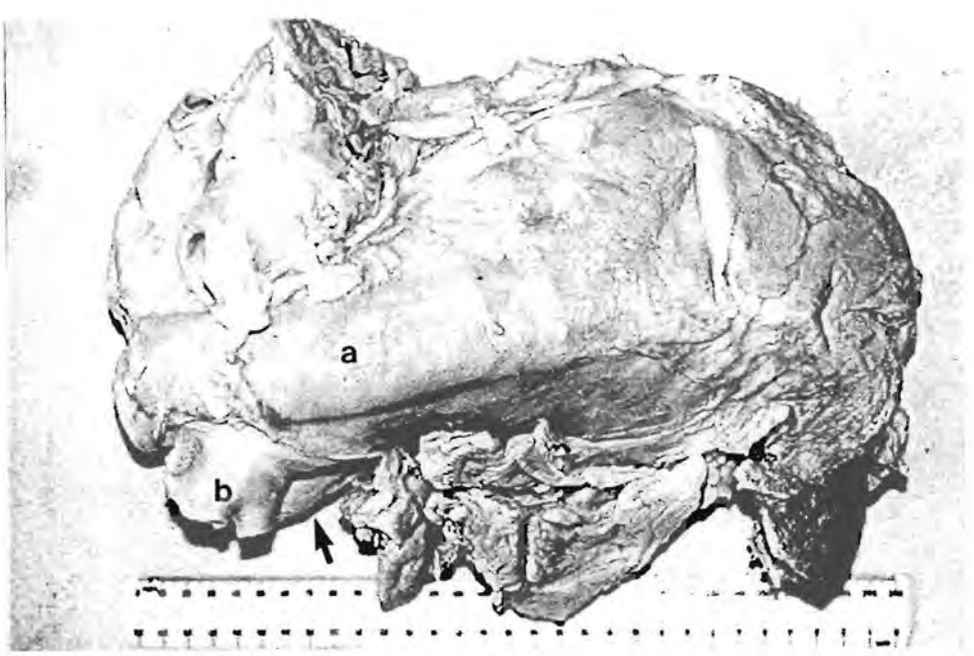

Plate 69: Dorsal view of the laryngeal apparatus of a juvenile female *C. marginata* (# 90/12). Note (a) oesophagus, (b) tracheal rings, (arrow) laryngeal sac.

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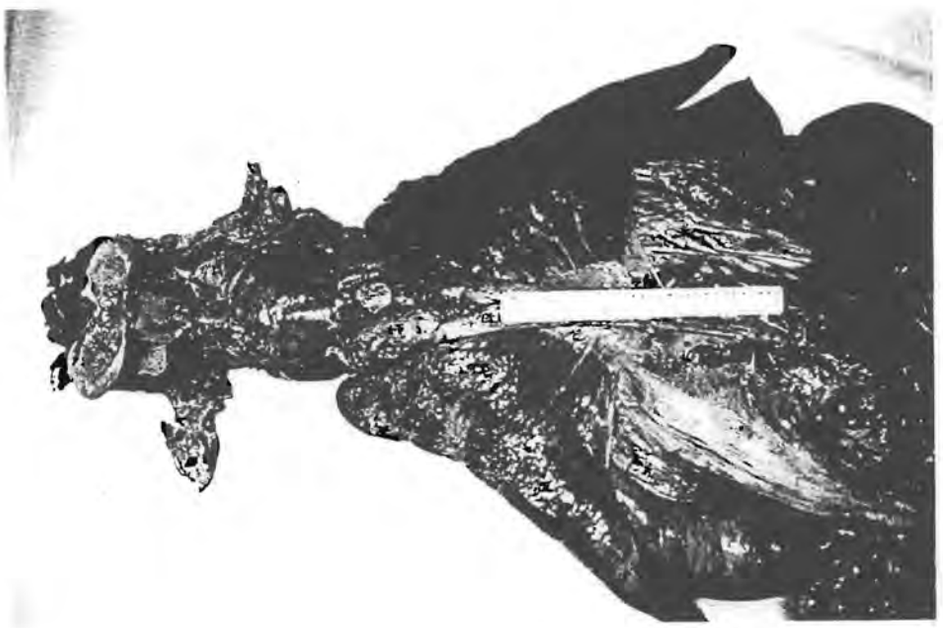

Plate 70: Dorsal view of the laryngeal apparatus of a juvenile female *C. marginata* (# 89/3).

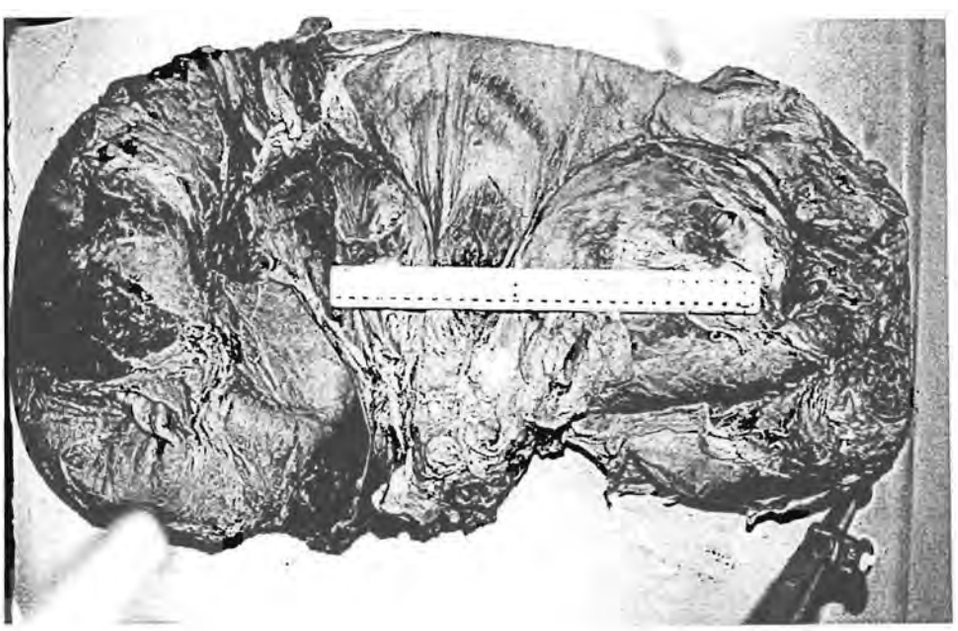

Plate 71: Lateral view of the laryngeal apparatus of an adult male C.marginata (ventral surface facing upwards).

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Plate 72: Ventral view of the hyoid bones attached to the laryngeal apparatus of a juvenile female *C. marginata* (# 89/3).

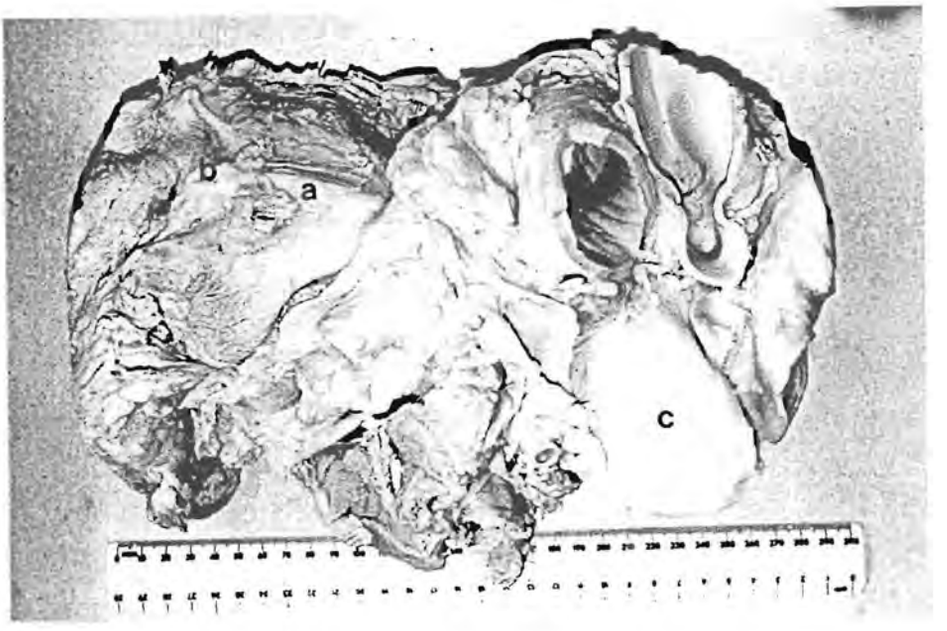

Plate 73: Ventral view of the laryngeal apparatus of a juvenile female *C. marginata* (# 90/12) showing the ducti arteriosus and ligamentum. Note (a) thyroid cartilage, (b) epiglottic protrusion, (c) laryngeal sac.

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i) Hyoid bones

The styloid bones of the juvenile female (89/3) are articulated with the os hyoides via small interconnecting pieces of cartilage, which are attached on each side of the posterior "v" of the os hyoides (Plate 74). The cartilage pieces themselves are not contiguous with either the os hyoides or the styloid bones and are attached to both only by small clumps of soft connective tissue, measuring approximately 3 cm in length.

The styloid bones are curved and lie attached to the apparatus in the form of horizontal "s"-shapes. The measurements of the various bones are given in Table 5.

b) Cartilages

The single thyroid, cricoid, epiglottic and the paired arytenoid cartilages are present in *C. marginata* and the positioning of these cartilages in the laryngeal apparatus of this species correlates with the positioning of these cartilages in *B. acutorostrata*. As in *B. acutorostrata*, the softness and flexibility of the various cartilages is a notable characteristic. The measurements of the various cartilages are given in Table 5.

i) Thyroid cartilage

The thyroid cartilage itself is soft, but is much sturdier compared to the extremely flexible thyroid cartilages found in *B. acutorostrata*. Distinct tubercles are present and in one of the juvenile females (89/3), form a distinctive shape (Plate 75). An anterior view of this specimen's cartilage shows that the tubercles possess dorsal cartilaginous peaks, which continue basally as two forward/ventral facing protrusions (Plate 75). All the specimens of this species possess a distinct ridge on the mid-ventral line of the thyroid cartilage which seems to provide attachment for the epiglottal protrusion (Plate 76).

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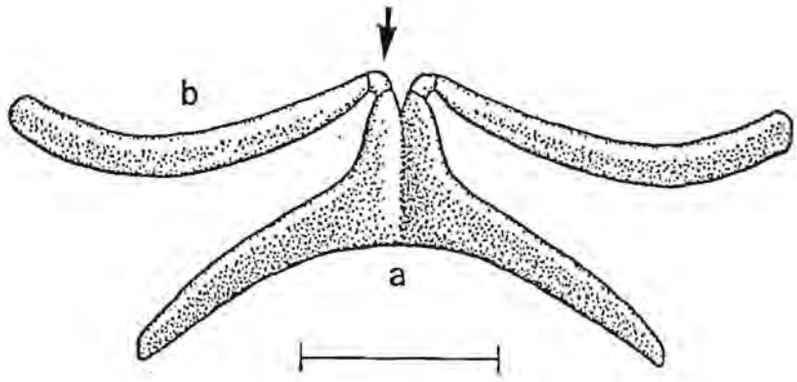

Plate 74: Ventral view of (a) the os hyoides and (b) the styloid bones of a juvenile female *C. marginata* (# 89/3) showing the cartilaginous pieces connecting the os hyoides to the styloid bones (arrow). Note scale bar = 10 cm.

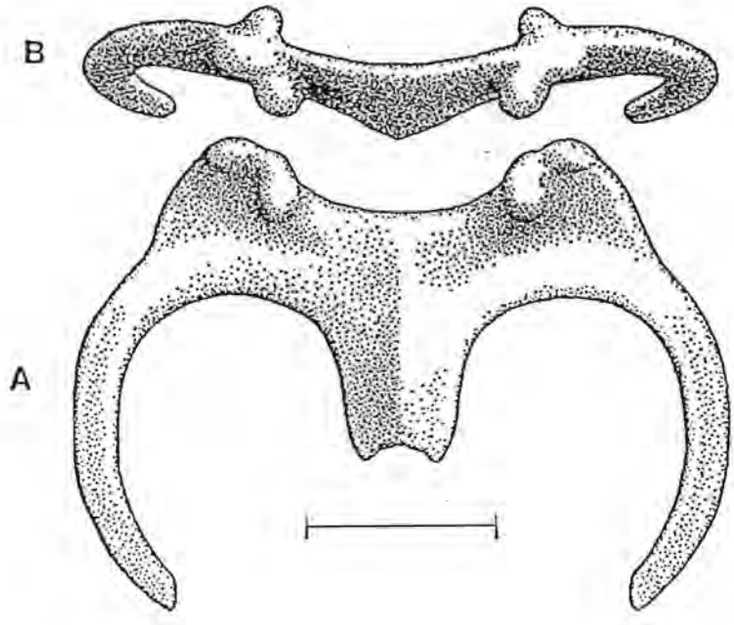

Plate 75: (A) Ventral view and (B) top view of the thyroid cartilage of a juvenile female *C. marginata* (# 89/3) showing distinct tubercles. Note the distinctive shape of the tubercles. Note scale bar = 5 cm.

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Table 5 Measurements associated with the laryngeal apparatus of *C. marginata*.

Cartilage/Bone Measurements (cm) (specimen #)		Juvenile ♀ (89/3)	Juvenile ♀ (90/12)	Juvenile ♂ (91/27)	*Adult ♂ (93/07)
Entire apparatus	Total length	37.2	29.2	23.4	60.9
Thyroid cartilage	Total length	11.7	13.7	12.5	25.0
	Body width	12.4	14.1	11.0	30.0
Cricoid cartilage	Length	10.4	11.3	9.9	18.2
	Width	9.4	9.5	9.5	21.3
Epiglottic cartilage	Total length	8.5	7.9	4.4	15.2
Arytenoid cartilages	Ave total length	15.2	14.5	15.2	28.2
Os hyoides	Length cornua LHS	22.6	-	-	-
	Length cornua RHS	tip cut	-	-	-
	Width of body - measured from bottom of "v" notch	3.7	-	-	-
Styloid bones	Total length (LHS)	19.5	-	-	-
	Total length (RHS)	21.0	-	-	-
	Average width - at thickest region	2.1	-	-	-

* Deformed character of specimen resulted in measurements differing from one side of a cartilage/bone to another, therefore, where applicable, averages are quoted.

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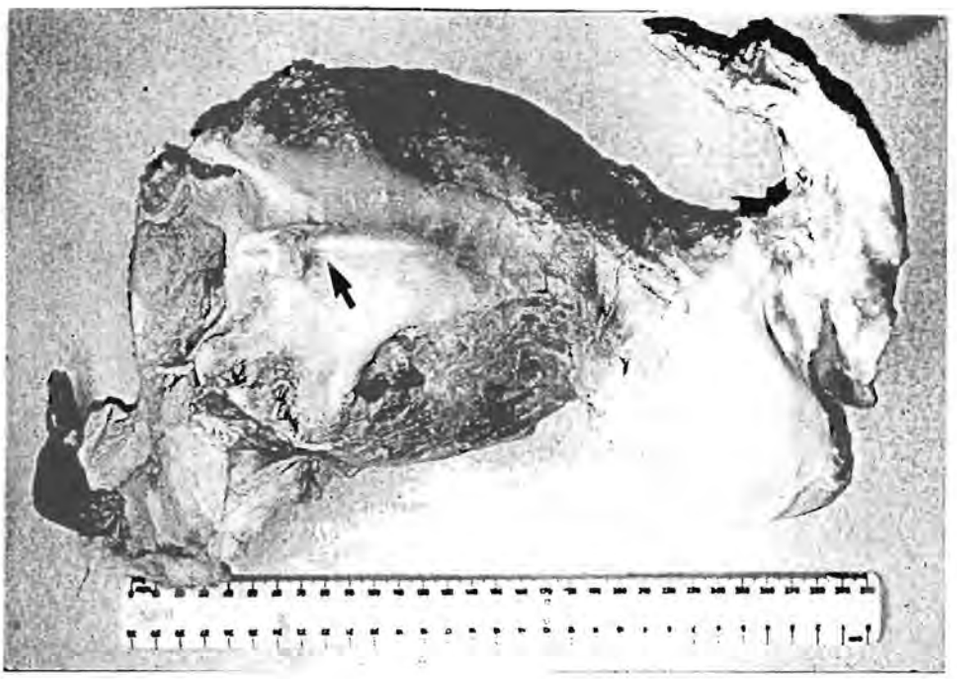

Plate76: Ventral view of the thyroid cartilage on the ventral surface of the laryngeal apparatus of a juvenile female *C. marginata* (# 90/12). Note distinct ridge along the mid-line of the thyroid cartilage being attached to the epiglottic protrusion (arrow).

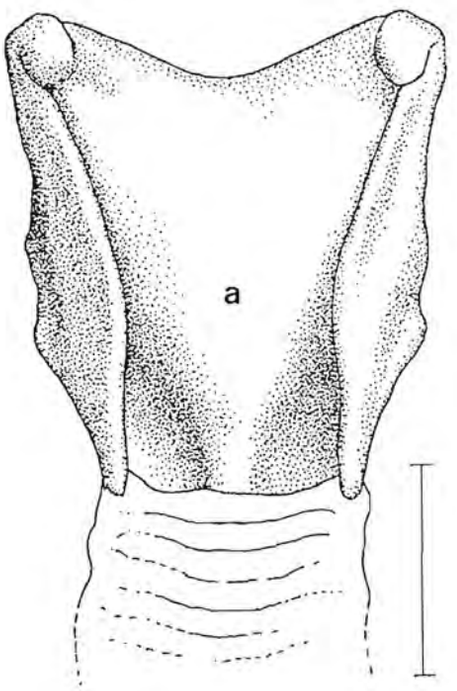

Plate 77: Ventral view of the cricoid cartilage of a juvenile female *C. marginata* (# 90/12) attached to the tracheal rings. Note (a) the distinct cushion on the anterior ventral surface of the cricoid body. Note scale bar = 5 cm.

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ii) Cricoid cartilage

The general structure and situation of the cricoid cartilage in *B. acutorostrata* is reflected in *C. marginata*, forming a cartilaginous plate dorsally and being incomplete ventrally (Plate 77). The major difference in the cricoid cartilage of *C. marginata* is that although the body does continue laterally and ventrally (forming raised arches) no distinct cornua are present (Plate 78). The "cushion" on the inside, anterior region of the body is well developed, continuing posteriorly as a ridge towards the tracheal rings (Plate 77). In this species, the middle region of the dorsal surface of the cricoid plate is indented (Plate 79). Foramina are also present in the posterior region. In the juvenile male, the cricoid cartilage is continuous with the first tracheal ring, while in the two juvenile females the cricoid is continuous with the third (90/12) and fourth (89/3) tracheal rings, respectively. Unfortunately in the adult male, the tracheal rings as well as the cricoid cartilage's association with them was indeterminable.

iii) Epiglottic cartilage

As in *B. acutorostrata*, a portion of the epiglottic cartilage protrudes through the ventral, muscular surface of the laryngeal apparatus, anterior to the thyroid cartilage (Plate 80). This protrusion was initially thought to be an extension of the thyroid cartilage, forming a "peak" between the tubercles. On closer inspection it was evident that this "peak" is in fact the epiglottic protrusion which is attached by connective tissue to the mid-ventral ridge of the thyroid cartilage (Plate 76). However, in the adult male it is evident that the thyroid and the epiglottic cartilage are continuous with each other, a union which presumably occurs as the animals age (Plate 81).

The epiglottic cartilage is reduced compared to that of *B. acutorostrata*. The comparable hood, which is tightly associated with the arytenoid bodies, only extends to the top of these bodies and is not as conspicuous as that of *B. acutorostrata* (Plate 82). A middle ridge and aryteno-epiglottic folds are barely discernible (Plate 83). The shape of the cartilage can be compared to a spoon, i.e. anteriorly it is convex, from a ventral perspective, continuing posteriorly into a broad rod

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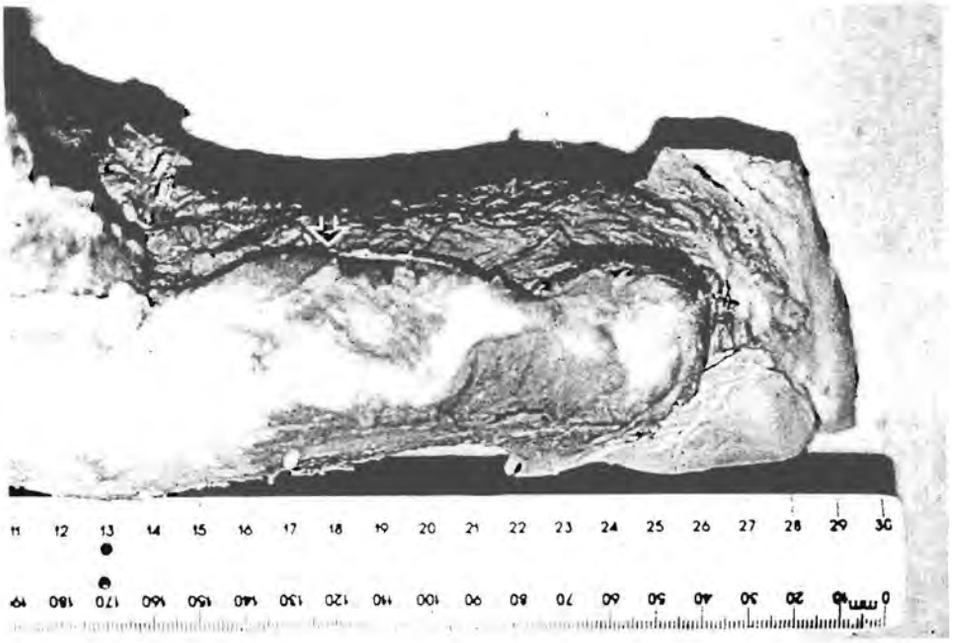

Plate 78: Lateral view of the cricoid cartilage of a juvenile female *C. marginata* (# 90/12) showing the lack of distinct ventral cornua (arrow).

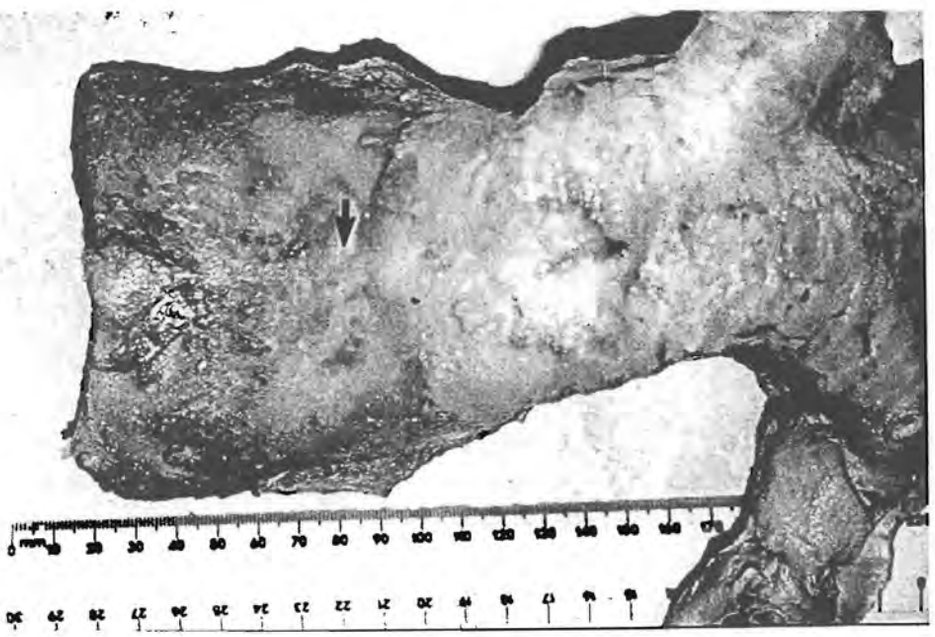

Plate 79: Dorsal view of the cricoid cartilage attached to the tracheal rings of a juvenile female *C. marginata* (# 89/3) indicating the indented nature of the middle region of the cricoid cartilage (arrow).

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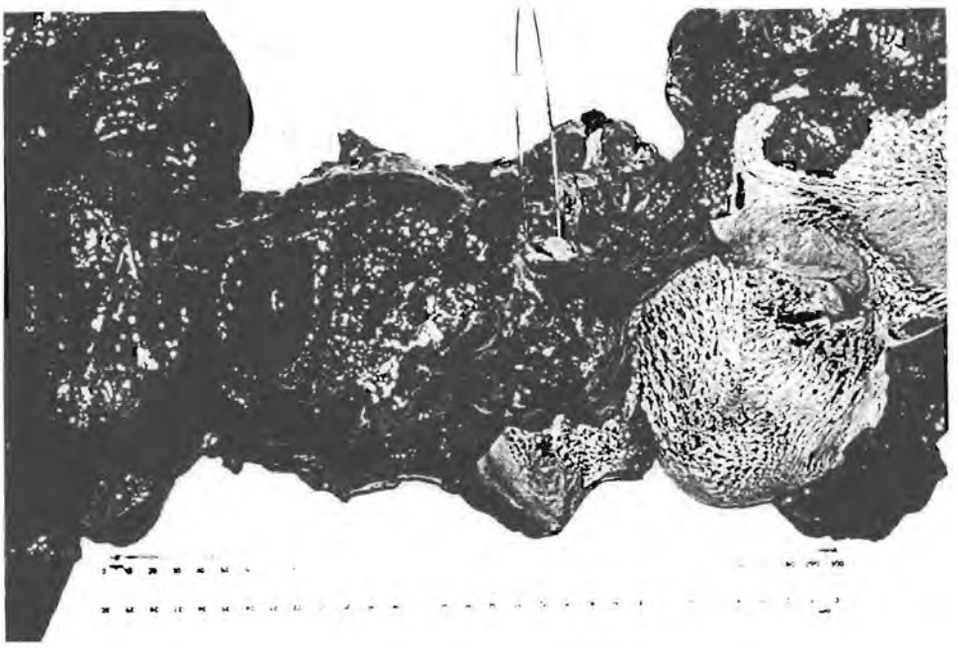

Plate 80: Ventral view of the laryngeal apparatus of a juvenile female *C. marginata* (# 89/3) with the epiglottic protrusion being indicated by the forceps.

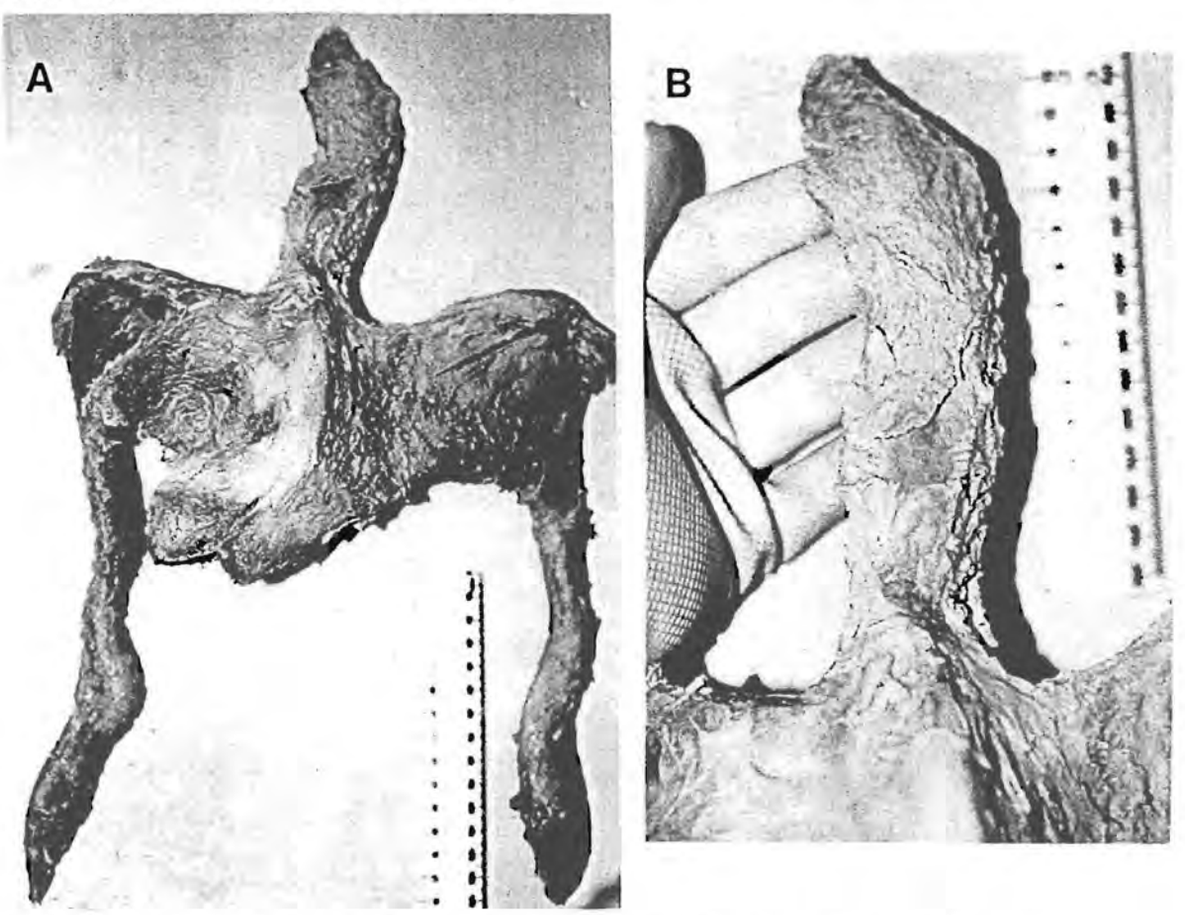

Plate 81: (A + B) Ventral view of a thyroid and epiglottic cartilage of an adult C.marginata (distorted during preservation). Note the continuation of the thyroid cartilage with the epiglottic cartilage (tip of the epiglottic protrusion has been cut off).

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Plate 82: Lateral view of (a) the anterior region of the epiglottic cartilage and (b) the arytenoid bodies of a juvenile female *C. marginata* (# 90/12). Note reduced size of the epiglottic "hood".

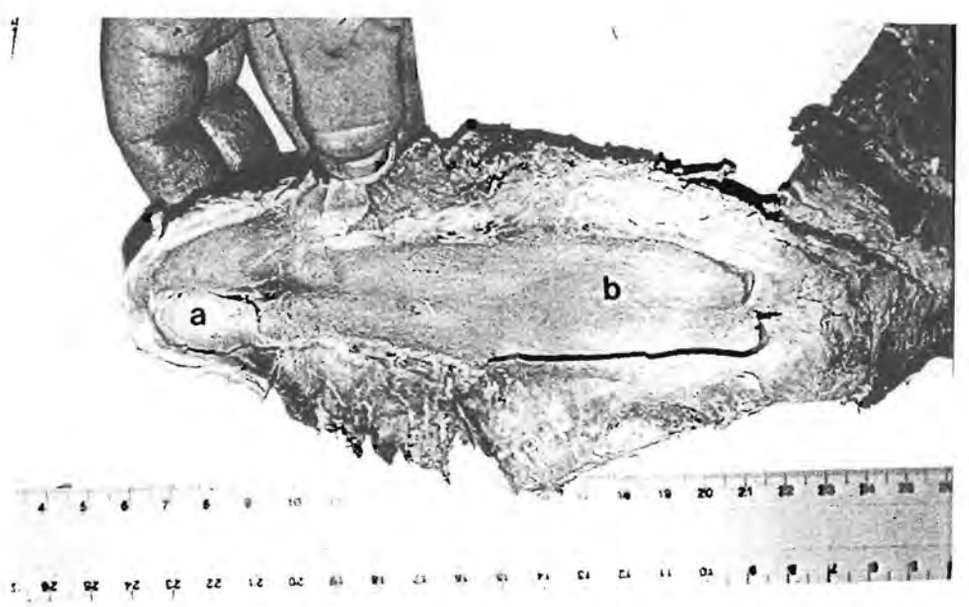

Plate 83: Dorsal view of (a) the epiglottis and (b) the trachea (arytenoid cartilages have been removed) of a juvenile male *C.marginata*. Note the lack of a middle ridge and of distinct aryteno-epiglottic folds of the epiglottis.

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(which is slightly convex) (Plate 84), which eventually becomes continuous with the thyroid cartilage in the adult male. The anterior and posterior margins of the cartilage are slightly extended, forming small wings (Plate 84).

iv) Arytenoid cartilages

The arytenoid cartilages of *C. marginata* are more compact in structure than those of *B. acutorostrata* i.e. the arytenoid bodies are continuous posteriorly with the processi vocales so that on first inspection there is no clear point of separation between the two aforementioned components of these cartilages (Plate 85). The tips of the arytenoid bodies are broad and are laterally flattened. In the juveniles, the right hand side tip generally projects 0.6 cm further than the left hand side tip (Plate 82) and in the adult, the left hand side tip projects 2.2 cm further than the right hand side tip. The crescentic "wings" are reduced in size and no connective tissue "lips" are present (Plates 82 and 85). The anterior tips of the arytenoids are firmer compared to those found in *B. acutorostrata* and the inner surface of the arytenoid bodies are pitted and have a rough texture, reminiscent of the tongue of a cat (Plate 86). This coarse epithelium is covered with crypts and joins the bodies on their interior, medial sides. The processi vocales are joined posteriorly by connective tissue and they are not continuous with the dorsal wall of the laryngeal sac, but they do provide the connection between the laryngeal sac and the rest of the apparatus (Plate 87). Approximately three-quarters of the way down the ventral side of the processi vocales, there is a small opening (approximately 1 cm in length) which leads into the laryngeal sac, thus making the opening between the arytenoid wings the connection between the trachea and the laryngeal sac (Plate 88).

Once dissected out, each arytenoid cartilage seemed to consist of two components which merge to form the arytenoid bodies i.e. the ventral margins of the arytenoid bodies continue posteriorly as narrow rods which are closely associated, but separate, to the interior margin of the processi vocales. The processi vocales and processi musculares continue anteriorly along their interior margins to form the dorsal margins of the arytenoid bodies (Plate 89).

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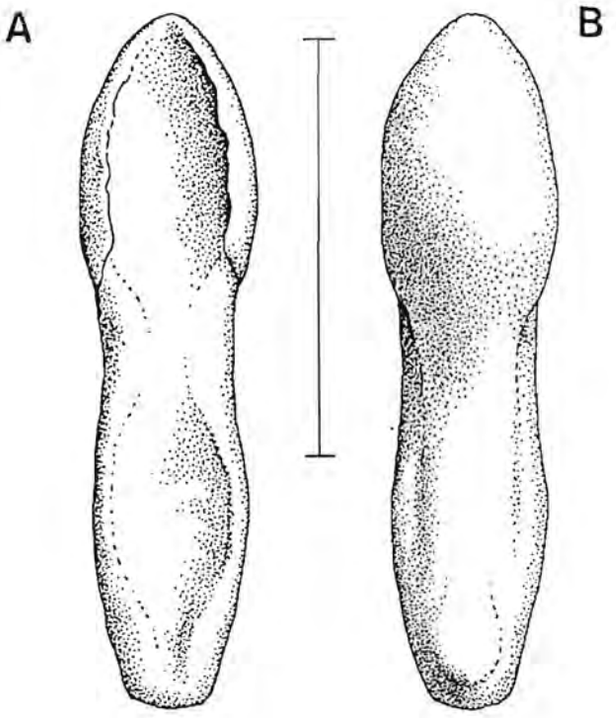

Plate 84: Dorsal (A) and ventral (B) view of the epiglottic cartilage of a juvenile female *C. marginata* (# 89/3). Note scale bar = 5 cm.

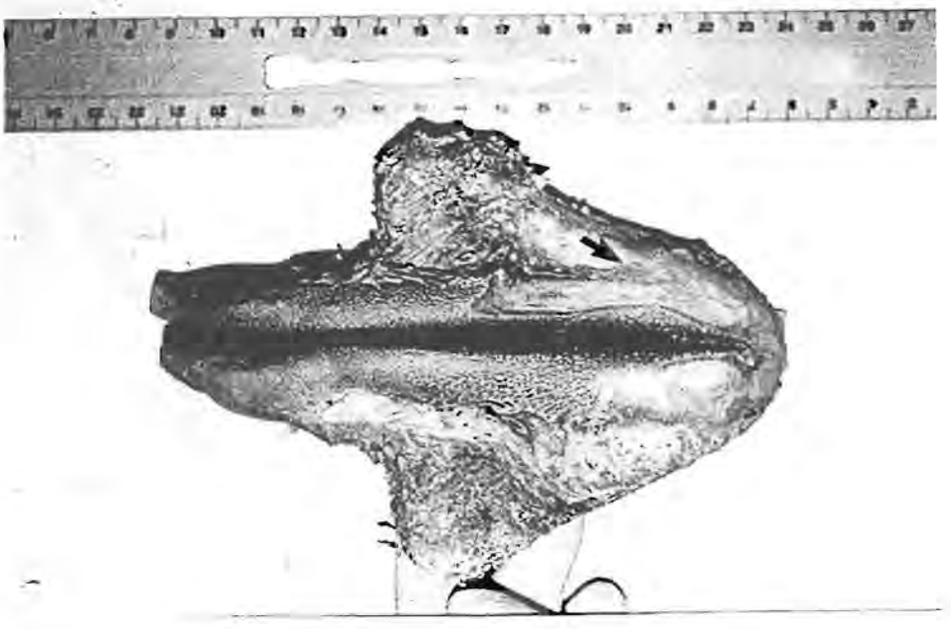

Plate 85: Ventral view of the arytenoid cartilages of a juvenile male *C. marginata*. On first inspection there is no clear point of separation between the arytenoid bodies and the processi vocales (arrow).

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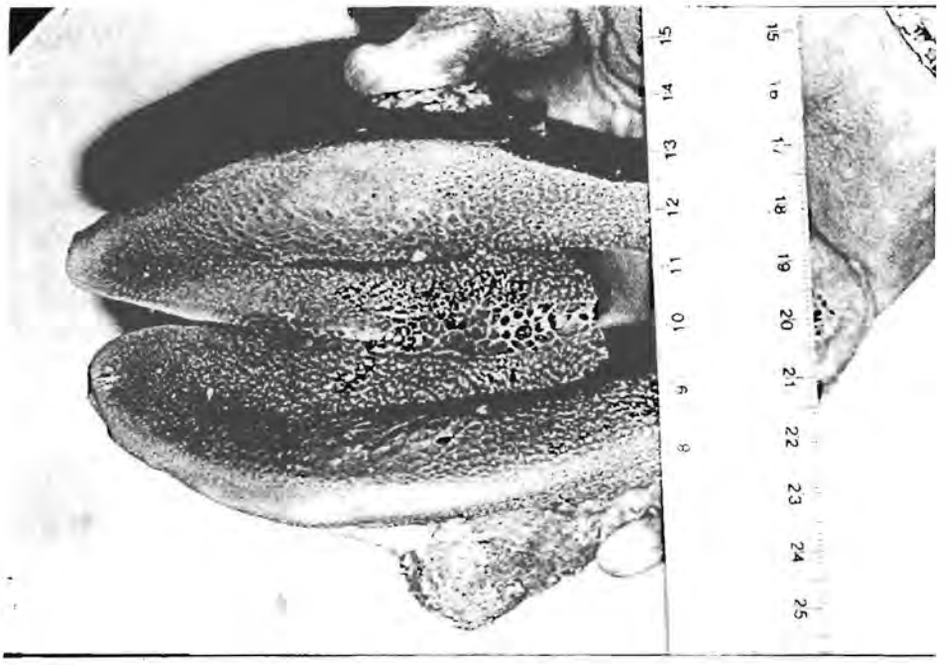

Plate 86: Ventral view of the arytenoid cartilages of a juvenile male *C. marginata*. Cartilages have been parted to expose the pitted, rough texture of the inner surface of the mucosa.

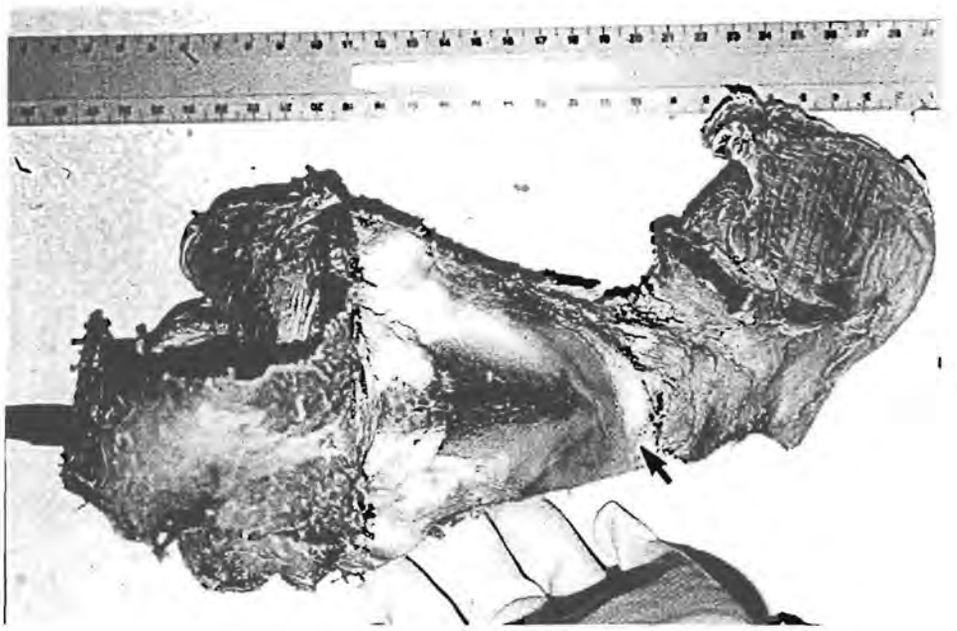

Plate 87: Dorsal view of the laryngeal apparatus of a juvenile male *C. marginata*. The oesophagus has been lifted to expose the arytenoid cartilages which are not continuous with the dorsal wall of the laryngeal sac, but act as a connection between the laryngeal sac and the rest of the apparatus (arrow).

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Plate 88: Ventral view of the arytenoid cartilage of a juvenile female *C. marginata* (# 90/12) indicating the opening to the laryngeal sac (arrow).

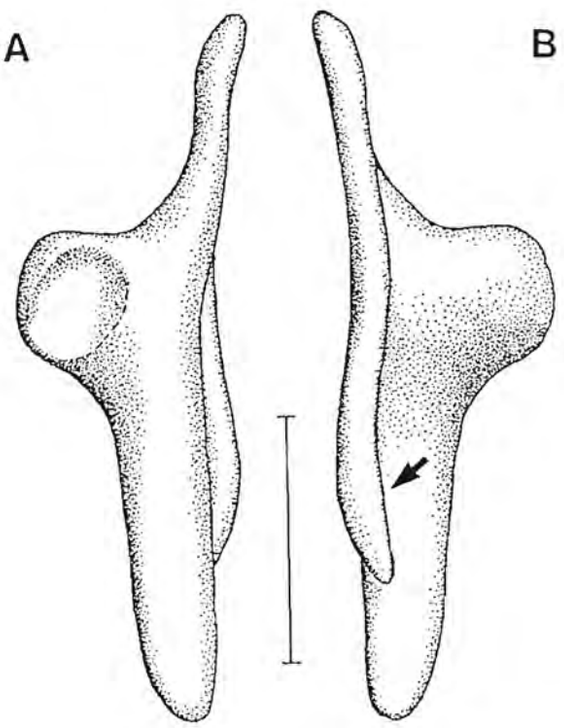

Plate 89: Dorsal (A) and ventral (B) views of the arytenoid cartilage of an adult *C. marginata* showing the separation of the cartilage into two components (arrows) which fuse to form the arytenoid bodies. Note scale bar = 10 cm.

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c) Myology of the laryngeal apparatus

The musculature of the laryngeal apparatus of *C. marginata* is similar to that described for *B. acutorostrata* by Benham (1901), so the terminology used by this author for the major muscles of the laryngeal apparatus is applied to this description (Plates 27a and 27b).

As already mentioned, sternum bones, complete nasopharynges and, consequently, their surrounding musculature were not recorded in any of the *C. marginata* specimens. Also, the condition of the specimens was such that the insertions/terminations of the various extrinsic and intrinsic muscles were not clearly defined.

i) Extrinsic muscles

The only extrinsic muscle identified was found in the adult male. The **sterno-thyroid** muscle was found on the right hand side body of the thyroid cartilage, situated approximately 2 cm from the interior, right hand side arch of the thyroid cartilage (Plate 90).

ii) Intrinsic muscles

The thyro-cricoid, the crico-thyroid, the accessory crico-thyroid (Plate 91) and the crico-arytenoid muscles (Plate 92), being the only discernible muscles, seemed to be present in the same aspect as in *B. acutorostrata*. The accessory crico-thyroid muscles seemed to continue to form the muscular base of the epiglottis. All of the identified muscles were skeletal muscles, being well vascularised and innervated (Plates 93 and 94).

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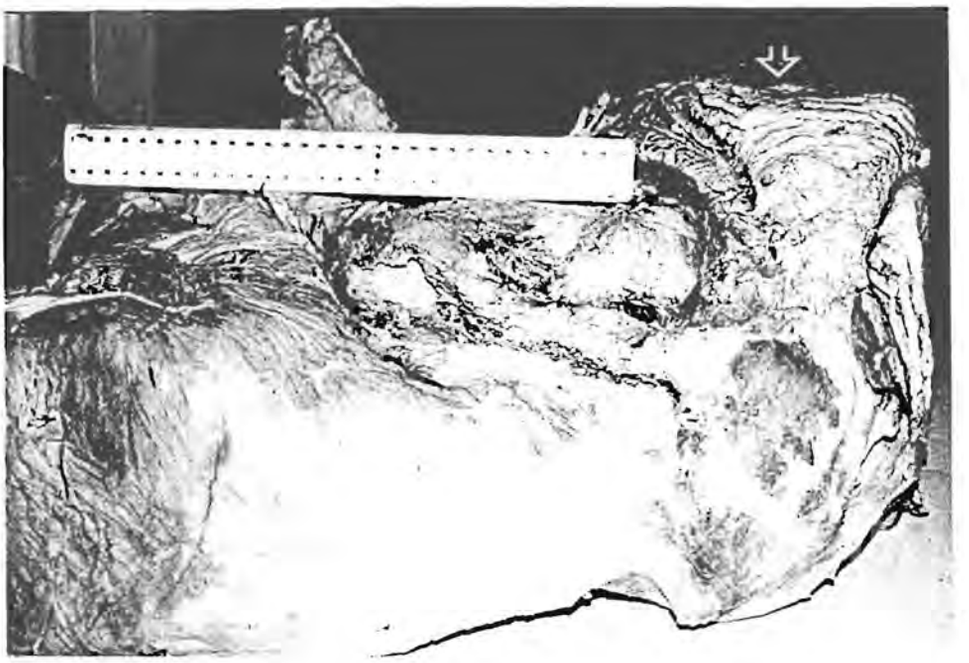

Plate 90: Lateral view of the laryngeal apparatus of an adult male *C. marginata*. Note the sterno-thyroid muscle above the thyroid cartilage (arrow).

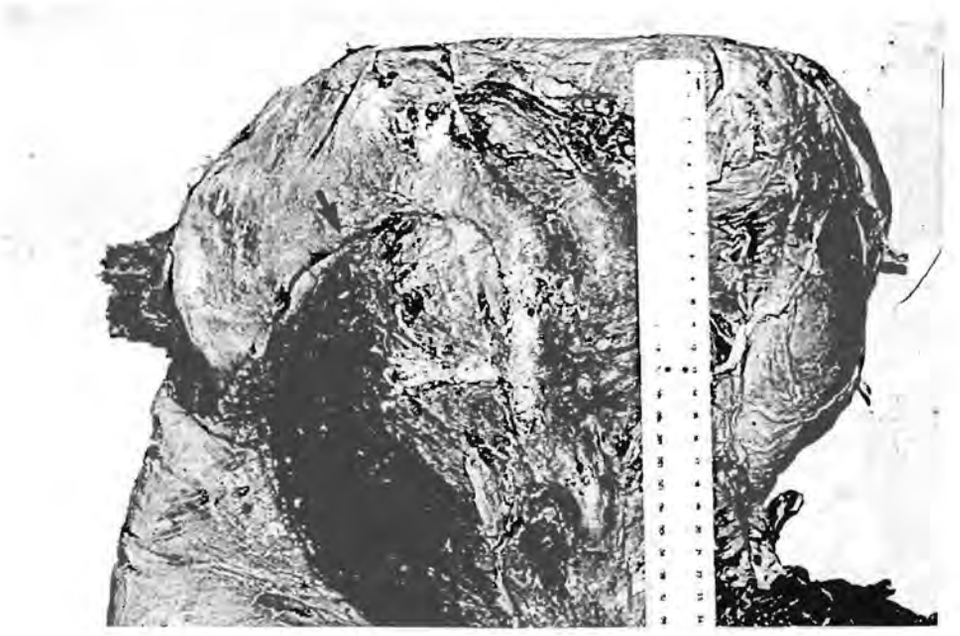

Plate 91: Lateral view of the laryngeal apparatus of an adult male *C. marginata* exposing the accessory crico-thyroid muscle between the thyroid cornu arch (arrow) (ventral surface of the laryngeal apparatus is on the left hand side of the Plate).

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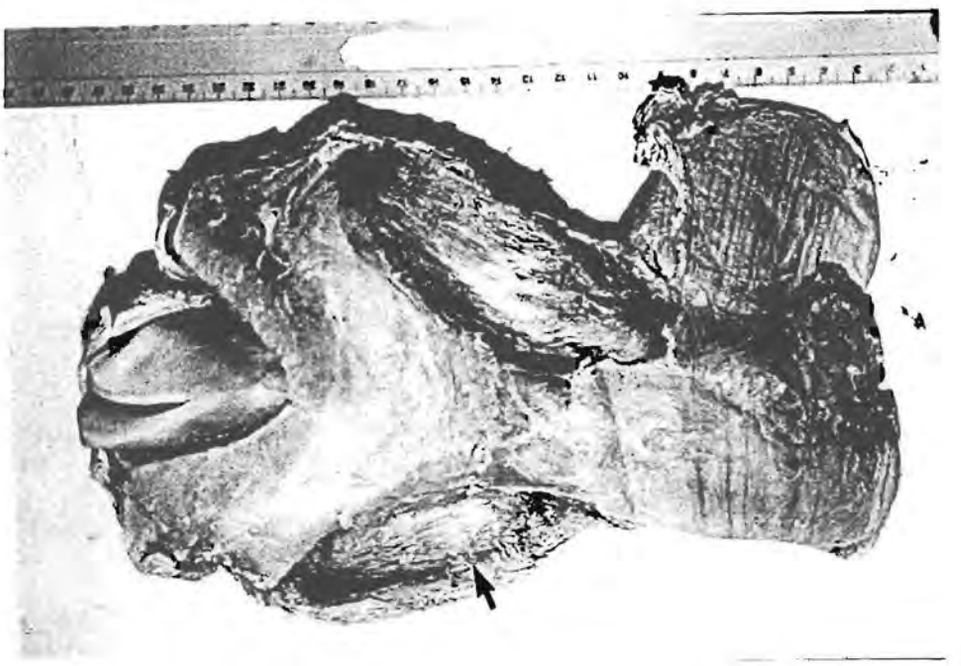

Plate 92: Dorsal view of the laryngeal apparatus of a juvenile male *C. marginata* indicating the position of the crico-arytenoid muscles (arrows) (muscles have been removed).

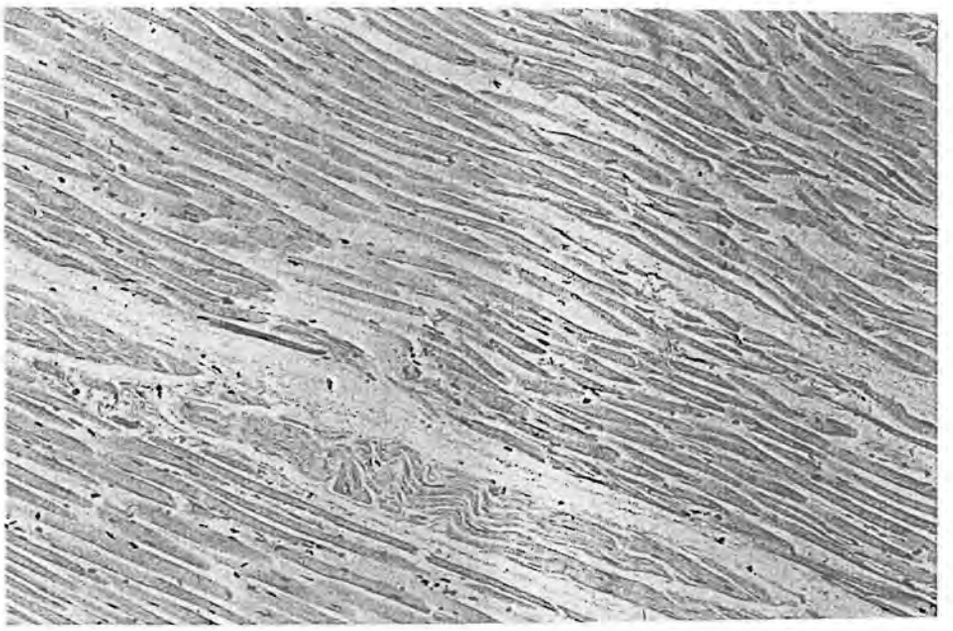

Plate 93: Longitudinal section of the crico-arytenoid muscle of a juvenile female *C. marginata* (# 89/3) showing typical skeletal muscle as well as the presence of nerves. (Mag. 100x, H/E).

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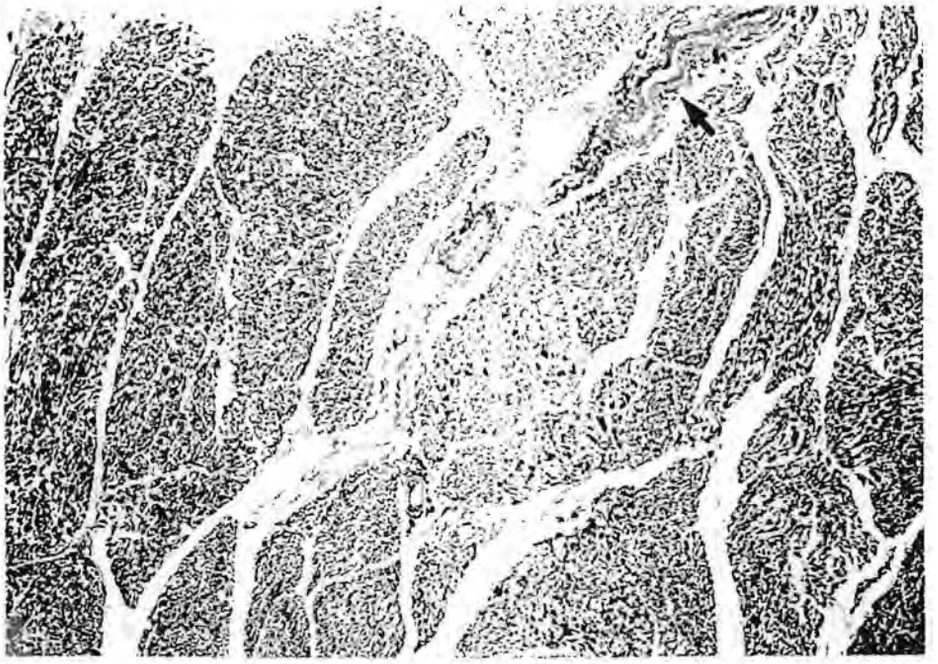

Plate 94: Transverse section of the thyro-cricoid muscle of a juvenile female *C. marginata* (# 89/3) showing skeletal muscle bundles interwoven with connective tissue and spiralling blood vessels (arrow). (Mag. 25x, MT).

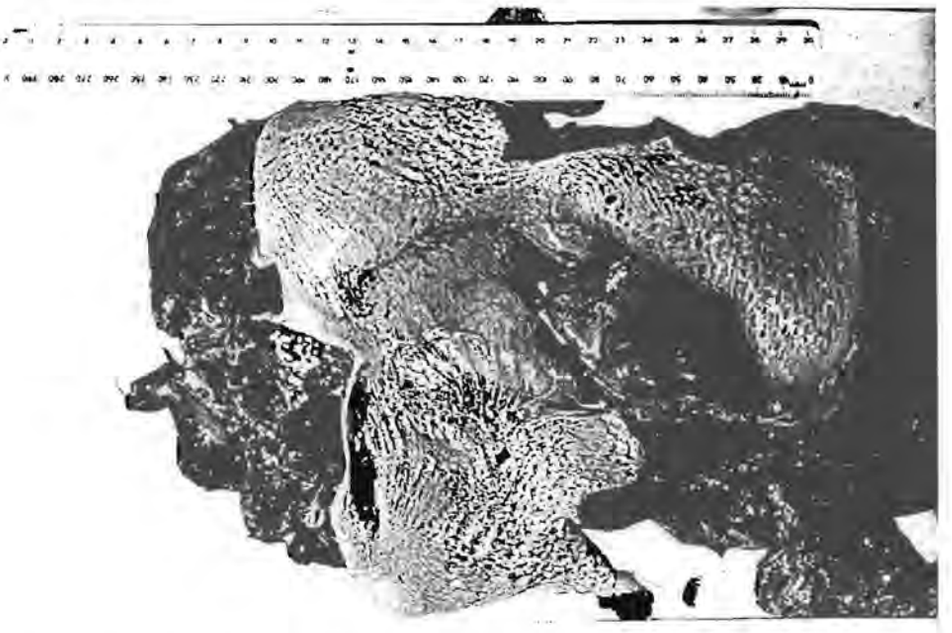

Plate 95: Remnants of the nasopharynx of a juvenile female *C. marginata* (# 89/3). Note the abundance of crypts.

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3.4 Histology of the laryngeal apparatus of *C. marginata*

Unfortunately, the nasopharynx had been removed from three of the four *C. marginata* specimens during their initial dissection, but one female specimen (89/3) did possess remnants of the nasopharynx, upon which an abundance of crypts occurred on either side (Plate 95). Histological analysis revealed that these crypts open into the nasopharyngeal space, providing avenues for secretions from the many serous glands present. Glands are also scattered throughout the underlying skeletal muscle layer, which is innervated and vascularised (Plate 96). The nasopharyngeal epithelial layer was only discernible by the presence of a basement membrane which was succeeded by a collagenous connective tissue layer. The posterior nare occurs at the posterior end of the nasopharynx, directly anterior to the epiglottis (Plate 97). The lining of the nare is a soft, membranous flap of skin - which, when pulled away (in an anterior direction) from the epiglottis, reveals the narial opening (Plate 97). The lining of the margin of the opening is smooth, having a slightly "gathered" nature with no crypts occurring on its exterior surface. This area is well innervated, glands occur and are surrounded by skeletal muscle bundles (Plate 98).

The epiglottic mucosa is smooth with many tiny holes dispersed throughout. Histologically the epithelium is made up of stratified squamous cells. The tip of the epiglottic "hood" is white in colour and is composed predominantly of adipose tissue (Plate 99). Blood vessels are strangely arranged in coils and richly supply the region, reaching directly beneath the epithelium (Plate 99). The Masson's trichrome stain revealed cytoplasm-filled adipocyte-type cells occurring throughout the mucosa (Plate 100).

The pharyngeal mucosa is of a grey colour, being more pronounced in the juvenile male specimen, although no melanocytes were seen during histological analysis (Plate 101). The pharyngeal epithelium is made up of stratified squamous cells, most of which had pulled away from the rest of the tissue during preparation (Plate 102). In one of the juvenile females, (89/3), besides the peeling away of the epidermal layer being evident, two distinct groups of elongated cells occurred on the left and right hand sides of the pharynx (Plate 103). Masses of dense connective tissue with skeletal muscle occur beneath the epithelium (Plate 104) and very large nerves in close apposition to many blood vessels occur throughout the pharyngeal region. Many crypts line the

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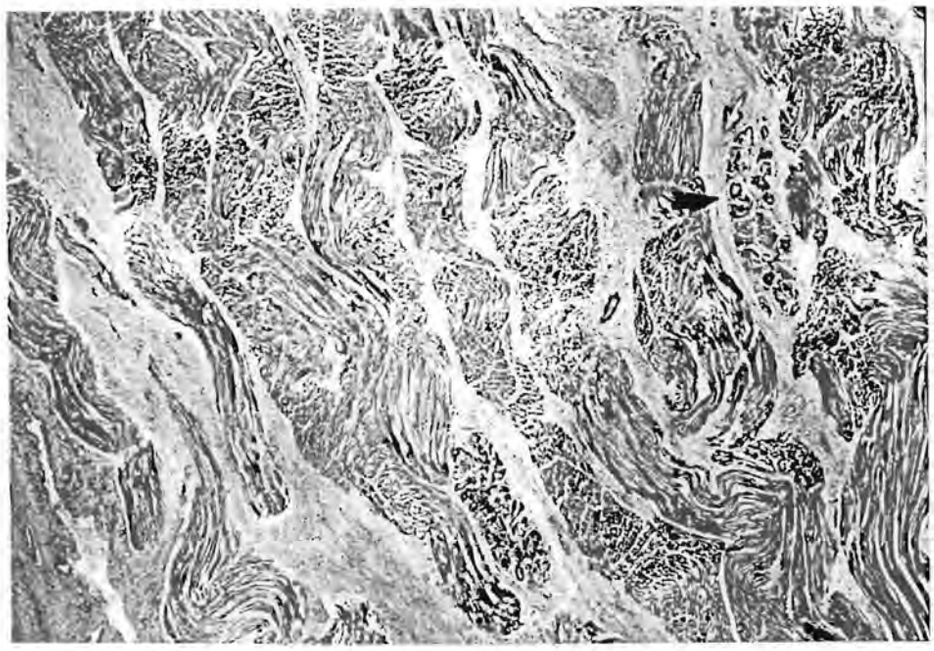

Plate 96: Longitudinal section of the nasopharynx of a juvenile female *C. marginata* (# 89/3). Note scattering of glands throughout skeletal muscle (arrow). (Mag. 25x, H/E)

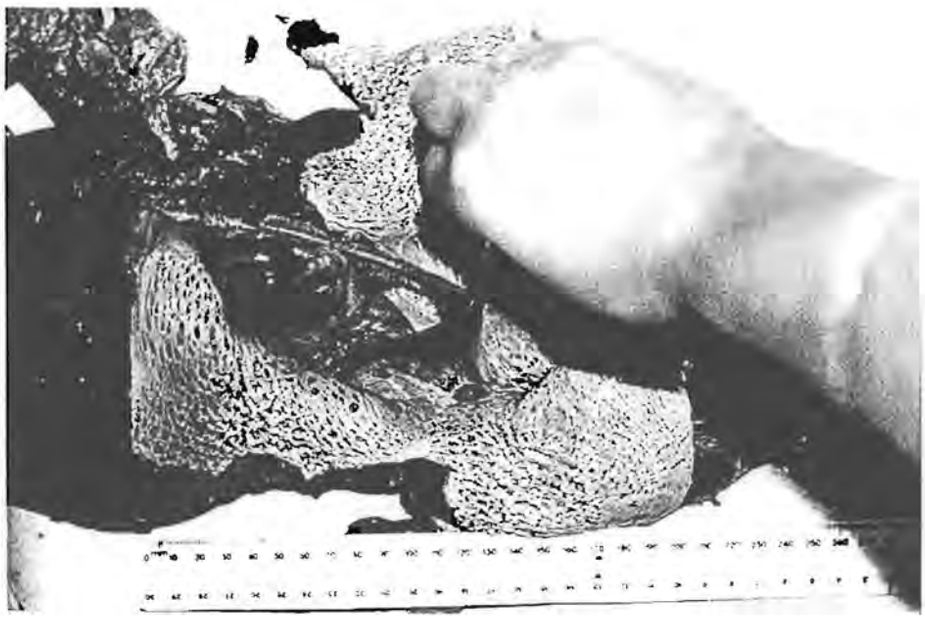

Plate 97: Dorsal view of the laryngeal apparatus of a juvenile female *C. marginata* (# 89/3). Posterior nare is pulled back to reveal the narial opening (anterior to the epiglottis).

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Plate 98: Transverse section of the margin of the narial opening of a juvenile female *C. marginata* (# 89/3). Note the muscular layer which surrounds the glands and nerves. (Mag. 50x, MT).

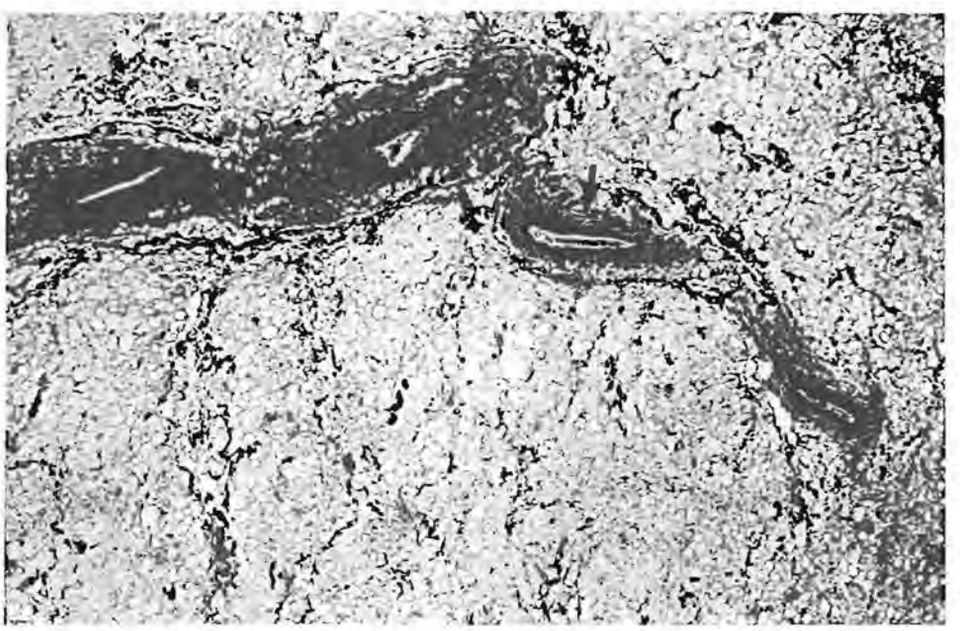

Plate 99: Transverse section of the epiglottic "hood". Note spiralling blood vessels (arrow) coursing through the adipocytes. (Mag. 25x, MT).

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Plate 100 Longitudinal section of the epiglottic "hood" mucosa of a juvenile female *C. marginata* (# 89/3). Cytoplasm-filled adipocyte-type cells (reddish-brown) are scattered throughout the mucosa . (Mag. 100x, MT).

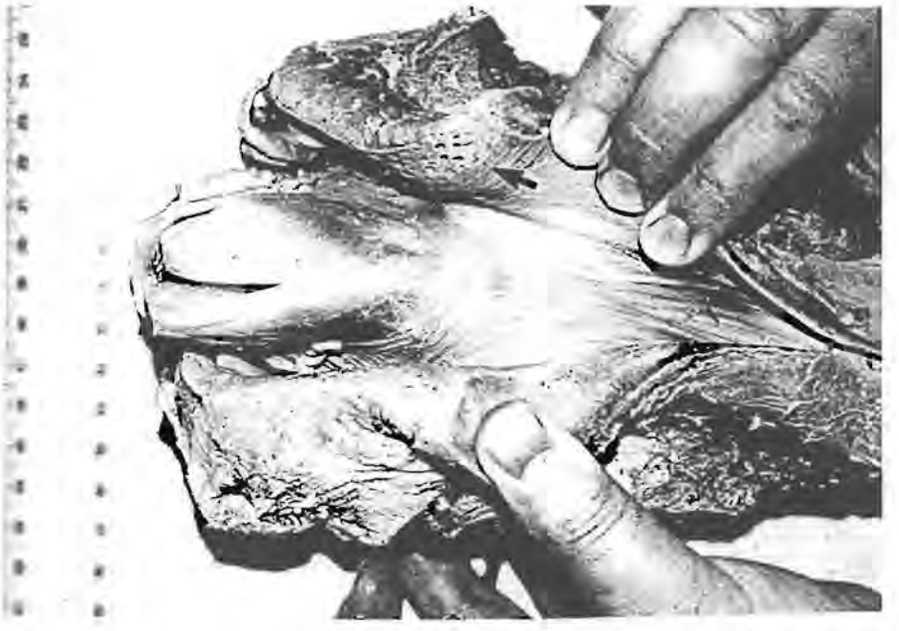

Plate 101 Dorsal view of the exposed oesophageal mucosa of a juvenile male *C. marginata*. Note that the pharyngeal region around the arytenoid bodies and the oesophageal mucosa is grey in colour and that crypts are present on the dorsal wall of the pharynx (arrow).

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Plate 102: Longitudinal section of the pharyngeal lining of a juvenile female *C. marginata* (# 89/3). Note the epithelium pulling away from the rest of the tissue (arrow), as well as large glands. (Mag. 25x, H/E).

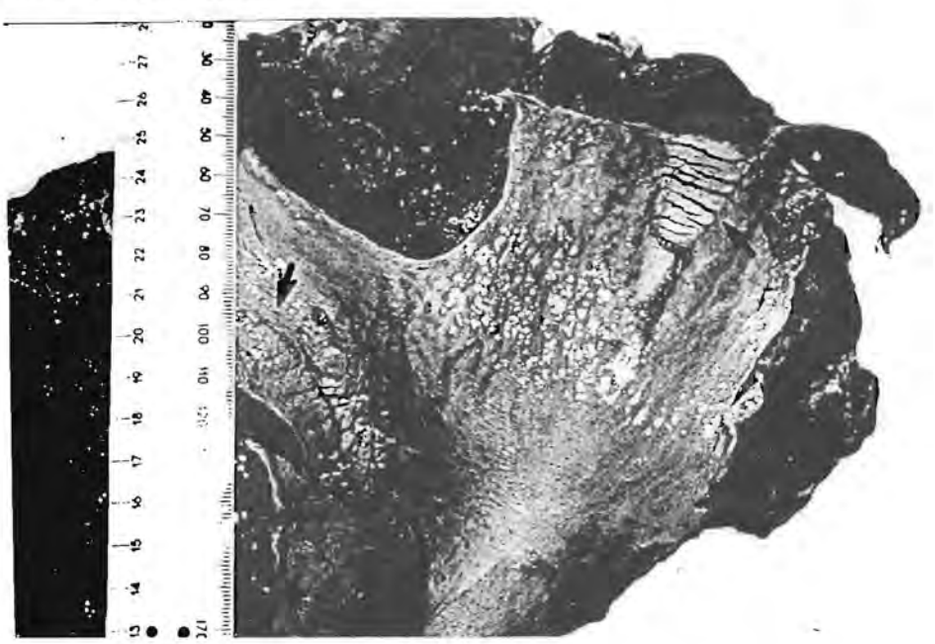

Plate 103: Ventral view of the pharynx of a juvenile female *C. marginata* (# 89/3). Note the two distinctive groups of elongated cells (arrows).

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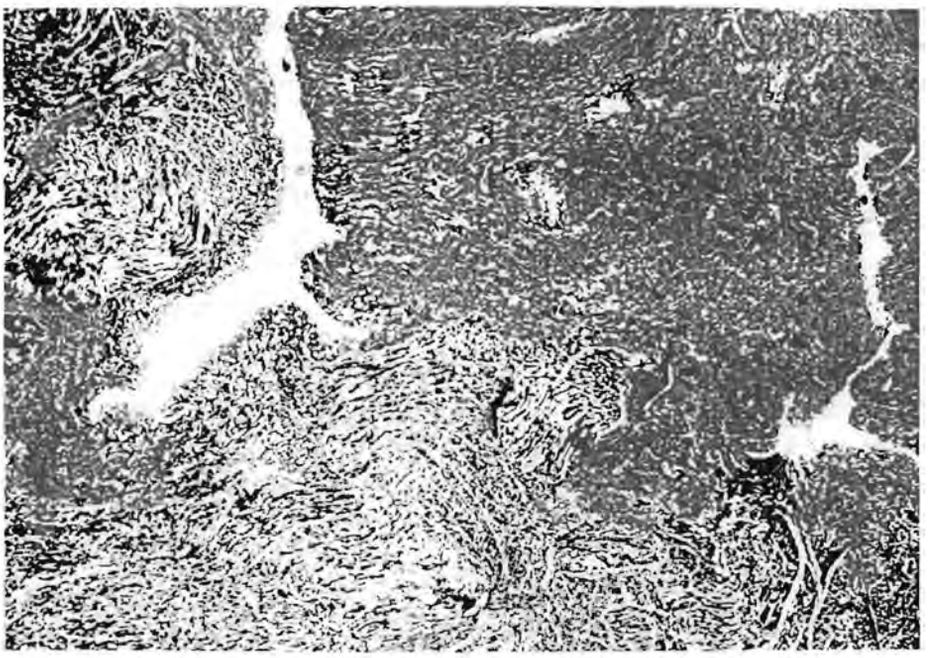

Plate 104: Transverse section of the pharynx of a juvenile female *C. marginata* (# 89/3). Note the mass of connective tissue (blue/green) and muscle. (Mag. 25x, MT).

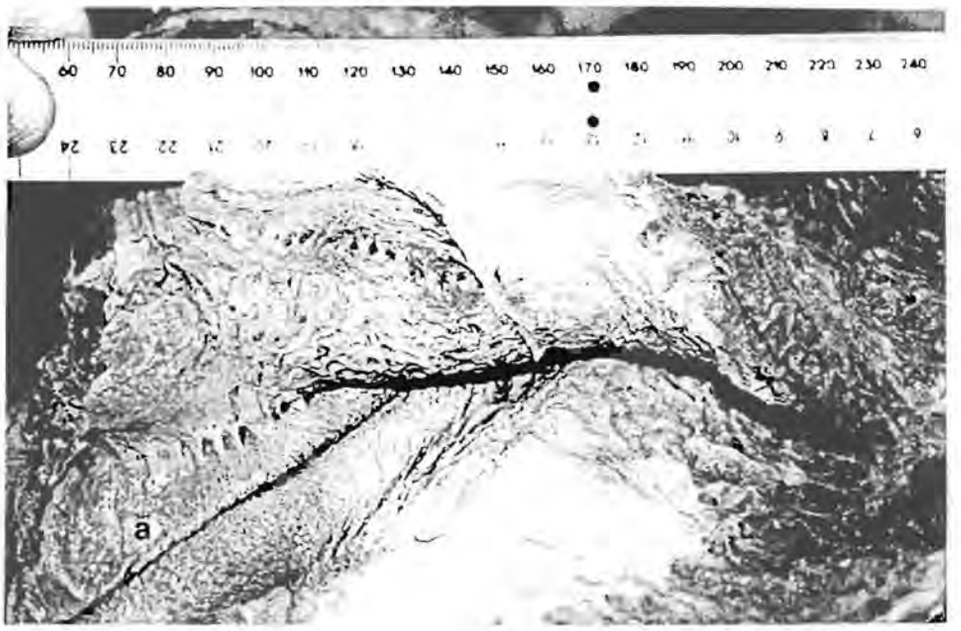

Plate 105: Ventral view of (a) the arytenoid bodies and part of the pharynx of an adult male *C. marginata*. Note crypts.

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pharynx, with an isolated group of crypts occurring on the internal, dorsal wall. Large groups of crypts and glands are present on the ventral surface of the pharynx, on the left and right hand sides of, and anterior to, the arytenoid tips (Plates 101 and 105).

The structure of the mucosa of the arytenoid tips is composed mainly of dense connective tissue which is interspersed with serous glands (Plate 106). In longitudinal section, the undulations of the stratified squamous epithelium show the coarse nature of the mucosal surface (Plate 106).

As in *B. acutorostrata*, the arytenoid cartilages form the ventral margin of the entrance to the laryngeal area, which then leads into the trachea, whose margins are made up by the cricoid and arytenoid cartilages. The tracheal mucosa lines the ventral surface of the body of the cricoid cartilage (Plate 107) and continues to the tracheal rings, where it splits to form the bronchi.

Only one juvenile female (89/3) specimen had an uninterrupted set of tracheal rings. From the few available on the other specimens, as well as from the former set, the rings were found to be incomplete ventrally, with no other definable features, besides for the flexible character of the cartilage. The tracheal mucosa consists of stratified columnar epithelium which overlays large amounts of adipose tissue and elastin in conjunction with connective tissue (Plate 108). This region is well vascularised and innervated. Small serous glands and ducts are visible.

From a dorsal perspective, the arytenoid mucosa (the pharyngeal mucosa that is continuous with the arytenoid tips) continues posteriorly into the oesophagus and the number of crypts decreases in this direction (Plate 109).

The oesophageal mucosa is smooth and longitudinally folded (Plate 110). The epithelium is stratified squamous, which overlies dense connective tissue and lower lying skeletal muscle (Plate 111). Lymphocytes are present, dispersed in between the muscle. Two small lines of crypts pass from the pharynx, continuing down either side of the oesophagus and small nodules or knobs protrude from the lining, occurring throughout the oesophagus (Plate 110). Tiny stones were found between the arytenoid bodies and continued to be found in the trachea and the oesophagus. The stones were a mixture of sand grains and small pieces of shell. Most of the stones occurred

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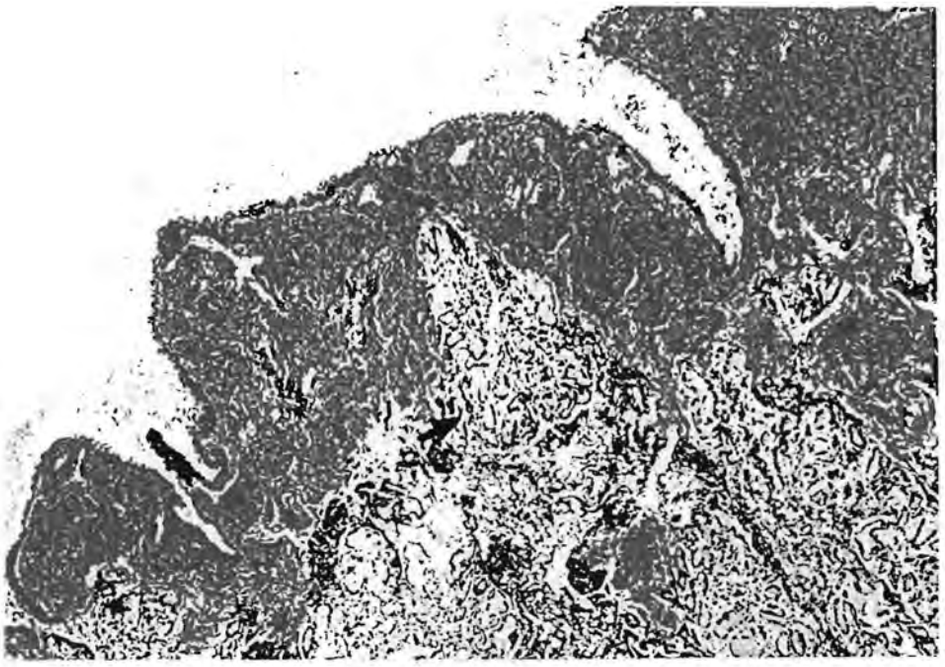

Plate 106: Longitudinal section of the lining of the arytenoid bodies. Note the connective tissue (blue/green) and the serous glands (arrow). (Mag. 50x, MT).

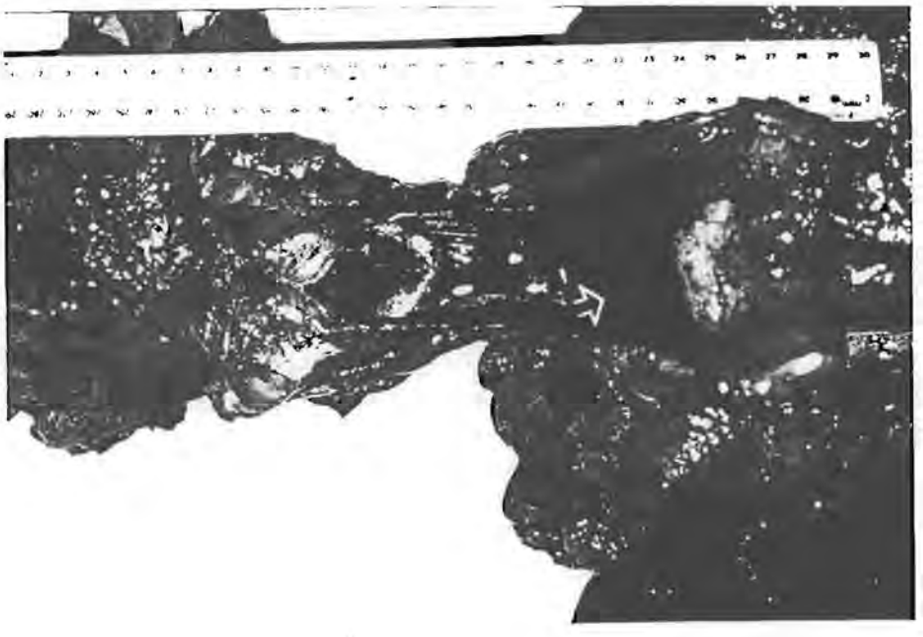

Plate 107: Dorsal view of the laryngeal apparatus of a juvenile female *C. marginata* (# 89/3) with the cricoid cartilage pulled away from the apparatus to reveal its ventral surface. Note that the tracheal mucosa lines the ventral surface of the body of the cricoid cartilage (arrow).

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Plate 108: Longitudinal section of the tracheal lining of a juvenile female *C. marginata* (# 89/3). Note elastin (black), adipocytes, connective tissue and blood vessels. (Mag. 25x, VB).

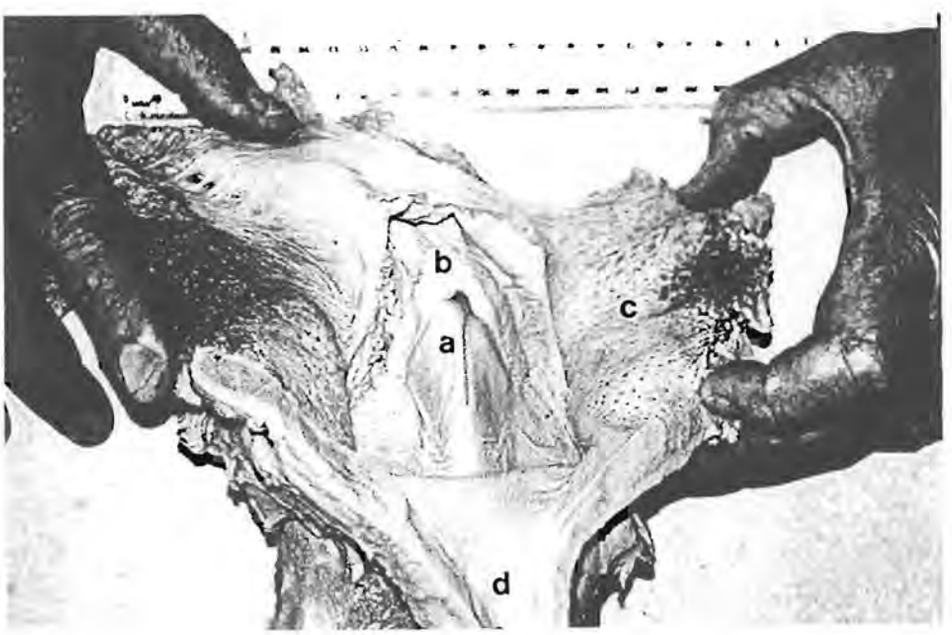

Plate 109: Dorso-lateral view of the (a) arytenoid bodies, (b) epiglottic "hood", (c) parts of the pharynx and (d) the oesophagus of a juvenile female *C. marginata* (# 90/12). Note that the arytenoid mucosa is continuous posteriorly with the oesophagus.

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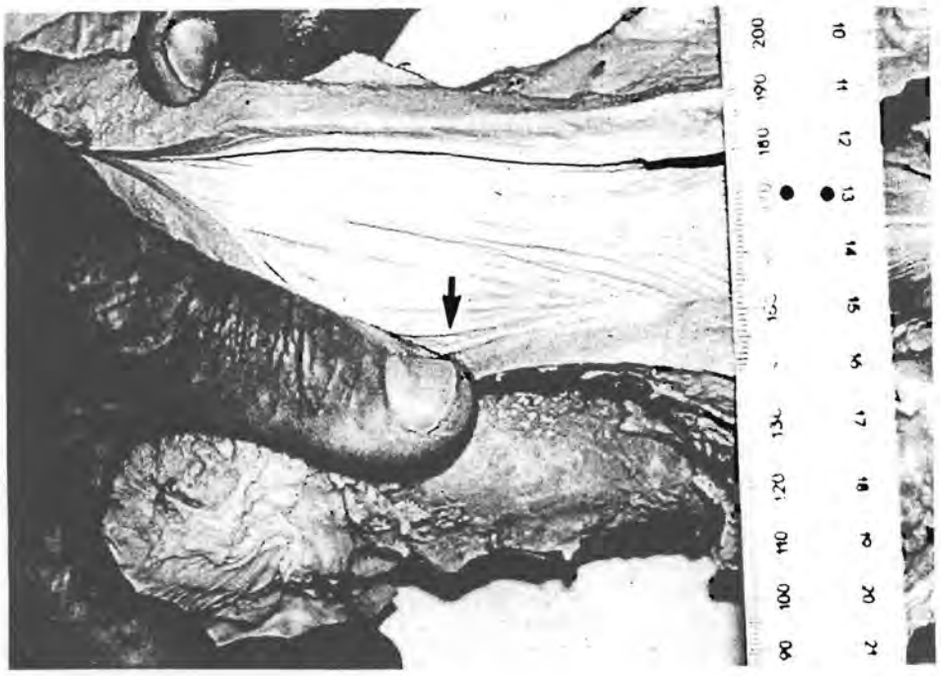

Plate 110: Dorsal view of the oesophagus of a juvenile female *C. marginata* (# 90/12). A sagittal incision reveals the folded oesophageal mucosa. Note knobs/nodules on the mucosa (arrow) (not very clear in Plate).

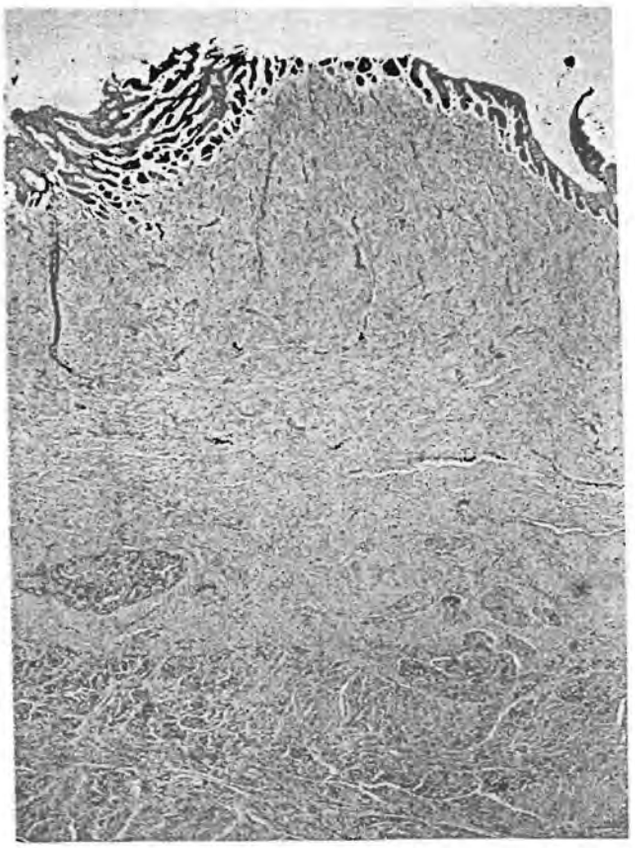

Plate 111: Longitudinal section of the oesophageal mucosa of a juvenile female *C. marginata* (# 89/3) showing epithelial layer, dense connective tissue layer and skeletal muscle layer.

freely, lying on the mucosa, but some stones found in relation to the arytenoid bodies seemed to be fixed in some of the crypts. The stones were probably introduced into the animals during stranding.

Ventrally, the arytenoidal mucosa continues in a posterior direction to form a blind-ending laryngeal sac (Plate 112). This is accomplished through a small opening (approximately 1-1.5 cm wide in the juveniles and approximately 2.5-3 cm in the adult) which occurs on the left hand side of the animal, to the left of the arytenoid bodies (Plates 88 and 113).

On the ventral side of the apparatus, the laryngeal sac protrudes half-way between the "v" notch of the thyroid cartilage and the tracheal rings, in between the cricoid cornua (Plate 114). The sac lies to the right hand side of the apparatus, lateral to the side of the tracheal rings, essentially being separate from the rest of the apparatus (Plate 114).

The wall of the laryngeal sac is very muscular and nerves are clearly visible coursing around the exterior of this structure. Within the mucosa, some nerves and blood vessels seemed to form a distinctive pattern whereby adipose tissue occurs on the outer margins, and moving inwards, blood vessels occur on either side of a nerve (Plate 115). It is not unusual to find nerves and blood vessels in close relation to each other, as blood vessels do tend to shadow the pathways of nerves. The adipose layer may perform an insulating function.

The sac is also well supplied with spiralling blood vessels in conjunction with spiralling nerves (Plate 115). Serous glands together with connective tissue and elastin fibres, underlain by parallel rays of muscle (Plate 116), compose the rest of the upper external layers of the laryngeal sac.

A ventral, longitudinal incision to inspect the internal character of the sac reveals two distinct rows of crypts, which stretch down either side of the laryngeal sac (Plate 117). The crypts diminish in size as they approach the posterior end of the sac. Other smaller crypts are present throughout the sac. The mucosal lining of the interior, in the two preserved juvenile specimens, is grey and yellowish in colour (Plates 117 and 118), whilst the (89/3) frozen female specimen showed no distinctive colouration of the interior laryngeal sac lining (Plate 119).

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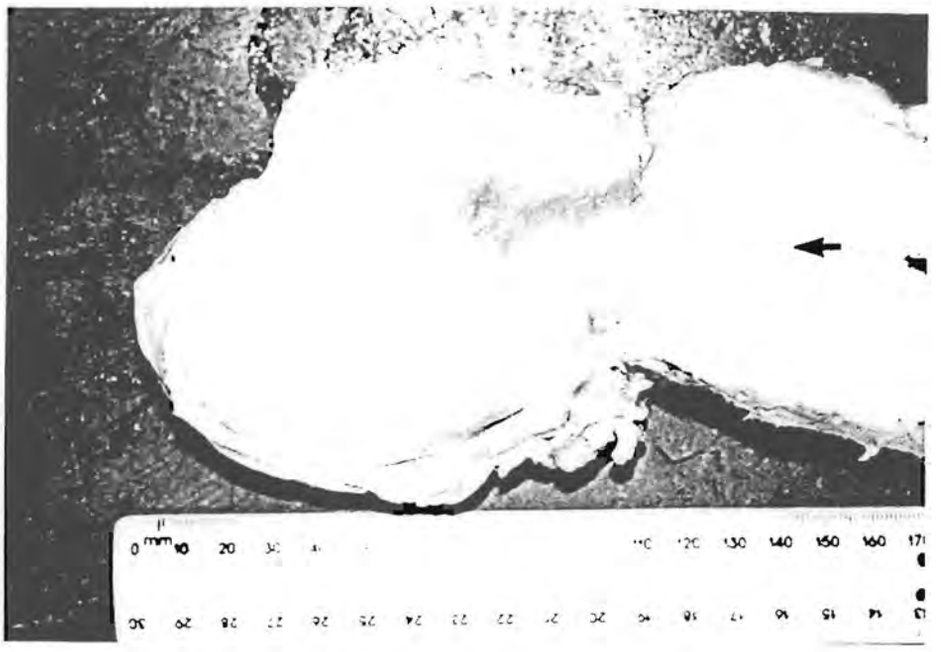

Plate 112: Dorsal view of the posterior tips of the processi vocales of a juvenile female *C. marginata* (# 90/12), providing attachment for the laryngeal sac (arrow). The arytenoid mucosa is continuous with the cavum of the laryngeal sac.

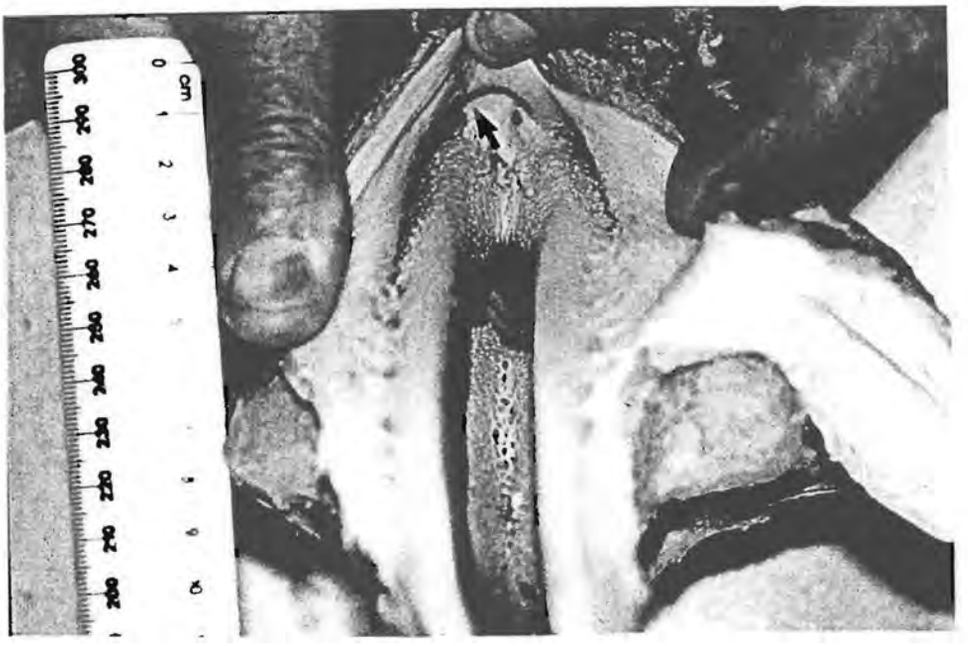

Plate 113: Antero-ventral view of the arytenoid cartilages and the small opening which leads into the laryngeal sac of a juvenile female *C. marginata* (# 90/12) (arrow).

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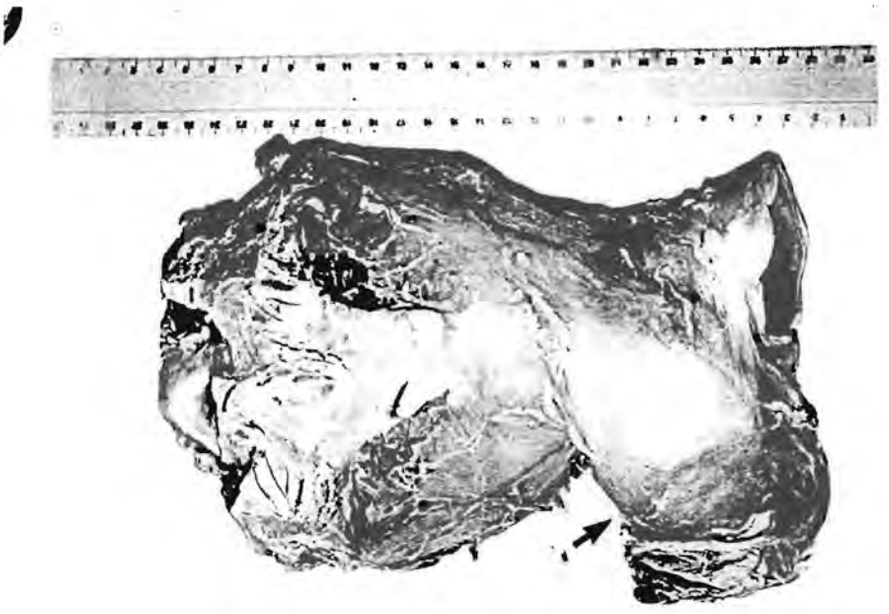

Plate 114: Ventral view of the laryngeal apparatus of a juvenile male *C. marginata* indicating the position of the laryngeal sac (arrow).

Plate 115: Transverse section of the laryngeal sac of a juvenile female *C. marginata* (# 89/3) showing the distinctive pattern formed by (a) adipose tissue, (b) spiralling blood vessels and (c) nerves. (Mag. 50x, H/E).

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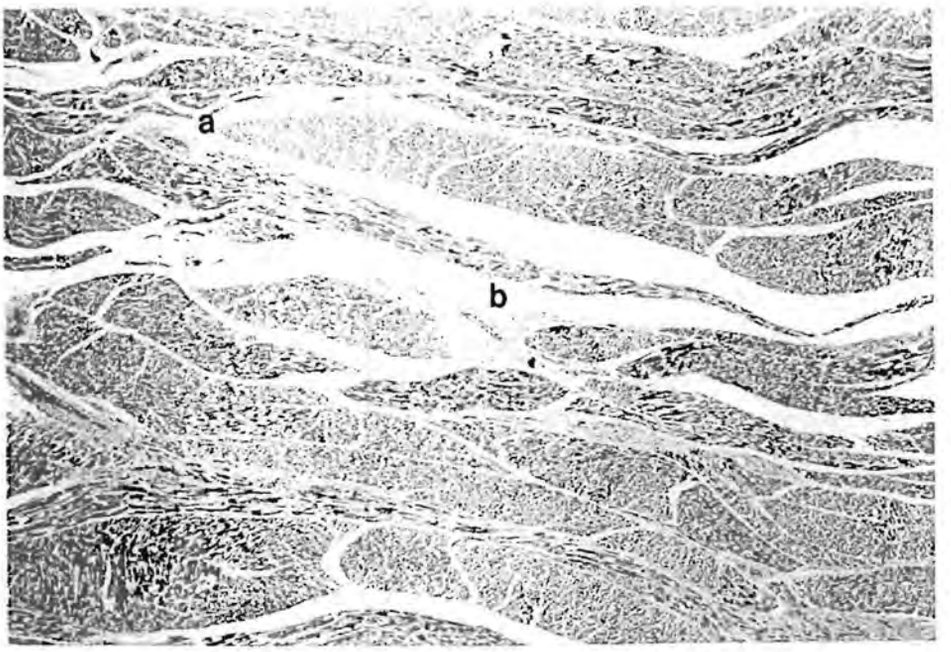

Plate 116: Longitudinal section of the parallel rays of skeletal muscle of the upper external layer of the laryngeal sac of a juvenile female *C. marginata* (# 89/3). Note (a) nerves and (b) blood vessels. (Mag. 25x, H/E).

Plate 117: The laryngeal sac of a juvenile male *C. marginata* (# 91/27). A longitudinal incision reveals two distinct rows of crypts. Note coloration of the mucosa.

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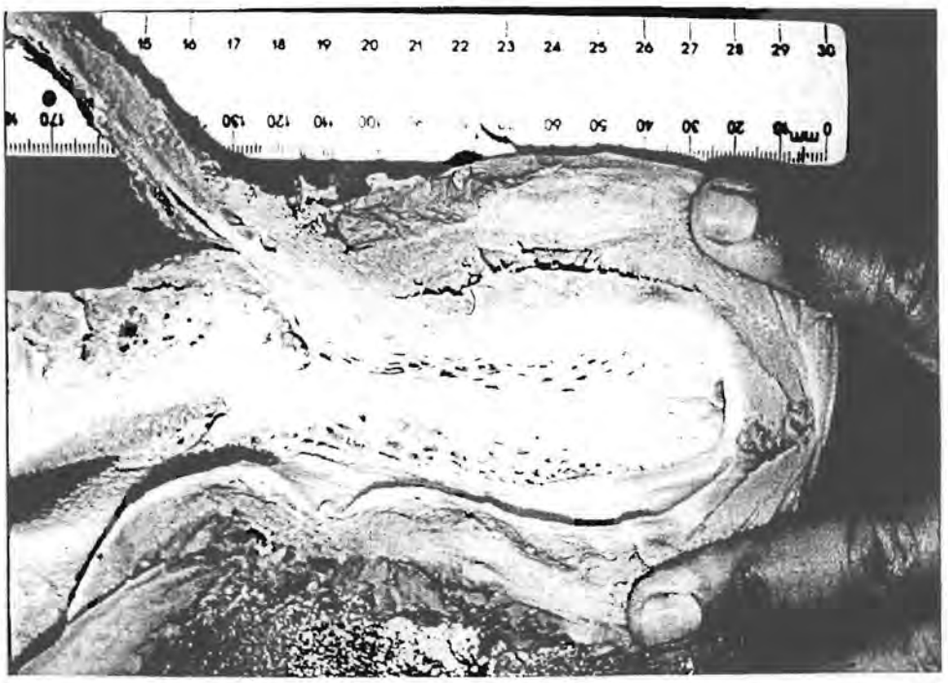

Plate 118: The laryngeal sac of a juvenile female *C. marginata* (# 90/12). A longitudinal incision reveals two distinct rows of crypts. Note coloration of the mucosa.

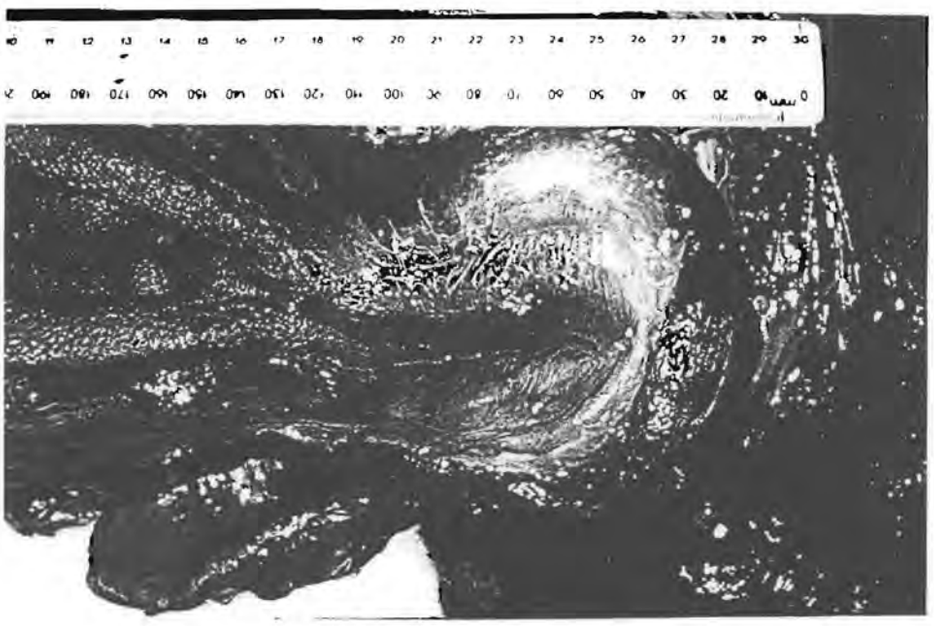

Plate 119: The laryngeal sac of a juvenile female *C. marginata* (# 89/3). A longitudinal incision reveals two distinct rows of crypts. Note lack of grey coloration of mucosa.

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These are compared to the definite grey colouration seen in the adult (Plate 120). The colouration of the laryngeal sac mucosa may be the result of preservation. The epithelium itself was not well preserved; in certain areas it seems that the epithelium is two layers thick and in other areas, only basement membranes remained. Lymphatic tissue was detected beneath the epithelial layer (Plate 121). Large serous glands filled with proteinaceous secretions are associated with ducts which visibly pierce the epithelium (Plate 122). Large nerves and large blood vessels, as well as connective tissue, course through the thick, parallel rays of skeletal muscle, which form the wall of the laryngeal sac (Plate 116). Undulating chains of adipocyte-type cells occur near the epidermis (Plate 123).

Measurements of the lengths of the sacs were taken from their opening, near the arytenoids, to their posterior ends, while the widths were measured across their widest points. The sacs of the juvenile females (89/3) and (90/12) were 10 cm long x 5.2 cm wide (Plate 68) and 11.5 cm long x 6.7 cm wide (Plate 69), respectively, and the juvenile male's (91/27) sac measured 12 cm x 8 cm (Plate 70). The adult male's sac measured 51.2 cm in length and 36.1 cm in width (Plate 120).

The introduction of air down the pharynx of the juvenile female (89/3) specimen, which was attached to the viscera, caused the laryngeal sac as well as the lungs to inflate (Plates 124 and 125).

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Plate 124: Lateral view of the laryngeal apparatus of a juvenile female *C. marginata* (# 89/3) showing (a) the laryngeal sac and (b) lungs before inflation. Arrow indicates hose insertion.

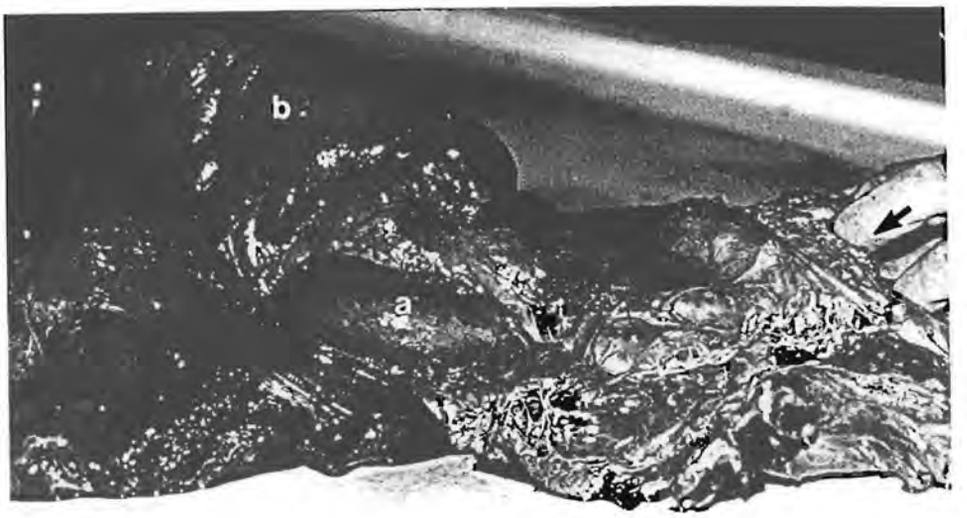

Plate 125: Lateral view of the laryngeal apparatus of a juvenile female *C. marginata* (# 89/3) showing (a) the laryngeal sac and (b) lungs during inflation. Arrow indicates hose insertion.

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CHAPTER 4: DISCUSSION

4.1 Comparison with previous descriptions of *B. acutorostrata*

Generally speaking, the structure of the laryngeal apparatus of *B. acutorostrata* agrees with previous descriptions for both this and other mysticetes (Eschricht & Reinhardt 1866; Carte & Macalister 1868; Turner 1872; Beauregard & Boulart 1882; Dubois 1886; Benham 1901; Schulte 1916; Hosokawa 1950; Yablokov, Bel'kovich & Borisov 1974; Quayle 1991; Haldiman & Tarpley 1993; Schoenfuss & Hillmann 1996).

The most detailed structural accounts of the laryngeal apparatus of *B. acutorostrata* are found in the works of Carte & Macalister (1868) and Benham (1901), which served as invaluable guides for the present study. Both accounts concerned subadult or juvenile animals only. Although Benham (1901) refers to Carte & Macalister's (1868) specimen as an adult, the whale measured 18'5" (approx. 5.6 m) in length, which indicates that it was sexually immature (Lockyer 1984) and cannot therefore be regarded as an adult, but rather as a sub-adult.

In this study the nature and positioning of the hyoid bones on the ventral surface of the laryngeal apparatus are shown to be similar to those previously described for *B. acutorostrata* (Carte & Macalister 1868; Benham 1901; Satake & Omura 1974), but the association of the hyoid bones with each other is not entirely consistent with previous descriptions. Drawings by Carte & Macalister (1868) indicate that the styloid bones are closely related to and occur on either side of the anterior notch of the os hyoides. This was found to be the case here in the adult female and the juvenile male. In the adult male and the juvenile female, however, the styloid bones were found with their internal margins in close proximity to each other while the anterior notch of the os hyoides lay beneath the mid-ventral meeting of these bones. The internal extremities of the styloid bones did seem to be attached to the anterior apex of the os hyoides via connective/fibrous tissue. Satake & Omura (1974) noted that the minke whale in the Antarctic has longer styloid bones (stylohyals) than the minke in the North Pacific, though there is rather wide individual variation. Extreme variation in both size and shape of the hyoid bones was also described by Perrin (1975) in the spotted dolphin, *Stenella attenuata*.

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The sternum bone described by Carte & Macalister (1868) as somewhat "heart-shaped" differed considerably in shape from those found in this study, although this shape has been described by previous authors (Tomilin 1967; True 1983). Much individual variation is also known to occur in specimens from the North Pacific and Antarctic Oceans, but the form of the sternum is generally cruciate (Omura 1975). Individual variation of the sternum did exist between the juvenile male and female specimens examined in this study, but the general "cross-like" form, also described by Flower (1864), is expressed in these specimens.

The five typical mammalian cartilages described in this study generally conform with previous descriptions for balaenopterids (Carte & Macalister 1868; Benham 1901). The cartilaginous framework is joined by muscles most of which could be identified as following the general mammalian pattern.

The existence of the sterno-thyroid, an extrinsic muscle, was denied by Carte & Macalister (1868) in their description of a specimen of *B. acutorostrata*. Benham (1901), without denying its existence, was unable to determine the relationships of a sterno-thyroid muscle in his specimen of *B. acutorostrata*. Unfortunately I was not able to isolate this muscle in any of the *B. acutorostrata* specimens to which I had access.

The finger-like projections of the dermis which reach into the epidermis of the nasopharynx may possibly provide an anchoring mechanism for the epidermis, which may be placed under considerable strain during respiration. Ling (1974) describes what seems to be the same arrangement (which he terms "rete ridges") on the underside of the epidermis in the integument of *B. physalus*. The presence of melanocytes in the nasopharynx seems to be related to the dark epidermal lining found in the blowholes (Quayle 1991).

The "elasticated" or "gathered" character of the narial opening, together with the presence of muscles directly beneath the epithelium, seem to indicate that this region is structured in order to encourage movement. With the epiglottis being inserted into the posterior narial opening and the whole larynx possibly being pulled upwards during the process of respiration (Benham 1901), movement of this structure seems essential.

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Haldiman & Campbell (1985) stated that the fusion of the tracheal rings with the posterior prominence of the cricoid cartilage in *B. mysticetus* may offer a method for aging harvested animals. Although the number of tracheal rings fused with the cricoid cartilage in *B. acutorostrata* varies according to the size of the animal, the difference in the number of fused tracheal rings between the juvenile female and the adult male is very small, although the age difference is quite substantial (Table 2). Clearly this species does not lend itself to the aging method proposed by the abovementioned authors.

Although the illustrations in Benham's (1901) paper do not show the clearly defined tubercles on the anterior margin of the thyroid cartilage as were seen in this study in both the juveniles and the adults, he does note their presence. The distinctive or well-developed structure of the tubercles found in this study may support Benham's (1901) theory that they are representative of anterior cornua.

Carte & Macalister (1868) describe a strong crescentic hood-like fold of mucous membrane two inches (5 cm) in front of the root of the epiglottis. They state that this fold is capable of being drawn over the orifice of the glottis when the margins of that opening are approximated, and that its use appears to be to cover and protect the superior opening of the larynx during the act of degluttition. Benham (1901) did not find any comparable structure except for the epiglottis itself and as such maintained that, although Carte & Macalister's (1868) account in the text was precise, the hood-like fold was in fact the epiglottis. In this study, no evidence of any such hood-like fold was found in any of the specimens. Consequently, I support Benham's (1901) opinion that Carte & Macalister (1868) must have been referring to the epiglottis itself.

Carte & Macalister (1868) did not regard the epiglottic cartilage as a true cartilage as they found that the cartilagenous portion of the epiglottis formed only a small part of the entire organ. Later, Benham (1901) described a 3 inch (7.6 cm) long, horse-shoe shaped cartilage in his juvenile specimen. This study extends Benham's (1901) finding to larger and older animals, where predictably, the epiglottic cartilages are better developed. Therefore, contrary to Carte & Macalister's (1868) findings, the epiglottic cartilage can be considered as one of the five cartilages that form the framework of the cetacean larynx.

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The arytenoid "lips", as they were observed in this study, have not been previously described in this species. In other mysticetes (e.g. *E. australis*), two lips formed by the extension of the laryngeal mucosa over the horns of the arytenoids are described (Beauregard & Boulart 1882). These authors also state that in *B. physalus* and *B. musculus* "the lips of the orifice communicating between the sac and the larynx are formed by the arytenoids covered by mucosa". Purves (1967) described "semidiscoid flanges projecting forward from the apex of each arytenoid cartilage" in *B. physalus*, with "closely-packed, warty rugosities" on the mesial surface in the more adult specimens. Haldiman & Campbell (1985) describe elongated "lips" of the laryngeal sac in *B. mysticetus*, whose sac in longitudinal section forms the shape of an anchor. It is the left and right hooks of the "anchor" which the authors refer to as "lips". Consequently these are not comparable to the "lips" described in *B. acutorostrata*. However, apart from the presence of warty rugosities, both the flanges of *B. physalus* and the "lips" (described in the present study) of *B. acutorostrata* occur at the anterior tip of the arytenoid bodies and are composed entirely of connective/elastic tissue, indicating that they are probably analogous structures. The descriptions of the lips found in *E. australis* (Beauregard & Boulart 1882) also seem to match that of the lips of *B. acutorostrata* as described in this study.

In humans, the vocal cords are the only part of the lower respiratory tract which is not lined by respiratory epithelium; they are lined by stratified squamous epithelium which is better adapted to withstand frictional stress (Wheater, P.R., Burkitt, H.G. & Daniels, V.G. 1979).

Being part of the anterior opening to the larynx, it is not unreasonable to expect that the arytenoid bodies, as well as their "lips", would be exposed to considerable strain caused by the movement of air that occurs during respiration. This fact is corroborated by the presence of a stratified squamosal epithelial lining which lines the arytenoid bodies and which continues to form the lip-structures.

Benham (1901) stated that in *B. mysticetus*, the two processi vocales of the arytenoid cartilages were continuous at their distal ends, forming a posterior support for the laryngeal opening, while in *B. acutorostrata*, the posterior ends of the processi vocales were connected by dense connective tissue. These findings for *B. acutorostrata* are echoed in this study, although, contrary

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to what Benham (1901) suggests, the connective tissue did not become replaced by cartilage in the older animals.

According to Fanning & Harrison (1974), both Simpson & Gardner (1972) and Slijper (1962) maintained that all the epithelial cells in cetacean trachea were ciliated. After consulting Slijper's (1962) original document, however, it is clear that he has been misquoted; in fact he explains that, based on the investigations of other researchers, the trachea is said to be lacking in cilia in various species of odontocetes. The researchers to whom Slijper (1962) refers were also surprised to note the complete absence of mucous (goblet) cells in the epithelium of the trachea and bronchi, and their almost complete absence in the glands terminating in the air passages. However, a pseudostratified ciliated columnar epithelium, containing goblet cells, has since been found lining the crypts on the ventral aspect of the cranial end of the trachea in *Tursiops truncatus* by Fanning & Harrison (1974). On the dorsal wall of the laryngotracheal junction in *B. mysticetus*, stratified squamous epithelium with lymphatic nodules in the lamina propria changed abruptly to ciliated pseudostratified columnar epithelium with diffuse lymphatic tissue underneath (Haldiman, Abdelbaki, Al-Bagdadi, Duffield, Henk & Henry 1981). In this study, changes in the composition of the epithelium of the dorsal wall of the tracheal mucosa from thick stratified squamous cells to thin pseudostratified columnar cells were evident. Small aggregations of goblet cells, together with lymphocytes, were found in the tracheal lining, although no goblet cells were found along any other air passage. No ciliated epithelia nor any remnants thereof were detected at all at the laryngotracheal junction nor in the middle of the trachea.

According to Slijper (1962), the alleged absence of cilia in cetacean trachea may have been due to the poor state of the histological material available. However, the relatively short post-mortem times of the specimens used in this study (Table 2), together with the cool nature of the environment in which they were obtained, should not have produced significant degeneration of the tissue and none was obvious in the histological material examined. As another explanation, Slijper (1962) speculates that ciliated structures may have been dispensed with by cetaceans, as they live in a moist and dust-free environment. Evans (1987) has suggested that secretions produced at the bottom of the junction between the larynx and the trachea are removed by the violent coughing movement of the blow, making the need for cilia redundant. In view of the fact

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that ciliated structures have been described in at least some mysticetes and odontocetes (see above), these theories do not seem plausible.

The laryngeal sacs described in other mysticetes namely, *Balaena mysticetus* (Eschricht & Reinhardt 1866; Haldiman & Campbell 1985), *Balaenoptera musculus* (Turner 1872; Beauregard & Boulart 1882; Dubois 1886), *B. physalus* (Beauregard & Boulart 1882), *B. borealis* (Hosokawa 1950) and *M. novaeangliae* (Quayle 1991), seem to follow the same general pattern, *B. acutorostrata* included (Carte & Macalister 1868; Benham 1901). All have the laryngeal sac on the ventral aspect of the larynx, between the thyroid and cricoid cartilages, in a post-thyroideal position. This study concurs with these descriptions.

The ventral muscular wall of the laryngeal sac consists partly of the crico-thyroid, accessory crico-thyroid and thyro-cricoid muscles, together with the circular muscular fibres which immediately surround the cavity of the sac. Benham (1901) only describes the circular muscular fibres, which he says differ from the musculature described by Eschricht & Reinhardt (1866) for *B. mysticetus*. As the latter authors' description concurs with the intrinsic muscle component described in this paper, I am inclined to believe that Eschricht & Reinhardt (1866) were describing the exterior character of the wall of the laryngeal sac, whereas Benham (1901) was describing the interior character of the wall of the laryngeal sac.

All the abovementioned authors seem to agree that the muscular structure of the wall of the laryngeal sacs found in the species of mysticetes studied could allow for extension and contraction of the sacs. Dubois (1886) describes the *N. laryngeus inferior* which innervates the sac and Hosokawa (1950) states that the muscular sheets of the sac are innervated by the vagus. The present histological study revealed nerves coursing through the muscular wall of the sac, as well as an abundance of blood vessels and lymphatic tissue in the mucosa. This, together with the definite ontogenetic development of the laryngeal sac further supports the theory that the structure is functional, at least in adults.

The interior of the laryngeal sac of *B. physalus* has been described as having "numerous voluminous fasciculate folds, subdivided into secondary folds with corrugated edges" as well as

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longitudinally directed folds which occupy the sides of the laryngeal sac, and numerous crypts (Beauregard & Boulart 1882). These authors found no such folds in *B. musculus* or in *E. australis*, although crypts were present in both species, being particularly abundant in the former. The arrangement of the crypts in *B. musculus* does seem to most closely represent that found in *B. acutorostrata*. In *B. borealis*, Hosokawa (1950) found many longitudinal folds in the upper part of the sac, while the lower parts showed many reticulated grooves. No evidence of folds was found in the laryngeal sac of *B. acutorostrata*. Apart from the absence of cilia, the pseudo-stratified, columnar nature of the epithelial cells lining the laryngeal sac is consistent with the epithelia almost exclusively confined to the larger airways of the respiratory system in other mammals (Wheater *et al.* 1979).

4.2 Comparison of the laryngeal apparatus in *B. acutorostrata* and *C. marginata*

The gross morphology of the laryngeal apparatus of *C. marginata* generally conforms with that of *B. acutorostrata*. A few structural differences do occur, and these are discussed below.

The identification of the various muscles of *C. marginata* is based on relating their positions to those found in *B. acutorostrata*. On this basis, it is found that the general myology of all the specimens studied is very similar, with a definite sterno-thyroid muscle being discernible in *C. marginata*. I hesitate to infer exactness of the attachment and insertions of the various musculature due to the condition of preservation of the *C. marginata* specimens.

The notable difference in the thyroid cartilage of *C. marginata* is that a distinct ridge was present on the mid-ventral line of the thyroid cartilage in all the specimens examined, whereas in *B. acutorostrata* a slight ridge was only visible in the adult female. The ridge seemed to provide attachment for connective tissue in the juveniles, but which became continuous with the epiglottic protrusion, and hence the epiglottic cartilage itself, in the adult male *C. marginata*.

The general structure of the cricoid cartilage of *C. marginata* differs slightly from that found in *B. acutorostrata*. Although the body does continue laterally and ventrally, no distinctive cornua

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are visible. Foramina were not definable along the mid-dorsal line of the body but there is a distinct hump on the anterior, dorsal surface of the body of the cricoid cartilage.

Once again the tracheal rings are seen to be continuous with the rounded prominence on the posterior margin of the dorsal side of the body of the cricoid cartilage. Although (as mentioned for *B. acutorostrata*) the numbers of tracheal rings continuous with the cricoid cartilage varied, the smaller of the juvenile females (89/3) had the highest number of such tracheal rings. This species therefore did not display a correlation between size of the animal and number of tracheal rings continuous with the cricoid prominence. Unfortunately the tracheal rings of the adult male could not be counted.

In *C. marginata*, the epiglottic cartilage is much reduced compared to that found in *B. acutorostrata*. The comparable hood, which is tightly associated with the arytenoid bodies, only extends to the top of these bodies and is not as conspicuous as that found in the latter species. A central ridge (as well as the aryteno-epiglottic folds) is barely discernible. The association which occurs between the epiglottic and thyroid cartilages in the adult male *C. marginata* did not occur in any of the *B. acutorostrata* specimens available. The absence of cartilaginous connections to the epiglottic cartilage is consistent with the findings of Schoenfuss & Hillmann (1996) for *B. mysticetus*.

The arytenoid cartilages of *C. marginata* differ from those of *B. acutorostrata* in that the whole structure is more compact in the former species. On first inspection, the arytenoid bodies in *C. marginata* are continuous posteriorly with the processi vocales, with no thinning or tapering evident. However, once dissected out, each arytenoid cartilage seemed to consist of two components which merge to form the arytenoid bodies. The broader tips of the arytenoid bodies of *C. marginata* are ventrally flattened against each other, with the right tip projecting further than the left tip in the juveniles and vice versa in the adult. This difference between the juveniles and the adult may be due to a difference in the rate of growth of the arytenoid cartilages. No "lips" are present at the tips of the arytenoid bodies and the processi vocales do not extend to form the dorsal wall of the laryngeal sac.

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In *D. leucas*, Watson & Young (1879) describe the apices and processes of the arytenoid cartilages as being covered with mucous membrane, forming the posterior thickened lip of the superior aperture of the larynx. The thickened character of the arytenoids in *C. marginata* and the nature of the mucosa which was found to cover the tips of the arytenoids suggest that this species may also be evolving towards a similar non-involvement of the arytenoid lip structure in the process of phonation.

In *B. acutorostrata* and *C. marginata* the oesophageal mucosa is folded, but distinct left and right groups of crypts occur on each side in the former, whereas they are almost absent in the latter species.

The laryngeal sac found in *C. marginata* is also present on the ventral surface of the larynx but differs in position from that in *B. acutorostrata* (or any other mysticete so far described) in that it is completely separate from the tracheal rings and lies to the right hand side of the animal (Plates 126 and 127). As such, the dorsal wall of the sac is not made up by the processi vocales and, unlike *B. acutorostrata*, the sac opens into the laryngeal area through a small opening (approx. 1 cm in length) on the ventral side of the processi vocales. The arrangement as well as the nature of the crypts inside the sacs also differ. As in *B. acutorostrata*, there is distinct ontogenetic development of the laryngeal sac, but to a much greater extent, with the adult's sac being almost five times the size of those found in the juveniles. This together with the notable vascularisation and innervation detected in the muscular lining, as well as the presence of lymphatic tissue in the mucosa of the laryngeal sac imply that the structure is functional. The coiled nerves and blood vessels also lend support to the theory which advocates that the laryngeal sac undergoes expansion and contraction.

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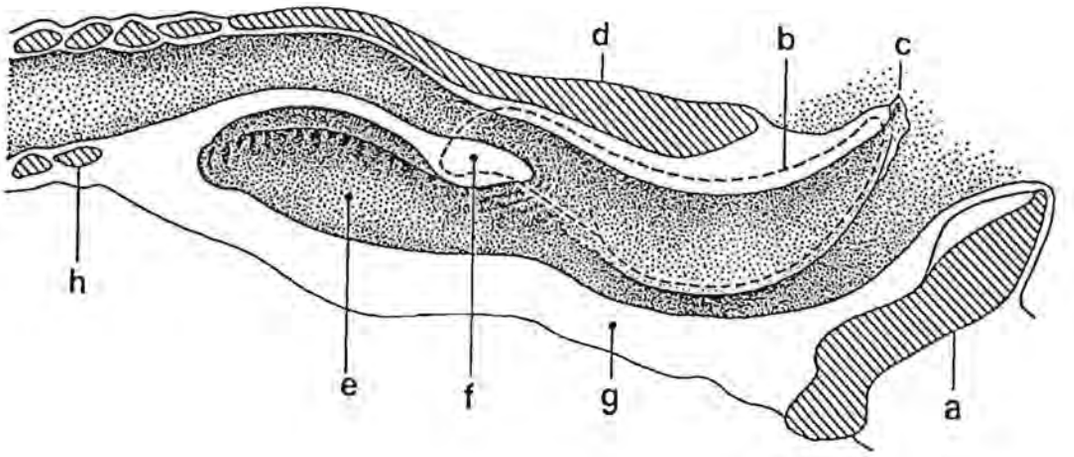

Plate 126: Sagittal section through the laryngeal apparatus of *B. acutorostrata* showing the position of the laryngeal sac. Labelling as follows: a: epiglottic cartilage; b: lateral view of the arytenoid cartilage (dashed line); c: arytenoid lip at anterior tip of the arytenoid cartilage; d: cricoid cartilage; e: laryngeal sac; f: inter-arytenoid connective tissue connection; g: thyro-arytenoid muscle; h: tracheal rings. Shaded area represents the air passage. Drawing not to scale.

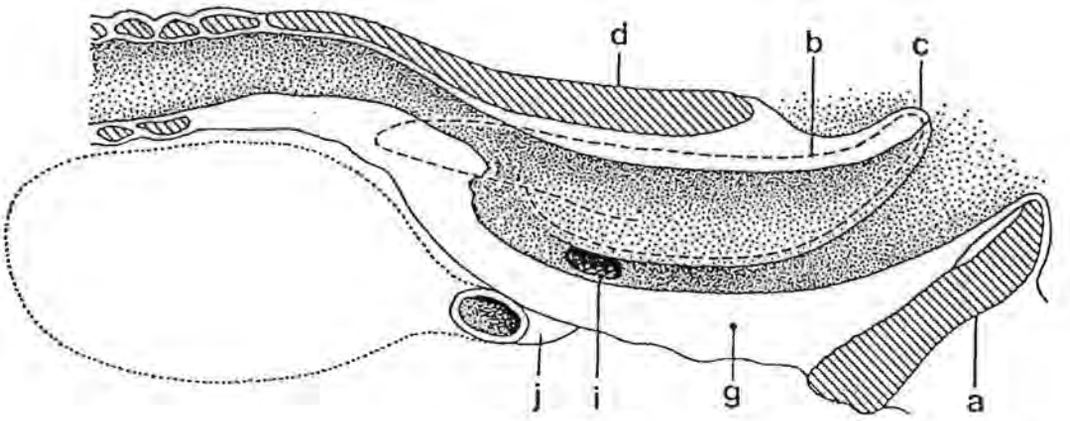

Plate 127: Sagittal section through the laryngeal apparatus of *C. marginata* showing the position of the laryngeal sac. Labelling as above except for i: opening on the left hand side of the arytenoid cartilages which opens into the laryngeal sac; j: neck of the laryngeal sac. Drawing not to scale.

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The most outstanding difference between the laryngeal sacs in the two species is the much greater development of the sac in adult *C.marginata* (at least for males). Table 6 lists the lengths of laryngeal sacs of some species of mysticetes as described by different authors.

Table 6 Lengths of laryngeal sacs of mysticetes as described by various authors.

Author	Species described	Sex	Length of animal (m)	Length of laryngeal sac (cm)	Relative age of specimen
Turner (1872)	*B. musculus*	♀	23.9	25.4	Adult
Beauregard & Boulart (1882)	*B. musculus*	♂	3.6	1.0	Foetus
	B. physalus	♀	12.0	≥35.0	Adult
	E. australis	♀	0.6	-*	Foetus
Purves (1967)	*B. physalus*	♀	17.8	120.0	Subadult
Quayle (1991)	*M. novaeangliae*	♂	4.2	10.0	Juvenile
Reeb (present study)	*B. acutorostrata*	♂	5.3	11.0	Juvenile
		♂	8.5	22.5	Adult
		♀	5.7	11.3	Juvenile
		♀	8.9	28.8	Adult
	C. marginata	♀	3.3	10.0	Juvenile
		♀	3.7	11.5	Juvenile
		♂	3.2	12.0	Juvenile
		♂	5.9	51.2	Adult

* "quite rudimentary with respect to Balaenopterids".

Although the methods of measurement used by the different authors are not known, it seems from Table 6 that (with the possible exception of *B. physalus*) the ontogenetic development of the laryngeal sac in *C. marginata* is not matched by any other mysticete species examined to date.

The size and position of the laryngeal sac are not the only unique anatomical features of *C. marginata*. The structure of the thorax, for instance, is quite different to that seen in any other mysticete. Beddard (1901) found that the ribs of *C. marginata* are only, and not very firmly, attached to the transverse processes of their vertebrae. Davies & Guiler (1957) pointed out that compared to other mysticetes the number and flattening of the ribs are much greater in *C.marginata*. The number of vertebrae (based on seven *C. marginata* specimens) ranges as

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follows: cervical, 7; thoracic, 17-18; lumbar, 1-4; caudal, 14-16; total, 40-44 (Baker 1985). The vertebral formula for *B. acutorostrata* (based on two adult specimens from the Antarctic and two juveniles from the North Pacific) was found by Omura (1975) to be cervical, 7; thoracic, 11-12; lumbar, 10-12; caudal, 18-20; total, 48-50.

The marked development of the thorax in *C. marginata* is perhaps best demonstrated by the proportion that the thoracic vertebrae constitute of the whole vertebral column. The data given by Baker (1985) show that thoracic vertebrae composed 41-43% of the vertebral count, compared to 22-25% for *B. acutorostrata* (Omura 1975). The thoracic development in *C. marginata* is in fact unmatched by any cetacean: vertebral counts listed by Watson (1981) show that in no cetacean family, apart from Neobalaenidae, do the thoracic vertebrae make up more than 26% of the total number of vertebrae (Table 7). This thoracic development is perhaps achieved at the expense of the lumbar vertebrae which only make up 2.5-9% in Neobalaenidae (Omura 1975) compared to 21-23% for other mysticetes (Omura 1975, Watson 1981) (Table 7).

Beddard (1901) compares the arrangement of the ribs to armature for the protection of the viscera and explains that if *C. marginata* is capable of longer submersion compared to some other mysticetes, "the lax attachment of the ribs may conceivably allow of a greater expansion of the contained lungs". Davies & Guiler (1957) however doubt whether this hypothesis is consistent with the knowledge of the physiology of diving in whales. These authors believe that the peculiar structure of its skeleton suggests that *C. marginata* may not be an especially deep diver, but rather that it spends abnormally long periods of time beneath the surface and may actually rest on the bottom for much of this time, the ribs acting as support at this time. Bonner (1980) raises the question what a filter-feeder, such as *C. marginata*, would be doing in such bottom-lying activities (except meditating).

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Table 7 Percentage that thoracic and lumbar vertebrae constitute of the total vertebral column in mysticete and odontocete families.

Family	Ave. Total No.of vertebrae	Percentage (%) of thoracic vertebrae	Percentage (%) of lumbar vertebrae
Balaenidae	55-57	24-25	21-22
Neobalaenidae	41	42	5
Eschrichtiidae	56	25	21
Balaenopteridae	48-64	23	21-23
Ziphidae	46-49	20-22	20-29
Platanistidae	40-51	23-26	13-16
Monodontidae	53-54	22-23	15
Physeteridae	50-56	22-23	16
Stenidae	51-67	19-24	22-24
Phocoenidae	62-97	17-19	18-27
Globicephalidae	50-82	17-20	21-26
Delphinidae	63-92	16-19	22-32

It is possible that the unique development and construction of the thorax of *C. marginata* are associated with the unusual positioning and ontogenetic development of the laryngeal sac (as seen in the adult male). Beddard's (1901) hypothesis in fact could be modified to refer to expansion of the laryngeal sac rather than the lungs.

4.3 Phylogeny and functions of the laryngeal sac

Barnes & McLeod (1984) are of the opinion that the order Cetacea is a monophyletic evolutionary group, in which the two modern suborders Odontoceti and Mysticeti both have their ultimate ancestries within the primitive suborder Archaeoceti. Unfortunately there are no fossils of *C. marginata* and therefore no palaeontological data on its familial interrelationships are available. However the fossil record of Balaenopteridae, the family under which *B. acutorostrata* falls, extends back about 10-12 million years ago to the Late Miocene (Barnes & McLeod 1984). According to Arnason & Best (1991), *C. marginata* is genetically closer to Balaenopteridae than to Balaenidae.

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The positioning and marked ontogenetic development of the laryngeal sac in *C. marginata* differ significantly from the laryngeal apparatus of *B. acutorostrata* (or any other mysticete described to date). This leads one to speculate whether the functioning of the apparatus differs in *C. marginata* compared to other mysticete species.

"We must consider any such supposition as that the water may penetrate from the cavity of the mouth into the respiratory canals, and particularly into the tracheal bag on the larynx, and be again expelled from thence through the nostrils, to be quite improbable" (Eschricht & Reinhardt 1866). The direct contact the laryngeal sac has with the trachea implies that water entering this sac would inevitably also enter the trachea and thus the lungs. The function of the laryngeal sac, as mentioned in the introduction, has therefore been narrowed down to three main possibilities involving the movement or storage of air.

4.3.1 Exclusion of water and food from the respiratory canal

The justification given for the sac as a mechanism to preclude the entrance of water and food into the respiratory canal (*vide* page 8) is quite improbable; in order to successfully execute a "blast of air" at the same time that food and water are ingested, as proposed by Rawitz (1900 in Hosokawa 1950), the timing of these activities would have to be very precise. It would also mean that feeding would be a function of sac inflation. Exhalation of air during feeding is also not an obvious feature in Balaenopterids (Best, pers. comm.). Finally, this proposed function of the sac is generally believed to be undertaken by the epiglottis.

4.3.2 Complete utilization of oxygen in inspired air

Schulte's (1916) speculation that contraction and relaxation of the laryngeal sac during submergence would cause a circulation of air in the trachea and bronchi and thereby encourage oxygen absorption (*vide* page 9) is unlikely when Scholander's (1940) proposal that alveolar collapse occurs in diving mammals at about 100m, is taken into consideration. Such collapse prohibits gaseous exchange during deep dives and possibly reduces the risk of the bends and nitrogen narcosis. Although there are no available data on the dive depths of *B. acutorostrata* and *C. marginata*, data have been collected for a few mysticete species. Maximum dive depths of

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272-306 m have been recorded for *E. glacialis* for a period of 2 minutes (Mate, Nieukirk, Mesecar & Martin 1992) and a harpooned *B. physalus* has been recorded diving to 355 m for a period of 20 minutes (Scholander 1940). As the vertical distribution of zooplankton in the world's oceans extends well below 100 m (Van der Spoel & Heyman 1983), it is not unreasonable to assume that most mysticetes can dive to depths greater than 100m.

Based on the laryngeal sac's position as an integral part of the ventral tracheal wall that bulges dorsally into the tracheal lumen, Haldiman & Tarpley (1993) concluded that enlargement of the sac in *B. mysticetus* would close off the trachea. Except for *C. marginata*, this positioning of the laryngeal sac has been found in all studies conducted on mysticetes (Eschricht & Reinhardt 1866; Carte & Macalister 1868; Turner 1872; Beauregard & Boulart 1882; Dubois 1886; Benham 1901; Schulte 1916; Hosokawa 1950; Yablokov, Bel'kovich & Borisov 1974; Quayle 1991; Schoenfuss & Hillmann 1996). The "re-breathing" of air as suggested by Negus (1962), whereby the sac might act as an air reservoir so that when the animal is submerged for a prolonged period of time, the used-up air which has been in contact with the pulmonary epithelium would mix with the relatively unused air which has lain in the sac, is therefore questionable in mysticetes due to the fact that the used-up air which has been in contact with the pulmonary epithelium would not be able to move into the laryngeal sac if it was inflated.

The size of the laryngeal sac should also be considered. Although previous studies of the laryngeal apparatus of various mysticete species involved foetal or juvenile material, the adult *B. acutorostrata* specimens available for this study had laryngeal sacs averaging 25.7 cm in length and 18.2 cm in width. Keeping Boyle's Law in mind (that the volume of a given quantity of gas varies inversely as the pressure on it), it seems that this structure would have to be a lot larger if it was to play any significant role in air storage for more efficient oxygen usage.

4.3.3 Phonation

Several authors support the hypothesis that the laryngeal sac is involved in phonation, and have suggested many possible ways in which this is achieved (*vide* page 9). Turner (1872) hypothesised that the processi vocales of the approximated arytenoid cartilages might be made

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to vibrate, using air forced from the laryngeal sac. However, although the posterior ends of the processi vocales are united only by connective tissue, while at the same time forming the dorsal wall of the laryngeal sac with the rest of the arytenoid cartilages, it is not clear how much vibration can actually be achieved by the portions of these cartilages.

Experiments performed by Schoenfuss & Hillmann (1996) with laminar air flow through the laryngeal diverticulum, and histological as well as anatomical investigations of this structure, in *B. mysticetus*, strongly suggest a sound-producing function. These authors found that when air is forced out of the laryngeal diverticulum, by contraction of the powerful *M. diverticulum laryngeus* (proposed), vibration along the leading edge of the flap (arytenoid lips) produces a high pitched sound. Unfortunately no sounds were recorded during the laminar air flow experiment conducted in this study, but the laryngeal sac inflated, as did the lungs, when air was introduced down the nasopharynx of *C. marginata*, an occurrence which can safely be expected to occur in *B. acutorostrata* considering the large glottis which opens directly into both the laryngeal sac and the trachea.

Schulte (1916) and Carte & Macalister (1868) describe the arytenoid lips as being homologous with the phonatory "arytenoid fold" of ungulate mammals. Purves (1967) states that the functionality of the arytenoid lips in the adult is indicated by "its isogonic [sic] growth in diameter and thickness, and its situation at the apex of the glottis gives support to the notion that the phonatory region of the larynx, is, as in the Odontocete, at the tip of the epiglottic spout".

Quayle (1991) states that the larynx seems too complicated a structure to be a simple valve and the remarkable similarity between the human and whale glottic inlets suggests that it is morphologically capable of phonation.

Sound is produced by the interruption of the flow of air which results in sound waves consisting of alternating compressions and rarefactions that are related to the displacement of molecules (Denny 1993). As such, the larynx provides animals, which possess a pulmonary system, with a convenient method of phonation (Negus 1962). This is also true for cetaceans. However, since most underwater sounds in cetacea are produced without the release of air, the passage of air

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across the larynx must involve some means of temporary storage and recycling of air back into the lungs/trachea.

In this study of the laryngeal apparatus of *B. acutorostrata*, the stratified squamosal nature of the epithelium lining the arytenoid lips, the highly vascularised and innervated muscles of the laryngeal sac and the spiral nature of its blood vessels and nerves, indicate that (a) the arytenoid lips are able to withstand strain caused by the movement of air over the larynx, and (b) the laryngeal sac is able to expand and contract when air is passed into it, allowing for the recycling of air to occur.

Clark (1983) noted that songs of *B. mysticetus*, which may be produced continuously for 154 seconds, have alternating louder and softer portions that may represent recycling of air. *M. novaeangliae* do not produce bubbles (Silber 1986) during the 7-30 minutes that they are submerged and singing (Payne & McVay 1971); so that recycling of air is likely in that species (Edds, Odell & Tershy 1993). Ridgway (in Edds *et. al.* 1993) observed that during vocalisation, young *T. truncatus* often released bubbles, but that adults do so rarely. He suggested that dolphins learn to recycle air. Although bubble release during vocalisation is not common in *M. novaeangliae* (Watkins 1967; Silber in Edds *et. al.* 1993) and *E. australis* (Clark 1982), bubble production has been observed in specific behavioural contexts as well as during particular sound types (Clark 1983; Silber 1986). Edds *et. al.* (1993) observed air being expelled during phonation by a juvenile *B. edeni*. These authors state that if recycling of air is common during repeated or extended vocalisations by mysticetes, it is possible that the skill is learned, and had not been mastered by the captive juvenile *B. edeni*, or alternatively, the recycling mechanism may not be functional in young animals for developmental or maturational reasons.

In *B. acutorostrata* as well as in *C. marginata* the components of the laryngeal apparatus were all present in the juveniles, but were larger and better developed in the adults. For this reason it seems likely that the apparatus is functional making it possible for the juveniles to begin the process of learning how to phonate. This skill might be developed with age as the laryngeal apparatus matures.

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The sounds reported for *C. marginata* by Dawbin & Cato (1992) were of very low frequency, with no high pitched sounds being reported. Although this recording was made from a juvenile (with a possibly reduced repertoire compared to an adult), it is perhaps significant that this study has shown that adult male (and presumably adult female) *C. marginata* do not possess arytenoid lips.

I therefore agree in principle with Purves (1967) and Schoenfuss & Hillmann (1996), that the laryngeal sac as well as the arytenoid lips are implicated in phonation (in animals in which they are found). The force of air from the laryngeal sac through the arytenoid lips may produce high pitched sounds, as indicated by the latter authors, but may well be responsible for other sounds in the live animals, for example, as the air is recycled or redirected to the trachea/lungs. However, I am also of the opinion that Quayle's (1991) hypothesis whereby the air columns in the laryngeal sac and nasopharynx vibrate due to air from the laryngeal sac (as well as the recycled air redirected to the trachea/lungs) being forced between the arytenoids, causing pressure fluctuations that are transmitted through the soft tissues of the whale into the surrounding water, could also provide for the majority of the mysticete repertoire. This hypothesis is supported by the incredible development of the laryngeal sac in adult *C. marginata* which, through expansion and contraction, would allow the recycling of large amounts of air for phonation.

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CHAPTER 5: CONCLUSION

This study adds to the previous descriptions of the laryngeal apparatus of *B. acutorostrata* by including both adult and juvenile specimens of both sexes, and by adding descriptions of the fine anatomy. The larynx of *C. marginata* has not been described previously, nor has the striking ontogenetic development of the laryngeal sac in any baleen whale, as most dissections have been of foetal or juvenile material.

"We know how unsafe it is , both generally and in respect to these organs in particular, to make inferences from parts as they appear in the dead body, as to their appearance during the life of the animal" (Eschricht & Reinhardt 1866).

The above statement summarises one of the greatest hurdles that anatomists have to overcome. For cetacean biologists, due to the inaccesibility of live mysticetes and the general difficulty of obtaining fresh mysticete material, it makes the hurdle an even more daunting one.

Certainly this is the case with regards to the laryngeal sac found in both *B. acutorostrata* and *C. marginata*. The ontogenetic development of the laryngeal sac seen in both species indicates that this structure is functional. The muscular nature and histological organisation of the laryngeal sac in both species is such that it also seems very probable that this structure undergoes expansion and contraction.

Although the positioning of the laryngeal sac differs markedly between *B. acutorostrata* and *C. marginata*, in both of these species the sac is associated with the trachea through non-valvular openings, necessitating that water not be introduced into the sac. This association together with all the characteristics listed above strongly suggest that the laryngeal sac plays a role in sound production, probably through the recycling of air during submergence. The force of air from the laryngeal sac through the arytenoid lips may produce high pitched sounds, but may well be responsible for other sounds in the live animals, for example, as the air is recycled or redirected to the trachea/lungs. The vibrations of the air columns in the laryngeal sac and nasopharynx caused by air from the laryngeal sac (as well as the recycled air redirected to the trachea/lungs)

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being forced between the arytenoids, causing pressure fluctuations that are transmitted through the soft tissues of the whale into the surrounding water, could also provide for the majority of the mysticete repertoire.

The size of the sac also differs in the respective species. This feature is directly related to the positioning of the sac i.e. the integration of the sac into the laryngeal apparatus of *B. acutorostrata* limits the extent to which the sac can grow and expand, whereas in *C. marginata*, the "separation" of the laryngeal sac from the tracheal rings as well as the thoracic development of this species allows the sac to grow to the enormous size it attains in the adult male. This development in *C. marginata*, unmatched by any other cetacean may also allow the sac of the adult animal to expand more freely.

Experimental confirmation of the role of the laryngeal sac in phonation really requires access to a live animal that is temporarily or permanently in captivity. Juvenile *C. marginata* have been caught from the beach with seine nets on several occasions in South African waters (Ross, Best & Donnelly 1975), and are nutritionally independent at 3 m in length (Sekiguchi, Best & Kaczmaruk 1992). Adults only grow to 6 m in length; many captive killer whales are substantially larger than this. Furthermore, recordings of sounds from adult free-living *C. marginata* are needed, and these might throw some light on the reason for the unique development of the laryngeal sac and thorax in this species.

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SUMMARY

The larynx of the pygmy right whale, *Caperea marginata* (Gray 1846), has not been described previously. The acquisition of laryngeal material from four pygmy right whales, *C. marginata*, revealed marked differences in structure from that of other mysticetes, including the separation of the laryngeal sac from the tracheal wall and its striking development in the adult male. To investigate the ontogeny and possible sexual dimorphism of the laryngeal apparatus in another mysticete, material from adult and juvenile minke whales, *B. acutorostrata* (Lacépède 1804) of both sexes was obtained.

The most essential peculiarity of the larynx of mysticetes, as compared with that of the odontocetes, is in its allowing the mucous membrane of the respiratory canals, by means of an opening on the ventral surface, to appear in the form of a sac with an exterior covering of a strong layer of muscles.

The laryngeal sacs described in other mysticetes by various authors seem to follow the same general pattern, *B. acutorostrata* and *C. marginata* included, and all agree that the well vascularised and innervated muscular structure of the wall of the laryngeal sacs could allow for extension and contraction of the sacs.

The laryngeal sac found in *C. marginata* differs in position from that in *B. acutorostrata* (or any other mysticete so far described) in that it is completely separate from the tracheal rings, does not open into the trachea through the glottis and lies to the right hand side of the animal.

The ontogenetic development of the laryngeal sac of *B. acutorostrata* supports the theory that the structure is functional, at least in adults. Distinctive ontogenetic development of the laryngeal sac occurs in *C. marginata*, with the adult's sac being almost five times the size of those found in the juveniles. Coiled nerves and blood vessels found in the laryngeal sac of *C. marginata* further support the expansion and contraction theory.

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The function of the laryngeal sac has been narrowed down to involving the movement or storage of air. Laminar air flow experiments through the laryngeal sac, and histological as well as anatomical investigations of this structure, strongly suggest a role in sound production, probably through the recycling of air during submergence.

The most likely site of sound production is considered to be the arytenoid cartilages and associated structures. The arytenoid lips as observed in minke whales in this study, formed by continuation of the mucosa which lines the arytenoid cartilages, have not been previously described in this species. The stratified squamosal character of the epithelial layer of the lips suggests that these structures may be exposed to considerable strain possibly during respiration or phonation.

The arytenoid cartilages of *C. marginata* are each made up of two components, which fuse anteriorly to form the arytenoid bodies, no arytenoid lips are present at the tips of the arytenoid bodies and the processi vocales do not extend to form the dorsal wall of the laryngeal sac. The absence of arytenoid lips suggest that this species may be evolving towards a non-involvement of the arytenoid lip structure in the process of phonation (similar to what has occurred in odontocetes).

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SAMEVATTING

Die larinks van die dwergnoordkaper, *Caperea marginata* (Gray 1846) is nog nie voorheen beskryf nie. Die verkryging van larinks-materiaal van vier dwergnoordkapers het aansienlike verskille in struktuur van dié van ander Mysticeti getoon, insluitende die skeiding tussen die larinkssak en die tragea wand en die opvallende ontwikkeling daarvan in die manlike volwassene.

Ten einde die ontogenie en moontlike seksuele tweevormigheid van die larinks-apparatuur in h ander lid van die Mysticeti te bestudeer, is materiaal van ander volwasse en jong minke-walvisse, Balaenoptera acutorostrata (Lacépède 1804) van albei gelsagte verkry.

Die mees wesentlike besonderheid van die larinks van die Mysticeti, soos vergelyk met dié van die Odontoceti, is die feit dat die slymvleis van die asemhalingskanale, deur middel van h opening in die ventrale oppervlakte, verskyn as h sak met h buitenste deklaag van sterk spiere.

Die larinkssak by ander Mysticeti, soos deur verskillende skrywers beskryf, skyn dieselfde patroon te volg, *B. acutorostrata* en *C. marginata* ingesluit, en almal stem saam dat die vaatryke en besenude spierstruktuur van die wande van larinkssakke die verlenging en saamtrekking van die sakke moontlik kan maak.

Die larinkssak by *C. marginata* verskil in posisie van dié by *B. acutorostrata* (of enige ander lid van die Mysticeti tot dusver beskryf) omdat dit heeltemaal van die lugpypringe geskei is, nie deur die stemspleet in die lugpyp open nie, en aan die regterkant van die dier voorkom.

Die ontogeniese ontwikkeling van die larinkssak van *B. acutorostrata* steun die teorie dat die struktuur funksioneel is, ten minste by volwassenes. Kenmerkende ontogeniese ontwikkeling van die larinkssak kom voor by *C. marginata* en die volwassene se sak is byna vyf keer groter as die van jong diere. Gekrulde senuwees en bloedvate in die larinkssak van *C. marginata* steun die uitsettings en sametrekkings teorie verder.

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Die funksie van die larinkssak kom neer op die beweging en berging van lug. Egalige lugvloei-eksperimente deur die larinkssak en histologiese sowel as anatomiese studies van hierdie struktuur, dui sterk op 'n rol in klankproduksie, waarskynlik deur middel van die hersirkulering van lug wanneer die dier onder water duik.

Die mees waarskynlike ligging van klankproduksie is die gieterkraakbene en geassosierde strukture. Die gieterkraakbeen-"lippe" wat by minke-walvisse in hierdie studie waargeneem is, en gevorm word deur die voortsetting van die slymvleis waarmee die gieterkraakbeen gevoer is, is nog nie voorheen vir hierdie spesie beskryf nie. Die gelaagde skubagtige aard van die epiteel en die "lippe" dui daarop dat hierdie strukture aan aansienlike spanning blootgestel word, waarskynlik tydens asemhaling of fonering.

Die gieterkraakbene van *C. marginata* bestaan elk uit twee komponente wat voor saamsmelt om the gieterkraakbeen-liggaampies to vorm. Geen "lippe" kom op die punte van die liggaampies voor nie en die "processi vocales" is nie verleng om die dorsale wand van die larinkssak te vorm nie. Die afwesigheid van gieterkraakbeen-"lippe" dui daarop dat hierdie spesie mag ontwikkel tot soortgelyke onbetrokkenheid van die gieterkraakbeen lipstrukture by die proses van fonering (soon wat by die Odontoceti gebeur het).

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