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FUNCTIONAL ANATOMY AND HISTOLOGY OF THE NASAL SAC SYSTEM IN ODONTOCETES
(SUPERFAMILY DELPHINOIDEA)

(Original title: Anatomia e histologia funcionales
del sistema de sacos nasales en los odontocetos (superfamilia Delphinoidea))

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Although this PhD thesis was defended in the year 1998, personal commitments and limitations have led me to postpone publishing the different scientific advances the dissertation carried at that time. Since then, several papers have appeared, giving light to the complex anatomy of the odontocete sound production structures and mechanisms, while the main topics of what was presented in this dissertation are still unrevealed. This, and the fact that it was originally written in Spanish, made me decide to translate it into English to allow the scientific community access to its content. I would like to point out that there was no digital imagery at that time, as all was based on conventional photography and slides. I really think and hope the current publication will be useful for other anatomists or scientists in general, looking for a deeper insight into this subject. I would like to thank Steffen de Vreese for the translation, h, as he did not just translate the dissertation into English but needed a comprehensive understanding of the morphology and function described for a better interpretation and writing.

I. INTRODUCTION

Marine mammals have attracted attention since ancient times, and they are embedded part of culture and tradition of people that live by the sea. These animals have been shown to have an enormous appeal on human beings, and, although they are abundantly present or referred to in stories, it has been noted that there still is a lot we do not know about the many different species of aquatic mammals, mainly because they live in an aquatic environment, relatively out of our reach.

Thus, although these animals are present in our culture, they only seem to get attention whenever there are incidents involving the death of numerous individuals or even the extinction of a species. These saddening and unfortunate situations highlight the concern about aquatic mammals, and more specifically cetaceans, and suppose an endless number of positive points to elaborate the knowledge about these great unknowns. These kind of alarm bells create a social impact that leads to a collective awareness of their existence, and likewise, the general interest grows, entailing a shift in the dedication of the animals' state, both in number of people as in time spent, which means, ultimately, achieving results in all areas that involve a large increase in the knowledge of this group of species.

A similar situation occurred in the western Mediterranean Sea where there was an outbreak of morbillivirus in striped dolphins (*Stenella coeruleoalba*) in 1990-91. This thesis can be considered to be a direct fallout of the death of hundreds of dolphins on the Catalan coast during the two years of the epidemic. The interest in the disease allowed to get a glimpse of the lack of profound knowledge, in our case anatomical knowledge, of this species and cetaceans in general.

How the anatomical mechanisms in delphinids have adapted to an underwater environment is truly remarkable, especially when looking at the development of the nasal region and how it is involved in (ultrasonic) sound production. The lack of knowledge of this anatomical region and its relation to sound production has led us to raise this doctoral thesis as an eminent morphological study on the nasal region in delphinids and more specifically in the striped dolphin, studying the functionality from an anatomical point of view. Moreover, we also take into account other odontocete species from a comparative point of view.

During the last decade there has been an abrupt development of equipment and techniques used in cetacean research. Anatomical studies have changed from conventional necropsy as the only source of information to the use of modern techniques like electron microscopy imaging, radiology and medical image processing. However, most investigations have been carried out without a deep analysis of structures and tissues that make up the different anatomical regions. This is the case for the nasal region, hence this study's approach of being an anatomical study based on both conventional dissection techniques and modern techniques of the structures in this region. It is not solely a quantitative or qualitative comprehensive study of the structure and ultrastructure of every corner of the entire nasal sac system, but rather an overview of the different structural properties of the region aiming to establish a link between anatomy and function.

As the nasal region comprises many different anatomical structures, so is this report structured in different chapters. We try to discuss each of these structures separately, while knowing that in

order to understand the functionality of the entire nasal sac system it should be regarded as one very complex unit. Only as such, we can draw conclusions that contribute to a better understanding of the functional morphology of the production of ultrasonic sounds in odontocetes.

Unfortunately, one of the major limiting factors in the development of this work has been the difficulty of obtaining study material. All species in this study are in the Delphinoidea superfamily of the Odontoceti suborder. Moreover, we intend to look for homologies within the entire suborder, but only in literature (Table 1).

II. LITERATURE REVIEW

1. SHORT HISTORICAL REVIEW

Over the last two centuries, the nasal region of toothed whales has drawn the attention of many anatomists and cetologists. The first anatomical descriptions of this region date back to the seventeenth century, when Ray (1671) reported the anatomical features that were observed during dissection of the harbour porpoise (*Phocoena phocoena*) and shined the first light on the complexity of the nasal tract of these marine mammals, mentioning a possible olfactory function. Although there are other works of that time (Tyson, 1680; Hunter 1787), the first detailed descriptions were not made until the nineteenth century (Von Baer, 1826; Cuvier, 1836; Sibson, 1846; Murie 1870, 1873; Kükenthal, 1893). These works were the fruit of painstaking dissections and according anatomical drawings, where the nasal region was almost every time a part of the general study, and in some cases the anatomy of the nasal region was the main focus (Von Baer, 1928). In the 20th century, research looked at possible homologies between species, taking ideas from the evolution of the nasal structures of cetaceans in comparison to the nasal tract of land mammals. Some authors considered the odontocete nasal sac system to have originated from the ethmoturbinalia, present in these species as vestigial olfactory structures (Von Baer, 1826; Sibson, 1846; Kükenthal, 1893). On the other hand, Murie (1873) expressed his disagreement with these theories as he did not find any structures that allowed for such a deduction. Moreover, all research that was published in the nineteenth century considered the nasal sac system to be only a part of the respiratory system, closed during apnoea and open during in- and expiration. Regarding the closing of the nasal tract, there was controversy on whether this occurred actively through muscle contractions (Cuvier, 1836), or passively as an elastic system (Von Baer, 1826). This discrepancy marked the studies that were conducted during the first half of the twentieth century, in which work was continued in the same vein as in the previous century. Detailed dissections allowed for gaining an in depth knowledge of the nasal sacs' morphology, their nature and their arrangement, as well as of the surrounding muscle layers (Rawitz, 1900. Gruhl, 1911; Gallardo, 1913; Keman and Schulte 1918; Kellogg, 1928; Raven and Gegory, 1933; Huber, 1934; Kellogg, 1938). However, despite the large number of studies conducted, there was great variation among the authors regarding the description of the nasal tract, nasal sacs and associated musculature, not bequeathing to common results, nor in odontocetes in general, nor in a single species. During this first half of the century, in search for an evolutionary pattern in the nasal sac system, more homologies between marine and terrestrial mammals were found. Notably, Huber (1934) proposed that the nasal musculature of odontocetes had its origin in the maxillonasolabial muscular complex of quadrupeds. Later, Lawrence and Schevill (1956) conducted a comprehensive comparative study of the nasal anatomy of the dolphin family, with the bottlenose dolphin (*Tursiops truncatus*) as benchmark. They described the arrangement of the nasal musculature in depth, identifying the different muscles and attempting a description of the muscle origins and insertions, thereby contributing to a leap forward regarding the conclusions of the functionality of the nasal sac system. Moreover, an *in vivo* study on the opening and closing of the system concluded that this is a resilient seal system, which was reinforced by the pneumatic effect of the air sacs (Lawrence and Schevill, 1956).

It is surprising that up to that point, there were few references about the possible functional implications of the nasal system, which, just by looking at its complexity, should give rise to the consideration that it serves more elaborate purposes than being a simple locking mechanism in between breaths.

There are many works that attribute a sound production capacity to different species of toothed whales (reviewed by Schevill, 1964), but it was not until 1947, when a flight response of a group of common dolphins was seen as a reaction to the echo-sounder of a boat, that research would start to consider that odontocetes could emit sounds with frequencies above the human receptive capabilities (Fraser, 1947).

In the fifties, research on anatomy of the upper respiratory tract became especially important. Following the works published by Kellog and Kohler in 1952 and Schevill and Lawrence in 1953, in which it became evident that toothed whales produce and are sensitive to ultrasonic sound, it was also discovered that they have the ability to echolocate. This use of high frequency sounds for 'seeing' objects was demonstrated in experiments with the bottlenose dolphin, in which the blindfolded animal was capable of locating and recognizing different targets by using ultrasonic sound (Norris, 1961). Once the hypothesis was confirmed, ruminations arose about the place of sound production, which marked a controversy that continued during the second half of the twentieth century until present work. There were two main streams of hypotheses on the organ of sound origin: the larynx, as forwarded by Schevill and Lawrence in 1956, and the nasal region, as advocated by Norris (1961).

Once this controversy was established, studies followed, analyzing different aspects of anatomy, functionality and bio-acoustic properties of the nasal region in different species of odontocetes, and all of them using new technologies as they became available. So from then on, the anatomy of the nasal region was extensively studied by macroscopic dissection techniques to that covered many species of toothed whales (Schenkkan, 1972, 1973; Mead, 1975; Heyning, 1989; Curry, 1992). The works of Schenkkan (1973) and Mead (1975) are of particular relevance, as they presented results of a broad comparative study. Also noteworthy was the emergence of a considerable number of different methodologies for anatomical studies, from the use of serial sections (Hosokawa, 1965; Green et al., 1980), molds of the nasal sacs (Schenkkan, 1973; Dormer, 1979; Gurevich, 1980) and cryosections with subsequent three-dimensional computer recomposition using image analysis (Amundin et al, 1992), to the most advanced imaging technologies, such as computer tomography (CT) (Brouwers et al., 1990; García Hartmann et al, 1996) and (nuclear) magnetic resonance imaging ((n)MRI) (Cranford, 1988, 1992; Cranford et al., 1996). A compendium of all methodologies used for studying the odontocete facial anatomy can be found in Cranford et al. (1996). Likewise, measuring things such as air pressure in the cavities, muscular electrical activity, movement, etc., in combination with simultaneous recordings of the sound that was being produced, deepened the knowledge of the functional mechanisms; in this way, experimental studies have, for example, been able to measure the pressure conditions that occur in the upper respiratory tract during phonation and respiration processes (Ridgway and Carder, 1998); electromyography has been used to detect muscle contractions during sound production (Amundin and Andersen, 1983; Ridgway et al, 1980), and X-rays (Hollien et al., 1976;. Dormer, 1979) and ultrasonography (Mackay, 1980, 1988; Mackay and Liaw, 1981) to observe movements of the nasal sacs.

The anatomy of the laryngeal region has also received special attention and was studied mainly by means of dissections and serial sections (Hosokawa, 1950; Lawrence and Schevill, 1965; Purves, 1966. Green et al, 1980; Reidenberg and Laitman, 1987, 1988); Furthermore, several experimental studies attributed the birthplace of phonation to this region (Purves, 1966; Busnel and Fish, 1980; Pilleri, 1983; Purves and Pilleri, 1983).

The arguments that allocate either the nasal region or the larynx as the place of sound production, have been reviewed extensively by Schenkkan (1973), Morris (1986) and Amundin (1991a). Although there have been several acoustic studies on sound production and transmission mechanisms of the larynx (Purves, 1966; Purves and Pilleri, 1983), experimental studies demonstrating motility and pressure distribution of the nasal tract and nasal sacs (Norris et al, 1971;. Hollien et al, 1976; Dormer, 1979; Ridgway et al., 1980; Liaw Mackaw and 1981; Amundin and Andersen, 1983; Ridgway and Carder, 1988) provided strong evidence to support the hypothesis, raised by the latter group of authors, among others, that phonation phenomena originate in the nasal tract itself.

While there have been several studies on the functional anatomy of the upper respiratory tract in relation to sound production, there have been many more on the physics and acoustic field of the actual ultrasonic sound that is being produced by different species, including how it could be used to detect a target and discriminate between targets, and how these sounds could be used for communication (reviewed by Morris, 1983; Au, 11993; Richardson et al., 1995). However, this knowledge of the physics of sound has not been used extensively to understand the function of the nasal region. Until present work, it is maybe only Cranford et al. (1996) who have established the first relationship between the sound characteristics and morphology of the presumed production / emission areas from a comparative point of view between species. Despite the growing number of studies on this matter, there is still a gap in the relation between anatomical and physics studies.

On the other hand, the facial anatomy of a variety of species of toothed whales has been described (Schenkkan, 1973; Mead, 1975), but only few studies refer to the species *Stenella coeruleoalba* (Hosokawa and Kamiya, 1965) and none of them carried out a histological study. One can say that in general there is a lack of both histological and ultrastructural studies to facilitate the understanding of and integration between different macroscopic structures observed in conventional dissections and through imaging techniques such as ultrasonography, radiographic techniques and MRI scans, as has been demonstrated in recent studies by Cranford et al. (1996).

There are a considerable number of species in the odontocete suborder (Table 1), and depending on the species, there is a great discrepancy among the results of the anatomical, functional or acoustic studies, which are a source of controversy (Schenkkan, 1973; Mead, 1975; Cranford et al, 1996).

CLASIFICACION DE LOS ODONTOCETOS		Estudios Anatómicos
Suborden Odontoceti		
Superfamilia Physeteroidea		
Familia Physeteridae		
Physeter	(Cachalote)	11,20,21,34,39
Kogia	(Cachalote pigmeo, C. enano)	10,11,20,21,30,34,39
Superfamilia Ziphioidea		
Familia Zhipiidae		
Zhipius	(Zifio común)	34
Tasmacetus	(Mesoplodon de Sheperd)	
Berardius	(Berardio de Baird...)	34
Hyperoodon	(Ballenato de hocico...)	20
Mesoplodon	(Mesoplodon	20,34,39
Superfamilia Platanistoidea		
Familia Platanistidae		
Platanista	(Delfín del Indo,D. del Ganges)	23,28
Superfamilia Inoidea		
Familia Iniidae		
Lipotes	(Baiji)	23,27
Inia	(Boto) 23,24,34	
Pontoporia	(Franciscana)	18,20,23,34,39
Superfamilia Delphinoidea		
Familia Monodontidae		
Delphinapterus	(Beluga)	8,23,34,39
Monodon	(Narval)	12,23
Familia Phocoenidae		
Phocoena	(Marsopa común,Vaquita...)	1,2,6,7,8,16,17,20,22,23,29,34,35,37,39
Australophocaena	(Marsopa de anteojos)	9
Neophocaena	(Marsopa sin aleta)	23,32,33
Phocoenoides	(Mardopa de Dall)	23,34,37,39
Familia Delphinidae		
Lagenorhynchus	(D. flancos blancos...)	4,9,13,17,20,23,34,36,39
Grampus	(Calderón común)	3,23,34,39
Globicephala	(Calderón común, C. tropical)	5,13,23
Tursiops	(Delfín mular)	8,12,13,17,19,20,22,23,25,26,34,38,39
Steno	(Delfín de dientes rugosos)	15,23,28
Stenella	(Delfín listado,de hocico largo...)	14,18, 20, 23,25,31,39
Delphinus	(Delfín común)	8,13, 17,20, 22,23,34,39
Lagenodelphis	(Delfín de Fraser)	23
Peponocephala	(Falsa orca cabeza de melón)	
Orcinus	(Orca)	20, 23,34,39
Pseudorca	(Falsa orca)	22,34,39
Orcaella	(Delfín de Irrawaddy)	
Feresa	(Orca pigmea)	
Sotalia	(Tucuxi)	17,20, 23
Lissodelphys	(Delfín sin aleta del norte/sur)	20, 23,34,39
Cephalorhynchus	(Delfín de Commerson...)	20,23,29,34,35

TABLA 1.Clasificación de los odontocetos existentes en la actualidad según Heyning (1989) y revisión bibliográfica de los trabajos sobre la morfología de la región nasal para los diferentes géneros. Lista de autores:1- Von Baer (1826); 2- Sibson (1848); 3- Murie (1870a); 4- Murie (1870b); 5- Murie (1873); 6- Kükenthal (1893); 7- Rawitz (1900); 8- Gruhl (1911); 9- Gallardo (1913); 10- Kernan y Schulte (1918); 11- Raven y Gregory (1933); 12- Huber (1934); 13- Lawrence y Schevill (1956); 14- Hosokawa y Kamiya (1965); 15- Purves (1966); 16- Moris (1969); 17- Schenkkan (1971); 18- Schenkkan (1972); 19- Evans y Maderson (1973); 20- Schenkkan (1973); 21- Schenkkan y Purves (1973); 22- Khomenko (1974); 23- Mead (1975); 24- Schenkkan (1977); 25- Dormer (1979); 26- Green y col. (1980); 27- Peixun y col. (1980); 28- Purves y Pillery (1983); 29- Amundin y col. (1988); 30- Carvan (1988); 31- Cranford (1988); 32- Gao y Zhou (1988); 33- Gao y Zhou (1989); 34- Heyning (1989); 35- Amundin y Cranford (1990); 36- Browers y col. (1990); 37- Curry (1992); 38- Rodionov y Markov (1992); 39- Cranford y col. (1996).

2. DESCRIPTIVE ANATOMY

The nasal region is structured around a single nasal tract in the centre, surrounded by a variable number of paired blind diverticula or nasal sacs, which collectively is called the nasal sac system. These sacs are located on both sides of the nasal tract, between the single outer nasal aperture or blowhole, and the paired external bony nares. The nasal tract runs perpendicular to the longitudinal axis of the body so that the blowhole is positioned dorsal to the nasal region, and caudal to the melon, which consists mainly of adipose tissue and gives the odontocetes their characteristic 'bulged forehead' look. This part of the upper respiratory tract is surrounded by a well-developed facial musculature and a complex arrangement of structures of connective and adipose tissue. In this review, we will present the current state of knowledge on this region together with a description of the nasal sac system; then follows the musculoskeletal system, of which not only the morphology is a subject of this study, but also the evolutionary changes this region has been subjected to in adaptation to the aquatic environment; thirdly we will describe the different adipose and connective tissue structures that play a substantial role in the functionality of the region; furthermore, the epithelial lining of the nasal spaces and the innervation and nasal asymmetry will be discussed, as these are unique for odontocetes. Finally, we will discuss the frequently used terminology that differs from the terminology used in Veterinary Anatomy as reflected in the Nomina Anatomica Veterinaria.

It is not the intention of this thesis to deal with interspecific differences in extent, inter alia because of the inability to provide functional evidence for previously described interspecific differences.

2.1. Nasal sac system

A paramedian section reveals the complexity of the nasal tract, which is closed in resting position, and takes on a sinuous path with interdigitating pro- and recesses. During respiration, the anterograde movement of the tract's anterior wall minimizes the windings and curves, leading to an apparently smooth walled cylindrical conduit between blowhole and external bony nares (Plate 19).

It is difficult to understand and describe the functional morphology of this system as it is very complex and shows significant interspecific differences. Excluding the early descriptions made during the first half of the century, we must mention the importance of the works of Lawrence and Schevill (1956), Schenkkan (1973) and Mead (1975).

The nasal tract opens to the exterior via the blowhole, a crescent-shaped hole that features two lips, one anterior and one posterior, which have intimate contact with each other when the blowhole closes during apnoea. The anterior lip has a convex border, is highly mobile and able to move in rostroventral direction, producing the opening of the blowhole, which then becomes an approximately circular hole, while on the other hand, the posterior lip is much more stationary**.**

Delphinids have four pairs of nasal sacs that surround and open into the central nasal tract. From superficial to deep there are the vestibular sacs, the nasofrontal sacs, the accessory sacs and the premaxillary sacs (Plate 1, Figs. A and B; Plate 3, Figs. 1, 3 and 4). Some families of odontocetes feature an additional pair of diverticula, the posterior nasal sacs.

Once passed the blowhole, the nasal tract is centrally delimited by the anterior and posterior lip of the opening, heads in caudoventral direction and features two lateral openings to the vestibular sacs. Some authors have referred to this area as the superior vestibule (Gallardo, 1916) or simply vestibulum (Schenkkan, 1973).

The **vestibular sacs** (Lawrence and Schevill, 1956) are presented in delphinids as lateral expansions of nasal tract and the entrances to the sacs have no clearly defined borders. In most species they are located caudolaterally to the blowhole, although there are exceptions (Schenkkan, 1973). The sacs are flattened dorsoventrally as they follow the facial contours. The sacs' interiors are lined by an epithelium with many small folds that are predominantly oriented transversely to the major axis of the sacs. This property, in combination with the very thin wall of the sac, makes the sacs capable of expanding extensively.

The anterior wall of each sac incurves into the lumen and makes up the **anterior fold of the vestibular sac** (Plate 2, Fig. 2; Plate 4, Fig. 1). This horizontally arranged fold divides the sac and creates a small ventral recess (Mead, 1975). Likewise, in the posterior wall there is the **posterior fold of the vestibular sac**, which is smaller than the anterior fold and fits into the recess created by the latter.

The nasal tract bends towards rostral and runs underneath the bottom of the vestibular sacs, at least underneath their medial halves, until reaching a structure called the **slit-like opening** (Lawrence and Schevill, 1956). This opening is covered dorsally by the anterior folds of the vestibular sacs, and consists of a narrowing of the lumen of the tract by the apposition of the anterior and posterior walls, shaped like a transversely orientated buttonhole (Plate 4, Fig. 3).

Within the slit-like opening there are the **phonic lips** ("museau de singe", Pouchet and Beauregard, 1885), situated on both sides of the midsagittal plane that is marked by the nasal septum and the anterior and posterior walls of the tract (Plate 4, Fig. 6). The lips are visible as a slight prominence of the wall, distinguishable because of a characteristic whitish line that runs transversely in between darker epithelial bands. Over the entire surface of the phonic lips, there are undulations arranged in a straight angle to the transverse axis of the lips (Plate 4, Fig. 6; Plate 16, Fig. 4). The anterior and posterior phonic lips are in intimate contact with each other when the nasal system is closed, or in rest. The phonic lips were first described in the pygmy sperm whale (*Kogia breviceps*) by Pouchet and Beauregard (1885), who named them "museau de singe" which literally means 'monkey muzzle' but got translated to English as "monkey lips", which made a clear reference to their appearance. Schenkkan and Purves (1973) noted the obvious homology between the 'museau de singe' of sperm whales (*Physeter catodon/macrocephalus*) and the phonic lips of delphinids. Recently, Cranford et al. (1996) have described these structures as "monkey lips", referring to a similar shape in all species, and it was proposed that the lips are directly involved in the production of ultrasonic sounds by letting pressurized air pass in between the lips.

The slit-like opening makes up the dorsal limit of the middle part of the nasal tract, which is also referred to as the **spiracular cavity** (Mead, 1975) (Pl. 2, Fig. 2). Here, the tract gets divided by the cartilage of the nasal septum. Further down, the tracts curve in ventrocaudal direction. The axis of rotation of this curve is less for each side the more medial it is, resulting in a tract with an approximately conical shape with its apex situated near the ridge of the nasal septum. The

posterior wall of the cavity is convex and complementary to the concave anterior wall, which allows this cavity to seal perfectly when the muscles relax.

Ventral to the posterior wall, in the most caudoventral part of the curve, are the openings to the **nasofrontal sacs** (Murie, 1871), shaped like transverse buttonholes, and located approximately in the centre of both tracts as they are covered by a ventral prominence of the wall. This space, where the sacs open into the lower part of the nasal tract is called the **inferior vestibule** (Gallardo, 1916), which is a term that is used in reference to phocenids, which feature a common space in which the nasofrontal and posterior nasal sacs open (Gallardo, 1913).

The nasofrontal sacs extend transversely from their inlet towards lateral as they curve around the nasal tract, thus taking on the shape of a horseshoe with a medial aperture, and the anterior part reaching up to the level of the slit-like opening. As such, from the entrance of the sacs, each cavity extends into two directions, making a distinction between the small caudal or posterior part, which has a blind ending close to the sagittal plane and the vertex of the skull, and a rostral or anterior part that extends laterally around the commissures of the slit-like opening and ends in a central cul-de-sac (Plate 1). The walls of the nasofrontal sacs feature many small folds parallel to the major axis of the sac, which is tubular in its posterior part and flattened dorsoventrally in the anterior part.

Lateral to the openings of the nasofrontal sacs, and in most species present in the same indent in the ventral part of the posterior wall of the spiracular cavity, there are the openings to the accessory sacs**.** The **accessory sacs** (Schenkkan, 1973) are the smallest diverticula of the entire nasal sac system, and are shaped like a comma (Plate 1) with a length of just over twice the height of the inlet. The sacs are flattened towards lateral and end blindly in direction of the rostrum.

More ventral, the anterior walls of the spiracular cavities transition into two compact masses that project caudolaterally into the lumen, shaped like a wave with the concave side dorsal, it forms the transverse crest or caudal border of the **nasal plugs** (Pl. 2, Fig. 2) (Lawrence and Schevill, 1956). These nasal plugs are fixed medially to the nasal septum, while they feature a free lateral end or lateral extension. The ventral borders of the plugs head in rostral direction where they lie over the external bony nares, and as such the plugs can act as a shutoff valve for the bony nasal tracts, hence some authors have named them **nasal valves** (Moris, 1969; Rodionov and Markov, 1992). The caudal borders of the nasal plugs, together with their lateral extensions, fit into the indents that contain the openings to the nasofrontal and accessory sacs, so that when the lateral extensions protrude into this indent, the movement of the nasal plugs is restricted, particularly towards lateral and dorsal. During respiration, the nasal plugs retreat in rostral direction so that the nasal tract opens up.

The **premaxillary sacs** (Murie, 1870) have their opening rostral and immediately dorsal to the external bony nares. These sacs are the largest diverticula of the entire nasal sac system, and are located very deep, lining the surface of the premaxillary bones, with a shape that is flattened dorsoventrally (Pl. 1B; Pl. 2; Pl. 3, Fig. 3). As the bottom of the nasal plugs continues in rostral direction, they make up the roof of the premaxillary sacs.

In each of the external bony nares there is a membranous fold that slightly abridges the lumen of the tract in its caudolateral angle (Plate 4, Fig. 6; Plate 14, Fig. 1). These folds are called the

diagonal membranes (Lawrence and Schevill, 1956) and they make contact with the bottom of the nasal plugs when the nasal system is closed (Plate 8, Fig. 5).

The nasal sac system has been studied in detail from a comparative point of view in the family Delphinidae (Lawrence and Schevill, 1956) and other families of odontocetes (Schenkkan, 1973; Mead, 1975) such as Phocoenidae (Moris, 1969; Amundin and Cranford, 1990; Curry, 1992) and Ziphiidae (Heyning, 1989). With the exception of the family of Physeteridae (Pouchet and Beauregard, 1885, Kernan and Schulte, 1918; Schenkkan and Purves 1973), in which few homologies have been found (Cranford et al, 1996) due to the large morphological and evolutionary differences (Milinkovich, 1995), there are generally great similarities between different odontocete families albeit with clear family-specific characteristics.

This thesis does not have the intention of being a comparative study. We only mention the most significant interspecific differences, particularly in cases with quantitative and remarkably qualitative differences and cases with possible functional implications. All of which will be considered for the families of Delphinidae, Phocoenidae and Ziphiidae (see Table 3).

The most significant morphological differences between the nasal sac system of phocenids and delphinids are the compactness of the phocenid nasal system that features relatively large vestibular sacs and the presence of an additional pair of diverticula, the posterior nasal sacs.

In porpoises, the vestibular sacs are very large and feature many transversely orientated folds and complementary deep grooves in the floor of the sac as well as that they are arranged like an arch around the medially located communicative opening to the nasal tract. The folds give a particular rigidity to the ventral half of the sac. Furthermore, the vestibular sacs opens into the nasal tract through several openings that form a narrow communication channel. The main part of the sacs is situated on both sides rostrolateral to the nasal tract.

The **posterior nasal sacs** (Morris, 1969) are two diverticula located caudal to the inlets and posterior parts of the nasofrontal sacs. They open ventrally into the inferior vestibule, a space that is shared with the opening of the nasofrontal sacs (Gallardo, 1916). Towards dorsal, these sacs extend over the mesethmoid bone, but not as high as the nasal bones or vertex of the skull. In between the posterior nasal sacs and the nasofrontal sacs there is a mass of dens connective tissue, the "hintereklappe" (Kükenthal, 1889), which is heart-shaped in a sagittal section. Hintereklappe is a German word which, although to date has not been translated (Curry, 1992), could amount to "posterior valve". Heyning (1989) also describes the presence of the posterior nasal sacs in species of the family Monodontidae, which include only the narwhal (*Monodon monoceros*) and beluga (*Delphinapterus leucas*). According to this author, these species bear some similarity with porpoises, especially when regarding the morphology of the vestibular sacs.

The Ziphiidae family has received very little attention in research, particularly from an anatomical point of view; with the exception of a couple of references alluding to the anatomy of the nasal region of this little known family (Norris and Harvey, 1972; Schenkkan, 1973) there is only one full comparative study between members of the family, and it also includes a systematic review that compares them to other toothed whales (Heyning, 1989). According to this author the most characteristic features of these species, regardless of the osseous features of the skull, is that the vestibular sacs are absent and the nasal tract is widened transversely and shaped like a

trapezoid from the blowhole down in caudoventral direction. The nasofrontal sacs were encountered in species of the genus Mesoplodon, but only the anterior parts of the sacs could be identified; however, all genera did possess posterior nasal sacs, attached to the spiracular surface of the vertex (Heyning, 1989) or to dome-shaped bony back wall that is formed by the premaxillary and nasal bones, which are very prominent in Ziphiidae.

2.2. Musculoskeletal system

The odontocete skull underwent an elongation in the evolutionary process, also known as "telescoping" (Miller, 1923), which consisted of a lengthening of the maxillary and premaxillary bones as the rostrum attained its characteristically sharp shape (Pl. 3, Fig. 1). Moreover, the caudal part of the maxillary bones regressed in caudodorsal direction, covering the frontal bones up to near the nuchal crest, while its lateral edge is situated dorsal to the orbit.

The nasal region of the skull is arranged as a flat surface, shaped liked an inclined screen with its slope facing rostral, because the entire caudodorsal expansion of the maxillary bones overlays the frontal bones. The entire facial region of the skull is limited centrally and dorsally by the vertex, laterally by the facial border (Heyning, 1989) that is formed by the nuchal crests, temporal crests and supraorbital processes, and rostrally by the lateral sides of the maxillae. In between the supraorbital process and the lateral edge of the maxilla on each body side, that features the antorbital notch and antorbital process (Heyning, 1989; Rommel, 1990).

The nasal sac system, and particularly the adjacent muscles, have been well described by Lawrence and Schevill (1956) and later also by Mead (1975). Overall we estimated that the most accurate muscular pattern to use as a guide, is based on divisions and terminology of Schevill and Lawrence (1956), without prejudicing the latter. The development of the odontocete nasal sac system coincided with a parallel development of related musculature, which has been considered to be homologous to the maxilonasolabial musculature in other mammals (Huber, 1934).

According to Mead (1975), the nasal portion of the maxilonasolabial musculature can be divided into five muscles, which are most easily distinguishable in their insertions. From superficial to deep there are the following **muscular layers: posteroexternal part, intermediate part, anteroexternal part, posterinternal part** and **anterointernal part** (Plate 3, Figs. 5 and 6). As it is difficult to distinguish these different layers in their origin, they are not considered to be independent muscles, but rather to be parts of a maxilonasolabial muscle complex.

The **posteroexternal part** is situated beneath the subcutaneous fatty tissue posterior to the transverse line that is marked by the blowhole. This part extends from the temporal crest and supraorbital process and inserts in an area caudal to the blowhole, specifically in the connective tissue underlying the posterior lip, where it mixes with the fibres of the contralateral part.

The **intermediate part** is situated medial to the posteroexternal part, and protrudes the latter's rostral free border as a thin muscle layer heading towards a subcutaneous transverse streak of fibrous connective tissue that runs between the blowhole and the melon. The origin of this part is not easily discernible as its muscle fibres mingle with the surrounding muscle parts.

Next, the **anteroexternal part** is the most widespread of the superficial facial musculature; it is shaped like a hand-fan whose wide side presents the origin that spans across the entire facial

border, from the vertex to approximately the antorbital notch, and as such it covers the caudolateral edges of the maxilla. The muscle insertions envelop the vestibular sac, but also insert into the connective tissue of the anterior lip of the blowhole, as well as into the lateral side of the nasal tract, and into the dorsal edge of the slit-like opening. Rostral to the blowhole and dorsal to the anterior parts of the nasofrontal sacs, the left and right anteroexternal muscle parts form a common aponeurosis. Lawrence and Schevill (1956) also mention the insertion of muscle fibres in the anterior fold of the vestibular sac.

The origin of the **posterointernal part** is smaller and situated more caudally that the anteroexternal part**.** It inserts into nasal tract from caudal, rostral to the vertex.

Finally, the **anterointernal part** is the thickest muscle layer who's origin occupies the entire concave surface of the ascending process of the maxilla, a.k.a. facial fossa (*fossa facialis*) (Heyning, 1989). This muscle part inserts in a widespread area, rostral to the nasal tract, where it commingles with the most rostral aponeuroses of the anteroexternal part.

There are other muscles in the nasal region that are not related with nor homologous to the maxilonasolabial musculature. These include the nasofrontal sac intrinsic muscles, the diagonal membrane muscles and the nasal plug muscles.

The **nasofrontal sac intrinsic muscles** (Lawrence and Schevill, 1956) envelop the posterior parts of the sacs (Plate 7, Fig. 3; Plate 8, Fig. 5). On both sides there are two parts, one *major intrinsic muscle*, caudodorsal to the posterior part of the sac, and a *minor intrinsic muscle*, situated rostroventrally. Mead (1975) considered both to be parts of the same muscle. Caudal to the posterior part of nasofrontal sac and the major intrinsic muscle there is the **diagonal membrane muscle** (Plate 8, Fig. 4; Plate 10, Fig. 1) with vertically oriented muscle fibres that span from a zone lateral to the nasal bones, covering the entire bony wall caudal to the tract*,* and attaining the area dorsal to the diagonal membrane.

Rostral to the nasal tract, inserting into the nasal plugs, there are the **nasal plug muscles**, which span over the premaxillary sacs (Plate 2, Fig. 1 and 2). They originate on the premaxilla, rostral to the blind ends of the sacs.

The discussed muscular pattern is based on the anatomy of the family of Delphinidae but does show certain variations in some of the genera that were part of this study. The most significant differences, in our view, are the absence of certain muscle formations in Phocoenidae (Curry, 1992) and Ziphiidae (Heyning, 1989), although they do not seem to have enough functional meaning for us to elaborate on the description of these families.

2.3. Adnexal structures

In this section we will describe structures that are related to the nasal sac system, but are not a direct part of the nasal tract. They contain fibrous connective tissue, adipose tissue and/or glandular tissue with unique characteristics that show a potential importance to the entire nasal complex. Strictly speaking, the musculature would also be included in this description, however, we regard it to have sufficient entity to be treated in a separate section (see section 2.2).

The melon is the adipose tissue that gives the odontocete head their characteristic prominent shape, and has been known and described since antiquity (Slijper, 1962). While other adnexal structures have also been described over the years, they were not regarded to be important until recent, either as individual structures or being part of an anatomical functional unit in sound production, emission or reception mechanisms.

a) Melon

The melon is a mass of adipose tissue situated dorsal to the rostrum (Pl. 2, Fig. 2; Pl. 3, Fig. 2; Pl. 4, Fig. 4), in between the skin and the alveolar parts of the maxillae and premaxillae. Depending on the species it extends up to the front end of the rostrum (in Phocoenidae, Monodontidae etc.) or up to halfway the facial extension (in Delphinidae, Platanistidae and Ziphiidae). The lateral sides of the melon are anchored to the rostrum by the rostral muscles or labial part of the maxillolabial musculature. It consists mainly of adipose tissue, with the centre of the melon, a.k.a. internal melon or core (Wedmid et al., 1973), containing only fat of a specific composition (Plate 2), which is surrounded by heterogeneous lipid gradations and fibrous tissue that infiltrates from the periphery. The core is positioned centrally in the entire facial prominence, extending from caudal, near the slit-like opening, to rostral where it contacts the epithelium of the skin, while the rest of the melon is surrounded by mixed fibrous and adipose tissue. The lipid composition of the melon has been studied in depth, and excellent sound transmission properties have been attributed to the melon and especially to the melon core (Malins and Varanasi, 1971; Varanasi and Malins, 1971, 1972; Litchfield et al, 1973), which is possible due to high concentrations of glycerol esters of long chain triglycerides with a high presence of isovaleric acid and saturated acids in general (Litchfield et al., 1973). In comparison to the blubber, the "acoustic" fatty tissues contain higher concentrations of branched and saturated acids and alcohols (Varanasi and Malins, 1971), which makes them difficult to metabolize (Malins and Varanasi, 1972, 1975). The high concentration of such fatty acids in the central part of melon implies the existence of a central channel of lower molecular weight through which sound can travel at lower speeds than in the more peripheral parts of the melon. This has been confirmed experimentally by measuring the propagation speed in different parts of melon (Norris and Harvey, 1974), with the impedance of the melon core similar to that of water.

This acoustic channel or 'core' has been identified by means of tomography as a low density pathway called PAP (potential acoustic pathway) (Cranford et al., 1996). Towards caudal, the melon continues as the same low density tissue inside the nasal plugs, gets surrounded by the nasal plug muscle, and reaches the anterior wall of the spiracular cavity (Pl. 2, Fig. 2). First described by Norris (1968) in the common dolphin, *Delphinus delphis*, this lipid extension is part of the acoustic pathway and commonly only occurs on the right side (Pl. 2, Fig. 1) although it may be bilateral in some species such as *Lagenorrhynchus obliquidens* and *L. albirostris* (Cranford et al., 1996). These authors also describe the lack of a complete caudal extension of the melon in porpoises.

b) Elliptical adipose bodies

The elliptical adipose bodies, called "elliptical bodies" by Mead (1975) and "dorsal bursae" by Cranford (1986), are ellipsoid shaped masses of adipose tissue that are present bilaterally at the level of the slit-like opening, both anterior and posterior to the nasal tract. They are related to the

four respective phonic lips (Plate 4, Fig. 3) in that the anterior bodies are situated slightly ventral to the anterior phonic lips and the posterior ones are at same level as the posterior phonic lips.

Mead (1975) and Heyning (1989) only mentioned the presence of the posterior elliptical adipose bodies, and it was not until the work of Cranford (1988) and Amundin and Cranford (1990) that all four structures were described in relation to the slit-like opening and spiracular cavity. The elliptical adipose bodies could be the transduction site for vibratory impulses (Cranford, 1988). Cranford et al. (1996) analysed these structures with imaging techniques, in particular by means of CT scans, and described their size and relative position to the nasal tract and also their possible functional implications. Besides the evident homologies of these structures in several species, these authors also justified a relationship between size, orientation and symmetry of the bodies and the ultrasonic sounds emitted by those species.

c) Nasal ligaments

The nasal ligaments (Rodionov and Markov, 1992), previously called blowhole ligaments by Lawrence and Schevill, 1956) are situated inside the posterior walls of the spiracular cavities, in between the nasal tract and nasofrontal sac. Each ligament consists of a sheet of dense connective tissue spanning between the caudal ends of the premaxilla lateral to the external bony nares, and attaches medially to the rostral part of the vertex of the skull (Plate 8, Fig. 1, 3, 4 and 5; Plate 13, Fig. 1). Dorsally they reach up to the level of the slit-like opening while the ventral parts of the ligaments form two arches over the openings of the nasofrontal and accessory sacs of the respective sides. It has been regarded as the anchoring structure of the posterior wall of the nasal tract and slit-like opening as it maintains the elongated shape of this latter opening. Inside each ligament there is an ovoid cartilaginous tissue band (Lawrence and Schevill, 1956; Heyning, 1989; Curry, 1992).

d) Glands

The cetacean epidermis lacks any associated glandular structures (Palmer and Weddell, 1964; Sokolov, 1970; Geraci et al., 1986). In contrast, other aquatic mammals have specializations of the integument such as hair, with the consequent presence of associated glands (Ling, 1974).

Maderson (1968) and Evans and Maderson (1973) demonstrated the presence of glands in the nasal sac system of *Tursiops truncatus* and similar structures in the genus Kogia (without specifying the species). Besides these, there are only few references mentioning the appearance of glandular epithelia (Mead, 1972). In porpoises, mineral deposits were noticed, which could indicate a possible presence of glands (Curry, 1994), however, no such structures were found in the histological studies that she carried out.

The glands have been described as exocrine, compound acinar glands, of apocrine secretion type and product without mucous properties. They secrete into the nasal tract at the level of the inferior vestibule and the opening of the nasofrontal sac by means of up to 20 tiny crescentic pores (Evans and Maderson 1973).

2.4. Epithelium of the nasal sac system

Before moving onto a description of the epithelial lining of the nasal tract and sacs we believe it is useful to explain the morphology of cetacean skin, as it will help to understand the former.

The skin of cetaceans, like the nasal system, has been poorly studied by histological means (Evans and Maderson, 1973; Harrison and Turley, 1974; Ling, 1974; Bryden and Molyneux, 1986). Although the blowhole region has been studied by Bryden and Molyneux (1986), who put particular emphasis on nerve endings in the form of mechanoreceptors, there is lack of detailed microscopic descriptions of the dermal-epidermal relationship and of the epithelium of the most dorsal part of the nasal tract.

e) Skin of the blowhole

In all cetaceans, the skin consists of an **epidermis, dermis and hypodermis**; It is in the latter where the adipose tissue or "**blubber**", typical for these mammals, is situated (Ling, 1973). The outer side of the skin consists of parallel and uniformly spaced epidermal folds, called **cutaneous ridges** (Geraci et al., 1986; Shoemaker and Ridgway, 1991). The interrelation between epidermis and underlying dermis is remarkably well developed when compared to terrestrial mammals: the dermis features uniformly arranged high **dermal ridges** with long tongue-shaped projections (**dermal papillae**) into the epidermis, while the complementary crests of the epidermis are called **epidermal ridges** (PI. 18a, Fig. 1). Together, they are arranged like the fingers of a hand in a glove, forming the **dermal-epidermal junction** (Becker, 1946; Palmer and Weddell, 1964, Giacometti, 1967; Sokolov, 1982, Stromberg., 1989; Meyer et al, 1995). This connection between the layers is reinforced by a series of small **epidermal protuberances** as an extension of the epidermal ridges (PI. 18a, Fig. 3a) (Stromberg 1989). In mammals with hair, these formations are only found in areas with sparse or no hair coverage, such as the snout, digital pads, eyelids, perineum, etc., which are areas that are subjected to high mechanical stress (Calhoun and Stinson, 1976; Nickel et al., 1981; Scott, 1988; Al-bagdadi, 1993). The management, arrangement and size of the dermal ridges and papillae could be related to the distinct pressures and mechanical stresses that act on the skin, mucous membranes and underlying tissues (Sokolov et al., 1969).

f) Transition zone between epidermis and nasal tract epithelium

The cetacean epidermis consists of a stratified, squamous, parakeratinized epithelium that is made up of four strata: stratum germinativum, spinosum, intermedium and externum (Ling, 1973). The latter is formed by flattened cells that maintain their nucleus and some of the cellular organelles.

The transition between the skin and the nasal mucosa in domestic mammals is characterized by a gradual loss of the keratinized layer (Plopper and Adams, 1987; Banks, 1993) and a decrease in length of the papillae. This transition is less clear in odontocetes as there is an incomplete keratinization in the entire skin (Harrison and Thurley, 1974) and furthermore, the nasal tract external epithelium remains parakeratinized over the entire system. In this study, we opted for the generic term ‘epithelium’ to reference the tissue lining of the nasal tract ventral to the blowhole, with different epithelial and subepithelial layers to differentiate with the outer coating of the skin. Due to its characteristics, this nasal tract lining cannot be considered to be a true mucosa (Schenkkan, 1973).

g) Epithelium of the upper nasal tract

The entire nasal system is lined by a stratified squamous parakeratinized epithelium, which is basically similar to the skin epithelium but with a less pronounced epithelial-subepithelial connection (Evans and Maderson, 1973; Schenkkan, 1973; Khomenko, 1974). Maderson and Evans

(1973) studied the histological structure and divided the epithelium of the nasal spaces into three categories:

Areas lined with smooth epithelium and undeveloped dermal papillae, which suggests a relative immobility of the tissues. This type of epithelium is present in the lower part of the nasal tract, ventral to the nasal plugs.

Areas of highly folded epithelium with well-developed dermal papillae, oriented perpendicular to the epithelial surface. This type of lining is characteristic for the vestibular sacs, which are capable of great expansion (Lawrence and Schevill, 1956; Evans and Prescott, 1962).

Finally, the rest of epithelium is considered to be an intermediate between the previous two, as in the accessory sacs, the inferior vestibules and the nasofrontal sacs.

h) Pigmentation

The epithelium of the odontocete nasal sac system is characterized by a pigmentation, albeit with a highly irregular distribution both within and between species. Thus, although the general appearance of the epithelium is dark, parts of it appear depigmented (Schenkkan, 1973; Heyning, 1989; Curry, 1992). There are no references in the literature to the possible origin and function of the presence of this pigmentation.

2.5. Innervation

The odontocete facial region is mainly innervated by branches of the facial and infraorbital nerve (Huber, 1934; Mead, 1975).

The facial nerve, which is responsible for the motor innervation, passes ventral to the orbit, revolves around the antorbital notch towards dorsocaudal and branches into smaller nerves (Pl. 6). At this level it is difficult to distinguish the single nerves as they are surrounded by a web of connective tissue (Mead, 1975). The nerve branches are distributed among the different parts of the maxilonasal musculature. Some terminal branches turn rostrally to innervate the nasal plug muscles (Huber, 1934).

The infraorbital nerve, a branch of the maxillary nerve, which in turn is a branch of the trigeminal nerve, provides sensory innervation and spans across the nasal region after dividing and exiting through several infraorbital foramina of the skull (Pl. 6). These branches innervate the structures of the nasal tract and the skin of the rostrum.

The cutaneous innervation of the facial region, like the skin of the rest of the body, is considered to be rich and specialized, as this region is endowed with high sensitivity, as was demonstrated in the bottlenose dolphin, *Tursiops truncatus* (Palmer and Weddell, 1964 ; Khomenko and Khadzhinzkiy, 1974) and the fin whale, *Balaenoptera physalus* (Giacometti, 1967). These authors describe the presence of many nerve endings located in the dermis and even penetrating the epidermis. Some of these endings are encapsulated, similar to Vater-Pacinian bodies. The presence of these nerve receptors, which are distributed irregularly over the body, does presuppose a possible involvement in hydrodynamic control of the skin.

As for the nasal sac system, Khomenko (1974) described the afferent innervation in which he identified three groups of recipients. A large and widely distributed first group consists of diffuse,

free and dendritic nerve fibres that are present in connective tissue, blood vessels, glands and even epithelium; the polyvalent receptors are responsible for regulating the local blood circulation and internal tissue metabolism functions. Another group consists of fibrillar discs or muscle spindles**,** arranged between muscle tissue and stimuli recipients that are associated with muscle contraction and relaxation during respiratory movements. A third group encompasses encapsulated nerve endings that can be differentiated according to the structure of the capsule and the number of terminations per corpuscle, as end-bulbs of Krause (a.k.a. bulboid corpuscles) or Golgi-Mazzoni corpuscles. The latter are found in large numbers in the area of the blowhole (Bryden and Molineux, 1986), and are situated immediately below the basal end of the epithelium and are considered, like the majority of nerve endings of the nasal sacs, as mechanoreceptors in broadest sense of the word.

Ultrastructural studies of the encapsulated nerve endings have shown that these are almost certainly mechanoreceptors when regarding the structural characteristics of this type of receptor (Andres, 1974). In short, Bryden and Molineux (1986) observed several axons for each receptor, which were surrounded by a capsule of up to six Schwann cell layers. These receptors organs are found in the dermis, close to the epithelium, and are numerously present in the anterior lip of the blowhole. According to these authors, who did not differentiate between different types of nerve endings, the encapsulated receptors described by Khomenko (1974) may well be the same type of mechanoreceptors than those found in the region of the blowhole; these receptors would also be found in the vestibular sacs where they could sense contact with water, as is also postulated for the blowhole, allowing for a reflex closure of the tract.

Unfortunately, Khomenko (1974) is the only author to describe the innervation of the entire nasal system, although it was done very generally and without distinguishing between different areas. His findings are consistent with the general innervation of the integument (Palmer and Weddell, 1964), but they do not allow to analyse any functional implications in this part of the upper respiratory tract.

2.6. Asymmetry

The odontocete splanchnocranium and soft tissues of the nasal region feature a high degree of asymmetry, which is variable depending on the species (Miller, 1923; Ness, 1967) (Pl. 3, Fig. 1). Much has been written about the basics of odontocete-specific development (reviewed extensively by Mead (1975)). Most of the theories lack a sufficient anatomo-functional basis and moreover, the interspecific variability within the suborder makes it difficult to draw conclusions.

The first observations of the possible relationship between the cranial asymmetry and a well-developed nasal complex (Howell, 1930) subsequently led to assign a possible function of sound production to this region (Norris, 1964). So, we are faced with an asymmetric nasal region that reflects an evolutionary development of structures involved in the mechanisms of sound production, which has led to alterations in bone structures (Wood, 1967; Mead, 1975). However, it has been shown that the asymmetry does not significantly affect the facial musculature, which has similar mass and weight on both sides (Howell, 1930; Mead, 1975); nonetheless, this soft tissue asymmetry is primarily pronounced in the deeper parts of the nasal sac system (Schenkkan, 1973;

Mead, 1975) and related structures such as the caudal extensions of the melon, which normally enter deep into the right nasal plug (Norris, 1968; Cranford, 1996).

2.7. Nomenclature

In this doctoral thesis, the nomenclature used to refer to the anatomical structures of the head of the toothed whales, has been based mainly on the works of Lawrence and Schevill (1956) and Mead (1975). We have used a terminology as simple and direct as possible in cases where the translation was consistent, and new terms had to be created, we opted for a morphologico-functional point of view or a terminology that was based on homologies, as will be discussed later in the section "Discussion on the nomenclature". Still, we found it convenient to emphasize certain general terms that refer to the anatomical area that is the subject of this work. As such, we called 'nasal region' of odontocetes the rostrodorsal area of the facial region in which the nasal tract and melon are present. The name of 'nasal tract' is due to a lack of a true nasal cavity, as is described in this paper, while the diverticula surrounding the tract were altogether called the 'nasal sac system'. The qualification of the nasal tract and sacs as a *system* was due to the widespread use of this terminology in the literature, and was based primarily on the anatomical changes and the functional specialization of the nasal region.

Table 2 features a comparative list of nomenclature that is used in this work ('Presente trabajo').

TABLA 2. Nomenclatura de las estructuras del sistema de sacos nasales

Presente trabajo	Lawrence & Schevill (1956)	Schenkkan (1973)	Mead (1975)	Rodionov & Markov (1992)	Cranford y col. (1996)
saco vestibular	vestibular sac	vestibular sac	vestibular sac	saccus dorsalis	vestibular sac
saco nasofrontal (SNF)	nasotubular sac	nasofrontal sac	nasofrontal sac (NFS)	saccus nasofrontalis	nasofrontal sac
saco accesorio	connecting sac	accesory sac	accesory sac	saccus accessorius	accesory sac
saco premaxilar	premaxillary sac	premaxillary sac	premaxillary sac	saccus premaxillaris	premaxillary sac
saco nasal posterior	--------	caudal section of NFS	posterior portion of NFS	-------	caudal sac
tracto nasal*	nasal tract/passage	nasal canal	espiracular cavity	meatus nasi mollis	espiracular cavity
abertura SNF	opening of nasotub.s.	NFS entrance	inferior vestibule	inferior vestibule	inferior vestibule
pliegues nasales	nasal plugs	nasal plugs	nasal plugs	valva ventralis	nasal plugs
extensión lateral	liplike extension	lateral flaps	lateral extension	lip of valva ventralis	nasal plug node
ligamento nasal	blowhole ligament	blowhole ligament	blowhole ligament	ligamentum nasi	blowhole ligament
membrana diagonal	diagonal membrane	diagonal membrane	diagonal membrane	diagonal membrane	diagonal membrane
constricción transversal	slit-like opening	--------	--------	ventral opening s.dorsalis	slit-like opening
labios vocales	----------	--------	--------	--------	monkey lips
cuerpos elípticos grasos	----------	--------	eliptical bodies	--------	dorsal bursae
Complejo Laminar Fibroso (CLF)	----------	--------	--------	--------	connective tissue theca**
posteroexterno	posteroexternus		posteroexternus	maxillonasalis dorsalis	----------
intermedio	intermedius		intermedius	maxillonasalis intermedia	----------
anteroexterno	anteroexternus		anteroexternus		----------
posterointerno	posterointernus	m. maxilonasalis	posterointernus	maxillonasalis ventralis	----------
anterointero	anterointernus		anterointernus	maxillonasalis inferior	----------
	profundus			dilator nasi	----------
m. pliegues nasales	nasal plug muscle	nasal plug muscle	nasal plug muscle	nasolabialis profundus	----------
porción mayor m.intrins.	major intrinsic muscle	--------	NFS intrinsic muscle	bucconasolabialis ant.	----------
porción menor m.intrins.	minor intrinsic muscle	--------			----------
m. membrana diagonal	--------	--------	diagonal membrane m.	bucconasolabialis post.	----------

*También cavidad espiracular, aunque en el presente trabajo sólo se emplea para hacer referencia a la porción media del tracto nasal.

**Cabe puntualizar que no se trata exactamente de las mismas estructuras.

3. ACOUSTICS

In this doctoral thesis about the morphology of the region where the sound is presumably being generated, a reference to acoustics as the science of the study of sound is in place. Moreover, sound is the odontocete's primary sense in relation to their underwater environment. However, we must declare ourselves to be a neophyte in this highly technical and specialized field, so we will only offer an outline of the acoustical principles that are important for a good understanding and application of the odontocete functional anatomy, without the intention of laying scientific foundations or to be a benchmark to be followed in the study of sound. Please grant us certain license and we apologize to the experts for intruding into their field.

3.1. Physical principles of sound and sound transmission

Basically, sound is a longitudinal pressure wave that is produced by a vibration creating a phenomenon of alternating compression and rarefaction of the molecules in the medium in which the wave moves (Greene, 1995). Every sound wave is characterized by its amplitude and frequency. Amplitude gives an idea of the sound intensity and is expressed in decibels (dB). Frequency is the number of oscillations or wave cycles per time and is measured in Hertz (Hz) (Johnson, 1986). Depending on the frequency of the sound it can be classified into audible sound, infrasound and ultrasound. Audible sounds are those whose frequencies lie within the human ear detection range, between 20 Hz and 20,000 Hz (20 kHz). Sounds below 20 Hz are called infrasonic, and at the opposite end, with a frequency above 20 kHz, ultrasonic sounds. This classification only takes into regard the physical capacities of human beings, while many sounds across the animal kingdom and especially in cetaceans, lie outside of the human hearing range (Greene, 1995).

Sound propagates through a medium at a rate that depends on the volumetric mass density of that medium; thus, if the density of a medium is constant, the speed of sound in that medium will be constant. Each medium has a specific resistance of radiation, or acoustic impedance, which is directly related to the medium's density. A higher density is associated with a higher sound propagation speed. For example, water has a higher density than air, and as such sound travels faster in the former than in the latter.

Moreover, when sound travels from one medium to another, it follows the same physical laws as does light. As such, at the boundary between two media, part of the sound gets reflected and another gets refracted, with the consequent loss of energy. The amount of reflected energy depends on the steepness of the angle of incidence (i.e. the angle between the direction of the wave propagation and the perpendicular axis of the surface between the two media) and on the difference in acoustic impedances between the two media. The greater the angle of incidence and / or the difference between the acoustic impedances of the two media, the greater the reflection. When looking at the impedance difference between water ($R= 1.5*10^5$) and air ($R= 40$), the difference is so big that the angle of incidence can be neglected, and sound will hardly pass the boundary between these two media, and will be almost totally reflected (Purves and Pilleri, 1983). Conversely, if the two media should have a similar acoustic impedance, the amount of reflection and related energy loss would be minimal.

For an object to produce an echo it must be physically larger than the wavelength of the emitted sound and at the same time have a sufficiently different impedance value than the

medium in which the sound propagates. These concepts explain why a dolphin is not able to detect nets with mesh sizes and structures smaller than the wavelength used (Goodson and Sturtivant, 1995). Similarly it is hard to detect a jellyfish with an impedance very similar to that of salty water, and as such hardly produces any echoes (Morris, 1986).

3.2. Study of sounds produced by odontocetes: Experimental contributions

Ever since ancient times, odontocetes have been known to produce a wide variety of sounds (Schevill, 1964), from audible to ultrasonic sounds.

Amongst the audible sounds of odontocetes are the continuous tonal whistles, and other sounds such as squeaks or grunts. Whistles are used for communicative purposes (reviewed by Hermann and Tavolga, 1980; Nachtigall, 1986). Nevertheless, there are odontocetes that do not emit whistles, such as the Physeteroidea superfamily, the Phocoenidea family, the Cephalorhynchus genus and La Plata Dolphin (*Pontoporia blainvillei)*, as comprehensively reviewed by Thomson and Richardson (1995). This has led scientist to hypothesize on other acoustic communicative methods, such as the rhythm of the vocalizations (André, 1997).

Although there are several odontocete vocalization categories within ultrasonic sounds, they can be summarized as either clicks or pulsed sounds (Evans, 1973; Au, 1983). The main difference between the two is that clicks are individual pulses with low repetition rates (1-90 per second) that can vary in duration, while pulsed sounds are chained pulses with a high repetition rate (up to 5000 per second) and the entire chain is short in duration, mostly between 0.5 and 1.5 seconds (Thomson and Richardson, 1995). For both clicks and pulsed sounds, the frequency characteristics vary among species.

Of all vocalizations, the most important ones for echolocation purposes are sounds of high and very high frequency, i.e. primarily clicks. In this regard, it was initially shown that a bottlenose dolphin (*Tursiops truncatus*) with eyes covered, could orient itself by means of echolocation clicks (Norris, 1964). In the seventies, the harbour porpoise (*Phocoena phocoena*) showed this ability as well (Evans, 1973), and since then it has been observed in all odontocete species studied and considered to be a common feature for the suborder (Au, 1993). Despite this common capability, the different species have evolved their vocalizations to a characteristic frequency range. Thus, it is possible to relate the characteristics of the emitted sounds to the natural habitat of the species. As such, coastal and riverine species basically make use of very high frequency sounds, which is understandable, considering the need for a high resolution in a relatively limited space.

Within this functional group of species there are descriptions of the sounds used for echolocation, among others, in the beluga, *Delphinapterus leucas* (Au et al., 1985, 1987; Au, 1993), the harbour porpoise, *Phocoena phocoena* (Möhl and Andersen, 1973; Kamminga and Wiersma, 1981), Dall's porpoise, *Phocoenoides dalli* (Evans and Awbrey, 1984; Hatakeyama and Soeda, 1990; Hatakeyama et al., 1995), the Commerson's dolphin, *Cephalorhynchus commersoni* (Evans and Awbrey, 1984; Au, 1993), Hector's dolphin, *Cephalorhynchus hectori* (Dawson, 1991), the tucuxi, *Sotalia fluviatilis* (Wiersma, 1982; Kamminga et al., 1993) and the boto, *Inia geoffrensis* (Evans, 1973; Kamminga et al., 1993). On the contrary, pelagic species make use of high frequency signals to cover larger distances, where depths usually exceed 200 meters, and in which the echolocation signal entail less detailed information. Among these are species such as the Pacific white-sided

dolphin, *Lagenorhynchus obliquidens* (Evans, 1973), the common dolphin, *Delphinus delphis* (Au, 1993) and the short-finned pilot whale, *Globicephala macrorhynchus* (Evans, 1973), among others. There are also species such as the bottlenose dolphin, *Tursiops truncatus*, that make use of all sorts of habitats and these produce sounds in a wider frequency band (Au et al., 1974; Moore, 1988; Kamminga and Beitsma, 1990; Au, 1993).

The process by which toothed whales make use of their ability to echolocate, can be summarized in a prey capture situation, demonstrated in the following sequence proposed by Morris (1986):

a) Swimming and scanning of the surroundings, during which lower frequency clicks are produced with steady pulse repetition rates (PRR), thereby allowing for a general scan and detection of possible targets.

b) Once a possible target is detected, fast series of broadband pulses are emitted to determine the scope and direction and to obtain information on the target.

c) Next, the sound beam is focused on the target to obtain more details. For this, there is a concentration of energy in the higher frequency components of the broadband click, and the sound beam is more directional.

d) As the distance between the dolphin and the target reduces, even higher frequencies are used with higher PRR's.

e) Finally, over short distances, thanks to the emission of very high frequency sounds at PRR's that can exceed 400-500 clicks per second, the animal can obtain complete information on the target, possibly even on the texture and composition of the target.

To conclude, each species makes use of a characteristics ultrasonic sound field, which is determined by the scope and breadth of their emissions. Echolocation sounds are emitted in a highly directional beam projection whose central region contains the highest intensity and scope (on-axis), decreasing as distance from the centre (=angle) increases (off-axis) in a field with undefined boundaries (Thomson and Richardson, 1995). These authors presented a review on the characteristics of the sound field and its amplitude in various species, as well on the directionality of the echolocation beam. Moreover, they explored the characteristics of this sound field in order to establish the sound production location and the transmission pathway through the head. For this, sound was recorded and measured with help of microphones or hydrophones in series, either in cadavers in which a whistle was inserted into the larynx for sound production (Purves, 1966; Pilleri et al., 1976, 1980a -b, 1983a-b; Purves and Pilleri, 1983) or by injection of pressurized air from the larynx (Prescott and Evans, 1962), or in live animals (Diercks et al, 1971). The anatomy of the head of the common dolphin and the corresponding sound beam formation through it, was depicted in several computer models (Aroyan et al., 1992). All these contributions, and many other experimental studies, opened the door to the integration of anatomy and acoustics.

III. PROBLEM STATEMENT / WORK HYPOTHESIS

In the initial phase of this doctoral thesis, during the literature study, it was surprising to find a large number of both profound and superficial studies related to the odontocete nasal sac system in many species. However, overall findings and conclusions were far from coherent and led to controversy.

Although the odontocete facial morphology has been studied and reviewed extensively, including explanations and hypotheses regarding the functionality, there are only few thorough descriptions of the gross anatomy of the nasal sacs and the facial musculature. Regarding the latter, there are uncertainties on the specific locations of the insertions of the nasal part of the maxilonasolabial musculature, and even less clear is the morphology and distribution of the small muscles that are not related to the maxilonasolabial musculature. These small muscles are located in the ventral half of the nasal system, where sound production takes places, and thus are situated in a particularly relevant location. Recently, there is deep knowledge of structures that are involved in sound production mechanisms, such as the phonic lips, the elliptical adipose bodies and the melon and melon core. These studies were performed using imaging techniques, gaining a better insight in these structures as they could be differentiated according to their density. However, it was not possible do delineate them clearly or to identify in detail the relationships between them and other small components of the nasal tract. Moreover, taking into account the wide variety of species studied, the striped dolphin (*Stenella coeruleoalba*), species of greater casuistry on the Mediterranean coast, has been barely described in the literature.

Most studies on the anatomy of the nasal region of odontocetes have been carried out by dissection techniques and, more recently, using advanced techniques. However, in our view, there are still certain gaps in the knowledge of structural interrelations within this region, mainly due to a lack of studies on the morphology of highly specialized structures that could exert a major role in the physiological mechanisms of the nasal sac system.

Therefore, we regarded it important to study the odontocete facial region from a structural point of view, because starting from the fact that the nasal system is the organ of ultrasonic sound production, we consider the following:

a) The supposed location where the necessary vibrations are generated for the production of ultrasonic sound, should present certain characteristics. As it is an epithelium, which is apparently delicate, only a certain specialized area could support the energy generated during the process of phonation.

b) As the sound is being generated within' the odontocete head, there should be an associated transmission pathway. The melon features the qualities of being a good sound transmitter (Litchfield et al., 1973; Norris and Harvey, 1974), so it is conceivable that the transmission pathway starts in the nasal system and makes its way to the exterior through the melon. However, sound does not travel unidirectional through the facial structures. Besides the path of least resistance through the melon core, there are focusing mechanisms that would allow for the elimination of not useful vibrations.

c) Additional to the respiratory function of the nasal system, which in itself put a strain on the epithelium, there are the frictional forces caused by phonation mechanisms, and therefore the epithelial lining should feature a morphology that is adapted to these forces. Moreover, the nasal system's glandular structures (Evans and Maderson, 1973) should be part of a complex system that allows for a functioning of all the activities that are being carried out in this region.

d) This would also imply very fine control mechanisms, so we expect a very finely developed and specialized innervation.

e) The complex nasal sac system should act as a unit, not based on individual movements of the different components. It would therefore feature some kind of structure that would allow for an integrated interplay of movements and functions of the different structures within the entire system.

f) When looking at cetacean evolution, the findings on the nasal anatomy of the striped dolphin should be extrapolatable to other related species, particularly to other odontocetes.

g) This evolution has led to extraordinary anatomical and functional adaptions to the aquatic environment, especially when looking the nasal structures. This could provide clues about the origin and the evolutionary path of the structures as they should have homologues in terrestrial mammals.

IV.OBJECTIVES

There are numerous theories and controversies regarding the odontocete nasal region and the lack of a structural knowledge has led us to pose as main objective of this study to perform a complete study of nasal sac system from a purely anatomical point of view. Unfortunately, as the field of bio-acoustics is very complicated, and there were only limited resources of study material available, we did not perform any experimental studies in this regard, although we did strive to approach the matter in a way that was as functional as possible. Thus, taking into account these issues, we established the following objectives:

1) To study the structural elements of the nasal sac system and to describe them as detailed as possible.
2) To identify and describe both the place of ultrasonic sound production, with all of its specialized characteristics, as well as the sound emission pathway through the melon.
3) To determine the structural relationships between the described components as a foundation for the description of the integrated functions of the entire nasal sac system.
4) To perform a detailed study on the glandular structures in order to hypothesize on their relevance in the functionality of the nasal system.
5) To establish a degree of homology between species by studying the comparative anatomy.
6) To assess the evolutionary links between land mammals and odontocetes.

V. MATERIAL AND METHODS

1. APPROACH

Our work plan consisted of carrying out conventional dissections, serial sections and plastic injections to obtain moulds of the nasal cavities.

Next, several histological staining techniques were used to study the tissue properties of the nasal system, as this aided in understanding the relationships between the different components of the system, and could help us identify the structures with specialized characteristics. Once the structures of major possible relevance to the functionality of the system were determined, different study methods were applied according to the properties that were observed previously.

For example, the study of the epithelium was primarily based on histology and through observing the surface through stereoscopic and scanning electron microscopy. The possible importance of the relation between the epithelial and subjacent layers in phenomena such as friction and tension has led us to develop a suitable technique for obtaining adequate study samples. The relationship between the tissue layers was particularly interesting in the region of the blowhole, so this region received particular attention although this was not included as an initial objective.

Finally, ultrastructural studies by means of transmission electron microscopy were performed whenever possible.

2. SOURCE MATERIAL

In this doctoral thesis we studied the facial anatomy of the striped dolphin thoroughly (*Stenella coeruleoalba*) with a diversity of available techniques, as outlined in the work plan. This species served as a reference and basis for a comparative study with other species of odontocetes. The reason this study focused on striped dolphins was primarily because of their abundance in the north-western Mediterranean, and the according predominance in stranding events.

The dolphins used in this study were mainly stranded along the Catalan coast. The work started in August 1993 by collecting stranded animals, which was carried out by 'el Servei de Pesca Maritima de la Generalitat de Catalunya'. Early 1994 there was a transfer of power to 'la Fundación para la Conservación y Recuperación de Animales Marinos' (CRAM) (*Foundation for the Conservation and Recovery of Marine Animals*), that up to this date has been responsible for managing the local stranding network, collecting stranded animals and arranging transport to the Faculty of Veterinary Medicine of the Autonomous University of Barcelona (UAB), where all mandatory necropsies and post mortem investigations take place.

All other study material, i.e. heads of species that did not occur stranded on the Catalan coast, could be obtained thanks to a collaboration with foreign institutions. Information on these animals, their general data (size, sex, etc.), together with the corresponding partner institution, is featured in Table 3.

The primary factor for the limited amount of samples was a shortage of available material, and most material came from stranded wild animals. This meant that sampling depended on the occurrence of the stranding events, which, by the way, declined over the four year duration of this work.

The second limiting factor was the difficulty of obtaining high quality samples. Either because the animal was not fresh, and/or because there was often a lag between the deceasing of the animal and it being spotted, after which it still had to be transported to the necropsy facilities.

As for the material obtained from other countries, it must be considered that dolphins are animals included in Appendix I of the Washington Convention on International Transport of Endangered Species (CITES). This implied that transport was restricted, and that specimens usually had to be preserved frozen, thus hampering the study.

3. MATERIAL SELECTION

The material had been selected based on the condition of the animals, and depending on the condition code different investigation methods could be applied.

Only the very fresh or fresh specimens, considered as grade M1 or M2 respectively, have been used for microscopic examination. These codes come from classifications that were established in general autopsy reports (Kuiken and García Hartmann, 1991; Geraci and Lounsbury, 1993), and were adapted by the necropsy team of the Department of Histology and Pathology, Faculty of Veterinary Medicine, Autonomous University of Barcelona led by Dr. Mariano Domingo. The adaptations consisted mainly of a freshness classification under perspective of a greater demand.

As such, only dolphin heads that were not in a state of decomposition nor were affected by disease, were used for structural studies and, of those, only M1 material or live stranded dolphins, were used for ultrastructural analysis.

Animals with higher condition codes, such as corpses under moderate decomposition, have also been used for the macroscopic anatomical study, as these contributed to the large sample size that allowed for a better understanding of the nasal sac system.

Last, part of the material was preserved by freezing, as this proved to be useful for dissections, serial sections, plastic injections or maceration of the epithelium. Some of this material was also used in the microscopy studies, particularly for species that had to be transported from abroad. Although the tissue presentations were less than acceptable, the specimens did prove to be very useful in comparative studies with other species.

Especies	Codigo	Sexo	Edad	Proc	Fuente	Técnicas
F. DELPHINIDAE						
Stenella coeruleoalba	MGH1			V/c		dis
Stenella coeruleoalba	MGH3			V/c		dis
Stenella coeruleoalba	930826Sc	Hg	A	V/f	CRAM	hist
Stenella coeruleoalba	MGH5		J	V/c	CRAM	dis
Stenella coeruleoalba	940207					dis/hist
Stenella coeruleoalba	N295-90			V/c		cort.ser.trans.
Stenella coeruleoalba	95CET002	H	A	V/f	CRAM	hist/MET
Stenella coeruleoalba	95CET003	M	A	V/f	CRAM	hist/MET
Stenella coeruleoalba	95CET005	M	J	V/f	CRAM	iny
Stenella coeruleoalba	95CET006	M	A	V/f	CRAM	iny
Stenella coeruleoalba	95CET011	H	J	V/f	CRAM	dis
Stenella coeruleoalba	95CET014	M	J	Vv/f	CRAM	hist/MET
Stenella coeruleoalba	95CET020	M		V/f	CRAM	dis/MEB
Stenella coeruleoalba	95CET023	M	A	V/f	CRAM	cort.ser.sagit.
Stenella coeruleoalba	SC96/001	H	A	V/f	CRAM	hist
Stenella coeruleoalba	SC96/010	H	A	V/f	CRAM	MEB
Stenella coeruleoalba	SC96/011	M	A	Vv/f	CRAM	hist/MET
Stenella coeruleoalba	SC96/012	H	A	V/f	CRAM	iny
*Stenella coeruleoalba**	SC96/013	M	J	V/f	CRAM	dis
Stenella coeruleoalba	SC97/004	M	J	V/f	CRAM	MEB
Stenella coeruleoalba	SC97/010	H	A	V/f	CRAM	TAC
Stenella coeruleoalba	SC97/016	H	A	V/f	CRAM	MEB
Lagenorhynchus albirostris	La950120				NMNH	dis/hist
Lagenorhynchus albirostris					NMNH	dis
Lagenorhynchus acutus	1-950925Lac	H	A	E/c	RIVO-DLO	dis/hist
Lagenorhynchus acutus	2-950925Lac	M	A	E/c	RIVO-DLO	dis
Lagenorhynchus acutus			A	V/f	NMNH	dis/hist
Lagenorhynchus acutus	3-950925Lac	H	J	E/c	RIVO-DLO	dis
Lagenorhynchus obliquidens	SCB0072			E/c	LACM	dis/hist
Grampus griseus	94CET002	H	A	V/c	CRAM	dis
Grampus griseus	95CET008	H	A	Vv/f	CRAM	MEB
Grampus griseus	GG96/019	M	A	V/f	CRAM	dis
Grampus griseus	GG96/021	M	N	V/f	CRAM	dis
Tursiops truncatus	94CET008	H	A	V/c	CRAM	dis
Tursiops truncatus	TT97/007	H	A	E/f	CRAM	dis
Delphinus delphis	95CET009	Hg	A	V/c	CRAM	dis/hist
Lyssodelphis borealis	DN-LB-0318			/c	LACM	dis
F. PHOCOENIDAE						
Phocoena phocoena	B90-3		J	V/c	NMNH	dis/hist
Phocoena phocoena	B91-18			V/c	NMNH	hist
Phocoena phocoena	PPG40121			V/c	NMNH	dis/MEB
Phocoena phocoena	PP940318			V/c	NMNH	cortes sagit
Phocoenoides dalli	LACM SLH064			/c	LACM	hist

TABLA 3. Relación de especímenes estudiados. En la tabla se incluyen, además de la especie de pertenencia, el código de identificación individual, el sexo (M:macho, H:hembra,Hg:hembra gestante), la edad (A:adulto, J:juvenil, N:neonato), la forma de obtención y conservación (V:varado, Vv:varado vivo, E: enmallado/f:fresco, c:congelado), la fuente (CRAM: fundación para la Conservación y Recuperación de Animales Marinos, Premià de Mar; NMNH:National Museum of Natural History, Leiden; RIVO-DLO:Netherlands Institute for Fisheries Research, Ijmuiden; LACM: Natural History Museum of Los Angeles County) y las técnicas de estudio utilizadas (dis:disección; hist:histología, MET:microscopía electrónica de transmisión, MEB:microscopía electrónica de barrido, cort.ser.sagit/trans:cortes seriados sagitales /transversales, iny:inyección de plástico).

4. PROTOCOL: DISSECTION AND SAMPLING

Once the cadaver was in the necropsy room and the necropsy started, the head was separated from the body by a transverse cut at the level of the cervical region.

Dissection of the head was performed following a consistent protocol: First, the dorsal area of the nasal region was accessed by removing all the skin surrounding the blowhole. Next, an incision was made on each side of the blowhole from the apex of the skull down to the level of the antorbital notches. Continuing in caudal direction, the skin was lifted while cutting through loose subcutaneous tissue, which transitioned into fibrous tissue as the bistoury progressed in rostral direction.

Next, the superficial external musculature was prepared up to the anterior border of the anteroexternal part. After this step all the skin surrounding the melon was removed (Pl. 3, Fig. 5 and 6).

The external musculature was dissected in layers, exposing the vestibular sac, and opening, inspecting and measuring it up to the slit-like opening (Pl. 4, Figs. 1 and 2). At this point, after removal of the vestibular sacs, the connective tissue dorsal to the nasofrontal sacs was cut to gain access to the sacs and all related structures such as the elliptical adipose bodies, the nasofrontal sacs' internal musculature and the caudal sides of the nasal ligaments (Pl. 4, Fig. 3).

After removing the anterior part of the nasofrontal sacs, transverse serial sections of the nasal plugs were made, together with sections of the adipose acoustic channels that formed the inner part of the melon, a.k.a. melon core (Pl. 3, Fig. 3). After the nasal plugs and the anterior wall of the spiracular cavities were displaced in rostral direction (Pl. 22, Fig. 2), two separate incisions were made in the lateral sides of said cavities and one in the nasal septum located in the centre. These incisions continued along the lateral curves of the premaxillary sacs to open these and to expose the external bony nares. The posterior wall of the spiracular cavities was left *in situ*, together with the openings to the nasofrontal sacs and the diagonal membranes, thus allowing inspection and possible resection of these tissues.

To continue the study and intricate dissection of other structures, both of grossly dissected pieces of tissue, as well as tissue still present on the skull such as the posterior wall of the nasal tract, as well as for the fine dissection of nerve branches, a stereoscopic microscope (Zeiss®) was used.

Concurrent with the dissection protocol and sampling, there was a photographic report on all structures that were removed from the head. Images were taken with a conventional SLR camera equipped with a 55mm F2.8 macro lens.

To obtain samples for histology we followed the same dissection sequence, but avoided contact with the walls of the nasal sac system; as such it was possible to sample the wall and surrounding tissues. In some cases the sampling succeeded the en bloc resection of the entire nasal sac system.

We tried to sample in a way that was as representative as possible for the various parts of the system; this required a sampling protocol that allowed to obtain sections of all structures. This protocol consisted of making multiple approximately sagittal cuts that included both the sacs and

the nasal tract, as well as cutting perpendicular to the first cuts. In addition, once possible structures of interest, such as glands, cartilage and specialized nerve endings, were observed in the general cuts, further samples were taken from different locations depending on the objective pursued in each case.

5. SAMPLE PROCESSING

5.1. Processing for optical microscopy

After obtaining the samples, they were **fixed** for a minimum of 24 hours in a solution of 10% neutral-buffered formalin. Next, the samples were sectioned into smaller fragments, leaving them prepared for embedding in paraffin.

Embedding in paraffin was used for studying the general histology, whereas in cases in which a higher resolution was required, the Technovit® (Kulzer) inclusion technique in methacrylate was used. Paraffin embedding was performed on a tissue embedder Hypercenter XP (Shandon) and block preparation on a Histocentre 2 (Shandon). Once the blocks were assembled, a MICROM HM330 (Heidelberg) microtome was used to obtain sections of 4-5 µm thickness.

Inclusion in methacrylate was performed after dehydration in increasing gradations of ethanol, followed by a pre-infiltration and infiltration in a solution based on Technovit 7100® more Hardener I (100 ml + 1g). After which the samples were finally included in an inclusion solution formed by an infiltration solution plus Hardener II (15 ml + 1 ml), forming blocks in a Histoform® plate at 37 ° C for 48h. The mounting of the blocks was performed in a specimen holder (Histobloc®), in which they were attached by adhesive Technovit 3040®. Sections of a thickness of about 2 µm were made with an Autocut (ReicherJung) 1150 microtome.

a) Staining for histology

Before the paraffin sections were stained, they were deparaffinised in xylene and rehydrated in alcohol baths of decreasing concentration.

Different general standard staining techniques were used such as Hematoxylin/Eosin (H/E) and Masson's Trichrome, and besides this, in order to stain elastic fibres, specific staining techniques were applied, such as Orcein/Van Gieson and Giemsa/Orcein Acid staining according McManus and Mowry (1960). The H/E staining was performed automatically with the help of a Varystain XY (Shandon) device. After appropriate staining, all sections were mounted on Eukitt® and kept at 37° C for 24h.

The sections that were obtained from specimens that were embedded in methacrylate, were stained with an aqueous solution of 1% Toluidine Blue for 1 minute on a hot plate (80° C). After the subsequent wash in running and distilled water (15 minutes), the sections were mounted on Eukitt®.

After staining the paraffin and methacrylate sections, these were observed under a microscope (Leitz® Dialux 20 EB) equipped with photography equipment (Wild®, Photoautomat MPS 45).

b) Staining for histochemistry

In order to determine the nature of the glandular structure secretions, and particularly to check for mucopolysaccharides, the following histochemical techniques (Pearse, 1985) were applied prior to the embedding in paraffin:

+ Periodic Acid-Schiff (PAS)

The sample was subjected to an oxidation of 10 minutes in an aqueous solution of periodic acid at 1%, followed by a 1 minute bath in Schiff's reagent (Sigma Chemical Co.).

+ Alcian Blue (AB) pH 1.0 8GX

Preparation of Alcian Blue (AB, Sigma Chemical Co.) with pH 1.0 (1% of Alcian Blue in 0.1 N HCl solution), proceeded by staining the sections for 30 minutes.

+ Alcian Blue (AB) pH 2.5 8GX

Preparation of Alcian Blue (AB, Sigma Chemical Co.) with pH 2.5 (1% in a solution of acetic acid 3%) proceeded by staining the sections for 30 minutes.

+ Alcian Blue (AB) pH 2.5 8GX - periodic acid-Schiff (PAS)

In this technique a combination of two of the above techniques was carried out, which consisted of staining the sections with Alcian Blue (AB, Sigma Chemical Co.) pH 2.5 (1% in acetic acid 3%) for 60 minutes, followed by a 10 minute oxidation in a 1% solution of Periodic Acid and a subsequent 1 minute bath in Schiff's reagent (Sigma Chemical Co.).

The PAS technique was used to detect the presence of nonspecific mucosubstances. Alcian Blue pH 1.0 was used to locate sulphomucins, and pH 2.5 was used to reveal the combination of sialomucins and sulphomucins. PAS staining of sections that were prestained with Alcian Blue pH 2.5 allowed for differentiation of neutral mucins and the acidic AB-positive mucins.

5.2. Processing for Electron Microscopy

Ultrastructural studies were only performed on samples obtained from very fresh material, at most three hours post-mortem. Cubic shaped samples of about 5 mm^3 were obtained, and fixed for 12 hours in a solution of 2.5% glutaraldehyde in 0.1 M phosphate buffer over 0.85% sucrose, adjusted to pH 7.4. Subsequently, samples were placed in phosphate buffer for 30 minutes and then fragmented for transmission electron microscopy (TEM) (block size 1 mm^3) or scanning electron microscopy (SEM) (3-5 mm^2). They were then post-fixated in 1% osmium tetroxide (OsO_4) for 60 minutes. After a second wash that consisted of two 15 minute baths in phosphate buffer, standard procedures were followed for both techniques.

The samples that were destined for the TEM study were included in Spurr (TAAB®) after dehydration and infiltration, as was defined in the protocol set by the manufacturer of this product. The blocks were sectioned using an ultramicrotome (LKB^{R}Nova) with glass blades. Initially, semi-thin sections of 1 µm thickness were obtained and stained with toluidine blue 1% on a hot plate and then examined under the microscope to select the areas of interest. Once these areas were located, and the sample was pyramided, they were sectioned to a thickness of 0.1 µm, and collected on copper grids of 3.05 mm in diameter G-200c (EBS). Finally, after staining the samples with lead citrate for 45 minutes, they were scanned with the transmission electron microscope (Hitachi 7100).

After fixating the samples that were selected for the morphologic studies of the surface, they were processed for scanning electron microscopy by routine procedures: dehydration in ethanol series of rising gradation, critical point drying, mounting in aluminium brackets, surface plating in a gold bath and ultimately scanning with SEM (Hitachi S-570) at an acceleration voltage of 15 kV.

5.3. Processing for the study of the junction of epidermis-dermis and epithelium-subepithelial layer

For this study we used samples of the entire nasal region. These samples were studied by optical microscopy and SEM.

In order to obtain representative samples of the blowhole region, both the anterior and posterior lip were divided into six parts of equal size (about 1 cm^2). Thus, on each side of the sagittal plane we considered three areas: a medial area, a lateral area where the commissures are, and an intermediate zone between these two.

As for the nasal sac system, routine samples were taken in the following locations: central zone in the roof of the vestibular sacs, anterior and posterior walls of the main nasal tract at the level of the spiracular cavity, the ventral aspects of the nasal plugs, and the entrances to the nasofrontal sacs (i.e. inferior vestibule according to Cranford (1988)).

For control samples, used to investigate the differences with the samples of the nasal region, skin was sampled from the dorsal neck region about ten centimetres caudal to the blowhole.

In carrying out the first steps of this research, it was noticed that, in order to preserve a good quality of the tissue, the processing of delphinid skin for SEM imaging required some adaptation of the standard procedures. The intricate connection between dermis and epidermis or between epithelial and subjacent layers of the nasal region, implied that separating the two without creating artefacts was more difficult than for similar tissues in other mammals. Moreover, as the skin decomposed quickly after death, which can be seen in a rapid desquamation of the epithelia, the latter was relatively fragile and could therefore be damaged easily. So to examine the corresponding surfaces of the dermal-epidermal junction by SEM, this required a smooth separation of the union. Considering the overall delicate nature of the tissues, standard procedures were adapted and carried out as followed:

- To obtain complete samples of the epidermis in both fresh and frozen specimens, the procedure as described by Stromberg (1989) was modified; as such, the dermis was separated from the epidermis by bathing the specimen in tap water of 57°C for 36 hours. Next, the epidermis was fixed by immersion in 2.5% glutaraldehyde in 0.1M phosphate buffer for 12 hours, followed by a post-fixation in 1% osmium tetroxide for 60 minutes. The same methodology was applied for studying the nasal tract epithelium.

- To obtain complete samples of the dermis, we experimented with standardized maceration procedures using potassium hydroxide (KOH) (Tsugane and Yasuda, 1995) or sodium bromide (NaBr) (Giacommeti, 1967), but these damaged the dermis to an extent that results were not acceptable, even after applying modifications to this procedure. However, we did obtain good quality samples of the dermis by immersing the fresh samples in 4% sodium hydroxide (NaOH) at 37°C for three hours. To process the fixated material, the maceration period was extending to 24

hours. Afterwards, the epidermis or what was left of it, was removed by applying a gentle massage of the tissue in 0.1M phosphate buffer under visual control with a stereoscopic microscope. After a complete removal of the pigmented epithelial debris and parts of epidermal protuberances, the sample was immersed in 2.5% glutaraldehyde and post-fixated in OsO_4, applying the same procedure as mentioned above. This methodology was also valid for studying the subepithelial layer of the nasal tract. Finally, the fixated samples were processed using routine procedures for SEM as mentioned above.

The general arrangement of the dermal ridges and the reciprocal epidermal structures was observed using a stereoscopic microscope (Zeiss). The observation of the skin surface was facilitated by a prior staining of the dermis by immersion in a solution of 1% toluidine blue for at least 15 seconds (cfr. Okijima, 1991).

5.4. Serial Sections

In order to gain a reference for the spatial distribution of the different structures that make up the facial region, two frozen striped dolphin heads were serial sectioned over their full diameter.

The cuts were made with a circular saw, parallel to each other and one centimetre thick. One head was sectioned transversely, the other longitudinally.

5.5. Methacrylate moulds

As an alternative method to obtain a three dimensional representation of the air spaces within the nasal region, the nasal sac system was injected with methacrylate in order to obtain moulds of the aerial spaces. For this, four heads were used, three of striped dolphins and one of a harbour porpoise.

Prior to injection, the nasal system was flushed with a phosphate buffer solution. The plastic used for injection was Araldite (Ciba Geigy), consisting of 70% monomer and 30% Cy223 hardener Hy2967. The injection was performed through the larynx using an automatic injector (Harvard Apparatus Pump 22) at constant speed. To control the plastic escaping dorsally, the blowhole was sealed with silicone paste, but two small tubes allowed for the release of air from the vestibular sacs as pressure increased during injection. To avoid a retrograde loss of plastic through nasopharynx, an 18” Foley catheter was used with the balloon inflated to the maximum.

To achieve maximum pressure inside the sacs and also to maintain this pressure until the plastic was polymerized, the injected amount was approximately 120cc. At this point, the tubes in the blow hole started to fill with plastic and they were pinched with a Kocher clamp to close the outlet.

Once the injection was completed, the heads were refrigerated for at least 24h followed by a maceration bath until the soft tissues were completely decomposed.

VI. RESULTS

1. ANATOMY AND GENERAL HISTOLOGY OF THE NASAL SAC SYSTEM

Although the anatomy of the nasal region of odontocetes has been studied in depth, we believe that certain aspects have not been adequately considered in the literature, especially in regards to a functional analysis of this region. And precisely because of this lack of structural studies, there is a corresponding lack of deep understanding of tissue formation and interrelations between the various components of the nasal sacs system. In this section we present those details of the gross anatomy that we consider to be insufficiently described, as well as the results of a histological study of the sacs and their surrounding tissue. We also describe structures of dense connective tissue that appear to form a functional unit, which we have called Laminar Fibrous Complex (LFC).

1.1. Nasal sac system

The nasal sac system of the superfamily of Delphinoidea is comprised of a nasal passage and four blind, paired diverticula. These nasal sacs are arranged around the main nasal tract in different planes, and are the following, from superficial to deep: vestibular sacs, nasofrontal sacs, accessory sacs and premaxillary sacs. This nasal sac system showed great similarities between species within this superfamily. The species of the family of Phocenidae and Monodontidae had a fifth pair of sacs, the posterior sacs.

We observed variations in shape and size of the different components between different species, as has been described previously (Schenkkan, 1973; Mead, 1975; Cranford, 1996), in the same way we have found small differences between individuals of the same species that, from a purely anatomically point of view, were not considered to be relevant for a detailed description, except for some cases that were noteworthy because of their size or their possible functional implications.

In this section we describe certain details that we believe are important to mention, and even though we will repeat some of the morphology of the nasal sac system and its conformation, we are mainly focusing on the epithelial lining and the underlying tissues. However, the relationship of the epithelium with the underlying layers will be discussed in different section, since its complexity has sought to give it a distinct chapter.

The morphology of the vestibular sacs was dorsoventrally flattened, as if it formed the number eight in a transverse manner, and each of the sacs was marked with the presence of an anterior and posterior fold that formed a transverse, rostrocaudal narrowing of the lumen. The folds extended over the entire wall in both the anterior and posterior parts of the sacs, and delimited a larger dorsal area and a smaller ventral recess. Both folds rested on the slit-like opening, in a way that when we opened the roof of the vestibular sac, the anterior fold became visible (Pl. 4, Fig. 1). Once this fold was displaced, the posterior fold and the slit-like opening came into view (Pl. 4, Fig. 2).

The vestibular sacs were contoured by an epithelium with many folds that were oriented in all directions, giving the surface a netlike appearance. Their presence entailed changes in thickness of the epithelium which was variable between the ridges and grooves, and depended on the compression of the different cell layers (Pl. 7, Fig. 1). The subepithelial layer consisted of loose connective tissue with collagenous fibres and a web of elastic fibres that ran parallel to the general surface of the sac (Pl. 7, Fig. 2), and extended in all directions surrounding the sac. Spreading out from this network of elastic fibres, there were fibres oriented perpendicular to surface of the sac entering the folds and even the papillae of the subepithelial layer (Pl. 7, Fig. 2). In the loose connective tissue we also observed striated muscle fibres that belonged to the anteroexternal part of the external musculature. However, these muscles fibres did not attach to the wall of the sac, except for those muscle bundles that entered into the folds of the sac here presence of connective tissue was denser than in the rest of the sac wall.

In comparison to other species of the Delphinoidea superfamily, the Phocoenidae family showed some exceptions to the above general description. The large vestibular sacs extended laterorostral to the blowhole and depicted a floor that was retracted rostrocaudally, and formed a series of semi-circular grooves and transverse folds around a central opening. This opening was continued in a short and flattened duct that opened into the nasal tract, dorsal to the slit-like opening. As such, we did not encounter the typical vestibular sac posterior and anterior folds, nor did the sacs have a direct open communication with the nasal tract (Pl. 4, Fig. 5). The folds of bottom of the vestibular sac presented a thick underlying layer of connective tissue layer that was especially dense in both folds and grooves, even though there was a central channel of loose connective tissue in the folds through which a subepithelial capillary network extended and vascularized the bottom of the sac. The wealth of dense connective tissue, coupled with the scarcity of elastic fibres in comparison to other parts of the sac, gave the floor of the vestibular sac great consistency. Moreover, no musculature was observed to be related to the sac that could be considered to be intrinsic.

Ventral to the vestibular sacs, the lumen narrowed to shape like an eyelet. This is called the ‘slit-like opening’, and it closes the lumen during apnea. It also contains the phonic lips. This set of structures are described in chapter VI.3.

Ventral to the slit-like opening was the middle part of the nasal tract or spiracular cavity. Passed this unique point the nasal tract splat in two with the nasal septum in the middle. This area is also closed between breaths via an apposition of the anterior and posterior walls, which take on a concave and convex shape respectively to fit perfectly (Pl. 2, Fig. 2). The anterior wall of the spiracular cavity continued ventrally as the nasal plugs that formed a transverse relief that protruded in caudal direction, was shaped like an impeller or plug lip and ended laterally in a rounded bulge or lateral extension. The posterior wall of the spiracular cavity inclined in caudal direction, forming an arched ceiling above the space where there were the entrance to the nasofrontal sac on the medial side and the opening to the accessory sac on the lateral side. The plug lip and the lateral extension of the nasal plug fitted into the respective openings (Plate 8, Fig. 4 and 5; Plate 10, Fig. 1). This whole area was lined by a particularly thin and extremely smooth epithelium. The subjacent tissue layer consisted of dense connective tissue with an apparent greater density of collagenous fibres in the anterior wall, muscle fibres that belonged to the nasal plug muscle, and adipose tissue of the melon core (Pl. 2, Fig. 2). All of these tissues together made

the anterior wall of the nasal tract feel quite robust. The posterior wall comprised less dense connective tissue, without muscle or adipose tissue fibres and was reinforced by the nasal ligament (Pl. 8, Fig. 4 and 5), whose ventral border acted as the ceiling of the slot that contained the openings to the nasofrontal and accessory sacs. Although elastic fibres were observed, they were not abundant nor seemed to adopt any type of structural pattern.

The main parts of the nasofrontal sacs were located at the same height as the slit-like opening, and were arranged in a shape like a horizontally flattened horseshoe (Pl. 4, Fig. 3). The walls contained large folds, particularly in the posterior part of the nasofrontal sac, which drastically reduced the lumen of the sac. The posterior aspect of the entrance to the sac featured a protrusion of the wall that consisted mainly of glandular tissue. It was shaped like a sack, which in some cases took on a volume capable of occupying the entire lumen of the entrance. This prominence was much more developed in species *Lagenorhynchus albirostris* (Pl. 14, Fig. 1) and *L. obliquidens*, somewhat less pronounced in *Tursiops truncatus* and virtually nil in *Stenella coeruleoalba*.

The epithelium presented a relatively constant thickness over the entire nasofrontal sac and was slightly thicker than in the spiracular cavity. In the curved extension of the sac there were small folds oriented parallel along the long axis of the sac. In the posterior part of the nasofrontal sac, subjacent to the epithelium, there was a small area of loose connective tissue that was infiltrated by fibres of the nasofrontal sac intrinsic muscle (Pl. 7, Fig. 3). The anterior part of the nasofrontal sac comprised a thin layer of loose connective tissue that was surrounded by broad bands of dense connective tissue, both dorsal and in lesser extent also ventral to the sac. These bands of dense connective tissue were stained by Van-Giesson / acid orcein and showed an accumulation of elastic fibres that formed a continuous elastic band (Pl. 9, Fig. 1). This elastic sheet, together with bands of dense connective tissue of the aponeuroses of the medial and lateral parts of the pars anteroexternus, formed part of the laminar fibrous structure that will be described later on. Ventral to the anterior part of the sacs, the subepithelial layer consisted of connective tissue infiltrated by the most dorsal fibres of the nasal plug muscle.

There was a large intraspecific variation in the morphology of the nasofrontal sac, especially of the anterior part of the nasofrontal sac. Some of these differences have already been described, such as the typical large anterior part of the nasofrontal sacs in *Lagenorhynchus albirostris*, with a great capacity for expansion, or the absence of the same in *Grampus griseus*; however, an exceptional case was the partial absence of the anterior right nasofrontal sac in a specimen of *Stenella coeruleoalba* (MGH5) (Pl. 22, Fig. 3).

The lining of the accessory sacs featured characteristics similar to the nasofrontal sacs, especially to the anterior part of the nasofrontal sacs.

The diagonal membrane was located ventral to the opening of the nasofrontal and accessory sacs, embedded in the posterior wall of the nasal tract and in the caudolateral angle of the external bony nare, thereby reducing the lumen slightly (Pl. 14, Fig. 1). The diagonal membrane, disposed in a plane that formed a boundary between the membranous and bony nasal tracts, consisted of a small fold with an epithelial membrane similar to the posterior wall of the spiracular cavity, with the exception that it contained a greater number of elastic fibres. Since the diagonal membrane was located ventral to the opening of the nasofrontal and accessory sacs, where the lip

and lateral extension of the nasal plug were present, it made contact with the bottom of the nasal plug when the nasal tract was closed, leaving a distinct impression in the former (Pl. 10, Fig. 1).

Similarly, the rest of the border of the external bony nare also left an imprint on the bottom of the nasal plug as the nasal plug literally acts like a plug that closes off the nasal passage. The epithelium in this ventral part of the nasal plug did not show any structural changes over its course, was histologically the same over the entire bottom, and featured a set of folds without any specific orientation and a layer of dense connective tissue in the base of the plug where the ventral fibres of the nasal plug muscle inserted (Pl. 7, Fig. 5). In rostral direction, the bottom of the nasal plug continued as the roof of premaxillary sac that presented a thin epithelium with a smooth surface over the entire sac (Pl. 7, Fig. 4). The conformation of this epithelium was similar to that found in the walls of the spiracular cavity. While the roof of the sac comprised a subepithelial layer with loose connective tissue, as was clear by the ease to separate the sac from the muscle, the floor of the sac was resting directly on the periosteum of the premaxillary bone, which made it much more difficult to detach. Even though there were many elastic tissue fibres in the roof of the sac, they did not seem to be organized in a pattern; there were relatively few fibres in the floor of the sac.

Porpoises feature a fifth pair of nasal sacs, called the **posterior nasal sac**s, and have accompanying structures such as the 'hintereklappe'. Therefore, their nasal system seems very complex; however, after a comparative analysis of these parts of the tract, we did not observe any major changes that affected the entire disposition of the nasal system. The posterior nasal sacs were located transversely between the bony wall of the nasal tract and ventrolaterally to the nasal bones and nasofrontal sacs, and they were similar in structure to the latter. The dorsal blind ends of each sac formed trabecular structures that subdivided the lumen into multiple fingerings, although there were no special structures related to these caverns. The most notable conformation feature specific for this species, would be the presence of posterior septum or hintereklappe, which was formed by dense connective tissue in which fibres were arranged in wide bands that made contact with the walls of the posterior sacs and the posterior part of the nasofrontal sacs. This septum consisted of a thick sheet of connective tissue that was folded in rostrocaudal direction, thus forming a double layered vertical septum of dense connective tissue with a central part of loose connective tissue; in a sagittal section this depicted a heart-shaped profile.

The nasal sac system was lined by a **stratified squamous parakeratotic epithelium** similar to the odontocete skin (Pl. 18, Fig. 1). It consisted of four layers (stratum germinativum, stratum spinosum, stratum intermedium, and stratum externum) throughout the entire nasal sac system, showing parakeratosis (i.e. cells in the outer layers maintain their nucleus and parts of the organelles). In comparison to the skin epithelium, the epithelium of the nasal sacs system is thinner in a way that is proportional for all layers (Pl. 5, Figs. 1, 5 and 6).

Moreover, the epithelial lining of the nasal sacs showed an abundance of underlying capillaries and nerve plexuses that were arranged parallel to the surface; in between the papillary and subjacent reticular areas there was also an abundance of elastic fibres (Pl. 5, Figs. 5 and 6).

Unlike the skin, in which pigmentation patterns were observed, which varied not only interspecifically but also intraspecifically, the epithelium of the nasal complex was pigmented in a

very irregular way. This was mainly observed in the striped dolphin, of which we obtained the most samples, but was also present in other species studied. Even so, it could be considered that the more dorsal the observed zone, the more pigmentation was present, as we found vestibular sacs to be usually high in pigmentation, similar to the region of the blowhole, ranging from grey shades in *Stenella coeruleoalba* (Pl. 4, Fig. 2 and 6) or *Grampus griseus*, through darker shades in *Stenella coeruleoalba* (Pl. 4, Fig. 1) up to a blackish coloration in *Phocoena phocoena* (Pl. 4 Fig. 5) and *Phocoenoides dalli*. This coloration in the upper parts of the nasal system tended to progress to a depigmented epithelium in the deeper, ventral parts. These changes in coloration were usually abrupt and more obvious species with in darker epithelia, such as in the genus Lagenorhynchus, in which a sharp transition of pigmentation was found at the level of the nasal plugs (Pl. 22, Fig. 2), leaving the anterior part of the nasofrontal sacs completely depigmented (Pl. 8, Fig. 2).

The S.E.M. study of the epithelial surface of the nasal sac system showed the presence of completely flattened keratinocytes in the face of the lumen, shaped like polyhedrals. These cells fitted into each other to form a smooth mosaic surface (Pl. 18, Fig. 5). The same was seen in locations with folds, where the epithelial surface maintained its smooth undulating contour. Due to the desquamation process, we regularly noticed the presence of cells in the lumen, isolated or in groups. Moreover, on top of the outer cell layer, we could easily spot the presence of particles of different sizes and shapes, from amorphous to spherical, compatible with cellular debris or secretion products respectively.

The anterior and posterior lips of the blowhole, which delineated the semilunar opening, did not present any macroscopic changes on entering the interior of the upper respiratory tract. However, the epidermis was remarkably thick, particularly in the anterior lip, with the greatest thickness in the turning point where the lip inclined inwardly (Pl. 18c, Fig. 11). This increase in thickness occurred in all cell layers proportionally. The dermis of the blowhole lips consisted mainly of dense connective tissue infiltrated with adipose tissue and bundled muscle fibres that were oriented perpendicularly to the epidermal surface in the anterior lip and parallel to the surface in the posterior lip (Pl. 18c, Figs. 11 and 12). Scattered dermal elastic fibres were also observed.

The thickness of the epithelium reduced gradually away from the blowhole as we progressed deeper into the nasal tract and the vestibular sacs.

1.2. Muscular system

As there are some magnificent descriptions and reviews on the musculature of the nasal region in the literature, this was not the focus of this work. However, the complexity and particularity of the structures in this region, which are features that are characteristic for odontocetes, asked for a detailed observation to get a better understanding of the functional implications of the morphology of the entire nasal system. The only way to get a good comprehension of these structures was through a thorough dissection of a large number of dolphin heads.

The facial region presented a clear differentiation between the rostral muscles that were related to the melon, the lips of the mouth, and the muscles related to the nasal tract.

In order to facilitate both identification and understanding of the functionality of the different muscle groups, we differentiated between the more superficially located muscles that are related to the upper half of the nasal tract, and to which we refer as the **external musculature**, and on the other hand the deeper lying muscles that relate to the lower half of the system, which we called **internal musculature**. This division was based on the location of the muscles and the presence of the laminar fibrous complex (to which reference will be made later) that acted as a physical separation between the external and internal musculature.

Even being able to identify all parts that have been described as external musculature, there was still difficulty in distinguishing the muscle origins from their bellies. This was more pronounced in larger species such as *Lagenorhynchus albirostris*, than in tinier species such as *Stenella coeruleoalba*.

From superficial to deep, we first encountered the **pars posteroexternus** that covered the lateral side of the vestibular sacs and inserted in the midline, caudal to the vestibular sacs together with its contralateral part, caudal to the nasal passage and caudoventral to the posterior lip of the blowhole (Pl. 3, Fig. 5).

The thin **pars intermedius** was located underneath the pars posteroexternus and was orientated in rostral direction (Pl. 3, Fig. 5). It inserted rostrolaterally into the blowhole, in the cross band of fibrous tissue that it had on its rostral side, where the blubber stopped being present in the hypodermis and it was mainly composed of collagen fibres.

Although the **pars anteroexternus** did not present a great thickness, it was visible as the outermost muscle, covering the entire facial fossa like a fan, spanning from the vertex of the skull to the more caudal part of the maxillolabial muscle, a.k.a. rostral muscle, at the level of the antorbital process. In this arrangement the muscle followed different orientations and inserted on various locations in the nasal tract system. In general, it was considered that the insertion of the pars anteroexternus extended from right beneath the blowhole lips, in the area around the dorsal edge of the vestibular sacs, down to the dorsal edge of the slit-like opening, passing the vestibular sacs both rostrally and caudally, and encompassing them in this manner.

The caudal fascicles were oriented in rostromedial direction and inserted deeply into the posterior lip of the blowhole and in the entire posterior wall of the vestibular sacs, where its fibres spanned in rostral direction.

The central fascicles were oriented in medial direction, encompassing the vestibular sacs and attaining the main nasal passage, but did not insert into the walls of the sac, except for some isolated fibres that reached the subepithelial layer. The most central of the central fascicles formed a common tendinous insertion in the nasal ligament, forming the lateral angle of the slit-like opening (Pl. 3, Fig. 6).

Finally, the cranial fascicles were arranged in caudomedial direction and infiltrated the marginal lateral sides of the mass of dense connective tissue that formed the anterior lip of the blowhole, while its deep fibres inserted into the anterior fold of the vestibular sac. Anyway, it was more a case of identifying the area of insertion rather than clearly separating the different bellies of the fascicles.

It was common for the entire pars anteroexternus that the insertions were formed out of muscle fibres infiltrating the connective tissue of the subepithelial layer of the nasal tract and its diverticula, with the exception of the group of central fibres that headed towards the lateral angles of the slit-like opening where it formed the aforementioned tendinous insertion.

The **pars posterointernus**, from the caudal third of the facial fossa, was orientated in rostral direction to insert in the entire posterior border of the slit-like opening of the corresponding side. This insertion consisted of a wide aponeurosis that covered the posterior part of the nasofrontal sac and its intrinsic muscles (Pl. 3, Fig. 6), and was bounded dorsally by the elliptical adipose bodies before inserting in the subepithelial layer of the slit-like opening, immediately dorsal to the phonic lips (Pl. 9, Fig. 4).

Finally, the **pars anterointernus** was the most developed muscle, situated deeper in the nasal system than the previously mentioned muscles. It extended across the entire region rostral to the nasal passage, and dorsal to the anterior part of the nasofrontal sac. At this level it emitted several aponeurotic layers that intermingled with fibrous, elastic and adipose tissue and also with its contralateral aponeuroses, thus constituting a part of the LFC and as such forming part of the dome that covered the nasal plug muscle and melon core (Pl. 2, Fig. 1; Pl. 9, Fig. 3).

The internal musculature, or muscular configuration of the more ventral part of the nasal sac system, also included the nasal plug muscle, the intrinsic muscle of the nasofrontal sac and the muscle of the diagonal membrane.

The **nasal plug muscle** originated rostral to the blind ending of the premaxillary sacs, in two triangular areas on the premaxillary bones, and from there inclined in dorsocaudal direction covering the sacs and reaching the nasal tract and nasal plugs (Pl. 2, Fig. 2). The area of insertion stretched from the dense connective tissue in the bottom of the nasal plugs and its lateral extensions, going up the entire anterior wall of the nasal tract up to the level of the spiracular cavity. Thus, we noted that the muscle was not limited to an insertion in the nasal plugs, hence its name, but extended dorsally up to the level of the slit-like opening, which was ventral to the anterior part of the nasofrontal sacs (discussion). This means that a contraction of this muscle would act on the entire anterior wall of the nasal tract, from the premaxillary up to the vestibular sacs. The nasal plug muscle was infiltrated by both fibrous and adipose tissue, which was also visible macroscopically (Pl. 2, Fig. 1 and 2).

The **nasofrontal sac intrinsic muscle** surrounded the posterior part of the nasofrontal sac and its opening to the nasal tract. Because this muscle was located both rostrally and caudally to the nasofrontal sac, without a clear separation between the two (Mead, 1975), we divided it into a minor and a major part, as proposed by Schevill and Lawrence (1956).

The minor part of the nasofrontal sac intrinsic muscle was situated between the posterior part of the nasofrontal sac and the nasal ligament. Its fibres were oriented transversely, following the axis of the sac, slanting ventrally towards the entrance of the sac and the ventral border of the nasal ligament. This entire part of the muscle inserted into the posterior aspect of the nasal ligament and the rostral aspect of the sac (Pl. 7, Fig. 3). Laterally, from an area ventral to the entrance of the anterior part of the nasofrontal sac, the minor part of the muscle intermingled with the major part from where it originated in the form of a tendon, which was partly in the

lateral side of the nasal ligament and its ventral extension (Pl. 8, Fig. 3) and partly in the fibres of the nasal plug muscle that were situated lateral to the tendon.

The major part of the nasofrontal sac intrinsic muscle stretched horizontally across the entire area caudal to the sac. It extended medially to the insertion of the nasal ligament in the nasal bones, and laterally to the junction with the minor part of the muscle; moreover, a part of this mayor part of the muscle originated from a fibrous tissue band that anchored onto the suture between the maxillary bone and the caudal extension of the premaxillary bone. The entire muscle was closely connected to the walls of the posterior part of the nasofrontal sac, penetrating into its folds (Pl. 7, Fig. 3; Pl. 8, Fig. 5).

The **diagonal membrane muscle** covered the bony wall, caudodorsal to the external bony nares. It originated in an area between the caudal ends of the ascending process of the premaxillary bone and the nasal bone, where there is the suture between these two and the maxillary and ectethmoid bone. From there it descended vertically until reaching the area lateral to the diagonal membrane (Pl. 8, Fig. 4). The insertion of the muscle concentrated in the angle that was formed by the diagonal membrane and the medial border of the caudal extension of the premaxillary bone. As such, it formed the caudolateral angle of the external bony nare. Thus, the muscle took on a triangular shape with its apex pointing towards ventral.

There was a strong similarity between species in terms of the referred musculature. In every species we studied, we could identify all the different parts of the maxillofacial musculature. And although the arrangement of the muscles could display some modifications to some extent, which were due to variations in the overall morphology of the nasal sacs, their insertions were always equivalent.

Furthermore, we did not observe any notable differences between the outer musculature of both sides, as such, they seemed symmetrical. However, the internal musculature of the right side was remarkably larger, which corresponded the predominance of the right side of the nasal tract system.

Other muscles?

2. LAMINAR FIBROUS COMPLEX (LFC)

During dissection and histological studies of all specimens, we could observe a dense sheet of fibro-elastic tissue that domed over the lower half of the upper respiratory tract including the inferior nasal sacs, internal musculature, nasal plug muscle, melon core and nasal ligament (Pl. 8, Fig. 5). We called this formation of connective tissue the Laminar Fibrous Complex (LFC). It facilitated the interaction between the different anatomical structures of this part of the nasal region.

The edges of the LFC stretched along the lateral edge of the premaxillary bones and followed the sutures between these and the maxillary and frontal bones in caudal direction, ultimately also attaining the nasal bones (Pl. 8, Fig. 1). Furthermore, the insertions of the LFC in the borders of the slit-like opening formed the dorsal limit of the connective tissue layer (Pl. 8, Fig. 2). Moreover, it

formed a domed continuum that featured a rostral opening in which the adipose tissue of the melon and the nasal plug muscle were the primary presence (Pl. 8, Fig. 1).

The deeper external musculature covered the LFC and inserted into it with its aponeuroses. The rostral part of the anterior part of the nasofrontal sac was surrounded by these aponeuroses, where a well-developed fibro-elastic sheet and abundant elastic fibres intermingled with the rest of the connective tissue (Pl. 9, Fig. 1).

The posterior part of the LFC was constituted by the nasal ligament, which consisted of a thick band of dense connective tissue that had a cartilaginous centre with an ovoid profile when it was observed in a sagittal section (Pl. 9, Fig. 2). The medial side of the nasal ligament inserted onto the vertex of the skull, in between the nasal bones, and its lateral side inserted onto the caudal ends of the premaxillary bones through tendinous bands (Pl. 8 Figs. 3 and 4). The dorsal side of the nasal ligament extended in caudal direction as a sheet of fibro-elastic tissue that inserted onto the caudal ends of the ascending processes of the premaxillary bone, the suture between the maxillary and ectethmoid bones, and medially onto nasal bone. This elastic sheet covered the posterior part of the nasofrontal sac and its associated musculature (Pl. 9 Figs. 2 and 4).

We did not observe any significant morphological differences in the LFC between species of the same family, and even the differences between families were small. Only the species of Phocoenidae had a lesser developed LFC with a smaller thickness. Altogether, the area covered by the LFC reduced proportionately, as did the nasal sacs it covered, leaving a compact space that existed only caudal to the nasal tract, where it was considerably prominent because of the dorsal protrusion of the posterior sacs and septum (Pl. 9, Fig. 3). In contrast, the porpoises presented the characteristic, proportionally larger vestibular sacs with convoluted, fibrous and rigid ventral surface.

3. SLIT-LIKE OPENING

The four phonic lips were situated in the slit-like opening as left and right anterior, and left and right posterior lips. They were all characterized by a clearly defined, transverse white line with particularly dark epithelial stripes on both sides (Pl. 14, Fig. 1**;** Pl. 10, Fig. 1). In the anatomical specimens, the lips featured undulations or grooves arranged in straight angles to the transverse axis (Pl. 4, Fig. 6) and this was also visible in the histological slides (Pl. 10, Fig. 4). The anterior and posterior lips were relaxed and in close contact with each other (note that this situation was post-mortem). Moreover, the right phonic lips were larger than the left ones, corresponding to the asymmetry of the nasal tract system that was mentioned previously.

Each phonic lip was associated with an easily observable elliptical adipose body that was embedded in the underlying tissue (Pl. 13, Fig. 1). The elliptical adipose bodies on either side were more or less similar in length. Dorsal to the phonic lips, and covering the elliptical adipose bodies, there was the insertion of the LFC, reinforcing the dorsal margin of the phonic lips. The slit-like opening and the phonic lips were closely related to the nasal ligament, which was located caudal to the posterior elliptical adipose bodies, as all these tissues were in close contact at this level between the two commissures of the nasal tract (Pl. 4, Fig. 3).

As mentioned along the chapter of Results, the stratified squamous parakeratotic epithelium that lined the walls of the nasal sac system showed no significant variations along the entire nasal complex. However, the epithelium of the phonic lips mainly showed an increase in the thickness of the outer cell layer, which was more than four times larger while the underlying layers had a similar height than in the rest of the tract (Pl. 10, Fig 2; Pl. 11, Fig. 1). The cells of this layer showed specific cell junctions (Pl. 11, Figs. 2 and 3) that avoided a rapid flaking that normally occurs in other epithelia. A cross section of the phonic lips demonstrated the fusiform shape of the lips and their protrusion into the lumen of the nasal tract.

Even though the anterior and posterior phonic lips turned out to be structurally similar, and they were both related to their own elliptical adipose body, we did observe several differences. The posterior elliptical adipose body, also called elliptical fatty body, was embedded in connective tissue (PI. 10, Fig. 5), without direct contact with the sorrounding adipose tissues. It was bordered dorsally by the insertion of the LFC in the nasal tract, and caudally by the dorsal part of the nasal ligament (Pl. 8, Fig. 5). Moreover, we observed connective tissue fibres that ran between the elliptical adipose body and the epithelium, oriented parallel to the surface of the epithelium.

Furthermore, the anterior elliptical adipose bodies were covered dorsally by the anterior part of the nasofrontal sac and they were separated from the melon core by a thin sheet of connective tissue (Pl. 8, Fig. 5). The tissue fibres in between the elliptical adipose body and the respective phonic lip were aligned in a straight angle with the surface of the epithelium running from one structure to the other (Pl. 10, Fig. 6).

After maceration and removal of the epithelium, the surface of the phonic lip's subepithelial layer presented relatively small ridges that were arranged parallel to each other and perpendicular to the long axis of the phonic lip (Pl. 12, Figs. 1 and 2); on the other hand, the ridges that were in close proximity of the phonic lip were oriented differently, without the parallel character and at an oblique angle to the axis of the lip (Pl. 12, Figs. 1 and 3) until reaching places such as the vestibular sacs where the ridges were organized in a complex mesh (PI. 12, Fig. 4).

Moreover, SEM of the epithelial surface of the phonic lips, showed a slightly elevated plateau with a slope of desquamated cells on its dorsal and ventral side (Pl. 12, Fig. 6). The ventral slope was more gentle (Pl. 12, Fig. 7) in comparison to the steeper dorsal slope (Pl. 12, Fig. 8).

4. GLANDULAR STRUCTURES

During the overall histological inspection of the nasal sac system, we encountered glandular structures with morphological similarities to those that have been described in the literature. A more detailed investigation showed that there were two types of glandular tissue as a constant feature, both in *Stenella coeruleoalba* as in all other species that were analysed. On one hand, there was a large glandular structure located in the posterior wall of the spiracular cavity, and on the other hand, there were smaller glandular structures located under the epithelium that lined the nasofrontal sacs. These structures were present on both sides of the nasal tract. These two conformations were the only glandular structures that were present in the upper part of the respiratory tract, which is located dorsal to the external bony nares.

Before present work, there was no specific nomenclature for these glandular structures. Based on anatomical location and comparative studies we opted to name the larger glands '**nasal glands'** and the smaller glands '**nasofrontal glands**'.

4.1. "Nasal glands"

The nasal gland glandular tissue was grouped and aligned transversely to the longitudinal axis of the body, and situated in the ventral part of the posterior wall of the spiracular cavity. In this wall the glandular tissue was located in an area that spanned from close to the membranous nasal septum medially, to the entrances of the nasofrontal and accessory sacs laterally. Each of the two glands was situated between the aforementioned lining of the posterior wall and the ventral side of the nasal ligament where the posterior lips of the nasal plugs made contact and could execute pressure onto the openings of the nasofrontal and accessory sacs (Pl. 13, Fig. 1). The glandular tissue, embedded in dense connective tissue, extended in ventral direction along the posterior wall of the nasal tract and the anterior wall of the posterior part of the nasofrontal sac (Pl. 8, Fig 5; Pl. 13, Fig. 1).

The nasal glands were oval-shaped, and their size varied depending on the overall dimensions of the nasal tract, the size of the posterior wall, the age, size and sex of the individual. There was also a significant difference between the nasal gland of the left and right nasal tract, related to the marked asymmetry present in certain species, which made it difficult to obtain representative values. However, here are some guidance measurements of the right nasal gland of an adult striped dolphin: ±16 mm long, equal to the width of the entire posterior wall, 2.5 mm high and 4 mm wide. The left nasal gland exhibited the same morphology as its contralateral homologue, but was smaller in size, corresponding to the overall asymmetry.

The gland was surrounded by a thin fibrous capsule with sheets or strands dividing up the interior and therefore segmenting the gland into several lobes, while it appeared compact (Pl. 13, Fig. 5). We did not encounter any myoepithelial cells nor an intrinsic muscles associated to the gland.

Microscopic examination showed that the parenchyma consisted of spherical acini (diameter 80 µm) with a single layer of cuboid or cylindrical cells (Pl. 13, Fig. 6; Pl. 15, Fig. 1). The conically shaped cells had a basally displaced core (Pl. 15, Fig. 6), a well-developed rough endoplasmic reticulum (RER) and abundant mitochondria that were visible through ultrastructural research (Pl. 15, Fig. 7). The apical part of the cytoplasm was usually occupied by numerous electron-dense vesicles of varying size. These vesicles deposited their content into the acinar lumen by means of apocrine secretion. On the other hand, there were cells that did not contain these vesicles, but instead showed the presence of electron-lucid vacuoles that were larger than the vesicles (Pl. 15, Fig. 1).

The acinar cells showed a positive reaction for all histochemical stains that were applied. Thus, we observed an intense purple blue reaction to the PAS/AB staining (Pl. 16, Fig. 1 and 5) in the acini, but not in the discharge ducts (Pl. 16, Fig. 2); similarly, a reddish colour appeared in the acinar cells when applying the PAS technique (Pl. 16, Fig. 3); regarding the AB staining, there was an intense blue coloration with the method at pH 2.5 (Pl. 16, Fig. 4) and a similar but weaker colour reaction with pH 1.0 (Pl. 16, Fig. 6).

The blue-purple colouration obtained with the PAS/AB staining technique indicated the presence of alcianophilic components, reagents to periodate, while mucosubstances containing hexoses were stained by the PAS process. The strong positive reaction to AB with pH 2.5 and the weak reaction with AB pH 1.0 pointed out that the secretion products were characterized as weakly acidic, sulphated mucosubstances. To conclude, the histochemical study showed that the secretion products consisted of acid mucopolysaccharides.

The excretory ducts were lined with a two-layered cuboidal epithelium. These cells had microvilli on their apical cell surface (Pl. 15, Fig. 9). As several of these ducts converged, the size of the lumen increased and so did the number of cell layers, until the lining was a multilayered squamous epithelium such as in the rest of the nasal tract. The main ducts opened into the nasal tract where the latter bent in caudal direction and was in contact with the dorsal surface of the nasal plugs (Pl. 13, Fig. 1; Pl. 14, Fig. 1). There were two main pairs of excretory holes for each segment of the gland. Near these main orifices there were minor openings that we considered to be accessory. The main excretory holes were arranged in transverse rows, reaching up to 35 holes on the right side and approximately 20 on the left side.

The duct orifices were macroscopically hardly noticeable in the fresh material. However, they were easily visible in the subjacent layer once the epithelium was removed (Pl. 13, Fig. 2). The duct outlets opened into the nasal tract at a sharp angle of 20 to 40 degrees, with the opening partially covered by an extension of the wall in the form of a sheet that could operate like a valve. The contour of these openings was shaped like the letter 'D' with a diameter of about 200-300 µm valve (Pl. 13, Fig. 3 and 4).

In the stroma surrounding the acini there were plasma cells (type B lymphocytes) that were dispersed, never appearing in clumps or any structured organization (Pl. 15, Fig. 8; Pl. 13, Fig. 6).

The nasal glands were present in all species that were part of this study, and the degree of similarity of their arrangement, size and location was remarkable. It was only the family of Phocoenidae that featured slight variations in size and arrangement of the glands. In these species, the ventral angle of the posterior wall of the spiracular cavity was sharper and thus presented a triangular profile in a paramedian cut, thanks to a transverse groove formed by an epithelial fold in between the anterior wall of the nasofrontal sac and the lower part of the nasal tract, which was characteristic for this family. This entire ventral part of the fold was taken in by these glandular structures (Pl. 13, Fig. 7). The structural, ultrastructural and histochemical characteristics of the glands were similar to those of other species that were part of this study, although the entire glands were proportionally larger.

4.2. Glands of the nasofrontal sac: “Nasofrontal glands”

The smaller nasofrontal glands in *Stenella coeruleoalba* were composed of numerous individual glandular units in the walls and especially the ceiling of the nasofrontal sacs around the opening. They presented themselves scattered along the wall of the nasofrontal sac at the level of its opening into the nasal tract, and also occupying a small part of the anterior and posterior parts. The glands were not observed in any of the blind ends of the anterior or posterior part of the nasofrontal sacs. They had a rounded dendritic shape and a size of approximately 2 to 3 mm in

diameter each. They were located within connective tissue of the subepithelial layer, and were connected to the lumen of the sac through a very short duct.

The nasofrontal glands were similar in morphology, size, dispersion and distribution on both sides. However, the average estimated number of glandular units that secreted through a single opening was approximately 40 on the right side and 15 on the left. These numbers were obtained from studying *Stenella coeruleoalba*, and were also valid for *Delphinus delphis* and *Lissodelphis borealis*.

In the genus Lagenorhynchus there was a fold in the form of a pouch, situated in the posterior wall of the nasofrontal sac. The size of this corresponded to the width of the opening to the nasal tract and could therefore be used to close of this opening (Pl. 14, Fig. 1). The nasofrontal glands were situated within this fold, as they formed a glandular complex that was larger than in all other species (Pl. 14, Fig. 3). Despite the difference in size, each individual featured the same morphologic structure that drained into the lumen of the sac or, in some cases, directly into the nasal tract through several ducts, one for each glandular unit. *Lagenorhynchus albirostris* featured a well-developed glandular fold, and so did *Lagenorhynchus obliquidens* but slightly less. On the other hand, in *Lagenorhynchus acutus* this crease was not observed, nor were the nasofrontal glands or the duct orifices. In other species such as *Tursiops truncatus* and *Grampus griseus* we observed a discrete fold with few scattered glandular formations. We did not encounter nasofrontal glands in Phocoenoides.

The nasofrontal gland acini were structurally similar to the nasal gland acini (Pl. 14, Fig. 4 and 5; Pl. 15, Figs. 4-6), as were the histochemistry results on their secretion products (Pl. 16, Fig. 2), and the presence of plasma cells in the immediate vicinity of the acini (Pl. 14, Fig. 4).

5. INNERVATION

5.1. Facial nerve

The facial nerve (nervus facialis - VII), responsible for the motor innervation of the nasal region, featured nerve branches along the entire facial musculature. From its origin in the stylomastoid foramen the nerve ran ventral to the orbit, curved in dorsal direction in the antorbital notch, and continuing caudal along the maxilla to the nasal region (Pl. 6). At this level the nerve could hardly be distinguished or separated from the dense connective tissue surrounding it. Once surpassed the antorbital notch, the nerve branched into several fibres, innervating the external musculature in different planes as each branch branched further in a hand-fan-shaped way. We could not see a direct insertion of the nerve fibres into the different parts of the maxillonasal musculature, and there were a greater number of branches than there were muscle parts. The deepest branches perforated the LFC rostral to the accessory sac and immediately diverged into several rostral branches distributed along the nasal plug muscle and caudal branches situated between the accessory sacs and the lateral sides of the nasal tract in direction of the posterointernal part.

The left and right facial nerves presented similar arrangement and diameter, without macroscopically detectable differences between the two.

5.2. Infraorbital nerve

The sensitivity of the nasal region seemed to be managed by the branches of the infraorbital nerve (nervus infraorbitalis), which is a branch of the maxillar nerve (V_2), which in turn is a branch of the trigeminal nerve (V). The infraorbital nerve branched on its entrance into the facial region through several foramina in the maxilla, lateral to the external bony nares (Pl. 6).

The largest branch of the infraorbital nerve passed through the most caudal dorsal infraorbital foramen, which has the largest diameter, and was responsible for the innervation of the bulk of the nasal tract system. This thick branch lied on top of the pars anterointernus, and continued in medial direction to cross the LFC in between the lateral commissure of the slit-like opening and the anterior end of the accessory sac, a little more caudal and dorsal to the area where the facial nerve entered the LFC (Pl. 17, Fig. 1). Once inside the space that was limited by the LFC, the nerves forked into small branches that headed in rostral direction and intermingled with soft tissues dorsal to the premaxillary sac. Other branches spread towards the anterior wall of the nasal tract at the level of the spiracular cavity and passed through the nasal plug muscle to insert into the epithelial membranes of the nasal plugs. Finally, a considerably thicker branch surpassed the most lateral parts of the spiracular cavity and succeeded transversely and ventrally to the angle of the cavity between its posterior wall and the entrance to the nasofrontal sac, into an area called the peninsula by Cranford et al. (1996). From this last branch, a nervous network sprouted and spanned across the entire posterior wall of the nasal tract and nasofrontal sac. In the subepithelial layer of this area, there was a considerably higher concentration of nerve ramifications than in the rest of the nasal tract.

During the dissection of the infraorbital nerves, there was a marked asymmetry between the two sides. The right infraorbital nerve could easily be followed and dissected thanks to its diameter of approximately 3 mm in *Stenella coeruleoalba*, as it was also accompanied by the corresponding infraorbital blood vessels. The left nerve was smaller and virtually indistinguishable from the surrounding tissues and muscle aponeuroses. This difference between the right and left sides corresponded partly to the asymmetry in volume of the nasal sac system, but this relationship was not proportional. For example, for *Stenella coeruleoalba* the volume of the right half of the nasal sac system was about 1.5 times that of the left half, while the left infraorbital nerve was unnoticeable in comparison to the right.

5.3. Sensory receptors

General histology showed the presence of nerve endings in the form of encapsulated receptors. Each of these receptors was surrounded by a variable number of concentric cell layers which gave them an onion-like appearance. The receptors were either single and isolated, or gathered in compact groups or rows of usually about four receptors in each conglomeration (Pl. 17, Figs. 2-5).

These encapsulated receptors were situated in the subepithelial layer and in some places in the papillary layer. Although these receptors were present in all parts of the nasal sac system, they were not evenly distributed, but concentrated in certain areas such as in the ventral part of the nasal plugs, cranial to their caudal border but caudal to the region where they make contact with the external bony nares. Likewise, they were also abundant in the connective tissue caudal to the

nasal ligament, and where this tissue intermingled with the minor part of the nasofrontal sac intrinsic muscle (Pl. 17, Fig. 2). At the same level, they were present but less abundant in the subepithelial layer of the nasofrontal sac in the proximity of the nasal ligament.

6. PATTERNS OF THE INTERRELATION BETWEEN EPITHELIUM AND SUBJACENT LAYER

The morphology of epidermis and epithelium, and especially their interrelationship with dermis and subepithelial layer respectively, were certainly complex.

When looking at the skin of the dorsal cervical region in *Stenella coeruleoalba*, the **epidermal ridges** were large, quite regular in shape and had clearly visible **protuberances or 'knobs'**. The **dermal ridges** presented large, straight **papillae** arranged parallel to the longitudinal axis of the body (Pl. 18, Figs. 1-4). The length of the dermal papillae gradually decreased as samples were taken closer to the blowhole, and in the nasal tract the papillae were mostly small or even absent.

In the area of the blowhole, the alignment of the dermal ridges showed a characteristic pattern, as revealed by stereoscopic examination of the separated tunicae. Also, the dermal ridges were wider and the dermal papillae were shorter in this area compared to the neck region (Pl. 18b, Fig. 5), and they were very short to absent in the rostrolateral parts of the posterior blowhole lip, at the level of the commissure.

The dermal ridges in the area of the blowhole presented three characteristic patterns, which in some specimens were also found in other locations:

First, an elongated and curved arrangement of the ridges, with a flattening of the apical ends of the papillae parallel to the surface of the skin (PI. 18b, Fig. 6). This was seen in the anterior blowhole lip, halfway between the commissure and the centre.

Second, a compression of the papillae, which were pear-shaped with a thickened apical end of the papilla (Pl. 18b, Fig. 7); this pattern was observed in the anterior blowhole lip, near the commissures of the blowhole.

Third, an irregular tilting of the ridge towards either side so that the crest had an undulating appearance (Pl. 18b, Fig. 8). This arrangement was seen less frequent than the first two, and was present in the anterior walls of the nasal passages and in the lateral walls of the commissures of the blowhole.

In the nasal tract, ventral to the blowhole lips the **subepithelial ridges** featured a reduced height in comparison to the **dermal ridges**, undergirding vestigial papillae, if any. This morphological change occurred gradually.

The contact area between epithelium and the underlying subepithelial layer gradually decreased deeper into the nasal tract system. The subepithelial ridges appeared as smooth lines with low altitude and the papillae almost totally disappeared in some cases. The alignment of the ridges corresponded to the orientation of the surface they occupied, always dominating the arrangement along the main axis of said surface. So, on flat surfaces, such as the ventral aspect of the nasal plugs and the continuation into the roof of the premaxillary sacs, the dermal ridges were aligned parallel to each other in rostrocaudal direction. On curved surfaces, such as the anterior

and posterior walls of the middle part of the nasal tract, the ridges curved along with the tract, from an initial dorsoventral orientation in the upper part to a lateroventral direction in the lower part. The arrangement in the nasofrontal sacs was an intermediate between the two as the ridges followed the major axis of the sac without altering their parallelism. The alignment also followed the curvature, and as such was shaped like a 'C' in the lateral corner of the sacs. The ridges in the vestibular sacs, although similar in morphology to those in the rest of the nasal complex, featured a completely irregular pattern, which was also visible in the external epithelial folds. Another divergence was observed at the entrance of the nasofrontal sacs, where the overall arrangement was maintained but where the ridges did present small sized papillae.

> "Only by carefully planning the dissection as though it were for an operation could one expect to be successful in a study like this. But even if one would overcome all this technical difficulties and succeed in dissecting the facial musculature, one experiences great difficulty in interpreting the relationship of the various elements"
>
> Ernest Huber 1934

VII. DISCUSSION

This quote echoes the enormous difficulty inherit to dissection and concomitant interpretation of the facial region in odontocetes. Although there is an abundance of anatomical studies that have addressed this issue over several centuries, and that have provided new knowledge ever since, there are still new questions arising. Moreover, it is curious how the knowledge of this region has been based on large studies, as well as on small but valuable contributions, and on countless theories and controversies. We believe that this is due to the extreme complexity of this functional system, the difficulty of obtaining material, and lately also the contribution of new technologies; the complexity of the nasal sac system, both morphologically and functionally, is not yet fully understood, as we first need to comprehend every interrelated part of the whole.

1. ANATOMY AND GENERAL HISTOLOGY OF THE NASAL SAC SYSTEM

1.1. The Nasal Sac system

The results we obtained by studying the gross anatomy of the nasal sac system broadly coincided with descriptions by Schenkkan (1973) and Mead (1975), among others. However, we found certain variations that we consider important for understanding the functionality of this region.

The vestibular sacs were characterized by highly elastic walls, which, judging by the folds on the inner surface, were capable of extending considerably. The morphology of these large sacs was highly variable between species, wheras each sac did feature an anterior and posterior vestibular fold. These folds were virtually ignored by several authors except for Schevill and Lawrence (1956) and Evans and Maderson (1973), who considered the vestibular folds to be the dorsal limit of the spiracular cavity. As such, they described a recess ventral to the anterior fold that was considered to be part of the spiracular cavity. In our opinion, the vestibular folds would be a part of the vestibular sacs, as we consider the slit-like opening to be the dorsal limit of the spiracular cavity. These folds could be very important because of their strategic location in between the vestibular sac lumen and the slit-like opening. Indeed, because the walls are corrugated, they allow the surface of the sacs to extend substantially; moreover, the anteroexternal part covers the vestibular sacs but also has lateral insertions into this fold. This allows the muscle to reduce the

lumen of the sacs while they can put tension on the anterior fold that overlays the slit-like opening and the incorporated phonic lips so that these are not in direct contact with the main airspace of the vestibular sac, which could otherwise form a resonant cavity, considering that the phonic lips are the area of ultrasonic sound production.

Although it was not until very recent that the middle part of the nasal tract, or spiracular cavity, was considered to be important (Cranford et al., 1996), its relevance is out of question since it has the ability to lock the nasal tract system thanks to a perfect apposition of the anterior and posterior walls which feature a very smooth surface epithelium. Moreover, the coupling of the walls is made possible by their high rigidity, which was present even in post-mortem material.

The layout of the nasofrontal sacs around the slit-like opening is enough to indicate their role in the pneumatic closing of the nasal tract at this level, as initially advanced by Schevill and Lawrence (1956). This possibility is supported by the presence of creases in the walls of the sacs, which would allow a large increase of the volume. As the anterior part of the nasofrontal sacs is enveloped by a fibro-elastic sheet that is part of the LFC, it could inflate easily; the posterior part of each nasofrontal sac is surrounded by the nasofrontal sac intrinsic musculature, which inserts in the sac itself, as such they seem co-dependent, and they are covered by the elastic sheet of the posterior part of the LFC. The accessory sacs are similar to the nasofrontal sacs in that they open posterior to the nasal tract and curves towards rostral, although less pronounced than the latter. Moreover, they are significantly smaller and extend in a vertical plane lateral to the corners of the spiracular cavity. As these sacs are situated between the nasofrontal sacs and the premaxilla, they complete the lateral sides of the dome that all nasal sacs form together. They are relatively small e and present large interspecific variation, which could indicate a little important function. They probably contribute to the overall function of the sac system as a sound baffle, as do the nasofrontal sacs in their parts that are located rostral, lateral and caudal to the nasal tract.

Each common entrance to the nasofrontal and accessory sacs is bounded dorsally by the nasal ligament in the form of an arc. The characteristic shape of the nasal plugs allows them to act as a plug into the openings, hence the name, and they are held tight dorsally by the nasal ligaments**.** The diagonal membranes are in contact with the medioventral side of the nasal plugs at rest and could therefore act as valves that seal the external bony nares. This whole set of structures is lined with a similar epithelium as in the other nasal sacs, with a subjacent layer of predominantly dense connective tissue, which makes us think, that the diagonal membranes play a part in sealing of the airways, as proposed by Cranford (1992), rather than being vibrational diaphragms (Mead, 1975) or being the location of sound production (Ridgway et al., 1980).

Judging by the homogeneity of the epithelial lining of the nasal sac system, we assume that the slit-like opening is the only structure capable of performing such a complex function as is the mechanism of sound production.

We have observed considerable interspecific differences in the morphology of the nasal sac system, which have also been mentioned by other authors (Schenkkan, 1973; Mead, 1975; Heyning, 1989), and were explained by Schenkkan (1973) as a result of the process of evolution. According to this author, there is a predominance of more superficial nasal sacs, such as the vestibular and nasofrontal sacs, in species that are less evolved such as in the family Platanistidae, whereas in more modern species such as in the family Delphinidae, the premaxillary sacs have

gained volume. In general, the aforementioned pattern concurs with the entire suborder, and we believe this occurred as a complementary occupation of the space, so for example, the development of huge vestibular and nasofrontal sacs in Phocoenidae would come along with a reduced size of the premaxillary sacs, even with an absence of the accessory sacs, and as well with a proportional reduction in the size of the entire nasal sac system and supporting structures such as the LFC. On the other hand, the nasal sac system of delphinids seems to be more balanced, as all sacs are more similar in size, and the LFC is more developed as it also extends into the periphery of the nasal tract. The other extreme can be found in the family of Ziphiidae, where the nasal sacs have a reduced size but some bony structures such as the elevated vertex and the maxillary crests are well developed (Schenkkan, 1973; Heyning, 1989) and envelop the nasal tract together with the LFC.

1.2. Musculature

The musculature of the nasal sac system seems consistent with the writings of Huber (1934). According to him, the rostral and nasal tract musculature of odontocetes is homologue to the respective labial and nasal part of the maxilonasolabial musculature in terrestrial mammals.

The external musculature is shaped by different layers that altogether are homologue to the nasal part of the musculus maxilonasolabialis. As such, our findings conform to those of Mead (1975). We do note a lack of clear relation between the functions that were attributed by this author and the insertions of the different muscle parts.

The arrangement of the external muscles in different sheets is a clear indication of their functionality (Lawrence and Schevill, 1956). As these different muscles are organized in alternating layers, with the muscle fibres orientated in different directions, and insertions on specific places, they work together to open or close the lumen of the nasal tract. For example, as the posterolateral part attaches to the vertex and to its contralateral part, it provides some motility to the posterior lip of the blowhole, but can also put pressure on the vestibular sac (Mead, 1975; Rodionov and Markov, 1992), therefore aiding in its emptying.

The intermediate part is presumably of little importance although it was considered to be able to compress the tissues immediately beneath it, and also to create tension on the melon (Curry, 1992). Although it inserts on fibrous tissue in between the blowhole and the melon, we believe it is of little influence due to its relatively small size. On the other hand, when considering both sides of the muscle working together, it could act as a blowhole sphincter.

The anteroexternal part is considered to be the most important of the external muscles. It seems to have a more complex function because of its multiple orientations and different insertion sites, and some of the fascicles that are in close proximity of each other could even act antagonistically. This is the case for the more caudal and superficial fascicles that can pull the posterior lip of the blowhole and the posterior fold of the vestibular sac in ventral direction, therefore contributing to the opening of the nasal tract, while the rostral fascicles insert into the commissures of the blowhole and also slightly into its anterior lip, and could therefore aid in its closing (Mead, 1975). Because the anteroexternal part has many insertions that surround the vestibular sac and the nasal tract, it is considered to act as a potential dilator of the tract (Lawrence and Schevill, 1956; Mead, 1975; Rodionov and Markov, 1992). However, there are no

clear allusions on the insertion sites into the folds of the vestibular sacs, especially in the anterior fold of the sac, which is the only site where the muscle inserts deeply into the tissue. Besides the fact that this could aid in opening the nasal tract, it could also allow for a good control of the anterior vestibular fold, which acts both as a valve that covers the slit-like opening as well as a limit for the expansion of the sac. Likewise, the central fibres of the anteroexternal part insert into the dorsolateral angle of the nasal ligament and could therefore make it stretch, and as the muscles on either sides act on it simultaneously, this could result in a powerful tension on the slit-like opening. Finally, the deepest cranial fascicles of the anteroexternal part insert dorsally into the anterior part of the nasofrontal sac and form a part of the LFC. As they could exert a pulling force in rostroventral direction, which would pull on the nasal tract and put pressure on the sacs, probably contributing to the emptying of the sac.

There is some confusion about the posterointernal part and its insertion site. Our results agree with Curry's (1992) findings in the harbour porpoise in that this muscle inserts into the posterior wall of the nasal tract, thereby aiding in the opening of the tract, while exerting pressure on the sacs that are located caudal to the tract. It does not connect to its contralateral part, as has been claimed by Mead (1975) and Green et al. (1980), which would enable it to close the tract. However, rather than inserting in the posterior elliptical adipose bodies as was described by Curry (1992), the muscle inserts in the slit-like opening, more specifically in the dorsal edge of the posterior phonic lips, where it joins the dorsal edge of the nasal ligament (Pl. 2, Fig. 2; Pl. 9, Fig 2). Therefore, it can put dorsal tension on the nasal ligament and the associated tissues which causes the slit-like opening and the entrance of the nasofrontal sacs to move as well.

The anterointernal part makes up the bulk of the external musculature and presents a homogeneous arrangement of its fibres while its insertion site attributes to the constitution of the anterior half of the LFC. Although most authors like Rodionov and Markov (1992) agree that its role is to put pressure on the nasal plugs and the premaxillary sacs, so that the plugs move caudally and close off the nasal tract, the actual mechanism is not clearly established. Because the muscle inserts into the anterior part of the LFC, this implies that all forces are distributed over the entire area rostral to the nasal tract, thereby putting pressure on all tissues therein, including the nasal plugs and their musculature, the melon core and the premaxillary sacs. Considering that the closing of the system is automatic because of its elastic qualities (Von Baer, 1826), the anterointernal muscle should contribute to this.

The nasal plug muscle takes up the bulk of the internal musculature. Its contraction creates an anteversion of the nasal plugs, uncovering the external bony nares and opening the passage, as described by Lawrence and Schevill (1956), who hypothesized on a great mobility of the lateral extensions based on the orientation of the muscles fibres. Again, as with the external musculature, we did not find any clear boundaries of the extent of the nasal plug muscle. As it inserts into the entire anterior wall of the spiracular cavity, a contraction of the muscle would displace the wall in rostral direction, causing the nasal passage to open up; the nasal plug lining was rigid, particularly in the large lateral extensions, which would produce a uniform motion of the entire plug. By pulling the anterior wall of the spiracular cavity in rostral direction, this exerts force on all adjacent structures such as the anterior lip of the blowhole, and therefore collaborates in opening the latter. Moreover, the melon core is situated centrally in between the muscle fibres, with a characteristic infiltration of adipose tissue outwards into the muscle. This indicates that there are

certain limitations in regard to the independent movement of the tissues, and it affirms our idea about the movement of the nasal tract anterior wall as a whole.

While some authors (Lawrence and Schevill, 1956; Mead, 1975) consider the nasofrontal sac intrinsic muscle to be a structure on its own, for others (Schenkkan, 1973) it is simply a part of the posterointernal part of the maxilonasolabial muscle. Our results clarify this controversy in favour of the former, as we observed an elastic sheet as a derivation of the LFC, which separates the external musculature from the internal. However, there seems to be a relationship between the two muscle groups occurring lateral to the nasal tract, where fibres of the nasofrontal sac intrinsic muscle and nasal plug muscle intermingle to form a bridge between the anterior and posterior internal musculature. Moreover, the nasofrontal sac intrinsic muscle envelops the posterior half of the sac, and could therefore be used to empty the sac. Also, the minor part of the muscle has some influence on the ventral border of the nasal ligament in opening the sac, and can therefore relieve pressure that the ligament puts on the nasal plug.

The diagonal membrane muscle was described by Mead (1975), and has often been overlooked or considered to be of minor importance (Green et al., 1980). Later, Rodionov and Markov (1992) also identified it and assigned it with the function of acting as the elevator of the diagonal membrane and the posterior wall of the entrance to the nasofrontal sac. Despite its small size, the insertion in the fragile diagonal membrane would confer some movement to it. Moreover, when considering the function of the diagonal membrane to act as a seal of the ventral third of the nasal tract, the muscle could act as the control over this valve.

2. LAMINAR FIBROUS COMPLEX

The Laminar Fibrous Complex (LFC) is the connective tissue structure that comprises the lower half of the nasal tract in all odontocetes that were part of this study, and has not been described to date. The only reference there might be to this structure is made by Cranford et al. (1996), in which they describe a “connective tissue theca" that refers to a sheath of dense connective tissue that envelops the posterior part of the melon and has a configuration that resembles a megaphone or horn. This description could well match the anterior part of the LFC. It was discovered because of sudden changes in density in CT images. Since there is a lack of a description on the origins/insertions, the composition of the tissue and the extent of the sheath, along with some confusion concerning its identification on the CT images that were presented, that makes it difficult to assert if it is the same structure that forms the LFC.

The presence of the LFC in the nasal region of odontocetes has led us to propose a number of possible functions. Among several hypotheses, we will mention three: it could act as an elastic container, a bounding sheet and a rigid support structure for the lower nasal tract and its adjacent structures. In the first case, the LFC would maintain all structures in position, and likewise it would keep the nasal sacs and the nasal tract under pressure with a minimum expenditure of energy. This would leave the nasal sac system closed when at rest. In the second case, the LFC would significantly limit the elasticity of the tissues below, and would therefore allow for a pressure difference to be created within the system. Finally, as it acts as a rigid structure, the LFC could prevent excessive deformation of the nasal system. As the environment exerts pressure from

outside, while there is outward pressure from within' the pressurized nasal sacs, the dome-shaped LFC could take on both forces, distribute them and avoid tissue deformation.

In short, the LFC may allow for the external musculature to exert pressure upon the lower parts of the nasal system, yielding lateral movements and/or adding additional pressure on the slit-like opening to increase the contact forces between the phonic lips and shut the nasal passage more tightly. Furthermore, the LFC could act as a rigid support structure that supports normal respiration functions. We can conclude that the importance of the LFC probably lies in the integration of the functions of the different structures that it envelops, and for all to act as a single functional unit.

3. GLANDULAR STRUCTURES

Histological examination showed the constant presence of two glandular tissue complexes in the nasal system of the Delphinoid superfamily, i.e. the nasal and nasofrontal glands. These structures have been not been previously described. Although Evans and Maderson (1973) observed glandular structures in the upper airways of the bottlenose dolphin, these structures were obviated in subsequent descriptions (Mead, 1975). Considering there is a general lack of glands in the integument of odontocetes (Weddell and Palmer, 1964), finding organized glandular tissues in the nasal sac system that is lined by an epithelial layer with similar characteristics to the integument, and that is allegedly involved in the production of ultrasound, makes us think that both glandular complexes could play an important role in this process. Obviously, these structures could take part in other functions as well, such as respiration. And indeed, during the rapid in- and exhalations, the secreted fluid could be important to avoid desiccation of the mucous membranes, as does the serous lateral nasal gland (glandula nasalis lateralis) in terrestrial mammals (Bojsen-Möller, 1964).

Finding glandular structures in the nasal sac system is not surprising when we consider the existence of such structures in the nasal cavity of terrestrial mammals, as for example in the dog (Adams and Hotchkiss, 1983), the cat, and laboratory animals, such as rats, guinea pigs, rabbits and monkeys (Bojsen-Möller, 1964). These glands are distributed along the mucosa of the nasal cavity and they secrete into the most rostral part of the nose, the nasal vestibule (Bojsen-Möller, 1967). The largest of these glands is the lateral nasal gland, which is present in most domestic species (Nickel et al., 1979). In parallel, the morphology of the glands that we describe in this work, seems to be very similar to the structure of the lateral nasal gland of a dog (Adams et al., 1981), thus this suggests a possible homology of these structures between these two mammals.

The nasal glands are strategically positioned along the posterior wall of the spiracular cavity, and as the openings are distributed over the width of the cavity, this facilitates the secretion product to be spread out evenly at this level. We did not encounter myoepithelial cells, which is the same for the dog's lateral nasal glands (Adams et al., 1981), and leads us to suppose that the secretion occurs due to a pressure exerted by the surrounding structures. The transversely orientated lip of the nasal plug could exert caudorostral directed pressure on the gland, which is limited dorsally by the nasal ligament, therefore favouring secretion. As the nasal plug is shaped like the letter D, located ventral to the duct opening, the pressure it exerts would facilitate the secretion products to be directed towards the upper parts of the nasal tract. Moreover, as the

ventral border of the duct opening is less fixed than the dorsal border, it is therefore more mobile and could act as a valve, preventing the retrograde movement of the secrete during moments of increased pressure. Furthermore, the nasofrontal glands, situated in close proximity to the nasofrontal sac, probably depend on movements of this sac and its intrinsic muscle for secreting their product in the nasal tract.

The glands are exocrine, compound, acinar, and secrete in a apocrine way, while the secretion product has a serous composition, as has been forwarded by Evans and Maderson (1973). Furthermore, the histochemical staining indicates the presence of slightly acidic mucopolysaccharides, however, the properties of the secretion products have not been denoted**.** Nonetheless, these glands are similar, both in structure as in composition, to the lateral nasal glands in terrestrial mammals and to the orbital gland in the eye of the bottlenose dolphin (Tarpley and Ridgway, 1991; Bodyak and Stepanova, 1994), which allows us to approach a hypothesis on their functionality. The bottlenose dolphin orbital gland secretion products function as a potent lubricant with greater capacity than in non-aquatic mammals (Tarpley and Ridgway, 1991). Given the similarities with the glands in our study, we can conclude that these could function as a lubricant of the nasal sac system, especially if we consider that the nasal tract in odontocetes is not a fixed open tract, but its walls are mobile and can have intimate contact. The lack of knowledge about the presence of fluid in the nasal system and about a system that controls the fluids' distribution, may explain the inconsistencies that are present in many theories of about the odontocetes' capacity to echolocate. In every hypothesis on the issue of the mechanism of the echolocation click production, it has to be considered that the entire region in which this takes place, is subjected to considerable changes in pressure, muscle activity and associated straining of the tissues in the tract (Ridgway et al., 1980; Amundin and Andersen, 1983, Ridgway and Carder, 1988). If there were no adequate lubrication, the nasal passage would suffer direct negative implications, especially regarding the epithelium but also other tissues that are subjected to these forces (Ridgway et al., 1980). This would be particularly true if the physical origin of the echolocation clicks is indeed caused by a tissue-friction phenomena (Evans and Maderson, 1973) or a pneumatic mechanism that acts on vibrating structures (Cranford et al., 1987; Cranford, 1990, 1992, Cranford et al, 1996).

Another function of this glandular secretion would be to aid in the cooling of the nasal tract tissues, as has been shown for the lateral nasal gland in the dog (Schmidt-Nielsen, 1970; Blatt et al, 1972). However, the process is different in the two groups, while the dog uses panting for thermoregulation, cetaceans use other mechanisms for heat loss that are based on the effortlessness of heat change with water (Irving, 1969; Ridgway , 1972). During the production of ultrasonic sounds, the tissues that are involved in this process vibrate, and as this requires a large amount of energy (Richardson et al., 1995), it leads to the production of heat which could be dissipated with the cooling effect of the glandular secretion.

Moreover, the nasofrontal glands are situated inside the wall of a narrow sac with a anterior part of considerable length. This could imply that the secretion products could act as a surfactant, reducing the surface tension, and avoiding collapse of the sac.

Finally, there is the presence of plasma cells, which are also present between the acini of the lateral nasal gland in the dog (Adams et al., 1981), and seem to suggest that these glandular

formations cooperate as a premier immunological barrier of the respiratory system. Because we did not perform a biochemical analysis, we do not know if IgA is present in the secretion product, as is the case for the dog.

The nasal glands are present in all odontocete species that were studied, and moreover, there is a significant interspecific similarity in their morphology and location, which gives us an idea of their importance and also supports the theory of homology of the structures within the odontocete rostrum (Cranford et al., 1996). On the other hand, the nasofrontal glands are irregular in morphology and location, even being absent in phocenids, though it has been considered that this family is likely to have similar glands when looking at the morphology of the posterior sac (Mead, 1975) and the frequent presence of mineral deposits (Curry, 1994). The interspecific differences of the nasofrontal glands correspond in some way to the variation that exists in the nasal glands of terrestrial mammals, where the lateral nasal gland is in general constant, it is accompanied by minor, isolated nasal glands that are distributed over the wall of the nasal vestibule (Bojsen-Möller, 1964).

4. INNERVATION

The odontocete nasal region is richly innervated by the facial and infraorbital nerves, which coincides with the findings of Huber (1934), but is contrary to the findings of Mead (1975).

The facial nerve is responsible for the motor innervation of the nasal region and features a similar course and thickness on each side of the head. This supports our observations and those of Mead (1975) in the sense that the maxilonasolabial muscle distribution is approximately symmetrical. However, not all branches of the facial nerve were symmetrical in size as there is a dominance in the right nerve branches that innervate the right side of the nasal plug muscle, which is understandable as this part is bigger. Moreover, once the facial nerve passes the antorbital notch, it branches abundantly in between the different layers of external musculature. This notion makes us think about the independence and mobility between the different muscle parts, of the control of the upper airways and the phenomena that take place therein. This is, however, contrary to the view of Purves (1966), who claimed that an independent action of these muscle layers would cause a rupture of blood vessels and nerves.

As for the infraorbital nerve, first, we believe it is important to highlight the contradiction of maintaining that name, as the nerve is not situated below or beneath the eye's orbit in odontocetes. However, we have kept the original nomenclature as used in terrestrial mammals, because both nerves are responsible for the sensitivity of the nasal region and are therefore functional homologues (Nickel et al., 1984).

The infraorbital nerve, which is responsible for the sensory innervation of the walls of the nasal tract and sacs, can be prepared for visual demonstration despite the wideness of the principal branch that traverses the LFC deeply, and splits further into numerous branches of notable sizes across the entire posterior wall of the nasal tract. In contrary to the facial nerve distribution, the infraorbital nerves are completely disproportional when comparing the left and right sides of the nasal system. Although this disproportion might give us an idea of the wealth of sensory nerve endings on the right side, it does not correspond to the general degree of asymmetry between the

two sides. This big difference in size could reinforce the theory of unilateral sound production on the right side (Au, 1993).

Histological examination shows the presence of encapsulated nerve endings that are morphologically similar to those previously described by Bryden and Molineaux (1986) in the blowhole, Palmer and Weddell (1964) in the skin and Khomenko (1974) in the nasal sac system. We consider these nerve endings to be mechanoreceptors, as posed by Bryden and Molineux (1986). While the presence of mechanoreceptors in the nasal sac system was already described by Khomenko (1974), the author did not specify their precise location. Our observations locate the highest concentrations in precisely those places that have been considered important in the mechanisms of air control during phonation phenomena, as are the nasal plugs and the posterior wall of the spiracular cavity (Ridgway and al., 1980; Cranford et al, 1996). Moreover, the high number of receptors that related to the nasofrontal side of the nasal ligament could indicate the significance of the existence of a fine control mechanism that might regulate both the degree of tension on the ligament itself, as well as the pressure in the nasofrontal sac. Obviously, the presence of such control mechanisms should count for the entire nasal tract.

We do not rule out that there could be other areas in the nasal sac system where these receptors are concentrated, but according to our observations, they were only present as single isolated receptors; unfortunately, we did not subject the presence of these receptors, or other possible nerve endings in the nasal sac system, to a qualitative or quantitative study. Such a study would allow for a more accurate and reliable prediction of the areas where there is greater control of stresses and strains and of the actual control mechanisms that are operating, especially during the production of ultrasonic sound, for which a fine control and feedback mechanism is necessary.

5. INTERRELATION BETWEEN EPITHELIUM AND SUBJACENT LAYER

The alignment and direction of the cutaneous ridges in mice paws and primate fingers, which make them suitable for fingerprinting, are present because of the underlying dermal ridges (Okajima and Newell-Morris, 1988; Montagna et al., 1922; Tsugane and Yasuda, 1995). In the striped dolphin, *Stenella coeruleoalba,* the dermal ridges in the skin of the neck and around the blowhole are aligned longitudinally, similar to *Tursiops truncatus* (Stromberg, 1989), and arranged in straight angles to the cutaneous ridges (PI. 18, Fig. 4). The latter are arranged in a circumferential manner caudal to the blowhole, as has been previously described in other species such as *Cephalorhynchus commersoni, Orcinus orca, Delphinapteurus leucas* and *Physeter macrocephalus* (Shoemaker and Ridgway, 1991). This difference in orientation between the dermal ridges and cutaneous ridges, may have a functional significance as to reduce the drag (Hertel, 1969).

Both in the skin of the blowhole as in the lining of the nasal tract immediately below it, there are different types of interrelations of the epithelium with the underlying tissue layer.

During respiration, the anterior blowhole lip gets displaced ventrorostrally in an explosive, abrupt manner (Rodionov and Markov, 1991), and as such, there are considerably strong forces at work that require a good fixation of the musculature inserting in this region. We suppose that the forces that act on the anterior lip during its opening are not homogenously distributed over the

entire lip, and because the lip has a semi-circular shape, these forces range from straight tensile forces that follow a sagittal direction, as for example in the centre of the lip, up to more complex forces such as torsion and traction, for example in the commissures of the blowhole. As such, the orientation of muscle and collagen fibres appears to confirm the presence of the different force vectors in this region (PI. 18c, Figs. 10, 11 and 12).

The different connection patterns of the superficial layers with their subjacent layers could be related to the forces that exert on a specific place, especially with regard to the anterior lip and the blowhole commissures. The posterior blowhole lip is less mobile and features a much less developed and less variable dermal-epidermal junction than does the anterior lip. This supports our idea of a relationship between the type of union and the quality and quantity of action it is subjected to.

The dermal-epidermal junction in the centre of the anterior lip features long and narrow dermal papillae that are typical for dolphin skin (Pl. 18a, Fig. 2). Away from the centre, these papillae gradually shorten and widen as do the dermal ridges (Pl. 18b, Fig. 5 and 9) until the union is characterized by the presence of only very small dermal papillae. This adaptation in the centre of the anterior lip seems adequate to withstand strong and linear rostro-ventral forces parallel to the axis of the dermal papillae, as it offers a large attachment surface and allows for a rectilinear movement. This finding might let us infer that the dermal papillae play a minor role in areas with straight tensile forces. Perhaps their role could be related more to the influence by external mechanical forces that occur in the aquatic environment (Sokolov, 1962. Sokolov et al, 1969).

In the intermediate, paramedian zone of the anterior blowhole lip, halfway between the commissures and the central area, the orientation of dermal ridges and papillae changes from straight to curved in laterocaudal direction, and gradually changes until the ridges and papillae are orientated parallel to the skin surface. Possibly, the forces that act on this paramedian area are somewhat angular due to the displacement this area is subjected to and they require a greater tissue elasticity compared to the central area of the lip, while the observed orientation pattern may be associated with to the two basic vectors characteristic of these forces.

The small areas of the blowhole commissures are not that mobile as the other regions and they mainly move in a rotational manner, as they are subjected to different forces that act on a relatively small area. Here, the number of dermal ridges per surface area is larger than in other areas, but both the ridges and papillae seem compressed as the papillae appear as small pear-shaped buds. While the increase in surface area and in number of dermal ridges could be related to the need for a strong fixation, their size and arrangement would also need to resist torsional forces and allow for a certain elasticity in the skin. In the lateral walls of the commissures, for example in the more laterorostral parts of the posterior lip, the dermal ridges feature a wavy arrangement, which may be related to the impact of more indirect forces on the surface, as these can create a longitudinal tension in the structure. In other parts of the caudal lip, the dermal ridges and papillae run parallel to each other. Here, the ridges are less pronounced than in the rest of the skin and they do not display any special features. This corresponds to an area that is not subjected to a particular tension or movement, and it is here that we find a gradual transition to the inner part of the nasal sac system.

On entering the nasal tract, the characteristics of the skin gradually change without a clearly defined transition zone, as there is no visible difference between dermal ridges and subepithelial ridges. Considering that these structures lack an organized orientation in the vestibular sacs, we could deduct that the forces that occur in this area lack a common directionality, which concurs with the expansion capacities of these sacs (Lawrence and Schevill, 1956; Evans and Maderson, 1973).

In the spiracular cavity, the height of the subepithelial ridges decreases to a ´moderate´ height and the lateral walls show no signs of specialization in the epithelium-subepithelial connection. However, in the anterior and posterior walls of the cavity, the subepithelial ridges are shaped like waves, which could indicate that they are subjected to tensile forces over the entire area, rather than to forces of traction on a single location. This would concur with the postulated mechanism of the movement of the slit-like opening and the distension of the nasal tract during respiration (Schevill and Lawrence, 1956).

6. INTERPRETATION OF THE FUNCTIONALITY OF THE NASAL SAC SYSTEM

The functionality of the nasal sac system has been widely discussed in all the works that deal with the anatomy of the region (reviewed by Curry, 1992), and a large number of functional interpretations have been put forward.

A clear example of the diversity of ideas that revolve around this concept can be found in Purves and Pilleri (1983), who write the following as an argument in favour of the larynx as the

> "It has been our experience that whatever structure is responsible for a certain function in terrestrial mammals is also responsible for that function in cetaceans, except that the structure may have undergone profound modification for operation

structure of sound production:

In contradiction to the meaning the authors intended to formulate, this statement could also be perfectly valid to support the theories that were postulated by Norris (1961) in which the nasal sac system is considered the source of phonation, while the anatomical complexity of the odontocete nasal system has no evolutionary base unless for the use of acoustics in that it makes use of the properties of water and has transformed the nasal region for the production and emission of sounds in such an environment.

When looking at the results of experimental studies on the functionality of the upper respiratory system (Dormer, 1979. Ridgway et al, 1980; Mackay and Liaw, 1981; Amundin and Andersen, 1983, Ridgway and Carder, 1988) along with the findings we encountered and described in this PhD thesis, we firmly believe that the main function of the complex nasal sac system is production of sound, and more specifically ultrasonic sounds that the toothed whales use for orientation and echolocation. Therefore we do not consider it necessary to discuss the pros and cons on the theory of the larynx being the site of sound origin, as has been reviewed by Purves and Pilleri (1983) and Morris (1986).

In addition to this primary function, the nasal sac system also participates in respiration in that it opens and closes the nasal tract. The latter function is of particular importance to prevent water from entering the respiratory system (Lawrence and Schevill, 1956; Dormer, 1979; Garcia-Hartmann and Degollada, 1996). Moreover, it has been considered that there is a recycling of air within the system during sound production (Norris, 1972), while the air-filled nasal sacs could act as an acoustic baffle (Norris, 1964).

6.1. Mechanisms of opening and closing

During ex- and inspiration, the nasal tract opens to be a uniform channel (Schevill and Lawrence, 1956). The opening happens mainly because of a contraction of the nasal plug muscle, which displaces the entire rigid anterior wall of the spiracular cavity as a block in rostral direction. This movement would be accompanied by the anterior lip of the blowhole thanks to the participation of the anteroexternal part of the external musculature, whose fibres insert in the anterior folds of the vestibular sacs and the connective tissue in between them. While the anterior wall of the tract is displaced, the nasal plugs are situated within the premaxillary sacs that act as a repository for the former during breathing (Heyning and Mead, 1990).

The nasal tract closes as the surrounding structures fall back to their resting position thanks to the elastic forces (Howell, 1930). At the same time, the external musculature, and in particular the anteroexternal and anterointernal parts, contracts and exerts pressure on the LFC and indirectly onto the underlying tissues.

In addition to the passive elastic closure, the nasal sac system is provided with active locking mechanisms that are located in the blowhole, the slit-like opening and the nasal plugs (Garcia-Hartmann and Degollada, 1996). These closures or valvar complexes act with multiple purposes, and as such they prevent water from entering the system, regulate the amount of air that flows through the tract during sound production and play an important role in the recycling of air within the system.

The most superficial closing mechanisms are the blowhole lips, which collaborate with the vestibular sacs. The rostral fibres of the most superficial musculature, which contains the pars posteroexternus, pars intermedius and pars anteroexternus, tighten the blowhole in a way that it takes on a crescent-shape, increasingthe contact area between the lips (Rodionov and Markov, 1992). Moreover, as the vestibular sacs inflate (Dormer, 1979) and the pressure inside them gradually increases (Ridgway et al., 1980), they collaborate in the pneumatic closing of the blowhole in that it is more difficult for air to get out or water to get in (Lawrence and Schevill, 1956). This closing system is less efficient in comparison to the other, but a small ingress of water should not be a problem as the vestibular sacs are arranged to act as 'water traps' and allow for an expulsion of the fluid in the following exhalation.

The next point along the nasal tract that acts as a valve is the combination of the slit-like opening, the LFC that inserts into it and the nasofrontal sac that surrounds it. The latter is completely inflated between breaths and during periods of phonic activity (Hollien et al., 1976; Dormer, 1979), applying pressure on the slit-like opening and thus increasing the clamping force (Lawrence and Schevill, 1956). This locking mechanism requires little effort from the animal, as was evidenced by the methacrylate moulds that filled the nasofrontal sacs, exerted pressure on

the slit-like opening and limited the plastic from accumulating in this area of the tract and which is why it was extremely difficult to obtain a complete mould of the system. The rigid consistency of the insertions of the LFC in the slit-like opening and the nasal ligament, allowed for an elevated transverse tension executed by the insertion of muscle fibres of the anteroexternal part, forcing an intimate contact between the caudal and rostral surfaces. Moreover, the effect of dorsal coverage by the anterior vestibular folds, which was clearly visible in the methacrylate moulds, demonstrated that these folds did not unfold when the vestibular sacs were inflated, as was also observed by Dormer's (1979) cineradiography of the nasal sac system.

The nasal plugs play a dual role as the most ventral closure system of the nasal complex (Lawrence and Schevill, 1956). First, because they cover the external bony nares, the nasal plugs are subjected to pressure from the entire anterior external musculature that inserts in the anterior part of the LFC. And, secondly, when at rest, they are covered dorsally by the nasal ligament, which fits in the concavity between the anterior wall of the spiracular cavity and the nasal plug lip, and ventrally by the diagonal membrane. The air pressure in the nasopharynx (Ridgway et al., 1980; Amundin and Andersen, 1983) surely is the cause for the diagonal membrane to bulge dorsally, putting pressure on the bottom of the nasal plugs, as has been shown by the methacrylate moulds that accumulated under the membrane in a space that appears much smaller during dissections, when there is only atmospheric pressure.

6.2. Hypothesis on the functional anatomy of the nasal sac system during sound production

a) Experimental contributions

The chain of events that occurs in the nasal complex during sound production was initially studied using cineradiography by Norris et al. (1971) and later more in depth by Dormer (1979). According to these authors the process starts in the nasopharynx with the closing of the larynx and a contraction of the nasopharyngeal sphincter, and this increases the air pressure in the bony nasal tract, but does not alter the pressure in the trachea. This is consistent with the measurements of Ridgway et al. (1980) and Amundin and Andersen (1983).

Parallel to the increase in pressure in the bony nasal tract, a swelling of the premaxillary sacs occurs and when these are fully inflated, the nasofrontal and vestibular sacs follow (Dormer, 1979). While the former reach their maximum expansion halfway through the process, the latter continue to inflate gradually until the end of the cycle when the airspace of the bony nasal tract reduces. During this process the animal has been known to produce different types of sounds, in particular whistles that are accompanied by rapid pulsations of the left nasal plug, whereas during the production of echolocation clicks, these pulsations were not observed. In both cases, sound production commences once all the sacs reach a certain level of inflation. As the process finishes, the vestibular and nasofrontal sacs start to deflate, accompanied by a slight retraction of the nasal plugs and a recovery of the airspace of the bony nasal tract.

The described sequence is in accordance to the electromyographical and pressure changes registered in the upper airways. The beginning of the process of phonation leads to an increase in pressure in the lumen of the nasal tract, as discussed above, and this pressure is maintained throughout the sequence (Ridgway et al., 1980; Amundin and Andersen, 1983; Ridgway and Carder, 1988). The nasopharynx acts as a pneumatic pump in that when the sphincter contracts,

the nasopharyngeal space compresses and air is impelled into the nasal system for the use of sound production (Dormer, 1979; Cranford et al., 1996). As the consequential increase in volume of the premaxillary sacs is accompanied by an increase in pressure, there is a similar pressure rise in the nasopharyngeal space (Ridgway et al., 1980), which infers that it is a common space (Rodionov and Markov, 1992). The assumption that the premaxillary sacs could act as reservoirs for recycled air (Purves, 1967) does not quite fit with their empty state at the beginning of the process of phonation (Dormer, 1979) nor with our findings on the structure of the sacs' walls that do not seem suitable for large expansion. Instead, these sacs could provide a space for the nasal plugs to retract into, and they could be part of the acoustic baffle during sound emission (Norris et al., 1971).

Similarly, the movements of the nasal plugs coincide in time with recorded contractions of the nasal plug muscle (Ridgway et al., 1980; Amundin and Andersen, 1983), and this muscle also shows activity during the production of ultrasonic clicks, even without movement of the plugs (Dormer, 1979), which might be because of a reduced flow of necessary air, and as such it would entail only hardly noticeable movements. Ridgway et al. (1980) recorded pressures during sound production, and noted a slight increase in pressure in the vestibular sacs and muscle contractions in the anteroexternal part and the diagonal membrane muscle. The pressure in the vestibular sacs increased slightly, while the sacs' volume increased largely, and this is understandable when looking at the morphology of the walls, that allow for a great expansion without much force. This makes these sacs suitable as a reservoir for recycling air during production of ultrasonic clicks (Norris, 1964), during which time there is no leakage of air through blowhole, in contrary to what happens during the emission of whistles. The contraction of the anterointernal part exerts pressure on the LFC and indirectly onto the anterior wall of the nasal tract, including the nasal plugs, thereby aiding in its closing mechanism, which would also allow for a more controlled passage of air during phonation. This air passage may also be controlled by movements of the diagonal membrane that coincide with the respective muscle contractions (Ridgway et al., 1980). Achieving a more elaborate electromyographic study would very probably show the activity and thus the involvement of each of the different muscles during the different stages of sound production, although it would be tremendously difficult to identify all the different muscle layers.

The fact that all nasal sacs are inflated during phonation (Dormer, 1979) substantiates the idea of their function as acoustic baffle, as has been noted by Norris (1964). Furthermore, this implication is reinforced by the interspecific homologies in odontocetes (Cranford et al., 1996) and by our own observations on the arrangement of the sacs in which they are balanced in a way that the downsizing of certain sacs, or the differences in asymmetry between some species, requires an equivalent enlargement of other sacs, as has been well described from an evolutionary point of view (Schenkkan, 1973). As such they would always work together as a functional unit. While the LFC acts as a physical barrier and a structure that constrains the system, it also maintains the relationship between different structures and keeps them grouped into a functional whole.

The theory about the nasal sac system as the place of sound production has been backed up by experimental studies, such as by Ridgway et al. (1980) and Ridgway and Carder (1988). They showed that the organ of sound origin was located between the bony nasal tract and the vestibular sacs, and that the intrusion of an open catheter into the nasal tract of a living animal prevented it from producing sound, whereas if the catheter lumen was sealed the animal could

produce sounds. The possible direct involvement of the premaxillary and vestibular sacs was also ruled out, as the animal could produce sound while an inflated balloon catheter was present in the lumen of these sacs.

These results, together with other morphologic and experimental findings have led many authors to target the nasal plugs as sound generators in odontocetes (Evans and Prescott, 1962. Diercks et al, 1971; Norris et al. 1971; Evans and Maderson, 1973; Mead, 1975; Dormer, 1979; Ridgway et al., 1980; Liaw and Mackay, 1981; Amundin and Andersen, 1983; Ridgway and Carder, 1988; Heyning, 1989). However, this theory presents certain inconsistencies. For example, interspecific anatomical differences such as the absence of the lateral extensions of the plugs in the family of Platanistidae and in certain species of the family Ziphiidae (Schenkkan, 1973), or the absence of the accessory sacs in Phocoena, Kogia, Physeter, Pontoporia and Inia (Schenkkan, 1972), is enough to exclude the nasal plugs as the organ of phonation; moreover, the lack of an acoustic pathway that originates in the nasal plugs together with the existence of a sonic field, ventral to the mandibula, which is characteristic for the family Platanistidae (Purves and Pilleri, 1983), and which is why the authors of that study argue for a laryngeal origin of sound production, stands in contrast to the dorsal sonic field in all other odontocetes studied.

Moreover, the ultrasonic sounds are created and transmitted through vibrating tissue, not air (Amundin, 1991b). Considering the melon to be able to function as an acoustic lens (Norris and Harvey, 1974), the finding of an emission channel that starts from the elliptical adipose bodies (Cranford, 1988) points to the area of the slit-like opening as the organ of sound production. This new theory was supported experimentally by a computerized model of the acoustic beam formation in *Delphinus delphis* (Aroyan et al., 1992). Thus, the nasal plugs regulate the amount of air passing through the tract, as proposed by Dormer (1979) and subsequently by Cranford et al. (1996), although the former authors also allotted the plugs as the organ of sound production. We believe that the diagonal membrane also has an important function in this air passing mechanism.

b) Anatomy of the ultrasonic sound production complex. Implication of the phonic lips

> "If on the other hand, the nasal sac system were assumed to be the source of sounds, one would predict anatomical and histological diversity, with specializations appropriate to the various roles of the component elements"

Paraphrasing these authors, the lack of structural evidence in the literature has led us to study the nasal sac system as a whole. We consider that the tissues that are responsible for the production of high energy sounds perform a physical effort that should entail a specific adaption of their architecture.

This study has allowed us to gain knowledge of the general morphology of the system and, what is more important, has led us to find structures with special characteristics that open doors for discussion on the exact location that is responsible for phonation in odontocetes. Moreover, our findings are consistent with recent hypotheses that point to the slit-like opening and in

particular to the phonic lips as the generators of sound (Cranford et al, 1987; Cranford, 1990, 1992; Cranford et al, 1996). These structures have overcome the dispute that considered the larynx (Purves and Pilleri, 1983) or the lower nasal tract, and more specifically the nasal plugs (Norris and Harvey, 1974), as the potential production sites of ultrasonic sounds.

The structural findings in the area of the slit-like opening, which in its simplified form contains folds that are arranged perpendicular to the major axis of the phonic lips, together with the protrusion of the mobile phonic lips in the nasal tract, allowed us to propose a working hypothesis on the functional anatomy of the production and emission of sound in the species that were studied.

The slit-like opening, the phonic lips, the elliptical adipose bodies and the insertion of the LFC, feature specialized morphological features that are suitable to carry out specific activities in comparison to the rest of the nasal tract.

It is likely that the small folds, oriented perpendicular to the major axis of the phonic lip, and which are a constant feature in all the specimens studied, are also present in the animal in vivo when the slit-like opening is relaxed. Since these small folds include both the epithelium and the underlying tissue in histological slides of a horizontal cut where there is no alteration of the tissues (Pl. 10, Fig. 4), we suggest that their presence is a normal feature for all the individuals studied.

The external musculature, and in specific the anteroexternal part, which inserts into the lateral commissures of the slit-like opening, could produce a transverse tension on this structure, which is also favoured by the insertion of the LFC. This would imply a disappearance of these small folds in vivo in the same way that they disappear when applying this tension manually during dissection. Therefore, we believe that the constant presence of these folds across the phonic lips, is what allows for a transverse tension and stretch of the slit-like opening.

Meanwhile, when there is tension on the slit-like opening, this also exerts traction on the epithelium dorsal and ventral to the phonic lips in direction of the lips. Thus, the width of the phonic lips reduces and the epithelium increases in thickness, which means that they protrude more into the lumen of the tract than when the system is relaxed (Pl. 20). This, in turn, enhances the contact pressure between the anterior and posterior lips. In this regard we also have to consider the morphological differences between the wall of the spiracular cavity and elliptical surfaces of the phonic lips in that the latter is presented with a seemingly tougher but also highly mobile epithelium. This mobility is possible due to the relationship between the phonic lips and their respective elliptical adipose bodies, since the proximity of these adipose bodies together with the underlying narrow strip of connective tissue may allow the lip to move inward and outward.

The presented structural findings have led us to develop a hypothesis about the possible mechanism of sound production, especially for high frequency sounds, given the permeation through tissues (Amundin, 1991b), in the phonic lips of the nasal sac system in members of the delphinid superfamily, and as will be discussed later, while this is should also count for all odontocetes.

Our present hypothesis is, first, that the pressurized air in the nasopharynx, as described by numerous authors (Amundin et al., 1983; Aroyan et al. 1992; Cranford, 1992; Cranford et al, 1996)

is released into the upper parts of the nasal tract under control of the conjunction of the nasal plug with the diagonal membrane and the nasal ligament, as described in the previous section. The amount of air that is released and the side of the nasal tract in which it is released would be regulated mainly by the movements of the diagonal membrane and the nasal ligament itself than by the respective nasal plug, which, when looking at the morphology, is not equipped with a control mechanism in any place of its caudal border. The diagonal membrane could exert this control by contracting its muscle, and similarly the nasal ligament could do this with help of the minor part of the nasofrontal sac intrinsic muscle.

Our working hypothesis, which has been undergoing testing in further research, suggests that an air bubble, rising through the spiracular cavity under pressure of the walls, produces a bend of the phonic lips, which protrude in the nasal tract (Plate 21, Figs. 1 and 2). This movement could be possible because of, as previously mentioned, a special provision of the related structures and the presence of loose tissue underlying the lips.

This bend of the phonic lips, with a displacement of the subjacent tissues, is followed by a clash between the anterior and posterior lips after the air bubble has passed, which is possible because of the elasticity of the tissues and the tension that acts on this zone (cfr. supra), and produces a strong and rapid vibratory movement. This vibration that may spread through all peripheral tissues, while especially following the path of least resistance through those tissues that have the best transmission properties**.** While this vibration would be absorbed by a set of structures caudal to the tract, such as the nasal ligament, the nasofrontal sac, and the posterior nasal sac in the species where they are present, the vibration of the anterior phonic lips would propagate through the anterior elliptical adipose bodies, the melon core and the melon (Pl. 21, Fig. 3). This emission pathway is widely accepted in the literature (reviewed by Cranford et al., 1996).

The tension on the phonic lips in the slit-like opening, which is set within the LFC, could be considered to be similar to the stretching of the mouth of a rubber balloon. As such, we can compare our hypothesis on the production of sounds with the act of releasing air through the stretched entrance of a balloon. However, this oversimplified extrapolation does not take into account the sound transmission between two different media (Purves and Pilleri, 1983). Therefore it would only be comparable if the entrance of the balloon was surrounded and in contact with material with an impedance that is similar to water and a part of the balloon wall would cover the entrance as an equivalent to the anterior folds of the vestibular sac in order to prevent the formation of a resonant cavity.

The morphology of the slit-like opening is poorly known and therefore this structure is often overlooked, which has led critics of the hypothesis on the nasal system as the organ of sound production, to conclude that there was no way of emission (Purves and Pilleri, 1983). However, a detailed study of the region shows a clear continuity from the phonic lips and elliptical adipose bodies through the melon core to the melon, as shown in Figure 5 of Plate 8. The lack of an exclusively adipose melon core in Phocoenidae (Schenkkan, 1973) is most likely not a problem given that the area is highly infiltrated with adipose tissue of the nasal plug muscle. Perhaps the small size of the lower part of the nasal tract of these species did not require or allow for the creation of a purely adipose channel.

Finally, it should be mentioned that the species of the genus Lagenorhynchus possess two melon cores (Pl. 22, Fig. 1) while having little asymmetry in the phonic lips or the elliptical adipose bodies, as shown by the measurements of Cranford et al. (1996). This could mean that these species have two places for ultrasonic sound production and, as these authors predicted, if there is a relationship between the peak frequency of each individual animal and the size of the elliptical adipose bodies, this should able the animal to produce two pulses relatively close together or one pulse compound of two, which in that case are hardly distinguishable. At the same time, a doubling of the sound production organ would allow for the understanding of the simultaneous production of whistles and clicks in the nasal region (Lilly and Miller, 1961; Evans and Prescott, 1962; Lilly, 1962, 1963, Busnel and Diedzic, 1966).

Unfortunately, intraspecific variations of the facial anatomy are generally small, and could only be really useful in case the individual vocalization features are available, which is very difficult considering that we work with stranded wildlife. It would be extremely interesting to conduct a detailed anatomical study of animals for which there are acoustic records available and, by taking representative samples, to find the relationships between the structures and the production and emission of sounds.

7. HOMOLOGY

When studying the facial anatomy of various odontocete species, both *in situ* and through descriptions found in literature, the first impression is always that of a very complex nasal sac system with a wide interspecific and even more interfamilial variability. However, a systematic approach of the different anatomical components demonstrates a clear parallelism between the structures. As Cranford et al. (1996) established a clear homology for the key structures for the production of sounds, a.k.a. "Monkey Lips-Dorsal Bursae" (MLDB)-complex, that are equivalents to our phonic lips-elliptical adipose bodies, we considered that there are homologies for each of the system's components, with some exceptions, such as the posterior sacs in the Phocoenidae and Ziphiidae families (Heyning, 1989). As such, there is also homology in the musculature, with a similar arrangement of the layers and locations of origin in all the genera that were part of this study. The same applies to the nasal tract and the diverticula, in which the locking systems of the slit-like opening, the nasal plugs, and the LFC function similarly in all species.

In the approach of the existing homologies, we also considered differential details such as the absence of the lateral extensions of the nasal plugs in the Platanistoidea, Ziphoidea and Physeteroidea superfamilies, which wielded as an argument against the hypothesis of sound production in the nasal plugs (Schenkkan, 1973). That same argument could serve to downplay the importance of anatomical sites with certain interspecific differences. We believe that these differences bear a part in the functionality of the whole system but they could be discarded as to having primary functions, such as sound production, if they are absent in other species. So, returning to the lateral extensions of the nasal plugs, we consider that their function is to assist in closing off the ventral end of the spiracular cavity, by plugging into the entrance areas of the accessory sacs, and making intimate contact with the nasal ligaments dorsolaterally (Plate 10, Fig. 1). In Ziphiidae, where the lateral extensions are absent (Heyning, 1989), a similar closure of the tract can be achieved thanks to the large size of the nasal plugs, or simply because the smaller

premaxillary sacs do not provide space for an extensive anterograde movement of the nasal plugs (Schenkkan, 1973; Heyning, 1989).

We could also transpose this hypothesis to the reduction or absence of sacs or parts thereof, such as for the absence of the left anterior part of the nasofrontal sac in *Grampus griseus* (Murie, 1870; Schenkkan, 1973; Mead, 1975). As all the different parts of the sacs are part of the whole nasal sac system that acts as a functional unit, small changes in this arrangement could influence differences in the phonation characteristics for each species and each individual without inhibiting the entire phonation process. For example, there was an absence of the right anterior part of the nasofrontal sac in a specimen of *Stenella coeruleoalba* (MGH5) (Pl. 22, Fig. 3), which was a pre-adult individual that was presented with pathologies provoked by morbillivirus as the cause of death, but whose anatomical anomaly did not appear to be of vital importance.

Moreover, in regard to the *de novo* described structures in this thesis, there is again a significant homology between the different species studied. First of all, the laminar fibrous complex was found present in all species, in the same way that Cranford et al. (1996) observed the "theca connective" in some species, which is the structure we have considered to be a possible part of the LFC. Similarly, the glandular structures, and especially the nasal glands, were particularly consistent in the different species, while there was a greater variability for the nasofrontal glands. All of these glands were also found in a specimen of Cuvier's beaked whale (*Ziphius cavirostris*), which was not included in this work.

8. ANATOMICAL CONSIDERATIONS ON THE EVOLUTIONARY ORIGIN OF THE NASAL REGION IN ODONTOCETES

In the literature there is no clear concept on the homology of the facial region of cetaceans and other mammals that considers the upper respiratory tract as a common space present throughout

> "In contrast to terrestrial and amphibious mammals, the epithelium of the upper respiratory passages of the dolphin does not have cilia…"

the animal kingdom, as becomes clear in the writings of Khomenko (1974):

That is why we analysed the evolution of the nasal region from a comparative point of view in order to find existing homologies between extant delphinids and mammals in general. This discussion is based on a study of the facial anatomy of certain species of odontocetes, work on the evolution of the formation and conformation of their skull, and new findings in the nasal system, which includes new descriptions as well as relationships between known structures.

A comparative analysis of the upper respiratory tract between odontocetes and land mammals reveals a number of correlations between soft tissues and bony structures. These possible homologies could explain the origin of the odontocete nasal sac system to be found in the nasal region of terrestrial mammals.

8.1. Nasal cavity

The nasal cavity of terrestrial mammals, which is covered dorsolaterally by the frontal, nasal, maxillar and incisive bones, extends from the nostrils to the choanae or internal bony nares. The most rostral part is called the nasal vestibule, which is in communication with the exterior, and is supported by cartilaginous bone (Nickel y col., 1979; Evans, 1993).

Meanwhile, the odontocete skull has undergone "telescoping", in which the bones that form the nasal cavity underwent a reduction, which is most visible in the nasal bones, while at the same time there was an elongation of the bones dorsal to the oral cavity, such as the premaxillae and maxillae. The nasal bones and nasal cavity have migrated caudally to be reduced, respectively to a pair of small bones and a nasal tract that winds perpendicularly through the skull through a few short cylindrical ducts (Mead, 1975; Rommel, 1990) (Plate 23, Fig. 1).

The nasal cavity of terrestrial mammals is mainly occupied by a series of thin bony plates that are arranged in a spiral manner, forming the nasal conchae (or turbinates) and ethmoturbinates, which are coated with respiratory and olfactory mucosae respectively (Calhoun and Stinson, 1976) (Pl. 23, Fig. 2). However, these mucosae do not continue in the nasal vestibule, which is covered by a skin-like epithelium (Adams and Hotchkiss, 1983). Herein, the rostral prolongation of the conchae ends in two bulbous enlargements, the alar folds (Evans, 1993) (Pl. 23, Fig. 3).

An important consequence of this bony migration is the fact that the external bony nares also moved caudally, together with the rest of the nasal tract. Dorsal to the external bony nares, between them and the blowhole or external nostril, there is the nasal sac system, surrounded by all structures associated with the nasal tract. The fact that the walls of this part of the airways are not made up of bone, but are membranous, suggests their homology with the nasal vestibule of terrestrial mammals. The rest of the nasal cavity, i.e. ventral to the external bony nares, has drastically decreased in size, which is associated with the reduction of the nasal bones and, with them, walls of the nasal cavity as it appears in terrestrial mammals, which leaves the nasal cavity limited to the short osseous channels between the external bony nares dorsally and the choanae ventrally. The reduction of the nasal cavity got accompanied by a complete loss of the conchae and ethmoturbinates, despite the homologies that can be found in the posterior parts of the nasal tract of porpoises, in particular the "hintereklappe" (Kükenthal, 1893).

8.2. Facial musculature

It has been considered that the facial musculature of odontocetes developed from the maxilonasolabial musculature of terrestrial mammals (Huber, 1934; Lawrence and Schevill, 1956; Schenkkan, 1973; Mead, 1975; Rodionov and Markov, 1991). One of the main reasons for this statement is that the facial nerve is responsible for the motor innervation of this muscle group in both marine and terrestrial mammals (Huber, 1934).

This homology can also be demonstrated by studying the relationship between the origins and insertions of the present muscles, especially when looking at the muscle origins in the maxilla, which has undergone a process of elongation (Miller, 1923) and moved caudally to the supraorbital region. The muscle origins have undergone the same migration, and are therefore located above the orbit as well. As for the muscle insertions, in terrestrial mammals they are

concentrated in the nasal vestibule and in the upper lip. Likewise, in marine mammals the entire facial musculature inserts into soft tissues, where the labial part of the maxilonasolabial musculature, a.k.a. rostral muscle, inserts in the melon, while the nasal part of the musculature inserts into the membranous nasal tract and into different parts of the nasal sac system (Huber 1934; Mead, 1975). Accordingly, the relationship between bone, muscle and nasal tract is maintained even though the nasal spaces evolved into the nasal sac system, this concurred with an increase in size of the facial musculature. The maxilla now features a larger region for muscle origins, called the facial fossa (Heyning, 1989).

The phylogenetic origin of the internal musculature still has to be clarified. The nasofrontal sac intrinsic musculature, the diagonal membrane muscle and the nasal plug muscle do not seem to correspond to any homologous structures of terrestrial mammals, although they could have been derived from the maxilonasolabial musculature.

8.3. Epithelial lining and pigmentation

Histological examination of the nasal tract shows the presence of a stratified squamous parakeratinized epithelium similar to that found in the skin, which is present throughout the nasal sac system. This is similar to the situation in terrestrial mammals, wherein the nasal vestibule also features the same epithelial structure as the skin (Adams and Hotchkiss, 1983). Where the epithelium transitions into respiratory epithelium is the caudal limit of the nasal vestibule.

The pigmentation and its distribution pattern is also homologue to terrestrial mammals. The dark, irregular pigmentation is present in all sacs, as has been described in the literature (Mead, 1975), but has not been reasoned. The completely irregular distribution pattern, with intraspecific variation, renounces any possible physiological reasoning such as the exposure to light. We noted that the pigmentation could be present anywhere, including in the blind endings of the sacs (Plate 9, Fig. 4; Plate 22, Fig. 2). The phonic lips are the only structures that have a consistent pigmentation.

Moreover, the pigmentation in the nasal cavity of terrestrial mammals corresponds to this description in that it is irregularly present in the nasal vestibule and stretches as far as its caudal limit (Plate 23, Fig. 3). This finding supports the idea of homology between the nasal vestibule of terrestrial mammals and the nasal sac system of odontocetes.

8.4. Nasal cartilage

Heyning and Mead (1990) described the presence of a cartilage located caudal to nasal tract in mysticetes, and they compared it to nasal cartilages of other mammals. If we consider that all of the odontocetes in this study feature a nasal ligament with a cartilaginous centre that reinforces the posterior wall of the spiracular cavity on both sides, we could argue for the nasal ligament being an evolutionary result of the nasal cartilage and possibly of the lateral nasal cartilage. The cartilage's arrangement, location and relationships with other structures support this hypothesis, as both cartilages are positioned laterodorsal to the nasal tract, which would be laterocaudal to the tract of odontocetes because of the vertical position of the tract, and they are related to the same outermost part of the upper respiratory system, as discussed in other points of this section.

Moreover, both insert medially onto the nasal bones and laterally onto the caudal parts of the premaxillary bone.

8.5. Glandular structures

The presence of glands in the nasal cavity is a general constant in all terrestrial mammals that have been studied so far (Bojsen-Möller, 1964, 1967; Adams et al., 1981). The structural characteristics of the glands in the nasal system in at least some of the odontocete species, once again depicts the similarity between these two kind of mammals; and as dogs and mice feature the openings of the lateral nasal glands in the alar folds, while the openings of the ducts of the minor glands are scattered along the nasal vestibule, these are new clues for the origin of the nasal sac system.

To conclude this section on the evolutionary origin of the nasal region of odontocetes, we note that the evolution of the nasal cavity, with the mentioned reduction of the bony walls, led to a disappearance of the olfactory properties. In addition, because the interrelations between bone, muscle and nasal tract are maintained, this helps us to point out the exact origin of the evolved facial structures. Moreover, there is an overlap between odontocetes and terrestrial mammals in the pattern of epithelialization and pigmentation of the epithelial layers with no other logical reasoning that the evolutionary origin. Finally, the nasal sac system has evolved as a specialized region that originated from the nasal vestibule, which means that any comparison or functional reasoning must be made based on the nasal region of odontocetes being the equivalent to the most rostral part of the upper respiratory system of non-cetacean mammals.

9. NOMENCLATURE

In search of Spanish nomenclature to describe the structures of the nasal region of odontocetes, we have been forced to create new terms for anatomical structures that are specific for these species of aquatic mammals. The lack of references in Spanish, in some cases absolute, has hampered this task. In a literature review we have only encountered Gallardo (1916), who uses Spanish to make a description of the facial region of the spectacled porpoise, *Australophocoena dioptrica*, and he used German words to refer to elements that had already been described (e.g. hintereklappe).Furthermore, he omitted reference to a large number of structures that would be described in subsequent work. Moreover, the use of the Nomina Anatomica Veterinaria has not been very helpful because of the many evolutionary adaptations in this location. Consequently, there is no clear equivalence between facial structures of cetaceans and other mammals, even though there are homologies such as in the facial musculature (Huber, 1934). In 1992, Markov and Rodionov made a review on the anatomy of the odontocete nasal region, assigning Latin names to all the structures that had been described until then, but it lacked scientific nomenclature due to an inadequate homology as was mentioned before. The result of the above work was an endless supply of Latin names, but we must not forget that the majority of these names were created by the authors, and that they possibly complicate the understanding of an already complex region even more.

So, being forced to use new terms, at least in terms of language, we have opted for a change in some cases (see Table 2). The terminology for the general nasal sac system has been maintained, but we used the term *nasal ligament* (Rodionov and Markov, 1992) instead of *blowhole ligament* as there is no clear link between the blowhole and the ligament. Furthermore, we have avoided using the term inferior vestibule for the site of the opening of the nasofrontal sacs in delphinids because this could not be considered to be an actual space. On the other hand, it could be considered to be an actual space in porpoises, hence the name (Gallardo, 1916), thanks to the confluence of the posterior nasal sac in the same place.

As for the organ of sound production, we have used the term phonic lips (prev. *labios vocales*) (vocal lips) as a clear reference to their function, instead of monkey lips, which originated from the appearance of these structures in the sperm whale, *Physeter macrocephalus / catodon* (Pouchet and Beauregard, 1885). The Physeteridae family features two of these lips, but the structure is doubled in delphinids who features four of them. We have also adapted the term “elliptical bodies” (Lawrence and Schevill, 1956) to elliptical adipose bodies as this gives a clearer picture on the form and composition of these structures that are very important in the process of sound production. This completely differs from the nomenclature used by Cranford (1996), who called these "dorsal bursae", a name that has no functional or morphological value, and was changed later to "bursae cantantes."

VIII. CONCLUSIONS

First.

The phonic lips of the nasal sac system feature a specialized anatomical arrangement and histological structure, and are well differentiated from the rest of the system and, in principle, would be compatible with the location of ultrasonic sound production in members of the Delphinoid superfamily.

Second.

The phonic lips, the elliptical adipose bodies and the posterior part of the melon are intimately related, both anatomically and histologically. The tight interrelationships between these structures and their well-known acoustical properties, would allow for these tissues to act as a transmission pathway for produced ultrasonic sounds.

Third.

The lower part of the nasal sac system and its associated structures are covered by a theca of dense connective tissue, which we have called “laminar fibrous complex” (LFC). This layer of connective tissue is formed by the aponeuroses of the different parts of the external musculature of the nasal region, and by their associated ligamentous structures, in particular the nasal ligament. By providing both rigidity and elasticity to the nasal tract, the LFC would be adequate to keep the system closed and could act as a baffle together with the nasal sacs.

Fourth.

The posterior wall of the spiracular cavity and the nasofrontal sac feature numerous well-organized glandular structures. The special arrangement of these accumulations, together with the fact that the glands appear constantly in all species studied, would assume that they play an important role in sound production processes.

Fifth.

The characteristics of the epithelium, the presence of pigmentation and glandular formations, the presence of cartilage of the nasal ligament, and the absence of any osseous boundary, are indications that the nasal sac system is homologue to the nasal vestibule of terrestrial mammals.

IX.SUMMARY

Although the acoustic and echolocation capacities of odontocetes have been studied intensely in the past decades, the specific anatomical location for the production of ultrasonic sound has not yet been identified. While some controversial theories considered the larynx or the lower nasal tract as the responsible structures, more recent hypotheses point to the superior nasal tract as the sound generator organ.

The lack of structural evidence has led us to study the microscopic morphology and architecture of the different structures that make up the nasal sac system, by conventional dissection, observation of transverse and longitudinal anatomical sections, histological techniques, and scanning and transmission electron microscopy in a set of individuals, with focus on the striped dolphin (*Stenella coeruleoalba*) but also including other representatives of the families of Delphinidae and Phocoenidae, which are both included in the Delphinoid superfamily.

The phonic lips of the slit-like opening are characterized by an architecture that distinguishes them from the rest of the nasal tract, as they feature a thickening of the epithelium, a protrusion of the surface into the lumen of the tract, and the presence of folds perpendicular to the major axis of the lips. The lower part of the nasal sac system and its associated structures are covered by a layer of dense connective tissue, rich in elastic fibres, which we have called the Laminar Fibrous Complex (LFC). The posterior wall of the spiracular cavity and nasofrontal sac features numerous and well organized glandular formations.

The anatomical conformation and histological structure of the slit-like opening suggest that this part of the nasal tract can be subjected to a transverse tension while it can move freely, thus showing the capacity for vibration and the production of ultrasonic sounds. The intimate relationship between the slit-like opening, the elliptical adipose bodies that are situated in its vicinity and the melon core, are elements that are all connected and linked by the LFC, which suggests that this unit acts as a pathway for the transmission of ultrasonic sounds.

X.FIGURES

PLATE 1

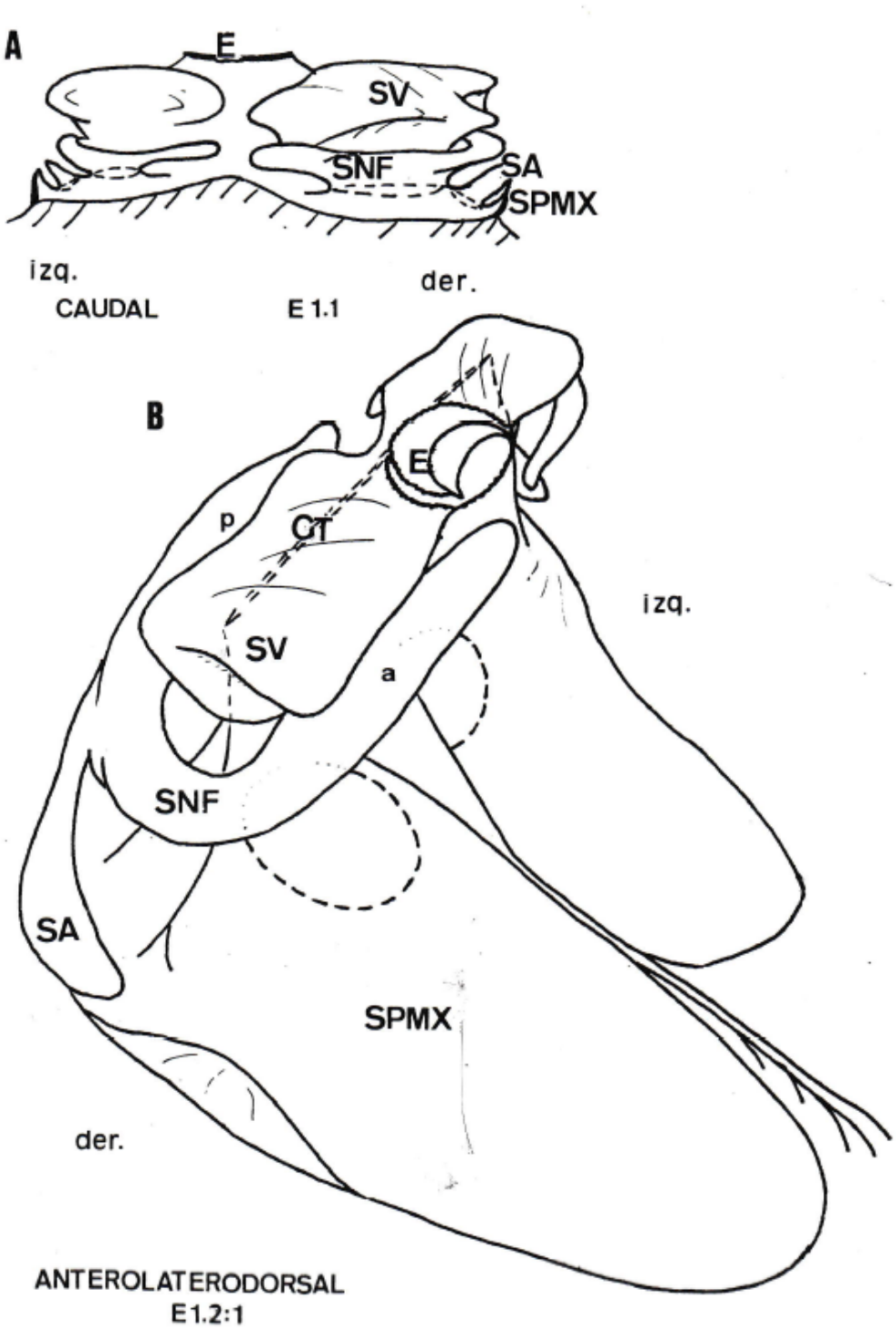

Plate 1.
General schematic representation of the nasal sac configuration. Caudal view (A) in which the communication of nasofrontal sacs (SNF) and accessory (SA) with the main tract is shown. Anterolaterodorsal view (B). E: blowhole; SV: vestibular sac; SPMX: premaxillary sac; CT: slit-like opening; SNFa/p: anterior/posterior portion of the nasofrontal sac.

PLATE 2

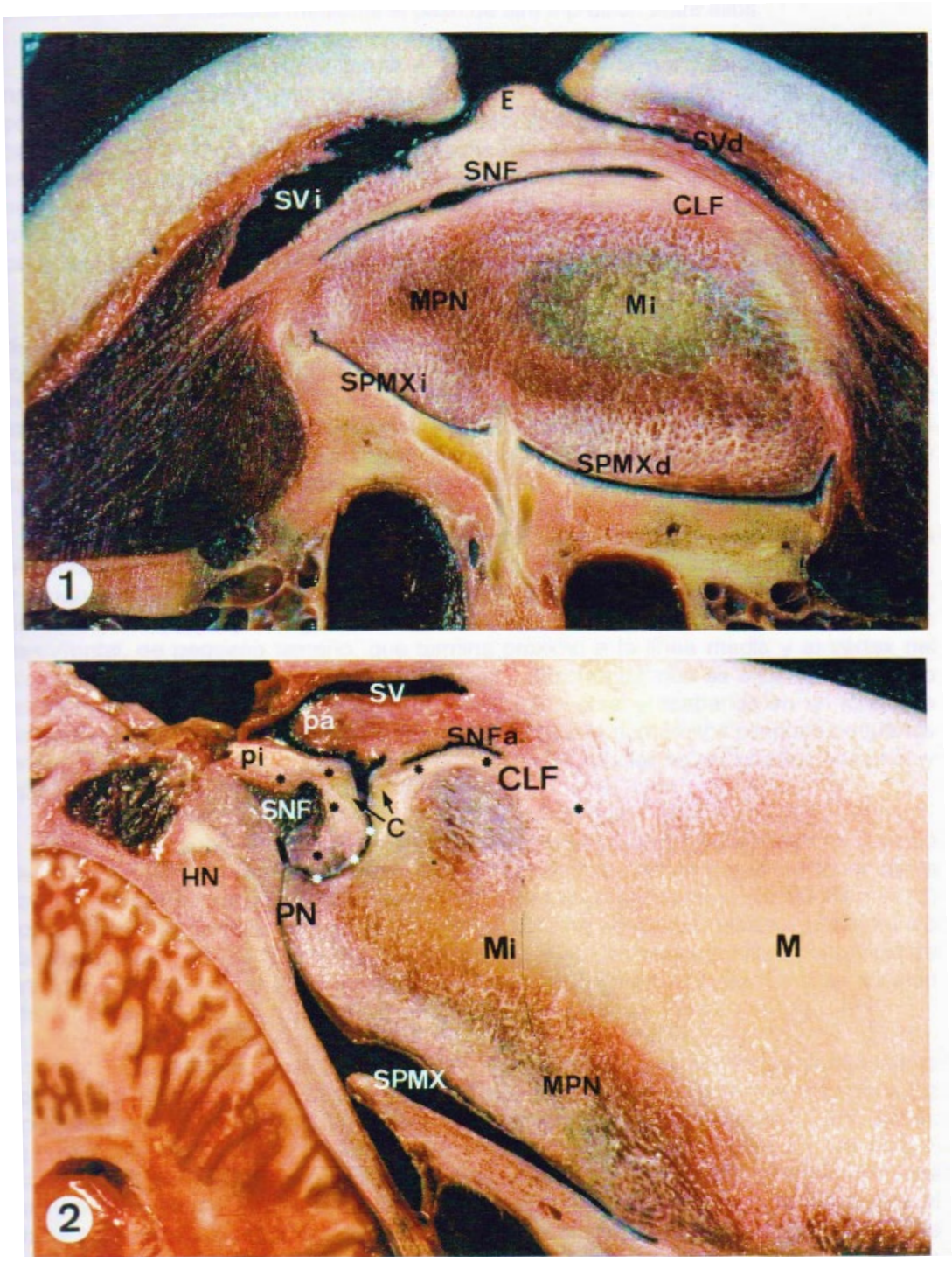

Plate 2.

Fig. 1. Caudal view of a transverse section of the head of a striped dolphin at the level of the blowhole (E). Notice the arrangement of the nasal sacs and the area covered by the laminar fibrous complex (CLF).

Fig. 2. Right parasagittal section through the head of a striped dolphin showing the nasal tract and its relation to the sacs. The laminar fibrous complex (CLF ***) covers the lower half of nasal system.

C (arrows) elliptical adipose bodies; E: blowhole; HN: nasal bone; M: melon; Mi: melon core; MPN: nasal plug muscle; pa: rostral fold of the vestibular sac; pi: insertion of the posterointernal part of the maxilonasolabial muscle; PN: nasal plug; SNF: nasofrontal sac, SNFa: anterior part of the nasofrontal sac; SPMX i/d: premaxillary sac left/right; SV i/d: vestibular sac left/right; white asterisks: nasal tract.

PLATE 3

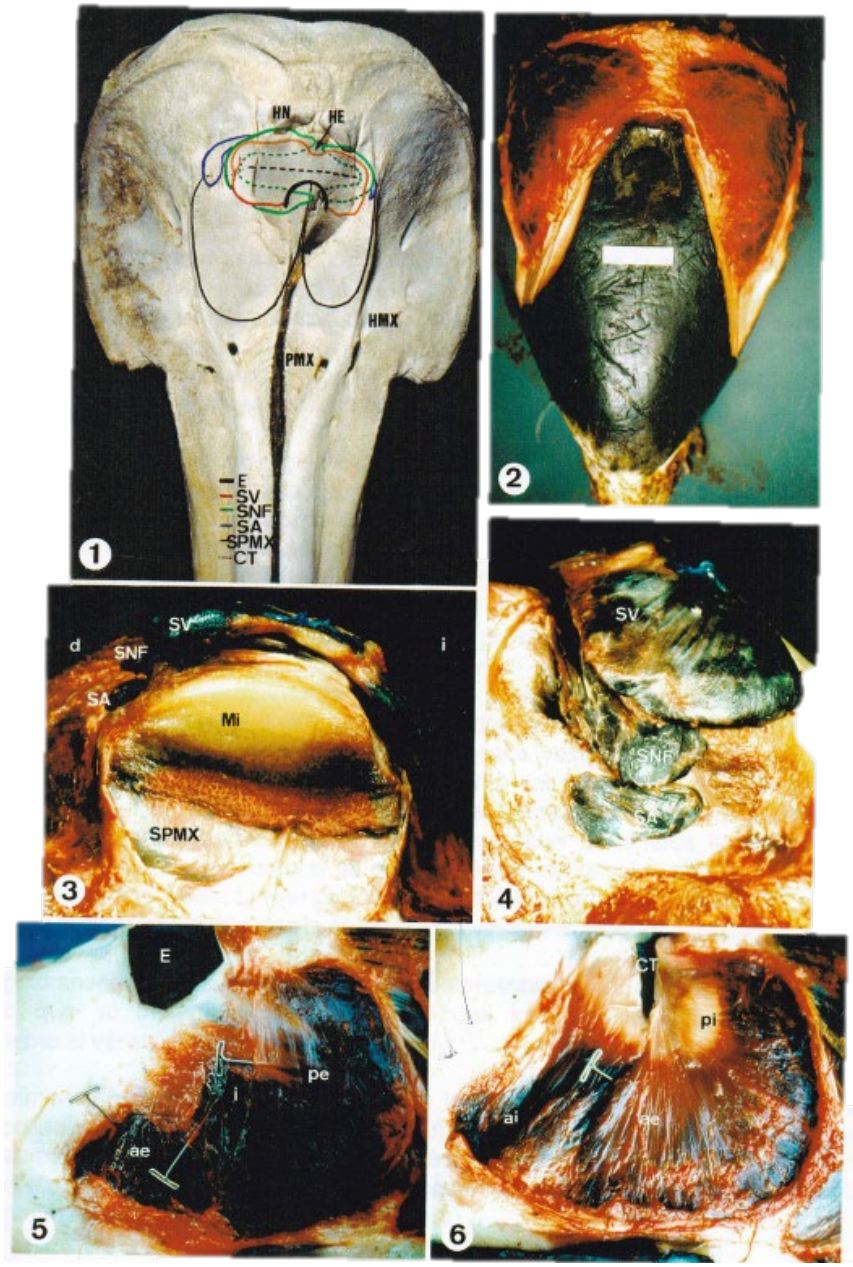

Plate 3.

Fig. 1. Dorsal view of a schematic drawing of the nasal sac configuration on top of a photo of a striped dolphin skull.

Fig. 2. Dorsal view of external musculature in a white-beaked dolphin head (*Lagenorhynchus albirostris)* after removal of the skin and subcutaneous tissues.

Fig. 3. Rostral view of the arrangement of the nasal sacs in a striped dolphin. It shows the different levels of the sacs which are shaped like a dome. d/i: right/left.

Fig. 4. Right lateral view of the same preparation as in Fig. 3.

Fig. 5-6. Left dorsolateral view of a dissection of the external musculature of a northern right whale dolphin (*Lissodelphis borealis)* in which was observed from superficial to deep: (5) musculus maxilonasolabialis anteroexternus and posteroexternus, m. maxilonasolabialis intermedius and (6) m. maxilonasolabialis anterointernus and posterointernus, separated by central fibres of the m. maxilonasolabialis anteroexternus (needle).

ae: anteroexternus; ai: anterointernus; CT: slit-like opening; E: blowhole; HE: ethmoid bone; HMX: maxilla; HN: nasal bone; HPMX: premaxilla; Mi: melon core; pe: posteroexternus; pi: posterointernus; SA: accessory sacs; SNF: nasofrontal sacs; SPMX: premaxillary sacs; SV: vestibular sacs.

PLATE 4

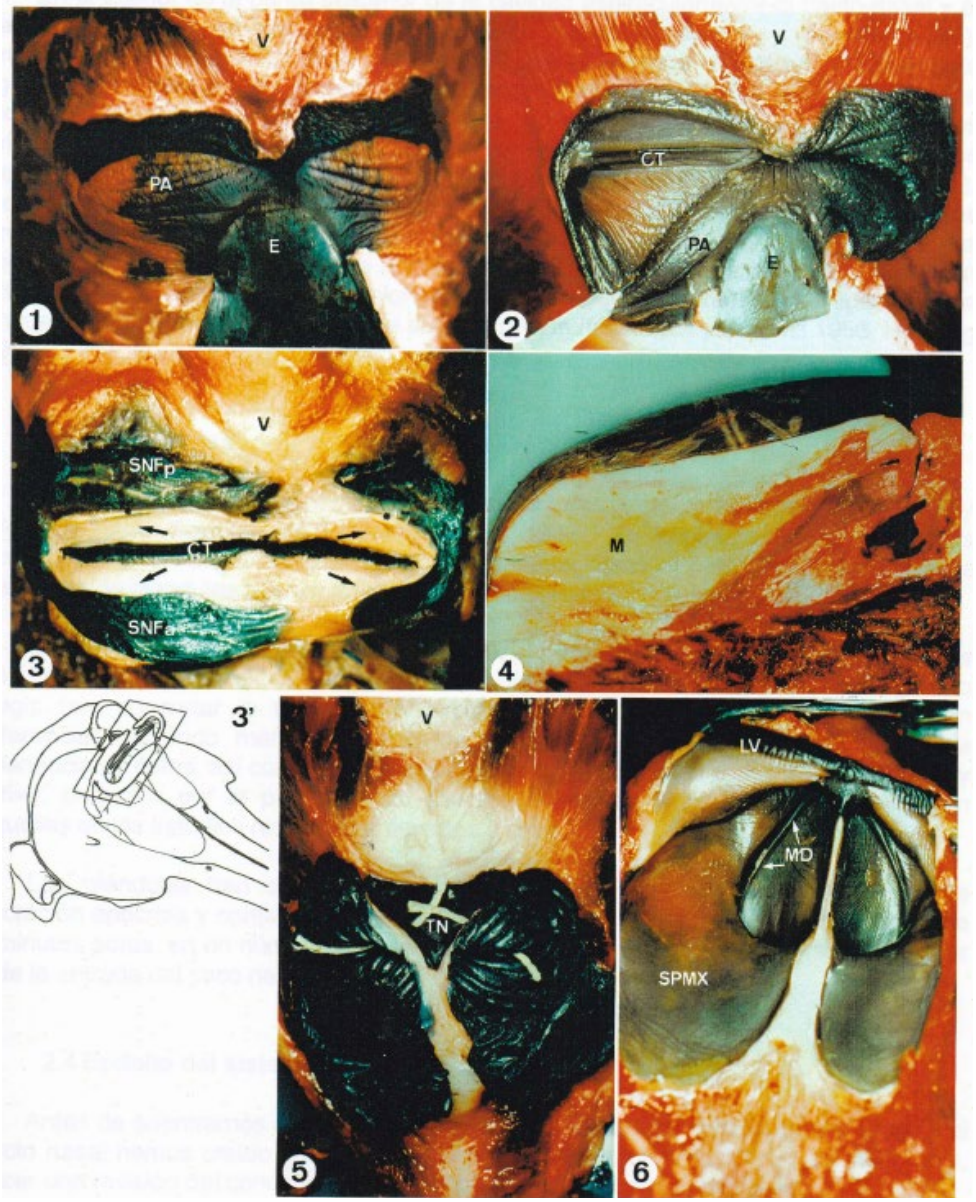

Plate 4.

Fig. 1. Dorsal view of the anterior vestibular folds of a white-beaked dolphin (*Lagenorhynchus albirostris*) after removal of the roof of the vestibular sacs.

Fig. 2. Same as Fig. 1, but the right anterior vestibular fold is manually pulled in rostral direction. By doing this, the slit-like opening (CT) and the right phonic lips come into view. Striped dolphin (*Stenella coeruleoalba*).

Fig. 3. Dorsal view of the nasofrontal sacs, after removal of the vestibular sacs. Note the location of the phonic lips (black arrows): the cranial ones are covered by the laminar fibrous complex, situated immediately ventral to the anterior nasofrontal sacs, while the caudal phonic lips are present in between the nasal ligament and the slit-like opening.

Fig. 3'. Dorsorostrolateral view of a schematic drawing of the skull, indicating the tissue plane that was prepared in Fig. 3.

Fig. 4. Median section through the melon of a Risso's dolphin (*Grampus griseus*). The melon adipose tissues take up almost all of the rostral pons and show obvious colour differences.

Fig. 5. Dorsal view of a preparation of the opened vestibular sacs in the harbour porpoise (*Phocoena phocoena*). Note the folds in the bottom of the sacs and the communication channels to the nasal tract (TN), indicated by two pieces of strings.

Fig. 6. Rostral view of the caudal wall of the nasal tract of a striped dolphin after removal of the rostral wall, the nasal plugs and the nasal plug muscle. Note the external bony nares and their respective diagonal membranes (MD).

PLATE 5

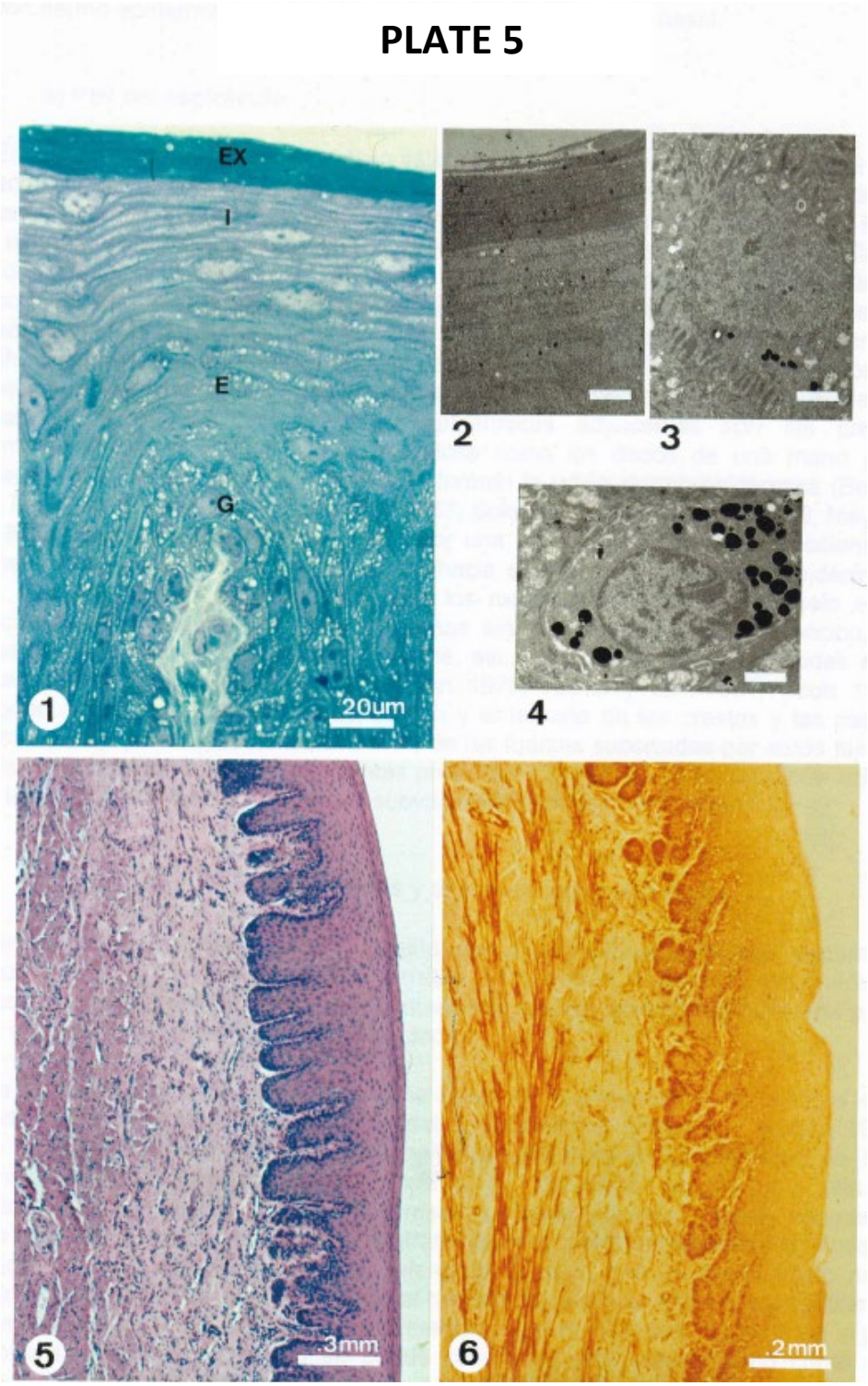

Plate 5.

Fig. 1. Histologic section of the epithelium of the nasal tract (Toluidine blue staining after metacrilate embedding). Note the different cellular layers. G: stratum germinativum; E: str. spinosum; I: str. intermedium; EX: stratum corneum. M.O.

Fig. 2. T.E.M.-image of the transition zone between the stratum intermedium and the stratum externum of the epithelium of the nasal tract. Scale bar: 5 µm.

Fig. 3. T.E.M.-image of a single cell in the stratum spinosum. Scale bar: 2 µm.

Fig. 4. T.E.M.-image of a melanocyte in between the cells of the stratum germinativum. Scale bar: 1.25 µm.

Fig. 5. Histological section of the wall of the nasal tract (H.E. staining). M.O.

Fig. 6. Histological section of the wall of the nasal sacs (Orcein-Van Gieson staining). Note the orange colored elastic fibres.

PLATE 6

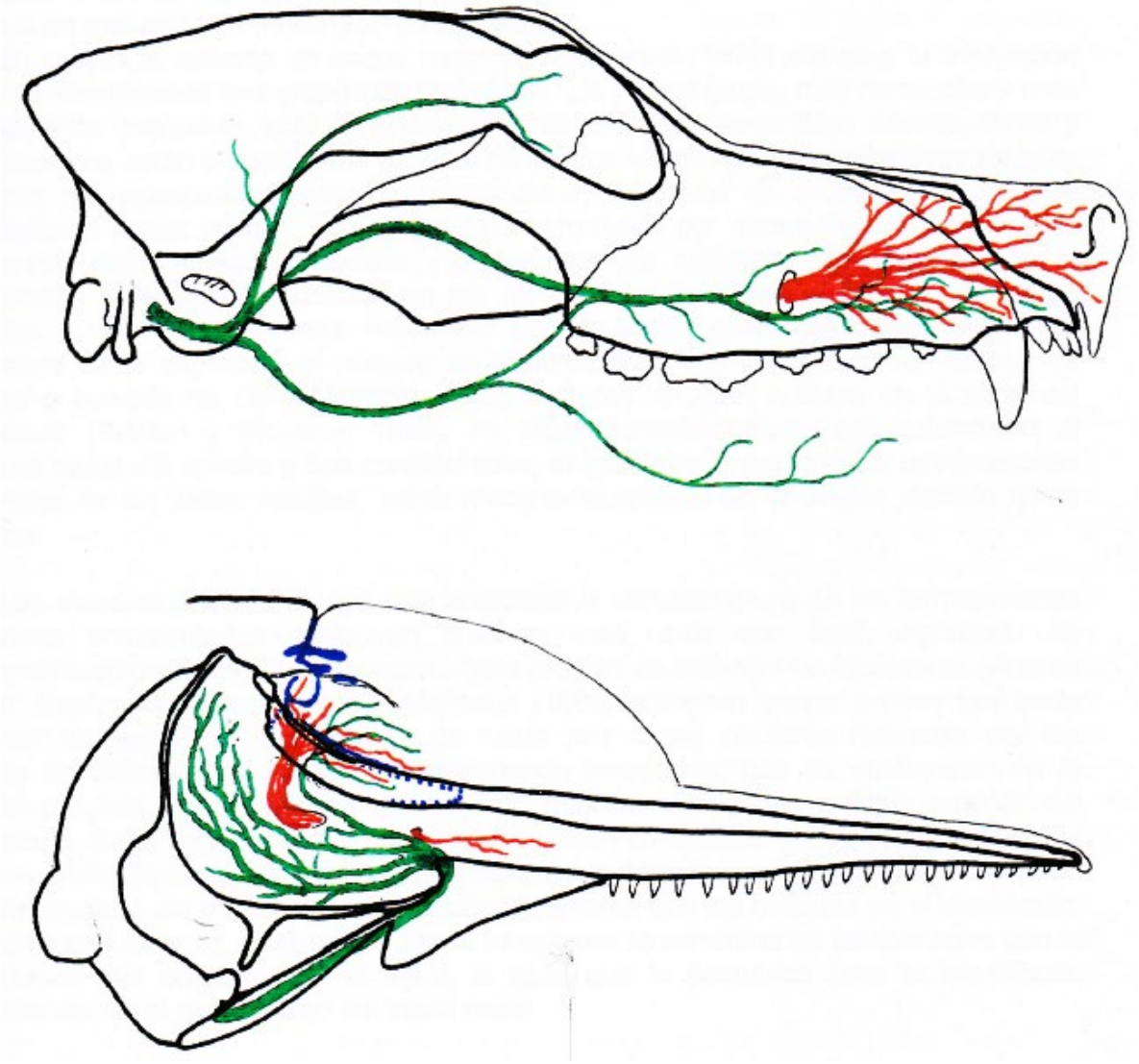

Plate 6.
Schematic drawing of a right lateral view of the distribution of the facial (green) and infraorbital (red) nerves in the dog (above) and the striped dolphin (below).

PLATE 7

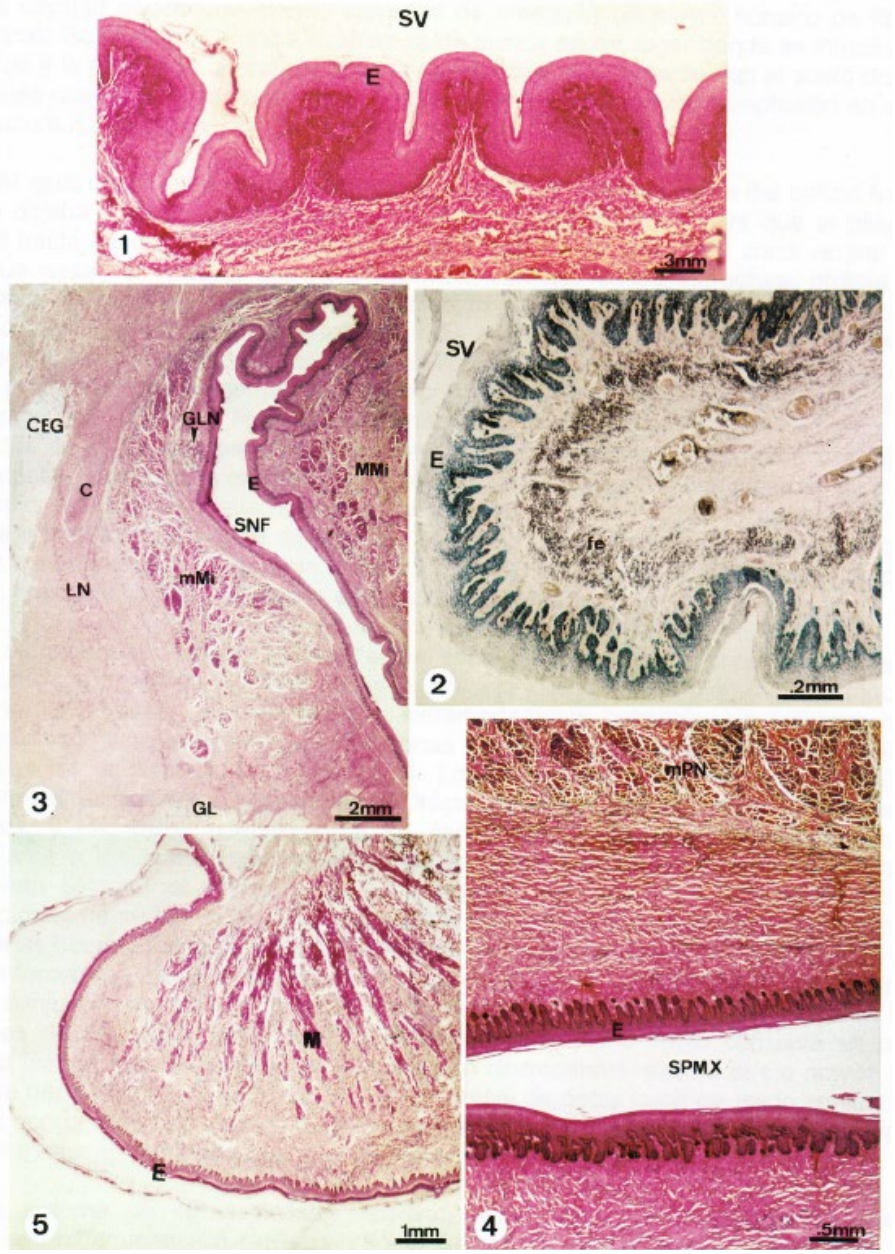

Plate 7.

Fig. 1. Histological section of the folded epithelium of the vestibular sacs (VS) (H.E. staining). E: epithelium. M.O.

Fig. 2. Histological section of a single fold in the wall of the vestibular sacs (VS) (Giemsa/Orcein staining). Note the abundant presence of elastic fibres in the loose connective tissue of the subepithelial tissue. E: epithelium. M.O.

Fig. 3. Histological section of a sagittal cut through the nasofrontal sac (NFS) at the level of the opening to the nasal tract (H.E. staining). The nasal ligament (LN) is located rostral to the sac. C: cartilage of the nasal ligament; CEG: elliptical adipose bodies; E: epithelium; GL: nasal gland; GLN: nasofrontal gland (arrow); m/M Mi: minor/major part of the NFS intrinsic muscle. M.O.

Fig. 4. Histological section of a sagittal cut through the premaxillary sac (SPMX) (H.E. staining). Note the epithelium overlaying the premaxilla (bottom) while the roof of the sac is covered by the nasal plug muscle (mPN). M.O.

Fig. 5. Histological section of a sagittal cut through the right nasal plug (H.E. staining). Note the arrangement of the fibres of the nasal plug muscle (M). E: epithelium. M.O.

Fig. 1-5. *Stenella coeruleoalba*

PLATE 8

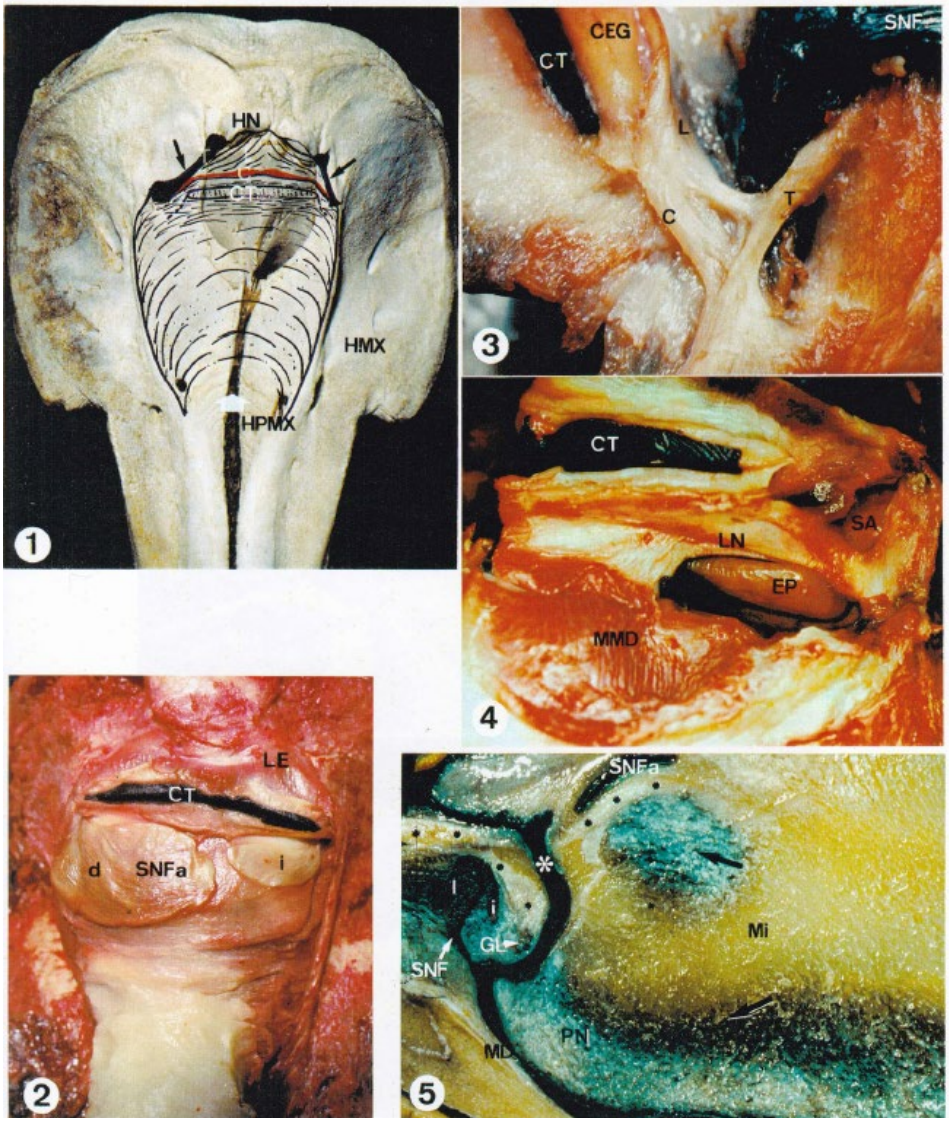

Plate 8.

Fig. 1. Dorsal view of a schematic drawing of the arrangement of the laminar fibrous complex (LFC) on top of a skull of a striped dolphin *(S. coeruleoalba)*. There is a rostral opening for the melon core (white arrow), and other opening for the passages of the nasofrontal and accessory sacs (black arrows).

Fig. 2. Dorsal view of the laminar fibrous complex of a Pacific white-sided dolphin (*L. obliquidens*) after removing the part of this complex that covered the anterior nasofrontal sacs.

Fig. 3. Left dorsolateral view of the lateral insertion of the nasal ligament (L) and the tendinous complex (T) in a striped dolphin (*S. coeruleoalba)*. Note the cartilage (C) that makes up the lateral commissure of the nasal tract.

Fig. 4. Caudodorsal view of the nasal ligament in the striped dolphin (*S. coeruleoalba)* after removal of the nasofrontal sacs and its intrinsic musculature. Note the presence of the nasal ligament, visible through the opening of the nasofrontal sacs. The diagonal membrane muscle (MMD) is visible caudally.

Fig. 5. Right lateral view of a paramedian section through a striped dolphin head (*S. coeruleoalba*) at the level of the melon core which is covered dorsally by the laminar fibrous complex (black asterisks) and the anterior part of nasofrontal sac (SNFa). At the level of the white asterisk, which is located inside the nasal tract, are the phonic lips and the elliptical adipose bodies on both sides of the tract.

C: cartilage of the nasal ligament; CEG: elliptical adipose bodies; CT: slit-like opening; EL: elastic sheet; GL: nasal gland; HMX: maxilla; HN: nasal bone; HPMX: premaxilla; I/i: major/minor part of the NFS intrinsic muscle; MD: diagonal membrane; Mi: melon core; PN: nasal plug and nasal plug muscle (black arrows); SA: accessory sac; SNFa d/i: anterior nasofrontal sac right/left.

PLATE 9

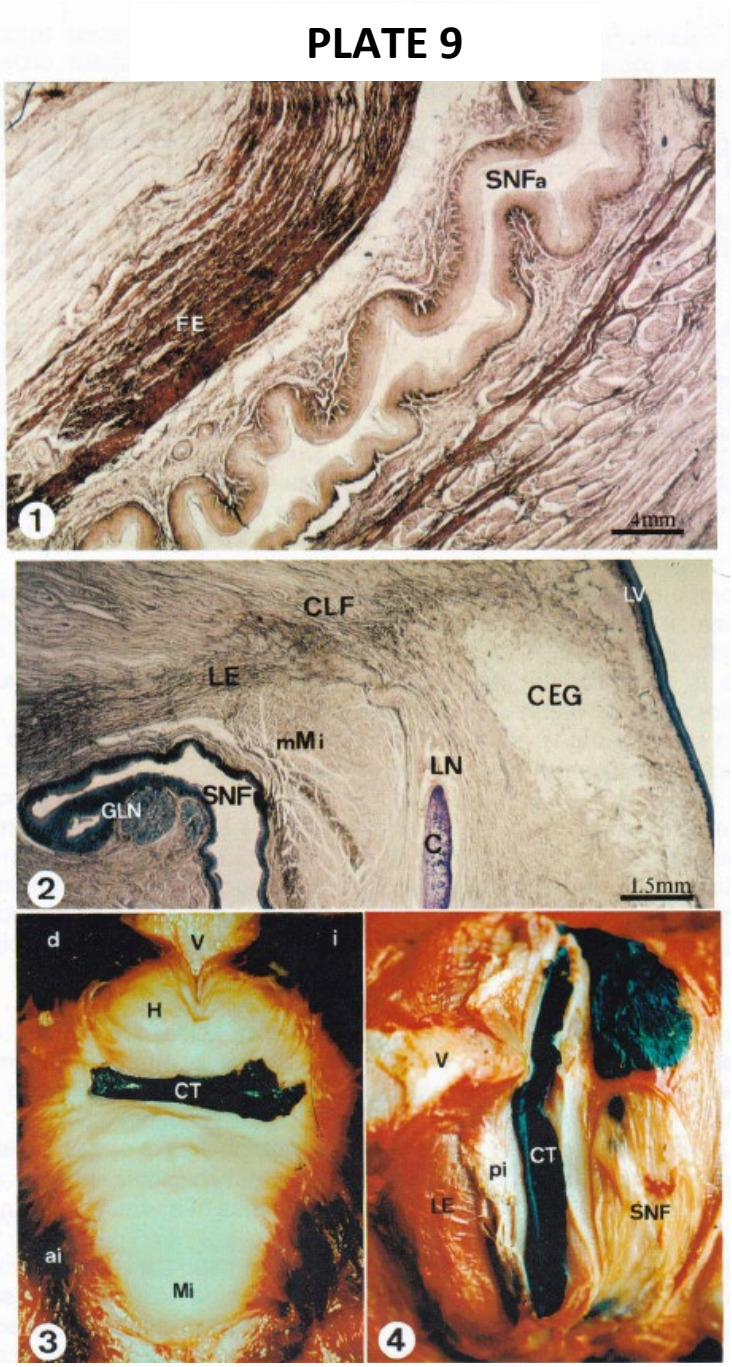

Plate 9.

Fig. 1. Histological section of a sagittal cut through the anterior nasofrontal sac (SNFa) of a striped dolphin (*S. coeruleoalba*) (Orcein/Van Gieson staining). Bands of dense connective tissue surrounding the sac make up a part of the laminar fibrous complex. Note the abundant presence of elastic fibres (FE), which is more pronounced dorsal to the sac. M.O.

Fig. 2. Histological section of a sagittal cut through the nasofrontal sac of a striped dolphin (*S. coeruleoalba*) (Giemsa/Orcein staining). The nasal ligament (LN) and its cartilage (C) make up the dorsal edge of the sac and continue in caudodorsal direction to form the elastic sheet (LE), which is the caudal part of the laminar fibrous complex. This part also covers the caudal elliptical adipose bodies (CEG) and the phonic lips (LV). M.O.

Fig. 3. Dorsal view of the prepared laminar fibrous complex of a harbour porpoise (*Phocoena phocoena*)

Fig. 4. Dorsal view of the laminar fibrous complex of an Atlantic white-sided dolphin where the LFC inserts onto the slit-like opening (CT). Caudally (left) there are tendinous bands that are cut loose from the m. maxilonasolabialis posterointernus (pi) which covers the elastic sheet (LE).

CT: slit-like opening; GLN: nasofrontal gland; H: hintereklappe; LE: elastic sheet; Mi: melon core; mMi: minor part of the nasofrontal sac intrinsic muscle; pi: insertion of the m. maxilonasolabialis posterointernus; V: vertex.

PLATE 10

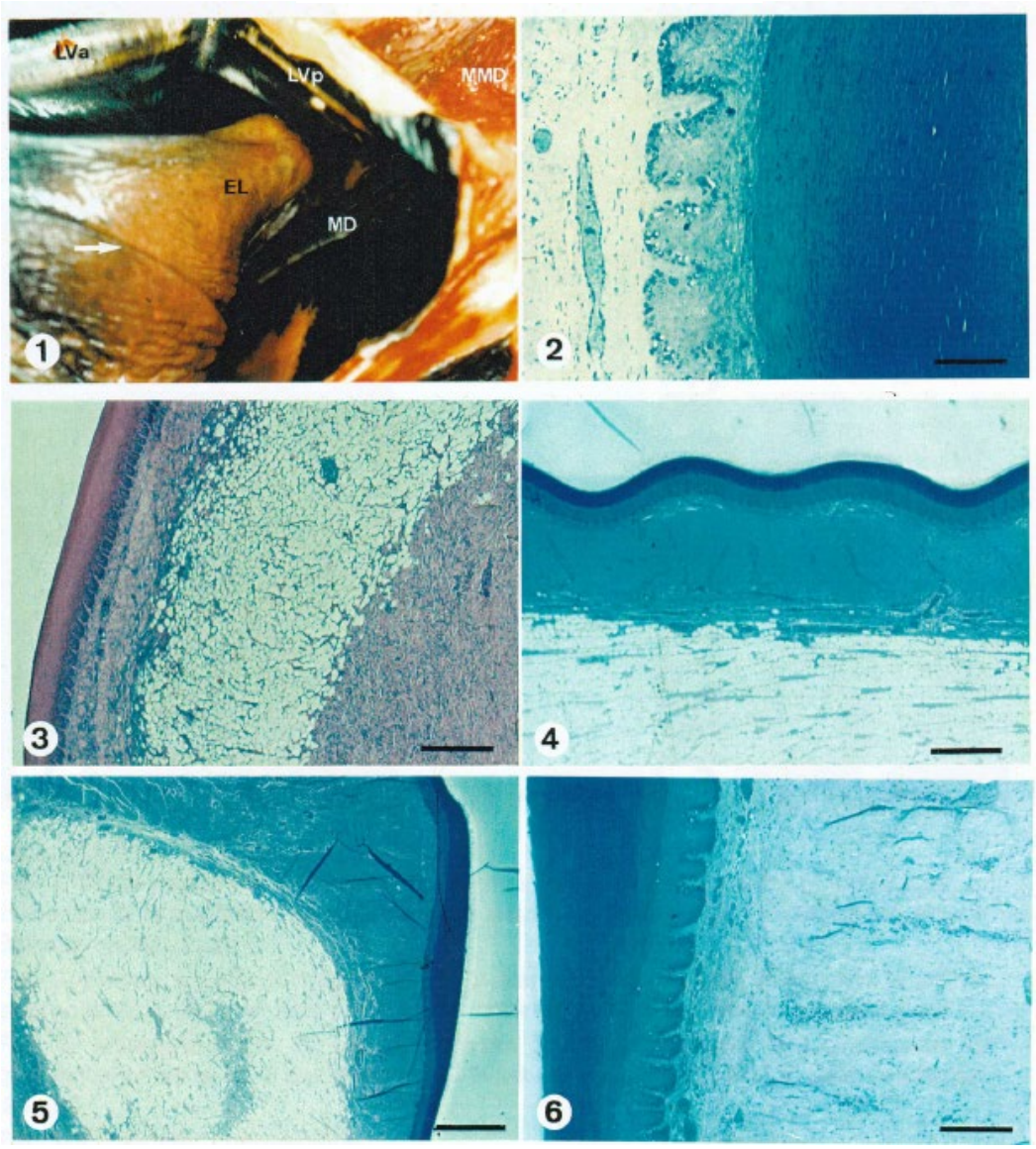

Plate 10.

Fig. 1. Medial view of the spiracular cavity after being opened with a **vertical (?)** cut. The nasal plug is displaced rostrally, as it maintains its lateral extension (EL). The diagonal membrane (MD) left an imprint on the surface of the nasal plug (white arrow). Lva/p: rostral/caudal phonic lip; MMD: diagonal membrane muscle.

Fig. 2. Histological section of a sagittal cut through the centre of the right caudal phonic lip (M.O.; Methacrylate embedding; Toluidine blue staining). Note the substantial thickness of the stratum externum. Scale bar: 20 µm.

Fig. 3. Histological section of a sagittal cut through the caudal wall of the spiracular cavity (M.O.; H.E. staining). The phonic lip has a direct relation with the subjacent elliptical adipose body. Scale bar: 2 mm. M.O.

Fig. 4. Histological section of a horizontal cut through the right caudal phonic lip (M.O.; Methacrylate embedding; Toluidine blue staining). Note the folds of the wall that form grooves without an alteration of the epithelial structure. Scale bar: 12 µm.

Fig. 5. Histological section of a sagittal cut through the right caudal phonic lip and elliptical adipose body (M.O.; Methacrylate embedding; Toluidine blue staining). The connective tissue in between the two did not show a specific arrangement, but if it did, it was parallel to the epithelium (The darker traces are artefacts). Scale bar: 2 mm.

Fig. 6. Histological section of a cut similar to the one in Fig. 5 but through the rostral phonic lip and at higher magnification (M.O.; Methacrylate embedding; Toluidine blue staining). Note the clear orientation of the connective tissue fibres running perpendicular to the surface of the epithelium. Scale bar: 30 µm.

Fig. 1-6. *Stenella coeruleoalba*

PLATE 11

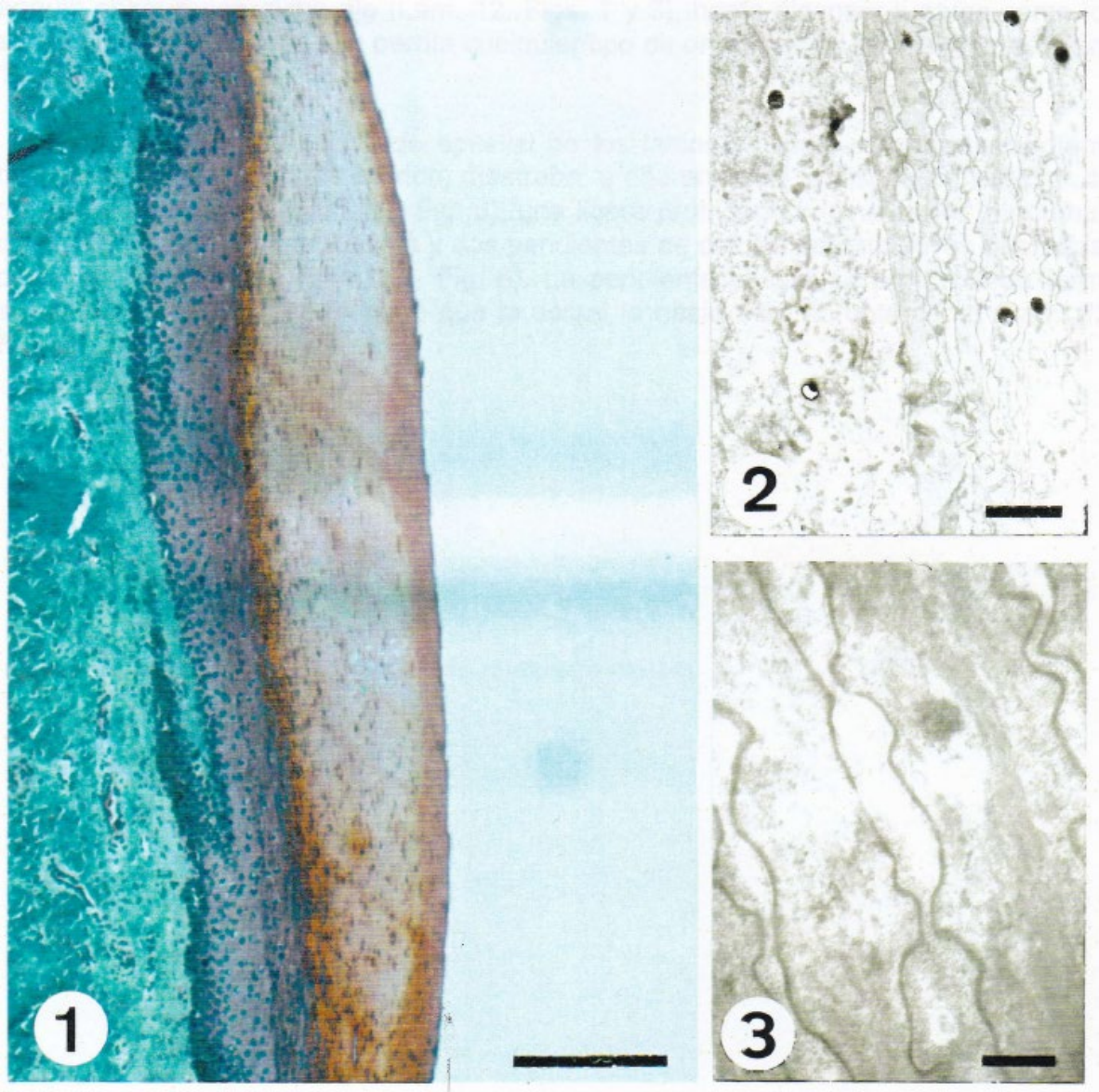

Plate 11.

Fig. 1. Histological section of a sagittal cut through the right phonic lip (M.O.; Mason's trichrome straining). Note the abundance of keratine in the stratum externum. Scale bar: 2 mm.

Fig. 2. T.E.M. image of the transition zone between the stratum intermedium and externum in the epithelium of the right caudal phonic lip. Scale bar: 1.5 µm.

Fig. 3. T.E.M. image of the numerous cell-cell junctions of the keratinocytes in Fig. 2, thus avoiding rapid desquamation. Scale bar: 0.25 µm

PLATE 12

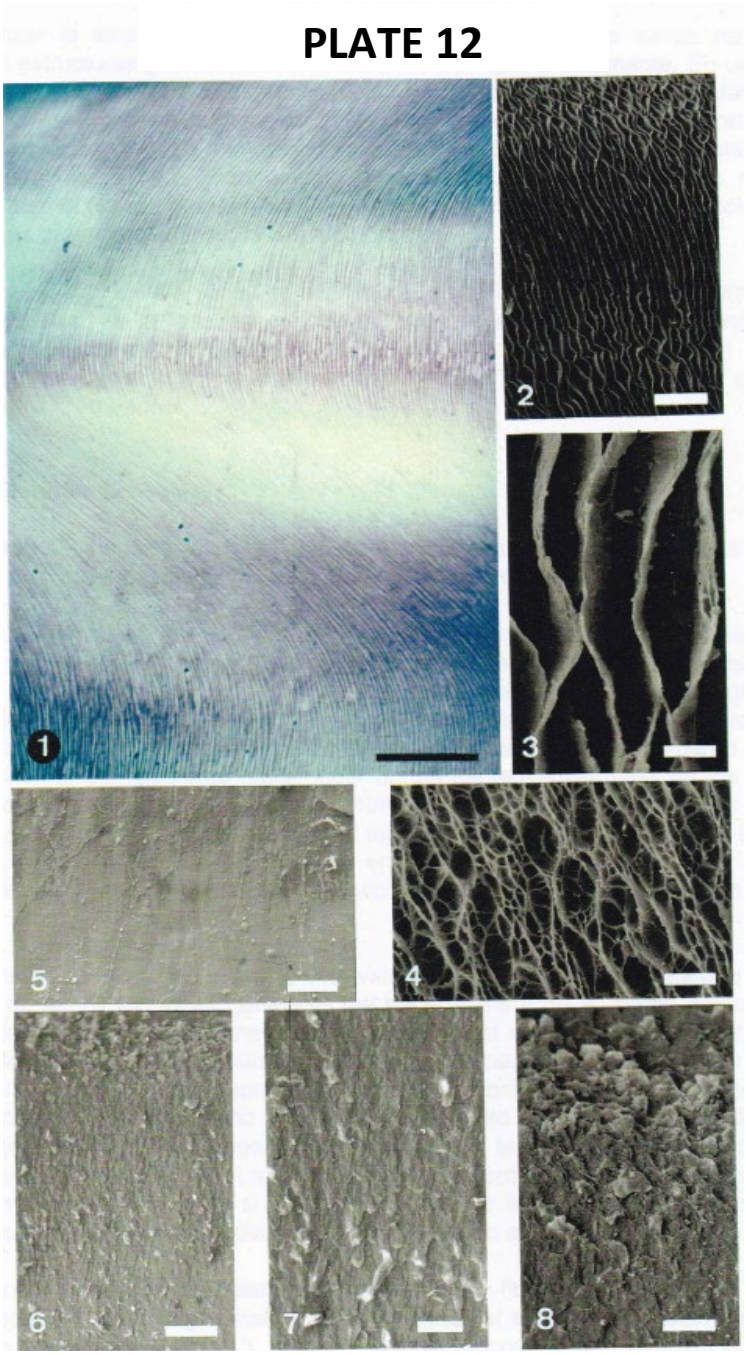

Plate 12.

Fig. 1. Stereoscopic image of half of the right caudal phonic lip after the epithelium has been removed and the subjacent surface was stained with Toluidine blue. Note the arrangement of the subepithelial ridges. Scale bar: 2 mm.

Fig. 2. T.E.M. image of the subepithelial ridges in the phonic lip. Note the parallel character of the ridges in the centre zone. Scale bar: 300 µm.

Fig. 3. T.E.M. image of the subepithelial ridges close to the phonic lip. Note the ridges display contact and they cross each other. Scale bar: 50 µm

Fig. 4. T.E.M. image of the subepithelial layer of the caudal fold of the vestibular sac. The ridges are absent, but there is an intricate framework of the epithelial protuberances. Scale bar: 90 µm.

Fig. 5. T.E.M. image of the surface of the wall of the spiracular cavity. Scale bar: 20 µm.

Fig. 6. . T.E.M. image of the surface of the posterior right phonic lip as it forms as plateau with a slope on both sides: dorsal (above) and ventral (below). Scale bar: 200 µm.

Fig. 7. . T.E.M. detail image of the ventral slope in Fig. 6. The cells present a gentle desquamation in ventral direction. Scale bar: 70 µm.

Fig. 8. T.E.M. detail image of the dorsal slope in Fig. 6. The cells present a sharp desquamation in dorsal direction. Scale bar: 70 µm.

PLATE 13

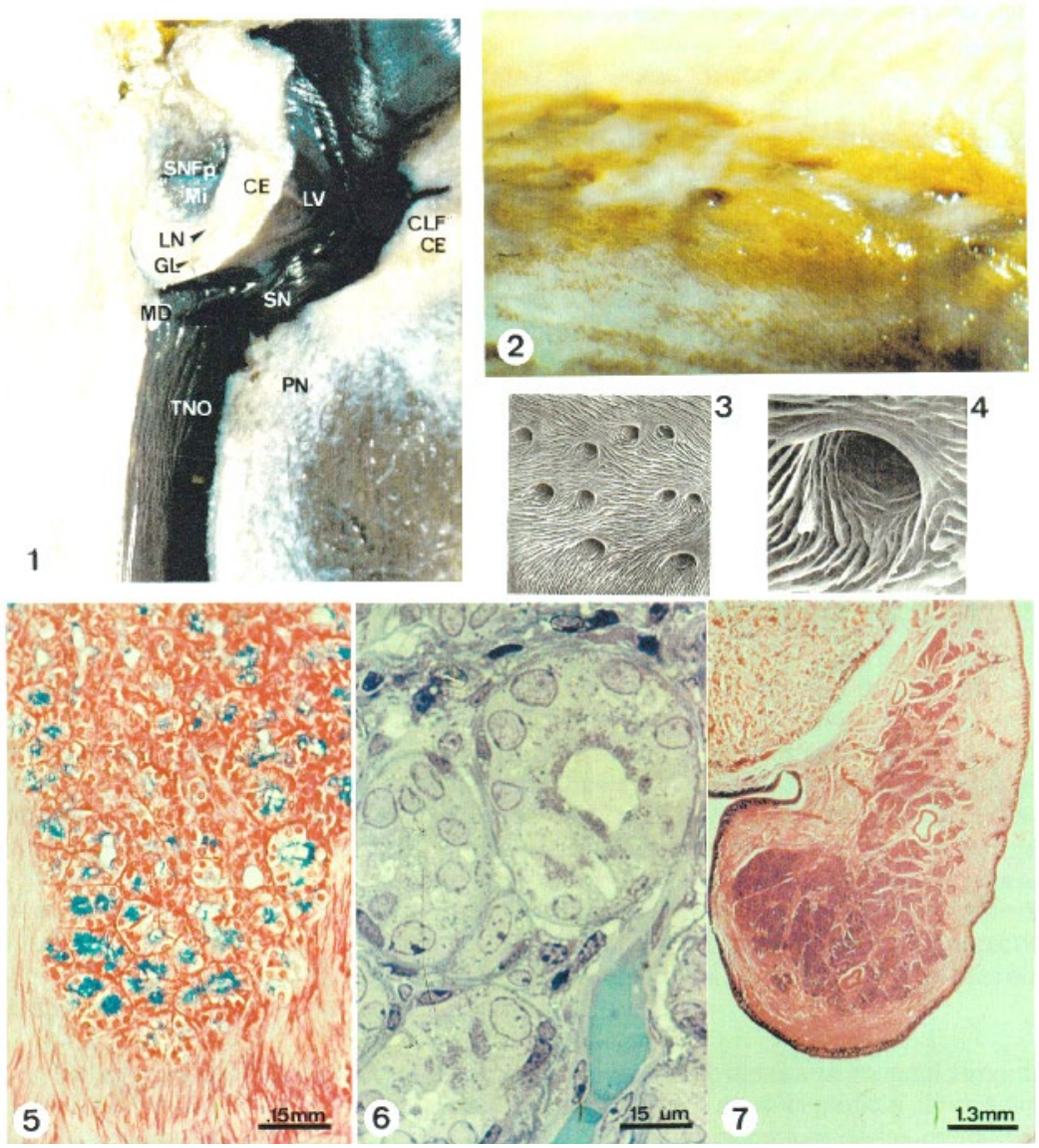

Plate 13.

Fig. 1. Right lateral view of a paramedian cut through the lower half of nasal complex. The spiracular cavity is surrounded by numerous structures from the ventral location of the diagonal membrane up to the slit-like opening dorsally. CE: bursa cantans; CLF: laminar fibrous complex; GL: nasal gland; LN: blowhole ligament; LV: phonic lip; Mi: intrinsic muscle of the nasofrontal sac; PN: nasal plug; SN: nasal septum; SNFp: posterior part of the nasofrontal sac; TNO: bony nasal tract.

Fig. 2. Stereoscopic image of the secretion orifices of the nasal gland after maceration of the epithelium.

Fig. 3. S.E.M. image of the same orifices as in Fig. 2

Fig. 4. S.E.M. detail image of a single orifice from Fig. 3.

Fig. 5. Histologic section of the nasal gland (M.O.; PAS staining).

Fig. 6. Histologic section of an acinus in the nasal gland (Semi-fine cut; Toluidine blue staining).

Fig. 1-6. *Stenella coeruleoalba*

Fig. 7. Histologic section of the nasal gland in a ventral fold of the caudal wall of the nasal tract in phocenids (*Phocoena phocoena*) (M.O.; H.E. staining).

PLATE 14

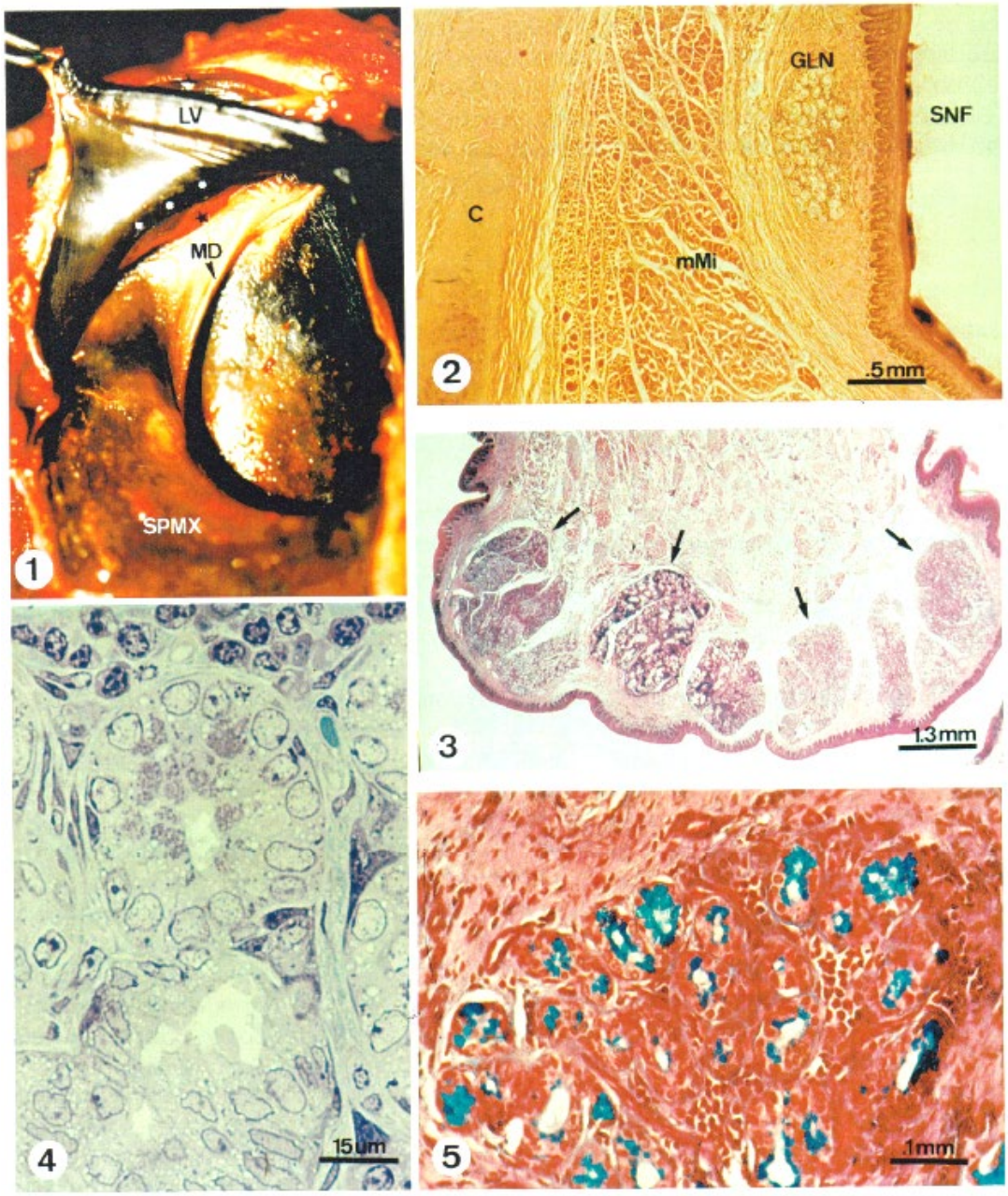

Plate 14.

Fig. 1. Rostral view of the right half of the nasal tract of a white-beaked dolphin (*Lagenorhynchus albirostris*) after removing the tissues that formed the rostral wall of the tract and the nasal plugs. Note the right caudal phonic lip (LV), the nasal gland and its orifices (white asterisks), the sack that contains the nasofrontal gland (black asterisk) and the diagonal membrane (MD) that confines the external bony nare. SPMX: premaxillary sac.

Fig. 2. Histological section of the nasofrontal gland (GLN) of a striped dolphin (*Stenella coeruleoalba*) (M.O.; H.E. staining). The nasofrontal gland is an isolated structure located centrally in the nasofrontal sac, and in proximity of the cartilage (C) of the blowhole ligament. mMi: minor part of the intrinsic muscle of the nasofrontal sac (SNF).

Fig. 3. Histological section of the nasofrontal glands in a white-beaked dolphin (*Lagenorhynchus albirostris)* (M.O.; H.E. staining). The glands are grouped in an epithelial pouch.

Fig. 4. Histological detail shot of an acinus in the nasofrontal gland of a striped dolphin (*Stenella coeruleoalba*) (M.O.; Semi fine cut;.Toluidine blue staining). Note the presence of an excretory duct (Bottom of the image)**.** Also note the presence of plasma cells (upper extreme). This image is very similar to the nasal gland histology (Plate 13, Fig. 6).

Fig. 5. Histological section of the nasofrontal gland of a striped dolphin (*Stenella coeruleoalba*) (M.O. AA2, 5Neutral red). Note the general morphology.

PLATE 15

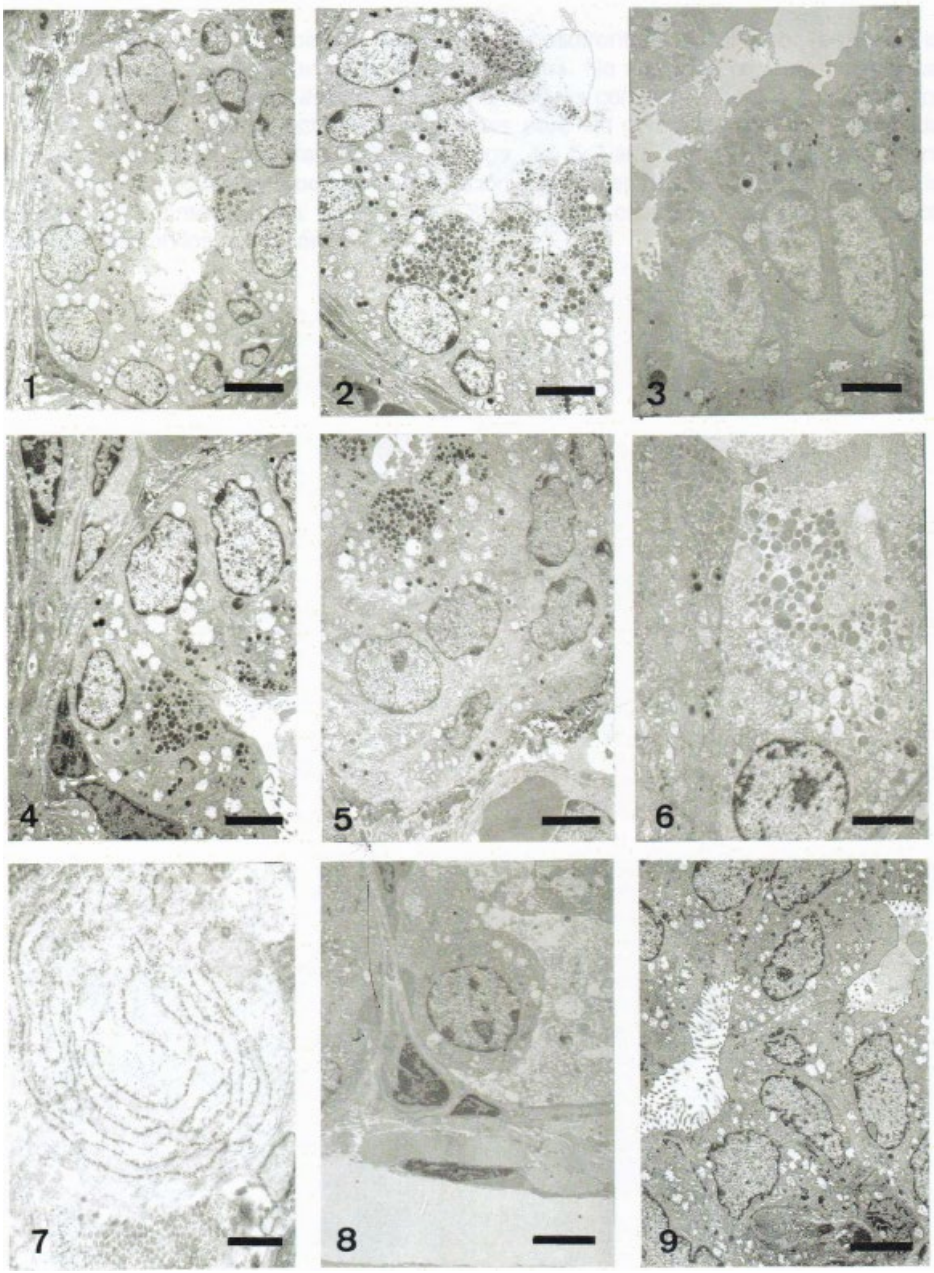

Plate 15.

Fig. 1. T.E.M. image of an acinus of the nasal gland in *Stenella coeruleoalba*. Note the moderate secretory activity. Scale bar: 7 µm.

Fig. 2. T.E.M. image of an acinus of the nasal gland in *Stenella coeruleoalba*. Note the elevated secretory activity with secretion vesicles of variable size and density in the lumen of the acinus. Scale bar: 5 µm

Fig. 3. T.E.M. image of the secretory cells in an acinus of the nasal gland in *Stenella coeruleoalba*. Note the homogeneity of the secretion products. Scale bar: 3 µm.

Fig. 4. T.E.M. image of an acinus of the nasofrontal gland in *Stenella coeruleoalba*. Scale bar: 2.5 µm.

Fig. 5. T.E.M. image of an acinus of the nasofrontal gland in *Stenella coeruleoalba*. Note the presence of the cytoplasmic organelles. Scale bar: 3.5 µm.

Fig. 6. T.E.M. image of a single secretory cell in an acinus of the nasofrontal gland in *Stenella coeruleoalba*. Note the basal displacement of the nucleus. Scale bar: 3.5 µm.

Fig. 7. T.E.M. image of the rough endoplasmic reticulum (RER) in a serous cell of the nasofrontal gland in *Stenella coeruleoalba*. Scale bar: 5 µm.

Fig. 8. T.E.M. image of a plasma cell in proximity of an acinus of the nasofrontal gland in *Stenella coeruleoalba*. Scale bar: 3 µm.

Fig. 9. T.E.M. image of duct cells of the nasofrontal gland in *Stenella coeruleoalba*. Note the apical villi projecting into the lumen. Scale bar: 5 µm.

PLATE 16

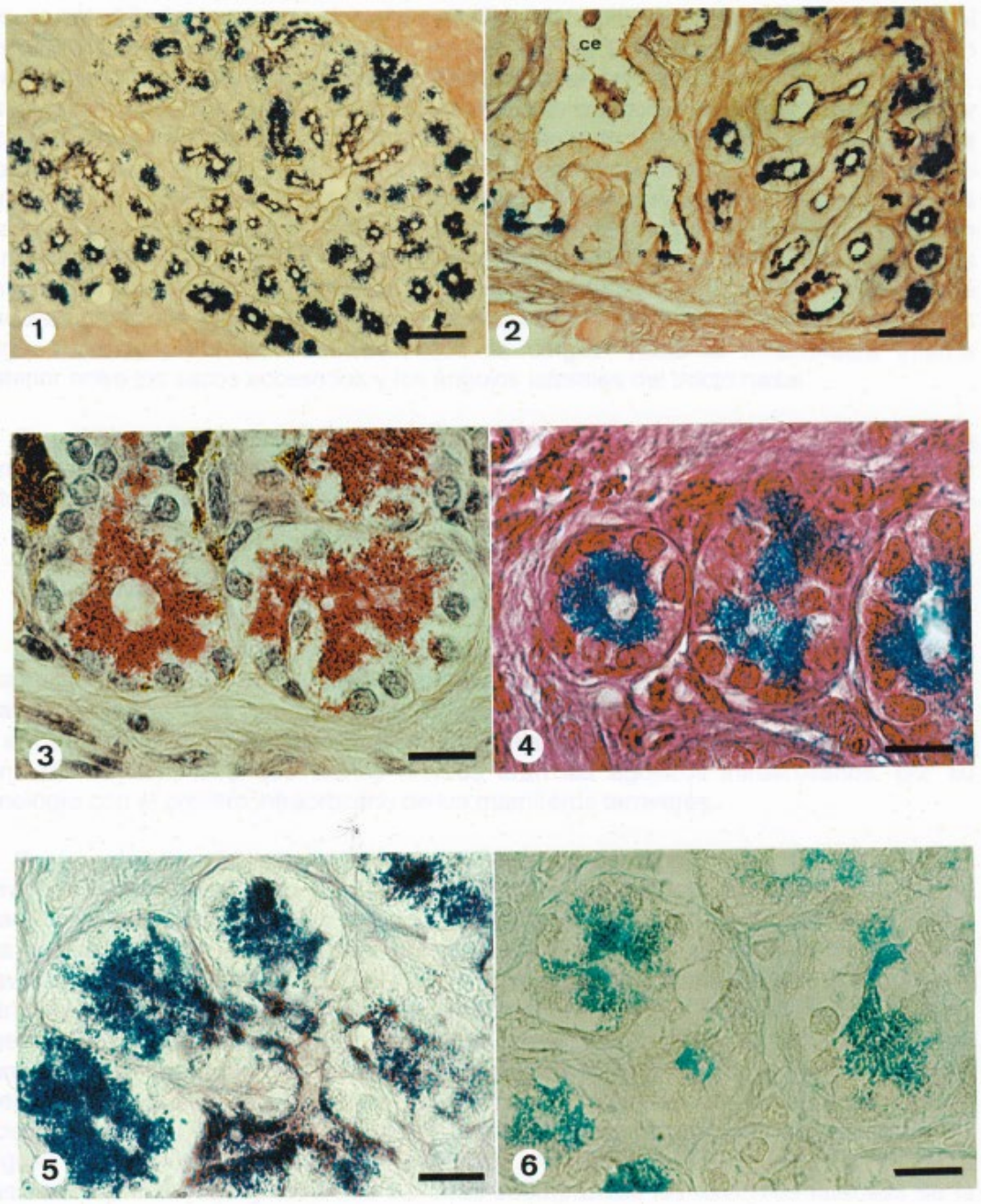

Plate 16.
Fig. 1. Histochemical section of the nasal gland (M.O.; PAS/AB staining). Scale bar: 30 µm.
Fig. 2. Histochemical section of the nasal gland (M.O.; PAS/AB staining). ce: excretory duct. Scale bar: 30 µm
Fig. 3. Histochemical section of the nasal gland (M.O.; PAS/AB staining). Scale bar: 12 µm.
Fig. 4. Histochemical section of the nasal gland (M.O.; Alcian Blue 2.5 staining/Neutral red). Scale bar: 12 µm.
Fig. 5. Histochemical section of the nasal gland (M.O.; PAS/AB staining). Scale bar: 12 µm.
Fig. 6. Histochemical section of the nasal gland (M.O.; Alcian Blue 1.0 staining). Scale bar: 12 µm.
Fig. 1-6. *Stenella coeruleoalba*

PLATE 17

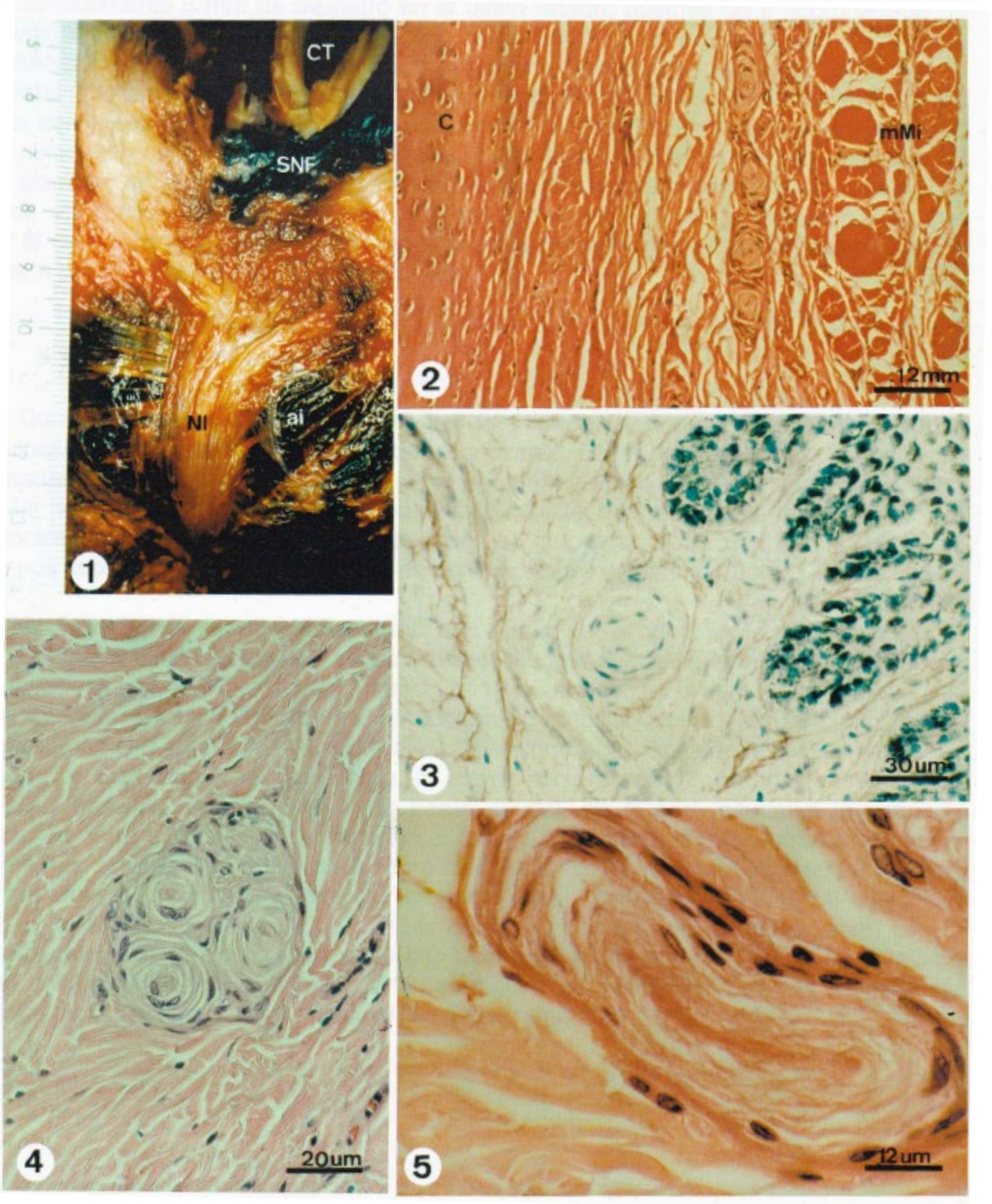

Plate 17.

Fig. 1. Lateral view on the infraorbital nerve (NI). Note how it runs over the anterointernal part of the maxilonasolabial muscle (ai) as it branches when entering the LFC. CT: slit-like opening; SNF: nasofrontal sac.

Fig. 2. Histological section of the encapsulated nerve endings (M.O.; H.E. staining). Note how they are lined up parallel to the caudal face of the cartilage of the nasal ligament.

Fig. 3. Histological section of an encapsulated nerve ending in the subepithelial tissue on the ventral side of the nasal plug (M.O.; Giemsa/Orcein acid staining).

Fig. 4. Histological section of a group of encapsulated nerve endings in connective tissue in between the intrinsic musculature of the nasofrontal sac (M.O.; H.E. staining).

Fig. 5. Histological section of a nerve ending (central) that is surrounded by cellular layers that form a capsule (M.O.; H.E. staining).

Fig. 1-5. *Stenella coeruleoalba*

PLATE 18a

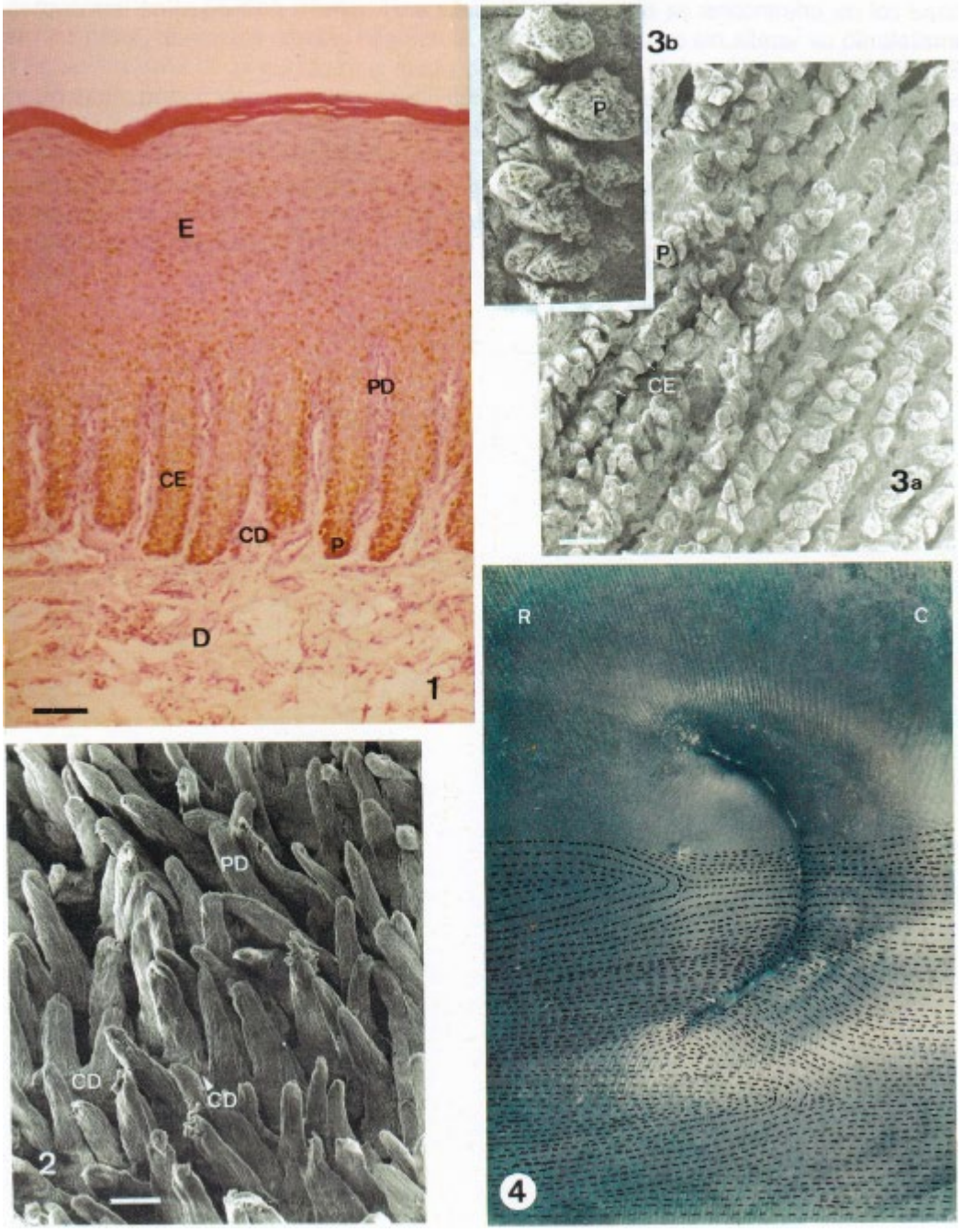

Plate 18a.

Fig. 1. Histological longitudinal section of the skin (M.O.; H.E. staining). Scale bar: 150 µm. CD: dermal ridge; CE: epidermal ridge; E: epidermis; P: epidermal protuberance; PD: dermal papilla.

Fig. 2. S.E.M. image of the dermal surface of the skin. Note the dermal ridges (CD) with large dermal papillae (PD). Scale bar: 100 µm.

Fig. 3. a) S.E.M. image of the basal epidermal surface of the skin after the epidermis and dermis were separated. Note the epidermal ridges (ER) and the epidermal protuberances (P). Scale bar: 100 µm. b) Detailed images of an epidermal ridge with the epidermal protuberances.

Fig. 4. Macroscopic image of the region of the blowhole view from dorsal. Note the cutaneous ridges at a straight angle to the longitudinal axis of the body. In the lower half of the image, the arrangement of the subjacent dermal ridges has been drawn on top of the image. (Left side of the image = rostral, R).

PLATE 18b

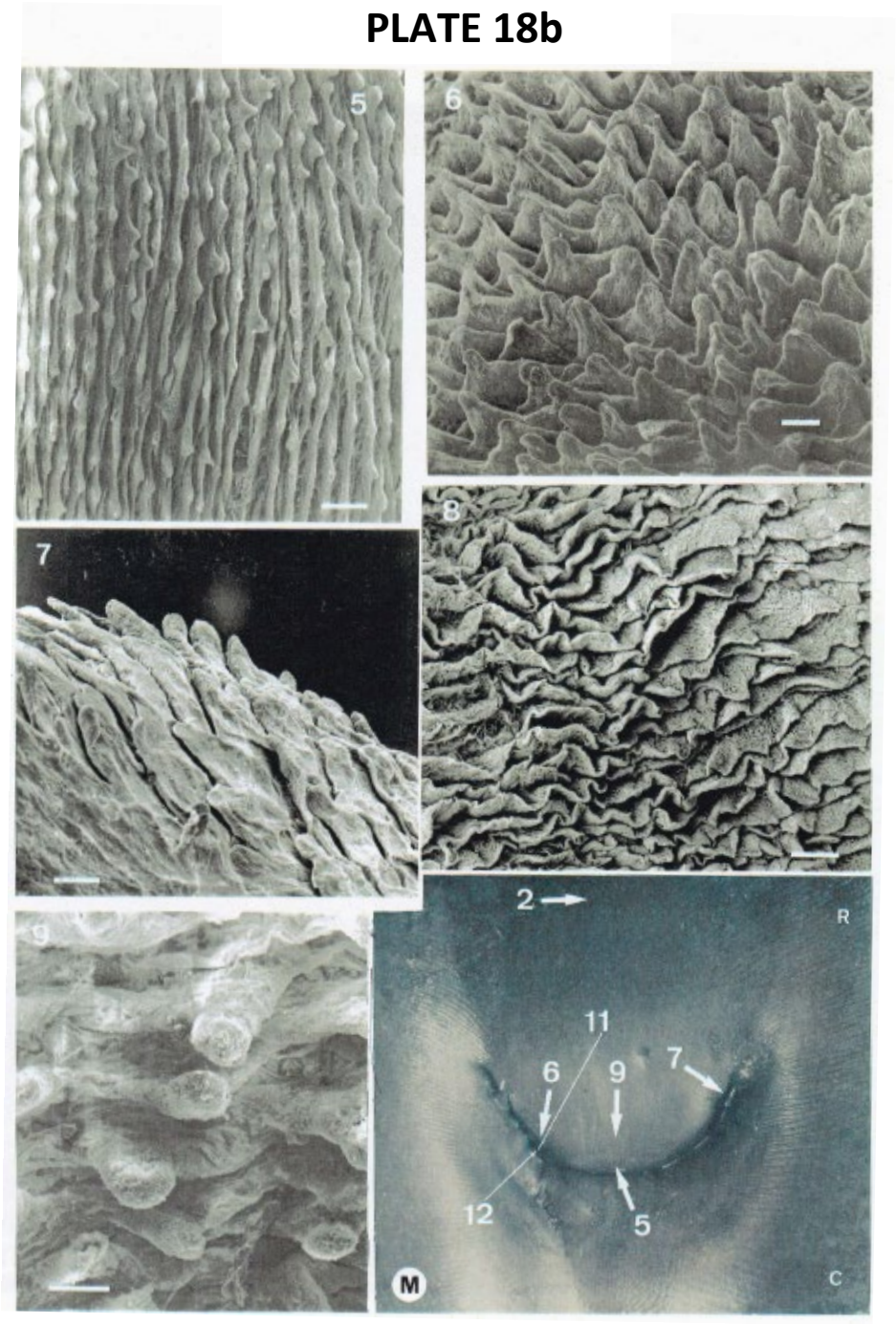

Plate 18b.

Fig. 5. S.E.M. image of the dermal surface of the rostral lip of the blowhole where it contacts the caudal lip. The dermal papillae are small. Scale bar: 200 µm.

Fig. 6. S.E.M. image of dermal surface in the centrolateral part of the rostral lip of the blowhole. The dermal structures are inclined and parallel to the surface of the skin. Scale bar: 100 µm.

Fig. 7. S.E.M. image of the dermal surface close to the right commissure of the blowhole. The dermal papillae are compressed onto each other. Scale bar: 100 µm.

Fig. 8. S.E.M. image of the dermal surface of the rostral wall of the nasal tract. The dermal ridges are arranged in an undulating manner. Scale bar: 200 µm.

Fig. 9. S.E.M. image of the dermal surface in the centre of the rostral blowhole lip. The dermal papillae are pear-shaped. Scale bar: 200 µm.

Fig. M. Macroscopic image of the blowhole, viewed from dorsal, in which the locations of the shown images are indicated.

Fig. 5-M. *Stenella coeruleoalba*

PLATE 18c

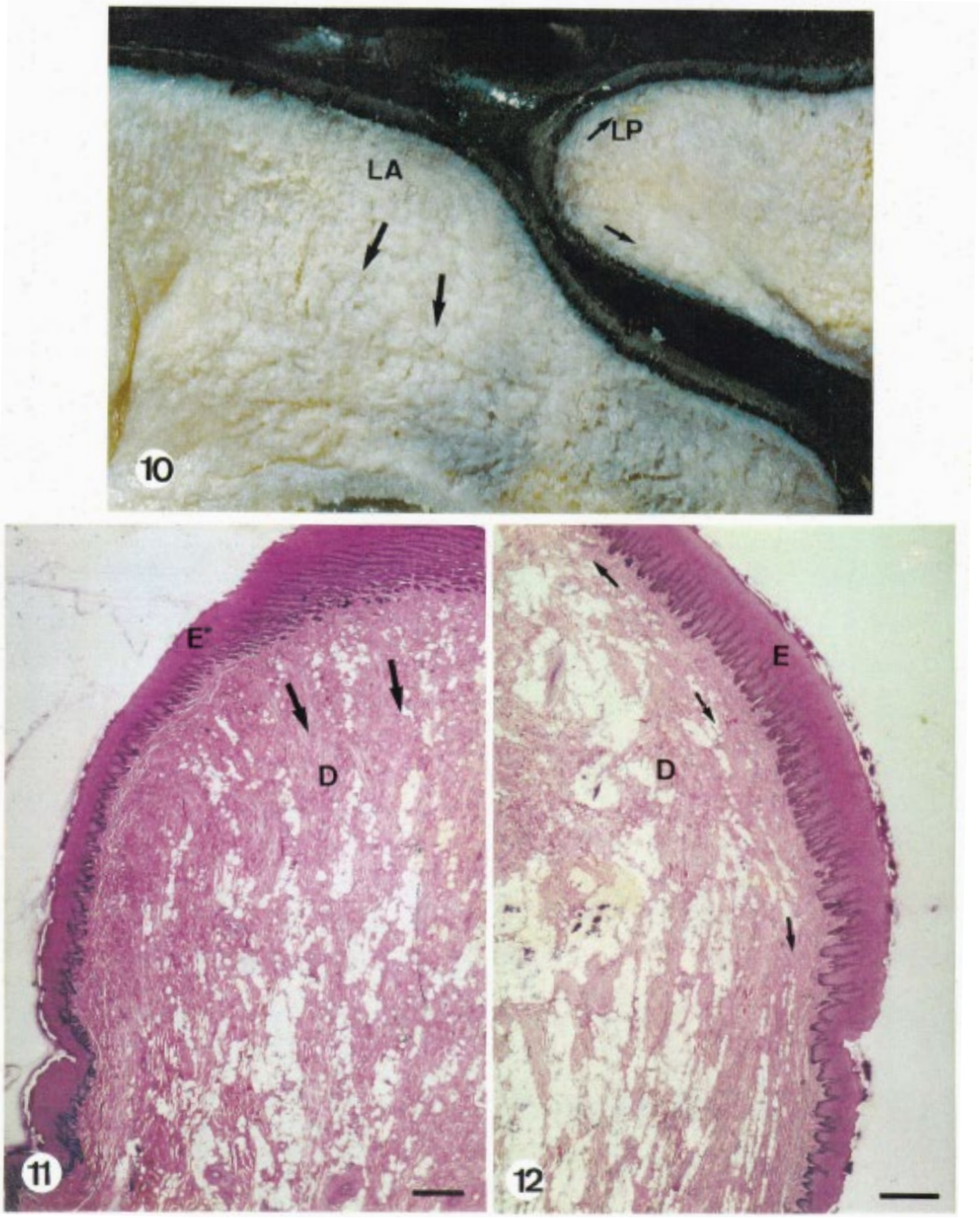

Plate 18c.

Fig. 10. Left lateral view of a median cut through the head at the level of the blowhole. Note the position of the rostral lip (LA) sliding under the caudal lip (LP) and the direction of the pulling forces that act upon this zone (arrows).

Fig. 11. Histological sagittal section of the anterior lip of the blowhole (M.O.; H.E. staining). The orientation of the muscle fibres coincides with the pulling forces (arrows) and the dermo-epidermal relation is arranged parallel to the surface of the skin and in a straight angle to the pulling forces. D: dermis; E: epidermis. Scale bar: 1 mm.

Fig. 12. Histological sagittal section of the caudal blowhole lip (M.O.; H.E. staining). Note the regular pattern of the skin and the fibres and pulling forces that run parallel to the surface of the skin. D: dermis; E: epidermis. Scale bar: 1 mm.

Fig. 10-12. *Stenella coeruleoalba.*

PLATE 19

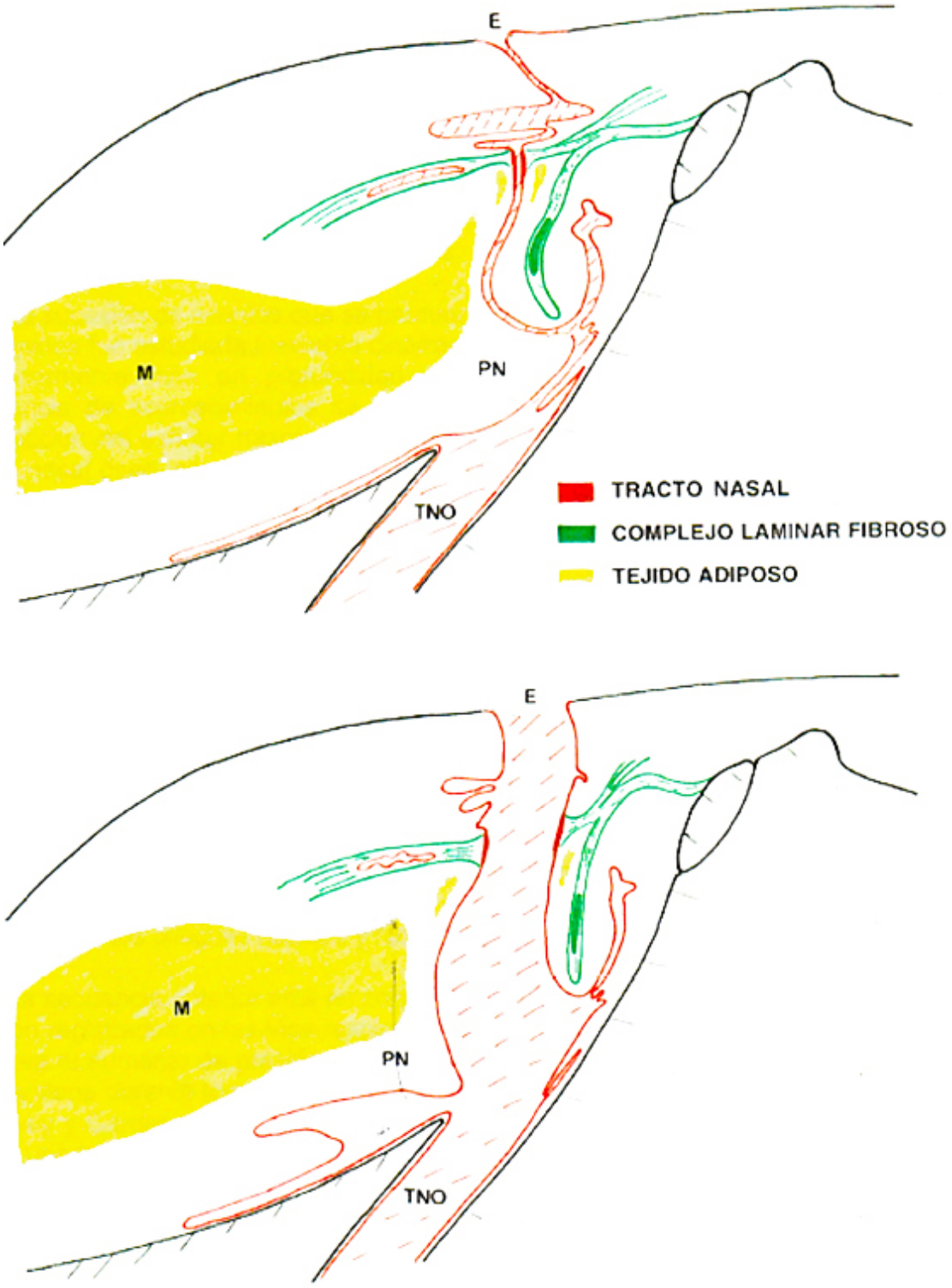

Plate 19.
Schematic drawing of a left lateral view of a parasagittal section through the nasal sac system in closed condition (above) and open condition (under).

PLATE 20

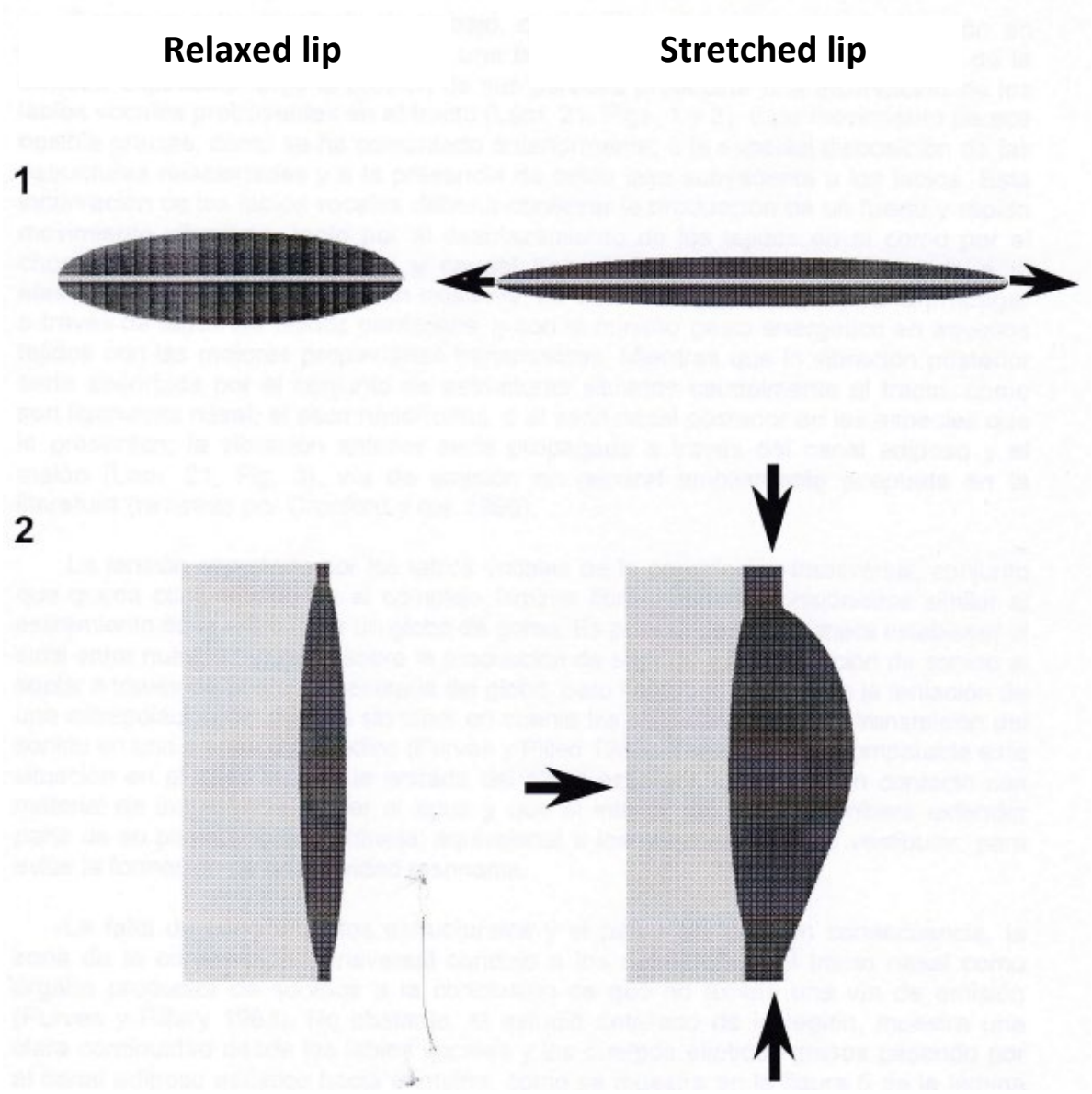

Plate 20.
Schematic representation of the modification of a phonic lip before and during there is tension on the slit-like opening. 1. View from within the lumen. The lip undergoes a transverse stretching (arrows) and this causes the disappearance of the small perpendicular folds. 2. Schematic representation of a left lateral view of a sagittal section through a caudal phonic lip. The arrows represent the exerted forces that occur during stretching of the slit-like opening as the lip protrudes into the lumen of the nasal tract.

PLATE 21

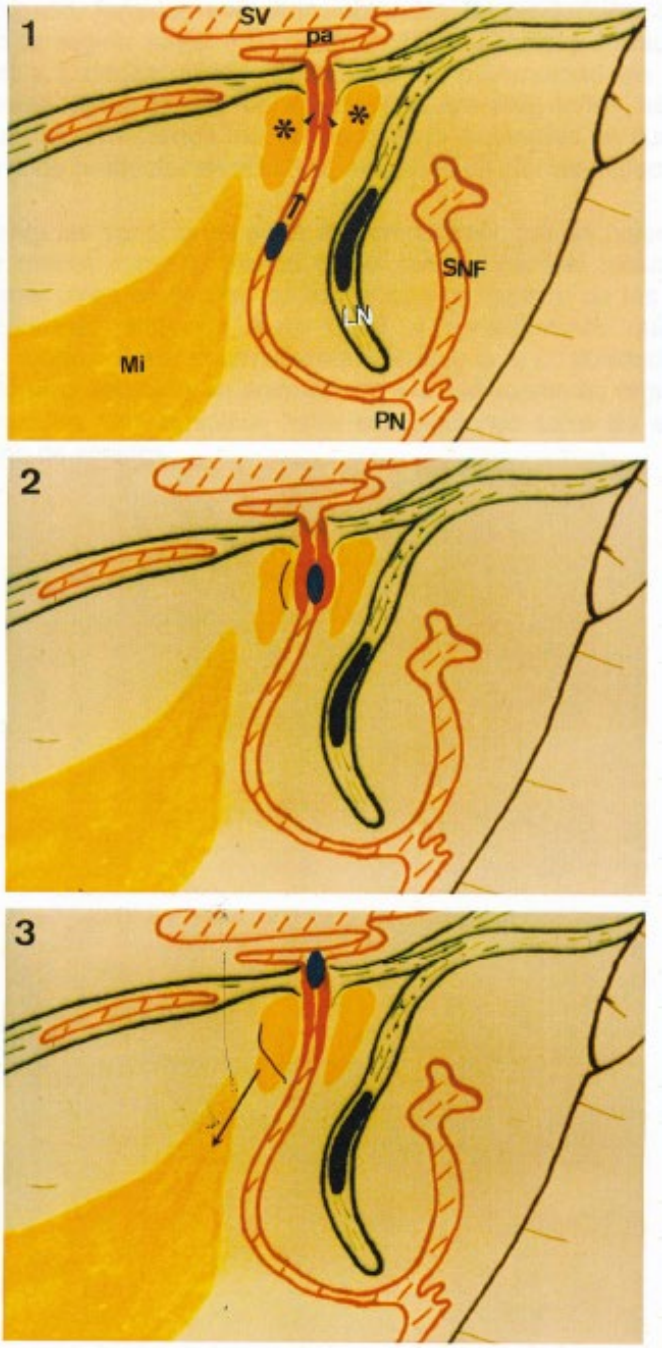

Plate 21.

Graphical representation of the hypothesis on sound production and emission in the nasal sac system of species of the Delphinoid superfamily.

Fig. 1. Air bubbles rise through the spiracular cavity under pressure of the walls.

Fig. 2. As bubbles pass between the (extremely tense) phonic lips, this causes a displacement of the tissues, which is feasible due to the elliptical adipose bodies.

Fig. 3. As the bubble surpasses the phonic lips, these regain their position with force. This collision of the phonic lips causes a vibration that is transmitted via the acoustic channel to the melon. The remaining vibrations are absorbed by the system. It is important to note that the nasal sacs would all be inflated during the process of sound production, which is not depicted in the representation.

PLATE 22

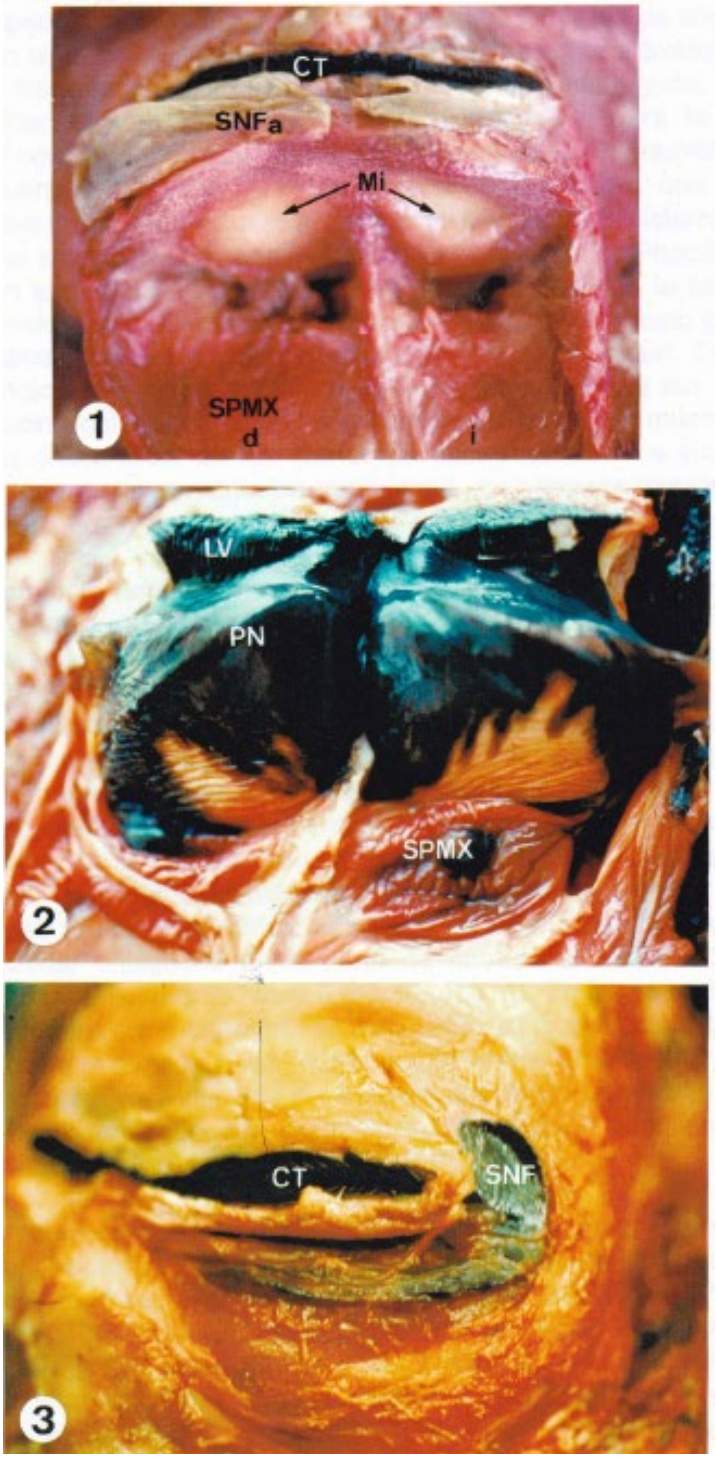

Plate 22.

Fig. 1. Dissection of the nasal sac system of a Pacific white-sided dolphin, *Lagenorhynchus obliquidens*. Rostral aspect of a transverse cut, rostral to the anterior parts of the nasofrontal sacs. There are two adipose channels as caudal projections from the melon.

Fig. 2. Caudal view of the rostral wall of the spiracular cavity with the nasal plugs displaced rostrally. The pigmentation changes abruptly and irregularly. *Lagenorhynchus obliquidens.*

Fig. 3. Laterodorsal view of a preparation of the right nasofrontal sac in a specimen of striped dolphin, *Stenella coeruleoalba*, showing an absence of the anterior part of the nasofrontal sac (SNF).

CT: slit-like opening; LV: phonic lip; Mi: melon core; PN: nasal plug; SNFA: anterior part of the nasofrontal sac; SPMX: premaxillary sac, d: right, i: left

PLATE 23

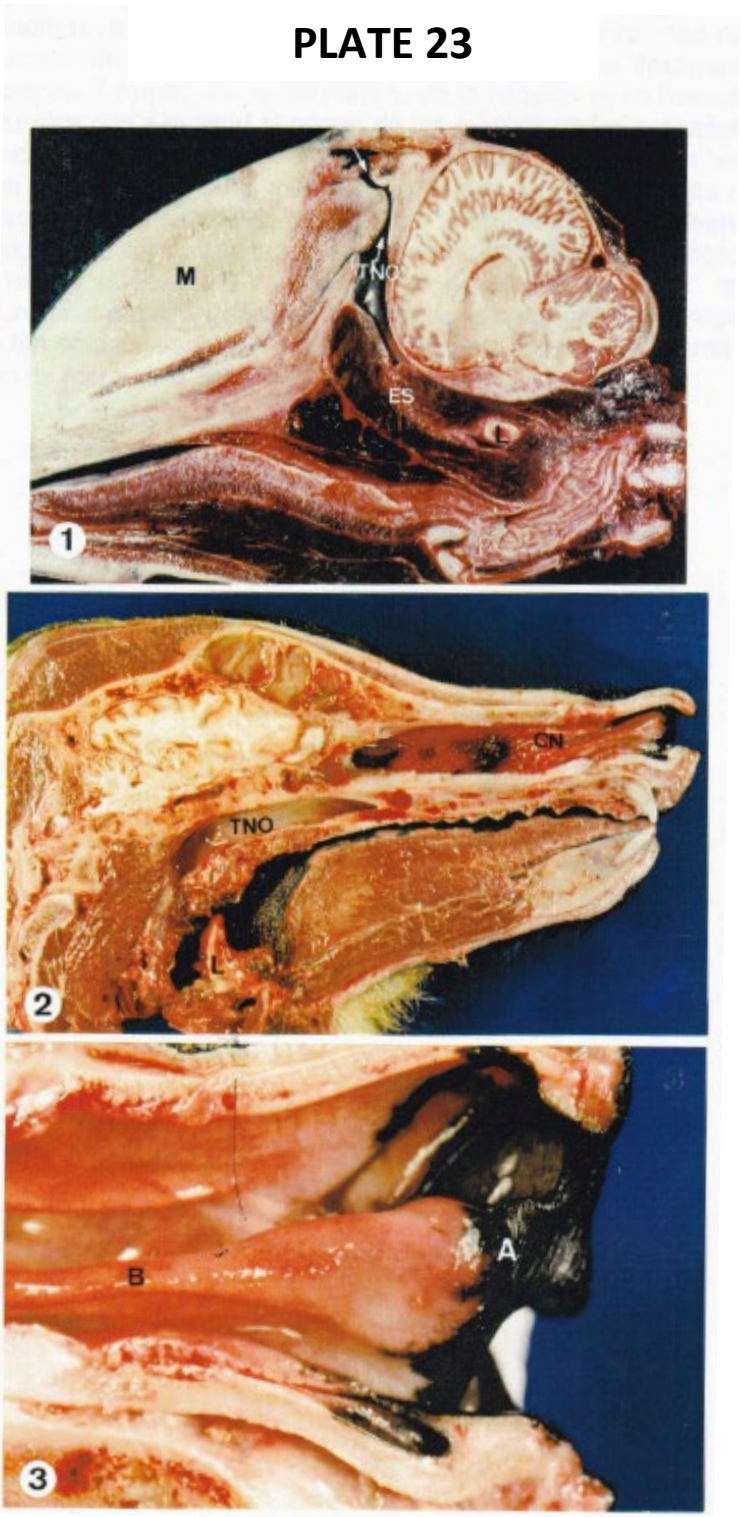

Plate 23.

Fig. 1. Paramedian section through the head of a striped dolphin (*Stenella coeruleoalba*). Note the relationship between the structures of the upper respiratory system. The arrows indicate the airflow during phonation (upward) and air recycling (descending). The laryngeal sphincter (ES) is situated between the larynx (L) and the bony nasal tract (TNO). M: melon.

Fig. 2. Paramedian section through the head of a dog. The nasal cavity (CN) presents structures that are typical for non-cetacean mammals. L: larynx.

Fig. 3. Detail of Fig. 2. Medial view of the nasal vestibule that is lined with stratified squamous epithelium (A) in contrast to the respiratory epithelium of the rest of the nasal tract (B).

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AGRAIMENTS (Acnowledgments, original version in Catalan and Spanish)

Aquesta Tesi I?0ctoral no és només el fruit d' uns quants anys d'esfor~os, dedicaci6, frustraci6 i petites
. recompenses. Es més l ' acompliment d 'un objectiu autoimposat de bon grat que ha marcat una etapa de la
. meva vida i que, sota un marc de responsabilitat, m'ha permes escollir lliurement cada una de les
_ decisioIis que m'han portat a aquest punt. Mostta d'això es aquesta Tesi, amb ella he compartit les
il.lusions i somnis que he viscut gracies a les persones que m'han envolt.at i han fet possible que jo portés
endavanpa meva "história personal". ..
Creo firmemente que en algún sitio estaba escrita mi entrada en el mundo marino y concretamente el de
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1 en la mateixa línia a totes aquelles personesil.lllrninades que vam coincidir en elinici d'aquest trajecte
atrets. per la fascinació pels dofins o que he trobat en el camí, al CRAM, o també dit Fernando, losep
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1 a tots aquell amics de sempre i més d'un de recent que han participat d'una manera o d'a1.tre en els meus
treballs i il.1usions, per ells tota la meva estima.

If you encounter difficulty,
don't change your decision to go.
Change your direction to get there.