

1. INTRODUCTION

The external ear canal in toothed whales has long been considered an evolutionary remnant that has lost its function as a sound conductor with the development of alternative acoustic pathways, such as the mandibular fat bodies (McCormick et al. 1970; Ketten 2000). Over the course of evolution, the canal has been reduced to a small string of soft tissues that are difficult to discern macroscopically. Nonetheless, microscopically, it has proven a complex structure with many active components (Yamada 1953; Reysenbach de Haan 1956; Fraser and Purves 1960). Therefore, the ear canal and its associated soft structures are bound to serve a certain function. However, up to date, such a function is still a conundrum, and even basic knowledge on the ear canal's morphology is largely incomplete.

The limited amount of morphological descriptions of the external ear canal dates mostly back to half a century ago, and exists only for a few toothed whale species (Hanke 1914; Yamada 1953; Fraser and Purves 1960; Purves 1966; Sassu and Cozzi 2007). Moreover, the results are sometimes inconsistent, associated with the limited number of specimens and/or species used, the tissue conservation state, or the thoroughness of the microscopical analyses. One of the main discrepancies concerns the continuity of the ear canal and whether or not it has an uninterrupted lumen from the external ear opening down to the tympanic membrane (e.g. Fraser & Purves, 1960; Ketten, 1997). There is also some controversy regarding the nature and conformation of glandular structures, the presence of sensory nerve formations, and the musculature that inserts into the connective tissue around the ear canal, all of which allegedly have a major influence on the function of the ear canal.

In this study, we provide a description of the common morphology of the external ear canal in toothed whales, derived from a variety of delphinid and beaked whale species. We present a generalized ear canal model that provides essential information for future morphology studies and contributes to the understanding of the function of the external ear canal in toothed whales.

2. MATERIALS AND METHODS

The external ear canals and associated soft tissues of a variety of odontocete species were assessed macroscopically (camera Sony Xperia Z5 Compact, 20 MP) and samples for histological analysis were taken during routine necropsy (see also Acknowledgements). The species included striped dolphin (*Stenella coeruleoalba*, 21 animals), bottlenose dolphin (*Tursiops truncatus*, 2 animals), common dolphin (*Delphinus delphis*, 1 animal), long-finned pilot whale (*Globicephala melas*, 1 animal), and Cuvier's beaked whale (*Ziphius cavirostris*, 1 animal), with post-mortem decomposition codes 2-3 (except for *M. melas*, which had code 4).

The ear canal samples were fixed in 10% neutral-buffered formalin, before being sliced into transverse slabs of 4-5 mm thickness, and embedded in paraffin blocks. Sections (4 μm thickness) of each block were stained according to hematoxylin-eosin (HE) and Masson's trichrome (with aniline blue) protocols. Sections for staining with HE were obtained from all slabs and dried overnight at 70°C, followed by automated staining using a Leica Autostainer XL (Leica Biosystems Nussloch GmbH). Slides for Masson's Trichrome staining were dried similarly, and staining was done manually. Slides were coverslipped using a mixture of Eukitt® (ORSAtec GmbH) and xylene. All slides were examined with an Olympus BX41 microscope (Olympus Italia S.r.l., Milan, Italy) at up to x600 magnification.

3. RESULTS

A. MACROSCOPIC COURSE

All species presented a very small external ear opening situated several centimeters caudoventral to the eye. From there, the ear canal travelled medially through the blubber layer in a horizontal course, slightly in caudoventral direction. The canal was visible as a minute, pigmented string, travelling through a mix of adipose and connective tissue into which various striated muscles inserted. It then curved and descended in anteroventral direction before turning back horizontal, entering the paraotic cavity in the caudodorsal margin of the trench created by the caudal ramus of the mandible rostrally, the retroarticular (postglenoid) and retrotympanic process of the squamosal dorsally, and the exoccipital bone caudally, before arriving at the lower tympanic aperture of the tympanic bone (Figure 1).

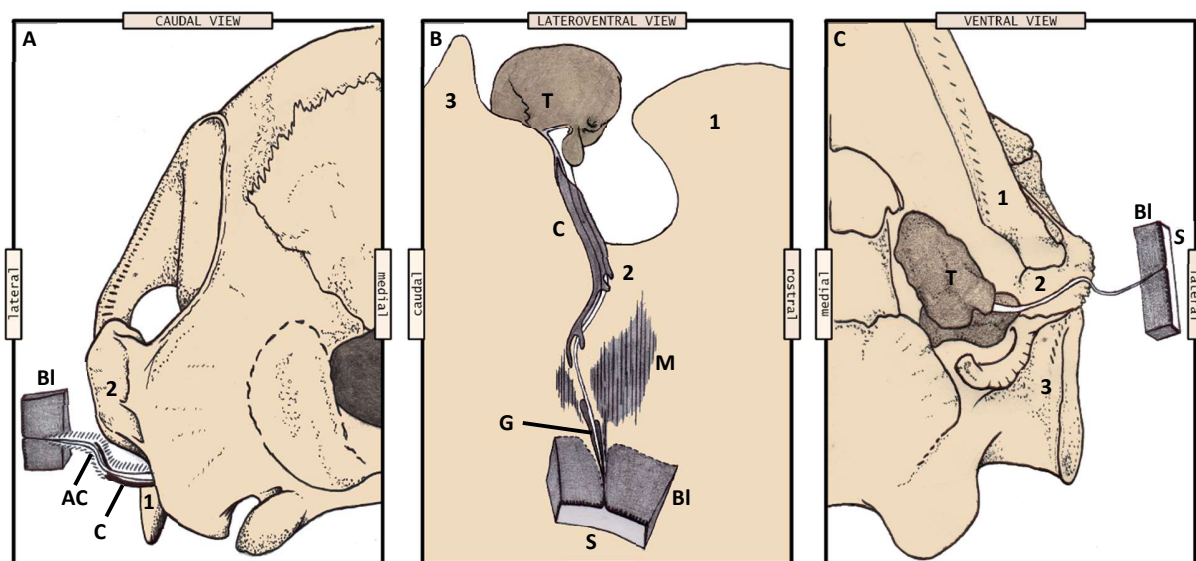


Figure 1. Drawings of the macroscopic course of the external ear canal in a striped dolphin from three different points of view: caudal, lateroventral and ventral. The drawings display the external ear opening in the skin (S) and the underlying blubber layer (Bl), and demonstrate the sigmoid course of the external ear canal (white), which is accompanied by an adipoconnective tissue capsule (AC, in A), local glands (G, in B) and musculature (M, in B), and a cartilage (C, in A & B), before reaching the tympanic bone (T, in B & C). 1: Mandible; 2: squamosal bone; 3: exoccipital bone.

B. MICROSCOPIC COURSE

The external ear canal varied in shape and size over the course of the canal from the external ear opening to the tympanic membrane (Figure 2). The ear opening was presented as a concavity in the skin that progressed through the blubber as a very narrow canal with a lumen that was either absent due to the collapse of opposing epithelial walls, was filled with desquamated epithelial cells and glandular product, or was presented with a minute diameter of a few tens of microns. In deeper sections, the canal opened up, and had a complex shape that extended into the excretory ducts of the neighboring mucinous glandular structures, which were situated in the adipoconnective tissue around the ear canal. From about halfway the course of the canal, medial to the glandular structures, at the level of the ventral curvature, the lumen opened up more, took on an oval to round shape, and had a gradually increasing diameter before arriving at the tympanic membrane.

The ear canal was lined by a stratified squamous epithelium, a continuation of the skin, which diminished in number of layers as the canal progressed deeper. It contained melanin, although not constantly present throughout the entire course.

The epithelium was encompassed by a relatively thin lamina propria with which it showed an intricate interaction, particularly in the superficial half of the canal. This layer was intensely vascularized, and presented a prominent innervation with the presence of sensory nerve formations, simple lamellar corpuscles, which were present throughout the entire course of the canal. The corpuscles were distributed around the ear canal in the superficial half down to the ventral curvature, while they appeared concentrated into a tissue bulge on one side of the canal in the inner half of the ear canal (Figure 3).

Surrounding the canal, there was an adipoconnective tissue capsule, which was present throughout the entire course although with varying ratios of fat and connective tissue. From superficial to deep, the adipose tissue of this capsule was gradually replaced by connective tissue until it was almost completely absent in the deepest sections. Various striped muscles inserted into the adipoconnective tissue, at least in the superficial two thirds of the canal, and were especially prominent at the level of the ventral curvature and the lateral projections of the cartilage.

The cartilage supported the ear canal from ventral and progressively enveloped the canal in deeper sections, although never fully closing. The cartilage ends at the level where the ear canal enters between the tympanic and petiotic bone, and the canal curves around the sigmoid process and ends as a blind sac at the tympanic membrane.

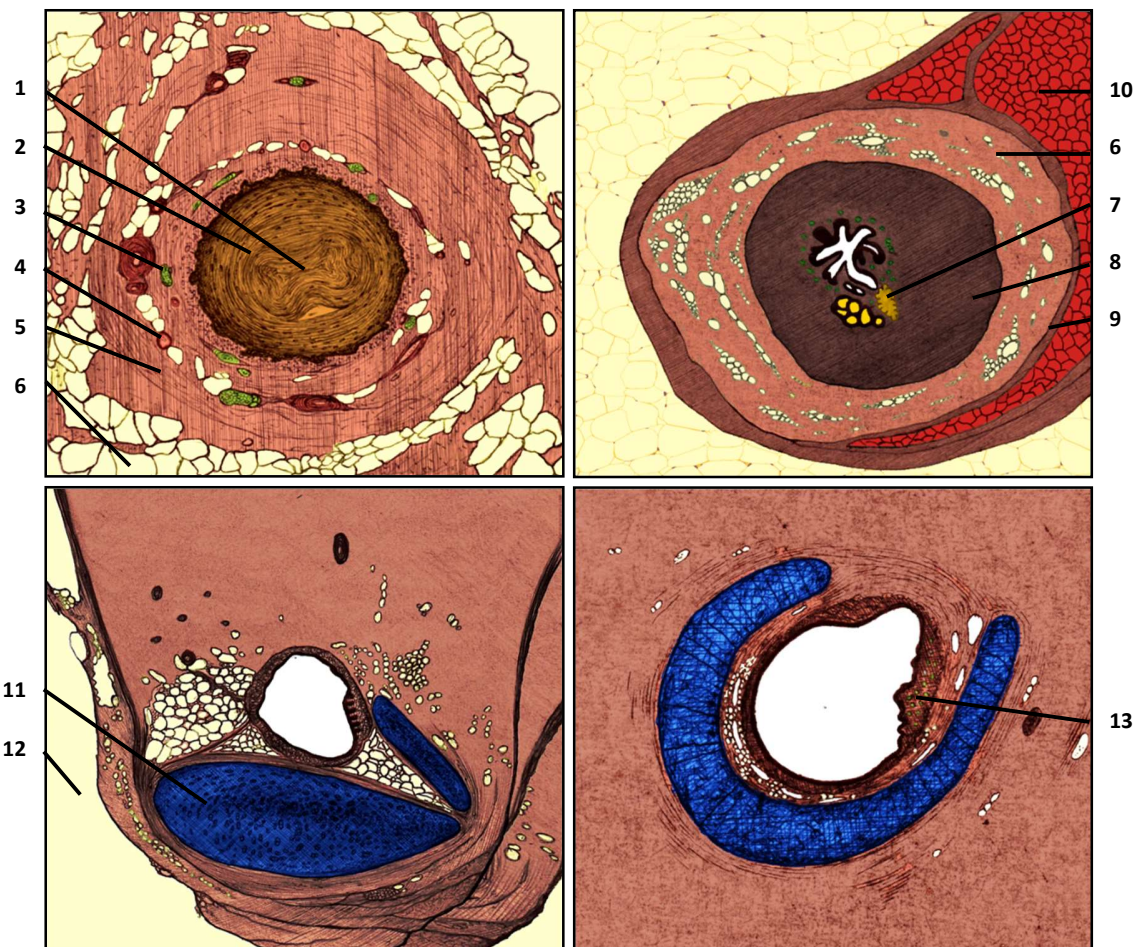


Figure 2. Schemato-artistic drawings of transverse sections through the external ear canal in four distinct regions in a generalized toothed whale, made to demonstrate the configuration of the canal lumen and the surrounding the soft tissues.

A. Section through the ear canal immediately medial to the external ear opening. The lumen (1) is collapsed and there is only a minute or even no physical connection between the external environment and the deeper ear canal lumen. The epithelium (2) is consists of many layers at this level. The subepithelial tissue contains many nervous structures (3), including lamellar corpuscles and small nerves, and vascular structures (4). The connective tissue is infiltrated by fat cells and forms an adipoconnective tissue sheath (5), which separates the ear canal from the surrounding blubber layer (6).

B. Section through the ear canal at the level of the glands. There is a true lumen with a complex shape that is connected to the surrounding glandular structures (7), situated in the subepithelial tissue, together with nervous and vascular structures (similar to A). The connective tissue in vicinity of the ear canal contains fewer fat cells (8). Surrounding this, although not always clearly distinguishable from the previous, there is the adipoconnective tissue sheath (6), which itself is surrounding by a fibro-elastic tissue capsule (9) in which striated muscles (10) insert.

C. Section through the ear canal proximal to the ventral curvature. The lumen widens and is oval to round in shape. The epithelium consists of only few layers and the subepithelial tissue containing nervous and vascular structures is sparse and gradually concentrates on one side of the canal. The ear cartilage (11) appears and consists of a main body with several 'fingers' in various configurations. The adipoconnective tissue sheath consist of a large amount of fat cells in the vicinity of the canal and dense connective tissue further peripheral. The entire structure is suspended from the skull dorsally and separated from acoustic fat (12) ventrally.

D. Section through the ear canal at the proximal end before reaching the tympanic membrane. At this level the lumen is round and large, the epithelium is thin and so is the subepithelial tissue layer, except for the location of the sensory ridge (13) (See Figure 3). The cartilage (10) is horseshoe-shaped and envelops the ear canal in an incomplete manner.

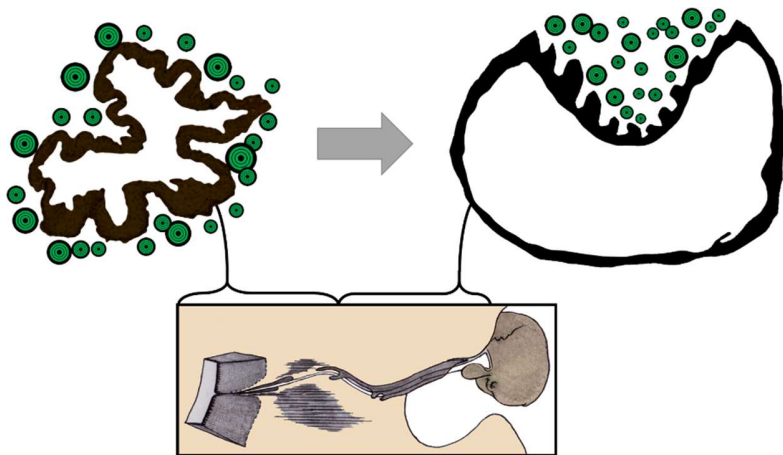


Figure 3. Schematic representation of the distribution of the lamellar corpuscles (green) associated with the external ear canal. In the superficial half of the canal (top left), the corpuscles are distributed in the subepithelial tissue around the ear canal, while in the proximal half of the ear canal (right), the corpuscles are situated on one side of the canal, in the subepithelial tissue that bulges together with the epithelium (black) into the lumen of the canal, and forms what we have a called a 'sensory ridge'.

4. DISCUSSION

This paper provides an overview of the morphological features of the external ear canal in toothed whales, and works through some of the long-standing ambiguities, such as the continuity of the ear canal, and its association with the tympanic membrane. As such, the canal presents a continuation between the exterior medium and the lumen of the ear canal, different from the situation in whalebone whales where there is a complete closure of the canal with the consequential presence of an ear plug. The ear plug is absent in toothed whales, but the presence of the glandular structures and the function of their product is not yet fully understood. Also, the various conformations in which the ear canal was presented between the exterior ear opening and the level of the glandular structures, could indicate either individual differences that share a common functionality, or they could be physiological variations of the same structure that adapt to different circumstances or physiological states. This might explain the differences reported in the literature. Similarly, the functional relations with the adipoconnective tissue, the musculature, the cartilage, and the tympanic membrane, are not yet fully understood, and further morphological studies would be needed to clarify those further, and clear up the associations with the obscure function of the external ear canal.

5. CONCLUSION

This paper highlights the characteristics of the ear canal that are common among toothed whales, and opens up perspectives for further research on its functional anatomy. Future comparative studies are essential to get a better understanding of the species-specific morphology and the function of the external ear canal in toothed whales.

6. ACKNOWLEDGEMENTS

The authors would like to acknowledge Prof. dr. Mariano Domingo (Department of Health and Anatomy, Faculty of Veterinary Medicine, Autonomous University of Barcelona, Bellaterra), and the Italian Zooprofilactic Institutes of Venezia, Lazio e Toscana, Liguria and Sardinia for collaboration in gathering samples. We would also like to thank Drs. Davide Trez, Enrico Gallo, Rosella Zanetti, Giuseppe Palmisano, Michele Povinelli, and Letizia Moro (Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro) for technical support.

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