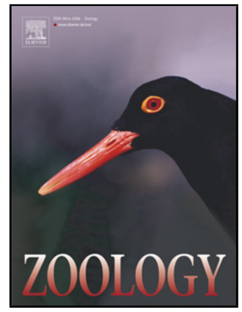

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Adaptations to a semiaquatic lifestyle in the external ear of southern pinnipeds (Otariidae and Phocidae, Carnivora): Morphological evidences

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Highlights

- Otariidae showed similar features along the entire extension of the cartilaginous OEC.
- The cartilaginous OEC in Phocidae showed three regions (distal, middle, proximal).
- The cartilage histology is different from the analyzed otariids.
- The cartilage of most phocids resembles a special elastic cartilage with myxoid tissue.
- *Mirounga leonina* OEC showed a composite of features similar to phocids and otariids.

Abstract

Pinnipeds are semiaquatic carnivorans that spend most of their lives in water and use coastal terrestrial, or ice pack, environments to breed, molt and rest. Certain characteristics of the ear have been linked to ecological aspects. In our contribution we focus on the study of the macroscopic and microscopic morphology of the external ear (with the exception of the osseous outer ear canal) of six species of Southern pinnipeds. In order to recognize the different components of tissues, sections were stained following several routine protocols. In addition, double-staining and enzymatic clearing (Alcian blue-alizarin red) was performed to assess the arrangement of skeletal elements in the OEC.The basic structure of the pinna in the southern otariids studied match those previously analyzed for Northern Hemisphere species. The cartilage macro anatomy of the OEC of *Mirounga leonina* and *Arctocephallus gazella* is different from that of the Northern Hemisphere species, with only one plate of cartilage, but markedly different between them. The histology of the otariids OEC is homogeneous along the entire extension, but phocids has three different regions (distal, middle, and proximal). The cartilage histology of most phocids is also different from that of analyzed otariids, with an elastic cartilage that resembles a myxoid-like tissue, but is not present in *M. leonina*, were the tissue around the OEC is very rich in adipocytes. The southern elephant seal *M. leonina* OEC has a combination of features similar to both the rest of the phocids and to the otariids. An auditory organ that is functional both over and under water could be essential for social behavior in these species.

Keywords: southern seals, fur seals, myxoid tissue, external ear, histology

1. Introduction

The external ear of mammals is formed by the pinna and the outer ear canal, which ends internally at the tympanic membrane. Its main function is receiving external sound and conveying it to the middle ear cavity (Cole, 2009).

The outer ear canal (OEC) is divided into a distal cartilaginous region and a proximal bony region (Nómina Anatómica Veterinaria, 2012). Internally, the canal is covered with skin, hair follicles, and sebaceous and ceruminous glands; the mix of both glandular secretions, and the cells that flake off from the skin, form the ear wax that acts as an internal protective coat. The morphology of the pinna is variable across mammals, and much of this variability is due to different life habits.

Pinnipeds are semiaquatic carnivorans that spend most of their lives in water, and use coastal terrestrial environments and ice packs to breed, molt and rest. They are currently classified into three families: Otariidae (fur seals and sea lions), Phocidae (seals and elephant seals), and Odobenidae (walruses). They probably evolved within group of arctoid carnivores in the Pacific Ocean, 27 million years ago (Berta and Adam, 2001). Although their origin is a matter of discussion, they are currently considered a monophyletic group, and the divergence between phocids and otariids was probably an early event within the history of the clade (Berta, 2009).

Because of the semiaquatic habits of these species, the ears are expected to be functional in two completely different media: on land, with sounds in a wide range of frequencies, and in water, with low-frequency sounds and a speed of sound nearly five times higher than in air (Hemilä et al. 2006, Nummela et al., 2007). Many species of pinnipeds often exhibit a high degree of sociability, mainly during the reproductive season, and sound communication between congeners seems crucial to establish either harems, or the mother/pup bond. Moreover, several species of pinnipeds vocalize underwater and elicit a behavioral response from their congeners, so we can assume that they do hear underwater. Examples of the last statement are mentioned to: among phocids,*Pusa hispida* (see Stirling, 1973), *Pagophilusgroenlandicus* (see Mohl et al., 1975), *Erignathus barbatus* (see Ray et al., 1969), *Leptonychotesweddellii* (see Thomas &Kuechle, 1982, Terhune 2017), *Hydrurgaleptonyx*, and *Lobodoncarcinophaga* (see Stirling and Sinnif, 1979, Rogers et al.1996, Rogers 2007,2017); and also for the only extant odobenid *Odobenus rosmarus* (see Schevill et al., 1966).In contrast, most members of Otariidae, and some phocids such as the elephant seal (Insley et al., 2003; Sanvito et al., 2007, 2008; Negrete et al., 2011), communicate more frequently through vocalizations when on land. In this regard, hearing is fundamental in these species both on land and in water. Nevertheless, the mechanisms for pinniped hearing underwater are still not fully understood (Hemilä et al., 2006; Nummela and Yamato, 2018).

One of the main differences between families is the degree of reduction of the pinna, which is reduced but still present in otariids and completely absent in phocids and odobenids. Phocids inhabits the Southern Hemisphere since at least the middle Miocene thus, a long period of evolutionary time separates southern species from those from the Northern Hemisphere, and this might have given rise to some morphological differences in the external ear. In turn, otariids reached the austral hemisphere during early Pliocene (see Berta et al., 2018).

Descriptive and comparative studies have been performed on the macroscopic morphology and histology of the pinna and OEC of several species of Northern Hemisphere pinnipeds (Böas, 1912; Huber, 1934; King, 1964; Ramprashad et al., 1971; Ramprashad, 1975; Ramprashad and Ronald, 1977; Solntseva, 2016). On the basis of comparative studies, certain characteristics of the ear have been linked to ecological aspects (Solntseva, 2007, 2016). Nevertheless, regarding Southern Hemisphere species, only *Leptonychotes weddellii*has been briefly described in this aspect,within the context of a histophysiological description of the entire ear (Welsch and Riedelsheimer, 1997).

In the present contribution, we studied the following Antarctic phocid species: *Leptonychoteswedde llii*(Weddell seal), *Lobodoncarcinophaga* (crabeater seal), and *Hydrurgaleptonyx* (leopard seal). Likewise, other two species were included whose distribution in the Southern Hemisphere includessubantarctic and antarctic islands: the otariid*Arctocephalusgazella* (Antarctic fur seal) and other phocid*Miroungaleonina* (southern elephant seal). The latter also breed in the American continent, at Península Valdés, Chubut, Argentina. In addition, we included *Arctocephalusaustralis* (South American fur seal), which is mainly distributed on the western South Atlantic coasts and islands, as well as in the eastern coasts and islands of the south Pacific (Crespo et al. 2015, Rodrigues et al. 2018).

Here we focus on the study of the macroscopic and microscopic morphology of the external ear (with the exception of the most internal osseous part) of several species of southern pinnipeds, in order to evaluate if differences between taxa are linked to ecological, biological, or behavioral parameters, and compare the results to Northern Hemisphere species.

2.Material and Methods

Heads of specimens were collected from freshly dead individuals found along the coast, which was regularly prospected (every one or two days approximately). Materialswere collected by the researchers of the Marine Mammals Laboratory of Instituto Antártico Argentino from different areas of the Argentine Antarctic sector, surveyed during five consecutive Antarctic field seasons between 2011 and 2015; finally, a specimen of *A. gazella* was donated from La Plata Zoo (Table 1).

A total of ten heads were fixed in 10%formaldehyde in seawater (to maintain osmolarity of soft tissues). The pinna and the non-osseous portion of the OEC were dissected. The osseous part of the OEC was not included in the study becauseit is strongly fused to the cranium and its study implies a series of techniques beyond the scope of this work. Relative age of all specimens was estimated based on characteristics of the fur, external morphology, teeth, and body size.

2.1 Macroscopic morphology, and double-staining and clearing technique

The pinna and OEC were studied under a binocular microscope Nikon SMZ645, and characters related to shape, hair density, and relative size of the canal were recorded. In order to assess the arrangement of the skeletal elements forming the OEC, a double-staining and enzymatic clearing technique was performed on one specimen of *A. gazella* and one *M. leonina*. The *A. gazella* specimen is a late fetus, while the*M. leonina*specimen is a lactating pup. The double-staining and clearing protocol was performed according to Prochel (2006), for which the soft tissues surrounding skeletal structures are cleared enzymatically (using trypsin) after the skeleton is double-stained using Alcian blue for cartilage and alizarin red for bone.

2.2 Routine techniques for histological analysis

During dissections, and after the macroscopic analysis, the external ear was extracted and processed for histological analysis. Samples were dehydrated using increasing concentrations of ethanol from 70% to 100% and embedded in paraffin using xylol as intermediate solvent. Histological sections were 5 µm to 8 µm thick (depending on tissue hardness). In order to recognize the different components of the tissues, the sections were stained following several routine protocols: Hematoxylin and Eosine (H-E), for general analysis; orcein, for determination of elastic fibers; reticulin, for reticular fibers;Gomoritrichrome, to differentiate collagen fibers from smooth muscle fibers; Periodic Acid-Schiff (PAS), for carbohydrates; Giemsa, to determine metachromasia; Alcian blue at pH 0.4, 1, and 2.5, to determine glycoconjugate components of the extracellular matrix.

Two variables were measured at the proximal and distal sections of the OEC for each genus. Total blood vessel count and total surface area were quantified from digital photographs to compare the degree of vascularization between the pinniped genera studied. The calibrated photographs were acquired with a Leica ICC50HD camera in a Leica microscope (DM-500) connected to a personal computer using LAS EZ software. 4X magnification was used for capturing individual images, and high-resolution composite images of the entire section of each OEC were generated. The blood vessel count and surface area were measured on the connective tissue adjacent to the epithelium surrounding the OEC using the software ImageJ.

3.Results

3.1 Macroscopic morphology

3.1.1 The pinna in studied pinnipeds

The pinna is present just in Otariidae, but reduced in relation to most terrestrial mammals; no one phocid is known having pinna. Both in *A. gazella* and *A. australis*, the pinna is long and sharpened distally, and folded showing an anterior concave surface, with sparse hair, and a convex posterior surface with abundant hair.

3.1.2 The outer ear canal (OEC) in otariids and phocids

The OEC in otariids show the same characteristics along the entire extension regarding luminal diameter, presence and thickness of cartilage, and absence of hair. Clearing after double-staining in *A. gazella* showed that the OEC is formed by an auricular cartilage plate that surrounds the canal partially, approximately half of the canal, but without cartilage in the portion of the wall that faces medially. Furthermore, the plate is incompletely segmented at regular intervals along the OEC (Fig. 1A). Unlike the case of otariid species, three regions are recognizable along the OEC of phocids, from outer to inner: distal, middle, and proximal regions.

The distal region lacks cartilage and presents a small lumen. The middle region is characterized by presence of cartilage and a wider lumen than the distal region. The proximal region contacts the osseous part of the OEC, its lumen is wider than in the distal and middle regions, it presents cartilage thicker than in the middle region, scarce hairs, and great muscular development as thick muscle bundles.

In the *M. leonina* specimen that was cleared after double-stained, the OEC shows an irregular cartilage plate arranged in a helicoidal path that partially surrounds the canal; different parts of the same cartilage plate are visible in both external and medial views (Fig.1 B).

3.2 Histological morphology

3.2.1 Otariidae

3.2.1.1 The pinna in *Arctocephalus gazella* and *A. australis*

In both species, the posterior surface of the pinna presents an epidermis with stratum basale, stratum spinosum (two or three cell layers thick), stratum granulosum, and stratum corneum (Fig. 2A, B, C). On the anterior surface the epidermis is thinner, with a stratum basale, stratum spinosum (one or two cell layers thick), and a poorly developed stratum corneum (Fig. 2A, D).

The superficial dermis both in the anterior and posterior surface has greater cell density, with collagen fibers arranged in different directions, while the deep dermis has less cell density, with thicker collagen bundles arranged parallel to the epidermis (Fig. 2C, D).

The hair follicles on the anterior surface are scarce and simple with few sebaceous glands (Fig. 3D), while the hair follicles on the posterior surface are abundant and grouped into sets arranged as one primary follicle, one secondary, and at least eight small follicles. Sebaceous glands are observed associated with the hair follicles in both species (Fig. 2E). Ceruminous glands (sweat apocrine glands) are present in *A. australis*, while in *A. gazella* these glands are absent. A central core of horseshoe-shaped elastic cartilage supports the pinna (Fig. 2 A, D, F).

3.2.1.2 The OEC in *Arctocephalusgazella* and *A. australis*

Both species showed similar features. The histological morphology of the OEC is similar along its entire extension, and has a wide lumen, oval-shaped in cross section in *A. australis*, and subcircular in *A. gazella* (Fig. 3A). The epidermis in both species is very thin and shows a stratum basale, stratum spinosum (two to three cell layers thick), and a poorly developed stratum corneum (Fig. 3, B, C).

The superficial dermis shows collagen fibers extended in diverse directions. The deep dermis, with thicker bundles of collagen fibers parallel to the epidermis (Fig. 3B), shows scarce hair follicles, and some of them are complex (formed by a main hair follicle, and 8 to 10 secondary ones) and associated to sebaceous glands (Fig. 3A, B, F). The ceruminous glands in *A. gazella* are scarce and associated to hair follicles (Fig. 3D), while in *A. australis* are more abundant and highly rolled (Fig. 3C).

Under the dermis of both species, the elastic cartilage plate is horseshoe-shaped in cross section and slightly thicker proximally. The plate is associated to striated muscle bundles that surround it (Fig. 3E).

3.2.2 The OEC in Phocidae

Macroscopic study of the cartilaginous part of the OEC allowed identification of three regions (distal, middle, and proximal), which will be described separately in the histological analysis of each species.

3.2.2.1 *Mirounga leonina*, In the distal region, the epidermis is highly folded, with several melanin granules, and shows a stratum basale, stratum spinosum (five to six cell layers thick), and a well-developed stratum corneum with pyknotic nuclei (Fig. 4A-C). The superficial dermis has collagen fibers arranged in different directions. The deep dermis shows collagen fibers arranged in thicker bundles, and abundant muscle fibers and groups of white adipocytes (Fig. 4A, B). Ceruminous glands are scarce (Fig. 4F).

The middle region of the OEC shows an almost collapsed lumen (Fig. 5A). The cornified epidermis is slightly thinner than in the previous region, less folded, and with a less developed stratum corneum (Fig. 5B). The dermis in this region is thinner when compared to the distal region. The deep dermis shows abundant white adipose tissue (Fig. 5B).The fibers become thicker towards the deep dermis. There are fewer simple hair follicles than in the distal region, and ceruminous glands are absent (Fig. 5B).The hypodermis shows abundant white adipose tissue and the cartilage is immersed within this layer (Fig. 5A). The elastic cartilage (Fig. 5C) surrounds the lumen incompletely and is attached to muscle bundles (Fig. 5A).

The lumen of the proximal region is wider than in the middle region (Fig. 5D).The epidermis is thinner, less folded, and less cornified than in the previous regions. The dermis shows abundant adipose tissue and an arrangement of fibers similar to that of the previous region (Fig. 5E). Furthermore, this region shows fewer simple hair follicles, the sebaceous glands that end within them are more numerous and larger (Fig. 5E). The hypodermis is similar to that in the middle region, with an incomplete elastic cartilage immersed in abundant adipose tissue (Fig. 5D, E, F).In addition, ceruminous glands are present, although very scarce, and abundant nerves and muscle fibers are present and irregularly arranged.

3.2.2.2 *Leptonychotes weddellii*.

In the distal region, the lumen is similar to that of *M. leonina* (Fig. 6A). The epidermis is also similar, but slightly thinner and with a less developed stratum corneum (Fig. 6B).In the dermis, the deep stratum presents less adipose tissue and the elastic bundles are thicker (Fig. 6A, C, D). The hair follicles are compound, formed by a primary follicle and at least three or four secondary follicles; towards the lumen the hair follicles share the pilary canal (Fig. 6A, C). Each group of hair follicles is associated to sebaceous glands, which are less developed than *M. leonina* (Fig. 6C), and to ceruminous glands (Fig. 6D).

The middle region shows a lumen similar to that of *M. leonina* (Fig. 7A, B). The dermis is thinner than in the distal region (Fig. 7B). The hair follicles are arranged in groups similar to those of the distal region, although fewer (Fig. 7B). The sebaceous glands are more developed than in the distal region and ceruminous glands are scarcer (Fig. 7B). Underlying the dermis, the hypodermis is well developed in the free-cartilage zones, while in zones with cartilage the hypodermis lies externally to it. The cartilage (Fig. 7C) in this region shows some special features: in some parts it is typical elastic cartilage, while in other parts the cells appear less ordered, and resemble myxoid tissue. In the latter portion, the matrix is positive for Alcian 2.5 and Giemsa, and shows scarce thin reticular fibers. In addition, unilocular adipocytes are present, either isolated or in small groups within the cartilage. The cartilage is more developed than in *M. leonina*.

The lumen in the proximal region is not collapsed (Fig. 7D). The epidermis is thin, similar to that of the middle region. The dermis is more vascularized than in the other regions (Fig. 7E). There are fewer hair follicles than in the previous regions, the sebaceous glands are well developed (Fig. 7E), and ceruminous glands are also observed. The elastic cartilage shows myxoid-like tissue, similar to the middle region (Fig. 7F).

3.2.2.3. *Lobodon carcinophaga*

In the distal region, the skin is similar to that of *L. weddellii* (Fig. 8A, E) with some differences. The dermis shows fewer muscle fibers (Fig. 8F). The hair follicles are compound although their arrangement is different: the set consists of one primary and two secondary follicles (Fig. 8D). Sebaceous and ceruminous glands are poorly developed (Fig. 8D-F). The hypodermis is similar to that of the previously described species (Fig. 8B).

In the middle region, the OEC lumen is wider than that of previously described species (Fig. 9A), and the epidermis is thinner than in the distal region (Fig. 9B). The dermis is similar to that of *L. weddellii* and *M. leonina*. The cartilage is similar to that of *L. weddellii*, and the muscle fibers are arranged in bundles surrounding the free-cartilage portion of the OEC (Fig. 9A, C). The hypodermis lies external to the cartilage, although in some parts it also appears internal to it (Fig. 9A, B).

The lumen of the OEC in the proximal region is wide (Fig. 9D), and the epidermis is thinner than in the middle region. The dermis shows fewer hair follicles than the previous regions. The sebaceous glands are numerous while ceruminous glands are scarce (Fig. 9E). The dermis is more vascularized than in the middle region, although less than in *L. weddellii*. Large bundles of striated muscle and nerves are closely related to the hypodermis (Fig. 9D). The cartilage shows features similar to those of *L. weddellii*, although is thicker (Fig. 9D, E). The hypodermis develops external to the cartilage (Fig. 9D).

3.2.2.4. *Hydrurga leptonyx*

The epidermis in the distal region is similar to that of previously described species (Fig. 10A, B). The dermis is relatively thin, with clearly differentiated superficial and deep dermis (Fig. 10A). The hair follicles are compound, formed by a primary and a secondary follicle (Fig. 10C), and the related sebaceous and ceruminous glands are similar to those of *L. carcinophaga* (Fig. 10D). The hypodermis is similar to that of previous described species (Fig. 10A).

In the middle region the epidermis is thinner than that of the distal region (Fig. 11A,B),some simple hair follicles are visible, and the number of sebaceous glands is greater (Fig. 11B). The dermis shows the same arrangement than in *L. carcinophaga*. The muscle bundles are arranged in a fashion similar to *L. weddellii* and *L. carcinophaga*, surrounding the cartilage plate, and reinforcing its free ends. Within the cartilage, unilocular adipocytes are present isolated or in small groups (Fig. 11C), similar to the condition in *L. weddellii* and *L. carcinophaga*. Below the muscular layer, the hypodermis is similar to that described for *L. carcinophaga* (Fig. 11A).

The OEC in the proximal region shows a thin epidermis (Fig. 11A, D). The dermis is similar to that from previous regions. The level of vascularization in the deep dermis is intermediate between the high irrigation observed in *L. weddellii* and the poor irrigation observed in *L. carcinophaga*. The numerous sebaceous glands drain into the hair follicles (Fig. 11D, E), and the ceruminous glands are scarce (Fig. 11 E). The cartilage is similar to that observed in the middle region (Fig. 11F), and the muscular tissue, which runs external to the cartilage, is arranged in a well-defined bundle (Fig. 11D).

The hypodermis is also external to the cartilage and shows numerous vessels and nerves (Fig. 11D).

3.3 Blood vessels quantification in the OEC

Figure 12 presents the number and total occupied surface area of blood vessels for each genus studied in this work. The graph illustrates the remarkable differences between *Hydrurga leptonyx* and *Mirounga leonina*, the genera with highest and lowest number and surface areaof blood vessels in both zones (proximal and middle), respectively. *M. leonina* consistently presents less vascularization in the external ear in comparison with the other genera studied.

4. Discussion

The basic structure of the pinna in the southern otariids studied in this contribution agrees with previous descriptions of Northern Hemisphere species like *Eumetopias jubatus* (Böas, 1912; Solntseva, 2007). However, previous authors did not investigate the arrangement of hair follicles. The species described here showed abundant hair follicles in the posterior surface of the pinna that were set in groups, and this arrangement is the same as in the rest of the head integument. In contrast, the anterior surface of the pinna, as the OEC, shows scarce hair follicles.

The cartilage macroanatomy of the OEC of phocids and otariids studied here is different from those described elsewhere for Northern Hemisphere species: *E. jubatus* (Böas, 1912), *Phoca vitulina* and *Pusa hispida* (Böas, 1912), and *Pagophilus groenlandicus* (Ramprashad, 1975). In *P. vitulina* and *P. hispida*, four cartilage plates were described (Böas, 1912). In a different paper describing the anatomy of the outer ear of *P. groenlandicus*, these four cartilage plates were named as lateral or helicoidal plate, postero-ventral plate, dorso-ventral plate, and medial plate (Ramprashad et al., 1971). Notwithstanding, here we observed only one plate in the two species that were analyzed using double staining and cleared, *A. gazella* and *M. leonina*. In addition, the morphology of the cartilage plate was markedly different between both species: in *A. gazella* the cartilage plate partially surrounds the OEC on its external side, leaving the medial side free of cartilage; in *M. leonina* the cartilage is helicoidal and both the medial and external side of the OEC are partially free and partially surrounded by the cartilage plate. This arrangement could be related to the mechanism of obturation of the OEC during diving. It is worth mentioning that in *Odobenus rosmarus* the cartilage plate (auricular plate) is also unique and similar to that of *M. leonina* (Kastelein et al., 1996).

Regarding the histology of the OEC, at its distal portion the hair follicles of otariids are scarce as in the anterior portion of the pinna, while in phocids they are abundant and the arrangement is similar to that of the rest of the skin (Krmpotic et al., 2017). Deeper in the OEC, hair follicles become scarcer and isolated (Table 2).

The thin layered epithelium (two to three layers) of the distal OEC in otariids when compared to phocids could be related to higher exposition of the OEC to the exterior in the latter. The absence of a pinna in phocids leaves the OEC exposed to external conditions; accordingly, the epithelium is more similar to the rest of the integument and is also folded in its most distal portion.

The dermis shows large amounts of collagen and elastic fibers. In phocids the fibers are thicker in the distal part of the OEC and become thinner towards the proximal part. This could be related to the opening and closure of the OEC in phocids (Table 2). Regarding the vascularization of the dermis in the OEC of phocids, *H. leptonyx* showed the highest vascularization both in total blood vessel count and total blood vessel surface area for the proximal and middle regions (Fig. 12).The total blood vessel count values for *H. leptonyx* are followed by those for *L. carcinophaga, L. weddellii*, and *M. leonina*. On the other hand, regarding total blood vessel surface area, *H. leptonyx* is followed by *L. weddellii*, and again *M. leonina* with the lowest value. This could be related to the presence of cavernous tissue in the middle ear, which has been described in other seals (Renouf, 1991; Welsch and Riedelsheimer, 1997). The presence of cavernous tissue in the OEC and middle ear has been related to withstand high pressures during diving (Stenfors et al., 2000) or, alternatively, to the modification of sound transmission properties of the ear when changing from aerial to aquatic environment (Mohl and Ronald, 1975). In any case, *M. leonina*, the deepest diver of all seals, showed the lowest values for vascularization in both middle and proximal OEC, suggesting poorly developed cavernous tissue. Furthermore, in this species the amount of adipose tissue surrounding the OEC is greater than that of the other species analyzed. In this regard, the adipose tissue may play a role similar to that of cavernous tissue in impeding collapse of the OEC. Alternatively, some studies on odontocete cetaceans have suggested that adipose tissue present in the mandible (called fat channel) is important for enhancement of sound transmission. In a similar way, the massive amount of adipose tissue in the OEC of *M. leonina* might improve sound transmission under water (Berta et al., 2005).

The number of sebaceous glands in phocids is greater at the most proximal region of the OEC, and their secretions probably act as a plug during diving. In this regard, the species with greatest number of sebaceous glands are *M. leonina* and *L. weddellii*, both deep divers. Riedman (1990) suggested that this wax covering in the OEC of pinnipeds greatly reduces their sound perception on land (Table 1).

The presence of a greater number of sweat glands in *A. australis* when compared to *A. gazella* could be related to its latitudinal distribution, since the former is widely distributed along South American coasts reaching Peru, while the latter is circumscribed to the Southern Ocean (Crespo et al. 2015, Rodrigues et al. 2018)(Table 2).

The cartilage histology of phocids is also different from that of the analyzed otariids. The characteristics of the cartilaginous tissue found in the proximal and middle regions of the OEC in *L. weddellii*, *L. carcinophaga*, *H. leptonyx* and, with lesser development, *M. leonina*, differ from the typical elastic cartilage described for other pinnipeds (except *P. groenlandicus*, for which hyaline cartilage was described in the proximal region of the OEC, by Ramprashad et al., 1971). This tissue resembles the cartilage classified by Hall(2005) as a special elastic cartilage with myxoid tissue. To our knowledge, the only places were this type of cartilage normally occur are the epiglottic cartilage of the cat, and the human palatal rugosities (Pedlar, 1987; Egerbacher et al., 1995), although in these cases the myxoid matrix and the adipose cells seem to occupy a greater proportion of the tissue than in the cartilage observed in the specimens studied here. To date, there are no data regarding the functional importance of this type of tissue, but it seems to be conserved in a great number of Southern Hemisphere phocids. This tissue is characterized by scarce fibroblast-like cells, poor vascularization, and a great amount of acidic mucopolysaccharides (hyaluronic acid) (Egerbacher and Bock, 1997). Pedlar (1987) suggested that the ability of the mucopolysaccharides to retain water plays a critical role in the recovering of the tissue after high mechanical pressure stress. Nevertheless, we did not find this type of tissue in *M. leonina*, although the tissue around the OEC was very rich in adipocytes. It is noteworthy that this type of cartilage (or adipose tissue in *M. leonina*) was observed in the same region in all phocids analyzed, regardlessof age of the specimen.

The muscular fibers are arranged in bundles along the entire OEC of otariids, while in phocids the organization in bundles occurs in areas where cartilage is present (middle and proximal parts of the OEC). This arrangement is probably related to the closure system of the OEC, which has been discussed by several authors (Solntseva, 2007).

5. Conclusion

In summary, the differences in sound communication modes between southern phocids and otariids could be reflected in the morphology of their external ear, since most phocid species studied here, with the exception of *M. leonina*, use vocal communication under water more often and covering a wider frequency range than do otariids (Rogers and Cato, 2002; Stirling and Thomas, 2003; Solntseva, 2007; Doiron et al., 2012). Some authors suggest that the OEC is completely closed during diving, and sound is transmitted to the middle ear by an area immediately ventral to the ear opening (e.g. Mohl and Ronald, 1975). Furthermore, despite the potential importance of the OEC during sound transmission in both water and air, most studies have focused on characteristics of the middle and inner ear.

Among Phocidae, the species from tribe Mirounguini *M. leonina* showed a combination of features in the OEC partly similar to the rest of the phocids (included in the Lobodontini tribe) and to the otariids (Fig. 13), probably related to three characteristics: the absence of a pinna (as in the rest of the phocids), which leads to a thicker epithelium and greater pilosity of the OEC; their having the deepest diving behavior, related to the formation of a ceruminous plug by the numerous glands in the OEC, and the presence of a massive amount of adipose tissue surrounding the OEC; and their in-land habits that include activities with frequent vocalizations, leading to a less vascularized OEC similar to that of otariids that suggests poor development of cavernous tissue (also absent in otariids).This behavior is displayed mostly during breeding season, and their inter- and intrasex communication has been related to territoriality and courtship behaviors (Rogers and Cato, 2002; Stirling and Thomas, 2003; Doiron et al., 2012). In this regard, a functional auditory organ both over and under water could be essential for social behavior in these species.

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Figure Captions

Figure 1. OEC cartilage in *Arctocephalus gazella* A, and *Mirounga leonina* B. The portion of the right OEC is on medial view. Abbreviations: (ca) cartilage.

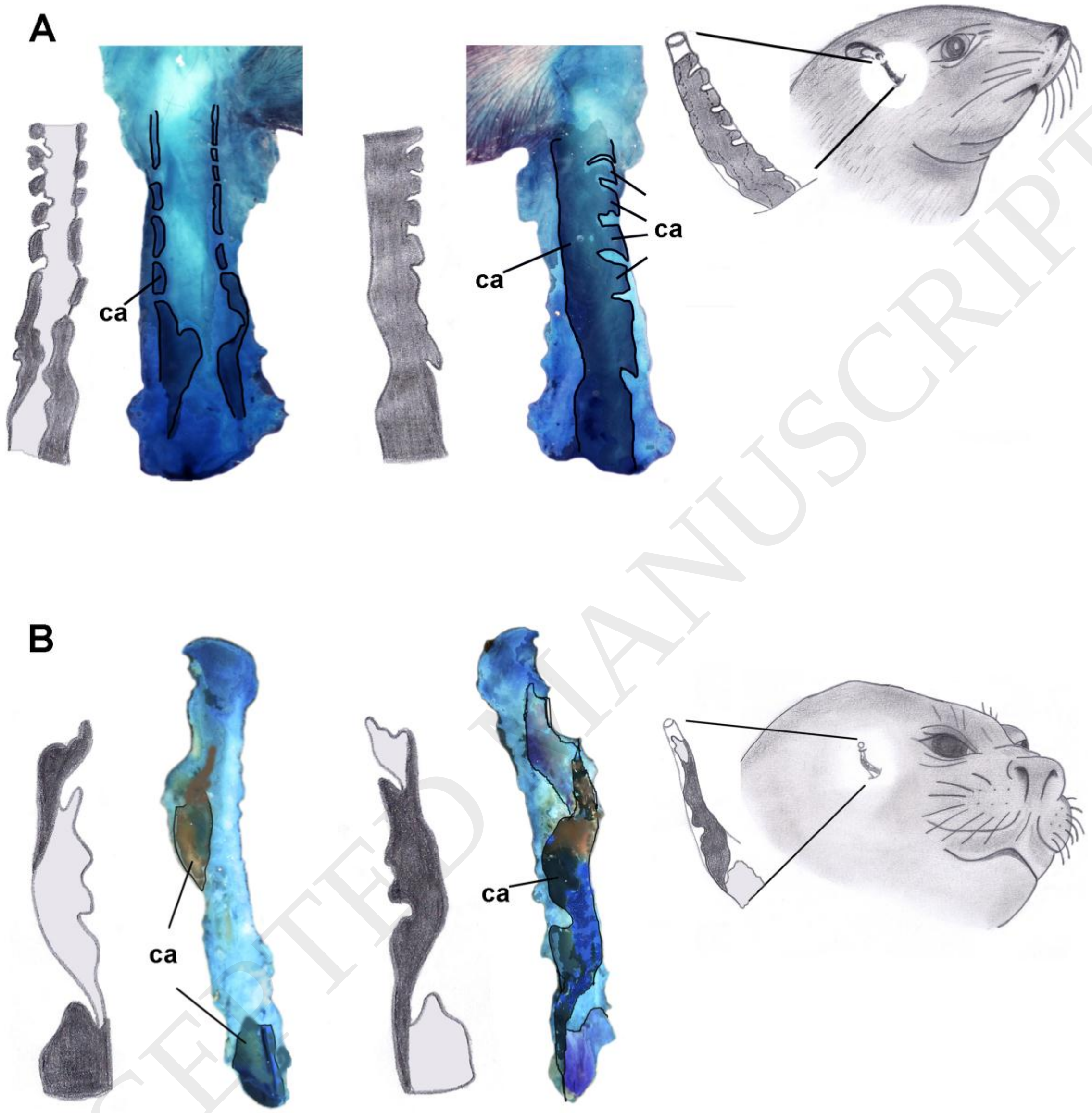

Figure 2. *Arctocephalus gazella*. Transversal section of the pinna (H-E stain). A- Panoramic view of the anterior and posterior surfaces with the central axe of cartilage (4X), B- Posterior surface (10x), C- Detail on the epidermis of the posterior surface (40x), D- Detail on the epidermis of the anterior surface (40x), E- Hair follicle group and sebaceous gland (10X), F- Elastic cartilage (40X, Gomori orcein technique). Abbreviations: (as) anterior side, (ca) cartilage, (dde) deep dermis, (de) dermis, (ep) epidermis, (hf) hair follicles, (ps) posterior side, (sbg) sebaceous glands, (sde) superficial dermis.

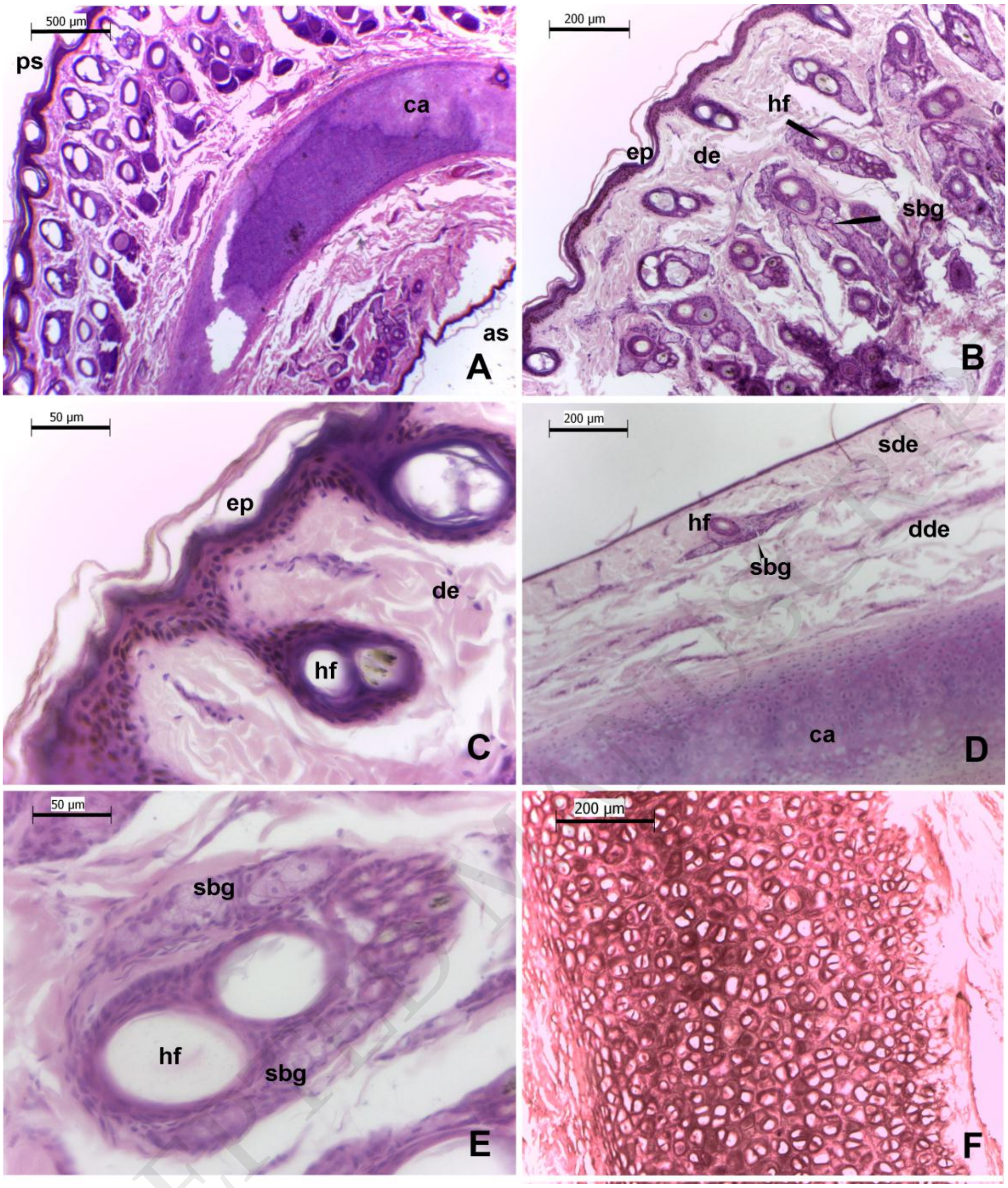

Figure 3. *Arctocephalus gazella* (A, B, D, E and F), *Arctocephalus australis* (C), transversal section of the outer ear canal (OEC). A- OEC reconstruction, transversal section of the proximal region (4x), B- panoramic view of the skin (10x), C- ceruminous gland (40x), D- ceruminous gland (40x), E- elastic cartilage, muscle fibers and collagen bundles, (10x), F- elastic cartilage (40x). A, D, E, Gomori trichrome stain; B, C, H-E stain; F, Gomori orcein technique. Abbreviations: (ca) cartilage, (cg) ceruminous glands (de) dermis, (dde) deep dermis, (ep) epidermis, (hf) hair follicles, (mf) muscular fibres, (sbg) sebaceous glands, (sde) superficial dermis.

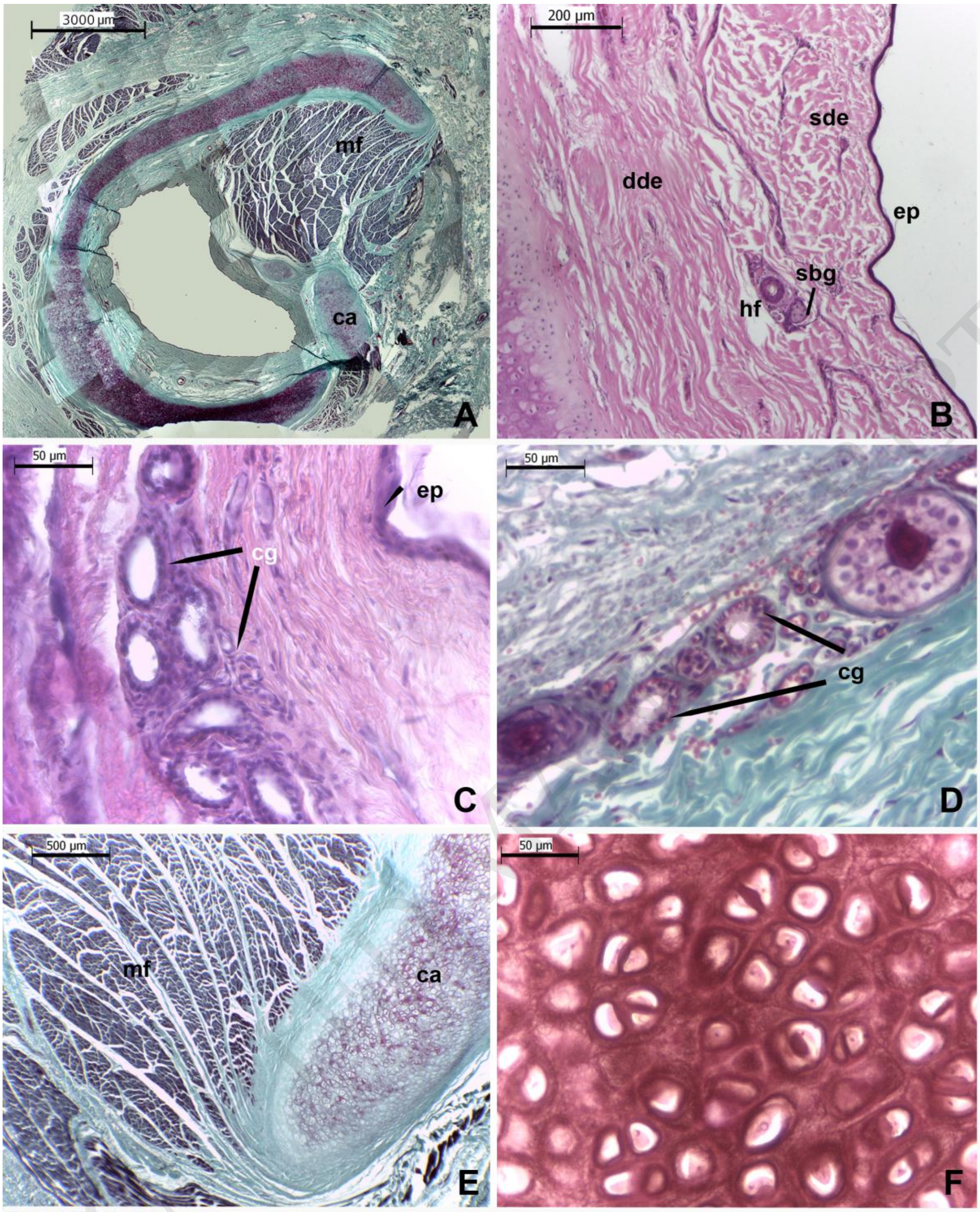

Figure 4. *Mirounga leonina*. Transversal section of the outer ear canal (OEC), distal region. A and B- panoramic view of the superficial and deep dermis (10x), C- epidermis and superficial dermis (40x), D- sebaceous gland (40x) (A, C, D, H-E stain; B, Gomori trichrome stain). Abbreviations: (ad) adipose tissue, (cg) ceruminous glands, (dde) deep dermis, (de) dermis, (ep) epidermis, (hf) hair follicles, (me) melanine, (pn) picnotic nuclei, (sbg) sebaceous glands, (sc) stratum corneum, (sde) superficial dermis.

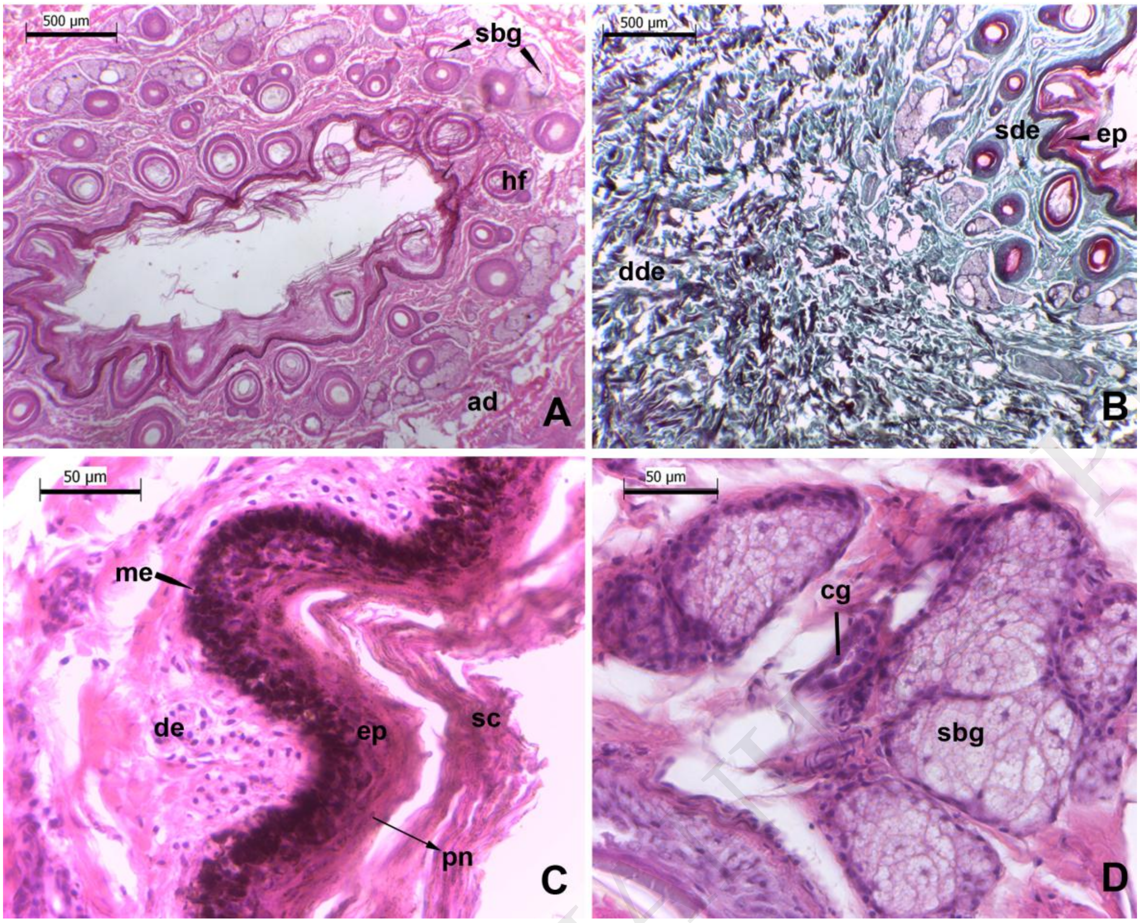

Figure 5. *Mirounga leonina*. Outer ear canal (OEC), transversal section of the middle (A, B, C) and proximal regions (D, E, F). A- general view of the reconstruction of the middle region, (4x, PAS stain), B- panoramic view of the epidermis-dermis (10x, H-E stain), C- detail of the elastic cartilage (40x, H-E stain), D- reconstruction of the proximal OEC (4x, Gomori trichrome stain), E- panoramic view of the epidermis-dermis (10x), F- elastic cartilage (10x). Abbreviations: (ad) adipose tissue, (ca) cartilage, (dde) deep dermis, (ep) epidermis, (hf) hair follicles, (sbg) sebaceous glands, (sde) superficial dermis, (mf) muscular fibres.

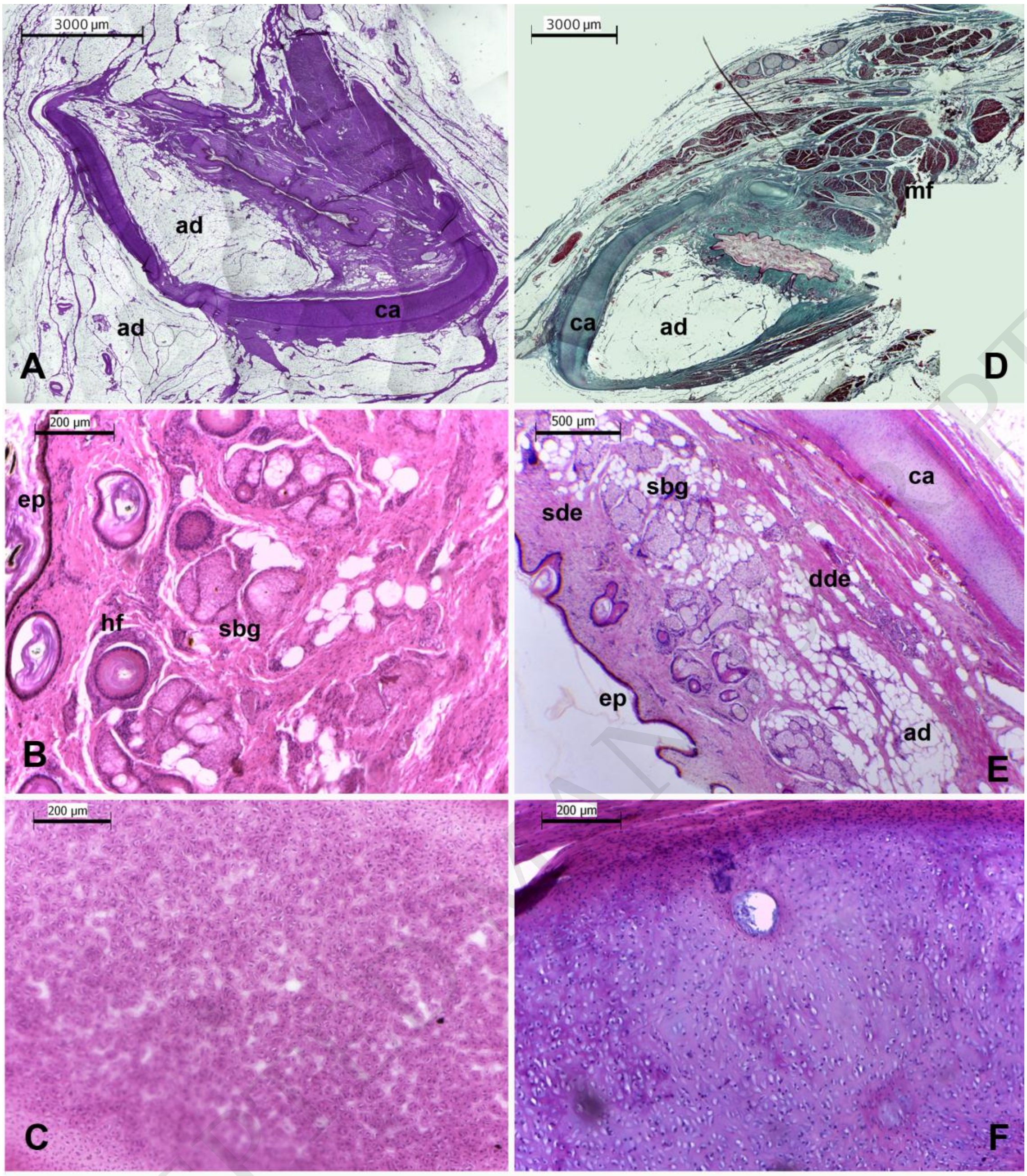

Figure 6. *Leptonychotes weddellii*. Transversal section of the outer ear canal (OEC), distal region. A- general view (4x), B- panoramic view epidermis-dermis, (10x), C- detail of a sebaceous gland and a group of hair follicles (40x), D- detail of a ceruminous gland (40x), (A, B, C, H-E stain, D, Gomori trichrome stain). Abbreviations: (cg) ceruminous gland, (dde) deep dermis, (hf) hair follicles, (sbg) sebaceous glands (sc) stratum corneum, (sde) superficial dermis.

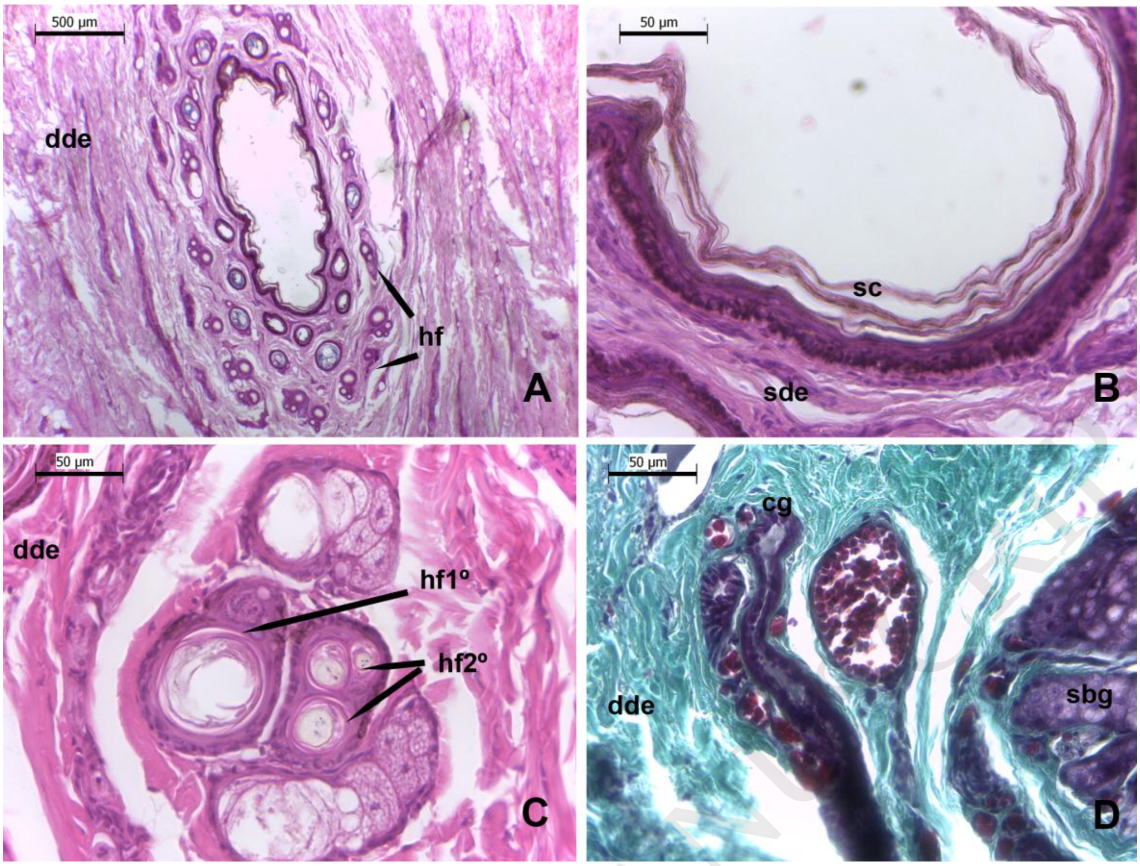

Figure 7. *Leptonychotes weddellii*. Outer era canal (OEC), middle (A, B, C) and proximal regions (D, E, F), transversal section. A- reconstruction of the middle OEC in general view (4x, Gomori trichrome stain), B- panoramic view of the epidermis-dermis (H-E stain), C- detail of the cartilage with myxoid tissue (Giemsa), D- reconstruction of the proximal OEC in general view (4x, Gomori trichrome stain), E- panoramic view of the epidermis-dermis, F- detail of the elastic cartilage (orcein stain). Abbreviations: (ad) adipose tissue, (ca) cartilage, (dde) deep dermis, (ep) epidermis, (hf) hair follicles, (mf) muscular fibres, (mt) myxoid tissue, (sbg) sebaceous glands, (sde) superficial dermis.

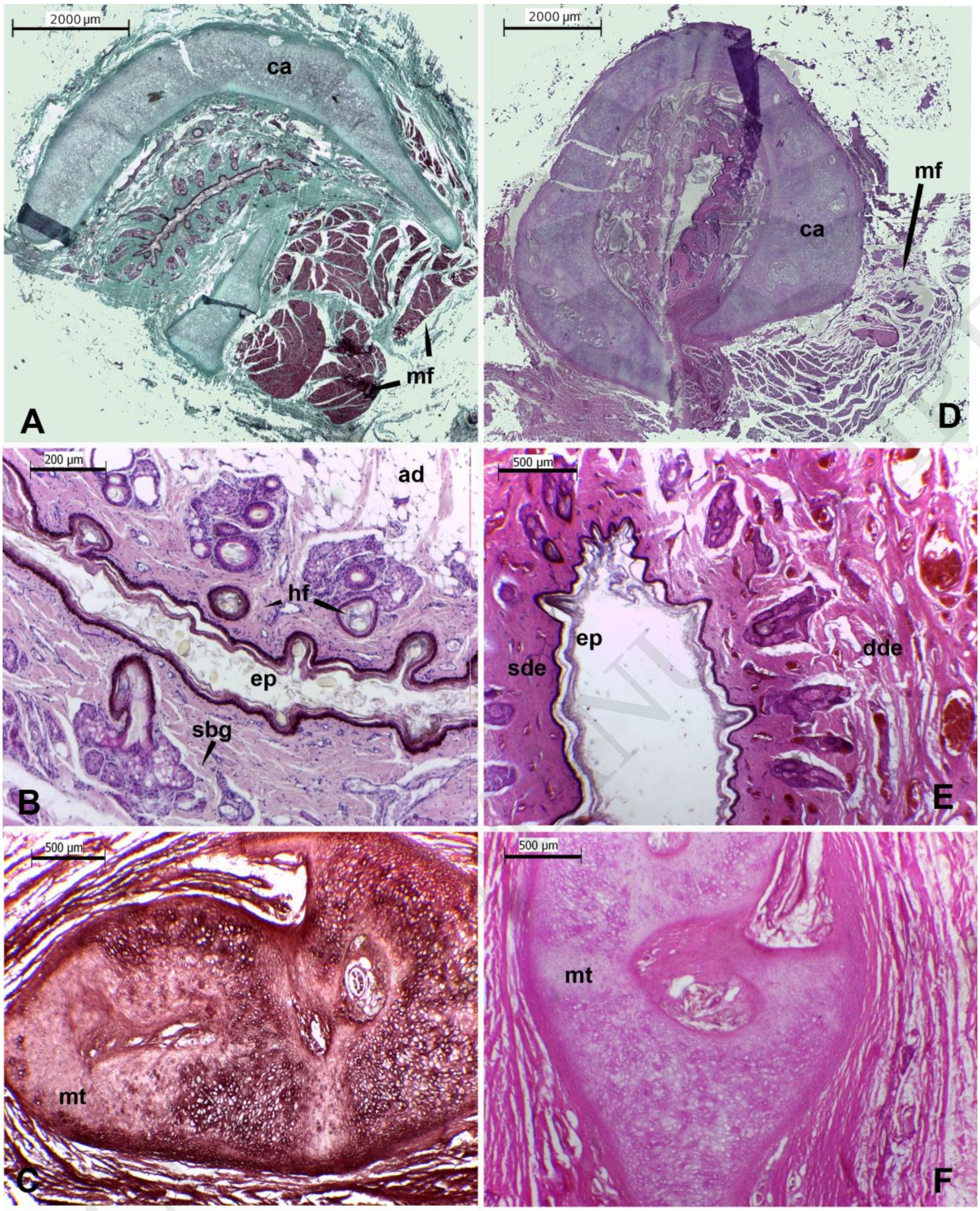

Figure 8. *Lobodon carcinophaga*. Outer ear canal (OEC) in transversal section, distal region (H-E stain). A- general view, B- panoramic view of the superficial and deep dermis, C- detail on the epidermis, D- detail on a sebaceous gland and follicular group, E- detail on a ceruminous gland, F- detail on a ceruminous gland (Gomori trichrome stain). Abbreviations: (cg) ceruminous gland, (dde) deep dermis, (ep) epidermis, (hd) hypodermis, (hf) hair follicles, (mf) muscular fibres, (sbg) sebaceous glands, (sde) superficial dermis.

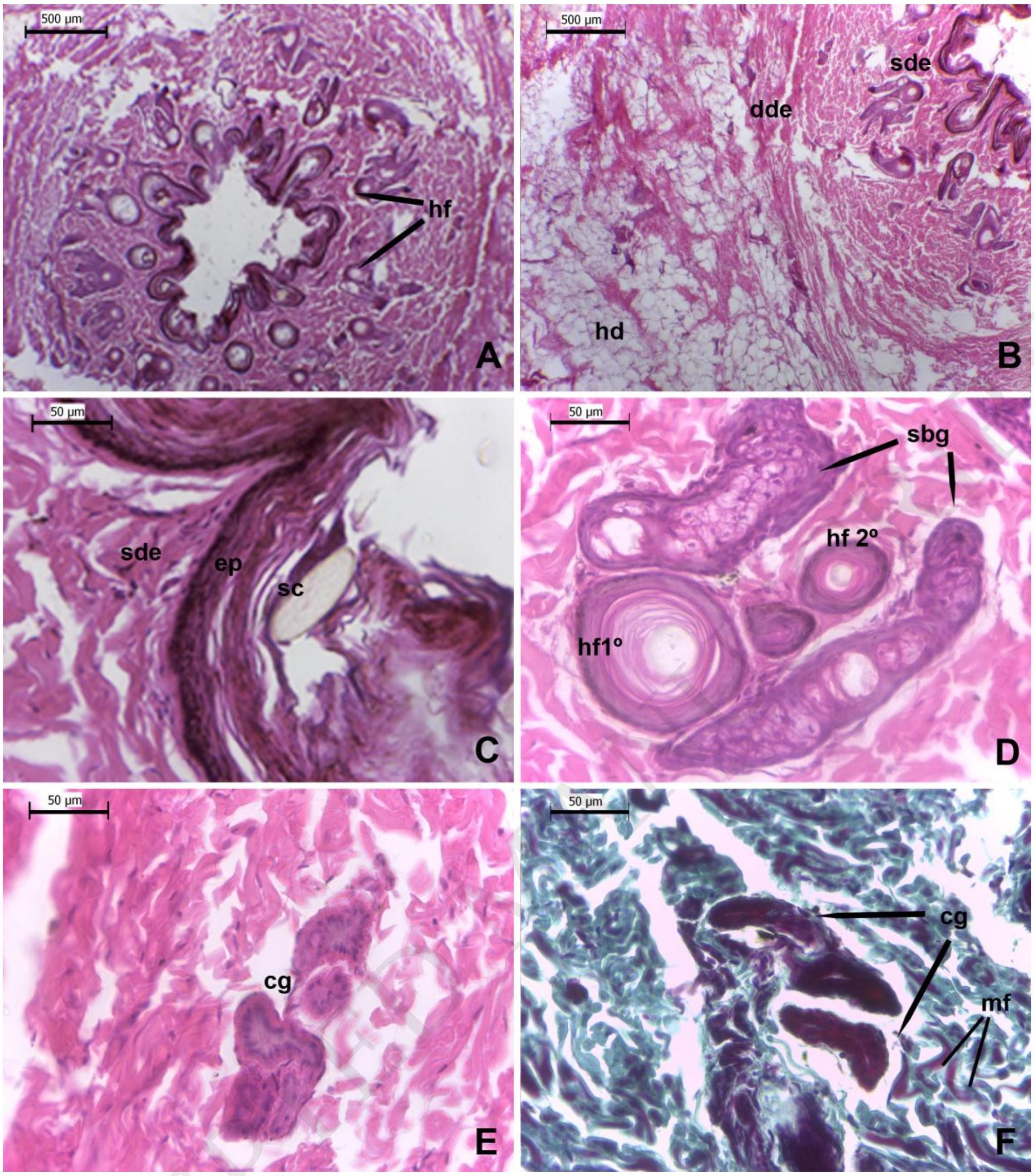

Figure 9. *Lobodon carcinophaga*. Outer ear canal (OEC), middle (A, B, C) and proximal (D, F) regions, transversal section. A- reconstruction of the middle OEC in general view (4x), B- panoramic view of the epidermis dermis (10x, Gomori trichrome stain), C- detail on the elastic cartilage (40x), D- reconstruction of the proximal OEC in general view (4x, Gomori trichrome stain), E- detail on the dermis (10x, H-E stain). Abbreviations: (ca) cartilage, (cg) ceruminous gland, (dde) deep dermis, (de) dermis, (ep) epidermis, (hd) hypodermis, (hf) hair follicles, (mf) muscular fibres, (mt) myxoid tissue, (sbg) sebaceous glands, (sde) superficial dermis.

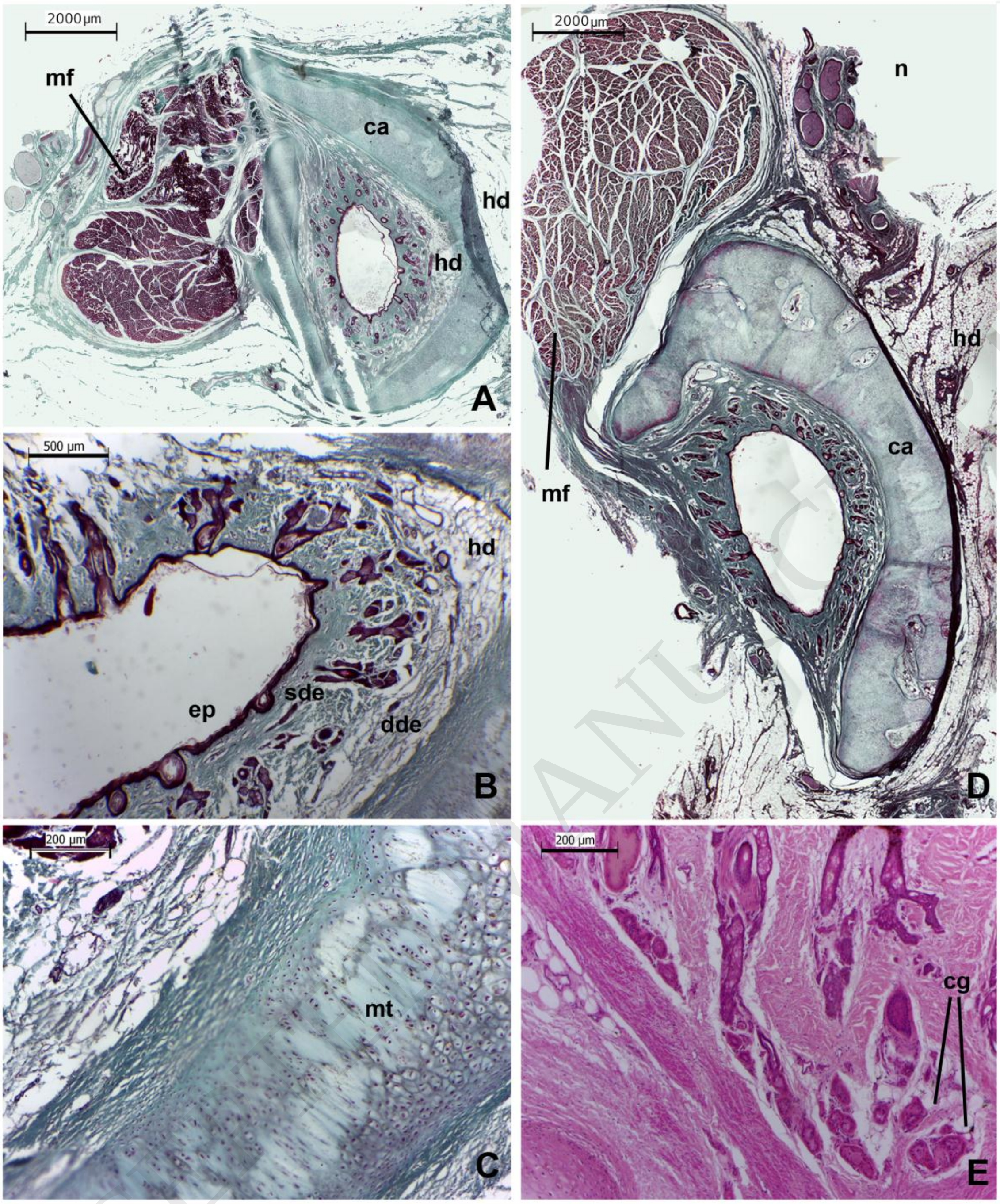

Figure 10. *Hydrurga leptonyx*. Outer ear canal (OEC) in transversal section, distal region (H-E stain). A- general view (4x), B- panoramic view of the epidermis and superficial dermis (10x), C- detail on a sebaceous gland and hair follicles (40x), D- detail on a ceruminous and a sebaceous gland (40x). Abbreviations: (cg) ceruminous gland, (dde) deep dermis, (de) dermis, (ep) epidermis, (hd) hypodermis, (hf) hair follicles, (sc) stratum corneum, (sbg) sebaceous glands, (sde) superficial dermis.

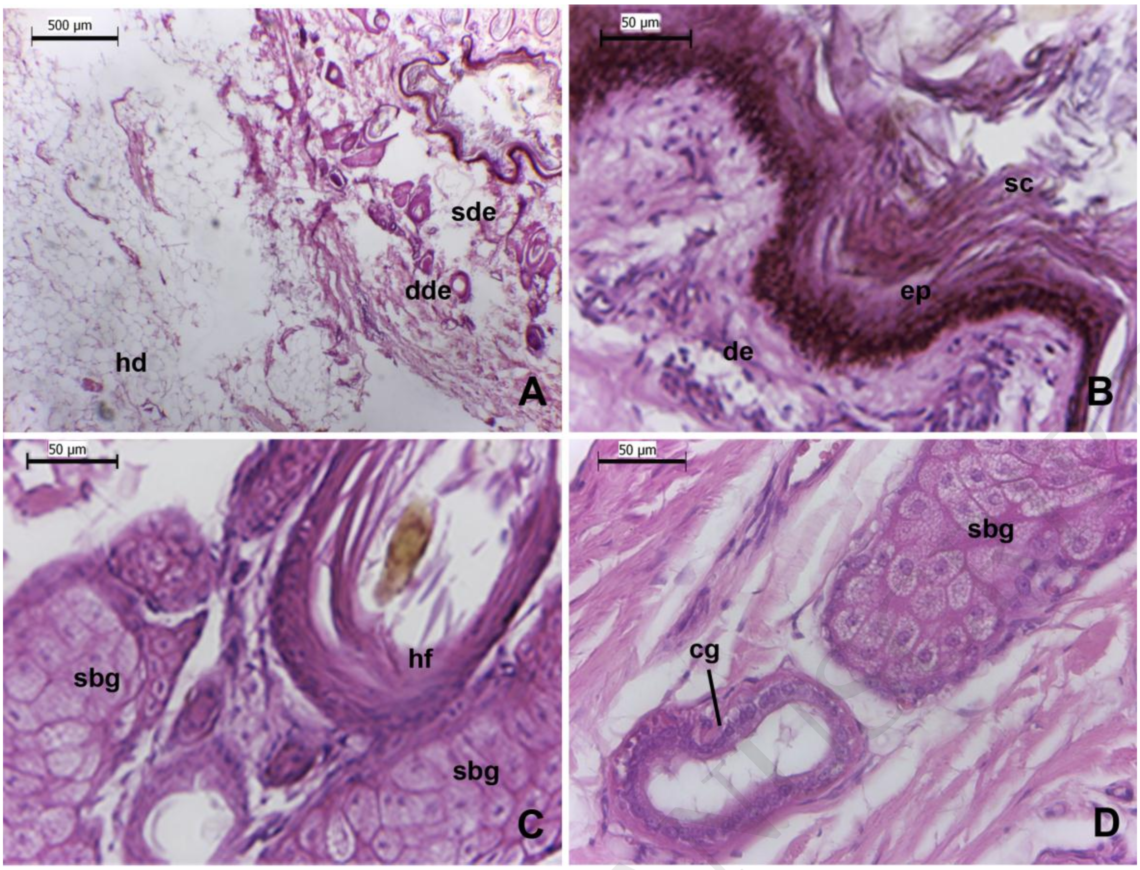

Figure 11. *Hydrurga leptonyx*. Outer ear canal (OEC), middle (A, B, C) and proximal (D, E, F) regions, in transversal section. A- reconstruction of the middle OEC in general view (4x, Gomori trichrome stain), B- panoramic view of the epidermis-dermis (10x, H-E stain), C- detail on the cartilage (40x, reticulin stain), D- reconstruction of the proximal OEC in general view (4x, H-E stain), E- panoramic view of the epidermis-dermis (10x), F- detail on the cartilage (40x, Alcian blue technique). Abbreviations: (ad) adipocite, (ca) cartilage, (cg) ceruminous gland, (dde) deep dermis, (de) dermis, (ep) epidermis, (hf) hair follicles, (mt) myxoid tissue, (sbg) sebaceous glands

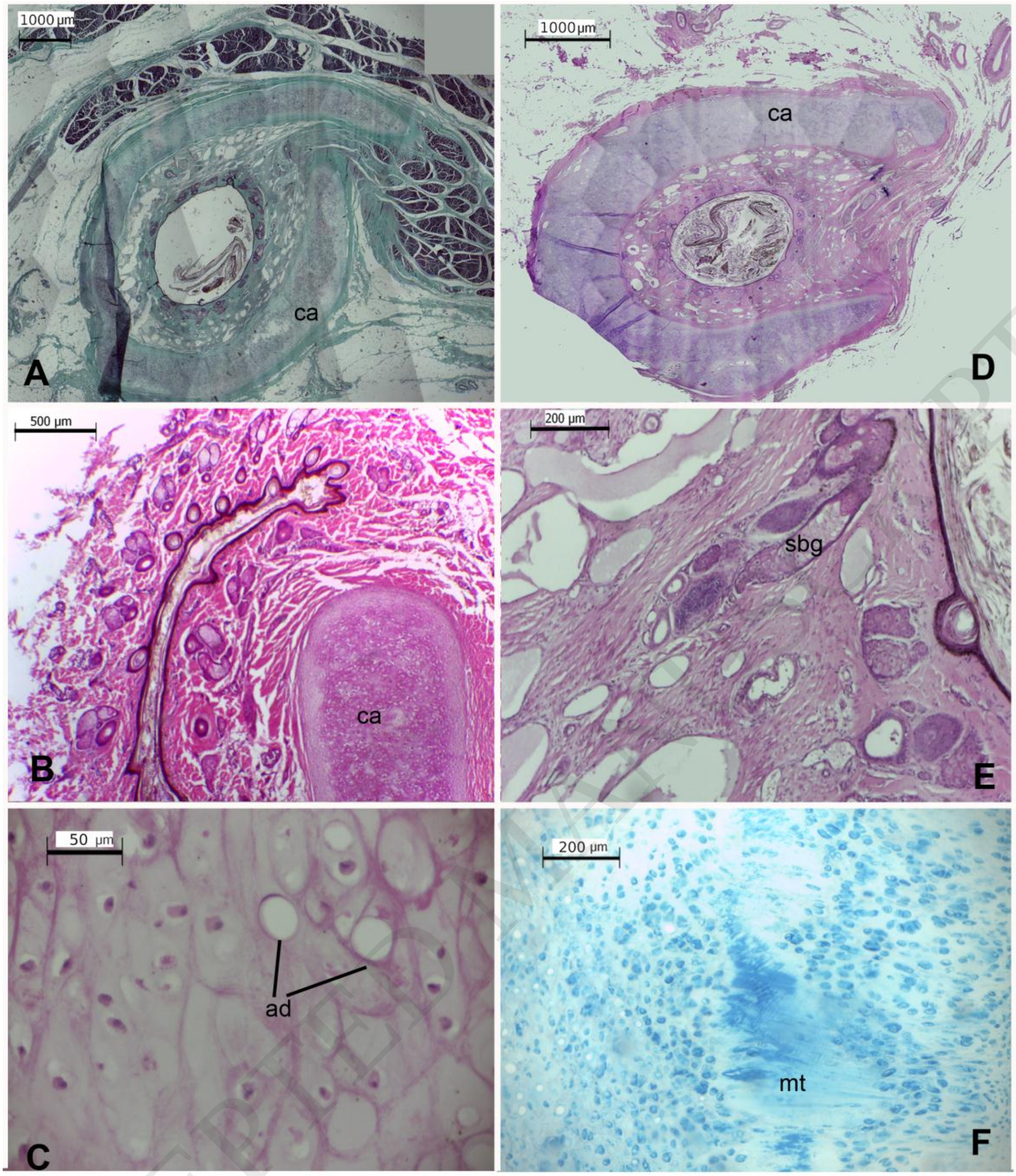

Figure 12: Barcharts comparing: A. Total blood vessel counts and, B. total blood vessels surface (in square microns) in Middle and Proximal regions for each genus.

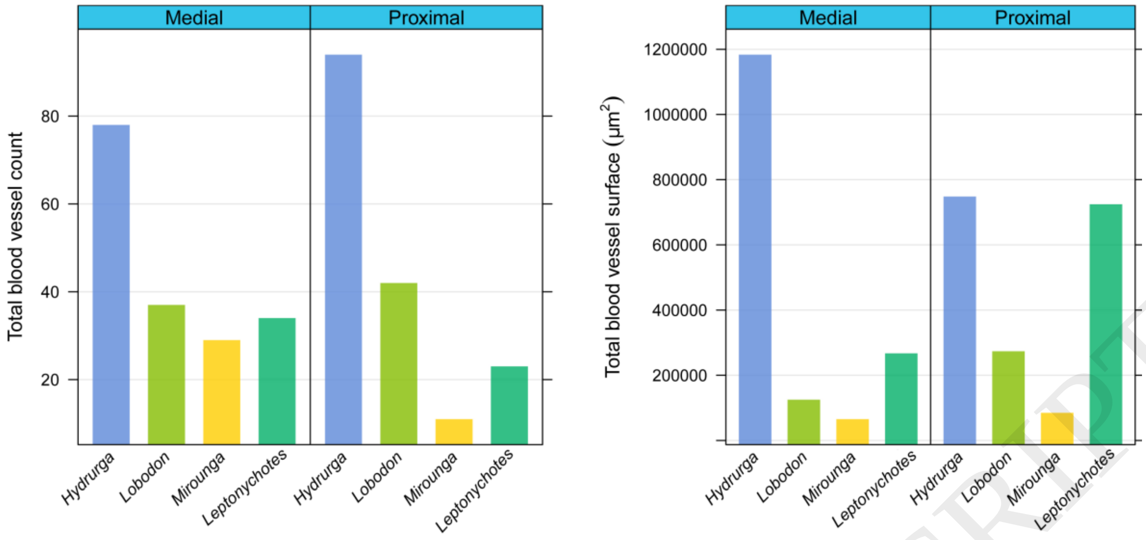

Figure 13: Histological sections of the outer ear canal (OEC), among Antarctic true seals (Lobodontini and Miroungini) in the context of the phylogenetic relationships among pinniped carnivorans, modified from Loza et al. (2017). A: Simplified phylogeny given by Arnason et al. (2006); B: medial histological sections; C: proximal histological sections; D: photographs of the cited species by C. M. Loza.

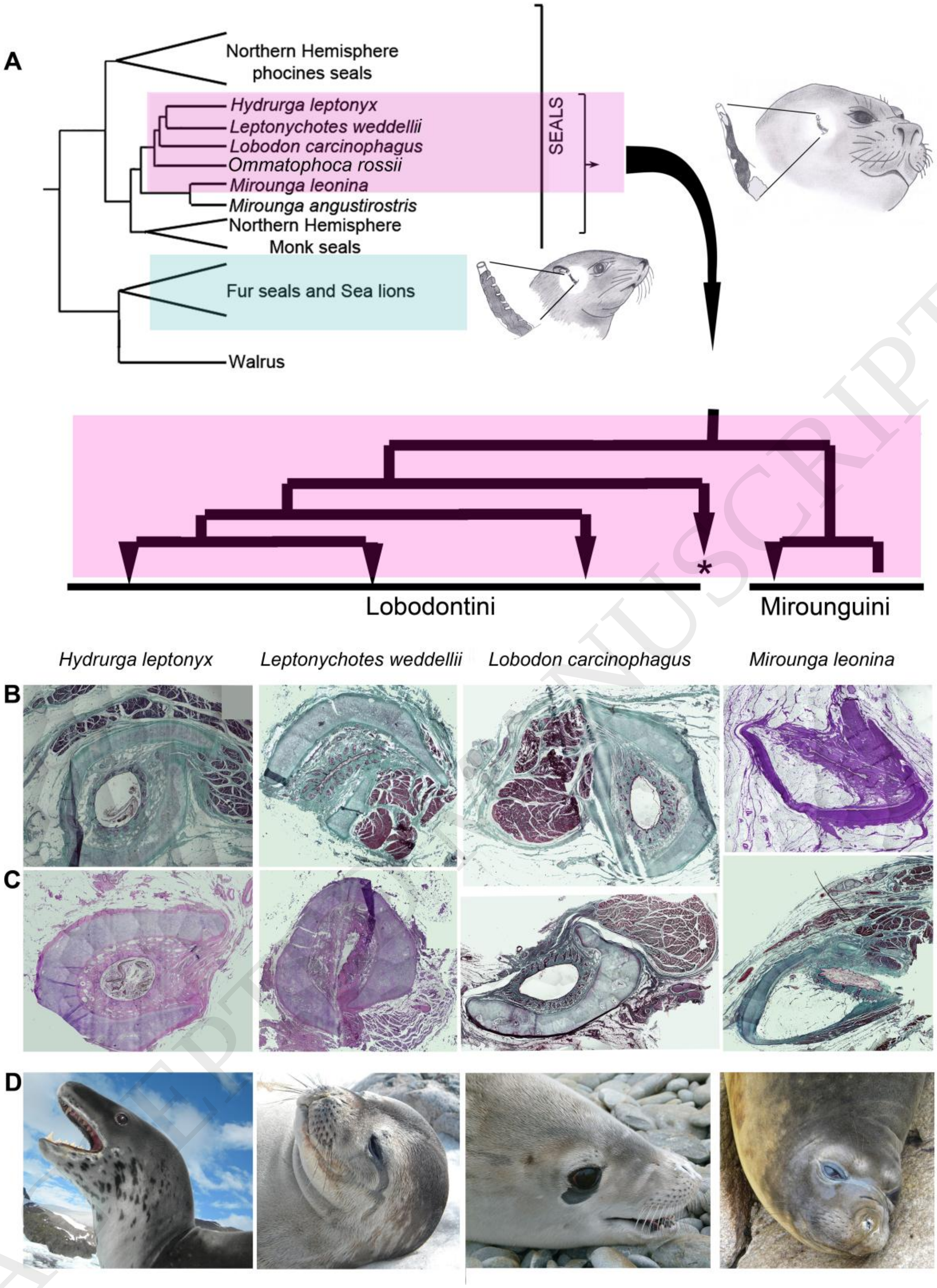
A
Northern Hemisphere
phocines seals
Hydrurga leptonyx
Leptonychotes weddellii
Lobodon carcinophagus
Ommatophoca rossii
Mirounga leonina
Mirounga angustirostris
Northern Hemisphere
Monk seals
SEALS
Fur seals and Sea lions
Walrus
*
Lobodontini
Mirounguini
Hydrurga leptonyx
Leptonychotes weddellii
Lobodon carcinophagus
Mirounga leonina
B
C
D

TABLE I: Studied specimens

Specimen	Age	Collection	Collecting place
Mirounga leonina	Lactating puppy	IAA_EL 18/01	Antarctic Specially Protected Area (ASPA) Nº 132 “Peninsula Potter”, on the Isla 25 de Mayo in the South Shetland Islands (62º 15’ S, 58º 37’ W)
Mirounga leonina	Weaned young during its first molting	IAA_EL 18/02	Antarctic Specially Protected Area (ASPA) Nº 132 “Peninsula Potter”,on the Isla 25 de Mayo in the South Shetland Islands (62º 15’ S, 58º 37’ W)
Leptonychotes weddellii	subadult	IAA_EL 18/03	Danco coast, west Antarctic Peninsula near the Argentine Antarctic Station “Primavera” (64º 09’ S, 60º57’ W)
Hydrurga leptonyx	adult	IAA_EL 18/04	Danco coast, west Antarctic Peninsula near the Argentine Antarctic Station “Primavera” (64º 09’ S, 60º57’ W)
Lobodon carcinophaga	adult	IAA_EL 18/05	Isla Marambio (64º 14’ S, 56º 37’ W).
Arctocephalus gazella	adult	IAA_EL 18/06	Isla Laurie, islas Orcadas del Sur (60º 30’ S, 44º 37’ W)
Arctocephalus gazella	late fetus	LMED-797	Coastal zone in San Clemente del Tuyú, Buenos Aires, Argentina
Arctocephalus australis	juvenile	LMED-796	Jardín Zoológico y Botánico de La Plata

TABLE II. Differences in the cartilaginous portion of the OEC between Otariidae and Phocidae.

	Otariidae	Phocidae
Regional differentiation	No	Distal, middle and proximal
Lumen caliper	Constant along the entire length (slightly narrower in the distal region).	Distally narrow, and increases towards the proximal region.
Cartilage presence	Along the entire canal.	Middle and proximal region only.
Type of cartilage	Elastic typical.	Elastic with myxoid like tissue (except *M. leonina*)
Epidermis	Thin (2-3 cell layers), no foldings, constant along the entire canal.	Thick (5-6 cell layers), folded in the distal region, the number of cell layers decreases towards the proximal region.
Hair follicles	Scarce, some of them are compound	Distally, they are similar to the rest of the integument.Towards the proximal region, the amount decreases.
Sebaceous glands	Scarce	Increase in size and number towards the proximal region.
Ceruminous glands	Scarce, although more abundant in *A. australis*.	Scarce.
Muscle fibers	Not observed within the dermis, only forming muscular bundles	Present in the dermis, unordered in the distal region and ordered in bundles in the middle and proximal region.
Vascularization and innervation	Poor	Rich, especially in the proximal region (except *M. leonina*).
Adipose tissue	Absent	External to the dermis (hypodermis) and adipocytes immerse in the dermis (mainly in *M. leonina*).