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Integument in Antarctic seals: A comparative study and its relation to extreme environments

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Abstract

Due to the semi-aquatic lifestyle of seals and the extreme climates they inhabit, their integumentary system has aroused the curiosity of several authors for more than a century. The aim of this contribution was to perform an exhaustive histological description of the integument Antarctic seals through different methodological approaches in a comparative framework. The species considered include the ice-obligate—Weddell (*Leptonychotes weddellii*) and leopard (*Hydrurga leptonyx*) seal—and the ice-tolerant, and southern elephant seal (*Mirounga leonina*). In addition, we attempted to link the histological features to their different lifestyles. All three species showed features related to their amphibious lifestyle (i.e., parakeratotic epidermis with abundant melanin granules, dermis with numerous arteriovenous anastomoses and a great amount of elastic fibres). In aquatic mammals, parakeratosis would decrease cellular replacement, the great amount of melanin is related to the high exposure to solar radiation, the presence of arteriovenous anastomoses is related to thermoregulatory mechanisms, and the presence of elastic fibres is due to variations of thickness in the adipose tissue that requires high elasticity of the dermis.

KEYWORDS

dermis, epidermis, histology, Pinnipedia, skin, thermoregulation

1 | INTRODUCTION

The vertebrate integument represents an evolutionary compromise between the needs for mechanical protection, and those of sensing the environment and regulating the exchange of materials and energy (Lillywhite, 2006). The skin of mammals is a multifunctional system that varies considerably with the lifestyle of the different species (Sokolov, 1982). Its integument includes the epidermis, dermis, subcutaneous fat and other structures like glands and hair. The pinnipeds, amphibious mammals that includes seals (Phocidae), fur seals and sea lions (Otariidae), and walruses (Odobenidae), spend most of their lives in open seas, reaching the coast (or pack ice) only to mate, give birth, moult, rest or even occasionally to escape from predators. Due to their semi-aquatic lifestyle and the extreme climates they live (mostly close to poles), the structure of their integumentary system (mainly associated with thermal insulation) has attracted considerable scientific interest for more than a century (see references, de Meijere, 1894; Erdsack et al., 2015; Gray, Canfield, & Rogers, 2006; Khamas, Smodlaka, Leach-Robinson, & Palmer, 2012; Ling, 1965, 1968, 1970, 2013; Sokolov, 1960).

The classical epidermis is mainly layered, including a deep basal stratum (stratum basale), followed (to the surface) by a stratum spinosum, stratum granulosum, stratum lucidum and finally a stratum corneum.

The epidermis in pinnipeds and cetaceans is also composed by multiple strata but just with three clearly defined of the five typical strata of most of mammals: stratum basale, stratum spinosum and stratum corneum (Berta, Sumich, & Kovacs, 2015); the presence of a fourth one, a true granular layer, in some of pinnipeds species is widely discussed (see e.g., Gray et al., 2006; Ling, 1968; Sokolov, 1982).

In turn, the characteristic thick pinniped′s dermis includes the papillary and the reticular layers, and bundles of smooth muscle fibres in the last one (Sokolov, 1982). In Phocidae, the sebaceous glands are very large, while the sweat glands are fewer than in other pinnipeds, and generally, its ducts open into the pilary canal below the sebaceous gland duct (Sokolov, 1982).

In the superficial dermis, the presence of a large number of arteriovenous anastomoses was observed, which are related to thermoregulatory mechanisms (Khamas et al., 2012).

The hair pattern in pinnipeds is made up of units, each one containing one coarse hair (primary hair or overhair guard) and generally a number of finer hairs (secondary hairs or fur hairs; Liwanag, Berta, Costa, Budge, & Williams, 2012; Scheffer, 1964). Primary and secondary hairs emerge from a common funnel or skin pore (Scheffer, 1964). Primary hairs in otariids have a pith or medulla, while in walrus and phocids, they do not (Scheffer, 1964). Unlike terrestrial carnivora, sea otters and pinnipeds lack erector muscles in their hair follicles and therefore have little physiological control over positioning of the hairs (Ling, 1968, 1970; Liwanag, Berta, Costa, Budge, et al., 2012; Montagna & Harrison, 1957; Sokolov, 1982). The integument of *Mirounga leonina* has been the most comprehensively studied one, particularly regarding its changes during the moulting period and postnatal development (Ling, 1965, 1968, 2013; Ling & Thomas, 1967; Montagna & Harrison, 1957; Spearman, 1968). Nevertheless, some questions about this species still remain unresolved, especially in relation to the epidermal layers. In contrast, the only contribution regarding the integument of *Leptonychotes weddellii* was done by Molyneux and Bryden (1978), on the dermal arteriovenous anastomoses. A similar situation occurs with *Hydrurga leptonyx*, as most of the studies on the integument of this species are broad and general descriptions (Gray et al., 2006). In the present contribution, we studied the integumentary system of specimens of three species of Antarctic phocids: southern elephant seal (*M. leonina*), leopard seal (*H. leptonyx*) and Weddell seal (*L. weddellii*; Figure 1). Leopard and Weddell seals are commonly known as pack-ice seals as they use the sea ice of Antarctica to rest, breed and moult, while the southern elephant seals breed and moult on island and continental coastal lands. All three species are present in Antarctica, and Weddell seal is the mammal with the most Austral distribution (Canevari & Vaccaro, 2007). The aim of the contribution is to perform a detailed descriptive and comparative study on the histology of the integument of these species, in an attempt to define characteristics that could be common to all of them and that may be extensive to most Phocidae, as well as those particular morphological features that are related to the extreme environments where they lives, and how ecological differences between species are reflected in the integument.

2 | MATERIAL AND METHODS

Samples were collected during the spring–summer Antarctic campaigns of 2012–2013 in the Antarctic Specially Protected Areas (ASPA) N° 132 and 134, also known as ‘Potter Peninsula’ (62°15′S and 58°40′W) and ‘Cierva Point’ (64°10′ S and 60°57′ W), respectively. Skin samples from the southern elephant seal were collected at ‘Potter Peninsula’, which is located on the South Shetland Islands, while samples from leopard seal and Weddell seal were collected at ‘Cierva Point’, located on the western sector of the Antarctic Peninsula.

All sampled animals were previously anesthetized with a combination of tiletamine (250 mg) and zolazepam (250 mg/ml), or with ketamine hydrochloride (50 mg/ml). A rigorous control of the anaesthetic protocol allowed us to ensure a good handling and well-being of the animal during the extraction of the skin samples. A total of 47 skin samples from adults of southern elephant seal ($n = 22$), leopard seal ($n = 15$) and Weddell seal ($n = 10$) were taken for the purpose of this work and others like trophic ecology and population genetics. Skin samples were obtained using an 8-mm biopsy punch. Only one sample was taken per specimen to avoid possible infections to the animal. All samples were taken just from the lateral body

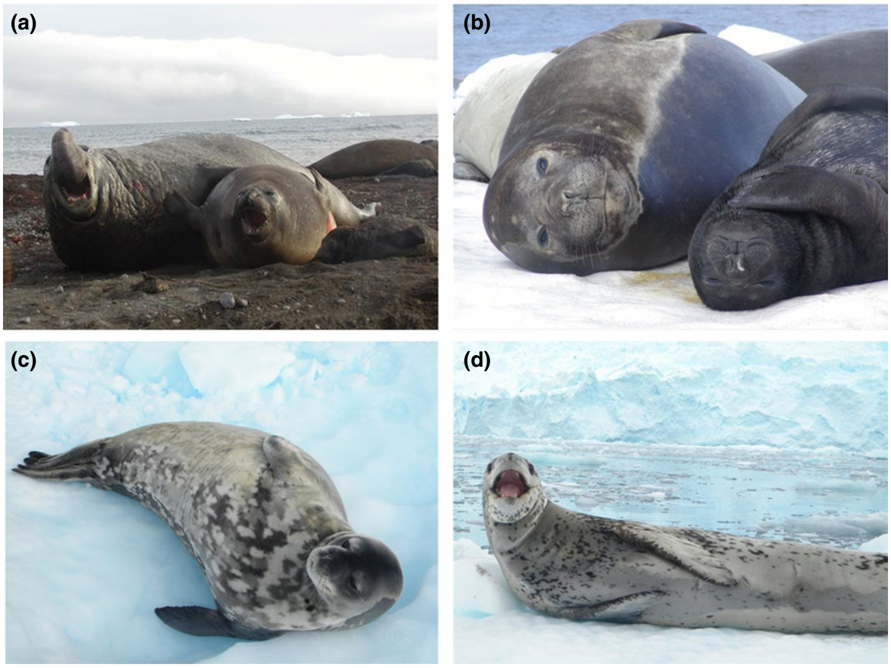

FIGURE 1 (a) Adult male, female and cub of *Mirounga leonina*. (b) Adult female and cub of *M. leonina*. (c) Adult of *Leptonychotes weddellii*. (d) Adult of *Hydrurga leptonyx*. [Colour figure can be viewed at wileyonlinelibrary.com]

of male and female adult specimens because the impossibility to roll them by its weight, and because this area was always exposed allowing us to compare homologous samples; samples from other areas were taken but just in few specimens (and, not in all the species). Afterwards, each skin sample was fixed in 10% formaldehyde on sea water. In these conditions, samples were shipped to the continent, to be further processed in the laboratories of the División Paleontología de Vertebrados in the Museo de La Plata, and the Histología y Embriología Descriptiva, Experimental y Comparada from the Facultad de Ciencias Veterinarias, both from the Universidad Nacional de La Plata, La Plata, Argentina.

2.1 | Sample processing for light microscopy

The samples were dehydrated in a series of increasing ethanol concentrations from 70% to 100% and later embedded in paraffin. Then, 5- to 8-µm-thick histological sections were obtained. To highlight the different components of the tissues, the slices were stained using haematoxylin and eosin (H-E), orcein (to determine elastic fibres), reticulin (to recognize reticular fibres) and Gomori's Trichrome (which will allow recognition of muscular from collagen fibres).

2.2 | Sample processing for transmission electron microscopy

Samples of southern elephant seal were also analysed using transmission electron microscopy (TEM). To this end, they were fixed in phosphate-buffered (pH 7.2–7.4) 2% glutaraldehyde for 2 h at 4°C and then in 1% osmium tetroxide for 1 h at 4°C. Afterwards, samples were dehydrated in an increasing ethanol series and embedded in epoxy resin. Semi-thin sections (1 µm thick) were stained with Toluidine blue. Ultra-thin (90 nm thick) sections were contrasted with uranyl acetate and lead citrate to be further examined in a TEM JEM 1200 EX II (JEOL Ltd., Tokyo, Japan). Pictures were taken with an Erlangshen ES1000W, 785 Model (Gatan Inc., Pleasanton, CA, USA) camera from the Servicio Central de Microscopía of the Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata.

2.3 | Sample processing for scanning electron microscopy

Samples of full and cut hairs of southern elephant seal, Weddell seal and leopard seal were also analysed on scanning electron microscopy (SEM).

Specimens were viewed and photographed using FEI SEM Quanta model with tungsten filament software, which belongs to CINDECA, Facultad de Ciencias Exactas, Universidad Nacional de La Plata (UNLP). The critical point drying was realized with an Emitech K850 dryer and the coated with gold with a SPI Module Sputter Coater.

2.4 | Hairs measurements

Different hair measurements were taken on cross-sectional views of leopard seal, Weddell seal and southern elephant seal. All measurements were taken using ImageJ 1.5 software (Schneider, Rasband, & Eliceiri, 2012) on calibrated digital images from scanning electron microscope (SEM). The measurements include the area of cross-sectional view of hairs, expressed in squared millimetres, used as a size descriptor. The circularity (values of 1 indicate a perfect circle and as the value approaches 0, it indicates an increasingly elongated shape), also measured on cross-sectional view of hairs and based on a threshold images, used as a shape descriptor. Also the number of hairs were measured, as the total number of hairs per square millimetre in each sample, and used as a density descriptor.

3 | RESULTS

3.1 | Southern elephant seal Epidermis

Light microscopy (LM) observations showed that the epidermis of this species has a stratum basale. TEM observations of the semi-thin sections showed that the morphology of the cells in this stratum is variable: some of them are cylindrical with oval or circular shaped nuclei, and other polygonal (Figure 2a,b). Some nuclei contained two nucleoli (Figure 2a,b). We observed melanin granules and melanocytes (Figures 2a,b and 3a) under LM and TEM.

The stratum spinosum is six to nine cell layers thick (Figures 2a and 3a). Nuclei with two nucleoli are present in some cells of the first and second layer as well as in the third and fourth layers (Figures 2a,b and 3a). The most superficial cells are flattened. Neither the semi-thin sections nor the TEM images allowed the detection of keratohyalin granules (Figure 2a,c). The stratum lucidum is absent (Figures 2a and 3a,b). The stratum corneum is well developed, very thick, and with some cells of it has pyknotic nuclei (Figure 2d).

3.2 | Dermis

The boundary between the dermis and the epidermis shows several epidermal pegs and dermal papillae (Figure 4a). The dermis has two strata: the superficial and the deep. The former is rich in cells, with a lower amount of collagen fibres, arranged in an irregular pattern, thin elastic fibres and abundant reticular fibres (Figure 4a–c). Also, a large number of arteriovenous anastomoses were recorded (Figure 4a,b). In the semi-thin slice sections, we were able to identify the three segments of the arteriovenous anastomoses: an arterial segment, a venous segment and an intermediate segment composed of an external layer of circular smooth muscle cells and an internal layer of polygonal cells (epithelioid cells), which correspond to modified smooth muscle cells (Figure 4b). The deep stratum shows three zones. The first zone, underlying the superficial

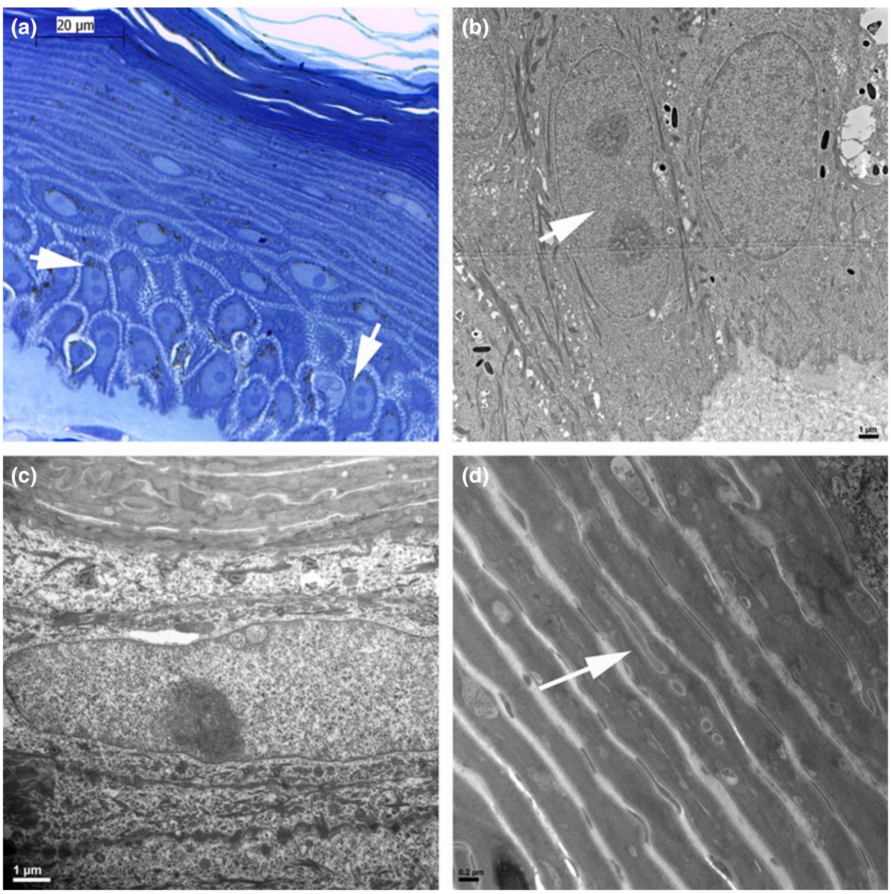

FIGURE 2 Epidermis of *Mirounga leonina*. (a) Semi-thin sections, the arrows show the keratinocytes with two nucleoli, Toluidine blue stain. (b) TEM micrographs of the stratum basale, the arrows show the keratinocytes with two nucleoli. (c) Keratinocyte underlying the stratum corneum without keratohyalin granules. (d) Stratum corneum, the arrows show pyknotic nuclei. [Colour figure can be viewed at wileyonlinelibrary.com]

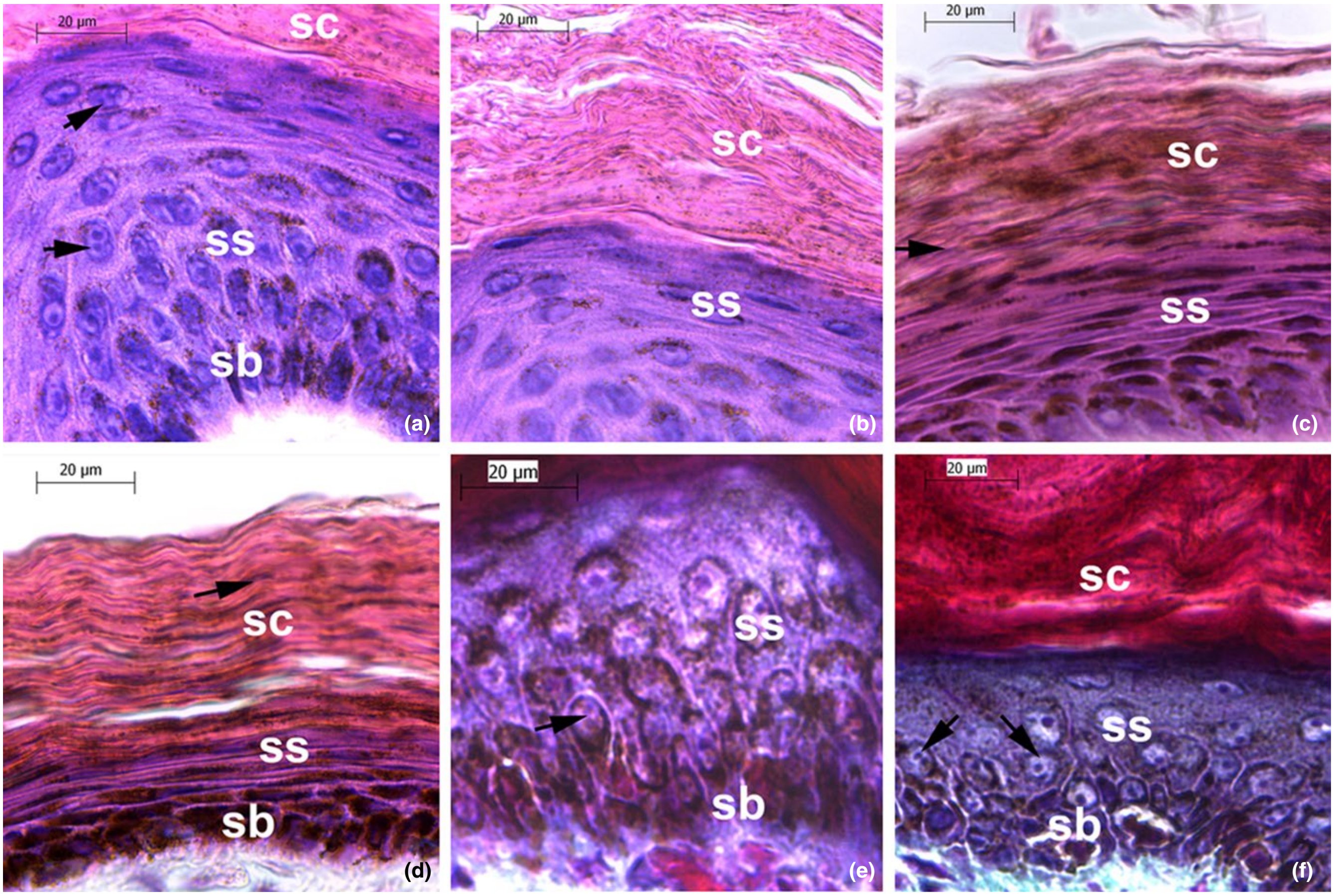

FIGURE 3 Epidermis. (a–b). *Mirounga leonina*, H-E stain. (a) The three layers of epidermis, arrows show the keratinocytes with two nucleoli. (b) More superficial layers of the stratum spinosum with stratum corneum. Note the absence of stratum granulosum and stratum lucid. (c) *Leptonychotes weddellii*, the arrows show pyknotic nuclei, H-E stain. (d) *Hydrurga leptonyx*, the arrows show pyknotic nuclei, H-E stain. (e) *Leptonychotes weddellii*, arrows show the keratinocytes with two nucleoli, Gomori trichrome stain. (f) *H. leptonyx*, arrows show the keratinocytes with two nucleoli, Gomori trichrome stain. sb, stratum basale; sc, stratum corneum; ss, stratum spinosum. [Colour figure can be viewed at wileyonlinelibrary.com]

dermis, shows thick bundles of collagen fibres (Figure 4d) and scarce arteriovenous anastomoses. The second zone is deeper, being at the same level that the basal part of the sebaceous glands, shows collagen bundles and is rich in adipocytes (adipocytes that are much more numerous in the very thick underlying hypodermis; Figure 4e,f). In the deepest zone, the bundles of muscular fibres are abundant (Figure 4g,h). Elastic fibres from the superficial stratum and those between the hair follicles are thin, but those from the deep stratum, underlying the hair follicles and glands, are much thicker (Figure 4c,i).

3.3 | Sebaceous and sweat glands

Highly voluminous sebaceous glands are associated with the hair follicles. They are bilobed and multisaccular (Figure 5a,e–g). The area around the glands is highly vascularized (Figure 5f). Sweat glands are apocrine and relatively little bulky. The adenomeres are present from the proximal to the middle part of the hair follicles (Figure 5g,h).

3.4 | Hair follicles

Only guard hairs are developed and lack the medulla. The follicles are not associated with hair erector muscles (Figure 5a–d). As a peculiarity of this species, we observed that cornified cells from the cuticle show hook-like extensions that lie between the cells from the external radicular sheath (Figure 5b,c).

The MEB images show that the guard hairs are more flattened, and they are in a lower density, than in the other two studied species (Figures 6a,b and 7a,b).

3.5 | Weddell seal epidermis

The epidermis consists of a stratum basale, and a stratum spinosum made up of 5–6 layers, therefore, is thinner than that of southern elephant seal (Figure 3c). It lacks the stratum granulosum and the stratum lucidum. The stratum corneum is well developed and shows pyknotic nuclei (Figure 3c). In all strata of the epidermis, including

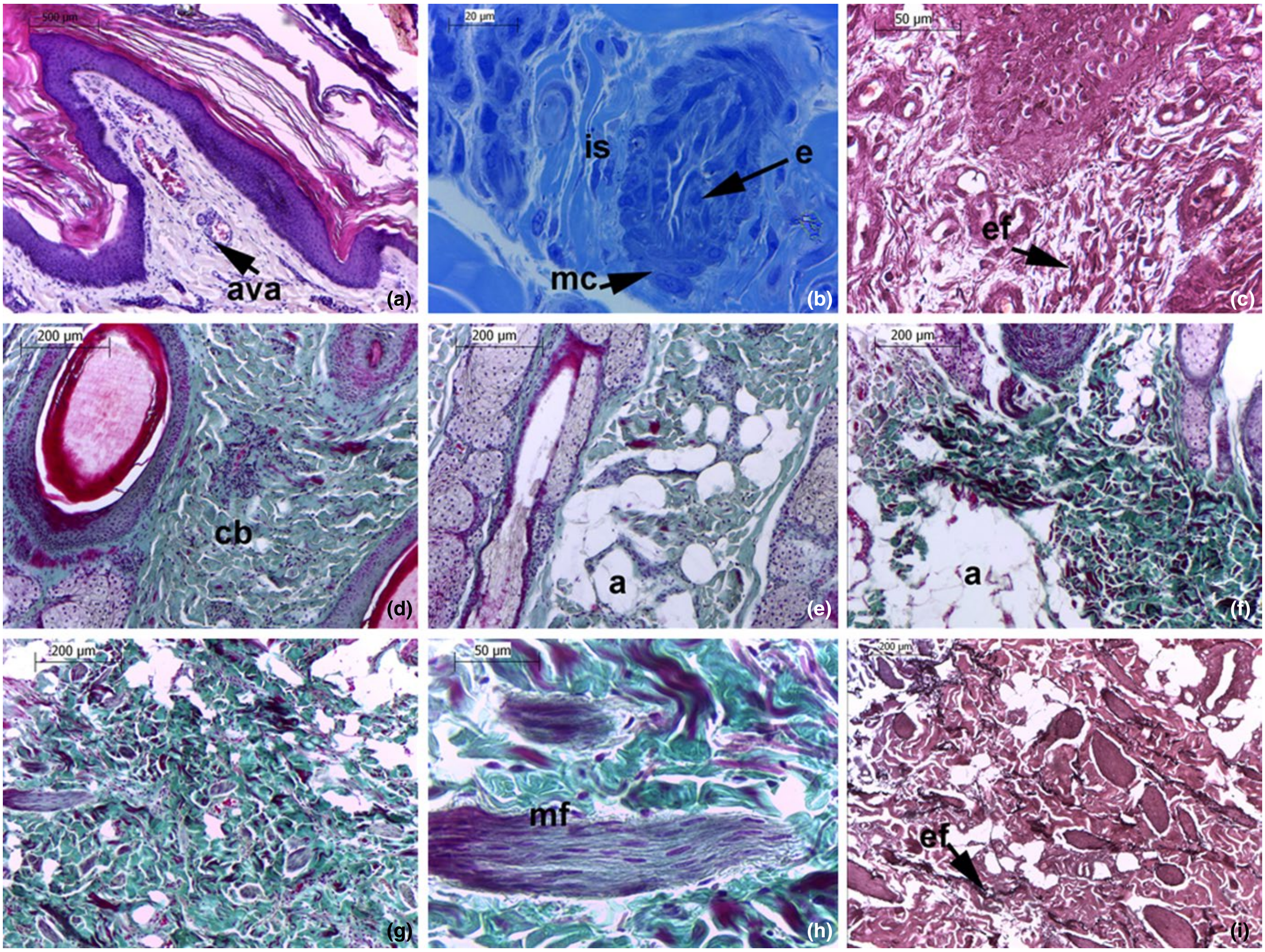

FIGURE 4 Dermis of *Mirounga leonina*. (a) Superficial layer of dermis, H-E stain. (b) Semi-thin sections of intermediate segment of AVA, Toluidine blue stain. (c) Superficial layer of dermis, Orcein stain. (d–h) Deep layer of the dermis, Gomori trichrome stain. (d) More surface zone of the deep dermis. (e,f) Middle zone of deep dermis. (g–i) Internal zone of deep dermis. (i) Orcein stain. a, adipose tissue; ava, arteriovenous anastomoses; cb, collagen bundles; e, "epithelioid smooth cell"; ef, elastic fibres; mc, circular layer of smooth muscle; mf, muscular fibres; is, intermediate segment of AVA. [Colour figure can be viewed at wileyonlinelibrary.com]

the stratum corneum, melanin granules are abundant, even more abundant than in southern elephant seal. Some nuclei contained two nucleoli in the stratum basale and stratum spinosum (Figure 3e).

3.6 | Dermis

Two strata are differentiated: the superficial and the deep stratum. The superficial stratum is poorly developed, shows numerous cells and irregularly arranged collagen fibres. It is highly vascularized, filled with arteriovenous anastomoses (Figure 8a–c).

The deep dermis shows much more organized collagen bundles and scarce arteriovenous anastomoses. In addition, a large amount of muscular fibres were observed. These muscular bundles, near the proximal part of the hair follicles (the most superficial part of the deep dermis), are arranged perpendicular or parallel to the epidermis (Figure 8d,e). Those observed in a much more internal position are parallel (Figure 8f–h). Unlike those observed in southern elephant seal, adipocytes are absent in this stratum. Elastic fibres are scarce in the superficial dermis and in large amount in the deep dermis (Figure 8c,i).

3.7 | Sebaceous and sweat glands

Similarly to those observed in southern elephant seal, the sebaceous glands of Weddell seal are bilobed and multisaccular, although they are proportionally smaller than southern elephant seal (Figure 9a,b,f,h). These glands are associated with pilary canal and located at both sides of the distal shaft of the follicles.

Apocrine sweat glands are associated with pilary canal. They are always in the deepest region of the dermis,

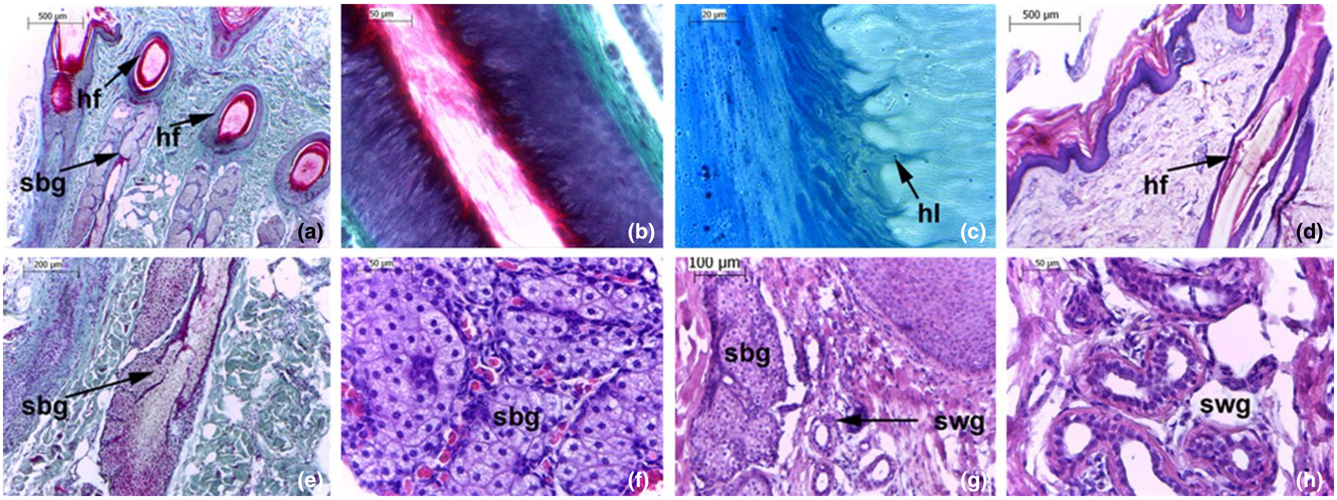

FIGURE 5 Integumentary glands and hair follicles of *Mirounga leonina*. (a) Hair follicles and sebaceous glands, Gomori trichrome stain. (b) Details of hair follicle, Gomori trichrome stain. (c) Semi-thin sections showing hook-like keratinized cells of cuticle which form attachments to the outer sheath cells, Toluidine blue stain. (d) Pilary canal of hair follicle, H-E stain. (e) Sebaceous gland, Gomori trichrome stain. (f) Details of sebaceous glands, H-E stain. (g) Sebaceous and sweat gland, H-E stain. (h) Details of sweat gland, H-E stain. hl, hook-like keratinized cells of cuticle; hf, hair follicle; sbg, sebaceous gland; swg, sweat gland. [Colour figure can be viewed at wileyonlinelibrary.com]

deeper than the sebaceous glands (Figure 9c,d,e,g). These glands are less bulky compared to those of southern elephant seal.

3.8 | Hair follicles

Hair follicles are arranged in bundles comprised of one guard or principal hair and secondary hair (generally two of them; Figure 9a–d). The guard hair and secondary hair lack the medulla. The hair follicles are not associated with erector pili muscles. The hairs within each bundle emerging from one pilary canal (Figure 9b). Guard hairs are flattened (Figure 6c,d) although less than southern elephant seal (Figure 7a). Secondary hairs are flattened too but they show a smaller diameter (0.19 mm guard hair, 0.072 mm secondary hair; Figure 6d). The hairs′ density is higher compared with the two other studied species (Figure 7b).

3.9 | Leopard seal epidermis

This species shows an epidermis with a basal-single stratum, stratum spinosum (5–6 layers), and a thick stratum corneum (Figure 3d). No stratum granulosum or lucidum were observed. The stratum corneum showed pyknotic nuclei. Like in Weddell seal, all strata were filled with melanin granules. Some cells of the stratum spinosum showed two nucleoli (Figure 3f).

3.10 | Dermis

The dermis is divided into two strata: a superficial and a deep one. The superficial stratum, like in the other species, shows a great number of cells and thin collagen fibres (Figure 10a–c). In addition, thin elastic fibres and arteriovenous anastomosis are present (Figure 10a–c).

The deep dermis shows thick bundles of collagen (Figure 10d). The elastic fibres in between the hair follicles are thin, whereas the ones in the deepest dermis are thick, like in the other two species (Figure 10e,i). Many bundles of smooth muscle fibres are present (Figure 10f–h). Although adipocytes are present, especially in association with the dermal papillae of the hair follicles, they are proportionally lesser than in southern elephant seal (Figure 10d).

3.11 | Sebaceous and sweat glands

The sebaceous glands are bilobed, multisaccular, and are associated with each hair canal (Figure 11a–c,g,h). They are proportionally less developed than in southern elephant seal, and more similar to those of Weddell seal. Sweat glands are in a position similar to that in Weddell seal, but less developed (Figure 11e,f).

3.12 | Hair follicles

Hairs are grouped in pairs composed by a guard hair and a secondary hair, which are proximally separated but share pilary canal. As in the other two species, the hairs lack the medulla, and no erector muscle was observed (Figure 11a–e).

The guard hairs are flattened (Figure 6e,f) but less flattened in comparison with the other species (Figure 7a). Secondary hairs are flattened too and have a lower density in comparison with the Weddell seal (Figure 7b).

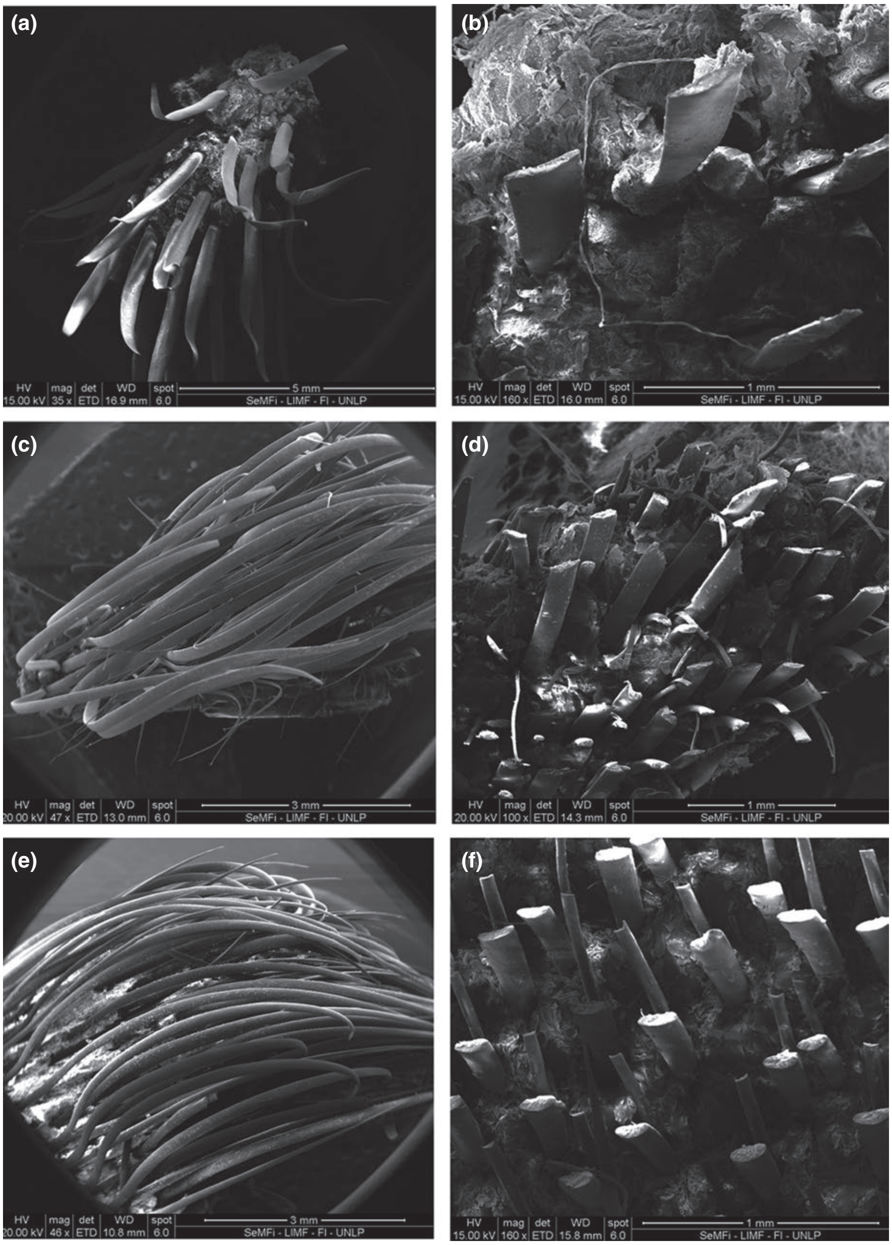

FIGURE 6 MEB images of hairs of three species of phocids. (a) Guard hairs of *Mirounga leonina*. (b) Cross-sectional cuts of guard hairs of *Mirounga leonina*. (c) Primary (or guard) and secondary hairs of *Leptonychotes weddelli*. (d) Cross-sectional cuts of guard and secondary hairs of *Leptonychotes weddelli*. (f) Guard and secondary hairs of *Hydrurga leptonyx*. (g) Cross-sectional cuts of guard and secondary hairs of *H. leptonyx*

4 | DISCUSSION

4.1 | Epidermis

In the three studied species, the epidermis lacks both the stratum granulosum and the stratum lucidum, and the stratum corneum shows pyknotic nuclei, which are features of a parakeratotic epidermis. With the exception of few particular cases, the epidermis of mammals is usually orthokeratotic. A parakeratotic epidermis is found in animals that possess epidermal scales (e.g., the tail of the house mouse *Mus musculus*, Alibardi, 2004; and armadillos, Krmpotic et al., 2014) and also described in aquatic mammals (e.g., cetaceans, Meyer, Neurand, & Klima, 1995; Reeb, Best, & Kidson, 2007). Montagna and Harrison (1957) suggested that this kind of parakeratotic epidermis in aquatic mammals would be beneficial for them because it would avoid detachment of the corneal scales when coming into contact with water, associated with an epidermis with slow cellular replacement as suggests the low amount of mitotic cells within it. In phocids, this is a matter of controversy. Montagna and Harrison (1957) stated that the stratum granulosum is mostly absent in the epidermis of *Phoca vitulina*, and that its presence is limited to a single discontinuous layer in few restricted areas; regarding to southern elephant seal, Spearman (1968) reported the presence of a single and discontinuous layer of stratum granulosum, and Ling (1968) suggested that a stratum granulosum as such is absent, and that just only scattered cells with granules are present in some areas, and both authors pointed out the absence of pyknotic nuclei within the stratum corneum. In contrast

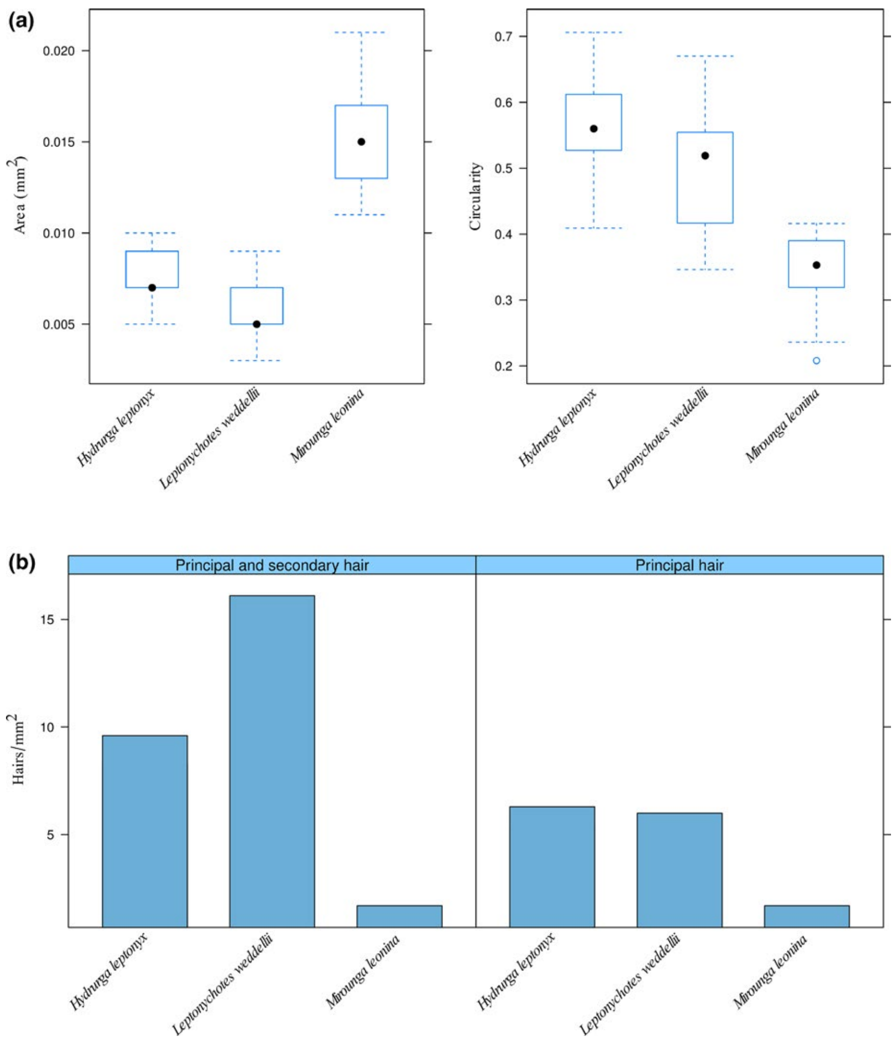

FIGURE 7 (a) Box-and-whisker plots of the area (in mm²) and circularity of Guard hairs of three species of phocids. (b) Barplot of density of hairs (number of hairs/mm²) in three species of phocids. [Colour figure can be viewed at wileyonlinelibrary.com]

with the statement of the cited authors, in southern elephant seal (using LM and TEM), we were not able to find granulosus cells forming a stratum or a single layer, not even scattered ones, but we did observe pyknotic nuclei within the stratum corneum. Regarding leopard seal, Gray et al. (2006) observed a discontinuous stratum granulosum, but they did not mention the presence of pyknotic nuclei in the stratum corneum. However, our observations revealed the absence of the stratum granulosum and the presence of pyknotic nuclei within the stratum corneum. In turn, the epidermis of Weddell seal has not been previously described; it lacks the stratum granulosum and shows pyknotic nuclei in the stratum corneum. Erdsack et al. (2015) describe in the seals skin soft flexible overlapping lobes that cover the hair follicles and proximal parts of the hair shafts. According to these authors, this structural configuration might play a role in drag reduction in one or several ways. Perhaps the type of parakeratotic cornification could be related to this structural change.

Among the described species, southern elephant seal comparatively shows the thickest epidermis. According to Sokolov (1982), the thickening of the stratum corneum should be directly correlated with a decreasing fur density. Given that during the terrestrial phases, southern elephant seal usually gather in large groups where aggressive interactions are frequent (Negrete et al., 2011), a thick epidermis could improve the protection that is lacking because of the low density of hairs.

The great amount of melanin granules in the two pack-ice seals studied here is probably due to a cellular protective system against the highly intense solar radiation they are exposed to, because melanin acts like an efficient shield against highly solar exposure (Sokolov, 1982). As solar radiation in Antarctica could be higher than in other ecosystems because of the high albedo of the snow and ice surfaces, it seems reasonable that the concentration of this pigment is higher in species that spend most of their non-aquatic phase on pack ice rather than on the continent.

Two nucleoli were frequently observed in both the stratum basale and the first layers of the stratum spinosum in all three species. The presence of two nucleoli could be related to an endoreplication process during epidermal differentiation. Several authors have pointed out that the keratinocytes above

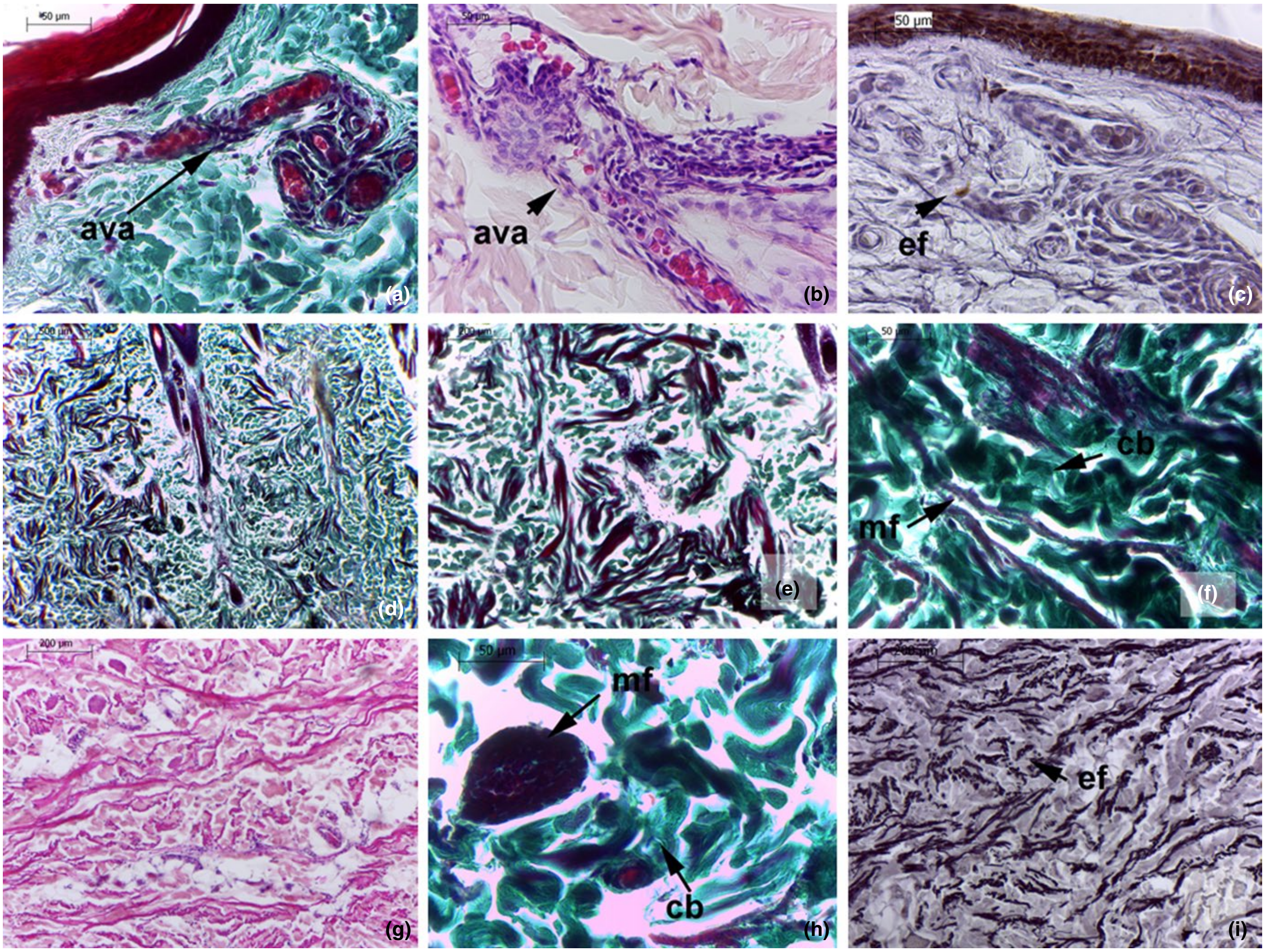

FIGURE 8 Dermis of *Leptonychotes weddellii*. (a–c) Superficial layer of dermis. (a) Gomori trichrome stain. (b) H-E stain. (c) Orcein stain. (d–i) Deep layer of the dermis. (d–f) More surface zone of the deep dermis, Gomori trichrome stain. (g–i) Internal zone of deep dermis. (g) H-E stain. (h) Gomori trichrome stain. (i) Orcein stain. ava, arteriovenous anastomoses; cb, collagen bundles; ef, elastic fibres; mf, muscular fibres. [Colour figure can be viewed at wileyonlinelibrary.com]

the basal layer could undergo endoreplication (Gandarillas & Freije, 2014; Trakala & Malumbres, 2014; Zanet et al., 2010). Endoreplication implies that keratinocytes from the stratum spinosum could replicate their DNA without undergoing cellular division. As a result, the nuclei would still grow as polyploid (Gandarillas & Freije, 2014). These supra basal keratinocytes would be larger and smaller number of cells would be necessary to cover the surface, therefore decreasing the rate of cell division in the basal cells and accordingly decreasing the probability of mutations. In addition, the number of gene copies would be amplified, causing cells to produce RNA and differentiation proteins more efficiently (Gandarillas & Freije, 2014). Although only sections for LM were available for the other two species, we observed keratinocytes that probably possess two nucleoli. As the endoreplication process could be widely spread in mammals, it would not be unexpected to find it in animals frequently exposed to high solar radiation, like those studied in the present work.

4.2 | Dermis

In the three species studied, we recorded common features regarding the dermis. A superficial and a deep dermis were recognized. The superficial dermis is thin with several arteriovenous anastomoses. The presence of arteriovenous anastomoses would be related to thermoregulation. It has been suggested that arteriovenous anastomoses are more developed in regions like flippers (regions with extremely thin hypodermal adipose tissue), operating as thermal windows (Khamas et al., 2012). Nevertheless, other authors like Molyneux and Bryden (1978) suggested that the density of AVAs in Weddell and elephant seals is approximately eight times greater than those reported in other animals and the superficial position of anastomoses over the whole body surface is the characteristic of phocid seals. In cold water, or very low temperature conditions, seals would keep warm by constriction of superficial blood vessels, and in warmer

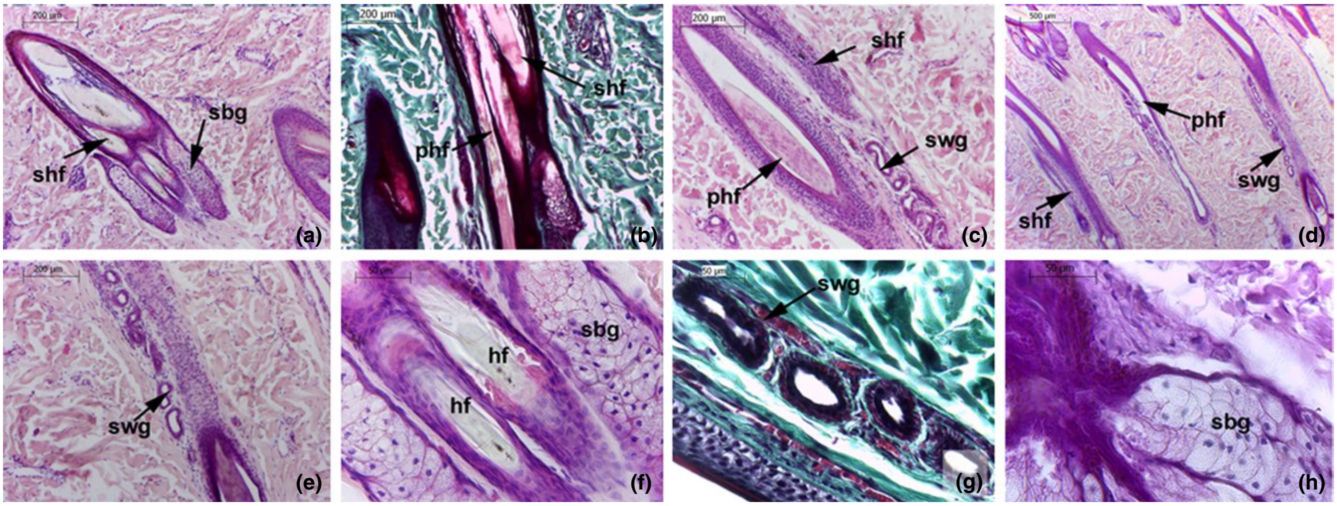

FIGURE 9 Integumentary glands and hair follicles of *Leptonychotes weddellii*. (a) Hair bundle and sebaceous glands, H-E stain. (b) Pilary canal of primary (guard) and secondary hair, Gomori trichrome stain. (c–f) H-E stain. (c–e). Primary and secondary hair follicles with sweat gland. (f) Hair follicles and sebaceous glands. (g) Details of sweat gland, Gomori trichrome stain. (h) Details of sebaceous gland, H-E stain. hf, hair follicles; sbg, sebaceous gland; swg, sweat gland; phf, primary hair follicle; shf, secondary hair follicle. [Colour figure can be viewed at wileyonlinelibrary.com]

conditions, heat can be dissipated by pumping blood into the peripheral system (Molyneux & Bryden, 1978). This may also be related to diving, as, during this activity, circulation is restricted only to vital organs. We record a great amount of arteriovenous anastomoses in the superficial dermis of all three species, without remarkable differences between them.

In the deep dermis, we recorded interspecific differences. *Mirounga leonina* showed a larger proportion of adipose tissue, which may buffer metabolic demands during the long fasting periods experienced by this species (Best, Bradshaw, Hindell, & Nichols, 2003; Lambert, Meynier, Donaldson, Roe, & Morel, 2013; Samuel & Worthy, 2004). Weddell seal showed comparatively little fat tissue in the dermis. In the deep dermis, muscular fibres were highly developed and the arrangement varied according to the zone. This arrangement was not observed in the other species studied. Leopard seal has adipose tissue. In the deep dermis of the three species, many muscular fibres were unrelated to hair follicles. These muscular fibres would play an important role improving skin motility during swimming (Sokolov, 1982). This would also be related to the great amount of elastic fibres that allow increasing skin elasticity (Sokolov, 1982). These muscular fibres would probably be important also when the animals are on land, given that, during terrestrial locomotion, all phocids perform creeping movements with most of their ventral side in contact with the soil.

4.3 | Glands

Only apocrine sweat glands were observed in the three studied species; these were poorly developed in general but comparatively more developed in Weddell seal. These glands have virtually no thermoregulatory function (Matsuura & Whittow, 1974), are commonly related to interspecific recognition (e.g., sexual attraction, mother–offspring recognition; Kovacs, 1995; McCann, 1982) and are responsible for the strong odour of phocids (Riedman, 1990). Differences in development of sweat glands are presumably related to behavioural differences such as recognition, type and duration of lactation, parental care and amount of hair follicles. Recent studies have suggested that mother pup recognition in Weddell seals, at least at close range, could be a combination of olfactory and visual cues rather than acoustic cues (Opzeeland, Parijs, Frickenhaus, Kreiss, & Boebel, 2012). On the other hand, southern elephant seal is the species with the least amount of hair follicles and would have less developed sweat glands.

With respect to the sebaceous glands, those of southern elephant seal showed the greatest sizes. According to Montagna and Harrison (1957), the sebum of *Phoca vitulina* lacks cholesterol, and the absence of this substance would be an adaptation to increase the impermeability of the epidermis. In contrast, Ling (1968) found 9% of cholesterol in the sebaceous gland secretion of southern elephant seal.

4.4 | Hair follicles

Spearman (1968) observed a peculiar feature in southern elephant seal, where hair follicles in telogen phase are firmly united to the external radicular sheath by means of hook-like projections of cornified cells from the cuticle, which interdigitate with the cells from the radicular external sheath in the base of the hair. This observation was confirmed in the present work.

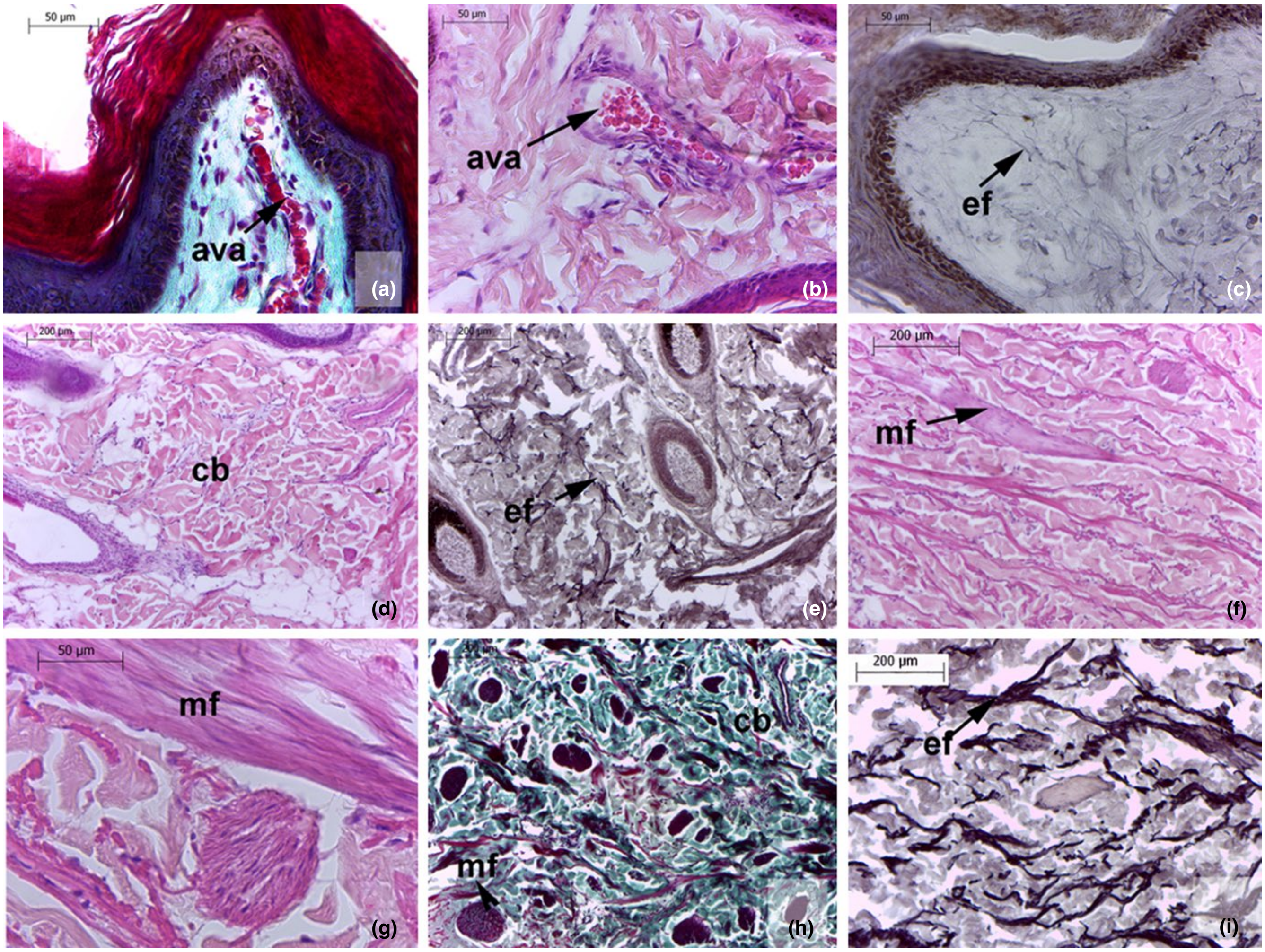

FIGURE 10 Dermis of *Hydrurga leptonyx*. (a–c) Superficial layer of dermis. (a) Gomori trichrome stain. (b) H-E stain. (c) Orcein stain. (d–i) Deep layer of the dermis. (d–e). More surface zone of the deep dermis. (d) H-E stain. (e) Orcein stain. (f–i) Internal zone of deep dermis. (f–g) H-E stain. (h) Gomori trichrome stain. (i) Orcein stain. ava, arteriovenous anastomoses; cb, collagen bundles; ef, elastic fibres; mf, muscular fibres. [Colour figure can be viewed at wileyonlinelibrary.com]

Regarding hair follicles, southern elephant seal is the only species studied that possesses only primary follicles that emerge individually to the exterior. In the other two species, one primary hair and one secondary hair (leopard seal) or one primary and two secondary hairs (Weddell seal) emerging from one pilary canal together. In all cases, the primary follicles have no medulla or erector muscles. Erector muscles act to straightening the hair and form a still air chamber between the epidermis and the external flowing air, reducing heat loss. Previous works have suggested that absence of these muscles is related to the loss of thermoregulatory function of the hair in Phocidae (Ling, 1968; Sokolov, 1982). This function is probably replaced by a thick subcutaneous fat layer. Regarding other functions of the hair, Montagna and Harrison (1957) suggested that secondary follicles allow phocids to keep an air bubble between the hair and the epidermis, which maintains the skin dryness. Nevertheless, this situation would only occur in Weddell and leopard seals where secondary follicles were observed. Sokolov (1982) suggested that air retention is lost in the water and that the main functions of the fur are to protect against injuries and to improve movements both in the water and on the ice. The fur can even modify the water turbulence.

A recent contribution suggests that guard hairs in aquatic species, including the semi-aquatic river otter, are flattened and maintain the air layer trapped by the fine fur hairs (Liwanag, Berta, Costa, Budge, et al., 2012). When the animal is submerged, the absence of hair erector muscles increases the extent to which the hairs lie flat against the body, and the overlapping arrangement of guard hairs protects the underlying air layer by preventing penetration by water (Kuhn & Meyer, 2009). The flat shape of the guard hair facilitates both laying flush when the animal is submerged and natural lifting as the fur dries (Liwanag, Berta, Costa, Budge, et al., 2012). Using SEM, we confirm that

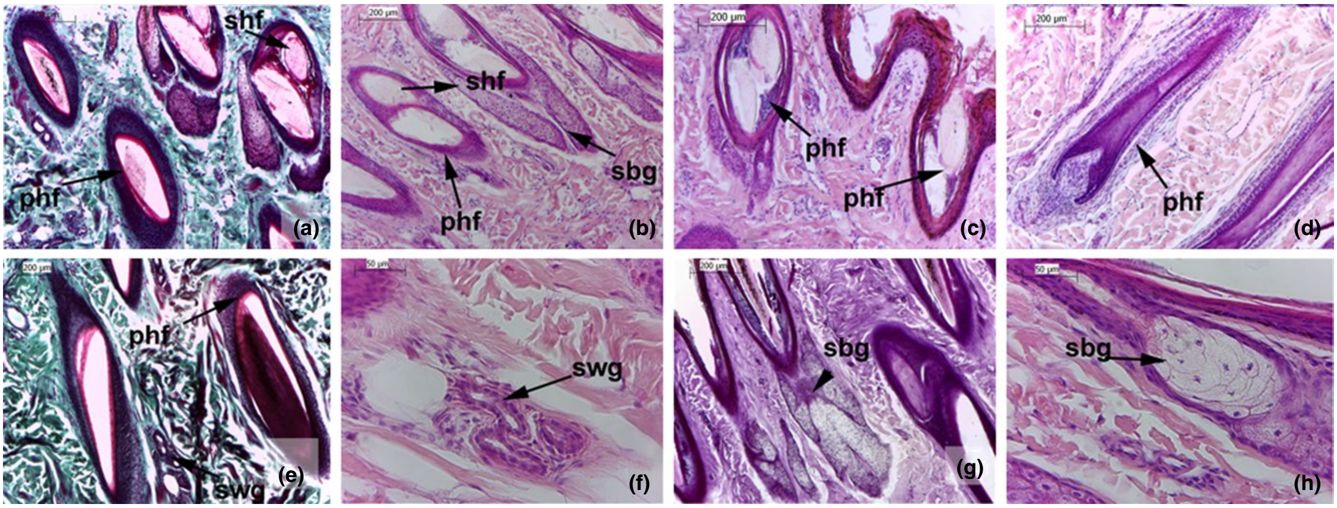

FIGURE 11 Integumentary glands and hair follicles of *Hydrurga leptonyx*. (a) Primary and secondary hair follicles and sebaceous glands, Gomori trichrome stain. (b–d) H-E stain. (b) Primary and secondary hair follicles and sebaceous glands. (c) Pilary canal of primary hair follicle. (d) Primary hair follicle. (e) Primary hair follicle, Gomori trichrome stain. (f) Details of sweat gland, H-E stain. (g) Sebaceous gland, H-E stain. (h) Details of sebaceous gland, H-E stain. hf, hair follicles; sbg, sebaceous gland; shf, secondary hair follicle; swg, sweat gland; phf, primary hair follicle. [Colour figure can be viewed at wileyonlinelibrary.com]

both guard and secondary hairs are flattened. The southern elephant seal presents only guard hairs and a minor hairs density than the other species. These characteristics could be related to a reduced thermoregulatory function of the hair. This could be compensated by a larger body size and an associated thicker blubber layer (Liwanag, Berta, Costa, Budge, et al., 2012; Liwanag, Berta, Costa, Abney, & Williams, 2012). The marked flattening of the guard hairs in the southern elephant seal probably represents a better hydrodynamic adaptation. This fact is related to the ecological characteristics of this species, whose diving activities are displayed in deeper locations. Weddell and leopard seals have secondary hairs that could have a thermoregulatory function. Weddell seal is the species with more secondary hairs and the largest hairs density. These characteristics could be explained by their austral distribution.

5 | CONCLUSIONS

The stratum granulosum is not present in the three studied species. Although these results are contradict to previous ones in southern elephant seal and leopard seal (Gray et al., 2006; Ling, 1968), it is worth noting that we only studied the lateral skin of the body, and that we did not explore the skin during the moulting period. The absence of the stratum granulosum, and the presence of pyknotic nuclei in the three species, implies the existence of a parakeratotic epidermis, which is beneficious for an amphibious aquatic life, like pinnipeds, and for strictly aquatic ones like cetaceans. The southern elephant seal shows a thicker epidermis, probably related to fur reduction.

The presence of two nucleoli in the stratum spinosum of southern elephant seal and their likely presence in the other two species might be related to an epidermal endoreplication process.

The amount of melanin in leopard seal and Weddell seal was higher than that in southern elephant seal. Melanin granules were uniformly distributed in all layers of the epidermis, and forming a protective barrier against solar radiation, which is necessary in mammals that live on ice.

The three species showed arteriovenous anastomoses in the superficial dermis related to thermoregulatory functions. The deep dermis of the three species studied was very rich in elastic and muscular fibres, a feature probably related to both swimming and on-land activity.

The hair follicles in the three species lacked medulla and erector muscles. All these species present flattened hairs, probably an adaptation to the aquatic habitat. This hair morphology associated with the absence of erective muscles would favour both a thermoregulatory, especially when the animal dives, and hydrodynamic functions. The southern elephant seal shows only primary follicles with histological features related to the moulting process. In this species, skin impermeability is probably regulated by the secretion of sebaceous glands, which are more developed than those of other studied species.

Finally, deep integument with a resistant epidermal level, a very thick adipose layer and an arteriovenous anastomoses are structural arrangement associated with extremely cold environment. The richness of elastic and muscular fibres helps to ease the undulatory movements on land and ice, and the richness of melanin protect them from the UV radiation.

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