scientific reports

Check for updates

OPEN Comparative genomics of sirenians reveals evolution of filaggrin and caspase-14 upon adaptation of the epidermis to aquatic life

Julia Steinbinder, Attila Placido Sachslehner, Karin Brigit Holthaus & Leopold Eckhart 🖂

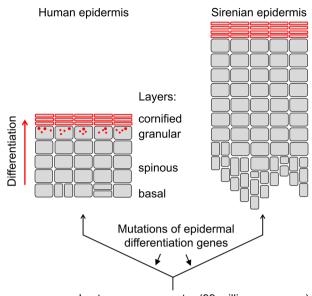
The mammalian epidermis has evolved to protect the body in a dry environment. Genes of the epidermal differentiation complex (EDC), such as FLG (filaggrin), are implicated in the barrier function of the epidermis. Here, we investigated the molecular evolution of the EDC in sirenians (manatees and dugong), which have adapted to fully aquatic life, in comparison to the EDC of terrestrial mammals and aquatic mammals of the clade Cetacea (whales and dolphins). We show that the main subtypes of EDC genes are conserved or even duplicated, like late cornified envelope (LCE) genes of the dugong, whereas specific EDC genes have undergone inactivating mutations in sirenians. FLG contains premature stop codons in the dugong, and the ortholog of human CASP14 (caspase-14), which proteolytically processes filaggrin, is pseudogenized in the same species. As FLG and CASP14 have also been lost in whales, these mutations represent convergent evolution of skin barrier genes in different lineages of aquatic mammals. In contrast to the dugong, the manatee has retained functional FLG and CASP14 genes. FLG2 (filaggrin 2) is truncated in both species of sirenians investigated. We conclude that the land-to-water transition of sirenians was associated with modifications of the epidermal barrier at the molecular level.

Life on land depends on the protection against excessive loss of water from the body in a dry environment^{1,2}. The control of water loss is mediated in part by the outermost, epithelial compartment of the skin, the epidermis. Within this stratified epithelium, keratinocytes proliferate in the basal layer and differentiate during their movement towards the skin surface while passing through the suprabasal layers (Fig. 1). Keratinocyte differentiation involves the accumulation of cytoskeletal proteins and enzymes that are required for establishing the barrier against the environment which mainly resides in the granular layer and the cornified layer of the epidermis. The transition of keratinocytes from the granular to the cornified layer is associated with programmed cell death and cross-linking of proteins to form a mechanically and chemically resilient protein envelope to which lipids are attached³⁻⁶. The integrity of cornified cell envelopes is essential for the barrier function of the epidermis^{7,8}.

A cluster of genes encoding protein components of the cornified envelope of epidermal keratinocytes is known as the epidermal differentiation complex (EDC). The EDC is located on human chromosome 1q21.3, and homologous gene clusters have been identified in other mammalian species⁹⁻¹² as well as non-mammalian tetrapods¹³⁻¹⁸. The EDC is bordered by genes of the \$100A family, which are considered the evolutionarily ancestors of other EDC gene types^{13,19}. Genes in the core region of the EDC can be classified into peptidoglycan recognition protein genes, single-coding-exon EDC (SEDC) genes and S100 fused-type protein (SFTP) genes. Peptidoglycan recognition protein 3 (PGLYRP3) and PGLYRP4 genes have antimicrobial functions²⁰ and differ substantially from other EDC genes¹³. SEDCs contain one protein-coding exon and one non-coding exon, whereas SFTPs comprise one non-coding and two protein-coding exons¹³. Gene families, such as small proline-rich proteins (SPRRs) and late-cornified envelope (LCE) genes, are crucial for the formation of the cornified envelope and belong to the SEDCs. Loricrin, involucrin, keratinocyte proline rich protein (KPRP), KPRP N-terminal and LCE C-terminal like protein (KPLCE), which was formerly known as LEP7, XP32 or Clorf68 (chromosome 1 open reading frame 68), proline rich 9 (PRR9) and late cornified envelope like proline rich 1 (LELP1) are SEDCs that exist as single-copy genes in the human genome.

SFTPs contain an S100 domain at their N-terminus and a long, sequence repeat-rich domain at the C-terminus^{9,10}. Additionally, a short C-terminal sequence motif is conserved in most SFTPs²¹. Seven SFTP genes, i.e.

Department of Dermatology, Medical University of Vienna, Vienna, Austria. 🖾 email: leopold.eckhart@meduniwien. ac.at



Last common ancestor (99 million years ago)

Figure 1. Keratinocyte differentiation and epidermal structure in humans and sirenians. The structure of the epidermis is schematically depicted. Cells are shown as squares with rounded corners. Red borders indicate the cornified envelope, consisting of cross-linked proteins. Red dots indicate keratohyalin granules in the granular layer of human epidermis. Differentiation of keratinocytes leads to the passive movement of cells from the inner to the outer layers and involves cornification, leading to flattening and death of keratinocytes at the surface of the skin.

cornulin (*CRNN*), filaggrin (*FLG*), filaggrin 2 (*FLG2*), hornerin (*HRNR*), repetin (*RPTN*), trichohyalin (*TCHH*) and trichohyalin-like1 (*TCHHL1*), are present in the human EDC. The best characterized genes among the latter are *FLG* and *TCHH*. FLG contributes to keratin filament aggregation in the epidermis, hydration of the stratum corneum and UV protection of the skin²². On histological sections, FLG forms, together with other proteins, basophilic keratohyalin granules in late differentiated but not yet cornified keratinocytes which form the granular layer of the epidermis. Mutations of the *FLG* gene are linked to skin barrier diseases, such as ichthyosis vulgaris and atopic dermatitis²³. TCHH interacts with keratins and is expressed in the inner root sheath of the hair follicle, the tongue filiform papillae and the nail isthmus²⁴.

Two clades of mammals have adapted to a fully aquatic lifestyle, cetaceans and sirenians. The former comprise whales, dolphins and porpoises and, together with artiodactyls, form the clade Cetartiodactyla within the superorder Laurasiatheria. Sirenians comprise manatees and dugongs and belong to the superorder of Afrotheria, with proboscideans (elephants) being their closest extant relatives²⁵. Land-dwelling ancestors of cetaceans and sirenians independently underwent the evolutionary transition to life in the sea.

The skin of sirenians differs histologically from that of terrestrial mammals and shows some similarities to that of cetaceans, as it contains a subcutaneous fat layer called blubber and lacks sweat glands, and the epidermis is thicker than that of terrestrial mammals^{26,27} (Fig. 1). Furthermore, the epidermis lacks a granular layer and contains a thickened cornified layer of incompletely characterized structure in sirenians and cetaceans^{27–29}. Specialized epithelial structures, namely vibrissae and keratinized pads that replace incisors have evolved as an adaptation of sirenians to feeding on seagrass³⁰.

The genes encoding many epidermal proteins have been studied in detail in cetaceans, but only very incompletely in sirenians. Among EDC genes, *LOR*, *IVL*, *SPRRs* and *CRCT1* have been conserved in cetaceans, whereas *KPRP*, *KPLCE* and *LCEs* with the exception of *LCE7A* are absent in all cetaceans and *PRR9* and *LELP1* have been lost in subclades of cetaceans¹¹. Keratins forming the cytoskeleton in the suprabasal epidermis of land-dwelling mammals, i.e. KRT1, KRT2, KRT9 and KRT10, are not conserved in cetaceans and they are also inactivated by mutations in the manatee^{31,32}. Additional genes with functions in the epidermis were lost in cetaceans^{33,34}.

In the present study, we analyzed the EDC of two species of sirenians in comparison to their homologs in humans and other mammals. We report that the coding sequence of the important skin barrier gene *FLG* is truncated and the FLG-processing protease, caspase-14, is inactivated by mutations in the dugong. However, we also demonstrate that most other EDC genes are conserved in sirenians and encode functional proteins, indicating roles of EDC genes that are not associated with the barrier to a dry environment.

Results

Identification of the EDC in the genomes of sirenians

We investigated the EDC in the partly annotated genome sequence of the manatee and the not-yet-annotated genome sequence assembly of the dugong (Supplementary Tables S1, S2; Supplementary Figs. S1, S2). The gene organization of the EDC of sirenians was compared to the EDC in the Asian elephant (*Elephas maximus indicus*)

(Supplementary Table S3; Supplementary Fig. S3), as a representative member of the phylogenetically closest terrestrial clade of mammals, the order Proboscidea. Furthermore, the human EDC was included in comparative analyses. The sequence of the EDC of the dugong was available as a continuous scaffold without sequence gaps, whereas genes of EDC of the manatee were identified on different sequence contigs that were not finally assembled at the time of this study (December 2023) (Fig. 2).

The EDC of both species of sirenians is comprised of S100A, PGLYRP, SEDC and SFTP genes in an arrangement homologous to that in other mammals^{9–11}. We focused on the genes located between *S100A9* and *S100A11*. *PGLYRP3* is free of disruptive mutations, whereas *PGLYRP4* contains inactivating mutations in its coding sequence (Supplementary Fig. S4). Conservation of *PGLYRP3* and loss of functional *PGLYRP4* was also detected in the elephant, suggesting that the inactivation of *PGLYRP4* has occurred in a common ancestor of sirenians and elephants. Both intact genes and pseudogenes were also identified among the main types of EDC genes, that is, SEDCs and SFTPs, as will be described in detail below.

Late cornified envelope (LCE) genes have been amplified in the dugong

Comparative analysis showed that sirenians have orthologs of all subtypes of SEDC genes (Fig. 2). *Loricrin, PRR9, LELP1, involucrin (IVL), SMCP, KPRP, KPLCE* and *CRCT1* are present as single copy genes in both manatee and dugong (Fig. 2, Supplementary Tables S1 and S2). Multiple paralogs of SPRRs and LCE genes are arranged in gene clusters in sirenians, similar to their homologs in elephants and humans. Due to gaps in the genome sequence of the manatee, the precise arrangement and the numbers of SPRR and LCE genes could not be determined for the manatee. In the dugong, twenty-one protein-coding SPRR genes and additional pseudogenized *SPRRs* are located between the *LELP1* and *IVL* genes. This number of SPRRs is smaller than that in the elephant (n = 34), but larger than the number of human SPRR genes (n = 12).

Strikingly, the number of LCE genes is greatly increased in the dugong as compared to both elephant and humans. With 3 LCE genes in cluster 1 between *SMCP* and *KPRP* and 47 *LCE* genes in cluster 2 between *KPLCE* and *CRCT1*, the dugong has more than twice as many LCE genes as humans (n = 19) and the elephant (n = 15) (Figs. 2 and 3). The increase in the number of *LCEs* is due to the amplification of LCE2 paralogs which show slight variation at amino acid positions of the entire length of the protein (Fig. 3). Phylogenetic analysis confirmed that the main cluster of LCE genes of the dugong is monophyletic (Supplementary Fig. S5). Fewer LCE paralogs were identified in the genome of the manatee, which, however, contained several gaps in the region of the LCE genes (Fig. 2).

Divergent evolution of KPLCE in elephants and sirenians

KPLCE is a gene that has been recently re-named by GenBank after it was originally reported as *LEP7*, *XP32* or *C1orf68*. The KPLCE protein is characterized by a tripartite organization with an N-terminal segment, a central region with imperfect sequence repeats (Supplementary Fig. S6) and a C-terminal segment, which are largely conserved across species (Fig. 4). However, KPLCE of the manatee has an unusual organization as it contains more sequence repeats than its homologs in other species and lacks the C-terminal segment (Fig. 4). The EDC of the Asian elephant contains 7 copies of *KPLCE*, of which 6 encode proteins and one is a pseudogene (Fig. 2). The KPLCE proteins of the elephant are characterized by a shortened C-terminal segment, which lacks a subsegment of 59 amino acid residues present in human KPLCE (Fig. 4).

To estimate when in evolution the copies of *KPLCE* have emerged, we investigated the EDC of the African Savannah elephant (*Loxodonta africana*) and the rock hyrax (*Procavia capensis*). The African elephant has at least two intact copies of *KPLCE*, whereas the hyrax has only one (Fig. 4). This pattern suggests that the amplification of KPLCE has occurred in the phylogenetic lineage leading to elephants, and only one *KPLCE* gene was present in the common ancestor of sirenians, elephants and hyrax.

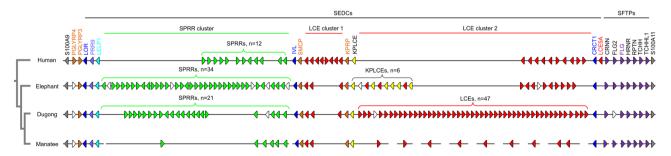


Figure 2. Comparison of the epidermal differentiation complex (EDC) in sirenians, elephant and human. The core region of EDC is represented by genes between *S100A9* to *S100A11*, which are schematically depicted as arrows pointing in the direction of transcription. Gene families are illustrated in identically colored arrows. White arrows indicate genes with a disrupted coding sequence by either premature stop codons or frameshifts. Gene family clusters are indicated by a bracket, where the "n" indicates the number of genes in the cluster. A cladogram shows the relation of the investigated species. Species: Human (*Homo sapiens*), elephant (*Elephas maximus indicus*), manatee (*Trichechus manatus latirostris*) and dugong (*Dugong dugon*). SEDC, simple EDC gene (1 coding exon); SFTP, S100 fused-type protein.

			minal segment			C-termina	1 segment rich in C/G/S	6 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
uman uman	LCE1A LCE1B	MSCQQSQQQCQ MSCQQNQQQCQ		TPKCPPKCPPKCPPVSS	CCSVSSGGCCGSSSGGGC	SSGGGGG	CLSHH-RRHRSHRHR-LQSSG <mark>CC</mark> SQPSGGS CLSHH-RRRRSH <mark>C</mark> HR-PQSSG <mark>CC</mark> SQPSGGS	-SCCGGDSGQHSGC
uman	LCE1C	MSC00S000C0			CCSVSSGGCCGSSSGGSCGSSSG	GCCSSGGGGC	CLSHH-RRRRSHCHR-PQSSGCCSQPSGS	- SCCGGGSGOHSG
uman	LCE1D	MSC00S000C		APKCPPKCPPVSS	CCSVSSGGCCGSSSGGGCGSNSG	GCC SSGGGGC	CLSHH-RRHRSHRRR-POSSDCCSOPSGGS	- SCCGGGSSOHSG
ıman	LCE1E	MSCQQSQQQC	РРРКСТРКСРРКСР	T <mark>PKCPPKCPPKCPP</mark> VSS	<mark>cc</mark> svssgg <mark>cc</mark> gsssggs <mark>c</mark> gsssg	G <mark>CC</mark> SSGGGG <mark>C</mark>	CLSHH-RRHRSHRRR- <mark>P</mark> QSSD <mark>CC</mark> SQPSGGS CLSHH-RHHRSHRHR-PQSSD <mark>CC</mark> SQPSGGS	- SCC GGGSGQHSGC
man	LCE1E	MCCOOCOOC	DDDVCTDVCDDVCD		n evecen n eccentration eccentr	CCC SSCCCCC		-sccccccoused
lephant	LCE1EL1	MS <mark>C</mark> QQSQQQ <mark>C</mark> Q	<mark>PPPKC</mark> TPKCPPKCP	T <mark>PKCPPKCPPKCP</mark> SASS	CCGSSGDW <mark>C</mark>	SSGGGG	CLSHH-RHRRS <mark>C</mark> RHR-HHSSE <mark>CC</mark> SQ <mark>P</mark> SGGSGG <mark>C</mark> GGS	-S <mark>CC</mark> GGGNGQSSG
ephant	LCE1EL2	MS <mark>C</mark> QQNQQQ <mark>C</mark> Q	DAS <mark>PKC</mark> AT <mark>KCPPK</mark> HPI	VSS	<mark>CC</mark> SISAGG <mark>CC</mark> G-SSGG <mark>CC</mark> GSSSV	'R <mark>CC</mark> SSGGGG <mark>C</mark>	CLSHHGH <mark>P</mark> RSHHDRHQSSD <mark>CC</mark> GSSQHSRG	- SDR <mark>C</mark> SGGSTW <mark>C</mark> S(
lephant	LCE1EL4	MSCQQNQQQCQ	ASPKCATKCPPKHPI	VSS	<mark>CC</mark> SISAGG <mark>CC</mark> G-SSGG <mark>CC</mark> GSSSV	R <mark>CC</mark> SSGGGG	CLSHHGHPRSHHDRHQSSDCCGSSQHSRG	-SDR <mark>C</mark> SGGSTW <mark>C</mark> SG
igong	LCE1	MSCQQNQQQC	ODSPKCTPKCPPKCP	TTKCPPKYPPVSS	CCSVSSGGCCGPSSEGCCGS	SSGGGG	CLSHH-RRHRSYRHR-HQSSDCWSQP	
ıman	LCE2A	MSCQQNQQQC	OBBERCEDERC b	- PKCPPKCRPQCPAPCPPPVSS	ccgpssggccgsssggcc	SSGGGG	CLSHH-RPRLFHRHR-HQSPDCCEC	- EPSGGSGCCHSSC
iman	LCE2B	MSCQQNQQQCQ			CCGP1SGGCCGPSSGGCC	NSGAGG	CLSHH-RPRLFHRRR-HQSPDCCES	-EPSGGSGCCHSSG
man	LCE2C	MSCQQNQQQC				SSGAGG		
uman	LCE2D	MSCQQNQQQC			ccsc			
onhant	LCE2AL2	MSC00S000C			CC30	SSGAGSC		
enhant	LCE2AL2	MSC00S000C			ccsv	SSGAGSC		
gong	LCE2ALD	MSWOONOOC				-CCSSGGGSC	CI SHH-RHHTEHRRRWHOSPOCSEC	
gong	LCE2AL2	MSWOONOOOC			CCSVSCGDCYGSSSG	-ccscggggg	CLSHH-RHHIFHRRRWHOSPOCCEC	-DSCGHSGCCMGS
gong	LCE2AL3	MSWOONOOOC	LPANCTPKCPPRCP	IPTCPPKCPOKCPPVSP	CCSVSCGDCCGPSSG	-CCRSGGGSC	CLSHH-RHHLFHRRRWHOSPDCCNC	-DPCGHSGCCSGSG
gong	LCE2AL5	MSWQQNQQQC	LPAKCTPKCPPKCP	I <mark>PKFPPKCPPKCP</mark> AVS <mark>P</mark>	CCSVSCGDCCGPSSG	-CCSSGGGRC	CLSHH-KHHVFHRRRWHQSPDCCEC	-DPCGHSGCCSGSF
gong	LCE2AL6	MSWQQNQQQC	FPAKCTPKYPPKCP	I <mark>PKCPPKCPPKC</mark> LPVSP	CCSVSCGDCCGPSSG	-CCSSGAGSC	CLSHH-RYHIFHRRRWHQS <mark>P</mark> DCCEC	-DPCGHSGCCSGSC
gong	LCE2AL7	MSWQQNQQQC	LPAKCTPKCPPKCP	I <mark>PMCPPKCPPKCPP</mark> VS <mark>P</mark>	<mark>cc</mark> svs <mark>c</mark> gD <mark>cc</mark> g <mark>P</mark> SSG	-ccsseess	CLSHH-RHHIFHRRRRHQS <mark>P</mark> DCCEC	-DPCGHSGCCSGP
gong	LCE2AL8	MSWQQNQQQC	<u>EPAKCTPKCPPKCP</u>	I <mark>P</mark> T <mark>CPPKCPPKCPP</mark> VS <mark>P</mark>	CCNVS <mark>C</mark> GD <mark>CC</mark> G <mark>P</mark> SSG	- <mark>CC</mark> SSGGSS <mark>C</mark>	CLSHH-RHHIFHRHRRHQS <mark>P</mark> DCCEC	-D <mark>PC</mark> GHSG <mark>CC</mark> SGS(
gong	LCE2AL9	MSW-QNQQQC	QL <mark>PAKC</mark> T <mark>PKCPPKCP</mark>	I <mark>PQCPPKCPP</mark> NCPP VS <mark>P</mark>	<mark>CC</mark> SVS <mark>C</mark> RD <mark>CC</mark> G <mark>P</mark> SSG	- F <mark>C</mark> SSGGGG <mark>C</mark>	CLSHH-RHHLFHRRRWHQS <mark>P</mark> DCCEC	-D <mark>PC</mark> GHSG <mark>CC</mark> SGS(
gong	LCE2AL10	MSWQQNQQQ <mark>C</mark> Q	2L <mark>PAKC</mark> T <mark>PKCPPKC</mark> P	I <mark>PKFPPKCPPKCPP</mark> VS <mark>P</mark>	<mark>CC</mark> SVS <mark>C</mark> ED <mark>CC</mark> G <mark>P</mark> SSG	- <mark>CC</mark> SSGGGS <mark>C</mark>	CLSHH-QHHVFHRRRWHQS <mark>P</mark> DC	<mark>C</mark> GHSG <mark>CC</mark> SGSF
gong	LCE2AL11	MSWQQNQQQC	DLPAKCTPKCPPKCP	IPT <mark>CLPMCPPKCPP</mark> VA <mark>P</mark>	<mark>CC</mark> SVS <mark>C</mark> GD <mark>CC</mark> G <mark>P</mark> SSG	- <mark>CC</mark> SSGGGG	CLSHH-RHHLFHRRRWHQNPDCCEC	-DPCGHSG <mark>CC</mark> LGSC
gong	LCE2AL12	MSWQQNQQQ <mark>C</mark> Q	DE <mark>PAKC</mark> NPKCPPKCP	IPT <mark>CPPKCPPKCPP</mark> VS <mark>P</mark>	CYSVS <mark>C</mark> GNG <mark>C</mark> GSSSG	- <mark>CC</mark> SSGGGG	SLSHH-RHHIFHQRRWHQS <mark>P</mark> D <mark>CC</mark> ES	-DPCGHSGCSSGS(
gong	LCE2AL13	MSWQQNQQQ <mark>C</mark> Q	DLPAKCTPRCPPKCP	LPM <mark>CPPKCPPKCPP</mark> VSP	CCSVSCGDCCGPSSG	-CCSSGGSSC	CLSHH-RHHIFHRRRRHQSPDCCEC	-DPCGHSGCCSGSC
gong	LCE2AL14	MSWQQNQQQC		LPKCPPKCPPKCPPVSP				
gong	LCE2AL15	MSHIDDNDDCC				-00000000000000000000000000000000000000		
gong	LCE2AL16					-005500550		
gong	LCE2AL1/							
gong	LCE2AL18	MSWOONOOO						
gong gong	I CE2AL 19	MSWHONKOOC				-CCSSGGGGG	LUSHIN HINKING (RINR. HINSSEC) SOCIAS (SQ) SGGS GGG GGG GGG CLUSHING RISHIDR HIGSSOC (SS) (HSRG CLISHI-RINRING RISHIRA HIGSSOC (SS) (HSRG CLISHI-RINRI FURRIR HIGSSOC (SS) (HSRG CLISHI-RINRI FURRIR HIGSSOC (SS) (HSRG CLISHI-RINRI FURRIR HIGSSOC (SC) (HSRG CLISHI-RINRI FURRIR HIGSSOC (SC) (HSRG CLISHI-RINRI FURRIR HIGSSOC (SC) (HSRG CLISHI-RINI FURRIR HIRKSSOC (SC) (HSRG CLISHI-RINI FURR HIRKSSOC (SC) (
gong	LCE2AL21	MSWOONOOOCO			CCSVTCGDCCGPSSG	-CCSSGGGGG	CLSHH-RHHIFHRHRRHOSPDCCEC	-DPCGHSCCCSGS
gong	LCE2AL22	MSWHONKOOC			CCNVSCGDCCGPSSG	-CCSSGGGGG	CLSHH-RHHLFHRRRWHOGPDCCEC	-DPCGHSGCCSGS
gong	LCE2AL23	MSWOONOOOC			CCSVSCGDCCGPSSG	-ccsseess	CLSHH-RHHIFHRRRRHOSPDCCEC	-DPCGHSGCCSGS
gong	LCE2AL24	MSWOONOOOC		IPT <mark>CPPKCPPKCPP</mark> VSP	ccsvscgdccgsssg	-ccsseeee	SLSHH-RHHIFHRRRWHOSPDCCEC	-DPCGHSGCSSGS
gong	LCE2AL25	MSW-QNQQQC	Ι. <mark>ΡΑΚ</mark> ΟΤΡΚΟΡΡΚΟΑΙ	IPT <mark>CPPKCPPKCPP</mark> VS <mark>P</mark>	ccsvscgdccgpssg	-ccsseeeec	CLSHH-RHHLFHRRRWHQSPDCCEC	-DPCGHSGCCSGSC
gong	LCE2AL26	MSWQQNQQQC	LPAKCTPKCPPKCP	I <mark>PKFPPKCPPKCP</mark> AVS <mark>P</mark>	<mark>cc</mark> svs <mark>c</mark> gD <mark>cc</mark> g <mark>P</mark> SSG	-ccsseeesc	CLSHH-QHHVFHRRRWHQS <mark>P</mark> DCCEC	-DPCGHSGCCSGSI
gong	LCE2AL27	MSWQQNQQQC	EPAKCTPKYPPKCP	I <mark>PTCLPKCPPKCPP</mark> VS <mark>P</mark>	<mark>CC</mark> SVS <mark>C</mark> GD <mark>CC</mark> G <mark>P</mark> SSG	- <mark>CC</mark> SSGGGS <mark>C</mark>	CLSHH-RHHIFHRRRWHQS <mark>P</mark> DCCEC	-DSCGHSGCCSSS\
gong	LCE2AL28	MSWHQNQQQC	<u>OLPAKCTPKCTPKCP</u>	I <mark>PK<mark>CPPKC</mark>PP<mark>KCPPK</mark>C-PPVPP</mark>	<mark>CC</mark> NVS <mark>C</mark> GD <mark>CC</mark> G <mark>P</mark> RSG	-CCSSGGGGG	CLSHH-RHHLFHRRCWHQSPDCCEC	-DPCGHS-CCSGSG
gong	LCE2AL29	MSWQQNQQQ <mark>C</mark> Q	<u>EPAKCTPKCPLKCP</u>	I <mark>PKCPPKCPP</mark> VS <mark>P</mark>	<mark>CC</mark> SVS <mark>C</mark> RD <mark>CC</mark> G <mark>P</mark> SSG	- <mark>CC</mark> SSGGGS <mark>C</mark>	CLSHH-RHHIFHRRRWHQS <mark>P</mark> DCCEC	-D <mark>PC</mark> GHSG <mark>CC</mark> SGS(
gong	LCE2AL30	MSWQQNQQQ <mark>C</mark> Q	ŨL <mark>PSKC</mark> TS <mark>KCPPKCP</mark>	I <mark>P T CPPK CPPK CPP</mark> VS <mark>P</mark>	<mark>CC</mark> SVS <mark>C</mark> GD <mark>CC</mark> G <mark>P</mark> SSG	- <mark>CC</mark> SSGGGG	CLSHH-RHHIFHRRHRHQS <mark>P</mark> DCCEC	-D <mark>PC</mark> GHYG <mark>CC</mark> SGS(
gong	LCE2AL31	MSWQQNQQQ <mark>C</mark> Q	2LPAKCTPKCPPKCP	I <mark>PNCPPKCPPKCPP</mark> VSP	<mark>CC</mark> SVS <mark>C</mark> GD <mark>CC</mark> G <mark>P</mark> SSG	- <mark>CC</mark> SSGGGG	CLSHH-RHHLFHRRRWHQS <mark>P</mark> DCCEC	-D <mark>PC</mark> GHSG <mark>CC</mark> SG <mark>P</mark> (
gong	LCE2AL32	MSWQQNQQQC	OLPYKCTSKCPPKCP	IPTCPPKCPPKCPPVSP	CCSVTCGDCCGPSSG	- <mark>CC</mark> SSGGGG	CLSHH-RHHIFHRRRRHQSPDCCEC	-DPCGHSGCCSGSG
gong	LCE2AL33	MSWQQNQQQC			CCSVTCGDCCGPSSG	-ccsseeeee	CLSHH-RHHIFHRHRRHQSPDCCEC	-DPCGHSCCCSGSC
gong	LCE2AL34	MSWHQNKQQCQ						
igong	LCE2AL35	MSWQQNQQQC						
igong	LCE2AL30	MSHOUNDOOC						
igong igong	LCE2AL37	MSWOONOOOC						
igong	LCE2AL39	MSWOONOOOC						
gong	LCE24140	MSW-0N000C			CSVSCGDCCGPSSG			
gong	LCE2AL41	MSWOONOOOC	LPAKCTPKCPPKCP	IPSCPPKCPPKCPPVSP	CCSVSCGDCCGTSSG	-ccsseeee	CLSHH-RHHLFHRRRWHKSPDCCEC	-DPCGHSGCCSGP
gong	LCE2AL42	MSWQQNQQQC	LPAKCTPKCPPKCP	IPT <mark>CLPKCPPKCPP</mark> VS <mark>P</mark>	ccsvscgdccgpssg	-ccsseeeec	CLSHH-RHHLFHRRRWHQS <mark>P</mark> DCCEC	-DPCGHSGCCSGP
gong	LCE2AL43	MSWHQNKQQC	DE <mark>PAKCTPKYPPKCP</mark>	I <mark>PKCPPKCPPKCPP</mark> VPP	CCNVS <mark>C</mark> GD <mark>CC</mark> GPSSG	- <mark>CC</mark> SSGGGG	CLSHH-RHHLFHRRRWRS9DCCEC CLSHH-RHHLFHRRRWRS9DCCEC CLSHH-RHHLFHRRRWRS9DCCEC CLSHH-RHHLFHRRRWRS9DCCEC CLSHH-RHHLFHRRRWRS9DCCEC CLSHH-RHHLFHRRRWRS9DCCEC CLSHH-RHHLFHRRRWRS9DCCEC CLSHH-RHHLFHRRRWRS9DCCEC CLSHH-RHHLFHRRRWRS9DCCEC CLSHH-RHLFHRRRWRS9DCCEC CLSHH-RHLFHRRRWRS9DCCEC	-DPCGHSGCCSGS
gong	LCE2AL44	MSWQQNQQQC	<u>D</u> LPAKCTPKCPPKCP	I <mark>PT<mark>CPPKCPPKCPP</mark>VS<mark>P</mark></mark>	<mark>CC</mark> NVS <mark>C</mark> GD <mark>CC</mark> G <mark>P</mark> SSG	- <mark>CC</mark> SSGGSS <mark>C</mark>	CLSHH-RHHIFHRRRRHQS <mark>P</mark> DCCEC	-D <mark>PC</mark> GHSG <mark>CC</mark> SGS
gong	LCE2AL45	MSW-QNHQQ <mark>C</mark> Q	<u>Į</u> L <mark>PAKC</mark> T <mark>PKCPPKCP</mark> I	I <mark>PQCPPKCPPKCPP</mark> VS <mark>P</mark>	<mark>CC</mark> SLS <mark>C</mark> GD <mark>CC</mark> G <mark>P</mark> SSG	- <mark>CC</mark> SSGGGG <mark>C</mark>	CLSHH-RHHLFHRRRWHQS <mark>P</mark> DCCEC	-D <mark>PC</mark> GHSG <mark>CC</mark> SGS
gong		MSWQQNQQQ <mark>C</mark> Q	<u>2</u> LPAKCTPKCPPKCP	IPT <mark>CPPKCPPKCPP</mark> VS <mark>P</mark>	CCSVS <mark>C</mark> GD <mark>CC</mark> GPSSG	- <mark>CC</mark> SSGGGG	CLSHH-KHHIFHRRRWHQSPDCCEC	-D <mark>PC</mark> GHSG <mark>CC</mark> SGS(
natee	LCE2AL1		2L <mark>PAKC</mark> TPE <mark>CPPKC</mark> P	I PKCPPKCPPKCPP VSP	CCSVNSADCCGPSSG	- <mark>CC</mark> SSGGGG	CLSHH-RQRLFH	
natee	LCE2AL2			IPKCPPKCPPKCPP VSP	CCSVSCGDCCGPSSG	-CCSSGGGGG	CLSHH-RHHFHRRHAHTIGOGEE CLSHH-RHHFHRRHAHTIGOGEE CLSHH-RHHFHRRHAHTIGOGEE CLSHH-RGRXSHRGR-RGSSNSCDRGSG CLSHH-RGRXSHRGR-RGRSNSCDRGSG CLSHH-RGRXSHRGR-RGRSNSCDRGSG CLSHH-RGRXSHRGR-RGRSNSCDRGSG CLSHH-RRKSHRGR-RSSNCSDOGSG CLSHH-RRKSHRGR-RSSNCSDOGSG CLSHH-RRKSHRGR-RSSNCSDOGSG CLSHH-RRKSHRGR-RSSNCSDOGSG CLSHH-RRKSHRGR-RSSNCSDOGSG CLSHH-RRKSHRGR-RSSDCSDOGRG CLSHH-RRKSHRGR-RSSDCSDOGRG CLSHH-RRKSHRGR-RSSDCSDOGRG CLSHH-RRKSHRGR-RSSDCSDOGRG CLSHH-RRKSHRGR-RSSDCSDOGRG CLSHH-RRKSHRGR-RSSDCSDOGRG CLSHH-RRKSHRGR-RSSDCSDOGG	-DPCGHSGC
natee	LCE2AL3				CLSVSCODCCGPSSG	- <u>vc</u> ss66666		-DPCGHSGC
nan	LCE3A	MSCQQNQQQCQ	PPKCP/	A <mark>KSP</mark> AQ <mark>CLPP</mark> ASS		SSERS-C		-QQGGSSSCGHSS/
man man	LCE3B LCE3C		PLPKCP	SPKCPPKSSAQCLPPASS SPKCPPKSPAQCLPPPSS				-QQUGASUCGYGSG
nan nan	LCE3C	MSCQQNQQQCQ MSCQQNQQQCQ		SPKCPPKSPAQCLPPPSSI SPKCPPKSPVQCLPPASSI				- QQGGGSCCKGHGS
nan	LCE3D	MSCQQNQKQCQ MSCQQNQKQCQ		SPKCPPKNPVOCLPPASS	GCAPSSGGCG-P	SSF66-0	FLNHH-RRHHRCR-RORSNSCDRGSG	-006665666
	LCE3CL1	MSCQQNQQQYQ		SPKCPPKSPAQCLPKVSS	GCALTSGGCHGP	SSETG-C	CLRHH-RRRRSHRCR-RRSSNCSDDGSG	-00SGGSHCGHSS
	LCE3CL2	MSCQQNQQQYQ		SPKCPPKSPAQCLPKVSS	GCALTSGGCHGP	SSETG-C	CLSHH-RHHRSHRCR-RRSSNCSDDGSG	-QQSGGSHCGHSS
	LCE3CL3	MSCQQNQQQC		L <mark>PKCPPK</mark> SPAQ <mark>C</mark> SPKVSS	G <mark>C</mark> ALSSGG <mark>C</mark> HG <mark>P</mark>	RSEAG-C	CLSHH-RRCRSHRCQ-HQNSDCSDNGSG	-QQSGGSRCDHSS
phant		MSCQQNQQQY	A <mark>PPKCP</mark>	S <mark>PKCPPK</mark> SPAQ <mark>C</mark> LPKVSS	G <mark>C</mark> ALTSGG <mark>C</mark> HG <mark>P</mark>	SSETG-C	CLRHH-RRRRSHRCR-RRSSNCSDDGSG	-QQSGGSHCGHSS
ong	LCE3DL2	MSCQQNQQQC	PPPKCP	S <mark>PKCPPK</mark> SPAQ <mark>C</mark> WPPVSS	G <mark>C</mark> A <mark>P</mark> SSGG <mark>C</mark> HG <mark>P</mark>	GSEGG-C	CLSPH-RCRRSHRCR-CQSSDCSDNGRG	-QQSGGSHCGHSS
atee	LCE3DL1	MSCQQNQQQC)H <mark>PPKCP</mark> :	S <mark>PKCPPK</mark> S <mark>P</mark> AQ <mark>C</mark> WPPVSS	G <mark>C</mark> A <mark>P</mark> SSGG <mark>C</mark> HG <mark>P</mark>	GSEGG- <mark>C</mark>	CLSPH-RRRRSHRCR-RQSSNCSDDGRG	-QQSGGSH <mark>C</mark> GHSS
atee	LCE3DL2	MSYQQNQQQC	H <mark>bbkCb</mark> ;	S <mark>PKCPPK</mark> S <mark>P</mark> AQ <mark>C</mark> WPPVSS	G <mark>C</mark> A <mark>P</mark> SSGG <mark>C</mark> HG <mark>P</mark>	GSEGG- <mark>C</mark>	CLSPH-RRHRSHRCR-RRSSDCSDDGHG	-QQSGGSH <mark>C</mark> GHSS
atee	LCE3DL3	MS <mark>C</mark> QQNQQQ <mark>C</mark> Q	<mark>P</mark> P <mark>PPKCP</mark> :					
atee	LCE3DL4	MSGQQNQQQC)H <mark>PPKCP</mark> :	S <mark>PKCPQK</mark> SPAQ <mark>C</mark> WPPVSS	G <mark>C</mark> A <mark>P</mark> SSGG <mark>C</mark> HG <mark>P</mark>	GSEGG- <mark>C</mark>	CLSPH-RHRRSHR <mark>C</mark> R-RQSSD <mark>C</mark> SDDGRG	-QQSGGSH <mark>C</mark> GHSS
atee	LCE3DL5	MS <mark>C</mark> QQNQQQ <mark>C</mark> Q	<u>HPPKCP</u>	S <mark>PKCPPK</mark> SPVQ <mark>C</mark> WPPVSS	G <mark>C</mark> T <mark>P</mark> SSGG <mark>C</mark> HG <mark>P</mark>	SSERS- <mark>C</mark>	CLS <mark>P</mark> H-RHRRSHR <mark>C</mark> R-RQSSN <mark>C</mark> SDDGRG	-QQSRGSH <mark>C</mark> GHSS
atee	LCE3DL6	MSGQQNQQQC	<mark>P</mark> PPKCP	S <mark>PKCPQKSP</mark> AQ <mark>C</mark> WPPVSS	G <mark>C</mark> A <mark>P</mark> SSGG <mark>C</mark> HG <mark>P</mark>	GSEGG- <mark>C</mark>	CLSPH-RHRRSHRCR-RQSSDCSDDGRG	-QQSRGSH <mark>C</mark> GHSS
nan	LCE4A	MS <mark>C</mark> QQNQQQ <mark>C</mark> Q	<mark>)PPPKCP</mark>	I <mark>PKYPPKCPSKC</mark> ASS <mark>CPPP</mark> ISS	<mark>CC</mark> GSSSGG <mark>C</mark> G	- <mark>CC</mark> SSEGGG <mark>C</mark>	CLSPH-RHRRSHRCR-RQSSDCSDDGRG CLSPH-RHRRSHRCR-RQSSNCSDDGRG CLSPH-RHRRSHRCR-RQSSDCSDDGRG CLSHH-RHRSHCHR- <mark>P</mark> KSSNCYGSGSG	-QQSGGSG <mark>CC</mark> -SG
ıan	LCE5A	MSCQQSQQQC	PPPKCTPKCPPKCT	- PKCPPKCPPKCPPQCSAP	CPPPVSS <mark>CC</mark> GSSSG	G <mark>CC</mark> SSEGGG <mark>C</mark>	CLSHH-RPRQSLRRR-PQSSSCCGSGSGQQSGGSSC	CHSSGGSGCCHSS
ian	LCE6A	MS-QQKQQSW	(PPNVPKCS	PPQRSNPCLAPYSTPCGAP		HSEG	CHSSSQR <mark>P</mark> EVQKPRRARQ <mark>K</mark> LR <mark>C</mark> LSRGTT	-YH <mark>CK</mark> EEE <mark>C</mark> EGD-
	LCE6A	MS - QQ <mark>K</mark> QR <mark>P</mark> C	L P DA <mark>PKC</mark> S	PPQCPNPGFARCCSTC		SGGY	CLHS-QR-SAQNPGRPRRTRRKPRCLRGGTI	-YH <mark>CK</mark> EEE <mark>C</mark>
gong	LCE6A	MS-Q <mark>KK</mark> QQS <mark>C</mark> Q	PPDTPKCS	LQCPNPYLAPS <mark>CAPC</mark>		SGS <mark>C</mark>	CLGS-QRPRAQSPVCPKRAHWKPRCLLGGTI	-YH <mark>CK</mark> EEE <mark>C</mark>
natee	LCE6A	MS-QKKQQSCE	PLDTPKCS	LQCPNPYPAPCCTPC		SGSC	CLGS-QRPRAQSPVCPKRAHWKPHCLRGGTI	-YHCKEEEC
ian	LCE7A	MSYQKHQQKWQ				-CISGEGGHC	CLSHH-RHRISHCHR-WESSNCYSSGSG CLSHH-RRGXCRRR-DCSSSCGSGSGSGSGSG CHS-SQRQEVQUBRRARQ	SRCSTC-YSS
	LCE7A LCE7A	MSVOOCKOKCK				-CISGEGGNC		
gong		INSTUUSKUKCK		VALUAPAPULPPAPS	CCMP ST	-CISOFOOKC	CLASUALLE TELLA CLASS	LLTSSCCHSSE

Figure 3. Amplification of late cornified envelope (LCE) proteins in dugong. Amino acid sequence alignment of LCE proteins in sirenans, elephant and human. Cysteine (C), glutamine (Q), lysine (K) and proline (P) are highlighted in yellow, grey, blue and green, respectively. Species: Human (*Homo sapiens*), elephant (*Elephas maximus indicus*), manatee (*Trichechus manatus latirostris*) and dugong (*Dugong dugon*).

Filaggrin and trichohyalin-like 1 genes contain premature stop codons in sirenians

SFTP genes form a cluster in the EDC of sirenians like in other mammals. All of the SFTP genes present in humans and elephants have homologs in sirenians (Fig. 2). However, due to premature stop codons the proteins encoded by *FLG*, *FLG2* and *TCHHL1* are more than 50% shorter in sirenians than in elephants and humans (Fig. 5A, Supplementary Fig. S7). A characteristic short amino sequence motif, that has been suggested to mediate binding of SFTPs to keratins³⁵, is conserved in 6 out of 7 SFTPs of humans and elephants (Fig. 5B), but only in 4 and 5 proteins encoded by SFTP genes of the dugong and manatee, respectively. Both species of sirenians lack the C-terminal motif in the predicted FLG2 and TCHHL1 proteins (Fig. 5B). The C-terminal motif of SFTPs²¹ is

			<u>N-terminal segment</u> >< <u>Repeat</u> >< <u>Re</u>
Human	([M <mark>C</mark> DQQKQPQF PPSCVKGSGLGAGQGSNGASVK<mark>CP</mark>VPCQTQTVCVTGPAPCPTQTVVKYQV<mark>PCQ</mark>TQTYVKCPAPCQ-RTYVKYPTPCQ-TY MCDQQEQQRFPPTCVKGLRLGSVQSTKCTSVKCAVPCETKTVSVVCPDPCQTQTYVKCPVPCKT-TCVKCPPPCQTQTYVKCPVPCQTTY
			MCDQQEQQYFPPTCVKGLKLGSVQSTKCTSVKCAAPCETKAVSVVCPDPCQTQTVVKCPAPCKT-TCVKCPPPCQTQTTVKCPVPCQTT MCDQQEQQYFPPTCVKGLKVGSVQSTKCTSVKCAAPCETKAVSVVCPDPCQTQTVVKCPAPCKT-TCVKCPTPCQTQSVVKCPPPCQTTY
Elephant	(Em)	KPLCE5	MCD00E00HFPPSCVKGLKVGSVOSTKCTSMKCAAPCKTKTVSVVCPDPC0T0TYVECSVPC0T-TCVKCPTPC0T0TYVKCPAPC0TTY
Elephant	(Em)	KPLCE3	M <mark>C</mark> DQQEQQHF PP S <mark>CVKGLK</mark> VGSVQSTK <mark>C</mark> TSMK <mark>C</mark> AA <mark>P</mark> CETKTVSVVC <mark>P</mark> D <mark>PC</mark> QTQTYVKCSVL <mark>C</mark> QT-M <mark>CVKCP</mark> TLCQTQTYVKCPAACQTTY
Elephant	(Em)	KPLCE6	M <mark>C</mark> DQQEQQRF PP S <mark>CVKGLK</mark> LGSVQSTKCTSMKCAAPCETKTVSVVCPDPCQTQTYVKCSVLCQT-MCVKCPTLCQTQTYVKCPAACQTTY
			M <mark>C</mark> DQQEQQYF <mark>PP</mark> T <mark>CVK</mark> GLRLGSVQSTK <mark>C</mark> TSVKCAA <mark>PC</mark> ETKAVSVVCPDPCQTQTYVKCPA <mark>PCK</mark> T-T <mark>CVKCPTPC</mark> QTQSYVKCPPPCQTTY
			M <mark>C</mark> DQQEQQRF PPTCVK GLRLGSAQSTK <mark>C</mark> TSVKCSVPCETKTVSVVCPDPCQTQTYVKCPAPCKT-TCVKCPPPCQTQTYVKCPVPCQTTY MCDQQEQQYF PPTCVKGLK LGSVRSTKCTSVKCAAPCETKAVSVVCPDPCQTQTYVKCPAPCKT-TCVKCPTPCQTQSYVKCPPPCQTTY
Hyrax	(La)		MCDQQEQQYPPPTCVKGLKLGSVRSTKCTSVKCAAPCETKAVSVVCPDPLQTQTVKCPAPCKT-TCVKCPTPCQTQSVKCPPPCQTTY MCEQQKQQQFPPSCVKGSGLGSAKYATSQEAKTGSVIGPAPCQTQTV-VKCPAPCPTQTVVKYQIPCQTQTVVKCPAPCQTQTYVKCPAPCQTQTYVKCPAPCQTTY
Dugong		KPLCE	MCD00K000FP05CVKG5GLGFV05IKGKFVKCAAPCETKTV5VTCPDPC0M0TLVKCFAPCFT0TVV0Y0VPC0T0TCVKCPVPY0TTC
Manatee		KPLCE	M <mark>C</mark> DQQKQQEF <mark>PQSCvK</mark> GSGLGFVQSTKGKFVK <mark>C</mark> AA <mark>PC</mark> ETKTVSVT <mark>CP</mark> APCQTQTLVKCPAPCPTQTYVQYEV <mark>PC</mark> QTQTCVKYPAPYQMT <mark>C</mark>
Human		KDI CE	Repeat X Repat X Rep
	(Fm)	KPLCE KPLCE1	VKCPAPCQTTTVKCPIPCQTTVVKCPPPCQTTVVKCPAPCQTTVVKCPVQCQMT <mark>CVKCP</mark> VPCQTTVVKCPVPCQT
Flenhant	(Fm)	KPI CE3	
Elephant	(Em)	KPLCE4	VKCPTPCQTQSYVKCPPPCQTTYVKCPAPCQTTYVKCPAPCQTTYVKCPAPCQTTCVKCPV
Elephant	(Em)	KPLCE6	
Flenhant	(Em)	KPLCE7	ϒ <mark>ϗϹϼͳϷ</mark> ϲϙͳϙϿϔϒϒ <mark>ϗϹϷϼϷ</mark> ϲϙͳϮϒϒ <mark>ϗϹϷ</mark> ϼϷϲϙͳϮϒϒϗϹϷϙϷϲϙͳϮϔϒϗϹϷϒϷϲϙͳϮϾϒϗϹϷϒϷϲϙͲϮϾϒϗϹϷϒϷϲϙͲϮϾϒϗϹϷϒϷϲϙͲϯϾϒϗϹϷϒϷ ϒϗϹϼͳϷϲϙͲϙϛϒϒϗϹϷϷϷϲϙͳϮϒϒϗϲϷϷϷϲϙͳϮϒϒϗϹϷϒϷϲϙͲϮϾϒϗϹϷϒϷϲϙͳϮϾϒϗϹϷϒϷϲϙͲϮϾϒϗϹϷϒϷϲϙͳ
		KPLCE2	
Hyrax	()	KPLCE	VK <mark>CPAPCQK</mark> -TYV <mark>KCPPPC</mark> QTMYVK <mark>CPVP</mark> RQTM <mark>C</mark> VKCPPPCQT
Dugong		KPLCE	VKCPTPCOT-TYVKCPTPCOTTYVKCPTPCOTTCVKCPTPCOTTYVKCPTPCOTTCVKCPTPCOTTCVKCPTPCOTTYVKCPTPCOT
Manatee		KPLCE	Ÿ <mark>ĸĊŖŦ₽</mark> ĊŎŦŦ <mark>ĊŸĸĊŖŦ₽Ċ</mark> ŎŦŦ <mark>ĊŴĊŖŦ₽Ċ</mark> ŎŦŦ <mark>ĊŸĸĊŖŦ₽Ċ</mark> ŎŦŦĊŸ <mark>ĸĊŖŦ₽Ċ</mark> ŎŦŦŸ <mark>ŸĸĊŖŦ₽Ċ</mark> ŎŦŦĊ <mark>ŴĸĊŖŦ₽Ċ</mark> ŎŦŦŶ <mark>ŴĸĊŖŦ</mark> ₽ĊŎŦŦŶŴĸĊ <mark>Ŗ</mark> Ŧ₽
			>< Repeat ><
Human		KPI CE	TYIKSPAPCQTQTCYVQGASPCQS
	(Em)		ΤΥΥΚ <mark>C</mark> ΡΤΡCQTQTYYVQC <mark>F</mark> SPCQT
Elephant	(Em)	KPLCE3	TYVK <mark>CP</mark> TPCQTQTCYVQCPSLCQT
Elephant	(Em)	KPLCE4	<mark>c</mark> QTTYVK <mark>CPTPC</mark> QTQTYYVQC <mark>P</mark> S <mark>PC</mark> QT
Elephant	(Em)	KPLCE5	TYVKCPTPCQTQTCYVQCPSPCQT
Elephant	(Em)	KPLCE6	TYVKCPTPCQTQTC CQTTYVKCPTPCQTQTC YVQCPSPCQT
Elephant	(La)	KPLCE1	IVVGTTPCQTQTVVQCPSPCQT
Elephant	(La)	KPLCE2	
Hyrax		KPLCE	
Dugong		KPLCE	TVVKCPTPCKTQTVYVQCPSPCHT
Manatee		KPLCE	ĊQTTYV <mark>KCPTPC</mark> QTTYV <mark>KCPTPCQTTC</mark> VKCPT <mark>P</mark> CQTT <mark>C</mark> VKCPTPCQTTCVKCPTPCQTTCVKCPTPCQTTYVKCPTPCQTTCVKCPTPCQTTC
			Repeat >< Repeat
Human		KPLCE	
Elephant	(Em)	KPLCE	
Elephant Elephant	(Em)	KPLCE KPLCE3	
Elephant Elephant Elephant Elephant	(Em) (Em) (Em)	KPLCE KPLCE3 KPLCE4 KPLCE5	
Elephant Elephant Elephant Elephant Elephant	(Em) (Em) (Em) (Em)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6	
Elephant Elephant Elephant Elephant Elephant Elephant	(Em) (Em) (Em) (Em) (Em)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7	
Elephant Elephant Elephant Elephant Elephant Elephant Elephant	(Em) (Em) (Em) (Em) (Em) (La)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE1	
Elephant Elephant Elephant Elephant Elephant Elephant Elephant	(Em) (Em) (Em) (Em) (Em) (La)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE1 KPLCE2	
Elephant Elephant Elephant Elephant Elephant Elephant Elephant Hyrax	(Em) (Em) (Em) (Em) (Em) (La)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE1 KPLCE2 KPLCE	
Elephant Elephant Elephant Elephant Elephant Elephant Elephant	(Em) (Em) (Em) (Em) (Em) (La)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE1 KPLCE2 KPLCE KPLCE	
Elephant Elephant Elephant Elephant Elephant Elephant Elephant Hyrax Dugong	(Em) (Em) (Em) (Em) (Em) (La)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE1 KPLCE2 KPLCE KPLCE	Ċ₽Ŧ₽ĊQŦŦĊŸĸĊ₽Ŧ₽ĊQŦŦŸŸĸĊ₽Ŧ₽ĊQŦŦŸŸĸĊ₽Ŧ₽ĊQŦŦŸŸĸĊ₽Ŧ₽ĊQŦŦŸŸĸĊ₽Ŧ₽ĊQŦŦŸŸĸĊ₽Ŧ₽ĊQŦŦŸŸĸĊ₽ŦQŎŦŢŸŸĸĊ₽ŦQŎŦŢ
Elephant Elephant Elephant Elephant Elephant Elephant Hyrax Dugong Manatee	(Em) (Em) (Em) (Em) (Em) (La)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE1 KPLCE2 KPLCE KPLCE KPLCE	CPTPCQTTCVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTQTPC >< Repeat >< Repeat >< Repeat >< Repeat >< C-terminal segment
Elephant Elephant Elephant Elephant Elephant Elephant Elephant Hyrax Dugong Manatee Human	(Em) (Em) (Em) (Em) (Em) (La) (La)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE1 KPLCE KPLCE KPLCE KPLCE	CPTPCQTTCVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTQTPC >< Repeat >< Repeat >< Repeat >< Repeat >< C-terminal segment
Elephant Elephant Elephant Elephant Elephant Elephant Hyrax Dugong Manatee Human Elephant Elephant	(Em) (Em) (Em) (Em) (La) (La) (Em) (Em)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE1 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE	CBTPCQTTCVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTQTPC >< Repeat >< Repeat >< Repeat >< C-terminal segment
Elephant Elephant Elephant Elephant Elephant Elephant Hyrax Dugong Manatee Human Elephant Elephant Elephant	(Em) (Em) (Em) (Em) (La) (La) (Em) (Em) (Em)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE1 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE3 KPLCE3	CPTPCQTTCVKCPTPCQTTVVK
Elephant Elephant Elephant Elephant Elephant Elephant Hyrax Dugong Manatee Human Elephant Elephant Elephant Elephant	(Em) (Em) (Em) (Em) (La) (La) (Em) (Em) (Em) (Em)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE1 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE3 KPLCE4 KPLCE4 KPLCE4	CPTPCQTTCVKCPTPCQTTVVKCPTPCQTTCVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTQC >< Repeat >< Repeat >< Repeat >< Repeat >< Repeat >< C-terminal segment
Elephant Elephant Elephant Elephant Elephant Elephant Hyrax Dugong Manatee Human Elephant Elephant Elephant Elephant Elephant Elephant	(Em) (Em) (Em) (La) (La) (Em) (Em) (Em) (Em) (Em) (Em)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE5 KPLCE7 KPLCE1 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE5	CPTPCQTTCVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTQTPC >< Repeat >< Repeat >< Repeat >< Repeat >< C-terminal segment
Elephant Elephant Elephant Elephant Elephant Elephant Elephant Hyrax Dugong Manatee Human Elephant Elephant Elephant Elephant Elephant Elephant	(Em) (Em) (Em) (Em) (La) (La) (La) (Em) (Em) (Em) (Em) (Em) (Em) (Em)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE6 KPLCE6	CFTPCQTTCVKCFTPCQTTVVKCFTQCFCC >
Elephant Elephant Elephant Elephant Elephant Elephant Elephant Hyrax Dugong Manatee Human Elephant Elephant Elephant Elephant Elephant Elephant Elephant	(Em) (Em) (Em) (La) (La) (La) (Em) (Em) (Em) (Em) (La)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE7	CPTPCQTTCVKCPTPCQTTVVKCPTQQAPassrVQSsGSQCNPDCCDC
Elephant Elephant Elephant Elephant Elephant Elephant Elephant Hyrax Dugong Manatee Human Elephant Elephant Elephant Elephant Elephant Elephant Elephant	(Em) (Em) (Em) (La) (La) (La) (Em) (Em) (Em) (Em) (La)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE4 KPLCE5 KPLCE1 KPLCE1 KPLCE2	CETECQTTCVKCETECQTTVVVQAASSTVQSSGSQCCNPCCCC
Elephant Elephant Elephant Elephant Elephant Elephant Elephant Hyrax Dugong Manatee Human Elephant Elephant Elephant Elephant Elephant Elephant Elephant Elephant Elephant Elephant Elephant Elephant	(Em) (Em) (Em) (La) (La) (La) (Em) (Em) (Em) (Em) (La)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE4 KPLCE5 KPLCE1 KPLCE2 KPLCE2 KPLCE	CETECQTTCVKCETECQTTVVKCETQCA
Elephant Elephant Elephant Elephant Elephant Elephant Elephant Hyrax Dugong Manatee Human Elephant Elephant Elephant Elephant Elephant Elephant Elephant Elephant	(Em) (Em) (Em) (La) (La) (La) (Em) (Em) (Em) (Em) (La)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE4 KPLCE5 KPLCE1 KPLCE1 KPLCE2	CFTPCQTTCVKCFTPCQTTVVKCFTQCAAPSSTVQSSGSQCKNPCCDC
Elephant Elephant Elephant Elephant Elephant Elephant Elephant Hyrax Dugong Manatee Human Elephant Elephant Elephant Elephant Elephant Elephant Elephant Elephant Elephant Elephant Elephant Elephant	(Em) (Em) (Em) (La) (La) (La) (Em) (Em) (Em) (Em) (La)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE4 KPLCE5 KPLCE1 KPLCE2 KPLCE2 KPLCE	CPTPCQTTCVKCPTPCQTTVVKCPTPCQTTCVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTQTPC >< Repeat >< Repeat >< Repeat >< Repeat >< C-terminal segment
Elephant Elephant Elephant Elephant Elephant Elephant Elephant Hyrax Dugong Manatee Human Elephant Elephant Elephant Elephant Elephant Elephant Elephant Elephant Elephant Elephant Elephant Elephant	(Em) (Em) (Em) (La) (La) (La) (Em) (Em) (Em) (Em) (La)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE3 KPLCE4 KPLCE4 KPLCE5 KPLCE6 KPLCE1 KPLCE1 KPLCE2 KPLCE KPLCE	C#TPCQTTCVKCPTPCQTTVVKCPTPCQTTCVKCPTPCQTTVVQ
Elephant Elephant	(Em) (Em) (Em) (Em) (La) (Em) (Em) (Em) (Em) (La) (La)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE7 KPLCE7 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE5 KPLCE4 KPLCE5 KPLCE1 KPLCE1 KPLCE1 KPLCE1 KPLCE4 KPLCE4 KPLCE4 KPLCE4 KPLCE4 KPLCE4 KPLCE4 KPLCE4 KPLCE4 KPLCE4 KPLCE4 KPLCE4 KPLCE4 KPLCE4 KPLCE4 KPLCE4 KPLCE4 KPLCE4 KPLCE4 KPLC24 KPLC44 KPL	CPTPCQTTCVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTPCQTTVVKCPTQTPC >< Repeat >< Repeat >< Repeat >< Repeat >< C-terminal segment
Elephant Elephant	(Em) (Em) (Em) (Em) (La) (Em) (Em) (Em) (Em) (La) (La) (La) (Em) (Em) (Em) (Em)	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE3 KPLCE4 KPLCE4 KPLCE5 KPLCE6 KPLCE4 KPLCE KPLCE KPLCE KPLCE KPLCE	C@TPCQTTCVKC@TPCQTTVVKC@TPCQTTCVKC@TPCQTTVVKC@TPCCLC C-terminal segment > @RTFGVS@LRRWIQR@QNCNTGSSGCCENSGSSGCCGSGCGCSGCGCGSCGCGCGSCGCCLGIIPMSSR@ACCDLEDDDCCC
Elephant Elephant	(Em) (Em) (Em) (Em) (La) (Em) (Em) (Em) (Em) (Em) (La) (Em) (Em) (Em) (Em) (Em) (Em) (Em) (Em	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE6 KPLCE7 KPLC27 K	CETPCQTTCVKCETPCQTTVVKCETPCQTTCVKCETPCQTTVVKCETCQTCVKCETCQTTVVKCETCQCLGITPMSSRGPACCDLEDDDCCC
Elephant Elephant	(Em) (Em) (Em) (Em) (La) (La) (Em) (Em) (La) (La) (Em) (La) (Em) (Em) (Em) (Em) (Em) (Em) (Em) (Em	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE1 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE3 KPLCE4 KPLCE4 KPLCE4 KPLCE4	C#TPCQTTCVKC#TPCQTTVVKCPTPCQTTCVKCPTPCQTTVVKCPTPCQTCCC
Elephant Elephant	(Em) (Em) (Em) (Em) (La) (La) (Em) (Em) (Em) (La) (La) (Em) (Em) (Em) (Em) (Em) (Em) (Em) (Em	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE6 KPLCE7 KPLCE6 KPLCE7 KPLCE4 KPLCE7 KPLCE4 KPLCE5 KPLCE4 KPLCE3 KPLCE3 KPLCE4 KPLCE3 KPLCE4 KPLCE3 KPLCE4 KPLCE4 KPLCE5 KPLCE4 KPLCE4 KPLCE4 KPLCE5 KPLCE4 KPLCE5 KPLCE4 KPLCE5 KPLCE4 KPLCE5 KPLCE4 KPLCE5 KPLCE4 KPLCE5 KPLCE4 KPLCE5 KPLCE5 KPLCE5 KPLCE5 KPLCE5 KPLCE5 KPLCE6 KPLCE5 KPLCE6 KPLCE5 KPLCE6 KPLCE5 KPLCE6 KPLC55 K	CBTPCQTTCVKCBTPCQTTVVKCBTPCCCC
Elephant Elephant	(Em) ((Em) ((Em)) ((La) ((Em))	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE1 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE5 KPLCE4 KPLCE5 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE5 KPLCE5 KPLCE4 KPLCE5 KPLCE4 KPLC24 KPLC25 KPLC24 KPLC25 KPLC24 KPLC24 KPLC24 KPLC24 KPLC24 KPLC24 KPLC24 KPLC25 KPLC24 KPLC25 KPLC24 KPLC24 KPLC24 KPLC24 KPLC24 KPLC25 KPLC24 KPLC25 KPLC26 KP	CBTPCQTTCVKCBTPCQTTVVKCBTPCXDC >< Repeat >< Repeat >< Repeat >< Repeat >< C-terminal segment YVQAPASSTVQSSGSQCCNDPCCDC YVQAPASSTVQSSGSQCNDPCCDC YVQAPASSTVQSSGSQCCDPCSA YVQAPASSTVQSSGSQCCDPCSA YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCDPCSA YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCDPCSA YVQAPASSTVQSSGSQCCDPCSA YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCDPCSA YVQAPASSTVQSSGSQCCDPCSA YVQAPASSTVQSSGSQCCDPCSA YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCCDC YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCCCC YVQAPASSTVQSSGSQCCDCCC YVQAPASSTVQSSGSQCCCDC YVQAPASSTVQSSGSQCCCDC YVQAPASSTVQSSGSQCCCDC YVQAPASSTVQSSGSQCCCDC YVQAPASSTVQSSGSQCCCDC YVQAPASSTVQSSGSQCCCD YVQAPASSTVQSSGSQCCCD YVQAPASSTVQSSGSQCCD YVQAPASSTVQSSGSQCCCD YVQAPASSTVQSSGSQCCCD YVQAPASSTVQSSGSQCCCD YVQAPASSTVQSSGSQCCD YVQAPASSTVQSSGSQCCD YVQAPASSTVQSSGSQCCD YVQAPASSTVQSSGSQCCD YVQAPASSTVQSSGSQCCD YVQAPASSTVQSSGSQCCD YVQAPASSTVQSSGSQCCD YVQAPASSTVQSSGSQCCD YVQAPASSTVQSSGSQCCD YVQAPASSTVQSSGSQCCD YVQAPASSTVQSSGSQCCD YVQAPASSTVSSGSQCCD YVQAPASSTVSSGSQCCD YVQAPASSTVSSGSQCCD YVQAPASSTVSSGSQCCD YVQAPASSTVSSGSQCCD YVQAPASSTVSSGSQCCD YVQAPASSTVSSGSQCCD YVQAPASSTVSSGSQCCD YVQAPASSTVSSGSQCCD YVQAPASSTVSSGSQCCD YVQAPASSTVSSGSQCCD YVQAPASSTVSSGSQCCD YVQAPASSTVSSGSQCCD YVQAPASSTVSSG YVQAPASSTVSSG YVQAPASSTVSSG YVQAPASSTVSSG YVQAPASSTVSSG YVQAPASSTVSSG YVQAPASSTVSSG YVQAPASSTVSSG YVQAPASSTVSS YVQAPA
Elephant Elephant	(Em) ((Em) ((Em)) ((La) ((Em))	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE3 KPLCE4 KPLCE2 KPLCE	CETECQTTCVKCETECQTTVVKCETECQTTCVKCETECQTTVVCCETECQTTVVCCC
Elephant Elephant	(Em) ((Em) ((Em)) ((La) ((Em))	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE4 KPLCE5 KPLCE4 KPLCE	C#TPCQTTCVKC#TPCQTTVVKC#TPCQTTCVKC#TPCQTTVVC#TPC
Elephant Elephant	(Em) ((Em) ((Em)) ((La) ((Em))	KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE6 KPLCE7 KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE KPLCE3 KPLCE4 KPLCE5 KPLCE4 KPLCE5 KPLCE4 KPLCE	CETECQTTCVKCETECQTTVVKCETECQTTCVKCETECQTTVVCCETECQTTVVCCCT

Figure 4. Amplification of KPLCE in elephants but not in sirenians and hyrax. Amino acid sequence alignment of KPLCE proteins of two elephant species compared to sirenians, hyrax and human. The positions of sequence repeats and the N-terminal and the C-terminal segments of the proteins are indicated above the alignment. Colored dashes indicate deletions in the C-terminal segment of KPLCE proteins in elephants and manatee. Cysteine (C), glutamine (Q), lysine (K) and proline (P) are highlighted by yellow, grey, blue and green shading, respectively. Species: Human (*Homo sapiens*), elephant (Em) (*Elephas maximus indicus*), elephant (La) (*Loxodonta africana*), hyrax (*Procavia capensis*), manatee (*Trichechus manatus latirostris*) and dugong (*Dugong dugon*).

Α

Number of amino acid residues							
Species	CRNN	FLG	FLG2	HRNR	RPTN	TCHH	TCHHL1
Human	495	4061	2391	2850	784	1943	904
Elephant	849	3361	1650	996	984	1647	912
Manatee	487	1605	497	749	647	1343	321
Dugong	1073	435	67	749	767	1236	223

Β

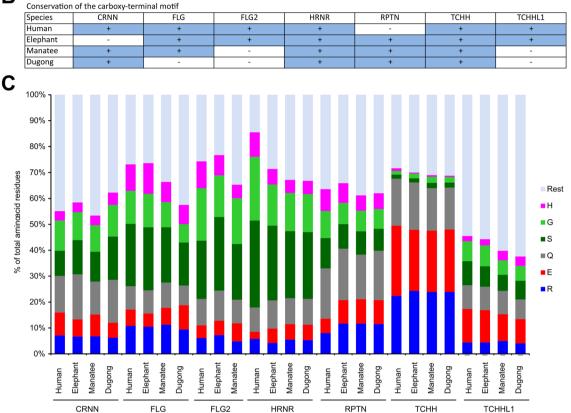


Figure 5. Comparison of SFTP genes of sirenians to placental mammals. Some SFTP genes of sirenians lack the carboxy-terminal motif and differ significantly in size compared to SFTPs in placentals. (**A**) Number of amino acid residues of sirenian SFTPs compared to SFTP proteins of elephant and human. (**B**) Comparison of the conservation of the carboxy-terminal motif in sirenians to placental mammals. (**C**) Amino acid contents of SFTP proteins of sirenians in comparison to elephant and human SFTPs. Alignments of the SFTPs are provided in Supplementary Fig. S7. Species: Human (*Homo sapiens*), elephant (*Elephas maximus indicus*), manatee (*Trichechus manatus latirostris*) and dugong (*Dugong dugon*).

present in FLG of the manatee but absent in FLG of the dugong. FLG2 of the dugong is predicted to be extremely short because of an in-frame stop codon in the currently available genome sequence. The sequence downstream of this predicted stop codon does not contain further stops for more than 2000 codons, suggesting that this gene has acquired the premature stop only recently in evolution.

SFTPs of sirenians and other species contain an N-terminal S100 domain of around 90 amino acid residues, followed by a long highly repetitive sequence that is strongly biased to only few amino acid residues. This leads to an extreme enrichment of few amino acids in many SFTPs. In line with this notion, only two amino acids, i.e. arginine (R) and glutamic acid (E), account for approximately 50% of all residues of TCHH in sirenians, strongly resembling TCHH in elephant and humans (Fig. 5C). Likewise, the high glycine and serine contents are conserved in HRNR of sirenians (Fig. 5C). Overall, the SFTPs of sirenians have a similar amino acid composition as their homologs in terrestrial mammals.

Caspase-14 is inactivated by mutations in the dugong

As FLG is an important skin barrier protein and mutations of the human *FLG* gene are associated with ichthyosis vulgaris and atopic dermatitis^{36,37}, we investigated FLG-interacting proteins in the manatee, which has retained FLG, and the dugong, which has lost the C-terminal portion of FLG (Fig. 5A,B). Two proteases, aspartic peptidase retroviral like 1 (ASPRV1) and caspase-14 (CASP14), are expressed specifically in terminally differentiated keratinocytes where they are involved in the proteolytic processing of filaggrin^{38,39}. ASPRV1 is conserved in the manatee, whereas it is disrupted by a premature stop codon and a frameshift mutation in the dugong (Fig. 6). All disruptive

A Human CASP14 Manatee CASP14 Dugong CASP14	Q F Q E E L E K F Q Q A I D S R E D P V S C A F V V L M A CTCTCACTCCAGCAATTCCAGGAAGAGCTGGAAAAATTCCAGCAGGCCATCGATTCCCGGGAAGATCCCGTCAGTTGTGCCTTCGTGGTACTCATGGCTC TTGTCCCTCCAGCAATTCCAGGAAGAGTTGGATAAATTCCGGGAGGCCATGGAATCCCGGACAGACCCCATCAGCTGTGCCTTTGTGGTGCTGATGGCGC CTCTCCCTCCAGCAATTCCCGGAAGAGTTGGATTAATTCCGGGAGGCCATGGAAGCCCGGACAGACCCCATCAGCTGTGCCTTTGTGGTGCTCATGGCGT Q F P E E L D - F R E A M E A R T D P I S C A F V V L M A stop
Human CASP14 Manatee CASP14 Dugong CASP14	H G R E G F L K G E D G E M V K L E N L F E A L N N K N C Q A L R A ACGGGAAGGCAAGGCTTCCTCAAGGGAGAAGATGGGGAGAATGGTCAAGCTGGAGAATCTCTTCGAGGCCCTGAACAACAAGAACTGCCAGGCCCTGCAGAC ATGGGTCAGAAAGGTCTTCTCAAGGGTGAGGATGAGCAGATGGTCGAGGTGGATGACCTCTTTGAGGTCCTGAACAACAAGAACTGCCGAGCCCTGAGAGC ATGGGTCAGAAAGCCTTCTCAAGGGTGAGGATGAGCAGATGGTCGAGGTGGATGACCTCTTTGAGGTCTGAACAACAAGAACTGCCGAGCCCTGAGAGC ATGGGTCAGAAAGCCTTCTCAAGGGTGAGGATGAGCAGATGGTCGAGCTGGATGACCTCTCTGAGGTCTTGAACAACAAGAAATGCTGGGCCCTGAGAGC Y G S E S L L K G E D E Q M V E L D D L S E V L N N K K C W A L R A
Human CASP14 Manatee CASP14 Dugong CASP14	K P K V Y I I Q A C R G TAAGCCCAAGGTGTACATCATACAGGCCTGTCGAGGAG CAAACCCAAAGTGTACATCGTGCAGGCCTGTCGAGGAGGTGAGGACAGAG CAAACCCAAAGTGTACATCGTGCAGGCCTGTCGAGGAGGGTGAGGACAGAG K P K V Y I V Q A C R G
B Human CASP14 Manatee CASP14 Dugong CASP14	V T R R M A E A E L V Q E G K A R K T N P E I Q S T L R K CTGTTGCTGCAGGTGACCCGGCGGATGGCAGAAGCAGAGCTGGTTCAAGAAGGAAAGCAAGGAAAACGAACCCTGAAATCCAAAGCACCCTCCGGAAAC CTGTCATTGCAGGTGACACGGAGGATGGCAGAAGGAAGGA
Human CASP14 Manatee CASP14 Dugong CASP14	R L Y L Q - GGCTGTATCTGCAGTAG AGCTCTATCTGCAGTAG AGCTCTATCTGCAGTAG S S I C S

Figure 6. Mutations of *CASP14* in the dugong. Nucleotide sequence alignment of exon 4 (**A**) and exon 7 (**B**) of the *CASP14* gene in human (*Homo sapiens*), manatee (*Trichechus manatus latirostris*) and dugong (*Dugong dugon*) genomes. Intronic nucleotide sequences flanking the exons are marked by grey shading. Frame-shift mutations and premature stop codons are highlighted by yellow shading. Nucleotides conserved in all species are indicated by blue fonts. Amino acid sequences, derived by translation of exonic nucleotide sequences, are shown for human and dugong above and below the alignment, respectively. Nucleotide sequences in panel (**A**) Human (GenBank accession number NC_000019.10, nucleotides 15,053,721–15,053,970), manatee (GenBank accession number NW_004444058.1, nucleotides 8,025,835–8,025,586), dugong (GenBank accession number JASCZL010000003.1, nucleotides 19,502,339–19,502,451). Nucleotide sequences in panel (**B**) Human (GenBank accession number NC_000019.10, nucleotides 15,055,973–15,056,089), manatee (GenBank accession number NW_004444058.1, nucleotides 8,024,311–8,024,195), dugong (GenBank accession number NW_004444058.1, nucleotides 8,024,311–8,024,195), dugong (GenBank accession number JASCZL010000003.1, nucleotides 19500820–19,501,069).

.....

mutations of *CASP14* were present in three dugong genome sequences that were available in GenBank as results of independent projects (Supplementary Fig. S9).

Discussion

The main function of keratinocyte differentiation is the establishment of the body's interface with the environment^{3,40}. Accordingly, adaptations to different environments are expected to involve adaptations of keratinocyte differentiation. Our results support this hypothesis with regard to mutations of genes, such as *FLG* and *CASP14*, implicated in the epidermal barrier formation in land-dwelling mammals. However, the extent of gene loss in the keratinocyte differentiation program is less pronounced than that in the other major group of aquatic mammals, the cetaceans^{11,41,42} (Fig. 7).

Sirenians have apparently intact *KPRP*, *KPLCE*, *PRR9*, *LELP1* and *LCEs*, the orthologs of which have been lost in cetaceans¹¹. Our analysis shows that *LCE* genes are even amplified in the dugong, whereas the incompleteness of the current genome sequence assembly of the manatee does not allow to conclude on the number of LCE genes in this species. The increase of LCE gene copy numbers in the dugong has likely occurred through gene duplications by the mechanism of unequal crossing over⁴³. The retention of the duplicated genes suggests that they have provided a selective advantage, for example by increasing the dosage of the encoded proteins or by facilitating subfunctionalization⁴⁴. However, the possibility of neutral evolution of gene copy numbers needs to be considered⁴⁵, and even potentially deleterious effects of large tandemly arrayed gene clusters have been discussed⁴⁶. In humans, LCE proteins are components of cornified envelopes⁴⁷. Their expression is increased upon exposure of the skin to ultraviolet radiation⁴⁸ and during the repair of the skin barrier⁴⁹, whereas lack of LCE3B and LCE3C due to gene loss predisposes to psoriasis⁵⁰. LCEs have antimicrobial activities⁵¹ and interact with the antimicrobial cysteine-rich tail protein 1 (CYSRT1)⁵⁰. It remains to be investigated which function of LCEs has been retained in sirenians whereas it is dispensable in cetaceans. Another antimicrobial protein encoded by an EDC gene, PGLYRP4, is absent in both sirenians and cetaceans¹¹, indicating that this protein is dispensable for fully aquatic mammals.

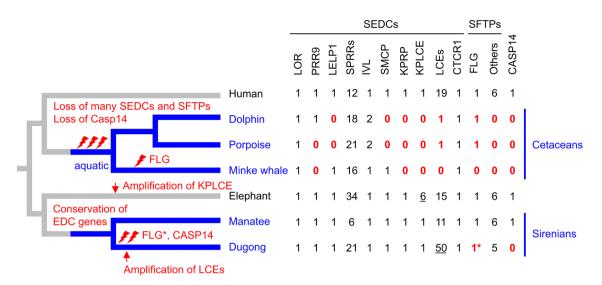


Figure 7. Schematic model showing the evolution of the EDC in mammals after the land-to-water transition. The matrix illustrates the numbers of genes belonging to the gene families in the species indicated. The cladogram shows the relation of the investigated species. Species colored in blue underwent the land to water transition. The asterisk indicates the loss of the C-terminal portion of FLG due to a premature stop. Underlines mark genes that are amplified in individual phylogenetic lineages. Species: Human (*Homo sapiens*), dolphin (*Tursiops truncatus*), porpoise (*Phocoena sinus*), Minke whale (*Balaenoptera acutorostrata scammoni*), elephant (*Elephas maximus indicus*), manatee (*Trichechus manatus latirostris*) and dugong (*Dugong dugon*).

.....

In contrast to SEDC genes, the SFTP gene clusters of sirenians are affected by several mutations which are predicted to impair the normal function of the encoded proteins. The proteins encoded by the genes *FLG*, *FLG2* and *TCHHL1* are much shorter in sirenians than their orthologs in other mammalian species. Interestingly, the manatee has a potentially functional FLG including the characteristic C-terminal sequence motif of SFTPs (Fig. 5B), whereas FLG of the dugong is truncated and lacks this motif. Human *FLG* is probably the most-investigated EDC gene because polymorphisms of *FLG* affect skin barrier properties^{21,52} and *FLG* mutations are associated with the highly prevalent inflammatory skin disease, atopic dermatitis³⁷. Both *FLG2* and *TCHHL1* are truncated by premature stop codons in sirenians (Fig. 5A,B). FLG2 is a component of cornified envelopes⁵³ and mutations of the *FLG2* gene cause peeling skin syndrome type A⁵⁴. *TCHHL1* is expressed in hair follicles⁵⁵, and TCHHL1 protein was detected by mass spectrometry-based proteomics in mature hair shafts of mice⁵⁶. As sirenians have a few hairs with putative mechanosensory functions, hair-related genes are not generally lost³¹. Accordingly, the main SFTP of the inner root sheath of hair follicles, TCHH, is conserved in both manatee and dugong. The comparison of SFTP genes in cetaceans⁴¹ and sirenians (this study) reveals striking differences, because all SFTPs have been lost in whales and only FLG is conserved in dolphins, whereas many SFTPs are conserved in sirenians.

Our finding of parallel loss of *FLG* and *CASP14* in the dugong suggests that a common pathway involving both proteins has been lost in the dugong. Caspase-14 is co-expressed with FLG⁵⁷ and proteolytically processes FLG in murine and human keratinocytes^{39,58}. However, *FLG* and *CASP14* have not been strictly interdependent during the evolution of mammals. *CASP14* is present in monotremes (platypus and echidna), whereas an SFTP with amino acid sequence features characteristic for FLG is missing¹². *CASP14* has been lost in cetaceans, whereas *FLG* has been conserved, as mention above, in a subgroup of cetaceans⁴¹. Deletions in the human *CASP14* gene have been linked to a defect in cornification that manifests as autosomal recessive inherited ichthyosis⁵⁹. The cellular features of the epidermis in manatees, which have *FLG* and *CASP14*, and dugongs, which lack *FLG* and *CASP14*, remain to be investigated in future studies.

Although the availability of genome sequences has provided insights into changes of keratinocyte differentiation genes, it is important to notice the limitations of the present study. First, the expression of EDC genes of sirenians remains to be investigated in situ, that is, in skin samples of manatees and dugongs. As protein sequences can be faithfully predicted now, proteomic analysis appears to be straightforward. Second, keratinocyte differentiation could not be studied in an in vitro model, because the culture of skin cells of sirenians is only in its infancy^{60,61}, and fresh biosamples were not available to us. Finally, the interpretation of sequence data must be done cautiously because errors of DNA-sequencing and sequence assembly cannot be excluded.

Material and methods

Ethics statement

Genome and transcriptome data were obtained from public databases. This study involved neither humans nor animals.

Identification of EDC genes in genomic sequences

Homologs of human EDC genes were identified by searches with the basic local alignment search tool (BLAST) at the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi, last accessed on 21 December 2023) and analysis of the genomic region between the genes S100A9 and S100A11 in the genomes of the dugong (Dugong dugon, mDugDug1.hap1, GenBank accession number GCA_030035585.1, submitted by Vertebrate Genomes Project)⁶², manatee (Trichechus manatus latirostris, GenBank accession number GCA_030013775.1, submitted by Consejo Superior de Investigaciones Cientificas, Valencia, Spain) and elephant (Elephas maximus indicus, GenBank accession number GCF_024166365.1, submitted by Vertebrate Genomes Project). Sequences of the dugong that were considered important for the conclusions of this study, were analyzed in two additional dugong genome sequences (Dugong dugon assembly, WGS project CAJQER01, GenBank accession number GCA_905400935.1, submitted by Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany; Dugong dugon genome assembly D_dugong, WGS project BMBL01, GenBank accession number GCA_015147995.1, submitted by National Institute for Environmental Studies, Japan). The EDC region around KPLCE was analyzed in the genome sequence of another species of Proboscidea, the African savannah elephant (Loxodonta africana, GenBank accession number GCA_030014295.1, submitted by Vertebrate Genomes Project), and the hyrax (Procavia capensis, GenBank accession number GCA_000152225.2, submitted by Baylor College of Medicine, Houston, Texas). For some EDC genes, annotations were available in the genome sequence assemblies of NCBI GenBank, as indicated in Supplementary Tables S1-S3. Other EDC genes were identified by tBLASTn searches using proteins encoded in the EDC of humans or Afrotherian species as queries. To avoid false elimination of hits with biased amino acid composition characteristic for EDC proteins^{13,24}, the filter for low sequence complexity was deactivated. Criteria for gene orthology were shared local synteny and reciprocal best hits in BLAST searches⁶³.

Analysis of amino acid sequences encoded by EDC genes

Amino acid sequences were aligned with MUSCLE⁶⁴ and MultAlin⁶⁵. The alignments were manually adjusted. Amino acid contents of proteins were calculated with the ProtParam tool at the ExPASy portal⁶⁶. For the visualization of sequence repeats in KPLCE proteins, sequence logos were generated using the Weblogo software⁶⁷.

Molecular phylogenetics

Sequences belonging to the LCE family were collected from NCBI GenBank for each species of interest. The phylogenetic analysis was performed with PhyML (version 3.3.20220408)⁶⁸ according to an approach described previously¹⁹. Phylogenetic trees were visualized and edited with FigTree (http://tree.bio.ed.ac.uk/software/figtr ee/, last accessed on December 17, 2023) and inkscape (version: 1.0.0.0; https://inkscape.org/de/, accessed on December 17, 2023).

Data availability

All data generated or analyzed during this study are included in this published article and its Supplementary Information files.

Received: 23 December 2023; Accepted: 18 April 2024 Published online: 23 April 2024

References

- Alibardi, L. Adaptation to the land: The skin of reptiles in comparison to that of amphibians and endotherm amniotes. J. Exp. Zool. B Mol. Dev. Evol. 298, 12–41 (2003).
- 2. Matsui, T. & Amagai, M. Dissecting the formation, structure and barrier function of the stratum corneum. *Int. Immunol.* 27, 269–280 (2015).
- 3. Watt, F. M. Terminal differentiation of epidermal keratinocytes. Curr. Opin. Cell Biol. 1, 1107-1115 (1989).
- 4. Candi, E., Schmidt, R. & Melino, G. The cornified envelope: A model of cell death in the skin. *Nat. Rev. Mol. Cell Biol.* 6, 328–340 (2005).
- 5. Sachslehner, A. P. *et al.* Transglutaminase activity is conserved in stratified epithelia and skin appendages of mammals and birds. *Int. J. Mol. Sci.* 24, 2193 (2023).
- Crumrine, D. et al. Mutations in recessive congenital ichthyoses illuminate the origin and functions of the corneocyte lipid envelope. J. Investig. Dermatol. 139, 760–768 (2019).
- Matsuki, M. et al. Defective stratum corneum and early neonatal death in mice lacking the gene for transglutaminase 1 (keratinocyte transglutaminase). Proc. Natl. Acad. Sci. U. S. A. 95, 1044–1049 (1998).
- Jonca, N. & Simon, M. The cornified envelope: A versatile contributor to the epidermal barrier. J. Investig. Dermatol. 143, 1335–1337 (2023).
- 9. Henry, J. et al. Update on the epidermal differentiation complex. Front. Biosci. 17, 1517-1532 (2012).
- Kypriotou, M., Huber, M. & Hohl, D. The human epidermal differentiation complex, cornified envelope precursors, S100 proteins and the "fused genes" family. *Exp. Dermatol.* 21, 643–649 (2012).
- Holthaus, K. B., Lachner, J., Ebner, B., Tschachler, E. & Eckhart, L. Gene duplications and gene loss in the epidermal differentiation complex during the evolutionary land-to-water transition of cetaceans. Sci. Rep. 11, 12334 (2021).
- 12. Steinbinder, J., Sachslehner, A. P., Holthaus, K. B. & Eckhart, L. Comparative genomics of monotremes provides insights into the early evolution of mammalian epidermal differentiation genes. *Sci. Rep.* **14**, 1437 (2024).
- Strasser, B. *et al.* Evolutionary origin and diversification of epidermal barrier proteins in amniotes. *Mol. Biol. Evol.* 31, 3194–3205 (2014).
- 14. Holthaus, K. B. *et al.* Comparative genomics identifies epidermal proteins associated with the evolution of the turtle shell. *Mol. Biol. Evol.* **33**, 726–737 (2016).
- 15. Holthaus, K. B. *et al.* Identification and comparative analysis of the epidermal differentiation complex in snakes. *Sci. Rep.* 7, 45338 (2017).
- Holthaus, K. B. et al. Comparative analysis of epidermal differentiation genes of crocodilians suggests new models for the evolutionary origin of avian feather proteins. Genome Biol. Evol. 10, 694–704 (2018).

- 17. Holthaus, K. B., Alibardi, L., Tschachler, E. & Eckhart, L. Identification of epidermal differentiation genes of the tuatara provides insights into the early evolution of lepidosaurian skin. *Sci. Rep.* **10**, 12844 (2020).
- 18 Davis, A. & Greenwold, M. J. Evolution of an epidermal differentiation complex (EDC) gene family in birds. *Genes (Basel)* 12, 767 (2021).
- 19. Sachslehner, A. P. & Eckhart, L. Evolutionary diversification of epidermal barrier genes in amphibians. Sci. Rep. 12, 13634 (2022).
- Kashyap, D. R. et al. Peptidoglycan recognition proteins kill bacteria by activating protein-sensing two-component systems. Nat. Med. 17, 676–683 (2011).
- 21. Mlitz, V., Hussain, T., Tschachler, E. & Eckhart, L. Filaggrin has evolved from an "S100 fused-type protein" (SFTP) gene present in a common ancestor of amphibians and mammals. *Exp. Dermatol.* **26**, 955–957 (2017).
- 22. Kezic, S. & Jakasa, I. Filaggrin and skin barrier function. Curr. Probl. Dermatol. 49, 1-7 (2016).
- 23. McLean, W. H. Filaggrin failure—From ichthyosis vulgaris to atopic eczema and beyond. Br. J. Dermatol. 175, 4–7 (2016).
- Mlitz, V. et al. Trichohyalin-like proteins have evolutionarily conserved roles in the morphogenesis of skin appendages. J. Investig. Dermatol. 134, 2685–2692 (2014).
- 25. Heritage, S. & Seiffert, E. R. Total evidence time-scaled phylogenetic and biogeographic models for the evolution of sea cows (Sirenia, Afrotheria). *PeerJ* 10, e13886 (2022).
- 26. Horgan, P. *et al.* Insulative capacity of the integument of the dugong (*Dugong dugon*): Thermal conductivity, conductance and resistance measured by in vitro heat flux. *Mar. Biol.* **161**, 1395–1407 (2014).
- Graham, A. Histological examination of the Florida manatee (Trichechus manatus latirostris) integument. Dissertation, The University of Florida, pp 175. http://ufdcimages.uflib.ufl.edu/UF/E0/01/33/43/00001/graham_a.pdf (2005).
- Menon, G. K., Elias, P. M., Wakefield, J. S. & Crumrine, D. Cetacean epidermal specialization: A review. Anat. Histol. Embryol. 51, 563–575 (2022).
- Elias, P. M., Menon, G. K., Grayson, S., Brown, B. E. & Rehfeld, S. J. Avian sebokeratocytes and marine mammal lipokeratinocytes: Structural, lipid biochemical, and functional considerations. *Am. J. Anat.* 180, 161–177 (1987).
- Hautier, L. et al. From teeth to pad: Tooth loss and development of keratinous structures in sirenians. Proc. Biol. Sci. 290, 20231932 (2023).
- Ehrlich, F. *et al.* Differential evolution of the epidermal keratin cytoskeleton in terrestrial and aquatic mammals. *Mol. Biol. Evol.* 36, 328–340 (2019).
- Eckhart, L., Ehrlich, F. & Tschachler, E. A stress response program at the origin of evolutionary innovation in the skin. Evol. Bioinform. Online 15, 1176934319862246 (2019).
- 33. Huelsmann, M. *et al.* Genes lost during the transition from land to water in cetaceans highlight genomic changes associated with aquatic adaptations. *Sci. Adv.* **5**, eaaw6671 (2019).
- Zhang, X. et al. Parallel independent losses of G-type lysozyme genes in hairless aquatic mammals. Genome Biol. Evol. 13, evab201 (2021).
- Takase, T. & Hirai, Y. Identification of the C-terminal tail domain of AHF/trichohyalin as the critical site for modulation of the keratin filamentous meshwork in the keratinocyte. J. Dermatol. Sci. 65, 141–148 (2012).
- 36. Smith, F. J. *et al.* Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. *Nat. Genet.* **38**, 337–342 (2006).
- Palmer, C. N. et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat. Genet. 38, 441–446 (2006).
- Matsui, T. et al. SASPase regulates stratum corneum hydration through profilaggrin-to-filaggrin processing. EMBO Mol. Med. 3, 320–333 (2011).
- 39. Hoste, E. *et al.* Caspase-14 is required for filaggrin degradation to natural moisturizing factors in the skin. *J. Investig. Dermatol.* 131, 2233–2241 (2011).
- 40. Eckhart, L. & Zeeuwen, P. L. J. M. The skin barrier: Epidermis vs environment. Exp. Dermatol. 27, 805-806 (2018).
- Strasser, B., Mlitz, V., Fischer, H., Tschachler, E. & Eckhart, L. Comparative genomics reveals conservation of filaggrin and loss of caspase-14 in dolphins. *Exp. Dermatol.* 24, 365–369 (2015).
- 42. Espregueira, T. G. et al. Losing genes: The evolutionary remodeling of cetacea skin. Front. Mar. Sci. 7, 592375 (2020).
- 43. Reams, A. B. & Rothm, J. R. Mechanisms of gene duplication and amplification. Cold Spring Harb. Perspect. Biol. 7, a016592 (2015).
- 44. Kuzmin, E., Taylor, J. S. & Boone, C. Retention of duplicated genes in evolution. *Trends Genet.* 38, 59–72 (2022).
- Wagner, A. Decoupled evolution of coding region and mRNA expression patterns after gene duplication: Implications for the neutralist-selectionist debate. *Proc. Natl. Acad. Sci. U. S. A.* 97, 6579–6584 (2000).
- 46 Schiffer, P. H., Gravemeyer, J., Rauscher, M. & Wiehe, T. Ultra large gene families: A matter of adaptation or genomic parasites?. *Life (Basel)* **6**, 32 (2016).
- Ishitsuka, Y. et al. Lce1 family members are Nrf2-target genes that are induced to compensate for the loss of loricrin. J. Investig. Dermatol. 136, 1656–1663 (2016).
- Jackson, B. et al. Late cornified envelope family in differentiating epithelia–response to calcium and ultraviolet irradiation. J. Investig. Dermatol. 124, 1062–1070 (2005).
- de Koning, H. D. et al. Expression profile of cornified envelope structural proteins and keratinocyte differentiation-regulating proteins during skin barrier repair. Br. J. Dermatol. 166, 1245–1254 (2012).
- Niehues, H. et al. CYSRT1: An antimicrobial epidermal protein that can interact with late cornified envelope proteins. J. Investig. Dermatol. 143, 1498–1508 (2023).
- Niehues, H. et al. Antimicrobial late cornified envelope proteins: The psoriasis risk factor deletion of LCE3B/C genes affects microbiota composition. J. Investig. Dermatol. 142, 1947–1955 (2022).
- Brown, S. J. et al. Intragenic copy number variation within flaggrin contributes to the risk of atopic dermatitis with a dosedependent effect. J. Investig. Dermatol. 132, 98–104 (2012).
- Albérola, G., Schröder, J. M., Froment, C. & Simon, M. The amino-terminal part of human FLG2 is a component of cornified envelopes. J. Investig. Dermatol. 139, 1395–1397 (2019).
- Mohamad, J. et al. Filaggrin 2 deficiency results in abnormal cell-cell adhesion in the cornified cell layers and causes peeling skin syndrome type A. J. Investig. Dermatol. 138, 1736–1743 (2018).
- Wu, Z., Latendorf, T., Meyer-Hoffert, U. & Schröder, J. M. Identification of trichohyalin-like 1, an S100 fused-type protein selectively expressed in hair follicles. J. Investig. Dermatol. 131, 1761–1763 (2011).
- 56. Sukseree, S. et al. Autophagy controls the protein composition of hair shafts. J. Investig. Dermatol. 144, 170-173 (2024).
- 57. Fischer, H. *et al.* Caspase-14 but not caspase-3 is processed during the development of fetal mouse epidermis. *Differentiation* **73**, 406–413 (2005).
- Denecker, G. *et al.* Caspase-14 protects against epidermal UVB photodamage and water loss. *Nat. Cell Biol.* 9, 666–674 (2007).
 Kirchmeier, P., Zimmer, A., Bouadjar, B., Rösler, B. & Fischer, J. Whole-exome-sequencing reveals small deletions in CASP14 in patients with autosomal recessive inherited ichthyosis. *Acta Derm. Venereol.* 97, 102–104 (2017).
- Nascimento, M. B. *et al.* The initial steps toward the formation of somatic tissue banks and cell cultures derived from captive Antillean manatee (*Trichechus manatus* manatus) skin biopsies. *Zoo Biol.* 42, 709–722 (2023).
- 61 Tavares, F. D. S. et al. Establishment and characterization of a primary fibroblast cell culture from the Amazonian manatee (Trichechus inunguis). Animals 14, 686 (2024).

- 62. Baker, D. N. et al. A chromosome-level genome assembly for the dugong (Dugong dugon). J. Hered. 115, 212-220 (2024).
- Moreno-Hagelsieb, G. & Latimer, K. Choosing BLAST options for better detection of orthologs as reciprocal best hits. *Bioinformatics* 24, 319–324 (2008).
- 64. Edgar, R. C. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**, 1792–1797 (2004).
- 65. Corpet, F. Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res. 16, 10881–10890 (1988).
- 66. Artimo, P. et al. ExPASy: SIB bioinformatics resource portal. Nucleic Acids Res. 40, 597-603 (2012).
- Crooks, G. E., Hon, G., Chandonia, J. M. & Brenner, S. E. WebLogo: A sequence logo generator. *Genome Res.* 14, 1188–1190 (2004).
 Guindon, S. & Gascuel, O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* 52, 696–704 (2003).

Acknowledgements

We thank Bettina Ebner, Julia Lachner, Veronika Mlitz and Florian Ehrlich for helpful discussions during early stages of the project. This work was supported by the Austrian Science Fund (FWF): P32777.

Author contributions

J.S., A.P.S., K.B.H, and L.E. conceived the study, J.S., and A.P.S. performed phylogenetic analyses, J.S., A.P.S., K.B.H. and L.E. analyzed genome and transcriptome sequences, J.S., A.P.S., K.B.H. and L.E. wrote the manuscript.

Funding

The funding was supported by Austrian Science Fund, https://doi.org/10.55776/P32777.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/ 10.1038/s41598-024-60099-2.

Correspondence and requests for materials should be addressed to L.E.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2024