

Supplementary Data: Supplementary Figures and Tables

Evolutionary diversification of epidermal barrier genes in amphibians

Attila Placido Sachslehner, Leopold Eckhart

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A

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QLLTIKDIINTFYSYCNKED--LHGKLNKEAFNLLIQNEFADIIEDSEKPTIAAIMQALDENNDGEVDFKEFLDLFFKVAVAYCEAL
>Xt_SFTP2
KLTGVINEIIGIFLSYSEGTC--APHKLNKNQMNRLIQNEFSDVIKNPKDPKTVEALIKVLDQNKDEEVDFDEFSDLLCKVLKAIYRAM
>Am_SFTP3
TIVSALQDIVDVFLRYTAQEC--DRDKLNRAELISIIQNEFTDVIKNPKDPQTPATMLIVLDEDDKGQVDFNEFMNLVCKVAKAYYKTF
>Rb_SFTP2
SIIGAIQDLIDVFHSYSKEQC--KL--LNKDELKSLQKELANVIKNPNPQTVETVMKILDDNQDGGVDFNEFTDLMCKIISKAYYNTF
>Mu_SFTP2
FLISAIQDIIEVLYSYAHREC--NLQKLNKEELKSLIQNEFADVIVKNPNPQTAVTVMKTLDDNRDGEVDFEEFMDLMSKVSAYYKTF
>Gs_SFTP2
SLINVIEDTIQVYFKYSQRSC--NHQKLNNEELKSLIQNEFADVIVKNPNPQTAETVLKILDENKDGKVDFFEEFMDLLAKVAKAYFKTF

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Figure S3. Alignment of S100 domain sequences used for phylogenetic analysis. The S100 domains of SFTPs and S100A16 were aligned with MAFFT. The sequences are shown in fasta_aln format. Dashes were introduced to optimize the alignment. The complete amino acid sequences of SFTPs of amphibians are shown in Figure S1. GenBank accession numbers of other proteins: Rb S100A16, XP_029435257; Mu S100A16, XP_030042730; Gs S100A16, XP_033780380; Xt S100A16, XP_031747447; Homo sapiens S100A16, NP_001303936; Gg S100A16, XP_040508506; Hs Filaggrin, NP_002007; Hs Cornulin, NP_057274; Hs Trichohyalin, NP_009044; Gg Cornulin, NP_001165847; Gg Scaffoldin, NP_001338424. Am, *Ambystoma mexicanum*; Gg, *Gallus gallus*; Gs, *Geotrypetes seraphini*; Hs, *Homo sapiens*; Mu, *Microcaecilia unicolor*; Rb, *Rhinatrema bivittatum*; Xt, *Xenopus tropicalis*.

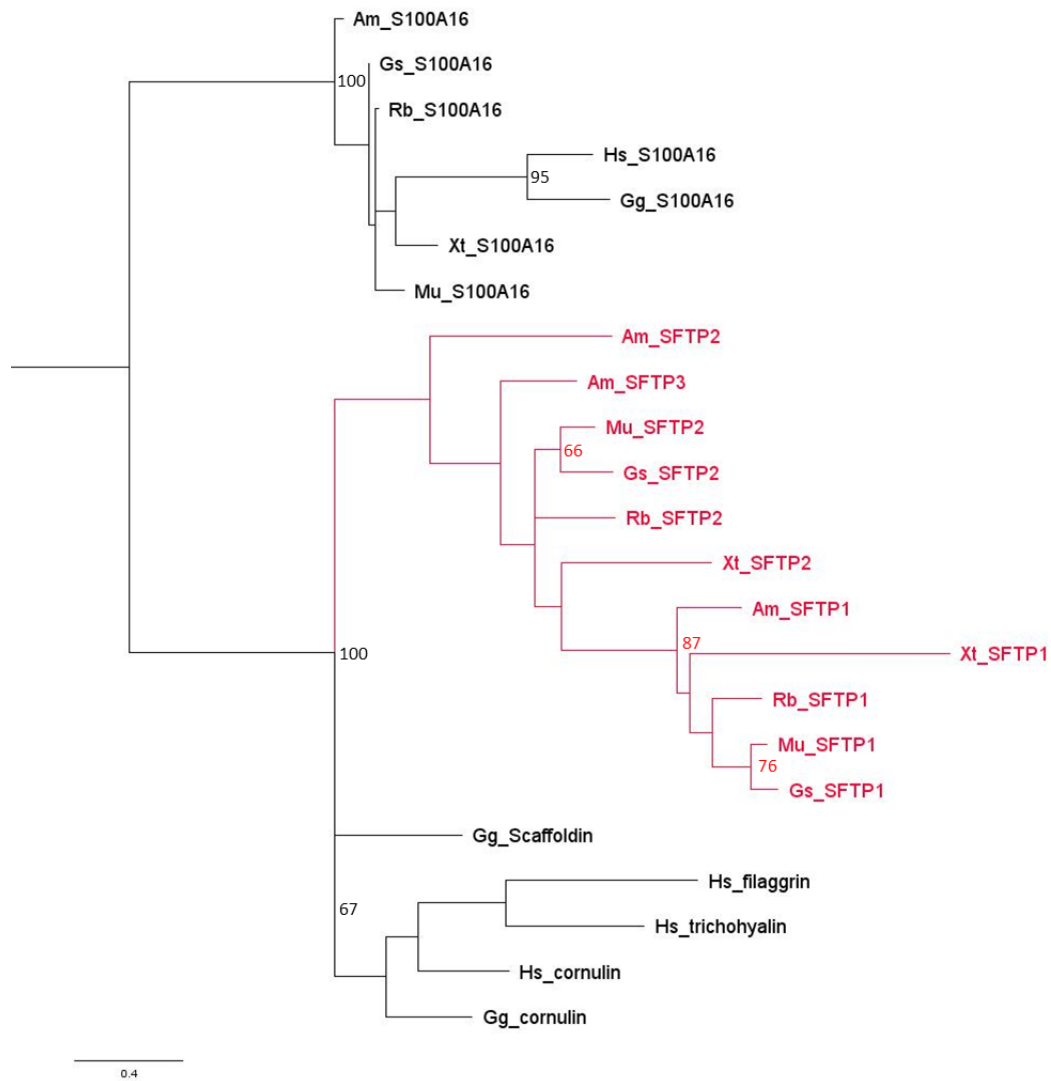


Figure S4. Phylogenetic analysis of SFTPs. The sequences of the S100 domains were aligned and used to construct the phylogenetic tree. S100A16 proteins were used as outgroup. Bootstrap values >60 are indicated. SFTP, S100 fused-type protein. Species: Am, *Ambystoma mexicanum*; Gg, *Gallus gallus*; Gs, *Geotrypetes seraphini*; Hs, *Homo sapiens*; Mu, *Microcaecilia unicolor*; Rb, *Rhinatrema bivittatum*; Xt, *Xenopus tropicalis*.

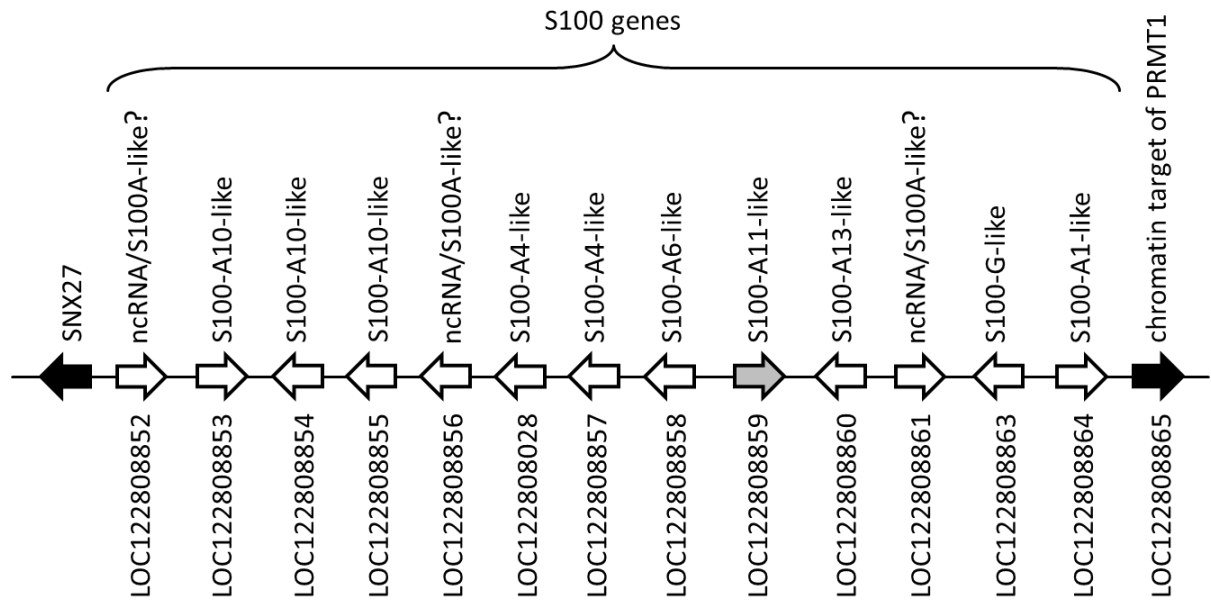


Figure S5. The S100A gene cluster on chromosome 8 of the African lungfish (*Protopterus annectens*) is predicted to contain neither *SEDC* nor *SFTP* genes. A segment of chromosome 8 of *Protopterus annectens*, corresponding to genome sequence assembly PAN1.0 (GCF_019279795.1), GenBank accession number NC_056741.1, nucleotide positions 430933198 – 444670046, was analyzed. Genes are depicted by arrows pointing in the direction of gene transcription. Annotations are from GenBank. Three genes are annotated as non-coding RNAs (ncRNAs) but their nucleotide sequences are similar to those of *S100A* genes. Note that no *SEDC* or *SFTP* genes are predicted in the region flanking the putative ortholog of *S100A11* (grey arrow). BLAST searches using *SEDC* proteins and *SFTPs* as queries were negative.

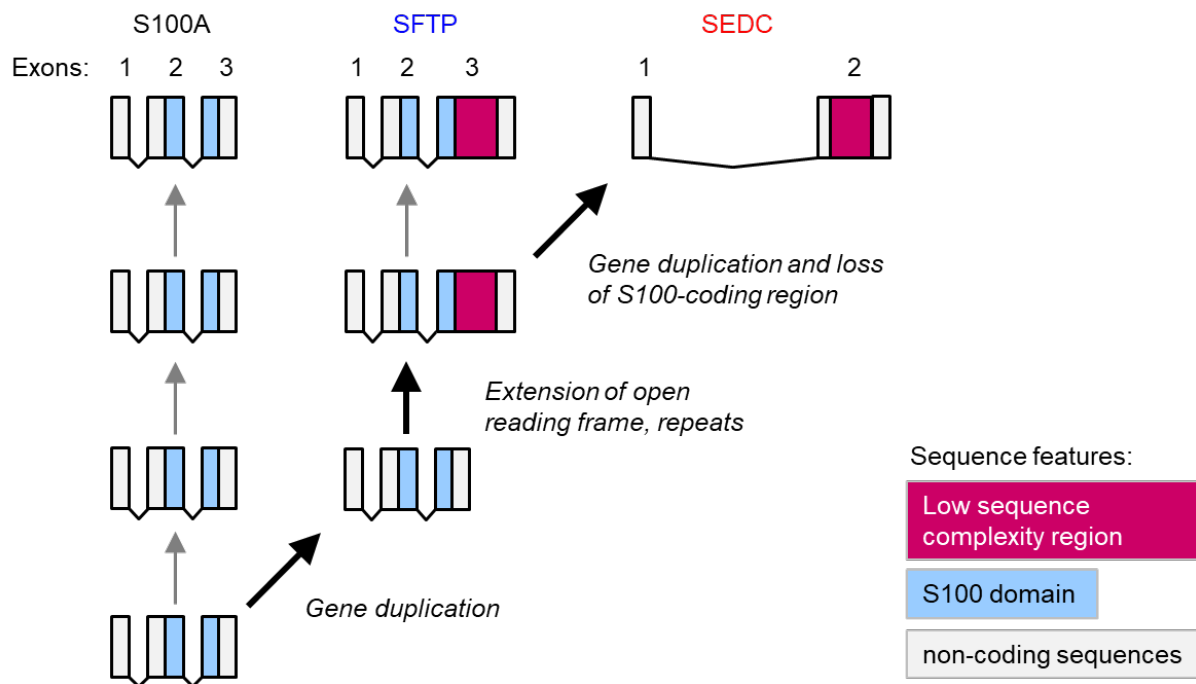


Figure S6. Evolutionary model for the origin of EDC gene types. The schematic depicts a hypothetical pathway of gene recombination and mutation events leading to the evolution of SFTP and SEDC genes from an S100A gene that was present in a fish ancestor of tetrapods. This model was developed from previous hypotheses (Strasser et al. 2014) taking into consideration the organisation of the EDC in newly available genome sequences and the absence of a *PGLYRP3* gene from the EDC of amphibians. Specifically, this model is a modification of scenario 4 of Supplementary Figure S13 in Reference Strasser et al. 2014.

Table S1. SEDC genes of caecilians

Species	Gene	Accession number (GenBank)	exon 1 end	exon 2 start	coding sequence start (exon 2)	coding sequence end (exon 2)	Orientation of gene	Confirmation by RNA-seq of skin	GenBank prediction of protein, accession number
<i>Rhinatrema bivittatum</i>	SEDC1	NC_042630.1	26179523	26189856	26189873	26190476	forward	yes	n.a.
<i>Rhinatrema bivittatum</i>	SEDC2	NC_042630.1	26289781	26291655	26291677	26297277	forward	yes	n.a.
<i>Microcaecilia unicolor</i>	SEDC1	NC_044044.1	37001319	36999093	36999073	36998210	reverse	yes	n.a.
<i>Microcaecilia unicolor</i>	SEDC2	NC_044044.1	36977283	36974970	36974887	36973724	reverse	yes	n.a.
<i>Microcaecilia unicolor</i>	SEDC3	NC_044044.1	36983767	37011504	37011524	37012123	forward	yes	XP_030043654.1
<i>Microcaecilia unicolor</i>	SEDC4	NC_044044.1	37037770	37038316	37038334	37041519	forward	yes	n.a.
<i>Geotrypetes seraphini</i>	SEDC1	NC_047099.1	25747663	25803662	25803682	25807008	forward	n.a.	XP_033781152.1
<i>Geotrypetes seraphini</i>	SEDC2	NC_047099.1	25784522	25781003	25780977	25779778	reverse	n.a.	XP_033781154.1

Abbreviations: RNA-seq, RNA sequencing; n.a., not available.

Table S2. SFTP genes of caecilians, axolotl and *Xenopus tropicalis*

Species	Gene	Accession number (GenBank)	exon 1 end	exon 2 start	coding sequence start (exon 2)	exon 2 end	exon 3 start	coding sequence end (exon 3)	Orientation of gene	GenBank prediction of protein, accession number
<i>Rhinatrema bivittatum</i>	SFTP1	NC_042630.1	26115567	26119185	26119200	26119337	26127989	26135020	forward	n.a.
<i>Rhinatrema bivittatum</i>	SFTP2	NC_042630.1	26161849	26164493	26164496	26164627	26168371	26171161	forward	n.a.
<i>Microcaecilia unicolor</i>	SFTP1	NC_044044.1	36896683	36902355	36902370	36902507	36910886	36922597	forward	n.a.
<i>Microcaecilia unicolor</i>	SFTP2	NC_044044.1	36954485	36958497	36958500	36958637	36961012	36962602	forward	XP_030043648.1
<i>Geotrypetes seraphini</i>	SFTP1	NC_047099.1	25955132	25940297	25940282	25940145	25926622	25911266	reverse	n.a.
<i>Geotrypetes seraphini</i>	SFTP2	NC_047099.1	25849649	25842556	25842541	25842404	25840504	25838156	reverse	n.a.
<i>Ambystoma mexicanum</i>	SFTP1	CM010927.2	n.a.	718074216	718074201	718074064	718047919	718047326	reverse	n.a.
<i>Ambystoma mexicanum</i>	SFTP2	CM010927.2	n.a.	717769809	717769804	717769667	717768256	717766007	reverse	n.a.
<i>Ambystoma mexicanum</i>	SFTP3	CM010927.2	n.a.	717517780	717517757	717517620	717516583	717515684	reverse	n.a.
<i>Xenopus tropicalis</i>	SFTP1	NC_030684.2	143977508	143969175	143969161	143969024	143963079	143950288	reverse	n.a.
<i>Xenopus tropicalis</i>	SFTP2	NC_030684.2	143901787	143897478	143897469	143897332	143896135	143893862	reverse	XP_031747944.1

Abbreviations: n.a., not available.