Castro *et al. BMC Evolutionary Biology* 2011, **11**:132
http://www.biomedcentral.com/1471-2148/11/132

BMC
Evolutionary Biology

RESEARCH ARTICLE Open Access

The evolutionary history of the stearoyl-CoA desaturase gene family in vertebrates

L Filipe C Castro[1*], Jonathan M Wilson[1], Odete Gonçalves[1], Susana Galante-Oliveira[1], Eduardo Rocha[1,2] and Isabel Cunha[1]

Report: Zhang Yuru

Content

- **Background**
- **Results and discussion**
- **Method**

Background

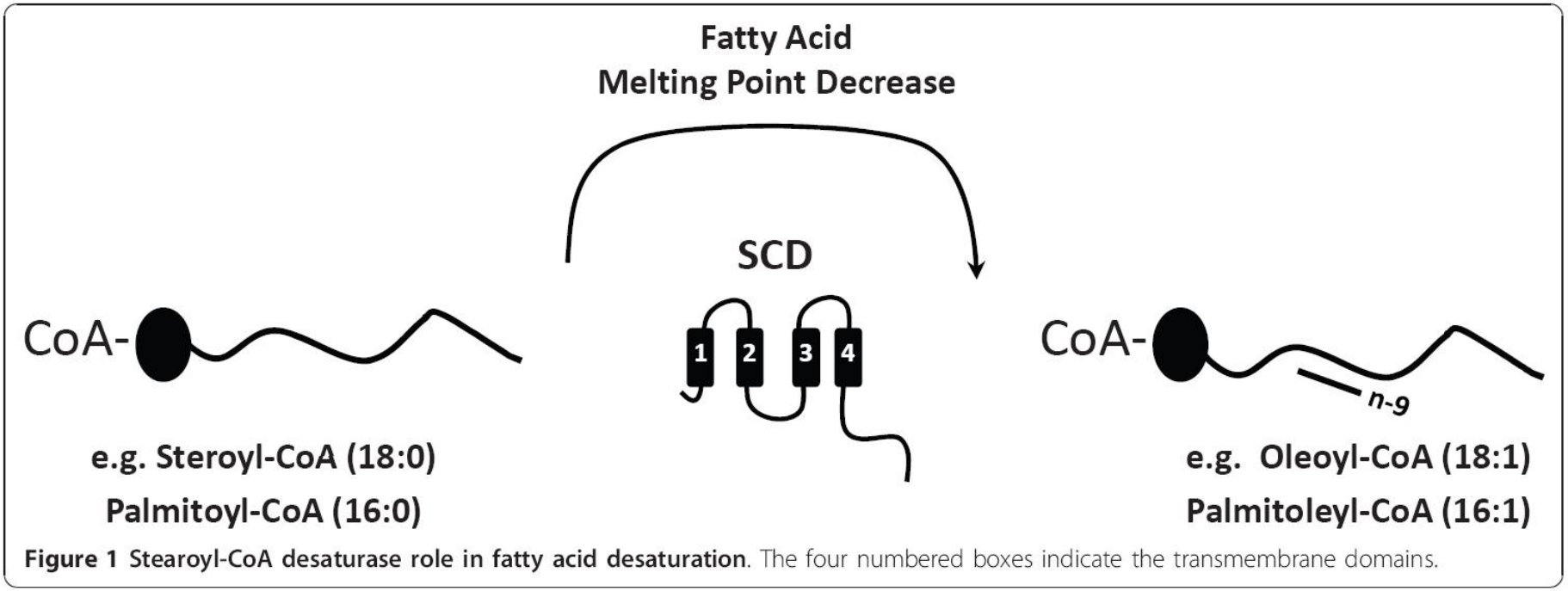
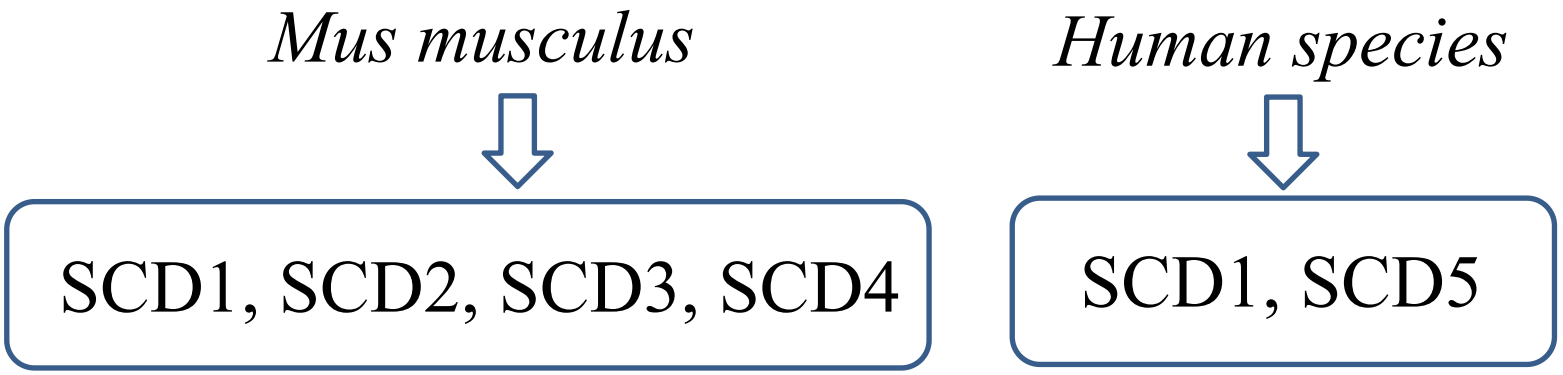


Figure 1 Stearoyl-CoA desaturase role in fatty acid desaturation. The four numbered boxes indicate the transmembrane domains.

Stearoyl-CoA desaturases (SCDs) are key enzymes involved in de novo monounsaturated fatty acid synthesis. They catalyze the desaturation of saturated fatty acyl-CoA substrates at the delta-9 position, generating essential components of phospholipids, triglycerides, cholesterol esters and wax esters.

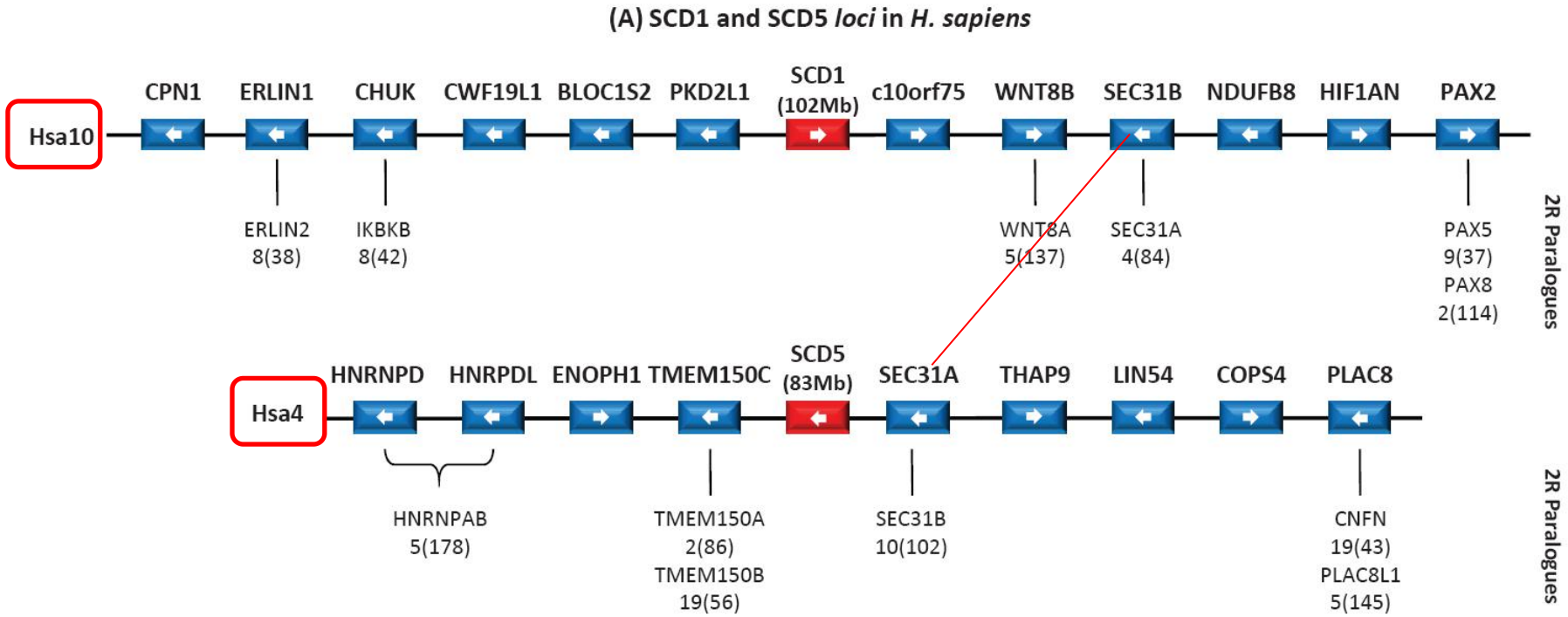


SCDs variability in vertebrates

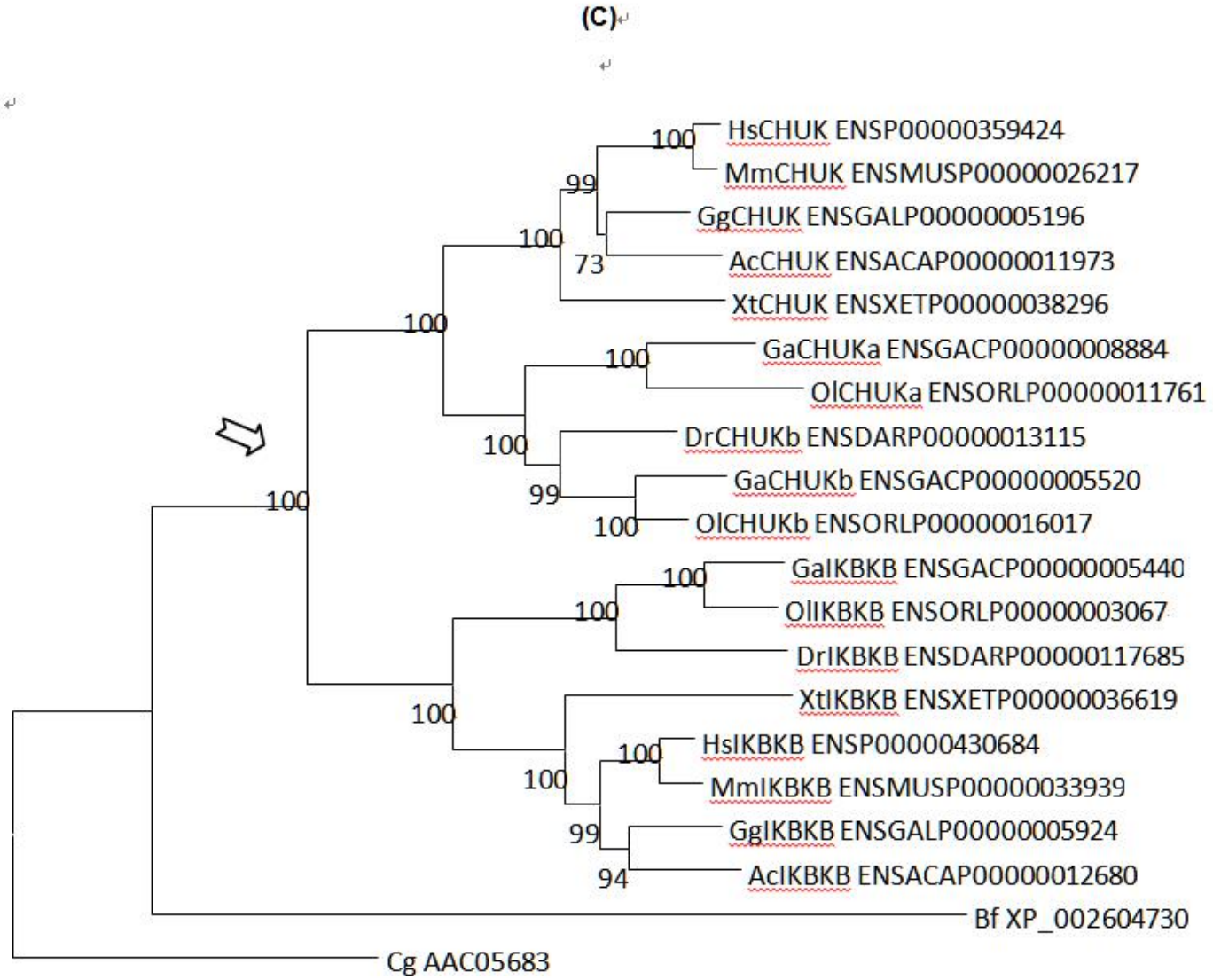
- The integration of the reported gene diversity with the functional physiological impacts requires the clarification of the SCD evolutionary path.
- Here, we provide a clear insight into SCD genes in vertebrate history by means of comparative genomics, phylogenetics and gene expression.

Results and Discussion

- 1, Human SCD1 and SCD5 map to the NK-linked paralogon



Chromosomal location of the SCD1 and SCD5 genes in Homo sapiens, and their neighbouring genes.



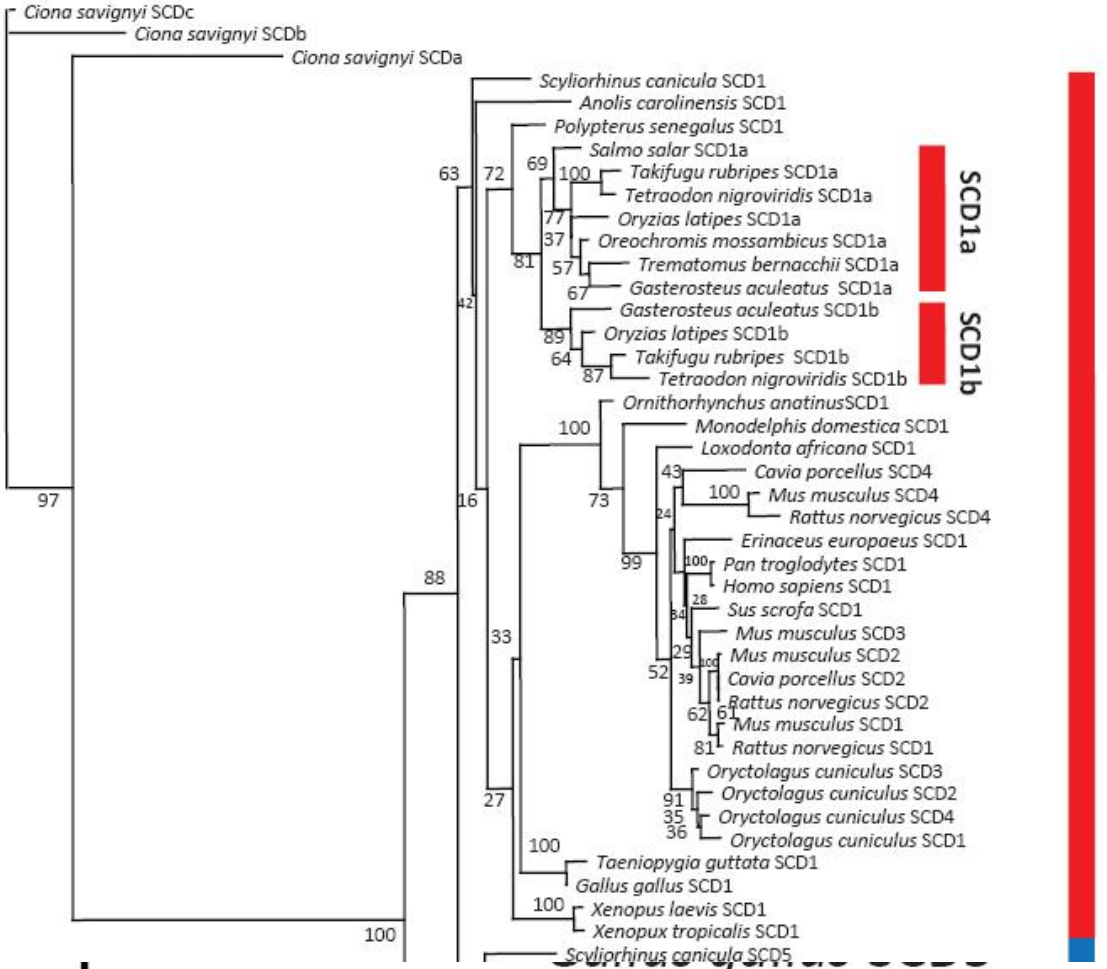
Evolutionary relationships of WNT8 (A), HNRNP (B), CHUK (C), ERLIN (D), SEC31 (E), and TMEM150 (F).

Both sites are highly indicative of a potential involvement of 2R genome duplications in the origin of these two genes.

The localization of the human SCD gene isoforms in a 2R-generated paralogon implies two testable predictions

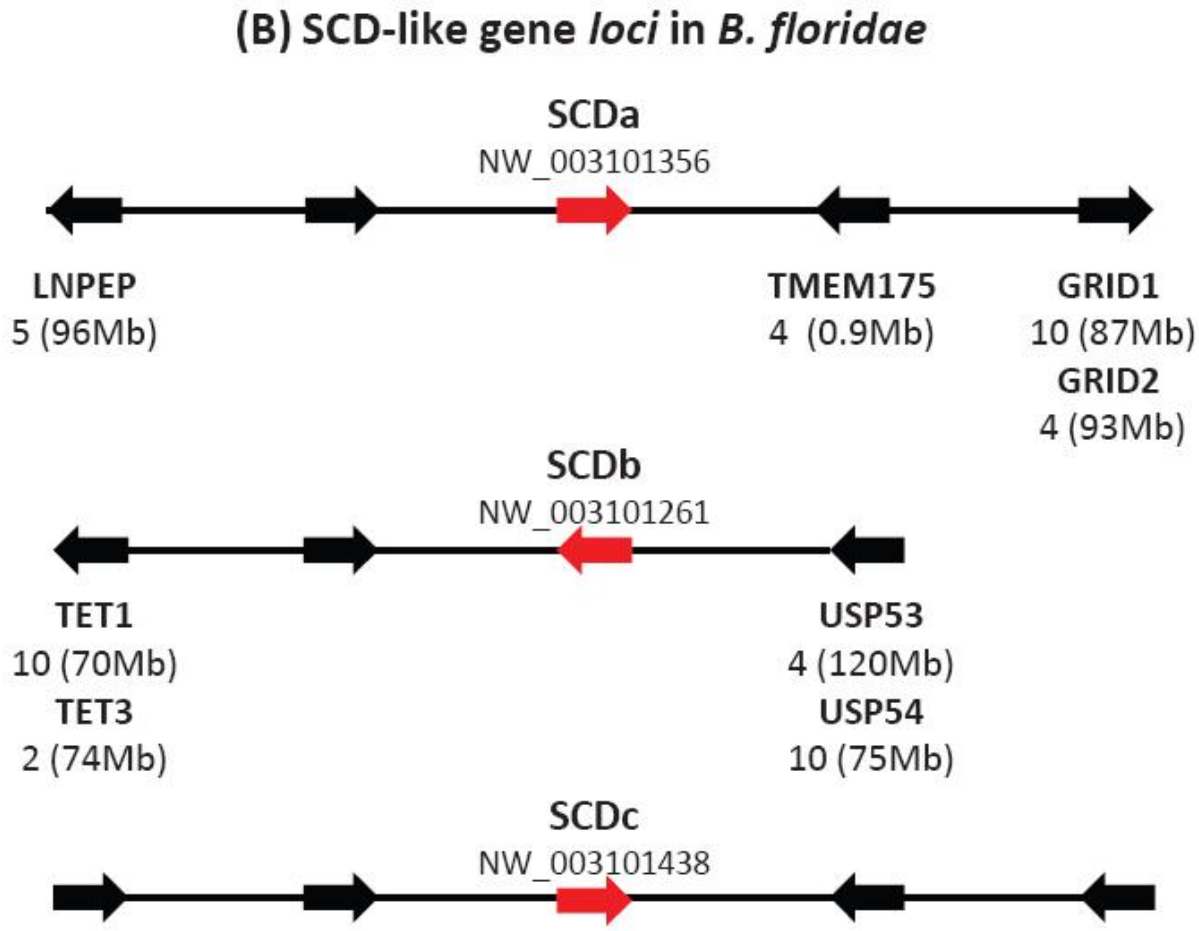
Unless independent gene expansions have taken place, invertebrate chordates should have a single SCD gene equally related to their vertebrate counterparts

Invertebrate SCD genes should be flanked by gene families that have their human orthologues/paralogues localising to regions of SCD paralogy (Hsa4, Hsa10, Hsa5 and Hsa2/8), even if conserved micro-synteny (conservation of immediately adjacent neighbours) is not observed.



Although we expected to find a single SCD isoform, our search retrieved three distinct SCD-like genes. Nevertheless, these represent an independent gene expansion in the amphioxus lineage, since they group together outside of the vertebrate SCD1/SCD5 clade.

Maximum likelihood tree of SCD genes

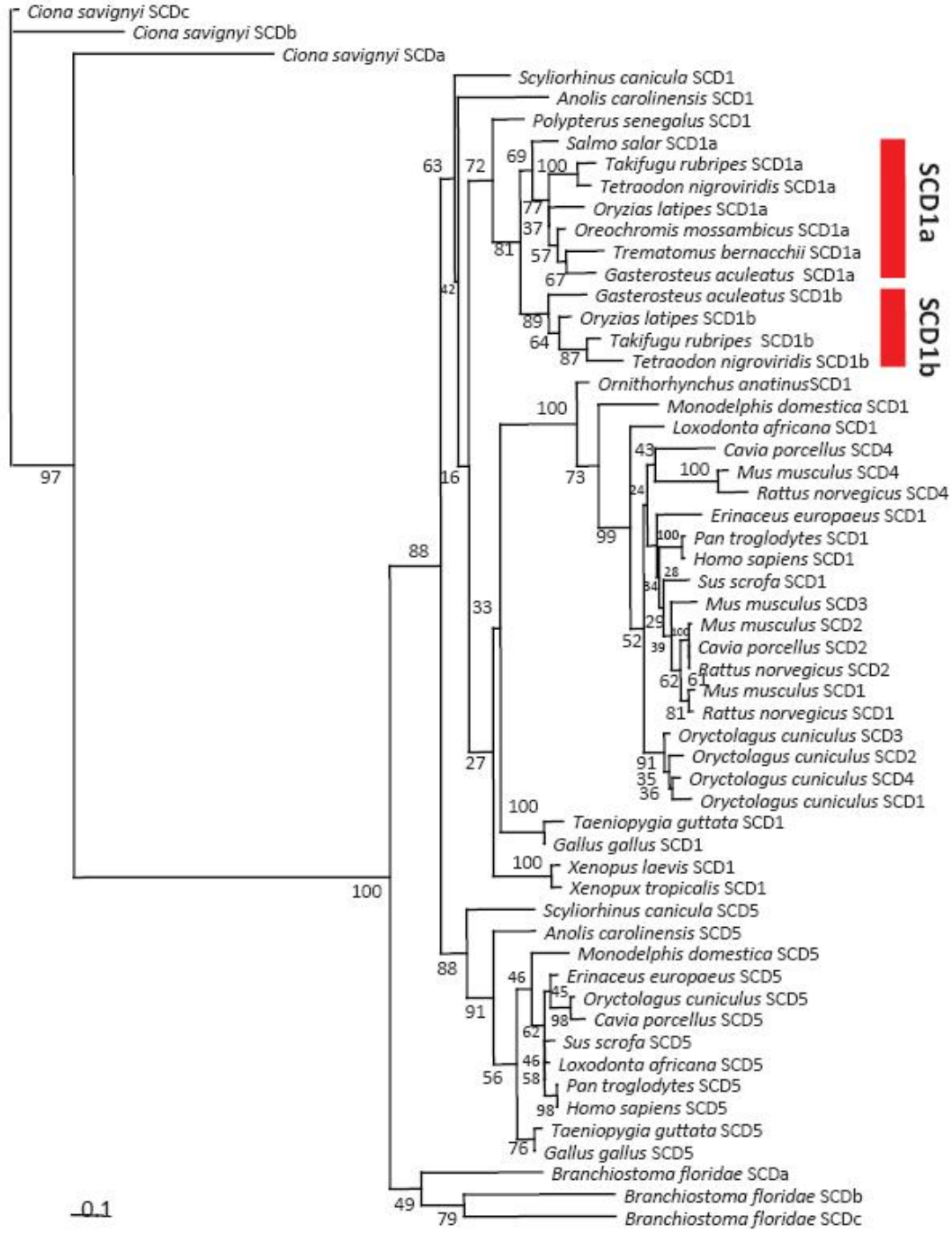


Genomic locus of *Branchiostoma floridae* SCD-like genes and the neighbouring gene families whose human paralogues localise to expected regions of human SCD paralogy (Hsa10, Hsa4, Hsa5 and Hsa2/8).

2,Gene loss and tandem duplications illustrate the tetrapod SCD repertoire

The evolutionary setting emerging from the paralogy analysis creates some important repercussions. For Example, the absence of SCD genes (either 1 or 5) in vertebrate classes would mean gene loss and not a different timing of the SCD1/SCD5 gene duplication.

To elucidate these matters, we started by analysing tetrapod species using two strategies. Firstly, by determining the duplication timing through phylogenetics, and secondly by investigating the SCD gene loci in available tetrapod genomes representing various lineages.

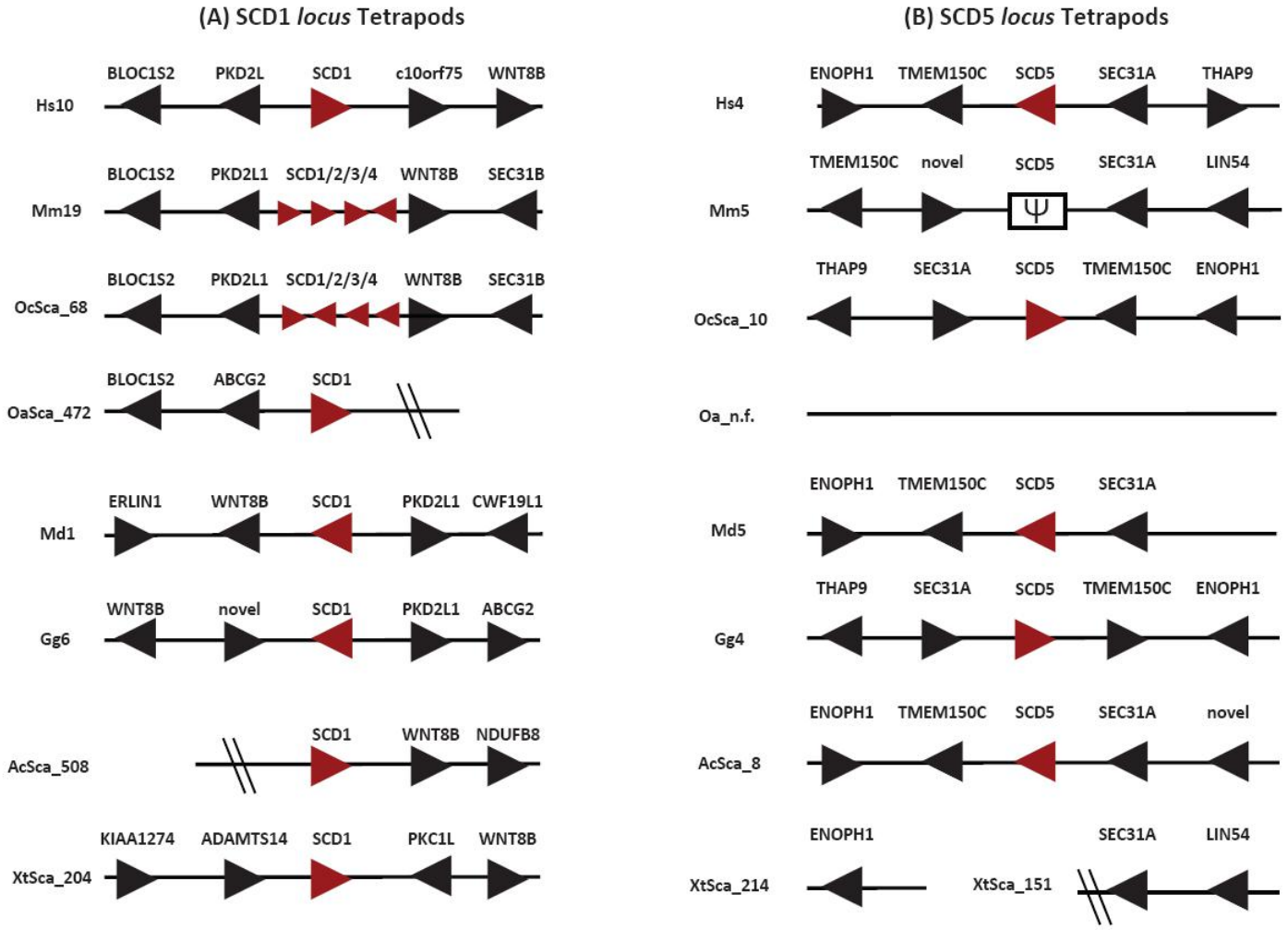


In tetrapod species, the separation of SCD1 and SCD5 lineages.

SCD1-type gene expansion in *M. musculus, Rattus norvegicus* and *O. cuniculus.*

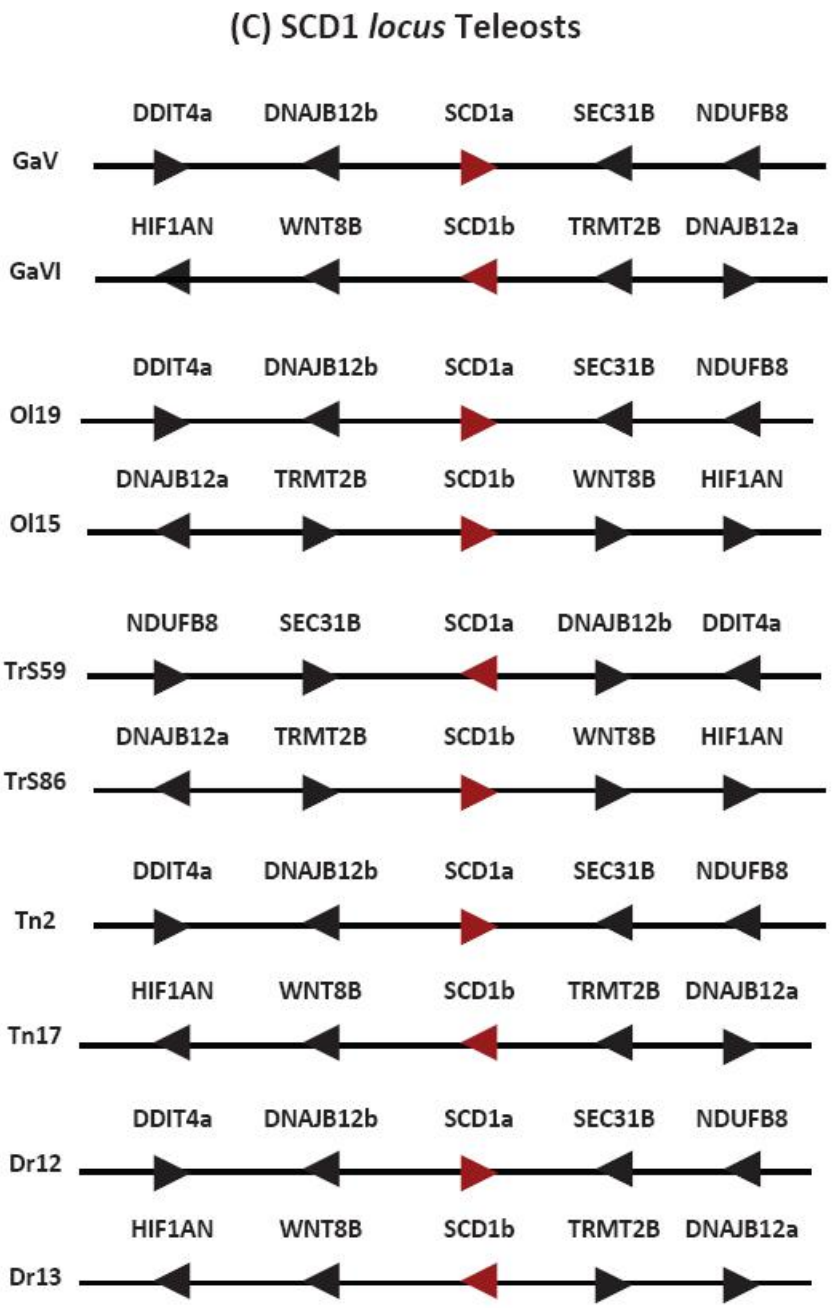
Within tetrapods we find no SCD5-like sequence in the *X. tropicalis*. In contrast to mice, *Cavia porcellus* (guinea pig, Rodent) and *O. cuniculus* have SCD5 Orthologues.

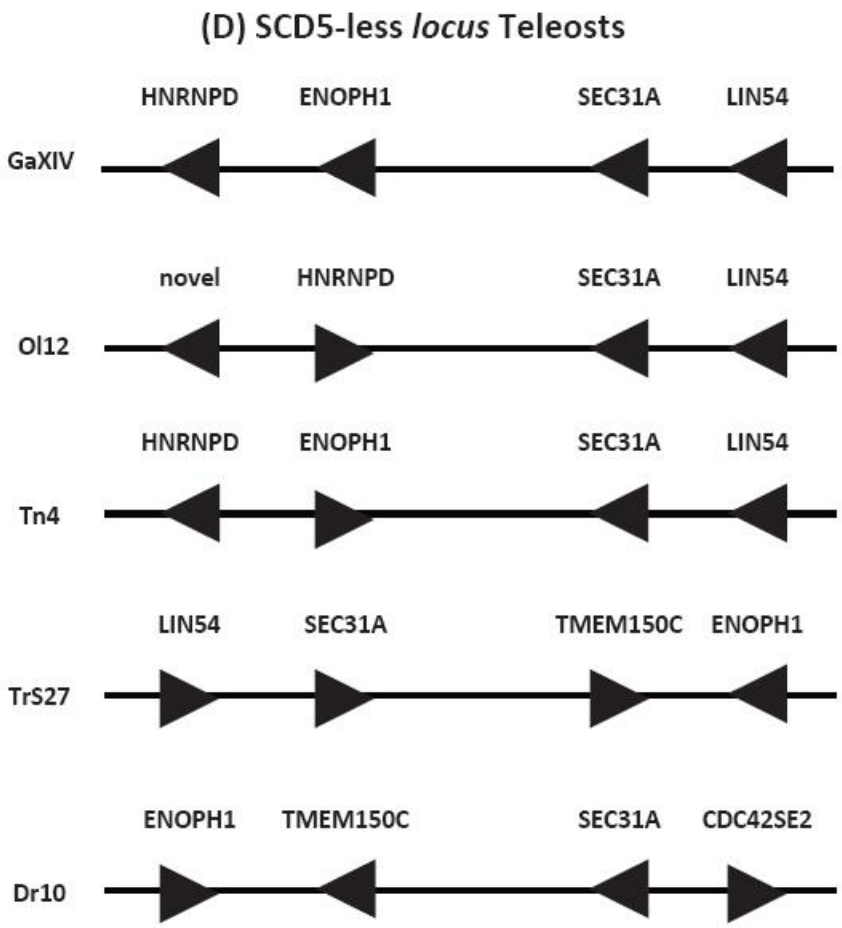
2,Gene loss and tandem duplications illustrate the tetrapod SCD repertoire



SCD1 **(A),** ***SCD5*** **(B) gene loci in tetrapod species**

SCD1 **gene tandem expansion**

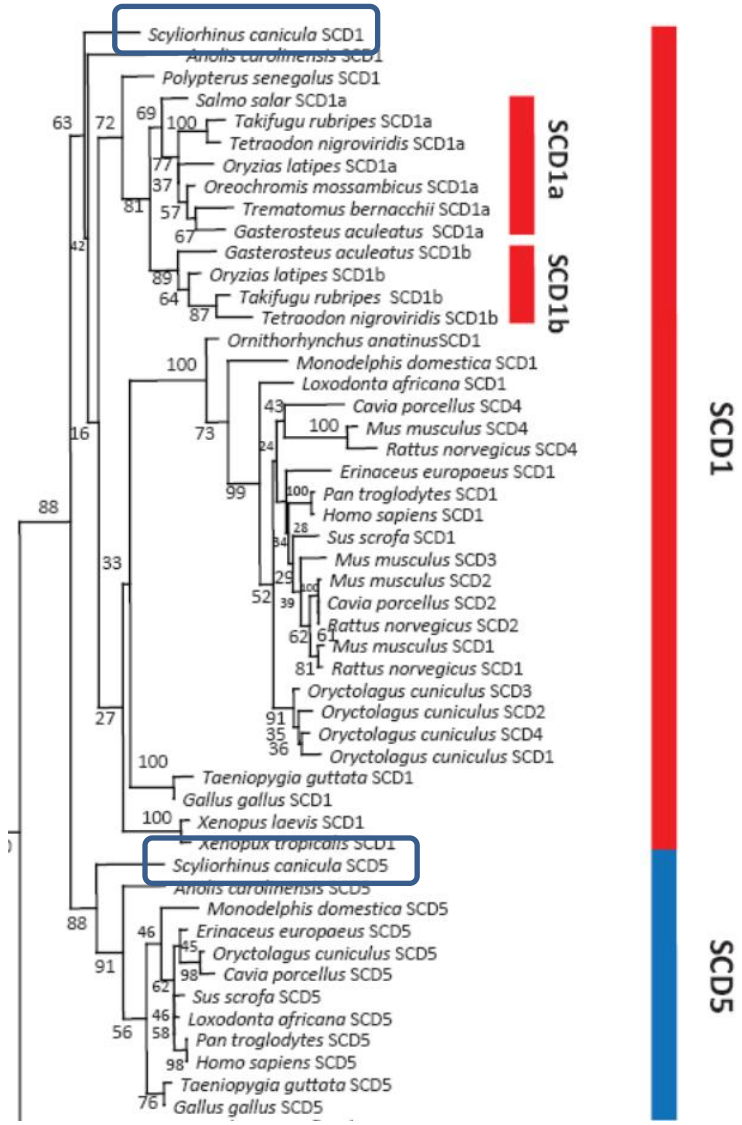




SCD1a/SCD1b (C) and SCD5 (D) gene loci in teleost species

Teleosts have lost SCD5 and SCD1a/SCD1b are 3R paralogues

3,SCD1 and SCD5 orthologues are present in the cartilaginous fish *Scyliorhinus canicula*



To determine whether SCD1 and SCD5 have been preserved in the oldest group of jawed vertebrates, using a degenerate PCR strategy we aimed at isolating orthologues of the SCD gene family from the *S. canicula*.

Sequence extension was achieved with various PCR strategies, resulting in two sequences coding for proteins with 341 and 325 amino acids when finally isolated.

ScSCD1 with the SCD1 clade (bootstrap63)
ScSCD5 with SCD5 genes (bootstrap 88)

4, SCD tissue expression suggests the conservation of an ancestral function

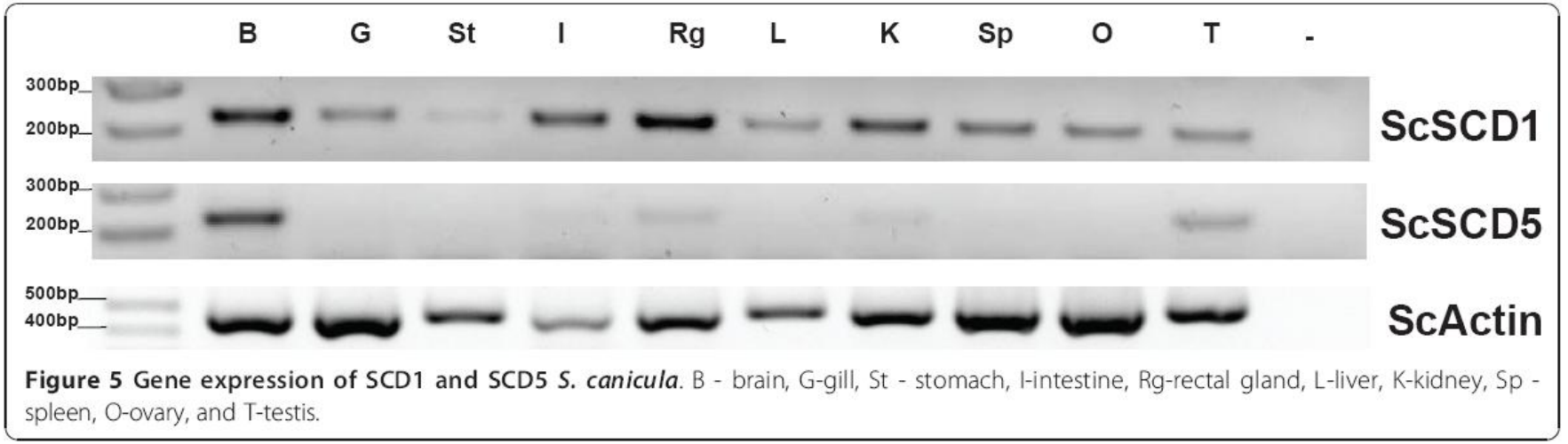


Figure 5 Gene expression of SCD1 and SCD5 *S. canicula*. B - brain, G-gill, St - stomach, I-intestine, Rg-rectal gland, L-liver, K-kidney, Sp - spleen, O-ovary, and T-testis.

ScSCD1 expressed at variable levels in all the tested tissues while ScSCD5 has a clear expression in the brain, and a minor expression in the testis and salt secreting rectal gland.

conserved expression site between birds and mammals

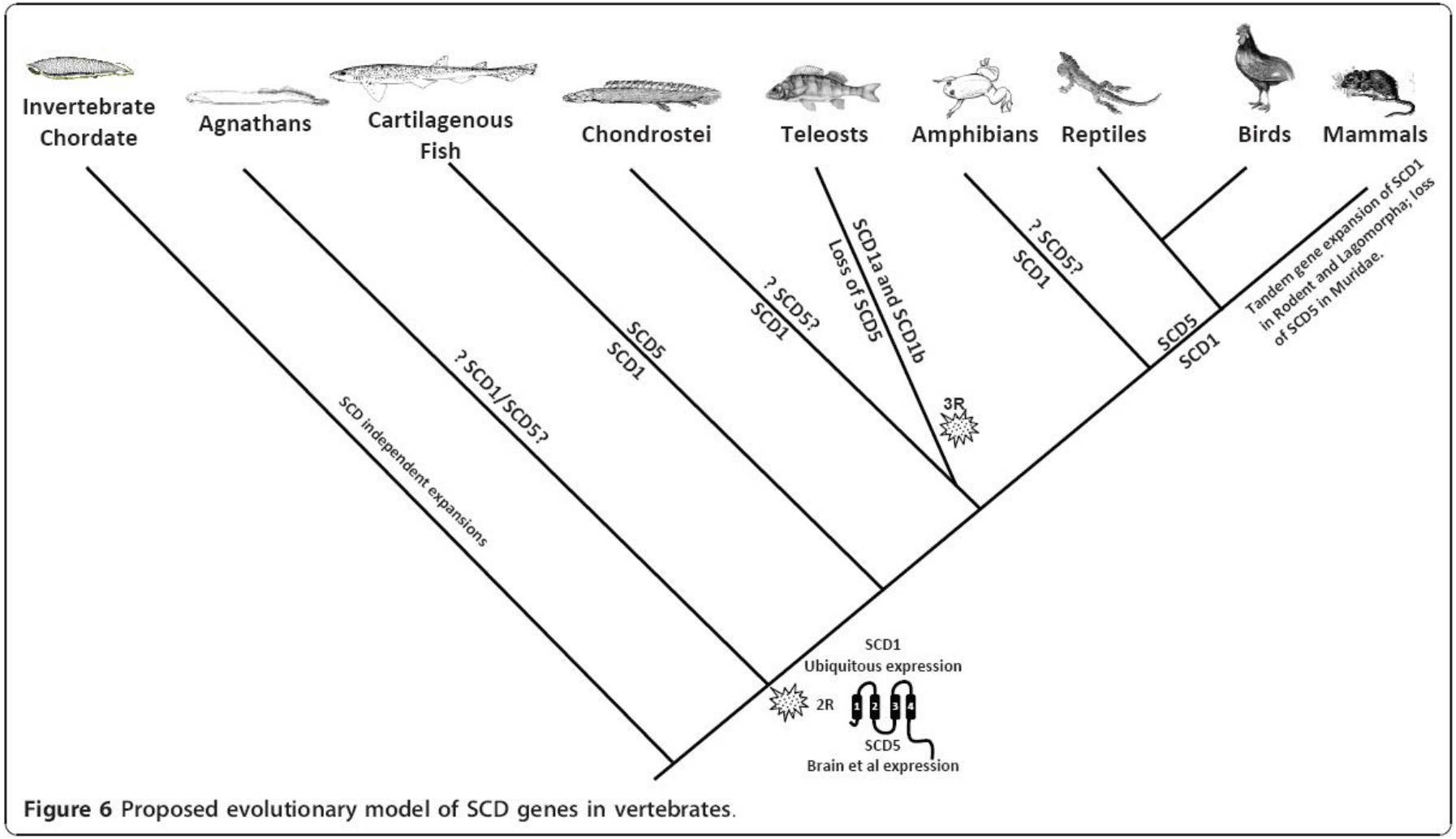


Figure 6 Proposed evolutionary model of SCD genes in vertebrates.

SCD1 and SCD5 orthologues were present in basal jawed vertebrate, and loss and duplication events took place during vertebrate evolution

Methods

- **Sample collection and storage**
  - weir fishing
  - fed mackerel
- **Synteny and Paralogy examination**
  - Ensembl genome database
  - MEGA4
- **Molecular phylogenetic analysis**
  - CLUSTALW in Bioedit
  - PHYML(Maximum Likelihood tree)

- **SCD1 and SCD5 isolation in *S. canicula* and gene expression**

  RACE PCR

  RT-PCR

RACE PCR

- **RACE(rapid-amplification of cDNA ends)是通过PCR进行cDNA末端快速克隆的技术。cDNA完整序列的获得对基因结构、蛋白质表达、基因功能的研究至关重要。**

RT-PCR简介

- 逆转录PCR（reverse transcription PCR）或者称反转录PCR（reverse transcription-PCR, RT-PCR），是聚合酶链式反应（PCR）的一种广泛应用的变形。在RT-PCR中，一条RNA链被逆转录成为互补DNA，再以此为模板通过PCR进行DNA扩增。

- RT-PCR注意事项

（1）引物的特异性决定PCR反应特异性。因此引物设计是否合理对于整个实验有着至关重要的影响。在引物设计时要充分考虑到可能存在的同源序列，同种蛋白的不同亚型，不同的mRNA剪切方式对引物的特异性的影响。尽量选择覆盖相连两个内含子的引物，或者在目的蛋白表达过程中特异存在而在其他亚型中不存在的内含子。

（2）实验所用的接触样品的耗材如冻存管、枪头、EP管之类事先都需经过0.1%DEPC水浸泡处理，除去RNA酶，防止操作过程中RNA降解。然后经高压灭活（灭菌和灭活DEPC）。

Thanks