

SUPPLEMENTARY TABLES

Table S1. Additional conserved protein domains identified on sirtuin proteins in our dataset.

Domain	Found in species	Sirtuin Clade	gene ID (short)	Position to SIR2 domain
CAP_C	<i>Acropora millepora</i>	SIR8	XP_029214425.1	Upstream
CAP_C	<i>Actinia tenebrosa</i>	SIR8	XP_031565139.1	Upstream
CAP_C	<i>Amphimedon queenslandica</i>	SIR8	XP_011406188.1	Upstream
CAP_C	<i>Orbicella faveolata</i>	SIR8	XP_020631316.1	Upstream
CAP_C	<i>Pocillopora damicornis</i>	SIR8	XP_027046210.1	Upstream
CAP_C	<i>Stylophora pistillata</i>	SIR8	XP_022790004.1	Upstream
DUF592	<i>Puccinia graminis</i>	SIR1	KAA1082764.1	Upstream
DUF592	<i>Rhizophagus irregularis</i>	SIR1	PKC08513.1	Upstream
DUF592	<i>Saccharomyces cerevisiae</i>	SIR1	4IAO	Upstream
DUF592	<i>Saccharomyces cerevisiae</i>	SIR1	NP_014573.1	Upstream
DUF592	<i>Schizosaccharomyces pombe</i>	SIR1	NP_001018840.1	Upstream
Img2	<i>Dendronephthya gigantea</i>	SIR2	XP_028398492.1	Upstream
Mob_synth_C	<i>Pleurobrachia bachei</i>	SIR7	sb 2669295	Downstream
Oxysterol_BP	<i>Mnemiopsis leidyi</i>	SIR3	ML002624a	Upstream
Radical_SAM	<i>Pleurobrachia bachei</i>	SIR7	sb 2669295	Downstream

Table S2: Probable contaminating sequences / horizontal gene transfer events in our dataset. See Figure S6 for the relevant gene tree.

Scientific name	gene ID (short)	Species with most similar protein
<i>Aurelia coerulea</i>	TCONS_00008113_Seg122.19.p1	<i>Aquifex aeolicus</i>
<i>Capitella teleta</i>	ELT91158.1	<i>Methanopyrus kandleri</i>
<i>Clytia hemisphaerica</i>	TCONS_00049926	<i>Thermus thermophilus</i>
<i>Leucosolenia complicata</i>	lcpid67440_lcgid21395	<i>Aquifex aeolicus</i>
<i>Salpingoeca rosetta</i>	XP_004993987.1	<i>Pyrobaculum aerophilum</i>
<i>Strongylocentrotus purpuratus</i>	XP_030829218.1	<i>Caldanaerobacter subterraneus</i>
<i>Stylissa carteri</i>	scaffold70346-gene-0.28	<i>Thermococcus kodakarensis</i>
<i>Sycon ciliatum</i>	scpid89809_scgid18507	<i>Saccharolobus solfataricus</i>

SUPPLEMENTARY FIGURE LEGENDS

Figure S1: A cartoon demonstrating the difference between orthologs and paralogs, and why the distinction shifts at different levels of evolutionary analysis. (A) A hypothetical gene tree of “family 3” sirtuins, based on the results of Opazo et al. (2020) Note that the fish and frog have two copies of the gene, which are distinguished as “Sir3” and “Sir 3-like”. From the vantage of vertebrates, Sir3 and Sir3-like are paralogs of each other, as signified by the colored boxes. A scientist who assumes that, for example, “Frog Sir3-like” and “Mouse Sir3” play similar roles in their respective organisms is likely to generate a flawed hypothesis. However, from the vantage of all animals (including invertebrates like sea urchins and jellyfish), vertebrate Sir3 and Sir3-like are both orthologs of their invertebrate counterpart. (B) The gene tree described in (A) reconciled with the species tree. In this scenario, the two genes in fish and frogs originate from a single gene duplication event that occurred early in vertebrate history. The mouse lost one of these two paralogs over the course of evolution. It is worth noting that other scenarios of gene duplication and loss could explain the gene tree seen in (A).

Figure S2. The full phylogeny used to design Figure 2. Clades are colored based on their assignment in Figure 2. See GitHub file “9b_Domain_NOTUNG_Final.tree” for the relevant tree file.

Figure S3. Partial alignment of SIR6 and SIR8 sequences. A conserved nine amino acid motif is identified at the top of the figure, although additional differences between SIR6 and SIR8 exist. See GitHub file “9_SIR2_Domains.vetted.mafft.fasta” for the full data. Image made in Geneious v.9.1.8.

Figure S4. A partial protein alignment showing a unique SIR9 motif and its distribution across animals. Additional SIR9 proteins from animals that were not queried in the original analysis are shown at the bottom of the alignment. Image made in Geneious v.9.1.8.

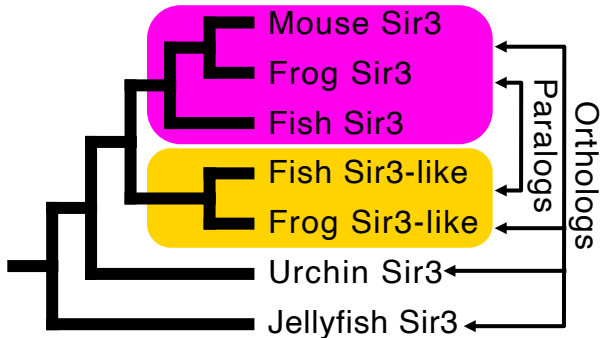
Figure S5. The full phylogeny used to design Figure 5. See GitHub file “4_NOTUNG-SpongeFirst-Final_Tree.tree” for the relevant tree file.

Figure S6. Initial phylogeny of sirtuin protein domains in our study. Likely contaminants / possible horizontal gene transfers are colored red and were excluded from further analysis. See GitHub file “8_SIR2_Domains.mafft.fasta.contree.tree” for the relevant tree file.

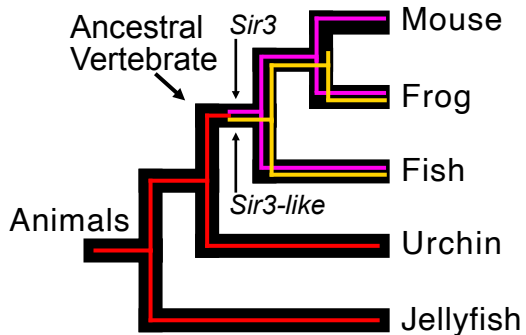
Figure S7. The full phylogeny used to inform Figure 6. Probable losses of PNC1 and NAMPT are labeled on the tree.

Figure S1

A Gene tree



B Gene tree reconciled with species tree



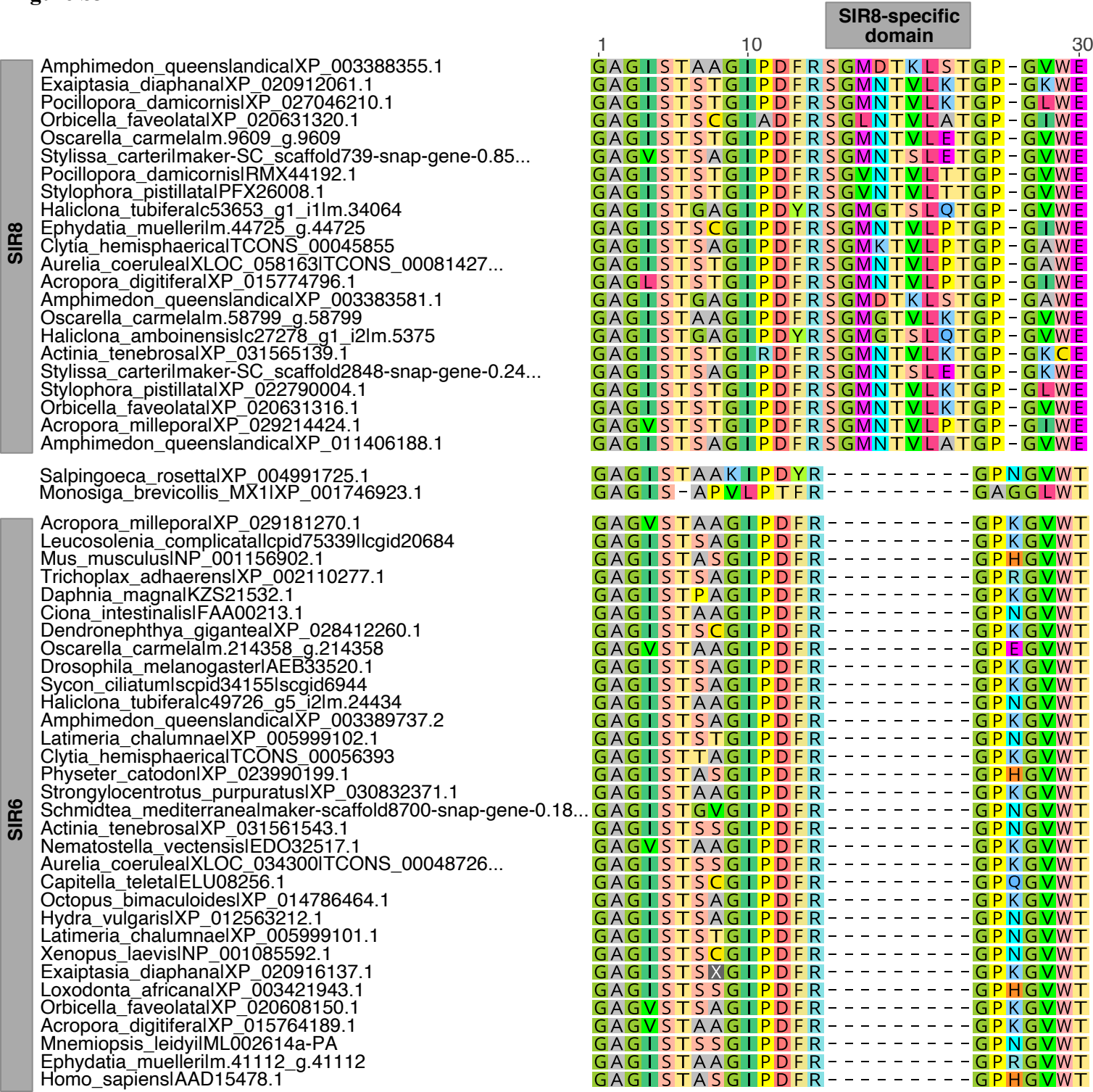


Figure S4

		SIR9-specific domain
SIR7	Drosophila_melanogaster AAL28953.1	N S V Y W R Q F D T T E M - - - - - T A R Y
	Homo_sapiens BAF82954.1	N R E Y V R V F D V T E R - - - - - T A L H
	Mus_musculus AAP83960.1	N R E Y V R V F D V T E R - - - - - T A L H
SIR6	Drosophila_melanogaster AEB33520.1	R R Q F V S P S A V E T V - - - - - G Q K S
	Caenorhabditis_elegans NP_491733.2	F S E Y V R E E I V M S V - - - - - G L C -
	Acropora_digitifera XP_015764189.1	G T E Y I R Q N A V - - - - - G T V G
	Mus_musculus NP_001156902.1	K T Q Y V R D T V V - - - - - G T M G
	Homo_sapiens AAD15478.1	K T Q Y V R D T V V - - - - - G T M G
SIR8	Leucosolenia_complicata lcpid182756...	G K Q Y L R D F H C Y R L - - - - - K K G R
	Acropora_digitifera XP_015774796.1	G M K Y M R D F R T R T S - - - - - R E V H

Proteins in original analysis

SIR9	Haliclona_tubifera c40065_g1_i1 m.9948	N H D Y Y R P Y Y V L D D E L S Q Y Y E D I N D N G T T D I I K P A H A L Q C G Q C K L
	Ephydatia_muelleri m.286671_g.286671	G R K Y Y R P F Y V L D D Q A S E Y Y E E L E D N G T T D L V K P R H A L R C G L C G L
	Amphimedon_queenslandica XP_003385596.1	D T K Y A R P Y Y V L D D D C S Q Y Y E D I N D C G K S S I K K P T Y G S Q C P Q C S L
	Stylissa_carteri maker-SC_scaffold144383...	D H R Y Y R P F Y V M D D R A S Q Y F E E L A D Y G T T D V P K P K H A A Q C R Q C G L
	Leucosolenia_complicata lcpid110268 ...	G K E H V R N Y Y T L D D A A D A V L D - - - - - A P G G I P A D S H V E F C P E C S C
	Mnemiopsis_leidyil ML200240a-PA	G T R Y S R E M Y T P D D I A G E Y Y D D L L T Y G K T D V K L P K H A I R C K T C S L
	Salpingoeca_roseotal XP_004993416.1	R T I Y M C S Q Y V L D D E S E A V F E - - - - - S G K I P K G S H V E V C P T C G L
	Actinia_tenebrosa XP_031565494.1	G T R Y E R D F Y V L D D N G S Y Y F E E L N E C G A S D I K K P R N A R Q C D L C G L
	Orbicella_faveolata XP_020604327.1	G T R Y E R P F Y V L D D H A S L Y Y E E L N D L G A T T V K K P R H A K R C D L C G L
	Capitella_teletal ELU05314.1	G H R Y E R S F Y V M D D V A C E Y F E E K A E L G H T D I I R P K H A K E C T T C G L

Additional proteins in NCBI

SIR9	Crassostrea_gigas XP_011416941.2	H H R Y E R T F Y V M D D A G S Q Y F E D I E D Y G K S E V K K P R H A K R C D T C G L
	Crassostrea_gigas XP_011416940.2	H H R Y E R T F Y V M D D A G S Q Y F E D I E D Y G K S E V K K P R H A K R C D T C G L
	Mizuhopecten_yessoensis OWF48853.1	G K R Y E R D F Y V M D D I S S Q Y F E E L E D F G K T D I R K P K H A V K C E R C E L
	Mizuhopecten_yessoensis XP_021356835.1	G K R Y E R D F Y V M D D I S S Q Y F E E L E D F G K T D I R K P K H A V K C E R C E L
	Crassostrea_virginical XP_022313135.1	G H H Y A R S F Y V M D D T S S Q Y F E D I E D Y G K S D V K K P R H A K Q C D T C G L
	Pecten_maximus XP_033729257.1	G K R Y E R D F Y V M D D I S S Q Y Y E E L E D F G K T D I R K P K H A V K C E R C G L
	Mytilus_coruscus CAC5374474.1	K R Q Y Y R H F Y V M D D V G S Q Y F E E L D D N G K T D M K K P K Y A V K C K K C G L
	Elysia_chlorotical RUS72897.1	G A R Y E R S F Y V C D D I A D I Y L E E L E D H G K T D V I P P K F M F K C R K C G L
	Aplysia_californical XP_005094645.1	G A M Y S R P Y Y V C D D I G D M Y L E E V A D F G K S T I I A P K F M K K C R K C G L

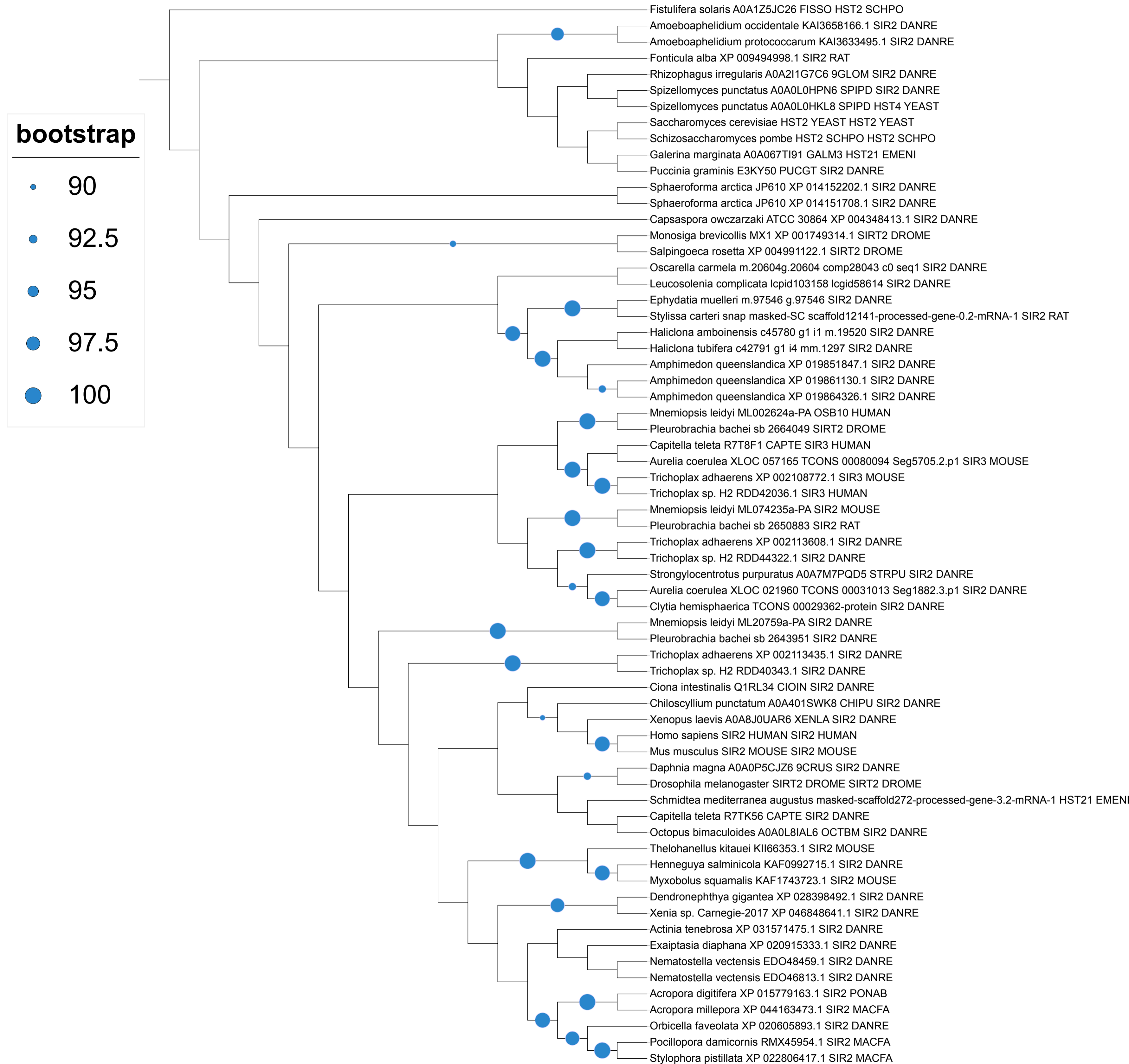


Figure S7

