Published datasets searched for TNF analysis
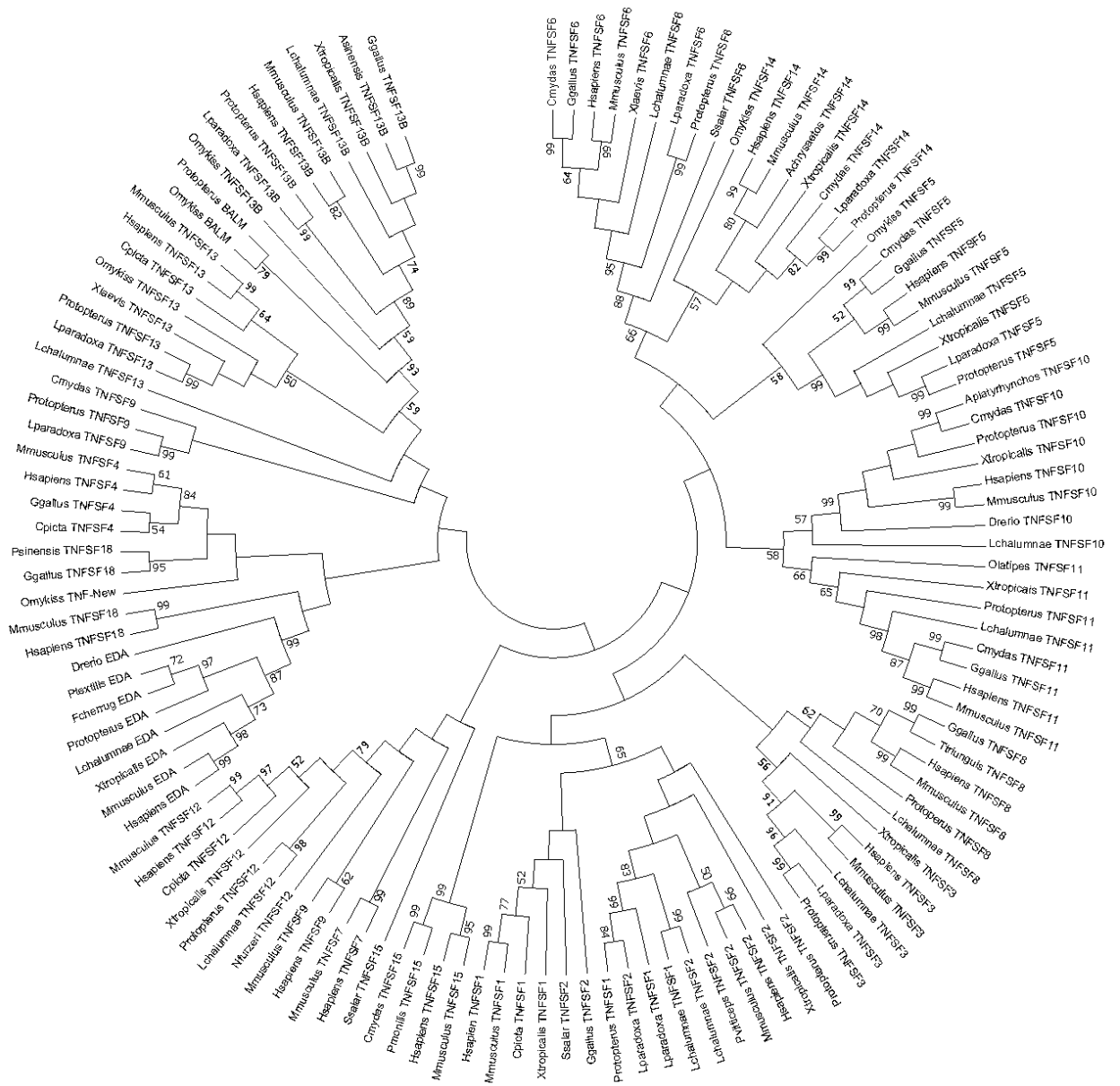
Organism	Genomes/Transcriptomes	Date Published
Mammals	Human	2001
	Mouse	2002
Aves	Zebra Finch	2010
	Chicken	2004
	Turkey	2011
	Mallard Duck	2013
Reptile	Western Painted Turtle	2013
	American Alligator	2012
	Chinese Alligator	2013
Amphibian	Western Clawed Frog	2010
	North American Bullfrog	2017
	High Himalaya Frog	2015
Sarcopterygii	Coelacanth	2013
	West African Lungfish	2013
	South American Lungfish	2015
Salmonids	Rainbow Trout	2014
	Salmon	2016
	Coho Salmon	2017

Supplementary Table I. Comprehensive list of genomes analyzed for the presence of TNFSF members in this study.

Statistical analysis of assembled transcriptomes

Transcriptome Statistics	Mouse Peyer's patch	Mouse skin	Trout interbranchial lymphoid tissue	Trout Muscle	Turkey cecal tonsil	Turkey muscle	Lungfish lymphoid aggregate	Lungfish skin
Organism	*Mus musculus*	*Mus musculus*	*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Meleagris gallopavo*	*Meleagris gallopavo*	*Protopterus dolloi*	*Protopterus dolloi*
Accession Numbers	SRR8114138	SRR6884615	SRR8112130	DRR046645	SRR7771850	SRR478418	SRR7879313	SRR8137461
Raw total sequences	68,691,990	57,486,984	31,379,578	157,592,662	37,217,902	25,983,202	57,607,080	37,794,958
Reads mapped and paired	67,068,330	56,347,246	29,359,424	148,341,764	35,589,842	24,619,708	56,232,156	36,707,530
% Reads mapped and paired	97.64%	98.02%	93.56%	94.13%	95.63%	94.72%	97.61%	97.12%
Reads unmapped	1,278,627	889,825	1,587,745	6,703,289	1,255,693	1,085,299	1,088,166	845,117
n_scaffolds	280,740	246,275	286,901	176,993	164,478	247,098	162,353	128,529
scaf_bp	201,556,902	306,900,459	212,361,256	110,893,564	176,631,903	163,148,464	114,958,573	101,050,590
scaf_N50	38,844	32,520	43,533	36,610	20,275	30,971	21,735	16,845
scaf_L50	1,265	2,778	1,336	882	2,521	1,275	1,398	1,468
scaf_N90	196,215	142,164	198,077	129,778	97,765	175,721	112,489	87,167
scaf_L90	280	425	283	274	349	250	258	295
scaf_max	16,461	35,601	15,868	9,880	18,221	18,940	10,356	47,530
gc_avg	0.46728	0.49312	0.45933	0.47307	0.4534	0.44589	0.39734	0.40296
gc_std	0.07262	0.07403	0.06972	0.0802	0.09034	0.09161	0.05759	0.05204

Supplementary Table II. Statistical analysis of assembled transcriptomes.

Supplementary Figure 1. Phylogenetic tree showing the evolutionary relationship of vertebrate TNFSF proteins. The analysis was performed on homologous sequences aligned with Muscle using MEGA 10 software (Kumar et al. 2018). The evolutionary distances were computed using a Poisson substitution model and the tree was constructed with the Neighbor-Joining method. Tree was bootstrapped 1000 times. Value lower than 50% were removed from the tree. Note that Lparadoxa TNFSF10, Lchalumnae TNFSF14 and Drerio TNFSF18 are not included in the phylogenetic tree. Species in the tree are abbreviated as follows: Hsapiens: human (Hs); Mmusculus: mouse (Mm); Ggallus: chicken (Gg); Aplatyrhynchos: mallard duck (Ap); Fcherrug: saker falcon (Fc); Pmonilis: band-tailed pigeon (Pm); Asinensis: Chinese alligator (As); Pvitticeps: bearded dragon (Pv); Ttriunguis: turtle; Cmydas: sea turtle (Cm); Psinensis: Chinese soft shelled turle (Ps); Cpicta: painted turtle (Cp); Ptextilis: brown snake (Pt); Achrysaetos: golden eagle (Ac); Xtropicalis: Western clawed frog (Xt); Xlaevis: African clawed frog (Xl); Protopterus: African lungfish; Lchalumnae: coelacanth (Lc); Lparadoxa: South American lungfish (Lp); Omykiss: rainbow trout (Om); Ssalar: salmon (Ss); Drerio: zebrafish (Dr); Olatipes: Japenese rice fish (Ol); Nfurzeri: killifish (Nf).

Supplementary Figure 2. Phylogenetic tree showing the evolutionary relationship of vertebrate TNFRSF proteins. The analysis was performed on homologous sequences aligned with Muscle using MEGA 10 software (Kumar et al. 2018). The evolutionary distances were computed using a Poisson substitution model and the tree was constructed with the Neighbor-Joining method. Tree was bootstrapped 1000 times. Value lower than 50% were removed from the tree. Note that TNFRSF 4, 7, 25, 17 are not included in the phylogenetic tree for clarity purposes. Species in the tree are abbreviated as follows: Hsapiens: human (Hs); Mmusculus: mouse (Mm); Ggallus: chicken (Gg); Scamelus australis: Wild ostrich (Sca); Xtropicalis or Xlaevis: frog (Xt or Xl); Protopterus: African lungfish; Acarolinensins: lizard (Ac); Amississippiensis: alligator (Am); Pbivittatus: python (Pb); Lchalumnae: coelacanth (Lc); Lparadoxa: South American lungfish (Lp); Drerio: zebrafish (Dr); Ssalar: salmon (Ss); Fheteroclitus: mummichog killifish (Fh); Omykiss: rainbow trout (Om).