Supplementary Figure 1. Results of a protein BLAST search against NCBI GenBank (2.2.26), excluding *L. calcarifer* (directs to this study) and *Salmo salar* (numerous identical entries). The red arrows indicate sequences that were non-specific on the subunit type or incorrectly annotated.

-	Accession	Description	Max score	Total score	Query coverage	E value	Max Ident
	ADF80517.1	ferritin M subunit [Sciaenops oceliatus]	357	357	99%	9e-130	95%
	ACY75476.1	ferritin M subunit [Larimichthys crocea]	353	353	99%	2e-128	94%
	AEI87383.1	ferritin middle subunit [Epinephelus bruneus]	352	352	99%	1e-127	94%
	ADI24354.1	ferritin middle subunit (Scophthalmus maximus)	350	350	99%	5e-127	93%
\rightarrow	CAG02064.1	unnamed protein product [Tetraodon nigroviridis]	344	344	97%	1e-124	92%
	ACQ57875.1	Ferritin, middle subunit [Anoplopoma fimbria]	343	343	99%	4e-124	91%
	ACQ57862.1	Ferritin, middle subunit [Anoplopoma fimbria]	342	342	99%	5e-124	91%
	AC008179.1	Ferritin, middle subunit [Oncorhynchus mykiss]	340	340	99%	őe-123	89%
	AC010415.1	Ferritin, middle subunit [Calicus rogercressev]	339	339	99%	8e-123	89%
	P85836.1	RecName: Full=Ferritin, liver middle subunit; Short=Ferritin M	338	338	99%	1e-122	88%
	AC007744.1	Ferritin, middle subunit [Oncorhynchus mykiss]	338	338	99%	3e-122	89%
	P85835.1	RecName: Full=Ferritin. middle subunit: Short=Ferritin M	337	337	99%	7e-122	87%
\rightarrow	NP_001118021.1	ferritin H-3 [Oncorhynchus mykiss] >dhilBAA13148.1] ferritin H-3 [Oncorhynchus mykiss]	337	337	99%	7e-122	89%
	XP_003446457.1	PREDICTED: ferritin middle subunit-like [Oreochromis nilaticus]	337	337	99%	8e-122	89%
	XP 003446470.1	PREDICTED: ferritin middle subunit-like isoform 1 [Oreochromis niloticus]	330	330	99%	1e-121	89%
	AC008329.1	Ferritin middle subunit (Oncorbynchus mykiss)	330	330	99%	1e-121	89%
\rightarrow	NP_001118019.1	ferritin H-1 [Oncorbynchus mykiss] >dbt/BAA13146 1 ferritin H-1 [Oncorbynchus mykiss]	330	330	99%	2e-121	89%
\rightarrow	NP 001118020.1	ferritin H-2 [Oncorhynchus mykiss] >dbi[BAA13147.1] ferritin H-2 [Oncorhynchus mykiss]	335	335	99%	5e-121	88%
	P85839.1	DerName: Full-Ferritin, enlage middle subunit: Short-Ferritin M	334	334	99%	9e-121	86%
	XP 003446471.1	PREDICTED: ferritin_middle_subunt-like isoform 2 [Oreochromis niloticus]	332	332	99%	9e-120	89%
	NP 001187268.1	ferritin middle subunit [Ictalurus nunctatus] >nb1ADE09345.11 ferritin middle subunit [Ictalurus nunctatus]	330	330	98%	3e-119	86%
	AD029006.1	farritin middle subunit [retaiurus punctatus]	329	329	98%	8e-119	86%
	AC009733 1	Ferritin middle subunit [Actain's particaus]	327	327	97%	6e-118	87%
	AD028388 1	feetile middle cubunit (Ictalurus fuscatus)	327	327	98%	6e-118	85%
	AEB71787.1	ferritin Michael Subanic (reclain as farcacas)	326	326	99%	1e-117	88%
	AD028147.1	ferritin middle subunit [Ictalurus furcatus]	320	320	99%	0e-115	83%
	AAG13315 1	feetin midde suburit (cillektive mickile)	316	316	98%	7e-113	85%
	AC009343.1	remain mode subunit (amonunys mirabilis)	215	215	97%	26.113	0504
$ \rightarrow $	NP 001007278 1	rentur, induce suburin [Osnielus inducax] farritin based chain [Osniel coria] pabliktu/75970 11 Zac:02066 [Osniel coria]	204	204	97%	5e-109	0.376
	ARV81252 1	feetilin neavy chain [Danio reno] sgu (Ani/ 36/9.1] 2gc.92006 [Danio reno]	304	304	97.70	9e-109	9179
	ABV20007 1	territin subunit (Acipenser sinensis)	204	200	0.6%	50-105	02.70
$\overline{}$	ND 001120225 1	remain neavy chain (Pintephales prometas)	222	200	2076	0c-107	0070
$\overline{}$	AAV06093 1	uncharacterized protein LOCIOU145388 (Xenopus (Silurana) tropicaiis) >gd(AAI54147.1) 2gc:109934 pl	222	200	01%	26-107	0070
$\overline{}$	AA100532.1	Terrin H-3 (Ictaurus punctatus)	220	220	5170	20100	0.376
	P83838.1	Rechame: Full=Perficin, neavy subunit; Snort=Perficin H	238	238	3/70	26-100	7.3%
$\overline{}$	NP_001103324.1	uncharacterized protein LOC100126128 [Danio reno] > rer[NP_001123766.1] uncharacterized protein LO	200	250	2076	20105	7379
$\overline{}$	NP_001018307.1	uncharacterized protein LOC553552 (Danio reno) >gb AAH95061.1 2gc:L09934 (Danio reno)	223	200	2076	20103	7376
	XP_001921731.1	PREDICIED: territin, middle sudunit-like (Danio reno)	294	294	90%	00-100	/9%
$\overline{}$	<u>P83837.1</u>	RecName: Full=Ferritin, heavy subunit; Short=Ferritin H	292	292	97%	36-104	/4%
	<u>XP_08/1/5.1</u>	PREDICIED: Territin, middle sudunit-like (Danio reno)	291	291	93%	86-104	/8%
	NP_001103175.1	uncharacterized protein LOC559768 [Danio rerio] >ref[NP_001120189.1] uncharacterized protein LOC10	288	288	93%	16-102	/8%
\leq	AC009911.1	Ferritin, heavy subunit [Osmerus mordax]	287	287	33%	46-102	/2%
	AAI71092.1	Wu:ŋ24c01 [Danio reno]	280	280	93%	98-102	78%
$\overline{}$	NP_001107131.1	uncharacterized protein LOC100006523 [Danio rerio] >gb AAI54747.1 Wu:fj24c01 protein [Danio rerio]	280	280	92%	26-101	77%
\rightarrow	NP_001124139.1	uncharacterized protein LOC100170833 [Danio reno] >gb AAI62709.1 Zgc:194125 [Danio reno] >gb A	283	280	92%	36-101	//%
	CAR00080.1	territin high chain [Notothenia corliceps]	281	281	99%	0e-100	71%
	CAL92185.1	remin neavy chain [Chlonodraco rastrospinosus] >emb[CAR66074.1] ferritin high chain [Chlonodraco ha	281	281	99%	8e-100	71%
\rightarrow	CAR66076.1	rerritin nign chain [Parachaenichthys charcoti] >emb[CAR66077.1] ferritin high chain [Gymnodraco acutik	281	281	99%	1e-99	70%
\rightarrow	NF_001187267.1	territin heavy chain subunit [Ictalurus punctatus] >gb ADE09343.1 ferritin heavy chain subunit [Ictaluru	280	280	99%	36-99	70%
\rightarrow	ALN80998.1	territin heavy polypeptide [Dicentrarchus labrax]	280	280	99%	36-99	70%
\rightarrow	ACY75475.1	territin H subunit [Larimichthys crocea]	280	280	99%	4e-99	70%
\rightarrow	CAR66078.1	ferritin high chain [Trematomus bernacchii]	279	279	99%	4e-99	70%
\rightarrow	P07229.3	RecName: Full=Ferritin, higher subunit; Short=Ferritin H >gb AAA49523.1 ferritin, higher subunit [Rana	279	279	99%	6e-99	74%
\rightarrow	CAR66075.1	ferritin high chain [Chaenocephalus aceratus]	279	279	99%	6e-99	70%
\rightarrow	ABD75379.1	ferritin H [Bufo gargarizans]	278	278	99%	1e-98	74%
\rightarrow	AAA49532.1	ferritin [Rana catesbelana]	277	277	99%	3e-98	73%

Supplementary Figure 2. Multiple sequence alignment showing the conserved ferroxidase centres (green) and nucleation sites (yellow) in selected vertebrate sequences. Latca_1, Dre2, Tru1, Tni1, Xtr4, HsaFTL, and MmuFTL represent putative middle/light-chain sequences while Latca_2, Dre4, Tru2, Tni2, Xtr2, HsaFTH1 and MmuFTH1 represent putative H-chains. The teleost M-chains appear to possess all of the conserved ferroxidase sites found in vertebrate H-chains.

		···· ···· >	···· ····) 30	···· ····	···· ····	\cdots
latca 1						
Tru1						
Trui Trii						
Vtr1						
MmuETI						
						FERHOSHEER
Dre4		EACEAAVNRO				FERHOSHEER
Tru2	MSSOVRONEH					FERHOSHEER
Tni2	-SSOVRONEH			YI SMAYYEDR	DDOAL HNEAK	FERHOSHEER
Xtr2	MNSOVRONYH	OFCEAAINRO	VNMELYASYV			YELHOSHEER
HsaFTH1	MTSOVRONYH	ODSEAAINRO			DDVAL KNEAK	YELHOSHEER
MmuFTH1	MPSOVRONYH	ODAFAAINRO		YLSMSCYEDR	DDVAL KNEAK	YELHOSHEER
		QUILINIIII		1 ESWSCIT DR		
	70) 80) 90) 100) 110) 120
Latca_1	EHAEKLLSFQ	NKRGGHIFLQ	DIKKPERDEW	GSGLEAMQCA	LQLEKNVNQA	LLDLHKLASD
Dre2	EHAEKFMEFQ	NKRGGRIVLQ	DIKKPERDEW	DNGLTAMQCA	LQLEKNVNQA	LLDLHKVASQ
Tru1	EHAEKLLSFQ	NKRGGRIFLQ	DIKKPERDEW	GSGLEAMQCA	LQLEKKVNQA	LLDLHKLASD
Tni1	EHADKLLSFQ	NKRGGRIFLQ	DIKKPERDEW	GSGLEAMQCA	LQLEKKVNQA	LLDLHKLASD
Xtr4	<mark>EH</mark> AEKFLKYQ	NKRGGRAVLQ	DIKKPERDEW	GNTLEAMQAA	LQLEKTVNQA	LLDLHKLASD
HsaFTL	<mark>E</mark> GYERLLKMQ	NQRGGRALFQ	DIKKPAEDEW	GKTPDAMKAA	MALEKKLNQA	LLDLHALGSA
MmuFTL	EGAERLLEFQ	NDRGGRALFQ	DVQKPSQDEW	GKTQEAMEAA	LAMEKNLNQA	LLDLHALGSA
Latca_2	EHAEKLMKLQ	NQRGGRIFLQ	DVRKPDRDEW	GSGVEALECA	LQLEKSVNQS	LLDLHKLCSD
Dre4	EHAEKLMKFQ	NQRGGRIFLQ	DVKKPEKDEW	GSGVEALECA	LQLEKSVNHS	LLELHKLASQ
Tru2	EHAEKLMKMQ	NQRGGRIFLQ	DVRKPERDEW	GSGMEALECA	LQLEKSVNQS	LLDLHKMCSD
Tni2	EHAEKLMKMQ	NQRGGRIFLQ	DVRKPERDEW	GSGTEALECA	LQLEKSVNQS	LLDMHKMCSD
Xtr2	EHAEKLMKMQ	NQRGGRIFLQ	DVKKPDRDEW	ANGLEALECS	LQLEKSVNQS	LLELHKLSTD
HsaFTH1	EHAEKLMKLQ	NQRGGRIFLQ	DIKKPDCDDW	ESGLNAMECA	LHLEKNVNQS	LLELHKLATD
MmuFTH1	E <mark>H</mark> AEKLMKLQ	NQRGGRIFLQ	DIKKPDRDDW	ESGLNAMECA	LHLEKSVNQS	LLELHKLATD
						[
	13() 140) 150) 160) 170)
Latca_1	HGDPHLCDFL	ETHYLNEQVE	AIKKLGDYIS	NLSRMDAQKN	KMAEYLFDKH	SLGGK
Dre2	KGDPHLCDFL	eshylne <mark>q</mark> ve	AIKKLGDHIT	NLSKMDAGNN	RMAEYLFDKH	TLDS-
Tru1	HVDPHLCDFL	eshylne <mark>q</mark> ve	AIKKLGDYIT	NLSRMDAQNN	KMAEYLFDKH	TLGSK
Tni1	HVDPHLCDFL	ethylne <mark>q</mark> ve	AIKKLGDYIT	NLSRMDAQNN	KLAEYLFDKH	TLGSK
Xtr4	KTDPHLCDFL	ESEYLEE <mark>Q</mark> VK	AMKELGDYIT	NLKRLGVPQN	GMGEYLFDKH	TLGES
HsaFTL	RTDPHLCDFL	ETHFLDEEVK	LIKKMGDHLT	NLHRLEA	GLGEYLFERL	TLKHD
MmuFTL	RADPHLCDFL	ESHYLDKEVK	LIKKMGNHLT	NLRRVGAPQG	SLGEYLFERL	TLKHD
Latca_2	HNDPHLCDFI	ETHYLDEQVK	SIKELADWVT	NLRRMGAPQN	GMAEYLFDKH	TLGKE
Dre4	HNDPHMCDFI	ethylde <mark>q</mark> vk	SIKELGDHVT	NLRRMGAPQN	GMAEYMFDKL	TLGKE
Tru2	HNDPHLCDFI	ethflde <mark>q</mark> vk	SIKELADWVT	NLRRMGAPQN	GMAEYLFDKH	TLGKV
Tni2	HNDPHMCDFI	ethflde <mark>q</mark> vk	SIKELADWVA	NLRRMGAPQN	GMAEYLFDKH	TLGKE
Xtr2	HNDPHLCDFL	ESHYLDE <mark>Q</mark> VK	SMKELGDHIT	NLRRMGAPSN	GMAEYLFDKH	TLGEH
HsaFTH1	KNDPHLCDFI	ethylne <mark>q</mark> vk	AIKELGDHVT	NLRKMGAPES	GLAEYLFDKH	TLGDS
MmuFTH1	KNDPHLCDFI	etyylse <mark>q</mark> vk	SIKELGDHVT	NLRKMGAPEA	GMAEYLFDKH	TLGHG

Supplementary Figure 3. Initial Maximum Likelihood analysis conducted using all sequences mined from Ensembl and the three annotated Rana sequences, with *C. savignyi* **as the outgroup.** No clear pattern of grouping was observed other than the clustering of the mammalian mitochondrial sequences and many branches lacked bootstrap support (values indicated at branches). Sequences that are indicated with * were removed from subsequent analysis.



Supplementary Figure 4. Bayesian tree constructed from the amino acid sequences of metazoan ferritins. The mammalian (*H. sapiens*, *M. musculus*) and non-vertebrate (*D. melanogaster*) mitochondria sequences are highlighted in brown and clustered separately. Values at branches indicate the posterior probabilities.



Supplementary Figure 5. Bayesian tree constructed from amino acid sequences of vertebrates ferritins. Values at branches indicate the posterior probabilities.



0.05

Supplementary Figure 6. Maximum Likelihood tree constructed from the amino acid sequences of vertebrate ferritin, including the annotated *R. catesbiana* L-chain. The inclusion of *R. catesbiana* L-chain (highlighted in red), clustered with the ancestral vertebrate sequences and decreases the bootstrap values (1000 replications; indicated at the branches).



0.05

Supplementary Figure 7. Bayesian tree constructed from the amino acid sequences of vertebrate ferritin, including the annotated *R. catesbiana* L-chain. The position of *R. catesbiana* L-chain in the tree was unresolved. Values at branches indicate the posterior probabilities.



0.2

Supplementary Table 1: Primers used to generate the complete putative cDNA sequences.

Primer	Sequence
FerM-F	5'-GAGCCTGGTTGACGTTCTTCTCC
FerM-R	5'-TCCTCCAGGACATCAAGAAACCG
FerH-F	5'-AGGCGTACAGCTCCAGGTTGATCTG
FerH-R	5'-GCAGATCAACCTGGAGCTGTACGC

Supplementary Table 2: Sequences used in the study.

Sequences marked with * were subsequently discarded after the initial analysis.

Species	Sequence name	Ascension number
Danio rerio	Dre1	ENSDARG00000079938
	Dre2	ENSDARG0000031776
	Dre3	ENSDARG00000078342
	Dre4	ENSDARG00000015551
	Dre5	ENSDARG0000073936
	Dre_79846*	ENSDARG00000079846
	Dre_77360*	ENSDARG00000077360
	Dre_76621*	ENSDARG00000076221
	Dre_07975*	ENSDARG0000007975
Takifugu rubripes	Tru1	ENSTRUG0000014292
	Tru2	ENSTRUG0000017266
Oryzias latipes	Ola1	ENSORLG0000016761
	Ola2	ENSORLG0000005872
	Ola_06156*	ENSORLG0000006156
Tetraodon nigroviridis	Tni1	ENSTNIP00000014079
	Tni2	ENSTNIP0000006889
Gasterosteus aculeatus	Gac1	ENSGACG0000005975
	Gac2	ENSGACG0000005990
	Gac3	ENSGACG00000015484
	Gac_05970*	ENSGACG0000005970
Xenopus tropicalis	Xtr1	ENSXETG00000021234
	Xtr2	ENSXETG0000006737
	Xtr3	ENSXETG00000021237
	Xtr4	ENSXETG00000021236
	Xtr_21235*	ENSXETG00000021235
	Xtr_08682*	ENSXETG0000008682
Anolis carolinensis	Aca1	ENSACAG00000004685
	Aca2	ENSACAG00000017578
	Aca_13348*	ENSACAG00000013348

Taeniopygia guttata	Tgu1	ENSTGUG0000005949
	Tgu2	ENSTGUG0000015659
Homo sapiens	HsaFTH1	ENSG00000167996
	HsaFTMtf	ENSG00000181867
	HsaFTL	ENSG0000087086
	Hsa_30962*	ENSG00000130962
	Hsa_32446*	ENSG00000132446
	Hsa_34733*	ENSG00000234733
Mus musculus	MmuFTH1	ENSMUSG0000024661
	MmuFTMtf	ENSMUSG0000024510
	MmuFTL	ENSMUSG0000050708
	Mmu_57829*	ENSMUSG0000057829
	Mmu_78208*	ENSMUSG0000078208
	Mmu_71815*	ENSMUSG0000071815
	Mmu_72249*	ENSMUSG0000072249
	Mmu_60685*	ENSMUSG0000060685
	Mmu_62382*	ENSMUSG0000062382
	Mmu_78206*	ENSMUSG0000078206
Rattus norvegicus	RnoFTH1	ENSRNOG0000022619
	RnoFTMtf	ENSRNOG0000014988
	RnoFTL	ENSRNOG0000020843
	Rno_28887*	ENSRNOG0000028887
	Rno_29241*	ENSRNOG0000029241
	Rno_38393*	ENSRNOG0000038393
	Rno_29583*	ENSRNOG0000029583
	Rno_39298*	ENSRNOG0000039298
	Rno_29880*	ENSRNOG0000029880
	Rno_30405*	ENSRNOG0000030405
	Rno_38188*	ENSRNOG0000038188
	Rno_29714*	ENSRNOG0000029714
	Rno_25351*	ENSRNOG0000025351
	Rno_32875*	ENSRNOG0000032875
	Rno_32593*	ENSRNOG0000032593
	Rno_18804*	ENSRNOG0000018804
	Rno_14913*	ENSRNOG0000014913

	Rno_07820*	ENSRNOG0000007820
	Rno_23094*	ENSRNOG0000023094
	Rno_22941*	ENSRNOG0000022941
	Rno_15960*	ENSRNOG0000015960
	Rno_22407*	ENSRNOG0000022407
	Rno_03783*	ENSRNOG0000003783
Bos taurus	BtaFTH1	ENSBTAG00000011184
	BtaFTMtf	ENSBTAG0000005779
	BtaFTL	ENSBTAG0000013343
	Bta_12533*	ENSBTAG0000012533
	Bta_32496*	ENSBTAG0000032496
	Bta_35206*	ENSBTAG0000035206
	Bta_40242*	ENSBTAG0000040242
	Bta_40327*	ENSBTAG0000040327
	Bta_38772*	ENSBTAG0000038772
	Bta_23560*	ENSBTAG0000023560
	Bta_09359*	ENSBTAG0000009359
	Bta_38111*	ENSBTAG0000038111
	Bta_30507*	ENSBTAG0000030507
	Bta_19709*	ENSBTAG00000019709
	Bta_37818*	ENSBTAG0000037818
Rana catesbiana	RcaFerH	M15655.1/P07229
	RcaFerM	J02724.1/P07798
	RcaFerL	J02725.1/P07797
Drosphila melanogaster	DmeFer1HCh	Fbgn0015222/FBpp0084995
	DmeFer2LCh	Fbgn0015221/FBpp0084986
	DmeFer3Mtf	Fbgn0030449/FBpp0073576
Eriocheir sinensis	Esi1	GU002544.1/ADF87490.1
	Esi2	GU002545.1/ADF87491.1
	Esi3	GU475115.2/ADD17345.1
Crassostrea gigas	CgiGF1	AY321299.1/Q7YZR9
	CgiGF2	AY321300.1/Q7YZR8
Haliotis discus	Hdi1	DQ821493.1
	Hdi2	DQ821494.1

Branchiostoma floridae	Bfl	BW844971.1
Ciona intestinalis	Cin	BW450247.1
	Cin_06943*	ENSCING0000006943
Ciona savignyi	Csa [contig]	BW577874.1; BW520036.1
	Csa_04271*	ENSCSAVG0000004271
Eptatretus burgeri	Ebu [contig]	BJ655837.1; BJ667150.1