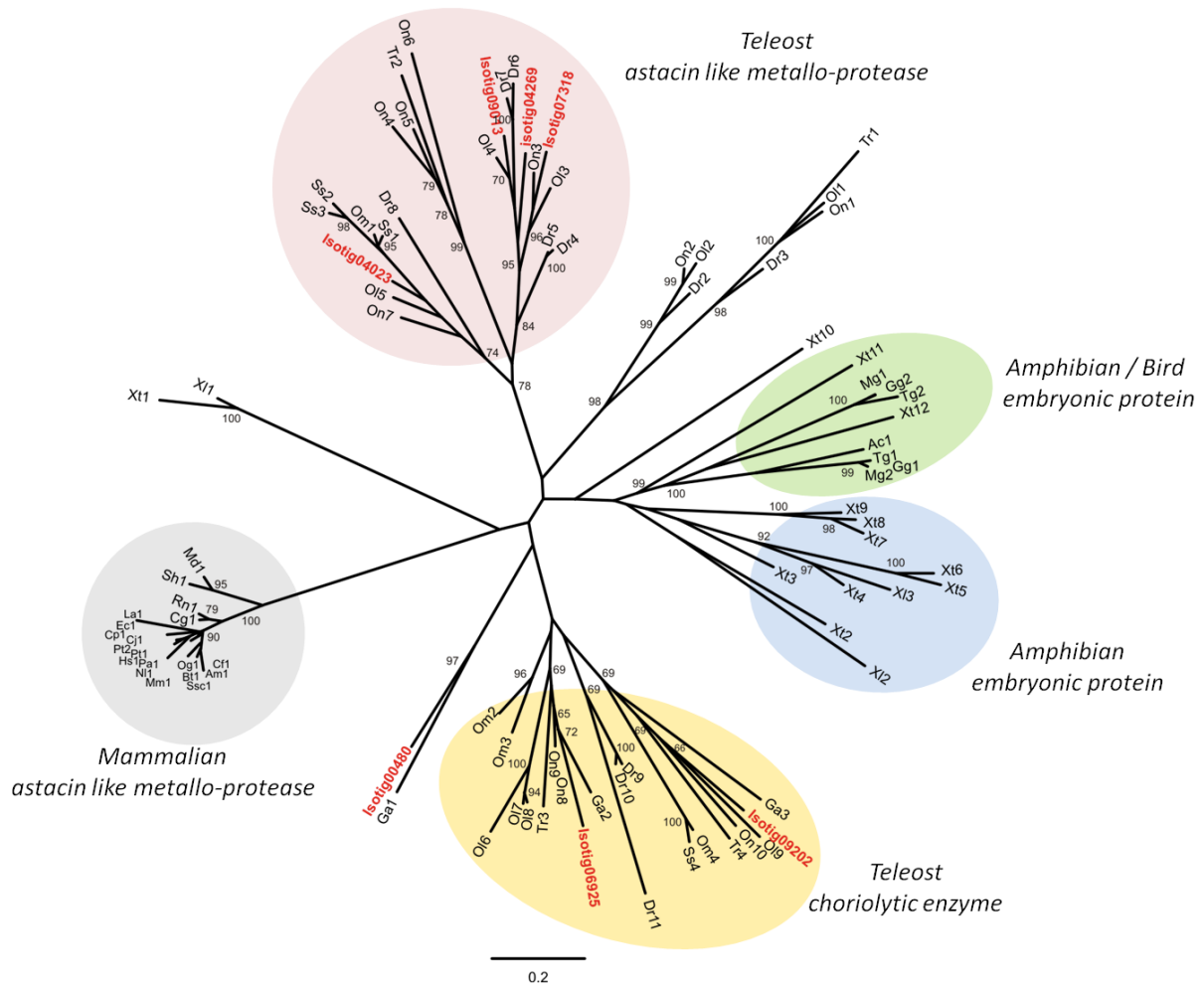


Phylogenetic analysis of “hatching” enzymes



Phylogenetic tree showing the evolutionary relationships between *S. solea* sequences (indicated with Isotig name) and all available astacin-like metalloproteases from vertebrate genomes. Vertebrate sequences are defined by the two-letter species followed by a number, correspondence of this “code” with each gene description and accession number on GenBank is reported on the Table below. Bootstrap values are assigned to each interior branch; bootstrap values less than 60 are not shown.

Methods

Translated *S. solea* hatching enzymes sequences were used as query for Blastp searches in NCBI nr protein database in order to find putative orthologs in other species. A total of 89 sequences (see below) were then employed for phylogenetic analysis, 4 sequences were later excluded since too divergent and barely alignable.

S. solea hatching enzymes and all published protein sequences were aligned using MUSCLE (MUltiple Sequence Comparison by Log- Expectation) software, applying default settings. The maximum likelihood analysis was performed with the Phyml 3.0 programme by applying the LG+I+G evolutionary model; non-parametric bootstrap resampling was also performed to evaluate the robustness of tree topology.

List of sequences employed for phylogenetic tree construction.

Code in phylogenetic tree	GenBank Acc.	Description
AC1	XP_003214688.1	PREDICTED: embryonic protein UVS.2-like [Anolis carolinensis]
AM1	XP_002926533.1	astacin-like metalloendopeptidase-like [Ailuropoda melanoleuca]
BT1	XP_588342.2	PREDICTED: astacin-like metalloendopeptidase [Bos taurus]
CF1	XP_003639630.1	PREDICTED: astacin-like metalloendopeptidase-like [Canis lupus familiaris]
CG1	XP_003497858.1	PREDICTED: astacin-like metalloendopeptidase [Cricetulus griseus]
CJ1	XP_002757421.1	PREDICTED: astacin-like metalloendopeptidase [Callithrix jacchus]
CP1	XP_003471753.1	PREDICTED: astacin-like metalloendopeptidase-like [Cavia porcellus]
DR10	NP_001038639.1	hatching enzyme 1a precursor [Danio rerio]
DR11	NP_001091658.1	hatching enzyme 2 precursor [Danio rerio]
DR2	XP_001919316.2	PREDICTED: high choriolytic enzyme 1-like [Danio rerio]
DR3	XP_001342763.2	PREDICTED: high choriolytic enzyme 1-like [Danio rerio]
DR4	NP_991145.1	nephrosin-like precursor [Danio rerio]
DR5	NP_001071247.1	nephrosin isoform 1 precursor [Danio rerio]
DR6	NP_001013544.1	six-cysteine containing astacin protease 3 precursor [Danio rerio]
DR7	NP_001020351.1	six-cysteine containing astacin protease 4 precursor [Danio rerio]
DR8	NP_001036784.1	six-cysteine containing astacin protease 1 precursor [Danio rerio]
DR9	NP_998800.2	hatching enzyme 1b precursor [Danio rerio]
EC1	XP_001916411.2	astacin-like metalloendopeptidase [Equus caballus]
GA1	ENSGACP00000004002 (Ensembl acc. Number)	
GA2	NP_001254602.1	hatching enzyme precursor [Gasterosteus aculeatus]
GA3	NP_001254603.1	hatching enzyme precursor [Gasterosteus aculeatus]
GG1	XP_426424.3	PREDICTED: embryonic protein UVS.2-like [Gallus gallus]
GG2	XP_421101.2	PREDICTED: astacin-like metalloendopeptidase [Gallus gallus]
HS1	NP_001002036.3	astacin-like metalloendopeptidase precursor [Homo sapiens]
LA1	XP_003422064.1	PREDICTED: astacin-like metalloendopeptidase-like [Loxodonta africana]
MD1	XP_001382060.1	PREDICTED: astacin-like metalloendopeptidase-like [Monodelphis domestica]
MG1	XP_003206449.1	PREDICTED: astacin-like metalloendopeptidase-like [Meleagris gallopavo]
MG2	XP_003206490.1	PREDICTED: embryonic protein UVS.2-like [Meleagris gallopavo]
MM1	XP_002799475.1	PREDICTED: astacin-like metalloendopeptidase-like [Macaca mulatta]
NL1	XP_003281094.1	PREDICTED: astacin-like metalloendopeptidase [Nomascus leucogenys]
OG1	XP_003799356.1	PREDICTED: astacin-like metalloendopeptidase [Otolemur garnettii]
OL1	NP_001098208.1	astacin like metallo-protease precursor [Oryzias latipes]
OL2	NP_001098332.1	astacin like metallo-protease precursor [Oryzias latipes]
OL3	NP_001098331.1	astacin like metallo-protease precursor [Oryzias latipes]
OL4	NP_001098207.1	astacin like metallo-protease precursor [Oryzias latipes]
OL5	NP_001098206.1	nephrosin precursor [Oryzias latipes]
OL6	NP_001098205.1	hatching enzyme like protease precursor [Oryzias latipes]
OL7	NP_001098293.1	high choriolytic enzyme 2 precursor [Oryzias latipes]
OL8	NP_001188427.1	high choriolytic enzyme 1 precursor [Oryzias latipes]
OL9	NP_001098292.1	low choriolytic enzyme precursor [Oryzias latipes]
OM1	NP_001158583.1	High choriolytic enzyme 1 precursor [Oncorhynchus mykiss]
OM2	NP_001165879.1	hatching enzyme 2 precursor [Oncorhynchus mykiss]

OM3	NP_001165880.1	hatching enzyme 1 precursor [Oncorhynchus mykiss]
OM4	NP_001165878.1	hatching enzyme precursor [Oncorhynchus mykiss]
ON1	XP_003447393.1	PREDICTED: low choriolytic enzyme-like [Oreochromis niloticus]
ON10	XP_003440698.1	PREDICTED: low choriolytic enzyme-like [Oreochromis niloticus]
ON2	XP_003447395.1	PREDICTED: high choriolytic enzyme 2-like [Oreochromis niloticus]
ON3	XP_003449256.1	PREDICTED: hypothetical protein LOC100697799 [Oreochromis niloticus]
ON4	XP_003459646.1	PREDICTED: high choriolytic enzyme 1-like, partial [Oreochromis niloticus]
ON5	XP_003456934.1	PREDICTED: high choriolytic enzyme 1-like [Oreochromis niloticus]
ON6	XP_003456946.1	PREDICTED: low choriolytic enzyme-like [Oreochromis niloticus]
ON7	XP_003458522.1	PREDICTED: low choriolytic enzyme-like [Oreochromis niloticus]
ON8	XP_003440757.1	PREDICTED: high choriolytic enzyme 2-like [Oreochromis niloticus]
ON9	XP_003440875.1	PREDICTED: high choriolytic enzyme 1-like [Oreochromis niloticus]
PA1	XP_002811693.1	PREDICTED: astacin-like metalloendopeptidase [Pongo abelii]
PT1	XP_003309175.1	astacin-like metalloendopeptidase isoform 2 [Pan troglodytes]
PT2	XP_001144288.1	astacin-like metalloendopeptidase isoform 1 [Pan troglodytes]
RN1	NP_001099974.1	astacin-like metalloendopeptidase precursor [Rattus norvegicus]
SH1	XP_003758159.1	PREDICTED: uncharacterized protein LOC100929627 [Sarcophilus harrisii]
SS1	NP_001134276.1	High choriolytic enzyme 1 precursor [Salmo salar]
SS2	NP_001140025.1	High choriolytic enzyme 2 precursor [Salmo salar]
SS3	NP_001158733.1	Low choriolytic enzyme precursor [Salmo salar]
SS4	NP_001156553.1	hatching enzyme precursor [Salmo salar]
SSc1	XP_003481181.1	PREDICTED: astacin-like metalloendopeptidase-like [Sus scrofa]
TG1	XP_002198927	embryonic protein UVS.2-like [Taeniopygia guttata]
TG2	XP_002198668	astacin-like metalloendopeptidase-like [Taeniopygia guttata]
TR1	NP_001072095.1	astacin like metallo-protease precursor [Takifugu rubripes]
TR2	NP_001072094.1	astacin like metallo-protease precursor [Takifugu rubripes]
TR3	NP_001072106.1	hatching enzyme precursor [Takifugu rubripes]
TR4	NP_001072065.1	hatching enzyme precursor [Takifugu rubripes]
XL1	NP_001108257.1	uncharacterized protein LOC100137635 precursor [Xenopus laevis]
XL2	NP_001121238.1	uncharacterized protein LOC100158314 [Xenopus laevis]
XL3	NP_001088112.1	uncharacterized protein LOC494813 precursor [Xenopus laevis]
XT1	XP_002932488.1	PREDICTED: bone morphogenetic protein 1-like [Xenopus tropicalis]
XT10	XP_002938079.1	PREDICTED: embryonic protein UVS.2-like [Xenopus tropicalis]
XT11	XP_002937346.1	PREDICTED: embryonic protein UVS.2-like [Xenopus tropicalis]
XT12	XP_002934133.1	PREDICTED: embryonic protein UVS.2-like [Xenopus tropicalis]
XT2	XP_002937348.1	PREDICTED: embryonic protein UVS.2-like [Xenopus tropicalis]
XT3	XP_002934115.1	PREDICTED: embryonic protein UVS.2-like [Xenopus tropicalis]
XT4	NP_001008039.1	tolloid-like 1 precursor [Xenopus tropicalis]
XT5	XP_002934134.1	PREDICTED: embryonic protein UVS.2-like [Xenopus tropicalis]
XT6	XP_002934137.1	PREDICTED: embryonic protein UVS.2-like [Xenopus tropicalis]
XT7	XP_002937467.1	tolloid-like protein 2-like [Xenopus tropicalis]
XT8	XP_002937432.1	PREDICTED: embryonic protein UVS.2-like [Xenopus tropicalis]
XT9	XP_002937431.1	PREDICTED: embryonic protein UVS.2-like [Xenopus tropicalis]