

Additional file 2: Sequence alignment and phylogenetic analysis

FigureS1A.

Multiple sequence alignments of mud loach LEAP-2 isoforms (mature peptide region) along with orthologs from other representative teleost species. In the mature peptide region, conserved amino acid residues are shown in boldface letters. Two cysteines to form a disulfide bond are joined by a solid line.

Figure S1B.

Multiple sequence alignments of LEAP-2 mature peptides in the cypriniform lineage (upper) and molecular phylogenetic relationships among cypriniform LEAP-2s (lower). In the alignment, conserved amino acid residues are bolded and Cys residues forming a putative disulfide bond are joined by a solid line. In the phylogenetics, neighbor-joining tree was drawn with complete amino acid sequence using MEGA7 software (7.0.26). Tree topology was tested by bootstrap test (1000 replicates). Only bootstrap values higher than 60% are shown.

	1	10	20	30	40																			
<i>Ctenopharyngodon idella</i> (ACR54299)	MTPL	WRIMG	TKPHGAY	CQNHYEC	STGIC	CRKGHCSYSQP	INS-----	LEAP-2A																
<i>Megalobrama amblycephala</i> (AF084708)	MTPL	WRIMG	TKPHGAY	CQNHYEC	STGIC	CRRGHCSYSQP	INS-----																	
<i>Danio rerio</i> (NP_001122249)	MTPL	WR	TVG	TKPHGAY	CQNNYEC	STGIC	CRMGHCSYSQP		VNS-----															
<i>Sinocyclocheilus graham</i> (XP_016096416)	MTPL	WRIMG	TKPHGAY	CQNNYEC	STGIC	CRKGHCSYSQP	INS-----																	
<i>Cyprinus carpio</i> (AGK89728)	MTPL	WRIMG	TKPHGAY	CQNNYEC	STGIC	CRKGHCSYSQP	IHS-----																	
<i>Cyprinus carpio</i> (KTG45496)	TTPL	WRIMG	TKPHGAY	CQNNYEC	STGIC	CRKGHCSYSQP	INS-----																	
<i>Misgurnus mizolepis</i> (APZ76684)	MTPL	WRIMG	TKPHGAF	CQNNYEC	STGIC	CRKGHCSYSQP	INS-----																	
<i>Tachysurus fulvidraco</i> (ACT33044)	MTPL	WR	I	VG	TKPHGAY	CQNNYEC	STGIC		CRKGHCSFSQP	IIS-----														
<i>Ictalurus punctatus</i> (AAX45791)	MTPL	WRIMG	TKPHGAY	CQNNYEC	STGIC	CRKGHCSFSQP	IIS-----																	
<i>Ictalurus furcatus</i> (AAX45792)	MTPL	WRIMG	TKPHGAY	CQNNYEC	STGIC	CRKGHCSFSQP	IIS-----																	
<i>Salmo salar</i> (XP_014062864)	MTPL	WR	TMGT	KPYGAY	CQNNYEC	STGIC	CRGGHCFMFSQA		IKS-----															
<i>Salmo salar</i> (XP_014039283)	MTPL	WR	TMGT	KPYGAY	CLHNYEC	STGIC	CRGGHCFMFSQP		IKS-----															
<i>Oncorhynchus mykiss</i> (AAR11766)	MTPL	WR	TMGT	KPYGAY	CLNNYEC	STGIC	CRGGHCFMFSQP		IKS-----															
<i>Oncorhynchus mykiss</i> (AAS49157)	MTPL	WR	TMGT	KPYGAY	CLNNYEC	STGIC	CRGGHCFMFSQP		IKS-----															
<i>Anguilla japonica</i> (ALB07167)	MTPL	WRIMG	SKPHGAY	CKNNYEC	STGIC	CSGGHCSFSQP	IKS-----																	
<i>Plecoglossus altivelis</i> (AIZ00779)	MTPL	WR	VMGNK	PF	GAYCQ	QDHVEC	STGIC		CKGGHCIYSQP	IKS-----														
<i>Fundulus heteroclitus</i> (XP_012730440)	MTPL	WR	IMSSK	PSGAF	CQNNFEC	ATGL	CREGR		CST	SQRPSLEPVKY														
<i>Boleophthalmus pectinirostris</i> (ANO39624)	MTPL	WR	I	LNSK	PF	GAYCQ	NNYEC		STGL	CRAGFCATMHR	SATVSVTN													
<i>Takifugu rubripes</i> (XP_011604919)	MTPL	WRIMNS	KPF	GAYCQ	N	SYEC	STGL		CRAGH	CSFSQR	SPSQIANY													
<i>Tetraodon nigroviridis</i> (CAF87882)	MSPL	WRIMSS	KPF	GAYCQ	N	SYEC	STGL		CREGH	CSISQR	SPPLIANY													
<i>Paralichthys olivaceus</i> (ACB97648)	MTPL	WRIMSS	KPF	GAYCQ	N	NYEC	STGL		CRAGH	CSTSHR	SPAEPVKY													
<i>Oreochromis niloticus</i> (XP_003457771)	MTPL	WRIMNS	KPF	GAYCQ	N	NYEC	STGL		CRAGH	CSTMNR	SASQPVNY													
<i>Larimichthys crocea</i> (KKF28325)	MTPL	WRIMNS	KPF	GAYCQ	N	NYEC	STGL		CRAGH	CSTSHR	ATSETVNY													
<i>Larimichthys crocea</i> (AFC90192)	MTPL	WRIMNS	KPF	GAYCQ	N	NYEC	STGL		CRAGH	CSTSHR	ATSETVNY													
<i>Miichthys miiuy</i> (AHN13905)	MTPL	WRIMNS	KPF	GAYCQ	N	NYEC	STGL		CRAGH	CSTSHR	ATSETVNY													
<i>Esox lucius</i> (XP_010893038)	-TPL	WR	FIG	TKPM	GAYC	RDHFEC	STQI		CRRGH	CALSHADH	S-----													
<i>Oncorhynchus mykiss</i> (AAR11767)	MTPL	WR	F	MGT	KPT	GAYC	RDHFEC		STQI	CRRGH	CALSGAVHS	-----												
<i>Salmo salar</i> (XP_013985003)	MTPL	WR	F	MGT	KPT	GAYC	RDHFEC		STQI	CRRGR	CARNGAVHS	-----												
<i>Astyanax mexicanus</i> (XP_015462576)	MSPV	WR	I	FASK	PP	GAYC	HDHIE		CTGL	CRRGF	CSFNQPVHS	-----												
<i>Misgurnus mizolepis</i> (APZ76685)	MSPL	WR	I	MAFK	PK	YGAYC	QDNIE		CTTGL	CRNGH	CSFN	EPVHA-----												
<i>Danio rerio</i> (XP_003200926)	MSPL	WR	I	MGYK	PK	YGAYC	HDNIE		CTNTGF	CRNGQ	CSFN	EAVHS-----												
<i>Cyprinus carpio</i> (KTG40386)	MSPL	WR	I	MGYK	PK	YGAYC	YDNIE		CTTGL	CRKGH	CSFN	EPVHS-----												
<i>Cyprinus carpio</i> (AGK89729)	MSPL	WR	I	MGYK	PK	YGAYC	HDNIE		CTTGL	CRNGH	CSFN	EPVHS-----												
<i>Cyprinus carpio</i> (XP_018963048)	MSPL	WR	I	MGYK	PK	YGAYC	HDNIE	CTTGL	CRNGH	CSFN	EPVHS-----													
<i>Cyprinus carpio</i> (XP_018932490)	-SLL	WR	W	N	T	L	K	P	V	G	S	CRDHYEC	GTNY	CRKHT	CSFYKA	QQA-----	LEAP-2B							
<i>Cyprinus carpio</i> (XP_018919135)	-SLL	WR	W	N	T	L	K	P	V	G	S	CRDHYEC	GTNY	CRKPH	LL	Q-----								
<i>Danio rerio</i> (XP_009293338)	-SLL	WR	W	N	T	L	K	P	V	G	S	CRDHYEC	GTNY	CRKQT	CSFN	R		QAQA-----						
<i>Oncorhynchus mykiss</i> (ADN34603)	-SLL	WR	W	N	T	L	K	P	V	G	T	S	CREHDEC	G	T	K		Y	CRKKI	CSFQ	V	F	I	S-----
<i>Oncorhynchus mykiss</i> (XP_021478258)	-SLL	WR	W	N	T	L	K	P	V	G	T	S	CREHDEC	G	T	K		Y	CRKKI	CSFQ	V	F	I	S-----
<i>Salmo salar</i> (ADN34604)	-SLL	WR	W	N	T	L	K	P	V	G	S	CREHDEC	G	T	K	Y		CRKKI	CSFQ	V	F	I	S-----	
<i>Takifugu rubripes</i> (XP_011603261)	-SLL	WR	W	N	S	M	K	P	L	G	A	S	CREHSEC	G	T	K		Y	CRKNI	CSF	Y-----			
<i>Paralichthys olivaceus</i> (XP_019937478)	-SLL	WR	W	N	S	M	K	A	V	G	A	S	CRDHSEC	A	T	Q		Y	CRKNI	CSF	W	I	S	T-----
<i>Fundulus heteroclitus</i> (XP_012731865)	-SLL	WR	L	N	S	L	K	P	V	G	A	S	CRDPSEC	G	T	K		H	CRKNI	CSF	-----			
<i>Oreochromis niloticus</i> (XP_013126243)	-SLL	WR	W	N	S	L	K	P	V	G	A	S	CRDHLEC	G	T	K		Y	CRKNI	CSF	W	I	T-----	
<i>Larimichthys crocea</i> (AHY01377)	-SLL	WR	W	N	S	L	K	P	V	G	A	S	CRDHAEC	G	T	K	Y	CRKNI	CSF	W	I	S	S-----	
<i>Larimichthys crocea</i> (XP_010734883)	-SLL	WR	W	N	S	L	K	P	V	G	A	S	CRDHAEC	G	T	K	Y	CRKNI	CSF	W	I	S	N-----	LEAP-2C

	1	10	20	30	40	aa	pI	Da	
XP_016096416	MTPL	LWRIMGTKPHGAY	CQNNYECSTGI	CRK	GHCSYSQPINS	41	8.86	4634.3	LEAP-2A
AGK89728	MTPL	LWRIMGTKPHGAY	CQNNYECSTGI	CRK	GHCSYSQPIHS	41	8.68	4657.3	
KTG45496	TTPL	LWRIMGTKPHGAY	CQNNYECSTGI	CRK	GHCSYSQPINS	41	8.86	4604.2	
AF084708	MTPL	LWRIMGTKPHGAY	CQNHYECSTGI	CR	RGHCSYSQPINS	41	8.88	4685.3	
ACR54299	MTPL	LWRIMGTKPHGAY	CQNHYECSTGI	CRK	GHCSYSQPINS	41	8.86	4657.3	
NP_001122249	MTPL	LWRTVGTKPHGAY	CQNNYECSTGI	CR	MGHCSYSQPVNS	41	8.48	4579.2	
APZ76684	MTPL	LWRIMGTKPHGAF	CQNNYECSTGI	CRK	GHCSYSQPINS	41	8.88	4618.3	LEAP-2B
APZ76685	MSPL	LWRIMAFKPY	GAYCQDNI	ECTTGL	CRNGHCSFNEPVHA	41	6.68	4662.4	
XP_003200926	MSPL	LWRIMGYKPY	GAHCHDNI	ECNTGF	CRNGQCSFNEAVHS	41	6.79	4675.3	
KTG40386	MSPL	LWRIMGFKPY	GAYCYDNI	ECTTGL	CRKGHCSFNEPVHS	41	7.69	4713.4	
XP_018963048	MSPL	LWRIMGFKPY	GAYCHDNI	ECITGL	CRNGHCSFNEPVHS	41	6.79	4685.4	
AGK89729	MSPL	LWRIMGFKPY	GAYCHDNI	ECITGL	CRNGHCSFNEPVHS	41	6.79	4685.4	
XP_009293338	-SLL	LWRWNTLKPVGSP	CRDHYECGTNY	CRKQT	CSFNRAQQA	40	9.20	4719.3	LEAP-2C
XP_018919135	-SLL	LWRWNTLKPVGSG	CRDHYECGTNY	CRKPHLL	LQ-----	35	9.06	4145.8	
XP_018932490	-SLL	LWRWNTLKPVGSG	CRDHYECGTNY	CRKHT	CSFYKAQQA	40	9.13	4709.3	

