

Cloning and characterization of *Vasa* gene expression pattern in adults of the Lusitanian toadfish *Halobatrachus didactylus*

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Supplement. Lusitanian toadfish cDNA *Vasa* products and Genbank accession numbers of the sequences used to perform the molecular phylogenetic analysis

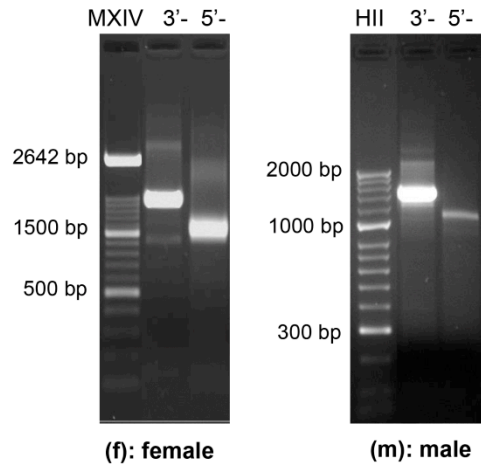
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Fig. S1. Nucleotide and predicted amino acid sequences of Lusitanian toadfish *Vasa* cDNA (GenBank accession number JX849133), shown in lower and upper case letters, respectively. Nucleotides are numbered to the right. Arginine/glycine-rich regions in the N-terminal region are boxed with a discontinuous line. Eight consensus sequences for the DEAD-protein family are framed by a solid black line. The region amplified by qPCR and used as probe in *in situ* hybridization (ISH) is emphasized in gray, primers OLIGOVAR and OLIGOVAR are represented by a solid black line above them

a 3'- and 5'-RACE-PCR *Vasa* products



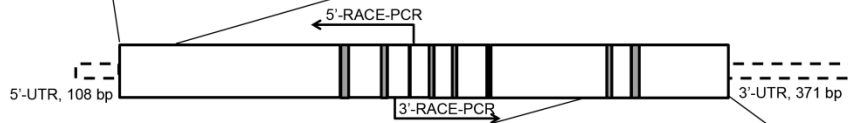
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B (m)  MDDWEEEGTATTITTLTSQPANEG-----
C (f,m) MDDWEEEGTATTITTLTSQPANEG-----GEDRWNDGE
D (m)  MDDWEEEGTATTITTLTSQPANEGGDDRWN-----GGEDRWNDGE
E (m)  MDDWEEEGTATTITTLTSQPANEGGDDRWDNDES LNSTGGRRGFRGRGGRGGEDRWNDGE
  
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B (m)  -----FRGGNRRGSQGSFDKGG-----NQDSRGAFGGGYRGKDE
C (f,m) SLNKTGGGCGFRGRGGRGFRGGNRRGSQGSFDKGG-----NQDSRGAFGGGYRGKDE
D (m)  SLNKTGGGCGFRGRGGRGFRGGNRRGSQGSFDKGG-----NQDSRGAFGGGYRGKDE
E (m)  SLNKTGGGCGFRGRGGRGFRGGNRRGSQGSFDKGGDEEMYENGNQDSRGAFGGGYRGKDE
  
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G (f)  HGDREQREREQALKDFRSGTAGGANLVGGVCI-----
st      QLARSLITVLSKAQQGVPTWLEESAFNDHGNTSFNPPRRDFGATDSRKGRAFPDNSVVTQPNDPAVADDDWE
F (f)  QLARSLITVLSKVTVLPKIGFYRKPHKSGLSQRPLSL-----
G (f)  -----
  
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Fig. S2. (a) Lusitanian toadfish 3'- and 5'-RACE *Vasa* products from ovary and testis. MXVI: molecular weight Marker XIV (Roche Applied Science). HII: molecular weight Marker Hyperladder II (Bioline). (b) Diagram of the standard *Vasa* transcript with the 8 conserved domains for DEAD-box proteins in gray. The alignments of protein sequences are shown in the regions in which the minority products A (from JX849140), B (JX849137), C (JX849136), D (JX849139), E (JX849138), F (JX849141), and G (JX849142) differ from the standard sequence (st). Hyphens represent deleted amino acids

Table S1. Genbank accession numbers of the amino acid sequences from the DEAD-box protein family used to perform the molecular phylogenetic analysis

	Species	Accession	Species	Accession
Vasa (DDX4)	<i>Danio rerio</i>	AAI29276	<i>Ctenopharyngodon idella</i>	ACR61400
	<i>Gobiocypris rarus</i>	AFA45124	<i>Kryptolebias marmoratus</i>	AGA16734
	<i>Cyprinus carpio</i>	AAL87139	<i>Leucopsarion petersii</i>	BAD04052
	<i>Carassius gibelio</i>	AAV70960	<i>Osphronemus goramy</i>	ACV69940
	<i>Carassius auratus</i>	AAX22126	<i>Dicentrarchus labrax</i>	ADK79106
	<i>Seriola quinqueradiata</i>	ADD91316	<i>Trachurus japonicus</i>	BAG72093
	<i>Nibeia mitsukurii</i>	ACV32355	<i>Oreochromis aureus</i>	AEO36953
	<i>Oreochromis niloticus</i>	BAB19807	<i>Salvelinus leucomaenis</i>	ACA33927
	<i>Thunnus orientalis</i>	ABY77970	<i>Auxis thazard</i>	ADD81194
	<i>Katsuwonus pelamis</i>	ADD81192	<i>Scomber australasicus</i>	ADD81190
	<i>Auxis rochei</i>	ADD81193	<i>Euthynnus affinis</i>	ADD81191
	<i>Scomber japonicus</i>	ACV32356	<i>Oryzias latipes</i>	NP_001098146
	<i>Sebastes schlegelii</i>	AEP68013	<i>Silurus meridionalis</i>	ACD62525
	<i>Clarias gariepinus</i>	ADK94762	<i>Monopterus albus</i>	ABA54551
	<i>Cynoglossus semilaevis</i>	ADX41681	<i>Paralichthys olivaceus</i>	AEY68604
	<i>Solea senegalensis</i>	AFN89212	<i>Scophthalmus maximus</i>	AFQ38974
	<i>Pagrus major</i>	BAJ25759	<i>Oncorhynchus mykiss</i>	NP_001117665
	<i>Gadus morhua</i>	ADV36250	<i>Culter ilishaeformis</i>	AGG53839
	<i>Lateolabrax japonicus</i>	AFI61840	<i>Xenopus laevis</i>	NP_001081728
	<i>Salmo salar</i>	AFH41530	<i>Homo sapiens</i>	NP_077726
<i>Larimichthys crocea</i>	AFW17056	<i>Gallus gallus</i>	NP_990039	
<i>Misgurnus anguillicaudatus</i>	BAJ19133			
PL10 (DDX3)	<i>Danio rerio</i>	NP_571016	<i>Xenopus laevis</i>	BC044972
	<i>Homo sapiens</i>	AAC34298		
DDX17	<i>Gallus gallus</i>	XP_416260	<i>Danio rerio</i>	XP_001923830
	<i>Homo sapiens</i>	CAG30318	<i>Xenopus laevis</i>	NP_001082679
DDX5	<i>Homo sapiens</i>	NP_004387	<i>Gallus gallus</i>	NP_990158
	<i>Xenopus laevis</i>	AAH82849	<i>Danio rerio</i>	NP_997777
	<i>Salmo salar</i>	ACN11269		
DDX41	<i>Homo sapiens</i>	NP_057306	<i>Meleagris gallopavo</i>	XP_003210383
	<i>Salmo salar</i>	NP_001133799		