

***Hox* gene expression profiles during embryonic development of common
sole**

**Menelaos Kavouras^{1,*}, Emmanouil E. Malandrakis¹, Eleni Golomazou¹, Ioannis
Konstantinidis¹, Ewout Blom², Arjan P. Palstra³, Konstantinos Anastassiadis⁴,
Panagiota Panagiotaki¹ and Athanasios Exadactylos¹**

¹ Department of Ichthyology and Aquatic Environment, School of Agricultural Sciences,
University of Thessaly, Fytokou Str., Volos, Greece

² Wageningen Marine Research, Wageningen University & Research, IJmuiden, The
Netherlands

³ Wageningen University & Research Animal Breeding and Genomics, Wageningen
Livestock Research, Wageningen, The Netherlands

⁴ Biotechnology Centre (BIOTEC), TU Dresden, Dresden, Germany

Submitted: September 29, 2017. Final revision received: June 14, 2018. Accepted: July 9,
2018

*) Corresponding author; e-mail: menelaoskavouras@gmail.com

Supplementary material

Table S1.

Sequences used for primer design, annealing temperatures and size of the PCR products of genomic DNA by the primers pairs used for cloning.

Target gene / GenBank accession number	GenBank accession number (sequences used for primer design)	Primer	Priming sequences (5'-3')	Product length (bp)	T (°C) annealing
<i>hoxa1a</i> / KX235355	AB232918 (<i>Oryzias latipes</i>)	hoxA1aF	GAYTAYWCKGTGATGAGC	986	58.0
	DQ481663 (<i>Takifugu rubripes</i>)	hoxA1aR	GTACTTGTTRAAGTGGAAAYTC		
<i>hoxa2a</i> / KX235356	EF594313 (<i>Astatotilapia burtoni</i>)	hoxA2aF ^a	TATCAATAGTCAGCCGTCGCT	1402	60.0
	JQ976896 (<i>Anguilla japonica</i>)	hoxA2aR ^a	GGGRAAATGTTTCAGATTKYT		
<i>hoxa13a</i> / KX235358	FJ824598 (<i>Callorhinchous milii</i>)	hoxA13aF	KGTRATGTTTCSTSTACGAC	1220	58.0
	AGRG01012345 (<i>C. semilaevis</i>)	hoxA13aR	TMGTGACYTGTCCKCTCRGA		
<i>hoxa2b</i> / KX235357	AB232919 (<i>Oryzias latipes</i>)	hoxA2bF	CDTTCCARAGTTCRTCAATCAAG	1001	60.0
	DQ481664 (<i>Takifugu rubripes</i>)				
	EF594311 (<i>Astatotilapia burtoni</i>)				
	JQ976897 (<i>Anguilla japonica</i>)				
	FJ823133 (<i>O. niloticus</i>)				
AGRG01020967 (<i>C. semilaevis</i>)	hoxA2bR	TGRAACCAVACYTTSACYTG			
<i>hoxb1a</i> / KX235359	AB232920 (<i>Oryzias latipes</i>)	hoxB1aF	TGGATGAARGTVAARAGRA	1480	57.0
	DQ481665 (<i>Takifugu rubripes</i>)	hoxB1aR	BCGYTTYTTCTGYTTCAT		

JQ976898 (*Anguilla japonica*)

AF071251 (*Danio rerio*)

^a Primer pair also suitable for *hoxa2b* (product length: 1342 bp).

Table S2.

Sequences used for primer design, annealing temperatures and size of the PCR products by the primer pairs used for qPCR.

Target gene / GenBank accession number		Priming sequences (5'-3')	Product length (bp)	T (°C) annealing
<i>hoxa1a</i> / KX235355	qPCR-hoxA1aF	GCTTGTTGTTACCTCTGTCT	95	61.4
	qPCR-hoxA1aR	CCTGACCTGCCTGTCTTTG ^b		
<i>hoxa2a</i> / KX235356	qPCR-hoxA2aF	ACCTGCCAACCTCCACTG	88	61.4
	qPCR-hoxA2aR	CCACTGCTCTCCTCGATCTC		
<i>hoxa13a</i> / KX235358	qPCR-hoxA13aF	TGCTGCCTATGGAGACTTAC	125	59.4
	qPCR-hoxA13aR	CACCGTGTCTGGTATGGA ^b		
<i>hoxa2b</i> / KX235357	qPCR-hoxA2bF	CTGACTCCTCTCCGCTCTAC	98	61.4
	qPCR-hoxA2bR	GTTGGTGTAGGCAGTCCTCA		
<i>hoxb1a</i> / KX235359	qPCR-hoxB1aF	CCGCCCAAGACAGTGAAG ^b	119	60.2
	qPCR-hoxB1aR	TTGCTGAAGTGGAACCTCCTT		
	qPCR-hoxB1bF	GGATTCAGAGCATGTGGAGGAAGT		
<i>hoxb1b</i> / unigene360987 ^a	qPCR-hoxB1bR	ACGGCTGTTTTTCGGAGGATTTCT ^b	78	59.4
	qPCR-elov15R	ACGGCTGGGATGGCAGAG		
<i>actb</i> / unigene224282 ^a	qPCR-act-bF	GCATCACACCTTCTACAA	113	60.0
	qPCR-act-bR	ACATAATCTGGGTCATCTTC ^b		

<i>ef1a</i> / HM246511	qPCR-ef1aF	TCGGAGTCAACAAGATGGA	135	60.0
	qPCR-ef1aR	CAGAGATGGGCACAAAGG		
<i>rps4</i> / GAAQ01019940	qPCR-rps4F	CCGGAGGTGCTAACTTGGGT	92	60.2
	qPCR-rps4	TGTGCTGTCCTTGACGTGGA		
<i>ubq</i> / GAAQ01003061	qPCR-ubqF	CGATGAGACCACCACGCAGA	78	60.2
	qPC-RubqR	GACGCACACTGTCCGACTAC		

^a Accession number of Solea DB, (*Solea solea* v1.0: http://www.juntadeandalucia.es/agriculturaypesca/ifapa/soleadb_ifapa/).

^b Primers are designed for the exons boundaries.

Table S3.

Correlation matrix of *hox* gene expression for the time period 30 ± 6–102 ± 6 hpf.

<i>a1a</i>	0.502				
<i>a2a</i>	0.685	0.899			
<i>a2b</i>	0.556	0.898	0.911		
<i>b1a</i>	0.405	0.860	0.850	0.950	
<i>b1b</i>	0.730	0.664	0.877	0.711	0.674
	<i>a13a</i>	<i>a1a</i>	<i>a2a</i>	<i>a2b</i>	<i>b1a</i>

Table S4A.Collinearity diagnostics of *hox* gene expression for the time period 30 ± 6 – 102 ± 6 hpf.

Model	Dimension	Eigenvalue	Condition index	Variance proportions						
				Constant	Z-score (a1a)	Z-score (a2a)	Z-score (a2b)	Z-score (a13a)	Z-score (b1a)	Z-score (b1b)
1	1	4.767	1.000	0.00	0.00	0.00	0.00	0.01	0.00	0.00
	2	1.000	2.183	1.00	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.784	2.466	0.00	0.01	0.00	0.00	0.22	0.01	0.02
	4	0.242	4.440	0.00	0.05	0.00	0.01	0.35	0.00	0.27
	5	0.150	5.641	0.00	0.35	0.02	0.04	0.08	0.11	0.00
	6	0.039	11.095	0.00	0.37	0.31	0.26	0.24	0.38	0.09
	7	0.020	15.598	0.00	0.22	0.67	0.69	0.10	0.49	0.61

Table S4B.Collinearity diagnostics of *hox* gene expression for the time period 54 ± 6 – 102 ± 6 hpf.

Model	Dimension	Eigenvalue	Condition index	Variance proportions						
				Constant	Z-score (a1a)	Z-score (a2a)	Z-score (a2b)	Z-score (a13a)	Z-score (b1a)	Z-score (b1b)
1	1	5.258	1.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	1.000	2.293	1.00	0.00	0.00	0.00	0.00	0.00	0.00
	3	0.462	3.375	0.00	0.00	0.00	0.00	0.28	0.04	0.00
	4	0.221	4.877	0.00	0.10	0.00	0.00	0.09	0.07	0.00
	5	0.037	11.077	0.00	0.14	0.02	0.05	0.10	0.84	0.06
	6	0.017	17.632	0.00	0.15	0.64	0.00	0.02	0.00	0.22
	7	0.005	32.800	0.00	0.60	0.33	0.95	0.42	0.05	0.72