

Supplementary Material

**Clustered brachiopod Hox genes are not expressed collinearly and
are associated with lophotrochozoan novelties**

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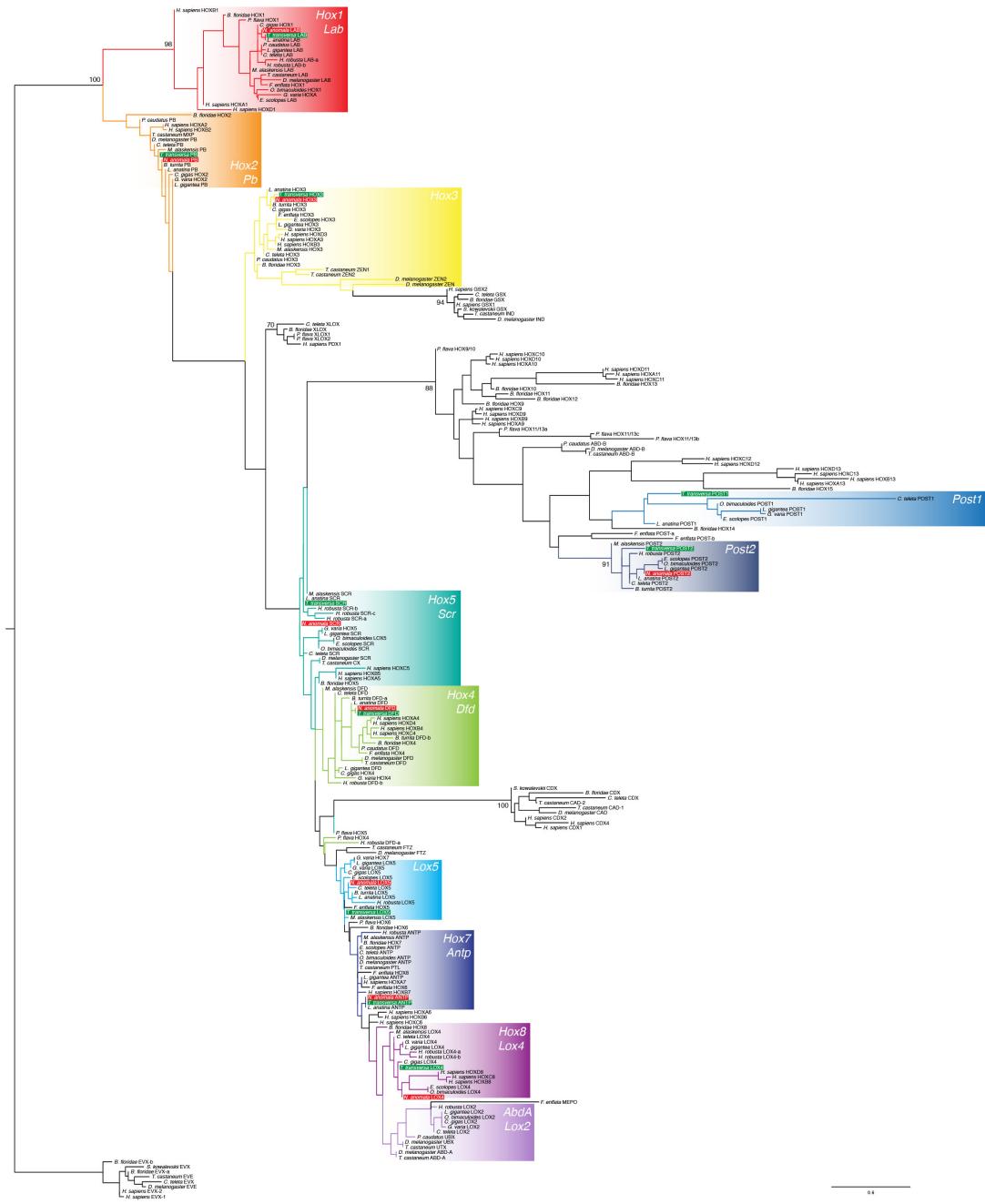


Figure supplementary 1. Orthology analysis of *T. transversa* and *N. anomala* Hox genes. Maximum likelihood phylogenetic analysis of bilaterian Hox and ParaHox genes, using as outgroup the *even-skipped* (EVX) subfamily. Colored boxes indicate Hox ortholog groups present in spiralian representatives. *T. transversa* sequences are highlighted by green boxes and *N. anomala* sequences by red boxes. Only high bootstrap values are shown.

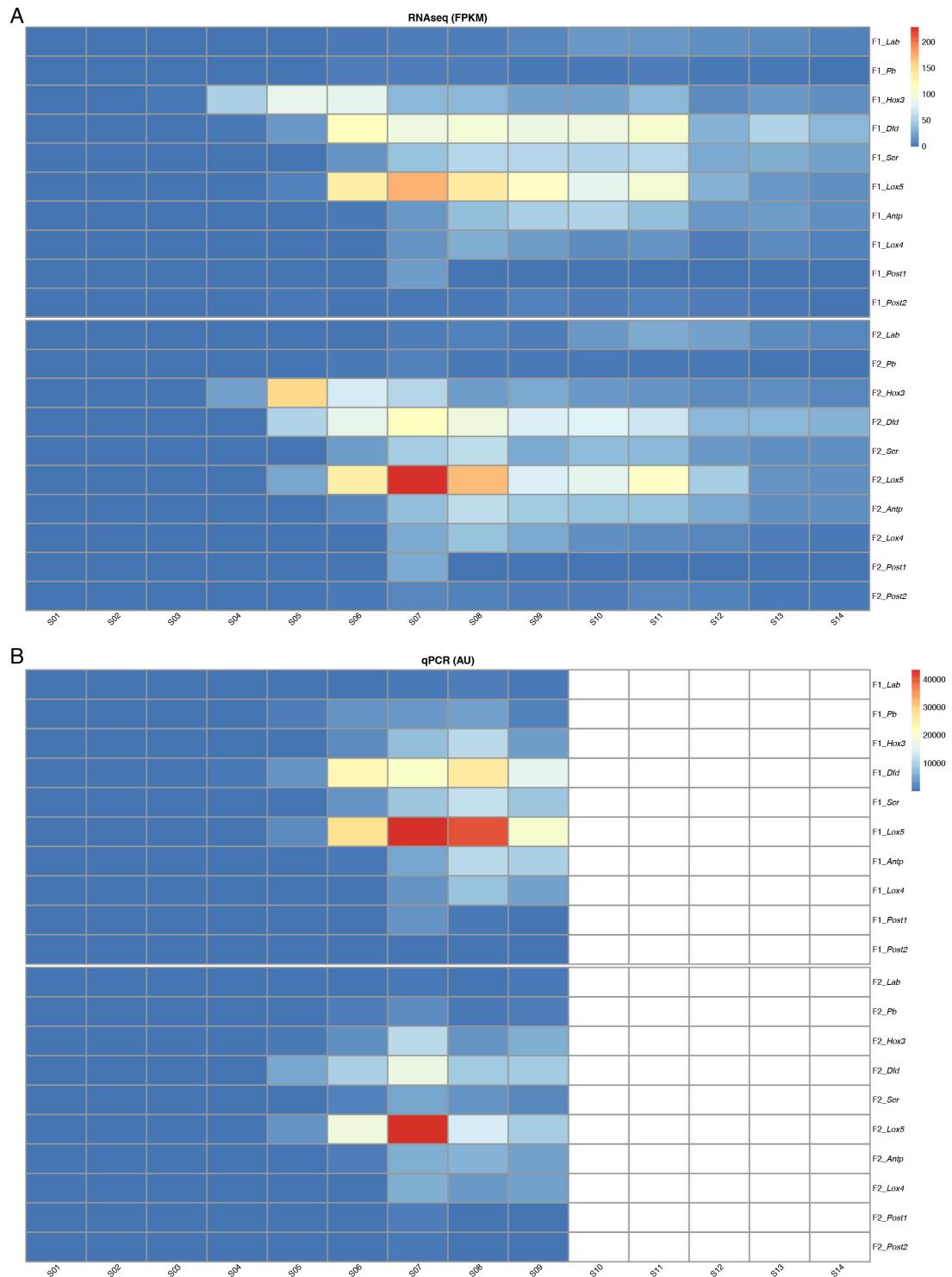


Figure supplementary 2. Quantitative expression of Hox genes in *T. transversa* developmental stages. (A) RNAseq expression levels calculated by fragments per kilobase of exon per million reads mapped (FPKM). As observed by whole-mount *in situ* hybridization, *Hox3* is the first gene up-regulated in the two biological replicates

(female 1, F1; female 2, F2). **(B)** quantitative real-time PCR (qPCR) expression levels based on absolute quantification units (AU). PCR was not performed for stages 10–14 (white cells). qPCR confirms the absence of temporal collinearity, although we do not detect higher levels of *Hox3* at late blastula (S04), as observed by RNAseq and *in situ* hybridization. Stages: S01, oocytes; S02, 8h mid blastula; S03, 19h late blastula; S04, 24h moving late blastula; S05, 26h early gastrula; S06, 37h mid gastrula; S07, 51h late gastrula; S08, 59h bilobed late gastrula; S09, 68h trilobed late gastrula; S10, 82h early larva; S11, 98h late larva; S12, competent larva; S13, 1 day juvenile; S14, 2 days juvenile.

	Hox gene complement	Hox number	Cluster	Lox2 Lox4	Spatial collinearity	Temporal collinearity	Mesodermal expression
Ctenophora	-	-	-	-	-	-	-
Porifera	-	-	-	-	-	-	-
Placozoa	-	-	-	-	-	-	-
Cnidaria	█	3/4	Yes (O)	-	-	-	-
Xenacoelomorpha	█	3	No	-	Yes	No	No
Echinodermata	█	11	Yes (D)	-	No	No	Yes
Hemichordata	█	12	Yes (O)	-	Yes	No	No
Cephalochordata	█	15	Yes (O)	-	Yes	Yes	Yes
Urochordata	█	9	No	-	Yes	Yes	Yes
Craniata	█	47*	Yes (O)	-	Yes	Yes	Yes
Chaetognatha	█	≥10	?	-	?	?	?
Bryozoa	█	6	?	?	?	?	?
Entoprocta	?	?	?	?	?	?	?
Cyclophora	?	?	?	?	?	?	?
Annelida	█	11	Yes (S)	Lox2/Lox4	Yes	Yes	Yes
Mollusca	█	11	Yes (O/S)/No	Lox2/Lox4	Yes/No	No	Yes
Nemertea	█	9	?	Lox4	Yes	Yes	?
Brachiopoda	█	10	Yes (S)	Lox4	No	No	Yes
Phoronida	?	?	?	?	?	?	?
Gastrotricha	?	?	?	?	?	?	?
Platyhelminthes	█	13	No	Lox4	No	?	Yes*
Gnathostomulida	?	?	?	?	?	?	?
Micrognathozoa	?	?	?	?	?	?	?
Rotifera	█	24	No	?	?	?	?
Priapulida	█	10	?	-	?	?	?
Loricifera	?	?	?	-	?	?	?
Kinorhyncha	?	?	?	-	?	?	?
Nematoda	█	6/7	Yes	-	Yes	No	Yes
Nematomorpha	█	8	?	-	?	?	?
Tardigrada	█	5/7	No	-	Yes	?	Yes
Onychophora	█	10	?	-	Yes	?	Yes
Arthropoda	█	11	Yes (O/S)/No	-	Yes	No	Yes

Figure supplementary 3. Evolution of Hox organization and expression across

Metazoa. Table depicting the features of the Hox gene complement of each animal lineage in a phylogenetic framework. The Hox complement is summarized by the presence of at least one representative of the anterior, Hox3, central and posterior ortholog groups. The Hox number indicates the possible ancestral number, but can vary between species (in Craniata, the number corresponds to the human Hox complement, which consists of four clusters; asterisk). The cluster organization can be of three types: organized (O), disorganized (D), split (S). When there are species with an atomized cluster we write that the cluster is absent (No presence). Question marks indicate unknown data and dashes indicate absences. See main text for references.

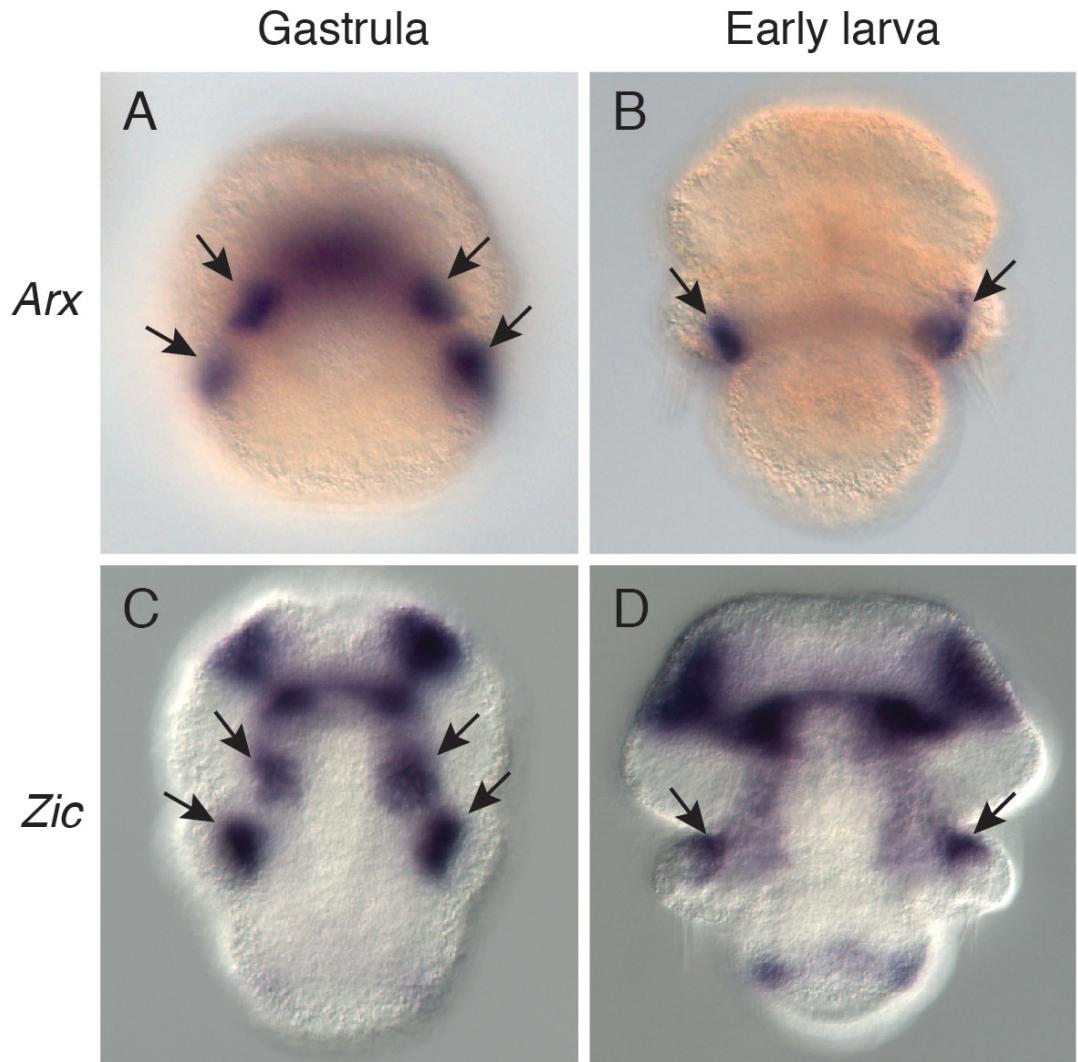


Figure supplementary 4. Expression of *Arx* and *Zic* during *T. transversa* embryogenesis. (A–D) Whole mount *in-situ* hybridization of *Arx* and *Zic* in gastrula embryos and early larvae of *T. transversa*. (A) In mid gastrulae, *Arx* is expressed in the ectoderm of the prospective chaetae sac territories (black arrows) and in a ventral domain. (B) In early larvae, *Arx* is expressed in the chaetae sacs (black arrows). (C) In late gastrulae, *Zic* is expressed in the mesoderm of the chaetae sacs (black arrows), apical lobe mesoderm and anterior ectoderm. (D) In early larvae, *Zic* is detected in the chaetae sacs (black arrows), in a domain in the pedicle lobe, and in the anterior

mesoderm and anterior ectoderm. In all panels, the images are dorsal views, with the anterior pole to the top.

Table supplement 1. Sequences and accession numbers used for Hox orthology assignment

Organism	Gene	Database	Accession number
<i>H. sapiens</i>	HoxA1	GenBank	AAB35423.2
	HoxB1		AAH99633.1
	HoxD1		AAG44444.1
	HoxA2		NP_006726.1
	HoxB2		NP_002136.1
	HoxA3		NP_705895.1
	HoxB3		AAD10852.1
	HoxD3		CAA71102.1
	HoxA4		NP_002132.3
	HoxB4		AAG45052.1
	HoxC4		AAG42145.1
	HoxD4		NP_055436.2
	HoxA5		CAG47052.1
	HoxB5		NP_002138.1
	HoxC5		EAW96748.1
	HoxA6		NP_076919.1
	HoxB6		NP_061825.2
	HoxC6		CAG33235.1
	HoxA7		CAA06713.1
	HoxB7		NP_004493.3
	HoxB8		AAG42143.1
	HoxC8		AAG42146.1
	HoxD8		AAG42152.1
	HoxA9		NP_689952.1
	HoxB9		AAG42144.1
	HoxC9		AAG42151.1
	HoxD9		NP_055028.3
	HoxA10		AAH07600.1
	HoxC10		NP_059105.2
	HoxD10		NP_002139.2
	HoxA11		NP_005514.1
	HoxC11		NP_055027.1
	HoxD11		AAF79045.1
	HoxC12		AAK16717.1
	HoxD12		AAF79044.1
	HoxA13		AAC50993.1
	HoxB13		AAH70233.1
	HoxC13		AAF73439.1
	HoxD13		AAC51635.1
	Gsx1		NP_663632.1
	Gsx2		NP_573574.1
	Pdx1		NP_000200.1
	Cdx-1		NP_001795.2
	Cdx-2		NP_001256.3

	Cdx-4		NP_005184.1
	Evx-1		NP_001291448.1
	Evx-2		NP_001073927.1
<i>B. floridae</i>	Hox1	GenBank	BAA78620
	Hox2		BAA78621
	Hox3		X68045
	Hox4		BAA78622
	Hox5		CAA84517
	Hox6		CAA84518
	Hox7		CAA84519
	Hox8		CAA84520
	Hox9		CAA84521
	Hox10		CAA84522
	Hox11		AAF81909
	Hox12		AAF81903
	Hox13		AAF81904
	Hox14		AAF81905
	Hox15		ACJ74394.1
	Gsx		AAC39015.1
	Xlox		AAC39016.1
	Cdx		AAC39017
	Evx-a		AAK58953.1
	Evx-b		AAK58954.1
<i>P. flava</i>	Hox1	GenBank	AAR07634.1
	Hox4		AAR07635.1
	Hox5		AAR07636.1
	Hox6		AAR07637.1
	Hox9/10		AAR07638.1
	Hox11/13a		AAR07639.1
	Hox11/13b		AAR07640.1
	Hox11/13c		AAR07641.1
	Xlox1		AAR07643.1
	Xlox2		AAR07644.1
<i>S. kowalevskii</i>	Gsx	Uniprot	A0A0U2UDE9
	Cdx	GenBank	NP_001158415.1
	Evx		NP_001164694.1
<i>F. enflata</i>	Hox1	GenBank	ABS18809.1
	Hox3		ABS18810.1
	Hox4		ABS18811.1
	Hox5		ABS18812.1
	Hox6		ABS18813.1
	Hox8		ABS18814.1
	MedPost		ABS18817.1
	Post-a		ABS18815.1
	Post-b		ABS18816.1
<i>P. caudatus</i>	Lab	GenBank	AAD40640.1
	Pb		AAD40641.1
	Hox3		AAD40642.1
	Dfd		AAD40643.1
	Ubx		AAD40647.1

	Abd-B		AAD40649.1
<i>D. melanogaster</i>	Lab	GenBank	CAB57787
	Pb		CAA45271
	Zen		AAF54087.1
	Zen2		P09090.2
	Dfd		P07548
	Scr		NP_524248
	Ftz		NP_477498
	Antp		CAA27417
	Ubx		CAA29194
	Abd-A		P29555
	Abd-B		CAB57859
	Ind		NP_996087.2
	Cad		AAA28409.1
	Eve		NP_523670.2
<i>T. castaneum</i>	Lab	GenBank	EEZ99257.1
	Mxp		NP_001107807.1
	Zen1		NP_001036813
	Zen2		AAK16425.1
	Dfd		AAK16423.1
	Cx		NP_001034523.1
	Ftz		AAK16421.1
	Ptl		NP_001034505.1
	Utx		EEZ99249.1
	Abd-A		EEZ99248.1
	Abd-B		EEZ99247.1
	Ind		AAW21974.1
	Cad-1		NP_001034498.1
	Cad-2		XP_008191732.1
	Eve		NP_001034538.1
<i>C. teleta</i>	Lab	GenBank	ABY67952
	Pb		ABY67953
	Hox3		ABY67954
	Dfd		ABY67955
	Scr		ABY67956
	Lox5		ABY67957
	Antp		ABY67962
	Lox4		ABY67958
	Lox2		ABY67959
	Post1		ABY67961
	Post2		ABY67960
	Gsx		AAZ23124.1
	Cdx		AAZ95508
	Xlox		AAZ95509.1
	Evx		ABG82164
<i>H. robusta</i>	Lab-a	Simakov <i>et al.</i> 2013	
	Lab-b	Simakov <i>et al.</i> 2013	
	Scr-a	Simakov <i>et al.</i> 2013	
	Scr-b	Simakov <i>et al.</i> 2013	
	Scr-c	Simakov <i>et al.</i> 2013	

	Dfd-a	Simakov <i>et al.</i> 2013	
	Dfd-b	Simakov <i>et al.</i> 2013	
	Lox5	Simakov <i>et al.</i> 2013	
	Antp	Simakov <i>et al.</i> 2013	
	Lox4-a	Simakov <i>et al.</i> 2013	
	Lox4-b	Simakov <i>et al.</i> 2013	
	Lox2	Simakov <i>et al.</i> 2013	
	Post2	Simakov <i>et al.</i> 2013	
<i>L. anatina</i>	Lab	ENSEMBL	g10891
	Pb		g10890
	Hox3		g10889
	Dfd		g10888
	Scr		g10887
	Lox5		g10886
	Antp		g10892
	Post1		g12396
	Post2		g12399
<i>C. gigas</i>	Hox1	ENSEMBL	CGI_10024083
	Hox2		CGI_10024086
	Hox3		CGI_10024087
	Hox4		CGI_10024091
	Lox5		CGI_10026565
	Lox2		CGI_10018592
	Lox4		CGI_10026562
<i>L. gigantea</i>	Lab	Simakov <i>et al.</i> 2013	
	Pb	Simakov <i>et al.</i> 2013	
	Hox3	Simakov <i>et al.</i> 2013	
	Dfd	Simakov <i>et al.</i> 2013	
	Scr	Simakov <i>et al.</i> 2013	
	Lox5	Simakov <i>et al.</i> 2013	
	Antp	Simakov <i>et al.</i> 2013	
	Lox2	Simakov <i>et al.</i> 2013	
	Lox4	Simakov <i>et al.</i> 2013	
	Post1	Simakov <i>et al.</i> 2013	
	Post2	Simakov <i>et al.</i> 2013	
<i>O. bimaculoides</i>	Hox1	ENSEMBL	Ocbimv22030263
	Scr		Ocbimv22018468
	Lox5		Ocbimv22010205
	Antp		Ocbimv22036189
	Lox2		Ocbimv22033340
	Lox4		Ocbimv22009726
	Post1		Ocbimv22015181
	Post2		Ocbimv22031197
<i>G. varia</i>	HoxA	GenBank	ACX84671.1
	Hox2		ADJ18233.1
	Hox3		ADJ18232.1
	Hox4		ACX84672.1
	Hox5		ADJ18234.1
	Lox5		ADJ18235.1
	Hox7		ADJ18236.1

	Lox2		ADJ18238.1
	Lox4		ADJ18237.1
	Post1		ACX84673.1
<i>E. scolopes</i>	Lab	GenBank	AY330184
	Hox3		AY330185
	Scr		AY330186
	Lox5		AY330187
	Antp		AY330188
	Lox4		AY330189
	Post1		AY330190
	Post2		AY330191
<i>M. alaskensis</i>	Lab	GenBank	KP762174
	Pb		KP762176
	Hox3		KP762173
	Dfd		KP762180
	Scr		KP762177
	Lox5		KP762179
	Antp		KP762171
	Lox4		KP762175
<i>B. turrita</i>	Post2		KP762178
	Pb	GenBank	AAS77225
	Hox3		AAS77226
	Dfd-a		AAS77227
	Dfd-b		AAS77228
	Lox5		AAS77229
<i>L. squamata</i>	Post2		AAS77230
	Lox4 ¹	This study	

¹>Lepidodermella_squamata_Lox4

IITNAVTGANNGSSGKLMGAHRTAPMYAWMAVVGPNSSQKRRGRQTYTRHQ^{TIEKEF}AFCHYLARK
RRIELAAALSLSERQVKIWFQNRRM^{KL}KKEKQQIADMNHISTSTTSN^{SSHS}KSNRHDDYNDVNDASS
SDEDHLD

Table supplement 2. Primers used for qPCR experiments

	Forward	Reverse
<i>Lab</i>	CAAAGCTCCGTAGCCACTTA	TCGAGCTCTGTCAATTGCTT
<i>Pb</i>	AACAAATCGGATGGCTCTG	TTCATGGTCTGCTTCCTCTG
<i>Hox3</i>	ACTTCGCGTTAGCCAATCA	TGCAGGAACCCTTCAGAAA
<i>Dfd</i>	ATGCCGAGTATAAGCCGTT	TATACCC GTGGATGAAACGA
<i>Scr</i>	ACGTCTGATGCCTGGTAG	ATAGCCATGAACAAATGCCA
<i>Lox5</i>	GTGTACGTTGCCTGGTACG	GCATGTCGCAAGCGTATAGT
<i>Antp</i>	TCTCAAGCTCGAGTGGTGG	GGAGACGCAGATAACGACAG
<i>Lox4</i>	GTTTGTGACCGCGTCTT	AAATGGATACGGGTCTGCTC
<i>Post2</i>	GCTCCTGTGGCATTGTGTAG	AGCAAGCAAGCCCTGTAGAT
<i>Post1</i>	AACGTTGTCCCCATTCTCTCC	CGATATACTATGCGGACCCA