

Supplementary Materials

Table S1. Primer sequences used to clone fragments of the *Plo*- and *Nco-Parahox* genes presented in the paper

<i>Plo-Gsx</i>, <i>Plo-Xlox</i>, and <i>Plo-Cdx</i> short fragments		
forward		5'-GBCARYTNGTHGARYTVGARAARG-3'
forward		5'-GGNAARACNMGNACNAARGAYAARTAYMG-3'
forward		5'-CARYTKYTDGARYTHGARMGRG-3'
reverse		5'-CKNCKRTTYTGRAACCA-3'
<i>Plo-Gsx</i>, <i>Plo-Xlox</i>, and <i>Plo-Cdx</i> RACE fragments		
<i>Plo-Gsx</i> 3'RACE	forward	5'-GTCACITTCGGACGAACGAGTACC-3'
	forward nested	5'-GGTGAACGCCGCGCATCGAAATG-3'
<i>Plo-Gsx</i> 5'RACE	reverse	5'-GATTTGCTGTTGAGACAGGTTTAAAGCG-3'
	reverse nested	5'-CCATTCGATGCGCGGCGTTCGAC-3'
<i>Plo-Gsx</i> 3'RACE	forward	5'-GCTAGAGCTTGAGCGTCAGTTC AAC-3'
	forward nested	5'-CCAACATGTACCTCTCGAGATTGC-3'
<i>Plo-Gsx</i> 5'RACE	reverse	5'-GATCTGTTTTTCGAAAGCTGGAGGCAG-3'
	reverse nested	5'-CTGCAATCTCGATCCGGCGCAATCTC-3'
<i>Plo-Xlox</i> 3'RACE	forward	5'-GCTACTTGAGCTCGAGAAAGAAATTCAC-3'
	forward nested	5'-CTACGACAAATATATATCCAGAGCTCGAC-3'
<i>Plo-Xlox</i> 5'RACE	reverse	5'-GTGACGCTCTGTGAGTCGAAGAACTTG-3'
	reverse nested	5'-GGCCATTTCCAGACGTCGAGCTC-3'
<i>Plo-Cdx</i> 3'RACE	forward	5'-CGCAGTTAGTCGAGTTCGAAAAGGAA-3'
	forward nested	5'-CAATTCAACGTAAGGCTGAGCTGGCTGG-3'
<i>Plo-Cdx</i> 5'RACE	reverse	5'-CGGCTGTTTGAACAGATCTTA ACTTG-3'
	reverse nested	5'-CTGTCTGAGAGTCCGATAGTGCCAGCC-3'
<i>Plo-Cdx</i> 3'RACE	forward	5'-CCAGCTCGTCGAGTTGGAGAAGG-3'
	forward nested	5'-CCAGTACATCACAATACAACGCAAAGC-3'
<i>Plo-Cdx</i> 3'RACE	reverse	5'-GGTTTTGGAACAGATCTTGACCTGACGG-3'
	reverse nested	5'-GGTCTGATAGACCAATATGTCCAGCAAG-3'
<i>Plo-Gsx</i>, <i>Plo-Xlox</i>, and <i>Plo-Cdx</i> fragments used as templates for RNA probes *		
<i>Plo-Gsx1</i>	forward	5'-GAAGATGCCCCGACATTCGTTCTGTTG-3'
	reverse	5'-GTCCCGTGTATAAGTTGCGATAATGC-3'
<i>Plo-Gsx2</i>	forward	5'-GAATTATCCAGCCGTCGACTGCCATC-3'
	reverse	5'-CAGTCCACGATGTTCTTGCCGACAAC-3'
<i>Plo-Xlox</i>	forward	5'-CCGTCACTCAGAGGATTGATGGAAAAC-3'
	reverse	5'-GACGTTGTGAAGTGGGCGGATGTG-3'
<i>Plo-Cdx1</i>	forward	5'-GCAATCGTTCGTTACCTGCCGCTG-3'
	reverse	5'-CACTCTATCGTCAGGCAATGTAACC-3'
*Previously cloned 5'partial CDS fragment of <i>Plo-Cdx2</i> (1507 bp, GenBank JQ685130) was used as a template for <i>Plo-Cdx2</i> RNA probe.		
<i>Nco-Gsx</i>, <i>Nco-Xlox</i>, and <i>Nco-Cdx</i> fragments used as templates for RNA probes		
<i>Nco-Gsx1</i>	forward	5'-CAGTCATTCCTCGTCGATTCACTCC-3'
	reverse	5'-GGTGGTTGTTGCTGTAGCTGATGTC-3'
<i>Nco-Gsx2</i>	forward	5'-CTGCCCTGTCGTCTCTGGTAGCAC-3'
	reverse	5'-GTAGTGTGGTGGACGAATCAGTTGC-3'
<i>Nco-Xlox</i>	forward	5'-GACTGGTTACAGCAGCAGGGACG-3'
	reverse	5'-CAGCAACTCCATCAATCTCCGAC-3'
<i>Nco-Cdx1</i>	forward	5'-GGTCACGGGTCAACTTATAGTCATC-3'

<i>Nco-Cdx2</i>	reverse	5'-GAACCAAATCTTGACTTGACGCTCG-3'
	forward	5'-GGTACACCATATTGACGATAGCATCAG-3'
	reverse	5'-CCGTTCCGCTTGATGATCCACTAC-3'

Table S2. GenBank accession numbers for sequences used for Gsx, Xlox, and Cdx amino acid alignments

Phylum	Gene name in tree	Species	GenBank #
Annelida	Cte_Antp	(outgroup) <i>Capitella teleta</i> , Antp Hox	EU196547
	Nco-Gsx1	<i>Nais communis</i>	OR050790
	Nco-Gsx2	<i>Nais communis</i>	OR050791
	Plo-Gsx1	<i>Pristina longiseta</i>	OR050795
	Plo-Gsx2	<i>Pristina longiseta</i>	OR050796
	Avi_Gsx	<i>Alitta virens</i>	ABB59695.1
	Cte_Gsx	<i>Capitella teleta</i>	AAZ23124.1
	Pdu_Gsx	<i>Platynereis dumerilii</i>	ACH87538.1
	Nco-Xlox	<i>Nais communis</i>	OR050792
	Plo-Xlox	<i>Pristina longiseta</i>	OR050797
	Cte_Xlox	<i>Capitella teleta</i>	AAZ95509.1
	Hro_Xlox	<i>Helodbella robusta</i>	XP_009020396.1
	Pdu_Xlox	<i>Platynereis dumerilii</i>	ACH87541.1
	Nco-Cdx1	<i>Nais communis</i>	OR050793
	Nco-Cdx2	<i>Nais communis</i>	OR050794
	Plo-Cdx1	<i>Pristina longiseta</i>	OR050798
	Plo-Cdx2	<i>Pristina longiseta</i>	OR050799
	Avi_Cdx	<i>Alitta virens</i>	AAN11403.2
	Cte_Cdx	<i>Capitella teleta</i>	AAZ95508.1
	Pdu_Cdx	<i>Platynereis dumerilii</i>	ACH87546.1
	Pex_Gsx	<i>Perionyx excavatus</i>	AAX09760.1
Mollusca	Aca_Gsx	<i>Aplysia californica</i>	XP_005093203.2
	Aen_Gsx	<i>Antalis entalis</i>	ALM30865.1
	Sof_Gsx	<i>Sepia officinalis</i>	ALC76021.1
	Ema_Xlox	<i>Elysia marginata</i>	GFR99919.1
	Pma_Xlox	<i>Pecten maximus</i>	XP_033742737.1
	Sof_Xlox	<i>Sepia officinalis</i>	AHY00652.1
	Pvu_Cdx	<i>Patella vulgata</i>	CAD57266.1
Insecta	Tca_Gsx	<i>Tribolium castaneum</i>	NP_001034494.1
	Tca_Cdx	<i>Tribolium castaneum</i>	XP_008191732.1
Echinodermata	Spu_Gsx	<i>Strongylocentrotus purpuratus</i>	XP_784486.3
	Spu_Xlox	<i>Strongylocentrotus purpuratus</i>	XP_003728715.2
	Spu_Cdx	<i>Strongylocentrotus purpuratus</i>	XP_789158.3
Hemichordata	Sko_Gsx	<i>Saccoglossus kowalevskii</i>	XP_002732050.2
	Sko_Xlox	<i>Saccoglossus kowalevskii</i>	XM_002741106.1
	Sko_Cdx	<i>Saccoglossus kowalevskii</i>	NP_001158415.1
Chordata	Mmu_Gsx1	<i>Mus musculus</i>	NP_032204.1
	Xla_Gsx1	<i>Xenopus laevis</i>	XP_018103198.1
	Mmu_Gsx2	<i>Mus musculus</i>	NP_573555.1
	Xla_Gsx2	<i>Xenopus laevis</i>	XP_018098177.1
	Bfl_Xlox	<i>Branchiostoma floridae</i>	XP_035682017.1
	Mmu_Xlox	<i>Mus musculus</i>	NP_032840.1
	Xla_Xlox	<i>Xenopus laevis</i>	XP_018103197.1
	Bfl_Cdx	<i>Branchiostoma floridae</i>	XP_035680992.1

	Mmu_Cdx1	<i>Mus musculus</i>	AAA37412.1
	Mmu_Cdx2	<i>Mus musculus</i>	NP_031699.2
	Mmu_Cdx4	<i>Mus musculus</i>	NP_031700.1
	Xla_Cdx1	<i>Xenopus laevis</i>	XP_018105758.1
	Xla_Cdx2	<i>Xenopus laevis</i>	AAI46638.1
	Xla_Cdx4	<i>Xenopus laevis</i>	AAH55999.2
Cnidaria	Nvi_Gsx	<i>Nematostella vectensis</i>	AAD39349.1
	Nvi_ParaHox	<i>Nematostella vectensis</i>	ACT36590.1

Figure S1. CLUSTAL W 2.0 multiple alignment of ParaHox sequences used for phylogenetic analysis (Figure 1)

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Cte_Antp      QFGPERKGRQTYTRYQTLELEKEFEHFNRYLTRRRRIEIAHALCLTERQIKIWFQNRRMKWKKE
Nvi_ParaHox   SSAQVRSRARTAYTASQQLELEKEFLYSRYITRTRRKELANTLDLSEKHIKIWFQNRRMKKKKTD
Cte_Gsx       ESSDAVKRMRTAFSSTQLELEREFASNMYLSRLRRIE IATYLSLSEKQVKIWFQNRRVKFKKE
Pdu_Gsx       MEDANGKRIRTAFTSTQLELEREFSNMYLSRLRRIE IATYLNSEKQVKIWFQNRRVKYKKE
Avi_Gsx       IEDANGKRIRTAFTSTQLELEREFSNMYLSRLRRIE IATYLNSEKQVKIWFQNRRVKYKKE
Sko_Gsx       NLXTSCKRIRTAFTSTQLELEREFASNMYLSRLRRIE IATYLNSEKQVKIWFQNRRVHKHKE
Spu_Gsx       QIRESSKRIRTAFTSTQLELEREFASNMYLSRLRRIE IATYLNSEKQVKIWFQNRRVKYKKE
Nvi_Gsx       XXXSRSKRIRTAFTSMQLELEKEFSQNYLSRLRRIQIAALLDLSEKQVKIWFQNRRVKWKDK
Tca_Gsx       XXXASSKRIXTAFTSTQLELEREFASNMYLSRLRRIE IATCLRLSEKQVKIWFQNRRVKYKKE
Aca_Gsx       DLXPSSKRMRTAFSTQLELEREFAGTNMYLSRLRRIE IATCLNLSEKQVKIWFQNRRVHKHKE
Xla_Gsx1      QLXPSSKRMRTAFSTQLELEREFASNMYLSRLRRIE IATYLNSEKQVKIWFQNRRVHKHKE
Aen_Gsx       DLXISSKRIRTAFTSTQLLDLEREFNSNMYLSRLRRIE IATYLNSEKQVKIWFQNRRVKYKKE
Sof_Gsx       XXXQSSKRIRTAFTSTQLELEREFASNMYLSRLRRIE IATYLNSEKQVKIWFQNRRVKYKKE
Mmu_Gsx1      QLXPSSKRMRTAFSTQLELEREFASNMYLSRLRRIE IATYLNSEKQVKIWFQNRRVHKHKE
Xla_Gsx2      QAXQNGKRMRTAFSSTQLELEKEFEHFNKYISRPRIELASMLSLTERHIKIWFQNRRMKWKKE
Mmu_Gsx2      QVXPNGKRMRTAFSTQLELEREFSNMYLSRLRRIE IATYLNSEKQVKIWFQNRRVHKHKE
Pex_Gsx       XXXXXSKRIRTAFTDSRQLDLEREFAGNMYLSRLRRIE IANSLSLTEKQVKIWFQNSRXXXXXX
Nco_Gsx1      SHAATKRRMRTAFSTRQLELEKEFGANMYLSRLRRIE IAACLQLSEKQIKIWFQNRRVKYKKE
Nco_Gsx2      DKPPGGKRARTSFTSEQLIELERHFNSNEYLGRTPRVEMALALNLSEKQIKIWFQNRRMKQKKE
Plo_Gsx1      HHHVTRRRMRTAFSTRQLELEKEFGANMYLSRLRRIE IAACLQLSEKQIKIWFQNRRVKYKKE
Plo_Gsx2      TDGAAAKRRRTIFSSEQLIELERHFRTNEYLGRTPRIEMALALNLSEKQIKIWFQNRRMKQKKE
Pdu_Xlox      DFTDENKRTRTAYTRSQLELEKEFEHFNKYISRPRIELASMLSLTERHIKIWFQNRRMKWKKE
Bfl_Xlox      AVEDENKRTRTAYTRGQLELEKEFEHFNKYISRPRIELAAMLNLTERHIKIWFQNRRMKWKKE
Sko_Xlox      QDLDENKRTRTAYTRSQLELEKEFEHFNKYISRPRIELAAMLNLTERHIKIWFQNRRMKYKKE
Spu_Xlox      ADFDENKRTRTAYTRGQLELEKEFEHFNKYISRPRIELAAMLNLTERHIKIWFQNRRMKWKKE
Pma_Xlox      PIEDDENKRTRTAYTRGQLELEKEFEHFNKYISRPRIELAAMLNLTERHIKIWFQNRRMKWKKE
Xla_Xlox      MEQEENKRTRTAYTRAQLELEKEFEHFNKYISRPRIELAVMLNLTERHIKIWFQNRRMKWKKE
Mmu_Xlox      AEPEENKRTRTAYTRAQLELEKEFEHFNKYISRPRIELAVMLNLTERHIKIWFQNRRMKWKKE
Cte_Xlox      QTFSENKRTRTAYTRAQLELEKEFEHFNRYITRPRRVELAAHLNLTEQHIIKIWFQNRRMKWKDV
Hro_Xlox      HILDDNKRTRTAYSRQLELEKEFEHFDKYISRPRIELASSLNLTERHIKIWFQNRRMKWKKE
Nco_Xlox      RMFDENKRTRTAYTRAQLELEKEFEHFDKYISRRLELANLLRLTERHIKIWFQNRRMKWKKE
Plo_Xlox      RTFDENKRTRTAYTRAQLELEKEFEHFDKYISRRLEMAKVLRRLTERHIKIWFQNRRMKWKKE
Cte_Cdx       GKTRTKDKYRIVYSEYQKVELEKEYLSKYITIQRKAELSRISGLSERQVKIWFQNRRAKERKQK
Pdu_Cdx       XKTRTKDKYRVVYTDHQRLLEKEFEHYSRYITIRKAE LAQTLNLSEKQVKIWFQNRRAKERKQN
Avi_Cdx       XKTRTKDKYRVVYTDHQRLLEKEFEHYSRYITIRKAE LAQNLNLSEKQVKIWFQNRRAKERKQN
Bfl_Cdx       GKTRTKDKYRVVYSDHQRLLEKEFEHYSRYITIRKAE LAQNLNLSEKQVKIWFQNRRAKERKQN
Sko_Cdx       GKTRTKDKYRVVYTDHQRLLEKEFEHYSRYITIRKAE LAHALGLSERQVKIWFQNRRAKERKQN
Spu_Cdx       GKTRTKDKYRVVYTDHQRLLEKEFEHYSRYITIRKAE LAHALGLSERQVKIWFQNRRAKERKMA
Nco_Cdx1      GKTRTRDKYRTVYTEPQVELEAEFQRTTYISAQRKADIASMVGLSERQVKIWFQNRRAKERDHRD
Nco_Cdx2      GKTRTKDKYRIVYTELQKVELEKEFLNQYITIQRKAELAGTIGLSRQVKIWFQNRRAKERKHK
Plo_Cdx2      GKTRTKDKYRIVYTELQKVELEKEFLNQYITIQRKAELAGHIGLSRQVKIWFQNRRAKERKHK
Plo_Cdx1      GKTRTKDKYRTVYTDKQRLLEAEYIRATYVSMNRKLNLSCTTGLSERQVKIWFQNRRAKERDHRQ
Pvu_Cdx       GKTRTKDKYRVAYTDHQRLLEKEFEHYSRYITIRKAE IAAQLALSERQVKIWFQNRRAKERKQN
Tca_Cdx       GKTRTKDKYRVVYTDHQRLLEKEFEHYSRYITIRKAE LANSLGLSERQVKIWFQNRRAKERKQV
Mmu_Cdx1      GKTRTKDKSRVVYTDHQRLLEKEFEHYSRYITIRKAE LANLGLSERQVKIWFQNRRAKERKVN
Mmu_Cdx2      VKTRTKDKYRVVYTDHQRLLEKEFEHYSRYITIRKAE LAATLGLSERQVKIWFQNRRAKERKIK
Mmu_Cdx4      GKTRTKDKYRVVYTDHQRLLEKEFEHFNRYITIRKAE LAVNLGLSERQVKIWFQNRRAKERKMI
Xla_Cdx1      TKTRTKDKYRVVYTDQRLLEKEFEHYSRYITIRKAE LAVNLGLSERQVKIWFQNRRAKERKIN
Xla_Cdx2      SKTRTKDKYRVVYTDQRLLEKEFEHYSRYITIRKAE LAVGLGLSERQVKIWFQNRRAKERKIN
Xla_Cdx4      GKTRTKDKYRVVYTDHQRLLEKEFEHYSRYITIRKAE LANLRLSERQVKIWFQNRRAKERKLF
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