

## Article

# Supplementary Materials for Germ Line/Multipotency Genes Show Differential Expression during Embryonic Development of the Annelid *Enchytraeus coronatus*

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**Table S1.** Primer sequences used to clone fragments of the *Eco-vasa1*, *Eco-vasa2*, *Eco-pl10-1*, *Eco-pl10-2*, *Eco-piwi1A*, *Eco-piwi1B*, *Eco-nanos1*, *Eco-nanos2*, *Eco-myc*, *Eco-pumilio2*, *Eco-pumilio3*, *Eco-tudor1*, *Eco-tudor2*, *Eco-tudor3*, *Eco-boule1*, *Eco-boule2*, *Eco-bruno1*, *Eco-bruno2*, *Eco-bruno3*, and *Eco-bruno4* genes presented in the paper.

<i>Eco-vasa1</i>	forward	5'-CCCAACGCTGATAAGATGCCTGTC-3'
	reverse	5'-GGTTCTCGAGGAAGTTTGAATCTC-3'
<i>Eco-vasa2</i>	forward	5'-GAGAAGATGGTAAGCCGAAGCCC-3'
	reverse	5'-CCACTCTGTATCATCGTTAGCAGC-3'
<i>Eco-pl10-1</i>	forward	5'-GGTGGAATCGTTTGAGGGAGGAGGAG-3'
	reverse	5'-CACCATTACCTCCCCCTCTCCTGTC-3'
<i>Eco-pl10-2</i>	forward	5'-CGAATGATTGGACTAAACCACTGCCC-3'
	reverse	5'-GGTAATCCCGCGAGGCAAATCCAGTG-3'
<i>Eco-piwi1A</i>	forward	5'-GGCGAGCACGTTGAGAAAGAAAG-3'
	reverse	5'-GGCTGGAGTCTAGTCGTGCTGTC-3'
<i>Eco-piwi1B</i>	forward	5'-GTTTGATGGGATGGGTCTGCTGTTTC-3'
	reverse	5'-GAGGTTAGAGTTGTCGTAGATGAC-3'
<i>Eco-nanos1</i>	forward	5'-CTCGCCGATGTCTAGCCGTTCTTC-3'
	reverse	5'-CTCAGAGAATCGCCTGTGTTGTATG-3'
<i>Eco-nanos2</i>	forward	5'-CCCTCCACTAATTCTTCCACTAATCC-3'
	reverse	5'-CTACAGTTTGGCCCCAGTAGATTAAACC-3'
<i>Eco-myc</i>	forward	5'-CTCTAAACTGGCTGGAAGACGGAC-3'
	reverse	5'-CCCTGTCGAGCCTTGGCATTCTC-3'
<i>Eco-pumilio2</i>	forward	5'-CAATACGCTCTGCCTACTGACCAAATAC-3'
	reverse	5'-CCGTATGGGTGCATGGCTAGGTTTC-3'
<i>Eco-pumilio3</i>	forward	5'-CAATGCAGGATTTCAAGGAGGCG-3'
	reverse	5'-GCTGTAGGGTAGTGCTGCCACCTG-3'
<i>Eco-tudor1</i>	forward	5'-CCAACGCTAGACAGCTGGATAACC-3'
	reverse	5'-GTCAGTGTAGCTCTGTACCAGGAC-3'
<i>Eco-tudor2</i>	forward	5'-CAAAGAGTTCAGCAGCAGTTGTGTG-3'
	reverse	5'-GTCCCCAGACACCGGTCTCAACTC-3'

<i>Eco-tudor3</i>	forward	5'-GATGACATCACTGCGGAGGCTGAG-3'
	reverse	5'-CTTTGAGGCCACATCTTCCCCATC-3'
<i>Eco-boule1</i>	forward	5'-CTACATTCTATCCGTCTAGACAGCAAAC-3'
	reverse	5'-CTGCATATCCACAGCTGATGACGG-3'
<i>Eco-boule2</i>	forward	5'-CTTCATTGTCGTCGCTGTGAGTCC-3'
	reverse	5'-CATCAGCTGAACATGACCGCCTG-3'
<i>Eco-bruno1</i>	forward	5'-GAACACTCAACCAATCACAGCAGAG-3'
	reverse	5'-CACAGACCGTTAGCGAGAGCAGC-3'
<i>Eco-bruno2</i>	forward	5'-CTGAATGCAGCAACCAGTGGGTCC-3'
	reverse	5'-GTAGAGGGTGGTATCAGACTTGGG-3'
<i>Eco-bruno3</i>	forward	5'-GTTCTGTTCCGTGCAACCGTATCG-3'
	reverse	5'-GTACGGTCGATTGGCGTCTTTAGG-3'
<i>Eco-bruno4</i>	forward	5'-GCATGTTTCATCGGCTGCGACTC-3'
	reverse	5'-CTCTTCAGTTGGACTTTCAACCTCTTC-3'

**Table S2.** GenBank accession numbers for sequences used for Pl10 and Vasa amino acid alignments.

Phylum	Species	Gene name in tree	GenBank #
Annelida	<i>Alitta virens</i>	Avi_pl10	AJW77404.1
	<i>Capitella teleta</i>	Cte_pl10	ELU14120.1
	<i>Enchytraeus coronatus</i>	Eco_pl10_1	OR750674
	<i>Enchytraeus coronatus</i>	Eco_pl10_2	OR750675
	<i>Helobdella robusta</i>	Hro_pl10	XP_009010523.1
	<i>Platynereis dumerilii</i> ,	Pdu_pl10	CAJ15140.1
	<i>Pristina longiseta</i>	Plo_pl10	JX264564
	<i>Alitta virens</i>	Avi_vasa	AJW77403.1
	<i>Capitella teleta</i>	Cte_vasa	ELU18906.1
	<i>Enchytraeus coronatus</i>	Eco_vasa1	OR750672
	<i>Enchytraeus coronatus</i>	Eco_vasa2	OR750673
	<i>Enchytraeus japonensis</i>	Eja_vasa	BAF76795.1
	<i>Helobdella robusta</i>	Hro_vasa	XP_009030586.1
	<i>Platynereis dumerilii</i>	Pdu_vasa	CAJ38803.1
	<i>Pristina leidy</i>	Ple_vasa	AIM52096.1
	<i>Pristina longiseta</i>	Plo_vasa	JX264563
	<i>Tubifex tubifex</i>	Ttu_vasa	BAD90012.1
Platyhelminthes	<i>Macrostomum lignano</i>	Mli_pl10	PAA56642.1
	<i>Schistosoma japonicum</i>	Sja_pl10	KAH8852518.1
Mollusca	<i>Crassostrea gigas</i>	Cgi_pl10	XP_011446932.2
	<i>Patella vulgata</i>	Pvu_pl10	XP_050413768.1
	<i>Crassostrea gigas</i>	Cgi_vasa	XP_034310873.1
	<i>Lottia gigantea</i>	Lgi_vasa	XP_009057808.1
	<i>Patella vulgata</i>	Pvu_vasa	XP_050411929.1

Arthropoda	<i>Pecten maximus</i>	Pma_vasa	P_033738811.1
	<i>Drosophila melanogaster</i>	Dme_belle	NP_536783.1
	<i>Tribolium castaneum</i>	Tca_belle	NP_001153721.1
	<i>Drosophila melanogaster</i>	Dme_vasa	CAA31405.1
Echinodermata	<i>Tribolium castaneum</i>	Tca_vasa	EFA07550.1
	<i>Strongylocentrotus purpuratus</i>	Spu_pl10	XP_030838128.1
	<i>Lytechinus variegatus</i> ,	Lva_pl10	XP_041483221.1
	<i>Patiria miniata</i>	Pmi_pl10	XP_038050100.1
	<i>Strongylocentrotus purpuratus</i>	Spu_vasa	NP_001139665.1
	<i>Lytechinus variegatus</i>	Lva_vasa	XP_041475118.1
Hemichordata	<i>Patiria miniata</i>	Pmi_vasa	XP_038056422.1
	<i>Ptychodera flava</i>	Pfl_vasa	QOW95195.1
	<i>Saccoglossus kowalevskii</i>	Sko_vasa	XP_006825896.1
Chordata	(outgroup) <i>Mus musculus</i>	Mmu_p68	AAB53236.1
	<i>Mus musculus</i>	Mmu_pl10	NP_149068.1
	<i>Danio rerio</i>	Dre_pl10	AAH59794.1
	<i>Mus musculus</i>	Mmu_vasa	NP_001139357.1
	<i>Danio rerio</i>	Dre_vasa	AAI29276.1
	<i>Branchiostoma floridae</i>	Bfl_vasa	XP_035672419.1
Cnidaria	<i>Actinia tenebrosa</i>	Ate_pl10	XP_031554871.1
	<i>Clytia hemisphaerica</i>	Che_pl10	AFD28592.1
	<i>Hydra vulgaris</i>	Hvu_pl10	XP_047130892.1
	<i>Nematostella vectensis</i>	Nve_pl10	XP_048589030.1
	<i>Actinia tenebrosa</i>	Ate_vasa	XP_031562414.1
	<i>Nematostella vectensis</i>	Nve_vasa	AFP87471.1

**Table S3.** GenBank accession numbers for sequences used for Piwi amino acid alignments.

Phylum	Species	Gene name in tree	GenBank #
Annelida	<i>Aeolosoma viride</i>	Avr_piwi	AQV09901.1
	<i>Alitta virens</i>	Avi_piwi1	AJW77405.1
	<i>Alitta virens</i>	Avi_piwi2	AJW77406.1
	<i>Capitella teleta</i>	Cte_piwi1	DAA34898.1
	<i>Capitella teleta</i>	Cte_piwi2	ELU02261.1
	<i>Eisenia fetida</i>	Efe_piwi2	AUS83924.1
	<i>Enchytraeus coronatus</i>	Eco_piwi1A	OR750676
	<i>Enchytraeus coronatus</i>	Eco_piwi1B	OR750677
	<i>Pristina leidy</i>	Ple_piwi	AIM52094.1
	<i>Pristina leidy</i>	Ple_piwi2	AIM52095.1
	<i>Pristina longiseta</i>	Plo_piwi_A	JX264566
	<i>Pristina longiseta</i>	Plo_piwi_1	OR203685
	<i>Pristina longiseta</i>	Plo_piwi_2	JX264567

	<i>Platynereis dumerilii</i>	Pdu_piwi	CAJ28986.1
	<i>Platynereis dumerilii</i>	Pdu_piwiB	AZG02837.1
Mollusca	<i>Crassostrea virginica</i>	Cvi_piwi	XP_022338755.1
	<i>Patella vulgata</i>	Pvu_piwi	XP_050410271.1
Brachiopoda	<i>Lingula anatina</i>	Lan_piwi	XP_013405373.1
Arthropoda	<i>Drosophila melanogaster</i>	Dme_piwi	AAD08705.1
Echinodermata	<i>Strongylocentrotus purpuratus</i>	Spu_piwi	XP_030846820.1
	<i>Lytechinus variegatus</i>	Lva_piwi	XP_041456323.1
	<i>Patiria miniata</i>	Pmi_piwi	XP_038059144.1
Chordata	(outgroup) <i>Mus musculus</i>	Mmu_Ago1	NP_700452.2
	<i>Mus musculus</i>	Mmu_piwi1	NP_067286.1
	<i>Mus musculus</i>	Mmu_piwi2	NP_067283.1
	<i>Mus musculus</i>	Mmu_piwi4	BAF65667.1
	<i>Danio rerio</i>	Dre_piwi1	NP_899181.1
	<i>Danio rerio</i>	Dre_piwi2	ACF35261.1
	<i>Branchiostoma floridae</i>	Bfl_piwi1	XP_035691448.1
	<i>Branchiostoma floridae</i>	Bfl_piwi2	AGI95997.1
Cnidaria	<i>Actinia tenebrosa</i>	Ate_piwi	XP_031558248.1
	<i>Hydra vulgaris</i>	Hvu_piwi	AFQ20832.1
	<i>Nematostella vectensis</i>	Nve_piwi	ASW22510.1
	<i>Nematostella vectensis</i>	Nve_piwi2	ASW22511.1
	<i>Podocoryna carnea</i>	Pca_piwi	AAS01181.1

**Table S4.** GenBank accession numbers for sequences used for Nanos amino acid alignments.

Phylum	Species	Gene name in tree	GenBank #
Annelida	<i>Alitta virens</i>	Avi_nanos	UFQ91327.1
	<i>Capitella teleta</i>	Cte_nanos	DAA06318.1
	<i>Enchytraeus coronatus</i>	Eco_nanos1	OR750678
	<i>Enchytraeus coronatus</i>	Eco_nanos2	OR750679
	<i>Helobdella robusta</i>	Hro_nanos	AAB63111.1
	<i>Helobdella robusta</i>	Hro_nanos1a	XP_009018920.1
	<i>Paranais litoralis</i>	Pli_nanos	ADE44351.1
	<i>Platynereis dumerilii</i>	Pdu_nanos	CAJ28985.1
	<i>Pristina leidy</i>	Ple_nanos	ADE44350.1
	<i>Tubifex tubifex</i>	Ttu_nanos	BAQ21630.1
Mollusca	<i>Crassostrea virginica</i>	Cvi_nanos	XP_022345706.1
	<i>Dreissena polymorpha</i>	Dpo_nanos	XP_052227042.1
	<i>Mizuhopecten yessoensis</i>	Mye_nanos1	OWF49530.1
	<i>Mizuhopecten yessoensis</i>	Mye_nanos3	OWF55054.1
	<i>Pecten maximus</i>	Pma_nanos	XP_033757476.1
Echinodermata	<i>Lytechinus variegatus</i>	Lva_nanos	ANT70531.1

Chordata	<i>Strongylocentrotus purpuratus</i>	Spu_nanos1a	XP_001177221.2
	<i>Strongylocentrotus purpuratus</i>	Spu_nanos2	NP_001073023.1
	<i>Mus musculus</i>	Mmu_nanos1	NP_848508.2
	<i>Mus musculus</i>	Mmu_nanos2	NP_918953.2
	<i>Mus musculus</i>	Mmu_nanos3	NP_918948.1
	<i>Xenopus laevis</i>	Xla_nanos1	NP_001081503.1
	<i>Xenopus tropicalis</i>	Xtr_nanos3	XP_004919225.1
	<i>Danio rerio</i>	Dre_nanos1	NP_001292590.1
	<i>Danio rerio</i>	Dre_nanos2_2	XP_009300191.1
	<i>Danio rerio</i>	Dre_nanos3	NP_571953.1
Cnidaria	<i>Hydra vulgaris</i>	Hvu_nanos1	BAB01491.1
	<i>Hydra vulgaris</i>	Hvu_nanos2	BAB01492.1
	<i>Nematostella vectensis</i>	Nve_nanos1	AAY67907.1
	<i>Nematostella vectensis</i>	Nve_nanos2	AAY67908.1
Porifera	<i>Amphimedon queenslandica</i>	Aqu_nanos	XP_003384296.1
	<i>Ephydatia muelleri</i>	Emu_nanos	AJE59349.1

**Table S5.** GenBank accession numbers for sequences used for Myc amino acid alignments.

Phylum	Species	Gene name in tree	GenBank #
Annelida	<i>Capitella teleta</i>	Cte_myc	ATN39075.1
	<i>Enchytraeus coronatus</i>	Eco_myc	OR750680
	<i>Helobdella robusta</i>	Hro_myc	XP_009030581.1
	<i>Owenia fusiformis</i>	Ofu_myc	CAH1774206.1
	<i>Platynereis dumerilii</i>	Pdu_myc	AGS55451.1
Mollusca	<i>Crassostrea virginica</i>	Cvi_myc	XP_022318424.1
	<i>Patella vulgata</i>	Pvu_myc	XP_050400774.1
	<i>Pecten maximus</i>	Pma_myc	XP_033738863.1
Brachiopoda	<i>Lingula anatina</i>	Lan_myc	XP_013410679.1
Arthropoda	<i>Drosophila melanogaster</i>	Dme_myc	AAD00517.1
	<i>Tribolium madens</i>	Tma_myc	XP_044270248.1
	<i>Daphnia pulex</i>	Dpu_myc	EFX79343.1
	<i>Limulus polyphemus</i>	Lpo_myc	XP_013793685.1
Echinodermata	<i>Patiria miniata</i>	Pmi_myc	XP_038057555.1
	<i>Strongylocentrotus purpuratus</i>	Spu_myc	NP_999744.1
Hemichordata	<i>Saccoglossus kowalevskii</i>	Sko_myc	NP_001158444.1
Chordata	<i>Mus musculus</i>	Mmu_myc	NP_034979.3
	<i>Danio rerio</i>	Dre_mycA	NP_571487.2
	<i>Danio rerio</i>	Dre_mycB	NP_956466.1
	<i>Branchiostoma floridae</i>	Bfl_myc	XP_002609915.1
Cnidaria	<i>Actinia tenebrosa</i>	Ate_myc	XP_031551646.1
	<i>Nematostella vectensis</i>	Nve_myc	XP_001627619.2

Porifera	<i>Amphimedon queenslandica</i>	Aqu_myc	XP_011408076.1
	<i>Halisarca dujardini</i>	Hdu_myc	QSX72220.1
Protista	(outgroup) <i>Monosiga brevicollis</i>	Mbr_myc	XP_001747958.1

**Table S6.** GenBank accession numbers for sequences used for Pumilio amino acid alignments.

Phylum	Species	Gene name in tree	GenBank #
Annelida	<i>Capitella teleta</i>	Cte_pum2	ELT98839.1
	<i>Capitella teleta</i>	Cte_pum3	ELU16136.1
	<i>Enchytraeus coronatus</i>	Eco_pum2	OR750681
	<i>Enchytraeus coronatus</i>	Eco_pum3	OR750682
	<i>Helobdella robusta</i>	Hro_pum2	XP_009016071.1
	<i>Helobdella robusta</i>	Hro_pum3	XP_009029294.1
	<i>Owenia fusiformis</i>	Ofu_pum3	CAH1774281.1
Mollusca	<i>Crassostrea gigas</i>	Cgi_pum2	XP_011422894.2
	<i>Crassostrea gigas</i>	Cgi_pum3	XP_034313950.1
	<i>Lottia gigantea</i>	Lgi_pum2	XP_009063233.1
	<i>Lottia gigantea</i>	Lgi_pum3	XP_009047841.1
	<i>Patella vulgata</i>	Pvu_pum3	XP_050403075.1
	<i>Pecten maximus</i>	Pma_pum3	XP_033737990.1
Brachiopoda	<i>Lingula anatina</i>	Lan_pum3	XP_013407952.1
Arthropoda	<i>Drosophila melanogaster</i>	Dme_pum	NP_731314.1
	<i>Tribolium castaneum</i>	Tca_pum	EFA12956.2
	<i>Tribolium madens</i>	Tma_pum2	XP_044266097.1
	<i>Ixodes scapularis</i>	Isc_pum2	XP_029825330.2
	<i>Limulus polyphemus</i>	Lpo_pum2	XP_013786730.1
Echinodermata	<i>Lytechinus variegatus</i>	Lva_pum2	XP_041466796.1
	<i>Lytechinus variegatus</i>	Lva_pum3	XP_041475303.1
	<i>Strongylocentrotus purpuratus</i>	Spu_pum2	XP_030830065.1
	<i>Strongylocentrotus purpuratus</i>	Spu_pum3	XP_030851927.1
	<i>Patiria miniata</i>	Pmi_pum2	XP_038051474.1
	<i>Patiria miniata</i>	Pmi_pum3	XP_038067463.1
Chordata	<i>Mus musculus</i>	Mmu_pum1	NP_109647.2
	<i>Mus musculus</i>	Mmu_pum2	XP_017170746.1
	<i>Mus musculus</i>	Mmu_pum3	NP_001390013.1
	<i>Danio rerio</i>	Dre_pum1	NP_001264048.1
	<i>Danio rerio</i>	Dre_pum2	NP_001096040.2
	<i>Danio rerio</i>	Dre_pum3	NP_001340777.1
Cnidaria	<i>Actinia tenebrosa</i>	Ate_pum2	XP_031566465.1
	<i>Actinia tenebrosa</i>	Ate_pum3	XP_031550523.1
	<i>Hydra vulgaris</i>	Hvu_pum2	XP_047145598.1
	<i>Hydra vulgaris</i>	Hvu_pum3	XP_002156370.2

	<i>Nematostella vectensis</i>	Nve_pum2	XP_032240209.1
	<i>Nematostella vectensis</i>	Nve_pum3	XP_001634611.1
Porifera	<i>Amphimedon queenslandica</i>	Aqu_pum2	XP_011402944.1
	<i>Amphimedon queenslandica</i>	Aqu_pum3	XP_003387437.1
Protista	(outgroup) <i>Monosiga brevicollis</i>	Mbr_pum	XP_001749459.1

**Table S7.** GenBank accession numbers for sequences used for Tudor amino acid alignments.

Phylum	Species	Gene name in tree	GenBank #
Annelida	<i>Capitella teleta</i>	Cte_Tdrd_ELU14575	ELU14575.1
	<i>Enchytraeus coronatus</i>	Eco_tudor1	OR750683
	<i>Enchytraeus coronatus</i>	Eco_tudor2	OR750684
	<i>Enchytraeus coronatus</i>	Eco_tudor3	OR750685
	<i>Owenia fusiformis</i>	Ofu_Tdrd_CAH1803308	CAH1803308.1
	<i>Platynereis dumerilii</i>	Pdu_Tdrd_1	CCK33035.1
	<i>Platynereis dumerilii</i>	Pdu_Tdrd_2	CCK33037.1
	<i>Platynereis dumerilii</i>	Pdu_Tdrd_3	CCK33036.1
Mollusca	<i>Crassostrea gigas</i>	Cgi_Tdrd_XP_011447159	XP_011447159.3
	<i>Patella vulgata</i>	Pvu_Tdrd_6	XP_050402777.1
	<i>Pecten maximus</i>	Pma_Tdrd_XP_033738269	XP_033738269.1
Brachiopoda	<i>Lingula anatina</i>	Lan_Tdrd_XP_013386808	XP_013386808.1
Arthropoda	<i>Drosophila melanogaster</i>	Dme_tudor	NP_476773.1
	<i>Drosophila melanogaster</i>	Dme_papi	NP_722773.1
	<i>Drosophila melanogaster</i>	Dme_tejas	NP_610950.2
	<i>Limulus polyphemus</i>	Lpo_Tdrd_1	XP_013776272.2
Echinodermata	<i>Strongylocentrotus purpuratus</i>	Spu_Tdrd_XP_782950	XP_782950.2
	<i>Strongylocentrotus purpuratus</i>	Spu_Tdrd_15_XP_780689	XP_780689.2
	<i>Strongylocentrotus purpuratus</i>	Spu_Tdrd_XP_797685	XP_797685.2
Hemichordata	<i>Saccoglossus kowalevskii</i>	Sko_Tdrd_XP_002737010	XP_002737010.1
Chordata	<i>Mus musculus</i>	Mmu_Tdrd_1	NP_001002238.1
	<i>Mus musculus</i>	Mmu_Tdrd_5	AAH99972.1
	<i>Mus musculus</i>	Mmu_Tdrd_6	AAI45249.1
	<i>Mus musculus</i>	Mmu_Tdrd_7	NP_666254.1
	<i>Xenopus laevis</i>	Xla_Tdrd_5	NP_001090599.1
	<i>Xenopus laevis</i>	Xla_Tdrd_7	NP_001084569.2
	<i>Xenopus tropicalis</i>	Xtr_Tdrd_6	XP_002938001.1
	<i>Xenopus tropicalis</i>	Xtr_Tdrd_7	NP_001011355.2
	<i>Danio rerio</i>	Dre_Tdrd_1	NP_001157500.1
	<i>Danio rerio</i>	Dre_Tdrd_5	AAI34986.1
	<i>Danio rerio</i>	Dre_Tdrd_6	NP_001182210.1
	<i>Danio rerio</i>	Dre_Tdrd_7	ABR24798.1
	<i>Branchiostoma floridae</i>	Bfl_Tdrd_XP_002608013	XP_002608013.1

Cnidaria	<i>Branchiostoma floridae</i>	Bfl_Tdrd_XP_002609625	XP_002609625.1
	<i>Branchiostoma floridae</i>	Bfl_Tdrd_7_XP_002590816	XP_002590816.1
	<i>Actinia tenebrosa</i>	Ate_Tdrd_XP_031549775	XP_031549775.1
	<i>Hydra vulgaris</i>	Hvu_Tdrd_1	XP_047129251.1
	<i>Hydra vulgaris</i>	Hvu_Tdrd_6	XP_047142151.1
	<i>Hydra vulgaris</i>	Hvu_Tdrd_7	XP_047145945.1
	<i>Nematostella vectensis</i>	Nve_Tdrd_XP_001627217	XP_001627217.1
	<i>Nematostella vectensis</i>	Nve_Tdrd_XP_001628171	XP_001628171.1
Porifera	<i>Nematostella vectensis</i>	Nve_Tdrd_EDO36108	EDO36108.1
	<i>Amphimedon queenslandica</i>	Aqu_Tdrd_1_XP_003388077	XP_003388077.1
	<i>Amphimedon queenslandica</i>	Aqu_Tdrd_XP_003384366	XP_003384366.1
	<i>Oscarella lobularis</i>	Olo_Tdrd_1_AQX83031	AQX83031.1
Fungi	(outgroup) <i>Schizosaccharomyces pombe</i>	Spo_snd1	NP_588117.1

**Table S8.** GenBank accession numbers for sequences used for Boule amino acid alignments.

Phylum	Species	Gene name in tree	GenBank #
Annelida	<i>Enchytraeus coronatus</i>	Eco-boule1	OR750686
	<i>Enchytraeus coronatus</i>	Eco-boule2	OR750687
	<i>Helobdella robusta</i>	Hro_boule	XP_009028824.1
	<i>Owenia fusiformis</i>	Ofu_boule	CAH1789320.1
Platyhelminthes	<i>Echinococcus multilocularis</i>	Emu_boule	CDS41941.1
	<i>Macrostomum lignano</i>	Mli_boule1	ADL09421.1
	<i>Macrostomum lignano</i>	Mli_boule2	AEK69206.1
	<i>Macrostomum lignano</i>	Mli_boule3	AEK69207.1
	<i>Schmidtea mediterranea</i>	Sme_boule1	ANE21796.1
	<i>Schmidtea mediterranea</i>	Sme_boule2	ANE21797.1
Mollusca	<i>Aplysia californica</i>	Aca_boule	XP_005103136.2
	<i>Crassostrea gigas</i>	Cgi_boule	XP_034310513.1
	<i>Crassostrea virginica</i>	Cvi_boule	XP_022305710.1
	<i>Octopus vulgaris</i>	Ovu_boule	CAI9742253.1
	<i>Patella vulgata</i>	Pvu_boule	XP_050418578.1
	<i>Pecten maximus</i>	Pma_boule	XP_033761447.1
	<i>Sepia pharaonis</i>	Sph_boule	CAE1318733.1
Brachiopoda	<i>Lingula anatina</i>	Lan_boule	XP_013418885.1
Arthropoda	<i>Drosophila melanogaster</i>	Dme_boule	AAF50316.3
	<i>Aedes albopictus</i>	Aal_boule	XP_029725578.1
	<i>Anopheles arabiensis</i>	Aar_boule	XP_040155388.1
	<i>Apis mellifera</i>	Ame_boule	XP_003250213.1
	<i>Culex quinquefasciatus</i>	Cqu_boule	XP_038118889.1
	<i>Nasonia vitripennis</i>	Nvi_boule	XP_001599398.1
	<i>Tribolium castaneum</i>	Tca_boule	EFA05679.2

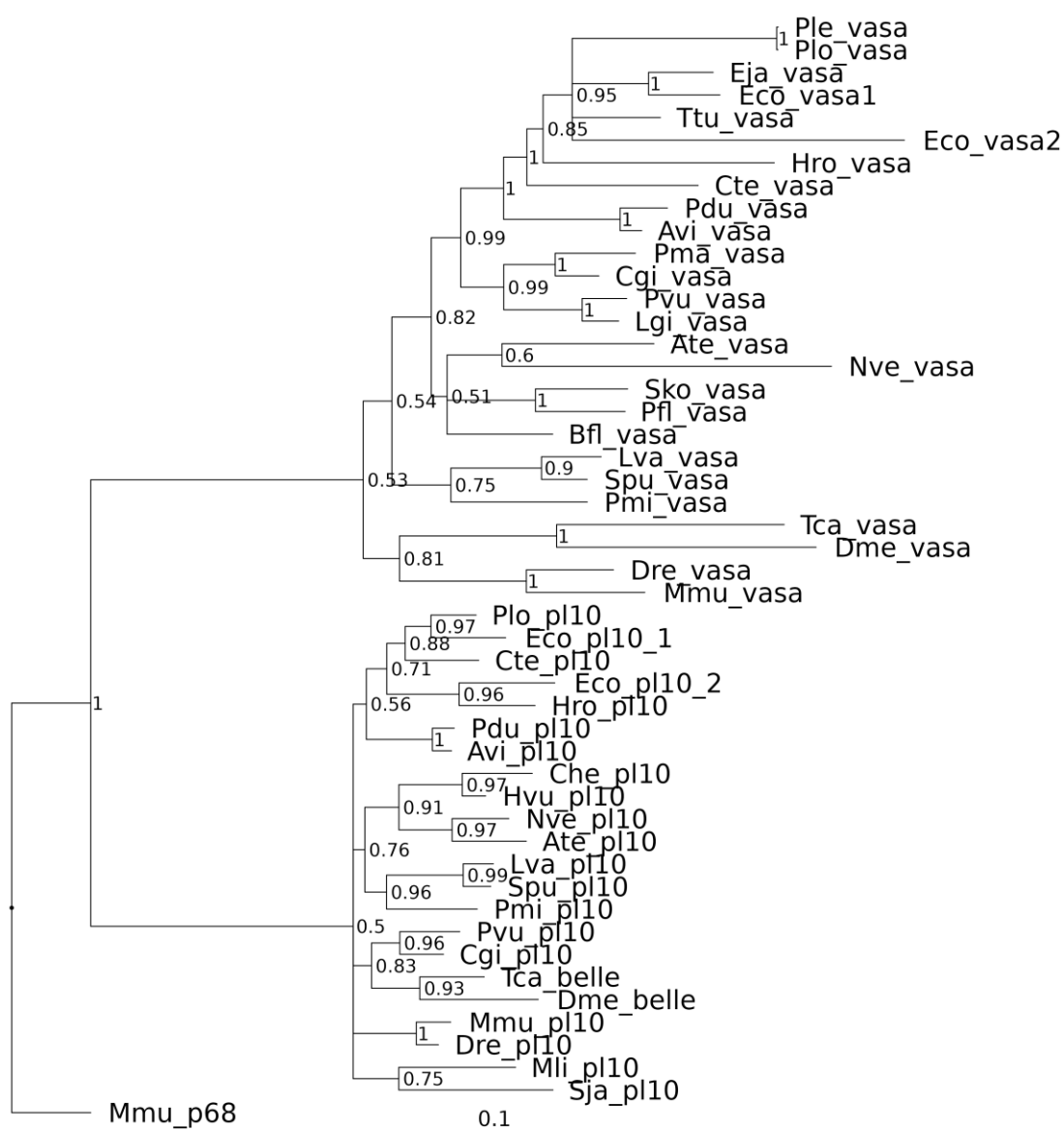


Echinodermata	<i>Strongylocentrotus purpuratus</i>	XP_030846218.1	Spu_boule
	<i>Patiria miniata</i>	Pmi_boule	XP_038044814.1
Hemichordata	<i>Saccoglossus kowalevskii</i>	Sko_boule	XP_006818817.1
Chordata	<i>Mus musculus</i>	Mmu_boule	NP_001354816.1
	<i>Oryzias latipes</i>	Ola_boule	ACU31026.1
Cnidaria	<i>Actinia tenebrosa</i>	Ate_boule	XP_031560060.1
	<i>Nematostella vectensis</i>	Nve_boule	XP_032243176.2
Fungi	(outgroup) <i>Saccharomyces cerevisiae</i>	Sce_HRP1	CAA99142.1

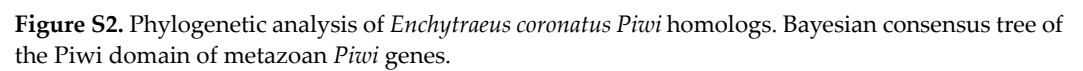
**Table S9.** GenBank accession numbers for sequences used for RRM domain protein Bruno amino acid alignments.

Phylum	Species	Gene name in tree	GenBank #
Annelida	<i>Capitella teleta</i>	Cte_ELU11974	ELU11974.1
	<i>Capitella teleta</i>	Cte_ELT93212	ELT93212.1
	<i>Enchytraeus coronatus</i>	Eco_bruno1	OR750688
	<i>Enchytraeus coronatus</i>	Eco_bruno2	OR750689
	<i>Enchytraeus coronatus</i>	Eco_bruno3	OR750690
	<i>Enchytraeus coronatus</i>	Eco_bruno4	OR750691
	<i>Helobdella robusta</i>	Hro_XP_009029960	XP_009029960.1
	<i>Helobdella robusta</i>	Hro_XP_009009284	XP_009009284.1
	<i>Helobdella robusta</i>	Hro_ESN89902	ESN89902.1
	<i>Helobdella robusta</i>	Hro_XP_009031985	XP_009031985.1
	<i>Malacoceros fuliginosus</i>	Mfu_CUGBP_elav_like_3	QNN94692.1
	<i>Owenia fusiformis</i>	Ofu_celf	QWY04388.1
	<i>Platynereis dumerilii</i>	Pdu_bruno	CCK33023.1
Mollusca	<i>Crassostrea gigas</i>	Cgi_CUGBP_elav_like_2	XP_034333010.1
	<i>Crassostrea gigas</i>	Cgi_CUGBP_elav_like_3B	XP_034317472.1
	<i>Lamellibrachia satsuma</i>	Lsa_CUGBP_elav_like_4	KAI0219664.1
	<i>Lottia gigantea</i>	Lgi_XP_009047452	XP_009047452.1
	<i>Lottia gigantea</i>	Lgi_XP_009055917	XP_009055917.1
	<i>Patella vulgata</i>	Pvu_CUGBP_elav_like_2	XP_050392146.2
	<i>Patella vulgata</i>	Pvu_CUGBP_elav_like_3B	XP_050395857.1
	<i>Pecten maximus</i>	Pma_CUGBP_elav_like_2	XP_033740337.1
Brachiopoda	<i>Pecten maximus</i>	Pma_CUGBP_elav_like_4	XP_033725903.1
	<i>Lingula anatina</i>	Lan_CUGBP_elav_like_2	XP_013383614.1
	<i>Lingula anatina</i>	Lan_CUGBP_elav_like_3A	XP_013408611.1
Arthropoda	<i>Drosophila melanogaster</i>	Dme_bruno1_isoformB	NP_723737.1
	<i>Drosophila melanogaster</i>	Dme_bruno2_isoformE	NP_001036356.1
	<i>Drosophila melanogaster</i>	Dme_bruno3_isoformA	NP_729923.1
	<i>Tribolium castaneum</i>	Tca_CUGBP_elav_like_2	XP_015838769.1
	<i>Tribolium castaneum</i>	Tca_CUGBP_elav_like_4	XP_015839523.1
	<i>Ixodes scapularis</i>	Isc_XP_002436197	XP_002436197.1

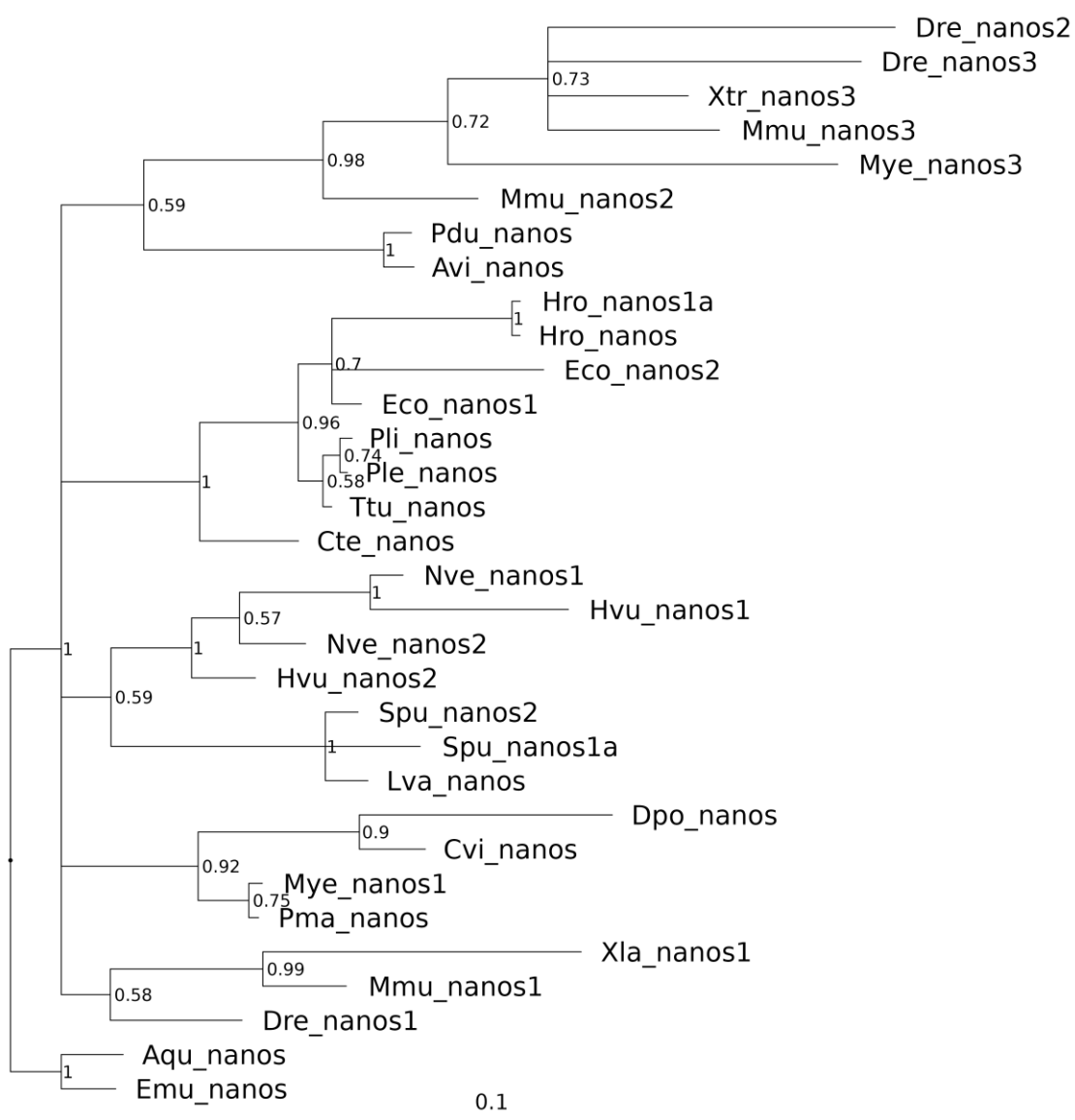
	<i>Ixodes scapularis</i>	Isc_XP_002404307	XP_002404307.1
	<i>Limulus polyphemus</i>	Lpo_CUGBP_elav_like_2	XP_022236600.1
	<i>Limulus polyphemus</i>	Lpo_CUGBP_elav_like_4	XP_013783377.2
Echinodermata	<i>Lytechinus variegatus</i>	Lva_CUGBP_elav_like_3B	XP_041459854.1
	<i>Strongylocentrotus purpuratus</i>	Spu_CUGBP_elav_like_1A	XP_782270.3
	<i>Strongylocentrotus purpuratus</i>	Spu_CUGBP_elav_like_2	XP_030844245.1
	<i>Strongylocentrotus purpuratus</i>	Spu_CUGBP_elav_like_3	XP_030829134.1
	<i>Strongylocentrotus purpuratus</i>	Spu_CUGBP_elav_like_4	XP_781047.2
Hemichordata	<i>Saccoglossus kowalevskii</i>	Sko_bruno_like	NP_001161510.1
	<i>Saccoglossus kowalevskii</i>	Sko_bruno2_like	XP_002736280.1
Chordata	(outgroup) <i>Mus musculus</i>	Mmu_elav_1	NP_034615.2
	<i>Mus musculus</i>	Mmu_CUGBP_elav_like_1	NP_059064.2
	<i>Mus musculus</i>	Mmu_CUGBP_elav_like_2	NP_001103698.1
	<i>Mus musculus</i>	Mmu_CUGBP_elav_like_3	NP_766022.1
	<i>Mus musculus</i>	Mmu_CUGBP_elav_like_4	XP_036016831.1
	<i>Mus musculus</i>	Mmu_CUGBP_elav_like_5	NP_795928.2
	<i>Mus musculus</i>	Mmu_CUGBP_elav_like_6	NP_780444.2
	<i>Xenopus tropicalis</i>	Xtr_CUGBP_elav_like_3	NP_001016491.1
	<i>Xenopus tropicalis</i>	Xtr_CUGBP_elav_like_4	NP_001072378.1
	<i>Xenopus tropicalis</i>	Xtr_CUGBP_elav_like_5	NP_001090639.1
	<i>Danio rerio</i>	Dre_CUGBP_elav_like_1	NP_571688.1
	<i>Danio rerio</i>	Dre_CUGBP_elav_like_2	NP_919382.1
	<i>Danio rerio</i>	Dre_CUGBP_elav_like_3	NP_571569.2
	<i>Danio rerio</i>	Dre_CUGBP_elav_like_4	NP_001002562.1
	<i>Danio rerio</i>	Dre_CUGBP_elav_like_5A	NP_001124260.1
	<i>Danio rerio</i>	Dre_celf3	XP_001335316.2
	<i>Danio rerio</i>	Dre_CUGBP_elav_like_6	XP_001334980.2
	<i>Branchiostoma floridae</i>	Bfl_CUGBP_elav_like_2	XP_035664982.1
	<i>Branchiostoma floridae</i>	Bfl_CUGBP_elav_like_4	XP_035668685.1
	<i>Branchiostoma floridae</i>	Bfl_CUGBP_elav_like_3A	XP_035668681.1
	<i>Branchiostoma floridae</i>	Bfl_XP_002588859	XP_002588859.1
	<i>Branchiostoma floridae</i>	Bfl_XP_002588860	XP_002588860.1
Cnidaria	<i>Nematostella vectensis</i>	Nve_CUGBP_elav_like_2	XP_048578496.1
	<i>Nematostella vectensis</i> , ,	Nve_XP_001627659	XP_001627659.1
	<i>Nematostella vectensis</i>	Nve_XP_001622676	XP_001622676.1
	<i>Nematostella vectensis</i>	Nve_XP_001621619	XP_001621619.1
Protista	<i>Monosiga brevicollis</i>	Mbr_XP_001745306	XP_001745306.1



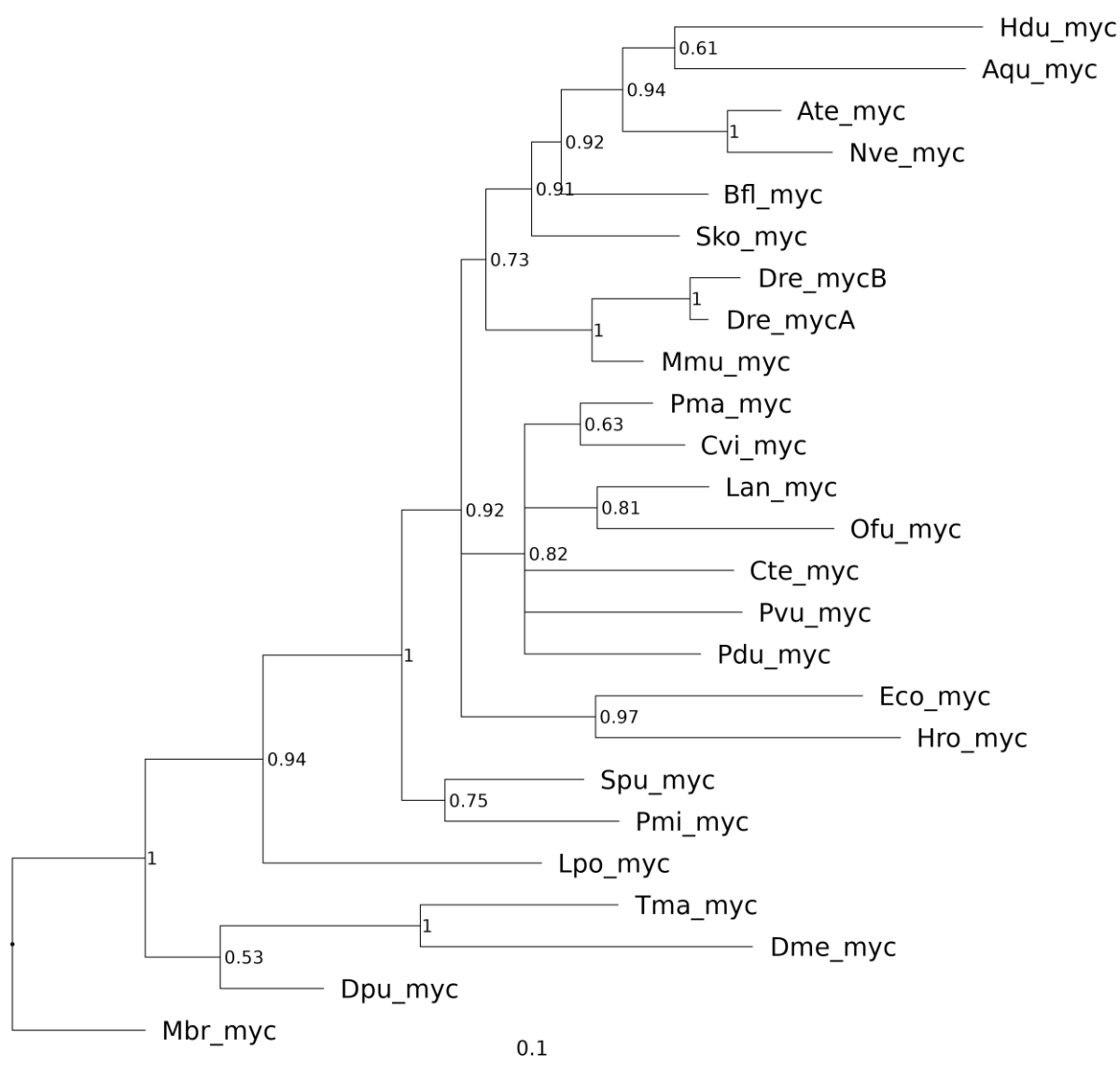
**Figure S1.** Phylogenetic analysis of *Enchytraeus coronatus Vasa* and *Pl10* homologs. Bayesian consensus tree of the Helicase domains of metazoan *Vasa* and *Pl10* genes.



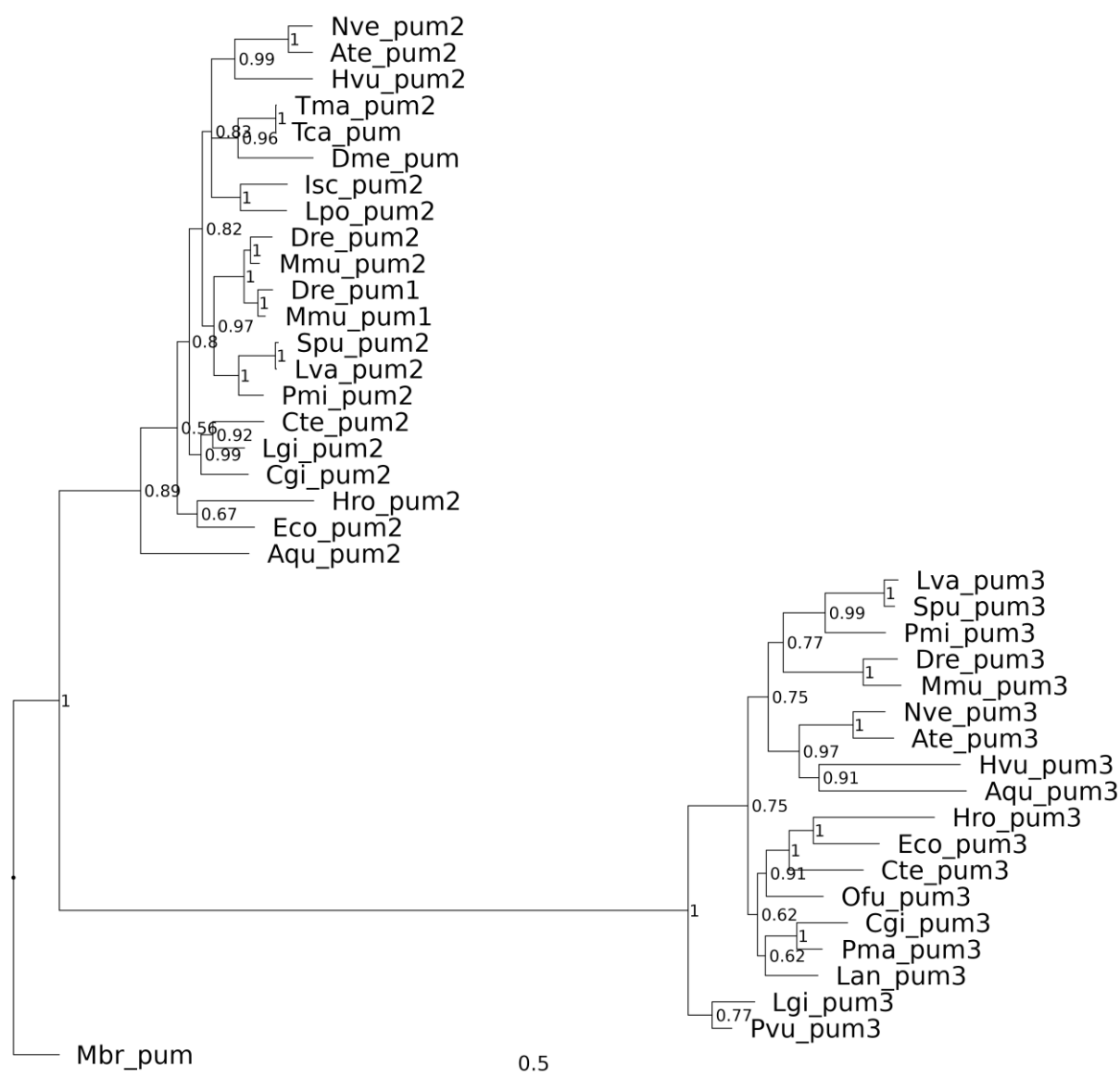
**Figure S2.** Phylogenetic analysis of *Enchytraeus coronatus* *Piwi* homologs. Bayesian consensus tree of the *Piwi* domain of metazoan *Piwi* genes.



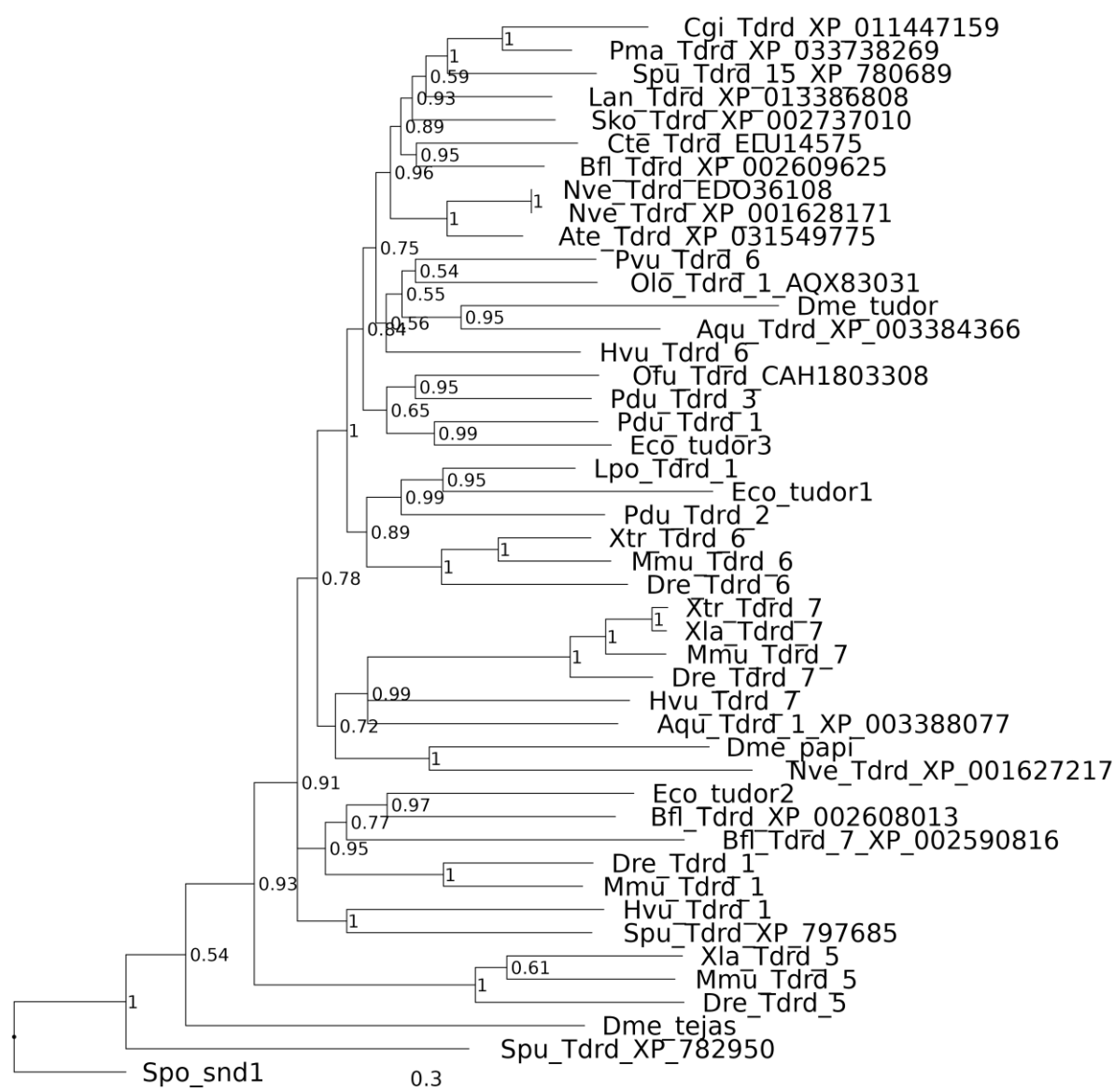
**Figure S3.** Phylogenetic analysis of *Enchytraeus coronatus* *Nanos* homologs. Bayesian consensus tree of the *Nanos* (CCHC)-type zinc-finger domain of metazoan *Nanos* genes.



**Figure S4.** Phylogenetic analysis of *Enchytraeus coronatus* Myc homolog. Bayesian consensus tree of the Myc-type basic helix-loop-helix (bHLH) domain of metazoan Myc genes.

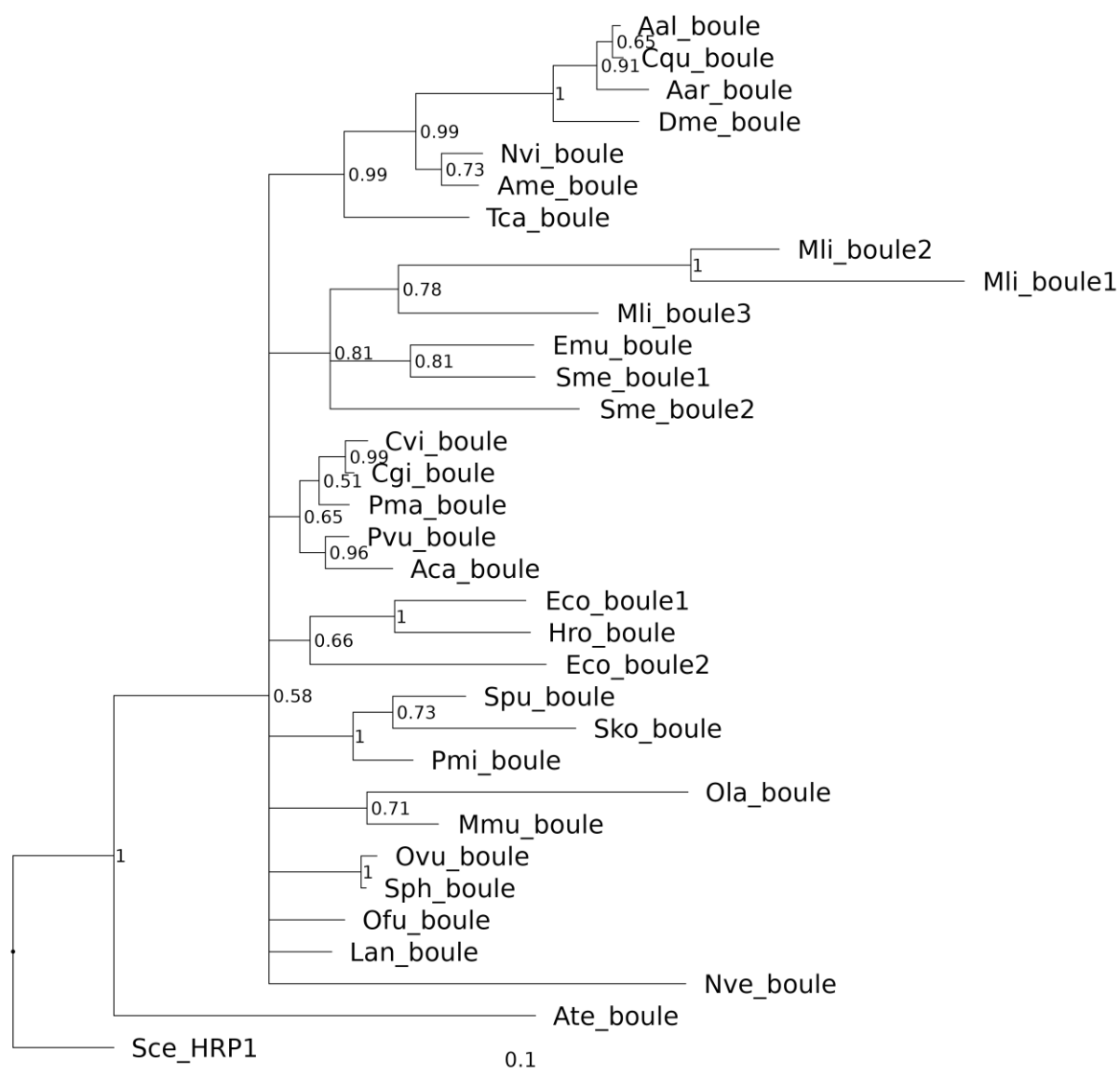


**Figure S5.** Phylogenetic analysis of *Enchytraeus coronatus* *Pumilio* homologs. Bayesian consensus tree of the *Pumilio* domain of metazoan *Pumilio* genes.

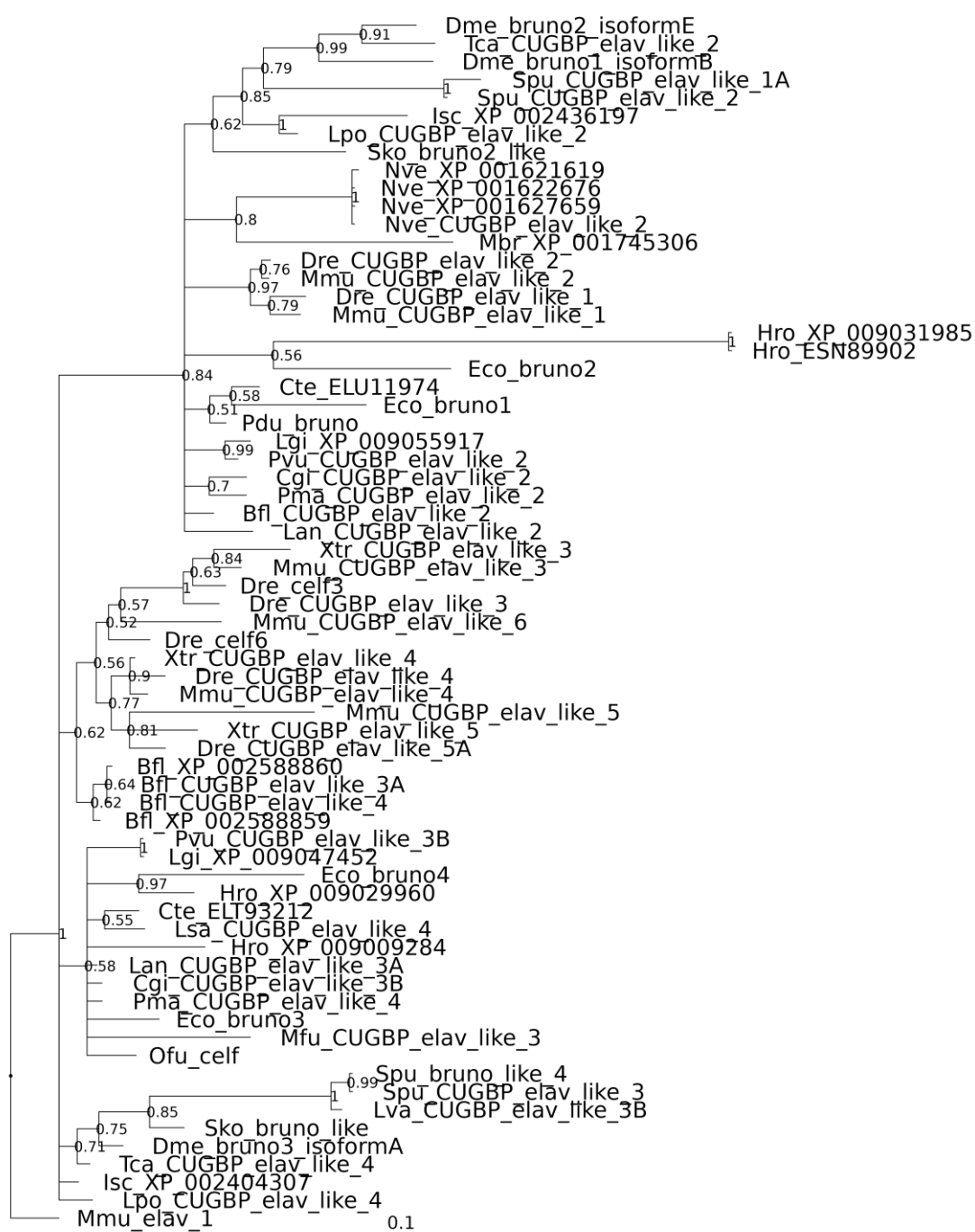


**Figure S6.** Phylogenetic analysis of *Enchytraeus coronatus* Tudor homologs. Bayesian consensus tree of the full-length alignment of metazoan Tudor genes.

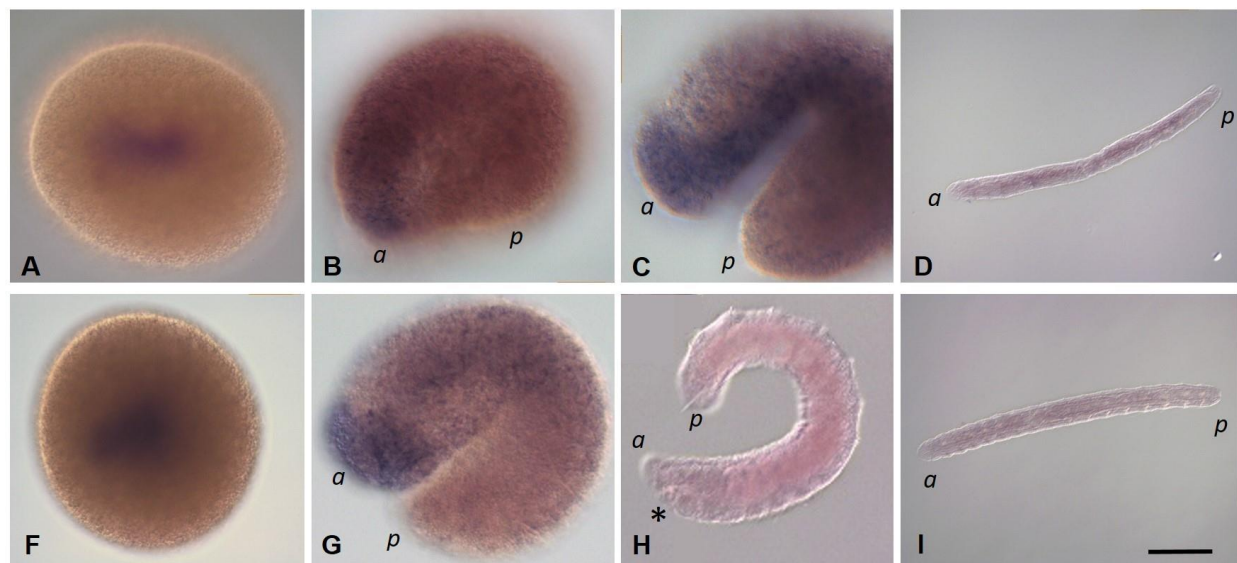




**Figure S7.** Phylogenetic analysis of *Enchytraeus coronatus* Boule homologs. Bayesian consensus tree of the Boule RRM domain of metazoan Boule genes.



**Figure S8.** Phylogenetic analysis of *Enchytraeus coronatus* Bruno homologs. Bayesian consensus tree of the Bruno RRM domain of metazoan Bruno/CUGBP-Elav-like genes.



**Figure S9.** *Eco-boule1* and *Eco-boule2* expression patterns. *Eco-boule1* (A–D) and *Eco-boule2* (F–I) expression patterns. (A,F) mRNA of both *Boule* orthologs is detected in oocytes. (B) *Eco-boule1* expression in the descendants of the animal micromeres and germband cells. (C) *Eco-boule1* expression becomes particularly prominent in cells of the animal pole and the ventral side of the anterior part of the embryo. (G) *Eco-boule2* expression in superficial cells is very weak, except for a domain in the most anterior part of the embryo. (D,I) In juvenile, no expression of identified *Boule* homologs is detected by WMISH. Asterisk marks the mouth position; *a* and *p* mark the anterior and posterior end, respectively. Scale bar, 50  $\mu\text{m}$  for all panels except D, H and I. Scale bar in D and I, 170  $\mu\text{m}$ ; in H, 80  $\mu\text{m}$ .