Molecular Searching for Gill Slits in Echinoderms:

Hox1 expression in *Strongylocentrotus purpuratus*

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Abstract:

The formation of gill slits is a homologous trait in most deuterostomes (including hemichordates, tunicates, lancelets, and vertebrates), however is not present in the Phylum Echinodermata. Thus, there may be a difference in gene order or gene expression. The anterior *Hox1* gene is responsible for gill slit formation (among other things), and is present in all deuterostomes, but perhaps in a different context. In this study, I performed phylogenetic analysis to determine how similar *Hox1* was in echinoderms and hemichordates. My results show that they are divergent genes and therefore a laboratory experiment is possible. The next step would be to observe and compare *Hox1* expression in both an echinoderm and a hemichordate.

Introduction:

Homologous structures provide support to the theory of evolution. They also help piece together the mysteries of the fossil record by providing connections and indicating when branching has occurred (Ou et al. 2012). One examined subject is the evolution of pharyngeal gill slits in the phylum Chordata–deuterostome animals that evolved a unique body plan a little before the Cambrian period (Brown et al. 2008). Chordates share many of the same morphologies including notochord, a dorsal hollow neural tube, an endostyle and pharyngeal gill slits. These gill slits seem to have originated with filter feeding when food capture in chordates internalized (Ogasawara et al. 1999). Interestingly enough, of the two Chordata out-groups, hemichordates have pharyngeal gill slits while echinoderms lack them (Rychel et al 2007). However, it has been suggested that pharyngeal gill slits are primitive features of deuterostomes and have simply been lost in echinoderms (Gillis et al. 2012, Swalla 2006). I want to examine the developmental differences between echinoderms and hemichordates, and why pharyngeal gill slits do/do not appear in echinoderms. I'm also interested in whether a transcription factor from another species (a hemichordate) that has pharyngeal gill slits or increasing one from the same species (echinoderm) could induce formation in echinoderms.

There are morphological similarities between the three phyla (chordates, hemichordates, and echinoderms), but the *Hox* genes solidify sister groups (Rottinger et al. 2012). The *Hox* gene cluster (*Hox1-13*) has a pivotal role in development, but *Hox1*, a gene present in all three groups, specifically regulates the position or appearance of the gill slits (Swalla 2006). However, the gene clusters are not all arranged the same. Hemichordates and chordates share the same genetic order and transcriptional orientation as chordates, but echinoderms do not (Freeman et al. 2012). Their *Hox1-5* genes have inversions, translocations and even a loss of gene *Hox4* (Freeman et al. 2012). Pharyngeal gill slits may not form in echinoderms because of this rearrangement. Another possibility is that in developmental systems, change can occur without a mutation in the overall *Hox* gene organization, but rather the regulation of the genes themselves (Holland et al. 1996). So, because other researchers have found the *Hox* gene cluster of echinoderms to be practically the same as that of chordates, instead it may be the cis-regulatory elements that have changed activity (Martinez et al. 1999). Therefore, I am interested in the regulation of *Hox* genes between these two phyla. What transcription factor(s) have been lost, or how has the expression of these genes been turned off and, in consequence, stopped pharyngeal gill slit formation?

Gill slit formation in hemichordates resembles that of vertebrates as both utilize a number of the same transcription factors (for example, *Pax1*, *Pax9 Eya1*, *Six1*, *Hox1*, *Hox3*, *FoxC* and *Tbx1*) during early development (Gillis et al. 2012). In mammalian experiments, mutants without one or more of these regulatory elements can lose pharyngeal arch formation (Gillis et al. 2012). In another animal, amphioxus, a vertebrate-like invertebrate, retinoic acid signaling has been found to regulate the expression of *Hox1* and thus development in the pharynx (Schubert et al. 2004). In these lancelets, RA signaling activates *Hox1*, which in turn represses *Pax1/9* (Schubert et al. 2004). *Pax1/9* is normally expressed in the endodermal cells of the pharynx after hatching and then later in the pharyngeal gill; so it has been suggested that these genes may play a pivotal part in the formation of the gill (Ogasawara et al. 1999). In fact, *Pax1/9* have been detected immediately before pharyngeal gill formation shown by the increased amount of transcripts during metamorphosis in both urochordates and hemichordates. Thus, it is expressed in adult gill tissues, a similarity shared with amphioxus and echinoderms (Ogasawara et al. 1999). However, as echinoderms do not develop gill slits, expression of *Pax1/9* occurs in the gill tissues, but also in the adult muscle and intestine (Ogasawara et al. 1999). Consequently, I wonder if *Hox1* and RA signaling can regulate the repression of *Pax1/9* in echinoderms. In vertebrates and hemichordates, *Hox1* is active right after the first gill slit forms (Swalla, 2006), allowing different morphological changes to occur. However, perhaps echinoderms have *Hox1* turned on before gill slit formation so *Pax1/9* is never even present in the pharynx region and no gill slits form.

There is also a possibility that these genes have become too genetically divergent to analyze effectively. It is much easier to study a recently derived trait between two groups, as it may have evolved too long ago and similarities will be difficult to connect and differences hard to quantify (Holland et al. 1996). If that were the case, this could instead prove more interesting, as it would not only indicate the starker difference between the phyla, but that the loss of pharyngeal gill slits has happened so long ago that we cannot find molecular signatures any longer.

Methods:

I used online gene databases genBank (http://www.ncbi.nlm.nih.gov/genbank/) and Spbase (http://www.spbase.org/SpBase/) to obtain protein sequences and then the program BLAST to look for similar sequences. I compared and aligned *Hox1* of *Strongylocentrotus purpuratus* to a variety of organisms on the program Mega5.1 using either Muscle or Clustal techniques. The program MacVector was also used, but produced inconsistent results so the trees presented in this paper are derived from Mega alignments. The *Hox1* gene sequence is not highly conserved, so instead transcribed protein sequences were used to achieve a successful alignment. It was difficult to find a range of animals because *Hox1* is not as extensively sequenced for invertebrates. Many of the original sequences I obtained were only partials or negatively affected the rest of the alignment and therefore were deleted. After an alignment for *Hox1* was generated, a tree could also be created using Mega5.1 with 1000 bootstrap replications. Aligned protein sequences were converted to a visible format using Clustalw (http://www.genome.jp/tools/clustalw/). A *Hox6* gene tree was also generated using the same techniques to provide a control comparison. There were less organisms available for the *Hox6* gene tree.

Results:

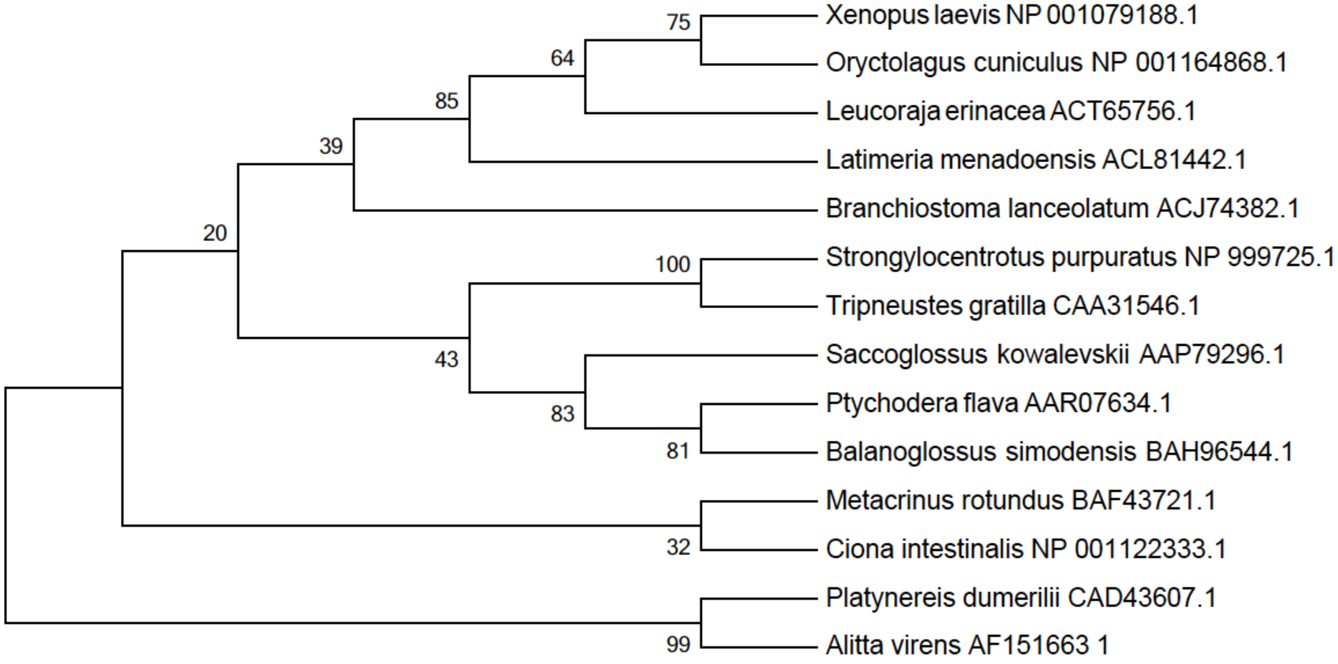

Figure 1: Above tree generated by Mega5.1 before the deletion of echinoderm *Metacrinus rotundus*. Deemed inaccurate because *Metacrinus rotundus* should not be grouping with the tunicate *Ciona intestinalis*. Bootstrap values are relatively low at some nodes, specifically at the node branching echinoderms and hemichordates from the vertebrates, suggesting inaccuracies in the tree. Improvement seen in Figure 2.

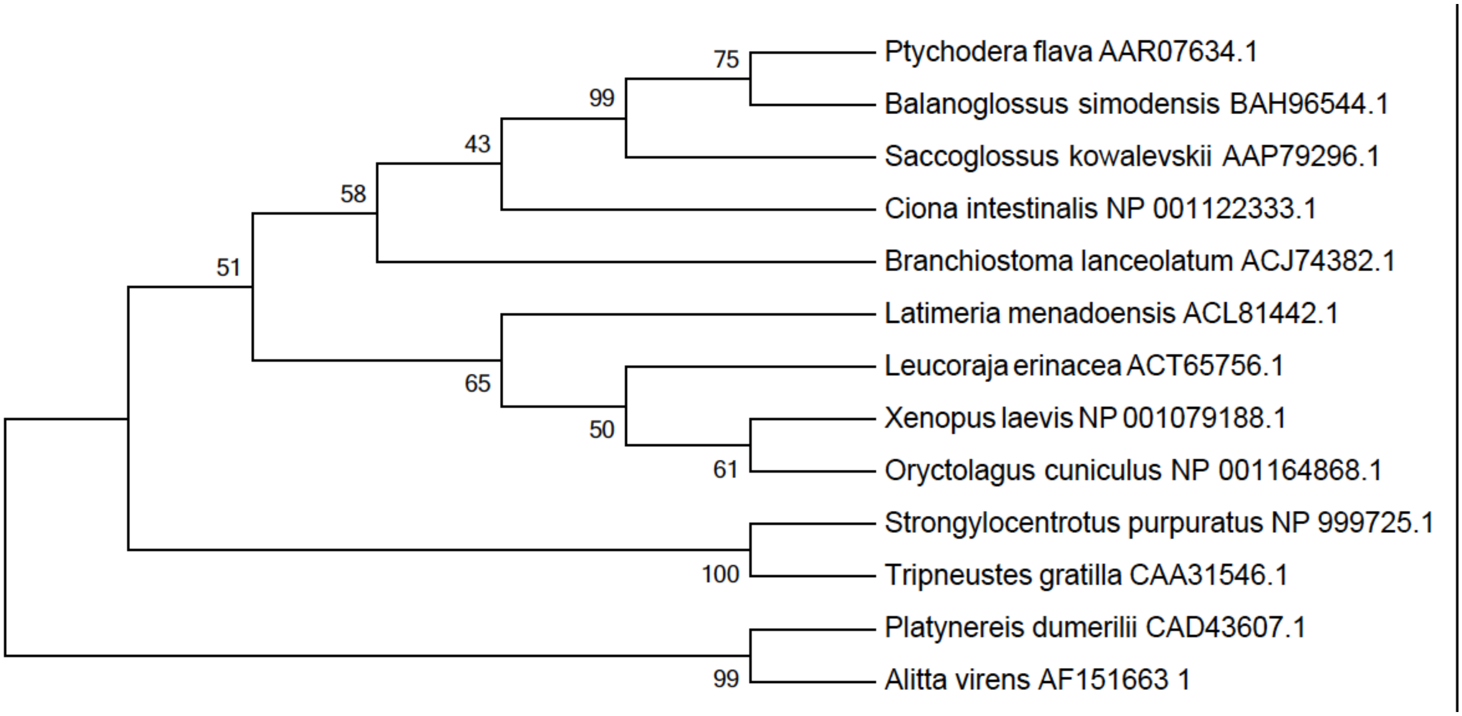

Figure 2: Tree after deletion of *M. rotundus*. The annelid worms are consistently shown as outgroups, and the echinoderms branch off at the next node. The vertebrates (*Latimeria menadoensis, Leucoraja erinacea*, etc.) appear in their own monophyletic group and the hemichordates (*Ptchodera flava, Balanoglossus simodensis, Saccoglossus kowalevski*) are also grouped together. The bootstrap values are improved, though still not completely reliable (<75).

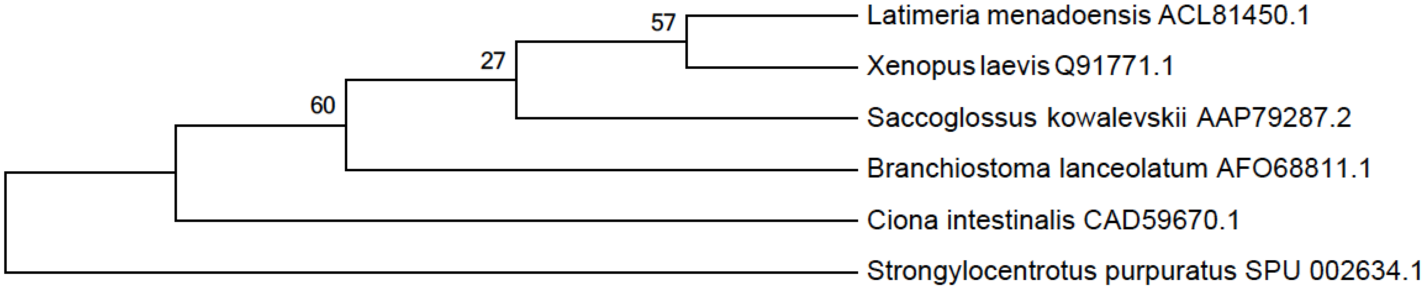

Figure 3: Tree of control phylogenetic analysis of *Hox6*. Not all organisms in Figure 2 had *Hox6* sequenced, tree is much smaller and rather incomplete. Bootstrap values indicate huge inaccuracies (60, 27, 57).

In Figure 2, I expected the lancelet species (*Branchiostoma lanceolatum*) and the tunicate (*Ciona intestinalis*) to branch closer with the vertebrates instead of the hemichordates (Brown et al. 2008). However, the echinoderms are seen as an out-group to the rest of the deuterostomes, including the hemichordates. This is good news for my project because that means the *Hox1* gene differs between echinoderms and hemichordates and a laboratory experiment is possible.

Discussion:

Conclusions can be drawn from the phylogenetic trees made using Mega5.1. Figure 2 clearly shows the echinoderms branching off from the hemichordates, chordates, and lancelets. Since echinoderms do not develop gill slits, activated by anterior *Hox1*, they may have an altered gene and therefore appear distinct from the chordates, hemichordates, tunicates, and lancelets (animals that have this morphology). The hemichordates are grouped closer toward the vertebrates and thus that implies that they have similar *Hox1* genes, and could explain why both groups develop gill slits. The bootstrap values for the echinoderms are very strong (100, Figure 2), however the rest of the tree including hemichordates, vertebrates, tunicates, and lancelets don't give very high bootstrap results. This has to be taken into account when drawing information from the tree as some of the results might not be as valid.

The phylogenetic trees produced by Mega5.1 in the results section are valuable; however, make some incorrect assumptions. This could possibly be due to insufficient sequencing. Many of the sequences found on GenBank and a BLAST search, were only partials. Though they were originally placed in the alignments, they disrupted the rest of the sequences aligning and thus they were deleted. Some of these include: *Oikopleura dioica, Podocoryna carnea, Symsagittifera roscoffensis, Holopneustes purpurascens, Balanoglossus misakiensis, Halocynthia roretzi, Polyandrocarpa misakiensis, Flaccisagitta enflata, Holothuria glaberrima, Branchiostoma floridae, Squaliobarbus curriculus, Heliocidaris erythrogramma,* and *Mus musculus*. After these deletions, a more logical tree was created. Because of scientific bias, vertebrates are often better studied and clearly better sequenced as the GenBank online database reflected that. Many of the organisms most applicable for my trees, in theory, were unhelpful because the sequences didn't line up. This could be part of the experimental error because many of the unusable organisms were closely related and this went underrepresented on my tree. Therefore, low bootstrap values could be due to a lack of data.

The next step would be to implement an actual experiment. I would need to first gather *Strongylocentrotus purpuratus,* as the echinoderm, from the Friday Harbor Laboratories dock, located on San Juan Island, and *Saccoglossus bromophenolosus*, as the hemichordate, obtained from Anacortes, Washington. The *S. bromophenolosus Hox1* is not located on GenBank and is therefore probably not sequenced, so I would need to design primers and perform PCR to sequence the gene. Eventually, I would spawn the organisms and fertilize the eggs to create embryos. These would be utilized for in sitiu hybridization to determine where and when *Hox1* expression is occurring. I would then compare the results of each organism and determine differences. These differences could indicate why pharyngeal gill slits are not formed in echinoderms. I'm also interested in the expression of *Pax1/9* and when this is expressed in both echinoderms and hemichordates. Past studies indicate that *Pax1/9* can influence *Hox1* expression and gill formation (Ogasawara et al. 1999) so this would be another important gene expression to analyze in echinoderms to explain the lack of gill slit formation.

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Supplemental Material

S1-Figure 1: Below pictured is the alignment of *Hox1* using Clustalw for formatting

(http://www.genome.jp/tools/clustalw/).

```
Xenopus_laevis_NP_001079188.1       ---MDYARRMSSFLEYP---GLMSGDLGTCSSRPF-
HPDHGITTF-QSCA
Oryctolagus_cuniculus_NP_00116      ---MDNA-RMSSFLEYP---ILSSGDSGTCSARTY-
SSDHGITTF-QSCA
Leucoraja_erinacea_ACT65756.1       ---MDNA-RMNSFLDY----SIISGETGTCSSRSY-
HADQGITTY-QSCA
Latimeria_menadoensis_ACL81442      ---MDNT-RMNSFLEY----
AICNRGTSAYSPKGYHHLDQGIPSFPPCSG
Ptychodera_flava_AAR07634.1         ---MDTS-KMGSYVDYGTESTLYPAVRNLNYPTEV-
PVNATTVHY-QQSY
Balanoglossus_simodensis_BAH96      ---MDTS-KMGSYVDYGSDSSMYPGVRSLNYPVEV-
PVNPSVAHY-QQSY
Saccoglossus_kowalevskii_AAP79      ---MDTS-KMAAYVDYGSDSGMYPAVRNLNYGSEV-
AVNSAPSHYQHTAY
Branchiostoma_lanceolatum_ACJ7      MEQMDTA-RMNSYVDY----SLCNGDQNTCSPRSY-
GQDYGVPAY-QSCA
Ciona_intestinalis_NP_00112233      ---------MNSYMKY-----
PVHASNSNSYTNHITPQGPFPTSHANKPT
Metacrinus_rotundus_BAF43721.1      ---MDKA-RISVDTPY----
NLCLSEHSNMYSTNAATAATDLSYH---SL
Platynereis_dumerilii_CAD43607      ---MPTA------------------------------------
-------
Alitta_virens_AF151663_1            -------------------------------------------
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Strongylocentrotus_purpuratus_      ---MSSS----
SYFVNPAFFPTYPHAGEQFYATPSGSYELSSCAF-----
Tripneustes_gratilla_CAA31546.      -------------------------------------------
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Xenopus_laevis_NP_001079188.1       VSANNCNGDDRFVVGRGVQISS---------------------
-------
Oryctolagus_cuniculus_NP_00116      VSANSCGGDDRFLVGRGVQISSPHHHHHH--------------
-------
Leucoraja_erinacea_ACT65756.1       VGNNNSNVDDRYIVSRGVQIGA---------------------
-------
Latimeria_menadoensis_ACL81442      TTNDSYNGDGRFLFGGSAPVSN---------------------
-------
Ptychodera_flava_AAR07634.1         GVPGSENVDGRMLSAVNSRTAM---------------------
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Balanoglossus_simodensis_BAH96      GVSSSENVDGRMLNSRTAM------------------------
-------
Saccoglossus_kowalevskii_AAP79      GVGNTDAIDARMLSGVNSRASL---------------------
-------
Branchiostoma_lanceolatum_ACJ7      MNNVDRHVTM---------------------------------
-------
Ciona_intestinalis_NP_00112233      VLPHETSAYGIYRTVESIRNHN---------------------
-------
Metacrinus_rotundus_BAF43721.1      PLSHSGSYHGHYTSRQDS-------------------------
-------
Platynereis_dumerilii_CAD43607      -------------------------------------------
-------
Alitta_virens_AF151663_1            -------------------------------------------
-------
Strongylocentrotus_purpuratus_      -------------------------------------------
-------
Tripneustes_gratilla_CAA31546.      -------------------------------------------
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Xenopus_laevis_NP_001079188.1       ---------HTHHHHQPGAFQHHSNLGMSY-AHPSC-
GSNYGMQNFSPGY
Oryctolagus_cuniculus_NP_00116      --------HHHHHHPQPATYQTSGNLGVSY-SHSSC-
GPSYGAQNFGAPY
Leucoraja_erinacea_ACT65756.1       ---------PLHHHHHQHASYTHNNLSIPYSAHPNC-
GTGYPAQSFNTGY
Latimeria_menadoensis_ACL81442      --------QPQHQNSSYHHHPHHSNMGIPYAST----
GSGYTPQTCNPGY
Ptychodera_flava_AAR07634.1         ---------NSYGHPHDSTVAGTAAMYSNSPATPQI-
GAANAYSFTDSMY
Balanoglossus_simodensis_BAH96      ---------NSYGHPHDSTVAGTAAMYSNSPATPQI-
GAANAYSFSDSMY
Saccoglossus_kowalevskii_AAP79      ---------
NTYQHSHDPSIAGPPSMYSSAPVTPQMNTAANAYSYPDSVY
Branchiostoma_lanceolatum_ACJ7      ---------
GPSGQLPPSAGPGPGPVPGPSPYDPPVIMSNGDPQNFTTYS
Ciona_intestinalis_NP_00112233      ---------ESTVTPQNEGSPGRSSR-ENSPTGP---
VGTTVHSTDNSPY
Metacrinus_rotundus_BAF43721.1      ---------
DSRLMNYPNQTAGSYSSWNGVSNNPECTASSLSSSSSPTGY
Platynereis_dumerilii_CAD43607      -------------------------------------------
----GGY
Alitta_virens_AF151663_1            -------------------------------------------
-------
Strongylocentrotus_purpuratus_      -----------
SKNPKTSSYSSSSSPSLVATSKPPCTQQLGAATFYGGGT
Tripneustes_gratilla_CAA31546.      -------------------------------------------
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Xenopus_laevis_NP_001079188.1       S---------------HFPINQEADVSAGF-----------
PQSVYSGNI
Oryctolagus_cuniculus_NP_00116      S---------------PYGLNQEADVSGGY---PPCAPAV---
--YSGNL
Leucoraja_erinacea_ACT65756.1       SH--------------HYSLNQETDGNGGY---PQCNPAV---
--YPGNL
Latimeria_menadoensis_ACL81442      GH--------------QYYFGQEPDG------------
MYFQSSGYSNSI
Ptychodera_flava_AAR07634.1         YNQN------------
HYNTAAAAAINRGSYLEPQCATATSLTTYSNTST
Balanoglossus_simodensis_BAH96      YNQT------------
HYSSAAAATINRGGYLESQCATATSLTSYNNSSP
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Saccoglossus_kowalevskii_AAP79      YN--------------
QNHYHNTSPLTRGTYLEPQCGNTTISPYNNN---
Branchiostoma_lanceolatum_ACJ7      YN--------------HYSHPGGHHMGNGY---GTNNHAA---
-MYSGSF
Ciona_intestinalis_NP_00112233      ----------------AY-SPACLNYNTST---
PYCNQLVGADLNYVHQS
Metacrinus_rotundus_BAF43721.1      HSYSN----------AVYSYNMSLNPYHADSEHAAVQEY---
PHIQRNIQ
Platynereis_dumerilii_CAD43607      -------------------------------------------
-------
Alitta_virens_AF151663_1            -------------------------------------------
-------
Strongylocentrotus_purpuratus_      LSNFST----------TAGYGDHSTTSAGY-------------
-GSMSQP
Tripneustes_gratilla_CAA31546.      -------------------------------------------
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Xenopus_laevis_NP_001079188.1       ASS-VVQHHQQHQS-------------------------
YIEGSAHYIHH
Oryctolagus_cuniculus_NP_00116      SSP-MVQHHHHHQGYAG----------------------
GTVGSPQYIHH
Leucoraja_erinacea_ACT65756.1       ASAISPQHHSYG---------------------------
GMVRPGQYNHH
Latimeria_menadoensis_ACL81442      GSNTSSLPDGYC---------------------------
GVSGPGQYQQQ
Ptychodera_flava_AAR07634.1         GHYTNHHHHHHHHHHDQ----------------------
HHHHHPQQQQP
Balanoglossus_simodensis_BAH96      GHYTNHHHHHHNHEQQQQQHH------------------
HHHQHHQQQQP
Saccoglossus_kowalevskii_AAP79      GHYTSHHDHHHHQHH----------------------------
-PQQQHS
Branchiostoma_lanceolatum_ACJ7      GAELAGSYSSYNSGMNG----------------------
TVAPPPLDSQY
Ciona_intestinalis_NP_00112233      YSI-NESYPTSNPLYTS----------------------
TFSNNSLYSQY
Metacrinus_rotundus_BAF43721.1      LQSATCQAVYTSRDS------------------------
NVNGRDTTGQV
Platynereis_dumerilii_CAD43607      -------------------------------------------
------P
Alitta_virens_AF151663_1            -------------------------------------------
-------
Strongylocentrotus_purpuratus_      ISPTSAWDSRMAAT-------------------------
YNSASWGSTAA
Tripneustes_gratilla_CAA31546.      -------------------------------------------
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Xenopus_laevis_NP_001079188.1       SYGPD----------HNIS------------
VANYNNNNVSSLHS-HREV
Oryctolagus_cuniculus_NP_00116      SYGQE----------HQSL-----------ALATY-
NNPLSPLHASHQEA
Leucoraja_erinacea_ACT65756.1       PYRQE----------QQGL------------
VLAAGCHPLSPAPGSHQES
Latimeria_menadoensis_ACL81442      PYPHE----------HQGF------------
LQGTYTNASSPQAGEKETT
Ptychodera_flava_AAR07634.1         PHSHH----------HPQQQQQHQL-DSINHNNADDSPD-
NPHLNQSDKD
Balanoglossus_simodensis_BAH96      THHHH----------HHPL-------HDVNHSTAEDSPD-
NSHLNQSDKE
Saccoglossus_kowalevskii_AAP79      QPSHH----------HLEV--------
SPNHSVADGSPEPSQHNGNSDKE
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Branchiostoma_lanceolatum_ACJ7      GYMHH----------HTGQ------------------------
DPMISTS
Ciona_intestinalis_NP_00112233      NPCIL----------GPNVSRDETN--
DSVINSCGVQDRISPPNSAYDTP
Metacrinus_rotundus_BAF43721.1      PVPES----------NGTT-------------
SAMGIDRDSPPPTLTNLD
Platynereis_dumerilii_CAD43607      PHGHH--------------------------------------
-MNMGNG
Alitta_virens_AF151663_1            -------------------------------------------
-------
Strongylocentrotus_purpuratus_      ELGDGS---------YRGR---------
VSALTAGTGCLVSAAAEPNNNH
Tripneustes_gratilla_CAA31546.      -------------------------------------------
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Xenopus_laevis_NP_001079188.1       CRSPASETSPG--------------PTQTFDWMKVKRNPP---
-----KT
Oryctolagus_cuniculus_NP_00116      CRSPALETSS---------------PAQTFDWMKVKRNPP---
-----KT
Leucoraja_erinacea_ACT65756.1       CCSPSAETSP---------------PAQTFEWMKVKRNPP---
-----KT
Latimeria_menadoensis_ACL81442      CQSEQTS------------------TGQTFEWMKVKRNPP---
-----KT
Ptychodera_flava_AAR07634.1         CDDHKSSG-----------------ETTVYNWMKVKRNPP---
-----KT
Balanoglossus_simodensis_BAH96      CDDQKSTG-----------------ETTVYNWMKVKRNPP---
-----KT
Saccoglossus_kowalevskii_AAP79      SSDPKSGGE----------------TPAMYNWMKIKRNPP---
-----KT
Branchiostoma_lanceolatum_ACJ7      CNPPAPSP-----------------PVATYDWMKLKRNPP---
-----RT
Ciona_intestinalis_NP_00112233      TASPQCTNTL---------------PSNTYDWMKIKRNPP---
-----KS
Metacrinus_rotundus_BAF43721.1      HQHPSG-------------------EAAIYSWMKVKRNPP---
-----KT
Platynereis_dumerilii_CAD43607      GQDQA--------------------
PVTTYKWMTVKRSTAQKRTQGYTQS
Alitta_virens_AF151663_1            -------------------------------------------
-------
Strongylocentrotus_purpuratus_      CSQVMSPCK----------------STSGYPWMPV--SGP---
-----NV
Tripneustes_gratilla_CAA31546.      ---------------------------------------P---
-----NV


Xenopus_laevis_NP_001079188.1       -----GKAGEYGYV-
GQPNTARTNFTTKQLTELEKEFHFNKYLTRARRVE
Oryctolagus_cuniculus_NP_00116      -----GKVGEYGYV-
GQPNSVRTNFTTKQLTELEKEFHFNKYLTRARRVE
Leucoraja_erinacea_ACT65756.1       -----GKAGEYGFA-
GQPNTVRTNFTTKQLTELEKEFHFNKYLTRARRVE
Latimeria_menadoensis_ACL81442      -----AKVAEYGVN-
GQQNTIRTNFTTKQLTELEKEFHFNKYLTRARRVE
Ptychodera_flava_AAR07634.1         -----GKSGEYGFT-
GSPNNGRTNFTNKQLTELEKEFHFNKYLTRARRVE
Balanoglossus_simodensis_BAH96      -----GKSGEYGFT-
GSPNNGRTNFTNKQLTELEKEFHFNKYLTRARRVE
Saccoglossus_kowalevskii_AAP79      -----GKSGEYGFT-
GSPANGRTNFTNKQLTELEKEFHFNKYLTRARRVE
Branchiostoma_lanceolatum_ACJ7      -----
GKPGEYGFTTSGPNNGRTNFTTKQLTELEKEFHYNKYLTRARRVE
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Ciona_intestinalis_NP_00112233      IYFSQNKMVEYTYG-
VTGNNGRTNFTTKQLTELEKEFHFNKYLTRARRVE
Metacrinus_rotundus_BAF43721.1      ----VKPANDFGTI-
SANNNGRTNFTNKQLTELEKEFHFNKYLTRARRVE
Platynereis_dumerilii_CAD43607
PWGGKVKPGEFTYTPGQPNMGRTNFTNKQLTELEKEFHFNKYLTRARRIE
Alitta_virens_AF151663_1            ------
KPGEFTYTPGQPNMGRTNFTNKQLTELEKEFHFNKYLTRARRIE
Strongylocentrotus_purpuratus_      -----------GLE-
VGRKRCRQTYTRYQTLELEKEFHFNRYLTRRRRIE
Tripneustes_gratilla_CAA31546.      -----------GLE-
VGRKRCRQTYTRYQTLELEKEFHFNRYLTRRRRIE
                                                         * .:*  *
*******:*:**** **:*

Xenopus_laevis_NP_001079188.1       IAAALQLNETQVKIWFQNRRMKQKKREKE--
GHLPISPSASTGSDEKSEE
Oryctolagus_cuniculus_NP_00116      IAASLQLNETQVKIWFQNRRMKQKKREKE--
GLLPISPATPPGSEEKAEE
Leucoraja_erinacea_ACT65756.1       IAAALQLNETQVKIWFQNRRMKQKKREKE--GLTSASPATPG-
SEANTED
Latimeria_menadoensis_ACL81442      IAATLELNETQVKIWFQNRRMKQKKRERE-----
GITSTFPNISTKEAGE
Ptychodera_flava_AAR07634.1         IAAMLGLNETQVKIWFQNRRMKQKKRYKE--------
PAFGQGINSMIGG
Balanoglossus_simodensis_BAH96      IAAMLGLNETQVKIWFQNRRMKQKKRYKE--------
PTFGQGITSMLGG
Saccoglossus_kowalevskii_AAP79      IAAMLGLNETQVKIWFQNRRMKQKKRFKD-------
GPSYSHSINDALGG
Branchiostoma_lanceolatum_ACJ7      IAAALNLNETQVKIWFQNRRMKQKKREKE------------
NGFSTPGSG
Ciona_intestinalis_NP_00112233      IAAALRLNETQVKIWFQNRRMKQKKRDKE--
AEKLNGNKMSQSKSETLHG
Metacrinus_rotundus_BAF43721.1      IASQLGLNETQVKIWFQNRRMKEKKKMKE-------------
CISSIHIS
Platynereis_dumerilii_CAD43607      IAAALGLNETQVKIWFQNRRMKQKKRMKE------------
TNVSPTTNG
Alitta_virens_AF151663_1            IAAALGLNETQVKIWFQNRRMKQKKRMKE------------
TNVSPTTNG
Strongylocentrotus_purpuratus_      LSHLLGLTERQIKIWFQNRRMKYKKESKN----------
KEEGGSGEGEG
Tripneustes_gratilla_CAA31546.      LSHLLGLTERQIKIWFQNRRMKYKKESKN----------
KEEGVSGEGDG
                                    ::  * *.* *:********** **. ::

Xenopus_laevis_NP_001079188.1       LSEKSNSSPCAPSPASSTSDHLSASG-----------------
-------
Oryctolagus_cuniculus_NP_00116      SSEKSSSSPCVPSPGSSTSDTLPTSH-----------------
-------
Leucoraja_erinacea_ACT65756.1       TSDKSSSTSSTPSPASSTSETLNTSG-----------------
-------
Latimeria_menadoensis_ACL81442      ASDQSNSTSPDASPNSATS------------------------
-------
Ptychodera_flava_AAR07634.1         KNADIVSITSCPT------------------------------
-------
Balanoglossus_simodensis_BAH96      KNSDIVSITSCPT------------------------------
-------
Saccoglossus_kowalevskii_AAP79      KNADTVGVTVSTS------------------------------
-------
Branchiostoma_lanceolatum_ACJ7      GSPAGEDSPSKST------------------------------
-------
Ciona_intestinalis_NP_00112233
LKQTSTSSSTNPMYLASSSSNKSTGVTNYNSLMLSHQFQKPAAEADMNIV
```

```
Metacrinus_rotundus_BAF43721.1      GSPELKSHLVYTS------------------------------
-------
Platynereis_dumerilii_CAD43607      STENSEHSIASDDNS----------------------------
-------
Alitta_virens_AF151663_1            STENSEHSIASDDNS----------------------------
-------
Strongylocentrotus_purpuratus_
ENESESTGTENAQPQSAVHGVTILEKPSSLVLHVDDTVGLNAVRHT----
Tripneustes_gratilla_CAA31546.
ENETESTGTENAQTQNAVHGVTILEKPSSLVLHVDDTIALNTVRHS----
                                    .

Xenopus_laevis_NP_001079188.1       ---------
Oryctolagus_cuniculus_NP_00116      ---------
Leucoraja_erinacea_ACT65756.1       ---------
Latimeria_menadoensis_ACL81442      ---------
Ptychodera_flava_AAR07634.1         ---------
Balanoglossus_simodensis_BAH96      ---------
Saccoglossus_kowalevskii_AAP79      ---------
Branchiostoma_lanceolatum_ACJ7      ---------
Ciona_intestinalis_NP_00112233      T--------
Metacrinus_rotundus_BAF43721.1      ---------
Platynereis_dumerilii_CAD43607      ---------
Alitta_virens_AF151663_1            ---------
Strongylocentrotus_purpuratus_      ---------
Tripneustes_gratilla_CAA31546.      ---------
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S1-Figure 2: Names and phylum from phylogenetic tree (Results Figure 2).

	Scientific Name	**Phylum**
1	*Alitta Virens*	Annelid Worm
2	*Platynereis dumerilii*	Annelid worm
3	*Tripneustes gratilla*	Echinoderm
4	*Strongylocentrotus purpuratus*	Echinoderm
5	*Oryctolagus cuniculus*	Vertebrate
6	*Xenopus laevis*	Vertebrate
7	*Leucoraja erinacea*	Vertebrate
8	*Latimeria menadoensis*	Vertebrate
9	*Branchiostoma lanceolatum*	Lancelet
10	*Ciona intestinalis*	Lancelet
11	*Saccoglossus kowalevskii*	Hemichordate
12	*Balanoglossus simodensis*	Hemichordate
13	*Ptychodera flava*	Hemichordate