

Supplementary data:

Domain combination of the vertebrate-like TLR gene family: implications for their origin and evolution

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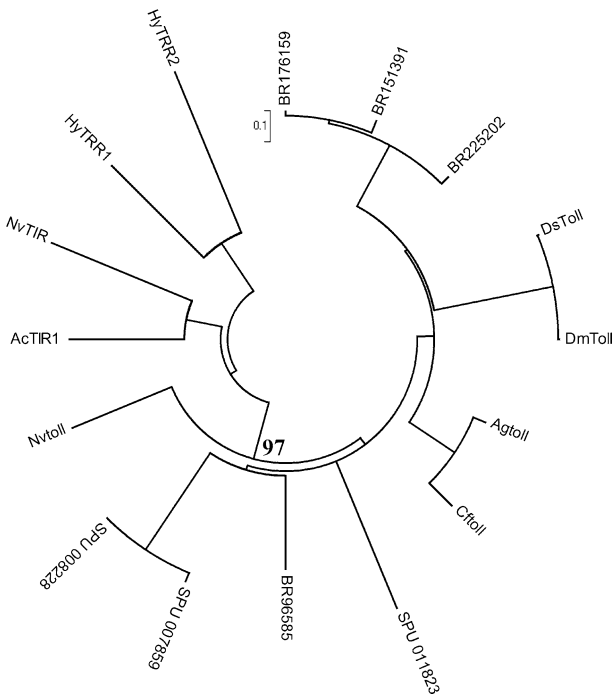


Figure 1. Unrooted NJ phylogenetic tree of P-Toll proteins from selected phyla based on TIR domains. The putative V-TIR protein from Cnidaria was used as an outgroup. The accession IDs are listed in table 2 of [electronic supplementary material](#). SPU, *Strongylocentrotus purpuratus*; Br, *Branchiostoma floridae*; Ds, *Drosophila simulans*; Dm, *Drosophila melanogaster*; Nv, *Nematostella vectensis*; Cf, *Chlamys farreri*; Ag, *Anopheles gambiae*; Ac, *Acropora millepora*; Hy, *Hydra magnipapillata*; Nv, *Nematostella vectensis*.

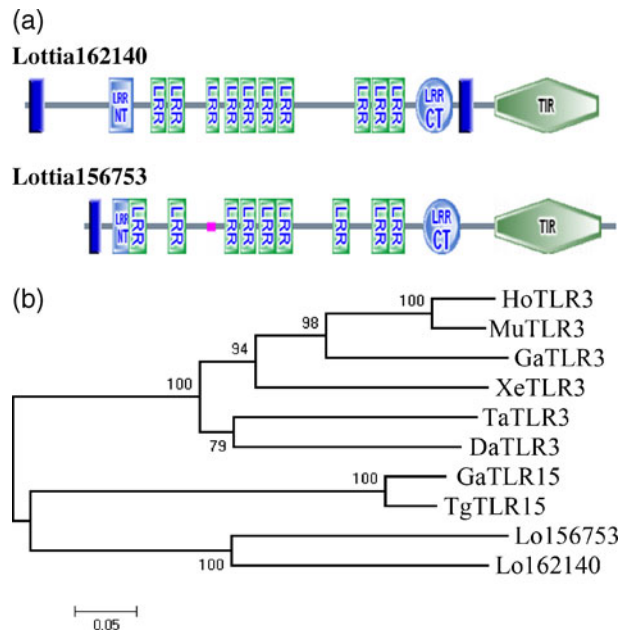


Figure 2. (a) Structures of the predicted *Lottia gigantean* TLR proteins. Domains in the protein were predicted by the SMART program. LRR-NT, LRR, LRR-CT and TIR domains are indicated in the picture. Blue-filled rectangle is a transmembrane domain. (b) Unrooted NJ phylogenetic tree of *Lottia gigantean* TLR15 based on TIR domains. Ho, *Homo sapiens*; Mu, *Mus musculus*; Ga, *Gallus gallus*; Xe, *Xenopus tropicalis*; Da, *Danio rerio*; Ta, *Takifugu rubripes*; Tg, *Taeniopygia guttata*.

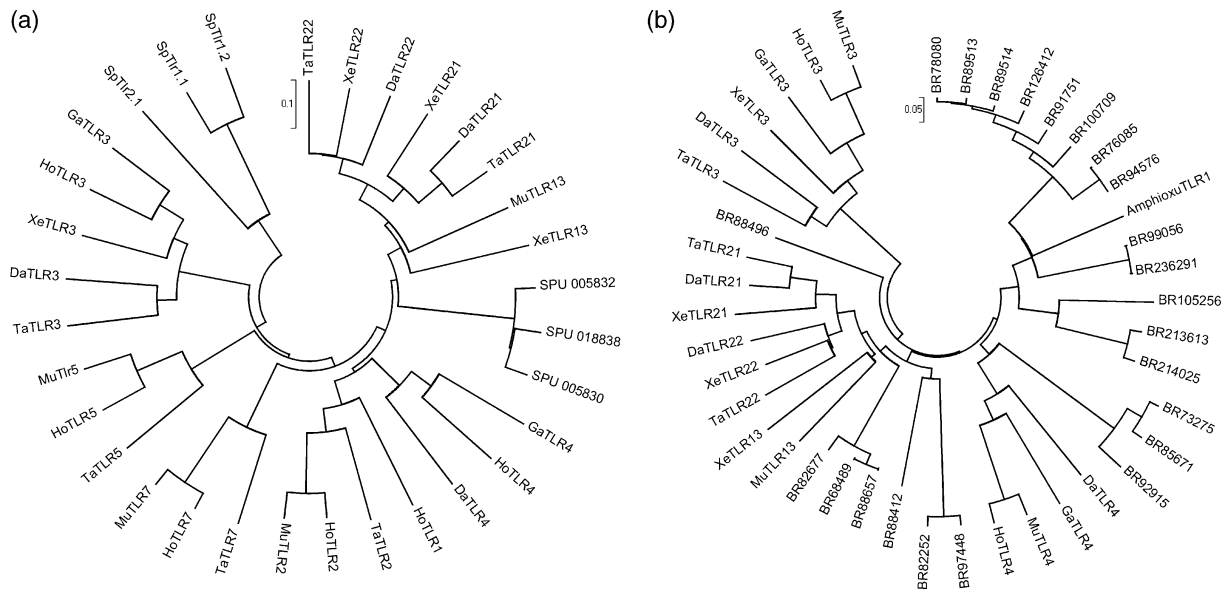


Figure 3. (a) Unrooted NJ phylogenetic trees revealing the evolutionary relationship between V-TLR of *Strongylocentrotus purpuratus* and vertebrate TLRs. (b) Unrooted NJ phylogenetic trees revealing the evolutionary relationship between V-TLR of *Branchiostoma floridae* and V-TLRs. All *Branchiostoma floridae* TLRs and TIRns are labelled with Br; *Strongylocentrotus purpuratus* TLRs and TIRns with Sp or SPU. The database accession IDs of these sequences are listed in table 2 of [electronic supplementary material](#).

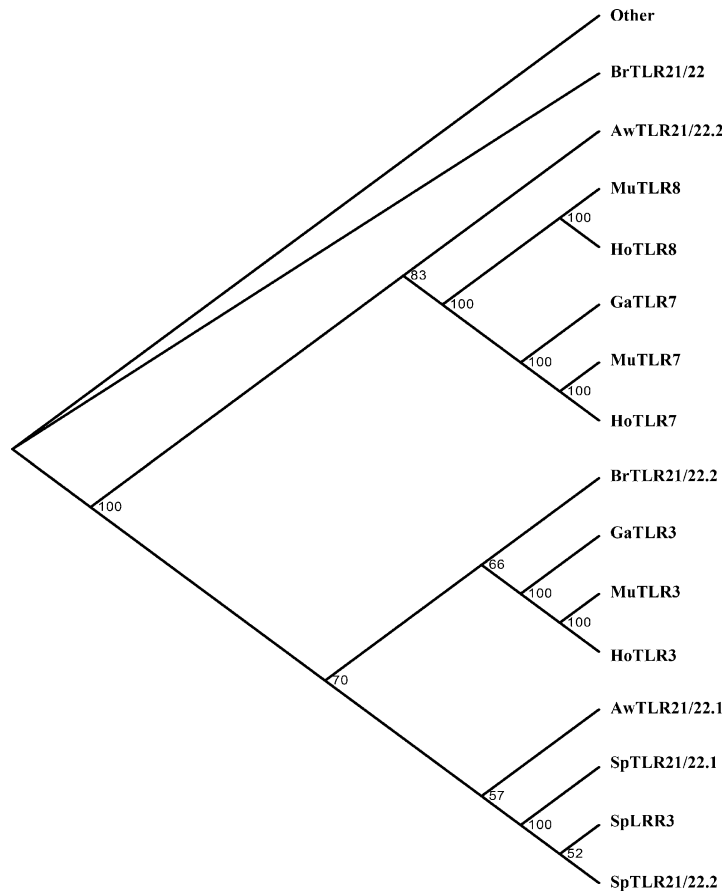


Figure 4. Unrooted ML phylogenetic tree of LRRn proteins based on LRR domains. Abbreviations are detailed in figure legend of [electronic supplementary material](#) figures 1 and 3.

Table 1. Database accession numbers of V-TLRs, V-TIRs, V-LRRs and P-Tolls from three basal deuterostomes.**Sea urchin, *Strongylocentrotus purpuratus* (NCBI)**

The TIR-only proteins are similar to TIR domains of SpTLRs.

Tlr1.1gi|72144119|ref|XP_789453.1|
 Tlr1.1gi|115655494|ref|XP_792653.2|
 Tlr1.1gi|115662465|ref|XP_001203683.1|
 Tlr1.1gi|115666630|ref|XP_001184021.1|
 Tlr1.1gi|115687984|ref|XP_001198591.1|
 Tlr1.1gi|115692339|ref|XP_001198327.1|
 Tlr1.1gi|115903706|ref|XP_799277.2|
 Tlr1.1gi|115644878|ref|XP_793575.2|
 Tlr1.1gi|115786374|ref|XP_796597.2|
 Tlr1.1gi|72011835 |ref|XP_783612.1|
 Tlr1.1gi|72046494|ref|XP_794773.1|
 Tlr1.1gi|72053023|ref|XP_799228.1|
 Tlr1.1gi|72081403|ref|XP_784221.1|
 Tlr1.1gi|115923327|ref|XP_001175608.1|
 Tlr1.1gi|115623255|ref|XP_001187560.1|
 Tlr1.1gi|115946727|ref|XP_001196733.1|
 Tlr1.1gi|115939085|ref|XP_001194205.1|
 Tlr1.1gi|115935113|ref|XP_001196567.1|
 Tlr1.1gi|115971342|ref|XP_001204468.1|
 Tlr1.1gi|115946733|ref|XP_001196810.1|
 Tlr1.1gi|115617875|ref|XP_001201037.1|
 Tlr1.1gi|115616977|ref|XP_001203115.1|
 Tlr1.1gi|115632194|ref|XP_001203368.1|
 Tlr1.1gi|115636026|ref|XP_001204423.1|
 Tlr1.1gi|115719114|ref|XP_001204024.1|
 Tlr1.1gi|115941541|ref|XP_001204488.1|
 Tlr1.1gi|115960259|ref|XP_001193119.1|
 Tlr1.1gi|115967866|ref|XP_001182822.1|
 Tlr1.1gi|115645632|ref|XP_001201924.1|
 Tlr1.1gi|115730125|ref|XP_001200908.1|
 Tlr1.2gi|72200768|ref|XP_782019.1|
 Tlr1.2gi|115715998|ref|XP_001199161.1|
 Tlr1.2gi|115710047|ref|XP_784915.2|
 Tlr1.2gi|115676874|ref|XP_784422.2|
 Tlr1.2gi|72138081|ref |XP_787073.1|
 Tlr1.2gi|72132341 |ref|XP_788291.1|
 Tlr1.2gi|115683689|ref|XP_791546.2|
 Tlr1.2gi|115760453|ref|XP_793544.2|
 Tlr1.2gi|72042738 |ref|XP_795086.1|
 Tlr1.2gi|115903698|ref|XP_001179202.1|
 Tlr1.2gi|115946329|ref|XP_001181859.1|
 Tlr1.2gi|115945375|ref|XP_001189457.1|
 Tlr1.2gi|115940626|ref|XP_001190191.1|
 Tlr1.2gi|115655242|ref|XP_001191129.1|
 Tlr1.2gi|115934978|ref|XP_001192009.1|
 Tlr1.2gi|115936749|ref|XP_001192817.1|
 Tlr1.2gi|115945053|ref|XP_001197670.1|
 Tlr1.2gi|115751056|ref|XP_001203570.1|
 Tlr1.2gi|115949634|ref|XP_001181423.1|
 Tlr1.2gi|115971334|ref|XP_001189255.1|
 Tlr1.2gi|115746526|ref|XP_788018.2|
 Tlr1.2gi|72159339|ref|XP_794559.1|
 Tlr1.2gi|72086600|ref|XP_791054.1|
 Tlr1.2gi|72115183|ref|XP_790668.1|
 Tlr1.2gi|115770630|ref|XP_793041.2|
 Tlr1.2gi|115967611|ref|XP_001190326.1|
 Tlr1.2gi|72005503|ref|XP_787551.1|
 Tlr1.2gi|115926925|ref|XP_001176051.1|
 Tlr1.2gi|115930332|ref|XP_001187412.1|
 Tlr1.2gi|115964182|ref|XP_001182113.1|
 Tlr1.2gi|115959329|ref|XP_001177860.1|

Table 1 (*contd.*)

The TIR-only proteins are similar to TIR domains of SpTLRs.

Tlr1.2gi|115959388|ref|XP_001180647.1|
 Tlr1.2gi|115723540|ref|XP_001200879.1|
 Tlr1.2gi|115974312|ref|XP_001180448.1|
 Tlr1.2gi|115638598|ref|XP_780143.2|
 Tlr1.2gi|115638694|ref|XP_001185401.1|
 Tlr1.2gi|115645575|ref|XP_001201720.1|
 Tlr2.1gi|72088004|ref|XP_786124.1|
 Tlr2.1gi|115842569|ref|XP_789945.2|
 Tlr2.1gi|72135673|ref|XP_790275.1|
 Tlr2.1gi|115676802|ref|XP_001179919.1|
 Tlr2.1gi|115928802|ref|XP_001188223.1|
 Tlr2.1gi|115657970|ref|XP_001182416.1|
 Tlr2.1gi|115951654|ref|XP_001194434.1|
 Tlr2.1gi|115955390|ref|XP_001194826.1|
 Tlr2.1gi|115621232|ref|XP_001196589.1|
 Tlr2.1gi|115955431|ref|XP_001177057.1|
 Tlr2.1gi|115609937|ref|XP_001189838.1|
 Tlr2.1gi|115931885|ref|XP_001182891.1|
 Tlr2.1gi|115931887|ref|XP_001182977.1|
 Tlr2.1gi|115942255|ref|XP_001189820.1|
 Tlr2.1gi|115955392|ref|XP_001194857.1|
 Tlr2.1gi|115677830|ref|XP_001204013.1|
 Tlr2.1gi|115653990|ref|XP_001201897.1|
 Tlr2.1gi|115657936|ref|XP_791697.2|
 Tlr2.1gi|115657938|ref|XP_001181167.1|
 Tlr2.1gi|115943842|ref|XP_001196070.1|
 Tlr2.1gi|115676804|ref|XP_796118.2|
 Tlr2.1gi|115678824|ref|XP_001198879.1|
 Tlr2.1gi|115682270|ref|XP_001199462.1|
 Tlr2.1gi|115720046|ref|XP_001199600.1|
 Tlr2.1gi|115721661|ref|XP_001197270.1|
 Tlr2.1gi|115701802|ref|XP_787628.2|
 Tlr2.1gi|115711284|ref|XP_001203467.1|
 Tlr2.1gi|115760394|ref|XP_781893.2|
 Tlr2.1gi|115760540|ref|XP_001177955.1|
 Tlr2.1gi|115815535|ref|XP_797281.2|
 Tlr2.1gi|115904035|ref|XP_789060.2|
 Tlr2.1gi|115926676|ref|XP_001188567.1|
 Tlr2.1gi|115959824|ref|XP_001194737.1|
 Tlr2.1gi|72155525|ref|XP_794606.1|
 Tlr2.1gi|115965197|ref|XP_001180872.1|
 Tlr2.1gi|72140505|ref|XP_797156.1|
 Tlr2.1gi|72076551|ref|XP_797924.1|
 Tlr2.1gi|72175030|ref|XP_795253.1|

The LRR-only proteins are similar to LRR domains of V-TLRs.

SPU_005831 (LRR-only) similar to Gallus TLR3
 gi|115970589|ref|XP_001181920.1|similar to toll-like receptor Tlr2.1
 gi|115741848|ref|XP_786707.2|similar to toll-like receptor Tlr1.2
 gi|115935525|ref|XP_001191564.1|similar to toll-like receptor Tlr1.2
 gi|115948328|ref|XP_001179139.1|similar to toll-like receptor Tlr1.2
 gi|115974788|ref|XP_001180486.1|similar to toll-like receptor Tlr1.2
 gi|115658880|ref|XP_001189650.1|similar to toll-like receptor Tlr1.1
 gi|115660877|ref|XP_001186010.1|similar to toll-like receptor Tlr1.1
 gi|115686080|ref|XP_001199005.1|similar to toll-like receptor Tlr1.1

Acorn worm, *Saccoglossus kowalevskii* (NCBI)

The TIR-only proteins are similar to TIR domains of vertebrate TLRs.

TIR6 XP_002732630.1
 TIR2 XP_002741808.1
 Toll P08953.1

Table 1 (contd.)

Amphioxus, <i>Branchiostoma floridae</i> (JGI)		
The TIR-only proteins are similar to TIR domain of vertebrate TLR2. We name them BrTIR2.		
H, Human	M, mouse	
Amphioxus JGI ID	Similar species	Identity to TLR2 TIR
87577	H	48%
87578	M	41%
87584	M	45%
89683	H	50%
91177	M	43%
The TIR-only proteins are similar to TIR domain of TLR14. We named it as BrTIR14.		
84739		TLR14
The TIR-only proteins are similar to TIR domain of vertebrate TLR22. We named them as BrTIR21/22. D, <i>Danio rerio</i> ; A, amphioxus		
Amphioxus JGI ID	Identity to DTLR22 TIR	Identity to ATLR22 TIR
57373	54%	60%
61417	54%	60%
The TIR-only proteins are similar to TIR domain of Toll.		
96585		Toll
92915		Toll
The LRR-only proteins are similar to LRR domain of vertebrate TLR.		
89518	similar to <i>Lethenteron japonicum</i> TLR14	
67184	similar to <i>Oncorhynchus mykiss</i> TLRs	
81224	similar to <i>Strongylocentrotus purpuratus</i> TLR3 precursor	
67867	similar to <i>Oncorhynchus mykiss</i> TLRs	
74180	similar to <i>Takifugu rubripes</i> TLR9	
102021	similar to <i>Strongylocentrotus purpuratus</i> TLR3 precursor	

Table 2. Database accession numbers of V-TLRs, V-TIRs, V-LRRs and P-Tolls used for phylogenetic analysis. The accession numbers of NCBI are listed. *Branchiostoma floridae* V-TLRs and V-TIRs are indicated by JGI ID.**Vertebrate TLRs (NCBI)**

Human, *Homo sapiens* TLR2 (U88878) TLR3 (U88879) TLR4 (U88880) TLR5 (NM 003268) TLR7 (NM 016562) TLR8 (NM 016610)
 Mouse, *Mus musculus* TLR2 (NM 011905.3) TLR3 (NM 126166) TLR4 (NM 021297) TLR5 (NM 016928) TLR7 (NM 133211)
 TLR8 (NM 133212)
 Chicken, *Gallus gallus* TLR2 (NM 204278) TLR3 (NM 001011691) TLR4 (NM 001030693)
 TLR5 (NM 001024586) TLR7 (NM 001011688) TLR15 (NM 001037835)
 Taeniopygia guttata TLR15 (XP_002197105.1)
 Xenopus, *Xenopus tropicalis* TLR2 (jgi320872) TLR3 (jgi271893) TLR14.1 (jgi190020) TLR21 (jgi349648) TLR22 (jgi414791)
 Zebrafish, *Danio rerio* TLR2 (NM 212812.1) TLR3 (AY616582) TLR4 (NM 001131051) TLR14 (NM 001089350)
 TLR21 (XP 001923227.1) TLR22 (XM 692565)
 Fugu, *Takifugu rubripes* TLR2 (AAW69370) TLR3 (AC156436)
 TLR14 (AC156431) TLR21 (NM 001032579) TLR22 (NM 001113193)

Cnidarian TIR genes (NCBI)

Coral, *Acropora millepora* TIR1 (ABK78770.1)
 Hydra, *Hydra magnipapillata* TRR1 (ABE26987.1) TRR2 (ABE26988.1)
 Sea anemone, *Nematostella vectensis* (XP_001623880.1)

Invertebrate deuterostomes (TLRs)

Sea urchin, *Strongylocentrotus purpuratus*-(NCBI)
 SpTLR21/22(1–3): SPU_018838 SPU_005830 SPU_005832; SpLRR3 (SPU_005831) TIR1.1
 (XP_789453.1) TIR1.2 (XP_782019.1) TIR2.1 (XP_786124.1) TLR1.1 (NP_999670.1) TLR1.2 (NP_999671.1)
 TLR2.1 (XP_001182083.1)
 Acorn worm, *Saccoglossus kowalevskii*-(NCBI)
 TLR2 (XP_002733943.1) TIR6 (XP_002732630.1) TIR 2 (XP_002741808.1) TLR21/22.1 (XP_002732314.1)
 TLR21/22.2 (XP_002732817.1)

Amphioxus, *Branchiostoma floridae*-(JGI)

TLR21/22 1–4: JGI 68489 JGI 88496 JGI 82677 JGI 88657
 TIR 2.1–2.5: JGI87577 JGI87578 JGI87584 JGI89683 JGI91177 TIR14 JGI84739 TIR21/22.1–2: JGI57373 JGI61417

Insect Toll (NCBI)

Drosophila melanogaster (NP_733166.1) *Anopheles gambiae* (AAL37901.1) *D. simulans* (EDX14589.1) *Chlamys farreri* (ABC73693.1)