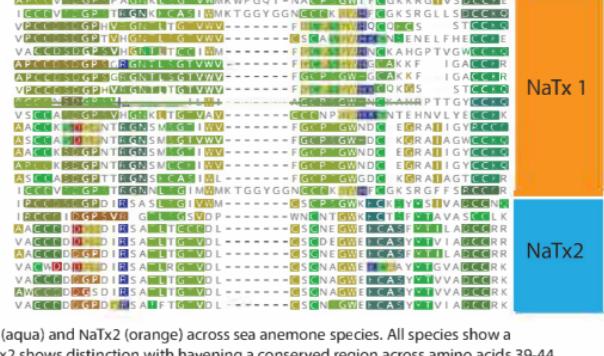


Transcriptome assembly	<ul style="list-style-type: none"> -Downloadsampling using SRA -FA STQC -De-novoassembly, Trinityv2.2 -Trimmomaticoption -BUSCO v5.3.1 Metazoa_odb10 lineage dataset -TransDecoder v5.5.0 <p>Minimum seq length of 50 amino acids</p>
Candidate toxin identification	<ul style="list-style-type: none"> -Blast+2.8.1 (e-value 0.001) -ToxProt animal venom database, keywords ("Cnidaria"), downloaded 2021 -NCBI Protein database, keywords ("Cnidaria AND ((Toxin) OR (Venom))" -HMMER 3.1b2 -Using all Cnidarian venom protein classes from VenomZone.
Filtering data	<ul style="list-style-type: none"> -Signal P V5.0 (70%)> https://services.healthtech.dtu.dk/service.php?SignalP-5.0 -CD-HIT v4.6.8 (cutoff 0.95) https://sites.google.com/view/cd-hit -Reciprocal blastsearch against a concatenated transcriptome
Annotation & Confirmation	<ul style="list-style-type: none"> -Blastp -ToxProt Animal venom database (e-value 1e-5) -NCBI non-redundant protein database (NR DB) -Hmmersearch PFAM (e-value 1e-5) -Manual curation to ensure all results matched -Assigned venom categories based on uniprot/prior research -Create alignment of select toxins

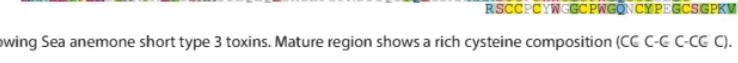
Supplementary figure s1. Annotation pipeline for project.

M. doreensis Natx1 [TRINITY_DN36294_c0_g2_i1_p1]
S. haddoni Natx1 [TRINITY_DN18025_c0_g1_i1_p1]
S. haddoni Natx1 [TRINITY_DN82418_c0_g1_i1_p1]
S. haddoni Natx1 [TRINITY_DN35483_c0_g1_i1_p1]
H crispa Natx1 [comp241777_c0_seq1_p1]
Oulactis_sp. Natx1
Oulactis_muscosa NaTx1
Stichodactyla gigantea_NaTx1
Rhodactis_sp. Natx1
Heteractis_aurora_NaTx1
Anemonia_sulcata_Natx1
Anemonia_sulcata_Natx
Anemonia_sulcata_Natx
Anemonia_viridis_Natx
Actinia_elegans_Natx
Actinia_elegans_Natx
Actinia equina_Natx
N. vectensis_Natx2
Urticina_crassicornis_Natx2
Stichodactyla_gigantea_Natx2
Thalassinithus_aster_Natx2
S.haddoni Natx2 [TRINITY_DN2118_c0_g2_i1_p1]
He_hemprichii_Natx2 [TRINITY_DN3697_c2_g1_i2_p1]
He_hemprichii_Natx2 [TRINITY_DN208_c0_g1_i10_p1]
He_hemprichii_Natx2 [TRINITY_DN6098_c0_g1_i7_p1]
C.adhaesum_Natx2 [TRINITY_DN1545_c0_g1_i6_p1]



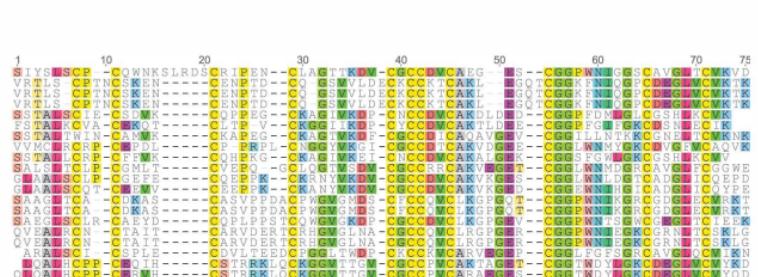
Supplementary figure s2. Alignment shows both NaTx1 (aqua) and NaTx2 (orange) across sea anemone species. All species show a conserved cysteine pattern across both NaTx types. NaTx2 shows distinction with having a conserved region across amino acids 39-44.

1. S.haddoni [TRINITY_DN5216_c0_g1_i19_p1]
2. He.hemprichii [TRINITY_DN73169_c0_g1_2_p1]
3. A.tenebrosa [STX3_ACTTE]
4. A.anemona [STX3_ANESU]



Supplementary figure s3. Alignment showing Sea anemone short type 3 toxins. Mature region shows a rich cysteine composition (CC-C-C-CG-C).

Lynchnia_mucronatus [VP302]
C.adhaesum [TRINITY_DN281_c0_g1_20_p1]
C.adhaesum [TRINITY_DN281_c0_g1_14_p1]
C.adhaesum [TRINITY_DN2381_c0_g1_i8_p1]
Hydra_vulgaris [A0AB8BX68]
Hydra_vulgaris [A0AB8D707]
Hydra_vulgaris [A0AB8D704]
Hydra_vulgaris [A0AB8BX26]
Benchistoma_belenchi [A0AEP4Z566]
Branchiostoma_belenchi [A0AEP4VYMA0]
Takifugu_flavidus [A0A5C9P8K3]
Oryzias_melastigma [A0A834FQW8]
Channa_argus [A0A6G1K41]
Pararuncinus_davidi [A0A6S7J357]
Pararuncinus_davidi [A0A6S7JM2]
Pararuncinus_davidi [A0A6S7JW8]



Supplementary figure s4. Alignment showing the insulin like toxin Venom protein 302 across all known species. Alignment shows conserved and rich cysteine region (CX5-CC CX4).

1. Ha. hemprichii [TRINITY_DN346_c0_g1_i2_p1]
2. N. vectensis [P0DQR6]



Supplementary figure s5. Alignment showing Shk-like-1 mature toxin. Alignment shows 7 conserved cystines across both samples.