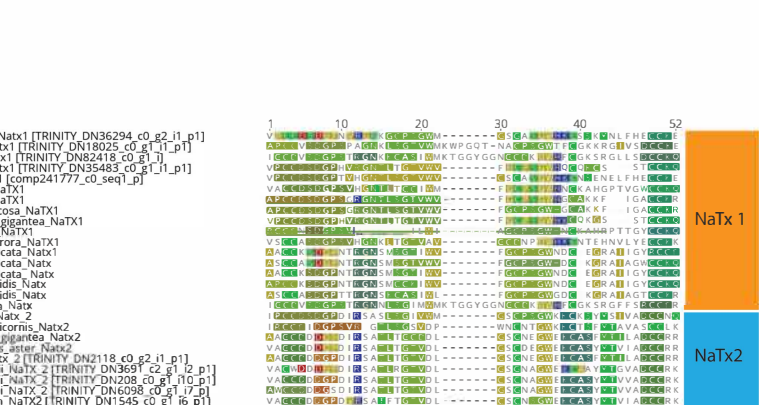
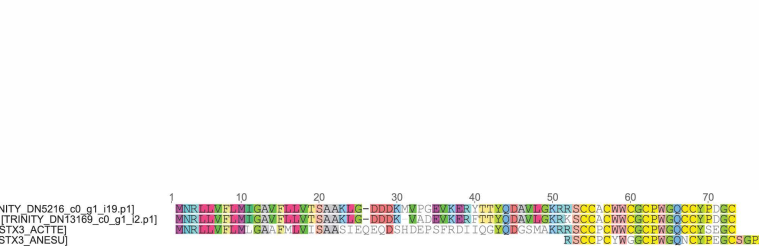


Transcriptome assembly	-Download sampling using SRA -FASTQC -De novo assembly, Trinityv2.2 -Trimmomatic -BUSCO v5.3.1 -Metazoa_odb10 lineage dataset -TransDecoder v5.5.0 Minimum seq length of 50 amino acids
Candidate toxin identification	-Blast+2.8.1 (e-value 0.001) -Tox:prot animal venom database, keywords ("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	-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	-Blastp -Tox-Prot Animal venom database (e-value 1e-5) -NCBI non-redundant protein database (NR DB) -Hmmssearch 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

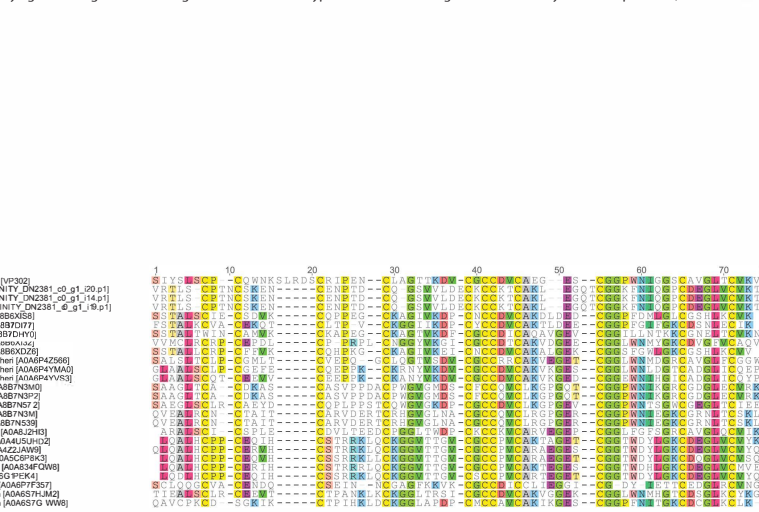
Supplementary figure s1. Annotation pipeline for project.



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.



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



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).



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