

Figure S1. Bioinformatic pipeline for the annotation of venom-like genes for four cerianthid transcriptomes.

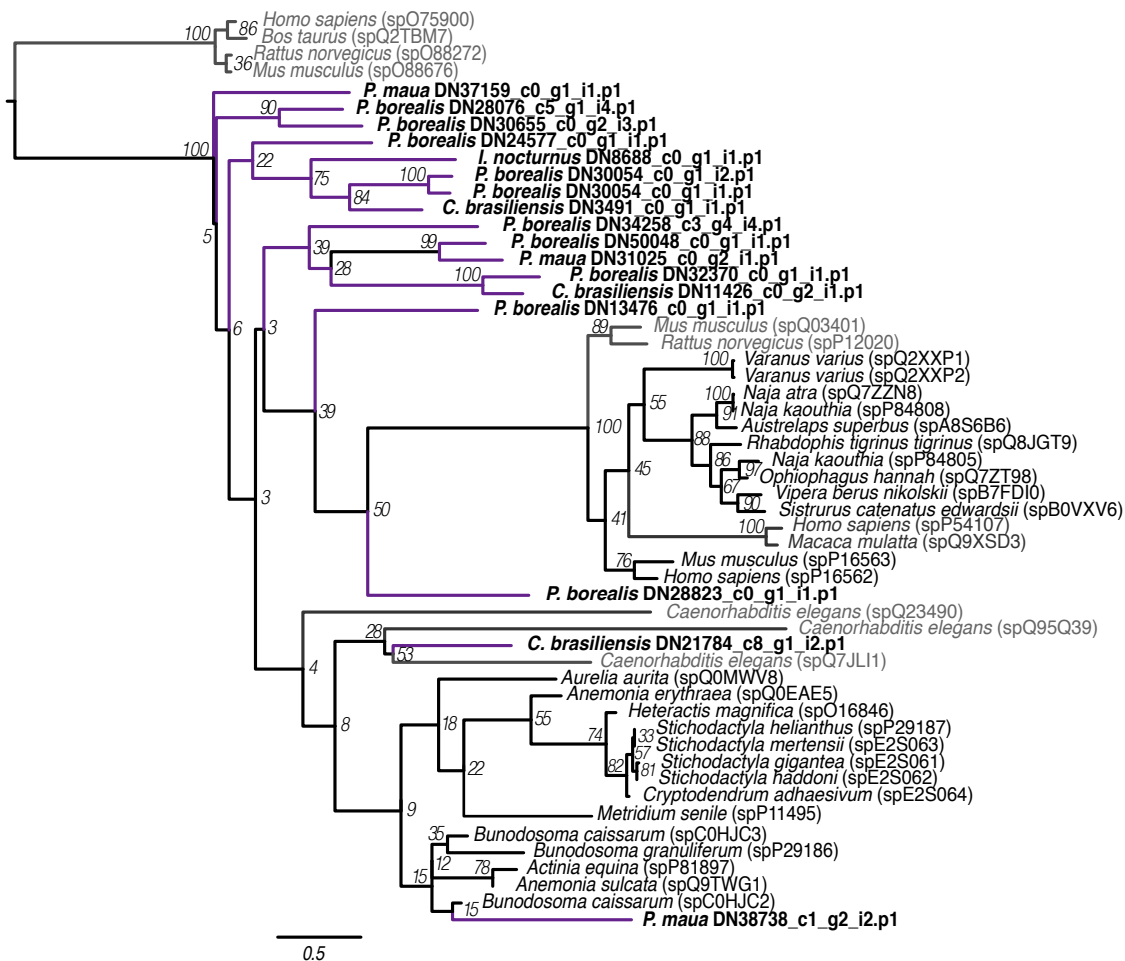


Figure S2. Phylogenetic gene tree of cystine-rich protein family sequences. The cysteine-rich protein gene tree was constructed using RAxML with the WAG+G model. Bootstrap support based on 500 rapid bootstrap replicates, and all support values are shown. Sequence names in bold with purple branches are those from cerianthids. Sequences in gray are non-venomous representatives. Phylogeny modified from [81].

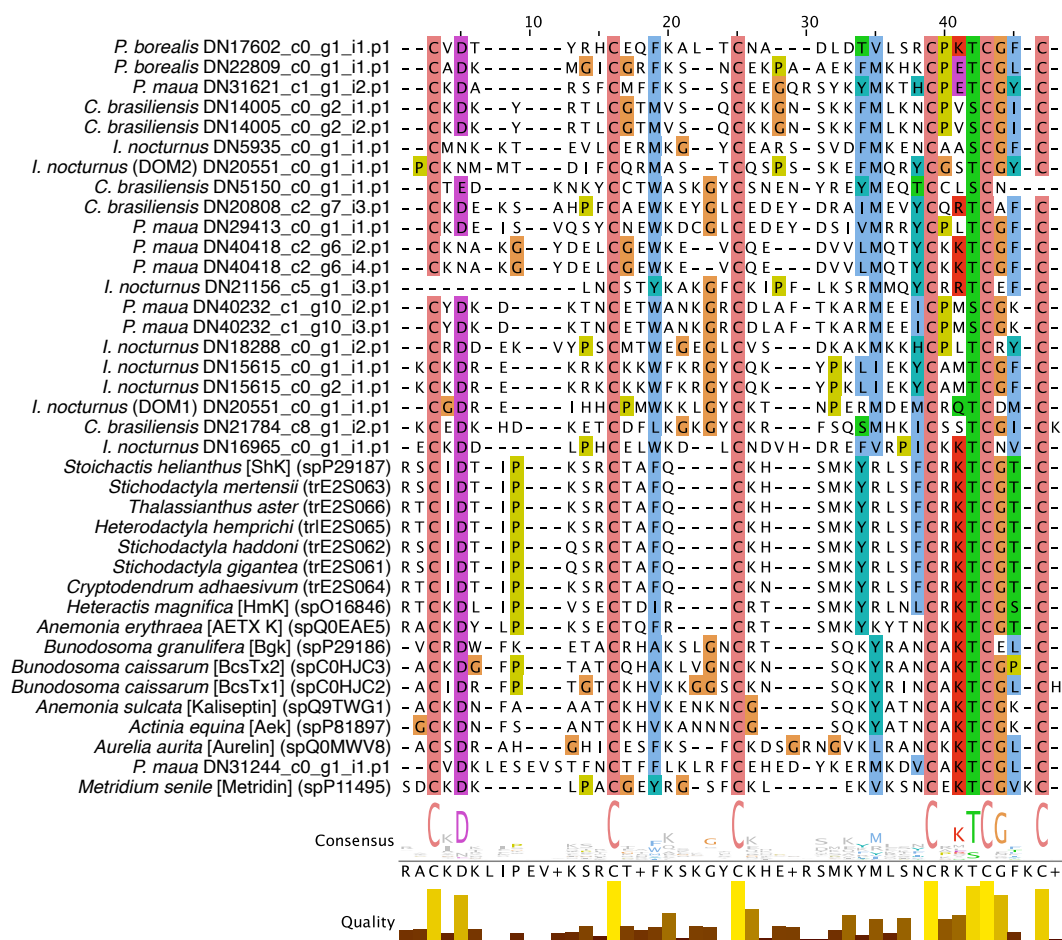


Figure S3. Multiple sequence alignment of ShK domains in cerianthid venom-like genes and other toxin representatives. Sequences alignment constructed using the L-INS-I algorithm in MAFFT [153] and viewed in a Clustal color scheme using Jalview v2.11.1 [155]. Six conserved cysteine residues characteristic of ShK domains are shown across all taxa. Various SHK toxin domains modified from [81].

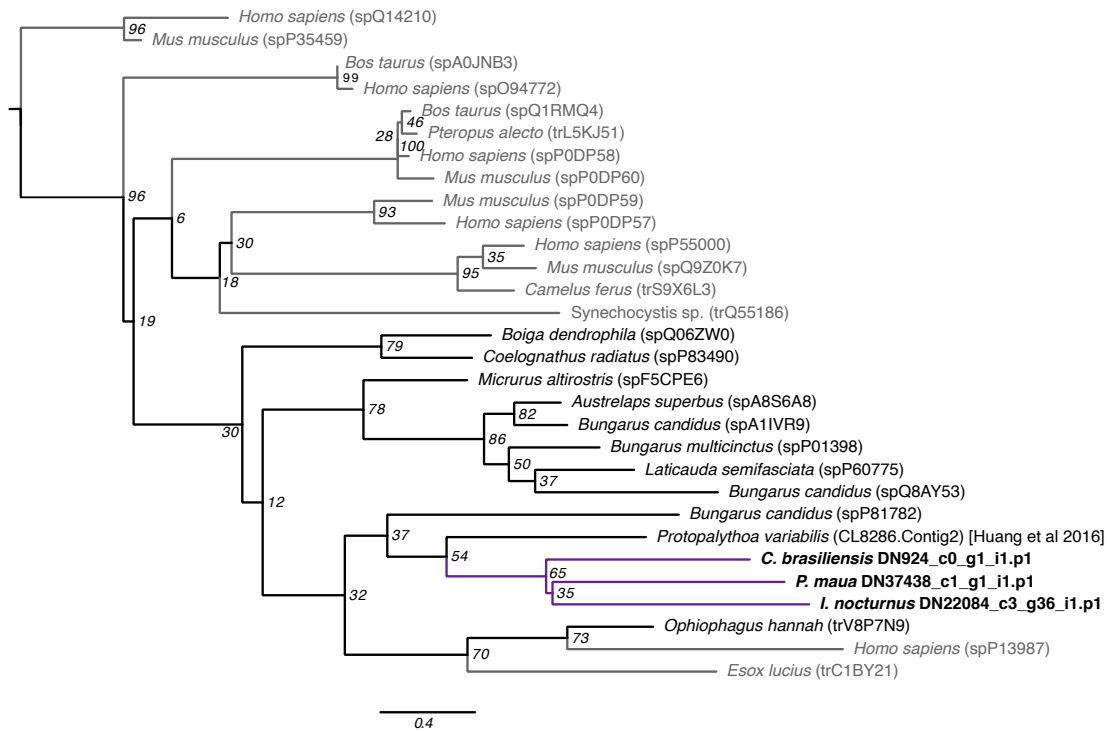


Figure S4. Phylogenetic gene tree of three-finger toxin family sequences. The three-finger gene tree was constructed using RAXML with the the WAG+I+G model. Bootstrap support based on 500 rapid bootstrap replicates, and all support values are shown. Sequence names in bold with purple branches are those from cerianthids. Sequences in gray are non-venomous representatives. Phylogeny modified from [47].

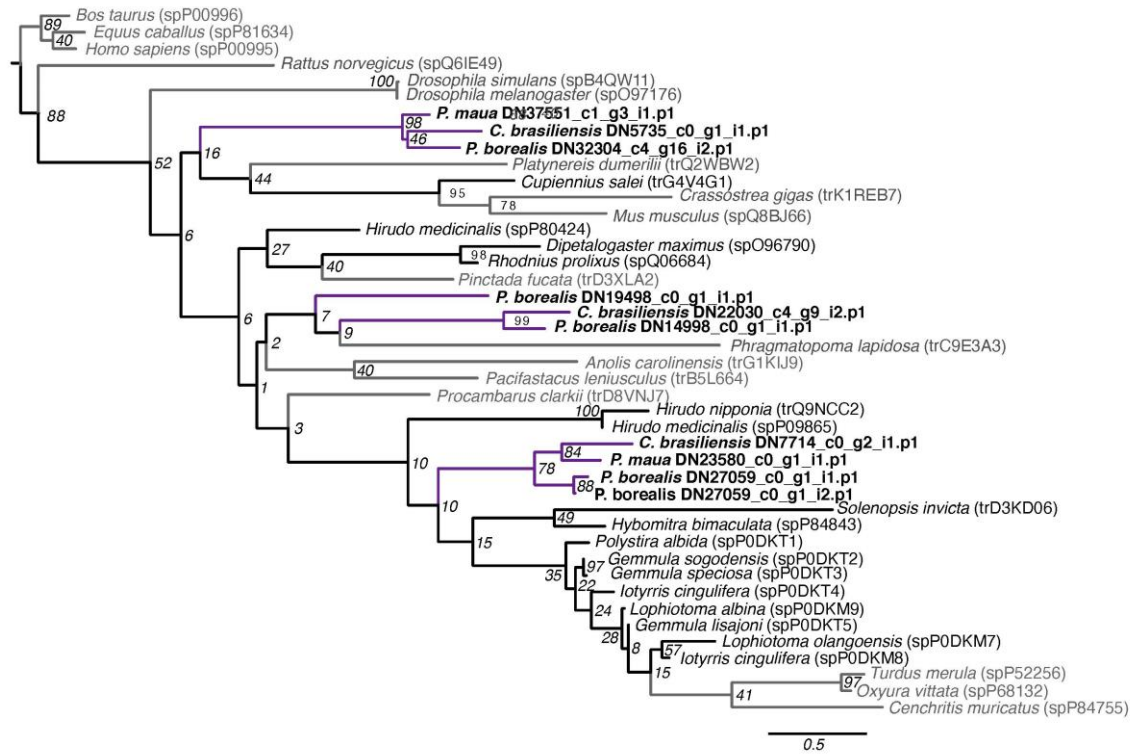


Figure S5. Phylogenetic gene tree of kazal family sequences. The kazal gene tree was constructed using RAxML with the WAG+I+G model. Bootstrap support based on 500 rapid bootstrap replicates, and all support values are shown. Sequence names in bold with purple branches are those from cerianthids. Sequences in gray are non-venomous representatives. Phylogeny modified from [81].

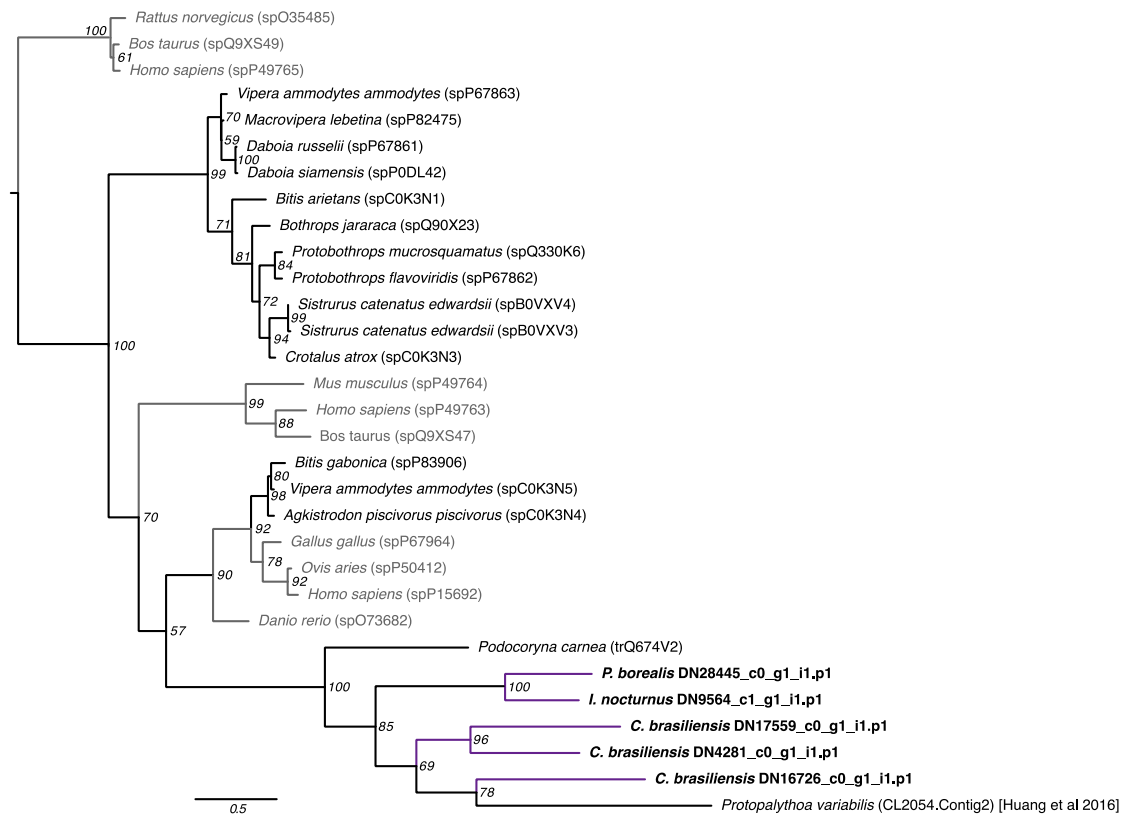


Figure S7. Phylogenetic gene tree of venom vascular endothelial growth factors (venom VEGF) family sequences. The venom VEGF gene tree was constructed using RAXML with the WAG+G model. Bootstrap support based on 500 rapid bootstrap replicates, and all support values are shown. Sequence names in bold with purple branches are those from cerianthids. Sequences in gray are non-venomous representatives. Phylogeny modified from [47].

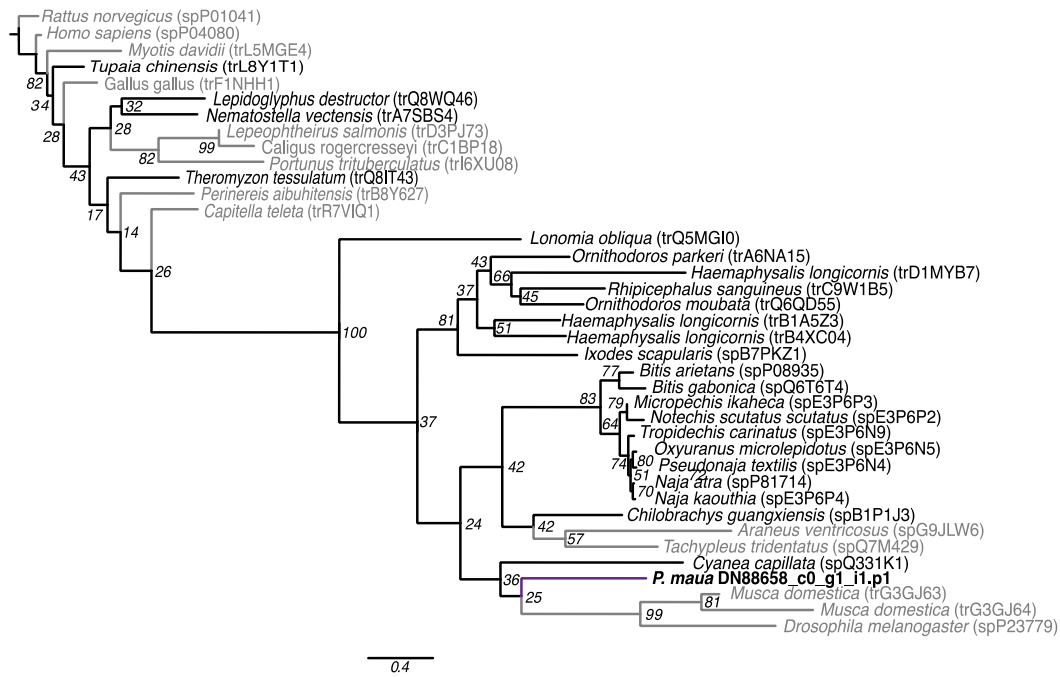


Figure S8. Phylogenetic gene tree of cystatin family sequences. The cystatin gene tree was constructed using RAxML with the WAG+I+G model. Bootstrap support based on 500 rapid bootstrap replicates, and all support values are shown. Sequence names in bold with purple branches are those from cerianthids. Sequences in gray are non-venomous representatives. Phylogeny modified from [81].

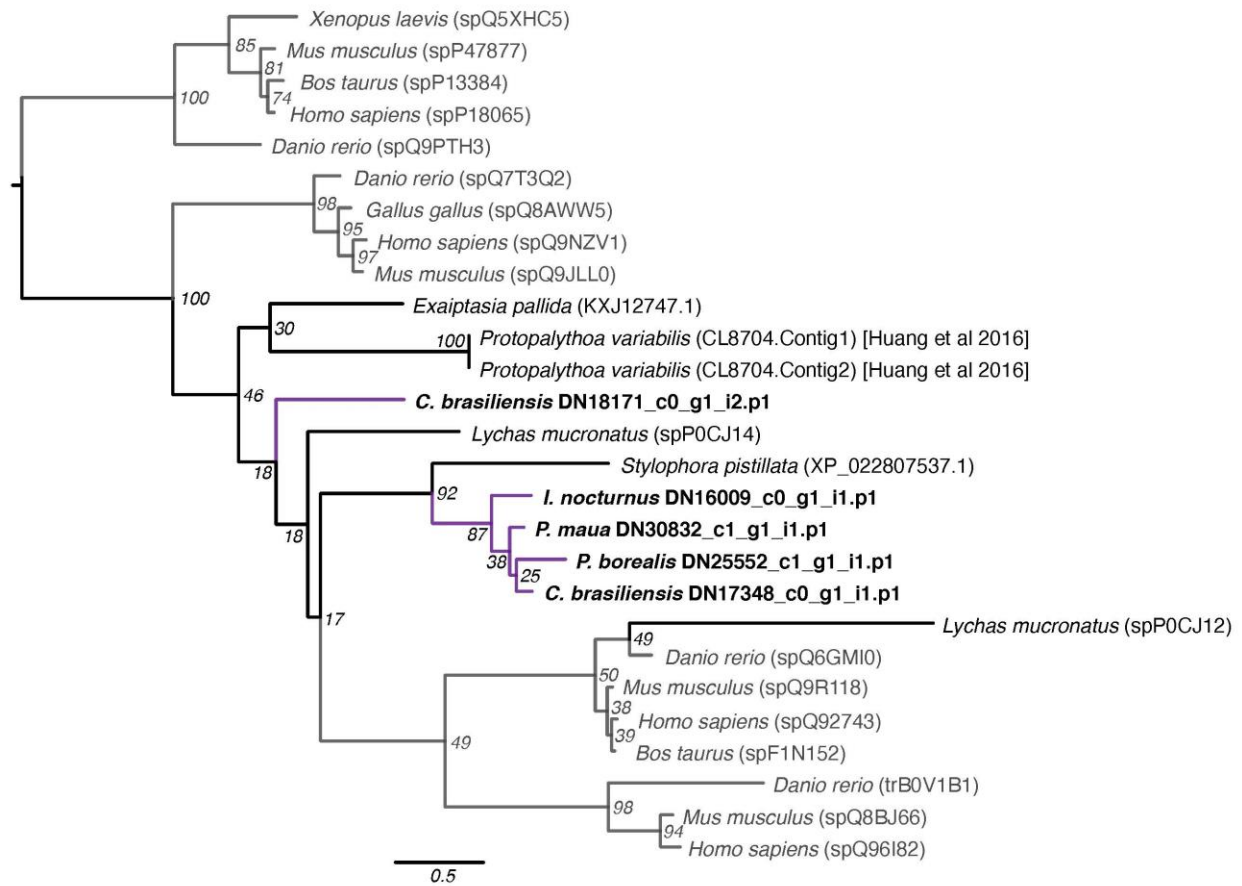


Figure S9. Phylogenetic gene tree of venom protein 302 family sequences. The venom protein 302 gene tree was constructed using RAXML with the JTT+I+G model. Bootstrap support based on 500 rapid bootstrap replicates, and all support values are shown. Sequence names in bold with purple branches are those from cerianthids. Sequences in gray are non-venomous representatives. Phylogeny modified from [47].



Figure S10. Phylogenetic gene tree of metalloprotease M12A family sequences. The metalloprotease M12A gene tree was constructed using RAxML with the WAG+I+G model. Bootstrap support based on 500 rapid bootstrap replicates, and all support values are shown. Sequence names in bold with purple branches are those from cerianthids. Sequences in gray are non-venomous representatives. Phylogeny modified from [81].

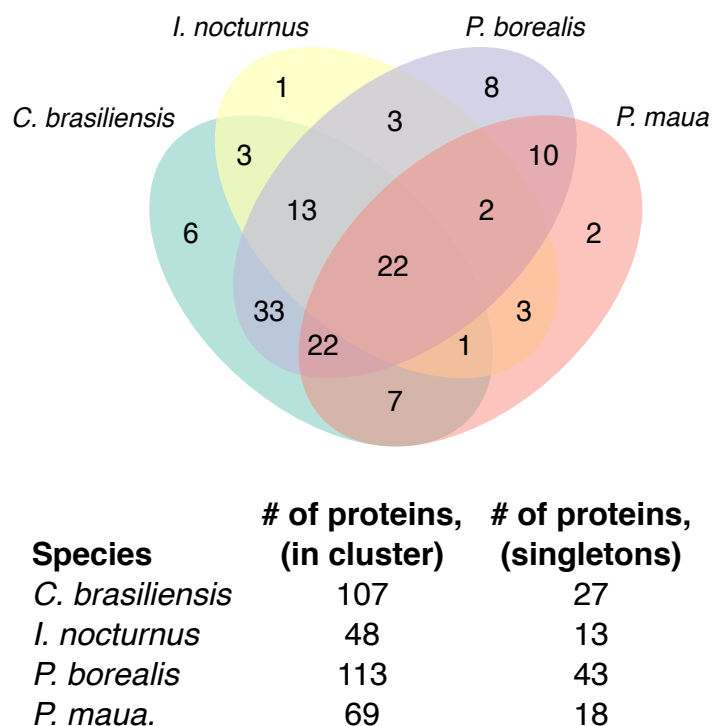


Figure S11. Orthologous gene clusters of the putative venom-like genes for all four cerianthids. Venn diagram shows overlap of gene clusters for all four species. Table shows the number of venom-like genes within each species that fall within a cluster or are singletons, which as genes that cannot be placed into a cluster (unique to each species). Gene clusters were analyzed using the OrthoVenn2 server under default setting except evalule changed to 1e-5 (<https://orthovenn2.bioinfotoolkits.net/>) [157].

Supplemental References

[157] Xu, L.; Dong, Z.; Fang, L.; Luo, Y.; Wei, Z.; Guo, H.; Zhang, G.; Gu, Y.Q.; Coleman-Derr, D.; Xia, Q.; et al. OrthoVenn2: A web server for whole-genome comparison and annotation of orthologous clusters across multiple species. *Nucleic Acids Res.* 201