

Supplementary Figures

Comparative venomomics of *C. flavidus* and *C. frigidus* and closely related vermivorous cone snails.

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| | 1 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 81 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| FLA_102_NSF1 | M | K | I | G | L | I | C | L | L | L | I | A | F | M | N | G | D | - | G | S | L | G | N | T | M | H | S | R | K | S | A | G | V | V | A | A | I | K | R | Y | Q | K | N | F | L | R | R | S | C | N | N | C | P | E | E | P | C | C | Y | G | D | Q | C | M | A | D | P | G | H | E | P | F | C | G | N | | |
| FLA_103_NSF1 | M | M | K | I | G | L | I | C | L | L | L | I | A | F | M | N | G | D | - | G | S | L | G | N | T | M | H | S | R | K | S | A | G | V | V | A | A | I | K | R | F | Q | K | N | F | L | R | R | T | C | T | N | C | P | E | E | P | C | C | Y | G | D | Q | C | M | P | D | P | G | H | E | P | F | C | G | N | |
| FLA_104-FRI_54_NSF1 | M | M | K | I | S | L | I | C | L | L | L | I | A | F | M | N | G | D | - | G | S | L | G | N | T | M | H | S | R | K | S | A | G | V | V | A | A | I | K | R | F | Q | K | N | F | L | R | R | T | C | T | N | C | P | E | E | P | C | C | Y | G | D | Q | C | M | P | D | P | G | H | E | P | F | C | G | N | |
| FLA_105_NSF2 | M | R | V | Y | L | L | T | V | T | L | L | T | S | F | T | G | A | D | A | G | P | M | P | A | N | G | H | F | Y | R | R | D | C | Q | M | G | C | V | - | - | - | - | - | G | C | H | N | R | A | G | C | C | G | D | Q | V | C | V | D | N | N | R | C | E | P | R | P | S | Q | T | | | | | | | |
| FLA_106-FRI_56_NSF2 | M | R | V | Y | L | L | T | V | T | L | L | T | S | F | T | G | A | D | A | G | P | M | P | A | N | G | H | F | Y | R | R | D | C | Q | M | G | C | V | - | - | - | - | - | G | C | R | N | R | A | G | C | C | G | D | Q | V | C | V | D | N | N | R | C | E | R | R | P | S | Q | T | | | | | | | |
| FLA_107_NSF2 | M | R | V | Y | L | L | T | V | T | L | L | T | S | F | T | G | A | D | A | G | P | M | P | A | N | G | H | F | Y | R | R | D | C | Q | M | G | C | V | - | - | - | - | - | G | C | R | N | R | A | G | C | C | G | D | Q | V | C | V | D | N | N | R | C | E | S | R | S | S | Q | T | T | R | P | T | Q | H | L |
| Consensus | m | r | v | y | l | l | t | ! | t | l | l | t | s | f | t | g | a | d | a | g | p | \$ | p | a | n | g | h | f | v | r | r | d | c | q | m | g | c | ! | | g | c | . | n | R | a | g | c | c | g | # | # | v | c | v | d | n | # | r | c | e | . | r | p | s | q | t | | | | | | | | | | | |

Figure S1: Sequence alignment of the New Superfamily 1 (NSF1) and 2 (NSF2) precursors

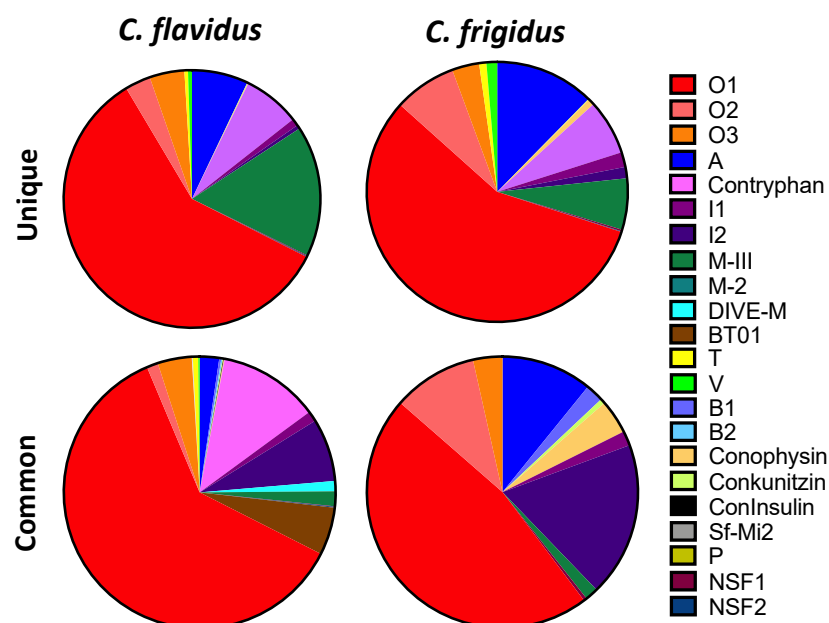


Figure S2: The superfamily expression profile of the unique (only found in one species) and common (found in both species) sequences in *C. flavidus* and *C. frigidus* transcriptomes.

O1 conotoxin Sub-Group 1

O1 conotoxin Sub-Group 2

O1 conotoxin Sub-Group 3

Known Toxins

| | 1 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 93 |
|----------------|------|------|-------|--------|-----------|----|----|----|----|----|----|
| FLA_116-FRI_75 | MKLT | CVLI | ISVFL | LAASQL | STADYPRDN | | | | | | |
| FLA_117-FRI_74 | MKLT | CVLI | ITVFL | LAASQL | ITADFSIDN | | | | | | |
| FLA_118 | MKLT | CVLI | ISVFL | LAASQL | ITADYTKDN | | | | | | |
| FLA_127-FRI_59 | MKLT | CVLI | ISVFL | LAASQL | ITADYTKDN | | | | | | |
| FLA_119 | MKLT | CVLI | ISVFL | LAASQL | ITADYTRDN | | | | | | |
| FLA_137 | MKLT | CVLI | ISVFL | LAASQL | ITADHSRDN | | | | | | |
| FLA_123 | MKLT | CVLI | ISVFL | LAASQL | ITADYTKDN | | | | | | |
| FLA_126-FRI_67 | MKLT | CVLI | ISVFL | LAASQL | ITADYTKDN | | | | | | |
| FLA_124 | MKLT | CVLI | ISVFL | LAASQL | ITADYTKDN | | | | | | |
| FLA_125 | MKLT | CVLI | ISVFL | LAASQL | ITADYTKDN | | | | | | |
| FLA_134 | MKLT | CVLI | ISVFL | LAASQL | ITADYSRDN | | | | | | |
| FLA_135 | MKLT | CVLI | ISVFL | LAASQL | ITADHSRDN | | | | | | |
| FLA_138 | MKLT | CVLI | ISVFL | LAASQL | ITADYSRDN | | | | | | |
| FLA_139-FRI_61 | MKLT | CVLI | ISVFL | LAASQL | ITADYPRDN | | | | | | |
| FRI_63 | MKLT | CVLI | ISVFL | LAASQL | ITADYPRDN | | | | | | |
| FLA_140-FRI_66 | MKLT | CVLI | ISVFL | LAASQL | ITADYTRDN | | | | | | |
| FLA_136 | MKLT | CVLI | ISVFL | LAASQL | ITADYSRDN | | | | | | |
| FLA_129 | MKLT | CVLI | ISVFL | LAASQL | ITADYTKDN | | | | | | |
| FLA_145-FRI_64 | MKLT | CVLI | ISVFL | LAASQL | ITADYTKDN | | | | | | |
| FLA_146-FRI_60 | MKLT | CVLI | ISVFL | LAASQL | ITADYTKDN | | | | | | |
| FRI_70 | MKLT | CVLI | ISVFL | LAASQL | ITADYTKDN | | | | | | |
| FLA_130-FRI_69 | MKLT | CVLI | ISVFL | LAASQL | ITADYPRDN | | | | | | |
| FLA_156-FRI_71 | MKLT | CVLI | ISVFL | LAASQL | ITADYPRDN | | | | | | |
| FRI_68 | MKLT | CVLI | ISVFL | LAASQL | ITADYPRDN | | | | | | |
| FLA_133-FRI_62 | MKLT | CVLI | ISVFL | LAASQL | ITADYPRDN | | | | | | |
| FLA_141 | MKLT | CVLI | ISVFL | LAASQL | ITADYSRDN | | | | | | |
| FLA_142 | MKLT | CVLI | ISVFL | LAASQL | ITADYSRDN | | | | | | |
| FLA_143 | MKLT | CVLI | ISVFL | LAASQL | ITADYSRDN | | | | | | |
| FLA_131 | MKLT | CVLI | ISVFL | LAASQL | ITADYSRDN | | | | | | |
| FLA_132 | MKLT | CVLI | ISVFL | LAASQL | ITADYSRDN | | | | | | |
| FLA_155-FRI_65 | MKLT | CVLI | ISVFL | LAASQL | ITADYTKDN | | | | | | |
| FLA_120 | MKLT | CVLI | ISVFL | LAASQL | ITADFSRDK | | | | | | |
| FLA_122 | MKLT | CVLI | ISVFL | LAASQL | ITADFSRDK | | | | | | |
| FLA_121 | MKLT | CVLI | ISVFL | LAASQL | ITADFSRDK | | | | | | |
| FLA_144 | MKLT | CVLI | ISVFL | LAASQL | ITADFSRDK | | | | | | |
| FRI_73 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| FLA_128 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| FLA_147-FRI_72 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| FRI_76 | MKLT | CVLI | ITVFL | LAASQL | ITADFSRDK | | | | | | |
| FLA_157 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| FLA_158 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| FLA_159 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDN | | | | | | |
| FRI_82 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDN | | | | | | |
| FLA_148 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| FLA_149 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| FLA_151 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| FLA_153-FRI_78 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| FLA_154-FRI_79 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| FRI_77 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| FLA_150 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| FLA_152 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| FRI_81 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| FRI_80 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| FRI_57 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| FRI_58 | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| PnVIA | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| TxVIA | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| TxVIA | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| PVIA | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| MVIA | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| MVIA | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| CVIA | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| GVIA | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| TVIA | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| MoVIA | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| MoVIB | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| TsVIA | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| SuVIA | MKLT | CVLI | ITVFL | LAASQL | ITADYSRDK | | | | | | |
| Consensus | MKLT | CVLI | ISVFL | LAASQL | ITADYSRDK | | | | | | |

Figure S3 : Alignment of O1 conotoxin precursors found in *C. flavidus* and *C. frigidus* transcriptomes. The precursor sequences were classified into three subgroups based on the sequence similarity. Known O1 superfamily ω - and δ -conotoxins from fish, mollusk and worm hunting cone snails were aligned with the *C. flavidus* and *C. frigidus* O1 precursors using Clustal Omega. Conserved amino acids are highlighted and the consensus sequence shown at the bottom.

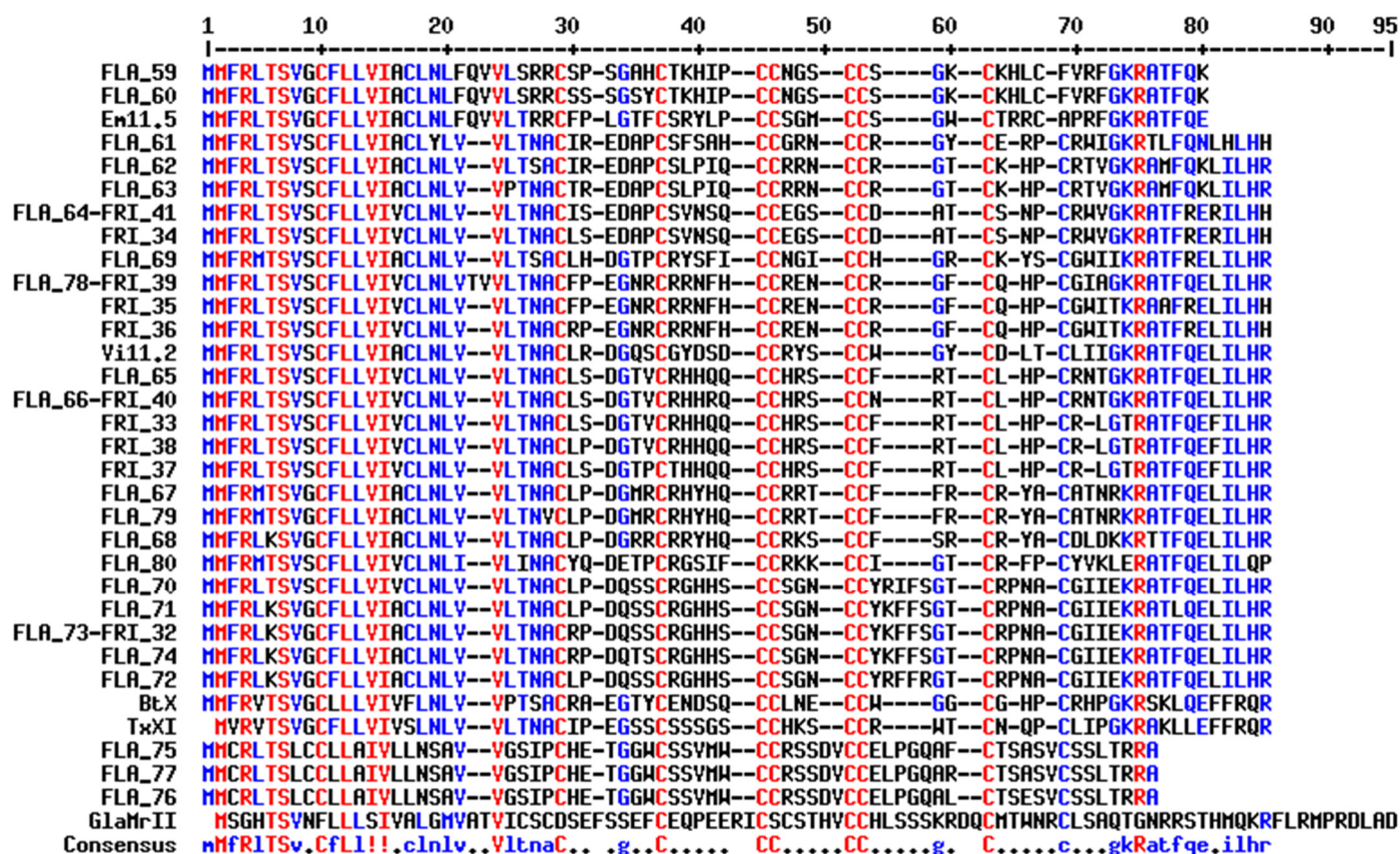


Figure S4 : 12 conotoxin precursors aligned with known 12 conotoxin precursors from other Cone snails (Vi11.2, Btx, TxxI and GlaMrII).

| | 1 | 10 | 2022 |
|---------------|-----------------------|----|------|
| | -----+-----+----- | | |
| FLA_01 | DCCNDPACAVNHQDLCT | | |
| FLA_02-FRI_01 | DCCNDPACAVNNPGLCT | | |
| FLA_03 | DCCNDPACTVNNPGLCT | | |
| FRI_02 | DCCNLPACAASNPGLCT | | |
| FRI_03 | DCCNFPACAASNPGLCT | | |
| Vc1.1 | GCCSDPRCNYDHPEIC | | |
| PeIA | GCCSHPACSVNHPELC | | |
| LvIA | GCCSHPACNVDHPEIC | | |
| LsIA | SGCCSNPACRYNNPNIC | | |
| TxIA | GCCSRPPCIANNPDLC | | |
| GIC | GCCSHPACAGNNQHIC | | |
| FLA_04-FRI_04 | DCCSSAACAHHTPSC | | |
| FLA_05 | DCCSSVACAHHTPSC | | |
| FLA_07-FRI_14 | GCCSDPPCAHHNPAC | | |
| FRI_08 | GCCSDPPCAHHNPSC | | |
| FLA_13 | GCCSNPPCAHHNPAC | | |
| FRI_11 | GCCSNPPCAHHNPDC | | |
| FRI_13 | GCCSDPPCAHHNPDC | | |
| FLA_10 | GCCSDPPCAHHNPAC | | |
| FLA_12 | GCCSDPPCARHNPAC | | |
| FLA_11-FRI_05 | GCCSDPPCAHHNPDC | | |
| FLA_16-FRI_12 | GCCSNPPCAHHNPDC | | |
| FLA_19-FRI_06 | DCCSDPPCAHHNPDC | | |
| ViIA | RDCCSNPPCAHHNPDCR | | |
| FRI_10 | DCCSDSPCAHHNPDC | | |
| FLA_14 | GCCSNPPCAHINPDC | | |
| FLA_15 | GCCSNPPCAHLNPAC | | |
| FLA_17 | GCCSNPPCALNPNDC | | |
| FLA_18 | GCCSSPPCARLNPAC | | |
| FLA_06 | GCCSDP-CAHKHC | | |
| FLA_08-FRI_09 | GCCSDPPCAHKHC | | |
| FLA_09-FRI_07 | GCCSDPPCAHKYC | | |
| RgIA | GCCSDPRCRYRCR | | |
| ImI | GCCSDPRCAWRC | | |
| P1169 | TVIMHNCCTRSFCRIYPDLCS | | |
| P1170 | GIGGSCCVIRSCAIKFSTLCG | | |
| SI | ICCNPACGPKYSC | | |
| MI | GRCCHPACGKNYSC | | |
| CIA | NGRCCHPACGKHFSC | | |
| Consensus | .gcCs.p.Ca..np.... | | |

Figure S5: A conotoxins aligned with known A conotoxins from other fish (SI, MI, CIA, GIC), mollusk (TxIA) and worm hunters (PeIA, LvIA, LsIA, ViIA, RgIA, ImI, P1169, P1170).

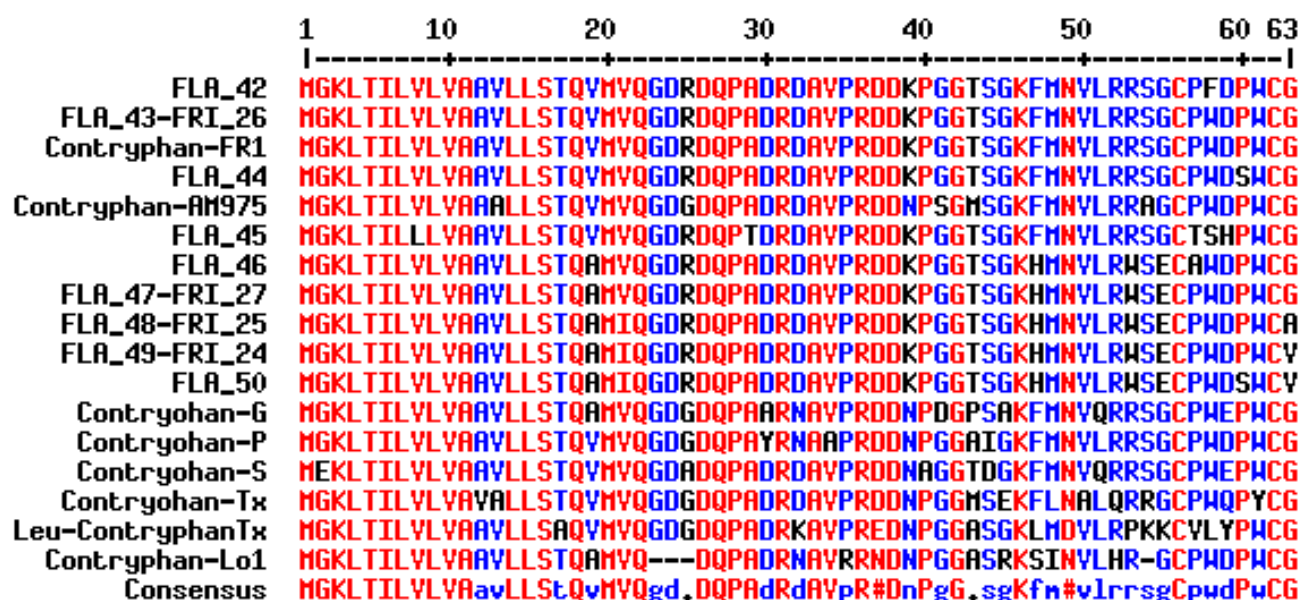


Figure S6: Contryphan precursors aligned with known Contryphan precursors from other fish (Contryphan-G, Contryphan-S Contryphan-P), mollusk (Contryphan-Tx, Leu-Contryphan-Tx) and worm (Contryphan-Lo1) hunters.

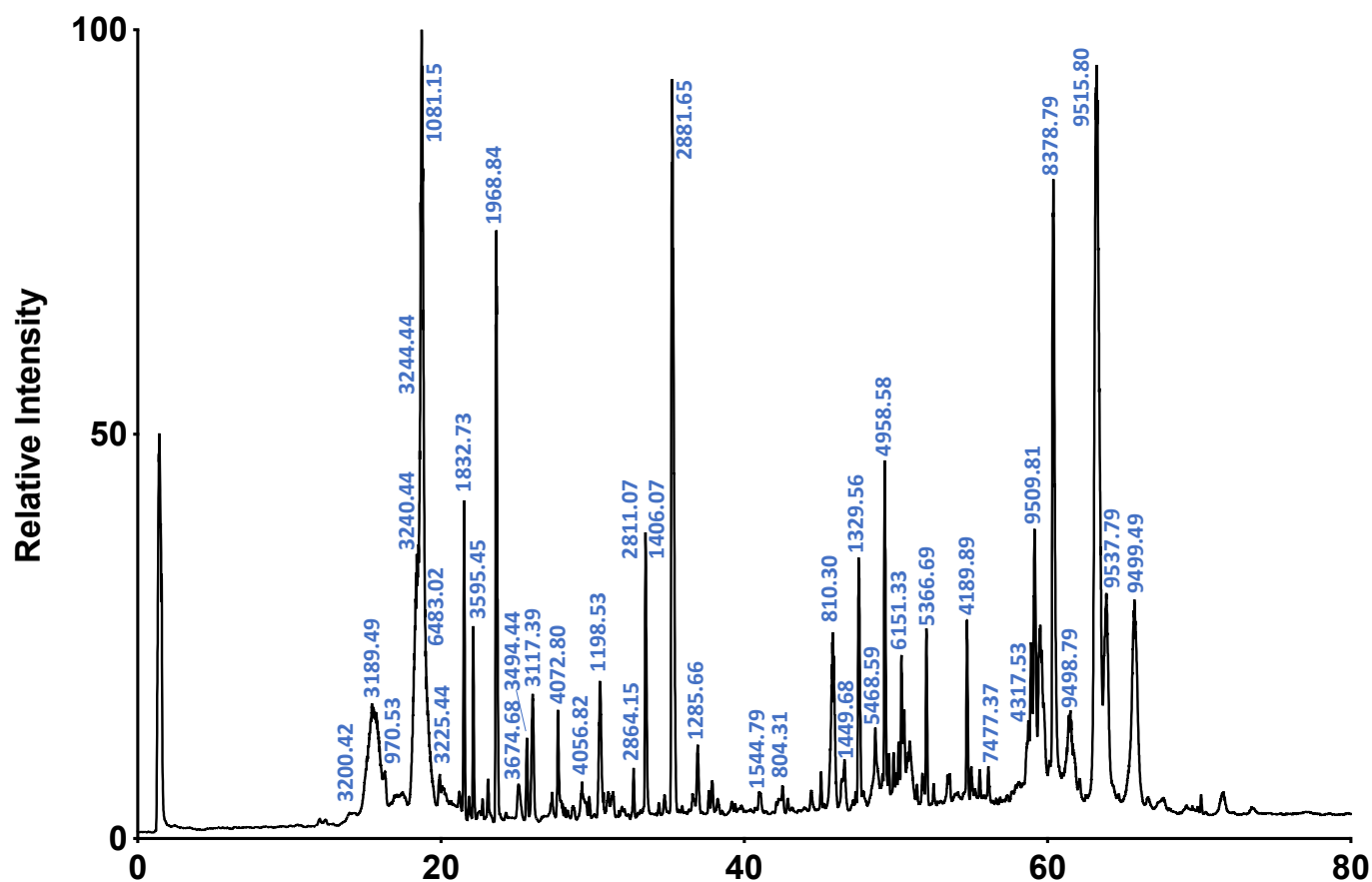


Figure S7: TIC of the *C. flavidus* Proximal venom duct showing dominant masses eluting under major peaks.

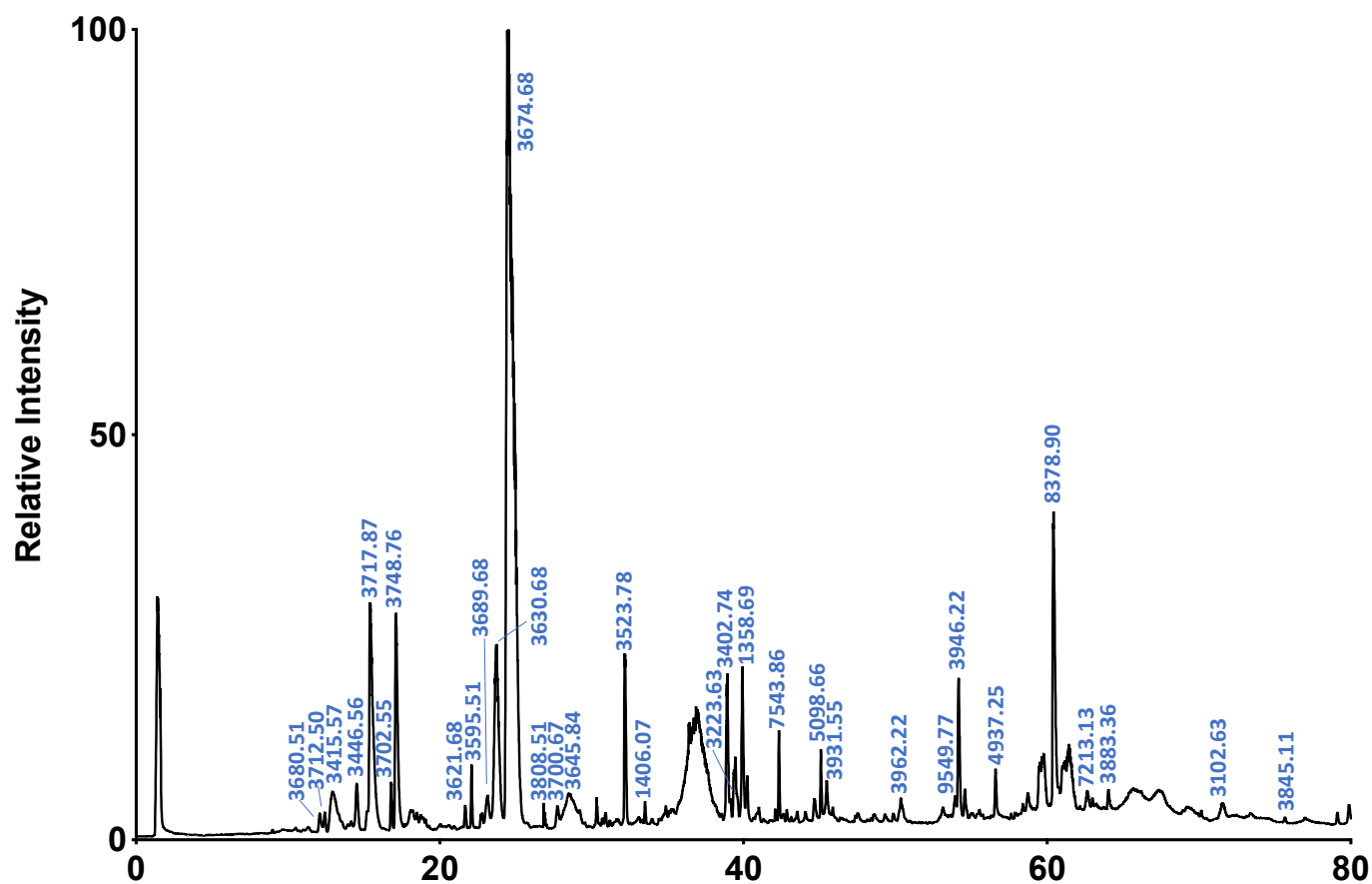


Figure S8: TIC of the *C. flavidus* Distal venom duct showing dominant masses eluting under major peaks.

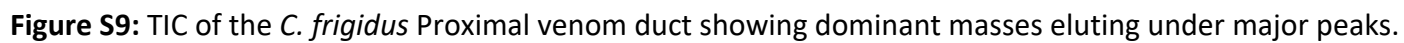


Figure S9: TIC of the *C. frigidus* Proximal venom duct showing dominant masses eluting under major peaks.

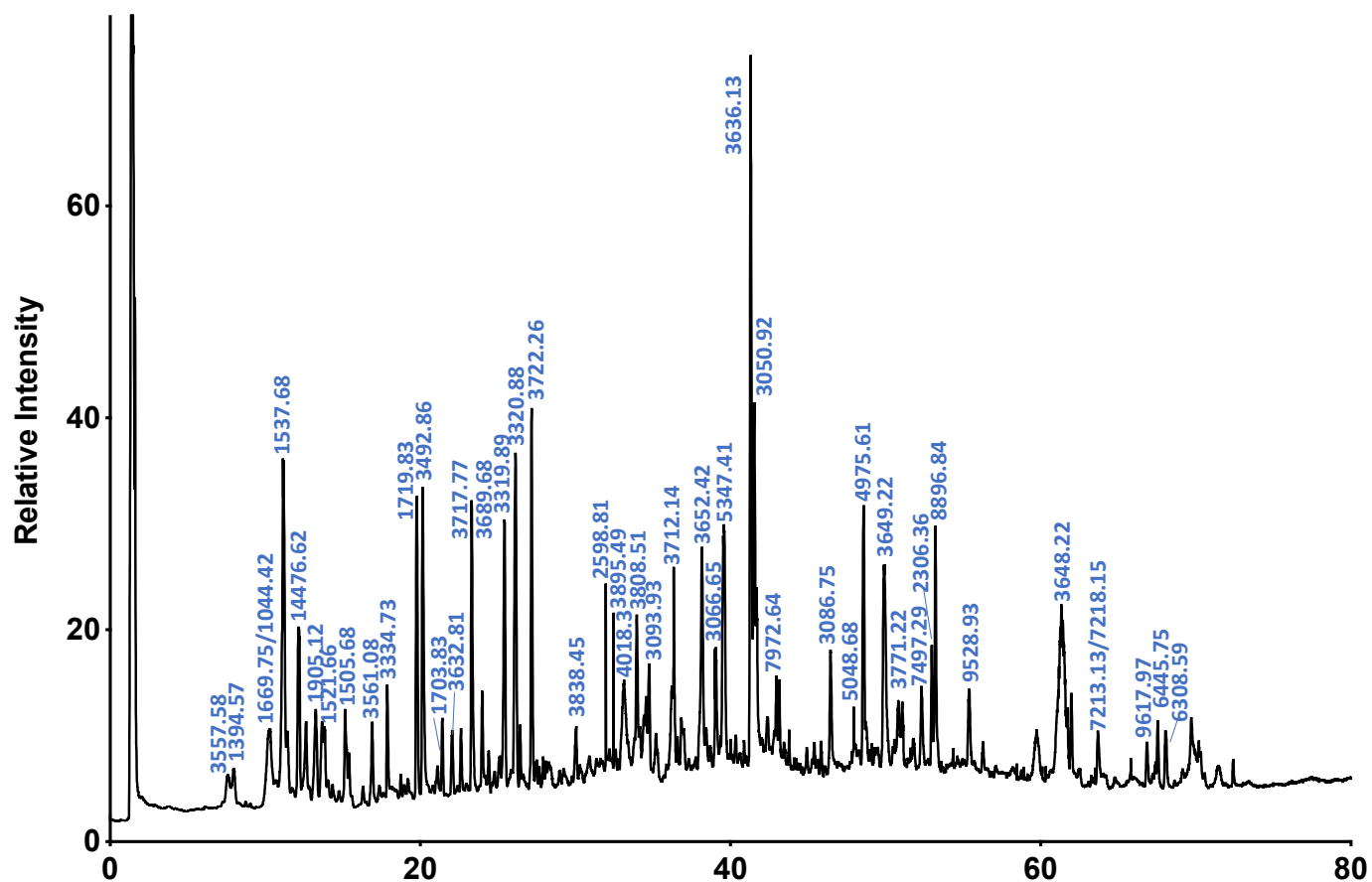


Figure S10: TIC of the *C. frigidus* Distal venom duct showing dominant masses eluting under major peaks.

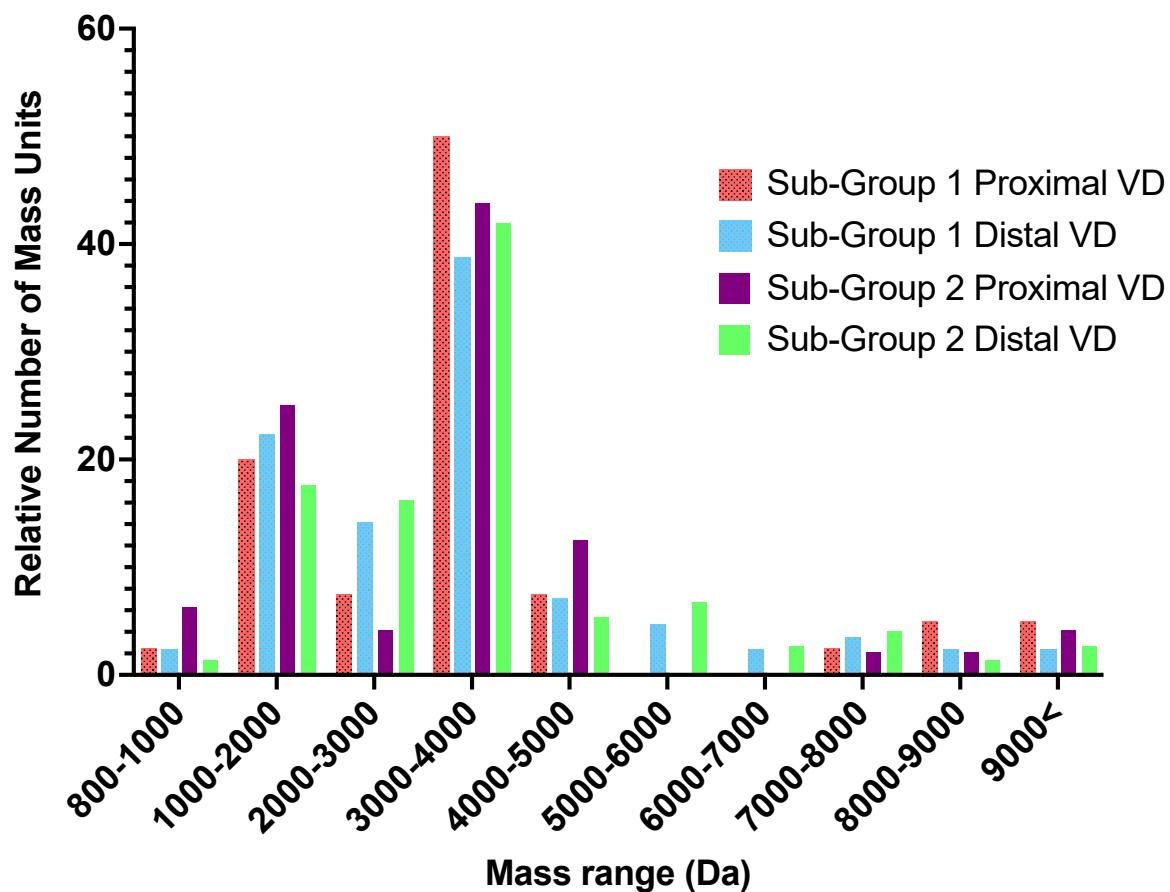


Figure S11: Mass distribution ranges in the reconstructed major masses of proximal and distal venom duct extracts of sub-groups 1 and 2.

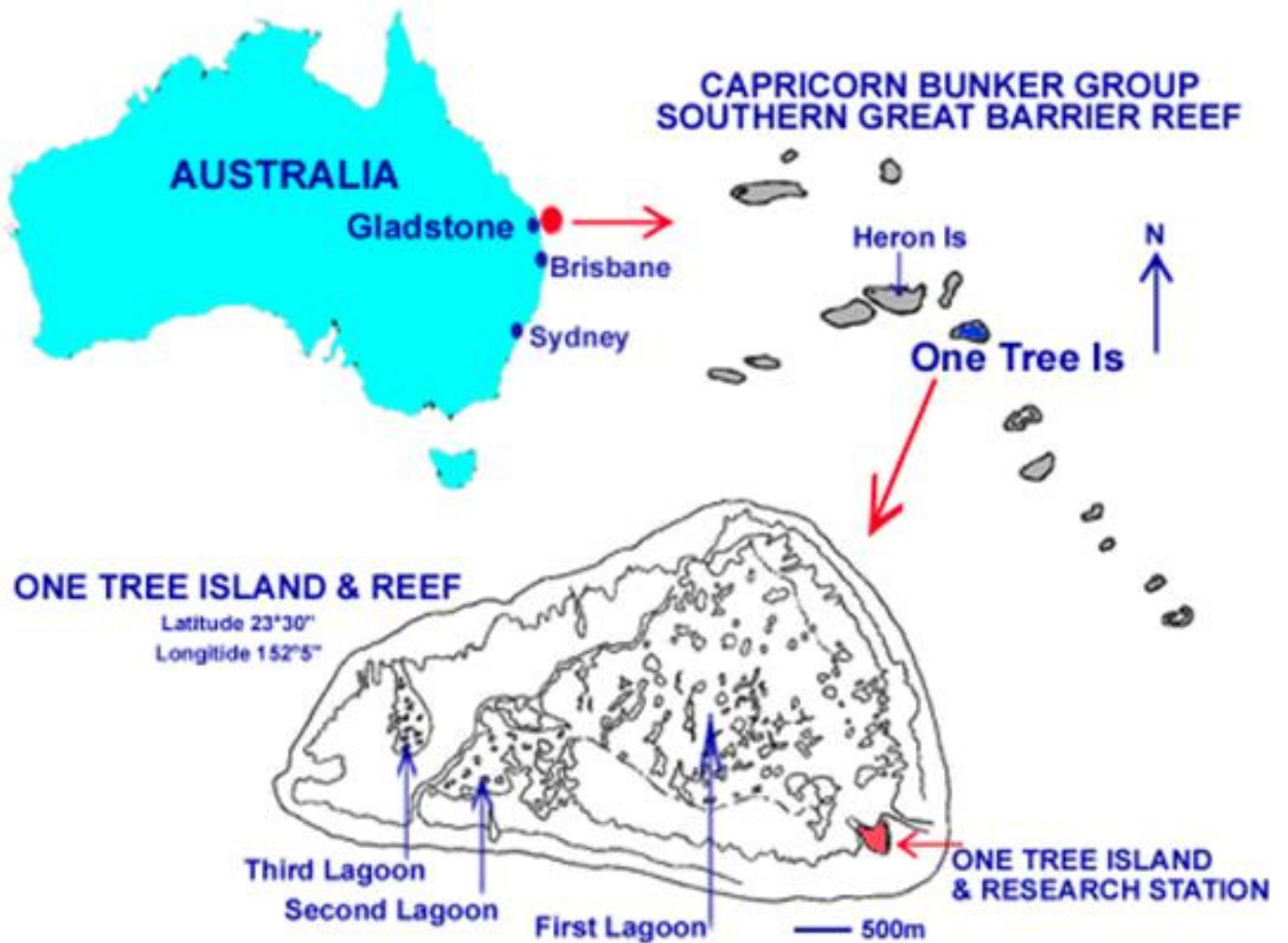


Figure S12. One Tree Island Map. The map is taken from the One Tree Island Research Station resources.