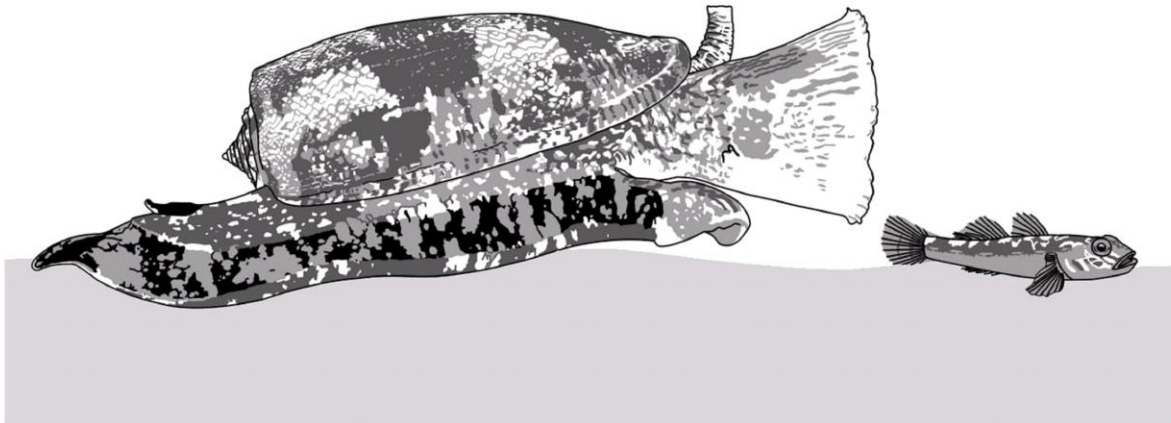
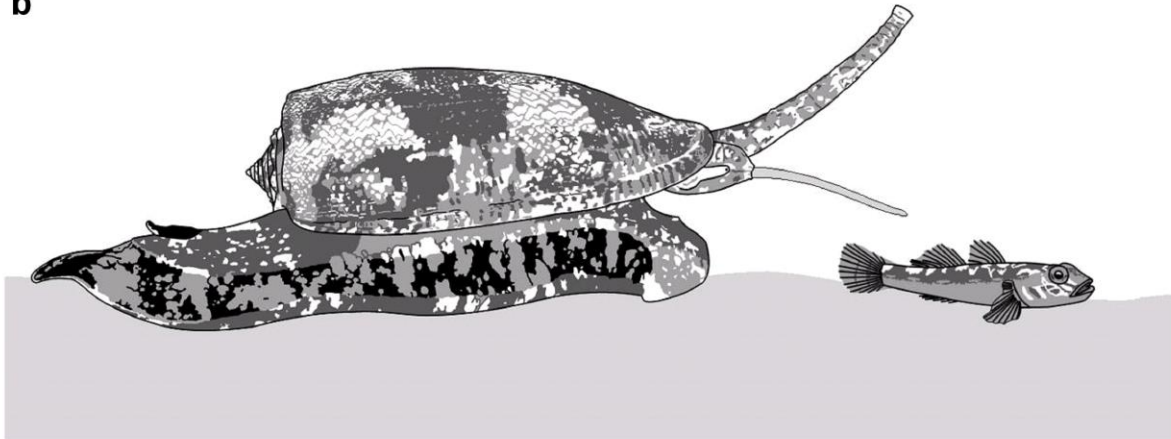


a

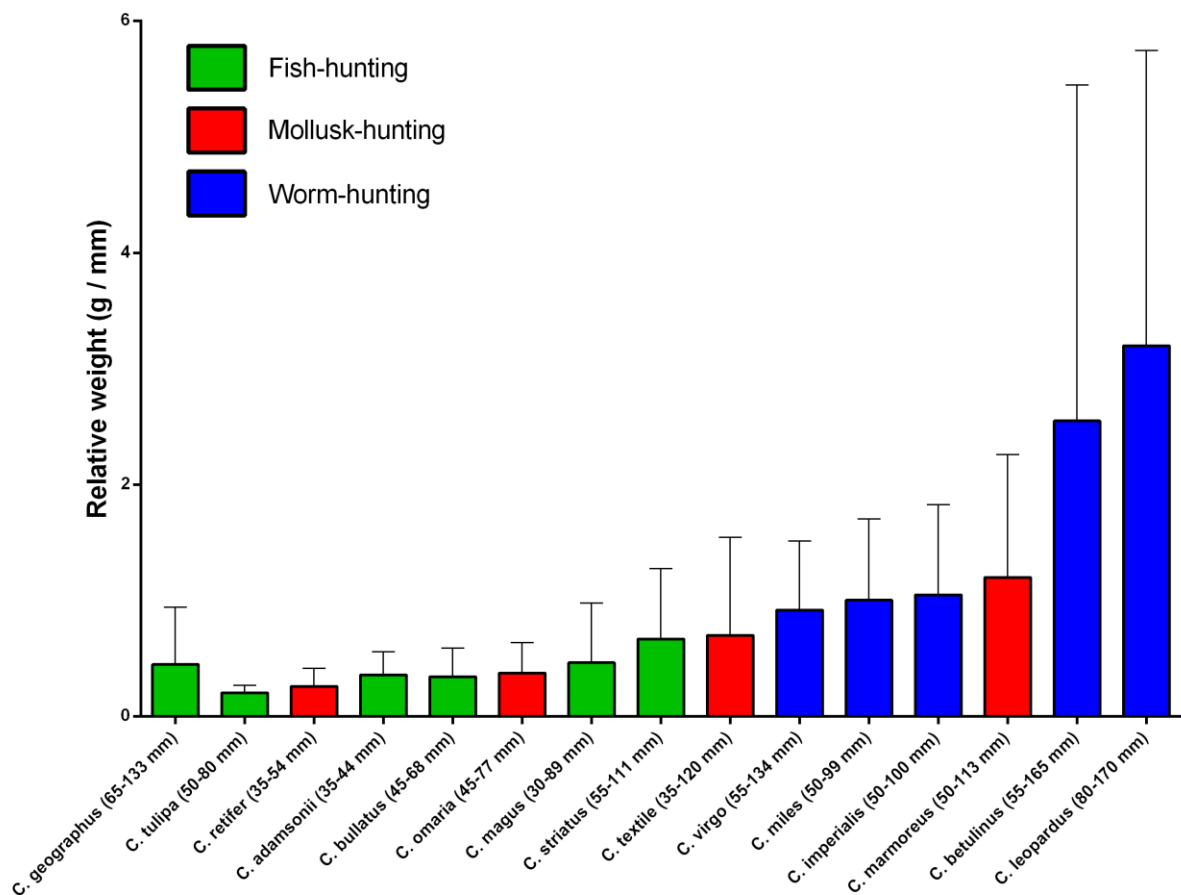


b

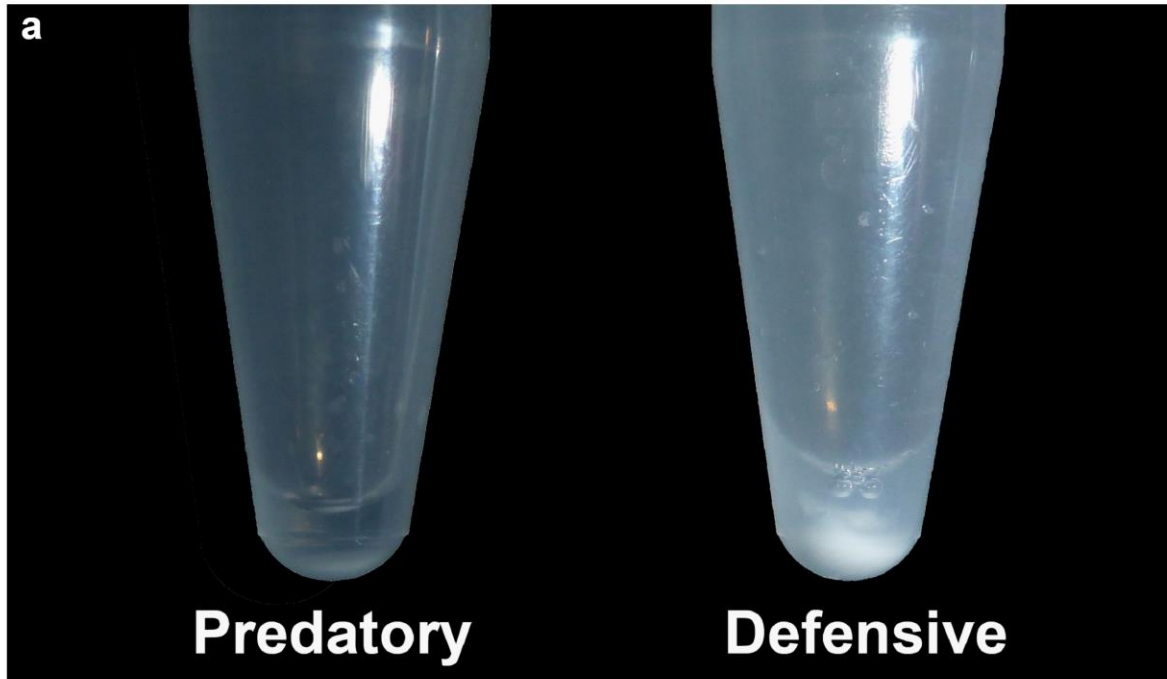


Supplementary Figure 1. *Conus geographus* prey-capture strategies.

a, While most piscivorous cone snails rely on the injection of venom to capture fish, *C. geographus* has evolved a different predatory strategy, using its distended rostrum as a “net” to capture fish sedated by a “nirvana cabal” released into the water. Observations on the feeding behaviour of *C. geographus* suggest that venom may be injected once the fish is securely imprisoned in its false mouth, however, this has never been directly proven. **b**, On occasions some *C. geographus* specimens use their proboscis to directly inject fish with venom, providing the opportunity to collect the predation-evoked venom as demonstrated in this study.



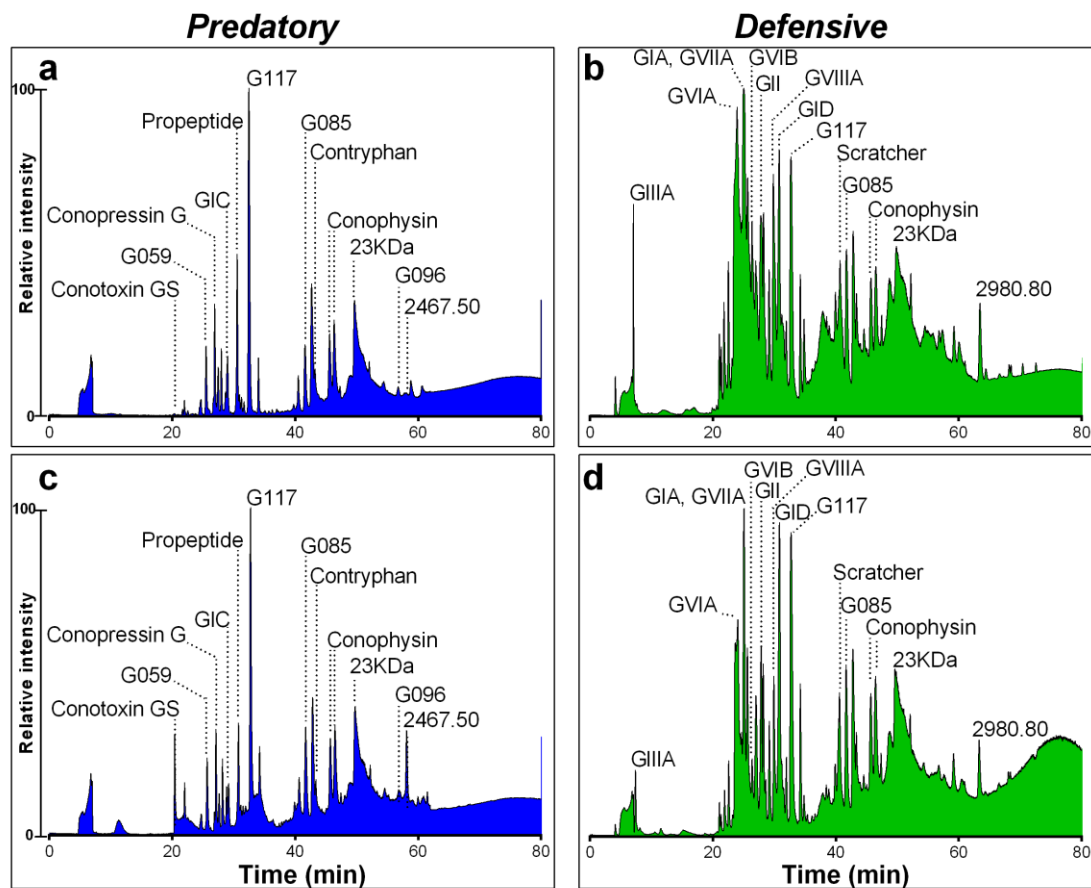
Supplementary Figure 3. Relative weight of *Conus* species. Relative weight (weight of shell/size of shell) provides a convenient estimate of shell thickness, reflecting the relative level of protection offered. Interestingly, *C. geographus* is the most dangerous cone snail but possesses one of the thinnest and lightest shells, with the largest aperture of all *Conus* species. We hypothesize that *C. geographus* evolved an aggressive behaviour and potent defensive venom to compensate for reduced shell protection. In support of this hypothesis, the second species thought to be dangerous to human is *C. tulipa*, which belongs to the same phylogenetic clade, and displays similar aggressive behaviour, shell characteristics and potent venom on vertebrate receptors. In contrast, *Conus* species with strong heavy shell such as *C. leopardus* have less potent venom on vertebrates and less diverse venom peptides compared to other species. Therefore, less potent venom may correlate with reduced dependence on defensive venom. (data from the Manual of the Living Conidae¹, are presented as a range of measurements, usually from at least 10 specimens).



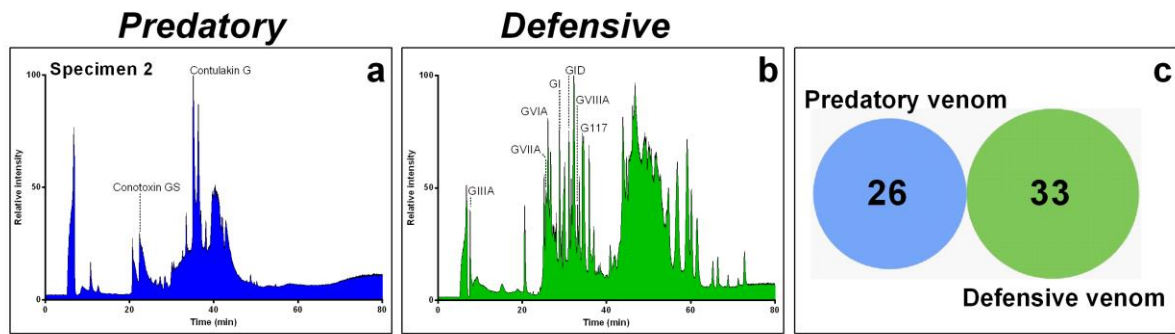
b

| Specimen Size (mm) | Predatory Volume (μl) | Defensive Volume (μl) | P / D ratio |
|---------------------------|------------------------------|------------------------------|--------------------|
| 1 (92.5) | 29 | 54 | 0.53 |
| 2 (95.5) | 34 | 61 | 0.55 |
| 3 (94) | 26 | 51 | 0.51 |
| 4 (112) | 33 | 66 | 0.50 |

Supplementary Figure 4. Predation- and defence-evoked venoms of *C. geographus*. **a**, The predatory venom of *C. geographus* appears translucent, whereas the defensive venom from the same specimen contains insoluble material comprising white secretory granules that quickly settles to the bottom. **b**, The volume of predatory venom injected is consistently ~50% the volume of defensive venom injected (predatory/defensive venom volume ratio 0.50–0.55).

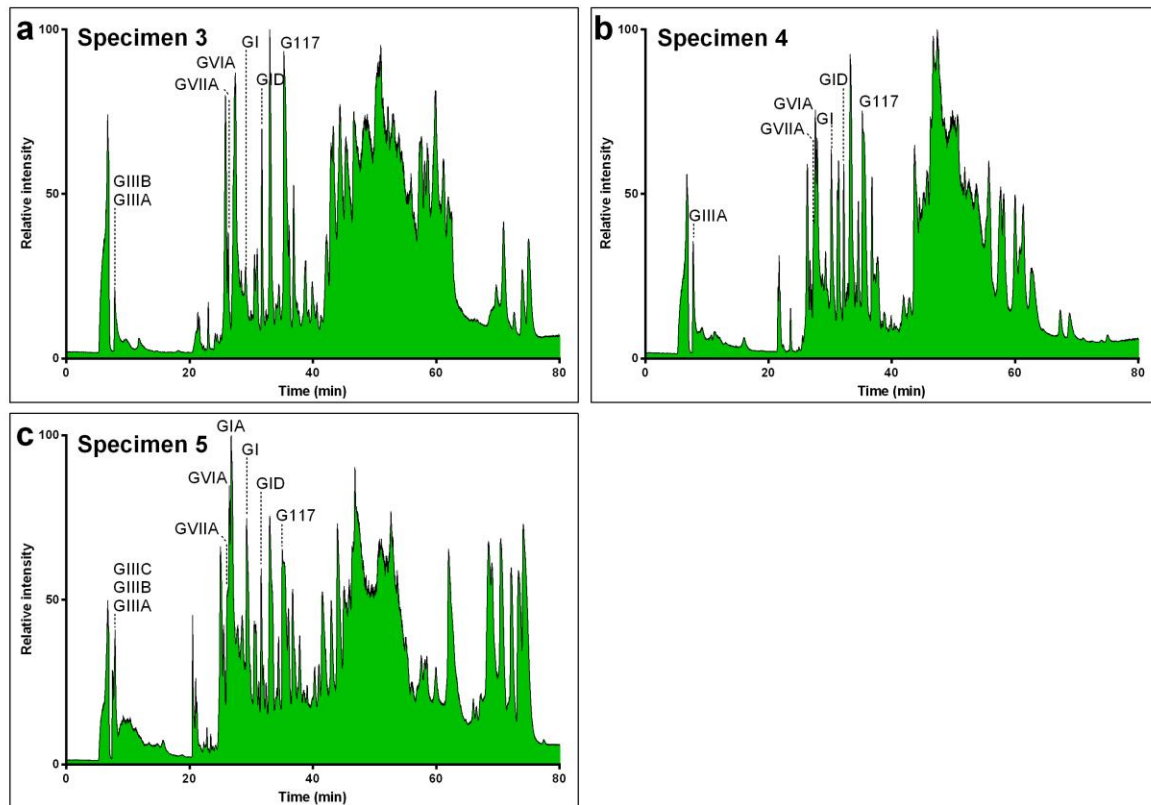


Supplementary Figure 5. Predation- and defence-evoked venoms of *C. geographus* can be generated reproducibly over time. **a** and **c**, Shown are LC-MS profiles and composition for two independent predatory stings collected from the same specimen 8 days apart (and interrupted by a defensive sting in **b**), and (**b** and **d**) two defensive stings also collected 8 days apart. This experiment demonstrates that discrete sets of toxins can be generated in response to predatory or defensive stimuli and remain unaltered by the type of sting previously deployed.



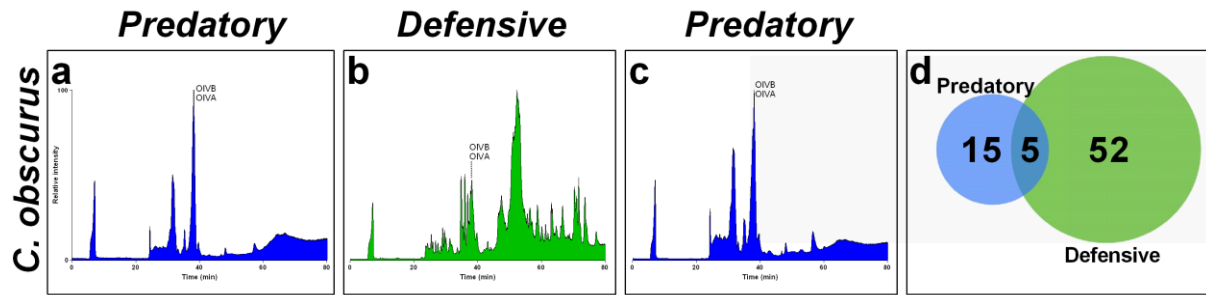
Supplementary Figure 6. Predation- and defence-evoked venoms from a second *C.*

geographus specimen. **a**, Consistent with results obtained for the *C. geographus* specimen shown in Fig. 1 and Fig. S5, predation-evoked venom from a second *C. geographus* specimen again show a simple LC-MS trace, where the fish-specific conotoxin GS is a major conotoxin, together with the NMDA antagonist conantokin G. **b**, The defence-evoked venom in contrast is highly complex and similar to the *C. geographus* specimen shown in Fig. 1 and Fig. S5, again with paralytic peptides such as GI, GVI, GVIIA and GIIIA dominating. **c**, Remarkably for this specimen, the two venoms show no overlap for the major peptide masses detected, supporting the independent release of distinct sets of conotoxins upon predatory or defensive stimuli.



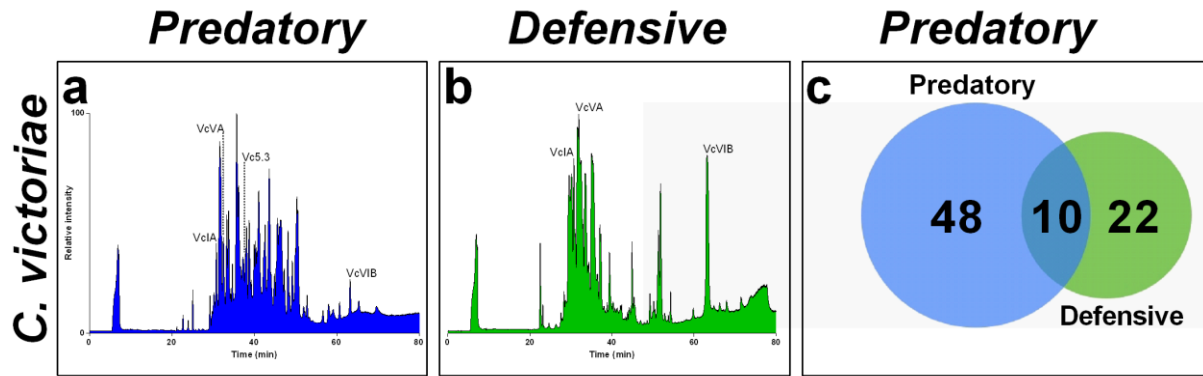
Supplementary Figure 7. Defence-evoked venoms from three specimens of *C.*

geographus. a-c, The defence-evoked venom was collected from three other specimens of *C. geographus*. While minor intraspecific variations (particularly variations in the intensity level of specific toxins) are observed, especially for the most hydrophobic peptides, the composition remains consistent across all specimens examined, with paralytic toxins such as GI, GVIA, GVIIA and GIIIA dominating in this venom.



Supplementary Figure 8. LC-MS profiles of predation- and defence-evoked venoms of *C. obscurus*.

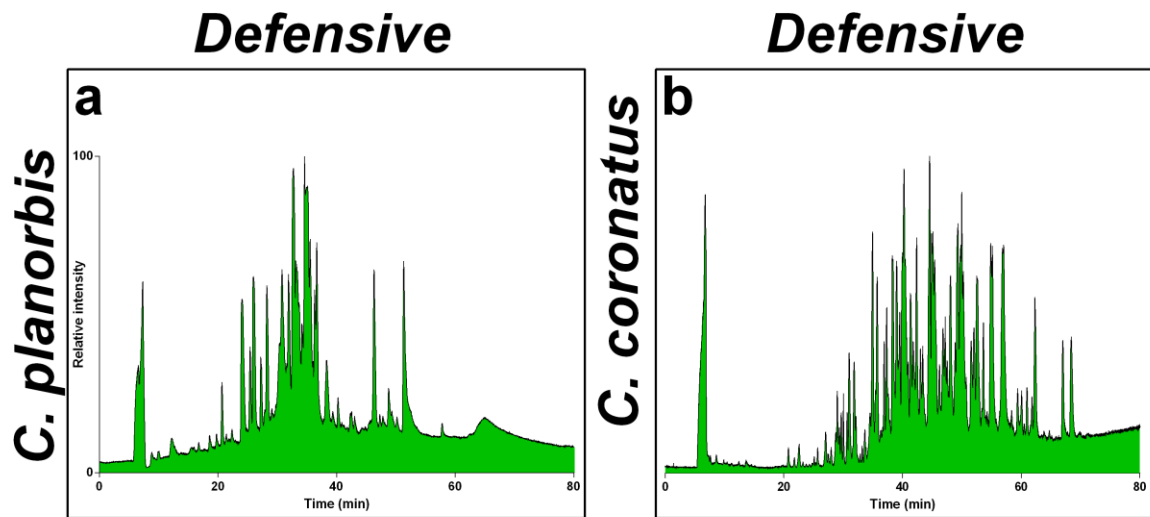
C. obscurus is also a piscivorous species like *C. geographus*, but belongs to a different phylogenetic clade (Protostricoonus) and uses a “hook-and-line” prey capture strategy rather than a net strategy (see supplementary Movie 6). Predation- and defence-evoked venoms were collected from the same individual at 2–7 days intervals (**a-c**). Again, the predation-evoked venom appears relatively simple in composition (20 major masses detected), and largely dominated by two characterised peptides, OIVA and OIVB. These excitatory toxins are part of the lightning-strike cabal, and induce a fast immobilisation of fish. The predation-evoked venom of *C. obscurus* resembles other hook-and-line piscivorous cones snails including *C. striatus* and *C. consors*. In contrast, the defence-evoked venom of *C. obscurus* is more complex (57 major masses detected) and comprises mostly unknown toxins, with only 5 of the predatory venom masses (20%) identified as being common to the defensive venom, including the previously identified α A-conotoxins OIVA and OIVB (**d**).



Supplementary Figure 9. LC-MS profiles of predation- and defence-evoked venoms of

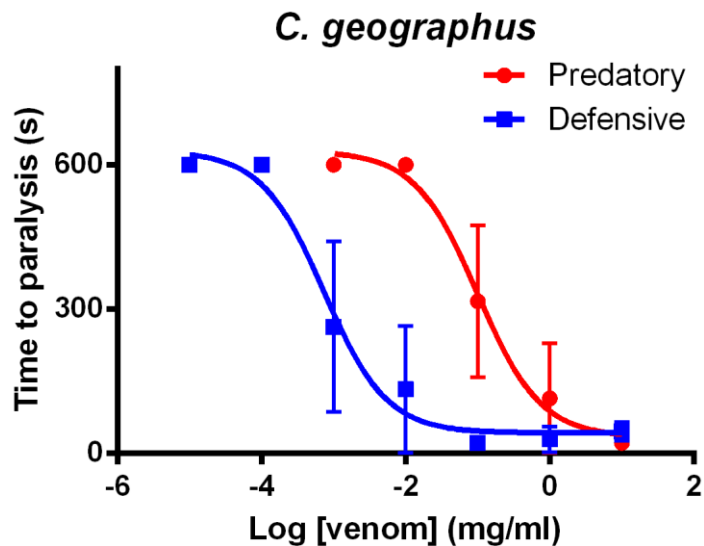
C. victoriae.

Predation- and defence-evoked venoms were also collected from *C. victoriae* (**a** and **b**), a mollusc-hunting cone snail like *C. marmoreus* (*Conus* Clade) but which belongs the Cylinder clade. Surprisingly, the predatory venom of *C. victoriae* (58 major masses detected) was more complex than its defensive venom (32 major masses detected) in contrast to other species examined. However, only 20% of the predation-evoked toxins were also present in the defensive-evoked venom (**c**). A number of minor predatory venom peptides were seen as major component in the defensive venom, including the conotoxins VcVIB, VcVA and VcIA.



Supplementary Figure 10. LC-MS profiles of defence-evoked venoms of *C. planorbis* and *C. coronatus*.

The defence-evoked venoms of two worm-hunting species could also be obtained despite their small radula, which made the milking challenging. Both *C. planorbis* (Vituliconus clade) and *C. coronatus* (Miliariconus clade) generated complex defence-evoked venoms (**a**, and **b**), where the 40-60 min time period appears dramatically different between the two species. These likely reflect the already well known interspecific variations in venom complexity that was demonstrated using dissected venom duct extracts. *C. coronatus* venom contains > 100 major masses, indicating that complex defensive-evoked venom can be generated by fish-, mollusc- but also worm-hunting cone snails.



Supplementary Figure 11. Effective dose of *C. geographus* predation- and defence-evoked venoms on adult zebrafish.

Incremental doses of both venoms were injected intramuscularly into adult zebrafish and time of paralysis (see method section) was plotted against venom concentration. The IC_{50} values obtained for the curves were then used to determine the ED_{50} . The IC_{50} for the defence- and predation-evoked venom were 0.0005 mg/ml and 0.175 mg/ml, respectively. Based on the calculated average weight of our adult zebrafish (0.5 g) and quantity effectively injected per fish (5 μ l), the defence-evoked venom of *C. geographus* has an ED_{50} of 10 μ g/kg, whereas the predation-evoked venom was much less potent, with an ED_{50} of 3.5 mg/kg. These results are consistent with the defence-evoked venom being used to deter much larger predators compared to the usual prey of *C. geographus*.

Con-ikot-ikot

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Conantokin

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Conopressin – conophysin

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Contulakin

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Superfamily A

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Superfamily J

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Superfamily M

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Superfamily O1

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Superfamily O1-variant 1

```
G092/50 MNLTCVLLI IAVLFLTACQLI AADSRDQKRR AVRRMDALKNFKDSRACSGRSGRCP PPGCCMGLTCCREYPPRCG-----
G093/10 MNLTCVLLI IAVLFLTACQVI AADSRDQKIYRAVRSRDMRNFSSRPPCANLGRACDTPVCCLVGRCFESRTPICRLKORGV
G094/5 MNLTCVLLI IAVLFLTACQVI AADSRDQKIYRAVRSRDMRNFSSRPPCANLGRACDTPVCCLVGRCFESRTPICRLKORGV
```

Superfamily O1-variant 2

```
G095/4 MKLAVVVI IAVLLLTACQLI TALDSRSPVTKCSDGTHPTLVKHKATGEYCRRYVTRCCCKGNNNRCT
```

Superfamily O2

```
G096/9 MEKLTIVLLVAAVL MSTOAT IGGGENRPMENI KYLSKSORSAERGWSECSDWLAGCSSPSECCEKCDTFRLWR
G097/6 MEKLTIVLLVAAVL MSTOAM IGGGENRPMENI KYLSKSORSAERGWSECSDWLAGCSSPSECCEKCDTFRLWR
G098/4 MEKLTIVLLVAAVL MSTOAM IGGGENRPMENI KYLSKSORSAERGWSECSDWLAGCSSPSECCEKCDTFRLWR
```

Superfamily O3

```
G099/163 MSLRIMVLTIVLLVLTMTSHODAGEKQAMORDAKNFRRRRLGIRKPKTRCEMELCEQEEKHCCRIRNENIQCAPRCLGIGV--
G101/36 MSLGIMVLTIVLLVLSMAISHRYAREKQATRRDVNIRRRS---KPKTPECKRICKLEKKCCVRSSEKPSRLC-GLPMFC
G100/80 MSLRIMVLTIVLLVLTMTSHODAGEKQAMORDAKNFRRRRLGIRKPKQGSACKCVSRRRNTAAA-----
G102/8 MSLRIMVLTIVLLVLTMTSHODAGEKQAMORDAKNFRRRRLGIRNQGSAKCCVSRNRNTAAA-----
```

Superfamily S

```
G103/128 MMSKMGAMFVLLLLLFTLASSLQEGDVGARKTRKSDFYRALARDVGECTH-CGGADCTGSCCTCTNWSSQVCMNFSSEEGECGGCTCFR--
G105/10 MMSKMGAMFVLLLLLFTLASSLQEGDVGARKTRKSDFYRALARDVGECTH-CGGADCTGSCCTCTNWSSQVCMNFSSEEGECGGCTCFR--
G101/36 MSLGIMVLTIVLLVLSMAISHRYAREKQATRRDVNIRRRS---KPKTPECKRICKLEKKCCVRSSEKPSRLC-GLPMFC
G106/9 MMSKMGAMFVLLLLLFTLASSLQEGDVGARKTRKSDFYRALPRSGSTCTG-FITSTNCGGSCGELSPPGYCSNNGIRQPG-CSTCGPTG
```

Superfamily T

```
G107/80 MCCLPVFVILLLLLITSAFVSDALPKTRDDVPLASFGGYNARRILQRRQGWCKENIACCI-----
G108/52 MPCLPVFVILLLLLISSAPSVDAARPKTKYDVSRAFSHFVNAKRDRQSRMERCCEERWCCFR-----
G109/26 MPCLPVFVILLLLLISSAPSVDAARPKTKYDVSRAFSHFVNAKRDRQSRMERCCEERWCCFR-----
G110/11 MLCPLPVFVILLLLLSPAAPNPFERRIQSDSIRAALEDADFNPDERSFNSIKALGEL--IRAKIFPVIIDMLPGDKK
G111/7 MLCPLPVFVILLLLLSPAAPNPFERRIQSDSIRAALEDADFNPDERSFNSIKALGEL--IRAKIFPVIIDMLPGDKK
G112/7 MCCLPVFVILLLLLITSAFVSDALPKTRDDVPLASFGGYNARRILQRRQGWCKENIACCI-----
G113/3 MLCPLPVFVILLLLLSPAAPNPFERRIQSDSIRAALEDADFNPDERSFNSIKALGEL--IRAKIFPVIIDMLPGDKK
```

Superfamily I1

```
G114/26 MKTVAVFLVVALAVAYGQFFCPSSKEDSLNCIETMATTATCMKSNKGEIYSYACGYCGKKKESCFDQKPKVTDYQCOTRNIIPNCGGAAAL
G115/14 MKTVAVFLVVALAVAYGQFFCPSSKEDSLNCIETMATTATCMKSNKGEIYSYACGYCGKKKESCFDQKPKVTDYQCOTRNIIPNCGGAAAL
G116/8 MKTVAVFLVVALAVAYGQFFCPSSKEDSLNCIETMATTATCMKSNKGEIYSYACGYCGKKKESCFDQKPKVTDYQCOTRNIIPNCGGAAAL
```

Superfamily I3

```
G117/16 MKLFLAIVLILMLQFLSTGAEITSDNHASRSTALRDWLLGPKAKRCAVTHEKCSDDYDCCGSLCCVGI CAKTIAPCK
```

New superfamily – 1

```
G118/491 MSRLFLVLLVIVSALTLTHTDSTQGHGGTDMSSRPVARAARDHASLARFHKFRAHARSORIGRLVEDQSGDDEEEENDEN
G119/426 MSRLFLVLLVIVSALTLTHTDSTQGHGGTDMSSRPVARAARDHASLARFHKFRAHARSORIGRLVEDQSGDDEEEENDEN
G120/35 MSRLFLVLLVIVSALTLTHTDSTQGHGGTDMSSRPVARAARDHASLARFHKFRAHARSORIGRLVEDQSGDDEEEENDEN
```

New superfamily - 2

```
G121/110 MTTSFYFLLVALGLLLYVQSSSFGNOHTRNSDTPKHRGSEELADQYVQLCHGKRNDAGKKRGRASPLWQRGGFLSMLKAKRNEAFFLHRDGRIVEVCCDNPCTVATLRTFCH
G124/19 MTTSFYFLLVALGLLLYVQSSSFGNOHTRNSDTPKHRGSEELADQYVQLCHGKRNDAGKKRGRASPLWQRGGFLSMLKAKRNEAFFLHRDGRIVEVCCDNPCTVATLRTFCH
G122/27 MTTSFYFLLVALGLLLYVQSSSFGNOHTRNSDTPKHRGSEELADQYVQLCHGKRNDAGKKRGRASPLWQRGGFLSMLKAKRNEAFFLHRDGRIVEVCCDNPCTVATLRTFCH
G123/19 MTTSFYFLLVALGLLLYVQSSSFGNOHTRNSDTPKHRGSEELADQYVQLCHGKRNDAGKKRGRASPLWQRGGFLSMLKAKRNEAFFLHRDGRIVEVCCDNPCTVATLRTFCH
```

New superfamily – 3

```
G125/41 MGSMKIYLLAFLLLLPFTIVDSGLLDKIEIIRNMRREDESKDGRCAELRSSRQTEAMFQLTPELCTPSPVSCPTGECRCKFHQSRCTRFEEGVNPKCRDA
G126/33 MGSMKIYLLAFLLLLPFTIVDSGLLDKIEIIRNMRREDESKDGRCAELRSSRQTEAMFQLTPELCTPSPVSCPTGECRCKFHQSRCTRFEEGVNPKCRDA
```

New superfamily – 4

```
G127/11 MGATLVTKL LLLAAALLGLCHEMAANPEAWDECYERMHMLIAPTWHAMYNCHEILEDKRRAALVLLKQLGVIHLDHGGWVVOHVKLLKYLGVNN
```

Supplementary Figure 12. Alignment of conotoxin sequences from a *C. geographus*

venom duct transcriptome. The 454 sequencing raw data (reads) of the venom duct

transcriptome were sorted using Conosorter and conotoxin sequences were classified by gene

superfamilies. Each sequence is identified by an identifier number (GXXX), with the number

of reads corresponding to the full length precursor indicated by the extension (GXXX/XX).

The number of reads provides an estimate of the level of expression of a particular sequence

at the time of RNA extraction.

Superfamily O1

GEORS003 MKLT¹⁰CVMI VAALFLT²⁰ACQLSTAA³⁰SFARDKEEYPAV⁴⁰RSSDGMQDSKDLT⁵⁰LAKKCKEQSQF⁶⁰GPNHKCCTSTCTDGI⁷⁰CPIVPV⁸⁰TAD⁸⁵LY

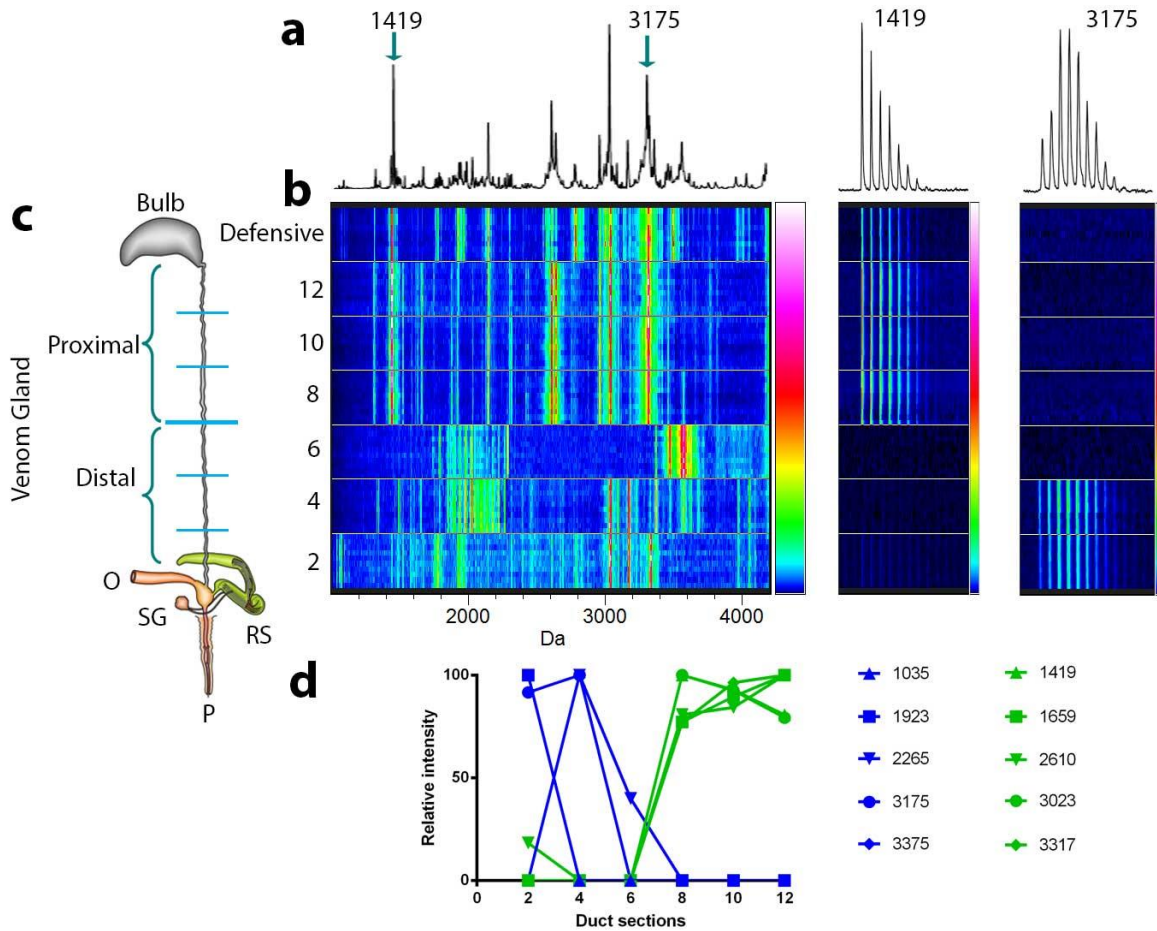
Superfamily A

GEORS001 MGMR¹⁰MMFTV²⁰LLVLAAT³⁰VSFTSDRAS⁴⁰GRNVAAKAFHRIGRT⁵⁰RDECCSNPA⁶⁰CRVNNPHV⁶⁵CRRR

Superfamily M

GEORS002 MMSK¹⁰LGVFL²⁰TICLLLFPI³⁰TALPLDEDQLA⁴⁰ERMQDDNSAAND⁵⁰PWFNPV⁶⁰KRCCEIC⁶⁵YGC⁷⁰SGN⁷⁵C⁸⁰CCG

Supplementary Figure 13. Conotoxin sequences from a *C. geographus* radular sac transcriptome. Only 3 sequences corresponding to conotoxin precursors were retrieved from the transcriptome of the radular sac, and all were expressed at very low levels (GEORS003 with 6 reads, GEORS002 with 2, and GEORS001 with 1).



Supplementary Figure 14. Distribution of toxins in *C. geographus* venom duct. Extracts from 6 sections of the venom duct (**a**) of a second specimen of *C. geographus* were spotted on MALDI plate together with the defensive venom from the same specimen. **b**, The average spectrum is again highly complex in the range 1000-4000 Da, corresponding to the size of the most common conotoxins. **c**, The gel-view representation shows distinct regionalization of many components, consistent with the data obtained for a separate specimen shown in Fig. 3. **d**, Furthermore, quantification of five selected predatory (including Conopressin G (1035) and Conantokin G (2265)) and defensive toxins (including GII (1419), GIIIA (2610) and GVIIA (3317)) clearly shows a tight correlation between distal duct producing predation-evoked venom peptides and the proximal duct producing defensive-evoked venom peptides.

Supplementary Table 1: MS/MS sequence coverage of conotoxin transcripts.

| Superfamily | ID | MS/MS Sequence (in bold) | Source | PTM |
|-----------------------|------|---|------------|------------|
| Con-ikot-ikot | G001 | MAMSMSMTLSVFMVMAATVTGFTQLKKPDLSRMKRDN ACCTNAIQ CLKRNPQESYNTPPCHAAATTRCPGSHIDGCCPKY AMCMSVNAQN NLEAAHTSCLNRPCFDPCE | PEV | O |
| Con-ikot-ikot | G003 | MAMSMSMTLSVFMVMAATVTGFTLQVPLNSRMRERDEPLDCCEMKK CCVRSMECLQDHR GNEYDMATECYQK AGDICGSYNEIVGCCQGYEICIL KNVPHTGLQ RAHDLCESTDCYNPCQ | PEV | |
| Con-ikot-ikot | G005 | MAMSMSMTLSVFMVMAATVTGFTQLKKPDLSRMKR NNKVCCTNAIQ CLKRNPQESYYAHP CQQAETVSCPGSDIDGCCPGYTM CMSTNAQNNV HTAHTSCLNRPCFGPKC | PEV | O |
| Conkunitzin | G037 | MEGRRVAALVITICMLALEAGAL LRDPPLCNLLPDTG PLAHERFYFDR FSKECKVFIYGGC PGNANNFTAKEYKRCGNK | DEV | O |
| Conkunitzin | G038 | MEGRRLAVLIVTISLTSALTVDTR SVPDVCLQPM DVGPCEQLPRYYFN AVQVTCKRFDYGGCGGNQRFNSKDDCLKKCLYSLT | DEV PEV | |
| Conophysin_conopresin | G039 | MTRSAMQMGRLLVCLLLLLLTTQACFIRNCPKGGKRDVDER YLFKAC FGQC VGPRI CCGPRGCEMGTAEANRCIEEDEDPIPCQVVGQHCDL NNPGNIHGNCVANGICVDDTCTHTGCL | DEV PEV | |
| Contryphan | G040 | MGKLTILVLAALLSTQAMVQGGDQPAAR NAVPRDDNDGPSAKFMN VQRRSGCPWEPWCG | DEV | |
| Superfamily_A | G044 | MGMRMMFTVFLVLAATTVVSPSERASDGR DDTAKDEGSDMDKLEVK ECCNPACGRHYSCGR | DEV | |
| Superfamily_A | G045 | MGMRMMFTVFLVLAATTVVSTSRGPKSRRGEPVPTVINYGECKD PSCWVKVDFQCPGASPPN | PEV | O |
| Superfamily_A | G043 | MGMRMMFTVFLVLAATTVVSPSERASDGRDDTAKDEGSDMEKLEVK KECCNPACGRHYSCGGR | DEV | * |
| Superfamily_A | G059 | MGMRMMFTVFLVLAATTVVSTDRASDRR NAAVKAFDLISSTVKKGC CSPACSGNNPEYCRQGR | PEV | * Y-So4 |
| Superfamily_A | G059 | MGMRMMFTVFLVLAATTVVSTDRASDRR NAAVKAFDLISSTVKKGC SHPACSGNNPEYCRQGR | DEV | * |
| Superfamily_A | G050 | MGMRMMFTVFLVLAATTVVSPSERASDGRDDTAKDEGSDMEKLEVEEK RCCNPACGRHYSCGGR | DEV | |
| Superfamily_A | G047 | MGMRMMFTVFLVLAATTVVSTDRASDRNVAAKAFHRIGRT IRDECC SNPACRVNPHVCRRR | DEV | O |
| Superfamily_A | G061 | MGMRMTFTVFLVLAATTVVSTDRASDRR NAAVKAFDLISSTVKKGC SHPACSGNNPEYCRQGR | DEV | |
| Superfamily_B | G066 | MLRLIIAAVLASACLAYPQRDGVPEASNL QGFQGMQAMPNMQGMQ GMPMPG MAGG QLFPNPNMAMPYK REDELEKRR HRYKFN GDSDAS PFQSEDFENFDMFKD NSNENLPLQQRGRRRQ | DEV | |
| Superfamily_B | G064 | MLRLIIAAVLASACLAYPQRDGVPEASNL QGFQGMQAMPNMQGMQ PMPGMTGGQLFPNPNMAMPYK REDELEKRR HRYKFN GDSDAS PF QSEDFDNFDMFKD NSNENL PFNNVEGA ANDLGQFEPSAENEDGKF RFFDKQQ | PEV | |
| Superfamily_J | G068 | MTSVQSVTCCLLWMLSVQIPITGSPGPAQLSRER SFKFLSGGFEIVC HRYCAK IA KEFCNCPDK RDVVSPRIRRRKSKAM | DEV | |
| Superfamily_M | G069 | MMSKLGVLLTICLLFLPTALPMDGDEPADRPVERMQDNISSEYQPLFEKR RDCCTPPKCKDRQCKPQCCAGR | DEV | * O |
| Superfamily_M | G069 | MMSKLGVLLTICPLFLPTAL PMDGDEPADRP VERMQDNISSEYQPLFEKR RDCCTPPKCKDRQCKPQCCAGR | DEV | |
| Superfamily_O1 | G084 | MNLTCVLIIVLFLTACQLLTADDSRDQKYRAVRLGDAMRIFKTRE KLCE LYDGCHDQRC CPGL CTDLFQCVRHS | PEV | |
| Superfamily_O1 | G085 | MKLTVCVIVAVLLLTACQLITADSRGTQKHHLRSTTKLSMLRTR SRDWCG DAGDACGTLKLRCCSGLCNQYSGTCTG | PEV | * W-Br |
| Superfamily_O1 | G081 | MKLTVCVIVAVLLLTACQLITADSRGTQKHHLRSTTKLTLST RCVPSGG SCSR TA YSCCHGSCSGGRCG | DEV | |
| Superfamily_O1 | G086 | MKLTVCVIVAVLLLTACQLITADCGGTQKHHLRSTIKLSLLRQHR GWCG DPGATCGKLR LYC CSGFCD CYTKT | DEV | |
| Superfamily_O1 | G087 | MKLTVCVIVAVLLLTACQLITAEDSRGMLKHLRSTKVK SPPCLVAGSSC RG TTRVCCGFCSHFYKCRDRPTS | DEV | O |
| Superfamily_O1 | G089 | MKLTVCVIVAVLLLTACQLITADSRGTQKHHLRSTTKLSMLRTR SRDWCG DAGDACGTLKLRCCSGLCNQYSGTCTG | DEV | |
| Superfamily_O1 | G091 | MKLTVCVIVAVLLLTARQLITADSRGTQKHHLRSTTKLTLSTRCKSPGTP CSR GMRD CT SCLLYSNK CRRY | DEV | |
| Superfamily_O1 | G083 | MKLTVCVIVAVLLLTACQLITADSRGTQKHHLRSTTKLTLSTRCK SPGS SCSPTS YN CCRS CPYTK RKCSG | PEV | O |

| | | | | |
|----------------|------|--|------------|-----------|
| Superfamily O1 | G080 | MKLTGVVIVAVLLLTACQLITADDSRGTQKHRALGSTTELSLSTRCKSPGS <i>SCSPTSYNCCRSCNPYTKRCYG</i> | DEV | O |
| Superfamily O1 | G079 | MKLTGVVIVAVLLLTACQLITADDSRGTQKHRALRSSTKLTSTRCKSPGTP <i>CSRGMRDCTSCLLYSNKCRRY</i> | DEV | |
| SF O1-var1 | G092 | MNLTGVIIIAVFLFTACQLIAADSRDNQKHRAVRMRDALKNFKDSRACSGR <i>GSRCPPQCCMGLTCGREYPPRCG</i> | DEV | O |
| SF O1-var2 | G095 | MKLACVVIVAVLLLTACQLITALDSRSPVTKCSDGTHPTLVKHCKATGEYCRYVTRCCCKGNNNRCT | PEV | O |
| Superfamily O2 | G098 | MEKLTILLVAAVLMSTQAMI <i>QGGGENRPKN</i> IKNYLSKRSQSAESGIWGECS DFLTGCTSPSQCCSENCETYCRAW | DEV | D-pyr |
| Superfamily O2 | G096 | MEKLTILLVAAVLMSTQAMIQGGGENRPMENIKYLSKRSQSAERGV <i>WSECSDWLAGCSSPSECCSEKCDTFCRLWR</i> | PEV | O W-Br |
| Superfamily O2 | G096 | MEKLTILLVAAVLMSTQAMI <i>QGGGENRPMENIKYLSKRSQSAERGVWSECSDWLAGCSSPSECCSEKCDTFCRLWR</i> | DEV | O W-Br |
| Superfamily S | G104 | MMSKMGAMFVLLLLFTLASSLQEGDVQARKTRLSDFYRALARDDRGCTRTCGGPKCTGTCTCTNSSKCGCRYNVHPSG <i>WGGCGCACSG</i> | PEV | * W-Br |
| Superfamily T | G111 | MLCLPVFIILLLLASPAAPNPLERRIQSDSIRALEDADFNLDER <i>SFNSIIKALGELIRAKIPVIVDMVPGGKK</i> | DEV | * |
| Superfamily T | G113 | MLCLPVFIILLLLASPAAP <i>KPFETKLPSDLTRADVIDMAVFEKLQDACCKNAPEFGCCTR</i> | DEV | |
| Superfamily T | G112 | MQCLPVFTILLLLASTAAPNPLETRIQLDTR <i>ADLESDTKTDERFITGLLGLLSAVGGITSLASRICCAITDSCC</i> | DEV | |
| Superfamily T | G110 | MLCLPVFIILLLLASPAAPNPFERRIQSDSIRALEDADFNPDERS <i>SFNSIIKALEELIRAKFPVIVDMLPGDKK</i> | DEV | |
| Superfamily I3 | G117 | MKLFLAIVLILMLQFLSTGAETSDNHSRSTTALRDWLLGPKAKR <i>CAVTHEKC</i> <i>SDDYCCGSLCCVGCIAKTIAPCK</i> | PEV DEV | |
| New 2 | G121 | MTTSFYFLLVALGLLLYVCQSSFGNQHTR <i>NSDTPKHRCGSELADQYVQLCHGKRNDAGKKRGRASPLWQRQGLSMLKA</i> <i>KRNEAFFLHRDGRGIVECCDNPCTVATLRTFCH</i> | PEV | |
| New 3 | G125 | MGSMKIYLCLAFLLLPFTIVDSGLLDKIETIRNRR <i>DESKDGCNCAELRSSRCTEAMFCLTPELCTPSVSCPTGECRCK</i> <i>FHQSRCTRFEECVFNKCRDA</i> | PEV | |
| New 3 | G126 | MGSMKIYLCLAFLLLPSTIVDSGLLDKIETIRNRR <i>DESKDRCNCAELRSSRCTQAI FCLTPELCTPSISCTPTEGECRCKFH</i> <i>QSRCTRFEVCPNKTDA</i> | PEV | |
| New 4 | G127 | MGATLVTKLLAAALLGLCHEMANPEAWDECYER <i>MHMHLIAPTWHAMYNCHIELEDRKRAALVLKQLGVIHLDHGG</i> <i>WVQHVKLLKYLGVNN</i> | PEV | |

To determine their toxin composition, both predation- and defence-evoked venoms were analysed by LC-MS/MS. Only MS/MS coverage (in bold/italic) of transcriptomic sequences with a confidence value of 99 are reported. Amino acids identified with post-translational modifications (PTMs) are underlined (O, hydroxyproline; Y-So4, sulfotyrosine; *, C-terminal amidation; W-Br, Bromotryptophan; D-pyr, pyroglutamate). Predation-evoked venom (PEV) and defence-evoked venom (DEV) highlighted blue and green, respectively, whereas conotoxins detected in both venom types are shown in pink.

Supplementary Table 2. Molecular evolution of *C. geographus* conotoxin gene superfamilies.

| | | FUBAR ^a | | PAML | | MEME ^b | BSR ^c |
|-----------------------|----------------|--------------------|----------------|------------|--------|-------------------|------------------|
| | | | | M8 | M2a | | |
| Con-ikot-ikot | $\omega > 1^d$ | 11 | $\omega > 1^f$ | 23 | 20 | 7 | 5 |
| | $\omega < 1^e$ | 2 | | (13+10) | (13+7) | | |
| | | | | $\omega =$ | | | |
| Conantokin | $\omega > 1^d$ | 5 | $\omega > 1^f$ | 17 | 15 | 31 | 43 |
| | $\omega < 1^e$ | 2 | | (16+1) | (15+0) | | |
| | | | | $\omega =$ | | | |
| Conkunitzin | $\omega > 1^d$ | 0 | $\omega > 1^f$ | 26 | 17 | 0 | 2 |
| | $\omega < 1^e$ | 0 | | (8+18) | (6+11) | | |
| | | | | $\omega =$ | | | |
| Contulakin | $\omega > 1^d$ | 1 | $\omega > 1^f$ | 0 | 0 | 0 | 1 |
| | $\omega < 1^e$ | 0 | | | | | |
| | | | | $\omega =$ | | | |
| Sumerfamily A | $\omega > 1^d$ | 1 | $\omega > 1^f$ | 6 | 4 | 5 | 1 |
| | $\omega < 1^e$ | 0 | | (3+3) | (1+3) | | |
| | | | | $\omega =$ | | | |
| Sumerfamily M | $\omega > 1^d$ | 2 | $\omega > 1^f$ | 14 | 14 | 6 | 7 |
| | $\omega < 1^e$ | 0 | | (12+2) | (11+3) | | |
| | | | | $\omega =$ | | | |
| Sumerfamily O1 | $\omega > 1^d$ | 11 | $\omega > 1^f$ | 25 | 23 | 4 | 5 |
| | $\omega < 1^e$ | 1 | | (22+3) | (21+2) | | |
| | | | | $\omega =$ | | | |
| Sumerfamily O2 | $\omega > 1^d$ | 0 | $\omega > 1^f$ | 0 | 0 | 0 | 0 |
| | $\omega < 1^e$ | 2 | | | | | |
| | | | | $\omega =$ | | | |
| Sumerfamily S | $\omega > 1^d$ | 4 | $\omega > 1^f$ | 30 | 30 | 1 | 3 |
| | | 1 | | 28+2 | 28+2 | | |

| | | FUBAR ^a | PAML | | MEME ^b | BSR ^c |
|----------------------|----------------|--------------------|----------------|-------|-------------------|------------------|
| Sumerfamily T | $\omega < 1^e$ | | $\omega =$ | 28.14 | 28.26 | |
| | $\omega > 1^d$ | 0 | $\omega > 1^f$ | 3 | 1 | |
| | $\omega < 1^e$ | 0 | | (0+3) | (0+1) | 1 |
| | | | $\omega =$ | 1.54 | 2.73 | 0 |

a: Fast, Unconstrained Bayesian Approximation (FUBAR)

b: Sites detected as experiencing episodic diversifying selection (0.05 significance) by the Mixed Effects Model Evolution (MEME)

c: Number of branches detected as episodically diversifying by branch-site REL

d: Number of sites detected by FUBAR as under pervasive diversifying selection at the posterior probability ≥ 0.9

e: Number of sites detected by FUBAR as under pervasive purifying selection at the posterior probability ≥ 0.9

f: Number of positively selected sites detected using the Bayes Empirical Bayes approach implemented in M8 and M2a. Sites detected at 0.99 and 0.95 significance are indicated in the parenthesis.

ω : mean dN/dS

Supplementary Table 3. Molecular evolution of *C. marmoreus* conotoxin gene superfamilies.

| | FUBAR ^a | | PAML | | MEME ^b | BSR ^c | |
|-----------------------|--------------------|---|----------------|--------------|-------------------|------------------|---|
| | | | M8 | M2a | | | |
| Sumerfamily H | $\omega > 1^d$ | 2 | $\omega > 1^f$ | 14 (7+7) | 9 (6+3) | 1 | 2 |
| | $\omega < 1^e$ | 1 | $\omega =$ | 4.98 | 4.98 | | |
| Sumerfamily I2 | $\omega > 1^d$ | 4 | $\omega > 1^f$ | 16 (11+5) | 12 (7+4) | 6 | 7 |
| | $\omega < 1^e$ | 1 | $\omega =$ | 2.40 | 2.0 | | |
| Sumerfamily M | $\omega > 1^d$ | 8 | $\omega > 1^f$ | 10 (9+1) | 10 (9+1) | 4 | 7 |
| | $\omega < 1^e$ | 4 | $\omega =$ | 2.43 | 2.38 | | |
| Sumerfamily O1 | $\omega > 1^d$ | 8 | $\omega > 1^f$ | 9 (8+1) | 9 (7+2) | 6 | 4 |
| | $\omega < 1^e$ | 2 | $\omega =$ | 2.23 | 2.25 | | |
| Sumerfamily O2 | $\omega > 1^d$ | 5 | $\omega > 1^f$ | 13 (10+3) | 11 (8+3) | 4 | 3 |
| | $\omega < 1^e$ | 2 | $\omega =$ | 2.76 | 2.76 | | |
| Sumerfamily T | $\omega > 1^d$ | 9 | $\omega > 1^f$ | 7 (6+1) | 6 (6+0) | 1 | 8 |
| | $\omega < 1^e$ | 1 | $\omega =$ | 4.97 | 4.89 | | |

a: Fast, Unconstrained Bayesian Approximation (FUBAR)

b: Sites detected as experiencing episodic diversifying selection (0.05 significance) by the Mixed Effects Model Evolution (MEME)

c: Number of branches detected as episodically diversifying by branch-site REL

d: Number of sites detected by FUBAR as under pervasive diversifying selection at the posterior probability ≥ 0.9

e: Number of sites detected by FUBAR as under pervasive purifying selection at the posterior probability ≥ 0.9

f: Number of positively selected sites detected using the Bayes Empirical Bayes approach implemented in M8 and M2a. Sites detected at 0.99 and 0.95 significance are indicated in the parenthesis.

ω : mean dN/dS.

Supplementary References

¹Rockel, D., Korn, W. and Kohn, A. J. (1995) Manual of the Living Conidae. Volume 1: Indo-Pacific Region. Verlag Christa Hemmen (ed.), Grillparzertr., Germany.