

Supplementary Materials: Bottom-up Proteomic Analysis of Polypeptide Venom Components of the Giant Ant *Dinoponera quadriceps*

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Contig164_B2-2 *D. quadriceps* venom dipeptidyl peptidase 4
 [Corrected] 5'3' Frame +1 insertion G position 359

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1  gcgatatattagctctgtcgcggactactcgcgggatcggcgaggcgtcgcgctgcagcggtatnttctgaacatc
1  5' UTR Start M A G H T V L P L L
76  gtccccggagctcgcgatccacgaatcggccccgacggtgatccacATGGCAGGACACACGGTTCTCCCACTTTTG
151 GCGCTGTTCAGCTGTCTGTTTCGTCATCGGGCAAACACGAACAGTCTCGCGCTCGACGAAAGCAGCATCGATGTT
36  P V Y K D S D V K K P F L L E E I Y F H F Y T P F
226 CCCGTTTACAAGGATTCGGATGTAAAAAAGCCGTTCCCTCTGGAGGAGATTTATTTCCACTTCTACACACCGTTC
61  G F N G T W I S D D E I L M E N T I L G D I T K Y
301 GGGTTCACAGCAGTGGATCTCCGACGATGAAATCTTAATGGAGAACACGATCTCTCGCGATATCACAAGTAC
86  N V M T G E T T T M F D G K N L P L E Y K F G S G
376 AACGTAATGACGGGGAGACGACAACGATGTTTCGACGGGAAGAACTTACCGCTGGAATACAAATTCGGTTCGGGA
111 T F S P D K Q N I L F T Y N V K H V F R H S K A I M
451 ACTTTTCCAGCACAACAGAATCTCTTTACTTACAACGTCAAACACGTCAGGCTTCAGGACGATCATG
136 S I V V Y D V K R G T F E K V A N E S R V L L A K
526 AGTATCGTGGTGTATGACGTGAAACGGGGCAGCTTCGAAAAGTTCGCGAACGAATCCCGTGTCTGCTGGCGAAG
161 W L P N G N G L V Y V R N N D I Y H A V F K D G K
601 TGGTTGCGCAAGCGCAACGGATTGGTCTACGTTCCGTAACGACATTTATCAGCGGTTTTCAGGACGGCAAG
186 S I V R R L T K S G K S G I V F N G I S D W V Y E
676 AGCATAGTCGCAAGACTGACGAAGAGCGGCAATCCGGAATTTGTTCAACGGAAATTCAGACTGGGTGTATGAA
211 E E V Y A S S T A M W H S P H G R Y L A F A T F N
751 GAGGAAGTATACGCTTCGTCGACCGGATGTGGCATTTCGCGCATGGACGGTATCTGGCCTTCGCAACCTCAAC
236 D T Q V R D M E Y S H Y G V P G S L R D Q Y P T Q
826 GACACTCAAGTGAGGGATATGGAGTATCCCATACCGCGTGCCTGGTCTCTAGGGACCAATACCCACGCAA
261 V K L K Y P K V G T P N P I V S L S V I D L T D P
901 GTGAAGTCAAGTATCCCAAGTGGGCACGCCGATCCGATAGTGTCTTGTGCGGTGATCGATCGACGATCCA
286 S S E A V T L E A P V H V V G S D N I L Y T V S W
976 TCGTCGAGGCGGTACGTTAGAAGCGCCGTCACGTTGGTTCGGCAGCGACAACATCCGTATACCGTGTGCTGG
311 W N L T H V T A T W T N R V Q N Q S Q L V M Y D A
1051 TGAACCTGACGACGTCACCGCAACGTCGACGCAACCGGTCGAGAAATCAATCCAGCTGGTGATGTACGATCGC
336 Q G T A K L V W Y D E E R E G W L Q P N S P V K V
1126 CAGGGCACCGTAAAGCTGTTTGGTACGACGAGGAGGGAAGGCTGGCTTACGCCAACAGCCCTGTGAAAGTC
361 G N Y A L L L R Q E D S G T S A G G K F R H I V R Y
1201 GGCAATTACGCGTTGCTCTACGCAAGAGGATTCGCGCACGAGCGCCGGAAGTTCGGGCACATAGGAGATAC
386 E C D D G R F T S R V D L T P G P S E V H S I Q A
1276 GAGTGCACGACGCGCGGTTCACTTCGCGAGTGGATCTCACGCCCGGTCCTCCGAGGTGCACTCGATCCAGGCC
411 V D S R R R G R V Y Y F A T A P G G E P S R R N L Y S
1351 GTCGATTCGCGAGAGCCGAGTACTACTCTGCTACGCGACCGGAGCCAGCCGAGGAAATTTGATTCG
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486 T I H D E N H R R I T T W E D N K L I R D M L S K
1576 ACCATACACGACGAGAACCATCGCGAATCAGACGTTGGGAAGATAACAATGATAAGGACATGCTGTCCAAG
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1801 GACGTAGTAAAGTTCGGATACGAGCATTACATGACGACCAACAACCGGTCATTTACGGTGGATCGACGGACGA
586 G S A F K G S K M L F E I Y R R I G T V E V E D T
1876 GGATCAGCCTTCAAGGGCAGTAAGATGCTGTTTCGAGATATACAGGCGCATAGGAACCGTTCGAGGTGCAAGACAG
611 I A V T K I L Q E R Y S W I D P D R T G I W G W
1951 ATCGCTTTACCAAGATTTCTACAGAACGGTACAGCTGGATCGACCCGACAGGACCGGATATAGGGGCTGGAGT
636 Y G G F T T G M V L A T D V E S V F K C G I S V A
2026 TACGGTGGTTACCACCGGCATGGTTCTCGCCACGGAGCTAGAATCCGTGTTCAAGTGGGCTTTCAGTCCGG
661 P V T S W I Y Y D S I Y T E R F M G L P T V D D N
2101 CCCGTAACCTCTGGATCTATTACGACTCCATTTACACAGAGCGGTTTCATGGGTTGCCAACCGTGGACGACAAC
686 L A G Y N A T D I S R V R G I R G K K Y M L I H
2176 CTAGCCGGCTACAACGCCACGGATATCTCGCGACGAGTGGAGGATAACGCCGAAGAAGTACATGCTGATACAC
711 G T E D N V H Y Q Q A M A L A K S L E R R D I L
2251 GGTACCAGGACGACAACGTGCATATCAGCAGGCGATGGCGCTCGCAAGTCCGTCGGAGCCGGGACATCCTA
736 F E Q V T Y T D E V H A L L R V S P H L Y H T M D
2326 TTTGAACAGGTACGTCACGACGAGGTGCACGCTCTTTCGCGGTGTCCCCCACCTCTATCACACGATGGAC
761 K F W S E C F G L D R T R *Stop 3' UTR
2401 AAATTTGGAGCGAGTGTTCGGCTCGACCCGCTGAcctgggtgctccgcccgcgacgagtcgacgcggt
2476 gagaatcgttcgcagcgcgagcggattcgtcgtccccgaaacgcaccagtttctccgcgaaacgatcgagaata
2551 cactctcggggatccgttcggacgatctccgtgacattacgtcgcactctcttcttgggagaatatttgacgt
2626 aacgtgtcctcggcaacgtcaatgacgacgggatgttccccgtggaaattcaggacgacgacagttccgagatac
2701 actctgtatccacgcagatatcctgcagacagctccgaacgcattaaaaactcgatcgtacaaagaaaaa
2776 gaaaaaaa polyadenylation signal
    
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◆ Active Site (Charge relay system)

polyadenylation signal

Figure S1. Structural nucleotide organization of venom dipeptidyl peptidase-4 of *D. quadriceps* expressed transcript contig164_B2-2. The product of this transcript is found in SDS-PAGE, gel band 1. (Figure 2, in the main text). In purple, part of the 5'UTR is shown, followed by the start codon that initiates the signal peptide of 21 amino acids predicted by SignalP 5.0 (in yellow). Followed by the coding region of a mature protein with 752 amino acids (pink), the active site is indicated by the catalytic triad (green diamonds). Followed by the stop codon, the 3' UTR region (purple) and the polyadenylation signal (ATTAAG), indicated by a yellow box.

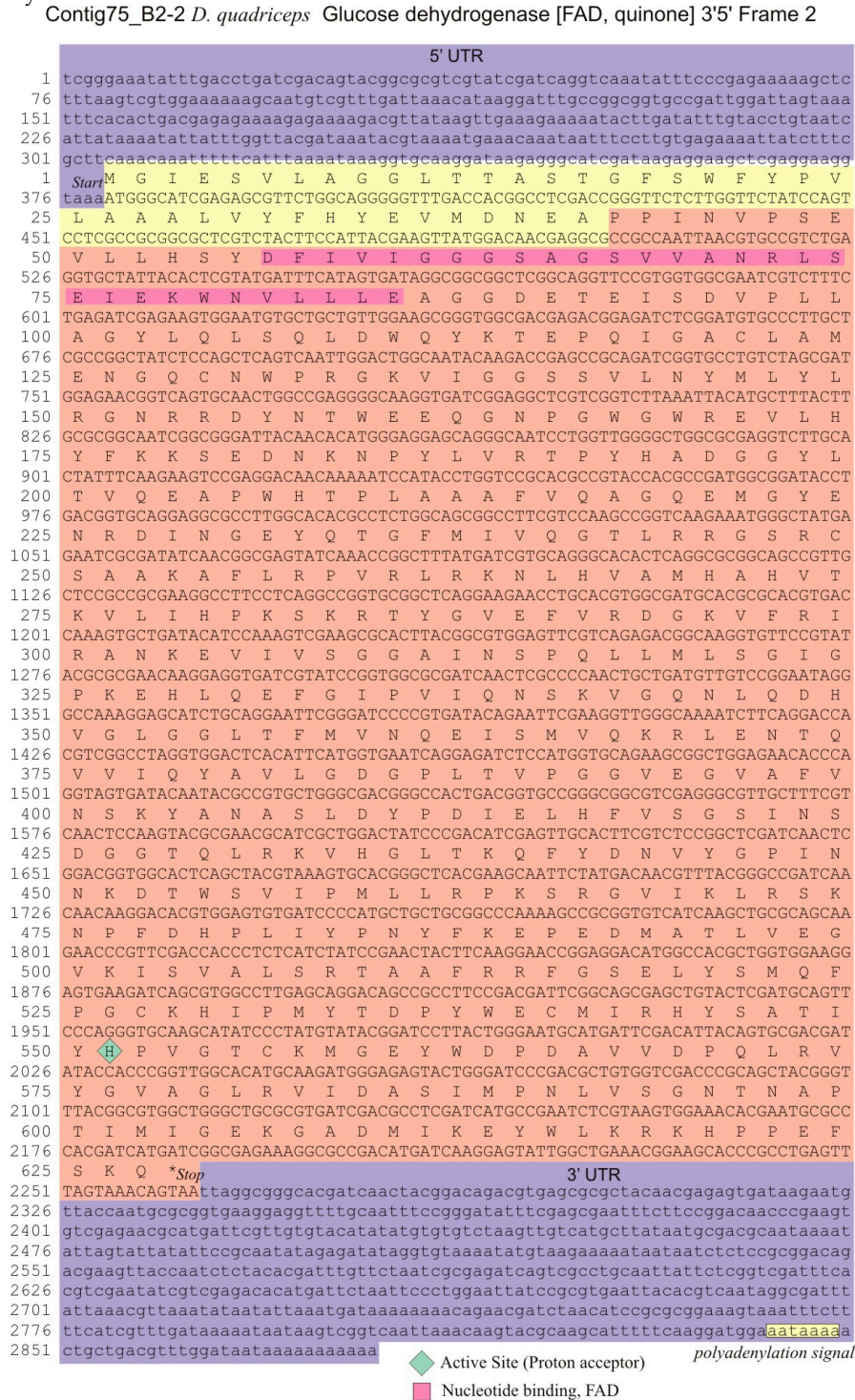


Figure S2. Glucose dehydrogenase precursor [FAD, quinone] encoded by Contig75_B2-2. The mature peptide was found in the venom proteomics, according to explanation in the main text. The cDNA encodes a precursor with 627 amino acids, the mature part of which is indicated in salmon. The nucleotide binding site is indicated by the pink box. The 5' and 3'-untranslated regions (UTRs) are indicated in purple. The polyadenylation signal is indicated by the yellow box. The proton acceptor of the active site indicated by a green diamond.

Contig98_B2-2 *Dinoponera quadriceps* transcribed RNA sequence
 Yellow like protein precursor [Major royal jelly protein domain] 430 aa

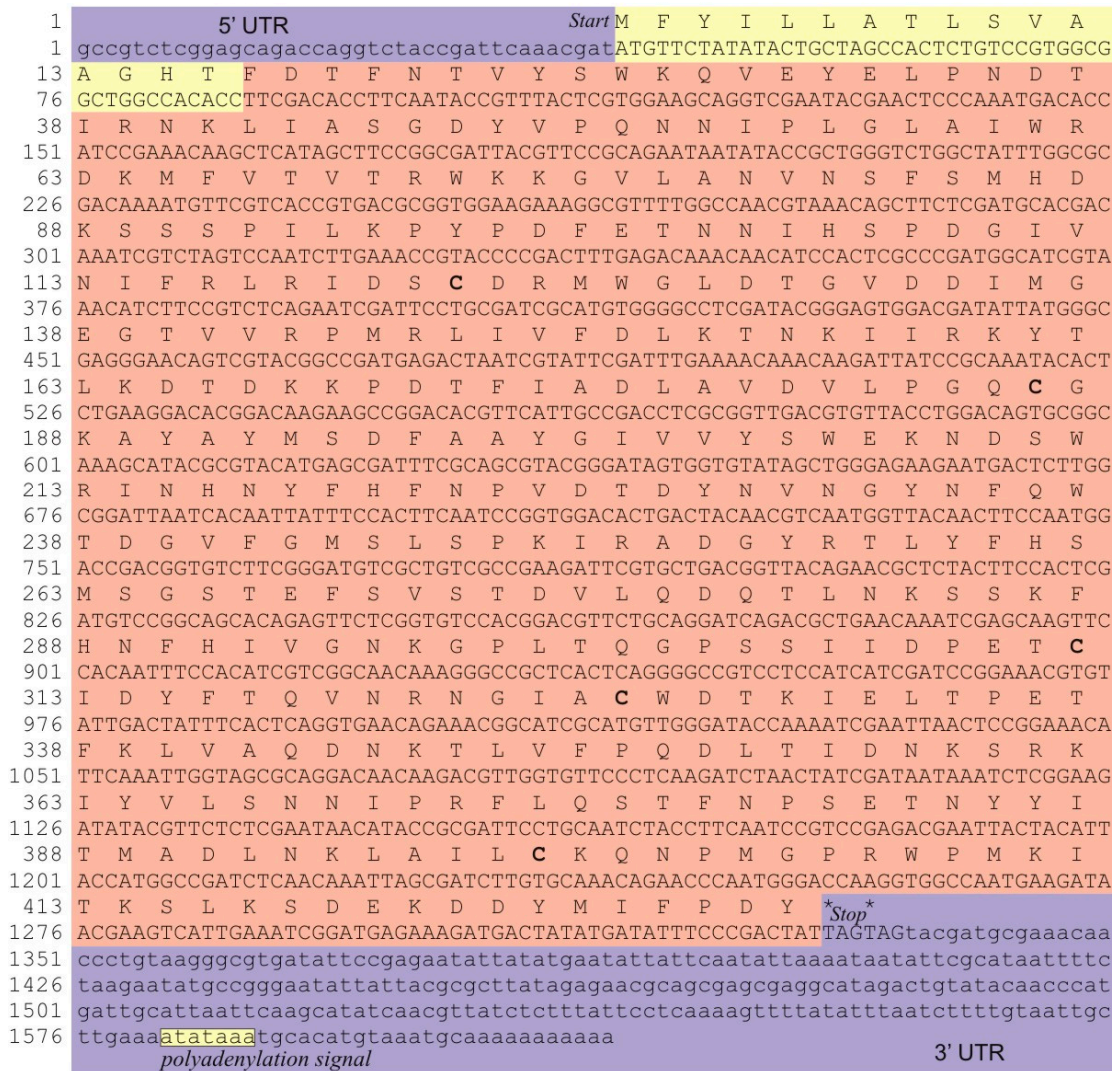


Figure S3. Structural nucleotide organization of the precursor of *D. quadriceps* MRJP-like venom protein. The contig98_B2-2 cDNA code for major royal jelly protein-like in giant ant venom gland. The mature toxins were found in the venom by the proteomic analysis (this study). Non-coding regions 5' and 3'-UTRs are indicated in purple. The predicted signal peptide is shown in yellow. The coding region for the mature protein (detailed in the main text) is indicated in beige/orange. Start and stop codons are indicated with asterisks. The putative polyadenylation site is indicated by the yellow box.

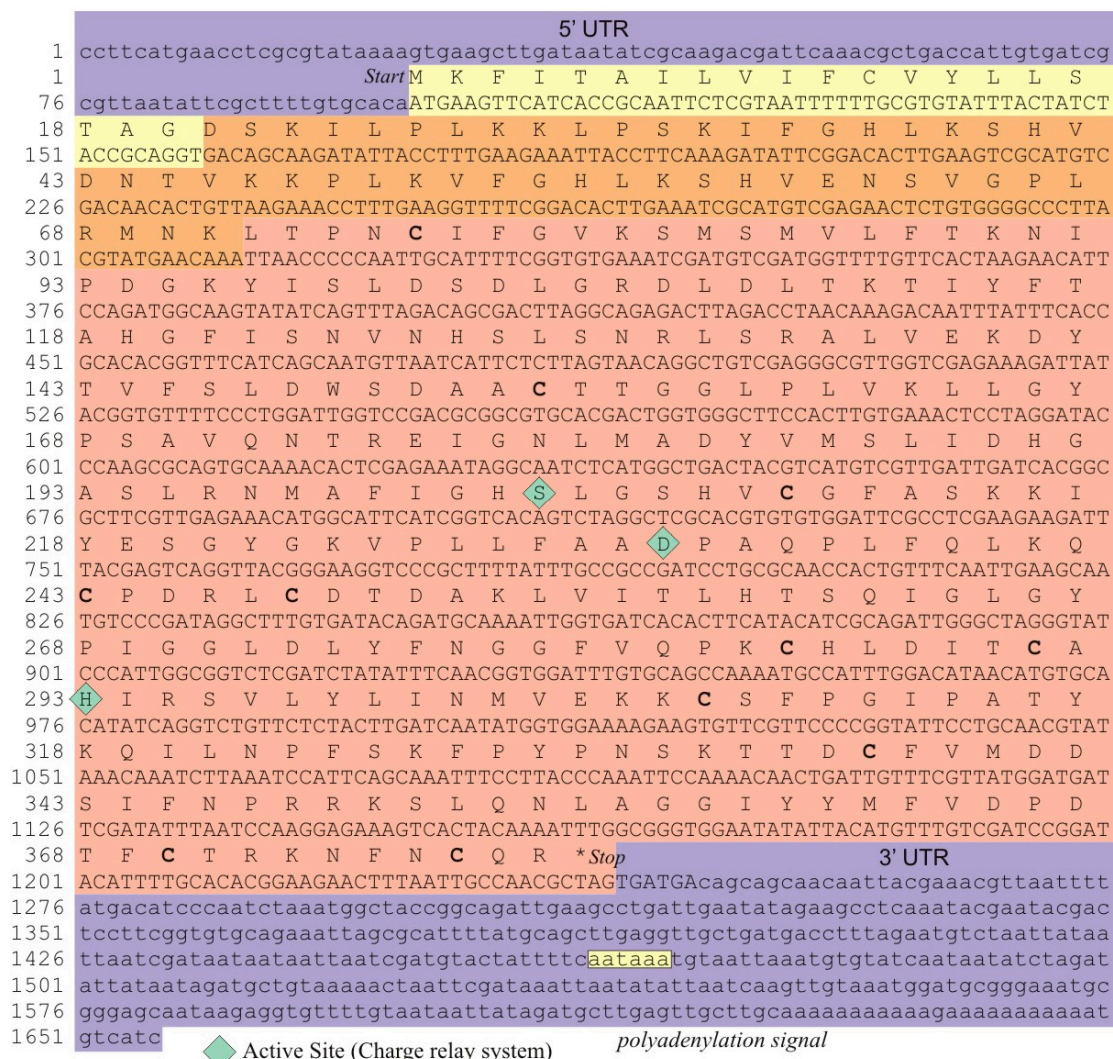
Contig27_B2-2 transcribed RNA sequence, phospholipase A1 2-like, *Dinoponera quadriceps*

Figure S4. Structural organization of the Contig27_B2-2 cDNA that code for phospholipase A1 in the *D. quadriceps* venom. The full-length precursor is 379-amino acid residues long. A signal peptide with 20 amino acids, as predicted by SignalP 5.0, is indicated in yellow. The 5' and 3'-untranslated regions are indicated in purple. The mature sequence is indicated in light pink and the putative propeptide is visible in the region indicated in orange. The mature peptide has 11 cysteines of which 10 are conserved among the PLA1s from the venom of hymenopterans. The mature peptide is found in the venom as detected by proteomic analysis (this study). The amino acids in the active site are indicated by green diamonds. The predicted polyadenylation signal is indicated by a yellow box

Contig385_B2-2 *Dinoponera quadriceps* venom Hyaluronidase

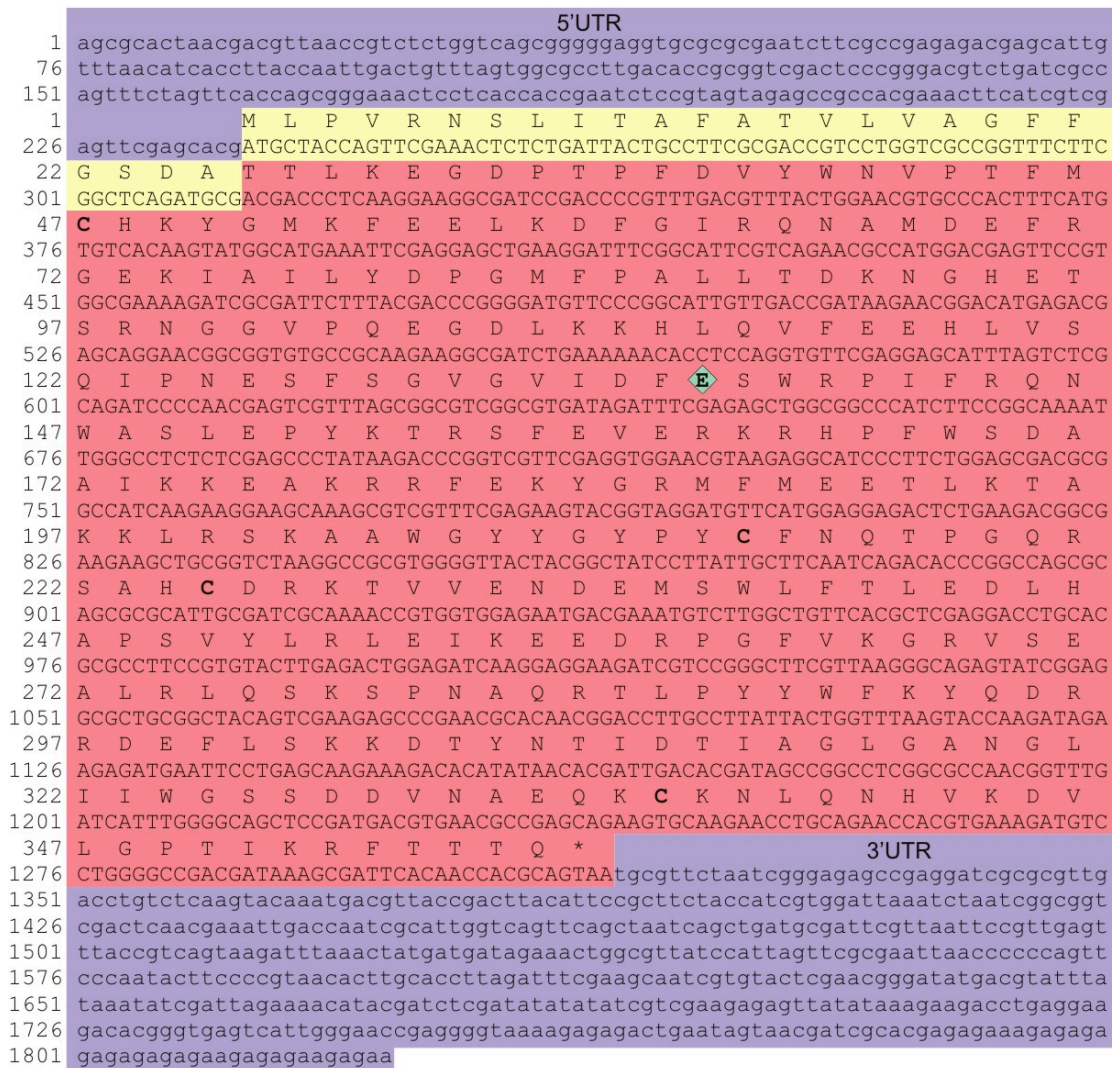


Figure S5. The venom hyaluronidase precursor, transcript contig385_B2-2, from *D. quadriceps* venom gland. Venom hyaluronidase was identified in proteomic analysis (this study) and confirmed the presence of the transcript product in the venom. Structurally, the untranslated regions, 5'-UTR and 3'-UTR, are indicated in purple. The coding region comprises a precursor of 368 amino acids, containing a signal peptide of 25 amino acids, as indicated in yellow, and a mature protein with 343 amino acids, as initiated in rose. This venom component belongs to the family of glycosyl hydrolases 56. The conserved cysteine residues, that form intramolecular disulfide bonds are indicated in bold and the proton donor of the active site is indicated by a green diamond. These characteristics are typical of a catalytically active hyaluronidase, in *D. quadriceps* venom.

Contig12_B2-2 *Dinoponera quadriceps* Major Allergen-3 Precursor (CAP/SPC)

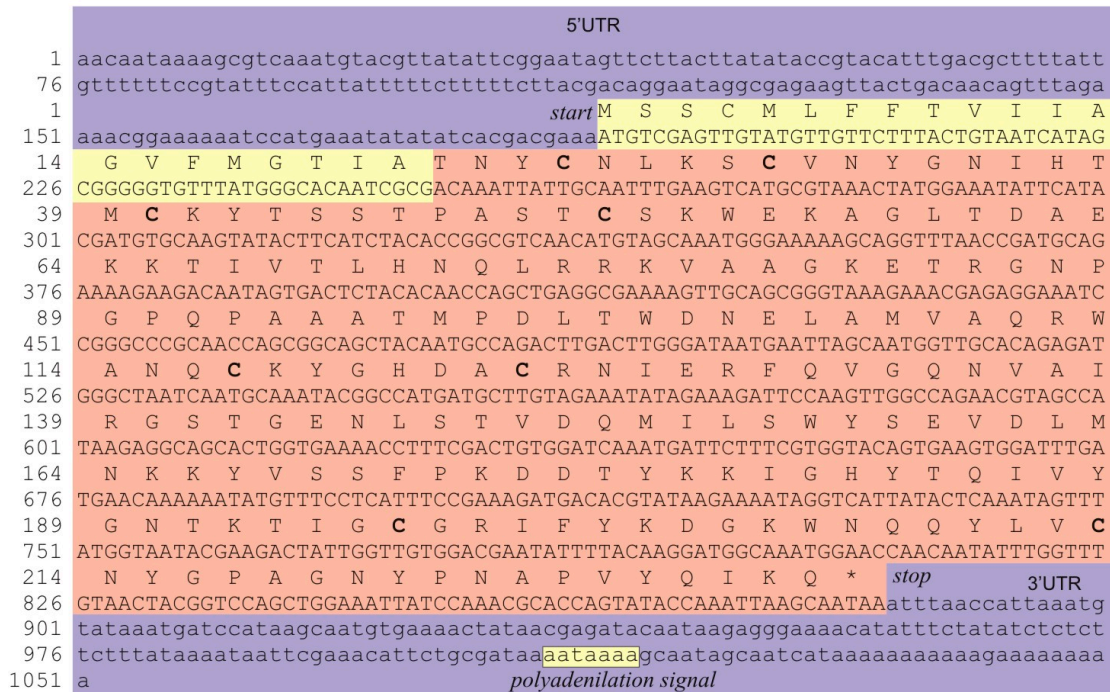


Figure S6. Structural nucleotide organization of transcript contig12_B2-2 that code for major allergen-3 in *D. quadriceps*. The major allergen-3 was identified in the proteomic, in this study, as one of the predominant components in the crude venom. In orange, the mature polypeptide is evidenced with its conserved 8 cysteine residues (in bold). This protein structure is typical of the V5-SCP/CAP (CRISP) family. Highlighted in purple, is indicate the 5'- and 3'-untranslated regions. In yellow the putative signal peptide as predicted by SignalP5.0. In yellow, the polyadenylation signal is boxed.

Contig8_B2-2 Ant Venom Allergen 2/4 (OBP/PBP)precursor *Dinoponera quadriceps*



Figure S7. Structural organization of *D. quadriceps* venom gland transcript for Venom allergen 2/4 (Contig29_B2-2) and respective protein precursor product. The sequence is 675 bp-long. In purple the untranslated regions (5 'and 3' UTRs) are shown. In yellow the signal peptide region predicted by SpecialP 5.0 (19aa) and in pink the mature peptide (112aa). A polyadenylation signal is indicated by a yellow box. Four conserved cysteines in the Ant venom Allergen 2/4 family are indicated in bold type and may form two disulfide bonds by similarity.

Contig511_B2-2 BPTI/Kunitz serine protease inhibitor

Dinoponera quadriceps

3'5' Frame 2

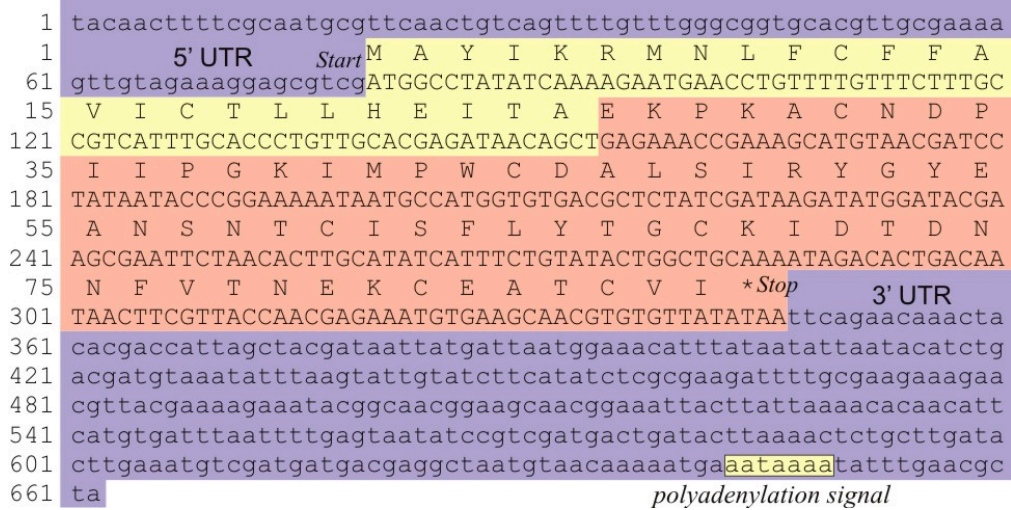
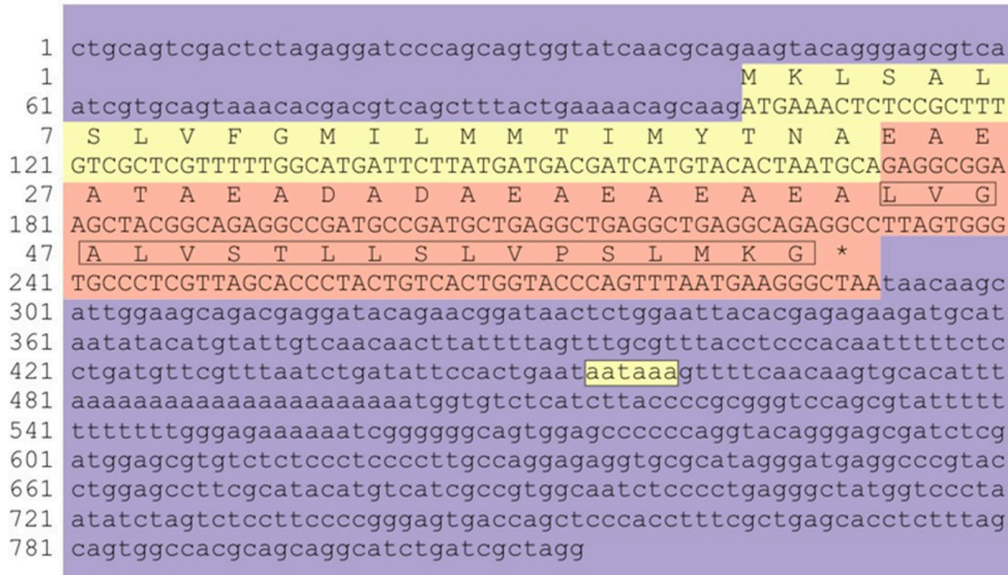


Figure S8. The *D. quadriceps* venom component BPTI/Kunitz-serine protease inhibitor. The structural organization of transcript Contig511_B2-2 from the *D. quadriceps* venom gland, identified by peptide mass fingerprint mass spectrometry analysis is shown. The signal peptide is shown in yellow; The mature part in salmon. The yellow box indicates the polyadenylation signal. In purple the untranslated regions.

Dinoponeratoxin pilusulin-like [Dq-1969.45]

Contig 1_A12_G5_1_DVC1



(A)

Dinoponeratoxin pilusulin-like peptide [Dq 2532.04 Da]
 Contig 1_F07_C7_2_DVB1

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1  tgctgtcgactctagaggatcccagcagtggtatcaacgcagagtacaggggaagcatta
1  5'UTR                               Start M K L S A L
61  atcgtgcggtaaatacaacgtagctttattgaaaacagcaagATGAAACTGTCCGCTTT
7  S I I F G M I L V M T I M Y T K A E A E
121 GTCGATCATTTTTGGCATGATCCTTGTGATGACGATCATGTACACTAAAGCAGAAGCGGA
27  A E A E A D A D A D A K A E A E A F W G
181 AGCTGAGGCAGAAGCCGATGCTGATGCCGATGCGAAAGCTGAAGCTGAGGCCTTCTGGGG
47  T L A K W A L K A I P A A M G T K Q N K
241 AACCCTAGCCAAGTGGGCGTTGAAAGCTATACCCGCAGCTATGGGCACGAAACAAAATAA
67  * Stop
301 GTGAaggaatgatgattatggaacaatatggaagaaaatgaataattcttgactatta
361 aaatttctttttgtttacgtttatttctgattatctagtgattctgatgatttaattatt
421 atcttgatatcgtttgacatttctgaataatttttcagcaagcataaaaaaaaaa
481 aaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa
541 taccgactcaaatttttcccggggagca 3'UTR
    
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(B)

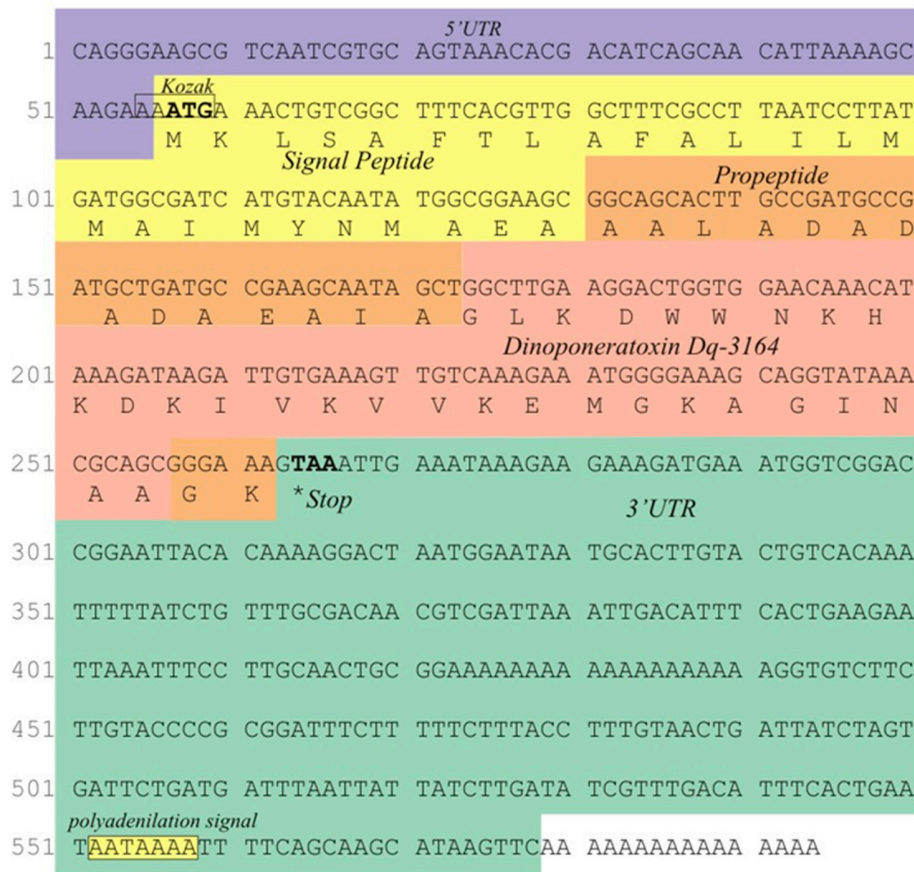
Dinoponeratoxin ponericin-like precursor [Dq-2562]
 Contig 1_F12_E3_DVA2

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                    5'UTR
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1  start M K L S A L
61  atcgtgcggtaaatacaacgtagctttattgaaaacagcaagATGAAACTGTCCGCTTT
7  S I I F G M I L V M T I M Y T K A E A E
121 GTCGATCATTTTTGGCATGATCCTTGTGATGACGATCATGTACACTAAAGCAGAAGCGGA
27  A E A E A D A D A D A K A E A E A F W G
181 AGCTGAGGCAGAGGCCGATGCTGATGCCGATGCGAAAGCTGAAGCTGAGGCCTTCTGGGG
47  T L A K W A L K A I P A A M G M K Q N K
241 AACCCTAGCCAAGTGGGCGTTGAAAGCTATACCCGCAGCTATGGGCATGAAACAAAATAA
67  * stop
301 GTGAaggaatgatgattatggaacaatatggaagaaaatgaataattcttgactatta
361 aaatttctttttgtttacgtttatttctgattatctagtgattctgatgatttaattatt
421 atcttgatatcgtttgacatttctgaataatttttcagcaagcaaaaaaaaaaaaa
481 aaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa
541 gaatttttcccccccccccccttgcccccaaaagatttaagggaaaagaaaaaaaac
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661 aaaagg 3'UTR
    
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(C)

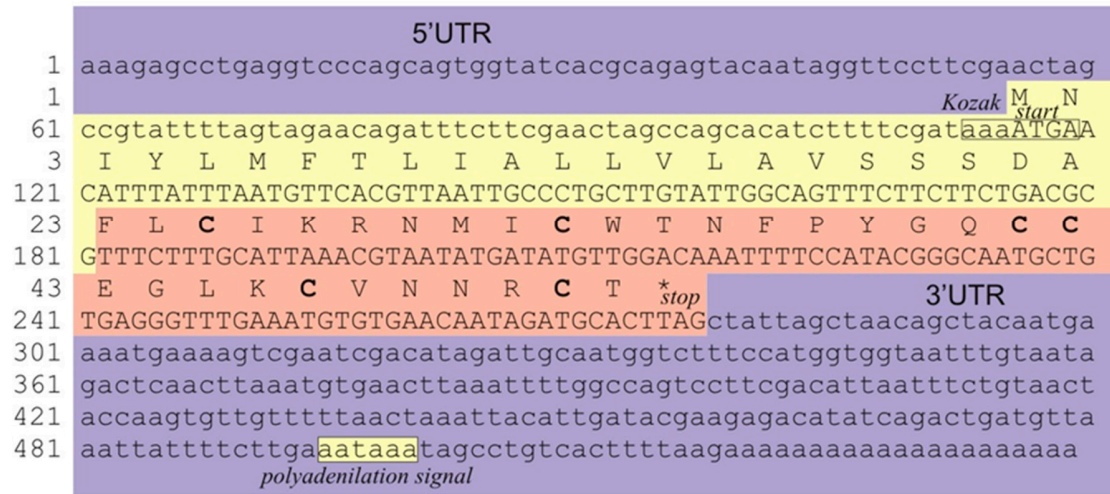
Dinoponeratoxin pilosulin-like peptide [Dq-3164.76]
 Contig Consensus_34 TX09_DIQUA



(D)

Figure S9. *D. quadriceps* venom gland transcript precursors for dinoponeratoxins (pilosulin- and poneracin-like peptides) with their respective peptide products, identified by proteomic analysis. The structural organization of transcripts and respective mature peptide, are as follow: **A.** Contig 1_A12_G5_1_DVC1 Pilosulin-like Precursor Peptide [Dq-1969]; **B.** Contig 1_F07_C7_2_DVB1 Pilosulin-like Precursor Peptide [Dq-2532]; **C.** Contig Consensus 34 Pilosulin-like Precursor Peptide; **D.** Contig 1_F12_E3_DVA2 Pilosulin-like precursor peptide [Dq-2562]. In purple, the transcript untranslated regions (UTR) are shown; in yellow the signal of peptides as predicted by the SignalP 5.0 algorithm and in salmon the regions of the pro-peptide. A yellow box indicates the signal of polyadenylation convenience in every 3'-UTR sequence. The amino acid sequence of the mature peptide, of which fragments were identified by mass spectrometry analysis (this study) are indicated by open box or underlined.

D. quadriceps poneritoxin ICK-like



(A)

D. quadriceps Knottin-like Contig516_B2-2



(B)

Figure S10. Structural organization of transcripts encoding knottin (ICK)-like toxins from the venom gland of *D. quadriceps*, of which identifiers of mature peptides were found in the venom proteomics. A. Contig 1_D07_A11_4_DVB1 coding for an ICK-like toxin (U1-poneritoxin-Dq5a) similar to neurotoxins from honey bee and *Conus* sea snail. B. structural organization of the transcript contig516_B2-2 gi|578895399| encoding a toxin similar to neurotoxins from honey bee and the ponerine ant *O. monticola*. The 5' and 3'-UTR transcript termini are shown in purple. The signal peptides were predicted by SignalP 5.0 and are indicated by yellow boxes. The mature peptides, of which the full sequences matched several peptide fragments identified by in-solution proteomics, are enlightened in pink color. The polyadenylation signal (AATAAA) are indicated by yellow boxes. The start codon and the stop codon are indicated the conserved cysteines which may form disulfide bonds are indicated in bold.