

SUPPLEMENTAL INFORMATION

Venom chemistry underlying the painful stings of velvet ants (Hymenoptera: Mutillidae)

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Fig S1. *D. klugii* venom enzymes. a) *D. klugii* venom metalloprotease aligned with *D. melanogaster* neprilysin (Q9W436). b) *D. klugii* venom PLA₂ aligned with *Apis mellifera* venom PLA₂ (P00630). Active site residues are boxed in red. The cytoplasmic and transmembrane domains of *D. melanogaster* neprilysin are underlined in green and orange, respectively. Signal peptides are underlined in purple.

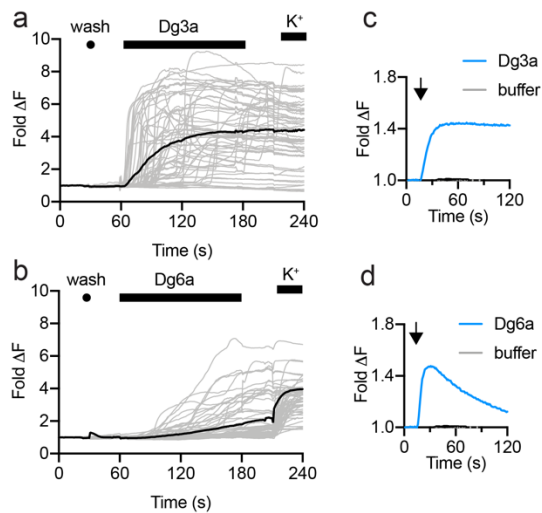


Fig S2. Defensive peptides of *D. gloriosa* venom. (a) Application of Dg3a (10 μ M) to DRG cells produced a rapid, non-cell-specific increase in $[Ca^{2+}]_i$. (b) Application of Dg6a (10 μ M) to DRG cells produced a gradual, non-cell-specific increase in $[Ca^{2+}]_i$. Each trace represents the fluorescence of a single cell in the field of view, with the average of all traces displayed in color. (c) Activity of Dg3a (10 μ M) on F11 cells. (d) Activity of Dg6a (30 μ M) on F11 cells. Traces corresponding to the change in $[Ca^{2+}]_i$ and representing the mean of 2–3 experiments, are displayed.

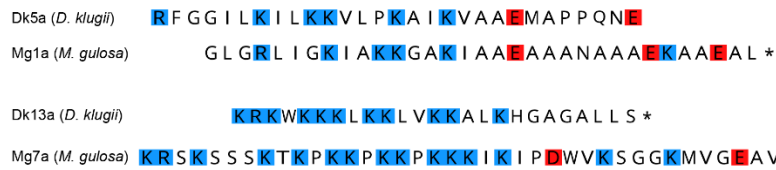


Fig S3. Defensive venom peptides of *Dasymutilla klugii* (Mutillidae) and *Myrmecia gulosa* (Formicidae). *, C-terminal amidation; [^], O-linked glycosylation. Lysine/arginine and aspartate/glutamate residues are highlighted in blue and red, respectively.

Table S1: venom components of *D. gloriosa*. TPM, transcripts per million.

	TPM	Sequence
Dg1a	575499 [†]	IKHRICKRRPNSPWCQ*
Dg2a	163727	SRVCNRPNLRWCR*
Dg3a	44430 [‡]	KKKKKWRKLLKLLKALKHGAGAVLS*
Dg3b[‡]	203	KKKKKWRKLLKTPQESSEAWSRSS
Dg3c[‡]	108	KKKKKWRKLLKLLKALKHGAGAILSE
Dg4a[‡]	42811	LSPAVIASLG*
Dg5a	9787	LVGALLGAVELGKLIYDLVKN*
Dg6a	6590	GIGGLLKVLGKVPKAVKVA AHLAPSQDE
Dg6b	3946	GIGGLLKVLGKVLPAIKVA AHLAPPQNE
Dg7a	6460 [†]	HLGGVLTDLVNKAL*
Dg8a	6377	KKGSRFSKLCCKKGGCKIFKNFLKNFGRGAAEAAGGA AVEVA ADEAASE
Dg8b	1558	KKGSIFSCLKCKKGGCKIFKNFLKNFGRGAAEAAGGA AVEVAA DEAASE
Dg9a[‡]	6365	LTPTRTRPTTPRTSRRFIVRVVPAGRL
Dg10a[‡]	6317	KRPPLTPRI*
Dg11a	5410	SKPGICKLVPHIPSCR*
Dg11b[‡]	1339	SKPGICKLVPHVPPCR*
Dg11c[‡]	1034	FKPGICKLVPHIPSCR*
Dg12a[‡]	2029	KKGRSRGGGGKRGKKTKNKIVSKIGDMIKDTLNKVGVAAGET AVEKTVEAVKDALQSEEEEEAPSE
Dg12b[‡]	1390	KRGRSRGGGGKRGKKTDKIASKIGDMIKDTLNKVGVAAGET AVEKTVEAVKDALQSEEEEEAPSE
Dg12c[‡]	581 [†]	KKGRSRGGGGKRGKKTKNKIASKIGDMIKDTLNKVGVAAGET AVEKTVEAVKDALQSEEEEEAPSE
Dg13a[‡]	191 [†]	RRAPPLPY
Dg14a[‡]	105 [†]	LLGGLVKVLAKVLPVVGAINPK
Dg14b	67	LFGGLVKVLAKVLPVVGAINPK
Kunitz domain proteins[‡]	409	
M13-metalloproteases[‡]	2826	
Phospholipase-A₂[‡]	161	
Hyaluronidase[‡]	84	

* C-terminal amidation; [†] The TPM value is the sum of more than one assembled transcript encoding the same mature peptide; [‡] Complete primary structure not confirmed by MS/MS.

Table S2: venom components of *D. bioculata*. TPM, transcripts per million.

	TPM	Sequence
Db1a	360958 [†]	SIPRVCKIKPSLPLCR*
Db1b	22892	SIPRICKIKPRLPLCR*
Db2a	123131	FLSKYGICKIKPLLQCRRQNW
Db2b	12167 [†]	LLSKYGICKIKPWLQCRRQNW
Db3a	40503	VFVPDHLMCKVRPYLPWCK*
Db4a	22112	SKPGICKLVPHIPPCR*
Db4b[‡]	11611	SKPGICRLMPHIPPCR*
Db5a	21091	HAPKRRICKTNPISPLCR*
Db5b[‡]	5382	HAPIRRICKINPMSPLCM*
Db6a	17026	SKTSICKLNPMLPMCR*
Db7a	14126	RRDGNRVRHDDLEIPDGVYNPQSPGVDVGKRSNDIFMDAAKR ELEAALRQ
Db8a	13940	LFIPRNVICKIRPYLSICK*
Db9a[‡]	13780	KKSKFKGSSGTGGKKSCKDKEPSKMDDILKFVSQ
Db10a[‡]	12132	RRAPPLY
Db11a[‡]	11368	KKRKWKKLKLIRKGLKHGAGALLT*
Db12a	10398	HLGGVLTDLVNKAL*
Db13a	8913	KKSKFKGSSDTGGKKSNDKKSSKMDDILKNTLKKVGVAVGKA AGEAAAKTTIEAIKDALQPGEESAEGEAPSE
Db13b	6117	KKSKFKGSSGTGGKKSCKDKEPSKMDDILKNTLKKVGVAAAGKA AGEAAAKTTIEAIKDALQPGEESAEGEAPSE
Db14a[‡]	7670	RALPRVGGYRVRHDDLAIPDGVYNPQSPGLDVGKLSDDIFIQA ARREFQEA VRQE QREKARRR*
Db14b[‡]	3915	RALPRGGGYRVRHDDLEIPDGVYNPQSPGLDVGKLSDDIFIQAA RREFQEA VRQE QREKARRR*
Db14c[‡]	1023	RALPRGGGYRVRHDDLEIPDGVYNPQSPGLDVGKLSDVVISFTS F
Db15a[‡]	7252	
Db15b[‡]	3600	
Db16a[‡]	5711	GPPGRIGAATSFVRASKTTTTKRPTTPKPKPKPKPKS*
Db17a[‡]	5030	FPSKHRICKIKPKLPWCR*
Db18a[‡]	3118	LSPAVIASLA*
Db19a	2928	RIGALIRVLRKVIPKAVKVAAHMAPQNEE
Db20a	2433	SKRGICKIYPRLSRCRRRMVGNIIAKT
Db21a	256	KRNQELPQCPPGYRRVLGRCRQML
Db22a[‡]	152	IIGKIKKMIKALKNL*
Kuntiz domain proteins[‡]	1551	
M13-metalloproteases[‡]	3123	
Phospholipase-A₂[‡]	61	
Hyaluronidase[‡]	29	

* C-terminal amidation; [†] The TPM value is the sum of more than one assembled transcript encoding the same mature peptide; [‡] Complete primary structure not confirmed by MS/MS.

Table S3: venom components of *D. sicheliana*. TPM, transcripts per million.

	TPM	Sequence
Ds1a	395479 [†]	SKHRICKIKPKLPWCR*
Ds1b	13306 [†]	SKRRICKIKPKLPWCR*
Ds2a	106089 [†]	LSPAVIASLA*
Ds3a	66353 [†]	VGPLTPR
Ds3b	64994 [†]	AGPLIPRI*
Ds4a	44253	VRVKPGICKIMPHIPPCKM
Ds5a [‡]	41625	EVKADPEPDPQFYPSR*
Ds6a [‡]	20222 [†]	GLPGRPGAATVFKATKTTTTKRPTKRKS*
Ds7a	18242 [†]	GISGLVKVLGKVLPKVAKVAAHLAAASQDQQ
Ds7b [‡]	2114	GISGLAKILGKVLPKVAKVAAHLAAASQDQQ
Ds8a [‡]	17313 [†]	KRGYRGGGKRRKQP
Ds9a	17009 [†]	RRGRPLPY
Ds10a	13861	KRGYRGGGKRRKQPKNKESKIGDAIKNALNEAGAAVGE TVAEKAVEAVKDALQPGEESAEGEAPSE
Ds11a	12944 [†]	FLGGLVKMLKKVPAAV*
Ds12a	10949 [†]	KKRKWRKLLKLLKKGLKHGAGVLLS
Ds13a	10590	HLGGVLTDLVNRAL*
Ds13b	2268	HLGDVLTDLVNRAL*
Ds14a	7144	ENPPQKPL
Ds15a [‡]	6136 [†]	GRRSRGNRGSERIYGGVYNPQSRGVDVGKLSDDIFIQARQE LADALARERAERAKAQRAR
Ds15b [‡]	1154	GRRSRGNRGSERIYGGVYNPQSRGVDVGRSDDIFIQARQE LADALARERAERAKAQRAR
Ds16a	5722	GRRSRGNRGSERIKIYGGVYNPQSRGVDVGKLSDDIFIQARQE FADALARAR
Ds17a	3945 [†]	VIKGFKLLKEALKKL*
Ds17b [‡]	3805	VIKEFKLLKEALKKL*
Ds17c	2063	VIKGFKLLKEAMKKL*
Ds18a [‡]	586 [†]	RTTTSTTIRPRIMVRIPLNRLGPLGRPPAAGA
Ds18b [‡]	513	RTTTSTTIRPRIMVRIRTIRTSSGRGIK
Ds18c [‡]	216	RTTTSTTVRPRIMVRIPLNRLGPLGRPPAAGA
Ds19a [‡]	489	
Ds20a	259	RDIILLIRRPKKKRHYIKIHKPKIRHIYI
Kuntiz domain proteins [‡]	9228	
M13-metalloproteases [‡]	12496	
Phospholipase-A₂ [‡]	170	
Hyaluronidase [‡]	76	

* C-terminal amidation; [†] The TPM value is the sum of more than one assembled transcript encoding the same mature peptide; [‡] Complete primary structure not confirmed by MS/MS.

Table S4: venom components of *D. occidentalis*. TPM, transcripts per million.

	TPM	Sequence
Do1a	165723 [†]	DADTVAALKELIELAKKGLERI*
Do2a[‡]	121053 [†]	VFVLPDICNERPHIPQCR*
Do3a	118842 [†]	VFTKPDICKVKPRFPPCR*
Do3b	74784	VFTKPNICKVKPRFPPCR*
Do3c[‡]	27559	VFTKPDICKLRPLLPCW*
Do3d[‡]	15025	VFFTKPDICKVKPLFRPCR*
Do4a	94692 [†]	AIYNICKIKPHLPCR*
Do5a[‡]	38566	VFDIPNICKKRPHILRCR*
Do6a	34292	LSPAVIASLVG*
Do7a[‡]	22025	LIFEPGICKVKPRLPLCR*
Do8a[‡]	18397	SELRICRTKPRSPLCR*
Do9a	17031	SIYNICKIKPRLPCRGRMIGKIF
Do10a[‡]	16884	KRKWRKKLKKLVKALKHAGALLS*
Do11a	16516	SKPSICKLVPFIPLCR*
Do11b	10607	SKPSICKLVPYIPLCR*
Do11c	9242	SKPSICKLMPHIPLCR*
Do11d	864	SKPTICKLVPHIPLCR*
Do12a	15126	KRKKRKGSKFGRNILTSFGKGAAEAAGEATVNAAVDQILEA QGEGEY
Do13a	14701	RIGGILKILKKVLPKAIKAAAEMAPPQNE
Do14a[‡]	9362	KRGRSRGDGKKGKKPKDKIASKIGDIIKNSLNKFGVVAGETI VEKTVEAVKDALQSGEASAESEAPSE
Do15a	5160	LVGAALGAVELGELIHHLIKK*
Do16a[‡]	3969 [†]	RRRGFGNENLDKIYGGVINPQSPGVDVGKLPDDIFIQARRE FAEALAREKAERAR*
Do17a	3341	HLGDVITDLVNKALNSL*
Do18a	2640	RRAPPLPY
Kuntiz domain proteins[‡]	840	
M13-metalloproteases[‡]	684	
Phospholipase-A₂[‡]	483	
Hyaluronidase[‡]	166	

* C-terminal amidation; [†] The TPM value is the sum of more than one assembled transcript encoding the same mature peptide; [‡] Complete primary structure not confirmed by MS/MS.