

Supplementary Materials: Functional Elucidation of *Nemopilema nomurai* and *Cyanea nozakii* Nematocyst Venoms' Lytic Activity Using Mass Spectrometry and Zymography

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1. Supplementary Information

Table S1. Identification of enzymatic constituents in jellyfish *C. nozakii* nematocysts venom (CnNV) indicated in Figure 4A by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Band No.	Venom Family	Protein Name/Source Organism	Mass (Da)	Accession	Protein Score	Sequence Coverage
CnNV-1~66 KDa	Phospholipase D	Phospholipase D(Fragments)/Loxosceles cf. spinulosa	31,968	C0JB40	28	8%
		Phospholipase D SpeSicTox-betaIB4 (Fragment) OS = Sicarius peruensis	31,259	C0JB34	27	13%
	Phospholipase A1	Phospholipase D LbSicTox-betaIA1a OS = Loxosceles boneti	31,921	Q5YD76	19	9%
		Phospholipase A1 OS = Polistes annularis	34,203	Q9U6W0	24	13%
		Probable phospholipase A1 magnifin OS = Vespa magnifica	38,504	P0CH47	14	5%
		Phospholipase A1 OS = Solenopsis invicta	39,190	Q68KK0	13	5%
	Phospholipase A2	Basic phospholipase A2 homolog zhaoermiatoxin O = Protobothrops mangshanensis	14,774	P84776	21	12%
		Basic phospholipase A2 DAV-N6 OS = Deinagkistrodon acutus	16,641	Q1ZY03	18	5%
		hospholipase A2 homolog OS = Echis coloratus	14,495	P0DMT3	17	6%
		Basic phospholipase A2 DsM-b1/DsM-b1' OS	16,688	A0CG82	16	6%
	Zinc metalloproteinase-disintegrin-like	Zinc metalloproteinase-disintegrin-like OS = Cerberus rynchops	71,078	D8VNS0	20	2%
		Zinc metalloproteinase-disintegrin-like brevilysin H2a	48,693	P0DM89	16	2%
	Other	Thrombin-like enzyme TLBm OS = Bothrops marajoensis	33,903	P0DJE9	19	7%
		Thrombin-like enzyme RP34 (Fragment) OS = Cerastes cerastes	3783	Q9PS28	17	36%
		Hyaluronidase A OS = Vespula vulgaris	39,137	P49370	17	9%

CnNV-2~55 kDa	Phospholipase D	Phospholipase D SpeSicTox-betaIB2a (Fragment) OS = Sicarius peruensis	31,305	C0JB37	40	16%
	Zinc Metalloproteinase- disintegrin-like	Zinc metalloproteinase-disintegrin-like VAP2A = Crotalus atrox	70,157	A4PBQ9	45	7%
		Zinc metalloproteinase-disintegrin-like kaouthiagin-like OS	68,187	D3TTC1	18	2%
		Zinc metalloproteinase-disintegrin-like OS = Cerberus rynchops	71,078	D8VNS0	16	1%
		Snake venom metalloproteinase kistomin OS = Calloselasma rhodostoma	48,157	P0CB14	15	3%
	Phospholipase A1	Phospholipase A1 OS	34,203	Q9U6W0	20	8%
	Phospholipase A2	Phospholipase A2 homolog OS = Echis coloratus	14,495	P0DMTS	20	6%
		Basic phospholipase A2 Bs-N6 OS	16,325	Q6EER4	17	15%
		Basic phospholipase A2 nigroxin B OS = Micrurus nigrocinctus	14,158	P81167	16	16%
	Other	Snake venom serine protease HS114 OS	28,509	Q5W959	17	10%
		Hyaluronidase OS = Echis ocellatus	53,137	A3QVN2	21	7%
		Hyaluronidase conohyal-ad1 (Fragment) OS	39,842	I0CME8	16	4%
		Thrombin-like enzyme TLBm OS = Bothrops marajoensis	33,903	P0DJE9	20	6%
		Thrombin-like enzyme cerastotin	11,329	P81038	13	24%
CnNV-3~45 kDa	Serine protease	Venom serine carboxypeptidase OS = Apis mellifera	53,783	C9WMM5	24	11%
		Snake venom serine protease 3 OS = Trimeresurus gramineus	28,700	O13063	22	4%
		Phospholipase D LamSicTox-alphaIC1 (Fragment) OS = Loxosceles amazonica	31,020	C0JAZ9	22	2%
	Phospholipase D	Phospholipase D LiSicTox-betaIA1i OS = Loxosceles intermedia	34,520	Q2XQ09	20	15%
		Phospholipase D SpaSicTox-betaIIA1 (Fragment) OS = Sicarius patagonicus	31,847	C0JB68	16	8%
		Phospholipase D LspiSicTox-betaIE4i (Fragment) OS = Loxosceles spinulosa	31,591	C0JB46	14	17%
		Zinc metalloproteinase-disintegrin-like EoVMP2 OS	71,605	Q2UXQ5	20	1%
	Zincmetalloproteinase- disintegrin-like	Zinc metalloproteinase-disintegrin-like = Cerberus rynchops	71,078	D8VNS0	17	2%
		Zinc metalloproteinase-disintegrin-like BfMP (Fragment) OS = Bungarus fasciatus	70,381	A8QL48	16	6%

Phospholipase A1/A2	Phospholipase A1 OS = Polistes annularis PE	34,203	Q9U6W0	19	20%
	Phospholipase A2 Scol/Pla OS = Scolopendra viridis PE	17,461	C1JAR9	20	11%
	Acidic phospholipase A2 BA2 OS = Gloydus halys	14,590	O42190	16	5%
	Phospholipase A2 (Fragment) OS = Bothrops jararaca PE	1752	Q9PRZ0	14	73%
Peptidase-like enzymes	Venom dipeptidyl peptidase	89,267	B1A4F7	16	2%
Other	L-amino-acid oxidase (Fragment) OS = Naja atra	51,805	A8QL58	15	2%
	Thrombin-like enzyme cerastotin (Fragments) OS = Cerastes cerastes PE	11,329	P81038	13	11%

Table S2. Identification of the enzymatic constituents in jellyfish *N. nomurai* nematocysts venom (NnNV) indicated in Figure 4A by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Band No.	Venom Family	Protein Name/Source Organism	Mass (Da)	Accession	Protein Score	Sequence Coverage
NnNV-1 > 110 kDa	Metalloprotease	Astacin-like metalloprotease toxin 5 (Fragment) OS = Loxosceles gaucho	21,858	P0DM62	29	10%
	Phospholipase A1/A2	Phospholipase A1 3 (Fragment) OS = Polistes dominula	35,738	Q6Q250	28	6%
		Acidic phospholipase A2 PA4 OS = Heloderma suspectum	16,200	P80003	18	5%
		Phospholipase A2 homolog OS = Echis coloratus PE	14,495	PODMT3	17	6%
		Phospholipase A2 OS = Mesobuthus tamulus	19,188	Q6T178	17	5%
		Hyaluronidase	Inactive hyaluronidase B OS = Vespula vulgaris	40,275	Q5D7H4	17
	L-amino acid oxidase	L-amino acid oxidase OS = Cerastes cerastes	58,805	X2JCV5	14	6%
		L-amino-acid oxidase OS = Calloselasma rhodostoma PE	58,583	P81382	13	10%
	NnNV-2~66 kDa	Metalloprotease or protease	Venom protease OS = Bombus pensylvanicus	27,633	Q7M4I3	25
Astacin-like metalloprotease toxin 1 OS = Loxosceles intermedia			30,650	A0FKN6	21	10%
Snake venom metalloproteinase H5 (Fragment) OS = Deinagkistro don acutus			46,518	Q9IAY2	14	5%
Zinc metalloproteinase-disintegrin-like EoVMP2 OS = Echis ocellatus GN			71,605	Q2UXQ5	14	4%
Phospholipase A2 1 (Fragment) OS = Micrurus tener microgalbineus			1397	P25072	37	100%
Phospholipase A2/A1		Phospholipase A2 OS = Mesobuthus tamulus	19,188	Q6T178	19	5%
		Basic phospholipase A2 PA-13 OS = Pseudechis australis	14,002	P04057	14	14%

NnNV-3~46 kDa	Serine proteinase	Basic phospholipase A2 bothropstoxin-2 OS = Bothrops jararacussu	16,553	P45881	15	5%
		Phospholipase A1 1 OS = Polistes dominula	38,390	Q6Q252	19	6%
		Snake venom serine proteinase 4a OS = Crotalus adamanteus	29,589	J3SDX0	17	10%
		Snake venom serine proteinase 9 OS = Crotalus adamanteus	30,195	J3RYA3	16	3%
		L-amino-acid oxidase OS = Calloselasma rhodostoma	58,583	P81382	21	12%
	L-amino-acid oxidase	L-amino-acid oxidase OS = Pseudechis australis	59,049	Q4JHE1	15	3%
	Metalloproteinase	Zinc metalloproteinase carinactivase-1 catalytic subunit (Fragment) OS = Echis carinatus PE	3748	Q9PRP9	64	37%
		Astacin-like metalloprotease toxin 1 OS = Loxosceles intermedia	30,650	A0FKN6	20	13%
		Snake venom metalloproteinase-disintegrin-like mocrhagin OS = Naja mossambica	70,412	Q10749	18	1%
		Zinc metalloproteinase-disintegrin-like HF3 OS = Bothrops jararaca	69,818	Q98UF9	14	3%
		Snake venom metalloproteinase HT-1 (Fragment) OS = Crotalus ruber ruber	25,182	Q9PSN7	13	5%
	Phospholipase A2/A1	Phospholipase A2 1 (Fragment) OS = Micrurus tener microgalbineus	1397	P25072	37	100%
		Basic phospholipase A2 homolog Pgo-K49 OS = Cerrophidion godmani	16,303	Q8UVU7	21	10%
		Acidic phospholipase A2 BA1 OS = Gloydus halys PE	14,847	O42189	19	5%
		Phospholipase A2 (Fragment) OS = Bunodosoma caissarum	4378	P86780	18	33%
	Phospholipase D	Phospholipase D LspiSicTox-betaIE4i (Fragment) OS = Loxosceles spinulosa	31,591	C0JB46	19	10%
		Phospholipase D SdSicTox-betaIF1 (Fragment) OS = Sicarius cf. damarensis	32,023	C0JB55	15	5%
	Serine proteinase	Snake venom serine proteinase 9 OS = Crotalus adamanteus	30,195	J3RYA3	17	8%
	Hyaluronidase	Hyaluronidase A OS = Vesputia vulgaris	39,137	P49370	17	4%
		Inactive hyaluronidase B OS = Vesputia vulgaris	40,275	Q5D7H4	15	12%
	L-amino-acid oxidase	L-amino-acid oxidase OS = Oxyuranus scutellatus scutellatus	59,374	Q4JHE3	22	1%
		L-amino-acid oxidase OS = Vipera ammodytes ammodytes	55,056	P0DI84	17	4%
	Other	Thrombin-like enzyme ancrod-2 OS = Calloselasma rhodostom	29,867	P47797	16	7%

NnNV-4~35 kDa	Metalloproteinase	Zinc metalloproteinase carinactivase-1 catalytic subunit (Fragment) OS = Echis carinatus	3748	Q9PRP9	32	37%	
		Astacin-like metalloprotease toxin 1 OS = Loxosceles intermedia	30,650	A0FKN6	19	10%	
		Zinc metalloproteinase/disintegrin (Fragment) OS = Trimeresurus gramineus	50,111	P0C6E8	15	4%	
		Zinc metalloproteinase-disintegrin-like brevilysin H2a	48,693	P0DM89	15	2%	
		Zinc metalloproteinase-disintegrin-like Eoc1 OS = Echis ocellatus	70,873	Q2UXR0	13	1%	
		Phospholipase A1	Phospholipase A1 OS = Solenopsis invicta	39,190	Q68KK0	19	4%
	Hyaluronidase	Inactive hyaluronidase B OS = Vesputia vulgaris	40,275	Q5D7H4	1	20%	
NnNV-5~27 kDa	Metalloproteinase	Zinc metalloproteinase carinactivase-1 catalytic subunit (Fragment) OS = Echis carinatus	3748	Q9PRP9	24	37%	
		Venom metalloproteinase 2 OS = Eulophus pennicornis	46,696	B5AJT3	21	4%	
		Zinc metalloproteinase-disintegrin-like stejnihagin-A OS = Trimeresurus stejnegeri	69,984	Q3HTN1	20	3%	
		Zinc metalloproteinase-disintegrin-like berythracivase OS = Bothrops erythromelas	70,767	Q8UVG0	17	1%	
		Zinc metalloproteinase-disintegrin-like OS = Cerberus rynchops PE	71,078	D8VNS0	17	2%	
		Zinc metalloproteinase-disintegrin-like brevilysin H2a OS = Gloydus brevicaudus	48,693	P0DM89	15	12%	
	Phospholipase D	Phospholipase D LamSicTox-alphaIC1 (Fragment) OS = Loxosceles amazonica	31,020	C0JAZ9	32	2%	
		Phospholipase D SpaSicTox-betaIIA1 (Fragment) OS = Sicarius patagonicus	31,847	C0JB68	18	2%	
		Phospholipase D LiSicTox-betaIA1i OS = Loxosceles intermedia	34,520	Q2XQ09	14	7%	
	Phospholipase A2	Phospholipase A2 1 (Fragment) OS = Micrurus tener microgalbineus	1397	P25072	36	50%	
		Basic phospholipase A2 homolog zhaoermiatoxin OS = Protobothrops mangshanensis	14,774	P84776	22	26%	
		Basic phospholipase A2 homolog Cax-K49 OS = Crotalus atrox	16,385	Q8UVZ7	19	10%	
		L-amino-acid oxidase	L-amino-acid oxidase OS = Notechis scutatus scutatus	59,363	Q4JHE2	15	8%

NnNV-6~18 kDa	Phospholipase A2	Phospholipase A2 1 (Fragment) OS = Micurus tener microgalbineus	1397	P25072	37	50%
		Basic phospholipase A2 homolog OS = Protobothrops mucrosquamatus	14,598	P22640	14	12%
	Phospholipase D	Phospholipase D SpeSicTox-betaIB1a (Fragment) OS = Sicarius peruensis	31,246	C0JB35	30	9%
		Phospholipase D LiSicTox-alphaIA2ai OS = Loxosceles intermedia	34,402	P0CE83	24	17%
		Phospholipase D StSicTox-betaIC1 (Fragment) OS = Sicarius terrosus	31,271	C0JB39	19	12%
		Phospholipase D SpaSicTox-betaIF1 (Fragment) OS = Sicarius patagonicus	30,636	C0JB52	19	2%
		Sphingomyelin phosphodiesterase D OS = Ixodes scapularis GN	41,826	Q202J4	13	5%
	Hyaluronidase	Hyaluronidase OS = Loxosceles intermedia	46,852	R4J7Z9	17	1%
		Hyaluronidase A OS = Vespula vulgaris	39,137	P49370	16	5%
	Other	Zinc metalloproteinase-disintegrin-like brevilysin H2a OS = Gloydius brevicaudus	48,693	P0DM89	14	4%
		L-amino-acid oxidase (Fragment) OS = Naja atra	51,805	A8QL58	13	5%
		Trehalase OS = Pimpla hypochondriaca GN	66,587	Q8MMG9	13	5%

Notes: In Tables S1 and S2, a discrepancy between the observed molecular weights and predicted molecular weights was noticed, which was partially attributed to absence of enough similar sequences with jellyfish-derived enzymes in animal toxin annotation project.

Table S3. CfTX-like toxins identified from two scyphozoans *N. nomurai* and *C. nozakii* by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Toxins	Jellyfish Species	Gel Band No. ^a	Observed Mass ^b (kDa)	MS ²			
				Matches ^c (n)	Coverage (%)	Score	Access Number
CfTX-1	<i>N. nomurai</i>	NnNV-3	~46	4(1)	8	18	A7L0035
	<i>N. nomurai</i>	NnNV-4	~35	2(1)	3	14	A7L0036
CfTX-2	<i>C. nozakii</i>	CnNV-2	~50	7(1)	11	15	A7L0036
		CnNV-3	~46	8(2)	9	25	

^a Gel band No. is indicated in Figure 4A; ^b Observed mass was evaluated from 12% SDS-PAGE gel under non-reducing conditions; ^c match peptides, number in bracket indicated the number of statistically significant unique peptides.