

Supplementary Materials: Biological Activities and Proteomic Profile of the Venom of *Vipera ursinii* ssp., a very Rare Karst Viper from Croatia

Maja Lang Balija, Adrijana Leonardi, Marija Brgles, Dora Sviben, Tihana Kurtović, Beata Halassy * and Igor Križaj *

Table S1. Identification of the structures of the Croatian *V. ursinii* ssp. venom proteins. The venom was separated by gel electrophoresis in one (1-DE) or in two (2-DE) dimensions. Cys in proteins, separated in bands and spots (labelled as in Figures 4A and 4B), were first carbamidomethylated and then digested in-gel by trypsin. The resulting peptides were analysed on LC-ESI-MS/MS. Modified (apart from Cys) or mutated amino acid residues are typed in bold letters. Abbreviations: AtnI, ammodytin I; CRISP, cysteine-rich secretory protein; FIXa, activated factor IX; FX, factor X; KSPI, Kunitz-type serine protease inhibitor; n.d., not determined; sPLA₂, secreted phospholipase A₂; SVMP, snake venom metalloproteinase; SVSP, snake venom serine protease; *Vaa*, *V. a. ammodytes*; VNGF, venom nerve growth factor; *Vu*, *V. ursinii*; *, by similarity. →, substitution of amino acid residue on the left with that on the right.

1-DE-Band/ 2-DE-Spot	Mol. Mass (kDa)	MS/MS-Derived Peptide Sequence	Predicted Sequence Modification	Peptide Mass (Da)	Protein Identity (Snake Species)	Protein ID	Protein Score	Protein Activity (Reference)	Protein Family
1DE-1	80	IPCAPQDVK	Ala→Gly	1041.54	VaH3 (<i>Vaa</i>)	AGL45259	178	haemorrhagin [1]	SVMP
		LGNEYGYCR		1131.49					
		SCIMSGTLSCEASIR		1673.73					
		ATVAEDSCFQENQK		1626.71	VLFXA heavy chain (<i>M. lebetina</i>)	AAQ17467	129.5	FX activator [2]	SVMP
		KIPCAPQDVK		1169.64					
		CILNPPLR		982.55					
1DE-2	75	KIPCAPQDIK	Met oxidation	1155.62	RVV-X heavy chain (<i>D. russelii</i>)	Q7LZ61	34.0	FX activator [3]	SVMP
		DCQNPPCNAATCK		1598.58					
		KIPCAPQDVK		1155.62					
		LGNEYGYCR		1131.49	VaH3 (<i>Vaa</i>)	AGL45259	528	haemorrhagin [1]	SVMP
		LTPGSQCADGECCDQCK		1985.75					
		MPQCILNKPLK		1357.73					

1DE-3	48	NPCQIYYTPR		1369.62	VLFXA heavy chain (<i>M. lebetina</i>)	AQ17467	172	FX activator [2]	SVMP
		SCIMSGTLSCEASIR	Ile->Val	1657.73					
		YLVELGEDCDCGSPR		1769.75					
		ATVAEDSCFQENQK		1626.71	RVV-X heavy chain (<i>D. russelii</i>)	Q7LZ61	48.8	FX activator [3]	SVMP
		DCQNPCCDAATCK	Asp->Asn	1598.58					
		KIPCAPQDIK		1155.62					
		IYEMVNTLNVVFR		1597.84	Vaa-MPIII-1 (<i>Vaa</i>)	AMB36352	321.0	n.d.	SVMP
		LVATSEQQSYDR		1559.73					
		LVIVVDHSMVTK		1340.76					
		VATSEQQSYDR		1446.65	HR1a (<i>P. mucrosquamatus</i>)	Q8JIR2	166.9	haemorrhagin [4]	SVMP
		YNNNSTAIR	Ala->Val	1080.54					
		LHSWVECESGECCEQCR		2283.85					
		SWVECESGECCEQCR		1975.70	SVMP (<i>E. c. sochureki</i>)	ADI47594	150.8	n.d.	SVMP
		GEECDGSPANCQDPCC	Ala->Thr	2677.90					
		DAASCK							
		KGKSYFYCR		1208.59	carinactivase-1 (<i>E. carinatus</i>)	Q9PRP9	46.3	prothrombin activator [5]	SVMP
		SECDLPEYCTGQ		1458.55					
		LVIVVDHSMVEK		1368.76					
1DE-4	45	LVATSEQQSYDR		1559.73	Vaa-MPIII-1 (<i>Vaa</i>)	AMB36352	306.1	n.d.	SVMP
		SQLVATSEQQSYDR		1774.82					
		VATSEQQSYDR		1446.65					
		IYEMVNTLNVVFR		1597.84	SVMP (<i>E. c. sochureki</i>)	ADI47595	132.5	n.d.	SVMP
		FLT NF KP DCT LIR		1624.85					
		FLT NF KP DCT LIR PSR		1965.99					
		TDIVSPPVCGNDLLER	Leu->Val	1770.87	HR1a (<i>P. mucrosquamatus</i>)	Q8JIR2	124.0	haemorrhagin [4]	SVMP
		LHSWVECESGECCEQCR		2283.85					
		SWVECESGECCEQCR		1975.70					
		LVIVVDHSMVEK		1367.89	carinactivase-1 (<i>E. carinatus</i>)	Q9PRP9	45.4	prothrombin activator [5]	SVMP

1DE-5	35	MEWYPEAAAANAER		1537.67	Vaa-CRISP-1 (<i>Vaa</i>)	KT148819	153.6	n.d.	CRISP
		VIGGIECGENIYMSTSPMK		2086.93					
		VIGGDECNINEHR		1512.69	Vaa-SP-4 (<i>Vaa</i>)	KT148827	49.5	n.d.	SVSP
		EKFFCLSSK	Glu->Gln	1144.58	Vaa-SPH-1 (<i>Vaa</i>)	KT148824	310.6	FIXa antagonist [6]	SVSP
		IMGWGTITTTK		1208.64					
		NVPNEDEQMR	Met oxidation	1247.53					
		NVPNEDEQMRVPK		1556.74					
		SLPSSPPSVGSVCR		1429.71					
		TLCAGILQGGIDSCK		1675.85					
1DE-6	27	QKFFCLSSK		1145.57	Vaa-SP-6 (<i>Vaa</i>)	MG958495	97.5	FV, FX activator [Latinović et al., in preparation]	SVSP
		VVCAGIWQGGK		1174.60					
		NIRNEDEQIR		1286.65	RVV-V alpha (<i>D. siamensis</i>)	P18964	85.7	FV activator [7]	SVSP
		YFCLNTK		929.45					
		SVDFDSESPR		1138.50	Vaa-CRISP-1 (<i>Vaa</i>)	KT148819	50.6	n.d.	CRISP
		CTYDHSPR	Thr->Ile	1047.47	Vaa-CRISP-1 (<i>Vaa</i>)	KT148819	635.0	n.d.	CRISP
		DFVYGQGASPAVAVVGH		1688.80					
		DFVYGQGASPAVAVVGH		1952.91					
		YT							
		KPEIQNEIIDLHNSLR		1920.02					
		MEWYPEAAAANAER	Met oxidation	1553.67					
		RSVNPTASNMLK		1316.71					
		SVDFDSESPR		1138.50					
		SVNPTASNMLK		1161.59					
		VIGGIECGENIYM		1454.67					
		VIGGIECGENIYMSTSPMK		2085.97					
		YTQIVWYK		1100.58					
		DFVYGQGASPAVAVVGH		1688.80	Dr-CRIPK (<i>D. russelii</i>)	ACE73567	445.6	n.d.	CRISP

		DFVYGQGASPANAVVGH YT	1952.91					
		KDFVYGQGASPANAVVG H	1816.90					
		KDFVYGQGASPANAVVG HYT	2081.01					
		MEWYPEAAAANAER	1553.67					
		RSVTPTASNMLK	1333.73					
		SVDFDSESPR	1138.50					
		YTQIVWYK	1100.58					
		FYCAGTLINQEWVLTAAR	2113.05	Vaa-SPH-1 (<i>Vaa</i>)	KT148824	173.6	FIXa antagonist [6]	SVSP
		IMGWGTITTTK	1208.64					
		TLCAGILQGGIDSCK	1592.78					
1DE-7	14	CCFVHDCCYGR	1533.55	AtnI ₁ (B) isoform (<i>Vu</i>)	CAE47156	298.0	indirect haemolytic activity * [8]	sPLA ₂
		NLSQFGDMINK	1266.62					
		VAAICFGENMNTYDK	1732.77					
		VAAICFGENMNTYDKK	1861.85					
		YMLYSLFDCK	1339.61					
		IYEMVNTLNVVFR	1613.84	Vaa-MPIII-1 (<i>Vaa</i>)	KT148834	128.0	n.d.	SVMP
		LVIVVDHSMVTK	1340.76					
1DE-8	60	IYEMVNTLNVVFR	1597.84	Vaa-MPIII-1 (<i>Vaa</i>)	KT148834	306.7	n.d.	SVMP
		LVATSEQQSYDR	1559.73					
		LVIVVDHSMVTK	1340.76					
		VATSEQQSYDR	1446.65					
		YNNNSTAIR	1080.54					
		LVATSEQQSYDR	1559.73	Vaa-MPIII-4 (<i>Vaa</i>)	MG958500	240	n.d.	SVMP
		VATSEQQSYDR	1446.65					
		VNILNEMYLPLNIR	1701.94					
		YIKLVIVVDHR	1355.77					

		YDYSEDPDYGMVDHGTK	1990.81	Vaa-MPIII-3 (<i>Vaa</i>)	MG958499	47.2	n.d.	SVMP
1DE-9	18	ALTMEGNQASWR	1379.64	VNGF (<i>Vu</i>)	AEH59582	130	promotes nerve growth * [9]	VNGF
		IDTACVCVISR	1293.63					
		LVIVVDHSMVTK	1340.76	Vaa-MPIII-1 (<i>Vaa</i>)	KT148834	118.8	n.d.	SVMP
		VATSEQQSYDR	1446.65					
		NYPSECTETEQC	1604.58	AtnI ₂ (D) isoform (<i>Vu</i>)	CAE47222	106.4	anticoagulant, antiplatelet* [10]	sPLA ₂
		VAAICFGENLNTYDK	1714.81					
1DE-10	13	FIYGGCR	872.41	chymotrypsin inhibitor (<i>Vaa</i>)	0909196A	78.0	protease inhibitor [11]	KSPI
		FYYNPASNK	1103.52					
		EFFYGGCGGNANNFK	1681.71	Vur-KIn (<i>V. renardi</i>)	P0DKL8	55.0	protease inhibitor [10]	KSPI
2DE-1	53	IYEMVNTLNVVFR	1597.84	Vaa-MPIII-1 (<i>Vaa</i>)	KT148834	112.7	n.d.	SVMP
		LVIVVDHSMVTK	1340.76					
		KENDVPIPCAPEDIK	1724.85	Vaa-MPIII-3 (<i>Vaa</i>)	MG958499	69.52	n.d.	SVMP
		YDYSEDPDYGMVDHGTK	2007.79					
				Met oxidation				
2DE-2	53	YIKLVIVVDHR	1355.77	Vaa-MPIII-4 (<i>Vaa</i>)	MG958500	64.7	n.d.	SVMP
		ATSEQQSYDR	1347.58	Vaa-MPIII-1 (<i>Vaa</i>)	KT148834	355.4	n.d.	SVMP
		IYEMVNTLNVVFR	1613.84					
				Met oxidation				
		LVATSEQQSYDR	1559.73					
		LVIVVDHSMVTK	1340.76					
		TDIVSPVCGNELLEK	1770.90					
		VATSEQQSYDR	1446.65					
		KIPCAPQDVK	1155.62	VaH3 (<i>Vaa</i>)	AGL45259	96.8	haemorrhagin [1]	SVMP
		NPCQIYYTPR	1311.62					
		LVIVVDHSMVLK	1368.79	carinactivase-1 (<i>E. carinatus</i>)	AAB36410	42	prothrombin activator [5]	SVMP
				Met oxidation				

2DE-3	120	YDYSEDPDYGMVDHGTK	Met oxidation	2007.79	Vaa-MPIII-3 (<i>Vaa</i>)	MG958499	125.6	n.d.	SVMP
		KENDVPIPCAPEDIK	Ala->Ser	1740.85					
		LHSWVECESGECCEQCR		2283.85	HR1a (<i>P. mucrosquamatus</i>)	Q8JIR2	64.5	haemorrhagin [4]	SVMP
2DE-5	120	IYEMVNTLNVVFR	Met oxidation	1613.84	Vaa-MPIII-1 (<i>Vaa</i>)	KT148834	243.0	n.d.	SVMP
		LVIVVDHSMVTK	Met oxidation	1340.76					
		SQLVATSEQQSYDR		1774.82					
		TDIVSPPVCGNELLEK		1770.90					
		FRYIKFVIVVDHSMVEK		2091.1	SVMP (<i>E. p. leakeyi</i>)	ADI47674	163	n.d.	SVMP
		FVIVVDHSMVEK		1368.76					
		SQLVATSEQQSYDR		1774.82					
		KENDVPIPCAPEDIK		1724.85	Vaa-MPIII-3 (<i>Vaa</i>)	MG958499	73.6	n.d.	SVMP
		YDYSEDPDYGMVDHGTK		1991.80					
		LVIVVDHSMVEK	Met oxidation	1384.75	carinactivase-1 (<i>E. carinatus</i>)	AAB36410	43.8	prothrombin activator [5]	SVMP
		IMGWGTITTTK		1224.63	Vaa-SPH-1 (<i>Vaa</i>)	KT148824	103.2	FIXa antagonist [6]	SVSP
		TLCAGILQGGIDSCK		1592.78					
2DE-7	120	IYEMVNTLNVVFR		1613.84	Vaa-MPIII-1 (<i>Vaa</i>)	KT148834	51.6	n.d.	SVMP
2DE-10	120	IYEMVNTLNVVFR		1613.84	Vaa-MPIII-1 (<i>Vaa</i>)	KT148834	166.3	n.d.	SVMP
		LVIVVDHSMVTK		1340.76					
		TDIVSPPVCGNELLEK		1770.90					
		KENDVPIPCAPEDIK		1724.85	Vaa-MPIII-3 (<i>Vaa</i>)	MG958499	135.3	n.d.	SVMP
		YDYSEDPDYGMVDHGTK		1990.81					
		LVIVVDHSMVTK		1340.76					
		FLTDFKPDCTLRPSR		1966.02	Vaa-MPIII-4 (<i>Vaa</i>)	MG958500	91.8	n.d.	SVMP
		VNILNEMYLPLNIR		1701.94					
		LHSWVECESGECCEQCR		2283.85	HR1a (<i>P. mucrosquamatus</i>)	Q8JIR2	74.4	haemorrhagin [4]	SVMP
		IMGWGTITTTK		1208.64	Vaa-SPH-1 (<i>Vaa</i>)	KT148824	101.2	FIXa antagonist [6]	SVSP
		TLCAGILQGGIDSCK		1593.74					
		KPEIQNEIIDLHNSLR		1920.02	Vaa-CRISP-1 (<i>Vaa</i>)	KT148819	84.3	n.d.	CRISP

		MEWYPEAAAANAER	Met oxidation	1553.67					
2DE-16	54	DFVYGQGASPAVAVVGH		1688.80	Vaa-CRISP-1 (<i>Vaa</i>)	KT148819	255.4	n.d.	CRISP
		DFVYGQGASPAVAVVGH		2770.36					
		YTQIVWYK							
		KPEIQNEIIDLHNSLR		1920.02					
		MEWYPEAAAANAER		1562.74					
		VIGGIECGENIYMSTSPMK		2085.97					
		IYEMVNTLNVVFR	Met oxidation	1613.84	Vaa-MPIII-1 (<i>Vaa</i>)	KT148834	101.7	n.d.	SVMP
		TDIVSPPVCGNELLEK		1770.90					
		TLCAGILQGGIDSCK		1592.78	Vaa-SPH-1 (<i>Vaa</i>)	KT148824	81.4	FIXa antagonist [6]	SVSP
		VTYPDVPHCADINMFDYS		2659.6					
		VCQK							
2DE-21	48	AENPWLPAQSR		1294.65	Vaa-SP-4 (<i>Vaa</i>)	KT148827	291.6	n.d.	SVSP
		IAPLSLPSSPPR		1234.72					
		INILNYAVCR		1235.66					
		STHIAPLSLPSSPPR		1559.85					
		SYTLWNKDIMLIK		1625.86					
		TLCAGILQGGIDTCK		1606.79					
		SYTLWDKDIMLIK	Met oxidation	1641.21					
2DE-22	38	LHSWVECESGECCEQCR		2226.83	HR1a (<i>P. mucrosquamatus</i>)	Q8JIR2	53.7	haemorrhagin [4]	SVMP
		AENPWLPAQSR		1310.65	Vaa-SP-4 (<i>Vaa</i>)	KT148827	125.9	n.d.	SVSP
		TLCAGILQGGIDTCK		1606.79					
		TLCAGILQGGIDSCK		1592.78	Vaa-SPH-1 (<i>Vaa</i>)	KT148824	82.4	FIXa antagonist [6]	SVSP
2DE-23	50	FRYIKLVIVVDHSMVTK		2050.8	Vaa-MPIII-1 (<i>Vaa</i>)	KT148834	133.8	n.d.	SVMP
		LVIVVDHSMVTK		1340.76					
		TDIVSPPVCGNELLEK		1770.90					
2DE-24	48	IYEMVNTLNVVFR		1613.84	Vaa-MPIII-1 (<i>Vaa</i>)	KT148834	116.4	n.d.	SVMP
		LVIVVDHSMVTK		1340.76					
		KENDVPIPCAPEDIK		1724.85	Vaa-MPIII-3 (<i>Vaa</i>)	MG958499	94.3	n.d.	SVMP

2DE-25	48	LVIVVDHSMVTK	Met oxidation	1340.76					
		IYEMVNTLNVVFR	Met oxidation	1613.84	Vaa-MPIII-1 (<i>Vaa</i>)	KT148834	160.7	n.d.	SVMP
		LVIVVDHSMVTK		1340.76					
		TDIVSPPVCGNELLEK		1770.90					
		LHSWVECESGECCQQCR	deamidation	2226.83	SVMP (<i>E. coloratus</i>)	ADI47644	112.7	n.d.	SVMP
2DE-26	37	LVIVVDHSMVTK		1340.76					
		LVIVVDHSMVEK		1368.76	carinactivase-1 (<i>E. carinatus</i>)	Q9PRP9	45.5	prothrombin activator [5]	SVMP
		KENDVPIPCAPEDIK	deamidation	1725.81	Vaa-MPIII-3 (<i>Vaa</i>)	MG958499	35.9	n.d.	SVMP
		IMGWGTITTTK		1208.64	Vaa-SPH-1 (<i>Vaa</i>)	KT148824	154.4	FIXa antagonist [6]	SVSP
		TLCAGILQGGIDSCK		1592.78					
2DE-30	35	VTYPDVPHCADINMFDYS		2659.6					
		VCQK							
		IYEMVNTLNVVFR	Met oxidation	1613.84	Vaa-MPIII-1 (<i>Vaa</i>)	KT148834	101.2	n.d.	SVMP
		TDIVSPPVCGNELLEK		1770.90					
		VAAICFGENLNTYDK		1714.81	AtnI ₂ (D) isoform (<i>Vu</i>)	CAE47222	43.7	anticoagulant, antiplatelet* [10]	sPLA ₂
2DE-31	30	SLPSSPPSVGSVCR		1429.71	Vaa-SPH-1 (<i>Vaa</i>)	KT148824	101.8	FIXa antagonist [6]	SVSP
		TLCAGILQGGIDSCK		1592.78					
		SEWSDGSSVSYDNLLK	Leu->His	1810.79	RVV-X light chain 2 (<i>D. siamensis</i>)	ADK22819	55.5	FX activator [3]	snaclec
		LTIYSYSFENGDIVCGDD		2656.7	Vur-PL2 (<i>V. renardi</i>)	ADG86231	147.4	anticoagulant, anti-platelet [10]	sPLA ₂
		SCKR		1714.81					
		VAAICFGENLNTYDK		1724.85	Vaa-MPIII-3 (<i>Vaa</i>)	MG958499	95.6	n.d.	SVMP
		KENDVPIPCAPEDIK							
		YDYSEDPDYGMVDHGTK	Met oxidation	2007.79					

		TLCAGILQGGIDSCK		1592.78	Vaa-SPH-1 (<i>Vaa</i>)	KT148824	45	FIXa antagonist [6]	SVSP
2DE-32	35	IMGWGTITTTK	Met oxidation	1224.63	Vaa-SPH-1 (<i>Vaa</i>)	KT148824	99.9	FIXa antagonist [6]	SVSP
		TLCAGILQGGIDSCK		1592.78					
		LVIVVDHSMVTK		1356.76	Vaa-MPIII-1 (<i>Vaa</i>)	KT148834	70.2	n.d.	SVMP
		YNNNSTAIR	Ala->Val	1080.54					
2DE-33	30	VAAICFGENLNTYDK		1714.81	AtnI ₂ (D) isoform (<i>Vu</i>)	CAE47222	59	anticoagulant, antiplatelet* [10]	sPLA ₂
		TLCAGILQGGIDSCK		1592.78	Vaa-SPH-1 (<i>Vaa</i>)	KT148824	48.3	FIXa antagonist [6]	SVSP
		MEWYPEAAANAER		1553.67	Vaa-CRISP-1 (<i>Vaa</i>)	KT148819	46	n.d.	CRISP
2DE-38	20	VAAICFGENLNTYDK		1714.81	AtnI ₂ (D) isoform (<i>Vu</i>)	CAE47222	122	anticoagulant, antiplatelet* [10]	sPLA ₂
		VAAICFGENLNTYDKK		1842.91					
		SEWSDGSSVSNDLLK	Leu->His	1809.84	RVV-X light chain 2 (<i>D. siamensis</i>)	ADK22819	62.2	FX activator [3]	snaclec
2DE-41	14	LTIIYSFENGDIVCGGDD SCKR		2657.5	Vur-PL2 (<i>V. renardi</i>)	ADG86231	196.0	anticoagulant, anti-platelet [10]	sPLA ₂
		VAAICFGENLNTYDK		1714.41					
		VAAICFGENLNTYDKK		1900.91					
2DE-43	14	LTIIYSFENGDIVCGGDD SCKR		2656.7	Vur-PL2 (<i>V. renardi</i>)	ADG86231	225.0	anticoagulant, anti-platelet [10]	sPLA ₂
		VAAICFGENLNTYDK		1714.81					
		VAAICFGENLNTYDKK		1842.91					
		VEAICFGENLNTYDK		1715.80	AtnI ₂ (A) isoform (<i>V. a. ruffoi</i>)	CAE47218	177	anticoagulant, antiplatelet* [10]	sPLA ₂
		VEAICFGENLNTYDKK		1842.94					
		VAAICFGENMNTYDK	Met oxidation	1748.76	AtnI ₁ (B) isoform (<i>Vu</i>)	CAE47156	89.7	indirect haemolytic activity* [8]	sPLA ₂
		VAAICFGENMNTYDKK	Met oxidation	1876.86					

2DE-44	12	IYYGCIYCGWGGK		1483.61	AtnI ₁ (B) isoform (<i>Vu</i>)	CAE47156	275.7	indirect haemolytic activity* [8]	sPLA ₂
		MGTYSYSFQNGDIVCGG	deamidati	2613.07					
		DDPCLR	on						
		NLSQFGDMINK		1266.62					
		VAAICFGENMNTYDK	Met	1748.76					
		VAAICFGENMNTYDKK	oxidation	1860.86					
		LTIYSYSFENGDIVCGGDD		2657.5	Vur-PL2 (<i>V. renardi</i>)	ADG86231	193.8	anticoagulant, anti-platelet [10]	sPLA ₂
		SCKR		1714.81					
		VAAICFGENLNTYDK		1900.91					
		VAAICFGENLNTYDKK							
2DE-46	14	LTIYSYSFENGDIVCGGDD		2681.62	Vur-PL2 (<i>V. renardi</i>)	ADG86231	227.9	anticoagulant, anti-platelet [10]	sPLA ₂
		SCKR							
		SYSFENGDIVCGGDDSC		2165.89					
		R							
		VAAICFGENLNTYDK		1714.81					
		VAAICFGENLNTYDKK		1884.92					
		IALFSYSDYGCYCGWGGQ		2514.28	AtnI ₂ (D) isoform (<i>Vu</i>)	CAE47222	182.1	anticoagulant, antiplatelet* [10]	sPLA ₂
		GKPK		1714.81					
		VAAICFGENLNTYDK		1884.92					
		VAAICFGENLNTYDKK		1919.04	Vaa-CRISP-1 (<i>Vaa</i>)	KT148819	100.7	n.d.	CRISP
		KPEIQNEIIDLHNSLR							
		MEWYPEAAANAER	Met	1553.67					
			oxidation						
2DE-47	12	VAAICFGENLNTYDK		1713.51	AtnI ₂ (D) isoform (<i>Vu</i>)	CAE47222	73.5	anticoagulant, antiplatelet * [10]	sPLA ₂

Table S2. Identification of proteins with higher abundance in the venom of the Croatian *V. ursinii* ssp. snakes living in captivity (*Vu*Cro-c) than in the venom of those living in the wild (*Vu*Cro). Using comparative 2-DE (Figure 5), the dominant spots of the *Vu*Cro-c venom, as compared to those of the *Vu*Cro venom, were excised (labelled as in Figure 5A). Cys of proteins in spots were carbamidomethylated and digested in-gel by trypsin. The resulting peptides were extracted and analysed by LC-ESI-MS/MS. Apart from Cys, modified or mutated amino acid residues are presented by bold letters. Abbreviations: AtnI, ammodytin I; CRISP, cysteine-rich secretory protein; FX, factor X; n.d., not determined; sPLA₂, secreted phospholipase A₂; SVSP, snake venom serine protease; *Vaa*, *V. a. ammodytes*; *Vu*, *V. ursinii*; *, by similarity; →, substitution of amino acid residue on the left to that on the right.

2-DE-Spot	Protein Mass (kDa)	MS/MS-Derived Sequence	Predicted Sequence Modification	Peptide Mass (Da)	Identified Protein (Snake Species)	Protein ID	Protein Score	Protein Activity (Reference)	Protein Family
1	40	AENPWLPAQSR		1268.64	VaaSP-4 (<i>Vaa</i>)	KT148827	167.8	n.d.	SVSP
		SYTLWNKDIMLIK	deamidation	1625.86					
		TLCAGILQGGIDTCK		1606.79					
		AA Y PWLLER	Tyr→Phe	1102.60	VaaSP-3 (<i>Vaa</i>)	KT148826	91.0	n.d.	SVSP
		GQLQGIVSWG Y R		1363.71					
2	30	CCFVHDCCYGR		1533.55	AtnI ₂ (D) isoform (<i>Vu</i>)	CAE47222	166.0	anticoagulant, antiplatelet * [10]	sPLA ₂
		NYPSSSECTETE Q C		1604.58					
		VAAICFGENLNTYDK		1714.81					
		KPEIQNEIIDLHNSLR		1919.98	VaaCRISP-1 (<i>Vaa</i>)	KT148819	108.9	n.d.	CRISP
		RSVNPTASNMLK		1317.69					
		SVNPTASNMLK		1161.59					
		TLCAGILQGGIDTCK		1606.79	VaaSP-4 (<i>Vaa</i>)	KT148827	92.7	n.d.	SVSP
		LNR P VTY		862.48					
3	18	KVLNEDEETREPTEK		1816.89	VaaSP-3 (<i>Vaa</i>)	KT148826	63.8	n.d.	SVSP
		VAAICFGENLNTYDK		1714.81	AtnI ₂ (D) isoform (<i>Vu</i>)	CAE47222	225.1	anticoagulant, antiplatelet * [10]	sPLA ₂
		VAAICFGENLNTYDKK		1842.91					
		YKNYPSSSECTETE Q C		1895.74					
		CCFVHDCCYGR		1533.55					
		SEWSDGSSVS Y DNLLK	Leu→His	1810.79	RVV-X light chain 2 (<i>D. siamensis</i>)	ADK22819	79.4	FX activator [3]	snaclec

4	14	SEWSDGSSVSYDNLLK	Leu->His	1810.79	RVV-X light chain 2 (<i>D. siamensis</i>)	ADK22819	161.9	FX activator [3]	snaclec
		FITHFWIGLR		1289.72					
		YFCYR		808.34					
		VAAICFGENLNTYDK		1714.81					
5	18	NYPSSQCTETEQC	Leu->His	1604.58	AtnI ₂ (D) isoform (<i>Vu</i>)	CAE47222	59.7	anticoagulant, antiplatelet * [10]	sPLA ₂
		NYPSSQCTETEQC		1604.58					
		NYPSSQCTETEQC		1604.58					
		NYPSSQCTETEQC		1604.58					
6	18	VAAICFGENLNTYDK	Leu->His	1714.81	RVV-X light chain 2 (<i>D. siamensis</i>)	ADK22819	104.8	FX activator [3]	snaclec
		VAAICFGENLNTYDKK		1842.91					
		SEWSDGSSVSYDNLLK		1810.79					
		YFCYR		808.34					
7	20	CCFVHDCCYGR	Leu->His	1533.55	AtnI ₂ (D) isoform (<i>Vu</i>)	CAE47222	164.7	anticoagulant, antiplatelet * [10]	sPLA ₂
		VAAICFGENLNTYDK		1714.81					
		VAAICFGENLNTYDKK		1842.91					
		SEWSDGSSVSYDNLLK		1810.79					
7	20	TWEEAER	Leu->His	920.41	RVV-X light chain 2 (<i>D. siamensis</i>)	ADK22819	130.1	FX activator [3]	snaclec
		YFCYR		808.34					
		CCFVHDCCYGR		1533.55					
		CCFVHDCCYGR		1533.55					
7	20	IALFSYSDYGCYCGWGGQGKPK	Leu->His	2514.12	AtnI ₂ (D) isoform (<i>Vu</i>)	CAE47222	279.7	anticoagulant, antiplatelet * [10]	sPLA ₂
		NYPSSQCTETEQC		1604.58					
		VAAICFGENLNTYDK		1714.81					
		VAAICFGENLNTYDKK		1842.91					

References

1. Sajevec, T.; Leonardi, A.; Kovačič, L.; Lang-Bališa, M.; Kurtović, T.; Pungerčar, J.; Halassy, B.; Trampuš-Bakija, A.; Križaj, I. VaH3, one of the principal hemorrhagins in *Vipera ammodytes ammodytes* venom, is a homodimeric P-IIIc metalloproteinase. *Biochimie* **2013**, *95*, 1158–1170.
2. Siigur, E.; Tõnismägi, K.; Trummal, K.; Samel, M.; Vija, H.; Subbi, J.; Siigur, J. Factor X activator from *Vipera lebetina* snake venom, molecular characterization and substrate specificity. *Biochim. Biophys. Acta Gen. Subj.* **2001**, *1568*, 90–98.
3. Takeya, H.; Nishida, S.; Miyata, T.; Kawada, S.; Saisaka, Y.; Morita, T.; Iwanaga, S. Coagulation factor X activating enzyme from Russell's viper venom (RVV-X). A novel metalloproteinase with disintegrin (platelet aggregation inhibitor)-like and C-type lectin-like domains. *J. Biol. Chem.* **1992**, *267*, 14109–14117.
4. Kishimoto, M.; Takahashi, T. Molecular cloning of HR1a and HR1b, high molecular hemorrhagic factors, from *Trimeresurus flavoviridis* venom. *Toxicon* **2002**, *40*, 1369–1375.
5. Yamada, D.; Sekiya, F.; Morita, T. Isolation and characterization of carinactivase, a novel prothrombin activator in *Echis carinatus* venom with a unique catalytic mechanism. *J. Biol. Chem.* **1996**, *271*, 5200–5207.
6. Latinović, Z.; Leonardi, A.; Kovačič, L.; Koh, C.; Šribar, J.; Bakija, A.; Venkateswarlu, D.; Kini, R.; Križaj, I. The first intrinsic tenase complex inhibitor with serine protease structure offers a new perspective in anticoagulant therapy. *Thromb. Haemost.* **2018**, *118*, 1713–1728.
7. Tokunaga, F.; Nagasawa, K.; Tamura, S.; Miyata, T.; Iwanaga, S.; Kisiel, W. The factor V-activating enzyme (RVV-V) from Russell's viper venom. Identification of isoproteins RVV-V alpha, -V beta, and -V gamma and their complete amino acid sequences. *J. Biol. Chem.* **1988**, *263*, 17471–17481.
8. Jan, V.M.; Guillemin, I.; Robbe-Vincent, A.; Choumet, V. Phospholipase A₂ diversity and polymorphism in European viper venoms: Paradoxical molecular evolution in Viperinae. *Toxicon* **2007**, *50*, 1140–1161.
9. Paalme, V.; Trummal, K.; Samel, M.; Tõnismägi, K.; Järvekülg, L.; Vija, H.; Subbi, J.; Siigur, J.; Siigur, E. Nerve growth factor from *Vipera lebetina* venom. *Toxicon* **2009**, *54*, 329–336.
10. Tsai, I.-H.; Wang, Y.-M.; Cheng, A.C.; Starkov, V.; Osipov, A.; Nikitin, I.; Makarova, Y.; Ziganshin, R.; Utkin, Y. cDNA cloning, structural, and functional analyses of venom phospholipases A₂ and a Kunitz-type protease inhibitor from steppe viper *Vipera ursinii renardi*. *Toxicon* **2011**, *57*, 332–341.
11. Ritonja, A.; Turk, V.; Gubenšek, F. Serine proteinase inhibitors from *Vipera ammodytes* venom. Isolation and kinetic studies. *Eur. J. Biochem.* **1983**, *133*, 427–432.