

Supplemental info to the work:

Dubovskii, P.V.; Utkin, Y.N. Specific Amino Acid Residues in the Three Loops of Snake Cytotoxins Determine Their Membrane Activity and Provide a Rationale for a New Classification of These Toxins. *Toxins* **2024**, *16*, 262. <https://doi.org/10.3390/toxins16060262>.

Q8UUK0	RKCHNSPLSLVYQTCPIGQNICFKINVKEAP-SIPVKRACAATCPKSSALVKVVCCKTDKCN-
P24778	LICHNRPLPFLHKTCEGQNICYKMTLKKTPMKLSVVRGCAATCPSERPLVQVECCKTDKCNW
P01473	IKCHNTPLPFIYKTCPEGNNLCFKGTL-KFPKKITYKRGCADACPKTSALVKYVCCNTDKCN-
P01474	IKCHNTLLPFIYKTCPEGQNLCKFKGTL-KFPKKTTYNRGCAATCPKSSLLVKYVCCNTNKC-
P49122	LKCHNTQLPFIYNTCEGKNLCFKATL-KFPVKFVVRGCAATCPRSSSLVKVVCCKTDKCN-
A0A8C6XPA7	LKCHNTQLPLIYKTCPEGKNLCFKMTLKRFPVKFVVRGCAATCPKSNALVKVVCCKTDKCN-
P62394	LKCHNTQLPFIYKTCPEGKNLCFKTTLLKKLPVKIPIKRGCAATCPKSSALLKVVCCSTDKN-
P14541	LKCHNTQLPFIYKTCPEGKNLCFKATLKKFPLVKIPIKRGCADNCPKNSALLKYVCCSTDKN-
Q91126	LKCHNTQLPFIYKTCPEGKNLCFKATLKKFPLVKFVVRGCADNCPKNSALLKYVCCSTEKCN-
Q91137	LKCHNTQLPFIYKTCPEGKNLCFKATLKKFPLVKFVVRGCADNCPKNSALLKYVCCSSDKCN-
P0DUK7	LKCHNTQLPFIYKTCPEGKNLCFKATLKKFPLVKFPFKRGCADNCPKNSALLKYVCCSTDKN-
Q9W716	LKCHNTQLPFIYKTCPEGKNLCFKATLRKFPLVKFVVRGCADNCPKNSALLKYVCCSTDKN-
P62375	LKCHNTQLPFIYKTCPEGKNLCFKATLKKFPLVKFVVRGCADNCPKNSALLKYVCCSTDKN-

Figure S1. Aligned amino acid sequences of CLBP listed in Table S1. The program CLUSTAL O(1.2.4) for multiple sequence alignment was used.

Table S1. Physico-chemical and bioinformatics data on three-finger cytotoxins (cardiotoxins).

ID	Length	Molecular weight (Da) ¹	Charge ¹ (pH 7.0)	Isoelectr. Point ¹	Group ²	GenBank/ UniProt (Swiss-Prot) ³	Snake	Name	Spatial structure/ Chemical shifts ⁴	Similarity ⁵
1	60	6791.11	6.45	8.80	1	None/ P01458	N. nivea	Cytotoxin 3	None	None
2	60	6802.3	9.45	9.63	1	None/ P01461	N. annulifera	Cytotoxin 4/ Toxin CM-11	None	None
3	60	6773.09	5.55	8.54	1	None/ P01457	N. haje	Cytotoxin 5/ Toxin CM-8	None	None
4	60	6839.27	6.45	8.78	1	None/ P01459	N. annulifera	Cytotoxin 5/ Toxin CM-8 (CM-8A)	None	None
5	60	6812.26	7.55	9.08	1	765048C/ P01460	N. annulifera	Cytotoxin 8/ Toxin CM-7	None	None
6	60	6696.09	6.55	8.8	1	731687A/ P01455	N. annulifera	Cytotoxin 1/ Toxin V(II)1	None	None
7	60	6697.08	5.55	8.55	1	None/ P01456	N. nivea	Cytotoxin 1/ Toxin V(II)1	None	None
8	60	6871.37	5.55	8.54	3	None/ P01463	N. nivea	Cytotoxin 2/ Toxin V(II)2	None	None
9	60	6856.4	7.55	9.08	3	770226C/ P01464	N. annulifera	Cytotoxin 5/ Toxin CM-6	None	None
10	60	6858.37	5.55	8.54	3	754240A/ P01462	N. annulifera	Cytotoxin 2/ Toxin V(II)2/ V(II)2A	None	None
11	60	6857.38	6.55	8.79	3	770226A/ P01465	N. annulifera	Cytotoxin 6/ Toxin CM-2H	None	None
12	60	6856.4	7.55	9.08	3	770226B/ P01466	N. annulifera	Cytotoxin 7/ Toxin CM-4B	None	None
13	61	6910.32	7.45	9.1	4	None/ Q98965	N. atra	Cytotoxin 6	None	None
14	60	6689.15	7.45	9.09	4	CAB42058/ CAA07687/ AAB86638/ P80245	N. atra	Cytotoxin (cardiotoxin) 6/ Cardiotoxin A6 (N)	1UG4/None	Three-finger toxin –N. atra -E2IU05; P49124; Q9PWQ7

15	60	6632.09	7.45	9.09	4	CAA90965/ P49123	N. atra	Cytotoxin (cardiotoxin) 8	None	None
16	60	6827.4	8.45	9.43	4	None/ P01468	N. pallida	Cytotoxin 1/ Cardiotoxin gamma	1CXO/None; 1CXN/None; 1TGX/None	None
17	60	6800.31	9.45	9.7	4	740421B/ P01469	N. mossambica	Cytotoxin 2/ Cardiotoxin 2A/ Cytotoxin V(II)2	None	None
18	60	6697.02	6.55	8.79	4	CAB41507/ Q9W6W6	N. atra	Cytotoxin (cardiotoxin) 10	None	None
19	60	6826.42	9.45	9.72	4	740421A/ P01467	N. mossambica	Cytotoxin 1/ Cardiotoxin IIB/ Cytotoxin V(II)1	2CCX/None	None
20	60	6894.47	9.45	9.72	4	740421C/ P01470	N. mossambica	Cytotoxin 3/ V(II)3	None	None
21	60	6895.45	8.45	9.43	4	None/P0DSN1	N. nigricollis	Naniproin	None	None
22	60	6764.12	6.45	8.78	5	AAB35381.1/None	N. naja	CLBP ⁶	None	None
23	60	6723.23	7.55	9.12	5	CAA90962.1/ None	N. naja	Cardiotoxin 1	None	None
24	60	6701.18	6.45	8.81	5	AAB25732.1/ None	N. naja	Cardiotoxin 1	None	None
25	59	6724.17	5.45	8.54	5	AAB24495.1/ Q9PS33	N. oxiana	Cytotoxin Vc-1	None	None
26	60	6791.19	7.45	9.06	5	None/ P01447	N. naja	Cytotoxin 1	None	Q9PSN2
27	60	6792.18	6.45	8.78	5	None/ P86382	N. naja	Cytotoxin 7	None	None
28	60	6745.21	8.45	9.38	5	None/ P24780	N. naja	Cytotoxin 3	None	None
29	60	6745.21	8.45	9.38	5	None/ P01445	N. kaouthia	Cytotoxin 2	7QFC, 7QHI, 7O2K/ 27877	A0A8C6XLA1_N. Naja; Uncharacterized protein; Cytotoxin 13 N. naja;
30	60	6717.16	8.45	9.38	5	None/ P01446	N. kaouthia	Cytotoxin 3	6RC7/ 34392	None
31	60	6807.28	8.45	9.38	5	None/ P0CH80	N. kaouthia	Cytotoxin 1	None	None
32	60	6792.29	9.45	9.63	5	None/ P60306	N. atra	Cytotoxin SP13b	None	None
33	60	6795.29	8.45	9.38	5	None/ Q98961	N. atra	Cytotoxin 5	None	None

34	60	6750.18	7.45	9.09	5	AAB18377.1 (AAB18381.1; CAB42054.1)/ P01442	N. atra	Cytotoxin 2/ Cardiotoxin 1A/ Cardiotoxin 2/ Cardiotoxin A2/ Cardiotoxin II/ Cardiotoxin analog II	1CRE, 1CRF/ None; 4OM4/None	Cytotoxin 5a N. sputatrix- AAC27691.1/AAC 61317.1/O73857; Cytotoxin 5b N. sputatrix-P60310
35	60	6821.28	5.45	8.54	5	PRF: 229587/ P01451	N. oxiana	Cytotoxin I	1RL5, 5NPN/ 5989; 1ZAD/None; 5NQ4/ 34125;	None
36	60	6701.18	6.45	8.81	5	AAB01539.1/ P60304	N. atra	Cytotoxin 1/ Cardiotoxin 1/ Cardiotoxin A1/ Cardiotoxin I/ Cardiotoxin analog I	2CDX/ None	Cytotoxin 1, N. kaouthia - P60305
37	60	6701.13	5.45	8.55	5	AAB18380/ Q98958	N. atra	Cytotoxin (cardiotoxin) 1d/1e	None	None
38	60	6725.16	6.55	8.81	5	AAB36927/ P79810	N. atra	Cytotoxin (cardiotoxin) 1c	None	None
39	60	6704.2	5.45	8.55	5	CAA63978/ Q91135	N. atra	Cytotoxin (cardiotoxin) I –like P-15	None	None
40	60	6685.13	6.45	8.81	5	AAB18379/ Q98957	N. atra	Cytotoxin (cardiotoxin) 1a	None	None
41	60	6705.16	6.45	8.81	5	CAA63977/ Q91136	N. atra	Cytotoxin (carditoxin) I-like T- 15	None	None
42	60	6715.25	9.45	9.63	5	None/ P01452	N. mossambica	Cytotoxin (cardiotoxin) 4/V(II)	1CDT/None	None
43	60	6668.94	3.56	8.12	5	765048A/ P01454	N. annulifera	Cytotoxin 9/ Toxin CM-2e	None	None

44	60	6681.94	3.56	8.12	5	765048B/ P01453	N. annulifera	Cytotoxin 10/ Toxin CM-4a	None	None
45	60	6793.21	8.45	9.4	5	AAB86637, CAA73098, CAA73096/ P01443	N. atra	Cytotoxin 4/ Cardiotoxin A4/ Cardiotoxin analog IV	1KBT, 1KBS/ None; 4OM5/None	None
46	60	6765.2	8.45	9.38	5	None/ O73858	N. sputatrix	Cytotoxin (cardiotoxin) 6	None	None
47	60	6797.26	8.45	9.38	5	AAC61319/ O73859	N. sputatrix	Cytotoxin (cardiotoxin) 7	None	None
48	60	6753.1	5.45	8.54	5	None/ P60311	N. sputatrix	Cytotoxin KJC3	None	None
49	60	6793.16	5.45	8.53	5	None/ P86540	N. naja	Cytotoxin 8	None	None
50	60	6775.15	7.45	9.06	5	None/A0A0U5AL91	N. naja	Cytotoxin 17	None	None
51	60	6791.19	7.45	9.06	5	BAU24671/ A0A8C6XG05	N. naja	Cytotoxin 14/ Uncharacterized protein	None	A0A8C7DYP2
52	60	6711.06	4.46	8.33	5	None/P86538	N. naja	Cytotoxin 2a	None	None
53	60	6765.16	7.45	9.06	5	None/A0A0U4W6K7	N. naja	Cytotoxin 16	None	
54	60	6761.21	8.45	9.34	5	None/A0A8C6XQ33	N. naja	Uncharacterized protein	None	None
55	60	6681.08	5.45	8.55	5	BAU24666/ A0A0U5ARS4	N. naja	Cytotoxin 11	None	None
56	60	6791.15	6.45	8.78	5	None/A0A0U5AR60	N. naja	Cytotoxin 12	None	None
57	60	6747.18	8.45	9.34	5	None/A0A0U4W6H0	N. naja	Cytotoxin 15	None	None
58	60	6810.35	8.45	9.38	6	None/ P07525	N. atra	Cytotoxin 5/ Cardiotoxin A4b/ Cardiotoxin T/ Cardiotoxin analog V/ Cytotoxin D-1/ Membrane toxin D1	1CHV/None	None
59	60	6603.07	5.45	8.55	6	AAB18378/ Q98956	N. atra	Cytotoxin (cardiotoxin) 1b	None	None

60	60	6682.02	5.55	8.55	6	740419A/ P01448	N. melanoleuca	Cytotoxin 1/V(II)1	None	None
61	60	6808.27	8.45	9.4	6	CAB42057/ Q9W6W9	N. atra	Cytotoxin (cardiotoxin) 4N	None	None
62	60	6780.25	6.45	8.79	6	None/A0A8C6XFH6	N. naja	Uncharacterized protein	None	None
63	60	6654.05	5.45	8.55	6	None/A0A8C6XFM5	N. naja	Uncharacterized protein	None	None
64	60	6654.14	8.45	9.38	7	None/ P24779	N. kaouthia	Cytotoxin 5	None	None
65	60	6774.29	8.45	9.38	7	CAB42053 (AAB18383)/ Q98959	N. atra	Cytotoxin 3a/ Cardiotoxin-31/ Cardiotoxin 3a	None	None
66	60	6636.13	8.45	9.43	7	AAB18386/ Q98962	N. atra	Cytotoxin (cardiotoxin) 3d	None	None
67	60	6732.21	8.45	9.38	7	AAB18384/ Q98960	N. atra	Cytotoxin (cardiotoxin) 3b	None	None
68	60	6714.17	8.45	9.38	7	AAG02235/ Q9DGH9	N. kaouthia	Cytotoxin 2	None	None
69	60	6790.29	9.45	9.58	7	-	N. naja	newCT	None	None
70	60	6747.27	8.45	9.38	8	AAA49386.1/ Q02454	N. sputartix	Cytotoxin	1XT3/None; 2BHI/None; 1H0J, 1I02, 2CRT, 2CRS/4966, 15305	Cytotoxin 3 N. sputatrix-P60302; cytotoxin 3 N. atra-P60301; cytotoxin 4 N. kaouthia-P60303; P01444; Q9PS23; Q9W6W7
71	60	6683.27	9.45	9.68	8	None/ P60307	N. atra	Cytotoxin SP15a	None	None
72	60	6802.31	9.45	9.56	6	None/ P83345	N. sagittifera	Cytotoxin sagitoxin	None	None
73	60	6763.27	8.45	9.34	8	None/ P01440	N. naja	Cytotoxin 2/ Cobramine-B/ Cytotoxin II/ Cytotoxin-9	None	None

74	60	6763.27	8.45	9.34	8	AAC27686/ Q9PST3	N. sputatrix	Cytotoxin (cardiotoxin) 2b	None	Cytotoxin 2a – N. sputatrix-Q9PST4; cytotoxin 9, N. naja - A0A0U5AUY6; cytotoxin 1, N. sumatrana - A0A7T7DMY7
75	60	6793.36	8.45	9.34	8	AAC27690/ O73856	N. sputatrix	Cytotoxin (cardiotoxin) 4b	None	None
76	60	6609.1	8.45	9.43	8	AAB01542/ Q91124	N. atra	Cytotoxin (carditoxin) 8/VIII	None	None
77	60	6777.36	8.45	9.38	8	AAC27689/ O93473	N. sputatrix	Cytotoxin (cardiotoxin) 4a	None	None
78	60	6729.23	8.45	9.38	8	AAC27684/ O93471	N. sputatrix	Cytotoxin (cardiotoxin) 1	None	None
79	60	6664.18	8.55	9.44	8	AAB24494/ Q9PS34	N. oxiana	Cytotoxin Vc-5	None	None
80	60	6636.17	8.55	9.42	8	None/ P01441	N. oxiana	Cytotoxin 2 (II)	1CCQ/4418,4419 1CB9/4419, 1FFJ/4815	None
81	60	6652.26	10.45	9.9	8	None/ P60309	N. atra	Cytotoxin SP15d	None	None
82	60	6755.33	8.45	9.4	8	None/ P60308	N. atra	Cytotoxin SP15c	None	None
83	60	6845.33	7.46	9.1	8	None/ P25517	N. mossambica	Cytotoxin 5	None	None
84	60	6543.09	10.45	9.9	8	None/ E2ITZ7	N. atra	Three-finger toxin	None	None
85	61	6844.45	9.55	9.72	8	None/ P01471	Hemachatus haemachatus	Cytotoxin 1/ hemolytic protein 12B	None	None

86	61	6844.45	9.55	9.72	8	None/ B3EWH9	Hemachatus haemachatus	Three-finger hemachatoxin	3VTS/None	None
87	61	6793.27	6.55	8.81	8	None/ P24777	Hemachatus haemachatus	Cytotoxin 3/ Toxin 11(11A)	None	None
88	61	6800.33	9.55	9.72	8	None/ P24776	Hemachatus haemachatus	Cytotoxin 2/ Toxin 12A	None	None
89	60	6764.25	7.45	9.06	8	None/ P86541	N. naja	Cytotoxin 10	None	None
90	60	6823.3	8.52	9.53	8	None/O93472	N. sputatrix	Cytotoxin 2c	None	None

¹ - Molecular weight, electrical charge and isoelectric point were calculated using program modlamp (<https://github.com/alexarnimueller/modlAMP>);

² - The groups are listed in the separate Table, according to the special amino acid residues within the three loops;

³ - Databank identifiers;

⁴ - PDB-code in the PDB-bank (www.rcsb.org)/ chemical shifts in the Biological Magnetic Resonance Databank (BMRB, <http://www.bmrb.wisc.edu/>);

⁵ - Identical polypeptides – Name/Snake/SwissProte identifier;

⁶ - CLBP, Cardiotoxin-Like Basic Polypeptide. Here, the term “cytotoxin” is more relevant.

Table S2. Physico-chemical data and database codes for Cardiotoxin-like Basic Polypeptides (CLBP) from Elapid venoms.¹

Id	Length ²	M.W.(Da) ²	Charge ² (pH 7.0)	Isoelectr. point	Uniprot/NLM ³ code	Snake	Name	Similarity
1	61	6608.89	7.55	9.15	sp Q8UUK0 3SOF3_NAJAT	N. atra	Cytotoxin homolog Clbp-3	None
2	63	7192.62	6.66	8.82	sp P24778 3SOE_HEMHA	Hemachatus haemachatus	Cytotoxin homolog	None
3	61	6833.1	7.55	9.07	sp P01473 3SOF3_NAJME	N. melanoleuca	Cytotoxin homolog 3	None
4	61	6850.13	8.55	9.38	sp P01474 3SOF2_NAJME	N. melanoleuca	Cytotoxin homolog 2	None
5	61	6795.14	8.55	9.5	sp P49122 3SOF7_NAJAT	N. atra	Cytotoxin 7	None
6	62	6974.51	10.55	9.98	tr A0A8C6XPA7 A0A8C6XPA7_NAJNA	N. naja	Snake toxin/toxin-like domain-containing protein	None
7	62	6842.33	9.55	9.75	sp P62394 3SOFB_NAJHH	N. haje haje	Cytotoxin 11	P62390
8	62	6994.39	8.55	9.4	sp P14541 3SOFH_NAJKA	N. kaouthia	Cytotoxin homolog	None
9	62	7028.41	8.55	9.4	sp Q91126 3SOFA_NAJAT	N. atra	Cardiotoxin 7a	None
10	62	7000.36	8.55	9.4	sp Q91137 3SOFL_NAJAT	N. atra	Cytotoxin homolog 5	None
11	62	7062.43	8.55	9.4	sp P0DUK7 3SOF1_NAJAT	N. atra	Mu-elapitoxin-Na1a	None
12	62	7042.39	8.55	9.42	sp Q9W716 3SOFV_NAJAT	N. atra	Cytotoxin homolog 5V	None
13	62	7014.38	8.55	9.4	sp P62375 3SOF5_NAJAT	N. atra	Cytotoxin A5	P62377 Q91996 A0A8C6XRN7

¹ Aligned amino acid sequences of the toxins (without signal peptides) are given in Figure S1.

² Molecular weight, electrical charge and isoelectric point were calculated using program modlamp (<https://github.com/alexarnimueller/modlAMP>);

³ NLM, US National Library of Medicine (<https://www.ncbi.nlm.nih.gov/>).

Table S3. List of short neurotoxins used to build up the logo-sequence in Figure 3c.

No	Identifier			Amino acid sequence ¹
1	sp	C1IC48	3S14_	LLCHNQQSSTSPTTTTCCSGGESKCYKKRWPTHRTGTERGCGCPTVKKGIELHCCTTDQC�NL
2	sp	P01432	3S13_	LNCHNQMSAQPPTTTTRCSTRWETNCYKKRWRDHRGYKTERGCGCPTVKKGIQLHCCTSDNCNN
3	sp	P34076	3S11_	MECHNQQSSQPPTTTTHCSGGETNCYEKRWDHRGTIIERGCGCPTVKPGVKLNCCTTDKCNN
4	sp	C0HM08	3S12_	LECHNQQSSQTPTTTTDCSGGETNCYKKWWS DHRGTIIERGCGCPTVKKGIELNCCTTDRCNN
5	tr	A0A8C6XDY8	A	LECHNQQSSQPPTTTTCC-SGETNCYKKRWRDHRGYRTERGCGCPSVRNGIEINCCTTDRCNN
6	sp	P82849	3S1B2	LECHNQQSSQTPTTTTGCSGGENNCYKKEWRDNRGYRTERGCGCPSVKKGIGINCCTTDRCNN
7	sp	P01431	3S11_	LECHNQQSSEPPTTTTRCSGGETNCYKKRWRDHRGYRTERGCGCPTVKKGIELNCCTTDRCNN
8	sp	O57327	3S1A4	LECHNQQSSQAPTTTTCGSGGETNCYKKGWRDHRGYRIERGCGCPSVKKGIEINCCTTDRCNN
9	sp	Q9YGJ5	3S1A2	LECHNQQSSQAPTTTTCGSGGETNCYKKS WRDHRGYRIERGCGCPSVKKGIEINCCTTDRCNN
10	sp	O57326	3S1A3	LECHNQQSSETPTTTTGCSGGETNCYKKRWRDHRGYRIERGCGCPSVKKGIEINCCTTDRCNN
11	sp	Q9YGJ6	3S1A1	LECHNQQSSETPTTTTGCSGGETNCYKKS WRDHRGYRIERGCGCPSVKKGIEINCCTTDRCNN
12	sp	E2ITZ3	3S11A	LECHNQQSSQTPTTTTGCSGGETNCYKKRWRDHRGYRTERGCGCPSVQTAIEINCCTTDRCNN
13	sp	P14613	3S1B1	LECHNQQSIQTPTTTTGCSGGETNCYKKRWRDHRGYRTERGCGCPSVKNGIEINCCTTDRCNN
14	sp	Q9PTT0	3S1CB	LECHNQQSSQTPTTTTGCSGGETNCYKKRWRDHRGYRTERGCGCPSVKNGIEINCCTTDRCNN
15	sp	Q9PSN6	3S13_	LECHDQSSQTPTTTTGCSGGETNCYKKRWRDHRGYRTERGCGCPSVKNGIEINCCTTDRCNN
16	sp	P01420	3S13_	MICYKQSSLQFPITTV C-PGEKNCYKKQWSGHRGTIIERGCGCPSVKKGIEINCCTTDKCNR
17	sp	P01421	3S14_	MICYKQRS LQFPITTV C-PGEKNCYKKQWSGHRGTIIERGCGCPSVKKGIEINCCTTDKCNR
18	sp	P34075	3S11_	KICYNQPSSQHPTTKAC-PGEKNCYRKQWS DHRGTIIERGCGCPTVVKPGVKLHCCTTEKCNN
19	sp	P01433	3S12_	LECHNQQSSQTPTTQTC-PGETNCYKKQWS DHRGSRTERGCGCPTVVKPGIKLKCCTTDRCNK
20	sp	P25675	3S12_	MICHNQQSSQPPTIKTC-PGETNCYKKQWRDHRGTIIERGCGCPSVKKGVGIYCCKTDKCNR
21	sp	P01422	3S12_	MICHNQQSSQPPTIKTC-PGETNCYKKRWRDHRGTIIERGCGCPSVKKGVGIYCCKTNKCNR
22	sp	P01423	3S12_	MICHNQQSSQRPTIKTC-PGETNCYKKRWRDHRGTIIERGCGCPSVKKGVGIYCCKTDKCNR
23	sp	P68417	3S11_	LECHNQQSSQPPTTKTC-PGETNCYKKRWRDHRGSITERGCGCPSVKKGIEINCCTTDKCNN
24	sp	P01425	3S11_	LECHNQQSSQPPTTKSC-PGDTNCYNKRWRDHRGTIIERGCGCPTVVKPGINLKCCTTDRCNN
25	sp	P01424	3S11_	MECHNQQSSQPPTTKTC-PGETNCYKKQWS DHRGTIIERGCGCPSVKKGVKINCCTTDRCNN
26	sp	P01426	3S11_	LECHNQQSSQPPTTKTC-PGETNCYKKVWRDHRGTIIERGCGCPTVVKPGIKLNCCTTDKCNN
27	sp	P80958	3S1CC	LECHNQQSSQTPTTKTC-SGETNCYKKWWS DHRGTIIERGCGCPVKVPGVNLNCCTTDRCNN
28	sp	P59275	3S1B_	LECHNQQSSQTPTTKTC-SGETNCYKKWWS DHRGTIIERGCGCPVKVPGVNLNCCRRDRCNN
29	sp	P60774	3S11_	LECHNQQSSQAPTTKTC-SGETNCYKKWWS DHRGTIIERGCGCPVKVPGVKLNCCTTDRCNN
30	sp	P60773	3S11_	LECHNQQSSQAPTTKTC-SGETNCYKKWWS DHRGTIIERGCGCPVKVPGVKLNCCTTDRCNN
31	sp	P01427	3S11_	LECHNQQSSQPPTTKTC-SGETNCYKKWWS DHRGTIIERGCGCPVKVPGVNLNCCRTDRCNN
	sp	P59276	3S1C_	LECHNQQSSQAPTTKTC-SGETNCYKKWWS DHRGTIIERGCGCPVKVPGVNLNCCRTDRCNN

32	sp	P01416	3S11_	RICYNHQSTTRATTKSCE--ENSCYKKYWRDHRGTIIERGCGCPKVKPGVGIHCCQSDKCNY
33	sp	P01418	3S11_	RICYNHQSTTPATTKSCG--ENSCYKKTWSDHRGTIIERGCGCPKVKRGVHLHCCQSDKCNN
34	sp	P01417	3S11_	RICYNHQSTTPATTKSCG--ENSCYKKTWSDHRGTIIERGCGCPKVKQGIHLHCCQSDKCNN
35	sp	F5CPD8	3S157	MICYNQQSSQPPTTTTCS--EGQCYKQRWRDHRGWRTERGCGCPKAIPEVKLNCCKTDRONG
36	sp	P80548	3S11_	MICHNQQSSQPPTIKTCS--EGQCYKKTWRDHRGTIIERGCGCPTVKPGIHIISCCASDKCNA
37	sp	K9MCH1	3S11_	RICYNQQSSQPPTTKTCS--EGQCYKKTWRDHRGTIIERGACPNVKPGIQISCCTSDDKCNG
38	sp	P86420	3S11_	MICYNHQSSSEPPTTKTCS--EGQCYKKSWSDHRGTIIERGACPNVKPGVKIICCRSC----
39	sp	P0CAR1	3S1D1	MICYNQQSSQPPTTKTCS--EGQCYKKTWSDHRGTIIERGACPNVKPGVKISCCSSDDKCNG
40	sp	F5CPD5	3S151	MICYNQQSSQPPTTTTCS--EGQCYKKTWSDHRGTIIERGACPNVKPGVKISCCSSDDKCNG
41	sp	P86095	3S11_	MICYNQQSTEPPTTKTCS--EGQCYKKTWSDHRGTIIERGACPNVKPGVKISCCSSDDKCR-
42	sp	C0HLK3	3S11_	MICYNQQSSEPPTTKTCS--EGQCYKKTWSDHRGTIIERGACPNVKPGVKISCCSSDDK---
43	sp	Q7T2I5	3S1EC	RICFNHQSSQPQTTKTCSPGESSCYHKQWSDFRGTIIERGCGCPTVKPGINLSCCESEVCNN
44	sp	P10457	3S12_	RRCYNQQSSQPKTTKSCPPGENSCYNKQWRDHRGSITERGCGCPTVKPGIKLRCCSEDCNN
45	sp	Q9YGC7	3S15_	RRCFNQQSSQPKTTKSCPLGENSCYNKQWRDHRGSITERGCGCPKVKPGIKLRCCSEDCNN
46	sp	Q9YGX0	3S14_	RRCFNQQSSQPKTTKSCPLGENSCYNKQWRDHRGSITERGCGCPKVKPGIKLRCCSEDCNN
47	sp	P10455	3S1C_	RRCYNQQSSQPKTTKSCPPGENSCYNKQWRDHRGSITERGCGCPKVKPGIKLRCCSEDCNN
48	sp	P10456	3S1D_	RRCFNQQSSQPKTTKSCPPGENSCYNKQWRDHRGSITERGCGCPKVKPGIKLRCCSEDCNN
49	sp	P10460	3S1C_	RRCFNQQSSQPQTNKSCPPGENSCYRKQWRDHRGTIIERGCGCPTVKPGVKLRCCQSEDCNN
50	sp	Q7T2I1	3S110	RRCFNQQSSEPQTNKSCPPGENSCYRKQWRDHRGTIIERGCGCPTVKPGVKLRCCSEDCNN
51	sp	Q9YGX1	3S1ED	RRCFNQQSSEPQTNKSCPPGENSCYRKQWRDHRGTIIERGCGCPTVKPGIKLRCCSEDCNN
52	sp	Q9YGW8	3S14_	RRCFNQQSSEPQTNKSCPPGENSCYNKQWRDHRGTIIERGCGCPQVKSGIKLTCCQSDDCNN
53	sp	Q9YGW9	3S12_	RRCFNQQSSEPQTNKSCPPGENSCYNKQWRDHRGTITERGCGCPQVKSGIKLTCCQSDDCNN
54	sp	P25496	3S1B_	RRCFNHPSSQPQTNKSCPPGENSCYNKQWRDHRGTIIERGCGCPQVKSGIKLRCCQSDDCNN
55	sp	P25495	3S1A_	RRCFNHPSSQPQTNKSCPPGENSCYNKQWRDHRGTIIERGCGCPTVKPGIKLRCCQSDDCNN
56	sp	Q9YGC2	3S11_	RRCFNHPSSQPQTNKSCPPGENSCYNKQWRDHRGTIIERGCGCPTVKPGIKLTCCQSEDCNN
57	sp	P19959	3S12_	LTCCNQQSSQPKTTTDCA--DNSCYKMTWRDHRGTIERGCGCPQVKPGIKLECCCKTNECNN
58	sp	P19960	3S14_	LTCCNQQSSQPKTTTDCA--DDSCYKKTWKDHRGTIERGCGCPQVKPGIKLECCCKTNECNN
59	sp	P32879	3S11_	LTCCNQQSSQPKTTTDCA--DNSCYKKTWQDHRGTIERGCGCPQVKPGIKLECCCKTNECNN
60	sp	P19958	3S13_	LTCCNQQSSQPKTTTDCA--DNSCYKKTWKDHRGTIERGCGCPQVKPGIKLECCCKTNECNN
61	sp	Q5UFR8	3S11_	MTCCNQQSSQPKTTTNCA--GNSCYKKTWSDHRGTIIERGCGCPQVKSGIKLECCCKTNECNN
62	sp	P62388	3S1A_	MTCCNQQSSQPKTTTNCA--ESSCYKKTWSDHRGTIERGCGCPQVKSGIKLECCCKTNECNN
63	sp	P25494	3S11_	MTCCNQQSSQPKTTTNCA--ESSCYKKTWSDHRGTIERGCGCPQVKKGIKLECCCKTNECNN
64	sp	Q8UW27	3S116	MTCCNQQSSQPKTTTNCA--ESSCYKKTWSDHRGTIERGCGCPQVKRGIKLECCCKTNECNN
65	sp	Q8UW26	3S12_	MTCCNQQSSQPKTTTNCA--ESSCYKKTWSDHRGTIERGCGCPQVKHGIKLECCCKTNECNN
65	sp	A8HDJ8	3S12_	MTCCNQQSSQPKTITTCA--ESSCYKKTWKDHHGTIERGCGCPKVKPGVGLECCCKTDECNN

66	sp	F8J2G3	3S12A	MTCYNQQSSQPQTTTTCA--ESSCYKKTWRDHRGTIIERGCGCPTVKPGIQRVCCATDKCNN
67	sp	A8HDJ6	3S12_	MTCCNQQSSQPKTTTPCA--ESSCYKKTWKDHRGTIIERGCGCPNVKPGIDLMCCRTDECNN
68	sp	A8HDJ5	3S11_	MTCCNQQSSQPKTTTPCA--ESSCYKKTWKDNRGTTIERGCGCPNVKPGIDLMCCCKTDECNN
69	sp	A8HDJ9	3S11_	MTCCNQQSSQPKTTKTCA--ESSCYKKTWRDHRGTITERGCGCPSVKPGIQLCCCKTNECNN
70	sp	A8S6A4	3S11_	MTCCNQQSSQPKTTTTCA--ESSCYKKTWRDHRGTIIERGCGCPNVKPGIQLVCCETNECNN
71	sp	A7X4R5	3S13_	MTCYNQQSSQAKTTTTCSGGVSSCYRKTWSDIRGTIIERGCGCPSVKKGIERICCGTDKCNN
72	sp	P0CB06	3S12_	MTCYNQQSSEAKTTTTCSGGVSSCYKKTWSDIRGTIIERGCGCPSVKKGIERICCRTDKCNN
73	sp	Q45Z11	3S11_	MTCYNQQSSEAKTTTTCSGGVSSCYKKTWSDGRGTIIERGCGCPSVKKGIERICCRTDKCNN
74	sp	P01434	3S11_	MQCCNQQSSQPKTTTTCPGGVSSCYKKTWRDHRGTIIERGCGCPRVKPGIRLICCKTDECNN
75	sp	A8HDJ4	3S11_	MTCCNQQSSQPKTTTTCAGGESSCYKKTWSDHRGSRTERGCGCPHVKPGIKLTCCETDECNN

¹ – Aligned with CLUSTAL W (1.83) multiple sequence alignment.

Table S4. Distribution of CT across groups, determining their membrane activity¹.

Group	Amino acid sequence	Database identifier	ID # in Table S1
1, PSX	LKCNQLIP P FWKTCPKGKNLCYNMYMV ST STVPVKRGCIDVCPK N SALVKYVCCNTDRCN LKCNKLIP P FWKTCPKGKNLCYKMYMV ST LTVPVKRGCIDVCPK N SALVKYVCCNTNKC LKCHQLV P FWKTCPEGKNLCYKMYMV SS STVPVKRGCIDVCPK N SALVKYVCCNTDKCN LKCYKL V PFWKTCPEGKNLCYKMYMV ST LTVPVKRGCIDVCPK N SALVKYVCCNTDKCN LKCHKL V PFWKTCPEGKNLCYKMYMV ST LTVPVKRGCIDVCPK N SALVKYVCCNTNKC LKCHKL V P V WKTCEGKNLCYKMFV ST STVPVKRGCIDVCPK N SALVKYVCCSTDKC LKCHKL V P V WKTCEGKNLCYKMFV ST STVPVKRGCIDVCPK D SALVKYVCCSTDKC In total: 7 items	P01458 P01461 P01457 P01459 P01460 P01455 P01456	1 2 3 4 5 6 7
2, PSO	This group is empty In total: 0 items		
3, POX	LKCHQLIP P FWKTCPEGKNLCYKMYVATPMIPVKRGCIDVCPK N SALVKYMCCNTDKCN LKCHKL V PFWKTCPEGKNLCYKMYVATPMIPVKRGCIDVCPK N SALVKYMCCNTNKC LKCHKL V PFWKTCPEGKNLCYKMYVATPMLPVKRGCIDVCPK D SALVKYMCCNTDKCN LKCHKL V PFWKTCPEGKNLCYKMYVATPMLPVKRGCIDVCPK D SALVKYMCCNTNKC LKCHKL V PFWKTCPEGKNLCYKMYVATPMLPVKRGCIDVCPK D SALVKYMCCNTNKC In total: 5 items	P01463 P01464 P01462 P01465 P01466	8 9 10 11 12
4, POO	LKCNQLIP P FYKTCAGKNLCYKMFVAAQRFVPVKRGCIDVCPKSSLLVKYVCCNTDRCN LKCNQLIP P FYKTCAGKNLCYKMFVAAPKVPVKRGCIDVCPKSSLLVKYVCCNTDRCN LKCNQLIP P FYKACAAGKNLCYKMFVAAPKVPVKRGCIDVCPKSSLLVKYVCCNTDRCS LKCNQLIP P FWKTCPKGKNLCYKMTMRAAPMVPVKRGCIDVCPKSSLLIKYMCCNTDKCN LKCNQLIP P FWKTCPKGKNLCYKMTMRGASKVPVKRGCIDVCPKSSLLIKYMCCNTDKCN LKCNQH I P P FYKTCAGKNLCYKIFMVAAPKVPVKRGCIDVCPKSSDLVKYVCCNTDRCN LKCNQLIP P FWKTCPKGKNLCYKMTMRAAPMVPVKRGCIDVCPKSSLLIKYMCCNTNKC LKCNRLIP P FWKTCPEGKNLCYKMTMRLAPKVPVKRGCIDVCPKSSLLIKYMCCNTNKC	Q98965 P80245 P49123 P01468 P01469 Q9W6W6 P01467 P01470	13 14 15 16 17 18 19 20

	LKCNRLIP P FWKTCPEGKNLCYKMTMRLAPKVPVKRGCIDVCPKSSLLIKYMCCTNDKCN In total: 9 items	PODSN1	21
5, OSX	LKCNKLIPLAYKTCPAGKNLCYKMYMV S NKTVPVKRGCIDVCPK N SLLVTYECCNTDRCN	AAB35381.1	22
	LKCNKLIPIASKTCPAGKNLCYKMFMM S DLTIPVKRGCIDVCPK N SLLVKYVCCNTHRCN	CAA90962.1	23
	LKCNKLIPIASKTCPAGKNLCYKMFMM S DLTIPVKRGCIDVCPK N SLLVKYVCCNTDRCN	AAB25732.1	24
	LKCNKLVPIAYKTCE - GKNLCYKMFMM S DLTIPVKRGCIDVCPK N SLLVKYVCCNTDRCN	<u>Q9PS33</u>	25
	LKCNKLIPLAYKTCPAGKNLCYKMYMV S NKTVPVKRGCIDVCPK N SLVLKYECCNTDRCN	P01447	26
	LKCNKLIPLAYKTCPAGKDLCYKMYMV S NKTVPVKRGCIDVCPK N SLLVKYECCNTDRCN	P86382	27
	LKCNKLIPLAYKTCPAGKNLCYKMFMV S NKTVPVKRGCIDVCPK N SLVLKYVCCNTDRCN	P24780	28
	LKCNKLIPLAYKTCPAGKNLCYKMFMV S NKTVPVKRGCIDVCPK N SLLVKYVCCNTDRCN	P01445	29
	LKCNKLIPLAYKTCPAGKNLCYKMFMV S NKTVPVKRGCIDACP N SLLVKYVCCNTDRCN	P01446	30
	LKCNKLVPLFYKTCPAGKNLCYKMFMV S NKTVPVKRGCIDVCPK N SLVLKYVCCNTDRCN	P0CH80	31
	LKCNKLKPLAYKTCPAGKNLCYKMFMM S NKTVPVKRGCIDVCPK N SLLVKYVCCNTDRCN	P60306	32
	LKCNKLVPLFYKTCPAGKNLCYKMFMV S NKMVPVKRGCIDVCPK N SALVKYVCCNTDRCN	Q98961	33
	LKCNKLVPLFYKTCPAGKNLCYKMFMV S NLTVPVKRGCIDVCPK N SALVKYVCCNTDRCN	P01442	34
	LKCNKLVPIAYKTCEGKNLCYKMFMM S DLTIPVKRGCIDVCPK N SLLVKYVCCNTDRCN	P01451	35
	LKCNKLIPIASKTCPAGKNLCYKMFMM S DLTIPVKRGCIDVCPK N SLLVKYVCCNTDRCN	P60304	36
	LKCNQLIPIASKTCPAGKNLCYKMFMM S DLTIPVKRGCIDVCPK N SLLVKYVCCNTDRCN	Q98958	37
	LKCNKLIPIASKTCPAGKNLCYKMFMM S DLTIPVKRGCIDVCPK N SHLVKYVCCNTDRCN	P79810	38
	LKCNKLIPIASKTCPAGMNL CYKMFMM S DLTIPVKRGCIDVCPK N SLLVKYVCCNTDRCN	Q91135	39
	LKCNKLPPIASKTCPAGKNLCYKMFMM S DLTIPVKRGCIDVCPK N SLLVKYVCCNTDRCN	Q98957	40
	LKCNKLIPIASKTCTAGKNLCYKMFMM S DLTIPVKRGCIDVCPK N SLLVKYVCCNTDRCN	Q91136	41
	LKCNKLIPIAYKTCEGKNLCYKMMLA S KKMVPVKRGGINVCPK N SALVKYVCCSTDRCN	P01452	42
	LECNKLVPIAHKTCEGKNLCYKMFMV S TSTVPVKRGCIDVCPK D SALVKYVCCNTDRCN	P01454	43
	LECNQLIPIAHKTCEGKNLCYKMFMV S TSTVPVKRGCIDVCPK N SALVKYVCCNTDRCN	P01453	44
	RKCNKLVPLFYKTCPAGKNLCYKMFMV S NLTVPVKRGCIDVCPK N SALVKYVCCNTDRCN	P01443	45
	LKCNKLVPLFYKTCPAGKNLCYKMFMV S NKTVPVKRGCIDVCPK N SALVKYVCCNTDRCN	O73858	46
	LKCNKLVPLFYKTCPAGKNLCYKMFMM S NKTVPVKRGCIDVCPK N SALVKYVCCNTDRCN	O73859	47

	DKCNKLVPLFYKTCPAGKNLCYKMFVMSDLTVPVKGRCIDVCPKNSALVKYVCCNTDRCN LKC NKLIPLAYKTCPAGKDL CYKMYMVSDKTVPVKGRCIDVCPKNSLLVKYECCNTDRCN LKC NKLIPLAYKTCPAGKNLCYKMYMVSNKTVPVKGRCIDVCPKNSPLVKYECCNTDRCN LKC NKLIPLAYKTCPAGKNLCYKMYMVSNKTVPVKGRCIDVCPKNSLLVKYECCNTDRCN LQCNKLVPIASKTCPPGKNLCYKMFVMSDLTIPVKGRCIDVCPKNSLLVKYECCNTDRCN LKC NKLIPLAYKTCAGKNLCYKMYMVSNKTVPVKGRCIDVCPKNSLLVKYECCNTDRCN LKC NKLIPLAYKTCPAGKNLCYKMYMVSNKTVPVKGRCIDVCPKNSLLVKYVCCNTDRCN LQCNKLVPIASKTCPPGKNLCYKMFVMSDLTIPVKGRCIDVCPKNSLLVKYVCCNTDRCN LQCNKLIPLAYKTCPAGKNLCYKMYMVSNKTVPVKGRCIDVCPKNSLLVKYECCNTDRCN LKC NKLVPLAYKTCPAGKNLCYKMYMVSNKTVPVKGRCIDVCPKNSLLVKYVCCNTDRCN In total: 36 items	P60311 P86540 A0A0U5AL91 A0A8C6XG05 P86538 A0A0U4W6K7 A0A8C6XQ33 A0A0U5ARS4 A0A0U5AR60 A0A0U4W6H0	48 49 50 51 52 53 54 55 56 57
6, OSO	LKC NKLVPLFYKTCPAGKNLCYKMFVMSNKMVPVKGRCIDVCPKSSLLVKYVCCNTDRCN LKC NKLVPIASKTCPAGKNLCYKMFMSDLTVPVKGRCIDVCPKSSLLVKYVCCNTDRCN LEC NKLVPIAHKTCPAGKNLCYQMYMVSKSTIPVKGRCIDVCPKSSLLVKYVCCNTDRCN RKC NKLVPLFYKTCPAGKNLCYKMFVMSNLTVPVKGRCIDVCPKSSLLVKYVCCNTDRCN LKC NKLVPLFYKTCPAGKNLCYKMFVMSDLTIPVKGRCIDVCPKSSLLVKYVCCNTDRCN LQCNKLVPIASKTCPPGKNLCYKMFVMSDLTIPVKGRCIDVCPKSSLLVKYVCCNTDRCN In total: 6 items	P07525 Q98956 P01448 Q9W6W9 A0A8C6XFH6 A0A8C6XFM5	58 59 60 61 62 63
7, OOX	LKC NKLIPLAYKTCPAGKNLCYKMFVMAAPKVPVKGRCIDACPKNSLLVKYVCCNTDRCN LKC NKLVPLFYKTCPAGKNLCYKMFVATPKVPVKGRCIDVCPKNSLLVKYVCCNTDRCN LKC NKLIPIASKTCPAGKNLCYKMFVATPKVPVKGRCIDVCPKNSLLVKYVCCNTDRCN LKC NKLVPLFYKTCPAGKNLCYKMFVATPKVPVKGRCIDVCPKNSALVKYVCCNTDRCN LKC NKLVPLFYKTCPAGKNLCYKIFVATPKVPVKGRCIDVCPKNSALVKYVCCNTDRCN LKC NKLVPLFYKTCPKGKNLCYKMYMVAAPTVPVKGRCINVC PKNSLV LKYECCNTNKC N In total: 6 items	P24779 Q98959 Q98962 Q98960 Q9DGH9 newCT ²	64 65 66 67 68 69
8, OOO	LKC NKLVPLFYKTCPAGKNLCYKMFVATPKVPVKGRCIDVCPKSSLLVKYVCCNTDRCN LKCKKLVPLFSKTCPPGKNLCYKMFVATPKVPVKGRCIDVCPKSSLLVKYVCCNTDKCN LKC NKLVPLAYKTCPAGKNLCYKMYVANKKVPVKGRCIDVCPKSSLLVKYECCNTDRCN	Q02454 P60307 P83345	70 71 72

LKCNKLVPLFYKTCPAGKNLCYKMYMVATPKVPVKGRCIDVCPKSSLLVKYVCCNTDRCN	P01440	73
LKCNKLVPLFYKTCPAGKNLCYKMYMVATPKVPVKGRCIDVCPKSSLLVKYVCCNTDRCN	Q9PST3	74
LKCNKLVPLFYKTCPAGKNLCYKMYMVAMPKVPVKGRCIDVCPKSSLLVKYVCCNTDRCN	O73856	75
LKCNKLIPIASKTCPAGKNLCYKMFVATPKVPVKGRCIDVCPKSSLLVKYVCCNTDRCN	Q91124	76
LKCNKLVPLFYKTCPAGKNLCYKMFVAMPKVPVKGRCIDVCPKSSLLVKYVCCNTDRCN	O93473	77
LKCNKLVPLFYKTCPAGKNLCYKIFMVATPKVPVKGRCIDVCPKSSLLVKYVCCNTDRCN	O93471	78
LKCKKLVPLFSKTCPAGKNLCYKMFVAAPHVPVKGRCIDVCPKSSLLVKYVCCNTDRCN	Q9PS34	79
LKCKKLVPLFSKTCPAGKNLCYKMFVAAPHVPVKGRCIDVCPKSSLLVKYVCCNTDKCN	P01441	80
LKCKKLVPLFSKTCPPGKNLCYKMFVAAPKVPVKGRCINVCPSLLVKYVCCNTDKCN	P60309	81
LKCKKLVPLFSKTCPPGKNLCYKMFVATPKVPVKRECIDVCPKSSLLVKYVCCNTDKCN	P60308	82
LKCKKLIPFSKTCPEGKNLCYKMTMLAPKVPVKGRCIDVCPKSSFLVKECCDTDRCN	P25517	83
LKCNKLVPLFYKTCPAGKNLCYKMFVATPKVPVKGCAATCPKSSALVKVCCCKTDKCN	E2ITZ7	84
LKCHNKLVPLFSKTCPEGKNLCYKMTMLKMPKIPKRGCTDACPKSSLLVKVCCNKDKCN	P01471	85
LKCHNKLVPLFSKTCPEGKNLCYKMTMLKMPKIPKRGCTDACPKSSLLVKVCCNKDKCN	B3EWH9	86
LKCHNKLVPLFSKTCPDGKNLCYKMSMEVTPMIPKRGCTDTCPSLLVKVCCCKTDKCN	P24777	87
LKCHNKVPLFSKTCPEGKNLCYKMTLKKVPKIPKRGCTDACPKSSLLVNMCCCKTDKCN	P24776	88
LKCNKLVPLFYKTCPAGKDLCYKMYMVATPKVPVKGRCIDVCPKSSLLVKYVCCNTDRCN	P86541	89
LKCNKLVPLFYKTCPAGKNLCYKMYMVATPKVPVKGRCIDVYPKSSLLVKYVCCNTDRCN	O93472	90
In total: 21 items		

¹- Specific residues determining assignment of a CT to the certain group are marked in bold;

²- This toxin has not been deposited to any database yet.