Table 1. Maximum-likelihood parameter estimates for the plesiotypic 3FTX from 'non-front-fanged' advanced snakes

Model	Likelihood (ι)	ω_0[a]	Parameters	Sign.[b]	No. of Sites with $\omega > 1$[c]
					B.E.B
M0 (One ratio)	-3678.383646	1.29	$= \omega_0$		-
M1 (Neutral)	-3585.956425	0.72	P_0: 0.314 ω_0: 0.126 P_1: 0.685 ω1:1.0		-
M2 (Selection)*	-3540.037278	1.71	P_0: 0.218 ω_0: 0.13 P_1: 0.328 ω_1:1.0 P_2: 0.452 ω_2: 3.0	P << 0.001	18 (PP ≥ 0.99) 8 (P ≥ 0.95)
M3 (Discrete)*	-3539.925471	1.67	P_0: 0.206 ω_0: 0.11 P_1: 0.330 ω_1: 0.91 P_2: 0.462 ω_2: 2.91	P << 0.001	-
M7 (beta)	-3588.471551	0.71	p: 0.34252 q: 0.13966		-
M8 (beta and ω)*	-3539.994764	1.63	p_0: 0.534 p: 0.447 q: 0.316 p1: 0.465 ω: 2.84	P << 0.001	27 (PP ≥ 0.99) 10 (P > 0.95)

Legend:

a: dn/ds (weighted average)

b: Significance of the model in comparison with the null model

c: Number of sites with ω > 1 under the Bayes empirical Bayes approach with a posterior probability (PP) more than or equal to 0.99 and 0.95

* Models which allow ω > 1

Table 2. Maximum-likelihood parameter estimates for the plesiotypic 3FTX from Viperidae

Model	Likelihood (ι)	ω_0[a]	Parameters	Sign.[b]	No. of Sites with $\omega > 1$[c]
					B.E.B
M0 (One ratio)	-1195.940485	1.79	$= \omega_0$		-
M1 (Neutral)	-1178.683529	0.67	P_0: 0.344 ω_0: 0.05 P_1: 0.655 ω1: 1.0		-
M2 (Selection)*	-1155.587491	3.28	P_0: 0.454 ω_0: 0.37 P_1: 0.0 ω_1:1.0 P_2: 0.545 ω_2: 5.70	$P << 0.001$	13 ($PP \geq 0.99$) 11 ($P \geq 0.95$)
M3 (Discrete)*	-1153.672936	3.93	P_0: 0.427 ω_0: 0.33 P_1: 0.485 ω_1: 4.86 P_2: 0.086 ω_2: 16.4	$P << 0.001$	-
M7 (beta)	-1179.346712	0.71	p: 0.03813 q: 0.01410		-
M8 (beta and ω)*	-1155.588330	3.28	p_0: 0.454 p: 58.52 q: 99.0 p1: 0.545 ω: 5.71	$P << 0.001$	18 ($PP \geq 0.99$) 12 ($P > 0.95$)

Legend:

a: dn/ds (weighted average)

b: Significance of the model in comparison with the null model

c: Number of sites with $\omega > 1$ under the Bayes empirical Bayes approach with a posterior probability (PP) more than or equal to 0.99 and 0.95

* Models which allow $\omega > 1$

Table 3. Maximum-likelihood parameter estimates for the plesiotypic 3FTX from Elapidae

Model	Likelihood (ι)	ω_0[a]	Parameters	Sign.[b]	No. of Sites with ω > 1[c]
					B.E.B
M0 (One ratio)	-1885.178988	1.30	$= \omega_0$		-
M1 (Neutral)	-1833.771080	0.60	P_0: 0.419 ω_0: 0.05 P_1: 0.580 ω1: 1.0		-
M2 (Selection)*	-1807.546142	1.79	P_0: 0.355 ω_0: 0.08 P_1: 0.249 ω_1:1.0 P_2: 0.395 ω_2: 3.83	P << 0.001	15 (PP ≥ 0.99) 9 (P ≥ 0.95)
M3 (Discrete)*	-1807.476797	1.75	P_0: 0.313 ω_0: 0.24 P_1: 0.279 ω_1: 0.81 P_2: 0.407 ω_2: 3.70	P << 0.001	-
M7 (beta)	-1835.536623	0.61	p: 0.03086 q: 0.01787		-
M8 (beta and ω)*	-1807.523245	1.75	p_0: 0.594 p: 0.236 q: 0.325 p1: 0.405 ω: 3.72	P << 0.001	22 (PP ≥ 0.99) 6 (P > 0.95)

Legend:

a: dn/ds (weighted average)

b: Significance of the model in comparison with the null model

c: Number of sites with ω > 1 under the Bayes empirical Bayes approach with a posterior probability (PP) more than or equal to 0.99 and 0.95

* Models which allow ω > 1

Table 4. Maximum-likelihood parameter estimates for type I (short-chain) α-neurotoxins

Model	Likelihood (ι)	ω_0 [a]	Parameters	Sign. [b]	No. of Sites with ω > 1 [c]
					B.E.B
M0 (One ratio)	-3446.886907	1.92	$= \omega_0$		-
M1 (Neutral)	-3372.647732	0.57	P_0: 0.485 ω_0: 0.12 P_1: 0.514 ω1: 1.0		-
M2 (Selection)*	-3285.128559	1.61	P_0: 0.349 ω_0: 0.16 P_1: 0.369 ω_1:1.0 P_2: 0.280 ω_2: 4.24	P << 0.001	11 (PP ≥ 0.99) 2 (P > 0.95)
M3 (Discrete)*	-3264.912926	2.16	P_0: 0.487 ω_0: 0.33 P_1: 0.410 ω_1: 2.67 P_2: 0.102 ω_2: 8.86	P << 0.001	-
M7 (beta)	-3380.125103	0.57	p:0.28637 q:0.21125		-
M8 (beta and ω)*	-3287.175727	1.72	p_0: 0.703 p: 0.303 q: 0.193 p1: 0.296 ω: 4.35	P << 0.001	13 (PP ≥ 0.99) 6 (P > 0.95)

Legend:

a: dn/ds (weighted average)

b: Significance of the model in comparison with the null model

c: Number of sites with ω > 1 under the Bayes empirical Bayes approach with a posterior probability (PP) more than or equal to 0.99 and 0.95

* Models which allow ω > 1

Table 5. Maximum-likelihood parameter estimates for type II (long-chain) α-neurotoxins

Model	Likelihood (ι)	ω_0[a]	Parameters	Sign.[b]	No. of Sites with ω > 1[c]
					B.E.B
M0 (One ratio)	-7338.701541	2.01	$= \omega_0$		-
M1 (Neutral)	-7080.814722	0.62	P_0: 0.405 ω_0: 0.06 P_1: 0.594 ω1: 1.0		-
M2 (Selection)*	-6845.160604	1.43	P_0: 0.384 ω_0: 0.10 P_1: 0.356 ω_1:1.0 P_2: 0.259 ω_2: 4.008	P << 0.001	17 (PP ≥ 0.99) 2 (P > 0.95)
M3 (Discrete)*	-6801.590700	2.37	P_0: 0.402 ω_0: 0.18 P_1: 0.461 ω_1: 2.54 P_2: 0.136 ω_2: 8.28	P << 0.001	-
M7 (beta)	-7078.743858	0.53	p: 0.18047 q: 0.16001		-
M8 (beta and ω)*	-6853.164183	1.45	p_0: 0.736 p: 0.210 q: 0.168 p1: 0.263 ω: 3.97	P << 0.001	19 (PP ≥ 0.99) 2 (P > 0.95)

Legend:

a: dn/ds (weighted average)

b: Significance of the model in comparison with the null model

c: Number of sites with ω > 1 under the Bayes empirical Bayes approach with a posterior probability (PP) more than or equal to 0.99 and 0.95

* Models which allow ω > 1

Table 6. Maximum-likelihood parameter estimates for type III α-neurotoxins

Model	Likelihood (ι)	ω_0[a]	Parameters	Sign.[b]	No. of Sites with ω > 1[c]
					B.E.B
M0 (One ratio)	-3312.727326	2.59	$= \omega_0$		-
M1 (Neutral)	-3210.492742	0.58	P_0: 0.428 ω_0: 0.04 P_1: 0.571 ω_1: 1.0		-
M2 (Selection)*	-3089.176251	2.59	P_0: 0.333 ω_0: 0.03 P_1: 0.247 ω_1:1.0 P_2: 0.418 ω_2: 5.57	P << 0.001	26 (PP ≥ 0.99) 1 (P > 0.95)
M3 (Discrete)*	-3079.356207	3.27	P_0: 0.413 ω_0: 0.12 P_1: 0.272 ω_1: 2.58 P_2: 0.314 ω_2: 8.0	P << 0.001	-
M7 (beta)	-3215.119806	0.42	p: 0.01764 q: 0.02272		-
M8 (beta and ω)*	-3089.367982	2.61	p_0: 0.577 p: 0.019 q: 0.025 p1: 0.422 ω: 5.61	P << 0.001	26 (PP ≥ 0.99) 4 (P > 0.95)

Legend:

a: dn/ds (weighted average)

b: Significance of the model in comparison with the null model

c: Number of sites with ω > 1 under the Bayes empirical Bayes approach with a posterior probability (PP) more than or equal to 0.99 and 0.95

* Models which allow ω > 1

Table 7. Maximum-likelihood parameter estimates for *Oxyuranus* / *Pseudonaja* Type II (long-chain) α-neurotoxin with cysteine doublet

Model	Likelihood (ι)	ω_0[a]	Parameters	Sign.[b]	No. of Sites with ω > 1[c]
					B.E.B
M0 (One ratio)	-953.657356	0.97	= ω_0		-
M1 (Neutral)	-943.204575	0.62	P_0: 0.375 ω_0: 0.0 P_1: 0.624 ω1: 1.0		-
M2 (Selection)*	-918.331297	3.57	P_0: 0.157 ω_0: 0.0 P_1: 0.529 ω_1: 1.0 P_2: 0.312 ω_2: 9.75	P << 0.001	10 (PP ≥ 0.99) 7 (P > 0.95)
M3 (Discrete)*	-916.570131	4.82	P_0: 0.625 ω_0: 0.62 P_1: 0.341 ω_1: 8.51 P_2: 0.032 ω_2: 47.15	P << 0.001	-
M7 (beta)	-943.258977	0.60	p: 0.00754 q: 0.00500		-
M8 (beta and ω)*	-918.427424	3.67	p_0: 0.674 p: 17.55 q: 6.79 p_1: 0.325 ω: 9.77	P << 0.001	11(PP ≥ 0.99) 7 (P > 0.95)

Legend:

a: dn/ds (weighted average)

b: Significance of the model in comparison with the null model

c: Number of sites with ω > 1 under the Bayes empirical Bayes approach with a posterior probability (PP) more than or equal to 0.99 and 0.95

* Models which allow ω > 1

Table 8. Maximum-likelihood parameter estimates for *Oxyuranus* / *Pseudonaja* Type II (long-chain) α-neurotoxin without cysteine doublet

Model	Likelihood (ι)	ω_0[a]	Parameters	Sign.[b]	No. of Sites with ω > 1[c]
					B.E.B
M0 (One ratio)	-843.272386	2.69	$= \omega_0$		-
M1 (Neutral)	-845.722868	0.76	P_0: 0.237 ω_0: 0.0 P_1: 0.762 ω1: 1.0		-
M2 (Selection)*	-832.929158	3.41	P_0: 0.416 ω_0: 0.06 P_1: 0.0 ω_1:1.0 P_2: 0.583 ω_2: 5.80	P << 0.001	2 (PP ≥ 0.99) 12 (P > 0.95)
M3 (Discrete)*	-832.929158	3.41	P_0: 0.416 ω_0: 0.06 P_1: 0.476 ω_1: 5.80 P_2: 0.107 ω_2: 5.80	P << 0.001	-
M7 (beta)	-846.563004	0.90	p: 0.04683 q: 0.00500		-
M8 (beta and ω)*	-832.929285	3.41	p_0: 0.416 p: 6.49 q: 98.96 p1: 0.583 ω: 5.80	P << 0.001	9 (PP ≥ 0.99) 11 (P > 0.95)

Legend:

a: dn/ds (weighted average)

b: Significance of the model in comparison with the null model

c: Number of sites with ω > 1 under the Bayes empirical Bayes approach with a posterior probability (PP) more than or equal to 0.99 and 0.95

* Models which allow ω > 1

Table 9. Maximum-likelihood parameter estimates for kappa three-finger toxins

Model	Likelihood (ι)	ω_0[a]	Parameters	Sign.[b]	No. of Sites with $\omega > 1$[c]
					B.E.B
M0 (One ratio)	-745.132731	1.64	$= \omega_0$		-
M1 (Neutral)	-743.698028	0.66	P_0: 0.344 ω_0: 0.03 P_1: 0.655 ω_1: 1.0		-
M2 (Selection)*	-728.968534	2.11	P_0: 0.782 ω_0: 1.0 P_1: 0.124 ω_1:1.0 P_2: 0.09 ω_2: 12.96	$P << 0.001$	3 ($PP \geq 0.99$) 2 ($P > 0.95$)
M3 (Discrete)*	-728.457588	2.30	P_0: 0.871 ω_0: 0.95 P_1: 0.1 ω_1: 7.61 P_2: 0.2 ω_2: 25.61	$P << 0.001$	-
M7 (beta)	-743.727286	0.70	p: 0.01191 q: 0.00500		-
M8 (beta and ω)*	-728.968534	2.11	p_0: 0.906 p: 3.212 q: 0.005 p1: 0.093 ω: 12.96	$P << 0.001$	3 ($PP \geq 0.99$) 2 ($P > 0.95$)

Legend:

a: dn/ds (weighted average)

b: Significance of the model in comparison with the null model

c: Number of sites with $\omega > 1$ under the Bayes empirical Bayes approach with a posterior probability (PP) more than or equal to 0.99 and 0.95

* Models which allow $\omega > 1$

Table 10. Maximum-likelihood parameter estimates for cytotoxic three-finger toxins

Model	Likelihood (ι)	ω_0[a]	Parameters	Sign.[b]	No. of Sites with $\omega > 1$[c]
					B.E.B
M0 (One ratio)	-970.316160	0.32	$= \omega_0$		-
M1 (Neutral)	-952.107186	0.33	P_0: 0.712 ω_0: 0.06 P_1: 0.287 ω1: 1.0		-
M2 (Selection)*	-945.995693	0.57	P_0: 0.675 ω_0: 0.06 P_1: 0.294 ω_1:1.0 P_2: 0.029 ω_2: 8.18	$P << 0.001$	1 ($PP \geq 0.99$) 1 ($P \geq 0.95$)
M3 (Discrete)*	-945.501674	0.52	P_0: 0.518 ω_0: 0.0 P_1: 0.446 ω_1: 0.62 P_2: 0.034 ω_2: 7.02	$P < 0.01$	-
M7 (beta)	-952.219533	0.31	p: 0.13885 q: 0.29569		-
M8 (beta and ω)*	-945.530089	0.53	p_0: 0.969 p: 0.173 q: 0.387 p1: 0.03 ω: 7.53	$P < 0.01$	2 ($PP \geq 0.99$) 0 ($P > 0.95$)

Legend:

a: dn/ds (weighted average)

b: Significance of the model in comparison with the null model

c: Number of sites with $\omega > 1$ under the Bayes empirical Bayes approach with a posterior probability (PP) more than or equal to 0.99 and 0.95

* Models which allow $\omega > 1$

$p > 0.05^{N.S}$: Not significant at 0.05

Supplementary Table 11. Nucleotide and complementary amino acid-level selection assessment

Site		CodeML		TreeSAAP		ASA
Codon	AA	M2a[a]	M8[b]	Property[c]	Magnitude[d]	
Type I α neurotoxins						
22	M	4.428±0.412 (0.989)*	4.456±0.247 (0.998)**	–	–	35.5 NA
37	**M**	**4.466±0.209 (1.0)****	**4.461±0.201 (1.0)****	α*c*	6	**39.9 Part. Exposed**
39	**A**	**4.444±0.341 (0.994)****	**4.456±0.243 (0.999)****	α*c*	6	**95.5 Exposed**
40	**-**	**4.290±0.783 (0.950**	**4.407±0.479 (0.985)***	α*c*	6	**100 Exposed**
43	**S**	**4.274±0.813 (0.945)**	**4.424±0.411 (0.990)***	*Hnc*, α*c*	7, 6	**69.5 Exposed**
49	**T**	**4.466±0.208 (1.0)****	**4.461±0.201 (1.0)****	*Hnc*, α*c*	7, 6	**66.4 Exposed**
51	**R**	**4.466±0.208 (1.0)****	**4.461±0.201 (1.0)****	*Hnc*, R_α	8, 6	**96.4 Exposed**
53	**H**	**4.466±0.210 (1.0)****	**4.461±0.202 (1.0)****	*Hnc*, R_α	8, 6	**90.2 Exposed**
56	**T**	**4.224±0.900 (0.931)**	**4.403±0.493 (0.984)***	*Hnc*, α*c*, R_α	8, 8, 6	**77.9 Exposed**
57	**I**	**4.428±0.414 (0.989)***	**4.455±0.253 (0.998)****	*Hnc*, α*c*, R_α	8, 8, 6	**72.6 Exposed**
58	**I**	**4.147±1.014 (0.909)**	**4.392±0.527 (0.981)***	*Hnc*, α*c*, R_α	8, 8, 6	**8.7 Buried**
66	**K**	**4.466±0.208 (1.0)****	**4.461±0.201 (1.0)****	*Hnc*, α*c*, R_α	8, 6, 6	**40.5 Part. Exposed**
69	**P**	**4.466±0.208 (1.0)****	**4.461±0.201 (1.0)****	*Hnc*, α*c*, R_α	8, 6, 6	**99.7 Exposed**
70	**G**	**4.455±0.283 (0.997)****	**4.459±0.219 (0.999)****	*Hnc*, α*c*, R_α	8, 7, 6	**98 Exposed**
71	**I**	**3.812±1.360 (0.814)**	**4.289±0.782 (0.952)***	α*c*	7	**23.5 NA**
72	**K**	**4.466±0.208 (1.0)****	**4.461±0.201 (1.0)****	α*c*	7	**65.9 Exposed**
73	**L**	**3.990±1.202 (0.864)**	**4.310±0.741 (0.958)***	α*c*	7	**14.2 Buried**
74	**E**	**4.466±0.208 (1.0)****	**4.461±0.201 (1.0)****	*Hnc*, α*c*	7, 7	**69.8 Exposed**
77	**K**	**4.466±0.208 (1.0)****	**4.461±0.201 (1.0)****	*Hnc*, α*c*	7, 7	**41.3 Part. Exposed**
Type II α neurotoxins						
22	**L**	**4.248±0.434 (1.0)****	**3.612±0.649 (0.965)***	*h*	6	**17.7 Buried**

Site		CodeML		TreeSAAP		ASA
26	M	4.248±0.434 (1.0)**	3.735±0.424 (1.0)**	*h*	6	0 Buried
29	P	4.248±0.434 (1.0)**	3.735±0.424 (1.0)**	*h*	6	100 Exposed
30	K	4.235±0.477 (0.996)**	3.733±0.428 (1.0)**	*h*	6	46 Part. Exposed
31	T	4.248±0.434 (1.0)**	3.735±0.424 (1.0)**	*h*	6	38.3 NA
33	R	4.248±0.434 (1.0)**	3.735±0.424 (1.0)**	*h*	6	45.7 Part. Exposed
39	E	4.217±0.529 (0.991)**	3.730±0.434 (0.999)**	pK', *h*	7, 6	62.4 Exposed
40	N	4.247±0.436 (1.0)**	3.735±0.424 (1.0)**	pK', *h*	7, 6	70.4 Exposed
41	L	4.245±0.445 (0.999)**	3.734±0.426 (1.0)**	pK', *h*	7, 6	18.4 Buried
50	P	4.248±0.434 (1.0)**	3.735±0.424 (1.0)**	pK', *h*	8, 8	57.7 Exposed
51	R	4.248±0.434 (1.0)**	3.735±0.424 (1.0)**	pK', *h*	8, 8	50.9 Exposed
53	S	4.248±0.434 (1.0)**	3.735±0.424 (1.0)**	pK', *h*	8, 8	56.7 Exposed
54	S	4.248±0.434 (1.0)**	3.735±0.424 (1.0)**	pK', *h*	8, 8	80.2 Exposed
58	L	4.248±0.434 (1.0)**	3.735±0.424 (1.0)**	pK', *h*	7, 8	41.8 Part. Exposed
69	I	4.248±0.434 (1.0)**	3.735±0.424 (1.0)**	pK', *h*	7, 6	84.6 Exposed
70	P	4.248±0.434 (1.0)**	3.735±0.424 (1.0)**	pK', *h*	8, 6	–
72	S	4.200±0.575 (0.986)*	3.726±0.445 (0.997)**	pK'	8	100 Exposed
73	Y	3.548±1.343 (0.799)	3.613±0.650 (0.964)**	pK'	8	75.4 Exposed
74	E	4.145±0.698 (0.971)*	3.719±0.460 (0.996)**	pK'	8	23.8 NA
75	D	4.248±0.435 (1.0)**	3.735±0.424 (1.0)**	pK'	8	61.4 Exposed
77	T	4.248±0.434 (1.0)**	3.735±0.424 (1.0)**	pK'	8	36.6 NA
Type III α neurotoxins						
23	T	5.542±0.211 (1.0)**	5.519±0.166 (1.0)**	Esm	6	51.1 Exposed
26	K	5.539±0.237 (0.999)**	5.518±0.178 (1.0)**	Esm	6	49.5 Exposed

Site		CodeML		TreeSAAP		ASA
27	**G**	**5.542±0.209 (1.0)****	**5.519±0.165 (1.0)****	**Esm**	**6**	**61.9 Exposed**
28	**Y**	**5.542±0.209 (1.0)****	**5.519±0.165 (1.0)****	**Esm**	**6**	**88.6 Exposed**
29	**H**	**5.542±0.209 (1.0)****	**5.519±0.165 (1.0)****	**Esm**	**6**	**52.7 Exposed**
30	**D**	**5.536±0.262 (1.0)****	**5.517±0.195 (0.999)****	**Esm**	**6**	–
35	**K**	**5.542±0.211 (1.0)****	**5.519±0.167 (0.999)****	**Esm**	**6**	**53.4 Exposed**
36	P	5.541±0.224 (1.0)**	5.518±0.173 (1.0)**	–	–	99.7 Exposed
37	H	5.270±1.084 (0.941)	5.394±0.757 (0.973)*	–	–	75.4 Exposed
43	E	5.542±0.209 (1.0)**	5.519±0.165 (1.0)**	–	–	28.8 NA
45	F	5.542±0.209 (1.0)**	5.519±0.165 (1.0)**	–	–	22.8 NA
46	I	5.542±0.209 (1.0)**	5.519±0.165 (1.0)**	–	–	58.7 Exposed
47	P	5.540±0.232 (0.999)**	5.518±0.177 (0.999)**	–	–	96.4 Exposed
48	A	5.542±0.209 (1.0)**	5.519±0.166 (1.0)**	–	–	73.7 Exposed
49	T	5.542±0.210 (1.0)**	5.519±0.166 (1.0)**	–	–	69.1 Exposed
50	H	5.360±0.904 (0.961)*	5.438±0.620 (0.983)*	–	–	58.1 Exposed
51	G	5.214±1.172 (0.930)	5.393±0.758 (0.973)*	–	–	77.1 Exposed
52	N	5.542±0.209 (1.0)**	5.519±0.165 (1.0)**	–	–	90.3 Exposed
53	A	5.536±0.269 (0.999)**	5.516±0.201 (0.999)**	–	–	47.6 Part. Exposed
54	I	5.542±0.209 (1.0)**	5.519±0.165 (1.0)**	–	–	11.7 Buried
55	L	5.542±0.209 (1.0)**	5.519±0.165 (1.0)**	–	–	35.9 NA
56	A	5.542±0.209 (1.0)**	5.519±0.165 (1.0)**	–	–	45.2 Part. Exposed
57	R	5.196±1.205 (0.925)	5.367±0.829 (0.967)**	–	–	16 Buried
60	**G**	**5.542±0.211 (1.0)****	**5.519±0.166 (1.0)****	**Esm**	**6**	**22.8 NA**

Site		CodeML		TreeSAAP		ASA
65	**G**	**5.542±0.209 (1.0)****	**5.519±0.165 (1.0)****	**Esm**	**6**	**77.9 Exposed**
66	**G**	**5.542±0.209 (1.0)****	**5.519±0.166 (1.0)****	**Esm**	**6**	**100 Exposed**
67	**I**	**5.539±0.243 (0.999)****	**5.518±0.181 (1.0)****	**Esm**	**6**	**60.4 Exposed**
68	**R**	**5.542±0.209 (1.0)****	**5.519±0.165 (1.0)****	**Esm**	**6**	**12 Buried**
69	**P**	**5.542±0.209 (1.0)****	**5.519±0.165 (1.0)****	**Esm**	**6**	–
79	K	5.542±0.209 (1.0)**	5.519±0.165 (1.0)**	–	–	–
Plesiotypic α-neurotoxins from ‘non-front-fanged’ advanced snakes						
21	H	3.082±0.722 (0.926)	2.484±0.206 (0.986)*	–	–	38.5 NA
22	G	3.259±0.429 (1.0)**	2.507±0.086 (1.0)**	–	–	13.4 Buried
23	F	3.246±0.460 (0.994)**	2.505±0.106 (0.998)**	–	–	46.3 Part. Exposed
25	L	3.255±0.438 (0.998)**	2.506±0.092 (0.999)**	–	–	0.4 Buried
29	–	3.039±0.773 (0.907)	2.462±0.278 (0.973)*	–	–	–
30	R	3.258±0.431 (0.999)**	2.507±0.089 (1.0)**	–	–	31.7 NA
32	T	3.161±0.619 (0.958)*	2.486±0.203 (0.987)*	–	–	58.3 Exposed
33	W	3.206±0.542 (0.977)*	2.493±0.172 (0.991)**	–	–	99 Exposed
34	S	3.258±0.431 (0.999)**	2.507±0.090 (1.0)**	–	–	52.8 Exposed
37	S	3.259±0.428 (1.0)**	2.507±0.084 (1.0)**	–	–	92.7 Exposed
38	I	3.259±0.428 (1.0)**	2.507±0.085 (1.0)**	–	–	46.6 Part. Exposed
39	G	3.257±0.433 (0.999)**	2.507±0.089 (1.0)**	–	–	9.2 Buried
40	H	3.258±0.430 (0.999)**	2.507±0.087 (1.0)**	–	–	60.6 Exposed
41	R	2.998±0.810 (0.891)	2.477±0.229 (0.982)*	–	–	82.2 Exposed
43	L	3.259±0.429 (1.0)**	2.507±0.086 (1.0)**	–	–	82.7 Exposed
44	P	3.126±0.664 (0.945)	2.492±0.175 (0.991)**	–	–	50.8 Exposed

Site		CodeML		TreeSAAP		ASA
46	H	3.032±0.776 (0.906)	2.482±0.214 (0.984)*	–	–	26.2 NA
47	M	3.259±0.429 (1.0)**	2.507±0.086 (1.0)**	–	–	52.7 Exposed
48	T	3.187±0.574 (0.970)*	2.496±0.158 (0.993)**	–	–	22.5 NA
53	Y	3.149±0.634 (0.954)*	2.491±0.179 (0.990)**	–	–	31.6 NA
54	K	3.190±0.568 (0.971)*	2.499±0.142 (0.995)**	–	–	41.3 Part. Exposed
55	P	**3.258±0.432 (0.999)****	**2.507±0.088 (1.0)****	***V°***	**6**	40.9 **Part. Exposed**
56	D	**3.197±0.556 (0.974)***	**2.500±0.136 (0.995)****	***V°***	**6**	46.2 **Part. Exposed**
57	E	**2.879±0.907 (0.843)**	**2.473±0.241** (0.979)*	***V°***	**6**	88.3 **Exposed**
58	N	**3.215±0.524 (0.981)***	**2.499±0.140** (0.995)**	***V°***	**6**	86.5 **Exposed**
64	A	**3.241±0.471 (0.992)****	**2.504±0.110** (0.998)**	***V°***	**6**	32.4 NA
70	R	3.259±0.429 (1.0)**	2.507±0.086 (1.0)**	–	–	52.2 Exposed
71	M	3.073±0.732 (0.922)	2.485±0.203 (0.986)*	–	–	90.6 Exposed
74	T	**3.217±0.521 (0.982)***	**2.501±0.130** (0.996)**	***V°***	**6**	100 **Exposed**
76	K	**3.258±0.432 (0.999)****	**2.507±0.089 (1.0)****	***V°***	**6**	82 **Exposed**
77	S	**3.084±0.720 (0.927)**	**2.483±0.210** (0.985)*	***V°***	**6**	100 **Exposed**
80	R	**2.457±1.092 (0.670)**	**2.426±0.353** (0.951)*	***V°***	**6**	64.8 **Exposed**
85	T	**3.256±0.437 (0.998)****	**2.506±0.092** (0.999)**	***V°***	**6**	83.4 **Exposed**
86	G	**3.257±0.434 (0.999)****	**2.506±0.091 (1.0)****	***V°***	**6**	49.7 **Exposed**
88	S	3.131±0.658 (0.947)	2.493±0.171 (0.991)**	–	–	34.8 NA
91	S	3.259±0.428 (1.0)**	2.507±0.085 (1.0)**	–	–	64.7 Exposed
92	D	2.905±0.896 (0.850)	2.432±0.351 (0.954)**	–	–	34.9 NA
Plesiotypic α-neurotoxins from Elapidae						
7	S	3.499±0.841 (0.919)	3.474±0.525 (0.969)*	–	–	–

Site		CodeML		TreeSAAP		ASA
28	S	3.485±0.864 (0.913)	3.451±0.581 (0.961)*	–	–	77.6 Exposed
30	Y	3.692±0.532 (0.986)*	3.549±0.296 (0.996)**	–	–	63.0 Exposed
33	P	3.731±0.433 (0.999)**	3.56±0.245 (1.0)**	–	–	66.5 Exposed
34	N	3.694±0.529 (0.986)*	3.549±0.297 (0.996)**	–	–	87.7 Exposed
35	S	3.597±0.707 (0.953)*	3.518±0.407 (0.985)*	–	–	85.8 Exposed
39	P	3.69±0.54 (0.985)*	3.545±0.314 (0.994)**	–	–	85.3 Exposed
40	D	3.724±0.455 (0.996)**	3.558±0.255 (0.999)**	–	–	66.1 Exposed
44	I	3.718±0.47 (0.994)**	3.556±0.264 (0.998)**	–	–	14.8 Buried
48	R	3.68±0.561 (0.981)*	3.543±0.32 (0.994)**	–	–	57.5 Exposed
50	W	3.549±0.776 (0.937)	3.498±0.461 (0.978)*	–	–	41.3 Par. exposed
52	T	3.729±0.44 (0.998)**	3.559±0.248 (1.0)**	–	–	65.8 Exposed
53	A	3.724±0.453 (0.997)**	3.558±0.254 (0.999)**	–	–	91.8 Exposed
54	V	3.733±0.426 (1.0)**	3.56±0.242 (1.0)**	–	–	96.8 Exposed
55	R	3.712±0.486 (0.992)**	3.553±0.276 (0.998)**	–	–	100.0 Exposed
57	R	3.721±0.463 (0.995)**	3.557±0.259 (0.999)**	–	–	42.2 Par. exposed
58	E	3.695±0.526 (0.986)*	3.549±0.294 (0.996)**	–	–	87.0 Exposed
59	I	3.399±0.948 (0.885)	3.43±0.616 (0.954)*	–	–	9.3 Buried
60	R	3.733±0.427 (1.0)**	3.56±0.242 (1.0)**	–	–	91.7 Exposed
69	P	3.708±0.495 (0.991)**	3.553±0.277 (0.997)**	–	–	91.7 Exposed
70	S	3.715±0.477 (0.993)**	3.555±0.27 (0.998)**	–	–	11.8 Buried
72	L	3.712±0.486 (0.992)**	3.554±0.275 (0.998)**	–	–	88.4 Exposed
73	G	3.65±0.622 (0.971)*	3.533±0.358 (0.990)**	–	–	54.0 Exposed

Site		CodeML		TreeSAAP		ASA
74	L	3.687±0.543 (0.984)*	3.546±0.306 (0.995)**	–	–	25.2 NA
75	T	3.722±0.459 (0.996)**	3.557±0.258 (0.999)**	–	–	39.8 Par. exposed
77	F	3.725±0.451 (0.997)**	3.558±0.254 (0.999)**	–	–	42.7 Par. exposed
83	N	3.602±0.697 (0.955)*	3.523±0.389 (0.987)*	–	–	69.0 Exposed
86	H	3.733±0.428 (1.0)**	3.56±0.243 (1.0)**	–	–	100.0 Exposed
Viperidae 3FTxs						
7	I	6.037±0.994 (0.983)*	5.932±0.85 (0.991)**	–	–	–
18	**S**	**5.874±1.314 (0.953)***	**5.825±1.11 (0.971)***	***El*, α*c***	**7, 6**	–
23	**E**	**5.796±1.430 (0.939)**	**5.784±1.186 (0.963)***	***El*, α*c***	**7, 6**	–
25	**Y**	**6.123±0.76 (0.999)****	**5.979±0.705 (1.0)****	***El*, α*c***	**7, 6**	–
28	**N**	**5.995±1.086 (0.975)***	**5.908±0.914 (0.986)***	***El, Pr***	**7, 8**	–
29	**M**	**6.128±0.744 (1.0)****	**5.981±0.697 (1.0)****	***El, Pr***	**7, 8**	–
30	**T**	**6.128±0.745 (1.0)****	**5.981±0.698 (1.0)****	***El, Pr***	**7, 8**	–
31	**F**	**6.12±0.77 (0.998)****	**5.977±0.71 (0.999)****	***El, Pr***	**6, 8**	–
34	**L**	**6.128±0.745 (1.0)****	**5.981±0.698 (1.0)****	***El, Pr***	**6, 8**	–
36	**R**	**6.122±0.766 (0.999)****	**5.978±0.709 (0.999)****	***El, Pr***	**6, 8**	–
40	**E**	**6.004±1.065 (0.977)***	**5.913±0.9 (0.987)***	***El, Pr***	**8, 7**	**100 Exposed**
42	**L**	**6.07±0.913 (0.989)***	**5.952±0.792 (0.995)****	***El, Pr***	**8**	**32.0 NA**
49	**K**	**6.042±0.984 (0.984)***	**5.934±0.844 (0.991)****	***El***	**8**	**48.8 Par. Exposed**
51	–	**6.126±0.753 (0.999)****	**5.98±0.702 (1.0)****	***El***	**8**	**100 Exposed**
54	L	5.757±1.510 (0.932)	5.716±1.325 (0.951)*	–	–	–
55	F	6.106±0.815 (0.996)**	5.969±0.738 (0.998)**	–	–	16.1 Buried
56	P	6.037±0.995 (0.983)*	5.932±0.851 (0.991)**	–	–	100 Exposed

Site		CodeML		TreeSAAP		ASA
57	V	5.757±1.485 (0.932)	5.754±1.241 (0.958)*	–	–	91.3 Exposed
58	L	5.965±1.156 (0.969)*	5.878±0.996 (0.980)*	–	–	42.9 Par. Exposed
59	K	5.818±1.400 (0.943)	5.795±1.166 (0.965)*	–	–	64.2 Exposed
61	E	5.780±1.449 (0.936)	5.775±1.199 (0.962)*	–	–	55.2 Exposed
70	**Q**	**5.939±1.205 (0.965)***	**5.863±1.029 (0.978)***	***Pr***	**7**	**100.0 Exposed**
72	**W**	**6.127±0.75 (1.0)****	**5.98±0.7 (1.0)****	***Pr*, α*c***	**7, 6**	**98.5 Exposed**
73	**T**	**6.119±0.775 (0.998)****	**5.977±0.712 (0.999)****	***Pr*, α*c***	**7 , 6**	**43.5 Par. Exposed**
74	**D**	**6.069±0.913 (0.989)***	**5.953±0.788 (0.995)****	***Pr*, α*c***	**7 , 6**	**2.8 Buried**
75	**K**	**5.802±1.430 (0.940)**	**5.773±1.212 (0.961)***	***El*, *Pr*, α*c***	**8, 7 , 6**	**92.4 Exposed**
78	**E**	**6.109±0.804 (0.996)****	**5.972±0.728 (0.998)****	***El*, *Pr*, α*c***	**8, 7 , 6**	**70.8 Exposed**
80	**N**	**5.885±1.287 (0.955)***	**5.848±1.053 0.975***	***El*, α*c***	**8, 6**	**98.7 Exposed**
81	**K**	**6.112±0.795 (0.997)****	**5.973±0.724 (0.999)****	***El*, α*c***	**8, 6**	**83.2 Exposed**
84	I	6.127±0.747 (1.0)**	5.981±0.698 (1.0)**	–	–	–
κ-bungarotoxins						
37	Q	8.983±1.705 0.982*	8.708±1.708 (0.989)*	–	–	49.1 Par. Exposed
44	L	9.069±1.500 0.993**	8.761±1.593 (0.995)**	–	–	40 Par. Exposed
47	Q	9.121±1.355 (0.999)**	8.792±1.515 (0.999)**	–	–	57.6 Exposed
50	K	8.956±1.761 0.979*	8.692±1.74 (0.987)*	–	–	39.8 Par. Exposed
53	S	9.126±1.341 (1.0)**	8.795±1.507 (1.0)**	–	–	62.7 Exposed
Cytotoxins						
50	T	7.473±1.990 (0.996)**	6.602±2.042 (0.999)**	*P*α, K^0	6, 7	75.2 Exposed
51	P	7.435±2.057 (0.988)*	6.590±2.062 (0.996)**	*P*α, K^0	6, 7	45.7 Part. exposed

Amino-acid property symbols used: α–helical tendencies (*P*α), Compressibility (K^0),Equalibrium constant (ionization of COOH) *(pK^1)*, Hydropathy *(h)*, Long-range n.b. energy *(El)*, Normalized consensus hydrophobicity *(Hnc)*, Partial specific volume *(V^0)*, Polar requirement *(Pr)*, Power to be at C-terminus of α-helix (αc), Short and medium-range n.b. energy *(Esm)*, Solvent accessible reduction ratio *(R*α*)* and Surrounding hydrophobicity *(Hp)*.

Legend:

a: M2a Bayes Empirical Bayes (BEB) posterior probability (* ≥ 0.95; ** ≥ 0.99) and post-mean omega indicated in brackets

b: M8 Bayes Empirical Bayes (BEB) posterior probability (* ≥ 0.95; ** ≥ 0.99) and post-mean omega indicated in brackets

c: amino acid property under selection

d: magnitude of selection on the amino acid property

ASA: Accessible surface area (50% ≥ Side chains completely exposed; 20% ≤ Side chains buried)

Part. exposed: Partially exposed side-chains (ASA: 40%-50%)

Sites detected as positively selected by both nucleotide and amino acid-level analyses are indicated in bold.

Supplementary Table 12. Surface accessibility of Three-finger toxins

Type I α-neurotoxin

Total Residues	62
Total Exposed	33
Total Buried	10
Exposed PS	15 (24%)
Buried PS	2 (3%)
Frequency of exposed PS sites (a)	45%
Frequency of buried PS sites (b)	20%
Exposure Ratio (a/b)	**2.3%**

Type II α-neurotoxin

Total Residues	74
Total Exposed	29
Total Buried	20
Exposed PS	14 (19%)
Buried PS	3 (4%)
Frequency of exposed PS sites (a)	48%
Frequency of buried PS sites (b)	15%
Exposure Ratio (a/b)	**3.2%**

Type III α-neurotoxin

Total Residues	57
Total Exposed	28
Total Buried	14
Exposed PS	20 (35%)
Buried PS	3 (5%)
Frequency of exposed PS sites (a)	71%
Frequency of buried PS sites (b)	21%
Exposure Ratio (a/b)	**3.3%**

'Non-front-fanged' Advanced Snakes

Total Residues	74
Total Exposed	32
Total Buried	17
Exposed PS	25 (34%)
Buried PS	3 (4%)
Frequency of exposed PS sites (a)	78%
Frequency of buried PS sites (b)	18%
Exposure Ratio (a/b)	**4.4%**

Viperidae

Total Residues	46
Total Exposed	23
Total Buried	8
Exposed PS	15 (33%)
Buried PS	2 (4%)
Frequency of exposed PS sites (a)	65%
Frequency of buried PS sites (b)	25%
Exposure Ratio (a/b)	**2.6%**

Elapidae

Total Residues	65
Total Exposed	32
Total Buried	14
Exposed PS	23 (35%)
Buried PS	3 (5%)
Frequency of exposed PS sites (a)	72%
Frequency of buried PS sites (b)	21%
Exposure Ratio (a/b)	**3.4%**

κ-neurotoxins

Total Residues	132
Total Exposed	47
Total Buried	42
Exposed PS	3 (2%)
Buried PS	0
Frequency of exposed PS sites (a)	6%
Frequency of buried PS sites (b)	0
Exposure Ratio (a/b)	–

Cytotoxins

Total Residues	60
Total Exposed	28
Total Buried	15
Exposed PS	2 (3%)
Buried PS	0
Frequency of exposed PS sites (a)	7%
Frequency of buried PS sites (b)	0
Exposure Ratio (a/b)	–

Legend: PS: Positively selected

Note:

- Type I α-neurotoxins had 39.4% of the exposed residues and 20% of the buried residues under positive selection. Thus, the exposed residues being **1.9 times** more likely to be positively selected than buried residues.
- Type II α-neurotoxins had 36.8% of the exposed residues and 15% of the buried residues under positive selection. Thus, the exposed residues being **2.5 times** more likely to be positively selected than buried residues.
- Type III α-neurotoxin had 58.8% of the exposed residues and 21.4% of the buried residues under positive selection. Thus, the exposed residues being **2.7 times** more likely to be positively selected than buried residues.
- The plesiotypic 'non-front-fanged' advanced snake 3FTx had 62.5% of the exposed residues and 17.6% of the buried residues under positive selection. Thus, the exposed residues being **3.5 times** more likely to be positively selected than buried residues.
- The plesiotypic Viperidae 3FTx had 51.7% of the exposed residues and 25% of the buried residues under positive selection. Thus, the exposed residues being **2.1 times** more likely to be positively selected than buried residues.
- The plesiotypic Elapidae 3FTx had 58.9% of the exposed residues positively selected while 21.4% of the buried residues under positive selection. Thus, the exposed residues being **2.8 times** more likely to be positively selected than buried residues.
- κ-neurotoxins had 5% of the exposed residues under positive selection, while none of the buried residues were positively selected.
- Cytotoxic 3FTx had 5.8% of the exposed residues under positive selection, while none of the buried residues were positively selected.

Supplementary Table 13. Structural and functional residues in 3FTx

3FTx	Structurally and/or functionally important residue	Reference
	α-neurotoxins	
Type I (short-chain)	Q6 (87%), S8 (95%), S9 (95%), Q10 (87%), Y25 (invariant), K27 (94%), W29 (95%), D31 (91%), R33 (89%), G34 (94%), E38 (99%), G40 (invariant), P44 (99%), K47 (93%)	(Pillet et al. 1993; Tremeau et al. 1995; Antil et al. 1999; Barber et al. 2013)
Type II (long-chain)	Y21 (72%), K23 (84%), W25 (98%), C26 (94%), D27 (91%), A28 (50%; PS), F29 (49%; PS), C30 (94%), R33 (83%), K35 (73%), R36 (very low; PS), G40 (invariant), P46 (98%), K49 (70%), F65 (very low; PS)	(Antil et al. 1999; Antil-Delbeke et al. 2000; Barber et al. 2013)
Type III	L1 (invariant), T20 (98%), S43 (90%), V51 (91%), S54 (91%), T55 (93%), D56 (invariant), N59 (invariant)	Predicted in this study as putative functional/structural residue
Plesiotypic 'non-front-fanged' advanced snakes	E64 (95%), N75 (95%)	Predicted in this study as putative functional/structural residue
Plesiotypic Elapidae 3FTx	K28 (invariant), R42 (invariant), P49 (invariant), V57 (96%), T62 (93%), D63 (invariant), N66 (96%)	Predicted in this study as putative functional/structural residue
Viperidae 3FTx	P20 (90%), G45 (invariant), K59 (90%), T66 (90%), N70 (invariant)	Predicted in this study as putative functional/structural residue
κ-bungarotoxin	R32 (invariant), P45 (invariant), F47 (invariant), L55 (invariant)	(Dewan et al. 1994)
Cytotoxin	K5 (83%), Y11 (72%), K12 (invariant), K18 (97%), Y22 (invariant), K23 (invariant), M24 (94%), M26 (invariant), K31 (56%), K35 (invariant), G37 (invariant), P43 (invariant), K44 (invariant), K50 (invariant), Y51 (invariant)	(Kumar et al. 1997)

PS: Positively selected.

Percent identity: has been indicated in parenthesis for each site.

Very low: very few sequences have the structurally/functionally important residue at this site.

Note: All site numbering corresponds to H8PG58 for short-chain, FJ752458.1for long-chain, AF082975.1 for Type III, DQ366293.1 for basal 3FTx from 'non-front-fanged' advanced snakes, AY611643.1 for basal 3FTx from Elapidae, AY057872.1 for kappa and U42585.1 for cytotoxins.

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