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RNA-seq and high-definition mass spectrometry reveal the complex and divergent venoms of two rear-fanged colubrid snakes

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Table 1 Expression levels of full-length toxin clusters for ***Bij*** based on 10 million reads mapped to coding sequences

Rank	Cluster name	Cluster size	CDS length	Median coverage	% Toxin reads	% Total reads
1	SVMPIII-2a	2	1,836	32,071	15.106	5.464
2	3FTx-9a	7	318	116,501	12.923	4.674
3	3FTx-4a	4	318	101,232	11.848	4.286
4	3FTx-3a	11	336	96,068	9.737	3.522
5	3FTx-2a	7	330	69,343	7.058	2.553
6	3FTx-10a	9	318	68,918	6.897	2.495
7	3FTx-8a	6	330	63,436	6.398	2.314
8	3FTx-1a	6	330	60,327	6.242	2.258
9	3FTx-7a	4	330	46,069	4.852	1.755
10	CRISP-1a	4	720	19,816	3.835	1.387
11	SVMPIII-3a	4	1,872	8,563	3.647	1.319
12	SVMPIII-4a	2	1,842	8,043	3.471	1.256
13	SVMPIII-1	1	1,842	6,274	2.630	0.951
14	NP-1a	2	534	9,116	1.400	0.507
15	3FTx-6a	3	255	14,984	0.948	0.343
16	3FTx-5	1	255	8,863	0.572	0.207
17	CTL-8a	2	495	4,283	0.497	0.180
18	CTL-9a	2	483	2,536	0.289	0.105
19	CTL-5	1	498	2,088	0.243	0.088
20	CTL-6	1	591	1,891	0.219	0.079
21	Ficolin-1a	2	996	953	0.196	0.071
22	Ficolin-3	1	996	667	0.146	0.053
23	CTL-3	1	501	1,076	0.123	0.044
24	KUN-1	1	765	623	0.100	0.036
25	AChE-1	1	1,818	268	0.100	0.036
26	CTL-7	1	483	808	0.090	0.033
27	Ficolin-2	1	999	403	0.083	0.030
28	AChE-2a	2	1,650	186	0.063	0.023
29	PLA_2-1a	2	456	408	0.042	0.015
30	CFVII	1	1,278	117	0.032	0.012
31	AChE-3	1	1,689	54	0.027	0.010

27

(24)-7039.2(KUN-1)AChE-1 (1) (,689)-5103(808)-77990875.0,808)-7799.0(948)-7592.740.043 0.010

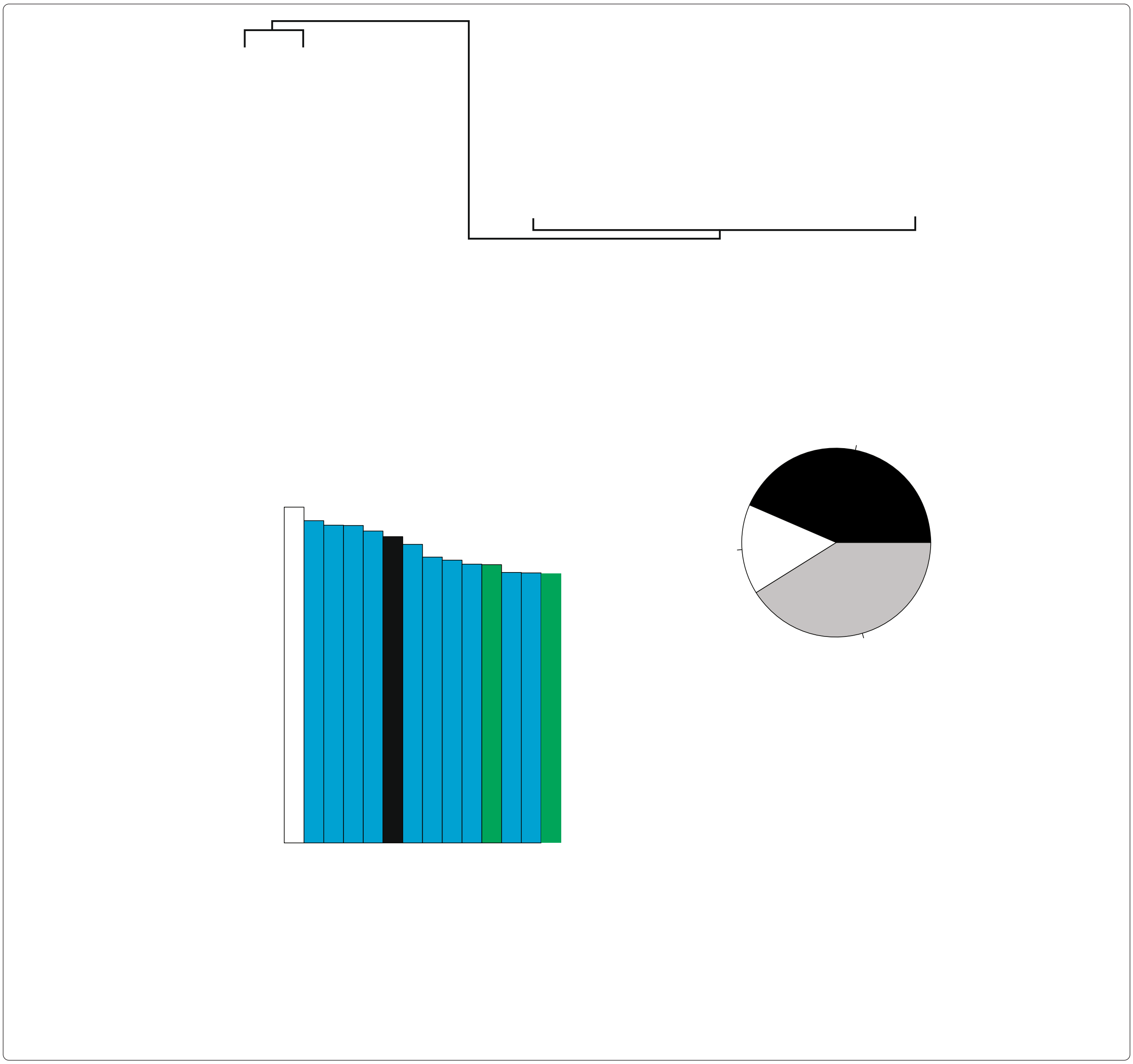


Table 2 Expression levels of full-length toxin clusters for ***Hy*** sp. based on 10 million reads mapped to coding sequences

Rank	Cluster name	Cluster size	CDS length	Median Coverage	% Toxin reads	% Total reads
1	CRISP-1a	5	720	92,411	16.684	7.256
2	SVMPIII-4	1	1,836	30,460	11.462	4.985
3	SVMPIII-9a	2	1,833	27,697	10.112	4.398
4	SVMPIII-13a	2	1,836	25,321	10.024	4.360
5	SVMPIII-12a	2	1,830	22,893	8.593	3.737
6	NP-1a	12	474	56,846	7.359	3.201
7	SVMPIII-3a	4	1,860	16,209	5.943	2.585
8	SVMPIII-7a	2	1,857	11,689	4.173	1.815
9	SVMPIII-11a	3	1,845	10,816	3.835	1.668
10	SVMPIII-8a	5	1,845	9,629	3.430	1.492
11	CTL-7a	5	471	32,519	3.382	1.471
12	SVMPIII-6a	3	1,854	7,559	2.727	1.186
13	SVMPIII-5	1	1,833	7,058	2.693	1.171
14	CTL-8a	7	480	25,070	2.633	1.145
15	SVMPIII-10a	4	1,827	4,941	1.687	0.734
16	SVMPIII-2	1	1,827	4,305	1.553	0.675
17	SVMPIII-14a	3	1,839	4,236	1.484	0.645
18	Fused	1	582	8,844	0.960	0.418
19	SVMPIII-1	1	1,857	2,406	0.950	0.413
20	3FTx	1	255	1,357	0.070	0.030
21	CTL-6a	2	480	640	0.060	0.026
22	KUN	1	759	423	0.056	0.024
23	CTL-5	1	477	464	0.042	0.018
24	CTL-1	1	483	304	0.029	0.013
25	CTL-3	1	495	239	0.022	0.010
26	VEGF	1	579	108	0.012	0.005
27	CTL-4	1	483	66	0.006	0.003
28	Waprin	1	405	75	0.006	0.002
29	Ficolin-1	1	1,032	21	0.004	0.002
30	Ficolin-2	1	999	21	0.004	0.002
31	Vespryn	1	558	25	0.003	0.001
32	PDE4	1	1,362	9	0.002	0.001
33	CTL-2	1	531	17	0.002	0.001

Abbreviations: 3FTx…three-finger toxin, CDS…coding sequence, CTL…C-type lectin, CRISP…cysteine-rich secretory protein, KUN-Kunitz-type protease inhibitor, PDE…phosphodiesterase, NP…natriuretic peptide, SVMPIII…snake venom metalloproteinase (P-III), VEGF…vascular endothelial growth factor.

sP-:othes36factor.

Table 3 ***Big*** LC/MS^E protein identifications

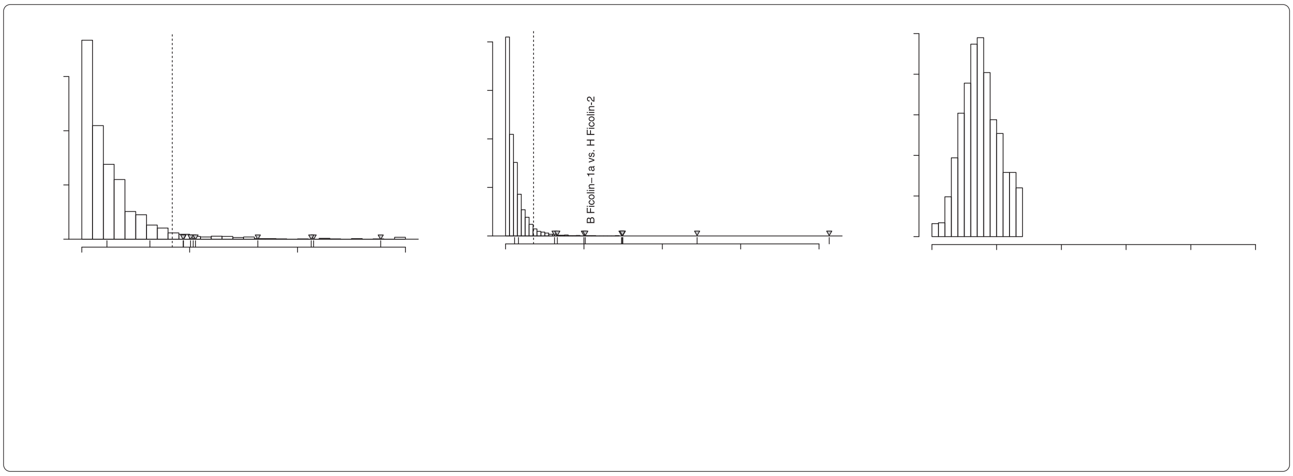
Transcript name	PLGS score score	Peptide matches	% Seq. coverage	Group
SVMPIII-1	896.04	29	57.84	1
SVMPIII-2b	2,805.59	26	62.77	2
SVMPIII-2a	2,694.91	29	66.50	3
SVMPIII-4a	900.64	29	64.92	3
SVMPIII-4b	890.08	30	64.92	3
CRISP-1b	6,457.39	23	93.67	4
CRISP-1d	6,198.10	21	85.52	4
CRISP-1a	6,248.59	21	87.33	5
CRISP-1c	6,507.89	23	95.48	5
NatA-10	327.40	5	7.92	6
3FTx-1a	4,423.76	10	78.89	7
3FTx-1b	6,549.43	10	78.89	7
3FTx-1c	4,423.76	10	78.89	7
3FTx-1d	6,549.43	10	78.89	7
3FTx-1e	6,549.43	10	78.89	7
3FTx-1f	4,423.76	8	74.44	7
3FTx-3a	8,140.22	6	40.22	8
3FTx-3b	8,140.22	6	40.22	8
3FTx-3c	8,140.22	6	40.22	8
3FTx-3d	2,067.74	4	20.65	8
3FTx-3e	8,140.22	6	40.22	8
3FTx-3f	8,140.22	6	40.22	8
3FTx-3g	8,140.22	6	40.22	8
3FTx-3h	8,140.22	6	40.22	8
3FTx-3i	8,140.22	6	40.22	8
3FTx-3j	6,072.48	2	19.78	8
3FTx-3k	2,067.74	4	20.65	8
3FTx-4a	7,234.41	15	100.00	9
3FTx-4b	6,945.58	13	87.21	9
3FTx-4c	5,137.73	14	94.19	9
3FTx-4d	7,234.41	14	92.94	9
3FTx-5	480.09	2	53.97	10
3FTx-6b	480.09	2	53.97	10
3FTx-6a	1,252.34	3	53.97	11
3FTx-6c	1,252.34	3	54.84	11
3FTx-7a	6,785.76	11	66.67	12
3FTx-7b	6,815.80	12	78.89	12
3FTx-7c	6,785.76	11	66.67	12
3FTx-7d	2,506.53	8	62.22	12
3FTx-8a	3,911.73	9	51.11	13
3FTx-8b	3,911.73	9	51.11	13
3FTx-8c	3,911.73	9	51.11	13
3FTx-8d	3,911.73	9	51.11	13
3FTx-8e	3,911.73	9	51.11	13
3FTx-8f	3,911.73	8	39.33	13
3FTx-9a	9,625.12	14	81.40	14
3FTx-9b	9,625.12	14	81.40	14
3FTx-9c	9,625.12	14	81.40	14
3FTx-9d	9,625.12	14	81.40	14
3FTx-9e	9,625.12	13	69.41	14
3FTx-9f	9,625.12	13	69.41	14
3FTx-9g	9,625.12	14	81.40	14
3FTx-10a	3,753.53	10	75.58	15
3FTx-10b	4,914.06	13	91.86	15
3FTx-10c	3,753.53	10	79.27	15
3FTx-10d	4,914.06	12	84.71	15
3FTx-10e	4,914.06	12	84.71	15
3FTx-10f	3,530.34	10	69.77	15
3FTx-10g	4,914.06	13	91.86	15
3FTx-10h	4,914.06	13	91.86	15
3FTx-10i	4,914.06	13	76.85	15
3FTx-2a	6,549.43	9	55.56	…
3FTx-2b	6,549.43	9	55.56	…
3FTx-2c	6,549.43	9	55.56	…
3FTx-2d	4,423.76	9	55.56	…
3FTx-2e	6,549.43	9	55.56	…
3FTx-2f	5,175.20	6	34.44	…
3FTx-2g	3,049.53	6	34.44	…

Transcripts were grouped on the basis of shared, unique peptides. Identifications without a group designation lacked unique identifying peptides but still had peptide matches. Abbreviations: 3FTx…three-finger toxin, CRISP…cysteine-rich

Table 4 *Hypsiglena* sp. LC/MS^E protein identifications

Transcript name	PLGS score	Peptide matches	% Seq. coverage	Group
CRISP-1b	90,678.90	28	97.29	1
CRISP-1d	90,678.90	28	97.29	1
CRISP-1a	83,532.05	29	97.29	2
CRISP-1c	83,532.05	29	97.29	2

evidence of 3FTxs. The transcriptome of *Sistrurus catenatus edwardsii* showed evidence for 3FTxs, but this evidence consisted of five distinct transcripts at extremely low abundances [33]. A 3FTx was also detected at low levels in the transcriptome of *Protobothrops flavoviridis* [43] and in the venom proteome of


B Ficolin−1a vs. H Ficolin−2

high-definition mass spectrometry. As previously seen for both elapids and viperids, venom expression was strongly biased towards toxin production in both *B. irregularis* and *Hypsiglena* sp., suggesting that venom plays an important function in the feeding ecology of these species.

sites within alignments using HyPhy [68]. We used the same maximum-likelihood tree as for the codeml analysis and ran the SLAC, FEL, and REL methods [65]. In each case we used the substitution model most closely resembling the model selected with MrModelTest. For the SLAC and methods, we looked for site under selection with $P <$ 0.05, and for the REL methods, we looked for sites with Bayes factors 100.

Abbreviations

3FTx: Three-finger toxin; AChE: Acetylcholinesterase; CDS: Coding sequence; CF: Coagulation factor; CTL: C-type lectin; CRISP: Cysteine-rich secretory protein;dN

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