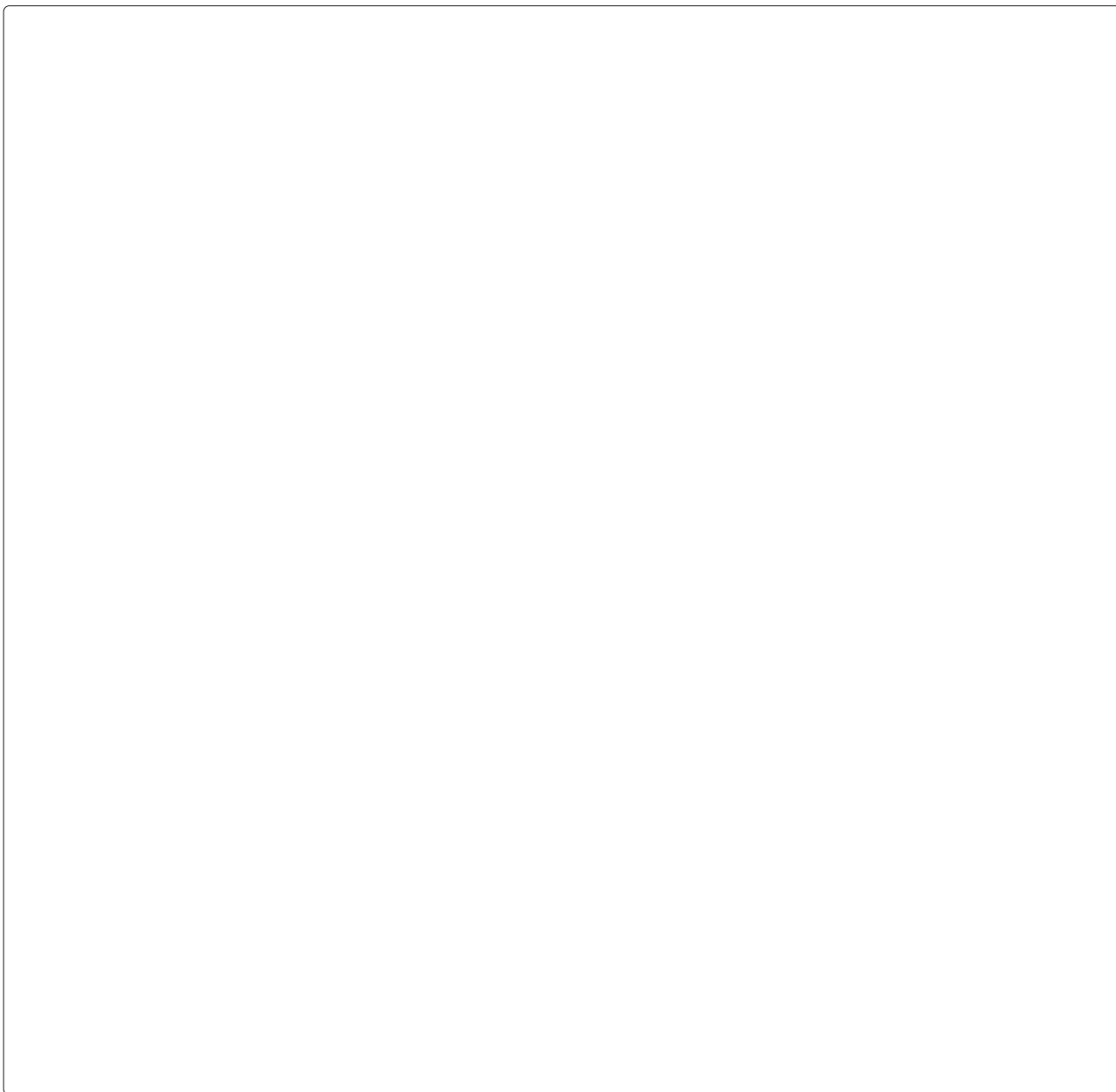


RESEARCH ARTICLE

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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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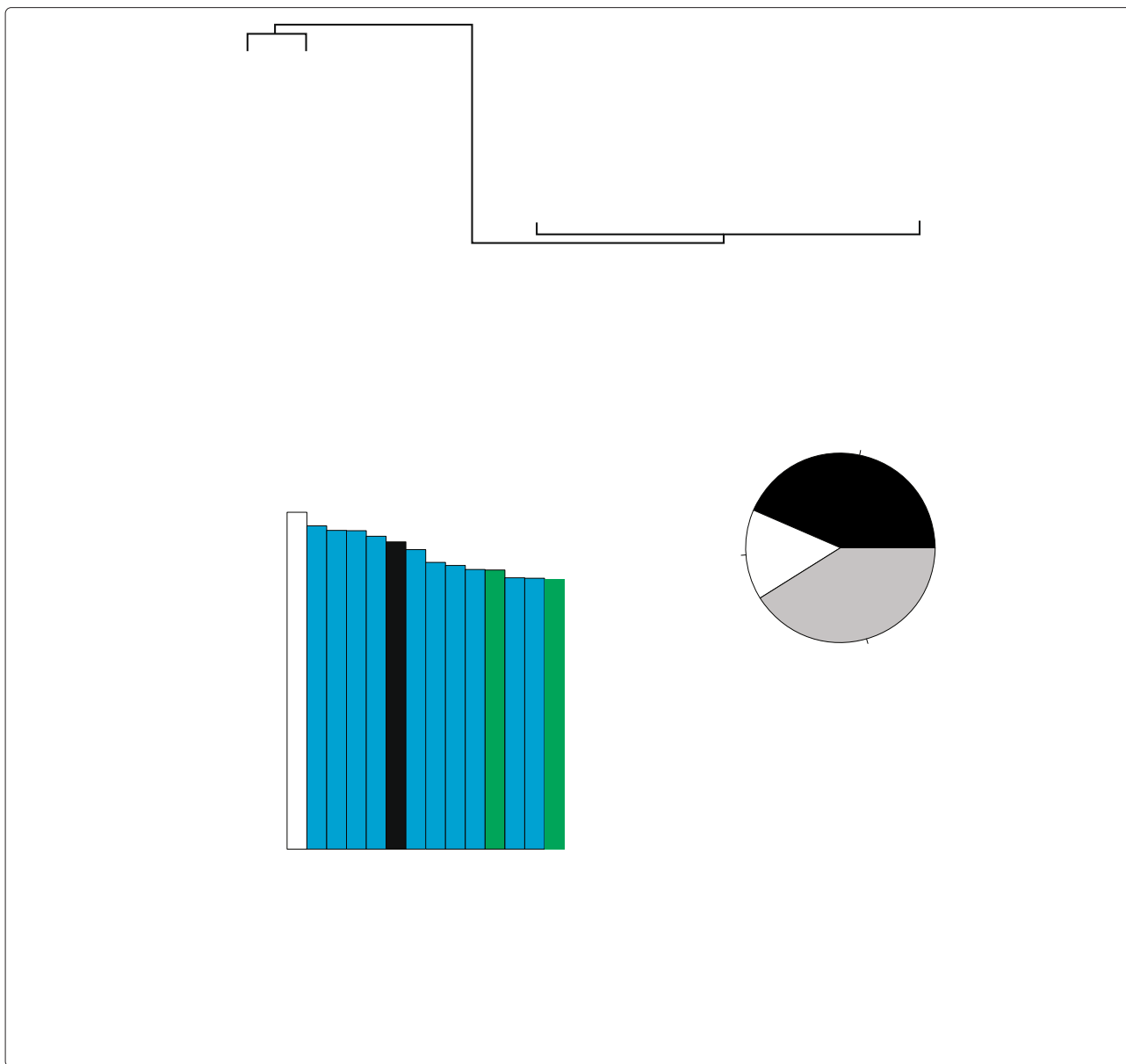


Table 2 Expression levels of full-length toxin clusters for *Hb* sp. based on 10 million reads mapped to coding sequences

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 **B** LC/MS^E protein identifications

Transcript name	PLGS 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

Table 3 **B** (Continued) LC/MS^E protein identifications

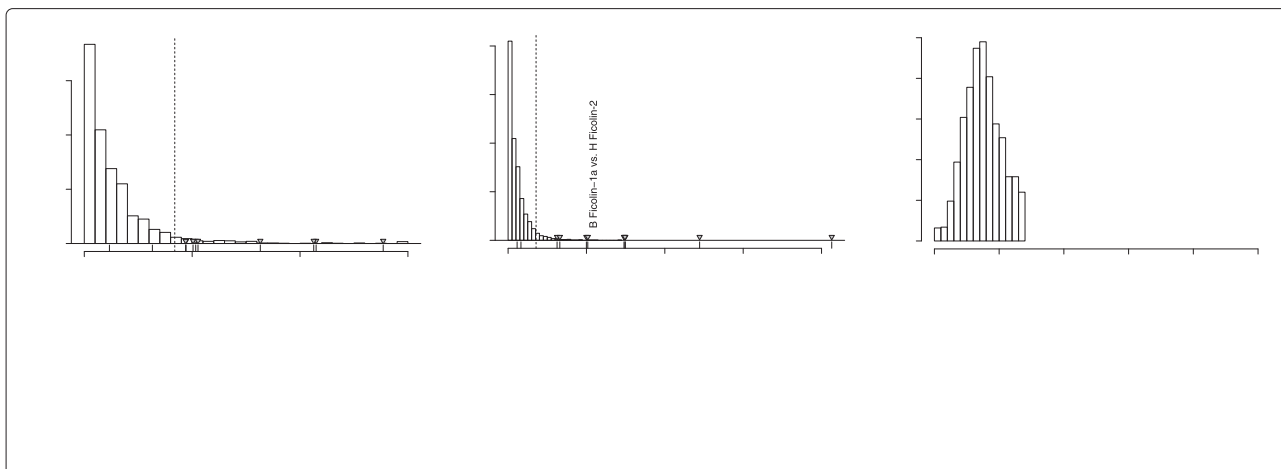
3FTx-8e	3,911.73	9	51.11	13
3FTx-8f	3,911.73	8	39.33	13
3FTx-9a	9,625.12	14	81.40	14
3FTx-9b	9,625.12	14	81.40	14
3FTx-9c	9,625.12	14	81.40	14
3FTx-9d	9,625.12	14	81.40	14
3FTx-9e	9,625.12	13	69.41	14
3FTx-9f	9,625.12	13	69.41	14
3FTx-9g	9,625.12	14	81.40	14
3FTx-10a	3,753.53	10	75.58	15
3FTx-10b	4,914.06	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 **Hb** 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 edwardsi* 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



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 *Hypsiglenasp.*, 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

29. Calvete JJ, Fasoli E, Sanz L, Boschetti E, Righetti PG: Expanding the venom proteome of the Western Diamondback Rattlesnake, *Crotalus atrox*, via snake venomomics and combinatorial peptide ligand library approaches. *J Proteome Res* 2009, 8:3055-3067.
30. Calvete JJ, Sanz L, Cid P, de la Torre P, Flores-Diaz M, Santos MCD, Borges A, Breimo A, Angulo Y, Lomonte B, Alape-García, Gutiérrez JM: Snake venomomics of the central american rattlesnake *Crotalus simus* and the south american *Crotalus durissus* complex points to neurotoxicity as an adaptive pedomorphic trend along *Crotalus* dispersal in South America. *J Proteome Res* 2010, 9:528-544.
31. Margres MJ, McGovern JJ, Wray KP, Seavy M, Calvin K, Rinkby DR: the transcriptome and proteome to characterize the venom of the eastern diamondback rattlesnake (*Crotalus adamanteus*). *J Proteomics* 2014, 96:145-158.
32. Fry BG: From genome to venom: molecular origin and evolution of the snake venom proteome inferred from phylogenetic analysis of toxin sequences and related body proteins. *Genome Res* 2005, 15:403-420.
33. Pahari S, Mackessy SP, Kintanum: The venom gland transcriptome of the Desert Massasauga Rattlesnake (*Sistrurus catenatus edwardsii*): towards an understanding of venom composition among advanced snakes (Superfamily Colubroidea). *BMC Mol Biol* 2007, 8:115.
34. Doley R, Pahari S, Reza MA, Mackessy SP, Kintanum: The structure and evolution of ku-wap-fusin (Kunitz Waprin fusion protein), a novel evolutionary intermediate of the Kunitz serine protease inhibitors and waprins from *Sistrurus catenatus* (Massasauga Rattlesnake) venom glands. *Open Evol Biol* 2010, 4:31-41.
35. Tsetlin V: Snake venom neurotoxins and other three-finger toxins. *Eur J Biochem* 1999, 264(2):281-286.
36. Karlsson E, Jolkkonen M, Mulugeta E, Onali P, Åsman A: Snake toxins with high selectivity for subtypes of muscarinic acetylcholine receptors. *Biochim Biophys Acta* 2000, 82(9-10):793-806.
37. Koivula K, Rondineli S, Nasmith: The three-finger toxin MT is a selective 2B-adrenoceptor antagonist. *Toxicol* 2010, 56(3):440-447.
38. Mackessy SP, Sixberry NM, Heyborne WH, Kintanum: Evolution of the brown treesnake, *Boiga irregularis*: ontogenetic shifts and taxa-specific toxicity. *Toxicol* 2006, 47:537-548.
39. Fry BG, Scheib H, van der Weerd L, Young B, McNaughtan J, Ramjan SFR, Vidal N, Poelmann RE, Norman EA: Evolution of an arsenal. *Mol Cell Proteomics* 2008, 7(2):215-246.
40. Pawlak J, Mackessy SP, Sixberry NM, Stura EA, DeMott IR, Foo CS, Menez A, Nirthanan S, Kintanum: A novel covalently linked heterodimeric three-finger toxin with high taxon-specific neurotoxicity. *FASEB J* 2009, 23(2):534-545.
41. Siang AS, Doley R, Vonk FJ, Kintanum: Transcriptomic analysis of the venom gland of the red-headed krait (*Bungarus flaviceps*) using expressed sequence tags. *BMC Mol Biol* 2010, 11:24.
42. Jia Y, Cantu BA, Sanchez EE, Perez JC: Complementary DNA sequencing and identification of mRNAs from the venomous gland of *Agkistrodon piscivorus leucostoma*. *Toxicol* 2008, 51:1457-1466.
43. Aird SD, Watanabe Y, Villar-Briones A, Poy MC, Terada K, Mikheyev AS: Quantitative high-throughput profiling of snake venom gland transcriptomes and proteomes (*Ovophis okinavensis* and *Protobothrops mucroserpens*). *BMC Microbiol* 2011, 11:115.

71. Greene HV. Snakes: the evolution of mystery in nature. Berkeley: University of California Press; 1997.
72. Secor SM, Diamond J. Evolution of regulatory responses to feeding in snakes. *Physiol Biochem Zool* 2000, 73:123...141.
73. Fitch H, Twining H. Feeding habits of the Pacific Rattlesnake. *Copeia* 1946, 1946:64...71.
74. Nogueira C, Sawaya RJ, Martins C. Ecology of the pitviper, *Bothrops moojeni*, in the Brazilian Caerrado. *J Herpetol* 2003, 37(4):653...659.
75. Tsai W. When prey acts as a lever: prey-handling behavior of the Chinese Green Tree Viper, *Trimeresurus stejnegeri stejnegeri* (Viperidae: Crotalinae). *Zool Stud* 2007, 46:631...637.
76. Greene HV. Defensive behavior and feeding biology of the Asian mock viper, *Psammodynastes pulverulentus* (Colubridae), a specialized predator on scincid lizards. *Chinese Herpetol* 1989, 2:21...32.
77. Shine R, Harlow P, Branch W, Webb J. On the lowest branch: sexual dimorphism, diet, and reproductive biology of an African twig snake, *Thelotornis capensis* (Serpentes, Colubridae). *Copeia* 1996, 2:290...299.
78. Greene HV. Ecological, evolutionary, and conservation implications of feeding biology in Old World cat snakes, genus *Boiga* (Colubridae). *Proc Calif Acad Sci* 1998, 46:193...207.
79. Hill RE, Mackessy SP. Venom yields from several species of colubrid snakes and differential effects of ketamine. *Toxicol* 1997, 35(5):671...678.
80. Rotenberg D, Bamberger ES, Koch S. Studies on ribonucleic acid synthesis in the venom glands of *Vipera palaestinae* (Ophidia, Reptilia). *Biochem J* 1971, 121:609...612.
81. Kosakovsky P, Posada D, Gravenor MB, Woelk CH, Frost SDW. Automated phylogenetic detection of recombination using a genetic algorithm. *Mol Biol Evol* 2006, 23(10):1891...1901.
82. Bendtsen JD, Nielsen H, von Heijne G, Brunak S. Improved prediction of signal peptides: SignalP 3.0. *J Mol Biol* 2004, 340:783...795.
83. Thompson JD, Higgins DG, Gibson TP. B1bhompsom0D6a.8(H)-.volutionBplImpeeding6he