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Research Article

Proteomic profiling of liver from *Elaphe taeniura*, a common snake in eastern and southeastern Asia

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Abstract

Snake liver has been implicated in the adaptation of snakes to a variety of habitats. However, to date, there has been no systematic analysis of snake liver proteins. In this study, we undertook a proteomic analysis of liver from the colubrid snake *Elaphe taeniura* using a combination of two-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption/ionization time of flightmass spectrometry (MALDI-TOF MS). We also constructed a local protein sequence database based on transcriptome sequencing to facilitate protein identification. Of the 268 protein spots revealed by 2-DE 109 gave positive MS signals, 84 of which were identified by searching the NCBInr, Swiss-Prot and local databases. The other 25 protein spots could not be identified, possibly because their transcripts were not be stable enough to be detected by transcriptome sequencing. GO analysis showed that most proteins may be involved in binding, catalysis, cellular processes and metabolic processes. Forty-two of the liver proteins identified were found in other reptiles and in amphibians. The findings of this study provide a good reference map of snake liver proteins that will be useful in molecular investigations of snake physiology and adaptation.

Keywords: 2D electrophoresis, *Elaphe taeniura*, MALDI-TOF mass spectrometry, protein.

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Introduction

Proteomics has been widely used for the global analysis of protein profiles and has provided insights into mechanisms related to development, physiology and pathology (Yanes *et al.*, 2007). Proteomic studies have been reported for various organisms, such asbacteria, mouse, rat, guinea pig (*Cavia porcellus*), worms (Nematoda), Leguminosae (*Medicago sativa Linn*), rice (*Oryza sativa*) and humans (Klose, 1975; O'Farrell, 1975; Scheele, 1975; Emmert-Buck *et al.*, 2000; Kaji *et al.*, 2000; Ahram *et al.*, 2002; Li *et al.*, 2003), as well as amphibians, *e.g.*, the African clawed frog *Xenopus laevis* (Jelaso *et al.*, 2005; Gillardin *et al.*, 2009), and snakes (Campbell and Campbell,2001). Although proteomic analyses of venoms from more than 55 snake genera have been reported (Fry *et al.*, 2003; Serrano *et al.*, 2005; Menezes *et al.*, 2006; Yanes *et al.*, 2007; Fox and Serrano, 2008), to date there has been no proteomic analysis of snake tissues.

Snakes are highly adapted to a variety of terrestrial, arboreal, semiaquatic and marine habitats (Conant and Collins,1991). This adaptability has been attributed to the development of a special metabolic system in snakes. The liver plays a major role in metabolism, including glycogen storage, red blood cell degradation, plasma protein synthesis, hormone production and detoxification (Dardevet *et al.*, 2006; Reddy and Rao, 2006), and also contains disease-associated proteins (Zeindl-Eberhart *et al.*, 2001; Yokoyama *et al.*, 2004). Recent studies have shown that snake liver has a strong metabolic capacity (Ladyman *et al.*, 2003; Starck *et al.*, 2004) that may have an important role ensuring survival in nature. Previous studies of snake liver have focused mainly on plasma proteins (Chiu and Lam,1994), as well as histological and biochemical analyses (Chiu and Wong, 1974; Wong and Chiu, 1979; Chang and Zheng, 2003); there have been no molecular biology or proteomic analyses.

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In this report, we describe the first proteomic study of snake liver using 2-DE and MALDI-TOF MS. The source of liver was *Elaphe taeniura* (Cope, 1861; Pike *et al.*, 2008), a colubrid snake with a wide distribution in China, Russian, Japan, India and southeastern Asia. Since there are no reports on snake genomes, we also constructed a local database of predicted protein sequences based on transcriptome sequencing data; this database significantly facilitated protein identification. A reference map of the *E. taeniura* liver protein profile was successfully constructed and 84 of 268 protein spots were identified.

Materials and Methods

Preparation

Adult *E. taeniura* were provided by Nanjing Jinlin Snake Industry Co. Ltd. (Jiangsu province, China). After anesthesia by an intraperitoneal injection of an overdose (45 mg/kg) of sodium pentobarbital, we dissected the body of snakes. Samples of the liver tissue were immediately frozen in liquid nitrogen and stored at -70 °C until use. All of the procedures conformed to ethical standards for the treatment of animals and were designed to minimize animal suffering. The protocol was approved by the ethics committee of the Laboratory for Animal Research Center at Jiangsu University, China. All reagents were from Sigma unless stated otherwise.

Protein sample preparation

Hepatic tissue (0.15 g) was homogenized with a precooled mortar and pestle in 1.5 mL of lysis buffer containing 7 M urea, 2 M thiourea and 4% Chaps followed by sonication (twice) for 10 s on ice. The homogenate was transferred to an Eppendorf tube and centrifuged (10,000 *g*, 20 min, 4 °C). Protein samples were treated with a 2-DE clean-up kit (GE Healthcare), vacuum-dried and dissolved in 120 μL of buffer A containing 7 M urea, 2 M thiourea, 4% Chaps, 0.2% Bio-Lyte pH 3-10 and 65 mM dithiotreitol (DTT). The protein concentration was determined using the Bradford assay with bovine serum albumin (BSA) as standard. The protein samples were stored at -20 °C for later use.

Two-dimensional electrophoresis

Isoelectric focusing (IEF) was done using an IPGphor focusing system (Bio-Rad) according to the manufacturer's instructions. IPG strips (linear pH 3-10 gradient; 17 cm) were run at 20 °C. The protein sample (1.4 mg) was first rehydrated in buffer using active rehydration (13 h with 50 V) in a total volume of 400 μL after which IEF was done with a voltage gradient of 250 V (0.5 h), 1,000 V (1 h) and 10,000 V (5 h), and then continued for a total of 60 kVh at 10 kV. The focused strip was equilibrated for 15 min with equilibration solution (6 M urea, 0.375 M Tris-HCl, pH 8.8, 20% (v/v) glycerol, 2% (w/v) SDS and 0.002% (w/v) bromophenol blue) containing 2% (w/v) DTT and for another 15 min with same solution containing 2.5% (w/v) iodoacetamide. SDS-PAGE was done using 12% gels at 30 mA (constant) until the dye front reached the bottom of the gel. The gel was stained with 0.1% coomassie brilliant blue G-250 (Bio-Safe^{TM}, Bio-Rad) and photographed with a digital single lens reflex (Nikon D5000) and standard lens (AF 50 mm f/1.4D).

In situ digestion

268 protein spots in the 2D gel that were detected by coomassie brilliant blue staining were manually excised, transferred to Eppendorf tubes and then destained, reduced, alkylated and digested with trypsin. Briefly, the gel slices were immersed in double-distilled water, sonicated (twice) for 10 min, washed with 100 mM NH_4HCO_3/acetonitrile (1:1, v/v), dehydrated in acetonitrile and dried in a Speedvac vacuum concentrator (Eppendorf AG, Hamburg, Germany). The protein slices were then reduced with 10 mM DTT in 100 mM NH_4HCO_3 for 1 h at 56 °C and then incubated with 40 mM iodoacetamide in 100 mM NH_4HCO_3 for 45 min at room temperature. The protein spots were washed sequentially with 25 mM ammonium bicarbonate, 50% acetonitrile and 100% acetonitrile, and then incubated overnight in 25 mM NH_4HCO_3 containing 20 μg of trypsin/mL at 37 °C (Liang *et al.*, 2007; Qin *et al.*, 2009).

Mass spectrometry analysis

After digestion, the protein peptides were extracted twice in 0.5% trifluoroacetic acid (TFA) and 2.5% TFA/50% acetonitrile. A one microliter sample was then spotted onto an MTP Anchor Chip board (Bruker) and analyzed with a MALDI-TOF mass spectrometer. Peptide mass fingerprints of 1,000-4,000 Da were obtained. Standard peptides were used as external standards. The peak value of the trypsin peptide and matrix were used as internal parameters (Kim *et al.*, 2007).

Liver transcriptome of *E. taeniura*

The liver transcriptome of *E. taeniura* was sequenced by Hanyu Biological Co., Ltd. (Shanghai, China). Briefly, liver RNA was extracted and mRNA was purified by standard procedures followed by cDNA synthesis. RNA was fragmented by incubation at 95 °C for 5 min and annealed with biotinylated random primers that contained a Solexa adapter sequence. The RNA fragments were then captured with streptavidin coupled to biotinylated random primers. Finally, a double-strand Solexa library was produced by PCR amplification, with clean data being obtained from the raw data using the FASTX software package. *De novo* assembling was then done using the Velvet and Oases software packages.

Bioinformatics analysis

Open reading frames were identified by using an in-house program based on 'GetORF' from EMBOSS (Rice *et al.*, 2000). Gene annotation was done by BLASTP searching against the Swiss-Prot and GenBank databases with an E value cutoff of $1e^{-3}$. The predicted proteins were used to construct a local MASCOT protein database and to analyze the peptide finger prints of snake liver proteins.

To identify the proteins, the MS fingerprints were screened against the NCBInr and Swiss-Prot sequence databases with the search engine MASCOT. Unidentified proteins were searched in a local database constructed specifically for this purpose. Fixed modification was set to be carbamidomethyl (C) and variable modification was set to be oxidation (M). The mass tolerance was set as ± 0.2 to ± 0.8 Da. The species were set as Chordata and a CI score > 62 was considered to be a positive match.

The resulting protein sequences were aligned with Interpro Scan to obtain the gene ontology (GO) identifications and the collected information was then analyzed by WEGO (Ye *et al.*, 2006).

Configuration and installation of local database

The Configuration Editor/Database Maintenance of the MASCOT homepage was used for the custom database configuration. In this process, the translated protein sequence in sequence directory file is parsed and compressed by the MASCOT program, producing homonyms a00, i00, s00 and a statistics database retrieval file in which the MS data perform a retrieval step. Subsequently, the new database name (cldata) was entered in the EST sequence file path in the configuration page, options and parsing rules were defined and applied to complete a new database definition. Details on the settings can be provided by the authors on request.

Results

Protein profile of *E. taeniura* liver

Figure 1 shows a reference 2-DE map of *E. taeniura* liver in which 268 spots were detected. The pIs of 196 protein spots ranged from 5 to 9 (73.1% of spots); 21 spots had a pI < 4 (7.83% of spots) and 26 had a pI > 9 (9.7% of spots). The molecular masses of most proteins were between 29 kDa and 97.2 kDa. 109 protein spots had a good MS signal, 84 of which were identified. Of these 84 proteins, 54 were represented in the NCBInr and Swiss-Prot databases for Chordata and 30 were in the local database. Table 1 summarizes the data for these proteins.

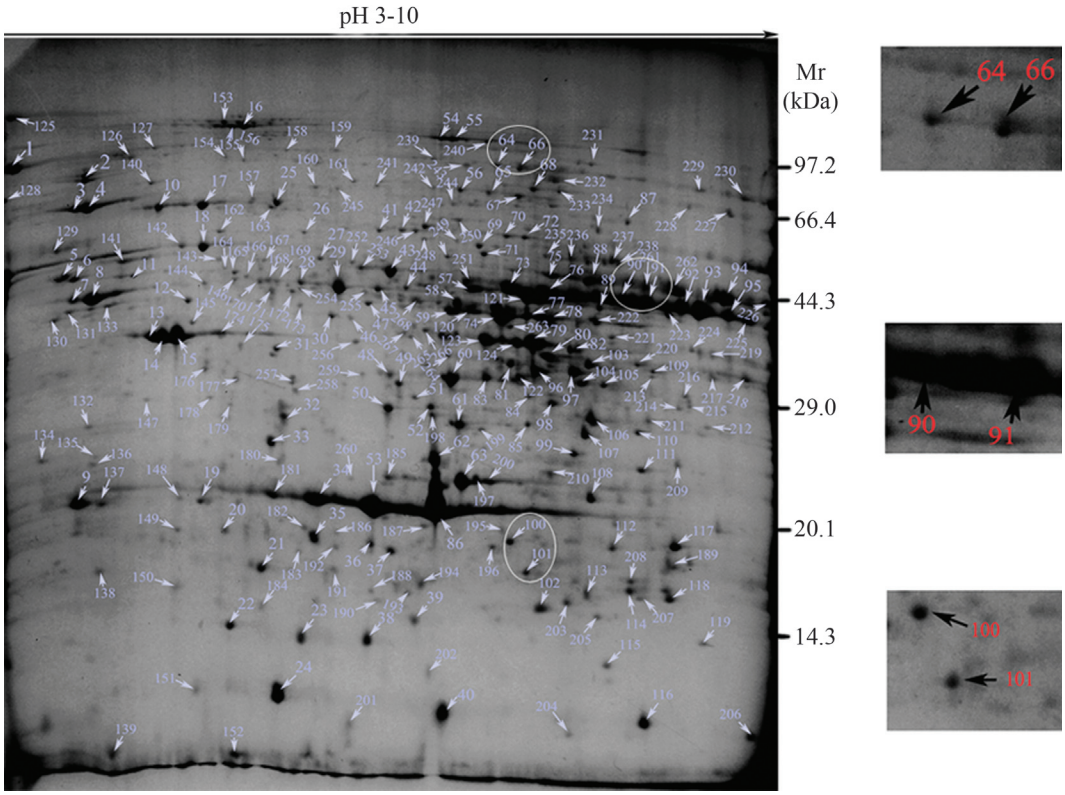

Figure 1 - Two-dimensional electrophoretic reference map of *E. taeniura* liver. A total of 268 protein spots were observed, of which 109 gave a good MS signal; 84 of the latter proteins were identified in subsequent analysis. A sample of liver protein (1.4 mg) was separated by IEF (linear pH gradient from pH 3-10) and SDS-PAGE on a 12% polyacrylamide gel. Proteins were visualized by coomassie brilliant blue (CBB) G-250 staining. Expanded views of spots 64, 66, 90, 91, 100 and 101 are shown on the right.

Table 1 - Proteins identified by MALDI-TOF mass spectrometry and MASCOT.

Spot	Protein name	Accession no.	Database	Species	pI/kDa	p value	Coverage (%)	Score
1	N-terminal plus middle domains (N+m) of Grp94	gi|159794958	NCBInr	*Canis lupus familiaris*	6.38/58167	5.40E-03	26	84
4	Hypothetical protein BRAFLDRAFT_71661	gi|260823156	NCBInr	*Branchiostoma floridae*	8.39/147395	5.40E-02	10	74
5	Heat shock protein HSP 90-α-like	gi|327278721	NCBInr	*Anolis carolinensis*	4.98/84451	4.30E-09	36	146
6	Tubulin β-4 chain-like	gi|327263983	NCBInr	*Anolis carolinensis*	4.82/50080	2.70E-11	47	184
7	Mitochondrial ATP synthase β subunit	gi|47575824	NCBInr	*Xenopus* (*Silurana*) *tropicalis*	5.38/56328	1.30E-06	38	120
8	Mitochondrial ATP synthase β subunit	gi|148223359	NCBInr	*Xenopus laevis*	5.25/56395	2.10E-10	44	158
9*	Nicotinamide N-methyltransferase-like	-	Local	*Elaphe taeniura*	5.10/32099	1.60E-04	29	82
12*	Cytochrome β-c1 complex subunit 1, mitochondrial-like	-	Local	*Elaphe taeniura*	5.64/53739	5.60E-08	33	122
13	Unnamed protein product	gi|47207906	NCBInr	*Tetraodon nigroviridis*	6.68/50199	7.40E-03	23	82
14	POTE ankyrin domain family member F	A5A3E0	Swiss-Prot	*Homo sapiens*	5.83/123020	3.80E-02	12	63
15	Actin, cytoplasmic type 5	gi|288541396	NCBInr	*Xenopus laevis*	5.30/42165	4.60E-04	44	95
17	Heat shock cognate 71 kDa protein-like	gi|74211333	NCBInr	*Anolis carolinensis*	5.37/71027	5.90E-04	25	121
18	Heat shock protein60 kDa	gi|296439571	NCBInr	*Mesocricetus auratus*	4.73/29133	2.20E-02	33	78
19*	Putative prohibitin variant 1	-	Local	*Elaphe taeniura*	5.59/31571	4.00E-03	38	74
20*$^{\Delta}$	Hypothetical protein VC0395_0781	-	Local	*Elaphe taeniura*	9.05/4233	3.30E-03	86	74
21*	Thioredoxin-dependent peroxide reductase, mitochondrial-like	-	Local	*Elaphe taeniura*	8.72/28255	8.70E-03	28	70
24	Putative protein FAM157A	C9JC47	Swiss-Prot	*Homo sapiens*	11.26/43211	1.60E-02	32	67
25	Heat shock 70 kDa protein 9 (mortalin)	gi|148228693	NCBInr	*Xenopus laevis*	5.68/72800	3.00E-03	19	86
27	40S ribosomal protein S6	P47838	Swiss-Prot	*Gallus gallus*	10.83/28808	4.10E-02	37	63
28*	4-Trimethylaminobutyraldehyde dehydrogenase-like	-	Local	*Elaphe taeniura*	8.66/41807	2.00E-05	36	96
29	Predicted: hypothetical protein	gi|194685097	NCBInr	*Bos taurus*	9.03/183966	1.90E-02	16	78
30	Leucine-rich repeat and coiled-coil domain-containing protein 1	Q69ZB0	Swiss-Prot	*Mus musculus*	5.67/120577	5.80E-03	19	72
31	Interleukin 15 receptor α chain isoform 1A	gi|29028294	NCBInr	*Mus musculus*	9.51/24907	5.30E-02	32	74
32	Predicted: low quality protein: prohibitin-like	gi|334323093	NCBInr	*Monodelphis domestica*	5.57/29882	8.30E-03	37	82
35	Protein Wnt-2b	Q98SN7	Swiss-Prot	*Gallus gallus*	9.17/44464	1.80E-02	33	67
37*	Heme oxygenase 1-like	-	Local	*Elaphe taeniura*	9.26/36906	3.50E-09	46	134
39*	Glutathione S-transferase 2-like	-	Local	*Elaphe taeniura*	5.63/17123	4.80E-05	46	93
40	2-Hydroxyacyl-CoA lyase 1	Q8CHM7	Swiss-Prot	*Rattus norvegicus*	7.08/64431	4.30E-02	18	63
46	Keratin 10	gi|186629	NCBInr	*Homo sapiens*	4.72/39832	4.10E-03	33	85
50	PMCA4	gi|170524482	NCBInr	*Caviaporcellus*	7.66/36538	4.20E-02	34	75
52	Keratin, type II cytoskeletal 1	gi|119395750	NCBInr	*Homo sapiens*	8.15/66170	4.30E-03	34	85

Table 1 (cont.)

Spot	Protein name	Accession no.	Database	Species	pI/kDa	p value	Coverage (%)	Score
53	Novel protein with part of an AIG1 family domain	gi|220672712	NCBInr	*Danio rerio*	7.77/26642	9.80E-03	47	81
54	Pyruvate carboxylase, gene 1	gi|148227386	NCBInr	*Xenopus laevis*	6.47/130809	1.20E-03	16	90
58	Predicted: hypothetical protein	gi|114641340	NCBInr	*Pan troglodytes*	11.59/32713	5.20E-02	32	74
59	Alpha-enolase	Q9W7L0	Swiss-Prot	*Python regius*	6.97/47940	1.10E-02	26	69
60	Guanine nucleotide binding protein (G protein)	gi|291394810	NCBInr	*Oryctolagus cuniculus*	4.76/8647	5.00E-02	64	74
61	Unnamed protein product	gi|189053805	NCBInr	*Homo sapiens*	6.00/35047	3.40E-02	26	76
64*	Cytoplasmic aconitate hydratase	-	Local	*Elaphe taeniura*	6.30/99709	3.50E-07	24	114
66*	Cytoplasmic aconitate hydratase	-	Local	*Elaphe taeniura*	6.30/99709	6.70E-04	17	81
67*$^{\Delta}$	Altered inheritance of mitochondria protein 36, mitochondrial	-	Local	*Elaphe taeniura*	8.30/34827	2.50E-02	45	73
68*	Trifunctional purine biosynthetic protein adenosine-3-like	-	Local	*Elaphe taeniura*	6.49/89044	3.50E-07	25	114
70	Ribosomal RNA large subunit methyltransferase L	Q0I4C6	Swiss-Prot	*Haemophilus somnus* 129PT	7.96/82748	2.20E-02	25	74
71	Predicted: hypothetical protein	gi|114672613	NCBInr	*Pan troglodytes*	7.26/227989	5.20E-02	10	74
73$^{\Delta}$	Protease	gi|308187502	NCBInr	*Pantoea vagans* C9-1	5.96/52002	1.30E-02	33	90
74	Tau-crystallin protein	gi|21325980	NCBInr	*Crocodylus palustris*	6.23/47879	1.60E-02	38	79
75*	Delta-1-pyrroline-5-carboxylate dehydrogenase, mitochondrial	-	Local	*Elaphe taeniura*	6.99/31850	1.00E-05	39	100
76	Primosomal protein N'	Q9WY22	Swiss-Prot	*Thermotoga maritima*	8.71/85017	4.90E-02	25	70
77	Alpha-enolase	gi|17367189	NCBInr	*Sceloporus undulatus*	6.64/47806	3.40E-02	28	76
78	Alpha-enolase	gi|17367183	NCBInr	*Python regius*	6.97/47940	2.90E-02	39	87
79	Serine/threonine-protein kinase DCLK1	Q9JLM8	Swiss-Prot	*Mus musculus*	9.00/84671	3.10E-02	13	64
80	Unnamed protein product	gi|47210960	NCBInr	*Tetraodon nigroviridis*	9.92/5919	4.00E-02	60	75
82	Immunoglobulin heavy chain variable region	gi|10636766	NCBInr	*Homo sapiens*	8.60/11954	5.30E-02	86	74
83	Predicted: centrosomal protein of 72 kDa-like	gi|297674852	NCBInr	*Pongo abelii*	6.36/12737	3.90E-02	58	75
87*$^{\Delta}$	MURB_BRASB	-	Local	*Elaphe taeniura*	6.33/32561	1.90E-02	40	74
88	Atherin	Q6SPF0	Swiss-Prot	*Homo sapiens*	7.12/56189	1.40E-02	18	68
90*	Glutamate dehydrogenase 1, mitochondrial-like	-	Local	*Elaphe taeniura*	6.82/59370	2.50E-02	25	66
91	Predicted: glutamate dehydrogenase 1	gi|327277111	NCBInr	*Anolis carolinensis*	8.21/60994	1.70E-06	31	119
92	Structure of glutamate dehydrogenase complexed with bithionol	gi|239781822	NCBInr	*Bos taurus*	7.01/55945	4.00E-04	29	95
93	Hypothetical protein BRAFLDRAFT_237370	gi|260811424	NCBInr	*Branchiostoma floridae*	9.28/22817	2.40E-03	61	88
94*	Catalase-like	-	Local	*Elaphe taeniura*	7.79/62201	4.50E-10	33	143
95*	Retinal dehydrogenase 1-like isoform 2	-	Local	*Elaphe taeniura*	7.47/56628	2.80E-03	16	75

Table 1 (cont.)

Spot	Protein name	Accession no.	Database	Species	pI/kDa	p value	Coverage (%)	Score
96*	Adenylosuccinate lyase	-	Local	*Elaphe taeniura*	6.17/58590	3.50E-07	34	114
97*	Fumarylacetoacetase-like	-	Local	*Elaphe taeniura*	6.87/24136	2.00E-02	36	66
98	Predicted: aconitate hydratase, mitochondrial	gi|297261176	NCBInr	*Macaca mulatta*	6.65/73986	4.50E-03	24	85
99*	Sorbitol dehydrogenase	-	Local	*Elaphe taeniura*	6.21/37677	2.20E-10	37	146
100*	4-Hydroxyphenylpyruvate dioxygenase-like	-	Local	*Elaphe taeniura*	6.02/27951	1.10E-06	42	109
101*	4-Hydroxyphenylpyruvate dioxygenase-like	-	Local	*Elaphe taeniura*	6.02/27951	1.90E-04	27	87
103*	ZYRO0D06578p	-	Local	*Elaphe taeniura*	10.86/4600	4.90E-02	90	63
105*	Sulfotransferase 6B1-like	-	Local	*Elaphe taeniura*	8.10/40274	2.70E-03	28	75
106	Predicted: similar to electron transfer flavoprotein	gi|114658278	NCBInr	*Pan troglodytes*	8.75/30249	2.10E-03	35	88
107	L-Lactate dehydrogenase B chain (LDH-B)	gi|17433148	NCBInr	*Sceloporus undulatus*	6.50/36850	4.80E-02	31	74
108*	S-Formylglutathione hydrolase-like	-	Local	*Elaphe taeniura*	6.58/32218	4.50E-10	50	143
109	Acyl-CoA thioesterase 8, isoform CRA_c	gi|149042921	NCBInr	*Rattus norvegicus*	6.38/22691	1.90E-02	37	78
110*	Uncharacterized oxidoreductase C663.06c-like	-	Local	*Elaphe taeniura*	8.19/31028	2.00E-02	20	66
111	Probable ATP-dependent RNA helicase DHX40	Q6PE54	Swiss-Prot	*Mus musculus*	8.91/89728	3.30E-02	12	64
112*	Uncharacterized oxidoreductase C663.06c-like	-	Local	*Elaphe taeniura*	8.19/31028	1.90E-02	26	67
113*	Proteasome subunit beta type-2-like	-	Local	*Elaphe taeniura*	6.73/25119	1.10E-02	49	69
114	Uncharacterized protein C11orf86	A6NJI1	Swiss-Prot	*Homo sapiens*	11.47/13164	5.40E-02	34	62
116	Ribose-phosphate pyrophosphokinase 2 isoform 2	gi|4506129	NCBInr	*Homo sapiens*	6.00/35431	2.10E-02	37	78
117*	Abhydrolase do-main-containing protein 14B	-	Local	*Elaphe taeniura*	7.22/23842	1.80E-06	58	107
118	Unnamed protein product	gi|47218712	NCBInr	*Tetraodon nigroviridis*	5.77/101753	1.90E-02	9	78
120	Histone-lysine N-methyltransferase	Q96L73	Swiss-Prot	*Homo sapiens*	8.40/302109	2.70E-02	6	65
123	Unnamed protein product	gi|313233113	NCBInr	*Oikopleura dioica*	7.46/83911	3.30E-02	14	76
124*	Glutamine synthetase-like	-	Local	*Elaphe taeniura*	8.00/45741	1.60E-04	26	88

*Proteins that matched the local database for the liver transcriptome of *E. taeniura*. [Δ]Proteins associated with bacteria. The pI/kDa values shown in the Table are theoretical.

Characteristics of the transcriptome assembly

DNA sequencing by Illumina HiSeq 2000 resulted in a cDNA library containing 29,614,448 reads and 2,754,143,664 bases. The number of paired-end reads was 14,807,224, with an average length of 93 bp. A total of 88,907 contigs > 100 bp in size (which reflected the quality of the data) was assembled using Velvet and Oases softwares, and 23,285 predicted proteins had clear biological annotations.

Protein identification and GO analysis

Of the 84 identified proteins, 42 were matched to amphibian and reptile protein sequence databases. Interestingly, the amphibian and reptile databases contained no information for the remaining 42 proteins, although there were matches in databases of other species. The presence of these protein spots in the 2D gel indicates that many snake proteins remain to be identified. For instance, protein spot 74 was identified as tau-crystallin, a homologue of which

has been found mainly in crocodiles. Figure 2 shows the peptide fingerprint and peptide match for spot 74 obtained with the MASCOT database. GO analysis showed that these proteins are mainly involved in binding, catalysis, cellular processes and metabolic processes (Figure 3).

Discussion

In this study, we successfully constructed a reference map for the liver protein profile of the colubrid snake *E. taeniura.* To date, liver proteomic analyses have been reported for mammals and fish (Karim *et al.*, 2011; Molette *et al.*, 2012). As far as we know, the present report is the first to describe the protein profile for snake liver. Preliminary experiments showed that common protein extraction methods such as alcohol phenyl, TCA/acetone, schizolysis were not applicable to snake liver (unpublished results). Instead, a combination of a 2D clean-up kit with the method described here significantly improved the resolution, and 268 protein spots were detected in 2D gels after staining with Coomassie blue. Further optimization of the protein extraction and visualization method could be helpful in identifying even more proteins.

As the snake genome has not yet been described and many proteins could not be identified by searching the NCBInr and Swiss-Prot databases, we constructed a local database based on transcriptome sequencing data. As a result, 84 of the 109 protein spots with high MS scores were identified. Interestingly, the remaining 25 proteins could not be identified although they were present in the 2D gels (Figure1); these proteins may be exclusive to snakes. In addition, some protein spots, *e.g.*, spots 64 and 66, and spots 90 and 91, were found to be the same proteins, although they migrated at different positions in 2-DE. This is a common finding in proteomic studies, as some proteins vary in their post-translational modifications (Meri and Baumann, 2001; Bretteville *et al.*, 2009; Merkley *et al.*, 2009). For example, protein spots 64 and 66 were identified as aconitate hydratase, which is predicted to have 11 phosphorylation sites. Similarly, protein spots 100 and 101 corresponded to dimeric 4-hydroxyphenylpyruvate dioxygenase (Lindblad *et al.*, 1970; Ruetschi *et al.*, 1997), the dimeric nature of which could contribute to variation in the migration pattern in 2-DE. GO analysis showed that most of the proteins identified were involved in catalytic activity, metabolic processes and binding activity, a finding consistent with liver proteomic data for other species (Mi *et al.*, 2007; Wei *et al.*, 2007).

Heat shock proteins (Hsps) Hsp60, Hsp70 and Hsp90 (spots 18, 25 and 5, respectively, in Figure 1) were highly expressed in liver tissue of *E. taeniura*. Hsps are a family of proteins induced by diverse stress factors, such as an increase in temperature, infection, inflammation, starvation,

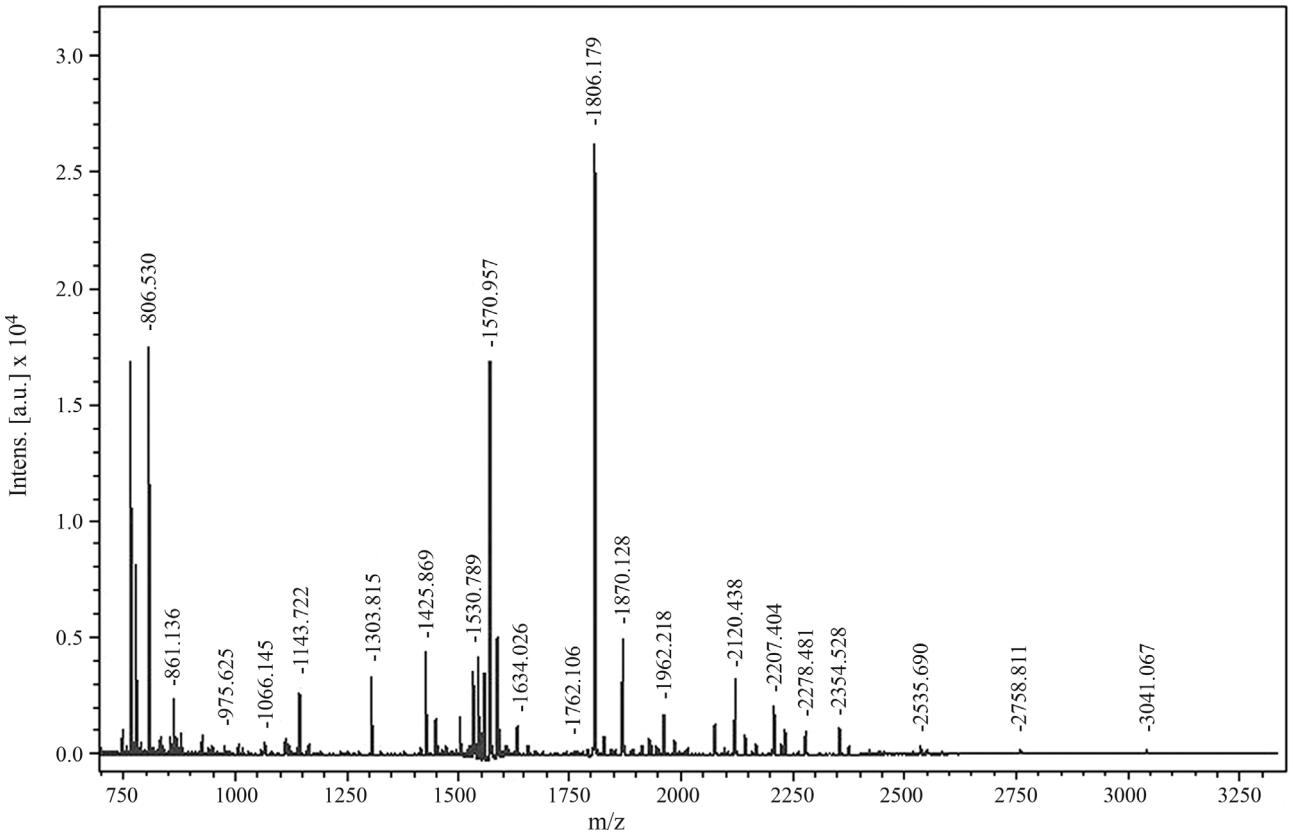

Figure 2 - MALDI-TOF mass spectrum for spot 74.

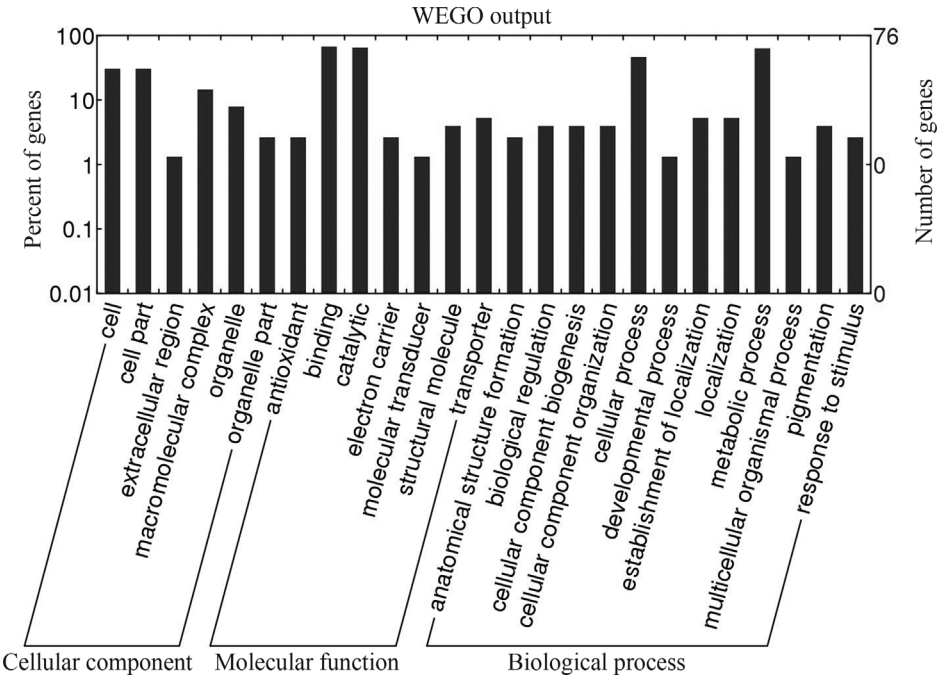

Figure 3 - GO classification of the proteins identified in *E. taeniura* liver. The proteins were grouped into three main categories and 25 subcategories. The total number of matched GOs corresponding to identified proteins was 77.

hypoxia and water deprivation. These proteins play important roles in protein folding, translocation and the assembly of intracellular proteins that may protect against environmental stress (Gray *et al.*, 1999; Nollen and Morimoto, 2002; Sun *et al.*, 2008; Di Domenico *et al.*, 2010). In the face of increasing habitat destruction, many animals, including reptiles, are facing severe environmental challenges that require physiological adaptations. The elevated expression of Hsps in snake liver may help to protect this species from such stress and could contribute to the ability of snakes to adapt to different environments.

Some of the proteins identified here, *e.g.*, tau-crystallin (spot 74) and α-enolase (spots 59, 77 and 78), have also been found in amphibians and reptiles. Tau-crystallin is a taxon-restricted crystallin found in the eye lenses of reptiles and a few avian species but is presumably absent in mammals (Mishra *et al.*, 2002). The level of tau-crystallin expression in the lens varies among species, *e.g.*, in crocodiles it is the least abundant crystallin and is present in trace amounts (Williams *et al.*, 1985; Mishra *et al.*, 2002). Interestingly, tau-crystallin had a relatively high expression in *E. taeniura* liver.

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Internet Resources

Interpro Scan, http://www.ebi.ac.uk/Tools/pfa/iprscan/ (accessed August 28, 2012).

WEGO, http://wego.genomics.org.cn/cgi-bin/wego/index.pl (accessed August 30, 2012).

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