

Supplementary Materials

The Unusual Metalloprotease-Rich Venom Proteome of the Australian Elapid Snake *Hoplocephalus stephensii*

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Table S1. Protein families detected by in-solution digestion of the whole venom. Asterisks indicate protein families not detected by venom fractionation and in-gel trypsin digestion.

3FTx
PLA2
SVSP
SVMP
CRISP
CTL
LAAO
Natriuretic peptide*
NGF
5'Nucleotidase*
Glutamyl cyclase*
Hyaluronidase*
Phosphatase*
Phospholipase B*
Sulfhydryl oxidase*
Waprin*
Aminopeptidase*

LAO-elapitoxin-Hs1	1	11	21	31	41	51
LAO-elapitoxin-Hs1 (proteoform)	MNV F I I F S L L F L A A L E S	C A D D R R R P L E E C F Q E A D Y E E F L E I A R N G L N E T S N P K H V V V V G A				
L-amino-acid oxidase (<i>N. scutatus</i>)	MNV F I I F S L L F L A A L E S	C A D D R R R P L E E C F Q E A D Y E E F L E I A R N G L N E T S N P K H V V V V G A				
L-amino-acid oxidase (<i>P. australis</i>)	MNV F F M F S L L F L A A L G S	C A D D R R R P L E E C F R E A D Y E E F L E I A K N G L K K R T S N P K R V V V V G A				
L-amino-acid oxidase (<i>B. fasciatus</i>)	MNV F S I F S L V F L A A F G S	C A D D R R S A L E E C F R E A D Y E E F L E I A R N G L K K R T S N P K H V V V V G A				
L-amino-acid oxidase (<i>C. rhodostoma</i>)	MNV F F M F S L L F L A A L G S	C A D D R R N P L A E C F Q E N D Y E E F L E I A R N G L K A T S N P K H V V I V G A				
LAO-elapitoxin-Hs1	61	71	81	91	101	111
LAO-elapitoxin-Hs1 (proteoform)	GMA G L S A A Y V L A G A G H K V T L L E A S E R V G G R V N T Y R N E T E G W Y V N M G P M R L P E R H R I I R E Y					
L-amino-acid oxidase (<i>N. scutatus</i>)	GMA G L S A A Y V L A G A G H K V T L L E A S E R V G G R V N T Y R N E T E G W Y V N M G P M R L P E R H R I I R E Y					
L-amino-acid oxidase (<i>P. australis</i>)	GMA G L S A A Y V L A G A G H N V T L L E A S E R V G G R V N T Y R N E T E G W Y V N L G P M R L P E R H R I I R E Y					
L-amino-acid oxidase (<i>B. fasciatus</i>)	GMA G L S A A Y V L A G A G H Q V T L L E A S E R V G G R V N T Y R N E K D G W Y V N L G P M R L P E R H R I I R E Y					
L-amino-acid oxidase (<i>C. rhodostoma</i>)	GMA G L S A A Y V L A G A G H R V T L L E A S D R V G G R V N T Y R D E K E G W Y V N M G P M R L P E R H R I V R T Y					
LAO-elapitoxin-Hs1	121	131	141	151	161	171
LAO-elapitoxin-Hs1 (proteoform)	IRK F G L E L N E F L Q E N E N A W Y F I K N I R K R V W E V K K D P G V F K Y P L N P S E E G K S A S Q L Y R E S L					
L-amino-acid oxidase (<i>N. scutatus</i>)	IRK F G L E L N E F L Q E N E N A W Y F I K N I R K R V W E V K K D P G V F K Y P L N P S E E G K S A S Q L Y R E S L					
L-amino-acid oxidase (<i>P. australis</i>)	IRK F G L K L N E F L Q E N E N A W Y F I R N I R K R V W E V K K D P G V F K Y P V E P S E E G K S A S Q L Y R E S L					
L-amino-acid oxidase (<i>B. fasciatus</i>)	IRK F G L E L N E F I Q E N D N A W Y F I K N I R K R V S E V K K D P G V F K Y P V K P S E E G K S A S Q L Y R E S L					
L-amino-acid oxidase (<i>C. rhodostoma</i>)	IRK F G L K L N E F F Q E N E N A W Y F I R N I R K R V W E V K K D P G V F K Y P V K P S E E G K S A S Q L Y R E S L					
LAO-elapitoxin-Hs1	181	191	201	211	221	231
LAO-elapitoxin-Hs1 (proteoform)	KKV I E E L K R T S C S Y I L N K Y D T Y S T K E Y L I K E G N L S R G A V D M I G K L L N E D S S Y Y L S F I E S L					
L-amino-acid oxidase (<i>N. scutatus</i>)	KKV I E E L K R T S C S Y I L N K Y D T Y S T K E Y L I K E G N L S R G A V D M I G K L L N E D S S Y Y L S F I E S L					
L-amino-acid oxidase (<i>P. australis</i>)	EKV I E E L K R T N C S Y I L N K Y D T Y S T K E Y L I K E G N L S R G A V D M I G K L P N E D S S Y Y L S F I E S L					
L-amino-acid oxidase (<i>B. fasciatus</i>)	KKV I E E L K R T N C S Y I L N K Y D T Y S T K E Y L I K E G N L S P G A V D M I G D L L N E D S S Y Y L S F I E S L					
L-amino-acid oxidase (<i>C. rhodostoma</i>)	KKV I E E L K R T N C S Y I L D K Y D T Y S T K E Y L I K E G N L S R G A V D M I G D L L N E D S S Y Y L S F I E S L					
LAO-elapitoxin-Hs1	241	251	261	271	281	291
LAO-elapitoxin-Hs1 (proteoform)	KSD D I F S Y E K R F D E I V G G F D Q L P I S M Y Q A I A E M V H L N A Q V I K I Q H N A E E V R V A Y Q T P A K T					
L-amino-acid oxidase (<i>N. scutatus</i>)	KSD D I F S Y E K R F D E I V G G F D Q L P I S M Y Q A I A E M V H L N A Q V I K I Q H N A E E V R V A Y Q T P A K T					
L-amino-acid oxidase (<i>P. australis</i>)	KSD D I F S Y E K R F D E I V G G F D Q L P R S M Y Q A I A E M V H L N A Q V I K I Q H N A E E V R V A Y Q T P A K T					
L-amino-acid oxidase (<i>B. fasciatus</i>)	KND D I F S Y E K R F D E I S D G F D Q L P K S M H Q A I A E M V H L N A Q V I K I Q R D A E K V R V A Y Q T P A K T					
L-amino-acid oxidase (<i>C. rhodostoma</i>)	KHD D I F A Y E K R F D E I V D G M D K L P T A M Y R D I Q D K V H F N A Q V I K I Q N D Q K V T V Y E T L S K E					
LAO-elapitoxin-Hs1	301	311	321	331	341	351
LAO-elapitoxin-Hs1 (proteoform)	LSY V T A D Y V I V C S T S R A T R R I Y F E P P L P P K K A H A L R S I H Y R S G T K I F L T C T R K F W E A D G I					
L-amino-acid oxidase (<i>N. scutatus</i>)	LSY V T A D Y V I V C S T S R A T R R I Y F E P P L P P K K A H A L R S I H Y R S A T K I F L T C T K F W E D D G I					
L-amino-acid oxidase (<i>P. australis</i>)	LSY V T A D Y V I V C S T S R A A R R I Y F E P P L P P K K A H A L R S I H Y R S G T K I F L T C T R K F W E A D G I					
L-amino-acid oxidase (<i>B. fasciatus</i>)	LSY V I A D Y V I V C S T S R A A R R I H F E P P L P P K K A H A L R S I H Y R S T K I F L T C S Q K F W E A D G I					
L-amino-acid oxidase (<i>C. rhodostoma</i>)	LSY V T A D Y V I V C A T S R A V R R I S F E P P L P P K K A H A L R S I H Y K S A T K I F L T C T R K F W E A D G I					
LAO-elapitoxin-Hs1	361	371	381	391	401	411
LAO-elapitoxin-Hs1 (proteoform)	TPS V T A D Y V I V C T T S R A V R L I K F N P P L L P K K A H A L R S V H Y R S G T K I F L T C T T K F W E D D G I					
L-amino-acid oxidase (<i>N. scutatus</i>)	HGG K S T T D L P S R F I Y Y P N H N F T S G I G V I V A Y T L A D D A D F F Q A L D I K T S A D I V I N D L S L I H					
L-amino-acid oxidase (<i>P. australis</i>)	HGG K S T T D L P S R F I Y Y P N H N F T S G I G V I V A Y T L A D D A D F F Q A L D I K T S A D I V I N D L S L I H					
L-amino-acid oxidase (<i>B. fasciatus</i>)	HGG K S T T D L P S R F I Y Y P N H N F T S G I G V I V A Y T L A D D T D F F Q A L D I E T S A D I V I N D L S L I H					
L-amino-acid oxidase (<i>C. rhodostoma</i>)	HGG K S T T D L P S R F I Y Y P N H N F T S G V G V I V A Y V L A D D S D F F Q A L D I K T S A D I V I N D L S L I H					
LAO-elapitoxin-Hs1	421	431	441	451	461	471
LAO-elapitoxin-Hs1 (proteoform)	QLP K E E I Q A L C Y P S M I Q K W S L D K Y A M G A I T S F T P Y Q F Q D F I E T V A A P V G R I Y F A G E Y T A S					
L-amino-acid oxidase (<i>N. scutatus</i>)	QLP K E E I Q A L C Y P S M I Q K W S L D K Y A M G A I T S F T P Y Q F Q D F I E T V A A P V G R I Y F A G E Y T A S					
L-amino-acid oxidase (<i>P. australis</i>)	QLP K E E I Q A L C Y P S M I K K W S L D K Y A M G A I T S F T P Y Q F Q D F I E T V A A P V G R I Y F A G E Y T A R					
L-amino-acid oxidase (<i>B. fasciatus</i>)	QLP K E Q I Q A L C Y P S K I Q K W S L D E Y A M G A I T S F T P Y Q F Q D F E I V A A P V G R I Y F A G E Y T A S					
L-amino-acid oxidase (<i>C. rhodostoma</i>)	QLP K N E I Q A L C Y P S L I K K W S L D K Y T M G A L T S F T P Y Q F Q D Y I E T V A A P V G R I Y F A G E Y T A T					
LAO-elapitoxin-Hs1	481	491	501	511		
LAO-elapitoxin-Hs1 (proteoform)	VHGW L D N T I K S G L T A A R D V N R A S Q K P S R I Q L I S D N Q L					
L-amino-acid oxidase (<i>N. scutatus</i>)	VHGW L D N T I K S G L T A A R D V N R A S Q K P S R I Q L I S D N Q L					
L-amino-acid oxidase (<i>P. australis</i>)	VHGW L D S T I K S G L T A A R D V N R A S Q K P S R R Q L S N D N E L					
L-amino-acid oxidase (<i>B. fasciatus</i>)	VHGW L D S T I K S G L T A A R D V N L A S Q K P S R I Q L S N D N E L					
L-amino-acid oxidase (<i>C. rhodostoma</i>)	VHGW L D S T I K S G L T A A R N V N R A S Q K P S R I H L I N D N Q L					
	AHGW I D S T I K S G L R A A R D V N L A S E N P S G I H L S N D N E L					

Figure S1. Deduced amino acid sequences for a new LAO toxin (LAO-elapitoxin-Hs1; row 1) and a proteoform of this differing by four residues (yellow shaded; row 2) found in *H. stephensii* venom compared to the previously identified L-amino-acid-oxidases in *N. scutatus* venom (Q4JHE2; row 3), *P. australis* venom (Q4JHE1; row 4), *B. fasciatus* (A8QL52; row 5) and *Calloselasma rhodostoma* (P81382; row 6). The signal sequence is shaded orange, conserved cysteine residues are shaded in blue and shaded grey areas indicate the same residue compared to LAO-elapitoxin-Hs1.

	1	11	21	31	41	51
Hopsarin	MAPQ L L L C L I L T F L W S V P E A E S N V F L K S K V A N R F L Q R T K R S N S L F E E I R P G N I E R E C I E E					
Shannon_62829	MAHQ L L L C L I L T F L W S V P E A E S N V F L K S K V A N R F L Q R T K R S N S L F E E I R P G N I E R E C I E E					
Shannon_117774						
Shannon_195406	MAHQ L L L C L I L T F L W S V P E A E S N V F L K S K V A N R F L Q R T K R S N S L F E E I R P G N I E R E C I E E					
Shannon_80160	MAHQ L L L C L I L T F L W S V P E A E S N V F L K S K V A N R F L Q R T K R S N S L F E E I R P G N I E R E C I E E					
Shannon_25096	MAHQ L L L C L I L T F L W S V P E A E S N V F L K S K V A N R F L Q R T K R S N S L F E E I R P G N I E R E C I E E					
Shannon_44872						
Shannon_147785						
	61	71	81	91	101	111
Hopsarin	K C S K E E A R E V F E D N E K T E T F W N V Y V D G D Q C S S N P C H Y H G T C K D G I G S Y T C T C L P N Y E G K N					
Shannon_62829	K C S K E E A R E A F E D N E K T E T F W N V Y V D G D Q C S S N P C H Y H G T C K D G I G S Y T C T C L P N Y E G K N					
Shannon_117774						
Shannon_195406	K C S K E E A R E A F E D N E K T E T F W N V Y V D G D Q C S S N P C H Y H G T C K D G I G S Y T C T C L P N Y E G K N					
Shannon_80160	K C S K E E A R E A F E D N E K T E T F W N V Y V D G D Q C S S N P C H Y H G T C K D G I G S Y T C T C L P N Y E G K N					
Shannon_25096	K C S K E E A R E A F E D N E K T E T F W N V Y V D G D Q C S S N P C H Y H G T C K D G I G S Y T C T C L P N Y E G K N					
Shannon_44872						
Shannon_147785						
	121	131	141	151	161	171
Hopsarin	C E K V L F K S C R A F N G N C W H F C K R V Q S E T Q C S C A E S Y R L G V D G H S C V A E G D F S C G R N I K A R N					
Shannon_62829	C E K V L F K S C R A F N G N C W H F C K R V Q S E T Q C S C A E S Y R L G V D G H S C V A E G D F S C G R N I K A R N					
Shannon_117774						
Shannon_195406	C E K V L F K S C R A F N G N C W H F C K R V Q S E T Q C S C A E S Y R L G V D G H S C V A E G D F S C G R N I K A R N					
Shannon_80160	C E K V L F K S C R A F N G N C W H F C K R V Q S E T Q C S C A E S Y R L G V D G H S C V A E G D F S C G R N I K A R N					
Shannon_25096	C E K V L F K S C R A F N G N C W H F C K R V Q S E T Q C S C A E S Y R L G V D G H S C V A E G D F S C G R N I K A R N					
Shannon_44872						
Shannon_147785						
	181	191	201	211	221	231
Hopsarin	K R E A S L P D F V Q S Q K A T L L K K S D N P S P D I R I V N G M D S K L G E C P W Q A V L I N E K G E V F C G G T I					
Shannon_62829	K R E A S L P D F V Q S Q K A T L L K K S D N P S P D I R I V N G M D S K L G E C P W Q A V L I N E K G E V F C G G T I					
Shannon_117774						
Shannon_195406	K R E A S L P D F V Q S Q K A T L L K K S D N P S P D I R I V N G M D S K L G E C P W Q A V L I N E K G E V F C G G T I					
Shannon_80160	K R E A S L P D F V Q S Q K A T L L K K S D N P S P D I R I V N G M D S K L G E C P W Q A V L I N E K G E V F C G G T I					
Shannon_25096	K R E A S L P D F V Q S Q K A T L L K K S D N P S P D I R I V N G M D S K L G E C P W Q A V L I N E K G E V F C G G T I					
Shannon_44872						
Shannon_147785						
	241	251	261	271	281	291
Hopsarin	L S P I H V L T A A H C I N Q T K S V S V I V G E I D I S R K E T R R L L S V D K I Y V H T K F V P P N Y Y Y G H Q N F					
Shannon_62829	L S P I H V L T A A H C I N Q T K S V S V I V G E I D I S R K E T R R L L S V D K I Y V H T K F V P P N Y Y Y G H Q N F					
Shannon_117774						
Shannon_195406	L S P I H V L T A A H C I N Q T K S V S V I V G E I D I S R K E T R R L L S V D K I Y V H T K F V P P N Y Y Y G H Q N F					
Shannon_80160	L S P I H V L T A A R S E E R R V G K E C R S R W S					
Shannon_25096	L S P I H V L T A A H C I N Q T K S V S V I V G E I D I S R K E T R R L L S V D K I Y V R S E E R R V G K E C R S R W S					
Shannon_44872						
Shannon_147785	L S P I H V L T A A H C I N Q T K S V S V I V G E I D I S R K E T R R L L S V D K I Y V H T K F V P P N Y Y Y G H Q N F					
	301	311	321	331	341	351
Hopsarin	D R V A Y D Y D I A I I R M K T P I Q F S E N V V P A C L P T A D F A N E V L M K Q D S G I V S G F G R I R F K E P T S					
Shannon_62829	D R V A Y D Y D I A I I R M K T P I Q F S E N V V P A C L P T A D F A N E V L M K Q D S G I V S G F G R I R F K E P T S					
Shannon_117774						
Shannon_195406	D R V A Y D Y D I A I I R M K T P I Q F S E N V V P A C L P T A D F A N E V L M K Q D S G I V S G F G R I R F K E P T S					
Shannon_80160	D R V A Y D Y D I A I I R M K T P I Q F S E N V V P A C L P T A D F A N E V Q I G R A S C R E R V					
Shannon_25096						
Shannon_44872						
Shannon_147785						
	361	371	381	391	401	411
Hopsarin	N T L K V I T V P Y V D R H T C M L S S D F R I T Q N M F C A G Y D T L P Q D A C E G D S G G P H I T A Y G D T H F I T					
Shannon_62829	N T L K V I T V P Y V D R H T C M L S S D F R I T Q N M F C A G Y D T L P Q D A C E G D S G G P H I T A Y G D T H F I T					
Shannon_117774						
Shannon_195406	N T L K V I T V P Y V D R H T C M L S S D F R I T Q N M F C A G Y D T L P Q D A C E G D S G G P H I T A Y G D T H F I T					
Shannon_80160						
Shannon_25096						
Shannon_44872						
Shannon_147785						
	421	431	441	451		
Hopsarin	G I V S W G E G C A R K G K Y G V Y T K V S R F I P W I K K I M S L K					
Shannon_62829	G I V S W G E G C A R K G K Y G V Y T K V S R F I P W I K K I M S L K					
Shannon_117774						
Shannon_195406						
Shannon_80160						
Shannon_25096						
Shannon_44872						
Shannon_147785						
Hopsarin	G I V S W G E G C A R K G Q I G R A S C R E R V					
Shannon_62829	G I V S W G E G C A R K G K Y G V Y T K V S R F I P W I K K I M S L K					
Shannon_117774						
Shannon_195406						
Shannon_80160						
Shannon_25096						
Shannon_44872						
Shannon_147785						

Figure S2. Seven deduced protein sequences from the transcriptome after matching with fragments from MS/MS, which were then matched using a BLAST search in UniProt to the previously identified hopsarin (P83370). The domains are shaded as follows: signal peptide (red), propeptide (yellow), light chain (blue), activation peptide (grey) and heavy chain (green), with non-shaded areas indicating different residues, importantly residues 3 and 70 that differ to hopsarin. Regions of the deduced sequence where MS/MS protein fragments matched are in bold blue (light chain) and bold green (heavy chain).

Tree

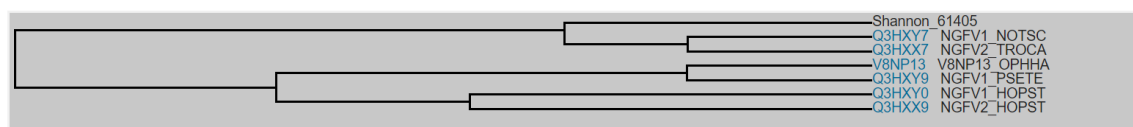


Figure S3. Tree diagram of the seven sequences for NGFs from Figure 10 using ALIGN in UniProt. This indicates that the new NGF-elapitoxin-Hs1 (transcript id: Shannon_61405) we identified is most similar to two previously identified NGFs in the closely related species, *N. scutatus* (NGFV1_NOTSC) and *T. carinatus* (NGFV2_TROCA). The previously identified NGFs in *H. stephensii* venom (NGFV1_HOPST and NGFV2_HOPST), have sequences closer to *P. textilis* (NGFV1_PSETE) and *O. hannah* (V8NP13_OPHHA), species in a different clade and a different subfamily, respectively. .

The crude venom of *H. stephensii* (pooled from two individuals), was first subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis (Figure S4). The electrophoretic venom profile was compared with an Asian elapid – the monocled cobra *Naja kaouthia*, and two other species of Australian elapids – Southern death adder *Acanthophis antarcticus* and the Papuan black snake *Pseudechis papuanus* (Figure S4). A remarkable feature of the venom profile of *H. stephensii* was the unusually high abundance of high molecular weight toxins in the mass range of 60 to 90kDa. This was calculated by densitometry to be 45% of the whole venom (Table S2).

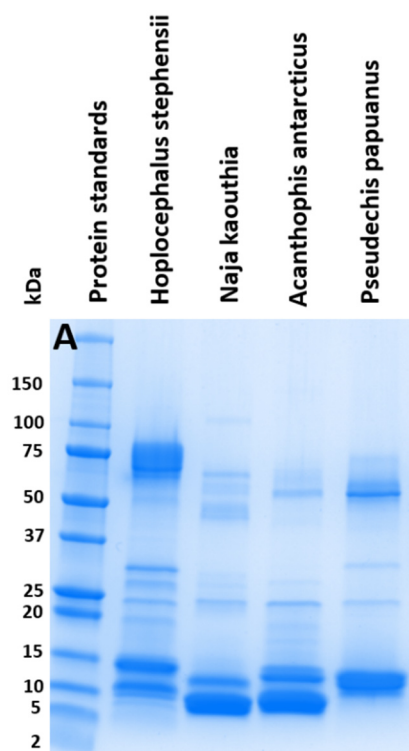


Figure S4. SDS-PAGE analysis of four elapid snake venoms shows the unusually high proportion of high molecular weight toxins (60 to 90kDa), in the venom of *Hoplocephalus stephensii* (pooled from two individuals), compared to elapid species with “typical” elapid 3FTx or PLA₂ (5 to 15kDa) dominant venoms; Monocled Cobra *Naja kaouthia*, Southern death adder *Acanthophis antarcticus*, and Papuan black snake *Pseudechis papuanus*.

Table S2. Densitometry of the whole venom of *Hoplocephalus stephensii* (taken from Figure S3), showing the high molecular weight toxins SVMP and LAAO make up 45% of the whole venom when fractionated electrophoretically.

Densitometry of WV Pooled 2 Individuals				
Lane	Band No.	Band %		
1	1	44.62928	45%	high molecular weight
1	2	3.842845		
1	3	2.24096		
1	4	2.058681		
1	5	2.122584		
1	6	22.29095		
1	7	18.2846		
1	8	4.530104		