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The venom gland transcriptome of the Desert Massasauga Rattlesnake (Sistrurus catenatus edwardsii): towards an understanding of venom composition among advanced snakes (Superfamily Colubroidea)

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Abstract

Background: n e eno s re co p e . res of ph r co og. c y c . e pro e. ns nd pep . des h. ch e ong o s n er of s perf . . es, o c og. ng of he eno r nscr. p o e f c. . es he . den . f. c . on of ne f . . es of o . ns s e s he ps . n nders nd. ng he e o . on of eno pro eo es,

Results: e h e cons r c ed cDNA . r ry of he eno g nd of hre ened r esn e p. . per ***Sistrurus catenatus edwardsii*** Deser M ss s g nd seq enced E s, O r res s de ons r e h. gh nd nce of ser. ne pro e. n se nd e opro e. n se r nscr. p s . nd. c . ng h he d. sr p . on of he os s. s . s pr. nc. p e ech n. s of c . on of he eno , n dd. . on o he r nscr. p s encod. ng co on eno pro e. ns e de ec ed o r. e . es of o nd nce n. q e r nscr. p s . n he . r ry. hese encode for hree f. nger o . ns nd no e o . n poss. y gener ed fro he f s. on of o genes, e so o ser ed po y deny ed r. oso RNAs . n he eno g nd . r ry n . n eres . ng pre . . n ry o se . on of h. s n s pheno enon . n rep . . n sys e ,

Conclusion: he hree f. nger o . ns re ch r c er. s . c of os e p. d eno s re r re . n . per. d eno s, e de ec ed se er E s encod. ng h. s gro p of o . ns . n h. s s dy, e so o ser ed he presence of r nscr. p encod. ng f sed pro e. n R f sons of r

o ser ed . n h. s nd o her s d. es . nd. c es gre er co pos. . on s. . r. y of eno s

ho gh po ency . d. ffer ong d nced sn es h n h s een pre . o s y

recogn. zed、

Background

he d nced sn es s perf . y Co ro. de cons. s of onophy e . c gro p of fo r f . . es A r c sp. d. d e Co r. d e." E p. d e nd . per. d e , hese sn es h e e o ed . oche . c e pon o . ns r her h n ech n. c e ns of h nd . ng prey, Phy ogene . c s d. es sho h he eno g nd here o . ns re prod ced e o ed once he se of he Co ro. de o . . on ye rs go nd h s ndergone e ens. e e o on ry . n er. ng" of de . ery sys e s nd co pos. . ons of eno , Phy ogene . c recons r c . on e een o . n genes nd sn e f . . es sho ed h he recr en of o . n f . . es . n o he eno g nd h s occ rred . p e . es y o h s e.g., e opro e. n ses CR P n. z ype ser. ne pro e se . nh. . ors N F nd . ndependen e.g., PLA n r. re . c pep . des recr en e en s , Appro . e y f . . es of o . ns h e een c og ed . n sn e eno pro eo es nd se er f . . es ppe r o e spec. f. c o p r . c r f . y of eno o s sn es Add. . on d f. e , r fo o . ns re fo nd on y . n eno s of A r c sp. d. d e., ser. ne pro e. n ses re ed o ood co g . on f c ors X co r eno f c or pr. ns nd A pro . ne . c. n f . y pep . des ppe r o e . . ed o he E p. d e., nd sc r endo he . gro h f c or E F d. s. n egr. ns g er. ns d. pep . dy pep . d se nd cro . ne occ r pr. r. y . n eno s of he . per. d e Add. . on d f. e , he occ rrence re . e nd nce nd ph r co og. c po ency of r. o s e ers of hese o . n f . . es . n eno e en eno . on re r y co p e , En eno . on y e p. d sn es . s s y ch r c er. zed y r p. d ne ro o . c co p . c . ons d e o presence of rge o n s of pos syn p . c ne ro o . ns h. e en eno . on y . per. d sn es e o es co p e he orrh g. c hypo ens. e nd . nf ory effec s c sed y he c . ons of n ero s ser. ne pro e. n ses e opro e. n ses nd C ype ec . ns CLP , Effec s of

en eno .on y sn es .n he gen s ***Atractaspis*** c n .nc de socons r.c .on res .ng

.n c rd. c rres 、 Desp. e o er s. . r. y .n c .n.c sy p o s e h. . ed f er

, Popu ons of ***S. catenatus*** gener y re hre ened or dec n ng r nge de pr r y s res of h oss nd h n encro ch en nd herefore end ngered spec es s s h s een reco ended , n sys e c s dy o ycross nd M c essy sho ed h ong Co or do Ar zon nd Ne Me co popu ons of ***S. c. edwardsii*** z rds re he or prey fo o ed y s s nd cen pedes, n he presen or he eno g nd h s een co ec ed fro sn es or g n ng fro he Co or do popu on,

ener sy p o s of en eno on resu ng fro ny Nor h A er c n p pers e re p n oc ssu e effec s progress e ede ery he nd necros s h co g op hy hypof r nogene nd pro ong on of pro hro n e nd hro ocy open s sys e c effec s, o e er here s no spec f c repor o d e n he er re concern ng en eno on y ***S. c. edwardsii***, Prof ng of o n e press on of h s hre ened sn e spec es g e g o e for he e press on of genres of o ns nc d ng r on n cod ng noncod ng seq ences nd he r e o on ry rends, he res s of h s s dy so he p n he nders nd ng of en eno on processes of r esn e es h ch n rn e por n for ore effec e c n c re en nd n eno n ge en n c ses of sn e e,

Results and discussion

A o of o of E s prod ced re d e seq ences, he s zes of seq ences sho ed d s r on e een nd se p rs h n er ge of se p rs d no sho n, A o of c s s for o he pr od c e e c , s e ,

Homo sapiens ce s nd . .s proposed o h e q . y con ro ro e .n rRNA degr d .on . h.s .s pre . .n ry repor sho .ng he poss. . . y of po y deny .on of r. oso RNA .n rep . . n sys e . On c oser e .n .on e fo nd p . e po y deny .on s.gn AA AAA Add. .on d f. e seq ence s. ses ps re of he po y A . .

Identification of toxin families

Serine proteinase: he ser. ne pro e.n ses .n he eno g nd . r ry of ***S. c. edwardsii*** re e pressed . h he h.ghes r nscr.p nd nce o E s F.g re nd e ong o c s ers. M . p e c ones ppe red .n c s ers h. e ere s.ng e ons Add. .on d f. e . One represen . e E fro e ch c s er s co p e e y seq enced DQ DQ DQ . One of he c s ers DQ con .ns on y , R E s . h.s c s er sho s s. . r. y . h he , R of ser. ne pro e.n se fro ***Bothrops jararaca*** eno g nd .

Mos sn e eno ser. ne pro e.n ses Ps o d e re s.ng e po ypep .de ch .ns e cep for o f. r.no y .c enzy es fro he eno of ore n .per ***Agkistrodon blomhoffi brevicaudus*** re .n se A nd s on se AF . n o h c ses f rds rdno e c s.ng e ch n n prec rsor .s os . e y c e e d o d . he p . . s e

.n.nogen nd p e e recep ors ، o e Ps e h. . ore h n one c . . y، For e p e .n dd. .on o he.r hro .n . e c. . y o hro .n cro se nd LM▲L .nd▁ce p e e ggreg .on .n.n re e se nd gyr ory c . . .es respec . e y ، e cons r▁c ed ne.gh or o.n.ng N phy ogene .c ree . h ne y .den .f.ed P .sofor s fro ***S. c. edwardsii,*** o ss.gn p▁ . e f▁nc .ons nd o e .ne rends .n he e o▁ .on of ne .sofor s F.g▁re ، ▲he phy ogene .c ree sho ed sc ered d.s r. ▁ .on of r.o▁s .sofor s . h d.fferen ph r co og.c c . . .es fro se er spec.es of p. .pers، ▲h.s p ern .nd.c es h Ps d. erged f er sn e .ne ges spec. ed، M ny Ps re co on y cons.dered s hro .n . e enzy es ▲LEs ec▁se hey . .c he f. r.nogeno y .c f▁nc .on of hro .n pro o .ng ood co g▁ .on، ▲herefore .n os c ses on y f. r.nogeno y .c f▁nc .on of Ps .s es ed nd he P .s c egor.zed s ▲LE، o e er so e hro .n . e enzy es .n dd. .on o re e s.ng f. r.nopep .de A nd or B fro f. r.nogen so c . e pro e.n C co p e en ❴ nd p e e s ❵ ، ▲herefore . o▁d e .n eres .ng o de er .ne he spec.f.c ph r co og.c proper .es of r.o▁s P .sofor s . h.n e ch gro▁p nd p hese on he.r e o▁ .on ry re .onsh.ps،

P genes e ong o ▁ .gene f . y nd he pro e.n cod.ng reg.ons h e een sho n o e e per.enc.ng cce er ed e o▁ .on . h.n he eno g nds of p. .pers ، ▁ch cce er ed e o▁ .on co▁d e d o he ch nges .n s▁rf ce oops s▁rro▁nd.ng he s▁ s r e .nd.ng s. e res▁ .ng .n he r. .on of s▁ s r e recogn. .on nd hence he f▁nc .on of he pro e.n، ▲he r .o e een nonsynony o▁s nd synony o▁s s▁ s . ▁ .on d_N d_S of he pro e.n cod.ng seq▁ences of ser.ne pro e.n se .sofor s of h.s spec.es s fo▁nd o e ، .nd.c .ng rend o rd cce er ed e o▁ .on nd herefore d. ergence .n ph r co og.c f▁nc .on d▁r.ng en eno .on،

Metalloproteinase and Disintegrin: A o of E s f n o c s ers nd s ng e ons for h s f y of pro e ns r nscr p nd nce F g re Add on d f e One represen e E fro e ch c s er s seq enced DQ DQ n e eno e opro e n se MP prec rsors re c ss f ed n o fo r gro ps ccord ng o s ze nd do n co pos on P e opro e n se do n on y P e opro e n se nd d s n egr n do ns P e opro e n se d s n egr n nd cys e ne r ch do ns nd P P ype do ns n ed o ec n e do n y d s f de onds None of he c s ers encode P ype MPs

he P sofor fro ***S. c. edwardsii*** DQ ches den y he

ono er.c d.s.n egr.ns r o r.n nd erge .n.n ere ch r c er.zed pre .o s y fro
he eno of ***S. miliarius barbouri*** nd ***S. c. tergeminus*** respec . e y

he .n .n egr.n recep or .nd.ng o .f of d.s.n egr.ns R D .s fo nd o e he .p of
f e . e h .rp.n oop r. .on of .no c.d res.d es .n h.s o .f R M D
MLD M D or R on he f e . e oop confers spec.f.c. y o rds spec.f.c
recep ors e.g. rep ce en of R . h .n R D o .f of r o r.n nd ss r.s .n
s.gn.f.c n y .ncre ses he se ec . . y for α β f. r.nogen recep or . ho ffec .ng . s
.nd.ng o $\alpha_5\beta_1$ f. ronec .n recep or or $\alpha\beta_3$. ronec .n recep or
Add. .on y he res.d es . ed. e y d cen o he R D oop so .nf ence o h
se ec . . y nd ff.n. y for .n egr.n recep ors For e p e d.s.n egr.ns . h
R D nd R DNP h e se ec . e y h.gher ff.n. y for α β nd α β respec . e y
he R DNP con .n.ng d.s.n egr.ns re fo d ore po en h n R D
con .n.ng d.s.n egr.ns .n oc .ng he dhes.on of ce s ed. ed y α β he p e
d.s.n egr.n fro ***S. c. edwardsii*** h s R DNP co p red o R D nd D .n
erge .n.n nd r o r.n respec . e y herefore f r her s d.es of he phys.o og.c
re e nce of r. .on .n recep or se ec . . y ong d.s.n egr.ns fro r s nog e y s f n ee

edwardsii eno ho gh sn e eno PLA .s one of he os r p.d y e o .ng enzy e f . .es n os spec.es se er .sofor s of PLA re o ser ed .n cDNA . r r.es nd eno s nd hese h e cq .red d. erse phys.o og.c f nc .ons h.s o ser .on .s so s ppor ed y pro eo .c n ys.s of ***S. c. edwardsii*** eno h. e eno s fro .nd. .d s of o her spec.es of ***Sistrurus*** con .n .p e PLA .sofor s

Phosphodiesterase eq ence of p r . s.ng e on E r nscr.p nd nce Add. .on d f. e F.g re DQ sho s .den . y o he C er .n reg.on of he phosphod.es er se gene fro ch. p nzee XP h.s .s he f.rs cDNA seq ence for phosphod.es er se fro sn e eno Phosphod.es er se c . . y h s een o ser ed .n eno s of E p.d e .per.d e nd Co r.d e sn es ho e er he ro e of h.s enzy e .n en eno .on .s no ye c e r eno phosphod.es er ses hydro yze phosphod.es er nd pyrophosph e onds .n n c eo .des nd n c e.c c.ds nd re e se d.phosph es onophosph es nd p r.nes Free p r.nes re so presen .n sn e eno s nd hey y con r. e o en eno .on seq e e for de . s see

L-amino acid oxidase:

C-type lectin: n o r . r ry CLP cco n for ppro . e y , nd nce nd h e one c s er DQ nd o s.ng e ons DQ nd DQ Add. .on d f. e F.g re , On BLA P se rch hey ch . h he β s n. of sh.g.n Q Y , .den . y C B P .! .den . y nd he A ch .n of F c or X F c or X .nd.ng pro e.n X X p ! A. .den . y respec . e y, M sh.g.n C B nd X X p re he erod. er.c. ho e er .n o r . r ry e d.d no f.nd ny ch o E s encod.ng he correspond.ng co p e en ry s n. s, herefore . y e .n eres .ng o e .ne he CLP re ed pro e.ns .n h.s eno nd de er .ne he.r .o og.c proper .es,

Growth factors: , e o .ned one c s er r nscr.p nd nce encod.ng sc r endo he . gro h f c or E F Add. .on d f. e F.g re , eq enc.ng of c ones fro h.s c s er sho ed here re o .sofor s DQ nd DQ . h on y o .no c.d res.d e n c eo .de d.fferences pos. .ons Q CA E A nd AA E A , , e so seq enced s.ng e on DQ encod.ng ner e gro h f c or N F , Ano her s.ng e on DQ ched . h he C er .n s of connec . e .ss e gro h f c or re ed pro e.n C F , h.s .s he f.rs repor of C F re ed pro e.n .n eno cDNA . r ry, s or.g.n .n he eno g nd .ns e d of o her s rro nd.ng .ss es needs o e er.f.ed,

Cysteine-rich secretory protein: , e o .ned one c s er r nscr.p nd nce Add. .on d f. e F.g re for CR P DQ ! h.ch ches . h C r.n AAO .den . y fro ***C. atrox*** eno , CR Ps re .de y d.s r. ed .n s rep . es ph. . ns r hropods ne odes cone sn . s nd p n s nd hey e h. . d. erse .o og.c f nc .ons , hey re s.ng e ch .n M of - ! D

d.s f.de r.dge .s .n oop F.g re 、A .sofor s h e he po en. ***N*** g ycosy .on o .f N X. F.g re 、

E s ere ho gh o e fo nd on y .n e p.d hydroph..d eno s ho gh he or.g.n of recr en o he e p.d hydroph.d eno pro eo e .s no c e r 、A po ypep .de o .n D h.ch crossre c s .h α ng ro o .n nd .nds .h h.gh ff.n. y o n.co .n.c ce y cho .ne recep or d of M .n co pe . .on .h α ng ro o .n s .so ed fro he eno of ***A. halys*** p. .per 、o e er no seq ence .nfor .on of h.s pro e.n .s . e、Recen y hree c ones DY DY nd DY ere o .ned fro cDNA . r ry of ***L. muta*** eno g nd h.ch po en. y encode po ypep .des s. . r c E fo d pro e.ns 、o e er on y one c one DY h s he s r nd s op codons co p e e ORF . he o her o do no、 hese seq ences do no h e ny ho o ogy e. her he n c eo .de or pro e.n e e s o hose o .ned fro ***S. c. edwardsii*** h.s s dy、

Phy ogene .c n ys.s of E Xs fro hree f . .es of sn es E p.d e Co r.d e nd .per.d e s ch.e ed s.ng PA P 、 、rees o .ned s.ng Ne.gh or o.n.ng oo s r pp.ng or P rs. ony An ys.s s r.c consens s ree .s no sho n ere so e h d.fferen or opo og.c fe res ere re .ned F.g re 、One r nscr.p fro ***S. c. edwardsii*** DQ does no c s er .h he o her fo r f s .h.n sep r e c de con .n.ng ***Naja*** nd ***Bungarus*** o h e p.ds E s、Fo r o her r nscr.p s of ***S. c. edwardsii*** for onophy e .c c de .h.n n e c s. e y e p.d c de、n eres .ng y o h e hods p ce ***L. muta*** .per.d c ones DY nd DY nd ***Coelognathus radiatus*** co r.d E s s o o her E s

s gges ng co on or g n fo o ed y d ers f c on of s ong d nced

pro e.n .s r.ch .n Cys res.d es s. . r o ny o her sn e eno o .ns, s N er .n do .n ches .h n. z BP o .ns .den .y nd he .dd e do .n ches .h pr.ns .den .y nd he no e r nscr.p h s n e ended C er .n s F.g re , Bo h n. z BP nd pr.ns re fo nd sep r e y s s.ng e do .n pro e.ns .n sn e eno s, o of he Cys res.d es h.ch for one of he fo r d.s f.de onds .n pr.ns re .ss.ng .n he ne r nscr.p F.g re , R PCR s.ng fresh RNA o her h n sed o e cDNA . r ry s e p e nd seq enc.ng e per. en s sho he presence of h.s f sed r nscr.p .n he eno g nd nd hence . .s no n r.f c d e o e p e s .ch.ng y he Re erse r nscr.p se sed for .ng he cDNA . r ry , A ho gh n er of cDNA seq ences of n. z BP fro sn e eno s h e een co p e ed none of he h e he pr.n do .n nd he C er .n e ens.on, C rren y cDNA seq ences of pr.ns re no no n, o e er h.s .s he f.rs e per. en e .dence for he presence of pr.n do .n ho gh f sed .h no her o .n .n .per.d eno ,

he onger ORF h .ng n. z BP nd pr.n do .ns oge her co d e d e o he f s.on of o .nd. .d genes encod.ng n. z BP nd pr.n, ene f s.on ed. ed y e on sh ff .ng .n ron ed. ed reco .n .on or re ro r nspos. .on h s een es .shed s n essen . gene .c ech n.s for he or.g.n of ne genes .n .n er e r es er e r es nd p n s , Recen y ne gene .c process r nscr.p .on .nd ced ch. er.s C .n c ses of nde y oc ed gene p .rs h s een sho n o e respons. e for gene f s.on .n he h n geno e prod c.ng ch. er.c

no e o .n . h o d.s .nc do .ns nd h .ng ne .o og.c f.nc .on、 h s een o ser ed h ne genes of en g. e r.se o ne .o og.c f.nc .ons dr. en y d p . e D r .n. n se ec .on 、 he ech n.s of f.s.on of hese pp ren y .ndependen genes he e o .on ry r ec ory of h.s f.sed gene nd he po en . ne o .c f.nc .on of he ch. er.c pro e.n re re s for f. re .n es .g .on、

Iron-binding protein Fo r E s Add. .on d f. e F.g re d E CE YPO r nscr.p nd nce 、 sho ed ho o ogy . h n .ron .nd.ng pro e.n . h po en . s.gn pep .de、 A ho gh os .ron .nd.ng pro e.ns re gener y c egor.zed s s or ge pro e.n so e of he s ch s o o r nsferr.n nd c oferr.n h e n . .cro . c . . .es 、 .s no c e r he her or no h.s pro e.n .s fo nd .n he eno 、 o e er o pr.n e er of he pr.n pro e.n f . y nd he C er .n reg.on of yo o .c PLA ere o h sho n o h e n . .cro . c . . y 、

Identification of cellular transcripts

、 e o .ned c s ers r nscr.p nd nce seq ences h.ch re .n o ed .n r.o s ce r f.nc .ons .nc d.ng r nscr.p .on nd r ns .on secre .on pos r ns .on od.f.c .on gener e o .s nd o her f.nc .ons Add. .on d f. e F.g re 、 . . r ho se eep.ng pro e.n prod c s h e een o ser ed .n o her sn e eno g nds 、 One of he E s CE ches c c. nd .n egr.n .nd.ng pro e.n h.ch ss.s s p e e spre d.ng 、 A ho gh od .on of p e e nd .n egr.n f.nc .ons .s ey c . . y of se er sn e eno co ponen s e do no e .e e h h.s pro e.n .s presen .n eno s . c s he s.gn pep .de、

recr[illegible] en e en s y e d o n .ncre se .n he spec r[illegible] of no n nd[illegible]n no n o .n f . .es decre s.ng he co pos. .on spec.f.c. .es ong eno o[illegible]s sn es, o e er d.fferen . con r. [illegible] .on of spec.f.c o .ns o he o er e pressed pro eo e of eno o[illegible]s sn es does e d o s.gn.f.c n d.fferences .n eno co pos. .on e een spec.es,

A cen r he e .n he e o[illegible] .on of eno sys e s .s co p e e d[illegible]p .c .on of o .n genes fo o ed y cce er ed e o[illegible] .on h.ch f ors nonsynony o[illegible]s .no c.d s[illegible] s . [illegible] .on o rds neof[illegible]nc .on .z .on, Mod.f.c .on of se ec ed s[illegible]rf ce re s of o .ns .s respons. e for prod[illegible]c.ng he f[illegible]nc .on d. ers. y .n n. .n er e r es sn . s nd scorp.ons. er e r es sn es o .n [illegible] .gene f . .es , o e er one . por n o ser .on .n he presen repor .s he occ[illegible]rrence of no e o .n . e r nscr.p gener ed y f[illegible]s.on of o .nd. .d[illegible] o .n genes [illegible]n. z BP nd pr.n .n sn e eno g nd, ho[illegible]gh he ech n.s for cre .on of h.s f[illegible]sed gene needs o e s[illegible]d.ed f[illegible]r her . c e r y .nd.c es h o her gene .c processes gene sh[illegible]ff .ng or C re so oper .ng .n he eno g nd o cre e no e o .n genes, enes or.g.n .ng y o her gene .c processes s[illegible]ch s e on sh[illegible]ff .ng re recen nd herefore he dd. .on of h.s f[illegible]sed o .n . e r nscr.p o he eno pro eo e .s perh ps ne , A h.s s ge . .s e p .ng o spec[illegible] e h he or.g.n of od[illegible] r org n.z .on of d.fferen c sses of MPs h.ch ppe rs o e he res[illegible] of gene f[illegible]s.on e en s y e d[illegible]e o gene .c process o her h n gene d[illegible]p .c .on, MPs re ery [illegible]nd n o .ns nd c rry o[illegible] pr.nc.p ro e .n en eno .on y .per.d sn es nd herefore s[illegible]d.es of he.r gene .c or.g.n nd org n.z .on . e of gre .n eres , C.rc[illegible] s n . e .dence of r ns sp .c.ng for he gener .on of ser.ne pro e.n se .sofor s .n he eno g nd of ***V. lebetina*** h s een presen ed . ope n e , h e sho n h ern . e sp .c.ng nd gene d[illegible]p .c .on re .n erse y corre ed e o[illegible] .on ry ech n.s s, Accord.ng o P rr

ne o .ns nd pro .des ech n.s .c e p n .ons for he.r e o .on nd d. ers.f.c .on, An nreso ed q es .on .n o es he re .onsh.p e een he eno g nd r nscr.p o e nd ho h.s .s . e y r ns ed o he f.n pro eo e, h.s r. e pro eo .c co pos. .on .n rn de er .nes he co p e nd of en d.ff.c o reso e seq e e h.ch freq en y de e op fo o .ng en eno .on y he d.fferen spec.es of eno o s sn es,

Methods

Venom extraction and collection of venom glands

pec. ens of ***Sistrurus c. edwardsii*** Deser M ss s g ere co ec ed .n L.nco n Co n y Co or do A nder per . s gr n ed y he Co or do D. .s.on of, . d .fe o PM per . s P , eno s e r c ed fro d sn es s.ng s nd rd n e hods , eno s ere hen cen r.f ged o re o e p r .c es frozen nd yoph. .zed, Pr.or o g nd re o sn es ere e r c ed of eno , Fo r d ys er hen RNA e e s re pres ed . o sn es ere nes he .zed .h .sof or ne nd hen s cr.f.ced y dec p. .on, nds ere hen r p.d y d.ssec ed fro

.nco p e e cDNAs ere re o ed y p ss.ng he . r ry hro gh C ROMA P N co n he . r ry s p c ged s.ng .g p c go d p c g.ng e r c r gene Ced r Cree e s A nd. .d c ones ere resc ed fro r ndo y se ec ed h. e p q es nd gro n .n L r. ro h p.c. .n ed. P s .ds ere p r.f.ed s.ng he Q Aprep sp.n .n.prep . Q. gen . den er ny P r.f.ed p s .ds ere seq enced y cyc e seq enc.ng re c .ons s.ng he B.gDye er .n or . App .ed B.osys e Fos er C. y C .forn. A nd n o ed DNA seq encer Mode A App .ed B.osys e Fos er C. y C .forn. A

RT-PCR

R PCR s perfor ed .n order o se rch for .sofor s of E seq ences .n he eno g nd n r.ef o RNA s .so ed fro eno g nds s o e nd s sed s e p e he fo o .ng pr. ers ere sed for p .f.c .on for rd pr. er ; A A AAAC C C N A CC N ; N A C . re erse pr. er ; AAA A ACCA CC A AAA G ; Re erse r nscr.p .on nd s seq en p .f.c .on re c .ons ere done s.ng he one s ep R PCR pro oco of Q. gen . den er ny he p .f.ed prod c s c oned .n o pDr. e ec or Q. gen . den er ny nd r ndo c ones ere seq enced R PCR s so perfor ed o conf.r he presence of f sed o .n r nscr.p .n he eno s.ng s e proced re . h he fo o .ng pr. ers for rd pr. er ; A A C C A C C C ; . re erse pr. er ; CCA ACA AA AA C C A ;

Bioinformatic analysis

C s er.ng of he E s s perfor ed s.ng he CAP progr f er re o .ng poor q . y seq ences nd ec or seq ences s.ng ec creen fro NCB e oo ed for f. A B recogn. .on seq ences .n he E s nd n y re o ed ps re nd

do ns re seq ences of hese s. es s e s po y A . s e s A s .n ro fro he ends A .n. o er p of p nd .den . y .n he o er p reg.on ere se ec ed s cr. er. for he c s er.ng A c s ers nd s.ng e ons ere s ec ed o BLA se rches BLA N nd BLA X s req red g .ns he non red nd n d se of NCB e es c off nd h .ng good co er ge of .n. se p .rs nd . .den . y for he p . e .den .f.c .on of he genes Presence of s.gn pep .des s pred.c ed .nd. .d y y s .ss.on of he seq ences o he .gn P ser er s . e .n he E p sy e s. e ene nd pro e.n .gn en s ere done s.ng he progr s C s nd DNAMAN Lynnon Corpor .on dre Dor.on Q e ec C n d he r .o e een nonsynony o s d_N nd synony o s s s . .on d_S ere c c ed s.ng he NAP progr he progr NAP h s een de e oped sed on he e hod of h .ncorpor .on of s .s .c n ys.s de e oped y

Phylogenetic analysis

Phy ogene .c n ys.s s c rr.ed o s.ng he progr ME A ers.on s.ng Po.sson correc ed d.s nces nd rees ere cons r c ed pp y.ng oo s r ps of rep .c es PA P s so sed for Boo s r p Ne.gh or o.n.ng nd P rs. ony n yses For he B yes. n .nferences of phy ogeny sed pon he pos er.or pro . . y d.s r. .on of he rees M r o ch .n Mon e C r o e hods MrB yes s sed he n ys.s s r n for gener .ons .n fo r ch .ns nd s p ed e ery gener .ons res .ng .n s p e rees he og . e .hood score of e ch s ed ree s p o ed g .ns he n er of gener .ons o de er .ne he po.n h.ch he og . e .hood scores of he n ys.s re ched he sy p o e he

pos er.or pro . . .es for he c des ere es .shed y cons r.c .ng consens.s ree of rees gener ed f er he co p e .on of he .rn .n ph se.

e .nc.ded he fo o .ng seq.ences fro hree f . .es of sn es .n o.r n yses he ne y .den .f.ed F. seq.ences o. of h.s s.dy fro ***S. c. edwardsii*** .per.d e enB n DQ DQ DQ DQ nd DQ ***L. muta*** .per.d e enB n DY DY α co.r. o .n .ss Pro P fro ***Coelognathus radiatus*** Co.r.d e nd non con en .on F. seq.ences .ss Pro P O nd P F. e seq.ences .ss Pro Q P P P P P P P P P Q nd Z nd F seq.ences .ss Pro P P P Q Y C P Q Y P P P P nd P fro E p.d e. A hese seq.ences e ong o he shor ch .n F. f . y. A BLA P se rch .s.ng enB n DY fro ***L. muta*** eno fo.nd h r pep .de seq.ence enB n AA sho ed he h.ghes ho o ogy o Ly n .gen h.ch h s een

List of abbreviations

E e pressed seq ence g. ORF open re d.ng fr e. PLA phospho .p se A F hree f.nger o .n. CR P cys e.ne r.ch secre ory pro e.n. CLP C ype ec .ns. BPP r dy .n.n po en . .ng pep .des. CNP C ype n r. re .c pep .des. LAO L .no c.d o .d se. BP o .ne p ncre .c ryps.n .nh. . or. E F sc r endo he . gro h f c ors. N F ner e gro h f c or. P sn e eno ser.ne pro e.n ses. LE hro .n . e enzy es. CRD cys e.ne r.ch do .n. N ne.gh or o.n.ng. C r nscr.p .on .nd ced ch. er.s 、

Authors' contributions

P h s des.gned nd c rr.ed o e per. en s n ysed d de e oped he concep nd ro e he n scr.p 、 PM h s co ec ed he eno g nd s p e、RM nd PM h e ed. ed he n scr.p o . pro e . s q . y、 PM h s he ped .n he phy ogene .c n ys.s of F s seq ences、A he hors h e ppro ed he f.n for of he n scr.p 、

Acknowledgments

h.s or s s ppor ed fro he gr n s fro B.o ed.c Rese rch Co nc. Agency for c.ence nd echno ogy Rese rch .ng pore RM 、Per . s nd s ppor for co ec .on of sn es s pro .ded y he Co or do D. .s.on of . d .fe P PM 、he ss.s nce of 、 、 ey orne nd s n Moore . h so e spec s of phy ogene .c n yses s gre y pprec. ed、

References

、 .d N **Colubroid systematics: Evidence for an early appearance of the venom apparatus followed by extensive evolutionary tinkering.** ***J Toxicol Toxin Rev*** **21:** 、

、 M c essy P **Biochemistry and pharmacology of colubrid snake venoms.** ***J Toxicol-Toxin Rev*** **21:** 、

、 .d N edges B **Higher-level relationships of snakes inferred from four nuclear and mitochondrial genes.** ***C R Biol*** **325:** 、

、 Fry B s er **Assembling an arsenal: origin and evolution of the snake venom proteome inferred from phylogenetic analysis of toxin sequences.** ***Mol Biol Evol*** **21:** 、

、 odgson C .c r r n C **In vitro neuromuscular activity of snake venoms.** ***Clin Exp Pharmacol Physiol*** **29:** 、

、 Me.er oc er **Effects of snake venoms on hemostasis.** ***Crit Rev Toxicol*** **21:** 、

、 Br d Bon C sner A **Snake venom proteins acting on hemostasis.** ***Biochimie*** **82:** 、

、 M s F r Y n. **Snake venom proteases affecting hemostasis and thrombosis.** ***Biochim Biophys Acta*** **1477:** 、

sh. Ro er o P o res AM As o f. F. ho Pere. r O *et al*

Analysis of *Bo o c* venomous gland transcriptome focusing on structural and functional aspects: I--gene expression profile of highly expressed phospholipases A_2. *Biochimie* 86:

the toxins potentially involved in coagulopathy. ***Biochem Biophys Res Commun***

C.d de DA . o.A D . AM , gner de LM Lee P *et al*, ***Bo _o***

_ _ *c* venom gland transcriptome: Analysis of the gene expression pattern.

Toxicon **48:** . ***c o o c.d . . d onn o M on _ C y _***

e.ge AR

h e E hn **The mechanism of 3' cleavage and polyadenylation of eukaryotic pre-mRNA.** ***Prog Nucleic Acid Res Mol Biol*** **57:**

g ch g r g ch Y M r y N Oh F Y C rgo AC ***et al*** **Molecular cloning of serine proteinases from**

e.r AM M g h es A D.n.z CR de O . e.r EB **Purification and properties of the thrombin-like enzyme from the venom of *L c* . *. Int J Biochem* 21:**

.s.e ondo . h McM en BA . h LF **Characterization of a protein C activator from *A* . *odon con o* *con o***

pRm

Zho Q N d M Broo s PC enson D R. er MR Argo no ***et al*** **Contortrostatin, a homodimeric disintegrin, binds to integrin alphavbeta5.** ***Biochem Biophys Res Commun*** **267:**

C e e rgens M M rc.n .e .cz C Ro ero A chr der M N.e . ro s . **Disulphide-bond pattern and molecular modelling of the dimeric disintegrin EMF-10, a potent and selective integrin alpha5beta1 antagonist from** ***oco . c on.*** **venom.** ***Biochem J*** **345 Pt 3:**

C e e Moreno M rc. no MP he s on RD .s.e D M rc.n .e .cz C **Snake venom disintegrins: novel dimeric disintegrins and structural diversification by disulphide bond engineering.** ***Biochem J*** **372:**

Fo err no M **Structural considerations of the snake venom metalloproteinases, key members of the M12 reprolysin family of metalloproteinases.** ***Toxicon*** **45:**

c r oro gh RM Rose s MA Ph. .ps DR Fr.ed A C p e AM ***et al*** **Barbourin. A GPIIb-IIIa-specific integrin antagonist from the venom of** ***o . J Biol Chem*** **266:**

Osh er d **Ussuristatin 2, a novel KGD-bearing disintegrin from** ***A odon n .*** **venom.** ***J Biochem (Tokyo)*** **125:**

y c.n egr c rRGro gh RM Rose d f gr d .n nR s r f d B . oc f d A g c odon d M n n RC

Ao N g . Y d M **Characterization of regions of fibronectin besides the arginine-glycine-aspartic acid sequence required for adhesive function of the cell-binding domain using site-directed mutagenesis.** ***J Biol Chem*** **266:**

R os h . E P.ersch cher MD **New perspectives in cell adhesion: RGD and integrins.** ***Science*** **238:**

o R .n. RM op r.shn one P **A novel prothrombin activator from the venom of** ***M.c_o c*** **: isolation and characterization.** ***Arch Biochem Biophys*** **408:**

B rn son B Fo **Hemorrhagic metalloproteinases from snake venoms.** ***Pharmacol Ther*** **62:**

Cos EP C .ss PB e. e.r CF Mo r d . AM **Importance of metalloproteinases and macrophages in viper snake envenomation-induced local inflammation.** ***Inflammation*** **26:**

L .ng D Mo r d . AM **Jararhagin and its multiple effects on hemostasis.** ***Toxicon*** **45:**

ng h.h C ng F **Primary structure and antiplatelet mechanism of a snake venom metalloproteinase, acurhagin, from** ***A _odon c*** **venom.** ***Biochimie*** **87:**

metalloproteinase isolated from the venom of *Bo o c*. *Biochem Biophys Res Commun* 322:

metalloprotease from *n* venom induces human endothelial cell apoptosis. *Toxicon* 46:

Phospholipase A(2) with platelet aggregation inhibitor activity from *A* venom: protein purification and cDNA cloning. *Arch Biochem Biophys* 375:

Venom phospholipases A_2 of bamboo viper ( ): molecular characterization, geographic variations and evidence of multiple ancestries. *Biochem J* 377:

Characterization of three edema-inducing phospholipase A_2 enzymes from habu (*o d*) venom and their interaction with the alkaloid aristolochic acid. *Toxicon* 25:

Accelerated evolution and molecular surface of venom phospholipase A_2 enzymes. *J Mol Evol* 48:

Lynch **Inventing an arsenal: adaptive evolution and neofunctionalization of snake venom phospholipase A_2 genes. *BMC Evol Biol* 7:**

Og N sh No h.s Desh r M h oh.g sh. Y F .
Y *et al*, **Accelerated evolution of snake venom phospholipase A_2 isozymes for acquisition of diverse physiological functions.** ***Toxicon*** **34:**

nz L . s L M c essy P C e e **Venom proteomes of closely related . _ _ rattlesnakes with divergent diets.** ***J Proteome Res*** **5:**

Bo n e **Identity of the phosphodiesterase and deoxyribonuclease in rattle-snake venom.** ***Nature*** **178:**

M c essy P **Phosphodiesterases, DNases and RNases.** ***In*** n e eno Enzy es B . ey , Ed, , F , Co .ns CO A, A en Press nc,

R sse FE **Phosphodiesterase of some snake and arthropod venoms.** ***Toxicon*** **4:**

F rs en CR ren .n D B rre o Ch es ML r .s **Ecto-nucleotide pyrophosphatase/phosphodiesterase as part of a multiple system for nucleotide hydrolysis by platelets from rats: kinetic characterization and biochemical properties.** ***Platelets*** **17:**

A.rd D **Ophidian envenomation strategies and the role of purines.** ***Toxicon*** **40:**

n N **L-amino acid oxidases and lactate dehydrogenases.** n ***Enzymes from Snake Venoms*** B ey Ed F Co ns CO A A en nc

Y z Y Mor **Structure and function of snake venom cysteine-rich secretory proteins.** ***Toxicon*** **44:**

o M eng M N L L Q ng Q o Q **Crystal structure of the cysteine-rich secretory protein stecrisp reveals that the cysteine-rich domain has a K+ channel inhibitor-like fold.** ***J Biol Chem*** **280:**

ng hen B o M Lo X D n Y Cheng XP ***et al*** **Blocking effect and crystal structure of natrin toxin, a cysteine-rich secretory protein from *N* venom that targets the BKCa channel.**

orres AM ong Y Des M Moochh che P n. RM **Identification of a novel family of proteins in snake venoms. Purification and structural characterization of nawaprin from *N n. co* snake venom. *J Biol Chem* 278:**

O h o ch M Brody EN **Temperature-dependent template switching during in vitro cDNA synthesis by the AMV-reverse transcriptase. *Nucleic Acids Res* 20:**

Zeng XC ng X **Evidence that BmTXK beta-BmKCT cDNA from Chinese scorpion *B n c* is an artifact generated in the reverse transcription process. *FEBS Lett* 520:**

Z ph. ropo os P **Template switching generated during reverse transcription? *FEBS Lett* 527:**

Long M **A new function evolved from gene fusion. *Genome Res* 10:**

Long M Be r n E horn on ng **The origin of new genes: glimpses from the young and old. *Nat Rev Genet* 4:**

A P opor. A Ede he. Pere z Y D. er A he esh R ***et al*** **Transcription-mediated gene fusion in the human genome. *Genome Res* 16:**

、 N . P N . M **Association of CIB with GPIIb/IIIa during outside-in signaling is required for platelet spreading on fibrinogen.** ***Blood*** **102:**

、 M c essy P . erry NM ey orne Fr. s **Venom of the Brown Treesnake,** ***Bo*** **: ontogenetic shifts and taxa-specific toxicity.** ***Toxicon*** **47:** 、

、 P ng YF ong P r PP odgson C .n. RM **Ohanin, a novel protein from king cobra venom, induces hypolocomotion and hyperalgesia in mice.** ***J Biol Chem*** **280:** 、

、 Zh Lee Zh ng Y **Cloning of cDNAs encoding C-type lectins from Elapidae snakes** ***B n c.*** **and** ***B n c.nc*** **.** ***Toxicon*** **39:** 、

、 ord.s D ense F **Adaptive evolution of animal toxin multigene families.** ***Gene*** **261:** 、

、 .. g r E A spo A .. g r **Sequence diversity of** ***n*** **snake venom gland serine proteinase homologs--result of alternative-splicing or genome alteration.** ***Gene*** **263:** 、

、 ope n NM L nce D Y n . **Alternative splicing and gene duplication are inversely correlated evolutionary mechanisms.** ***Nat Genet*** **37:** 、

M c essy P B er LM **Bioweapons synthesis and storage: the venom gland of front-fanged snakes** ***Zoologischer Anzeiger*** **245:**

Ch pp P Boche Co r o s B **Electrophoretic patterns of the venoms from a litter of** ***B on c*** **snakes.** ***Toxicon*** **20:**

D ry C s er horpe R **Diet and snake venom evolution.** ***Nature*** **379:**

y n h P o d **Geographical variation in India in the composition**

or er B **HIV signature and sequence variation analysis.** n ***Computational and evolutionary analysis of HIV molecular sequences***, Dordrech he Ne her nds er Ac de c P shers.

Ne. M o o or. **Simple methods for estimating the numbers of**

FIGURE LEGENDS

Figure 1

he r nscr. p o e prof. e of he eno g nd of ***S. c. edwardsii.*** A nd nce of o .ns nd o .n . e r nscr. p c s ers nd ce r pro e. ns nd hypo he .c seq ences c s ers, Percen ge of o E s for e ch c egory re sho n,

Figure 2

Phy ogene .c N ree of Ps, eq ences co p e e ORF . e fro o her p. .pers nd .sofor s DQ DQ fro h. s s dy f. ed c. rc e ere sed, . ss e . re. n P .s sed s o gro p, he n ers on he r nches .nd. c e he oo s r p s ppor es for nodes nd he hor. zon r represen s n er of s s . . ons per s. e, LE hro .n . e enzy es. N .n. nogen se. PA p s .nogen c . or. PA p e e ggreg .on .nd cer. BCD ood c o d. spers. on. X c . . y n no n, E per. en y er. f. ed c . . .es re sh ded,

Figure 3

Phy ogene .c N ree for c ss P e opro e. n ses of .per. d eno s, D se co p e e ORF sed fro .n dd. .on o one .sofor DQ o .ned .n h. s s dy f. ed c. rc e , ADAM fro ***Danio rerio*** Q PE nd ADAM fro ***Mus musculus*** ere sed s o gro p, A .gn en of he d. s. n egr. n do .n of c ss P MPs sho .ng C C C nd C r ed .n grey h. ch re proposed o e .n o ed .n he for .on of o h ono er. c M nd d. er. c D d. s. n egr. ns .n he eno , On y re e n por .ons of he seq ences re sho n,

Figure 4

A .gn en of .no c. d seq ences of he p . e prec rsors of E s, Cys res. d es h. ch re sh ded .n grey re co on y presen .n shor ch .n E nd for d. s f. de

r. dges so . d c . nes 、 Cys res. d es sh ded . n c poss. y for he dd. . on d. s f. de r. dge do ed c . ne presen . n he non con en . on F f . y、 Po en . N g ycosy . on s. es re nder . ned、

Figure 5

Ne. gh or o. n. ng c dogr of F seq ences、 N ers preceed. ng e ch spec. es n e refer o en n ccess. on n ers nd n ers efore os nodes . nd. c e oo s r p es rep . c es 、

Figure 6

A . gn en of he no e o . n . e r nscr. p . h sn e eno n. z BP pro e. ns nd pr. ns、 ABD ***Daboia russellii russellii).*** Q , e . . n. n ***Pseudonaja textilis textilis .*** P C c. c d. n ***Dendroaspis angusticeps .*** P Dendro o . n ***Dendroaspis polylepis polylepis*** nd BP fro Bo . ne、 P O pr. n ***Oxyuranus microlepidotus).*** P N pr. n ***Naja nigricollis)***、 he presence of conser ed d. s f. de onds re . nd. c ed y so . d c . nes、 he d. s f. de ond h. ch . s . ss. ng . n he no e o . n conser ed . n pr. ns . s . nd. c ed y do ed . nes、 he e ended C er . n s of he no e o . n . s nder . ned、

-

Additional data files

Additional data file 1

ype PDF s.ze B s e sho ng he d.s r. on of sn e eno o n f es ong perf y Co ro.d e

Additional data file 2

ype PDF s.ze B s e sho ng he c s ers of E s n er of c ones n e ch c s er nd he.r p e.den y

Additional data file 3

ype PDF s.ze B s e sho ng he c s ers of E s encod.ng ce r pro e.ns

Additional data file 4

ype PDF s.ze B C s gn en e een r. oso RNA seq ence DQ fro h.s s dy nd AF sed for ono c.den f.c on of ***S. c. edwardsii*** Po y deny on s.gn seq ence s nder ned

Additional data file 5

ype PDF s.ze B C s gn en of P e opro e.n ses on y pro e.n se do n s sho n Cys e.ne res.d es h.ch re conser ed re r ed n grey nd r. e n c Access.on n ers of he sed seq ences re s fo o s AP enB n BAB enB n BAB ys se enB n LA P A enB n LA P B enB n o h. g.n ss Pro P Bery hr c se ss Pro Q Ec r.n ss Pro Q r rh g.n ss Pro Q Bo hrop s.n ss Pro O Ac rh g.n ss Pro Q Q C roco s n ss Pro Q A ro ys.n ss Pro Q e n.h g.n A ss Pro Q N e n.h g.n B ss Pro Q N R A

.ss Pro Q R R B .ss Pro P E enB n 、P

s.gn pep.de do .n. PRO pro do .n. sp cer. D N d.s.n egr.n do .n. CRD

cys e.ne r.ch do .n

Additional data file 6

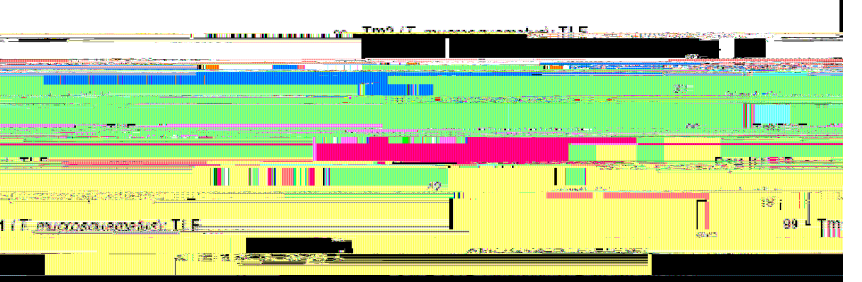
.ype PDF s.ze 、 B 、B yes. n ree gener ed fro .gned F seq.ences s

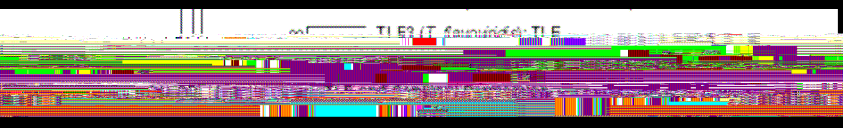
descr. ed .n M er. s nd Me hods、N. ers on r nches .nd.c e percen ge of

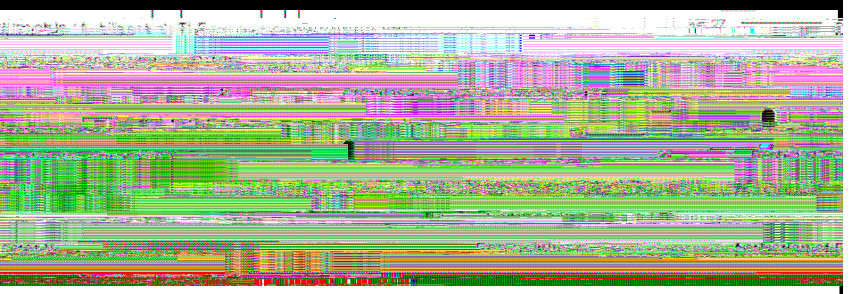
pos er.or c de pro . . y、 F seq.ences fro ***S. c. edwardsii*** nd ***L. muta*** . r r.es

re r ed .h f. ed c.rc e nd r. ng e respec . e y、

Figure 1







0.1

• F

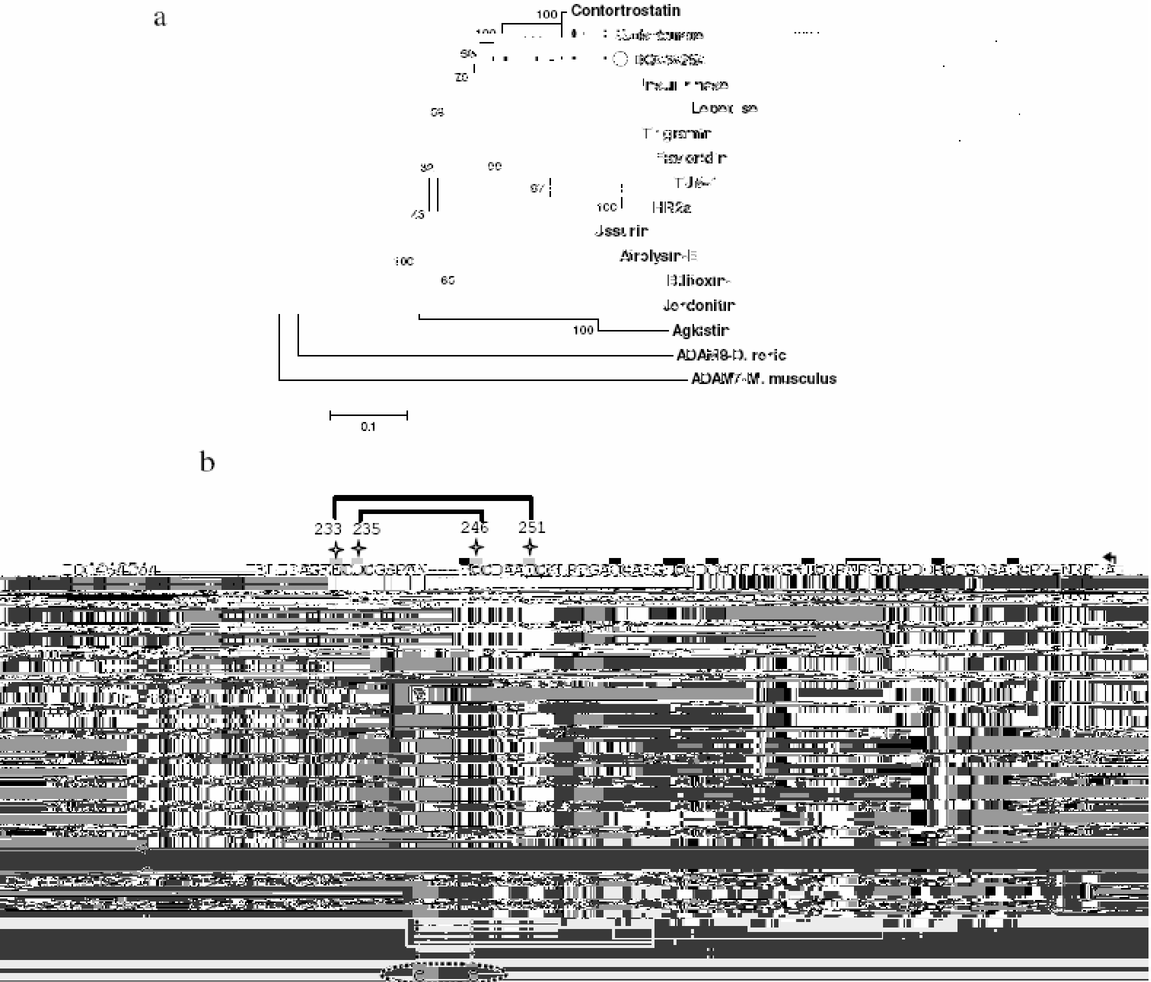

a
Contortrostatin
Agkistin
ADAM7-M. musculus
0.1
b
233
235
246
251

Figure 3

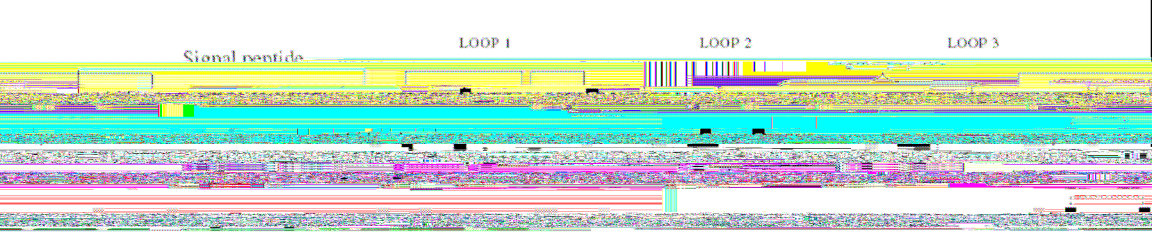


Figure 4

Figure 5

Figure 6

Additional files provided with this submission:

Additional file 1: additional data file 1.pdf, 23K