

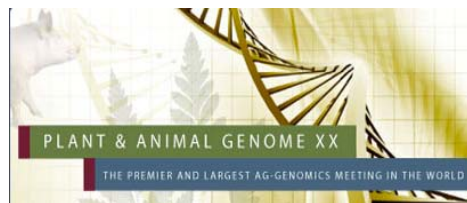


Sequencing the Snake Venom Transcriptome for its Applications in Biomedicine

Ana Conesa
Genomics of Gene Expression Lab
Centro de Investigación Príncipe Felipe
Valencia
aconesa@cipf.es



PRINCIPE FELIPE
CENTRO DE INVESTIGACION



Snake envenomation

Global evaluation of snakebites [8]

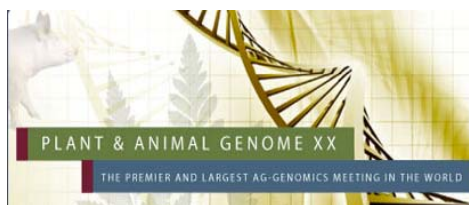
Landmasses	Population (x10 ⁶)	Total number of bites	No. of envenomations	No. of fatalities
Europe	730	25,000	8,000	30
Middle East	160	20,000	15,000	100
USA and Canada	310	45,000	6,500	15
Central and South America	400	300,000	150,000	5,000
Africa	760	1,000,000	500,000	20,000
Asia	3,500	4,000,000	2,000,000	100,000
Oceania	20*	10,000	3,000	200
Total	5,840	5,400,000	2,682,500	125,345



*Population at risk



PRINCIPE FELIPE
CENTRO DE INVESTIGACION



Snake envenomation



Symptoms snake envenomation

- Flaccid Paralysis
- Systemic myolysis
- Coagulopathy
- Cardiotoxicity
- Local tissue injury

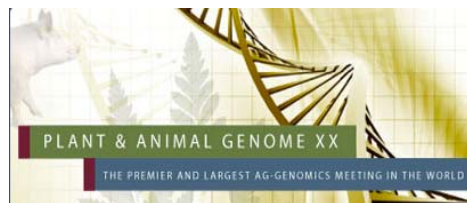


Biomedical interest of snake venom toxins

Anti-venoms development:
Venoms are protein cocktails

Biomedical research:
Molecular studies on
venom targets

Pharmaceutical:
Treatment of diseases caused
by target alterations





Familia Colubridae
Hembra de color: *Atroxia ornata*
Hembra de color: *Crotalus*



Familia Colubridae
Hembra de color: *Crotalus ornatus*
Hembra de color: *Crotalus*



Familia Colubridae
Hembra de color: *Crotalus ornatus*
Hembra de color: *Crotalus*



Familia Colubridae
Hembra de color: *Crotalus ornatus*
Hembra de color: *Crotalus*



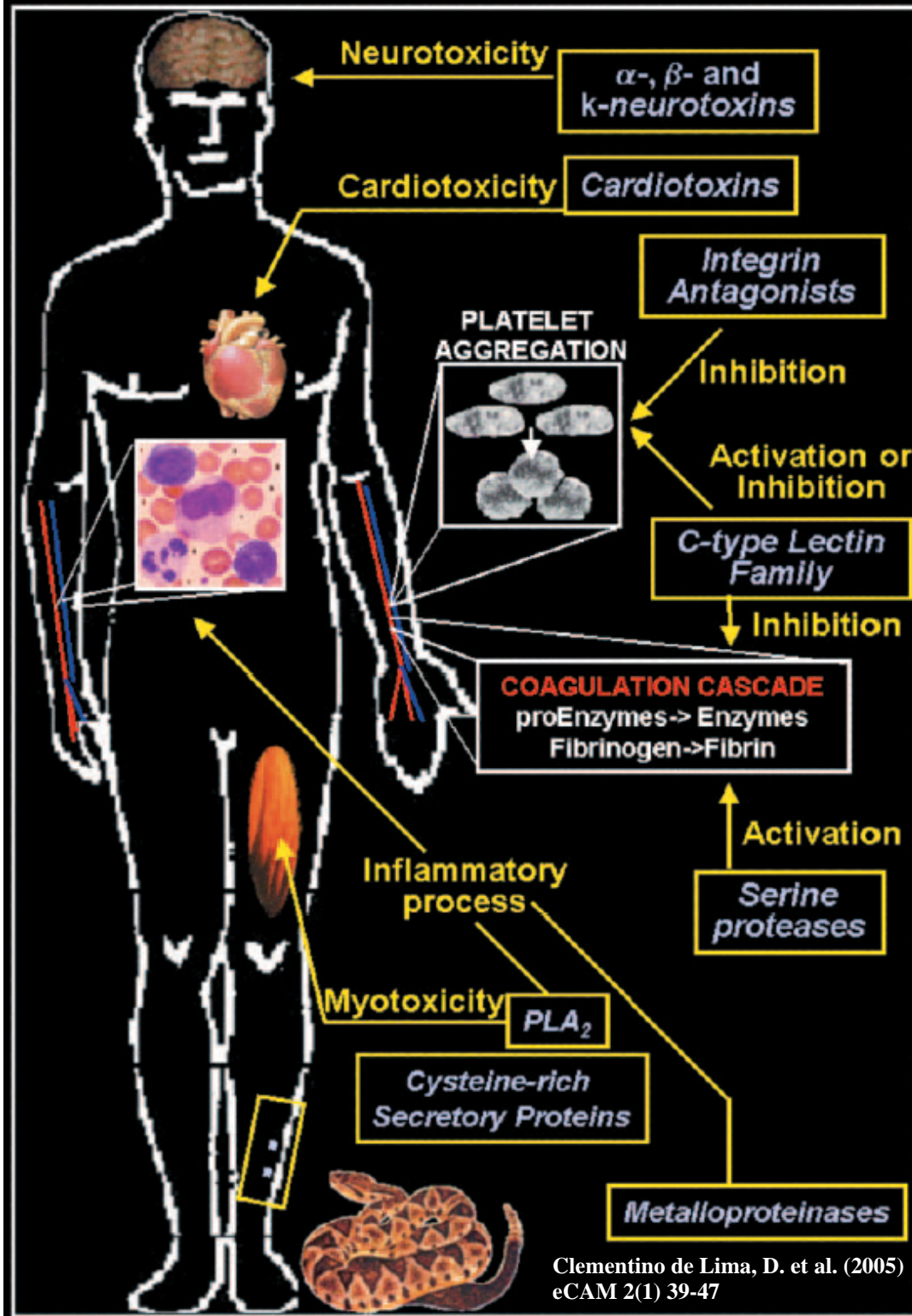
Familia Colubridae
Hembra de color: *Crotalus ornatus*
Hembra de color: *Crotalus*



Familia Colubridae
Hembra de color: *Crotalus ornatus*
Hembra de color: *Crotalus*



Familia Colubridae
Hembra de color: *Crotalus ornatus*
Hembra de color: *Crotalus*



Clementino de Lima, D. et al. (2005)
eCAM 2(1) 39-47



Familia Colubridae
Hembra de color: *Crotalus ornatus*
Hembra de color: *Crotalus*



Familia Colubridae
Hembra de color: *Crotalus ornatus*
Hembra de color: *Crotalus*



Familia Colubridae
Hembra de color: *Crotalus ornatus*
Hembra de color: *Crotalus*



Familia Colubridae
Hembra de color: *Crotalus ornatus*
Hembra de color: *Crotalus*



Familia Colubridae
Hembra de color: *Crotalus ornatus*
Hembra de color: *Crotalus*



Familia Colubridae
Hembra de color: *Crotalus ornatus*
Hembra de color: *Crotalus*



Familia Colubridae
Hembra de color: *Crotalus ornatus*
Hembra de color: *Crotalus*



Familia Colubridae
Hembra de color: *Crotalus ornatus*
Hembra de color: *Crotalus*



Familia Colubridae
Hembra de color: *Crotalus ornatus*
Hembra de color: *Crotalus*



Familia Colubridae
Hembra de color: *Crotalus ornatus*
Hembra de color: *Crotalus*

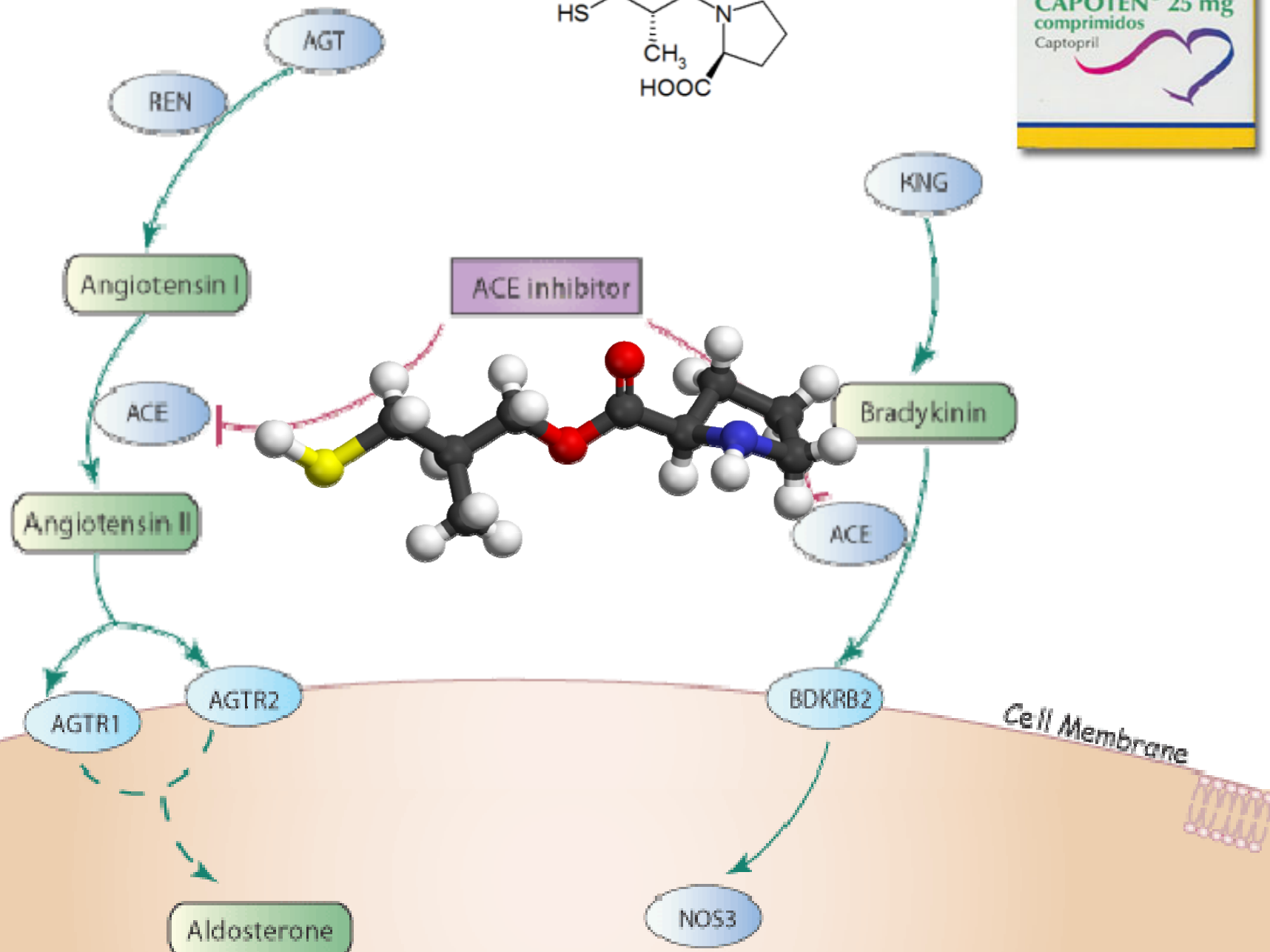
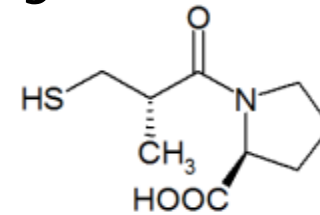


Familia Colubridae
Hembra de color: *Crotalus ornatus*
Hembra de color: *Crotalus*



Familia Colubridae
Hembra de color: *Crotalus ornatus*
Hembra de color: *Crotalus*

CAPTOPRIL (Bothrops jararaca): Angiotensin-converting enzyme inhibitor



Biomedical applications

Review

Snake venom components and their applications in biomedicine

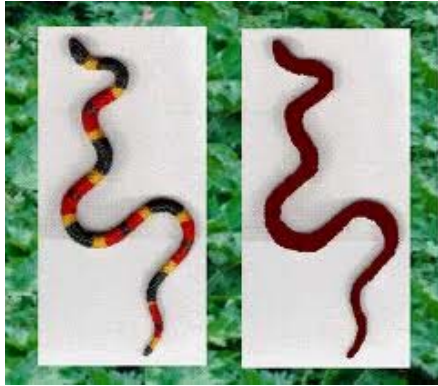
D. C. I. Koh., A. Armugam and K. Jeyaseelan*

Cell. Mol. Life Sci. 63 (2006) 3030–3041

Table 3. Drugs/clinical diagnostic kits from snake venoms.

Drug/trade name®	Target and function/treatment	Source
Captopril; enalapril	ACE inhibitor/high blood pressure	<i>Bothrops jaracusa</i> (Brazilian arrowhead viper)
Integrilin (eptifibatide)	platelet aggregation inhibitor/acute coronary syndrome	<i>Sistrurus miliaris barbouri</i> (south-eastern pigmy rattlesnake)
Aggrastat (tirofiban)	GPIIb-IIIa inhibitor/myocardial infarct, refractory ischaemia	<i>Echis carinatus</i> (African saw-scaled viper)
Ancrod (Viprinex)	Fibrinogen inhibitor/stroke	<i>Agkistrodon rhodostoma</i> (Malayan pit viper)
Defibrase	thrombin and prothrombin inhibitor/acute cerebral infarction, unspecific angina pectoris	<i>Bothrops moojeni</i>
Hemocoagulase	thrombin-like effect and thromboplastin activity/prevention and treatment of haemorrhage	<i>Bothrops atrox</i>
Protac/protein C activator	protein C activator/clinical diagnosis of haemostatic disorder	<i>Agkistrodon contortix contortix</i> (American copperhead)
Reptilase	diagnosis of blood coagulation disorder	<i>Bothrops jaraca</i> (South American lance adder)
Ecarin	prothrombin activator/diagnostic	<i>E. carinatus</i>
Exanta; ximelagatran	blood thinner/anti-coagulant, thrombin inhibitor	Cobra

Costa Rica Crotaline snakes



Bothrops asper (Pac)
Bothrops asper (Atl)
1.5-2 m, “terciopelo”



Cerrophidion godmani
viviper



Bothriechis lateralis
Bothriechis schlegelii
70-100 cm, tree snakes



Atropoides picadoi
Atropoides mexicanus
“mano de piedra”, vivipar



Crotalus simus
rattlesnake



Available information

- Extensive Proteome studies
- No genome available
- Presence in GenBank: 37000 sequences, 13000 of which are mitochondrial



Snake Venomics of the Lancehead Pitviper *Bothrops asper*: Geographic, Individual, and Ontogenetic Variations

Alberto Alape-Girón,^{†,‡} Libia Sanz,[§] José Escolano,[§] Marietta Flores-Díaz,[†] Marvin Madrigal,[†]
Mahmood Sasa,[†] and Juan J. Calvete^{*,§}

*Instituto Clodomiro Picado, Universidad de Costa Rica, San José, Costa Rica, Departamento de Bioquímica,
Escuela de Medicina, Universidad de Costa Rica, San José, Costa Rica, and Instituto de Biomedicina de
Valencia, C.S.I.C., Jaume Roig 11, 46010 Valencia, Spain*

Received April 30, 2008



Snake Venomics of Central American Pitvipers: Clues for Rationalizing the Distinct Envenomation Profiles of *Atropoides nummifer* and *Atropoides picadoi*

Yamileth Angulo,[†] José Escolano,[‡] Bruno Lomonte,[†] José María Gutiérrez,[†] Libia Sanz,[‡] and
Juan J. Calvete^{*,†}

*Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica, and
Instituto de Biomedicina de Valencia, Consejo Superior de Investigaciones Científicas (CSIC), Jaume Roig 11,
46010 Valencia, Spain*

Received September 20, 2007; Accepted October 29, 2007



Snake Venomics and Antivenomics of the Arboreal Neotropical Pitvipers *Bothriechis lateralis* and *Bothriechis schlegelii*

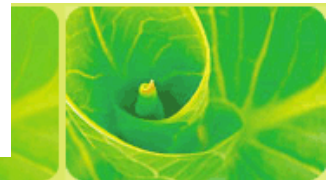
Bruno Lomonte,[†] José Escolano,[‡] Julián Fernández,[†] Libia Sanz,[‡] Yamileth Angulo,[†]
José María Gutiérrez,[†] and Juan J. Calvete^{*,†}

*Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica,
and Instituto de Biomedicina de Valencia, C.S.I.C., Jaume Roig 11, 46010 Valencia, Spain*

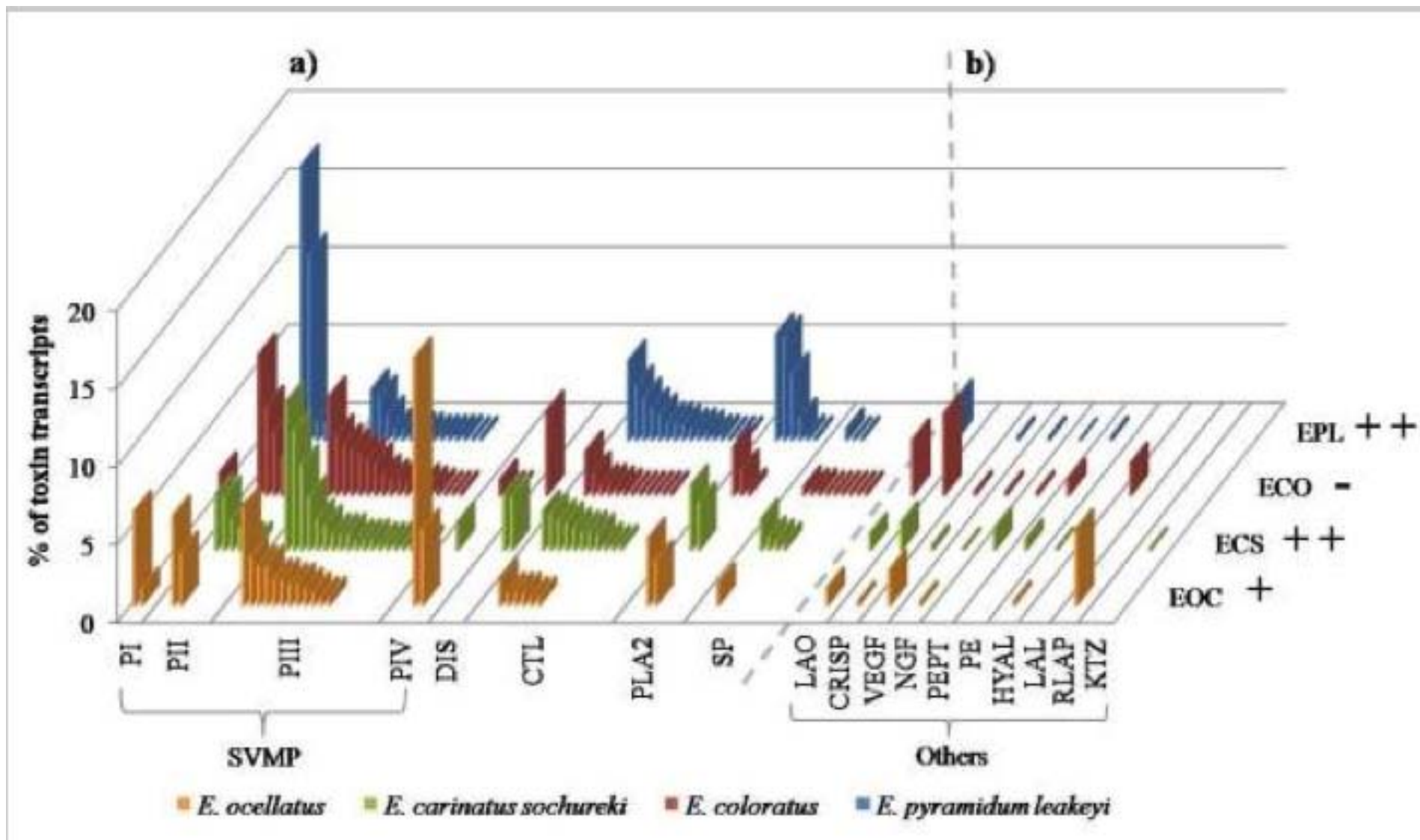
Received January 9, 2008



PRINCIPE FELIPE
CENTRO DE INVESTIGACION



Complexity venom gland composition



Casewell et al. , BMC Genomics 2009



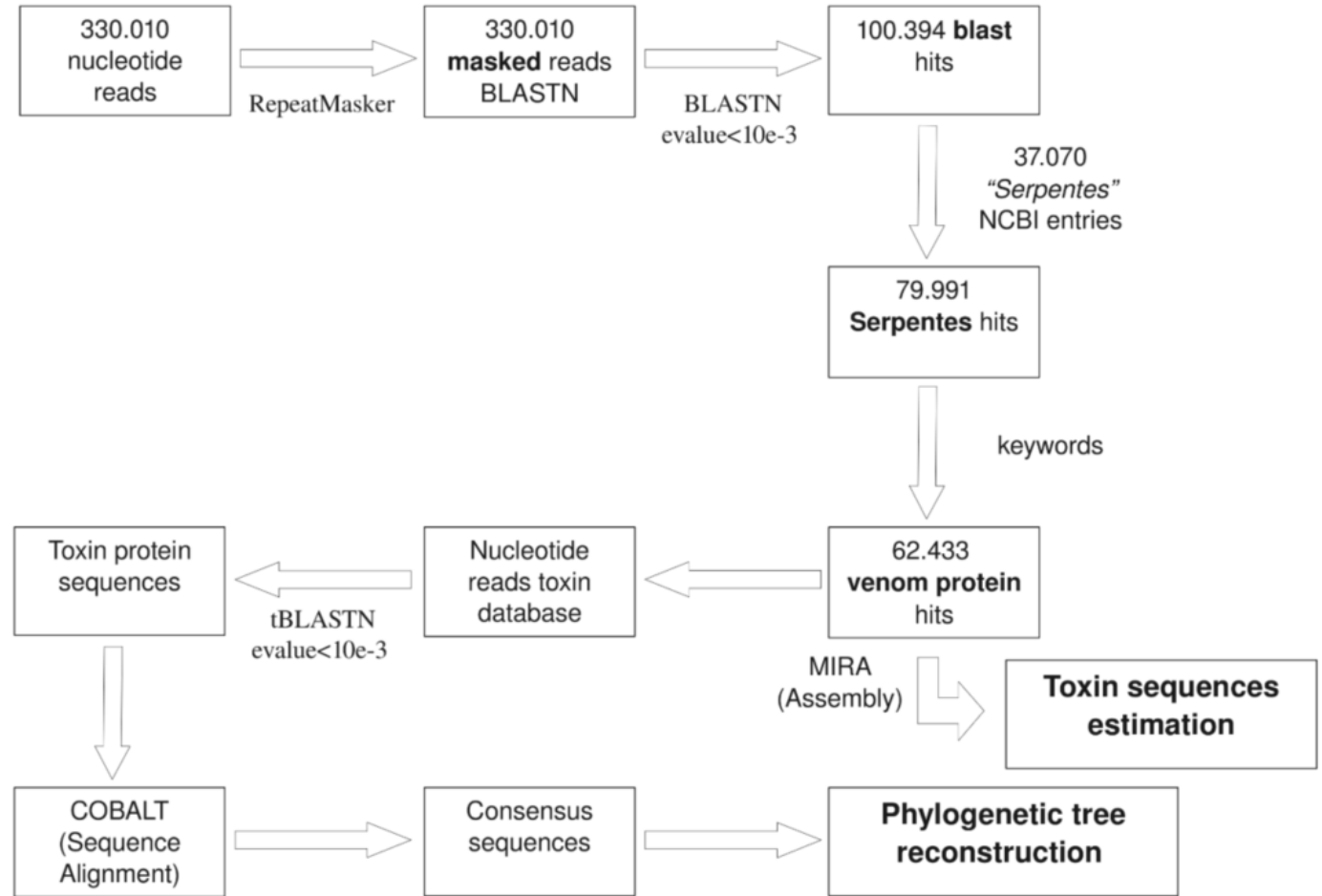
Transcriptomics analysis snake venom

Figure 1

454 Pyrosequencing

8 snake venom transcriptomes

Multiplexed libraries



454 sequencing stats

About 70% of the snake affiliated transcripts are toxins

Snake species	Total reads	Mean length (nt)	BLAST hits	(% of total hits)	<i>Serpentes</i> hits	(% of total hits)	Venom protein	(% <i>Serpentes</i> hits)
<i>C. simus</i>	22389	193.6	3608	(16.1)	2320	(64.3)	1327	(57.2)
<i>B. asper</i> (Car)	123485	185.6	43818	(35.5)	35655	(81.4)	28220	(79.1)
<i>B. asper</i> (Pac)	16076	184.8	2848	(17.7)	2078	(72.9)	1378	(66.3)
<i>C. godmani</i>	44843	182.1	13743	(30.7)	11252	(81.9)	9824	(87.3)
<i>A. picadoi</i>	31027	188.8	13295	(42.8)	11350	(85.4)	9951	(87.7)
<i>A. mexicanus</i>	27080	192.1	6418	(23.7)	4070	(63.4)	3109	(76.4)
<i>B. schlegelii</i>	33276	191.5	6826	(20.5)	4525	(66.2)	2893	(63.8)
<i>B. lateralis</i>	31833	186.1	9838	(30.9)	7970	(81.0)	5731	(71.9)
TOTAL	330010	187.3	100394	(30.4)	79991	(79.7)	62433	(78.0)



High abundance of repetitive elements

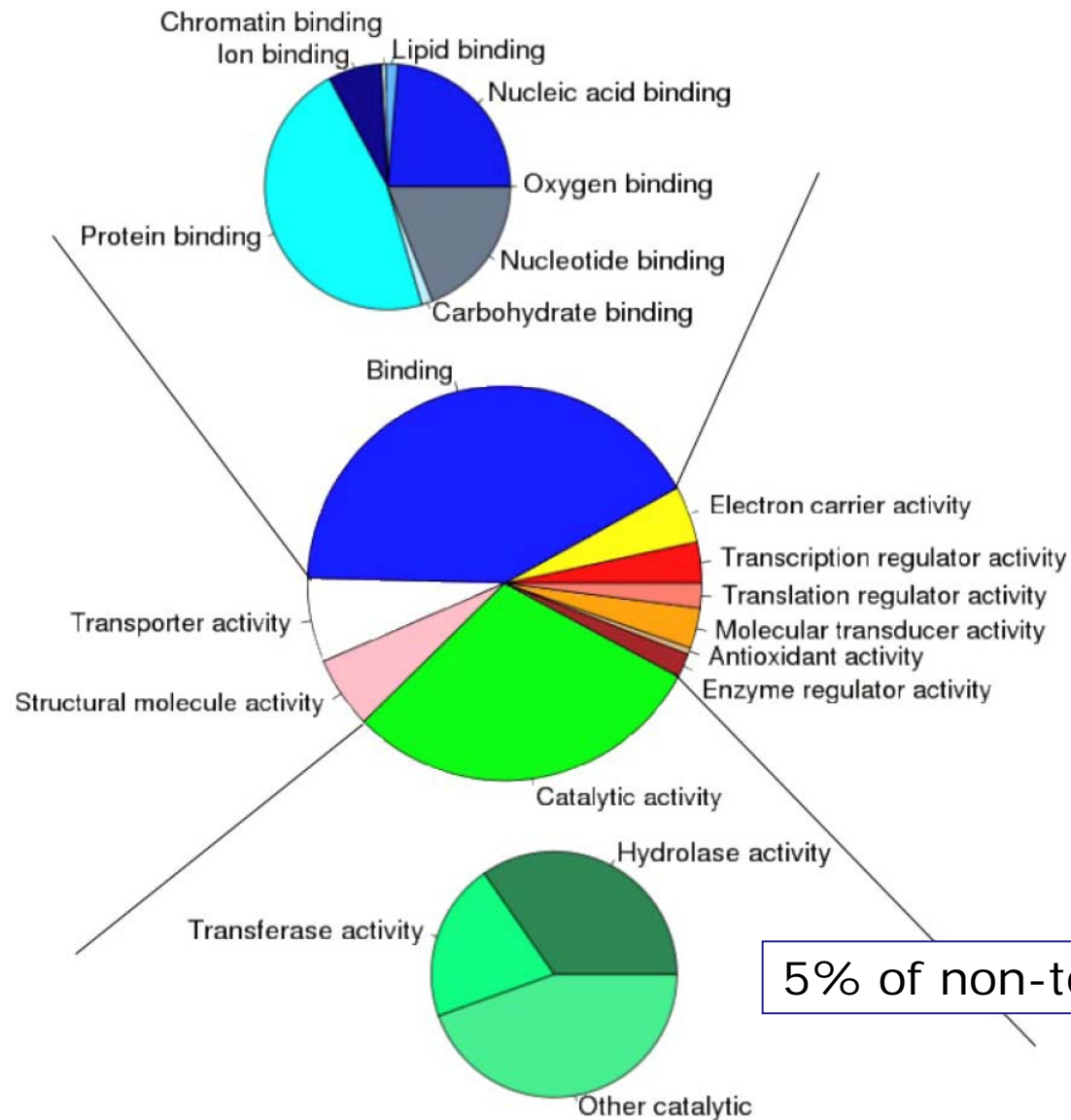
5% Repetitive Elements

	number of elements	length occupied (nucleotides)
Retroelements	28609	3312010
<u>SINEs:</u>	816	85079
Penelope	1263	137377
<u>LINES:</u>	22125	2709376
CRE/SLACS	0	0
L2/CR1/Rex	6322	761398
R1/LOA/Jockey	2	170
R2/R4/NeSL	295	34226
RTE/Bov-B	13880	1742940
L1/CIN4	363	33265
<u>LTR elements:</u>	5668	517555
BEL/Pao	0	0
Ty1/Copia	19	2164
Gypsy/DIRS1	1102	142726
Retroviral	4461	363048
DNA transposons	2749	238354
hobo-Activator	1390	96605
Tc1-IS630-Pogo	630	97585
En-Spm	59	3109
MuDR-IS905	5	350

Many retrotransposons
within toxin protein genes



Functional annotation non-toxin proteins

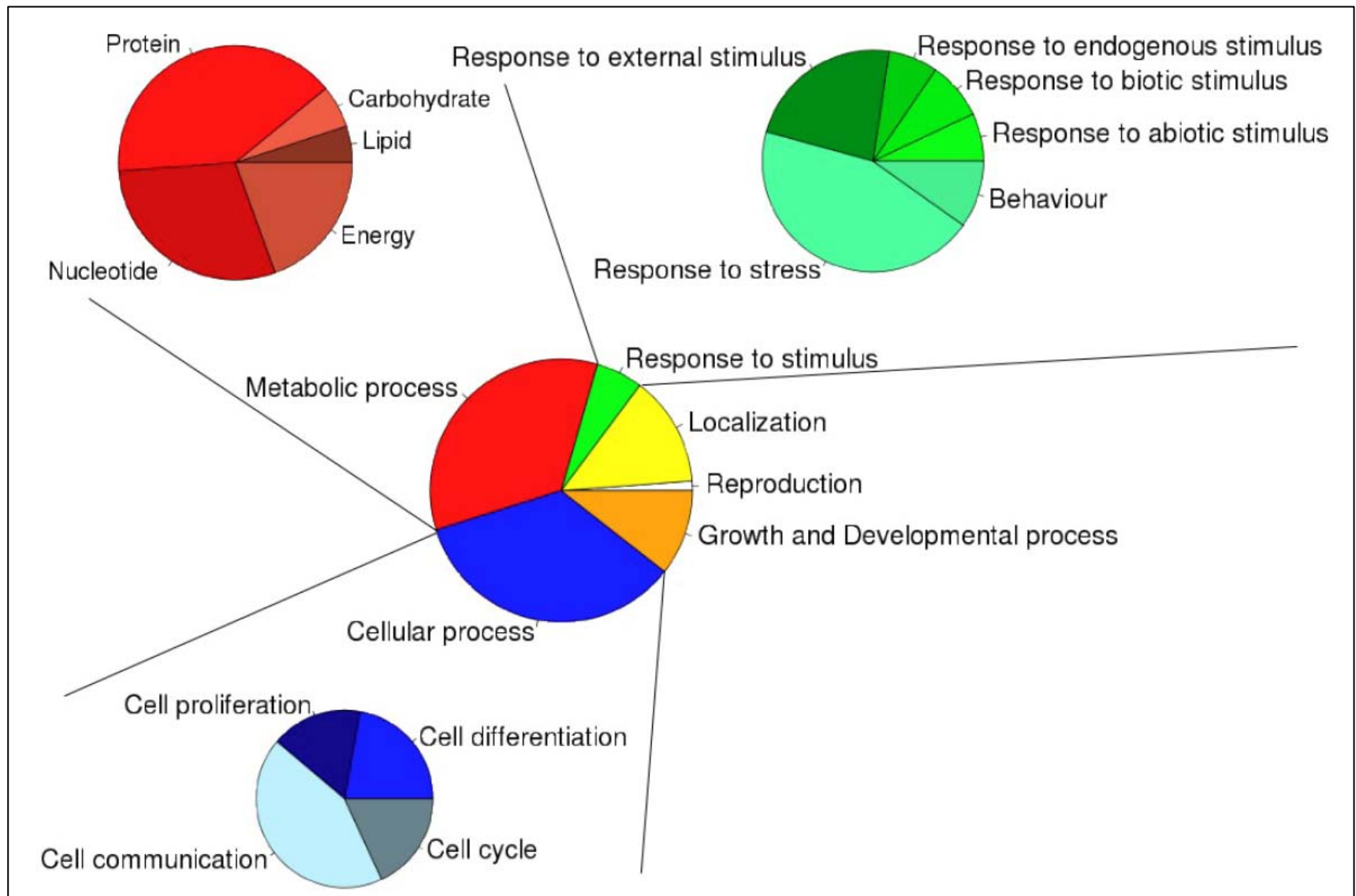


Blast2GO analysis of the Non toxin fraction

5% of non-toxin reads are PDI



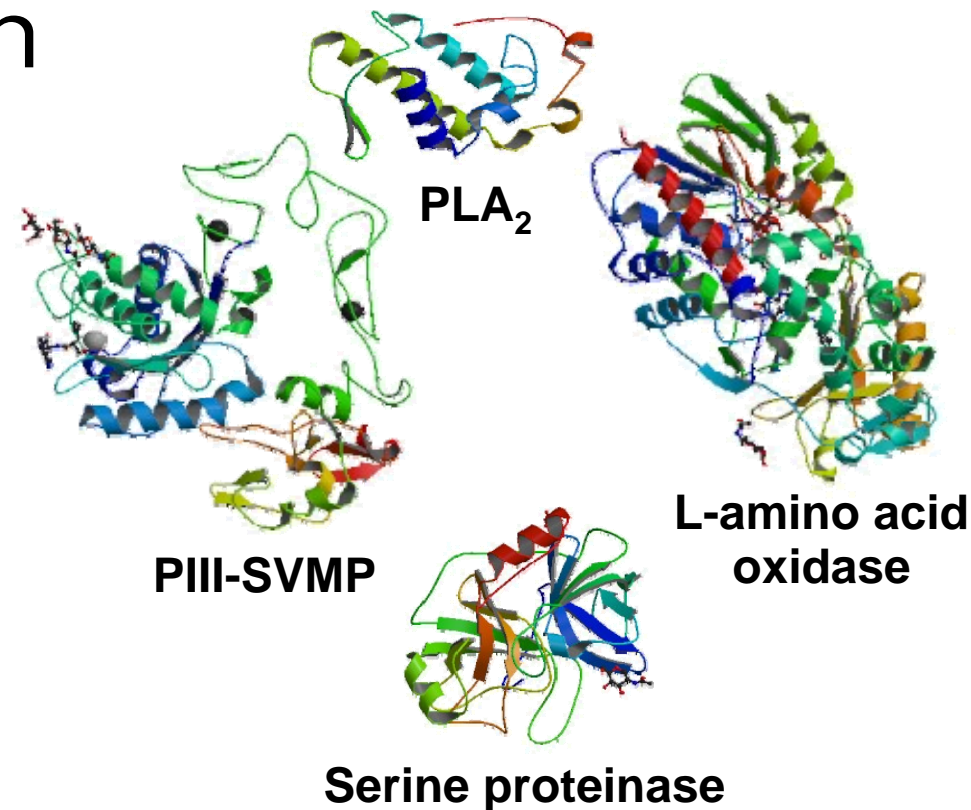
Functional annotation non-toxin proteins



Analysis Toxin fraction

	Number of reads	% of total venom protein entries
Bradykinin potentiating peptide (BPP)	9231	14.8
Cysteine-Rich Secretory Peptide (CRISP)	1066	1.7
C-type lectin-like protein (CTL)	1039	1.6
Growth factor (GF)	789	1.2
L-amino acid oxidase (LAO)	2535	4.0
Phospholipase A ₂ (PLA ₂)	7065	11.3
Metalloproteinase (SVMP)	26646	42.7
Serine Proteinase (SP)	10019	16.0

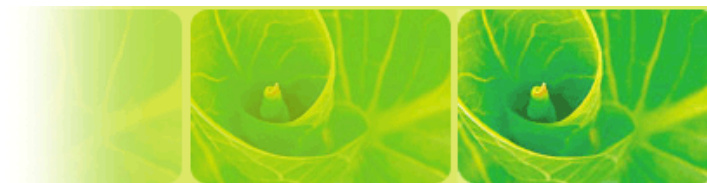
5'-nucleotidase (5'-NTase)	374	0.6
Phosphodiesterase (PDE)	119	0.2
Glutaminyl cyclase (GC)	170	0.3
Cobra Venom Factor (CVF)	8	0.01
Crotamine (CRO)	22	0.04
Sarafotoxin (SARA)	3	0.005
Waprin (WAP)	26	0.04
Kunitz-type inhibitor (KUN)	21	0.03
Kazal-type inhibitor (KAZ)	21	0.03
Hyaluronidase (HYA)	24	0.04
Ohanin (OHA)	2412	3.9
Three-Finger Toxin (3FTx)	845	1.3



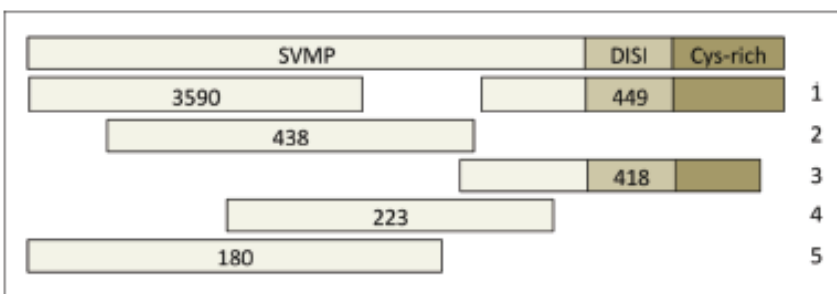
Low expression proteins, never identified in the venom of these snakes.

Most reads are at 3' UTR regions

Pseudogenes? Residual genes of venom speciation??



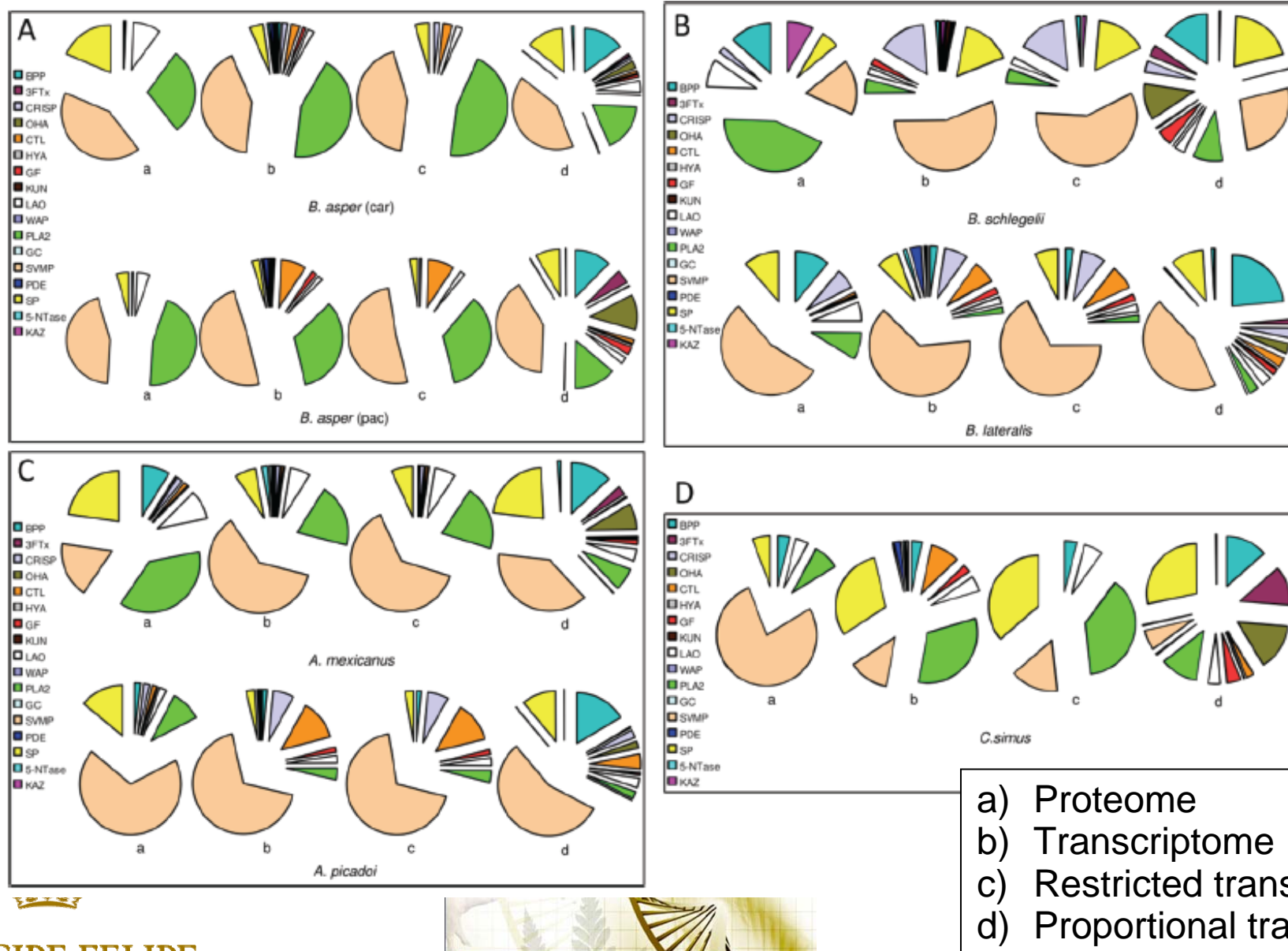
Estimation number of toxin genes



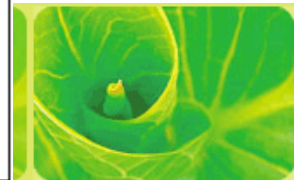
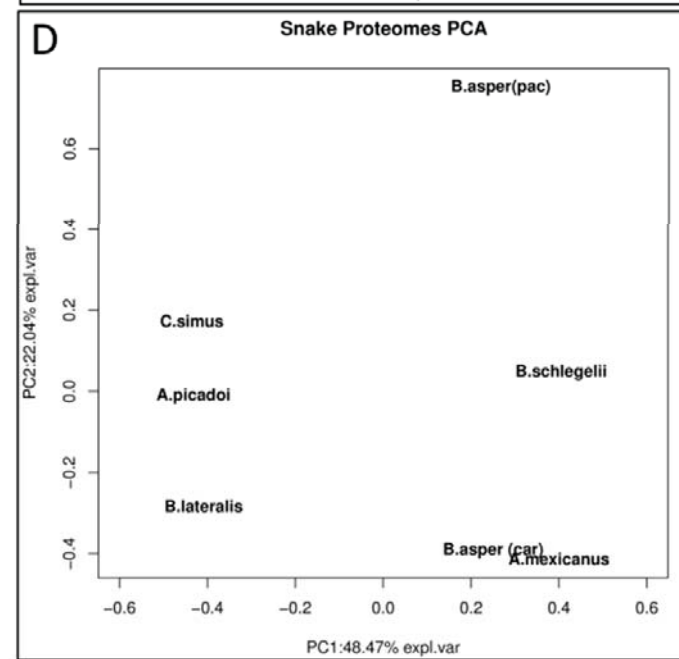
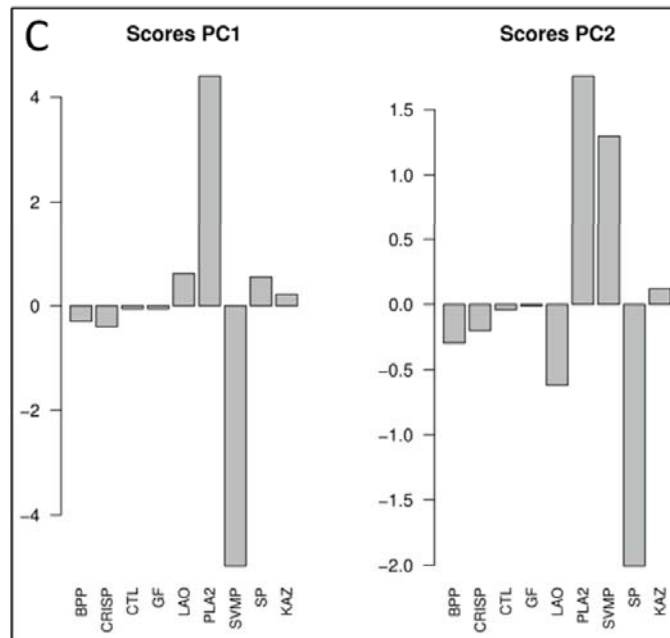
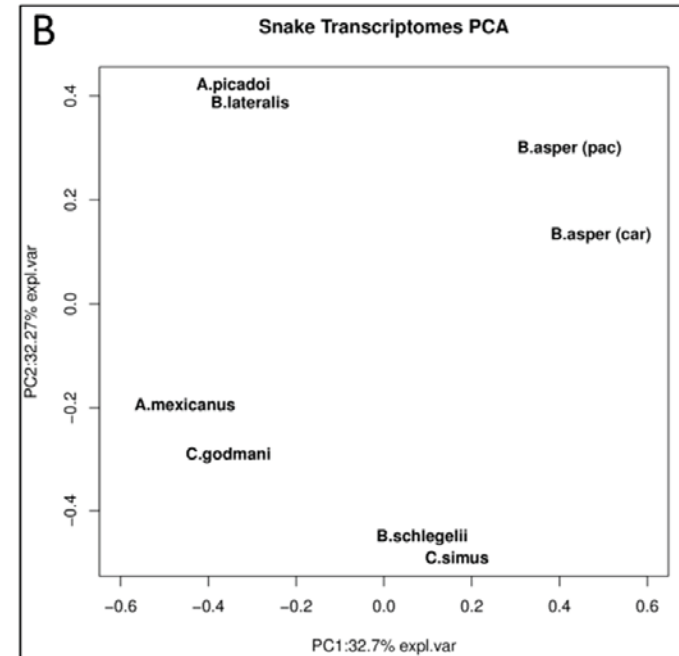
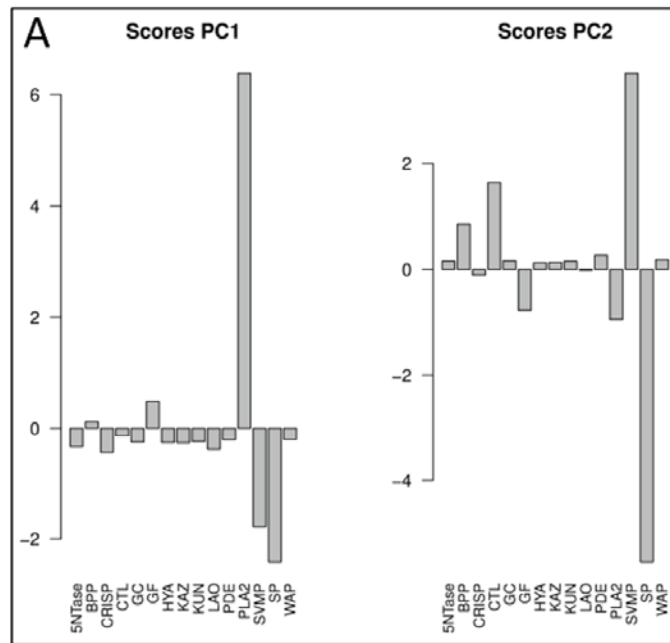
Reference-guided alignment of contigs
+
quantification of topologically equivalent fragments

	C.simus	B.asper(Car)	B.asper(Pac)	C.godmani	A.picadoi	A.mexicanus	B.schlegelii	B.lateralis
BPP	1	1	1	2	1	1	4	2
CRISP	0	2	1	2	4	1	2	1
CTL	2	5	2	3	9	1	0	5
GF	2	5	1	3	3	1	1	1
LAO	3	2	2	4	5	2	3	3
PLA2	3	9	4	4	2	2	1	3
SVMP	9	29	5	19	15	4	14	20
SP	6	15	1	13	7	6	8	11
5'-NTase	1	3	1	2	2	2	1	3
PDE	1	1	1	2	2	0	1	2
GC	1	2	1	1	1	1	0	1
WAP	0	2	0	0	0	0	0	0
HYA	2	2	0	1	1	1	0	1
OHA	0	0	0	0	1	1	0	0
3FTx	1	0	0	0	0	0	0	0
KUN	0	0	0	0	0	0	1	0
KAZ	0	0	0	0	0	0	1	1
TOTAL	32	78	20	56	53	23	37	54
PROTEOME	20	30	27	NA	25-27	25-27	27	29

Transcriptome vs. proteome comparison suggests differential translational rates

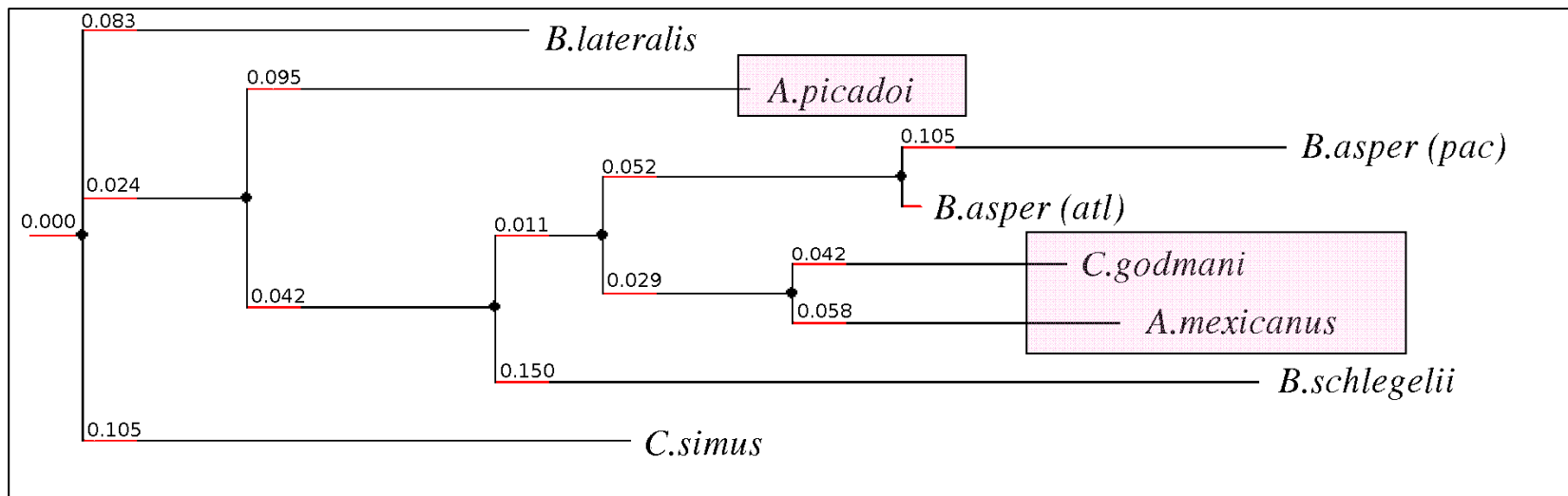


PCA snake venom transcriptome vs. proteome

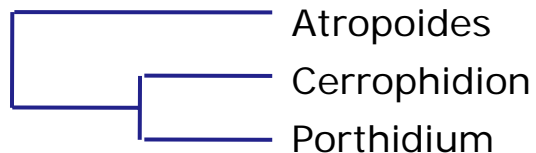


Phylogenetic analysis based on transcriptomics* data

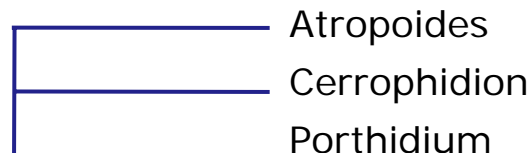
*Based on BPP, LAO, PLA2, SVMP, and SP consensus translated protein sequences



Castoe et al., 2005

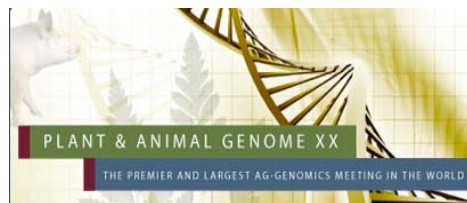


Castoe et al., 2006



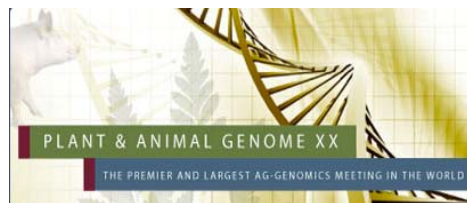
Angulo et al., 2008:

15% divergence between *A.picadoi* and *A.mexicanus*



Conclusions

- *Characterization of the Costa Rican snake venoms transcriptomes reveal the diversity of the venom composition. Dissection of venom complexity is a requirement for efficient exploitation of this chemical pool towards biomedical applications.*
- *Comparison of transcriptome and proteome profiles suggests additional mechanisms of translational control of snake venoms*
- *Venom transcriptomics data reveal interesting features on snake speciation and divergence*



Acknowledgements



Durban et al. *BMC Genomics* 2011, **12**:259
<http://www.biomedcentral.com/1471-2164/12/259>



RESEARCH ARTICLE

Open Access

Profiling the venom gland transcriptomes of Costa Rican snakes by 454 pyrosequencing

Jordi Durban¹, Paula Juárez¹, Yamileth Angulo², Bruno Lomonte², Marietta Flores-Díaz², Alberto Alape-Girón^{2,3}, Mahmood Sasa², Libia Sanz¹, José M Gutiérrez², Joaquín Dopazo⁴, Ana Conesa^{4*} and Juan J Calvete^{1*}

