Legends for File S1

Figure S1: The distribution of EST sequences in different GO categories

Figure S2: Distribution of the length of identified transcripts

Figure S3: Scatter plots of RPKM distribution of genes in the KEGG classes.

Figure S4: The mapping of identified transcripts/proteins (marked as red) in the spliceosome pathway of KEGG database

Figure S5: The mapping of identified transcripts /proteins (marked as red) in the pathway of protein process in endoplasmic reticulum in KEGG database.

Figure S6: Sequence characteristics of members in orphan families. Sequence characteristics of members in orphan families. The potential toxins, which haven't homologue's function annotations, are classified into orphan families including two groups: one comprises toxins predicted from Cys patterns, and the other is based on sequence homology with known toxins containing domains. Within Cys patterns, the char "#" represents any three amino acids other than Cys. For other toxins, the domain architectures were predicted by the SMART and Pfam servers [59,60]. The character "-F" appended to protein ID numbers indicates that these sequences are fragments but not full-length proteins. The abbreviations of domain names are as follow: EGF (SMART ID: SM00181); KU (SMART ID: SM00131), glyco_hydro_56 (Pfam ID: PF01630); crust_neurohorm (Pfam ID: PF01147).

Figure S7: The abundance of toxin families in different functional categories. Bars represent toxin families clustered based on their functional characteristics. The sum of RPKM values for each class and category are labeled. Neurotoxins including the ANK superfamily, the SCP family and the lycotoxin family; Assistant toxins including theriditoxin family; Proteases including ctenitoxin family; Function unknown toxins including scorpion toxin like family and the orphan family.

Figure S8: Phylogenomic trees for trypsin, scorpion toxin-like, lycotoxin, ctenitoxin, SCP family. Phylogenomic trees of trypsin, scorpion toxin-like, lycotoxin, ctenitoxin and SCP families. A. Ctenitoxin family; B. Trypsin family; C. Scorpion toxin-like family; D. SCP family; E. Lycotoxin family. The members of family and their homologues from other spiders are colored as blue and red on branches. For spider species that have transcriptomic data were highlighted by a green line.

Figure S9: Phylogenetic tree of ANK superfamily toxins and their homologues from other 45 species. Phylogenetic tree of ANK superfamily toxins and their homologues from other 45 species. Color code: pink for α-LTX-Lt1a family1; blue for α-LTX-Lt1a family2; green for δ-LIT-Lt1a family; red for α-LIT-Lt1a family; brown for ANK family. All phylogeny analyses are performed with MEGA 5.2 using Maximum Likelihood algorithm and 1000 bootstrap tests. The numbers on the branches are the supporting percentages of 1000 bootstrap tests.

Table S1: RPKM distribution in the top ten of three GO namespaces.

Table S2: RPKM statistics of Ion channel in *Latrodectus tredecimguttatus*.

Table S3: The statistics of RPKM in KEGG pathway superclass.

Table S4: The RPKM list of sub-classes of the “Genetic information processing” category in KEGG database.

Table S5: List of toxins identified by sequence analyses.

Table S6: Known ion channel toxins in five venomous species.

Table S7: Full names/abbreviations’ and taxonomic classification of 18 species in phylogenetic analysis.

Table S8: Full names/abbreviations and taxonomic classification of 54 arthropod species.

Table S9: Full names/abbreviations and taxonomic classification of species shown in Figure S8.

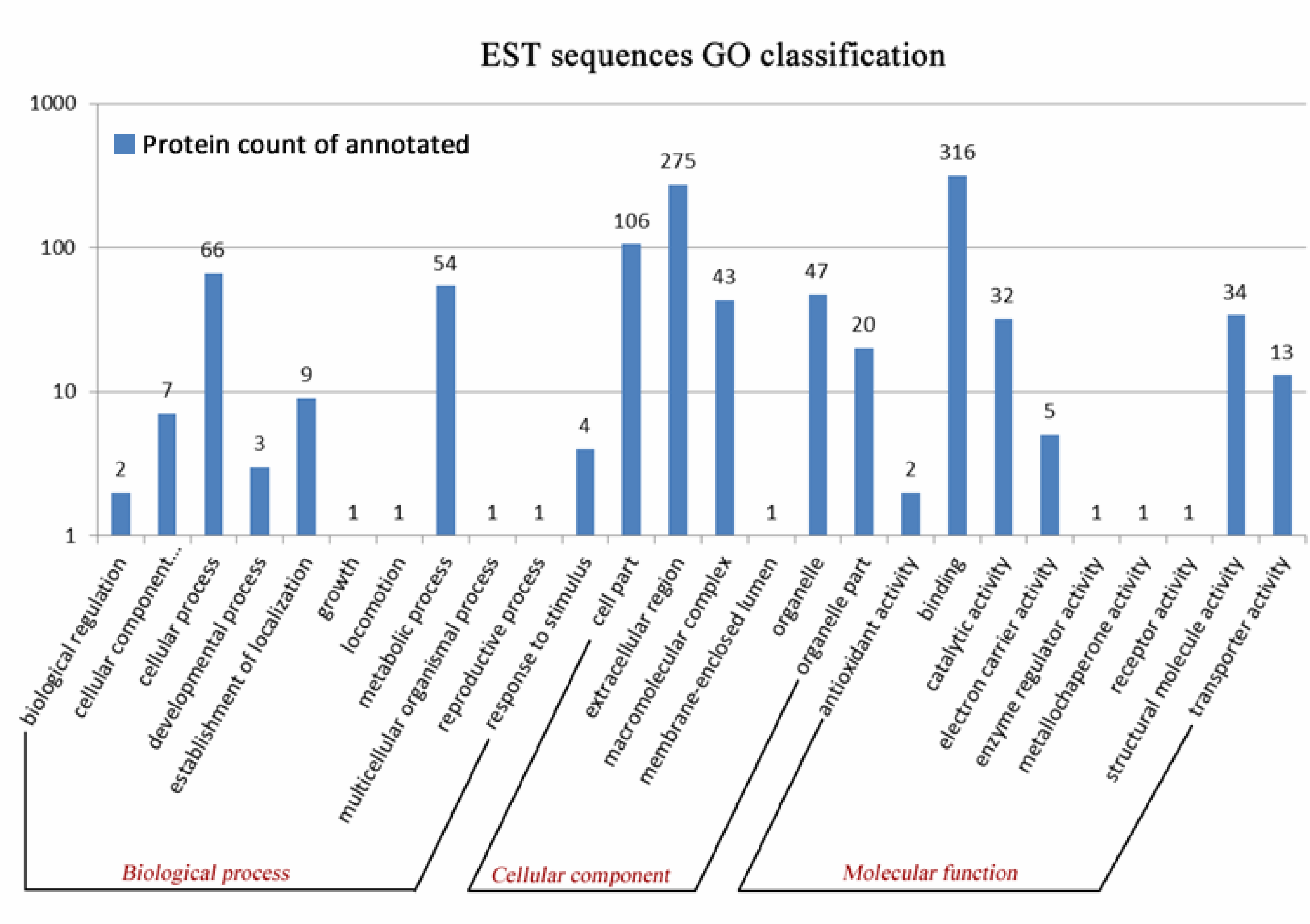

EST sequences GO classification
1000
100
10
1
Protein count of annotated
2
7
66
3
9
1
1
54
1
1
4
106
275
43
1
47
20
2
316
32
5
1
1
1
34
13
biological regulation
cellular component...
cellular process
developmental process
establishment of localization
growth
locomotion
metabolic process
multicellular organismal process
reproductive process
response to stimulus
cell part
extracellular region
macromolecular complex
membrane-enclosed lumen
organelle
organelle part
antioxidant activity
binding
catalytic activity
electron carrier activity
enzyme regulator activity
metallochaperone activity
receptor activity
structural molecule activity
transporter activity
Biological process
Cellular component
Molecular function

Figure S1

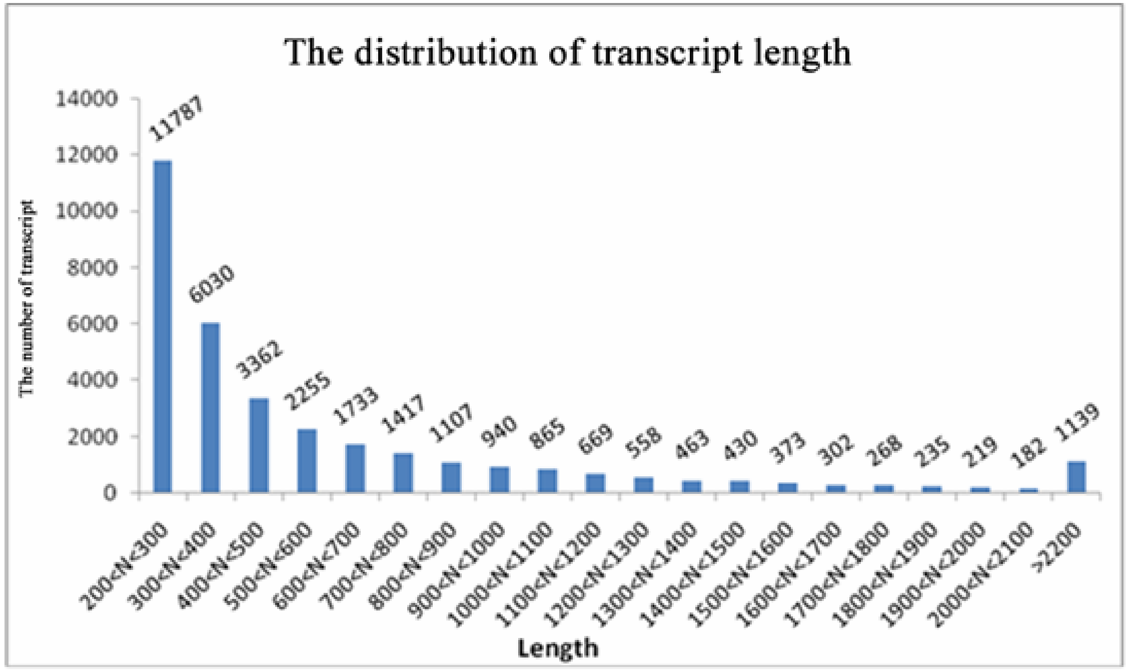

The distribution of transcript length
The number of transcript
14000
12000
10000
8000
6000
4000
2000
0
11787
6030
3362
2255
1733
1417
1107
940
865
669
558
463
430
373
302
268
235
219
182
1139
200<N<300
300<N<400
400<N<500
500<N<600
600<N<700
700<N<800
800<N<900
900<N<1000
1000<N<1100
1100<N<1200
1200<N<1300
1300<N<1400
1400<N<1500
1500<N<1600
1600<N<1700
1700<N<1800
1800<N<1900
1900<N<2000
2000<N<2100
>2200
Length

Figure S2

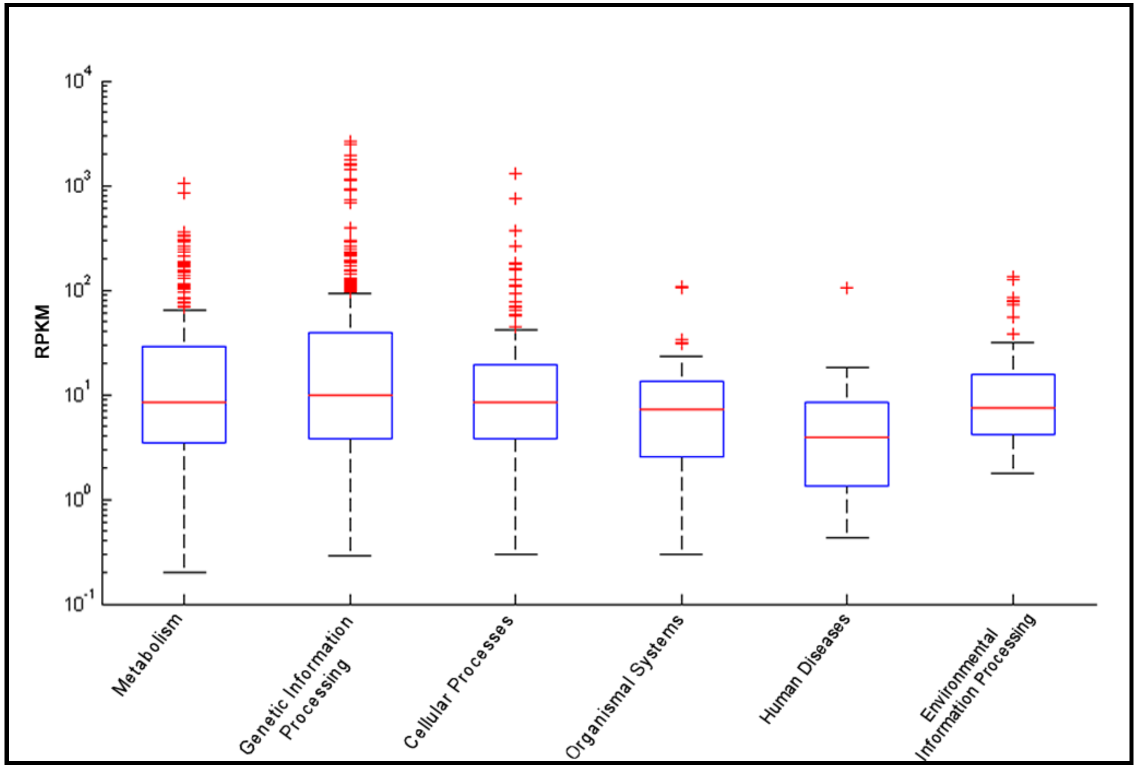

RPKM
10^4
10^3
10^2
10^1
10^0
10^{-1}
Metabolism
Genetic Information Processing
Cellular Processes
Organismal Systems
Human Diseases
Environmental Information Processing

Figure S3

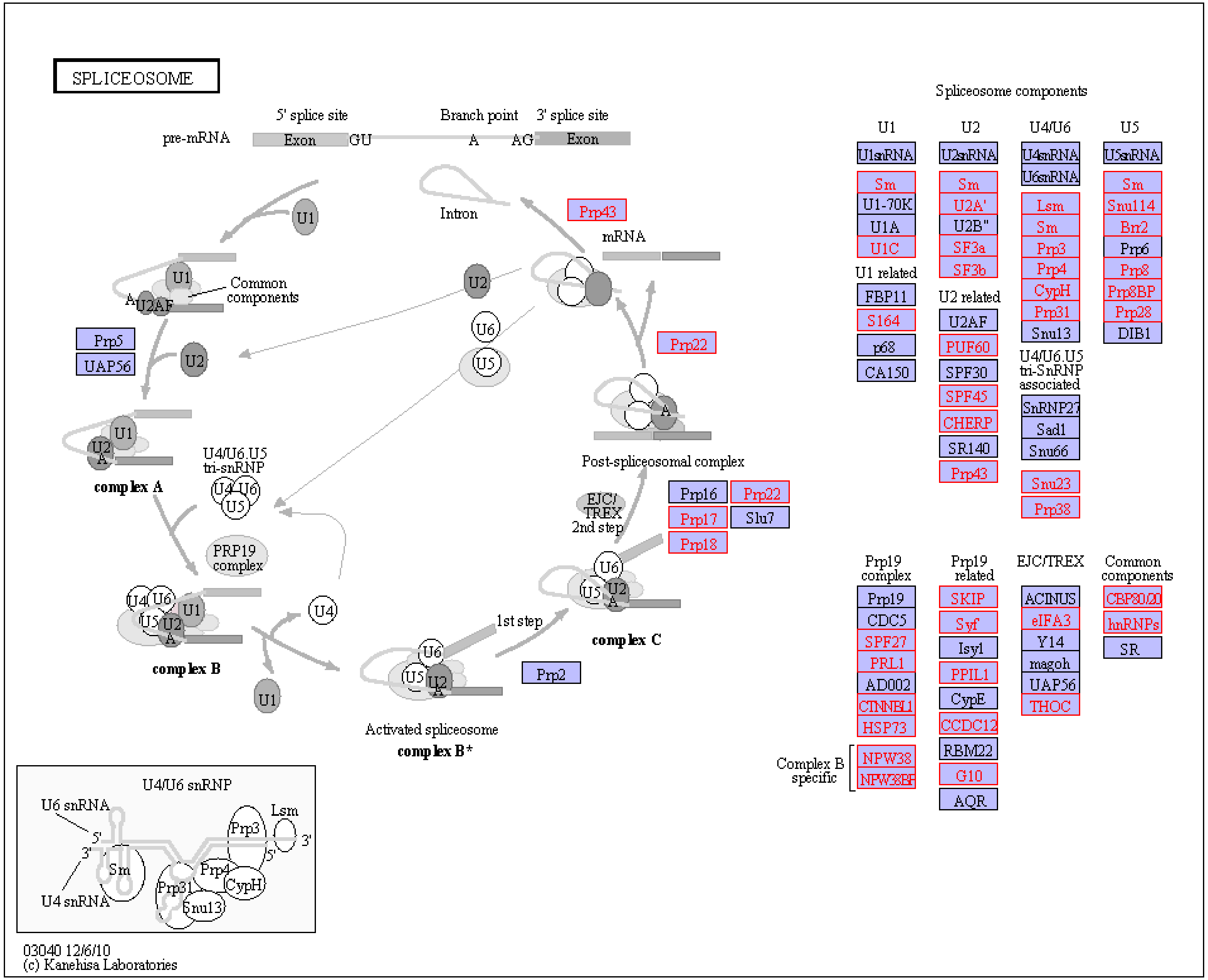
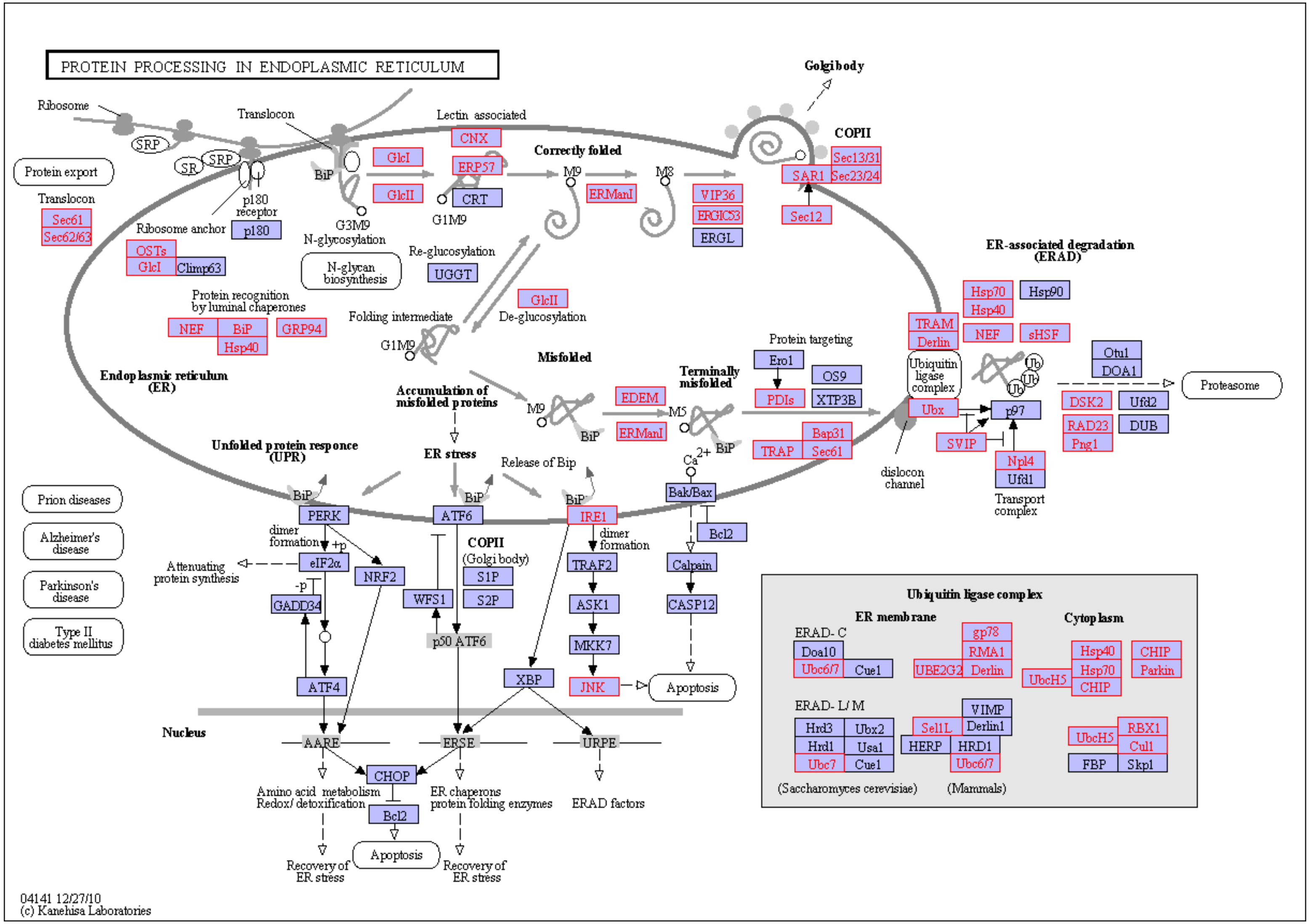

SPLICEOSOME
pre-mRNA
5' splice site
Branch point
3' splice site
Exon
GU
A
AG
Exon
Intron
mRNA
Prp43
U1
U2
U6
U5
Common components
U2AF
Prp5
UAP56
Prp22
complex A
U4/U6.U5 tri-snRNP
U4
PRP19 complex
Post-spliceosomal complex
EJC/TREX
2nd step
Prp16
Prp17
Prp18
Slu7
complex B
complex C
1st step
Prp2
Activated spliceosome
complex B*
U4/U6 snRNP
U6 snRNA
U4 snRNA
Lsm
Prp3
Prp4
Prp31
CypH
Snu13
Sm
5'
3'
03040 12/6/10
(c) Kanehisa Laboratories
Spliceosome components
U1
U1snRNA
Sm
U1-70K
U1A
U1C
U1 related
FBP11
S164
p68
CA150
U2
U2snRNA
Sm
U2A'
U2B"
SF3a
SF3b
U2 related
U2AF
PUF60
SPF30
SPF45
CHERP
SR140
Prp43
U4/U6
U4snRNA
U6snRNA
Lsm
Sm
Prp3
Prp4
CypH
Prp31
Snu13
U4/U6.U5 tri-SnRNP associated
SnRNP27
Sad1
Snu66
Snu23
Prp38
U5
U5snRNA
Sm
Snu114
Brr2
Prp6
Prp8
Prp8BP
Prp28
DIB1
Prp19 complex
Prp19
CDC5
SPF27
PRL1
AD002
CTNNBL1
HSP73
Complex B specific
NPW38
NPW38BP
Prp19 related
SKIP
Syf
Isy1
PPIL1
CypE
CCDC12
RBM22
G10
AQR
EJC/TREX
ACINUS
eIFA3
Y14
magoh
UAP56
THOC
Common components
CBP80/20
hnRNPs
SR

Figure S4



PROTEIN PROCESSING IN ENDOPLASMIC RETICULUM
Ribosome
Translocon
Lectin associated
Golgi body
COPII
SRP
SR
SRP
Protein export
Translocon
Sec61
Sec62/63
p180 receptor
p180
Ribosome anchor
OSTs
GlcI
Climp63
BiP
G3M9
N-glycosylation
N-glycan biosynthesis
GlcI
GlcII
CNX
ERP57
CRT
G1M9
Correctly folded
M9
M8
ERManI
VIP36
ERGIC53
ERGL
SAR1
Sec13/31
Sec23/24
Sec12
Re-glucosylation
UGGT
Protein recognition by luminal chaperones
NEF
BiP
Hsp40
GRP94
Folding intermediate
G1M9
GlcII
De-glucosylation
Endoplasmic reticulum (ER)
Accumulation of misfolded proteins
Misfolded
M9
BiP
EDEM
ERManI
Terminally misfolded
M5
BiP
Protein targeting
Ero1
PDIs
OS9
XTP3B
Bap31
TRAP
Sec61
ER-associated degradation (ERAD)
Hsp70
Hsp40
Hsp90
TRAM
Derlin
NEF
sHSF
Ubiquitin ligase complex
Ub
Ub
Ub
Ubx
p97
SVIP
Npl4
Ufd1
Transport complex
dislocon channel
Otu1
DOA1
Proteasome
DSK2
RAD23
Png1
Ufd2
DUB
Unfolded protein responce (UPR)
ER stress
Release of Bip
BiP
BiP
BiP
PERK
ATF6
IRE1
Ca2+
Bak/Bax
Bcl2
Calpain
CASP12
dimer formation
+p
eIF2α
-p
GADD34
NRF2
Attenuating protein synthesis
ATF4
WFS1
COPII (Golgi body)
S1P
S2P
p50 ATF6
dimer formation
TRAF2
ASK1
MKK7
JNK
Apoptosis
XBP
Nucleus
AARE
ERSE
URPE
CHOP
Bcl2
Amino acid metabolism Redox/ detoxification
ER chaperons protein folding enzymes
ERAD factors
Apoptosis
Recovery of ER stress
Recovery of ER stress
Prion diseases
Alzheimer's disease
Parkinson's disease
Type II diabetes mellitus
Ubiquitin ligase complex
ER membrane
Cytoplasm
ERAD- C
Doa10
Ubc6/7
Cue1
gp78
RMA1
UBE2G2
Derlin
UbcH5
Hsp40
Hsp70
CHIP
CHIP
Parkin
ERAD- L/ M
Hrd3
Ubx2
Hrd1
Usa1
Ubc7
Cue1
SelIL
VIMP
Derlin1
HERP
HRD1
Ubc6/7
UbcH5
RBX1
Cul1
FBP
Skp1
(Saccharomyces cerevisiae)
(Mammals)
04141 12/27/10
(c) Kanehisa Laboratories

Figure S5

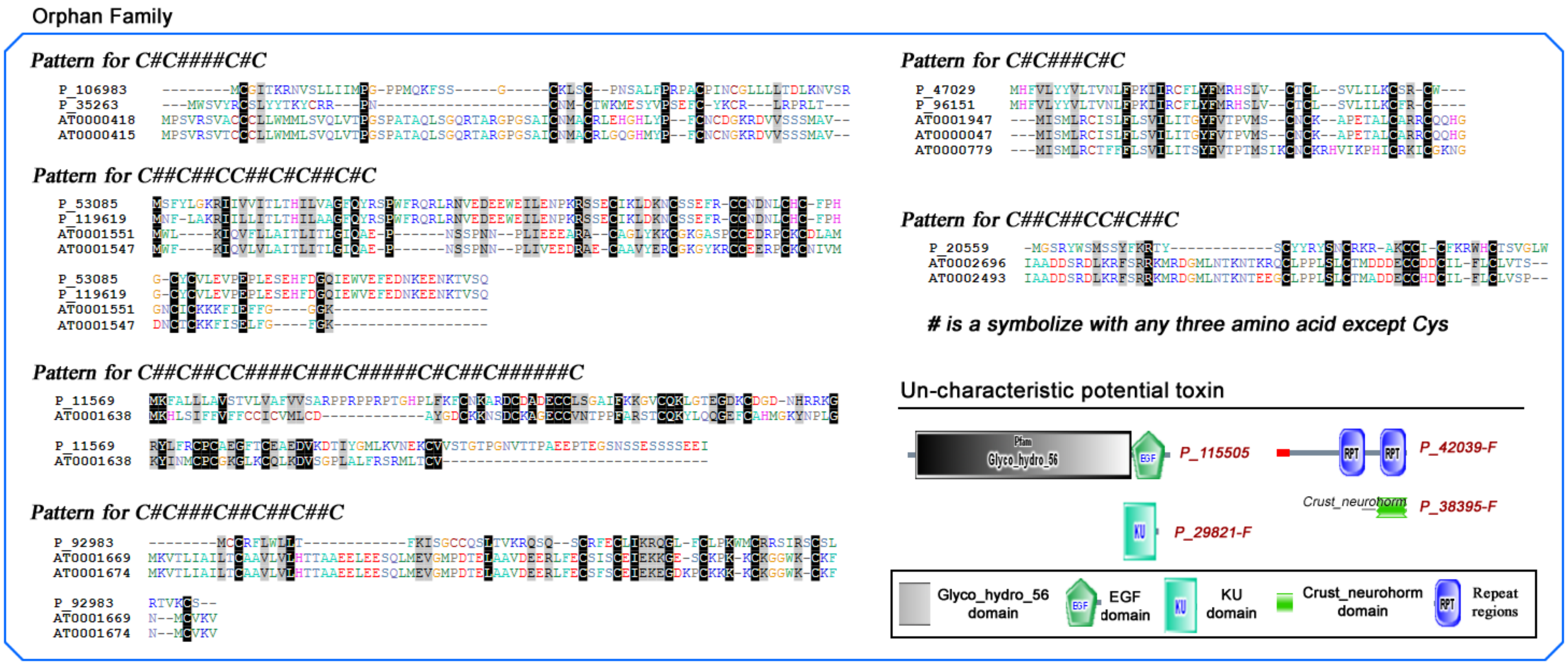

Orphan Family
Pattern for C#C####C#C
P_106983 --------MCGITKRNVSLLIIMPG-PPMQKFSS-----G-----CKLSC--PNSALFPRPACPINCGLLLLTDLKNVSR
P_35263 ---MWSVYRCSLYYTKYCRR---PN-------------------CNM-CTWKMESYVPSEFC-YKCR---LRPRLT---
AT0000418 MPSVRSVACCCLLWMMLSVQLVTPGSPATAQLSGQRTARGPGSAICNMACRLEHGHLYP--FCNCDGKRDVVSSSMAV--
AT0000415 MPSVRSVTCCCLLWMMLSVQLVTPGSPATAQLSGQRTARGPGSAICNMACRLGQGHMYP--FCNCNGKRDVVSSSMAV--
Pattern for C##C##CC##C#C##C#C
P_53085 MSFYLGKRIIVVITLTHILVAGFQYRSPWFRQRLRNVEDEEWEILENPKRSSECIKLDKNCSSEFR-CCNDNLCHC-FPH
P_119619 MNF-LAKRIILLITLTHILAAGFQYRSPWFRQRLRNVEDEEWEILENPKRSSECIKLDKNCSSEFR-CCNDNLCHC-FPH
AT0001551 MWL----KIQVFLLAITLITLGIQAE-P------NSSPNN--PLIEEEARA--CAGLYKKCGKGASPCCEDRPCKCDLAM
AT0001547 MWF----KIQVLVLAITLITLGIQAE-P------NSSPNN--PLIVEEDRAE-CAAVYERCGKGYKRCCEERPCKCNIVM
P_53085 G-CYCVLEVPEPLESEHFDGQIEWVEFEDNKEENKTVSQ
P_119619 G-CYCVLEVPEPLESEHFDGQIEWVEFEDNKEENKTVSQ
AT0001551 GNCICKKKFIEFFG----GGK------------------
AT0001547 DNCTCKKFISELFG----FGK------------------
Pattern for C##C##CC####C###C#####C#C##C######C
P_11569 MKFALLLAVSTVLVAFVVSARPPRPPRPTGHPLFKFCNKARDCDADECCLSGAIFKKGVCQKLGTEGDKCDGD-NHRRKG
AT0001638 MKHLSIFFVFFCCICVMLCD------------AYGDCKKNSDCKAGECCVNTPPFARSTCQKYLQQGEFCAHMGKYNPLG
P_11569 RYLFRCPCAEGFTCEAEDVKDTIYGMLKVNEKCVVSTGTPGNVTTPAEEPTEGSNSSESSSSEEI
AT0001638 KYINMCPCGKGLKCQLKDVSGPLALFRSRMLTCV-------------------------------
Pattern for C#C###C##C##C##C
P_92983 --------MCCRFLWLLT------------FKISGCCQSLTVKRQSQ--SCRFECLIKRQGL-FCLPKWMCRRSIRSCSL
AT0001669 MKVTLIAILTCAAVLVLHTTAAEELEESQLMEVGMPDTELAAVDEERLFECSISCEIEKKGE-SCKPK-KCKGGWK-CKF
AT0001674 MKVTLIAILTCAAVLVLHTTAAEELEESQLMEVGMPDTELAAVDEERLFECSFSCEIEKEGDKPCKKK-KCKGGWK-CKF
P_92983 RTVKCS--
AT0001669 N--MCVKV
AT0001674 N--MCVKV
Pattern for C#C###C#C
P_47029 MHFVLYYVLTVNLFPKIIRCFLYFMRHSLV--CTCL--SVLILKCSR-CW---
P_96151 MHFVLYYVLTVNLFPKIIRCFLYFMRHSLV--CTCL--SVLILKCFR-C----
AT0001947 ---MISMLRCISLFLSVILITGYFVTPVMS--CNCK--APETALCARRCQQHG
AT0000047 ---MISMLRCISLFLSVILITGYFVTPVMS--CNCK--APETALCARRCQQHG
AT0000779 ---MISMLRCTFFFLSVILITSYFVTPTMSIKCNCKRHVIKPHICRKICGKNG
Pattern for C##C##CC#C##C
P_20559 -MGSRYWSMSSYFKRTY------------SCYYRYSNCRKR-AKCCI-CFKRWHCTSVGLW
AT0002696 IAADDSRDLKRFSRRKMRDGMLNTKNTKRQCLPPLSLCTMDDDECCDDCIL-FLCLVTS--
AT0002493 IAADDSRDLKRFSRRKMRDGMLNTKNTEEGCLPPLSLCTMADDECCHDCIL-FLCLVSP--
is a symbolize with any three amino acid except Cys
Un-characteristic potential toxin
Pfam
Glyco_hydro_56
EGF
P_115505
RPT
RPT
P_42039-F
Crust_neurohorm
P_38395-F
KU
P_29821-F
Glyco_hydro_56 domain
EGF domain
KU domain
Crust_neurohorm domain
RPT
Repeat regions

Figure S6

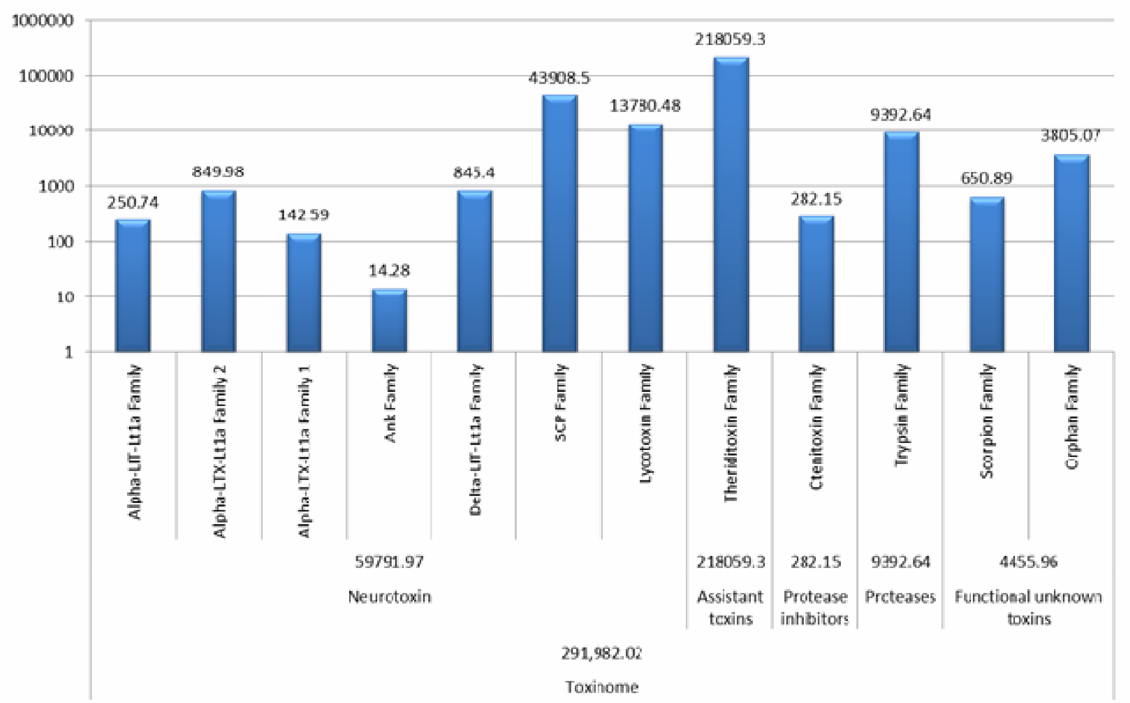

1000000
100000
10000
1000
100
10
1
250.74
849.98
142.59
14.28
845.4
43908.5
13780.48
218059.3
282.15
9392.64
650.89
3805.07
Alpha-LIT-Lt1a Family
Alpha-LTX-Lt1a Family 2
Alpha-LTX-Lt1a Family 1
Ank Family
Delta-LIT-Lt1a Family
SCP Family
Lycotoxin Family
Theriditoxin Family
Cteitoxin Family
Trypsin Family
Scorpion Family
Orphan Family
59791.97
Neurotoxin
218059.3
Assistant toxins
282.15
Protease inhibitors
9392.64
Proteases
4455.96
Functional unknown toxins
291,982.02
Toxinome

Figure S7

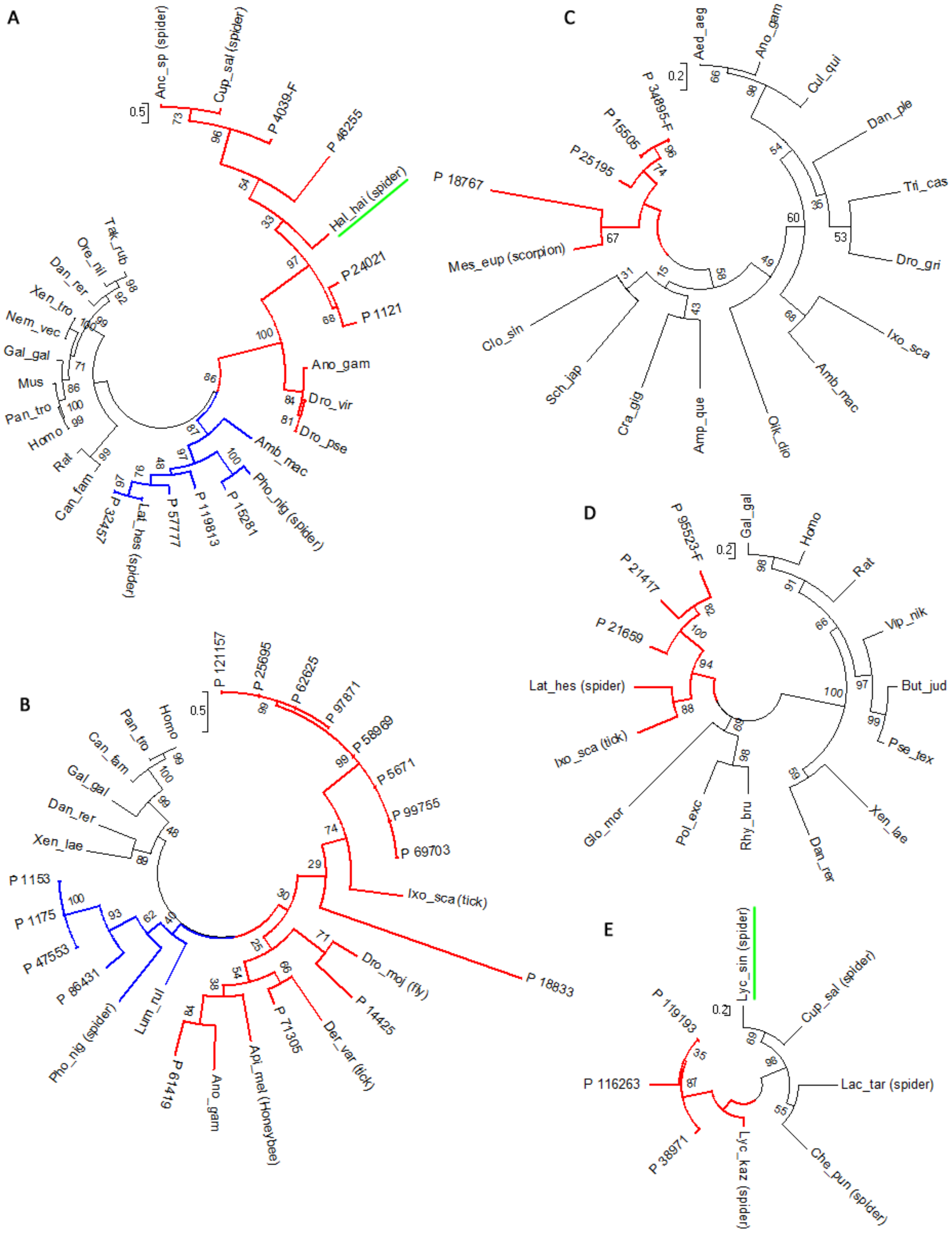

A
0.5
Anc_sp (spider)
Cup_sal (spider)
P 4039-F
P 46255
Hal_hal (spider)
P 24021
P 1121
Ano_gam
Dro_vir
Dro_pse
Amb_mac
Pho_nig (spider)
P 15281
P 119813
P 57777
Lat_hes (spider)
P 32457
Can_fam
Rat
Homo
Pan_tro
Mus
Gal_gal
Nem_vec
Xen_tro
Dan_rer
Ore_nil
Tak_rub
B
0.5
P 121157
P 25695
P 62625
P 97871
P 58969
P 5671
P 99755
P 69703
Ixo_sca (tick)
P 18833
Dro_moj (fly)
P 14425
Der_var (tick)
P 71305
Api_mel (Honeybee)
Ano_gam
P 61419
Lum_rul
Pho_nig (spider)
P 86431
P 47553
P 1175
P 1153
Xen_lae
Dan_rer
Gal_gal
Can_fam
Pan_tro
Homo
C
0.2
Aed_aeg
Ano_gam
Cul_qui
Dan_ple
Tri_cas
Dro_gri
Ixo_sca
Amb_mac
Oik_dio
Amp_que
Cra_gig
Sch_jap
Clo_sin
Mes_eup (scorpion)
P 18767
P 25195
P 15505
P 34895-F
D
0.2
Gal_gal
Homo
Rat
Vip_nik
But_jud
Pse_tex
Xen_lae
Dan_rer
Rhy_bru
Pol_exc
Glo_mor
Ixo_sca (tick)
Lat_hes (spider)
P 21659
P 21417
P 95523-F
E
0.2
Lyc_sin (spider)
Cup_sal (spider)
Lac_tar (spider)
Che_pun (spider)
Lyc_kaz (spider)
P 38971
P 116263
P 119193

Figure S8

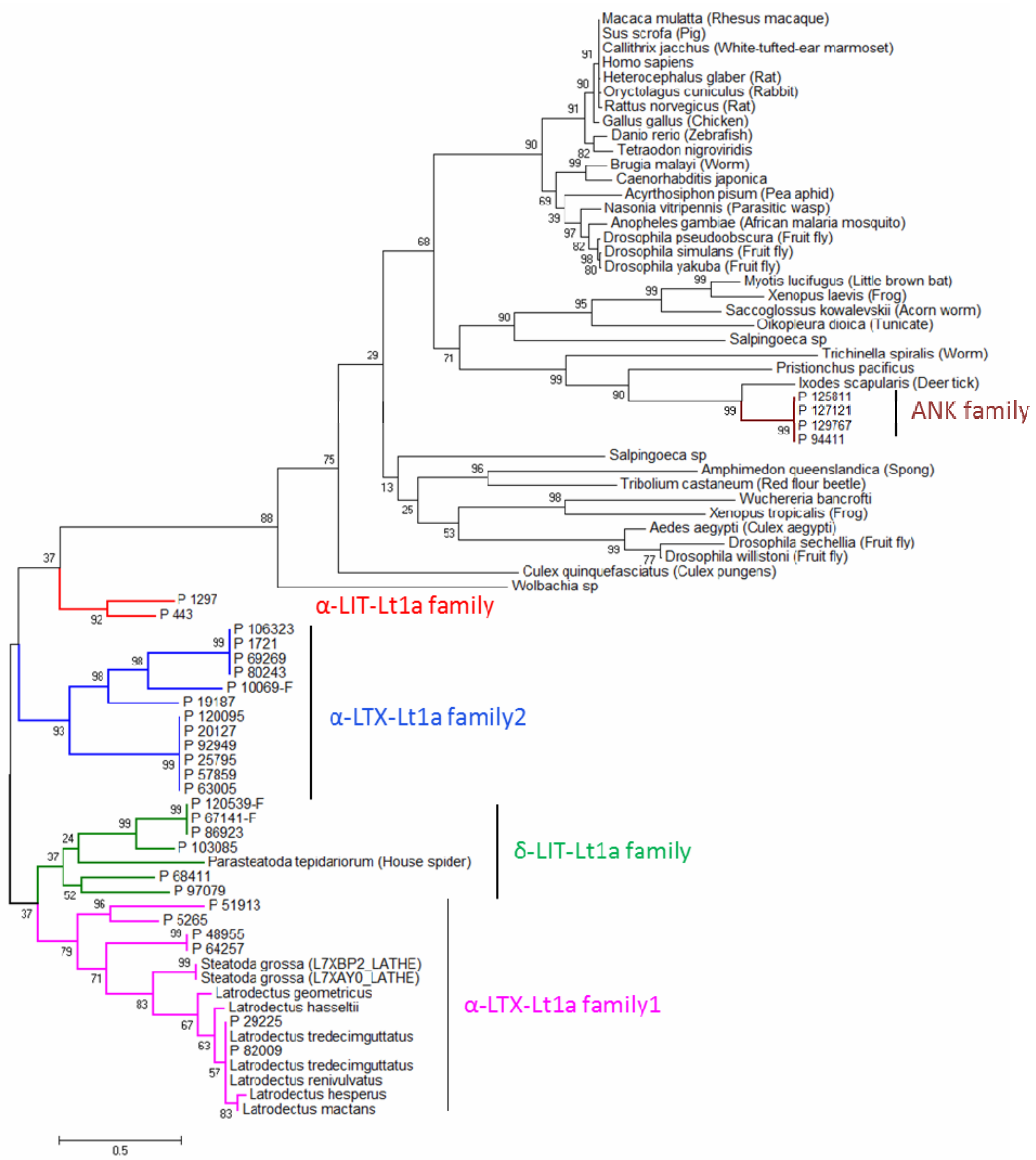

Macaca mulatta (Rhesus macaque)
Sus scrofa (Pig)
Callithrix jacchus (White-tufted-ear marmoset)
Homo sapiens
Heterocephalus glaber (Rat)
Oryctolagus cuniculus (Rabbit)
Rattus norvegicus (Rat)
Gallus gallus (Chicken)
Danio rerio (Zebrafish)
Tetraodon nigroviridis
Brugia malayi (Worm)
Caenorhabditis japonica
Acyrthosiphon pisum (Pea aphid)
Nasonia vitripennis (Parasitic wasp)
Anopheles gambiae (African malaria mosquito)
Drosophila pseudoobscura (Fruit fly)
Drosophila simulans (Fruit fly)
Drosophila yakuba (Fruit fly)
Myotis lucifugus (Little brown bat)
Xenopus laevis (Frog)
Saccoglossus kowalevskii (Acorn worm)
Oikopleura dioica (Tunicate)
Salpingoeca sp
Trichinella spiralis (Worm)
Pristionchus pacificus
Ixodes scapularis (Deer tick)
P 125811
P 127121
P 129767
P 94411
ANK family
Salpingoeca sp
Amphimedon queenslandica (Spong)
Tribolium castaneum (Red flour beetle)
Wuchereria bancrofti
Xenopus tropicalis (Frog)
Aedes aegypti (Culex aegypti)
Drosophila sechellia (Fruit fly)
Drosophila willistoni (Fruit fly)
Culex quinquefasciatus (Culex pungens)
Wolbachia sp
P 1297
P 443
α-LIT-Lt1a family
P 106323
P 1721
P 69269
P 80243
P 10069-F
P 19187
P 120095
P 20127
P 92949
P 25795
P 57859
P 63005
α-LTX-Lt1a family2
P 120539-F
P 67141-F
P 86923
P 103085
Parasteatoda tepidariorum (House spider)
P 68411
P 97079
δ-LIT-Lt1a family
P 51913
P 5265
P 48955
P 64257
Steatoda grossa (L7XBP2_LATHE)
Steatoda grossa (L7XAY0_LATHE)
Latrodectus geometricus
Latrodectus hasseltii
P 29225
Latrodectus tredecimguttatus
P 82009
Latrodectus tredecimguttatus
Latrodectus renivulvatus
Latrodectus hesperus
Latrodectus mactans
α-LTX-Lt1a family1
0.5

Figure S9

Table S1. RPKM distribution in the top ten GO items in three GO namespaces

Biological process namespace			**Cellular component namespace**			**Molecular function namespace**		
GO Item	**Count**	**RPKM**	**GO Item**	**Count**	**RPKM**	**GO Item**	**Count**	**RPKM**
Translation	222	101254.5	Extracellular region	69	87229.42	Neuropeptide hormone activity	4	218072.3
Proteolysis	183	29747.34	Ribosome	202	99301.79	Structural constituent of ribosome	214	106616
Electron transport chain	6	11005.62	Integral to membrane	617	54733.82	ATP binding	622	24843.5
Transmembrane transport	96	10427.08	Cytoplasm	272	23632.59	Metalloendopeptidase activity	48	17527.55
ATP synthesis coupled proton transport	16	10399.12	Mitochondrial inner membrane	29	17699	Oxygen transporter activity	16	17100.38
Transport	47	7432.82	Respiratory chain	6	12817.73	Electron carrier activity	82	16007.03
Aerobic respiration	1	6966.76	Small ribosomal subunit	16	8400.76	Calcium ion binding	158	15046.09
Chitin metabolic process	12	5714.95	Eukaryotic translation elongation factor 1 complex	7	8236.08	Oxidoreductase activity	117	14029.13
Translational elongation	9	5654.64	Mitochondrion	36	8214.48	Binding	333	13876.24
GTP biosynthetic process	5	5213.21	Membrane	239	7967.85	RNA binding	182	13527.49

Table S2. RPKM statistics of ion channels in *Latrodectus tredecimguttatus*

Class Name	Ion channel Name	Attacked by spider toxin	RPKM	RPKM Count
Cation Channel	Novel protein similar to vertebrate calcium channel voltage-dependent alpha 2/delta subunit 2 (CACNA2D2)	Unknown	4.13	***162.86***
	Potassium channel protein putative	Unknown	12.08	
	Potassium channel putative	Unknown	2.26	
	Regulator of ATP-sensitive K+ channels putative	Unknown	91.89	
	Cyclic nucleotide-gated cation channel	Unknown	1.05	
	Transient receptor potential cation channel protein 4	Unknown	1.45	
	Trimeric intracellular cation channel type A putative	Unknown	40.27	
	Voltage-dependent calcium channel subunit alpha-2/delta-3	Unknown	4.68	
	Inward rectifier K+ channel putative	Unknown	2.54	
	Voltage-gated hydrogen channel putative	Unknown	2.51	
Anion Channel	Chloride channel putative	Unknown	10	***970.65***
	Chloride channel protein 3	Unknown	4.26	
	Glutamate gated chloride channel	Unknown	6.23	
	pH-sensitive chloride channel	Unknown	4.16	
	Voltage-dependent anion-selective channel putative	Unknown	946	
Other	Potassium intermediate/small conductance calcium-activated channel subfamily N member 2	Unknown	1.05	***6.12***
	Cyclic nucleotide-gated ion channel LCNG1	Unknown	5.07	

Table S3. The statistics of RPKMs in KEGG pathway superclass

KEGG pathway superclass	Pathway Number	RPKM (%)	Protein Number	Average RPKM
Genetic Information Processing	21	32142.33 (48%)	522	61.57
Metabolism	75	19924.51 (30%)	588	33.88
Cellular Processes	11	6168.96 (9%)	161	38.31
Human Diseases	21	5182.66 (8%)	40	129.56
Environmental Information Processing	14	3086.34 (4%)	86	133.27
Organismal Systems	22	764.83 (1%)	58	13.18

Table S4. The RPKM list of sub-classes of the "Genetic information processing" category in KEGG database

Sub-class Name	**RPKM**
Ribosome	11166.9
Protein processing in endoplasmic reticulum	5193.12
RNA transport	4974.49
Protein export	2689.95
Proteasome	1720.84
Spliceosome	1183.85
Ubiquitin mediated proteolysis	1168.3
Ribosome biogenesis in eukaryotes	712.82
mRNA surveillance pathway	581.5
Aminoacyl-tRNA biosynthesis	421.24
Nucleotide excision repair	412.8
RNA polymerase	397.21
RNA degradation	373.73
Basal transcription factors	327.53
Homologous recombinatio	303.85
SNARE interactions in vesicular transport	257.33
DNA replication	79.05
Mismatch repair	67.74
Base excision repair	53.79
Sulfur relay system	47.58
Non-homologous end-joining	8.71

Table S5: List of toxins identified by sequence analyses

Family Name	Homologue/Conserved Domain	Protein ID	RPKM
Alpha-LIT-Lt1a Family	Homologue: Alpha-LCT-Lt1a	P_443	250.74
	Homologue: Alpha-LIT-Lt1a	P_1297	
Theriditoxin Family	Homologue: Alpha-latrotoxin-associated LMWP2	P_66861	218059.3
	Homologue: Alpha-latrotoxin-associated LMWP	P_89055	
Alpha-LTX-Lt1a Family 2	Homologue: Alpha-LTX-Lt1a	P_51913, P_29225, P_124863-F, P_64257, P_48955, P_82009, P_5265	849.98
Alpha-LTX-Lt1a Family 1	Homologue: Alpha-LTX-Lt1a	P_120095, P_25795, P_1721, P_57859, P_20127, P_63005, P_92949, P_19187, P_10069-F, P_69269, P_106323, P_80243	142.59
Ank Family	Domain matching	P_63979-F, P_77051, P_36165-F, P_73235, P_97059, P_87955, P_116129, P_127121, P_129767, P_125811, P_94411, P_77485	153.24
SCP Family	Homologue: Cysteine-rich secretory protein	P_21417, P_21659, P_95523-F, P_67749	44017.56
Delta-LIT-Lt1a Family	Homologue: Delta-LIT-Lt1a	P_67141-F, P_97079, P_103085, P_86923, P_68411, P_120539-F	845.4
Ctenitoxin Family	Domain matching	P_46255	282.15
	Homologue: U24-ctenitoxin-Pn1a	P_32457, P_15281, P_119813, P_57777	
	Homologue: U3-ctenitoxin-Asp1a	P_4039-F	
	Homologue: U9-ctenitoxin-Pr1a	P_24021, P_86613-F	
	Homologue: U19-ctenitoxin-Pn1a	P_1121	
Trypsin Family	Domain matching	P_5671, P_121157, P_99755, P_18833, P_14425, P_61419, P_25695, P_97871, P_62625, P_58969, P_69703, P_71305, P_1153	9392.64
	Homologue: Fibrinolytic enzyme	P_1175	
	Homologue: Trypsin-7	P_47553	
	Homologue: U21-ctenitoxin-Pn1a	P_86431	
Lycotoxin Family	Homologue: U1-aranetoxin-Av1a	P_29821-F	13781.7
	Homologue: U1-lycotoxin-Ls1hh	P_116263	
	Homologue: U4-lycotoxin-Ls1a	P_119193, P_38971	
Scorpion Family	Homologue: Venom protein-2	P_18767	650.89
	Homologue: Venom toxin-like peptide-6	P_15505, P_34895-F, P_25195	
Orphan Family	Scaffold matching	P_92983 , P_96151, P_106983, P_35263, P_47029, P_11569, P_53085, P_119619, P_20559	3816.79
	Homologue: Orcokinin-like peptide	P_42039-F	
	Homologue: Ion transport peptide	P_38395-F	
	Homologue: Venom hyaluronidase	P_115505	

Note, known toxins are colored by blue; Toxins and toxin families that were firstly reported in are colored by red and green, respectively

Table S6. Known ion channel toxins in five venom species

	Sodium channel	Potassium channel	Calcium channel
Scorpion	263	188	47
Snake	20	32	50
Spider	188	62	101
Sea anemone	67	29	0
Cone	108	39	76

Table S7. Full names/abbreviations' and taxonomic classification of 18 species

Amphibia	Xenopus tropicalis (Western clawed frog) [Xen_tro]
Arthropoda	Anopheles gambiae (African malaria mosquito) [Ano_gam]
	Apis mellifera (Honeybee) [Api_mel]
	Drosophila melanogaster (Fruit fly) [Dro_mel]
	Latrodectus tredecimguttatus (Lat_tre)
Aves	Gallus gallus (Chicken) [Gal]
Fungi	Dictyostelium discoideum (Slime mold) [Dic_dis]
	Dictyostelium fasciculatum (strain SH3) (Slime mold) [Dic_fas]
Mammalia	Canis familiaris (Dog) (Canis lupus familiaris) [Can_fam]
	Mus musculus (Mouse) [Mus]
	Pan troglodytes (Chimpanzee) [Pan_tro]
	Homo sapiens (Human) [Homo]
	Pan troglodytes (Chimpanzee)[Pan_tro]
	Rattus norvegicus (Rat) [Rat]
Nematoda	Caenorhabditis elegans [Cae_ele]
Teleostei	Danio rerio (Zebrafish) (Brachydanio rerio) [Dan_rer]
	Takifugu rubripes (Japanese pufferfish) (Fugu rubripes) [Tak_rub]
Other	Ciona intestinalis (Transparent sea squirt) [Cio_int]

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Table S8. Full names/abbreviations and taxonomic classification of 54 arthropod species

Lineage				**Species**
Arthropoda	**Mandibulata**	**Pancrustacea**	**Hexapoda**	Metajapyx subterraneus (Jap); Eumesocampa frigilis (Efr); Orchesella imitari (Oim); Ischnura verticalis(Ive); Plathemis lydia (Lly); Hexagenia limbata (May); Periplaneta americana (Pam); Acheta domesticus (Ado); Nicoletia meinerti (Nme); Ctenolepisma lineata (Cli); Pedetontus saltator (Psa); Machiloides banksi (Mba); Ephemerella inconstans (Ein);
			Crustacea	Neogonodactylus oerstedii (Neo); Libinia emarginata (Lem); Armadillidium vulgare (Avu3); Eurytemora affinis (Eaf); Acanthocyclops vernalis (A369); Daphnia magna (Dma); Lynceus sp. (Lyn); Artemia salina (Asa3); Streptocephalus seali (ufs); Loxothylacus texanus (Lox); Semibalanus balanoides (Bba); Chthamalus fragilis (Cfr); Lepas anserifera (Lean)
			Oligostraca	Skogsbergia lerneri (Skle); Argulus sp. (Arg2); Armillifer armillatus (Aar)
		Myriapoda		Scutigera coleoptrata (Scol); Eurypauropus spinosus (Eury); Craterostigmus tasmanianus (Ctas2); Hanseniella sp.(Han2); Scutigerella sp.(Scu3); Polyxenus fasciculatus (Pol2); Abacion magnum (Ama); Polyzonium germanicum (Pge2);
	Chelicerata		**Arachnida & Merostomata**	Prokoenenia wheeleri (Pwh); Dinothrombium pandorae (Din2); Leiobunum verrucosum (Lnig); Amblyomma sp. (Amb2); Idiogaryops pumilis (Ipum); Carcinoscorpius rotundicauda (Cro2); Cryptocellus centralis (Crp2); Eremocosta gigasella (Egig); Hadrurus arizonensis (Hari); Heterometrus spinifer (Hsp); Aphonopelma chalcodes (Ach); Phrynus marginemaculatus (Pma2); Stenochrus portoricensis (Stp); Latrodectus tredecimguttatus (Lat_tre)
			Pycnogonida	Achelia echinata (Aeli); Endeis laevis (Ele); Ammothea hilgendorfi (Ahi)

Table S9. Full names/abbreviations and taxonomic classification of species shown in Figure S8.

Amphibia	Xenopus tropicalis (Xen_tro)
Arthropoda	Anopheles gambiae (Ano_gam), Apis mellifera (Api_mel), Drosophila melanogaster (Dro_mel), Drosophila pseudoobscura pseudoobscura (Dro_pse), Amblyomma maculatum (Amb_mac), Haplopelma hainanum (Hap_hai), Phoneutria nigriventer (Pho_nig), Cupiennius salei (Cup_sal), Ancylometes sp (Anc_sp), Latrodectus hesperus (Lat_hes), Lycosa singoriensis (Lyc_sin), Lachesana tarabaevi (Lac_tar), Cheiracanthium punctorium (Che_pun), Lycosa kazakhstanicus (Lyc_kaz), Aedes aegypti(Aed_aeg), Tribolium castaneum(Tri_cas), Drosophila grimshawi(Dro_gri), Danaus plexippus(Dan_ple), Anopheles gambiae str (Ano_gam), Culex quinquefasciatus(Cul_qui), Ixodes scapularis(Ixo_sca), Amblyomma maculatum(Amb_mac), Crassostrea gigas(Cra_gig), Mesobuthus eupeus(Mes_eup), Buthotus judaicus(But_jud), Glossina morsitans morsitans (Glo_mor), Rhynchium brunneum (Rhy_bru), Polistes exclamans(Pol_exc)
Aves	Gallus gallus (Gal)
Mammalia	Pan troglodytes (Chimpanzee), Mus musculus (Mus), Pan troglodytes (Chimpanzee), Rattus norvegicus (Rat), Homo sapiens (Homo), Canis familiaris (Dog)
Trematoda	Schistosoma japonicum(Sch_jap), Clonorchis sinensis(Clo_sin)
Teleostei	Danio rerio (Dan_rer)[Zebrafish], Oreochromis niloticus (Ore_nil), Takifugu rubripes (Tak_rub)
Other	Nematostella vectensis (Nem_vec), Oikopleura dioica(Oik_dio), Amphimedon queenslandica (Amp_gue), Pseudonaja textilis(Pse_tex), Vipera nikolskii(Vip_nik)