Spiders and venom

MASARYK UNIVERSITY

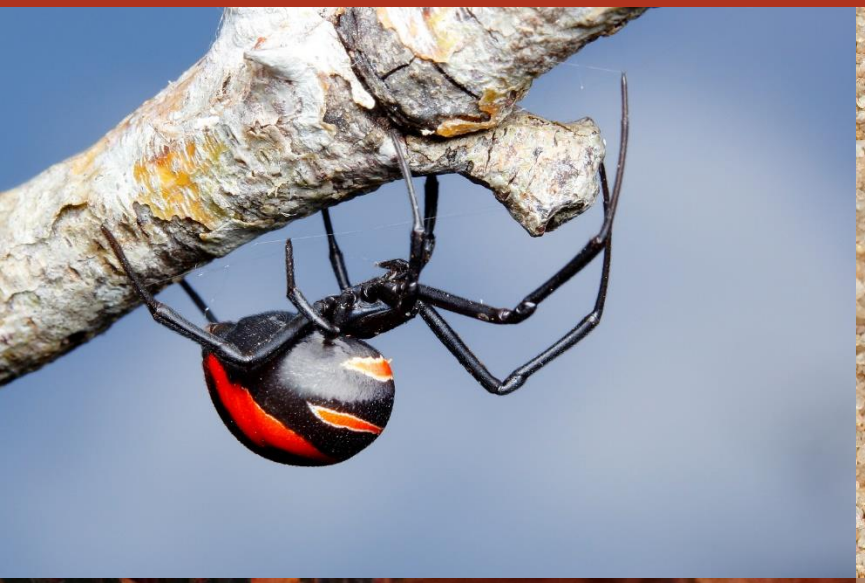
Ondřej Michálek

FACULTY OF SCIENCE · DEPARTMENT OF BOTANY AND ZOOLOGY · MASARYK UNIVERSITY · BRNO

OUTLINE

1) Introduction to venom & venom in spiders
2) Venom apparatus morphology
3) Venom chemistry
4) Venom evolution
5) Venom ecology
6) Methods to study venoms & venom applications
7) Sum-up

Part 1: Introduction to venom






What is venom?

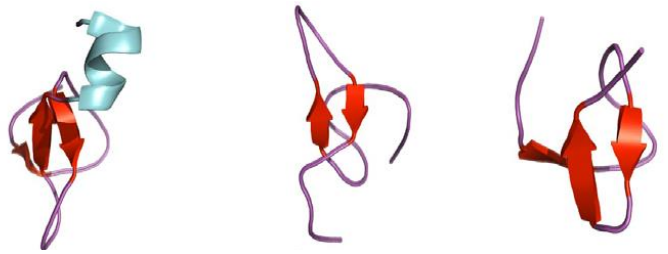
- physical warfare -> chemical warfare

- „A biological substance produced by an organism that contains molecules (“**toxins**”) which **interfere with physiological or biochemical processes** in another organism, which has evolved in the venomous organism **to provide a benefit** to itself once introduced to the other organism. The venom is **produced** and/or stored in **a specialised structure** and **actively transferred to another organism** through **an injury** by means of **a specialised delivery system**.”

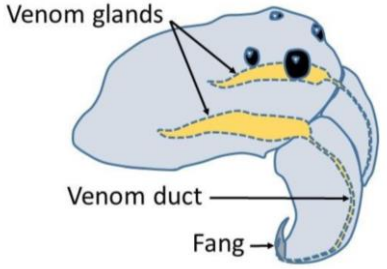
  (Arbuckle, 2017)

What is venom?

- **Key aspects of venom:**
  - Toxins
  - Venom gland/tissue
  - Specialised venom apparatus
  - Transfer to target animal through injury
  - Alter physiological or biochemical processes in target animal (paralysis)
  - A benefit to venomous animal

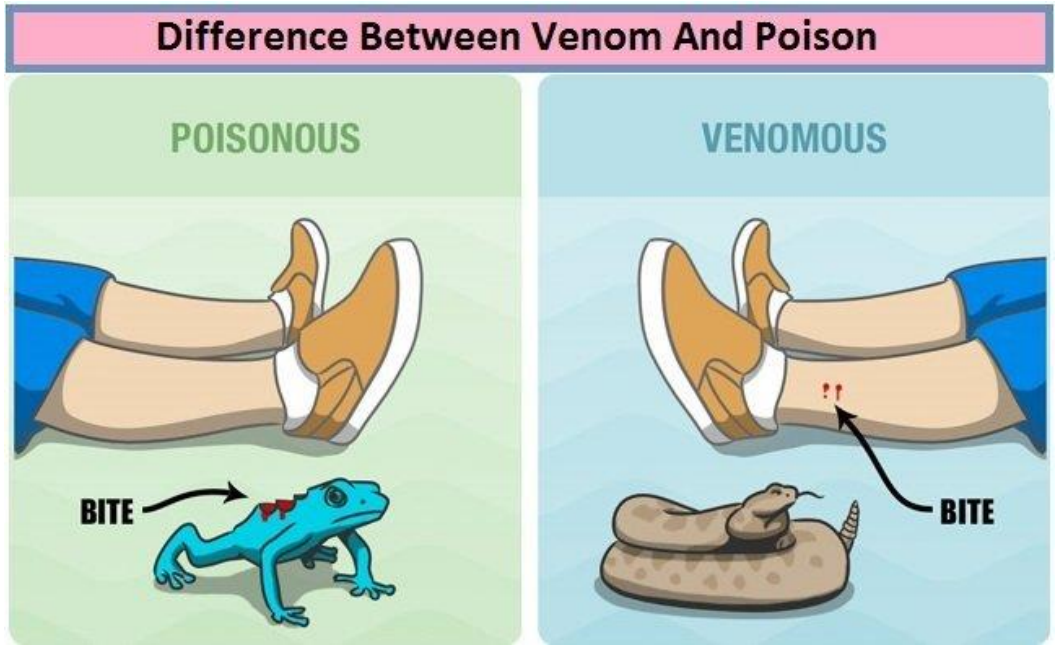


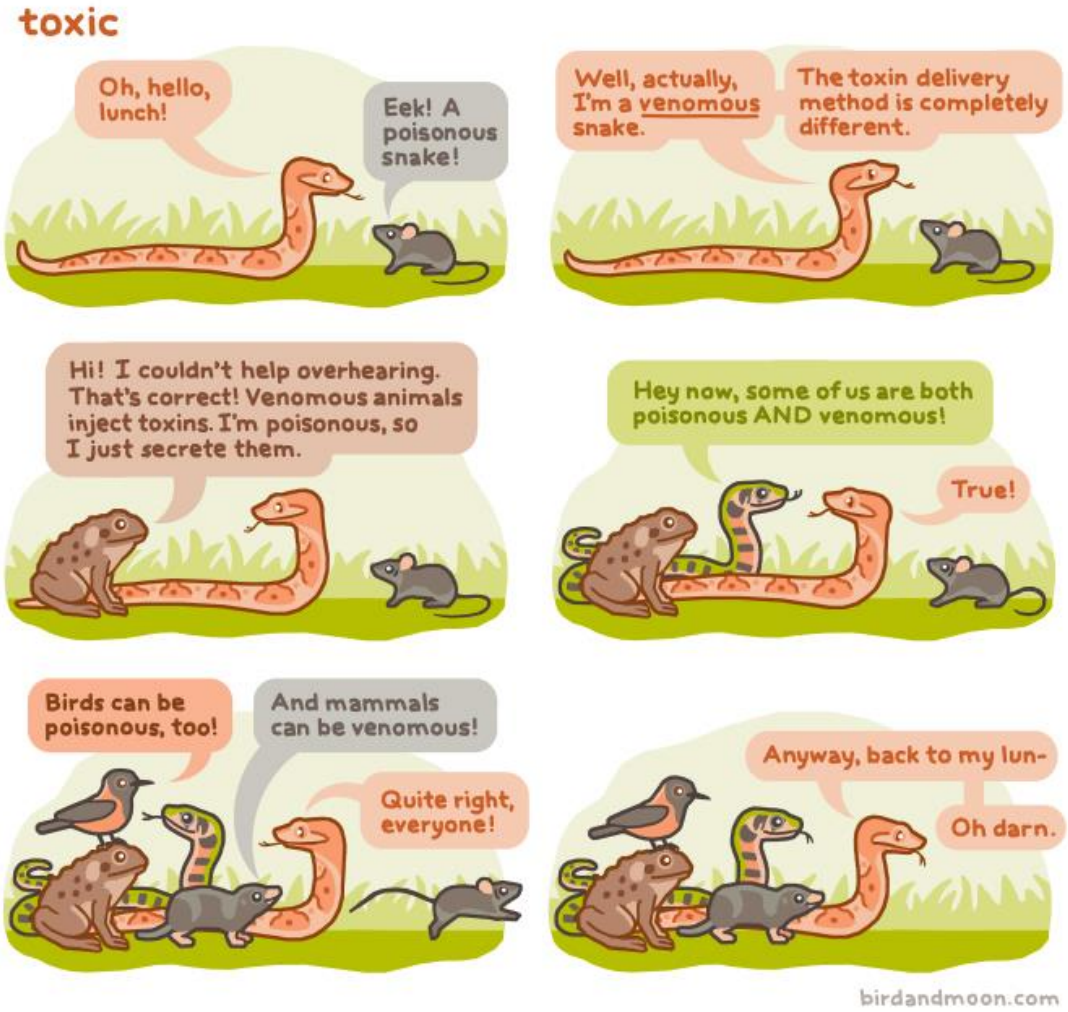
Lüddecke et al., 2019



Koua et al., 2020

Venom *vs* poison





Venom *vs* poison *vs* toxungen

Table 2. Critical components and features that distinguish the three major categories of biological toxins

Biological toxin	Delivery mechanism	Penetration wound	Mechanism of transfer or deployment
Poison	No	No	Ingestion, inhalation, or absorption across body surface
Toxungen	Yes	No	Delivered to body surface without accompanying wound
Venom	Yes	Yes	Delivered to internal tissues *via* wound

Nelsen et al., 2014

Photo: Wolfgang Wuster

Venom function

- Predation
- Defence

Venom function – not just predation and/or defence…

Table 1. Functional diversity of venom. Examples of uses of venom beyond predation, defense, and blood-feeding.

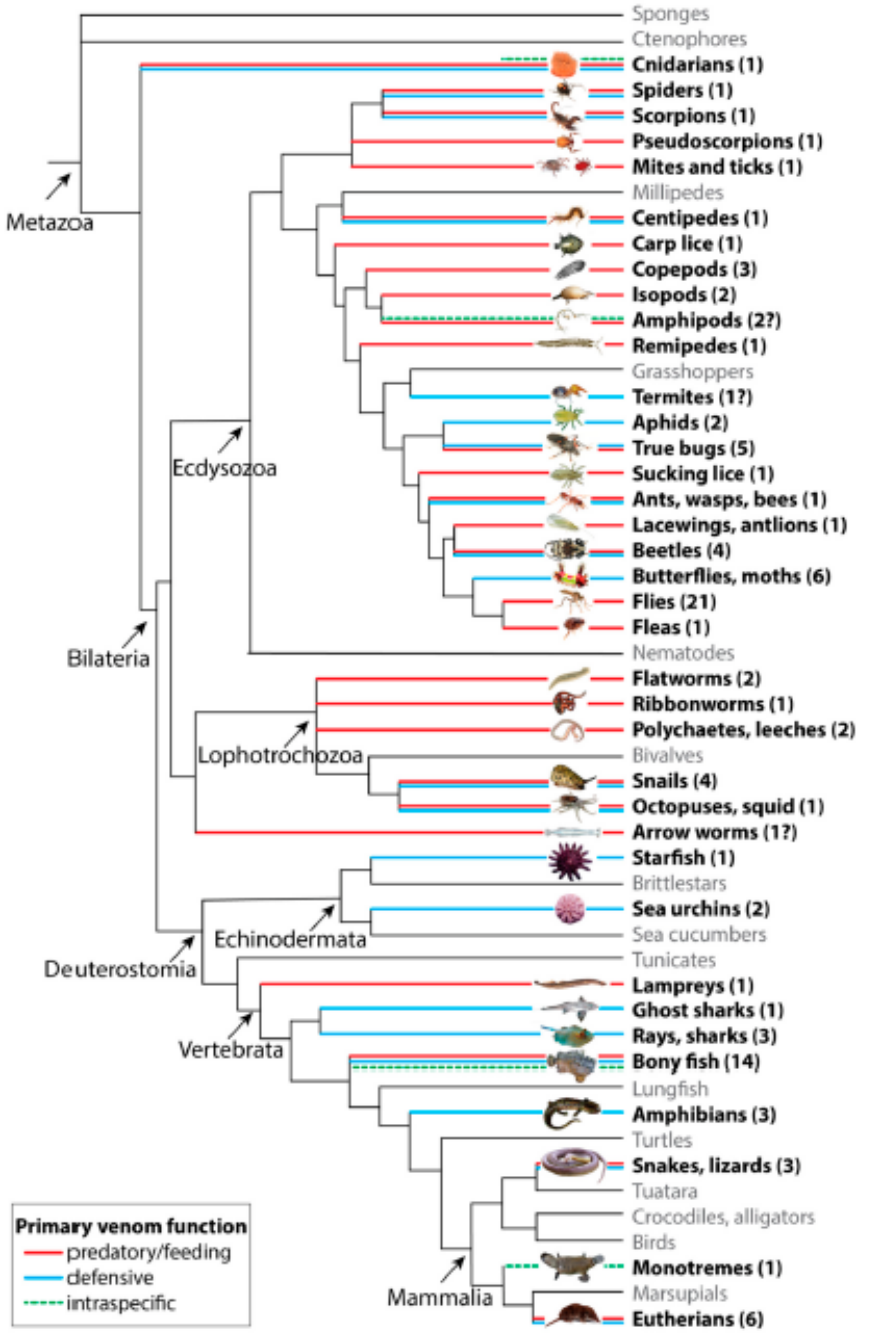
Function	Example of Venomous Animal	References
Intraspecific competition	Platypus, sea anemones, slow loris	[51–53]
Food storage	Moles, shrews, parasitoid wasps	[54,55]
(Pre-)Digestion	Sea anemones, assassin bugs, centipedes, remipedes, vipers	[56–60]
Offspring care	Sea anemones, cubozoan jellyfish, parasitoid wasps, saw flies	[55,61–63]
Mating	Scorpions	[27]
Habitat creation	Ants	[64]
Antimicrobial ointment	Ants, wasps	[65]
Ectoparasite deterrent	Slow loris	[66]
Antivenom	Tawny crazy ant (*Nylanderia fulva*)	[28]
Prey homing device	Rattlesnakes	[67]
Intraspecific communication	Ants, wasps	[68–70]

Schendel et al., 2019

How widespread is venom?

- Evolved independently more than 100 times
- Eight separate phyla

(Schendel et al., 2019)

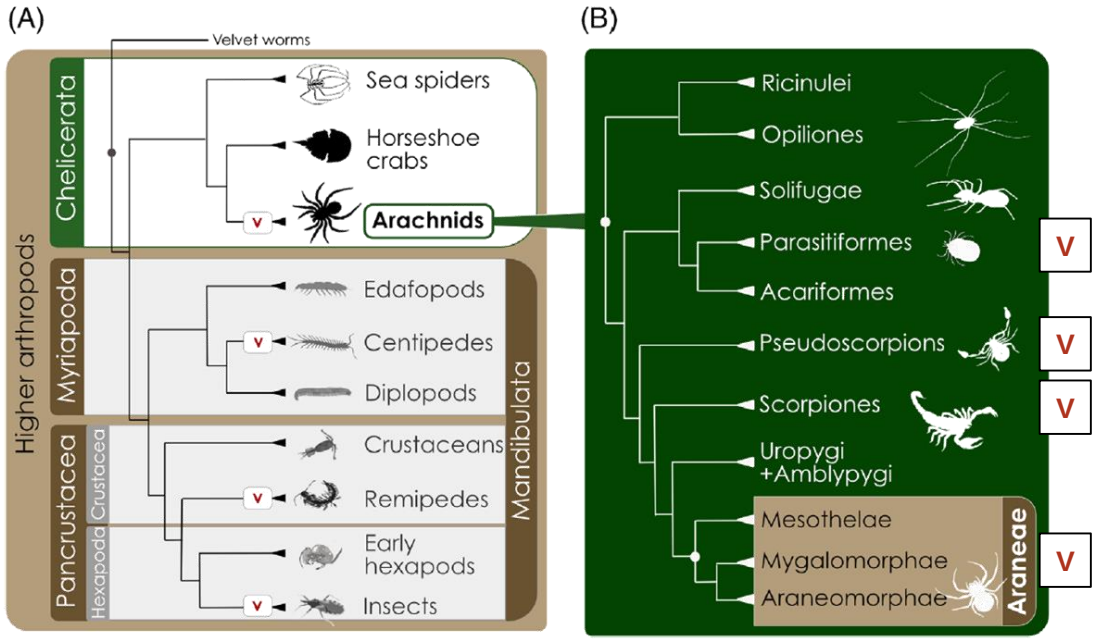


How widespread is venom?

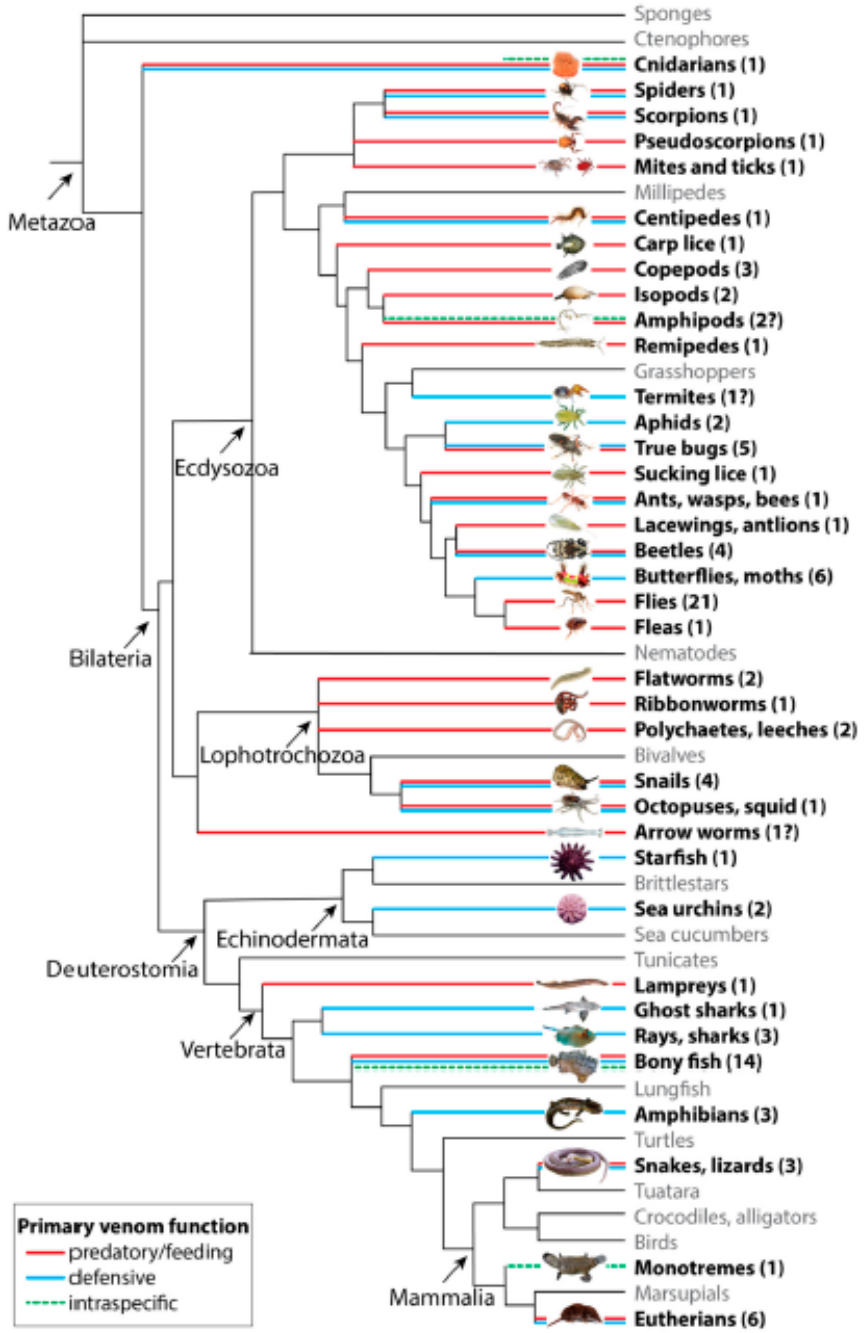
- Evolved independently more than 100 times
- Eight separate phyla

(Schendel et al., 2019)

- Arthropods and arachnids:



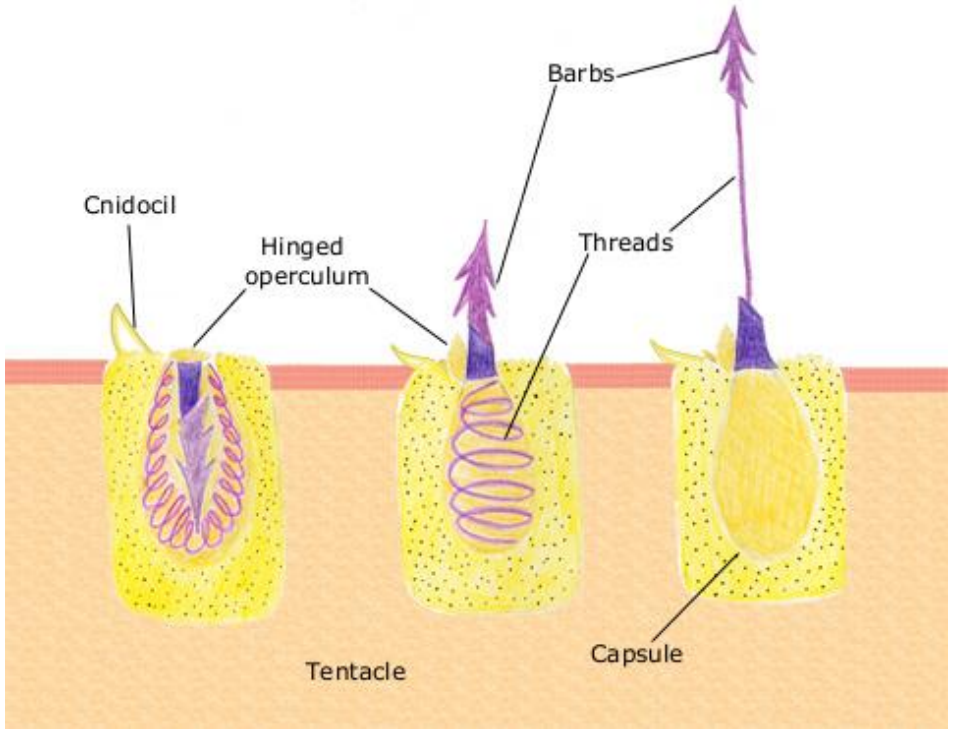
(Lüddecke et al., 2021)



Part 2: Venom apparatus morphology

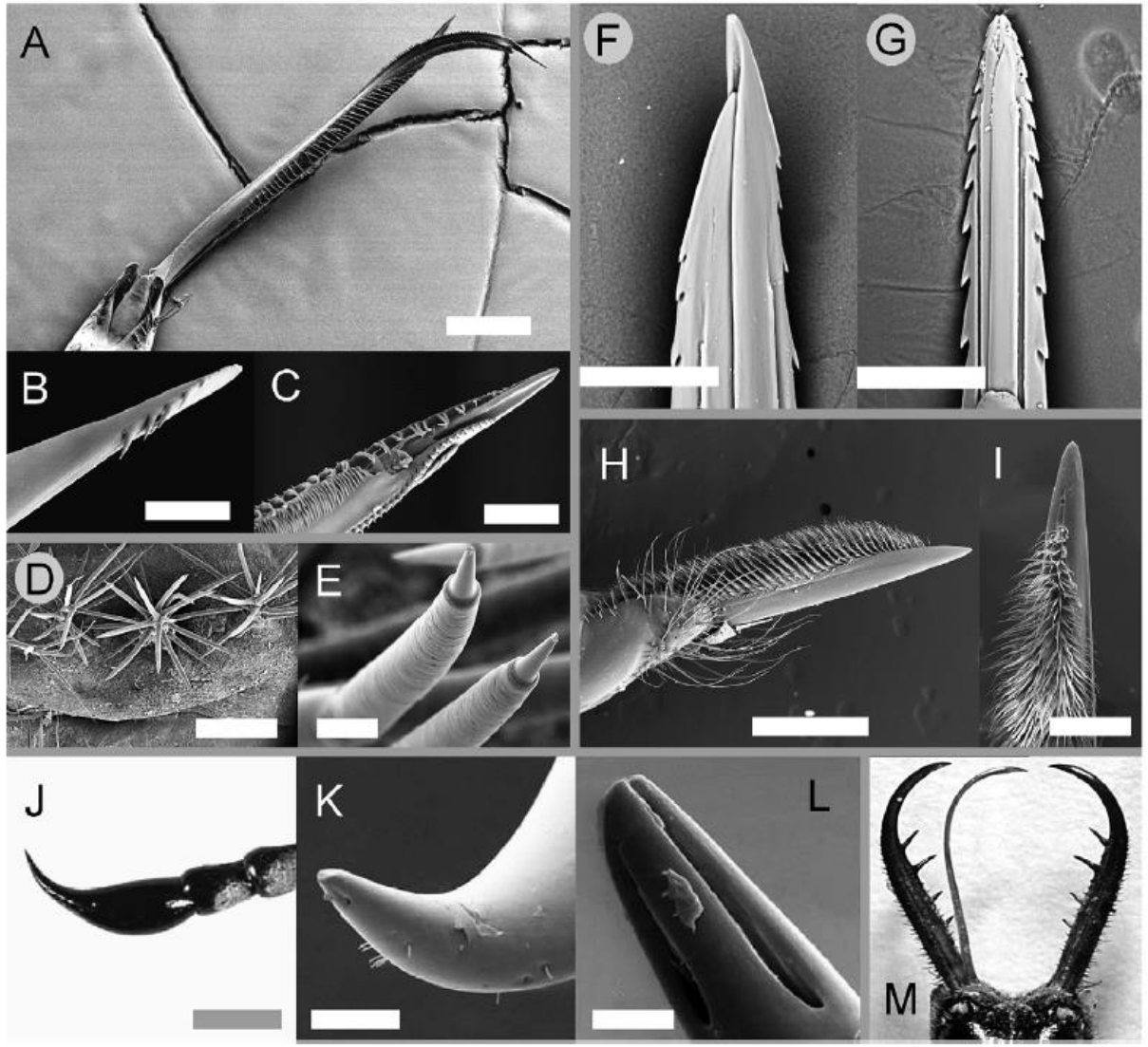
Gereral morphology

- Venom glands or organelles
- Venom apparatus



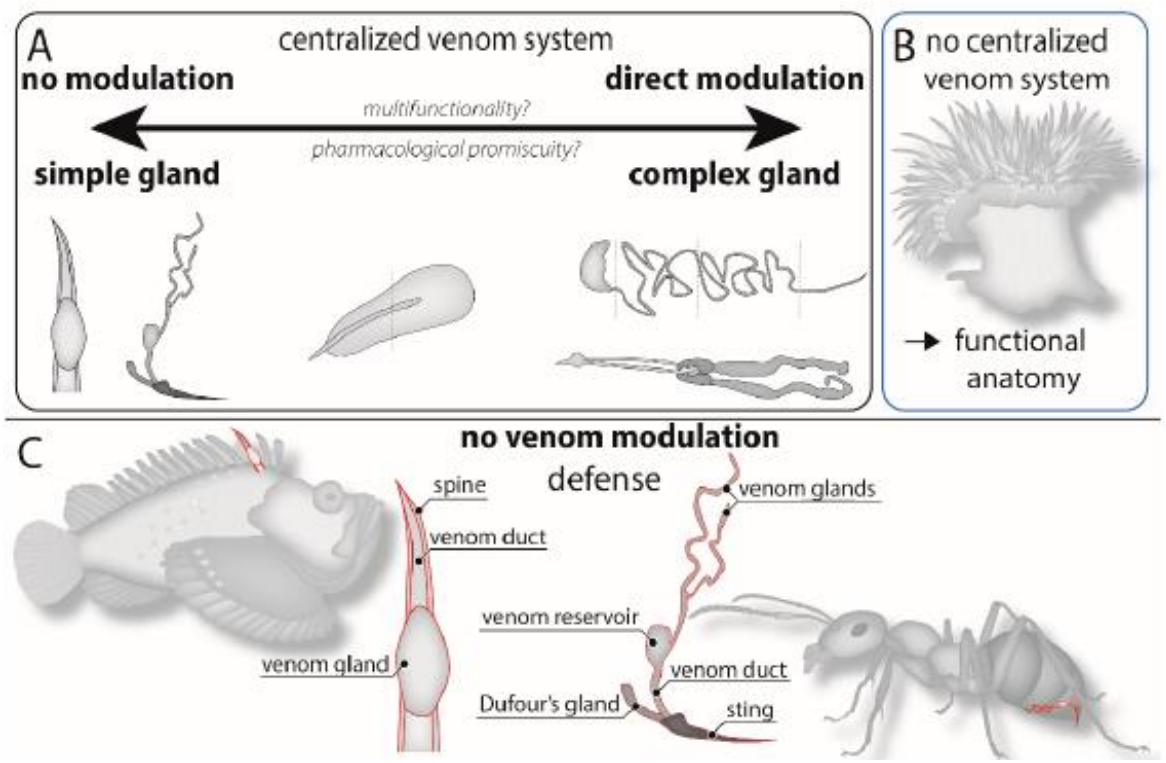
Nematocyst of cnidarians

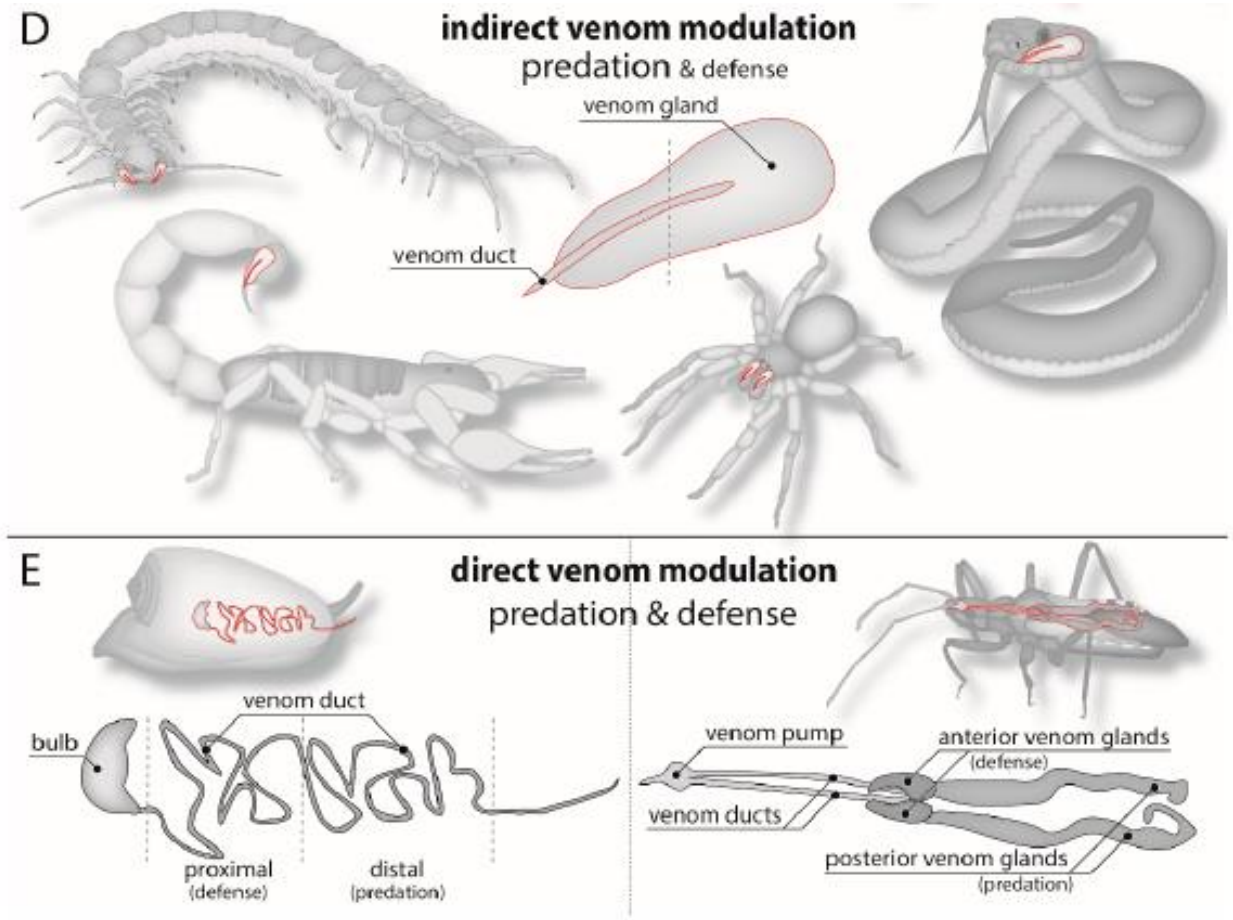
Insect venom apparatus



Walker al., 2018

Gereral morphology





Schendel et al., 2019

Spider venom apparatus morphology

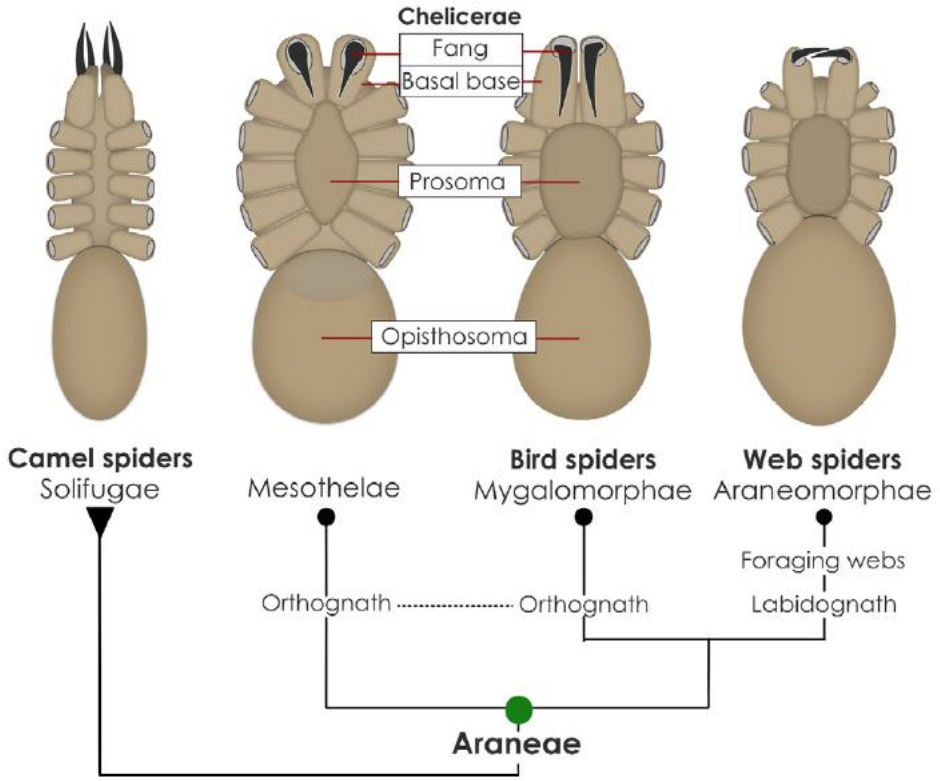


Fig 2. The chelicerae of the three major lineages within Araneae. The abstract ventral sides illustrate the different chelicerae configurations and major traits are plotted on the phylogram. Solifugae are added as an older chelicerate group that still feature the more ancient (plesiomorphic) state of chelicerae, which were originally three-segmented and scissor-like.

Lüddecke et al., 2021

Spider venom apparatus morphology

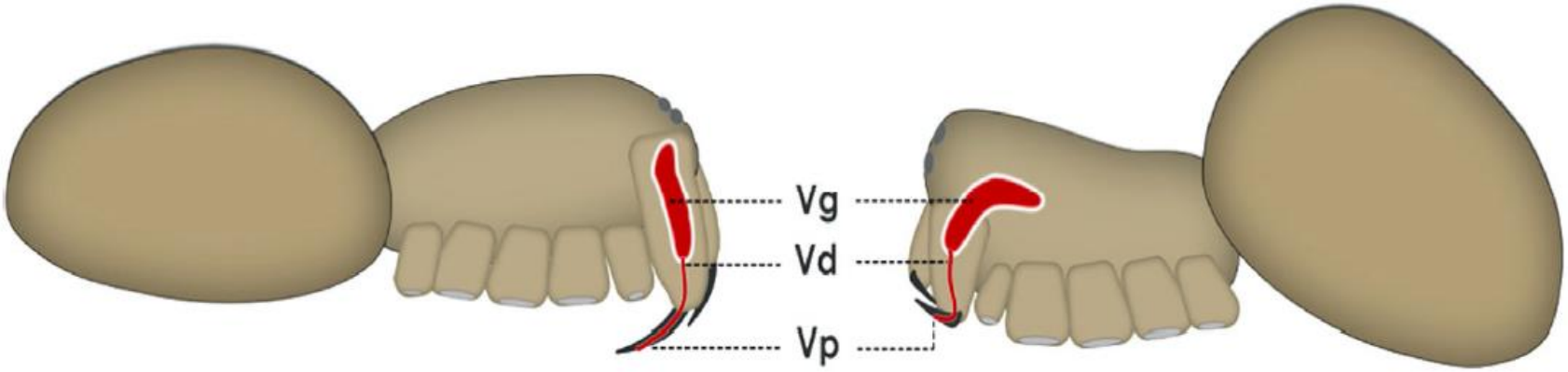


Fig 3. Comparison of the venom apparatus in orthognath and labidognath spiders. Venom glands (Vg) and venom duct (Vd) that leads to the venom pore (Vp) at the outer side of the fang tip are shown in red. The muscle layer surrounding the gland is illustrated in white.

Lüddecke et al., 2021

Part 3: Venom chemistry

THE CHEMISTRY OF SPIDER VENOM

Spider venoms are complex mixtures with a large number of components. This graphic looks at the chemical identities of some of these components, and their roles in venoms.

TYPES OF VENOM

Venoms are grouped into two main categories, necrotic or neurotoxic, though some spider venoms can exhibit both types of effect.

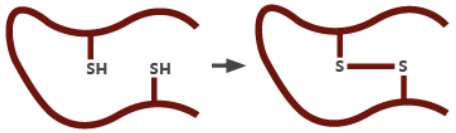
NECROTIC

Also referred to as 'cytotoxic'. Damages and kills the cells and tissue around the site of the bite, causing blisters, inflammation, and lesions to appear. Recluse spiders and South African Sand spiders have necrotic venom.

NEUROTOXIC

These venoms act directly on the nervous system. They interfere with nerve signals; in extreme cases, this can lead to death from respiratory or cardiac failure. Black Widow & Funnel Web spiders have neurotoxic venom.

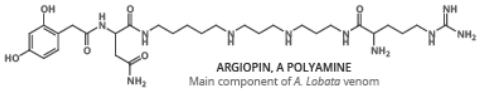
LINEAR & DISULFIDE-CONTAINING PEPTIDES



DISULFIDE BONDS IN A PEPTIDE
Disulfide bonds form between thiol (-SH) groups on peptide chains

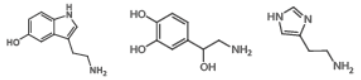
Cytolitic peptides are linear molecules, responsible for the necrotic activities of some venoms. They show activity against a broad range of target cells, have a function in aiding external digestion, and can also be synergistic with neurotoxins. Larger peptides containing disulfide bonds are neurotoxic, exerting effects by acting on certain ion channels. They are the major toxic components of the majority of spider venoms.

LOW MOLECULAR WEIGHT COMPOUNDS



ARGIOPIN, A POLYAMINE
Main component of *A. Lobata* venom

These are defined as compounds with a molecular weight of less than 1000, and include salts, carbohydrates, amino acids, amines, and acylpolyamines, some of which act synergistically with other venom components. Amines can contribute pain, and also help the venom spread from the bite site. Acylpolyamines help paralyse invertebrate victims by blocking ion channels that are activated by glutamate, but can also be effective against vertebrate nervous systems.



BIOGENIC AMINES
L to R: Serotonin, noradrenaline, histamine

43,244

Number of different spider species catalogued (as of 2011)

150,000

Estimated number of different spider species in existence

174

Number of species for which venom components described

Almost all spiders produce venom, but very few produce venom that is harmful to humans. Venoms are often investigated for potential agricultural and medicinal uses.

PROTEINS & ENZYMES

Enzymes in the venom play an important role in external digestion, and some can also act as spreading agents for the venom. In Recluse spider venoms, atracoxins are the primary toxic component. In Widow spiders, high molecular weight neurotoxins called latrotoxins cause release of neuromediators, resulting in blockade of nerve signal transmission.

NEURONS, SYNAPSES & AXONS
Many spider venom proteins bind to various molecular targets on neurons. Other venom components can assist them in reaching these. Greek letters in front of a toxin often denote its target; α = nictotine, acetylcholine and glutamate receptors, κ = potassium ion channels, μ = sodium ion channels, and ω = calcium ion channels.

Type of toxins

- **Haemotoxins**
  - disrupt haemostatic system
  - not common in spiders

- **Cytotoxins**
  - impair the structure and function of cell membranes
  - not common in spiders

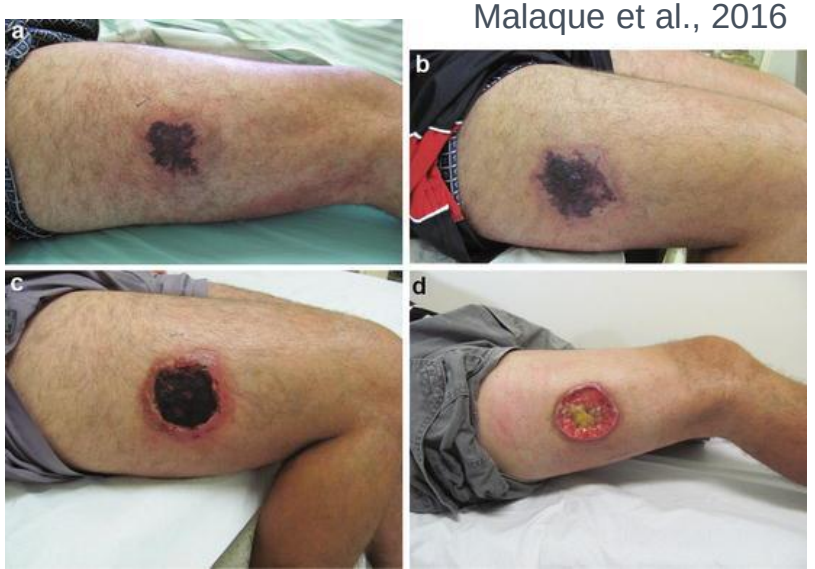
Type of toxins

- **Haemotoxins**
  - disrupt haemostatic system
  - not common in spiders

recluse spiders (*Loxosceles* spp.)

- **Cytotoxins**
  - impair the structure and function of cell membranes
  - not common in spiders

Malaque et al., 2016



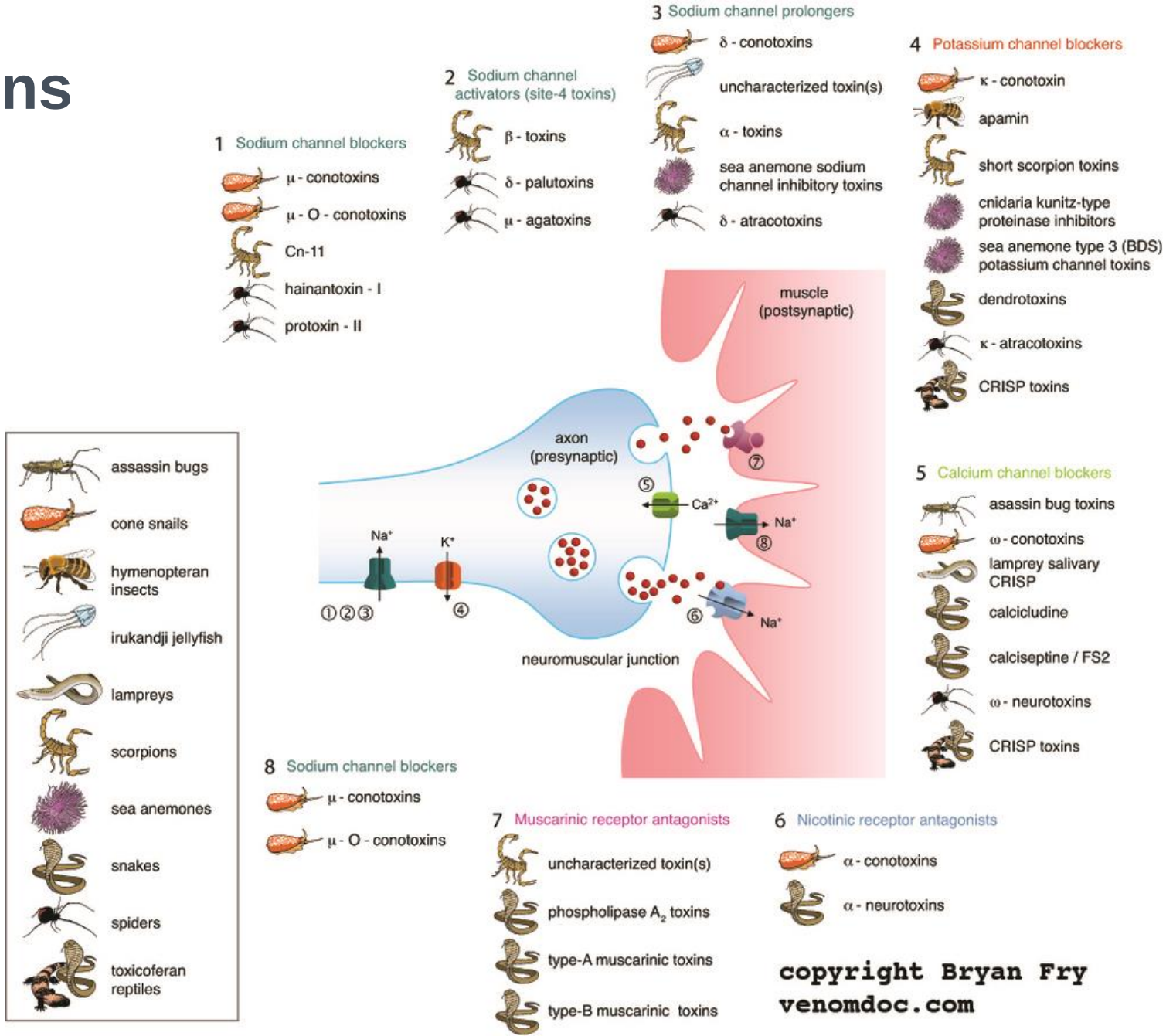
Type of toxins

- **Haemotoxins**
  - disrupt haemostatic system
  - not common in spiders

- **Cytotoxins**
  - impair the structure and function of cell membranes
  - not common in spiders

- **Neurotoxins**
  - presynaptically or postsynaptically affect neurotransmission
  - main components of spider venom

Neurotoxins

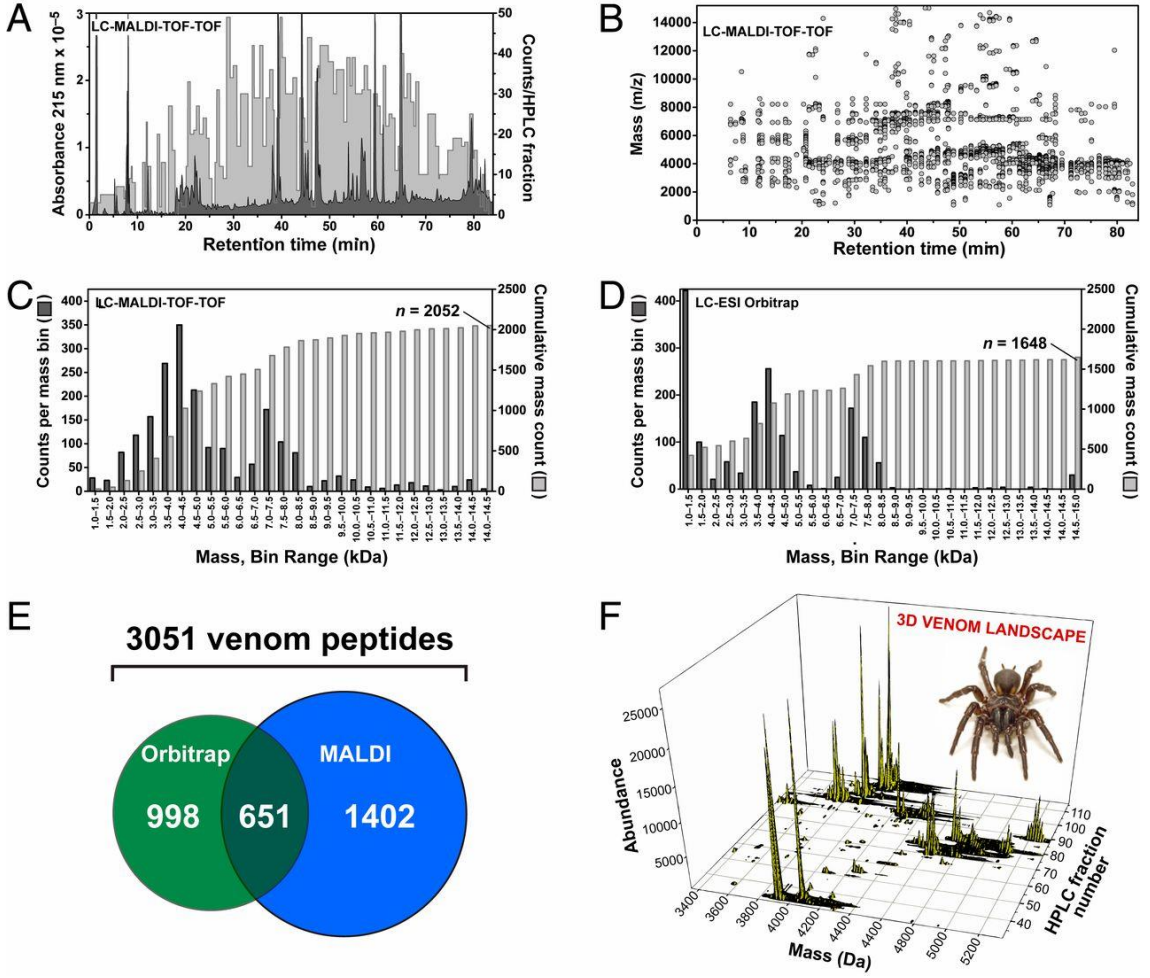


Venom biochemistry

- Tens to thousands of components in single species
- Hereafter emphasis on spider venom
- **Types of molecules:**
  - Small molecules
  - Peptides
    - Antimicrobial Peptides
    - Cysteine-rich Peptides
  - Proteins

Photo: David Wilson

Hadronyche infensa



Pineda et al., 2020

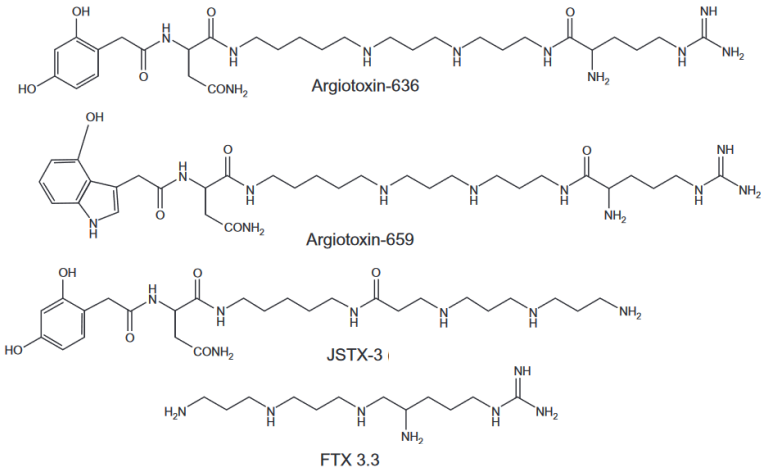
Small molecules

- < 1 kDa

- **various compounds:**
  ions, salts, organic acids, nucleotides, nucleosides, amino acids, amines, alkaloids and polyamines

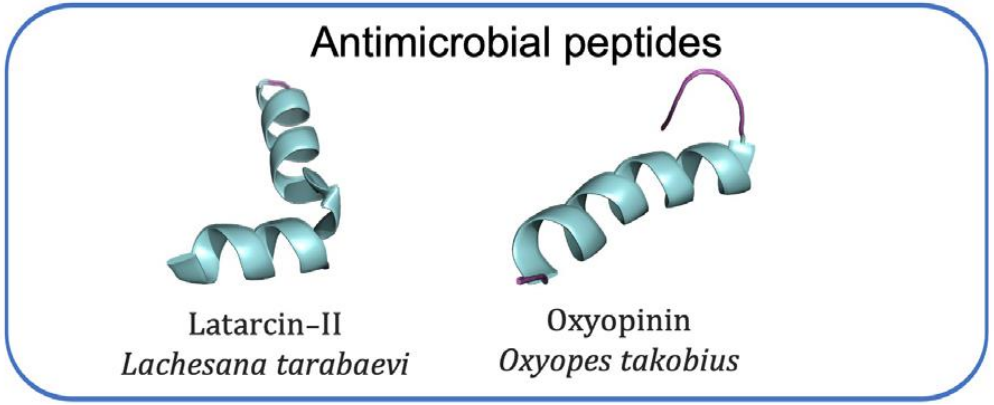
- **various functions:**
  - neurotransmitters
  - co-factors facilitating the folding and activity of toxins
  - insecticidal neurotoxins

examples of spider acylpolyamines



reviewed in Lüddecke et al., 2021

Peptides



- < 10 kDa
- **Antimicrobial Peptides**
  - linear, α-helical peptides without disulfide bonds
  - dual function:
    antimicrobial activity or lytic peptides
  - most of those identified AMPs found in Lycosidae and Theraphosidae;
    also Zodariidae or Oxyopidae
  - more than 50 such peptides in the venom of *Lycosa sinensis*

reviewed in Lüddecke et al., 2021

Peptides

- < 10 kDa
- **Antimicrobial Peptides**

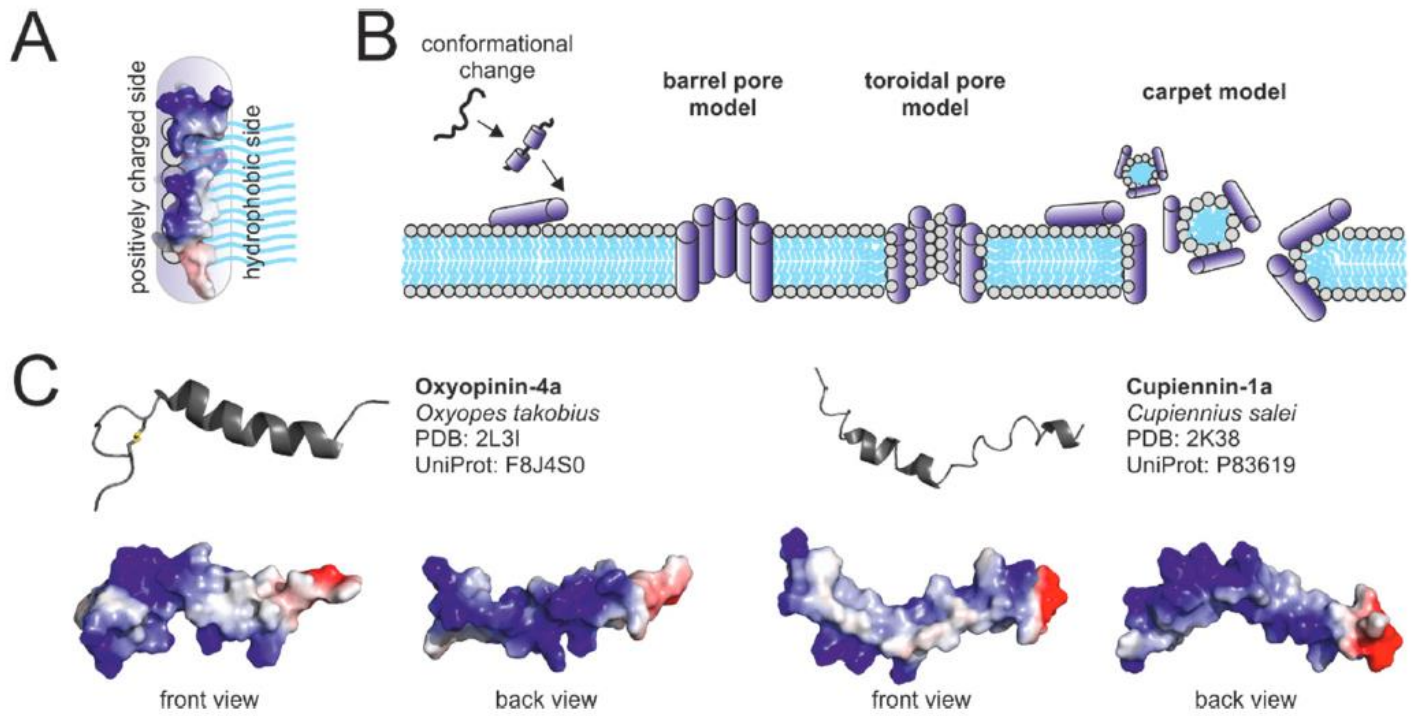
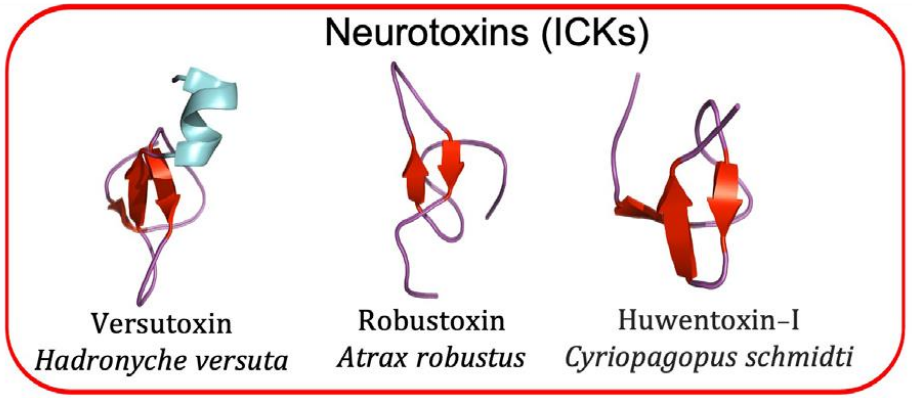


Figure 1. Antimicrobial peptides (AMPs) and their proposed mechanism of action. (**A**) Model of interaction between an AMP and phospholipids. AMPs assume an amphipathic α-helical structure in proximity to cellular membranes. The hydrophobic side of the helix (white) inserts into the membrane and interacts with the phospholipid side chains. The positively charged side (blue) interacts with negatively charged lipid head groups. (**B**) Models of membranolytic actions of AMPs. (**C**) NMR-based 3D structures of two antimicrobial peptides from spider venom. Electrostatics were computed using PDB2PQR [70]. Blue surfaces represent positively charged surfaces; red negative charged; and white neutral.

Langenegger, Nentwig & Kuhn-Nentwig, 2019

Peptides

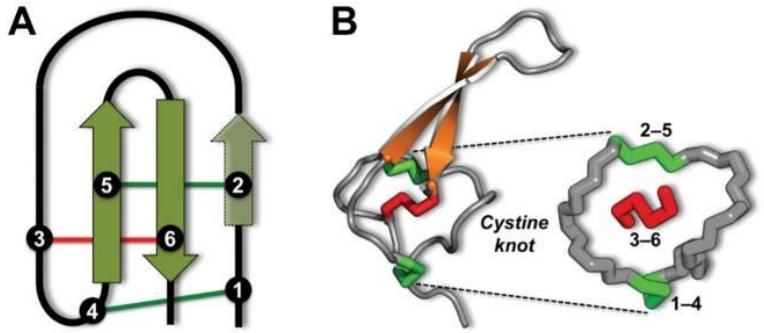


- < 10 kDa
- **Antimicrobial Peptides**
- **Cysteine-rich Peptides**
  - functionally most important group of components
  - principal neurotoxic components
  - typically with molecular masses below 10 kDa
  - rich in disulfide bonds
  - different families:
    Kunitz peptides, HAND peptides, DDH peptides, **ICK peptides**

reviewed in Lüddecke et al., 2021

Peptides

Inhibitor cystine knot (ICK)



Herzig & King, 2015

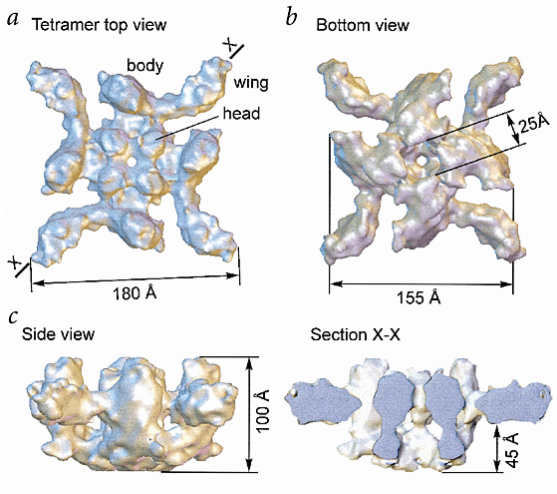
- **ICK (inhibitor cysteine knot) peptides**
  - **structure:**
    (triple-stranded) antiparallel β-sheets
    at least 6 cysteine residues (forming 3 disulfide bridges)
    -> pseudoknot motif
  - expanded cysteine scaffolds and/or double ICK (dICK) motifs
  - exceptional **stability**
  - **mode of action:** stable complexes with prey receptors, disrupting their normal function
  - **targets:**
    voltage-gated sodium, potassium and calcium channels
    acid-sensing ion channels, glutamate receptors
    transient receptor potential channels

reviewed in Lüddecke et al., 2021

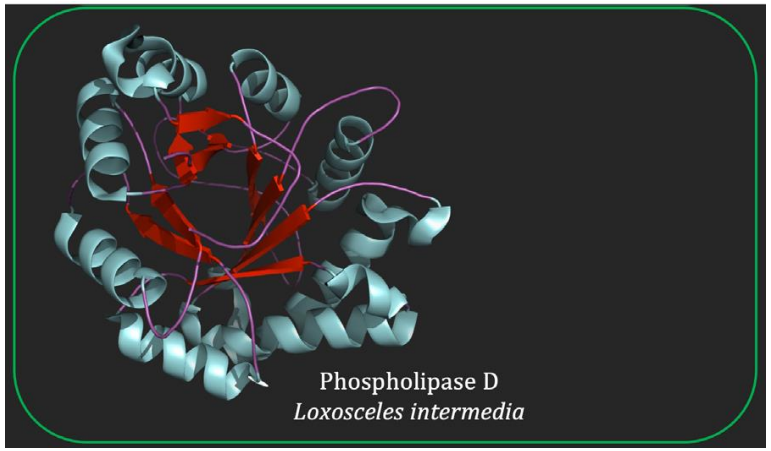
Proteins

- > 10 kDa
- key venom components in some taxa (e.g., black widows)

α-latrotoxin



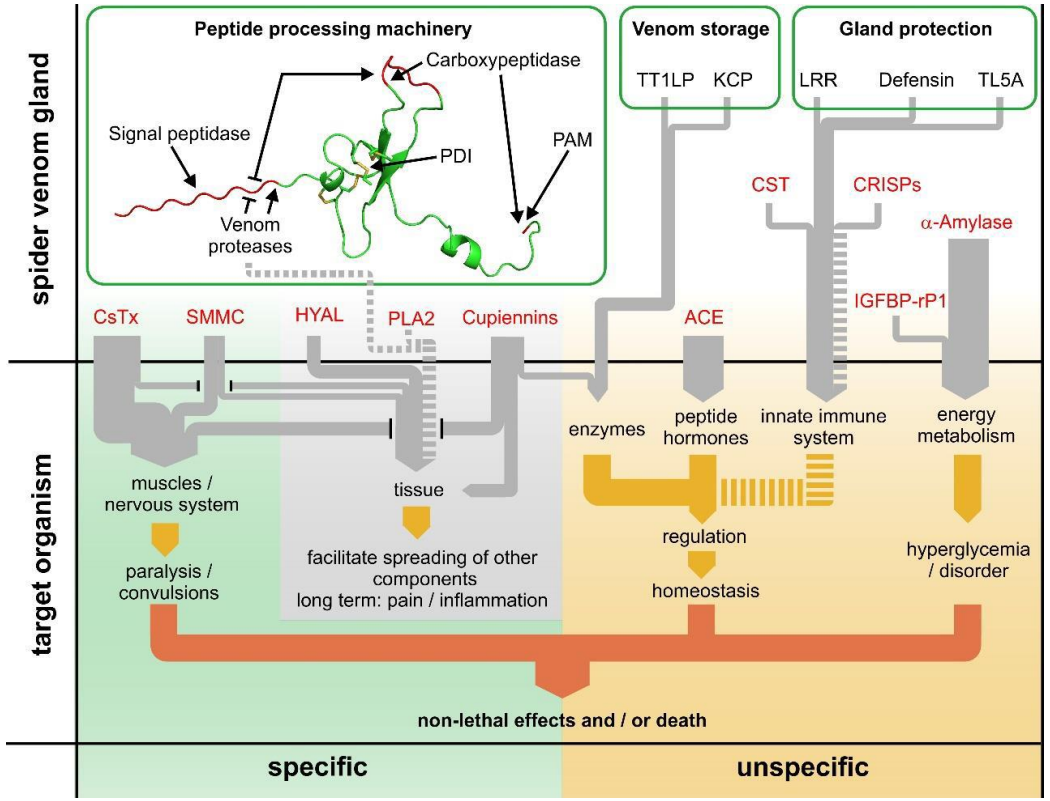
- **latrotoxins**
  - homotetrameric pores in the presynaptic neuronal membranes
- **phospholipase D**
  - highly cytotoxic sphingomyelin-hydrolysing enzyme
- **neprilysin metalloproteases, CAP proteins**
  - unclear function



reviewed in Lüddecke et al., 2021

Mode of action - synergistic effects of venom compounds

- temporally and spatially regulated interactions
- dual prey inactivation strategy (Kuhn-Nentwig et al., 2019)



reviewed in Lüddecke et al., 2021

Mode of action - synergistic effects of venom compounds

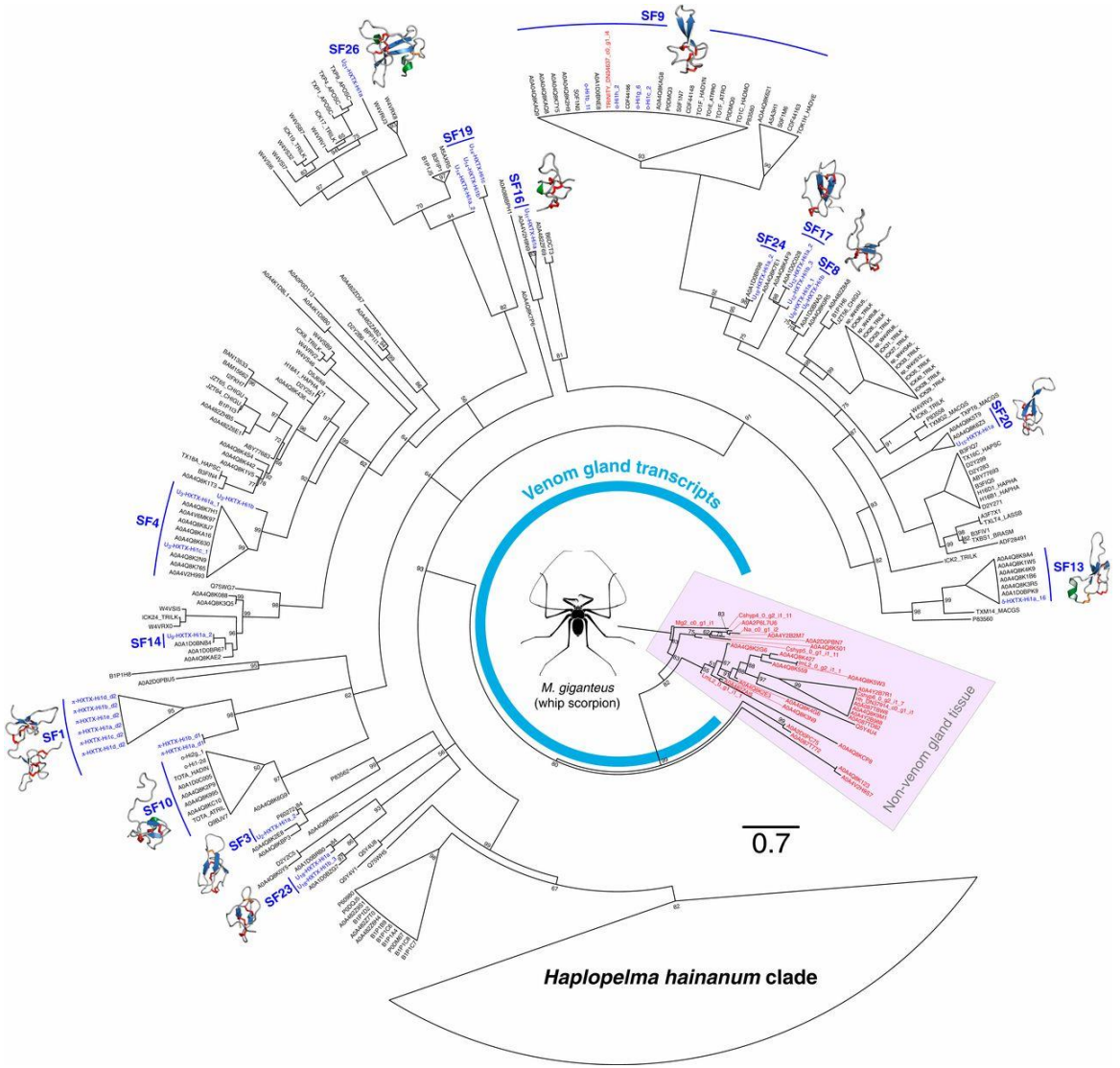
- temporally and spatially regulated interactions
- dual prey inactivation strategy (Kuhn-Nentwig et al., 2019)

> *Some of the large proteins immediately disrupt prey physiology and metabolism, while others act to spread the neurotoxins and thus trigger a subsequent wave of paralysis.*

- some peptides mediate or enhance the bioactivity of others
- rapid paralysis followed by long-term immobilization
- neurotransmitters making binding sites accessible for other toxins

reviewed in Lüddecke et al., 2021

Part 4: Venom evolution



Pineda et al., 2020

Evolution of the delivery system in spiders

- **origin not clear**
  - salivary glands, similar to those of ticks
  - silk-producing glands present in early chelicerates
- **the size and placement of the venom apparatus**
  - modern araneomorphs vs Mesothelae and Mygalomorphae
  - migration of the venom glands from the basal part of the chelicerae into the prosoma
  - reflected during ontogenesis

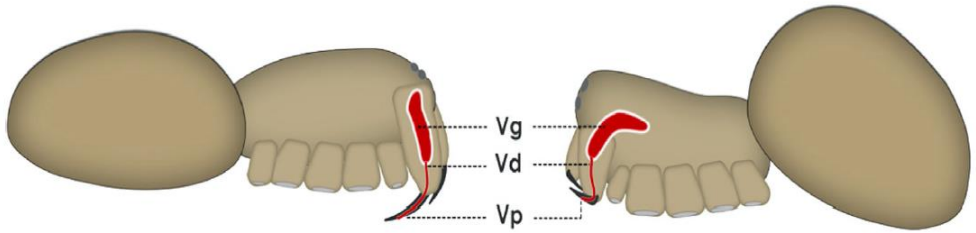


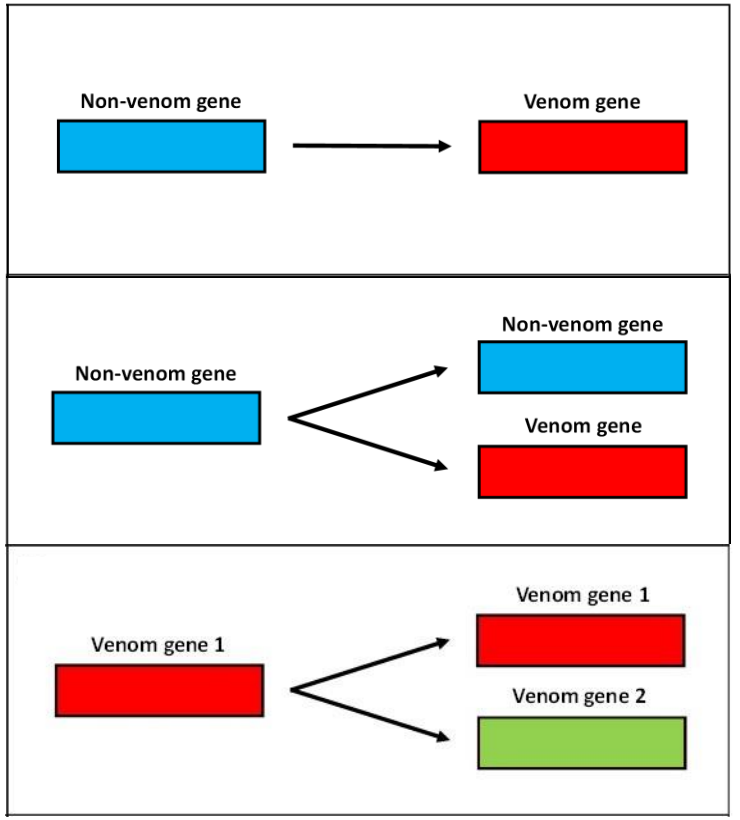
Fig 3. Comparison of the venom apparatus in orthognath and labidognath spiders. Venom glands (Vg) and venom duct (Vd) that leads to the venom pore (Vp) at the outer side of the fang tip are shown in red. The muscle layer surrounding the gland is illustrated in white.

reviewed in Lüddecke et al., 2021

Recruitment and neofunctionalization

Evolution of venom compounds

- recruitment and weaponization
  - signalling molecules into unregulated agonists or inhibitors
- frequent duplications
- existing toxins can also undergo neofunctionalization
  - new activities and functions



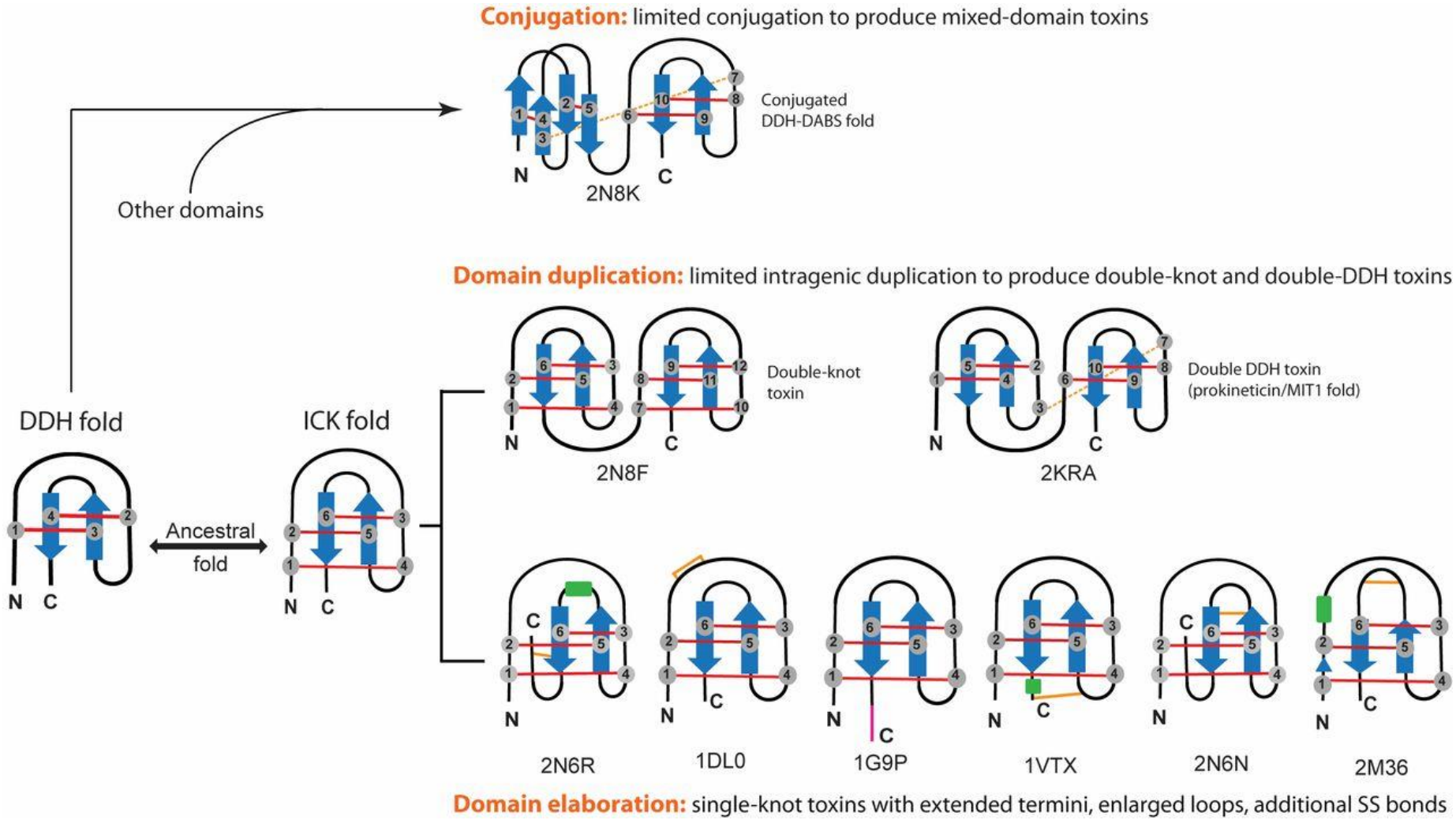
reviewed in Casewell et al., 2012; Lüddecke et al., 2021

Gene duplication

- **ICK peptides**
  - pseudoknot motif
    - amino acid substitutions can accumulate with little impact on structure
  - descendants of a single weaponized ICK lineage
  - duplication and structural diversification
  - domain duplication - dICK peptides
- also proposed for latrotoxins

reviewed in Lüddecke et al., 2021

Overview of spider ICK peptides evolution



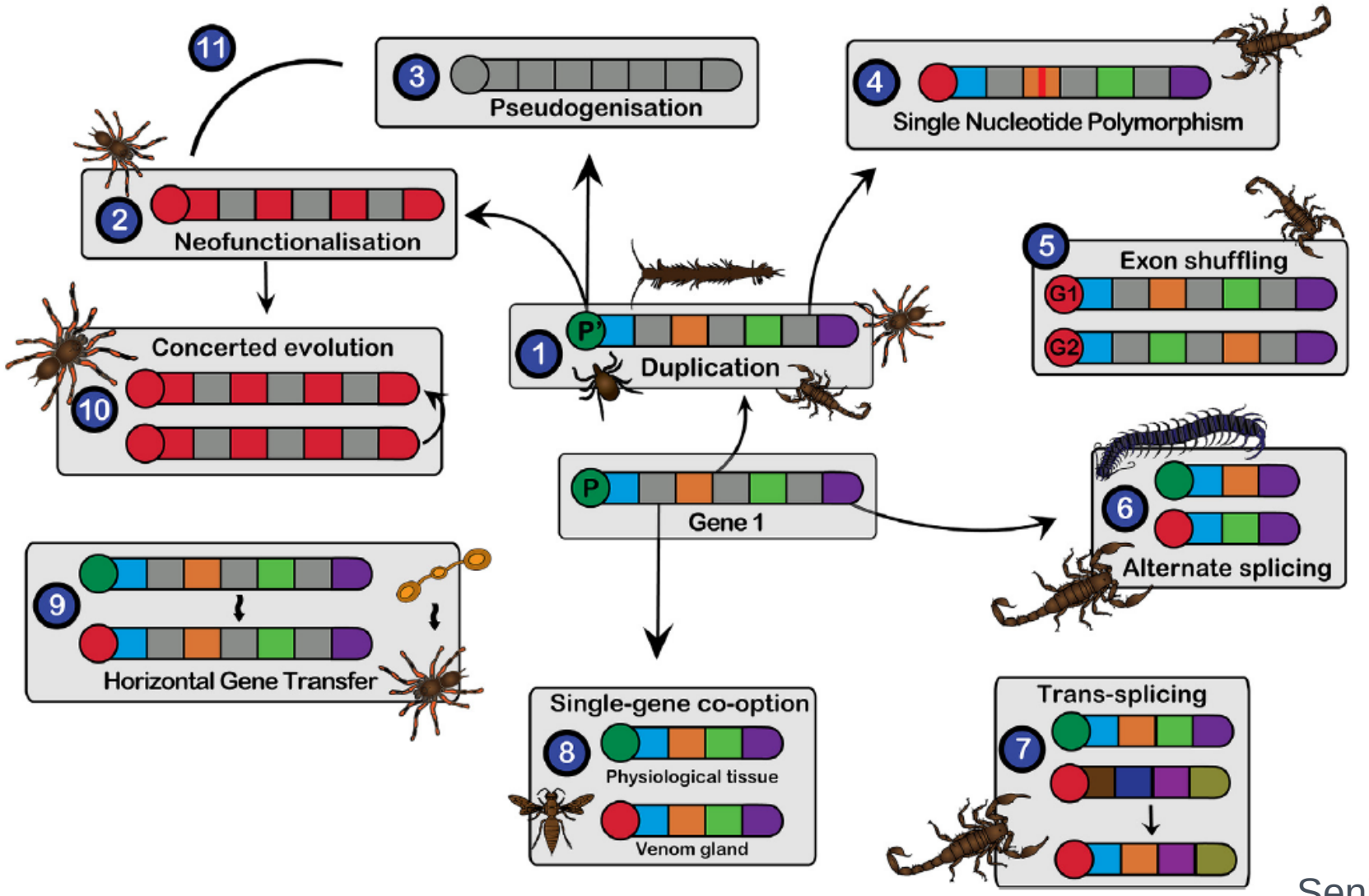
Pineda et al., 2020

Horizontal gene transfer

- recruitment of toxins from bacterial and fungal donors
- phospholipase D
  - in the family Sicariidae
  - a single proteobacterial ancestor
- also proposed to explain the origin of αLTX of *Parasteatoda tepidariorum*
- otherwise documented in other venomous taxa, such as centipedes (Undheim & Jenner, 2021)

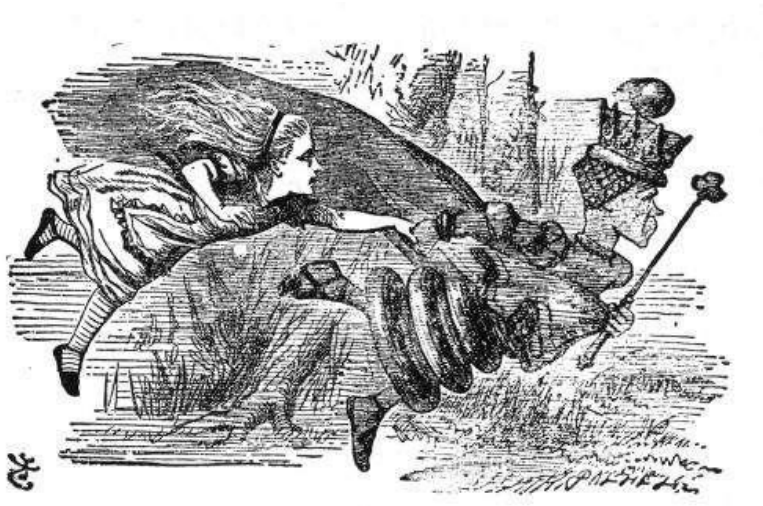
reviewed in Lüddecke et al., 2021

Molecular mechanisms of venom evolution - overview



Senji Laxme et al. 2019

Selection pressures acting on venom



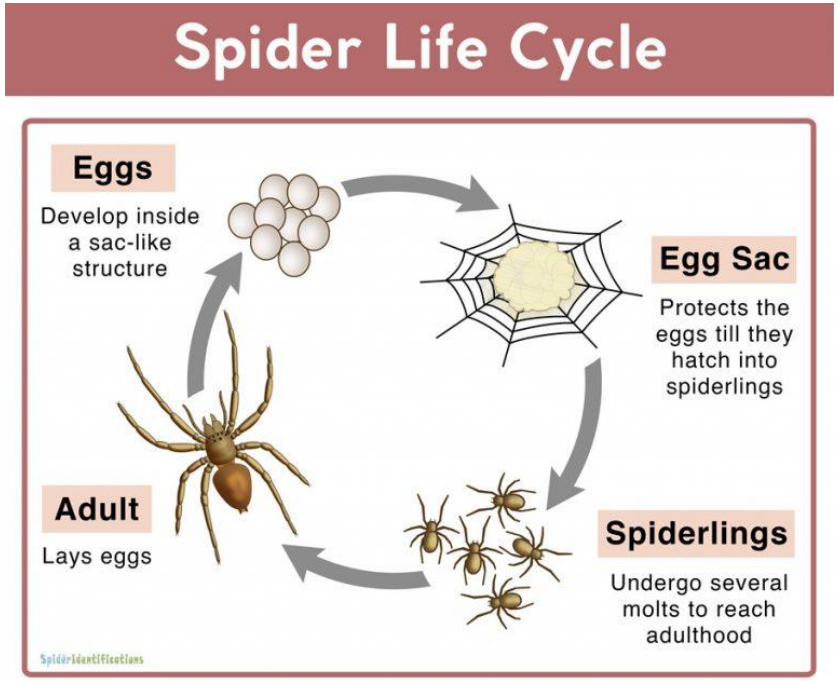
- strong positive selection
- ‘Red Queen hypothesis’ – predator *vs* prey
- but older lineages such as spiders show signatures of purifying selection
- a two-speed model of venom gene evolution
  - positive selection mostly acts during the early stages of ecological specialization
  - followed by an extended stage of purifying selection

reviewed in Lüddecke et al., 2021

Part 5: Venom Ecology

Ontogeny

- venom properties linked to life-history stages
- venom yield increases as the spider ages
- venom yield declines prior to ecdysis
- compositional alterations as the spider ages



reviewed in Lüddecke et al., 2021

Geograpic variation

- the variability of venom profiles
  between allopatric populations of the same species
- well documented in other venomous taxa, such as snakes
- little attention in spiders (contrasting results)

- ***Eratigena agrestis*** (Agelenidae)
  - Europe *vs* North America
  - no differences
- ***Loxosceles rufescens*** (Sicariidae)
  Latrodectus spp. (Theridiidae)
  - differences in venom profiles and toxicity

Eratigena agrestis

Photo: Rudolf Macek

Loxosceles rufescens

reviewed in Lüddecke et al., 2021

Sexual dimorphism

- well documented in spiders

- **australian funnel-web spiders**
  - fatal males bites in humans, females much less toxic

- ***Phoneutria nigriventer*** (Ctenidae)
  Loxosceles intermedia (Sicariidae)
  - females more toxic than males
  - sex-specific components

Hadronyche infensa

Photo: David Wilson

Phoneutria nigriventer

Photo: Graham Wise

reviewed in Lüddecke et al., 2021

Individual variability

- well documented in other venomous taxa, less attention in spiders
- only recently documented in *Hadronyche valida*

Photo: David Wilson

reviewed in Lüddecke et al., 2021

Envenomation strategies

- envenomation as major strategy
  to incapacitate prey in most spiders
  - some **exceptions**: Araneidae, Uloboridae, Scytotidae

Argiope bruennichi

Photo: Christine Hanrahan

- toxins on the silk strands
  of the web (Esteves et al., 2020)

Uloborus walckenaerius

Scytodes thoracica

Photo: Fritz Geller-Grimm

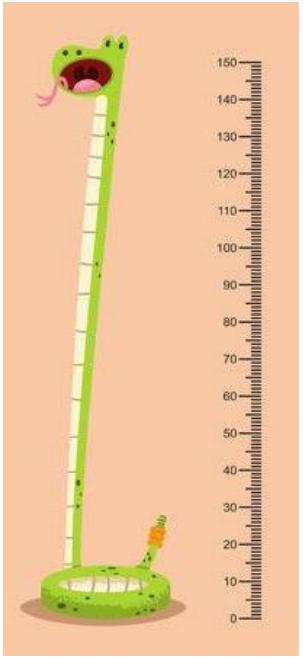
- venom in defence
  - escape prioritized
  - dry bites
  - aposematism
    (supported by pain-inducing components)

reviewed in Lüddecke et al., 2021

Venom optimization

- venom - physiologically **expensive**

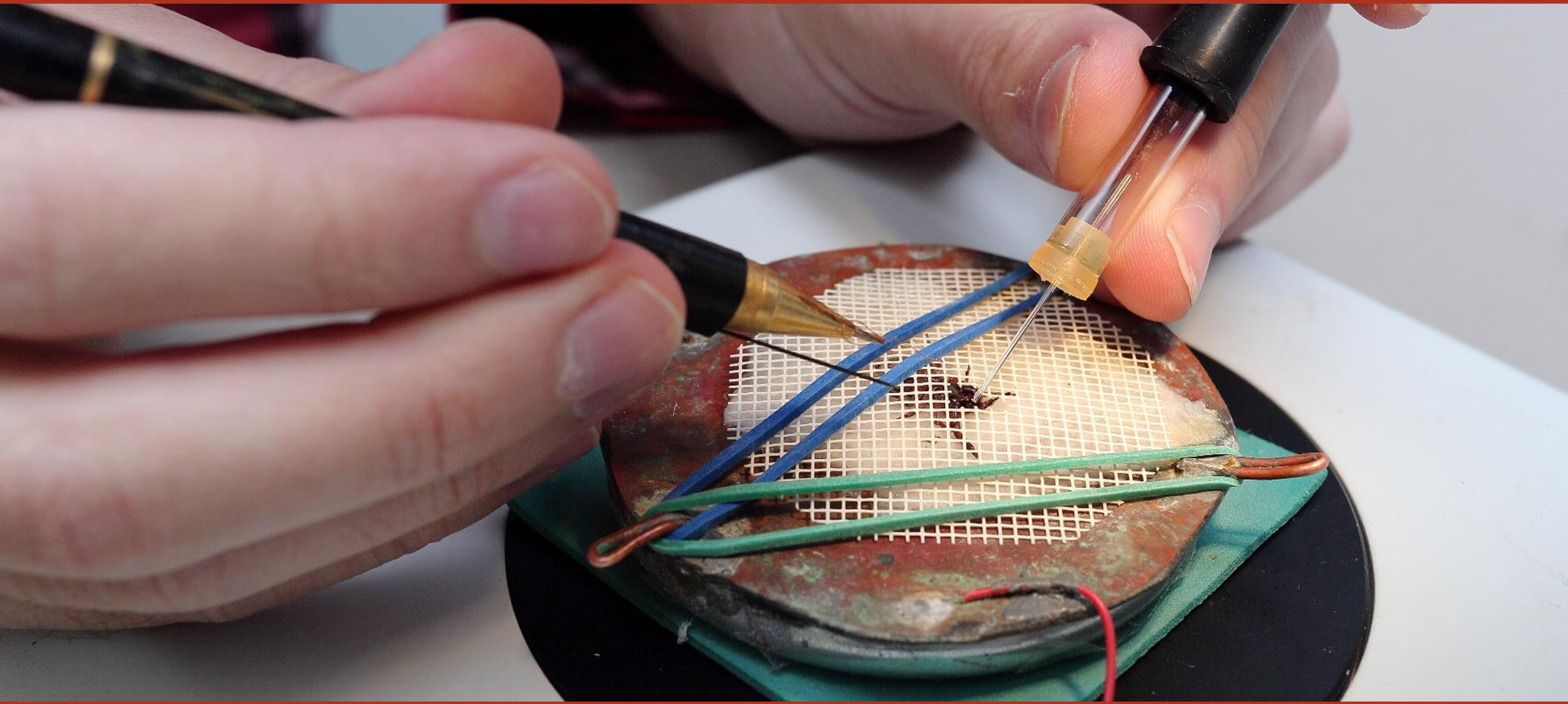
- **venom optimisation (or venom metering)**
  - conservation of venom resources
  - economical delivery of venom
    - weak prey -> small amount of venom
    - resistant/dangerous prey -> larger ammount of venom



- **trophic specialisation**
  - highly effective, but simpler venom
  - dispensable venom components purged from the venom to save resources

Lüddecke et al., 2021; Morgenstern & King 2013; Pekár et al., 2018a,b

Part 6: Methods & Applications

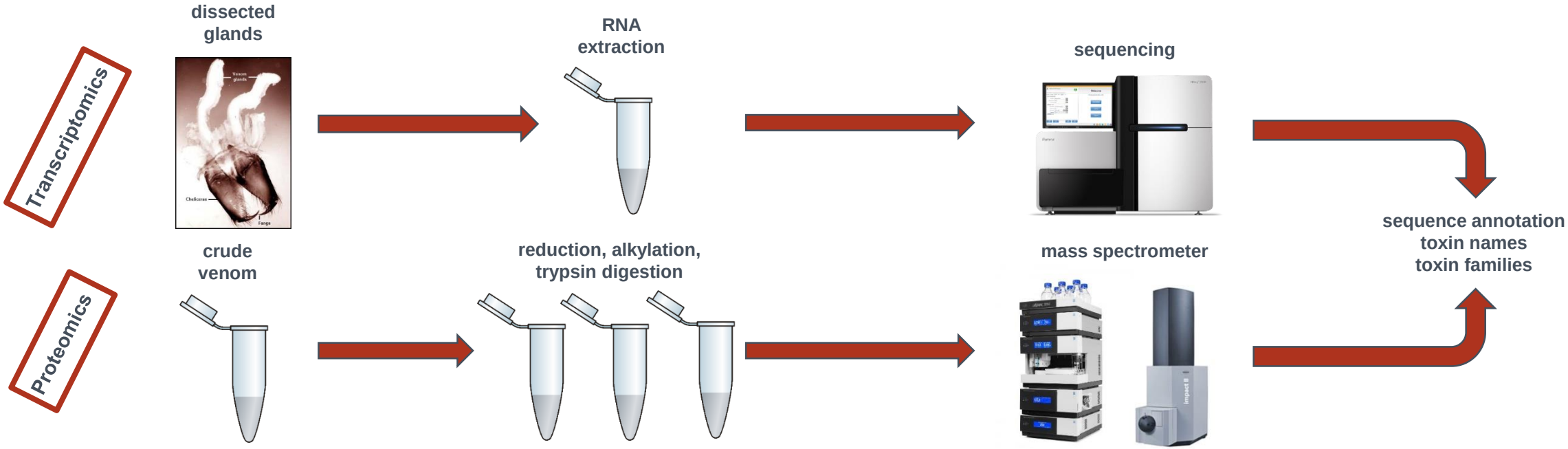


Behavioural experiments

- laboratory experiments with living specimens
- for example, observation of prey paralysis after bite (e.g., Pekár et al, 2018b)

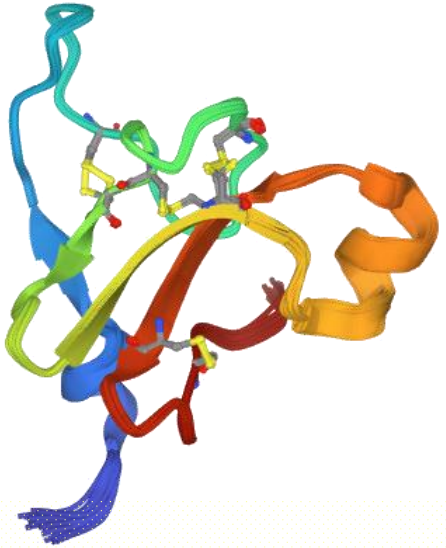
Venom composition - venomics

- integration of **transcriptomic**, **proteomic** (and genomic) approaches
- transcriptomics of the venom-producing tissue
- mass spectrometry (MS) based proteomics
- bioinformatic integration of the data



Structure of venom compotents

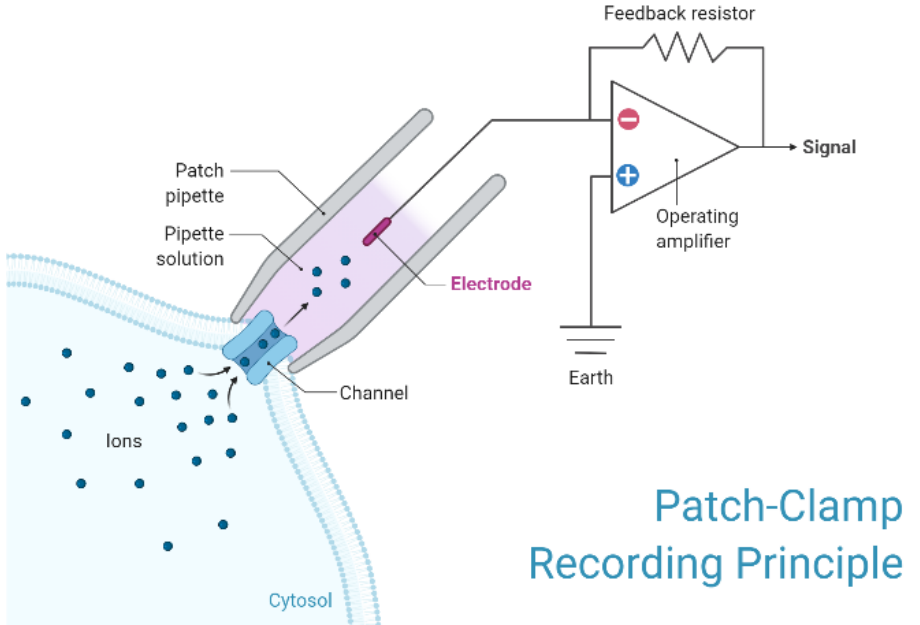
- **X-ray crystallography**
  - rarely used for spider toxins
  - larger proteins
- **nuclear magnetic resonance (NMR)**
  - most used for spider toxins
  - peptides
- **cryoelectron microscopy (EM)**
  - rarely used for spider toxins



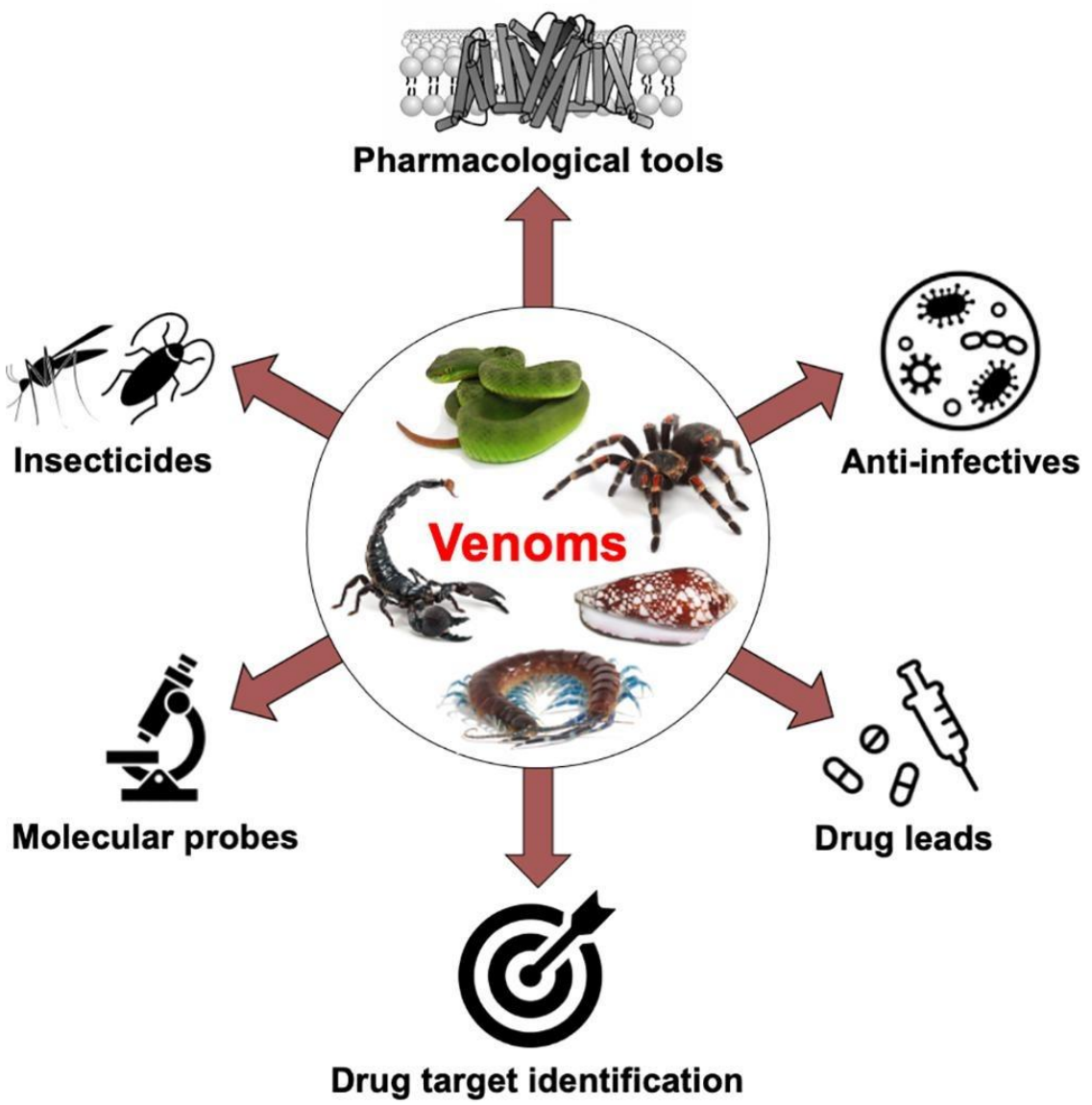
Venom physiology bioassays

- **venom efficiency**
  – venom injection bioassays
  – crude venom, venom fractions, isolated recombinant toxins

- **venom physiology**
  – patch clamp technique
  – target Ion Channels synthesized and expressed in *Xenopus* oocytes
  – toxins added (recombinant toxins)
  – electrophysiological two-electrode voltage-clamp recordings



Venom applications



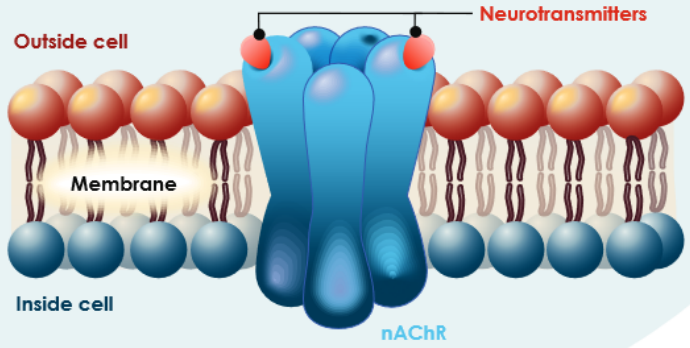
Venom applications - pesticides

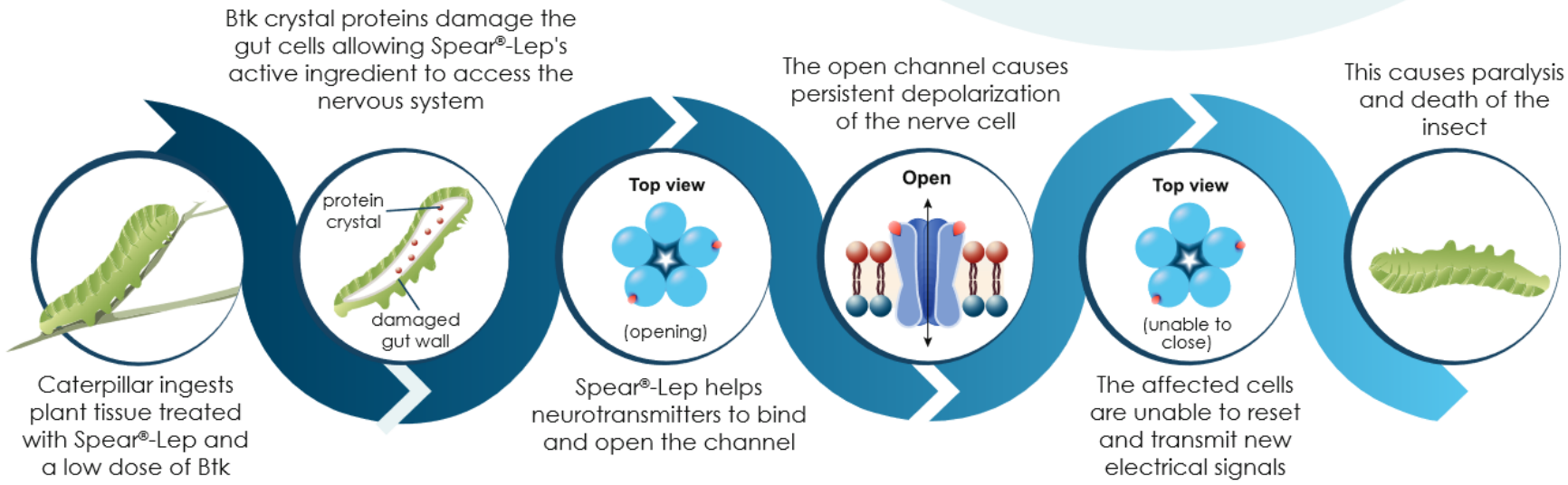
- eco-friendly bioinsecticides

Venom applications - pesticides

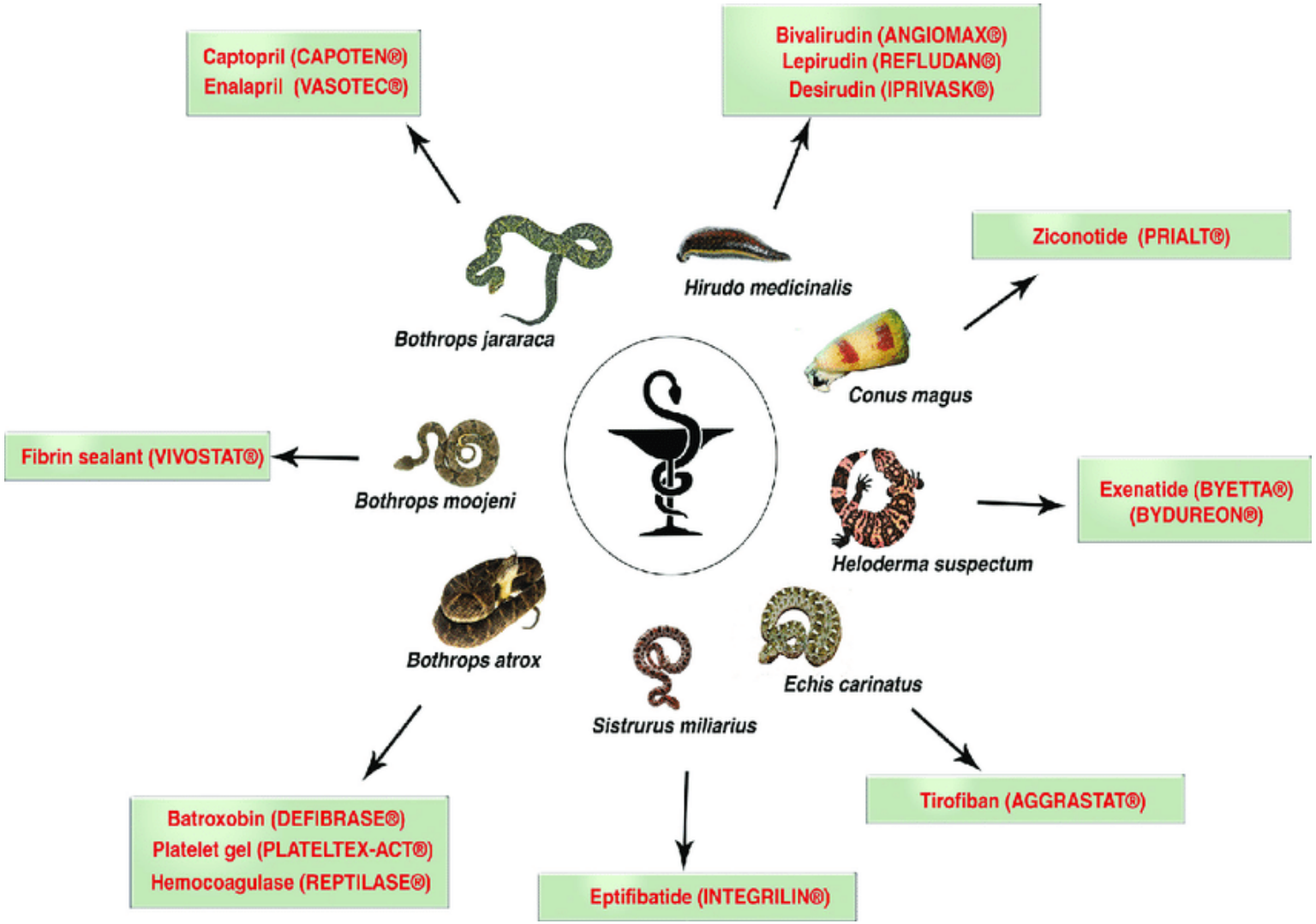
The active ingredient in Spear® is GS-omega/kappa-Hxtx-Hv1a. Spear® products are the first peptide-based insecticides, and the first bioinsecticides that affect a specific neuromuscular target. Spear® delivers an entirely new mode of action for crop protection (IRAC group 32), which means no cross resistance to any other active ingredient, and a novel tool for insecticide resistance management. Because of its biological origins, Spear® is lethal to insect and mite pests, but non-toxic to bees, fish and mammals.

Nicotinic acetylcholine receptors (nAChR) are channels found in the nerves of insects that respond to neurotransmitters. These receptors are essential for transducing certain electrical signals, such as the muscle contraction.





Venom applications - drug leads



Ma, Mahadevappa & Kwok, 2015

Venom applications - drug leads

Photo: David Wilson

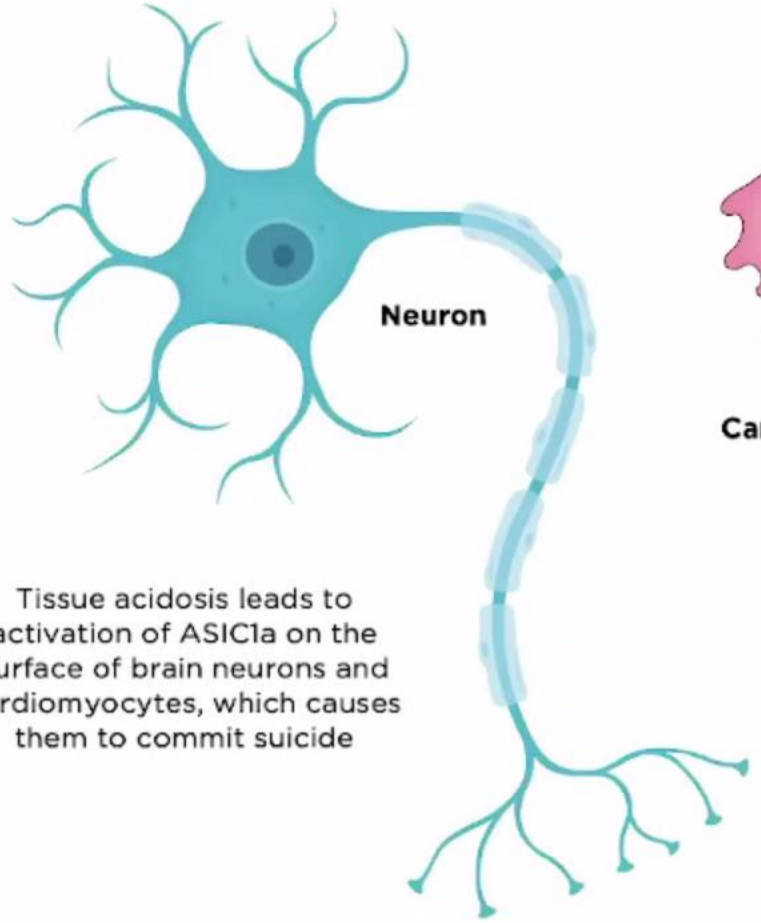
funnel-web spider
Hadronyche infensa

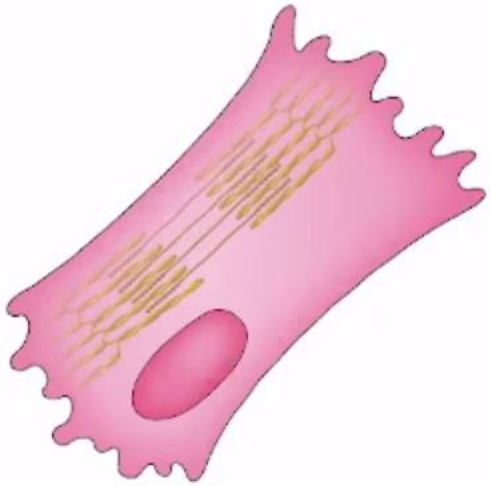
prof. Glenn King

The University Of Queensland AUSTRALIA | IMB
Institute for Molecular Bioscience

-> cure for stroke

ASIC1a MEDIATES CELL DEATH AFTER STROKE & MI





Tissue acidosis leads to activation of ASIC1a on the surface of brain neurons and cardiomyocytes, which causes them to commit suicide

Arterial blockage

↓

Decreased oxygen supply

↓

Tissue acidosis

↓

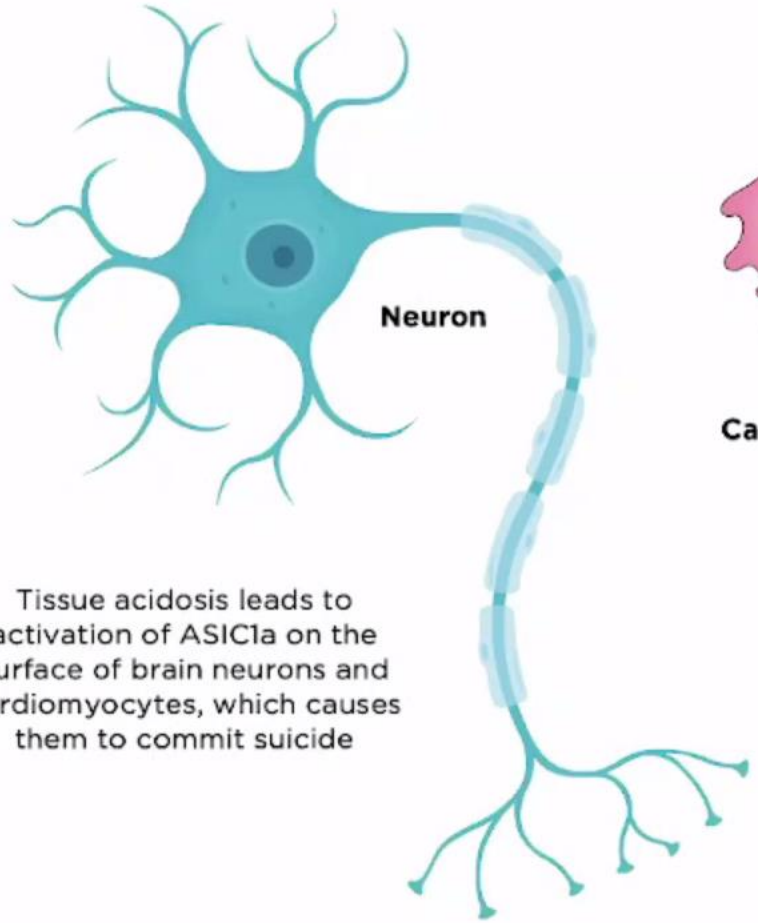
Activation of ASIC1a

↓

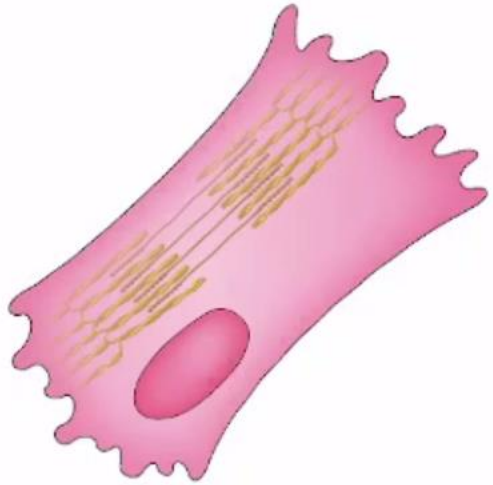
Injury to brain or heart

Glenn King: Deadly cures: a spider-venom peptide for treating ischemic injuries of the heart and brain (1st EUVEN CONGRESS)

ASIC1a MEDIATES CELL DEATH AFTER STROKE & MI



Tissue acidosis leads to activation of ASIC1a on the surface of brain neurons and cardiomyocytes, which causes them to commit suicide



Cardiomyocyte

Does pharmacological inhibition of ASIC1a stop suicide signals being sent to brain neurons and heart muscle cells?

Arterial blockage

↓

Decreased oxygen supply

↓

Tissue acidosis

↓ ⊗

Activation of ASIC1a

↓ ⊗

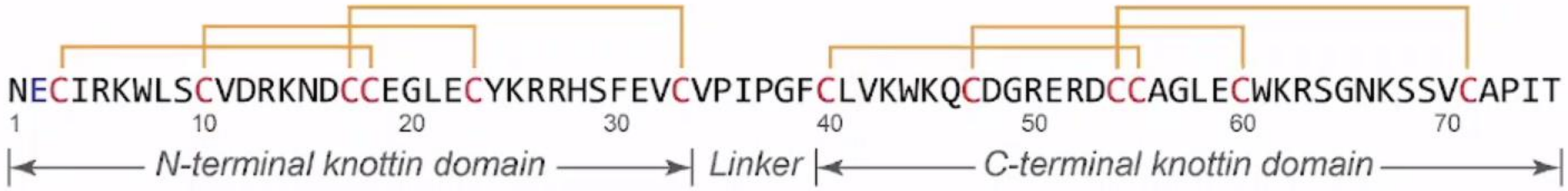
Injury to brain or heart

Glenn King: Deadly cures: a spider-venom peptide for treating ischemic injuries of the heart and brain (1st EUVEN CONGRESS)

SERENDIPITOUS DISCOVERY OF Hi1a

Sandy Pineda Gonzalez

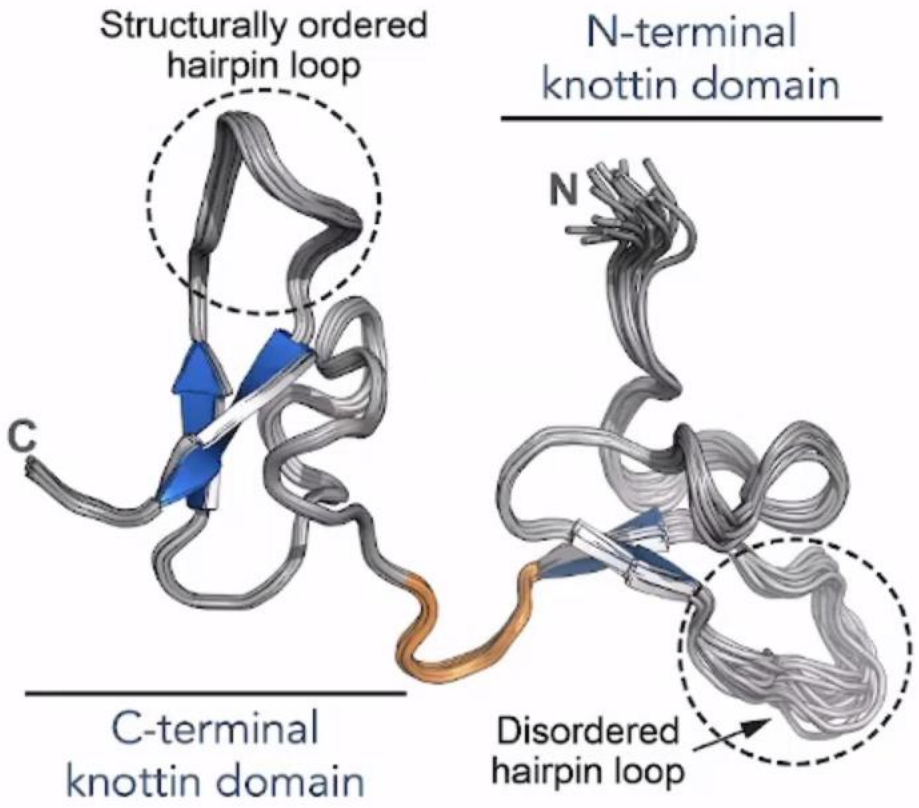
Irène Chassagnon

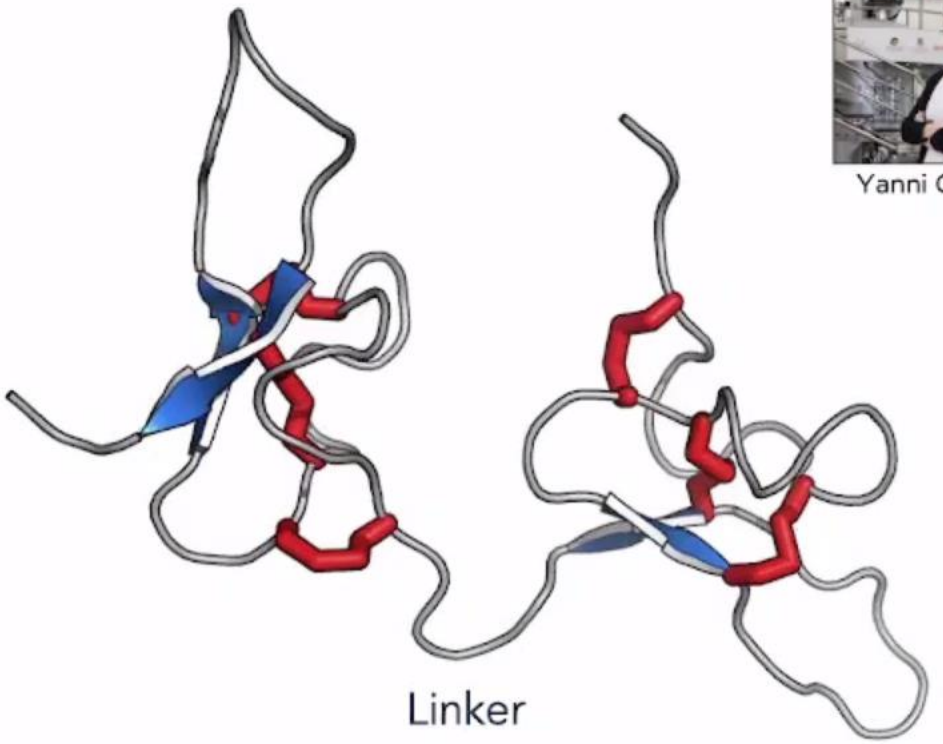


Hi1a: 75 residues; 6 disulfide bonds = 10,395 disulfide isomers

Glenn King: Deadly cures: a spider-venom peptide for treating ischemic injuries of the heart and brain (1st EUVEN CONGRESS)

Hi1a IS A COMPLEX DOUBLE-KNOTTIN PEPTIDE





C-terminal knottin domain

N-terminal knottin domain

Yanni Chin

Glenn King: Deadly cures: a spider-venom peptide for treating ischemic injuries of the heart and brain (1st EUVEN CONGRESS)

Summarization

Summarization

- one of the key traits of spiders
- mainly for predation and defence
- hundreds to thousand compounds per species
- neurotoxins, mainly ICK peptides
- evolved through duplication and neofunctionalization
- positive selection followed by purifying selection
- sexual dimorphism, perhaps some geographic and individual variability
- costly substance – venom optimisation
- various methods to study venoms; promising applications (drugs and pesticides)

REFERENCES

Arbuckle, K. (2017). Evolutionary context of venom in animals. *Evolution of venomous animals and their toxins*, *24*, 3-31.

Casewell, N. R., Wüster, W., Vonk, F. J., Harrison, R. A., & Fry, B. G. (2013). Complex cocktails: the evolutionary novelty of venoms. *Trends in ecology & evolution*, *28*(4), 219-229.

Esteves, F. G., dos Santos-Pinto, J. R. A., Ferro, M., Sialana, F. J., Smidak, R., Rares, L. C., ... & Palma, M. S. (2020). Revealing the Venomous Secrets of the Spider's Web. *Journal of Proteome Research*, *19*(8), 3044-3059.

Langenegger, N., Nentwig, W., & Kuhn-Nentwig, L. (2019). Spider venom: components, modes of action, and novel strategies in transcriptomic and proteomic analyses. *Toxins*, *11*(10), 611.

Lüddecke, T., Herzig, V., Von Reumont, B. M., & Vilcinskas, A. (2021). The biology and evolution of spider venoms. *Biological Reviews*.

Malaque, C. M. S., Chaim, O. M., Entres, M., & Barbaro, K. C. (2016). Loxosceles and loxoscelism: biology, venom, envenomation, and treatment. *Spider venoms. Dordrecht: Springer Nature*, 419-44.

Morgenstern, D., & King, G. F. (2013). The venom optimization hypothesis revisited. *Toxicon*, *63*, 120-128.

Nelsen, D. R., Nisani, Z., Cooper, A. M., Fox, G. A., Gren, E. C., Corbit, A. G., & Hayes, W. K. (2014). Poisons, toxungens, and venoms: redefining and classifying toxic biological secretions and the organisms that employ them. *Biological Reviews*, *89*(2), 450-465.

Pekár, S., Bočánek, O., Michálek, O., Petráková, L., Haddad, C. R., Šedo, O., & Zdráhal, Z. (2018a). Venom gland size and venom complexity—essential trophic adaptations of venomous predators: a case study using spiders. *Molecular ecology*, *27*(21), 4257-4269.

Pekár, S., Líznarová, E., Bočánek, O., & Zdráhal, Z. (2018b). Venom of prey-specialized spiders is more toxic to their preferred prey: A result of prey-specific toxins. *Journal of Animal Ecology*, *87*(6), 1639-1652.

Pineda, S. S., Chin, Y. K. Y., Undheim, E. A., Senff, S., Mobli, M., Dauly, C., ... & King, G. F. (2020). Structural venomics reveals evolution of a complex venom by duplication and diversification of an ancient peptide-encoding gene. *Proceedings of the National Academy of Sciences*, *117*(21), 11399-11408.

Senji Laxme, R. S., Suranse, V., & Sunagar, K. (2019). Arthropod venoms: Biochemistry, ecology and evolution. *Toxicon*, *158*, 84-103.

Schendel, V., Rash, L. D., Jenner, R. A., & Undheim, E. A. (2019). The diversity of venom: the importance of behavior and venom system morphology in understanding its ecology and evolution. *Toxins*, *11*(11), 666.

Walker, A.A., Robinson, S.D., Yeates, D.K., Jin, J., Baumann, K., Dobson, J., Fry, B.G. and King, G.F. (2018). Entomo-venomics: The evolution, biology and biochemistry of insect venoms. *Toxicon*, 154, 15-27.