Why do we study animal toxins?

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ABSTRACT

Venom (toxins) is an important trait evolved along the evolutionary tree of animals. Our knowledges on venoms, such as their origins and loss, the biological relevance and the coevolutionary patterns with other organisms are greatly helpful in understanding many fundamental biological questions, i.e., the environmental adaptation and survival competition, the evolution shaped development and balance of venoms, and the sophisticated correlations among venom, immunity, body power, intelligence, their genetic basis, inherent association, as well as the cost-benefit and trade-offs of biological economy. Lethal animal envenomation can be found worldwide. However, from foe to friend, toxin studies have led lots of important discoveries and exciting avenues in deciphering and fighting human diseases, including the works awarded the Nobel Prize and lots of key clinic therapeutics. According to our survey, so far, only less than 0.1% of the toxins of the venomous animals in China have been explored. We emphasize on the similarities shared by venom and immune systems, as well as the studies of toxin knowledge-based physiological toxin-like proteins/peptides (TLPs). We propose the natural pairing hypothesis. Evolution links toxins with humans. Our mission is to find out the right natural pairings and interactions of our body elements with toxins, and with endogenous toxin-like molecules. Although, in nature, toxins may endanger human lives, but from a philosophical point of view, knowing them well is an effective way to better understand ourselves. So, this is why we study toxins.

Keywords: Toxins; Survival competition; Evolution; Disease mechanism; Drug development

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INTRODUCTION

Struggle for existence in nature created toxins

A basic issue for a living organism is that how to adapt to the environments, to keep "homeostasis" facing various ecological conditions and noxious stimuli, and to win in the survival competitions (Darwin, 1859). Prey-predator interaction and prevention of pathogenesis while maintaining symbiosis in coexistence with enormous microbes are the key biological challenges (Cortez & Weitz, 2014; Lazzaro & Rolff, 2011; Yoshida et al, 2003). Accordingly, toxins are widely produced by all forms of life, including animals, plants and microbes, to interfere and disrupt the physiological processes of other organisms and on the other hand, favor their own struggles for existence. Toxins can be small molecular compounds, proteins and peptides. Toxic animals can be broadly classified into two categories (Mebs, 2002): (1) venomous species possess a specialized venom system and are able to produce their own venom, which is a mixture of gene-encoded proteins and peptide toxins; (2) species acquire and accumulate small molecular and poisonous metabolites and toxins from their environments while maintaining relative resistance to the toxins' poisonous effects, such as poison-dart frogs (Daly et al, 2005), New Guinean Pitohui birds (Dumbacher e al, 1992) and African crested rats (Kingdon et al, 2012). In this review, we focused on the venomous animals and their gene-encoded proteins and peptide toxins.

Evolution links animal toxins with humans

Humans originated and live together with venomous animals. During evolution, the ancestors of humans and animal toxins were tightly associated with each other in terms of evolutionary conservation as well as mutual interactions. The natural and inherent links of animal toxins with humans were determined by the origin, biological relevance and biochemical properties of animal toxins. Although, at present stage, humans are generally neither the prey nor the predator of venomous animals, each year, numerous cases of animal envenomation are reported worldwide, which have caused substantial morbidity and mortality and has become a serious global public health problem (Balhara & Stolbach, 2014; Isbister & Bawaskar, 2014; Kasturiratne et al, 2008).

Molecular diversities of animal key physiological elements

Genome sequences of animals, including those of humans, have revealed huge molecular diversity of key physiological elements, such as cell membrane ion channels and receptors, non-membrane factors, etc. For example, human genome comprises approximately 400 genes encoding pore-forming ion channels of plasma membranes, which can be broadly classified as either voltage or ligand gated depending on the primary factors determing channel opening and/or closing. Receptors and ion channels of cell membranes play vital roles in various malfunctions and diseases, and function as major drug targets (Bagal et al, 2013; Bradley et al, 2014; Wickenden et al, 2012; Wootten et al, 2013).

Coevolution results in the huge molecular diversities of animal toxins

Living strategies for prey capture and defense have evolved venom from venomous animals. Venom, typically a mixture of proteins and peptide toxins, can be broadly defined as a secretion, produced in a specialized gland in one animal and delivered to a target animal through the infliction of a wound, which contains molecules that disrupt normal physiological or biochemical processes so as to facilitate feeding or defense by the producing animal (Casewell et al, 2013; Fry et al, 2009a). Long-term coevolution has created extensively diversified proteins and peptide toxins, which specifically act on key physiological elements of the target organisms, such as cell membrane ion-channels and receptors. Selective pressure and long-term coevolution have endowed animal toxins with strong activity (act in pmol/L and nmol/L), high specificity (effective on the subtypes of receptors and ion channels) and huge molecular diversity (multiple-gene copy families).

Animal toxin study was originally driven by the motivation of understanding animal envenomation and cinical treatments. As early as 1781, an Italian naturalist Felice Fontana investigated the disturbances of snake venoms on blood coagulation. However, from foe to friend, venom toxins are being treated as invaluable and powerful pharmacological research tools, as well as important clinic therapeutics both in history and nowadays. By reviewing the historical contributions and the impacts of animal toxins on life sciences, in this article, we addressed the major aspects of toxin study and particularly, we summarized the known venomous animals in China, and emphasized on the studies of toxin knowledge-based physiological toxin-like proteins/peptides (TLPs), the patho-physiological relevance, as well as the similarities shared between toxins and immune effectors from the natural attack and defense systems.

BIOLOGY OF ANIMAL TOXINS

Venom system (apparatus) as a special trait in animals

Venoms have evolved on numerous occasions in animals. The venom (mixture of toxins) of a venom system typically stores in a discrete gland and a specialized delivery system. The common and well-known venomous animals include cnidarians (jellyfishes, sea anemones and hydra), molluscs (cone snails), annelids (leeches), arthropods (spiders, scorpions, centipedes, bees and wasps, ants, ticks and horseflies, crustaceans), echinoderms (sea urchins and starfishes), vertebrates (fishes, snakes and lizards, as well as mammals) (Figure 1). A wide

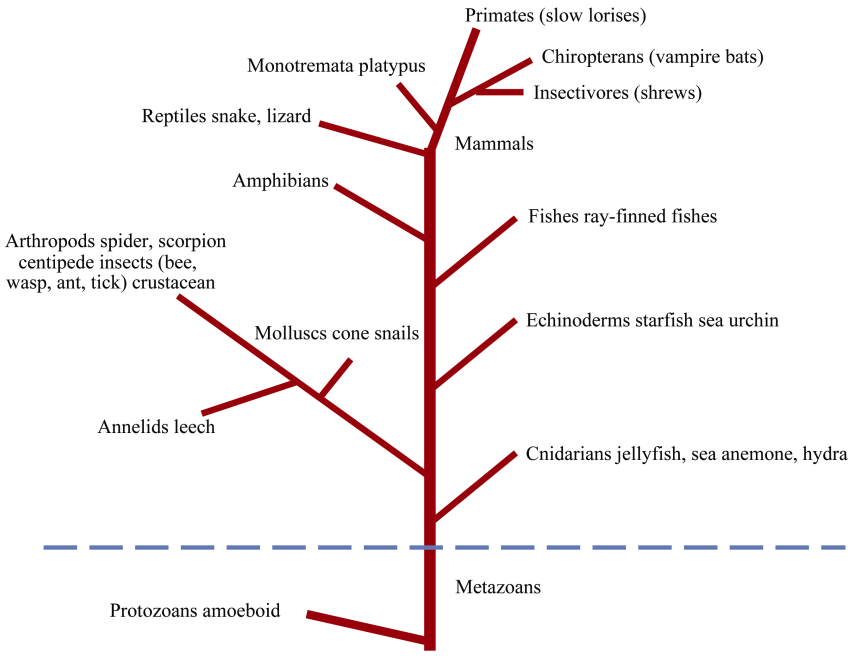

Figure 1 Venom (a mixture of toxins) evolved along the evolutionary tree of animal kingdom

As a special trait in animal kingdom, venom system has evolved in nature for survival competition, which plays important roles in predation, defense, competition, antimicrobial and even communication in given ecological contexts. The common and well-known venomous animals are shown. Toxins are produced from single cell protozoans to metazoan primates.

range of innovative structures (venom delivery systems) have evolved to facilitate the delivery of venoms, including fangs or modified teeth, harpoons, nematocysts, pincers, proboscises, spines, sprays, spurs and stings.

Nematocysts of cnidarians

Cnidarians (corals, sea anemones, jellyfish and hydra) are morphologically simple animals surviving in an aquatic environment with potential predators, competitors and pathogenic microbes. Most of the cnidarians are also active venomous predators feeding on arthropods and fish. Their diversified proteins and peptide toxins are stored and delivered into the preys through the highly developed and specialized stinging cells, the nematocysts. In spite of the large variations in size and morphology, nematocysts share a common organelle, which comprises a cylindrical capsule containing a long hollow thread attached to it. During the discharge of nematocysts following a chemical or mechanical stimulus, the thread is expelled from within the capsule matrix in a harpoon-like fashion (Beckmann & Ozbek, 2012; Mariottini & Pane, 2013; Ozbek et al, 2009; Rachamim & Sher, 2012). Although representing one of the most complex organelles in animals, the evolutionary origin of the nematocyst remains largely unknown.

Molluscs (cone snails)

The molluscs (cone snails) include more than 750 species of venomous predatory marine gastropods. During the past 50 million years, cone snails have evolved into three general feeding groups based on their prey preference: fish-hunters, worm-hunters and mollusc-hunters (Duda et al, 2001). The proboscis, which is a long, flexible, hydrostatically-supported appendage, is used by cone snails to sense and locate preys (Greene & Kohn, 1989) and is subsequently functions as a conduit to deliver immobilizing venom. To envenomate preys, cone snails inject a harpoon-like radular tooth into their preys, allowing toxins to be delivered through the hollow central canal of the tooth (Salisbury et al, 2010).

Arthropods

Except for their ingenious exploitation of silk, another remarkable evolutionary success of spiders is the evolution of pharmacologically complex venom that ensures rapid subjugation of preys. Spiders produce venom in paired glands that reside either in the basal segment of the chelicerae in primitive mygalomorph spiders or in the anterior of the prosoma in modern araneomorph spiders. A duct from each venom gland leads to a small opening near the tip of the corresponding fang. Compression of the muscles encircling each venom gland forces venom along the duct and out through the opening in the fang tip (King, 2004; King & Hardy, 2013).

Scorpions are one of the most ancient groups of terrestrial animals belonging to the class Arachnida within the phylum Arthropoda. Scorpions represent a basal branch of arachnids and have a relatively distant relationship with Acari (mites) and Araneae (spiders), the other two groups of the class Arachnida. Scorpion stings are specialized tools that are sharp enough to penetrate cutaneous tissue, and strong enough to withstand the

stress of making the puncture. The sharply pointed aculeus of the telson inflicts the wound. The expanded bulb houses a pair of venom glands, each with an exit duct leading to an aperture just before the tip of the aculeus (Berkov et al, 2008; Hjelle, 1990).

Centipedes have the modification of the first pair of walking legs into venomous appendages often called poison claws, forcipules, or maxillipeds. Venom is secreted through a pore located on the outer curvature near the tip of each claw, which again is connected to each maxilliped's venom gland through a chitinous venom duct. The venom-injecting forcipules of centipedes represent an evolutionary novelty that appeared in the centipede stem lineage more than 400 million years ago (MYA). No other lineage of arthropods (or indeed of animals) has evolved claws for injecting venom from a pair of walking legs (Dugon & Arthur, 2012; Undheim & King, 2011).

Hymenoptera are the large group of insects which includes bees, wasps and ants. Female hymenoptera possess specialized stinging apparatus with which they inject their venom into preys or intruders. Hymenopteran venom glands are epidermal glands that have evolved from female accessory reproductive glands. The venom apparatus of European honey bees (*Apis mellifera*) comprises a sting and a venom gland. A honey bee venom gland is a simple, long, thin, distally bifurcated structure, opening into an ovoid reservoir (Bridges & Owen, 1984; Kheyri et al, 2013). The basic morphology of the venom apparatus is quite uniform among Vespidae. The proper glandular portion consists of two relatively long tubules that drain, independently or through a short common tract, into a muscular sac-like structure, the gland reservoir. A single duct eventually conveys the venom from the reservoir to the sting. The elongated accessory Dufour's gland, directly connected with the sting, completes the venom apparatus (Petrocelli et al, 2014). The venom apparatus of the fire ant (*Solenopsis saevissima*) has been described with the aid of light and electron microscopy techniques, which mainly consists of a sting and venom sac (Fox et al, 2010).

Crustaceans are the only major traditional arthropod group of which no venomous species were known. Recently, von Reumont et al (2014a) provided the first conclusive evidence that the aquatic, blind, and cave-dwelling remipede crustaceans are venomous, indicating the evolving of venoms in all four major arthropod groups. Analysis of the venom delivery apparatus of the remipede *Speleonectes tulumensis* showed that remipedes can inject venom in a controlled manner. Synchrotron radiation micro-computer tomography (SR-μCT) was used to prepare the first three-dimensional reconstruction of the venom delivery apparatus of the remipede *S. tulumensis*. The anterior trunk of *S. tulumensis* contains two equally sized venom glands, which connect via ducts to reservoirs located in the terminal segments of a robust pair of legs (maxillules) in the head (von Reumont et al, 2014a).

Vertebrate venom systems

In vertebrates, venom systems have evolved several times independently. Besides well-known venomous snakes, lizards and fishes, venom systems can also be found in mammals. However, the venom systems in mammals were neglected by scientists for centuries. The mammalian animals known or suspected to be venomous come from the species of Insectivora, Monotremata, Chiroptera, as well as primates, including Haitian solenodons (*Solenodon paradoxurus*), European water shrews (*Neomys fodiens*), American short-tailed shrews (*Blarina brevicauda*), platypus (*Ornithorhynchus anatinus*), vampire bats (such as *Desmodus rotundus*) and the slow lorises of Southeast Asia (*Nycticebus spp.*) (Ligabue-Braun et al, 2012; Nekaris et al, 2013).

Fish venomous spines Venomous ray-finned fishes are diverse and with habitats ranging from freshwater to seas. The known venomous fishes are mainly distributed among the catfishes (*Siluriformes*) and six groups of "acanthomorphs" or spiny-rayed fishes, like toadfishes and scorpionfishes, in which several thousand of species are presumed to be venomous (Smith & Wheeler, 2006; Wright, 2009). So, venomous fish may outnumber the combined diversity of all the other venomous vertebrates. Diverse phylogenetic distribution of venomous fishes results in variation in the morphology of fish venom apparatuses. Many fish species with venomous dorsal spines have distinct anterolateral grooves on the lateral surfaces of the fin spines, where the venom gland is situated. While venomous toadfishes have distinct venom glands surrounding their dorsal spines, the anterolateral grooves are absent. There are venomous grooved teeth in the lower jaw of saber-toothed blenny fishes, which deliver the venom (Smith & Wheeler, 2006). The venom glands of catfishes are found in association with sharp, bony spines along the leading edge of the dorsal and pectoral fins. When a spine enters a potential predator, the integument surrounding the venom gland cells is torn to deliver venom into the wound (Wright, 2009). It was proposed that the venom glands of fishes are originated from epidermal secretory cells. The toxic peptides of fish venoms may be derived from and are highly homologous to protein components in epidermal secretions (Tamura et al, 2011; Wright, 2009).

Snake fangs Snakes are the masters of venom delivery systems in terms of sophistication, efficiency, and diversity (Jackson, 2003). Elapids, viperids, and atractaspidids possess a large post-orbital gland in which venom is secreted and stored. It is enclosed in a fibrous sheath for the attachment of muscles. It has been suggested that all venom glands are the homologs of the Duvernoy's gland coined to refer to the venom gland of colubrid snakes, which appeared early in colubroid evolution and subsequently specialized independently into venom glands (Jackson, 2007). Many venomous snakes use tubular fangs, which are specialized teeth associated with a venom gland and are positioned either anterior or posterior in the upper jaw. Tubular fangs have a completely enclosed venom canal for the conduction of venom into a bite wound. An elegant study has been carried out by using the sonic hedgehog gene as a marker, and by three-dimensionally reconstructing the development of snake embryos from different species. Their findings put forward a new model for the evolution of snake fangs. The

developmental uncoupling of the posterior from the anterior tooth region could have allowed the posterior teeth to evolve independently and in close association with the venom gland. Subsequently, the posterior teeth and venom gland could have become modified and formed the fang-gland complex (Vonk et al, 2008).

Grooved teeth of lizards and insectivores The closest relatives of snakes are the anguimorphs (which include the venomous helodermatids) and iguanian lizards. The anguimorphs, iguanians and snakes, which form a well-resolved clade, are shown to be the only lineages possessing protein-secreting mandibular and/or maxillary glands (Fry et al, 2012). In contrast to venomous snakes, the venom of the venomous lizards in the genus Heloderma is produced by multi-compartmentalised glands on the lower jaw from which ducts lead onto grooved teeth along the length of the mandible. Recently, anguimorph lizards other than helodermatids and iguanian lizards have been shown to be venomous (Fry et al, 2009b; 2010a). This new perspective revealed that Heloderma and snake venom systems are homologous but highly differentiated descendants of an early-evolved venom system in squamates which possessed incipient venom glands in both the mandibular and maxillary regions, with snakes favouring the development of the maxillary venom gland and secondarily reducing the mandibular components, while the anguimorph lizards did the reverse (Fry et al, 2009b; 2012). In mammals, mildly toxic salivary secretions are associated with grooved teeth in some insectivores. The venomous species of insectivora have significantly enlarged and granular submaxillary salivary glands from which the toxic saliva is produced.

Platypus spurs and slow loris brachial glands Mammalian platypus has the bizarre crural venom system. Rather than delivering venom through a bite, as do shrews and vampire bats, male platypuses have venomous spurs on each hind leg, which is connected via a duct to venom glands evolved from modified sweat glands. The study on the platypus reveals strong convergence between reptile and mammal venomous systems (Whittington et al, 2008). The slow lorises are the only primates, which harbour toxins. It has been proposed that the venom is a mixture of fluid of its brachial gland located in the ventral side of the elbow with saliva, and is applied to the top of the head for defense or kept in the mouth to bite (Nekaris et al, 2013). Knowledge of mammalian venom is only in its infancy, and that even more species of mammals may harbour venomous adaptations. The study of chemical and genetic aspects of venom can help to elucidate the evolution of this trait in mammals (Ligabue-Braun et al, 2012; Nekaris et al, 2013).

Biological roles of venoms

The ecological advantages conferred by the possession of a venom system are evident from the extraordinarily diverse range of animals that have evolved venoms. Animals' venoms serve a variety of functions. The three most common uses are predation or resource acquisition, defense and reduction of competition.

Predation

The evolution of animal venoms is thought to be a typical predatory adaptation (Daltry et al, 1996; Fry et al, 2009a). First, selection for immobilization favors venoms that are fast acting and directly influence mobility and coordination. For this reason, many types of venom include neurotoxic components that disrupt information transfer in nerves or muscles. Snakes, scorpions, spiders, centipedes, and cone snails all produce different neurotoxins that act on key physiological elements of neurotransmission, such as cell membrane ion channels and receptors.

Second, disrupting blood coagulation system is another effective way to disturb the key physiological process and facilitate predation, which is a strategy used by many viperid snakes. Accordingly, their venoms contain numerous haemotoxins, which act on almost all the elements of blood coagulation and fibrinolytic systems (Kini, 2011). Vampire bats are highly specialized mammals, with their entire physiology modified to use blood as their only source of food and water. To do so, the bats have modified sharp teeth, anticoagulants in their saliva and a specialized tongue. The evolution of anticoagulants in the saliva of the three different vampire bat species revealed transitions in their preferred preys (Ligabue-Braun et al, 2012).

Third, excessive and uncontrolled proinflammatory and immune reactions can also cause heavy toxicity, even death (Medzhitov, 2010a; Palm et al, 2012). Even often neglected, the immune system of prey must be an important target of venoms. This is the strategy of some type of venomous animals used, and the related components called "immunotoxins" exist in their venoms. In fact, manipulating host defense mechanisms by venoms has been reported for some venomous animals like ticks (Cabezas-Cruz & Valdés, 2014). For successfully and effectively sucking blood, horsefly and leech venoms contain many components acting on blood coagulation and immune systems (Ma et al, 2009; Min et al, 2010). This notion is further supported by natterins and nattering-like toxins in fishes, and the action of these toxins on immune system (Lopes-Ferreira et al, 2014).

Defense

Venoms serve a defensive role, but this function is thought to be secondary (Brodie, 2009; Fry et al, 2009a). A range of venom components could be used for defensive purpose, like peptide toxins, alkaloids, protease inhibitors that prevent digestion, and other compounds that cause organism insult. Interestingly, some compounds elicit specific behaviors in predators, like the peptides in the skin mucus of *Xenopus* frogs that stimulate uncontrollable yawning and gaping that allow the frogs to crawl out of the mouth of snakes (Brodie, 2009).

Traditionally, venomous animals are thought to inject the same combination of toxins for both predation and defense. However, recent studies showed that cone snails can rapidly switch between distinct venoms in response to predatory or defensive stimuli. Predation- and defense-evoked venoms

originate from the distal and proximal regions of the venom duct, respectively, explaining how different stimuli can generate two distinct venoms (Dutertre et al, 2014a). Geography cone (*Conus geographus*) is the most dangerous cone snail species known, with reported human fatality rates as high as 65%. To study the venom that is directly relevant to human envenomation, the defense-evoked venom of several specimens of *C. geographus* was analyzed. The molecular composition of individual defense-evoked venom showed significant intraspecific variations (Dutertre et al, 2014b).

Competition

The role of ant venoms in ecological competition among ant species has been reported. The rasberry crazy ant (*Nylanderia fulva*) applies abdominal exocrine gland secretions to detoxify fire ant (*Solenopsis invicta*) venom. This capacity to detoxify a major competitor's venom probably contributes substantially to its ability to displace fire ant populations (Lebrun et al, 2014).

The platypus venom gland is seasonally active and secretes venom only during the short annual breeding season, suggesting that it has evolved primarily as an offensive weapon for use in conspecific aggression to assert dominance over other male platypuses (Grant & Temple-Smith, 1998). Most of the evidence now supports the proposition that the venom is used by males as a weapon when competing for females, taking part in sexual selection (Ligabue-Braun et al, 2012).

The venom of slow lorises can cause death in small mammals and anaphylactic shock and death in humans. Wild field and laboratory studies have been conducted for attempting to understand the function and ecological role of loris venom. The least evidence is found for the hypothesis that loris venom is evolved to kill preys. It was suggested that the venom's primary function in nature seems to be as a defense against parasites and conspecifics. It may also serve to threat olfactory-orientated predators (Nekaris et al, 2013). Further detailed studies on the ecology, habitat use and phylogenetic relationships of slow lorises may shed light on this topic.

Concerning the biological function of the venoms in insectivores, the venom as a weapon for intraspecific competition should be considered (Ligabue-Braun et al, 2012). Scleractinian coral colonies and many actiniarians (anemones) use venom for predation and defense, but also possess specialized tentacles to attack other nearby colonies, thereby protecting and expanding their own territory in the context of intraspecific and interspecific competition for space (Nelsen et al, 2014; Williams, 1991)

Antimicrobial defense

Recent studies showed that there are many venom components that possess strong antimicrobial activity. Defensin and cathelicidin are the two main families of naturally occurring antimicrobial peptides, which exhibit potent microbicidal properties against bacteria, fungi, and some viruses. Cathelicidin-type antimicrobial peptides have been identified from elapid snake venoms (Zhao et al, 2008). OH-CATH30 peptide exerted potent antibacterial activity, selective immunomodulatory properties, and low toxicity to eukaryotic cells (Li et al, 2012; 2013). In scorpion venoms, many peptides with antimicrobial, antiviral, antimalarial, immuno-modulating activities were also identified (Almaaytah & Albalas, 2014; Ortiz et al, 2015). In predation, numerous microbes exist in preys. It is reasonable to speculate that the existence of antimicrobial agents in the venom may function to prevent the potential infection caused in the process of predation. In addition to the use of venom for self and/or colony defense, some hymenopterans also spray their 'venom' to keep their broods free of parasites in the context of hygiene (Oi & Pereira, 1993).

Communication

Ants use many different chemical compounds to communicate with their nestmates. Foraging success depends on how efficiently ants communicate the presence of food and thus recruit workers to exploit the food resource. Trail pheromones, produced by different exocrine glands, are a key part of ant foraging strategies. In the subfamily *Myrmicinae*, trail pheromones are mostly produced in the venom gland (Cerdá et al, 2014). Fire ant venom components act as key attractants for the parasitic phorid fly, *Pseudacteon tricuspis* (Diptera: Phoridae), indicating the role of ant venom as attractants for their natural enemies (Chen et al, 2009). Many trail pheromones identified in the venom glands of ants are small molecular organic compounds, such as alkaloids, etc. The possible role of venom proteins and peptide toxins in ant communication is an interesting open question for further investigation.

Venom loss

Because venoms are protein-rich, they come with a considerable metabolic and biochemical price. The "venom optimization hypothesis" postulates that venom is metabolically expensive and therefore is used frugally through behavioral control (Wigger et al, 2002). The metabolic cost of venom is sufficiently high to result in secondary loss of venom whenever its use becomes non-essential to survival of the animal (Morgenstern & King, 2013). There are multiple examples of secondary loss of venom in the evolution processes of animals. It has been reported that a dinucleotide deletion in the only expressed toxin gene in sea snakes (*Aipysurus eydouxii*), resulting in an inactive form of the toxin. This is a result of the change in its dietary habit from fish to fish eggs, showing how the change in ecology subsequently significantly affected the composition of the venom (Li et al, 2005). All spiders are predators and have venom glands, with the exception of the hackled orbweavers (*Uloboridae*) and certain species of primitive mesothelids. Venom has also been secondarily lost in uloborid spiders which instead kill their prey by wrapping them tightly in hackled silk (King, 2004).

Toxins in animal venoms

Selection pressures and animal toxins

Ecological conditions play important roles in the natural selection of toxin compositions. The evolution of venom molecular components is often linked to diet and trophic

ecology through an evolutionary arms race between predators and preys. Specific resistance to snake venoms has evolved in both natural preys and predators of snakes (Biardi & Coss, 2011; Heatwole & Powell, 1998; Jansa & Voss, 2011). Venom resistance in natural preys and predators provides a selective pressure on snakes to develop venom that is of higher toxicity, which in turn selects for increased resistance in sympatric prey populations. This continuum results in a predator-prey co-evolutionary 'arms race' synonymous with Van Valen's "red queen hypothesis" (Richards et al, 2012).

As mentioned above, an additional evolutionary challenge for venomous animals is that toxins synthesis appears to carry an appreciable metabolic cost, which leads to the optimization of venom toxins to adapt different preys and predators in different ecological conditions. Thus, the variations of ecological context and long-term coevolution have created extensive diversified proteins and peptide toxins, which specifically act on targeted organisms. Significant variation in venom compositions and toxin molecules in the same animal species has been often detected from the venom samples collected from different places and/or times.

Evolutionary origin and genetic basis of animal toxins

In the immune system of vertebrates, three major gene families, namely the MHC, T cell receptor (TCR), and Ig gene families, play an important role in identifying and removing invading microbes like virus, bacteria, and eukaryotic parasites. These immune genes are believed to evolve via the 'birth and death' process of gene evolution (Nei, 1969; Nei et al, 1997). In this model of evolution, duplicate genes are produced by various mechanisms, including tandem and block gene duplication, and some of the duplicate genes diverge functionally but others become pseudogenes owing to deleterious mutations or are deleted from the genome. The end result of this mode of evolution is a multigene family with a mixture of divergent groups of genes and highly homologous genes within groups plus a substantial number of pseudogenes (Nei et al, 1997).

Similarly, many venom toxins are believed to be originated through evolutionary process by which a gene encoding a normal 'physiological' body protein, usually one involved in key regulatory processes or bioactivity, is duplicated and a duplicate copy selectively expressed in the venom gland, resulting in large multilocus gene families that encode toxins exhibiting a variety of functional activities and potencies (Casewell et al, 2013; Fry et al, 2009a).

Venom toxins are often characterized by accelerated evolution and positive selection, especially on amino acid residues that are surface-exposed on the protein macromolecular structure (Jiang et al, 2011; Kordis & Gubenšek, 2000). Thus, gene duplication, positive selection, and protein neofunctionalization are major genetic elements to work in unison to provide the evolutionary novelty that allows adaptation of venom toxins to different requirements under various biological contexts. Gene duplication is not a prerequisite for toxin recruitment. Some identified toxins are simply modified, alternatively spliced, or generated through alterations in the structure of domains of gene loci that are physiologically expressed in non-venomous taxa and therefore appear to have been 'hijacked' for a role in venom (Casewell et al, 2013).

Huge molecular diversity of animal toxins

In recent years, the information on molecular diversity of animal toxins is explosively increasing because the applications of modern techniques. It has become clear that the animals' proteins and peptide toxins are considerably more complex than previously realized via proteomic and transcriptomic analyses of the venom compositions of venomous animals. The extensive diversification of toxins may have been driven by extreme diversification of physiological elements of potential preys and predators in evolutionary processes (Figure 2).

Cnidarians The toxicity of *Cnidaria* is a subject of concern for its influence on human activities and public health, as well as a potential source of natural bioactive compounds useful to develop new drugs or biomedical materials (Mariottini & Pane, 2013).

Jellyfish Jellyfish *Stomolophus meleagris* is one of the most dangerous jellyfish in China sea. People stung by the jellyfish would suffer itch, edema, myalgia, dyspnea, hypotension, shock, and even death. The venom of *S. meleagris* contains various toxins including serine protease inhibitors, PLA2, potassium channel inhibitors, metalloproteases, C-type lectins, hemolysins, cytotoxins, cardiotoxins and neurotoxins. The identified toxins are probably related to the sting caused by the jellyfish (Li et al, 2014a).

Sea anemone Sea anemone toxins comprise mainly proteins and peptides, including different ion channel modulators, cytolysins, protease inhibitors and PLA2s, which are efficient in targeting different preys (Frazão et al, 2012). The neurotoxic fractions from the exudates of *Stichodactyla helianthus* and *Bunodosoma granulifera* were analyzed by reversed-phase chromatography and mass spectrometry. The resulting fractions were analyzed by their toxicity to crabs. The first peptide fingerprints of these sea anemones were assessed, revealing the largest number of peptide components (about 156 peptides) so far found in sea anemone species (Rodríguez et al, 2012).

Hydra Like in other cnidarians, hydra polypeptide toxins are expressed mainly in nematocysts and represent a highly complex array of effector molecules aimed at paralyzing a prey and disintegrating its tissue (Rachamim & Sher, 2012). The proteome of nematocysts from the freshwater polyp *Hydra magnipapillata* has been reported, which revealed an unexpectedly complex secretome of 410 proteins, from which 55 toxin-related sequences were found to be homologous with toxins in other venomous animals. These include neurotoxins, cytolysins, toxic phospholipases, many peptidases, and proteins of the SCP_GAPR-1-like family. The molecular masses of the toxins mainly range from 25 to 100×10^3

(Balasubramanian et al, 2012). Small peptide toxins affecting ion channels identified in many sea anemones have not been determined in this analysis.

Molluscs (cone snails) Cone snails (*Conus* species) are predatory molluscs that inhabit tropical and subtropical shallow seawater. The systematic mining of fish-hunting cone snail toxins began 30 years ago. Extensive studies revealed that their venom ducts produce a mixture of peptides, generally known as conotoxins, having exquisite specificity for different ion channels, receptors, and transporters (Olivera et al, 1985; 1990). They are mostly short disulfide-rich peptides of 10 to 40 amino acids with remarkable structural diversities. An emerging enigma concerning conotoxins is their striking diversity. It was estimated that each *Conus* species could produce more than 1 000 different conotoxins (Biass et al, 2009; Davis et al, 2009). Conotoxin-encoding transcripts are diversified by hypermutation, fragment insertion/deletion, and mutation-induced premature termination, and a single mRNA species can produce multiple toxin products through alternative post-translational modifications and alternative cleavages of the translated precursor (Lu et al, 2014).

Annelids (leeches) Leeches are hematophagous annelids. They penetrate the body surface of the host and have to take measures to inhibit the normal reactions in host tissues to blood vessel damage, including blood coagulation, swelling, pain and inflammation. Long term evolution made leeches have acquired the ability to control these processes in their hosts by transferring various bioactive substances to the host through tiny salivary ductile (Baskova et al, 2008; Lemke et al, 2013). An expressed sequence tag (EST) library-based analysis of the salivary transcriptome of the North American medicinal leech (*Macrobdella decora*) revealed a complex cocktail of anticoagulants and other bioactive secreted proteins, including saratin, bdellin, destabilase, hirudin, decorsin, endoglucoronidase, antistatin, and eglin, as well as to other previously uncharacterized serine protease inhibitors, lectoxin-like c-type lectins, ficolin, disintegrins and histidine-rich proteins (Min et al, 2010).

Arthropods

Spiders Spiders (order *Araneae*) are the most successful venomous animals in term of their species and toxin diversification, and spider venoms have been intensively investigated. The major components of most spider venoms are small disulfide-bridged peptides, and more than 1 000 spider toxins have been characterized from about 90 species (Herzig et al, 2011). From Chinese bird spider (*Ornithoctonus huwena*), 626 toxin precursor sequences in total were retrieved from the transcriptomic data and were clustered into 16 gene superfamilies, including six novel superfamilies and six novel cysteine patterns (Zhang et al, 2014). Many spider toxins described to date contain an unusual structural motif known as an inhibitor cystine knot, which is typically highly resistant to proteases, acidic pH, high temperatures and organic solvents (Saez et al, 2010). Spider toxins mainly target on various ion channels and exhibit a range of pharmacological activities, including Ca^{2+}, K^+, Na^+ channels, transient receptor potential (TRP) channels, mechanosensitive channels, acid-sensing ion channels (ASICs), glutamate receptors and glutamate transporters (King & Hardy, 2013).

Scorpions Though scorpions are a small arachnid group, they constitute a very well adapted order of predatory animals that have been living in the Earth for nearly 400 million years (Polis, 1990). Individual scorpion venoms often contain as many as several hundred components (Almeida et al, 2012; Xu et al, 2014), and by coupling with measures of taxonomic diversities of scorpions, this has led to estimates of ~100 000 bioactive peptides in the venoms of scorpions (King, 2011). Scorpion cysteine-stabilised α/β (CSα/β) toxins are disulfide-bridged peptides with a significantly constrained structure, possess pharmacological action on ion channels, including Ca^{2+}, Na^+, K^+, Cl^- channels (Ortiz et al, 2015). Non-disulfide-bridged peptides constitute an important group of scorpion venom components. The pharmacological properties of these linear peptides include antimicrobial, cytolytic, antiviral, antimalarial, bradykinin potentiating and immuno-modulating activities (Almaaytah & Albalas, 2014). Interestingly, it has been shown that a majority of CSα/β toxin scaffolds have experienced episodic influence of positive selection, while most non-CSα/β linear toxins evolve under the extreme influence of negative selections (Sunagar et al, 2013).

Centipedes Centipedes are excellent predatory arthropods. Recently, centipede *Scolopendra subspinipes dehaani* venom was systematically investigated by transcriptomic and proteomic analysis coupled with biological function assays. In total, 543 venom proteins and peptides were cloned, and 50 proteins/peptides were purified from the venom (Liu et al, 2012). In another report, 26 neurotoxin-like peptides belonging to 10 groups were identified from the venom of *Scolopendra subspinipes mutilans* (Yang et al, 2012). The purified toxins mainly possessed various ion channel modulating properties. Most of them showed no significant sequence similarity to other proteins and peptides deposited in the known public database. These works provide a novel reservoir of mining ion channel modulating agents. Furthermore, a selective $Na_V1.7$ inhibitor (named μ-SLPTX-Ssm6a) with analgesic efficacy as assayed in rodent pain models was discovered, which might be a promising lead molecule for the development of novel analgesics targeting $Na_V1.7$ (Yang et al, 2013).

Bees and wasps An in-depth study of honeybee (*Apis mellifera*) venom proteome revealed an unexpectedly rich venom composition, in which in total of 102 proteins and peptides were found. A group of 33 putative toxins is proposed to contribute to venom activity by exerting toxic functions or by playing a role in social community (Van Vaerenbergh et al, 2014). There are two major forms of honeybee venom used in pharmacological applications: manually extracted glandular venom, and venom extracted through the use of electrical

stimulation. A proteome comparison data demonstrated that these two venom forms are different in their compositions, which are important in their use as pharmacological agents (Li et al, 2013a). An optimized experimental protocol was used for the detection of peptides in the venom of the social wasp *Polybia paulista*. The results revealed a surprisingly high level of intra- and inter-colonial variability for the same wasp species, which detected 78-108 different peptides in the venom of different colonies of *P. paulista* with molecular mass range from 400 to 3 000×10^3; among those, only 36 and 44 common peptides were observed in the inter- and intra-colony comparisons, respectively (Dias et al, 2014).

Ants Ants (Hymenoptera, Formicidae) represent a taxonomically diverse group of arthropods comprising more than 10 000 of species. Ant venom components exhibit a variety of biological activities, including antimicrobial, haemolytic, cytolytic, paralytic, insecticidal and pain-producing activities (Aili et al, 2014). Transcriptomic analysis for Brazilian ant (*Tetramorium bicarinatum*) venom revealed a high diversification of the venom components, including venom allergens, distinct isoforms of PLA1 and PLA2, serine proteases, hyaluronidases, protease inhibitors, secapin, waprin-like and agatoxins (Bouzid et al, 2014). About 40% of the generated sequences have no hits in the databases, emphasizing the existence of many new unknown molecules. From the venom gland of the predatory giant ant *Dinoponera quadriceps*, inhibitor cysteine-knot (ICK)-like toxins, insect allergens, enzymes, and lethal toxins were determined (Torres et al, 2014). Ant venoms, similar to those of bees and wasps, contain many allergens, which are the most frequent elicitors of anaphylaxis in humans.

Sharing some common toxins in venoms, each species of ants appears to have a number of unique components. Interestingly, the nesting habits of ants have deeply influenced their venom toxicity and composition. In ant genus Pseudomyrmex, the venom of the ground-dwelling species, *Pseudomyrmex termitarius* is composed of 87 linear peptides. However, the venoms of the arboreal and the plant-ant species, *P. penetrator* and *P. gracilis*, contain 26 and 23 peptides with disulfide bonds, respectively (Touchard et al, 2014). The large number of peptides in *P. termitarius* venom is likely related to potential prey diversity plus the antibacterial peptides required for nesting in the ground.

Ticks and horseflies As haematophagous arthropods and for biological success, ticks use their salivary constituents to successfully obtain a blood meal by targeting major physiological pathways involved in host defense mechanisms. The resulting feeding site also becomes a favorable environment for many pathogens to exploiting ticks to facilitate their transmission to the host (Wikel, 2013). It has been reported that tick salivary gland extract inhibits host complement activation and depresses macrophage function by inhibiting lipopolysaccharide (LPS)-induced nitric-oxide synthesis and proinflammatory cytokine production (Cabezas-Cruz & Valdés, 2014; Stibrániová et al, 2013).

In traditional Eastern medicine, horseflies are used as anti-thrombosis material for hundreds of years. Similar to other hematophagous arthropods, such as mosquitoes (Arcà et al, 1999), several families of proteins or peptides, which act mainly on the hemostatic system or immune system of the host, were identified in the horsefly *Tabanus yao* salivary glands. These include fibrinogenolytic enzymes, RGD-containing anti-platelet aggregation disintegrins, thrombin inhibitors, vasodilator peptides, peroxidase and apyrase (Ma et al, 2009; Xu et al, 2008). The diversity of anti-thrombosis components in horsefly saliva reflects the molecular basis of its blood-sucking living strategy.

Echinoderms

Starfishes and sea urchins

Starfishes and sea urchins are the popular name for marine invertebrates that belong to the phylum Echinodermata. Comparatively speaking, studies on their venoms are still in a primitive stage. Some species of starfishes and sea urchins are dangerous to humans. When stung by the venomous spines on the surface of crown-of-thorns starfish (*Acanthaster planci*), various pathological symptoms, such as severe pain, redness, swelling, and protracted vomiting, are induced (Sato et al, 2008). The crude venom extracted from the spines exhibits diverse biological effects, including hemolytic, mouse lethal, edema-forming, PLA2, anticoagulant and cytotoxic activities (Butzke & Luch, 2010; Lee et al, 2014). In the case of sea urchins, envenomations are caused by stings from either pedicellariae or spines (Balhara & Stolbach, 2014). A galactose-binding lectin SUL-I was isolated from the venom of sea urchin *Toxopneustes pileolus*, which showed mitogenic, chemotactic, and cytotoxic activities through binding to carbohydrate chains on cells (Hatakeyama et al, 2014). Cathepsin B/X was found to be secreted by *Echinometra lucunter* sea urchin spines, a structure rich in granular cells and toxins, which was thought to participate in the the inflammatory response to the accident (Sciani et al, 2013).

Venomous vertebrates

Fishes Despite the large number of species, compared with other groups of venomous organisms, the study on fish venoms is still in a relatively preliminary state and fish venoms are neglected source of bioactive proteins and peptides. Protein toxins natterins were characterized from Brazilian venomous fish *Thalassophryne nattereri* (Magalhães et al, 2005). Natterins and their analogues might be widely distributed in the fish venom glands, thereby forming one family of fish venom toxins (Tamura et al, 2011). The difficulty in the study of fish venoms is that the venoms are sensitive to heat, pH, and lyophilization, as well as are often contaminated with mucus components. A novel protein-handling protocol has been developed recently, upon which the investigation of fish venom composition using barb tissue from the blue-spotted stingray (*Neotrygon kuhlii*) was carried out. The results revealed a variety of protein types that are novel to animal toxins. Putative venom toxins identified include cystatin, peroxiredoxin and galectin (Baumann et al, 2014).

Amphibians Amphibians might not be considered as typical venomous animals due to the lack of a venom delivery system. Amphibian skin is naked to fulfill special physiological requirements, such as respiration and water-salt balance (Campbell et al, 2012; Duellman & Trueb, 1994). Thus, the skin has to form a special defense system to withstand constantly confronted injurious mechanical, chemical and biological factors. Defensive (innate immunity) responses against potential invading of pathogens and repairing capacity of the disrupted surface layer of cells are essential (Voyles et al, 2009). Amphibian skin contains an arsenal of bioactive molecules to fulfill the related functions (König et al, 2014; Zhang, 2006). Indeed, there are many poisonous frogs, including *Dendrobatidae*, *Mantellidae*, *Bufonidae*, and *Myobatrachidae*, which are very "toxic" to mammals and caused by alkaloids sequestered from dietary alkaloid-containing arthropods (Daly et al, 2005; Hantak et al, 2013). The toxicity of some amphibian species to mammals results from physiological proteins and peptides secreted in the skin mucus (Lai et al, 2002a; 2002b; Liu et al, 2008; Qian et al, 2008a; 2008b). Many amphibian skin peptides are related to mammalian hormones or neurotransmitters, as well as antimicrobial peptides (Xu & Lai, 2015; Zhang, 2006).

Several hundreds of peptides were identified from Chinese odorous frogs (Li et al, 2007; Yang et al, 2012b). The function of frog skin peptides are diverse, including antimicrobial, antioxidant, immunomodulatory, and metabolic regulatory activities (Conlon et al, 2014; Yang et al, 2012b). Under environmental pressure, gene duplication, rapid mutation at the amino acid level, domain shuffling and conversion are among the major forces in the formation of heavy diversification of peptides in frog skin (Duda et al, 2002; Lee et al, 2005; Roelants et al, 2013). This evolution pattern is very similar to those of toxins in venomous animals.

Snakes Snake venoms comprise a diverse array of toxins that have a variety of pharmacological and toxicological effects, and are conveniently classified as hemotoxic and neurotoxic (Du, 2006; Kini, 2011; Kularatne & Senanayake, 2014). Most of the snake toxins were recruited or derived from the normal body proteins in the common ancestor of venomous squamates (Toxicofera) or advanced snakes (Caenophidia) during 100–200 MYA (Fry, 2005; Fry et al, 2009b; 2012). By using cutting-edge proteomic and transcriptomic approaches, the venomics of various venomous snake species have been conducted (Brahma et al, 2015; Calvete, 2014).

The toxin profiles of elapid snakes *Naja naja* and *Bungarus multicinctus* were analyzed by sequencing their venom gland transcriptomes (Jiang et al, 2011). Totally 1 092 valid expressed sequences tags (ESTs) for *B. multicinctus* and 1 172 ESTs for *N. atra* were generated. The major components of *B. multicinctus* venom are neurotoxins, including long chain alpha-neurotoxins and recently originated beta-bungarotoxin, whereas, *N. atra* venom mainly contains 3FTs with cytotoxicity and neurotoxicity (short chain alpha-neurotoxins). A recent expansion of alpha-neurotoxins genes in *N. atra* was observed. Tandem duplications contributed the most to the expansion of toxin multigene families. Furthermore, not only the multigene toxin families but also the less abundant toxins were under rapid adaptive evolution (Jiang et al, 2011).

Lizards The lizards of genus Heloderma, which live in the south-western part of the North American continent, have been recognized as venomous for more than a century. Envenomations of humans by helodermatid lizards may cause complicated symptoms including extreme pain, acute local swelling, nausea, fever, hypotension, and inhibition of blood coagulation (Koludarov et al, 2014). Lizard venoms contain a cocktail of different proteins and peptides including hyaluronidase, PLA2s, kallikrein-like proteases, helokinestatin, helofensin, as well as bioactive peptides including hormone-like exendin peptides (Fry et al, 2010a; 2010b). In a recent study attempting to characterize the gila monster (*Heloderma suspectum suspectum*) venom proteome, a total of 39 different proteins were identified out of the 58 selected spots that represent the major constituents of the venom. A neuroendocrine convertase 1 homolog was identified, which is likely to converts the proforms of exendins into the mature and active forms (Sanggaard et al, 2015).

Venomous mammals

The northern short-tailed shrew (*Blarina brevicauda*) saliva contains blarina toxin (Kita et al, 2004) showing kallikrein-like protease activity. This toxin cleaves kininogens to release kinins, including bradykinin, which are inflammation mediators. Blarina toxin shows sequence homologous to gila toxin and horridum toxin, two toxins from the Mexican beaded lizard. Blarina toxin and gila toxin have served as nice molecular models to study the structural basis of transition from a non-toxic to a toxic kallikrein, which is also a good example of convergent evolution at the molecular level (Aminetzach et al, 2009). Two distinct classes of anticoagulants are found in the saliva of vampire bats, i.e., plasminogen activators and inhibitors of proteinases (Ligabue-Braun et al, 2012).

The platypus venom contains natriuretic peptides, defensin-like peptides, nerve growth factors, isomerases, hyaluronidase, proteases, mammalian stress response proteins, cytokines, and other immune molecules (Wong et al, 2012). Gene duplication and subsequent functional diversification of beta-defensins gave rise to platypus *Ornithorhynchus* venom defensin-like peptides (Whittington et al, 2008). The brachial gland exudates of primate slow lorises contain a new member of the secretoglobin family, which is a 17.6×10^3 heterodimeric protein homologous to Fel 1d, the major allergen from domestic cat (Nekaris et al, 2013). This is in accordance with the variable sensitivity to loris bites and the onset of anaphylaxis caused.

Neglected Venomous animals

Recent technological advances dramatically accelerate research into neglected or even completely unstudied venomous taxa. A transcriptomic profile analysis of the venom glands of the remipede crustaceans (*Speleonectes tulumensis*) showed that they express a unique cocktail of transcripts coding for known venom toxins, including a diversity of enzymes and a

probable paralytic neurotoxin very similar to one described from spider venom (von Reumont et al, 2014a). Glycerids are marine annelids commonly known as bloodworms, which prey on invertebrates, and their venom glands produce compounds that can induce toxic effects in animals. The transcriptomic profiles of the venom glands of three species of bloodworm, *Glycera dibranchiata*, *G. fallax* and *G. tridactyla* have been reported (von Reumont et al, 2014b). The toxins represent five functional categories: pore-forming and membrane-disrupting toxins, neurotoxins, protease inhibitors, other enzymes, and CAP domain toxins. The vast majority of neglected venomous taxa are invertebrates. The study of neglected venomous taxa is necessary both for understanding the full diversity of venom systems that have evolved in the animal kingdom, and to robustly answer fundamental questions about the biology and evolution of venoms (von Reumont et al, 2014c).

Genome of venomous animals

Recently, the whole genome information of many venomous animals has become available. These include the genome of sea anemone *Nematostella vectensis* (Putnam et al, 2007), *Hydra magnipapillata* (Chapman et al, 2010), leech *Helobdella robusta* (Simakov et al, 2013), Western honey bee (*Apis mellifera*), Asian honey bee (*Apis cerana*) (Park et al, 2015), five ant species and three solitary hymenopterans in the parasitoid jewel wasp genus (Fischman et al, 2011), scorpion *Mesobuthus martensii* (Cao et al, 2013b), king cobra snake (*Ophiophagus Hannah*) (Vonk et al, 2013) and centipede *Strigamia maritime* (Chipman et al, 2014). The advances should greatly help in understanding the molecular diversity of animal venomics. However, it should be emphasized that in the annotation of the genome sequence, the information from venom transcriptoms and proteomics is very important and even crucial, especially in the cases that many potential venom proteins and peptides are complete new. In addition, data collected from transcriptomic approach are needed to be validated whether the transcripts indeed code for active venom toxins.

Venomous animals in China

China has a vast territory with highly diversified topography, climate and vegetation, and a wealth of animal and plant resources. China is ranked eighth in the world and first in the Northern Hemisphere on richness of biodiversity. China is the home for approximately 10% of the world's biodiversity (Zhang & Ge, 2007), which provides rich resources for studying venomous animals.

Marine venomous animals

Jellyfish belongs to the phylum Cnidarians. The phylum is subdivided into five classes: *Staurozoa* (*Stauromedusae*), *Scyphozoa* (true jellyfish), *Hydrozoa* (fire corals and hydroids), *Cubozoa* (box jellyfish), and *Anthozoa* (sea anemones and true corals), and is composed of about 10 000 species, with 100 of them known to be dangerous to humans (Kayal et al, 2013; Cegolon et al, 2013). Sea anemones (order *Actiniaria*) are among the most diverse and successful members of the anthozoan subclass *Hexacorallia*, occupying benthic marine habitats across all depths and latitudes. *Actiniaria* comprises approximately 1 200 species of solitary and skeleton-less polyps and lacks any anatomical synapomorphy (Rodríguez et al, 2014). The investigation of the actual distribution and species diversification of cnidarians in China is still in its infancy, and according to the present data, there are roughly 200 and 110 species described for jellyfishes and sea anemones, respectively (Li, 2013; Liu, 2008; Liu, 2013a; Pei, 1998;).

The gastropod family *Conidae*, commonly known as cone snails, includes the widely distributed, mainly tropical *Conus*, a relatively young genus first appearing in the Early Eocene. *Conidae* is one of the most diverse animals in the marine environment, with more than 760 valid species currently recognized in the World Register of Marine Species (WoRMS, 2013) (Puillandre et al, 2014). All cone snails whose feeding biology is known inject venom into large prey animals and swallow them whole. Works based on cone snail specimen collected along Chinese coastal waters described roughly 100 species, and most of them were found in the South China Sea (Li, 1999; Liu, 2008). Obviously, the record is largely incomplete due to the lack of systematic investigation of marine biodiversity in China seas (Liu, 2011).

Land venomous animals

Spiders are common in daily human experience because of their biodiversity, wide distribution and abundance in favorable seasons. Emerged about 400 MYA and developed along the long evolutionary course of insects, spiders prey essentially on insects for subsistence. Spiders are the most speciose venomous animals and are the most successful terrestrial predators, with over 45 000 extant species described to date according to the record in World Spider Catalog (version 16) (http: //www.wsc.nmbe.ch) of American Museum of Natural History. Until now, there are about 2 600 spider species described in China, in which about 700 are found in Yunnan Province (Song et al, 1999; Yang, 2006).

Scorpions are a small arachnid group. Until now, there are 15 families, 197 genera and 2 069 species recorded in the world. About 30 of them are recognized as potentially dangerous for humans. They all belong to the family of *Buthidae* which includes nearly 80 genera distributed in both the old and new worlds (Chippaux & Goyffon, 2008). The recorded scorpion fauna of China consists of 53 species and subspecies belonging to 12 genera, 5 families, including 33 species (62.3%) and 1 genus recorded as endemic (Di et al, 2014).

Centipedes (*Chilopoda*), one of the four major lineages of myriapods, are an important group of predatory arthropods in many terrestrial habitats. They comprise approximately 3 300 species belonging to the five extant orders: *Scutigeromorpha*, *Lithobiomorpha*, *Craterostigmomorpha*, *Geophilomorpha* and *Scolopendromorpha* (Edgecombe & Giribet, 2007). In China, 30 species belonging to 5 genera of 3 families in the order *Scolopendromorpha* have been recorded (Song, 2004). In the order *Lithobiomorpha*, 83 species belonging to 15 genera, among which one genus and 25 species are new to science, have been described (Ma, 2007).

Bees are arguably the most important group of angiosperm-pollinating insects. They arose in the early to mid-Cretaceous approximately 140 to 110 MYA, roughly coincident with the origins and early diversification of flowering plants. Bees comprise nearly 20 000 described species (Danforth et al, 2013). There are about 4 200 described vespid species currently classified into 6 subfamilies based on morphological evidence (Hines et al, 2007). The current known species of bees and wasps (*Vespidae*) in China are about 1 000 and 200, respectively (Li, 1985; Wu, 2000).

According to The Reptile Database website (http: //reptile-database.reptarium.cz/), there are about 3 500 snake species that exist in the world, in which about 750 are venomous. In China, there are about 220 snake species recorded, and about 60 are venomous snakes (Cai et al, 2012; Zhao, 2006). Currently, there are 360 known amphibian species distributed in China, including 210 species (58.3%) are recorded as endemic. Three Chinese regions are particularly rich in amphibian diversity: Hengduan, Nanling, and Wuyi mountains, and habitat loss, pollution, and over-harvesting are the most serious threats to Chinese amphibians (Fei, 1999; Xie et al, 2007).

Less than 0.1% of toxins have been explored

The approximate number of venomous animals known in China at present time is listed for each group, as well as the estimated number of proteins and peptides in their venoms based on present data of venom proteomic and/or transcriptomic analysis (Table 1). Exploring molecular diversities of animal toxins in China has been expanded rapidly. According to the 2014 report of Chinese 973 project term, the venomics of 49 venomous animals have been investigated by transcriptomic and proteomic analysis in the past five years, in which about 5 000 toxin sequences of good quality were obtained and about 1 000 proteins and peptide toxins were purified and characterized biochemically. An online Animal Toxin Database has been established (ATDB 2.0, http: //protchem.hunnu.edu.cn/toxin), which embodies the updated information concerning toxin structure, biological function and their targets. In terms of the protein and peptide molecules estimated in Chinese venomous animals (Table 1), less than 0.1% of toxins have been explored.

FROM FOE TO FRIEND, TOXINS AND HUMAN HEALTH

Animal envenomation of humans is a serious public health hazard

Envenomation by venomous reptiles, scorpions, and insects are a common worldwide occurrence, which is an important, but neglected, public health hazard in many parts of the world, particularly in the tropics. It has been estimated that the actual numbers, as the authors suggested, could be as high as 1.8 million envenomings and 94 000 deaths each year due to snakebite worldwide, and the highest burden exists in South Asia, Southeast Asia, and sub-Saharan Africa (Kasturiratne et al, 2008). The envenomations by venomous fishes cause at least 50 000 reported injuries annually with symptoms, such as blisters, intense pain, fever, and even death (Church & Hodgson, 2002; Lopes-Ferreira et al, 2014). Each year more than a million cases of scorpion envenomation occur worldwide, causing substantial morbidity and, among children, a risk of death (Isbister & Bawaskar, 2014). The bite by northern short-tailed shrew causes burning sensation at the wound, swelling and intense pain in humans. Platypus envenomation results in immediate and acute pain and swelling. Slow loris bites have a wide variety of effects upon humans, from none to death, with most of the reported cases resembling allergic reactions (Ligabue-Braun et al, 2012).

Toxicology of animal envenomation in humans

Block neurotransmission

Snakebites caused by the families Viperidae and Elapidae snakes are very dangerous to humans. The fatal effects include widespread bleeding, muscle paralysis, and tissue necrosis around the bite site. Neurotoxins are particularly important (Chippaux, 2008; Harrison et al, 2009). The venom of elapid snakes is rich in PLA2 and 3FTs, which are potent neurotoxins affecting the neuromuscular transmission at either pre- or post-synaptic levels. Pre-synaptic-acting neurotoxins (β-neurotoxins) inhibit the release of acetylcholine, while post-synaptic-acting neurotoxins (α-neurotoxins) cause a reversible blockage of acetylcholine receptors (Jiang et al, 2011). Recent data have challenged the traditional concept of neurotoxicity in snake envenoming, and highlighted the rich diversity of snake neurotoxins (Ranawaka et al, 2013). Though the disruption of blood coagulation system is a common envenomation outcome in victims bitten by viperid snakes like *Trimeresurus spp.*, neurotoxicity has also been well described in the victims (White, 2005; Warrell, 2010). So far, antivenom (mixtures of antibodies that neutralize venoms) is the only validated treatment for snakebite (Gutiérrez et al, 2014).

The venom of the bark scorpion (*Centruroides sculpturatus*) can cause serious and potentially fatal neurotoxicity, with young children most vulnerable to its effects. The most common symptoms of envenomation of patients included local pain, restlessness, and roving eye movements (Skolnik & Ewald, 2013). Highly species-specific antivenom is needed to treat severe envenomation, which is lacking for resource-limited areas, and poorly refined antivenom may induce severe side effects (Megarbane et al, 2014). Latrodectism resulted from bites by widow spiders (*Latrodectus spp*) causes local, regional, or generalized pain associated with non-specific symptoms and autonomic effects. Antivenoms are an important treatment for spider envenomation but have been less successful than have those for snake envenomation (Isbister & Fan, 2011). Envenomation by centipedes such as *Scolopendra subspinipes* typically leads to extreme localized pain, erythema, induration, and tissue necrosis. Mortality is uncommon and may result from secondary infection or anaphylaxis (Veraldi et al, 2014).

Disrupt blood coagulation system

Hematologic abnormalities are the most common effects of snake envenoming, especially in victims bitten by viperid snakes (Warrell, 2010; White, 2005). Venom-induced coagulopathy is a venom-induced activation of the clotting

Table 1 Major venomous animals in China and the estimated number of proteins/peptides in their venoms

Animals	Numbers of species (*n*)	Numbers of Proteins/peptides (*n*)	References
Cnidarians			
Jellyfishes	200	40 000	Li et al, 2014; Liu, 2008; Liu, 2013;
Sea anemones	110	20 000	Frazao et al, 2012; Li, 2013; Pei, 1998; Rodríguez et al, 2012
Hydra (*Hydridae*)	10	1 000	Balasubramanian et al, 2012; Rachamim & Sher, 2012; Zhang et al, 2012
Molluscs			
Cone snails	100	100 000	Biass et al, 2009; Davis et al, 2009; Li, 1999; Liu, 2008
Annelids			
Leeches	90	10 000	Lemke et al, 2013; Min et al, 2010; Yang, 1996
Arthropods			
Spiders	2 500	750 000	Song et al, 1999; Yang, 2006; Zhang et al, 2014
Scorpions	50	10 000	Cao et al, 2013; Di et al, 2014
Centipedes	110	30 000	Liu et al, 2012; Ma, 2007; Song, 2004
Bees	1000	50 000	Dias, 2014; Li, 1985; Wu, 2000
Wasps	200	20 000	Dias, 2014; Li, 1985; Wu, 2000
Ants	200	20 000	Bouzid et al, 2014; Torres et al, 2014; Zhou, 2012
Ticks	120	20 000	Cabezas-Cruz & Valdés, 2014; Yang, 2007
Horseflies	300	30 000	Ma et al, 2009; Wang, 1994; Xu et al, 2008
Crustaceans*	ND	ND	
Echinoderms			
Starfishes	150	ND	Liu, 2011
Sea urchins	100	ND	Liu, 2011
Vertebrates			
Mammals	ND	ND	
Snakes	60	20 000	Jiang et al, 2011; Zhao, 2006
Amphibians**	360	25 000	Fei, 1999; Xie et al, 2007; Yang, 2012; Zhang, 2006
Fishes	500	20 000	Baumann et al, 2014; Liu, 2008; Smith & Wheeler, 2006; Wright, 2009

The approximate number of venomous species known in China at present time is listed for each group of animals, and the estimated number of proteins and peptides in their venoms based on venom proteomic and/or transcriptomic analysis. *: Crustaceans are considered as non-venomous, but a recent report has described the venom system and venom components of a crustacean (von Reumont et al, 2014a); **: Amphibians are not typical venomous animals, but they are listed here because that their naked skin forms a special defense system and rich proteins and peptides exist in their skin secretions (Xu & Lai, 2015; Zhang et al, 2006); ND: no actual and reliable data available.

pathway by procoagulant toxins, resulting in clotting factor consumption and coagulopathy. It is a significant cause of both morbidity and mortality in these patients, either directly, or indirectly. The enzymatic toxins interfering with coagulation are procoagulant proteases (prothrombin activator, thrombin-like enzymes, factor X and factor V activators) and anticoagulant proteases (factor IX and X inhibitors, protein C activator, anticoagulant PLA2s). The venom components acting on fibrinolysis are fibrinolytic enzymes and plasminogen activators (Du et al, 2006; Kini, 2011). The major complication of venom-induced consumption coagulopathy is hemorrhage, including intracranial hemorrhage which is often fatal (Maduwage & Isbister, 2014). Metalloproteinases are widely distributed in snake venoms and play important roles in haemostatic disorders and local tissue damage that follows snakebite. Some metalloproteinases induce hemorrhage by directly affecting capillary blood vessels. They also induce skeletal muscle damage and myonecrosis (Gutiérrez et al, 2014).

Trigger type 2 immunity

Most of the hymenoptera stings are well tolerated and result in only small local reactions with erythema, swelling, and pain in humans. However, the stings can result in severe systemic medical complications, including toxic and potentially fatal allergic reactions, mediated by venom-specific IgE antibodies (Brehler et al, 2013; Mingomataj et al, 2014). Bee and wasp

stings cause various types of allergic reactions, which contribute to the fatal outcome. Proteome and allergenome analysis of Asian wasp (*Vespa affinis*) venom and IgE reactivity of the venom components has been conducted. The results showed that the major allergenic proteins that reacted to IgE of >50% of the wasp allergic patients included PLA1, arginine kinase, heat shock protein (70×10^3), venom allergen-5, enolase, magnifin, glyceraldehyde-3-phosphate dehydrogenase, hyaluronidase, and fructose-bisphosphate aldolase (Sookrung et al, 2014). When there is a history of anaphylaxis from a previous hymenoptera sting and the patient has positive skin tests to venom, at least 60% of adults and 20%-32% of children will develop anaphylaxis with a future sting (Koterba & Greenberger, 2012). Though carrying a small but significant risk of systemic adverse reaction, venom immunotherapy is commonly used for preventing further allergic reactions to insect stings in people who have had a sting reaction (Incorvaia et al, 2011).

Contributions of toxins in deciphering human patho-physiology

Animal toxins show high specificity and potency for particular molecular targets. These features, which are difficult to replicate in the form of small molecules, have made animal toxins extremely valuable pharmacological tools. With animal toxins as irreplaceable molecular probes and research tools, many exciting discoveries that have significantly influenced life science and medical fields were made (Figure 3).

Discovering non-membrane physiological elements

Snake venom played an important and fortuitous role in the discovery of nerve growth factors (Cohen & levi-Montalcini, 1956). By utilizing snake venom and mouse salivary gland extract, purification of nerve growth factor and production of the antibodies against it became possible. For this pioneering work,Stanley Cohen and Rita levi-Montalcini were awarded the 1986 Nobel Prize. When studying the toxicology of *Bothrops jararaca* snake envenoming, bradykinin was discovered (Rocha e Silva et al, 1949), which contributed greatly to our understanding of human path-physiology in cardiovascular and immune systems.

Probing ion channels

The flow of ions across the cell membrane is essential to many life processes, and ion channels are transmembrane pore-forming proteins that create a gated, water-filled pore to allow the movement of ions across cell membranes (Gouaux & Mackinnon, 2005). Given the essential functions of ion-channels in neuronal signaling and muscle contractility, it is not surprising that many toxins have evolved to block or activate ion channels. Animal venoms provide a virtually untapped reservoir of millions of bioactive peptides with highly diverse structures to target on ion-channels (Dutertre & Lewis, 2010).

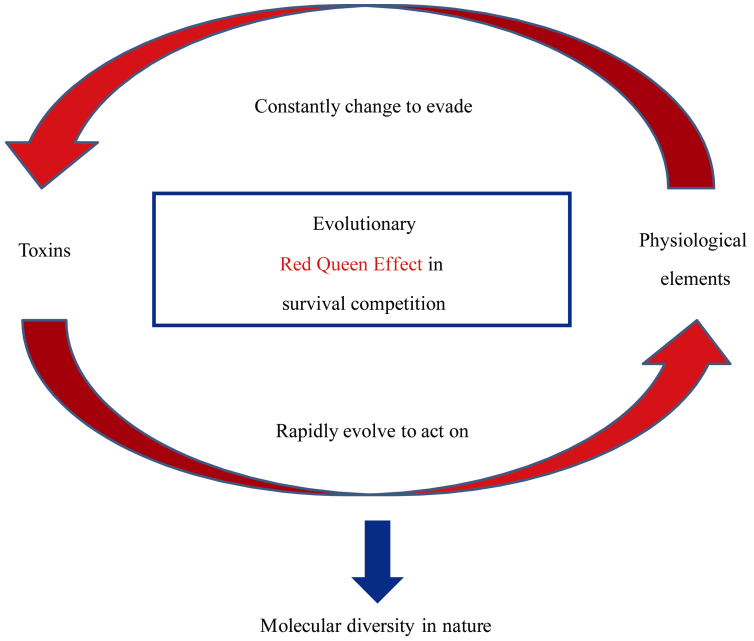

Figure 2 Evolutionary diversification of toxins

Dobzhansky (1973) stated in a classic article that nothing in biology makes sense, except in the light of evolution. The extensive diversification of toxins may have been driven by extreme diversification of physiological elements of potential preys and predators in evolutionary processes. Toxins may be subject to evolutionary Red Queen Effect (Van Valen, 1974), in which toxins must evolve rapidly to effectively act on diversified biological targets. On the other hand, it is possible that the physiological elements, which are critical for the survival of organisms, have to constantly change them to evade being targeted by toxins.

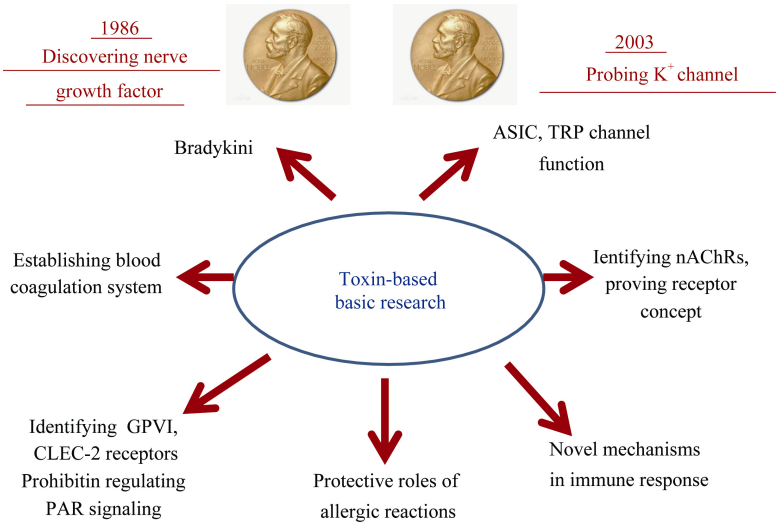

Figure 3 Contribution of toxins in deciphering human patho-physiology and diseases mechanisms

With animal toxins as irreplaceable molecular probes and research tools, many exciting discoveries have been made, which have significant impact on life sciences and medical fields. See description in detail for each story and references cited in the text.

Potassium (K^+) channels Pore-blocking toxins from scorpion venoms, such as charybdotoxin, have profoundly impacted research in the K^+ channel field primarily in two ways. First, they have enabled purification of specific novel K^+ channels such as the BK channel, a Ca^{2+} and voltage-gated K^+ channel (Banerjee et al, 2013). Second, they provided knowledge about channel subunit stoichiometry and the shape of the extracellular K^+ pore entryway at a time when no 3-D structure was available for any ion channel (Hidalgo & MacKinnon, 1995; MacKinnon, 1991). In 2003, Prof MacKinnon was awarded the Nobel Prize for the structural and mechanistic study of ion channels. In his Nobel lecture, Prof Mackinnon emphasized the role of scorpion charybdotoxin in his studies. The toxin was used to probe the "pore" of K^+ channels, leading to important conclusion concerning the architecture of the channels (MacKinnon, 2003).

Acetylcholine receptors Acetylcholine receptors (AChRs) consist of two major subtypes: the metabotropic muscarinic receptors (mAChRs) and the ionotropic nicotinic receptors (nAChRs). Both could be activated by the endogenous neurotransmitter acetylcholine. The muscarinic receptors are G protein-coupled seven-transmembrane proteins, which are activated by muscarine, a toxin from the mushroom *Amanita muscaria*, and inhibited by atropine, a toxin from *Atropa belladonna* as well as a widely used clinic drug (Albuquerque et al, 2009). α-bungarotoxin is the first snake venom 3FT that binds muscle-type nAChRs with near covalent affinity to inhibit their function and promote debilitating paralysis (Chang & Lee, 1963). At the time of the discovery of α-bungarotoxin, the nAChR, although physiologically and pharmacologically well defined, was a molecular enigma. Even the question of whether it was a protein was disputed (Hall, 1999). Affinity columns of α-bungarotoxin allowed separation of nAChRs from other proteins in detergent-solubilized electric organs, which led to the identification, cloning, and sequencing of genes responsible for encoding these receptors. These advance resulted in nAChR at the most advanced stage for any type of receptor (Dolly & Barnard, 1984). The α-conotoxins from marine cone snails were used for discriminating among the subtypes of nAChRs (Lewis et al, 2012).

Acid-sensing ion channels Acid-sensing ion channels (ASICs) are voltage-independent proton-gated cation channels that are largely expressed in the nervous system as well as in some non-neuronal tissues. Six protein isoforms exist in rodents: ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3 and ASIC4 (Deval et al, 2010). Several toxins targeting ASICs can discriminate between the subtypes of ASIC1- and ASIC3-containing channels (Baron et al, 2013). Snake toxin MitTx consists of a heteromeric complex between Kunitz- and PLA2-like proteins that together function as a potent, persistent and selective agonist for ASICs, eliciting robust pain-related behaviors in mice (Bohlen et al, 2011). A new class of 3FTs (mambalgins) from snake black mamba is able to abolish pain through inhibition of ASICs expressed either in central or peripheral neurons. Blockade of heteromeric channels made of ASIC1a and ASIC2a subunits in central neurons and of ASIC1b-containing channels in nociceptors is involved in the analgesic effect of mambalgins (Diochot et al, 2012). Taken together, these findings highlight an unexpected contribution of ASIC channels to nociception and identify new potential therapeutic targets for pain. The cocrystal structure of chicken ASIC1a with MitTx has been determined, which defines the structure of the selectivity filter of voltage-independent, sodium-selective ion channels, and captures the open state of the ASIC (Baconguis et al, 2014).

TRP channels The mammalian transient receptor potential (TRP) channel family consists of >30 members, many of which

are known to form tetrameric cation channels. Though several TRP channels are known to contribute to sensory signaling, like thermosensation, nociception, and pain, the physiological roles of many TRP channels remain enigmatic (Venkatachalam & Montell, 2007). A peptide toxin from the earth tiger tarantula spider that selectively and irreversibly activates the capsaicin- and heat-sensitive channel, TRPV1. This “double-knot” toxin (DkTx) traps TRPV1 in the open state by interacting with residues in the presumptive pore-forming region of the channel, which highlights the importance of conformational changes in the outer pore region of TRP channels during activation (Bohlen et al, 2010). The toxin was further used as a probe to determine structures of two activated states of TRPV1. The study revealed that TRPV1 opening is associated with major structural rearrangements in the outer pore, including the pore helix and selectivity filter, suggesting a dual gating mechanism. These findings revealed differential gating mechanisms for TRPs and voltage-gated ion channels (Cao et al, 2013a).

Discovering and probing membrane receptors

Glycoprotein VI (GPVI) Snake venoms contain a vast number of toxins, in which C-type lectins are fascinating due to their diverse binding specificities to platelet surface proteins and their complex targeting mechanisms. These proteins have made great contribution to the understanding of thrombosis and haemostasis (Du et al, 2006; Lee et al, 2003). Platelets play key roles in haemostasis and thrombus formation. From 1980s, GPVI emerged as a candidate receptor for collagen through investigation of patients with an auto-immune thrombocytopenia, as well as the signaling events that underlie platelet activation by collagen (Watson et al, 2010). However, the molecular identity of GPVI remained elusive at that time. Convulxin is a snake venom C-type lectin purified from the tropical rattlesnake *Crotalus durissus terrificuswas* (Prado-Franceschi & Vital-Brazil 1981; Vargaftig et al, 1983). It is able to activate platelets by binding specifically to GPVI (Polgár et al, 1997). As a critical step, GPVI was isolated from platelets using affinity chromatography of convulxin, which identified that GPVI is actually a member of the immunoglobulin superfamily (Clemetson et al, 1999). The downstream signaling cascade, which leads to the activation of αIIbβ3 and thrombus formation was elucidated (Watson et al, 2010).

C-type lectin-like receptor 2 (CLEC-2) Rhodocytin (also called aggretin) is a heterodimeric C-type lectin and was purified from snake *Calloselasma rhodostoma* venom in 1990s (Huang et al, 1995; Shin & Morita, 1998). It stimulates platelet aggregation independently of the collagen receptor GPVI/FcRγ-chain complex (Navdaev et al, 2001). Using rhodocytin affinity chromatography, a novel C-type lectin receptor (CLEC-2) in platelets was identified, which represents the first C-type lectin receptor found on platelets and represents a novel signaling pathway in platelets (Suzuki-Inoue et al, 2006). Soon afterwards, podoplanin, a type I transmembrane sialomucin-like glycoprotein, was identified as an endogenous ligand for CLEC-2. Subsequent works illustrated that platelets regulate tumour metastasis, lymphangiogenesis, and dissemination of HIV through interaction between CLEC-2 and its endogenous ligand podoplanin (Suzuki-Inoue et al, 2011).

Interaction of trefoil factors with protease-activated receptors (PARs) Trefoil factors (TFFs) are characterized by one to four trefoil domains, which are highly conserved among TFF proteins, from frogs to humans. TFFs are believed to be initiators of mucosal healing and being greatly involved in tumorigenesis (Lefebvre et al, 1996; Mashimo et al, 1996). However, the first hand actions and the mechanisms involved by which TFFs exert their biological activities are still largely unknown (Kjellev, 2009; Zhang et al, 2011). Bm-TFF2 is a two-domain single chain TFF isolated from frog *B. maxima*, which is able to trigger human platelet activation (Zhang et al, 2005). Unexpectedly, it bound and activated PAR1 on human platelets, which is independent of the receptor cleavage and tethered-ligand unmasking. Further results showed the capacity of human TFF2 to act on PAR4 to promote cell migration *in vitro*. The findings suggested the interaction of a PAR with a TFF (Zhang et al, 2011). Their possible interaction *in vivo*, with TFFs acting as either agonists or antagonists of PARs and physiological relevance are certainly worthy of further studying.

Prohibitins as novel regulators of PAR signaling In the process of identifying Bm-TFF2 receptor(s) in human platelets, both prohibitin 1 (PHB1) and PHB2 were detected on the surface of human platelets and were found to be involved in PAR1-mediated platelet aggregation (Zhang et al, 2012b). PHBs are ubiquitously expressed and highly conserved. The membrane PHBs have been reported to be involved in inflammation, obesity and cancer metastasis (Thuaud et al, 2013). The finding uncovered that PHBs are hitherto unknown regulators of PAR1 signaling. Targeting PHBs might be a useful therapeutic approach for anti-platelet therapy. Further study revealed that PHB1 participates in PAR1 activated internalization, Erk1/2 phosphorylation and degradation, but these regulatory roles are aberrant in cancer cells (Wang et al, 2014a). A crucial role of PHB1 in IgE-mediated activation and degranulation of mast cells was also identified afterwards (Hajime & Krishnaraj, 2013).

Establishment of basic concept in blood coagulation system

Snake venoms were used to obtain data that was the basis for considering that blood coagulation is primarily promoted by proteolytic enzymes. In the development of the general understanding of blood coagulation, venom proteases were proved very useful in clarifying some basic concepts (Serrano, 2013). These intriguing toxins are generally variants of normal mammalian physiological proteins. TSV-PA is a specific plasminogen activator from Chinese snake *Trimeresurus stejnegeri* venom (Zhang et al, 1995; 1997). Its 3D-structure, as the first one determined for snake venom serine proteases, was elucidated by the group of Profs Bode and Huber (the 1988 Nobel Laureate) (Parry et al, 1998), which displays a typical trypsin-like fold.

Deep understanding of immunity

Revealing novel mechanisms in immune responses Venoms frequently trigger host immune responses. The illustration of their action may provide insight into novel inflammatory and immune pathways. It was found that bee venom-derived PLA2 activates T cells through generation of small neoantigens, such as free fatty acids and lysophospholipids, from common phosphodiacylglycerides. Subsequent studies in patient showed that injected PLA2 generates lysophospholipids within human skin *in vivo*, and polyclonal T cell responses are dependent on CD1a protein and the PLA2. These findings support a previously unknown skin immune response based on T cell recognition of CD1a proteins and lipid neoantigen generated *in vivo* by phospholipases, revealing mechanisms underlying phospholipase-dependent inflammatory skin disease (Bourgeois et al, 2013).

Elucidating protective roles of allergic reactions in innate immunity Diverse components from animal venoms, plants, parasites, foods and environments can activate allergic responses, including fatal anaphylaxis (Gutierrez & Rodewald, 2013). Allergies have been considered misguided T helper type 2 cell responses (Artis et al, 2012). The mechanisms of innate immune recognition of parasitic worms, as well as allergens, are largely unknown (Medzhitov, 2010b; Licona-Limon et al, 2013). Bee venom PLA2 induces a T helper type 2 cell-type response and group 2 innate lymphoid cell activation. Interestingly, the IgE response to PLA2 could protect mice from future challenge with a near-lethal dose of PLA2, indicating that the innate immune system can detect the activity of a conserved venom component and induce a protective immune response against a venom toxin (Palm et al, 2013). Marichal et al (2013) also found protective rather than allergic immune responses in mice repeatedly challenged by bee venom or its components. These findings support the hypothesis that IgE, which also contributes to allergic disorders, has an important function in the protections of host against noxious substances.

Toxin knowledge guides physiological toxin-like protein/peptide (TLP) studies

The idea that most toxin molecules have evolved from endogenous genes operating in normal physiological processes and cellular pathways suggests the existence of endogenous counterparts of toxin genes. Rapid expansion of gene and protein information, uncovered especially by 3D-structural determination, revealed that numerous TLPs are expressed in non-venomous animals and/or in non-venom systems with unknown physiological functions, including in mammals. Knowledge obtained in the study of toxins could certainly help to illustrate the role and mechanism involved of these endogenous TLPs (Figure 4).

Ly6/neurotoxin family

Snake venom 3FTs and lymphocyte antigen 6 (Ly6) proteins have a variety of biological activities, but their three-finger folding combines them in one Ly6/neurotoxin family (Tsetlin, 2014). Identifying TLPs by applying homology search methods have mostly failed, and ClanTox (classifier of animal toxins) was developed for identifying TLPs from mammalian complete proteomes (Tirosh et al, 2013). In the murine proteome, there are about 60 such proteins that belong to the Ly6/neurotoxin family. They are either secreted or anchored to the cell membrane.

Mammalian Lynx1 in nervous system Lynx1, a murine protein of Ly6/neurotoxin family, is highly expressed in several discrete neuronal populations in the brain. Based on the well characterized properties of snake venom 3FTs in nAChR, the possible action of lynx1 was tested. The results showed that lynx1 is a novel protein modulator for nAChRs (Miwa et al, 1999). Their works further indicate that lynx1 colocalizes with nAChRs on CNS neurons and physically associates with nAChRs. These results established direct interaction of lynx1 with nAChRs, indicating that this endogenous TLP plays important roles *in vivo* by modulating functional properties of their cognate CNS receptors. Lynx1 expression maintains stability of mature cortical networks in the presence of cholinergic innervations (Morishita et al, 2010).

Mammalian SLURPs in immunity Secreted mammalian Ly-6/uPAR-related protein 1 (SLURP-1) is another mammalian Ly6/neurotoxin family member. Structural similarity between SLURP-1 and snake venom 3FTs suggests that this protein might interact with nAChRs. This hypothesis led to the demonstration that SLURP-1 regulates epidermal calcium homeostasis and cutaneous inflammation though nAChRs (Chimienti et al, 2003). SLURP-1 binds to the conventional ligand binding site on keratinocyte 7 nAChRs and exhibits a proapoptotic effect (Chernyavsky et al, 2010). SLURP-2, another member of Ly6 family, was then shown to bind to 3 nAChRs, thereby delaying keratinocyte differentiation and preventing apoptosis (Arredondo et al, 2006). Both SLURP-1 and SLURP-2 are expressed in various immune cells and organs (Moriwaki et al, 2007). These findings illustrated that SLURPs act as an autocrine and/or paracrine factor via AChRs on epithelial cells and immune cells to modulate immune function.

Mammalian β-defensins

Many toxins share a striking degree of conservation with defensin-like antimicrobial peptides that contain a ganna-core motif (Yeaman & Yount, 2007). Crotamine is a toxin from the snake *Crotalus durissus terrificus* venom. Computational docking suggests direct interactions of the peptide with Kv channels in eukaryotic but not prokaryotic cells (Yount et al, 2009). Later, it was shown that crotamine selectively inhibits Kv1.1, Kv1.2, and Kv1.3 channels with an IC_{50} of ～300 nmol/L (Peigneur et al, 2012). Human β-defensin 2 (hBD2) is an antimicrobial peptide that protects hosts from microbial infection by killing bacteria, fungi and viruses, and recruits memory T cells through interacting with CCR6 (Pazgier et al, 2006). 3D-alignment between hBD2 and crotamine revealed a striking degree of identity (Yount et al, 2009), suggesting that hBD2 might be a toxin-like modulator of Kv channels. hBD2 was then found to be able to inhibit human Kv1.3 channel (Yang et al, 2014). In another work, hBD2 was found to be a novel opener via

interacting with human β1 subunit coexpressed with mouse α subunit of large conductance Ca^{2+}-activated K^+ channel (Liu et al, 2013b). These studies opened new possibilities to explore the path-physiological roles of hBD2 via actions through K^+ channels.

Apoptotic Bcl2 proteins

Bacteria have developed sophisticated virulence factors such as pore-forming toxins (PFTs) to mount their attack against their hosts. An essential feature of PFTs is their ability to convert from a water-soluble form to a transmembrane form via oligomerization step that is followed by membrane insertion and channel formation (Bischofberger et al, 2012; Iacovache et al, 2008). PFTs are classified into two large families, the α- and the β-PFT according to the type of structures they use to insert into the lipid bilayer upon pore formation via α-helices (α-PFT) or β-sheets (β-PFT), respectively (Iacovache et al, 2010). The Bcl-2 family proteins regulate programmed cell death. Despite their physiological importance, the biochemical functions of Bcl-2-related proteins had remained elusive until the structure determination. The arrangement of the α-helices in Bcl-xL, a member of Bcl-2 family member, was found to reminiscent of the membrane translocation domain of bacterial toxins, in particular diphtheria toxin and the colicins (Muchmore et al, 1996). Subsequent investigation illustrated that Bcl-xL and other Bcl-2 members can insert into either synthetic lipid vesicles or planar lipid bilayers and form an ion-conducting channel, uncovering the possible mechanism involved of mammalian Bcl2 family members via inserting into membranes to favor or inhibit apoptosis (Minn et al, 1997; Schendel et al, 1997).

Venomous animals are important medicinal animals Traditional medicine and modern practice

Medicinal animals have a long history as a source of clinic therapeutics worldwide, in which venomous animals take a key position. Tarantulas were used by indigenous populations of Mexico and Central and South America to treat a variety of ailments ranging from asthma to cancer (Machkour-M'Rabet et al, 2011). Cobra snake venom has been used to treat cancer and moderate to severe pain, as well as multiple sclerosis and rheumatism (Reid, 2007; 2011). Medicinal leech therapy became less used toward the end of 19th century but now has emerged again as a widely useful therapy. Leech therapy is effective in establishing venous outflow in congested flaps and replants, and has been shown to be effective for symptomatic treatment of osteoarthritis of the knee (Michalsen et al, 2008). Some modern indications for leech therapy have been proposed by American Food and Drug Administration (FDA) (Nouri et al, 2012). Apitherapy is an effective and safe treatment for recalcitrant localized plaque psoriasis, when other topical or physical therapies have failed (Eltaher et al, 2014). Dried toad (*Bufo bufo*) skin secretions (Chan Su) has been used in traditional Chinese medicine as a cardiotonic, analgesic and anesthetic agent, and as a remedy for ulcers, as well as an anti-cancer agent (Meng et al, 2009; Wang et al, 2014b).

Snakes (*Zaocys dhumnades*, *B. multicinctus*, *Agkistrodon acutus*), amphibians (*Rana temporaria*, *B. bufo*), fishes (*Hippocampus histrix*, *Solenognathus hardwickii*), scorpions (*Buthus martensii*), insects (*Eupolyphaga sinensis*, *Mylabris phalerata*), centipedes (*S. subspinipes*), leeches (*Whitmania pigra*, *W. acranulata*, *Hirudo nipponica*) are listed in national Chinese Pharmacopoeia (National Pharmacopoeia Committee, 2010). They are used in treatments of various cardiovascular, nervous and immune related diseases. The first version of the pharmacopoeia was published in 1953. After about 60 years and several major revisions, these medicinal animals are still emboded in the pharmacopoeia, indicating their confirmed treatment effects in clinic. These long-term clinic practice and success are invaluable experience and resources for modern drug development, especially upon the challenges of complicated human diseases caused by muti-element disorders.

Toxins and TLPs in medicinal animals play key roles in their pharmacological effects

Although effectively used in clinic, the material basis of medicinal animals is still enigmatic. Many toxins and TLPs are low molecular weight peptides rich in disulfide bridges, which are highly stable molecular scaffolds resistant to heat and degradation by proteases (Fry et al, 2009a; Harvey, 2014; King, 2011) Numerous endogenous counterparts of toxin genes, namely physiological TLPs, are expressed in non-venomous animals and/or in non-venom systems. Centipedes are important venomous medicinal animals in traditional Chinese medicine, and the whole animal is used in clinic. Approximately 400 novel protein/peptide molecules have been found from the venom of centipede *S. subspinipes* (Liu et al, 2012). In addition, comprehensive transcriptomic analysis of body (without venom glands) and venom glands of the centipede revealed a substantial overlapping of transtripts expressed (Lee et al, unpublished observation). It is reasonable to speculate that peptide toxins in the venom glands and TLPs in the body tissues are mainly responsible for the pharmacological effects obtained in clinic because of their biochemical stability and biological activity.

Modern clinic drugs

The potency, specificity, and stability of toxins have made them a valuable source of natural products for drug discovery (Harvey, 2014; King, 2011). In the 1970s, antihypertensive drug captopril was developed from a bradykinin potentiating peptide (BPP) discovered in the venom of the Brazilian viper *Bothrops jaracaca* (Cushman & Ondetti, 1991). This important achievement marked the beginning of modern toxins-based drug discovery. Several important drugs derived from venom peptides or proteins (Figure 5) have been approved and widely used in clinic. There are still tons of molecules in clinical trials and many more in various stages of preclinical development.

Cardiovascular diseases

Hypertension In 1965, BPPs were identified by Prof Sergio Ferreira (Ferreira, 1965) from snake venoms, which are inhibitors of angiotensin-converting enzymes (Camargo et al, 2012). The structure determination and synthesis of BPP9a, namely teprotide, were performed by Ondetti et al (1971). The teprotide, when injected, was able to lower blood pressure.

However, developing classical peptides for oral antihypertensive drugs were proved difficult. The strategy to develop non-peptidic inhibitors from peptides directed the synthesis of captopril by Cushman et al (1977). Based upon the model proposed by Byers & Wolfenden (1972), when a succinyl radical was added to the carboxy terminal proline of BBP5a, a weak specific angiotensin-converting enzyme inhibitor with oral activity resulted, leading to the invention of captopril and a new class of antihypertensive clinic therapeutics (McCleary & Kini 2013). Profs Cushman and Ondetti shared the 1999 Albert Lasker Award in clinical medical research.

Thrombosis and haemostasis Excessive platelet aggregation is associated with myocardial infarction and other thrombotic diseases. Integrin αIIbβ3 plays key roles in platelet aggregation, serving as a rational target for antithrombotic therapy (Bledzka et al, 2013). In order to discover αIIbβ3 antagonists, 62 snake venoms were screened, leading to the identification of barbourin, a 73-amino acid disintegrin from the venom of *Sistrurus miliarius barbouri* (Scarborough et al, 1991). Eptifibatide (Integrilin) is a cyclic heptapeptide (6 amino acids) designed from barbourin (Scarborough et al, 1993; Scarborough, 1999). It has a relatively long half-life in plasma (about 2.5 hours), and cyclizes the peptide via a disulfide bond greatly enhancing its potency. Tirofiban (Aggrastat) is a non-peptide mimetic of αIIbβ3 inhibitor, which was designed based on a RGD peptide from snake venom (Lynch et al, 1995). Based on the distance separating the side chains of Arg and Asp in the RGD motif of echistatin, a disintegrin isolated from the venom of *Echis carinatus* (Saudek et al, 1991), a lead was identified and optimized (Egbertson et al, 1994) to produce tirofiban. These two drugs received FDA approval in clinic for antithrombotic therapy, like acute coronary syndromes since 1998.

Thrombin-like enzymes (TLEs) are serine proteinases reported from many different crotalid, viperid and colubrid snakes that share some functional similarity with thrombin. Unlike thrombin, most TLEs are neither inhibited by heparin-antithrombin III complex, nor are they able to activate FXIII. Ancrod and batroxobin are the most well-known examples of TLEs from the venoms of *Agkistrodon rhodostoma* and *Bothrops atrox*, respectively (Nolan et al, 1976; Stocker & Barlow, 1976). They rapidly catalyze the formation of soluble clot (that can be easily broken down by plasmin) and deplete the level of circulating fibrinogen, preventing formation of insoluble clots in acute thrombosis events. These TLEs are used in clinic for treatment of many thrombosis events with beneficial outcomes. A mixture of two enzymes from the venom of *B. atrox*, a TLE and a thromboplastin-like enzyme, forms a clot-promoting product called Haemocoagulase, which has procoagulant effects only at the sites of injury or surgery and is used as a haemostatic agent in clinic primarily in China (Koh & Kini, 2012).

Hirudin, consisting of 65 amino acids, is a direct thrombin inhibitor from the saliva of the medicinal leech *H. medicinalis* (Petersen et al, 1976). By molecular modeling and design, a novel class of bivalent peptide inhibitors of thrombin hirulogs were developed (Maraganore et al, 1990). Bivalirudin (20 amino acids) was developed from hirulog-1, which combines a C-terminal segment of 12 amino acids derived from native hirudin binding site to an active site-binding tetrapeptide sequence at its N-terminus, linked together by four glycine residues. It specifically binds to both the active catalytic site and anion binding exosite of thrombin, with a short half-life of 25 min *in vivo*. Bivalirudin lacks immunogenicity and has a wider therapeutic index than recombinant hirudin. It has become one of most widely used antithrombotics in clinic (Coppens et al, 2012).

Neurogenic diseases

Pain relief Omega-conotoxin MVIIA from *Conus magus* selectively blocks N-type Ca^{+} ion channels (Olivera et al, 1987). Ziconotide, a 25-amino acid polypeptide, is the synthetic version of the peptide. When administered intrathecally, it produces potent analgesia by interruption of Ca^{2+} channel-dependent transmission of pain signals in the spinal cord, and was approved by the FDA in 2004 for treating patients with intractable pain (Pope & Deer, 2013). Early in the 1930s, the relief of pain was found to be the dominant pharmacological activity when cobra snake venom was used in cancer patients in clinic. Using cobra venom as an analgesic in clinic was adopted by Macht in the United States (Macht, 1936). In the early 1980s, a cobra venom neurotoxin preparation isolated from Chinese cobra (*N. naja atra*) venom, named ketongning or cobratide, was developed as a drug in clinic for pain killing by Kunming Institute of Zoology of the Chinease Academy of Sciences, which has been primarily used in China for almost 35 years. Later, the investigators from the same institute developed the oral tablets "Keluoqu" by combining cobratide with tramadol hydrochloride and ibuprofen, which was approved by the Chinese FDA in 1998 for the treatment of moderate to severe pain (Lu et al, 2010).

Metabolic diseases

Glucagon-like peptide 1 (GLP-1) is a hormone that stimulates insulin and suppresses glucagon secretion. It exerts its actions by acting on G-protein-coupled GLP-1 receptor (Drucker & Nauck, 2006). The pleiotropic actions of GLP-1 and GLP-1 receptor on the control of blood glucose have fostered considerable interests in the use of GLP-1 and GLP-1 receptor agonists for the treatment of type 2 diabetes. GLP-1 has a very short half-life *in vivo*. So, GLP-1 receptor agonists with peptide degradation-resistant and more suitable pharmacokinetic properties should be better for the long-term treatment of type 2 diabetes. Exendins are hormone-like peptides found in the lizard Heloderma venoms (Irwin, 2012). Exendins-3 and -4 exhibit sequence similarity (56%) and have biological functions most similar to mammalian GLP-1 by acting on mammalian GLP-1 receptors (Eng et al, 1992; Göke et al, 1993). Exenatide (synthetic exendin-4) has a circulating half-life of 60–90 min, leading to its development as an anti-diabetic agent in 2005. Recently, evidence suggested that agonists for GLP-1 receptors may have biological properties relevant to Parkinson's disease. Exenatide may serve as a neuroprotective candidate and be used in the treatment of Parkinson's disease in clinic (Foltynie & Aviles-Olmos, 2014).

Infectious diseases

Peptide antibiotics Traditional antibiotics have been widely used, resulting in the emergence of many antibiotic-resistant strains worldwide. Thus, there is a vital need for new effective therapeutics to conquer infections caused by drug-resistant bacteria (Fischbach & Walsh, 2009). Naturally occurring antimicrobial peptides, owing to their unique mechanisms that differ from the conventional clinical drugs, are considered to be excellent templates for the design of novel antibiotics with promising therapeutic effects, especially for drug-resistant microbes (Hancock & Sahl, 2006). Cathelicidins are cationic host-defense peptides that play important roles in innate immune system, which have also been identified from elapid snake venoms (Zhao et al, 2008). In animal models, OH-CATH30, isolated from king cobra venom, protects mice from lethal sepsis due to its direct antimicrobial activity and selective immune-modulatory properties. Treatment with OH-CATH30, alone or in combination with levofloxacin, significantly improves the clinical outcomes of rabbit antibiotic-resistant *Pseudomonas aeruginosa* keratitis. These results suggest that OH-CATH30 is an excellent candidate for infectious disease caused by drug-resistant bacteria (Li et al, 2012; 2013b; 2014b).

SIMILARITIES SHARED BY VENOMS AND IMMUNE SYSTEMS

Numerous species of microbes, plants and animals are able to produce toxins. Besides well-developed venom systems in metazoan animals, some amoeboid protozoans are facultative or obligate parasites in humans and they produce PFTs for invasion (Leippe, 2014). On the other hand, all organisms have developed immune systems to defend against the threats of potential parasites and pathogens early during evolution. Besides well-known innate and adaptive immunity, the ancient and ubiquitous cell-autonomous immunity operates across all three domains of life (Randow et al, 2013). The basic similarities of venoms and immune systems are reflected by their primary biological tasks, attacks and defenses (Figure 6). They may also function as ways of peaceful co-existence among organisms. However, the biological significance and mechanisms involving these two systems are not well appreciated and understood nowadays, i.e., the prevention of pathogenesis while maintaining symbiosis in the coexistence of enormous microbes.

Toxins and immune effectors share similar evolutionary patterns

In animals, venom system is considered to evolve for feeding or defense by toxin producing animals, which mainly functions among prey-predator interactions of animals. Defenses against microbial invasions and malignant cells are major missions of immune systems. As discussed earlier in this review, the genetic and evolution origination of venom toxins and host immune effectors are believed to evolve via the 'birth and death' process of gene evolution (Casewell et al, 2013; Nei et al, 1997). Recruitment of a proper gene and duplication and rapid mutation created diversified innovative toxins or immune effective molecules, which are selectively expressed in venom glands or immune related organs (Figure 6).

Toxins and immune effectors share common protein folds

Accumulated evidence have uncovered a fact that many similar proteins and common protein folds, which were previously identified by primary sequences but now by 3D-structures, have been used and engineered into conductors of immune effectors as well as venom toxins. This is exemplified by cases

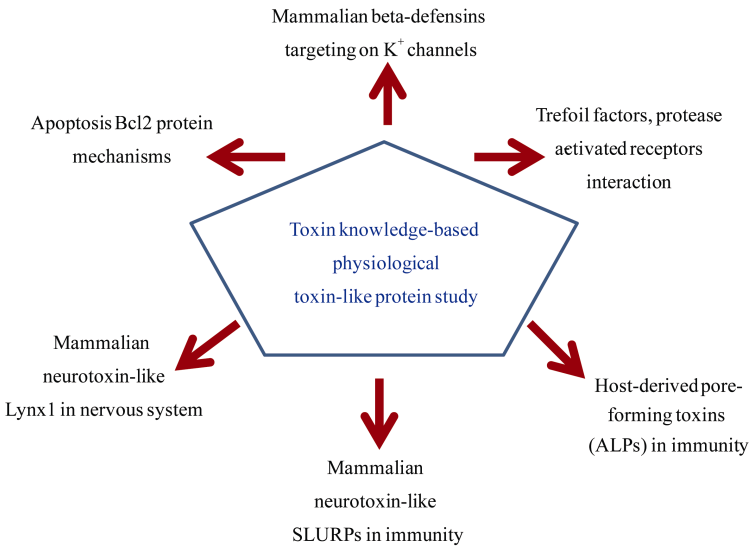

Figure 4 Toxin knowledge guides physiological toxin-like protein/peptide (TLP) study

There are numerous TLPs expressed in non-venomous animals and/or in non-venom systems with unknown physiological functions, including those in mammals. Knowledge obtained in the study of toxins has greatly facilitated uncovering the functions and mechanisms involved of these endogenous TLPs. See description in detail for each story and references cited in the text.

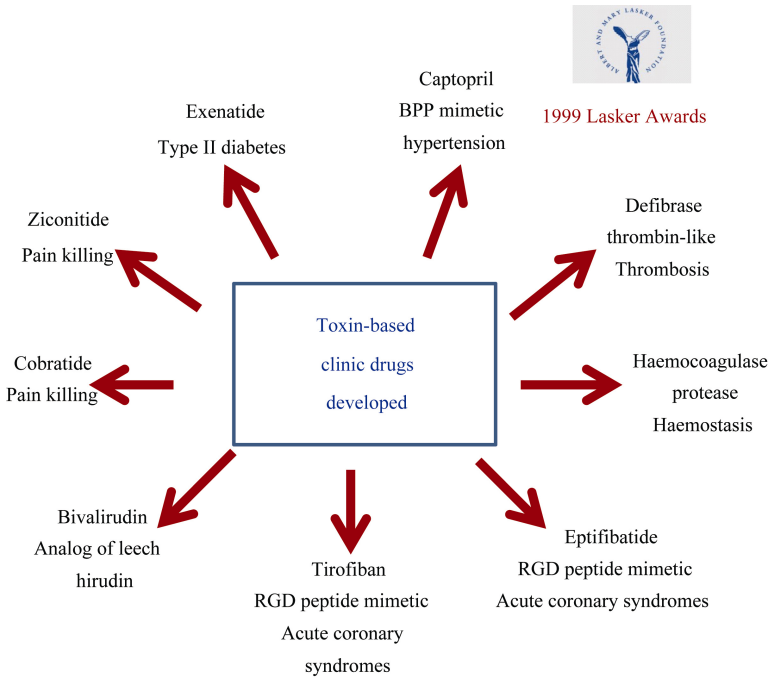

Figure 5 Modern toxin-based drugs developed

The potency, specificity, and stability of toxins have made them an invaluable source of natural products for drug discovery. The approved and widely used drugs derived from venom peptides or proteins are listed here. See description in detail for each example and references cited in the text. There are still tens of molecules in clinical trials and many more in various stages of preclinical development.

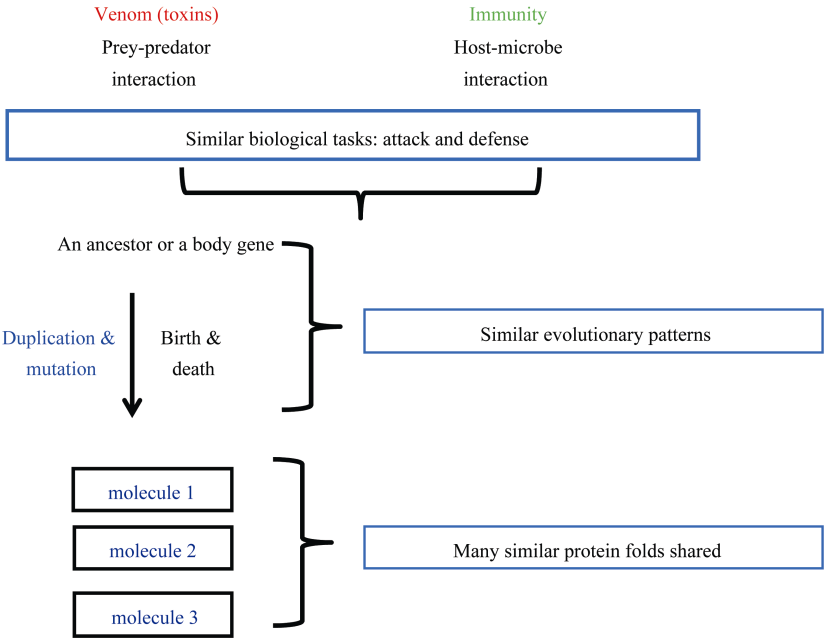

Figure 6 Similarity shared by venom and immune systems

The basic similarity of venom and immune systems is reflected by their primary biological tasks, attacks and defenses. The genetic and evolution origin of toxins and immune effectors are believed to evolve via the 'birth and death' process of gene evolution (Casewell et al, 2013; Fry, 2005; Nei, 1997). Recruitment of a proper gene and duplication and rapid mutation created diversified innovative toxin or immune effective molecules, which are selectively expressed in the venom glands or immune related organs.

mentioned earlier in this review about the structural similarity and conversion between snake neurotoxins and immune active SLURPs, crotamine toxins and defensins and their targeting on ion channels. These molecules were once thought to be distinct in form and function now appear to be members of a same family, probably descended from archetype predecessors that emerged in the early time of life on earth (Yeaman & Yount, 2007).

Cellular membranes are crucial for the survival of organisms. Pore-formation is frequently used in toxic arrack on cells, as it can lead to efficient disruption of cell functions and even cell death. Many major pathogenic bacteria employ PFTs as virulence factors, representing some 30% of all known bacterial toxins (van der Goot, 2014). Aerolysin, a toxin produced by the Gram-negative bacterium *Aeromonas hydrophila* and related species, belongs to the β-PFT group and shares a common mechanism of action involving β-barrel structures resulting from the assembly of β-hairpins from individual toxin monomers into a heptamer (Bischofberger et al, 2012; Iacovache et al, 2008). The aerolysin domain is defined according to its structural similarity to the transmembrane domain of aerolysin toxins (Szczesny et al, 2011).

As discussed below, aerolysin domain with membrane activity and pore-forming capacity has been discovered in virulence factors of pathogenic microbes for attacking, immune effectors of vertebrates (Xiang et al, 2014), as well as venom toxins in fishes (Magalhães et al, 2005) and centipedes (Liu et al, 2012) for prey-predator interaction.

Aerolysin-like proteins (ALPs) in venoms and immunity

Large-scale sequencing and bioinformatics analyses have revealed that proteins with an aerolysin fold, namely aerolysin-like proteins (ALPs), can be found in all froms of life. Particularly, a diverse array of proteins harboring an aerolysin domain fused with other domains has been identified in various animal and plant species (Szczesny et al, 2011; Xiang et al, 2014). In vertebrate species, ALPs are widely expressed in various body tissues of animals, including embryonic epidermis, skin, blood, gastrointestinal tract, spleen and kidney (Liu et al, 2008). However, little is known about their biological functions and mechanisms involved. The vast majority of these proteins have low sequence similarity (<20%). 3D-structures are crucial in revealing structural conservation that is elusive at sequence level (Moran et al, 2012).

ALPs in venoms

Aerolysin fold has been recruited to venom systems in venomous animals as toxin components in their venoms, as revealed in those of fishes and centipedes (Liu et al, 2012; Szczesny et al, 2011). Natterins, which are a class of protein toxins characterized from *T. nattereri* fish venom (Magalhães et al, 2005). In-depth bioinformatics analyses indicated that natterins are actually ALPs (Szczesny et al, 2011). Their toxicological effects are known to cause nociception and edema (Lopes-Ferreira et al, 2014). Two nattering-like proteins have been characterized from skin secretions of oriental catfish (*Plotosus lineatus*). In the same fish, which also possess a venom system, immunocytochemical approaches have established that the venom gland toxins of oriental catfish are natterin-like proteins (Tamura et al, 2011). The origin of these fish venoms was closely associated with skin glands and epidermal secretions, which normally play physiological roles in wound healing and innate immune defense (Al-Hassan et al, 1985; Wright, 2009).

ALPs in immunity

Direct killing/inhibiting pathogens Biomphalysin is an ALP protein in the snail *Biomphalaria glabrata*. The exclusive expression of Biomphalysin in hemocytes, the immune cells of *B. glabrata*, consolidates the role of Biomphalysin in immunity. In the presence of plasma, recombinant Biomphalysin is highly toxic toward parasitic *Schistosoma mansoni*, suggesting that one or more unknown plasma factors could act together with Biomphalysin. These results provide the first functional description of a mollusk immune effector protein involved in killing *S. mansoni* (Galinier et al, 2013).

Lysenin or lysenin-like proteins are ALPs from earthworm. One subgroup of earthworm immune cells (so called coelomocytes) express the highest amount of lysenin, and its expression can be enhanced by Gram-positive bacterial exposure. It has been suggested that lysenin appears to display sphingomyelin-dependent and sphingomyelin-independent activities to kill various foreign intruders of the earthworm's coelomic cavity (Bruhn et al, 2006; Opper et al, 2013). But the direct killing or inhibiting effects of lysenin on microbes have not been well-characterized, and further study is necessary to well illustrate its real actions in antimicrobial responses.

Acting together with TFF to trigger host innate immunity

Frog *B. maxima* lives in very harsh environments, such as pools containing microorganism-rich mud, and its skin is very “toxic”. Comprehensive transcriptome analysis of the frog skin and blood suggested that the frog can live well in harsh environments owing to its nearly parallel innate and adaptive immune systems to mammals (Zhao et al, 2014). Interestingly, a heteromeric protein complex was recently identified and isolated from the frog skin secretions, which is responsible for the lethal toxicity of the frog on mammals (Gao et al, 2011; Liu et al, 2008; Qian et al, 2008a;). This heteromeric protein consists of a βγ-crystallin fused ALP, namely *B. maxima* aerolysin-like protein 1 (Bm-ALP1), and a three domain trefoil factor (Bm-TFF3). It was named βγ-CAT to reflect its domain composition (Figure 7A insert).

The rich expression of Bm-ALP1 and Bm-TFF3 in the frog blood and immune-related tissues, and the induction of its presence in peritoneal lavage by bacterial challenge were detected, raising the possibility of their involvement in anti-microbial infections. Indeed, subsequent *in vivo* assays illustrated that the complex of Bm-ALP1 and Bm-TFF3 (βγ-CAT) was able to significantly accelerate bacterial clearance, thus reduce the mortality rate in frog *B. maxima* and mouse peritonitis models (Xiang et al, 2014). In contrast to small molecular-weight antimicrobial peptides from the same frog species (Lai et al, 2002b; Lee et al, 2005), βγ-CAT neither directly kills bacteria nor inhibits their growth. The rapid

maturation and release of IL-1β triggered by βγ-CAT were detected both *in vivo* and *in vitro* and may have resulted from the oligomerization of and pore formation by Bm-ALP1 within cellular endo-lysosomes, which partially explain the robust and effective antimicrobial responses observed (Xiang et al, 2014). Figure 7A shows the mechanisms involved and action models proposed for βγ–CAT in host antimicrobial responses. The mechanism by which the complex of Bm-ALP1 and Bm-TFF3 (βγ-CAT) activates inflammasome is completely different from that of aerolysin, which was found to activate inflammasome via pore formation on the plasma membrane (Gurcel et al, 2006).

Importantly, preliminary assays revealed that the biological activities of βγ–CAT were inhibited by free sialic acids, and were largely attenuated by eliminating sialic acid residues in cell membranes with sialidases (Guo & Zhang, unpublished observation), which suggests that either Bm-TFF3 or Bm-ALP1 βγ-crystallin domains interact with oligosaccharide chains (glycans), and sialic acids are essential in its binding to cells. Sialic acids are a diverse family of monosaccharides widely expressed on all cell surfaces of vertebrates and "higher" invertebrates, and on certain bacteria. Different modified forms of sialic acids can be attached to underlying glycans by means of various linkages from the C2 position. The remarkable diversity is expressed in a cell-type and developmentally regulated manner, and often changes in response to environmental cues, which plays important roles in pathogen infection, inflammation and immunity (Hart & Copeland, 2010; Varki, 2006, 2007). The in-depth studies of the interaction of βγ-CAT with glycans containing sialic acids, which might act as its putative membrane receptor(s), and biological relevance will certainly help to illustrate the roles of ALPs and TFFs in host immunity as well as the mechanisms involved.

ALPs and TFFs may consist of novel pathways and effectors in immunity The eradication of invading microorganisms is essential for the survival of multicellular organisms. Innate antimicrobial responses play a key role in host defense against many infections (Beutler, 2004). Profs Bruce Beutler and Jules Hoffmann were awarded the Nobel Prize in 2011 for the discovery of toll-like receptors (TLRs), sensors of microbes and a kind of pattern-recognition receptors (PRRs), which made great progress in our understanding of innate immunity. On the other hand, the interface of animals with the microbial world is characterized by the necessity to peacefully coexist with symbiotic microorganisms (the microbiota) (Chu & Mazmanian, 2013; Duerkop & Hooper, 2013). Thus, the basic puzzle here is how the host does to prevent pathogenesis while maintaining symbiosis. The strategies and molecular effectors of host endogenous regulators that ensure rapid, effective and controllable antimicrobial responses are incompletely understood. In addition, parasitic worms and allergens induce type 2 immune responses through mechanisms that appear to be independent of PRRs and remain largely unknown (Iwasaki & Medzhitov 2015; Licona-Limon et al, 2013; Medzhitov, 2010b; Medzhitov et al, 2012; Sansonetti, 2014; Strowig et al, 2012).

Interestingly, the membrane attack complex of the complement system shares a common core fold with bacterial cholesterol-dependent cytolysin-like PFTs (Rosado et al, 2008). The complement system is an evolutionarily well-conserved system, which constitutes a highly sophisticated body defense machinery. The current complexity in mammals consists of more than 30 components, while some components of the most primitive complement system can be identified in cnidaria (Nonaka, 2014). Three activation pathways of complement system are well-defined: the classical pathway, the lectin pathway, and the alternative pathway (Holers, 2014).

Here comes ALPs, another kind of bacterial PFT-like proteins and their possible roles in host immunity. βγ-CAT is the first example of an ALP and a TFF complex. Present data suggests that in contrast to microbial TLR ligands, which represent first signal of potential microbial infection, host-derived ALP and TFF complex primarily acts as a secondary signal, which might be necessary to initiate and trigger rapid and effective immune actions for eliminating dangerous microbial infections (Figure 7A). Thus, we hypothesized that ALPs and TFFs may consist of novel pathways and effectors in inflammation and immunity. The composition, regulation and effective actions of the pathways with ALPs and/or TFFs as potential sensors and effectors should have evolved diversification and variation along evolutionary processes in different lineages of organisms including vertebrates, which are certainly interesting and important subjects for future investigation.

In light of many well-defined innate immune pathways, such as those mediated by TLRs, the inflammasome-related NOD-like receptors (NLRs) as well as the components of complement system, which might be the functional positions and biological necessities of ALPs and TFFs in immunity? There are many possibilities, but in our opinion, the following aspects should be emphasized and studied in detail: (1) the clearance of intracellular microbial invasion of bacteria, virus or parasites; (2) the initiating of type II immunity to against parasitic infection as well as in allergic reactions; (3) the wound healing and tissue repairing. Further studies on their actions upon noxious stimuli, regulatory networks and underlying mechanisms will elucidate unknown pathways and effectors in inflammation and immunity, and eventually help to illustrate human patho-physiology, disease mechanisms and to provide novel drug targets and to develop novel therapeutics for related diseases.

Numerous ALPs mainly contain a membrane active and pore-forming aerolysin domain that undergoes fusion with agglutinin, jacalin, tachylectin, DM9, βγ-crystallin, and Ig-like domains have been found (Szczesny et al, 2011; Xiang et al, 2014), and could be readily identified by blast in Genbank from diverse plant and animal species, such as rice, grapes, fishes, amphibians, reptiles as well as birds (Figure 7B). Some of these additional domains fused to aerolysin membrane insertion domain might be carbohydrate-related, such as agglutinin, jacalin, tachylectin domains. Whether the ALPs containing these lectin-like domains represent an unknown type of Sugar-binding Oligomerization Proteins (SOPs), which could be regulated by sugar recognition and binding, are worthy of further elucidating in detail. Interestingly, TFF domains are found to widely exist in diverse glycosidases (Genbank data),

implying their interaction with sugar, which might represent their original and ancient functions. These domains have been recruited in immune system in host defense, and the sugar binding capacity of human TFF2 has been identified recently (Hanisch et al, 2014).

FUTURE DIRECTIONS AND CHALLENGES

Toxins are natural evolutionary products of living organisms for special biological purposes. They are often gene-coded proteins and peptides, and are different from simple chemical toxic substances (toxicants). People may often be confounded by "toxinology" and toxicology. Although there is some overlapping, significant difference exists between them. Toxinology, or more accurately toxin biology and toxin medicine, is the specialized area that deals scientific disciplines with microbial, plant and animal venoms, poisons and toxins. Besides the chemistry and mode of action, toxicological effects in other organisms, it deals also with the biology of toxin-producing and toxin-targeting organisms, the venom apparatus, as well as the ecological roles and bio-economy of toxins.

Toxins to answer basic biological questions

The primary biological role of venom system concerns struggle for existence and environmental adaptation. Consequently, venom toxins are tightly associated with specific ecological contexts and environmental conditions. The formation and loss of venom systems and coevolutionary patterns with other organisms and biological relevance related in various animal classes provide nice models to understand fundamental biological questions concerning the strategies for environmental adaptation, genetic basis, evolutionary mechanisms and biological economy. It has been suggested that the genetic and functional diversity of animal toxins make them ideal systems for testing the models postulated to underlie gene evolution and adaptive change in organisms (Innan & Kondrashov, 2010).

Zoological and ecological issues

Investigations concerning the distributions and ecological environments and living conditions of venomous animals, the species taxonomy as well as venom system anatomy and characterization are no doubt the basic fields needed to be substantially reinforced. This is particularly important in areas that are rich in biodiversity, such as China and countries in Southeast Asia. Even though there has been a strong research community in China in documenting the flora and the fauna of animals distributed in China, and remarkable achievements have been made over the past decades, the actual animal diversity, especially venomous animals, in China are still incomplete and many new venomous animals are waiting for discovery. We are now pressed for time and the situation is very serious facing continued decline and extinction of animal species worldwide. Data on animal feeding behavior, prey-predator relationship, distinct microbiota should be collected, which are important clues for directing the related toxin study.

Structure-function of toxins

The elucidation of molecular diversity of toxins and their biochemical properties, including post-translational modification, 3D-structure and family classification are another basic aspect of toxin study. The biological activity and toxicological effect of toxins in other organisms should be carefully assayed. Living strategy of a given animal is an important guiding principle in the assays. Blood coagulation and neuromuscular transmission are well-known key physiological networks targeted by venomous animals for rapid and effective immobilization of prey and/or defense. The haematotoxic and neurotoxic effects associated with venom exposure are widely recognized. In addition, immune and metabolic systems are also important targets of animal toxins, which is the strategy used by many venomous animals.

Natural pairing hypothesis

The most concerned issue right now and in the future is the molecular targets of toxins. It is quite difficult to fully understand the mutual interactions and mechanisms involved of the novel toxin molecules. Needless to say, this is a long-lasting task. The evolutionary origin and conservation of animals determine the inherent links among animals. Despite the huge species diversification, significant similarities and conservations share among animal physiological elements. Here, we propose the "natural pairing hypothesis" is that: (1) each animal key physiological element has been targeted by toxins in evolution process, and there has been at least one toxin molecule acting on it; (2) for the interactions of physiological elements and toxins, there are endogenous similarities and conservation, which occur among physiological elements and endogenous toxin-like molecules and play roles in physiological processes. This speculative idea is supported by accumulated and emerging identification of the interactions among animal physiological elements with toxins, as well as with endogenous toxin-like molecules. Traditionally, the study of toxin targets is mainly focused on protein components. In light of the critical roles of oligosaccharide chains (glycans) in physiological processes (Hart & Copeland 2010; Varki 2007), the specific glycans as toxin targets *in vivo* and biological relevance are worthy of noting and studying, especially for venom lectin-like proteins.

Although humans are generally neither the prey nor the predator of venomous animals, numerous toxins are able to actively and specifically interact with human physiological elements. This phenomena is hard to be either simply explained by conservations between humans and other animals or be seen as an interesting event by chance. The basic biological principles, which tightly link humans with toxins, are not recognized and understood well yet. We are now simply viewing a brief window of biological time, which represents the present status of trade-offs reached by currently living organisms subject to a number of evolutionary forces (Varki, 2006).

As reviewed above, it has been revealing that numerous physiological body proteins and peptides of various animals, including those of mammals, are homologous of toxins (TLPs). It would be speculated that the natural pairing and interaction of toxins and animal physiological elements are imitated and

conserved in normal physiological processes, which are conducted by endogenous interactions between body TLPs with their pairing physiological elements, especially in mammals, including humans. The similarity shared among normal endogenous interactions (TLPs with their pairing elements) in humans and exogenous interactions (toxins with their pairing elements) might be another explanation for unexpected tight links of toxins and humans.

Origin and loss, evolution and economy of toxins

As discussed extensively above, venom system is a special and complex trait shaped by evolution in struggles for existence in animals. However, the biological strategies of different animals, the origin and related genetic basis, the developmental regulation, as well as the underlying evolutionary mechanisms are not fully understood yet and are needed to be addressed in detail. Furthermore, the influence of specific prey-predator interaction (including those against microbial invasion) on the variation of toxins and coevolutionary patterns between toxins and body key physiological elements are interesting and important future challenges.

The possession of a venom system obviously has conferred the animal ecological advantages. However, there are also numerous non-venomous species in the same animal class, like non-venomous snakes. Many examples of venom loss in animals have been observed, supporting the notion that venom system occurs at a considerable cost in animals. The cost-benefit trade-offs of a given trait is optimized by evolutionary process, like that of inflammatory response in innate immunity (Okin & Medzhitov, 2012). Similarly, the trade-offs between beneficial and cost aspects of venoms may account for the origin and loss of a venom system, which is associated with the physiology and living strategy of a specific animal species. The biological philosophy and secrets underlying are important subjects worthy of investigating.

These studies, combining with principles and knowledge obtained in social and economic sciences, should certainly help biologists to better understand how evolution shaped development, change and balance among key factors (complex traits) in the struggle for existence of animals, such as venom (toxins), immunity, body power and intelligence, as well as genetic basis involved, inherent association and cost-benefit trade-offs of biological economy (Figure 8).

Toxins in fighting human diseases

Toxin related study in biomedicine may generally be conducted through two ways depending on the working fields, interests and technique skills of researchers. People mainly working in basic research fields or pharmaceutical industry focus on scientific questions of human patho-physiology and/or a clinic disease, and the works with the help of toxins have contributed substantially to human health. Studies starting with toxins are an alternatively way. Though being much more difficult, working with novel toxins or TLPs, which have novel actions and mechanisms, may eventually lead to novel clues and/or ways for fighting human diseases.

Deciphering human patho-physiology and diseases mechanisms

One of the most fascinating and important works is that using toxins as molecular probes to decipher the physiological functions and patho-physiological relevance of human physiological elements, and eventually disease mechanisms.

First, generally speaking, the issue on one hand is the determination of molecular interaction of a toxin molecule with a human physiological element, which could be an ion-channel, a receptor or a non-membrane protein. Work on the other hand is pharmacological and toxicological activities of the toxin, especially those of *in vivo*. The data obtained provide first indication and suggestion on the biological function of the human physiological elements. These assays should be conducted in both cellular level and in animal models. The obtained results should preferably be validated in animal model deficient in the targeted proteins. Mechanisms involved in the interactions between the toxin and the targeted protein should be investigated in detail. Collectively, these works will hopefully illustrate the unknown function and patho-physiological relevance of the targeted protein, and eventually the disease mechanisms. There are extensive molecular diversities of potential molecular targets of animal toxins, such as approximately 400 genes for pore-forming ion channels in human genome. Only a small part of them have been characterized until now. Screening of specific toxin molecules that act on those "unknown" or "orphan" ion-channels and/or receptors may be very interesting and important. Very often, the *in vivo* target of a given toxin is totally elusive, and data obtained from animal models should serve as an important guiding principle. In addition, the signs and symptoms during animal envenoming in humans could provide nice suggestions.

Second, immune system is an important target of animal toxins. Animal envenomation in humans often leads serious inflammatory responses and allergic reactions, which are one of the major causes of death. As reviewed above, the using of bee venom PLA2 revealed the protective roles of allergic reactions in innate immunity, which is contrast to the traditional view of allergy as a misguided and detrimental immune response (Palm et al, 2013). There are accumulative evidences showing conservation and similarity in venom toxins and immune effectors from these two systems. Thus, to understand immune systems, future studies are necessary to witness the facts from venom toxins.

Third, many components in animal venoms are mammalian hormone-like peptides, which could deeply affect metabolism systems. A nice example is exendins from the venom of lizards, which are homologous of human GLP-1 and are able to stimulate insulin and suppress glucagon secretions. Amphibian skin is also enriched with bioactive peptides, which may stimulate or inhibit metabolic activities. Starting with a focused metabolic disease, like diabetes, the screening of venom toxins preferably in animal models may lead to the discovery of novel metabolic active components modulating metabolic systems. Such works may result in uncovering the unknown regulatory pathways in human metabolism and disease mechanisms involved.

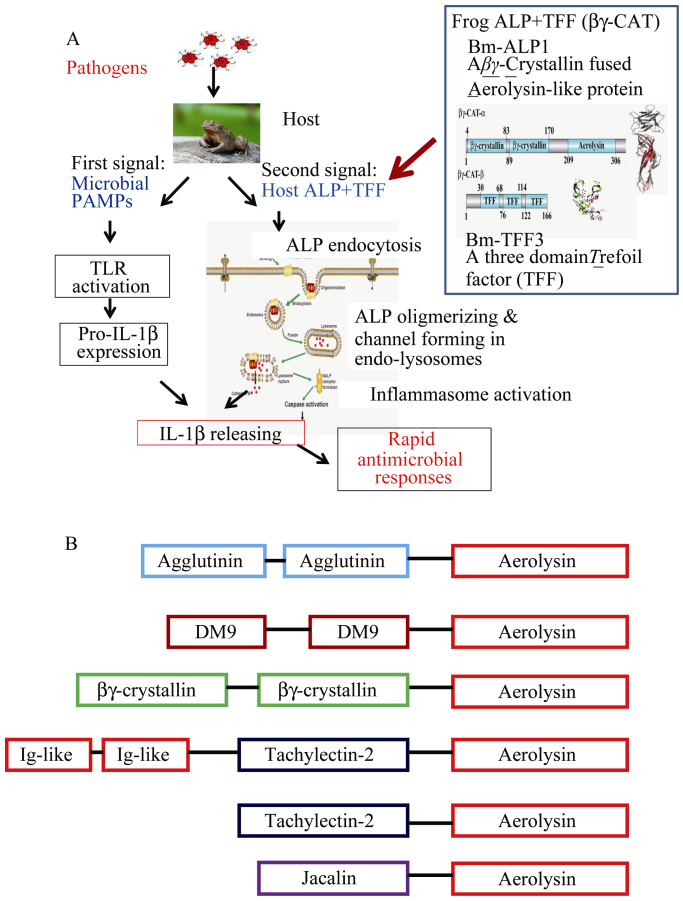

Figure 7 Aerolysin-like proteins (ALPs) and trefoil factors (TFFs) may consist of novel pathways and effectors in immunity

A: βγ-CAT, a heteromeric complex consists of Bm-ALP1 and Bm-TFF3 (a three domain TFF), was identified from *Bombina maxima* (insert). Upon bacterial infection, pathogen-associated molecular patterns (PAMPs) induce the activation of Toll-like receptors (TLRs), which subsequently trigger the intracellular production of pro-IL-1β. Additionally, βγ-CAT was endocytosed via membrane receptor mediation. Bm-ALP1 was found to oligomerize along endo-lysosome pathways to trigger lysosome destabilization, and led to IL-1β maturation and secretion via inflammasome activation, resulted in host rapid and effective antimicrobial responses (Xiong et al, 2014). B: ALPs mainly contain a pore-forming aerolysin domain that undergoes fusion with agglutinin, jacalin, tachylectin, DM9, βγ-crystallin, and Ig-like domains have been found (Szczesny et al, 2011; Xiang et al, 2014), and could be readily identified by blast in GenBank from diverse plant and animal species, such as rice, grapes, fishes, amphibians, reptiles as well as birds. The schematic domain composition of representative ALPs is cited and modified from Szczesny et al (2011). We speculated that some of these ALPs might be sugar-binding oligomerization proteins (SOPs), which could be regulated by sugar recognition and binding.

Fourth, numerous physiological TLPs, especially in terms of their 3D-structures, with unknown functions are expressed in non-venomous animals and in tissues not related to venom systems, such as ALPs. Knowledge obtained in toxin study are helping to illustrate their functions *in vivo*, the mechanisms involved as well as the path-physiological relevance in humans. Technological advances on the determination of protein 3D-structure will greatly accelerate the uncovering of body physiological TLPs. Another important work is that once the possible function of an "unknown" or "orphan" ion-channel or a receptor has been elucidated via its interaction with toxins, people will immediately search for its potential unknown endogenous ligand(s). In many cases, these works are difficult due to the lack of significant structural similarities.

Drug development

Based on their interactions with human targets, toxin molecules have stimulated many drug development projects. Thus, another attractive aspect in toxin study is that as the clinic therapeutics, toxins may be used in either direct or after toxin-based drug designs. Focusing on a properly selected disease and its clinic indications, the evaluation of druggability and pharmaceutical properties of a given toxin molecule is the most important work. Even though with high costs, these assays should preferably be conducted on animal models of human diseases, which are more productive than been simply

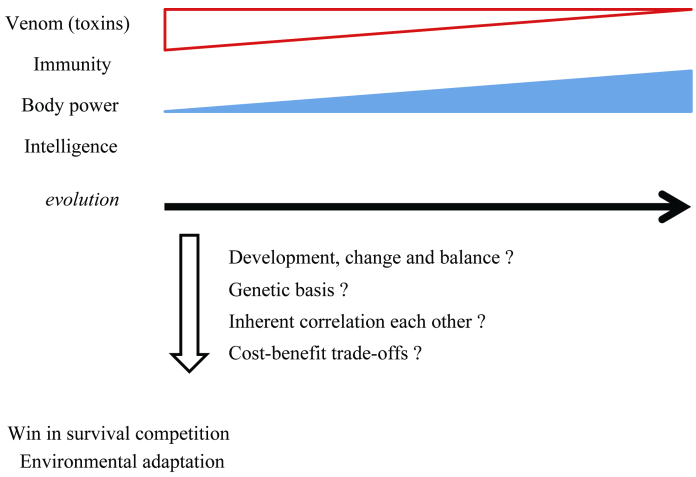

Figure 8 Venom systems provide nice models to understand fundamental biological questions

The cost-benefit trade-offs of a given trait is optimized by evolutionary process. In depth understanding of the origin and loss of venoms, biological relevance and coevolutionary patterns with other organisms provides nice models to investigate how evolution has shaped development, change and balance among key factors in the struggles for existence of animals, such as venom (toxins), immunity, body power and intelligence, as well as cost-benefit trade-offs of biological economy and the genetic basis related.

analyzed on molecular and cellular levels. The *in vivo* efficacy, potential antigenicity, unfavorable pharmacokinetics, costs, side-effects and advantages compared with present clinic drugs are major concerns in toxin-based drug development. In many cases, toxin molecules serve as templets in small molecule design for optimizing drug development directed by structural information of the mutual interaction between a toxin in its native or modified forms and its specific targets, and the pharmacological activity resulted.

There are many medicinal animals widely and effectively used in clinic as therapeutics, which are often venomous animals. The traditional practice and experience of these medicinal animals, especially clinic indications related, provide invaluable information in modern drug development. We should not forget that there are many TLPs expressed in the non-venom tissues of both venomous and non-venomous medicinal animals. It is highly interesting to investigate in detail whether the effective components in these medicinal animals are venom toxins and/or body TLPs. Such work will greatly promote the establishment of medicinal animal standards in industry and markets and enhance the modernization of traditional medicine.

As reviewed by Harvey (2014), drug discovery and development are an inherently risky business. Although with some notable successes, there have been many more disappointments on the road from toxin discovery to approval of a new medicine. Some products have been dropped in clinic trials due to side-effects and toxicity in human encountered. The failure may also be often caused by the discrepancy of toxin targets determined *in vitro* and those actually targeted by the compound *in vivo*, leading to unexpected and unwanted effects in clinic. After efforts of many years, US FDA did not approve marketing of pexiganan, an analog of magainin-2 from forg skin for treatment of infected foot ulcers in diabetic patients because of not enough efficacies demonstrated in clinic. Consequently, alternative therapeutic applications need to be explored (Conlon et al, 2014).

Problems encountered

First, toxins are traditionally isolated from crude venoms by classic chemical techniques. Obtaining pure enough toxin sample with sufficient quantity is the first key factor that limits toxin study. Minor contamination often resulted in wrong conclusions in the interpretation of pharmacological and toxicological activities of a specific toxin. On the other hand, the majority of toxins revealed by genome and transcriptomic analysis are almost impossible to obtain by classical purification processes. New advances in chemical synthesis and recombinant expression of a toxin polypeptide have been greatly accelerating toxin study, which allow sufficient and pure enough toxin sample obtained (Cui et al, 2013; Schroeder et al, 2014).

Second, a serious concern of animal toxins in biomedical research and drug development is the specificity of a toxin on its molecular targets. Facing the diversity of physiological elements in prey and/or predator, selection pressure and coevolution have made creation of diversified toxins with relatively high specificity and potency, especially compared with those of small compounds from plants. The interaction of a toxin with an ion channel or a receptor depends on its binding affinity. Taking K^+ channels as an example, it was estimated that there are nearly 100 K^+ channel members (Ashcroft, 2006) in humans. High specificity means the difference of binding affinity to a kind of K^+ channels or to the subtypes of K^+ channels is high, which does not exclude the possible action on other K^+ channels or channel subtypes. Furthermore, the interaction of a toxin with an ion channel was usually determined *in vitro*, and often assayed or screened with targets in hands, which are unfortunately only corresponding to a small part of those *in vivo*. The interactions discovered might not really reflect its optimal targets, especially *in vivo*. This discrepancy of toxin action

determined *in vitro* and those actually conducting *in vivo* may either mislead the use of the toxin as a tool to deciphering patho-physiology or result in the failure of toxin-based drug development (Harvey, 2014).

We should recognize that to human health, venomous animals are neglected strategic resources. The exploitation involves investigation and collection of animals, the well-organized preservation of toxin genes before the extinction of specific animal species, and toxin-related works, which is a typical systematic engineering. Obviously, tight collaboration of researchers in different fields, including with people in industry is a preferable way for effective outcome prospected. Establishment of toxin center(s) or working networks, if possible in national level and international level, should greatly prompt the advance of toxin studys. If we plan to obtain the genome sequences of 10 000 people, why not try to obtain those of 100 venomous animals too. These works will substantially help to illustrate human patho-physiology and disease mechanisms, potential drug finding, rational new drug design, as well as clinic utilization.

CONCLUDING REMARKS

As a special trait in animals, venom system has evolved in nature for survival competitions, which plays important biological roles in predation, defense, competition and even communication in given ecological contexts. Facing the huge molecular diversity of key physiological elements of animals including humans, such as cell membrane ion channels and receptors, long-term coevolution has evolved extensive and diverse peptide toxins in the venom of venomous animals, which are able to specifically target on these key physiological elements.

Defenses against microbial invasion and self-malignant cells are major missions of immune systema. Attack and defense are the basic commonality of venom and immune systems. It would be reasonable to predict and hypothesize that both systems share substantial conservation and similarity in their biological strategies and molecular effectors, as supported by many conserved protein folds used in both systems. The investigation with this notion in mind should certainly benefit mutually and facilitate the in-depth understanding of these two systems. Venom (toxins), immunity, body power and intelligence are evolved along the evolutionary tree of animals and play key roles in animal survival competitions. Toxin study will hopefully provide useful information and will help to illustrate the basic biological issue that how a living organism adapt environments, keep “homeostasis” facing various ecological conditions and noxious stimuli, and win in struggles for existence.

According to "The Medical Classic of the Yellow Emperor (Huangdi Neijing)", one of the earliest theoretical classics on Chinese medicine, a disease is an unbalanced state of human body. Drugs *per se* are agents causing another unbalanced state, which are used to recover the given unbalanced state of a human disease. So, drugs are inherently more or less toxic, and can be viewed as special poisons/toxins. Theoretically, each poison/toxin molecule created by nature could potentially serve as a pharmacological tool and/or a clinic therapeutics in either its native or modified forms in conditions that the toxin is used at a right time, in a right place, with a right dosage and for proper purposes or clinic indications.

Evolution links animal toxins with humans, providing natural basis of animal envenomation as well as for animal toxins being used as pharmacological research tools and/or clinic therapeutics. Our goal is to reveal the right natural pairings and interactions between our body elements and toxins. Starting with focused scientific questions and/or a clinic disease, or starting with toxins themselves, through diligent work and/or serendipity, work with toxin molecules has led, and will lead in future, to new discoveries and exciting avenues for deciphering and fighting human diseases. Biomedical researches in humans and in model animals have made great advances in the understanding of our physiology and diseases. In depth understanding of toxins is an effective way to better know ourselves. All in all, this is why we study toxins (Figure 9).

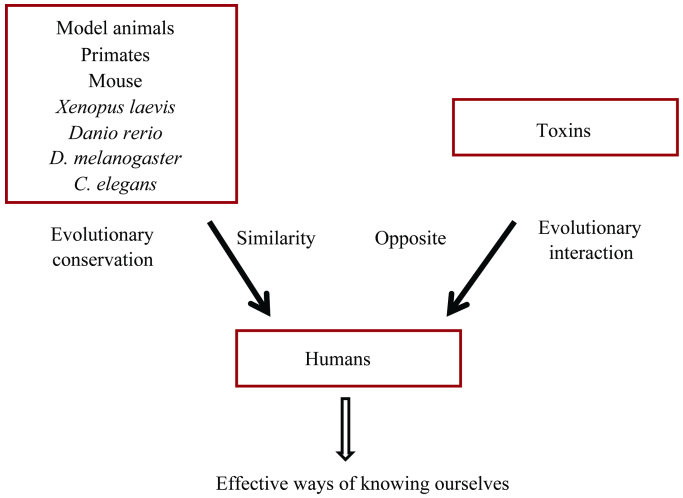

Figure 9 Recognizing us from toxins is an effective way to better understand ourselves

To understand human physiology and diseases, studies in model animals rely on the similarity and conservation shared by humans and model animals in evolution. In nature, toxins are agianst humans, and studies depending on the evolutionary interaction between humans and toxins are an alternative and effective way to better understand ourselves.

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REFERENCES

Aili SR, Touchard A, Escoubas P, Padula MP, Orivel J, Dejean A, Nicholson GM. 2014. Diversity of peptide toxins from stinging ant venoms. *Toxicon,* **92**: 166-178.

Albuquerque EX, Pereira EFR, Alkondon M, Rogers SW. 2009. Mammalian nicotinic acetylcholine receptors: from structure to function. *Physiological Reviews,* **89**(1): 73-120.

Al-Hassan JM, Thomson M, Criddle KR, Summers B, Criddle RS. 1985. Catfish epidermal secretions in response to threat or injury. *Marine Biology,* **88**(2): 117-123.

Almaaytah A, Albalas Q. 2014. Scorpion venom peptides with no disulfide bridges: a review. *Peptides,* **51**: 35-45.

Almeida DD, Scortecci KC, Kobashi LS, Agnez-Lima LF, Medeiros SRB, Silva-Junior AA, Junqueira-De-Azevedo Ide LM, Fernandes-Pedrosa Mde F. 2012. Profiling the resting venom gland of the scorpion *Tityus stigmurus* through a transcriptomic survey. *BMC Genomics,* **13**: 362.

Aminetzach YT, Srouji JR, Kong CY, Hoekstra HE. 2009. Convergent evolution of novel protein function in shrew and lizard venom. *Current Biology,* **19**(22): 1925-1931.

Arcà B, Lombardo F, De Lara Capurro M, Della Torre A, Dimopoulos G, James AA, Coluzzi M. 1999. Trapping cDNAs encoding secreted proteins from the salivary glands of the malaria vector *Anophelesgambiae*. *Proceedings of the National Academy of Sciences of the USA,* **96**(4): 1516-1521.

Arredondo J, Chernyavsky AI, Jolkovsky DL, Webber RJ, Grando SA. 2006. SLURP-2: A novel cholinergic signaling peptide in human mucocutaneous epithelium. *Journal of Cellular Physiology,* **208**(1): 238-245.

Artis D, Maizels RM, Finkelman FD. 2012. Forum: Immunology: Allergy challenged. *Nature,* **484**(7395): 458-459.

Ashcroft FM. 2006. From molecule to malady. *Nature,* **440**(7083): 440-447.

Baconguis I, Bohlen CJ, Goehring A, Julius D, Gouaux E. 2014. X-ray structure of acid-sensing ion channel 1-snake toxin complex reveals open state of a Na^+-selective channel. *Cell,* **156**(4): 717-729.

Bagal SK, Brown AD, Cox PJ, Omoto K, Owen RM, Pryde DC, Sidders B, Skerratt SE, Stevens EB, Storer RI, Swain NA. 2013. Ion channels as therapeutic targets: a drug discovery perspective. *Journal of Medicinal Chemistry,* **56**(3): 593-624.

Balasubramanian PG, Beckmann A, Warnken U, Schnölzer M, Schüler A, Bornberg-Bauer E, Holstein TW, Özbek S. 2012. Proteome of Hydra nematocyst. *The Journal of Biological Chemistry,* **287**(13): 9672-9681.

Balhara KS, Stolbach A. 2014. Marine envenomations. *Emergency Medicine Clinics of North America,* **32**(1): 223-243.

Banerjee A, Lee A, Campbell E, Mackinnon R. 2013. Structure of a pore-blocking toxin in complex with a eukaryotic voltage-dependent K^+ channel. *eLife,* **2**: e00594.

Baron A, Diochot S, Salinas M, Deval E, Noël J, Lingueglia E. 2013. Venom toxins in the exploration of molecular, physiological and pathophysiological functions of acid-sensing ion channels. *Toxicon,* **75**: 187-204.

Baskova IP, Kostrjukova ES, Vlasova MA, Kharitonova OV, Levitskiy SA, Zavalova LL, Moshkovskii SA, Lazarev VN. 2008. Proteins and peptides of the salivary gland secretion of medicinal leeches *Hirudo verbana*, *H. medicinalis*, and *H. orientalis*. *Biochemistry (Moscow),* **73**(3): 315-320.

Baumann K, Casewell NR, Ali SA, Jackson TNW, Vetter I, Dobson JS, Cutmore SC, Nouwens A, Lavergne V, Fry BG. 2014. A ray of venom: Combined proteomic and transcriptomic investigation of fish venom composition using barb tissue from the blue-spotted stingray (*Neotrygon kuhlii*). *Journal of Proteomics,* **109**: 188-198.

Beckmann A, Özbek S. 2012. The nematocyst: a molecular map of the cnidarian stinging organelle. *The International Journal of Developmental Biology,* **56**(6-8): 577-582.

Berkov A, Rodríguez N, Centeno P. 2008. Convergent evolution in the antennae of a cerambycid beetle, *Onychocerus albitarsis*, and the sting of a scorpion. *Naturwissenschaften,* **95**(3): 257-261.

Beutler B. 2004. Innate immunity: an overview. *Molecular Immunology,* **40**(12): 845-859.

Biardi JE, Coss RG. 2011. Rock squirrel (*Spermophilus variegatus*) blood sera affects proteolytic and hemolytic activities of rattlesnake venoms. *Toxicon,* **57**(2): 323-331.

Biass D, Dutertre S, Gerbault A, Menou JL, Offord R, Favreau P, Stocklin R. 2009. Comparative proteomic study of the venom of the piscivorous cone snail *Conus consors*. *Journal of Proteomics,* **72**(2): 210-218.

Bischofberger M, Iacovache I, Van Der Goot FG. 2012. Pathogenic pore-forming proteins: function and host response. *Cell Host & Microbe,* **12**(3): 266-275.

Bledzka K, Smyth SS, Plow EF. 2013. Integrin αIIbβ3: from discovery to efficacious therapeutic target. *Circulation Research,* **112**(8): 1189-1200.

Bohlen CJ, Priel A, Zhou S, King D, Siemens J, Julius D. 2010. A bivalent tarantula toxin activates the capsaicin receptor, TRPV1, by targeting the outer pore domain. *Cell,* **141**(5): 834-845.

Bohlen CJ, Chesler AT, Sharif-Naeini R, Medzihradszky KF, Zhou S, King D, Sánchez EE, Burlingame AL, Basbaum AI, Julius D. 2011. A heteromeric Texas coral snake toxin targets acid-sensing ion channels to produce pain. *Nature,* **479**(7373): 410-414.

Bourgeois EA, Subramaniam S, Cheng TY, De Jong A, Layre E, Ly D, Salimi M, Legaspi A, Modlin RL, Salio M, Cerundolo V, Moody DB, Ogg G. 2015. Bee venom processes human skin lipids for presentation by CD1a. *Journal of Experimental Medicine,* **212**(2): 149-163.[Epub ahead of print]

Bouzid W, Verdenaud M, Klopp C, Ducancel F, Noirot C, Vétillard A. 2014. *De Novo* sequencing and transcriptome analysis for *Tetramorium bicarinatum*: a comprehensive venom gland transcriptome analysis from an ant species. *BMC Genomics,* **15**(1): 987.

Bradley SJ, Riaz SA, Tobin AB. 2014. Employing novel animal models in the design of clinically efficacious GPCR ligands. *Current Opinion in Cell Biology,* **27**: 117-125.

Brahma RK, McCleary RJ, Kini RM, Doley R. 2015. Venom gland transcriptomics for identifying, cataloging, and characterizing venom proteins in snakes. *Toxicon,* **93**: 1-10.

Brehler R, Grundmann S, Stöcker B. 2013. Cross-reacting carbohydrate determinants and hymenoptera venom allergy. *Current Opinion in Allergy and Clinical Immunology,* **13**(4): 360-364.

Bridges AR, Owen MD. 1984. The morphology of the honey bee (*Apis mellifera* L.) venom gland and reservoir. *Journal of Morphology,* **181**(1): 69-86.

Brodie ED. 2009. Toxins and venoms. *Current Biology,* **19**(20): R931-R935.

Bruhn H, Winkelmann J, Andersen C, Andrä J, Leippe M. 2006. Dissection of the mechanisms of cytolytic and antibacterial activity of lysenin, a defence protein of the annelid *Eisenia fetida*. *Developmental & Comparative Immunology,* **30**(7): 597-606.

Butzke D, Luch A. 2010. High-molecular weight protein toxins of marine invertebrates and their elaborate modes of action. *Molecular, Clinical and Environmental Toxicology,* **100**: 213-232.

Byers LD, Wolfenden R. 1972. A potent reversible inhibitor of carboxypeptidase A.*The Journal of Biological Chemistry,* **247**(2): 606-608.

Cabezas-Cruz A, Valdés JJ. 2014. Are ticks venomous animals? *Frontiers in Zoology,* **11**: 47.

Cai B, Huang Y, Chen YY, Hu JH, Guo XG, Wang YZ. 2012. Geographic patterns and ecological factors correlates of snake species richness in China. *Zoological Research,* **33**(4): 343-353. (In Chinese)

Calvete JJ. 2014. Next-generation snake venomics: protein-locus resolution through venom proteome decomplexation. *Expert Review of Proteomics,* **11**(3): 315-329.

Camargo ACM, Ianzer D, Guerreiro JR, Serrano SMT. 2012. Bradykinin-potentiating peptides: beyond captopril. *Toxicon,* **59**(4): 516-523.

Campbell CR, Voyles J, Cook DI, Dinudom A. 2012. Frog skin epithelium: electrolyte transport and chytridiomycosis. *The International Journal of Biochemistry & Cell Biology,* **44**(3): 431-434.

Cao E, Liao MF, Cheng YF, Julius D. 2013a. TRPV1 structures in distinct conformations reveal activation mechanisms. *Nature,* **504**(7478): 113-118.

Cao ZJ, Yu Y, Wu YL, Hao P, Di ZY, He YW, Chen ZY, Yang WS, Shen ZY, He XH, Sheng J, Xu XB, Pan BH, Feng J, Yang XJ, Hong W, Zhao WJ, Li ZJ, Huang K, Li T, Kong YM, Liu H, Jiang DH, Zhang BY, Hu J, Hu YT, Wang B, Dai JL, Yuan BF, Feng YQ, Huang W, Xing XJ, Zhao GP, Li X, Li YX, Li WX. 2013b. The genome of *Mesobuthus martensii* reveals a unique adaptation model of arthropods. *Nature Communications,* **4**: 2602.

Casewell NR, Wüster W, Vonk FJ, Harrison RA, Fry BG. 2013. Complex cocktails: the evolutionary novelty of venoms. *Trends in Ecology & Evolution,* **28**(4): 219-229.

Cegolon L, Heymann WC, Lange JH, Mastrangelo G. 2013. Jellyfish stings and their management: a review. *Marine Drugs,* **11**(2): 523-550.

Cerdá X, Van Oudenhove L, Bernstein C, Boulay R. 2014. A list of and some comments about the trail pheromones of ants. *Natural Product Communications,* **9**(8): 1115-1122.

Chang CC, Lee CY. 1963. Isolation of neurotoxins from the venom of Bungarus multicinctus and their modes of neuromuscular blocking action. *Archives Internationales de Pharmacodynamie et de Thérapie,* **144**: 241-257.

Chapman JA, Kirkness EF, Simakov O, Hampson SE, Mitros T, Weinmaier T, Rattei T, Balasubramanian PG, Borman J, Busam D, Disbennett K, Pfannkoch C, Sumin N, Sutton GG, Viswanathan LD, Walenz B, Goodstein DM, Hellsten U, Kawashima T, Prochnik SE, Putnam NH, Shu S, Blumberg B, Dana CE, Gee L, Kibler DF, Law L, Lindgens D, Martinez DE, Peng JS, Wigge PA, Bertulat B, Guder C, Nakamura Y, Ozbek S, Watanabe H, Khalturin K, Hemmrich G, Franke A, Augustin R, Fraune S, Hayakawa E, Hayakawa S, Hirose M, Hwang JS, Ikeo K, Nishimiya-Fujisawa C, Ogura A, Takahashi T, Steinmetz PRH, Zhang XM, Aufschnaiter R, Eder MK, Gorny AK, Salvenmoser W, Heimberg AM, Wheeler BM, Peterson KJ, Böttger A, Tischler P, Wolf A, Gojobori T, Remington KA, Strausberg RL, Venter JC, Technau U, Hobmayer B, Bosch TCG, Holstein TW, Fujisawa T, Bode HR, David CN, Rokhsar DS, Steele RE. 2010. The dynamic genome of *Hydra*. *Nature,* **464**(7288): 592-596.

Chen L, Sharma KR, Fadamiro HY. 2009. Fire ant venom alkaloids act as key attractants for the parasitic phorid fly, *Pseudacteon tricuspis* (Diptera: Phoridae). *Naturwissenschaften,* **96**(12): 1421-1429.

Chernyavsky AI, Arredondo J, Galitovskiy V, Qian J, Grando SA. 2010. Upregulation of nuclear factor-κB expression by SLURP-1 is mediated by α_7-nicotinic acetylcholine receptor and involves both ionic events and activation of protein kinases. *American Journal of Physiology Cell Physiology,* **299**(5): C903-C911.

Chimienti F, Hogg RC, Plantard L, Lehmann C, Brakch N, Fischer J, Huber M, Bertrand D, Hohl D. 2003. Identification of SLURP-1 as an epidermal neuromodulator explains the clinical phenotype of Mal de Meleda. *Human Molecular Genetics,* **12**(22): 3017-3024.

Chipman AD, Ferrier DE, Brena C, Qu JX, Hughes DST, Schröder R, Torres-Oliva M, Znassi N, Jiang HY, Almeida FC, Alonso CR, Apostolou Z, Aqrawi P, Arthur W, Barna JCJ, Blankenburg KP, Brites D, Capella-Gutiérrez S, Coyle M, Dearden PK, Du Pasquier L, Duncan EJ, Ebert D, Eibner C, Erikson G, Evans PD, Extavour CG, Francisco L, Gabaldón T, Gillis WJ, Goodwin-Horn EA, Green JE, Griffiths-Jones S, Grimmelikhuijzen CJP, Gubbala S, GuigóR, Han Y, Hauser F, Havlak P, Hayden L, Helbing S, Holder M, Hui JHL, Hunn JP, Hunnekuhl VS, Jackson L, Javaid M, Jhangiani SN, Jiggins FM, Jones TE, Kaiser TS, Kalra D, Kenny NJ, Korchina V, Kovar CL, Bernhard Kraus F, Lapraz F, Lee SL, Lv J, Mandapat C, Manning G, Mariotti M, Mata R, Mathew T, Neumann T, Newsham I, Ngo DN, Ninova M, Okwuonu G, Ongeri F, Palmer WJ, Patil S, Patraquim P, Pham C, Pu LL, Putman NH, Rabouille C, Ramos OM, Rhodes AC, Robertson HE, Robertson HM, Ronshaugen M, Rozas J, Saada N, Sánchez-Gracia A, Scherer SE, Schurko AM, Siggens KW, Simmons D, Stief A, Stolle E, Telford MJ, Tessmar-Raible K, Thornton R, Van Der Zee M, Von Haeseler A, Williams JM, Willis JH, Wu YQ, Zou XY, Lawson D, Muzny DM, Worley KC, Gibbs RA, Akam M, Richards S. 2014. The First Myriapod Genome Sequence Reveals Conservative Arthropod Gene Content and Genome Organisation in the Centipede *Strigamia maritima*. *PLoS Biollogy,* **12**(11): e1002005.

Chippaux JP. 2008. Estimating the global burden of snakebite can help to improve management. *PLoS Medicine,* **5**(11): e221.

Chippaux JP, Goyffon M. 2008. Epidemiology of scorpionism: a global appraisal. *Acta Tropica,* **107**(2): 71-79.

Chu HT, Mazmanian SK. 2013. Innate immune recognition of the microbiota promotes host-microbial symbiosis. *Nature Immunology,* **14**(7): 668-675.

Church JE, Hodgson WC. 2002. The pharmacological activity of fish venoms. *Toxicon,* **40**(8): 1083-1093.

Clemetson JM, Polgar J, Magnenat E, Wells TNC, Clemetson KJ. 1999. The platelet collagen receptor glycoprotein VI is a member of the immunoglobulin superfamily closely related to FcαR and the natural killer receptors. *The Journal of Biological Chemistey,* **274**(41): 29019-29024.

Cohen S, Levi-Montalcini R. 1956. A nerve growth-stimulating factor isolated from snake venom. *Proceedings of the National Academy of Sciences of the USA,* **42**(9): 571-574.

Conlon JM, Mechkarska M, Lukic ML, Flatt PR. 2014. Potential therapeutic applications of multifunctional host-defense peptides from frog skin as anticancer, anti-viral, immunomodulatory, and anti-diabetic agents. *Peptides,* **57**: 67-77.

Coppens M, Eikelboom JW, Gustafsson D, Weitz JI, Hirsh J. 2012. Transla-

tional success stories: development of direct thrombin inhibitors. *Circulation Research,* **111**(7): 920-929.

Cortez MH, Weitz JS. 2014. Coevolution can reverse predator-prey cycles. *Proceedings of the National Academy of Sciences of the USA,* **111**(20): 7486-7491.

Cui HK, Guo Y, He Y, Wang FL, Chang HN, Wang YJ, Wu FM, Tian CL, Liu L. 2013. Diaminodiacid-based solid-phase synthesis of peptide disulfide bond mimics. *Angewandte Chemie International Edition,* **52**(36): 9558-9562.

Cushman DW, Cheung HS, Sabo EF. 1977. Design of potent competitive inhibitors of angiotensin converting enzyme: Carboxyalkanoyl and mercaptoalkanoyl amino acids. *Biochemistry,* **16**: 548–591.

Cushman DW, Ondetti MA. 1991. History of the design of captopril and related inhibitors of angiotensin converting enzyme. *Hypertension,* **17**(4): 589-592.

Daltry JC, Wüster W, Thorpe RS. 1996. Diet and snake venom evolution. *Nature,* **379**(6565): 537-540.

Daly JW, Spande TF, Garraffo HM. 2005. Alkaloids from amphibian skin: a tabulation of over eight-hundred compounds. *Journal of Natural Products,* **68**(10): 1556-1575.

Danforth BN, Cardinal S, Praz C, Almeida EAB, Michez D. 2013. The impact of molecular data on our understanding of bee phylogeny and evolution. *Annual Review Entomology,* **58**(1): 57-78.

Darwin CR. 1859. On the Origin of Species. London: John Murray.

Davis J, Jones A, Lewis RJ. 2009. Remarkable inter- and intra-species complexity of conotoxins revealed by LC/MS. *Peptides,* **30**(7): 1222-1227.

Deval E, Gasull X, Noël J, Salinas M, Baron A, Diochot S, Lingueglia E. 2010. Acid-sensing ion channels (ASICs): pharmacology and implication in pain. *Pharmacol & Therapeutics,* **128**(3): 549-558.

Di ZY, Yang ZZ, Yin SJ, Cao ZJ, Li WX. 2014. History of study, updated checklist, distribution and key of scorpions (Arachnida: Scorpiones) from China. *Zoological Research,* **35**(1): 3-19. (In Chinese)

Dias NB, De Souza BM, Gomes PC, Palma MS. 2014. Peptide diversity in the venom of the social wasp *Polybia paulista* (Hymenoptera): a comparison of the intra- and inter-colony compositions. *Peptides,* **51**: 122-130.

Diochot S, Baron A, Salinas M, Douguet D, Scarzello S, Dabert-Gay AS, Debayle D, Friend V, Alloui A, Lazdunski M, Lingueglia E. 2012. Black mamba venom peptides target acid-sensing ion channels to abolish pain. *Nature,* **490**(7421): 552-555.

Dobzhansky T. 1973. Nothing in biology makes sense, except in the light of evolution. *American Biology Teacher*, **35**: 125-129.

Dolly JO, Barnard EA. 1984. Nicotinic acetylcholine receptors: an overview. *Biochemical Pharmacology,* **33**(6): 841-858.

Drucker DJ, Nauck MA. 2006. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *The Lancet,* **368**(9548): 1696-1705.

Du XY, Sim DS, Lee WH, Zhang Y. 2006. Blood cells as targets of snake toxins. *Blood Cells, Molecules, and Diseases,* **36**(3): 414-421.

Duda TF Jr, Kohn AJ, Palumbi SR. 2001. Origins of diverse feeding ecologies within *Conus*, a genus of venomous marine gastropods. *Biological Journal of the Linnean Society,* **73**(4): 391-409.

Duda TF Jr, Vanhoye D, Nicolas P. 2002. Roles of diversifying selection and coordinated evolution in the evolution of amphibian antimicrobial peptides. *Molecular Biology and Evolution,* **19**(6): 858-864.

Duellman WE, Trueb L. 1994. Relationship with the environment. *In*: Duellman WE, Trueb L. Biology of Amphibians. Maryland: The Johns Hopkins University Press, 197-228.

Duerkop BA, Hooper LV. 2013. Resident viruses and their interactions with the immune system. *Nature Immunology,* **14**(7): 654-659.

Dugon MM, Arthur W. 2012. Comparative studies on the structure and development of the venom-delivery system of centipedes, and a hypothesis on the origin of this evolutionary novelty. *Evolution Development,* **14**(1): 128-137.

Dumbacher JP, Beehler BM, Spande TF, Garraffo HM, Daly JW. 1992. Homobatrachotoxin in the genus Pitohui: chemical defense in birds? *Science,* **258**(5083): 799-801.

Dutertre S, Lewis RJ. 2010. Use of venom peptides to probe ion channel structure and function. *The Journal of Biological Chemistry,* **285**(18): 13315-13320.

Dutertre S, Jin AH, Alewood PF, Lewis RJ. 2014a. Intraspecific variations in *Conus geographus* defence-evoked venom and estimation of the human lethal dose. *Toxicon,* **91**: 135-144.

Dutertre S, Jin AH, Vetter I, Hamilton B, Sunagar K, Lavergne V, Dutertre V, Fry BG, Antunes A, Venter DJ, Alewood PF, Lewis RJ. 2014b. Evolution of separate predation- and defence-evoked venoms in carnivorous cone snails. *Nature Communications,* **5**: 3521.

Edgecombe GD, Giribet G. 2007. Evolutionary biology of centipedes (Myriapoda: Chilopoda). *Annual Review of Entomology,* **52**: 151-170.

Egbertson MS, Chang CT, Duggan ME, Gould RJ, Halczenko W, Hartman GD, Laswell WL, Lynch JJ Jr, Lynch JR, Manno PD. 1994. Non-peptide fibrinogen receptor antagonists.2.Optimization of a tyrosine template as a mimic for Arg-Gly-Asp. *Journal of Medicinal Chemistry,* **37**(16): 2537-2551.

Eltaher S, Mohammed GF, Younes S, Elakhras A. 2014. Efficacy of the apitherapy in the treatment of recalcitrant localized plaque psoriasis and evaluation of tumor necrosis factor-alpha (TNF-α) serum level: A double-blind randomized clinical trial. *Journal of Dermatological Treatment,* **30**: 1-5. [Epub ahead of print]

Eng J, Kleinman WA, Singh L, Singh G, Raufman JP. 1992. Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. *Journal of Biological Chemistry,* **267**(11): 7402-7405.

Fei L. 1999. Atlas of Amphibians of China. Zhengzhou: Henan Science and Technology Press. (In Chinese)

Ferreira SH. 1965. A Bradykinin-potentiating factor (BPF) present in the venom of *Bothrops Jararca*. *British Journal of Pharmacology,* **24**(1): 163-169.

Fischbach MA, Walsh CT. 2009. Antibiotics for emerging pathogens. *Science,* **325**(5944): 1089-1093.

Fischman BJ, Woodard SH, Robinson GE. 2011. Molecular evolutionary analyses of insect societies. *Proceedings of the National Academy of Sciences of the USA*, **108**(Suppl 2): 10847-10854.

Foltynie T, Aviles-Olmos I. 2014. Exenatide as a potential treatment for patients with Parkinson's disease: first steps into the clinic. *Alzheimer's & Dementia,* **10**(Suppl 1): S38-S46.

Fox EGP, Bueno O, Yabuki AT, De Jesus CM, Solis DR, Rossi ML, Nogueira Nde L. 2010. General morphology and ultrastructure of the venom apparatus and convoluted gland of the fire ant, Solenopsis saevis-

sima. *Journal of Insect Science,* **10**: 24.

Frazão B, Vasconcelos V, Antunes A. 2012. Sea anemone (Cnidaria, Anthozoa, Actiniaria) toxins: an overview. *Marine Drugs*, **10**(8): 1812-1851.

Fry BG. 2005. From genome to 'venome': molecular origin and evolution of the snake venom proteome inferred from phylogenetic analysis of toxin sequences and related body proteins. *Genome Research,* **15**(3): 403-420.

Fry BG, Vidal N, Van Der Weerd L, Kochva E, Renjifo C. 2009b. Evolution and diversification of the Toxicofera reptile venom system. *Journal of Proteomics,* **72**(2): 127-136.

Fry BG, Casewell NR, Wüster W, Vidal N, Young B, Jackson TNW. 2012. The structural and functional diversification of the Toxicofera reptile venom system. *Toxicon,* **60**(4): 434-448.

Fry BG, Roelants K, Winter K, Hodgson WC, Griesman L, Kwok HF, Scanlon D, Karas J, Shaw C, Wong L, Norman JA. 2010a. Novel venom proteins produced by differential domain-expression strategies in beaded lizards and gila monsters (genus *Heloderma*). *Molecular Biology and Evolution,* **27**(2): 395-407.

Fry BG, Roelants K, Champagne DE, Scheib H, Tyndall JD, King GF, Nevalainen TJ, Norman JA, Lewis RJ, Norton RS, Renjifo C, De La Vega RC. 2009a. The toxicogenomic multiverse: convergent recruitment of proteins into animal venoms. *Annual Reviews of Genomics and Human Genetics*, **10**: 483-511.

Fry BG, Winter K, Norman JA, Roelants K, Nabuurs RJA, Van Osch MJP, Teeuwisse WM, Van Der Weerd L, McNaughtan JE, Kwok HF, Scheib H, Greisman L, Kochva E, Miller LJ, Gao F, Karas J, Scanlon D, Lin F, Kuruppu S, Shaw C, Wong L, Hodgson WC. 2010b. Functional and structural diversification of the Anguimorpha lizard venom system. *Molecular & Cellular Proteomics,* **9**(11): 2369-2390.

Galinier R, Portela J, Moné Y, Allienne JF, Henri H, Delbecq S, Mitta G, Gourbal B, Duval D. 2013. Biomphalysin, a new β pore-forming toxin involved in *Biomphalaria glabrata* immune defense against *Schistosoma mansoni*. *PLoS Pathogens,* **9**(3): e1003216.

Gao Q, Xiang Y, Zeng L, Ma XT, Lee WH, Zhang Y. 2011. Characterization of the βγ-crystallin domains of βγ-CAT, a non-lens βγ-crystallin and trefoil factor complex, from the skin of the toad *Bombina maxima*. *Biochimie,* **93**(10): 1865-1872.

Göke R, Fehmann HC, Linn T, Schmidt H, Krause M, Eng J, Göke B. 1993. Exendin-4 is a high potency agonist and truncated exendin-(9-39)-amide an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting beta-cells. *Journal of Biological Chemistry,* **268**(26): 19650-19655.

Gouaux E, Mackinnon R. 2005. Principles of selective ion transport in channels and pumps. *Science,* **310**(5753): 1461-1465.

Grant TR, Temple-Smith PD. 1998. Field biology of the platypus (*Ornithorhynchus anatinus*): historical and current perspectives. *Philosophical Transactions the Royal Society Lond B Biological Sciences,* **353**(1372): 1081-1091.

Greene JL, Kohn AJ. 1989. Functional morphology of the *Conus* proboscis (Mollusca: Gastropoda). *Journal of Zoology,* **219**(3): 487-493.

Gurcel L, Abrami L, Girardin S, Tschopp J, Van Der Goot FG. 2006. Caspase-1 activation of lipid metabolic pathways in response to bacterial pore-forming toxins promotes cell survival. *Cell,* **126**(6): 1135-1145.

Gutierrez DA, Rodewald HR. 2013. A sting in the tale of TH2 immunity. *Immunity,* **39**(5): 803-805.

Gutiérrez JM, Burnouf T, Harrison RA, Calvete JJ, Kuch U, Warrell DA, Williams DJ. 2014. A multicomponent strategy to improve the availability of antivenom for treating snakebite envenoming. *Bulletin of the World Health Organization,* **92**(7): 526-532.

Hajime Y, Krishnaraj R. 2013. A role for prohibitin in mast cell activation: location matters. *Science signaling,* **6**(292): pe29.

Hall ZW. 1999. Alpha neurotoxins and their relatives: foes and friends? *Neuron,* **23**(1): 4-5.

Hancock RE, Sahl HG. 2006. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nature Biotechnology,* **24**(12): 1551-1557.

Hanisch FG, Bonar D, Schloerer N, Schroten H. 2014. Human trefoil factor 2 is a lectin that binds α-GlcNAc-capped mucin glycans with antibiotic activity against Helicobacter pylori. *Journal of Biological Chemistry*, **289**(40): 27363-27375.

Hantak MM, Grant T, Reinsch S, McGinnity D, Loring M, Toyooka N, Saporito RA. 2013. Dietary alkaloid sequestration in a poison frog: an experimental test of alkaloid uptake in *Melanophryniscus stelzneri* (Bufonidae). *Journal of Chemical Ecology,* **39**(11-12): 1400-1406.

Harrison RA, Hargreaves A, Wagstaff SC, Faragher B, Lalloo DG. 2009. Snake envenoming: a disease of poverty. *PLoS Neglected Tropical Diseases,* **3**(12): e569.

Hart GW, Copeland RJ. 2010. Glycomics hits the big time. *Cell*, **143**: 672-676.

Harvey AL. 2014. Toxins and drug discovery. *Toxicon,* **92**: 193-200.

Hatakeyama T, Ichise A, Yonekura T, Unno H, Goda S, Nakagawa H. 2014. cDNA cloning and characterization of a rhamnose-binding lectin SUL-I from the toxopneustid sea urchin *Toxopneustes pileolus* venom. *Toxicon,* **94**: 8-15.

Heatwole H, Powell J. 1998. Resistance of eels (*Gymnothorax*) to the venom of sea kraits (*Laticauda colubrina*): a test of coevolution. *Toxicon,* **36**(4): 619-625.

Herzig V, Wood DLA, Newell F, Chaumeil PA, Kaas Q, Binford GJ, Nicholson GM, Gorse D, King GF. 2011. ArachnoServer 2.0, an updated online resource for spider toxin sequences and structures. *Nucleic Acids Research,* **39**(Suppl 1): D653-D657.

Hidalgo P, MacKinnon R. 1995. Revealing the architecture of a K+ channel pore through mutant cycles with a peptide inhibitor. *Science,* **268**(5208): 307-310.

Hines HM, Hunt JH, O'Connor TK, Gillespie JJ, Cameron SA. 2007. Multigene phylogeny reveals eusociality evolved twice in vespid wasps. *Proceedings of the National Academy of Sciences of the USA*, **104**(9): 3295-3299.

Hjelle JT. 1990. Anotomy and morphology. *In*: Polis GA (ed) The biology of scorpions. Stanford: Stanford University Press, 9-63.

Holers VM. 2014. Complement and its receptors: new insights into human disease. *Annual Review of Immunology*, **32**: 433-459.

Huang TF, Liu CZ, Yang SH. 1995. Aggretin, a novel platelet-aggregation inducer from snake (Calloselasma rhodostoma) venom, activates phospholipase C by acting as a glycoprotein Ia/IIa agonist. *Biochemical Journal,* **309**(3): 1021-1027.

Iacovache I, Van Der Goot FG, Pernot L. 2008. Pore formation: an ancient yet complex form of attack. *Biochimica et Biophysica Acta(BBA)-Biomembranes,* **1778**(7-8): 1611-1623.

Iacovache I, Bischofberger M, Van Der Goot FG. 2010. Structure and

assembly of pore-forming proteins. *Current Opinion in Structural Biology,* **20**(2): 241-246.

Incorvaia C, Frati F, Dell'Albani I, Robino A, Cattaneo E, Mauro M, David M, Qualizza R, Pastorello E. 2011. Safety of hymenoptera venom immunotherapy: a systematic review. *Expert Opin Pharmacother,* **12**(16): 2527-2532.

Innan H, Kondrashov F. 2010. The evolution of gene duplications: classifying and distinguishing between models. *Nature Reviews Genetics,* **11**(2): 97-108.

Irwin DM. 2012. Origin and convergent evolution of exendin genes. *General and Comparative Endocrinology,* **175**(1): 27-33.

Isbister GK, Fan HW. 2011. Spider bite. *The Lancet,* **378**(9808): 2039-2047.

Isbister GK, Bawaskar HS. 2014. Scorpion envenomation. *The New England Journal of Medicine,* **371**(5): 457-463.

Iwasaki A, Medzhitov R. 2015. Control of adaptive immunity by the innate immune system. *Nature Immunology*, **16**(4): 343-353.

Jackson K. 2003. The evolution of venom-delivery systems in snakes. *Zoological Journal of the Linnean Society,* **137**(3): 337-354.

Jackson K. 2007. The evolution of venom-conducting fangs: insights from developmental biology. *Toxicon,* **49**(7): 975-981.

Jansa SA, Voss RS. 2011. Adaptive evolution of the venom-targeted vWF protein in opossums that eat pitvipers. *PLoS One,* **6**(6): e20997.

Jiang Y, Li Y, Lee WH, Xu X, Zhang Y, Zhao R, Zhang Y, Wang W**.** 2011. Venoms of two Elapid snakes (*Bungarus multicinctus* and *Naja atra*) and their evolution patterns. *BMC Genomics,* **12**: 1.

Kasturiratne A, Wickremasinghe AR, De Silva N, Gunawardena NK, Pathmeswaran A, Premaratna R, Savioli L, Lalloo DG, De Silva HJ. 2008. The global burden of snakebite: a literature analysis and modelling based on regional estimates of envenoming and deaths. *PLoS Medicine,* **5**(11): e218.

Kayal E, Roure B, Philippe H, Collins AG, Lavrov DV. 2013. Cnidarian phylogenetic relationships as revealed by mitogenomics. *BMC Evolutionary Biology,* **13**(1): 5.

Kheyri H, Cribb BW, Merritt DJ. 2013. Comparing the secretory pathway in honeybee venom and hypopharyngeal glands. *Arthropod Strucuture & Development,* **42**(2): 107-114.

King GF. 2004. The wonderful world of spiders: preface to the special Toxicon issue on spider venoms. *Toxicon,* **43**(5): 471-475.

King GF. 2011. Venoms as a platform for human drugs: translating toxins into therapeutics. *Expert Opinion on Biological Therapy,* **11** : 1469-1484.

King GF, Hardy MC. 2013. Spider-venom peptides: structure, pharmacology, and potential for control of insect pests. *Annual Review Entomology,* **58**: 475-496.

Kingdon J, Agwanda B, Kinnaird M, O'Brien T, Holland C, Gheysens T, Boulet-Audet M, Vollrath F. 2012. A poisonous surprise under the coat of the African crested rat. *Proceedings of the Royal Society B: Biological Sciences,* **279**(1729): 675-680.

Kini RM. 2011. Toxins in thrombosis and haemostasis: potential beyond imagination. *Journal of Thrombosis and Haemostasis,* (Suppl 1): 195-208.

Kita M, Nakamura Y, Okumura Y, Ohdachi SD, Oba Y, Yoshikuni M, Kido H, Uemura D. 2004. Blarina toxin, a mammalian lethal venom from the short-tailed shrew *Blarina brevicauda*: isolation and characterization. *Proceedings of the National Academy of Sciences of the USA,* **101**(20): 7542-7547.

Kjellev S. 2009. The trefoil factor family - small peptides with multiple functionalities. *Cellular and Molecular Life Sciences,* **66**(8): 1350-1369.

Koh CY, Kini RM. 2012. From snake venom toxins to therapeutics-Cardiovascular examples. *Toxicon,* **59**(4): 497-506.

Koludarov I, Jackson TNW, Sunagar K, Nouwens A, Hendrikx I, Fry BG. 2014. Fossilized venom: the unusually conserved venom profiles of *Heloderma* species (beaded lizards and gila monsters). *Toxins (Basel),* **6**(12): 3582-3595.

König E, Bininda-Emonds ORP, Shaw C. 2014. The diversity and evolution of anuran skin peptides. *Peptides,* **63**: 96-117.

Kordiš D, Gubenšek F. 2000. Adaptive evolution of animal toxin multigene families. *Gene,* **261**(1): 43-52.

Koterba AP, Greenberger PA. 2012. Chapter 4: Stinging insect allergy and venom immunotherapy. *Allergy and Asthma Proceedings,* **33**(Suppl 1): S12-S14.

Kularatne SAM, Senanayake N. 2014. Venomous snake bites, scorpions, and spiders. *Handbook of Clinical Neurology,* **120**: 987-1001.

Lai R, Zhao Y, Yang DM, Zha HG, Lee WH, Zhang Y. 2002a. Comparative study of the biological activities of the skin secretions from six common Chinese amphibians. *Zoological Research,* **23**(2): 113-119. (In Chinese)

Lai R, Zheng YT, Shen JH, Liu GJ, Liu H, Lee WH, Tang SZ, Zhang Y. 2002b. Antimicrobial peptides from the skin secretions of Chinese red belly toad *Bombina maxima. Peptides,* **23**(3): 427-435.

Lazzaro BP, Rolff J. 2011. Danger, microbes, and homeostasis. *Science,* **332**(6025): 43-44.

Lebrun EG, Jones NT, Gilbert LE. 2014. Chemical warfare among invaders: a detoxification interaction facilitates an ant invasion. *Science,* **343**(6174): 1014-1017.

Lee CC, Hsieh HJ, Hsieh CH, Hwang DF. 2014. Spine venom of crown-of-thorns starfish (*Acanthaster planci*) induces antiproliferation and apoptosis of human melanoma cells (A375. S2). *Toxicon,* **91**: 126-134.

Lee WH, Du XY, Lu QM, Clemetson KJ, Zhang Y. 2003. Stejnulxin, a novel snake C-type lectin-like protein from Trimeresurus stejnegeri venom is a potent platelet agonist acting specifically via GPVI. *Thrombosis and Haemostasis,* **90**(4): 662-671.

Lee WH, Li Y, Lai R, Li S, Zhang Y, Wang W. 2005. Variety of antimicrobial peptides in the *Bombina maxima* toad and evidence of their rapid diversification. *European Journal of Immunology,* **35**(4): 1220-1229.

Lefebvre O, Chenard MP, Masson R, Linares J, Dierich A, LeMeur M, Wendling C, Tomasetto C, Chambon P, Rio MC. 1996. Gastric mucosa abnormalities and tumorigenesis in mice lacking the pS2 trefoil protein. *Science,* **274**(5285): 259-262.

Leippe M. 2014. Pore-forming toxins from pathogenic amoebae. *Applied Microbiology and Biotechnology,* **98**(10): 4347-4353.

Lemke S, Müller C, Lipke E, Uhl G, Hildebrandt JP. 2013. May salivary gland secretory proteins from hematophagous leeches (*Hirudo verbana*) reach pharmacologically relevant concentrations in the vertebrate host? *PLoS One,* **8**(9): e73809.

Lewis RJ, Dutertre S, Vetter I, Christie MJ. 2012. Conus venom peptide pharmacology. *Pharmacological Reviews,* **64**(2): 259-298.

Li FL. 1999. Supplementary studies on the species of Conidae of the China seas. *Studia Marina Sinica,* **41**: 221-237.

Li JX, Xu XQ, Xu CH, Zhou WP, Zhang KY, Yu HN, Zhang YP, Zheng YT, Rees HH, Lai R, Yang DM, Wu J. 2007. Anti-infection peptidomics of amphibian skin. *Molecular & Cellular Proteomics,* **6**(5): 882-894.

Li M, Fry BG, Kini RM. 2005. Eggs-only diet: its implications for the toxin profile changes and ecology of the Marbled Sea Snake (*Aipysurus eydouxii*). *Journal of Molecular Evolution,* **60**(1): 81-89.

Li RL, Zhang L, Fang Y, Han B, Lu XS, Zhou T, Feng M, Li JK. 2013a. Proteome and phosphoproteome analysis of honeybee (*Apis mellifera*) venom collected from electrical stimulation and manual extraction of the venom gland. *BMC Genomics,* **14**(1): 766.

Li RF, Yu HH, Xue W, Yue Y, Liu S, Xing R, Li PC. 2014a. Jellyfish venomics and venom gland transcriptomics analysis of *Stomolophus meleagris* to reveal the toxins associated with sting. *Journal of Proteomics,* **106**: 17-29.

Li SA, Lee WH, Zhang Y. 2012. Efficacy of OH-CATH30 and its analogs against drug-resistant bacteria *in vitro* and in mouse models. *Antimicrobial Agents and Chemotherapy,* **56**(6): 3309-3317.

Li SA, Xiang Y Lee W, Zhang Y. 2013b. Naturally occurring antimicrobial peptide OH-CATH30 protects against sepsis by regulating host innate immune responses. *Journal of Medicinal Chemistry,* **56**: 9136-9145.

Li SA, Liu J, Xiang Y, Wang YJ, Lee WH, Zhang Y. 2014b. The antimicrobial peptide OH-CATH30 as a potential therapy for antibiotic-resistant *Pseudomonas aeruginosa* keratitis. *Antimicrobial Agents and Chemotherapy,* **58**(6): 3144-3150.

Li TS. 1985. Economic Insect Fauna of China Fasc.30 Hymenoptera Vespoidea. Beijing, China: Science Press. (In Chinese)

Licona-Limón P, Kim LK, Palm NW, Flavell RA. 2013. T_H2, allergy and group 2 innate lymphoid cells. *Nature Immunology,* **14**(6): 536-542.

Ligabue-Braun R, Verli H, Carlini CR. 2012. Venomous mammals: a review. *Toxicon,* **59**(7-8): 680-695.

Liu P, Zhang XL, Song HJ, Pang M. 2013a. Taxonomy key of the venomous jellyfishes in China sea. *Advances in Marine Science,* **31**(2): 290-294.

Liu R, Zhang Z, Liu H, Hou PP, Lang J, Wang S, Yan HL, Li PC, Huang ZG, Wu HB, Rong MQ, Huang J, Wang H, Lv LB, Qiu MF, Ding JP, Lai R. 2013b. Human β-defensin 2 is a novel opener of Ca^{2+}-activated potassium channels and induces vasodilation and hypotension in monkeys. *Hypertension,* **62**(2): 415-425.

Liu RY. 2008a. Checklist of marine biota of China seas. Beijing: Science Press.

Liu RY. 2011. Progress of marine biodiversity studies in China seas. *Biodiversity Science,* **19**(6): 614-626.

Liu SB, He YY, Zhang Y, Lee WH, Qian JQ, Lai R, Jin Y. 2008b. A novel non-lens βγ-crystallin and trefoil factor complex from amphibian skin and its functional implications. *PLoS One,* **3**(3): e1770.

Liu ZC, Zhang R, Zhao F, Chen ZM, Liu HW, Wang YJ, Jiang P, Zhang Y, Wu Y, Ding JP, Lee WH, Zhang Y. 2012. Venomic and transcriptomic analysis of centipede *Scolopendra subspinipes dehaani. Journal of Proteome Research,* **11**(12): 6197-6212.

Lopes-Ferreira M, Grund LZ, Lima C. 2014. *Thalassophryne nattereri* fish venom: from the envenoming to the understanding of the immune system. *Journal of Venomous Animals and Toxins Including Tropical Diseases,* **20**(1): 35.

Lu AP, Yang LJ, Xu SQ, Wang CG. 2014. Various conotoxin diversifications revealed by a venomic study of *Conus flavidus*. *Molecular & Cellular Proteomics,* **13**(1): 105-118.

Lu QM, Lai R, Zhang Y. 2010. Animal toxins and human disease: from single component to venomics, from biochemical characterization to disease mechanisms, from crude venom utilization to rational drug design. *Zoological Research,* **31**(1): 2-16. (In Chinese)

Lynch JJ Jr, Cook JJ, Sitko GR, Holahan MA, Ramjit DR, Mellott MJ, Stranieri MT, Stabilito II, Zhang G, Lynch RJ. 1995. Nonpeptide glycoprotein IIb/IIIa inhibitors.5.Antithrombotic effects of MK-0383. *Journal of Pharmacology and Experimental Therapeutics,* **272**(1): 20-32.

Ma DY, Wang YP, Yang HL, Wu J, An S, Gao L, Xu XQ, Lai R. 2009. Antithrombosis repertoire of blood-feeding horsefly salivary glands. *Molecular & Cellular Proteomics,* **8**(9): 2071-2079.

Ma HQ. 2007. Taxonomic Study of Lithobiomorpha from China (Myriapoda: Chilopoda). Ph. D. thesis, Hebei University, Baoding. (In Chinese)

Machkour-M'Rabet S, Hénaut Y, Winterton P, Rojo R. 2011. A case of zootherapy with the tarantula *Brachypelma vagans* Ausserer, 1875 in traditional medicine of the Chol Mayan ethnic group in Mexico. *Journal of Ethnobiology and Ethnomedicine,* **7**(1): 12.

Macht DI. 1936. Experimental and clinical study of cobra venom as an analgesic. *Proceedings of the National Academy of Sciences of the USA,* **22**(1): 61-71.

MacKinnon R. 1991. Determination of the subunit stoichiometry of a voltage-activated potassium channel. *Nature,* **350**(6315): 232-235.

MacKinnon R. 2003. Potassium channels. *FEBS Letters,* **555**(1): 62-65.

Maduwage K, Isbister GK. 2014. Current treatment for venom-induced consumption coagulopathy resulting from snakebite. *PLoS Neglected Tropical Diseases,* **8**(10): e3220.

Magalhães GS, Lopes-Ferreira M, Junqueira-De-Azevedo ILM, Spencer PJ, Araújo MS, Portaro FCV, Ma L, Valente RH, Juliano L, Fox JW, Ho PL, Moura-Da-Silva AM. 2005. Natterins, a new class of proteins with kininogenase activity characterized from *Thalassophryne nattereri* fish venom. *Biochimie,* **87**(8): 687-699.

Maraganore JM, Bourdon P, Jablonski J, Ramachandran KL, Fenton JW. 1990. Design and characterization of hirulogs: a novel class of bivalent peptide inhibitors of thrombin. *Biochemistry,* **29**(30): 7095- 7101.

Marichal T, Starkl P, Reber LL, Kalesnikoff J, Oettgen HC, Tsai M, Metz M, Galli SJ. 2013. A beneficial role for immunoglobulin E in host defense against honeybee venom. *Immunity,* **39**(5): 963-975.

Mariottini GL, Pane L. 2013. Cytotoxic and cytolytic cnidarian venoms. A review on health implications and possible therapeutic applications. *Toxins,* **6**(1): 108-151.

Mashimo H, Wu DC, Podolsky DK, Fishman MC. 1996. Impaired defense of intestinal mucosa in mice lacking intestinal trefoil factor. *Science,* **274**(5285): 262-265.

McCleary RJR, Kini RM. 2013. Non-enzymatic proteins from snake venoms: a gold mine of pharmacological tools and drug leads. *Toxicon,* **62**: 56-74.

Mebs D. 2002. Venomous and Poisonous Animals: A Handbook for Biologists, Toxicologists and Toxinologists, Physicians and Pharmacists. Boca Raton: CRC Press.

Medzhitov R. 2010a. Inflammation 2010: new adventures of an old flame. *Cell,* **140**(6): 771-776.

Medzhitov R. 2010b. Innate immunity: *quo vadis*? *Nature Immunology,* **11**(7): 551-553.

Medzhitov R, Schneider DS, Soares MP. 2012. Disease tolerance as a defense strategy. *Science,* **335**(6071): 936-941.

Mégarbane B, Abroug F, Soulaymani R. 2014. Scorpion envenomation. *The New England Journal of Medicine,* **371**(16): 1557-1560.

Meng ZQ, Yang PY, Shen YH, Bei WY, Zhang Y, Ge YQ, Newman RA,

Cohen L, Liu LM, Thornton B, Chang DZ, Liao ZX, Kurzrock R. 2009. Pilot study of Huachansu in patients with hepatocellular carcinoma, nonsmall-cell lung cancer, or pancreatic cancer. *Cancer,* **115**(22): 5309-5318.

Michalsen A, Lüdtke R, Cesur Ö, Afra D, Musial F, Baecker M, Fink M, Dobos GJ. 2008. Effectiveness of leech therapy in women with symptomatic arthrosis of the first carpometacarpal joint: a randomized controlled trial. *Pain,* **137**(2): 452-459.

Min GS, Sarkar IN, Siddall ME. 2010. Salivary transcriptome of the North American medicinal leech, *Macrobdella decora*. *Journal of Parasitology,* **96**(6): 1211-1221.

Mingomataj EÇ, Bakiri AH, Ibranji A, Sturm GJ. 2014. Unusual reactions to hymenoptera stings: what should we keep in mind? *Clinical Reviews in Allergy & Immunology,* **47**(1): 91-99.

Minn AJ, Vélez P, Schendel SL, Liang H, Muchmore SW, Fesik SW, Fill M, Thompson CB. 1997. Bcl-x(L) forms an ion channel in synthetic lipid membranes. *Nature,* **385**(6614): 353-357.

Miwa JM, Ibañez-Tallon I, Crabtree GW, Sánchez R, Sali A, Role LW, Heintz N. 1999. Lynx1, an endogenous toxin-like modulator of nicotinic acetylcholine receptors in the mammalian CNS. *Neuron,* **23**(1): 105-114.

Moran Y, Fredman D, Szczesny P, Grynberg M, Technau U. 2012. Recurrent horizontal transfer of bacterial toxin genes to eukaryotes. *Molecular Biology and Evolution,* **29**(9): 2223-2230.

Morgenstern D, King GF. 2013. The venom optimization hypothesis revisited. *Toxicon,* **63**: 120-128.

Morishita H, Miwa JM, Heintz N, Hensch TK. 2010. Lynx1, a cholinergic brake, limits plasticity in adult visual cortex. *Science,* **330**(6008): 1238-1240.

Moriwaki Y, Yoshikawa K, Fukuda H, Fujii YX, Misawa H, Kawashima K. 2007. Immune system expression of SLURP-1 and SLURP-2, two endogenous nicotinic acetylcholine receptor ligands. *Life Sciences,* **80**(24-25): 2365-2368.

Muchmore SW, Sattler M, Liang H, Meadows RP, Harlan JE, Yoon HS, Nettesheim D, Chang BS, Thompson CB, Wong SL, Ng SL, Fesik SW. 1996. X-ray and NMR structure of human Bcl-xL, an inhibitor of programmed cell death. *Nature,* **381**(6580): 335-341.

National Pharmacopoeia Committee, 2010. Chinese Pharmacopoeia, Chemical Industry Press, Beijing,

Navdaev A, Clemetson JM, Polgár J, Kehrel BE, Glauner M, Magnenat E, Wells TNC, Clemetson KJ. 2001. Aggretin, a heterodimeric C-type lectin from *Calloselasma rhodostoma* (malayan pit viper), stimulates platelets by binding to $\alpha_2\beta_1$ integrin and glycoprotein Ib, activating Syk and phospholipase Cγ2, but does not involve the glycoprotein VI/Fc receptor γ chain collagen receptor. *The Journal of Biological Chemistry,* **276**(24): 20882-20889.

Nei M. 1969. Gene duplication and nucleotide substitution in evolution. *Nature,* **221**(5175): 40-42.

Nei M, Gu X, Sitnikova T. 1997. Evolution by the birth-and-death process in multigene families of the vertebrate immune system. *Proceedings of the National Academy of Sciences of the USA,* **94**(15): 7799-7806.

Nekaris KAI, Moore RS, Rode EJ, Fry BG. 2013. Mad, bad and dangerous to know: the biochemistry, ecology and evolution of slow loris venom. *Journal of Venomous Animals and Toxins Including Tropical Diseases,* **19**(1): 21.

Nelsen DR, Nisani Z, Cooper AM, Fox GA, Gren ECK, Corbit AG, Hayes WK. 2014. Poisons, toxungens, and venoms: redefining and classifying toxic biological secretions and the organisms that employ them. *Biological Reviews,* **89**(2): 450-465.

Nolan C, Hall LS, Barlow GH. 1976. Ancrod, the coagulating enzyme from Malayan pit viper (*Agkistrodon rhodostoma*) venom. *Methods in Enzymology,* **45**: 205-213.

Nonaka M. 2014. Evolution of the complement system. *Subcellular Biochemistry*, **80**: 31-43.

Nouri M, Karimi-Yarandi K, Etezadi F, Amirjamshidi A. 2012. Leech therapy for pain relief: Rational behind a notion. *Surgical Neurology International*, **3**: 159.

Oi DH, Pereira RM. 1993. Ant behavior and microbial pathogens (Hymenoptera, Formicidae). *The Florida Entomologist,* **76**(1): 63-74.

Okin D, Medzhitov R. 2012. Evolution of inflammatory diseases. *Current Biology,* **22**(17): R733-R740.

Olivera BM, Gray WR, Zeikus R, McIntosh JM, Varga J, Rivier J, De Santos V, Cruz LJ. 1985. Peptide neurotoxins from fish-hunting cone snails. *Science,* **230**(4732): 1338-1343.

Olivera BM, Cruz LJ, De Santos V, LeCheminant GW, Griffin D, Zeikus R, McIntosh JM, Galyean R, Varga J, Gray WR. 1987. Neuronal calcium channel antagonists. Discrimination between calcium channel subtypes using omega-conotoxin from Conus magus venom. *Biochemistry,* **26**(8): 2086-2090.

Olivera BM, Rivier J, Clark C, Ramilo CA, Corpuz GP, Abogadie FC, Mena EE, Woodward SR, Hillyard DR, Cruz LJ. 1990. Diversity of Conus neuropeptides. *Science,* **249**(4966): 257-263.

Ondetti MA, Williams NJ, Sabo EF, Pluscec J, Weaver ER, Kocy O. 1971. Angiotensin-converting enzyme inhibitors from the venom of Bothrops jararaca. Isolation, elucidation of structure, and synthesis. *Biochemistry,* **10**(22): 4033-4039.

Opper B, Bognár A, Heidt D, Németh P, Engelmann P. 2013. Revising lysenin expression of earthworm coelomocytes. *Developmental & Comparative Immunology,* **39**(3): 214-218.

Ortiz E, Gurrola GB, Schwartz EF, Possani LD. 2015. Scorpion venom components as potential candidates for drug development. *Toxicon,* **93**: 125-135.

Özbek S, Balasubramanian PG, Holstein TW. 2009. Cnidocyst structure and the biomechanics of discharge. *Toxicon,* **54**(8): 1038-1045.

Palm NW, Rosenstein RK, Medzhitov R. 2012. Allergic host defences. *Nature,* **484**(7395): 465-472.

Palm NW, Rosenstein RK, Yu S, Schenten DD, Florsheim E, Medzhitov R. 2013. Bee venom phospholipase A2 induces a primary type 2 response that is dependent on the receptor ST2 and confers protective immunity. *Immunity,* **39**(5): 976-985.

Park D, Jung JW, Choi BS, Jayakodi M, Lee J, Lim J, Yu Y, Choi YS, Lee ML, Park Y, Choi IY, Yang TJ, Edwards OR, Nah G, Kwon HW. 2015. Uncovering the novel characteristics of Asian honey bee, *Apis cerana*, by whole genome sequencing. *BMC Genomics,* **16**(1): 1.

Parry MAA, Jacob U, Huber R, Wisner A, Bon C, Bode W. 1998. The crystal structure of the novel snake venom plasminogen activator TSV-PA: a prototype structure for snake venom serine proteinases. *Structure,* **6**(9): 1195-206.

Pazgier M, Hoover DM, Yang D, Lu W, Lubkowski J. 2006. Human β-

defensins. *Cellular and Molecular Life Sciences,* **63**(11): 1294-1313.

Pei ZN. 1998. Fauna Sinica, Coelenterata, Actiniaria, Ceriantharia, Zoanthidae. Beijing, China: Science Press.

Peigneur S, Orts DJB, Prieto Da Silva AR, Oguiura N, Boni-Mitake M, De Oliveira EB, Zaharenko AJ, De Freitas JC, Tytgat J. 2012. Crotamine pharmacology revisited: novel insights based on the inhibition of K_V channels. *Molecular Pharmacology,* **82**(1): 90-96.

Petersen TE, Roberts HR, Sotrup-Jensen L, Magnusson S. 1976. Primary structure of hirudin, a thrombin-specific inhibitor. *In*: Peeters H. Protides of the Biologic Fluids. Oxford: Pergamon Press, 145-149.

Petrocelli I, Turillazzi S, Delfino G. 2014. The venom apparatus in stenogastrine wasps: subcellular features of the convoluted gland. *Arthropod Structure Development,* **43**(5): 457-468.

Polgár J, Clemetson JM, Kehrel BE, Wiedemann M, Magnenat EM, Wells TNC, Clemetson KJ. 1997. Platelet activation and signal transduction by convulxin, a C-type lectin from Crotalus durissus terrificus (tropical rattlesnake) venom via the p62/GPVI collagen receptor. *The Journal of Biological Chemistry,* **272**(21): 13576-13583.

Polis GA. 1990. The Biology of Scorpions. California, Palo Alto: Stanford University Press.

Pope JE, Deer TR. 2013. Ziconotide: a clinical update and pharmacologic review. *Expert Opinion Pharmacotherapy,* **14**(7): 957-966.

Prado-Franceschi J, Vital-Brazil O. 1981. Convulxin, a new toxin from the venom of the South American rattlesnake *Crotalus durissus terrificus*. *Toxicon,* **19**(6): 875-887.

Puillandre N, Bouchet P, Duda TF Jr, Kauferstein S, Kohn AJ, Olivera BM, Watkins M, Meyer C. 2014. Molecular phylogeny and evolution of the cone snails (Gastropoda, Conoidea). *Molecular Phylogenetics and Evolution,* **78**: 290-303.

Putnam NH, Srivastava M, Hellsten U, Dirks B, Chapman J, Salamov A, Terry A, Shapiro H, Lindquist E, Kapitonov VV, Jurka J, Genikhovich G, Grigoriev IN, Lucas SM, Steele RE, Finnerty JR, Technau U, Martindale MQ, Rokhsar DS. 2007. Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. *Science,* **317**(5834): 86-94.

Qian JQ, Liu SB, He YY, Lee WH, Zhang Y. 2008a. Acute toxicity of βγ-CAT, a naturally existing non-lens βγ-crystallin and trefoil factor complex from frog *Bombina maxima* skin secretions. *Toxicon,* **52**(1): 22-31.

Qian JQ, Liu SB, He YY, Lee WH, Zhang Y. 2008b. βγ-CAT, a non-lens βγ-crystallin and trefoil factor complex from amphibian skin secretions, caused endothelium-dependent myocardial depression in isolated rabbit hearts. *Toxicon,* **52**(2): 285-292.

Rachamim T, Sher D. 2012. What *Hydra* can teach us about chemical ecology -how a simple, soft organism survives in a hostile aqueous environment. *The International Journal of Developmental Biology,* **56**(6-8): 605-611.

Ranawaka UK, Lalloo DG, De Silva HJ. 2013. Neurotoxicity in snakebite-the limits of our knowledge. *PLoS Neglected Tropical Diseases,* **7**(10): e2302.

Randow F, MacMicking JD, James LC. 2013. Cellular self-defense: how cell-autonomous immunity protects against pathogens. *Science,* **340**(6133): 701-706.

Reid PF. 2007. Alpha-cobratoxin as a possible therapy for multiple sclerosis: a review of the literature leading to its development for this application. *Critical Reviews in Immunology,* **27**(4): 291-302.

Reid PF. 2011. Cobra venom: A review of the old alternative to opiate analgesics. *Alternative Therapies in Health and Medicine,* **17**(1): 58-71.

Richards DP, Barlow A, Wüster W. 2012. Venom lethality and diet: differential responses of natural prey and model organisms to the venom of the saw-scaled vipers (*Echis*). *Toxicon,* **59**(1): 110-116.

Rocha E Silva M, Beraldo WT, Rosenfeld G. 1949. Bradykinin, a hypotensive and smooth muscle stimulating factor released from plasma globulin by snake venoms and by trypsin. *The American Journal of Physiology,* **156**(2): 261-273.

Rodríguez AA, Cassoli JS, Sa F, Dong ZQ, De Freitas JC, Pimenta AMC, De Lima ME, Konno K, Lee SMY, Garateix A, Zaharenko AJ. 2012. Peptide fingerprinting of the neurotoxic fractions isolated from the secretions of sea anemones *Stichodactyla helianthus* and *Bunodosoma granulifera*. New members of the APETx-like family identified by a 454 pyrosequencing approach. *Peptides,* **34**(1): 26-38.

Rodríguez E, Barbeitos MS, Brugler MR, Crowley LM, Grajales A, Gusmão L, Häussermann V, Reft A, Daly M. 2014. Hidden among sea anemones: the first comprehensive phylogenetic reconstruction of the order Actiniaria (Cnidaria, Anthozoa, Hexacorallia) reveals a novel group of hexacorals. *PLoS One,* **9**(5): e96998.

Roelants K, Fry BG, Ye L, Stijlemans B, Brys L, Kok P, Clynen E, Schoofs L, Cornelis P, Bossuyt F. 2013. Origin and functional diversification of an amphibian defense peptide arsenal. *PLoS Genetics,* **9**(8): e1003662.

Rosado CJ, Kondos S, Bull TE, Kuiper MJ, Law RH, Buckle AM, Voskoboinik I, Bird PI, Trapani JA, Whisstock JC, Dunstone MA. 2008. The MACPF/CDC family of pore-forming toxins. *Cell Microbiology*, **10**(9): 1765-1774.

Saez NJ, Senff S, Jensen JE, Yan Er S, Herzig V, Rash LD, King GF. 2010. Spider-venom peptides as therapeutics. *Toxins,* **2**(12): 2851-2871.

Salisbury SM, Martin GG, Kier WM, Schulz JR. 2010. Venom kinematics during prey capture in Conus: the biomechanics of a rapid injection system. *J Experimental Biology*. 213(5):673-682.

Sanggaard KW, Dyrlund TF, Thomsen LR, Nielsen TA, Brøndum L, Wang T, Thøgersen IB, Enghild JJ. 2015. Characterization of the gila monster (*Heloderma suspectum suspectum*) venom proteome. *Jopurnal of Proteomics,* 117: 1-11.

Sansonetti PJ. 2014. To be or not to be a pathogen: that is the mucosally relevant question. *Mucosal Immunology,* **4**: 8-14.

Sato H, Tsuruta Y, Yamamoto Y, Asato Y, Taira K, Hagiwara K, Kayo S, Iwanaga S, Uezato H. 2008. Case of skin injuries due to stings by crown-of-thorns starfish (*Acanthaster planci*). *The Journal of Dermatology,* **35**(3): 162-167.

Saudek V, Atkinson RA, Pelton JT. 1991. Three-dimensional structure of echistatin, the smallest active RGD protein. *Biochemistry,* **30**(30): 7369-7372.

Scarborough RM, Rose JW, Hsu MA, Phillips DR, Fried VA, Campbell AM, Nannizzi L, Charo IF. 1991. Barbourin. A GPIIb-IIIa-specific integrin antagonist from the venom of Sistrurus m. barbouri. *Journal of Biological Chemistry,* **266**(15): 9359-9362.

Scarborough RM, Naughton MA, Teng W, Rose JW, Phillips DR, Nannizzi L, Arfsten A, Campbell AM, Charo IF. 1993. Design of potent and specific integrin antagonists. Peptide antagonists with high specificity for glycoprotein IIb-IIIa. *Journal of Biological Chemistry*, **268**(2): 1066-1073.

Scarborough RM. 1999. Development of eptifibatide. *American Heart*

Journal, **138**(6): 1093-1104.

Schendel SL, Xie ZH, Montal MO, Matsuyama S, Montal M, Reed JC. 1997. Channel formation by antiapoptotic protein Bcl-2. *Proceedings of the National Academy of Sciences of the USA,* **94**(10): 5113-5118.

Schroeder CI, Rash LD, Vila-Farrés X, Rosengren KJ, Mobli M, King GF, Alewood PF, Craik DJ, Durek T. 2014. Chemical synthesis, 3D structure, and ASIC binding site of the toxin mambalgin-2. *Angewandte Chemie International Edition,* **53**(4): 1017-1020.

Sciani JM, Antoniazzi MM, Neves Ada C, Pimenta DC. 2013. Cathepsin B/X is secreted by *Echinometra lucunter* sea urchin spines, a structure rich in granular cells and toxins. *Journal of Venomous Animals and Toxins Including Tropical Diseases,* **19**(1): 33.

Serrano SMT. 2013. The long road of research on snake venom serine proteinases. *Toxicon,* **62**: 19-26.

Shin Y, Morita T. 1998. Rhodocytin, a functional novel platelet agonist belonging to the heterodimeric C-type lectin family, induces platelet aggregation independently of glycoprotein Ib. *Biochemical and Biophysical Research Communications,* **245**(3): 741-745.

Simakov O, Marletaz F, Cho SJ, Edsinger-Gonzales E, Havlak P, Hellsten U, Kuo DH, Larsson T, Lv J, Arendt D, Savage R, Osoegawa K, de Jong P, Grimwood J, Chapman JA, Shapiro H, Aerts A, Otillar RP, Terry AY, Boore JL, Grigoriev IV, Lindberg DR, Seaver EC, Weisblat DA, Putnam NH, Rokhsar DS. 2013. Insights into bilaterian evolution from three spiralian genomes. *Nature,* **493**(7433): 526-531.

Skolnik AB, Ewald MB. 2013. Pediatric scorpion envenomation in the United States: morbidity, mortality, and therapeutic innovations. *Pediatric Emergency Care,* **29**(1): 98-103.

Smith WL, Wheeler WC. 2006. Venom evolution widespread in fishes: a phylogenetic road map for the bioprospecting of piscine venoms. *Journal of Heredity,* **97**(3): 206-217.

Song DX, Zhu MS, Chen J. 1999. The spiders of China. Shijiazhuang: Hebei Science and Technology Publishing House.

Song ZS. 2004. Taxonomic Study on Chinese Centipedes of the Order *Scolopendromorpha* (Myriapoda: Chilopoda). Master thesis, Hebei University, Baoding China. In Chinese.

Sookrung N, Wong-din-Dam S, Tungtrongchitr A, Reamtong O, Indrawattana N, Sakolvaree Y, Visitsunthorn N, Manuyakorn W, Chaicumpa W. 2014. Proteome and allergenome of Asian wasp, *Vespa affinis*, venom and IgE reactivity of the venom components. *Journal of Proteome Research,* **13**(3): 1336-1344.

Štibrániová I, Lahová M, Bartíková P. 2013. Immunomodulators in tick saliva and their benefits. *Acta Virologica,* **57**(2): 200-216.

Stocker K, Barlow GH. 1976. The coagulant enzyme from Bothrops atrox venom (batroxobin). *Methods Enzymology,* **45**: 214-223.

Strowig T, Henao-Mejia J, Elinav E, Flavell R. 2012. Inflammasomes in health and disease. *Nature,* **481**(7381): 278-286.

Sunagar K, Undheim EAB, Chan AHC, Koludarov I, Muñoz-Gómez SA, Antunes A, Fry BG. 2013. Evolution stings: the origin and diversification of scorpion toxin peptide scaffolds. *Toxins,* **5**(12): 2456-2487.

Suzuki-Inoue K, Fuller GLJ, García Á, Eble JA, Pöhlmann S, Inoue O, Gartner TK, Hughan SC, Pearce AC, Laing GD, Theakston RDG, Schweighoffer E, Zitzmann N, Morita T, Tybulewicz VLJ, Ozaki Y, Watson SP. 2006. A novel Syk-dependent mechanism of platelet activation by the C-type lectin receptor CLEC-2. *Blood,* **107**(2): 542-549.

Suzuki-Inoue K, Inoue O, Ozaki Y. 2011. Novel platelet activation receptor CLEC-2: from discovery to prospects. *Journal of Thrombosis Haemostasis,* **9**(Suppl 1): 44-55.

Szczesny P, Iacovache I, Muszewska A, Ginalski K, Van Der Goot FG, Grynberg M. 2011. Extending the aerolysin family: from bacteria to vertebrates. *PLoS One,* **6**(6): e20349.

Tamura S, Yamakawa M, Shiomi K. 2011. Purification, characterization and cDNA cloning of two natterin-like toxins from the skin secretion of oriental catfish *Plotosus lineatus*. *Toxicon,* **58**(5): 430-438.

Thuaud F, Ribeiro N, Nebigil CG, Désaubry L. 2013. Prohibitin ligands in cell death and survival: mode of action and therapeutic potential. *Chemistry & Biology,* **20**(3): 316-331.

Tirosh Y, Ofer D, Eliyahu T, Linial M. 2013. Short toxin-like proteins attack the defense line of innate immunity. *Toxins,* **5**(7): 1314-1331.

Torres AFC, Huang C, Chong CM, Leung SW, Prieto-Da-Silva ÁRB, Havt A, Quinet YP, Martins AMC, Lee SMY, Rádis-Baptista G. 2014. Transcriptome analysis in venom gland of the predatory giant ant Dinoponera quadriceps: insights into the polypeptide toxin arsenal of hymenopterans. *PLoS One,* **9**(1): e87556.

Touchard A, Labrière N, Roux O, Petitclerc F, Orivel J, Escoubas P, Koh JMS, Nicholson GM, Dejean A. 2014. Venom toxicity and composition in three *Pseudomyrmex* ant species having different nesting modes. *Toxicon,* **88**: 67-76.

Tsetlin VI. 2014. Three-finger snake neurotoxins and Ly6 proteins targeting nicotinic acetylcholine receptors: pharmacological tools and endogenous modulators. *Trends in Pharmacological Sciences,* **36**(2): 109-123.

Undheim EAB, King GF. 2011. On the venom system of centipedes (Chilopoda), a neglected group of venomous animals. *Toxicon,* **57**(4): 512-524.

Van Der Goot GF. 2014. Introduction: brief historical overview. *Subcellular Biochemistry,* **80**: 3-6.

Van Vaerenbergh M, Debyser G, Devreese B, De Graaf DC. 2014. Exploring the hidden honeybee (*Apis mellifera*) venom proteome by integrating a combinatorial peptide ligand library approach with FTMS. *Journal of Proteomics,* **99**: 169-178.

Van Valen L. 1974. Two modes of evolution. *Nature*, **252**: 298-300.

Vargaftig BB, Joseph D, Wal F, Marlas G, Chignard M, Chevance LG. 1983. Convulxin-induced activation of intact and of thrombin-degranulated rabbit platelets: specific crossed desensitisation with collagen. *European Journal of Pharmacology,* **92**(1-2): 57-68.

Varki A. 2006. Nothing in glycobiology makes sense, except in the light of evolution. *Cell*, **126**: 841-845.

Varki A. 2007. Glycan-based interactions involving vertebrate sialic-acid-recognizing proteins. *Nature*, **446**: 1023-1029.

Venkatachalam K, Montell C. 2007. TRP channels. *Annual Review of Biochemistry,* **76**(1): 387-417.

Veraldi S, Çuka E, Gaiani F. 2014. Scolopendra bites: a report of two cases and review of the literature. *International Journal of Dermatology,* **53**(7): 869-872.

von Reumont BM, Blanke A, Richter S, Alvarez F, Bleidorn C, Jenner RA. 2014a. The first venomous crustacean revealed by transcriptomics and functional morphology: remipede venom glands express a unique toxin cocktail dominated by enzymes and a neurotoxin. *Molecular Biology and Evolution,* **31**(1): 48-58.

von Reumont BM, Campbell LI, Richter S, Hering L, Sykes D, Hetmank J,

Jenner RA, Bleidorn C. 2014b. A Polychaete's powerful punch: venom gland transcriptomics of *Glycera* reveals a complex cocktail of toxin homologs. *Genome Biology and Evolution,* **6**(9): 2406-2423.

von Reumont BM, Campbell LI, Jenner RA. 2014c. *Quo vadis* venomics? A roadmap to neglected venomous invertebrates. *Toxins,* **6**(12): 3488-3551.

Vonk FJ, Casewell NR, Henkel CV, Heimberg AM, Jansen HJ, McCleary RJR, Kerkkamp HME, Vos RA, Guerreiro I, Calvete JJ, Wüster W, Woods AE, Logan JM, Harrison RA, Castoe TA, Jason De Koning AP, Pollock DD, Yandell M, Calderon D, Renjifo C, CurrierRB, Salgado D, Pla D, SanzL, HyderAS, RibeiroJMC, Arntzen JW, Van Den Thillart GEEJM, Boetzer M, Pirovano W, Dirks RP, Spaink HP, Duboule D, McGlinn E, Manjunatha Kini R, Richardson MK. 2013. The king cobra genome reveals dynamic gene evolution and adaptation in the snake venom system. *Proceedings of the National Academy of Sciences of the USA,* **110**(51): 20651-20656.

Vonk FJ, Admiraal JF, Jackson K, Reshef R, De Bakker MAG, Vanderschoot K, Van Den Berge I, Van Atten M, Burgerhout E, Beck A, Mirtschin PJ, Kochva E, Witte F, Fry BG, Woods AE, Richardson MK. 2008. Evolutionary origin and development of snake fangs. *Nature,* **454**(7204): 630-633.

Voyles J, Young S, Berger L, Campbell C, Voyles WF, Dinudom A, Cook D, Webb R, Alford RA, Skerratt LF, Speare R. 2009. Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. *Science,* **326**(5952): 582-585.

Wang YJ, Guo XL, Li SA, Zhao YQ, Liu ZC, Lee WH, Xiang Y, Zhang Y. 2014a. Prohibitin is involved in the activated internalization and degradation of protease-activated receptor 1. *Biochimica et Biophysica Acta(BBA)-Molecular Cell Research,* **1834**(7): 1393-1401.

Wang ZJ, Sun LQ, Heinbockel T. 2014b. Resibufogenin and cinobufagin activate central neurons through an ouabain-like action. *PLoS One,* **9**(11): e113272.

Wang ZM. 1994. Economic Insect Fauna of China Fasc.45 Deptera Tabanidae. Beijing: Science Press. (In Chinese)

Warrell DA. 2010. Snake bite. *The Lancet,* **375**(9708): 77-88.

Watson SP, Herbert JMJ, Pollitt AY. 2010. GPVI and CLEC-2 in hemostasis and vascular integrity. *Journal of Thrombosis and Haemostasis,* **8**(7): 1456-1467.

White J. 2005. Snake venoms and coagulopathy. *Toxicon,* **45**(8): 951-967.

Whittington CM, Papenfuss AT, Bansal P, Torres AM, Wong ESW, Deakin JE, Graves T, Alsop A, Schatzkamer K, Kremitzki C, Ponting CP, Temple-Smith P, Warren WC, Kuchel PW, Belov K. 2008. Defensins and the convergent evolution of platypus and reptile venom genes. *Genome Research,* **18**(6): 986-994.

Wickenden A, Priest B, Erdemli G. 2012. Ion channel drug discovery: challenges and future directions. *Future Medicinal Chemistry,* **4**(5): 661-679.

Wigger E, Kuhn-Nentwig L, Nentwig W. 2002. The venom optimisation hypothesis: a spider injects large venom quantities only into difficult prey types. *Toxicon,* **40**(6): 749-752.

Wikel S. 2013. Ticks and tick-borne pathogens at the cutaneous interface: host defenses, tick countermeasures, and a suitable environment for pathogen establishment. *Frontiers in Microbiology,* **4**: 337.

Williams RB. 1991. Acrorhagi, catch tentacles and sweeper tentacles: a synopsis of 'aggression' of actiniarian and scleractinian Cnidaria. *Hydrobiologia,* **216-217**(1): 539-545.

Wong ESW, Morgenstern D, Mofiz E, Gombert S, Morris KM, Temple-Smith P, Renfree MB, Whittington CM, King GF, Warren WC, Papenfuss AT, Belov K. 2012. Proteomics and deep sequencing comparison of seasonally active venom glands in the platypus reveals novel venom peptides and distinct expression profiles. *Molecular & Cellular Proteomics*, **11**(11): 1354-1364.

Wootten D, Christopoulos A, Sexton PM. 2013. Emerging paradigms in GPCR allostery: implications for drug discovery. *Nature Reviews Drug Discovery,* **12**(8): 630-644.

Wright JJ. 2009. Diversity, phylogenetic distribution, and origins of venomous catfishes. *BMC Evolutionary Biology,* **9(1)**: 282.

Wu YR. 2000. Fauna Sinica Insecta. Vol 20. Hymenoptera. Milittidae, Apidae. Beijing, China: Science Press. (In Chinese)

Xiang Y, Yan C, Guo XL, Zou KF, Li SA, Gao Q, Wang X, Zhao F, Liu J, Lee WH, Zhang Y. 2014. Host-derived pore-forming toxin-like protein and trefoil factor complex protects the host against microbial infection. *Proceedings of the National Academy of Sciences of the USA,* **111**(18): 6702-6707.

Xie F, Lau MWN, Stuart SN, Chanson JS, Cox NA, Fischman DL. 2007. Conservation needs of amphibians in China: a review. *Science in China Series C: Life Sciences,* **50**(2): 265-276.

Xu XQ, Yang HL, Ma DY, Wu J, Wang YP, Song YZ, Wang X, Lu Y, Yang JX, Lai R. 2008. Toward an understanding of the molecular mechanism for successful blood feeding by coupling proteomics analysis with pharmacological testing of horsefly salivary glands. *Molecular & Cellular Proteomics,* **7**(3): 582-590.

Xu XB, Duan ZG, Di ZY, He YW, Li JL, Li ZJ, Xie CL, Zeng XZ, Cao ZJ, Wu YL, Liang SP, Li WX. 2014. Proteomic analysis of the venom from the scorpion *Mesobuthus martensii*. *Journal of Proteomics,* **106**: 162-180.

Xu XQ, Lai R. 2015. The chemistry and biological activities of peptides from amphibian skin secretions. *Chemical Reviews,* **115**(4): 1760-1846.

Yang SL, Liu ZH, Xiao Y, Li Y, Rong MQ, Liang SP, Zhang ZY, Yu HN, King GF, Lai R. 2012a. Chemical punch packed in venoms makes centipedes excellent predators. *Molecular & Cellular Proteomics,* **11**(9): 640-650.

Yang SL, Xiao Y, Kang D, Liu J, Li Y, Undheim EAB, Klint JK, Rong MQ, Lai R, King GF. 2013. Discovery of a selective $Na_V1.7$ inhibitor from centipede venom with analgesic efficacy exceeding morphine in rodent pain models. *Proceedings of the National Academy of Sciences of the USA,* **110**(43): 17534-17539.

Yang T. 1996. Fauna Sinica, Annelida, Hirudinea. Beijing: Science Press.

Yang WS, Feng J, Xiang F, Xie ZL, Zhang GY, Sabatier JM, Cao ZJ, Li WX, Chen ZY, Wu YL. 2014. Endogenous animal toxin-like human β-defensin 2 inhibits own K^+ channels through interaction with channel extracellular pore region. *Cellular and Molecular Life Sciences,* **72**(4): 845-853.

Yang XJ, Chen Z, Liu JZ. 2007. Advances in systematics of ticks. *Acta Entomologica Sinica,* **50**(9): 941-949.

Yang XW, Lee WH, Zhang Y. 2012b. Extremely abundant antimicrobial peptides existed in the skins of nine kinds of Chinese Odorous frogs. *Journal of Proteome Research,* **11**(1): 306-319.

Yang ZZ. 2006. On the Spider Fauna of Yunnan, China, and its Evolution. Ph.D. thesis, Hebei University, Baoding China. (In Chinese)

Yeaman MR, Yount NY. 2007. Unifying themes in host defence effector polypeptides. *Nature Reviews Microbiology,* **5**(9): 727-740.

Yoshida T, Jones LE, Ellner SP, Fussmann GF, Hairston NG Jr. 2003. Rapid evolution drives ecological dynamics in a predator-prey system. *Nature,* **424**(6946): 303-306.

Yount NY, Kupferwasser D, Spisni A, Dutz SM, Ramjan ZH, Sharma S, Waring AJ, Yeaman MR. 2009. Selective reciprocity in antimicrobial activity

versus cytotoxicity of hBD-2 and crotamine. *Proceedings of the National Academy of Sciences of the USA,* **106**(35): 14972-14977.

Zhang DS, Zhao CX, Wang AT, Deng L, Ma LA. 2012a. Hydra vulgaris (Anthoathecatae, Hydridae): a new record species in China. *Sichuan Journal of Zoology,* **31**(5): 821-824. (In Chinese)

Zhang J, Zhang Y, Wan SG, Wei SS, Lee WH, Zhang Y. 2005. Bm-TFF2, a trefoil factor protein with platelet activation activity from frog *Bombina maxima* skin secretions. *Biochemical and Biophysical Research Communications,* **330**(4): 1027-1033.

Zhang Y, Wisner A, Xiong YL, Bon C. 1995. A novel plasminogen activator from snake venom, purification, characterization, and molecular cloning. *Journal of Biology Chemistry,* **270**(17): 10246-10255.

Zhang Y, Wisner A, Maroun RC, Choumet V, Xiong YL, Bon C. 1997. *Trimeresurus stejnegeri* snake venom plasminogen activator, site-directed mutagenesis and molecular modeling. *Journal of Biology Chemistry,* **272**(33): 20531-20537.

Zhang Y. 2006. Amphibian skin secretions and bio-adaptive significance — implications from *Bombina maxima* skin secretion proteome. *Zoological Research,* **27**(1): 101-112. (In Chinese)

Zhang Y, Yu GY, Wang YJ, Xiang Y, Gao Q, Jiang P, Zhang J, Lee WH, Zhang Y. 2011. Activation of protease-activated receptor (PAR) $_1$ by frog trefoil factor (TFF) $_2$ and PAR4 by human TFF_2. *Cellular and Molecular Life Sciences,* **68**(22): 3771-3780.

Zhang Y, Wang Y, Xiang Y, Lee W, Zhang Y. 2012b. Prohibitins are involved in protease-activated receptor 1-mediated platelet aggregation. *Journal of Thrombosis and Haemostasis,* **10**(3): 411-418.

Zhang YY, Huang Y, He QZ, Liu JY, Luo J, Zhu L, Lu SS, Huang PF, Chen XY, Zeng XZ, Liang SP. 2014. Toxin diversity revealed by a transcriptomic study of *Ornithoctonus huwena*. *PLoS One,* **9**(6): e100682.

Zhang YP, Ge S. 2007. Molecular evolution study in China: progress and future promise. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **362**(1482): 973-986.

Zhao EM. 2006. Chinese Snakes. Hefei: Anhui Science & Technology Publishing House. (In Chinese)

Zhao F, Yan C, Wang X, Yang Y, Wang GY, Lee WH, Xiang Y, Zhang Y. 2014. Comprehensive transcriptome profiling and functional analysis of the frog (*Bombina maxima*) immune system. *DNA Research,* **21**(1): 1-13.

Zhao H, Gan TX, Liu XD, Jin Y, Lee WH, Shen JH, Zhang Y. 2008. Identification and characterization of novel reptile cathelicidins from elapid snakes. *Peptides,* **29**(10): 1685-1691.

Zhou SY. 2012. Progress of taxonomic study on Formicidae (Hymenoptera) in China. *Journal of Guangxi Normal University: Natural Science Edition,* **30**(3): 244-251. (In Chinese)

About the author

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Dr Yun ZHANG is a Chinese biologist. He is currently the Director of the Toxin Committee of the Chinese Society of Toxicology, the Chief of the Academic Committee of Kunming Institute of Zoology, the Chinese Academy of Sciences, and serves as a novel drug reviewer of the China Food and Drug Administration, the executive editor-in-chief of *Zoological Research*, and the editorial member of *Toxicon*, the official journal of the International Society on Toxinology (IST)

Dr ZHANG was born in Kunming, Yunnan Province, China in July, 1963. He got his bachelor's degree from East China University of Science and Technology in 1984 and received his Ph.D from the Chinese Academy of Sciences (CAS) in 1992. From 1991 to 1995, he studied in Pasteur Institute at Paris as a Ph.D candidate and later a postdoctoral fellow of the joint training program of CAS and Pasteur Institute. Dr ZHANG's work follows two key lines of enquiry, including deciphering human diseases mechanisms using animal toxins as natural probes, and exploring new animal toxins and developing them into clinical drugs. He has published more than 100 papers in international journals, including *PNAS*, *Nature communication*, *Journal of Biological Chemistry,* among others. Host-derived bacterial pore-forming toxin Aerolysin-like Proteins (ALPs) are widely expressed in various animal and plant species with unknown functions, and trefoil factors (TFFs) are well conserved from frog to human. By using frog *Bombina maxima* as an animal model, his group discovered βγ-CAT, a complex Bm-ALP1 and Bm-TFF3. They further illustrated that this protein complex is able to protect the frog and mice against microbial infection, which may represent novel immune pathways in host immunity.