Phylogenetics of Scorpions of Medical Importance

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Abstract

This chapter assesses the phylogenetic relationships between scorpions and sodium channel-active scorpion toxins (NaScTx) of medical significance, almost entirely contained within the family Buthidae, with the exception of *Hemiscorpius lepturus* (Hemiscorpiidae). Within Buthidae, venom capable of severe and lethal scorpionism appears to have evolved multiple times among and within major morphological groups. Published mitochondrial sequence data from two markers (*COI* & 16S) were used to construct a partial maximum likelihood phylogeny for Buthidae. The resulting topology is largely congruent

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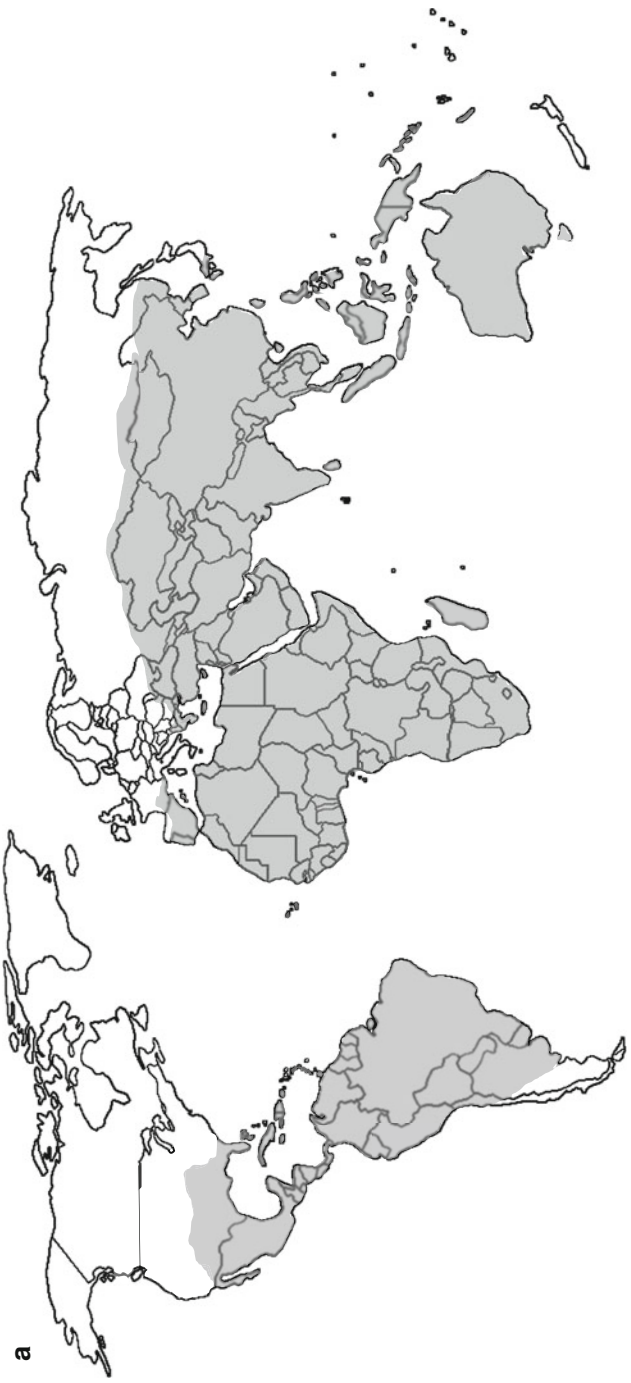
with results from comparative analysis of morphological data. Old World and New World buthids appear to be split, suggesting that some of the higher-level patterns in Buthidae can be explained by the breakup of Pangea. Provided that the venom composition should be more similar among closely related than distant species, the phylogeny can be used to predict which of the less dangerous species could also produce potent venoms. Clinical, phylogenetic, and toxinological evidence were also used to interpret the evolution and biogeography of these medically significant venomous taxa and the evolution of their toxic molecules. The existence of species-specific NaScTx repertoires in scorpions is probably the consequence of coevolution and arms races at the molecular and biochemical levels to overcome the ever-evolving structure of receptor sites (including sodium channels) in their predators and preys.

Introduction

Scorpions are an ancient and widespread group, mostly known for the extreme toxicity of some species to humans. The approximately 2,140 extant (Recent) scorpion species are contained within 14–18 families (see "Phylogeny of Buthidae" section), although Buthidae is the largest and most widely distributed family, with ~1,023 spp. (Fig. 1a). Scorpions of medical importance are considered hereby as those with a sting that produces severe or lethal envenomation in humans. Table 1 presents an updated list of the world's scorpion species positively identified in the literature as responsible for severe/lethal scorpionism (based only on reports including taxonomical verification), together with their range of distribution and clinical manifestations. The majority of medically relevant species belong to family Buthidae, the only exception being the Indo-Arabian *Hemiscorpius lepturus* (Hemiscorpiidae). Envenomations by the latter are generally characterized by coagulation disorders and local necrosis, which markedly differ from the neurotoxic and cardiotoxic symptoms characteristic of buthids. The 45 buthid taxa identified in Table 1 responsible for severe/lethal scorpionism comprise less than 8% of recognized species in this family and represent groups with both Old World and New World origins (see section "Toxinological Diversity in Buthidae: Old World Versus New World"). The broad range of genera containing these noxious species, however, explains the distribution of areas with the highest incidence of scorpionism in the world. In particular, many regions in Central and South America, the Middle East, Asia, and northern and southern Africa harbor species that are responsible for significant morbidity and pediatric mortality (Fig. 1b and Table 1).

The number of noxious genera ($n = 6$) is higher in the Old World (Asia, Africa, Europe), whereas the number of toxic species ($n = 30$) is higher in the New World (the Americas) (Table 1), a trend that applies to the distribution of scorpion diversity in general (Nenilin and Fet 1992). In the Old World, buthid genera containing medically relevant species include *Androctonus* (northern Africa, Middle East), *Leiurus* (northern Africa up to Algeria, Middle East), *Buthus* (Mediterranean basin including northern Africa and southern Europe, in Africa



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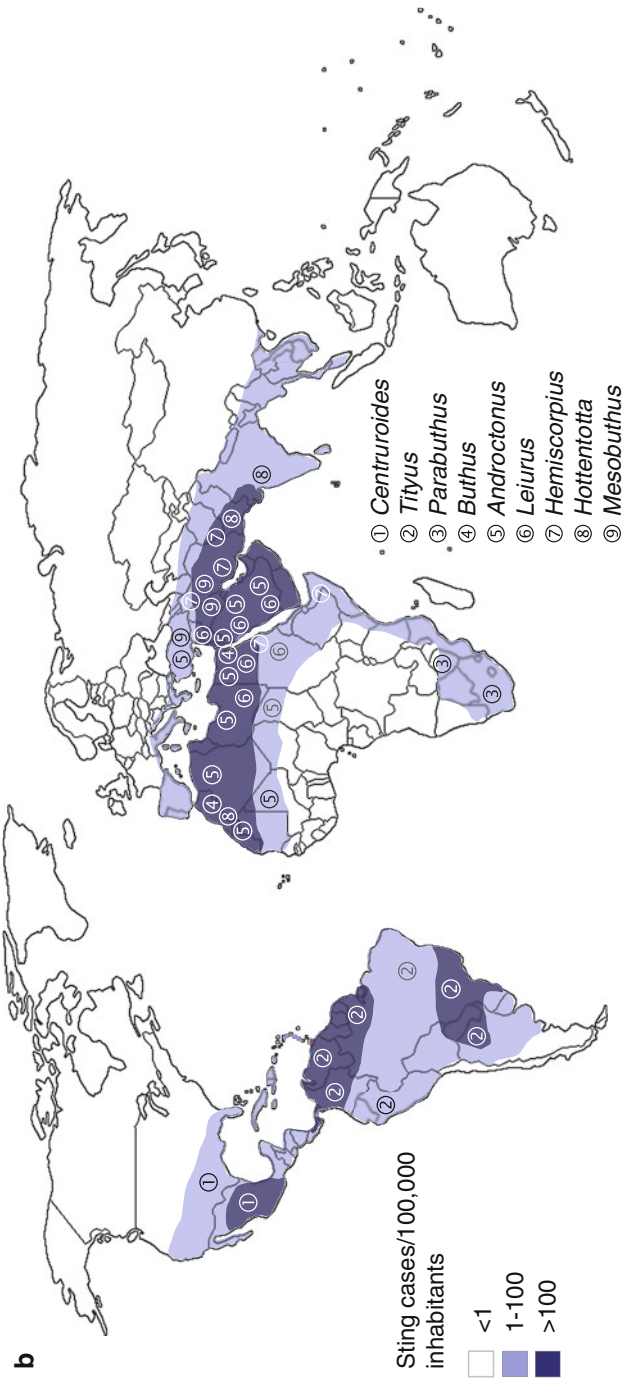

Fig. 1 (**a**) Worldwide distribution of the family Buthidae. (**b**) Areas of incidence of scorpionism in the world (based on Chippaux and Goyffon 2008), together with the distribution of medically significant buthid genera and including genus *Hemiscorpius* (family Hemiscorpiidae)

Table 1 List of scorpion species documented as medically significant

Species	Distribution	Manifestations in severe scorpionism	References
Androctonus australis	Chad, Egypt, Libya, Mauritania, Somalia, Sudan, Tunisia, India, Israel, Pakistan, Saudi Arabia, Yemen	Encephalopathy and cardiovascular collapse	Amitai (2005)
Androctonus bicolor	Algeria, Egypt, Eritrea, Libya, Morocco, Tunisia, Israel, Jordan, Syria	Tachycardia and respiratory distress	Amitai (2005)
Androctonus crassicauda	Armenia, Azerbaijan, Bahrain, Egypt, Iran, Iraq, Israel, Jordan, Kuwait, Oman, Saudi Arabia, Syria, Turkey, United Arab Emirates, Yemen	Parasympathetic and sympathetic manifestations, characterized by pain, hyperemia and edema, sinus tachycardia, pallor and cold extremities, nausea, vomiting, and restlessness	Amitai (2005)
Androctonus mauritanicus	Mauritania, Morocco	Not described but fatalities reported from Morocco	Amitai (2005)
Buthus occitanus complex	Algeria, Burkina Faso, Djibouti, Egypt, Ethiopia, Gambia, Gabon, Guinea-Bissau, Libya, Mauritania, Morocco, Nigeria, Senegal, Somalia, Sudan, Tunisia, Cyprus, Iraq, Israel, Jordan, Lebanon, France (southern), Greece, Portugal, Spain	Priapism, restlessness, vomiting, siderosis, abdominal pain and/or hyperdistention, tachycardia, polypnea/dyspnea, cyanosis, arterial hypotension, bradycardia	Amitai (2005)
Centruroides infamatus	Mexico (States of Aguascalientes, Colima, Durango, Guanajuato, Jalisco, Michoacán, Nayarit, Oaxaca, Puebla, Sinaloa, Veracruz, and Zacatecas)	Heart failure, pulmonary edema, circulatory shock, convulsions	Chávez-Haro and Ortiz (2014)
Centruroides limpidus	Mexico (States of Guerrero, México, Michoacán, Morelos, Querétaro, Puebla)	*Globus pharyngeus*, blurry vision, temporary blindness, nystagmus, dysarthria, muscle ataxia, abdominal distension, opisthotonos, convulsions, and priapism	Chávez-Haro and Ortiz (2014)
Centruroides noxius	Mexico (Nayarit state)	*Globus pharyngeus*, blurry vision, temporary blindness, nystagmus, dysarthria, muscle ataxia, abdominal distension, opisthotonos, convulsions, and priapism	Chávez-Haro and Ortiz (2014)
Centruroides sculpturatus[a]	USA (States of Arizona, California, Nevada, New Mexico); Mexico (Sonora)	Neurotoxic, predominantly cholinergic manifestations. Cranial nerve abnormalities, neuromuscular hyperactivity, and dysautonomias	Skolnik and Ewald (2013)

(*continued*)

Table 1 (continued)

Species	Distribution	Manifestations in severe scorpionism	References
Centruroides suffusus	Mexico (Durango state)	*Globus pharyngeus*, blurry vision, temporary blindness, nystagmus, dysarthria, muscle ataxia, abdominal distension, opisthotonos, convulsions, and priapism	Chávez-Haro and Ortiz (2014)
Centruroides tecomanus	Mexico (States of Colima, Michoacán)	*Globus pharyngeus*, blurry vision, temporary blindness, nystagmus, dysarthria, muscle ataxia, abdominal distension, opisthotonos, convulsions, and priapism	Chávez-Haro and Ortiz (2014)
Centruroides villegasi	Mexico (Guerrero state)	Not described	Chávez-Haro and Ortiz (2014)
Grosphus palpator[b]	Madagascar (Tananarive province)	Neurotoxic (predominantly cholinergic), *globus pharyngeus*, apnea	Bergman (1997)
Hemiscorpius lepturus	Iran (Khuzestan province), Iraq, Pakistan, Yemen	Severe and fatal hemolysis, secondary renal failure, deep and necrotic ulcers, ankylosis of the joints, thrombosis, cardiovascular failure	Jalali et al. (2010)
Hottentotta franzwerneri	Morocco	Not described but fatalities reported from Morocco	Amitai (2005)
Hottentotta tamulus	India, Pakistan	Myocarditis, pulmonary edema, but no raised circulating catecholamines	Strong et al. (2014)
Leiurus hebraeus	Israel, Jordan, Lebanon, Syria, Saudi Arabia, Yemen	Sympathetic manifestations including tachycardia, hypertension, ventricular arrhythmia as well as parasympathetic manifestations, including priapism, hypersalivation, and muscular twitching	Amitai (2005)
Leiurus quinquestriatus	Algeria, Chad, Egypt, Ethiopia, Libya, Mali, Niger, Somalia, Sudan, Tunisia	Encephalopathy, pulmonary edema, seizures, and clinical features suggestive of myocarditis, e.g., heart failure, cyanosis, cardiogenic shock, and dysrhythmia	Amitai (2005)

Mesobuthus eupeus	Afghanistan, Turkey, Armenia, Azerbaijan, China, Georgia, Iran, Iraq, Kazakstan, Kyrgyzstan, Mongolia, Pakistan, Syria, Tajikistan, Turkmenistan, Uzbekistan, Russia (Astrakhan region)	Autonomic manifestations including hyperemia and edema	Amitai (2005)
Mesobuthus gibbosus	Cyprus, Syria, Turkey, Albania, Greece, Macedonia, Montenegro	Abdominal pain, muscle contractions, nausea, hypertension, hypotension, bradycardia, dyspnea, pulmonary edema, convulsion, and shock	Amitai (2005)
Parabuthus granulatus	Angola, Botswana, Namibia, South Africa	Predominantly sympathetic manifestations, respiratory distress hypertension, and pulmonary edema	Bergman (1997), Müller et al. (2011)
Parabuthus transvaalicus	Botswana, Mozambique, South Africa, Zimbabwe	Predominantly parasympathetic manifestations, with neuromuscular and cardiac alterations; profuse sialorrhea	Bergman (1997), Müller et al. (2011)
Tityus asthenes	Northern Peru, Ecuador (*cis*- and *trans*-Andean distribution), Pacific coast of Colombia, and Panama	Cardiopulmonary complications, respiratory distress (including tachypnea), vomits, edematous, or hemorrhagic acute pancreatitis; fatalities reported from Panama and Colombia, also probably from Ecuador	Borges et al. (2012), Otero et al. (2004)
Tityus bahiensis	South-eastern Brazil, Argentina (Misiones, Corrientes, and Santa Fé provinces)	Predominantly autonomic manifestations, including vomiting, agitation, sweating, dyspnea, bradycardia, tachycardia, tachypnea, somnolence/lethargy, cutaneous paleness, hypothermia, and hypotension. Manifestations less severe than in the case of *T. serrulatus*	Pucca et al. (2014)
Tityus breweri	Venezuela (Bolívar state)	Sympathetic and parasympathetic manifestations comprising tachycardia, arrhythmia, respiratory complications including tachypnea, and pancreatic alterations; muscle fasciculations	Borges et al. (2010b)
Tityus caripitensis	Venezuela (Monagas state)	Sympathetic and parasympathetic manifestations comprising tachycardia, arrhythmia, respiratory complications including tachypnea, and pancreatic alterations	Borges and De Sousa (2006)

(*continued*)

Table 1 (continued)

Species	Distribution	Manifestations in severe scorpionism	References
Tityus cerroazul	Panama (Coclé and Panamá provinces), Costa Rica (Limón province)	Not described but at least one death reported from Panama	Borges et al. (2012)
Tityus championi	Costa Rica (Puntarenas province), Panama (Chiriquí province)	Not described but at least one death reported from Costa Rica	Borges et al. (2012)
Tityus confluens	Argentina (Corrientes, Santa Fé, La Rioja, Córdoba, Tucumán, Salta, and Jujuy provinces)	Not described but several deaths reported from Argentina	de Roodt et al. (2009)
Tityus discrepans	Venezuela (Capital District, Miranda and Aragua states)	Neurotoxic, mainly cholinergic, including gastrointestinal manifestations, with scarce cardiopulmonary alterations	Borges and De Sousa (2006)
Tityus falconensis	Venezuela (Falcón and Lara states)	Diaphoresis, vomits, arterial hypertension, irritability, tachycardia, myocarditis, but no alterations of blood glucose or amylase levels	Borges and De Sousa (2006)
Tityus festae	Eastern Panama, Northern Colombia	Not described but at least one death reported from Panama	Borges et al. (2012)
Tityus isabelceciliae	Venezuela (Capital District)	Not described but fatalities reported	Borges and De Sousa (2006)
Tityus metuendus	Amazonian regions of Peru, Brazil, Bolivia	Not described but at least one death reported from Peru	Lourenço (1997)
Tityus neoespartanus	Venezuela (Margarita Island)	Adrenergic and cholinergic manifestations comprising tachycardia, arrhythmia, respiratory complications including tachypnea, and pancreatic alterations	De Sousa et al. (2007)
Tityus nororientalis	Venezuela (Sucre, Monagas and Anzoátegui states)	Adrenergic and cholinergic manifestations comprising tachycardia, arrhythmia, respiratory complications including tachypnea, and pancreatic alterations	Borges and De Sousa (2006)
Tityus obscurus	Brazil (Amapá, Pará, and Amazonas states), French Guiana, Suriname	Mainly central neurotoxicity, including general paresthesia, ataxia, dysarthria, myoclonus, and dysmetria	Pardal et al. (2014)

Tityus pachyurus	Costa Rica (Limón Province), central and western Panama, Colombia (Antioquia, Boyacá, Caldas, Cundinamarca, Huila, and Tolima departments)	Pulmonary edema, hypertension, heart arrest associated with ventricular tachycardia	Borges et al. (2012), Otero et al. (2004)
Tityus perijanensis	Western Venezuela (Zulia State), Northeastern Colombia	Cardiopulmonary manifestations and abdominal distress; central neurotoxicity	Borges and De Sousa (2006)
Tityus sabineae	Colombia (Caldas, Boyacá, and Cundinamarca departments)	Not described but at least one death reported	Lourenço (2000)
Tityus serrulatus	Southeastern Brazil	Predominantly autonomic manifestations, including vomiting, agitation, sweating, dyspnea, bradycardia, tachycardia, tachypnea, somnolence/lethargy, cutaneous paleness, hypothermia and hypotension, pulmonary edema. Manifestations like severe hypertension, seizures, priapism, and coma are less prevalent	Pucca et al. (2014)
Tityus stigmurus	Northeastern Brazil	Vomiting, nausea, abdominal pain, sialorrhea, neurological disorders (tremor, agitation, dizziness, difficulty in walking, contracture, blurred vision, pallor, and somnolence), cardiovascular disorders (tachycardia, hypertension, and hypotension), and breathing disorders (dyspnea). Manifestations less severe that in the case of *T. serrulatus*	Pucca et al. (2014)
Tityus trinitatis	Trinidad and Tobago	Neurotoxic, mainly cholinergic, including acute hemorrhagic pancreatitis and myocarditis	Borges (2014)
Tityus trivittatus	Argentina (Tucumán, Santa Fé, Catamarca, Santiago del Estero, San Juan, Mendoza, Formosa, Chaco, Entre Ríos, Buenos Aires, Corrientes, Córdoba, Misiones, La Rioja provinces)	Adrenergic hyperstimulation. In severe cases: shock, congestive cardiac insufficiency and arrhythmia, pulmonary edema, bradycardia, bradypnea, apnea, and coma	de Roodt (2014)
Tityus zulianus	Western Venezuela (south of Lake Maracaibo)	Cardiopulmonary manifestations as a result of massive adrenergic stimulation, pulmonary edema	Borges and De Sousa (2006)

[a]*C. sculpturatus* (range southern US and northern Mexico), synonymized with *C. exilicauda* (range Baja California), differs from the latter genetically and toxinologically (Fet et al. 2005)

[b]The *Grosphus* species associated with the accident has not been verified

from Senegal to Ethiopia), *Mesobuthus* (in Asia, from Lebanon to Korea; in the eastern Mediterranean basin), *Hottentotta* (throughout Africa; in Asia, from Lebanon to China, including the Arabian peninsula), and *Parabuthus* (southern and eastern Africa, the Arabian peninsula). In the New World, all reported scorpionism cases are due to envenomation by *Centruroides* (North, Central and South America, and the Caribbean) and *Tityus* (from northern Costa Rica to northern Argentina, including the Caribbean area) species (Fig. 1b). Clinical, phylogenetic, and toxinological evidence continue to elucidate the evolution and biogeography of these medically significant venomous taxa and the evolution of their toxic molecules.

Phylogeny of Buthidae

Higher-level scorpion systematics has recently experienced extensive revisions and heated controversy, with different authors proposing taxonomies with between 14 and 18 families (Prendini and Wheeler 2005; Soleglad and Fet 2003). Despite the conflicting taxonomic viewpoints, family Buthidae has remained relatively stable and contains all medically significant species except *Hemiscorpius lepturus* (family Hemiscorpiidae). A cladistic analysis of Buthidae, based on the relative position of the d_3 trichobothrium and DM_c carina on the pedipalp patella, suggests six main groups of buthid scorpions: *Buthus* group, *Ananteris* group, *Isometrus* group, *Charmus* group, *Uroplectes* group, and the *Tityus* group (Fet et al. 2005). Two main evolutionary lineages are represented by this approach: (1) the *Buthus* group, containing 39 predominantly arid-adapted Palearctic genera (especially from North Africa and Middle East), including all Old World noxious taxa (*Androctonus*, *Buthus*, *Leiurus*, *Hottentotta*), and (2) a second clade encompassing 43 predominantly Afrotropical genera, separated into the five remaining groups, which includes a few Oriental and Australian genus-level endemics (*Lychas*, *Isometrus*) and a separate Neotropical clade of nine genera, including all noxious taxa in the New World (*Centruroides*, *Tityus*) (Fet et al. 2005). The monophyly of the New World buthid clade has been confirmed by several lines of evidence, including the anatomy of book lungs and ovary uterine structure (Kamenz and Prendini 2008; Volschenk et al. 2008), molecular phylogenetics (Soleglad and Fet 2003), and toxinological data (Rodríguez de la Vega and Possani 2005). The latter have indicated that toxic peptides produced by New World scorpions, at least within the group of toxins altering voltage-gated sodium channel activation (β-NaScTx, see section "Physiopathology and Molecular Diversity in Scorpion Toxins"), belong to a different evolutionary lineage than Old World scorpions, indicating that toxinological divergence parallels results from the cladistic analysis. Thus, within Buthidae, severe/lethal scorpionism is not restricted to a single group but appears to have evolved multiple times among and within major buthid groups.

To examine the relationships among medically significant buthids, published mitochondrial sequence data from two markers (*COI* & 16S) gleaned from GenBank were used to construct a partial maximum likelihood (ML) phylogeny for Buthidae (Fig. 2). Data were available for 114 buthid taxa and the phylogeny

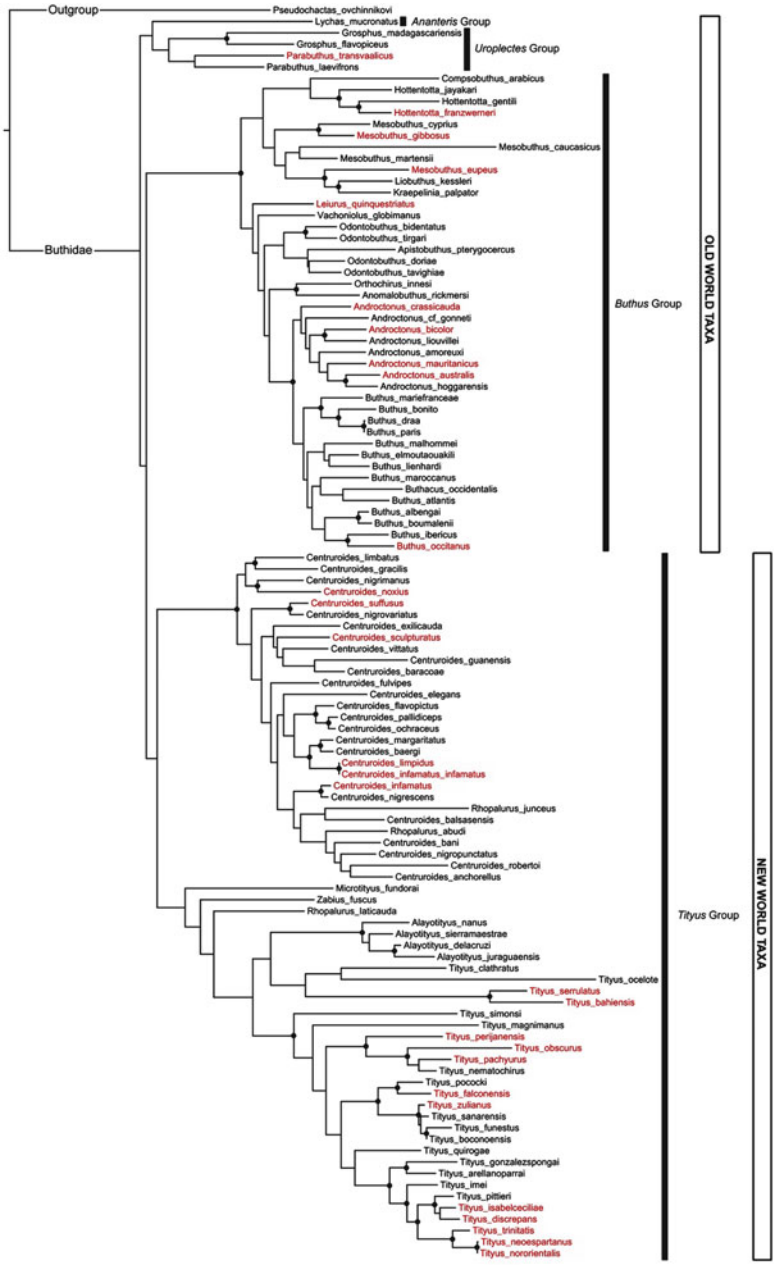

Fig. 2 Partial maximum likelihood (ML) phylogeny of Buthidae based on concatenated mitochondrial (*COI* & 16S) sequence data. Species considered to be medically significant are in *red*. *Black* dots indicate strongly supported nodes (bootstrap ≥ 70). *Black* bars represent morphological groupings (Fet et al. 2005). The ML phylogeny was generated using RAxML (Stamatakis 2006)

was rooted with *Pseudochactas ovchinnikovi*, a unique taxon currently considered to represent the sister group of Buthidae (Prendini et al. 2006). Although many nodes across the phylogeny are not strongly supported, especially at higher levels likely due to genetic saturation, the ML topology is congruent with results from comparative analysis of morphological data (Fet et al. 2005); the *Ananteris*, *Buthus*, *Tityus*, and *Uroplectes* groups are all monophyletic, although with varying degrees of support. Furthermore, Old World and New World buthids appear to be split, suggesting that some of the higher-level patterns in Buthidae can be explained by the breakup of Pangea.

Of the Old World taxa, all of the medically significant taxa except *Parabuthus transvaalicus* are members of the *Buthus* group. Within the group, species responsible for severe/lethal scorpionism are not monophyletic and are instead scattered across the phylogeny. Interestingly, medically significant species do not form monophyletic groups within *Mesobuthus* and *Androctonus* and are instead polyphyletic with respect to less dangerous congenerics. A similar trend occurs among New World taxa, of which only *Centruroides* and *Tityus* contain medically significant species. However, assuming that there is some phylogenetic signal to venom toxicity in scorpions, the Buthidae phylogeny can be used to make some important predictions regarding buthids not considered medically significant. In other words, if the composition of venoms is more similar among closely related than distantly related species, then the phylogeny can be used to predict which of the less dangerous species could also potentially possess potent venoms. Using this reasoning, envenomation by Old World *Mesobuthus cyprius* and *Buthus ibericus* should be taken seriously as both are strongly supported as sister to medically significant species. Of the New World taxa, *Centruroides nigrovariatus*, *C. nigrescens*, *Tityus nematochirus*, *T. pococki*, *T. sanarensis*, *T. funestus*, *T. boconoensis*, and *T. pittieri* are potentially dangerous as they are closely related to medically significant sister taxa.

Although many buthid genera are not represented in the above phylogeny, medically significant species are clearly the result of many independent evolutionary events that lead to venom capable of severe/lethal scorpionism. Sampling of additional taxa, as well as new genomics tools, will undoubtedly resolve some of the higher-level relationships among buthid taxa and further precipitate the understanding of relationships among medically important species.

Physiopathology and Molecular Diversity in Scorpion Toxins

Exacerbation of presynaptic neurotransmission is the main mechanism employed by buthid scorpions to subdue prey or deter predators by using toxins that affect voltage-gated sodium (Nav) and potassium channels (NaScTx and KScTx, respectively). These low-molecular-mass (3–8 kDa) proteins, collectively known as the CSα/β superfamily (cysteine-stabilized α/β scaffold, containing 3–8 cysteine residues forming three or four intramolecular disulfide bridges), act either by altering the channel's gating mechanism or blocking its selectivity filter (Rodríguez de la Vega et al. 2013). Considering that the Nav-active toxins (NaScTx) are responsible

for the most dangerous neurotoxic effects observed during human envenomation (Guerrero-Vargas et al. 2012), those scorpions producing the highest abundance and/or more active NaScTx variants toward skeletal/cardiac muscle and neuronal Nav isoforms are expected to produce the most toxic venoms against mammals. In fact, less toxic species within the Neotropical genus *Centruroides*, such as *C. margaritatus*, appear to produce venoms richer in potassium channel-active toxins (KScTx) and pore-forming (antimicrobial) peptides than their toxic congenerics from the Mexican Pacific versant (reviewed in Borges 2015). In the case of the Old World genus *Parabuthus*, it was originally assumed that all 22 species endemic to southern Africa were equally noxious, but a differential toxicity is observed both experimentally and clinically, with *P. granulatus* being the most toxic, followed by *P. transvaalicus* and more distantly by *P. capensis*, *P. raudus*, *P. villosus*, *P. kalaharicus*, and *P. schlechteri* (Müller et al. 2011). Significantly, mass spectral evidence indicates that *P. granulatus* venom only contains peptides in the NaScTx molecular mass range, whereas other *Parabuthus* species are richer in KScTx peptides (Dyason et al. 2002). A similar situation probably occurs in other genera such as the speciose genus *Tityus*, where envenomation by *T.* (*Atreus*) *brazilae*, *T.* (*Tityus*) *neglectus* (northern Brazil), *T.* (*Tityus*) *obtusus* (Puerto Rico), *T.* (*Archaeotityus*) *mattogrossensis*, and *T.* (*Tityus*) *adrianoi* only produces local symptomatology (Borges 2014; Pucca et al. 2014), whereas syntopic *Tityus* spp. from northern and southern South America can be life-threatening (see Table 1). Thus, mammalian toxicity of scorpion venoms is directly related to NaScTxs content.

Notwithstanding the fact that scorpion venom toxicity is the result of several variables including the age of the victim and the amount and delivery route of injected venom, differences in the toxin repertoires expressed by individual species are evident from the differential symptomatology elicited in envenomed humans. *Leiurus hebraeus*, which is the main problem species in Israel, exerts both neurotoxicity and cardiotoxicity. Myocarditis and pulmonary edema have been frequently reported from India following envenomation by *Hottentotta tamulus*. In Tunisia, severe envenomation has been caused by *Androctonus amoreuxi* and *Androctonus australis* resulting in encephalopathy and cardiovascular collapse. The Arizona bark scorpion, *Centruroides sculpturatus,* is mainly neurotoxic, of a predominantly cholinergic nature (Amitai 2005). As mentioned above, the clinical picture of envenomation by *Tityus*, the most diverse scorpion genus, is dependent on the species involved. *T. serrulatus*, from the Brazilian southeast, elicits autonomic manifestations, whereas envenomation by *T. obscurus*, in the Amazon basin including French Guiana, involves mainly central neurotoxicity, including general paresthesia, ataxia, dysarthria, myoclonus, and dysmetria (Pardal et al. 2014). In addition, envenomation by *T. discrepans* in northcentral Venezuela is characterized by gastrointestinal manifestations, contrasting with the predominantly cardiopulmonary symptomatology caused by *T. zulianus* in the Mérida Andes (Borges et al. 2010b). In the southern African genus *Parabuthus*, envenomation by *P. transvaalicus* elicits mainly cholinergic manifestations, whereas stings by *P. granosus* produces predominantly sympathetic manifestations (Bergman 1997; Table 1).

Underlying the generation of such different physiopathological effects is the fact that NaScTxs alter Nav gating either affecting the channel threshold of activation (β-toxins) or the inactivation process (α-toxins). Furthermore, β- and α-toxins, which differ in concentration in crude venoms among species and even among individuals, exhibit marked selectivity for different Nav isoforms (Gilles et al. 2000; Leipold et al. 2006). Despite the general similarity among vertebrate Nav subtypes (currently nine isoforms are known), these are expressed differentially depending on the tissue and cell type and their receptor-binding sites for NaScTxs vary (Gurevitz et al. 2014). There is evidence suggesting that minor changes in NaScTx primary structure promote dramatic changes in their pharmacological properties. For instance, the 64-residue-long NaScTx Tz1 from *Tityus zulianus* is 92% identical to toxin Tc48b from *T. obscurus* but for only five surface-located residues; however, Tz1 acts as a β-toxin and Tc48b as an α-toxin (Borges et al. 2004; Murgia et al. 2004). Since α- and β-toxins differ in their ability to promote in vivo release of different amounts and types of neurotransmitters (Vasconcelos et al. 2005), differences in their abundance in crude venoms can have physiopathological implications. Even toxins produced by phylogenetically related species can exhibit different profiles of Nav modification. Thus, toxin Ts1 from *Tityus serrulatus* produces both an excitatory (shifting of activation threshold) and depressant (Na^+ current blockade) effect on the Nav1.4 (skeletal muscle-specific) isoform (Leipold et al. 2007). In contrast, toxin Tt1g from *Tityus trivittatus* (95% identical to Ts1, differing in three surface-located residues) only produces a depressant effect on the same isoform, not affecting the channel's threshold potential (Coronas et al. 2014). Accordingly, predicted surface accessibility of hypermutable sites in α- and β-NaScTxs suggests that most point mutations, via positive selection, are located in the molecular surface and the loops connecting secondary structure elements, areas involved in receptor recognition and antibody binding. The rapid accumulation of non-synonymous replacements in exposed residues has therefore been suggested to play a significant role in the neofunctionalization of these toxins through variations in their surface chemistry (Sunagar et al. 2013).

As seen in other venomous taxa (Casewell et al. 2012), such species-specific toxin repertoires in scorpions are probably the consequence of coevolution and arms races at the molecular and biochemical levels to overcome the ever-evolving structure of receptor sites (including Navs) in predators and preys of scorpions. For example, alternative splicing in insects renders more Nav variants (from a single Nav background) in comparison with their vertebrate counterparts, producing a broader spectrum of both drastic and subtle differences in channel expression and gating properties (e.g., 69 Nav splice variants in *Blattella germanica* (Orthoptera) versus the nine vertebrate Nav isoforms) (Dong et al. 2014). In one such arms race, there are also examples of counter selection in predators to elude the scorpion venom effect: the Nav1.8 variant involved in nociception in the southern US desert mouse *Onychomys torridus* contains mutations that render this channel susceptible of inhibition by the NaScTxs of its prey, the North American scorpion *Centruroides sculpturatus*. While scorpion peptides that target Nav channels typically activate the channel (β-NaScTxs) or block inactivation (α-NaScTxs), prolonging channel

activity and increasing neuron excitability, *C. sculpturatus* NaScTxs inhibit *O. torridus* Nav1.8 Na^+ current and decrease neuron excitability, blocking neuronal signaling and inducing analgesia instead of pain (Rowe et al. 2013).

Vicariance and Scorpion Venom Evolution

The origins of higher-level diversity among scorpions are often attributed to vicariance due to the fragmentation of Pangea and subsequent continental drift (e.g., Soleglad and Fet 2003; Lourenço 1996), although new studies suggest that Recent scorpions diversified with the fragmentation of Gondwana ca. 180–165 Myr (Sharma and Wheeler 2014). Under either scenario, vicariance has likely been the predominant force driving venom evolution in scorpions through the emergence of different toxin repertoires, as suggested by recent work on the Chinese buthid *Lychas mucronatus* (Ruiming et al. 2010). The venom composition of *L. mucronatus* from Hainan and Yunnan provinces, 1,000 km apart, was evaluated at the transcriptome level. Although no significant difference was observed in the abundance of α- and β-NaScTx types, toxins from each population differed in their primary structures. Based on primary structures and cysteine pairing, KScTxs, which alter the gating and/or block the various K^+ channel selectivity filters, are classified into four subfamilies: α, β, γ, and κ (Bergeron and Bingham 2012). α-KScTxs in *L. mucronatus* from Yunnan were twofold more abundant than in the Hainan-sourced population and possessed more diverse primary structures: the most abundant α-type from Yunnan (GT028663) has no homologue in the Hainan population. Also, transcripts encoding antimicrobial (pore-forming) peptides accounted for 21% of all toxin-like peptides in the Yunnan population, whereas their abundance reached 40% in the Hainan population (Ruiming et al. 2010).

The forces driving these intraspecific toxinological differences in *L. mucronatus* are not known at present, nor for any other scorpion species. However, divergence of toxins from one species to the next, or even among populations of the same species, suggests that venoms are sensitive to environmental selection. The interrelations between natural selection and the genetic and molecular processes responsible for generating variation in toxins have been most extensively studied in snakes and gastropods, where diet is a crucial component, but have not yet been elucidated in scorpions (Casewell et al. 2012). Studies of the gastropod genus *Conus* suggest that venom peptide genes rapidly diversify because genes whose products act on other animals in the environment, termed exogenes (*sensu* Olivera 2006), will evolve quickly. For other diverse metazoan lineages, such as scorpions, similarly rapidly diversifying genes should be a major genetic foundation for generating biodiversity. RNA editing, gene duplication, and posttranslational modification have been presented as the mechanisms responsible for NaScTx diversity (Zhu et al. 2004; Zhu and Gao 2006a). In particular, RNA editing has been suggested as the mechanism in scorpions producing gene variants encoding different disulfide-bridge linking patterns ((Zhu and Gao 2006a) see below).

Phylogenetic Scale of Venom Variation in Scorpions

Of the 14–18 recognized scorpion families, those that have been investigated for toxin data using venom gland transcriptomes include Bothriuridae (genus *Cercophonius*), Buthidae (genera *Australobuthus*, *Centruroides*, *Isometroides*, *Isometrus*, *Hottentotta*, *Lychas*, and *Tityus*), Chaerilidae (genus *Chaerilus*), Iuridae (genus *Hadrurus*), Liochelidae (genera *Opisthacanthus*), Scorpionidae (genera *Heterometrus* and *Pandinus*), Scorpiopidae (genus *Scorpiops*), and Urodacidae (genus *Urodacus*). These studies have provided information to infer the phylogenetic scale of scorpion venom variation, including the evolution of vertebrate toxicity in Buthidae (Ma et al. 2012; Sunagar et al. 2013).

The toxin types currently found in one or several of these scorpion families may have been the result of individual recruitments. For instance, pore-forming peptides with antimicrobial and hemolytic activity are widely distributed in bothriurids, chaerilids, scorpionids, liochelids, and iurids, but lacking in buthids (Ma et al. 2012; Sunagar et al. 2013). Toxin sequences originally thought to be exclusive of Buthidae venoms include bacterial cell-wall-disrupting peptides, BPPs (bradykinin-potentiating peptides), and NaScTxs. Extensive sequencing of venom gland cDNA libraries in bothriurids, iurids, liochelids, scorpionids, and scorpiopids has not revealed thus far the existence of NaScTxs (Ma et al. 2012), but a distant homologue (Ctricontig80) has been found recently in the venom of the chaerilid *Chaerilus tricostatus* (He et al. 2013). Upon phylogenetic analyses, this 23-residue-long peptide, putatively packed by three disulfide bridges, clusters with birtoxin, an also three disulfide-bridged peptide from the southern African buthid *Parabuthus transvaalicus*; the latter is a potent anti-mammalian β-toxin (Inceoglu et al. 2001) but with nontoxic (insect-specific) homologues in *Androctonus* species (Abbas et al. 2011). All members in the birtoxin subfamily contain only three disulfides with a slightly smaller size (about 58 residues) relative to the NaScTxs with four disulfides. They share 40–60% sequence identity to β-toxins but display more diverse pharmacological activities, ranging from those with a characteristic β-toxin effect, altering sodium channel activation, to others serving as potassium channel blockers due to the development of a putative functional dyad in their α-helical region (Abbas et al. 2011). The *Chaerilus tricostatus* (Ctricontig80) sequence also clusters with phaiodotoxin, a NaScTx-like depressant insect toxin from *Anuroctonus phaiodactylus* (family Iuridae), with four disulfide bridges and an eight C-terminal cysteine that imposes a different disulfide-bridge pattern (Valdez-Cruz et al. 2004; Fig. 3).

A crucial feature in NaScTxs is that 3 (out of 4) disulfide bridges are buried in the structure, stabilizing the CSα/β scaffold, and are conserved across the family, whereas the fourth disulfide corresponds to an exposed wrapper disulfide bridge (WDB) which varies in position among different toxins (Rodríguez de la Vega and Possani 2005). Alteration of the WDB linkage pattern can lead to a functional switch of the NaScTxs via adjusting the conformation of key residues associated with toxin function. Whereas in most NaScTxs the fourth bridge links the N- and

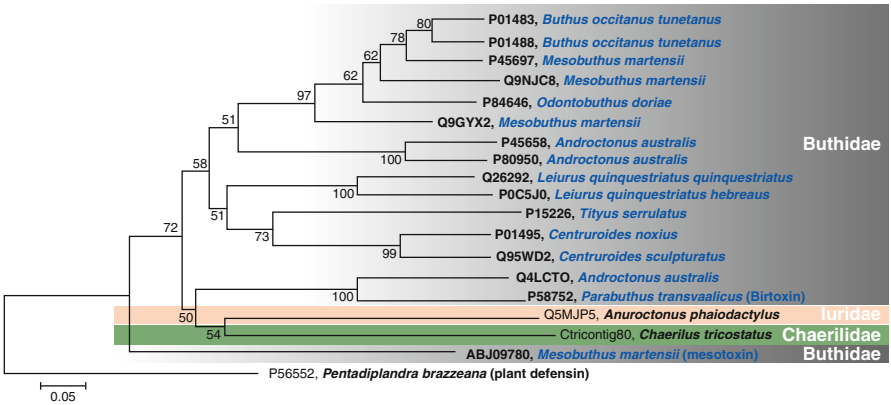

Fig. 3 Evolutionary analysis of NaScTxs sequences retrieved from scorpion venoms in the families Chaerilidae, Iuridae, and Buthidae (Adapted from He et al. 2013). Ctricontig80 is the NaScTx-like peptide sequence from *Chaerilus tricostatus* (Chaerilidae). P58752 is birtoxin from the scorpion *Parabuthus transvaalicus* (Buthidae), Q5MJPG is phaiodotoxin from the scorpion *Anuroctonus phaiodactylus* (Iuridae), and Q9GYX2 is toxin BmKα1 and ABJ09780 is mesotoxin, both from the scorpion *Mesobuthus martensii* (Buthidae). A plant defensin-like protein is used to root the phylogenetic tree

C-terminus, in the three disulfide-bridged members, the WDB is lacking. In phaiodotoxin the fourth bridge is located in the C-tail region (Zhu and Gao 2006a). In another group of NaScTx-like peptides (LVPs), which induce lipolytic responses in adipose cells and were originally thought to be exclusive of buthids, the evolutionary loss of the first cysteine removed the intramolecular WDB and allowed the assembly of an interchain WDB, resulting in a dimeric toxin with lipolytic instead of neurotoxic activity (Zhu and Gao 2006a). LVP-like sequences have been identified in *Cercophonius squama* (Bothriuridae) and *Urodacus manicatus* (Urodacidae) (Sunagar et al. 2013), rendering the buthid LVPs non-monophyletic. Thus, it has been suggested that an ancient peptide with three disulfide bridges might be the ancestor of NaScTx given that its scaffold can easily serve as a template for assembly of different types of toxins via evolution of the WDB in different positions (Zhu and Gao 2006b). NaScTx from buthids and non-buthids might have diverged from a common ancestor similar to mesotoxin, a three disulfide peptide from the buthid *Mesobuthus martensii* with a compatible size to typical NaScTx and considered evolutionary basal to α- and β-NaScTx (He et al. 2013). Considering the homology of the chaerilid Ctricontig80 peptide with mesotoxin and birtoxin-like peptides, and also taking into account the isolation of bothriurid and urodacid LVP-like sequences, NaScTx were probably recruited into the venom prior to the lineage split between buthids and non-buthids, which imply that toxins targeting Navs are among the most ancient components of scorpion venoms (He et al. 2013).

Toxinological Diversity in Buthidae: Old World Versus New World

Old World and New World buthids probably diverged during the Early Cretaceous (~140 Mya) as South America began to drift away from Africa, setting the stage for structural and functional divergent evolution of their venoms. NaScTxs of New and Old World buthids now differ in amino acid sequences, pharmacological action, and antigenic properties, although they still share characteristics suggestive of their common ancestry and conserved function, such as disulfide-bridge linkage pattern (Loret and Hammock 2001). Whereas α-NaScTxs, affecting Nav inactivation, have been found in scorpions throughout the world, anti-mammalian β-NaScTxs were originally assigned to New World scorpions in genera *Centruroides* and *Tityus*, and anti-insect selective β-toxins (depressant and excitatory) had been described only in the Old World. This suggested that diversification of β-NaScTxs into distinct pharmacological groups occurred after the splitting of Gondwana (Gordon et al. 2003). More recent research revealed, however, the existence of Old World toxins Lqhβ1 (from *Leiurus quinquestriatus*), BmK AS and BmK AS-1 (from *Mesobuthus martensii*), and AahIT4 (from *Androctonus australis*), all with a β-toxin fingerprint. The fact that Lqhβ1 displays both anti-mammalian (against Nav1.4) and anti-insect (against *para* channels from *Drosophila*) toxicity and that it is 41 to 50% identical to New World β-NaScTxs has suggested that the group of toxins represented by Lqhβ1 and AahIT4 evolved into the anti-insect selective depressant toxins in the Old World, and into β-toxins currently found in New World scorpions (Gordon et al. 2003).

Additional diversification of the β-NaScTx clade in the New World proceeded toward those with affinities to mammals (e.g., toxins Cn2, Css2, and Css4, from *Centruroides noxius* and *C. suffusus*), crustaceans (e.g., toxins Cn5, Cn11, and Cll1, from *C. noxius* and *C. limpidus*), and a group that acquired α-like activity while maintaining the structural features of β-NaScTxs (toxin CsE variants 1–3, from *C. sculpturatus*) (Gordon et al. 2003). *Tityus* β-NaScTxs from southern South America, such as Ts1, Tst1, and Tb1 (from *Tityus serrulatus* and *Tityus bahiensis*), are highly active on mammals and insects and thus seem to preserve ancient properties of Lqhβ1 in the New World (Rodríguez de la Vega and Possani 2005). Notwithstanding this effect of southern *Tityus* β-toxins, northern South American *Tityus* toxins might diverge from this scheme as toxin Bact-2 from *Tityus discrepans* (and also possibly Tz1 from *Tityus zulianus*, which is 98% identical to Bact-2) is a true anti-mammalian β-NaScTx that does not modify gating of the insect DmNav1 channel (Peigneur et al. 2012). The phylogenetic divergence existing among *Tityus* taxa across its distributional range in South America, illustrated by the split between *T. serrulatus* and *T. bahiensis* and northern *Tityus* species, is therefore paralleled by functional, structural, and immunological differences among their toxins (Borges et al. 2010a). Although not yet supported by a cladistic analysis, the abrupt split in the toxicity to humans that occurs within the spatial distribution of *Centruroides* (when noxious species, restricted to the

Mexican highlands, are compared with congenerics from other regions) also probably has a phylogenetic basis associated with the landscape history of Central America and the Caribbean (Borges 2015).

The *Isometrus* and *Uroplectes* groups make for interesting comparisons to the *Tityus* group from a toxinological standpoint. As mentioned above, *Parabuthus* species (in the *Uroplectes* group) produce β-NaScTxs, such as birtoxin, structurally and functionally related to North American *Centruroides* toxins (Inceoglu et al. 2001). The Eastern African *Babycurus centrurimorphus* (in the *Isometrus* group) produces toxin Babycurus-1 (Ben Khalifa et al. 1997), in which the 30-residue-long N-terminal sequence is most homologous (57–65%) to putative β-NaScTx RjAa14f from the Cuban *Rhopalurus junceus* (F2YLA3) and the North American Cex9 (*Centruroides exilicauda*; Q68PG6), CsEv5 (*Centruroides sculpturatus*; P58779), and CssIX (*Centruroides suffusus*; ADY17426), also in the β-NaScTx group. Homology of *Parabuthus* and *Babycurus* toxins to North American/Caribbean toxins suggests a common NaScTx ancestor to Caribbean/North American and southern/eastern African scorpions which is not shared by the genus *Tityus*. Importantly, upon the comparison of NaScTxs sequenced worldwide, β-NaScTxs from *Tityus* species cluster apart, together with several NaScTxs that affect inactivation (belonging to the α-class, Tf4 and Tc48a, from *Tityus fasciolatus* and *Tityus obscurus*), from clusters assigned to excitatory and depressant β-NaScTxs from Old World (Africa, Asia, and Europe) and the New World classical β-NaScTxs from scorpions of the genus *Centruroides* (Rodríguez de la Vega and Possani 2005). This indicates that toxins affecting Nav activation are not monophyletic and that New World buthid toxins represent multiple evolutionary lineages. Clearly, the analysis of more toxins from Afrotropical genera should shed light on the evolutionary relationships between South/North American and African buthid scorpions.

Fet et al. (2005) provide a biogeographical explanation for the generation of such toxin diversity between Old and New World scorpion peptides, as the result of the arms race between scorpions and predators. Mammal-specific NaScTxs, which constitute the most abundant components of venoms from extant, noxious Old World taxa, could have evolved during aridification of the Palearctic in the Tertiary period, when small burrowing mammals (mostly rodents) radiated into arid landscapes. In addition to being important nocturnal predators (Rowe et al. 2013), an increase in the number of rodents would have facilitated direct competition for space (burrows). Such a scenario explains the emergence of specific mammal-targeting toxins (used for defense, not for foraging) in predominantly burrow-living Palearctic buthids, as opposed to largely vegetation-inhabiting New World buthids such as *Tityus* and *Centruroides* species. The deserts of the New World are much younger, and competition with other arid-adapted scorpions – such as Bothriuridae, Caraboctonidae, and Vaejovidae – could have prevented New World taxa from diversifying as much as the Old World species. Venom, as it evolved, appears to have changed to serve a more of a defensive function in Old World buthids.

The Hemiscorpiidae

Hemiscorpius lepturus (family Hemiscorpiidae) is the sole member of order Scorpiones with venom that can cause severe and fatal hemolysis, secondary renal failure, deep and necrotic ulcers, ankylosis of the joints, thrombosis, and cardiovascular failure. These symptoms are clearly divergent from the typical neurotoxic/cardiotoxic buthid envenomation, resembling envenomations by spiders in genera *Loxosceles* and *Sicarius* (Araneae: Sicariidae) (Jalali et al. 2010). Biochemical characterization has indicated that neurotoxic components of *H. lepturus* venom are minor constituents, including Hemicalcin (a 33-mer peptide active on ryanodine-sensitive calcium channels representing 0.6% of the total protein content) and Hemitoxin (a 35-mer KScTx, representing 0.1% of the total protein). On the contrary, Heminecrolysin, a 33-kDa SMaseD (sphingomyelin D-degrading enzyme), is the main protein responsible for the pathological effects observed following *H. lepturus* envenomation. Although the SMaseD activity of Heminecrolysin is low compared to *Loxosceles* dermonecrotic enzymes, its lysophospholipase D catalytic efficiency is up to three orders of magnitude greater than spider SMaseDs, which explains the strong hemolytic capacity of *H. lepturus* venom (Borchani et al. 2013). Based on de novo sequencing, Heminecrolysin shares only limited sequence homology with *Loxosceles* SMaseDs, but its partial primary structure precludes any comparison with enzymes from other taxa, including ticks and bacteria (Borchani et al. 2011). Enzyme activities have been reported in venoms from several scorpion families: hyaluronidases and phospholipases (in families Buthidae, Scorpionidae, Caraboctonidae, Chactidae, and Liochelidae) and Zn^{+2}-dependent metalloproteases (e.g., antarease), only from buthids (Rodríguez de la Vega et al. 2013). Scorpion SMaseD activity, however, appears to be restricted to Hemiscorpiidae as it has only been reported from *H. lepturus*.

Scorpions in the family Hemiscorpiidae comprise a divergent group of species ranging from Somalia to Iran and Pakistan. Hemiscorpiids are a sister group to Hormuridae from which are thought to have diverged as a result of climatic changes in Gondwana during the Permian global warming (290–250 Myr) (Monod and Lourenço 2005). The genus comprises 14 species, but only *H. lepturus*, which is particularly abundant in southwestern Iran (Khuzestan province) is considered dangerous and potentially lethal. Iranian *Hemiscorpius* species are morphologically very similar and difficult to distinguish, so it is probable that *H. lepturus* is not the only species accountable for all severe envenomations. The fact that the African congenerics do not show the extreme sexual dimorphism and cytotoxic venom of *H. lepturus* indicates that Iranian species have probably been separated from the other hemiscorpiids for a considerable length of time (Monod and Lourenço 2005). The venom evolution (defensive?) strategy in *Hemiscorpius*, also an arid-adapted genus, clearly diverges from that of burrow-living Palearctic buthids, a probable result of a quite different arms race. Modern phylogenetic analyses will no doubt elucidate the enigmatic position of this genus from both evolutionary and toxinological standpoints in the near future.

Conclusion and Future Directions

In summary, venom capable of severe/lethal scorpionism is rare among scorpions, currently known from only about 2% of the 2,140 extant species. All but 1 of the 45 medically significant species are members of Buthidae, the largest and most widely distributed of the scorpion families, found in a variety of Old World and New World habitats. The presented phylogenetic analysis of Buthidae (Fig. 2), based on mitochondrial sequence data, is largely congruent with morphology-based cladistic analyses and suggests that venoms with the potential to produce severe/lethal scorpionism evolved multiple times in both hemispheres. Furthermore, if there is a phylogenetic signal to venom composition, then the following species, which are currently not considered to be medically significant, are predicted to also possess particularly dangerous venoms: *Mesobuthus cyprius*, *Buthus ibericus*, *Centruroides nigrovariatus*, *C. nigrescens*, *Tityus nematochirus*, *T. pococki*, *T. sanarensis*, *T. funestus*, *T. boconoensis*, and *T. pittieri*.

Structural and functional divergence of the toxins from one species to the next, or even among populations of the same species, suggest that venoms are sensitive to selective pressures among different environments. Multiple lines of evidence suggest that aridification-induced radiations of rodent predators could have been a primary selective pressure driving venom evolution in scorpions. In addition, rodent radiations could also be responsible for the emergence of noxious toxins, as mammalian toxicity of scorpion venoms is directly related to mammal-specific NaScTxs content. Although natural selection has been most extensively studied in snake and gastropod venoms, scorpions are also proposed as an ideal model system for venom research. Medically significant scorpion species are clearly the result of many independent evolutionary events that lead to venom capable of severe/lethal scorpionism. Additional sampling and novel tools from genomics could usher in a new era of discovery for scorpions of medical importance.

Cross-References

► Scorpion Venom Gland Transcriptomics and Proteomics: An Overview

References

Abbas N, Rosso J, Céard B, et al. Characterization of three “Birtoxin-like” toxins from the *Androctonus amoreuxi* scorpion venom. Peptides. 2011;32:911–9.

Amitai Y. Scorpions. In: Brent J, Wallace KL, Burkhart KK, Phillips SD, Donovan JW, editors. Critical care toxicology: diagnosis and management of the critically poisoned patient. Maryland Heights: Elsevier Mosby; 2005. p. 1213–20.

Ben Khalifa R, Stankiewicz M, Pelhate M, et al. Action of babycurus-toxin 1 from the east African scorpion *Babycurus centrurimorphus* on the isolated cockroach giant axon. Toxicon. 1997;35:1069–80.

Bergeron ZL, Bingham J. Scorpion toxins specific for potassium (K^+) channels: a historical overview of peptide bioengineering. Toxins (Basel). 2012;4:1082–119.

Bergman NJ. Clinical description of *Parabuthus transvaalicus* scorpionism in Zimbabwe. Toxicon. 1997;35:759–71.

Borchani L, Sassi A, Shahbazzadeh D, et al. Heminecrolysin, the first hemolytic dermonecrotic toxin purified from scorpion venom. Toxicon. 2011;58:130–9.

Borchani L, Sassi A, Ben Gharsa H, et al. The pathological effects of Heminecrolysin, a dermonecrotic toxin from *Hemiscorpius lepturus* scorpion venom are mediated through its lysophospholipase D activity. Toxicon. 2013;68:30–9.

Borges A. Scorpionism and dangerous scorpions in Central America and the Caribbean area. In: Gopalakrishnan P, Rodriguez de la Vega R, Schwartz EF, Possani LD, editors. Handbook of toxinology: scorpion venoms. Dordrecht: Springer Science + Business Media; 2015. p. 215–44.

Borges A, De Sousa L. Escorpionismo en Venezuela: Una aproximación molecular, inmunológica y epidemiológica para su estudio. Rev Fac Farm (Caracas). 2006;69:15–27.

Borges A, Alfonzo MJ, García CC, Winand NJ, Leipold E, Heinemann SH. Isolation, molecular cloning and functional characterization of a novel beta-toxin from the Venezuelan scorpion, *Tityus zulianus*. Toxicon. 2004;43:671–84.

Borges A, Bermingham E, Herrera N, Alfonzo MJ, Sanjur OI. Molecular systematics of the neotropical scorpion genus *Tityus* (Buthidae): the historical biogeography and venom antigenic diversity of toxic Venezuelan species. Toxicon. 2010a;55:436–54.

Borges A, Rojas-Runjaic FJM, Diez N, Faks JG, Op den Camp HJM, De Sousa L. Envenomation by the scorpion *Tityus breweri* in the Guayana Shield, Venezuela: report of a case, efficacy and reactivity of antivenom, and proposal for a toxinological partitioning of the Venezuelan scorpion fauna. Wilderness Environ Med. 2010b;21:282–90.

Borges A, Miranda RJ, Pascale JM. Scorpionism in Central America, with special reference to the case of Panama. J Venom Anim Toxins Incl Trop Dis. 2012;18:130–43.

Casewell NR, Wüster W, Vonk FJ, Harrison RA, Fry BG. Complex cocktails: the evolutionary novelty of venoms. Trends Ecol Evol. 2012;28:219–29.

Chávez-Haro AL, Ortiz E. Scorpionism and dangerous species around the world: Mexico. In: Schwartz EF, Rodríguez de la Vega RC, Possani LD, editors. Handbook of toxinology – scorpion venoms. Berlin: Springer; 2014.

Chippaux JP, Goyffon M. Epidemiology of scorpionism: a global appraisal. Acta Trop. 2008;107:71–9.

Coronas F, Diego-García E, Restano-Cassulini R, de Roodt AR, Possani LD. Biochemical and physiological characterization of a new Na^+−channel specific peptide from the venom of the Argentinean scorpion *Tityus trivittatus*. Peptides. 2014. doi:10.1016/j.peptides.2014.05.002.

de Roodt AR. Comments on environmental and sanitary aspects of the scorpionism by *Tityus trivittatus* in Buenos Aires City, Argentina. Toxins. 2014;6:1434–52.

de Roodt A, Lago N, Salomón O, et al. A new venomous scorpion responsible for severe envenomation in Argentina: *Tityus confluens*. Toxicon. 2009;53:1–8.

De Sousa L, Boadas J, Kiriakos D, et al. Scorpionism due to *Tityus neoespartanus* (Scorpiones, Buthidae) in Margarita Island, northeastern Venezuela. Rev Soc Bras Med Trop. 2007;40:681–5.

Dong K, Du Y, Rinkevich F, et al. Molecular biology of insect sodium channels and pyrethroid resistance. Insect Biochem Mol Biol. 2014;50:1–17.

Dyason K, Brandt W, Prendini L, et al. Determination of species-specific components in the venom of *Parabuthus* scorpions from southern Africa using matrix-assisted desorption time-of-flight mass spectrometry. Rapid Commun Mass Spectrom. 2002;16:768–73.

Fet V, Soleglad M, Lowe G. A new trichobothrial character for the high-level systematics of Buthoidea (Scorpiones: Buthida). Euscorpius. 2005;23:1–40.

Gilles N, Chen H, Wilson H, et al. Scorpion alpha and alpha-like toxins differentially interact with sodium channels in mammalian CNS and periphery. Eur J Neurosci. 2000;12:2823–32.

Gordon D, Ilan N, Zilberberg N, et al. An 'Old World' scorpion β-toxin that recognizes both insect and mammalian sodium channels: a possible link towards diversification of β-toxins. Eur J Biochem. 2003;270:2663–70.

Guerrero-Vargas JA, Mourao CBF, Quintero-Hernández V, Possani LD, Schwartz EF. Identification and phylogenetic analysis of *Tityus pachyurus* and *Tityus obscurus* novel putative Na^+ channel scorpion toxins. PLoS One. 2012;7:e30478.

Gurevitz M, Gordon D, Barzilai MG et al. Molecular Description of Scorpion Toxin Interaction with Voltage-Gated sodium Channels. In: Gopalakrishnakone P, Ferroni Schwartz E, Possani LD, Rodriguez de la Vega RV, editors. Handnook of Toxinology – Scorpion Venoms. Berlin: Springer; 2014.

He Y, Zhao R, Di Z, et al. Molecular diversity of Chaerilidae venom peptides reveals the dynamic evolution of scorpion venom components from Buthidae to non-Buthidae. J Proteomics. 2013;89:1–14.

Inceoglu B, Lango J, Wu J, Hawkins P, Southern J, Hammock BD. Isolation and characterization of a novel type of neurotoxic peptide from the venom of the South African scorpion *Parabuthus transvaalicus* (Buthidae). Eur J Biochem. 2001;268:5407–13.

Jalali A, Pipelzadeh MH, Sayedian R, Rowan EG. A review of epidemiological, clinical and in vitro physiological studies of envenomation by the scorpion *Hemiscorpius lepturus* (Hemiscorpiidae) in Iran. Toxicon. 2010;55:173–9.

Kamenz C, Prendini L. An atlas of book lung ultrastructure in the order Scorpiones (Arachnida). Bull Am Mus Nat Hist. 2008;316:1–259.

Leipold E, Hansel A, Borges A, Heinemann SH. Subtype specificity of scorpion beta-toxin Tz1 interaction with voltage-gated sodium channels is determined by the pore loop of domain 3. Mol Pharmacol. 2006;70:340–7.

Leipold E, De Bie H, Zorn S, et al. μO-conotoxins inhibit NaV channels by interfering with their voltage sensors in domain-2. Channels. 2007;1:e1–9.

Loret E, Hammock B. Structure and neurotoxicity of venoms. In: Brownell P, Polis G, editors. Scorpion biology and research. Oxford: Oxford University Press; 2001. p. 204–20.

Lourenço WR. The biogeography of scorpions. Revue Suisse de Zoologie. 1996;hors. série:437–48.

Lourenço WR. Additions á la faune de scorpions néotropicaux (Arachnida). Rev Suisse Zool. 1997;104:587–604.

Lourenço WR. Synopsis of the Colombian species of *Tityus* Koch (Chelicerata, Scorpiones, Buthidae), with descriptions of three new species. J Nat Hist. 2000;34:449–61.

Ma Y, He Y, Zhao R, Wu Y, Li W, Cao Z. Extreme diversity of scorpion venom peptides and proteins revealed by transcriptomic analysis: implication for proteome evolution of scorpion venom arsenal. J Proteomics. 2012;75:1563–76.

Monod L, Lourenço WR. Hemiscorpiidae (Scorpiones) from Iran, with descriptions of two new species and notes on biogeography and phylogenetic relationships. Rev Suisse Zool. 2005;112:869–941.

Müller G, Modler H, Wium C, Veale D, van Zyl J. *Parabuthus granulatus* identified as the most venomous scorpion in South Africa: motivation for the development of a new antivenom. Clin Toxicol. 2011;49:226.

Murgia AR, Batista CV, Prestipino G, Possani LD. Amino acid sequence and function of a new alpha-toxin from the Amazonian scorpion *Tityus cambridgei*. Toxicon. 2004;43:737–40.

Nenilin AB, Fet V. Zoogeographical analysis of the world scorpion fauna (Arachnida, Scorpiones). Arthropoda Selecta. 1992;1:3–31.

Olivera BM. *Conus* peptides: biodiversity-based discovery and exogenomics. J Biol Chem. 2006;281:31173–7.

Otero R, Navío E, Céspedes FA, et al. Scorpion envenoming in two regions of Colombia: clinical, epidemiological and therapeutic aspects. Trans R Soc Trop Med Hyg. 2004;98:742–50.

Pardal PP, Ishikawa EA, Vieira JL, et al. Clinical aspects of envenomation caused by *Tityus obscurus* (Gervais, 1843) in two distinct regions of Para state, Brazilian Amazon basin: a prospective case series. J Venom Anim Toxins Incl Trop Dis. 2014;20:3.

Peigneur S, Sevcik C, Tytgat J, Castillo C, D'Suze G. Subtype specificity interaction of bactridines with mammalian, insect and bacterial sodium channels under voltage clamp conditions. FEBS J. 2012;279:4025–38.

Prendini L, Wheeler WC. Scorpion higher phylogeny and classification, taxonomic anarchy, and standards for peer review in online publishing. Cladistics. 2005;21:446–94.

Prendini L, Volschenk ES, Maaliki S, Gromov AV. A living fossil from central Asia: The morphology of *Pseudochactas Ovchinnikovi* Gromov, 1998 (Scorpiones: Pseudochactidae), with comments on its phylogenetic position. Zool Anz. 2006;245:211–248

Pucca MB, Neves Oliveira F, Schwartz EF, Arantes EC, Lira-da-Silva RM. Scorpionism and dangerous species of Brazil. In: Gopalakrishnakone P, Ferroni Schwartz E, Possani LD, Rodriguez de la Vega RV, editors. Handbook of Toxinology – Scorpion Venoms. Berlin: Springer; 2014.

Rodríguez de la Vega RC, Possani LD. Overview of scorpion toxins specific for Na^+ channels and related peptides: biodiversity, structure-function relationships and evolution. Toxicon. 2005;46:831–44.

Rodríguez de la Vega RC, Vidal N, Possani LD. Scorpion peptides. In: Abba K, editor. Handbook of biologically active peptides. 2nd ed. Boston: Academic; 2013. p. 423–9.

Rowe AH, Xiao Y, Rowe MP, Cummins TR, Zakon HH. Voltage-gated sodium channel in grasshopper mice defends against bark scorpion toxin. Science. 2013;342:441–6.

Ruiming Z, Yibao M, Yawen H, et al. Comparative venom gland transcriptome analysis of the scorpion *Lychas mucronatus* reveals intraspecific toxic gene diversity and new venomous components. BMC Genomics. 2010;11:452.

Sharma PP, Wheeler WC. Cross-bracing uncalibrated nodes in molecular dating improves congruence of fossil and molecular age estimates. Front Zool. 2014;11:57.

Skolnik AB, Ewald MB. Pediatric scorpion envenomation in the United States: morbidity, mortality, and therapeutic innovations. Pediatr Emerg Care. 2013;29:98–103.

Soleglad ME, Fet V. High-level systematics and phylogeny of the extant scorpions (Scorpiones: Orthosterni). Euscorpius. 2003;11:1–175.

Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics. 2006;22:2688–90.

Strong PN, Mukherje S, Shah N, Chowdhary A, Jeyaseelan K. The Indian red scorpion. In: Schwartz EF, Rodríguez de la Vega RC, Possani LD, editors. Handbook of toxinology – scorpion venom. Berlin: Springer; 2014.

Sunagar K, Undheim E, Chan A, et al. Evolution stings: the origin and diversification of scorpion toxin peptide scaffolds. Toxins (Basel). 2013;5:2456–87.

Valdez-Cruz NA, Batista CVF, Zamudio FZ, Bosmans F, Tytgat J, Possani LD. Phaiodotoxin, a novel structural class of insect-toxin isolated from the venom of the Mexican scorpion *Anuroctonus phaiodactylus*. Eur J Biochem. 2004;271:4753–61.

Vasconcelos F, Lanchote VL, Bendhack LM, Giglio JR, Sampaio SV, Arantes EC. Effects of voltage-gated Na^+ channel toxins from *Tityus serrulatus* venom on rat arterial blood pressure and plasma catecholamines. Comp Biochem Physiol C. 2005;141:85–92.

Volschenk ES, Mattoni CI, Prendini L. Comparative anatomy of the mesosomal organs of scorpions (Chelicerata, Scorpiones), with implications for the phylogeny of the order. Zool J Linn Soc. 2008;154:651–75.

Zhu S, Gao B. Molecular characterization of a new scorpion venom lipolysis activating peptide: evidence for disulfide bridge-mediated functional switch of peptides. FEBS Lett. 2006a;580:6825–36.

Zhu S, Gao B. Molecular characterization of a possible progenitor sodium channel toxin from the Old World scorpion *Mesobuthus martensii*. FEBS Lett. 2006b;580:5979–87.

Zhu S, Bosmans F, Tytgat J. Adaptive evolution of scorpion sodium channel toxins. J Mol Evol. 2004;8:145–153.