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Updating knowledge on new medically important scorpion species in Mexico

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ABSTRACT

The increment in the number of scorpion envenoming cases in Mexico is mainly associated to the rapid growth of the urban areas, and consequently, to the invasion of natural habitats of these arachnids. On the other hand, there is a great diversity of scorpion species, so it is indispensable to identify those of medical importance, which we now know are many more than the 7–8 previously reported as dangerous to humans. Because different LD_{50} values have been reported for the venom of the same species, probably due to variations in the experimental conditions used, in this work we determined the LD_{50} values for the venoms of 13 different species of scorpions using simple but systematic procedures. This information constitutes a referent on the level of toxicity of medically important scorpion species from Mexico and establishes the bases for a more comprehensive assessment of the neutralizing capacity of current and developing antivenoms.

1. Introduction

Mexico, is one of the countries with the greatest diversity of scorpion species (Santibáñez-López et al., 2015). Although about 280 species have been reported, this number may increase since new species continue to be described. A study of the diversity of venomous species is in itself an important source of information on the components of venoms related to their therapeutic potential (antimicrobial peptides, analgesics, immune response inhibitors and some anticancer drugs among others) (Ortiz et al., 2015; Rodríguez de la Vega et al., 2010). From the medical point of view, it is important to identify the toxic components that affect mammalian sodium channels (Rodríguez De La Vega and Possani, 2005).

In Mexico, it is estimated that approximately 300,000 envenomations occur per year, some of which lead to loss of human lives (Secretaria de Salud, 2016). In 1995, 285 deaths were reported, while 49 deaths were reported in the year 2012. Fortunately, the number of deaths has followed a downward trend due to the timely use of the antivenom. The Mexican states with the highest number of stings are Guanajuato, Guerrero, Morelos, Jalisco and Michoacán, where several dangerous species of scorpions coexist with humans (Table 1). Table 1 shows the number of scorpion stings reported in each entity by the Ministry of Health of the Mexican government in the year 2016 (Secretaria de Salud, 2016). All dangerous scorpions to humans belong to the genus *Centruroides*, hereafter abbreviated *C*.

Currently the treatment against scorpion sting in Mexico, consists of the application of a polyvalent F (ab)-antivenom that is produced from the hyperimmunization of horses with the venom of four dangerous species of scorpions (Espino-Solis et al., 2009). The term “Fab-therapeutics” is related to the pepsin processing of the immunoglobulins present in the plasma of the immunized horses giving as products the F(ab') 2 fragment and the Fc fragment which is removed from the preparation. Although this antivenom is very efficient, it continues to be of equine origin and since the venoms contain up to 70 different components, this Fab-antivenom contains antibodies directed against all these components and the successful production of good antivenom will depend on the

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Table 1
Report of the incidence of *Centruroides* scorpion stings in some states of the Mexican Republic during 2016.

State	Species dangerous to humans	Reported envenomations
Aguascalientes	*C. infamatus*	1931
Colima	*C. elegans, C. hirsutipalpus, C. infamatus, C. tecomanus*	12 318
Durango	*C. infamatus, C. suffusus*	8364
Guanajuato	*C. infamatus, C. ornatus, C. suffusus*	42 989
Guerrero	*C. balsasensis, C. elegans, C. limpidus, C. meisei, C. villegasi*	37 730
Jalisco	*C. elegans, C. infamatus, C. ornatus, C. tecomanus*	50 426
México	*C. balsasensis, C. limpidus*	10 962
Michoacán	*C. balsasensis, C. infamatus, C. ornatus, C. tecomanus, C. limpidus*	31 012
Morelos	*C. balsasensis, C. limpidus*	29 161
Nayarit	*C. infamatus, C. noxius, C. ornatus, C. suffusus*	14 003
Oaxaca	*C. limpidus, Centruroides* sp. *nov.* "A" (Huajuapan)	4664
Puebla	*C. balsasensis, C. limpidus, Centruroides* sp. *nov.* "A" (Huajuapan)	13 275
Querétaro	*C. infamatus, C. limpidus*	4792
Sinaloa	*C. infamatus, C. noxius, C. suffusus*	11 346
Sonora	*C. sculpturatus, Centruroides* sp. *nov.* "B" (Cumpas)	4929
Zacatecas	*C. infamatus, C. suffussus*	4447

Only states with more than 1000 cases are included as well as the corresponding scorpion species.

corresponding immune response of the horses immunized with the venom mixture. Alternatively, it is possible to identify the medically important toxins of the venoms from poisonous scorpions and to develop recombinant antibody fragments of human origin capable of neutralizing those toxins. The set of antibody fragments being capable of neutralizing the venoms of the medically important scorpion species would form part of a recombinant antivenom of human origin. Using phage displayed libraries of scFv fragments and in vitro maturation of the best isolated scFvs we have generated several antibody fragments from human origin which are capable of neutralizing the main toxins of several venoms and the whole venom of some species (Riaño-Umbarila et al., 2013, 2011, 2005; Rodríguez-Rodríguez et al., 2016). However, we need to complete the set of neutralizing scFvs in order to cover the neutralization of all venomous species. With the purpose of reaching this goal, we need to know how many toxic species there are in Mexico and how many lethal toxins are in each venom. As the next step towards that goal, in this contribution we determined the LD_{50} values for the venoms of 13 different species of scorpions using simple but systematic procedures.

Although several studies have reported taxonomic descriptions for toxic scorpions, many of them lack the determination of lethal dose 50 (LD_{50}) and no mention is made on the composition of the venom. In the cases where the LD_{50} has been determined, there is a significant variation in this parameter for the same species. For example, in the case of *C. limpidus*, doses between 21 μg and 66 μg/20 g of mouse have been reported (Alagón et al., 1988; Padilla et al., 2003). This is probably due to the use of different strains of mice and/or to the quality of the venom (fresh or lyophilized venom, storage time and temperature, etc.). In addition, depending on the source of the venom (scorpions collected in the field and milked immediately or kept in captivity and milked afterwards), the quality of the venom can change. We have observed a decrease in toxicity. Additionally, large variations were detected in the composition of *Tityus serrulatus* scorpion venom, depending on the diet used to feed them (Pucca et al., 2014). For these reasons, it has been difficult to determine which of the species are the most toxic. To cope with this limitation, groups of scorpions, mainly adults from each of the species included in this study, were collected. The scorpions were taxonomically identified, photographed and later used for the extraction and evaluation of the fresh venom (immediately after being extracted). Determination and/or confirmation of the LD_{50} were done using mice of the CD1 strain. This is the first time that the toxicity of this number of species is compared using fresh venom with the exception of *C. sculpturatus* where venom had already been lyophilized. Although the available amount of *C. balsasensis* venom was not enough to determine the LD_{50}, the lethality was confirmed in mice by using 15 μg/20 g of mouse. This work is important to be aware of the demanding challenge which implies the generation of new recombinant antivenom of wide spectrum with the capacity of neutralizing all the venoms from the dangerous species of Mexican scorpions.

2. Materials and Methods

2.1. Scorpion venoms

Groups of scorpions freshly collected, by personnel authorized by SEMARNAT (SGPA/DGVS/06721/16), were milked by electrical stimulation as previously reported (Dent et al., 1980). The amount of venom obtained usually goes from 150 μg to 650 μg, which depends on the species and size of the scorpion. Since electrical stimulation is a harsh treatment, we assume that in natural incidents the scorpions inject a lower amount of venom. The species collected were: *C. noxius* (Pantanal, Nayarit), *C. suffusus* (El Salto, Durango), *C. villegasi* (Tlacoaxtla, Guerrero), *C. meisei* (Acapulco, Guerrero), *C. elegans* (Chamela, Jalisco), *C. hirsutipalpus* (Minatitlán, Colima), *C. ornatus* (Morelia, Michoacan), *C. infamatus* (San Antonio de Padua, Guanajuato), *C. balsasensis* (Churumuco, Michoacán), *C. tecomanus* (Coquimatlán, Colima), *C. sp nov* "A" (Huajuapan, Oaxaca), *C. sp* nov "B" (Cumpas, Sonora), *C. limpidus* (Santa Catarina, Xochitepec, Yautepec in Morelos; Taxco de Alarcón, Santa cruz, Mayanalan, Xochimilco in Guerrero and Acatlán, Puebla). Medically important toxins (Cll1, Cll2, Cll13, Cn2, Css2, Ct1a and Cell9, were separated and purified following the methods already reported (Alagón et al., 1988; Espino-Solis et al., 2011; Ramírez et al., 1994, 1988; Zamudio et al., 1992).

Venom samples from 10 to 12 scorpions were dissolved in water and centrifuged at 12,000 rpm and 4 °C, for 10 min. Insoluble material was discarded, whereas the toxin-containing supernatant was recovered and spectrometrically quantified at $\lambda = 280$ nm (GENESYS 10S UV-VIS spectrophotomer - Madison, WI 53711 USA), assuming that 1 unit of absorbance is equivalent to 1 mg mL^{-1} of protein (Dehesa-Dávila et al., 1996; Olamendi-Portugal et al., 2017). The soluble venom was stored in ice, kept protected from light and used during the same day. Sometimes, we detected a decrease and even a loss of biological activity in only 24 h of storage. Only in the case of *Centruroides sculpturatus,* lyophilized venom was used. This species is located between the border of Mexico and the USA (Sonora and Arizona, respectively). Due to the difficulty of

collecting scorpions in the Sonora desert, we chose to buy lyophilized venom from a company located in the USA. (Spider Pharm).

2.2. Determination of lethal dose 50 (LD_{50}), method "up and down"

The protocol and use of mice was approved by the Animal Welfare Committee of our Institute 290 (PDCPN 2014-01 246924). The minimum doses used to reach death and survival maximums for each venom administered intraperitoneally in CD1-ICR mice were established. Based on this information, it was possible to establish dose intervals between the minimum and the maximum. Following the procedure described (Dixon and Mood, 1948), the dose corresponding to the established intervals was administered and depending on whether the animal survived or died, the dose of

Fig. 1. Photographs of scorpions from the genus *Centruroides* which are toxic to mammals. In each pair of pictures, females are shown at left and males at right. All the scorpions shown were adults. Sizes are not comparable because a different close up was applied to each picture. Scorpions from Guerrero: *C. balsanesis, C. meisei, C. villegasi, and C. limpidus*; scorpions from Colima: *C. hirsutipalpus, C. elegans, C. tecomanus*; *C. noxius* of Nayarit; *C. suffusus* of Durango, *C. infamatus* of Guanajuato, *C. ornatus* of Morelia, *C. sp. nov.* "A" of Oaxaca and *C. sp. nov.* "B" of Sonora.

venom was increased or reduced respectively. In this way, it is possible to minimize the number of mice (between 12 and 15 mice) that allows determination of the LD_{50} for each venom. The results are processed following the proposed statistical treatment (Dixon and Mood, 1948).

2.3. Electrophoretic profiles

Samples of pure toxins and scorpion venoms were evaluated by electrophoresis following the methodology described (Reisfeld et al., 1962) with some modifications. Polyacrylamide gel electrophoresis at pH 3.6 of scorpion venoms was performed using slab minigels (8x7x0.075 cm) instead of rod gels. The separating gel contained 11.64% acrylamide, 0.36% N,N′-methylenebisacrylamide, 0.1% N,N,N′,N′-tetramethyethylenediamine, 0.001% Riboflavin-5′-phosphate, and 0.042 M potassium acetate brought to pH 4.2 with acetic acid. This gel solution was polymerized by exposure to fluorescent light. The stacking gel contained 3.62% acrylamide, 0.13% N,N′-methylene-bisacrylamide, 10% glicerol, 0.1% N,N,N′,N′-tetramethyl-ethylenediamine, 0.001% Riboflavin-5′-phosphate, 0.01% ammonium persulfate and 0.090 M potassium acetate brought to pH 6 with acetic acid. This solution was poured on top of the separating gel and a wells former ("comb") inserted. Exposure to fluorescent light was continued until complete polymerization was observed. The indicated acrylamide monomer concentrations were obtained by appropriate dilution of a stock solution containing 29 g acrylamide and 1 g N,N′-methylene-bisacrylamide in 100 ml distilled water. A buffer containing 0.060 M alanine and 0.1 M acetic acid in both upper and lower reservoirs with the anode into the upper buffer and the cathode in the lower buffer. Samples were mixed with an equal volume (2X) loading buffer containing 25% V/V glycerol, 0.18 M potassium acetate pH 6, 0.01% methyl green. Electrophoresis was carried out at ambient temperature, at constant current of 5 mA in the staking gel and 9 mA in the resolving gel until the tracking dye approaches the lower end of the gel. Gels were stained with 0.05% w/v Coomassie Blue R in 10% v/v methanol, 7% v/v acetic acid for no more of 30 min, and faded in the same solution without dye. One microgram of purified toxins produces well defined, intensely stained bands. It is recommended to load soluble venom samples between 10 μg and 30 μg to observe abundant as well as rare components. Continuous inspection of the gel staining was performed to control the process because some bands clearly visible during the first minutes, can disappear with longer staining times. Due to this time depending results, 30 min staining was established as the recommended staining time to obtain the best results.

3. Results

3.1. Scorpion species

The groups of scorpions brought from different states were analyzed to confirm the identity of the species by observing the main morphological characteristics of the individuals, using the available literature (Quijano-Ravell and Ponce-Saavedra, 2016a; Ponce-Saavedra and Francke, 2014; Santibáñez-López et al., 2015; Santibáñez-López and Contreras-Félix, 2013; Santibáñez-López and Ponce-Saavedra, 2009). Among the discriminatory features stand out: the shape of the pectineal plate, counts of pectineal teeth, the proportion of the length and thickness of the fifth metasomal segment and the size of the subaculear spine of the telson. In the photographs (Fig. 1), it is possible to observe the shape of males and females of the species enlisted in Table 1. Compared with species from other genera, those of the genus *Centruroides* cannot easily be identified with a naked eye. For example, *C. limpidus* (formerly *C. limpidus limpidus*), *C. tecomanus* (formerly *C. limpidus tecomanus*) and *C. balsasensis* (formerly *C. limpidus balsasensis*) due to their great resemblance, had been considered subspecies. Because of the same reason, *C. infamatus* (formerly *C. infamatus infamatus*) and *C. ornatus* (formerly *C. infamatus ornatus*) had been classified as a subspecies. Nowadays, it is known that all these are independent species. Several species described as potentially toxic, are located in unsafe areas in Guerrero and Michoacán, so we have not been able to evaluate them.

3.2. Determination of the lethal dose

Determination of LD_{50} was a meticulous process. Results are summarized in Table 2, where the venoms are shown in descending order of toxicity. In this sense, scorpion with the most toxic venom was the one from Nayarit (*C. noxius*) and the least toxic was the one from Sonora, Mexico and Arizona, USA (*C. sculpturatus*). Within this list, in previous report of our group the LD50 of *C. noxius* and *C. suffusus* had been confirmed several times (Riaño-Umbarila et al., 2016, 2011; Rodríguez-Rodríguez et al., 2016).

3.3. Analysis of the electrophoretic profile of the venoms

The main focus of the study of venoms of medical importance, lies in the identification of the main toxins, which in this genus correspond to β-type toxins that modify the activity of voltage-dependent sodium channels (Restano-Cassulini et al., 2017; Rodríguez De La Vega and Possani, 2005). This process is demanding specially when many and very similar species are being analyzed. The electrophoretic profiles of the venoms can help to discriminate whether the animals collected correspond to the same species.

The advantage of using acid electrophoresis in non-denaturing conditions (see Materials and Methods) is reflected by the ability to separate individual toxins despite corresponding to similar molecular masses. This neat separation of the toxins is impossible to accomplish by means of regular SDS-PAGE. Previously purified toxins characterized by mass spectrometry, migrate differently despite having molecular weights, structure and similar properties (Table 3, Fig. 2 A). In the control, samples of venom from 5 species

Table 2
Determination of the LD_{50} using mice of CD1 strain.

Toxicity Rank	*Centruroides* species	LD_{50}	LD_{50} published	
1	*C. noxius*	2.50[a]	5.2[f]	6.2[e]
2	*C. suffusus*	8.75[a]	8.6[e]	
3	*C. infamatus*	9.6 ± 0.8	25.4[f]	54.0[e]
4	*C. tecomanus*	10.2 ± 0.3	13.0[f]	
5	*C. elegans*	11.5 ± 1.9		
6	*C. hirsutipalpus*	11.7 ± 1.9		
7	*C. villegasi*	12.2 ± 0.9		
8	*C. sp. nov.* "A" (Huajuapan)	13.0 ± 2.0		
9	*C. ornatus*	13.3 ± 1.7		
10	*C. meisei*	14.0 ± 0.9		
11	*C. limpidus*	15.0 ± 0.5[d]	40.0[f]	13.8–100[e]
12	*C. sp. nov.* "B" (Cumpas)	19.2 ± 2.0		
13	*C. sculpturatus*	22.7 ± 0.8[b]	22.4[f]	
ND	*C. balsasensis*	[c]		

The LD_{50} **values** correspond to μg of venoms/20 g of mouse.
[a] Determination previously performed.
[b] Assays performed with lyophilized venom.
[c] Sample containing insufficient venom to make the determination, although toxicity was confirmed.
[d] Determinations for several samples of *C. limpidus* scorpion venom. The list of venoms is shown from higher to lower toxicity.
[e] Data taken from: (Francisco, n.d.).
[f] Data taken from: (Carboney and Jasso Villaazul, 2012).

(30 μg each) and their respective main toxic components (1 μg) are showed. It is possible to locate the toxins in the middle part of the migration profile of the complete venom.

Electrophoretic separation of the venoms in acidic conditions, allowed comparing their profiles. Different patterns are observed for each venom (Fig. 2). It was possible to detect significant differences in the venom profiles of scorpions previously considered subspecies, such as *C. limpidus* and *C. tecomanus* (Ponce-Saavedra et al., 2009), *C. limpidus* and *C. balsasensis*, and between *C. ornatus* and *C. infamatus*, for example (Fig. 2 Gel 2). Since toxins present in the venoms correspond to proteins of low molecular weight, they diffuse quickly into the gel so that staining time must be controlled to prevent band spreading. These aspects may explain some of the variations observed in the intensity of the bands in the control (venom from *C. limpidus*). These observations also show the variability in the venoms of individuals of the same species distributed in the States of Guerrero, Morelos and Puebla. Despite these variations, no other venom is identical to the one of *C. limpidus* and it is possible to observe the position of the main toxins, especially those corresponding to Cll1 toxin, and the close bands in which Cll2 and Cl13 toxins are present.

4. Discussion

As mentioned above, species of the genus *Centruroides* in Mexico are numerous and currently about 45 species are known. In this work, we studied 14 Mexican species that are toxic to mammals, from which 2 of them had not been described previously. Considering the diversity of species studied in this work, as well as recent publications of new species of the genus *Centruroides* (*C. ruana* (Quijano-Ravell and Ponce-Saavedra, 2016a), *C. bonito* (Quijano-Ravell and Ponce-Saavedra, 2016b), *C. huichol* (Teruel et al., 2015)), the number of toxic species is expected to increase. For many of these species, their level of toxicity to mammals, as well as the evaluation of neutralizing capacity of the commercial antivenom or the human antibodies (scFvs) that we are developing (Riaño-Umbarila et al., 2013, 2011; Rodríguez-Rodríguez et al., 2016), are unknown. It is important to note that for a long time only 7 *Centruroides* species were considered toxic to mammals: *C. noxius, C. suffusus, C. limpidus, C. tecomanus, C. elegans, C. infamatus* and *C. balsasensis*.

For the determination of lethal doses 50 it must be considered that there are many factors that affect the toxicity of the venoms. One of them is the manipulation of wild scorpions (captivity, diet, number and frequency of milking, among others). Other factors can be the processing and general storage conditions of the venoms obtained. In order to know some of the determinants of this variability, it was decided to work with freshly collected scorpions and freshly extracted venom. It was also decided to work with the CD1 strain of mice as they show greater sensitivity to scorpion venom than mice strains BALB/C or C57 (data not shown). It was interesting to note that most of fresh venoms showed a lower LD_{50} as compared to those previously reported, except for *C. sculpturatus* and *C. suffusus* which are in the same range (see Table 2). It was determined that the majority of the species of scorpions showed a toxicity between 10 μg and 15 μg/20 g of mouse. *C. noxius* and *C. suffusus* besides being the most toxic species, they are implicated in a significant number of envenomation (Tables 1 and 2). It was also observed that the least toxic species (*C. sculpturatus*) is 10 times less toxic than *C. noxius* (the most toxic species in Mexico), but it should be noticed that lyophilized venom was used. It is important to mention that variations in LD_{50} values can be observed in groups of scorpions of the same species. As an example, *C. limpidus* scorpions collected in different places of the States of Morelos and Guerrero, located at distances of 30 km–70 km apart, show the average lethal dose ranging from 13 μg to 15 μg/20 g of CD1 mouse, whereas for some *C. suffusus* scorpions collected in the suburbs of Durango City (State of Durango) their venom killed 100% of mice using an amount of venom corresponding to 1 LD_{50} previously determined. These results correlate with observations reported by other authors for scorpions such as *Tityus serrulatus* collected in two Brazilian states (Oliveira et al., 2013) and for venom from *C. edwarsii* (Estrada-Gómez et al., 2014) in Colombia as well as the variation in electrophoretic profiles of *C. limpidus* venom collected at different locations (Fig. 2).

The diversity of the components of the venoms is reflected in the different electrophoretic patterns. Although there are some similar components present in the different venom species, each venom exhibit different electrophoretic but in general consistent patterns. It is widely accepted that the best way of distinguishing differences in venom composition of species of scorpions is HPLC separation followed by molecular weight determination by mass spectrometry, as usually done by our group (Alagón et al., 1988; Dent et al., 1980; Olamendi-Portugal et al., 2017; Zamudio et al., 1992). However, this procedure is time consuming and requires hard work like collecting individual fractions, quantification and loading each sample into the mass spectrometer. We are aware that the discriminatory power of gel electrophoresis in normal conditions, is not the best manner to perform the identification of species by biochemical methods. However, when a quick method needs to be used, in our opinion the gel electrophoresis system described here is adequate and satisfies the expectancy. This can be clearly seeing in Fig. 2. Using this methodology allows prompt identification of small differences in the venom composition of similar species of scorpions belonging to the same genus. These results highlight the complexity of the venoms, reflecting that the experimental strategy here used represents a simple tool that allows discerning in cases of difficult identification of the species. However, the separation of the components of the venoms and their individual analysis remains an inevitable process to achieve a

Table 3
Summary of major toxic components identified in venoms of Mexican scorpions.

Toxin	Sequence	MW Da	Pi	Ref
Ct1a	KEGYLVNHSTGCKYECFKLGDNDYCLRECRQQYGKGAGGYCYAFGCWCTHLYEQAVVWPLPKKTCN	7590.6	8.16	(Ramírez et al., 1988)
CeII9	KEGYLVNHSTGCKYECFKLGDNDYCLRECRQKYGKGAGGYCYAFGCWCTHLYEQAVVWPLPKKTCN	7590.7	8.44	(Vandendriessche et al., 2010)
Cll2	KEGYLVNHSTGCKYECFKLGDNDYCLRECKQQYGKGAGGYCYAFGCWCNHLYEQAVVWPLPKKTCN	7575.6	8.15	(Dehesa-Dávila et al., 1996)
Cll1	KEGYIVNLSTGCKYECYKLGDNDYCLRECKQQYGKGAGGYCYAFGCWCTHLYEQAVVWPLPKKTCT	7541.6	8.15	(Ramírez et al., 1994)
Cii1	KEGYLVNHSTGCKYECYKLGDNDYCLRECKQQYGKGAGGYCYAFGCWCTHLYEQAVVWPLPKKTCN	7578.6	8.15	(Dehesa-Dávila et al., 1996)
Css2	KEGYLVSKSTGCKYECLKLGDNDYCLRECKQQYGKSSGGYCYAFACWCTHLYEQAVVWPLPNKTCN	7538.6	8.15	(Martin et al., 1987)
Cn2	KEGYLVDKNTGCKYECLKLGDNDYCLRECKQQYGKGAGGYCYAFACWCTHLYEQAIVWPLPNKRCS	7589.7	8.15	(Zamudio et al., 1992)
Cl13	KEGYLVDYHTGCKYTCAKLGDNDYCVRECRLRYYQSAHGYCYAFACWCTHLYEQAVVRPLPNKRCR	7845.9	8.66	(Olamendi-Portugal et al., 2017)

Description of the primary sequences, molecular weights and theoretical isoelectric points of the main toxins from the scorpion venoms studied in this work.

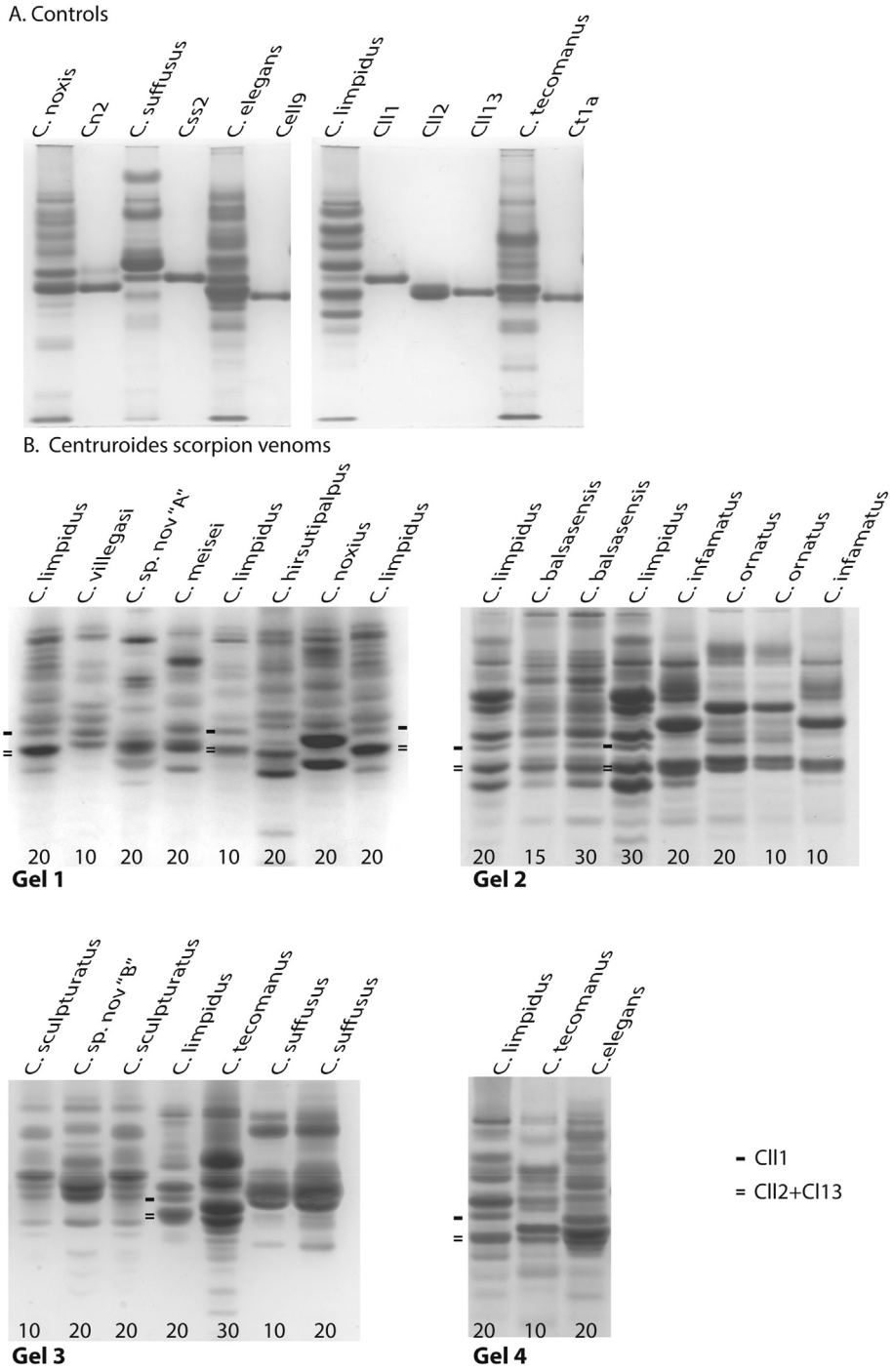

Fig. 2. Electrophoretic profiles of pure toxins and whole venoms under acidic conditions. A) Profiles of purified toxins and their respective venoms, where approximately 1 μg of toxins and 30 μg of each soluble venom were loaded. *C. limpidus* scorpions were collected in Morelos State. B) Profiles of the components of the venoms. The quantity of venoms (μg) are indicated in each lane. In all cases *C. limpidus* venom was used as a standard reference in which the location of the three main toxic components are indicated. Gel 1, *C. limpidus* from Acatlán, Puebla; Gels 2 and 4, *C. limpidus* from Guerrero; Gel 3, *C. limpidus* from Yautepec, Morelos.

precise characterization. From the scorpion species that have been characterized in depth and defined as medically important are: *C. noxius*: toxins Cn2 and Cn8 (Dent et al., 1980; Schiavon et al., 2012; Zamudio et al., 1992); *C. suffusus*: toxins Css2 and Css4 (Martin et al., 1987), *C. limpidus*: CII1, CII2 and CI13 (Dehesa-Dávila et al., 1996; Olamendi-Portugal et al., 2017; Ramírez et al., 1994) and *C. tecomanus*: Ct1a (Ramírez et al., 1988), whereas important advances have been made with *C. elegans* CeII9 (Vandendriessche et al., 2010), and *C. infamatus*: Cii toxin (Dehesa-Dávila et al., 1996).

5. Conclusions

This work has allowed us to size the biodiversity of dangerous scorpions in Mexico and their relation to the tremendous efforts associated to the characterization of the venoms of all toxic species. This information would let people know which of them are toxic.

The results obtained in this work also help us to establish new priorities for the development of recombinant antivenom of human origin. A more indepth characterization of the venoms that are not yet neutralized by the fragments of antibodies obtained so far (Riaño-Umbarila et al., 2011; Rodríguez-Rodríguez et al., 2016), help us to implement better strategies that will allow to contend with the variability of the toxicity. This work emphasizes the importance of implementing systematized assay strategies that include: the use of the same strain of mice and the use of fresh venoms obtained from freshly collected scorpions avoiding to the maximum a captivity and/or feeding conditions that would be affecting the quality of the venom produced by scorpions maintained under these conditions.

Author contributions

LRU and BB conceived and designed the experiments; LRU, ERRR, LG, SJUR, IVGR, CESL and EDN performed the experiments; LRU, ERRR, LDP and BB analyzed the data and wrote the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.toxicon.2017.08.022.

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