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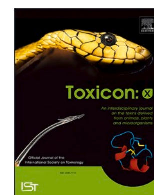

Fast venomic analysis of *Crotalus durissus terrificus* from northeastern Argentina

Luciano S. Fusco[a,*], Emidio B. Neto[b], Aleff F. Francisco[f], Jorge Alfonso[c,d], Andreimar Soares[c,e], Daniel C. Pimenta[b], Laura C. Leiva[a,**]

[a] Laboratorio de Investigación en Proteínas (LabInPro), Instituto de Química Básica y aplicada del Nordeste Argentino (IQUIBA-NEA, CONICET), Universidad Nacional del Nordeste (UNNE), Corrientes, Argentina

[b] Laboratorio de Bioquímica y Biofísica, Instituto Butantan, São Paulo, SP, Brazil

[c] Centro de Estudos de Biomoléculas Aplicadas à Saúde (CEBio), Fundação Oswaldo Cruz (FIOCRUZ), Fiocruz Rondônia, Porto Velho-RO e Universidade Federal de Rondônia (UNIR), Porto Velho, RO, Brazil

[d] Centro Para el Desarrollo de la Investigación Científica (CEDIC), Asunción, Paraguay

[e] Centro Universitário São Lucas (UniSL), Porto Velho, RO, Brazil

[f] Departamento de Física e Biofísica, Instituto de Biociências, São Paulo State University, UNESP, Botucatu, SP, Brazil

A B S T R A C T

The complete knowledge of the toxins that make up venoms is the base for the treatment of snake accidents victims and the selection of specimens for the preparation of venom pools for antivenom production. In this work, we used a fast and direct venomics approach to identify the toxin families in the *C.d. terrificus* venom, a Southern American Neotropical rattlesnake. The RP-HPLC separation profile of pooled venom from adult specimens followed by mass spectrometry analysis revealed that *C.d. terrificus'* venom proteome is composed of 12 protein families, which are unevenly distributed in the venom, e.g., there are few major proteins in the venom's composition phospholipase A_2, serine proteinase, crotamine and L-amino acid oxidase. At the same time, the proteome analysis revealed a small set of proteins with low quantity (less than 1.5%), both enzymes (metaloprotease, phospholipase B and 5′-nucleotidase) and proteins (Bradykinin potentiating and C-type natriuretic peptides, C-type lectin convulxin and nerve growth factor). To sum up, this research is the first venomic report of *C.d.terrificus* venom from Argentina. This proved to be crotamine positive venom that has a lower metalloprotease content than *C.d. terrificus* venoms from other regions. This information could be used in the discovery of future pharmacological agents or targets in antivenom therapy.

1. Introduction

Accidents with *Crotalus durissus terrificus* (*C.d. terrificus*) are an important public health issue in South America (Calvete et al., 2010). In Argentina, envenomation caused by this species is of medical importance due to the lethality of their venom (Garcia Denegri et al., 2019). Such cases are often fatal, when treatment with specific antivenom is not quickly administered (Acosta de Perez et al., 1997; Baudou et al., 2017; de Roodt et al., 1998).

Neurotoxic paralysis is the major clinical effect in envenomation by *C.d.terrificus,* whereas local effects are small or even absent (Rosenfeld, 1971). The envenomation causes neurotoxic, myotoxic, and coagulopathic effects (Acosta de Perez et al., 1997). Regarding systemic myotoxicity, it could lead to acute renal failure, also with tubular necrosis (Amaral et al., 1980; Azevedo-Marques et al., 1985).

In general terms, the venomic of *C.d.terrificus* comprises toxins from different protein families: phospholipases A_2, serine proteinases, 5′nucleotidases, metalloproteinases, nerve growth factors, phosphodiesterases, glutaminylcyclase, C-type lectin, crotamine, L-amino acid oxidase and disintegrins (Boldrini-França et al., 2010; Georgieva et al., 2010; Gutierrez et al., 2009; Wiezel et al., 2018). Additionally, using different bottom-up approaches, Melani and collaborators (2015) provided more information about the proteomic characterization of this venom. They also describe the presence of CRISP, phospholipase-B, snake venom vascular endothelial growth factor (svVEGF) and other proteins involved in the regulation of toxin synthesis and processing (Melani et al., 2015).

Venomic analysis is a tool to evaluate new sources of biologically compounds of therapeutic value (Radha and Kumaran, 2018). It is also important for better understanding the ecological and evolutionary relationships among snake species, and for the preparation of more effective toxin-specific antivenoms. Regarding evolution, some authors

* Corresponding author.

** Corresponding author.

E-mail addresses: flucianosebastian@gmail.com (L.S. Fusco), lauraleiva2004@yahoo.com.ar, lcleiva@exa.unne.edu.ar (L.C. Leiva).

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show the distributional history of the neotropical rattlesnake in South America and the Amazon. The phylogeographic pattern of *C.durissus sp.* is best explained by a Pleistocene trans-Amazonian corridor of continuous distribution: past savanna or dry forest vegetation Adrian Quijada-Mascareñas et al., 2007). *C. d .terrificus* is a subspecies that it is found only in South America and its geographical distribution is shown in Fig. 1 (http://www.reptile-database.org/).

C.d. terrificus whole venom as well as some isolated toxins (PLA_2, SVSP) from specimens that inhabit in Argentina, were further studied with respect to the toxicological properties (Acosta de Perez et al., 1997; Fusco et al., 2015a, 2015b; Marunak et al., 2004; Rodríguez et al., 2006, 2009, 2012; Ruiz de Torrent et al., 2007), but no previous proteomic reports have been found. Knowledge about the toxins from venoms is the basis for the treatment of victims, and the selection of specimens for the preparation of venom pools for antivenom production (Gutierrez et al., 2009). Here we used a fast and direct venomics approach (Calvete et al., 2007, 2009; Gutierrez et al., 2009) to identify the toxin families in the *C. d. terrificus* rattlesnake.

2. Material and methods

2.1. Venom

Crotalus d. terrificus venom was pooled from 25 specimens of adult snakes held in the serpentarium of the local Zoo (CEPSAN), Corrientes, Argentina. The venom was lyophilized and kept frozen at −20 °C.

2.2. Reverse phase (RP) HPLC

Five mg of *C.d. terrificus* venom were dissolved in 200 mL of solvent A

Fig. 1. C.d. *terrificus* distribution in South America (green area), reptile area follows the electronic database accessible at http://www.reptile-database.org accessed on 14 May 2020. Symbols indicate the approximate regions of origin of the snakes sampled in previous and present work: ■;-Wiezel et al. (2018),•- Melani et al. (2015), ▲- Boldrini-França et al. (2010), O – Georgieva et al. (2010), Φ Present work.

(0.1% (v/v) trifluoroacetic acid; TFA). The resulting solution was clarified by centrifugation at 5000×*g* for 5 min and the supernatant was applied to a Discovery C18 column (4.6 mm × 25 cm; Akta Purifier system). Proteins were eluted with a gradient (0–20%, 20–60%, 60–100%) of 66% (v/v) acetonitrile in solvent B, at a flow rate of 1 mL/min. The elution profile was monitored at 215 nm and the fractions were collected, dried in speed vac and stored at −20 °C. Values of area under the curve were used to estimate the relative abundance of each protein family (%).

2.2.1. Protein mass determination by mass spectrometry

The dried samples from RP-HPLC fractions were re-dissolved (in 0.1% acetic acid) and analyzed using a matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometer (Axima Performance, Shimadzu). One microliter of each sample was co-crystallized with 1 μL of sinapic acid matrix (saturated solution prepared in 50% ACN/0.1% acetic acid) in the plate and dried at room temperature. The mass spectrum was obtained in the 50–50,000 mass/charge (m/z) range, in linear positive mode.

2.2.2. Protein identification by proteomic analysis

The samples from RP-HPLC fractions were buffered in ammonium bicarbonate 50 mM, pH7), and then dithiothreitol (10 mM final concentration) was added and left for 1 h at 60 °C; after that, iodoacetamide (IAA) (10 mM final concentration) was added and incubated for 1 h, at room temperature and protected from light. Next, 10 μL of trypsin (40 ng μL^{-1} in 100 mMTris-HCl, pH 8.5) was added. The incubation was performed overnight, at room temperature. The reaction was stopped by adding 50% ACN/5% TFA.

The samples were then analyzed by liquid chromatography-mass spectrometry using an ESI-IT-TOF (LCMS-IT-TOF/Shimadzu Co., Kyoto, Japan) system coupled to a binary ultra-fast liquid chromatography system (UFLC) (20A Prominence, Shimadzu). Each sample was loaded in a C18 column (Discovery C18, 5 μm; 50 × 2.1 mm) in a binary solvent system: (A) water: acetic acid (999:1, v:v) and (B) ACN: water: acetic acid (900:99:1, v:v:v). The column was eluted at a constant flow rate of 0.2 mL min^{-1} with a 0–40% gradient of Solvent B over 35 min. The eluates were monitored by a Shimadzu SPD-M20A PDA detector before checking on the mass spectrometer. The interface voltage was 4.5 kV; the capillary voltage was 1.85 kV, at 200 °C; and the fragmentation was induced by argon collision, at 55% 'energy'. MS spectra were acquired under positive mode and collected in the 350–1400 mass/charge (m/z) range. MS/MS spectra were collected in the 50–1950 m/z range.

LCD Shimadzu raw data were converted (LCMS Protein Postrun, Shimadzu) to Mascot Generic Format (MGF) files prior to analyses. Peaks Studio V7.0 (BSI, Toronto, ON, Canada) was used for data processing (de novo peptide sequencing and proteomic identification) (Ma et al., 2003). Peaks Studio V7.0 (BSI, Toronto, ON, Canada) was used for data processing (Ma et al., 2003). Proteomic identification was performed according to the following parameters: error mass (MS and MS/MS) set to 0.2 Da; methionine oxidation and carbamidomethylation as variable and fixed modification, respectively; trypsin method; maximum missed cleavages (3), maximum variable Post-Translational Modifications (PTMs) per peptide (3) and non-specific cleavage (one). The samples were analyzed against a provide specific information on UniProt database (UniProtKB/Swiss-Prot, 2020- Total entries 562,253).

2.3. Proteolytic activity

Caseinolytic activity was measured colorimetrically by a modified procedure described (Kreger and Lockwood, 1981) with slight modifications. The reaction mixture, consisting of 142 μL azocasein (5 mg/mL) in PBS (phosphate-buffered saline, pH 7.2), 25 μL venom dilution (5, 2.5, 1.25, 0.6 mg/mL) in PBS buffer, was incubated for 90 min at 37 °C. The reaction was quenched by the addition of 334 μl of 5% trichloroacetic acid and rest for 30 min at room temperature. After centrifugation at 13000×*g* for 5 min, 100 μL of the supernatant was mixed with the equal volume of 0.5 M NaOH and absorbance was measured at 450 nm and the percentage of activity was calculated. *B. alternatus* venom was used as positive control (100% of caseinolytic activity). Assays were run in triplicate including appropriate blanks.

3. Results and discussion

The combination of RP-HPLC and mass spectrometric analyses was used to identify the major protein components from the crude venom that comes from specimens that inhabit the Northeast region of Argentina. The RP-HPLC separation profile of pooled venom from adult specimens (Fig. 2), followed by mass spectrometry analysis (Supplementary Table 1 and Fig. 1), revealed that *C.d. terrificus* venom proteome is made up of 12 protein families, which are unevenly distributed in the venom, e.g., there are few major proteins in the venom composition (Table 1).

Our protocol, however, differs from others previously described (Calvete, 2011; Calvete et al., 2007, 2009; Gutierrez et al., 2009). The first step was the separation of one (or several) protein components from the whole venom by reversed-phase chromatography. This was carried out to increase the detection sensitivity for minor components of the protein mixture in the subsequent identification steps. In sequence the mass and complexity of the protein samples were analyzed by MALDI-TOF, and the proteins were identified by ESI-IT-TOF and proteomic analysis.

The venom of *C.d.terrificus* from Brazil has been extensively studied by proteomic methods. In this sense, at least five proteomic studies were found (Boldrini-França et al., 2010; Calvete et al., 2010; Georgieva et al., 2010; Melani et al., 2015; Wiezel et al., 2018) four of them as listed in Table 2. The first proteomic *C.d. terrificus* study was performed by Calvete et al. (2009), but the geographical origin of the venom is unknown because the venom was purchased from Latoxan (Valence, France). Later, other studies (Table 2) were performed, and increased information on the composition of venom components, and their relations with geographic and ontogenetic changes of specimens belonging to this subspecies (Fig. 1). In this sense, variations in the relative quantity of CTX were determined in venoms from different geographic regions and, in general, exceeded 50% of the total venom protein composition. Serine (SVSP) and metaloprotease (SVMP) families were present in all venoms analyzed. Other components were also present in small percentages (≤3%).

In this work, the *C.d. terrificus* from Argentina venom proteins were analyzed, and our results showed similar composition to other rattlesnake venoms from Brazilian snakes. However, as discussed below, some differences were detected.

Crotoxin is abundant in the venom of *C.d. terrificus* (60.7%), and this figure is close to information from Melani and collaborators (2015). As we have previously reported (Fusco et al., 2015a, 2015b), PLA_2 from crotoxin complex (crotoxin basic chain, CTX B-chain) is present in different molecular forms in the venom. This enzyme could be detected in fractions 13 and 15 to 21 in the RP-HPLC separation (Fig. 2) and accounted for 30.7% of total venom proteins (Table 1). The molecular masses vary between 13.551,1 and 14.259,7 Da. With a similar abundance (30.0%), crotoxin acid chain (CTX A-chain) was detected in fractions 7 to 12 and their molecular masses were around 10 kDa. Hence, we were able to detect eight molecular forms of CTX B-chain and six of CTX A-chain. This is in agreement to previous studies reported by other authors, who informed that crotoxin is a mixture of variant forms of each component (Faure et al., 1993, 1994).

Serine proteinases constitute another large protein family of the *C. d. terrificus* venom. They were present in about 1–25% in all *C.d. terrificus* venom studied (Table 2). The quantity observed was intermediate (18.3%) in the venom here studied. In RP-HPLC profile the SVSP was detected in a fraction 14 closed to CTX B-chain fractions and in a non-resolved fraction (19–23). This proteinases group featured a molecular

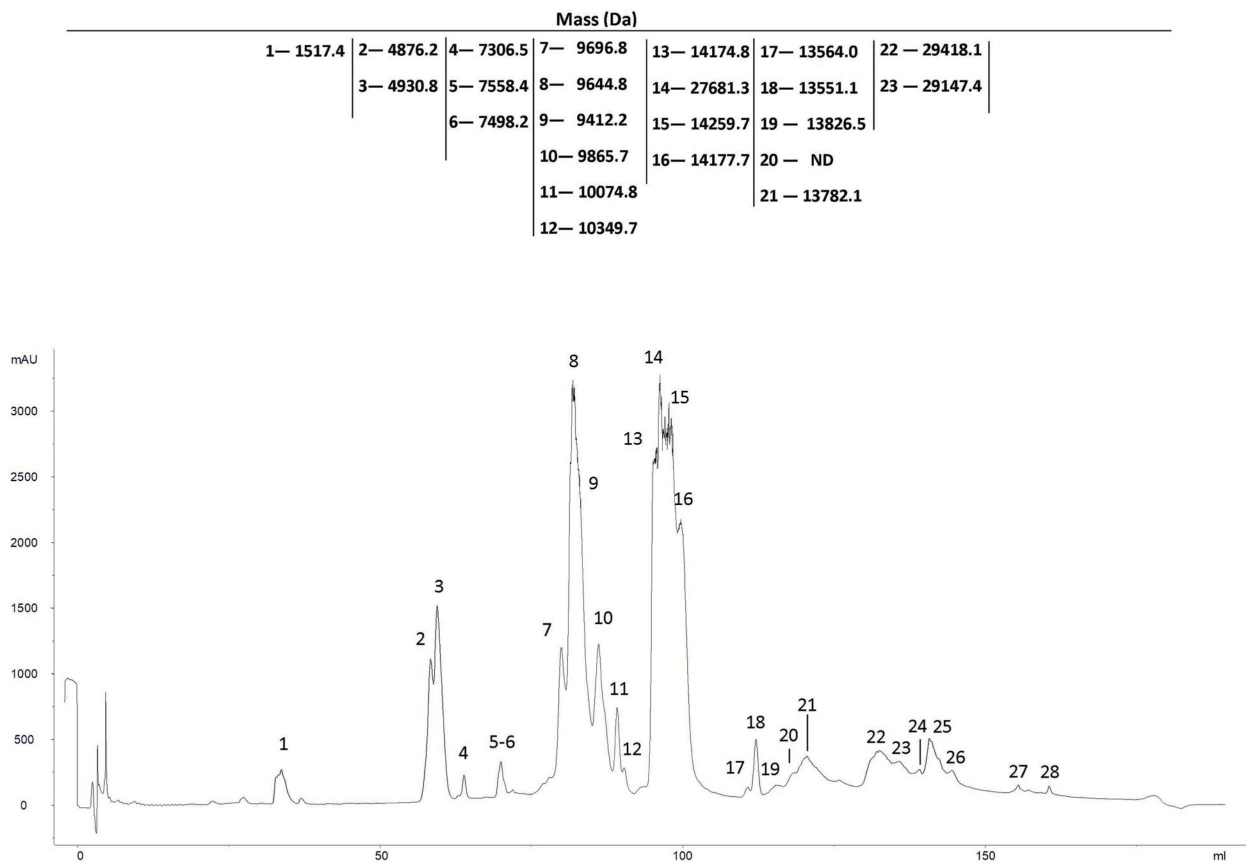

Fig. 2. Characterization of the venom proteome of *C.d. terrificus* from Argentina. Reverse-phase HPLC separations of the proteins from venom of specimens of Crotalus (lower in the panel). Protein Mass Determination by mass spectrometry (upper in the panel).

Table 1
Assignment of the proteins isolated by reversed-phase HPLC of the *Crotalus durissus terrificus* venom to protein Families by ESI-IT-TOF and proteomic analysis.

HPLC Peak	Protein Family	%	
13,15-19	Crotoxin basic chain	30.7	CTX B-chain
7–12	Crotoxin acid chain	30.0	CTX A-chain
14,19-23	Serine Proteinase	18.3	SVSP
2–3	Crotamine	7.7	CTM
24–26	L-amino-acid oxidase	~3.0	LAAO
26	Phosphodiesterase	~2.6	PDE
18	Vascular endothelial growth factor	<1.5	svVEGF
4,5,6,25	Zinc metalloproteinase-disintegrin	<1.4	SVMP
1	Bradykinin potentiating and C-type natriuretic peptides	<1.2	BBP/C-NP
27	C-type lectin convulxin	<1	CTL
28	Snake venom 5′-nucleotidase	<0.2	5′NT
28	Phospholipase B	<0.2	PLB
28	Venom nerve growth factor	<0.2	VNGF
	Unidentified	2.2	NI

mass around 29 kDa, similar to what was reported by Calvete et al. (2009). Some SVMPs detected by MS/MS analysis belonging to the fibrinogenases subgroup (peak 21, Supplementary Data Table 1). All this evidence agrees with Maruñak and collaborators works, who isolated a 33 kDa (by SDS-PAGE) SVMP from *C.d. terrificus* venom of Argentina. The isolated enzyme exhibited a thrombin-like activity and strong hydrolyzing activity on fibrinogen and fibrin (Marunak et al., 2004). These venom serine proteinases may be part of a family with multiple molecular forms (Yonamine et al., 2009).

Table 2
Overview of the relative occurrence and comparison with previously studies of protein families identified of *Crotalus durissus terrificus* venom.

Protein Family	Present Work	1	2	3	4
CTX A/B-chain	60.7	59.5	48.5	69.3	89.4
SVSP	18.3	8.2	25.3	13.4	1.43
CTM	7.7	19.0	ND	1	ND
LAAO	~3.0	4.5	ND	3	ND
PDE	~2.6	ND	1.9	1.2	0.39
svVEGF	<1.5	ND	ND	ND	2.2
SVMP	<1.4	4.8	3.9	2.9	6
BBP/C-NP	<1.2	2.8	ND	0.2	0.04
CTL	<1	1.7	ND	2.7	0.02
5′-NT	<0.2	ND	7.8	0.4	0.39
PLB	<0.2	ND	ND	0.2	0.04
VNGF	<0.2	ND	1.9	0.4	0.15
Non identify	2.2	ND	9.7	ND	ND
Glutaminyl cyclase	ND	ND	1	N	ND
CRISP	ND	ND	ND	0.2	ND
Hyaluronidase	ND	ND	ND	0.8	0.45

1.(Calvete et al., 2010); The origin of the venom was not determined (ND).
2.(Georgieva et al., 2010); San Maru Serpentarium, Taquaral, São Paulo.
3.(Melani et al., 2015); Butantan Institute, São Paulo.
4.(Wiezel et al., 2018); Ribeirão Preto, São Paulo.

Regarding the metalloproteinases, rattlesnake venom from Brazilian specimens present SVMPs in low relative quantities (Calvete et al., 2010). The mass spectrometry analysis of RP-HPLC fractions of pooled venom from adult *C.d. terrificus* specimens from Argentina (Fig. 2), revealed the presence of one peptide belonging to the catalytic domain of SVMP (Supplementary Data, Table 1). This evidence was found in fraction 25, a pool of several proteins (LAOO, SVSP, 5′ND, PLB) all in

very low quantity. The 25 peak area from RP-HPLC profile represents minor of 2% of total chromatogram area. In agreement with this observation, *C.d.terrificus* venom exhibited a low proteolytic activity. Only the highest concentrations tested (2.5–5 mg venom/mL) were capable of hydrolyze casein, and the activity was 5% of that for *B. alternatus* venom (a well-known venom by its high proteolytic activity) under similar conditions. The activity was not detected at lower venom concentrations assayed. In light of these results, it can be inferred that *C.d. terrificus* venom has SVMP only as traces. This confirms observations that have been reported in previous works. At the same time, the very low level of SVMP detected may explain the lack of hemorrhagic activity, as it was described for this venom. (Acosta de Perez et al., 1997; Garcia Denegri et al., 2019).

Disintegrins are a family of polypeptides present in the venoms of Viperidae and Crotalidae snakes (vipers and rattlesnakes) (Calvete, 2013). In the present work, zinc metalloproteinase/disintegrins were detected at the beginning of the chromatogram in fraction 5 (Fig. 2). Our results are agreement to Wiezel and collaborators who detected two disintegrins isoforms in *C.d. terrificus* venom from Brazilian specimens (Table 2), while Calvete et al., 2010, Georgieva et al., 2010 and Melani et al., 2015, did not find these proteins in the venoms analyzed. Disintegrins detected in this work showed molecular masses around 7.4 kDa and this value similar to disintegrins isolated from venoms of *Crotalus durissus* subspecies as *C.d.collilineatus* and *C.d.cascavella* (Boldrini-França et al., 2010), as well as other Crotalus species and subspecies, such as those studied by Scarborough et al. (1993), *C. atrox* and *C.d. durissus*. Additionally, Sánchez et al. (2006) also reported the characterization of mojastin 1 (7.436 kDa) and 2 (7.636 kDa) with masses around 7.5 kDa present in the venom of *C.s. scutulatus* (Sánchez et al., 2006). These reports of the presence of disintegrins are from venoms containing SVMPs. The presence of disintegrins could be due to the direct synthesis from short-coding mRNAs (Calvete, 2010).

On the other hand, a peptide belonging to the prodomain of SVMP was identified by MS/MS analysis of fraction 3 (Supplementary Data Table 1). Simultaneously the MALDI-TOF analyses of this fraction showed the presence of proteins with molecular mass around 5 kDa. Although SVMP prodomain peptides are very rare in vipers venome, they have been described in some venoms (Moura-da-Silva et al., 2016).

Taken together, these results could be indicating that the SVMP molecule "pool" in the *C.d. terificus* venom is a mixture of fully- and partially processed (active) metalloproteinases, as well as well as unprocessed zymogens, as described Nicolau and cowokers in *Bothrops jararaca* venom proteopeptidomic analysis (Nicolau et al., 2017). In Table 2 the percentual content of this proteins family is shown. This value (<1.4%) is lesser between the obtained values for all *C.d. terrificus* venom studied.

Crotamine represents approximately 8% of the protein composition of the total venom. Here, it was detected in fractions 2 and 3, i.e., variants forms showing molecular masses close to 5 kDa. It is well known that Crotamine is a small basic myotoxin (Falcao and Radis-Baptista, 2019; Rádis-Baptista and Kerkis, 2011). Johara Boldrini-França et al. (2010) reported the increased frequency of crotamine-positive populations from Southeast to Southern Brazil for *C.d. terrificus*. In this sense, some authors describe crotalid venoms as crotamine-positive or –negative specimens and correlating with different toxicological properties (Cameron and Tu, 1978; Schenberg, 1959). The *C.d. terrificus* venom from Argentina is crotamine positive as was demonstrated not only in the identification proteomic analysis, but was also previously reported by Echeverría et al. (2016).

Studies from other South American rattlesnakes have pointed increased neurotoxicity southwards along the North-South axis of the species dispersal into South America (Boldrini-França et al., 2010; Calvete et al., 2010). Our results indicate that *C.d.terrificus* venom has a high content of neurotoxins (crotoxin and crotamin). This finding are in concordance with those reported by Calvete and collaborator (2010) who showed the gain of neurotoxicity and lethal venom activities to mammals venom toxicity has evolved during *Crotalus durissus* invasion of South America. This analysis was supported by mapping a "lethal neurotoxicity coefficient" (LNC), which these authors define as the ratio between the average LD50 (μg/kg) of the venom and the crotoxin + crotamine concentration (Supplementary Table 2).The overall analysis of venom composition, LD50 and LNC of *C. d. cascavella, collilineatus and terrificus* subspecies venoms closely resemble that of *C. d. terrificus* venom from Argentina, supporting the hypothesis that these taxa can be considered geographical variations of the same species (Boldrini-França et al., 2010).

L-amino acid oxidase and PDE are usually minor components in the mixture of protein and represent between 1.2 and 3% (Table 2) of the venom composition.

LAAO was detected at the beginning of RP-chromatography (fraction1), as well as at the end (fractions 24–26) co-eluting with minor protein components, as CTL (<1%), 5′NT (<0.2%), PLB (<0.2%) or VNGF (<0.2%). In comparison with studies from other authors, this protein was not always detected. In this present work, LAAO represents around three percent of the whole venom. Since it is a large protein, this atypical chromatographic behavior could be due to isoforms or to protein cleavage (either artifactual or already present in the venom). Bordonein-L, the L-amino acid oxidase from *C.d. terrificus*, has been previously studied and characterized (Bordon et al., 2015; Wiezel et al., 2019).

Phosphodiesterases (PDE) are one of the lesser abundant enzymes in snake venoms, they hydrolyze phosphodiester bonds from the 3' terminus of polynucleotides producing 5-mononucleotides (Fox, 2013). Although PDEs account for only around 2 percent of total venom proteins, it was postulated that this enzyme would induce important clinical disorders during the intoxication process (Mackessy, 2016). In this work, PDE was detected in fraction 26 and represents circa 2.6 percent of the venom proteins. In a previous study, we have identified two PDEs from *C.d. terrificus* from Argentina, with molecular mass of ~ 100 kDa (Fusco et al., 2016). Our findings are in accordance with Georgieva and collaborators which identified 2 phosphodiesterase spots thorough proteomic research of the 2D-separated venom (Georgieva et al., 2010).

The proteome analysis revealed a small set of proteins with a low quantity (less than 1.5%), both enzymes (PLB, 5′NT) and proteins (BBPs, CTL and svVEGF). Their presence in this venom is discussed below.

The first RP-HPLC fraction contained bradykinin potentiating peptides (BBPs). BBPs are prolinerich peptides which structural determinants were recently reviewed by Sciani and Pimenta (2017). BBPs have already been isolated from *Crotalus* genera (Gomes et al., 2007; Lopes et al., 2014). From *C.d. terrificus* two homologous cDNAs encoding the BPPs precursor protein were cloned (Gomes et al., 2007). These peptides, together with crotamine, represent the two families of *C.d. terrificus* venom small molecules (Wermelinger et al., 2005).

The glycosylated C-type lectin from venom of *C.d. terrificus* (CVX) (Murakami et al., 2003) could be identified by reverse-phase separation as the C-type lectinconvulxin, and it was one of the most hydrophobic proteins fractionated. Similar observations were made by other authors when studying the venom of Brazilian snakes (Boldrini-França et al., 2010; Calvete et al., 2010; Melani et al., 2015; Wiezel et al., 2018).

In the present work 5′ nucleotidase, phospholipase B and venom nerve growth factor were detected as the most hydrophobic proteins from the *C.d. terrificus* venom (fraction 28) and co-eluted together at the end of RP-HPLC profile. These proteins represent less than 0.2 percent of the proteome.

As it was mentioned, toxicological properties of *C.d. terrificus* venom from Argentina as well as of isolated toxins were further studied. The venomic results here obtained are largely agreeing with previous biochemical venom characterization, and they provide novel information about the presence of proteins (e.g. desintegrins) that, even in low levels, are interesting to isolate and study them.

In conclusion, this proteomic analysis allowed us to identify proteins from *C.d. terrificus* venom from adult snakes that inhabit the north

region of Argentina, as well as the comparison to the previously data obtained by authors who studied crotalic venom from Brazil. The results reveal that, despite the geographical proximity, the venom from *C.d. terrificus* presents many similarities and some differences to the Brazilian snake venom. The proteome here described shows, in general, the typical and expected protein composition for *C.d. terrificus* venom. Nevertheless, this crotamine positive venom has a lower metalloprotease content than *C.d. terrificus* venoms from other regions. This information is relevant for the design of pan-south American *C.d.terrificus* antivenom.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Luciano S. Fusco: Conceptualization, Investigation, Writing - original draft. **Emidio B. Neto:** Formal analysis, Methodology, Software. **Aleff F. Francisco:** Resources, Validation. **Jorge Alfonso:** Resources. **Andreimar Soares:** Funding acquisition, Supervision. **Daniel C. Pimenta:** Funding acquisition, Project administration, Writing - original draft. **Laura C. Leiva:** Writing - review & editing, Project administration.

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Appendix A. Supplementary data

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