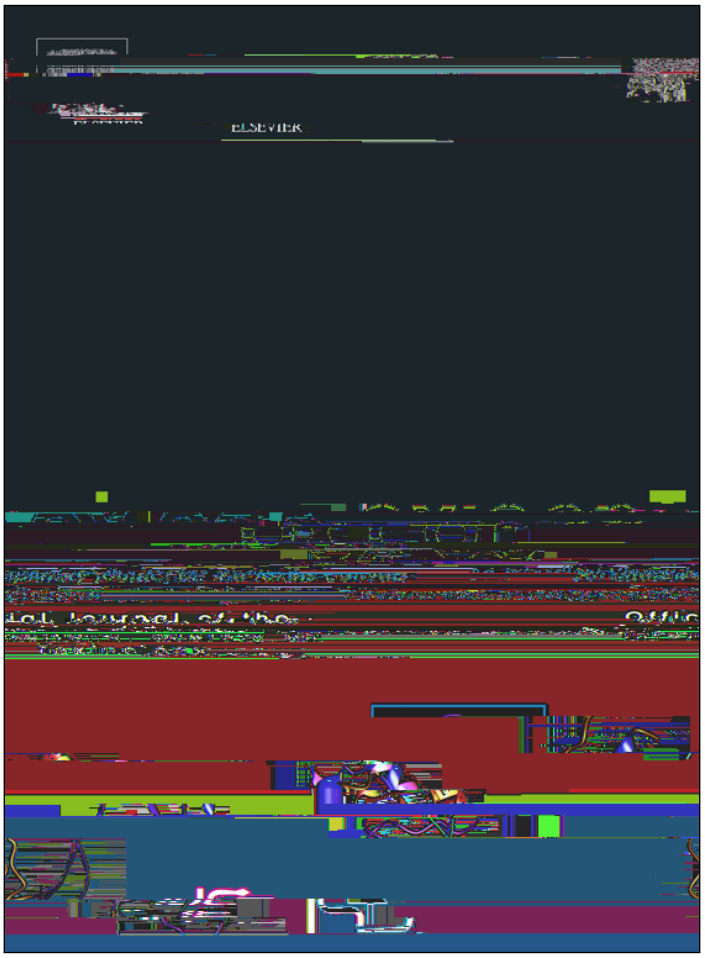

(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

Assessment of the potential toxicological hazard of the Green Parrot Snake (Leptophis ahaetulla marginatus): Characterization of its venom and venom-delivery system

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[a] Consejo Nacional de Investigaciones Científicas y Téc9

venom containing a simple to complex mixture of biologically active compounds, some of which can exhibit prey-specific toxicity (Heyborne and Mackessy, 2013; Mackessy, 2010; Mackessy and Saviola, 2016; Oliveira et al., 2016; Zelanis et al., 2010). Although the DVG is homologous with the venom glands of families Viperidae, Elapidae and Atractaspididae, it is anatomically and functionally distinct (Arikan et al., 2005). Duvernoy's venom glands typically lack any significant storage capacity and usually have no direct striated muscle insertion to pressurize the fundus of the gland. As a consequence, these glands constitute a low-pressure venom injection system (Kardong and Lavín-Murcio, 1993).

As a trophic adaptation, the primary function of venom is to facilitate prey capture, and it is known that composition is closely associated with the snake's diet (Calvete et al., 2009; Daltry et al., 1996; Mackessy, 1988; Pawlak et al., 2009). Although the number of published works investigating the complexity of venoms from front-fanged snakes is quite high to date, the composition and biochemical properties of rear-fanged snake venoms remain relatively poor studied, mainly because of the exceedingly small quantities of raw material that is possible to obtain for investigation (Hill and Mackessy, 2000; Mackessy, 2002; Weldon and Mackessy, 2010). Despite this disadvantage, rear-fanged snake venoms constitute a largely untapped source of bioactive compounds and potential drug leads (King, 2011; Koh et al., 2006; Saviola et al., 2014). For this reason, and because there is no toxicological information about many genera of colubrid snakes, investigation of these venoms has accelerated somewhat in the last several years (Junqueira-de-Azevedo et al., 2016).

Leptophis ahaetulla marginatus, the Southern Green Parrot

(Antunes et al., 2010). One unit of enzymatic activity toward collagen was defined as the amount of protein that causes an increase of 0.003 units of absorbance per min at 540 nm and the specific activity was expressed as U/mg protein. Activity toward casein was determined by a previously reported method (Quintana et al., 2017). The amount of protein that causes an increase of 0.005 units of absorbance per min at 450 nm was defined as one unit of enzymatic activity and the specific activity was expressed as U/mg protein. Acetylcholinesterase (AChE) activity was assayed according to Ellman et al. (1961) and activity was expressed as micromoles of product formed per minute per milligram of protein. Phospholipase A_2 (PLA

membranes in a tank transfer system (Hoeffer mini VE, Amersham Biosciences) at 25 V for 1.5 h. Membranes were then blocked with 5% nonfat dry milk and incubated with bivalent or tetravalent anti-Bothrops or anti-Micrurus serum (kindly donated by the INPB) diluted 1:500, and subsequently with 1:10,000 peroxidase-conjugated anti-horse IgG (Sigma A9292). The reaction was developed with 3,3′-diaminobenzidine tetrahydrochloride hydrate (DAB, Sigma D5637) as reported elsewhere (Antunes et al., 2010).

2.3.7. Reverse-phase high performance liquid chromatography (RP-HPLC)

Venom was dissolved in buffer A (0.1% trifluoroacetic acid, TFA, in ultrapure water) and then injected onto an Innoval C18 column (5 μm, 100 Å, 4.6 250 mm, Agela Technols5mDs) hpr14.1(neviousl)-276.8(cq)23.3.(uil)]TJ0-8.686-1.3159TD[inra)8(ed)-842.42in tre 1sam 8uffer

abnormal behavior characterized by an unusual apathy and a rapid rate of breathing (tachypnea), with marked contraction of the intercostal muscles. Initially, all of the animals became anorexic but returned to normal by 24 h post-injection.

3.6. Myotoxicity

When venom from L. a. marginatus was locally injected into mouse gastrocnemius muscle, the macroscopic appearance of the treated muscle was only slightly different from the control. No evidence of macroscopic hemorrhage was observed. In addition, the histopathological analysis revealed only a slight myonecrotic

structural proteins and extracellular matrix components
(

proteins is widely distributed among snake venoms. Although to

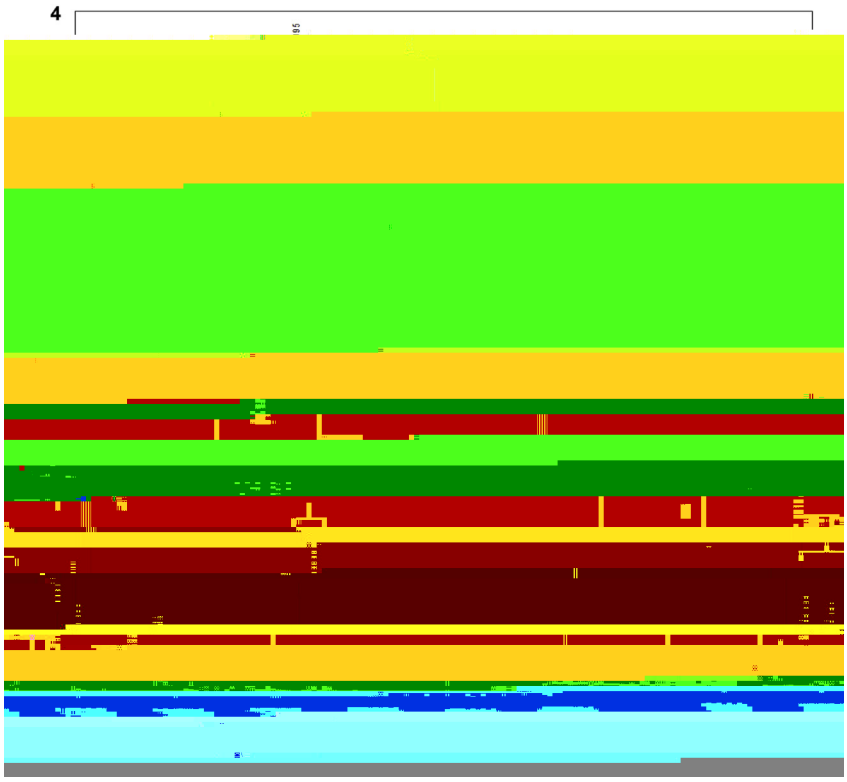

Fig. 8. MALDI-TOF mass spectrogram of L. a. marginatus venom. Tentative protein identifications, based on published masses for rear-fanged snake venoms, are given for three clusters of peaks. CTL, C-type lectin; CRiSP, cysteine-rich secretory protein; 3FTx, three-finger toxin.

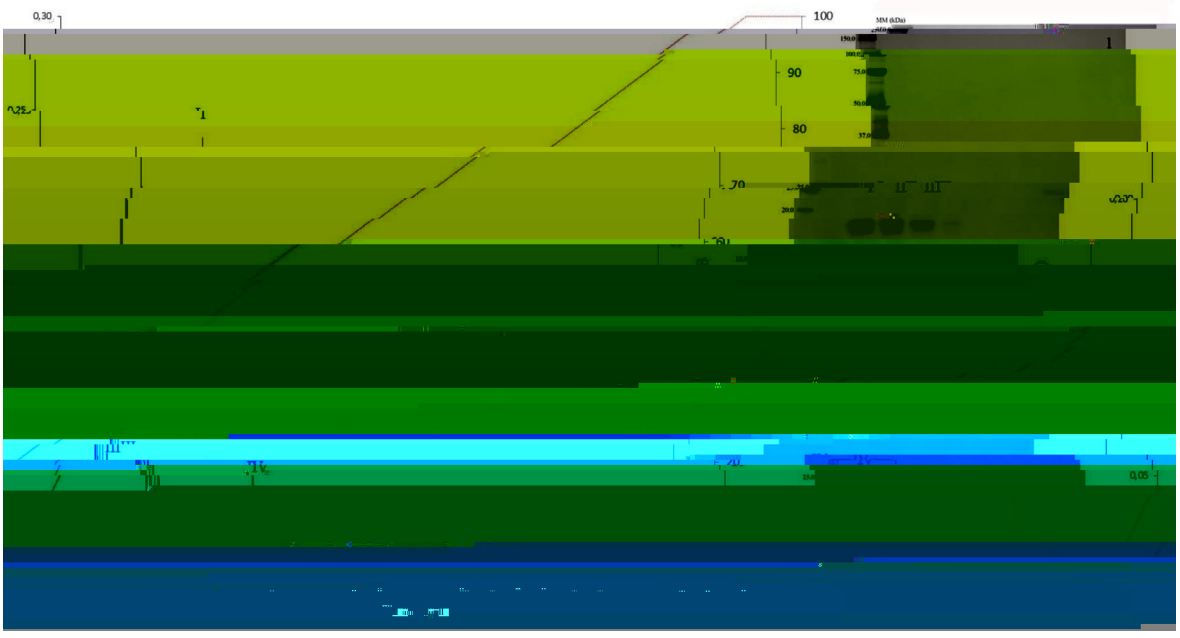

Fig. 9. Reverse-phase HPLC chromatogram of Duvernoy's venom proteins from L. a. marginatus. Insert shows SDS-PAGE of the eluted protein peaks on 12% gel run under non-reducing conditions.

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Conflicts of interest

The authors have no conflicts of interest with this work.

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