Size matters: an evaluation on the molecular basis of ontogenetic modifications in the composition of *Bothrops jararacussu* snake venom

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INTRODUCTION

Ontogenetic changes in venom composition have been described in *Bothrops* snakes but only a few studies have attempted to identify the targeted paralogues or the molecular mechanisms involved in venom modifications of gene expression during ontogeny. In this study we use a comprehensive dataset of *B. jararacussu* venom gland transcripts and venom composition from 19 specimens of different sex, size, reproductive status, or geographical location and identify the dynamic changes in toxin families and isoforms among these life history stages.

METHODOLOGY

Serpent collection and characterization of reproductive status by histology and Extraction of venom and venom glands

Identification of mRNA sequences encoding venom toxins by Illumina Plataform

Evaluation of biological activities and main enzymatic activities present in individual venoms

Identification of proteins in individual venoms by LC-MS/MS analysis

RESULTS AND DISCUSSION

According to the proteomic data, we noticed a tendency for a higher abundance of PLA_2s in larger individuals, whereas the PIII-class SVMPs are the most abundant toxins in smaller individuals.

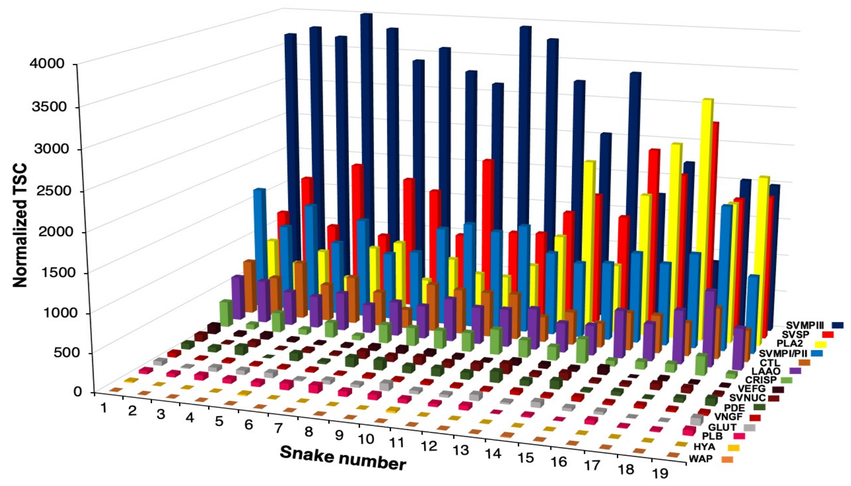

Figure 1. Distribution of the toxin families in the venom of 19 specimens of *Bothrops jararacussu* identified from proteome analysis. Relative expression is indicated by the normalized Total Spectrum Count (TSC) in each snake.

Taking this into account, our next step was to evaluate if there was any correlation between the expression levels of the most abundant toxin families in *B. jararacussu* venoms to the snake size, reproductive status, sex, and locality of collection individual snakes (Figure 2).

Ontogenetic variability was linearly related to snake size and did not correspond to the maturation of the reproductive stage.

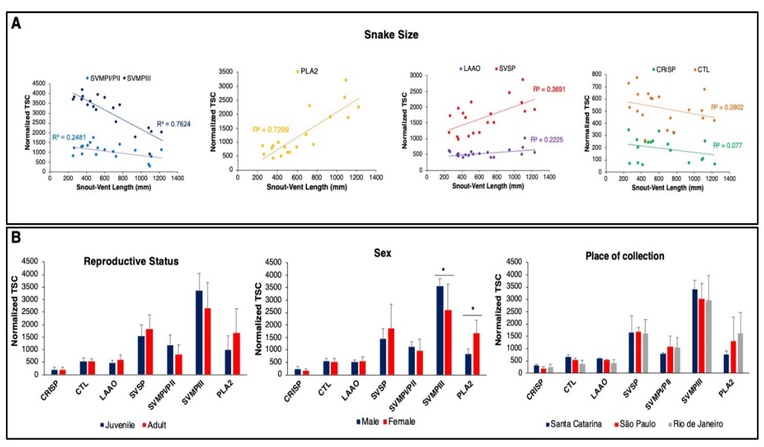

Figure 2 . Correlation of the main toxin families of *Bothrops jararacussu* venom with the individuals' snout-vent length (SVL), reproductive status, sex, and place of collection. Relative expression is indicated by the normalized Total Spectrum Count (TSC) in each snake. * $p < 0.05$.

We searched for the expression of transcription factors (i.e., NFIA, NFIB, and NFIX) in the transcriptome of *B. jararacussu* samples to understand if these regulatory mechanisms may be controlling the expression profile observed in the toxin genes. The NFIB has a higher expression in small snakes and a lower expression in larger snakes and showed a negative correlation with the individuals' SVL ($R^2 = 0.6821/p = 0.042$/slope b = −50.16).

B. jararacussu venom shows up-regulation in the expression of myotoxic PLA_2s and down-regulation of PIII-class SVMPs paralogues along the snake lifecycle, which is proportional to the snake size and not related to reproductive stage or geographical location

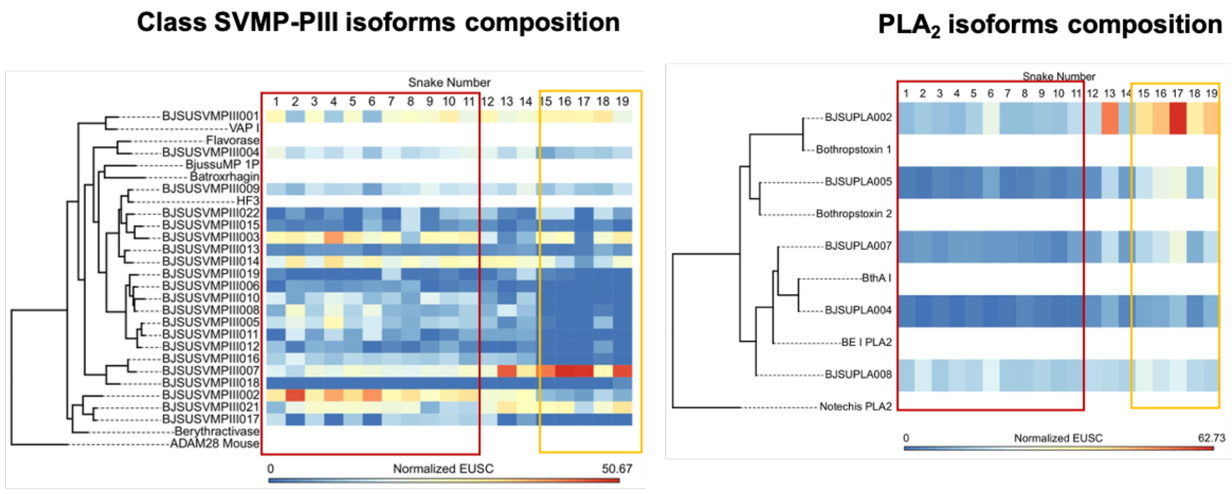

Figure 3. Distribution of SVMPIII isoforms and PLA_2present in the venoms of 19 individuals from *B. jararacussu* and functional inferences.

The SVMPs identified corresponded to novel sequences and conferred higher pro-coagulant and hemorrhagic functions to the venom of small snakes, while in large snakes, venoms were more myotoxic.

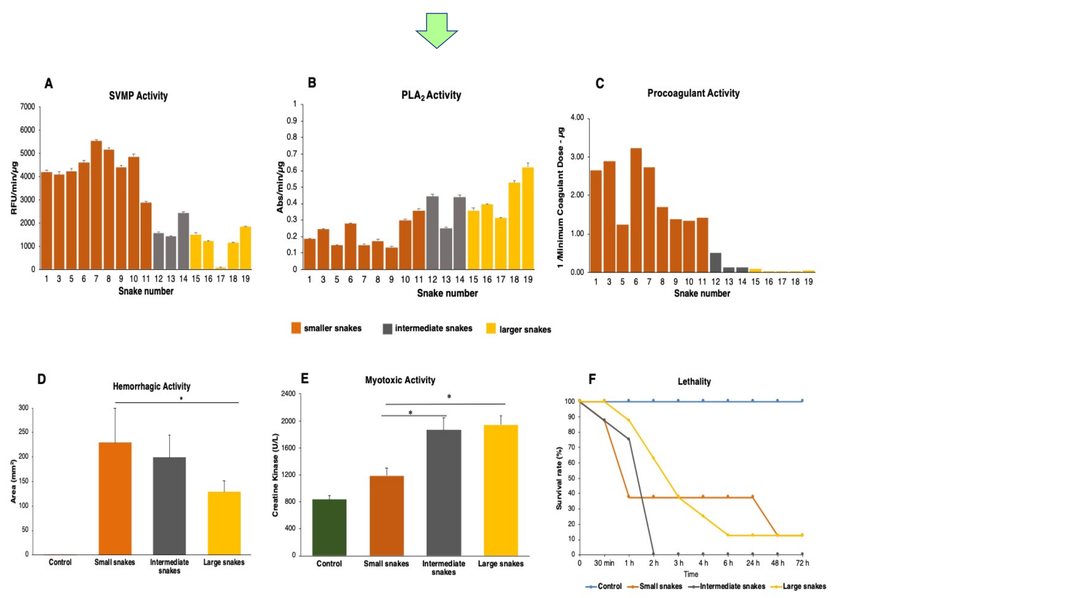

Figure 4. Functional activities of *Bothrops jararacussu* venom samples. The results are representative of two independent experiments. * $p < 0.05$.

CONCLUSION

We present here a comprehensive study on *Bothrops jararacussu* snake venom composition based on data obtained from 19 individuals of different sex, size, reproductive status, or geographical location. We carried out the first next-generation sequencing transcriptome (NGS) of venom glands from six individuals, which allowed a label-free characterization and quantification of venom toxins at the isoform level and evidencing the great diversity of PIII-class SVMPs in this venom. Venom variability was modulated mostly at transcriptional levels of a limited number of paralogues. As result, coagulant and hemorrhagic venoms from small snakes might be related to predatory function, while the venom from large snakes seems more related to immobilizing by high tissue-damaging myotoxic activity.