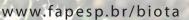
BIOTA-FAPESP PROGRAM



BIOTA

DIMENSIONS US-BIOTA-SÃO PAULO: SCALES OF BIODIVERSITY – INTEGRATED STUDIES OF SNAKE VENOM EVOLUTION AND FUNCTION ACROSS MULTIPLE LEVELS OF DIVERSITY

Inácio de Loiola Meirelles Junqueira de Azevedo & Lisle Gibbs

Butantan Institute and Ohio State University

Co-PIs: Erika Zaher, Ana Moura-da-Silva, Hussam Zaher, Darin Rokyta, Christopher Parkinson FAPESP Process 2016/50127-5 | Term: Sep 2016 to Aug 2021

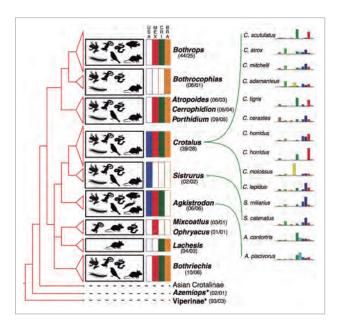


Figure 1. Phylogeny illustrating one of the target clades (Viperidae family). Colored boxes indicate occurrence in the U.S. (blue), Mexico (red), Costa Rica (green), and Brazil (orange). Taxa in bold will be sampled, if available, and taxa with an asterisk (*) indicate outgroups for comparative analyses. Numbers in parentheses (S/C) describe number of species within each clade (S) and the number of species with a high probability of being captured (C) based on museum records and personal experience. Animal silhouettes indicate documented prey in each clade (amphibians, squamates, birds, arthropods, small mammals, fish, and soft-bodied invertebrates). Histograms are preliminary transcriptomic data for different toxin-gene families, measured as percentage of reads mapped. (Source: draw provided by Felipe Graziotin based on unpublished preliminary data).

This recently started project supported by the partnership between FAPESP and NSF (National Science Foundation) involves researchers from Brazil (Instituto Butantan and Museu de Zoologia da USP) and US (Ohio State University, Florida State University and Florida Central University). As part of the Dimensions of Biodiversity Program, it aims to investigate the interface between genetic, phylogenetic and functional diversities, focusing on the evolution of advanced (Caenophidia) snakes.

The first step to understand the rapid diversification within an evolutionary lineage requires the determination of the factors that promote diversification. Traits that are key innovations certainly have a role in adaptive radiations, but few systems exist with one trait that is readily identifiable and genetically tractable such that the precise mutational pathways to species diversification can be ascertained. In snakes, venom has been hypothesized to be an innovation that initiated the radiation of species by expanding trophic opportunities, with subsequent toxin recruitment and toxin-gene neofunctionalization, promoting further diversification.

The relationships between function and properties of venoms, including complexity, composition, and enzymatic activities, and cladogenic patterns across the advanced snakes will be addressed, as shown in the Viperidae family phylogeny in figure 1. The genetic pathways underlying specific cases of rapid functional evolution will be characterized to test for generalities in the processes leading to the observed patterns of diversification. Based on that, it will be possible to determine how secondary key innovations within the venom system contributed to diversification patterns in the advanced snakes.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The first part of the project will be dedicated to test for relationships between venom diversity and function and diversification rates using data from more than 100 species belonging to three families of venomous snakes. The specimens used here will be collected in areas of exceptionally high biodiversity in North and Central America and in different domains of Brazil. Using data from venom-gland transcriptomics, quantitative mass spectrometry, and functional assays we will estimate phylogeny, quantify venom function and complexity and test for a link between clade diversification and venom composition. The second part will focus on the evaluation of the genetics of phenotypic divergence and mutational biases in the generation of venom as an adaptive trait. This will be achieved by comprehensively investigating species pairs that show recent divergence and significant differences in venom function. For these species, we will assess the importance of pre-transcriptional and post-transcriptional mechanisms as determinants of venom protein diversity.

The molecular details of species divergence and any inherent mutational biases will be determined since they could be as important in generating macroevolutionary patterns as the ecological context and the nature of the selective pressures driving species divergence. This will provide unprecedented detail about microevolutionary

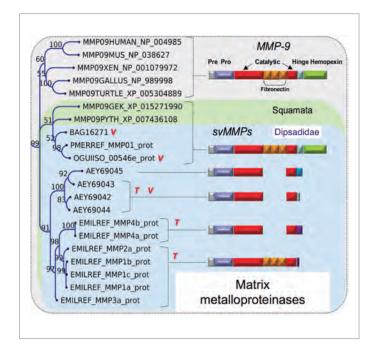


Figure 2. Relationship among sequences of snake venom Matrix Metalloproteinases (svMMPs), a class o toxin identified only in Dipsadidae snakes. On the right, schemes representing the structural organization of protein domains indicate processes of domain loss in venom proteins in comparison to non-venom orthologous protein (MMP 9). Evidences for the presence in venoms are indicated by "T" (transcribed) and "V" (detected in venom proteome). processes that underlie a key trait that influences macroevolutionary patterns, providing an integrated perspective from the molecular to organismal level on the fundamental processes generating biodiversity. The results will also substantially improve our knowledge about the toxins produced by medically important species or by less studied ones, as exemplified by the recent discovery of a new family of toxins in Dipsadidae snakes (Figure 2).

As broad impacts, the project aims to promote undergraduate research through the partnership between the institutions involved, and by offering a series of technical symposia and lectures for distinct audiences. Moreover, an interactive traveling display and educational pamphlets about snake diversity and evolution are planed to be used in public outreach to local schools in São Paulo. All results and events such as field trips and courses will also be available in a website designed to engage the public and promote public understanding of science.

INÁCIO DE L. M. JUNQUEIRA DE AZEVEDO

Laboratório Especial de Toxinologia Aplicada Instituto Butantan Av. Vital Brasil, 1500 CEP 05503-900 – São Paulo, SP – Brasil

+55-11-2627-9731 inacio.azevedo@butantan.gov.br