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# Peptide diversity in the venom of the social wasp *Polybia paulista* (Hymenoptera): A comparison of the intra- and inter-colony compositions

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ABSTRACT

The venoms of the social wasps evolved to be used as defensive tools to protect the colonies of these insects against the attacks of predators. Previous studies estimated the presence of a dozen peptide components in the venoms of each species of these insects, which altogether comprise up to 70% of the weight of freeze-dried venoms. In the present study, an optimized experimental protocol is reported that utilizes liquid chromatography coupled to electrospray ionization mass spectrometry for the detection of peptides in the venom of the social wasp *Polybia paulista*; peptide profiles for both intra- and inter-colonial comparisons were obtained using this protocol. The results of our study revealed a surprisingly high level of intra- and inter-colonial variability for the same wasp species. We detected 78–108 different peptides in the venom of different colonies of *P. paulista* in the molar mass range from 400 to 3000 Da; among those, only 36 and 44 common peptides were observed in the inter- and intra-colony comparisons, respectively.



## 1. Introduction

The evolution of venoms and their injection apparatus among Insecta represents an evolutionary trend that contributed to their adaptation to many different terrestrial environments. As predators, the stinging wasps have evolved to use their venoms as a chemical weapon for both the defense of their colony and for capturing prey [3].

The venoms of wasps are complex mixtures of biologically active compounds, such as low molecular mass compounds, peptides, and proteins [14,18]. Different types of inflammatory peptides are reported in these venoms, including mastoparans [7], protonectin-like peptides, chemotactic peptides, and wasp kinins [6].

Recent studies have demonstrated the analytical capabilities of various mass spectrometry approaches in the detection and identification of compounds present in very low concentrations in animal venoms [6]. A combination of techniques such as high-performance liquid chromatography (HPLC) and mass spectrometry contributed to the discovery of many components of animal venoms [10,11,17,21]. Venom profiling constitutes the basic approach in global venom exploration by mass spectrometry. A venom profiling data set generated with or without chromatographic separation provides a global picture of the venom showing both its complexity and an overview of the molecular nature of the venom components [11].

The conventional experimental approaches, such as large-scale fractionation techniques with off-line fraction collection, simply concentrate the most abundant components and do not account for the presence of minor constituents. Research involving proteomics and peptidomics using advanced techniques such as LC–ESI-MS and nano-ESI-MS/MS allows for a rapid and sensitive identification and characterization of proteins and peptides with high efficiency. Snake venoms are known to contain approximately 100 different peptide components [2], while venoms of scorpions, spiders, and marine snails of the genus *Conus* have been shown to contain between 300 and 1000 different components [1,5,12,19].

Differences in the number and composition of the peptide components in animal venoms have been reported for snails [15,25], spiders [12,31] and scorpions [11,13,24]. The origin of these differences may be multi-factorial, including differential processing of the zymogens, differential gene expression, genetic polymorphism, and altered post-translational processing. The chemical diversity of peptides from the Hymenoptera venoms is currently poorly understood; in the venom of the social wasp *Polybia paulista* as example, only six peptides are structurally and functionally known: the mastoparans Polybia-MP-I and -II, that are known to induce mast cell degranulation [7–9]; the Polybines-I and -II that are

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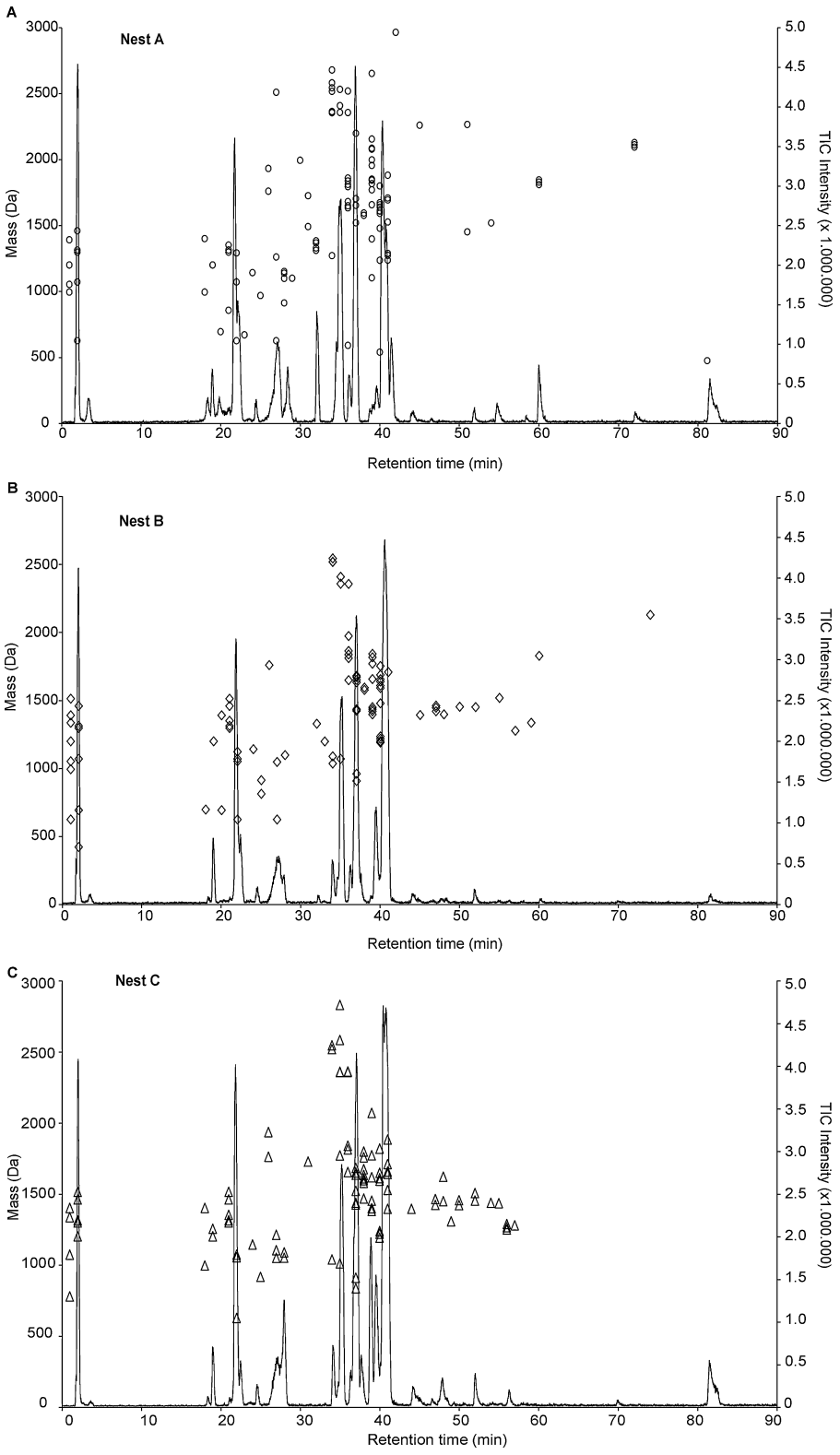


**Fig. 1.** LC–ESI-MS total ion chromatograms and peptide profiles (peptide mass vs. retention time) of *P. paulista* venom from nests A (108 peptides), B (92 peptides), and C (98 peptides). The reconstructed masses of each peptide are represented as different symbols according to the sample.

N-terminally acetylated peptides involved with a series of inflammatory actions [22]; the Polybia-CP, that is a chemotactic peptide for polymorphonucleated leukocytes [7,9,29], and the peptide presenting a disulfide bridge, that promotes insulin secretion from β-cells [20]. Hence, to shed some light on the subject, peptide diversity of the venom toxins from the social wasp *P. paulista* was investigated in our lab. Wasp workers were collected from same nest at different seasons (intra-nest study), as well from different nests in the same season (inter-nest study), and samples were characterized using an optimized LC–ESI-MS protocol.