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High accuracy mass spectrometry comparison of *Conus bandanus* and *Conus marmoreus* venoms from the South Central Coast of Vietnam

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ABSTRACT

Cone snail (genus *Conus*) venoms provide a rich source of small bioactive peptides known as conopeptides or conotoxins, which are highly interesting in pharmacological studies for new drug discovery. *Conus* species have evolved expressing a variety of conopeptides, adapted to the biological targets of their own specific preys at their living environments. Therefore, the potential proteomic evaluation of *Conus* venom components, poorly studied, is of great interest. Early studies supposed about 5% overlap in venom peptides from different *Conus* species. In this study, we compare using nano-liquid chromatography coupled with electrospray ionisation-mass spectrometry and bioinformatics, the molluscivorous *Conus bandanus* venom to that of its close-relative *Conus marmoreus* of the South Central Coast of Vietnam. With this approach, we demonstrate with high precision that 92 common conopeptides are present in the venom of the two mollusc-hunting cone snails, representing 24.4% (out of 376 peptides) and 18.4% (out of 499 peptides) of *C. bandanus* and *C. marmoreus* components, respectively. The proteomic comparison of the two close-relative interspecies suggests both common and different strategies for mature conopeptide production in the two species. The overall estimation of putative conopeptide disulphide bridges reveals 75% and 61% of "disulphide-rich" peptides in *C. bandanus* and *C. marmoreus* venom components, respectively. The same amino acid sequence for Bn1.1 and Mr1.1, determined at the genomic level, was also found in the two venoms, besides other common conopeptides. Confidently, the broader distribution of *C. bandanus* compared to *C. marmoreus* guarantee new opportunities for discovering conopeptides with original pharmacological properties.

Abbreviations: IAA, iodoacetamide; nanoLC–ESI-MS or MS/MS, nano-liquid chromatography–electrospray ionization-mass spectrometry or tandem mass spectrometry; PD, Proteome Discoverer; TFA, trifluoroacetic acid; TIC, total ion current.

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1. Introduction

Cone snails are predator marine gastropods that use a highly complex cocktail of specific bioactive peptides (named conopeptides or conotoxins) for prey capture, defence and competition. The conopeptides have been of particular interest to pharmacological studies for drug discovery over the past 35 years (Endean et al., 1974; Lewis et al., 2012; Olivera and Teichert, 2007) because of their relative small size and their stable structure diversity, such as highly diverse cysteine frameworks and numerous post-translational modifications. They are well known to modulate potential targets in the nervous system including neurotransmitter receptors, ion channels and transporters, with high specificity (Favreau and Stocklin, 2009; Lewis and Garcia, 2003; Olivera, 1997; Terlau and Olivera, 2004).

There are more than 700 known venomous *Conus* species, divided generally into three types according to their major prey: piscivorous, molluscivorous and vermivorous (Röckel et al., 1995). Each species can produce a range of 50 to more than 1000 distinct conopeptides, mostly disulphide-rich peptides, and inter-species similarity was estimated to ~5% by using LC–MS (Davis et al., 2009; Jones et al., 1996), or ~27% by using MALDI-TOF mass spectrometry (Kauferstein et al., 2011). Despite the putative high number of bioactive compounds, only ~2% of conopeptides have been so far studied, most of them from the venoms of fish hunter cone snails (Kaas et al., 2010). In addition, some mollusc-hunting species were well investigated (such as *Conus textile* and *Conus marmoreus*) at both transcriptomic and proteomic levels (Dutertre et al., 2013; Garrett et al., 2005; Han et al., 2005; Luo et al., 2006b; Tayo et al., 2010).

The present work focussed on estimating and comparing the conopeptides of the mollusc-hunting *Conus bandanus* venom to that of the better-known *C. marmoreus* from the same geographical region (Nha Trang bay in the South Central Coast of Vietnam) using a comparative proteomic approach (ConoServer database). Interestingly, some previous reports indicated that *C. bandanus* and *C. marmoreus* are close-relative species from both conchological and phylogenetic points of view, but some ecological differences remain between the two species (Nam et al., 2009; Röckel et al., 1995). So far, the information regarding the overall protein content of dissected *C. bandanus* venom is missing, may be because of difficulties in sample collection procedure required to obtain sufficient amounts of venom material from this cone snail. The nano-liquid chromatography and electrospray ionisation-mass spectrometry (nanoLC–ESI-MS) was used to estimate the conopeptide content of the two close-relative mollusc-hunting species. The interest of this method is that it can be performed with minimal amounts of *Conus* venom with a very accurate mass measurement (less than 5 ppm). In this work, we demonstrate an important similarity and cysteine distribution in the conopeptide content between *C. bandanus* and *C. marmoreus*. This comprehensive approach gives not only a preliminary overview about dissected *C. bandanus* venom composition, but also provides information about new peptides candidates for characterisation.

2. Materials and methods

2.1. Venom sample preparation

The specimens of each *C. bandanus* and *C. marmoreus* (Fig. 1) were collected in sea water of the Nha Trang bay in the South Central Coast of Vietnam and were frozen at −80 °C at the Institute of Biotechnology and Environment (Nha Trang University, Vietnam). The crude venom of the whole venom apparatus of each species (from 3 adults with length ≥ 72 mm) was carefully dissected, extracted with 0.1% trifluoroacetic acid (TFA) four times, lyophilised and then stored at −80 °C.

Eight mg of each lyophilised crude venom was dissolved in 1 mL distilled water containing 0.1% TFA and centrifuged for 1 min at 20 × *g* to separate the fine visible granular material. Their supernatant was recovered, filtered by 0.5 mL Amicon Ultra 10 kDa device (Millipore's Amicon® Ultra-0.5 centrifugal filter devices), and spun at 12,000 × *g* for 20 min at 4 °C. Protein concentration assays were carried out using the Biorad Protein Assay Reagent (Cat. No 500-0006, Marnes-la-Coquette, France) following the manufacturer's instructions and adapted from the method of Bradford (Bradford, 1976). Protein concentration estimates were obtained using NanoDrop 2000c Spectrophotometer Thermo Scientific, and comparison was made with bovine serum albumin and insulin standard. For the comparison, 25–50 μL volume of each quantified venom extract (0.354 μg/μL for *C. bandanus* and 0.169 μg/μL for *C. marmoreus*) were centrifuged and dried under vacuum by a

Fig. 1. Shells of two mollusc-hunting cone snails from Nha Trang bay, on the South Central Coast of Vietnam. *C. bandanus* (top left) exhibits two coloured bands, and is sculptured with prominent tubercles on its shell. In contrast, these two coloured bands are absent on *C. marmoreus* (down right).