Review of: "Evolution of Venom Production in Marine Predatory Snails"

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Potential competing interests: No potential competing interests to declare.

The manuscript reports transcriptomics data on oesophagus-associated glands from marine predatory gastropods, with a focus on venoms from marine predatory cone snails. The approaches proposed by the authors were developed to bring new insights into the genetic basis of functional divergences and to highlight the plasticity of the digestive system in gastropods. They addressed the three following points: (1) the link between the oesophageal glands and the venom profiles, (2) the contribution of novel genes to the evolution of venom production, and (3) the link between the evolution of the transcriptomes of the glands and the divergence of the venom gland functions. They observed that venom glands have high expression levels and a shift of function of the mid-oesophageal gland for toxin production rather than for a digestive purpose.

One comment: there are several papers that clearly evidence that venoms may be excellent contributors, facilitators, or enhancers in the digestion of prey and tissues. This is not so discussed in the version submitted.

The manuscript is properly presented and documented; it is a well-conducted study with robust data generated from appropriate methods. The bibliography is of quality and helpful for the reader to get a feel for the topic and objective of this work.

General comments

A “conclusive remarks” section may bring to the reader a summary of all results obtained in a brief description and what will be the future. Do the authors expect to go further in this genomic transcriptomic work, such as in the proteome of the different glands investigated in this work? This would bring an additional dimension to this work by performing proteomics studies on each type of gland to better understand the maturation of the peptides from their precursors (e.g., prodomains cleavages, C-terminal amidation, glycosylation), modifications that are essential to generate bioactive compounds and their stability in the gland or following secretion. They used SignalP (which version?) to identify signal peptides, which is not the end point in precursor maturation of bioactive peptides. To define what the sequence of the functional matured peptide in venoms is, a precursor form is subjected to additional modifications (see above). In addition, unconventional protein secretion may occur without a signal peptide (doi: 10.1016/j.tcsw.2020.100045). This may significantly contribute to understanding the evolution and specificities of the glands. Nothing is discussed in their manuscript on these points, as well as on the immune pathways, etc. There are transcriptomics and proteomics analyses, at least on cone snails including *Conus imperialis* and *C. marmoreus*. The authors should benefit from this to make the link with their results.

Results section

The illustration is properly organised to provide a rather clear vision of the GO terms and biological process functions and cellular compartments for each type of gland. The text reporting these results may be a little bit more “enriched” by more examples than only one (see, e.g., the AP-5 story).

Regarding the Methods section

The authors referred to Supp Data 1 (section 4.1), while the Supplementary Information does not provide this information, or the name of the file is not the same. What is the content of the information provided? The number of specimens per species was not mentioned in the Methods section (only in the results section); please provide the information also in the Methods section.

It is not so clear what the color code used in figure 1 represents. What is the orange organ? It is written "gland" in black, while all the other organs have their names written with the color code of the organ. From my understanding, “gland” in black corresponds to the series of gland names proposed in the phylogeny part. So a suggestion would be to have the orange organ in black. The idea to keep the same color for the different organs all around the manuscript is very useful.

Minor comments

According to the classical writing policy, Latin names should be in italic except if the entire sentence is in italic. Is the journal policy adjusted to this or not? If yes, then the species names in the references should not be in italic. As well, *De novo* etc. should be in italic

Some typos to adjust.

In the Supp info, Supp figure 1, it is not clear from the figure and its legend what the different abbreviations listed in the PCAs are (e.g., F, SAG, is MB the muscular bulb (not mentioned in Fig. 1), is OE = OEG as mentioned in Fig. 1, etc.). Adjust, if possible, to simplify reading. In figure 2, enriched GO terms VG, what is L-ascorbic acid binding? Do the authors mean L-ascorbic acid binding?

This manuscript may be acceptable after minor revisions.