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 a comparative transcriptomics analysis of the oesophagus-associated glands in marine predatory gastropods, focusing on the evolution of cone snail venom production. This study evaluated gene expression in the mid-oesophageal glands and other tissues across 12 marine caenogastropod species to understand the genetic basis of venom gland evolution. The analysis investigates whether 1) oesophageal glands have similar overall gene expression profiles, 2) the venom gland transcriptome has evolved faster than other mid-oesophageal glands due to its specialized role, and 3) novel genes played a role in the development of venom production. The esophagus of venomous species showed higher levels of gene expression, which may indicate that it is more active in the production of substances, possibly associated with the secretion of auxiliary compounds in the capture and digestion of prey. Other parts of the digestive system also showed higher rates in venomous species, suggesting that the evolution of these organs accompanied the specialization of the venom gland, suggesting concerted evolution across the digestive system. The results revealed that each gland not only has specific functions, but that these functions reflect the evolutionary adaptations of each gland to the species' environment and lifestyle (possible lineage-specific adaptations linked to diet).

The study design is appropriate to answer the research question, and the conclusions are supported by the evidence presented. The references provided are appropriate and up to date.

General comments:

- The use of a different color for each tissue, maintained throughout the manuscript, made it easier to read and understand the figures and text. I suggest using the same color scheme in Supplementary Figure 1 (and the same different color for each tissue should be used across the 12 plots to avoid misinterpretation).

- Captions should provide a more complete and detailed description, allowing readers to better understand the elements represented in the graphs and the meaning of each main component. The meaning of each abbreviation should be written in the caption (salivary glands, foot, columellar muscle, oesophagus, oesophageal gland, gland of Leiblein, dorsal glandular folds, venom gland, muscular bulb).

- In the introduction, cite venom enzymes and toxins that degrade specific tissues, allowing the predatory sea snails to capture and digest their prey.

There are a few points that need attention:

- In section 2.1, Cymatiidae (typo - Cymatidae).

- Italicize *de novo* (in the titles of sections 2.2 and 4.2).

- Supplementary Data 1 and 2 cited in sections 2.2 and 4.1 were not shown.

- In sections 2.3.2 and 4.2 - was SignalP 6.0 used?

- Prodomain cleavages and post-translational modifications such as C-terminal amidation and glycosylation were not discussed. Further proteomic studies may increase knowledge about how peptides mature within each gland. What future approaches can be used to overcome the underestimation of the toxic potential of gastropod salivary glands mentioned in the last paragraph of section 2.3.3? It would be interesting to add a “Future Perspectives” section.

- Supplementary Note 1 - Fold change (FC)

- Supplementary Note 2 - Gene Ontology (GO). I suggest that the information “we found arginine kinase activity, as it was previously found in a proteomics investigation” be added to the manuscript. Comparing the data obtained with previously published proteomics results could enrich the discussion.

- Supplementary Figure 1 - The following caption is suggested:

"**Supplementary Figure 1. Principal component analysis (PCA) plots of gene expression profiles for each of the 12 gastropod species after quality filtering.** Each plot shows the variation along the first principal component (PC1, x-axis), and the second principal component (PC2, y-axis). Tissue types are color-coded and labeled with abbreviations (…). These PCA plots illustrate tissue-specific clustering patterns within each species."

- Supplementary Figure 18 is the same as Figure 4, and Suppl. Fig. 18b, d, and e are not even cited in the manuscript. I suggest excluding Suppl. Fig. 18 and inserting additional information in the caption of Fig. 4 or in section 4 (Methods).

Recommendation: This manuscript may be accepted after minor revisions.