of the colonial hydrozoan *Hydractinia symbiolongicarpus*

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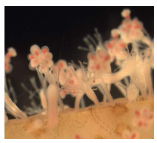


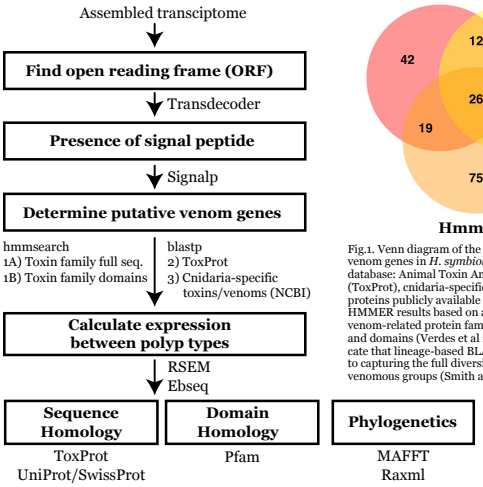
1 Abstract

Cnidaria (jellyfish, hydra, sea anemones, etc.) represent the earliest diverging venomous animal lineage. Venom is deployed in cnidarians for predation, defense, competition, and digestion. Recent evidence suggests venom composition can be influenced by age, diet, geography, and the presence of predators or prey. Although venom production and maintenance are central to the life history of cnidarians, little is known about their venom composition with respect to biological or ecological function. Hydractiniid hydrozoans are an ideal system for studying venom function and evolution due to their functionally specialized tissue types and complex life cycles. The hydractiniid *Hydractinia symbiolongicarpus* displays a division of labor among its polyps that comprise the colony: dactylozooids (defense and predation), gastrozooids (feeding and digestion), and gonozooids (reproduction). Using an existing transcriptome of the different functional polyp types of *H. symbiolongicarpus*, we characterized the putative venom components and venom expression between these tissues. By using functionally specific polyps of *H. symbiolongicarpus*, we can determine how the venom arsenal varies for specific tasks. Understanding how venom composition is influenced by various developmental and ecological factors will lead to a better understanding of venom diversity and function in cnidarians.

2 Specific Aims

- **Characterize putative venom composition in whole-animal transcriptome of *Hydractinia symbiolongicarpus***
- **Determine differential expression of venom genes between functionally specialized polyp types**

3 Methods



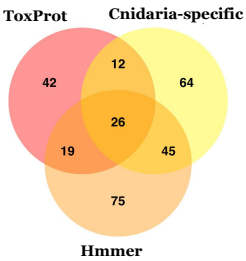


Fig.1. Venn diagram of the number of identified venom genes in *H. symbiolongicarpus* based on database: Animal Toxin Annotation Project (ToxProt), cnidaria-specific toxin and venom proteins publicly available through NCBI, and HMMER results based on alignments of known venom-related protein families, both full sequences and domains (Verdes et al 2017). These results indicate that lineage-based BLAST methods are critical to capturing the full diversity of poorly explored venomous groups (Smith and Undheim 2018).

4 Results

Differentially Expressed Venom Genes in Polyps of *H. symbiolongicarpus*

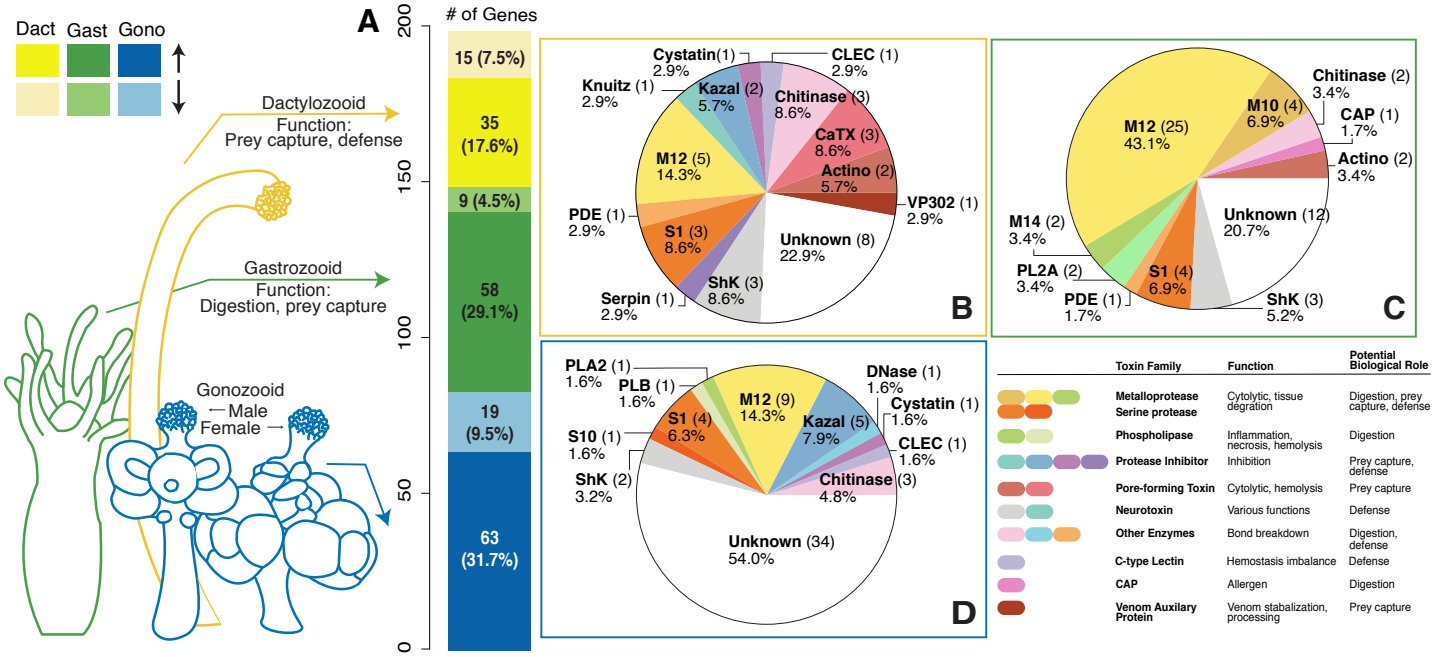


Fig 2. Visualization of differentially expressed putative venom genes of *H. symbiolongicarpus*. The bar graph (A) indicates the total number of putative venom genes upregulated (darker colors) and downregulated (lighter colors) within each tissue type: dactylozooids (orange), gastrozooids (green), and gonozooids, male and female (blue). The pie charts indicate the composition of putative venom genes upregulated in the dactylozooid (B), gastrozooid (C), and gonozooid (D). Abbreviations as followed: Actino, actinoporins; CAP, cystine-rich secretary proteins; CaTX, CaTX-like (or jellyfish toxins); CLEC, C-type lectins, M10/12/14, metalloproteases; PL2A/B, phospholipase 2A/B; PDE, phosphodiesterase; S1/10 serine protease 1/10 family; ShK, ShK-domain containing protein.

5 Conclusions

- **266 putative venom genes were identified, 199 of which are differentially expressed with respect to polyp type**
- ***H. symbiolongicarpus* polyps display unique venom composition profiles, reflecting variation associated with function**
  - Pore-forming toxins in dactylozooid = prey capture
  - Metalloproteases in gastrozooid = digestion of prey
  - Maternally deposited venom in gonozooid = protection of eggs

6 Future Aims

- Nematocyst-type specific venom (single-cell transcriptomics, proteomics, Fig. 3A)
- Functional characterization of candidate genes using CRISPR knockouts
- Comparative venomics using life stages of *Podocoryna carnea* (Fig. 3BC)

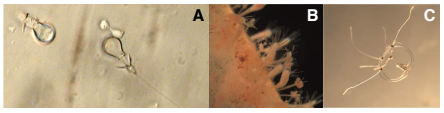


Fig 3: Isolated nematocytes (stenoteles) from *Hydra* (A). *P. carnea* polyps (B) and medusa (C).

References

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