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## Abstract

Cnidaria (jellyfish, hydra, sea anemones, etc) represent the earliest diverging venomous animal lineage. Cnidarians deploy venom 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: the gastrozooid (feeding and digestion), dactylozooid (defense and predation), and gonozooid (reproduction). *Podocoryna carnea*, a related hydractiniid hydrozoan, displays a complex life history that includes a benthic colonial stage and a pelagic medusa. Using publicly available RNA-seq data of the functionally specific polyp types of *H. symbiolongicarpus* and life cycle stages of *P. carnea*, we characterized the putative venom components and venom expression between these tissues. Based on this analysis, we can determine how the venom arsenal varies for specific tasks and between life cycle stages. Future work will include lineage-based transcriptomics on the stinging cells of *H. symbiolongicarpus* as well as functional characterization of specific venom components using CRISPR. 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.

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## Specific Aims

- Characterize putative venom composition in whole-animal transcriptomes of *Hydractinia symbiolongicarpus* and *Podocoryna carnea*
- Determine differential expression of venom genes between functionally distinct tissues (i.e. polyp type, life stage)

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## Results

### Differentially Expressed Venom Genes in Polyps of *H. symbiolongicarpus* and Life Stages of *P. carnea*

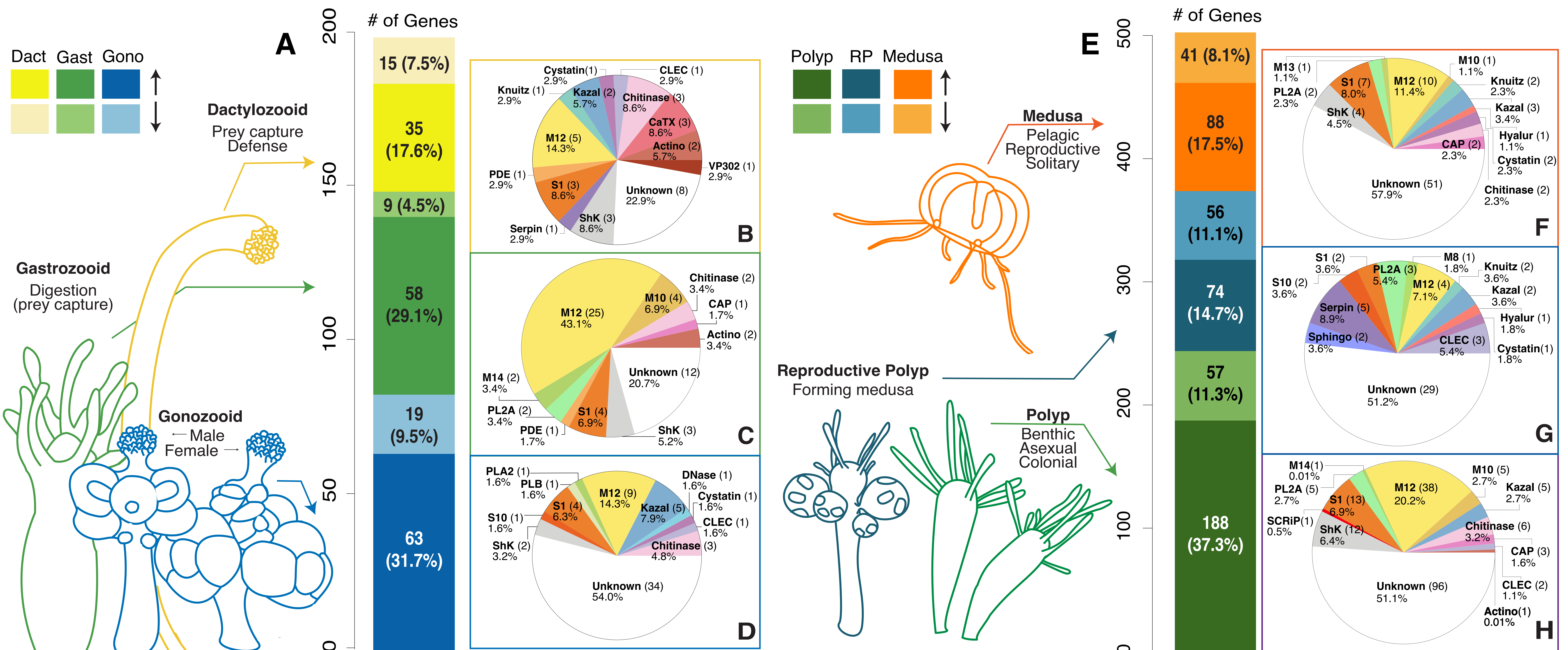


Fig 2. Visualization of differentially expressed putative venom genes of *H. symbiolongicarpus* and *P. carnea*. The bar graphs (A,E) indicate the total number of putative venom genes upregulated (darker colors) and downregulated (lighter colors) within each tissue type and life stage. The pie charts indicate the composition of putative venom genes upregulated in the *H. symbiolongicarpus* dactylozooid (B), gastrozooid (C), and gonozooid (D) and in the *P. carnea* medusa (F), reproductive polyp (G), and nonreproductive polyp (H). Abbreviations as followed: Actino, actinoporins; CAP, cysteine-rich secretory proteins; CaTX, CaTX-like (or jellyfish toxins); CLEC, C-type lectins; Hyalur, hyaluronidase; M8/10/12/13/14, metalloproteases; PL2A/B, phospholipase 2A/B; PDE, phosphodiesterase; S1/10 serine protease 1/10 family; Sphingo, Sphingomylinase; ShK, ShK-domain containing protein.

Toxin Family	Function	Potential Biological Role
Metalloprotease Serine protease	Cytolytic, tissue degradation	Digestion, prey capture, defense
Phospholipase	Inflammation, necrosis, hemolysis	Digestion
Protease Inhibitor	Inhibition	Prey capture, defense
Pore-forming Toxin	Cytolytic, hemolysis	Prey capture
Neurotoxin	Various functions	Defense
Other Enzymes	Bond breakdown	Digestion, defense
C-type Lectin	Hemostasis imbalance	Defense
CAP	Allergen	Digestion
Venom Auxiliary Protein	Venom stabilization, processing	Prey capture

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## Conclusions

### *H. symbiolongicarpus*

- 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

### *P. Carnea*

- 599 putative venom genes were identified, 504 of which are differentially expressed with respect to life stage
  - Change in metalloprotease composition throughout life stages
  - Single pore-forming toxin identified in polyp stage
  - Distinct composition from *H. symbiolongicarpus* despite close evolutionary relationship

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## Future Aims

- Determine how venom composition varies with respect to nematocyst type using FACS and single-cell transcriptomics in *H. symbiolongicarpus*
- Functional characterization of candidate venom genes using CRISPR knockouts in *H. symbiolongicarpus*
- Using proteomic approaches to further characterize the venom composition within these two species
- Further exploration into how venom composition changes over the complex life history of various cnidarian species using transcriptomics and proteomics

## References

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