



Carcinonemertidae: family of worms seeking a secure home and phylogenetic closure



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Introduction

The phylum Nemertea consists of approximately 1,300 species of unsegmented, bilaterally symmetric worms characterized by an eversible proboscis housed in a fluid filled cavity called a rhynchocoel. A molecular systematic study of approximately 50 species, using sequence data from five genes has cleared up several long-standing questions about the evolutionary relationships among the major groups of Nemerteans, but raised new, lower-level questions. (Thollesson & Norenburg 2003, Andrade et al. 2014a). A surprising result of both of those studies was the position of Carcinonemertes as a sister taxon to the suborder Distromatanemertea, whereas consensus understanding of anatomy and biology of carcinonemertids suggests that they should be highly derived within that suborder. Andrade et al. (2014b) recently strengthened the understanding of deep Nemertean phylogeny by applying new genetic tools- transcriptome sequencing (i.e. the set of thousands of genes actively being expressed in an organism at the time it is preserved). The aim of the current research project is to obtain a transcriptome from a representative carcinonemertid, and, by comparing it to the existing Nemertean transcriptomes, test the hypothesis that Carcinonemertidae are not derived members of Distromatanemertea.

Members of the family Carcinonemertidae are obligate egg predators that live on a wide variety of crab species. The family contains twenty described species known from approximately seventy species of crabs (Kajihara and Kuris 2013) and at least forty more unnamed species are known from forty additional crab species (Norenburg unpubl. obs.). The 6,700 known species of crab hold promise for many more undiscovered carcinonemertids. While their life cycle is similar to that of a parasite, the worms are technically egg predators, since they consume single embryos (Santos et al. 2006). The representative specimen used in this study is Carcinonemertes cf. epialti, collected from the pacific rock crab, Romaleon antennarium.



Figure 1: The Pacific rock crab (Romaleon antennarium), left. Right, a member of the family Carcinonemertidae, Ovicides paralithoidis, pictured with crab eggs (Kajihara & Kuris 2013).

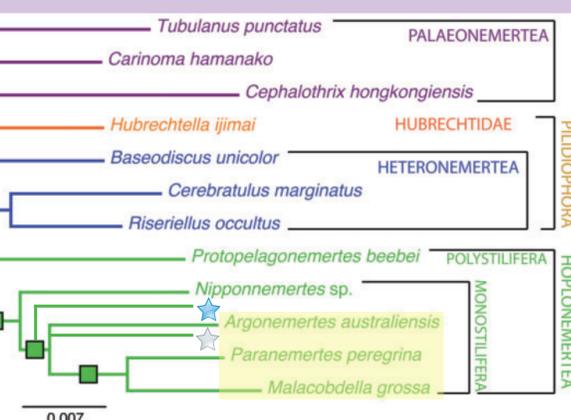
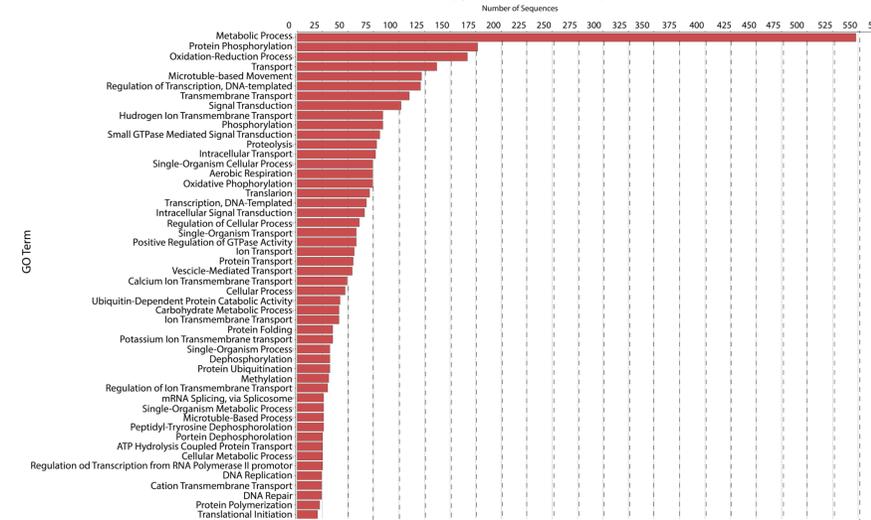
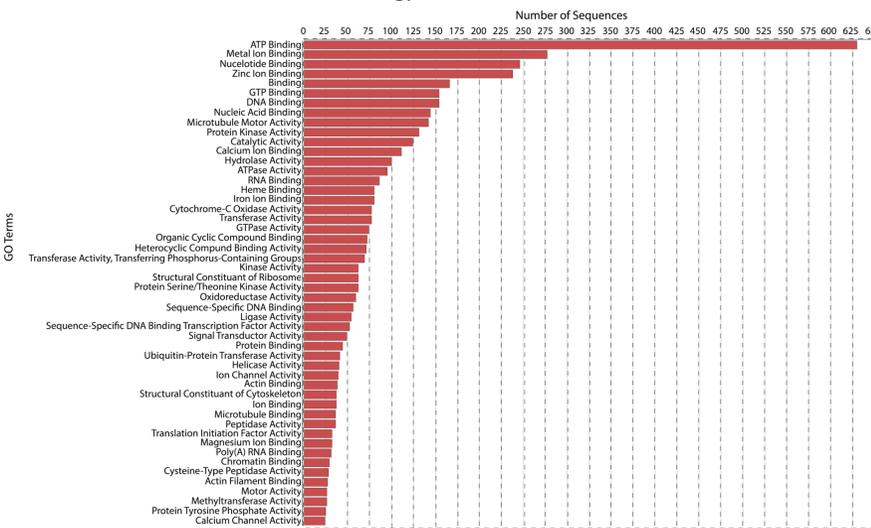


Figure 2: Hypothetical placement of Carcinonemertes cf. epialti based on previous molecular work (blue star) and on consensus understanding of Nemertean biology (silver star). The yellow highlighted area represents the suborder Distromatanemertea.

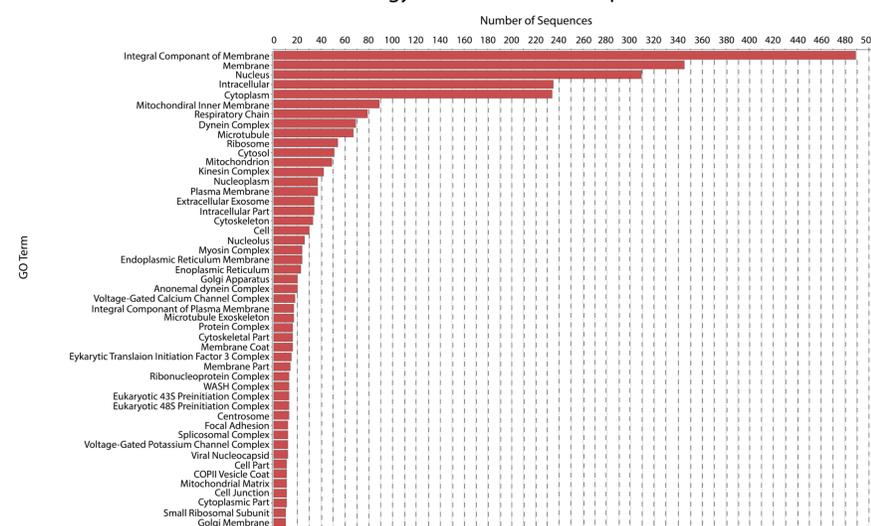
Gene Ontology Terms: Biological Processes



Gene Ontology Terms: Molecular Function



Gene Ontology Terms: Cellular Components



Discussion

Agalma and other metrics demonstrate that our transcriptome data is of suitable quality to yield a robust tree. Though remaining analyses may take weeks of computer time, we anticipate that analysis of the combined nemertean transcriptome data will provide strong support for a phylogeny that places Carcinonemertidae either as 1) sister to or 2) within the suborder Distromatanemertea. If option 1 is supported, we would infer a relatively ancient radiation as the primary source for high genetic diversity of the group. That inference could be corroborated if studies of symbiont-host co-evolution show deep divergences within Carcinonemertidae that parallel the calibrated deep divergences in host lineages. If option 2 – the currently assumed topology – is supported, we would strongly suspect that carcinonemertids experienced a rapid radiation, with an associated accelerated molecular clock. We anticipate testing both phylogenetic hypotheses with new anatomical study of the rhynchocoel musculature via confocal microscopy and – if needed – transmission electron microscopy. We will test molecular clock models via recently developed simulation methods (e.g., Duchêne et al 2015).

Gene Annotation

The three graphs to the left show the break-down of the function of each transcript that was sequenced by Agalma. Gene Ontology (GO) terms are assigned to transcripts to denote the function of that transcripts gene product. GO terms are assigned in one of three categories: Biological processes (top; a series of events accomplished by molecular functions), molecular functions (middle; activities that occur from the molecular level), and cellular components (bottom; components of a cell that are part of something larger, like a cellular structure or gene product group) (Altenhoff et al 2015).

Materials and Methods

Carcinonemertes cf. epialti specimens were collected from Coose Bay, Oregon and shipped live to the Smithsonian's National Museum of Natural History in Washington, DC. The worm used for sequencing was flash frozen and processed immediately. RNA was extracted from the worm using the Dynabeads[®] mRNA DIRECT[™] Purification Kit from Life Technologies following the manufacturer's instructions. RNA was then converted to cDNA using the ScriptSeq[™] v2 RNA-Seq Library Preparation Kit from Illumina, following the manufacturer's instructions. The cDNA was sequenced on the Illumina MiSeq in the National Museum of Natural History's Laboratory of Analytical Biology.

After obtaining reads from the MiSeq, a FastQC (Andrews S.) report was created to assess the amount and quality of the reads. Next, we used the Agalma v0.5.0 (Dunn et al 2013) transcriptome pipeline to assemble the reads on the Smithsonian's high performance computing cluster, Hydra-3, using 30 GB of memory on 2 CPUs. At the conclusion of the transcriptome pipeline, an Agalma report summarized the sequence quality and generated graphs to illustrate several attributes of the assembly. Once Agalma was finished, the assembled nucleotide contigs from the Agalma transcriptome pipeline were blasted (blastx) to NCBI non-redundant (nr) database. Blastx output was used in Blast2GO (Conesa et al 2005) to annotate gene functions and assign Gene Ontology (GO) terms. Blast2GO generated graphs to easily visualize gene function in three areas: biological processes, molecular processes, and cellular components (Figures 3-5).

To produce a phylogenetic tree, the twelve previously sequenced transcriptomes from Andrade et al (2014), along with our new Carcinonemertes transcript were processed by OMA (Orthologous Matrix, Altenhoff 2015) to identify homologous sequences (sequences of common ancestry). However, this process remains unfinished at his point.

References

Altenhoff et al., The OMA orthology database in 2015: function predictions, better plant support, synteny view and other improvements. Nucleic Acids Research, 2015, 43 (D1): D240-D249 (doi:10.1093/nar/gku1158).
Andrade, S. C. S., Montenegro, H., Strand, M., Schwartz, M. L., Kajihara, H., Norenburg, J. L., Turbeville, J. M., Sundberg, P., Gribbet, G. (2014). A Transcriptomic Approach to Ribbon Worm Systematics (Nemertea): Resolving the Plidiophora Problem. Molecular Biology and Evolution, 31 (12), 3206-3215. http://doi.org/10.1093/molbev/msu253
Andrews, Simon. FastQC - A Quality Control application for FastQ files. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
Conesa, A., Götz, S., García-Gómez, J. M., Terol, J., Talón, M., & Robles, M. (2005). Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics, 21(18), 3674-3676. http://doi.org/10.1093/bioinformatics/bt1610
Duchêne, D. A., Duchêne, S., Holmes, E. C., & Ho, S. Y. W. (2015). Evaluating the adequacy of molecular clock models using posterior predictive simulations, 1-28.
Dunn, C. W., Howison, M., & Zapata, F. (2013). Agalma: an automated phylogenomics workflow. BMC Bioinformatics, 14, 330. http://doi.org/10.1186/1471-2105-14-330
Kajihara, H., & Kuris, A. M. (2013). Ovicides paralithoidis (Nemertea, Carcinonemertidae), a new species of symbiotic egg predator of the red king crab Parolithodes camtschaticus (Tilesius, 1815) (Decapoda, Anomura). ZooKeys, 258, 1-15. http://doi.org/10.3897/zookeys.258.4260
Thollesson, M., & Norenburg, J. L. (2003). Ribbon worm relationships: a phylogeny of the phylum Nemertea. Proceedings. Biological Sciences / The Royal Society, 270(1513), 407-415. http://doi.org/10.1098/rspb.2002.2254

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