Biodiversity and mitogenomics of deep-sea Euphrosinidae (Annelida)

By Elizabeth Kantzler, Karsyn Whitman, Kevin Kocot
The University of Alabama Deparment of Biological Sciences

Introduction

The deep sea is the least studied habitat on Earth. In remote regions such as the Arctic and Antarctic, little is known about the biodiversity of deep sea organisms, especially those that are small bodied. We have been characterizing the biodiversity of deep sea marine invertebrates from Iceland and Antarctica in order to improve the understanding of deep-sea marine invertebrates living in these remote regions. A specific group of invertebrates that we are researching are the segmented worms (Annelida) from the kigndom Animalia. There is not much present research on annelids today, so phylogenetic relationships among the groups are poorly understood, which is why it is important to research them. In order to improve understanding on the biodiversity of this group, we have been imaging deep-sea specimens of the annelid family *Euphrosinidae* using light and electron microscopy and conducting "DNA barcoding," to sequence the mitochondrial 16S rRNA gene. Further, we sequenced and annotated the first mitochondrial genome from a euphrosinid, the Antarctic *Euphrosinopsis horsti*.

Methods

We extracted the DNA from E. horsti and sent out the DNA for genome sequencing. Once the sequence was obtained, we used Mitos for annotation of the genome and Cogview for images of what it would look like. NCBI blast was used to compare the unannotated intergenic sequences in the NCBI nucleotide data base to find similarities among other organisms.

For the phylogenetic analysis, we extracted DNA, conducted PCR on the 16s gene, and sent the products to UAGC for sequencing. Sequences were aligned and the phylogenetic analysis was conducted in RAxML.

Euphrosinopsis horsti

Figure 1. Scanning electron micrographs of the *E. horsti* anterior (A) and chaetae, spines that cover the body (C). Microscopic image of *E. horsti* ventral body.

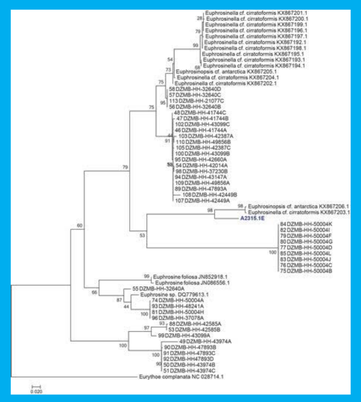

Figure 2. Phylogenetic analysis of 16s sequences from deep sea Icelandic and Antarctic Euphrosinids. Samples collected from the Ice AGE project begin with DZMB. Boot strap values are shown at each node

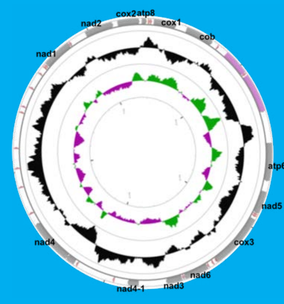

Figure 3. *Euphrosinopsis* complete mitochondrial genome. 24,275 bp

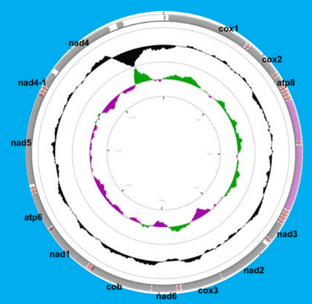

Figure 4. *Eurythoe* complete mitochondrial genome. 15,829 bp.

Results

The phylogenetic analysis of16s (Figure 1) shows severao genetically distinct lineages of Euphrosinidae, based on mitochondrial genomic data. This likely corresponds to undescribed species. Many new clades have been identified, indicating that many new species exist in the arctic waters. We conducted comparative genomic analyses to *Eurythoe complanata,* a close relative of *E. horsti* which has laready existing published data on its genome (Figure 3 and Figure 4) The miitochondrial genome size varies from 15,829 bp (*Eurythoe)* to 24,275 bp *(Euphrosinidae).* All 13 protein coding genes and 2 rRNAs are found in the same order in both genomes. A characteristic of *E. horsti's* genome is that it contains more intergenic sequences than *Eurythoe's.* We hypothesized that these noncoding sequences are a result of viral conservation in the genome over evolutionary history. These sequences were searched against public data bases using NCBI Blast. The data bases showed that these were highly conserved sequences among many organisms, which means that they could potentially be viral. However, there is no conclusive answer as of right now what these requences are. Although is unclear what these sequences are because they are unannotated, we will continue to explore these unknown regions in the future.

Conclusion

Remarkably, *E. horsti* has the largest sequenced mitochondrial genome of any known annelid, which is around 22,000 base pairs. Studies on other complete mitochondrial genomes for annelid families have shown that these families have highly conserved mitochondrial gene orders. The reason for this is because rearrangements rarely occur independently. Both genomes contain the same thirteen protein coding genes. However, their gene arrangement differs. Presently, only forty complete genomes are availble for annelids, when over 9,000 species of annelids exist. This study provides a first step towards research of *E. horsti's* mitochondrial genome. Our results not only give insight towards Euphrosinid's evolutionary history but also provides annotated and sequenced DNA for future researchers. By continuing further research into these species, we can provide more information into mitochondrial genome rearrangements within annelids and help provide additional data to determine the pattern of gene order within annelids.

References

http://mitos2.bioinf.uni-leipzig.de/index.py
http://stothard.afns.ualberta.ca/cgview
https://www.ncbi.nlm.nig.gov/nuccore/KT726962.1
Weigert, Anne. 2015."Evolution of mitochondrial gene order in Annelida". 94. 196-206. *Elsevier.*
Kudenov, Jerry. 1993. "Biology of the Antarctic Seas". 58. 93-150. *Antarctic Research Series.*

Icelandic marine Animals: Genetics and Ecology

ALABAMA MUSEUM OF NATURAL HISTORY
THE UNIVERSITY OF ALABAMA