The Biodiversity of Deep-Sea Icelandic Aplacophoran Molluscs

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Introduction

Aplacophorans are a group of small, spiny, worm-like animals that are closely related to snails and other molluscs. Roughly 415 species have been described, but estimates place the total diversity at over 4000[1]. These animals, though ecologically important in deep-sea habitats and significant to molluscan phylogeny, are difficult to study due to their small size, morphological similarity, and habitat restricted largely to the deep sea. As a result, only five labs worldwide study this group and little is known about its evolutionary history.

To perform this phylogenetic analysis, we sampled 75 specimens spanning the diversity of Aplacophora, imaged them using light and scanning electron microscopy (SEM), and developed a library of sequences for the mitochondrial COI gene. COI encodes a protein subunit essential for eukaryotic metabolism, and thus experiences mutations at a slower rate than most of the mitochondrial genome. Using these sequences, we inferred evolutionary relationships among genera in this group.

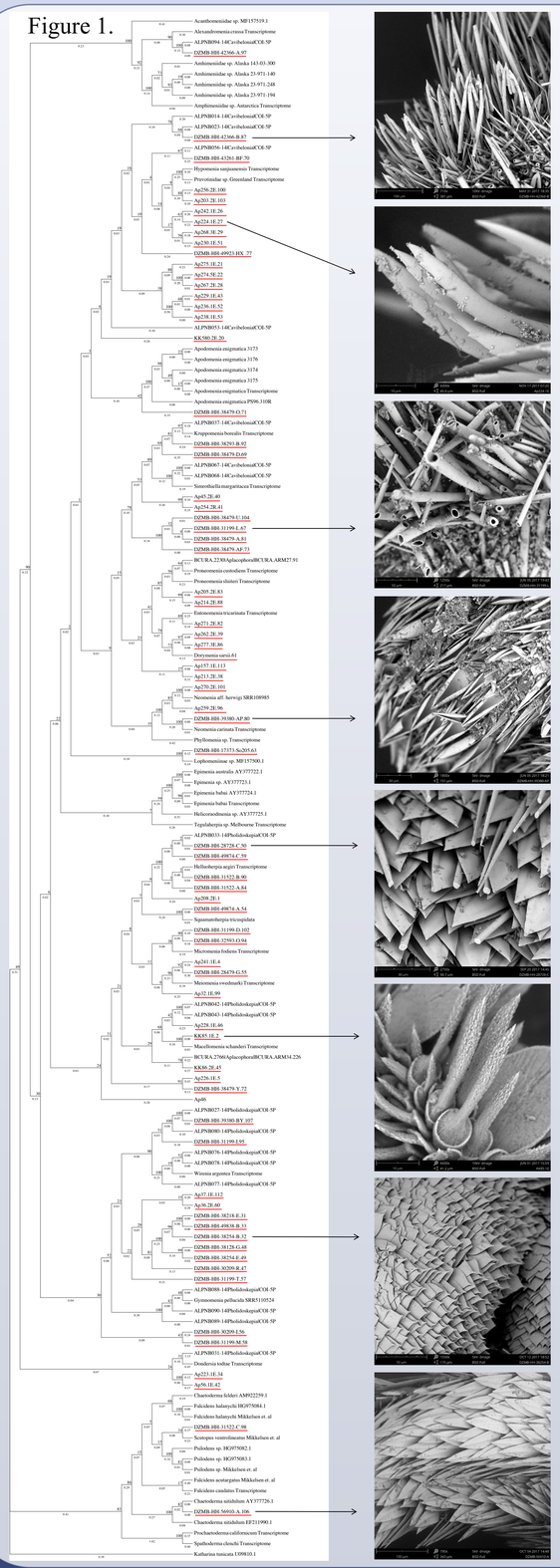
Objectives

The primary objectives of this project were to investigate the biodiversity of Aplacophora and build a DNA barcode database of samples that can be used by scientists in other fields to identify unknown specimens.

Materials and Methods

Samples were collected on the IceAge cruise and selected based on morphological characteristics elucidated by light and SEM. DNA was extracted using the Omega Bio-Tek E.Z.N.A. MicroElute Genomic DNA Kit. A polymerase chain reaction (PCR) was performed on each sample using custom COI primers. PCR products were gel purified using the Omega Bio-Tek E.Z.N.A. MicroElute Gel Extraction Kit. Purified PCR products were sent to the University of Arizona Genetics Core for sequencing. Sequence data were assembled into contiguous sequences and then corrected to form consensus sequences using Sequencher. Consensus sequences were trimmed and aligned to existing COI sequences using MEGA7[2]. A maximum likelihood phylogeny was built using MEGA7 and the GTR-G model with 100 bootstrap replicates.

Figure 1.



Results

Our data (Figure 1, underlined) nearly double the existing number of COI sequences for Aplacophora from 75 to 146, from which the adjacent phylogenetic tree was built. The tree produced from these data (Figure 1), though well-supported at shallow nodes, has weak support for most deeper regions. This is expected, as COI mutates rapidly in comparison to most of the genome, and is therefore valuable for determining diversity on short time-scales, but is less informative for addressing more ancient divergences. To resolve the deeper nodes of our tree, we must include sequence data from more conserved regions of the genome that evolve more slowly.

Although COI sequences fail to create a complete phylogenetic tree without the addition of sequence data from more-conserved nuclear genes, they remain invaluable for specimen identification purposes. These data can serve as "DNA barcodes" for different species that can be used to help scientists in other fields identify unknown specimens.

For example, DNA barcoding confirmed the identity of sample KK85.1E.2, which was hypothesized to be a juvenile of *Macellomenia schanderi* based on initial SEM. Our tree placed *M. schanderi* sister to KK85.1E.2 with a branch length of only 0.01 separating them and a bootstrap value of 100, strongly supporting this hypothesis. Additionally, KK85.1E.2 was run through BLAST (Basic Local Alignment Search Tool) via the NCBI database (National Center for Biotechnology Information), which resulted in a 99% match to *M. schanderi*, further supporting the identification based on phylogenetics and morphology.

Of the 75 samples that were initially sequenced, 71 were shown to be members of Aplacophora. These four outlying samples were other aquatic organisms such as cnidarians and ribbon worms. Although these were not the intended PCR products, these results might actually provide avenues for future research. It is known that some aplacophorans consume cnidarians as part of their diets[1]. It is quite possible that COI sequences representing other clades were not exogenous contamination but rather indicative of that organism's gut contents and therefore its diet. Through continued sequencing of various aplacophorans, the accumulation of "undesired" PCR products might allow for a more in-depth look at the feeding habits of different genera.

Conclusions

Aplacophora, a group of small, worm-like bottom-feeders found mainly in deep-sea habitats, remains one of the lesser-known molluscan classes. Fortunately, recent increases in the amount of available specimens has allowed us to broaden our understanding of the biodiversity of this group.

Initially, the phylogeny of Aplacophora, when viewed via COI sequence data, only contained 75 representative specimens of a class that may contain as many as 4000 distinct species. As a direct result of this work, our phylogenetic understanding of this group via the COI gene has nearly doubled in depth to contain 146 specimens, many of which are likely undescribed.

This large DNA barcode database, combined with our microscopy data, will serve as an important resource for future specimen identification. KK85.1E.2 and *Macellomenia schanderi* is just one example of an integrative taxonomic approach to identification made possible through the development of this DNA barcode database

Future Directions

Future research will focus on increasing the depth and diversity of our DNA barcode database through the addition of 16S rRNA sequences from the same specimens used for this project. 16S is a more conserved region than COI, allowing us to resolve the deeper nodes of our phylogenetic tree and increase the types of sequences that can be used to identify unknown specimens.

Additionally, DNA extractions of key taxa spanning of the diversity of Aplacophora acquired through this project will be selected and used for a target-capture phylogenetic approach. Rather than sequencing a single gene, hundreds of genes will be sequenced. This will allow us to increase our understanding of many different regions of the genome and possibly create and publish the first ever complete aplacophoran genome.

References

1. Todt, C. 2013. Aplacophoran Molluscs—Still Obscure and Difficult?. *American Malacological Bulletin* 31(1): 181-187.
2. Kumar, S. et al. 2016. MEGA7: Molecular Evolutionary genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* 33(7): 1870-1874.