The genome of the chiton *Acathopleura granulata*:

preliminary work toward understanding biomineralization of teeth as tough as tank armor

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Introduction

Chitons (Polyplacophora; **Figure 1**) have a toothed, tongue-like organ called a radula (**Figures 2-3**), which they use to feed by rasping food from hard substrates.

Figure 1: *Acanthopleura granulata,* an emerging model system for biomineralization

Unlike most other molluscs, chiton teeth are further hardened with a coating of iron in the form of magnetite[1]. These remarkably strong teeth have an abrasion resistance comparable to that of tank armor and thus are of great interest to researchers in fields ranging from evolutionary biology to biomechanics.

Acanthopleura granulata is an ideal organism for study, as in addition to an iron-coated radula it bears shell valves with aesthetes and sclerites. The genomic basis of biomineralization in the species is unknown.

Methods

A. granulata were collected from the Florida Keys, dissected, and subsampled. Tissues were stored at -80°C.

DNA was extracted from foot tissue via CTAB/ Phenol/ Chloroform and the Zymo Clean and Concentrator Kit was used to clear samples of low molecular weight DNA and residual chemicals. DNA was sheared with a Covaris M220 to an average size of around 350 bp and an Illumina PCR-free library was prepared. Sequencing was conducted using one lane of Illumina HiSeq X (2X150bp paired-end reads).

Resulting reads were trimmed with TrimGalore[2] and assembled with SPAdes[3] using the "–careful" and "–cov-cutoff auto" flags, assessed via QUAST[4]. K-mer-based estimation of genome size was conducted using estimate_genome_size.pl[5].

For gene model prediction, transcriptomes were sequenced from demineralized shell and radula. Libraries were prepared with the IlluminaTruSeq RNA v2 kit (polyA enrichment) and sequenced on an Illumina HiSeq 2500. Genome assembly was assessed with BUSCO[6]. Genome annotation is currently underway with the MAKER pipeline[7].

Phylogenetic analysis was conducted in RAxML using the PROTGAMMAAUTO amino acid substitution model and optimal number of rapid bootstraps.

The Chiton Radula

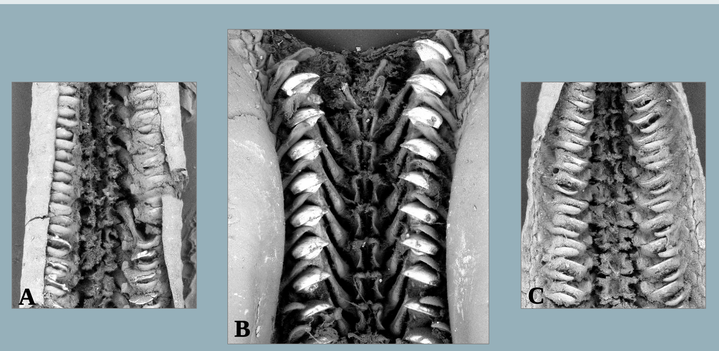

Figure 2: Scanning electron micrographs of the chiton radula from posterior (A), anterior (B), and mid-region (C).

Figure 3: Light microscopy of the chiton radula (D) and the transitional zone of iron mineralization (E). Scale bar = 1 mm.

Preliminary Genomic Data

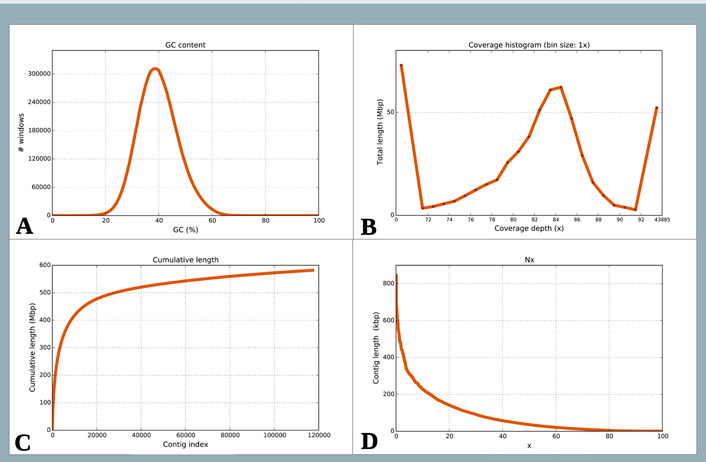

Figure 4: GC content (A), coverage (B), cumulative length (C) and contig length (D).

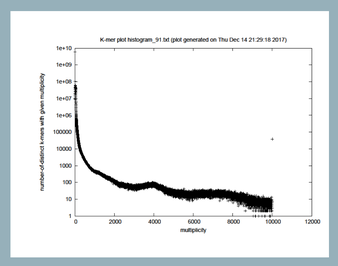

Figure 5: Histogram of k-mer frequency (91 bp), showing likely high heterozygosity

Size (flow cytometry)	743 Mbp
Size (k-mer-based)	1.1 Gbp
Total reads	410,620,976
Reads after trimming	408,299,582
Average coverage	85X
Scaffolds (>500 bp)	117,286
Contigs (>500 bp)	141,977
Longest scaffold	846,176
Longest contig	482,223
Scaffold N50	36,064
Scaffold N75	7,648
BUSCO assessment	901/978

Table 1: Genome assembly and annotation statistics

Iron Transport

As the major iron transport protein in chitons, ferritin is hypothesized to play a significant role in localizing iron to the radula.

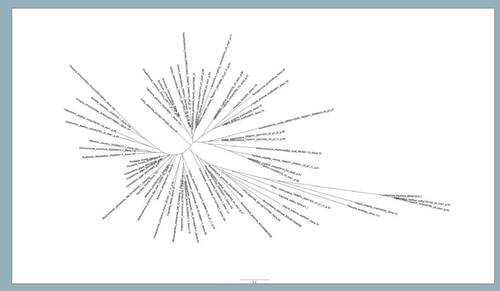

Figure 6: RAxML tree of ferritin sequences from available genomes, transcriptomes, and *A. granulata*

Comparison of gene expression between radula and shell indicated several highly expressed transcripts with potential roles in iron biomineralization, notably multivesicular body protein 6 (FPKM = 28438.21), known to be involved in endosome formation.

Future Work

Long-read sequencing data will be obtained using the Oxford Nanopore GridIon platform.

Differential gene expression will be examined among four regions of the radula (posterior, transitional zone of iron mineralization, fully magnetite-coated, and anterior active teeth) to identify candidate genes involved in iron biomineralization.

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Acknowledgements

We thank Kyle Roebuck for providing genome size estimates, Kim Lackey for critical point dryer training, and the Keys Marine Lab for facilitating field work.