Metagenomics reveals diverse host-specific photosymbionts in Okinawan large benthic foraminifera

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Background

Large benthic foraminifera (LBF) are single celled eukaryotes thriving in reef flats. They can create a cm-wide calcium carbonate shell which makes them some of the largest single cell organisms. LBF can host a wide range of symbionts (archaea, cyanobacteria, bacteria, eukaryotes…) likely involved in calcification, UV protection and metabolism.

Dinoflagellate-hosting Amphisorus *spp., red-algae hosting* Peneroplis *spp.,* Baculogypsina sphaerulata, Calcarina gaudichaudii *and* Amphistegina lobifera *(diatom hosting)*

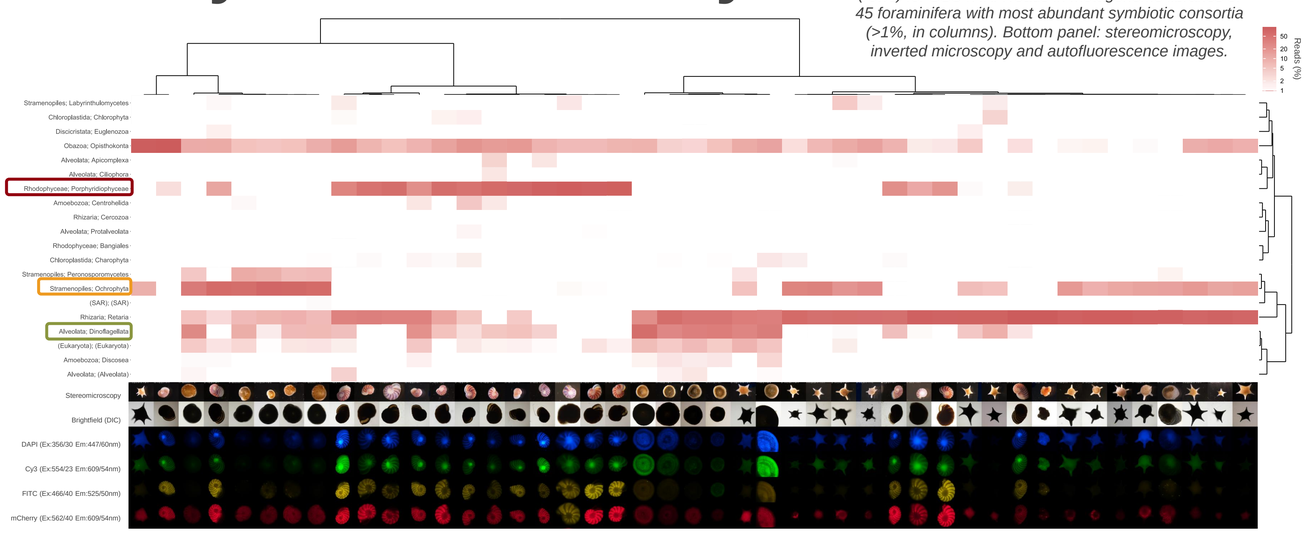
Sand particles in Kouri Island dominated by LBF's shells

Abstract

Metagenomics confirmed that the symbiosis in LBF is species-specific with different foraminifera hosting distinct clades of symbionts. Surprisingly, it also revealed that some dinoflagellates can co-exist with other symbionts in diatoms- and red algae-hosting species. Phylogenetic analysis confirmed that dinoflagellates belong to the Symbiodiniaceae family, red algal symbionts were part of the *Porphyridium* genus and that diatoms from different host species belonged to multiple *Nitzschia* clades.

1 - Symbiont diversity

Fig. 1 – top panel: heatmap of taxonomic assignments (rows) for SSU rRNA reads in metagenome libraries of 45 foraminifera with most abundant symbiotic consortia (>1%, in columns). Bottom panel: stereomicroscopy, inverted microscopy and autofluorescence images.



Beside Opisthokonta (mainly dominated by Fungi), red algae (Porphyridiophycae), diatoms (Ochrophyta) and dinoflagellates (Dinoflagellata) were the most abundant eukaryotic symbionts found in Okinawan LBF. Interestingly, diatoms and red algae rarely co-occurred in the same host, but could be found together with dinoflagellates. Fluorescence imaging in red-algae hosting *Peneroplis* spp. showed different autofluorescence patterns than those observed in diatom and dinoflagellate hosting species suggesting that the photo-symbionts harbor diverse photosynthetic pigments.

2 - Symbiont phylogeny

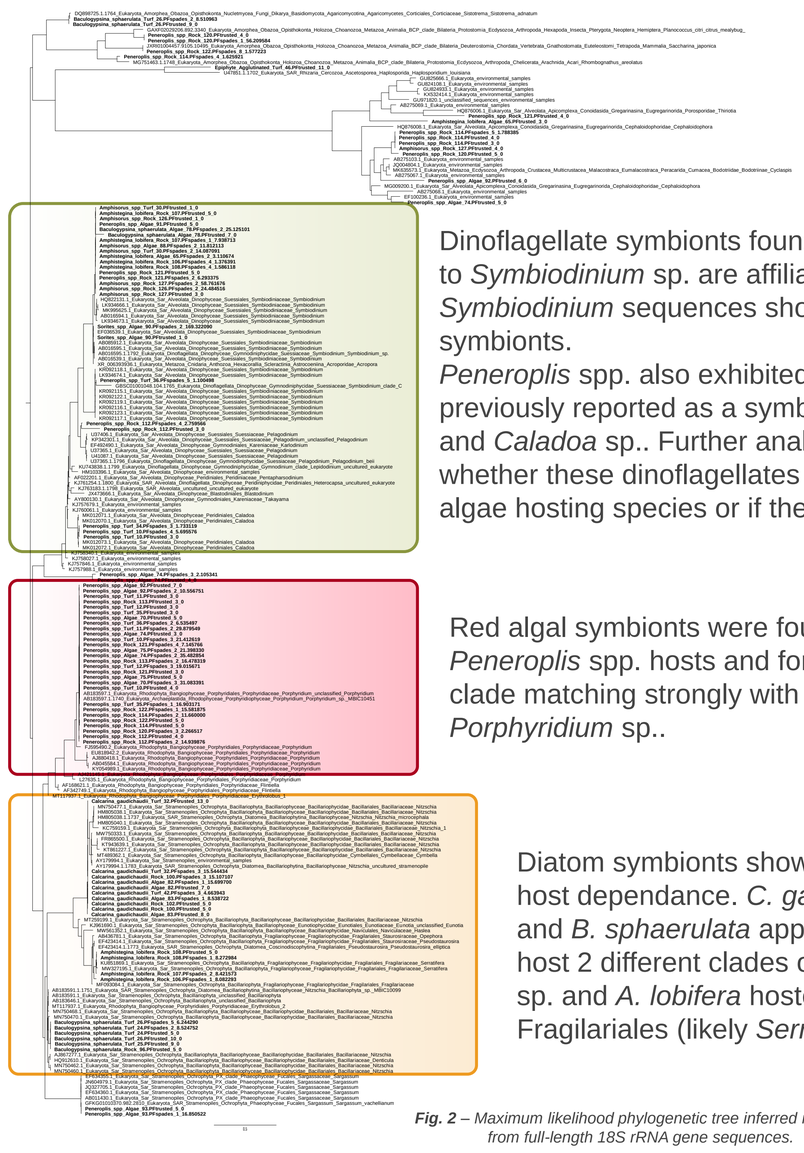
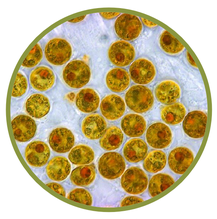


Fig. 2 – Maximum likelihood phylogenetic tree inferred in IQTree from full-length 18S rRNA gene sequences.



Symbiodinium (Baums et al., 2014)

Dinoflagellate symbionts found in Okinawan LBF mainly belong to *Symbiodinium* sp. are affiliated to *Symbiodinium* clade C. *Symbiodinium* sequences showed proximity to *Acropora* symbionts.
Peneroplis spp. also exhibited *Pelagodinium* sp. - which was previously reported as a symbiont of planktonic foraminifera – and *Caladoa* sp.. Further analyses are needed to determine whether these dinoflagellates are symbionts in diatom and red algae hosting species or if they were only present as prey.

Red algal symbionts were found solely in *Peneroplis* spp. hosts and form a single clade matching strongly with *Porphyridium* sp..

Porphyridium purpureum (Unknown source)

Diatom symbionts showed a strong host dependance. *C. gaudichaudii* and *B. sphaerulata* appeared to host 2 different clades of *Nitzschia* sp. and *A. lobifera* hosted Fragilariales (likely *Serratifera* sp.).

Nitzschia sp. (landcareresearch.co.nz)

3 - Single-cell metagenomes

Amphisorus sp.

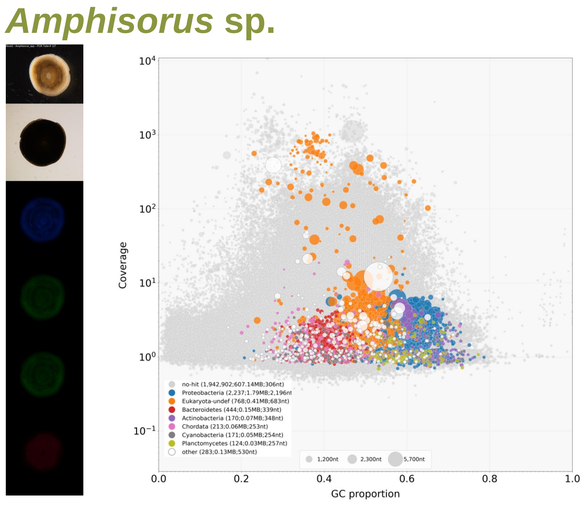
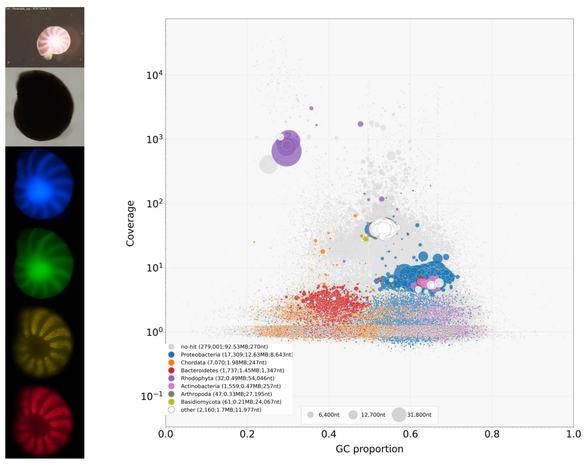


Fig. 3 – blobplot showing read coverage, GC content and size of each scaffold. Size of the blobs corresponds to size of the scaffold and color corresponds to taxonomic assignment based on a blast search against the nt database.

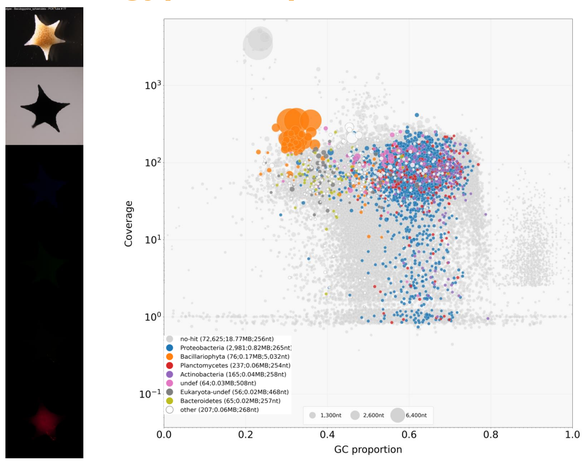
Symbiodiniaceae contain secondary plastids that are highly reduced and fragmented into minicircles. Their identification and assembly is therefore challenging and in progress.

Peneroplis sp.



Red algal plastid genomes are large and gene-rich. The purple blobs in the top left corner are well-assembled plastid and mitochondrial contigs.
Their complete assembly will later allow us to better understand their photosynthesis functioning.

Baculogypsina sphaerulata

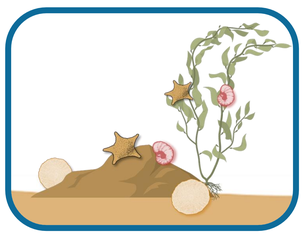


The grey and orange blobs in the top left corner are the plastid and mitochondrial contigs of the diatom symbiont.

We have also identified several eukaryotic DNA viruses likely infecting the diatom symbionts or the foraminiferal host.

Perspectives

To better understand the functional role of the LBF symbionts we are now studying their effects on:



Diversity and distribution *in situ*

Metabolic response to light

Behavior (phototaxis and geotaxis)

Methods

Sampling: Foraminifera were sampled from the Ikei Island reef flat in Oct. 2021. Living specimens were isolated, cleaned in microfiltered seawater, imaged (brightfield and autofluorescence) and frozen in microtubes at -70°C.
DNA isolation: Single-cells were crushed using a sterile stainless-steel pestle and lysed with a series of freeze/thaw cycles in liquid nitrogen. DNA was isolated using Lucigen's MasterPure complete DNA and RNA Purification Kit.
Sequencing: Sequencing libraries were prepared with the NEB NextUltra II kit and sequenced with 150bp paired-end reads on a single lane of the Illumina NovaSeq6000 sequencer.
Bioinformatics: Symbiont diversity was analyzed with PhyloFlash using 16S and 18S rRNA gene reads. Metagenomes were assembled with SPAdes using default metagenome settings and analyzed with Blobtools.

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