EUKARYOTIC DIVERSITY OF MARINE SPONGES IN BIDONG ISLAND

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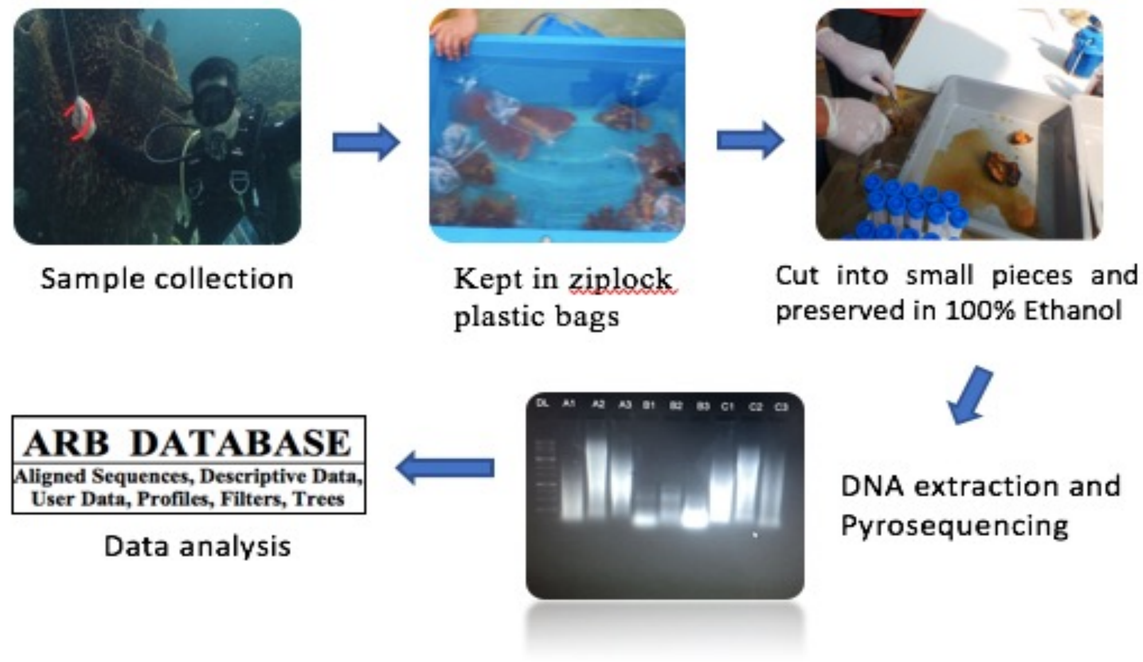
INTRODUCTION

Marine sponges have attracted substantial research interest because of their ecological importance and their production of a wide range of bioactive compounds for biotechnological applications. There is evidence that the majority of these compounds are probably not produced by the sponge itself, but rather by microorganisms that are associated with their host (Indraningrat et al., 2016). Sponges are sessile, benthic organisms that are known to host diverse microorganisms including Archaea, Bacteria and Eukarya. Prokaryotic microbial communities associated with sponges have been well studied by both cultivation-dependent and cultivation-independent approaches (Hentschel et al. 2006), whereas eukaryotic microbial are the last frontier of microbial diversity yet to be fully characterized. The aim of this research was to explore the eukaryotic diversity of marine sponges from Bidong Island, based on molecular analysis using Hiseq 2500 Illumina Sequencing out in order to better explore the diversity of eukaryotes associated with different sponge species from Bidong island. We also compared the sponge-associated eukaryotic communities with those present in the surrounding seawater to assess the influence of environmental conditions. Through this research, the eukaryotic community structure was able to be determined and assessed.

METHODOLOGY



FINDINGS

Most of the early studies on sponge–microbe associations heavily relied on morphological descriptions using microscopy technique and numerical taxonomy based on cultivation. These studies are generally limited by the fact that microbes are largely indistinguishable by morphology and most of them are not cultivable. However, the advent of next generation sequencing have made it possible to study sponge-associated fungi by generating millions of raw sequence reads and separating between the sponge reads and fungal reads (Naim et al., 2015).

After DNA sequence quality filtering, a total of 896880 partial 18S rRNA non-chimeric reads were retained. For deeper phylogenetic analysis, OTUs obtained as singletons and those were only found in the seawater were disregarded, leaving a final set of 102 eukaryotic OTUs detected in sponges and seawater which consist of fungi (21%), Stramenopiles (16%), Rhizaria (16%), Metazoa (15%), Holozoa (13%), Animalia (11%), Alveolata (3%) and unclassified OTUs (5%) (**Figure 1**).

Comparison between the relative abundance of sponges, fungi and other eukaryotes 18S rRNA gene sequences in sponge specimens and seawater, showed that most of the sequence reads were identified as sponge (**Figure 2**). For most sponges, fungi were present at very low relative abundance of the 18S rRNA gene reads compared to the seawater.

Further data analysis using Arb software can identified the eukaryotic OTUs found in sponges into deeper phylogeny. Based on the phylogenetic tree (**Figure 3**), OTU82 and OTU102 can be potential novel fungi as they formed separate branches from the closest strains. Among the fungal OTUs detected in the sponges were dominated with the phylum Ascomycota and Basidiomycota (**Table 1**). Xylariales (Ascomycota) and Malasseziales (Basidiomycota) were found relatively abundance in Sponge B1.

RESULTS

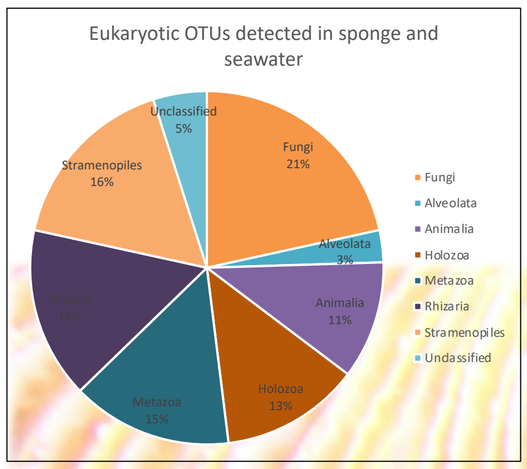


Figure 1: The eukaryotic OTUs in sponge and seawater.

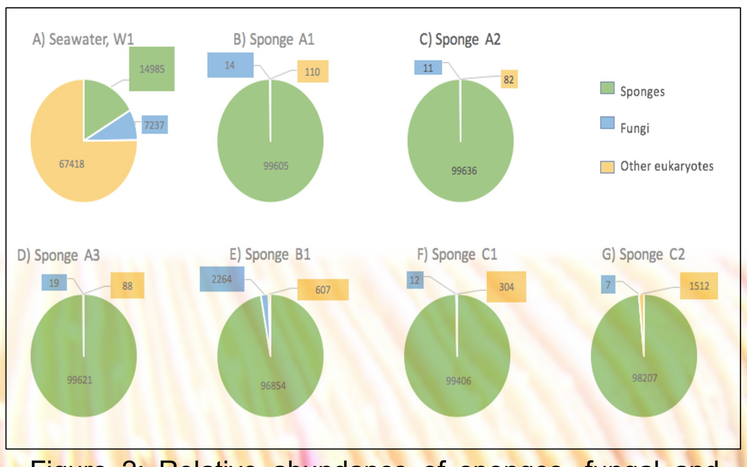


Figure 2: Relative abundance of sponges, fungal and other eukaryotic 18S rRNA gene sequences for sample types from Bidong Island.

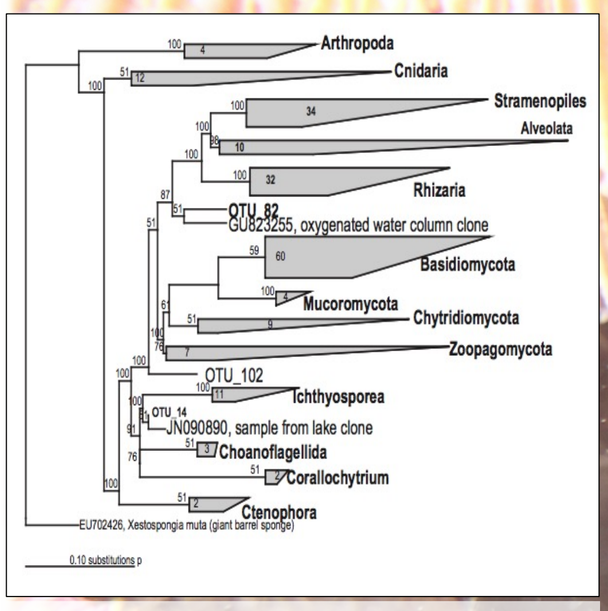
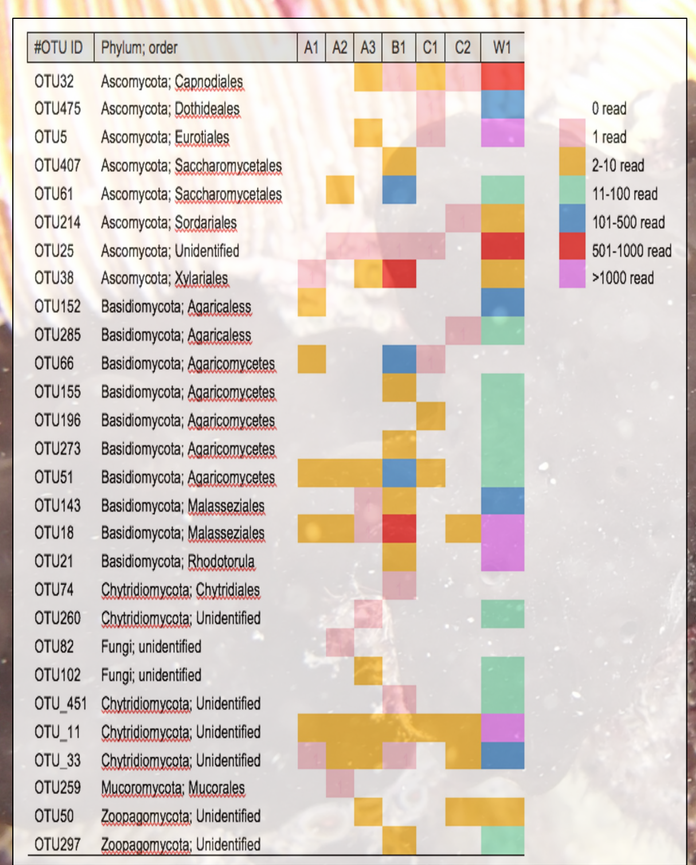


Figure 3: Bayesian Phylogram of eukaryotic OTUs foung in sponges based on 18S rRNA gene sequences.



#OTU ID	Phylum; order	A1	A2	A3	B1	C1	C2	W1
OTU32	Ascomycota; Capnodiales							
OTU475	Ascomycota; Dothideales							
OTU5	Ascomycota; Eurotiales							
OTU407	Ascomycota; Saccharomycetales							
OTU61	Ascomycota; Saccharomycetales							
OTU214	Ascomycota; Sordariales							
OTU25	Ascomycota; Unidentified							
OTU38	Ascomycota; Xylariales							
OTU152	Basidiomycota; Agaricales							
OTU285	Basidiomycota; Agaricales							
OTU66	Basidiomycota; Agaricomycetes							
OTU155	Basidiomycota; Agaricomycetes							
OTU196	Basidiomycota; Agaricomycetes							
OTU273	Basidiomycota; Agaricomycetes							
OTU51	Basidiomycota; Agaricomycetes							
OTU143	Basidiomycota; Malasseziales							
OTU18	Basidiomycota; Malasseziales							
OTU21	Basidiomycota; Rhodotorula							
OTU74	Chytridiomycota; Chytridiales							
OTU260	Chytridiomycota; Unidentified							
OTU82	Fungi; unidentified							
OTU102	Fungi; unidentified							
OTU_451	Chytridiomycota; Unidentified							
OTU_11	Chytridiomycota; Unidentified							
OTU_33	Chytridiomycota; Unidentified							
OTU259	Mucoromycota; Mucorales							
OTU50	Zoopagomycota; Unidentified							
OTU297	Zoopagomycota; Unidentified							

0 read
1 read
2-10 read
11-100 read
101-500 read
501-1000 read
>1000 read

Table 1: Heatmap fungal OTUs in marine spones and seawater from Bidong island.

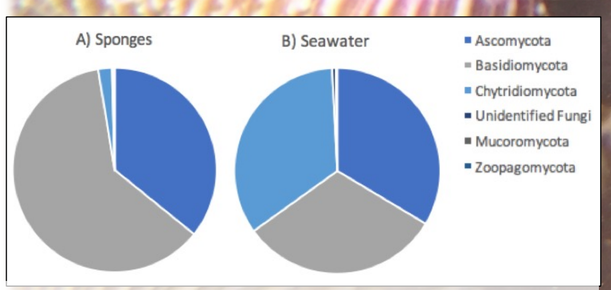


Figure 4: Relative abundance of fungal phyla in the cumulative sponge and seawater samples.

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CONCLUSION & FUTURE WORK

In conclusion, we detected diverse eukaryotes in marine sponges of Bidong island using molecular approach. Based on the data shown, there are indications that we know very little about the sponge-associated eukaryotes because there is little to be known. A better understanding of sponge-eukaryotic relationships would benefit from further exploring these two known relationships.

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