Annals of Microbiology (2019) 69:861–866
https://doi.org/10.1007/s13213-019-01476-5

SHORT COMMUNICATION

# A comparison of microeukaryote communities inhabiting sponges and seawater in a Taiwanese coral reef system

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Received: 15 February 2019 / Accepted: 12 April 2019 / Published online: 16 May 2019


## Abstract

**Purpose** Assess microeukaryote community composition in seawater and sponge samples from Taiwanese coral reefs.

**Methods** In the present study, we used Illumina sequencing to explore the microeukaryote communities of seven biotopes (six sponge species and seawater) sampled in the Penghu archipelago of Taiwan.

**Result** Microeukaryote communities were dominated by Dinoflagellates with Dinophyceae and Syndiniales well represented in all biotopes. Other abundant taxa included metazoa, red and green algae and Radiolaria. The only significant differences were a significantly higher relative abundance of Picobiliphyta and Stramenopiles_X in seawater and Metamonada in the sponge *Acanthostylotella cornuta*. There was also a significant difference in composition among biotopes with samples from sponges and seawater forming distinct clusters. There was, however, no congruence between prokaryote and microeukaryote community composition. After removing all OTUs < 100 sequences, more than 90% of remaining OTUs representing > 99.5% of sequences were shared between sponge and seawater samples.

**Conclusion** This data in the present study would appear to suggest that marine microeukaryote communities in sponges are largely derived from the surrounding seawater. Abundant OTUs were also related to organisms previously retrieved from seawater. A number of these OTUs though had relatively low sequence similarity to organisms in GenBank suggesting that more research of the microeukaryote communities in the Penghu archipelago may yield novel organisms in this relatively unexplored area.

**Keywords** Agelasidae · Composition · Coral reefs · Illumina · Penghu islands

## Findings

Sponges are sessile, benthic and mainly filter-feeding organisms (Diaz and Rützler 2001) that can be found in several aquatic habitats (freshwater, marine, warm or cold water). They are involved in the bioerosion and consolidation of the coral reef carbonate framework, modification of the water column due to filtration and secondary metabolite emanation and benthic-pelagic coupling (Bell 2008; Maldonado et al. 2016). Sponges are also noteworthy for the range and abundance of natural products (Hochmuth et al. 2010) and their prokaryote diversity and abundance (Taylor et al. 2007), which are widely accepted to be inter-connected (Piel 2009). In addition to being involved in the biosynthesis of secondary metabolites (Fan et al. 2012; Hentschel et al. 2012), sponge microbial communities also play a critical role in the sponge metabolism (Hentschel et al. 2006; Taylor et al. 2007). Prokaryotes are not the only inhabitants of the sponge holobiont. Another important, but largely overlooked group is the sponge microeukaryote community. Relatively little research has focused on this group compared to the relatively well-known prokaryote community (Rodríguez-Marconi et al. 2015).

In the present study, we used Illumina sequencing to explore and compare the microeukaryote communities of 6 sponge species and seawater collected in the Penghu archipelago, Taiwan from 25 to 29 of July, 2014 (Supplementary

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s13213-019-01476-5) contains supplementary material, which is available to authorized users.

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Table 1; see Huang et al. (2016) and Coelho et al. (2018) for detailed descriptions of the sampling area). The sponge species sampled were *Acanthostylotella cornuta*, *Agelas cavernosa*, *Echinodictyum asperum*, *Hyrtios erectus*, *Stylissa carteri* and *Suberites diversicolor*. Two to three replicates were sampled per species. Sponges were photographed in situ, collected using scuba diving, brought back to the laboratory and preserved in 95% ethanol for further identification and molecular work. All specimens have been deposited at the Naturalis Biodiversity Center, the Netherlands. Our main objectives were to (1) test for compositional differences among samples from different biotopes (sponge species and seawater) and (2) test for significant compositional congruence between prokaryote and microeukaryote communities.

Prokaryotes and microeukaryotes were sequenced, but the present study will mainly focus on microeukaryotes and only use the prokaryote data to test for compositional congruence between both groups. Briefly, for prokaryotes, the 16S rRNA gene V3V4 variable region was amplified with PCR primers 341F 5′-CCTACGGGNGGCWGCAG-3′ and 785R 3′-GACT ACHVGGGTATCTAATCC-5′ and with barcode on the forward primer. For microeukaryotes, 18S rDNA gene fragments were amplified using primers TAReuk454FWD1 (5′-CCAG CA(G/C)C(C/T)GCGGTAATTCC-3′ and TAReukREV3 (5′-ACTTTCGTTCTTGAT(C/T)(A/G)A-3′ (Stoeck et al. 2010). DNA extraction and sequencing using an Illumina MiSeq device followed Coelho et al. (2018) and Swierts et al. (2018). Resultant files were analysed using QIIME (Quantitative Insights Into Microbial Ecology; (Caporaso et al. 2010) (http://www.qiime.org/; last checked 2017-01-20) and UPARSE (Edgar 2013) with OTU selection at 97% similarity cut-off. Taxonomy for microeukaryotes was assigned using the PR2 database (http://ssu-rrna.org/pr2) (Guillou et al. 2013) and for prokaryotes using the SILVA_128_QIIME_release database (Quast et al. 2013). All statistical analyses were performed in the R environment (R Core Team 2013). For a more detailed description of the sequencing and statistical analyses, see Coelho et al. (2018), Cleary et al. (2018) and Cleary and Polónia (2018). The sequences generated in this study can be downloaded from the NCBI SRA: SRP109605 (SUB2790757; SUB4184084).

Sequencing yielded 1093775 sequences binned into 2882 microeukaryote OTUs after quality control. OTU richness varied widely among and within biotopes with the most prominent feature, a higher richness for samples collected in the southern Penghu islands including a much higher richness for the water sample collected in the southern islands versus both water samples collected in the northern islands (Supplementary Table 1). The most abundant taxa overall was the phylum Dinophyta (dinoflagellates, 545564 sequences; 1593 OTUs) followed by the kingdom Metazoa (302301 sequences; 286 OTUs), phylum/division Chlorophyta (green algae, 89355 sequences; 96 OTUs), phylum Rhodophyta (red algae, 88745 sequences; 244 OTUs), the subphylum Radiolaria (phylum Retaria, 24724 sequences; 109 OTUs) and the phylum Apicomplexa (16205 sequences; 71 OTUs) (Supplementary Fig. 1).

Relative abundance varied considerably among and within biotopes, but this variation was only significant for Picobiliphyta (Fig. 1h), Stramenopiles_X (Fig. 1j) and Metamonada (Fig. 1u) after applying a Bonferroni correction for multiple comparisons. The relative abundance of OTUs assigned to the Picobiliphyta and Stramenopiles_X was significantly higher in seawater than other biotopes, whereas the relative abundance of OTUs assigned to the Metamonada was significantly higher in *A. cornuta* than all other biotopes (Supplementary Table 2). Microeukaryote OTU richness was highest in *E. asperum* and lowest in *S. diversicolor* (Fig. 1x). Evenness, in turn, was highest in seawater and lowest in *A. cornuta* (Fig. 1w).

There was a highly significant difference in microeukaryote composition among biotopes (Adonis, $F_{6,11} = 1.73$; $P < 0.001$; $R^2 = 0.486$; Fig. 2a). The first axis separated samples of sponges and water collected from the northern Penghu islands at positive axis 1 values from samples of sponges and water collected in the southern Penghu islands at negative axis 1 values (Supplementary Table 1). The only exception was a single specimen of *H. erectus* collected in Si Ji Yu (southern Penghu). The second axis separated samples of water from samples of sponges. There was no significant congruence between prokaryote (Fig. 2b) and microeukaryote (Fig. 2a) composition (Fig. 2c; Procrustes correlation = 0.386, $P = 0.130$).

A number of OTUs significantly discriminated (Simper analysis; $P < 0.01$) between pairs of biotopes (Supplementary Fig. 2). By far, the most abundant OTU was OTU-3, which significantly discriminated between *A. cornuta* and *Suberites sp.* (Simper analysis, $P < 0.001$; Supplementary Table 3). OTU-3 was assigned to the coral genus *Montipora* (Supplementary Table 4). OTUs 36 and 306 significantly discriminated between seawater and all sponge species (Simper analysis, $P < 0.05$). OTUs 36 and 306 were assigned to the Dinophyceae and closely related to organisms obtained from seawater (Supplementary Table 4). OTU-59, assigned to the Dinophyceae, significantly discriminated between the sponge species *A. cornuta* and all other biotopes (Simper analysis; $P < 0.05$; Supplementary Table 3). OTU-8, assigned to the Syndiniales, significantly discriminated between *H. erectus* and the sponge species *A. cavernosa* and *S. diversicolor* in addition to seawater. The highly variable nature of OTUs within biotopes can be seen in Supplementary Figs. 2 and 3.

The microeukaryote OTU subset of all OTUs < 100 sequences ($OTUs_{100}$) included 390 $OTUs_{100}$ and 1059147 sequences (96.8% of all sequences). Of the 390 $OTUs_{100}$, 363 (93.1%) representing 1053869 sequences (99.5%) were found in seawater samples and 27 (278 sequences; 0.5% of total sequences) were only found in sponge samples. After

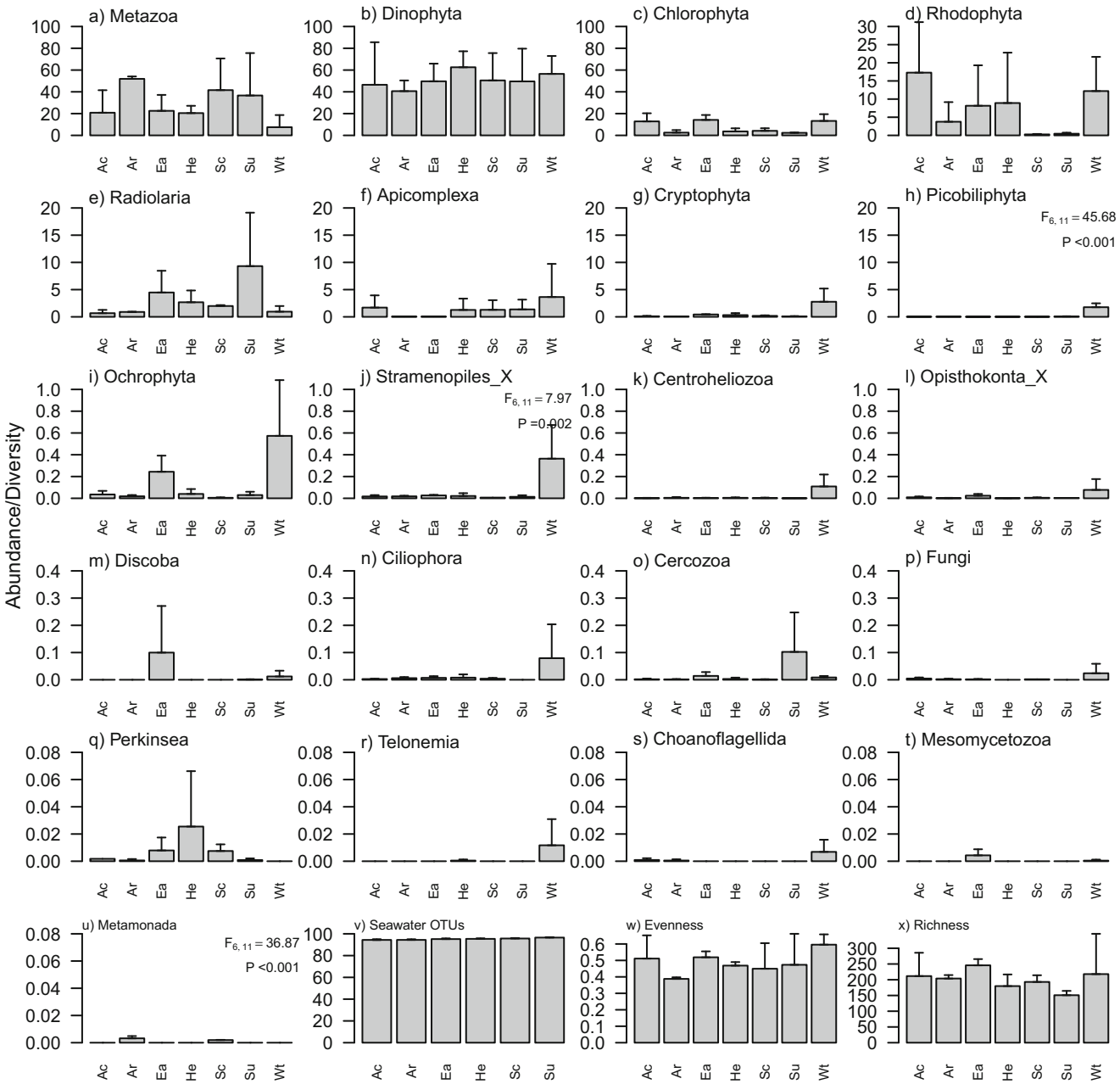


**Fig. 1** Relative abundance and diversity (evenness and rarefied OTU richness) of the most abundant eukaryote taxa for the sponges: Ac, *Agelas cavernosa*; Ar, *Acanthostylotella cornuta*; He, *Hyrtios erectus*; Xt, *Xestospongia testudinaria*, Ea, *Echinodictyum asperum*; Sc, *Stylissa carteri*; Su, *Suberites diversicolor* and Wt water. **a** Metazoa. **b** Dinophyta. **c** Chlorophyta. **d** Rhodophyta. **e** Radiolaria. **f** Apicomplexa. **g** Cryptophyta. **h** Picobiliphyta. **i** Ochrophyta. **j** Stramenopiles_X. **k** Centroheliozoa. **l** Opisthokonta_X. **m** Discoba. **n** Ciliophora. **o** Cercozoa. **p** Fungi. **q** Perkinsea. **r** Telonemia. **s** Choanoflagellida. **t** Mesomycetozoa. **u** Metamonada. **v** Seawater OTUs. **w** Evenness. **x** Richness

controlling for multiple tests, there was no significant difference in the relative abundance of seawater OTUs among sponge species (Fig. 1v).

Dinoflagellates were by far the most abundant eukaryote taxon in terms of relative abundance and number of OTUs. Both of the major taxa within the dinoflagellates, the Dinophyceae and Syndiniales were well represented in all biotopes. Dinoflagellates are dominant unicellular members of marine and freshwater plankton with high morphological and trophic diversity that play critical roles as predators, primary producers, parasites and symbionts. They are also responsible for toxic algal blooms with deleterious effects to aquatic and human life (Gómez 2012; Price and Bhattacharya 2017). Only one abundant OTU, OTU-28, was assigned to the genus *Symbiodinium*, and was highly similar to an organism identified as *Symbiodinium goreaui* strain CCATM-210 obtained

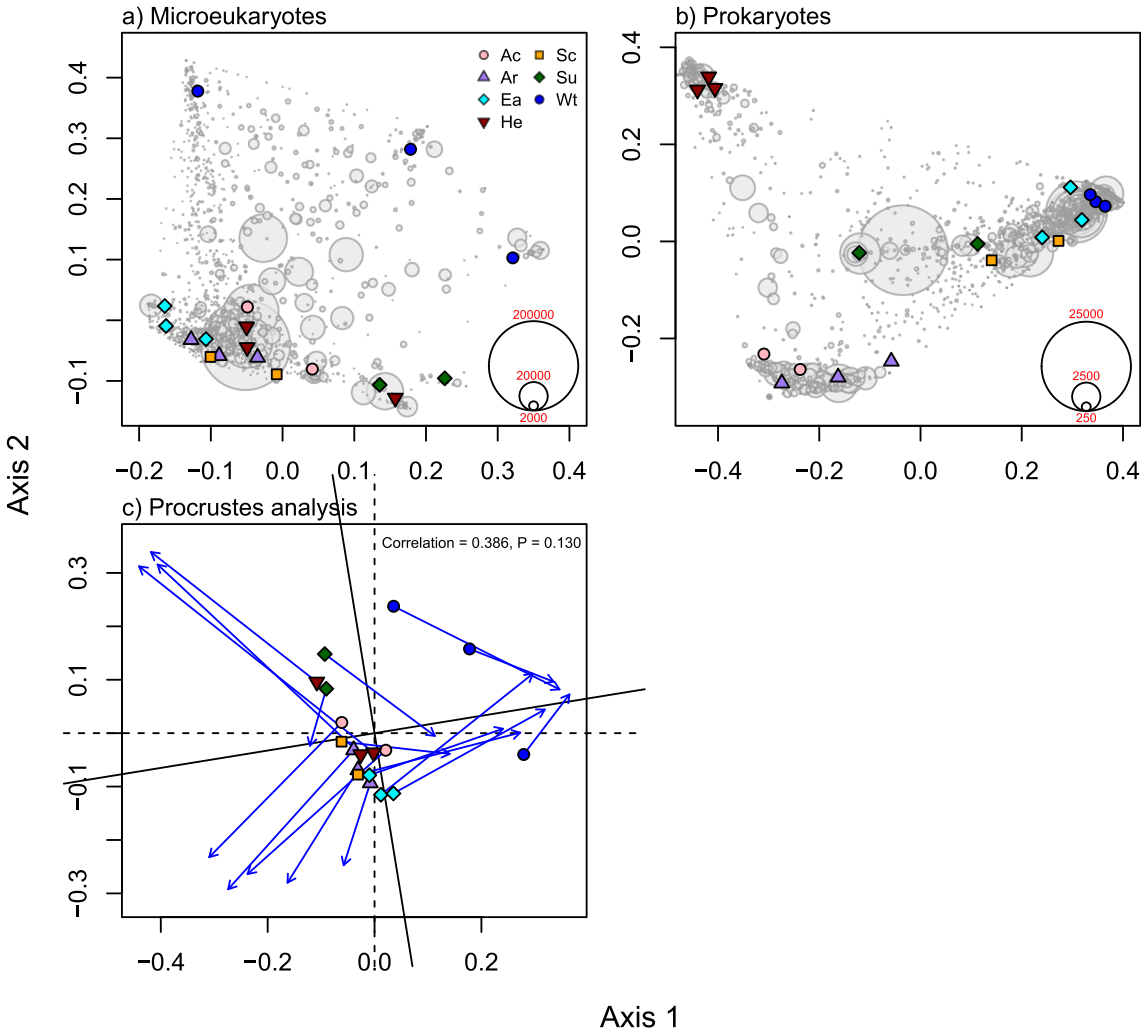


**Fig. 2** Ordination showing the first two axes of the principal coordinates analysis (PCO) of **a** microeukaryote OTU composition, **b** prokaryote composition and **c** Procrustes analysis comparing (a) microeukaryote and (b) prokaryote OTU composition with the procrustes() function in the vegan package in R. The arrow bases indicate the corresponding positions of the samples in the microeukaryote map while the arrowheads indicate the corresponding positions of the samples in the prokaryote map. Ac, *Agelas cavernosa*; Ar, *Acanthostylotella cornuta*; He, *Hyrtios erectus*; Xt, *Xestospongia testudinaria*, Ea, *Echinodictyum asperum*; Sc, *Stylissa carteri*; Su, *Suberites diversicolor*; Wt, water

from Iranian seawater (Supplementary Table 4). Although well known as symbionts of scleractinian hosts, species of *Symbiodinium* inhabit a wide range of metazoan hosts (including foraminifera, black corals and sponges) where they perform important functional tasks including transmembrane transport and translocation of photosynthesis products to their host; they have also been shown to be enriched for functions including reactive oxygen species response and UV radiation protection (González-Pech et al. 2017; Ramsby et al. 2017). Although stable associations with species are known from just a few sponge species, Strehlow et al. (2016) showed that normally non-symbiont-bearing sponge species were able to acquire and maintain *Symbiodinium* for a number of days after experimental inoculation. Wecker et al. (2015) also recorded dinoflagellates including *Symbiodinium* from a number of nudibranchs and sponges in French Polynesia. *Symbiodinium* Clade C was found in the sponge species *Lamellodysidea herbacea* while other Dinophyceae members were recorded in the sponge species *Haliclona* sp., *Phycopsis* sp. and *Flabellina* sp. Dinoflagellates have also been recorded in freshwater sponges inhabiting lake Baikal including sequences assigned to the order Suessiales, known for establishing symbiotic relationships with other invertebrates (Annenkova et al. 2011). Other numerically important taxa included metazoa, red and green algae and Radiolaria.

Although there was compositional variation in eukaryote composition among biotopes, the degree of differentiation was less pronounced than that observed for prokaryote communities. In contrast to prokaryote communities, there was a pronounced effect of sampling location (northern versus southern

islands). This would appear to suggest that microeukaryote communities in sponges are more susceptible to local environmental conditions than prokaryote communities. A more detailed and well-designed sampling strategy is, however, needed to explicitly test this hypothesis.

Our results are also in line with Chaib De Mares et al. (2017) who also found no significant congruence between bacterial and microeukaryote composition and noted that the compositional structure of dinophytes, ciliophores (alveolates) and stramenopiles could not be explained by host sponge or geographic location at large geographic scales (Caribbean versus Mediterranean). In the present study, more than 90% of microeukaryote $OTUs_{100}$ representing more than 99.5% of all sequences were also recorded in the seawater samples. This would appear to suggest that microeukaryote communities in sponges are largely derived from seawater and may just represent environmental DNA. That being said, microeukaryote communities are far less known than prokaryote communities and the relatively low sequence similarities of abundant OTUs sampled in this study to organisms in GenBank suggest that novel taxa remain to be discovered.

**Acknowledgements** Support in the field and lab was provided by Julian Cleary, Floris Cleary, Yusheng Huang, Kate, Ana R.M. Polónia and Nicole J de Voogd.

**Funding** Financial support was provided to CESAM (UID/AMB/50017 – POCI-01-0145-FEDER-007638) and for the project LESS CORAL (PTDC/AAC-AMB/115304/2009) by FCT/MEC through national funds, and co-funding by FEDER, within the PT2020 Partnership Agreement and Compete 2020.

## Compliance with ethical standards

**Conflict of interest** The author declares that he has no conflict of interest.

**Research involving human participants and/or animals** This article does not contain any studies with human participants or laboratory animals.

**Informed consent** Not applicable.

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