

The sponge microbiome project

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Data Note

DATA NOTE

The sponge microbiome project

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Abstract

Marine sponges (phylum Porifera) are a diverse, phylogenetically deep-branching clade known for forming intimate partnerships with complex communities of microorganisms. To date, 16S rRNA gene sequencing studies have largely utilised different extraction and amplification methodologies to target the microbial communities of a limited number of sponge species, severely limiting comparative analyses of sponge microbial diversity and structure. Here, we provide an extensive and standardised dataset that will facilitate sponge microbiome comparisons across large spatial, temporal, and environmental scales. Samples from marine sponges ($n = 3569$ specimens), seawater ($n = 370$), marine sediments ($n = 65$) and other environments ($n = 29$) were collected from different locations across the globe. This dataset incorporates at least 268 different sponge species, including several yet unidentified taxa. The V4 region of the 16S rRNA gene was amplified and sequenced from extracted DNA using standardised procedures. Raw sequences (total of 1.1 billion sequences) were processed and clustered with (i) a standard protocol using QIIME closed-reference picking resulting in 39 543 operational taxonomic units (OTU) at 97% sequence identity, (ii) a *de novo* clustering using Mothur resulting in 518 246 OTUs, and (iii) a new high-resolution Deblur protocol resulting in 83 908 unique bacterial sequences. Abundance tables, representative sequences, taxonomic classifications, and metadata are provided. This dataset represents a comprehensive resource of sponge-associated microbial communities based on 16S rRNA gene sequences that can be used to address overarching hypotheses regarding host-associated prokaryotes, including host specificity, convergent evolution, environmental drivers of microbiome structure, and the sponge-associated rare biosphere.

Keywords: marine sponges; archaea; bacteria; symbiosis; microbiome; 16S rRNA gene; microbial diversity

Data Description

Purpose of data acquisition

Sponges (phylum Porifera) are an ancient metazoan clade [1], with more than 8500 formally described species [2]. Sponges are benthic organisms that have important ecological functions in aquatic habitats [3, 4]. Marine sponges are often found in symbiotic association with microorganisms, and these microbial communities can be very diverse and complex [5, 6]. Sponge symbionts perform a wide range of functional roles, including vitamin synthesis, production of bioactive compounds, and biochemical transformations of nutrients or waste products [7–9]. The diversity of microorganisms associated with sponges has been the subject of intense study (the search of "sponge microbial diversity" returned 348 publications in the Scopus database) [10]. Most of these studies were performed on individual species from restricted geographic regions [e.g., 11, 12]. A comparative assessment of these studies is often hindered by differences in sample processing and 16S rRNA gene sequencing. However, 2 recent studies incorporating a large number of sponge microbiomes (>30) [5, 13] revealed the potential of large-scale, standardised, high-throughput sequencing for gaining insights into the diversity and structure of sponge-associated microbial communities. The purpose of this global dataset is to provide a comprehensive 16S rRNA gene-based resource for investigating and comparing microbiomes more generally across the phylum Porifera.

Sample collection, processing, and 16S rRNA gene sequencing

Sample collection and processing, species identification, and DNA extractions were conducted as previously described [13]. A total of 3569 sponge specimens were collected, representing at least 268 species, including several yet unidentified taxa (hereafter collectively referred to as species) (Supplementary Table S1). Of all species, 213 were represented by at least 3 specimens. *Carteriospongia foliascens* had the highest replication, comprising

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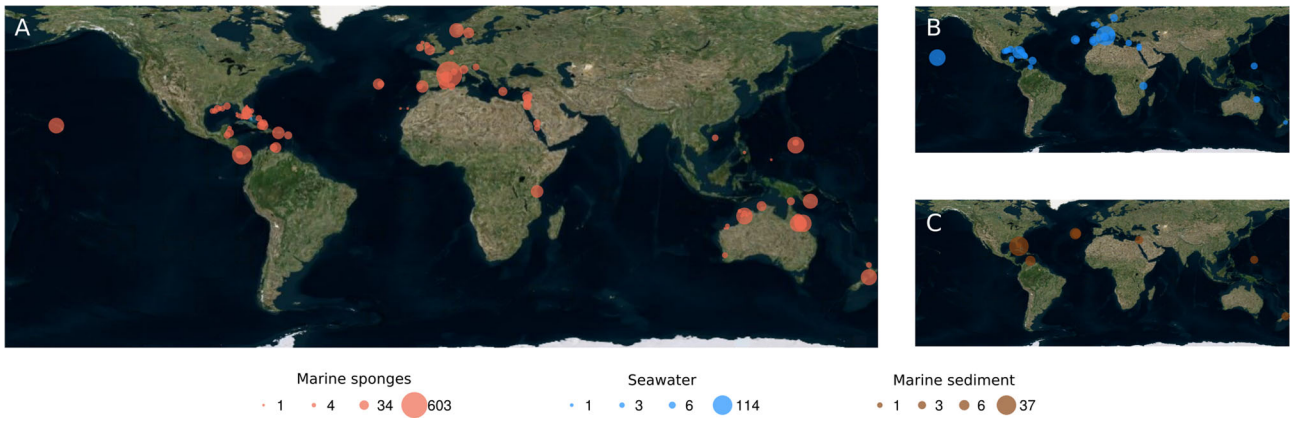


Figure 1: Global sample collection sites. Bubbles indicate collection sites of (**A**) marine sponges, (**B**) seawater, and (**C**) marine sediment samples. Bubble sizes are proportional to number of samples as indicated.

150 individuals. Seawater ($n = 370$), sediment ($n = 65$), algae ($n = 1$), and echinoderm ($n = 1$) samples as well as biofilm swabs ($n = 21$) of rock surfaces were collected in close proximity to the sponges for comparative community analysis. Six negative control samples (sterile water) were processed to identify any potential contaminations. Of the samples included in this current dataset, 973 samples had been analysed previously [13]. Samples were collected from a wide range of geographical locations (Fig. 1; Supplementary Table S1). Total DNA was extracted as previously described [13] and used as templates to amplify and sequence the V4 region of the 16S rRNA gene using the standard procedures of the Earth Microbiome Project (EMP) [14, 15].

Processing of sequencing data

Clustering using the EMP standard protocols in QIIME

Raw sequences were demultiplexed and quality controlled following the recommendations of Bokulich et al. [16]. Quality-filtered, demultiplexed fastq files were processed using the default closed-reference pipeline from QIIME v. 1.9.1 (QIIME, RRID:SCR_008249). Briefly, sequences were matched against the GreenGenes reference database (v. 13_8 clustered at 97% similarity). Sequences that failed to align (e.g., chimeras) were discarded, which resulted in a final number of 300 140 110 sequences. Taxonomy assignments and the phylogenetic tree information were taken from the centroids of the reference sequence clusters contained in the GreenGenes reference database (Greengenes, RRID:SCR_002830). This closed-reference analysis allows for cross-dataset comparisons and direct comparison with the tens of thousands of other samples processed in the EMP and available via the Qiita database [17].

Clustering using Mothur

Quality-filtered, demultiplexed fastq files were also processed using Mothur v. 1.37.6 (Mothur, RRID:SCR_011947) [18] and Python v. 2.7 (Python Programming Language, RRID:SCR_008394) [19] custom scripts with modifications from previously established protocols [13]. Detailed descriptions and command outputs are available at the project notebook (see Availability of supporting data). Briefly, sequences were quality-trimmed to a maximum length of 100 bp. To minimize computational effort, the dataset was reduced to unique sequences, retaining total sequence counts. Sequences were aligned to the V4 region of the 16S rRNA gene sequences from the SILVA v. 123 database (SILVA, RRID:SCR_006423) [20]. Sequences that aligned at the expected positions were kept, and this dataset was again reduced to unique sequences. Further, singletons were removed from the dataset, and the remaining sequences were preclustered if they differed by 1 nucleotide position. Sequences classified as eukaryote, chloroplast, mitochondria, or unknown according to the Greengenes (v. 13_8 clustered at 99% similarity) [21] and SILVA taxonomies [22] were removed. Chimeras were identified with UCHIME (UCHIME, RRID:SCR_008057) [23] and removed. Finally, sequences were *de novo* clustered into operational taxonomic units (OTUs) using the furthest neighbour method at 97% similarity. Representative sequences of OTUs were retrieved based on the mean distance among the clustered sequences. Consensus taxonomies based on the SILVA, Greengenes, and RDP (v. 14_03 2015; Ribosomal Database Project, RRID:SCR_006633) [24] databases were obtained based on the classification of sequences clustered within each OTU. The inclusion of these taxonomies is helpful considering that they have substantial differences, as recently discussed [25]. For example, Greengenes and RDP have the taxon Poribacteria, a prominent sponge-enriched phylum [26], which did not exist in the SILVA version used.

De-noising using Deblur

Recently, sub-OTU methods that allow views of the data at single-nucleotide resolution have become available. One such method is Deblur [27], which is a de-noising algorithm for identification of the actual bacterial sequences present in a sample. Using an upper bound on the polymerase chain reaction and read-error rates, Deblur processes each sample independently and outputs the list of sequences and their frequencies in each sample, enabling single nucleotide resolution. For creating the deblurred biom table, quality-filtered, demultiplexed fasta files were used as input to Deblur using a trim length of 100 and min-reads of 25 (removing sOTUs with <25 reads total in all samples combined). Taxonomy was added to the resulting biom table using QIIME [28], RDP classifier [29], and Greengenes v. 13.8 [21].

Database metadata category enrichment

For enrichment analysis of metadata terms in a set of sequences, each unique metadata value is tested using both a binomial test and a ranksum test. All analysis is performed on a randomly subsampled (5000 reads/sample) table.

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Figure 2: Microbial taxonomic profile of marine sponge samples processed with Mothur. (**A**) SILVA, (**B**) Greengenes, and (**C**) RDP taxonomies are shown. OTU sequence counts were grouped according to phylum and class. Taxa with relative abundances ≤0.5% were grouped as "others." Classes with relative abundances >1% are shown in the legend (phylum ";" class). Relative abundances are represented on the x-axes.

The binomial (presence/absence) P-value for enrichment calculated as follows

For a bacterial sequence s and metadata value v, denote N the total number of samples, O(s) the number of samples where s is present, K_v(s) the number of samples with value v where s is present, and T(v) the total number of samples with value v.

$$P\text{-value} = binomial_cdf\,(\mathrm{T(v)} - \mathrm{K_v(s)}, \mathrm{T(v)}, P_{\mathrm{Null}}(\mathrm{s}))$$

where $P_{\mathrm{Null}}(\mathrm{s}) = \mathrm{O(s)}/N$

The ranksum (frequency aware) P-value is calculated using the Kruskal-Wallis test (implemented in scipy 0.19) as follows.

For a bacterial sequence s and metadata value v, denote by $F_v(s)$ the vector of relative frequencies of bacteria s in all samples with metadata value v, and denote by $\widehat{F_v(s)}$ the vector of relative frequencies of bacteria s in all samples with metadata other than v. The ranksum P-value is then calculated using the Kruskal-Wallis test for $F_v(s)$ and $\widehat{F_v(s)}$ and shown only if significantly enriched in samples containing v (i.e., rank difference of $F_v(s)$—$\widehat{F_v(s)} > 0$).

We have set up a webserver [30] that performs this enrichment analysis for user-defined sequence submissions. The code for the webserver is also available in Github for a local installation.

Data description

The dataset covers 4033 samples with a total of 1 167 226 701 raw sequence reads. These sequence reads clustered into 39 543 OTUs using QIIME's closed-reference processing, 518 246 OTUs from *de novo* clustering using Mothur (not filtered for OTU abundances), and 83 908 sOTUs using Deblur (with a filtering of at least 25 reads total per sOTU). We recommend that data users consider the differences in sequencing depths per sample and abundance filtering for certain downstream analyses, such as when calculating diversity estimates [16] and comparing OTU abundances across samples [31]. In terms of taxonomic diversity, most Mothur OTUs were assigned to the phylum Proteobacteria, although more than 60 different microbial phyla were recovered from the marine sponge samples according to SILVA ($n = 63$) and Greengenes classifications ($n = 72$) (Fig. 2).

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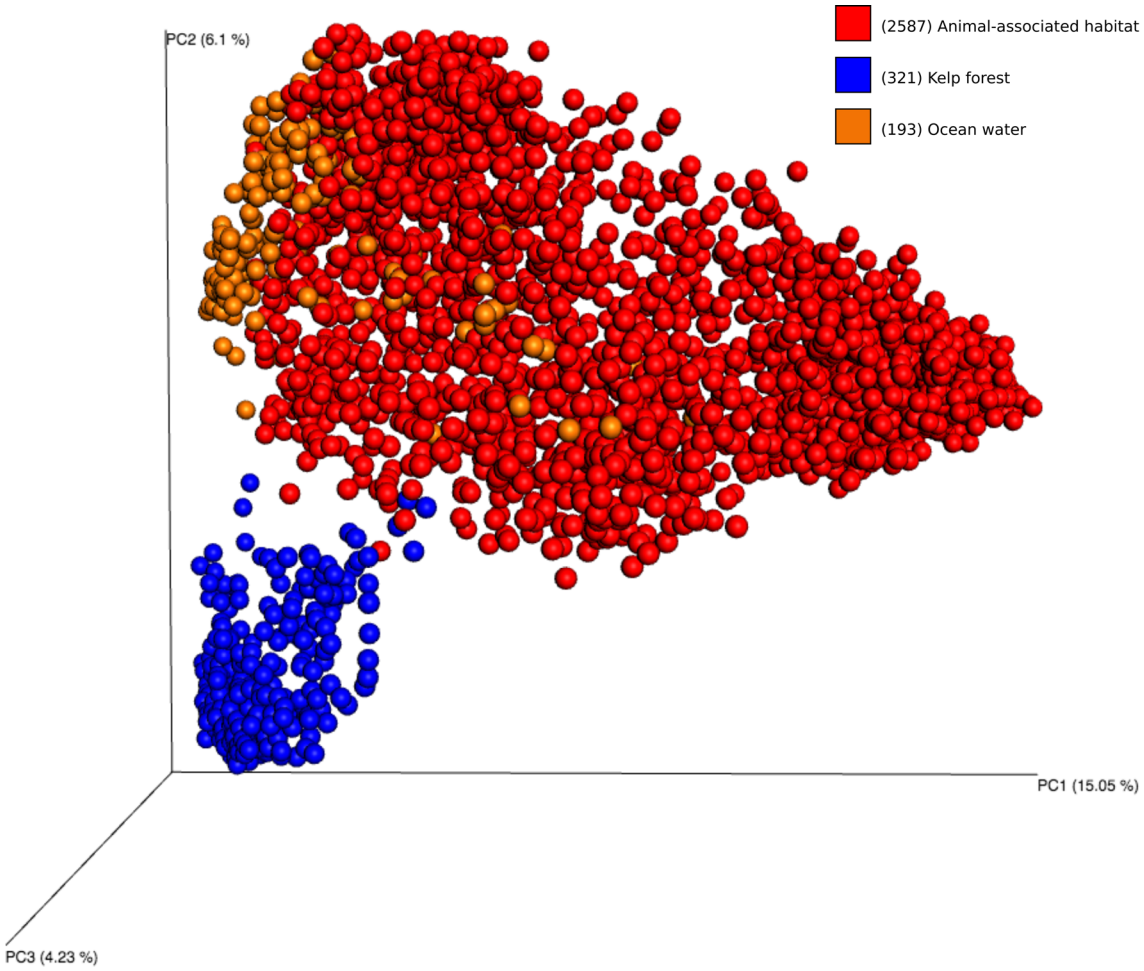


Figure 3: Unweighted UniFrac Principal Coordinates Analysis (PCA) of samples from sponges ("animal-associated habitat"), kelp forest, and ocean water. Samples were rarefying to 10 000 sequences per sample. A movie showing the PCA plot in 3D is provided in the supporting information.

Potential uses

This dataset can be utilised to assess a broad range of ecological questions pertaining to host-associated microbial communities generally or to sponge microbiology specifically. These include: (i) the degree of host specificity, (ii) the existence of biogeographic or environmental patterns, (iii) the relation of microbiomes to host phylogeny, (iv) the variability of microbiomes within or between host species, (v) symbiont co-occurrence patterns, and (vi) assessing the existence of a core sponge microbiome. An example of this type of analysis is shown in Fig. 3, where samples were clustered using unweighted UniFrac data [10] with a Principal Coordinates Analysis and visualization in Emperor [15] based on their origins from sponges, seawater, or kelps [17].

Availability and requirements

Project name: The Sponge Microbiome Project

Project home page: www.spongeemp.com; https://github.com/amnona/SpongeEMP

Operating system(s): Unix

Programming language: Python and R

Other requirements: Python v. 2.7, Biopython v. 1.65, Python 3.5, R v. 3.2.2, Mothur v. 1.37.6, QIIME v. 1.9.1, Deblur

License: MIT

Any restrictions to use by non-academics: none

Availability of supporting data

Raw sequence data were deposited in the European Nucleotide Archive (accession number: ERP020690). Quality-filtered, demultiplexed fastq files, QIIME resulting OTU tables are available at the Qiita database (Study ID: 10 793) [17]. The additional datasets that support the results of this article are available in the *GigaScience* repository, *Giga*DB [32] and include an OTU abundance matrix (the output ".shared" file from Mothur, which is tab delimited), an OTU taxonomic classification table (tab delimited text file), an OTU representative sequence FASTA file, a table of samples' metadata, the biom files from QIIME and Deblur analyses, and the QIIME-generated tree file. The project workflow, Mothur commands, and additional scripts are available as HTML in *Giga*DB [32].

The deblurred dataset has also been uploaded to an online server [19] that supplies both html and REST-API access for querying bacterial sequences and obtaining the observed

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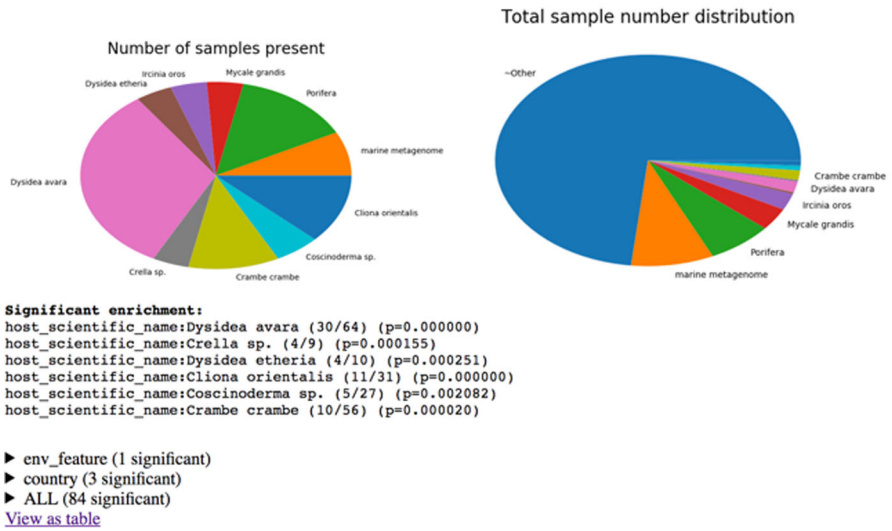


Figure 4: Output of the enrichment analysis through the online server www.spongeemp.com. Top line shows taxonomic assignment for the user-submitted sequence in the second line. Pie charts below show the total number of samples (right) and the number of samples where the submitted sequence is present (left) based on the scientific names of the host, followed by the significantly enriched host names containing the submitted sequence (using either presence/absence binomial test or relative frequency–based ranksum test). At the bottom, fields can be opened to show results of the enrichment analyses for other metadata types (e.g., country).

prevalence and enriched metadata categories where the sequence is observed (Figure 4). This allows an interactive view of which sequences are associated with which specific parameters, such as depth or salinity.

Additional file

sample.metadata

Abbreviations

EMP: Earth microbiome project; bp: base pairs; OTU: operational taxonomic unit; rRNA: ribosomal RNA.

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Competing interests

The authors declare that they have no competing interests.

Author contributions

L.M.-S., N.S.W., and T.T. designed the study. C.A.G., D.S., F.L., G.S., G.K., G.McC., G.-F. F, J.J.B., J.V., J.R.B., J.M.M., J.R., L.S., M.C.P, M.V.M., M.W.T., N.S.W., P.P., P.M.E., P.J.S., R.L.S, R.W.T., R.C., R.T.H., S.L-L., T.D., T.R., U.H., and Z-Y. L. collected samples. C.A.G., D.S., J.V., J.R.B., L.S., M.C.P., M.W.T., N.S.W., P.M.E., R.L.S, R.W.T., S.L-L., and U.H. extracted DNA. G.L.A. and R.K. sequenced DNA. L.M.-S., S.N., A.A., A.G., G.L.A., and T.T. performed data processing and analysis. L.M.-S., N.S.W., and T.T. wrote the manuscript. All authors contributed to the writing of the manuscript.

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