

**Please cite the Published Version**

Griffiths, Sarah , Antwis, Rachael, Lenzi, Luca, Lucaci, Anita, Behringer, Donald, Butler, Mark J and Preziosi, Richard  (2019) Host genetics and geography influence microbiome composition in the sponge *Ircinia campana*. *Journal of Animal Ecology*, 88 (11). pp. 1684-1695. ISSN 0021-8790

**DOI:** <https://doi.org/10.1111/1365-2656.13065>

**Publisher:** Wiley

**Version:** Accepted Version

**Downloaded from:** <https://e-space.mmu.ac.uk/623612/>

**Additional Information:** This is an Author Accepted Manuscript of a paper in *Journal of Animal Ecology* published by Wiley.

**Enquiries:**

If you have questions about this document, contact [openresearch@mmu.ac.uk](mailto:openresearch@mmu.ac.uk). Please include the URL of the record in e-space. If you believe that your, or a third party's rights have been compromised through this document please see our Take Down policy (available from <https://www.mmu.ac.uk/library/using-the-library/policies-and-guidelines>)

# Journal of Animal Ecology

DR SARAH M GRIFFITHS (Orcid ID : 0000-0003-4743-049X)  
DR RACHAEL ANTWIS (Orcid ID : 0000-0002-8849-8194)

Article type : Research Article  
Editor : Ann Tate  
Section : Molecular Ecology

## **Host genetics and geography influence microbiome composition in the sponge *Ircinia campana***

Sarah M. Griffiths<sup>1#</sup>, Rachael E. Antwis<sup>2</sup>, Luca Lenzi<sup>3</sup>, Anita Lucaci<sup>3</sup>, Donald C. Behringer<sup>4, 5</sup>,  
Mark J. Butler IV<sup>6</sup> and Richard F. Preziosi<sup>1</sup>

<sup>1</sup> Ecology and Environment Research Centre, Manchester Metropolitan University, Chester Street, Manchester, UK.

<sup>2</sup> School of Environment and Life Sciences, University of Salford, Salford, UK.

<sup>3</sup> Centre for Genomic Research, Institute of Integrative Biology, University of Liverpool, Liverpool, UK.

<sup>4</sup> Fisheries and Aquatic Sciences, University of Florida, Gainesville, Florida, USA.

<sup>5</sup> Emerging Pathogens Institute, University of Florida, Gainesville, Florida, USA.

<sup>6</sup> Department of Biological Sciences, Old Dominion University, Norfolk, Virginia, USA.

Corresponding author:

# Sarah M. Griffiths, griffiths.sarahm@gmail.com, John Dalton Building, Chester Street, Manchester, M1 5GD

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1365-2656.13065

This article is protected by copyright. All rights reserved.

**Keywords:** Bacteria; microsatellites; Porifera; genetic diversity; eco-evolutionary dynamics; host-microbe interactions; core microbiome

## ABSTRACT

1. Marine sponges are host to large, diverse communities of microorganisms. These microbiomes are distinct among sponge species and from seawater bacterial communities, indicating a key role of host identity in shaping its resident microbial community. However, the factors governing intraspecific microbiome variability are underexplored, and may shed light on the evolutionary and ecological relationships between host and microbiome.
2. Here, we examined the influence of genetic variation and geographic location on the composition of the *Ircinia campana* microbiome.
3. We developed new microsatellite markers to genotype *I. campana* from two locations in the Florida Keys, USA, and characterised their microbiomes using V4 16S rRNA amplicon sequencing.
4. We show that microbial community composition and diversity is influenced by host genotype, with more genetically similar sponges hosting more similar microbial communities. We also found that although *I. campana* was not genetically differentiated between sites, microbiome composition differed by location.
5. Our results demonstrate that both host genetics and geography influence the composition of the sponge microbiome. Host genotypic influence on microbiome composition may be due to stable vertical transmission of the microbial community from parent to offspring, making microbiomes more similar by descent. Alternatively, sponge genotypic variation may reflect variation in functional traits that influence the acquisition of environmental microbes. This study reveals drivers of microbiome variation within and among locations, and shows the importance of intraspecific variability in mediating eco-evolutionary dynamics of host-associated microbiomes.

## INTRODUCTION

A recent paradigm shift in biology has been the discovery of the breadth, diversity and importance of microbial communities associated with multicellular animals and plants. Termed the "microbiome", these communities influence a number of traits associated with host health, physiology and development (Blaser, Bork, Fraser, Knight, & Wang, 2013; Gilbert, Jansson, & Knight, 2014), and as such have been the focus of attention in fields as diverse as human medicine and wildlife conservation (Kashyap, Chia, Nelson, Segal, & Elinav, 2017; Trevelline, Fontaine, Hartup, & Kohl, 2019). Host-microbiome systems are complex ecological communities encompassing an array of host-microbe and microbe-microbe interactions (Bauer, Kainz, Carmona-Gutierrez, & Madeo, 2018; Coyte, Schluter, & Foster, 2015). Understanding the ecological and evolutionary nature of the relationship between hosts and their microbiome requires an understanding of the forces structuring these microbial communities, driven by both host and environment (Antwis et al., 2017).

Sponges (phylum Porifera) are considered valuable model systems in host-microbiome research due to the abundance and diversity within their associated microbial communities (Pita, Fraune, & Hentschel, 2016), with a total of 52 bacterial phyla and candidate phyla discovered among sponge hosts (Thomas et al., 2016). Sponge-microbiome interactions are numerous and complex, and microbial symbionts may confer a number of benefits to their host including nutrition and waste metabolism (Freeman, Thacker, Baker, & Fogel, 2013; Karimi et al., 2018; Moitinho-Silva et al., 2017; Thomas et al., 2010), acclimation to ocean acidification (Ribes et al., 2016), reduction in host surface fouling (On, Lau, & Qian, 2006), and production of compounds that deter predation of the sponge host (Garate, Blanquer, & Uriz, 2015). Sponge-associated microbes are also of significant biotechnological interest due to their potential for production of novel, pharmaceutically-active secondary metabolites (Thomas, Kavlekar, & Loka Bharathi, 2010).

Sponge microbiome composition is predominantly host-driven, with only a small degree of overlap with seawater microbial communities (Hentschel et al., 2002; Schmitt et al., 2012). Similarly, relatively few microbial taxa are shared across the phylum, and host species is a key determinant of microbiome composition (Blanquer, Uriz, & Galand, 2013; Pita, Turon, López-Legentil, & Erwin, 2013; Schmitt et al., 2012; Thomas et al., 2016; Turon, Cáliz, Garate, Casamayor, & Uriz, 2018). This strong association is thought to be driven by a combination of vertical transmission of microbial associates (i.e., parent to offspring transmission) and horizontal transmission of seawater microbes to highly-selective host environments (Fieth, Gauthier, Bayes, Green, & Degnan, 2016; Thacker & Freeman, 2012; Turon et al., 2018).

Despite the strong effect of host species identity, significant variation in microbiome composition is still present within host sponge species (Thomas et al., 2016; Turon et al., 2018). Intra-specific microbiome variation has been associated with environmental variation, such as geographic location (Fiore, Jarett, & Lesser, 2013; Luter et al., 2015; Swierts, Cleary, & de Voogd, 2018), depth (Morrow, Fiore, & Lesser, 2016), habitat (Cleary et al., 2013; Weigel & Erwin, 2017) and water quality (Luter et al., 2015). However, given the strength of host identity in structuring the microbiome, genetic variation within the species may also be significant. Indeed, host genotype influences microbiome composition in several systems, including plants (Wagner et al., 2016), fish (Uren Webster, Consuegra, Hitchings, & Garcia de Leaniz, 2018), amphibians (Griffiths et al., 2018), birds (Pearce et al., 2017), and mammals (Benson et al., 2010; Goodrich et al., 2014). However, host genotype and microbial variation have not yet been linked in sponges.

Noyer and Becerro (2012) found no significant relationship between host microsatellite diversity and bacterial communities analysed using denaturing gradient gel electrophoresis (DGGE) in *Spongia lamella*. However, DGGE has a lower resolution than current sequencing techniques, giving less information on community composition at lower

taxonomic ranks. In a later study, Marino et al. (2017) assessed correlations between latitude, sponge mitochondrial haplotype and microbiome composition in *Ircinia campana* from Florida and the Caribbean. However, cytochrome oxidase I haplotype correlated with location as well as microbiome composition, preventing the two variables from being disentangled. The role of host sponge genotype in structuring microbial communities is therefore still to be determined. This study addresses this gap in the literature, using highly polymorphic microsatellite markers and 16S rRNA Illumina sequencing to characterise *Ircinia campana* populations and their associated microbial communities in two locations in the Florida Keys, USA.

## MATERIALS AND METHODS

### Sample collection and DNA extraction

In July 2014, we sampled *Ircinia campana* (Caribbean vase sponge) individuals at two shallow (< 2 m) nearshore hard bottom sites in the Florida Keys (FL, USA) separated by approximately 70 km: Long Key (24.81437, -80.8307) and Kemp Channel (24.6768, -81.4757). We took samples in a single collection instance at each site to eliminate temporal variability, with 20 individuals sampled per site. We cut a piece of tissue from each individual and immediately preserved it in 99% ethanol upon surfacing. We then replaced the ethanol, firstly to act as a rinse, removing loosely attached seawater bacteria, and secondly to prevent dilution of the ethanol to aid DNA preservation. We stored samples at -80°C until processing. Prior to DNA extraction, we dissected the tissue under a stereomicroscope using aseptic technique to remove commensal macro-organisms. We then extracted total DNA with the DNeasy® Blood and Tissue Kit (Qiagen), and normalised it to 1 ng/μl.

### Microsatellite development and host genotyping

We developed a suite of 10 tri- and tetra-nucleotide polymorphic microsatellite markers for *I. campana* using the pipeline implemented in the Palfinder Galaxy service (Griffiths et al., 2016) (Table S1, see Supporting Information for full details of methods). One locus (Icam34)

performed well in individuals sampled from other localities (Griffiths et al. in prep, data not shown); however, it did not amplify well in the study populations. We therefore excluded Icam34 from further analysis in this study, thus using a total of nine loci. To fluorescently label PCR products, we used a three-primer PCR method, using a fluorescently labelled universal primer and tagging the 5' end of the forward primer with the universal primer sequence, as described by Culley et al. (2013). We carried out multiplex PCR amplifications using the Type-it® Microsatellite Kit (Qiagen) using the following PCR thermal cycling conditions: 95°C initial denaturation for 5 minutes, 28 cycles of 95°C for 30 seconds, 60°C/63°C for 90 seconds and 72°C for 30 seconds, and a final extension at 60°C for 30 minutes. PCR products were sized using the DNA Analyzer 3730 at the DNA Sequencing Facility at the University of Manchester, using the GeneScan™ LIZ® 1200 size standard. We scored alleles with Genemapper v3.7 (Thermo Fisher Scientific) and binned alleles in MsatAllele v1.03 (Alberto, 2009) in RStudio 1.1.442 for R 3.3.3 (RStudio Team, 2016; R Core Team, 2017).

### **Microbiome characterisation**

#### *PCR, library preparation and sequencing*

PCR, sequencing and OTU taxonomic assignment were carried out at the Centre for Genomics Research, University of Liverpool, UK. We carried out amplification of the V4 region of the 16S rRNA gene in a two-stage nested PCR in 5µl reaction volumes using primers described in Caporaso et al. (2011). We used the following thermal cycling conditions: 15 x 95°C for 20 seconds, 65°C for 15 seconds and 70°C for 30 seconds; 1 x 72°C for 5 minutes. We purified PCR products using AMPure SPRI beads (Beckman Coulter), before entering into a second stage of PCR (conditions as above, 20 cycles) to incorporate Illumina sequencing adapter sequences containing indexes (i5 and i7) for sample identification. Following PCR, we purified the samples again, and quality checked the amplicon libraries using a Qubit and an Agilent Fragment Analyzer. We pooled the final libraries in equimolar amounts and used a Pippin Prep (Sage Science) to carry out size

selection of 300-600 bp. We assessed quantity and quality of the library pool using a Bioanalyzer (Agilent) and qPCR with the Illumina® Library Quantification Kit (Kapa Biosystems) on a LightCycler® (Roche). We then conducted paired-end (2 x 250 bp) sequencing on the Illumina MiSeq, with fragmented PhiX bacteriophage genome added to increase sequence complexity.

#### *Quality filtering and pre-processing*

We used CASAVA v1.8.2 (Illumina) to base call and de-multiplex indexed reads, and cutadapt v1.2.1 (Martin, 2011) to remove Illumina adapter and PCR primer sequences. We trimmed low quality bases from the reads using Sickle v1.200 (Joshi & Fass, 2011) (minimum window quality score 20), and removed reads under 10 bp in length. Sequencing errors were corrected using the error-correct module in SPAdes v3.1.0 (Bankevich et al., 2012). We aligned read pairs using USEARCH8 (Edgar, 2010) with the 'fast-mergepairs' command, and selected merged sequences of between 200 and 600 bp. We used BLASTN (Altschul, Gish, Miller, Myers, & Lipman, 1990) to search for PhiX sequences (GenBank GI:9626372) in each sample; matching sequences (E-value  $<10^{-5}$ ) were then filtered out. Sequences containing Ns were discarded to remove low-quality reads.

We clustered sequences into operational taxonomic units (OTUs) with 99% sequence similarity. Two different clustering algorithms were used for OTU picking; the first implemented in VSEARCH 1.1.3 (Edgar, 2010) using the function '-cluster-smalmem' with 99% identity threshold, and the second in Swarm (Mahé, Rognes, Quince, de Vargas, & Dunthorn, 2014). We removed clusters containing fewer than two sequences to reduce error, and merged the results from both clustering steps to create a non-redundant sequence set. We conducted chimera detection in VSEARCH using both a *de novo* approach and a reference-based approach with the SILVA 119 database. The reference-based step found 12% of the sequences to be chimeras, which were removed for subsequent analyses, while none were found using the *de novo* approach. We used the

'usearch\_global' function in VSEARCH to define the abundance of each OTU, and taxonomically classified these in QIIME 1.9.0 (Caporaso et al., 2010) using pick\_rep\_set.py to select the most representative sequence in the OTU, and assign\_taxonomy.py to match sequences to those in the SILVA 119 database (Quast et al., 2013). We produced an OTU count table for all samples, and exported this and the taxonomic classification as a biom file. We imported this into RStudio using the phyloseq package (McMurdie & Holmes, 2013) for subsequent statistical analyses. We converted OTU count data to relative abundance for subsequent compositional and beta-diversity analyses.

We also created a rarefied dataset for use in alpha-diversity analyses, as sequencing depths among samples were uneven. Repeated subsampling (33 repetitions) was carried out on the OTU count table at sampling depths from 2000 to 350,000 in QIIME (multi\_rarefaction.py), following which we calculated Chao1 alpha diversity and plotted rarefaction curves (Fig. S1).

We then created a rarefied dataset (single\_rarefaction.py) by repeatedly subsampling (without replacement) at a depth of 173,000 sequences; samples with fewer sequences were removed from subsequent analysis (leaving  $n = 12$  for Kemp Channel and  $n = 13$  for Long Key). We used this dataset for alpha-diversity analyses as described below; we also repeated our beta-diversity analyses using this dataset, which produced similar results to the non-rarefied dataset (data not shown).

## Statistical analyses

### *Host genetics*

We tested for linkage disequilibrium between microsatellite loci using Genepop on the Web v.4.2 (Rousset, 2008), correcting the significance threshold for multiple tests using Benjamini and Yekutieli's (2001) correction with the R function *p.adjust*. We estimated null allele frequencies in FreeNA (Chapuis & Estoup 2007) using the EM algorithm (Dempster, Laird, & Rubin, 1977). We calculated  $F_{ST}$  between the two sites, and corrected for null allele

presence using the ENA method as described in Chapuis & Estoup (2007) and implemented in FreeNA.

We calculated pairwise Euclidean genetic distances between individuals from the multi-locus genotypes using GenoDive v2.0 b27 (Meirmans & Van Tiendener, 2004). In distance-based calculations, null alleles and missing data can bias results, overestimating differences between samples (Chapuis & Estoup, 2007). Thus, we first filled in missing data based on overall allele frequencies (11.8% in Long Key and 7.6% in Kemp Channel). We then used these distances to conduct a principle coordinates analysis (PCoA) in GenAIEx v.6.503 (Peakall & Smouse, 2012).

#### *Microbiome composition*

We conducted analyses in RStudio using the phyloseq (McMurdie & Holmes, 2013), vegan (Oksanen et al. 2018) and microbiome (Lahti 2017) packages. We used a PERMANOVA (adonis) to test for significant differences in microbiome composition between sites using Bray-Curtis dissimilarities. We calculated the core microbiome of individual samples using a detection threshold of 0.001% and a prevalence threshold of 100% (i.e. a given OTU must be present in all individuals, with a relative abundance of at least 0.001%). We identified the core OTUs and then calculated the proportion of the total microbiome that these represented. We then repeated the core microbiome analysis with the data agglomerated to genus level.

#### *Host genotype-microbiome analyses*

We produced pairwise microbial community distance matrices between individuals across both sites, and for each site individually, using distance matrices based on Chao1 values as a measure of alpha-diversity, and Jensen-Shannon divergence (JSD) and Bray-Curtis dissimilarity as measures of beta-diversity. We tested for correlations between each microbial distance matrix (Chao1, JSD and Bray-Curtis) and host genetic distance matrix

(Euclidean) using Mantel tests with 999 permutations. We repeated the analyses with a further, more conservative genetic data file, removing loci with high null allele frequencies and high proportions of missing data. Following removal of loci with high ( $>0.16$ ) null allele frequencies (Icam24, Icam26 and Icam10 in both sites, Icam3 in Kemp Channel samples), missing data was only present in Icam23 (0.077) and Icam3 (0.462) in Long Key. We then removed Icam3 at Long Key, giving final datasets of 5 loci for each site.

We also extracted Bray-Curtis distance matrices for core microbiota across and within sites at both the OTU and genus levels, and used Mantel tests to test for correlation with the full and reduced genetic distance matrices. As the taxonomic composition of the core does not vary across individuals, this metric describes variation in relative abundances of these core taxa.

## RESULTS

### *Population genetics*

We found no identical multi-locus genotypes in the dataset, indicating no clones were present amongst the sampled individuals. Across all sites, all loci were polymorphic, ranging up to 18 alleles per locus, but two of the loci were monomorphic in Kemp Channel (Icam32 and Icam4) (Table S2). No significant linkage disequilibrium occurred among any loci pairs. Null allele frequencies and the proportion of missing alleles (genotyping failures) were high for many loci, and heterozygosity deficiencies were observed in many cases (Table S2).

Pairwise genetic differentiation between Long Key and Kemp Channel was low ( $F_{ST} = 0.021$ ). The first and second principle coordinates of the PCoA explained only 15.81% of the total variation among the samples, and the individuals are not separated by site (Fig. 1). The sites can therefore be considered to be well-mixed genetically.

### Microbial community composition

We successfully conducted PCR amplification and Illumina sequencing on 34 samples (17 each from Long Key and Kemp Channel). Between 97.10 and 99.61% of reads were assembled per sample. One sample from Kemp Channel yielded a far fewer assembled sequences than the remaining samples (28,163) and was removed from the analyses, leaving samples with between 118,370 and 426,014 assembled sequences (mean =  $241,448 \pm 13,758$  S.E., Fig. S1). Between 80.77 and 87.57% of the filtered sequence set could be aligned to a taxon, with 31,567 OTUs found across all samples, and individual sponges ranging from 4,165 to 14,503 OTUs (Fig. S1). Among all OTUs, we detected a total of 22 bacterial phyla and one archaeal phylum. The most abundant phylum was Chloroflexi (62.6% of the total reads), followed by Proteobacteria (17.5%), Acidobacteria (6.4%), PAUC34f (4.3%), SBR1093 (3.8%), Gemmatimonadetes (1.6%) and Actinobacteria (1.5%) (Fig. S2). The remaining phyla formed less than 1% of the total reads. Within Chloroflexi, Anaerolineae was the most dominant class, forming large proportions of the microbiomes of all samples (Fig. S3).

There was a statistically significant difference in microbiome composition between sponges at Long Key and Kemp Channel (adonis,  $F_{1,31} = 4.391$ ,  $R^2 = 0.124$ ,  $p = 0.001$ ; Fig. 2), with 12.4% of the variation in microbiome composition explained by site. These compositional differences are evident, albeit subtle, at both the phylum and class levels (Figs. S2 and S3).

At the genus level, the core microbiome comprised 69.6% ( $\pm 1.7$  S.E.) of the total reads for sponges in Long Key, and 63.0% ( $\pm 1.6$ ) for sponges in Kemp Channel. These genera included *Desulfovibrionales*, *Chloroflexi*, *Pseudospirillum*, *PAWS52f*, *Nitrosococcus*, *Rhodovulum*, *Defluviicoccus*, *Acidobacteria*, *OM75 clade*, *Granulosicoccus*, *Nitrospira*, *Cerasicoccus*, *Actinobacterium MSI70*, *Candidatus Nirtosopumilus*, *Acidobacterium*, *PAUC32f*, *Truepera*, *PAUC43f*, *Synechococcus*, and a number of unidentified Proteobacteria genera. Out of a total of 31,567 OTUs, we only identified two in the core

microbiome; one assigned to Proteobacteria and the other an unidentified Bacteria. Together these comprised 1.2% ( $\pm 0.2$ ) of the total microbiome for sponges in Long Key, and 1.3% ( $\pm 0.1$ ) in Kemp Channel, and had the third (Proteobacteria sp.) and eleventh (Bacteria sp.) highest relative abundances of all OTUs in the total microbiome.

#### *Relationship between host genotype and microbiome composition*

We found statistically significant, positive relationships between genetic distance and microbial community dissimilarity when considering both alpha-diversity (Chao1) and beta-diversity (Bray-Curtis and JSD distances), and when using the full and reduced microsatellite datasets (Table 1; Fig. 3). However, there were no significant relationships between genetic distance and core microbiome distance at either the OTU or genus level when using either of the genetic datasets (all  $p > 0.100$ ).

## DISCUSSION

Host genotype had a significant effect on microbiome diversity and composition in *I. campana*, both across and within sites. More genetically similar sponges hosted more similar microbial communities, in terms of both richness and composition. Between sites, sponge microbiomes significantly differed in composition, despite no genetic differentiation between the sponge populations. These results indicate that both environment and host genetics influence intraspecific microbiome variability in *I. campana*, and that these drivers vary in influence by spatial scale.

#### *Host genotype*

Host genetic similarity and microbiome similarity were positively correlated, both in terms of microbiome alpha- and beta-diversity. This relationship may be driven by vertical transmission of microbial communities, with sponges that are more genetically similar by descent hosting more similar microbiomes. Evidence for vertical transmission of the microbiome has been observed in the sympatric congener *Ircinia felix* (Schmitt, Weisz,

Lindquist, & Hentschel, 2007), as well as other sponge species (Ereskovsky, Gonobobleva, & Vishnyakov, 2005; Lee, Chui, Wong, Pawlik, & Qian, 2009; Sharp, Eam, Faulkner, & Haygood, 2007; Sipkema et al., 2015), and is thought to be a significant driver of the high host-species fidelity of microbiomes in sponges. Many evolutionary advantages can be gained from the inheritance of parental microbiomes, as favourable symbionts that are important for host health and physiology are already present in growing larvae.

Horizontal transmission of microbes from the environment also contributes to the sponge microbiome (Fieth et al., 2016; Maldonado & Riesgo, 2009; Sipkema et al., 2015; Turon et al., 2018). As such, ecological interactions with seawater microbes could be key in shaping the microbiome. The relationships observed in this study may therefore be driven by host genotype-specific selection of seawater microbes.

Selection of environmental microbes imposed by host genetic variation could result from secondary metabolites produced by the sponge, which are highly diverse (Genta-Jouve & Thomas, 2012) and include antimicrobial compounds (Kelman et al., 2001). Using an in-situ experimental approach, Tout et al. (2017) showed that seawater bacteria exhibit chemotaxis to cellular extracts isolated from a sponge, with particular enrichment of bacterial taxa that are commonly found in sponges. As sponge secondary metabolites can be intra-specifically variable (Noyer, Thomas, & Becerro, 2011; Puyana et al., 2015), the production of genotype-specific compounds could attract varying seawater microbes to the sponge microbiome.

Alternatively, genetic variation may encode variable responses in the host immune system to microbes in the environment. In other species, polymorphism in immunity related genes has been found to affect microbiome composition (Bolnick et al., 2014; Kubinak et al., 2015; Pearce, Hoover, Jennings, Nevitt, & Docherty, 2017) and responses to pathogenic bacteria (Lazzaro, Sceurman, & Clark, 2004). In addition, genotype-specific immune response, and varying gene expression patterns in response to a potentially pathogenic bacteria, have

been observed in the coral *Acropora millepora* (Wright et al., 2017). Although they do not have an acquired immune system, sponges have a relatively sophisticated innate immune system (Müller & Müller, 2003), which has been speculated to aid the maintenance of distinct extracellular microbial communities in the mesohyl tissue where phagocytosis of food bacteria takes place (Wehrl, Steinert, & Hentschel, 2007; Wilkinson, Garrone, & Vacelet, 1984). This system includes receptor proteins at the interface between the organism and the environment that can recognise and differentiate bacteria (Wiens et al., 2005, 2007). There is currently no evidence that immune response varies intra-specifically in sponges. However, there is evidence of polymorphism of the *Amphimedon queenslandica* *AqNLR* (nucleotide-binding domain and Leucine-rich repeat containing) genes, which are pattern recognition receptors involved in detecting and binding a range of microbial ligands (Degnan, 2015).

Further to these potential mechanisms, the sponge itself cannot be considered in isolation; selection of seawater bacteria is likely to be performed by the entire holobiont. The timing and order in which microbes join a sponge microbiome may have secondary effects on determining succession and ultimately community composition (historical contingency; Costello et al., 2012), with competitive interactions occurring among community members (Esteves, Cullen, & Thomas, 2017). Because of this, influence of the host genotype on even a relatively small proportion of the microbiome could increase its reach in shaping community composition.

#### *Geographic location*

Location was the largest driver of microbiome structure in this study. Although the sampling sites are only approximately 70 km apart, location accounted for 12.4% of the total microbiome variation observed across samples. Microbiomes vary within species by geographic location in a number of benthic marine organisms (Pantos, Bongaerts, Dennis, Tyson, & Hoegh-Guldberg, 2015; Rubio-Portillo, Kersting, Linares, Ramos-Esplá, & Antón,

2018; van de Water, Allemand, & Ferrier-Pagès, 2018), including in some sponge species (Fiore et al., 2013; Luter et al., 2015; Marino et al., 2017; Swierts et al., 2018), although this finding is not universal (Pita, López-Legentil, & Erwin, 2013; Pita, Turon, et al., 2013).

Marino et al. (2017) showed a latitudinal gradient in microbiome composition in *I. campana* in the Caribbean, which also correlated with host mitochondrial haplotype. Our results show that even on a relatively local spatial scale (i.e., within the Florida Keys), microbiomes of *I. campana* can vary among sampling sites.

In this study, we did not sample seawater bacterial communities or collect environmental data, as investigating environmental effects was not the objective of this study. Instead, we sampled two sites as a form of replication to investigate host genetics. However, because there was effectively no genetic differentiation between the sponge populations at each site, the microbiome differences found between sites indicate that environmental variation drives *I. campana* microbiome composition at larger spatial scales. The environmental parameters responsible for this pattern remain unknown. However, host genotype also had a significant effect on microbiome composition when considered across locations. In addition, there appears to be between-site variation in the strength of host genotype-microbiome relationships. Mantel test statistic values were mostly higher for Long Key than those for Kemp Channel; this could be the result of genotype x environment interactions, and could extend the influence of genotype at larger spatial scales.

#### *Core microbiome*

Despite a significant effect of host genotype on total microbiome composition, we did not find any effect of host genotype on core microbiome composition at the genus or OTU level. As the core was defined in this study as taxa found in all individuals, composition refers here to variation in relative abundance of the same microbial phylotypes among individuals. Therefore, our results show that while genotype exerts an effect on non-core microbiome taxa, it does not drive abundances of core taxa. This lends support to the theory that

horizontal transmission has an important role in forming the core microbiome (Turon et al., 2018), as we may expect vertical transmission to produce significant genotype-core microbiome relationships. However, some refinement of the core microbiome concept and, in particular, the associated methodologies to define and identify ‘core’ taxa may be required to further our understanding of their significance, role and transmission.

At the 99% OTU level, the core microbiome in our study was comprised of two OTUs, which made up 1.2% and 1.3% of the total microbiome in Long Key and Kemp Channel, respectively. This core community appears small compared to results reported in some previous studies on sponges (Marino et al., 2017; Turon et al., 2018). Marino et al. (2017) found 119 core OTUs among 18 *I. campana* individuals, comprising 79.2 – 87.0% of the total microbiome. However, this study used 97% OTU clustering, which means individual OTUs are likely to encompass wider microbial taxonomic variation in contrast to 99% clustering of OTUs used in this study. The core microbial genera in our study formed 63% and 69% of the total microbiome in Long Key and Kemp Channel respectively, suggesting that the differences observed relative to previous work primarily reflect methodical differences (Astudillo-García et al., 2017). Furthermore, the higher the number of replicates, the smaller the apparent ‘core’ microbiome appears (Turon et al., 2018), and in this study the number of within-species replicates we used ( $n = 33$ ) was substantially larger than in many previous studies.

The core microbiome concept aims to identify stable, functionally important members of the microbiome, rather than transient or opportunistic members (Hernandez-Agreda, Gates, & Ainsworth, 2017; Shade & Handelsman, 2012). *Ircinia campana* appears to have a strong, possibly symbiotic, relationship with the two core OTUs observed, indicating a potentially important role in holobiont function. However, the larger genus-level core observed may be due to a level of functional redundancy within microbial genera, with characteristics at higher taxonomic ranks being more important for successful transmission and stability than OTU-

level characteristics. As such, defining a core in terms of wider phylogenetic or functional groups may therefore be more useful than a strict OTU/ species-level approach (Turnbaugh et al., 2009). Furthermore, as our findings show that microbiomes vary by genotype, identifying stable associations using the host species-level core microbiome approach may obscure genotype-specific host-microbe symbioses.

#### *Concluding remarks*

We show that genetic diversity has an important influence in shaping microbiome composition in *I. campana*. These results highlight the potential for intraspecific genetic diversity to impact ecological dynamics within sponge-microbe relationships, and demonstrate an eco-evolutionary relationship between sponges and microbial communities. Further work on the mechanisms underlying host genotype-microbiome relationships will aid our understanding of the nature and eco-evolutionary dynamics of the sponge-microbiome relationship. Furthermore, understanding these drivers of interspecific microbiome variability is also important in the context of global climate change. Ocean warming and acidification are predicted to change microbial communities both within the environment and in host-associated microbiomes, with huge implications for health and survival of marine species and their ecosystems (Lesser, Fiore, Slattery, & Zaneveld, 2016; Qiu et al., 2019). For example, ocean warming in the Mediterranean triggered microbial imbalances in *I. fasciculata*, which have been implicated in disease and mass mortalities (Blanquer, Uriz, Cebrian, & Galand, 2016). In Florida Bay, *I. campana* populations have already suffered numerous mass mortality events (Butler et al., 1995; Stevely, Sweat, Bert, Sim-Smith, & Kelly, 2010) due to cyanobacterial blooms caused by decades of ecosystem instability (Butler & Dolan, 2017; Butler, Weisz, & Butler, 2018; Fourqurean & Robblee, 1999; Kearney et al., 2015; Robblee et al., 1991). Microbiomes potentially have a huge importance in acclimation and resilience to climate change scenarios in marine organisms (Ribes et al., 2016; Webster & Reusch, 2017). With this in mind, understanding individual-level drivers of

microbiome variation may assist in species management and conservation in the face of future stressors.

### Acknowledgements

This study was funded by a NBAF-NERC pilot project grant awarded to SMG and RFP, and a NERC PhD studentship awarded to SMG. Thanks to J. Rowntree and G. Fox for their helpful advice in study design, and to C. Freeman for providing a sample for microsatellite development. Thanks to students from University of Florida and Old Dominion University for their assistance in collecting samples, and to the staff at CGR Liverpool for their assistance in data generation. Thanks to P. Briggs and the University of Manchester Bioinformatics Core Facility for use of the Palfinder Galaxy Service and to P. Fullwood at the University of Manchester DNA Sequencing Facility for the use of lab equipment. Samples were collected with permission of the Florida Fish and Wildlife Commission under permit SAL-13-0582A-SR. The authors declare no conflict of interests.

### Author contributions

SMG and RFP conceived and designed the study; SG, RFP, MB and DCB secured funding; SG, MJB and DCB collected the samples; SMG and AL carried out the lab work; SMG, REA and LL conducted the analysis; SMG and REA wrote the manuscript; all authors revised the manuscript.

### Data accessibility

16s rRNA amplicon sequence data are available from the NCBI SRA (accession number PRJNA506340). Microsatellite loci sequences are logged in NCBI GenBank under accession numbers MF987878 to MF987887. Raw Illumina sequencing data from *Ircinia campana* used for microsatellite development is deposited in the NCBI SRA (accession number PRJNA528609). The code used for analyses in R is available as a RMarkdown file in the Supporting Information.

## REFERENCES

- Alberto, F. (2009). MsatAllele\_1.0: An R package to visualize the binning of microsatellite alleles. *Journal of Heredity*, 100(3), 394–7. doi:10.1093/jhered/esn110
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215(3), 403–410. doi:10.1016/S0022-2836(05)80360-2
- Antwis, R. E., Griffiths, S. M., Harrison, X. A., Aranega-Bou, P., Arce, A., Bettridge, A. S., ... Sutherland, W. J. (2017). Fifty important research questions in microbial ecology. *FEMS Microbiology Ecology*, 93(5). doi:10.1093/femsec/fix044
- Astudillo-García, C., Bell, J. J., Webster, N. S., Glasl, B., Jompa, J., Montoya, J. M., & Taylor, M. W. (2017). Evaluating the core microbiota in complex communities: A systematic investigation. *Environmental Microbiology*, 19(4), 1450–1462. doi:10.1111/1462-2920.13647
- Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., ... Pevzner, P. A. (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology*, 19(5), 455–77. doi:10.1089/cmb.2012.0021
- Bauer, M. A., Kainz, K., Carmona-Gutierrez, D., & Madeo, F. (2018). Microbial wars: Competition in ecological niches and within the microbiome. *Microbial Cell*, 5(5), 215–219. doi:10.15698/mic2018.05.628
- Benjamini, Y., & Yekutieli, D. (2001). The control of the false discovery rate in multiple testing under dependency. *The Annals of Statistics*, 29(4), 1165–1188. doi:10.1214/aos/1013699998
- Benson, A. K., Kelly, S. A., Legge, R., Ma, F., Low, S. J., Kim, J., ... Pomp, D. (2010). Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proceedings of the National Academy of Sciences*, 107(44), 18933–8. doi:10.1073/pnas.1007028107
- Blanquer, A., Uriz, M. J., Cebrian, E., & Galand, P. E. (2016). Snapshot of a bacterial

- microbiome shift during the early symptoms of a massive sponge die-off in the Western Mediterranean. *Frontiers in Microbiology*, 7, 752. doi:10.3389/fmicb.2016.00752
- Blanquer, A., Uriz, M. J., & Galand, P. E. (2013). Removing environmental sources of variation to gain insight on symbionts vs. transient microbes in high and low microbial abundance sponges. *Environmental Microbiology*, 15(11), 3008–3019. doi:10.1111/1462-2920.12261
- Blaser, M., Bork, P., Fraser, C., Knight, R., & Wang, J. (2013). The microbiome explored: recent insights and future challenges. *Nature Reviews Microbiology*, 11(3), 213–217. doi:10.1038/nrmicro2973
- Bolnick, D. I., Snowberg, L. K., Caporaso, J. G., Lauber, C., Knight, R., & Stutz, W. E. (2014). Major Histocompatibility Complex class IIb polymorphism influences gut microbiota composition and diversity. *Molecular Ecology*, 23(19), 4831–4845. doi:10.1111/mec.12846
- Butler, M. J., Hunt, J. H., Herrnkind, W. F., Childress, M. J., Bertelsen, R., Sharp, W., ... Marshall, H. G. (1995). Cascading disturbances in Florida Bay, USA: cyanobacteria blooms, sponge mortality, and implications for juvenile spiny lobsters *Panulirus argus*. *Marine Ecology Progress Series*, 129, 119–125. doi:10.3354/meps129119
- Butler, M. J., & Dolan, T. W. (2017). Potential impacts of Everglades restoration on lobster and hard bottom communities in the Florida Keys, FL (USA). *Estuaries and Coasts*, 40(6), 1523–1539. doi:10.1007/s12237-017-0256-8
- Butler, Mark J., Weisz, J. B., & Butler, J. (2018). The effects of water quality on back-reef sponge survival and distribution in the Florida Keys, Florida (USA). *Journal of Experimental Marine Biology and Ecology*, 503(February), 92–99. doi:10.1016/j.jembe.2018.03.001
- Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., ... Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 7(5), 335–336. doi:10.1038/nmeth.f.303
- Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh,

- P. J., ... Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences*, 108, 4516–22. doi:10.1073/pnas.1000080107
- Chapuis, M.-P., & Estoup, A. (2007). Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution*, 24(3), 621–31. doi:10.1093/molbev/msl191
- Cleary, D. F. R., Becking, L. E., de Voogd, N. J., Pires, A. C. C., Polónia, A. R. M., Egas, C., & Gomes, N. C. M. (2013). Habitat- and host-related variation in sponge bacterial symbiont communities in Indonesian waters. *FEMS Microbiology Ecology*, 85(3), 465–82. doi:10.1111/1574-6941.12135
- Costello, E. K., Stagaman, K., Dethlefsen, L., Bohannan, B. J. M., & Relman, D. A. (2012). The application of ecological theory toward an understanding of the human microbiome. *Science*, 336(6086), 1255–1262. doi:10.1126/science.1224203
- Coyte, K. Z., Schluter, J., & Foster, K. R. (2015). The ecology of the microbiome: Networks, competition, and stability. *Science*, 350(6261), 663–6. doi:10.1126/science.aad2602
- Culley, T. M., Stamper, T. I., Stokes, R. L., Brzyski, J. R., Hardiman, N. a., Klooster, M. R., & Merritt, B. J. (2013). An efficient technique for primer development and application that integrates fluorescent labeling and multiplex PCR. *Applications in Plant Sciences*, 1(10), 1300027. doi:10.3732/apps.1300027
- Degnan, S. M. (2015). The surprisingly complex immune gene repertoire of a simple sponge, exemplified by the NLR genes: A capacity for specificity? *Developmental & Comparative Immunology*, 48(2), 269–274. doi:10.1016/j.dci.2014.07.012
- Dempster, A. P., Laird, N. M., & Rubin, D. B. (1977). Maximum likelihood from incomplete data via the EM algorithm. *Journal of the Royal Statistical Society, Series B*, 39(1), 1–38. doi:10.1111/j.2517-6161.1977.tb01600.x
- Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, 26(19), 2460–2461. doi:10.1093/bioinformatics/btq461
- Ereskovsky, A. V., Gonobobleva, E., & Vishnyakov, A. (2005). Morphological evidence for

- vertical transmission of symbiotic bacteria in the viviparous sponge *Halisarca dujardini* Johnston (Porifera, Demospongiae, Halisarcida). *Marine Biology*, 146, 869–875. doi:10.1007/s00227-004-1489-1
- Esteves, A. I. S., Cullen, A., & Thomas, T. (2017). Competitive interactions between sponge-associated bacteria. *FEMS Microbiology Ecology*, 321(3), fix008. doi:10.1093/femsec/fix008
- Fieth, R. A., Gauthier, M.-E. A., Bayes, J., Green, K. M., & Degnan, S. M. (2016). Ontogenetic changes in the bacterial symbiont community of the tropical demosponge *Amphimedon queenslandica*: Metamorphosis Is a new beginning. *Frontiers in Marine Science*, 3(November), 1–20. doi:10.3389/fmars.2016.00228
- Fiore, C. L., Jarett, J. K., & Lesser, M. P. (2013). Symbiotic prokaryotic communities from different populations of the giant barrel sponge, *Xestospongia muta*. *MicrobiologyOpen*, 2(6), 938–52. doi:10.1002/mbo3.135
- Fourqurean, J. W., & Robblee, M. B. (1999). Florida Bay: A history of recent ecological changes. *Estuaries*, 22(2), 345–357.
- Freeman, C. J., & Thacker, R. W. (2011). Complex interactions between marine sponges and their symbiotic microbial communities. *Limnology and Oceanography*, 56(5), 1577–1586. doi:10.4319/lo.2011.56.5.1577
- Freeman, C., Thacker, R., Baker, D., & Fogel, M. (2013). Quality or quantity: is nutrient transfer driven more by symbiont identity and productivity than by symbiont abundance? *The ISME Journal*, 7(6), 1116–1125. doi:10.1038/ismej.2013.7
- Garate, L., Blanquer, A., & Uriz, M. (2015). Calcareous spherules produced by intracellular symbiotic bacteria protect the sponge *Hemimycale columella* from predation better than secondary metabolites. *Marine Ecology Progress Series*, 523, 81–92. doi:10.3354/meps11196
- Genta-Jouve, G., & Thomas, O. P. (2012). *Sponge Chemical Diversity. From Biosynthetic Pathways to Ecological Roles*. Advances in Marine Biology (Vol. 62). doi:10.1016/B978-0-12-394283-8.00004-7

- Gilbert, J. A., Jansson, J. K., & Knight, R. (2014). The Earth Microbiome project: successes and aspirations. *BMC Biology*, 12(1), 69. doi:10.1186/s12915-014-0069-1
- Goodrich, J. K., Waters, J. L., Poole, A. C., Sutter, J. L., Koren, O., Blekhman, R., ... Ley, R. E. (2014). Human genetics shape the gut microbiome. *Cell*, 159(4), 789–99. doi:10.1016/j.cell.2014.09.053
- Griffiths, S. M., Fox, G., Briggs, P. J., Donaldson, I. J., Hood, S., Richardson, P., ... Preziosi, R. F. (2016). A Galaxy-based bioinformatics pipeline for optimised, streamlined microsatellite development from Illumina next-generation sequencing data. *Conservation Genetics Resources*, 8(4), 481–486. doi:10.1007/s12686-016-0570-7
- Griffiths, S. M., Harrison, X. A., Weldon, C., Wood, M. D., Pretorius, A., Hopkins, K., ... Antwis, R. E. (2018). Genetic variability and ontogeny predict microbiome structure in a disease-challenged montane amphibian. *The ISME Journal*, 12, 2506–2517. doi:10.1038/s41396-018-0167-0
- Hentschel, U., Hopke, J., Horn, M., Friedrich, A. B., Wagner, M., Hacker, J., & Moore, B. S. (2002). Molecular evidence for a uniform microbial community in sponges from different oceans. *Applied and Environmental Microbiology*, 68(9), 4431–4440. doi:10.1128/AEM.68.9.4431-4440.2002
- Hernandez-Agreda, A., Gates, R. D., & Ainsworth, T. D. (2017). Defining the core microbiome in corals' microbial soup. *Trends in Microbiology*, 25(2), 125–140. doi:10.1016/j.tim.2016.11.003
- Karimi, E., Slaby, B. M., Soares, A. R., Blom, J., Hentschel, U., & Costa, R. (2018). Metagenomic binning reveals versatile nutrient cycling and distinct adaptive features in alphaproteobacterial symbionts of marine sponges. *FEMS Microbiology Ecology*, 94(6). doi:10.1093/femsec/fiy074
- Kashyap, P. C., Chia, N., Nelson, H., Segal, E., & Elinav, E. (2017). Microbiome at the frontier of personalized medicine. *Mayo Clinic Proceedings*, 92(12), 1855–1864. doi:10.1016/J.MAYOCP.2017.10.004
- Kearney, K. A., Butler, M., Glazer, R., Kelble, C. R., Serafy, J. E., & Stabenau, E. (2015).

Quantifying Florida Bay habitat suitability for fishes and invertebrates under climate change scenarios. *Environmental Management*, 55(4), 836–856. doi:10.1007/s00267-014-0336-5

Kelman, D., Kashman, Y., Rosenberg, E., Ilan, M., Ifrach, I., & Loya, Y. (2001). Antimicrobial activity of the reef sponge *Amphimedon viridis* from the Red Sea: evidence for selective toxicity. *Aquatic Microbial Ecology*, 24(1), 9–16. doi:10.3354/ame024009

Kubinak, J. L., Stephens, W. Z., Soto, R., Petersen, C., Chiaro, T., Gogokhia, L., ... Round, J. L. (2015). MHC variation sculpts individualized microbial communities that control susceptibility to enteric infection. *Nature Communications*, 6(1), 8642. doi:10.1038/ncomms9642

Lazzaro, B. P., Sceurman, B. K., & Clark, A. G. (2004). Genetic basis of natural variation in *D. melanogaster* antibacterial immunity. *Science*, 303(5665), 1873–1876. Retrieved from <http://science.sciencemag.org/content/303/5665/1873>

Lee, O. O., Chui, P. Y., Wong, Y. H., Pawlik, J. R., & Qian, P.-Y. (2009). Evidence for vertical transmission of bacterial symbionts from adult to embryo in the Caribbean sponge *Svenzea zeai*. *Applied and Environmental Microbiology*, 75(19), 6147–56. doi:10.1128/AEM.00023-09

Lesser, M. P., Fiore, C., Slattery, M., & Zaneveld, J. (2016). Climate change stressors destabilize the microbiome of the Caribbean barrel sponge, *Xestospongia muta*. *Journal of Experimental Marine Biology and Ecology*, 475, 11–18. doi:10.1016/j.jembe.2015.11.004

Luter, H. M., Widder, S., Botté, E. S., Abdul Wahab, M., Whalan, S., Moitinho-Silva, L., ... Webster, N. S. (2015). Biogeographic variation in the microbiome of the ecologically important sponge, *Carteriospongia foliascens*. *PeerJ*, 3, e1435. doi:10.7717/peerj.1435

Mahé, F., Rognes, T., Quince, C., de Vargas, C., & Dunthorn, M. (2014). Swarm: robust and fast clustering method for amplicon-based studies. *PeerJ*, 2, e593. doi:10.7717/peerj.593

Maldonado, M., & Riesgo, A. (2009). Gametogenesis, embryogenesis, and larval features of

- the oviparous sponge *Petrosia ficiformis* (Haplosclerida, Demospongiae). *Marine Biology*, 156(10), 2181–2197. doi:10.1007/s00227-009-1248-4
- Marino, C., Pawlik, J., López-Legentil, S., & Erwin, P. (2017). Latitudinal variation in the microbiome of the sponge *Ircinia campana* correlates with host haplotype but not anti-predatory chemical defense. *Marine Ecology Progress Series*, 565, 53–66. doi:10.3354/meps12015
- Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet*, 17(1), 10. doi:10.14806/ej.17.1.200
- Meirmans, P. G., & Van Tiendener, P. H. (2004). Genotype and Genodive: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes*, 4(4), 792–794. doi:10.1111/j.1471-8286.2004.00770.x
- Moitinho-Silva, L., Díez-Vives, C., Batani, G., Esteves, A. I., Jahn, M. T., & Thomas, T. (2017). Integrated metabolism in sponge–microbe symbiosis revealed by genome-centered metatranscriptomics. *The ISME Journal*, 11(7), 1651–1666. doi:10.1038/ismej.2017.25
- Morrow, K. M., Fiore, C. L., & Lesser, M. P. (2016). Environmental drivers of microbial community shifts in the giant barrel sponge, *Xestospongia muta*, over a shallow to mesophotic depth gradient. *Environmental Microbiology*, 18(6), 2025–2038. doi:10.1111/1462-2920.13226
- Müller, W. E. G., & Müller, I. M. (2003). Origin of the metazoan immune system: identification of the molecules and their functions in sponges. *Integrative and Comparative Biology*, 43(2), 281–92. doi:10.1093/icb/43.2.281
- Noyer, C., & Becerro, M. (2012). Relationship between genetic, chemical, and bacterial diversity in the Atlanto-Mediterranean bath sponge *Spongia lamella*. *Hydrobiologia*, 687, 85–99. doi:10.1007/s10750-011-0884-0
- Noyer, C., Thomas, O. P., & Becerro, M. A. (2011). Patterns of chemical diversity in the Mediterranean sponge *Spongia lamella*. *PLoS ONE*, 6(6), e20844. doi:10.1371/journal.pone.0020844

- On, O. L., Lau, S. C. K., & Qian, P. Y. (2006). Defense against epibiosis in the sponge *Mycale adhaerens*: Modulating the bacterial community associated with its surface. *Aquatic Microbial Ecology*, 43(1), 55–65. doi:10.3354/ame043055
- Pantos, O., Bongaerts, P., Dennis, P. G., Tyson, G. W., & Hoegh-Guldberg, O. (2015). Habitat-specific environmental conditions primarily control the microbiomes of the coral *Seriatopora hystrix*. *ISME Journal*, 9(9), 1916–1927. doi:10.1038/ismej.2015.3
- Peakall, R., & Smouse, P. E. (2012). GenAIEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research- an update. *Bioinformatics*, 28(19), 2537–2539. doi:10.1093/bioinformatics/bts460
- Pearce, D. S., Hoover, B. A., Jennings, S., Nevitt, G. A., & Docherty, K. M. (2017). Morphological and genetic factors shape the microbiome of a seabird species (*Oceanodroma leucorhoa*) more than environmental and social factors. *Microbiome*, 5(1), 146. doi:10.1186/s40168-017-0365-4
- Pita, Lucia, Fraune, S., & Hentschel, U. (2016). Emerging sponge models of animal-microbe symbioses. *Frontiers in Microbiology*, 7, 2102. doi:10.3389/fmicb.2016.02102
- Pita, Lucía, López-Legentil, S., & Erwin, P. M. (2013). Biogeography and host fidelity of bacterial communities in *Ircinia* spp. from the Bahamas. *Microbial Ecology*, 66(2), 437–47. doi:10.1007/s00248-013-0215-2
- Pita, Lucía, Turon, X., López-Legentil, S., & Erwin, P. M. (2013). Host rules: spatial stability of bacterial communities associated with marine sponges (*Ircinia* spp.) in the Western Mediterranean Sea. *FEMS Microbiology Ecology*, 86(2), 268–76. doi:10.1111/1574-6941.12159
- Puyana, M., Pawlik, J., Blum, J., Fenical, W., Puyana, M., Pawlik, J., ... Fenical, W. (2015). Metabolite variability in Caribbean sponges of the genus *Aplysina*. *Revista Brasileira de Farmacognosia*, 25(6), 592–599. doi:10.1016/j.bjp.2015.08.002
- Qiu, Z., Coleman, M. A., Provost, E., Campbell, A. H., Kelaher, B. P., Dalton, S. J., ... Marzinelli, E. M. (2019). Future climate change is predicted to affect the microbiome and condition of habitat-forming kelp. *Proceedings of the Royal Society B: Biological Sciences*

*Sciences*, 286(1896), 20181887. doi:10.1098/rspb.2018.1887

- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., ... Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41(Database issue), D590-6. doi:10.1093/nar/gks1219
- Ribes, M., Calvo, E., Movilla, J., Logares, R., Coma, R., & Pelejero, C. (2016). Restructuring of the sponge microbiome favors tolerance to ocean acidification. *Environmental Microbiology Reports*, 8(4), 536–544. doi:10.1111/1758-2229.12430
- Robblee, M., Barber, T., Carlson, P., Durako, M., Fourqurean, J., Muehlstein, L., ... Zieman, J. (1991). Mass mortality of the tropical seagrass *Thalassia testudinum* in Florida Bay (USA). *Marine Ecology Progress Series*, 71, 297–299. Retrieved from <http://www.resalliance.org/index.php?id=458>
- Rousset, F. (2008). genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*, 8(1), 103–6. doi:10.1111/j.1471-8286.2007.01931.x
- Rubio-Portillo, E., Kersting, D. K., Linares, C., Ramos-Esplá, A. A., & Antón, J. (2018). Biogeographic differences in the microbiome and pathobiome of the coral *Cladocora caespitosa* in the Western Mediterranean Sea. *Frontiers in Microbiology*, 9(JAN), 1–11. doi:10.3389/fmicb.2018.00022
- Schmitt, S., Tsai, P., Bell, J., Fromont, J., Ilan, M., Lindquist, N., ... Taylor, M. W. (2012). Assessing the complex sponge microbiota: core, variable and species-specific bacterial communities in marine sponges. *The ISME Journal*, 6(3), 564–76. doi:10.1038/ismej.2011.116
- Schmitt, S., Weisz, J. B., Lindquist, N., & Hentschel, U. (2007). Vertical transmission of a phylogenetically complex microbial consortium in the viviparous sponge *Ircinia felix*. *Applied and Environmental Microbiology*, 73(7), 2067–78. doi:10.1128/AEM.01944-06
- Shade, A., & Handelsman, J. (2012). Beyond the Venn diagram: The hunt for a core microbiome. *Environmental Microbiology*, 14(1), 4–12. doi:10.1111/j.1462-

- Sharp, K. H., Eam, B., Faulkner, D. J., & Haygood, M. G. (2007). Vertical transmission of diverse microbes in the tropical sponge *Corticium* sp. *Applied and Environmental Microbiology*, 73(2), 622–9. doi:10.1128/AEM.01493-06
- Sipkema, D., de Caralt, S., Morillo, J. A., Al-Soud, W. A., Sørensen, S. J., Smidt, H., & Uriz, M. J. (2015). Similar sponge-associated bacteria can be acquired via both vertical and horizontal transmission. *Environmental Microbiology*, 17(10), 3807–3821. doi:10.1111/1462-2920.12827
- Stevely, J. M., Sweat, D. E., Bert, T. M., Sim-Smith, C., & Kelly, M. (2010). Sponge mortality at Marathon and Long Key, Florida: Patterns of species response and population recovery. *Proceedings of the 63rd Gulf and Caribbean Fisheries Institute*, 63, 384–400.
- Swierts, T., Cleary, D. F. R., & de Voogd, N. J. (2018). Prokaryotic communities of Indo-Pacific giant barrel sponges are more strongly influenced by geography than host phylogeny. *FEMS Microbiology Ecology*, 94(12), 1–12. doi:10.1093/femsec/fiy194
- Thacker, R. W., & Freeman, C. J. (2012). Sponge-microbe symbioses: recent advances and new directions. *Advances in Marine Biology*, 62, 57–111. doi:10.1016/B978-0-12-394283-8.00002-3
- Thomas, T. R. A., Kavlekar, D. P., & LokaBharathi, P. A. (2010). Marine drugs from sponge-microbe association- a review. *Marine Drugs*, 8(4), 1417–68. doi:10.3390/md8041417
- Thomas, T., Rusch, D., DeMaere, M. ., Yung, P. ., Lewis, M., Halpern, A., ... Kjelleberg, S. (2010). Functional genomic signatures of sponge bacteria reveal unique and shared features of symbiosis. *The ISME Journal*, 4, 1577–1567. doi: 10.1038/ismej.2010.74
- Thomas, T., Moitinho-Silva, L., Lurgi, M., Björk, J. R., Easson, C., Astudillo-García, C., ... Webster, N. S. (2016). Diversity, structure and convergent evolution of the global sponge microbiome. *Nature Communications*, 7, 11870. doi:10.1038/ncomms11870
- Tout, J., Astudillo-García, C., Taylor, M. W., Tyson, G. W., Stocker, R., Ralph, P. J., ... Webster, N. S. (2017). Redefining the sponge-symbiont acquisition paradigm: sponge microbes exhibit chemotaxis towards host-derived compounds. *Environmental*

- Trevelline, B. K., Fontaine, S. S., Hartup, B. K., & Kohl, K. D. (2019). Conservation biology needs a microbial renaissance: a call for the consideration of host-associated microbiota in wildlife management practices. *Proceedings of the Royal Society B: Biological Sciences*, 286(1895), 20182448. doi:10.1098/rspb.2018.2448
- Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., ... Gordon, J. I. (2009). A core gut microbiome in obese and lean twins. *Nature*, 457, 480–485. doi:10.1038/nature07540
- Turon, M., Cáliz, J., Garate, L., Casamayor, E. O., & Uriz, M. J. (2018). Showcasing the role of seawater in bacteria recruitment and microbiome stability in sponges. *Scientific Reports*, 8(1), 15201. doi:10.1038/s41598-018-33545-1
- Uren Webster, T. M., Consuegra, S., Hitchings, M., & Garcia de Leaniz, C. (2018). Interpopulation variation in the Atlantic salmon microbiome reflects environmental and genetic diversity. *Applied and Environmental Microbiology*, 84(16), e00691-18. doi:10.1128/AEM.00691-18
- van de Water, J. A. J. M., Allemand, D., & Ferrier-Pagès, C. (2018). Host-microbe interactions in octocoral holobionts - recent advances and perspectives. *Microbiome*, 6(1), 64. doi:10.1186/s40168-018-0431-6
- Wagner, M. R., Lundberg, D. S., del Rio, T. G., Tringe, S. G., Dangl, J. L., & Mitchell-Olds, T. (2016). Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. *Nature Communications*, 7, 12151. doi:10.1038/ncomms12151
- Webster, N. S., & Reusch, T. B. H. (2017). Microbial contributions to the persistence of coral reefs. *The ISME Journal*. doi:10.1038/ismej.2017.66
- Wehrl, M., Steinert, M., & Hentschel, U. (2007). Bacterial uptake by the marine sponge *Aplysina aerophoba*. *Microbial Ecology*, 53(2), 355–365. doi:10.1007/s00248-006-9090-4
- Weigel, B. L., & Erwin, P. M. (2017). Effects of reciprocal transplantation on the microbiome and putative nitrogen cycling functions of the intertidal sponge, *Hymeniacidon*

*heliophila*. *Scientific Reports*, 7, 43247. doi:10.1038/srep43247

Wiens, M., Korzhev, M., Krasko, A., Thakur, N. L., Perović-Ottstadt, S., Breter, H. J., ...

Müller, W. E. G. (2005). Innate immune defense of the sponge *Suberites domuncula* against bacteria involves a MyD88-dependent signaling pathway. Induction of a perforin-like molecule. *The Journal of Biological Chemistry*, 280(30), 27949–59. doi:10.1074/jbc.M504049200

Wiens, M., Korzhev, M., Perović-Ottstadt, S., Luthringer, B., Brandt, D., Klein, S., & Müller, W. E. G. (2007). Toll-like receptors are part of the innate immune defense system of sponges (Demospongiae: Porifera). *Molecular Biology and Evolution*, 24(3), 792–804. doi:10.1093/molbev/msl208

Wilkinson, C. R., Garrone, R., & Vacelet, J. (1984). Marine sponges discriminate between food bacteria and bacterial symbionts: Electron microscope radioautography and in situ evidence. *Proceedings of the Royal Society B: Biological Sciences*, 220(1221), 519–528. Retrieved from <http://rsbp.royalsocietypublishing.org/content/220/1221/519>

Wright, R. M., Kenkel, C. D., Dunn, C. E., Shilling, E. N., Bay, L. K., & Matz, M. V. (2017). Intraspecific differences in molecular stress responses and coral pathobiome contribute to mortality under bacterial challenge in *Acropora millepora*. *Scientific Reports*, 7(1), 2609. doi:10.1038/s41598-017-02685-1

## FIGURE LEGENDS

### Figure 1

Principle coordinates analysis (PCoA) showing Euclidean genetic distances among *Ircinia campana* individuals at Long Key and Kemp Channel

### Figure 2

Principle coordinates analysis (PCoA) showing Bray-Curtis distances among microbiomes of *Ircinia campana* from Long Key and Kemp Channel.

### Figure 3

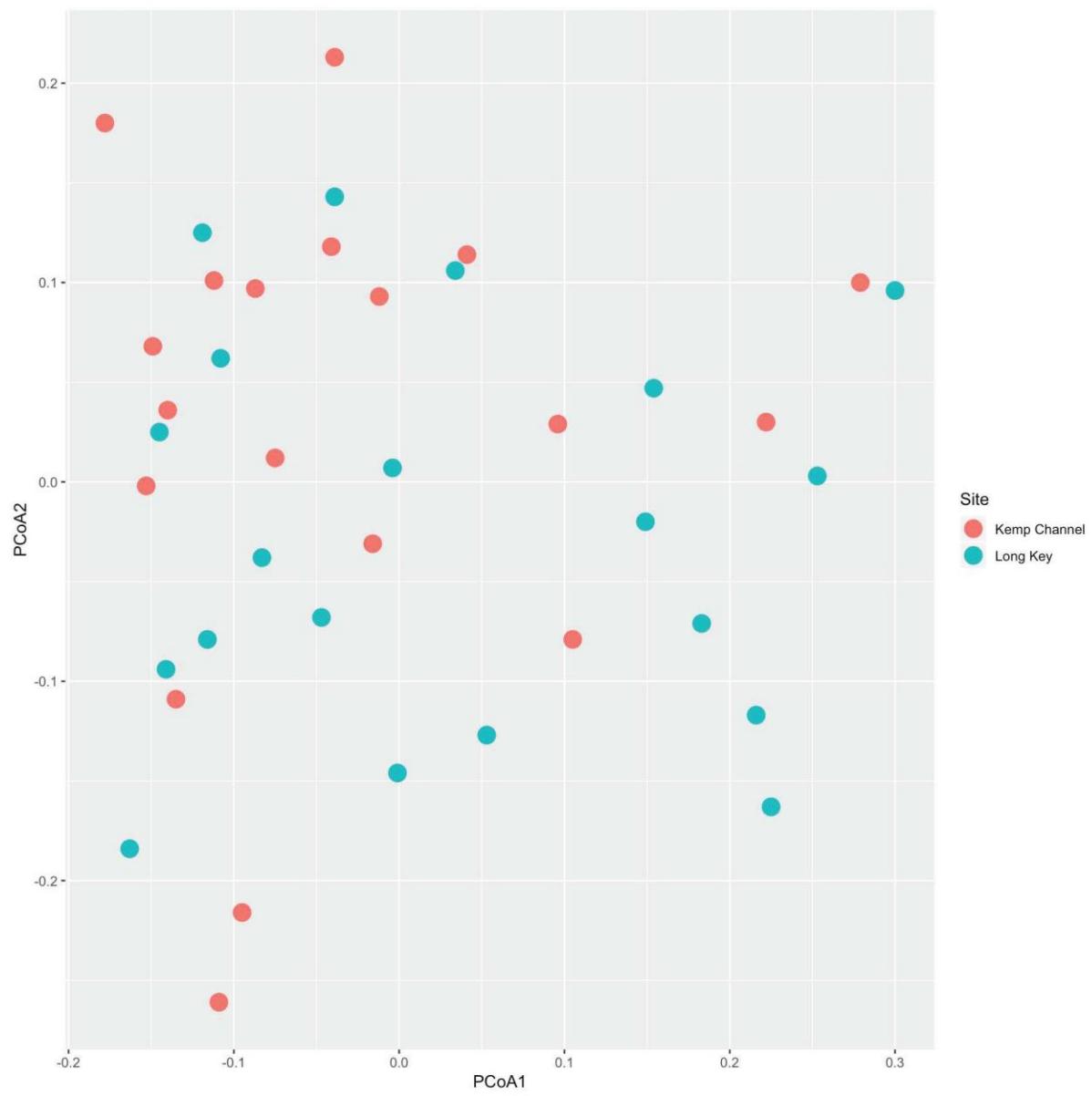
Scatter plot with regression lines showing correlations between pairwise host Euclidean genetic distances (using nine microsatellite genotypes) and microbial community Chao1 distances (alpha-diversity) in *Ircinia campana* at a) Long Key, and b) Kemp Channel, and host genetic Euclidean distances and microbial community Jensen-Shannon divergence values (beta-diversity) at c) Long Key, and d) Kemp Channel. Shaded areas show 95% confidence intervals.

**Table 1**

Results of Mantel tests between genetic distance matrices and microbiome distance matrices of *Ircinia campana* sponges. Genetic distances were based on either ten (“full genetic dataset”) or five (“reduced genetic dataset”) microsatellites.

Location	Microbiome dissimilarity metric	Genetic dataset	r value	p value
Across sites	Chao1	Full	0.410	0.001
		Reduced	0.347	0.001
	Bray-Curtis	Full	0.211	0.002
		Reduced	0.139	0.025
	JSD	Full	0.278	0.001
		Reduced	0.206	0.003
Long Key	Chao1	Full	0.503	0.001
		Reduced	0.264	0.005
	Bray-Curtis	Full	0.465	0.001
		Reduced	0.232	0.006
	JSD	Full	0.483	0.001
		Reduced	0.236	0.009
Kemp Channel	Chao1	Full	0.297	0.033
		Reduced	0.340	0.023
	Bray-Curtis	Full	0.280	0.013
		Reduced	0.228	0.039
	JSD	Full	0.307	0.014
		Reduced	0.277	0.033

# Accepted Article



# Accepted Article

This article is protected by copyright. All rights reserved.

