

Universiteit
Leiden
The Netherlands

Diversity in the globally intertwined giant barrel sponge species complex

Swierts, T.

Citation

Swierts, T. (2019, December 17). *Diversity in the globally intertwined giant barrel sponge species complex*. Retrieved from https://hdl.handle.net/1887/81578

Version:	Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/81578

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The following handle holds various files of this Leiden University dissertation:
http://hdl.handle.net/1887/81578

Author: Swierts, T.
Title: Diversity in the globally intertwined giant barrel sponge species complex
Issue Date: 2019-12-17

DIVERSITY IN THE GLOBALLY INTERTWINED GIANT BARREL SPONGE SPECIES COMPLEX

Thomas Swierts

Thomas Swierts. 2019. Diversity in the globally intertwined giant barrel sponge species complex.
Ph.D. Thesis at Leiden University, the Netherlands, 2019.

ISBN: 978-94-6182-988-7

Cover, layout, and printing: Off Page, Amsterdam

This Ph.D. research was made possible with financial support of:
The Dutch Research Council (NWO)
Royal Netherlands Academy of Arts and Sciences (KNAW)
Naturalis Biodiversity Center, Marine Biodiversity group, Leiden, the Netherlands

DIVERSITY IN THE GLOBALLY INTERTWINED GIANT BARREL SPONGE SPECIES COMPLEX

Proefschrift

Ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op dinsdag 17 december 2019
klokke 10:00 uur

door

Thomas Swierts
geboren te Amsterdam
in 1989

PROMOTOREN

Prof. Dr. N.J. de Voogd (Naturalis Biodiversity Center & Universiteit Leiden)
Prof. Dr. P.M. van Bodegom (Universiteit Leiden)

CO-PROMOTOR

Dr. D.F.R. Cleary (University of Aveiro)

PROMOTIECOMMISSIE

Prof. Dr. A. Tukker (Universiteit Leiden) - Voorzitter
Prof. Dr. Ir. M. G. Vijver (Universiteit Leiden) - Secretaris
Prof. Dr. J. C. Biesmeijer (Naturalis Biodiversity Center & Universiteit Leiden)
Dr. D. E. Rozen (Universiteit Leiden)
Prof. Dr. J. Pawlik (UNC Wilmington)
Dr. Ir. D. Sipkema (Wageningen University & Research)

TABLE OF CONTENTS

Chapter 1 General introduction 7

Section one: Genetics

Chapter 2 Globally intertwined evolutionary history of giant barrel sponges 21

Chapter 3 Two differently colored giant barrel sponge species in Tanzania merge when they grow against each other 41

Section two: Prokaryotes

Chapter 4 Prokaryotic communities of Indo-Pacific giant barrel sponges are more strongly influenced by geography than host phylogeny 47

Chapter 5 Impacts of host identity and geography on the prokaryotic community of giant barrel sponges at multiple spatial scales 69

Section three: Reef Interactions

Chapter 6 The sponge microbiome within the greater coral reef microbial metacommunity 117

Chapter 7 The giant barrel sponge facilitates the recovery of coral fragments after a tropical storm in Taiwan 147

Chapter 8 Synthesis and future directions 151

Chapter 9 References 166

Summary 198

Samenvatting 200

Acknowledgements 202

Curriculum Vitae 204

List of publications 205

1

General introduction

Coral reefs are among the most productive and diverse ecosystems on Earth, rivaling the biodiversity of rainforests (Knowlton et al. 2010). They provide numerous ecosystem services, such as in fisheries, shoreline protection, tourism and yielding compounds for the development of new medicine (Woodhead et al. 2019). Over 500 million people depend on coral reefs for their subsistence (Moberg and Folke 1999; Hughes et al. 2017). Over half of all coral reefs worldwide are threatened by climate change and other stressors, potentially affecting the livelihoods of millions of people (Burke et al. 2011). The large array of human-induced and natural pressures related to coral reef decline has been well studied (e.g. Wilkinson 1999; Hoegh-Guldberg et al. 2007; Perry et al. 2013). Among the major anthropogenic stressors are increased terrestrial runoff, coastal development, dredging, unsustainable fisheries and plastic waste (Bannister et al. 2012; Stender et al. 2014; Hughes et al. 2017; Lamb et al. 2018).

Corals are vulnerable to environmental changes, and other groups of organisms may profit from their demise (Mumby et al. 2016; Cruz et al. 2017). Amongst others, algae, cyanobacteria, and sponges have been reported to increase when corals decline (Hughes et al. 2007; Nörstrom et al. 2009; de Bakker et al. 2017). Of these groups, sponges (Porifera) may challenge both hard and soft corals in terms of species richness, abundance, and biomass on coral reefs (Diaz and Rützler 2001). They play a central role in regulating the carbonate budget of the reefs through bio-erosion, and their erosion rates can equal the calcification rates of reef-building corals (Perry et al. 2014; Webb et al. 2017). An equal balance between erosion and accretion is essential for the sustainability of coral reef ecosystems (Bell et al. 2008). Furthermore, through their pumping and feeding behaviour, sponges play central roles in the cycling of various nutrient elements including the nitrogen, sulphur, silicon, and phosphorus cycles (Southwell et al. 2008; Mohamed et al. 2010; Maldonado et al. 2012; Fiore et al. 2013a; Zhang et al. 2015) and contribute to the so-called benthic-pelagic coupling on coral reefs (Pile et al. 1997; Bak et al. 1998).

Benthic-pelagic coupling is the exchange of energy, mass, or nutrients between the seabed (i.e. the benthic environment) and the water column (i.e. the pelagic environment) (Griffiths et al. 2017). Sponges have relatively simple body plans and their cells are not organized in tissues or organs (van Soest et al. 2012). Instead, their bodies are designed to pump water through a system of channels and pores from which they filter food particles and dissolved organic matter (Pile et al. 1997; de Goeij et al. 2008; Koopmans et al. 2010). Water is drawn into the sponge through small surface openings called ostia and is then led through a narrowing system of incurrent canals to choanocyte chambers. The choanocyte chambers are covered with flagellates (choanocytes), which generate water flows by synchronized movements. From the choanocyte chambers, the water passes through microvilli where nutrients are filtered from the water and food particles are phagocytized by the sponge cells. The water then streams through a series of exhalant channels to the spongocoel, a large central cavity, and is then discharged through an osculum, a large opening on the sponge surface (Reiswig

1975; Larsen and Riisgard 1994). Sponge assemblages in natural densities can overturn the entire water column in a period ranging from 56 days to less than one day (Reiswig 1974; Pile et al. 1996; Patterson et al. 1997). The pumping system of sponges is efficient and it is estimated that less than 4% of the total metabolic expenditure in sponges is required for this activity (Riisgard and Larsen 1995).

The food extracted from the water mainly consists of organic matter. Particulate organic matter (POM), for example detritus or live picoplankton, forms an important part of their nutrition (Hadas et al. 2009). All POM in the seawater that is pumped through the sponge, passes through the choanocyte chambers and the observed variation in the uptake and release of different groups of picoplankton suggests that sponges are selectively feeding on the picoplankton (Frost 1980; Ribes et al. 1999; Yahel et al. 2006; Hanson et al. 2009; Maldonado et al. 2010; Riisgard and Larsen 2010). Due to the efficiency with which the sponges filter picoplankton from the sea, the number of bacteria in discharged seawater can be reduced by more than 99% (Wehrl 2007). However, in other sponge species dissolved organic matter (DOM) is the main source of organic carbon in their diets (Yahel et al. 2003; de Goeij et al. 2008; Mueller et al. 2014). The 'sponge loop' describes how sponges make DOM available to higher trophic levels by rapidly expelling filter cells as detritus (de Goeij et al. 2013). The sponge loop has been suggested to be the reason that coral reef ecosystems can exist in oligotrophic waters or 'marine deserts' (de Goeij et al. 2013).

SPONGE MICROBIAL COMMUNITY

Symbiotic microorganisms are believed to play key roles in the physiology of sponges, including many of the abovementioned processes (Osinga et al. 2001; Pita et al. 2018). Certain microorganisms can also harvest energy from light by photosynthesis making some sponges net primary producers (Southwell et al. 2008; Thacker and Freeman 2012; Fiore et al. 2013a). Others can produce bioactive compounds, some of which act as a chemical defense used to deter predators, pathogens and other harmful organisms (Pawlik 1993; Hentschel et al. 2012). The antimicrobial activities of sponge-associated microorganisms show great pharmaceutical potential and sponges are considered the most promising marine source for new therapeutic compounds (Fuerst 2014; Indraningrat et al. 2016; Mori et al. 2018). Of the 15.000 discovered marine natural products, around 30% have been derived from sponges (Leal et al. 2012; Mehbub et al. 2014; Blunt et al. 2018). The pharmaceutical potential of marine natural products in sponges, however, is still largely untapped (Romano et al. 2017). A better understanding of the nature of the symbiotic relationship between sponges and their associated microbiomes and the drivers of changes in this relationship is essential to accelerate these efforts (Taylor et al. 2007a; Webster and Taylor 2012; Valliappan et al. 2014; Marino et al. 2017). Due to their intricate relationship, sponges and their associated microorganisms are together often referred to as the 'sponge holobiont' (Taylor et al. 2007a; 2007b; Webster and Thomas 2016; Pita et al. 2018). New molecular techniques have accelerated the number of studies on the sponge holobiont, and have provided new

insights in the richness, functional roles and evolutionary history of the sponge-associated microbial community.

A dichotomy seems to exist between sponges harbouring dense communities of symbiotic microorganisms and sponges with much lower concentrations (Reiswig 1974). For these two groups, the names 'high microbial abundance' (HMA) and 'low microbial abundance' (LMA) are generally used (Hentschel et al. 2003). The differences in microbial abundance can be clearly observed with Transmission Electron Microscopy (Fig. 1.1). HMA sponges harbour 10^8 – 10^{10} microbial cells per gram of sponge wet weight, while LMA sponges contain only 10^5-10^6 prokaryotic cells per gram of sponge wet weight, which is similar to the concentration of microorganisms in seawater (Hentschel et al. 2006). In HMA sponges, the microbial biomass can account for more than 35% of the total sponge biomass (Vacelet 1975). HMA microbiomes are rich and diverse, whereas LMA microbiomes are mostly made up of Cyanobacteria and Proteobacteria (Hentschel et al. 2006; Weisz et al. 2007; Gloeckner et al. 2014; Moitinho-Silva et al. 2017a). Various physiological and metabolic differences between the groups have been found, and this is receiving more attention in recent studies (Weisz et al. 2008; Ribes et al. 2012; Moitinho-Silva et al. 2017b).

HMA sponges are capable of maintaining a unique microbial community, despite the constant influx of seawater (Glasl et al. 2017a). Sponge host species often have distinctive microbial fingerprints (Thomas et al. 2016) and the differences among hosts can originate at an early reproductive phase (Schmitt et al. 2008). Microorganisms can be assimilated in gametes by the host sponge ensuring the transmission of essential microorganisms to their offspring (Maldonado et al. 2005; Funkhouser and Bordestein 2013). However, the majority

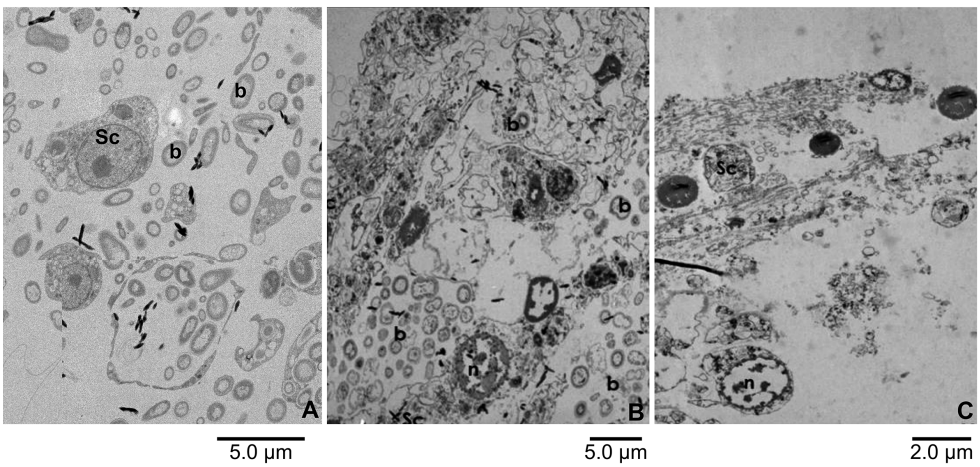


Figure 1.1. Transmission electron microscopy of three sponge species belonging to the order Haplosclerida. Two species are classified as HMA sponges (A, B) and one species is classified as a LMA sponge (C). A = *Xestospongia testudinaria*, B = *Xestospongia vansoesti*, C = *Haliclona fascigera*. Sc = sponge cell; b = bacteria; n = nucleus.

of the Operational Taxonomic Units (OTUs) are likely to be harvested from the surrounding environment, in which they tend to have much lower densities than in the host sponge (Reveillaud et al. 2014; Lynch and Neufeld 2015). As many as 41 different prokaryotic phyla have thus far been identified in sponges, many of which are shared among sponge species (Thomas et al. 2016). The sponge microbiome is thought to be stable across time and space, especially the core community that consists of OTUs that are present in most or all individuals of a certain host species (Erwin et al. 2012; Pita et al. 2013a; 2013b; 2018; Cárdenas et al. 2014; Thomas et al. 2016; Glasl et al. 2018). However, temporal and spatial variation did exist in the microbial communities of some sponge species (Wichels et al. 2006; White et al. 2012; Luter et al. 2015; Weigel and Erwin 2016; Pita et al. 2018).

CLASSIFICATION OF SPONGES

Sponges have evolved over 600 million years ago, placing them among the oldest animal lineages on Earth (Love et al. 2009; Simion et al. 2017). They are generally considered a sister group to all other multicellular animals (Fueda et al. 2017; Pett et al. 2019) and they are subdivided into four distinct classes, the Calcarea, Demospongiae, Hexactinellida and Homoscleromorpha (Gazave et al. 2011; van Soest et al. 2012; van Soest et al. 2012; 2015). The class Demospongiae is the largest and most diverse class, occurs in marine and freshwater environments, and sponges in this class have skeletons composed of spongin fibres and/or siliceous spicules. Approximately 81% of the 7,000 described species belong to this class, and more than 50 new species are described every year (Hooper and van Soest 2002; van Soest et al. 2019).

Demosponges exist in a wide variety of shapes and sizes, from small encrusting layers to large cups, barrels or branching forms (van Soest et al. 2012). Besides high interspecific morphological variation, sponges of the same species may adapt to local environmental conditions such as hydrodynamics, light, and turbidity, resulting in numerous morphologies (Palumbi 1984; Bell et al. 2002). Due to this high morphological variation, the classification of sponges at higher taxonomic levels has long been in debate. The Systema Porifera was a historic publication in 2002 and provided a large revision and comprehensive overview of the taxonomy of sponges (Hooper and van Soest 2002). The classification used in the Systema Porifera is largely based on sponge morphology, especially of the spicules. Although morphology-based classifications provided an excellent baseline, the use of molecular techniques revealed several weaknesses and inconsistencies in this morphology-based classification (Wörheide et al. 2012; Renard et al. 2018). Especially at the lower taxonomic levels in the class Demospongiae, molecular results did not support the existing classification (Redmond et al. 2013). Many scientists and other end users depend on the correct identification of their studied organisms to properly set up experiments and interpret results. Therefore, multiple attempts have been made to further improve the classification of the Demospongiae using molecular techniques, and this remains an

ongoing effort of the scientific community (Redmond et al. 2013; Morrow and Cárdenas 2015; Erpenbeck et al. 2016).

Despite their ecological importance, sponges have been underrepresented in coral reef research (de Voogd et al. 2006; Bell 2008). This is illustrated by the low attendance of international sponge conferences (909 individual participants between 1968 and 2013) compared to international coral reef symposia (over 1000 participants per edition) (Schönberg 2017). Furthermore, coral reef communities are generally assessed on the basis of benthic cover, while sponges are more abundant if the three-dimensional structure of reefs is taken into account, as they are often present in cryptic spaces (Zea 1993; Southwell et al. 2008). If we want to understand coral reefs better, we should look at sponges more.

GIANT BARREL SPONGES

A critical group within the demosponges consists of the giant barrel sponges (belonging to the genus *Xestospongia,* order *Haplosclerida)*. They have a large impact on coral reefs around the globe due to their abundance, size, and ecological relevance. They are among the most conspicuous reef members and occur in the tropical seas of the Atlantic Ocean, Indian Ocean, and western Pacific Ocean. They can reach sizes of over a meter in height and width and due to their slow growth rates of ±1.85 cm per year, large specimens in the Caribbean are thought to be over 1000 years old. One photographed specimen in Curaçao was estimated to be ±2,300 years old (Nagelkerken et al. 2000; McMurray et al. 2008). Due to their size and longevity, giant barrel sponges have been nicknamed 'Redwoods of the Reef' (McMurray et al. 2008). On Indo-Pacific reefs, however, giant barrel sponges grow at least twice as fast and are less long-lived, suggesting that they are more comparable to 'Pines in the Indo-Pacific' (McGrath et al. 2018).

In the Caribbean, giant barrel sponge populations may cover more than 9% of the available reef surface area and have a biomass and filtering capacity greater than any other benthic invertebrate (Zea 1993; McMurray et al. 2008). They are capable of pumping vast quantities of water per day and retain picoplankton at high efficiencies (McMurray et al. 2014; 2016). Giant barrel sponges alone can overturn a water column of 30 m deep every 2.8 to 6.0 days in the Florida Keys, and between 2.3 and 18 days in the Bahamas (McMurray et al. 2014). Their diet consists mostly of dissolved organic carbon (±60-70% of the total organic carbon) and detritus (±20-35%) (McMurray et al. 2017; Wooster et al. 2019). They can also offer shelter to corals (Hammerman and García-Hernández 2016) and harbour other organisms such as sea cucumbers, brittle stars, and lobsters (Hammond and Wilkinson 1985; Baba 1994).

Giant barrel sponges reproduce by broadcast spawning and do not reproduce by fragmentation (McMurray and Pawlik 2009). They release negatively buoyant egg cells and positively buoyant sperm cells during mass spawning events (Ritson-Williams et al. 2005).

Recordings of spawning events are rare but were made during multiple seasons in both the Indo-Pacific and the Caribbean (Ritson-Williams et al. 2005; McMurray et al. 2008; Swierts et al. 2013). They produce a large number of gametes (Fromont and Bergquist 1994) that are unpalatable to fish predators (Lindquist and Hay 1996). Not much is known about the larval behavior, but the dispersal of larvae is believed to be influenced by ocean currents (López-Legentil and Pawlik 2008). Recent increases in the abundance of giant barrel sponges are observed on multiple reefs throughout the Caribbean, with some reefs showing an increase in abundance of more than 300% in 12 years (McMurray et al. 2010; 2015).

Nowadays, three different species are described and generally accepted: *Xestospongia muta* occurs in the Caribbean region, *Xestospongia testudinaria* is spread across large parts of the Indo-Pacific and *Xestospongia bergquistia* is endemic to reefs in Australia (Schmidt 1870; Lamarck 1885; Fromont 1991). The species names of *X. muta* and *X. testudinaria* have been subject to many changes. According to the World Porifera Database, *Xestospongia muta* was formerly known as *Schmidtia muta* and *Petrosia muta*, and *Xestospongia testudinaria* was previously named *Alcyonium testudinarium*, *Reniera crateriformis, Reniera testudinaria* and *Petrosia testudinaria* (van Soest et al. 2015).

Xestospongia testudinaria and *X. bergquistia* are sympatric in Australia and the morphological differences between the species are subtle, with the most profound difference being the amount of spongin fibres present between the spicules (Fromont 1991). It is not possible to distinguish between the species based on visual characteristics in the field, although the strength and elasticity of the sponge is an indication of species identity when they are being pierced or cut (Fromont 1991). No unique characters distinguishing all three species have been found so far. The spicule size ranges of the three species overlap and *X. muta* and *X. testudinaria* have similar morphologies and skeletal structures, and the main distinction between the two is the ocean they live in (Setiawan et al. 2016a).

The giant barrel sponge species have a large intraspecific variability of spicule types and sizes (Subagio et al. 2017) and individuals within each species are also highly variable in size, shape, and external surface morphology. They can have a smooth external surface, or be covered with digitate or lamellate structures (Kerr and Kelly-Borges 1994). Previously, this external morphological variation was believed to represent multiple species (Wilson 1925), and the name *Xestospongia testudinaria* var. *fistulophora* was given to a variety of giant barrel sponge from the Philippines of which the outer surface had fistular structures instead of vertical ridges. Congruent patterns between external morphology, mitochondrial DNA markers and nuclear DNA markers on reefs around Lembeh Island in Indonesia support the hypothesis that multiple species co-exist in the Indo-Pacific (Swierts et al. 2013). Furthermore, in the Caribbean, distinct chemotypes of *X. muta* exist (Fromont et al. 1994) which are not correlated with sampling location, depth, or morphotype (Kerr and Kelly-

Borges 1994). These chemotypes were also suggested to represent different biological species. Furthermore, the three giant barrel sponge species cannot be differentiated using the I3-M11 partition of the CO1 mitochondrial gene (Setiawan 2016a; 2016b). This gene was suggested as a candidate marker for species-level phylogenies in sponges other than the standard barcoding marker using the Folmer primers (Erpenbeck et al. 2006a). Microsatellite data further suggests that the current taxonomy may not represent the true diversity in giant barrel sponges, potentially hindering our understanding of their evolutionary history and the correct interpretation of experimental results (Bell et al. 2014; Richards et al. 2016). Unfortunately, no DNA could be extracted from the dried syntypes of *X. muta* and the holotype of *X. testudinaria* has been lost, and it is, therefore, impossible to examine the genetics of these specimens.

Giant barrel sponges are HMA sponges, and their microbiome consists primarily of Chloroflexi, Proteobacteria, Acidobacteria and Actinobacteria (Montalvo et al. 2014; Montalvo and Hill 2011; Polonia et al. 2017; Cleary et al. 2015a; De Voogd et al. 2015). The bacterial communities of *X. muta* and *X. testudinaria* are very similar, but species-specific differences also exist (Montalvo and Hill 2011). Since the sponges live in different oceans, it is not clear whether the differences are a result of the different local environments or because the sponges are two different species. In the Caribbean local environmental differences significantly affected the microbial communities of giant barrel sponges (Lesser et al. 2016; Morrow et al. 2016; Villegas-Plazas et al. 2018). However, these differences may also represent the different giant barrel sponge species that have been suggested to exist in the Caribbean (Fromont 1994 et al. 1994). These examples illustrate the importance of proper identification of giant barrel sponge species and their evolutionary history.

THESIS OUTLINE

The state of coral reef ecosystems is precarious and it remains difficult to predict how they are affected by current and future environmental changes. The importance of sponges in coral reef ecosystems has long been neglected but is becoming more and more acknowledged. Nevertheless, some fundamental aspects of sponges and their microbiomes remain poorly understood. Inconsistencies between taxonomical classifications and phylogenies are unresolved, the interactions between individual drivers of the prokaryotic community of sponges are unexamined and the role of the sponge holobiont in the wider coral reef ecosystem is unclear. These gaps in our knowledge often even exist in sponges that are used as model groups, such as giant barrel sponges.

This thesis addressed these challenges and aims to answer the following questions: 1. How many giant barrel sponge species exist around the globe? 2. What is the evolutionary history of the giant barrel sponge species? 3. What are the drivers of variation in the prokaryotic community composition of giant barrel sponges? 4. How does the richness, diversity, and

evenness of the (giant barrel) sponge prokaryotic community relate to those of other coral reef organisms? This is studied through a series of in-situ observational studies and by multiple laboratory analyses on giant barrel sponge samples collected from the tropical regions of the Atlantic, Indian and Pacific Ocean.

The section *Genetics* of this thesis unravels the complex phylogeny and evolutionary history of giant barrel sponges. **Chapter 2** shows that at least three giant barrel sponge species exist in the Caribbean and at least three species exist in the Indo-Pacific. The species in each of the ocean basins are sympatric, difficult to distinguish morphologically in the field and do not form monophyletic lineages. In other words, a sponge from Curaçao can genetically be more closely related to a giant barrel sponge in Indonesia, than to another giant barrel sponge on the same reef. This suggests that multiple giant barrel sponge species already occurred before the Atlantic and Indo-Pacific realms were geographically separated by the closure of the Tethys Sea. Although the species diversity in giant barrel sponges seems cryptic, **Chapter 3** contains a photo with a short description of an observation in Tanzania, demonstrating two color morphologies of giant barrel sponges that correspond with different species identities.

The section *Prokaryotes* discusses the diversity of host associated prokaryotes in the giant barrel sponge species complex. **Chapter 4** shows that the prokaryotic community of giant barrel sponges is more strongly influenced by geography than host phylogeny in the Indo-Pacific. This is also true for the Caribbean, as is shown in **Chapter 5**, but this chapter also confirms the role of host identity as a driver of the prokaryotic community, particularly at smaller spatial scales - including giant barrel sponges - relates to other reef organisms such as corals, algae, holothurians, nudibranchs, sea urchins and sponge denizens. Prokaryotic microorganisms are often shared among multiple coral reef host organisms, and the sponge prokaryote community does not appear to be as sponge-specific as previously thought. The section *Reef interactions examines* two ways in which giant barrel sponges interact with other reef organisms. **Chapter 6** explains how the prokaryotic community of multiple sponge species. **Chapter 7** contains several photos with a short description illustrating how giant barrel sponges in Taiwan can facilitate the recovery of coral fragments after a tropical storm.

Chapter 8 synthesizes the main findings from the previous chapters and provides directions for future research on giant barrel sponges and their role in coral reef ecosystems.

METHODS USED IN THIS THESIS

Mitochondrial genes have proven useful for population genetic and phylogeographic studies as they are maternally inherited, have short coalescence times and are expected to undergo lineage sorting relatively fast (Avise et al. 1987; Palumbi et al. 2001). The results

of population genetic studies in sponges, however, showed low levels of genetic variation between populations with the standard mitochondrial DNA barcoding primers (Folmer et al. 1994), even when populations were more than 7,000 km apart (Duran et al. 2004; Wörheide 2006). Erpenbeck et al. (2006a) found that the I3-M11 partition of the CO1 mitochondrial gene in sponges presented more variability at the species level, making it a more suitable marker for taxonomic studies and DNA barcoding. However, this marker alone did not give enough resolution to fully understand the evolutionary history of giant barrel sponges on a global scale nor on regional scales (Setiawan et al. 2016a). Sponges from the Caribbean and the Indo-Pacific were sharing haplotypes, and the haplotypes from both oceans did not form separate monophyletic lineages (Setiawan et al. 2016a). In the Caribbean, the most pronounced morphologies were represented by different haplotypes of this mitochondrial gene (López-Legentil and Pawlik 2009). Since both haplotypes were also found in specimens presenting the typical vase-shaped morphology with an irregular, rough surface, it was not clear whether these different haplotypes represented different species. The ATP6 mitochondrial gene was found to also be useful in sponges and can be added to other mitochondrial markers to increase the genetic variation (Rua et al. 2011).

Nuclear genetic markers independently evolve from mitochondrial markers and congruency between the two suggests the existence of distinct biological species (Padial et al. 2010). For sponges, including giant barrel sponges, the nuclear gene ATPs provides considerable genetic variation (Bentlage and Wörheide 2007; Setiawan et al. 2016b). Combined sequencing of the I3-M11 partition of the CO1 mitochondrial gene, the ATP6 mitochondrial gene, and the ATPs nuclear gene greatly enhances the opportunities to reconstruct the evolutionary history of giant barrel sponges, especially in combination with morphological and ecological variation. These molecular markers are, together, used in Chapter 2.

Next generation sequencing techniques have allowed for the profiling of microbial communities in all environments, including sponges (Claesson et al. 2010; Buermans and Den Dunnen 2014). Illumina sequencing using the 16S rRNA gene V3V4 hypervariable region has been a preferred method in sponge microbial studies in recent years due to the high output, relatively low price and suitability to assess microbial community structures (Reveillaud et al. 2014; Gaikwad et al. 2016; Thomas et al. 2016). This method has been used in Chapters 4, 5 and 6.

Section one: Genetics

2

Globally intertwined evolutionary history of giant barrel sponges

Swierts, T., Peijnenburg, K. T. C. A., de Leeuw, C. A., Breeuwer, J. A.,
Cleary, D. F. R., & de Voogd, N. J.

(2017). Globally intertwined evolutionary history of giant barrel sponges. Coral reefs, 36(3), 933-945.

ABSTRACT

Three species of giant barrel sponge are currently recognized in two distinct geographic regions, the tropical Atlantic and the Indo-Pacific. In this study, we used molecular techniques to study populations of giant barrel sponges across the globe and assessed whether the genetic structure of these populations agreed with current taxonomic consensus or, in contrast, whether there was evidence of cryptic species. Using molecular data, we assessed whether giant barrel sponges in each oceanic realm represented separate monophyletic lineages. Giant barrel sponges from 17 coral reef systems across the globe were sequenced for mitochondrial (partial CO1 and ATP6 genes) and nuclear (ATPsβ intron) DNA markers. In total, we obtained 395 combined sequences of the mitochondrial CO1 and ATP6 markers, which resulted in 17 different haplotypes. We compared a phylogenetic tree constructed from 285 alleles of the nuclear intron ATPsβ to the 17 mitochondrial haplotypes. Congruent patterns between mitochondrial and nuclear gene trees of giant barrel sponges provided evidence for the existence of multiple reproductively isolated species, particularly where they occurred in sympatry. The species complexes in the tropical Atlantic and the Indo-Pacific, however, do not form separate monophyletic lineages. This rules out the scenario that one species of giant barrel sponge developed into separate species complexes following geographic separation and instead suggests that multiple species of giant barrel sponges already existed prior to the physical separation of the Indo-Pacific and tropical Atlantic.

INTRODUCTION

There has been much controversy over the processes driving evolution and speciation in marine environments (e.g., Mayr 1942; Rocha and Bowen 2008). Physical barriers are less obvious in seas and oceans than on land, and many marine organisms have long-range dispersal capabilities during early life stages. Taken together, these factors were believed to lead to fewer opportunities for allopatric speciation compared to terrestrial ecosystems (Palumbi 1997; Rocha and Bowen 2008). However, this is inconsistent with the high biodiversity found in coral reefs, which rivals numbers found in tropical rainforests (Reaka-Kudla et al. 1997). Coral reefs are currently among the most vulnerable of ecosystems (Bridge et al. 2013) due to the combined threat of climate change and anthropogenic stressors including pollution and overfishing (Hughes 1994; Pandolfi et al. 2005). It is, therefore, important to study and quantify the diversity of these systems and understand the evolutionary processes that have led to this diversity.

Marine speciation does not fundamentally differ from terrestrial speciation, but ecological partitions among populations are believed to be more important in the former, whereas geographic partitions are more important in the latter (Bowen et al. 2013). An increasing number of examples of non-allopatric speciation along ecological gradients (reviewed in Bowen et al. 2013) illustrate the evolutionary potential of tropical marine environments. Furthermore, numerous phylogenetic studies have provided evidence of cryptic species, i.e., species that are indistinguishable from congenerics in morphology and spatial distribution, but that are clearly differentiated genetically (Bickford et al. 2007). In contrast, certain species show strong genetic connectivity at a global scale (Horne et al. 2008; Reece et al. 2011) despite apparent morphological variation (Rocha et al. 2005).

Correct identification of species is a fundamental part of conservation and management, and misidentification of cryptic species may impair conservation efforts (Robinson et al. 2014). Genetic markers have become increasingly important tools to identify divergent cryptic species and have forced the rejection of the long-believed assumption of cosmopolitan distribution of certain species (Boury-Esnault et al. 1992; Knowlton 1993; Klautau et al. 1999). Molecular techniques have also helped to reconstruct the distributional patterns of invasive species, which have become major drivers of ecosystem change due to the increase in global shipping (Concepcion et al. 2010; Teske et al. 2011). Most studies that have focused on the distribution and evolution of marine species cover small spatial scales and become more useful when they are compared to more wide-ranging studies (Briggs and Bowen 2013; Cowman and Bellwood 2013a). A focus on wide-ranging studies within each marine phylum should therefore be a priority for the scientific community.

Sponges (Porifera) are an animal group with a relatively simple morphology and often pronounced morphological plasticity (Knowlton 2000). Hence, they can be notoriously

difficult to identify to species or even to a higher taxonomic level due to the lack of reliable morphological markers (Knowlton 2000). They are considered the oldest multicellular animal lineage (van Soest et al. 2012), having evolved more than 500 million yr ago (Love et al. 2009; Maloof et al. 2010), and are widespread in many aquatic systems. On tropical reefs, sponge diversity and abundance can be higher than that of corals (Diaz and Rützler 2001). Unfortunately, this large and important animal group has long been understudied in coral reef ecology (Diaz and Rützler 2001). Most genetic studies of sponges have indicated the existence of cryptic species and refuted ocean-wide distributions of several taxa (Duran and Rützler 2006; Swierts et al. 2013; Bell et al. 2014; Knapp et al. 2015). However, these studies were done at small spatial scales, and, to the best of our knowledge, there has been no global phylogenetic study of any sponge taxon.

Giant barrel sponges (genus *Xestospongia*, family Petrosiidae, order Haplosclerida) are widely distributed throughout multiple tropical oceans. Giant barrel sponges are large and long-lived and have therefore been nicknamed 'the redwoods of the reef' (McMurray et al. 2008). These conspicuous sponges can measure up to a base diameter of more than 2.5 m (Nagelkerken et al. 2000) and can cover up to 9% of some reefs (Zea 1993); one specimen from Curaçao was estimated to be over 2300 yr old (Nagelkerken et al. 2000). Three species have been described, with the species delineation mainly based on geographic distributions. *Xestospongia muta* occurs in the tropical Atlantic, *X. testudinaria* in the Indo-Pacific from the Red Sea to Taiwan and *X. bergquistia* is thought to be confined to inshore environments in northern Australia where it lives in sympatry with *X. testudinaria*. Recent molecular studies have suggested that these species delineations are incorrect and that both *X. muta* and *X. testudinaria* consist of multiple sympatric species that apparently do not interbreed (Swierts et al. 2013; Bell et al. 2014). This has important implications for a number of published studies on the demography and population genetics of giant barrel sponges which assumed a single population of giant barrel sponge (López-Legentil and Pawlik 2009; McMurray et al. 2010; Richards et al. 2016).

The congruent identification of a phylogenetic lineage by multiple unlinked genetic loci indicates that it is genetically isolated from other such lineages, and thus qualifies as a species, because only in separate species will the coalescent histories of the different markers agree (Avise and Ball 1990; Coyne and Orr 2004; Padial et al. 2010). Nuclear DNA (nDNA) evolves independently from mitochondrial DNA (mtDNA); thus, congruent patterns across these markers support the existence of biological species (Goetze 2010; Padial et al. 2010). In sponges, mitochondrial variation is typically low (Wörheide et al. 2005), but previous studies of giant barrel sponges have shown that the combination of the adenosine triphosphate synthase subunit 6 gene (ATP6) with the I3-M11 partition of the cytochrome oxidase 1 gene (CO1) was informative (Rua et al. 2011; Swierts et al. 2013). The nuclear adenine triphosphate synthesis-β intron (ATPsβ) is very variable in giant barrel sponges, and because it is unlinked

to the mtDNA, it serves as a good additional marker to identify potential species (Bentlage and Wörheide 2007; Swierts et al. 2013).

Molecular studies on giant barrel sponges using these mtDNA and nDNA markers have revealed some interesting results. For example, some haplotypes of the I3-M11 partition of the CO1 gene are shared between Indonesia (*X. testudinaria*) and Florida (*X. muta*); hence, two individuals from different ocean basins can be more closely related for this slowly evolving gene than two sympatric individuals on the same reef (Swierts et al. 2013; Setiawan et al. 2016a). Giant barrel sponges from the tropical Atlantic and the Indo-Pacific sharing the same CO1 haplotype have both been related to an exterior morphology consisting of digitate structures (López-Legentil and Pawlik 2009; Swierts et al. 2013). These studies imply that the giant barrel sponge is a classic example of a tropical marine animal in which poor identification at the species level has led to an oversimplified taxonomic classification. Due to its conspicuousness, geographic range and available genetic markers, this group of sponges is suitable as a model for global sponge evolution and phylogeography. A better understanding of these species helps in our understanding of the evolutionary history of tropical marine species in general and marine sponges in particular, which is essential to our understanding of marine diversity. In this study, we sequenced giant barrel sponges from reefs across the globe for a combination of the mtDNA genes ATP6 and CO1 and the nDNA intron ATPsβ. The first aim of this study was to assess how many species of giant barrel sponge are present globally and how they are distributed. Our second aim was to test whether the giant barrel sponges in the tropical Atlantic and the Indo-Pacific represent two monophyletic lineages. If this is the case, it would suggest that one species of giant barrel sponge in each ocean basin independently developed into different species and/or species complexes. However, if sponges do not form two distinct monophyletic groups in different ocean basins, it suggests that a species complex already existed prior to the ocean basins becoming separated. This information provides insight into genetic divergence among tropical reefs before physical barriers impeded gene flow between the Indo-Pacific and tropical Atlantic.

METHODS

Giant barrel sponges (*Xestospongia* spp.) were collected by SCUBA diving from 17 different locations (Table 2.1; Fig. 2.1). Sponge tissue for DNA extraction was immediately stored in absolute ethanol (98%) in a cool box. After 6–12 h, the ethanol was changed and samples were stored at −20 °C. Fifty-four sponge samples from Lembeh Island, Indonesia, were previously described in Swierts et al. (2013), but amplification and sequencing were repeated in this study to confirm haplotype assignment.

DNA was extracted from sponge tissue using the DNeasy Blood and Tissue kit (Qiagen) following the manufacturer's instructions. We sequenced 395 samples for a combination

of the mitochondrial CO1 (Erpenbeck et al. 2002) and ATP6 genes (Rua et al. 2011). For the CO1 gene, we used the primers C1-J2165 (5′-GAAGTTTATATTTTAATTTTACCDGG-3′) and C1-Npor2760 (5′-TCTAGGTAATCCAGCTAAACC-3′), which amplified a fragment of 544 base pairs (bp). Amplification was performed in a 25 µL total reaction volume with 15.5 µL sterile water, 5 µL dNTPs (2.5 mM), 2.5 µL coralload buffer (Qiagen), 0.4 µL of each primer (10 µM), 0.25 µL taq polymerase (Qiagen) and 1 µL DNA template (20 ng µL−1). For the ATP6 gene, we used the primers ATP6porF (5′-GTAGTCCAGGATAATTTAGG-3′) and ATP6porR (5′-GTTAATAGACAAAATACATAAGCCTG-3′), which amplified a product of 445 bp. Amplification was performed in a 25 µL total reaction volume with 14 µL sterile water, 5 µL dNTPs (2.5 mM), 2.5 µL coralload buffer (Qiagen), 1.5 µL BSA (Promega), 0.4 µL (10 µM) of each primer, 0.25 µL taq polymerase (Qiagen) and 1 µL DNA template (20 ng µL−1). For both genes, we used a PCR protocol that consisted of an initial denaturing step (95 °C for 5 min), followed by 35 cycles of denaturing (95 °C for 30 s), annealing (42 °C for 45 s) and extension (68 °C for 1.30 min), and a final extension step (72 °C for 10 min) executed in a T100 thermal cycler (Bio-Rad).

To test for congruent patterns at an independent genetic locus, the ATPsβ nuclear intron was amplified for a subset of 211 samples following Jarman et al. (2002). For this gene, we used the primers ATPSβ-F (5′-ATGAGATGATCACATCAGGTG-3′) and ATPSβ-R (5′-GGTTCGTTCATCTGTCC-3′), which amplified products in the range of 258–279 bp. Amplification was performed in a 25 µL total reaction volume with 14.55 µL sterile water, 4.2 µL dNTPs (2.5 mM), 2.6 µL buffer (Qiagen), 1.6 µL BSA (Promega) 0.4 µL of each primer (10 µM), 0.25 µL taq polymerase (Qiagen) and 1 µL DNA template (20 ng µL−1). The PCR protocol consisted of an initial denaturing step (95 °C for 5 min), followed by 35 cycles of denaturing (95 °C for 30 s), annealing (45 °C for 30 s) and extension (72 °C for 45 s), and a final extension step (72 °C for 4 min) executed in a T100 thermal cycler from Bio-Rad. All PCR products were sequenced in both directions by BaseClear, Leiden, the Netherlands or Macrogen Europe, Amsterdam, the Netherlands.

Sequences were checked using CodonCode Aligner version 3.7.1.2 (CodonCode Corporation). Double peaks were called when the height of the secondary peak was at least 60% of that of the primary peak in both the forward and reverse sequence reads. Samples that contained two nucleotide positions with double peaks were reconstructed using DnaSP v5.10.01 with the PHASE v2.1 algorithm (Stephens et al. 2001). Only reconstructed haplotypes with probabilities >0.9 were used for further analysis. Samples that contained many double peaks may have represented mixtures of multiple sequences and were therefore cloned using the pGEM-T Easy kit (Promega Corporation) or the TOPO-TA cloning kit (Thermo Fisher Scientific), following the manufacturers' protocols. Primer sequences were trimmed of the final sequences, and alignments were obtained using ClustalW (Larkin et al. 2007) in Geneious v9.04 (Kearse et al. 2012) for both the combined mtDNA and single nDNA markers using the default software settings.

Table 2.1. Overview of sampling locations and the number of samples from each region sequenced for mitochondrial DNA (mtDNA) genes CO1 and ATP6, and for the nuclear gene (nDNA) ATPsβ respectively. Abbreviations in region names: C= Central; W=Western.

Location	Abbreviation	Region	mtDNA	nDNA
Derawan Islands - Indonesia	Der	C Indo-Pacific	46	26
Jakarta Bay; Thousand Islands- Indonesia	Jak	C Indo-Pacific	20	11
Lembeh Island - Indonesia	Lem	C Indo-Pacific	54	21
Spermonde Archipelago - Indonesia	Spe	C Indo-Pacific	67	49
Tioman Island - Malaysia	Tio	C Indo-Pacific	9	7
St. John Island - Singapore	Sin	C Indo-Pacific	15	7
Penghu Islands - Taiwan	Tai	C Indo-Pacific	48	7
Pattaya - Thailand	Pat	C Indo-Pacific	14	9
Phuket - Thailand	Phu	C Indo-Pacific	13	5
Koh Tao - Thailand	Koh	C Indo-Pacific	10	4
Halong Bay - Vietnam	HB	C Indo-Pacific	2	1
Phu Quoc - Vietnam	PQ	C Indo-Pacific	10	3
Jeddah - Saudi Arabia	Sau	Red Sea	11	4
Santa Barbara - Curaçao	Cur	Tropical Atlantic	28	22
Sint-Eustatius – the Netherlands	SE	Tropical Atlantic	27	23
Mayotte – France	May	W Indian Ocean	10	5
Dar es Salaam - Tanzania	Tan	W Indian Ocean	11	6
Total			395	210

We made separate statistical parsimony networks for the combined mtDNA sequences (CO1 + ATP6) and the nDNA sequences with TCS v 1.21 (Clement et al. 2000). A maximum likelihood phylogenetic tree was constructed for the ATPsβ -intron in Geneious using the PHYML plugin (Guindon et al. 2010) with the GTR model, which was the best fit model according to jModelTest2 (Guindon and Gascuel 2003; Darriba et al. 2012) based on the Akaike information criterion (Akaike 1974). The number of bootstrap replications was set at 1000. We also calculated Bayesian support values with MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The analysis of every gene consisted of two independent runs of four Metropolis-coupled Markov chains, sampled at every 1,000th generation. Analyses were terminated after the chains converged significantly as indicated by an average standard deviation of split frequencies <0.01. Trees were visualized with FigTree v1.4.2 (Morariu et al. 2009).

Individuals were grouped based on a combination of mtDNA, nDNA and the geographic origin of the sample. Mean genetic distance was calculated between these groups for the mtDNA and nDNA genes in MEGA 7.0.21 (Kumar et al. 2016) using the Tamura–Nei model (Tamura and Nei 1993) with standard settings. We conducted an automated barcoding gap discovery (ABGD) analysis with standard settings to split our sequences into candidate

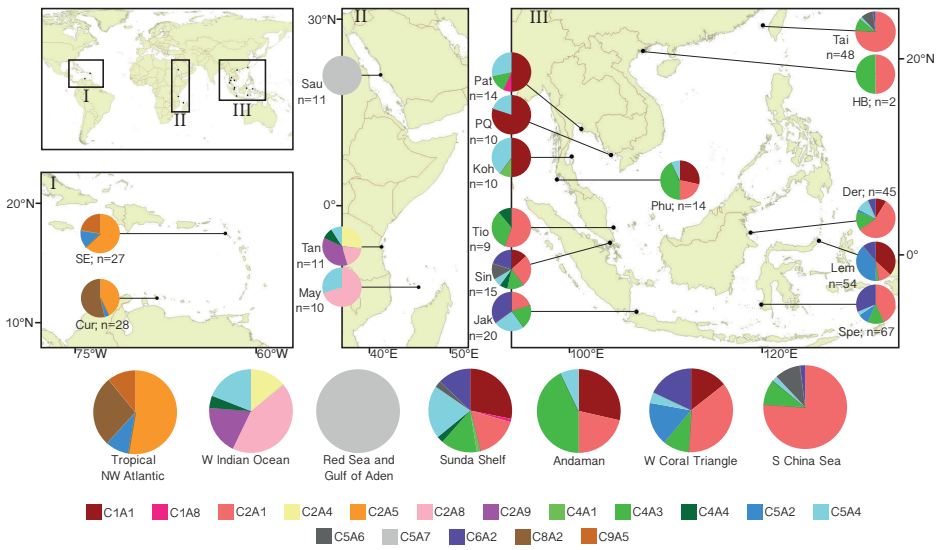


Figure 2.1. Location maps with haplotype frequencies of the mitochondrial DNA genes CO1 and ATP6 of giant barrel sponges. *SE* = Sint-Eustatius, the Netherlands; *Cur* = Santa Barbara, Curaçao; *Sau* = Jeddah, Saudi-Arabia; *Tan* = Dar es Salaam, Tanzania; *May* = Mayotte, France; *Pat* = Pattaya, Thailand; *PQ* = Phu Quoc, Vietnam; *Koh* = Koh Tao, Thailand; *Tio* = Tioman Island, Malaysia; *Sin* = St. John's Island, Singapore, *Jak* = Jakarta Bay and Thousand Islands, Indonesia; *Phu* = Phuket, Thailand; *Tai* = Taiwan; *HB* = Halong Bay, Vietnam; *Der* = Derawan Islands, Indonesia; *Lem* = Lembeh Island, Indonesia; *Spe* = Spermonde Archipelago, Indonesia

species and compare those to our identified groups based on congruence between mtDNA, nDNA and geography (Puillandre et al. 2012).

RESULTS

We obtained a total of 395 combined sequences of partial mitochondrial CO1 and ATP6 genes. In the final alignment of 989 base pairs, we found 13 variable sites: six were located in the CO1 gene and seven in the ATP6 gene, resulting in 17 different haplotypes (Table 2.2). Seven of the nine CO1-haplotypes (C1–C9) previously submitted to GenBank (López-Legentil and Pawlik 2009; Swierts et al. 2013; Setiawan et al. 2016) were present in this dataset. Re-analysis of the sample carrying the C3 haplotype from Lembeh Island showed that the sample was C2, and hence, this haplotype was wrongly identified (Swierts et al. 2013). Haplotype C7, described by Setiawan et al. (2016) from one sample from Tanzania, was not found in our dataset, and no new haplotypes were found for the CO1 gene. For the ATP6 gene, only three haplotypes were previously known (A1–A3; Swierts et al. 2013) and six new haplotypes were identified (A4–A9; GenBank accession numbers: KY381287–KY381292). Adding this gene to the CO1 gene expanded the number of haplotypes in our dataset from seven (C1, C2, C4–C6, C8, C9) to seventeen (Table 2.2). As is common in sponges

(Wörheide et al. 2005), mitochondrial variation was low (π = 0.0032). With one exception, connected haplotypes in the statistical parsimony network were differentiated by a single mutation (Fig. 2.2).

The tropical Atlantic haplotypes C2A5 and C9A5 are located at the opposite end of the network compared to the only other tropical Atlantic haplotype C8A2 and separated by 11 mutational steps. All 11 sequences from the Red Sea were identical and unique for the region (C5A7). In the western Indian Ocean, we found five different haplotypes in 21 sponges that were spread over the haplotype network; three of these haplotypes were only present in this region. Six haplotypes from the Indo-Pacific were represented by more than 25 individuals in our dataset, and the majority of these haplotypes were widespread and occurred at multiple sampling sites. Haplotype C5A2 was found in the central Indo-Pacific, but also in the tropical Atlantic. The four regions in which we sampled (central Indo-Pacific, tropical Atlantic, Red Sea, western Indian Ocean), which are geographically distant from one another, were characterized by different haplotype compositions, and all hosted unique haplotypes (Fig. 2.1).

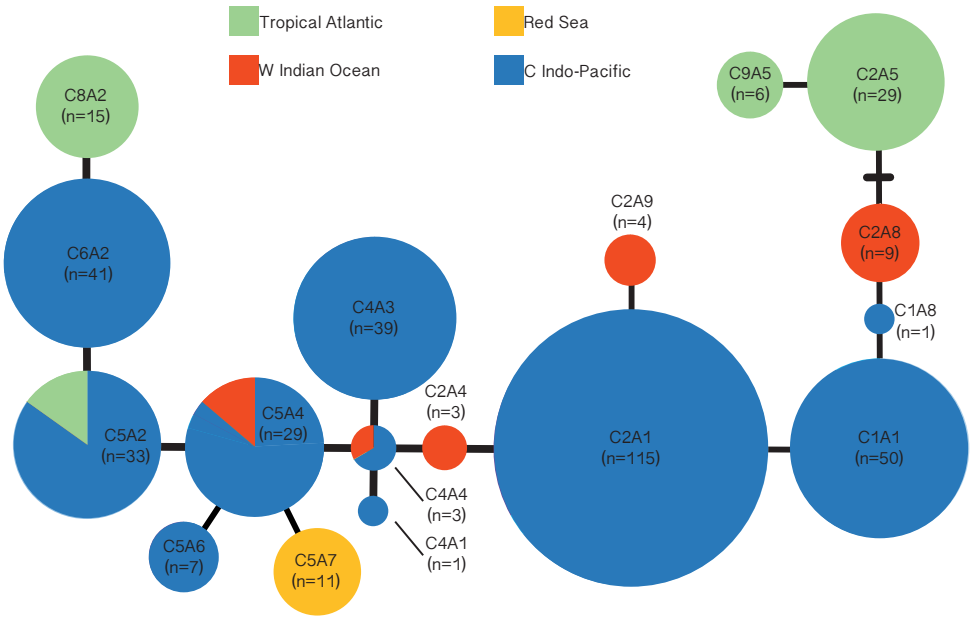


Figure 2.2. Haplotype network of the mitochondrial DNA genes CO1 and ATP6 of giant barrel sponges. *Pie chart size* is relative to the number of individuals with that haplotype. *Colors* indicate regions of origin. *Lines* connecting haplotypes represent one base substitution between two haplotypes; *additional crossbars* indicate an additional base substitution each. *Green* tropical Atlantic; *red* western Indian Ocean; *yellow* Red Sea; *blue* central Indo-Pacific.

Table 2.2. Nucleotide differences for mitochondrial markers CO1 and ATP6. Nucleotide differences in mitochondrial markers Cytochrome Oxidase I (CO1) and adenosine triphosphate synthase subunit 6 (ATP6). Seven haplotypes (C1, C2, C4-C6, C8, C9) are found for the CO1 fragment (base pairs 1-544) with a total of six variable sites. Nine haplotypes (A1-A9) are found for the ATP6 fragment (base pairs 545-989) with a total of seven variable sites. Seventeen different haplotypes are found when the CO1 and ATP6 markers combined (e.g. C1A1, base pairs 1-989).

mtDNA	**CO1**							**ATP6**						
CO1+ATP6	11	22	28	133	347	463	576	725	749	785	891	902	933	N
C1A1	A	T	C	A	G	T	T	T	G	T	G	G	T	50
C1A8	.	.	.	.	.	.	.	C	.	.	.	.	.	1
C2A1	.	.	.	.	.	C	.	.	.	.	.	.	.	115
C2A4	.	.	.	.	.	C	C	.	.	.	.	.	.	3
C2A5	.	.	.	.	.	C	.	C	.	.	A	A	.	29
C2A8	.	.	.	.	.	C	.	C	.	.	.	.	.	9
C2A9	.	.	.	.	.	C	.	.	.	C	.	.	.	4
C4A1	.	.	.	G	.	C	.	.	.	.	.	.	.	1
C4A3	.	.	.	G	.	C	C	C	.	.	.	.	.	39
C4A4	.	.	.	G	.	C	C	.	.	.	.	.	.	3
C5A2	.	A	.	G	.	C	C	.	.	C	.	.	.	33
C5A4	.	A	.	G	.	C	C	.	.	.	.	.	.	28
C5A6	.	A	.	G	.	C	C	.	A	.	.	.	.	7
C5A7	.	A	.	G	.	C	C	.	.	.	.	.	C	11
C6A2	G	A	.	G	.	C	C	.	.	C	.	.	.	41
C8A2	G	A	.	G	A	C	C	.	.	C	.	.	.	15
C9A5	.	.	T	.	.	C	.	C	.	.	A	A	.	6
Total														395

We successfully amplified the nuclear intron ATPsβ from 211 individuals. The nuclear intron ATPsβ provided much more genetic variation (157 segregating sites; $\pi = 0.0767$) than the mitochondrial genes (13 segregating sites; $\pi = 0.0032$). A circular phylogenetic tree consisting of 285 alleles (137 homozygotes, 74 heterozygotes) was constructed for the nuclear intron and compared to the 17 different mitochondrial haplotypes (Fig. 2.3); a larger and more detailed rectangular phylogenetic tree is provided in Appendix 2.1. All sequences were submitted to GenBank under accession numbers KY381293–KY381577. The nuclear sequences provided much more information than the mitochondrial markers, but were mostly phylogenetically congruent with the mtDNA. While not all branches in the nDNA tree were statistically supported and some mtDNA haplotypes were shared between regions, we could identify multiple groups that potentially operate as reproductively isolated populations.

Sponges were assigned to a separate group when they possessed unique mtDNA haplotypes within one of the geographic regions and also formed a separate cluster of unique nuclear

alleles (Fig. 2.3). Based on these criteria, the individuals from the central Indo-Pacific could be separated into three groups: group 1—haplotypes C1A1, C1A8 and C2A1; group 2—haplotypes C4A3 and C4A4; and group 3—haplotypes C5A2, C5A4 and C6A2. In the tropical Atlantic, the nuclear gene tree also contained three groups that were mostly congruent with mitochondrial haplotypes: group 7—haplotypes C2A5 and C9A5; group 8—haplotype C8A2; and group 9—haplotype C5A2. We found one group in the western Indian Ocean

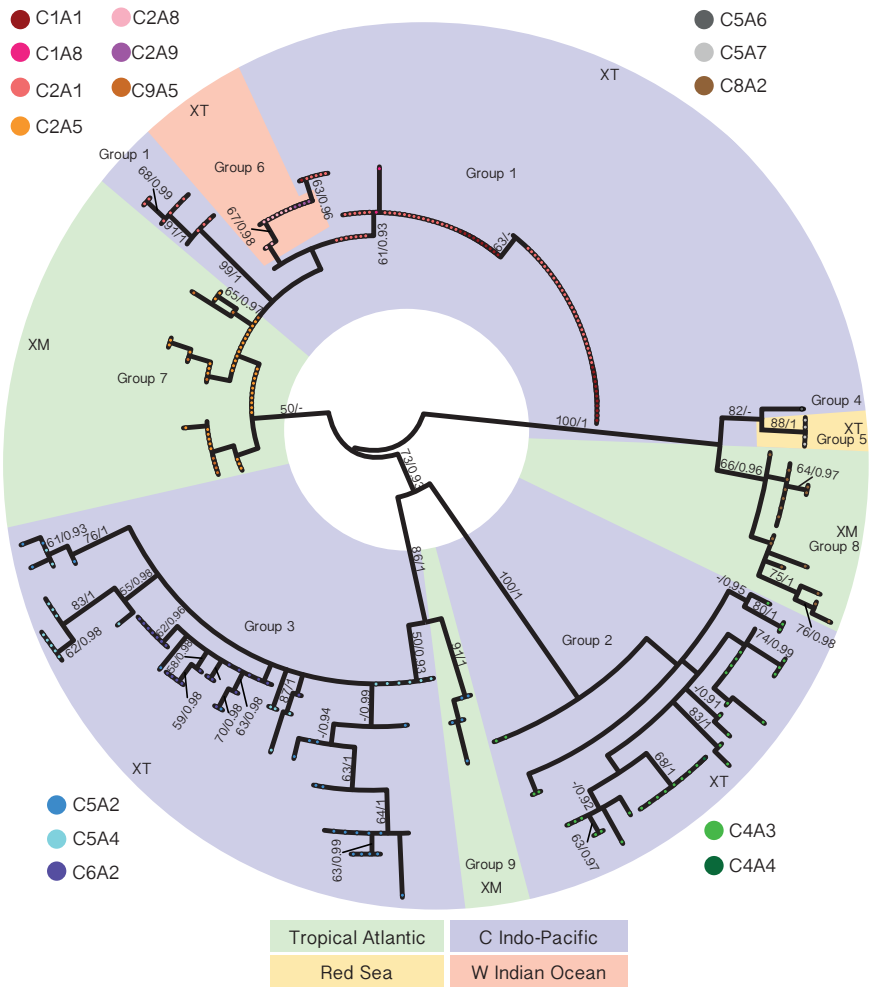


Figure 2.3. Unrooted circular maximum likelihood tree of 285 alleles of the nuclear DNA gene ATPsβ. Values on branches indicate bootstrap support (only shown when >50) and Bayesian support value (only shown when >0.90). Dots on the branches indicate the number of individuals with that allele, and the colors of the dots indicate the haplotype of the individual for the mitochondrial CO1 and ATP6 haplotypes. Background colors represent geographic origin of the lineages. Abbreviations in the legend of the background colors indicate the current species consensus (XT, *Xestospongia testudinaria*; XM, *Xestospongia muta*)

with mitochondrial haplotypes C2A8 and C2A9 (group 6), and one group in the Red Sea with mitochondrial haplotype C5A7 (group 5). The only individual with haplotype C5A6 was found in Taiwan; this individual had unique nuclear DNA and did not fit in any of the other three groups of the Indo-Pacific (group 4). Table 2.3 shows the mean genetic distances between these drafted groups. Mean genetic distance for the nDNA was considerably higher than for the mtDNA. An ABGD analysis on our nuclear data supported the groups 2, 3, 4, 5, 8 and 9 with recursive partitions at a prior maximal distance of 0.0046, while groups 1, 6 and 7 were not supported as separate groups (Appendix 2.2). The statistical parsimony network constructed with TCS from our nuclear data resulted in seven unconnected statistical parsimony networks. Group 8 represented one network, group 3 represented three networks, and the remaining three networks consisted of the combinations of groups 2 and 9, groups 1, 6 and 7, and groups 4 and 5, respectively (Appendix 2.3; 2.4).

The groups from the tropical Atlantic (7, 8, 9) and the Indo-Pacific (1, 2, 3) were not monophyletic per region for the nuclear marker, but rather were intertwined in a generally congruent pattern with the mtDNA (Fig. 2.3). They did not share any nuclear alleles, indicating that there has been no recent genetic exchange between giant barrel sponges from these areas. Also, heterozygotes were only found with both alleles within the same nuclear group providing further support that different groups are reproductively isolated (Appendix 2.1). Note that not all groups are monophyletic in the nDNA tree. In particular, groups 1, 6 and 7 are 'mixed' in the tree, but they do not share any alleles. Also, some groups are only partly congruent, for example groups 4 and 5. Both groups show congruent patterns in the sense that they both host unique mtDNA haplotypes and unique nDNA alleles and are closely related to each other for both markers. However, there is an inconsistency in their placement relative to the other groups, since they are most closely related to group 8 in the nDNA phylogenetic tree (Fig. 2.3) but most closely related to group 3 in the haplotype network for the mtDNA (Fig. 2.2).

DISCUSSION

Congruent patterns between mtDNA and nDNA markers of giant barrel sponges around the globe point to the existence of multiple genetically isolated taxa and support our hypothesis of the existence of additional species. The genetic differences between the nuclear clades are based on the variation of a single gene and the mitochondrial markers have low variation. Nevertheless, plotting mtDNA haplotypes to the nuclear phylogeny does not result in random distribution. All of the mtDNA haplotypes are confined to one nDNA group within a geographic region, suggesting biological species. Altogether, we identified at least eight potential giant barrel sponge species globally, yet the limitations of the markers make it impossible to exactly determine the number of species with the data presented in this study.

Table 2.3. Mean genetic distances of the mtDNA and the nDNA between identified groups of giant barrel sponges (*Xestospongia* spp.) Mean genetic distances between groups for the mtDNA are shown in the lower left section of the table, and mean genetic distances between groups for the nDNA are shown in the upper right section of the table.

Group	Haplotypes	Region	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Group 1[a]	C1A1, C1A8, C2A1	C Indo-Pacific		0.082	0.061	0.065	0.073	0.011	0.015	0.071	0.067
Group 2[b]	C4A3, C4A4	C Indo-Pacific	0.003		0.085	0.093	0.098	0.079	0.071	0.096	0.078
Group 3[c]	C5A2, C5A4, C6A2	C Indo-Pacific	0.005	0.003		0.094	0.099	0.058	0.059	0.096	0.036
Group 4[d]	C5A6	C Indo-Pacific	0.005	0.003	0.002		0.013	0.066	0.059	0.023	0.087
Group 5[d]	C5A7	Red Sea	0.005	0.003	0.002	0.002		0.070	0.064	0.028	0.092
Group 6[a]	C2A8, C2A9	W Indian Ocean	0.002	0.003	0.004	0.005	0.005		0.012	0.067	0.065
Group 7[a]	C2A5, C9A5	Tropical Atlantic	0.004	0.005	0.008	0.008	0.008	0.004		0.062	0.057
Group 8[e]	C8A2	Tropical Atlantic	0.007	0.005	0.002	0.004	0.004	0.006	0.010		0.090
Group 9[c]	C5A2	Tropical Atlantic	0.005	0.003	0.001	0.002	0.002	0.004	0.008	0.002	

[a] Indicates three groups that are not separated from each other with the automated barcode gap discovery analysis [ABGD analysis; Puillandre et al. (2012) and that did not form separate haplotype networks in the TCS analysis (Clement et al. 2000).

[b] Indicates a group that was separated by the ABGD analysis and formed three haplotype networks in the TCS analysis

[c] Indicates two groups that were separated by the ABGD analysis and formed one haplotype networks in the TCS analysis

[d] Indicates two groups that are separated from each other by the ABGD analysis and formed one haplotype network in the TCS analysis

[e] Indicates a group that was separated by the ABGD analysis and formed a separate network in the TCS analysis

We found three potential giant barrel sponge species in the central Indo-Pacific (groups 1, 2, 3), three in the tropical Atlantic (groups 7, 8, 9), one in the western Indian Ocean (group 6) and one in the Red Sea (group 5). The lack of hybridization between these taxa, especially between those that are sympatric, indicates complete reproductive isolation. A ninth group (group 4) consisted of a single sample from Taiwan. Some genetic groups (e.g., groups 1, 6 and 7) were not statistically supported in the nuclear gene tree, but these could still represent (incipient) species because they occur in different parts of the world's oceans and are thus geographically isolated. Certain other groups were statistically supported, which is, particularly in combination with their sympatric occurrence, a strong indication for speciation. It is important to note that some discrepancies exist in the results from our statistical analyses. Our own interpretation of nine groups differs from the results from the ABGD and TCS analyses, which both find only seven groups, but with partially different compositions. Also, the inconsistencies of the placement of certain groups relative to other groups illustrate that the phylogenetic relationships between the groups cannot be completely resolved with the combination of markers used in this study. The higher variation on the nDNA marker suggests that the evolutionary history of giant barrel sponges is better represented by this marker; however, additional genetic evidence and a thorough morphological analysis are required to delineate and describe distinct groups as species. Nevertheless, our data do indicate that the current taxonomic consensus with *X. muta* occurring in the tropical Atlantic and *X. testudinaria* in the Red Sea, western Indian Ocean and central Indo-Pacific, is incorrect.

Our results suggest the existence of three species in the tropical Atlantic, in line with previous suggestions based on a study of the sterol compositions of giant barrel sponges (Kerr et al. 1991a; 1991b). All CO1 haplotypes of samples from the tropical Atlantic in this analysis were previously described by López-Legentil and Pawlik (2009) from locations in the north (Florida) and west (Belize) of the tropical Atlantic and by de Bakker et al. (2016) from locations in the east (Saba Bank) of the tropical Atlantic. Therefore, these three groups seem to be distributed across the entire tropical Atlantic. Similarly, large geographic ranges can be observed for groups 1, 2 and 3 from the central Indo-Pacific, which have overlapping geographic ranges between locations more than 2000 km apart. Of particular interest is the finding that lineages in a given ocean basin were more closely related to lineages in another ocean basin than to lineages with which they co-occur. For instance, individuals from groups 3 and 9 can share the same mtDNA haplotype and are also closely related in the nuclear gene tree, but are found in the Indo-Pacific and tropical Atlantic, respectively. In other words, different species in each ocean basin do not form separate monophyletic clades. This suggests that distinct species of giant barrel sponges must have existed prior to the most recent physical separation of the tropical Atlantic and the Indo-Pacific.

At present, giant barrel sponges occur in the western Indo-Pacific (including the Red Sea and western Indian Ocean), the central Indo-Pacific and the tropical Atlantic. Giant barrel

sponges have not been found in the eastern Pacific and the eastern tropical Atlantic. Hence, the sponges from the tropical Atlantic and the Indo-Pacific are separated by several barriers that have developed at various times throughout history (Cowman and Bellwood 2013b). The Tethys Seaway may have provided a potential migration route throughout the first half of the Cenozoic era between 60 and 30 million yr ago between the tropical Atlantic and the Indo-Pacific (Vermeij 2001; Harzhauser and Piller 2007). At that time, the western Tethys was the center of global marine biodiversity, but these subsequently shifted eastwards to its present location in the Indo-Australian archipelago (Renema et al. 2008). In the early Miocene, the African-Arabian Plate moved northwards to adjoin the Eurasian Plate (Aitchison et al. 2007), dividing the Tethys realm into a western and an eastern part: the 'Terminal Tethyan Event' (TTE). Previously, the closure of the Isthmus of Panama, approximately 3 million yr ago (Keigwin 1978, was suggested as the final geographic separation between giant barrel sponges from the tropical Atlantic and the Indo-Pacific (Montalvo and Hill 2011; Swierts et al. 2013). However, since giant barrel sponges do not occur east of New Caledonia, the scenario in which the tropical Atlantic and the Indo-Pacific locations became isolated due to the TTE should also be considered. If isolation followed the TTE, the lineages now living in sympatry must have evolved into different species before the TTE occurred. This could be a result of sympatric speciation within the Tethys Sea, or perhaps due to a series of speciation events from periodic geographic isolation as a result of changing sea levels (Haq et al. 1987).

Reproductive mechanisms can explain how different species of *Xestospongia* are maintained in sympatry. Sympatric populations of *X. testudinaria* and *X. bergquistia* spawn at different times of the year near Australia, possibly triggered by water temperature (Fromont and Bergquist 1994). This is comparable to corals of the *Montastrea annularis* species complex (van Veghel et al. 1996) in the tropical Atlantic, which are unlikely to interbreed due to a combination of temporal differences in spawning, sperm aging, gamete dispersal and dilution, and gametic incompatibility (Levitan et al. 2004). In the tropical Atlantic, *X. muta* has been observed to spawn and recruit twice a year, in spring and in late summer (Ritson-Williams et al. 2005; McMurray et al. 2008). However, it is possible that all giant barrel sponges spawn just once a year, and these different times in fact correspond to different mass spawning events of distinct species. Spawning events of giant barrel sponges have also been reported at different times of the year in the Indo-Pacific (Röthing and Voolstra 2016; Swierts et al. 2013).

Some of the previously assumed 'morphological plasticity' of giant barrel sponges might actually be morphological differentiation between species. For instance, sponges with haplotype C5 for the CO1 gene are associated with a digitate outer morphology in both the tropical Atlantic and the Indo-Pacific (López-Legentil and Pawlik 2009; Swierts et al. 2013). Other morphological characteristics might also differentiate between 'cryptic' species. For example, the spicules of *X. muta* have a broad size range (length 290–435 µm, width 8.3–23 µm) and vary between geographic locations (van Soest 1980). With possibly three

species present in the tropical Atlantic, we have to consider that such a broad size range may be the result of lumping three different spicule size ranges and an uneven distribution of the various cryptic species among locations. Also in the tropical Atlantic, McMurray et al. (2014) found large differences in spongocoel morphology that were independent of sponge size and strongly influenced excurrent seawater velocity. Given that only three species have been described globally so far, scientific priority should be given to resolving this species complex further and differentiating between their potentially distinct ecological roles.

To our knowledge, the intertwined evolutionary history of tropical Atlantic and Indo-Pacific taxa we found for giant barrel sponges has never been found in other benthic reef animals. Usually, there is a general conformity between phylogeography and biogeography in marine animal groups, which suggests that geographic isolation is a starting point for divergences between species (Teske et al. 2011; Bowen et al. 2016). Most global marine species complexes are so-called 'sibling species complexes' that developed simultaneously in different ocean basins, potentially followed by cryptic invasions (Schwaninger 2008; Geller et al. 2010). The importance of geographic isolation, possibly related to sea currents, was suggested as a driving force in sponge speciation (DeBiasse et al. 2016). It may also be a driving force for giant barrel sponges, especially considering the groups that are confined to the Red Sea and the western Indian Ocean, which represent distinct biogeographic provinces (Briggs and Bowen 2012; Bowen et al. 2016). However, present-day ocean currents and geographic barriers cannot explain why giant barrel sponges in the Tropical Atlantic and the Indo-Pacific do not form monophyletic lineages, and why in each ocean basin multiple genetically isolated lineages exist in sympatry. Trumpet fish represent a so-called 'global ring species complex', in which different lineages have come into contact after three to four million years of isolation and appear to be merging (Bowen et al. 2001). Four highly divergent lineages have been identified for vase tunicates; these four were originally limited to a certain geographic range, but two are now considered invasive with global distributions (Zhan et al. 2010). Sponge larvae, however, are generally considered to have low survival under environmental stress, and their transport in the ballast water of ships is unlikely and has not yet been reported (Klautau et al. 1999). Our data do not support any recent invasions of giant barrel sponges from one ocean basin to another and none of the candidate species has a global distribution. Instead, our results show a unique evolutionary history, suggesting intertwined species complexes in different ocean basins. This pattern may, however, be found in other tropical marine species, especially those with a long evolutionary history such as other globally distributed sponge groups. Resolving accurate species boundaries in such groups is important for the conservation of tropical marine ecosystems.

ACKNOWLEDGEMENTS

We thank Friso Dekker, Maarten van Gemert, Christine Hörnlein, Yusheng Huang, Laurie van Reemst, Ee Crovetto, Ana-Rita Polonia, Rossana Freitas, Anne Bialecki, Mike Berumen, Jaaziel Garcia Hernandez, Sumaitt Putchakarn, Chad Scott, Nguyen Khac Bat, Swee Cheng Lim, Zarinah Waheed, Yosephine Tuti, Betsie Voetdijk, Peter Kuperus and Marcel Eurlings for logistic support, collecting and laboratory assistance. The feedback and constructive suggestions made during the review process are much appreciated and significantly improved this manuscript.

APPENDIX 2.1

Unrooted circular maximum likelihood tree of 285 alleles of the nuclear DNA gene ATPsβ. *Values* on branches indicate bootstrap support (only shown when >50) and Bayesian support value (only shown when >0.90). *Dots* on the branches indicate the number of individuals with that allele, and the *colors* of the dots indicate the haplotype of the individual for the mitochondrial CO1 and ATP6 haplotypes. *Background colors* represent geographic origin of the lineages. Abbreviations in the legend of the background colors indicate the current species consensus (XT, *Xestospongia testudinaria*; XM, *Xestospongia muta*)

APPENDIX 2.2

https://link.springer.com/article/10.1007/s00338-017-1585-6#SupplementaryMaterial

APPENDIX 2.3

https://link.springer.com/article/10.1007/MediaObjects/338_2017_1585_MOESM2_ESM.graph.log

APPENDIX 2.4

List of hypothetical species of giant barrel sponge species (*Xestospongia spp.*) in which all individuals are grouped according to an Automatic Barcode Gap Discovery (ABGD) analysis (Puillandre et al. 2012). Settings of the analysis: Partition with prior maximal distance P=7.74e-03 Distance JC69 Jukes-Cantor MinSlope=1.500000

Group[1] n: 16 ;id: CUR_XMC01_HETA CUR_XMC01_HETB CUR_XMC07_HETA CUR_XMC07_HETB CUR_XMC12_HETA CUR_XMC12_HETB CUR_XMC13_HOM CUR_XMC14_HETA CUR_XMC14_HETB CUR_XMC25_HETA CUR_XMC25_HETB CUR_XMC35_HETA CUR_XMC35_HETB CUR_XMC71_HOM CUR_XMC85_HETA CUR_XMC85_HETB
Group[2] n: 158 ;id: CUR_XMC05_HOM CUR_XMC18_HOM CUR_XMC22_HETA CUR_XMC22_HETB CUR_XMC32_HOM CUR_XMC33_HETA CUR_XMC33_HETB CUR_XMC45_HOM CUR_XMC54_HOM CUR_XMC55_HOM CUR_XMC77_HOM CUR_XMC78_HETA CUR_XMC78_HETB CUR_XMC81_HOM CUR_XMC83_HETA CUR_XMC83_HETB DER_583A_HETA DER_583A_HETB DER_584_HOM DER_585_HOM DER_586_HOM DER_587_HETA DER_587_HETB DER_588_HOM DER_589_HOM DER_592_HOM DER_593_HETA DER_593_HETB DER_594_HOM DER_595_HETA DER_595_HETB DER_628_HETA DER_628_HETB DER_630_HOM DER_634_HOM JAK_PS355_HETA JAK_PS355_HETB JAK_PS388_HOM KOH_PES200_HOM KOH_THA244_HOM KOH_THA257_HOM KOH_THA258_HOM LEM_XT014_HOM LEM_XT020_HOM LEM_XT021_HOM LEM_XT029_HOM LEM_XT084_HOM LEM_XT123_HOM LEM_XT157_HOM LEM_XT163_HETA LEM_XT163_HETB LEM_XT172_HOM LEM_XT175_HOM LEM_XT178_HOM May083_HETA May083_HETB May137_HETA May137_HETB May142_HOM May154_HOM May162_HOM PAT_THA030_HOM PAT_THA052_HOM PAT_THA054_HETA

PAT_THA054_HETB PES200_HOM PES471_HOM PES482_HOM PES530_HOM PHU_THA112_HOM PHU_THA113_HOM PHU_THA114_HOM PHU_THA140_HOM SE02_HETA SE02_HETB SE03_HOM SE04_HETA SE04_HETB SE07_HOM SE08_HETA SE08_HETB SE09_HOM SE10_HETA SE10_HETB SE11_HOM SE12_HOM SE13_HOM SE14_HETA SE14_HETB SE15_HETA SE15_HETB SE17_HETA SE17_HETB SE18_HETA SE18_HETB SE21_HOM SE23_HETA SE23_HETB SE24_HETA SE24_HETB SE27_HOM SE28_HETA SE28_HETB SE29_HETA SE29_HETB SIN021_HOM SIN026_HOM SIN030_HOM SPER_501_HOM SPER_502_HOM SPER_522_HOM SPER_526_HOM SPER_529_HOM SPER_530A_HETA SPER_530A_HETB SPER_531_HETA SPER_531_HETB SPER_534_HOM SPER_536_HETA SPER_536_HETB SPER_539_HETA SPER_539_HETB SPER_545_HETA SPER_545_HETB SPER_547_HETA SPER_547_HETB SPER_548_HOM SPER_549_HETA SPER_549_HETB SPER_550_HETA SPER_550_HETB SPER_551_HOM SPER_555_HOM SPER_562_HETA SPER_562_HETB SPER_563_HOM SPER_564_HOM SPER_567_HETA SPER_567_HETB SPER_568_HOM SPER_574_HOM SPER_576_HOM SPER_577A_HOM SPER_578_HOM TAN04_HOM TAN05_HOM TAN07_HOM TAN08_HOM TAN12_HOM TAN13_HOM TIO030_HOM TIO032_HOM TIO036_HOM VIE_PQ09_HOM VIE_PQ10_HETA VIE_PQ10_HETB VIE_PQ11_HETA VIE_PQ11_HETB **Group[3] n: 5 ;**id: CUR_XMC11_HOM SE05_HOM SE19_HETA SE19_HETB SE20_HOM **Group[4] n:65;**id: DER_598A_HOM DER_599_HETA DER_599_HETB DER_621_HETA DER_621_HETB DER_623_HETA DER_623_HETB DER_659_HOM DER_662_HOM JAK_PS197_HOM JAK_PS218_HOM JAK_PS219_HETA JAK_PS219_HETB JAK_PS225_HOM JAK_PS233_HETA JAK_PS233_HETB JAK_PS302_HETA JAK_PS302_HETB JAK_PS324_HOM JAK_PS336_HOM JAK_PS394_HOM LEM_XT005_HETA LEM_XT005_HETB LEM_XT007_HOM LEM_XT009_HETA LEM_XT009_HETB LEM_XT040_HETA LEM_XT040_HETB LEM_XT071_HETA LEM_XT071_HETB LEM_XT126_HETA LEM_XT126_HETB LEM_XT152_HETA LEM_XT152_HETB LEM_XT166_HETA LEM_XT166_HETB LEM_XT176_HOM PAT_THA031_HOM PAT_THA036_HOM PAT_THA070_HOM PAT_THA071_HOM SIN018_HOM SIN029_HOM SIN032_HOM SPER_503_HOM SPER_506_HOM SPER_507_HOM SPER_508_HETA SPER_508_HETB SPER_512_HOM SPER_521_HETA SPER_521_HETB SPER_524_HOM SPER_540A_HOM SPER_552_HOM SPER_554_HOM SPER_570_HOM SPER_572_HETA SPER_572_HETB SPER_573_HETA SPER_573_HETB SPER_575_HOM SPER_579_HOM SPER_580_HOM SPER_581_HOM **Group[5] n: 36 ;**id: DER_598B_HETA DER_598B_HETB DER_600_HOM DER_601_HETA DER_601_HETB DER_602_HOM DER_609_HETA DER_609_HETB DER_624_HOM DER_627_HETA DER_627_HETB JAK_PS310_HOM LEM_XT003_HETA LEM_XT003_HETB PAT_THA040_HETA PAT_THA040_HETB PAT_THA053_HETA PAT_THA053_HETB PES473_HOM PES480_HOM PES485_HOM PHU_THA085_HOM SIN023_HETA SIN023_HETB SPER_537_HOM SPER_538_HOM SPER_541_HOM SPER_560_HETA SPER_560_HETB SPER_561_HOM TIO037_HOM TIO038_HETA TIO038_HETB TIO039_HOM VIENORTH_VIE034_HETA VIENORTH_VIE034_HETB **Group[6] n: 4 ;**id: JED100_HOM JED135_HOM JED178_HOM JED188_HOM **Group[7] n: 1 ;**id: PES481_HOM

3

Two differently colored giant barrel sponge species in Tanzania merge when they grow against each other

Swierts, T

Submitted

Giant barrel sponges (*Xestospongia* spp.) form a species complex with a globally intertwined evolutionary history and multiple species often co-exist on the same reefs (Chapter 2 this thesis). Subtle species-specific variation in the outer morphology of giant barrel sponge species has been documented (López-Legentil and Pawlik 2009; Swierts et al. 2013), but due to the similarities in their main body plans it remains difficult to taxonomically distinguish between the species, especially in the field. However, in Tanzania (6°42'27S, 39°17'1E) a large population (n= >100) of giant barrel sponges was found that consisted of two clear morphologies. One morphology was purple with a relatively smooth to bumpy surface, and the other was bronze with digitate external structures (Fig. 3.1). DNA barcoding of three genes showed that these morphologies represented two different genetic groups that were described in Chapter 2 of this thesis. The bronze sponges corresponded with genetic group 2 which was omnipresent on Southeast Asian reefs, and the purple sponges corresponded with genetic group 4 which was previously only found in Taiwan and Singapore.

In addition, two individuals with different genetic identities were observed growing against each other, and at the contact zone, the color of the purple sponge diffused into the bronze sponge (Fig. 3.1). The color of sponges is often derived by the composition of the associated microbiota (Blanquer et al. 2011). It is, therefore, not clear whether this blended area is the result of the exchange of hybridizing sponge cells or an exchange of microsymbionts.



Figure 3.1. Two merged individuals of giant barrel sponge species (*Xestospongia* spp.) with distinct color morphologies in Tanzania.

II

Section two: Prokaryotes

4

Prokaryotic communities of Indo-Pacific giant barrel sponges are more strongly influenced by geography than host phylogeny

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), fiy194.

ABSTRACT

Sponges harbor complex communities of microorganisms that carry out essential roles for the functioning and survival of their hosts. In some cases, genetically related sponges from different geographic regions share microbes, while in other cases microbial communities are more similar in unrelated sponges collected from the same location. To better understand how geography and host phylogeny cause variation in the prokaryotic community of sponges, we compared the prokaryotic community of 44 giant barrel sponges (*Xestospongia* spp.). These sponges belonged to six reproductively isolated genetic groups from eight areas throughout the Indo-Pacific region. Using Illumina sequencing, we obtained 440 000 sequences of the 16S rRNA gene V3V4 variable region that were assigned to 3795 operational taxonomic units (OTUs). The prokaryotic community of giant barrel sponges was characterized by 71 core OTUs (i.e. OTUs present in each specimen) that represented 57.5% of the total number of sequences. The relative abundance of these core OTUs varied significantly among samples, and this variation was predominantly related to the geographic origin of the sample. These results show that in giant barrel sponges, the variation in the prokaryotic community is primarily associated with geography as opposed to phylogenetic relatedness.

INTRODUCTION

Sponges are among the oldest living multicellular animals and form symbiotic relationships with complex communities of microorganisms including archaea, bacteria and single-celled eukaryotes (Hentschel et al. 2012). These microbial symbionts are essential for the functioning and survival of marine sponges, and play key roles in processes such as CO2-fixation, nutrient cycling, secondary metabolite production and the conversion of dissolved organic matter into particulate organic matter (Schmidt et al. 2000; Fan et al. 2012; de Goeij et al. 2013, Zhang et al. 2015; Slaby et al. 2017). In high microbial abundance (HMA) sponges, microbes can make up 40% of the total weight (Friedrich et al. 2001). Cyanobacteria also provide more than half of the energy requirements of several sponge species by fixing carbon through photosynthesis (Wilkinson 1983). Due to this intricate relationship, sponges are often referred to as the 'sponge holobiont': the combination of the sponge host and all residing microorganisms (Webster and Thomas 2016; Pita et al. 2018).

Host species throughout the phylum Porifera often have characteristic microbial fingerprints (Thomas et al. 2016) and the differences among hosts can originate at an early reproductive phase (Schmitt et al. 2008). Certain microorganisms can be assimilated in gametes or other reproductive stages by the host sponge, and such vertical transmission ensures that essential bacteria, archaea, and even yeasts are transmitted to their offspring (Ereskovsky et al. 2005; Maldonado et al. 2005; Sharp et al. 2007; Funkhouser and Bordestein 2013). Another means of acquiring relevant microbes is through horizontal transmission, whereby microorganisms are recruited from the environment (Taylor et al. 2007b; Sipkema et al. 2015). These recruits are often harvested from the rare biosphere and tend to be found at much greater densities within the sponge host (Lynch and Neufeld 2015). Recent studies have found that certain microbes deemed 'sponge-specific' may indeed be found in the surrounding seawater as well, albeit in very low abundances (Taylor et al. 2013). Hence, the seawater may act as a reservoir for these microbes, from which related sponges in distant geographic regions are populated through horizontal transmission (Moitinho-Silva et al. 2014).

Microbial host specificity and stability across time and space is potentially a derivative of co-speciation (Erwin et al. 2012; Hardoim et al. 2012; Webster et al. 2013; Pita et al. 2013a; Cuvelier et al. 2014; Naim et al. 2014; Webster and Thomas 2016; Souza et al. 2017; Steinert et al. 2017). Related sponges from distant geographic regions can share microbial phylotypes that were not recorded in their respective non-sponge environments, suggesting that a common ancestor harbored these phylotypes and that they have been passed on by vertical transmission during speciation events into each lineage (Taylor et al. 2007b; Lafi et al. 2009). Similar microbial fingerprints among more related host species does not, however, necessarily require coevolution (Moran and Sloan 2015). Certain substructures of the sponge host (such as pores, channels, choanocytes, etc.) could provide distinct microenvironments, which have allowed niche differentiation resulting in similar host species specificity patterns (Webster and Thomas 2016).

It is apparent that host identity shapes the microbial community of many sponges, and that in some cases geographic origin is also an important driver (Erwin et al. 2012; Schmitt et al. 2012; Pita et al. 2013b; Easson and Thacker 2014; Marino et al. 2017; Souza et al. 2017). However, it is hard to assess whether geography or phylogeny are equally important drivers, or that one of the two is more important. At present, there is a dearth of studies that incorporate both geography and phylogeny, especially at a large geographic scale and with large sample sizes. To pinpoint the relative importance of host identity and geography on the microbial community, research should be expanded to large sample sizes from closely related sponges with broad distributions and a similar bauplan. Such a study can also help to define the species-specific core microbiota. Generally, the core is defined as the operational taxonomic units (OTUs) present in most, or all, samples within a certain taxonomic level, and which exact definition is chosen usually does not alter the interpretation of the results (Turnbaugh et al. 2006; Huse et al. 2012; Otani et al. 2014; Walke et al. 2014; Astudillo-García et al. 2017). While the core microbiota of sponges as a whole has been elaborately discussed by Schmitt et al. (2012), the OTUs considered to be species-specific are based on one individual per species. Without replicates, it is impossible to extrapolate which of the unique microbes occur in (almost) every specimen of that species, and are thus universal members of their microbiota.

Giant barrel sponges are a particularly suitable model for such research since they have a broad distribution on coral reefs around the globe and have an intricate phylogeny (Swierts et al. 2013; Chapter 2 of this thesis). While three giant barrel sponge species have been described so far, namely *Xestospongia muta* from the Caribbean, *Xestospongia testudinaria* from the Indo-Pacific and *Xestospongia bergquistia* from the north-eastern coast of Australia, molecular studies comparing these giant barrel sponge species were unable to find a separation that correlated with the species descriptions as they exist today (Setiawan et al. 2016a, Chapter 2 of this thesis). Recent studies have, furthermore, revealed that giant barrel sponges around the globe form a much broader species complex (Swierts et al. 2013; Bell et al. 2014; Setiawan et al. 2016b; Chapter 2 of this thesis). Some of the species occur over large geographic areas, while others are confined to smaller water bodies, but a remarkable feature of this species complex is the lack of correlation between phylogenetic affinity and geography on global scales. While it is nearly impossible to distinguish among groups based on morphological characters, the sister group of each genetic group appears to occur in a different ocean. In other words, two visually similar individuals living one meter apart can be genetically more distinct from one another than from individuals living on a reef at the other side of the world (Chapter 2 of this thesis).

Previous studies on the giant barrel sponge microbiota found that they are dominated by Chloroflexi, Proteobacteria, Acidobacteria, and Actinobacteria (Montalvo et al. 2005, 2014; Montalvo and Hill 2011; Polonia et al. 2014, 2017; Cleary et al. 2015a; De Voogd et al. 2015). However, these studies included a small number of replicates and sites and used

lower resolution sequencing methods. These restrictions hamper the ability to draw strong conclusions. Montalvo and Hill (2011) compared the microbiota of three *X. muta* specimens from a reef in Florida with three *X. testudinaria* specimens from a reef in Indonesia. They concluded that the bacterial communities associated with these sponges, although very similar, are highly specific to each of the species. However, since the sponges inhabit water bodies on opposite sides of the globe, it is hard to argue that the different microbial communities are a direct consequence of being two species, rather than being driven by their environments. On the other hand, Fiore et al. (2013b) found a significant effect of location on the symbiotic microbial communities in *X. muta*, but with the revelation of the existence of at least three giant barrel sponge species in the Caribbean, the differences linked to the environment could also be a consequence of sampling different cryptic species at different sites (Chapter 2 of this thesis). These examples illustrate the need to thoroughly examine how the microbial communities in giant barrel sponges vary with geography and phylogeny.

This is, to the best of our knowledge, the first study that includes intricate phylogenetic relationships within a single sponge genus at an ocean-wide scale in order to compare sponge microbiota. First, we characterize the core prokaryotic community within Indo-Pacific giant barrel sponges. Next, we test to what extent the variation in the prokaryotic community of giant barrel sponges can be explained by geography and host relatedness.

METHODS

Sample collection and study areas

Our dataset included 44 samples, unevenly collected by scuba diving from eight areas across the Indo-Pacific (Fig. 4.1). After collection, the material was immediately stored in absolute ethanol (98%) at -20°C. Sponge DNA extraction and the amplification of the mitochondrial genes CO1 and ATP6 were performed following the protocols described in Chapter 2 of this thesis.

For the 16S rRNA gene barcoded Illumina sequencing, we used the FastDNA SPIN Kit for Soil (MP Biochemicals) following the manufacturer's instructions. In brief, sponge samples were cut into small pieces containing both ectosome and choanosome, which were then added to a mixture of silica and ceramic particles in the manufacturer-provided Lysing Matrix E tubes. Cell lysis was performed in a Qiagen TissueLyser II during two sessions of 40 s at the maximum speed, with a 2-min interval between sessions to prevent the samples from overheating. Extracted DNA was eluted into DNase/Pyrogen-Free Water to a final volume of 40 µl and stored at −20°C until use.

Clade delineation, distribution, codes, and core

Recent studies have shown that what is currently considered *X. testudinaria* actually includes multiple reproductively isolated lineages (i.e. species) (Swierts et al. 2013; Bell et al. 2014; Chapter 2 of this thesis). In the absence of renewed species descriptions, we classified our samples into six clades, based on the CO1 and ATP6 mitochondrial genes, that correspond to the 'groups' or candidate species identified in Chapter 2 of this thesis. Some clades are found in different regions, with clade 3 being the most widespread with presence in the Indonesian Seas, Mozambique Channel, Gulf of Thailand and Singapore Strait (Fig. 4.1). Clades 5 and 6, on the other hand, are not widespread and are confined to the Red Sea and Mozambique Channel, respectively (Fig. 4.1).

Seven-symbol sample codes, as shown in certain figures and tables, contain the information of the location, clade and the sample number. The first two letters indicate the location (Pk = Phuket, Thailand; Rd = Red Sea; etc.), the next number indicates the genetic group (1 = clade 1; 2 = clade 2; etc.), and the following four symbols indicate the sample number (s001 = specimen 001; s004 = specimen 004; etc.). The location codes 'Mk' (Makassar) and 'Lm' (Lembeh) are both sublocations of 'Id' (Indonesian Seas).

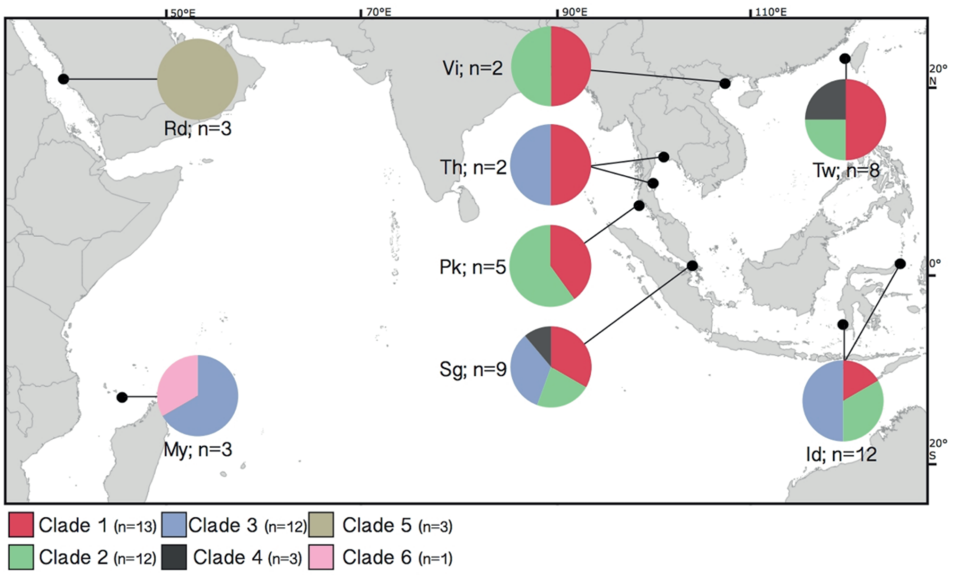


Figure 4.1. Map with the sampling sites per geographic region. Colors of the pie charts indicate the genetic clades of the sponge specimens. Abbreviations: Rd = Red Sea; My = Mayotte; Pk = Phuket, Thailand; Sg = Singapore; Th = Koh Tao and Pattaya, Gulf of Thailand; Vi = Vietnam; Id = Lembeh and Makassar, Indonesian seas; Tw = Taiwan.

Sequence analyses

While there is no consensus on which definition for the core microbiota should be used in sponges, limiting analyses to a core microbial community is a simple method to manage the complexity of the microbiota of marine sponges (Astudillo-García et al. 2017). In our analyses, we defined the core community as the sum of the OTUs present in every sponge specimen. This most stringent definition served as a good guideline, as our subject species are very closely related. However, changing the core definition of three species within the *Xestospongia* genus did not clearly influence the findings of beta-diversity (Astudillo-García et al. 2017).

The 16S rRNA gene V3V4 variable region PCR primers 341F 5'-CCTACGGGNGGCWGCAG-3' and 785R 3'-GACTACHVGGGTATCTAATCC-5' with barcode on the forward primer were used in a 28-cycle PCR assay (5-cycle used on PCR products) using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 min, followed by 28 cycles of 94°C for 30 s, 53°C for 40 s and 72°C for 1 min, after which a final elongation step at 72°C for 5 min was performed. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads. Pooled and purified PCR product was used to prepare the DNA library following the Illumina TruSeq DNA library preparation protocol. Next generation, paired-end sequencing was performed at mrDNA Molecular Research LP (http://www.mrdnalab.com/; last checked 18 November 2016) on an Illumina MiSeq device (Illumina Inc., San Diego, CA, USA) following the manufacturer's guidelines. Sequences from each end were joined following Q25 quality trimming of the ends followed by reorienting any 3'-5' reads back into 5'-3', and removal of short reads (<150 bp). The resultant files were analyzed using the Quantitative Insights Into Microbial Ecology (QIIME) (Caporaso et al. 2010) software package (http://www.qiime.org/; last checked 20 January 2017).

In QIIME, fasta and qual files were used as input for the split_libraries.py script. Default arguments were used except for the minimum sequence length, which was set at 250 bps after removal of forward primers and barcodes. In addition to user-defined cut-offs, the split_libraries.py script performs several quality filtering steps (http://qiime.org/scripts/split_libraries.html). OTUs were selected using the UPARSE pipeline (https://www.drive5.com/usearch/manual7/uparse_pipeline.html; last checked 5 July 2018; Cleary et al. 2017a; 2017b; 2018a; 2018b; 2018c) with usearch10 (Edgar 2010). The UPARSE pipeline (Edgar 2013) includes clustering, chimera checking and quality filtering on de-multiplexed sequences. Chimera checking was performed using the UCHIME algorithm (Edgar et al. 2011). The quality filtering as implemented in usearch10 filters noisy reads and results suggest its output is comparable with other denoisers such as AmpliconNoise, but is much

less computationally expensive (Edgar and Flyvbjerg 2015). First, reads were filtered with the -fastq_filter command and the following arguments: -fastq_trunclen 250, -fastq_maxee 0.5, -fastq_truncqual 15. Sequences were then dereplicated and sorted using the -derep_fulllength and -sortbysize commands. OTU clustering was performed using the -cluster_otus command followed by the -usearch_global command (using global alignment) with id set to 97% to map reads back to OTUs. AWK scripts were then used to convert the OTU files to QIIME format. In QIIME, representative sequences were selected using the pick_rep_set.py script in QIIME using the 'most_abundant' method. Taxonomy was assigned to reference sequences of OTUs using default arguments in the assign_taxonomy.py script in QIIME with the rdp method (Wang et al. 2007). In the assign_taxonomy.py function, we used a fasta file containing reference sequences from the SILVA 128 QIIME release and the uclust classifier method to map sequences to the assigned taxonomy. The make_otu_table.py script in QIIME was used to generate a square matrix of OTUs x SAMPLES followed by the single_rarefaction.py script to rarefy each sample to 10000 sequences. The rarefied table was used as input for further analyses using the R package (R Core Team 2013). We used the blastn command line tool in a Linux environment to query representative sequences of selected taxa including all of the most abundant (≥5000 sequences) OTUs against the online NCBI nucleotide database. Vectors were then generated containing sequence identifiers (GIs) of the 10 top hits of all representative sequences and the Entrez.efetch function in BioPython (Cock et al. 2009) was used with the retype argument set to 'gb' to download Genbank information of the aforementioned top hits including the isolation source of the organism and the host if relevant. The DNA sequences generated in this study can be downloaded from the NCBI SRA: SRP150943.

Statistical analyses

A table containing the presence and abundance per sample of all OTUs was imported into R using the read.csv() function. Plant organelles, mitochondria, known contaminants (Salter et al. 2014) and sequences not assigned to a domain, phylum or class were removed prior to statistical analysis. Singletons were not removed in contrast to other studies, but the rigorous approach above and quality control steps during sequence analyses were taken to minimize the problem posed by sequencing errors in order to enable us to compare rare and abundant OTUs in our dataset. Pielou's J (H/log(S)) was calculated to estimate evenness using the diversity() function in the VEGAN package (Oksanen et al. 2016) in R. The OTU abundance matrix was loge (x + 1) transformed (in order to normalize the distribution of the data) and distance matrices were constructed using the Bray-Curtis index with the vegdist() function in the VEGAN package. The Bray-Curtis index is one of the most frequently applied (dis)similarity indices used in ecology (Legendre and Gallagher 2001; Cleary 2003; Polónia et al. 2015, 2016). Variation in OTU composition was assessed with principal coordinates analysis (PCO) using the cmdscale() function in R with the Bray-Curtis distance matrix as input. We tested for significant variation among geography and phylogeny using an adonis() analysis.

In the adonis analysis, the Bray-Curtis distance matrix of OTU composition was the response variable with geographical area and haplotype as independent variables. The number of permutations was set at 999; all other arguments used the default values set in the function. Weighted averages scores were computed for OTUs on the first two PCO axes using the wascores() function in the vegan package.

In order to test for phylogenetic differences between abundant and rare species we constructed two phylogenetic trees consisting of the two most abundant classes (SAR202 and Caldilineae) of the Chloroflexi, which was the most abundant phylum in our study. For the purposes of this study, OTUs of the Caldilineae were considered abundant if they had >100 sequences in the total dataset. OTUs were considered rare if they had <5 sequences. For the SAR202, the numbers were >1000 sequences for abundant OTUs and <5 sequences for rare OTUs. With these cut-off values we obtained comparable amounts of 'rare' and 'abundant' OTUs per bacterial class. The ape (Paradis et al. 2004), phangorn (Schliep 2011) and picante (Kembel et al. 2010) libraries were used during phylogenetic construction and analysis. First, fasta files containing representative sequences of abundant and rare OTUs were imported into R using the read.DNA() function. Sequences <350 bps were subsequently removed and the remaining sequences aligned using the muscle() function with arguments -gapopen -400.0, -gapextend -0.1, -seqtype dna and -cluster1 neighbor-joining. The resultant dataset was transformed using the as.DNAbin() function. The modelTest() function was used to compare different nucleotide or amino acid substitution models including tests for the Gamma model and invariant sites. The best model selection was based on Akaike information criterion (AIC) model selection (Akaike 1974). For all three classes the GTR + G + I model gave the best result. Neighbor-joining tree estimation (Saitou and Nei 1987) with the dist.hamming() function was achieved using the NJ() function with the ratio argument set to TRUE and the exclude set to pairwise. The resultant tree was analyzed using the pml() function, which computed the likelihood of the phylogenetic tree with the sequence alignment and GTR + G + I model. The number of intervals of the discrete gamma distribution was set to 4 and the proportion of invariable sites to 0.2. The optim.pml() function was subsequently used to optimize the different model parameters with the optNni, optGamma and optInv arguments all set to TRUE and the model argument set to GTR. Finally, the bootstrap.pml() function was used to perform bootstrap analysis on the resultant tree with the number of bootstraps set to 100 and other arguments following the optim.pml() function. All OTUs were assigned to either 'abundant' or 'rare' and the phylo.d() function in the package caper was used to calculate the D value, a measure of phylogenetic signal in binary traits, and to test for significant departure from random association. D values of 1 indicate random association while D values <1 indicate clumping and values >1 indicate overdispersion. Detailed descriptions of the functions used here can be found in R (e.g. ?cmdscale) and online in reference manuals (http://cran.r-project.org/web/packages/vegan/index.html).

RESULTS

Core microbiota

Illumina sequencing of the 16S rRNA gene V3V4 variable region from 44 giant barrel sponges throughout the Indo-Pacific yielded 440 000 sequences. These sequences were assigned to 3795 OTUs after quality control. The OTUs were assigned to 48 phyla, 106 classes, and 145 orders. Proteobacteria was the most diverse and abundant phylum with 134 057 sequences from 1541 OTUs. Chloroflexi were almost equally abundant with 126 358 sequences, but with 448 OTUs they were less diverse than Proteobacteria. Other diverse phyla included Bacteroidetes (239 OTUs), Acidobacteria (178), Actinobacteria (171), Gemmatimonadetes (163), Planctomycetes (134), Cyanobacteria (111) and Poribacteria (62).

According to our definition, the core consisted of 71 OTUs (1.9% of all OTUs) which together yielded 252 988 sequence reads (57.5% of the total number of sequences) (Appendix 4.1). Hence, a small number of OTUs make up the majority of the giant barrel sponge microbiota, illustrating the core's importance. In our dataset of healthy Indo-Pacific giant barrel sponges, 38–69% of the sponge microbiota consisted of OTUs present in all giant barrel sponges. The sample with the lowest relative contribution of its core community (38.8%) was a sponge from Taiwan (Tw4s476) and the sample with the highest relative contribution of its core community (68.6%) was a sponge from Lembeh, Indonesia (Lm3s005).

The most diverse phylum in the core community was Chloroflexi (25 OTUs), which included two members of the class Caldilineae and 18 members of the class SAR202. Whereas the most abundant core OTU was a member of the Caldilineae (OTU 1; 17 592 sequences; 7% of the total amount of core sequences), the SAR202 members combined added up to 23.2% of the total core sequences and were the most abundant bacterial class in the giant barrel sponge core. Other phyla in the core were Proteobacteria (19 OTUs), Actinobacteria (7), Gemmatimonadetes (5), Acidobacteria (4), Nitrospirae (2) and Poribacteria(1). No archaeon was part of the core prokaryotic community; however, each giant barrel sponge harbored at least one OTU from the archaeal genus Candidatus Nitrosopumilus. Nearly half of the OTUs (49.9%) occurred in only one sponge individual, and many of these OTUs returned only one sequence read. The OTUs occurring in one specimen encompassed only a small proportion of the total amount of sequence reads (0.48%).

Host specificity compared to geography and host phylogeny

The results of our PCO analysis, based on all 3795 OTUs, are shown in Fig. 4.2. The samples visually cluster together based on geography. Samples from the Gulf of Thailand, Indonesia, Mayotte, Phuket, and Singapore are separated along the first PCO axis from samples from the Red Sea and Taiwan. This axis explained 19.7% of the variation in our PCO analysis. The second axis, which explained 13.3% of the variation, separated the sponges of clade 5, which were all collected in the Red Sea, from the other clades and locations. The third and

fourth axes, which explained 8.0% and 6.2% of the variation, respectively, followed the same pattern, with sample clustering based on geography rather than phylogeny (Appendix 4.2). Both geography (adonis: F5,41 = 3.00, P < 0.001, R2 = 0.368) and phylogeny (adonis: F5,41 = 1.86, P < 0.001, R2 = 0.197) were significant predictors of variation in the composition of the prokaryotic community. Due to the larger influence of geography, and the lack of obvious clustering in our PCO analysis based on phylogeny, we focused on the variation

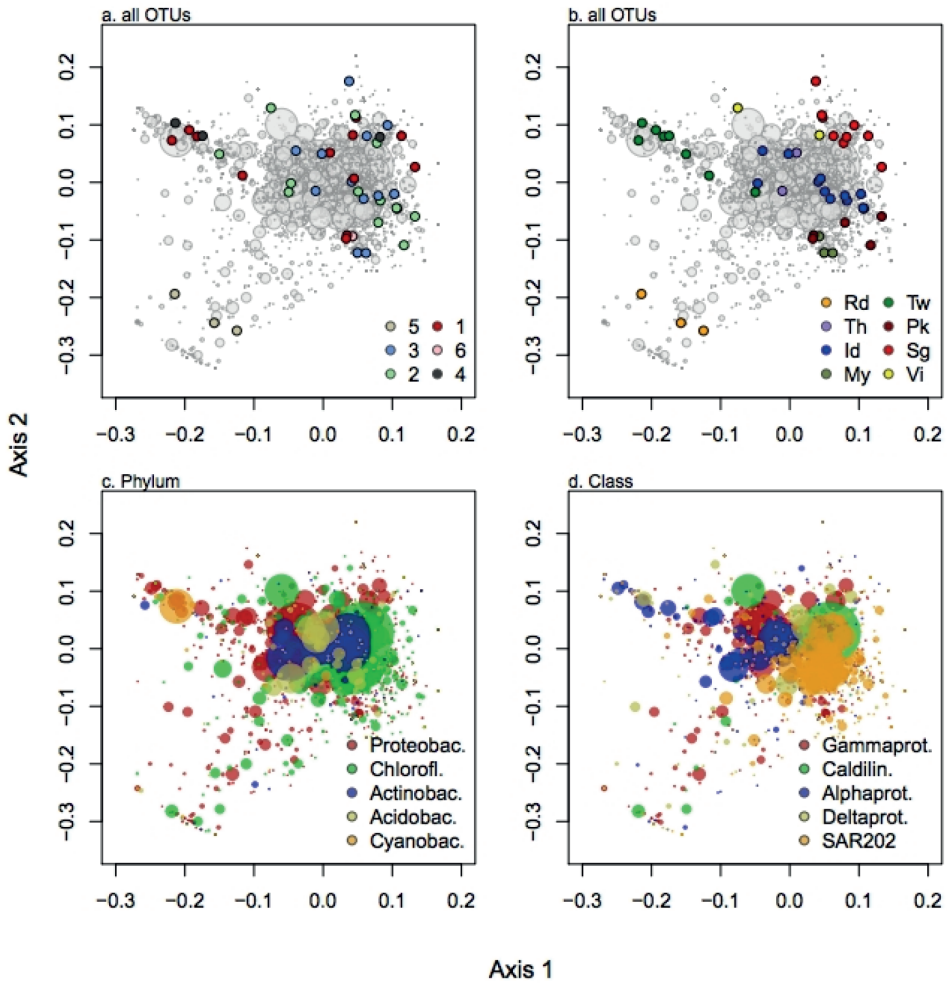


Figure 4.2. First and second axes of the Principle Coordinate Ordination based on our full dataset. Each dot in the (a) and (b) graphs represents one sponge individual, and their positioning in the ordination is identical for both (a) and (b), the only difference being the color scheme. Colors in (a) indicate clades and in (b) geographic origin. Abbreviations of geographic locations are: Rd = Red Sea; My = Mayotte; Pk = Phuket, Thailand; Sg = Singapore; Th = Koh Tao and Pattaya, Gulf of Thailand; Vi = Vietnam; Id = Lembeh and Makassar, Indonesian seas; Tw = Taiwan. The OTUs are color-coded for phylum in (c) and bacterial class in (d).

in prokaryotic communities of giant barrel sponges with regard to geography in subsequent analyses.

The abundance of some higher bacterial taxa among geographic locations varied significantly (Fig. 4.3). The Red Sea, Gulf of Thailand, Taiwan, and Vietnam were characterized by relatively high numbers of Proteobacteria and low numbers of Chloroflexi, while the opposite was true for sponges from the Indonesian Seas, Mayotte, Phuket and Singapore (Fig. 4.3a,b). The abundance of the phyla Actinobacteria, Acidobacteria, Gemmatimonadetes, Nitrospirae, Cyanobacteria, Bacteroidetes, Spirochaetae, Deinococcus – Thermus, and Planctomycetes differed significantly among groups from different geographic regions (Fig. 4.3c-g,j,k,m,n). In contrast, PAUC34f, SBR1093, and Poribacteria did not show a similar effect (Fig. 4.3h,i,l). In addition to phyla, certain bacterial classes also differed significantly among locations (Fig. 4.3o-r). For example, the bacterial classes SAR202 and Caldilineae showed a large variation in relative abundance, varying from 10.3 (± 3.6)% in Vietnam to 30.1 (± 5.0)% in Mayotte for SAR202, and from 1.9 (± 1.6)% in the Indonesian Seas to 12.7 (± 5.9)% in Phuket for Calidilineae (Fig. 4.3o,r). For these two bacterial classes, we tested whether abundant OTUs were phylogenetically related to one another. We found a significant phylogenetic clumping of abundant OTUs within the Caldilineae (estimated D: 0.365; $P < 0.001$), whereas this was not observed for SAR202 (estimated D: 1.583; $P = 1.000$), where abundant OTUs did not cluster together in the phylogenetic tree (Appendix 4.3). The evenness and rarefied richness per geographical location are shown in Fig. 4.3s,t.

The abundance of certain individual OTUs was also related to geography. The most abundant OTU (OTU 1; 15 592 sequences) in our dataset was assigned to the family Caldineaceae within the Caldilineae and was similar to an organism previously found in giant barrel sponges from Indonesia (sequence similarity = 100%; Appendix 4.4). Although this was the most abundant OTU in our total dataset, there was pronounced variation in its relative abundance among geographic locations, varying from an average abundance of <1% in Taiwan (0.72 ± 0.69%) to 12% in Phuket (11.86 ± 5.03%).

The second most abundant OTU in our dataset (OTU 2; 11 491 sequences) was assigned to the class Nitrospira and was closely related to an organism found in the coral *Porites lutea* (sequence similarity = 100%; Appendix 4.4). This OTU was most abundant in sponges from Singapore (4.0 ± 3.1%) and Vietnam (5.4 ± 1.35%), and it was often the dominant Nitrospira member in the giant barrel sponge microbiota with very low numbers of other OTUs assigned to the Nitrospira (Fig. 4.3).

The third most abundant OTU in our dataset (OTU 3; 18 996 sequences) was assigned to the class SAR202, within the Chloroflexi, and was closely related to an organism previously found in the sponge *Astrosclera willeyana* (Appendix 4.4). Each giant barrel sponge sample

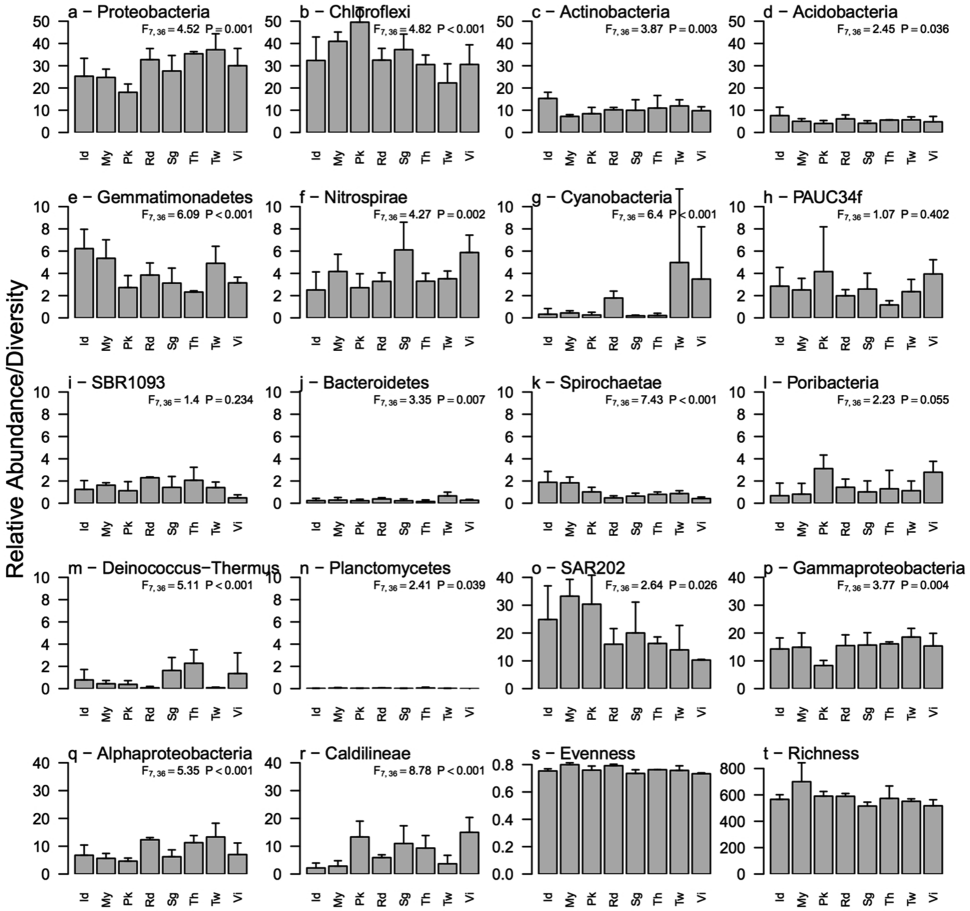


Figure 4.3. Mean relative abundance of all OTUs within the most abundant bacterial phyla (a-n) and classes (o-r) and the evenness (s) and richness (t) for giant barrel sponges from eight locations around the globe (Rd = Red Sea; My = Mayotte; Pk = Phuket, Thailand; Sg = Singapore; Th = Koh Tao and Pattaya, Gulf of Thailand; Vi = Vietnam; Id = Lembeh and Makassar, Indonesian seas; Tw = Taiwan). Error bars indicate the standard deviation. Results of GLM (General Linear Model) are shown in the top right corner of each graph.

hosted a fair number of sequences of OTU 3 (47–598 reads), but simultaneously also harbored a rich variety of 15 to 58 OTUs of other moderately abundant SAR202 members (>0.1%). One sponge from Phuket, Thailand (Pk2s085) even harbored 16 OTUs of SAR202 which each comprised at least 1% of its total community. This is different from the previously mentioned classes, Caldilineae and Nitrospira, in which one specific OTU of each of the respective bacterial classes was often abundant.

Figure 4.4. illustrates that some OTUs were strongly restricted to specific locations. The OTUs included in this graph were selected because their presence varied with location. For example,

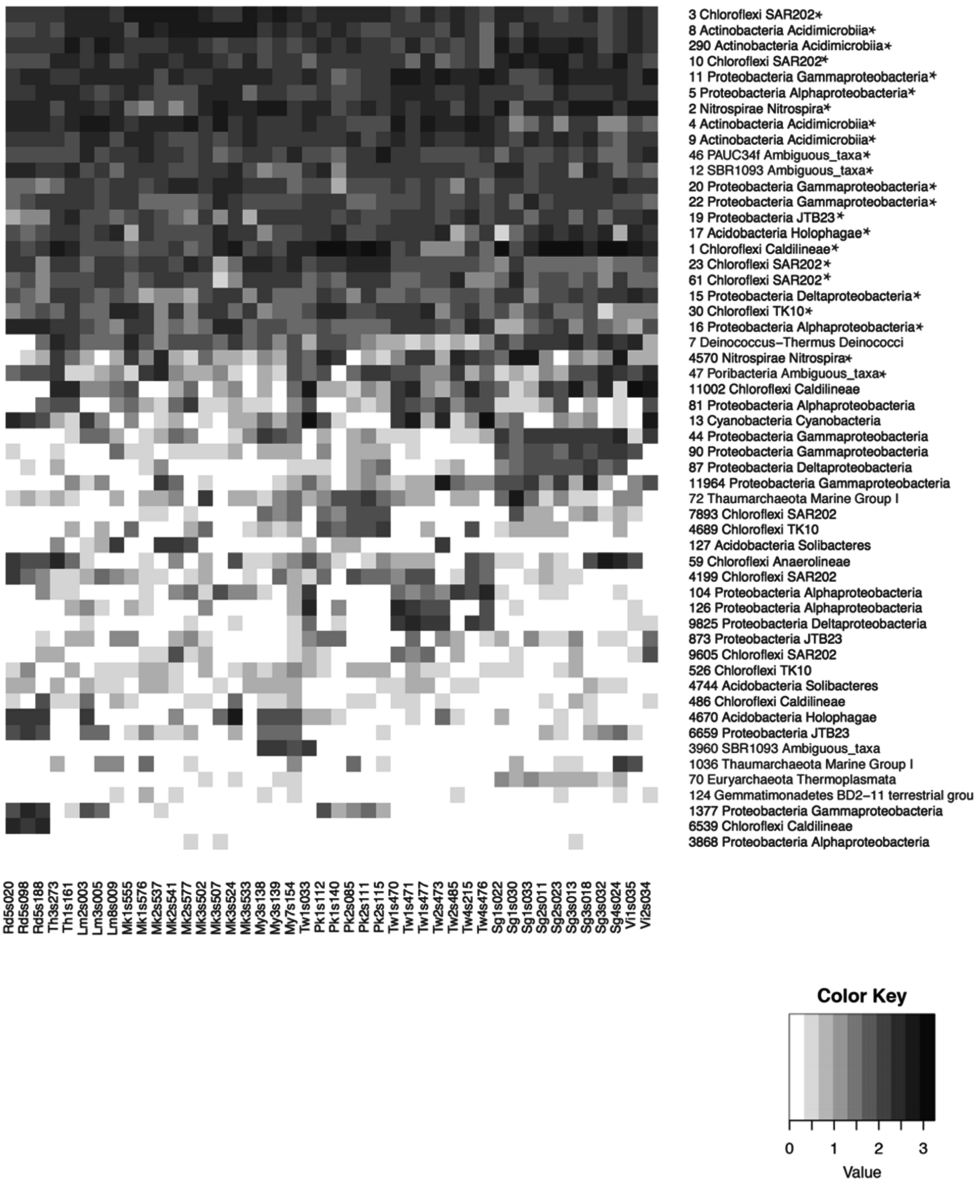


Figure 4.4. Heat map indicating the abundance of the 19 most abundant OTUs in our dataset and 35 handpicked OTUs in each giant barrel sponge sample. The handpicked OTUs are specified in Appendix 4.4. The sponges are ordered based on geography (Rd = Red Sea; My = Mayotte; Pk = Phuket, Thailand; Sg = Singapore; Th = Koh Tao and Pattaya, Gulf of Thailand; Vi = Vietnam; Lm and Mk = Lembeh and Makassar, Indonesian seas; Tw = Taiwan) and clade (numbers 1–6 after geography code). Scale is logarithmic. Asterisks indicate OTUs that are part of the core (i.e. OTUs present in each sample in our dataset).

OTU 3960 was predominantly found in samples from Mayotte. This OTU was assigned to the bacterial class EC214 and is related to a bacterium previously found in a sponge from the Red Sea (sequence similarity = 99.56%; Appendix 4.4), but remarkably enough this OTU is completely absent in our Red Sea samples. In Mayotte, the relative abundance of this OTU is 0.96 ± 0.26%, and besides being present in one Taiwanese specimen, it was virtually absent in all other sponges.

The Red Sea also had a distinct prokaryotic community. OTU 6539 made up 1.0–3.0% of the bacterial community of these specimens but was nearly absent in all other samples (Fig. 4.4). It was related to an organism obtained from *Ircinia strobilina* in Bahamian mangroves (sequence similarity = 99.53%; Appendix 4.4). Other characteristic OTUs for the Red Sea are the OTUs 1377, 4670 and 6659 (Fig. 4.4; Appendix 4.4). These specific OTUs, together with the high relative abundances of Alphaproteobacteria and Cyanobacteria (Fig. 4.3), give the Red Sea a distinct prokaryotic community as evidenced by the distinct cluster it forms in the PCO analysis (Fig. 4.2). Since all Red Sea samples belonged to clade 5, a clade that was not found in other locations, this distinct Red Sea prokaryotic community is likewise characteristic for clade 5.

DISCUSSION

Core microbiota

Focusing on a core microbiota is a straightforward approach to manage the complexity of the microbiota of marine sponges (Astudillo-García et al. 2017). The prokaryotic community of giant barrel sponges in the Indo-Pacific is characterized by a relatively high number of core OTUs (i.e. OTUs present in each specimen) that represent the majority of the total number of sequences. In five other sponge species, both LMA (Low Microbial Abundance) and HMA (High Microbial Abundance), the core microbiota varied between seven and 20 OTUs, with each of those OTUs present in at least 85% of the samples (Thomas et al. 2016). With our more stringent definition of a core OTU, we found that Indo-Pacific giant barrel sponges have a diverse core, with 71 OTUs occurring in each specimen. The main bacterial phyla in the core prokaryotic community were Proteobacteria, Chloroflexi, Actinobacteria, Gemmatimonadetes, Nitrospirae, Acidobacteria, PAUC34f, and Poribacteria. Members of Chloroflexi have been shown to be capable of harvesting energy from sunlight (Bryant and Frigaard 2006). The fact that 31 OTUs assigned to the Chloroflexi coexist in each giant barrel sponge in our Indo-Pacific dataset suggests that the giant barrel sponge holobiont is mixotrophic and that photosynthesis may be an important pathway in its physiology. The same bacterial phyla were also among the main groups found in previous studies of the microbiota of giant barrel sponges (Montalvo and Hill 2011; Fiore et al. 2013b; Morrow et al. 2016; Cleary et al. 2015a; De Voogd et al. 2015; Astudillo-García et al. 2017). Previously, members of the Actinobacteria were suggested to dominate the microbiota of *X. muta*, making up 12% of the community based on clone libraries (Montalvo et al. 2005). In line

with Olson and Gao (2013), and Morrow et al. (2016), our data indicates that they are not the largest group in the microbiota; however, they are still an important contributor to the prokaryotic community, particularly in absolute numbers of sequences.

Core OTUs may possess traits that are beneficial for the host's survival in the Indo-Pacific since they occur in all sampled giant barrel sponges irrespective of their geographical origin or phylogenetic position. To determine which of these OTUs are fundamental for the giant barrel sponge species complex as a whole, these core OTUs should be compared with those of giant barrel sponges from other locations not included in this study, particularly the Caribbean and Australia. For example, a BLAST search of one OTU returned an identical sequence from a Caribbean giant barrel sponge (Montalvo and Hill 2011). The associations with OTUs that are specific to giant barrel sponges, and that occur in each specimen around the globe, may have originated in a common sponge ancestor prior to the first speciation event, whereas the associations with OTUs that are only found in all Indo-Pacific specimens but not necessarily in specimens from the other locations may have co-diversified locally with the giant barrel sponge species complex after the first speciation events.

In contrast to the core OTUs, a large number of OTUs only occurred in a single individual sponge. Almost half of the OTUs were such singularly occurring OTUs and should therefore not be considered specific to giant barrel sponges in general. Host species specificity implies that the OTU is characteristic for sponges of a certain species, but this is not the case for these singularly occurring OTUs. They are potentially misleading in the interpretation of interspecies comparisons as they might be mistaken for host-specific OTUs, particularly when the comparisons are based on just one sample or only a few samples per host species. It is likely that the number of 70% of host-species specific OTUs that was identified by Schmitt et al. (2012) is an overestimation since this number probably contains such OTUs that were only found in one individual.

Host specificity compared to geography and host phylogeny

Previously, it was found that prokaryotic communities of sponges are generally stable across sampling events, seasonal shifts in temperature and irradiance, and across large spatial scales (Erwin et al. 2012; Björk et al. 2013; Reveillaud et al. 2014; Steinert et al. 2016; Thomas et al. 2016). This was also true for giant barrel sponges (Olson and Gao 2013; Morrow et al. 2016), but our results have led us to a different interpretation. The relative abundance of core OTUs and non-core OTUs varied considerably, and this variation was mostly related to the geographic origin of the sample, and to a lesser extent to the phylogeny. Samples from the same location had very similar prokaryotic communities, irrespective of the present genetic clades. In more isolated regions, such as the Red Sea and Mayotte, the sponges harbored specific OTUs that were orders of magnitude more abundant compared with sponges from other locations. In contrast to the Red Sea, multiple clades occur in Mayotte, and therefore the specificity of certain OTUs to several locations seems to be related to

geography rather than phylogeny. In addition to giant barrel sponge-specific OTUs, one could argue that geography-specific OTUs within giant barrel sponges also exist.

The giant barrel sponge microbiota is believed to play key roles in nutrient cycling, and these communities may adapt to local light conditions and nutrient availability (Webster and Taylor 2012; Morrow et al. 2016). Not all bacterial phyla and classes varied in a similar fashion or magnitude across the sampled locations. The groups that varied stronger, for example Chloroflexi, Cyanobacteria and Nitrospirae, might be more sensitive to local or regional environmental factors than other microbial groups with a more uniform distribution across the various areas. Many members of the class SAR202 within the Chloroflexi, for example, are associated with sulphite oxidation in aphotic conditions, and this could be an important function in certain populations of giant barrel sponges depending on the local conditions (Mehrshad et al. 2018). Other studies have also found that the abundances in the sponge microbiota of several bacterial groups may correlate with environmental factors such as depth, turbidity, available food sources, pH and temperature (Olson et al. 2014; Luter et al. 2015; Morrow et al. 2015; Lesser et al. 2016). The geographical variation in the giant barrel sponge microbiota is not a direct derivative of the local microbiota from the abiotic environment, since it has been shown that both the bacterial and archaeal communities of both sediment and seawater are highly dissimilar to the prokaryotic community of giant barrel sponges (Polónia et al. 2014; Cleary et al. 2015a; De Voogd et al. 2015).

While giant barrel sponges from the same location harbored more similar prokaryotic communities compared with giant barrel sponges from locations further away, phylogenetic relationships were also, albeit to a lesser extent, a predictor of prokaryotic community composition. However, these results were not visually detectable in the PCO analysis. This could simply be overshadowing of the phylogenetic signals by the stronger geographic signals in the analysis. However, this could also be the result of the genetic groups not being equally distributed over the geographic locations. For instance, all samples from the Red Sea belonged to one clade that was unique for that location (Chapter 2 of this thesis). The significant phylogenetic signal in our statistical test could, therefore, be a type I error as a result. This makes it difficult to confirm or reject hypotheses regarding the influence of phylogeny on the giant barrel sponge prokaryotic community.

Our results contradict the conclusions of a previous study comparing the microbiota of *X. muta* from Florida with *X. testudinaria* from Indonesia (Montalvo and Hill 2011). In this study, the authors concluded that the differences between the two species suggested vertical transmission and bacterial speciation within sponge hosts. However, after the recently exposed intricate and intertwined phylogenies of Caribbean and Indo-Pacific giant barrel sponges, it has become clear that the *X. testudinaria* samples used in their study were actually two different species (clade 1 and clade 3; Setiawan et al. 2016a; Setiawan et al. 2016b; Chapter 2 of this thesis). Therefore, it is more likely that the differences in the microbial

communities reflect the geographic locations they were sampled in. Some of the lineages within the giant barrel sponge species complex are suggested to have been diverging since a time before the closing of the Tethys Seaway, approximately 50 million years ago (Chapter 2 of this thesis). Nevertheless, while these clades have genetically grown apart for millions of years, the sponges have retained nearly identical body plans. This taxonomical similarity may have allowed prokaryotic lineages to move from one giant barrel sponge clade to another by horizontal transmission, limiting or preventing co-diversification between prokaryotes and individual giant barrel sponge species (Moran and Sloan 2015).

Whether the giant barrel sponge prokaryotic community composition adapts to local conditions, or that available OTUs in the surrounding seawater are driving the variation, remains unknown. This study, however, shows that the environment can be a more important driver of the prokaryotic community than is generally considered. Furthermore, this study underlines the importance of incorporating geographic variation in comparisons among the prokaryotic communities of multiple sponge species or taxa.

ACKNOWLEDGEMENTS

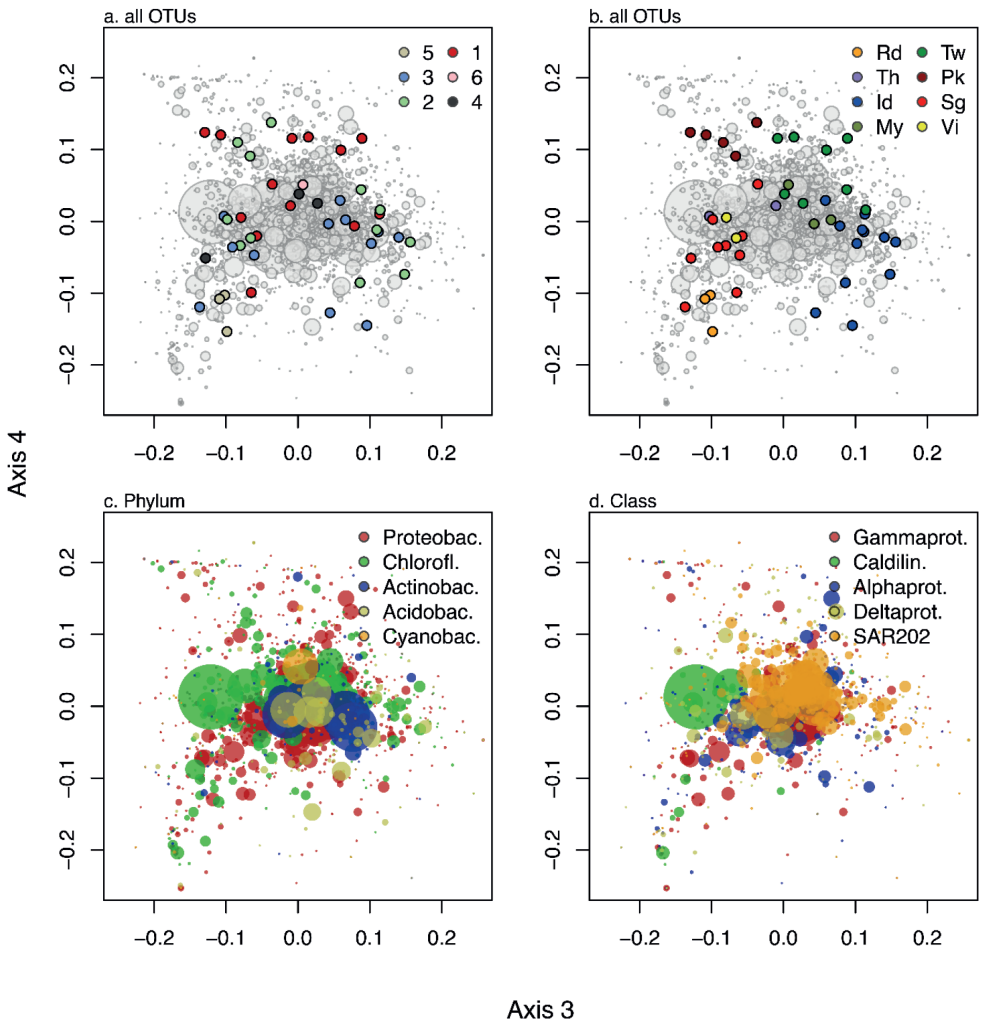
We thank Anne Bialecki, Cécile Debitus, Bruno Fichou, Stephan Aubert, Philippe Prost and Jean-Pierre Bellanger for their support. Research permits in Indonesia were issued by the Indonesian State Ministry for Research and Technology (RISTEK) and the Indonesian Institute of Sciences (PPO-LIPI). Fieldwork in Lembeh Strait (2012) took place during a Marine Biodiversity Workshop based at the Bitung Field Station of RCO-LIPI, co-organized by Universitas Sam Ratulangi. Field work in Makassar was supported by the Hasanuddin University. Fieldwork in Thailand was supported by the institute of Marine Sciences of the Burapha University and we thank Saowapa Sawatpeer, Sumaitt Putchakarn and Chad Scott for their support. The Economic Planning Unit, Prime Minister's Department Malaysia, and the Department of Marine Park Malaysia granted research permits to Z Waheed and we thank her for support. The study in Vietnam was made possible through collaborations with the Research Institute of Marine Fisheries and we especially thank Nguyen Khac Bat. We thank Swee Cheng Lim for his help in Singapore and Yusheng Huang in Taiwan.

APPENDIX 4.1

https://academic.oup.com/femsec/article/94/12/fiy194/5115559#supplementary-data

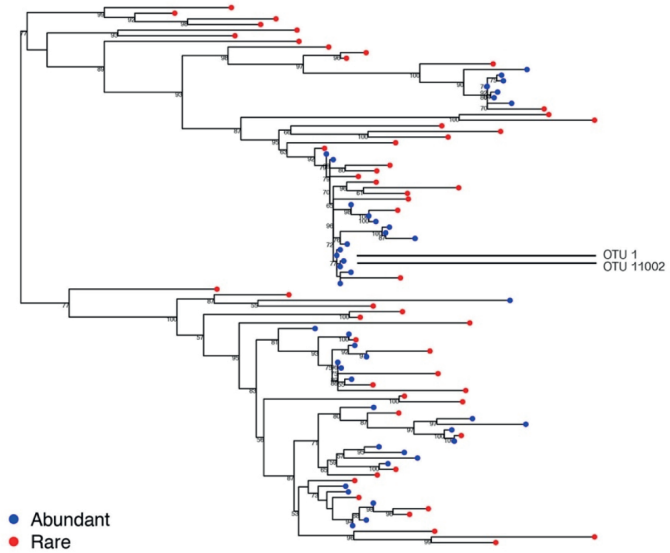
APPENDIX 4.2

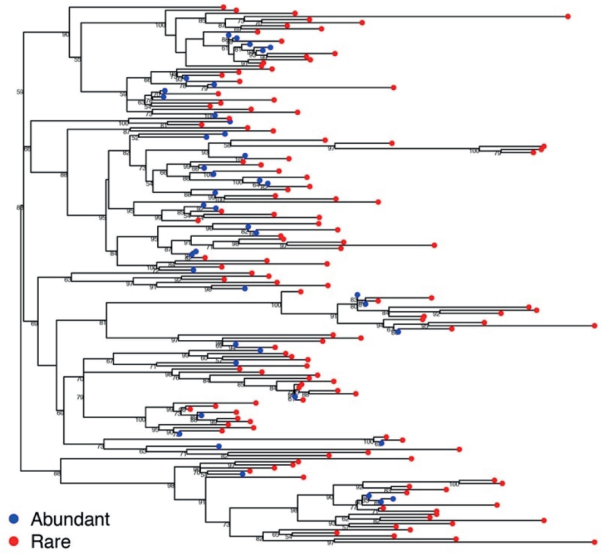
Third and fourth axes of the Principle Coordinate Ordination based on our full dataset. Each dot in the (**a**) and (**b**) graphs represents one sponge individual, and their positioning in the ordination is identical for both (a) and (b), the only difference being the color scheme. Colors in (a) indicate clades and in (b) geographic origin. Abbreviations of geographic locations are: Rd = Red Sea; My = Mayotte; Pk = Phuket, Thailand; Sg = Singapore; Th = Koh Tao and Pattaya, Gulf of Thailand; Vi = Vietnam; Id = Lembeh and Makassar, Indonesian seas; Tw = Taiwan. The OTUs are color-coded for phylum in (**c**) and bacterial class in (**d**).



APPENDIX 4.3

Phylogenetic trees of the OTUs from bacterial classes Caldilineae (top) and SAR202 (bottom). Colours at the end of the branch indicates the abundance of the OTU: Red = Rare; Blue = Abundant. OTUs of the Caldilineae were considered abundant if they had > 100 sequences in the total dataset. OTUs were considered rare if they had < 5 sequences. For SAR202, OTUs were considered abundant if they had > 1000 sequences and rare if they had < 5 sequences. With these cut-off values we obtained similar amounts of 'rare' and 'abundant' OTUs per bacterial class.





APPENDIX 4.4

https://academic.oup.com/femsec/article/94/12/fiy194/5115559#supplementary-data

5

Impacts of host identity and geography on the prokaryotic community of giant barrel sponges at multiple spatial scales

Swierts, T., Cleary, D. F. R., Clay, M., Abdelmalik, N.,
van der Ent, E., van Bodegom, P. M. & de Voogd, N. J.

Submitted

ABSTRACT

Sponge holobionts are model systems for marine host–microorganism interactions. The understanding of the drivers of the sponge microbiome, therefore, shapes the general views in marine microbial ecology. A broader understanding of these interactions, furthermore, enhances the potential to utilize these microbes for biotechnological purposes. Most studies aiming to identify the drivers of the sponge microbiome focus on a single driver or on one spatial scale, leaving a hiatus in our understanding of the interplay of the drivers. In the present study, we assessed the importance of host identity and geography on prokaryotic communities at multiple spatial scales by comparing 73 giant barrel sponges (*Xestospongia* spp.) from Curaçao, Martinique, and Thailand. Geographic distance was the main driver of prokaryote communities at the global and regional scales, whereas host identity was a minor, albeit significant, driver. At a local scale in Curaçao, Martinique, and Thailand, the phylogenetic variation in the prokaryotic community was not related to inter-site distance, but rather to unidentified local site conditions while depth was an important driver in Martinique. Phylogeny was a more influential driver at the three localities than at the larger spatial scales. Together, this study shows that the relative importance of drivers of the sponge microbiome shifts across different spatial scales. Our results are in contrast with the assumption that host identity is the principal driver of the sponge microbial community. Instead, biogeographical differences should be comprehensively considered. Dispersal limitations seem crucial at large scale, while the importance of depth and local site differences shows that the prokaryote community of giant barrel sponges is flexible. This raises the question of whether this translates into adaptability to environmental change, potentially making them resilient to such changes.

INTRODUCTION

Sponges are an ancient animal lineage (Simion et al. 2017), and their evolutionary success has been suggested to be, at least in part, due to their intimate relationship with microbial symbionts (Taylor et al. 2007b). Their long evolutionary history has made sponge holobionts an important model system for marine host–microorganism interactions. As hosts, sponges are an important contributor to the diversity of the coral reef prokaryotic metacommunity (Chapter 6 of this thesis). Sponge-associated microorganisms perform a wide range of functional roles within sponge hosts (Webster and Thomas 2016). Among other things, they produce various secondary metabolites that help the sponge defend itself against pathogens and other harmful organisms (Pawlik 1993; Hentschel et al. 2012). These secondary metabolites have great potential for the pharmaceutical industry and have gained much attention over the past decades (Munro et al. 1999; Sipkema et al. 2005; Thomas et al. 2010). Some microbes living in sponges, e.g. Cyanobacteria, are capable of photosynthesis and provide their hosts with an extra source of energy (Usher 2008; Thacker and Freeman 2012). Other microbes play important roles in nutrient cycling within the wider ecosystem (Hoffman et al. 2009; Mohamed et al. 2010; de Goeij et al. 2013; Zhang et al. 2015).

The scientific community has placed substantial effort into studying the drivers of compositional variation in sponge-associated microbial communities, such as host identity and the environment. Host identity has generally been found to be a much more important driver of sponge microbial communities than the environment, particularly at higher taxonomic ranks (Thomas et al. 2016; Souza et al. 2017; Steinert et al. 2017). This host specificity has regularly been hypothesized to originate in the vertical transfer of microorganisms from parent to offspring which instigated co-evolution or co-diversification (Schmitt et al. 2008). However, it is also possible that these OTUs are acquired from the rare biosphere, after which they thrive in their respective hosts (Taylor et al. 2013; Reveillaud et al. 2014). Because of its link to host identity, the sponge microbiome is often considered to be stable across space and time (Erwin et al. 2012; Pita et al. 2013a, 2013b, 2018; Cárdenas et al. 2014; Hardoim and Costa 2014; Thomas et al. 2016; Gantt et al. 2017; Glasl et al. 2018). However, seasonal and spatial variation have been observed in certain species (Wichels et al. 2006; Cao et al. 2012; Turque et al. 2012; White et al. 2012; Luter et al. 2015; Weigel and Erwin 2016; Pita et al. 2018; Chapter 4 of this thesis). Hence, host-identity and environmental drivers may both contribute to prokaryote composition.

Most studies of the sponge microbiome have focused on only one driver, and no studies have hitherto compared the importance of multiple drivers at multiple spatial scales. Our knowledge of how drivers act together on the sponge prokaryote community thus represents an important hiatus. This fundamental knowledge, however, is essential to work on the main future directions in sponge holobiont research, recently identified by Pita et al. (2018). They highlighted the importance of determining how environmental factors alter

microbiome-mediated processes and the need to develop management solutions, which will ensure the maintenance of sponge holobiont functions at the ecosystem level (Pita et al. 2018). Furthermore, understanding the interplay between the various drivers is not only relevant for sponges, but also for other reef organisms whose microbial communities may be directly affected by those of sponges (Chapter 6 of this thesis).

In this study, we hypothesize that the relative contribution of drivers such as host identity and environmental conditions to the sponge prokaryotic community can shift at different spatial scales. This hypothesis was tested with a sampling design that includes sampling closely related and sympatric sponge species across multiple spatial scales. The widespread occurrence and recently unraveled phylogeny of giant barrel sponges (*Xestospongia* spp.) make them an ideal group for this purpose (Chapter 2 of this thesis). The most dominant prokaryotic phyla in giant barrel sponges include Proteobacteria, Actinobacteria, Chloroflexi, Nitrospirae, and Cyanobacteria, which were also among the most dominant groups in other high microbial abundance (HMA) sponge species (Thomas et al. 2016, Moitinho-Silva et al. 2017a, Chapter 4 of this thesis). Various drivers have been suggested to impact the giant barrel sponge prokaryote community, including host identity, geography (the location at which a sponge is collected) and depth (Montalvo and Hill 2011; Fiore et al. 2013a; Morrow et al. 2016; Chapter 4 of this thesis). Hence, giant barrel sponges can serve as a model to study the interplay between spatial and phylogenetic variation as drivers of sponge prokaryote communities.

METHODS

Study object

Giant barrel sponges are found across the entire Caribbean and are known as *Xestospongia muta*. However, this species consists of three reproductively isolated groups that have different morphologies and sterol compositions, and are believed to act as separate species (Fromont et al. 1994; López-Legentil and Pawlik 2009, Deignan et al. 2018; Chapter 2 of this thesis). In the Indo-Pacific, giant barrel sponges occur from the east coast of Africa and the Red Sea to Taiwan and New Caledonia and are referred to as *Xestospongia testudinaria*. This species complex consists of at least six genetically isolated groups, in some places with distinct morphological differences (Swierts et al. 2013; Bell et al. 2014; Chapter 2 this thesis). Although the different species have not yet been named, we use the variation in this species complex for our host-phylogenetic analyses.

A third described species, *Xestospongia bergquistia*, is confined to the Australian coast and, in contrast to other giant barrel sponges, lacks spongin fiber between the skeletal spicules (Fromont 1991). All of our samples contained spongin fiber; hence *X. bergquistia* was not included in the specimens used in the present study. Giant barrel sponges occur along a large depth range, from just below the surface, to depths exceeding 120 m. They are found in both clear and turbid waters and seem resilient to external stressors (McMurray et al. 2015).

Sample collection

We assessed the importance of host identity and geography on the prokaryotic community of giant barrel sponges by comparing their prokaryotic communities at different spatial scales. On a global scale, we tested samples from three Caribbean giant barrel sponge species against two Indo-Pacific species. At a regional scale, we compared the prokaryotic communities of three giant barrel sponge species from the Caribbean islands Curaçao and Martinique. And at a local scale, we compared giant barrel sponges from multiple reef sites within Curaçao, Martinique, and Thailand, respectively. Samples were collected in Curaçao, Martinique, and Thailand between September 2016 and March 2017 (Fig. 5.1a). In Curaçao, we collected 32 samples from six sites, in Martinique 25 samples from five sites and in Thailand sixteen samples from four sites (Fig. 5.1b-d). We chose different experimental setups in each of these localities in order to additionally explore multiple potential nominally defined environmental drivers. This was given preference over a uniform setup to compare one single driver as a proxy for environmental variation across these three localities. In Thailand, our setup focused on distance to shore, in Curaçao on coastal development and in Martinique on depth and light availability. The sponges in shallow water (<40 m) were sampled with an apple corer by SCUBA diving and the sponges from deeper water in Martinique (>80 m) were collected by dredging. The collected tissue was immediately stored in ethanol (98%) and kept in a cooler until they were finally stored at -20°C in the laboratory. A detailed description of the sites in Curaçao, Martinique, and Thailand is available in Appendix 5.1.

DNA analysis

Each sponge was barcoded for the I3-M11 partition of the mitochondrial CO1-gene and assigned to a species following the protocol and classification described in Chapter 2 of this thesis. Prokaryotic DNA extraction was performed as described in Chapter 4 of this thesis.

The 16S rRNA gene V3V4 variable region PCR primers 341F 5'-CCTACGGGNGGCWGCAG-3' and 785R 3'-GACTACHVGGGTATCTAATCC-5' with barcode on the forward primer were used in a 28 cycle PCR assay (5 cycle used on PCR products) using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 minutes was performed. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads. Pooled and purified PCR product was used to prepare the DNA library following the Illumina TruSeq DNA library preparation protocol. Next-generation, paired-end sequencing was performed at mrDNA Molecular Research LP (http://www.mrdnalab.com/; last checked 2016 11 18) on an Illumina MiSeq device (Illumina Inc, San Diego, CA, USA) following the manufacturer's guidelines. Sequences from each end were joined following Q25 quality trimming of the ends followed by reorienting any 3'-5' reads

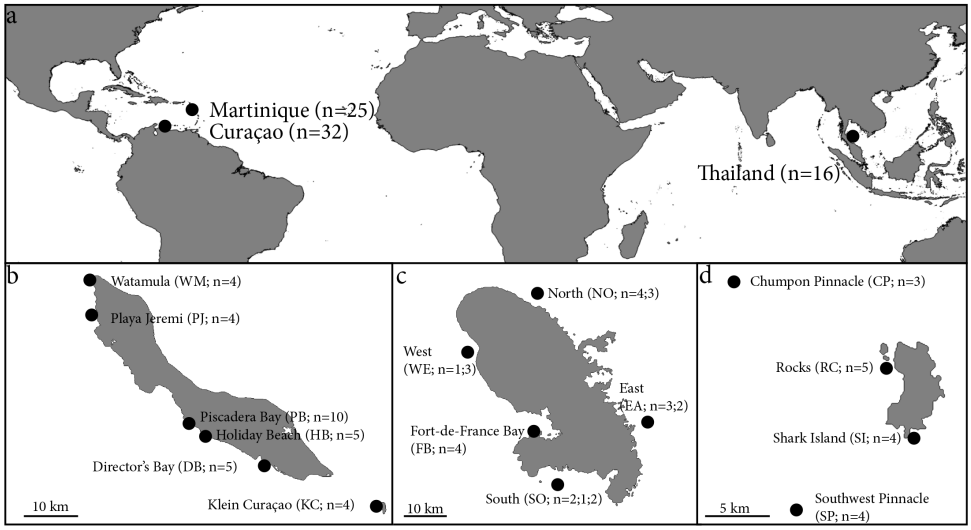


Figure 5.1. Maps indicating the sampled localities in the global oceans (a) and the sampling sites at the localities Curaçao (b), Martinique (c) and Thailand (d).

back into 5'-3', and removal of short reads (< 150 bp). The resultant files were analyzed using the QIIME (Quantitative Insights Into Microbial Ecology; (Caporaso et al. 2010) software package (http://www.qiime.org/; last checked 2017-01-20).

In QIIME, fasta and qual files were used as input for the split_libraries.py script. Default arguments were used except for the minimum sequence length, which was set at 250 bps after removal of forward primers and barcodes. In addition to user-defined cut-offs, the split_libraries.py script performs several quality filtering steps (http://qiime.org/scripts/split_libraries.html). OTUs were selected using the UPARSE pipeline (https://www.drive5.com/usearch/manual7/uparse_pipeline.html; last checked 2018 07 05) with usearch10 (Edgar 2013). The UPARSE pipeline (Edgar 2013) includes clustering, chimera checking and quality filtering on de-multiplexed sequences. Chimera checking was performed using the UCHIME algorithm (Edgar et al. 2011). Quality filtering in usearch10 filters noisy reads and results suggest its output is comparable to other denoisers such as AmpliconNoise, but is much less computationally expensive (Edgar and Flyvbjerg 2015). First, reads were filtered with the -fastq_filter command and the following arguments -fastq_trunclen 250 -fastq_maxee 0.5 -fastq_truncqual 15. Sequences were then dereplicated and sorted using the -derep_fulllength and -sortbysize commands. OTU (Operational Taxonomic Unit) clustering was performed using the -cluster_otus command followed by the -usearch_global command (using global alignment) with id set to 97% to map reads back to OTUs. AWK scripts were then used to convert the OTU files to QIIME format. In QIIME, representative sequences were selected using the pick_rep_set.py script in QIIME using the 'most_abundant' method.

Taxonomy was assigned to reference sequences of OTUs using default arguments in the assign_taxonomy.py script in QIIME with the rdp method (Wang et al. 2007). In the assign_taxonomy.py function, we used a fasta file containing reference sequences from the SILVA 128 QIIME release and the uclust classifier method to map sequences to the assigned taxonomy. The make_otu_table.py script in QIIME was used to generate a square matrix of OTUs x SAMPLES, followed by the single_rarefaction.py script to rarefy each sample to 25000 sequences. The rarefied table was used as input for further analyses. The DNA sequences generated in this study can be downloaded from the NCBI SRA: PRJNA554009.

Analyses

We performed our analyses on the prokaryotic community as a whole and on a subset that only included core Operational Taxonomic Units (OTUs) to assess what types of OTUs varied. In our dataset, an OTU was considered a core OTU when it occurred in each sample. Average relative abundances were calculated per bacterial phylum and class at global (Caribbean vs. Indo-Pacific; >15,000 km) and regional (Curaçao vs. Martinique; 800-1,000 km) scales. Mann-Whitney *U* tests were used to compare whether the abundances of these bacterial phyla and classes in the groups had similar distributions. The Mann-Whitney *U* tests were executed with the wilcox.test-function in the R package *stats*. The significance level was set at 0.05 and the p-values were corrected using the Benjamini-Hochberg procedure with an allowed false discovery rate of 0.1 (Benjamini and Hochberg 1995).

To compare beta diversity, we used principal coordinates analysis, an exploratory method, and mantel tests, an interpretative method (Paliy and Shankar 2016). Principal coordinates analysis is a preferred method in microbial ecology due to the capacity to use community composition measures and phylogenetic distances to calculate similarity or dissimilarity among microbial populations. The mantel test compares the microbial community distance matrix with an additional distance matrix of an independent set of variables. Both techniques complement each other in the analysis of microbial communities (Paliy and Shankar 2016). PCO ordinations were constructed for 'all samples' and for subsets of only 'Caribbean samples', 'Curaçao samples', 'Martinique samples' and 'Thailand samples'. PCO-values were calculated using the square root transformed data of 'all OTUs' and for a subset of 'core OTUs'. For this purpose, we used the ordinate-function in the R package *phyloseq* with the MDS method and Bray-Curtis distance (McMurdie and Holmes 2013). The PCO ordinations of the subset of core OTUs were plotted using different color codes to visualize differences regarding 'host-identity' and 'geography'. For each of the 'localities' (Curaçao, Martinique, Thailand), we also color-coded the samples for different local sites to explore which environmental factors are involved in driving compositional variation in the giant barrel sponge prokaryotic community. Using the adonis-function in the R package *phyloseq*, we performed PERMANOVA-tests comparing the PCO-values with location, host identity and, only for the 'local' subsets, different environmental factors. In Thailand, inshore

locations had a distance of <500 m to shore, and offshore locations of >5 km. In Curaçao, locations were considered city locations when they were located within 1 km of the urban area of Willemstad, the capital city of the island, and non-city locations when they were >20 km from the urban area of the city. In Martinique, samples from shallow reefs were sampled at a depth of <30 m, from deep reefs at a depth of >90 m and shallow caves at a depth of <30 m. We performed these PERMANOVA-tests on the PCO values of 'all OTUs' and of the subsets including only 'core OTUs' to compare which types of OTUs varied. The number of permutations was set at 9999 and the Benjamini-Hochberg Procedure was used as a post hoc test with an allowed false discovery rate of 0.1 (Benjamini and Hochberg 1995).

We tested for significant differences among local sites and host species in individual core OTUs in Curaçao, Martinique, and Thailand with an analysis of deviance using the glm-function in R-package *stats v3.6.0*. We first applied a generalized linear model (GLM) with the family argument set to 'quasibinomial'. In the 'quasibinomial' family, the dispersion parameter is not fixed at one so that it can model over-dispersion. Using the glm models, we tested for significant variation among local sites using the ANOVA-function in R with the F test, which is most appropriate when dispersion is estimated by moments, as is the case with quasibinomial fits.

In order to assess to what extent beta diversity or the variation in composition could be explained by geography, phylogeny or its combination, we constructed Bray-Curtis dissimilarity matrices of the prokaryotic communities using the vegdist-function in the R package *vegan,* geographic distance matrices using the distm-function in the R package *geosphere* with the Haversine-function and genetic distance matrices (phylogeny) using the dist.hamming-function in the R-package *phangorn* (Schliep 2011). The hamming-function is based on Hamming distance, which is a metric describing the minimum number of mutations required to convert one sequence into another sequence (Hamming 1950). For each set of samples, we used the multi.mantel-function from the R-package *phytools* (Revell 2012) to assess the amount of variation jointly explained by spatial and phylogenetic matrices. We then used the mantel.partial-function in the R-package *vegan* to obtain the amount of variation explained by distance only after partialing out the variation explained by phylogeny and the amount of variation explained by phylogeny only after partialing out the variation explained by distance. Through variance partitioning (Borcard et al. 1992), we subsequently obtained the amount of variation explained by distance only, phylogeny only, by distance and phylogeny combined (the spatially structured phylogenetic signal) and unexplained at all spatial scales from global to local.

Lastly, we used the simper-function in the R package *vegan* to identify significantly discriminating OTUs between sites, species, and environments within Curaçao, Martinique and, Thailand with the number of permutations set at 999 (Oksanen et al. 2007).

RESULTS

Dataset

In total, we retrieved 1,825,000 sequences, evenly distributed over 73 giant barrel sponge samples. These sequences were assigned to 8970 OTUs. The OTUs were assigned to 54 phyla, 135 classes and 194 orders (Appendix 5.2). The phylum Proteobacteria was the most abundant and diverse of all phyla with 921,510 sequences assigned to 4687 OTUs. Other diverse phyla included Bacteroidetes (760 OTUs), Planctomycetes (551), Acidobacteria (471), Chloroflexi (467), Actinobacteria (288) and Gemmatimonadetes (216) (Appendix 5.2). Although diverse, Planctomycetes and Bacteroidetes were not abundant with only 835 and 4039 sequence reads, respectively. In contrast, Nitrospirae (76), Cyanobacteria (137) and Tectomicrobia (46) were not diverse, but abundant with 274,497 (15.0% of all sequences), 76982 (4.2%) and 12288 (0.67%) sequence reads, respectively (Appendix 5.2). There was a clear positive relationship between OTU abundance and the number of samples in which the OTU was present (Appendix 5.3). In other words, abundant OTUs were also widespread.

Following our definition, there were 98 OTUs in the core (1.3% of the total number of OTUs), which together accounted for 1,447,732 sequences (79.3% of the total number of sequences; Appendix 5.2). The core consisted of OTUs assigned to the phyla Proteobacteria (63), Actinobacteria (11), Chloroflexi (10), Nitrospirae (7), Cyanobacteria (4), Acidobacteria (1), PAUC34f (1) and SBR1093 (1) (Appendix 5.2). The most abundant core members belonged to multiple bacterial phyla and classes (Appendix 5.4). In our dataset, between 49.8% and 85.5% of the giant barrel sponge prokaryotic community consisted of OTUs that were found in all of the sampled giant barrel sponges (Appendix 5.2).

Differences in total prokaryote community composition across scales

In the PCO analysis, samples from the Caribbean and the Indo-Pacific formed separate clusters (Fig. 5.2a). The first axis, which split the two oceans, explained 18.3% of the variation in the data set. The second axis, which explained 17.2% of the variation, was mostly related to the higher abundances of Chloroflexi (positive PCO values) or Cyanobacteria (negative PCO values) (Fig. 5.2e). The ocean of origin was a significant predictor of prokaryotic community composition and explained 19.6% of the variation in composition (Table 5.1). Dissimilarity between the prokaryotic communities of specimens was larger when they were spatially and genetically further apart (Fig. 5.3a,b). The relative abundances of Nitrospirae and SBR1093 were higher in the Indo-Pacific, whereas Gemmatimonadetes, PAUC34f, and Tectomicrobia were more abundant in the Caribbean (Table 5.2). Although 98 OTUs were present in all of our samples around the globe, 47 additional OTUs were present in all samples from the Caribbean but were not present in all samples from Thailand. An additional 65 OTUs were found in all samples from Thailand, but not in all Caribbean samples (Fig. 5.4). At this global scale, host identity explained more variance than geography (Fig. 5.2c; Table 5.1). This result, however, is inflated due to the fact that no species were shared between the two

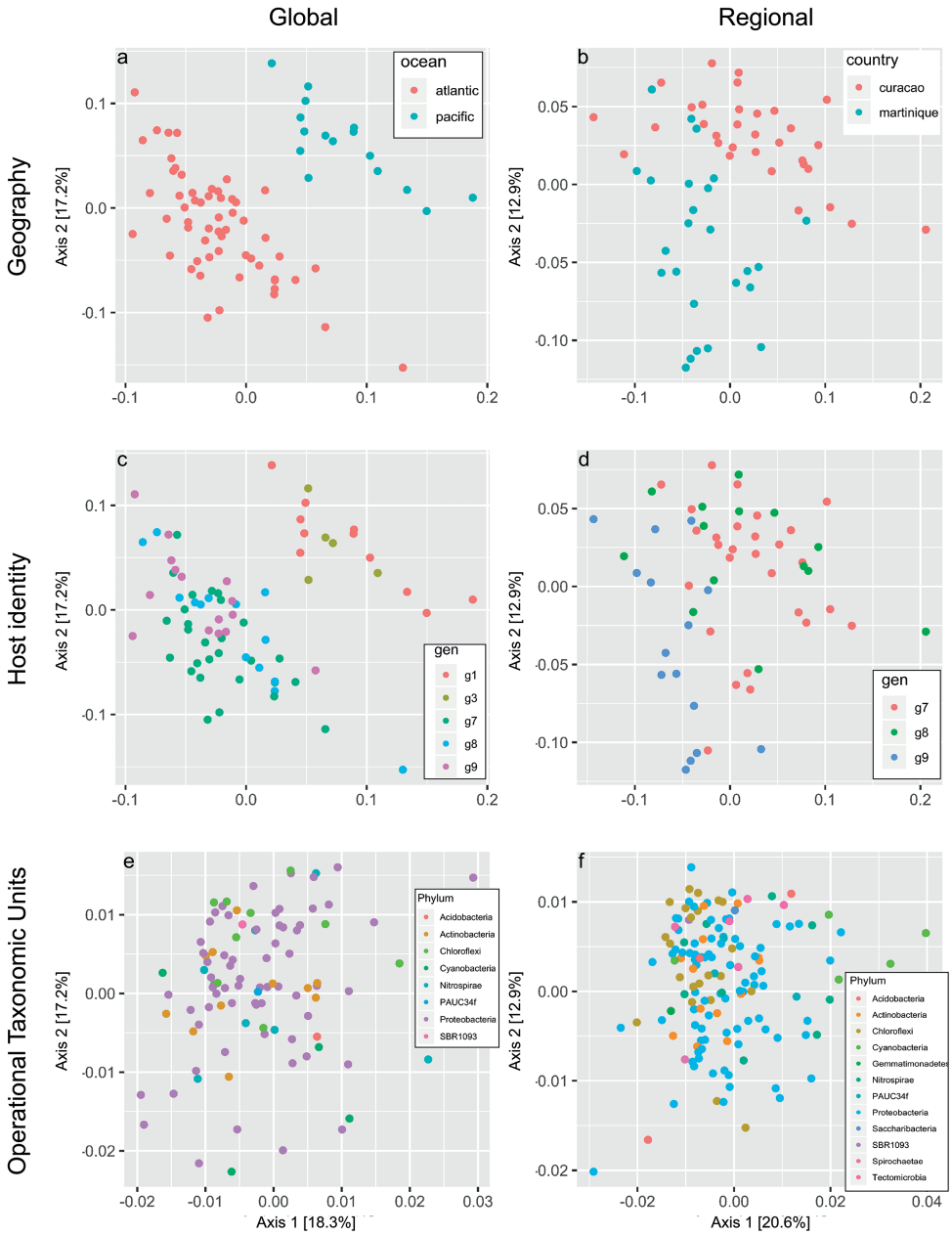


Figure 5.2. First and second axes of the Principal Coordinates Analysis based on our full dataset (a,c,e) and a subset including samples from the Caribbean (b,d,f). Each dot in the (a,b,c,d) graphs represents one sponge individual, and their positioning in the ordination is identical for (a) and (c) and for (b) and (d), the only difference being the color schemes. Colors in (a) indicate the ocean of origin and in (c) the species. Colors in (c) indicate the locality of origin and in (d) the species. In graphs (e) and (f) each dot indicates one core Operational Taxonomic Unit, color coded for phylum.

Table 5.1. Table including the statistical values from PERMANOVA test comparing the Principal Coordinates Analyses with geographic and phylogenetic variation. Asterisks indicate level of significance (* = <0.05; ** = <0.001; * = <0.001) after correcting for the number of tests using the Benjamini-Hochberg procedure with an allowed false discovery rate of 0.1.

	Geography				Phylogeny				Other			
Global	**F-value**	**R^2**	**p-value**		**F-Value**	**R^2**	**p-value**		**F-Value**	**R^2**	**p-value**	
Caribbean vs. Indo-Pacific	17.34	0.196	<0.001	***	6.81	0.286	<0.001	***				
Caribbean vs. Indo-Pacific core	10.51	0.129	<0.001	***	4.81	0.220	<0.001	***				
Regional												
Curaçao vs. Martinique	6.48	0.105	<0.001	***	3.27	0.108	<0.001	***				
Curaçao vs. Martinique core	6.50	0.106	<0.001	***	3.41	0.112	<0.001	***				
Local												
Thailand									**Inshore / Offshore**			
Thailand all	1.62	0.288	0.022	*	2.07	0.129	0.019	*	1.12	0.074	0.312	
Thailand all core	1.72	0.301	0.021	*	2.15	0.133	0.031	*	1.12	0.074	0.307	
Curaçao									**City / Non-city**			
Curaçao all	1.90	0.268	<0.001	***	2.02	0.123	0.017	*	1.37	0.044	0.149	
Curaçao all core	1.77	0.254	0.006	**	2.02	0.122	0.024	*	1.23	0.039	0.222	
Piscadera Bay all					2.13	0.379	0.033	*				
Piscadera Bay core					1.94	0.357	0.065					
Martinique									**Depth (deep / reef / cave)**			
Martinique all	1.21	0.194	0.112		1.34	0.109	0.077		1.76	0.138	0.006	**
Martinique all core	1.23	0.197	0.110		1.32	0.107	0.105		1.85	0.144	0.004	**
Martinique reef	1.63	0.419	0.015	*	1.12	0.170	0.289					
Martinique reef core	1.65	0.423	0.013	*	1.11	0.168	0.318					
Martinique deep all	0.95	0.363	0.555		0.96	0.243	0.543					
Martinique deep core	0.96	0.366	0.536		0.92	0.235	0.585					

oceans, which g1 and g3 occurring in the Pacific and g7, g8 and g9 occurring in the Atlantic. Within each ocean, no clustering by host identity seemed to occur (Fig. 5.2c).

At the regional scale, the giant barrel sponges from Curaçao and Martinique formed two separate clusters, primarily separated by the second axis which explained 12.9% of the variation (Fig. 5.2b). The island of origin was thus a significant predictor of the prokaryotic community composition and explained 10.5% of the variation in composition (Table 5.1). The first axis, which explained 20.6% of the variation was also related to the higher abundances of Chloroflexi (negative PCO values) or Cyanobacteria (positive PCO values) (Fig. 5.2f). Samples from Curaçao housed on average more than twice as many Cyanobacteria as samples from Martinique, while the latter harbored more Gammaproteobacteria and Nitrospinae (Table 5.2). In addition to the 145 OTUs that were present in each of the Caribbean samples, 36 other OTUs were also present in each sample from Martinique and five in each sample from Curaçao (Fig. 5.4). Host identity was also a significant predictor of the prokaryotic community and explained 10.8% of the variation in composition (Table 5.1). Species that were genetically and spatially closer had more similar prokaryotic community compositions (Fig. 5.3c,d). At this regional scale, host identity explained more variance than geography (Table 5.1), but also here this result is, at least partially, overestimated due to the unequal distribution of species among the localities.

In Curaçao, samples clustered by reef site along the first axis, which explained 32.0% of the variation (Fig. 5.5a). The second axis explained 8.7% of the variation and separated samples of the species 'g7' and 'g8' (Fid. 5.5d). The factors site and host identity were significant determinants of prokaryotic community composition (Table 5.1). Accordingly, sponges genetically (but not spatially) further apart had more dissimilar prokaryotic communities (Fig. 5.3e). The impacts of sampling site irrespective of geographic distance suggest that local environmental conditions play a role in structuring the prokaryote community (Fig. 5.3f). However, there was no apparent association between coastal development (i.e., whether a sponge was located near the city of Willemstad or in an area with low coastal development) and the variation in prokaryote composition (Fig. 5.5f; Table 5.1). Instead, the proportion of giant barrel sponge, coral, and algal covers varied among the sampled sites in Curaçao. A high abundance of giant barrel sponge individuals was found on reefs with high soft coral cover, high fleshy and calcareous algal cover, and a lower abundance of giant barrel sponges on reefs with low soft coral cover, low fleshy and calcareous algal cover, and high turf algal cover (Appendix 6.1). These factors, or other local environmental variables, which were not measured, may play a role in structuring the prokaryote community. Zooming in to one local community, at Piscadera Bay, showed that when removing most of the geographic variation, the sponges significantly clustered together based on host identity (Table 5.1; Appendix 5.5).

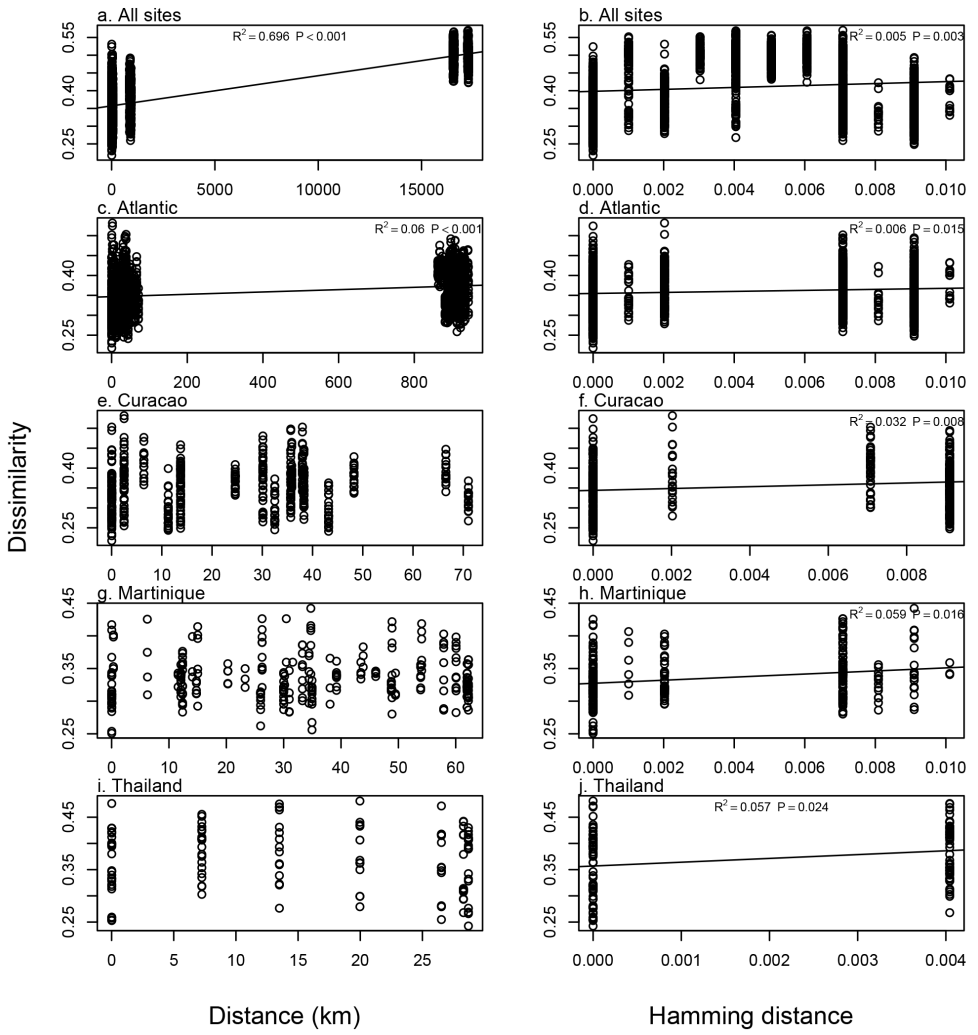


Figure 5.3. Scatterplots of dissimilarity x geographic distance and dissimilarity x genetic distance for 'all samples (global)' and for subsets of only 'Caribbean samples', 'Curaçao samples', 'Martinique samples' and 'Thailand samples'. We added regression lines that were calculated with the lm-function and the method set at "qr" to the plots if they had a significant fit, indicating a positive or negative relationship between geographic or genetic distance and dissimilarity to core prokaryotic community composition.

In Martinique, samples did not cluster according to sampling site or host identity (Fig. 5.5b,e; Table 5.1). Sponges genetically (but not spatially) further apart, however, had more dissimilar prokaryotic communities (Fig. 5.3g,h). Depth, however, proved a significant predictor of variation in prokaryote composition and explained 13.8% of the variation in composition (Fig. 5.5h; Table 5.1). Deeper waters were characterized by greater relative abundances of Nitrospirae, Tectomicrobia, and Spirochaetae, while sponges from shallower waters harbored

Table 5.2. Table including the average abundance of various bacterial phyla and classes in different regions and localities. Asterisks indicate significance level of the p–values after T-tests comparing the average abundance between the regions and localities and correcting for the number of tests using the Benjamini-Hochberg procedure with an allowed false discovery rate of 0.1 (* = <0.05; ** = <0.001; * = <0.001).

	Global			Regional		
Phylum/*Class*	**Caribbean**	**Pacific**		**Curaçao**	**Martinique**	
Proteobacteria	**50.28%**	**51.27%**		**49.05%**	**51.85%**	*
Gammaproteobacteria	34.48%	36.30%		32.71%	36.74%	***
Alphaproteobacteria	8.33%	9.13%		8.14%	8.58%	
Deltaproteobacteria	5.88%	4.48%	**	6.25%	5.41%	
JTB23	1.29%	1.04%		1.58%	0.91%	***
Betaproteobacteria	0.17%	0.09%		0.26%	0.05%	**
Epsilonproteobacteria	0.10%	0.14%	***	0.08%	0.13%	
Actinobacteria	**17.34%**	**17.66%**		**16.04%**	**18.99%**	**
Nitrospirae	**13.74%**	**19.68%**	***	**14.50%**	**12.76%**	
Chloroflexi	**6.94%**	**5.36%**	**	**7.28%**	**6.50%**	*
Caldilineae	4.01%	3.77%		4.15%	3.84%	
SAR202 clade	2.10%	1.09%	***	2.22%	1.93%	
Anaerolineae	0.61%	0.36%		0.67%	0.54%	
TK10	0.17%	0.12%		0.18%	0.15%	
Cyanobacteria	**4.84%**	**2.00%**	*	**6.34%**	**2.92%**	***
Gemmatimonadetes	**2.30%**	**0.58%**	***	**1.99%**	**2.69%**	*
Acidobacteria	**1.54%**	**1.36%**		**1.84%**	**1.16%**	
Holophagae	1.42%	1.22%		1.70%	1.06%	
Solibacteres	0.06%	0.07%		0.06%	0.06%	
Tectomicrobia	**0.86%**	**0.02%**	***	**0.78%**	**0.95%**	
Saccharibacteria	**0.36%**	**0.60%**		**0.39%**	**0.33%**	
PAUC34f	**0.46%**	**0.14%**	***	**0.50%**	**0.42%**	
Spirochaetae	**0.37%**	**0.33%**		**0.31%**	**0.45%**	**
SBR1093	**0.20%**	**0.31%**	**	**0.21%**	**0.20%**	
Bacteroidetes	**0.22%**	**0.22%**		**0.20%**	**0.25%**	
Bacteroidetes Incertae Sedis	0.10%	0.03%	***	0.07%	0.15%	
Flavobacteriia	0.07%	0.12%		0.05%	0.08%	
Poribacteria	**0.13%**	**0.05%**	*	**0.16%**	**0.11%**	
Nitrospinae	**0.09%**	**0.06%**		**0.06%**	**0.11%**	***
Parcubacteria	**0.08%**	**0.05%**	**	**0.10%**	**0.06%**	

more Cyanobacteria. When separating the dataset into shallow and deep locations, sampling site became a significant predictor of the prokaryotic community in shallow reef sponges, but not in deep reef sponges (Table 5.1). Host identity was not a significant driver at either depth range (Table 5.1).

In Thailand, samples from the four sites were separated by the first PCO axis (which explained 23.6% of the variation), although there was overlap among samples from different sites

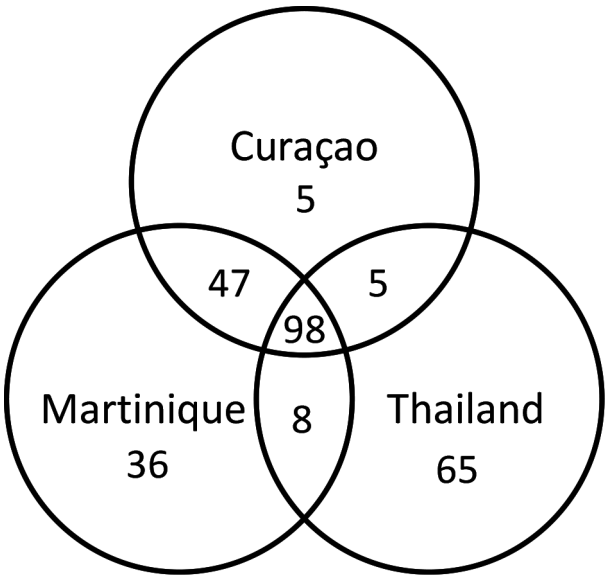


Figure 5.4. Venn-diagram indicating the number of core OTUs that are shared between Curaçao, Martinique and Thailand, or are unique to one of the sites.

(Fig. 5.5c). The second axis, which explained 18% of the variation, split the two species, although one sample from species g3 did not follow this pattern (Fig. 5.5f). Both sample site and host identity were significant predictors of the prokaryotic community and explained 28.8% and 12.9% of the variation in composition, respectively (Table 5.1). As in Curaçao, sponges spatially further apart did not have more dissimilar prokaryotic communities, suggesting that the sample site is a determinant of prokaryotic community composition due to local differences in environmental conditions that appear unrelated to geographic distance (Fig. 5.3i). Samples genetically further apart did have more dissimilar prokaryotic community compositions (Fig. 5.3j). Whether the sample originated in an inshore or offshore location did not appear to affect prokaryote composition (Fig. 5.5i; Table 5.1).

Patterns for the core community compared to the total community

Core and total prokaryote communities largely followed the same trends with respect to sample site, host identity or other drivers. The only exception was the subset of samples from Piscadera Bay in Curaçao, which showed a significant impact of host identity on the total prokaryote community, but not on the core community (Table 5.1). This subset, however, only consisted of ten samples, and this small number may have caused the lack of statistical support in the core community. Nonetheless, this general congruence of patterns between the core and total prokaryotic community indicates that the drivers not only affected the presence or absence of OTUs, but that they also affected the relative abundance of core OTUs.

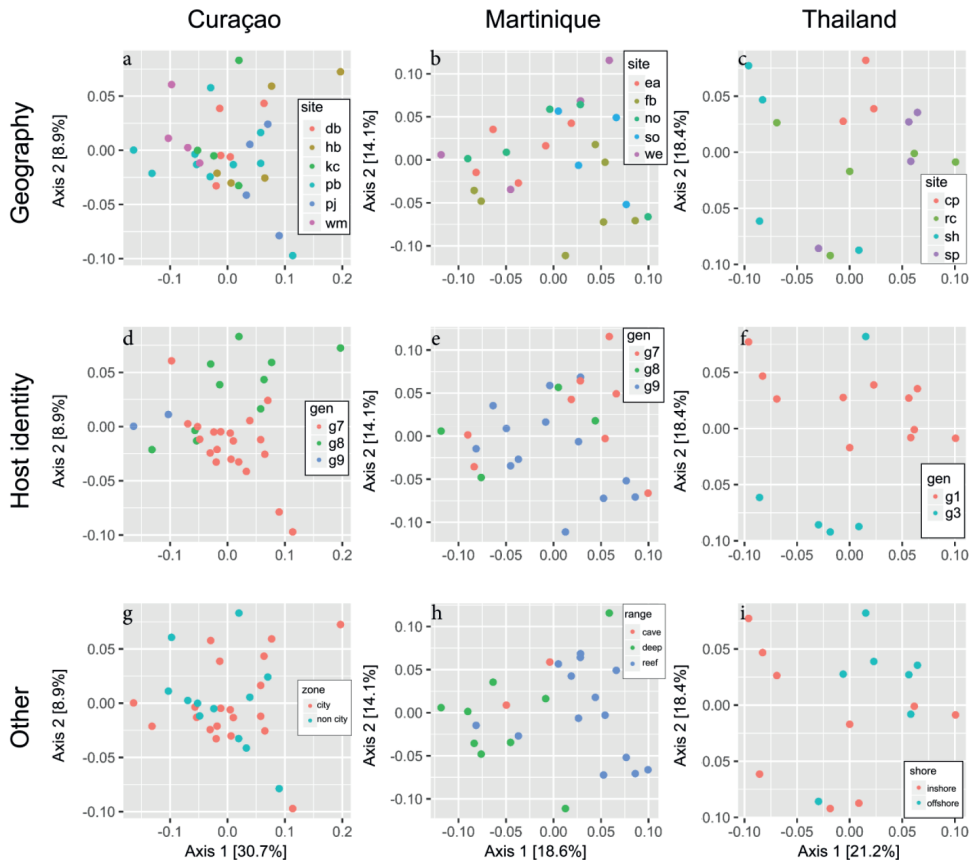


Figure 5.5. First and second axes of the Principal Coordinates Analyses based on the subsets subset including samples from Thailand (a,b,c), Curaçao (d,e,f) and Martinique (g,h,i). In each graph one dot represents one sponge individual, and their positioning in the ordination is identical for the graphs of Thailand (a,b,c), Curaçao (d,e,f) and Martinique (g,h,i), the only difference being the color schemes. Colors in (a), (d) and (g) indicate the site at which the specimens were sampled. Abbreviations of the sites are: cp = Chumpon Pinnacle, rc = Twin Rocks, sh = Shark Island, sp = Southwest Pinnacle, db = Director's Bay, hb = Holiday Beach, kc = Klein Curaçao, pb = Piscadera Bay, pj = Plaja Jeremy, wm = Watamula, ea = East, no = North, so = South, sw = Southwest, fb = Fort-De-France Bay. Colors in (b), (e) and (h) indicate the species identity of the host. Colors in (c) urban and rural environments. Colors in (f) indicate depth category or cave environment. Colors in (i) indicate whether the sponge sampled was from an inshore or offshore reef.

Core OTUs that differed with sample site and host identity

The abundance of some individual core OTUs in the localities Curaçao, Martinique and Thailand varied significantly with sample site and/or host identity based on the SIMPER analyses. In Curaçao, 2.6-19.0% of the core OTUs varied in abundance between two individual sites and 17.0-23.5% between two individual host species. In Martinique, these abundances varied between 4.4-9.9% and 7.2-7.7% respectively, and in Thailand, 4.9-9.8% and 17.2%.

Table 5.3. Table showing the amount of variation explained by 'distance only', 'phylogeny only', 'by distance and phylogeny combined (the spatially structured phylogenetic signal)' and 'unexplained' at all spatial scales from global to local according to our mantel tests. Asterisks indicate level of significance (* = <0.05; ** = <0.001; * = <0.001).

			F	R2	Pr(>|t|)	
All	Multi-mantel		2972.62	0.7	0.001	***
	Partial mantel	Distance only		0.696	0.001	***
		Phylogeny only		0.005	0.003	**
		Distance + Phylogeny		-0.002		
Atlantic	Multi-mantel		57.15	0.069	0.001	***
	Partial mantel	Distance only		0.06	0.001	***
		Phylogeny only		0.006	0.015	*
		Distance + Phylogeny		0.002		
Thailand	Multi-mantel		3.829	0.061	0.052	
	Partial mantel	Distance only		0	0.657	
		Phylogeny only		0.057	0.024	*
		Distance + Phylogeny		0.005		
Curaçao	Multi-mantel		9.46	0.037	0.025	*
	Partial mantel	Distance only		0	0.127	
		Phylogeny only		0.032	0.008	**
		Distance + Phylogeny		0.005		
Martinique	Multi-mantel		9.075	0.062	0.024	*
	Partial mantel	Distance only		0	0.176	
		Phylogeny only		0.059	0.016	*
		Distance + Phylogeny		0.004		

Eight OTUs varied significantly between at least two individual reef sites in each of the three localities. These OTUs were assigned to Proteobacteria (4), Nitrospira (2), SBR1093 (1) and Acidobacteria (1). OTU-108, a member of the order HOC36 within the Gammaproteobacteria, was the only core OTU that differed significantly with host identity in all three localities. This OTU was especially abundant in 'species g3' from Thailand and 'species g9' from Curaçao and Martinique, two genetically closely related species.

Twenty-six of the 181 core OTUs in Martinique significantly discriminated samples from different depths according to a SIMPER analysis. These OTUs were assigned to the Proteobacteria (16), Actinobacteria (3), Nitrospirae (2), Bacteroidetes (2), Chloroflexi (1), Spirochaetae (1) and Tectomicrobia (1).

Assessing the importance of distance and phylogeny using distance matrices

On a global scale there was a highly significant difference in composition between the total prokaryotic communities in different oceans. Geographic distance alone (the difference between sample sites after removing the variation attributable to phylogeny) was a significant predictor of compositional variation and explained 69.6% of this variation (Table 5.3; Fig. 5.3). Although phylogeny, when considered alone, was also a significant predictor, it only explained 0.5% of the variation in composition. The spatially structured phylogenetic component explained none of the variation in composition. At the regional scale, distance alone was also a significant predictor of compositional variation, but only explained 6% of the variation. Phylogeny alone was also a significant predictor at the regional scale, but only explained 0.6% of the variation with the spatially structured phylogenetic component explained 0.2% of the variation. In contrast to the global and regional scales, geographic distance was not a significant predictor of compositional variation between local sites in any of the three locations (Curaçao, Martinique, and Thailand). In contrast, the phylogeny alone component was a significant predictor at all three locations and explained between 3.2 and 5.9% of the variation in composition. The spatially structured phylogenetic component explained between 0.4 and 0.5% of the variation in composition.

DISCUSSION

The importance of geography vs phylogeny across scales

In the present study, we found that distance was a highly significant predictor of compositional variation in giant barrel sponge prokaryote communities at the global scale (distances >15,000 km) (Fig. 5.6). This finding is in line with a number of studies highlighting the importance of spatial processes in structuring communities of macrobes and microbes in sponges (e.g. Fiore et al. 2013a; Luter et al. 2015; Lesser et al. 2016; Morrow et al. 2016; Chapter 4 of this thesis). At the regional scale (distances 800-1,000 km), distance also proved a significant predictor of compositional variation albeit explaining much less variation (Fig. 5.6). At the local scale (distances 2-70 km), geographic distance did not contribute significantly to variation in the prokaryotic composition (Fig. 5.6). This pattern contrasts starkly with similar studies of terrestrial (Condit et al. 2002; Cleary et al. 2004; Cleary and Priadjati 2005; Keil et al. 2012) and marine (Becking et al. 2006; Watson et al. 2011) plants and animals, and marine microeukaryote communities (Zhang et al. 2018). These studies showed a rapid increase in dissimilarity at very small spatial scales followed by a very long tail where there was relatively little change in dissimilarity. One of the reasons for this relative lack of change is that similar environments are encountered at greater distances, for example, multiple mountain tops are encountered with similar communities. This relative lack of change is hypothesized to continue until, at very large scales, biogeographical barriers or climatic gradients further increase dissimilarity (Nekola and White 1999). The existence of a significant biogeographical barrier between Caribbean and Indo-Pacific populations of

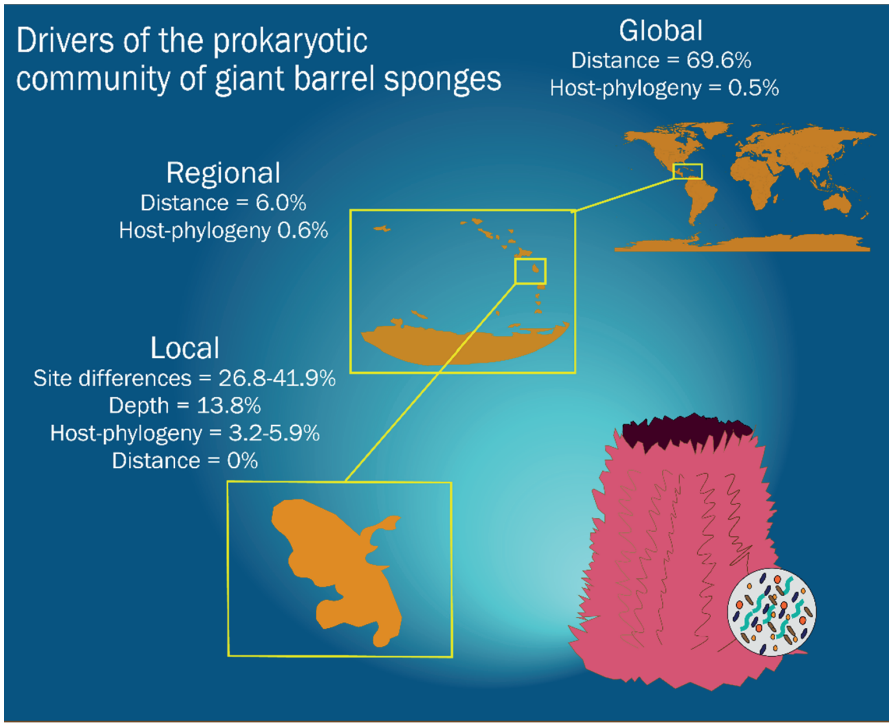


Figure 5.6. Overview of drivers of the prokaryotic community of giant barrel sponges (*Xestospongia* spp.) on different spatial scales.

giant barrel sponges is the most probable explanation for the pronounced distance-decay at a global scale. Although a biogeographical barrier is less obvious at a regional scale within the Caribbean basin, the significant geographic differences between Curaçao and Martinique might indicate that connectivity between Caribbean islands is somewhat more restricted than previously thought.

In contrast to the above, phylogenetic differences among host species explained only a very small amount of variation in composition at regional and global scales, after removing the spatial component. However, they were a more important determinant of compositional variation at local scales. These results indicate that the relative contribution of drivers such as distance and host phylogeny can shift at different spatial scales. Furthermore, other environmental parameters, for example, depth at local scales, can drive prokaryote community composition and obfuscate variation related to other spatial factors and host identity.

We used principal coordinates analyses and distance matrices to study the impact of geography and phylogeny on the prokaryote composition. Both techniques are

complementary and reveal different components of prokaryote community composition (Paliy and Shankar 2016). When geographic variation is inextricably entangled with variation in species identity, for example, it is difficult to infer to which driver the observed variation is truly related to (Marino et al. 2017). The co-variation in geography and host-phylogeny also affects the interpretation of drivers for giant barrel sponges, especially at the larger spatial scales. For example, giant barrel sponges from the Caribbean and the Indo-Pacific exist in different oceans and are assigned to different species. This can easily lead to the drawing of inaccurate conclusions. For example, Montalvo and Hill (2011) concluded that *X. muta* and *X. testudinaria* had different microbial communities due to being different species, but with the recent knowledge of their more complicated phylogeny, it has become clear that the differences in their microbial communities more likely reflect the different oceans they were sampled in (Chapter 4 of this thesis), although host identity is still expected to play a role. In the present study, we used distance matrices to show that distance alone, after removing the variation due to phylogeny, explained almost 70% of the variation in composition while phylogeny alone, after removing the impact of distance, explained less than 1%. Phylogeny seems to only affect specific prokaryotes such as OTU-108, a member of the order HOC36 that differed strongly with host identity in all three localities. This OTU was especially abundant in species g3 from Thailand and species g9 from Curaçao and Martinique. These genetic groups are both characterized by mitochondrial haplotype C5 and share a more common ancestor with one another than with any of the other genetic groups (Chapter 2 of this thesis).

At the smallest spatial scales, e.g., in Curaçao and Thailand, variation in the prokaryotic community was not related to distance, but rather due to differences among sampling sites. We were not able to identify which environmental variables were responsible for the differences. In Martinique, however, the differences between the prokaryotic communities were strongly correlated with depth. Depth-related shifts in the microbial community structure of giant barrel sponges have been previously detected at a depth range from 10 to 90 meters in sites surrounding the island Little Cayman in the Caribbean (Morrow et al. 2016). There, the researchers found a reduction in photosynthetic Cyanobacteria with depth, combined with an increase in Nitrospira. Although no cyanobacterial core OTUs and only two nitrospiral core OTUs differed with depth in Martinique, depth-related shifts for these taxa as a whole (i.e. including non-core OTUs) were also observed in this study. At first glance, this seems a perfect example of how different environmental conditions in deep *versus* shallow waters can structure prokaryotic communities. However, if only depth was important, we would expect shallow sponges from Martinique to be more similar to shallow sponges from other regions, like Curaçao, and this is not what we observed. Therefore, it is not merely the environmental differences related to depth that structure the distinct prokaryotic communities, but other factors too. Our results indicate that local site differences affect the abundance of bacterial phyla as a whole, rather than a change

in the relative abundance of various OTUs within each phylum that still add up to similar abundances at each location. Chlorofexi, Cyanobacteria, and Nitrospira are among the main phyla whose abundance varies strongly among locations. Chloroflexi are believed to play a key role in the degradation of dissolved organic matter from seawater (Bayer et al. 2018), Cyanobacteria are photosynthesizers (Burgsdorf et al. 2015) and members of the Nitrospira play a pivotal role in nitrification (Daims and Wagner 2018). The abundance of these phyla likely depends on the local availability of different resources.

The importance of drivers on the core community

Most of the observed patterns in the prokaryotic community of giant barrel sponges in relation to geographical variation, depth, and host identity were also present in the subset of core OTUs. This means that the variation is not necessarily only caused by the presence or absence of region-, depth range- or species-specific OTUs, but also by different relative abundances of core OTUs. So far, most evidence for environmental parameters that shape sponge microbiomes is derived from measured variation in the non-core microbial community (Schmitt et al. 2012; Pita et al. 2018). Differences in the presence and absence of certain non-core OTUs can be explained by the sponge hosts' ability to differentiate between alien and associated microbes, likely through the inherited immune system (Wilkinson et al. 1984; Wehrl et al. 2007). However, this concept fails to explain the differences in the relative abundances of core OTUs, which have to be regulated by different processes. The varying relative abundances of core OTUs between different giant barrel sponge species may be actively managed by the host sponge to fit specific environmental conditions or be a result of structural differences (such as pores, channels, choanocytes, etc.) between the giant barrel sponge species, which have yet to be identified.

In five different sponge species, Thomas et al. (2016) found a core community of seven to twenty OTUs per species, whereby they used a less stringent definition of a core OTU than we used (any OTU present in ≥ 85% of replicates). Despite our more stringent definition of a core OTU (any OTU present in 100% of replicates), by combining five closely related species distributed over multiple oceans, we found a core prokaryotic community that was five to fourteen times larger. This shows that the core prokaryotic community of giant barrel sponges is rich compared to other sponge species.

Implications for the functioning of coral reef ecosystems

This study illustrates the complex dynamics of the drivers that structure the composition of the giant barrel sponge prokaryotic community. It is important to translate how these complex dynamics cause differences in holobiont functioning and their cascading effects in the surrounding ecosystem (Pita et al. 2018). For example, the sponge holobiont can simultaneously perform nitrification and denitrification. The relative number of nitrifying microbes dictates whether the sponge acts as a source or sink of bioavailable nitrogen, often

one of the limiting resources in a marine ecosystem (Fiore et al. 2013b; 2015). With regards to photosynthesis, Cyanobacteria have a stronger association with giant barrel sponges in more oceanic environments or at elevated pCO_2 levels (Fiore et al. 2013b; Morrow et al. 2015; Lesser et al. 2016). Thus, the contribution of Cyanobacteria to the primary production of the ecosystem depends on the local environment of the sponge host (Wilkinson 1983). Besides changing the biochemistry, giant barrel sponges may also directly affect the prokaryotic community of sponge denizens or mobile organisms interacting with the sponges (Chapter 6 of this thesis).

Furthermore, the importance of depth and local site differences shows the flexibility of the prokaryote community of giant barrel sponges. Flexibility in host-symbiont interactions may translate into adaptability to environmental change, potentially making them more resilient or even acclimatized to such changes than other benthic coral reef organisms (Prazeres et al. 2017; Pita et al. 2018). The microbiome of corals, for example, is inflexible and adaptable (Pogoreutz et al. 2018). If the sponge holobiont is indeed better equipped to adapt to climate change, their increasing abundance on reefs worldwide may further accelerate (Bell et al. 2013; Deignan et al. 2018).

Pharmaceutical potential

The recent improvement of tools to study chemical compound profiles in sponges enables the identification of OTUs whose presence correlates with the production of such chemical compounds (Bayona et al. 2018). For example, Caribbean giant barrel sponges from the same location have been shown to harbor different sterol compositions (Fromont et al. 1994), and this chemical diversity may potentially be linked to OTUs that vary with host identity. As bacterial taxa in the sponge prokaryotic community can be influenced by different drivers, the production of chemical compounds can be similarly influenced by those drivers. It is especially appealing to try to identify (groups of) OTUs that are related to the production of secondary metabolites with pharmaceutical potential, such as members of the *Entotheonella,* which have been found in other marine sponges such as *Theonella swinhoei* and *Discodermia calyx* (Wilson et al. 2014; Wakimoto et al. 2014). This study shows that taking only host identity into account in such efforts may result in overlooking these OTUs and that spatial variation may also be important.

Conclusion

Considering the close and long-existing relationship between sponges and their microbial symbionts, we understand remarkably little about how this symbiosis is shaped by multiple drivers. The present study shows how the relative importance of drivers of microbial variation in sponges may shift across different spatial scales. We show that environmental drivers predominate at regional to global scales, while host identity is the dominant driver of prokaryote communities at local scales. With these findings, we expect that shortcomings in

the fundamental understanding of how these microbial communities develop can be solved and that the large pharmaceutical potential of sponges, in which their microbial symbionts are thought to play an essential role, can be better utilized.

ACKNOWLEDGEMENTS

We would like to thank Dr. Mark Vermeij of CARMABI and Roy Belderok for their support during field work in Curaçao. We thank Niels van der Windt for his advice about the field work in Thailand. We are grateful to the Muséum National d'Histoire Naturelle (MNHN) who invited us to participate in the MADIBENTHOS expedition, Martinique 2016 (Principal Investigator Philippe Bouchet). This work is part of the research programmes NWO-VIDI with project number 16.161.301 and ASPASIA (015.010.030), which are both (partly) financed by the Netherlands Organisation for Scientific Research (NWO). Furthermore, the fieldwork was supported by De Treubmaatschappij (Treub 2016b_De Voogd).

APPENDIX 5.1.

Detailed description of the sites in Curaçao, Martinique and Thailand.

Caribbean - Curacao

Curaçao is a Caribbean island that lies approximately 65 kilometers north of the Venezuelan coast. The Curaçaoan landmass is elongated in shape, with a long, sandy southern shoreline, and a rocky, long northern shoreline. The local bathymetry and oceanographic conditions mean that the southern side of the island hosts calm waters whose currents tend to run from East to West, whilst the northern coast has much rougher waters. The southern shoreline is more subject to human impact and is most populated in the capital city of Willemstad (population: 140 000), located to the Southeast of the island. The urbanization introduces large quantities of nutrients into the adjacent waters. It is expected that eutrophication diminishes as distance from the urban area decreases. The rest of the landmass is sparsely populated, with the eastern tip consecrated purely to nature conservation.

The Curacaoan section of the project involved sampling from six sites: Watamula (WM), Klein Curaçao (KC), Playa Jeremi (PJ), Piscadera Bay (PB), Holiday Beach (HB), and Director's Bay (DB). Klein Curaçao is a remote, uninhabited island with a leeward side with relatively high, yet patchy coral cover (Fig. 5.1b; Waitt Institute Report 2016). Director's Bay is a reef near a bay inlet with relatively high coral cover but relatively limpid water. Holiday Beach is a reef in a zone with high pressure from Willemstad, yet with relatively high coral cover, and low to medium turbidity. The reefs at Piscadera Bay receive considerable tidal sedimentation from the mangrove-lined bay. Playa Jeremi is a relatively secluded area, with intermediate to low coral cover and low turbidity. Watamula is located on the tip of the island, has a high abundance of giant barrel sponges, and intermediate to low coral cover. It is a site where the currents from the North and South sides mix. It is expected that Holiday Beach, Director's Bay, and Piscadera Bay were most affected by urbanization and associated eutrophication.

Caribbean - Martinique

Martinique is a volcanic island in The Lesser Antilles, stretching 70 km in length and 30 km in width (Fig. 5.1c). The five sampled sites around the island each have unique characteristics, derived from the surrounding environment. The northern part (NO) of the island is mountainous, heavily forested and catches most of the rainfall. The South (SO) is drier and more densely populated. Here 'Diamond Rock' is located, a small basalt island. Underwater is a ~30 m long tunnel, ending in a large overhang covered with sponges, 0 to 15 m deep, providing semi-dark conditions. The East (EA), or windward side, of Martinique, is exposed to the Atlantic Ocean and characterized by shallow coral reefs and cays. The West (WE), or leeward, side, is exposed to the Caribbean Sea and more sheltered. The reef slopes here descend steeply from the shore. The Fort-de-France Bay (WB) is a large inlet of the Caribbean Sea. The capital city of the island, Fort-de-France lies along the bay's northern coastline, but

most of the coastline is covered by extensive mangroves. The bay is shallow (<10 m) and very turbid. Deep samples (> 90 m) are collected in close proximity to the shallow samples, but usually further offshore.

Indo-Pacific - Thailand

Koh Tao is a small island in the Gulf of Thailand, approximately 60 kilometers from the mainland. It is a popular touristic destination, and sometimes called the diving capital of the world (Weterings 2011). Koh Tao has many regularly visited dive sites all around the island and a few sites that are more distant. In this study we compared two inshore sites, Shark Island (SH) and Twin Rocks (RC), with two offshore sites, Chumphon Pinnacle (CP) and Southwest Pinnacle (SP) (Fig. 5.1d). The sponges were collected from depths up to 25 meters.

Shark Island is a small uninhabited island situated in the Southeast of Koh Tao, approximately 300 m of the coast. Strong currents run along the fringes of the island, often resulting in heavy swell. The reef surrounding the island stretches from 5 until 25 meters deep, forming a mountainous underwater landscape. Twin Rocks lies northwest of Koh Tao, at a distance of approximately 0.5 km from the main island. Chumphon Pinnacle is a submerged pinnacle located around 8 km northwest of Koh Tao. The bottom of the pinnacle is located at 35 m depth and the pinnacle reaches its highest point at 14 m depth. Southwest Pinnacle includes of a series of seven submerged pinnacles approximately 8 km southwest of Koh Tao. The pinnacles start at a depth of 25-30 m and the shallowest point is reached at 5 m below the sea surface.

APPENDIX 5.2

Table 1. General information on phyla, classes and orders

Phylum	Seqs	OTUs	Class
Proteobacteria	921510	4687	Gammaproteobacteria
Actinobacteria	318055	288	Acidimicrobiia
Nitrospirae	274497	76	Acidimicrobiia
Chloroflexi	120321	467	Nitrospira
Cyanobacteria	76982	137	Alphaproteobacteria
Gemmatimonadetes	35052	216	Deltaproteobacteria
Acidobacteria	27426	471	Cyanobacteria
Tectomicrobia	12288	46	Caldilineae
Saccharibacteria	7511	63	BD2-11 terrestrial group
PAUC34f	7174	67	SAR202 clade
Spirochaetae	6614	128	Holophagae
SBR1093	4140	26	JTB23
Bacteroidetes	4039	760	uncultured bacterium
Poribacteria	2133	30	Anaerolineae
Nitrospinae	1459	24	uncultured Candidatus Saccharibacteria bacterium
Parcubacteria	1367	62	Spirochaetes
Planctomycetes	835	551	Ambiguous_taxa
Thaumarchaeota	691	19	TK10
Verrucomicrobia	585	160	uncultured delta proteobacterium
Firmicutes	529	148	Betaproteobacteria
Woesearchaeota (DHVEG-6)	431	30	Unassigned
Tenericutes	323	40	Epsilonproteobacteria
Marinimicrobia (SAR406 clade)	234	35	Bacteroidetes Incertae Sedis
Euryarchaeota	111	20	Flavobacteriia
Lentisphaerae	106	40	MD2898-B26
Deferribacteres	102	43	Solibacteres
Latescibacteria	89	64	Marine Group I
Chlamydiae	65	56	Subgroup 9
Peregrinibacteria	42	21	S085
Fusobacteria	35	12	ARKDMS-49
Ignavibacteriae	32	9	Cytophagia
Gracilibacteria	29	18	Clostridia
Omnitrophica	26	25	PAUC43f marine benthic group
Elusimicrobia	25	10	Mollicutes
Fibrobacteres	25	23	Sphingobacteriia
Chlorobi	15	11	Bacteroidia
Deinococcus-Thermus	12	9	Phycisphaerae
Unassigned	12	9	Planctomycetacia
Hydrogenedentes	11	9	Actinobacteria
BRC1	11	11	OPB35 soil group
TM6 (Dependentiae)	10	9	OM190

Seqs	OTUs	Order	Seqs	OTUs
636515	2170	uncultured	558023	303
317680	233	Acidimicrobiales	317680	233
317680	233	Nitrospirales	274497	76
274497	76	Desulfurellales	93123	109
155230	849	uncultured bacterium	93036	961
101746	1472	SubsectionI	75695	45
76923	102	Caldilineales	72299	84
72299	84	Rickettsiales	67365	122
34663	159	Rhodospirillales	51831	329
34208	188	KI89A clade	34342	185
25090	215	Rhodobacterales	32278	126
22508	36	Subgroup 10	25013	179
17240	285	Xanthomonadales	21402	184
10189	107	Unassigned	18336	522
7450	40	Ambiguous_taxa	11152	191
6614	128	Anaerolineales	10189	107
5632	52	uncultured Candidatus Saccharibacteria bacterium	7450	40
2881	36	HOC36	6771	53
2802	8	Oceanospirillales	6649	335
2736	62	Spirochaetales	6614	128
2086	138	Bdellovibrionales	3086	295
1970	29	Vibrionales	2847	88
1584	62	uncultured delta proteobacterium	2802	8
1396	254	Nitrosomonadales	2657	38
1266	18	Rhizobiales	2655	122
1106	44	uncultured Chloroflexi bacterium	2378	19
691	19	Cellvibrionales	2089	332
679	21	Campylobacterales	1970	29
646	8	Oligoflexales	1906	95
458	8	Order II	1552	53
426	151	Flavobacteriales	1396	254
390	122	Alteromonadales	1370	148
338	36	SubsectionIII	1201	42
323	40	Solibacterales	1106	44
304	209	NB1-j	892	276
286	55	Desulfobacterales	822	197
228	132	Unknown Order	791	68
228	136	SAR324 clade(Marine group B)	747	34
224	34	Gammaproteobacteria Incertae Sedis	563	114
215	18	SAR11 clade	462	6
210	153	Myxococcales	436	242

Table 1. (continued)

Phylum	Seqs	OTUs	Class
RBG-1 (Zixibacteria)	10	8	uncultured euryarchaeote
Aminicenantes	9	7	Subgroup 22
LCP-89	5	5	MD2896-B214
WS2	4	3	Blastocatellia
Armatimonadetes	3	3	SPOTSOCT00m83
WA-aaa01f12	3	3	Opitutae
Synergistetes	3	2	Thermoleophilia
Acetothermia	2	2	Bacilli
Candidatus Berkelbacteria	2	2	Verrucomicrobiae
AC1	1	1	Thermoplasmata
FCPU426	1	1	uncultured archaeon
Cloacimonetes	1	1	Subgroup 6
SR1 (Absconditabacteria)	1	1	Deferribacteres Incertae Sedis
Caldiserica	1	1	Chlamydiae
			Oligosphaeria
			vadinHA49
			Pla4 lineage
			Lentisphaeria
			Gemmatimonadetes
			R76-B128
			Pla3 lineage
			AEGEAN-245
			Tectomicrobia Incertae Sedis
			Proteobacteria Incertae Sedis
			Fusobacteriia
			Ignavibacteria
			ML635J-21
			Melainabacteria
			pltb-vmat-80
			Ardenticatenia
			WCHB1-41
			Elusimicrobia
			Dehalococcoidia
			Bacteroidetes BD2-2
			uncultured organism
			Subgroup 26
			KD4-96
			Fibrobacteria
			JG30-KF-CM66
			BD7-11
			Deferribacteres
			Subgroup 2
			Candidatus Campbellbacteria

Seqs	OTUs	Order	Seqs	OTUs
208	9	Cytophagales	426	151
192	122	Desulfovibrionales	420	48
186	2	Thiotrichales	419	64
172	7	E01-9C-26 marine group	400	43
167	16	Clostridiales	373	108
155	37	Chromatiales	306	50
140	13	Sphingobacteriales	304	209
128	18	Bacteroidales	278	50
122	52	Planctomycetales	227	135
111	20	uncultured euryarchaeote	208	9
108	6	Bradymonadales	193	90
99	29	BD7-8 marine group	182	10
85	40	Mycoplasmatales	180	13
65	56	Phycisphaerales	176	92
58	20	Blastocatellales	172	7
49	34	Arenicellales	155	34
49	42	PeM15	147	4
48	20	Gaiellales	137	11
48	18	Kordiimonadales	130	14
43	18	Puniceicoccales	127	27
42	28	uncultured archaeon	122	8
41	9	Verrucomicrobiales	122	52
37	6	Legionellales	121	63
36	6	Sphingomonadales	119	13
35	12	Thermoplasmatales	111	20
32	9	Bacillales	108	9
31	20	Entomoplasmatales	104	8
28	15	Parvularculales	103	16
27	7	Alphaproteobacteria Incertae Sedis	103	49
27	18	Caulobacterales	91	23
26	23	Chlamydiales	65	56
25	10	uncultured organism	63	49
24	11	Methylococcales	61	8
23	16	Subgroup 23	52	17
21	19	Salinisphaerales	49	11
20	7	Gemmatimonadales	48	18
20	2	Methylophilales	48	6
20	18	P.palmC41	46	11
18	5	Desulfarculales	40	27
18	18	Sva0071	37	11
17	3	Victivallales	36	13
16	3	Corynebacteriales	36	12
15	7	Fusobacteriales	35	12

Table 1. (continued)

Phylum	Seqs	OTUs	Class
			Chlorobia
			Subgroup 17
			Arctic97B-4 marine group
			Deinococci
			Subgroup 13
			Subgroup 21
			MACA-EFT26
			JdFBHP3
			Erysipelotrichia
			Skagenf62
			Subgroup 11
			Coriobacteriia
			Nitrospinia
			Spartobacteria
			Candidatus Falkowbacteria
			Chitinivibrionia
			Candidatus Adlerbacteria
			AT-s3-28
			Bacteroidetes vadinHA17
			OPB41
			Subgroup 18
			WCHB1-32
			Candidatus Moranbacteria
			Fimbriimonadia
			Synergistia
			Ktedonobacteria
			Chloroflexia
			Nitriliruptoria
			Belgica2005-10-ZG-3
			MD2902-B12
			uncultured crenarchaeote
			028H05-P-BN-P5
			ML602M-17
			Verrucomicrobia Incertae Sedis
			Candidatus Peribacteria
			Milano-WF1B-44
			Bacteroidetes VC2.1 Bac22
			Unknown Class
			SJA-15
			S0134 terrestrial group
			Negativicutes
			MD2896-B258
			Candidatus Azambacteria

Seqs	OTUs	Order	Seqs	OTUs
15	11	Sva0485	35	21
14	8	Order III	32	9
12	3	Ignavibacteriales	32	9
12	9	NB1-n	30	12
12	1	Aeromonadales	28	9
10	4	Sneathiellales	26	4
9	3	Acidithiobacillales	26	5
9	2	Propionibacteriales	25	9
9	6	Acanthopleuribacterales	24	18
7	2	CCM11a	24	21
7	4	uncultured Acidobacteria bacterium	21	17
6	4	Lactobacillales	20	9
5	2	Fibrobacterales	20	18
5	4	Deferribacterales	17	3
5	5	Syntrophobacterales	17	13
5	5	Hydrogenophilales	16	7
4	2	Halanaerobiales	16	13
4	3	Elusimicrobiales	15	1
3	1	Chlorobiales	15	11
3	2	SubsectionII	14	10
3	2	MB11C04 marine group	13	4
3	2	Lentisphaerales	12	7
3	2	SubsectionIV	11	3
3	3	Deinococcales	11	8
3	2	Micrococcales	11	7
2	1	CS-B046	10	5
2	2	HTA4	10	10
2	2	uncultured planctomycete	10	10
2	2	uncultured gamma proteobacterium	9	1
2	2	Pseudomonadales	9	1
2	2	Obscuribacterales	9	2
2	2	Gastranaerophilales	9	3
2	1	Erysipelotrichales	9	6
2	2	Vampirovibrionales	9	9
2	2	Bacteroidia Incertae Sedis	8	5
1	1	Desulfuromonadales	8	7
1	1	SS1-B-02-17	8	6
1	1	mle1-8	8	6
1	1	Emcibacterales	6	2
1	1	1013-28-CG33	6	3
1	1	Ardenticatenales	6	4
1	1	Coriobacteriales	6	4
1	1	Run-SP154	6	4

Table 1. (continued)

Phylum	Seqs	OTUs	Class
			MSBL8
			BJGMM-U56
			marine metagenome
			Candidatus Uhrbacteria
			Omnitrophica Incertae Sedis
			SB-5
			Latescibacteria Incertae Sedis
			uncultured Planctomycetales bacterium
			SGST604
			Caldisericia
			uncultured Microgenomates group bacterium
			SJA-68

Seqs	OTUs	Order	Seqs	OTUs
1	1	RS-B22	6	2
1	1	Lineage IIb	6	6
1	1	EC3	6	6
1	1	Nitrospinales	5	2
1	1	S-70	5	2
1	1	MSBL5	5	3
1	1	ss1-B-07-44	5	2
1	1	S26-47	5	3
1	1	Frankiales	5	2
1	1	vadinBA26	5	4
1	1	Rhodocyclales	5	4
1	1	Chthoniobacterales	5	4
		Enterobacteriales	5	5
		Acholeplasmatales	4	2
		Opitutales	4	2
		B103G10	4	2
		TRA3-20	4	2
		uncultured proteobacterium	4	3
		X35	4	3
		C86	4	3
		Oligosphaerales	4	3
		Chitinivibrionales	4	4
		A714019	3	1
		SS1-B-09-64	3	1
		F9P41300-M23	3	2
		Pasteurellales	3	1
		4-Org1-14	3	2
		uncultured Gemmatimonadetes bacterium	3	2
		MSBL9	3	2
		Fimbriimonadales	3	3
		uncultured Bacteroidetes bacterium	3	3
		Solirubrobacterales	3	2
		Synergistales	3	2
		Magnetococcales	3	3
		Mollicutes RF9	3	3
		marine metagenome	3	2
		43F-1404R	3	3
		Lineage IV	2	1
		SC-I-84	2	1
		MVP-21	2	1
		uncultured deep-sea bacterium	2	1
		Nitriliruptorales	2	2
		uncultured crenarchaeote	2	2

Table 1. (continued)

Phylum	Seqs	OTUs	Class

Seqs	OTUs	Order	Seqs	OTUs
		E6aD10	2	1
		OTU048-Xeno	1	1
		Neisseriales	1	1
		Kallotenuales	1	1
		Holophagales	1	1
		FW22	1	1
		DMI	1	1
		Caenarcaniphilales	1	1
		Selenomonadales	1	1
		uncultured Cytophagales bacterium	1	1
		possible order 07	1	1
		GB102	1	1
		Amsterdam-1B-07	1	1
		10bav-F6	1	1
		Thermales	1	1
		MSB-3A7 sediment group	1	1
		uncultured Planctomycetales bacterium	1	1
		GIF3	1	1
		Brocadiales	1	1
		MVP-88	1	1
		Mollicutes Incertae Sedis	1	1
		Caldisericales	1	1
		Tepidisphaerales	1	1
		Chloroflexales	1	1
		uncultured Microgenomates group bacterium	1	1
		Rs-M47	1	1
		Thermoanaerobacterales	1	1
		Sh765B-AG-111	1	1

APPENDIX 5.2

Table 2. Description of core OTUs

OTU	Sum	Number of samples	Domain	Phylum	Class	Order
6	125103	73	Bacteria	Nitrospirae	Nitrospira	Nitrospirales
8	84801	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
2	71140	73	Bacteria	Nitrospirae	Nitrospira	Nitrospirales
4	67225	73	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfurellales
1	61254	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
3	58869	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
22	47159	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
24	45009	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
35	42502	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
37	32052	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
18	30336	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
57	30213	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
27	28460	73	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI
61	26094	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
46	24932	73	Bacteria	Nitrospirae	Nitrospira	Nitrospirales
54	24022	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
32	22468	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
52	21982	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
36	21214	73	Bacteria	Acidobacteria	Holophagae	Subgroup 10
66	21186	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
44	20355	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
30	19280	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
33	19223	73	Bacteria	Proteobacteria	JTB23	uncultured bacterium
76	18561	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales
55	18245	73	Bacteria	Nitrospirae	Nitrospira	Nitrospirales
39	17919	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales
58	16580	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
53	16191	73	Bacteria	Chloroflexi	Caldilineae	Caldilineales
56	16040	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
147	13996	73	Bacteria	Chloroflexi	Caldilineae	Caldilineales
79	12896	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
51	12413	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
65	12376	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
133	12371	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
114	12361	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales
134	12035	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
73	11290	73	Bacteria	Chloroflexi	Caldilineae	Caldilineales
67	11092	73	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfurellales
112	11057	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
81	10212	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
82	9248	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales

Family	Genus	Species
Nitrospiraceae	Nitrospira	uncultured bacterium
Sva0996 marine group	Unassigned	Unassigned
Nitrospiraceae	Nitrospira	uncultured bacterium
Desulfurellaceae	G55	Unassigned
Sva0996 marine group	Unassigned	Unassigned
Sva0996 marine group	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Sva0996 marine group	Unassigned	Unassigned
Sva0996 marine group	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
FamilyI	uncultured	Ambiguous_taxa
Unassigned	Unassigned	Unassigned
Nitrospiraceae	Nitrospira	uncultured bacterium
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
TK85	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
uncultured bacterium	uncultured bacterium	uncultured bacterium
Rhodospirillaceae	uncultured	Unassigned
Nitrospiraceae	Nitrospira	uncultured bacterium
SAR116 clade	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Caldilineaceae	uncultured	uncultured Chloroflexi bacterium
Ambiguous_taxa	Ambiguous_taxa	Ambiguous_taxa
Caldilineaceae	uncultured	uncultured Chloroflexi bacterium
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Rhodospirillaceae	uncultured	uncultured bacterium
Sva0996 marine group	Unassigned	Unassigned
Caldilineaceae	uncultured	uncultured Chloroflexi bacterium
Desulfurellaceae	G55	Unassigned
Sva0996 marine group	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Rhodobacteraceae	uncultured	Unassigned

Table 2. (continued)

OTU	Sum	Number of samples	Domain	Phylum	Class	Order
84	8898	73	Bacteria	Nitrospirae	Nitrospira	Nitrospirales
145	8679	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
129	8568	73	Bacteria	Chloroflexi	Caldilineae	Caldilineales
179	8417	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
68	8326	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
63	8282	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
74	7980	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales
80	7966	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
70	7944	73	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI
90	7551	73	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales
1711	7233	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
104	6678	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
87	6511	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
89	6488	73	Bacteria	Nitrospirae	Nitrospira	Nitrospirales
105	6284	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales
271	6096	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales
150	5991	73	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales
1377	5610	73	Bacteria	Nitrospirae	Nitrospira	Nitrospirales
146	5601	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
188	5443	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales
160	5256	73	Bacteria	Chloroflexi	Caldilineae	Caldilineales
194	5165	73	Bacteria	Chloroflexi	SAR202 clade	Unassigned
121	4960	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales
144	4876	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
102	4828	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
182	4572	73	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales
156	4360	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales
125	4290	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales
154	4235	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales
140	3923	73	Bacteria	Chloroflexi	SAR202 clade	Unassigned
204	3739	73	Bacteria	Chloroflexi	Caldilineae	Caldilineales
164	3706	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
995	3673	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
247	3566	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
240	3414	73	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfurellales
176	3145	73	Bacteria	Proteobacteria	Gammaproteobacteria	KI89A clade
108	3075	73	Bacteria	Proteobacteria	Gammaproteobacteria	HOC36
321	3031	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
11	2942	73	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI
326	2907	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
187	2899	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
233	2779	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales

Family	Genus	Species
Nitrospiraceae	Nitrospira	uncultured bacterium
Unassigned	Unassigned	Unassigned
Caldilineaceae	uncultured	uncultured Chloroflexi bacterium
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
SAR116 clade	uncultured bacterium	uncultured bacterium
Unassigned	Unassigned	Unassigned
FamilyI	Synechococcus	uncultured bacterium
JTB255 marine benthic group	Unassigned	Unassigned
Ambiguous_taxa	Ambiguous_taxa	Ambiguous_taxa
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Nitrospiraceae	Nitrospira	uncultured bacterium
SAR116 clade	Unassigned	Unassigned
Rhodobacteraceae	Albidovulum	uncultured alpha proteobacterium
JTB255 marine benthic group	Unassigned	Unassigned
Nitrospiraceae	Nitrospira	uncultured bacterium
Unassigned	Unassigned	Unassigned
Rhodobacteraceae	uncultured	Unassigned
Caldilineaceae	uncultured	uncultured Chloroflexi bacterium
Unassigned	Unassigned	Unassigned
Rhodospirillaceae	uncultured	Unassigned
Ambiguous_taxa	Ambiguous_taxa	Ambiguous_taxa
Unassigned	Unassigned	Unassigned
JTB255 marine benthic group	Unassigned	Unassigned
SAR116 clade	uncultured bacterium	uncultured bacterium
SAR116 clade	uncultured bacterium	uncultured bacterium
SAR116 clade	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Caldilineaceae	uncultured	uncultured Chloroflexi bacterium
Sva0996 marine group	uncultured bacterium	uncultured bacterium
Unassigned	Unassigned	Unassigned
Sva0996 marine group	Unassigned	Unassigned
Desulfurellaceae	G55	Unassigned
Unassigned	Unassigned	Unassigned
uncultured bacterium	uncultured bacterium	uncultured bacterium
Unassigned	Unassigned	Unassigned
FamilyI	Prochlorococcus	uncultured bacterium
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
SAR116 clade	Unassigned	Unassigned

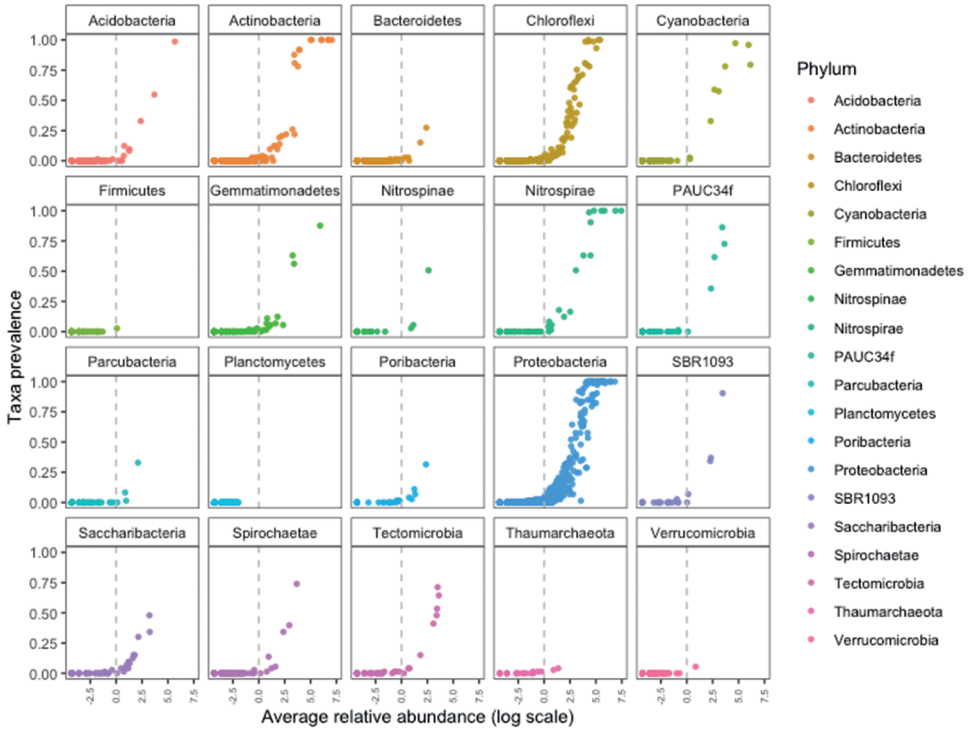
Table 2. (continued)

OTU	Sum	Number of samples	Domain	Phylum	Class	Order
3853	2535	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
130	2470	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
405	2377	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
221	2367	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
172	2328	73	Bacteria	SBR1093	Ambiguous_taxa	Ambiguous_taxa
207	2328	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
460	2249	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
190	2123	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales
250	1709	73	Bacteria	Chloroflexi	SAR202 clade	Unassigned
260	1621	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales
236	1572	73	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI
257	1283	73	Bacteria	Chloroflexi	SAR202 clade	Unassigned
25	1087	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
293	1057	73	Bacteria	PAUC34f	Unassigned	Unassigned
338	977	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured

Family	Genus	Species
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Ambiguous_taxa	Ambiguous_taxa	Ambiguous_taxa
Sva0996 marine group	Unassigned	Unassigned
Sva0996 marine group	uncultured actinobacterium	uncultured actinobacterium
Rhodospirillaceae	OM75 clade	Unassigned
Unassigned	Unassigned	Unassigned
SAR116 clade	Unassigned	Unassigned
FamilyI	Synechococcus	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned

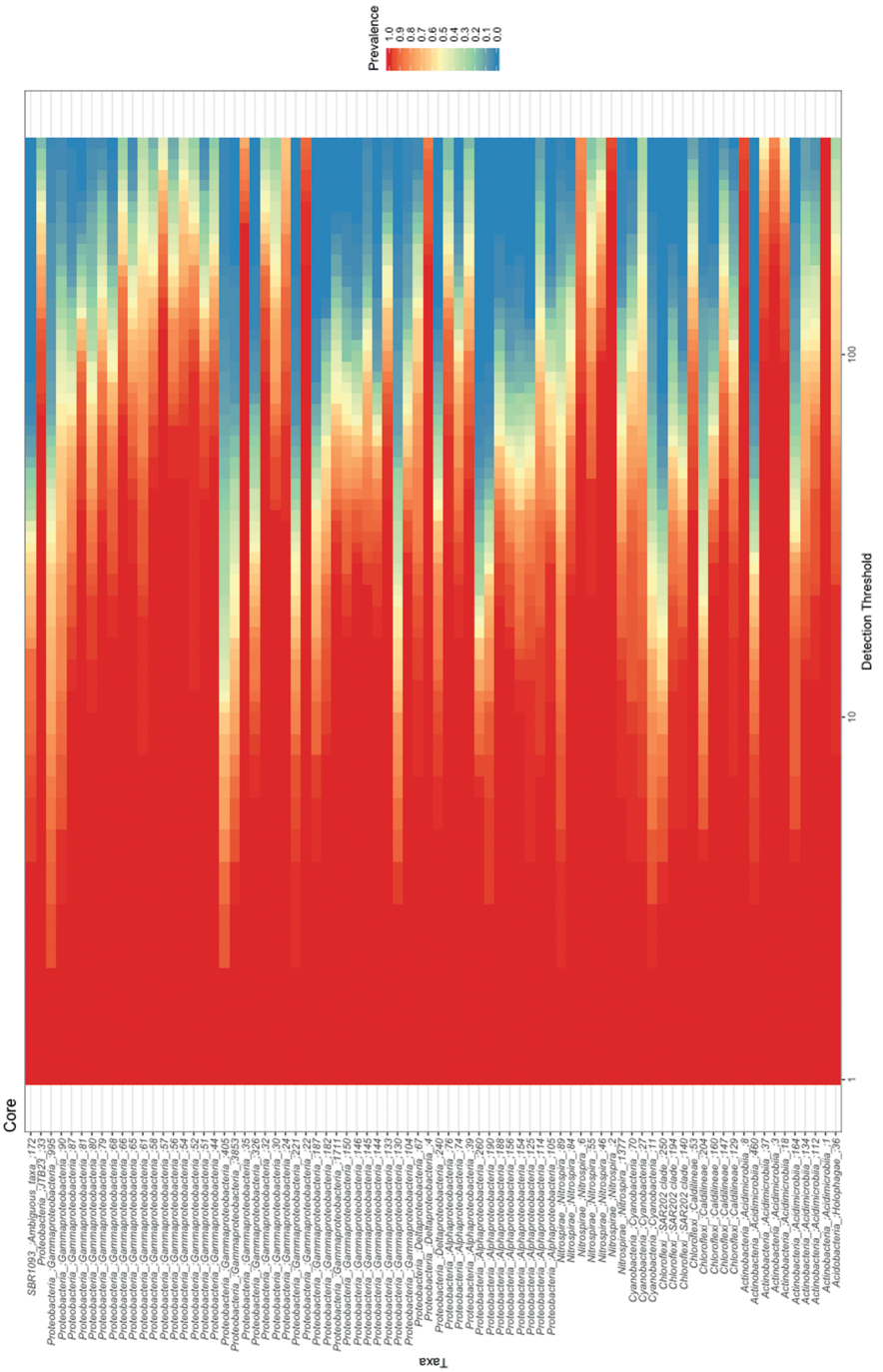
APPENDIX 5.3

Overview of OTU prevalences and their taxonomic affiliations.



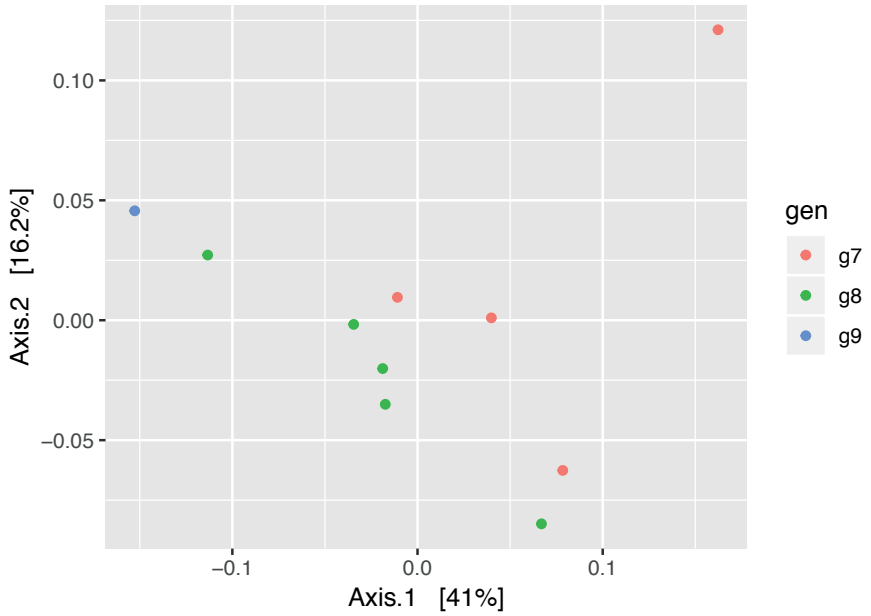
APPENDIX 5.4

Heat map of abundances of core OTUs.



APPENDIX 5.5

PCO of core community composition of 10 giant barrel sponge (Xestospongia spp.) samples from Piscadera Bay, Curaçao.



III

Section three: Reef Interactions

6

The sponge microbiome within the greater coral reef microbial metacommunity

*Cleary, D. F. R., *Swierts, T., Coelho, F. J., Polónia, A. R., Huang, Y. M., Ferreira, M. R.,Putchakarn, S., Carvalheiro, L., van der Ent, E., Ueng, J., Gomes, N. C. M. & de Voogd, N. J.

* = Both authors contributed equally

(2019). The sponge microbiome within the greater coral reef microbial metacommunity. Nature communications, 10(1), 1644.

ABSTRACT

Much recent marine microbial research has focused on sponges, but very little is known about how the sponge microbiome fits in the greater coral reef microbial metacommunity. Here, we present an extensive survey of the prokaryote communities of a wide range of biotopes from Indo-Pacific coral reef environments. We find a large variation in operational taxonomic unit (OTU) richness, with algae, chitons, stony corals and sea cucumbers housing the most diverse prokaryote communities. These biotopes share a higher percentage and number of OTUs with sediment and are particularly enriched in members of the phylum Planctomycetes. Despite having lower OTU richness, sponges share the greatest percentage (>90%) of OTUs with >100 sequences with the environment (sediment and/or seawater) although there is considerable variation among sponge species. Our results, furthermore, highlight that prokaryote microorganisms are shared among multiple coral reef biotopes, and that, although compositionally distinct, the sponge prokaryote community does not appear to be as sponge-specific as previously thought.

INTRODUCTION

In recent years, high-throughput sequencing methods have generated an unprecedented amount of information on the structural and functional diversity of microbial communities (Douglas 2014). Marine host-associated prokaryote communities, particularly those associated with sponges, have been reported to be highly diverse (Thomas et al. 2016). Despite the constant influx of seawater, sponges are able to sustain dense and diverse symbiotic communities, which can comprise up to 35% of sponge biomass (Taylor et al. 2007b; Hentschel et al. 2012). These associations, furthermore, appear to be consistent over different geographical areas and under different environmental conditions (Hentschel et al. 2002; Lee et al. 2011; Cleary et al. 2015b; de Voogd et al. 2015; Pólonia et al. 2015; 2017).

Much like the human gut, sponges are considered to be an important model to study host–prokaryote associations (Hentschel et al. 2012). Although much recent research has characterized the phylogenetic diversity and biogeography of sponge-associated microorganisms, relatively little is known about a range of other hosts in coral reef ecosystems. If, and to what extent, sponge-associated microorganisms occur in these other hosts is still largely unknown. This is an important hiatus in our understanding of coral reef microbial ecology given that the prokaryote communities of sponges are part of a wider prokaryote 'metacommunity' of host-associated and free-living (in sediment and seawater) microorganisms (Leibold et al. 2004). This metacommunity forms the regional pool of prokaryote species from which local (within a single host) host-associated communities of microorganisms are assembled. These local communities are presumably linked by dispersal, mainly between host organisms and the external environment, thus maintaining the intricate structure of the metacommunity (Adair and Douglas 2017). Occasionally, direct contact between different host taxa may also induce dispersal and shape the microbial community. Pratte et al. (2018), for example, showed that direct contact between turf algae and the coral species *Porites* sp. had a strong influence on the coral (but not the algal) bacterial community.

In the present study, we assess and compare prokaryote communities from a range of host taxa and the abiotic environment (sediment and seawater) in Indo-Pacific coral reef habitats. Our samples include high and lower diversity hosts. High diversity hosts include samples of algae, chitons, stony corals and the sea cucumber gut and mantle. Samples from these hosts are compositionally similar and have relatively high abundances of operational taxonomic units (OTUs) assigned to the phylum Planctomycetes and relatively high OTU richness and evenness. Prokaryote communities of this group also share significantly more $OTUs_{100}$ (OTUs with >100 sequences) with sediment (i.e. OTUs found in sediment but not seawater) than other biotopes. The lower diversity host group includes sponges, sponge denizens and the nudibranch gut and mantle biotopes. Compared to the first group, samples of this group have a relatively low OTU richness and evenness (with the exception of high microbial

abundance [HMA] sponges) and a relatively low percentage of sediment $OTUs_{100}$. The mean percentage of total environmental $OTUs_{100}$ (OTUs recorded in sediment and/or seawater), however, is highest in sponges. The main compositional differences observed in the present study appear to be driven by the apparent permeability of certain taxa (namely algae, sea cucumbers, and stony corals) to sediment prokaryotes and the concomitant high prokaryote richness found in these taxa. In turn, sponges, nudibranchs, flatworms, and sponge denizens have much fewer sediment prokaryotes $OTUs_{100}$ and a concomitantly lower prokaryote richness, despite having a sometimes very high contribution of environmental $OTUs_{100}$ to total $OTUs_{100}$ richness.

METHODS

Sampling locations

All host-associated, sediment and seawater samples were collected from various sites in Taiwan and Thailand (Appendix 6.1). All locations were coral reef habitat. A detailed description of the Taiwanese sampling sites can be found in Coelho et al. (2018) and Huang et al. (2016) and meta data for all samples including the sampling location and time of sampling can be found in Appendix 6.1. Fragments of host individuals were collected using SCUBA diving, or snorkelling, including the surface and interior or the whole organism (depending on the size) in order to sample as much as possible of the whole prokaryote community. Sediment was collected from the upper 5 cm surface layer using a plastic disposable syringe from which the end had been cut in order to facilitate sampling. Seawater was collected between the depths of 1–2 m with a 1.5 L bottle and subsequently 1 L (±50 ml) of water was filtered through a Millipore® White Isopore Membrane Filter (0.22 µm pore size) to obtain seawater prokaryote communities. All samples were subsequently preserved in 96% EtOH. All samples were kept cool (<4 °C) immediately after collection and during transport. In the laboratory, samples were stored at –20 °C until DNA extraction.

A total of 216 samples belonging to algae, chitons, stony corals, sea cucumbers, sponge denizens (organisms that live on or within sponges), nudibranchs, flatworms, soft corals, sponges, sea urchins, water and sediment were collected. In the present study, all samples were assigned to 14 biotopes, which included the guts and mantles of sea cucumbers and nudibranchs as separate biotopes. Certain biotopes were well represented, e.g. sponges (63 samples from 18 species) and nudibranchs (48 samples from 13 species) while others only consisted of a just few samples and/or a single species., e.g. soft corals (4 samples from the species *Cladiella* sp.), chitons (3 samples from the species *L. japonica*) and sea urchins (5 samples from the species *D. savignyi*). All the samples used in the present study can be found in Appendix 6.1 including the sampling site and taxonomic identification.

DNA extraction and next-generation sequencing analysis

PCR-ready genomic DNA was isolated from all samples using the FastDNA® SPIN soil Kit (MPbiomedicals) following the manufacturer's instructions. Briefly, the whole membrane filter (for seawater samples) and ±500 mg of sediment and host specimens (including parts of the surface and/or interior) were cut into small pieces (in the case of the membrane filter and host specimens) and transferred to Lysing Matrix E tubes containing a mixture of ceramic and silica particles. A blank control, in which no tissue was added to the Lysing Matrix E tubes, was also included. The microbial cell lysis was performed in the FastPrep® Instrument (Q Biogene) for 80 s at 6.0 ms−1. The extracted DNA was eluted into DNase/Pyrogen-Free Water to a final volume of 50 µl and stored at −20 °C until use. The 16S rRNA gene V3V4 variable region PCR primers 341F 5′-CCTACGGGNGGCWGCAG-3′ and 785R 5′-GACTACHVGGGTATCTAATCC-3′ (Klindworth et al. 2013) with barcode on the forward primer were used in a 30 cycle PCR assay using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94 °C for 3 min, followed by 28 cycles of 94 °C for 30 s, 53 °C for 40 s and 72 °C for 1 min, after which a final elongation step at 72 °C for 5 min was performed. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands; the blank control did not yield any bands. Multiple samples were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads. Pooled and purified PCR product was used to prepare the DNA library following the Illumina TruSeq DNA library preparation protocol. Next-generation, paired-end sequencing was performed at MrDNA (Molecular Research LP; http://www.mrdnalab.com/; last checked 18 November 2016) on an Illumina MiSeq device (Illumina Inc., San Diego, CA, USA) following the manufacturer's guidelines. Sequences from each end were joined following Q25 quality trimming of the ends followed by reorienting any 3′–5′ reads back into 5′–3′ and removal of short reads (<150 bp). The resultant files were analyzed using the QIIME (Quantitative Insights Into Microbial Ecology; Caporaso et al. 2010) software package (http://www.qiime.org/) and USEARCH10 19.

16S rRNA gene sequencing analysis

For a detailed description of the sequence analysis, see Coelho et al.(2018) and Cleary et al. (2018c). Briefly, in QIIME, fasta and qual files were used as input for the split_libraries.py script in QIIME. Default arguments were used except for the minimum sequence length, which was set at 250 base pairs (bps) after removal of forward primers and barcodes. Using USEARCH10 (https://www.drive5.com/usearch/; last checked 2019 02 11), reads were filtered with the -fastq_filter command and the following arguments: -fastq_trunclen 250 -fastq_maxee 0.5 -fastq_truncqual 15. Sequences were then dereplicated and sorted using the -derep_fulllength and -sortbysize commands. OTU clustering (97% sequence similarity threshold) was performed using the -cluster_otus command of USEARCH10 yielding 12025383 sequences assigned to 48880 OTUs. Potential contaminants were removed from

the OTU table if they occurred at least two times in the blank control. This conservative measure was chosen because of observations of bleeding between samples from Illumina sequencing and the appearance of abundant reads in blank controls with very low counts (Mitra et al. 2015; Sinha 2017). Based on this procedure, 958995 sequences and 77 OTUs were removed from the non-rarefied OTU table. OTUs not classified as Bacteria or Archaea or classified as chloroplasts and mitochondria were also removed. Taxonomy was assigned to reference sequences of OTUs using default arguments in the assign_taxonomy.py script in QIIME using the SILVA_128_QIIME_release database and the uclust classifier method (Quast et al. 2013). The make_otu_table.py script in QIIME was used to generate a square matrix of OTUs × SAMPLES and subsequently rarefied to 10,000 sequences per sample with the single_rarefaction.py script in QIIME yielding 2,160,000 sequences and 30,725 OTUs. This rarefied table was used as input for further analyses using the R language for statistical computing and has been included as a source data file (https://www.r-project.org/; last checked 2018–07–17).

Statistical analysis

A data matrix containing OTU counts per sample was imported into R using the read.csv() function. This table was used to compare community composition, estimate richness and assess the relative abundance of selected higher taxa and is included as a Source Data file. The OTU abundance matrix was loge (x + 1) transformed (in order to normalize the distribution of the data) and a distance matrix constructed using the Bray–Curtis index with the vegdist() function in the vegan package (Oksanen et al. 2019). The Bray–Curtis index is one of the most frequently applied (dis)similarity indices used in ecology (Legendre and Gallagher 2001; Cleary 2003; de Voogd et al. 2006; Cleary et al. 2016). Variation in prokaryote composition among biotopes was assessed with Principal Coordinates Analysis (PCO) using the cmdscale() function in R with the Bray–Curtis distance matrix as input. Variation among biotopes was tested for significance using the adonis() function in vegan. In the adonis analysis, the Bray–Curtis distance matrix of species composition was the response variable with biotope as independent variable. The number of permutations was set at 999; all other arguments used the default values set in the function. Weighted average scores were computed for OTUs on the first four PCO axes using the wascores() function in the vegan package. The simper() function in vegan was used to identify significantly discriminating OTUs between pairs of biotopes based on the loge (x + 1) transformed OTU table and 999 permutations. The discriminating OTUs contribute the most to differences between pairs of biotopes.

We tested for significant differences in the relative abundance of 18 of the most abundant phyla, the four most abundant proteobacterial classes, and the count and relative abundance of sediment and environmental OTUs among biotopes with an analysis of deviance using the glm() function in R. For the most abundant phyla, proteobacterial classes, and the relative abundance of sediment and environmental OTUs, we first applied a generalized

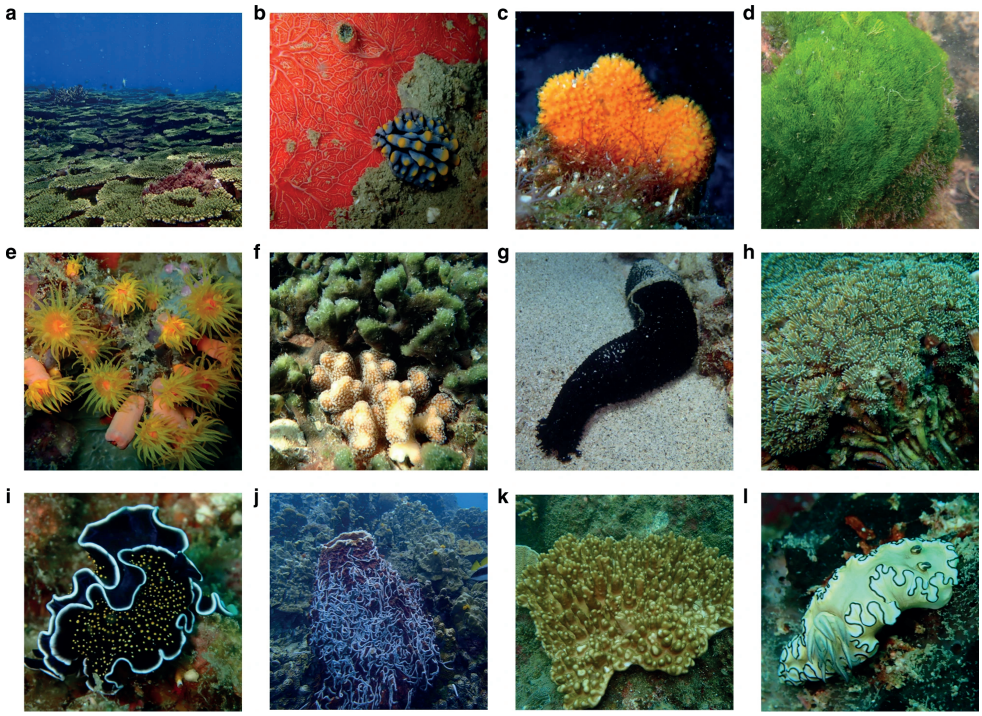


Figure 6.1. Pictures of sampling sites and organisms sampled during the present study. a Coral reef in the southern Penghu islands, Taiwan, b the nudibranch *Phyllidia* cf. *coelestis*, c the sponge *Ptilocaulis spiculifer*, d the green alga *Chlorodesmis fastigiata* in shallow water, e the sun coral *Tubastraea coccinea*, f the green sponge *Haliclona cymaeformis*, g the sea cucumber *Holothuria leucospilota*, h the stony coral *Galaxea astreata*, i the spotted flatworm *Thysanozoon nigropapillosum*, j the barrel sponge *Xestospongia testudinaria* covered by sea cucumbers (*Synaptula* sp.), k the soft coral *Cladiella* sp. and l the nudibranch *Doriprismatica atromarginata*. All photographs were taken by D.F.R. Cleary or N.J. de Voogd.

linear model (GLM) with the family argument set to binomial. The ratio, however, of residual deviance to residual d.f. in the models substantially exceeded 1 so we set family to 'quasibinomial'. In the 'quasibinomial' family, the dispersion parameter is not fixed at one so that it can model over-dispersion. For the counts of sediment and environmental OTUs, we set the family argument to 'quasipoisson'. For the least abundant phyla and the two least abundant proteobacterial classes, which included zero counts in the samples, we set the family argument to 'tweedie' (Tweedie 1984) with var.power = 1.5 and link.power = 0 (a compound Poisson–gamma distribution). Using the glm models, we tested for significant variation among biotopes using the anova() function in R with the F test, which is most appropriate when dispersion is estimated by moments as is the case with quasibinomial fits. We subsequently used the emmeans() function in the emmeans library (Lenth 2017) to perform multiple comparisons of mean abundance among biotopes using the false discovery rate (fdr) method in the adjust argument. Additional graphs were produced using

the ggplot (Wickham 2009) and limma (Ritchie et al. 2015) packages. Detailed descriptions of the functions used here can be found in R (e.g.?cmdscale) and online in reference manuals (http://cran.r-project.org/web/packages/vegan/index.html)

RESULTS

Approach

In this study, we applied high-throughput 16S rRNA gene sequencing analysis to simultaneously assess the diversity of 216 prokaryote communities (Appendix 6.1) from the following 14 biotopes: algae, chitons, stony corals, sea cucumber gut, sea cucumber mantle, sponge denizens (organisms that live on or within sponges), flatworms, nudibranch gut, nudibranch mantle, soft corals, sponges, sea urchins, seawater and sediment (Fig. 6.1). All host-associated biotopes consisted of multiple species, with the exception of chitons (only included the species *Liolophura japonica*), soft corals (only included the species *Cladiella* sp.) and sea urchins (only included the species *Diadema savignyi*). Samples were collected from coral reef sites in Taiwan and Thailand (Appendix 6.1).

General patterns

We recorded 30,725 OTUs assigned to 68 phyla over 2,160,000 sequences (after rarefying to 10,000 sequences per sample). The number of OTUs recorded per sample varied from only 103 for a gut sample of the nudibranch *Phyllidia picta* to 3704 for a sediment sample (Appendix 6.1). The richest host-associated sample (2997 OTUs) was from the gut of the sea cucumber *Holothuria hilla*. The richest (in terms of OTUs) and most abundant (in terms of sequences) prokaryote phyla sampled in the present study included Proteobacteria, Bacteroidetes, Planctomycetes, Acidobacteria, Chloroflexi, and Actinobacteria. Abundant phyla with relatively few OTUs, but numerous sequence reads, included Tenericutes, Cyanobacteria, Spirochaetae, Thaumarchaeota, and Nitrospirae (Appendix 6.1; 6.2).

The relative abundance of 18 of the most abundant phyla (with the exception of Proteobacteria) and the four most abundant proteobacterial classes (with the exception of Gammaproteobacteria), varied significantly among biotopes (Fig. 6.2; pairwise comparisons between pairs of biotopes are presented in Appendix 6.3). Some biotopes were strongly enriched by specific prokaryote phyla. The abundance of Planctomycetes, for example, was significantly higher in sediment, and the sea cucumber gut and mantle than the nudibranch gut and mantle and sponge biotopes (Fig. 6.2i and Appendix 6.3). The relative abundance of Chloroflexi, in turn, was highest in the sponge, sponge denizen and nudibranch mantle biotopes and significantly higher than in the algae and nudibranch gut biotopes. There was, however, pronounced variation in Chloroflexi abundance within these biotopes as shown by the large standard deviations in Fig. 6.2d. For example, the sponge species *Aaptos lobata, Hyrtios erectus,* and *Xestospongia testudinaria*, which have been previously identified as HMA sponges or have been shown to house prokaryote communities very similar to those

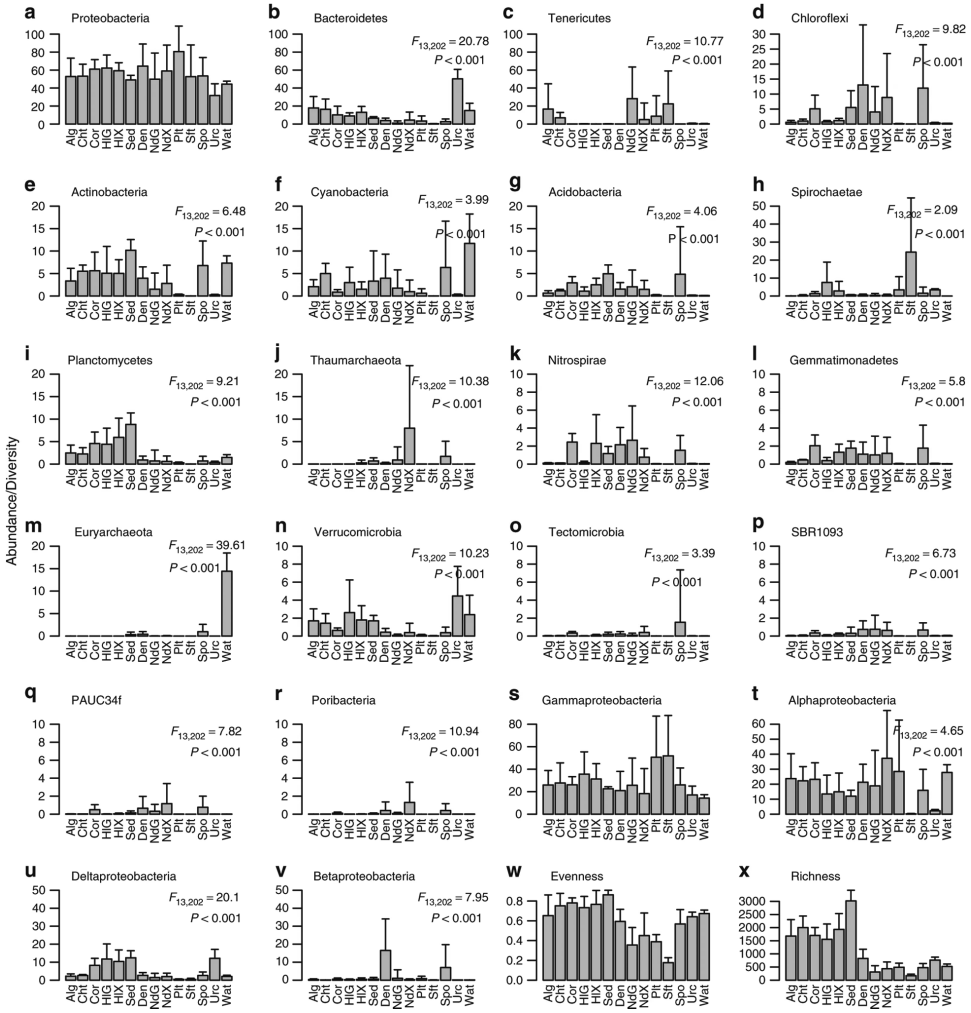


Figure 6.2. Mean relative abundance of the most abundant phyla, proteobacterial classes, OTU richness and evenness. Error bars represent a single standard deviation. a Proteobacteria, b Bacteroidetes, c Tenericutes, d Chloroflexi, e Actinobacteria, f Cyanobacteria, g Acidobacteria, h Spirochaetae, i Planctomycetes, j Thaumarchaeota, k Nitrospirae, l Gemmatimonadetes, m Euryarchaeota, n Verrucomicrobia, o Tectomicrobia, p SBR1093, q PAUC34f, r Poribacteria, s Gammaproteobacteria, t Alphaproteobacteria, u Deltaproteobacteria, v Betaproteobacteria and diversity components, w Evenness and x Richness in the following biotopes: algae (Alg), chitons (Cht), stony corals (Cor), sea cucumber gut (HlG), sea cucumber mantle (HlX), sediment (Sed), sponge denizens (Den), nudibranch gut (NdG), nudibranch mantle (NdX), flatworms (Plt), soft corals (Sft), sponges (Spo), sea urchins (Urc) and seawater (Wat). When significant ($P < 0.0023$; Bonferroni corrected α value), results of the GLM analyses are presented in the top right of the subfigures.

found in HMA sponges (Gloeckner et al. 2014; Cleary et al. 2015a; 2015b; 2018; Moitinho-Silva et al. 2017; Pólonia et al. 2018), had higher relative abundances of Chloroflexi, and

other taxa including SBR1093 (Fig. 6.2p) and Poribacteria (Fig. 6.2r), than all other sponge species (Appendix 6.1). At the class level, alphaproteobacterial abundance was highest in the nudibranch mantle and significantly higher than in the sea cucumber gut, soft coral, sponge and, sea urchin biotopes (Fig. 6.2t and Appendix 6.3). Deltaproteobacterial abundance was highest in the stony coral, sea cucumber gut and mantle, sediment, and sea urchin biotopes and significantly higher than in the algal, sponge denizen, nudibranch gut and mantle, flatworm, soft coral, sponge, and seawater biotopes (Fig. 6.2u). Betaproteobacterial abundance was highest in the sponge and sponge denizen biotopes and significantly more so than in the algae, sea cucumber gut, and nudibranch gut and mantle biotopes (Fig. 6.2v and Appendix 6.3).

OTU sample richness was highest in the sediment, chiton, algae, stony coral and sea cucumber gut and mantle biotopes and lowest in the flatworm, sponge, nudibranch gut and mantle, soft coral, sea urchin and seawater biotopes (Fig. 6.2x and Appendix 6.1). This same pattern also applied to cumulative OTU richness (Appendix 6.4). Histograms of OTU richness also showed largely non-overlapping distributions with samples of sponges and the nudibranch mantle clustered at low OTU richness values while samples of algae, the sea cucumber gut, and sediment were spread out over a larger range at higher OTU richness values (Appendix 6.5). This distinction also held after removing all OTUs <100 sequences (Appendix 6.6). Singletons are sometimes removed due to possible problems with sequencing errors associated with Illumina and other next-generation sequencing platforms (Edgar 2013). Removing all OTUs <100 sequences shows the robustness of the pattern and, thus, the apparent prevalence of high diversity and low diversity hosts in coral reef habitat.

Evenness was also high in biotopes with the highest richness and was lowest in the flatworm and nudibranch gut biotopes. Evenness was particularly low in prokaryote communities of the soft coral *Cladiella* sp. (Fig. 6.2w). For example, 95.5 ± 2.9% (mean ± standard deviation; n = 4) of the prokaryote community of *Cladiella* sp. consisted of just three OTUs (OTUs 4, 14 and 17).

Compositionally distinct but overlapping communities

There was a highly significant compositional difference among biotopes (Adonis test: $F_{13, 201} = 6.64$, $R2 = 0.293$, $P < 0.001$; Fig. 6.3a). The factor biotope, thus, explained almost 30% of the variation in OTU composition. The main axis of variation (axis 1) separated samples of algae, chitons, sediment, stony corals, and the sea cucumber gut and mantle from samples of sponges, sponge denizens, seawater and the nudibranch gut and mantle. Samples from the flatworm, soft coral and sea urchin biotopes were intermediate. The second axis of variation (axis 2 in Fig. 6.3a) separated a cluster of sponge and seawater samples at high axis 2 values from a cluster of sponge, nudibranch gut and mantle and sponge denizen samples at low axis 2 values. OTUs that significantly discriminated between pairs of biotopes are presented in Fig. 6.4 and Appendix 6.7.

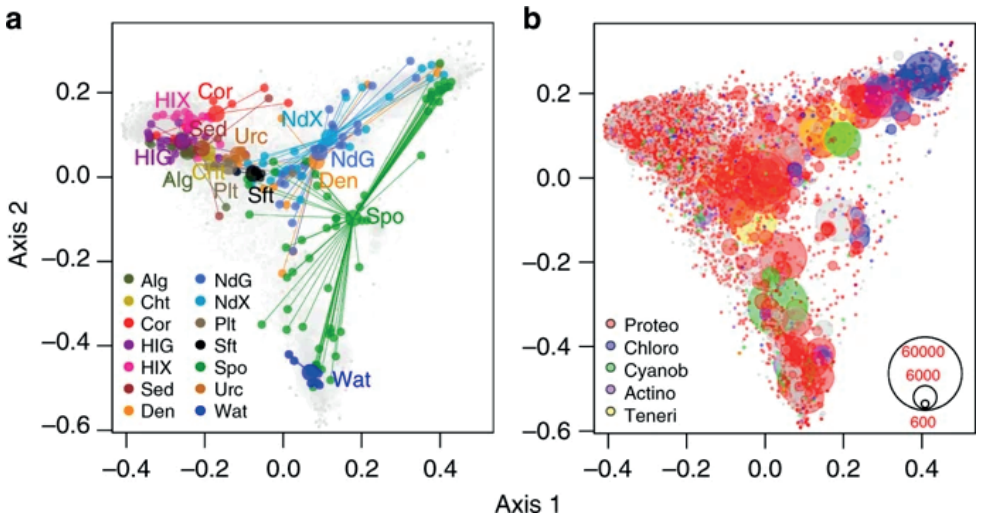


Figure 6.3. Ordination showing the first two axes of the PCO analysis. a Symbols represent samples of algae (Alg), chitons (Cht), stony corals (Cor), sea cucumber gut (HIG), sea cucumber mantle (HIX), sediment (Sed), sponge denizens (Den), nudibranch gut (NdG), nudibranch mantle (NdX), flatworms (Plt), soft corals (Sft), sponges (Spo), sea urchins (Urc) and seawater (Wat). Samples from biotopes are connected to group centroids; the figure was produced using the ordispider function in the vegan package. b OTU symbols color-coded according to their taxonomic assignment to selected phyla: Proteobacteria (Proteo), Chloroflexi (Chloro), Cyanobacteria (Cyanob), Actinobacteria (Actino) and Tenericutes (Teneri). The first two axes explain 22% of the variation in the data set. The circle size of the OTU is proportional to their abundance (number of sequences) as indicated by the symbol legend in the bottom right corner of b.

The most abundant OTUs observed in the present study were OTUs 1, 2, 9 and 25, all with >30,000 sequence reads. With the exception of OTU-25, the most abundant OTUs were not the most widespread (in terms of their occurrence in samples), but rather were very abundant in selected hosts (Fig. 6.4). OTU-2, assigned to *Mycoplasma* sp., and with only 92% sequence similarity to an OTU obtained from the oyster *Crassostrea gigas* from Australia (Gb-Acc: JF827444; Appendix 6.8), was mainly found in the nudibranch species *Halgerda willeyi* (although it was a rare constituent of the sea cucumber gut and mantle and stony coral biotopes). OTU-9, assigned to the Rhodospirillales order, and with 96% sequence similarity to an OTU obtained from seawater in the Northeast subarctic Pacific Ocean (Gb-Acc: HQ672247), was most abundant in the nudibranch species *Hypselodoris maritima* and *Mexichromis multituberculata*. OTU-1, assigned to the Rhizobiales order, and with 99% sequence similarity to an OTU obtained from the sponge *Tethya californiana* (Gb-Acc: EU290221), was abundant in various *Phyllidia* species. OTU-25, assigned to the genus Synechococcus, and with 100% sequence similarity to an OTU obtained from seawater in the Mediterranean Sea (Gb-Acc: MH076976), was the most widespread OTU and was found in 209 (of 216; 96.8% of all samples) samples and was most abundant in seawater samples (Fig. 6.4).

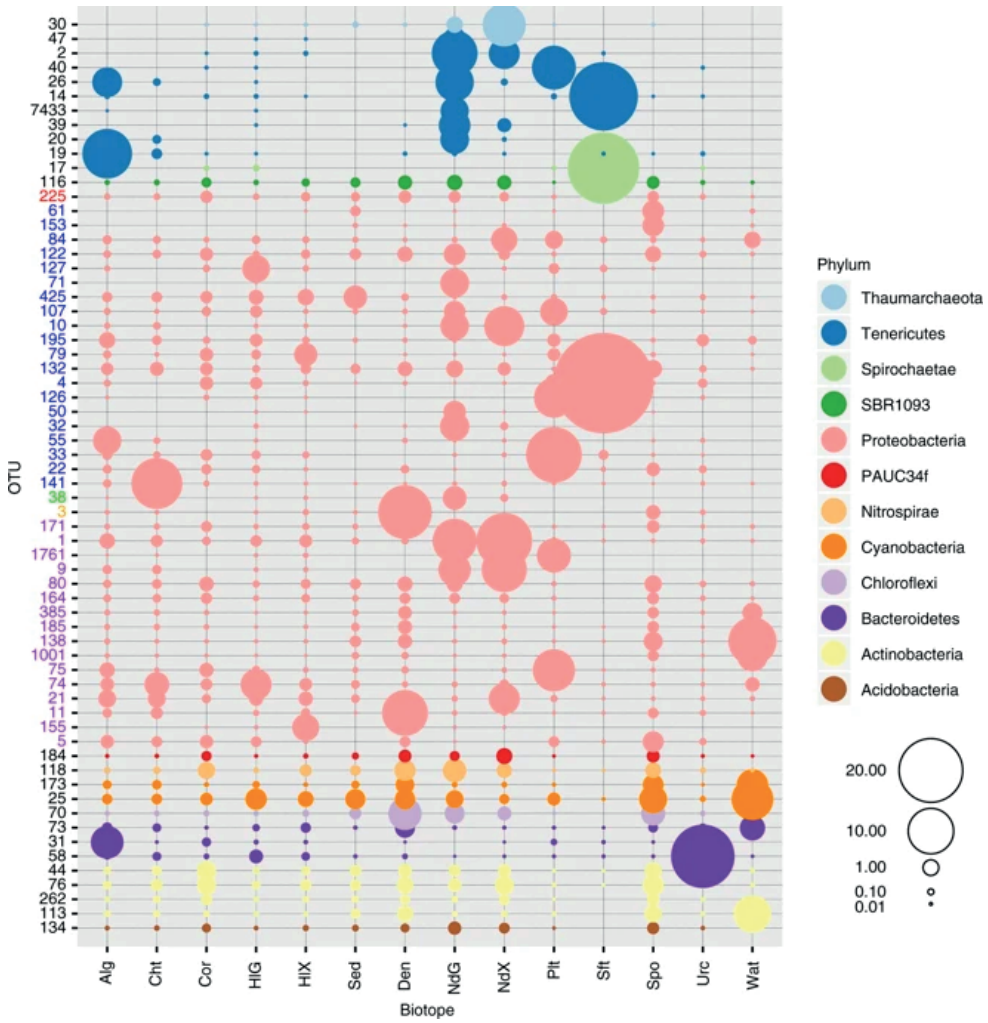


Figure 6.4. Relative abundance of significantly discriminating OTUs ($P < 0.001$) identified using Simper. Symbols are color-coded according to prokaryote phylum. Codes on the *x*-axis represent algae (Alg), chitons (Cht), stony corals (Cor), sea cucumber gut (HlG), sea cucumber mantle (HlX), sediment (Sed), sponge denizens (Den), nudibranch gut (NdG), nudibranch mantle (NdX), flatworms (Plt), soft corals (Sft), sponges (Spo), sea urchins (Urc) and seawater (Wat). The circle size of the OTU is proportional to the mean percentage of sequences per biotope as indicated by the symbol legend in the bottom right corner of the figure. The *y*-axis shows the OTU id number. The *y*-axis numbers have been color coded for the proteobacterial OTUs to identify class assignment; red: JTB23, blue: Gammaproteobacteria, green: Epsilonproteobacteria, orange: Betaproteobacteria and purple: Alphaproteobacteria.

As can be seen in Fig. 6.4 and Appendices 6.9, 6.10 and 6.11, most of the abundant OTUs, including significantly discriminating OTUs, were recorded in multiple biotopes, albeit oftentimes a rare component of these biotopes. Notable exceptions to this pattern were

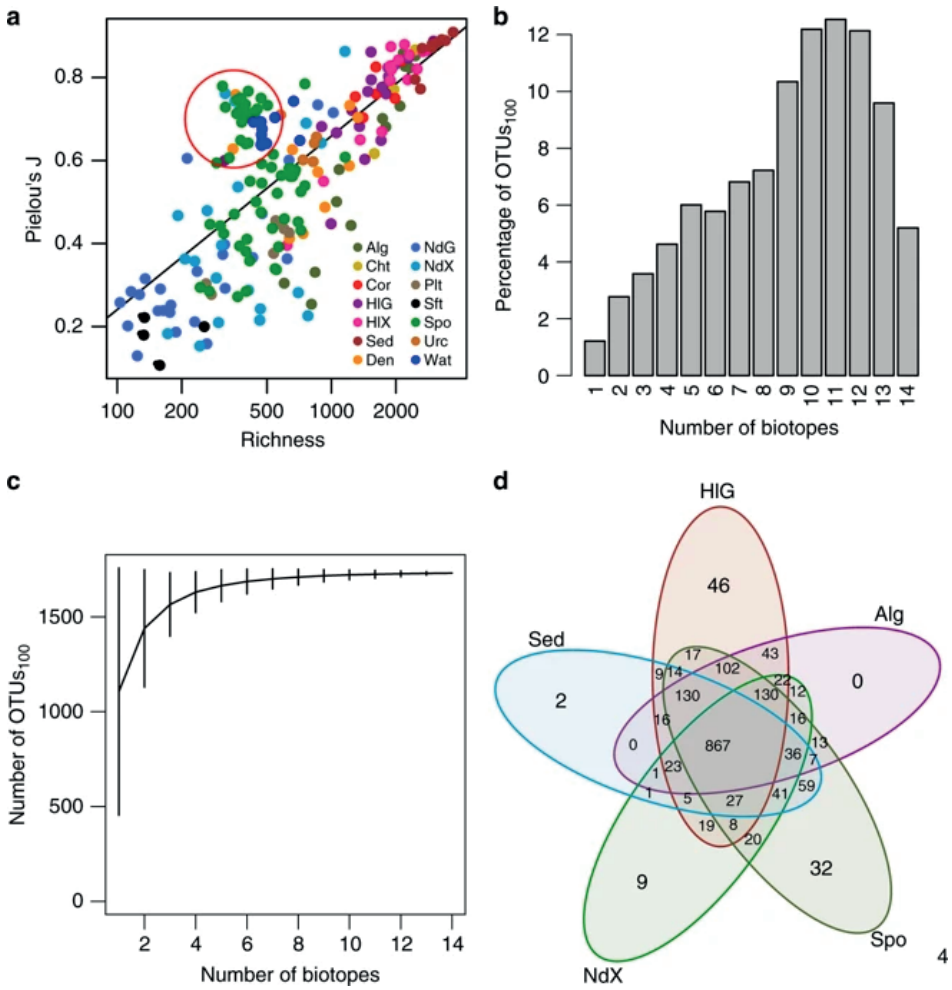


Figure 6.5. Diversity components and distribution of OTUs among biotopes. **a** Relationship between richness and evenness. OTUs representing HMA sponges have been encircled in red. **b**Percentage of $OTUs_{100}$ recorded in from 1 to 14 biotopes. For example, 1.2% of $OTUs_{100}$ (21 $OTUs_{100}$) were recorded in one biotope, 2.8% (48 $OTUs_{100}$) in two biotopes, 3.6% (62 $OTUs_{100}$) in three biotopes and 5.2% (90 $OTUs_{100}$) in all 14 of the main biotopes. **c** Rarefied OTU richness (error bars represent 95% confidence intervals) as a function of the number of biotopes sampled and estimated using the specaccum function in vegan with the 'method' argument set to 'random' and 999 permutations. **d** Venn diagram, obtained using the vennCounts and vennDiagram functions of the limma package in R, showing the number of OTUs shared among the following five biotopes: algae (Alg), holothurian gut (HIG), sponges (Spo), sediment (Sed) and nudibranch mantle (NdX).

OTUs assigned to the phylum Tenericutes (e.g. OTU-2), which were highly abundant in selected biotopes and often absent in other biotopes. OTUs found across a range of biotopes included OTUs assigned to phyla that have been deemed to be indicator phyla of HMA sponges, such as Chloroflexi, Acidobacteria, and Poribacteria (Schmitt et al. 2011a;

2011b; Moitinho-Silva et al. 2017). Despite, for example, the relatively high abundance of Chloroflexi in HMA sponges (Fig. 6.3b and Appendix 6.1), the most abundant Chloroflexi OTUs were also present in most biotopes, albeit at lower relative abundances (Appendix 6.9). This same pattern held for other abundant phyla, e.g. Acidobacteria and Actinobacteria, but also for less abundant phyla, including Poribacteria, of which OTUs were found in relatively low numbers in a large number of biotopes (Appendix 6.10). In the present study, OTUs assigned to phyla including Chloroflexi, Acidobacteria, Actinobacteria, and Poribacteria were present in most biotopes, although they were particularly abundant in HMA sponges, sponge denizens and nudibranchs (Appendix 6.1).

A large amount of variation in the adonis analysis (~70%) remained unexplained. This is, in part, due to the pronounced overlap among samples from different biotopes or a separation between different groups or species within the same biotope. Within algae, for example, specimens of *Halimeda* sp. were compositionally distinct from other algal species and had lower OTU richness and evenness (Appendix 6.1). Sponges, in turn, included samples of the species *Acanthella cavernosa*, *Echinodictyum asperum*, *Ptilocaulis spiculifer,* and *Stylissa carteri* that clustered with seawater samples (high axis 1 and low axis 2 values; Fig. 6.4). Species of these genera have been previously identified as low microbial abundance (LMA) sponges (Gloeckner et al. 2014). Other sponge samples clustered together with a subset of samples from the sponge denizens and nudibranch gut and mantle biotopes (high axis 1 and high axis 2 values). These were all from the HMA sponges *A. lobata*, *H. erectus,* and *X. testudinaria*. Other samples of sponges appeared to house prokaryote communities intermediate in composition between these two previous clusters (high axis 1 and intermediate axis 2 values). These included the agelasids *Agelas nemoechinata* and *Acanthostylotella cornuta*. Finally, a number of sponge samples were compositionally similar to samples from other host taxa with intermediate axis 1 and 2 values (Fig. 6.3 and (Appendix 6.1). These included samples of *Haliclona cymaeformis*, *Suberites diversicolor* and *Hymeniacidon* sp. (Appendix 6.1).

HMA sponges have low richness but high evenness

In general, there was a positive linear relationship between richness and evenness, among biotopes but also within biotopes (Fig. 6.5a). This figure also highlights that, although there was a continuous variation in prokaryote OTU richness among samples, there appear to be high and low diversity host species, in addition to host species of intermediate diversity. Species hosting some of the richest prokaryote communities included the sea cucumber *H. hilla* (2260 ± 383 OTUs; mantle; n = 7), the chiton *L. japonica* (2001 ± 439 OTUs; n = 3) and the alga *Padina* sp. (2099 ± 267 OTUs; n = 3). In contrast, some of the least diverse prokaryote communities were found in the soft coral *Cladiella* sp. (170 ± 58 OTUs; n = 4) and the gut (218 ± 182 OTUs; n = 3) and mantle (311 ± 114 OTUs; n = 4) of the nudibranch *P. picta*. Species of intermediate diversity included the sponge *E. asperum* (801 ± 311 OTUs; n = 3) and the sea urchin *D. savignyi* (764 ± 113 OTUs; n = 5). The large standard deviations in richness values

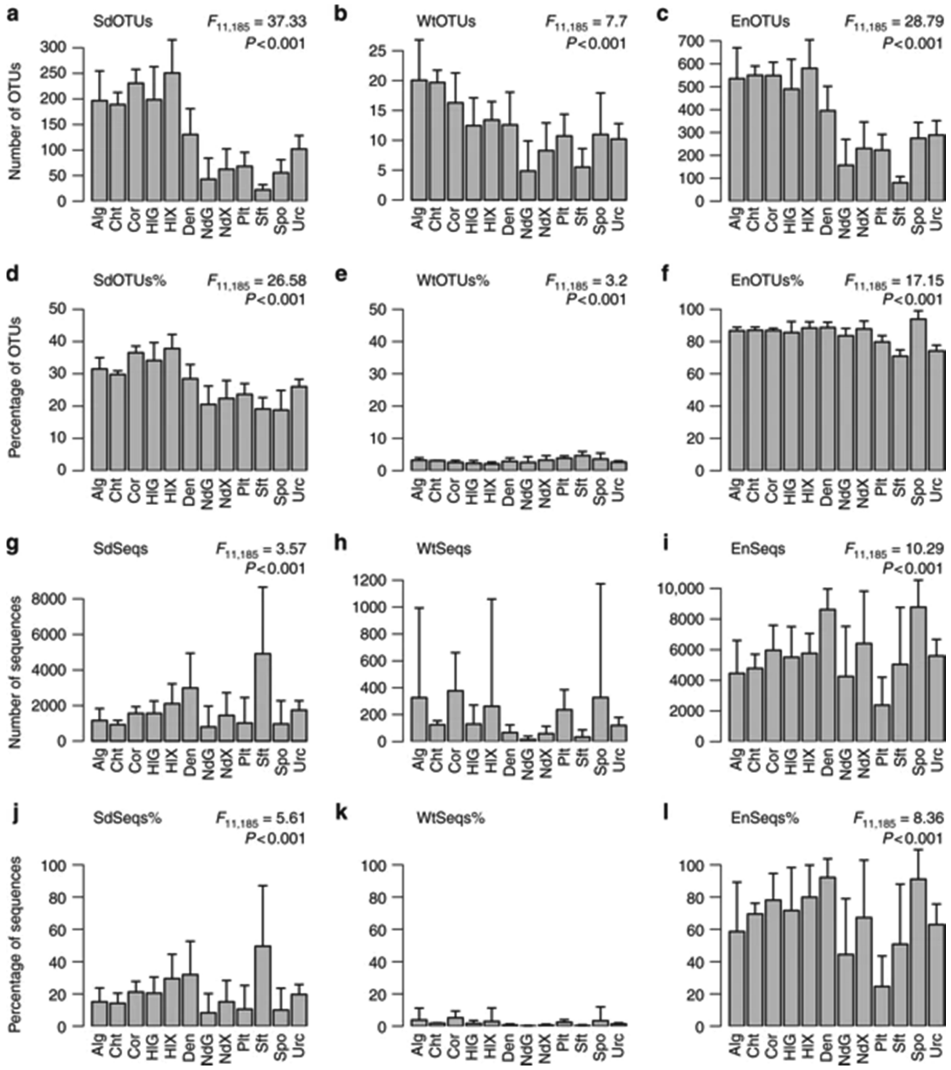


Figure 6.6. Mean counts and percentages of sediment, seawater and environmental OTUs in selected hosts. Error bars represent a single standard deviation. Codes on the *x*-axis represent algae (Alg), chitons (Cht), stony corals (Cor), sea cucumber gut (HlG), sea cucumber mantle (HlX), sponge denizens (Den), nudibranch gut (NdG), nudibranch mantle (NdX), flatworms (Plt), soft corals (Sft), sponges (Spo) and sea urchins (Urc). a Number of $OTUs_{100}$ shared with sediment only (SdOTUs), b number of $OTUs_{100}$ shared with seawater only (WtOTUs), cnumber of $OTUs_{100}$ shared with sediment and/or seawater (EnOTUs), d percentage of $OTUs_{100}$ shared with sediment only (SdOTUs%), e percentage of $OTUs_{100}$ shared with seawater only (WtOTUs%), f percentage of $OTUs_{100}$ shared with sediment and/or seawater (EnOTUs%), g number of sequences shared with sediment only (SdSeqs), h number of sequences shared with seawater only (WtSeqs), i number of sequences shared with sediment and/or seawater only (EnSeqs), j percentage of sequences shared with sediment only (SdSeqs%), k percentage of sequences shared with seawater only (WtSeqs%) and lpercentage of sequences shared with sediment and/or seawater (EnSeqs%). Results of the GLM analyses are presented in the top right of the subfigures when significant.

within species, particularly in high diversity hosts, highlights that there was also substantial variation within host species. Certain species also deviated from the general trend of increasing richness and evenness. This was most apparent with species in the 'HMA' cluster, *A. lobata*, *H. erectus* and *X. testudinaria*, in addition to certain nudibranch and sponge denizen samples, that were characterized by relatively low richness, but high evenness (Fig. 6.5a; encircled in red; (Appendix 6.1).

Is everything everywhere?

In order to study the distribution of OTUs among biotopes, we created a subset of the total dataset only including OTUs with >100 sequences (hereafter called $OTUs_{100}$; Appendix 7.12). This subset included 1731 $OTUs_{100}$ and 1,922,781 sequences (89% of all sequences). In this subset, only a very small percentage (1.2%; 21 $OTUs_{100}$) of $OTUs_{100}$ were restricted to a single biotope and less than 3.9% (69 $OTUs_{100}$) were restricted to one or two biotopes (Fig. 6.5b and Appendix 6.12). Of the 21 $OTUs_{100}$ restricted to a single biotope, all except three (restricted to the sea cucumber mantle) were only found in sponges. Thirty-four of the 48 $OTUs_{100}$ restricted to two biotopes were also found in sponges and another biotope. An additional 11 were found in the sea cucumber gut and/or mantle biotopes (Appendix 6.12).

The 21 $OTUs_{100}$ restricted to a single biotope, give us a new look into the rare members of the coral reef prokaryote metacommunity. The total abundance of those $OTUs_{100}$ varied from 102 (0.005% of $OTUs_{100}$ sequences) to 905 (0.11%) sequences (Appendix 6.12). The most abundant of these (OTUs-579) was restricted to sponges and assigned to the Latescibacteria phylum with only 81% sequence similarity to an organism previously obtained from a deep-sea octocoral (Gb-Acc: DQ395794). The most abundant $OTUs_{100}$ restricted to two biotopes included OTUs 71, 550 and 762. OTUs 550 and 762 were restricted to the sponge and sediment biotopes while OTU-71 was restricted to the sea cucumber and nudibranch gut biotopes. OTU-71, assigned to the gammaproteobacterial order HTA4, had 92% sequence similarity to an organism obtained from black deposit in a lava tube from a cave in the Canary Islands (Gb-Acc: LT702969). OTU-550, assigned to the Caldilineaceae (Chloroflexi), had 95% sequence similarity with an organism obtained from the sponge *Agelas dilatata* (Gb-Acc: EF076192). OTU-762, assigned to the Gemmatimonadetes, had 98% sequence similarity with an organism obtained from the sponge *Amphimedon compressa* (Gb-Acc: GU984210).

In Fig. 6.5c, it can be seen that there is both a wide variation in the number of $OTUs_{100}$ found in a single biotope and a rapid increase in the number of total $OTUs_{100}$ sampled as biotopes are added. To explore this further, we assessed the number of $OTUs_{100}$ shared among biotopes (Fig. 6.5d). Figure 6.5d shows the numbers of $OTUs_{100}$ shared among five biotopes, namely, sediment, the sea cucumber gut, algae, sponge and nudibranch mantle biotopes. All but 4 $OTUs_{100}$ (99.8% of all $OTUs_{100}$; Fig. 6.5d) were found in these five biotopes. These five biotopes shared 867 $OTUs_{100}$, while 2 $OTUs_{100}$ were only found in sediment, 0 in algae, 9

in the nudibranch mantle, 32 in sponges and 46 in the sea cucumber gut. Note that these $OTUs_{100}$ may be present in other biotopes. Sponges shared 59 $OTUs_{100}$ with sediment, which were not shared with the other biotopes compared to 9 $OTUs_{100}$ shared between sediment and the sea cucumber gut. Note that just three biotopes, namely, the sea cucumber gut, sponges and nudibranch mantle encompassed all but 6 $OTUs_{100}$ (99.7% of all $OTUs_{100}$).

Environmental OTUs in host-associated prokaryote communities

In order to study the influence of seawater, sediment and the broader surrounding environment (sediment and seawater) on prokaryote composition in our host biotopes, we assessed the number and percentage of $OTUs_{100}$ in each host that were also found in (1) sediment but not seawater (hereafter known as sediment $OTUs_{100}$), (2) seawater but not sediment (hereafter known as seawater $OTUs_{100}$) and (3) sediment and/or seawater (hereafter known as environmental $OTUs_{100}$). Note that category 3 (sediment and/or seawater) also includes all $OTUs_{100}$ of categories 1 and 2.

Significantly more sediment $OTUs_{100}$ were recorded in algae, chitons, stony corals and the sea cucumber gut and mantle than all other biotopes with the exception of the sponge denizen and sea urchin biotopes (Fig. 6.6a and Appendix 6.3). This also held as a percentage of total $OTUs_{100}$ (Fig. 6.6d). The number of seawater $OTUs_{100}$ was highest in the algae and chiton biotopes and significantly more so than in the nudibranch gut and mantle, sponge and soft coral biotopes (Fig. 6.6b and Appendix 6.3). Algae, chitons, stony corals and the sea cucumber gut and mantle also housed significantly more environmental $OTUs_{100}$ than the nudibranch gut and mantle, flatworm, soft coral and sponge biotopes (Fig. 6.6c). However, the percentage of environmental $OTUs_{100}$ was significantly higher in sponges than all other biotopes, except chitons (Fig. 6.6f). Sponges housed a mean of 93.8 ± 3.5% (representing 91.1 ± 18.3% of $OTUs_{100}$ sequences; Fig. 6.6l; n = 63) environmental $OTUs_{100}$ compared, for example, to just 71.0 ± 5.2% (50.8 ± 37.2% of $OTUs_{100}$ sequences; n = 4) for soft corals, 74.2 ± 3.5% (62.9 ± 12.7% of $OTUs_{100}$ sequences; n = 5) for sea urchins and 79.8 ± 3.9% (24.6 ± 18.9% of $OTUs_{100}$ sequences; n = 7) for flatworms. Four of the most abundant OTUs in flatworms (OTUs 33, 40, 126 and 1761) and two in soft corals (OTUs 14 and 17) were only found in host-associated biotopes and were not found in seawater or sediment, explaining the low percentages of environmental sequences in both biotopes (Fig. 6.4).

Although, on average, almost 94% of the $OTUs_{100}$ recorded in sponges were found in the surrounding environment (whether sediment or seawater), there was pronounced variation among sponge species. More than 97% of the $OTUs_{100}$ of *E. asperum*, and *S. carteri* were present in the surrounding environment compared to just 79.7 ± 5.2% of *H. cymaeformis* (n = 4), 86.0 ± 0.3% of *Paratetilla* sp. (n = 2) and 86.6 ± 2.6% of *Hymeniacidon* sp. (n = 4) $OTUs_{100}$. For the HMA sponges, 95.8 ± 1.9% of *A. lobata* (n = 2), 96.3 ± 1.1% of *H. erectus* (n = 9) and 91.5 ± 1.1% of *X. testudinaria* (n = 9) $OTUs_{100}$ were found in the surrounding environment.

The very high prevalence of 'environmental' OTUs in certain sponge species would appear to support the prevalence of horizontal transmission in sponge-prokaryote dynamics. However, sponges may also seed the abiotic environment with their prokaryote symbionts.

DISCUSSION

The present study revealed pronounced differences in composition and diversity among host-associated biotopes. The great majority of $OTUs_{100}$, however, were recorded in multiple biotopes and a large percentage of OTUs were shared with environmental samples (sediment and/or seawater) with the highest percentage found in sponges. Despite the prevalence of environmental OTUs in sponges, there was pronounced compositional variation between sponges and other host taxa and among sponge species. Certain species, for example, housed prokaryote communities similar to seawater (LMA sponges) while others (HMA sponges) housed communities similar to those found in certain samples of nudibranchs and sponge denizens.

A number of studies have previously remarked on the greater compositional similarity of the prokaryote communities of HMA as opposed to LMA sponges and the greater prevalence of transient (seawater) bacteria in the latter (Weisz et al. 2007; Kamke et al. 2010; Erwin et al. 2015; Ribes et al. 2015). LMA sponges have also been shown to be dominated by different sets of highly abundant OTUs and sometimes even a single dominant OTU (de Voogd et al. 2015; 2018; Cleary et al. 2015a; 2018; Croué et al. 2013; Giles et al. 2013; Knobloch et al. 2019). Compare this to the prokaryote communities of the HMA sponge species *X. testudinaria* where the core community of 44 specimens sampled across the vast expanse of the Indo-Pacific region consisted of 71 OTUs representing 57.5% of sequences on average (Chapter 4 of this thesis).

The greater evenness of HMA sponge species observed in the present study and other studies may help to explain the greater similarity and limited prevalence of transient bacteria in these sponges (Erwin et al. 2015). Importantly, species evenness has been shown to be positively related to invasion resistance, presumably by limiting the invaders access to available resources (De Roy et al. 2013). The question remains, however, as to why HMA sponges house more even (and compositionally similar) prokaryote communities than LMA sponges. Previous studies have shown that certain sponge species are able to transmit microorganisms through their larvae (vertical transmission) and suggested that this plays an important role in structuring the prokaryote community (Taylor et al. 2007b; Schmitt et al. 2008; Leite et al. 2017). Other studies have focused on horizontal transmission, e.g. from water column to sponge (Sipkema et al. 2015) and the ability of sponges to selectively recruit specific microbial symbionts from seawater (Taylor et al. 2007b; Adair and Douglas 2017; Webster and Thomas 2016). The actual degree to which the sponge prokaryote community is shaped by both forms of transmission, however, remains largely unknown.

The compositional similarity between certain sponge samples and samples of nudibranchs and sponge denizens suggests that sponges may influence the prokaryote composition of organisms that live on or within them or that feed on them. The sponge denizen biotope included sea cucumbers and barnacles that lived within or on the sponge, presumably for much of their life (Hammond and Wilkinson 1985; van Syoc and Newman 2010). Nudibranchs, however, are more mobile and may represent vectors carrying microorganisms from one sponge to the other. Nudibranchs also come into intimate contact with their sponge prey during feeding whereby certain species evert and extend their pharyngeal bulb deep into the sponge (van Alphen et al. 2011). During this process, they are also able to sequester toxins from the sponge for their own defence (Proksch 1994). Our results indicated that the gut and/or mantle prokaryote communities of specimens from certain nudibranch species (*Doriprismatica atromarginata*, *Phyllidiella pustulosa*, *Phyllidiella nigra*, *Phyllidia ocellata* and *Phyllidia elegans*) closely resembled that of sponge prokaryote communities. All of these nudibranch species have been recorded feeding on sponges (Fusetani et al. 1992; Fontana et al. 1999; van Alphen et al. 2011; Wright 2003). A number of these specimens were also collected from sponges while diving. Specimens of the sea cucumber *Synaptula* sp., a sponge denizen sampled from *X. testudinaria*, housed a prokaryote community similar to that of the 'HMA' sponge cluster, which included *X. testudinaria*. Members of the genus *Synaptula* are often common in coral reef habitat, particularly in association with sponges and can sometimes be so abundant that they cover the sponge's surface. They have also been shown to be able to exploit sponge exudates (Hammond and Wilkinson 1985). Interestingly, the barnacle *Acasta* sp., which was collected within *X. testudinaria*, was the only sponge denizen barnacle that also housed a prokaryote community similar to that of members of the 'HMA' sponge cluster. The other sponge barnacles were collected within samples of the sponge species *Dasychalina fragilis*, *Agelas cavernosa* and *Cinachyrella* sp.

The similarity between the prokaryote communities of sponges and the guts of certain nudibranch samples may be an indication that the nudibranch gut communities are dominated by transient microorganisms derived from their preferred food source, namely sponges (Proksch 1994; van Alphen et al. 2011). An individual's diet can have a profound effect on gut prokaryote composition (David et al. 2014; Carmody et al. 2015). This difference can extend to species, whereby there are marked differences in gut microbiome composition among mammal species with different diets (Groussin et al. 2017; Nishida and Ochman 2018). This distinction appears to apply to nudibranchs, whereby the gut and mantle prokaryote communities of species known to feed on sponges closely resembled that of certain sponge species (Fig. 6.3). It would be interesting to test how different diets (e.g. different sponge species) affect the nudibranch prokaryote community.

The very high number of OTUs shared among different biotopes would appear to lend support to the 'everything is everywhere but the environment selects' hypothesis of Baas

Becking (1934). In line with this, the very high richness and evenness of sediment suggest that it may function as a microbial seed bank. There was also considerable compositional similarity between sediment samples and high diversity host samples of algae, stony corals and sea cucumbers among others. In contrast, seawater samples were only compositionally similar to samples of certain sponge species. Previously, Cleary and Polónia (2018) also showed that populations of mussels inhabiting Indonesian marine lakes and mangroves shared much more OTUs with sediment than with seawater and were compositionally more similar to sediment than to seawater. Gibbons et al. (2013) previously suggested that the marine biosphere maintains a persistent microbial seed bank. In their scenario, all microbes are found everywhere due to the immensity and persistence of this seed bank, and apparent local or host-associated endemism is merely a result of insufficient sequencing. Community structure is, thus, a function of relative abundance rather than the presence or absence of certain microbial taxa. The presence of such a seed bank has repercussions for ecological theory, given the limited importance of long-distance dispersal and the ability of low abundance populations to rapidly expand when the appropriate environment is encountered (Gibbons et al. 2013). In the global marine environment, hydrographic parameters of seawater masses, furthermore, greatly contribute to the dispersion of sediment microbial communities at regional and global scales, although microbial cell dispersion is highly dependent on the ability to tolerate stress (Galand et al. 2009; De Rezende et al. 2013).

Although wide in scope, the present study only represents a small fraction of marine species in the coral reef environment and even in this dataset, there was considerable variation among species within biotopes. Much more research is needed to understand the variation in microbial composition of taxa such as sea cucumbers, flatworms, algae, and nudibranchs. A large amount of time and resources have been spent studying the prokaryote communities of a limited number of taxa leaving large gaps in our knowledge of the coral reef metacommunity. Sponges have been deemed major contributors to total microbial diversity in the world's oceans, and are considered to be reservoirs of exceptional microbial diversity2 without, however, having actually studied other host taxa in detail. In coral reefs, sponges do not appear to stand alone as the main contributors to total prokaryote diversity as this study highlights; other biotopes host more diverse prokaryote communities, e.g. sea cucumbers. The present study shows that sponges are only one, albeit an interesting, component of a much larger coral reef metacommunity.

ACKNOWLEDGEMENTS

This work was supported by European Funds through COMPETE [FCOMP-01-0124-FEDER-008657] and by National Funds through the Portuguese Foundation for Science and Technology (FCT) within the LESS CORAL [PTDC/AAC-AMB/115304/2009] and Ecotech-Sponge (PTDC/BIAMIC/6473/2014 – POCI-01-0145-FEDER-016531) projects. This work is also part of the research programmes NWO-VIDI with project number 16.161.301 and ASPASIA

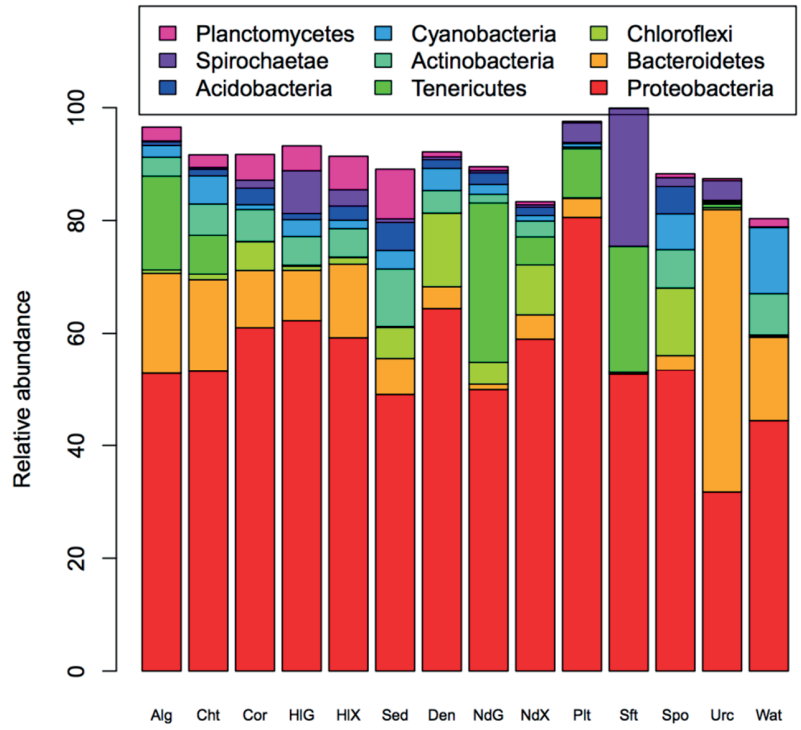
(015.010.030), which are both (partly) financed by the Netherlands Organisation for Scientific Research (NWO). Thanks are due for financial support to CESAM (UID/AMB/50017 - POCI-01-0145-FEDER-007638), to FCT/MCTES through national funds (PIDDAC), and co-funding by FEDER, within the PT2020 Partnership Agreement and Compete 2020. Francisco J.R.C. Coelho (postdoctoral scholarship: SFRH/BPD/92366/2013), Ana R.M. Polónia (postdoctoral scholarship: SFRH/BPD/117563/2016) and Marina R.S. Ferreira (Ph.D. scholarship: SFRH/BD/114809/2016), were supported by scholarships funded by FCT, Portugal within the Human Capital Operational Programme (HCOP), subsidized by the European Social Fund (ESF) and national funds (MCTES). This research was facilitated by the generous support of the Ministry of Science and Technology (MOST) and the Marine National Parks Headquarter (MNPH), Taiwan to Y.M.H. (MOST 105-2621-B-346-002 and MNPH 104403). All specimens collected in Taiwan were under a permit (No. 20160316364) issued by MNPH. The authors would also like to acknowledge Ana Cecilia Pires, Helder Gomes, Bastian Reijnen and Niels van der Windt for their support in the laboratory and Devrim Gunsel Zahir, Chad Scott, Gregory Hanigan, Floris Cleary, Julian Cleary, Yi-Chin Wu, Yuan-Hao Lin, Ming-Hong Chang, Miao-Yin Syu and You-Hua Lin for their help in the field.

APPENDIX 6.1

https://static-content.springer.com/esm/art%3A10.1038%2Fs41467-019-09537-8/MediaObjects/41467_2019_9537_MOESM5_ESM.xls

APPENDIX 6.2

Stacked barplot of the relative abundance of the nine most abundant phyla in algae (Alg), chitons (Cht), stony corals (Cor), sea cucumber gut (HlG), sea cucumber mantle (HlX), flatworms (Plt), sediment (Sed), sponges (Spo), sponge denizens (Den), nudibranch gut (NdG), nudibranch mantle (NdX), flatworms (Plt), soft corals (Sft), sponges (Spo), sea urchins (Urc) and seawater (Wat). Source data are provided as a Source Data file.

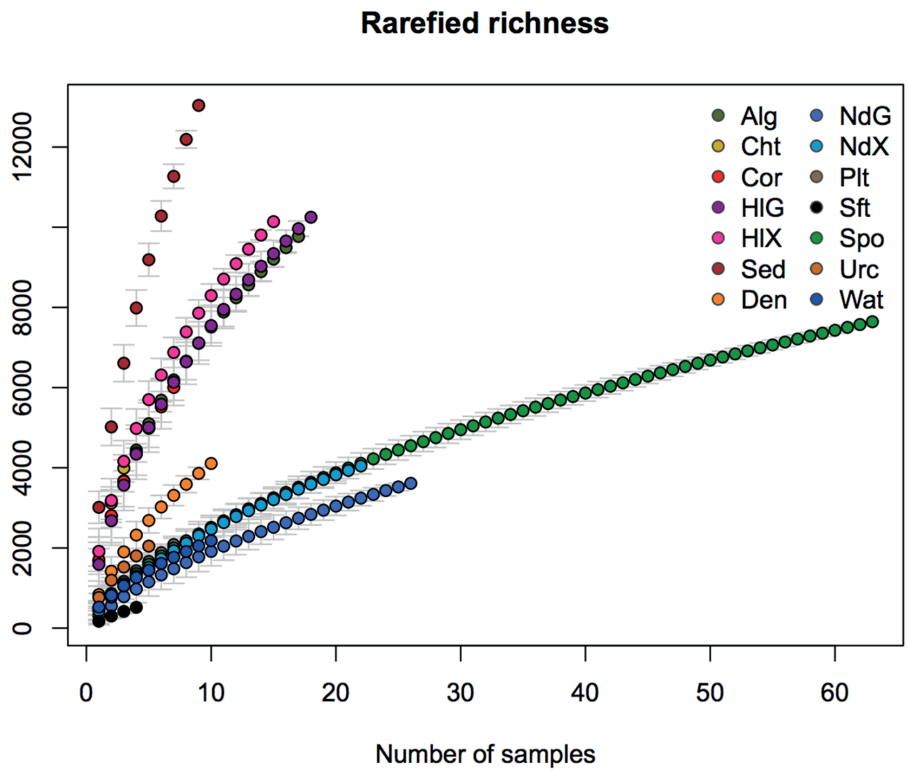


APPENDIX 6.3

https://static-content.springer.com/esm/art%3A10.1038%2Fs41467-019-09537-8/MediaObjects/41467_2019_9537_MOESM6_ESM.xls

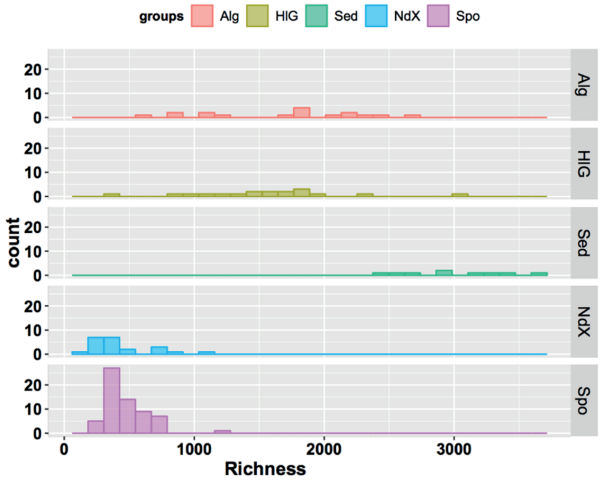
APPENDIX 6.4

Cumulative OTU richness (error bars represent 95% confidence intervals) of algae (Alg), chitons (Cht), stony corals (Cor), sea cucumber gut (HlG), sea cucumber mantle (HlX), flatworms (Plt), sediment (Sed), sponges (Spo), sponge denizens (Den), nudibranch gut (NdG), nudibranch mantle (NdX), flatworms (Plt), soft corals (Sft), sponges (Spo), sea urchins (Urc) and seawater (Wat) estimated using the specaccum function in vegan with the 'method' argument set to 'random' and 999 permutations . Error bars represent a single standard deviation. Each sample represents 10000 sequences. Source data are provided as a Source Data file.



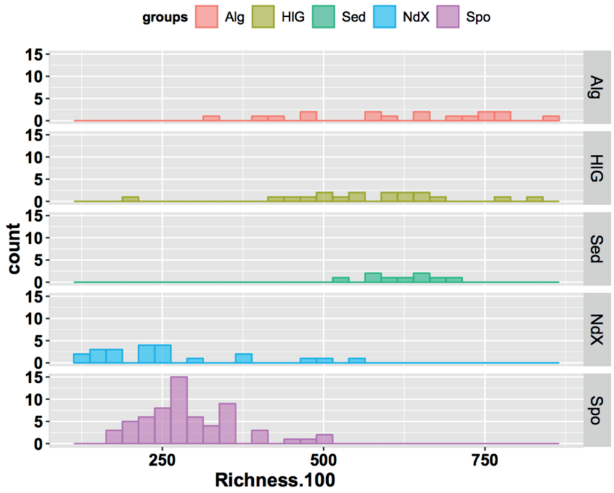
APPENDIX 6.5

Histograms of richness counts for samples of algae (Alg), sea cucumber gut (HlG), sediment (Sed), sponges (Spo) and the nudibranch mantle (NdX) made with the ggplot2.histogram function in the easyGgplot2 library in R. Source data are provided as a Source Data file.



APPENDIX 6.6

Histograms of richness counts, only including all OTUs > 100 sequences (OTUs100), for samples of algae (Alg), sea cucumber gut (HlG), sediment (Sed), sponges (Spo) and the nudibranch mantle (NdX) made with the ggplot2.histogram function in the easyGgplot2 library in R. Source data are provided as a Source Data file.



APPENDIX 6.7

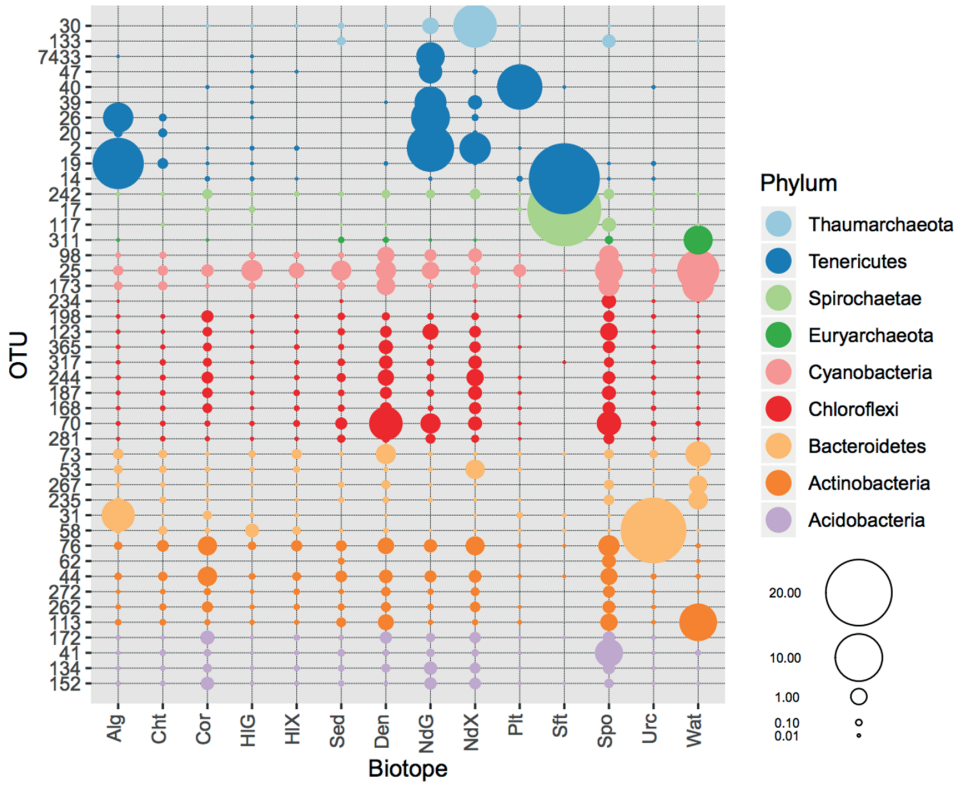
https://static-content.springer.com/esm/art%3A10.1038%2Fs41467-019-09537-8/MediaObjects/41467_2019_9537_MOESM7_ESM.xls

APPENDIX 6.8

https://static-content.springer.com/esm/art%3A10.1038%2Fs41467-019-09537-8/MediaObjects/41467_2019_9537_MOESM8_ESM.xls

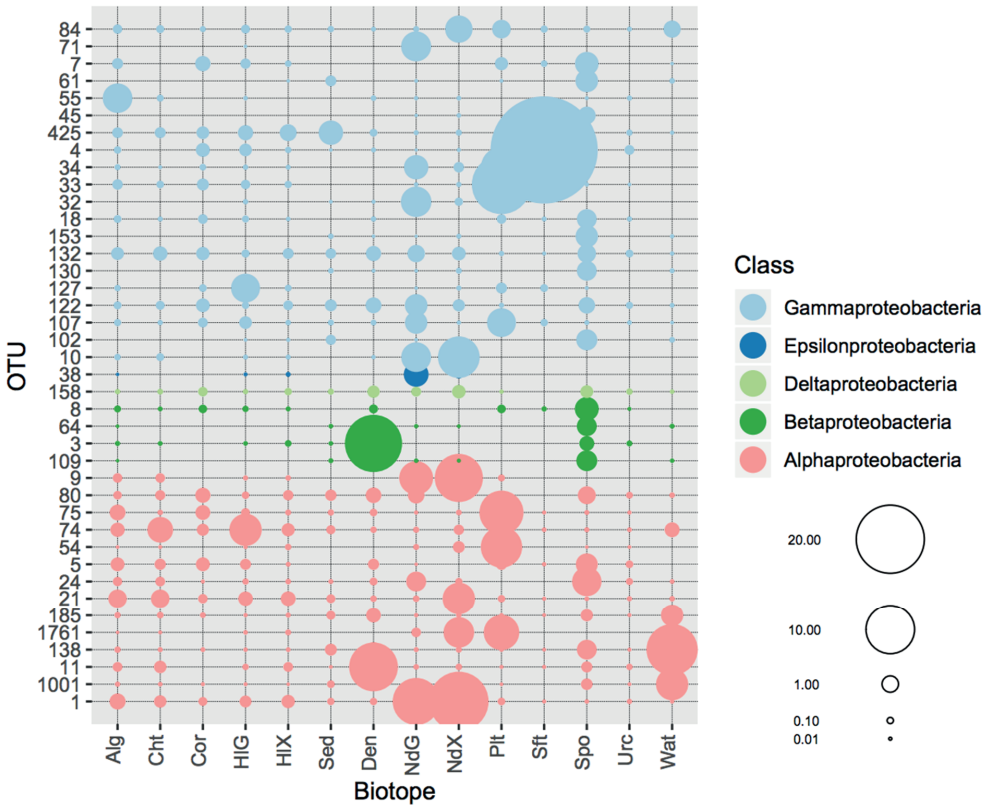
APPENDIX 6.9

Relative abundance of the most abundant OTUs, colour-coded according to prokaryote phyla, in algae (Alg), chitons (Cht), stony corals (Cor), sea cucumber gut (HlG), sea cucumber mantle (HlX), flatworms (Plt), sediment (Sed), sponges (Spo), sponge denizens (Den), nudibranch gut (NdG), nudibranch mantle (NdX), flatworms (Plt), soft corals (Sft), sponges (Spo), sea urchins (Urc) and seawater (Wat). The phyla included are: Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Euryarchaeota, Spirochaetae, Tenericutes and Thaumarchaeota. The size of the symbol is proportional to the relative abundance of sequences represented by a given OTU. Source data are provided as a Source Data file.



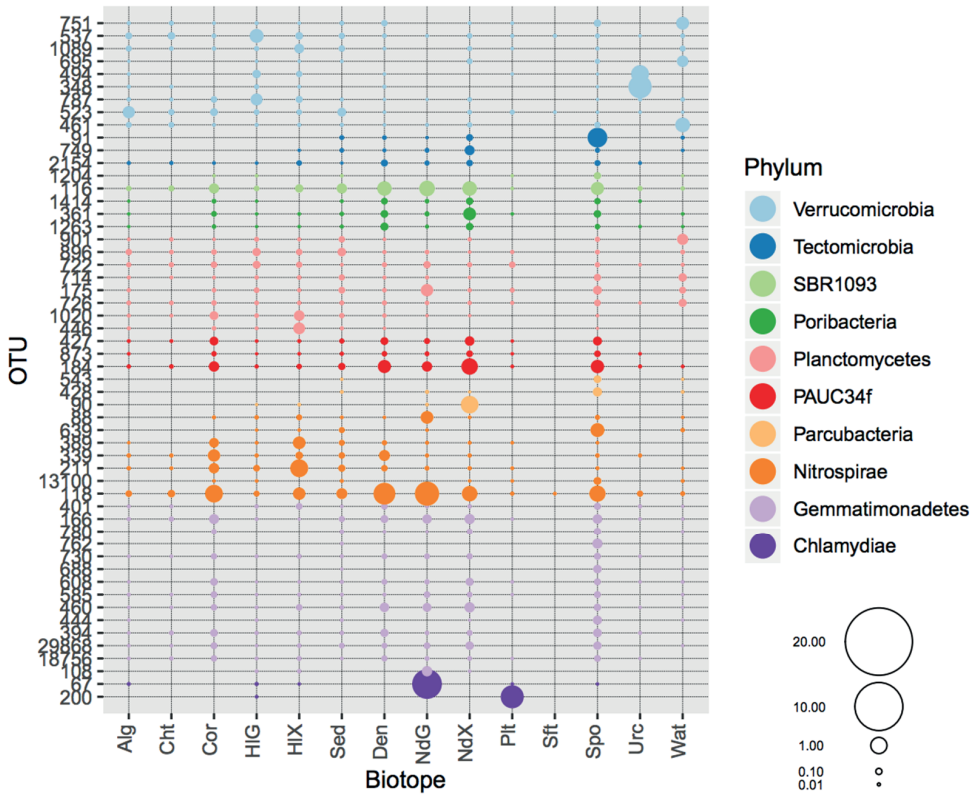
APPENDIX 6.10

Relative abundance of the most abundant OTUs, colour-coded according to proteobacterial class, in algae (Alg), chitons (Cht), stony corals (Cor), sea cucumber gut (HlG), sea cucumber mantle (HlX), flatworms (Plt), sediment (Sed), sponges (Spo), sponge denizens (Den), nudibranch gut (NdG), nudibranch mantle (NdX), flatworms (Plt), soft corals (Sft), sponges (Spo), sea urchins (Urc) and seawater (Wat). The classes included are: Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria and Gammaproteobacteria. The size of the symbol is proportional to the relative abundance of sequences represented by a given OTU. The y-axis shows the OTU id number. Source data are provided as a Source Data file.



APPENDIX 6.11

Relative abundance of OTUs, colour-coded according to prokaryote phyla, in algae (Alg), chitons (Cht), stony corals (Cor), sea cucumber gut (HlG), sea cucumber mantle (HlX), flatworms (Plt), sediment (Sed), sponges (Spo), sponge denizens (Den), nudibranch gut (NdG), nudibranch mantle (NdX), flatworms (Plt), soft corals (Sft), sponges (Spo), sea urchins (Urc) and seawater (Wat). The phyla included are: Chlamydiae, Gemmatimonadetes, Nitrospirae, Parcubacteria, PAUC34f, Planctomycetes, Poribacteria, SBR1093, Tectomicrobia and Verrucomicrobia. The size of the symbol is proportional to the relative abundance of sequences represented by a given OTU. The y-axis shows the OTU id number. Source data are provided as a Source Data file.



APPENDIX 6.12

https://static-content.springer.com/esm/art%3A10.1038%2Fs41467-019-09537-8/MediaObjects/41467_2019_9537_MOESM9_ESM.xls

SOURCE DATA

https://static-content.springer.com/esm/art%3A10.1038%2Fs41467-019-09537-8/MediaObjects/41467_2019_9537_MOESM10_ESM.xls

7

The giant barrel sponge facilitates the recovery of coral fragments after a tropical storm in Taiwan

Swierts, T., Huang, Y. M., & de Voogd, N. J.

(2018). The giant barrel sponge facilitates the recovery of coral fragments after a tropical storm in Taiwan. Coral Reefs, 37(3), 675-675.

Observations conducted in the aftermath of typhoon Soudelor (category 5) at the site Fongguie, Taiwan (23°32'16N, 119°32'41E), revealed extensive reef degradation. Corals and sponges were knocked over or broken, and many coral fragments were spread across the reef. Some of these fragments, mainly Porites, Acropora, and Pocillopora, had wound up inside the oscules of locally abundant giant barrel sponges (*Xestospongia testudinaria*). This phenomenon, where new coral fragments were lying in the sponges' osculum, was observed multiple times (Fig. 7.1a). Other giant barrel sponges had large adult branching and plating coral colonies growing in their osculum (Fig. 7.1b–d), presumably representing growth of fragments deposited inside the sponges during previous storms.

The sponges harboring large coral colonies were morphologically distorted but did not seem to be affected by the presence of these corals. Tests with fluorescent dye indicated the sponges still had a functioning pumping system, even when the osculum was entirely covered by the coral. In the Caribbean, *Xestospongia muta* has been observed to offer stony corals shelter from strong hydrodynamic forces (Hammerman and Garcia-Hernandez 2016). It seems that under the right circumstances coral larvae or coral fragments can find refuge in or on giant barrel sponges, and in the case of Taiwan the presence of the giant barrel sponges seems to enhance coral reef recovery after tropical storms.

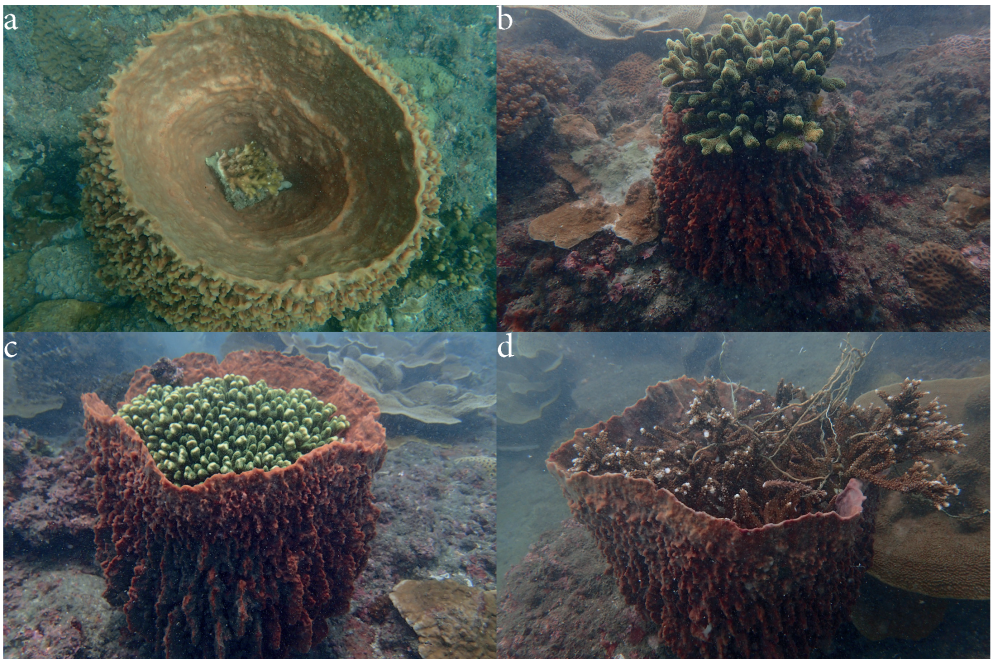


Figure 7.1. Photographs of new (a) and adult (b–d) coral fragments growing inside the Pacific giant barrel sponge *Xestospongia testudinaria.*

8

Synthesis and future directions

KEY FINDINGS

» At least nine giant barrel sponge species exist around the globe.

» The giant barrel sponge species in the Caribbean and the Indo-Pacific do not form separate monophyletic lineages.

» Different giant barrel sponge species vary in their morphological features and habitat preferences depending on location.

» Variation in the prokaryotic community composition of giant barrel sponges is primarily driven by geography. Depth, local site differences, and host-identity are also important.

» Prokaryotic microorganisms are shared among multiple coral reef biotopes and the sponge microbiome is less sponge-specific than previously thought.

EVOLUTIONARY HISTORY OF GIANT BARREL SPONGES

Sponges (phylum Porifera) have a special position in the tree of life as the sister taxa to all other multicellular animals (Morris 1993; Feuda et al. 2017; Simion et al. 2017). Despite this special position, we know remarkably little about the evolutionary history of the representatives within this phylum (Sperling et al. 2010). Sponges generally do not fossilize well, resulting in an incomplete fossil record and hampering the reconstruction of their evolutionary history (Carrera and Botting 2008). The reconstruction of the evolutionary history of sponges, therefore, depends mostly on studying the phylogenetic relationships between species presently existing on Earth, which is a method that involves a high error rate (Lieberman 2002). The identification of currently extant sponges in the Systema Porifera, the baseline publication for sponge classification, is predominantly based on the skeletal structures and spicule shapes and sizes (Hooper and van Soest 2002; Morrow and Cárdenas 2015). Molecular techniques have shown that using only morphological systematics in sponges indeed has clear shortcomings (Borchiellini et al. 2004; Gazave et al. 2010; Voigt et al. 2012; Wörheide et al. 2012; Thacker et al. 2013). Sponges with fundamentally different skeletons can be genetically closely related (Erpenbeck et al. 2006), and homoplasy can occur between unrelated species (Morrow et al. 2013). Molecular markers are thus essential to properly differentiate between sponge species and to increase the reliability of the molecular results they are ideally supported by independent markers such as chemical compound compositions or morphological characters (Slater et al. 2012). Only when species are correctly identified, accurate phylogenies can be constructed and the evolutionary history can be reconstructed. This thesis successfully identified the genetic variation in the global giant barrel sponge species complex (*Xestospongia* spp.), which are among the most studied tropical sponges and often serve as a model group in sponge research (e.g. McMurray et al. 2008; 2010; 2014; 2015; Bell et al. 2013; Swierts et al. 2013; Fiore et al. 2013; 2015;

Richards et al. 2016; McGrath et al. 2017; Villegas-Plazas et al. 2018). This attainment has led to the redefinition of the evolutionary history of this group, and a renewed understanding of their interactions with other organisms.

At least nine giant barrel sponge species exist in tropical oceans around the globe

Giant barrel sponges are among the largest reef sponges and can be found in tropical regions in the Atlantic Ocean, Red Sea, Indian Ocean and the Pacific Ocean westward of New Caledonia (McMurray et al. 2008; Setiawan et al. 2016b). They can be found from shallow reef environments of a couple of meters depth until mesophotic reefs at a depth of approximately 120 m. Three species have been described so far, one occurring across the Caribbean (*Xestospongia muta*), one throughout the Indo-Pacific region from the East coast of Africa to New Caledonia (*Xestospongia testudinaria*) and one confined to the eastern coast of Australia (*Xestospongia bergquistia*). No differentiating morphological characters have been identified between *X. muta* and *X. testudinaria* and their species delineation is solely based on the different oceans in which they live (Montalvo and Hill 2011). *Xestospongia bergquistia*, which occurs sympatrically with *X. testudinaria* in Australia, is morphologically distinct from the other two species, due to a lack of spongin fiber in their skeleton (Fromont et al. 1991). In this thesis, we assessed the phylogenetic structure of giant barrel sponges around the globe with three genetic markers and showed that these classifications are insufficient to describe the existing variation in the giant barrel sponge species complex (Swierts et al. 2013; Setiawan et al. 2016b; Chapter 2 of this thesis).

Based on the mitochondrial genes CO1 and ATP6 and the nuclear marker ATPsβ, it can be concluded that at least nine giant barrel sponge species exist around the globe (Chapter 2 of this thesis; Table 8.1). Three species occur in the Caribbean, five in the Central Indo-Pacific and/or the Western Indian Ocean and one is endemic to the Red Sea. Except for Species 5 in the Red Sea (following the classification of Chapter 2 of this thesis), all other species live sympatrically with other giant barrel sponge species.

It is not very straightforward to determine which of the species presented in this thesis represent the originally described giant barrel sponge species. The type material of *X. testudinaria* has been lost, and a sample from the Gulf of Mannar (Indian Ocean) was assigned as the neotype by Hooper and Wiedenmeyer (1994). Unfortunately, the DNA of this sample could not be successfully sequenced, however, sequencing of an associated sample from the same collection by Setiawan et al. (2016a) revealed that the sample had haplotype C2, which is characteristic for Species 1 and Species 6. It is, therefore, not possible yet to determine which of the species represents the originally described *X. testudinaria*.

For *X. muta* multiple specimens were assigned as syntypes of which Setiawan et al. (2016a) assigned one as lectotype. Unfortunately, they could not harvest any amplification product

Table 8.1. Overview of species identified in Chapter 2 of this thesis with their characterizing Cytochrome Oxidase 1 (CO1) and Adenine Triphosphate 6 (ATP6) haplotypes and their distribution.

	CO1 + ATP6	Distribution
Species 1	C1A1; C2A1	Central Indo-Pacific
Species 2	C4A3; C4A4	Central Indo-Pacific; Western Indian Ocean
Species 3	C5A2; C5A4; C6A2	Central Indo-Pacific; Western Indian Ocean
Species 4	C5A6	Central Indo-Pacific; Western Indian Ocean
Species 5	C5A7	Red Sea
Species 6	C2A8; C2A9	Western Indian Ocean
Species 7	C2A5; C9A5	Tropical Atlantic
Species 8	C8A2	Tropical Atlantic
Species 9	C5A2	Tropical Atlantic

from this specimen or from the other syntypes. Therefore, it is also not possible to assign any of the species to the original *X. muta* specimens.

The holotype of *X. bergquistia* was identified as CO1-haplotype C5 by Setiawan et al. (2016a), which is characteristic for Species 3, 4 and 5. Without sequencing additional markers it cannot be determined whether one of the identified species in this thesis represents *Xestospongia bergquistia*. This species is described from Australia, and no Australian samples have been included in this thesis, and also all specimens that were included from other locations contained spongin fiber, which *X. bergquistia* lacks. This could mean that *X. bergquistia* represents another species that was not included in this thesis. Alternatively, *X. bergquistia* could be included in this thesis, but then the lack of sponging fiber would not be a morphological character for the species outside of Australia.

The giant barrel sponge species in the Caribbean and the Indo-Pacific do not form monophyletic lineages in each of the ocean basins

Due to the absence of a fossil record of giant barrel sponges and the lack of clear species-specific morphological characteristics, the current phylogenetics of the giant barrel sponge species is the only starting point from which their evolutionary history can be reconstructed. Commonly, there is conformity between biogeography and phylogenetic patterns in marine animals, which suggests that geographic isolation is the starting point for divergence between species (Teske et al. 2011; Bowen et al. 2016). Geographic isolation was also suggested to be an important starting point for speciation in sponges, possibly in relation to ocean currents (DeBiasse et al. 2016). It was previously assumed that *X. muta* and *X. testudinaria* also diverged after geographic isolation, at least since the closing of the Isthmus of Panama three million years ago (Haug and Tiedeman 1998; Montalvo and Hill 2016; Deignan et al. 2018). Multiple geographic barriers currently exist between the Caribbean and the Indo-Pacific (Cowman and Bellwood 2013).

The prevailing geographic barriers and ocean currents cannot explain the phylogenetic relationships between the nine giant barrel sponge species that have been presented in this thesis. Remarkably, the species in the Indo-Pacific and the Caribbean do not form separate monophyletic lineages but are instead intertwined (Fig. 8.1). For instance, a Species 9 specimen from Curaçao is genetically more related to a Species 3 specimen from Indonesia, than to Species 7 and 8 on the same Curaçaoan reef. This intertwined phylogeny rules out that vicariance occurred from a single common ancestor after the geographic separation of the Caribbean and Indo-Pacific. Instead, multiple species already existed before these ocean basins were physically separated. After the physical separation of the Caribbean and Indo-Pacific, the already existing species developed into multiple lineages in each ocean basin. No giant barrel sponges occur eastwards of New Caledonia in the Pacific Ocean, and there is no evidence of recent stepping stones connecting the Caribbean and Indo-Pacific species. This implies that the general assumption that the most recent barrier, the closing of the Isthmus of Panama, was the instigating event of vicariance, is unsubstantiated (Chapter 2 of this thesis).

Considering the long evolutionary history of approximately 635 million years of sponges as a whole (Love et al. 2009), it is not out of the ordinary to look further back than three million years, when the closing of the Isthmus of Panama occurred, for events initiating speciation in giant barrel sponges. Events like the Terminal Tethyan Event, approximately 25 million years ago, should be considered as the starting point of vicariance between the giant barrel sponge species currently living in the Caribbean and Indo-Pacific. It is not clear which processes resulted in the existence of multiple sympatric giant barrel sponge species before the Indo-Pacific and the Caribbean became physically separated. The species may have diverged after the development of a geographic barrier that disappeared after the speciation was completed. This could, for example, be related to changing sea levels (Haq et al. 1987) or plate tectonics temporarily limiting genetic exchange between populations (Briggs 1999).

Alternatively, pre- or post-zygotic barriers may have prevented sympatric groups of individuals of a common ancestor from exchanging genes (Bickford et al. 2007). Reproductive asynchrony, for example, can result in limited gene flow between subpopulations of benthic marine organisms that release gametes at different times (Chamberland et al. 2017). In corals, closely related species can spawn at different seasons within years (Dai et al. 2000; Ohki et al. 2015; Rosser et al. 2015) or at different hours within a day (Levitan et al. 2004). Similar to many coral species, giant barrel sponges are reproducing during mass spawning events which they are believed to do once a year (Ritson-Williams et al. 2005). Such spawning events, however, have been recorded in different seasons in both the Caribbean and the Indo-Pacific. The spawning dates of *Xestospongia testudinaria* and *Xestospongia bergquistia* at the Great Barrier Reef in Australia were consistently separated by at least 15 days (Fromont and Bergquist 1994). Furthermore, in the Indo-Pacific, mass spawning events have been

documented in August of 1989 in the Banda Sea (Sarano 1991), in July 1997 in southwest Sulawesi (pers. comm. Prof. Dr. N de Voogd), in July 2015 in Komodo (Röthig & Voolstra 2016) and in February 2012 in Lembeh Strait (Swierts et al. 2013). These observations did not only occur in different seasons, but also at different times during the day. In the Caribbean, *Xestospongia muta* was observed to spawn in March in Belize (Ritson-Williams et al. 2005), but also in August in Florida (NOAA, 2006) and in May and November in Curaçao (pers. comm. Dr. Mark Vermeij). The timing of spawning events may thus act as a potential reproductive barrier between the different sympatric giant barrel sponge species (Fromont and Bergquist 1994). However, it is not clear whether the observed temporal variation in the spawning events of Caribbean and Indo-Pacific sponges corresponds with species identity, as is true for *X. testudinaria* and *X. bergquistia* in Australia. Furthermore, even if reproductive asynchrony is a more general phenomenon in sympatric giant barrel sponge species, it is not known whether this is a cause or a result of speciation.

The fact that no differentiating morphological characters were ever described between *X. muta* and *X. testudinaria* makes sense in the light of their previously unrecognized intertwined evolutionary history. Looking at the full range of giant barrel sponge species, however, some morphological differences become evident in specific locations. The spicule length and width of the giant barrel sponge species are variable among sites, resulting in large spicule size ranges for each of the species with much overlap between them. Looking at the ratio between the spicule length and spicule width, however, reduces the large range in spicule size and removes much overlap and shows some interesting patterns. Preliminary and unpublished data suggests that certain lineages contain spicules with a relatively large length:width ratio. These lineages are genetically closely related (Fig. 8.1). Furthermore, in Lembeh (Indonesia), Species 3 specimens were more associated with lamellar or digitate outer structures and occurred mostly in habitat with turbid water, whereas Species 1 specimens had a smooth outer morphology and were found in habitat with clearer water (Swierts et al. 2013). The habitat preferences of these two species were also observed in the Spermonde archipelago (SW Sulawesi) and the Berau region (East Kalimantan) (unpublished figures of the samples from Chapter 2 of this thesis). The digitate morphology of Species 3 specimens give them higher surface:volume ratios compared to the other morphotypes which could be beneficial in turbid environments. In Australia, the two occurring species were also found to have different habitat distributions. *Xestospongia bergquista* is only found inshore, whereas *X. testudinaria* can also be found on mid-shelf reefs (Fromont and Bergquist 1994). It is important to better understand what causes the uneven distribution of morphotypes among locations, as one of the species may be better adapted to disturbed environments. As coral reef environments are becoming more and more disturbed, the competitiveness of the different species may be altered. This could explain the shifting genetic structures among Caribbean giant barrel sponges that were showed by Deignan et al. (2018).

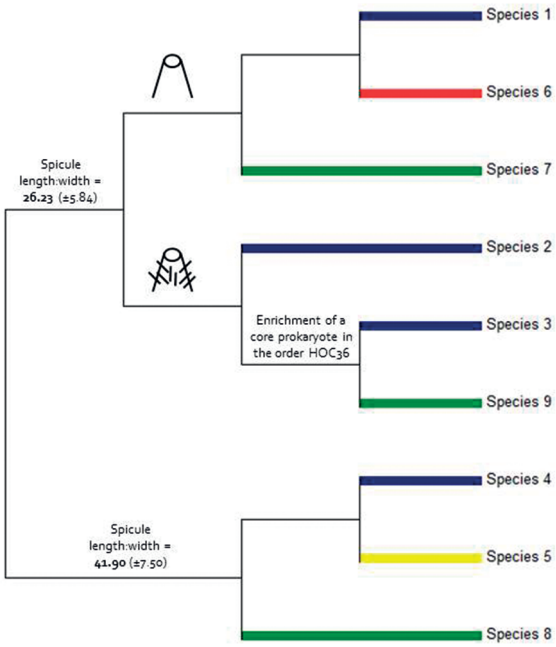


Figure 8.1. Topology of the global giant barrel sponge species complex. Branch color indicates the distribution of the species (blue = Indo-Pacific; red = Tropical Atlantic; Red = restricted to East Africa; Yellow = Red Sea).

Different giant barrel sponge species may have different morphological features and habitat preferences at specific localities

Digitate structures on giant barrel sponges were previously observed by Wilson (1925) who described them as a characteristic of an infraspecies that he named *Xestospongia testudinaria var. fistulophora* (originally *Petrosia testudinaria var. fistulophora*). Similar morphological features were present in Caribbean giant barrel sponges and these features were also corresponding with genetic variation (López-Legentil and Pawlik 2009). The Caribbean giant barrel sponges with the most pronounced digitate structures belonged to Species 9, which is most closely related to the Indo-Pacific Species 3 and Species 2, which are also associated with these digitate structures (Swierts et al. 2013; Chapter 3 of this thesis). The giant barrel sponges with the most pronounced smooth morphologies in the Caribbean belonged to Species 8, closely related to Species 4 from the Indo-Pacific (López-Legentil and Pawlik 2009). This latter species is also associated with a smooth outer surface in Tanzania, where it also has a distinctive purple color (Chapter 3 of this thesis). It seems that associations with certain morphological characters are shared between closely related lineages in both ocean basins (Fig. 8.1).

PROKARYOTIC DIVERSITY

Variation in the prokaryotic community composition of giant barrel sponges is primarily driven by geography

Sponges can maintain highly diverse and specific symbiont communities, despite the continuous influx of seawater resulting from their filter-feeding activities (Thomas et al. 2016). These associated microorganisms are known to play key roles in various metabolic processes within their sponge host, including CO2-fixation, nitrogen cycling, secondary metabolite production and processing dissolved organic matter (Hentschel et al. 2012). Due to the intricacy of their relationship, sponge hosts and their associated microbial communities are often regarded as 'sponge holobionts' (McFall-Ngai et al. 2013; Pita et al. 2018).

Several studies have focused on the drivers of the variation in the sponge microbial community compositions. Different sponge species harbor distinct microbial communities and host-identity is generally considered one of the most important drivers (Thomas et al. 2016; Souza et al. 2017; Steinert et al. 2017). This host-specificity is believed to be a result of vertical transmission in which microorganisms are passed on from parent to offspring (Schmitt et al. 2008). Many of these Operational Taxonomic Units (OTUs) were found to actually be rare members of the surrounding environment, from which they may be acquired through horizontal transfer (Reveillaud et al. 2014; Rua et al. 2018). The sponge prokaryote community is often stable in relation to spatial and temporal variation (Erwin et al. 2012; Pita et al. 2013a, 2013b, 2018; Cardenas et al. 2014; Hardoim & Costa 2014; Thomas et al. 2016; Glasl et al. 2018), although both space and time can also be drivers of the sponge microbiome (Cao et al. 2012; Turque et al. 2012; White et al. 2012; Luter et al. 2015; Weigel & Erwin 2016; Pita et al. 2018).

In giant barrel sponges, less related species from the same location have more similar prokaryotic communities, than more related specimens from different locations (Chapters 4 and 5 of this thesis). Distance was especially a strong driver of giant barrel sponge prokaryote communities on the global scale (distances >15,000 km). On a regional scale (distances 800-1,000 km), distance was also a significant driver of the prokaryotic community composition albeit less strong than on a global scale. At a local scale (distances 2-70 km), distance was not a driver of the prokaryotic community composition, however, samples from the same site harbored more similar prokaryotic communities. This suggests that local environmental differences unrelated to distance between the sites also influence the prokaryotic community. Depth is one of the drivers that influences the prokaryotic community of giant barrel sponges on a local scale, but it is expected that other unidentified environmental drivers play a role as well.

Host-identity is also a driver of the prokaryotic community composition of giant barrel sponges, although less strongly than geography (Chapter 5 of this thesis). The abundance

of most core OTUs was not related to host-identity, but one core OTU of the Proteobacterial order *HOC36* was enriched in the Indo-Pacific Species 3 and the Caribbean Species 9. These two species are characterized by mitochondrial haplotype C5 and share a more common ancestor than with any of the other lineages (Fig. 8.1). The strong affiliation between this OTU and these two giant barrel sponge species most likely originates from a time prior to their divergence. The fact that different giant barrel sponge species have different microbial community compositions, even though the variation is subtle, further supports the findings presented in this thesis that more giant barrel sponge species exist than previously thought.

Although giant barrel sponge species have most likely been genetically isolated for millions of years, many prokaryotic microorganisms are still present in all species, and giant barrel sponges maintain a rich core prokaryotic community. Previous research showed that in five other sponge species (*Carteriospongia foliascens, Cliona delitrix, Ircinia oros, Ircinia variabilis,* and *Sarcotragus fasciculatus*) a core community existed of seven to twenty core OTUs per species, defining any OTU present in ≥85% of replicates as a core OTU (Thomas et al. 2016). With the strictest definition of a core OTU (any OTU present in 100% of replicates), the core prokaryotic community of five giant barrel sponge species around the globe was composed of 71 OTUs (Chapter 5 of this thesis).

The different giant barrel sponge species have maintained similar body plans, including their skeletal and choanocyte structures. This similarity may have allowed the prokaryotes to thrive in all giant barrel sponge species (Webster and Thomas 2016). Our findings further suggest that these prokaryotic members can be transferred from one species to another by horizontal transmission, as strict vertical transmission would have resulted in co-diversification or co-evolution (Peek et al. 1998; Schmitt et al. 2008). The complex and intertwined evolutionary history of giant barrel sponges, combined with their rich microbial communities, makes them an ideal model group to study the evolution of the associations between sponges and their microbial communities.

Prokaryote microorganisms are shared among multiple coral reef biotopes and the sponge microbiome is less sponge-specific than previously thought

Compared to other marine organisms, the sponge microbiome is generally considered rich, diverse and sponge-specific (Hentschel et al. 2012; Reveillaud et al. 2014; Thomas et al. 2016). As shown by the results in Chapter 6 of this thesis, there are indeed pronounced differences between the composition and diversity of sponges and other host-associated organisms on coral reefs.

Nevertheless, were the majority of the common OTUs (i.e. the OTUs with a total abundance >0.005% of the total microbial metacommunity) on coral reefs recorded in multiple biotopes.

Many host organisms, especially sponges, shared OTUs with the sediment and/or seawater. Sponges also shared many OTUs with sponge denizens, including barnacles and sea cucumbers that live their entire life within or on the sponges, but also with nudibranchs that only interact with sponges on occasion. This compositional similarity suggests that sponges may influence the prokaryote composition of organisms that interact with them and that these microorganisms are not restricted to a sponge host for their endosymbiotic way of life.

FUTURE DIRECTIONS

I. Classification

The ability to identify specimens according to a correct species classification is essential for many biological sciences (Ebach et al. 2011). Nevertheless, the number of taxonomists is declining, potentially leading to the use of erroneous data for analyses, complicating the process of accurately linking new results to existing literature and creating extra challenges in the reproduction of experiments (Hopkins and Freckleton 2002; Joppa et al. 2011). The adoption of DNA barcodes to discover species and identify specimens has made the scientific community less dependent on taxonomists and is also less prone to errors than morphology-based taxonomy (Hebert et al. 2003; Bucklin et al. 2011). DNA barcoding has revealed many occurrences of cryptic speciation in taxa previously believed to be a single species (Hebert et al. 2004; Hajibabaei et al. 2006; Hou et al. 2018; Arroyave et al. 2019). However, many of these cryptic species complexes still await description, and multiple species thus remain pooled under one species name (Struck et al. 2018).

As shown by this thesis, giant barrel sponges are a species complex that is not yet properly resolved and described, hindering the interpretation of experimental results. This is illustrated by Montalvo and Hill (2011), who incorrectly concluded that giant barrel sponges from the Indo-Pacific and the Caribbean harbored different microbial communities due to being different species, instead of living in different oceans. To prevent misinterpretations in the future, the different species presented in this study should be given proper species names and be accompanied by clear instructions on the identification methods. This would especially benefit the large community studying the chemical compounds of giant barrel sponges and their antimicrobial potential (Zhou et al. 2010; Bayona et al. 2018).

II. Linking chemistry to microbial diversity

The majority of natural products (NPs) with pharmaceutical potential have been isolated from organisms in terrestrial environments, especially from plants and microorganisms (Chin et al. 2006; Romano et al. 2017). These organisms become increasingly depleted as a source for the discovery of new NPs, initiating a search for new NP sources (Pye et al. 2017). Since the 1950s much attention has been given to the marine domain as a source for novel NPs (Jaspars et al. 2016). The sessile nature of many benthic marine organisms has resulted in high concentrations of unique potent secondary metabolites (Pawlik 1993; Siegl et al.

2008), which are not know from the terrestrial realm (Gribble 2015; Reen et al. 2015; White et al. 2017). Within the marine domain, sponges (*Porifera*) are among the most important sources of marine NPs (Blunt et al. 2016).

A large part of these marine NPs are believed to actually be produced by sponge-associated microorganisms (Taylor et al. 2012; Fuerst 2014; Indraningrat et al. 2016; Mori et al. 2018). To develop a structured search for novel NPs from sponge-associated microbes, it is important to understand which bacterial taxa are linked to the production of bioactive NPs. Subsequently, knowledge of the inter- and intraspecific variation of these bacterial taxa and assessing how this variation translates into different chemical compound compositions helps to better target sponge-associated microorganisms as a source of novel NPs (Sacristan-Soriano & Beccero 2016).

Only a handful of peer-reviewed research papers have compared microbial and chemical variation in sponges (Hochmuth et al. 2010; Seacristan-Soriano & Beccero 2011b; 2016; Villegas-Plazes et al. 2018). Due to this hiatus, we do not understand under which conditions certain bacterial taxa may produce bioactive NPs, hindering the development of sampling designs that maximize the chance of finding novel NPs. For example, should many sponges be sampled from one site, or is it better to collect specimens from multiple sites? Such questions need to be addressed to accelerate the finding of novel NPs from sponge-associated microorganisms. Giant barrel sponges are an ideal model group to explore which drivers underlie the variation in the chemical compound composition of sponges, and which bacterial taxa may play a role in the production of these compounds. Many chemical compounds have already been isolated from giant barrel sponges around the globe (Zhou et al. 2011; Li et al. 2012; Ma et al. 2013; Ibrahim et al. 2014), some of which have pharmaceutical potential (Quah et al. 2018). Furthermore, the extraction process for metabolomics has been widely studied (Bayona et al. 2018) and they are one of the few sponge groups for which the relationships between the microbiome and the metabolome profile have partly been explored. Villegas-Plazas et al. (2018) showed that the differences of the microbial communities with respect to depth were mirrored in the profile of nine abundant metabolites. Giant barrel sponges are, thus, a proven and suitable model sponge for studies comparing the microbiome and metabolome and can help to develop a framework for future sampling designs in the search for novel NPs from sponge-associated microorganisms (Paul et al. 2019). The suitability of giant barrel sponges as a model species is greatly improved by the unraveled phylogenetic relationships between the giant barrel sponge species and the detailed assessment of the drivers of their prokaryotic community that were presented in this thesis.

III. Reproduction

This thesis shows that multiple giant barrel sponge species co-exist on many reefs in the Indo-Pacific and Caribbean. This raises questions about how the species became and remained reproductively isolated. One hypothesis is that the timing of spawning events may act as a potential reproductive barrier between the different sympatric giant barrel sponge species. This can be studied by monitoring the production and release of gametes with histological slides. This method was used to reveal temporal variation in the gamete production and release of *X. testudinaria* and *X. bergquistia* in Australia (Fromont 1994) and could be used to study whether temporal variation in the reproductive cycle is a general concept throughout the giant barrel sponge species complex.

Alternatively, gametes of the different species may be chemically incompatible. To study this, live gametes are necessary, but unfortunately, mass spawning events of giant barrel sponges cannot be predicted yet. Also, giant barrel sponges are difficult to rear in aquaria in which reproduction could be monitored. Therefore, no studies have been performed on live gametes or larvae of giant barrel sponges so far. The lack of knowledge of sponge larvae from mass spawning species as giant barrel sponges is especially striking compared to the general knowledge of the behavior of coral larvae from mass spawning coral species (Vermeij et al. 2011; Chamberland et al. 2015; Ritson-Williams et al. 2016; Richmond et al. 2018). The ability to collect live gametes and larvae from giant barrel sponges and rear them in laboratory conditions will facilitate studies focusing on the larval settlement cues, the vertical transmission of the prokaryotic community, the ability of larvae to be transported by ocean currents and the biological potential of the species to create hybrids. Questions related to these topics are among the most fundamental and are not only relevant to understanding the life history of giant barrel sponges, but also to sponges as a whole and their impact on the wider coral reef ecosystem.

IV. The sponge microbiome at mesophotic reef ecosystems

Mesophotic coral reef ecosystems are approximately located at depths between 30 and 150 m, and their ecology, composition and environmental conditions remain greatly understudied (Lesser et al. 2009; Bridge et al. 2013). They host habitat-building taxa such as corals, sponges, and algae, but more detailed information on the taxonomic composition and reef structuring processes remains scarce (Kahng et al. 2010). Sponges can be the most dominant taxa on mesophotic coral reefs, sometimes covering more than 80% of the benthos in the lower mesophotic zone (Lesser et al. 2010). Despite this abundance, even less is known about the role of sponges on mesophotic reefs than that of corals (Olson and Kellogg 2010; Kahng et al. 2014).

Mesophotic reefs experience lower temperatures and less light compared to shallow reefs and are often more isolated from anthropogenic stressors such as fishing, pollution, and

terrestrial run-off. This has led to the 'deep reef refugia hypothesis', which proposes that coral species at greater depths will not suffer mass mortalities caused by climate change related stressors (Riegl and Piller 2003). Mesophotic reefs could, therefore, act as refuges for shallow coral reefs that do suffer such mass mortalities (Lesser et al. 2009). However, recent studies have shown that many dominant shallow-water coral species are absent on mesophotic reefs and that these ecosystems have a lower species richness and diversity (Bongaerts et al. 2013; 2017; Rocha et al. 2018). This substantially lowers the potential of mesophotic reefs to act as a refuge.

Giant barrel sponges are one of the organisms that can be very abundant in mesophotic reef zones, particularly in the Caribbean (Bongaerts et al. 2015; Morrow et al. 2016). The island of Curacao, for example, is surrounded by large giant barrel sponge dominated patches at a depth of 90-120 m (pers. comm. Prof. Dr. Nicole de Voogd, Dr. Mark Vermeij). In some locations, giant barrel sponge abundance on mesophotic reefs is higher than on shallow reefs, raising questions about their ecological role in the mesophotic and whether they may actually be better adapted to mesophotic environments.

The giant barrel sponges which were collected from the mesophotic in Martinique included all three species which also occur on the shallow reefs of the Caribbean and, therefore, their adaptation to life in the mesophotic zone seems acquired by the group as a whole (Chapter 5 of this thesis). Their ability to live in such a wide range of habitat may be explained by the flexibility of their prokaryotic community. The prokaryotic community of giant barrel sponges differed significantly between shallow and mesophotic reefs (Chapter 5 of this thesis). In corals, however, most endosymbionts and prokaryotes are restricted to specific depths, and only the corals with a broad depth range revealed a high variability in their prokaryotic community (Glasl et al. 2017). Generally, the upper mesophotic zone hosts coral-endosymbionts that are shared with both the shallow and lower mesophotic reefs, whereas the lower mesophotic reef hosts corals with a specialized deepwater endosymbiont community (Bongaerts et al. 2015). In giant barrel sponges, however, many OTUs are present in both shallow and lower mesophotic specimens, while only their relative abundance differs (Morrow et al. 2016). Does this mean that mesophotic reefs can act as refuges for shallow water giant barrel sponges? Sponges have been suggested to be more resilient to climate change, and some coral reefs may, therefore, transform into sponge dominated reefs in the future (Bell et al. 2013). If mesophotic reefs have the ability to seed more sponges than corals to shallow reefs, this may further improve the competitive advantage of the former over the latter. Furthermore, does the role of the sponge prokaryotic community in the wider coral reef metacommunity change with depth? And what role do sponge holobionts on mesophotic reefs have in the various nutrient cycles? These are fundamental questions that need to be answered to understand the basic processes on mesophotic reefs. Only when we understand these processes we can predict how these reefs will be affected by the changing environment, and what their cascading effect may be on shallow reefs.

CONCLUSIONS

This thesis aimed to answer the following questions: 1. How many giant barrel sponge species exist around the globe? 2. What is the evolutionary history of the giant barrel sponge species? 3. What are the drivers of variation in the prokaryotic community composition? 4. How does the richness, diversity, and evenness of the (giant barrel) sponge prokaryotic community relate to those of other coral reef organisms? This was studied through a series of in-situ observational studies and by multiple laboratory analyses on giant barrel sponge samples collected from the tropical regions of the Atlantic, Indian and Pacific Ocean and the Red Sea. Based on molecular analysis, giant barrel sponges were found to exist of at least nine different species around the globe. It is difficult to distinguish between the species in the field based on morphology, but in some locations, the species have different morphological features. Interestingly, the giant barrel sponge species in the Caribbean and the Indo-Pacific do not form separate monophyletic lineages. In other words, a giant barrel sponge from Curacao can be genetically more related to a sponge in Indonesia, than to another specimen on the same reef. With the better resolved evolutionary history of giant barrel sponges, it became clear that the variation in their prokaryotic community composition is primarily driven by geography instead of host-phylogeny. Host-phylogeny, depth and local site difference, however, are also important drivers of their prokaryotic community. Prokaryotic microorganisms in the (giant barrel) sponge microbiome are shared among multiple coral reef biotopes and are less sponge-specific than previously thought.

REFERENCES

A

Adair, K. L., & Douglas, A. E. (2017). Making a microbiome: the many determinants of host-associated microbial community composition. *Current opinion in microbiology*, *35*, 23-29.

Akaike, H. (1974). A new look at the statistical model identification. In Selected Papers of Hirotugu Akaike (pp. 215-222). Springer, New York, NY.

Aitchison, J. C., Ali, J. R., & Davis, A. M. (2007). When and where did India and Asia collide?. Journal of Geophysical Research: Solid Earth, 112(B5).

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. Austral ecology, 26(1), 32-46.

Arroyave, J., Martinez, C. M., & Stiassny, M. L. J. (2019). DNA barcoding uncovers extensive cryptic diversity in the African long-fin tetra Bryconalestes longipinnis (Alestidae: Characiformes). Journal of fish biology.

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.

Avise J. C. & Ball R. M. (1990). Principles of genealogical concordance in species concepts and biological taxonomy. Oxford surveys in evolutionary biology, 7, 45-67.

Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., ... and Saunders, N. C. (1987). Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annual review of ecology and systematics, 18(1), 489-522.

B

Baas-Becking, L. G. M. (1934). Geobiologie; of inleiding tot de milieukunde. WP Van Stockum & Zoon NV.

Baba, K. (1994). Lauriea siagiani, a new galatheid (Decapoda: Anomura: Galatheidae) from Bali, Indonesia. Crustacean Research, 23, 40-45.

Bak, R. P. M., Joenje, M., De Jong, I., Lambrechts, D. Y. M., and Nieuwland, G. (1998). Bacterial suspension feeding by coral reef benthic organisms. Marine Ecology Progress Series, 175, 285-288.

Bannister, R. J., Battershill, C. N., and De Nys, R. (2012). Suspended sediment grain size and mineralogy across the continental shelf of the Great Barrier Reef: Impacts on the physiology of a coral reef sponge. Continental Shelf Research, 32, 86-95.

Bayer, K., Jahn, M. T., Slaby, B. M., Moitinho-Silva, L., & Hentschel, U. (2018). Marine Sponges as Chloroflexi Hot Spots: Genomic Insights and High-Resolution Visualization of an Abundant and Diverse Symbiotic Clade. MSystems, 3(6), e00150-18.

Bayona, L., Videnova, M., & Choi, Y. (2018). Increasing Metabolic Diversity in Marine Sponges Extracts by Controlling Extraction Parameters. Marine drugs, 16(10), 393.

Becking, L. E., Cleary, D. F., de Voogd, N. J., Renema, W., de Beer, M., van Soest, R. W., & Hoeksema, B. W. (2006). Beta diversity of tropical marine benthic assemblages in the Spermonde Archipelago, Indonesia. Marine Ecology, 27(1), 76-88.

Bell, J., Barnes, D., and Turner, J. (2002). The importance of micro and macro morphological variation in the adaptation of a sublittoral demosponge to current extremes. Marine Biology, 140(1), 75-81.

Bell, J. J. (2008). The functional roles of marine sponges. Estuarine, coastal and shelf science, 79(3), 341-353.

Bell, J. J., Davy, S. K., Jones, T., Taylor, M. W., and Webster, N. S. (2013). Could some coral reefs become sponge reefs as our climate changes? Global change biology, 19(9), 2613-2624.

Bell, J. J., Smith, D., Hannan, D., Haris, A., Jompa, J., and Thomas, L. (2014). Resilience to disturbance despite limited dispersal and self-recruitment in tropical barrel sponges: implications for conservation and management. PLoS One, 9(3), e91635.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the royal statistical society. Series B (Methodological), 289-300.

Bentlage, B., & Wörheide, G. (2007). Low genetic structuring among Pericharax heteroraphis (Porifera: Calcarea) populations from the Great Barrier Reef (Australia), revealed by analysis of nrDNA and nuclear intron sequences. Coral Reefs, 26(4), 807-816.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K., Meier, R., Winker, K., ... & Das, I. (2007). Cryptic species as a window on diversity and conservation. Trends in ecology & evolution, 22(3), 148-155.

Björk, J. R., Díez-Vives, C., Coma, R., Ribes, M., & Montoya, J. M. (2013). Specificity and temporal dynamics of complex bacteria–sponge symbiotic interactions. Ecology, 94(12), 2781-2791.

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.

Blunt, J. W., Carroll, A. R., Copp, B. R., Davis, R. A., Keyzers, R. A., and Prinsep, M. R. (2018). Marine natural products. Natural product reports, 35(1), 8-53.

Bongaerts, P., Frade, P. R., Ogier, J. J., Hay, K. B., Van Bleijswijk, J., Englebert, N., ... & Hoegh-Guldberg, O. (2013). Sharing the slope: depth partitioning of agariciid corals and associated Symbiodinium across shallow and mesophotic habitats (2-60 m) on a Caribbean reef. BMC Evolutionary Biology, 13(1), 205.

Bongaerts, P., Frade, P. R., Hay, K. B., Englebert, N., Latijnhouwers, K. R., Bak, R. P., ... & Hoegh-Guldberg, O. (2015). Deep down on a Caribbean reef: lower mesophotic depths harbor a specialized coral-endosymbiont community. Scientific reports, 5, 7652.

Bongaerts, P., Riginos, C., Brunner, R., Englebert, N., Smith, S. R., & Hoegh-Guldberg, O. (2017). Deep reefs are not universal refuges: reseeding potential varies among coral species. Science Advances, 3(2), e1602373.

Borcard, D., P. Legendre & P. Drapeau. 1992. Partialling out the spatial component of ecological variation. Ecology 73: 1045-1055.

Borchiellini, C., Chombard, C., Manuel, M., Alivon, E., Vacelet, J., & Boury-Esnault, N. (2004). Molecular phylogeny of Demospongiae: implications for classification and scenarios of character evolution. Molecular phylogenetics and evolution, 32(3), 823-837.

Boury-Esnault, N., Solé-Cava, A. M., & Thorpe, J. P. (1992). Genetic and cytological divergence between color morphs of the Mediterranean sponge Oscarella lobularis Schmidt (Porifera, Demospongiae, Oscarellidae). Journal of Natural History, 26(2), 271-284.

Bowen, B. W., Bass, A. L., Rocha, L. A., Grant, W. S., & Robertson, D. R. (2001). Phylogeography of the trumpetfishes (Aulostomus): ring species complex on a global scale. Evolution, 55(5), 1029-1039.

Bowen, B. W., Rocha, L. A., Toonen, R. J., & Karl, S. A. (2013). The origins of tropical marine biodiversity. Trends in ecology & evolution, 28(6), 359-366.

Bowen, B. W., Gaither, M. R., DiBattista, J. D., Iacchei, M., Andrews, K. R., Grant, W. S., ... & Briggs, J. C. (2016). Comparative phylogeography of the ocean planet. Proceedings of the National Academy of Sciences, 113(29), 7962-7969.

Bridge, T. C., Hughes, T. P., Guinotte, J. M., & Bongaerts, P. (2013). Call to protect all coral reefs. Nature Climate Change, 3(6), 528.

Briggs, J. C. (1999). Modes of speciation: marine Indo-west Pacific. Bulletin of Marine Science, 65(3), 645-656.

Briggs, J. C., & Bowen, B. W. (2012). A realignment of marine biogeographic provinces with particular reference to fish distributions. Journal of Biogeography, 39(1), 12-30.

Briggs, J. C., & Bowen, B. W. (2013). Marine shelf habitat: biogeography and evolution. Journal of Biogeography, 40(6), 1023-1035.

Brown, B. E. (1990). Damage and recovery of coral reefs affected by El Niño related seawater warming in the Thousand Islands, Indonesia. Coral reefs, 8(4), 163-170.

Bryant, D. A., & Frigaard, N. U. (2006). Prokaryotic photosynthesis and phototrophy illuminated. Trends in microbiology, 14(11), 488-496.

Bucklin, A., Steinke, D., & Blanco-Bercial, L. (2011). DNA barcoding of marine metazoa. Annual review of marine science, 3, 471-508.

Buermans, H. P. J., and Den Dunnen, J. T. (2014). Next generation sequencing technology: advances and applications. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1842(10), 1932-1941.

Burgsdorf, I., Slaby, B. M., Handley, K. M., Haber, M., Blom, J., Marshall, C. W., ... & Steindler, L. (2015). Lifestyle evolution in cyanobacterial symbionts of sponges. MBio, 6(3), e00391-15.

Burke, L., Reytar, K., Spalding, M., and Perry, A. (2011). Reefs at risk revisited.

C

Cao, H., Cao, X., Guan, X., Xue, S., & Zhang, W. (2012). High temporal variability in bacterial community, silicatein and hsp70 expression during the annual life cycle of Hymeniacidon sinapium (Demospongiae) in China's Yellow Sea. Aquaculture, 358, 262-273.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., ... & Huttley, G. A. (2010). QIIME allows analysis of high-throughput community sequencing data. Nature methods, 7(5), 335.

Cárdenas, C. A., Bell, J. J., Davy, S. K., Hoggard, M., and Taylor, M. W. (2014). Influence of environmental variation on symbiotic bacterial communities of two temperate sponges. FEMS Microbiology Ecology, 88(3), 516-527.

Carmody, R. N., Gerber, G. K., Luevano Jr, J. M., Gatti, D. M., Somes, L., Svenson, K. L., & Turnbaugh, P. J. (2015). Diet dominates host genotype in shaping the murine gut microbiota. Cell host & microbe, 17(1), 72-84.

Carrera, M. G., & Botting, J. P. (2008). Evolutionary history of Cambrian spiculate sponges: implications for the Cambrian evolutionary fauna. Palaios, 23(3), 124-138.

Chamberland, V. F., Vermeij, M. J., Brittsan, M., Carl, M., Schick, M., Snowden, S., ... & Petersen, D. (2015). Restoration of critically endangered elkhorn coral (Acropora palmata) populations using larvae reared from wild-caught gametes. Global Ecology and Conservation, 4, 526-537.

Chamberland, V. F., Snowden, S., Marhaver, K. L., Petersen, D., & Vermeij, M. J. (2017). The reproductive biology and early life ecology of a common Caribbean brain coral, Diploria labyrinthiformis (Scleractinia: Faviinae). Coral reefs, 36(1), 83-94.

Chin, Y. W., Balunas, M. J., Chai, H. B., & Kinghorn, A. D. (2006). Drug discovery from natural sources. The AAPS journal, 8(2), E239-E253.

Claesson, M. J., Wang, Q., O'sullivan, O., Greene-Diniz, R., Cole, J. R., Ross, R. P., and O'toole, P. W. (2010). Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. Nucleic acids research, 38(22), e200-e200

Cleary, D. F. (2003). An examination of scale of assessment, logging and ENSO-induced fires on butterfly diversity in Borneo. Oecologia, 135(2), 313-321.

Cleary, D. F., Mooers, A. Ø., Eichhorn, K. A., Van Tol, J., De Jong, R., & Menken, S. B. (2004). Diversity and community composition of butterflies and odonates in an ENSO-induced fire affected habitat mosaic: a case study from East Kalimantan, Indonesia. Oikos, 105(2), 426-448.

Cleary, D. F., & Priadjati, A. (2005). Vegetation responses to burning in a rain forest in Borneo. Plant Ecology, 177(2), 145-163.

Cleary, D. F., & De Voogd, N. J. (2007). Environmental associations of sponges in the Spermonde Archipelago, Indonesia. Journal of the Marine Biological Association of the United Kingdom, 87(6), 1669-1676.

Cleary, D. F. R., Oliveira, V., Gomes, N. C. M., Pereira, A., Henriques, I., Marques, B., ... & Lillebø, A. I. (2012). Impact of sampling depth and plant species on local environmental conditions, microbiological parameters and bacterial composition in a mercury contaminated salt marsh. Marine pollution bulletin, 64(2), 263-271.

Cleary, D. F., de Voogd, N. J., Polónia, A. R., Freitas, R., & Gomes, N. C. (2015a). Composition and predictive functional analysis of bacterial communities in seawater, sediment and sponges in the Spermonde Archipelago, Indonesia. Microbial ecology, 70(4), 889-903.

Cleary, D. F. R., Becking, L. E., & Polónia, A. R. M. (2015b). Composition and putative functional ecology of mussels inhabiting Indonesian marine lakes. A Van Leeuw J Microb, 107, 821-34.

Cleary, D. F., Becking, L. E., Polónia, A. R., Freitas, R. M., & Gomes, N. (2016). Jellyfish-associated bacterial communities and bacterioplankton in Indonesian Marine lakes. FEMS microbiology ecology, 92(5).

Cleary, D. F., Coelho, F. J., Oliveira, V., Gomes, N. C., & Polónia, A. R. (2017a). Sediment depth and habitat as predictors of the diversity and composition of sediment bacterial communities in an inter-tidal estuarine environment. Marine Ecology, 38(2), e12411.

Cleary, D. F., Polónia, A. R., & de Voogd, N. J. (2017b). Prokaryote composition and predicted metagenomic content of two Cinachyrella Morphospecies and water from West Papuan Marine Lakes. FEMS microbiology ecology, 94(2), fix175.

Cleary, D. F., Polónia, A. R., & de Voogd, N. J. (2018a). Bacterial Communities Inhabiting the Sponge Biemna fortis, Sediment and Water in Marine Lakes and the Open Sea. Microbial ecology, 1-15.

Cleary, D. F. R., & Polónia, A. R. M. (2018b). Bacterial and archaeal communities inhabiting mussels, sediment and water in Indonesian anchialine lakes. Antonie van Leeuwenhoek, 111(2), 237-257.

Cleary, D. F. R., Polónia, A. R. M., Becking, L. E., de Voogd, N. J., Gomes, H., & Gomes, N. C. M. (2018c). Compositional analysis of bacterial communities in seawater, sediment, and sponges in the Misool coral reef system, Indonesia. Marine Biodiversity, 48(4), 1889-1901.

Clement, M., Posada, D. C. K. A., & Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. Molecular ecology, 9(10), 1657-1659.

Cock, P. J., Antao, T., Chang, J. T., Chapman, B. A., Cox, C. J., Dalke, A., ... & De Hoon, M. J. (2009). Biopython: freely available Python tools for computational molecular biology and bioinformatics. Bioinformatics, 25(11), 1422-1423.

Coelho, F. J. R. C., Cleary, D. F., Gomes, N. C., Pólonia, A. R., Huang, Y. M., Liu, L. L., & de Voogd, N. J. (2018). Sponge Prokaryote Communities in Taiwanese Coral Reef and Shallow Hydrothermal Vent Ecosystems. Microbial ecology, 75(1), 239-254.

Concepcion, G. T., Kahng, S. E., Crepeau, M. W., Franklin, E. C., Coles, S. L., & Toonen, R. J. (2010). Resolving natural ranges and marine invasions in a globally distributed octocoral (genus Carijoa). Marine Ecology Progress Series, 401, 113-127.

Condit, R., Pitman, N., Leigh, E.G. et al. 2002. Beta-diversity in tropical forest trees. Science 295: 666 - 669.

Cowman, P. F., & Bellwood, D. R. (2013a). The historical biogeography of coral reef fishes: global patterns of origination and dispersal. Journal of biogeography, 40(2), 209-224.

Cowman, P. F., & Bellwood, D. R. (2013b). Vicariance across major marine biogeographic barriers: temporal concordance and the relative intensity of hard versus soft barriers. Proceedings of the Royal Society B: Biological Sciences, 280(1768), 20131541.

Coyne, J. A., & Orr, H. A. (2004). Speciation (Vol. 37). Sunderland, MA: Sinauer Associates.

Croué, J., West, N. J., Escande, M. L., Intertaglia, L., Lebaron, P., & Suzuki, M. T. (2013). A single betaproteobacterium dominates the microbial community of the crambescidine-containing sponge Crambe crambe. Scientific reports, 3, 2583.

Cruz, I. C., Waters, L. G., Kikuchi, R. K., Leão, Z. M., and Turra, A. (2018). Marginal coral reefs show high susceptibility to phase shift. Marine pollution bulletin, 135, 551-561.

Cuvelier, M. L., Blake, E., Mulheron, R., McCarthy, P. J., Blackwelder, P., Thurber, R. L. V., & Lopez, J. V. (2014). Two distinct microbial communities revealed in the sponge Cinachyrella. Frontiers in microbiology, 5, 581.

D

Dai, C. F., Fan, T. Y., & Yu, J. K. (2000). Reproductive isolation and genetic differentiation of a scleractinian coral Mycedium elephantotus. Marine Ecology Progress Series, 201, 179-187.

Daims, H., & Wagner, M. (2018). Nitrospira. Trends in microbiology, 26(5), 462-463.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. Nature methods, 9(8), 772.

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., ... & Biddinger, S. B. (2014). Diet rapidly and reproducibly alters the human gut microbiome. Nature, 505(7484), 559.

de Bakker, D. M., Meesters, E. H., van Bleijswijk, J. D., Luttikhuizen, P. C., Breeuwer, H. J., & Becking, L. E. (2016). Population genetic structure, abundance, and health status of two dominant benthic species in the Saba Bank National Park, Caribbean Netherlands: Montastraea cavernosa and Xestospongia muta. PloS one, 11(5), e0155969.

De Bakker, D. M., Van Duyl, F. C., Bak, R. P., Nugues, M. M., Nieuwland, G., and Meesters, E. H. (2017). 40 Years of benthic community change on the Caribbean reefs of Curaçao and Bonaire: the rise of slimy cyanobacterial mats. Coral Reefs, 36(2), 355-367.

De Goeij, J. M., Moodley, L., Houtekamer, M., Carballeira, N. M., and Van Duyl, F. C. (2008). Tracing 13C-enriched dissolved and particulate organic carbon in the bacteria-containing coral reef sponge Halisarca caerulea: Evidence for DOM-feeding. Limnology and Oceanography, 53(4), 1376-1386.

De Goeij, J. M., Van Oevelen, D., Vermeij, M. J., Osinga, R., Middelburg, J. J., de Goeij, A. F., and Admiraal, W. (2013). Surviving in a marine desert: the sponge loop retains resources within coral reefs. Science, 342(6154), 108-110.

De Rezende, J. R., Kjeldsen, K. U., Hubert, C. R., Finster, K., Loy, A., & Jørgensen, B. B. (2013). Dispersal of thermophilic Desulfotomaculum endospores into Baltic Sea sediments over thousands of years. The ISME journal, 7(1), 72.

De Roy, K., Marzorati, M., Negroni, A., Thas, O., Balloi, A., Fava, F., ... & Boon, N. (2013). Environmental conditions and community evenness determine the outcome of biological invasion. Nature communications, 4, 1383.

de Voogd, N. J., Cleary, D. F., Hoeksema, B. W., Noor, A., & van Soest, R. W. (2006). Sponge beta diversity in the Spermonde Archipelago, SW Sulawesi, Indonesia. Marine Ecology Progress Series, 309, 131-142.

De Voogd, N. J., & Cleary, D. F. (2008). An analysis of sponge diversity and distribution at three taxonomic levels in the Thousand Islands/Jakarta Bay reef complex, West-Java, Indonesia. Marine Ecology, 29(2), 205-215.

De Voogd, N. J., Cleary, D. F., Polónia, A. R., & Gomes, N. C. (2015). Bacterial community composition and predicted functional ecology of sponges, sediment and seawater from the thousand islands reef complex, West Java, Indonesia. FEMS Microbiology Ecology, 91(4), fiv019.

de Voogd, N. J., Gauvin-Bialecki, A., Polónia, A. R., & Cleary, D. F. (2018). Assessing the bacterial communities of sponges inhabiting the remote western Indian Ocean island of Mayotte. Marine Ecology, 39(6), e12517.

DeBiasse, M. B., Richards, V. P., Shivji, M. S., & Hellberg, M. E. (2016). Shared phylogeographical breaks in a Caribbean coral reef sponge and its invertebrate commensals. Journal of Biogeography, 43(11), 2136-2146.

Deignan, L. K., Pawlik, J. R., & López-Legentil, S. (2018). Evidence for shifting genetic structure among Caribbean giant barrel sponges in the Florida Keys. Marine biology, 165(6), 106.

Diaz, M. C., & Rützler, K. (2001). Sponges: an essential component of Caribbean coral reefs. Bulletin of Marine Science, 69(2), 535-546.

Douglas, A. E. (2014). Symbiosis as a general principle in eukaryotic evolution. Cold Spring Harbor Perspectives in Biology,

Duran, S., Pascual, M., & Turon, X. (2004). Low levels of genetic variation in mtDNA sequences over the western Mediterranean and Atlantic range of the sponge Crambe crambe (Poecilosclerida). Marine Biology, 144(1), 31-35.

Duran, S. & Rützler, K. (2006). Ecological speciation in a Caribbean marine sponge. Molecular Phylogenetics and Evolution, 40(1), 292-297.

E

Easson, C. G., & Thacker, R. W. (2014). Phylogenetic signal in the community structure of host-specific microbiomes of tropical marine sponges. Frontiers in microbiology, 5, 532.

Ebach, M. C., Valdecasas, A. G., & Wheeler, Q. D. (2011). Impediments to taxonomy and users of taxonomy: accessibility and impact evaluation. Cladistics, 27(5), 550-557.

Edgar, R. C., & Flyvbjerg, H. (2015). Error filtering, pair assembly and error correction for next-generation sequencing reads. Bioinformatics, 31(21), 3476-3482.

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. Bioinformatics, 27(16), 2194-2200.

Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature methods, 10(10), 996.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. Bioinformatics, 26(19), 2460-2461.

Erpenbeck, D., Breeuwer, J., van der Velde, H., & van Soest, R. (2002). Unravelling host and symbiont phylogenies of halichondrid sponges (Demospongiae, Porifera) using a mitochondrial marker. Marine Biology, 141(2), 377-386.

Erpenbeck, D., Hooper, J. N. A., & Wörheide, G. (2006a). CO1 phylogenies in diploblasts and the 'Barcoding of Life'—are we sequencing a suboptimal partition?. Molecular ecology notes, 6(2), 550-553.

Erpenbeck, D., Breeuwer, J. A. J., Parra-Velandia, F. J., & Van Soest, R. W. M. (2006b). Speculation with spiculation?—Three independent gene fragments and biochemical characters versus morphology in demosponge higher classification. Molecular Phylogenetics and Evolution, 38(2), 293-305.

Erpenbeck, D., Ekins, M., Enghuber, N., Hooper, J. N., Lehnert, H., Poliseno, A., ... & Van Soest, R. W. (2016). Nothing in (sponge) biology makes sense–except when based on holotypes. Journal of the Marine Biological Association of the United Kingdom, 96(2), 305-311.

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(5), 869-875.

Erwin, P. M., Pita, L., López-Legentil, S., & Turon, X. (2012). Stability of sponge-associated bacteria over large seasonal shifts in temperature and irradiance. Appl. Environ. Microbiol., 78(20),

Erwin, P. M., Coma, R., López-Sendino, P., Serrano, E., & Ribes, M. (2015). Stable symbionts across the HMA-LMA dichotomy: low seasonal and interannual variation in sponge-associated bacteria from taxonomically diverse hosts. FEMS Microbiology Ecology, 91(10).

Excoffier, L., Laval, G., & Schneider, S. (2005). Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evolutionary bioinformatics, 1, 117693430500100003.

F

Fan, L., Reynolds, D., Liu, M., Stark, M., Kjelleberg, S., Webster, N. S., & Thomas, T. (2012). Functional equivalence and evolutionary convergence in complex communities of microbial sponge symbionts. Proceedings of the National Academy of Sciences, 109(27), E1878-E1887.

Farris, J. S. (1972). Estimating phylogenetic trees from distance matrices. The American Naturalist, 106(951), 645-668.

Faulkner, D. J. (2001). Marine natural products. Natural product reports, 18(1), 1R-49R.

Feuda, R., Dohrmann, M., Pett, W., Philippe, H., Rota-Stabelli, O., Lartillot, N., ... & Pisani, D. (2017). Improved modeling of compositional heterogeneity supports sponges as sister to all other animals. Current Biology, 27(24), 3864-3870.

Fiore, C. L., Baker, D. M., & Lesser, M. P. (2013a). Nitrogen biogeochemistry in the Caribbean sponge, Xestospongia muta: a source or sink of dissolved inorganic nitrogen?. PLoS One, 8(8), e72961.

Fiore, C. L., Jarett, J. K., & Lesser, M. P. (2013b). Symbiotic prokaryotic communities from different populations of the giant barrel sponge, Xestospongia muta. MicrobiologyOpen, 2(6), 938-952.

Folmer, O., Black, M. B., Hoeh, W., Lutz, R., Vrijenhoek, R. C. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3:294–299.

Fontana, A., Cavaliere, P., Ungur, N., D'Souza, L., Parameswaram, P. S., & Cimino, G. (1999). New scalaranes from the nudibranch Glossodoris atromarginata and its sponge prey. Journal of natural products, 62(10), 1367-1370.

Friedrich, A. B., Fischer, I., Proksch, P., Hacker, J., & Hentschel, U. (2001). Temporal variation of the microbial community associated with the Mediterranean sponge Aplysina aerophoba. FEMS Microbiology Ecology, 38(2-3), 105-113.

Fromont, J. (1988, August). Aspects of the reproductive biology of Xestospongia testudinaria (Great Barrier Reef). In Proc of the 6th Coral Reef Symposium, Australia (Vol. 2, pp. 685-691).

Fromont, J. (1991). Descriptions of species of the Petrosida (Porifera: Demospongiae) occurring in the tropical waters of the Great Barrier Reef. Beagle: Records of the Museums and Art Galleries of the Northern Territory, The, 8, 73.

Fromont, J., & Bergquist, P. R. (1994). Reproductive biology of three sponge species of the genus Xestospongia (Porifera: Demospongiae: Petrosida) from the Great Barrier Reef. Coral Reefs, 13(2), 119-126.

Fromont, J., Kerr, S., Kerr, R., Riddle, M., & Murphy, P. (1994). Chemotaxonomic relationships within, and comparisons between, the orders Haplosclerida and Petrosida (Porifera: Demospongiae) using sterol complements. Biochemical Systematics and Ecology, 22(7), 735-752.

Frost, T. M. (1980). Selection in sponge feeding processes. In Nutrition in the Lower Metazoa (pp. 33-44). Pergamon.

Fueda, R., Dohrmann, M., Pett, W., Philippe, H., Rota-Stabelli, O., Lartillot, N., ... and Pisani, D. (2017). Improved Modeling of Compositional Heterogeneity Supports Sponges as Sister to All Other Animals. Curr Biol, 27, 1-7.

Fuerst, J. A. (2014). Diversity and biotechnological potential of microorganisms associated with marine sponges. Applied microbiology and biotechnology, 98(17), 7331-7347.

Funkhouser, L. J., & Bordenstein, S. R. (2013). Mom knows best: the universality of maternal microbial transmission. PLoS biology, 11(8), e1001631.

Fusetani, N., Wolstenholme, H. J., Shinoda, K., Asai, N., Matsunaga, S., Onuki, H., & Hirota, H. (1992). Two sesquiterpene isocyanides and a sesquiterpene thiocyanate from the marine sponge Acanthella cf. cavernosa and the nudibranch Phyllidia ocellata. Tetrahedron letters, 33(45), 6823-6826.

G

Gaikwad, S., Shouche, Y. S., and Gade, W. N. (2016). Microbial community structure of two freshwater sponges using Illumina MiSeq sequencing revealed high microbial diversity. AMB Express, 6(1), 40.

Galand, P. E., Potvin, M., Casamayor, E. O., & Lovejoy, C. (2010). Hydrography shapes bacterial biogeography of the deep Arctic Ocean. The ISME journal, 4(4), 564.

Gantt, S. E., López-Legentil, S., & Erwin, P. M. (2017). Stable microbial communities in the sponge Crambe crambe from inside and outside a polluted Mediterranean harbor. FEMS microbiology letters, 364(11), fnx105.

Gazave, E., Lapébie, P., Renard, E., Vacelet, J., Rocher, C., Ereskovsky, A. V., ... and Borchiellini, C. (2010). Molecular phylogeny restores the supra-generic subdivision of homoscleromorph sponges (Porifera, Homoscleromorpha). PloS one, 5(12), e14290.

Gazave, E., Lapébie, P., Ereskovsky, A. V., Vacelet, J., Renard, E., Cárdenas, P., and Borchiellini, C. (2011). No longer Demospongiae: Homoscleromorpha formal nomination as a fourth class of Porifera. In Ancient Animals, New Challenges (pp. 3-10). Springer, Dordrecht.

Geller, J. B., Darling, J. A., & Carlton, J. T. (2010). Genetic perspectives on marine biological invasions. Annual review of marine science, 2, 367-393.

Gibbons, S. M., Caporaso, J. G., Pirrung, M., Field, D., Knight, R., & Gilbert, J. A. (2013). Evidence for a persistent microbial seed bank throughout the global ocean. Proceedings of the National Academy of Sciences, 110(12), 4651-4655.

Giles, E. C., Kamke, J., Moitinho-Silva, L., Taylor, M. W., Hentschel, U., Ravasi, T., & Schmitt, S. (2013). Bacterial community profiles in low microbial abundance sponges. FEMS Microbiology Ecology, 83(1), 232-241.

Glasl, B., Webster, N. S., and Bourne, D. G. (2017a). Microbial indicators as a diagnostic tool for assessing water quality and climate stress in coral reef ecosystems. Marine biology, 164(4), 91.

Glasl, B., Bongaerts, P., Elisabeth, N. H., Hoegh-Guldberg, O., Herndl, G. J., & Frade, P. R. (2017b). Microbiome variation in corals with distinct depth distribution ranges across a shallow–mesophotic gradient (15–85 m). Coral Reefs, 36(2), 447-452.

Glasl, B., Smith, C. E., Bourne, D. G., & Webster, N. S. (2018). Exploring the diversity-stability paradigm using sponge microbial communities. Scientific reports, 8(1), 8425.

Gloeckner, V., Wehrl, M., Moitinho-Silva, L., Gernert, C., Schupp, P., Pawlik, J. R., ... and Hentschel, U. (2014). The HMA-LMA dichotomy revisited: an electron microscopical survey of 56 sponge species. The Biological Bulletin, 227(1), 78-88.

Goetze E (2010). Species discovery in marine planktonic invertebrates through global molecular screening. Molecular Ecology, 19(5), 952-967.

Gomes, N. C., Cleary, D. F., Pinto, F. N., Egas, C., Almeida, A., Cunha, A., ... & Smalla, K. (2010). Taking root: enduring effect of rhizosphere bacterial colonization in mangroves. PloS one, 5(11), e14065.

Gribble, G. W. (2015). Biological activity of recently discovered halogenated marine natural products. Marine drugs, 13(7), 4044-4136.

Griffiths, J. R., Kadin, M., Nascimento, F. J., Tamelander, T., Törnroos, A., Bonaglia, S., ... & Kotta, J. (2017). The importance of benthic–pelagic coupling for marine ecosystem functioning in a changing world. Global change biology, 23(6), 2179-2196.

Groussin, M., Mazel, F., Sanders, J. G., Smillie, C. S., Lavergne, S., Thuiller, W., & Alm, E. J. (2017). Unraveling the processes shaping mammalian gut microbiomes over evolutionary time. Nature communications, 8, 14319.

Guindon, S., & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic biology, 52(5), 696-704.

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic biology, 59(3), 307-321.

H

Hadas, E., Shpigel, M., and Ilan, M. (2009). Particulate organic matter as a food source for a coral reef sponge. Journal of Experimental Biology, 212(22), 3643-3650.

Hajibabaei, M., Janzen, D. H., Burns, J. M., Hallwachs, W., & Hebert, P. D. (2006). DNA barcodes distinguish species of tropical Lepidoptera. Proceedings of the National Academy of Sciences, 103(4), 968-971.

Hammerman, N. M., and García-Hernández, J. E. (2017). The sponge Xestospongia muta offers shelter to the stony coral Madracis auretenra (Northwest Puerto Rico). Marine Biodiversity, 47(1), 57-58.

Hamming, R. W. (1950). Error detecting and error correcting codes. The Bell system technical journal, 29(2), 147-160.

Hammond, L. S., and Wilkinson, C. R. (1985). Exploitation of sponge exudates by coral reef holothuroids. Journal of experimental marine biology and ecology, 94(1-3), 1-9.

Hanson, C. E., McLaughlin, M. J., Hyndes, G. A., and Strzelecki, J. (2009). Selective uptake of prokaryotic picoplankton by a marine sponge (Callyspongia sp.) within an oligotrophic coastal system. Estuarine, Coastal and Shelf Science, 84(2), 289-297.

Haq, B. U., Hardenbol, J. A. N., & Vail, P. R. (1987). Chronology of fluctuating sea levels since the Triassic. Science, 235(4793), 1156-1167.

Hardoim, C. C., Esteves, A. I., Pires, F. R., Gonçalves, J. M., Cox, C. J., Xavier, J. R., & Costa, R. (2012). Phylogenetically and spatially close marine sponges harbor divergent bacterial communities. PLoS One, 7(12), e53029.

Harzhauser, M., & Piller, W. E. (2007). Benchmark data of a changing sea—palaeogeography, palaeobiogeography and events in the Central Paratethys during the Miocene. Palaeogeography, Palaeoclimatology, Palaeoecology, 253(1-2), 8-31.

Hasegawa, M., Kishino, H., & Yano, T. A. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of molecular evolution, 22(2), 160-174.

Haug, G. H., & Tiedemann, R. (1998). Effect of the formation of the Isthmus of Panama on Atlantic Ocean thermohaline circulation. Nature, 393(6686), 673.

Hebert, P. D., Cywinska, A., Ball, S. L., & Dewaard, J. R. (2003). Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences, 270(1512), 313-321.

Hebert, P. D., Penton, E. H., Burns, J. M., Janzen, D. H., & Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. Proceedings of the National Academy of Sciences, 101(41), 14812-14817.

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. Appl. Environ. Microbiol., 68(9), 4431-4440.

Hentschel, U., Fieseler, L., Wehrl, M., Gernert, C., Steinert, M., Hacker, J., & Horn, M. (2003). Microbial diversity of marine sponges. In Sponges (Porifera) (pp. 59-88). Springer, Berlin, Heidelberg.

Hentschel, U., Usher, K. M., and Taylor, M. W. (2006). Marine sponges as microbial fermenters. FEMS microbiology ecology, 55(2), 167-177.

Hentschel, U., Piel, J., Degnan, S. M., & Taylor, M. W. (2012). Genomic insights into the marine sponge microbiome. Nature Reviews Microbiology, 10(9), 641.

Hess, P. N., & De Moraes Russo, C. A. (2007). An empirical test of the midpoint rooting method. Biological Journal of the Linnean Society, 92(4), 669-674.

Hochmuth, T., Niederkrüger, H., Gernert, C., Siegl, A., Taudien, S., Platzer, M., ... & Piel, J. (2010). Linking chemical and microbial diversity in marine sponges: possible role for Poribacteria as producers of methyl-branched fatty acids. ChemBioChem, 11(18), 2572-2578.

Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., ... and Knowlton, N. (2007). Coral reefs under rapid climate change and ocean acidification. science, 318(5857), 1737-1742.

Hoeksema, B. W. (2004). Biodiversity and the natural resource management of coral reefs in Southeast Asia. Challenging coasts: transdisciplinary excursions into integrated coastal zone development. Amsterdam University Press. Mare Publication Series, (1), 49-71.

Hoeksema, B. W. (2007). Delineation of the Indo-Malayan centre of maximum marine biodiversity: the Coral Triangle. In Biogeography, time, and place: distributions, barriers, and islands (pp. 117-178). Springer, Dordrecht.

Hoffmann, F., Radax, R., Woebken, D., Holtappels, M., Lavik, G., Rapp, H. T., ... & Kuypers, M. M. (2009). Complex nitrogen cycling in the sponge Geodia barretti. Environmental Microbiology, 11(9), 2228-2243.

Hooper, J. N. A. & Wiedenmayer, F. (1994) Porifera. In: Wells A (ed) Zoological Catalogue of Australia, pp1-62

Hooper, J. N., & Van Soest, R. W. (2002). Systema Porifera. A guide to the classification of sponges. In Systema Porifera (pp. 1-7). Springer, Boston, MA.

Hopkins, G. W., & Freckleton, R. P. (2002). Declines in the numbers of amateur and professional taxonomists: implications for conservation. Animal Conservation, 5(3), 245-249.

Horne, J. B., van Herwerden, L., Choat, J. H., & Robertson, D. R. (2008). High population connectivity across the Indo-Pacific: congruent lack of phylogeographic structure in three reef fish congeners. Molecular phylogenetics and evolution, 49(2), 629-638.

Hou, G., Chen, W. T., Lu, H. S., Cheng, F., & Xie, S. G. (2018). Developing a DNA barcode library for perciform fishes in the South China Sea: Species identification, accuracy and cryptic diversity. Molecular ecology resources, 18(1), 137-146.

Huang, Y. M., de Voogd, N. J., Cleary, D. F., Li, T. H., Mok, H. K., & Ueng, J. P. (2016). Biodiversity pattern of subtidal sponges (Porifera: Demospongiae) in the Penghu Archipelago (Pescadores), Taiwan. Journal of the Marine Biological Association of the United Kingdom, 96(2), 417-427.

Hughes, T. P. (1994). Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. Science, 265(5178), 1547-1551.

Hughes, T. P., Rodrigues, M. J., Bellwood, D. R., Ceccarelli, D., Hoegh-Guldberg, O., McCook, L., ... and Willis, B. (2007). Phase shifts, herbivory, and the resilience of coral reefs to climate change. Current Biology, 17(4), 360-365.

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics, 17(8), 754-755.

Hughes, T. P., Barnes, M. L., Bellwood, D. R., Cinner, J. E., Cumming, G. S., Jackson, J. B., ... and Palumbi, S. R. (2017). Coral reefs in the Anthropocene. Nature, 546(7656), 82.

Huse, S. M., Ye, Y., Zhou, Y., & Fodor, A. A. (2012). A core human microbiome as viewed through 16S rRNA sequence clusters. PloS one, 7(6), e34242.

I

Ibrahim, D., Nazari, T. F., Kassim, J., & Lim, S. H. (2014). Prodigiosin-an antibacterial red pigment produced by Serratia marcescens IBRL USM 84 associated with a marine sponge Xestospongia testudinaria. J. Appl. Pharm. Sci, 4, 1-6.

Indraningrat, A. A. G., Smidt, H., & Sipkema, D. (2016). Bioprospecting sponge-associated microbes for antimicrobial compounds. Marine drugs, 14(5), 87.

J

Jarman, S. N., Ward, R. D., & Elliott, N. G. (2002). Oligonucleotide primers for PCR amplification of coelomate introns. Marine Biotechnology, 4(4), 347-355.

Jaspars, M., De Pascale, D., Andersen, J. H., Reyes, F., Crawford, A. D., & Ianora, A. (2016). The marine biodiscovery pipeline and ocean medicines of tomorrow. Journal of the Marine Biological Association of the United Kingdom, 96(1), 151-158.

Joppa, L. N., Roberts, D. L., & Pimm, S. L. (2011). The population ecology and social behaviour of taxonomists. Trends in Ecology & Evolution, 26(11), 551-553.

K

Kahng, S. E., Garcia-Sais, J. R., Spalding, H. L., Brokovich, E., Wagner, D., Weil, E., ... & Toonen, R. J. (2010). Community ecology of mesophotic coral reef ecosystems. Coral Reefs, 29(2), 255-275.

Kahng, S. E., Copus, J. M., & Wagner, D. (2014). Recent advances in the ecology of mesophotic coral ecosystems (MCEs). Current opinion in environmental sustainability, 7, 72-81.

Kamke, J., Taylor, M. W., & Schmitt, S. (2010). Activity profiles for marine sponge-associated bacteria obtained by 16S rRNA vs 16S rRNA gene comparisons. The ISME journal, 4(4), 498.

Kayal, E., & Lavrov, D. V. (2008). The mitochondrial genome of Hydra oligactis (Cnidaria, Hydrozoa) sheds new light on animal mtDNA evolution and cnidarian phylogeny. Gene, 410(1), 177-186.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., ... & Thierer, T. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12), 1647-1649.

Keigwin Jr, L. D. (1978). Pliocene closing of the Isthmus of Panama, based on biostratigraphic evidence from nearby Pacific Ocean and Caribbean Sea cores. Geology, 6(10), 630-634.

Keil, P., Schweiger, O., Kühn, I., Kunin, W. E., Kuussaari, M., Settele, J., ... & Moustakas, A. (2012). Patterns of beta diversity in Europe: the role of climate, land cover and distance across scales. Journal of Biogeography, 39(8), 1473-1486.

Kelly-Borges, M., & Bergquist, P. R. (1988). Sponges from Motupore Island, Papua New Guinea. Indo-Malayan Zoology, 5, 121–159.

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., ... & Webb, C. O. (2010). Picante: R tools for integrating phylogenies and ecology. Bioinformatics, 26(11), 1463-1464.

Kerr, R. G., Kerr, S. L., & Djerassi, C. (1991). Biosynthetic studies of marine lipids. 26. Elucidation of the biosynthesis of mutasterol, a sponge sterol with a quaternary carbon in its side chain. The Journal of Organic Chemistry, 56(1), 63-66.

Kerr, R. G., & Baker, B. J. (1991). Marine sterols. Natural Product Reports, 8(5), 465-497.

Kerr, R., & Kelly-Borges, M. (1994). Biochemical and morphological heterogeneity in the Caribbean sponge Xestospongia muta (Petrosida: Petrosiidae). RWM van SoestTMG van KempenJC Braekman. Sponges in time and space. Rotterdam: Balkema, 65-73.

Klautau, M., Russo, C. A., Lazoski, C., Boury-Esnault, N., Thorpe, J. P., & Solé-Cava, A. M. (1999). Does cosmopolitanism result from overconservative systematics? A case study using the marine sponge Chondrilla nucula. Evolution, 53(5), 1414-1422.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic acids research, 41(1), e1-e1.

Knapp, I. S., Forsman, Z. H., Williams, G. J., Toonen, R. J., & Bell, J. J. (2015). Cryptic species obscure introduction pathway of the blue Caribbean sponge (Haliclona (Soestella) caerulea),(order: Haplosclerida) to Palmyra Atoll, Central Pacific. PeerJ, 3, e1170.

Knobloch, S., Jóhannsson, R., & Marteinsson, V. (2018). Bacterial diversity in the marine sponge Halichondria panicea from Icelandic waters and host-specificity of its dominant symbiont "Candidatus Halichondribacter symbioticus". FEMS microbiology ecology, 95(1), fiy220.

Knowlton, N. (1993). Sibling species in the sea. Annual review of ecology and systematics, 24(1), 189-216.

Knowlton, N. (2000). Molecular genetic analyses of species boundaries in the sea. Hydrobiologia, 420(1), 73-90.

Knowlton, N., Brainard, R. E., Fisher, R., Moews, M., Plaisance, L., and Caley, M. J. (2010). Coral reef biodiversity. Life in the world's oceans: diversity distribution and abundance, 65-74.

Koopmans, M., Martens, D., and Wijffels, R. H. (2010). Growth efficiency and carbon balance for the sponge Haliclona oculata. Marine biotechnology, 12(3), 340-349.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular biology and evolution, 33(7), 1870-1874.

L

Lafi, F. F., Fuerst, J. A., Fieseler, L., Engels, C., Goh, W. W. L., & Hentschel, U. (2009). Widespread distribution of Poribacteria in Demospongiae. Appl. Environ. Microbiol., 75(17), 5695-5699.

Lamarck, J.B.P.D.M., Comte De (1815) Suite des polypiers empâtés. In: Mémoires du Muséum d'Histoire naturelle, Paris, Paris, pp. 69–80, 162–168, 331–340

Lamb, J. B., Willis, B. L., Fiorenza, E. A., Couch, C. S., Howard, R., Rader, D. N., ... and Harvell, C. D. (2018). Plastic waste associated with disease on coral reefs. Science, 359(6374), 460-462.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., ... & Thompson, J. D. (2007). Clustal W and Clustal X version 2.0. bioinformatics, 23(21), 2947-2948.

Larsen, P. S., and Riisgard, H. U. (1994). The sponge pump. Journal of Theoretical Biology, 168(1), 53-63.

Leal, M. C., Puga, J., Serôdio, J., Gomes, N. C., and Calado, R. (2012). Trends in the discovery of new marine natural products from invertebrates over the last two decades–where and what are we bioprospecting?. PLoS One, 7(1), e30580.

Lee, O. O., Wang, Y., Yang, J., Lafi, F. F., Al-Suwailem, A., & Qian, P. Y. (2011). Pyrosequencing reveals highly diverse and species-specific microbial communities in sponges from the Red Sea. The ISME journal, 5(4), 650.

Leibold, M. A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J. M., Hoopes, M. F., ... & Loreau, M. (2004). The metacommunity concept: a framework for multi-scale community ecology. Ecology letters, 7(7), 601-613.

Leite, D. C., Leão, P., Garrido, A. G., Lins, U., Santos, H. F., Pires, D. O., ... & Peixoto, R. S. (2017). Broadcast spawning coral Mussismilia hispida can vertically transfer its associated bacterial core. Frontiers in microbiology, 8, 176.

Legendre, P., & Gallagher, E. D. (2001). Ecologically meaningful transformations for ordination of species data. Oecologia, 129(2), 271-280.

Lenth, R. (2018). Emmeans: Estimated marginal means, aka least-squares means. R package version, 1(1).

Lesser, M. P., Slattery, M., & Leichter, J. J. (2009). Ecology of mesophotic coral reefs. Journal of Experimental Marine Biology and Ecology, 1(375), 1-8.

Lesser, M. P., & Slattery, M. (2011). Phase shift to algal dominated communities at mesophotic depths associated with lionfish (Pterois volitans) invasion on a Bahamian coral reef. Biological Invasions, 13(8), 1855-1868.

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.

Levitan, D. R., Fukami, H., Jara, J., Kline, D., McGovern, T. M., McGhee, K. E., ... & Knowlton, N. (2004). Mechanisms of reproductive isolation among sympatric broadcast-spawning corals of the Montastraea annularis species complex. Evolution, 58(2), 308-323.

Li, D., Xu, Y., Shao, C. L., Yang, R. Y., Zheng, C. J., Chen, Y. Y., ... & Wang, C. Y. (2012). Antibacterial bisabolane-type sesquiterpenoids from the sponge-derived fungus Aspergillus sp. Marine drugs, 10(1), 234-241.

Lieberman, B. S. (2002). Phylogenetic biogeography with and without the fossil record: gauging the effects of extinction and paleontological incompleteness. Palaeogeography, Palaeoclimatology, Palaeoecology, 178(1-2), 39-52.

Lindquist, N., and Hay, M. E. (1996). Palatability and chemical defense of marine invertebrate larvae. Ecological Monographs, 66(4), 431-450.

López-Legentil, S., & Pawlik, J. R. (2009). Genetic structure of the Caribbean giant barrel sponge Xestospongia muta using the I3-M11 partition of COI. Coral Reefs, 28(1), 157-165.

Love, G. D., Grosjean, E., Stalvies, C., Fike, D. A., Grotzinger, J. P., Bradley, A. S., ... & Bowring, S. A. (2009). Fossil steroids record the appearance of Demospongiae during the Cryogenian period. Nature, 457(7230), 718.

Luter, H. M., Widder, S., Botté, E. S., Wahab, M. A., Whalan, S., Moitinho-Silva, L., ... & Webster, N. S. (2015). Biogeographic variation in the microbiome of the ecologically important sponge, Carteriospongia foliascens. PeerJ, 3, e1435.

Lynch, M. D., & Neufeld, J. D. (2015). Ecology and exploration of the rare biosphere. Nature Reviews Microbiology, 13(4), 217.

M

Ma, X., Li, L., Zhu, T., Ba, M., Li, G., Gu, Q., ... & Li, D. (2013). Phenylspirodrimanes with anti-HIV activity from the sponge-derived fungus Stachybotrys chartarum MXH-X73. Journal of natural products, 76(12), 2298-2306.

Maldonado, M., Cortadellas, N., Trillas, M. I., and Rutzler, K. (2005). Endosymbiotic yeast maternally transmitted in a marine sponge. The Biological Bulletin, 209(2), 94-106.

Maldonado, M., Zhang, X., Cao, X., Xue, L., Cao, H., and Zhang, W. (2010). Selective feeding by sponges on pathogenic microbes: a reassessment of potential for abatement of microbial pollution. Marine Ecology Progress Series, 403, 75-89.

Maldonado, M., Ribes, M., and van Duyl, F. C. (2012). Nutrient fluxes through sponges: biology, budgets, and ecological implications. In Advances in marine biology (Vol. 62, pp. 113-182). Academic Press.

Maloof, A. C., Rose, C. V., Beach, R., Samuels, B. M., Calmet, C. C., Erwin, D. H., ... & Simons, F. J. (2010). Possible animal-body fossils in pre-Marinoan limestones from South Australia. Nature Geoscience, 3(9), 653.

Marino, C. M., Pawlik, J. R., López-Legentil, S., & Erwin, P. M. (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.

Mayr, E. (1942) Systematics and the origin of species, from the viewpoint of a zoologist. Harvard University Press.

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C., Carey, H. V., Domazet-Lošo, T., Douglas, A. E., ... & Hentschel, U. (2013). Animals in a bacterial world, a new imperative for the life sciences. Proceedings of the National Academy of Sciences, 110(9), 3229-3236.

McGrath, E. C., Woods, L., Jompa, J., Haris, A., and Bell, J. J. (2018). Growth and longevity in giant barrel sponges: Redwoods of the reef or Pines in the Indo-Pacific?. Scientific reports, 8.

McKinney, M. L. (2002). Urbanization, Biodiversity, and ConservationThe impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. Bioscience, 52(10), 883-890.

McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS one, 8(4), e61217.

McMurray, S. E., Blum, J. E., and Pawlik, J. R. (2008). Redwood of the reef: growth and age of the giant barrel sponge Xestospongia muta in the Florida Keys. Marine Biology, 155(2), 159-171.

McMurray, S. E., and Pawlik, J. R. (2009). A novel technique for the reattachment of large coral reef sponges. Restoration Ecology, 17(2), 192-195.

McMurray, S. E., Henkel, T. P., and Pawlik, J. R. (2010). Demographics of increasing populations of the giant barrel sponge Xestospongia muta in the Florida Keys. Ecology, 91(2), 560-570.

McMurray, S. E., Pawlik, J. R., and Finelli, C. M. (2014). Trait-mediated ecosystem impacts: how morphology and size affect pumping rates of the Caribbean giant barrel sponge. Aquatic Biology, 23(1), 1-13.

McMurray, S. E., Finelli, C. M., and Pawlik, J. R. (2015). Population dynamics of giant barrel sponges on Florida coral reefs. Journal of Experimental Marine Biology and Ecology, 473, 73-80.

McMurray, S. E., Johnson, Z. I., Hunt, D. E., Pawlik, J. R., and Finelli, C. M. (2016). Selective feeding by the giant barrel sponge enhances foraging efficiency. Limnology and Oceanography, 61(4), 1271-1286.

McMurray, S. E., Pawlik, J. R., and Finelli, C. M. (2017). Demography alters carbon flux for a dominant benthic suspension feeder, the giant barrel sponge, on Conch Reef, Florida Keys. Functional Ecology, 31(11), 2188-2198.

Mehbub, M., Lei, J., Franco, C., and Zhang, W. (2014). Marine sponge derived natural products between 2001 and 2010: Trends and opportunities for discovery of bioactives. Marine drugs, 12(8), 4539-4577.

Mehrshad, M., Rodriguez-Valera, F., Amoozegar, M. A., López-García, P., & Ghai, R. (2018). The enigmatic SAR202 cluster up close: shedding light on a globally distributed dark ocean lineage involved in sulfur cycling. The ISME journal, 12(3), 655.

Mitra, A., Skrzypczak, M., Ginalski, K., & Rowicka, M. (2015). Strategies for achieving high sequencing accuracy for low diversity samples and avoiding sample bleeding using illumina platform. PloS one, 10(4), e0120520.

Moberg, F., and Folke, C. (1999). Ecological goods and services of coral reef ecosystems. Ecological economics, 29(2), 215-233.

Mohamed, N. M., Saito, K., Tal, Y., and Hill, R. T. (2010). Diversity of aerobic and anaerobic ammonia-oxidizing bacteria in marine sponges. The ISME journal, 4(1), 38.

Moitinho-Silva, L., Bayer, K., Cannistraci, C. V., Giles, E. C., Ryu, T., Seridi, L., ... & Hentschel, U. (2014). Specificity and transcriptional activity of microbiota associated with low and high microbial abundance sponges from the Red Sea. Molecular Ecology, 23(6), 1348-1363.

Moitinho-Silva, L., Steinert, G., Nielsen, S., Hardoim, C. C., Wu, Y. C., McCormack, G. P., ... and Hentschel, U. (2017a). Predicting the HMA-LMA status in marine sponges by machine learning. Frontiers in Microbiology, 8, 752.

Moitinho-Silva, L., Nielsen, S., Amir, A., Gonzalez, A., Ackermann, G. L., Cerrano, C., ... & Steinert, G. (2017b). The sponge microbiome project. GigaScience, 6(10), 1-7.

Montalvo, N. F., Mohamed, N. M., Enticknap, J. J., & Hill, R. T. (2005). Novel actinobacteria from marine sponges. Antonie Van Leeuwenhoek, 87(1), 29-36.

Montalvo, N. F., & Hill, R. T. (2011). Sponge-associated bacteria are strictly maintained in two closely related but geographically distant sponge hosts. Appl. Environ. Microbiol., 77(20), 7207-7216.

Montalvo, N. F., Davis, J., Vicente, J., Pittiglio, R., Ravel, J., & Hill, R. T. (2014). Integration of culture-based and molecular analysis of a complex sponge-associated bacterial community. PLoS One, 9(3), e90517.

Morariu, V. I., Srinivasan, B. V., Raykar, V. C., Duraiswami, R., & Davis, L. S. (2009). Automatic online tuning for fast Gaussian summation. In Advances in neural information processing systems (pp. 1113-1120).

Moran, N. A., & Sloan, D. B. (2015). The hologenome concept: helpful or hollow?. PLoS biology, 13(12), e1002311.

Mori, T., Cahn, J. K., Wilson, M. C., Meoded, R. A., Wiebach, V., Martinez, A. F. C., ... and Keren, R. (2018). Single-bacterial genomics validates rich and varied specialized metabolism of uncultivated Entotheonella sponge symbionts. Proceedings of the National Academy of Sciences, 115(8), 1718-1723.

Morris, S. C. (1993). The fossil record and the early evolution of the Metazoa. Nature, 361(6409), 219.

Morrow, C. C., Redmond, N. E., Picton, B. E., Thacker, R. W., Collins, A. G., Maggs, C. A., ... & Allcock, A. L. (2013). Molecular phylogenies support homoplasy of multiple morphological characters used in the taxonomy of Heteroscleromorpha (Porifera: Demospongiae). Integrative and Comparative Biology, 53(3), 428-446.

Morrow, C., and Cárdenas, P. (2015). Proposal for a revized classification of the Demospongiae (Porifera). Frontiers in Zoology, 12(1), 7.

Morrow, K. M., Bourne, D. G., Humphrey, C., Botté, E. S., Laffy, P., Zaneveld, J., ... & Webster, N. S. (2015). Natural volcanic CO 2 seeps reveal future trajectories for host–microbial associations in corals and sponges. The ISME journal, 9(4), 894.

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.

Mueller, B., de Goeij, J. M., Vermeij, M. J., Mulders, Y., van der Ent, E., Ribes, M., and van Duyl, F. C. (2014). Natural diet of coral-excavating sponges consists mainly of dissolved organic carbon (DOC). PloS one, 9(2), e90152.

Mumby, P. J., Steneck, R. S., Adjeroud, M., and Arnold, S. N. (2016). High resilience masks underlying sensitivity to algal phase shifts of Pacific coral reefs. Oikos, 125(5), 644-655.

Munro, H. G. (1994). From seabed to sickbed: what are the prospects. In proceedings of the 4th International Porifera Congress, Amsterdam, Netherlands (pp. 473-484). AA Balkema.

Munro, M. H., Blunt, J. W., Dumdei, E. J., Hickford, S. J., Lill, R. E., Li, S., ... & Duckworth, A. R. (1999). The discovery and development of marine compounds with pharmaceutical potential. Journal of Biotechnology, 70(1), 15-25.

N

Nagelkerken I, Aerts L and Pors L (2000). Barrel sponge bows out. Reef Encounter, 28, 14-15.

Naim, M. A., Morillo, J. A., Sørensen, S. J., Waleed, A. A. S., Smidt, H., & Sipkema, D. (2014). Host-specific microbial communities in three sympatric North Sea sponges. FEMS Microbiology Ecology, 90(2), 390-403.

Nekola, J. C., and White P. S. 1999. The distance decay of similarity in biogeography and ecology. Journal of Biogeography 26:867-878.

Nishida, A. H., & Ochman, H. (2018). Rates of gut microbiome divergence in mammals. Molecular ecology, 27(8), 1884-1897.

NOAA (2006) https://flowergarden.noaa.gov/education/spawningobservations.html

Norström, A. V., Nyström, M., Lokrantz, J., and Folke, C. (2009). Alternative states on coral reefs: beyond coral–macroalgal phase shifts. Marine ecology progress series, 376, 295-306.

O

Ohki, S., Kowalski, R. K., Kitanobo, S., & Morita, M. (2015). Changes in spawning time led to the speciation of the broadcast spawning corals Acropora digitifera and the cryptic species Acropora sp. 1 with similar gamete recognition systems. Coral Reefs, 34(4), 1189-1198.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., & Simpson, G. L. (2009). vegan: Community Ecology Package. R Package Version 1: 15-14. The comprehensive R Archive Network website.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., & McGlinn, D. (2016). vegan: community ecology package. R package version 2.4-0. 2016.

Oksanen, J. et al. vegan: Community Ecology Package. R package version 2.5-4 https://CRAN.R-project.org/package=vegan (2019)

Olson, J. B., & Kellogg, C. A. (2010). Microbial ecology of corals, sponges, and algae in mesophotic coral environments. FEMS microbiology ecology, 73(1), 17-30.

Olson, J. B., & Gao, X. (2013). Characterizing the bacterial associates of three Caribbean sponges along a gradient from shallow to mesophotic depths. FEMS microbiology ecology, 85(1), 74-84.

Olson, J. B., Thacker, R. W., & Gochfeld, D. J. (2014). Molecular community profiling reveals impacts of time, space, and disease status on the bacterial community associated with the Caribbean sponge Aplysina cauliformis. FEMS microbiology ecology, 87(1), 268-279.

Osinga, R., Armstrong, E., Burgess, J. G., Hoffmann, F., Reitner, J., and Schumann-Kindel, G. (2001). Sponge–microbe associations and their importance for sponge bioprocess engineering. Hydrobiologia, 461(1-3), 55-62.

Otani, S., Mikaelyan, A., Nobre, T., Hansen, L. H., Koné, N. G. A., Sørensen, S. J., ... & Poulsen, M. (2014). Identifying the core microbial community in the gut of fungus-growing termites. Molecular ecology, 23(18), 4631-4644.

P

Padial, J. M., Miralles, A., De la Riva, I., & Vences, M. (2010). The integrative future of taxonomy. Frontiers in zoology, 7(1), 16.

Paliy, O., & Shankar, V. (2016). Application of multivariate statistical techniques in microbial ecology. Molecular ecology, 25(5), 1032-1057.

Palumbi, S. R. (1984). Tactics of acclimation: morphological changes of sponges in an unpredictable environment. Science, 225(4669), 1478-1480.

Palumbi, S. R. (1997). Molecular biogeography of the Pacific. Coral Reefs, 16(1), S47-S52.

Palumbi, S. R., Cipriano, F., and Hare, M. P. (2001). Predicting nuclear gene coalescence from mitochondrial data: the three-times rule. Evolution, 55(5), 859-868.

Pandolfi, J. M., Jackson, J. B., Baron, N., Bradbury, R. H., Guzman, H. M., Hughes, T. P., ... & Sala, E. (2005). Are US coral reefs on the slippery slope to slime?.

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. Bioinformatics, 20(2), 289-290.

Patterson, M. R., Chernykh, V. I., Fialkov, V. A., and Savarese, M. (1997). Trophic effects of sponge feeding within Lake Baikal's littoral zone. 1. Insitu pumping rates. Limnology and Oceanography, 42(1), 171-178.

Paul, V. J., Freeman, C. J., & Agarwal, V. (2019). Chemical ecology of marine sponges: new opportunities through "-omics". Integrative and comparative biology.

Pawlik, J. R. (1993). Marine invertebrate chemical defenses. Chemical Reviews, 93(5), 1911-1922.

Peek, A. S., Feldman, R. A., Lutz, R. A., & Vrijenhoek, R. C. (1998). Cospeciation of chemoautotrophic bacteria and deep sea clams. Proceedings of the National Academy of Sciences, 95(17), 9962-9966.

Perry, C. T., Murphy, G. N., Kench, P. S., Smithers, S. G., Edinger, E. N., Steneck, R. S., and Mumby, P. J. (2013). Caribbean-wide decline in carbonate production threatens coral reef growth. Nature communications, 4, 1402.

Perry, C. T., Murphy, G. N., Kench, P. S., Edinger, E. N., Smithers, S. G., Steneck, R. S., and Mumby, P. J. (2014). Changing dynamics of Caribbean reef carbonate budgets: emergence of reef bioeroders as critical controls on present and future reef growth potential. Proceedings of the Royal Society B: Biological Sciences, 281(1796), 20142018.

Pett, W., Adamski, M., Adamska, M., Francis, W. R., Eitel, M., Pisani, D., and Wörheide, G. (2019). The role of homology and orthology in the phylogenomic analysis of metazoan gene content. Molecular biology and evolution, 36(4), 643-649.

Pile, A. J., Patterson, M. R., and Witman, J. D. (1996). In situ grazing on plankton. Marine Ecology Progress Series, 141, 95-102.

Pile, A. J., Patterson, M. R., Savarese, M., Chernykh, V. I., and Fialkov, V. A. (1997). Trophic effects of sponge feeding within Lake Baikal's littoral zone. 2. Sponge abundance, diet, feeding efficiency, and carbon flux. Limnology and Oceanography, 42(1), 178-184.

Pires, A. C., Cleary, D. F., Almeida, A., Cunha, Â., Dealtry, S., Mendonça-Hagler, L. C., ... & Gomes, N. C. (2012). Denaturing gradient gel electrophoresis and barcoded pyrosequencing reveal unprecedented archaeal diversity in mangrove sediment and rhizosphere samples. Appl. Environ. Microbiol., 78(16), 5520-5528.

Piola, R. F., & Johnston, E. L. (2008). Pollution reduces native diversity and increases invader dominance in marine hard-substrate communities. Diversity and Distributions, 14(2), 329-342.

Pita, L., Turon, X., López-Legentil, S., & Erwin, P. M. (2013a). Host rules: spatial stability of bacterial communities associated with marine sponges (Ircinia spp.) in the Western Mediterranean Sea. FEMS microbiology ecology, 86(2), 268-276.

Pita, L., López-Legentil, S., & Erwin, P. M. (2013b). Biogeography and host fidelity of bacterial communities in Ircinia spp. from the Bahamas. Microbial ecology, 66(2), 437-447.

Pita, L., Rix, L., Slaby, B. M., Franke, A., & Hentschel, U. (2018). The sponge holobiont in a changing ocean: from microbes to ecosystems. Microbiome, 6(1), 46.

Pogoreutz, C., Rädecker, N., Cárdenas, A., Gärdes, A., Wild, C., & Voolstra, C. R. (2018). Dominance of Endozoicomonas bacteria throughout coral bleaching and mortality suggests structural inflexibility of the Pocillopora verrucosa microbiome. Ecology and evolution, 8(4), 2240-2252.

Polónia, A. R., Cleary, D. F., Duarte, L. N., de Voogd, N. J., & Gomes, N. C. (2014). Composition of Archaea in seawater, sediment, and sponges in the kepulauan seribu reef system, Indonesia. Microbial ecology, 67(3), 553-567.

Polónia, A. R., Cleary, D. F., Freitas, R., de Voogd, N. J., & Gomes, N. C. (2015). The putative functional ecology and distribution of archaeal communities in sponges, sediment and seawater in a coral reef environment. Molecular ecology, 24(2), 409-423.

Polónia, A. R. M., Cleary, D. F. R., Freitas, R., da Costa Coelho, F. J. R., de Voogd, N. J., & Gomes, N. C. M. (2016). Comparison of archaeal and bacterial communities in two sponge species and seawater from an Indonesian coral reef environment. Marine genomics, 29, 69-80.

Polónia, A. R. M., Cleary, D. F. R., Freitas, R., Gomes, N. C. M., and de Voogd, N. J. (2017). Archaeal and bacterial communities of Xestospongia testudinaria and sediment differ in diversity, composition and predicted function in an Indonesian coral reef environment. Journal of sea research, 119, 37-53.

Polónia, A. R. M., Cleary, D. F. R., Coelho, F. J. R. D. C., Becking, L. E., de Voogd, N. J., Toha, A. H. A., & Gomes, N. C. M. (2018). Compositional analysis of archaeal communities in high and low microbial abundance sponges in the Misool coral reef system, Indonesia. Marine Biology Research, 14(6), 537-550.

Pratte, Z. A., Longo, G. O., Burns, A. S., Hay, M. E., & Stewart, F. J. (2018). Contact with turf algae alters the coral microbiome: contact versus systemic impacts. Coral Reefs, 37(1), 1-13.

Prazeres, M., Ainsworth, T., Roberts, T. E., Pandolfi, J. M., & Leggat, W. (2017). Symbiosis and microbiome flexibility in calcifying benthic foraminifera of the Great Barrier Reef. Microbiome, 5(1), 38.

Proksch, P. (1994). Defensive roles for secondary metabolites from marine sponges and sponge-feeding nudibranchs. Toxicon, 32(6), 639-655.

Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. (2012). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Molecular ecology, 21(8), 1864-1877.

Pye, C. R., Bertin, M. J., Lokey, R. S., Gerwick, W. H., & Linington, R. G. (2017). Retrospective analysis of natural products provides insights for future discovery trends. Proceedings of the National Academy of Sciences, 114(22), 5601-5606.

Q

Quah, Y., Ismail, N. I. M., Ooi, J. L. S., Affendi, Y. A., Manan, F. A., Wong, F. C., & Chai, T. T. (2018). Identification of Novel Cytotoxic Peptide KENPVLSLVNGMF from Marine Sponge Xestospongia testudinaria, with Characterization of Stability in Human Serum. International Journal of Peptide Research and Therapeutics, 24(1), 189-199.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., ... & Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic acids research, 41(D1), D590-D596.

R

Rachello-Dolmen, P. G., & Cleary, D. F. R. (2007). Relating coral species traits to environmental conditions in the Jakarta Bay/Pulau Seribu reef system, Indonesia. Estuarine, Coastal and Shelf Science, 73(3-4), 816-826.

Reaka-Kudla, M. L., Wilson, D. E., & Wilson, E. O. (1997). Biodiversity II: understanding and protecting our biological resources. Journal of Insect Conservation, 1, 247-250.

Redmond, N. E., Morrow, C. C., Thacker, R. W., Diaz, M. C., Boury-Esnault, N., Cárdenas, P., ... & Kayal, E. (2013). Phylogeny and systematics of Demospongiae in light of new small-subunit ribosomal DNA (18S) sequences. Integrative and comparative biology, 53(3), 388-415.

Reece, J. S., Bowen, B. W., & Larson, A. (2011). Long larval duration in moray eels (Muraenidae) ensures ocean-wide connectivity despite differences in adult niche breadth. Marine Ecology Progress Series, 437, 269-277.

Reen, F., Gutiérrez-Barranquero, J., Dobson, A., Adams, C., & O'Gara, F. (2015). Emerging concepts promising new horizons for marine biodiscovery and synthetic biology. Marine drugs, 13(5), 2924-2954.

Reiswig, H. M. (1974). Water transport, respiration and energetics of three tropical marine sponges. Journal of experimental marine Biology and Ecology, 14(3), 231-249.

Reiswig, H. M. (1975). The aquiferous systems of three marine Demospongiae. Journal of Morphology, 145(4), 493-502.

Renard, E., Leys, S. P., Wörheide, G., and Borchiellini, C. (2018). Understanding Animal Evolution: The Added Value of Sponge Transcriptomics and Genomics: The disconnect between gene content and body plan evolution. BioEssays, 40(9), 1700237.

Renema, W., Bellwood, D. R., Braga, J. C., Bromfield, K., Hall, R., Johnson, K. G., ... & O'dea, A. (2008). Hopping hotspots: global shifts in marine biodiversity. science, 321(5889), 654-657.

Reveillaud, J., Remerie, T., van Soest, R., Erpenbeck, D., Cárdenas, P., Derycke, S., ... & Vanreusel, A. (2010). Species boundaries and phylogenetic relationships between Atlanto-Mediterranean shallow-water and deep-sea coral associated Hexadella species (Porifera, Ianthellidae). Molecular phylogenetics and evolution, 56(1), 104-114.

Reveillaud, J., Van Soest, R., Derycke, S., Picton, B., Rigaux, A., & Vanreusel, A. (2011). Phylogenetic relationships among NE Atlantic Plocamionida Topsent (1927)(Porifera, Poecilosclerida): under-estimated diversity in reef ecosystems. PLoS One, 6(2), e16533.

Reveillaud, J., Maignien, L., Eren, A. M., Huber, J. A., Apprill, A., Sogin, M. L., & Vanreusel, A. (2014). Host-specificity among abundant and rare taxa in the sponge microbiome. The ISME journal, 8(6), 1198.

Revell, L. J. (2012). phytools: an R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution, 3(2), 217-223.

Ribes, M., Coma, R., and Gili, J. M. (1999). Natural diet and grazing rate of the temperate sponge Dysidea avara (Demospongiae, Dendroceratida) throughout an annual cycle. Marine Ecology Progress Series, 176, 179-190.

Ribes, M., Jiménez, E., Yahel, G., López-Sendino, P., Díez, B., Massana, R., ... and Coma, R. (2012). Functional convergence of microbes associated with temperate marine sponges. Environmental microbiology, 14(5), 1224-1239.

Ribes, M., Dziallas, C., Coma, R., & Riemann, L. (2015). Microbial diversity and putative diazotrophy in high-and low-microbial-abundance Mediterranean sponges. Appl. Environ. Microbiol., 81(17), 5683-5693.

Richards, V. P., Bernard, A. M., Feldheim, K. A., & Shivji, M. S. (2016). Patterns of population structure and dispersal in the long-lived "redwood" of the coral reef, the giant barrel sponge (Xestospongia muta). Coral Reefs, 35(3), 1097-1107.

Richmond, R. H., Tisthammer, K. H., & Spies, N. P. (2018). The effects of anthropogenic stressors on reproduction and recruitment of corals and reef organisms. Frontiers in Marine Science, 5, 226.

Riegl, B., & Piller, W. E. (2003). Possible refugia for reefs in times of environmental stress. International Journal of Earth Sciences, 92(4), 520-531.

Riesgo, A., & Maldonado, M. (2008). Differences in reproductive timing among sponges sharing habitat and thermal regime. Invertebrate Biology, 127(4), 357-367.

Riisgard, H. U., and Larsen, P. S. (1995). Filter-feeding in marine macro-invertebrates: Pump characteristics, modelling and energy cost. Biological Reviews, 70(1), 67-106.

Riisgård, H. U., and Larsen, P. S. (2010). Particle capture mechanisms in suspension-feeding invertebrates. Marine Ecology Progress Series, 418, 255-293.

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic acids research, 43(7), e47-e47.

Ritson-Williams, R., Becerro, M. A., & Paul, V. J. (2005). Spawning of the giant barrel sponge Xestospongia muta in Belize. Coral Reefs, 24(1), 160-160.

Ritson-Williams, R., Arnold, S. N., & Paul, V. J. (2016). Patterns of larval settlement preferences and post-settlement survival for seven Caribbean corals. Marine Ecology Progress Series, 548, 127-138.

Roberts, C. M., McClean, C. J., Veron, J. E., Hawkins, J. P., Allen, G. R., McAllister, D. E., ... & Vynne, C. (2002). Marine biodiversity hotspots and conservation priorities for tropical reefs. Science, 295(5558), 1280-1284.

Robinson, J. P., White, E. R., Wiwchar, L. D., Claar, D. C., Suraci, J. P., & Baum, J. K. (2014). The limitations of diversity metrics in directing global marine conservation. Marine Policy, 48, 123-125.

Rocha, L. A., Robertson, D. R., Roman, J., & Bowen, B. W. (2005). Ecological speciation in tropical reef fishes. Proceedings of the royal society B: biological sciences, 272(1563), 573-579.

Rocha, L. A., & Bowen, B. W. (2008). Speciation in coral-reef fishes. Journal of Fish Biology, 72(5), 1101-1121.

Rocha, L. A., Pinheiro, H. T., Shepherd, B., Papastamatiou, Y. P., Luiz, O. J., Pyle, R. L., & Bongaerts, P. (2018). Mesophotic coral ecosystems are threatened and ecologically distinct from shallow water reefs. Science, 361(6399), 281-284.

Romano, G., Costantini, M., Sansone, C., Lauritano, C., Ruocco, N., and Ianora, A. (2017). Marine microorganisms as a promising and sustainable source of bioactive molecules. Marine environmental research, 128, 58-69.

Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics, 19(12), 1572-1574.

Rosser, N. L. (2015). Asynchronous spawning in sympatric populations of a hard coral reveals cryptic species and ancient genetic lineages. Molecular ecology, 24(19), 5006-5019.

Röthig, T., & Voolstra, C. R. (2016). Xestospongia testudinaria nighttime mass spawning observation in Indonesia. Galaxea, Journal of Coral Reef Studies, 18(1), 1-2.

Rua, C. P., Zilberberg, C., & Solé-Cava, A. M. (2011). New polymorphic mitochondrial markers for sponge phylogeography. Journal of the Marine Biological Association of the United Kingdom, 91(5), 1015-1022.

Rua, C. P., de Oliveira, L. S., Froes, A., Tschoeke, D. A., Soares, A. C., Leomil, L., ... & Berlinck, R. G. (2018). Microbial and functional biodiversity patterns in sponges that accumulate bromopyrrole alkaloids suggest horizontal gene transfer of halogenase genes. Microbial ecology, 76(3), 825-838.

S

Sacristan-Soriano, O., Banaigs, B., & Becerro, M. A. (2011a). Relevant spatial scales of chemical variation in Aplysina aerophoba. Marine drugs, 9(12), 2499-2513.

Sacristán-Soriano, O., Banaigs, B., Casamayor, E. O., & Becerro, M. A. (2011b). Exploring the links between natural products and bacterial assemblages in the sponge Aplysina aerophoba. Applied and environmental microbiology, 77(3), 862-870.

Sacristán-Soriano, O., & Becerro, M. A. (2016). Publication impact in sponge chemical and microbial ecology. Scientia Marina, 80(4), 555-566.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution, 4(4), 406-425.

Salter, S. J., Cox, M. J., Turek, E. M., Calus, S. T., Cookson, W. O., Moffatt, M. F., ... & Walker, A. W. (2014). Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC biology, 12(1), 87.

Sarano, F. (1991) Synchronised spawning in Indonesian sponges. Coral Reefs 10 (3): 166-166

Schliep, K. P. (2010). phangorn: phylogenetic analysis in R. Bioinformatics, 27(4), 592-593.

Schmidt, E. O. (1870). Grundzüge einer Spongien-fauna des atlantischen Gebietes. W. Engelmann.

Schmidt, E. W., Obraztsova, A. Y., Davidson, S. K., Faulkner, D. J., & Haygood, M. G. (2000). Identification of the antifungal peptide-containing symbiont of the marine sponge Theonella swinhoei as a novel δ-proteobacterium,"Candidatus Entotheonella palauensis". Marine Biology, 136(6), 969-977.

Schmitt, S., Weisz, J. B., Lindquist, N., & Hentschel, U. (2007). Vertical transmission of a phylogenetically complex microbial consortium in the viviparous sponge Ircinia felix. Appl. Environ. Microbiol., 73(7), 2067-2078.

Schmitt, S., Angermeier, H., Schiller, R., Lindquist, N., & Hentschel, U. (2008). Molecular microbial diversity survey of sponge reproductive stages and mechanistic insights into vertical transmission of microbial symbionts. Appl. Environ. Microbiol., 74(24), 7694-7708.

Schmitt, S., Deines, P., Behnam, F., Wagner, M., & Taylor, M. W. (2011a). C hloroflexi bacteria are more diverse, abundant, and similar in high than in low microbial abundance sponges. FEMS Microbiology Ecology, 78(3), 497-510.

Schmitt, S., Hentschel, U., & Taylor, M. W. (2011b). Deep sequencing reveals diversity and community structure of complex microbiota in five Mediterranean sponges. In Ancient Animals, New Challenges (pp. 341-351). Springer, Dordrecht.

Schmitt, S., Tsai, P., Bell, J., Fromont, J., Ilan, M., Lindquist, N., ... & Webster, N. (2012). Assessing the complex sponge microbiota: core, variable and species-specific bacterial communities in marine sponges. The ISME Journal, 6(3), 564.

Schönberg, C. H. L. (2017). Culture, demography and biogeography of sponge science: From past conferences to strategic research?. Marine ecology, 38(2), e12416.

Schwaninger, H. R. (2008). Global mitochondrial DNA phylogeography and biogeographic history of the antitropically and longitudinally disjunct marine bryozoan Membranipora membranacea L.(Cheilostomata): another cryptic marine sibling species complex?. Molecular Phylogenetics and Evolution, 49(3), 893-908.

Setiawan, E., de Voogd, N. J., Swierts, T., Hooper, J. N., Wörheide, G., & Erpenbeck, D. (2016a). MtDNA diversity of the Indonesian giant barrel sponge Xestospongia testudinaria (Porifera: Haplosclerida)–implications from partial cytochrome oxidase 1 sequences. Journal of the Marine Biological Association of the United Kingdom, 96(2), 323-332.

Setiawan, E., De Voogd, N. J., Hooper, J. N., Woerheide, G., & Erpenbeck, D. (2016b). Bottomless barrel-sponge species in the Indo-Pacific. Zootaxa, 4136(2), 393-396.

Sharp, K. H., Eam, B., Faulkner, D. J., & Haygood, M. G. (2007). Vertical transmission of diverse microbes in the tropical sponge Corticium sp. Appl. Environ. Microbiol., 73(2), 622-629.

Siegl, A., Bayer, K., Kozytska, S., Hentschel, U., & Schmitt, S. (2008). Sponges and Microbes-New frontiers in an ancient symbiosis. Vie et Milieu, 58(2), 165-174.

Simion, P., Philippe, H., Baurain, D., Jager, M., Richter, D. J., Di Franco, A., ... and Lapebie, P. (2017). A large and consistent phylogenomic dataset supports sponges as the sister group to all other animals. Current Biology, 27(7), 958-967.

Sinha, R., Stanley, G., Gulati, G. S., Ezran, C., Travaglini, K. J., Wei, E., ... & Conley, S. D. (2017). Index switching causes "spreading-of-signal" among multiplexed samples in Illumina HiSeq 4000 DNA sequencing. BioRxiv, 125724.

Sipkema, D., Franssen, M. C., Osinga, R., Tramper, J., & Wijffels, R. H. (2005). Marine sponges as pharmacy. Marine biotechnology, 7(3), 142.

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.

Slaby, B. M., Hackl, T., Horn, H., Bayer, K., & Hentschel, U. (2017). Metagenomic binning of a marine sponge microbiome reveals unity in defense but metabolic specialization. The ISME journal, 11(11), 2465.

Slater, G. J., Harmon, L. J., & Alfaro, M. E. (2012). Integrating fossils with molecular phylogenies improves inference of trait evolution. Evolution: International Journal of Organic Evolution, 66(12), 3931-3944.

Southwell, M. W., Weisz, J. B., Martens, C. S., and Lindquist, N. (2008). In situ fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. Limnology and Oceanography, 53(3), 986-996.

Souza, D. T., Genuario, D. B., Silva, F. S. P., Pansa, C. C., Kavamura, V. N., Moraes, F. C., ... & Melo, I. S. (2017). Analysis of bacterial composition in marine sponges reveals the influence of host phylogeny and environment. FEMS microbiology ecology, 93(1).

Sperling, E. A., Robinson, J. M., Pisani, D., & Peterson, K. J. (2010). Where's the glass? Biomarkers, molecular clocks, and microRNAs suggest a 200-Myr missing Precambrian fossil record of siliceous sponge spicules. Geobiology, 8(1), 24-36.

Steinert, G., Taylor, M. W., Deines, P., Simister, R. L., De Voogd, N. J., Hoggard, M., & Schupp, P. J. (2016). In four shallow and mesophotic tropical reef sponges from Guam the microbial community largely depends on host identity. PeerJ, 4, e1936.

Steinert, G., Rohde, S., Janussen, D., Blaurock, C., & Schupp, P. J. (2017). Host-specific assembly of sponge-associated prokaryotes at high taxonomic ranks. Scientific reports, 7(1), 2542.

Stephens, M., Smith, N. J., & Donnelly, P. (2001). A new statistical method for haplotype reconstruction from population data. The American Journal of Human Genetics, 68(4), 978-989.

Stender, Y., Jokiel, P. L., and Rodgers, K. S. (2014). Thirty years of coral reef change in relation to coastal construction and increased sedimentation at Pelekane Bay, Hawai'i. PeerJ, 2, e300.

Struck, T. H., Feder, J. L., Bendiksby, M., Birkeland, S., Cerca, J., Gusarov, V. I., ... & Stedje, B. (2018). Finding evolutionary processes hidden in cryptic species. Trends in Ecology & Evolution, 33(3), 153-163.

Subagio, I. B., Setiawan, E., Hariyanto, S., and Irawan, B. (2017, June). Spicule size variation in Xestospongia testudinaria Lamarck, 1815 at Probolinggo-Situbondo coastal. In AIP Conference Proceedings (Vol. 1854, No. 1, p. 020034). AIP Publishing.

Suchanek, T. H., Carpenter, R. C., Witman, J. D., & Harvell, C. D. (1983). Sponges as important space competitors in deep Caribbean coral reef communities. The ecology of deep and shallow coral reefs, 1(1), 55-60.

Swierts, T., Peijnenburg, K. T., de Leeuw, C., Cleary, D. F., Hörnlein, C., Setiawan, E., ... & de Voogd, N. J. (2013). Lock, stock and two different barrels: comparing the genetic composition of morphotypes of the Indo-Pacific sponge Xestospongia testudinaria. PloS one, 8(9), e74396.

T

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular biology and evolution, 10(3), 512-526.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular biology and evolution, 28(10), 2731-2739.

Tavaré, S. (1986). Some probabilistic and statistical problems in the analysis of DNA sequences. Lectures on mathematics in the life sciences, 17(2), 57-86.

Taylor, M. W., Radax, R., Steger, D., & Wagner, M. (2007a). Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. Microbiol. Mol. Biol. Rev., 71(2), 295-347.

Taylor, M. W., Hill, R. T., Piel, J., Thacker, R. W., and Hentschel, U. (2007b). Soaking it up: the complex lives of marine sponges and their microbial associates. The ISME Journal, 1(3), 187.

Taylor, M. W., Tsai, P., Simister, R. L., Deines, P., Botte, E., Ericson, G., ... & Webster, N. S. (2013). 'Sponge-specific'bacteria are widespread (but rare) in diverse marine environments. The ISME journal, 7(2), 438.

Teske, P. R., Rius, M., McQuaid, C. D., Styan, C. A., Piggott, M. P., Benhissoune, S., ... & Cooke, G. M. (2011). " Nested" cryptic diversity in a widespread marine ecosystem engineer: a challenge for detecting biological invasions. BMC Evolutionary Biology, 11(1), 176.

Thacker, R. W., & Freeman, C. J. (2012). Sponge–microbe symbioses: recent advances and new directions. In Advances in marine biology (Vol. 62, pp. 57-111). Academic Press.

Thacker, R. W., Hill, A. L., Hill, M. S., Redmond, N. E., Collins, A. G., Morrow, C. C., ... & Hall, C. (2013). Nearly complete 28S rRNA gene sequences confirm new hypotheses of sponge evolution. Integrative and Comparative Biology, 53(3), 373-387.

Thomas, T. R. A., Kavlekar, D. P., & LokaBharathi, P. A. (2010). Marine drugs from sponge-microbe association—A review. Marine Drugs, 8(4), 1417-1468.

Thomas, T., Moitinho-Silva, L., Lurgi, M., Björk, J. R., Easson, C., Astudillo-García, C., ... & Chaves-Fonnegra, A. (2016). Diversity, structure and convergent evolution of the global sponge microbiome. Nature communications, 7, 11870.

Thorpe, J. P., & Solé-Cava, A. M. (1994). The use of allozyme electrophoresis in invertebrate systematics. Zoologica scripta, 23(1), 3-18.

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. nature, 444(7122), 1027.

Turque, A. S., Batista, D., Silveira, C. B., Cardoso, A. M., Vieira, R. P., Moraes, F. C., ... & Muricy, G. (2010). Environmental shaping of sponge associated archaeal communities. PLoS One, 5(12), e15774.

Tweedie, M. C. K., Gosh, J. K., & Roy, J. (1984). Statistics: Applications and New Directions, Proceedings of the Indian Statistical Institute Golden Jubilee International Conference.

U

Usher, K. M. (2008). The ecology and phylogeny of cyanobacterial symbionts in sponges. Marine Ecology, 29(2), 178-192.

V

Vacelet, J. (1975). Electron-microscope study of association between bacteria and sponges of genus Verongia (Dictyoceratida). Journal de microscopie et de biologie cellulaire, 23(3), 271.

Valliappan, K., Sun, W., & Li, Z. (2014). Marine actinobacteria associated with marine organisms and their potentials in producing pharmaceutical natural products. Applied microbiology and biotechnology, 98(17), 7365-7377.

Van Alphen, J., de Voogd, N. J., & Hoeksema, B. W. (2011). Differential feeding strategies in phyllidiid nudibranchs on coral reefs at Halmahera, northern Moluccas. Coral reefs, 30(1), 59-59.

Van Oppen, M. J. H., Wörheide, G., & Takabayashi, M. (2002, October). Nuclear markers in evolutionary and population genetic studies of scleractinian corals and sponges. In Proc 9th Int Coral Reef Symp (Vol. 1, pp. 131-138).

Van Soest, R. W. M. (1980). Marine sponges from Curaçao and other Caribbean localities Part II. Haplosclerida. Studies on the Fauna of Curaçao and other Caribbean Islands, 62(1), 1-173.

Van Soest, R. W. M. (1989). The Indonesian sponge fauna: a status report. Netherlands Journal of Sea Research, 23(2), 223-230.

Van Soest, R. W., Boury-Esnault, N., Vacelet, J., Dohrmann, M., Erpenbeck, D., De Voogd, N. J., ... & Hooper, J. N. (2012). Global diversity of sponges (Porifera). PLoS one, 7(4), e35105.

Van Soest, R. W. M., Boury-Esnault, N., Hooper, J. N. A., Rützler, K. D., De Voogd, N. J., Alvarez de Glasby, B., ... & Janussen, D. (2015). World porifera database. The World Register of Marine Species (WoRMS).

Van Soest, R. W. M., Boury-Esnault, N., Hooper, J. N. A., Rützler, K. D., De Voogd, N. J., Alvarez, B., ... & Downey, R. (2019). World porifera database. The World Register of Marine Species (WoRMS).

Van Syoc, R. J., & Newman, W. A. (2010). Morphology and evolutionary ecology of a sponge-barnacle symbiosis: Four new genera of barnacles (Archaeobalanidae, Bryozobiinae). Journal of Experimental Marine Biology and Ecology, 392(1-2), 65-88.

Van Veghel, M. L., Cleary, D. F., & Bak, R. P. (1996). Interspecific interactions and competitive ability of the polymorphic reef-building coral Montastrea annularis. Bulletin of Marine Science, 58(3), 792-803.

Vermeij, G. J. (2001). Community assembly in the sea: geologic history of the living shore biota. Marine community ecology, 39-60.

Vermeij, M. J., Marhaver, K. L., Huijbers, C. M., Nagelkerken, I., & Simpson, S. D. (2010). Coral larvae move toward reef sounds. PloS one, 5(5), e10660.

Villegas-Plazas, M., Wos-Oxley, M. L., Sanchez, J. A., Pieper, D. H., Thomas, O. P., & Junca, H. (2018). Variations in Microbial Diversity and Metabolite Profiles of the Tropical Marine Sponge Xestospongia muta with Season and Depth. Microbial ecology, 1-14.

Voigt, O., Wülfing, E., & Wörheide, G. (2012). Molecular phylogenetic evaluation of classification and scenarios of character evolution in calcareous sponges (Porifera, Class Calcarea). PLoS One, 7(3), e33417.

W

Wakimoto, T., Egami, Y., Nakashima, Y., Wakimoto, Y., Mori, T., Awakawa, T., ... & Abe, I. (2014). Calyculin biogenesis from a pyrophosphate protoxin produced by a sponge symbiont. Nature chemical biology, 10(8), 648.

Walke, J. B., Becker, M. H., Loftus, S. C., House, L. L., Cormier, G., Jensen, R. V., & Belden, L. K. (2014). Amphibian skin may select for rare environmental microbes. The ISME journal, 8(11), 2207.

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol., 73(16), 5261-5267.

Wang, X., Zhang, Y., Qin, G., Luo, W., & Lin, Q. (2016). A novel pathogenic bacteria (Vibrio fortis) causing enteritis in cultured seahorses, Hippocampus erectus Perry, 1810. Journal of fish diseases, 39(6), 765-769.

Watson, J. R., Hays, C. G., Raimondi, P. T., Mitarai, S., Dong, C., McWilliams, J. C., ... & Siegel, D. A. (2011). Currents connecting communities: nearshore community similarity and ocean circulation. Ecology, 92(6), 1193-1200.

Webb, A. E., van Heuven, S. M., de Bakker, D. M., van Duyl, F. C., Reichart, G. J., and de Nooijer, L. J. (2017). Combined effects of experimental acidification and eutrophication on reef sponge bioerosion rates. Frontiers in Marine Science, 4, 311.

Webster, N. S., & Taylor, M. W. (2012). Marine sponges and their microbial symbionts: love and other relationships. Environmental microbiology, 14(2), 335-346.

Webster, N. S., Luter, H. M., Soo, R. M., Botté, E. S., Simister, R. L., Abdo, D., & Whalan, S. (2013). Same, same but different: symbiotic bacterial associations in GBR sponges. Frontiers in microbiology, 3, 444.

Webster, N. S., & Thomas, T. (2016). The sponge hologenome. MBio, 7(2), e00135-16.

Wehrl, M., Steinert, M., and Hentschel, U. (2007). Bacterial uptake by the marine sponge Aplysina aerophoba. Microbial ecology, 53(2), 355-365.

Weigel, B. L., and Erwin, P. M. (2016). Intraspecific variation in microbial symbiont communities of the sun sponge, Hymeniacidon heliophila, from intertidal and subtidal habitats. Appl. Environ. Microbiol., 82(2), 650-658.

Weisz, J. B., Hentschel, U., Lindquist, N., and Martens, C. S. (2007). Linking abundance and diversity of sponge-associated microbial communities to metabolic differences in host sponges. Marine Biology, 152(2), 475-483.

Weisz, J. B., Lindquist, N., & Martens, C. S. (2008). Do associated microbial abundances impact marine demosponge pumping rates and tissue densities?. Oecologia, 155(2), 367-376.

White, J. R., Patel, J., Ottesen, A., Arce, G., Blackwelder, P., and Lopez, J. V. (2012). Pyrosequencing of bacterial symbionts within Axinella corrugata sponges: diversity and seasonal variability. PloS one, 7(6), e38204.

White, K. N., Tenney, K., & Crews, P. (2017). The Bengamides: A Mini-Review of natural sources, analogues, biological properties, biosynthetic origins, and future prospects. Journal of natural products, 80(3), 740-755.

Wichels, A., Würtz, S., Döpke, H., Schütt, C., and Gerdts, G. (2006). Bacterial diversity in the breadcrumb sponge Halichondria panicea (Pallas). FEMS microbiology ecology, 56(1), 102-118.

Wickham, H. (2016). ggplot2: elegant graphics for data analysis. Springer.

Wilkinson, C. R. (1983). Net primary productivity in coral reef sponges. Science, 219(4583), 410-412.

Wilkinson, C. R., Garrone, R., & Vacelet, J. (1984). Marine sponges discriminate between food bacteria and bacterial symbionts: electron microscope radioautography and in situ evidence. Proc. R. Soc. Lond. B, 220(1221), 519-528.

Wilkinson, C. R. (1999). Global and local threats to coral reef functioning and existence: review and predictions. Marine and Freshwater Research, 50(8), 867-878.

Wilson, H.V. (1925). Silicious and horny sponges collected by the U.S. Fisheries Steamer 'Albatross' during the Philippine Expedition,1907-10. pp. 273-532, pls 37-52. In: Contributions to the biology of the Philippine Archipelago and adjacent regions. Bulletin of the United States National Museum. 100 (2, part 4).

Wilson, M. C., Mori, T., Rückert, C., Uria, A. R., Helf, M. J., Takada, K., ... & Rinke, C. (2014). An environmental bacterial taxon with a large and distinct metabolic repertoire. Nature, 506(7486), 58.

Woodhead, A. J., Hicks, C. C., Norström, A. V., Williams, G. J., and Graham, N. A. (2019). Coral reef ecosystem services in the Anthropocene. Functional Ecology.

Wooster, M. K., McMurray, S. E., Pawlik, J. R., Morán, X. A., & Berumen, M. L. (2019). Feeding and respiration by giant barrel sponges across a gradient of food abundance in the Red Sea. Limnology and Oceanography.

Wörheide G, Solé-Cava AM and Hooper JN (2005). Biodiversity, molecular ecology and phylogeography of marine sponges: patterns, implications and outlooks. Integrative and Comparative Biology, 45(2), 377-385.

Wörheide, G. (2006). Low variation in partial cytochrome oxidase subunit I (COI) mitochondrial sequences in the coralline demosponge Astrosclera willeyana across the Indo-Pacific. Marine Biology, 148(5), 907-912.

Wörheide, G., Epp, L. S., & Macis, L. (2008). Deep genetic divergences among Indo-Pacific populations of the coral reef sponge Leucetta chagosensis (Leucettidae): founder effects, vicariance, or both?. BMC evolutionary biology, 8(1), 24.

Wörheide, G., Dohrmann, M., Erpenbeck, D., Larroux, C., Maldonado, M., Voigt, O., ... and Lavrov, D. V. (2012). Deep phylogeny and evolution of sponges (phylum Porifera). In Advances in marine biology (Vol. 61, pp. 1-78). Academic Press.

Wright, A. D. (2003). GC-MS and NMR analysis of Phyllidiellapustulosa and one of its dietary sources, the sponge Phakelliacarduus. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 134(2), 307-313.

X

Xavier, J. R., Rachello-Dolmen, P. G., Parra-Velandia, F., Schönberg, C. H. L., Breeuwer, J. A. J., & Van Soest, R. W. M. (2010). Molecular evidence of cryptic speciation in the "cosmopolitan" excavating sponge Cliona celata (Porifera, Clionaidae). Molecular phylogenetics and evolution, 56(1), 13-20.

Y

Yahel, G., Sharp, J. H., Marie, D., Häse, C., and Genin, A. (2003). In situ feeding and element removal in the symbiont-bearing sponge Theonella swinhoei: Bulk DOC is the major source for carbon. Limnology and Oceanography, 48(1), 141-149.

Yahel, G., Eerkes-Medrano, D. I., and Leys, S. P. (2006). Size independent selective filtration of ultraplankton by hexactinellid glass sponges. Aquatic Microbial Ecology, 45(2), 181-194.

Z

Zea, S. (1993). Cover of sponges and other sessile organisms in rocky and coral reef habitats of Santa Marta, Colombian Caribbean Sea. Caribb. J. Sci, 29(1-2), 75-88.

Zhan, A., Macisaac, H. J., & Cristescu, M. E. (2010). Invasion genetics of the Ciona intestinalis species complex: from regional endemism to global homogeneity. Molecular Ecology, 19(21), 4678-4694.

Zhang, F., Blasiak, L. C., Karolin, J. O., Powell, R. J., Geddes, C. D., & Hill, R. T. (2015). Phosphorus sequestration in the form of polyphosphate by microbial symbionts in marine sponges. Proceedings of the National Academy of Sciences, 112(14), 4381-4386.

Zhang, H., Huang, X., Huang, L., Bao, F., Xiong, S., Wang, K., & Zhang, D. (2018). Microeukaryotic biogeography in the typical subtropical coastal waters with multiple environmental gradients. Science of The Total Environment, 635, 618-628.

Zhou, X., Xu, T., Yang, X. W., Huang, R., Yang, B., Tang, L., & Liu, Y. (2010). Chemical and biological aspects of marine sponges of the genus Xestospongia. Chemistry & biodiversity, 7(9), 2201-2227.

Zhou, X., Lu, Y., Lin, X., Yang, B., Yang, X., & Liu, Y. (2011). Brominated aliphatic hydrocarbons and sterols from the sponge Xestospongia testudinaria with their bioactivities. Chemistry and physics of lipids, 164(7), 703-706.

SUMMARY

Sponges are the oldest multicellular animal lineage that occurs on Earth today. Despite their special place in evolutionary history, sponges have received relatively little attention in scientific research compared to other 'charismatic' organisms such as corals and fish. Among other things, this is due to the logistical problems of working under water and the lack of clear distinctive features between sponge species. However, it is becoming increasingly clear that sponges play a central role in marine ecosystems, such as coral reefs. Sponges, for example, are important in nutrient cycles, such as nitrogen, carbon, silicon and phosphorus. In addition to this, sponges provide habitat for other organisms and can erode and consolidate coral reef structures depending on the species, thereby influencing biodiversity in the system. In view of the changing conditions in oceans and seas, which are related to climate change and other human-induced stressors, it is important to thoroughly understand the role of sponges in coral reefs. This knowledge is essential in order to predict how coral reefs will respond to future conditions and to take appropriate measures to preserve these rich ecosystems. In this thesis, an attempt is made to better understand the evolutionary history of sponges and to gain a better understanding of the cooperation between sponges and the various microorganisms that they harbor. To achieve this goal, the giant barrel sponge has been used as a model organism. This group of sponges is widespread and occurs in the Caribbean and Indo-Pacific, from the east coast of Africa and the Red Sea to Taiwan and New Caledonia. Moreover, giant barrel sponges are abundant components of many coral reefs and are among the largest sponges in existence.

The central questions of this thesis are: 1. How many types of giant barrel sponges exist? 2. What is the evolutionary history of the extant giant barrel sponges? 3. What drives variation in the composition of the prokaryotic community of giant barrel sponges? 4. How do richness, diversity and composition of the prokaryotic communities in (giant barrel) sponges relate to those of other organisms in coral reef ecosystems?

To answer these questions, samples from a large number of giant barrel sponges (*Xestospongia* spp.) have been collected. A family tree of all specimens was constructed after DNA-analysis. Although it was originally believed that the giant barrel sponges in the Atlantic Ocean and the Indo-Pacific Ocean were two different species, our analyses showed that there are several species in both ocean basins. Even more remarkable, is that each species is most closely related to a species in the other ocean basin. The relationships and patterns in the family tree do not correspond with the geographical distribution of the giant barrel sponge species as presently understood. In other words, a giant barrel sponge from Curaçao, in the Caribbean can be more akin to a specimen in Indonesia, ±18,000 km apart, than to a specimen one meter away on the same reef. According to our analysis, there are three giant barrel sponge species in the Atlantic Ocean, and, at least, six in the Indo-Pacific Ocean. The intertwined evolutionary history of the species in both oceans shows us that there were

already several giant barrel sponges before the Atlantic Ocean and the Indo-Pacific Ocean became separated. Each of these species was split into two populations during the collision of the European and African tectonic plates about 40 million years ago and subsequently developed into different species.

Although the giant barrel sponge species look alike, there are subtle differences in some places. In North Sulawesi (Indonesia), one species has a smooth appearance, while the other is covered with spiky or digitate structures. In Tanzania, one type is bronze-colored and another blue-purple. However, these distinctions are location-specific, and there are no distinctive features that occur ocean-wide. This makes it impossible to distinguish between the species in the field without the ability to perform DNA analysis.

Sponges house a large and diverse community of microorganisms. It is generally believed that these microorganisms play a central role in the physiological processes in the host. Modern DNA-analysis has made it possible to make an extensive screening of the microorganisms that live in giant barrel sponges. At present, millions of microorganisms can be identified from a single individual. It appears that the region in which a giant barrel sponge occurs mainly determines the composition of their microbial community. However, if you look at a smaller scale within a region, for example within one reef or around an island such as Curaçao, it appears that not only is the geographical location important, but the host species to which an individual belongs also plays an important role. The composition of the microbial community in giant barrel sponges is, therefore, dependent on a combination of factors, and the influence of these vary across spatial scales.

Sponges were generally seen as one of the richest sources of microbial organisms on coral reefs, and the composition of sponges was believed to be very different from other hosts such as corals, sea cucumbers, fish and lobsters. An extensive analysis of 216 hosts, across a range of host taxa, showed that sponges are indeed a rich source of microorganisms on coral reefs, but not the richest. In addition to this, many microorganisms from sponges are also found in other host species. It appears that the microbial communities of different hosts on a coral reef are much more closely connected than previously thought.

SAMENVATTING

Sponzen vormen de oudste groep multicellulaire dieren die heden ten dage op Aarde voorkomen. Ondanks haar bijzondere plek in de evolutionaire geschiedenis is de spons in wetenschappelijk onderzoek een onderbelichte groep gebleven. Dit is met name te wijten aan de logistieke problemen van werken onder water en het gebrek aan duidelijke onderscheidende kenmerken tussen sponzensoorten. Het wordt echter steeds duidelijker dat sponzen een centrale rol spelen in mariene ecosystemen, bijvoorbeeld koraalriffen. Zo zijn sponzen belangrijk in de kringloop van nutriënten als stikstof, koolstof, silicium en fosfor. Bovendien bieden sponzen andere organismen habitat en kunnen ze stukken koraalrif eroderen wat biodiversiteit in het systeem stimuleert. Met het oog op de veranderende omstandigheden in de zeeën en oceanen die grotendeels gerelateerd zijn aan klimaatsverandering en andere menselijke invloeden, is het van belang de rol van sponzen in koraalriffen goed te doorgronden. Deze kennis is noodzakelijk om te kunnen voorspellen hoe koraalriffen zullen reageren op de omstandigheden van de toekomst en om passende beschermingsmaatregelen te nemen voor het behoud van deze rijke ecosystemen. In deze thesis wordt getracht de evolutionaire geschiedenis van sponzen beter te begrijpen en een beter inzicht te krijgen in de samenwerking tussen sponzen en de verschillende micro-organismen die zij herbergen. Om dit doel te bereiken is de reuzenbekerspons als modelorganisme gebruikt. Deze groep sponzen is wijdverspreid en komt voor in het Caraïbische gebied in de Atlantische Oceaan, en in de Indo-Pacifische Oceaan vanaf de oostkust van Afrika en de Rode Zee tot aan Taiwan en Nieuw-Caledonië. Bovendien hebben reuzenbekersponzen op veel koraalriffen een hoge abundantie en is het één van de grootste sponzen die op koraalriffen leven.

De centrale vragen in deze thesis zijn: 1. Hoeveel soorten reuzenbekersponzen bestaan er? 2. Wat is de evolutionaire geschiedenis van de bestaande reuzenbekersponssoorten? 3. Wat zijn de sturende factoren van de variatie in de samenstelling van de prokaryotische gemeenschap in reuzenbekersponzen? 4. Hoe verhouden de rijkdom, diversiteit en spreiding in de prokaryotische gemeenschap in (reuzenbeker-)sponzen zich tot die van andere organismen in koraalrifecosystemen?

Om deze vragen te beantwoorden is materiaal van een grote hoeveelheid reuzenbekersponzen (*Xestospongia* spp.) verzameld. Door middel van DNA-analyse kon een stamboom van alle exemplaren worden gereconstrueerd. Waar oorspronkelijk werd gedacht dat de reuzenbekersponzen in de Atlantische Oceaan en de Indo-Pacifische Oceaan twee verschillende soorten waren, blijkt uit onze analyses dat er in beide oceaanbekkens meerdere soorten voorkomen. Nog opmerkelijker is dat iedere soort het nauwst verwant is aan een soort in de andere oceaan. De verwantschappen en patronen in de stamboom komen dus niet overeen met de geografische verspreiding van de reuzenbekersponzen. Met andere woorden een reuzenbekerspons uit Curaçao kan meer verwant zijn aan

een exemplaar op een afstand van ongeveer 18.000 kilometer in Indonesië, dan aan een exemplaar op één meter afstand op hetzelfde rif. Volgens onze analyse zijn er drie soorten reuzenbekersponzen in de Atlantische Oceaan, en minstens zes in de Indo-Pacifische Oceaan. De vervlochten stambomen van beide oceanen tonen ons dat er meerdere reuzenbekersponssoorten bestonden voordat de Atlantische Oceaan en de Indo-Pacifische Oceaan van elkaar gescheiden raakten. Het meest voor de hand liggende scenario is dat ieder van deze soorten tijdens de botsing van Europa en Afrika - ongeveer 40 miljoen jaar geleden - in twee populaties is gesplitst die zich vervolgens hebben ontwikkeld tot verschillende soorten.

Hoewel de reuzenbekersponssoorten in eerste instantie veel op elkaar lijken, zijn er op sommige plaatsen toch subtiele verschillen zichtbaar. In Noord-Sulawesi (Indonesië) heeft de ene soort een glad uiterlijk, terwijl de andere met stekelige structuren is bedekt. In Tanzania is de ene soort bronskleurig, terwijl de ander blauwpaars is. Deze onderscheidingen zijn echter locatie gebonden, en er zijn geen onderscheidende kenmerken die algemeen zijn. Dit maakt het onmogelijk de soorten in het veld zonder de mogelijkheid om DNA-analyses uit te voeren van elkaar te onderscheiden.

Sponzen huisvesten een grote hoeveelheid en diversiteit aan micro-organismen. Het wordt algemeen aangenomen dat deze micro-organismen een centrale rol spelen in de fysiologische processen in de gastheer. Door middel van moderne DNA-analyses kon een uitgebreide screening worden gemaakt van de micro-organismen die in reuzenbekersponzen leven. Per individu kunnen tienduizenden bacteriën en archaea worden geïdentificeerd. Het blijkt dat de regio waarin een reuzenbekerspons staat bepalend is voor de samenstelling van deze microbiële gemeenschap. Als je echter op een kleinere schaal binnen een regio kijkt, bijvoorbeeld binnen één rif of rondom een eiland als Curaçao, dan blijkt dat niet alleen de geografische locatie belangrijk is, maar ook tot welke soort het individu behoort. De samenstelling van de microbiële gemeenschap in reuzenbekersponzen is dus afhankelijk van een samenspel van factoren, waarvan de invloed afhangt van de schaal waarop je kijkt.

Sponzen werden over het algemeen gezien als één van de rijkste bronnen van microbiële organismen op koraalriffen, en de samenstelling zou zeer sterk afwijken van andere gastheren zoals koralen, zeekomkommers, vissen en kreeften. Uit een uitgebreide analyse van 216 gastheren blijkt dat sponzen inderdaad een rijke bron zijn van micro-organismen op koraalriffen, maar niet de rijkste. Bovendien worden veel micro-organismen uit sponzen ook gevonden in andere gastheersoorten. Het blijkt dat de microbiële gemeenschappen van verschillende gastheren op een koraalrif veel sterker met elkaar verbonden zijn dan voorheen werd gedacht.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Nicole de Voogd for introducing me to sponges in general, and giant barrel sponges in particular. Nicole has been a great mentor in sponge biology to me over the past years. For nearly as many years, Daniel Cleary has been a wonderful guide in the mean world of statistics and analysis. Without Daniel, this thesis would not be the same and would have much lower significance to the scientific community. Although Peter van Bodegom became involved in this research later, his contribution was tremendous. Peter sharpened my mind, taught me by questioning, helped me structure thoughts and hypotheses and encouraged me to paint bigger pictures.

Before I started my Ph.D., many people showed me the beauty and magnificence of nature, without whom I would never have developed an interest in biology. My gratitude to them has no limits. The person who planted the first seed is my uncle Hugo de Haart, my personal Sir David Attenborough, who taught me how dandelions reproduce when I was three years old and who told me stories of the long journeys of migratory birds. Every ounce of biologist in me, is because of Hugo. The reason I submerged myself into marine biology, is my other uncle, Allard de Haart. Allard showed me the pictures and films he shot of coral reefs all over the world when I was twelve years old. He took me to the swimming pool for a practice dive and annoyingly made me wait until I was fifteen before I was allowed to get my Open Water diving license. As my diving instructor, Allard is the only reason I became comfortable enough underwater to make diving part of my profession.

Furthermore, I would like to thank Martijn Egas for his astonishing lecture on altruism that convinced me to study Biology. I am grateful to many other teachers from the University of Amsterdam, but especially to Hans Breeuwer for educating me so entertainingly about genetics and to Mark Vermeij for his fervent lectures on coral reefs ecosystems.

I would like to thank Bert Hoeksema for taking me on a marine expedition to Lembeh, Indonesia, where I could work with giant barrel sponges for the first time. I am also grateful to Christine Hörnlein for all the great dives together, to Bastian Reijnen and Sancia van der Meij for showing me what life as a Ph.D. student meant at Naturalis and to Ronald Vonk for being a supportive roommate and fieldwork partner at a personally difficult time.

I am honored that I was allowed to share a desk with Rob van Soest during the first year of my work at Naturalis. Once I was asked by a foreign visitor if I could stand up so he could sit on the chair on which Rob sat while writing 'the Sponge Bible'. I did not tell the visitor Rob wrote the Systema Porifera when he worked at the Zoological Museum in Amsterdam.

The valuable sponge-related discussions and brainstorm sessions with Esther van der Ent, Lina Bayona Maldonado and the other 'sponge team'-members often were catalysts for

new hypotheses and experiments. I feel blessed with all other colleagues from the Marine Biodiversity research group and I would like to extend a special thanks to Aleksandre Goghaladze and Lisette Mekkes. As the head of the Marine Biodiversity research group, Willem Renema has provided an ideal environment to do research. The work of the other lead scientists in our research group, José Joordens, Katja Peijnenburg, and Frank Wesselingh, was incredibly inspiring and I was filled with pride every time I saw one of their (many) appearances in various media.

I would like to thank Marcel Eurlings, Elza Duijm, Rob Langelaan, Bertie Joan van Heuven and Kees van der Berg for creating impeccable lab conditions and for their technical support. Furthermore, I am grateful to Nisma Abdelmalik, Gydo Geijer, Roy Belderok and Megan Clay for trusting me as their supervisor and for their great work in the field.

The data Christiaan de Leeuw has collected on giant barrel sponges and which has largely been incorporated in this thesis has incredible value. I also treasure the logistical support of many people during my various fieldwork trips, but especially that of Yusheng Huang in Taiwan, Gary Henden in Vietnam and Thailand and Christian Vaterlaus in Tanzania.

Furthermore, I am grateful for the support of all my friends and family throughout the years. A few of them, however, deserve to be spotlighted. Sem Aronson who I met on the first introduction day of our study and who has been a close friend and an inspiration to me ever since. Jenő Molnár who opened his house to me when I needed a home and who has put my interests over those of himself on many occasions. The 'Three Musketeers' - Maarten van Gemert, Milo de Baat and Daan Mes – my fellow biologists who live adventurous lives indeed. I feel very fortunate to act as their D'Artagnan, the main character, and most famous musketeer in Alexandre Dumas' novel who shares many adventures with the three lesser-known musketeers. And last but not least, Esper Postma, who has acted as a beacon in my life and who has supported me in many of my most important decisions.

I cannot finish differently than by thanking those who have been there since the start of my life. Anja Mol who has taken care of me for so many days during my first 12 years. My grandmother Oma Swierts who I always tried to make proud. My sisters Karin Swierts and Jasmijn Ruijter and my parents Eva de Haart and Frans Swierts for being a loving family.

And lastly I would like to thank Ayşen Şimşek for adding color to my life. I hope she believes sponges are animals and not plants after reading this thesis.

CURRICULUM VITAE

Thomas Swierts was born in Amsterdam, the Netherlands, on the 30th of May 1989. During his secondary education [Gymnasium] at the Sint-Nicolaaslyceum in Amsterdam, he was introduced to diving by his uncle at the age of fifteen. A year later, he obtained his Open Water diver certificate and started to discover what was hidden in the cold waters of the Netherlands. During his Bachelor's degree Biology at the University of Amsterdam (2007-2010), he picked as many water-related courses as possible and grew more and more passionate about the marine world. After he graduated with a B.Sc., he travelled for a year through the tropical regions of this planet and discovered the joys of warm water diving. Strongly believing his calling was "to count fish on an Indonesian reef" he enrolled in the Master's program 'Limnology and Oceanography' at the University of Amsterdam (2011-2014).

When Prof. Dr. Nicole de Voogd from Naturalis Biodiversity Center gave him the opportunity to do fieldwork in Indonesia for his first internship, he grabbed the opportunity with both hands. He had to abandon other plans to work in the Caribbean, but the opportunity to do exactly what he wanted was too good to let pass. That he was going to work with sponges instead of fish was just a detail to him at the time, but it turned out to be life-defining. His second internship was carried out with Dr. Mark Vermeij of the University of Amsterdam and involved the documentation of the competition between turf algae and coral colonies on a shallow reef in Vietnam. During this second internship and also during various courses of the Master's program, he kept working on various scientific projects about sponges.

After some successful and some more 'questionable' entrepreneurial adventures, he started his doctoral studies at the Institute of Environmental Sciences at Leiden University in 2015. During his doctoral studies, he worked in the Marine Biodiversity research group at Naturalis Biodiversity Center in Leiden, the Netherlands, under the daily supervision of Prof. Dr. Nicole de Voogd. His Ph.D. research focused on the genetic and microbial variation in giant barrel sponges. His thesis was submitted to the reading committee a week before the end of his contract.

LIST OF PUBLICATIONS

Swierts, T., Peijnenburg, K. T., de Leeuw, C., Cleary, D. F., Hörnlein, C., Setiawan, E., ... & de Voogd, N. J. (2013). Lock, stock and two different barrels: comparing the genetic composition of morphotypes of the Indo-Pacific sponge Xestospongia testudinaria. *PloS one*, *8*(9), e74396.

Swierts, T., & Vermeij, M. J. (2016). Competitive interactions between corals and turf algae depend on coral colony form. *PeerJ*, *4*, e1984.

Setiawan, E., de Voogd, N. J., **Swierts,** T., Hooper, J. N., Wörheide, G., & Erpenbeck, D. (2016). MtDNA diversity of the Indonesian giant barrel sponge *Xestospongia testudinaria* (Porifera: Haplosclerida)–implications from partial cytochrome oxidase 1 sequences. *Journal of the Marine Biological Association of the United Kingdom*, *96*(2), 323-332.

Swierts, T., Peijnenburg, K. T., de Leeuw, C. A., Breeuwer, J. A., Cleary, D. F., & de Voogd, N. J. (2017). Globally intertwined evolutionary history of giant barrel sponges. *Coral reefs*, *36*(3), 933-945.

Swierts, T., Huang, Y. M., & de Voogd, N. J. (2018). The giant barrel sponge facilitates the recovery of coral fragments after a tropical storm in Taiwan. *Coral reefs*, *37*(3), 675-675.

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), fiy194.

Cleary, D. F., **Swierts, T.,** Coelho, F. J., Polónia, A. R., Huang, Y. M., Ferreira, M. R., ... & Gomes, N. C. (2019). The sponge microbiome within the greater coral reef microbial metacommunity. *Nature communications*, *10*(1), 1644.