CARL VON OSSIETZKY universität OLDENBURG

Microbial Diversity of Temperate and Tropical Sponges

by Georg Steinert

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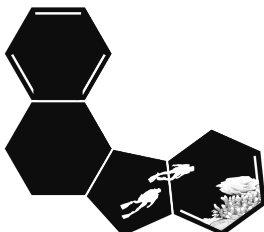

Erstgutachter: Prof. Dr. Peter J. Schupp
Zweitgutachterin: Prof. Dr. Ute Hentschel Humeida

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GENERAL INTRODUCTION

GENERAL INTRODUCTION

1.1 Sponge Introduction

Sponges (phylum Porifera - Box I) are benthic sessile, filter-feeding members of the aquatic environment, which inhabit a diverse variety of marine as well as freshwater habitats (Bell 2008; Gili and Coma 1998; van Soest et al. 2012). Sponge populations can be found in all climate zones: in polar regions and temperate benthic communities, as well as in subtropical and tropical reef ecosystems (Gili and Coma 1998; van Soest et al. 2012). Thereby, sponge populations vary in their diversity, abundance and richness. For example, more than 80% of the available benthic substrate is covered by sponges in an Irish lake (Bell 2008). By contrast, tropical Caribbean sponge populations can occupy more than 40% of the available benthic space (Bell 2008; Diaz and Rützler 2001), whereas sponges from Sulawesi can still cover up to 30% (Bell and Smith 2004). Furthermore, certain sponge populations show low abundances in reefs, but are abundant in caves and crevices in the Red Sea or Caribbean coral reefs (Diaz and Rützler 2001; Richter et al. 2001).

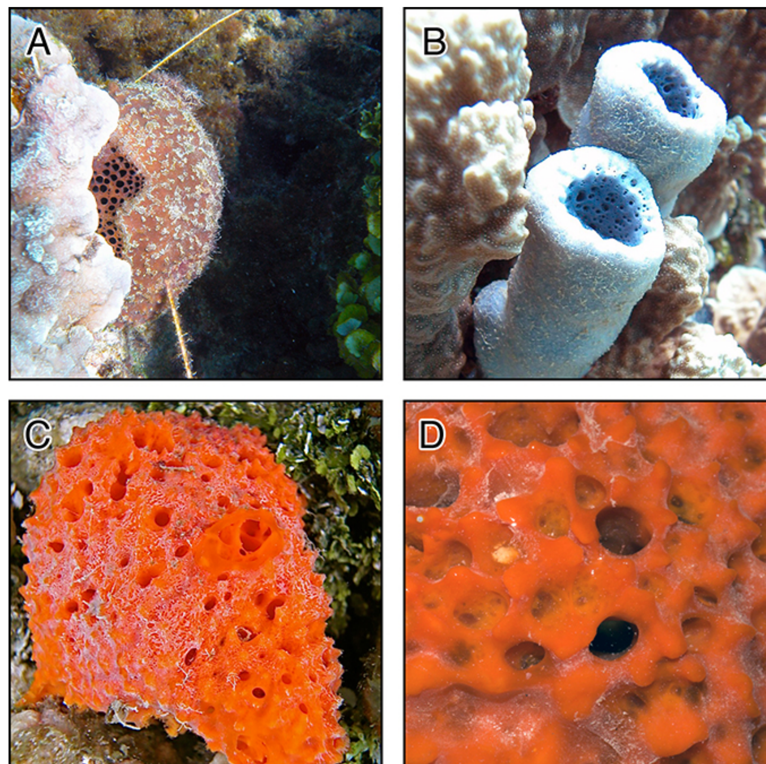

Figure 1.1 Examples of Guam reef sponges whose microbial community structure has been investigated in this thesis. A) *Rhabdastrella globostellata* was used in diffusion growth chamber implants (Chapter I), and B) *Callyspongia* sp. (Chapter II), C) *Acanthella* sp., D) *Acanthella* sp. close-up (photographs by Dr. Peter J. Schupp).

BOX I

The phylum **Porifera** is one of the most basal metazoan lineages, which dates back to the Precambrian Eon almost 600 million years ago. Molecular clocks estimated that the divergence event between sponges and their metazoan ancestors could have happened even earlier: around 1.5 billion years ago (Hedges et al. 2004). There are currently 8629 accepted marine and non-marine sponge species listed in The World Porifera Database (http://www.marinespecies.org/porifera/ - van Soest et al. 2012). These species are distributed into four classes: Calcarea (calcareous sponges), Demospongia (demosponges), Hexactinellida (glass sponges), and Homoscleromorpha. The largest class is Demospongia, comprising 83.4% of all known species. Out of > 8600 accepted species only ~ 250 are accepted freshwater sponge species (suborder Spongillina).

Sponges are filter feeders, which actively filter food particles, such as microbes, phytoplankton and small eukaryotes from the surrounding seawater through an aquiferous canal system, which serves as circulatory, digestive and excretory system (Taylor et al. 2007; Vacelet and Donadey 1977; Vogel 1977). Filtered particles get digested by phagocytosis, while metabolic waste is discharged by a constant water current, which is persistent throughout the whole sponge body, back into the surrounding water (Hentschel 2003; Taylor et al. 2007). Moreover, a small group of carnivorous sponges was found in the deep-sea and Mediterranean caves, lacking this aquiferous canal system (Vacelet and Boury-Esnault 1995).

The sponge morphology is highly diverse, with different growth strategies and shapes, held together by the mesohyl and sponge cells of various types (Box II). The morphological variety can range from massive statures, through vase and tubes to branching and encrusting types within a size range from a few millimeters up to two meters in height and / or diameter (Bell 2007). The individual skeletal components that give sponges their structural integrity, are composed of calcareous (calcium carbonate - $CaCO_3$) or siliceous (silicon dioxide - SiO_2) spicules, chitin, or the collagenous protein spongin (Hentschel et al. 2012; Taylor et al. 2007). The chemical composition, structural differences and size of the spicule are important morphological features for taxonomic identification (van Soest et al. 2012; Uriz et al. 2003). The classes Demospongia and Homoscleromorpha have spongin or silica spicule, or both. In addition, the class Homoscleromorpha has also representatives without spicules, which contain spongin skeletons instead. The classes Calcarea and Hexactinellida have calcareous or siliceous spicules, respectively (van Soest et al. 2012).

1.2 Ecological Functions of Marine Sponges

Sponge populations are usually diverse and structurally dominant in marine ecosystems, influencing ecosystem functioning (Bell 2008; Wulff 2006). For example, coral reef sponges can improve reef resilience and health by fulfilling several functional roles. First, coral reef sponges serve as a food source for other organisms, such as spongivore fishes; second sponge-specific microbial symbionts function as primary producers and nitrifiers; third, they alter the reef structure through calcification, cementation and bioerosion; fourth, they increase the resilience of coral reefs by stabilizing the carbonate rubble of physically destroyed reefs; fifth, sponges recycle nutrients, and sixth, they alter their surrounding water by filtering and water pumping activities, removing plankton and dissolved organic matter (DOM), exhaling secondary metabolites (Bell 2008; Diaz and Rützler 2001; Wulff 2001). While this list focuses on the functional roles of sponges in Caribbean coral reefs, many functional roles of sponges are equally prevalent in temperate, cold or deep water ecosystems. The pumping capability of sponges can reach up to 24000 L kg^{-1} d^{-1} in certain species (Hentschel 2003). Especially, the bentho-pelagic coupling, which describes the connection between benthic and pelagic environments is a functional aspect of sponges that is presumably universally applicable from tropical to cold water ecosystems (Cattaneo-Vietti et al. 1999; Lesser 2006; Maldonado et al. 2012; van Oevelen et al. 2009; Pile and Young 2006). The capability of filtering large volumes of water does not only remove nutrients (C, N, P, Si) from the water column. Conversely, it also returns processed nutrients or otherwise metabolized (in)organic compounds, such as siliceous spicules and other types of particular organic matter (POM), dissolved organic nitrogen (DON) and carbon (DOC), DOM or

BOX II - SPONGE CELL TYPES

Pinacocyte cells are together with **porocytes** part of the epidermis (pinacoderm). Closeable tube-like porocytes form the aquiferous canal system. **Choanocyte** cells are flagellated and form the internal choanoderm. They create the characteristic permanent water current, which enters the body through the ostia to the pinacoderm, and gets ejected from the osculum at the top.

Archaeocyte cells perform the food phagocytosis and can additionally differentiate into oocytes or gemmules for sexual and asexual reproductions, respectively. The **mesohyl** is not a cell type, but a collagenous matrix which fills the space between the pinacoderm and the choanoderm, in which archaeocytes, and further cell types and microbes are embedded.

secondary metabolites, back to the water column (Maldonado et al. 2012). Consequently, abundant sponge populations can significantly affect the bentho-pelagic coupling, by altering the complex carbon, nitrogen, and silicon cycles (Bell 2008; Maldonado 2015; Maldonado et al. 2005). Significant uptake rates of dissolved silicon by sublittoral sponges indicate that sponges may play a substantial role in the global silicon cycling (Maldonado et al. 2011; Tréguer and De La Rocha 2013). In marine ecosystems the central trophic pathway of DOM to higher trophic levels is the so called 'microbial loop' (Azam et al. 1983; Cho and Azam 1988). However, recent findings indicate relevant contributions of sponges to the marine food web as important connection between trophic levels by the release of cellular detritus (i.e., filter cells) as part of the 'sponge-loop' in oligotrophic habitats (Goeij et al. 2013; Maldonado 2015). The important role of sponges in ecosystem functioning in tropical coral reefs has recently received further attention, as reefs seem to undergo community shifts from coral to sponge dominance after anthropogenic and natural disturbances (McMurray et al. 2010; Norström et al. 2009; Pawlik et al. 2013). Therefore, sponges represent important marine benthic filter feeders and can be seen as ecosystem engineers, as they not only physically create habitats for microorganisms, but also chemically influence benthic and pelagic processes.

1.3 Sponge Natural Products and Chemical Ecology

In many marine habitats, sponge communities are affected by predation of spongivore organisms, such as turtles, fishes, or nudibranchs (Lesser 2006; Ruetzler 2003; Wulff 2006). Studies about sponge related predator-prey dynamics in coral reefs provided a profound understanding of the role of sponges as food sources, the impact of predation on sponge communities, and their intrinsic defense mechanisms to resist predation (Hill 1998; León and Bjorndal 2002; Pawlik 2011; Pawlik 1998). Certain sponge species are specifically adapted to an increased chance of predation by particular structural and chemical defense mechanisms (Burns et al. 2003; Burns and Ilan 2003; Rohde and Schupp 2011).

Herbivory defense mechanisms of terrestrial plant species are often used to describe the analogous defense strategies of sponges (Pawlik 2011; Rohde and Schupp 2011). For instance, the assumption that different internal chitin fibers, siliceous spicules, and spongin elements provide physical defense against potential predators, is similar to the roles of external spines of cactuses. However, the results of several studies on physical defenses of sponges vary considerably with no

consistent trend towards a general deterrent effect of these structural elements (Burns and Ilan 2003; Chanas and Pawlik 1996; Chanas and Pawlik 1995; Hill et al. 2005).

On the contrary, chemical defenses in sponges seem to have an overall effect in preventing predation (Burns et al. 2003; Pawlik 2011; Thacker et al. 1998; Thoms and Schupp 2007; Wilson et al. 1999). This is comparable to many terrestrial plants that depend on defenses induced by secondary products, which are inedible or toxic. Research on marine natural products established marine sponges as renowned sources of a wide range of bioactive natural products, with > 250 novel compounds isolated each year (e.g., Blunt et al. 2014; Blunt et al. 2013; Blunt et al. 2012). Feeding experiments with omnivorous or spongivorous reef fish, in which extracts and chemical compounds from sponges were added to artificial diets, revealed that these sponge-related metabolites play an important role in anti-predatory defenses (Pawlik 2011; Rohde and Schupp 2011; Waddell and Pawlik 2000). For example, an investigation of > 70 Caribbean sponge species showed that the majority of sponges examined (69%) are chemically defended (Pawlik et al. 1995). Correspondingly, the analyses of fish guts revealed that sponges without known chemical defenses are the primary diet of sponge-predatory fish (Pawlik 2011). With regards to the proposed structural defense mechanisms of sponges, most results indicate that chemical defense is the main anti-predatory mechanism in sponges, which can be complemented by structural defenses, like spicules (Pawlik 2011).

Besides anti-predatory functions of natural products, sessile sponges depend on a wide range of bioactive compounds to prevent diseases, infections, or parasitism (Dobson et al. 2015; Sipkema et al. 2005a; Thakur and Müller 2004). Another function of sponge natural products is the defense from competitive overgrowth by other benthic sessile organisms – e.g., corals, ascidians, bryozoans, other sponges, and algae (Engel and Pawlik 2000; Thacker et al. 1998). These compounds include a wide range of chemical classes with different bioactive properties, such as alkaloids, amino acid derivatives, lactones, macrolides, peptides, polyketides, or sterol terpenoids (Blunt et al. 2014; Blunt et al. 2013; Ebada et al. 2010; Molinski et al. 2009; Piel et al. 2004; Sánchez et al. 2006; Tasdemir et al. 2002). Consequently, many of these bioactive compounds derived from sponges have a high pharmacological or commercial potential (Fenical 2006; Proksch et al. 2003; Sipkema et al. 2005a). The biological activities and biotechnological applications of sponge derivatives span a broad spectrum: from antibacterial, antifungal, antitumor and antiviral applications, to antifouling and anti-inflammatory compounds (Dobson et al. 2015; Sipkema et al. 2005a). Besides the sponges

themselves, associated microbes are also important producers of marine natural products (Hentschel et al. 2012; Piel 2009; Wilson et al. 2014). For instance, chemical structures of natural products isolated from sponges closely resemble those, which have been previously derived from sponge-associated microbes (Bewley et al. 1996; Bewley and Faulkner 1998; Unson et al. 1994; Unson and Faulkner 1993). Furthermore, sponge-associated microbes have been identified as the actual sources of several bioactive compounds after they have been isolated from sponge extracts (Ebada et al. 2010; König et al. 2006; Piel et al. 2004; Rasyid and Adachi 2007).

However, the ongoing research on the biotechnological potential of sponge derived bioactive natural products as pharmacological lead compounds, drugs and agro chemicals faces the widely acknowledged 'supply issue', which prevents a comprehensive research approach of the potential large range of known and unknown bioactive products (Schippers et al. 2012). This is attributed to the fact that many bioactive products are only present at very low concentrations within the sponge. Different methods to enhance the concentration of many chemicals (e.g., aquaculture, direct chemical synthesis, or cloning of biosynthetic genes) have been applied, although with varying success (Schippers et al. 2012; Sipkema et al. 2005b; Taylor et al. 2007). One promising approach to overcome this issue is the cultivation of sponge-associated bacteria in the search for novel marine natural products (Dobson et al. 2015; Koopmans et al. 2009; Piel 2009; Schippers et al. 2012; Sipkema et al. 2011). However, currently most environmental microbes, from terrestrial to marine environments, are uncultivable with standard cultivation techniques (Alain and Querellou 2009; Bollmann et al. 2010; Dobson et al. 2015; Joint et al. 2010; Olson and McCarthy 2005). The true percentage of cultivable bacteria isolated from sponges is uncertain and the known range fluctuates greatly from 0.06 to 11% (Taylor et al. 2007). Sponge-associated bacteria are particularly difficult to cultivate, since their broad and often symbiotic relationship with sponges is very likely maintained by complex, host-specific biochemical and nutrient networks, which are influenced by the presence of other microbes, the environmental conditions of the sponge itself and its respective occupied habitat (Hentschel et al. 2012; Hoffmann et al. 2009; Liu et al. 2012; Radax et al. 2012a; Taylor et al. 2007; Thacker and Freeman 2012; Thomas et al. 2010).

1.4 The Sponge Microbiota

Sponges are acknowledged marine holobionts, hosting a diverse range of eukaryotic and prokaryotic (bacteria and archaea) organisms: up to 35% of the sponge biomass can made up of sponge-associated microbes (Taylor et al. 2007). Regardless of the genetic methods applied to investigate and characterize the sponge-associated microbiota, particular microbial phyla are usually found to be dominant within sponges (e.g., Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Firmicutes, Gemmatimonadetes, Nitrospirae, Proteobacteria (Alpha-, Beta-, Gamma-, and Delta-), Planctomycetes, Verrucomicrobia, Thaumarchaeota, as well as the candidate phylum Poribacteria). However, varying degrees of diversity, specificity and functional roles of the sponge-associated microbiota have been described (Hentschel et al. 2012; Taylor et al. 2007; Thacker and Freeman 2012; Webster and Taylor 2012), with further distinct differences between the microbial assemblages in sponges and the surrounding seawater (e.g., Erwin et al. 2012; Reveillaud et al. 2014; Taylor et al. 2013; Webster et al. 2010). Since the first massively parallel sequencing (MPS) study of the sponge-associated microbiome, the number of microbial phyla reported in individual sponge studies so far increased from initially 23 reported in Webster et al. (2010) to 47 in reported Reveillaud et al. (2014). Altogether, sponge-microbe associations comprise a large variety of beneficial or harmful interactions, which range from being a source of food, over parasitism and microbial pathogenesis, to mutualism and commensalism (Taylor et al. 2007). Immunological evidence dates the origin of bacterial symbioses in sponges back to the Precambrian Eon around 600 million years ago, whereas the first fossils that probably represent one of the first metazoan animals, found in 665 million year old rock layers in South Australia, were identified as early sponges (Love et al. 2009; Taylor et al. 2007). Therefore, sponge-microbe associations are probably the most ancient relationships between animals and microorganisms known to date. A growing body of literature focuses on the complex and diverse relationships of sponge-associated microbes. The mechanisms by which these associations are established and maintained are as diverse and manifold as the so far observed microbial diversity in sponges as a whole. The initial concept of abundant sponge-specific microbiota within the mesohyl has been introduced by first comparative bacterial cultivation studies and electron microscopy investigations conducted by Wilkinson et al. (1984) and Vacelet & Donadey (1977).

1.4.1 Ecological and Metabolic Functions of the Sponge Microbiota

Sponge-associated microbes posses a wide range of potential functional roles within their sponge hosts (Hentschel et al. 2012; Taylor et al. 2007; Thacker and Freeman 2012; Webster and Taylor 2012). For example, sponge hosts can benefit from the associated microbes in form of secondary metabolite (Fisch et al. 2009; Piel et al. 2004) and vitamin biosynthesis (Siegl et al. 2011; Thomas et al. 2010), the removal of metabolic waste (Hallam et al. 2006; Hoffmann et al. 2009), the stabilization of the sponge skeleton (Hentschel et al. 2002), or even UV protection (Regoli et al. 2000; Sarà 1971). As briefly introduced in Chapter 1.3, microbially synthesized secondary metabolites can be important factors of chemical defense mechanisms of sponges, protecting against spongivorous predators, pathogens or space-competing benthic sessile animals (e.g., corals or bryozoans) (Pawlik 2011; Thoms and Schupp 2007).

Many sponge-associated microbes are involved in complex nutrient cycles with interacting heterotrophic, autotrophic, aerobic and anaerobic processes between sponges and their environment (Hentschel et al. 2012; Webster and Taylor 2012). For instance, sponge-associated heterotrophic microorganisms can mediate chemoautotrophic and photoautotrophic transformation processes of dissolved organic and inorganic carbon (DOC and DIC respectively) as part of the sponge carbon cycle (Easson and Thacker 2014; Maldonado et al. 2012; Ribes et al. 2012). Especially the nitrogen metabolism in sponges added much to understanding of the functional roles of the sponge microbiota. Sponges are known sinks and sources of dissolved inorganic nitrogen (N_2), ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3), and particulate organic nitrogen (Maldonado et al. 2012; Ribes et al. 2005; Wilkinson and Fay 1979). Furthermore, certain microbial sponge symbionts may have important functions in the sponge nitrogen cycle, and are involved in the sponge metabolic nitrogen transformation processes of nitrogen fixation, nitrification, anaerobic ammonia oxidation, and denitrification (Fan et al. 2012; Hoffmann et al. 2009; Liu et al. 2012; Radax et al. 2012a). For instance, many tropical sponge species posses large abundances of cyanobacterial symbionts (e.g., genus *Synechococcus*) (Bayer et al. 2014; Hentschel et al. 2006; Unson et al. 1994). Sponges benefit from these photoautotrophic symbionts, which fix nitrogen as ammonia. They further benefit from supplemental nutrition, such as organic phosphate or glycerol (Taylor et al. 2007). Up to 50% of the energy requirements and 80% of the carbon budget of the host sponge can be provided by the

symbiotic cyanobacteria (Thoms and Schupp 2007; Wulff 2006). This may lead to the observed enhanced growth rates of certain sponges hosting abundant cyanobacterial communities (Erwin and Thacker 2008). In cold, temperate, and tropical sponges (e.g., *Phakellia ventilabrum*, *Geodia barretti*, or *Inflatella pellicula*) assemblages of ammonia oxidizing archaea (AOA, e.g., phylum Thaumarchaeota with the genera *Nitrosopumilus* or *Cenarchaeum*), ammonia oxidizing bacteria (AOB, e.g., phylum Planctomycetes with order Planctomycetales or class Betaproteobacteria with genus *Nitrosomonas*), and nitrite oxidizing bacteria (NOB, e.g., phylum Nitrospirae, genus *Nitrospira*) are present in sometimes substantial abundances (Bayer et al. 2008; Hoffmann et al. 2009; Mohamed et al. 2010; Radax et al. 2012a). Based on transcriptomic and metatranscriptomic analyses, ammonia-oxidizing archaea (e.g., *Cenarchaeum symbiosum*) are potentially important mediators of nitrification, at least in cold water sponges, such as *P. ventilabrum* or *G. barretti*, which accordingly exhibit high nitrification rates (Radax et al. 2012a; Radax et al. 2012b). Moreover, investigations of microbial symbionts in sponges with regards to the vast metabolic processes and nutrient exchange suggest that in divergent sponge hosts different microorganisms perform similar functions (Freeman et al. 2013; Ribes et al. 2012). The analysis of six different sponge microbiota by Fan et al. (2012) revealed that some of the sponge-specific functions are performed by phylogenetically distinct symbionts with analogous enzymes and biosynthetic pathways.

1.4.2 Microbial Abundance and Transmission

Sponge-associated microbial communities are differentiated based on the sponge-specific differences in abundance within the mesohyl, which organizes them into high microbial abundance (HMA) and low microbial abundance sponges (LMA) (Gloeckner et al. 2014; Hentschel et al. 2006). Generally, HMA sponges contain 10^8 to 10^{10} microbes g^{-1} tissue, which is three to four orders of magnitude higher than in the surrounding seawater. On the contrary, LMA sponges only possess 10^5 to 10^6 microbes g^{-1} tissue, which is close to the abundances of microorganisms in seawater (Hentschel et al. 2006). Together with differences in microbial abundances, molecular genetic analyses of the small subunit 16S ribosomal RNA (16S rRNA) gene showed differences in microbial diversity between HMA and LMA sponges. On phylum level, HMA sponges exhibited a higher diversity than their LMA counterparts, with Proteobacteria, Chloroflexi, Acidobacteria, Actinobacteria, and the candidate phylum 'Poribacteria', as primarily dominant phyla. Conversely, LMA sponges possess a lower

microbial diversity and different phylum associations, with dominant Proteobacteria (Alpha-, Beta-, or Gamma-) or Cyanobacteria (Bayer et al. 2014; Giles et al. 2013; Moitinho-Silva et al. 2014; Poppell et al. 2013; Schmitt et al. 2012a; Weisz et al. 2007).

Specific aspects of HMA and LMA differences intertwine with the fundamental question of the evolution and maintenance of the sponge-microbe relationships. For example, considering that both types of sponges are present in the same habitat and actively pump and filter microbes into their body from the surrounding seawater, the differences in abundances and composition are remarkable. Taylor et al. (2007) estimated that sponges with a pumping capacity of 24000 L kg^{-1} d^{-1} could filter (and release) up to 2.4 x 10^{13} bacterial cells per day with a given bacterial density of 10^6 cells ml^{-1} seawater. These differences may arise from the apparent differences in HMA and LMA sponge morphology (Schläppy et al. 2010; Weisz et al. 2008). Generally, HMA members have a denser mesohyl and more complex and narrow aquiferous canals (Vacelet and Donadey 1977), which may lead to pumping rates 52 - 94% slower than in their LMA counterparts (Weisz et al. 2008). The porous tissue of LMA sponges potentially allows higher pumping rates that result in larger quantities of filtered seawater. Their aquiferous channels, which are shorter and wider, together with the less dense mesohyl, could accelerate the exchange of nutrients and microbes. In contrast, the aquiferous system properties of HMA sponges may cause overall slower filter rates, therefore increasing the chance of nutrient uptake of their dense microbial communities (Weisz et al. 2008).

The discovery of large aggregations of microbes in the larvae of HMA sponges further supports that sponge-microbe associations are evolutionary linked and not only a result of arbitrary filtered and incorporated seawater microorganisms. In contrast, the larvae of LMA sponges are generally free of microbial activity (Gloeckner et al. 2013a; Gloeckner et al. 2013b; Lee et al. 2009a; Maldonado 2007; Schmitt et al. 2008; Schmitt et al. 2007; Sharp et al. 2007). The transfer of microbes from one generation to another via the sponge larvae is called 'vertical transmission' and it is assumed to be an ancient evolutionary mechanism for the maintenance of microbial symbioses in HMA sponges (Figure 1.2) (Hentschel et al. 2012; Taylor et al. 2007). This evolutionary scenario gained support when microbial sponge-specific phylogenetic clusters were found in distantly related and geographically separated sponges (Hentschel et al. 2002; Simister et al. 2012a; Taylor et al. 2007). Further, many of the sponge-specific microbes appeared not to be present in the surrounding seawater.

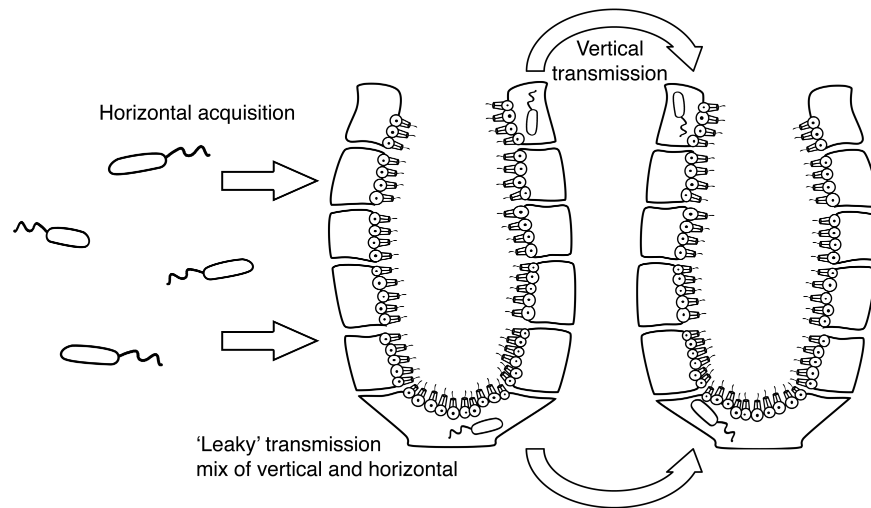

Figure 1.2 The three modes of microbial acquisition and transmission to establish and maintain sponge-associated microbial communities.

In contrast to the vertical transmission of putative microbial symbionts from parent to larvae, the 'horizontal transmission' scenario assumes that sponges acquire their microorganisms via specific or unspecific enrichment from the environment. In this scenario the sponge-specific microbiota gets unspecifically enriched, for instance via nutrition such as food bacteria from the seawater, or random uptake through injuries in the sponge epidermis. In theory, the specific enrichment of microbes in the horizontal transmission scenario comprises several mechanisms, with 1) an active absorption of specific microorganisms through the recognition of particular microbes by the immune system of the sponge, 2) subtractive enrichment, which is the accumulation of phagocytosis resistant microbes (Taylor et al. 2007). In these cases, the environmentally acquired microbes need to outcompete other potential colonizers in order to establish dominant sponge-associated communities. However, instead of just one of the two scenarios actively shaping the known sponge-microbe relationships, it is most likely that both scenarios are simultaneously present in sponges (Schmitt et al. 2008; Thacker and Freeman 2012; Webster and Taylor 2012). This has been recently termed as 'leaky vertical transmission' (Won et al. 2008) – with horizontal acquisition complementing the sponge-specific communities, which are established via vertical transmission.

1.4.3 Microbial Sponge Specificity and Diversity

One initially assumed key characteristic of sponge-microbe associations is the sponge-specificity of certain microbial organisms, which has been proposed due to early electron microscopy surveys and immunological experiments (Vacelet and Donadey 1977). The commencing use of molecular genetic

techniques for the investigation of microbial abundance, diversity, community structures or phylogenetic relationships improved the understanding of sponge-specific microbial community assemblages, and their potential establishment and maintenance mechanics (Diaz et al. 2003; Hentschel 2003; Hentschel et al. 2002; Taylor et al. 2005; Taylor et al. 2004; Webster et al. 2001). These methods cover a wide range of techniques, from cultivation and clone libraries to community analyses, such as denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP) or fluorescence in situ hybridization (FISH).

Phylogenetic reconstructions based on near-full-length 16S rRNA sequences suggested that a certain proportion of the sponge-associated microbiota can be divided into globally distributed sponge-specific sequence clusters (Hentschel et al. 2002; Simister et al. 2012a; Taylor et al. 2007). These sponge-specific clusters are monophyletic clades, which consist of at least three sponge-derived sequences from at least two specimen and are supported by three phylogenetic inference methods - usually maximum likelihood, neighbor-joining, and maximum parsimony (Hentschel et al. 2002). The comprehensive survey by Simister et al. (2012a) placed 27% of > 7500 near-full-length sponge-associated 16S rRNA sequences within 173 sponge-specific clusters. The *Synechococcus spongiarum* (phylum Cyanobacteria) cluster is a prime example that showed the complex entanglements of sponge-specificity and maintenance mechanisms. The cyanobacterial species *S. spongiarum* represents the largest sponge-specific cluster with 245 sequences from 40 globally distributed sponge species (Simister et al. 2012a). Although, while evidence of vertical transmission of these symbionts exists, phylogenetic analyses of 16S rRNA sequences of *S. spongiarum* lacked the phylogenetic evidence of co-speciation with the sponge hosts, which would have been expected to be present due to the vertical transmission mode from one generation to the next (Taylor et al. 2007). However, later phylogenetic reconstructions based on internal transcribed spacer (ITS) sequences, revealed not only the expected host specialization, but also a geographic pattern, which suggested a combination of vertical and horizontal transmission to maintain the presence of *S. spongiarum* in their specific hosts (Thacker and Freeman 2012). It is noteworthy that a recent phylogenetic mapping of 12 million short 16S rRNA sequence reads from the International Census of Marine Microbes (ICoMM - http://icomm.mbl.edu/) pyrosequencing project against the known 173 sponge-specific clusters from Simister et al. (2012a) revealed that many of the sponge-specific bacteria are also present within various marine ecosystems, but only in low abundances (Taylor et al. 2013). This result indicates that

many of the sponge-specific bacteria are able to survive in the seawater as part of the marine low abundance microbiome (i.e., rare biosphere), even though probably mostly metabolically inactive. Therefore, it adds to the hypothesis of 'leaky vertical transmission', that sponge-specific microbes are present in very low abundances throughout the marine environment, thus providing a 'seed bank' for a later uptake by sponges and subsequent colonization (Thacker and Freeman 2012; Webster et al. 2010).

As indicated by the study of Taylor et al. (2013), the advent of massively parallel sequencing technologies (e.g., 454 pyrosequencing or Illumina MiSeq) was a major development in the vast research field of sponge microbe relationships. In comparison to Sanger sequencing, the amplicon (i.e., polymerase chain reaction amplified sequence fragment) length of MPS amplified sequences are considerably shorter (~ 1200 base pairs vs. ~ 400 - 800 base pairs for Sanger and MPS respectively – depending on the respective primer position). On the one hand, short MPS amplicons contain less information about the respective gene evolution than their Sanger sequences based counterparts. While this reduces the phylogenetic and / or taxonomic resolution of each amplicon, their large quantity circumvents the constraints of sequencing depth and costs of classical Sanger sequencing. This development allowed conducting metagenomic studies (i.e., genetic analysis from environmental samples) on multiple samples with sequencing depths between mega- and gigabases, and thereby also allowed the investigation of the 'hidden diversity' (e.g., uncultivable low abundance microbes) of the microbiome with much greater detail and scale with an additional simultaneous approximation of microbial abundances (Huse et al. 2008; Sogin et al. 2006).

Webster et al. (2010) applied 16S rRNA tag pyrosequencing to investigate the diversity and abundance of sponge associated microbiota from three sponges (*Ianthella basta*, *Ircinia ramosa* and *Rhopaloeides odorabile*) and their larvae from the Great Barrier Reef in the North-East of Australia. Their results revealed a very high bacterial operational taxonomic unit (OTU) and phylum diversity (n = 23) within these three sponge species and larvae. The sequences obtained from sponges and larvae fell into 33 sponge-specific clusters, with almost half of the clusters exclusively associated with the hosts. Moreover, patterns of partial sponge species specificity among different hosts and seawater samples emerged, when the bacterial community diversity based on Bray-Curtis dissimilarity distances were plotted as pairwise similarity matrices. Bray-Curtis dissimilarity is a measure of the species turnover between two or more sites (i.e., beta diversity; Figure 2 in Webster et al. 2010, Figure 2 in

Schmitt et al. 2012b, and Box III). By applying 16S rRNA amplicon MPS to 32 sponge species (> 90 specimen) from eight globally distributed tropical and temperate locations, Schmitt et al. (2012b) described three general states of sponge associated microbial communities: 1) a small core community, present in at least 70% of all analyzed sponges, 2) a variable community, present in < 70% of all sponges, and in at least two specimens, and 3) a large species-specific group, in which the respective OTUs are only present within one single sponge species (Figure 1.3). Altogether, the use of a variety of molecular genetic methods to analyze the microbial community patterns of the sponge-associated microbiota, demonstrated apparent overlapping levels of sponge species specificity and an exceptional high diversity and richness for marine host-associated microbial communities (e.g., Jackson et al. 2013; Lee et al. 2011; Webster et al. 2010). Nevertheless, host-specificity already has become evident in studies that applied techniques such as T-RFLP, DGGE or clone library analyses (Erwin et al. 2012; Lee et al. 2009b; Olson and Gao 2013; Pita et al. 2013a; Pita et al. 2013b; Taylor et al. 2005; Taylor et al. 2004; Webster et al. 2012). Increasing numbers of recent studies, which utilize MPS data, emphasize the general accuracy of the variable and core microbial community patterns (Cleary et al. 2013; Lee et al. 2011; Reveillaud et al. 2014; Schmitt et al. 2012b; Webster et al. 2010).

The variability of sponge-associated communities over time, space as well as among different species was reviewed by Taylor et al. (2007), who took a comprehensive collection of studies into account. Altogether, based on their reviewed literature, the sponge-associated communities appeared

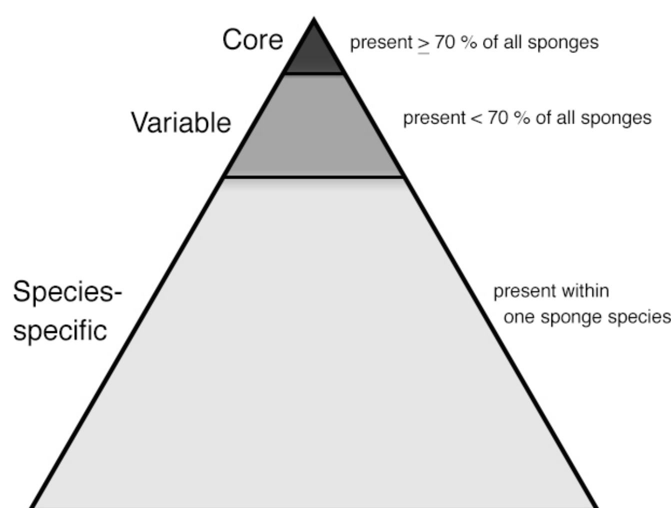

Figure 1.3 The three general states of sponge-microbe microbe associations. Figure modified from Schmitt et al. (2012b).

to be relatively stable and host- or species-specific over spatio-temporal gradients. Recent investigations of the species turnover of sponge-associated communities along temporal (Simister et al. 2013; Hardoim & Costa 2014; White et al. 2012; Erwin et al. 2012), spatial (Pita et al. 2013a; Pita et al. 2013b; Reveillaud et al. 2014; Schmitt et al. 2012b; Schöttner et al. 2013), and spatio-temporal gradients (Anderson et al. 2010) as well as along environmental differences (Becerro and Paul 2004; Cárdenas et al. 2014; Cleary et al. 2013; Olson and Gao 2013) emphasized the notion that microbial community structures are generally species-specific. In contrast, vertical (depth) and horizontal (biogeographic) differences seem to have only minor effects on the observed community patterns. However, the effect of local natural environmental gradients (e.g., depth, nutrient concentrations, salinity, or light gradients) or combined factors (e.g., species turnover along a depth gradient over time) on the sponge microbiota are less comprehensively studied, with only a few studies focusing specifically on these particular effects (e.g., Cárdenas et al. 2014; Olson & Gao 2013). In their review of the host-associated microbial ecology in mesophotic coral reefs, Olson & Kellogg (2010) discussed the lack of comparative molecular genetic community analyses and emphasized the need for further investigations on the effects of depth on the host associated microbial communities for a better understanding of species turnover between shallow and mesophotic sites.

An emerging aspect in microbial molecular ecology is the ecological relevance of high microbial taxonomic ranks (Fierer et al. 2007; Kent et al. 2007; Prosser et al. 2007). In present community ecology studies, which utilize MPS technologies to achieve high-resolution microbial diversity data, taxonomically classified OTUs (generally 97% sequence similarity) are used to display and to analyze existing community patterns. This allows us to observe diversity patterns and shifts of taxonomically similar and divergent OTUs over time and space and within or between different sponge species. Furthermore, for the description and comparison of sponge-microbe community patterns, individual OTUs are generally summed up and their relative abundance reported mainly on phylum level. As a general result, and as discussed above, the sponge-associated microbiota exhibit an exceptional range of specificity and variations in diversity and community assemblages among different sponge species (Schmitt et al. 2012b; Webster et al. 2010; White et al. 2012). Philippot et al. (2010) proposed that adaptation to different ecological niches would shape and differentiate microbial genetic lineages in such a way, that these evolutionary divergences should be observable also at higher taxonomic ranks than just on species (i.e., OTU) level. They further proposed that ecological relevance of deeply

branching microbial lineages should become discernable by biogeographic patterns at such high taxonomic ranks. In fact, many environmental studies on the microbiota of marine or terrestrial ecosystems appear to support the hypothesis of ecological coherence of microbial lineages at high taxonomic ranks (see Philippot et al. 2010). For example, Zinger et al. (2011) specifically addressed this question by analyzing bacterial beta diversity patterns on a global scale across seawater and marine soil communities on taxonomic ranks from OTU, over genus to phylum level. Beta diversity analyses provide an analytical framework for reproducible comparisons of diversity patterns and processes (Ramette 2007; Tuomisto 2010). They observed consistent patterns of bacterial community distribution across all taxonomic ranks, which supports the notion of ecological meaningful bacterial assemblages at high taxonomic ranks based on evolutionary adaptation processes. Since individual sponge species represent most likely very divergent microhabitats, with many intrinsic ecological and functional differences (e.g., habitat preferences, natural product synthesis and allocation, metabolic pathways, nutrient provision, and microbial assemblage), one could postulate that sponge-specific ecologically relevant deep branches of bacterial and archaeal lineages should result in coherent patterns at high taxonomic ranks, which reflect these mutualistic host-symbiont identities (Freeman et al. 2013; Liu et al. 2012; Ribes et al. 2012; Weisz et al. 2007). For example, Hardoim & Costa (2014) described an 'essential bacteriome' consisting of 27 OTUs distributed across nine bacterial phyla within the sponge *Sarcotragus spinosulus*. They interpreted the maintenance of these species-specific phyla and classes as an indication that at higher bacterial taxonomic ranks ecologically meaningful patterns are present, which are coherent with the functional roles in the particular microhabitat (i.e., sponges). Further indications for the potential relevance of this idea are the differences in dominant bacterial symbiont phyla observed in HMA (e.g., Chloroflexi or Poribacteria) and LMA (e.g., Cyanobacteria) sponges (Moitinho-Silva et al. 2014). This also extends to the dominance of functional important AOA (e.g., *Nitrosopumilus*) and AOB (e.g., *Nitrospira*) in certain sponge species, while they are almost absent in phylogenetically divergent sponges from the same geographic location and habitat (Chapter III). Finally, the analysis by Fan et al. (2012) of microbially mediated nitrogen metabolism pathways of six sponge-associated microbiomes demonstrated that in phylogenetically divergent sponge hosts complex symbiont communities possess analogous denitrification pathways. This provided evidence that different symbiotic microbial assemblages show a certain degree of functional equivalence in phylogenetically different sponge hosts, and therefore supports the idea that

this observed evolutionary convergence could be an important process, shaping the potential ecological coherent patterns in sponges-associated microbial communities at high taxonomic ranks.

BOX III - BIODIVERSITY can refer to a wide range of diverse meanings, from the taxonomic, morphological and genetic diversity within populations, the diversity between species, and the ecological diversity within ecosystems and between habitats. This all cumulates to the global biodiversity as the total diversity and variability of life.

SPECIES DIVERSITY is defined as the diversity of species within a community (i.e., location or sample). Species diversity (e.g., Shannon index) is composed of species richness, the number of species. Species evenness describes the relative abundance of different species within the respective community.

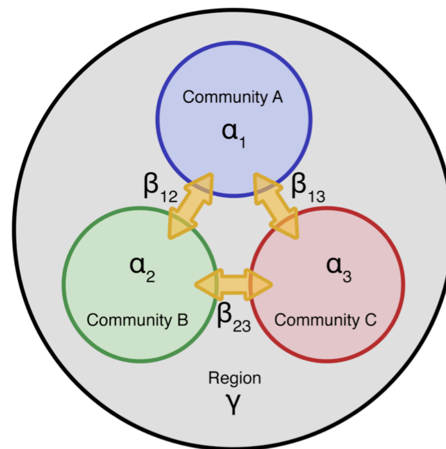

Figure 1.4 Schematic representation of alpha diversity as individually colored circles, beta diversity as arrows between the local alphas, and gamma diversity as space with the outer black frame. Figure modified from Zinger et al. (2012).

ALPHA DIVERSITY is the diversity of a local community. Measured as species richness, Shannon diversity, or species evenness.

BETA DIVERSITY is the difference in diversity between two or more communities. Measured as presence / absence community composition with Jaccard or Sorensen similarity indices & relative species abundance measured with Bray-Curtis or Morisita-Horn dissimilarity indices.

GAMMA DIVERSITY is the diversity of a region.

2. Aims and Outline

Given the often dense and diverse microbial communities and the complex symbiotic relationships with the sponge hosts, the general aim of this thesis was to further investigate the specificity and turnover of sponge-associated microbial communities among phylogenetically divergent sponges from temperate and tropical habitats.

Chapter I of this thesis describes the first time application of diffusion growth chambers (DGCs) for the isolation of cultivable and previously uncultivated bacteria derived from sponges (Figure 1.1A). While sponges are known sources of bioactive metabolites, it has been shown for at least some cases that not the sponge itself, but symbiotic microbes are the actual producers of these metabolites. However, because of the very low concentrations of many compounds within the source sponge, the cultivation of secondary metabolite synthesizing bacteria could circumvent the acknowledged 'supply issue'. Since most sponge-associated bacteria resist cultivation on artificial growth media, the diffusion growth chamber method is a promising approach to cultivate bacteria *in situ* by allowing interaction with other microorganisms and chemical compounds within the ambient environment.

Authors: Georg Steinert, Susanna Whitfield, Michael W. Taylor, Carsten Thoms, Peter J. Schupp.
I evaluated the data and wrote the manuscript with editorial help of Michael W. Taylor and Peter J. Schupp. This article was published in 2014 in Marine Biotechnology 16(5), 594-603, DOI 10.1007/s10126-014-9575-y

Chapter II deals with the analysis of sponge-associated microbial diversity in three different tropical sponge species from Guam coral reefs (Figure 1.1B-D) based on 454 pyrosequencing generated community data. The study investigates the community turnover (i.e., variances in beta diversity - Box III, Figure 1.4) between the different sponge species and environmental water samples. To assess the effect of vertical spatial differences, the variance of microbial community composition has been investigated along a local depth gradient from shallow to mesophotic reef sites within two sponge populations.

Authors: Georg Steinert, Michael W. Taylor, Peter Deines, Rachel L. Simister, Michael Hoggard, Peter J. Schupp
I shared the laboratory work with Peter Deines, Rachel L. Simister and Michael Hoggard. I evaluated the data and wrote the manuscript with editorial help of Michael W. Taylor and Peter J. Schupp. The manuscript is ready for submission.

Chapter III is an assessment of the microbial host-specificity and diversity among seven sponge taxa from temperate marine sponges collected in Sweden at depths from 2 to 158 meters. The data was extracted from the sponge dataset provided from the Earth Microbiome Project (http://www.earthmicrobiome.org/). The effect of host taxonomy and habitats along a depth gradient on the community turnover between the individual sponges is investigated. In addition, the consistency of the microbial community composition is analyzed at high taxonomic ranks and compared with the results of the indicator species analysis.

Authors: **Georg Steinert**, Sven Rohde, Peter J. Schupp
I shared the sampling work with Sven Rohde and Peter J. Schupp. I evaluated the data and wrote the manuscript with editorial help of Sven Rohde and Peter J. Schupp. The manuscript is ready for submission.

Chapter IV investigates the microbial diversity of one marine ascidian species, *Eudistoma toealensis*, collected from two Micronesian islands based on 454 pyrosequencing community data. Compared to sponges, marine ascidians have similar lifestyle traits and microbial associations, and they are also known as rich sources of bioactive natural products. Besides the microbial diversity assessment, the focus is especially on the diversity host-associated Actinobacteria as potential producers of natural products and on the evaluation of sponge-associated microbes in *E. toealensis*.

Authors: Georg Steinert, Michael W. Taylor, Peter J. Schupp
I performed the laboratory work, evaluated the data and wrote the manuscript with editorial help of Michael W. Taylor and Peter J. Schupp. This article was published online in February 2015 in Marine Biotechnology, DOI 10.1007/s10126-015-9622-3

Chapter V is the general discussion of **Chapter I** to **IV** integrating and discussing the results from all chapters as follows:

> **Chapters I** & **II** - the aim was the comparison of identified *R. globostellata*-associated microbial phylotypes derived from DGC / direct isolates and environmental 16S rRNA gen tag pyrosequencing.
>
> **Chapters II** & **III** - the aim was to investigate the effect of depth on microbial communities in tropical and temperate sponges and to discuss the potential causes for the observed patterns.
>
> **Chapters II** to **IV** - the aim was to investigate patterns of community assemblages among invertebrate-associated marine microbiomes. For this I performed a synthesis of the present MPS data and additional downloaded microbial community data to perform a meta-analysis of ecological meaningful microbial community patterns on a global scale, based on concatenated sponge, ascidian and coral datasets.

CHAPTER I

Application of Diffusion Growth Chambers for the Cultivation of Marine Sponge-Associated Bacteria

Abstract

Marine sponges contain dense and diverse microbial communities, which are renowned as a source of bioactive metabolites. The biological activities of sponge-microbe natural products span a broad spectrum, from anti-bacterial and anti-fungal to anti-tumor and anti-viral applications. However, the potential of sponge-derived compounds has not been fully realized, due largely to the acknowledged 'supply issue'. Most bacteria from environmental samples have resisted cultivation on artificial growth media and cultivation of sponge-associated bacteria has been a major focus in the search for novel marine natural products. One approach to isolate so-called 'uncultivable' microorganisms from different environments is the diffusion growth chamber method. Here, we describe the first application of diffusion growth chambers for the isolation of cultivable and previously uncultivated bacteria from sponges. The study was conducted by implanting diffusion growth chambers in the tissue of *Rhabdastrella globostellata* reef sponges. In total, 255 16S rRNA gene sequences were obtained, with phylogenetic analyses revealing their affiliations with the Alpha- and Gammaproteobacteria, Bacteroidetes, Actinobacteria and Firmicutes. Fifteen sequences represented previously uncultivated bacteria belonging to the Bacteroidetes and Proteobacteria (Alpha & Gamma classes). Our results indicate that the diffusion growth chamber approach can be successfully applied in a natural, living marine environment such as sponges.

Introduction

Many marine sponges contain dense and diverse microbial communities of both ecological and biotechnological importance (Hentschel et al. 2012; Webster and Taylor 2012). Sponge-microbe associations are renowned as a source of bioactive metabolites, with > 250 novel compounds isolated from sponges each year (e.g. Blunt et al. 2013; Blunt et al. 2012; Blunt et al. 2011). These compounds, which include alkaloids, macrolides, polyketides, terpenoids and other bioactive products, have generated much research and commercial interest due to their diverse pharmacological properties (Koopmans et al. 2009; Piel et al. 2004; Sipkema et al. 2005a; Wang 2006; Zhang et al. 2005). Biological activities of sponge natural products span a broad spectrum, from anti-bacterial and anti-fungal to anti-tumor and anti-viral applications (Ebada et al. 2010; Gandhimathi et al. 2008; Molinski et al. 2009; Sashidhara et al. 2009). However, the considerable potential of sponge-derived

compounds as lead structures and potential drugs has not been fully realized, due largely to the acknowledged ‘supply issue’ (Schippers et al. 2012). Many compounds are only present at very low concentrations within the source sponge. Various options for accessing these compounds, ranging from sponge aquaculture to direct chemical synthesis to cloning of biosynthetic genes, have been applied with differing levels of success (Schippers et al. 2012; Taylor et al. 2007). Given that at least some of these metabolites are believed to be made by symbiotic microbes (Piel 2009), cultivation of sponge-associated bacteria has been a major focus in the search for novel marine natural products (Koopmans et al. 2009; Schippers et al. 2012; Sipkema et al. 2011). However, most bacteria from environmental samples – including sponges – have resisted cultivation on artificial growth media (Alain and Querellou 2009; Bollmann et al. 2010; Joint et al. 2010; Olson and McCarthy 2005). Approximately 0.06 to 11% of the sponge-associated bacterial community could be cultivated to date (Taylor et al. 2007). In the case of sponge bacteria it is presumed that their growth is maintained by complex, host-specific biochemical and nutrient networks influenced by the presence of other microorganisms and the sponge itself (Hentschel et al. 2006; Taylor et al. 2007).

One promising approach to isolating so-called ‘uncultivable’ microorganisms is the diffusion growth chamber (DGC) method (Kaeberlein et al. 2002). While there are different variations to this approach (Bollmann et al. 2007; Kaeberlein et al. 2002; Nichols et al. 2010), the unifying feature is an attempt to cultivate bacteria *in situ* by allowing interaction with other microorganisms and chemical components within the ambient environment. During the first approach, Kaeberlein and co-workers deployed DGCs in aquarium sediments and succeeded in growing isolates in the presence of other microorganisms (Kaeberlein et al. 2002). This method led to the successful isolation of previously uncultivated, or rarely cultivated, bacteria from sediment samples (Bollmann et al. 2007; Gavrish et al. 2008; Kaeberlein et al. 2002). Following the initial cultivation success, Bollmann and colleagues repeatedly inoculated new DGCs with bacteria-medium mixes from previously incubated DGCs, then redeployed these new chambers in the aquarium sediments, thereby increasing the isolation success of rare bacterial species (Bollmann et al. 2007).

Here, we describe the first attempt at using DGCs for the isolation of cultivable and previously uncultivated bacteria from sponges. The study was conducted by implanting DGCs in the tissue of *Rhabdastrella globostellata* reef sponges. *R. globostellata* is a relatively common sponge on reefs inside Apra Harbor, Guam. It has a massive barrel- to ball-shaped growth form of up to 70 cm in

diameter and over 60 cm in height, making it a prime candidate for insertion and incubation of DGCs. Furthermore, previous reports of cytotoxic isomalabaricane triterpenes and sesterterpenes from *R. globostellata* (Fouad et al. 2006; Tasdemir et al. 2002) identify this as a sponge of chemical significance, while the microbial diversity of *R. globostellata* has also received attention (Lafi et al. 2009; Lafi et al. 2005). Hence, we chose this species for its production of bioactive secondary metabolites, its growth morphology and previous microbial diversity research.

Materials and Methods

Diffusion growth chambers (DGCs) were constructed from two combined centrifuge micro-filter sections (Whatman Centrex, pore size 0.2 μm) (Figure 2.1A). Earlier attempts using thin sections of PVC pipe (5 mm) with attached 0.2 μm membranes did not work well as they separated during autoclaving or during incubation in the sponge. The advantage of the microtube filters was that the membranes were embedded in the plastic. The original micro-filter tubes were cut with a hot sterile scalpel at the end of the filter sections, heated at the cuts and glued together. This provided an inner volume of approx. 1 ml for inoculation with media, while maintaining the 0.2 μm membranes at both sides of the chamber, allowing diffusion of nutrients and waste products in and out of the chambers. Assembly of the chambers was conducted under aseptic conditions and chambers were visually inspected after assembly, inoculation and recovery for possible leaks and the maintenance of intact filters, in order to assess their continuous operability during the experiment. Each chamber received a cable tie after inoculation for insertion and removal of the chambers from the sponges (Figure 2.1B & 2.1C).

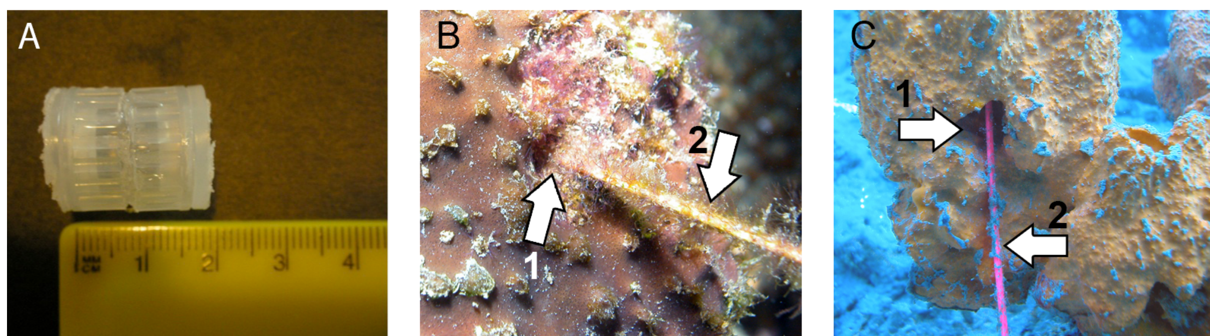

Figure 2.1 Diffusion growth chamber for in vivo cultivation of sponge- associated bacteria: A) DGC after assembly, B) incision is completely healed (left arrow), cable tie attached to incubating DGC protruding from an *R. globostellata* specimen after 4 weeks (right arrow), C) retraction of tissue in *Stylissa massa* after 4 weeks of DGC incubation. Upper arrow, tissue retracted around the DGC, creating a hole in the sponge; lower arrow, cable tie.

Tissue from *R. globostellata* was sampled from three individual sponges at depths of 1-3 m in August 2008 at Gab Gab reef (13°27'57"N 144°38'42"E) (Apra Harbor, Guam). Tissue samples (5 cm^3) were washed three times with filtered (0.2 μm), autoclaved seawater. Tissue portions were subsequently combined to maximize bacterial diversity and homogenized using a sterile mortar and pestle with an equal volume of sterile seawater. This homogenate was used for both the DGC and direct plating methods (Figure 2.2).

In order to inoculate the DGCs, homogenized tissue was diluted with one of three different media (0.1 ml sponge homogenate and 9.9 ml warm medium). For growth media, low-, medium- and high-nutrient compositions were used: M8 (1.8% agar & 80 μm-filtered seawater); MB 1:10 (1.5% agar, 3.75 g/L Difco Marine Broth 2216, filtered deionized water); M1 (1.8% agar, 1% starch, 0.4% yeast extract, 0.2% peptone, 80 μm-filtered seawater). After the media had cooled to 40°C the sponge homogenate was added, mixed with a pipette and injected into the respective DGCs. For field incubation, three different *R. globostellata* sponges in Gab Gab reef (same location and depth as described above) were chosen to incorporate the inoculated growth chambers *in situ*. Each individual sponge received three DGCs (one DGC per media type per sponge) approximately 5 cm deep within the sponge tissue. Cuts from the insertion of the chambers usually healed completely within 7-10 days (Figure 2.1B). Sponges were initially observed for signs of disease or other health impairments (e.g. oscula are open, no discoloration or bleaching, no soft decaying tissue on the sponge especially near the incision for the DGC) following the insertion of the DGCs. After four weeks the initial DGC chambers were cut out of the sponge tissue, placed in sterile seawater and returned to the laboratory. During removal of the chambers one could clearly observe that *R. globostellata* had integrated the chambers into the tissue, as pieces of tissue were firmly attached to the outside chamber and the chambers were not merely loose within the sponge tissue. However, initial trials with several sponge species revealed that not all sponges were suited for DGC experiments. The sponges need to be large enough to accommodate a 5 cm cut in order to squeeze the chambers deep inside the sponge tissue. We could do this successfully with the sponges *Hyrtios altum*, *Melophlus sarasinorum* and *R. globostellata*. However, *Stylissa massa,* although large enough to accommodate the chamber, did reject them, as the tissue retracted from the chambers to result in holes in the sponge tissue (Figure 2.1C). This led either to chambers being incubated in the water, or loss of the chamber as they were

washed out from the holes. Therefore, it seems advisable to test sponges for their healing ability by inserting dummy chambers prior to conducting a full-scale incubation experiment.

Four types of media were used for plating of the sponge bacteria (Figure 2.2), namely the same three media types used in the DGCs and described above, and additionally M1 media with *R. globostellata* crude extract (methanol/ethyl acetate 1:1) at 1/10th the natural concentration found in the sponge. The aim of this was to provide a medium which mimicked the chemical components of the host sponge. Extract concentration was diluted from natural concentration due to the large volume that would have otherwise been required. Extract was added to M1 medium after autoclaving. Additionally, the antibiotics Rifampicin (5 μg/ml) and Cycloheximide (100 μg/ml) were added to all four media types to inhibit fast growing Gram-negative bacteria and fungal growth. Because of media loss in each DGC during the *in situ* incubation, all corresponding DGC media (low, medium & high nutrient levels) were combined after extraction from the sponges and serially diluted to 10^{-4} to yield sufficient incubated media for subsequent plating. From these dilutions, 100 μl were used for plating on the corresponding media type (Figure 2.2). Only M1 DGC dilutions were plated onto M1 medium, and M1 medium with *R. globostellata* extract added. The remaining chamber material was mixed with the respective medium to a dilution of 10^{-3} and was used to inoculate the new DGCs, which were inserted back into the sponge individuals. This was repeated for four cycles each of plating and incubation (Figure 2.2). By re-using the initial inoculates from the first DGC generations in each cycle we took care of the possible lower doubling times of bacteria in nature. Hence, the total *in vivo* incubation time of the initial inoculates was 16 weeks. For direct plating of sponge-associated bacteria the aforementioned tissue homogenate was diluted in sterile seawater to 10^{-4}. For each dilution step 100 μl were spread-plated onto the four different plating types of media described above (Figure 2.2). Petri dishes were incubated at 27°C and checked every two days for bacterial colonies. Colonies were selected for re-streaking to purity based on unique growth morphology and pigment production. For each obtained pure isolate a slant and two -80°C cryo-stocks (in 15% glycerol) were created.

Genomic DNA was obtained from most isolates by re-plating cryo-stocks, picking a single colony, adding 40 μl sterile ddH_2O, vortexing and freezing overnight. For those isolates for which PCR initially failed, DNA was extracted using the QIAGEN DNeasy Tissue Kit following the manufacturer's protocol for Gram-negative bacteria and animal tissue. 16S ribosomal RNA genes were amplified by PCR using the eubacterial primers 27f (GAG TTT GAT CCT GGC TCA) and 1492r (TAC GGY TAC CTT GTT

ACG ACT T) (Lafi et al. 2005). The 50 μl PCR reaction mix contained approximately 100 ng of DNA, 60 pmol of each primer, 10x PCR buffer (Qiagen, Gaithersburg, MD), 1.5 U of TopTaq DNA polymerase (Qiagen, Gaithersburg, MD) and 200 μM deoxynucleoside triphosphate mixture. The cycling conditions for PCR were: 95°C for 5 min; 30 cycles of 95°C for 1 min, 55°C for 1 min, 72°C for 90 s; followed by 72°C for 10 min. PCR products were checked on 1% agarose gels stained with ethidium bromide and visualized under UV illumination. DNA was purified to remove excess primers and unused material using the QIAquick PCR Purification Kit (Qiagen, Gaithersburg, MD) following the manufacturer's protocol. PCR products were sequenced by Macrogen Inc. using the forward primer 27f. Sequence chromatograms were viewed and edited using Chromas Pro version 2.3 (Technelysium Pty Ltd). High-quality sequences (defined as > 600 bp) were compared to the National Center for Biotechnology Information (NCBI) database (http://blast.ncbi.nlm.nih.gov) by using BLAST. Sequences displaying $\leq$97% similarity to the closest sequence from a cultivated microorganism were then re-sequenced using the reverse primer 1492r to obtain near full-length 16S rRNA gene sequences.

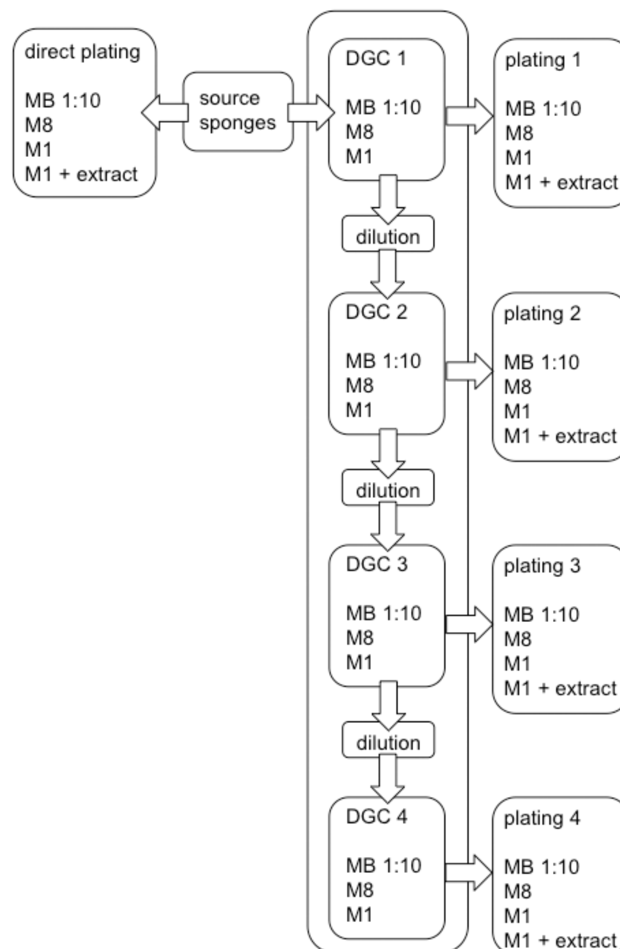

Figure 2.2 Cultivation and isolation workflow.

Obtained forward and reverse sequences were assembled and trimmed using Geneious (ver. 6.0.6) (Kearse et al. 2012). For phylogenetic analysis and reconstruction of possible monophyletic sponge and sponge/coral-specific sequence clusters, we used the sponge and coral specific ARB database established by Simister et al. (2012a). These data encompassed all 7451 sponge-derived 16S rRNA gene sequences (from bacteria and archaea) and 95 18S rRNA gene sequences (from eukaryotic microbes) which were available in February 2010 (excluding 454 pyrosequencing data obtained from sponges). In order to update this specific database with recent relevant sequences, which were not present in the ARB/SILVA database of Simister et al. (2012a), we retrieved for each DGC sequence the three most similar sponge, coral, and non-sponge/coral sequences from the NCBI BLAST database. Using the SINA Web Aligner these sequences and those obtained from the DGC and direct-plating experiments were aligned and imported into the SILVA database. To test for the existence of monophyletic sponge-specific clusters we followed the approach originally described by Hentschel et al. (2002) and re-evaluated by Simister et al. (2012a). For each bacterial phylum, multiple trees were constructed in ARB using long ($\geq$ 1200 bp) sequences with the implemented phylogenetic programs RAxML (maximum likelihood), DNAPARS (maximum parsimony), and Neighbor Joining. Short sequences (< 1200 bp) were added subsequently to all trees using the Parsimony Interactive tool. Conservation filters of 50% were applied for each phylum, and sequences representing a range of other bacterial phyla were used as outgroups. In order to obtain robust bootstrap values each maximum likelihood tree was exported and bootstrapped (rapid bootstrap, 1000 replicates) using the most recent RAxML stand alone version (RAxML version 7.5.3) (Stamatakis et al. 2008; Stamatakis et al. 2005). Sponge-specific clusters were only marked in the maximum likelihood trees if these clusters occurred with all three phylogenetic methods. For OTU-based community analysis and identification of previously uncultivated (i.e. 'novel') bacterial strains, sequences from DGC and direct-plating approaches were grouped into operational taxonomic units using mothur (v.1.30.2) (Schloss et al. 2009). Short and long sequences were pooled together, and subsequently aligned and trimmed by using mothur. Using the screen.seqs and filter.seqs command allowed retention of as many overlapping sequence fragments as possible with an overall sequence length sufficient for subsequent taxonomic classification (mothur command: classify.seqs). This also gave an OTU average neighbor clustering with a 0.03 cutoff level for a species-based study approach (mothur commands: dist.seqs, cluster, make.shared, classify.otu). The internal slayer algorithm was used to detect and remove

possible chimeric sequences. Finally, the command get.oturep was applied to retrieve representative sequences for each 0.03 cutoff OTU. These sequences were subjected to a BLAST search against the NCBI nucleotide collection (search query: 'all [filter] NOT(srcdb refseq model[prop] AND biomol rna[prop]) OR environmental samples[organism] OR metagenomes[orgn]') to identify DGC-based OTUs with $\leq$ 97% sequence similarity to closest cultured ones. The unaltered full length sequence dataset (without mothur treatment) was used for an additional BLAST search based on the same search conditions. In order to identify novel bacterial strains from sponges, we used two approaches: the 0.03 OTU and the full sequence length approach. The additional full sequence length approach was chosen because the OTU definition for 16S rRNA genes is still a subject of debate (Huse et al. 2010; Petrosino et al. 2009; Schloss and Westcott 2011). Furthermore, the assembling of OTUs from variable-length sequences requires a highly trimmed, and hence very shortened, alignment. This loss of genetic information can influence the BLAST search results significantly for novel strains**.** Therefore, the comparison of full length sequences with known sequences during BLAST similarity search can result in different identity values in comparison to trimmed OTU representatives.

Results

In total, 255 DGC-derived 16S rRNA gene sequences were obtained during this study (Supplementary Table 2.1). Fifty-eight of these sequences were obtained from the direct-plating approach and 197 from various DGC generations (Table 2.1). The distribution of the DGC-derived sequences ranged from a minimum of 34 sequences (13% of all sequences) in generation four (DGC4) to a maximum of 72 sequences (28%) in generation three (DGC3). The gap between the minimum number of gathered sequences (n = 34 sequences) in M1 medium with sponge extract and MB 1:10 (both 13%), and the maximum with 114 sequences in M8 medium (46%), is more prominent by taking the four different DGC media types into account (Table 2.1).

At 0.03 genetic distance cut-off level we retrieved 60 OTUs in total. Thirty-three (55% of all 0.03 OTUs) were derived from the four different DGC generations, 19 (31%, like above) via direct plating alone and 8 (5%, like above) were recovered from both the DGC and direct plating methods (Table 2.2). Only three 0.03 OTUs were $\leq$ 97% similar to previously cultured bacterial strains (Table 2.2). Nonetheless, by using full-length 16S rRNA sequences 15 sponge-derived 16S rRNA sequences were considered to represent novel cultured bacteria based on the same BLAST search procedure (Table 2.2). Both approaches (OTU and full length sequences) yielded BLAST results for Bacteroidetes and

Alphaproteobacteria which were $\leq$ 97% similar to known cultured strains. Only the full-length BLAST approach yielded novel strains for Gammaproteobacteria (Table 2.3). The phyla Actinobacteria and Firmicutes did not contribute any new strain with either approach. Current and future experiments will chemically and phenotypically characterize the putative novel strains by growing them under different media conditions and testing them in various bioassays for production of bioactive compounds (e.g. antimicrobial, antifouling and cytotoxic activities). Among the 255 obtained sequences, the most abundant taxa were the Gammaproteobacteria (n = 96 sequences, 37% of all sequences) and Alphaproteobacteria (n = 83, 32%), followed by Firmicutes (n = 43, 16%), Bacteroidetes (n = 19, 7%), and Actinobacteria (n = 14, 5%) (Table 2.1). One monophyletic sponge-specific 16S rRNA gene sequence cluster was identified among the isolates of this study and related sequences, within the Bacteroidetes phylum (Figure 2.3). Furthermore, one monophyletic sponge/coral-specific cluster was found within the Gammaproteobacteria (Figure 2.4). Neither of these two clusters contained novel uncultured DGC or direct plating-derived 0.03 OTUs.

Table 2.1 Taxonomic summary of 255 *Rhabdastrella globostellata*-derived 16S rRNA sequences. n seq = number of sequences per phyla; direct, DGC, and DGC1 to 4 = different cultivation generations; M1, M1 extract, M8, MB 1:10 = different media types; DGC = relative abundance.

Phylum	n seq	direct total	relative total	dgc total	relative dgc	dgc1	dgc2	dgc3	dgc4	M1	M1 extract	M8	MB1:10
Actinobacteria	14	2	0.034	12	0.061	3	4	5	0	7	0	4	3
Bacteroidetes	19	12	0.207	7	0.036	0	0	6	1	5	3	9	2
Firmicutes	43	4	0.069	39	0.198	13	11	13	2	11	4	14	14
Proteobacteria-Alpha	83	24	0.414	59	0.299	16	16	12	15	22	10	42	9
Proteobacteria-Gamma	96	16	0.276	80	0.406	21	7	36	16	28	17	45	6
Total	255	58		197		53	38	72	34	73	34	114	34

Table 2.2 Number of OTUs with 0.03 distance cutoff level, number of novel 0.03 OTUs with ≤ 97% similarity to cultivated bacteria, number of novel full sequences with ≤ 97% similarity to cultivated bacteria.

source	*n* 0.03	*n* novel 0.03 OTUs	*n* novel full sequences
direct	19	2	6
dgc	33	1	9
shared dgc/direct	8		
total	60	3	15

Discussion

Marine sponges are notable sources of bioactive compounds and also host a great variety of microorganisms, which are often quite specific to sponges and are frequently in high abundance (Blunt et al. 2013; Hentschel et al. 2012; Taylor et al. 2007). Given that microbes can comprise up to 35% of total sponge biomass, and that some of the compounds are thought to be produced by microbes rather than by the sponge itself, new feasible and cost-effective cultivation techniques are needed in order to access this large repository of microbes. Hence, this study sought to evaluate DGCs as a means of cultivating sponge-associated bacteria. Besides testing the proof of concept of the DGC technique in living marine invertebrates (i.e. sponges), we also assessed whether this approach would yield host-associated, hitherto uncultivated bacteria.

Phylum-level diversity of bacteria cultivated using the DGC approach

The phylum-level affiliations of the bacteria cultivated in this study by using the DGC approach are consistent with the findings of previous, non-DGC studies of sponges, in which the dominant bacterial taxa were Proteobacteria (Gamma & Alpha), Actinobacteria, Firmicutes and Bacteroidetes (Simister et al. 2012a; Taylor et al. 2007). For example, our DGC approach yielded similar results to previous cultivation efforts from two Australian sponge species (*Pseudoceratina clavata* and *Rhabdastrella globostellata)* using traditional plating techniques (Lafi et al. 2005). This is unsurprising considering that both studies used the same sponge species, *R. globostellata*. Using the DGC method in addition to direct plating, we were able to obtain additional 15 novel cultures from this sponge species.

Table 2.3 Number of sequences within 0.03 OTUs with ≤ 97% similarity to cultivated bacteria, number of full sequences with ≤ 97% similarity to cultivated bacteria.

Phylum	*n* novel 0.03 OTU sequences	n novel full sequences
Actinobacteria	0	0
Bacteroidetes	3	11
Firmicutes	0	0
Proteobacteria-Alpha	1	2
Proteobacteria-Gamma	0	2
total	4	15

However, we were not able to isolate the proposed phylum Poribacteria which was previously reported to be found (but not cultivated) from *R. globostellata* (Lafi at al. 2009). Furthermore, the sequences obtained from the bacteria isolated by Lafi et al. (2005) only appeared in two of our trees (sequences AY372898 and AY372927 in Actinobacteria and Firmicutes, respectively). Since we used for phylogenetic reconstruction only sequences which were closely related to our newly generated sequences, we conclude that our approach yielded a quite different cultivable bacterial community (at least, at genus/strain-levels) compared to that reported by Lafi et al. (2005). This difference could be attributed to various factors such as the additional application of DGCs in our study, different media types, or a different community due to the geographic distance between the sampling sites. Interestingly, at phylum level our results are also broadly congruent with the main microbial representatives isolated from DGCs in other, non-sponge habitats (Bollmann et al. 2007).

When comparing the two different cultivation methods employed in this study by relative abundance, the DGC approach yielded more cultures than direct plating for the Gammaproteobacteria, Actinobacteria and Firmicutes. Only for Alphaproteobacteria and Bacteroidetes did direct plating yield more isolates than the four DGC generations. While the surplus of cultivable strains from the DGCs for three of the four phyla can be explained by the ratio of plating/isolation generations (1:4 - direct vs. DGC), the higher amount of Bacteroidetes from direct plating implies that for this phylum the DGC approach may not be beneficial.

Sponge-specific bacteria

To assess whether the DGC isolates were affiliated with sponge-specific 16S rRNA gene sequence clusters, we followed the approach of Hentschel, Taylor and colleagues (Hentschel et al. 2002; Taylor et al. 2007) and utilized the recently established sponge-specific database of Simister et al. (2012a). As a result we were able to identify among our sequences one putative sponge-specific cluster in the Bacteroidetes phylum, and one sponge-coral-cluster in the Gammaproteobacteria. Considering the low number of new sequences gained during this study (n = 255) in comparison to the sponge-related sequences in the original database (> 7451), these two newly generated clusters were a promising result for using this method to isolate sponge-associated bacteria. Furthermore, both clusters were also unique given the fact that no monophyletic, sponge-specific clusters were found among the Bacteroidetes in a recent meta-analysis and no sponge- and coral-specific clusters were found within

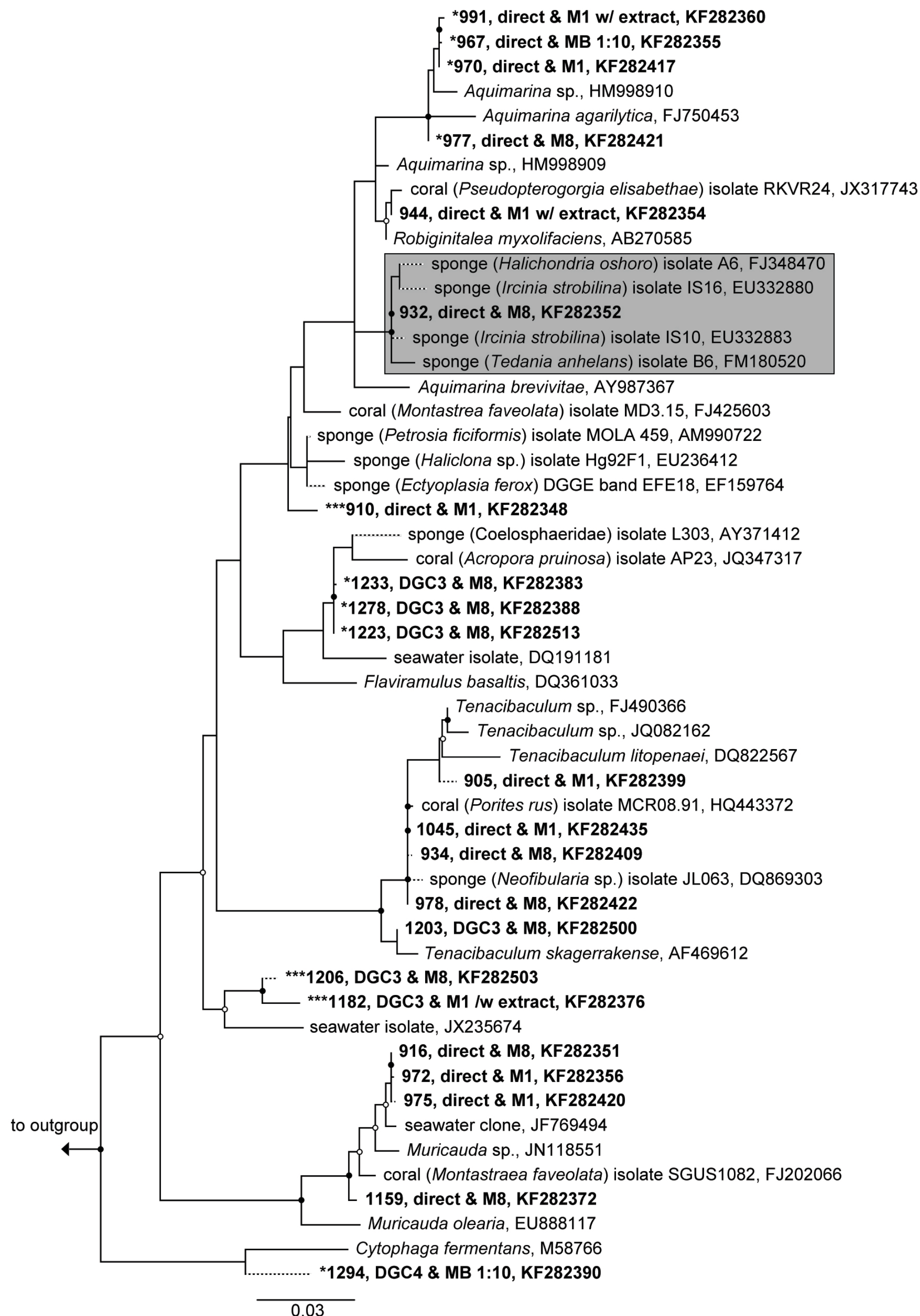

Figure 2.3 16SrRNA gene-based phylogeny of *Rhabdastrella globostellata*-associated *Bacteroidetes* organisms. All sequences derived during this study are shown in bold with related DGC/direct culture and media type information. The displayed tree is the maximum likelihood tree (RAxML, fast bootstrap, 1,000 replicates) constructed based on long (≥1,200 nucleotides) sequences only. Shorter sequences were added using the parsimony interactive tool in ARB and are indicated by dashed lines. The shaded box represents a sponge- specific monophyletic sequence cluster. Filled circles indicate bootstrap support of ≥ 90%, open circles represent ≥ 75% support. Asterisks indicate novel cultivated bacteria: one asterisk for full length, two asterisks for OTU, and three asterisks for both classification approaches.

the Gammaproteobacteria (Simister et al. 2012a). One notable observation was the influence of multiple DGC generations on parts of the phylogenetic outcome. For example, the obtained Bacteroidetes sequences were isolated exclusively either with the direct plating method or the DGC3 generation - with one exception from the DGC4 generation (Figure 2.3). This observation is even more prominent for Gammaproteobacteria in our study (Figure 2.4 & Supplementary Figure 2.1). It is notable that Bollmann et al. (2007) observed similar generation-based isolation clusters for Verrucomicrobia and Acidobacteria. The authors suggested two possible explanations: a) these representatives were too rare during the first generations and needed more DGC generations to allow sufficient enrichment for isolation, and b) that their acclimation to growth in petri dishes needed several growth events in the *in vivo* DGC environment. This observation overlaps with the remark of Sipkema et al. (2011), who pointed out that the doubling times of bacteria are much lower in nature than most cultivation experiments last. But contrary to Bollmann et al. (2007) we were able to obtain from nearly every DGC generation the four phyla of this present study, except for Actinobacteria (no strains from DGC4) and Bacteroidetes (no strains from DGC2 & DGC3). A possible explanation could be the use of different media and a greater number of isolated strains in our study. Hence, for future studies we suggest to extend the variety and composition of applied media in the DGCs for inoculation. For example, to further simulate a natural environment one could add structural components from sponges, such as spongin and chitin. Additionally, media with low concentrations of mucin or a mixture of peptone and starch are reported to be successful during sponge-associated cultivation experiments (Sipkema et al. 2011). Moreover, the use of sponge extract during the DGC phase could also contribute to an enhanced continuous presence of growth-promoting secondary metabolites. Furthermore, as mentioned above, longer incubation times of DGCs during future studies would probably achieve a wider spectrum of novel cultivable slow growing bacteria.

Concluding remarks

Our study showed that the DGC technique can be successfully applied in living marine organisms such as sponges. It enabled the cultivation and isolation of novel, previously uncultivated sponge-associated bacterial strains. The *R. globostellata* microbial community obtained by the DGC approach was comparable to the known main cultivable microbial phyla of this sponge species. Moreover, recent cultivation experiments with sponge-associated bacteria showed that cultivation methods specifically designed for the metabolic needs of sponge-associated bacteria (e.g. aerobic and microaerophilic

states) and highly specialized media compositions can enhance the yield of novel bacteria (Lavy et al. 2014; Sipkema et al. 2011). Hence, the application of DGCs seems to be a promising attempt for further *in vivo* experiments in combination with specific media types and cultivation techniques. We conclude that a broader experimental approach with more than one sponge species and a larger cultivation process with specifically adapted media types could extend our present yield of novel cultivated sponge-specific bacteria. Furthermore, future application of high-throughput sequencing for community analysis from sponge tissue parallel to full length 16S rRNA gene analysis of the cultivated bacteria *via* Sanger sequencing would allow us to analyze the cultivation success with respect to the natural sponge-associated bacterial community.

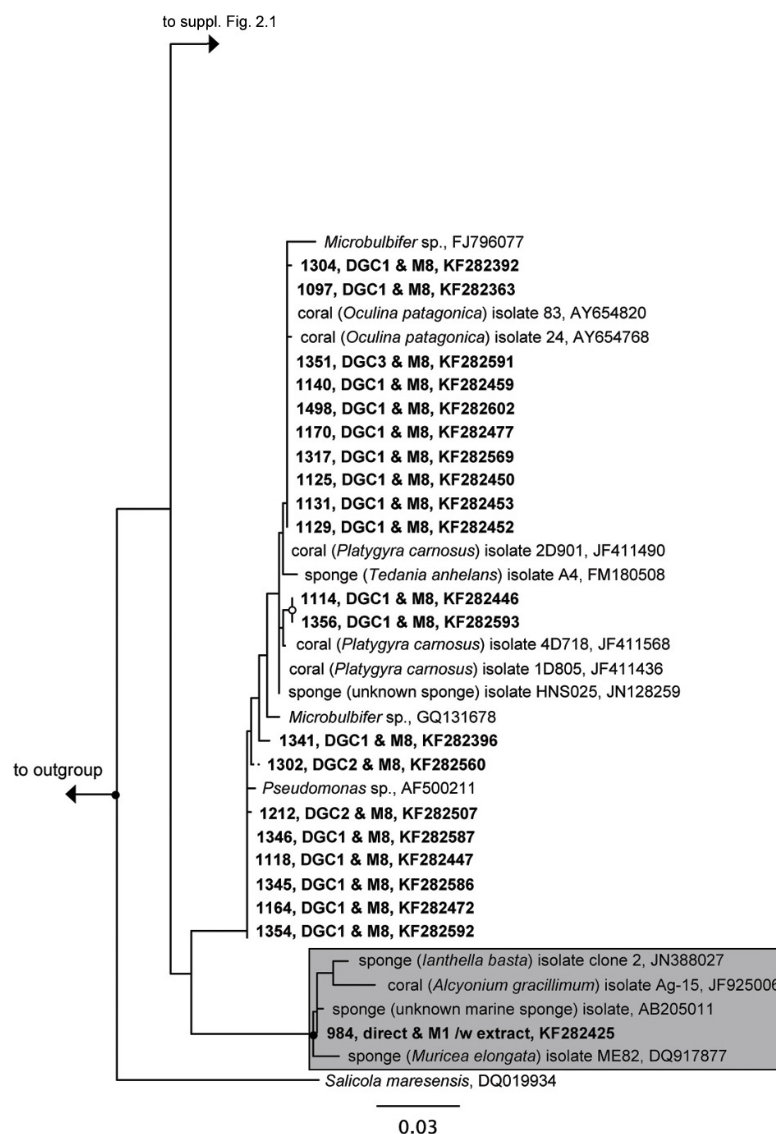

Figure 2.4 16SrRNA gene-based phylogeny of *R. globostellata*-associated *Gammaproteobacteria* organisms. Details are the same as those provided in Figure 2.3, with the following additions. The shaded box represents a sponge/coral- specific cluster.

CHAPTER II

Host Specificity and Spatial Variability in the Microbiota of Mesophotic Tropical Reef Sponges

Abstract

Sponges (phylum Porifera) are important members of almost all aquatic ecosystems, and are renowned for hosting dense and diverse microbial communities. While the specificity of the sponge microbiota seems to be closely related to host phylogeny, the environmental factors that could shape differences within local sponge-specific communities remain less understood. On tropical coral reefs, sponges are among the key invertebrate taxa and their habitat can span from shallow reef areas to deeper, mesophotic sites. These habitats differ in terms of environmental factors such as light, temperature, and food availability, as well as anthropogenic impact. In order to study the influence of depth on the sponge microbiota within a local area, three tropical reef sponges, *Rhabdastrella globostellata*, *Callyspongia* sp. and *Acanthella* sp., were collected from shallow and deeper mesophotic coral reef sites. Based on 16S rRNA gene pyrosequencing profiles, each sponge species possessed a specific microbiota that was significantly different to those of the other species and exhibited the known characteristics of high- and/or low-microbial-abundance sponges. In addition, the measured differences in beta diversity (community turnover) were large enough to group the *R. globostellata* and *Callyspongia* sp. microbial communities along a local depth gradient.

Introduction

In marine ecosystems, sponges represent common and versatile members of the benthos, with distribution ranges along large environmental gradients and across various habitats including deep sea benthos, seamounts, polar regions, temperate and tropical coral reefs (Bell 2008). Many sponges are notable for their diverse and abundant microbial biota, with up to 35% of sponge biomass being made up of microbes (Taylor et al. 2007). Sponge-microbe relationships can include microbial cells as a food source for filter-feeding sponges, carbon- and nitrogen-based nutritional interactions, and the synthesis of secondary metabolites for chemical defense mechanisms (Hentschel et al. 2012; Taylor et al. 2007). Accumulated evidence indicates that much of the sponge microbiota is specific to, or at the very least heavily enriched in, sponge hosts (Lee et al. 2011; Pita et al. 2013a; Schmitt et al. 2012a; Simister et al. 2012a). Even studies which detect so-called 'sponge-specific' microbes outside the sponge host only find these at very low abundances, with no evidence for these free-living microbes being metabolically active (Moitinho-Silva et al. 2014; Taylor et al. 2013). In addition to the

apparent host specificity, marine sponge-associated microbial communities exhibit relatively high temporal and biogeographic stability (e.g., Hardoim and Costa 2014; Pita et al. 2013b; Reveillaud et al. 2014; Simister et al. 2013; Taylor et al. 2004).

While sponges occupy a range of different depths, knowledge about the influence of depth on the composition of the sponge microbiota remains rather limited (Olson and Gao 2013; Olson and Kellogg 2010). Spatial dynamics of the host-associated microbiota within coral ecosystems, from shallow (0 – 30 m) to mesophotic (30 – 150 m) sites, are of great interest because of the potential role of the mesophotic coral ecosystem (MCE) as refugia for both microbial symbionts and their hosts facing threats of environmental change and anthropogenic disturbances (Kahng et al. 2014; Lesser et al. 2009; Olson and Kellogg 2010). Sponges in particular seem to be very important benthic members of MCEs, with increased growth rates, biomass and coverage compared to their shallow counterparts (Lesser et al. 2009). The habitat of sponges can span from shallow reef ecosystems into these mesophotic zones, which are less influenced by variable abiotic factors such as surface water temperature and salinity, or by direct human impact like overfishing and pollution (Kahng et al. 2014; Olson and Kellogg 2010). It has been suggested that inter-habitat connectivity of the host-associated microbial biota between the light flooded subsurface and the twilight areas of the MCEs exists because of larval migration, water circulation and the filtering activities of sessile benthic invertebrates that inhabit these zones (Kahng et al. 2014; Olson and Kellogg 2010; Slattery et al. 2011; Thacker and Freeman 2012). The first assessment of *in situ* sponge-associated communities along an MCE depth gradient suggested host-specific local variations in community structure, which are possibly influenced by prevailing biotic and abiotic factors (Olson and Gao 2013). However, patterns of local spatial dynamics in host-associated microbial communities could have been overlooked because of the use of particular molecular methods, such as terminal restriction fragment length polymorphism (T-RFLP) and clone library analysis, that could limit the resolution of fine-scale community assessments by the exclusion of low abundance species (van Dorst et al. 2014; Lee et al. 2011). Knowledge about the spatial dynamics of potential microbial refugia could yield new perspectives on the resilience and management of coral ecosystems, which are facing enormous pressures because of increasing global climatic disturbances and anthropogenic influences along highly populated and narrow land-sea transition zones (Ainsworth et al. 2010; Olson and Kellogg 2010). Research on thermal stress responses of sponge-associated microbial communities, for example, has already shown the drastic

effects of rising water temperatures on the microbial symbionts (Simister et al. 2012b; Webster et al. 2008).

In this study, we apply high-throughput 16S rRNA gene amplicon pyrosequencing to profile host specificity and variations in beta diversity patterns of sponge-associated microbial communities collected along a local depth gradient spanning from shallow locations to mesophotic reef areas. Three demosponge species*, Rhabdastrella globostellata*, *Callyspongia* sp., and *Acanthella* sp., were collected together with surrounding seawater samples in order to further compare sponge and seawater microbiota.

Table 3.1 Sample data with internal sample name, classified taxon, date and site of collection, depth and coordinates.

Samples	Taxonomic information	Date	Site	Depth [m]	Coordinates
RG1	*R. globostellata*	29-Jun-10	Blue Hole	80-90	N13.26.180;E144.37.504
RG2	*R. globostellata*	29-Jun-10	Blue Hole	80-90	N13.26.180;E144.37.504
RG3	*R. globostellata*	29-Jun-10	Blue Hole	80-90	N13.26.180;E144.37.504
RG4	*R. globostellata*	25-Jun-10	Western Shoals	1.5-3	N13.27.31;E144.39
RG5	*R. globostellata*	25-Jun-10	Western Shoals	1.5-3	N13.27.31;E144.39
RG6	*R. globostellata*	25-Jun-10	Western Shoals	1.5-3	N13.27.31;E144.39
RG7	*R. globostellata*	06-Jul-10	Western Shoals	3	N13.27.31;E144.39
RG8	*R. globostellata*	06-Jul-10	Western Shoals	3	N13.27.31;E144.39
RG9	*R. globostellata*	06-Jul-10	Western Shoals	3	N13.27.31;E144.39
RG10	*R. globostellata*	06-Jul-10	Blue Hole	80-90	N13.26.180;E144.37.504
RG11	*R. globostellata*	06-Jul-10	Blue Hole	80-90	N13.26.180;E144.37.504
RG12	*R. globostellata*	06-Jul-10	Blue Hole	80-90	N13.26.180;E144.37.504
RG13	*R. globostellata*	29-Jun-10	Blue hole	80-90	N13.26.180;E144.37.504
C1	*Callyspongia* sp.	06-Jul-10	Gab Gab	4.5	N13.26.35;E144.38.36
C2	*Callyspongia* sp.	06-Jul-10	Western Shoals	4.5	N13.27.31;E144.39
C3	*Callyspongia* sp.	06-Jul-10	Gab Gab	4.5	N13.26.35;E144.38.36
C4	*Callyspongia* sp.	23-Jan-08	Blue Hole	80-90	N13.26.180;E144.37.504
C5	*Callyspongia* sp.	23-Jan-08	Blue Hole	80-90	N13.26.180;E144.37.504
C6	*Callyspongia* sp.	29-Jun-10	Blue Hole	80-90	N13.26.180;E144.37.504
C7	*Callyspongia* sp.	06-Jul-10	Blue Hole	80-90	N13.26.180;E144.37.504
A1	*Acanthella* sp.	29-Jun-10	near Blue Hole	5	N13.26.180;E144.37.504
A2	*Acanthella* sp.	04-Nov-10	Tanguisson	77	N13.32.620;E144.48.265
A3	*Acanthella* sp.	17-Mar-08	Hospital Point	92	N13.30.126,E144.46.092
W1	waterfilter	06-Jul-10	Blue hole	80-90	N13.26.180;E144.37.504
W2	waterfilter	06-Jul-10	Blue hole	80-90	N13.26.180;E144.37.504
W3	waterfilter	06-Jul-10	Blue hole	80-90	N13.26.180;E144.37.504
W4	waterfilter	06-Jul-10	Western Shoals	3	N13.27.31; E144.39
W5	waterfilter	06-Jul-10	Western Shoals	3	N13.27.31; E144.39

Materials and Methods

Sample processing and sequencing

Samples of three different sponge species (*Rhabdastrella globostellata*, *Callyspongia* sp., and *Acanthella* sp.) and seawater were collected from closely connected Guam reef sites and depths via snorkeling and diving (Table 3.1). All samples were frozen, freeze-dried, then stored at -20°C prior to further processing. Species were identified by Peter Schupp and vouchers of each species were preserved in 70% ethanol and deposited by Dr. Nicole J. de Voogd at the Naturalis Biodiversity Center, Leiden, Netherlands.

Genomic DNA was extracted from sponge tissue and water filters (1 liter each, 0.22 μm filter) by bead-beating in an ammonium acetate buffer, as previously described (Taylor et al. 2004). 16S rRNA gene amplification with primers targeting the V4-V5 region (454MID_533F: GTGCCAGCAGCYGCGGTMA and 454_907RC: CCGTCAATTMMYTTGAGTTT) and purification for pyrosequencing was performed as previously described by Simister et al. (2012b). Pyrosequencing was performed by Macrogen Inc. (Seoul, South Korea) using the Roche GS FLX Titanium system. The obtained data can be accessed via the NCBI Sequence Read Archive under accession number SRX838037.

Raw sequence processing

Sequences were processed using mothur v.1.33.0 (Schloss et al. 2011; Schloss et al. 2009). Pyrosequencing flowgrams were filtered and denoised using the mothur implementation of AmpliconNoise (Quince et al. 2011). Adaptor, MID, and primer sequences were removed from raw sequences. Sequences were removed from the analysis if they were $\leq$ 200 bp or contained ambiguous characters, homopolymers longer than 8 bp, more than one MID mismatch, or more than two mismatches to the reverse primer sequence. Unique sequences were aligned against a SILVA alignment (available at http://www.mothur.org/wiki/Silva_reference_alignment). After chimera-checking with UCHIME (Edgar et al. 2011), unique sequences were identified using the Greengenes 'gg_13_8_99' reference taxonomy (available at http://www.mothur.org/wiki/Greengenes-formatted_databases). Non-target sequences (e.g. chloroplasts, mitochondria, eukaryotic 18S rRNA) were removed.

Sequence data analyses

After raw data processing, mothur was used to group the obtained high quality sequences into 97% sequence-similarity threshold operational taxonomic units (i.e., 97%-OTUs), for calculation of Chao1 richness, inverse Simpson diversity, and rarefaction curves. For visualization and interpretation of the microbial community data, we used normalized 97% OTU abundance information. Multivariate cut-off level analysis (MultiCoLA) was performed using permuted Mantel tests (vegan package) with Pearson's correlation coefficient between full and reduced datasets (stepwise exclusion of singletons) (Gobet et al. 2010; Oksanen et al. 2012). To estimate the variance of beta diversity, two hypothetical treatments were applied to the dataset: a) 'habitat' (shallow, mesophotic) and b) 'group' (*Rhabdastrella*, *Callyspongia*, *Acanthella*, water column) (Supplementary Table 3.1). These treatments were used for analysis of multivariate homogeneity of group dispersions (variances) (Anderson 2006) with the *betadisper* and *permutest* function from the vegan package in R (v. 3.0.2) (R Development Core Team, 2013). We used the *adonis* function (1000 permutations) from the vegan package to estimate the variances in beta diversity for both treatment groups (Anderson 2001). Visualization of variations in sponge composition among habitats (shallow and mesophotic) and sponge hosts (*R. globostellata*, *Callyspongia* sp. and *Acanthella* sp.) were assessed with multivariate non-metric multidimensional scaling (nMDS) using the *metaMDS* function from the vegan package (standardized with *decostand* & *method = normal*; Bray-Curtis dissimilarities for relative abundance & Jaccard dissimilarities for presence/absence analyses). Hypothesis-based treatments were added as dispersion ellipses to the ordination plots with the vegan function *ordieellipse* (0.95 confidence interval). All multivariate analyses were performed with relative abundance and presence/absence data. In addition to the full OTU dataset, two cut-off levels (n = 1 & n = 10 sequences/OTU) were tested. The contribution of OTUs to average overall pairwise sample dissimilarity in *R. globostellata* and *Callyspongia* sp. specific datasets ('habitat' treatment) was assessed using the vegan function *simper* for similarity percentages (SIMPER). Bray-Curtis dissimilarities between all samples, relative abundance of the 35 most abundant OTUs, and relative phylum abundance were visualized with JColorGrid (Joachimiak et al. 2006). Hierarchical clustering of OTUs was performed using the vegan package in R via the function *vegdist* (Bray-Curtis dissimilarity) and *hclust* (*method = average*) and subsequently added onto the OTU fingerprint. For analyses of depth-dependent differences (i.e.,

betadisper/adonis, nMDS, SIMPER), *Acanthella* sp. and water samples were excluded due to insufficient numbers of replicates in the dataset.

Results

In total, 204435 sequences were retained after denoising and quality control. Between all sampling groups (three sponge taxa and one seawater group), on average the seawater samples yielded the highest number of sequences (n = 13344) (Table 3.2). For the sponge groups the average numbers of sequences were lower: *R. globostellata* (n = 6631), *Acanthella* sp. (n = 6309), and *Callyspongia* sp. (n = 4654). Observed numbers of 97%-OTUs, as well as richness estimates based on Chao1 statistics, were higher overall within the seawater group in comparison to the sponge groups. Coverage was slightly higher for *Acanthella* sp. and *R. globostellata* compared to seawater and *Callyspongia* sp. samples (Table 3.2). These group-specific observations were also reflected in the rarefaction curves (Supplementary Figure 3.1). Across 28 samples (23 sponges and 5 seawater samples), 2310 OTUs (97% cut-off) were determined. After Greengenes classification, these OTUs were assigned to 33 bacterial and two archaeal phyla (Figure 3.1 & Supplementary Figure 3.2). Pooled seawater samples showed the highest phylum richness with 30 bacterial and two archaeal phyla, while *R. globostellata*

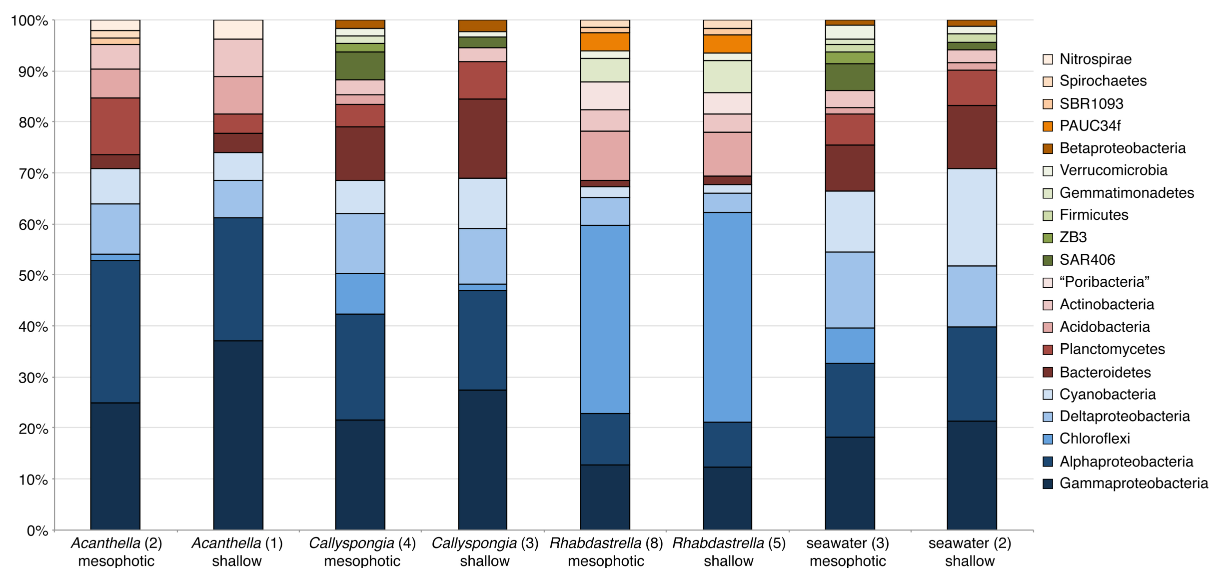

Figure 3.1 Relative abundance of 16S rRNA gene amplicon sequences classified to the level of phyla, pooled by sample group (sponge or seawater) and habitat (shallow or mesophotic sites). Numbers in parenthesis correspond to the number of pooled samples for each group. Only phyla with n $\geq$ 1% abundance within each group are presented here - for more details see Supplementary Figure 3.2.

and *Callyspongia* sp. each contained 24 bacterial phyla and one archaeal phylum. *Acanthella* sp. deviated notably from this composition, with only 13 bacterial phyla and one archaeal phylum (Supplementary Figure 3.2). Almost half of the occurring phyla were present in all four groups (e.g. Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Nitrospirae, Planctomycetes, Proteobacteria, Spirochaetes, Synergistetes, and Verrucomicrobia) (Figure 3.1).

Table 3.2 Sequence and OTU statistics with number of sequences, observed 97%-OTUs, Chao1 richness estimates, coverage and inverse Simpson diversity index.

Sample	Number of sequences	observed OTUs	Chao1	Coverage	inverse Simpson
A1 (shallow)	5500	56	87.63	0.996	5.609
A2 (mesophotic)	7076	78	107.00	0.996	4.040
A3 (mesophotic)	6351	86	193.63	0.993	6.040
Acanthella mean	6309.0	73.3	129.42	0.995	5.230
C1 (shallow)	5530	131	205.39	0.989	1.557
C2 (shallow)	3474	132	179.12	0.986	3.117
C3 (shallow)	4361	170	457.64	0.974	1.631
C4 (mesophotic)	5077	165	297.14	0.985	2.020
C5 (mesophotic)	7322	188	312.09	0.988	1.558
C6 (mesophotic)	2387	133	204.72	0.973	2.360
C7 (mesophotic)	4433	321	422.00	0.977	5.434
Callyspongia mean	4654.9	177.1	296.9	0.982	2.525
RG1 (mesophotic)	4917	206	267.75	0.992	62.763
RG2 (mesophotic)	4423	198	241.16	0.991	63.081
RG3 (mesophotic)	3635	199	238.06	0.990	66.076
RG4 (shallow)	9488	205	225.65	0.997	36.978
RG5 (shallow)	5803	206	226.31	0.996	64.769
RG6 (shallow)	10773	230	273.94	0.996	57.374
RG7 (shallow)	5596	228	309.67	0.991	74.629
RG8 (shallow)	7129	248	382.06	0.991	72.201
RG9 (mesophotic)	6262	289	471.81	0.984	43.345
RG10 (mesophotic)	7092	228	330.79	0.991	51.502
RG11 (mesophotic)	4175	205	277.07	0.989	60.650
RG12 (mesophotic)	7539	222	292.71	0.994	62.842
RG13 (mesophotic)	9372	217	288.75	0.996	56.485
Rhabdastrella mean	6631.1	221.6	294.3	0.992	59.438
W1 (mesophotic)	11606	555	788.72	0.982	5.086
W2 (mesophotic)	7740	592	983.51	0.966	4.163
W3 (mesophotic)	18007	782	1023.00	0.987	6.913
W4 (shallow)	19586	493	850.08	0.988	4.512
W5 (shallow)	9781	342	685.15	0.981	2.527
water mean	13344	552.8	866.1	0.981	4.640
total mean	7301.3	253.8	379.38	-	-
total	204435	-	-	-	-

The most abundant OTUs (n = 35) included members of the Proteobacteria (Alpha-, Beta-, Delta-, and Gamma-), Cyanobacteria, Acidobacteria, Chloroflexi, ‘Poribacteria’, Actinobacteria, Nitrospirae and PAUC34f (Figure 3.2). The distribution of the three most abundant OTUs (OTU0001: Betaproteobacteria, uncultured order EC94; OTU0002: Cyanobacteria, *Synechococcus*; OTU0003: Cyanobacteria, *Prochlorococcus*) was mostly limited to *Callyspongia* sp. and seawater samples. Among the abundant *R. globostellata* OTUs were representatives of Chloroflexi class SAR202, the candidate phylum ‘Poribacteria’, various Acidobacteria and Proteobacteria, and the uncultured sponge symbiont PAUC34f. Four OTUs were highly abundant only in the *Acanthella* sp. group: OTU0004 (Acidobacteria, PAUC26f), OTU0007 (Gammaproteobacteria, HTCC2089), OTU0027 (Alphaproteobacteria, Rhodobacteraceae), and OTU0033 (Acidobacteria, ii1-15) (Figure 3.2). Hierarchical clustering based on Bray-Curtis dissimilarity distances revealed group-specific clades with high between-group and low within-group dissimilarities (Figure 3.2). Additionally, individual sample by sample comparison also showed low within- and high between-group dissimilarities (Supplementary Figure 3.3).

Non-metric multidimensional scaling plots created with all samples showed high sample-specific pooling (Figure 3.3A). The variation among sample-specific groups was significant for both datasets (Table 3.3). The habitat-based nMDS and multivariate analysis of variance for *Rhabdastrella* and *Callyspongia* were both significant and showed intra-specific mesophotic/shallow community clusters (Figure 3.3BC & Table 3.3). In contrast, the same analyses, but with randomized relative abundance and presence/absence OTU tables, showed no significant mesophotic/shallow community clusters (Supplementary Table 3.2). The removal of dominant & rare OTUs and the application of two cut-off levels (OTUs with $n = 1$ & $n \leq 10$ sequences) had no effect on the resulting ordinations and spatial community structures (Table 3.3 & Supplementary Table 3.3). However, in most presence/absence tests the significant multivariate spread (*betadisper*/*permutest*) might contribute to the observed significant variance effects (Table 3.3).

The analysis of contribution of individual 97%-OTUs to the overall Bray-Curtis dissimilarity (*simper*) showed that OTUs with a high abundance among all samples are also the main contributors to the overall dissimilarity among the shallow/mesophotic sample groups (Table 3.4).

Table 3.3 Analysis of Bray-Curtis (relative abundance) and Jaccard (presence-absence) dissimilarities among all samples and truncated datasets with two cut off levels (n = 1 sequence & n $\leq$ 10 sequences). Results represent the three groups and ordination ellipses from Figure 3.3ABC with p-values for betadisper / permutest (1000 permutations) and p-values and R square for adonis analyses (1000 permutations).

OTU Dataset Type	all samples - 'group' betadisper / permutest	adonis	R2	*R. globostellata* - 'habitat' betadisper / permutest	adonis	R2	*Callyspongia* sp. - 'habitat' betadisper / permutest	adonis	R2
relative abundance	0.098	**0.001**	0.782	0.061	**0.003**	0.251	0.746	**0.027**	0.390
relative abundance cut off 1	0.087	**0.001**	0.785	0.065	**0.003**	0.252	0.677	**0.034**	0.394
relative abundance cut off 10	0.134	**0.001**	0.789	0.056	**0.004**	0.254	0.812	**0.023**	0.401
presence/absence	**0.001**	**0.001**	0.510	**0.008**	**0.011**	0.175	0.550	**0.001**	0.270
presence/absence cut off 1	**0.001**	**0.001**	0.590	**0.001**	**0.009**	0.221	0.534	**0.001**	0.308
presence/absence cut off 10	**0.001**	**0.001**	0.692	**0.002**	**0.008**	0.256	0.258	**0.025**	0.369

Discussion

Host specificity of the microbiota of three MCE sponges

In the present study spatial patterns along a depth gradient were visible in two tropical sponge microbiota (discussed further below). Each of the two sponges, *R. globostellata* and *Callyspongia* sp., appear to have an intrinsic microbiota, which is variable enough to significantly separate the intra-species communities by their shallow or mesophotic habitats. In addition, we could also observe significant microbial specificity across all analyzed sponge taxa.

Little is known about the *R. globostellata*-associated microbial community. While culture-dependent and culture-independent approaches found only Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria (Alpha- & Gamma-) and the candidate phylum 'Poribacteria' to be associated with this sponge (Lafi et al. 2009; Lafi et al. 2005; Steinert et al. 2014), we can increase the number to a total of 28 phyla found in *R. globostellata* by adding the results of the first high-throughput-sequencing amplicon screening to the data presented here (Schmitt et al. 2012b). In addition to the abundant phyla described previously, in the present study the candidate phylum PAUC34f and the class SAR202 (Chloroflexi) were also dominant representatives of these communities. While SAR202 is an ubiquitous bacterioplankton (Varela et al. 2008), PAUC34f is commonly found in close association with sponges (Fiore et al. 2013; Hardoim and Costa 2014; Moitinho-Silva et al. 2014). PAUC34f and SAR202 were also the two most abundant bacterial taxa in the high microbial abundance (HMA) demosponge *Xestospongia testudinaria* (Moitinho-Silva et al. 2014), while in *Xestospongia muta* PAUC34f exhibited only a low abundance (Fiore et al. 2013).

Recent transmission electron microscopy, DAPI cell-counting and 16S rRNA gene amplicon sequencing results assigned different members of the demosponge genera *Acanthella* and *Callyspongia* to the low microbial abundance (LMA) group, with abundant Proteobacteria (Alpha, Beta, & Gamma) and Cyanobacteria (*Synechococcus*) microbial community members (Giles et al. 2013; Gloeckner et al. 2014; Jeong et al. 2013).

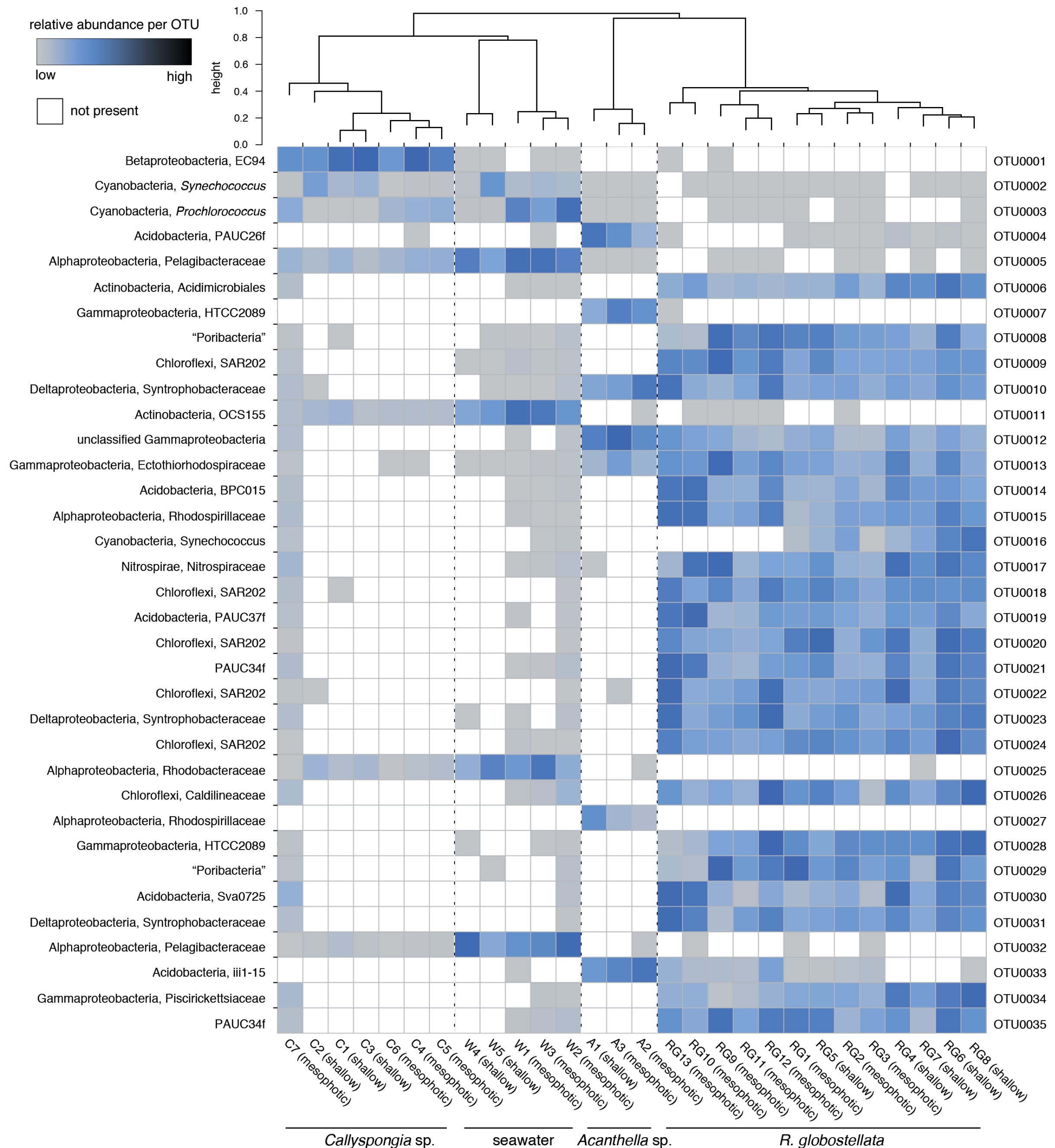

Figure 3.2 Fingerprint of the 35 most abundant 0.03 OTUs with relative abundance for each individual OTU. The above plotted dendrogram is based on Bray-Curtis dissimilarities (relative abundances of 97%-OTUs).

We also found that our *Callyspongia* sp. specimens exhibited an abundant occurrence of *Synechococcus* (Cyanobacteria), Actinobacteria, Bacteroidetes and Proteobacteria (Beta & Gamma). Within the Betaproteobacteria, the order EC94 exhibited the highest overall abundance in our *Callyspongia* sp. samples and among all OTUs, but in contrast was mostly absent in the other two sponge taxa and the seawater samples (values ranged between 0 - 0.2% - Figure 3.2). Reports on this order are rare, but recent community analyses on different demosponges found this microbial taxon to be dominant in the deep sea sponge *Inflatella pellicula* and several shallow water sponges from Korea.

In addition to the abundant microbial phyla associated with *R. globostellata* and/or *Callyspongia* sp., *Acanthella* sp. also possessed a relatively high abundance of members of the family Nitrospiraceae (phylum Nitrospirae*)*. This physiologically highly diverse family includes the chemolithoautotrophic aerobic nitrite-oxidizing genus *Nitrospira*, which is often associated with sponges (Schmitt et al. 2012b; Webster and Taylor 2012).

Depth-dependent microbial community patterns in MCE sponges

Knowledge about community variability of the sponge microbiota along depth gradients on local spatial scales is still scarce (Olson and Kellogg 2010). An initial T-RFLP and clone study on three MCE sponges observed a trend in community composition along a depth gradient, but could not identify the bacteria which caused these variations (Olson and Gao 2013). In the present study, *Callyspongia* sp. and *R. globostellata* microbial communities were significantly different when comparing shallow and mesophotic sampling sites for each sponge.

Within *Callyspongia* sp., mainly either Cyanobacteria or Proteobacteria OTUs were responsible for the differences in beta diversity observed by SIMPER*.* In particular, cyanobacterial *Synechococcus* OTUs were among the main contributors to the shallow waters microbiota, whereas only one *Prochlorococcus* OTU was a dominant cyanobacterium contributing to the mesophotic group. The predominance of photoautotrophs in tropical filter-feeding sponges is intuitive given the widespread prevalence of cyanobacteria in the ocean. Indeed, cyanobacteria comprise one of the most abundant sponge-associated phyla, with well-established sponge-specific symbionts (e.g. *Synechococcus spongiarum*) (Hentschel et al. 2006; Simister et al. 2012a; Taylor et al. 2007). Moreover, their occurrence within sponge hosts can be positively correlated with sponge growth rates (Erwin & Thacker 2008, Freeman & Thacker 2011), and LMA sponges in particular are known to harbor

cyanobacteria in high abundance (Bayer et al. 2014). Besides their potential phototrophic activities in the sponge pinacoderm (outer tissue) and mesohyl (inner sponge matrix), nitrogen-fixing cyanobacteria may inhabit a niche within the complex nitrogen cycle in sponges, with a mutual benefit due to nutrient supply by the sponge and secondary metabolite production by the Cyanobacteria (Arillo et al. 1993; Taylor et al. 2007; Wilkinson and Fay 1979). Given the dominance of Cyanobacteria in our *Callyspongia* sp. specimens, with *Synechococcus* dominant in the shallow water and *Prochlorococcus* in the mesophotic sponges, the spatial pattern between shallow and mesophotic MCE sites could be shaped predominantly by cyanobacterial lineages. This distribution pattern of microbes in *Callyspongia* sp. could also indicate a seasonal vertical distribution pattern, in which one genus dominates the shallow high-light water column, while the other genus is temporarily mainly present in the low-light area below. While temporal shifts of Cyanobacteria in sponges have been observed to varying degrees previously, the influence and effect of time and depth on these and other chlorophototrophs remains uncertain (White et al. 2012; Erwin et al. 2012; Hardoim & Costa 2014; Taylor et al. 2004).

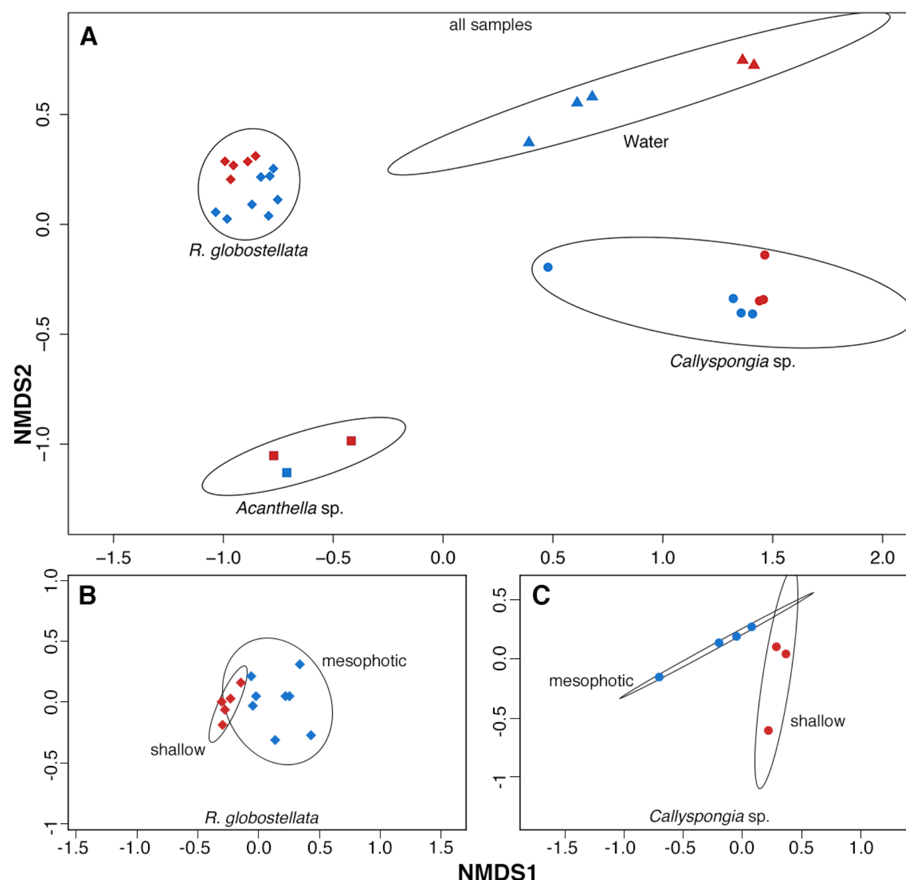

Figure 3.3 nMDS ordinations based on Bray-Curtis dissimilarities (97%-OTUs) - with A) all samples and ordination ellipse for each sample group, B) only *R. globostellata* with ordination ellipse for each habitat, and C) *Callyspongia* sp. with ordination ellipse for each habitat.

In *R. globostellata* one cyanobacterial OTU is predominant within shallow water communities (Table 3.4). This single *Synechococcus* OTU was present in all shallow *R. globostellata* samples, but undetected from five of the eight mesophotic samples. Moreover, it was also largely absent from all other sponge and seawater samples. Compared to the main cyanobacterial OTUs found in *Callyspongia* sp., which were also present in seawater and the other two sponge taxa, this is a further indication that each sponge taxon may host specific cyanobacterial communities, which may be maintained by different mechanisms. This corresponds to the theory that LMA sponges generally acquire their microbial symbionts via horizontal transmission from the surrounding environment, while HMA sponges possess a more individual microbial community that does not mirror the surrounding seawater microbiome as closely as their low abundance counterparts (Gloeckner et al. 2014; Hentschel et al. 2006). The detection of microbes within different reproductive stages of seven sponge species led to the hypothesis that HMA sponges can maintain parts of their symbiotic microbiota via vertical transmission (Schmitt et al. 2008).

While we can differentiate the present microbial community in *R. globostellata* by shallow and mesophotic sampling locations, the major microbial drivers contributing to the observed differences in beta diversity are almost equally distributed in shallow sites and mesophotic sites groups, thus providing no clear statement about depth coherence of any specific microbial taxon. Nonetheless, given that the abundant microbial phyla in *R. globostellata* (i.e., Acidobacteria, Chloroflexi, Cyanobacteria, Gemmatimonadetes, and Alphaproteobacteria) are known to contain (bacterio)chlorophyll-based phototrophic lineages (Zeng et al. 2014), it is still possible that photoheterotrophic bacteria play a considerable role in this host-specific microbiota. However, while the function of Chloroflexi in sponges is yet unclear, the distribution of members of this phylum within their hosts from different depths suggests that they may not be phototrophically active within the sponges (Olson et al. 2013). Therefore, other factors should be considered to explain the observed differences. Since the same sponge species show differences in growth rates and richness at different depths (Lesser et al. 2009), different biotic and abiotic niches may be available for symbionts depending on their habitat. Such ecologically-based niche differentiation is known for the tropical coral *Seriatopora hystrix* and its associated *Symbiodinium* symbiont (Bongaerts et al. 2010). In addition, recent oligotyping of Nitrospira symbionts associated with sponges collected along large horizontal

and vertical gradients provided further evidence for such patterns of differential enrichment of closely related microbial variants (Reveillaud et al. 2014).

Conclusion

The present study suggests that some sponge-specific communities in tropical coral ecosystems are predominantly characterized by bacteria with photoheterotrophic abilities. Moreover, the variance between shallow and mesophotic communities is large enough to see significant grouping along a local depth gradient in some sponge species. However, the actual environmental factors contributing to the observed depth-dependent variances remain uncertain. While sponge-microbe communities show an overall stability along large geographic and temporal gradients, local environmental factors likely have a considerable effect on the small-scale patterns observed here. To further test the hypothesis of differential sponge-associated microbial communities along local depth gradients, functional and temporal aspects should be considered in future *in-situ* studies and systematic sampling setups. Moreover, since temporal turnover of phytoplankton is faster in the tropics (Soininen 2010), tropical sponges with dominant phototrophic microbial communities most likely provide ideal conditions to design spatio-temporal studies on phototrophic host-symbiont dynamics.

Table 3.4 Mean percentage contribution of the dominant 97%-OTUs contributing at least 70% of the SIMPER contrast of Bray-Curtis dissimilarities among euphotic and mesophotic *Callyspongia* sp. and *R. globostellata* specimen - csum: ordered cumulative contribution; ava: average abundance group a - mesophotic; avb: average abundance group b - shallow; taxonomic rank I (highest classified level) & II (lowest classified level).

***Callyspongia* sp.**

OTU	csum	ava - mesophotic	avb - shallow	taxonomic rank I	taxonomic rank I
Otu0001	0.242	0.628	**0.684**	Betaproteobacteria	EC94
Otu0002	0.450	0.011	**0.159**	Cyanobacteria	*Synechococcus*
Otu0003	0.629	**0.133**	0.005	Cyanobacteria	*Prochlorococcus*
Otu0005	0.645	**0.027**	0.015	Alphaproteobacteria	Pelagibacteraceae
Otu0073	0.659	**0.008**	0.007	Proteobacteria	-
Otu0152	0.670	**0.008**	0.003	Gammaproteobacteria	Endozoicimonaceae
Otu0037	0.679	0.000	**0.006**	Cyanobacteria	*Synechococcus*
Otu0011	0.686	0.008	**0.011**	Actinobacteria	OCS155
Otu0041	0.693	0.000	**0.005**	Cyanobacteria	*Synechococcus*
Otu0025	0.699	0.001	**0.006**	Alphaproteobacteria	Rhodobacteraceae
Otu0232	0.705	0.002	**0.004**	Gammaproteobacteria	Endozoicimonaceae

Rhabdastrella globostellata

OTU	csum	ava - mesophotic	avb - shallow	taxonomic rank I	taxonomic rank II
Otu0016	0.053	0.004	**0.044**	Cyanobacteria	*Synechococcus*
Otu0006	0.094	0.019	**0.048**	Acidobacteria	PAUC26f
Otu0008	0.132	**0.042**	0.024	Poribacteria	-
Otu0054	0.154	0.001	**0.018**	Poribacteria	-
Otu0014	0.174	**0.029**	0.017	Acidobacteria	BPC015
Otu0009	0.195	**0.038**	0.026	Chloroflexi	SAR202

Table 3.4 (continued)

Rhabdastrella globostellata

OTU	csum	ava - mesophotic	avb - shallow	taxonomic rank I	taxonomic rank II
Otu0015	0.212	**0.028**	0.018	Alphaproteobacteria	Rhodospirillaceae
Otu0017	0.229	0.020	**0.026**	Nitrospirae	Nitrospiraceae
Otu0046	0.245	0.004	**0.016**	Gemmatimonadetes	Gemm-2
Otu0042	0.261	0.004	**0.016**	Gammaproteobacteria	Piscirickettsiaceae
Otu0034	0.277	0.006	**0.018**	Gammaproteobacteria	Piscirickettsiaceae
Otu0029	0.291	**0.017**	0.009	Poribacteria	-
Otu0043	0.306	0.006	**0.015**	Gammaproteobacteria	HTCC2089
Otu0020	0.319	0.016	**0.021**	Chloroflexi	SAR202
Otu0072	0.333	0.001	**0.011**	Gemmatimonadetes	Gemm-2
Otu0010	0.346	**0.028**	0.021	Deltaproteobacteria	Syntrophobacteraceae
Otu0013	0.359	**0.026**	0.018	Gammaproteobacteria	Ectothiorhodospiraceae
Otu0089	0.371	0.001	**0.010**	Bacteroidetes	Salisaeta
Otu0019	0.384	**0.020**	0.013	Acidobacteria	PAUC37f
Otu0049	0.396	**0.013**	0.005	Gammaproteobacteria	HTCC2089
Otu0028	0.407	0.015	0.015	Gammaproteobacteria	HTCC2089
Otu0056	0.419	**0.011**	0.002	Acidobacteria	iii1-15
Otu0030	0.430	**0.011**	0.010	Acidobacteria	Sva0725
Otu0093	0.441	0.001	**0.010**	Gemmatimonadetes	Gemm-2
Otu0026	0.451	0.013	**0.016**	Chloroflexi	Caldilineaceae
Otu0021	0.461	0.016	**0.017**	PAUC34f	-
Otu0023	0.470	**0.019**	0.015	Deltaproteobacteria	Syntrophobacteraceae
Otu0060	0.479	**0.007**	0.000	Gammaproteobacteria	-
Otu0038	0.488	0.009	**0.013**	Gemmatimonadetes	Gemm-2
Otu0035	0.496	**0.013**	0.010	PAUC34f	-
Otu0018	0.504	**0.021**	0.020	Chloroflexi	SAR202
Otu0022	0.513	**0.018**	0.015	Chloroflexi	SAR202
Otu0082	0.520	**0.009**	0.003	Alphaproteobacteria	-
Otu0036	0.528	0.009	**0.014**	Gemmatimonadetes	Gemm-2
Otu0068	0.535	**0.009**	0.003	Acidobacteria	iii1-15
Otu0031	0.543	**0.013**	0.010	Deltaproteobacteria	Syntrophobacteraceae
Otu0045	0.550	0.008	**0.012**	Chloroflexi	Caldilineaceae
Otu0077	0.557	**0.008**	0.003	Acidobacteria	iii1-15
Otu0052	0.564	**0.009**	0.005	Deltaproteobacteria	Entotheonellaceae
Otu0024	0.570	0.016	0.016	Chloroflexi	SAR202
Otu0048	0.577	**0.010**	0.008	AncK6	-
Otu0012	0.583	0.014	0.014	Gammaproteobacteria	-
Otu0084	0.589	0.004	**0.008**	Gammaproteobacteria	-
Otu0070	0.595	**0.008**	0.005	Bacteria	unclassified
Otu0055	0.601	**0.010**	0.005	Alphaproteobacteria	Rhodospirillaceae
Otu0057	0.607	**0.009**	0.006	Alphaproteobacteria	Rhodospirillaceae
Otu0065	0.613	**0.007**	0.006	Acidobacteria	B110
Otu0069	0.619	**0.008**	0.005	Chloroflexi	Caldilineaceae
Otu0051	0.624	0.008	**0.010**	Chloroflexi	SAR202
Otu0039	0.630	**0.012**	0.009	Chloroflexi	SAR202
Otu0050	0.635	**0.010**	0.007	Chloroflexi	SAR202
Otu0129	0.641	**0.004**	0.001	SBR1093	EC214
Otu0079	0.646	0.005	**0.008**	PAUC34f	-
Otu0127	0.651	**0.004**	0.003	Poribacteria	-
Otu0063	0.656	**0.009**	0.006	Chloroflexi	SAR202
Otu0159	0.661	**0.004**	0.000	Alphaproteobacteria	Rhodobacterales
Otu0086	0.665	**0.007**	0.004	Acidobacteria	PAUC26f
Otu0097	0.670	0.005	**0.006**	Bacteria	unclassified
Otu0047	0.675	0.009	0.009	Deltaproteobacteria	Entotheonellales
Otu0040	0.680	0.010	0.010	Spirochaetes	Spirochaetaceae
Otu0092	0.684	**0.007**	0.003	Chloroflexi	SAR202

CHAPTER III

Consistent Microbial Community Patterns of High Taxonomic Ranks and Microbial Indicator Species in Temperate Marine Sponges

Abstract

In most marine ecosystem, from the deep sea to tropical reefs, sponges (Porifera) are abundant and diverse members of the benthic filter feeding community. A characteristic feature are the associated dense and diverse microbial communities present within the mesohyl matrices. Previous molecular genetic studies on the sponge-associated microbiota revealed a variety of differential patterns of sponge-microbe relationships, with core, variable, and species-specific communities, or certain microbial phyla associated with sponge-ecological traits, such as containing high and low microbial abundances. These sponge-associated microbial communities are generally displayed and evaluated based on the relative abundances of operational taxonomic units at genus or phylum level. Since microbial symbionts exhibit functional convergence in phylogenetic divergent sponge hosts, deep branching microbial clades could be functionally coherent, which could be reflected as patterns across broad taxonomic ranks. However, little is known if sponge-associated microbial community structures observed at 97% sequence similarity could also be consistently observed at high taxonomic ranks. In the present study, we investigated the small-scale microbial community structure and variation of 24 sponge specimen and three water samples collected along a depth gradient (2 to 158 m) at the Atlantic coast of Sweden. Results show that the resemblance of communities on different taxonomic ranks are consistent with patterns present on 97% operational taxonomic unit level. In addition, subsequent indicator species analysis showed high agreement with the abundant microbial taxa influencing the community variation even at high taxonomic ranks.

Introduction

Sponges (Porifera) are important benthic filter feeding organisms, which inhabit a large range of environmentally different aquatic ecosystems, from the deep sea, tropical reefs, and continental shelves to temperate coastal areas, and freshwater lakes or rivers (Bell 2008). Moreover, sponges are renown hosts of diverse and complex microbial communities: up to 35% of the sponge biomass can be made up of microbes (Taylor et al. 2007). The relationships and interactions between sponge-hosts and their microbial communities ranges from being simply a source of food, over chemical defenses, to mutual metabolic interactions (Hentschel et al. 2012; Taylor et al. 2007). In addition, a growing

amount of data, generated by different molecular genetic techniques, shows that sponge-specific microbial communities are generally stable across small and large geographic and temporal gradients (Erwin et al. 2012; Hardoim and Costa 2014; Pita et al. 2013b; Reveillaud et al. 2014; Schöttner et al. 2013).

Molecular genetic community studies on the sponge-associated microbiota revealed apparent differential patterns of coexistence. Phylogenetic reconstruction based on almost full length 16S rRNA data (acquired with DGGE, cloning and cultivation approaches), showed sponge-specific clusters of bacterial clades that comprise only sponge-derived sequences (Hentschel et al. 2002; Simister et al. 2012a; Taylor et al. 2007). The subsequent mapping of metagenomic seawater data obtained from the International Census of Marine Microbes (ICoMM - http://icomm.mbl.edu/) against the sponge-specific clusters showed that sponge-specific bacteria are present within various marine ecosystems, but only in very low abundances (Taylor et al. 2013). Quantitative approaches using scanning electron microscopy (SEM) of microbial cell numbers in sponges provide a differentiation of high microbial abundance (HMA) and low microbial abundance (LMA) sponges (Gloeckner et al. 2014). As a general rule, HMA sponges contain 10^8 microorganisms / g sponge tissue, while LMA representatives possess 10^5 to 10^6 bacteria / g sponge tissue, which is similar to the microbial abundances in seawater (Gloeckner et al. 2014; Moitinho-Silva et al. 2014). Additionally, HMA sponges more likely host Proteobacteria, Chloroflexi, Acidobacteria, Actinobacteria and the candidate phylum Poribacteria in abundant numbers, while LMA sponges are commonly dominated by Proteobacteria (Alpha-, Beta-, or Gamma-) or Cyanobacteria. By applying 16S rRNA gene tag pyrosequencing on 32 globally distributed sponge species, Schmitt et al. (2012b) described three general states of microbial relationships within sponges: core (present in at least 70% of all sponges), variable (present in < 70% of all sponges), and species-specific. Correspondingly, the extensive use of massively parallel sequencing technologies emphasized the apparent sponge specificity of certain microbial phyla and the high diversity and richness of microbial communities associated with sponges (e.g., Jackson et al. 2013; Lee et al. 2011; Webster et al. 2010). For example, regardless of the genetic methods used to characterize the sponge-associated microbiota, some microbial phyla (i.e., Alpha-, Gamma-, Deltaproteobacteria, Chloroflexi, Actinobacteria, Acidobacteria, Nitrospirae and the candidate phylum Poribacteria) are usually found to be dominant within sponges with varying degrees of specificity (Hentschel et al. 2012). These sponge-associated microbial communities are generally displayed and

evaluated based on the relative abundances of operational taxonomic units at genus or phylum level. Since microbial symbionts exhibit functional convergence in phylogenetic divergent sponge hosts (Fan et al. 2012; Ribes et al. 2012), deep branching microbial clades could be functionally coherent, which could be reflected as patterns across broad taxonomic ranks. It is assumed, that the organization of microbial genetic sequences into ecological meaningful taxonomic ranks could provide a framework for a better understanding of complex microbial community compositions (Philippot et al. 2010). For example, ecologically coherent diversity patterns of different bacterial taxonomic ranks across global marine ecosystems as well as the human skin and gut microbiota, have been identified in recent years (Koeppel and Wu 2012; Zinger et al. 2011). However, the spatial turnover of microbial communities of high taxonomic ranks within a local sponge population and between individual sponge specimen has not been investigated yet. Moreover, while different sponge species apparently possess these specific dominant microbial phyla, little is known if the observed beta diversity patterns (i.e., spatial community variation among individual sponge samples - sponges are here defined as local habitats of microbial alpha diversities) are consistently detectable across taxonomic ranks from OTU to phylum level.

In the present study we investigate if microbial beta diversity patterns of high taxonomic ranks are consistent and meaningful for the sponge-associated microbiota. We hypothesize that even at this small scale, patterns of microbial beta diversity will reflect the putative functional traits and adaptation of sponge-specific deep branching microbial lineages at high taxonomic ranks. We use a small subset consisting of 24 temperate sponge-specimen (7 sponge taxa) and three water samples, collected from different depths in Sweden, provided by the first batch of the sponge-associated Earth Microbiome Project (http://www.earthmicrobiome.org/). For the analysis of beta diversity among different sponge-host, located along a depth gradient (categorized into three levels: shallow, upper twilight, and lower twilight), the available community data were collapsed at $OTU_{0.03}$ level (97% sequence identity) and integrated into high taxonomic ranks from genus to phylum level. In addition, we used indicator species analysis to assess host-specific associations of sponge-associated microbial communities.

Materials and Methods

We analyzed a subset of the of the sponge 2012 released EMP data, which consists of 7 species (24 individuals - Table 4.1) and one subsurface water sample triplicate collected from various depths (from ~ 2 m to ~ 157 m) in a confined area of Kosterjforden bay (Tjärnö, Sweden) for its host-associated

microbial diversity and the effect of host taxonomy and environment on microbial community patterns at high taxonomic ranks. Marine sponges were collected from several sampling sites and depths by snorkeling, SCUBA diving and by sampling using a remotely automated vehicle (Sperre Sub-Fighter ROV) at several sampling sites around the Kosterjforden bay (Tjärnö, Sweden, 58°52'53.1"N 11°07'17.5"E) in September 2012. Sponges were identified morphologically and dissected at the Tjärnö Marine Biology Laboratory field station. Obtained pieces of mesohyl and outer pinacoderm tissue from each specimen were immediately freeze-dried until further processing. DNA extraction, Illumina MiSeq sequencing (V4-V5 region of the 16S rRNA gene, using the 515F / 806R archaeal / bacterial primer pair) and raw sequence quality control of the samples analyzed in the present study

Table 4.1 Microbiota diversity for each host species and water samples based on 16S rRNA 97% OTUs – showing: number of sequence reads; OTU richness (S), Shannon index (H'), inverse Simpson index (D); OTU evenness (J); habitat and depth.

Core ID	Host/Sample	Reads	*S*	*H'*	*D*	*J*	Light Regime	Depth
SW.05	*Phakellia ventilabrum*	13054	566	3.41	7.64	0.54	lower twilight	155
SW.09	*Phakellia ventilabrum*	16500	668	3.32	7.93	0.51	lower twilight	150
SW.310	*Phakellia ventilabrum*	19731	449	2.77	5.59	0.45	lower twilight	117
SW.06	*Geodia barretti*	15178	1122	5.26	75.30	0.75	lower twilight	149
SW.08	*Geodia barretti*	12150	967	5.08	50.79	0.74	lower twilight	158
SW.312	*Geodia barretti*	10496	904	5.23	78.46	0.77	lower twilight	154
SW.35	*Axinella infundibuliformis*	5214	355	2.89	5.96	0.49	upper twilight	59
SW.37	*Axinella infundibuliformis*	8923	318	2.42	4.56	0.42	upper twilight	60
SW.38	*Axinella infundibuliformis*	7367	352	2.44	4.31	0.42	upper twilight	62
SW.10	*Axinella* sp.	17876	211	1.54	1.75	0.29	upper twilight	78
SW.16	*Axinella* sp.	18613	627	2.63	2.88	0.41	upper twilight	68
SW.19	*Axinella* sp.	17965	576	2.25	2.25	0.35	upper twilight	64
SW.30	*Mycale lingua*	6334	798	5.28	46.16	0.79	upper twilight	66
SW.32	*Mycale lingua*	8903	667	4.88	33.47	0.75	upper twilight	66
SW.31	*Mycale lingua*	8685	536	3.20	4.00	0.51	upper twilight	66
SW.227	*Myxilla* sp.	7171	160	1.51	2.80	0.30	shallow	25
SW.233	*Myxilla* sp.	5544	220	1.92	3.42	0.36	shallow	25
SW.234	*Myxilla* sp.	4432	138	1.32	2.19	0.27	shallow	25
SW.60	*Halichondria panicea*	18112	453	1.81	2.18	0.30	shallow	2
SW.241	*Halichondria panicea*	19154	692	1.86	1.78	0.28	shallow	7
SW.245	*Halichondria panicea*	19259	831	1.90	1.73	0.28	shallow	5
SW.25	*Axinella infundibuliformis*	7034	604	3.32	6.51	0.52	shallow	35
SW.26	*Axinella infundibuliformis*	6244	617	3.61	6.77	0.56	shallow	35
SW.27	*Axinella infundibuliformis*	3114	535	4.53	22.72	0.72	shallow	35
SW.H2O.4	marine metagenome	17426	1318	4.79	22.77	0.67	shallow	4
SW.H2O.5	marine metagenome	17981	1445	4.90	29.18	0.67	shallow	4
SW.H2O.6	marine metagenome	19784	1439	4.93	33.45	0.68	shallow	4

were carried out by the EMP collaborators – for further details on EMP quality control see Easson & Thacker (2014) and the online available EMP protocol (http://www.earthmicrobiome.org/emp-standard-protocols/dna-extraction-protocol/). We used a subset of 24 sponges and three water filter samples (332244 reads and 7201 OTUs in total) obtained from the EMP sponge dataset (R1.v2.4). Greengenes (0.03 OTUs, 60% identity cutoff) was used as reference taxonomy.

All data processing and subsequent analyses, e.g. alpha diversity estimates and sequence statistics (i.e., OTU richness, Shannon index, and inverse Simpson index, evenness) were executed in R v.3.0.3 (R Development Core Team, 2013) unless stated otherwise. For beta diversity analyses all samples were categorized into habitat definitions based on the approximate prevailing daylight conditions of the sampling sites (shallow illuminated zone: 1 - 35 m; upper twilight zone: 35 - 120 m; lower twilight zone: 120 - 160 m) and sampling locations (i.e., host taxon or water sample). In addition to the full $OTU_{0.03}$ table (referred as OTU from here), individual datasets for each taxonomic rank (from phylum to Genus) were compiled, based on the available Greengenes taxonomy. Community data were standardized using *decostand* (method = 'Hellinger'). Bray-Curtis distances were calculated using function *vegdist*. Multivariate analyses on the *a posteriori* groups and non-metric multidimensional scaling (NMDS) on all datasets (OTUs, genus, family, order, class, phylum) were performed using the functions *permutest.betadisper*, permutational multivariate analysis of variance (*adonis*) and *metaMDS* (Bray-Curtis distances) functions of the vegan package (Oksanen et al. 2012). Critical values for significance were corrected for *permutest* multiple pairwise comparisons following the Benjamini & Hochberg (1995) method (BH correction). The *envfit* function in vegan was subsequently applied to test for correlation with corresponding environmental factors (i.e., depth and habitat type) and sample location (sponge-hosts or seawater). Redundancy analysis (RDA) was performed to assess the variance and significance of the effect of depth, habitat, and sample type variables on the microbial communities. Significance tests were based on 1000 permutations for all performed analyses. The relative OTU abundance and distribution on phylum level was plotted as heatmap using JColorGrid (Joachimiak et al. 2006). Hierarchical clustering of OTUs was performed using *vegdist* (Bray-Curtis) and *hclust* (method='average') and added subsequently to the heatmap. For the indicator analysis based on the OTU data, which utilized the *multipatt* (func='IndVal.g', duleg=TRUE, 1000 permutations) algorithm in the indicspecies package (De Cáceres and Legendre

2009), each sample triplicate (sponges and marine metagenome) has been pooled and then subsequently analyzed.

Results and Discussion

Microbial community diversity

The analysis of 7201 OTUs (97% sequence similarity - comprising 332244 reads) revealed that 24 bacterial and two archaeal phyla are associated with the sponge and water samples (Figure 4.1 and Table 4.1). Seawater samples possessed the highest OTU richness followed by the *Geodia barretti* specimen, while *Myxilla* sp. showed the lowest OTU richness among all samples. OTU diversity was highest in *G. barretti* among all samples followed by seawater and *Mycale lingua* triplicates, which is consistent with the evenness patterns in the samples (Table 4.1). The most abundant microbial phyla among all samples belonged to Proteobacteria (Alpha- and Gamma-), Crenarchaeota, Bacteroidetes, Nitrospirae, Actinobacteria, Chloroflexi, Cyanobacteria and Acidobacteria (Figure 4.1). These phyla are known to be generally sponge-specific and abundant (Hentschel et al. 2012; Schmitt et al. 2012b). However, the comparison of microbial abundances between sponge and seawater samples revealed highly sponge-specific dominant phyla, such as Crenarchaeota, Nitrospira, Chloroflexi, or Acidobacteria, whereas the seawater contained mainly Proteobacteria (Alpha-, and Gamma-), Bacteroidetes, Actinobacteria, Verrucomicrobia and Cyanobacteria (Figure 4.1).

Host-consistency across high taxonomic ranks

Host-specificity of sponge-associated microbial communities is an overall acknowledged feature in the versatile and complex symbiotic sponge-microbe relationships (Hentschel et al. 2012; Taylor et al. 2007). In this study, hierarchical clustering, multivariate analysis of community variation and nMDS ordination based on OTU community dissimilarities confirmed this apparently significant host-specificity (Figure 4.1, Figure 4.2A, and Table 4.2). Based on 16S rRNA gene analysis Schmitt et al. (2012b) assigned three groups of sponge microbial communities: a microbial core community that is present in < 70% of all sponges, a variable community with changing presence among different sponge species, and a species-specific community, which is specific to its host-species. Especially the latter group is presumably marked by a highly symbiotic relationship to its host taxon and functionally involved in the sponge-specific metabolism (e.g., Fan et al. 2012; Liu et al. 2012; Reveillaud et al. 2014).

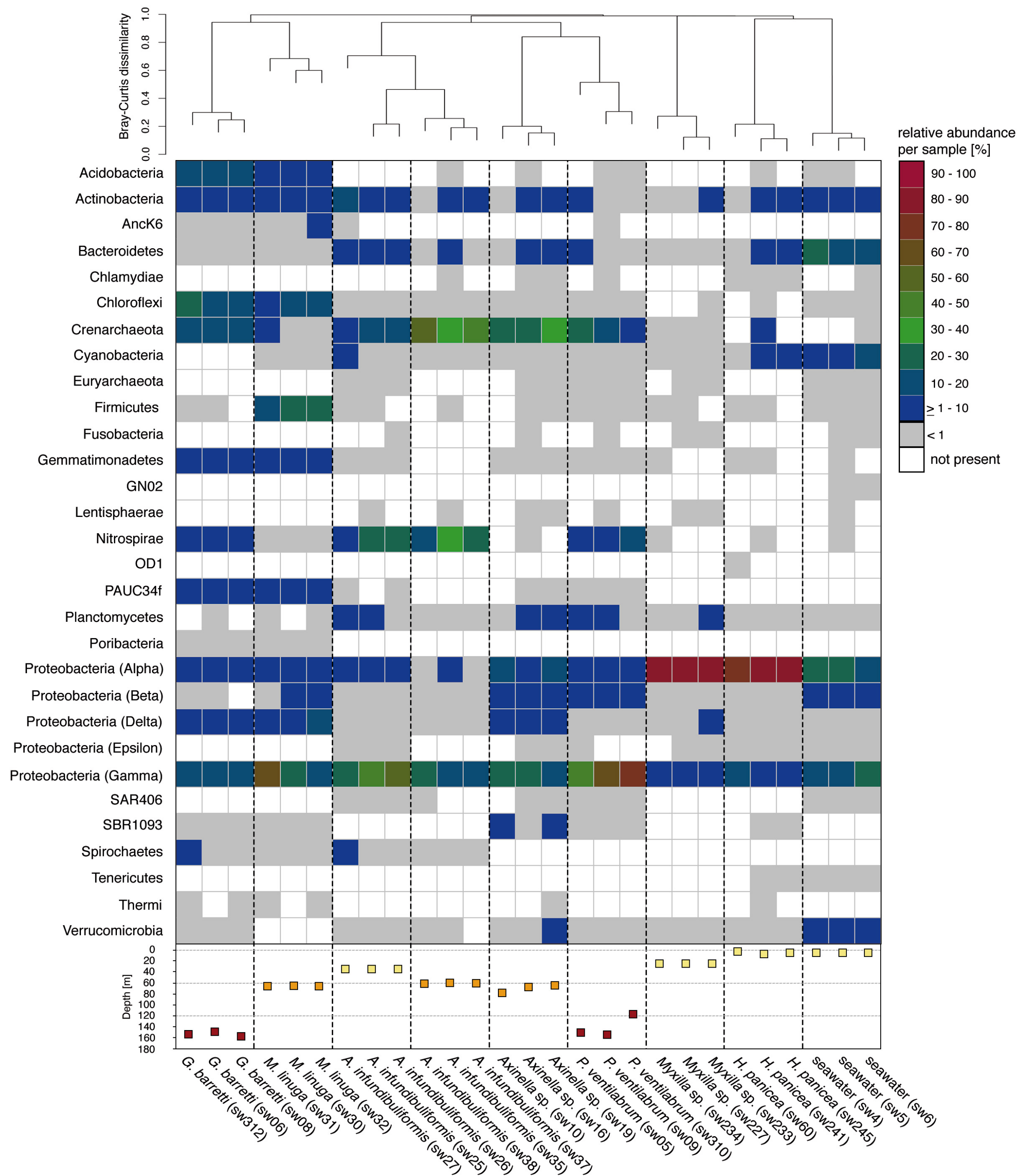

Figure 4.1 Taxonomic breakdown per sample at phylum level based on relative abundance of assigned 16S rRNA 97%. Samples arranged by Bray-Curtis dissimilarity as shown by the dendrogram on top. Sampling depth of individual samples is shown below – color code for the hypothetical habitat groups: red for lower twilight, orange for upper twilight, yellow for shallow sites.

Philippot et al. (2010) discussed the phylogenetic coherence of ecological traits, which is presumable responsible for the display of ecological similarities over large phylogenetic distance of certain microbial lineages. With the observed functional equivalence in complex sponge-associated symbiont communities (Fan et al. 2012; Ribes et al. 2012), we would expect to find such functional

(i.e., ecological) meaningful patterns at deep-branching microbial clades. However, little is known about the coherence and consistence of microbial associations across different taxonomic ranks. Collapsing the present OTU table into individual tables from genus to phylum to levels revealed that with each ascending taxonomic rank the number of available taxa decreased, while the number of classified OTUs available for each rank increased (Table 4.2). For example, at genus level 192 genera were available, while > 81% of the OTUs had to be discarded due to missing taxonomic information. Conversely, on phylum level only 26 phyla were available, while only 3% of the full OTU dataset had to be excluded due to missing taxonomic information.

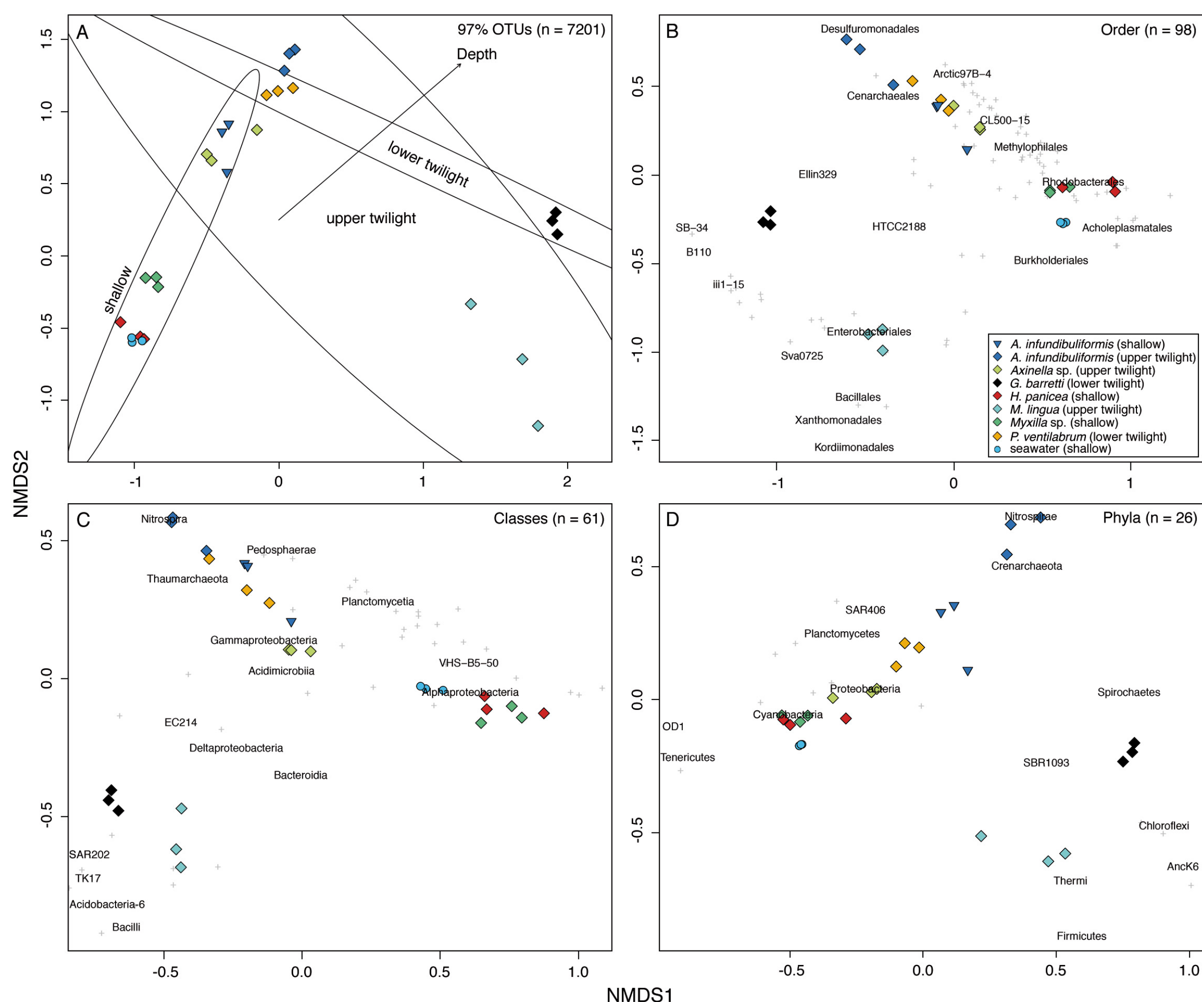

Figure 4.2 nMDS ordinations based on Bray-Curtis dissimilarities of all samples for four different taxonomic levels: 97% OTUs, order, class, and phylum. Available number of taxonomic data points of each dataset is plotted in the upper right of each individual plot - A) displays 97% OTUs, hypothetical groups are drawn as dispersion ellipses with a confidence interval of 95%, the significant influence of depth is overlaid as vector via *envfit*; B) ordination of orders, with gray points displaying each individual taxa and the most abundant taxa plotted with names based on abundance; C) ordination of classes, with the same options used as described in B; D) ordination of phyla, with the same options used as described in C.

Table 4.2 Individual effects of hosts or samples, light regimes, and depth on the structure of bacterial communities. For each taxonomic rank the number of available ‘species’ (n) and percentage of unclassified ‘species’ are given within the upper brackets, followed by nMDS stress values for each rank ordination. Pairwise comparisons of group mean dispersions (betadisper with permutest) are shown as *F* ratio and adonis as correlation coefficient R^2 with permuted *F* ratio significance indices attached. Environmental vector fit results (*envfit*) shown as permuted *r* ratios and correlation coefficient R^2. RDA model results are shown with degrees of freedom (df) explained variance with permuted *F* ratio significance indices attached. Significances of F and r ratios (1000 permutations) are indicated as ***, p<0.001; **, p<0.01; *, p<0.05

Taxonomic rank	97% OTU (n = 7201)		Genus (n = 192, 81.2%)		Family (n = 162, 56.3%)		Order (n = 98, 46%)		Class (n = 61, 12.8%)		Phylum (n = 26, 3%)	
nMDS stress	0.017		0.016		0.015		0.014		0.012		0.007	
Betadisper/Adonis	***F***	**R^2**	***F***	**R^2**	***F***	**R^2**	***F***	**R^2**	***F***	**R^2**	***F***	**R^2**
Sample	4.72	0.82***	3.18	0.80***	1.63	0.84***	1.53	0.87***	1.82	0.88***	2.24	0.90***
Light Regime	3.38	0.21***	4.53	0.33***	3.14	0.27***	1.32	0.25***	0.35	0.32***	3.10	0.26***
Envfit	**Pr(*r*)**	**R^2**	**Pr(*r*)**	**R^2**	**Pr(*r*)**	**R^2**	**Pr(*r*)**	**R^2**	**Pr(*r*)**	**R^2**	**Pr(*r*)**	**R^2**
Sample	< 0.001***	0.97	< 0.001***	0.91	< 0.001***	0.93	< 0.001***	0.95	< 0.001***	0.96	< 0.001***	0.94
Light Regime	< 0.001***	0.42	< 0.001***	0.47	< 0.001***	0.41	< 0.001***	0.39	< 0.001***	0.44	0.005**	0.29
Depth	< 0.001***	0.72	< 0.001***	0.59	0.002**	0.58	< 0.001***	0.62	< 0.001***	0.56	0.002**	0.44
RDA	**df**	**Variance**	**df**	**Variance**	**df**	**Variance**	**df**	**Variance**	**df**	**Variance**	**df**	**Variance**
Sample	7	0.75***	7	0.45***	7	0.51***	7	0.53***	7	0.35***	7	0.22***
Light Regime	2	0.18***	2	0.17***	2	0.16***	2	0.15***	2	0.12***	2	0.06**
Depth	1	0.11***	1	0.13***	1	0.13**	1	0.12***	1	0.09***	1	0.05***

Nevertheless, despite theses remarkable differences in phylotype richness (see Table 4.2), unconstrained and constrained multivariate analyses resulted in overall consistent significant patterns of host specificity from OTU up to phylum level (Figure 4.2A-D, Table 4.2). The present results suggest that within the large microbial community on OTU level (n = 7201 OTUs) coherent host-specific patterns are prominent enough to be visible at the lowest taxonomic resolution (n = 26 phyla). However, the redundancy analysis showed that with increasing taxonomic resolution the community variation explained by host-specificity also continuously increased from 22% on phylum to 75% on OTU level (Table 4.2). In contrast to this, multivariate tests on group variation showed similar coefficients of determination for sampling location related groups (R^2 = 0.82 on OTU level vs. R^2 = 0.90 on phylum level). This is slight increase in percentage of the explained variance with deeper nodes, is congruent with the drastic decrease of taxonomically unclassified community data from genus (> 81%) to phylum (> 26%) level. In addition to the increase of taxonomically unclassified data and the relatively low number of classified phylotypes (n = 192) on genus level, the related nMDS plot lacks the present distinct clusters of sponge species visible in the 97% OTU and in the remaining higher taxonomic ordination plots to some extent (Supplementary Figure 4.1AB). The change of microbial community patterns with lower taxonomic ranks (i.e., genus level, and to some extent on family level) presumably hints to different functional and ecological associations with host and / or

habitat among these specific phylogenetic deep branches (Koeppel and Wu 2012; Philippot et al. 2010). Moreover, Zinger et al. (2011) still observed important ecological signal at genus level in a comparable analysis, with 76% of the community data disregarded due to missing taxonomic annotation. Nonetheless, since the taxonomic levels, from 97% OTU to phylum, reproduced overall consistent community patterns, we can not exclude that the diverging multivariate ordinations at least on genus level could also presumably caused by the evident decrease of available taxonomic data.

Depth and habitat effects on the community structure

The environmental factors 'depth' and 'habitat type' (i.e., shallow, upper and lower twilight) had a significant effect on the community structure (Figure 4.2A and Table 4.2). However, depth, habitat type and sponge taxonomy may be (indirectly) inter-related. Since *A. infundibuliformis* is the only sponge species collected from two 'habitat types', the sponge-associated community patterns observed in the present study that are based on the significant microbial host-specificity are probably nested within depth and habitat, hence indirectly causing the observed effects. If certain sponge species, which possess sponge species-specific microbial communities, inhabit discrete aquatic habitats (e.g., marine and freshwater, or coral reef and deep sea sponge species), these habitat preferences may also indirectly generate such microbial patterns (Bell 2008; Maldonado and Young 1998; Van Wagoner et al. 1989). To better resolve such interactions, future studies on sponge-associated community structure and environmental effects should take these considerations into account.

So far, only a small number of sponge-microbe related studies assessed the influence of habitat characteristics such as depth, or light conditions on the microbial community structure (e.g., Cárdenas et al. 2014; Olson & Gao 2013; Schöttner et al. 2013). These studies consistently showed that host-taxonomy has the strongest effect on the associated microbial diversity structure, and that depth had only minor or even no observable effects on microbial community patterns. Since the particular EMP subset used in this study contained only one sponge taxon sampled from two different sampling zones (~ 35 m & ~ 60 m depth), present analysis of depth-relatedness was limited to *Axinella infundibuliformis*. The effect of depth on the *A. infundibuliformis* specific community structure was evident ($p < 0.001$) and did not show any significant dispersion (Supplementary Figure 4.1C). This suggests that within the same local sponge species population, significantly different microbial communities may exist, which are potentially influenced by environmental factors (e.g., light regime, temperature, salinity, or nutrient supply) of the habitat. For example, because of different light regimes

in ascending habitats photoautotrophic bacteria that are dominant in the upper layers, but rare in the twilight habitats, can hypothetically cause depth-differences in sponge species-specific communities (Olson and Kellogg 2010).

Indicator species analysis of the sponge-associated microbiota

To further examine the specific associations of microbial taxa with individual sponge species, an indicator species analysis has been performed to search for significant relationships of OTUs in the pooled sponge species. For this, the resulting indicator OTUs were combined at class level for each sample group (Figure 4.3). The resulting community diversity and host-specificity patterns in Figure 4.3 are mostly consistent with the observed host-specificity of the full microbial-community data in Figure 4.1 of this study (for a complete list of all indicator OTUs and related taxonomic ranks see Supplementary Table 4.1). Noticeably, only the sponges *Myxilla* sp. and *Haliclona panicea* possess an

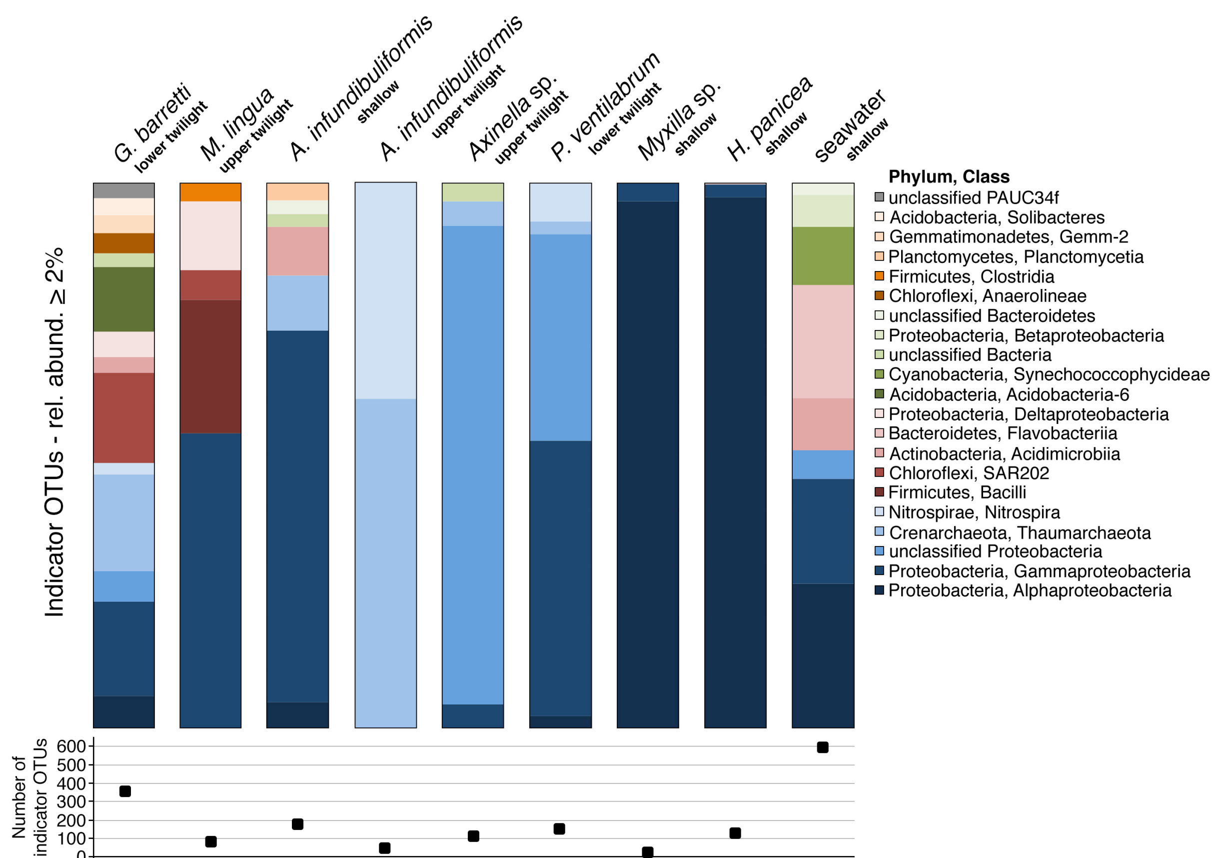

Figure 4.3 Abundance and diversity of significantly correlated indicator species collapsed to classes for each sample pool. For better clarity only classes with an abundance ≥ 2% are shown – a more detailed table with all individual significant OTUs and taxonomic classification down to Species level can be found in the Supplementary Table 4.1. The numbers of individual indicator OTUs for each sample pool plotted below.

overall similar group identity, while the abundances of indicator OTUs indicate differences of both sponge-associated microbial communities (Figure 4.3). With Proteobacteria (Alpha- and Gamma-), Firmicutes, Chloroflexi, Actinobacteria, Bacteroidetes, Acidobacteria, Cyanobacteria, Planctomycetes, and Gemmatimonadetes, almost every phylum, which is known to be associated with sponges, is represented here by significant indicator OTUs (Supplementary Table 4.1). Moreover, the indicator analysis seems to support the host-specificities of microbial communities shown in the multivariate ordination plots on phylum and class level (Figure 4.2CD & Figure 4.3). For example, the dominant indicator occurrences of Crenarchaeota and Nitrospirae within the mesophotic *A. infundibuliformis* is consistent with the abundant phyla close to *A. infundibuliformis* shown in the according ordination (Figure 4.2D). These consistencies among the two methods can also be observed for further detected sponge-microbe associations - for example: the class Bacilli (phylum Firmicutes) is abundant in *Mycale linguae* and the class Alphaproteobacteria is dominant in seawater, *H. panicea* and *Myxilla* sp. samples. *G. barretti* is significantly associated with the classes SAR202 (phylum Chloroflexi) and Acidobacteria-6 (phylum Acidobacteria) (Figure 4.2CD & Figure 4.3). Additionally, since *G. barretti* belongs to the HMA group, the latter observation is also in accordance with the notion that Chloroflexi and Acidobacteria are commonly abundant members of HMA sponges (Gloeckner et al. 2014; Schöttner et al. 2013).

Conclusion

The present study shows for the first time that sponge-specific communities exhibit consistent spatial patterns and variation in beta diversity across high microbial taxonomic ranks within a local community of taxonomic divergent sponge species. The resemblance of communities on different taxonomic ranks is mostly in agreement with structures present on $OTU_{0.03}$ level, with highly significant results for sample associations. This leads to the indication that microbial communities exhibit ecological meaningful patterns on high taxonomic ranks. However, the processes that shaped these patterns are still unknown. Therefore, future studies should focus on the intrinsic differences according to which sponges can be organized into ecological or physiological meaningful groups, such as HMA and LMA members, the abundance of ammonia-oxidizing-bacteria / archaea, environmental niches, or specific chemical compounds. For these analyses, large datasets with a long sampling depth gradient, across large geographic and environmental gradients, and an extensive collection of metadata, will certainly help to resolve the present observed consistent patterns in greater detail. Therefore, the sponge-

specific EMP data presents a unique opportunity for future ecological meta-studies on the complex sponge-microbe relationships on an unprecedented scale.

CHAPTER IV

Diversity of Actinobacteria Associated with the Marine Ascidian *Eudistoma toealensis*

Abstract

Ascidians have yielded a wide variety of bioactive natural products. The colonial ascidian *Eudistoma toealensis* from Micronesia has been identified as the source of a series of staurosporine derivatives, though the exact origin of these derivatives is still unknown. To identify known staurosporine-producing microbes associated with *E. toealensis* we analyzed with 16S rRNA gene tag pyrosequencing the overall bacterial community and focused on potential symbiotic bacteria already known from other ascidians or other marine hosts, such as sponges. The described microbiota was one of very high diversity, comprising 43 phyla: 2 from archaea, 34 described bacterial phyla, and 7 candidate bacterial phyla. Many bacteria, which are renowned community members of other ascidians and marine holobionts, such as sponges and corals, were also part of the *E. toealensis* microbial community. Furthermore, two known producers of indolocarbazoles, *Salinispora* and *Verrucosispora*, were found with high abundance exclusively in the ascidian tissue, suggesting that microbial symbionts and not the organism itself may be the true producers of the staurosporines in *E. toealensis*.

Introduction

Ascidians (Tunicata) are an important source of marine natural products, with over 1,000 natural products identified from ascidians so far (Schmidt and Donia 2010) and some 40 novel ascidian-derived natural products still isolated every year (Blunt et al. 2013). The colonial ascidian *Eudistoma toealensis* is a highly abundant species within mangrove root habitats in Micronesia and, despite lacking morphological defenses, only the flatworms *Pseudoceros indicus* and *P. tristiatus* are known to feed upon this species (Schupp et al. 2002; Schupp et al. 1999). A series of staurosporine derivatives, belonging to the group of indolocarbazole alkaloids, has been isolated from *E. toealensis*. Staurosporines have received considerable attention due to their pronounced cytotoxic activity resulting from inhibition of protein kinases (Blunt et al. 2012; Sánchez et al. 2006; Tamaoki et al. 1986). In addition, several staurosporine derivatives have entered phase I/II clinical trials for treating various cancer types (e.g. leukemia, lymphomas, advanced solid tumors, and melanoma), emphasizing their role as highly bioactive secondary metabolites (Sánchez et al. 2006). Besides being isolated from several marine macroorganisms (e.g. nudibranchs, ascidians), staurosporines have long

been known to be produced by terrestrial *Streptomyces* strains and, more recently, from various marine actinomycetes (Schmidt and Donia 2010). However, the source of the *E. toealensis*-associated staurosporines is still unknown. Since *E. toealensis* is a filter feeder and ingests diverse marine microbes from seawater, it is possible that these compounds are of microbial origin and are actually taken up via the food chain (Schupp et al. 2009; Schupp et al. 1999). Such metabolic associations and interactions between marine filter feeders and microbes are currently best known from marine sponges (Hentschel et al. 2006; Taylor et al. 2007; Webster and Taylor 2012; Wilson et al. 2014). Although sponges and ascidians are phylogenetically not closely related, the identical lifestyle of filter-feeding in often shared habitats has presumably led to similar symbiotic interactions with microorganisms. Recent studies highlighted the status of ascidians as marine holobionts capable of hosting highly diverse microbial communities with great potential for specific biosynthetic pathways and microbially derived secondary metabolites (Behrendt et al. 2012; Donia et al. 2011; Erwin et al. 2014; Erwin et al. 2013; López-Legentil et al. 2011; Schmidt and Donia 2010).

The aim of this study was to identify known staurosporine-producing microbes associated with *E. toealensis* from two Micronesian islands. While analyzing the overall bacterial community, a focus was set on a) potential symbiotic bacteria already known from other ascidians and sponges, and b) the ascidian-associated Actinobacteria, due to their possible staurosporine production in *E. toealensis*.

Materials and Methods

In 2006, whitish and slightly transparent *E. toealensis* specimens were collected via snorkeling on the Micronesian Islands of Chuuk (EtCI 1 - 3) (7°26′N, 151°51′E) and Pohnpei (EtPI 1 - 5) (6°51′N, 158°13′E) from mangrove roots at depths of 1 to 2 m. Ascidians were compared with previously collected vouchers from Schupp et al. (1999), which have been identified by ascidian taxonomists Monniot and Monniot at the Museum National d'Histoire Naturelle, Paris, France. During sampling on Pohnpei, environmental samples (rootPI 1- 3) were also collected from the surface of the mangrove roots by swabbing. All samples were frozen immediately, freeze-dried and stored at -20°C until sample analysis.

Genomic DNA was extracted from ascidian tissue and root surface swab samples using a bead-beating method previously described for sponges (Taylor et al. 2004). Additionally, root surface swab samples were incubated for 30 min at 94°C after initial bead-beating following a modified DNA extraction protocol for swab samples (modified after Waite et al. 2012). 16S rRNA gene amplification

with primers 454MID_533F (GTG CCA GCA GCY GCG GTM A) and 454_907RC (CCG TCA ATT MMY TTG AGT TT) and purification for pyrosequencing was performed as previously described (Simister et al., 2012b). The resulting flowgram data can be accessed via the Sequence Read Archive (SRA) of the National Center for Biotechnology Information under the accession number SRX682233.

Sequences were initially processed using Mothur v.1.33.0 (Schloss et al. 2011; Schloss et al. 2009). Pyrosequencing flowgrams were filtered and denoised using the Mothur implementation of AmpliconNoise (Quince et al. 2011). Sequences were removed from the analysis if they were <200bp, contained ambiguous characters, had homopolymers longer than 8 bp, more than one MID mismatch, or more than two mismatches to the reverse primer sequence. Denoised and trimmed sequences (Mothur v.1.33.0) were uploaded and processed via SILVAngs v.1.3.0 (https://www.arb-silva.de/ngs/) as described in Krupke et al. (2014). SILVAngs classification was performed two times, for each individual sample (*E. toealensis*: EtCI 1 - 2 & EtPI 1 - 5; rootPI 1 - 3) and additionally as a pooled dataset for each combination site/sample (EtCI, EtPI and rootPI).

The SILVAngs fingerprint results, which provided detailed comparative information about the classification of the 0.03 OTUs (i.e. >97% 16S rRNA gene sequence similarity) for each sample at maximum taxonomic depth (setting: max. taxonomic depth '20'), were subsequently used for multivariate non-metric multidimensional scaling (nMDS, Bray-Curtis dissimilarity) using the metaMDS command from the vegan package (Oksanen et al. 2012) in R (v. 3.0.2) (R Development Core Team, 2013). Hypothesis-based community treatments were drawn with the vegan command 'ordieellipse' (0.95 confidence interval). Treatments were as follows: a) source - A 'EtCI', B 'EtPI', and C 'rootPI', b) habitat - 'ascidian' and 'environmental', and c) location - 'Pohnpei' and 'Chuuk'. These treatments were used for hypothesis-based multivariate analysis of variance by the 'adonis' command from the vegan package. The same dataset was used to generate heatmaps with JColorGrid (Joachimiak et al. 2006) for *Actinobacteria*. Dendrograms were generated using the vegan package in R via the commands ‘vegdist’ (Bray-Curtis dissimilarity) and ‘hclust’ (method = average) and subsequently added onto the heatmaps. OTU and sequence statistics, taxonomic fingerprint and krona charts were provided by SILVAngs v.1.3 (Ondov et al. 2011; Quast et al. 2013). Eukaryotes and sequences classified as ‘no relatives’ found in our samples (n = 792) were excluded from all statistical analyses (Supplementary Table 4.1).

Results and Discussion

OTU statistics and microbial diversity

The analyzed ascidian and environmental microbiota displayed a very high operational taxonomic unit (OTU) diversity within all sites and samples. The microbial community associated with *E. toealensis* comprised 2967 OTUs (0.03 cutoff) in total among the three individuals from Chuuk Island (EtCI 1 - 3) and 3405 OTUs among the five individuals from Pohnpei Island (EtPI 1 - 5) (Table 5.1).

Overall, at phylum level the *E. toealensis* microbial composition is comparable to that described in other recent ascidian microbiology studies (Behrendt et al. 2012; Erwin et al. 2014; Erwin et al. 2013). Here, we report 43 ascidian-associated phyla: 2 from archaea, 34 described bacterial phyla, and 7 candidate bacterial phyla. The dominant phylum was Proteobacteria, which accounted for over 50 % of all classified sequences found in every sample (Figure 5.1 & Supplementary Table 5.1). Within Proteobacteria, the Alphaproteobacteria were, on average, most dominant (20.1% averaged across all samples), followed by Gammaproteobacteria (18.7%) and Deltaproteobacteria (11.2%). Other abundant phyla throughout all samples included Planctomycetes, Bacteroidetes, Actinobacteria, Acidobacteria, and Cyanobacteria (Figure 5.1 & Supplementary Table 5.1). In comparison to the known dominant phyla in *Eudistoma amplum* (Erwin et al. 2014), only the low abundance of Thaumarchaeota in *E. toealensis* deviates noticeably from the general dominant phyla within the two *Eudistoma* species. However, due to low sequence numbers and possible sequencing errors or primer biases in the targeted 16S rRNA region, caution is required in order to not overestimate the abundance and diversity for the archaeal lineages in our data.

Table 5.1 Sequence and OTU summary - with number of total sequence available for each individual sample and pooled samples, number of OTUs for individual and pooled samples, frequencies of classified sequences and sequences considered as unclassified (No Relative - BAST alignment coverage and alignment identity < 93%), and sampling coverage.

Sample Name	# Sequences	# OTUs	% Classified	% No Relative	Good's coverage
EtCI1	7891	675	95.08	4.82	0.95
EtCI2	14296	1932	99.02	0.85	0.95
EtCI3	8447	1243	97.67	2.24	0.93
EtCI pooled	30634	2967	98.19	1.70	0.96
EtPI1	4372	858	99.91	0.05	0.91
EtPI2	8785	1686	99.57	0.43	0.91
EtPI3	9128	1566	99.64	0.19	0.93
EtPI4	6327	751	99.83	0.08	0.94
EtPI5	6910	1252	99.58	0.23	0.92
EtPI pooled	35522	3405	99.68	0.21	0.96
rootPI1	9442	2112	99.75	0.13	0.89
rootPI2	6952	1806	99.61	0.07	0.87
rootPI3	7969	1868	99.77	0.06	0.89
rootPI pooled	24363	3953	99.73	0.07	0.93

In our study, 31 phyla are shared between *E. toealensis* and environmental samples (Supplementary Figure 5.1 & Supplementary Table 5.1). While 12 phyla were recovered exclusively from *E. toealensis*, two phyla were obtained from the environment only (BHI80-139 & *Synergistetes*). An example of the shared community (found in both *E. toealensis* and on the root surface) is a strictly anaerobic described Chloroflexi lineage which was also found in other recent studies of ascidian-associated microbiota and which has been described as a sponge and coral symbiont (Behrendt et al., 2012; Erwin et al., 2012, 2014; Simister et al., 2012a; Taylor et al., 2013). Two other sponge symbionts within the shared phyla dataset were the deltaproteobacteria Candidatus *Entotheonella* (Brück et al. 2008; Schmidt et al. 2000; Wilson et al. 2014) and *Nitrospina* (Hentschel et al. 2006; Schmitt et al. 2012b). The candidate genus *Entotheonella* is a renowned symbiotic genus in the marine sponge *Theonella swinhoei* with a remarkably diverse natural products repertoire. Almost all bioactive polyketides and peptides from *T. swinhoei* have been attributed to one of the two chemically distinct *Entotheonella* phylotypes inhabiting this sponge (Wilson et al. 2014). While *Entotheonella* spp. is widely distributed in sponges, we observed members of this candidate genus in *E. toealensis* from both locations (1% EtCI & 8% EtPI of all *Desulfobacterales*) and our environmental samples (0.2%). The presumed nitrite-oxidizing *Nitrospina* symbionts, which were recently found in some ascidians (Erwin et al. 2014), comprise 11% of all *Desulfobacterales* in EtCI, 3% in EtPI, and 0.6% in environmental root surface swabs.

Among the microbiota occurring exclusively within *E. toealensis* was the ammonia-oxidizing Thaumarchaeota (i.e. Marine Group I, *Candidatus* Nitrosopumilus, and the Soil Crenarchaeota Group), but apparently at lower abundance than that recently described by Erwin et al. (2014) (Figure 5.1 & Supplementary Table 5.1). However, finding evidence of Thaumarchaeota occurrence only in *E. toealensis* specimens and not in our environmental samples highlights this genus as a potential ascidian symbiont (Martínez-García et al. 2008). Moreover, 4% of the *E. toealensis* Gammaproteobacteria community from Chuuk Island (and 0.2% from Pohnpei Island) was associated with the genus Candidatus *Endoecteinascidia*, which was previously described as species-specific for the ascidian *Ecteinascidia diaphanis* (Great Barrier Reef) and *E. turbinata* (Mediterranean and Caribbean Sea) (Erwin et al. 2014; Moss et al. 2003; Pérez-Matos et al. 2007). To the best of our knowledge, this is the first time that this symbiont lineage, with an assumed role as a secondary metabolite producer (Rath et al. 2011), has been reported from another ascidian genus.

By using the 0.03 OTU community data for non-metric multidimensional scaling, the resulting ordination and multivariate analysis of variance (adonis) showed significant differences between ascidian and environmental samples (Supplementary Figure 5.2). The distinct grouping of environmental and *E. toealensis* samples supports recent findings that ascidians host very specific microbial communities with potential symbiotic relationships (Donia et al. 2011; Erwin et al. 2014; Martínez-García et al. 2007; Piel 2009; Schmidt and Donia 2010). As with sponges, the maintenance of symbiont communities presumably represents a combination of horizontal and vertical transmission (Erwin et al. 2014; Schmitt et al. 2012b). While vertical transmission is usually associated with colonial ascidians, horizontal acquisition from the environment is assumed for solitary ascidians, e.g. *Styela plicata* (Erwin et al. 2013). However, for the colonial ascidian *E. toealensis* the large number of microbial phyla that are shared with the environment suggests that the transmission of associated bacteria is presumably a mix of vertical and horizontal transmission, as observed and discussed for sponges (Reveillaud et al. 2014; Schmitt et al. 2012b; Taylor et al. 2013) and ascidians (Erwin et al. 2014; Erwin et al. 2013).

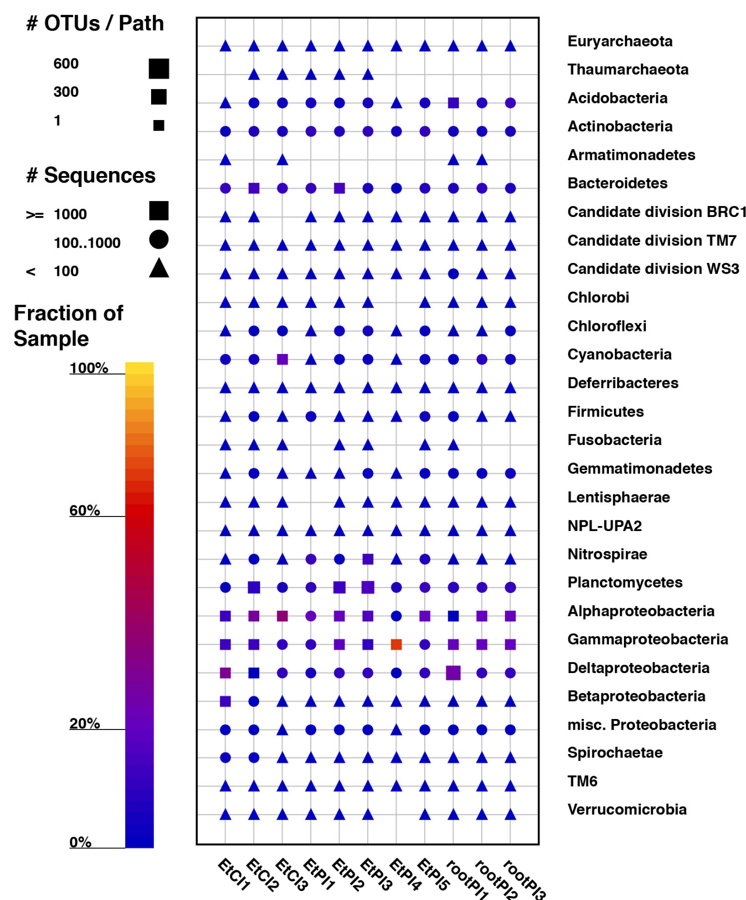

Figure 5.1 Taxonomic breakdown per sample at phylum level - showing only phyla with ≥ 10% relative abundance.

Actinobacteria diversity

Several staurosporine derivatives have been isolated from *E. toealensis* samples in the past (Proksch et al. 2003; Schupp et al. 2001; Schupp et al. 1999) with high structural similarity between compounds found in *E. toealensis* and in Actinobacteria suggesting a microbial origin (Schmidt and Donia 2010). Since Actinobacteria are well known producers of secondary metabolites (e.g. staurosporines and other indolocarbazoles) in marine eukaryotes and are, furthermore, often associated with marine sponge and coral holobionts, we focused on the diversity of Actinobacteria associated with *E. toealensis* (Blunt et al. 2013; Piel 2009; Sánchez et al. 2006; Schmidt and Donia 2010; Schmitt et al. 2012b; Simister et al. 2012a; Webster and Taylor 2012).

Actinobacteria constitute between 2 - 10% of all bacteria within the dataset and are comprised of 51 Actinobacteria genera (Figure 5.2 & Supplementary Table 5.2). The krona charts of the three pooled datasets showed distinct community structures, in which the *E. toealensis* samples from Chuuk and Pohnpei Island (Figure 5.3AB) exhibited greater diversity than the environmental samples (Figure 5.3c). Among the 51 Actinobacteria genera, 16 were shared between *E. toealensis* and environmental samples (Figure 5.2 & Supplementary Table 5.2). Additionally, nMDS analysis and adonis hypothesis testing based on the Actinobacteria community data revealed a significant difference between the *E. toealensis* and environmental samples, while the overlapping ordination of most of the ascidian samples tentatively suggests an *E. toealensis*-specific Actinobacteria community within geographically different sampling sites (Supplementary Figure 5.2). The shared Actinobacteria made the greatest contributions, with two dominant marine groups OCS155 and Sva0996 and two uncultured Acidimicrobiales and Gaiellales clades (Figure 5.2). Most notable were the genera *Salinispora* and *Verrucosispora*, which were only found in *E. toealensis* but not the environmental samples (Figure 5.2 & 5.3). Both are members of the Micromonosporaceae and these two have been described as potential indolocarbazole producers (Sánchez et al. 2006). Bacteria of the marine genus *Salinispora* have been cultured from sponges (e.g. Great Barrier Reef sponge *Pseudoceratina clavata*; Kim et al., 2005) and are known for their production of bioactive secondary metabolites, such as salinosporamide A, sporolide A, and also staurosporine derivatives (Blunt et al. 2013; Freel et al. 2011; Jensen et al. 2007; Udwary et al. 2007). *Verrucosispora* are known producers of numerous ascidian (Blunt et al. 2012) and sponge (Blunt et al. 2013; Jiang et al. 2007) secondary metabolites. Both *Salinispora* and

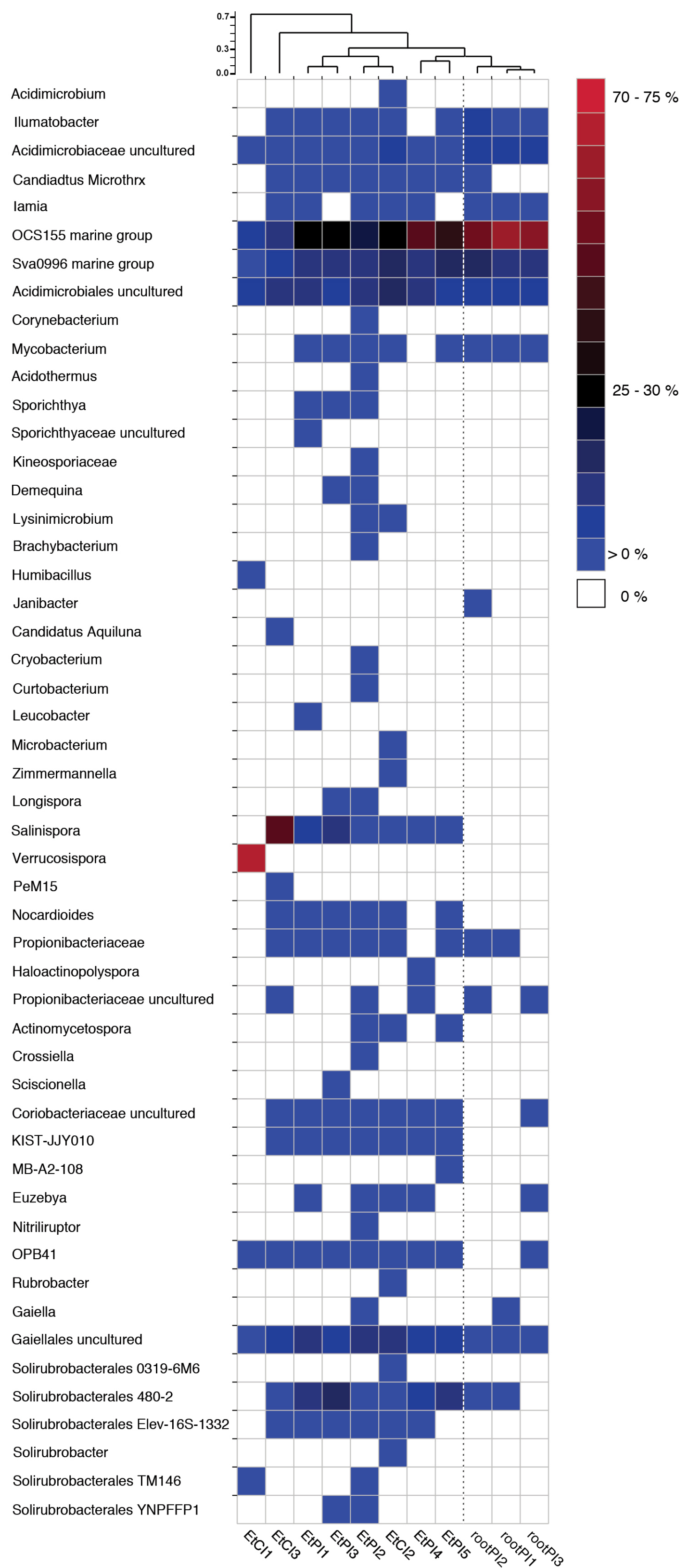

Figure 5.2 Occurrence of Actinobacteria in Ascidian and root surface samples. The grey scale code indicates relative abundance, ranging from light grey (low abundance) to black (high abundance). White indicates that no sequence was assigned to the specific Actinobacteria genera. Samples are clustered using Bray-Curtis dissimilarity and group averages.

Verrucosispora have also been recently cultured from the colonial ascidian *Lissoclinum patella* (Donia et al. 2011). Furthermore, two new staurosporine derivatives have been isolated from the Brazilian ascidian *Eudistoma vannamei* (Jimenez et al. 2012). Subsequently 20 actinomycetes strains were isolated from *E. vannamei,* indicating that ascidians of the genus *Eudistoma* seem to host diverse actinomycetes communities, which produce biologically highly active secondary metabolites (Jimenez et al. 2013).

The exclusive low abundance Actinobacteria members in our data (Figure 5.2) exhibit also an intriguing spectrum of marine-invertebrate associations. Many of them are known for potential symbiotic relationships and/or microbial secondary metabolite production within their hosts. For example, *Acidimicrobium*, *Brachybacterium*, *Corynebacterium*, *Leucobacter*, and *Solirubrobacter* representatives were found in various sponge species (Hentschel et al. 2006; Khan et al. 2012; Sfanos et al. 2005; Taylor et al. 2007). The genus *Microbacterium*, which already showed antitumor properties (Wicke et al. 2000), has been recovered from sponges (Lafi et al. 2005; Muscholl-Silberhorn et al. 2008; Sfanos et al. 2005; Taylor et al. 2007), sea anemones (Du et al. 2010) and sediments (Bollmann et al. 2010; Gavrish et al. 2008). Nitrogen-fixing *Sporichthya* are potential symbionts located in the nidamental glands of the squid *Sepia officinalis* (Grigioni et al. 2000). Finally, the genus *Nocardioides* (family Nocardiopsaceae) was found in culture-dependent and independent studies in the sponges *Haliclona* sp. and *Hymeniacidon perleve* (Khan et al. 2011; Sun et al. 2010).

Concluding remarks

This study revealed exceptionally high microbial diversity within the ascidian species *E. toealensis*. Many known symbiotic microbes, which previously had been described from sponges and ascidians (e.g. Candidatus *Entotheonella*, Nitrospina, Thaumarchaeota), were also part of the *E. toealensis*-associated microbiota. Some of these microbes may contribute to the ascidians' metabolic pathways, for example with nitrification abilities, while other are able to synthesize highly biologically active secondary metabolites, with bioactivities ranging from anticancer, antimicrobial and antiviral activities to chemical defenses. Altogether, *E. toealensis* seems to be an important holobiont, able to host a diverse and rich microbial biota with a great potential to act as a source of bioactive compounds of microbial origin. Moreover, with the occurrence of *Salinispora* and *Verrucosispora*, two known producers of indolocarbazoles, such as staurosporines, were found with high abundance exclusively in

the ascidian tissue, hinting that microbial symbionts and not the organism itself may be the true producers of these derivatives.

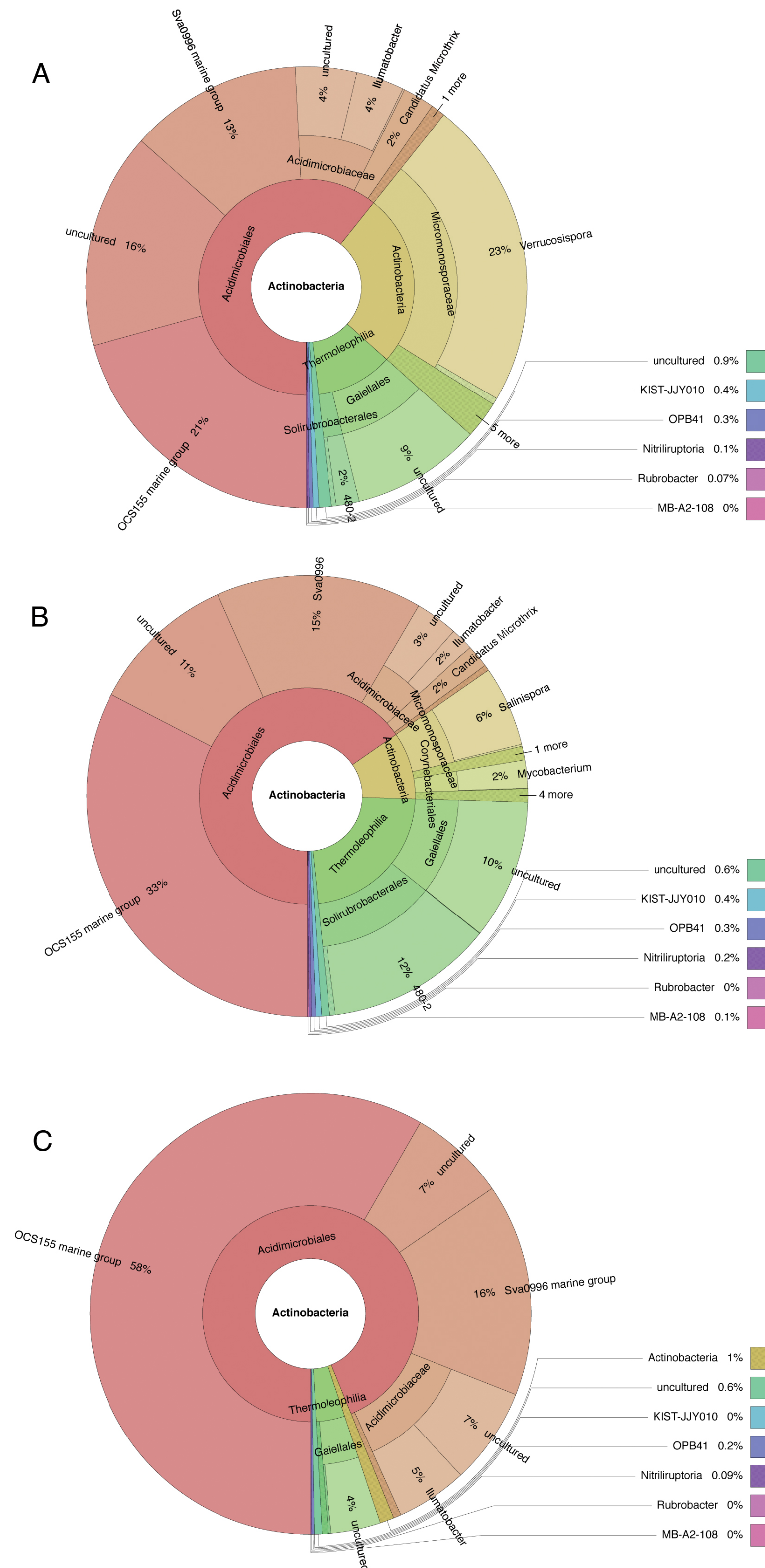

Figure 5.3 Overview of the diversity and relative abundance of Actinobacteria groups within the pooled Ascidian samples from A) Chuuk Island (EtCI), B) Pohnpei (EtPI), and C) root surface samples from Pohnpei (rootPI) visualized in a hierarchical structure.

CHAPTER V

GENERAL DISCUSSION

I. Diffusion Growth Chamber Applicability and Environmental Coverage in *R. Globostellata*

In Chapter I, I presented a method for the *in situ* inoculation of sponge microbes in DGCs by exposing them to their assumed natural chemical and physiological environment, which yielded 255 bacterial isolates; 15 of them presented previously uncultured bacteria. The general aim of this study was to acclimate sponge-associated bacteria to subsequent *in vitro* culturing conditions, since the vast majority of the environmental (sponge-associated) marine microbes are currently not cultivable with present cultivation methods (Bollmann et al. 2007; Kaeberlein et al. 2002; Schippers et al. 2012). The microbes associated to sponges are often symbiotic, possessing host-specific functions in nutrient cycling, as well as in biosynthetic processes (Fan et al. 2012). Thus, it is reasonable to assume that sponge-specific microbes are adapted to host-specific chemicals and a physiological environment in order to maintain their presence within the respective hosts. Different discoveries such as functionally equivalent metabolic roles of genetically divergent microbes, or the enriched presence of eukaryotic-like ankyrin-repeat proteins in sponge-microbes that modulate the phagocytosis in the sponge host support the theory of profound evolutionary mechanisms in sponge-microbe symbiosis (Fan et al. 2012; Hentschel et al. 2012; Nguyen et al. 2014; Ribes et al. 2012; Siegl et al. 2011). Application of the DGC method could circumvent the lack of biochemical networks in cultivation under laboratory conditions by enabling the inoculation and adaptation to the growth media in a natural environment. Overall, the analysis of the first application of this method with the Guam reef sponge *R. globostellata* proofed to be successful to some extent, through the finding of 16S rRNA sequences from so far uncultured bacteria and the phylogenetic reconstruction of one sponge- and one sponge-coral-specific sequence cluster with sequences of Bacteroidetes and Gammaproteobacteria.

In Chapter I, it was only possible to compare the DGC derived sequences with the microbial yields of another cultivation approach of *R. globostellata* associated bacteria (Lafi et al. 2005). Therefore, the overall efficiency and coverage of the DGC approach regarding the natural microbiota of the host could only be estimated. To circumvent this present lack of knowledge and to generate comparative analyses of the actual coverage and efficiency of the DGC technique in *R. globostellata*, I integrated the DGC sequence information from Chapter I and the MPS pyrosequencing data from Chapter II into one concatenated dataset. This combined the taxonomic information of environmental MPS amplified

and DGC isolated sequences from Guam *R. globostellata* reef sponges. Thereafter, I applied the method in Chapter III, for collapsing individually sequences or OTUs to higher taxonomic ranks. Thus achieving comparability at community level and estimating taxonomical coverage of the DGC method with regards to a *R. globostellata* microbial metagenome from the same sponge species and the same geographic location. Since MPS techniques made it possible to gain information about the diversity and structure of the rare biosphere (Reveillaud et al. 2014; Sogin et al. 2006; Webster et al. 2010), comparing this information with cultivation techniques could yield further insights into the microbial specificity and coverage of sponge-microbe cultivation approaches.

After merging the two datasets, a total of 21 phyla were found to be associated with the *R. globostellata* sponges of the Guam reef (Table 6.1). The number of classified phylotypes increased with decreasing rank from n = 43 on class to n = 93 on genus level. However, while the number of DGC / direct isolated phylotypes increased tenfold from phylum (n = 4) to genus (n = 41) level, the number of 454 derived phylotypes approximately tripled from n = 21 at phylum to n = 57 at genus level (Table 6.1). Moreover, while the numbers of classified phylotypes isolated during the DGC / direct study increased with each rank, the numbers of 454 obtained phylotypes, either as calculated for the full *R. globostellata* 454 data or exclusive (i.e., minus the shared ones with DGC / direct), declined or stopped increasing with decreasing taxonomic rank. Although MPS is known to achieve highly resolved community maps and also realized broad analyses of the so far putatively uncultivable rare biosphere based on 16S rRNA sequence data, the present results appear to slightly contradict this general point of view. Most notably is the low overlap of DGC / direct phylotypes when compared with 454 sequence data at lower taxonomic ranks (Figure 6.1 & Table 6.1).

Table 6.1 DGC cultivation vs. 454 environmental *R. globostellata* sequence data

Rank	Total Phylotypes	454 Metagenome	454 Metagenome exclusive	Direct & DGC	Direct & DGC exclusive	Isolates & 454 Shared	unclassified Metagenome [%]	unclassified Isolates [%]
Phylum	21	21	17	4	0	4	2.9	na
Class	43	41	36	7	2	5	5.4	na
Order	70	61	51	19	9	10	33.7	na
Family	79	60	52	27	19	8	52.7	na
Genus	93	57	52	41	36	5	88.3	3.9

Direct & DGC Isolates n = 255
454 Metagenome OTUs n = 793
454 Amplicons n = 93192

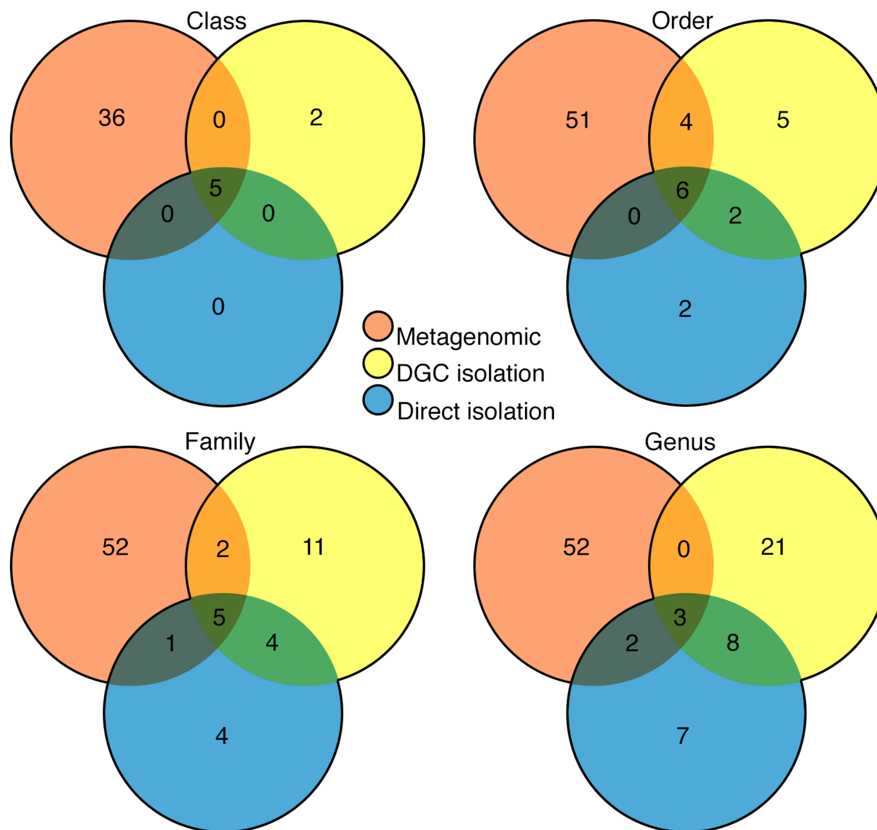

Figure 6.1 Venn diagrams displaying the shared and exclusive phylotype distributions and different high taxonomic ranks from class to phylum level. The sequence data was classified with a 60% similarity cut off in mothur v.1.35.0 (Schloss et al. 2009) based on the SILVA 119 reference database, and subsequently compiled with R v.3.2.0 (R Development Core Team, 2013), as presented in Chapter III.

The DGC / direct related phylotypes possess no exclusive members on phylum level and only two exclusive phylotypes at class rank (Bacteroidia and Rubrobacteria). On family and especially genus level the number of exclusive DGC / direct associated phylotypes was surprisingly high (DGC / direct n = 36 vs. 454 metagenome n = 52), with only 5 shared phylotypes between both datasets. The most likely explanation is that many of the 97% pyrosequencing OTUs were taxonomically not resolvable at these lower taxonomic ranks due to limited phylogenetic information available in short length sequences. In Chapter III, a massive increase of unclassified OTUs was visible with descending ranks (Chapter III, Table 4.2), which is also evident in the present analysis (Table 6.1). Consequently, the number of classified 454 sequencing related phylotypes could in fact steadily increase with decreasing taxonomic rank, if not constrained by the evolutionary uncertainty of short sequence reads in general, and at certain 16S rRNA gene regions even with strain specific differences in conserved and variable regions on the RNA molecule (Fox et al. 1992; Stackebrandt and Goebel 1994). Another potential cause for the observed low overlap could be primer biases, by which the primers are universally

specific at certain ranks and for particular microbes (Klindworth et al. 2013; Mizrahi-Man et al. 2013). Additionally, one should keep in mind that this comparison is based on different *R. globostellata* individuals and not on the exact same specimen and sampling site. Moreover, the collection dates in Guam were approximately two years apart, with DGC / direct tests in 2008 and the sampling for the metagenomic (i.e., 16S rRNA gen tag pyrosequencing) study performed in 2010. However, most sponge-associated microbial communities are stable along geographical and temporal gradients, with some fluctuations in rare symbiont taxa among individual hosts (e.g., Erwin et al. 2012; Hardoim et al. 2012). While the microbial communities associated with *R. globostellata, Callyspongia* sp. *and A. infundibuliformis* individually showed that on OTU level spatial differences can affect the observable community composition (Chapter II & Chapter III), in this present comparison environmentally caused differences in local community composition are not visible at this state of processed 454 amplicon data (Figure 6.2). Based on these considerations, I assume that the small variances in community composition over time and among individuals should have only minor effects on the actual (i.e., the theoretically available complete environmental sponge-associated microbiota) and observed community compositions.

Finally, it is possible that the DGC / direct cultivation approach is actually yielding quiet different results compared to metagenomic sequencing. The phylotype pattern at genus level (based on absolute abundances in a community heatmap) shows that the DGC / directly derived sequences mostly do not overlap with the abundant *R. globostellata* 16S rRNA metagenome (Figure 6.2). The few shared phylotypes (n = 7) between the metagenomic and the DGC / direct study mostly occurred among the rare members of the metagenomic sequence data (Figure 6.2). Exceptions are an unclassified Rhodobacteraceae (Alphaproteobacteria) phylotype and the genus *Endozoicomonas* (Gammaproteobacteria) (Figure 6.2). Besides the uncertainty introduced by the large number of unclassified OTUs at lower taxonomic ranks, these patterns of overlap could indicate that the DGC / direct approach is somehow more sensitive for the rare sponge microbiota, or that the *in situ* approach enables the acclimation and growth of none sponge-associated microbes within the implanted DGCs. However, a closer look at certain DGC / direct exclusive phylotypes showed that many, which were absent in the metagenomic data (Figure 6.2), are often common in sponges.

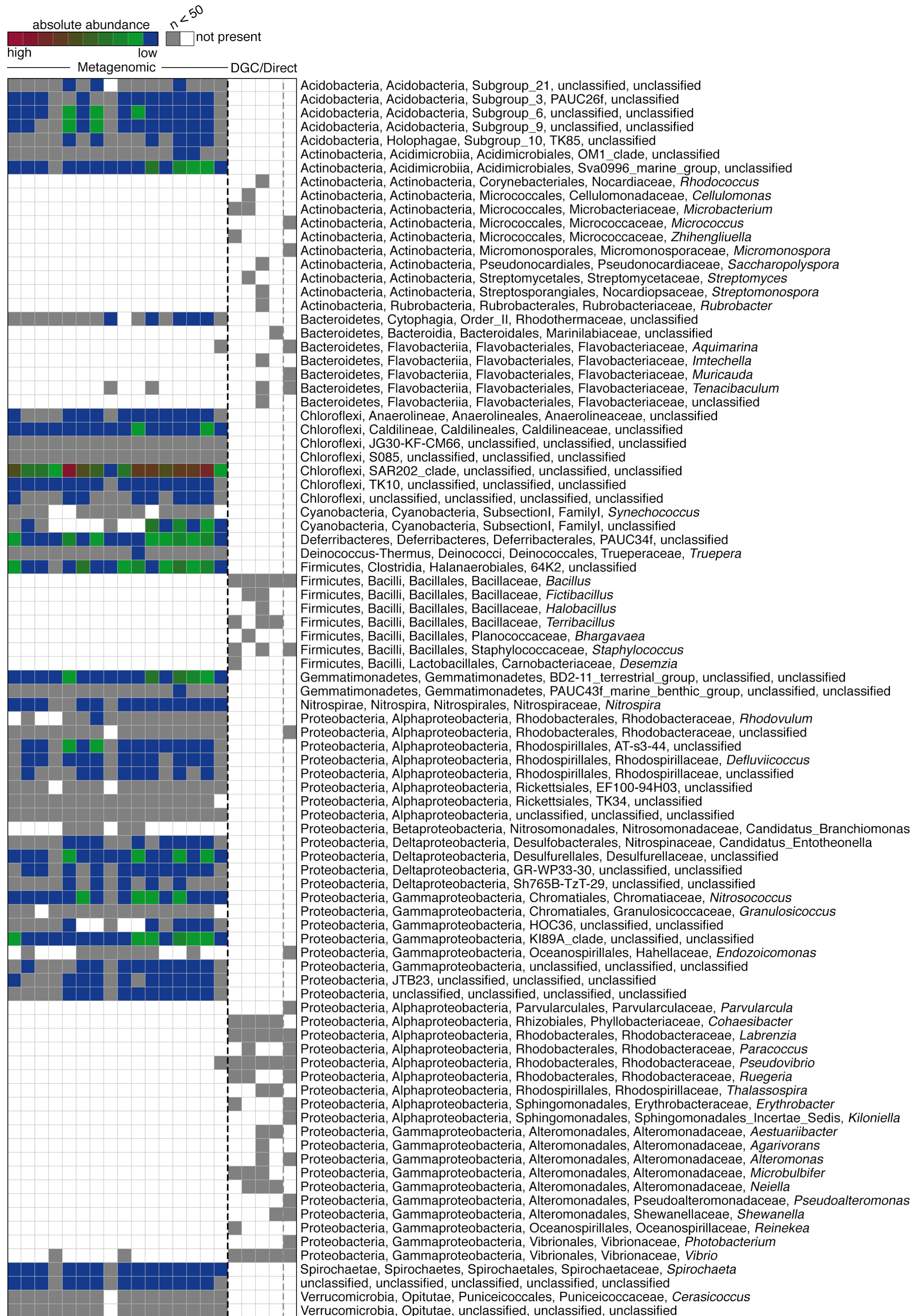

Figure 6.2 Taxonomical heatmap illustrating the distribution and absolute abundance (from 1 to ~ 2400 sequences) at genus level based on *R. globostellata* 'Metagenomic' pyrosequencing data and the DGC / direct cultivation yields. While all DGC / directly derived taxa are displayed, the metagenomic data displays only phylotypes with n $\geq$ 50 absolute abundance or if shared with DGC / direct phylotypes for the sake of visibility.

For instance, the genera *Pseudovibrio*, *Streptomyces* or *Pseudoalteromonas* are often found in sponge microbes in cultivation or isolation studies and are known for their antibacterial activities and secondary metabolite syntheses (Dobson et al. 2015; Hentschel et al. 2001; Kennedy et al. 2009; Khan et al. 2011; Lavy et al. 2014; Muscholl-Silberhorn et al. 2008). Therefore, while it is possible that the DGC method promotes microbial growth of less sponge specific microbes, many sponge related bacteria were present in the DGC / direct results.

In summary, most of the abundant genera in the metagenomic *R. globostellata* analysis were not covered by the DGC / direct isolation method and vice versa. While this should be interpreted with caution due to the large number of unclassified pyrosequencing OTUs (Table 6.1), the already emerging DGC / direct exclusive phylotypes at higher taxonomic ranks (e.g., class, order or family), indicate that *in situ* cultivation could yield sponge-associated microbes, which are not detected by metagenomic 16S rRNA short read sequencing methods. For instance, this integrated comparison yielded two exclusive classes derived from the DGC (Bacteroidia and Rubrobacteria), which are rarely mentioned in the sponge-microbe literature, indicating that these phylotypes are either uncommon in sponges or less discussed at this particular taxonomic rank. In addition, Chapter I already showed that the DGC / direct approach is able to yield uncultivated bacteria and with some sequences even generating sponge-specific clusters. The present analysis of DGC / direct and metagenomic 454 pyrosequencing data showed that specialized cultivation and isolation approaches were valuable complementary methods to gain greater knowledge about the actual environmental sponge microbiota and that they have potential to cultivate putative rare sponge-associated microorganisms.

II. Changes in Sponge-Specific Community Patterns along Local Depth Gradients

In Chapter II and Chapter III, I presented two studies focusing on the microbial taxonomic diversity and their beta diversity, which is here defined as the turnover of community composition from one sponge sample to another. In total, the microbial communities of 23 tropical and 24 temperate sponge specimen, were sequenced using either 454 pyrosequencing or Illumina MiSeq. Both studies presented apparent patterns of host-specificity among all sponge samples and the additional metagenomic seawater profiles (Chapter II Figure 3.3A & Chapter III Figure 4.2A), with significant effects of host types (Chapter II Table 3.3 & Chapter III Table 4.2). These sponge-specific (in comparison to seawater samples) and host or species-specific (compared among different sponge

taxa) patterns were observed in several recent multivariate community analyses (e.g., Erwin et al. 2012; Fiore et al. 2013; Reveillaud et al. 2014; Schmitt et al. 2012b; Schöttner et al. 2013). In addition, they are mostly stable over time and space (e.g., Erwin et al. 2012; Hardoim and Costa 2014; Lee et al. 2011; Pita et al. 2013b). The current hypothesis explaining the establishment and maintenance of these observed stable symbiotic relationships is that sponge-specific symbionts are passed from one generation to another via vertical transmission with assemblages of symbiotic microbes within the sponge larvae (Lee et al. 2009a; Schmitt et al. 2008; Sharp et al. 2007; Webster et al. 2010). In addition, horizontal acquisition (the uptake of microbes from the environment) presumably complements this process. This is indicated by the prominent lack of co-speciation between sponge hosts and microbial symbionts, and the detection of sponge-specific bacteria in seawater (Hentschel et al. 2012; Taylor et al. 2013; Thacker and Freeman 2012).

Besides the observed stability of sponge-specific microbial communities along horizontal spatial and temporal gradients, little is known about the effect of local depth gradients on the sponge microbe patterns. In a review about microbial ecology of sponges, corals and algae in mesophotic coral reefs, Olson and Kellogg (2010) emphasized the need to study the host-associated microbial relationships in this particular ecosystem. In particular, they suggest that investigations on microbial diversity and their functional roles, performed on various shallow sponge species and populations, should be extended to the mesophotic realm. In this habitat, mesophotic host-associated microbial communities could possibly act as a refuge for their shallow host-counterparts (Lesser et al. 2009). Thus, further knowledge about this is certainly needed to estimate its potential effects on the resilience capabilities of coral reefs and other coastal habitats that are presumably coupled along local depth gradients (Slattery et al. 2011). While the study by Olson & Gao (2013) on community differences in tropical mesophotic coral reefs already showed some variability of the microbial community along a depth gradient, this study investigated this effect for first time in a temperate mesophotic habitat.

In Chapter II and Chapter III, the two datasets contained three different sponge species with a sufficient number of replicates ($n \geq 3$) at different depths (*R. globostellata*, *Callyspongia* sp., *A. infundibuliformis*) to investigate the effect of depth on the distribution of microbial communities.

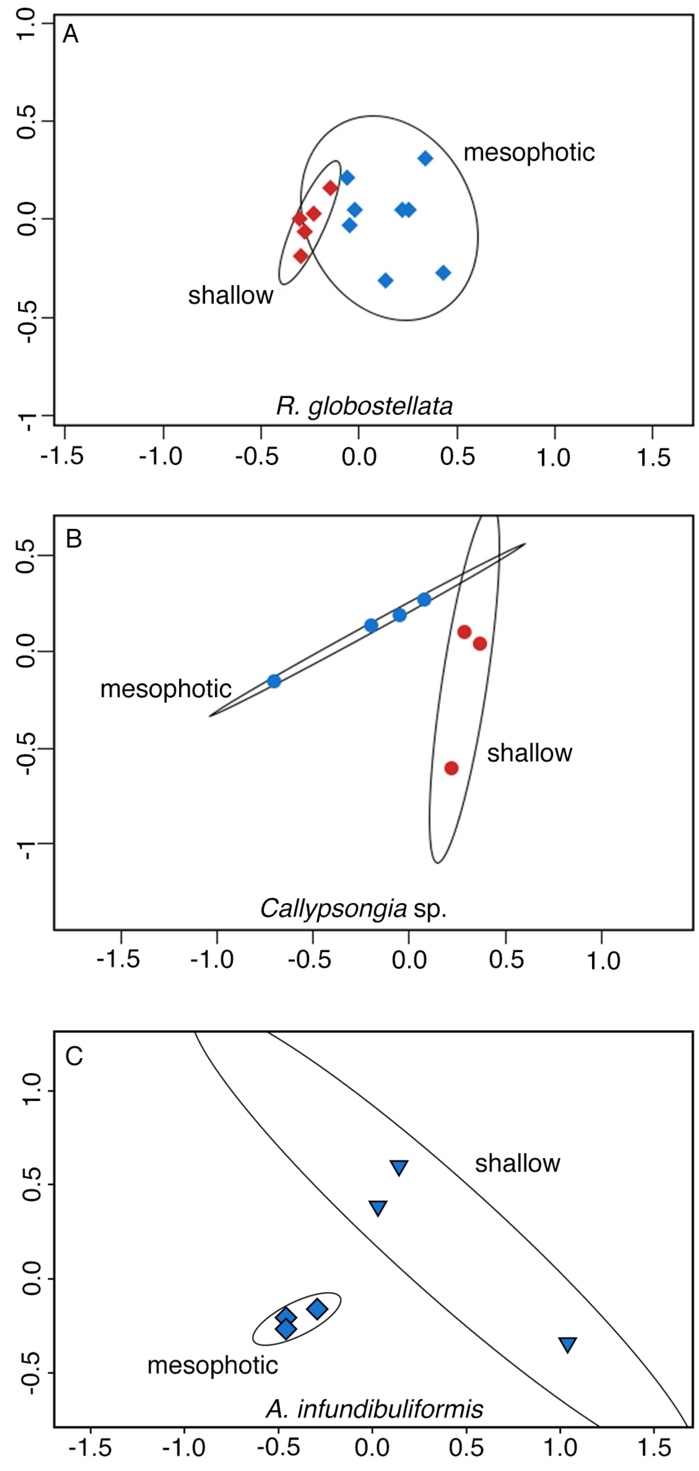

Figure 6.3 Two-dimensional nMDS ordinations based on a Bray-Curtis dissimilarity matrix. The figures were extracted from the Chapters II (Figure 3.3) & III (Suppl. Figure 4.1) - A & B) *R. globostellata* (blue and red diamonds) and *Callyspongia* sp. (blue and red dots) specimen collected in Guam, and C) *A. infundibuliformis* specimen from Sweden (diamonds and triangles).

Each of the sponges exhibited observable depth-dependent ordinations (Figure 3ABC) and significant groupings between shallow and mesophotic sites based on permutational multivariate analysis of variance: *Axinella infundibuliformis* ($p = 0.0014$), *Rhabdastrella globostellata* ($p = 0.003$), and *Callyspongia* sp. ($p = 0.027$). Both tropical sponges exhibited differences in dominant microbial species when compared with the similarity percentages (*simper*) function based on Bray-Curtis dissimilarities (Chapter II, Table 3.4). Cyanobacteria (genus *Synechococcus*) were dominant in *Callyspongia* sp. and unequally distributed among mesophotic and shallow sites. Thus, in the following, I will assess this apparent importance of Cyanobacteria for the depth-depending microbial community pattern (Figure 6.3B). Therefore, I removed all cyanobacterial OTUs from the *Callyspongia* data and repeated the multivariate analyses as described in Chapter II. As a result, the apparent significant differences disappear ($p > 0.05$) and both species-specific communities are overlapping in a two dimensional nMDS space (Figure 6.4). This indicates that sponge-specific microbial lineages, in this particular case Cyanobacteria, have a significant effect on the overall community structure along depth gradients in certain tropical sponges. It is known that Cyanobacteria form ecotypes, which occupy different niches in the water column (Lindell 2014; Moore et al. 1998; Rocap et al. 2003). If one considers that the mesophotic sponges had been collected in 'Blue Hole' in Guam (depth > 90m) and the shallow counterparts outside this location at Western Shoals, Guam, it could be that the observed differences in community similarity are caused by horizontal spatial differences.

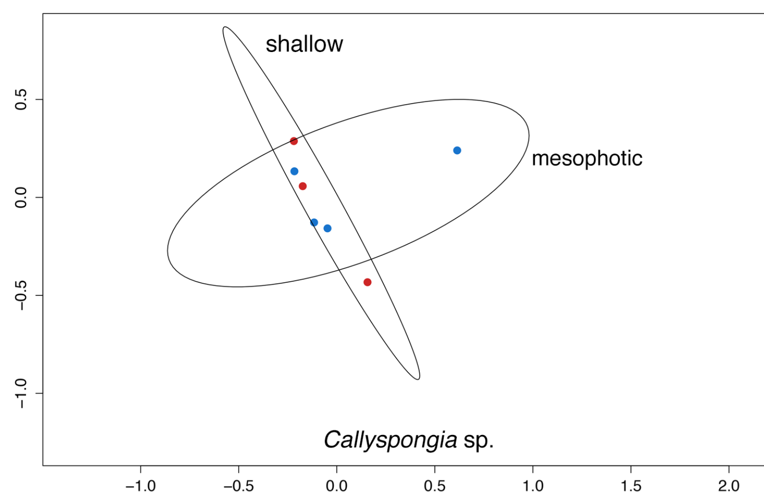

Figure 6.4 Two-dimensional nMDS ordinations based on a Bray-Curtis dissimilarity matrix of the *Callyspongia* sp. with all cyanobacterial OTUs removed. Red and blue dots indicate shallow or mesophotic *Callyspongia* specimen, respectively.

In contrast, the temperate *A. infundibuliformis* specimens were all collected at the same site and at the same day in Tjärnö (Sweden), minimizing the residual variation that may result from spatial and temporal differences in the community distribution. The indicator species analysis in Figure 6.3 of Chapter III showed apparent differences in community composition with dominant *Nitrosopumilus* and *Nitrospira* microbes present in the mesophotic specimen and a more divers community composition within the shallow specimen. Therefore, the depth-depending local species-specific community patterns could reflect metabolic differences in sponge nitrogen cycles, since *Nitrosopumilus* is ammonia-oxidizing and *Nitrospira* is nitrite-oxidizing (Hoffmann et al. 2009; Radax et al. 2012a). In addition, an analysis by Reveillaud et al. (2014) showed that *Nitrospira* 'oligotypes' (see Eren et al. 2013) can be differentially distributed within very closely related sponges. To test if either *Nitrospira* or *Nitrosopumilus* exhibit individual depth-specific patterns, I created two datasets that contained only OTUs from the respective genera, and subsequently performed the multivariate community analysis using *adonis* in R (as descripted in Chapter II and Chapter III). Both groups (shallow vs. mesophotic) showed no significant differences ($p > 0.05$). This indicates that the microbes involved in the nitrogen cycling are evenly distributed along this depth gradient and it further indicates that nitrogen cycling as a metabolic process is presumably not responsible for the *A. infundibuliformis* differences. Thus far, little is known about possible divergent functional roles in sponge metabolic processes along spatial depth gradients.

R. globostellata is a putative HMA sponge based on the presence and abundance of Acidobacteria, Chloroflexi and Poribacteria, which are known to be dominant in HMA members (Chapter II - Supplementary Figure 3.2 & see Gloeckner et al. 2014; Schmitt et al. 2012b; Schmitt et al. 2011). In contrast, *Callyspongia* and *Axinella* genera were identified as LMA sponges (Gloeckner et al. 2014). Since these present three sponge groups belong to two different climate zones and are probably members of both microbial abundance classes, neither specific climatic conditions, nor physiological differences (e.g., pumping behavior or mesohyl structure) can initially explain the observed depth-depending differences. However, there is evidence that many sponges possess extensive cryptic speciation (Borchiellini et al. 2000; Knowlton 2000). Hence, I cannot exclude that these patterns were observed due to phylogenetic differences of the sponge host.

In addition, abiotic factors are known to influence sponge health, which presumably influences the associated microbiota. For instance, Lesser et al. (2009) found that with increasing depth nutrient

concentrations increased and sponge growth was higher. This has also been documented for *Callyspongia vaginalis* (Trussell et al. 2006). Yet, it is still unclear, which process - either top-down (predation pressure) or bottom-up (food limitation) - has the biggest influence on the distribution and biodiversity of coral reef sponges (Pawlik et al. 2015; Slattery and Lesser 2015). Moreover, the contributions of the sponge-associated microbiota to these processes is still virtually unknown (Goeij et al. 2013). Investigations on the effect of temperature showed that sponge symbionts can survive short exposure to high temperatures (Simister et al. 2012c), while constant high water temperatures have a negative effect on the sponge and its microbial community (Fan et al. 2013; Simister et al. 2012b). Conversely, a transplantation experiment of sponges from light to dark sites (Cárdenas et al. 2014) as well as a study on seasonal shifts of irradiance and temperature (Erwin et al. 2012) both emphasized the general stability of sponge-associated microorganisms. Other abiotic factors which potentially influence the sponge-associated microbial community along a depth gradient are nutrient distribution in the water column and pollution (Olson and Kellogg 2010). However, environmental variables were not recorded during sampling, therefore the interpretation of environmental effects remains elusive at present and need to be included in future studies.

III. Global Patterns of Beta Diversity in Marine Host-Associated Microbial Communities

In Chapter III, I showed that local sponge communities possess consistent sponge-specific microbial assemblages across high taxonomic ranks. Philippot et al. (2010) discussed the phylogenetic coherence of ecological traits, which might be responsible for the display of ecological similarities over large phylogenetic distances of certain microbial lineages. Nemergut et al. (2013) further hypothesized that phylogenetically clustered microbial communities are affected by strong selection processes acting over broad phylogenetic scales. The renown 'everything is everywhere, but the environment selects' hypothesis by Baas-Becking and Beijerink is often used to explain distributional patterns of microbial populations (de Wit and Bouvier 2006). Microbial organisms are assumed to be ubiquitously distributed due to their high dispersal ability, small body size and short generation times (Martiny et al. 2006). However, Zinger et al. (2011) recently discussed contradicting patterns of simultaneous global and local occurrences (i.e., cosmopolitanism vs. provincialism) of certain microbial species. This could be either ascribed to the spatial scales and taxonomic resolutions of the particular studies, or to the

investigated ecosystems in which these microbial patterns have been observed. Assuming that ecological meaningful patterns exist at high microbial taxonomic ranks in host-associated microbial communities across large geographic and host phylogenetic distances, further investigations should address the above mentioned factors of taxonomic resolutions as possible causes for the simultaneous global and local patterns.

In addition to the investigation of the sponge-microbe diversity, I also presented a study about the microbial diversity of a marine ascidian species in Chapter IV. The collected *Eudistoma toealensis* individuals possessed biogeographic patterns while being simultaneously different to the environmental biofilm microbiome (Chapter IV Supplementary Figure 5.2). While sponges and ascidians are phylogenetically divergent marine organisms, both possess a sessile, filter-feeding lifestyle. Moreover, secondary metabolites isolated from ascidians are in some cases identical or very similar to compounds derived from microbes, a pattern which has been observed in sponge natural products research (Schmidt and Donia 2010). To further emphasize these similarities, ascidians possess high degrees of microbial diversity, and indications of microbial mediated ascidian nitrogen cycling processes were found in various recent ascidian-microbe studies (Behrendt et al. 2012; Erwin et al. 2014; Erwin et al. 2013; Martínez-García et al. 2008; Tianero et al. 2014). Considering these congruent observations, ascidians are a potentially appropriate choice to test if the hypothesis of ecological coherence of high taxonomic ranks can be transferred to other marine invertebrates.

Here, I present an initial brief synthesis of microbial community data from Chapter II to IV, collected data from the NCBI Sequence Read Archive and additional EMP data (Table 6.3). I compared microbial beta diversity patterns associated with sponges (n = 97 individuals), ascidians (n = 53 individuals), corals (n = 9), as well as supplementary environmental samples, such as filtered seawater (n = 21) and biofilm swabs (n = 7). The full dataset (Table 6.3) comprises 187 samples from 9 globally distributed locations (Guam, Gulf of Mexico, Mediterranean Sea, Micronesia, NE Australia, Red Sea, Sweden, Zanzibar). The additional sponge, ascidian, coral, and environmental samples were added to avoid or reduce the potential effects of nestedness. Nestedness could become a problem during the analyses of beta diversity, because samples comprised different geographic sampling locations (see Chapters II & III) and organisms (i.e., multiple sponge species versus a single ascidian species - Chapter IV).

Table 6.2 Permutational statistical analysis of the bacterial community structure (*adonis*, i.e., variance) and homogeneity of dispersion (*permutest.betadisper*, i.e., dispersion) among three different groups of marine benthic invertebrates (i.e., ascidians, sponges, and corals) from globally distributed sampling sites (Table 6.3 for more details) are shown.

All samples (n=187)	Analysis	Phylum (n=50)	Class (n=108)	Order (n=210)	Family (n=328)	Genus (n=714)
	nMDS stress	0.051	0.060	0.078	0.083	0.086
Origin	dispersion (*F* ratio)[1]	**20.492 ***	**21.655 ***	**21.145 ***	**17.183 ***	**16.69 ***
	variance (R^2)	**0.156 *****	**0.164 *****	**0.143 *****	**0.145 *****	**0.131 *****
Location	dispersion (*F* ratio)[1]	14.280	10.128	10.031	7.759	5.544
	variance (R^2)	**0.182 *****	**0.165 *****	**0.153 *****	**0.15 *****	**0.153 *****
Sponge samples (n=97)	**Analysis**	**Phylum (n=31)**	**Class (n=83)**	**Order (n=128)**	**Family (n=213)**	**Genus (n=391)**
	nMDS stress	0.035	0.045	0.053	0.064	0.062
Host	dispersion (*F* ratio)	2.268	2.271	2.785	2.593	2.711
	variance (R^2)	**0.579 *****	**0.583 *****	**0.583 *****	**0.58 *****	**0.553 *****
Location	dispersion (*F* ratio)	**7.204 ***	3.858	**5.672 ***	**5.736 ***	**5.212 ***
	variance (R^2)	0.060	**0.102 *****	**0.101 *****	**0.101 *****	**0.105 *****
Ascidian samples (n=53)	**Analysis**	**Phylum (n=44)**	**Class (n=99)**	**Order (n=186)**	**Family (n=257)**	**Genus (n=431)**
	nMDS stress	0.040	0.051	0.057	0.055	0.068
Host	dispersion (*F* ratio)	1.178	1.511	2.415	2.248	2.152
	variance (R^2)	**0.413 *****	**0.386 ****	**0.383 *****	**0.378 *****	**0.367 *****
Location	dispersion (*F* ratio)	**15.505 ****	**9.46 ****	**12.962 ****	**9.393 ****	**8.311 ***
	variance (R^2)	**0.096 ****	**0.083 ****	**0.067 *****	**0.065 ****	**0.068 *****

'Permutational Multivariate Analysis of Variance Using Distance Matrices' (variance) hypothesis tests of 'Origin' (Ascidian, Biofilm, Coral, Seawater, Sponge) or 'Host' - either 'Sponge' (n=18 sponge genera) or 'Ascidian' (n=14 ascidian genera) - see Table 6.3 - are shown, along with pairwise comparisons among locations with 'Multivariate homogeneity of groups dispersions' (dispersion). Locations are as follows: in 'Origin' (Guam, Gulf of Mexico, Micronesia, NE Australia, Red Sea, Spain, Sweden, Zanzibar), in 'Sponge' (Guam, Gulf of Mexico, Red Sea, Sweden, Zanzibar), and in 'Ascidian' (Micronesia, NE Australia, Spain). Significant comparisons following Benjamini-Hochberg correction for multiple hypothesis testing (Benjamini and Hochberg 1995) are indicated in bold, with asterisks denoting significance level ($p < 0.001$ ***, < 0.01 **, < 0.05 *)

I applied the method of collapsing 97% OTU datasets into individual taxonomically rank-specific datasets (see Chapter III for details). For this, I processed the raw pyrosequencing data obtained from the listed studies in Table 6.3 individually with mothur v.1.35.0 (Schloss et al. 2009) and classified the 97% OTUs based on the SILVA 119 reference taxonomy. Single datasets were merged and subsequently collapsed to individual datasets that present high taxonomic ranks from phylum to genus level (Table 6.2). Since beta diversity analyses provide an analytical framework for reproducible comparisons of diversity patterns and processes (Ramette 2007; Tuomisto 2010), I applied multivariate ordination, as well as dispersion and variance as a measure of beta diversity (see Chapter II – IV for details).

Initially, the resulting dataset of 187 samples, was analyzed with hypothetical identity groups based on the environmental 'Origin' (i.e., sponges, ascidians, corals, seawater, and biofilm - Table 6.2 & 6.3) at each high taxonomic rank (Table 6.2 & Figure 6.5AB). Subsequently, the sponge and ascidian samples were analyzed individually to investigate host-genus specific effects on microbial diversity patterns (Table 6.2 & Figure 6.5C-F). In all cases the group data (i.e., 'Origin' or 'Host') was nested within the 'Location' to constrain the permutations so that the randomizations only occurred within each location and not across all locations during the analysis of variance. This was done, since the experimental design of group identities (Table 6.2 'Origin' or 'Host') still might have been nested, due to missing host-replicates among the sampling locations in the final datasets.

The observed beta diversity patterns showed that marine microbial communities possess different overlapping patterns in the nMDS ordination plots at different high taxonomic ranks (Figure 6.5). Multivariate analyses of sample dispersions and group variances were overall consistent, varying only slightly in sample dispersion and variance in sponges and ascidians among high taxonomic ranks (Table 6.2). Overlaps in nMDS plots generally indicate community similarities. The 'Origin' groups in Figure 6.5AB exhibiting a shift in overlap in which the ascidian associated microbiota was less similar to the sponge microbial communities on order level when compared to the phylum level. Further notable were the mostly separate coral, seawater and biofilm patterns, which still overlapped with sponges, regardless of phylum or order level (Figure 6.5AB). For sponges-only samples a shift from close clusters on phylum to more distinct groups on order level was visible in Figure 6.5CD. Whereas, the ascidian-only groups showed a consistent community overlap in both plots (Figure 6.5EF).

The statistical analyses presented a mostly consistent distribution of patterns at high taxonomic ranks among the three experimental designs (Table 6.2). Both group designs in the full dataset analyses ('Origin' or 'Location' identities) had a significant effect on the microbial communities. This indicates that besides the 'Origin'-specific patterns (i.e., host organism or environmental identity) also biogeographic effects are present in the full dataset. In addition, the analyses of sponge- or ascidian-associated beta diversity patterns at high taxonomic ranks consistently exhibited significant effects of 'host' identities. However, the significant 'Location' (sponges & ascidians) and 'Origin' (all samples) identities may be affected by a significant multivariate spread. (Table 6.2). Hence, the results for the sponge and ascidian microbiota indicate that they are globally distributed while being host-specific among all high taxonomic ranks.

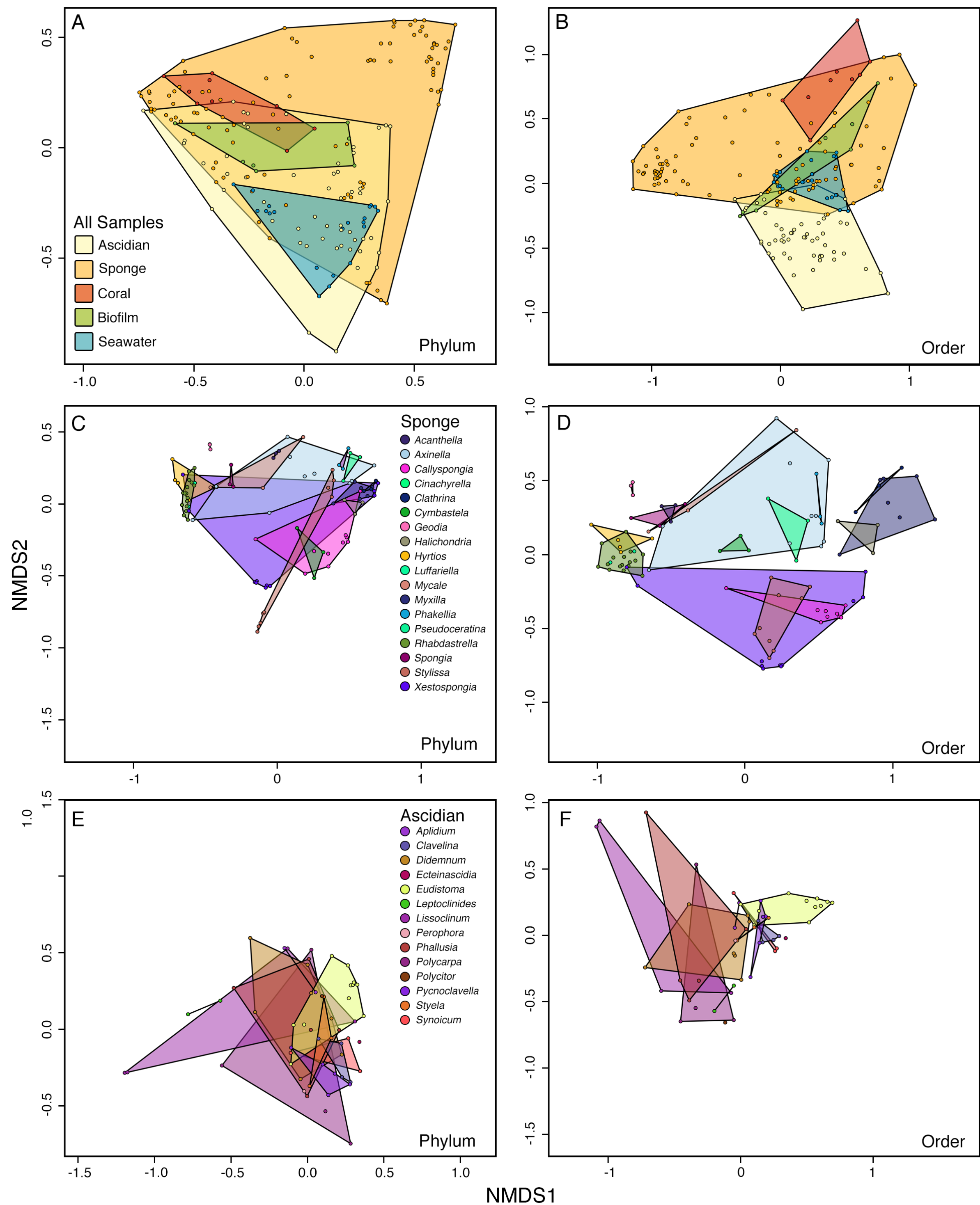

Figure 6.5 nMDS ordination plot (Bray–Curtis distance) of rank profiles of all samples at phylum and order level (A & B), of sponge samples at phylum and order level (C & D), and of ascidian samples at phylum and order level (E & F). Function *ordihull* in vegan (Oksanen et al. 2012) was used to encircle the sites in the 'Origin' or 'Host' hypotheses groups (Table 6.2). A close proximity between samples corresponds to high similarities of the respective communities. Stress values were overall very low and are shown in Table 6.2.

Altogether, the present results emphasize the notion that consistent microbial patterns achieved by the present high resolution MPS studies are visible and presumably ecologically meaningful at higher taxonomic ranks (Gobet et al. 2013). The synthesis of Chapter II, III and IV, in combination with additional integrated data (from NCBI and EMP), gives a first indication that the community assemblages and distributions observed between marine benthopelagic environments (Fuhrman et al. 2008; Gobet et al. 2012; Gobet et al. 2010; Zinger et al. 2014; Zinger et al. 2011), and between phylogenetically divergent sponges (e.g., Cárdenas et al. 2014; Schmitt et al. 2012b; Schöttner et al. 2013), ascidians (e.g., Erwin et al. 2014; Tianero et al. 2014), or corals (e.g., Lee et al. 2012; Schöttner et al. 2012; Schöttner et al. 2009), are based on ecological and evolutionary processes, which are reflected as patterns across broad taxonomic ranks. Regarding the differences of environmental seawater and biofilm samples (Figure 6.5AB) in the present analyses, Zinger et al. (2011) suggested that the consistent beta diversity patterns observed at high taxonomic ranks between seawater and seafloor could result from long-term processes. At least for sponges, these patterns may be ancient establishments of host-specific microbial symbionts caused by the complex modes of vertical transmission and horizontal acquisition. However, these ecological coherent patterns are not only visible in the marine but also in the terrestrial realm. The analysis of human skin and gut microbiota showed the presence of consistent habitat-associated patterns at high taxonomic ranks (Koeppel and Wu 2012). Koeppel & Wu (2012) further noted that the phylogenetic depth of ecological coherence may vary among certain microbial linages.

However, it is important to note that the observed patterns only determine the spatial microbial distribution without any further knowledge about the related functions of these putatively ecological coherent microbial lineages. Therefore, the indicated ecological coherence at these high taxonomic ranks should be investigated and interpreted with respect to environmental and host-specific factors which may have shaped these deep-branching ecological patterns. Indeed, research on the functional metabolic roles of microbial symbionts in sponges has already demonstrated the evolutionary convergence in complex symbiotic microbial communities (e.g., Fan et al. 2012; Liu et al. 2012; Ribes et al. 2012). Since sponge-associated microbial communities may mediate biochemical reactions of the sponge nutrient cycles and often comprise large parts of the sponge biomass (with very diverse and specific microbial assemblages), these sponge-microbe relationships are potentially fundamental to the various functional roles of sponges in the marine ecosystem. In addition, the present results

underline the observation of Zinger et al. (2011) that the ecological signal at high taxonomic ranks, such as genus level, is still meaningful regardless of the increasing number of unclassified and therefore disregarded sequences.

Table 6.3 Integrated samples from this present study and additionally collected data from the NCBI Sequence Read Archive as SRA files. Each single SRA file has been processed with mothur 1.35.0 (Schloss et al. 2009) (from raw reads, to quality control, and OTU clustering) as described in Chapter II & IV. Each individual dataset is classified based on the SILVA 119 reference taxonomy. The individual datasets were then collapsed to high taxonomic ranks, merged and subsequent multivariate analyses were performed in R as described in Chapter III.

Host Type	Environment	Associated Genera	Primer Region	Location	Depth [m]	Temperature [°C]	Data Source
Sponge (n=30)	Seawater (n=5)	*Acanthella, Axinella, Callyspongia, Hyrtios, Luffariella, Pseudoceratina*	V4-V5	Guam	~ 2 - 99	~ 26 - 29	**Chapter II**
Sponge (n=24)	Seawater (n=3)	*Axinella, Geodia, Halichondria, Mycale, Myxilla, Phakellia*	V4-V5	Sweden	~ 2 - 160	~ 7 - 12.5	**Chapter III**
Sponge (n=18)	na	*Callyspongia, Cinachyrella, Clathrina, Cymbastella, Spongia, Xestospongia*	V4-V5	Zanzibar	~ 2	~ 27	unpublished EMP data
Sponge (n=4)	Biofilm (n=4)	*Myxilla*	V6	Gulf of Mexico	~ 550	~ 7	Arellano et al. 2013
Sponge (n=7)	Seawater (n=4)	*Hyrtios, Stylissa*	V6	Red Sea	~ 8 - 19	~ 26 - 27	Lee et al. 2011
Sponge (n=11)	Seawater (n=6)	*Stylissa, Xestospongia*	V4-V5	Red Sea	~ 10	~ 26	Moitinho-Silva et al. 2014
Ascidian (n=8)	Biofilm (n=3)	*Eudistoma*	V4-V5	Micronesia	~ 1	~ 28	**Chapter IV**
Ascidian (n=3)	na	*Styela*	V6-V8	Spain	~ 2	~ 12	Erwin et al. 2013
Ascidian (n=39)	Seawater (n=3)	*Clavelina, Didemnum, Ecteinascidia, Eudistoma, Leptoclinides, Lissoclinum, Perophora, Phallusia, Polycarpa, Polycitor, Pycnoclavella, Synoicum*	V6-V8	Australia	~ 2 - 14	~ 25	Erwin et al. 2014
Coral (n=9)	na	*Astreopora, Pocillopora, Sarcopython, Stylopora*	V3-V4	Red Sea	~ 8 - 19	~ 26	Lee et al. 2012

SUMMARY

Sponges are important aquatic invertebrates that inhabit many different freshwater and marine habitats. They are acknowledged holobionts, host a wide range of dense, diverse and often symbiotic microbial communities. I investigated the diversity and specificity of sponge-associated microbial communities under different methodological and environmental aspects in this thesis.

Since sponge-associated microbes are often metabolically active and physiologically involved in the sponge metabolism, they are difficult to cultivate under laboratory conditions with standard cultivation methods. Therefore, I investigated in Chapter I, if the diffusion growth chamber (DGC) method can yield so far uncultivated and potential novel microbes. The DGC method presumably allows sponge-associated microbes to acclimate to the growth media under *in vivo* conditions when implanted within a sponge (in this case *Rhabdastrella globostellata*). After phylogenetic analyses of 255 16S rRNA gene sequences obtained from the DGC and directly cultivated isolates, I found four phyla (Actinobacteria, Bacteroidetes, Proteobacteria (Alpha- and Gamma-) and Firmicutes) to be affiliated with bacterial sequences. The phylogenetic analyses further identified one monophyletic sponge-specific and one monophyletic sponge-coral-specific cluster. In addition, 15 of the sequences were considered to represent novel cultured bacteria.

In Chapter II of my thesis I addressed the effect of host relatedness as well as a local spatial depth gradient on the microbial community turnover between the tropical sponges *Rhabdastrella globostellata*, *Callyspongia* sp., and *Acanthella* sp. by using 454 pyrosequencing generated data. Microbial communities were highly sponge specific when compared to the seawater microbiota, and sponge species-specific when compared among the three sponge taxa. Moreover, two sponge-specific microbial communities (*R. globostellata* and *Callyspongia* sp.) showed significant community patterns along a vertical depth gradient from shallow reef areas to mesophotic sampling sites. By discriminating species between two groups using Bray-Curtis dissimilarities (simper) analysis, I compared the contribution of individual species within both species-specific microbial communities along the spatial gradient. The results indicated that within both sponge-specific microbiota different microbial members were responsible for the observed significant depth-depending patterns.

In Chapter III of my thesis I assessed the microbial diversity and sponge / host-specificity of 7 sponge taxa and one seawater sample collected from a temperate Fjord in Tjärnö (Sweden). The

sponge-associated community showed significant host-relatedness, with further significant effects of water depth on the microbial community composition. The 97% OTU dataset was subsequently collapsed to individual datasets of community composition data at high taxonomic ranks from phylum to genus level. After repeating the multivariate analysis initially performed on the OTU data, the results showed that the microbial community patterns are consistent across high taxonomic ranks. These results indicated that sponge-associated microbial communities possess discernible ecologically meaningful patterns at high taxonomic ranks, which may reflect functional traits at deep branches of the microbial 16S rRNA phylogenetic tree.

Chapter IV deals with the analysis of the microbial diversity of the marine ascidian *Eudistoma toealensis* collected from two Micronesian islands. Ascidians possess similar lifestyle traits and host-associated microbial communities as sponges, and yield a wide variety of bioactive natural products. Since *E. toealensis* has been identified as the source of different bioactive staurosporine derivatives, I investigated the associated microbial community to identify potential staurosporine-producing microbes in this specific ascidian. The resulted microbiota comprised 43 phyla (bacteria and archaea). Many of the bacteria were identified as common members of the sponge or ascidian microbiota. Additionally, two staurosporine derivative producers, *Salinispora* and *Verrucosispora*, were found exclusively in the ascidian tissue.

In Chapter V, the general discussion, I have combined the individual datasets from each of the four chapters into three syntheses that re-evaluated the general results of this thesis under three aspects:

1.) I compared the *R. globostellata*-associated microbial data from DGC and direct cultures in Chapter I with the *R. globostellata* 16S rRNA gen tag pyrosequencing data from Chapter II. The results showed that the DGC study exhibited only a small community overlap at certain taxonomic ranks when compared pyrosequencing data. This indicated that the DGC *in vivo* cultivation, and the direct isolation, may be seen as complementary methods to cover the broad taxonomic range of the sponge-associated microbiota.

2.) I discussed the results of Chapter II and Chapter III concerning the depth-depending patterns apparent in sponge species-specific microbial communities along local depth gradients. When Cyanobacteria OTUs were removed in *Callyspongia* sp., previously observed significant depth-depending community patterns disappeared. This indicated that only certain dominant microbes are

putatively responsible (i.e., Cyanobacteria in *Callyspongia* sp.), while others (i.e., *Nitrosopumilus* and *Nitrospira* in *Axinella infundibuliformis*) are not responsible for the observed differentiating patterns.

3.) To further investigate if ecologically meaningful patterns reflect functional (ecological) traits in deep-branches of the microbial 16S rRNA tree, I integrated the MPS data from Chapters II - IV and combined this dataset with additional data obtained from the NCBI Sequence Read Archive and the Earth Microbiome Project. The meta-analysis showed that on a global scale marine invertebrate-associated and environmental microbial communities possess consistent patterns across high taxonomic ranks. Additionally, they were still significantly affected by their associated organism or environment (i.e., either sponge, ascidian, coral, or seawater and biofilm) and global distribution. Furthermore, consistent significant patterns of host-relatedness were present at high taxonomic ranks if assessed individually for either sponges or ascidians.

In summary, temperate and tropical sponge-associated microbial communities exhibit a significant sponge species specificity. Furthermore, these microbial community patterns are consistent among high taxonomic ranks, which indicates that long-term host-specific processes may have shaped the relationships between sponges and microbial symbionts. In addition, sponge-associated microbes possess significant variations in community structure within their particular hosts along local depth gradients, and are thus potentially influenced by environmental factors. On the contrary, marine invertebrate-associated microbial communities exhibit consistent significant community patterns on a global scale, which further emphasizes the idea of the ecological coherence of high microbial taxonomic ranks.

ZUSAMMENFASSUNG

Schwämme sind wichtige aquatische Invertebraten, die in verschiedenen limnischen und marinen Habitaten vorkommen. Weiterhin gelten sie als Holobionten, welche eine diverse und oft symbiotische mikrobielle Gemeinschaft aufweisen. Das zentrale Ziel meiner Dissertation war die Untersuchung der Schwamm-assoziierten mikrobiellen Diversität und Spezifizität mit Hinblick auf methodische und umweltbedingte Unterschiede.

Häufig sind die Schwamm-assoziierten Mikroben in den Stoffwechsel der Schwämme eingebunden, was die Kultivierung von Schwamm-assoziierten Bakterien unter normalen

Laborbedingungen erschwert. In Kapitel I meiner Dissertation habe ich daher die Ergebnisse eines Experimentes mit Wachstumskammern ausgewertet. Wachstumskammern, welche mit einem Kulturmedium gefüllt sind, wurden in lebende Schwämme (in dem Fall *Rhabdastrella globostellata*) implantiert, damit sich die Schwamm-assoziierten Mikroben in einer natürlichen (chemischen) Umgebung an die spätere Kultivierung unter Laborbedingungen gewöhnen können. Die phylogenetische Analyse der 255 16Sr RNA Sequenzen ergab, dass Bakterien von vier unterschiedlichen Phyla kultiviert werden konnten: Actinobacteria, Bacteroidetes, Proteobacteria (Alpha- und Gamma-) und Firmicutes. Die phylogenetische Analyse ergab weiterhin eine monophyletische Schwamm-spezifische Klade und eine Schwamm-Korallen-spezifische Klade innerhalb des 16S rRNA Stammbaumes, welcher mit allen vorhandenen Sequenzen berechnet wurde. Letztendlich wurden 15 Bakterien gefunden, deren Sequenzen nach Abgleich mit der NCBI Datenbank als bisher 'unkultiviert' eingestuft werden konnten.

In Kapitel II meiner Dissertation habe ich bei drei tropischen Schwämmen (*Rhabdastrella globostellata*, *Callyspongia* sp., *Acanthella* sp.) untersucht, ob ein lokaler Tiefengradient in einem mesophotischen Riffsystem einen Einfluss auf die mikrobielle Gemeinschaftsstruktur hat. Weiterhin habe ich untersucht, wie spezifisch die mikrobielle Gemeinschaft im jeweiligen Wirt ist. Es zeigte sich, dass die Schwamm-assoziierte mikrobielle Gemeinschaft strukturell von der des umgebenden Seewassers abweicht. Auch zwischen den unterschiedlichen Schwammarten waren die Unterschiede signifikant. Zusätzlich fand ich, entlang eines Tiefengradienten vom flachen Riffbereich zum mesophotischen Riffsystem, Unterschiede in den mikrobiellen Gemeinschaftsstrukturen zweier Schwämme. Eine Analyse, welche die mikrobiellen Arten für die jeweilige Tiefe und Schwammart identifiziert, zeigte, dass die mikrobiellen Gemeinschaftsstrukturen wahrscheinlich von unterschiedlich dominanten Mikroben innerhalb der beiden Schwammarten geprägt werden.

In Kapitel III meiner Dissertation habe ich die Diversität und Spezifität von Schwamm-assoziierten mikrobiellen Gemeinschaften aus einem Fjord in der Nähe von Tjärnö (Schweden) untersucht. Die Schwamm-assoziierte Gemeinschaft zeigte signifikante Strukturen, welche zum einen auf das jeweilige Taxon des Wirts zurückzuführen waren, und zum anderen signifikant für die unterschiedlichen Tiefen waren. Zusätzlich habe ich die ursprüngliche 97% OTU Tabelle auf individuelle Tabellen aufgeteilt, welche sich in ihrem Informationsgehalt bezüglich des jeweiligen taxonomischen Rangs (von Phylum zu Gattung) der ursprünglichen OTUs hin unterscheiden. Die

multivariaten Analysen wurden mit diesen Tabellen wiederholt und die Ergebnisse zeigten, dass mikrobielle Gemeinschaftsstrukturen, welche auf OTU Ebene sichtbar waren, übereinstimmend auch auf den höheren Rängen wiederzufinden waren. Dieses Ergebnis lässt vermuten, dass die Schwamm-assoziierten mikrobiellen Gemeinschaftsstrukturen auf ökologischen oder funktionellen Eigenschaften und Merkmalen basieren, welche tief verwurzelt sind im mikrobiellen phylogentischen Stammbaum.

Im Kapitel IV meiner Dissertation habe ich die mikrobielle Diversität der Seescheide *Eudistoma toealensis* untersucht. Seescheiden und Schwämme besitzen zum Teil sehr ähnliche Merkmale: sie sind benthisch, sessil und besitzen sehr diverse mikrobielle Gemeinschaften. *E. toealensis* ist eine bekannte Quelle für Staurosporine, wobei es jedoch nicht geklärt ist, ob diese von assoziierten Mikroben oder dem Wirt selbst produziert werden. Daher war ein Ziel dieser Analyse, Staurosporin-produzierende Mikroben innerhalb der Seescheide zu identifizieren. Die resultierende mikrobielle Gemeinschaft war mit 43 Phyla sehr hoch. Weiterhin wurden zwei mikrobielle Gattungen (*Salinospora* und *Verrucosispora*) gefunden, welche als Produzenten von Staurosporinen gelten.

Das Kapitel V ist die allgemeine Diskussion meiner Dissertation. Im Rahmen der allgemeinen Diskussion habe ich die Ergebnisse der vier vorherigen Kapitel so kombiniert, dass es mir möglich war, drei übergreifende Synthesen meiner Ergebnisse zu generieren:

1.) Ich habe die Ergebnisse der DGC & direkten Kultivierung aus Kapitel I mit den Pyrosequenzierungsdaten des Kapitel II zusammengeführt. Damit konnte ich die Effizienz der jeweiligen Methoden miteinander vergleichen. Es zeigte sich, dass beide Methoden ein sehr unterschiedliches Bild der mikrobiellen Gemeinschaft ergeben, wenn mehr taxonomische Ebenen als nur das Phylum betrachtet wird.

2.) In diesem Abschnitt habe ich die Ergebnisse bezüglich der Unterschiede von mikrobiellen Gemeinschaftsstrukturen entlang eines Tiefengradienten gemeinsam diskutiert. Zusätzlich konnte ich zeigen, dass in *Callyspongia* sp. vermutlich Cyanobakterien die tiefenspezifische Gemeinschaftstruktur verursachen. Während in *Axinella infundibuliformis* die mikrobiellen Gattungen *Nitrosopumilus* und *Nitrospira* keinen Einfluss auf die tiefenspezifische Gemeinschaftstruktur zu haben scheinen, was im Widerspruch zu dem Ergebnis Indikator-Arten-Analyse steht.

3.) Im letzten Teil der Diskussion untersuchte ich, ob die Beobachtung von konsistenten mikrobiellen Gemeinschaftstrukturen, welche ich in Kapitel III durchgehend auf höheren taxonomischen Rängen gefunden habe, auch auf andere Schwämme und weitere marine Invertebraten übertragbar ist. In

diesem Rahmen kombinierte ich die Datensätze aus den Kapiteln II bis IV und zusätzlichen Datensätzen aus dem 'Sequence Read Archive' des NCBI und des 'Earth Microbiome Projects'. Es zeigten sich konsistente signifikante Muster in den mikrobiellen Gemeinschaftsstrukturen basierend auf ihrem jeweiligen Ursprung (Schwamm, Koralle, Seescheide, Seewasser, Substrat) und der Herkunft für alle taxonomischen Ränge vom Phylum zur Gattung. Zusätzlich habe ich die Daten nach Schwamm und Seescheide aufgetrennt und unabhängig untersucht. Auch hier wurden diese signifikanten Strukturen, mit leichten Unterschieden, für alle untersuchten taxonomischen Ränge gefunden.

Zusammenfassend kann gesagt werden, dass die mikrobiellen Gemeinschaften signifikant mit den jeweiligen Schwammarten assoziiert sind. Weiterhin bleiben diese spezifischen mikrobiellen Gemeinschaftsstrukturen auch dann erhalten, wenn sie auf höheren taxonomischen Rängen abgebildet werden. Dies spricht dafür, dass die symbiontische Gemeinschaft zwischen den Schwämmen und Mikroben durch langfristige Prozesse geprägt wird. Zusätzlich zeigen die Gemeinschaftsstrukturen innerhalb von bestimmten Schwammarten, entlang von lokalen marinen Tiefengradienten, signifikante Unterschiede. Diese könnten von unterschiedlichen Umweltfaktoren entlang des Gradienten geprägt sein. Dem gegenüber sind die mikrobiellen Gemeinschaften auf globaler Ebene, und auf unterschiedlichen taxonomischen Rängen, durchgehend signifikant mit ihren wirbellosen Wirten assoziiert. Dies unterstützt die Theorie von einem Zusammenhang zwischen mikrobieller Taxonomie und ökologischer Funktion.

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SUPPLEMENTARY MATERIAL

Supplementary Table 2.1 Sequence information for all isolates. Novel cultured strains are highlighted in bold. It includes the GenBank accession number (ACC), the length of the sequence (long = >1199, short ≤1199), the isolate DGC source of the sequence (direct or DGC1 to DGC4), the different media types, and the GenBank blast results with the taxonomic classification from phylum to genus, the maximum identity and the related GenBank accession number.

Acc	length	strain	DGC isolate	media type	phylum	class	order	family	genus	blast results	max ident	blast Acc
KF282347	long	907	direct	M1	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Phaeobacter*	Phaeobacter gallaeciensis strain NBRC16654 16S ribosomal RNA gene, partial sequence	0.99	DQ915619.1
KF282348	**long**	**910**	**direct**	**M1**	**Bacteroidetes**	**Flavobacteria**	**Flavobacteriales**	**Flavobacteriaceae**	***Aquimarina***	**Flavobacteriaceae bacterium 04OKA-17-12 gene for 16S rRNA, partial sequence**	**0.95**	**AB270586.1**
KF282349	long	913	direct	M1	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Bacterium 3D802 16S ribosomal RNA gene, partial sequence	1	JF411518.1
KF282350	**long**	**915**	**direct**	**M1**	**Proteobacteria**	**Gammaproteobacteria**	**Vibrionales**	**Vibrionaceae**	***Vibrio***	**Bacterium M7(2011b) strain M7 16S ribosomal RNA gene, partial sequence**	**1**	**JN119259.1**
KF282351	long	916	direct	M8	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Muricauda*	Muricauda sp. sw027 16S ribosomal RNA gene, partial sequence	0.99	JN118551.1
KF282352	**long**	**932**	**direct**	**M8**	**Bacteroidetes**	**Flavobacteria**	**Flavobacteriales**	**Flavobacteriaceae**	***Aquimarina***	**Flavobacteria bacterium B6 ZZ-2008 partial 16S rRNA gene, strain B6**	**0.99**	**FM180520.1**
KF282353	long	942	direct	M1 w/ extract	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Alteromonas*	Alteromonas marina strain MR32c 16S ribosomal RNA gene, partial sequence	0.99	HQ439507.1
KF282354	**long**	**944**	**direct**	**M1 w/ extract**	**Bacteroidetes**	**Flavobacteria**	**Flavobacteriales**	**Flavobacteriaceae**	***Aquimarina***	**Flavobacteriaceae bacterium 04OKA-17-12 gene for 16S rRNA, partial sequence**	**0.99**	**AB270586.1**
KF282355	long	967	direct	MB 1:10	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Aquimarina*	Aquimarina sp. PSC33 16S ribosomal RNA gene, partial sequence	0.97	HM998910.1
KF282356	**long**	**972**	**direct**	**M1**	**Bacteroidetes**	**Flavobacteria**	**Flavobacteriales**	**Flavobacteriaceae**	***Muricauda***	**Muricauda sp. sw027 16S ribosomal RNA gene, partial sequence**	**0.99**	**JN118551.1**
KF282357	long	979	direct	M8	Proteobacteria	Alphaproteobacteria	Parvularculales	Parvularculaceae	*Parvularcula*	Parvularcula lutaonensis strain CC-MMS-1 16S ribosomal RNA, partial sequence	0.97	NR_044474.1
KF282358	**long**	**989**	**direct**	**M1 w/ extract**	**Proteobacteria**	**Alphaproteobacteria**	**Rhodobacterales**	**Rhodobacteraceae**	***Phaeobacter***	**Phaeobacter sp. 256-7 16S ribosomal RNA gene, partial sequence**	**0.98**	**FJ015019.1**
KF282359	long	990	direct	M1 w/ extract	Proteobacteria	Alphaproteobacteria	Kiloniellales	Kiloniellaceae	*Kiloniella*	Kiloniellaceae bacterium EF3B-B119 16S ribosomal RNA gene, partial sequence	0.99	KC545311.1
KF282360	**long**	**991**	**direct**	**M1 w/ extract**	**Bacteroidetes**	**Flavobacteria**	**Flavobacteriales**	**Flavobacteriaceae**	***Aquimarina***	**Aquimarina sp. PSC33 16S ribosomal RNA gene, partial sequence**	**0.97**	**HM998910.1**
KF282361	long	1043	direct	MB 1:10	Proteobacteria	Gammaproteobacteria	Alteromonadales	Shewanellaceae	*Shewanella*	Shewanella sp. RKVR22 16S ribosomal RNA gene, partial sequence	0.99	JX407255.1
KF282362	**long**	**1088**	**1**	**MB 1:10**	**Firmicutes**	**Bacilli**	**Bacillales**	**Bacillaceae_1**	***Bacillus***	**Bacillus sp. CNJ803 PL04 16S ribosomal RNA gene, partial sequence**	**0.99**	**DQ448744.1**
KF282363	long	1097	1	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Mucus bacterium 83 16S ribosomal RNA gene, partial sequence	0.99	AY654820.1
KF282364	**long**	**1101**	**1**	**M1 w/ extract**	**Proteobacteria**	**Alphaproteobacteria**	**Rhodobacterales**	**Rhodobacteraceae**	***Pseudovibrio***	**Pseudovibrio sp. FO-BEG1 strain FO-BEG1 16S ribosomal RNA, complete sequence**	**1**	**NR_074229.1**
KF282365	long	1105	1	M1	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Pseudovibrio*	Bacterium 1H216 16S ribosomal RNA gene, partial sequence	0.99	JF411477.1
KF282366	**long**	**1112**	**1**	**M8**	**Proteobacteria**	**Alphaproteobacteria**	**Rhodobacterales**	**Rhodobacteraceae**	***Ruegeria***	**Ruegeria sp. WJ45-6 16S ribosomal RNA gene, partial sequence**	**1**	**JX853816.1**
KF282367	long	1115	1	MB 1:10	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus sp. SCSSS01 16S ribosomal RNA gene, partial sequence	0.99	FJ461460.1
KF282368	**long**	**1124**	**1**	**M1**	**Proteobacteria**	**Alphaproteobacteria**	**Rhodobacterales**	**Rhodobacteraceae**	***Pseudovibrio***	**Bacterium 1H216 16S ribosomal RNA gene, partial sequence**	**0.99**	**JF411477.1**
KF282369	long	1126	1	M1	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Pseudovibrio*	Bacterium 1H216 16S ribosomal RNA gene, partial sequence	0.99	JF411477.1
KF282370	**long**	**1130**	**1**	**M8**	**Proteobacteria**	**Gammaproteobacteria**	**Oceanospirillales**	**Oceanospirillaceae**	***Reinekea***	**Reinekea blandensis MED297 16S ribosomal RNA, complete sequence**	**0.97**	**NR_043788.1**
KF282371	long	1145	2	M1	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio aerogenes partial 16S rRNA gene, strain FG1	0.99	HF558385.1
KF282372	**long**	**1159**	**direct**	**M8**	**Bacteroidetes**	**Flavobacteria**	**Flavobacteriales**	**Flavobacteriaceae**	***Muricauda***	**Muricauda sp. sw027 16S ribosomal RNA gene, partial sequence**	**0.98**	**JN118551.1**
KF282373	long	1160	direct	MB 1:10	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Silicibacter*	Bacterium BW3PhS19 16S ribosomal RNA gene, partial sequence	0.94	KC012887.1
KF282374	**long**	**1163**	**1**	**M1**	**Proteobacteria**	**Alphaproteobacteria**	**Rhodobacterales**	**Rhodobacteraceae**	***Pseudovibrio***	**Bacterium 1H216 16S ribosomal RNA gene, partial sequence**	**0.99**	**JF411477.1**
KF282375	long	1168	direct	M8	Proteobacteria	Alphaproteobacteria	Parvularculales	Parvularculaceae	*Parvularcula*	Parvularcula lutaonensis strain CC-MMS-1 16S ribosomal RNA, partial sequence	0.98	NR_044474.1
KF282376	**long**	**1182**	**3**	**M1 w/ extract**	**Bacteroidetes**	**Flavobacteria**	**Flavobacteriales**	**Flavobacteriaceae**	***Tamlana***	**Flavobacteriaceae bacterium E403 16S ribosomal RNA gene, partial sequence**	**0.94**	**JX502604.1**
KF282377	long	1188	3	M1 w/ extract	Firmicutes	Bacilli	Bacillales	Bacillaceae_2	*Halobacillus*	Firmicutes bacterium JS13 16S ribosomal RNA gene, partial sequence	1	AY372927.1
KF282378	**long**	**1207**	**3**	**M8**	**Proteobacteria**	**Alphaproteobacteria**	**Rhodobacterales**	**Rhodobacteraceae**	***Labrenzia***	**Stappia meyerae strain GA15 16S ribosomal RNA gene, partial sequence**	**0.98**	**EF101502.1**
KF282379	long	1211	2	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Ruegeria*	Bacterium 2D802 16S ribosomal RNA gene, partial sequence	0.99	JF411487.1
KF282380	**long**	**1218**	**1**	**MB 1:10**	**Proteobacteria**	**Alphaproteobacteria**	**Sphingomonadales**	**Erythrobacteraceae**	***Erythrobacter***	**Bacterium BW3PhG11 16S ribosomal RNA gene, partial sequence**	**0.99**	**KC012857.1**
KF282381	long	1219	1	M8	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	*Zhihengliuella*	Zhihengliuella sp. RS236(2010) 16S ribosomal RNA gene, partial sequence	0.99	GU968470.1
KF282382	**long**	**1231**	**4**	**M1**	**Proteobacteria**	**Alphaproteobacteria**	**Rhizobiales**	**Cohaesibacteraceae**	***Cohaesibacter***	**Cohaesibacter sp. SJH-001 16S ribosomal RNA gene, partial sequence**	**0.97**	**KC335135.1**
KF282383	long	1233	3	M8	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Gaetbulibacter*	Flavobacteriaceae bacterium DOKDO 020 16S ribosomal RNA gene, partial sequence	0.96	DQ191181.1
KF282384	**long**	**1239**	**4**	**M1**	**Proteobacteria**	**Gammaproteobacteria**	**Alteromonadales**	**Shewanellaceae**	***Shewanella***	**Alteromonadales bacterium fav-2-10-05 16S ribosomal RNA gene, partial sequence**	**0.95**	**FJ041083.2**
KF282385	long	1251	direct	MB 1:10	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Labrenzia*	Labrenzia alba partial 16S rRNA gene, isolate CMS163	0.99	FR750958.1

Supplementary Table 2.1 (continued)

Acc	length	strain	DGC isolate	media type	phylum	class	order	family	genus	blast results	max ident	blast Acc
KF282386	long	1257	3	M8	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiopsaceae	*Streptomonospora*	Streptomonospora sp. S05 16S ribosomal RNA gene, partial sequence	0.99	JX007947.1
KF282387	long	1271	3	M8	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. DQHS-21 16S ribosomal RNA gene, partial sequence	0.98	GQ200200.2
KF282388	long	1278	3	M8	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Gaetbulibacter*	Flavobacteriaceae bacterium DOKDO 020 16S ribosomal RNA gene, partial sequence	0.96	DQ191181.1
KF282389	long	1281	3	M8	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Bacterium 3H106 16S ribosomal RNA gene, partial sequence	0.99	JF411533.1
KF282390	long	1294	4	MB 1:10	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cytophagaceae	*Cytophaga*	Cytophaga sp. UDC369 16S ribosomal RNA gene, partial sequence	0.96	HM031970.1
KF282391	long	1298	4	M1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. DQHS-21 16S ribosomal RNA gene, partial sequence	0.98	GQ200200.2
KF282392	long	1304	1	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Microbulbifer sp. HB09007 16S ribosomal RNA gene, partial sequence	0.99	FJ796077.1
KF282393	long	1319	2	M8	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. DQHS-21 16S ribosomal RNA gene, partial sequence	0.98	GQ200200.2
KF282394	long	1327	4	M8	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. SJH-001 16S ribosomal RNA gene, partial sequence	0.98	KC335135.1
KF282395	long	1333	3	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Labrenzia*	Bacterium 1H204 16S ribosomal RNA gene, partial sequence	0.99	JF411465.1
KF282396	long	1341	1	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Bacterium 1H217 16S ribosomal RNA gene, partial sequence	0.99	JF411478.1
KF282397	long	1353	3	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Alteromonas sp. H91 16S ribosomal RNA gene, partial sequence	0.99	FJ903194.1
KF282398	long	1355	4	MB 1:10	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Labrenzia*	Labrenzia marina strain F84035 16S ribosomal RNA gene, partial sequence	0.99	HQ908720.1
KF282399	short	905	direct	M1	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Tenacibaculum*	Tenacibaculum sp. CNURIC 013 16S ribosomal RNA gene, partial sequence	0.99	FJ490366.1
KF282400	short	908	direct	M1	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	*Pseudoalteromonas*	Pseudoalteromonas luteoviolacea gene for 16S rRNA, partial sequence, strain: NBRC 103183	1	AB681981.1
KF282401	short	909	direct	M1	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Ruegeria*	Bacterium 1H121 16S ribosomal RNA gene, partial sequence	1	JF411460.1
KF282402	short	911	direct	MB 1:10	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Silicibacter*	Ruegeria lacuscaerulensis gene for 16S rRNA, partial sequence, strain: C128	0.99	AB719154.1
KF282403	short	926	direct	MB 1:10	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	Staphylococcus aureus M1 complete genome	1	HF937103.1
KF282404	short	927	direct	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Silicibacter*	Ruegeria lacuscaerulensis gene for 16S rRNA, partial sequence, strain: C128	0.99	AB719154.1
KF282405	short	928	direct	MB 1:10	Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	*Micromonospora*	Micromonospora sp. 204801 16S ribosomal RNA gene, partial sequence	0.99	EU437811.1
KF282406	short	929	direct	MB 1:10	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	*Micrococcus*	Micrococcus yunnanensis partial 16S rRNA gene, isolate Kongs-10	0.99	HE800816.1
KF282407	short	930	direct	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Silicibacter*	Bacterium 1H121 16S ribosomal RNA gene, partial sequence	1	JF411460.1
KF282408	short	931	direct	M8	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. EF4A-CB189 16S ribosomal RNA gene, partial sequence	1	KC545328.1
KF282410	short	937	direct	M1 w/extract	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio ponticus strain AN62	0.99	JQ409384.1
KF282411	short	939	direct	M1 w/ extract	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Photobacterium*	Photobacterium sp. JSA04 16S ribosomal RNA gene, partial sequence	0.99	KC012646.1
KF282412	short	940	direct	M1 w/ extract	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio communis strain F75020 16S ribosomal RNA gene, partial sequence	0.99	HQ161734.1
KF282413	short	943	direct	M1 w/ extract	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	Staphylococcus hominis strain FSHS5 16S ribosomal RNA gene, partial sequence	1	KC609740.1
KF282414	short	965	direct	MB 1:10	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	*Erythrobacter*	Erythrobacter sp. U1370-101126-SW175 16S ribosomal RNA gene, partial sequence	0.99	JQ082155.1
KF282415	short	968	direct	MB 1:10	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Labrenzia*	Bacterium 2H304 16S ribosomal RNA gene, partial sequence	0.99	JF411504.1
KF282416	short	969	direct	M1	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Ruegeria*	Bacterium 1H121 16S ribosomal RNA gene, partial sequence	1	JF411460.1
KF282417	short	970	direct	M1	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Aquimarina*	Aquimarina sp. PSC33 16S ribosomal RNA gene, partial sequence	0.97	HM998910.1
KF282418	short	971	direct	M1	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Silicibacter*	Ruegeria atlantica strain F77046 16S ribosomal RNA gene, partial sequence	1	HQ908706.1
KF282419	short	973	direct	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Oceanicola*	Mucus bacterium 112 16S ribosomal RNA gene, partial sequence	1	AY654757.1
KF282420	short	975	direct	M1	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Croceitalea*	Muricauda sp. sw027 16S ribosomal RNA gene, partial sequence	0.99	JN118551.1
KF282421	short	977	direct	M8	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Aquimarina*	Aquimarina sp. PSC33 16S ribosomal RNA gene, partial sequence	0.96	HM998910.1
KF282423	short	980	direct	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Ruegeria*	Bacterium 1H212 16S ribosomal RNA gene, partial sequence	1	JF411473.1
KF282424	short	983	direct	M1 w/ extract	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Pseudovibrio*	Pseudovibrio sp. JZ08IS8 16S ribosomal RNA gene, partial sequence	1	KC429790.1
KF282425	short	984	direct	M1 w/ extract	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Hahellaceae	*Endozoicomonas*	Spongiobacter nickelotolerans gene for 16S rRNA, partial sequence	1	AB205011.1
KF282426	short	985	direct	MB 1:10	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	*Pseudoalteromonas*	Pseudoalteromonas luteoviolacea gene for 16S rRNA, partial sequence, strain: NBRC 103183	0.99	AB681981.1
KF282427	short	986	direct	M1 w/ extract	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	Staphylococcus sp. SR24-1 16S ribosomal RNA gene, partial sequence	1	KC710225.1
KF282428	short	987	direct	M1 w/ extract	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio communis strain F75020 16S ribosomal RNA gene, partial sequence	1	HQ161734.1
KF282429	short	988	direct	M1 w/ extract	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Roseovarius*	Roseobacter sp. RED85 16S ribosomal RNA gene, partial sequence	0.99	AY136135.1
KF282430	short	994	direct	M1 w/ extract	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Ruegeria*	Ruegeria sp. JZ08ML31 16S ribosomal RNA gene, partial sequence	1	KC429856.1
KF282431	short	995	direct	M1 w/ extract	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. S1396 16S ribosomal RNA gene, partial sequence	0.99	FJ457399.1
KF282432	short	998	direct	M1 w/ extract	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. 08XMBC-14 16S ribosomal RNA gene, partial sequence	0.99	HM565993.1

Supplementary Table 2.1 (continued)

Acc	length	strain	DGC isolate	media type	phylum	class	order	family	genus	blast results	max ident	blast Acc
KF282433	short	1000	direct	M1 w/ extract	Proteobacteria	Alphaproteobacteria	Kiloniellales	Kiloniellaceae	*Kiloniella*	Kiloniellaceae bacterium EF3B-B119 16S ribosomal RNA gene, partial sequence	0.99	KC545311.1
KF282434	short	1044	4	M8	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus sp. CNJ803 PL04 16S ribosomal RNA gene, partial sequence	1	DQ448744.1
KF282436	short	1047	direct	M1	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Bacterium 1D807 16S ribosomal RNA gene, partial sequence	0.99	JF411438.1
KF282437	short	1049	direct	M1	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Bacterium 1D807 16S ribosomal RNA gene, partial sequence	0.99	JF411438.1
KF282438	short	1051	direct	M1 w/ extract	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus methylotrophicus strain LJ10 16S ribosomal RNA gene, partial sequence	1	KC880355.1
KF282439	short	1052	direct	M8	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	*Erythrobacter*	Erythrobacter sp. SMB20 16S ribosomal RNA gene, partial sequence	0.99	DQ868685.1
KF282440	short	1100	1	M1 w/ extract	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. TAV24 16S ribosomal RNA gene, partial sequence	0.99	GU580887.1
KF282441	short	1102	1	M1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. SJH-001 16S ribosomal RNA gene, partial sequence	0.98	KC335135.1
KF282442	short	1103	1	M1 w/ extract	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Pseudovibrio*	Pseudovibrio sp. JZ08IS8 16S ribosomal RNA gene, partial sequence	1	KC429790.1
KF282443	short	1107	1	M1	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus sp. L67 16S ribosomal RNA gene, partial sequence	1	KC466215.1
KF282444	short	1110	1	M8	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio coralliilyticus partial 16S rRNA gene, strain VC LPI 06_210	1	HF549288.1
KF282445	short	1113	1	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Silicibacter*	Ruegeria sp. WJ45-6 16S ribosomal RNA gene, partial sequence	1	JX853816.1
KF282446	short	1114	1	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Microbulbifer sp. HB09007 16S ribosomal RNA gene, partial sequence	1	FJ796077.1
KF282447	short	1118	1	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Microbulbifer epialgicus strain F-104 16S ribosomal RNA, partial sequence	0.99	NR_041493.1
KF282448	short	1121	1	M8	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	*Desemzia*	Desemzia incerta strain SB-B1 16S ribosomal RNA gene, partial sequence	1	KC577171.1
KF282449	short	1122	1	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Ruegeria*	Ruegeria lacuscaerulensis gene for 16S rRNA, partial sequence, strain: C128	1	AB719154.1
KF282450	short	1125	1	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Microbulbifer sp. HB09007 16S ribosomal RNA gene, partial sequence	1	FJ796077.1
KF282451	short	1127	1	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Silicibacter*	Ruegeria lacuscaerulensis gene for 16S rRNA, partial sequence, strain: C128	1	AB719154.1
KF282452	short	1129	1	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Microbulbifer sp. HB09007 16S ribosomal RNA gene, partial sequence	1	FJ796077.1
KF282453	short	1131	1	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Microbulbifer sp. HB09007 16S ribosomal RNA gene, partial sequence	1	FJ796077.1
KF282454	short	1132	1	MB 1:10	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus algicola strain CBW9 16S ribosomal RNA gene, partial sequence	0.99	JX005838.1
KF282455	short	1133	1	M8	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus algicola strain CBW9 16S ribosomal RNA gene, partial sequence	0.99	JX005838.1
KF282456	short	1136	1	M8	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus sp. L37 16S ribosomal RNA gene, partial sequence	1	KC466208.1
KF282457	short	1138	1	MB 1:10	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus thuringiensis strain Sn9 16S ribosomal RNA gene, partial sequence	1	KC683724.1
KF282458	short	1139	1	M1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. SJH-001 16S ribosomal RNA gene, partial sequence	0.98	KC335135.1
KF282459	short	1140	1	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Microbulbifer sp. HB09007 16S ribosomal RNA gene, partial sequence	1	FJ796077.1
KF282460	short	1141	2	MB 1:10	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus sp. CNJ803 PL04 16S ribosomal RNA gene, partial sequence	0.99	DQ448744.1
KF282461	short	1142	2	M1	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. PaH1.12 16S ribosomal RNA gene, partial sequence	1	GQ391933.1
KF282462	short	1143	2	M1	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus thuringiensis strain Sn9 16S ribosomal RNA gene, partial sequence	1	KC683724.1
KF282463	short	1146	2	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Pseudovibrio*	Pseudovibrio sp. FO-BEG1 strain FO-BEG1 16S ribosomal RNA, complete sequence	1	NR_074229.1
KF282464	short	1147	2	M1	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio aerogenes partial 16S rRNA gene, strain FG1	0.99	HF558385.1
KF282465	short	1148	2	MB 1:10	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus sp. CNJ803 PL04 16S ribosomal RNA gene, partial sequence	0.99	DQ448744.1
KF282466	short	1150	2	M1	Firmicutes	Bacilli	Bacillales	Planococcaceae	*Bhargavaea*	Bhargavaea cecembensis strain TRTYP8 16S ribosomal RNA gene, partial sequence	0.99	KC734528.1
KF282467	short	1151	2	M8	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. SJH-001 16S ribosomal RNA gene, partial sequence	0.98	KC335135.1
KF282468	short	1152	2	M8	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus sp. L74 16S ribosomal RNA gene, partial sequence	1	KC466220.1
KF282469	short	1153	2	M1	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. PaH1.12 16S ribosomal RNA gene, partial sequence	1	GQ391933.1
KF282470	short	1155	2	M1 w/ extract	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. EF3B-CB181 16S ribosomal RNA gene, partial sequence	0.99	KC545326.1
KF282471	short	1157	2	M1	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus sp. S50 16S ribosomal RNA gene, partial sequence	1	KC466255.1
KF282472	short	1164	1	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Bacterium VIII.10 16S ribosomal RNA gene, partial sequence	0.99	JN699195.1
KF282473	short	1165	1	MB 1:10	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus thuringiensis strain Sn9 16S ribosomal RNA gene, partial sequence	1	KC683724.1
KF282474	short	1166	1	M8	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	*Microbacterium*	Microbacterium aquimaris strain DT27 16S ribosomal RNA gene, partial sequence	0.99	HQ009858.1
KF282475	short	1167	direct	M8	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	*Erythrobacter*	Mucus bacterium 91 16S ribosomal RNA gene, partial sequence	0.99	AY654830.1
KF282476	short	1169	2	M1	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Paracoccus*	Paracoccus sp. C3305 16S ribosomal RNA gene, partial sequence	1	JX097010.1
KF282477	short	1170	1	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Microbulbifer sp. HB09007 16S ribosomal RNA gene, partial sequence	1	FJ796077.1

Supplementary Table 2.1 (continued)

Acc	length	strain	DGC isolate	media type	phylum	class	order	family	genus	blast results	max ident	blast Acc
KF282478	short	1171	2	MB 1:10	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus sp. CMJ3-7RA 16S ribosomal RNA gene, partial sequence	1	KC782848.1
KF282479	short	1172	1	MB 1:10	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	*Microbacterium*	Microbacterium aquimaris strain DT27 16S ribosomal RNA gene, partial sequence	0.99	HQ009858.1
KF282480	short	1173	2	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Silicibacter*	Ruegeria sp. JZ10ML52 16S ribosomal RNA gene, partial sequence	1	KC429889.1
KF282481	short	1174	3	M1	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. PaD1.27 16S ribosomal RNA gene, partial sequence	0.99	GQ391983.1
KF282482	short	1175	3	M1	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Alteromonas*	Alteromonas sp. MEBiC06033 16S ribosomal RNA gene, partial sequence	0.99	GU289642.1
KF282483	short	1176	3	M1	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Alteromonas sp. MOLA 365 partial 16S rRNA gene, culture collection MOLA:365	1	AM990642.1
KF282484	short	1177	3	M1	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Alteromonas*	Bacterium 3D801 16S ribosomal RNA gene, partial sequence	1	JF411517.1
KF282485	short	1178	3	M1	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	Staphylococcus sp. U1369-101122-SW168 16S ribosomal RNA gene, partial sequence	1	JQ082147.1
KF282486	short	1179	3	M1	Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	*Saccharopolyspora*	Saccharopolyspora gregorii strain OAct417 16S ribosomal RNA gene, partial sequence	1	KC514124.1
KF282487	short	1180	3	M1 w/ extract	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. S1396 16S ribosomal RNA gene, partial sequence	0.99	FJ457399.1
KF282488	short	1183	3	M1 w/ extract	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. S1396 16S ribosomal RNA gene, partial sequence	0.99	FJ457399.1
KF282489	short	1185	3	MB 1:10	Firmicutes	Bacilli	Bacillales	Bacillaceae_2	*Terribacillus*	Bacillus sp. A2-37c-20 16S ribosomal RNA gene, partial sequence	1	JX517226.1
KF282490	short	1186	3	MB 1:10	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. DQHS-21 16S ribosomal RNA gene, partial sequence	0.99	GQ200200.2
KF282491	short	1187	3	M1 w/ extract	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Alteromonas sp. MOLA 365 partial 16S rRNA gene, culture collection MOLA:365	0.99	AM990642.1
KF282492	short	1189	1	M1	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus sp. L74 16S ribosomal RNA gene, partial sequence	1	KC466220.1
KF282493	short	1192	3	M1 w/ extract	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Alteromonas sp. MOLA 365 partial 16S rRNA gene, culture collection MOLA:365	0.99	AM990642.1
KF282494	short	1193	3	M1	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. AKA07-10b gene for 16S rRNA, partial sequence	0.99	AB571952.1
KF282495	short	1194	3	M1	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus sp. S85 16S ribosomal RNA gene, partial sequence	1	KC466272.1
KF282496	short	1196	3	M1	Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	*Saccharopolyspora*	Saccharopolyspora gregorii strain OAct417 16S ribosomal RNA gene, partial sequence	1	KC514124.1
KF282497	short	1198	3	M1	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus algicola strain CBW9 16S ribosomal RNA gene, partial sequence	0.99	JX005838.1
KF282498	short	1201	3	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Gamma proteobacterium r61 gene for 16S rRNA, partial sequence	0.99	AB470941.1
KF282499	short	1202	3	M8	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. AKA07-10b gene for 16S rRNA, partial sequence	1	AB571952.1
KF282500	short	1203	3	M8	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Tenacibaculum*	Tenacibaculum sp. C28 16S ribosomal RNA gene, partial sequence	1	JX853817.1
KF282501	short	1204	3	M1 w/ extract	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. DQHS-21 16S ribosomal RNA gene, partial sequence	0.99	GQ200200.2
KF282502	short	1205	3	M8	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. PaD1.27 16S ribosomal RNA gene, partial sequence	0.99	GQ391983.1
KF282503	short	1206	3	M8	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Lutaonella*	Flavobacteriaceae bacterium E403 16S ribosomal RNA gene, partial sequence	0.94	JX502604.1
KF282504	short	1208	1	M8	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus sp. S50 16S ribosomal RNA gene, partial sequence	1	KC466255.1
KF282505	short	1209	2	M8	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. DQHS-21 16S ribosomal RNA gene, partial sequence	0.99	GQ200200.2
KF282506	short	1210	2	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Ruegeria*	Bacterium 1D710 16S ribosomal RNA gene, partial sequence	0.99	JF411431.1
KF282507	short	1212	2	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Microbulbifer epialgicus strain F-104 16S ribosomal RNA, partial sequence	0.99	NR_041493.1
KF282508	short	1213	2	M8	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillales bacterium Cul_0282 16S ribosomal RNA gene, partial sequence	1	JX990154.1
KF282510	short	1220	3	M1 w/ extract	Proteobacteria	Gammaproteobacteria	Alteromonadales	Ferrimonadaceae	*Ferrimonas*	Shewanella sp. j21421 16S ribosomal RNA gene, partial sequence	0.99	FJ357688.1
KF282511	short	1221	3	MB 1:10	Firmicutes	Bacilli	Bacillales	Bacillaceae_2	*Terribacillus*	Terribacillus goriensis strain JP44SK47 16S ribosomal RNA gene, partial sequence	0.99	JX155764.1
KF282512	short	1222	3	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aliagarivorans*	Alteromonadaceae bacterium 1ta3 16S ribosomal RNA gene, partial sequence	0.99	FJ952768.1
KF282513	short	1223	3	M8	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Gaetbulibacter*	Marine bacterium CS-24 16S ribosomal RNA gene, partial sequence	0.96	EF040545.1
KF282514	short	1224	3	M1	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. AKA07-10b gene for 16S rRNA, partial sequence	1	AB571952.1
KF282515	short	1228	4	MB 1:10	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Aestuariibacter sp. PX-1 16S ribosomal RNA gene, partial sequence	0.99	JF309276.1
KF282516	short	1229	4	MB 1:10	Proteobacteria	Gammaproteobacteria	Alteromonadales	Ferrimonadaceae	*Ferrimonas*	Gamma proteobacterium r61 gene for 16S rRNA, partial sequence	0.99	AB470941.1
KF282517	short	1230	4	M1	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Bacterium PII.1 16S ribosomal RNA gene, partial sequence	0.99	JN699135.1
KF282518	short	1232	3	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aliagarivorans*	Alteromonadaceae bacterium 1ta3 16S ribosomal RNA gene, partial sequence	0.99	FJ952768.1
KF282519	short	1234	4	M1	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Aestuariibacter sp. PX-1 16S ribosomal RNA gene, partial sequence	0.99	JF309276.1
KF282520	short	1238	4	M1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Thalassospira*	Thalassospira profundimaris strain S8-2 16S ribosomal RNA gene, partial sequence	0.99	KC420685.1
KF282521	short	1242	3	M8	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus sp. S50 16S ribosomal RNA gene, partial sequence	1	KC466255.1
KF282522	short	1243	3	M1	Firmicutes	Bacilli	Bacillales	Bacillaceae_2	*Halobacillus*	Halobacillus kuroshimensis strain HNS013 16S ribosomal RNA gene, partial sequence	1	JN128247.1

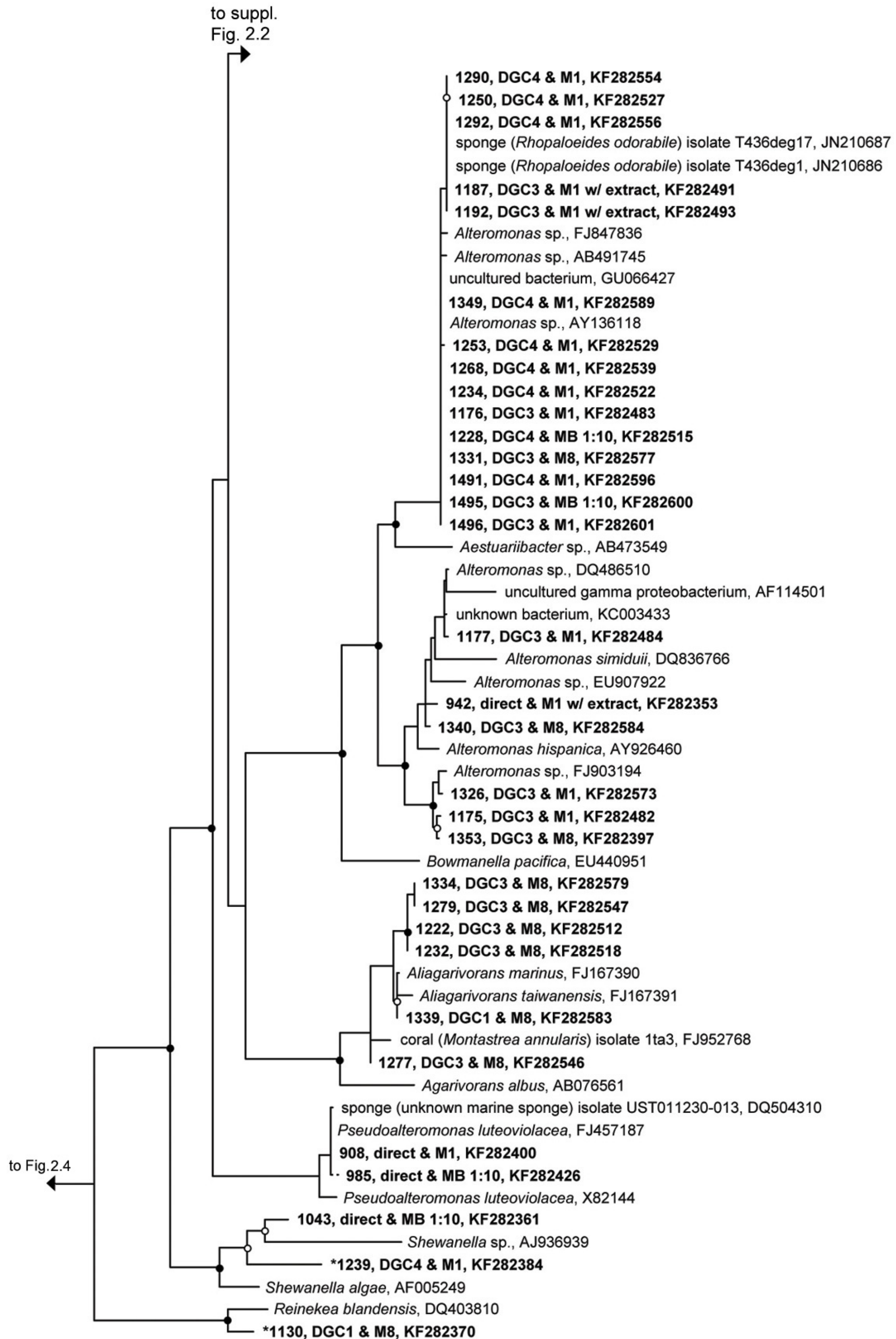
Supplementary Table 2.1 (continued)

Acc	length	strain	DGC isolate	media type	phylum	class	order	family	genus	blast results	max ident	blast Acc
KF282523	short	1245	3	M8	Firmicutes	Bacilli	Bacillales	Bacillaceae_2	*Halobacillus*	Halobacillus mangrovi strain MS10 16S ribosomal RNA, partial sequence	0.99	NR_044002.1
KF282524	short	1246	3	M8	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. AKA07-10b gene for 16S rRNA, partial sequence	0.99	AB571952.1
KF282525	short	1247	3	M8	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Lucibacterium*	Shewanella sp. j21421 16S ribosomal RNA gene, partial sequence	0.99	FJ357688.1
KF282526	short	1249	4	M1	Firmicutes	Bacilli	Bacillales	Bacillaceae_2	*Terribacillus*	Terribacillus goriensis strain JP44SK47 16S ribosomal RNA gene, partial sequence	0.99	JX155764.1
KF282527	short	1250	4	M1	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Aestuariibacter sp. PX-1 16S ribosomal RNA gene, partial sequence	0.99	JF309276.1
KF282528	short	1252	4	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Ferrimonadaceae	*Ferrimonas*	Shewanella sp. j21421 16S ribosomal RNA gene, partial sequence	0.99	FJ357688.1
KF282529	short	1253	4	M1	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Aestuariibacter sp. PX-1 16S ribosomal RNA gene, partial sequence	0.99	JF309276.1
KF282530	short	1254	3	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Labrenzia*	Labrenzia alba partial 16S rRNA gene, isolate CMS163	0.99	FR750958.1
KF282531	short	1255	3	M1 w/ extract	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Lucibacterium*	Shewanella sp. j21421 16S ribosomal RNA gene, partial sequence	0.99	FJ357688.1
KF282532	short	1261	2	M8	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. DQHS-21 16S ribosomal RNA gene, partial sequence	0.99	GQ200200.2
KF282533	short	1262	2	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Pseudovibrio*	Pseudovibrio sp. FO-BEG1 strain FO-BEG1 16S ribosomal RNA, complete sequence	1	NR_074229.1
KF282534	short	1263	2	M8	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. DQHS-21 16S ribosomal RNA gene, partial sequence	0.99	GQ200200.2
KF282535	short	1264	2	M8	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus sp. CNJ803 PL04 16S ribosomal RNA gene, partial sequence	0.99	DQ448744.1
KF282536	short	1265	2	M8	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. DQHS-21 16S ribosomal RNA gene, partial sequence	0.99	GQ200200.2
KF282537	short	1266	2	M8	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus sp. CNJ803 PL04 16S ribosomal RNA gene, partial sequence	0.99	DQ448744.1
KF282538	short	1267	2	M8	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. SJH-001 16S ribosomal RNA gene, partial sequence	0.98	KC335135.1
KF282539	short	1268	4	M1	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Alteromonadales bacterium HOT4G10 16S ribosomal RNA gene, partial sequence	0.99	HQ537367.1
KF282540	short	1269	4	M1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Thalassospira*	Thalassospira profundimaris strain S8-2 16S ribosomal RNA gene, partial sequence	0.99	KC420685.1
KF282541	short	1270	4	M1	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. DQHS-21 16S ribosomal RNA gene, partial sequence	0.99	GQ200200.2
KF282542	short	1272	3	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Labrenzia*	Bacterium 1H211 16S ribosomal RNA gene, partial sequence	0.99	JF411472.1
KF282543	short	1274	3	MB 1:10	Firmicutes	Bacilli	Bacillales	Bacillaceae_2	*Terribacillus*	Bacillus sp. A2-37c-20 16S ribosomal RNA gene, partial sequence	1	JX517226.1
KF282544	short	1275	3	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Pseudovibrio*	Bacterium 1H215 16S ribosomal RNA gene, partial sequence	1	JF411476.1
KF282545	short	1276	3	M1	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. PaD1.27 16S ribosomal RNA gene, partial sequence	0.99	GQ391983.1
KF282546	short	1277	3	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aliagarivorans*	Alteromonadaceae bacterium 1ta3 16S ribosomal RNA gene, partial sequence	0.99	FJ952768.1
KF282547	short	1279	3	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aliagarivorans*	Alteromonadaceae bacterium 1ta3 16S ribosomal RNA gene, partial sequence	0.99	FJ952768.1
KF282548	short	1282	3	M1 w/ extract	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Bacterium 3H106 16S ribosomal RNA gene, partial sequence	0.99	JF411533.1
KF282549	short	1283	3	M1 w/ extract	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Thalassospira*	Thalassospira profundimaris strain S8-2 16S ribosomal RNA gene, partial sequence	0.99	KC420685.1
KF282550	short	1284	2	M1	Actinobacteria	Actinobacteria	Actinomycetales	Cellulomonadaceae	*Cellulomonas*	Cellulomonas sp. m10-48 16S ribosomal RNA gene, partial sequence	0.99	HM587947.1
KF282551	short	1285	2	M1	Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	*Streptomyces*	Streptomyces sp. URI-F11 16S ribosomal RNA gene, partial sequence	0.99	JF939719.1
KF282552	short	1287	2	M8	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. SJH-001 16S ribosomal RNA gene, partial sequence	0.98	KC335135.1
KF282553	short	1289	2	M1	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	*Microbacterium*	Microbacterium aquimaris strain DT27 16S ribosomal RNA gene, partial sequence	0.99	HQ009858.1
KF282554	short	1290	4	M1	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Alteromonas sp. MOLA 365 partial 16S rRNA gene, culture collection MOLA:365	0.99	AM990642.1
KF282555	short	1291	4	M1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Thalassospira*	Thalassospira profundimaris strain mj01-PW1-OH20 16S ribosomal RNA gene, partial sequence	0.99	HQ425693.2
KF282556	short	1292	4	M1	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Alteromonas sp. MOLA 365 partial 16S rRNA gene, culture collection MOLA:365	0.99	AM990642.1
KF282557	short	1295	4	MB 1:10	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio brasiliensis strain CAIM 961 16S ribosomal RNA gene, partial sequence	0.99	HM584046.1
KF282558	short	1299	3	M8	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Lucibacterium*	Shewanella sp. j21421 16S ribosomal RNA gene, partial sequence	0.99	FJ357688.1
KF282559	short	1300	3	M8	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus thuringiensis strain Sn9 16S ribosomal RNA gene, partial sequence	1	KC683724.1
KF282560	short	1302	1	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Microbulbifer epialgicus strain F-104 16S ribosomal RNA, partial sequence	0.99	NR_041493.1
KF282561	short	1305	2	M8	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Lucibacterium*	Shewanella sp. j21421 16S ribosomal RNA gene, partial sequence	0.99	FJ357688.1
KF282562	short	1307	1	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Labrenzia*	Labrenzia sp. FGH455 16S ribosomal RNA gene, partial sequence	1	JQ342690.1
KF282563	short	1308	4	MB 1:10	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Labrenzia*	Labrenzia marina strain F84035 16S ribosomal RNA gene, partial sequence	0.99	HQ908720.1
KF282564	short	1311	4	M8	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Lucibacterium*	Shewanella sp. j21421 16S ribosomal RNA gene, partial sequence	0.99	FJ357688.1
KF282565	short	1312	4	M1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Thalassospira*	Thalassospira profundimaris strain mj01-PW1-OH20 16S ribosomal RNA gene, partial sequence	0.99	HQ425693.2
KF282566	short	1313	2	M8	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. DQHS-21 16S ribosomal RNA gene, partial sequence	0.98	GQ200200.2

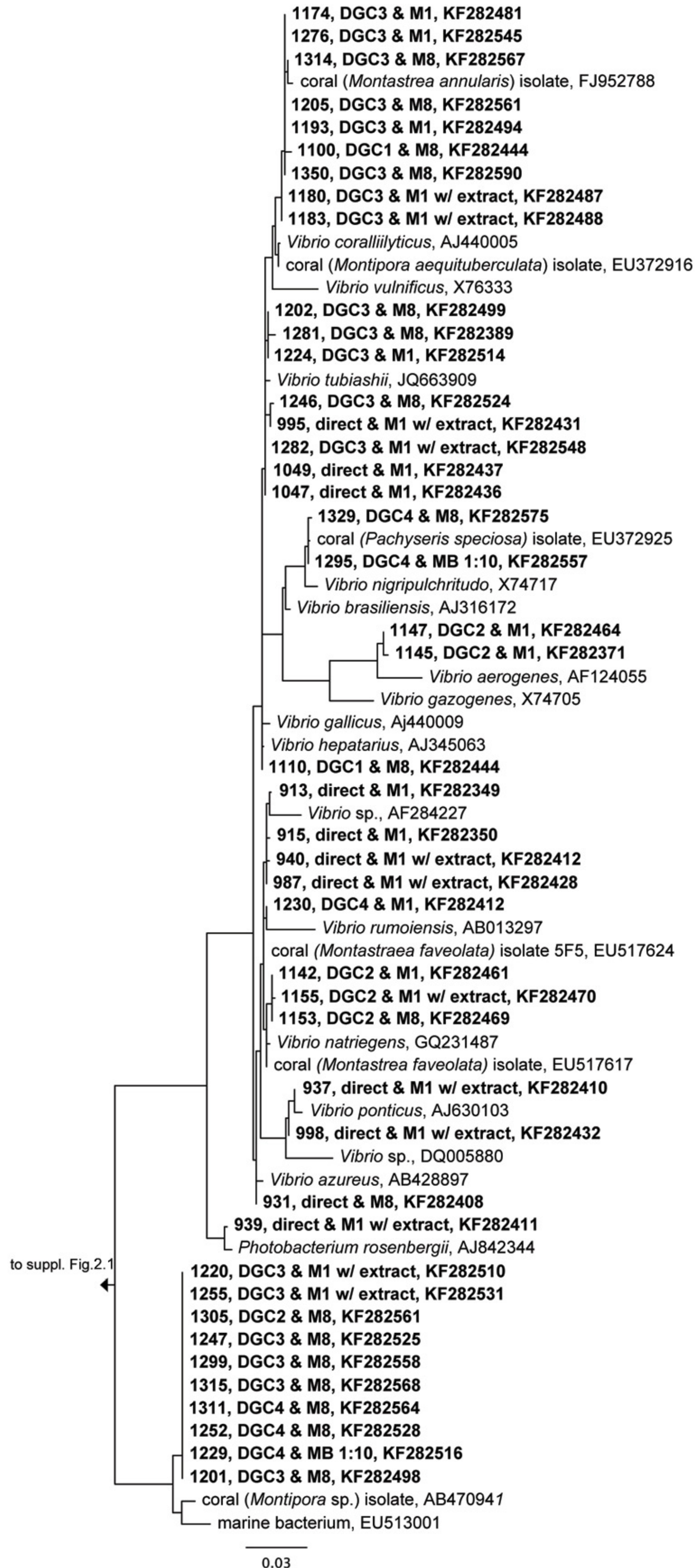
Supplementary Table 2.1 (continued)

Acc	length	strain	DGC isolate	media type	phylum	class	order	family	genus	blast results	max ident	blast Acc
KF282567	short	1314	3	M8	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. AKA07-10b gene for 16S rRNA, partial sequence	0.99	AB571952.1
KF282568	short	1315	3	M8	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Lucibacterium*	Shewanella sp. j21421 16S ribosomal RNA gene, partial sequence	0.99	FJ357688.1
KF282569	short	1317	1	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Microbulbifer sp. HB09007 16S ribosomal RNA gene, partial sequence	1	FJ796077.1
KF282570	short	1320	1	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Silicibacter*	Ruegeria lacuscaerulensis gene for 16S rRNA, partial sequence, strain: C128	1	AB719154.1
KF282571	short	1322	2	M1	Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	*Streptomyces*	Streptomyces sp. URI-F11 16S ribosomal RNA gene, partial sequence	1	JF939719.1
KF282572	short	1323	4	M1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Thalassospira*	Thalassospira profundimaris strain mj01-PW1-OH20 16S ribosomal RNA gene, partial sequence	0.99	HQ425693.2
KF282573	short	1326	3	M1	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Alteromonas sp. H91 16S ribosomal RNA gene, partial sequence	0.99	FJ903194.1
KF282574	short	1328	4	M1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Thalassospira*	Thalassospira profundimaris strain S8-2 16S ribosomal RNA gene, partial sequence	0.99	KC420685.1
KF282575	short	1329	4	M8	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. S10 16S ribosomal RNA gene, partial sequence	1	EU372920.1
KF282576	short	1330	4	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Pseudovibrio*	Pseudovibrio sp. FO-BEG1 strain FO-BEG1 16S ribosomal RNA, complete sequence	0.99	NR_074229.1
KF282577	short	1331	3	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Alteromonas sp. S89 16S ribosomal RNA gene, partial sequence	1	JN654450.1
KF282578	short	1332	3	M8	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. SJH-001 16S ribosomal RNA gene, partial sequence	0.98	KC335135.1
KF282579	short	1334	3	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aliagarivorans*	Alteromonadaceae bacterium 1ta3 16S ribosomal RNA gene, partial sequence	0.99	FJ952768.1
KF282580	short	1336	3	M8	Proteobacteria	Alphaproteobacteria	Rhizobiales	Cohaesibacteraceae	*Cohaesibacter*	Cohaesibacter sp. DQHS-21 16S ribosomal RNA gene, partial sequence	0.98	GQ200200.2
KF282581	short	1337	1	M8	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	*Staphylococcus*	Staphylococcus epidermidis isolate TD27 16S ribosomal RNA gene, partial sequence	1	DQ978252.1
KF282584	short	1340	3	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Alteromonas*	Alteromonas macleodii str. 'English Channel 673', complete genome	0.99	CP003844.1
KF282585	short	1344	1	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Silicibacter*	Ruegeria lacuscaerulensis gene for 16S rRNA, partial sequence, strain: C128	1	AB719154.1
KF282586	short	1345	1	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Bacterium VIII.10 16S ribosomal RNA gene, partial sequence	0.99	JN699195.1
KF282587	short	1346	1	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Microbulbifer epialgicus strain F-104 16S ribosomal RNA, partial sequence	0.99	NR_041493.1
KF282588	short	1347	direct	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Paracoccus*	Paracoccus sp. Bmb21 16S ribosomal RNA gene, partial sequence	1	JQ977226.1
KF282589	short	1349	4	M1	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Alteromonas sp. S89 16S ribosomal RNA gene, partial sequence	1	JN654450.1
KF282590	short	1350	3	M8	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	*Vibrio*	Vibrio sp. PaD1.27 16S ribosomal RNA gene, partial sequence	0.99	GQ391983.1
KF282591	short	1351	3	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Microbulbifer sp. HB09007 16S ribosomal RNA gene, partial sequence	1	FJ796077.1
KF282592	short	1354	1	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Microbulbifer epialgicus strain F-104 16S ribosomal RNA, partial sequence	0.99	NR_041493.1
KF282593	short	1356	1	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Microbulbifer sp. HB09007 16S ribosomal RNA gene, partial sequence	1	FJ796077.1
KF282594	short	1358	4	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Labrenzia*	Bacterium WP2ISO13 16S ribosomal RNA gene, partial sequence	1	DQ985840.1
KF282595	short	1359	4	M8	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Labrenzia*	Bacterium 1H211 16S ribosomal RNA gene, partial sequence	0.99	JF411472.1
KF282596	short	1491	4	M1	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Alteromonas sp. S89 16S ribosomal RNA gene, partial sequence	1	JN654450.1
KF282597	short	1492	3	MB 1:10	Firmicutes	Bacilli	Bacillales	Bacillaceae_2	*Terribacillus*	Terribacillus sp. A1 16S ribosomal RNA gene, partial sequence	1	KC310807.1
KF282598	short	1493	1	M8	Firmicutes	Bacilli	Bacillales	Bacillaceae_2	*Terribacillus*	Terribacillus goriensis strain JP44SK47 16S ribosomal RNA gene, partial sequence	1	JX155764.1
KF282599	short	1494	3	M1	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Thalassospira*	Thalassospira profundimaris strain S8-2 16S ribosomal RNA gene, partial sequence	0.99	KC420685.1
KF282600	short	1495	3	MB 1:10	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Alteromonas sp. MOLA 365 partial 16S rRNA gene, culture collection MOLA:365	1	AM990642.1
KF282601	short	1496	3	M1	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Aestuariibacter*	Aestuariibacter sp. PX-1 16S ribosomal RNA gene, partial sequence	0.99	JF309276.1
KF282602	short	1498	1	M8	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Microbulbifer*	Microbulbifer sp. HB09007 16S ribosomal RNA gene, partial sequence	1	FJ796077.1
KF282606	short	1559	2	MB 1:10	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus barbaricus strain SR2 16S ribosomal RNA gene, partial sequence	1	KC923289.1
KF282607	short	1564	3	M8	Actinobacteria	Actinobacteria	Rubrobacterales	Rubrobacteraceae	*Rubrobacter*	Rubrobacter bracarensis partial 16S rRNA gene, strain VFA70612_S5	0.99	HE672088.1
KF282608	short	1565	3	M1	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Bacillus*	Bacillus barbaricus strain SR2 16S ribosomal RNA gene, partial sequence	1	KC923289.1
KF282609	short	1566	3	M1	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	*Rhodococcus*	Rhodococcus kroppenstedtii strain ICN23 16S ribosomal RNA gene, partial sequence	1	KC346296.1
KF282610	short	1567	2	M1	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Labrenzia*	Stappia sp. JZ08IS43 16S ribosomal RNA gene, partial sequence	0.99	KC429796.1

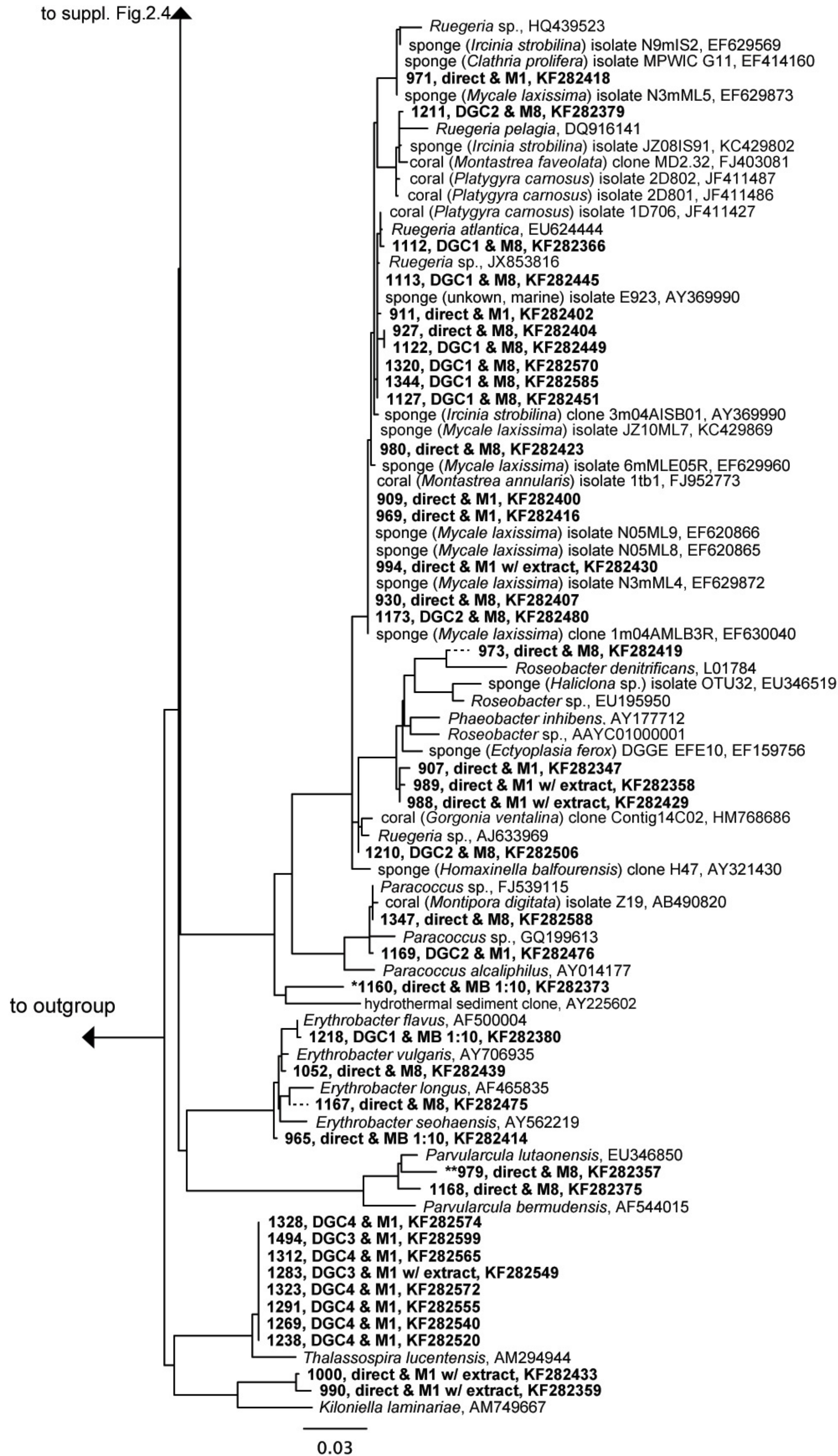
Supplementary Figure 2.1 16S rRNA-based phylogeny of *R. globostellata*-associated *Gammaproteobacteria* organisms. Details are the same as those provided in Figure 2.3



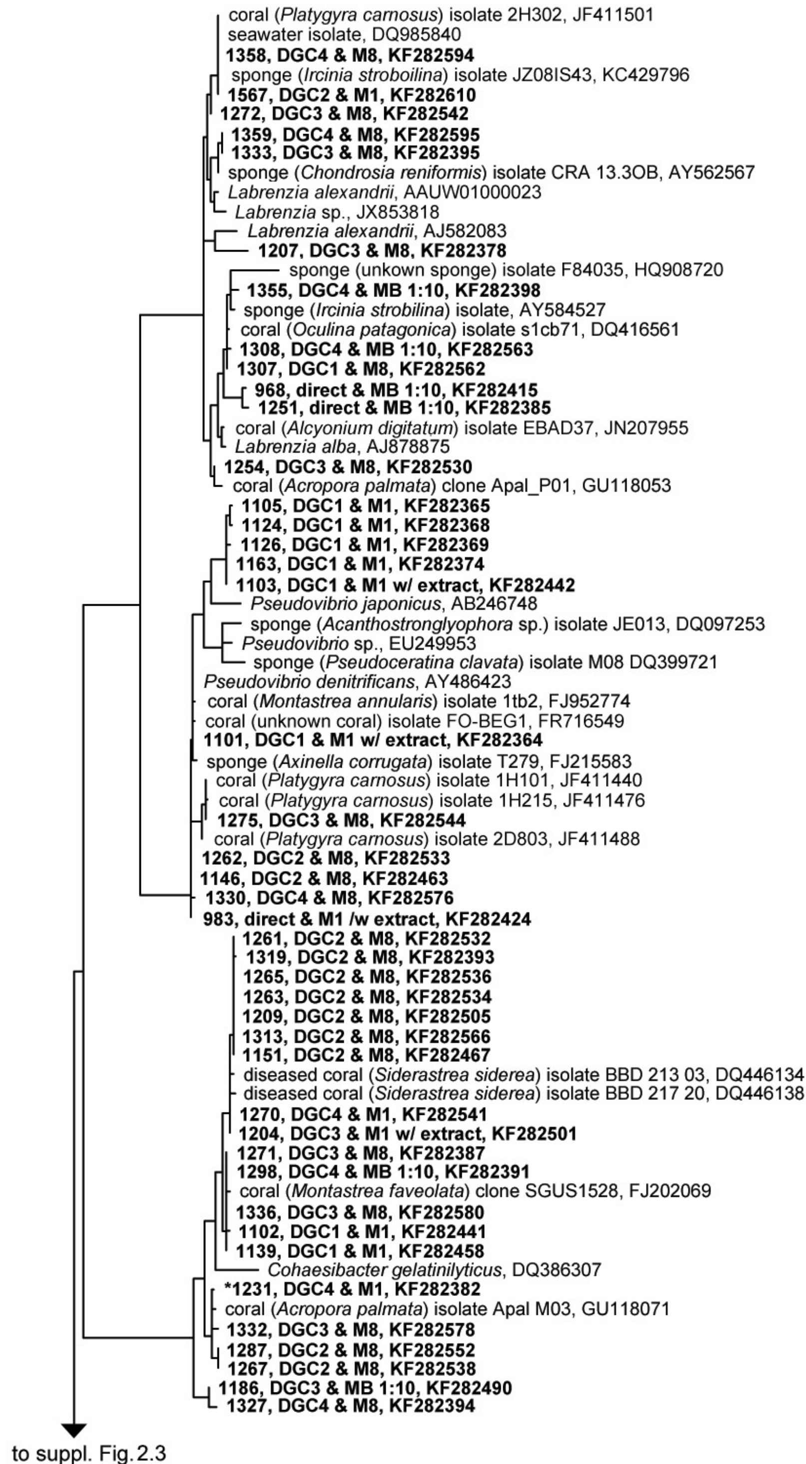
Supplementary Figure 2.2 16S rRNA-based phylogeny of *R. globostellata*-associated *Gammaproteobacteria* organisms. Details are the same as those provided in Figure 2.3



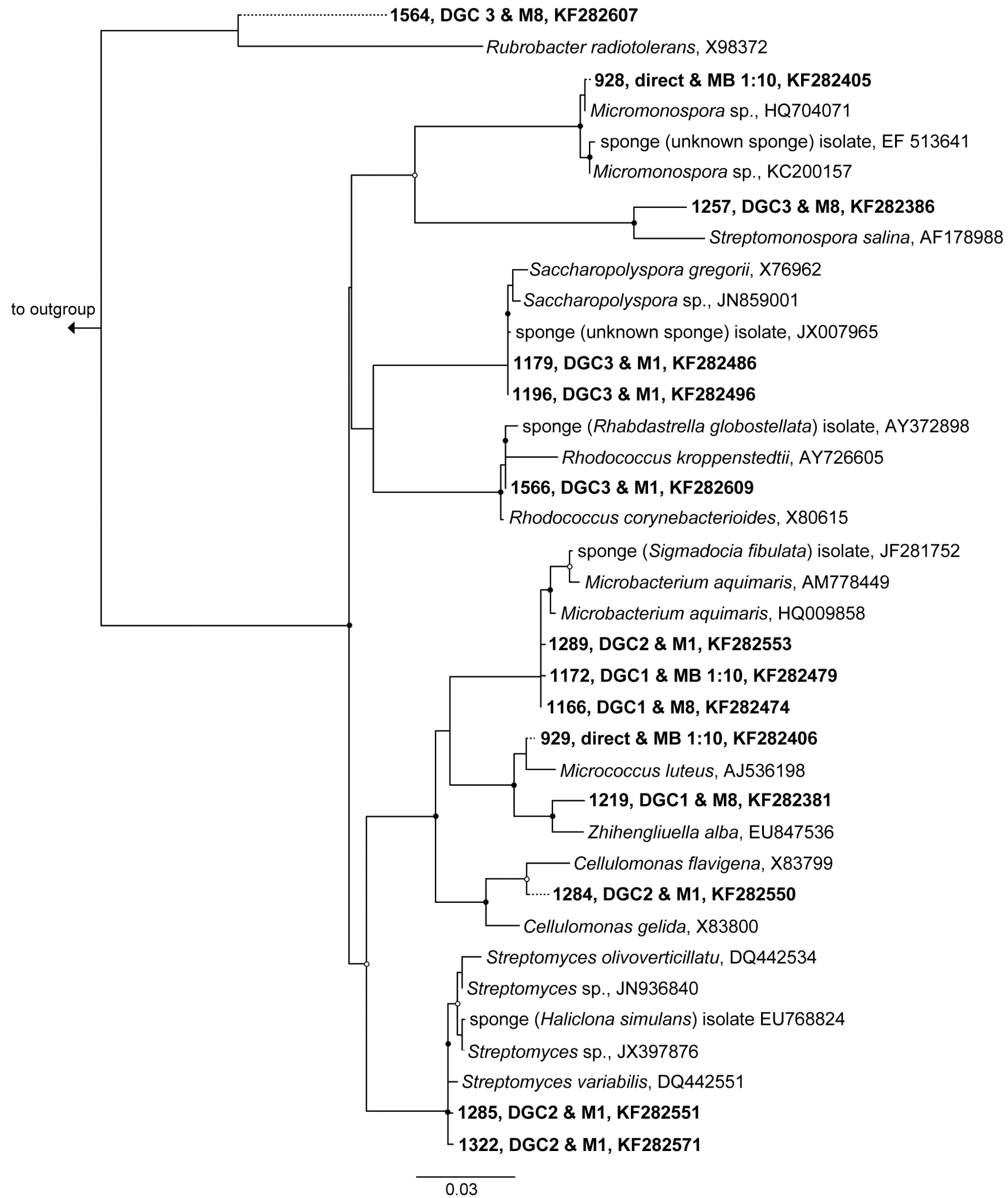
Supplementary Figure 2.3 16S rRNA-based phylogeny of *R. globostellata*-associated *Alphaproteobacteria* organisms. Details are the same as those provided in Figure 2.3



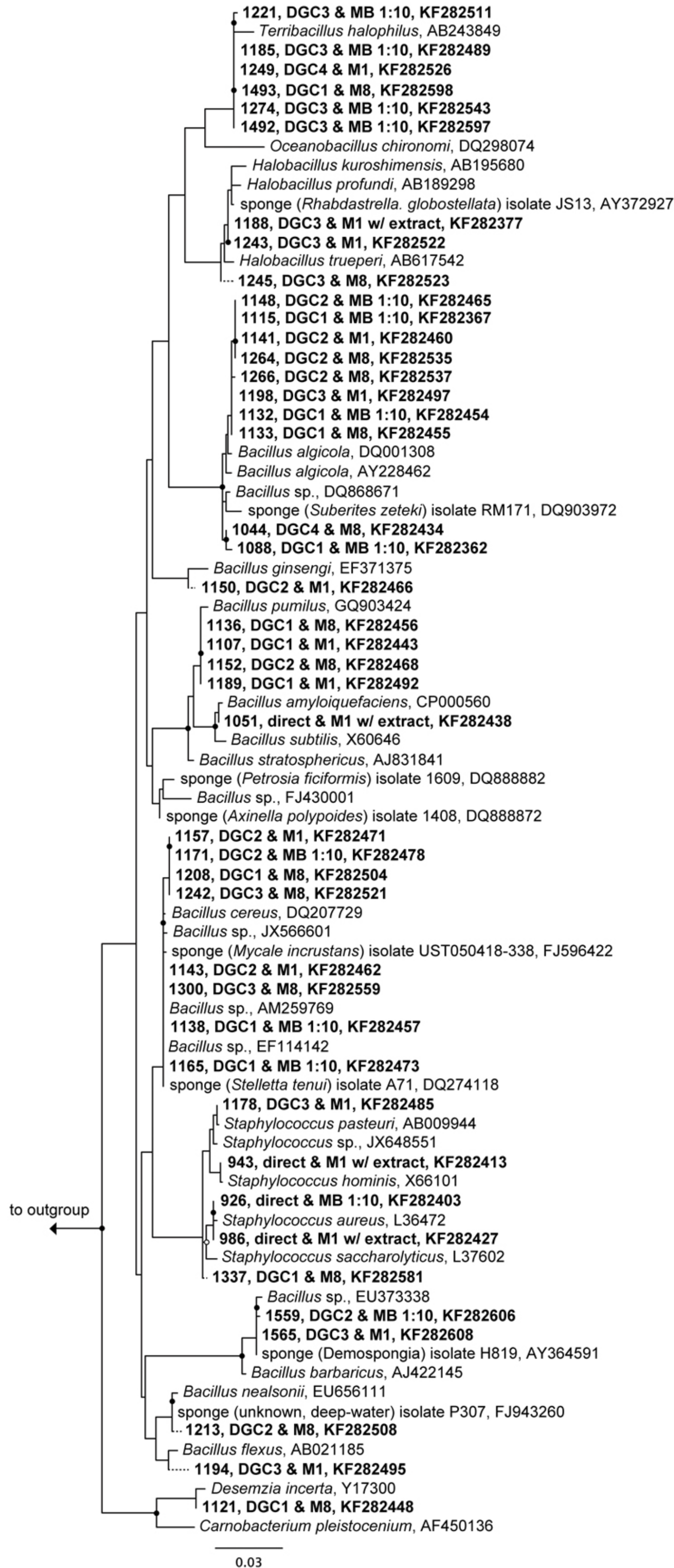
Supplementary Figure 2.4 16S rRNA-based phylogeny of *R. globostellata*-associated *Alphaproteobacteria* organisms. Details are the same as those provided in Figure 2.3



Supplementary Figure 2.5 16S rRNA-based phylogeny of *R. globostellata*-associated *Actinobacteria* organisms. Details are the same as those provided in Figure 2.3



Supplementary Figure 2.6 16S rRNA-based phylogeny of *R. globostellata*-associated *Firmicutes* organisms. Details are the same as those provided in Figure 2.3



Supplementary Table 3.1 Habitat file used for grouping of samples in multivariate analyses (nMDS & adonis).

Name	Habitat	Group	MID GeoB.sff	MID MikeH.sff
Acan_deep_PS508	mesophotic	*Acanthella*	ACTACTATGT	
Acan_deep_PS773	mesophotic	*Acanthella*	ACGCGAGTAT	
Acan_shallow_PS701	shallow	*Acanthella*	TAGAGACGAG	
Cally_deep_PS483	mesophotic	*Callyspongia*	CGAGAGATAC	
Cally_deep_PS484	mesophotic	*Callyspongia*		TAGTATCAGC
Cally_deep_PS700	mesophotic	*Callyspongia*	TACACACACT	
Cally_deep_PS719	mesophotic	*Callyspongia*	TACACGTGAT	
Cally_shallow_PSC1	shallow	*Callyspongia*		TCTCTATGCG
Cally_shallow_PSC2	shallow	*Callyspongia*	TGATACGTCT	
Cally_shallow_PSC3	shallow	*Callyspongia*		TGATACGTCT
Rhabda_deep_PS1	mesophotic	*Rhabdastrella*	ACATACGCGT	
Rhabda_deep_PS2	mesophotic	*Rhabdastrella*	ACTGTACAGT	
Rhabda_deep_PS4	mesophotic	*Rhabdastrella*	AGACTATACT	
Rhabda_deep_PS699	mesophotic	*Rhabdastrella*	TCTCTATGCG	
Rhabda_deep_PS718	mesophotic	*Rhabdastrella*		CTCGCGTGTC
Rhabda_deep_PS720	mesophotic	*Rhabdastrella*	ACGAGTGCGT	
Rhabda_deep_PS723	mesophotic	*Rhabdastrella*	ACGCTCGACA	
Rhabda_deep_PS724	mesophotic	*Rhabdastrella*	ATCAGACACG	
Rhabda_shallow_PS1	shallow	*Rhabdastrella*	AGCGTCGTCT	
Rhabda_shallow_PS2	shallow	*Rhabdastrella*	AGTACGCTAT	
Rhabda_shallow_PS3	shallow	*Rhabdastrella*	ATAGAGTACT	
Rhabda_shallow_PSRG1	shallow	*Rhabdastrella*		AGACGCACTC
Rhabda_shallow_PSRG2	shallow	*Rhabdastrella*		AGCACTGTAG
water_deep1	mesophotic	watercolumn		ACGCTCGACA
water_deep2	mesophotic	watercolumn		AGACGCACTC
water_deep3	mesophotic	watercolumn		AGCACTGTAG
water_shallow1	shallow	watercolumn		ATCAGACACG
water_shallow2	shallow	watercolumn		ATATCGCGAG

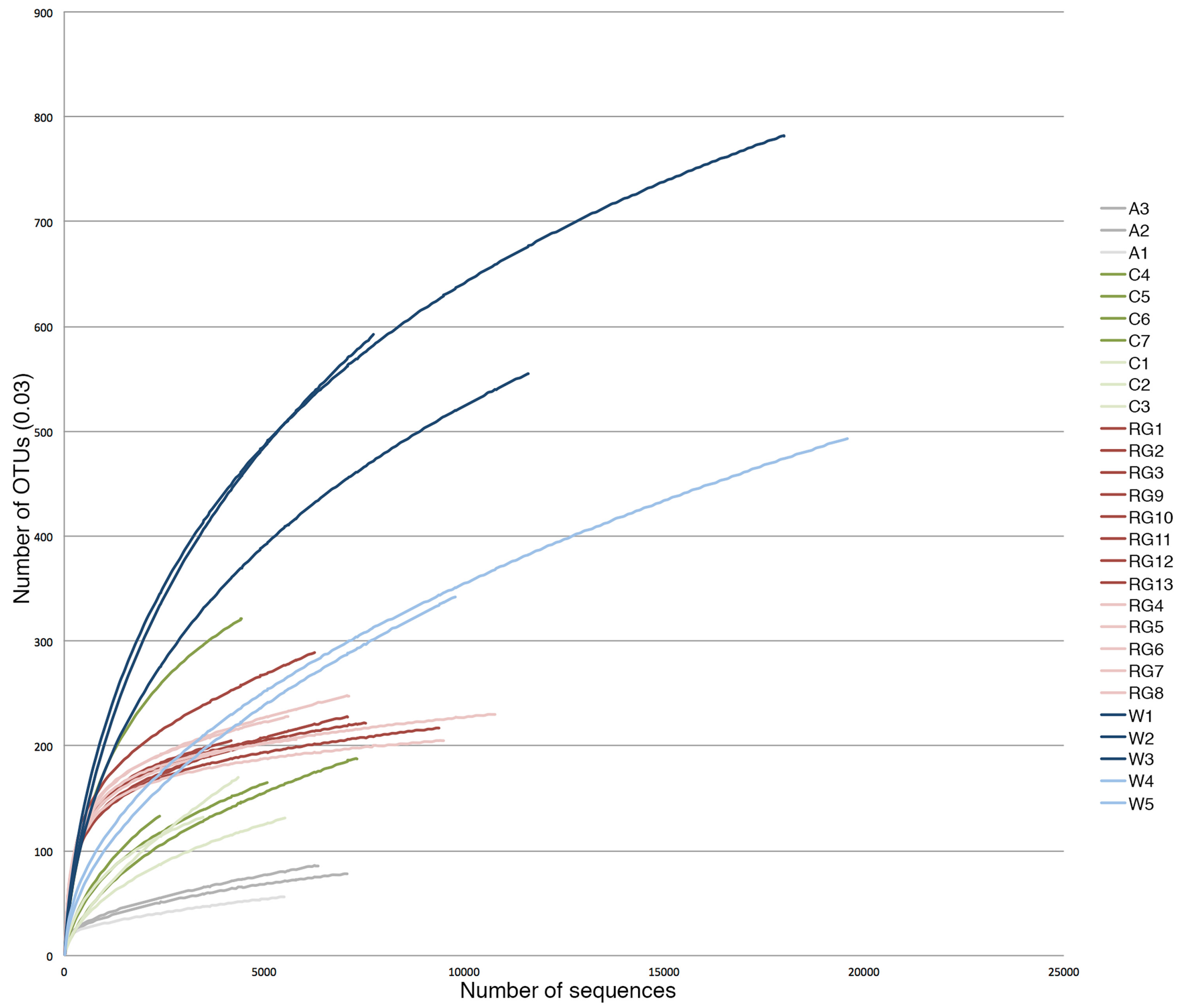
Supplementary Table 3.2 Analysis of Bray-Curtis (relative abundance) and Jaccard (presence-absence) dissimilarities among randomized *Rhabdastrella* and *Callyspongia* samples. Results represent the two randomized sponge groups with p-values for betadisper/permutest (1000 permutations) and p-values and R square for adonis analyses (1000 permutations).

Randomized Dataset	***R. globostellata*** **- 'habitat'**			***Callyspongia*** **sp. - 'habitat'**		
	betadisper / permutest	**adonis**	**R2**	**betadisper / permutest**	**adonis**	**R2**
relative abundance randomized #1	0.056	0.358	0.087	0.873	0.687	0.114
relative abundance randomized #2	0.180	0.589	0.068	0.064	0.373	0.175
relative abundance randomized #3	0.351	0.111	0.130	0.739	0.752	0.110
presence/absence randomized #1	0.345	0.554	0.073	0.220	0.635	0.153
presence/absence randomized #2	0.986	0.437	0.076	0.758	0.813	0.148
presence/absence randomized #3	0.788	0.677	0.069	0.373	0.151	0.185

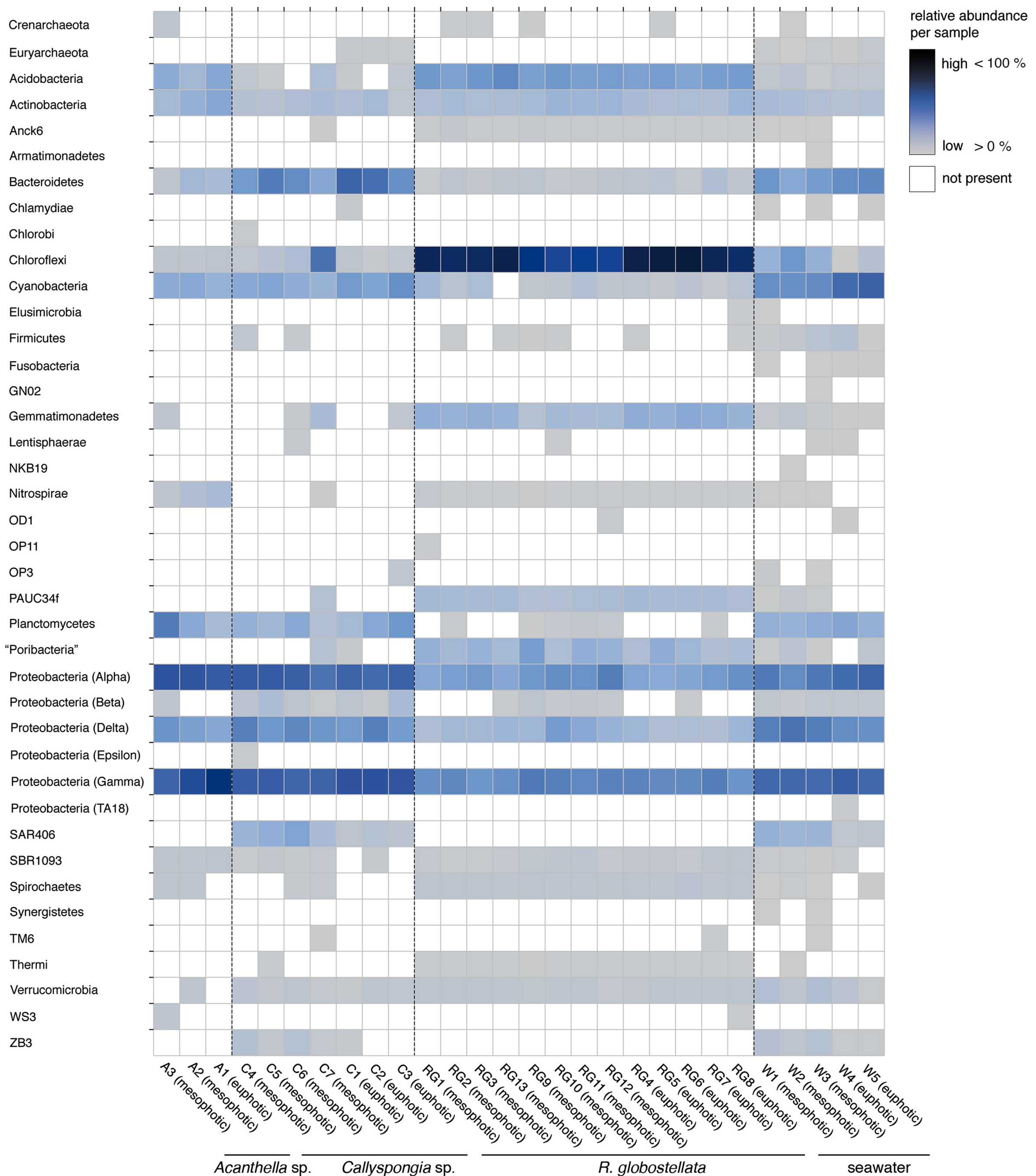
Supplementary Table 3.3 Multivariate cut off level analysis (MultiCoLA) using permuted Mantel tests with Pearson's correlation coefficient between full and reduced datasets.

	dominant OTUs			**rare OTUs**		
Cut off	**Procrustes**	**Non-par**	**Abundance**	**Procrustes**	**Non-par**	**Abundance**
0.01	0.998876245	0.999952675	202390	0.955929173	0.990847212	182344
0.05	0.998026749	0.999700943	194212	0.955929173	0.990847212	182344
0.1	0.997643621	0.999170372	183910	0.955929173	0.990847212	182344
0.15	0.998851376	0.997745964	173693	0.98654765	0.984225522	164124
0.2	0.998917036	0.996643441	163189	0.991355015	0.983824706	146106
0.25	0.997701669	0.994084126	153097	0.991355015	0.983824706	146106
0.3	0.997440442	0.993081641	143050	0.990759805	0.983008853	138567
0.35	0.994210387	0.991420608	132532	0.944929121	0.981574891	131578
0.4	0.993348484	0.988079435	121974	0.944721771	0.98080503	120438
0.45	0.99016401	0.98464139	111935	0.931181411	0.946154857	110696
0.5	0.991230448	0.978697577	101775	0.944180036	0.946765191	101095
0.55	0.98038228	0.965281782	91506	0.93263475	0.94471845	91200
0.6	0.951616248	0.913760673	81235	0.915612303	0.926259791	81470
0.65	0.914343455	0.771746681	69955	0.83228007	0.924876486	71119
0.7	-	-	58329	0.829616413	0.845048896	60749
0.75	-	-	40311	0.835164794	0.846246044	50792
0.8	-	-	40311	0.844161613	0.830130533	40827
0.85	-	-	22091	0.842720293	0.829341574	30493
0.9	-	-	-	0.841320168	0.819130931	20376
0.95	-	-	-	0.837169281	0.785577375	10170
0.99	-	-	-	0.357933116	0.247504821	2041

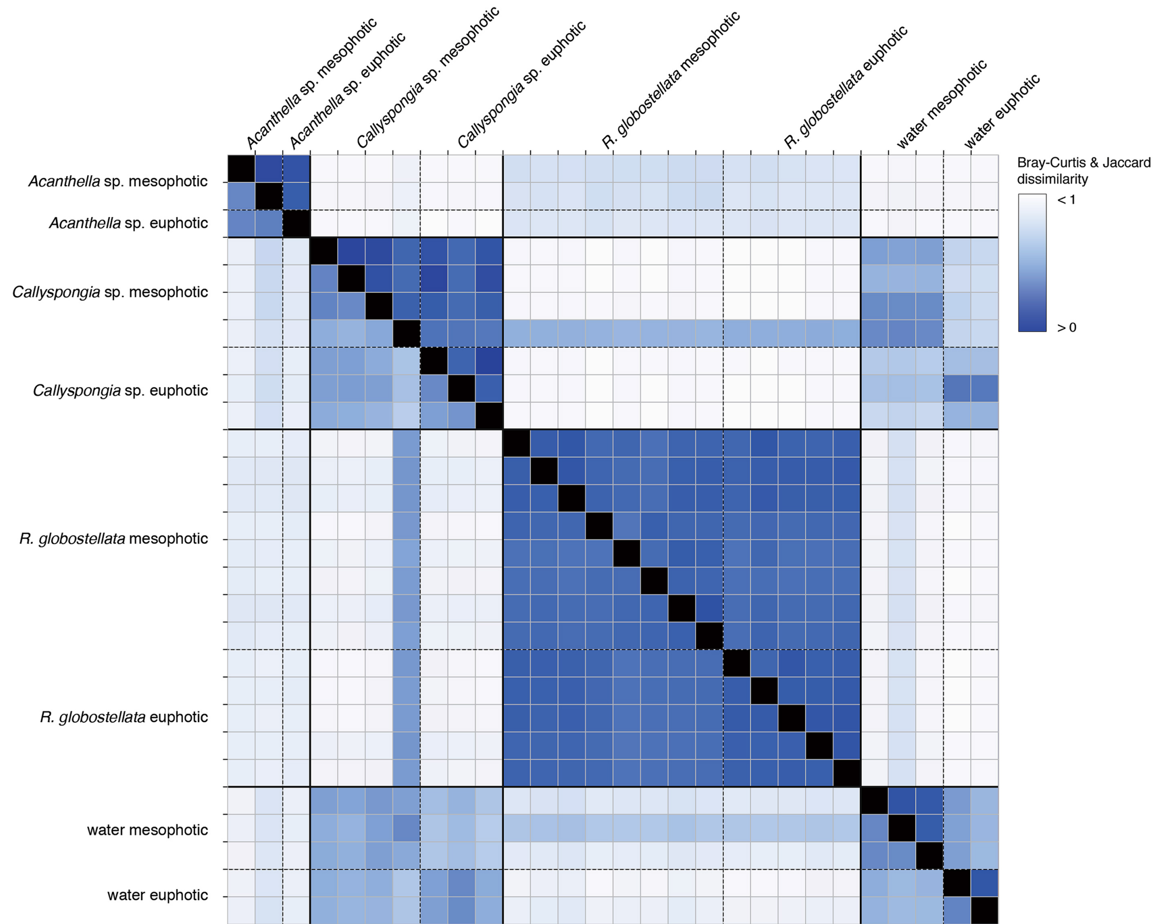
Supplementary Figure 3.1 Rarefaction curves of 97%-OTU 16S rRNA amplicon data for each sample.



Supplementary Figure 3.2 Taxonomic heat map based on relative abundance of assigned 97%-OTUs for each taxon on phylum level (i.e., either sponge specimen or seawater sample).



Supplementary Figure 3.3 Pairwise community dissimilarity heat map for each sample based on 97%-OTUs. The lower panel represents Jaccard (presence-absence) and the upper panel Bray-Curtis dissimilarities (relative abundance).



Supplementary Material - Chapter III

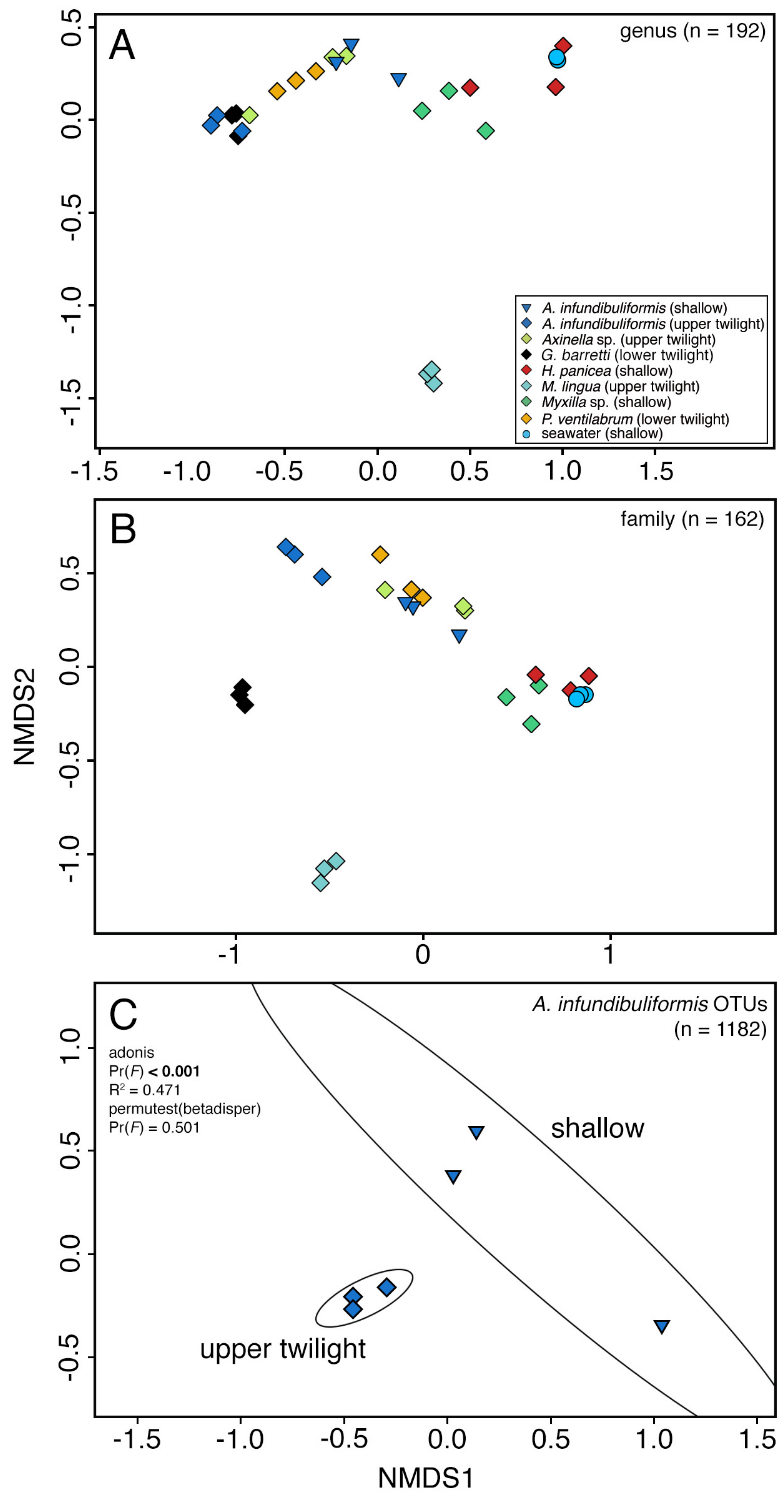
Supplementary Table 4.1 Summary of the indicator species (i.e., 97% OTUs) analysis performed by the function *multipatt.* Shown is 'stat' as minimum value of the statistic for selecting the indicator OTUs. Only OTUs with significant p values < 0.05 are summarized. In addition for each OTU, Greengenes classification results are added from phylum to species level if available. Only the top 10 results for each sponge taxon is shown.

OTU	stat	p.value	Domain	Phylum	Class	Order	Family	Genus	Species
***Phakellia ventilabrum* (n = 153)**									
Otu000121	1	0.001	Bacteria	Proteobacteria	Gammaproteobacteria	unclassified	unclassified	unclassified	unclassified
Otu000139	0.877	0.01	Bacteria	Proteobacteria	unclassified	unclassified	unclassified	unclassified	unclassified
Otu000342	1	0.003	Bacteria	Proteobacteria	Gammaproteobacteria	unclassified	unclassified	unclassified	unclassified
Otu000378	0.999	0.003	Bacteria	Proteobacteria	unclassified	unclassified	unclassified	unclassified	unclassified
Otu001422	0.667	0.05	Archaea	Crenarchaeota	Thaumarchaeota	Cenarchaeales	Cenarchaeaceae	Nitrosopumilus	unclassified
Otu001934	1	0.003	Bacteria	Proteobacteria	Alphaproteobacteria	unclassified	unclassified	unclassified	unclassified
Otu002228	1	0.003	Bacteria	Proteobacteria	unclassified	unclassified	unclassified	unclassified	unclassified
Otu002275	0.811	0.022	Bacteria	Proteobacteria	unclassified	unclassified	unclassified	unclassified	unclassified
Otu002524	0.752	0.018	Archaea	Crenarchaeota	Thaumarchaeota	Cenarchaeales	Cenarchaeaceae	Nitrosopumilus	unclassified
Otu002903	1	0.003	Bacteria	Proteobacteria	unclassified	unclassified	unclassified	unclassified	unclassified
***Geodia barretti* (n = 374)**									
OTU	**stat**	**p.value**	**Domain**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
Otu000087	0.775	0.009	Bacteria	Proteobacteria	Gammaproteobacteria	unclassified	unclassified	unclassified	unclassified
Otu000142	0.832	0.007	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	HTCC2188	HTCC	unclassified
Otu000218	0.92	0.004	Bacteria	Acidobacteria	PAUC37f	unclassified	unclassified	unclassified	unclassified
Otu000292	0.999	0.004	Bacteria	Acidobacteria	Acidobacteria-6	BPC015	unclassified	unclassified	unclassified
Otu000488	0.998	0.004	Bacteria	Chloroflexi	SAR202	unclassified	unclassified	unclassified	unclassified
Otu000527	0.968	0.004	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	unclassified	unclassified
Otu000531	0.892	0.009	Bacteria	Chloroflexi	SAR202	unclassified	unclassified	unclassified	unclassified
Otu000540	0.902	0.004	Bacteria	Proteobacteria	Gammaproteobacteria	unclassified	unclassified	unclassified	unclassified
Otu000543	1	0.004	Bacteria	Proteobacteria	Gammaproteobacteria	unclassified	unclassified	unclassified	unclassified
Otu000549	0.931	0.004	Bacteria	Chloroflexi	SAR202	unclassified	unclassified	unclassified	unclassified
***Axinella infundibuliformis* mesophotic (n = 62)**									
OTU	**stat**	**p.value**	**Domain**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
Otu000144	0.774	0.013	Bacteria	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	unclassified	unclassified
Otu000215	0.853	0.014	Bacteria	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	unclassified	unclassified
Otu000729	0.632	0.049	Archaea	Crenarchaeota	Thaumarchaeota	Cenarchaeales	Cenarchaeaceae	Nitrosopumilus	unclassified
Otu000742	0.909	0.005	Archaea	Crenarchaeota	Thaumarchaeota	Cenarchaeales	Cenarchaeaceae	Nitrosopumilus	unclassified
Otu002418	0.915	0.005	Archaea	Crenarchaeota	Thaumarchaeota	Cenarchaeales	Cenarchaeaceae	Nitrosopumilus	unclassified
Otu003072	0.912	0.005	Archaea	Crenarchaeota	Thaumarchaeota	Cenarchaeales	Cenarchaeaceae	Nitrosopumilus	unclassified
Otu003184	0.767	0.013	Bacteria	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	unclassified	unclassified
Otu006577	0.767	0.009	Bacteria	Proteobacteria	Alphaproteobacteria	unclassified	unclassified	unclassified	unclassified
Otu008996	0.934	0.005	Archaea	Crenarchaeota	Thaumarchaeota	Cenarchaeales	Cenarchaeaceae	Nitrosopumilus	unclassified
Otu009043	0.731	0.005	Archaea	Crenarchaeota	Thaumarchaeota	Cenarchaeales	Cenarchaeaceae	Nitrosopumilus	unclassified
***Axinella* sp. (n = 110)**									
OTU	**stat**	**p.value**	**Domain**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
Otu000129	0.943	0.003	Bacteria	Proteobacteria	unclassified	unclassified	unclassified	unclassified	unclassified
Otu001166	0.999	0.003	Bacteria	Proteobacteria	Gammaproteobacteria	unclassified	unclassified	unclassified	unclassified
Otu001175	0.98	0.003	Bacteria	unclassified	unclassified	unclassified	unclassified	unclassified	unclassified
Otu001317	0.99	0.003	Archaea	Crenarchaeota	Thaumarchaeota	Cenarchaeales	Cenarchaeaceae	Nitrosopumilus	unclassified
Otu001502	0.98	0.003	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Nitrospinaceae	Nitrospina	unclassified
Otu002672	0.957	0.003	Bacteria	Proteobacteria	Gammaproteobacteria	unclassified	unclassified	unclassified	unclassified
Otu003466	0.827	0.01	Bacteria	Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	unclassified	unclassified
Otu003540	0.964	0.003	Bacteria	Proteobacteria	Alphaproteobacteria	unclassified	unclassified	unclassified	unclassified
Otu004426	0.987	0.003	Bacteria	Proteobacteria	Alphaproteobacteria	unclassified	unclassified	unclassified	unclassified
Otu004850	0.773	0.018	Bacteria	Proteobacteria	Gammaproteobacteria	unclassified	unclassified	unclassified	unclassified
***Mycale lingua* (n = 80)**									
OTU	**stat**	**p.value**	**Domain**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
Otu000123	1	0.004	Bacteria	Chloroflexi	Anaerolineae	Caldilineales	Caldilineaceae	unclassified	unclassified
Otu000125	0.996	0.004	Bacteria	Proteobacteria	Gammaproteobacteria	unclassified	unclassified	unclassified	unclassified
Otu000143	1	0.004	Bacteria	Proteobacteria	Betaproteobacteria	unclassified	unclassified	unclassified	unclassified
Otu000162	0.998	0.004	Bacteria	Firmicutes	Bacilli	Bacillales	Alicyclobacillaceae	Alicyclobacillus	unclassified
Otu000230	0.997	0.004	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	haemolyticus
Otu000238	0.993	0.004	Bacteria	Proteobacteria	Deltaproteobacteria	Entotheonellales	Entotheonellaceae	Candidatus_Entotheonella	unclassified
Otu000240	0.999	0.004	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Erwinia	unclassified
Otu000263	0.964	0.011	Bacteria	AncK6	unclassified	unclassified	unclassified	unclassified	unclassified
Otu000277	1	0.004	Bacteria	Proteobacteria	unclassified	unclassified	unclassified	unclassified	unclassified
Otu000317	1	0.004	Bacteria	Chloroflexi	Anaerolineae	Caldilineales	Caldilineaceae	unclassified	unclassified
***Myxilla* sp. (n = 25)**									
OTU	**stat**	**p.value**	**Domain**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
Otu000022	1	0.003	Bacteria	Proteobacteria	Alphaproteobacteria	unclassified	unclassified	unclassified	unclassified
Otu000368	0.999	0.003	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	unclassified	unclassified	unclassified
Otu000638	0.794	0.006	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	unclassified	unclassified	unclassified
Otu002056	1	0.003	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Endozoicimonaceae	unclassified	unclassified
Otu002915	1	0.003	Bacteria	Proteobacteria	Gammaproteobacteria	unclassified	unclassified	unclassified	unclassified
Otu003504	1	0.003	Bacteria	Proteobacteria	Alphaproteobacteria	unclassified	unclassified	unclassified	unclassified
Otu012379	1	0.003	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	unclassified
Otu012690	0.748	0.01	Bacteria	Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	unclassified	unclassified
Otu016360	1	0.003	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	unclassified	unclassified	unclassified
Otu016422	1	0.003	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	unclassified	unclassified	unclassified

Supplementary Table 4.1 (continued)

OTU	stat	p.value	Domain	Phylum	Class	Order	Family	Genus	Species
Halichondria panicea **(n = 127)**									
Otu000089	1	0.005	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified	unclassified
Otu001049	0.778	0.013	Bacteria	Cyanobacteria	Synechococcophycideae	Synechococcales	Synechococcaceae	unclassified	unclassified
Otu001114	0.95	0.007	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	unclassified	unclassified	unclassified
Otu001339	0.813	0.008	Bacteria	Cyanobacteria	Synechococcophycideae	Synechococcales	Synechococcaceae	Synechococcus	unclassified
Otu001655	0.997	0.005	Bacteria	Proteobacteria	unclassified	unclassified	unclassified	unclassified	unclassified
Otu001971	0.757	0.02	Bacteria	Cyanobacteria	Synechococcophycideae	Synechococcales	Synechococcaceae	unclassified	unclassified
Otu002906	0.706	0.03	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Coxiellaceae	Rickettsiella	unclassified
Otu002976	0.77	0.018	Bacteria	Proteobacteria	unclassified	unclassified	unclassified	unclassified	unclassified
Otu003761	1	0.005	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Octadecabacter	unclassified
Otu003911	1	0.005	Bacteria	Proteobacteria	Gammaproteobacteria	unclassified	unclassified	unclassified	unclassified
Axinella infundibuliformis **euphotic (n = 190)**									
OTU	**stat**	**p.value**	**Domain**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
Otu000165	0.783	0.01	Bacteria	Proteobacteria	Gammaproteobacteria	unclassified	unclassified	unclassified	unclassified
Otu001070	0.758	0.001	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Aliivibrio	fischeri
Otu001374	0.885	0.03	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	TK06	unclassified	unclassified
Otu001422	0.788	0.009	Archaea	Crenarchaeota	Thaumarchaeota	Cenarchaeales	Cenarchaeaceae	Nitrosopumilus	unclassified
Otu002028	0.752	0.003	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	HTCC2188	HTCC	unclassified
Otu002431	0.939	0.003	Bacteria	Cyanobacteria	Synechococcophycideae	Synechococcales	Synechococcaceae	unclassified	unclassified
Otu002742	0.849	0.009	Bacteria	Planctomycetes	Phycisphaerae	Phycisphaerales	unclassified	unclassified	unclassified
Otu003242	0.852	0.026	Bacteria	unclassified	unclassified	unclassified	unclassified	unclassified	unclassified
Otu003584	0.63	0.038	Archaea	Crenarchaeota	Thaumarchaeota	Cenarchaeales	Cenarchaeaceae	Nitrosopumilus	unclassified
Otu003632	0.869	0.005	Bacteria	Proteobacteria	Gammaproteobacteria	unclassified	unclassified	unclassified	unclassified
Seawater (n = 590)									
OTU	**stat**	**p.value**	**Domain**	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
Otu000075	1	0.003	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	unclassified	unclassified
Otu000085	1	0.003	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	OCS155	unclassified	unclassified
Otu000092	1	0.003	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	unclassified	unclassified
Otu000095	1	0.003	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Psychrobacter	pacificensis
Otu000106	1	0.003	Bacteria	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Photobacterium	angustum
Otu000119	1	0.003	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Candidatus_Portiera	unclassified
Otu000146	1	0.003	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	OM60	unclassified	unclassified
Otu000148	1	0.003	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Candidatus_Portiera	unclassified
Otu000198	1	0.003	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	Candidatus_Portiera	unclassified
Otu000205	1	0.003	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Octadecabacter	unclassified

Supplementary Figure 4.1 nMDS ordinations based on Bray-Curtis dissimilarities of all samples for two taxonomic ranks: A genus and B family with number (n) of available 'species' in brackets. Plot C shows the ordination of shallow and upper twilight *A. infundibuliformis* sponges with *a posteriori* groups drawn as dispersion ellipses with a confidence interval of 95%. Adonis and permutest(betadisper) results of the effects of habitat on the community structure are added to this plot subsequently.



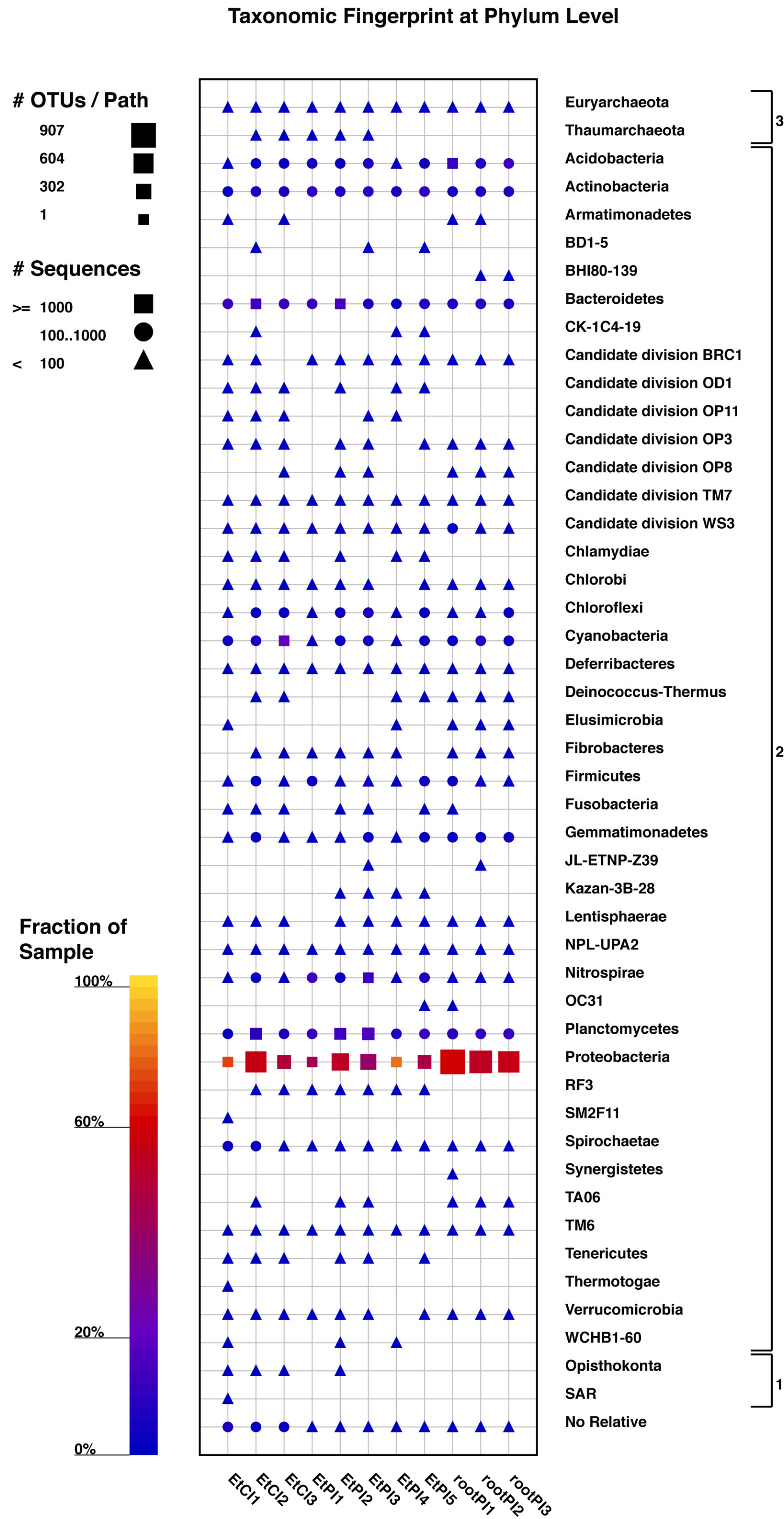
Supplementary Table 5.1 Fingerprint phyla - raw table for Figure 5.1 and Supplementary Figure 5.1.

# total	% total	EtCl1	EtCl2	EtCl3	# EtCl	% EtCl	EtPl1	EtPl2	EtPl3	EtPl4	EtPl5	# EtPl	% EtPl	rootPl1	rootPl2	rootPl3	# rootPl	% rootPl	Taxonomy
49772	55.49	5702	8007	4077	17786	59.45	1866	4617	3524	5117	3139	18263	51.53	5642	3670	4411	13723	56.38	Proteobacteria (total)
18059	20.13	1044	3718	2946	7708	25.76	908	1870	1529	432	1569	6308	17.80	1101	1334	1608	4043	16.61	Alphaproteobacteria
16790	18.72	1025	1984	714	3723	12.44	469	1465	1009	4430	692	8065	22.75	1794	1359	1849	5002	20.55	Gammaproteobacteria
10028	11.18	2124	1228	299	3651	12.20	362	668	726	211	672	2639	7.45	2399	665	674	3738	15.36	Deltaproteobacteria
1718	1.92	1060	308	22	1390	4.65	27	78	51	9	24	189	0.53	48	48	43	139	0.57	Betaproteobacteria
23	0.03	12	6	3	21	0.07	0	0	0	0	0	0	0.00	2	0	0	2	0.01	Epsilonproteobacteria
3154	3.52	437	763	93	1293	4.32	100	536	209	35	182	1062	3.00	298	264	237	799	3.28	misc. Proteobacteria
8522	9.50	129	1285	467	1881	6.29	462	1085	1402	393	851	4193	11.83	831	734	883	2448	10.06	Planctomycetes
8209	9.15	867	1867	929	3663	12.24	482	1219	498	112	552	2863	8.08	580	644	459	1683	6.91	Bacteroidetes
5364	5.98	145	868	410	1423	4.76	431	568	927	242	704	2872	8.10	369	277	423	1069	4.39	Actinobacteria
4321	4.82	15	276	223	514	1.72	199	215	450	97	294	1255	3.54	1047	561	944	2552	10.48	Acidobacteria
4230	4.72	268	601	1717	2586	8.64	63	178	220	56	165	682	1.92	142	557	263	962	3.95	Cyanobacteria
2610	2.91	8	228	54	290	0.97	519	162	1141	47	402	2271	6.41	17	28	4	49	0.20	Nitrospirae
1426	1.59	8	166	107	281	0.94	79	256	402	49	138	924	2.61	64	38	119	221	0.91	Chloroflexi
1183	1.32	16	151	69	236	0.79	45	75	170	31	141	462	1.30	174	108	203	485	1.99	Gemmatimonadetes
1120	1.25	52	153	21	226	0.76	139	85	85	85	255	649	1.83	187	45	13	245	1.01	Firmicutes
622	0.69	209	264	9	482	1.61	1	22	4	22	24	73	0.21	52	12	3	67	0.28	Spirochaetae
575	0.64	3	41	45	89	0.30	23	42	88	15	66	234	0.66	110	50	92	252	1.04	Candidate division WS3
287	0.32	9	14	3	26	0.09	33	93	81	14	33	254	0.72	3	1	3	7	0.03	Candidate division TM7
220	0.25	17	92	23	132	0.44	3	26	9	0	1	39	0.11	13	10	26	49	0.20	Verrucomicrobia
215	0.24	9	14	16	39	0.13	0	13	3	5	8	29	0.08	65	55	27	147	0.60	Lentisphaerae
144	**0.16**	**1**	**8**	**1**	**10**	**0.03**	**6**	**9**	**5**	**13**	**15**	**48**	**0.14**	**24**	**45**	**17**	**86**	**0.35**	**Euryarchaeota**
96	0.11	1	3	0	4	0.01	4	5	6	4	16	35	0.10	13	18	26	57	0.23	Candidate division BRC1
95	0.11	1	14	8	23	0.08	2	6	4	2	27	41	0.12	8	19	4	31	0.13	TM6
94	0.10	3	48	1	52	0.17	0	1	3	0	33	37	0.10	5	0	0	5	0.02	Fusobacteria
93	0.10	2	3	4	9	0.03	1	5	20	0	6	32	0.09	26	18	8	52	0.21	Chlorobi
86	0.10	1	10	16	27	0.09	6	4	12	1	4	27	0.08	19	7	6	32	0.13	Deferribacteres
64	0.07	1	3	1	5	0.02	2	7	9	5	1	24	0.07	19	10	6	35	0.14	NPL-UPA2
53	0.06	9	9	25	43	0.14	0	7	2	0	1	10	0.03	0	0	0	0	0.00	Tenericutes
50	**0.06**	**0**	**4**	**1**	**5**	**0.02**	**1**	**16**	**28**	**0**	**0**	**45**	**0.13**	**0**	**0**	**0**	**0**	**0.00**	**Thaumarchaeota**
40	0.04	0	11	2	13	0.04	0	0	0	1	1	2	0.01	4	8	13	25	0.10	Deinococcus-Thermus
35	0.04	6	4	3	13	0.04	0	5	2	0	6	13	0.04	1	7	1	9	0.04	Candidate division OP3
35	0.04	0	14	5	19	0.06	1	6	1	1	0	9	0.03	2	4	1	7	0.03	Fibrobacteres
21	0.02	0	0	1	1	0.00	0	2	1	0	0	3	0.01	3	10	4	17	0.07	Candidate division OP8
20	0.02	7	2	1	10	0.03	0	8	0	1	1	10	0.03	0	0	0	0	0.00	Candidate division OD1
15	0.02	0	3	0	3	0.01	0	2	1	0	0	3	0.01	5	2	2	9	0.04	TA06
14	0.02	2	3	1	6	0.02	0	0	7	1	0	8	0.02	0	0	0	0	0.00	Candidate division OP11
13	0.01	0	2	1	3	0.01	2	2	2	2	2	10	0.03	0	0	0	0	0.00	RF3
10	0.01	1	0	0	1	0.00	0	0	0	1	0	1	0.00	1	5	2	8	0.03	Elusimicrobia
9	0.01	1	3	1	5	0.02	0	2	0	1	1	4	0.01	0	0	0	0	0.00	Chlamydiae
6	0.01	3	0	1	4	0.01	0	0	0	0	0	0	0.00	1	1	0	2	0.01	Armatimonadetes
6	0.01	0	1	0	1	0.00	0	0	0	2	3	5	0.01	0	0	0	0	0.00	CK-1C4-19
5	0.01	0	0	0	0	0.00	0	1	2	1	1	5	0.01	0	0	0	0	0.00	Kazan-3B-28
5	0.01	2	0	0	2	0.01	0	2	0	1	0	3	0.01	0	0	0	0	0.00	WCHB1-60
4	0.00	0	1	0	1	0.00	0	0	1	0	2	3	0.01	0	0	0	0	0.00	BD1-5
3	0.00	0	0	0	0	0.00	0	0	0	0	0	0	0.00	0	2	1	3	0.01	BHI80-139
2	0.00	0	0	0	0	0.00	0	0	1	0	0	1	0.00	0	1	0	1	0.00	JL-ETNP-Z39
2	0.00	0	0	0	0	0.00	0	0	0	0	1	1	0.00	1	0	0	1	0.00	OC31
2	0.00	2	0	0	2	0.01	0	0	0	0	0	0	0.00	0	0	0	0	0.00	SM2F11
2	0.00	0	0	0	0	0.00	0	0	0	0	0	0	0.00	2	0	0	2	0.01	Synergistetes
1	0.00	1	0	0	1	0.00	0	0	0	0	0	0	0.00	0	0	0	0	0.00	Thermotogae
21	0.02	4	1	15	20	0.07	0	1	0	0	0	1	0.00	0	0	0	0	0.00	Opisthokonta
5	0.01	5	0	0	5	0.02	0	0	0	0	0	0	0.00	0	0	0	0	0.00	SAR
792	0.88	381	122	189	692	2.31	2	38	17	5	16	78	0.22	12	5	5	22	0.09	
89701		7501	14173	8243	29917		4370	8746	9111	6322	6894	35443		9430	6947	7964	24341		

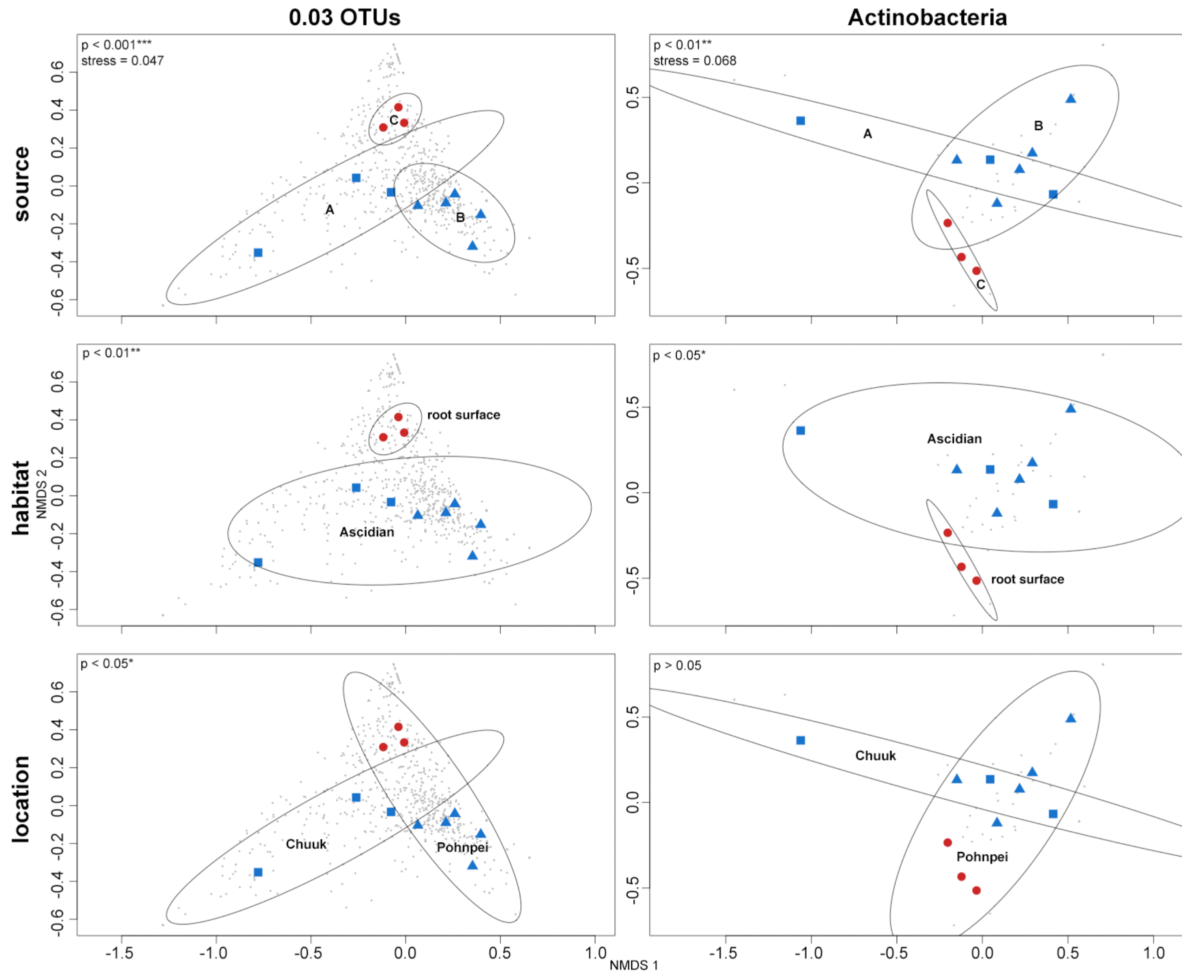
Supplementary Table 5.2 Actinobacteria diversity - raw table for Actinobacteria heatmap, extracted raw data with additional frequency information

			EtCl1		EtCl2		EtCl3		EtPl1		EtPl2		EtPl3		EtPl4		EtPl5		rootPl1		rootPl2		rootPl3	
Taxonomy	num seqs total	abund seqs actino	num seqs	abund seqs actino	num seqs	abund seqs actino	num seqs	abund seqs actino	num seqs	abund seqs actino	num seqs	abund seqs actino	num seqs	abund seqs actino	num seqs	abund seqs actino	num seqs	abund seqs actino	num seqs	abund seqs actino	num seqs	abund seqs actino	num seqs	abund seqs actino
Acidimicrobiaceae, Acidimicrobium	2	0.04	0.00	0.00	2.00	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Acidimicrobiaceae, Ilumatobacter	156	2.91	0.00	0.00	35.00	4.03	17.00	4.15	4.00	0.93	13.00	2.29	6.00	0.65	0.00	0.00	27.00	3.84	18.00	4.88	16.00	5.78	20.00	4.73
Acidimicrobiaceae, uncultured	230	4.29	5.00	3.45	47.00	5.41	12.00	2.93	16.00	3.71	18.00	3.17	22.00	2.37	4.00	1.65	28.00	3.98	21.00	5.69	22.00	7.94	35.00	8.27
Acidimicrobiales, Candidatus, Microthrix	83	1.55	0.00	0.00	30.00	3.46	3.00	0.73	11.00	2.55	10.00	1.76	15.00	1.62	5.00	2.07	8.00	1.14	0.00	0.00	1.00	0.36	0.00	0.00
Acidimicrobiales, Iamia	29	0.54	0.00	0.00	13.00	1.50	2.00	0.49	3.00	0.70	4.00	0.70	0.00	0.00	2.00	0.83	0.00	0.00	2.00	0.54	1.00	0.36	2.00	0.47
Acidimicrobiales, OCS155	1855	34.58	13.00	8.97	233.00	26.84	49.00	11.95	124.00	28.77	147.00	25.88	268.00	28.91	118.00	48.76	281.00	39.91	233.00	63.14	146.00	52.71	243.00	57.45
Acidimicrobiales, Sva0996	787	14.67	2.00	1.38	141.00	16.24	39.00	9.51	62.00	14.39	89.00	15.67	119.00	12.84	35.00	14.46	133.00	18.89	47.00	12.74	54.00	19.49	66.00	15.60
Acidimicrobiales, uncultured	602	11.22	12.00	8.28	153.00	17.63	56.00	13.66	49.00	11.37	86.00	15.14	80.00	8.63	28.00	11.57	62.00	8.81	27.00	7.32	22.00	7.94	27.00	6.38
Corynebacteriaceae, Corynebacterium	1	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mycobacteriaceae, Mycobacterium	78	1.45	0.00	0.00	12.00	1.38	0.00	0.00	9.00	2.09	21.00	3.70	25.00	2.70	0.00	0.00	5.00	0.71	1.00	0.27	4.00	1.44	1.00	0.24
Acidothermaceae, Acidothermus	1	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sporichthyaceae, Sporichthya	5	0.09	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.23	2.00	0.35	2.00	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sporichthyaceae, uncultured	1	0.02	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Kineosporiales, Kineosporiaceae	1	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Demequinaceae, Demequina	3	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00	0.35	1.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Demequinaceae, Lysinimicrobium	8	0.15	0.00	0.00	7.00	0.81	0.00	0.00	0.00	0.00	1.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dermabacteraceae, Brachybacterium	1	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Intrasporangiaceae, Humibacillus	1	0.02	1.00	0.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Intrasporangiaceae, Janibacter	1	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.36	0.00	0.00
Microbacteriaceae, Candidatus Aquiluna	1	0.02	0.00	0.00	0.00	0.00	1.00	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Microbacteriaceae, Cryobacterium	2	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00	0.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Microbacteriaceae, Curtobacterium	1	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Microbacteriaceae, Leucobacter	1	0.02	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Microbacteriaceae, Microbacterium	1	0.02	0.00	0.00	1.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Microbacteriaceae, Zimmermannella	1	0.02	0.00	0.00	1.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Micromonosporaceae, Longispora	4	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00	0.35	2.00	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Micromonosporaceae, Salinispora	396	7.38	0.00	0.00	36.00	4.15	191.00	46.59	24.00	5.57	24.00	4.23	103.00	11.11	1.00	0.41	17.00	2.41	0.00	0.00	0.00	0.00	0.00	0.00
Micromonosporaceae, Verrucosispora	103	1.92	103.00	71.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Actinobacteria, PeM15	1	0.02	0.00	0.00	0.00	0.00	1.00	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nocardioidaceae, Nocardioides	17	0.32	0.00	0.00	2.00	0.23	3.00	0.73	1.00	0.23	5.00	0.88	4.00	0.43	0.00	0.00	2.00	0.28	0.00	0.00	0.00	0.00	0.00	0.00
Propionibacteriales, Propionibacteriaceae	21	0.39	0.00	0.00	5.00	0.58	1.00	0.24	2.00	0.46	6.00	1.06	4.00	0.43	0.00	0.00	1.00	0.14	1.00	0.27	1.00	0.36	0.00	0.00
Propionibacteriaceae, Haloactinopolyspora	1	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Propionibacteriaceae, uncultured	6	0.11	0.00	0.00	0.00	0.00	1.00	0.24	0.00	0.00	2.00	0.35	0.00	0.00	1.00	0.41	0.00	0.00	0.00	0.00	1.00	0.36	1.00	0.24
Pseudonocardiaceae, Actinomycetospora	10	0.19	0.00	0.00	3.00	0.35	0.00	0.00	0.00	0.00	5.00	0.88	0.00	0.00	0.00	0.00	2.00	0.28	0.00	0.00	0.00	0.00	0.00	0.00
Pseudonocardiaceae, Crossiella	1	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pseudonocardiaceae, Sciscionella	3	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.00	0.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Coriobacteriaceae, uncultured	36	0.67	0.00	0.00	12.00	1.38	1.00	0.24	1.00	0.23	7.00	1.23	4.00	0.43	3.00	1.24	2.00	0.28	0.00	0.00	0.00	0.00	6.00	1.42
KIST-JJY010	18	0.34	0.00	0.00	5.00	0.58	1.00	0.24	1.00	0.23	5.00	0.88	2.00	0.22	2.00	0.83	2.00	0.28	0.00	0.00	0.00	0.00	0.00	0.00
MB-A2-108	1	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00
Euzebyaceae, Euzebya	8	0.15	0.00	0.00	2.00	0.23	0.00	0.00	1.00	0.23	3.00	0.53	0.00	0.00	1.00	0.41	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.24
Nitriliruptoraceae, Nitriliruptor	1	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
OPB41	15	0.28	1.00	0.69	1.00	0.12	2.00	0.49	1.00	0.23	2.00	0.35	1.00	0.11	4.00	1.65	1.00	0.14	0.00	0.00	0.00	0.00	2.00	0.47
Rubrobacteriaceae, Rubrobacter	1	0.02	0.00	0.00	1.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Gaiellaceae, Gaiella	3	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	2.00	0.54	0.00	0.00	0.00	0.00
Gaiellales, uncultured	468	8.72	7.00	4.83	104.00	11.98	24.00	5.85	51.00	11.83	86.00	15.14	85.00	9.17	20.00	8.26	52.00	7.39	13.00	3.52	7.00	2.53	19.00	4.49
Solirubrobacterales, 0319-6M6	1	0.02	0.00	0.00	1.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Solirubrobacterales, 480-2	381	7.10	0.00	0.00	19.00	2.19	5.00	1.22	67.00	15.55	10.00	1.76	179.00	19.31	16.00	6.61	80.00	11.36	4.00	1.08	1.00	0.36	0.00	0.00
Solirubrobacterales, Elev-16S-1332	10	0.19	0.00	0.00	1.00	0.12	1.00	0.24	1.00	0.23	5.00	0.88	1.00	0.11	1.00	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Solirubrobacteraceae, Solirubrobacter	1	0.02	0.00	0.00	1.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Solirubrobacterales, TM146	2	0.04	1.00	0.69	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Solirubrobacterales, YNPFFP1	3	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00	0.35	1.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
action seqs	5364.00		145.00			868.00		410.00		431.00		568.00		927.00		242.00		704.00		369.00		277.00		423.00
total bacteria seqs	89507.00		7500.00			14161.00		8241.00		4363.00		8721.00		9078.00		6309.00		6879.00		9406.00		6902.00		7947.00
abund actino seqs	5.99		1.93			6.13		4.98		9.88		6.51		10.21		3.84		10.23		3.92		4.01		5.32

Supplementary Figure 5.1 Taxonomic breakdown per sample at phylum level - showing all available taxonomic groups.



Supplementary Figure 5.2 non-metric multidimensional scaling (nMDS) for all 0.03 OTUs and Actinobacteria 0.03 OTUs for three treatments: source - A=EtCI (blue squares), B=EtPI (blue triangles), C=rootPI (red circles); habitat - ascidian or environmental; location - Chuuk or Pohnpei Island. Shown p values are from 'Permutational Multivariate Analysis of Variance Using Distance Matrices' (adonis) analysis. Shown ellipses are based on the treatments (A, B, C) used for the adonis hypothesis test.



Georg Steinert

Certified biologist and PhD student

Date of birth	08.13.1979
Place of birth	Wilhelmshaven
Citizenship	German

Email: georg.steinert@iba-science.de
Homepage: http://georg.iba-science.de

Studies

02l2013 - 05l2013	Guest research at the 'Centre for Microbial Innovation' University of Auckland, New Zealand
10l2012 - now	PhD student at Graduate School 'Science and Technology', 'Environmental Sciences and Biodiversity', University of Oldenburg
10l2011 - now	PhD student at the Environmental Biochemistry Working Group, Institute of Chemistry and Biology of the Marine Environment (ICBM)
05l2010	Diploma degree in Biology Diploma thesis: 'Molecular study of the blue mussel (*Mytilus edulis*) within the Wadden Sea of Lower Saxony'
10l2002 - 05l2010	Biology Carl von Ossietzky University of Oldenburg
10l2000 - 06l2004	Physics Carl von Ossietzky University of Oldenburg

Work experience

11l2011 - now	Research associate at the Environmental Biochemistry Working Group, Institute of the Chemistry and Biology of the Marine Environment (ICBM), University of Oldenburg
10l2010 - 10l2011	Research associate at the Planktology Working Group, Institute of Chemistry and Biology of the Marine Environment (ICBM), University of Oldenburg
06l2010 - 10l2011	Scientific assistant during the ICBM science exhibitions 'Understanding the Coasts' (2010) and 'Showcase Marine Sciences' (2011) & IdeenExpo Hannover 2011

Academic affiliations

Environmental Biochemistry Working Group, Institute of Chemistry and Biology of the Marine Environment (ICBM), 11l2011 - now

Planktology Working Group, Institute for Chemistry and Biology of the Marine Environment (ICBM), University of Oldenburg, 10l2010 - 10l2011

Systematics and Evolutionary Biology Working Group, Department of Biology and Environmental Sciences (IBU), University of Oldenburg, 9l2008 - 5l2010

Fellowships and grants

- Conference fellowship for the '2nd International Symposium on Sponge Microbiology 2014', German Academic Exchange Service DAAD
- Funding for the organization of the 'Computational Molecular Analysis Summer School 2014' - Graduate School 'Science and Technology', University of Oldenburg
- Funding for the organization of the 'Computational Molecular Analysis Summer School 2014' - International Relationship Office (IRO), University of Oldenburg
- Conference fellowship for the '9th World Sponge Conference 2013', German Academic Exchange Service DAAD
- Conference fellowship for the '9th World Sponge Conference 2013', Universitätsgesellschaft Oldenburg e.V. (UGO)
- Short term fellowship 'Microbial Symbiosis and Diversity in Marine Sponges', New Zealand from 02/2013 to 06/2013, Centre for Microbial Innovation, University of Auckland - German Academic Exchange Service DAAD
- Travel grant 2/2012 for the ISME14 conference in Copenhagen, Graduate school 'Science and Technology', University of Oldenburg
- Project funding of my diploma thesis ' Molecular study of the the blue mussel (*Mytilus edulis*) within the Wadden Sea of Lower Saxony' 2010 - EWE Foundation

Attended courses, workshops, summer schools

- Earth Microbiome Project, Bioinformatics Workshop at the Institute of Marine and Environmental Technology (IMET), Baltimore, 2014
- E.O. Wilson Symposium on Biodiversity at CvO University of Oldenburg, 2014
- 'Meta-analysis mini-workshop' at the ICBM Terramare, 2014
- 'Understanding adaptation to habitat fragmentation and climate - from genes to populations' Mini-Symposium at the University of Helsinki, 2014
- Science Software Day - Max Planck Institute for Marine Microbiology (MPI), Bremen, 2013
- 'Scientific Writing' - Graduiertenschule 'Science and Technology', Universität Oldenburg, 2012
- Computational Molecular Evolution - European Molecular Biology Organization (EMBO) Practical Course Institute of Marine Biology and Genetics (IMBG) - Hellenic Center for Marine Research (HCMR), Crete, 2012
- DZG Workshop on Next Generation Sequencing and Transcriptomics – Göttingen Center for Molecular Biosciences (GZMB), Georg-August-University Göttingen, 2011
- The Second White Sea Molecular Zoology Summer School – School of Molecular Zoology, White Sea Biological Station (Russia), Moscow State University, 2010
- Laboratory cooperation with the Ruhr-University of Bochum during my Diploma thesis (Dr. T. Huelsken), 2009
- 'Species in Space and Time – Biogeography' Workshop, Johannes Gutenberg University of Mainz, 2009

Teaching experience

- Organization of the 'Computational Molecular Analysis Summer School 2014' - Graduate School 'Science and Technology' (OLTECH), University of Oldenburg (2014)
- Tutor 'Concepts of Marine Ecology', ICBM (2014)
- Tutor 'Benthic Ecology', ICBM (2013)
- Tutor 'Chemical Ecology', ICBM (2013)
- Scientific assistant during the ICBM science exhibitions 'Understanding the Coasts' (2010) and 'Showcase Marine Sciences' (2011) & IdeenExpo Hannover (2011)
- Tutor 'Communicating Biology', University of Oldenburg (2005)

Field work experience

FS Sonne Cruise P7 - IFM-Geomar/ICBM, Kiel, 2014

ASSEMBLE (Association of European Marine Biological Laboratories) research project at the Sven Lovén Centre for Marine Sciences - Tjärnö (Sweden), 2012

Marine Biology Excursion – Giglio (Mediterranean Sea), 2011

Sampling at various *Mytilus edulis* mussel beds in the littoral zone of the Lower Saxonian Wadden Sea (Germany) during my diploma thesis, 2009

Field and sampling trips with the research vessel 'FS Senckenberg' (Senckenberg Institute, Wilhelmshaven) during my diploma thesis, 2009

Planktonic sampling assistant on the research vessel 'Navicula' (ICBM), 2008 – 2009

Publications

Steinert G, Taylor MW, Schupp PJ. (2015) 'Diversity of Actinobacteria associated with the marine ascidian *Eudistoma toealensis*' Marine Biotechnology, DOI: 10.1007/s10126-015-9622-3

Steinert G, Whitfield S, Taylor MW, Thoms C, Schupp PJ. (2014) 'Application of Diffusion Growth Chambers for the Cultivation of Marine Sponge-Associated Bacteria', Marine Biotechnology, DOI: 10.1007/s10126-0149575-y

Hillebrand H, **Steinert G**, Boersma M, Mahlzahn A, Meunier C, Plum C, Ptacnik R. (2013) 'Phytoplankton growth and N:P stoichiometry'. Limnology and Oceanography 58(6): 2076 - 2088, DOI: 10.4319/lo.2013.58.6.2076

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Conference proceedings

Talk at the 2nd International Symposium on Sponge Microbiology in Baltimore 2014. **Steinert G**, Deines P, Simister R, Taylor MW, Hoggard M, Schupp PJ. 'Sponge-associated microbial communities along a spatial gradient'

Poster at the 2nd International Symposium on Sponge Microbiology in Baltimore 2014. **Steinert G**, Deines P, Simister R, Taylor MW, Hoggard M, Schupp PJ. 'Are microbial community structures in three Guam reef sponges depth-independent?'

Talk at the 9th World Sponge Conference in Fremantle 2013. **Steinert G**, Whitfield S, Taylor MW, Schupp PJ. 'Phylogenetic analysis of bacteria isolated from sponges using a diffusion-growth-chamber'

Poster at the Youmares 4.0 in Oldenburg 2013. **Steinert G**, Whitfield S, Michael W. Taylor MW, Schupp PJ. 'Cultivation of novel sponge-associated bacteria by using the diffusion-growth-chamber approach'

Poster at the HBV Meeting in Oxford 2012, 119. DGIM Wiesbaden 2013 & GASL 2103 Hannover. Zacher B, Taranta A, Wiegand S, **Steinert G**, Deterding K, Manns MP, Wedemeyer H, Cornberg M, Wursthorn K. 'Early on treatment detection of nucleos(t)ide analogue resistant minor variants of Hepatitis B Virus (HBV) with ultra deep pyrosequencing (UDPS) in patients with chronic hepatitis B (CHB) infection.'

Poster at the ISME14 - The Power Of The Small Copenhagen 2012. **Steinert G**, Schupp PJ. 'Distribution and phylogeny of the Sponge-Symbiont '*Candidatus* Entotheonella palauensis' in selected sponges from Micronesia'

Talk at the 15th Evolutionary Biology Meeting at Marseilles 2011. **Steinert G**, Huelsken T, Bininda-Emonds ORP. 'Molecular phylogeny of *Mytilus edulis*: Dispersal and diversity of the blue mussel at the coast of Germany'

Poster at the DZG Workshop on Next Generation Sequencing and Transcriptomics Göttingen 2011. **Steinert G**, Huelsken T, Bininda-Emonds ORP. 'Population structure and phylogeny of the blue mussel (*M. edulis*) of the Wadden Sea of Lower Saxony (Germany)'

Talk at the BioSystematics Berlin 2011. **Steinert G**, Huelsken T, Bininda-Emonds ORP. 'Population structure and phylogeny of the Blue Mussel (*Mytilus edulis*) of the Wadden Sea of Lower Saxony (Germany)'

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ERKLÄRUNG

Hiermit bestätige ich, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit – einschließlich Abbildungen und Tabellen – die ich anderen Werken im Wortlaut oder dem Sinn nach entnommen habe, in jedem Einzelfall kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorlag; dass sie – abgesehen von den in der Outline angegebenen Teilpublikationen – noch nicht veröffentlicht wurde sowie, dass ich solche Veröffentlichungen vor Abschluss des Promotionsverfahren nicht vornehmen werde. Die Leitlinien guter wissenschaftlicher Praxis an der Carl von Ossietzky Universität Oldenburg sind mir bekannt und wurden befolgt. Es wurden im Zusammenhang mit dem Promotionsverfahren keine kommerziellen Vermittlungs- und Beraterdienste (Promotionsberatung) in Anspruch genommen. Die Bestimmungen der Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation wurde von Prof. Dr. Peter J. Schupp betreut.

Oldenburg, den 11.02.2016

(Georg Steinert)