Diversity in the globally intertwined giant barrel sponge species complex

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Synthesis and future directions

KEY FINDINGS

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

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

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

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

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

EVOLUTIONARY HISTORY OF GIANT BARREL SPONGES

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

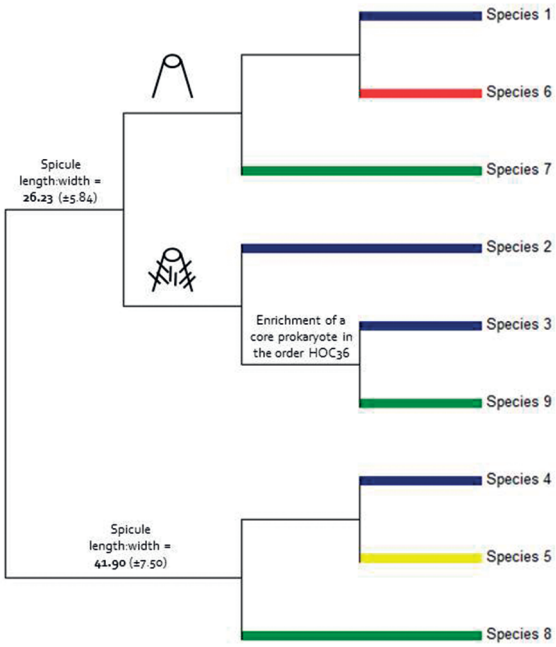

Figure 8.1. Topology of the global giant barrel sponge species complex. Branch color indicates the distribution of the species (blue = Indo-Pacific; red = Tropical Atlantic; Red = restricted to East Africa; Yellow = Red Sea).

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

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

PROKARYOTIC DIVERSITY

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

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

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

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

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

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

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

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

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

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

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

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

FUTURE DIRECTIONS

I. Classification

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

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

II. Linking chemistry to microbial diversity

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

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

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

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

III. Reproduction

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

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

IV. The sponge microbiome at mesophotic reef ecosystems

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

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

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

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

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

CONCLUSIONS

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