Original article

Characterization of archaeal symbionts of sponges from the coral reef ecosystems of the Gulf of Mannar, Southeast coast of India

Jasmin Chekidhenkuzhiyila, Abdulaziz Anasa,⇑, P.A. Thomasb, Balu Tharakan¹, Shanta Naira¹

ª CSIR-National Institute of Oceanography, Regional Centre, Cochin, Kerala 682 018, India
b Panachammoottil, Gandhipuram, Thiruvananthapuram, Kerala 695017, India

A R T I C L E   I N F O

Article history:
Received 24 December 2020
Revised 10 March 2021
Accepted 21 March 2021
Available online 27 March 2021

Keywords:
Archaea
Sponges
Gulf of Mannar
Coral reef
Nitrosopumilus

A B S T R A C T

Sponges accommodate a diverse group of microorganisms with varied metabolic capabilities. The bacterial associates of sponges are widely studied while our understanding of archaeal counterparts is scanty. In the present study, we report the archaeal associates of two sponges, Pseudoceratina purpurea (NCBI barcode: KX454492) and Cinachyra sp. (NCBI barcode: KX454495), found in the coral reef ecosystems of Gulf of Mannar, India. Archaea in the water column was predominated by members of class Halobacteria of Phylum Euryarchaeota (97%) followed by a minor fraction (3%) of Nitrospumilus sp. of phylum Thaumarchaeota. Interestingly, Thaumarchaeota was identified as the sole archaeal population associated with the two sponges studied, among which Nitrospumilus sp. occupied 80 and 100% of the sequences in the clone library of P. purpurea and Cinachyra sp. respectively. Other archaea found in the P. purpurea were Nitrosothphaera (10%) and unclassified ones (10%). The study identified Nitrospumilus sp. as a unique symbiotic archaeon of sponges, P. purpurea and Cinachyra sp. The existence of host driven factors in selecting specific associates from a diverse group of archaea in the environment may need further investigations.

© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Microorganism being the coevolved partner of higher organisms (host) in the marine environment contribute significantly to the existence of their hosts through providing secondary metabolites, enzyme etc (O’Brien et al., 2019; Vijayan et al., 2017; Vinokhumar and Parameswaran, 2013). Each host can be considered as a micro-niche where a consortium of microorganisms live and interact. Any alterations in the composition of this consortium can impart significant changes to the health of the host (Clemente et al., 2012; Cryan and Dinan, 2012). However, the discussions on such interactions were restricted to the discipline of pathogenesis and translocation, certain secondary metabolites of associated microorganisms enhance the chemical defence of sponges to predators and epibionts, which is thought to be the underlying mechanisms such as sponges towards environmental stressors are attributed to the microbial flora associated with them. Sponges are the ideal organisms to study the host-microbe interactions as a significant volume of their biomass are contributed by associated microorganisms (Hentschel et al., 2012). Many of the ecological functions such as remineralization of organic matter and participation in nitrification, which were originally thought to be carried out by sponge are actually performed by the microorganisms inhabiting in their body (Bayer et al., 2008; Erwin et al., 2011). Sponges accommodate a diverse community of microorganisms in their body representing all three domain of life viz Archaea, Bacteria and Eukarya and are recently documented to contain viruses as well (Chaib De Mares et al., 2017). A maximum of 52 phyla of bacteria, including candidate phyla such as Poribacteria and Tectomicroba and two archaeal phyla are reported from different sponges (Schmitt et al., 2012; Thomas et al., 2016; Webster and Taylor, 2012). The interactions of these microorganisms with respective sponges are quite diverse that ranged from mutualistic to commensalistic or exploitative (parasitism/pathogenesis) in nature. Apart from participating in nutrient cycling and translocation, certain secondary metabolites of associated microorganisms enhance the chemical defence of sponges to predators and epibionts, which is thought to be the underlying
factor to the ecological and evolutionary success of sponges (Webster and Thomas, 2016).

Although less diverse compared to bacteria, symbiotic archaea are known for performing specific functions in sponges, especially participation in nitrogen metabolism (Auguet et al., 2010; Radax et al., 2012; Turque et al., 2010; Zhang et al., 2014). Interestingly, the abundance of single archaeal lineage have been found exceeding the bacterial abundance in several sponges (Jackson et al., 2012; Preston et al., 1996). However, we know little about the diversity of archaea associated with marine sponges globally. Majority of the studies are restricted to sponges from Mediterranean, Caribbean and Pacific region with less reports from Indian subcontinent (Auguet et al., 2010; Chabi De Mares et al., 2017; Feng et al., 2016; Jackson et al., 2013, 2014; Radax et al., 2012; Turque et al., 2010; Zhang et al., 2014). Most of these studies reported the dominance of *Thaumarchaeota* and or *Crenarchaeota* in sponges while more diverse group of archaea were present in the environment in which they were grown. Investigations on archaean symbionts of sponges from different ecosystems across the globe will support in defining the role of host driven factors and archaeal symbionts of sponges from different ecosystems across the globe (Webster and Thomas, 2016).

2. Materials and methods

2.1. Sample collection and transportation

The sponge samples were collected by scuba diving from (5–10 m depth) the coral reef ecosystems of Gulf of Mannar, India. Once sampled, the sponge samples were handled with all precautions (wearing nitrile gloves) to minimize contamination, sand and debris found on the surface were removed by washing with sterile calcium-magnesium-free artificial seawater (CMF-ASW, pH7.2) and transported to the laboratory in an ice chest. Reef water (1000 ml) were collected from within 5 m periphery of the sponge and transported to the laboratory in an ice chest. The reef water was filtered through a mixed cellulose ester membrane filter and was subjected for spicule characterization. The molecular identification was carried out using 16S rDNA clone library method. The archaeal 16S rDNA gene was amplified from metagenomic DNA isolated from the reef water and two sponges; *Cinachyra* sp. and *Pseudoceratina purpurea* and *Cinachyra* sp in comparison with the water column in proximity in the coral reef ecosystems of Gulf of Mannar situated at the southeast coast of India.

2.2. Extraction of DNA from sponge holobiont and water

Genomic DNA was extracted from reef water and sponge tissue separately following the methods of Bostöm et al. (2004) and Ouyang et al. (2010) respectively with slight modifications. The sponge tissue (100 mg) was homogenized with 400 μl of lysis buffer (500 mM NaCl, 100 mM EDTA and10 mM Tris pH 8.0). Similarly, one half of the filter paper (cut into small pieces) were subjected for bead beating for 1–2 min (2 mm diameter beads) in the presence of 500 μl lysis buffer (400 mM NaCl, 750 mM sucrose, 20 mM EDTA and 50 mM Tris, pH 8.0). The lysed samples were further treated with lysozyme (25 U) for one hour at 37 °C followed by SDS (1%) and protease K (500 μgml−1) for 2 h (for tissue) or 5 h (for filter paper) at 55 °C. Both the reaction mixtures were further extracted twice with Chloroform:isoamyl alcohol (24:1). The aqueous phase was recovered into another microcentrifuge tube and supplemented with 0.6 times volume of isopropanol and was kept −20 °C for 60 min to precipitate the DNA. The precipitated DNA was washed thrice with ethanol (70%), dissolved in TE buffer (pH 7.4) and stored at −20 °C until used. The quality and purity of DNA was assessed using agarose gel electrophoresis (on 0.8% agarose gel) and spectrophotometry (absorbance at 260/280 nm in a ND-1000 spectrophotometer (NanoDrop, Thermo Scientific) respectively.

2.3. Identification of sponges

The sponge samples were identified following a polyphasic approach using both morphological and molecular tools. Morphological characterization includes recording the colour, morphology and spicule characterization. The molecular identification was done by amplifying the COI gene in a PCR using universal primers; LCO1490:5′–GGTCAACAAATCATAAAGATATTGG–3′ and coxl-R1:5′–TGGTGRGGGAAAAKGTAAAATT–3′ (Folmer et al., 1994). The reaction was conducted in a 25 μl reaction volume containing 1 μl template DNA (50–100 ng), 1 μl of each primers (10 pmicromoles μl−1), 10x Taq DNA polymerase buffer (2.5 μl), dNTPs (200 μM each) and DNA polymerase (0.5U Taq). The PCR was carried out in a Thermal cycler (Eppendorf, Germany) with recommended conditions for the primer (Folmer et al., 1994). The amplified PCR products were purified using a PCR cleanup kit (Geneti Biotech, India) and was cloned in to *pJET* 1.2 blunt vector using CloneJET PCR cloning kit (Thermo Scientific), following the instruction manual. Plasmids were extracted from ten randomly selected clones and were subjected for sequencing PCR with vector specific primers, *pJET* 1.2F and *pJET* 1.2R and ABI PRISM Bigdyne terminator v3.1cycle sequencing kit (Life Technologies, USA). The Products of sequencing PCR were purified and sequenced on Applied Biosystems ABI 3730xl DNA analyser.

2.4. Archaeal community structure

The community structure of archaea associated with sponges were studied using 16S rDNA clone library method. The archaean 16S rDNA gene was amplified from metagenomic DNA isolated from the reef water and two sponges; *Cinachyra* sp. and *P. purpurea* using universal primers [ARCH 21F: TCCGCGTATCAGGCACTGGCRG and ARCH 958R: TCCCGGTTAGGACTCAAAATT] (DeLong, 1992). The gene amplification was performed following standard PCR conditions (30 cycles) with annealing at 55 °C for 60 s and extension at 72 °C for 1.5 min. The PCR products were purified, cloned and sequenced following the same conditions and reagents mentioned in the above section. Nearly 270 to 330 clones were there in each of the three clone libraries, out of which 100 to 130 clones were randomly chosen and grown for plasmid isolation in 5 ml LB media for overnight at 37 °C degree under shaking conditions. The recombinant plasmids were purified and sequenced as mentioned in the earlier section.

2.5. Data analysis

The sequences with high quality were selected after screening using Sequencher V4.10.1 (GeneCodes Corporation, Ann Arbor, MI USA). Contaminating vector- and chimeric-sequences were removed from the dataset using VecScreen program of NCBI and DECIPHER online tool (http://decipher.cee.wisc.edu/FindChimeras.html) respectively. The sequences were further analysed in depth and classified into OTUs in the NGS analysis pipeline of SILVA (https://www.arb-silva.de/ngs/). From the three libraries with 100–120 sequences each, a total of 81 OTUs were obtained upon SILVA analyses. The OTU data available were used to depict the bar chart which describes the diversity and abundance of microbes in each of the three libraries analysed here. Also, the representative sequences (OTUs) were searched against NCBI Genbank and the sequence data of the nearest neighbours were downloaded to be used as reference sequences. Together, the OTUs and the reference sequences were aligned (using clustal W program) and a
Phylogenetic tree was constructed using neighbour-joining method in MEGA 5.2 (Tamura et al., 2011). The difference in microbial community structure in sponges *Cinachyra* sp, *Pseudoceratina purpurea*, and the reef water sample were analysed using Library compare tool in RDP (http://rdp.cme.msu.edu). The multivariate cluster analysis of the resulting data were performed in PRIMER v.6 (Plymouth Marine Laboratory, UK).

3. Results

Two sponges used in the study were identified based on morphological and molecular tools as *P. purpurea* (NCBI barcode: KX454492) and *Cinachyra* sp. (NCBI barcode: KX454495). The *P. purpurea* is described as an yellow coloured erect sponge with laminar branches and was found attached to seaweed Caulerpa racemosa (Fig. 1A). The surface colour changed to yellowish brown on removing from the substratum and exposed to air, while the interior remains yellow (inset in Fig. 1A). The texture was leathery and oscules and pores were untraceable. The spicules are blunt ended with terminal dome shaped structures. The COI gene sequence of the same showed 98.86% similarity with *Pseudoceratina purpurea* (KY565313) isolated from Oahu Island, Hawaii. The *Cinachyra* sp. was found as a massive sponge with bright yellow in color and occasional patches of greyish brown and green due to the settlement of silt/sand and algae (Fig. 1B). The *Cinachyra* sp. was found attached to the substratum with a broad base and the spherical body has unevenly arranged golf ball like depressions. *Cinachyra* sp. have tough and incompressible body with small oscules scattered over (inset in Fig. 1B). The long spicules were sharply pointed with a size of approximately 2.529 mm. The COI gene sequence showed only 92.7% of similarity with a distant relative Biemna fistulosa, in the same class Demospongiae.

The archaea was more diverse in the water column, dominated by phylum Euryarchaeota (97%) followed by Thaumarchaeota (3%) (Fig. 2). Members of class Halobacteria were predominant (95%) among Euryarchaeota, among which a significant fraction (35%) were grouped into unclassified. The remaining members of Halobacteria were identified as Halorussus and Halomicroarcula (9% each); Haloplanus, Halolamina, Halobellus, Haloarcula Halomicrobiun (6% each) and Genus Natronomonas (4%).

The Nitrosopumilus sp. coming under phylum Thaumarchaeota were poorly represented in the clone library of reef water (3%), while they dominated in the library prepared from sponges (Fig. 3). An 80 and 100% of sequences from the clone libraries of *P. purpurea* and *Cinachyra* sp respectively were identified as *Nitrosopumilus* sp coming under order Nitrosopumilales of Phylum Thaumarchaeota. Remaining sequences in the clone library (10% each) of *P. purpurea* were identified as *Nitrososphaera* sp (Phylum Thaumarchaeota, order Nitrososphaerales) and unclassified archaea respectively. The phylogenetic relationship between the sequences obtained from the three clone libraries are depicted as rooted neighbour joining tree using MEGA software (Fig. 3). The multivariate cluster analysis using Primer software showed nearly 40% similarity of archaeal diversity between the sponges, while they were highly distant from the reef water (Fig. 4).

4. Discussion

From the first description by Woese and Fox in 1978 and till recently, Archaea were known for their existence in extreme environments. Later they were reported from marine waters and sediments as planktonic or in association with other organisms (Auguet et al., 2010; DeLong, 1992; Dombrowski et al., 2019; Fuhrman et al., 1992; Santoro et al., 2019). Their association with sponges was first detected in *Cenarchaeum symbiosum* from the offshore waters of Santa Barbara in 1996 (Preston et al., 1996). Archaea associated with sponges were less diverse (Zhang et al., 2014) with only two lineages reported from many sponges studied (Hentschel et al., 2012). More studies from diverse marine environment are important to understand the diversity of archaea associated with sponges and the ecological significance of their interactions. Current study provides the first insight into the archaeal diversity associated with sponges from Indian waters. The archaea were less diverse in sponge *P. purpurea* and *Cinachyra* sp, compared to the water column in which they were living. Previous reports from Mediterranean and Caribbean waters showed that the archaeal community associated with sympatric sponges are different from that of seawater and varied between different sponges (Zhang et al., 2014). Studies from Atlantic, Mediterranean and Caribbean waters showed the higher diversity of archaea with phylum Euryarchaeota as ubiquitous in seawater (Garcia-Bonilla et al., 2019; Jackson et al., 2013; Zhang et al., 2014). The Thaumarchaeal reads from seawater and sponge were almost represented by order Nitrososphaerales and Nitrosopumilales. Among this, the Nitrosopumilus sp of Nitrosopumilales represented almost all the clones in *P. purpureae* and Cinachyra sp respectively.

Similar to the recent observations from Mediterranean sponges (Garcia-Bonilla et al., 2019), we also found higher abundance of Thaumarchaeota in *P. purpureae* and *Cinachyra* sp. Although there are diverse archaeal genera present in reef water, the unique genus...
**Nitrosopumilus** (contributed only ~3% of clone library of reef water) contributed nearly 80% of the clones in the library generated from both sponges. It is possible that the sponges catch the transient flora from water and enrich within the mesophyl tissue. A study based on the analysis of 440,000 sequence of associated microorganisms in *Xestospongia* sp from different geographical locations also reported the dominance (~60%) of core group of microorganisms in sponges (Swierts et al., 2018). Similarly, 4 and 6 OTUs were found contributing 88% of the 210 and 273 OTUs respectively of two individuals of *Inflataella pellicula* from deep sea (Jackson et al., 2013). Majority of the studies agrees to a point that archaea are less diverse in sponges compared to the surrounding seawater and certain archaeal species get enriched within sponges (Swierts et al., 2018). If the variable/core/species-specific concept of bacterial symbionts of sponges are taken into consideration, these dominant archaea can be considered as the core archaea associated with sponges.

*Nitrosopumilus* sp of archaea are reported from a wide range of sponges across geographical barriers and are known to participate in nitrification, specifically in the oxidation of ammonium to nitrite (Bayer et al., 2008; Moeller et al., 2019; Polónia et al., 2014; Rodríguez-Marconi et al., 2015). In a coral reef ecosystem, sponges ingest nitrogen along with their food and excrete ammonia (Brusca and Brusca, 1990). The *Nitrosopumilus* sp. colonized in sponge tissue can support the conversion of ammonium to nitrite and nitrate (Feng et al., 2016) and thus protect the host cells from ammonia toxicity. The numerical dominance of *Nitrosopumilus* sp. in sponges indicates that they could be the core archaea of sponges. However, this doesn’t mean that they are species specific and their growth is obligately depended on the host. The dominance of *Nitrosopumilus* sp. was reported from the sponge *Ircinia* sp from Mediterranean and Caribbean waters (Zhang et al., 2014). There must be a factor which promoted the selective enrichment of *Nitrosopumilus* sp. in sponge tissue from the surrounding environment, which is yet to be confirmed. There are different assumptions on microbiome and physiology of sponges, one of which says that the small sized pores, dense mesophyl and complicated canal systems inside the sponges like *Cinachyra* sp. may result in the circulation of water at high pressure through their canal system and which will promote the colonisation of species specific microorganisms (Jasmin et al., 2015). This could be a possible factor influencing the colonization of limited number of archaea in certain sponges. Based on the literature, these observations can also be connected with the functional role of sponges in reef ecosystems (Bell, 2008; James, 2007). The *Nitrosopumilus* sp. are known for their capabilities to oxidize ammonium and hence their interactions may be in support of the participation of those sponges in the biogeochemical cycling of nitrogen in the reef ecosystem. Or otherwise the enrichment of ammonia oxidizing archaea are also defined as an adaptation of sponge to increase their resistance to ammonium toxicity from eutrophication pressure (Baquiran and Conaco, 2018; Turque et al., 2010).

**5. Conclusion**

Present study reports the diversity of archaea associated with sponges, *P. purpurea* (NCBI barcode: KX454492) and *Cinachyra* sp. (NCBI barcode: KX454495), found in the coral reef ecosystems of Gulf of Mannar. Although the water column had a diverse group of archaea, the *Nitrosopumilus* sp. was found contributing more than 80% of the sequences in the clone library of both the sponges. This indicates the existence of a host driven factor in enriching selected group of archaea in sponges. Further studies may focus on the factors influencing this enrichment and its ecological significance.

**6. Compliance with Ethical Standards**

Authors declare no conflict of interest. The field collections carried out for the purpose of this manuscript did not involved endangered or protected species. Permission was received from Principal Chief Conservator of Forests and Chief Wildlife Warden for entering coral reef ecosystems of GoM. No specific permission was required to collect the analysed sponge samples.

**Author contribution**

The design of the work and the preparation of manuscript was done by AA, JC and SN. JC and BT contributed to the molecular biology analysis of the sample and TPA contributed to the morphological identification of sponges.
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank the Director, CSIR - National Institute of Oceanography, Goa and the Scientist-in-Charge, CSIR-NIO Regional Centre, Kochi, for extending all required support. The authors acknowledge Dr. Manikandan C, Scientist CSIR-NIO for the support rendered for sample collection and underwater imaging of...
sponges. JC is grateful to Department of Science and Technology, Govt of India for the research grant SR/WOS-A/LS-339/2009.

References

August, J.-C., Barberan, A., Casamayor, E.O., 2010. Global ecological patterns in uncultured Archaea. ISME J. 4, 182–190.

Baquiran, J.I.P., Conaco, C., 2018. Sponge-microbe partnerships are stable under eutrophication pressure from mariculture. Mar. Pollut. Bull. 136, 125–134.

Bayer, K., Schmitt, S., Hentschel, U., 2008. Physiology, phylogeny and in situ evidence for bacterial and archaeal nitrifiers in the marine sponge Aplysina aerophoba. Environ. Microbiol. 10, 2942–2955.

Bell, J.J., 2008. The functional roles of marine sponges. Estrau. Coast. Shelf Sci. 79, 341–353.

Boström, K.H., Simu, K., Hagström, Å., Riemann, L., 2004. Optimization of DNA extraction for quantitative marine bacterioplankton community analysis. Limonol. Oceanogr. Methods 2, 365–373.

Brusca, R., Brusca, G., 1990. Phylum Porifera: The Sponges. Sinauer Press, Sunderland, MA.

Chahb De Mares, M., Sipkema, D., Huang, S., Bunk, B., Overmann, J., van Elsas, J.D., 2017. Host specificity for bacterial, archaeal and fungal communities determined for high- and low-microbial abundance sponge species in two genera. Front. Microbiol. 8, 2560.

Clemente, J.C., Ursell, L.K., Parfrey, L.W., Knight, R., 2012. The impact of the gut microbiota on human health: an integrative view. Cell 148, 1258–1270.

Cryan, J.F., Dinan, T.G., 2012. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat. Rev. Neurosci. 13, 701–712.

Delong, E.F., 1992. Archaea in coastal marine environments. Proc. Natl. Acad. Sci. 89, 5685–5689.

Dombrowski, N., Lee, J.-H., Williams, T., 2008. The functional roles of marine sponges. Estuar. Coast. Shelf Sci. 79, 341–353.

Douglas, A.E., Dubilier, N., Eberl, G., Fukami, T., Gilbert, S.F., Hentschel, U., 2006. Molecular systematics of tropical and deep sea sponges. PloS one 8, e84438.

Feng, G., Sun, W., Zhang, F., Karthik, L., Li, Z., 2016. Inhabitancy of active amoeboid amoebobacteria in the sponge Theonella swinhoei. Sci. Rep. 6, 24966.

Ferrier, L.W., Knight, R., 2012. The impact of the gut microbiota on brain and behaviour. Nat. Rev. Neurosci. 13, 701–712.

Finn, G., Sun, W., Zhang, F., Karthik, L., Li, Z., 2016. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse.

Flemming, T., Bönisch, H., Wehling, U., Dussap, C.G., Seba, I., Stoecker, R., 2017. Stable and enriched Crenarchaeum symbiosum and uncultured Betaproteobacteria HFI in the microbiome of the Mediterranean sponge Halichondria juniperina (Demospongiae: Haplosclerida). Microb. Ecol. 77, 25–36.

Hentschel, U., Piol, J., Degnan, S.M., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: a review. Mar. Ecol. 33, 477–497.

Hentschel, U., 2015. Archaea in coastal marine environments. Proc. Natl. Acad. Sci. 89, 5685–5689.

King, N., Kjelleberg, S., Knorr, J., Duguid, J.P., Deibel, G., Felsenstein, J., 2013. Phylogenetic diversity, host-specificity and evolution of DPANN archaea. FEMS Microbiol. Lett. 366, fnz008.

Limonol. Oceanogr. Methods 2, 365–373.

Douglas, A.E., Dubilier, N., Eberl, G., Fukami, T., Gilbert, S.F., Hentschel, U., 2006. Molecular systematics of tropical and deep sea sponges. PloS one 8, e84438.

Feng, G., Sun, W., Zhang, F., Karthik, L., Li, Z., 2016. Inhabitancy of active amoeboid amoebobacteria in the sponge Theonella swinhoei. Sci. Rep. 6, 24966.

Finn, G., Sun, W., Zhang, F., Karthik, L., Li, Z., 2016. Inhabitancy of active Nitrosopumilus-like ammonia-oxidizing archaea and Nitrospira nitrite-oxidizing bacteria in the sponge Theonella swinhoei. Sci. Rep. 6, 24966.

Flemming, T., Bönisch, H., Wehling, U., Dussap, C.G., Seba, I., Stoecker, R., 2017. Stable and enriched Crenarchaeum symbiosum and uncultured Betaproteobacteria HFI in the microbiome of the Mediterranean sponge Halichondria juniperina (Demospongiae: Haplosclerida). Microb. Ecol. 77, 25–36.

Hentschel, U., Piol, J., Degnan, S.M., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: a review. Mar. Ecol. 33, 477–497.

Hentschel, U., Piol, J., Degnan, S.M., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: a review. Mar. Ecol. 33, 477–497.

Hentschel, U., Piol, J., Degnan, S.M., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: a review. Mar. Ecol. 33, 477–497.

Hentschel, U., Piol, J., Degnan, S.M., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: a review. Mar. Ecol. 33, 477–497.

Hentschel, U., Piol, J., Degnan, S.M., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: a review. Mar. Ecol. 33, 477–497.

Hentschel, U., Piol, J., Degnan, S.M., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: a review. Mar. Ecol. 33, 477–497.

Hentschel, U., Piol, J., Degnan, S.M., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: a review. Mar. Ecol. 33, 477–497.

Hentschel, U., Piol, J., Degnan, S.M., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: a review. Mar. Ecol. 33, 477–497.

Hentschel, U., Piol, J., Degnan, S.M., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: a review. Mar. Ecol. 33, 477–497.

Hentschel, U., Piol, J., Degnan, S.M., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: a review. Mar. Ecol. 33, 477–497.

Hentschel, U., Piol, J., Degnan, S.M., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: a review. Mar. Ecol. 33, 477–497.

Hentschel, U., Piol, J., Degnan, S.M., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: a review. Mar. Ecol. 33, 477–497.

Hentschel, U., Piol, J., Degnan, S.M., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: a review. Mar. Ecol. 33, 477–497.

Hentschel, U., Piol, J., Degnan, S.M., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: a review. Mar. Ecol. 33, 477–497.

Hentschel, U., Piol, J., Degnan, S.M., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: a review. Mar. Ecol. 33, 477–497.

Hentschel, U., Piol, J., Degnan, S.M., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: a review. Mar. Ecol. 33, 477–497.

Hentschel, U., Piol, J., Degnan, S.M., Taylor, M.W., 2012. Marine sponges and their microbial symbionts: a review. Mar. Ecol. 33, 477–497.