Symbiotic archaea in marine sponges show stability and host specificity in community structure and ammonia oxidation functionality

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Received 4 April 2014; revised 2 September 2014; accepted 10 September 2014. Final version published online 20 October 2014.

DOI: 10.1111/1574-6941.12427

Editor: Gary King

Keywords
Porifera; ammonia-oxidizing archaea; 16S rRNA gene; microbial biogeography; symbiosis; archaeal communities.

Abstract
Archaea associated with marine sponges are active and influence the nitrogen metabolism of sponges. However, we know little about their occurrence, specificity, and persistence. We aimed to elucidate the relative importance of host specificity and biogeographic background in shaping the symbiotic archaeal communities. We investigated these communities in sympatric sponges from the Mediterranean (Ircinia fasciculata and Ircinia oros, sampled in summer and winter) and from the Caribbean (Ircinia strobilina and Mycale laxissima). PCR cloning and sequencing of archaeal 16S rRNA and amoA genes showed that the archaeal community composition and structure were different from that in seawater and varied among sponge species. We found that the communities were dominated by ammonia-oxidizing archaea closely related to Nitrosopumilus. The community in M. laxissima differed from that in Ircinia spp., including the sympatric sponge I. strobilina; yet, geographical clusters within Ircinia spp. were observed. Whereas archaeal phylotypes in Ircinia spp. were persistent and belong to ‘sponge-enriched’ clusters, archaea in M. laxissima were closely related with those from diverse habitats (i.e. seawater and sediments). For all four sponge species, the expression of the archaeal amoA gene was confirmed. Our results indicate that host-specific processes, such as host ecological strategy and evolutionary history, control the sponge–archaeal communities.

Introduction
Marine sponges are one of the most significant groups in benthic ecosystems in terms of diversity, abundance, and function (Diaz & Rutzler, 2001; Ribes et al., 2012; De Goeij et al., 2013); notably, they are key players in the recycling of nutrients such as nitrogen (Jimenez & Ribes, 2007; Southwell et al., 2008). Interestingly, their contribution to nutrient cycles in the ecosystem relies on the complex microbiota associated with them, including bacteria and archaea (Mohamed et al. 2010; López-Legentil et al., 2010; Radax et al., 2012). Archaea have been detected in sponges from different oceans (Webster et al., 2001; Margot et al., 2002; Lee et al., 2003) and belong mostly to the newly defined kingdom Thaumarchaeota, previously known as Marine Group 1 Crenarchaeota (Brochier-Armanet et al., 2008; Pester et al., 2011). Based on genomic information and physiology experiments, members of this phylum of archaea are chemolithoautotrophic ammonia oxidizers; that is, they can convert ammonium to nitrite and fix CO2 using the energy obtained from ammonia oxidation. Archaeal members of sponge symbiotic microbial communities may play an important role in the nitrogen cycle of the ocean.

Despite the interesting metabolism of these organisms, the symbiotic archaeal communities in sponges have received less attention than bacterial communities and we know little about the specificity, persistence, or resilience of archaea in sponges. With one exception (Lee et al., 2011), sponge symbiotic archaeal communities have been
found in low diversity, are metabolically active, and may reach high densities in the mesohyl in some sponge species (Webster et al., 2001; Bayer et al., 2008; Radax et al., 2012). The first study of archaea in sponges found a sole archaeal phylotype persistently associated with Axinella mexicana that was named Cenaarchaeum symbiosum (Preston et al., 1996); and, since then, closely related phylotypes have been found in other sponge species (Hentschel et al., 2006). The presence of archaea in sponge larvae suggests a tight link between archaeal symbionts and the sponge host phylogeny (Schmitt et al., 2008; Steger et al., 2008). Conversely, environmental factors (e.g. seasonal changes in seawater conditions) or host biogeography may also affect the structure of archaeal symbionts in marine sponges (Turque et al., 2010).

Our study aims to elucidate the relative importance of host species-specific factors and biogeographic background in shaping sponge-associated archaeal communities. To achieve our objective, we investigated the archaeal communities in congeneric sponges and distantly related sympatric sponges. Two Mediterranean species, Ircinia fasciculata and Ircinia oros, and two Caribbean species, Ircinia strobilina and Mycale laxissima, were targeted. Mediterranean species were sampled in summer and winter, as this region shows a marked seasonality in seawater conditions (Erwin et al., 2012b). We used cloning and Sanger sequencing techniques to study archaeal 16S rRNA and amoA genes as phylogenetic markers as well as amoA transcripts to assess the active ammonia-oxidizing archaeal communities. The archaeal amoA gene encodes the $\alpha$-subunit of ammonia mono-oxygenase, which is essential in the nitrification process and is well conserved. The archaeal communities from seawater were also included in the analysis.

**Material and methods**

**Sampling strategy**

All sponge and seawater samples were collected by SCUBA diving. Tissue samples from three I. fasciculata individuals and three I. oros individuals were collected at Tossa de Mar (Girona), NE Spain, NW Mediterranean Sea (41°43.23′N, 2°56.45′E) during the summer (September 2012) and winter (March 2013) seasons. Three M. laxissima individuals and three I. strobilina individuals were collected from Conch Reef, Key Largo, FL, NE Caribbean (24°57.11′N, 80°27.57′W), in July 2011. Three seawater samples from Key Largo (3 L for each sample) were collected simultaneously in close proximity (1 m) to sampled sponges and filtered through 0.22 µm Sterivex filter units (Millipore, Billerica, MA). In all cases, intact sponges were held in the seawater in which they were collected, maintained at the ambient temperature, and transported rapidly (within 1 h) back to a shore laboratory for processing. Seawater was drained and the sponges were rinsed three times with sterile artificial seawater. Tissue samples (1-cm cubes) were steriley excised from sponges. Samples for DNA and RNA extraction were preserved in RNAlater stabilization solution (Qiagen, Valencia, CA) on board, transferred to a −20 °C freezer on site then kept in a −80 °C freezer for long-term storage.

**Genomic DNA/RNA extraction**

Total DNA and RNA from sponges and the seawater samples from Key Largo were extracted using a TissueLyser System (Qiagen) and AllPrep DNA/RNA Mini Kit (Qiagen) with RNase-free DNase treatment steps (Qiagen) for RNA samples following the manufacturer’s protocol. Reverse transcription of RNA was performed using RevertAid Reverse Transcriptase (Thermo Scientific, Waltham, MA) with random primers and following manufacturer’s protocol. RNA samples without the RT step were included as PCR template to check for residual DNA in the RNA samples.

**Archaeal 16S rRNA gene clone library construction**

We constructed archaeal 16S rRNA gene clone libraries from total DNA for each sponge specimen and seawater samples from Key Largo. Partial archaeal 16S rRNA gene sequences (c. 950 bp) were amplified using the archaea-specific primer set 21F (DeLong, 1992): (5′-TTC CGG TTAG ATC CYG CCG GA-3′) and 915R (Siboni et al., 2008): (5′-GTG CTC CCC CCG CAA TCC-3′) at 0.2 µM each. 50 µL of PCR mix included: 5 µL of 10× high-fidelity PCR buffer (Invitrogen Life Technologies, Carlsbad, CA), 2 µL MgCl₂, 1 µL of a mix of deoxyribonucleoside triphosphates (dNTP) at 0.2 mM, 0.2 µL of Platinum® Taq DNA Polymerase (Invitrogen), and 1 µL of each primer. Thermocycler parameters were set to 95 °C for 5 min; 30 cycles of 94 °C for 45 s, 56 °C for 60 s, and 72 °C for 60 s; then, a final extension at 72 °C for 15 min. Amplification products were analyzed by electrophoresis in 1.0% (w/v) agarose gels in 1× TAE buffer. PCR products were ligated into PCR-XL-TOPO vectors and transformed into ONEShot TOP10 chemically competent Escherichia coli cells using the TOPO XL PCR Cloning Kit (Invitrogen). Plasmid DNA was isolated from individual clones, purified using Mini prep spin kit (Qiagen), and sequenced using an ABI PRISM 3130XL genetic analyzer (Applied Biosystems, Foster City, CA). Sequences were screened for chimeras in Mothur software (Schloss et al., 2009).
Diversity and structure of 16S rRNA gene clone libraries

All archaeal 16S rRNA gene sequences were aligned in ARB (Ludwig et al., 2004) and ascribed to 97% operational taxonomic units (OTUs) using the MOTHUR software package (Schloss et al., 2009). Diversity metrics (observed OTUs, coverage, Chao1 estimator, Shannon index and Simpson’s inverse) were calculated for sponge species and seawater. The structure of the archaeal community in each sponge species was compared by nonmetric multidimensional scaling (nMDS). All analyses were performed using mother software package (Schloss et al., 2009).

Archaeal amoA gene and transcript analysis

Partial archaeal amoA gene sequences (c. 600 bp) were amplified from DNA and reverse transcribed cDNA using Arch-amoAF (5’-STA ATG GTC TGG CTT AGA CG-3’) and Arch-amoAR (5’-GCG GCC ATC CAT CCA TCT GTA TGT-3’) primers at 0.2 µM each (Francis et al., 2005). Except for the primers, all the reagents used for PCR were the same as for archaeal 16S rRNA gene amplification. Thermocycler settings for archaeal amoA gene amplification were 94 °C for 5 min; 42 cycles of 94 °C for 60 s, 60 °C for 1 min and 30 s, and 72 °C for 1 min and 30 s; followed by a final extension step at 72 °C for 15 min. Amplification products from DNA extracts (one sample per source) were cloned and sequenced as indicated above. We tried to clone from amplification products from cDNA from all sponge samples, but were only able to obtain clone libraries for two Caribbean sponges. All archaeal amoA gene and transcript sequences were aligned in ARB (Ludwig et al., 2004) and ascribed to 97% operational taxonomic units (OTUs) using MOTHUR software package (Schloss et al., 2009).

Phylogenetic analysis of archaeal 16S rRNA and amoA genes

All DNA sequences obtained here and their top BLASTN hits (GenBank database) were imported into ARB (Ludwig et al., 2004) for 16S rRNA and amoA gene phylogenetic analysis. For 16S rRNA phylogenetic analysis, we also included the archaeal sequences from Galand et al. (2010), who characterized the archaeal assemblages in coastal seawater over seasons in the NW Mediterranean Sea. Sequences were aligned based on homologous regions for each gene (dataset included in the software for the 16S rRNA gene, and based on c. 2000 environmental sequences kindly provided by Dr A. Santoro for the amoA gene). Multiple sequence alignments were visually checked and improved manually using the ARB editor. The aligned archaeal 16S rRNA gene (699 bp) or amoA gene (489 bp) was imported into PHYML 3.1 software package to construct a tree based on maximum likelihood method (Jukes–Cantor correction). The robustness of the resulting tree topologies was evaluated by 100 bootstrap replicates for archaeal 16S rRNA and 1000 for amoA gene (Guindon & Gascuel, 2003).

Quality-checked archaeal 16S rRNA and amoA gene sequences obtained in this study were deposited in GenBank under the accession numbers KJ504270–KJ504352, KJ526740–KJ526772, and KM042426–KM042428, respectively (Supporting Information, Tables S1 and S2).

Results

Diversity and structure of archaeal communities

Two eighty-one archaeal 16S rRNA gene sequences obtained in our study were ascribed to 14 OTUs (97% sequence identity). Archaeal communities in Caribbean sponges were less rich than in seawater samples, and not all the diversity in seawater was covered (Table 1, Supporting Information, Fig. S1). For the sponge species, rarefaction curves of Chao1 estimator reached the asymptote (Fig. S1), so we proceeded to compare the diversity metrics and structure of their archaeal communities. In terms of diversity (Shannon and inverse Simpson indices, Table 1), the community in I. fasciculata and I. oros was similar and stable over seasons. The archaeal 16S rRNA gene clone libraries derived from I. strobilina (three individuals and 74 clones analyzed) were composed of only one OTU (OTU001, Table 2) at the 97% cut-off level. In contrast, the sympatric Caribbean sponge M. laxissima presented the highest richness among sponge samples; yet the archaeal diversity in this sponge is low (Shannon and inverse Simpson indices, Table 1), due to the community being dominated by one OTU (OTTOM2, Table 2). In the nMDS plot, Ircinia-derived archaeal communities appeared closer to each other than to M. laxissima (Fig. 1). In each Mediterranean Ircinia species, archaeal communities from different seasons clustered together and closer to the other Mediterranean species than to the Caribbean I. strobilina sponges. The relative abundance of OTUs in sponge species is depicted in Fig. 2. Within the total 14 OTUs recovered in this study, 6 OTUs were retrieved in at least one sponge sample. Mediterranean Ircinia sponges showed the same archaeal community composition, although relative abundances of OTUs slightly varied from I. fasciculata to I. oros (Table 1, Fig. 2). The only OTU in I. strobilina (OTU001) was also present in the other two Ircinia species but absent in the...
sympatric sponge *M. laxissima* and surrounding seawater (Table 2). In contrast, the archaeal communities in *M. laxissima* were dominated by OTU002 (99.3% identity to the cultivated archaeon *Nitrosopumilus koreensis*), accounting for more than 90% of the sequences recovered from this species (Table 2). OTU002 was retrieved once from the NE Caribbean seawater (Table 2). Similarly, another common OTU in *M. laxissima* (OTU016) was also observed in Caribbean seawater samples (Table 2).

Archaeal community phylogeny

All the sponge-derived archaeal 16S rRNA gene sequences obtained in this study fell into the recently proposed kingdom *Thaumarchaeota*, previously known as Marine Group 1 Crenarchaeota (Brochier-Armanet et al., 2008; Pester et al., 2011) and were closely related to *Nitrosopumilus* sp. Specifically, the dominant OTU001 of archaeal 16S rRNA gene sequences recovered from the three Ircinia species formed a separate branch in the tree topology (Fig. 3), supporting the existence of sponge-enriched archaeal clusters, sensu Moitinho-Silva et al. (2014): sequences in this OTU clustered together with archaeal sequences (GenBank database, ≥ 98% identity) derived from other sponges species, including diverse genera and geographically distant locations (Great Barrier Reef, East China Sea, coastal of Indian Ocean). So far, this OTU has not been reported from seawater samples. OTU007, recovered from only Mediterranean Ircinia species, was

Table 1. Richness and dominance metrics for archaeal communities in sponges and seawater based on 16S rRNA gene sequences (OTU = 97% sequence identity)

<table>
<thead>
<tr>
<th>Sponge- Derived 97%-OTUs</th>
<th>Nb of sequences</th>
<th>Observed OTUs (Sobs)</th>
<th>Coverage</th>
<th>Expected OTUs (Chao1)</th>
<th>Inverse Simpson Index</th>
<th>Shannon Index</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>I. fasciculata</em></td>
<td>46</td>
<td>3</td>
<td>1</td>
<td>3 (3–3)</td>
<td>2.1 (1.8–2.6)</td>
<td>0.8</td>
</tr>
<tr>
<td>September</td>
<td>23</td>
<td>3</td>
<td>0.957</td>
<td>3 (0–3)</td>
<td>2.2 (1.8–2.9)</td>
<td>0.9</td>
</tr>
<tr>
<td><em>I. oros</em></td>
<td>46</td>
<td>3</td>
<td>0.957</td>
<td>3 (0–3)</td>
<td>2.1 (1.7–2.9)</td>
<td>0.9</td>
</tr>
<tr>
<td>September</td>
<td>22</td>
<td>3</td>
<td>1</td>
<td>3 (3–3)</td>
<td>1.7 (1.3–2.2)</td>
<td>0.7</td>
</tr>
<tr>
<td>March</td>
<td>24</td>
<td>3</td>
<td>1</td>
<td>3 (3–3)</td>
<td>1.5 (1.1–2.3)</td>
<td>0.7</td>
</tr>
<tr>
<td><em>I. strobilina</em></td>
<td>74</td>
<td>1</td>
<td>1</td>
<td>1 (1–1)</td>
<td>1 (1–1)</td>
<td>0</td>
</tr>
<tr>
<td><em>M. laxissima</em></td>
<td>78</td>
<td>4</td>
<td>0.974</td>
<td>5 (4–17)</td>
<td>1.1 (1.0–1.3)</td>
<td>0.4</td>
</tr>
<tr>
<td>Seawater</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Key Largo</td>
<td>37</td>
<td>10</td>
<td>0.865</td>
<td>13 (11–32)</td>
<td>4.9 (3.5–8.2)</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Lower and upper 95% confidence intervals are shown in parentheses where available.

Table 2. Archaeal OTUs (16S rRNA gene) found in at least one sponge sample and their closest BLAST sequence matches

<table>
<thead>
<tr>
<th>Sponge- Derived 97%-OTUs</th>
<th>No. of clones in each OTU per sponge species and seawater</th>
<th>Closest BLAST match (accession no., %identity, source)</th>
<th>Closest cultivated microorganism (accession no., %identity, source)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTU001</td>
<td>0 74 27 35 0</td>
<td>HM101089 (98.0%) sponge</td>
<td>NR_102913 (96%) <em>Nitrosopumilus maritimus</em></td>
</tr>
<tr>
<td>OTU002</td>
<td>73 0 2 4 1</td>
<td>JQ227250 (100%) seawater</td>
<td>NR_102904 (99.3%) <em>Nitrosopumilus koreensis</em></td>
</tr>
<tr>
<td>OTU007</td>
<td>0 0 17 7 0</td>
<td>AY192632 (98.4%) sponge</td>
<td>NR_102904 (96.9%) <em>N. koreensis</em></td>
</tr>
<tr>
<td>OTU016</td>
<td>3 0 0 0 2</td>
<td>AB611676 (99.8%) seawater</td>
<td>NR_102904 (95.5%) <em>N. koreensis</em></td>
</tr>
<tr>
<td>OTU023</td>
<td>1 0 0 0 0</td>
<td>EF069366 (99.5%) seawater</td>
<td>NR_102904 (93%) <em>N. koreensis</em></td>
</tr>
<tr>
<td>OTU025</td>
<td>1 0 0 0 0</td>
<td>EF367493 (97.0%) sediment</td>
<td>NR_102904 (94.6%) <em>N. koreensis</em></td>
</tr>
</tbody>
</table>


Archaeal community phylogeny

All the sponge-derived archaeal 16S rRNA gene sequences obtained in this study fell into the recently proposed kingdom *Thaumarchaeota*, previously known as Marine Group 1 Crenarchaeota (Brochier-Armanet et al., 2008; Pester et al., 2011) and were closely related to *Nitrosopumilus* sp. Specifically, the dominant OTU001 of archaeal 16S rRNA gene sequences recovered from the three *Ircinia* species formed a separate branch in the tree topology (Fig. 3), supporting the existence of sponge-enriched archaean clusters, sensu Moitinho-Silva et al. (2014): sequences in this OTU clustered together with archaean sequences (GenBank database, ≥ 98% identity) derived from other sponges species, including diverse genera and geographically distant locations (Great Barrier Reef, East China Sea, coastal of Indian Ocean). So far, this OTU has not been reported from seawater samples. OTU007, recovered from only Mediterranean *Ircinia* species, was
Stability and specificity of sponge-associated archaea

Ammonia-oxidizing archaea

A 635 bp fragment of the amoA gene was amplified from both DNA and cDNA extracts of all sponge species and seawater, which indicates the presence and activity of ammonia-oxidizing archaea (AOA) in sponge-derived archaeal communities. We subsequently constructed clone libraries from DNA extracts for all four species and cDNA clone libraries for each Caribbean species (one sample per sponge species). We were able to amplify amoA gene PCR products from cDNA for all Mediterranean sponges, but clone library construction was not successful. In total, 68 archaeal amoA gene and transcript sequences were obtained in our study and ascribed to 11 OTUs (97% sequence identity), with 6 total OTUs found in sponge samples (Table S3). In the case of the Caribbean sponges, the amoA genes found to be expressed fell into the same OTUs that dominated the amoA assemblage amplified from the corresponding DNA samples. Specifically, in M. laxissima, amoA gene fragments amplified from DNA all fell into OTU006; the cloned fragments from the cDNA sample also fell into OTU006. Similarly, for I. strobilina, almost all amoA gene fragments amplified from DNA fell into OTU004 and all clones derived from this cDNA sample also fell into OTU004 (see Table S3).

Those OTUs obtained from sponge samples in this study were closely related with amoA gene sequences found in other sponge species (BLASTN search, > 88.2% similarity), with the only exception of OTU003 (formed by one sequence retrieved from I. fasciculata) that was related to a sequence from a sand filter (87.9% similarity) (Table S3). Phylogenetic analysis placed amoA sequences from sponges in separate branches based on the host genus (Fig. 4). The AOA sequences obtained from Ircinia species fell into the same cluster, together with a sequence derived from the sponge Rhopaloepides odorabile (89% sequence identity), whereas the sequences from M. laxissima formed a different cluster together with sequences from the sponge Luffariella sp., a coral species and free-living archaea (Fig. 4).

Discussion

In this study, we analyzed the sponge-associated archaeal communities in sympatric sponges from the Mediterranean Sea (I. fasciculata and I. oros) and the Caribbean Sea (I. strobilina and M. laxissima) to elucidate the relative importance of host phylogeny and biogeographic background in structuring these communities. Although we did not capture all of the diversity of the archaeoplankton in the surrounding seawater (Fig. S1), our results show that sponge species harbor symbiotic archaeal communities different from the archaeoplankton, in agreement with previous studies (Holmes & Blanch, 2007). In all four sponge species, archaea closely related to Nitrosopumilus dominated the archaeal symbiotic communities and phylogenetic analysis detected ‘sponge-enriched’ archaeal clusters. Our results, based on archaeal 16S rRNA gene sequences, amoA gene and transcript sequences, showed low diversity and host genus-specificity of the sponge-derived archaeal communities in Ircinia spp. from the Mediterranean and the Caribbean Seas.

The expression of archaeal amoA genes was confirmed for all species, and sequence information from cDNA libraries of the two Caribbean sponges showed expression of the same OTUs as found in DNA libraries, which suggests that dominant members in some communities were metabolically active AOA. Interestingly, the amoA OTUs were almost all closely related with amoA gene sequences found in other sponges and were quite closely related to amoA gene sequences from the cultured microorganism Candidatus Nitrosopumilus sp., indicating that close relatives of this archaeon may be quite ubiquitous in sponges. However, the experimental difficulties in obtaining clone libraries from cDNA of the Mediterranean sponges limited our ability to further compare the active communities among these sponge species.
Fig. 3. Phylogenetic relationships of archaeal communities in sponges based on archaeal 16S rRNA gene. Sequences from sponges in this study are highlighted in bold and shaded rectangles represent sequences from same sponge species or locations. Tree topology constructed using maximum likelihood method, with bootstrap values (> 50%) indicated at the branch nodes. 16S ribosomal RNA gene from uncultured euryarchaeote clone B0803_E3A (GQ387923.1) was used as out-group.

Archaeal communities in Mediterranean species were persistently recovered from samples collected during different seasons (i.e. summer and winter) despite the marked seasonality in seawater conditions of temperature and irradiance (Erwin et al., 2012b), supporting the stability of sponge–archaea associations (Preston et al., 1996; Margot et al., 2002; Hardoim & Costa, 2014). Archaeal 16S rRNA gene OTU001 (present in all Ircinia spp) and OTU007 (present in Mediterranean Ircinia spp., but absent in I. strobilina) were consistently recovered over seasons and clustered with sponge-derived sequences in all our analysis, suggesting that these two OTUs correspond to sponge-enriched archaea.

Based on 16S rRNA gene analysis at community level, the archaeal community in sponges depended on the host species considered. The community in M. laxissima differed from those in Ircinia spp., including the sympatric Caribbean sponge I. strobilina, and presented the highest archaeal richness of all four sponge species, although dominated by a single archaeal OTU. Phylogenetic analysis of 16S rRNA and amoA gene sequences confirmed the host genus-specificity of archaea in sponges. In addition, the archaeal phylotypes dominant in Ircinia spp. were mostly closely related to archaea found in other sponge species, whereas archaeal phylotypes in M. laxissima seem to be more closely related to environmental samples. Within the same genus, the archaeal communities in Mediterranean Ircinia spp. (I. fasciculata and I. oros) were more similar to each other than to I. strobilina. Indeed, the communities in I. fasciculata and I. oros were composed by the same archaeal phylotypes, in contrast to the species-specificity of their bacterial communities (Erwin
et al., 2012a). Interestingly, \textit{I. strobilina} and \textit{I. fasciculata} are phylogenetically closer to each other than to \textit{I. oros} (Pita et al., 2013). It appears that the archaeal 16S rRNA gene did not reflect a coevolution of sponge hosts within a same genus with their archaeal symbionts. Our analysis suggests the influence of biogeographic background. However, the phylogeny based on \textit{amoA} gene showed a different picture. The AOA sequences from \textit{I. strobilina} and \textit{I. fasciculata} were more closely related to each other than to AOA from \textit{I. oros}, in accordance with their host phylogenetic relationships. The information discrepancy observed with these two phylogenetic markers suggests that genes involved with functional characters like ammonia oxidation might be subjected to higher selection pressure during host evolution than ribosomal genes.

The different host specificity of archaeal phylotypes found in \textit{Ircinia} species (dominated by sponge-enriched archaea) and \textit{M. laxissima} (more closely related to environmental archaea) suggests a tighter link in \textit{Ircinia} species that may reflect the different ecological strategies adopted by high microbial abundance (HMA) sponges like \textit{Ircinia} spp. (Erwin et al., 2012a; Poppell et al., 2013) and low microbial abundance (LMA) sponges like \textit{M. laxissima} (Reiswig, 1974), as shown for sponge bacterial communities (Giles et al., 2013; Poppell et al., 2013; Moitinho-Silva et al., 2014). Considering the low pumping rate in many HMA sponges (Weisz et al., 2007, 2008), the activity of AOA may provide an efficient way to remove ammonium waste secreted by the host and prevent toxic ammonium accumulation, strengthening the tie between symbionts and HMA sponges (Radax et al., 2012).

The archaeal communities in sponge hosts studied herein were specific and persistent within the same sponge species. At least some of the sponge archaeal symbionts were metabolically active AOA, suggesting that these symbionts play a key role in ammonium detoxification for their hosts, and could significantly impact the nitrogen cycle in the ecosystem. However, the archaeal community composition and structure varied depending on the sponge considered. Whereas archaeal phylotypes in \textit{Ircinia} spp. seemed to belong to sponge-enriched clusters, geographical clusters within the genus were also observed. Comparatively,
archaeal phylotypes in *M. laxissima* were closely related to sequences from diverse habitats (i.e. seawater, sediments). Our results indicated that host-specific processes, such as host ecological strategy and evolutionary history, determine the sponge–archaeal communities in some species. Persistent sponge-specific archaeal groups may provide a good target for future studies comparing sponge-associated and free-living AOA, and the interaction of bacteria and archaea within the sponge host.

**Acknowledgements**

We are grateful to J. Vicente for supplying samples from the Caribbean Sea, L. Blasiak for help and advice during laboratory work, A. Santoro for kindly providing ARB database of *amoA* gene, and S. Nazar for sequencing support. We acknowledge the National Undersea Research Center (NURC), University of North Carolina at Wilmington for providing lab facilities in Key Largo, Florida. This research was funded by U.S. National Science Foundation #IOS-0919728 to R.T.H., Fulbright S&T fellowship to F.Z. and a BE-DGR fellowship to L.P. This is contribution no. 4958 from UMCES and contribution no. 14–135 from IMET.

**Authors’ contribution**

F.Z. and L.P. contributed equally to this work.

**References**


### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** Rarefaction curves for Chao1 estimator of the archaeal 16S rRNA gene sequences obtained from *I. fasciculata* samples collected in March (IFmar), and September (IFsep), *I. oros* samples collected in March (IOmar) and September (IOsep), the Caribbean sponges *I. strobilina* (IST) and *M. laxissima* (ML), and seawater samples from Key Largo, Caribbean Sea (SWKL).

**Table S1.** Operational taxonomic units (OTUs) at 97% of sequence affiliation, isolation source and GenBank accession number for non-redundant archaeal 16S rRNA gene sequences derived from *I. fasciculata*, *I. oros*, *I. strobilina*, *M. laxissima* and seawater (Key Largo) samples collected during this study.

**Table S2.** Operational taxonomic units (OTUs) at 97% of sequence affiliation, GenBank accession numbers and isolation source for non-redundant archaeal *amoA* gene sequences derived from *I. fasciculata*, *I. oros*, *I. strobilina*, *M. laxissima* and seawater (Key Largo) samples collected during this study.

**Table S3.** Archaeal OTUs-97% similarity (*amoA* gene) found in sponge samples in this study and their closest BLAST sequence matches.