RESEARCH ARTICLE

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

Fan Zhang[1], Lucía Pita[2], Patrick M. Erwin[3], Summara Abaid[1], Susanna López-Legentil[2,3] & Russell T. Hill[1]

[1]Institute of Marine and Environmental Technology (IMET), University of Maryland Center for Environmental Science (UMCES), Baltimore, MD, USA; [2]Department of Animal Biology, Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona (UB), Barcelona, Spain; and [3]Department of Biology & Marine Biology, Center for Marine Science, University of North Carolina Wilmington, Wilmington, NC, USA

**Correspondence:** Russell T. Hill, 701 E Pratt St, Baltimore, MD 21202, USA.
Tel.: +1 410 234 8802;
fax: +1 410 234 8818;
e-mail: hill@umces.edu

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## 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 $CO_2$ 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 *Cenarchaeum 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 archaea communities. The archaeal *amoA* gene encodes the α-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 sterilely excised from sponges. Samples for DNA and RNA extraction were preserved in RNA*later* 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 RNAase-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 TTG ATC CYG CCG GA-3′) and 915R (Siboni *et al.*, 2008): (5′-GTG CTC CCC CGC 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 $MgCl_2$, 1 μL of a mix of deoxynucleoside 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 mothur 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 (OTU002, 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

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

| | Nb of sequences | Observed OTUs (Sobs) | Coverage | Expected OTUs (Chao1) | Inverse Simpson Index | Shannon Index |
|---|---|---|---|---|---|---|
| *I. fasciculata* | 46 | 3 | 1 | 3 (3–3) | 2.1 (1.8–2.6) | 0.8 |
| September | 23 | 3 | 0.957 | 3 (0–3) | 2.2 (1.8–2.9) | 0.9 |
| March | 23 | 3 | 0.957 | 3 (0–3) | 2.1 (1.7–2.9) | 0.9 |
| *I. oros* | 46 | 3 | 1 | 3 (0–3) | 1.7 (1.3–2.2) | 0.7 |
| September | 22 | 3 | 1 | 3 (3–3) | 1.5 (1.1–2.3) | 0.7 |
| March | 24 | 3 | 1 | 3 (3–3) | 1.9 (1.4–3) | 0.8 |
| *I. strobilina* | 74 | 1 | 1 | 1 (1–1) | 1 (1–1) | 0 |
| *M. laxissima* | 78 | 4 | 0.974 | 5 (4–17) | 1.1 (1.0–1.3) | 0.4 |
| Seawater | | | | | | |
| Key Largo | 37 | 10 | 0.865 | 13 (11–32) | 4.9 (3.5–8.2) | 2.1 |

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

| Sponge- derived 97%-OTUs | No. of clones in each OTU per sponge species and seawater | | | | | Closest BLAST match (accession no., %identity, source) | Closest cultivated microorganism (accession no., %identity, source) |
|---|---|---|---|---|---|---|---|
| | ML | IST | IF | IO | SWKl | | |
| OTU001 | 0 | 74 | 27 | 35 | 0 | HM101089 (98.0%) sponge | NR_102913 (96%) *Nitrosopumilus maritimus* |
| OTU002 | 73 | 0 | 2 | 4 | 1 | JQ227250 (100%) seawater | NR_102904 (99.3%) *Nitrosopumilus koreensis* |
| OTU007 | 0 | 0 | 17 | 7 | 0 | AY192632 (98.4%) sponge | NR_102904 (96.9%) *N. koreensis* |
| OTU016 | 3 | 0 | 0 | 0 | 2 | AB611676 (99.8%) seawater | NR_102904 (95.5%) *N. koreensis* |
| OTU023 | 1 | 0 | 0 | 0 | 0 | EF069366 (99.5%) seawater | NR_102904 (93%) *N. koreensis* |
| OTU025 | 1 | 0 | 0 | 0 | 0 | EF367493 (97.0%) sediment | NR_102904 (94.6%) *N. koreensis* |

ML, *M. laxissima*; IST, *I. strobilina*; IF, *I. fasciculata*; IO, *I. oros*; SWKl, Seawater from Key Largo, Caribbean Sea.

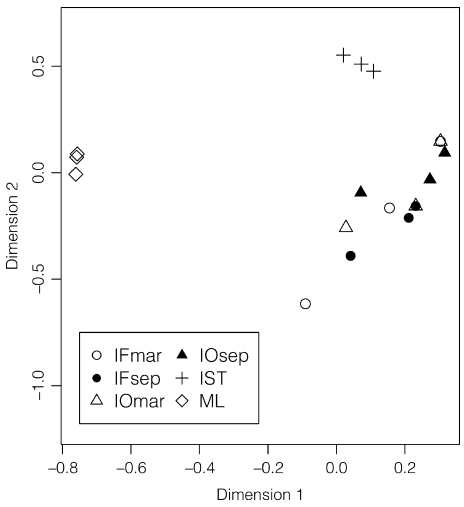


**Fig. 1.** Nonmetric multidimensional scaling (nMDS) plot based on Bray–Curtis distances between samples. IOsep, *Ircinia oros* from September (summer); IOmar; *I. oros* from March (winter); IFsep, *I. fasciculata* from September (summer); IFmar, *I. fasciculata* from March (winter); IST, *I. strobilina*; ML, *Mycale laxissima*; SWKL, seawater from Key Largo (NE Caribbean). Stress value = 0.08; $R^2 = 0.978$.

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

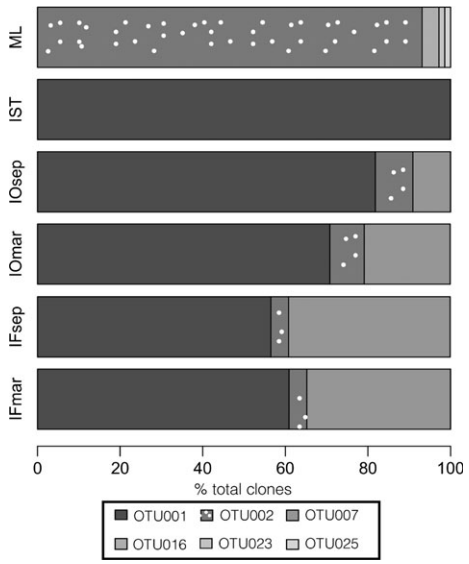


**Fig. 2.** Relative abundance of archaeal OTUs (97% identity) in sponge samples derived from 16S rRNA gene clone libraries. ML, *Mycale laxissima*; IST, *Ircinia strobilina*; IOsep, *I. oros* from September (summer); IOmar; *I. oros* from March (winter); IFsep, *I. fasciculata* from September (summer); IFmar, *I. fasciculata* from March (winter).

also related with a sponge-derived sequence (Fig. 3; Table 2). In the seawater samples analyzed in this study, archaeal communities were dominated by Marine Group 2 *Euryarchaeota*.

### 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 *Rhopaloeides 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.

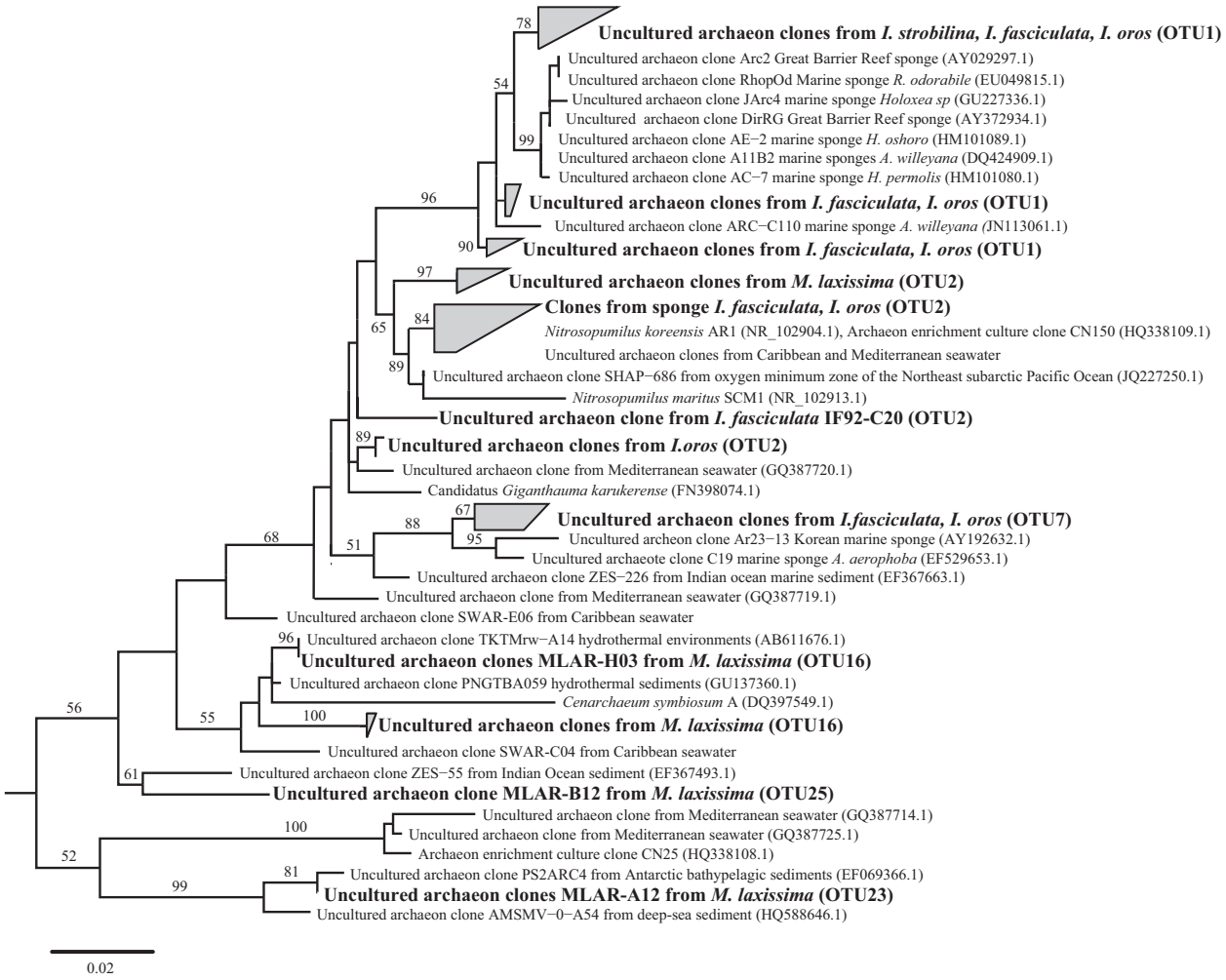


**Fig. 3.** Phylogenetic relationships of archaeal communities in sponges based on archaeal 16S rRNA gene. Sequences from sponges in this study are highlighted in bold and shaded rectangles represent sequences from same sponge species or locations. Tree topology constructed using maximum likelihood method, 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

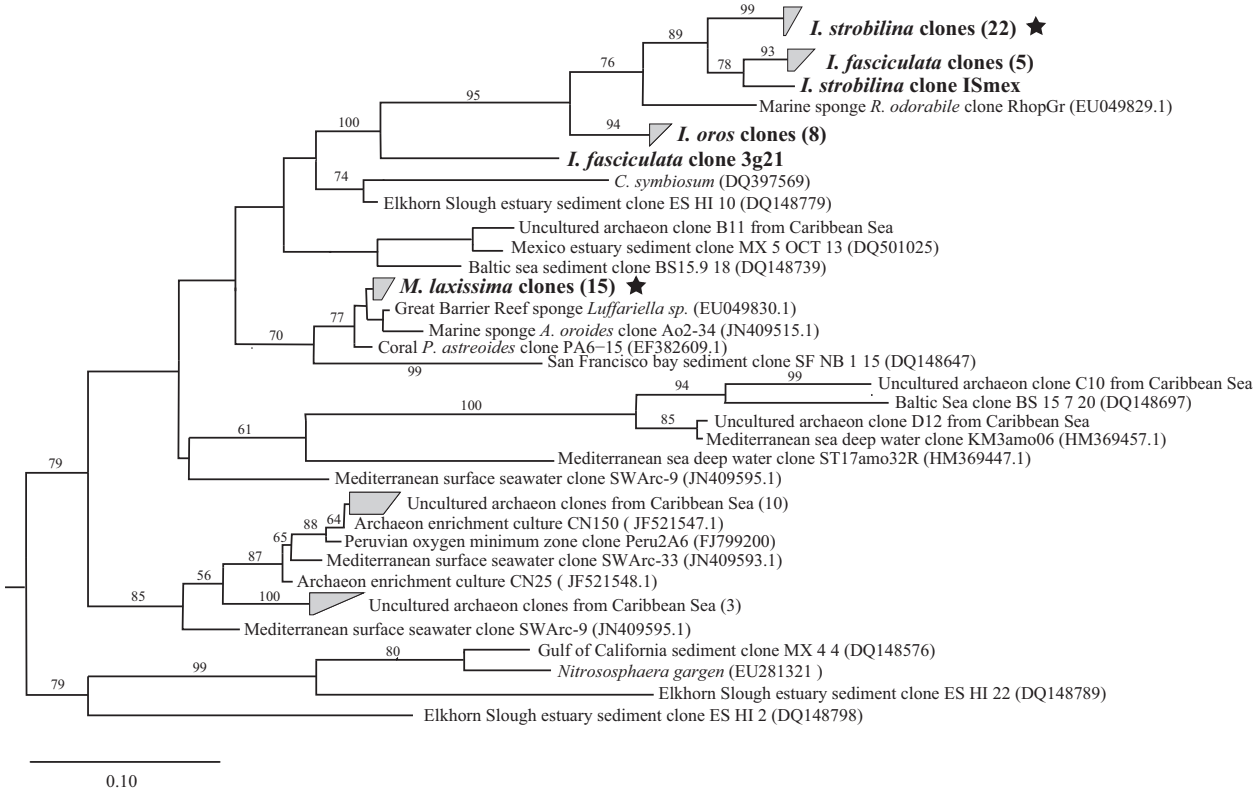


**Fig. 4.** Phylogenetic relationships of archaeal communities in sponges based on archaeal *amoA* genes and transcripts. Sequences from sponges in this study are highlighted in bold. Number in parentheses indicated the quantity of clones from corresponding DNA and/or cDNA clone libraries. The cluster marked with the star symbol included sequences from the corresponding cDNA libraries obtained from the same sponge source. Shaded rectangles represent samples from the same sponge species or locations. Tree topology constructed using maximum likelihood method with bootstrap values (> 50%) indicated at the branch nodes. Archaeal putative *amoA* gene sequence from candidatus *Nitrosocaldus yellowstonii* strain HL72 (EU239961.1) was used as out-group.

*et al.*, 2012a). Interestingly, *I. strobilina* and *I. fasciculata* are phylogenetically closer to each other than to *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 *amoA* gene showed a different picture. The AOA sequences from *I. strobilina* and *I. fasciculata* were more closely related to each other than to AOA from *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 *Ircinia* species (dominated by sponge-enriched archaea) and *M. laxissima* (more closely related to environmental archaea) suggests a tighter link in *Ircinia* species that may reflect the different ecological strategies adopted by high microbial abundance (HMA) sponges like *Ircinia* spp. (Erwin *et al.*, 2012a; Poppell *et al.*, 2013) and low microbial abundance (LMA) sponges like *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 *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.

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## Authors' contribution

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

## References

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.

Brochier-Armanet C, Boussau B, Gribaldo S & Forterre P (2008) Mesophilic *Crenarchaeota*: proposal for a third archaeal phylum, the *Thaumarchaeota*. *Nat Rev Microbiol* **6**: 245–252.

De Goeij JM, van Oevelen D, Vermeij MJA, Osinga R, Middelburg JJ, de Goeij AFPM & Admiraal W (2013) Surviving in a marine desert: the sponge loop retains resources within coral reefs. *Science* **342**: 108–110.

DeLong EF (1992) Archaea in coastal marine environments. *P Natl Acad Sci USA* **89**: 5685–5689.

Diaz MC & Rutzler K (2001) Sponges: an essential component of Caribbean coral reefs. *Bull Mar Sci* **69**: 535–546.

Erwin PM, López-Legentil S, González-Pech R & Turon X (2012a) A specific mix of generalists: bacterial symbionts in Mediterranean *Ircinia* spp. *FEMS Microbiol Ecol* **79**: 619–637.

Erwin PM, Pita L, López-Legentil S & Turon X (2012b) Stability of sponge-associated bacteria over large seasonal shifts in temperature and irradiance. *Appl Environ Microbiol* **78**: 7358–7368.

Francis CA, Roberts KJ, Beman JM, Santoro AE & Oakley BB (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *P Natl Acad Sci USA* **102**: 14683–14688.

Galand PE, Gutiérrez-Provecho C, Massana R, Gasol JM & Casamayor EO (2010) Inter-annual recurrence of archaeal assemblages in the coastal NW Mediterranean Sea (Blanes Bay Microbial Observatory). *Limnol Oceanogr* **55**: 2117–2125.

Giles EC, Kamke J, Moitinho-Silva L, Taylor MW, Hentschel U, Ravasi T & Schmitt S (2013) Bacterial community profiles in low microbial abundance sponges. *FEMS Microbiol Ecol* **83**: 232–241.

Guindon S & Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* **52**: 696–704.

Hardoim CP & Costa R (2014) Temporal dynamics of prokaryotic communities in the marine sponge *Sarcotragus spinosulus*. *Mol Ecol* **23**: 3097–3112.

Hentschel U, Usher KM & Taylor MW (2006) Marine sponges as microbial fermenters. *FEMS Microbiol Ecol* **55**: 167–177.

Holmes B & Blanch H (2007) Genus-specific associations of marine sponges with group I crenarchaeotes. *Mar Biol* **150**: 759–772.

Jimenez E & Ribes M (2007) Sponges as a source of dissolved inorganic nitrogen: nitrification mediated by temperate sponges. *Limnol Oceanogr* **52**: 948–958.

Lee E-Y, Lee HK, Lee YK, Sim CJ & Lee J-H (2003) Diversity of symbiotic archaeal communities in marine sponges from Korea. *Biomol Eng* **20**: 299–304.

Lee OO, Wang Y, Yang J, Lafi FF, Al-Suwailem A & Qian P-Y (2011) Pyrosequencing reveals highly diverse and species-specific microbial communities in sponges from the Red Sea. *ISME J* **5**: 650–664.

López-Legentil S, Erwin PM, Pawlik JR & Song B (2010) Effects of sponge bleaching on ammonia-oxidizing *Archaea*: distribution and relative expression of ammonia monooxygenase genes associated with the barrel sponge *Xestospongia muta*. *Microb Ecol* **60**: 561–571.

Ludwig W, Strunk O, Westram R *et al.* (2004) ARB: a software environment for sequence data. *Nucleic Acids Res* **32**: 1363–1371.

Margot H, Acebal C, Toril E, Amils R & Puentes JF (2002) Consistent association of crenarchaeal *Archaea* with sponges of the genus *Axinella*. *Mar Biol* **140**: 739–745.

Mohamed NM, Saito K, Tal Y & Hill RT (2010) Diversity of aerobic and anaerobic ammonia-oxidizing bacteria in marine sponges. *ISME J* **4**: 38–48.

Moitinho-Silva L, Bayer K, Cannistraci CV, Giles EC, Ryu T, Seridi L, Ravasi T & Hentschel U (2014) Specificity and transcriptional activity of microbiota associated with low and high microbial abundance sponges from the Red Sea. *Mol Ecol* **23**: 1348–1363.

Pester M, Schleper C & Wagner M (2011) The *Thaumarchaeota*: an emerging view of their phylogeny and ecophysiology. *Curr Opin Microbiol* **14**: 300–306.

Pita L, López-Legentil S & Erwin PM (2013) Biogeography and host fidelity of bacterial communities in *Ircinia* spp. from the Bahamas. *Microb Ecol* **66**: 437–447.

Poppell E, Weisz J, Spicer L, Massaro A, Hill A & Hill M (2013) Sponge heterotrophic capacity and bacterial community structure in high- and low-microbial abundance sponges. *Mar Ecol* doi:10.1111/maec.12098.

Preston CM, Wu KEY, Molinskit TF & Delong EF (1996) A psychrophilic crenarchaeon inhabits a marine sponge: *Cenarchaeum symbiosum* gen. nov, sp. nov. *P Natl Acad Sci USA* **93**: 6241–6246.

Radax R, Hoffmann F, Rapp HT, Leininger S & Schleper C (2012) Ammonia-oxidizing archaea as main drivers of nitrification in cold-water sponges. *Environ Microbiol* **14**: 909–923.

Reiswig HM (1974) Water transport, respiration and energetics of three tropical marine sponges. *J Exp Mar Biol Ecol* **14**: 231–249.

Ribes M, Jiménez E, Yahel G, López-Sendino P, Diez B, Massana R, Sharp JH & Coma R (2012) Functional convergence of microbes associated with temperate marine sponges. *Environ Microbiol* **14**: 1224–1239.

Schloss PD, Westcott SL, Ryabin T *et al.* (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* **75**: 7537–7541.

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

Siboni N, Ben-Dov E, Sivan A & Kushmaro A (2008) Global distribution and diversity of coral-associated *Archaea* and their possible role in the coral holobiont nitrogen cycle. *Environ Microbiol* **10**: 2979–2990.

Southwell MW, Weisz JB, Martens CS & Lindquist N (2008) *In situ* fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. *Limnol Oceanogr* **53**: 986–996.

Steger D, Ettinger-Epstein P, Whalan S, Hentschel U, de Nys R, Wagner M & Taylor MW (2008) Diversity and mode of transmission of ammonia-oxidizing archaea in marine sponges. *Environ Microbiol* **10**: 1087–1094.

Turque AS, Batista D, Silveira CB *et al.* (2010) Environmental shaping of sponge associated archaeal communities. *PLoS ONE* **5**: e15774.

Webster NS, Watts JE & Hill RT (2001) Detection and phylogenetic analysis of novel crenarchaeote and euryarchaeote 16S ribosomal RNA gene sequences from a Great Barrier Reef sponge. *Mar Biotechnol* **3**: 600–608.

Weisz JB, Hentschel U, Lindquist N & Martens CS (2007) Linking abundance and diversity of sponge-associated microbial communities to metabolic differences in host sponges. *Mar Biol* **152**: 475–483.

Weisz JB, Lindquist N & Martens CS (2008) Do associated microbial abundances impact marine demosponge pumping rates and tissue densities? *Oecologia* **155**: 367–376.

## 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.