

Archaeal communities at gas-venting shallow basins in the northern Gulf of California (Wagner and Consag basins)

Comunidades arqueales en cuencas someras con emisión de gas en el norte del Golfo de California (cuencas de Wagner y Consag)

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ABSTRACT

Deep-sea vents microorganisms have been well characterized with defined typical taxonomic groups, principally composed of archaea, while shallow hydrothermal vents are considered to host a different community with bacteria as predominant prokaryotes. This work focuses on two shallow basins in the northern Gulf of California: Wagner and Consag basins which show evidence of early stages of rifting processes and host numerous submarine vents with intense gas discharge. The exploratory study of archaea and bacteria in six sediment samples from the shallow vents in those basins (average depths of 100 m), demonstrate that similar archaea phyla inhabit these vents. The phylum Thermoproteota with *Nitrosopumilus* as the most abundant genera in five sites. However, in the sample with the highest temperature, the phylum Asgardarchaeota was predominant, and also the occurrence of archaeal lineages typical of deep sea vents like Nanoarchaeota, Thermoproteota, Euryarchaeota, and members from phylum Asgardarchaeota, were identified. This work is the first report of the presence of a typically deep vent community of archaea in this shallow vent environment.

Keywords: shallow submarine vents, extremophiles, Lokiarchaeia, Mexico.

RESUMEN

Los microorganismos que habitan las ventilas submarinas profundas han sido bien caracterizados con grupos taxonómicos típicos bien definidos y están integrados principalmente por arqueas, mientras que se considera que las ventilas someras se caracterizan por una comunidad de microorganismos representados principalmente por bacterias. Este estudio se enfoca en las comunidades de Arqueas en 6 sitios en las Cuencas de Wagner y Consag (con profundidades promedio de 100m), donde se han descubierto evidencias de que existen procesos iniciales de dispersión oceánica y que presentan numerosas ventilas submarinas someras con una intensa descarga de gas. El estudio para identificar la presencia de arqueas y bacterias, usando amplificación y secuenciación de 16S rDNA, se realizó en muestras de sedimento de ambas cuencas y se encontraron phyla de arquea similares a los de ventilas profundas. El phylum Thermoproteota del que *Nitrosopumilus* fue el más abundante en cinco sitios. Sin embargo, en la muestra proveniente del sitio con la temperatura más alta, el phylum Asgardarchaeota fue el predominante, también se identificaron los típicos de ventilas profundas como Nanoarchaeotas, Thermoproteota, Euryarchaeota, y miembros del phylum Asgardarchaeota. Este trabajo es el primer reporte de la presencia de estas comunidades en este ambiente de ventilas someras.

Palabras clave: ventilas submarinas someras, extremófilos, Lokiarchaeia, México.

1. Introduction

Submarine vents are sites where high temperatures and chemical conditions of the expelled fluids favour the occurrence of chemosynthetic communities of archaea and bacteria. The richness and abundance of microbes in deep vents have been studied for several decades and reported in numerous references (Pricur, 1997; Speth *et al.*, 2022; Takai and Nakamura, 2011; Zhou *et al.*, 2022). Specifically, archaea diversity in deep hydrothermal vents has been determined by DNA genome sequences that allowed the identification of thermophilic species of the phyla Thermoproteota and Euryarchaeota as well as methanogens and halophile species (Takai and Horikoshi, 1999; Rinke *et al.*, 2021). In contrast, less attention has been paid to the shallow submarine hydrothermal manifestations with lower temperatures <200°C. Shallow systems are characterized by the presence of a gas phase (absent in the deep-sea), low pressure, sunlight, and photosynthetic rates, which are remarkable differences that distinguish shallow from deep hydrothermal systems (Tarasov *et al.*, 2005). Those conditions define the features of the microbial species present in the shallow vents. Prokaryotic microbial species isolated from shallow hydrothermal vents correspond mainly to archaeal Phyla Thermoproteota and Euryarchaeota, and bacterial phyla Bacteroidetes, Chloroflexi, Firmicutes, Proteobacteria and Thermotogae. These organisms inhabit the subsurface and sediment around hydrothermal emissions, accumulating dense mats that can reach a thickness of up to 30 cm (Tarasov *et al.*, 2005; Rinke *et al.*, 2021).

The most studied shallow venting site is the Baia di Levante of Vulcano Island (Eolian Islands), where abundant information has been produced on the microbial communities of bacteria and archaea (Fiala & Stetter, 1986; Stetter, 1988; Caccamo *et al.* 2001; Maugeri *et al.*, 2001, 2002, 2009, 2010; Gugliandolo *et al.* 2003; Gugliandolo and Maugeri, 2019; Rizzo *et al.*, 2022). The microorganisms reported include aerobic and anaerobic,

thermophilic, and hyperthermophilic species. Archaea identified in Panarea vents are similar in community structure to those collected from submarine vents and geothermal wells in New Zealand and nearby Volcano Island (Maugeri *et al.*, 2009). A report for the Flegrean area, at Cape Palinuro and around the Eolian Islands (Maugeri *et al.*, 2010) confirms that the richness and abundance of bacteria is greater than for archaea, as observed in most shallow vent systems. Recent studies of bacterial communities in a low-temperature shallow vent site revealed the predominance of proteobacteria and bacteroidetes (Rajasabapathy *et al.*, 2018).

The second most studied area contains the shallow vents near Milos Island, where archaea represent only a minor part of the microbial community (Sievert *et al.*, 2000). On the other hand, in the shallow vents at the Kodakara-Jima Island in Japan, Hoaki *et al.* (1994) reported the presence of hyperthermophilic archaea closely related to the genus *Thermococcus*.

The 200-210 m deep Wagner and Consag Wagner and Consag Basins basins are the northernmost of the 8 active extensional basins within the Gulf of California (Figure 1). This is a particular zone where active rifting and intense gas discharge recently have been discovered, related with anomalously high heat flow (Prol-Ledesma *et al.*, 2013); the presence of extensive gas venting and heated sediments along the main faults indicates an estimated 15,000 CO₂-containing gas vents, each up to 100 m wide and giving rise to water column pH values as low as 6.3. This range of pH values and the higher than average sediment temperature in the studied area make this an excellent area for studying the combined effects of high temperature and low pH on microbial communities. In this work, we report the results of the characterization, using the 16S rRNA gene clone library approach, of the archaeal community found in the sediments from the Wagner and Consag Basins, in order to gain new insight into the archaeal communities of shallow vents.

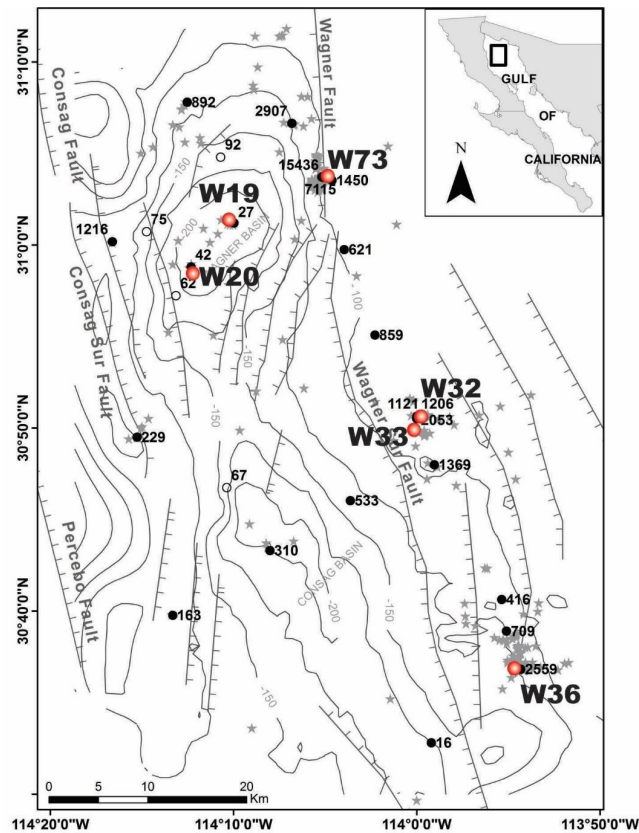


Figure 1 Bathymetric map and heat flow data of the Wagner and Consag basins; jagged lines denote faults, stars indicate vent location (after Prol-Ledesma *et al.*, 2013). Numbers indicate heat flow data (values in mWm^{-2} , the mean ocean heat flow is $105.4mWm^{-2}$). Sediment sampling sites shown with red dots and the station number: W19, W20, W32, W33, W36, W73, four of them are located nearby the largest vents in Figure 1 (W32, W33, W36, W73-highest heat flow values), site W73 is close to a mud volcano and stations W19 and W20 are in the Wagner Basin bottom with no evident features of gas discharge (low heat flow values).

2. LOCATION AND CHARACTERISTICS OF THE SAMPLING SITES

The Gulf of California is an example of the transition from continental rift to seafloor spreading. The extensional tectonics in the area separated the Baja California Peninsula from the North American plate by about 6 Ma, forming a series of basins separated by transformed faults related to the San Andreas fault system. There are 8 basins where active extensional features have been documented. Spreading centres maturity decreases towards the north, and new oceanic crust has been positively identified only in the southernmost basins (Lizarralde *et al.*, 2007; Paduan *et al.*, 2018); therefore, the studied area represents a nascent ocean basin

where hydrothermal activity is in its early stages. The studied basins (Wagner and Consag) had not been considered active spreading centres (Persaud *et al.*, 2003) until recently, when heat flow and seismic evidence revealed the occurrence of active spreading features and incipient hydrothermal manifestations (González-Escobar *et al.*, 2010; Prol-Ledesma *et al.*, 2013; Neumann *et al.*, 2017).

The shallow Wagner and Consag basins (200-210 m depth) have a large sedimentation rate that accounts for a great thickness of the sedimentary cover of about 7 km. In this area, mixing in the water column results in a homogeneous temperature of 15 °C at the sea bottom that remains practically constant the whole year (Álvarez-Borrego and Lara-Lara, 1991) except at the hydrothermal

vent discharge, where temperatures higher than 60 °C were measured at some vents (Prol-Ledesma *et al.*, 2013). The mean heat flow in the area is approximately 2 W/m², which is about 20 times the average seafloor heat flow (0.105 W/m²; Davies and Davies, 2010). The highest heat flow values are aligned with the Wagner fault that is the most relevant structure where the largest vents were discovered, and four sampling sites are positioned (Figure 1).

Seismic studies provide evidence of deep intrusions at the base of the sedimentary cover (González-Escobar *et al.*, 2010). Analyses of the gas sample showed that the gas from the sediment was predominantly CO₂ and methane (Prol-Ledesma *et al.*, 2013). Some of the deepest water samples collected within the plumes had Ba, As and Mn concentrations (30, 60, 12 µg/l, respectively, 20 m above the seabed), around 3 times higher than background (Canet *et al.*, 2010). The presence of extensive gas venting and heated sediments along the main faults indicates an estimated 15,000 CO₂-discharging gas vents, giving rise to water column pH values as low as 6.3 (Canet *et al.*, 2010). This range of pH values and the higher than average sediment temperature are expected to generate a specific ecosystem including a distinct microbial community. Biological studies in this area include benthic Foraminifera, Sipunculida and Kinorhyncha distribution in relation with venting, their abundance seems to be related with the gas venting occurrence and geochemistry (Hermoso-Salazar *et al.*, 2013; Pettit *et al.*, 2013; Álvarez-Castillo *et al.*, 2015, 2018, 2023). Twelve higher taxa were recorded in both basins, where meiofauna was dominated by Nematoda (73.1%) followed by Copepoda Harpacticoida (11.28%), Polychaeta (8.41%) and Kinorhyncha (4.71%) (Álvarez-Castillo *et al.*, 2018). Close to fluid outlets in the softer sediments, there was a community of chaetopterid species, with tubes up to 40 cm or more in length and one station contained 3 specimens of a bivalve *Solemya* (*Petrasma*) sp. with endosymbiotic bacteria in the gills (Canet *et al.*, 2010).

Organic matter makes up to 2% in the sediment samples and more than 20% is bitumen, which indicates a high level of organic matter maturation (Ángeles *et al.*, 2017). None of the recovered samples showed signs of hydrocarbon staining; however, evidence of the presence of hydrothermal activity in the Wagner and Consag basins is provided by the SOM alteration. The hydrothermal activity in the area generates the maturation of organic matter, as inferred from the presence of diverse compounds: polycyclic aromatic hydrocarbons (PAHs), mono and dimethylated alkanes, trimethylnaphthalene isomers and their relative proportions in the sediment samples (Ángeles *et al.*, 2017). The identification of the pentamethyl-icosane (PMI) isoprenoid in sediment samples from the Wagner- Consag basins indicated the probable presence of Thermoproteota that live at temperatures between 75 and 105°C (Thiel *et al.*, 1999; Madigan *et al.*, 2003), this is important evidence of the occurrence of hydrothermal activity even when the main manifestation is intense gas venting.

3. Methodology

3.1. SAMPLE COLLECTION AND CHEMISTRY

During the WAG-2 cruise of the O/V “El Puma” the seabed sediments of the Wagner and Consag Basins were sampled using a USNEL box corer and a Smith-McIntyre grab in 6 sites within and outside the basins (Figure 1). Temperature profiles were measured within the sediments to determine temperature gradient using a 6m long FIELAX probe with 11 thermistors, these measurements included sea bottom temperature determination (T_{sb}). Additionally, the temperature of recovered sediment was immediately measured on board with a digital thermometer on recovery (T_o). The pH was immediately measured on the water samples using a pH meter with an accuracy of 0.01 pH units and compared with buffers prepared in artificial seawater (Table 1).

Table 1. Sample location, T_{sb} -sea bottom temperature, T_{ob} -on board sample temperature.

Sample number	Longitude W	Latitude N	Depth (m)	T_{ob} (°C)	pH	T_{sb} (°C)
W-19	114.1660	31.0207	220	20.6	7.20	15
W-20	114.2070	30.9792	207	19.7	7.27	15
W-32	113.9940	30.8455	89	20.0	6.48	20
W-33	114.0000	30.8439	84	23.0	6.83	20
W-36	113.9010	30.6268	119	19.0	7.31	17
W-73	114.0900	31.0796	98	21.0	6.29	27

3.2. DNA EXTRACTION, CLONING AND SEQUENCING OF 16S RDNA GENES

Total DNA was extracted using the Power Soil® DNA Isolation Kit (Mo Bio). 16S rDNA genes were amplified using the universal 16S primers for bacteria 27F and 1492R (Lane, 1991), and A21F (5'-TTCCGGTTGATCCYGCCGGA-3') and A958R (5'-YCCGGCGTTG AMTCCAATT-3') for archaea. 1492R primer (5'-TACCTTGT-TACGACTT) and one of the following three 27f primer formulations: twofold-degenerate primer 27f-CM (5'-AGAGTTTGATCMTGGCTCAG, where M is A or C), fourfold-degenerate primer 27f-YM (5'-AGAGTTTGATYMTGGCTCAG, where Y is C or T), The reaction mixtures were incubated at 94°C for 4 min, followed by 33 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 15 s, and extension at 72°C for 45 s.

Purified PCR products were cloned into TOPO TA cloning vector pCR2.1 according to the manufacturer's instructions (Invitrogen, Carlsbad, CA) and transformed into *E. coli* XL1-Blue competent cells by electroporation. Transformants were transferred to plates containing LB broth (25 ug/ml kanamycin and 200 ug/ml ampicillin) and grown overnight at 37°C. Plasmids were isolated

using High Pure Plasmid kit (ROCHE, Indianapolis, IN); subsequently, clones were digested (2 h, 37°C) with EcoRI (BioLabs, New England) for evaluating the presence of inserts. Inserts were sequenced at the Sequencing Facility of the Instituto de Biotecnología, Universidad Nacional Autónoma de México, using a Taq FS Dye Terminator cycle fluorescence-based sequencing with an automated capillary sequencer (Perkin Elmer, model 3130x1, Applied Biosystems).

3.3. 16S RDNA SEQUENCES AND PHYLOGENETIC ANALYSIS

Forward and reverse sequences were pre-processed and assembled using free available software (Staden *et al.*, 2003). Potential chimeric sequences were identified using the software UCHIME (Edgar *et al.*, 2011) and DECIPHER (Wright *et al.*, 2012). The sequences identified by BLAST (available through the National Center for Biotechnology Information) as 16S rRNA were subsequently aligned using ClustalW (Thompson *et al.*, 1994). Aligned sequences having >97% of similarity were clustered into operational taxonomic units (OTUs) by the average neighbour algorithm (Legendre and Legendre, 1998), for each OTU

the sequence with the smallest maximum distance to the other sequences (representative sequence) was selected using Mothur (Schloss *et al.*, 2009). The representative sequence for each OTU was taxonomically assigned using the SILVA 128 Incremental Aligner (SINA) Online (Pruesse *et al.*, 2012; Quast *et al.*, 2013). Phylogeny was inferred using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980). The consensus phylogenetic tree was inferred from 500 bootstrap replicates, evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013).

Bacterial community was inspected, after raw sequence clean and processing there were obtained 38 bacterial 16S rRNA sequences with an average length of 756bp. The number of sequences for each sampled site ranged from 2 to 12. All the sequences were average neighbour clustering at a 0.03 distance level, in total 12 OTUs were produced. After clustering independently each sampled site's sequences 2 to 5 OTUs were produced. The 12 OTUs were taxonomically classified. Data available in <https://www.ncbi.nlm.nih.gov/popset/1345460006> , <https://www.ncbi.nlm.nih.gov/popset/1343668055>

4. Results

4.1. ARCHAEA DIVERSITY

The 41 OTUs were taxonomically classified (Figure 3); OTU1 and OTU2 are the most abundant OTUs with 48% of all sequences, those were affiliated to the candidate *Nitrosopumilus maritimus* from Thermoproteota Phylum, this archaea is capable to grow in environments with very low ammonium concentrations and is ubiquitous in aquatic ecosystems (Offre *et al.*, 2013). The high abundance of *Nitrosopumilus maritimus* in all the sampling sites may correspond to the predominance of seawater content in the sediments. Two OTUs could not be associated with any archaeal phyla and may belong to some novel archaeal phylum. The rest of the OTUs were classified into the novel proposed phyla: Asgardarchaeota, Thermoproteota

and Nanoarchaeota; to the uncultivable classes like, Nitrososphaeria from the phylum Thermoproteota, Thermococci from Euryarchaeota and Lokiarchaeia from the Asgardarchaeota phylum, all those archaeal lineages have previously been reported in marine sediments and near to deep marine hydrothermal vents (Takai and Horikoshi, 1999; Zaremba-Niedzwiedzka *et al.*, 2017; MacLeod *et al.*, 2019; Rinke *et al.*, 2021).

The relative abundance of the identified archaeal lineage for each sampled site is shown in Figure 3. About 50 % of the sequences were taxonomically assigned as *Nitrosopumilus maritimus* (phylum Thermoproteota), such microorganisms are one of the most common archaeon living in seawater. Two OTUs could not be associated with any archaeal phylum and may belong to some novel archaeal phyla (Figure 2). The rest of the OTUs were classified into the novel proposed phylum: Asgardarchaeota, Thermoproteota and Nanoarchaeota; to the uncultivable Group C3 Thaumarchaeota and the candidate's divisions WSA2 and Marine Hydrothermal Vent Group (MHVG), all these archaeal lineages have previously been reported in marine sediments and near to deep marine hydrothermal vents (Adam *et al.*, 2017; Spang *et al.*, 2017).

Asgardarchaeota was the second most abundant phylum identified, many of these archaea were previously classified as Marine Benthic Group B (Solden *et al.*, 2016); this group is related to the iron cycle (Aoki *et al.*, 2014) and some archaea of this group have been also found in marine sediments (Durbin and Teske, 2012; Fry *et al.*, 2008; Hugoni *et al.*, 2015; Jiao *et al.*, 2011; Na *et al.*, 2015; Teske *et al.*, 2002; Teske and Sorensen, 2008; Rinke *et al.*, 2021). In this study, the presence of Asgardarchaeota was observed in all sites; however, Asgardarchaeota was the most abundant archaeon in site W33 (Figure 3), at approximately 80 m depth, where the most intensive gas discharge and extremely high heat flow were detected (Figure 1).

The third most abundant phylum identified was Thermoproteota previously named Miscel-

laneous Crenarchaeota Group (Meng *et al.*, 2014; Rinke *et al.*, 2021), this archaeal group has capabilities for protein fermentation (Lloyd *et al.*, 2013; Meng *et al.*, 2014; Rinke *et al.*, 2021), homoacetogenesis (Lazar *et al.*, 2016; Meng *et al.*, 2014) and methane cycling (Evans *et al.*, 2015). The fourth most abundant archaeal lineage identified was the Thermoproteota phylum Nitrososphaeria class, in many reports identified as dominant phyla in deep

hydrothermal vents, and also abundant in W33 (Nunoura *et al.* 2018; Meng *et al.*, 2014; Rinke *et al.*, 2021).

Archaeal lineages Lokiarchaeia class and Thermococci class were less abundant and were present in two sites W33 and W73 (Figure 3). Lokiarchaeia class is a deep branch archaeal lineage detected near to deep marine hydrothermal vents and has been poorly described (Teske and Sorensen, 2008).

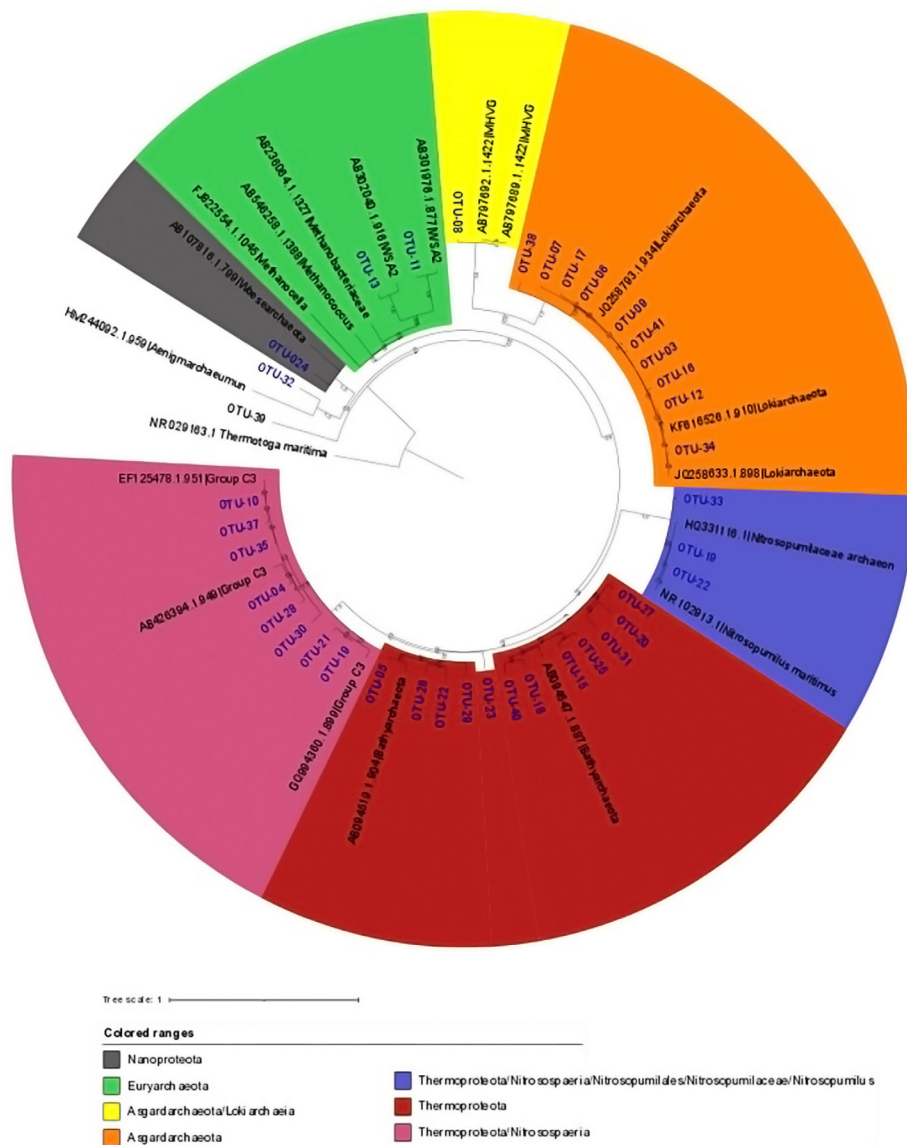


Figure 2 Phylogenetic tree of the archaeal sequences clones. The representative sequence for each archaeal OTU were aligned by ClustalW. Phylogeny was inferred using the Maximum Likelihood method based on the Kimura 2-parameter model, the consensus phylogenetic tree was inferred from 500 bootstrap replicates.

Members of Nanoarchaeota, a phylum which was previously named Deep-sea Hydrothermal Vent Euryarchaeota Group-6, might primarily be involved in anaerobic carbon cycling and is considered to have a symbiotic lifestyle (Castelle *et al.*, 2015; Rinke *et al.*, 2021), it was present only in site W36 (Figure 3). The Thermococci class archaea have been detected in a wide range of natural environments including deep marine hydrothermal sediments (Nobu *et al.*, 2016 ; Rinke *et al.*, 2021). Whole genome sequencing shows that these archaeal groups possess metabolic routes for methanogenesis (Dhillon *et al.*, 2005). In this study its occurrence was restricted to two sites W33 and W73 (Figure 3), where the most intense gas discharge occurs.

The appearance of some lineages like Nitroso- poeria class from the Thermoproteota phylum and Nanoarchaeota phylum (typically dominant archaea groups in deep hydrothermal vents) in the shallow vents studied; suggest that fluid chemistry could be one of the most important factors determining their presence in these environments, possibly more restricted by local temperature and fluid composition than by depth and pressure.

4.2. BACTERIA DIVERSITY

The identified Bacterial taxonomic clones for each sampled site are presented in Table 2. The Phylum Proteobacteria was found to be dominant with presence in all sites studied, Class Gammaproteobacteria (Teske and Salman, 2014) was the most abundant bacterial lineage identified to correspond to the genera *Psychrobacter*, *Shewanella* and *Amphritea*; bacteria that belong to these genera have previously been detected near to marine hydrothermal vents (Gärtner *et al.*, 2008; Sun *et al.*, 2015; Zhou *et al.*, 2016; Satomi, 2014).

5. Discussion

The formation of typical chimneys associated with hydrothermal activity at the sea bottom has not been documented in the Wagner and Consag basins. However, there is evidence of convective hydrothermal circulation based on the extremely high heat flow that was measured in the Wagner Basin (Prol-Ledesma *et al.*, 2013). Additionally, extensive gas discharge was observed and reported

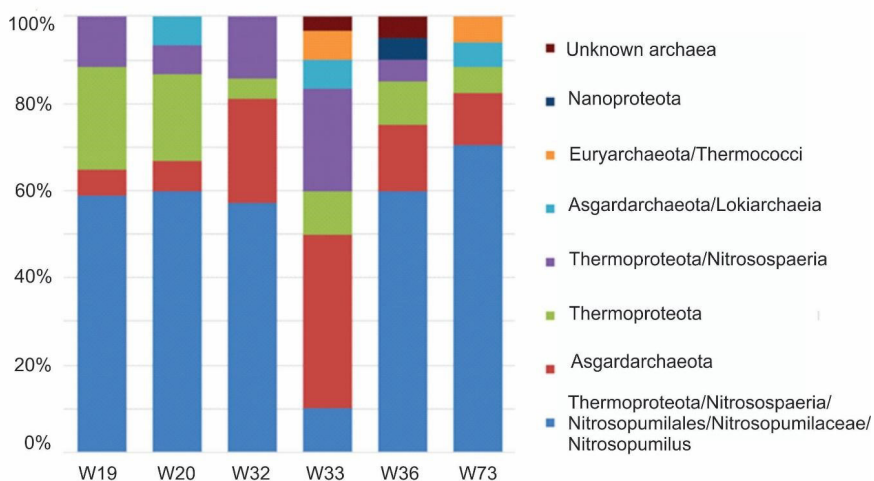


Figure 3 The plot presents the relative abundance of each identified archaeal lineage for each sampled site. The representative sequence for each OTU was taxonomically assigned using SILVA 128 Incremental Aligner (SINA) Online (Pruesse *et al.*, 2012; Quast *et al.*, 2013).

Table 2. Bacterial taxonomic clones identified per sampled site. The sequences from all sampled sites were average neighbour clustered, and the description of the number of sequences per OTU, sequence id and taxonomic assignment is shown.

OTU	Representative sequence	Sequences in the OTU	Sequences ids for each sampled site						Taxonomic Assignment
			19	20	32	33	36	73	
1	MG952508	8	-	MG952508 MG952491 MG952506A	-	MG952515	MG952518 MG952500 MG952522 MG952525	-	Shewanella
2	MG952517	9	-	-	MG952512	-	MG952517 MG952523 MG952520 MG952521 MG952516 MG952502 MG952501 MG952524	-	Amphritea
3	MG952498	6	-	MG952489	-	MG952498 MG952495 MG952497 MG952494 MG952514	-	-	Psychrobacter
4	MG952503	5	MG952488	MG952490	MG952513	-	-	MG952503 MG952504	Psychrobacter
5	MG952493	3	-	-	-	MG952493 MG952505	-	MG952526	Paenisporosarcina
6	MG952528	1	-	-	-	-	-	MG952528	Unknow Rhodobacteraceae
7	MG952507	1	-	MG952507	-	-	-	-	Sulfitobacter
8	MG952496	1	-	-	-	MG952496	-	-	Unknow Desulfobacteraceae
9	MG952527	1	-	-	-	-	-	MG952528	Ruegeria
10	MG952487	1	MG952487	-	-	-	-	-	Erythrobacter
11	MG952506	1	-	MG952506	-	-	-	-	Shewanella
12	MG952499	1	-	-	-	MG952499	-	-	Delftia

in the area, and the predominance of CO₂ and CH₄ was measured. The discharge of CO₂ is common in hydrothermal systems and in this case it is associated with the ocean acidification measured in this area, and the presence of CH₄ is frequently related with methanotrophs presence.

The results of this study present the composition of archaeal and bacterial communities at 6 different sites in an area characterized by incipient hydrothermal activity related with ocean spreading. Four samples represent the sites with the most intense fluid discharge and high heat flow, and two sites were sampled where flares have not yet been detected. A total of 120 archaeal sequences using amplification and sequencing of 16S rDNA gene were obtained. About 50 % of the sequences were identified as *Nitrosopumilus maritimus* (Phylum Thermoproteota, class Nitrososphaeria), such

microorganisms are one of the most common archaeon living in seawater. The rest of the OTUs were classified into the novel proposed phyla: Asgardarchaeota, Thermoproteota and Nanoarchaeota; and into the uncultivable classes like, Nitrososphaeria from the phylum Thermoproteota, Thermococci from Euryarchaeota phylum and Lokiarchaeia from the Asgardarchaeota phylum, all these archaeal lineages have previously been reported in marine sediments and near to deep marine hydrothermal vents (Teske and Sorensen, 2008). The presence of Asgardarchaeota in shallow vents indicates that they are more adaptable and not restricted exclusively to deep hot vents. High temperature seems to favour their abundance as the site where they were most abundant is one with strong venting activity. Apparently, the high temperature and intense venting activity boost up

its higher relative abundance in sites W32, W33, W36 and W73, compared to their low abundance in the inactive sites W19 and W20.

The recently discovered candidate Archaeal Phylum Asgardarchaeota was defined previously as part of the Deep Sea Archaeal Group (DSAG) and it is a likely candidate to stand as a link with the Eukaryotic domain with which they share specific genome features (Spang *et al.*, 2015; Rinke *et al.*, 2021). In this work we show that within the sediments affected by hydrothermal shallow vents, Asgardarchaeota represents an important part of the microbial composition and diversity and is the dominant phyla in the hottest sample at a depth of approximately 80m. The presence of Asgardarchaeota in shallow vents, indicates that they are more adaptable and not restricted exclusively to deep hot vents. Zaremba-Niedzwiedzka *et al.* (2017) reported a wide distribution of what they denominated the Aasgard clade in different environments; nonetheless, they did not find any representatives of the Aasgard clade in the shallow vent site that they studied.

The similarity of some groups like Thaumarchaeota in shallow and deep vents indicate that depth/pressure may not play a key role in the microbial diversity, and that fluid chemistry and temperature are the main factors in the species present in the shallow and deep sea bottom hydrothermal vents.

Recent studies demonstrated that the growth rate of *Archaeoglobus fulgidus* is not affected up to pressures equivalent to 4000 m depth (Oliver *et al.*, 2020); however, further work is required to evaluate the optimal pressure for the different phyla. Presently, the presented evidence suggests that deep-sea archaea occurrence is more restricted by temperature than by pressure.

In the case of bacteria, *Psychrobacter* and *Shewanella* genera have been detected in a wide range of marine habitats (Teske and Sorensen 2008; Satomi, 2014) and the Amphritea genus is closely associated with living marine organisms (Jang *et al.*, 2015).

Bacterial families Rhodobacteraceae and Planococcaceae and the genera *Erythrobacter*, *Delftia*

and *Sulfitobacter* were identified in less abundance. The clones that belong to the families Rhodobacteraceae and Planococcaceae may correspond to novel bacteria genera, both Planococcaceae and Rhodobacteraceae bacteria have been detected in a wide variety of habitats (Pujalte *et al.*, 2014; Shivaji *et al.*, 2014). The genera *Erythrobacter*, *Delftia* and *Sulfitobacter* also have been detected in a wide range of habitats; therefore, it is expected that the physiology of each genera species should be diverse (Rosenberg *et al.*, 2013).

The shallow vents in two sites in western Mexico, in Bahía Concepción and Punta Mita where microbial communities have been characterized, present different redox conditions that constrain the presence/absence of distinct species of bacteria: Gamma-, Delta-, and Epsilonproteobacteria as well as Bacteroidetes are present under the oxidizing conditions of Bahía Concepción, and Thermotogae, Aquificae, and Planctomycetes were identified in the shallow vents under strongly reduced conditions in Punta Mita (Dávila-Ramos *et al.*, 2015).

One of the best documented shallow hydrothermal areas is the Baia di Levante of Vulcano Island (Eolian Islands), where abundant information has been produced about the bacteria and archaea communities present on that site (Caccamo *et al.*, 2001; Fiala and Stetter, 1986; Gugliandolo *et al.*, 2003; Maugeri *et al.*, 2001, 2009; Segerer *et al.*, 1986; Stetter, 1988); the microorganisms reported include aerobic and anaerobic, thermophilic, and hyperthermophilic species. In the shallow vents near Milos Island, archaea represent less than 10% in average of the microbial community (Sievrt *et al.*, 2000), similarly to what was observed in shallow vents from Panarea, Italy and western Mexico (Dávila-Ramos *et al.*, 2015; Lentini *et al.*, 2014). In Panarea vents, the archaea identified are less abundant than bacteria and restricted to the hottest areas in this system (Euryarchaeota: Thermococci and Thermoplasmata; and Crenarchaeota: Thermoprotei) (Lentini *et al.*, 2014). The studies of microbial communities in shallow vents generated by serpentinization reactions like Prony Bay, reveal low archaeal diversity with a predominance of

methane metabolizing organisms, very similar to the diversity observed in deep vents with the same processes of serpentinization (Quéméneur *et al.*, 2014). Therefore, the presence of archaea and bacteria in the shallow vents studied here extends the conditions under which these communities have been reported.

6. CONCLUSIONS

In this work, we demonstrated the presence of archaea, typically associated with deep-sea hydrothermal vents, in sediments from shallow vents corresponding to the Wagner and Consag basins in the northern Gulf of California. We suggest that discharge and composition of hydrothermal fluids and local temperature, more than depth, are the determinant factors favouring the occurrence of specific microbial groups like Asgardarchaeota and Nanoarchaeota (previously named Deep-sea Hydrothermal Vent Euryarchaeota Group) and typically associated to deep sea vents and now also detected in the shallow vents studied. This work confirms the presence of the deep vent community of archaea and the Lokiarchaeia class of the Asgardarchaeota phylum in shallow hydrothermal vents.

The study of microbial communities in sediment samples from the shallow vents in the Wagner and Consag basins demonstrated that these archaea and bacterial communities are similar to those identified in deep sea hydrothermal vents. Altogether, these findings represent valuable information for understanding the microbial distribution and potential ecological roles in deep and shallow hydrothermal environments.

Author contribution

(1) conceptualization: RMPL, KJ; (2) analysis/data acquisition: FPV, SD; (3) methodological development: KJ, FVP, SD; (4) writing original manuscript: KJ, FVP, RMPL, SD; (5) writing revised manuscript: KJ, FVP, RMP, SD; (6) graphic

design: FPV, SD; (7) field work: RMPL; (8) interpretation: KJ, FVP, RMP, SD; (9) funding: RMPL.

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Conflict of Interest

The authors declare no financial/commercial conflict of interest.

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