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Expanding Metabolic Diversity of Two Archaeal Phyla: Nanoarchaeota and Korarchaeota

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Expanding Metabolic Diversity of Two Archaeal Phyla:
Nanoarchaeota and Korarchaeota

by

John Forad Kelley

A thesis submitted in partial fulfillment of the
requirements for the degree of

Master of Science
in
Biology

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Abstract

Culture independent studies have revealed a greater diversity of Archaea than the two kingdoms initially defined by Carl Woese. Culturing species from the newly discovered archaeal lineages, as with the majority of microbes, has been difficult. To overcome the culturing dilemma, metagenomics is being used to reconstruct environmental genomes. Two metagenomic studies are presented in this thesis, with the aim of recovering newly proposed archaeal genomes from the Nanoarchaeota and Korarchaeota.

In the first study, a sediment sample, NZ13, was collected from a terrestrial New Zealand hot spring. Along with the sediment sample, two complex enrichments were sequenced on an Illumina MiSeq platform. Assembly and differential binning recovered two nearly complete genomes of a nanoarchaeote and a korarchaeote. The NZ13 nanoarchaeote is similar to other terrestrial nanoarchaeotes, which lack an ATP synthase and encode genes for glycolysis/gluconeogenesis and archaeella. One notable difference is the NZ13 nanoarchaeote contains CRISPR genes, which are absent in other terrestrial nanoarchaeotes, although present in a marine nanoarchaeote, *Nanoarchaeum equitans*. The NZ13 korarchaeote mirrors *Candidatus Korarchaeum cryptofilum*, lacking genes for *de novo* synthesis of purines and several cofactors, while containing an abundance of peptide transporters and amino acid fermentation pathways.

The second study focused on sulfide samples collected from deep-sea hydrothermal vent fields in southwestern Pacific Ocean along the Eastern Lau Spreading Center. Ten sulfide samples were sequenced on an Illumina HiSeq platform. Small

subunit ribosomal RNA genes were extracted from the metagenome reads and aligned against the SILVA Ref NR 99 123 database. The preliminary results identified which samples could be prioritized for genome reconstruction of uncultured bacterial and archaeal lineages. Three uncultured bacteria, candidate division SR1, Gracilibacteria (GN02) and Parcubacteria (OD1) were identified in several samples. Many uncultured deep-sea hydrothermal archaeal lineages were identified in all samples. In particular, korarchaeotal sequences were in high relative archaeal abundances in two samples, ABE-1 and Vai Lili-2, while few nanoarchaeotal reads were classified.

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Chapter 1: Introduction

Microbes account for most of our planet's biological diversity, yet more than a century of cultivation approaches have isolated only a small fraction of the microbial life (Torsvik et al. 1990; Ward et al. 1990; Giovannoni et al. 1990). Through microscopy, early microbiologists knew the natural environment was more complex than their cultures. The advent of DNA sequencing unveiled the true complexities of the microscopic world, through identification of microbes using cultivation independent techniques (Barns et al. 1994; Barns et al. 1996).

The small subunit ribosomal RNA (SSU rRNA) is conserved evolutionarily and was first exploited by Carl Woese to redefine the Tree of Life (Fox et al. 1977). Woese's work resulted in the reclassification of prokaryotes from a single kingdom into two domains: Bacteria and Archaea (Fox et al. 1977; Woese et al. 1990). For the first time, a phylogenetic tree could be drawn from DNA sequences and subsequently, the SSU rRNA gene became a widespread method for prokaryotic identification. The SSU rRNA redefined evolutionary relationships, but the bulk of the uncultivated microbial genomic information was out of reach to researchers prior to whole genome sequencing. From Woese's rRNA-based reclassification, nearly two decades passed until the first bacterial genome, *Haemophilus influenza*, was fully sequenced from an isolate (Fleischmann et al., 1995). Soon thereafter, the requirement for lab cultures was bypassed as a precursor for genomic sequencing (Stein et al. 1996). Sequencing of all DNA isolated from environmental samples was demonstrated and termed metagenomics (Handelsman et al. 1998).

Pioneering Metagenomic Research

The first metagenomic studies cloned environmental DNA fragments to construct sequence libraries. Environmental DNA was extracted, digested into large fragments and randomly inserted into vectors. The recombinant DNA was then fragmented again for shotgun sequencing. Then overlapping sequences were reassembled to generate contiguous strands of DNA known as contigs (Figure 14). An initial study used fosmid vectors which would accept DNA inserts up to 40 kilobases (kb) (Stein et al. 1996). Subsequent studies used bacterial artificial chromosome (BAC) vectors with DNA inserts of up to 750 kb (Stone et al. 1996; Handelsman et al. 1998).

A metagenomic study by Bj et al., 2000 of oceanic surface waters revealed the existence of rhodopsin in a bacterial genome. A 130 kb BAC vector encoded for a rRNA operon belonging to SAR86, an uncultivated bacterium from the *-Proteobacteria* (Bj et al. 2000). The SAR86 genomic fragment contained a rhodopsin gene, a light dependent proton pump. Prior to this study, the rhodopsin genes were only known in Archaea and Eukarya. To investigate whether the bacterial rhodopsin gene was functional, it was cloned and expressed in *Escherichia coli*. In the presence of retinal, cells expressing rhodopsin developed pigmentation and showed a peak absorbance of 520 nm, a wavelength of light that travels far below the ocean's surface. This discovery explained why the SAR86 clade was localized to the marine euphotic zone, the region where sunlight penetrates. The small genomic fragment contained important information about microbial physiology of SAR86, but was still only a snapshot of the uncultured genome.

Genome Recovery from Environmental Samples

Some of the first nearly complete environmental genomes were reconstructed from a metagenome of a subsurface acid mine drainage (AMD) microbial community (Tyson et al. 2004). The AMD was selected for the low diversity, self-sustaining microbial community that received little to no external nutrients. With a low diversity microbial community, enough DNA fragments were sequenced, assembled into contigs and enabled genomic reconstruction. Contigs were grouped into single genomic bins using GC content and sequencing coverage. The results were two nearly complete genomes and three partial genomes, a near full recovery of the genomic DNA from the microbial community (Tyson et al., 2004).

The AMD microbial community required the machinery for assimilating carbon and nitrogen. As expected, several recovered genomes contained carbon fixation pathways that utilize the Calvin-Benson-Bassham cycle or reductive acetyl coenzyme A pathway. The primary nitrogen fixer was expected to be the dominant bacterial species, *Leptospirillum* group II. However, the nitrogen assimilation genes were located on a binned genome belonging to an uncultivated, relatively low abundance community member, *Leptospirillum* group III. Subsequently, the results led to the isolation of *Leptospirillum ferrodiazotrophum*, providing a cultured representative for a lineage in the Nitrospirae (Tyson et al. 2005).

The example above provided evidence that genome directed culturing was possible. However, there have only been a few examples where this method led to the successful isolation of novel microbes (e.g. Könneke et al. 2005; Bomar et al. 2011; Wurch et al. 2016). Instead, microbial ecology is still reliant on metagenomics or other

meta-omics to explore microbial diversity.

Archaeal Diversity Expansion: Korarchaeota and Nanoarchaeota

Analysis of SSU rRNA genes from cultured isolates supported the initial division of the Archaea, creating the Crenarchaeota and Euryarchaeota kingdoms (Woese et al. 1990). The Euryarchaeota contained methanogenic, thermophilic and halophilic species, while the Crenarchaeota mostly comprised of thermophiles. Environmental SSU rRNA gene surveys exposed a new archaeal lineage, the Korarchaeota, that was grouped at the phyla level (Barns et al. 1994). The korarchaeotal SSU rRNA genes are endemic to terrestrial and marine thermophilic hot springs, ranging from mildly acidic to neutral (Barns et al. 1994; Auchtung et al. 2006; Miller-Coleman et al. 2012). Phylogenetic analysis indicated that the Korarchaeota cluster into five groups separated by geographic location (Auchtung et al. 2006). Additionally, there has been difficulty in obtaining a member from the phylum in culture (Elkins et al. 2008).

The first korarchaeotal genome was obtained from an enrichment culture of a sample from Obsidian Pool, Yellowstone National Park (YNP) (Elkins et al. 2008). Korarchaeotal specific SSU rRNA probes showed long, thin ($>0.2\ \mu\text{m}$) filaments ranging in length from $15\ \mu\text{m}$ to $100\ \mu\text{m}$ (Elkins et al. 2008). From the genome, *Ca. K. cryptofilum* utilizes peptide fermentation pathways for carbon and energy (Elkins et al. 2008). Pathways for the biosynthesis of purines and several cofactors are absent, suggesting a dependency on another organism or nutrient scavenging. Protein homology analysis found many crenarchaeotal and euryarchaeotal specific genes which supports a deep-branching archaeal lineage.

Another proposed ancestral archaeal lineage, the Nanoarchaeota, was previously not detected using environmental SSU rRNA gene surveys. Several base pair changes in the highly conserved regions of the SSU rRNA gene prevented universal primers from binding (Huber et al. 2002). The Nanoarchaeota were discovered while isolating the hyperthermophilic crenarchaeote, *Ignicoccus hospitalis*, from a shallow hydrothermal vent off the coast of Iceland (Huber et al. 2002). Many tiny cocci, less than 400 nm in diameter, were attached to its outer membrane and could only be grown in a coculture with *I. hospitalis*. The nanoarchaeotal cells were physically isolated for sequencing, producing the genome of *Nanoarchaeum equitans* (Waters et al. 2003).

The genome of *N. equitans* is greatly reduced, consisting of only 490 kbp and has many split proteins and tRNAs (Waters et al. 2003; Randau et al. 2005). The genome lacks synthesis pathways for lipids, cofactors, amino acids and nucleotides. Therefore, *N. equitans* is dependent on its host for most of its nutrients, but has retained molecular machinery genes for genetic processing, repair and replication.

Two nanoarchaeotal genomes have recently been sequenced from YNP. *Nanopusillus acidilobi* genome has been fully sequenced (Wurch et al. 2016) and Nst1 consists of 7 contigs (Podar et al. 2013). *N. acidilobi* and Nst1 are characterized by three important differences from *N. equitans*: an absence of an ATP synthase complex, the presence of archaeal genes and a near complete gluconeogenesis/glycolysis pathway (Podar et al. 2013; Wurch et al. 2016). All three nanoarchaeotal genomes share in common a reduced genome, the lack of biosynthesis pathways and the dependency on a host for growth (Waters et al. 2003; Podar et al. 2013; Wurch et al. 2016).

All three nanoarchaeotal hosts are from different crenarchaeotal orders, *Desulfurococcales*, *Acidilobales* and *Sulfolobales* (Huber et al. 2002; Podar et al. 2013; Wurch et al. 2016). Furthermore, *N. equitans* host is an autotroph (Huber et al. 2000), likewise Nst1 proposed host is an autotroph (Podar et al. 2013), while *N. acidilobi* host is a heterotroph (Wurch et al. 2016). In the nanoarchaeotal cocultures, *N. equitans* reduced its host ability to reproduce (Jahn et al. 2008), while *N. acidilobi* did not have an apparent negative effect on its host growth (Wurch et al. 2016). Further nanoarchaeotal-host characterization, either through cultivation or single cell genomics, should reveal the diversity of hosts utilized by different nanoarchaeotal species.

Originally the Korarchaeota and Nanoarchaeota were proposed as sister phyla to the Crenarchaeota and Euryarchaeota (Barns et al. 1996; Huber et al. 2002). Since then, metagenomics has expanded the archaeal domain, with proposals that the Korarchaeota and Nanoarchaeota are members of the TACK and DPANN superphyla, respectively (Rinke et al. 2013). Recently, the SSU rRNA gene has been used in conjunction with full-length ribosomal protein sequences yielding higher-resolution phylogenetic trees, inclusive of uncultivated lineages. Still, the basis for these new superphyla has been controversial (Hug et al. 2016). Many proposed phyla like the Korarchaeota and Nanoarchaeota have limited genomic representation. Nonetheless, the number of archaeal phyla continues to expand and for a more robust analysis, additional genomic data, from metagenomics and cultivation, needs to be gathered from diverse locations before a phylogenetic consensus can be reached.

Scope of Thesis

The main goal of this study was to expand understanding of microbial diversity, with a specific focus on Korarchaeota and Nanoarchaeota from high temperature environments using metagenomic sequencing. In Chapter 2, I used differential binning to recover and refine a nanoarchaeote and a korarchaeote genome from a New Zealand hot spring. I annotated both genomes and compared their physiologies to available nanoarchaeotal and korarchaeotal genomes. In Chapter 3, I performed the initial metagenomic processing for ten hydrothermal vent sulfide samples from the Eastern Lau Spreading Center in the southwestern Pacific Ocean. I extracted the SSU rRNA reads from each metagenome and aligned the reads against a SSU rRNA reference database, creating a community profile for each sample. I also identified which samples would be best for genome recovery of uncultivated bacterial and archaeal lineages from the deep-sea hydrothermal vent samples.

Chapter 2: Genomic analysis of New Zealand nanoarchaeotal and korarchaeotal genomes reveals a conserved metabolism among terrestrial lineages

Abstract

The Nanoarchaeota and Korarchaeota represent two poorly characterized, deep-branching archaeal phyla, with few sequenced genomes. Both lineages have been detected globally in terrestrial and marine high temperature environments, but have been difficult to cultivate in the laboratory. All cultivated Nanoarchaeota form ectosymbiotic/parasitic associations with their crenarchaeotal hosts, while no Korarchaeota have been grown in pure culture. In order to further characterize and obtain additional insights into their potential physiologies, metagenomes of environmental and enrichment samples containing nanoarchaeotes and korarchaeotes were analyzed from a New Zealand terrestrial hot spring (NZ13). The NZ13 nanoarchaeotal genome is similar to other terrestrial nanoarchaeotes from Yellowstone National Park with similar metabolism, archaeellum genes and lack of an ATP synthase. The NZ13 korarchaeote mirrors *Ca. K. cryptofilum*, which lack genes for *de novo* synthesis of purines and several cofactors, while containing an abundance of peptide transporters and amino acid fermentation pathways.

Introduction

Since their discovery, the Nanoarchaeota and Korarchaeota have been detected globally in terrestrial and marine high temperature environments using small subunit (SSU) rRNA gene surveys (Barns et al. 1994; Huber et al. 2002; Auchtung et al. 2006; Casanueva et al. 2008; Miller-Coleman et al. 2012). Their phylogenetic placement within the Archaea has remained ambiguous and has become further muddled as more

archaeal phyla continue to be discovered (Brochier-Armanet et al. 2008; Rinke et al. 2013; Spang et al. 2015; Baker et al. 2016). SSU rRNA alignments show phylogenetic diversity and geographical clustering among each respective phylum, especially between terrestrial and marine lineages.

Currently, the Nanoarchaeota are represented by one marine and one terrestrial isolate, both in a coculture with their respective obligate crenarchaeotal host (Waters et al. 2003; Wurch et al. 2016). The first isolate to be sequenced was *Nanoarchaeum equitans*. *N. equitans* was obtained from an Icelandic hydrothermal vent and attached to its crenarchaeotal host, the autotrophic *Ignicoccus hospitalis* (Huber et al. 2002). The highly reduced *N. equitans* genome is 490 kbp (kilobase pair), with a large portion of the genome dedicated to genome processing. *De novo* pathways for lipids, cofactors, amino acids and nucleotides are absent from the genome. Nutrient transfer from host to *N. equitans* likely occurs through direct membrane to membrane contact that has been observed by electron microscopy (Huber et al. 2002; Jahn et al. 2008).

Two nanoarchaeotal genomes have recently been sequenced from Yellowstone National Park (YNP). *Nanopusillus acidilobi* genome has been fully sequenced (Wurch et al. 2016) and Nst1 consists of 7 contigs (Podar et al. 2013). *N. acidilobi* and Nst1 share extensive protein synteny and a SSU rRNA gene identity of 97-98%. (Wurch et al. 2016). The YNP nanoarchaeotes also have highly reduced genomes and share many split genes with *N. equitans*. Differing from the marine lineage, the terrestrial genomes contain archaeal genes and a near complete gluconeogenesis pathway, constituting about a 23% increase in genome size relative to *N. equitans*.

Unlike the Nanoarchaeota, no korarchaeote has been successfully cultured, but a complete genome was assembled from a stable complex enrichment from Obsidian Pool, YNP (Elkins et al. 2008). *Candidatus* Korarchaeum cryptofilum contains many incomplete pathways which may be part of the reason that it has eluded isolation (Elkins et al. 2008). Pathways for purines and several cofactors are incomplete. For carbon and energy, *Ca. K. cryptofilum* contains an abundance of peptide transporters and amino acid fermentation pathways.

A korarchaeotal draft genome from a terrestrial hot spring in Little Hot Creek (LHC), California was recently sequenced and used for comparisons in this study (Saw et al. 2015). Alignment of the LHC korarchaeotal SSU rRNA gene to *Candidatus* Korarchaeum cryptofilum revealed a 97% similarity in genomic sequence. Saw et al. (2015) reported a genome of similar size and GC content, but did not report any genomic insights from their assembled metagenome.

In order to expand the genomic diversity of Nanoarchaeota and Korarchaeota, I characterized a nanoarchaeotal and a korarchaeotal genome from a New Zealand hot spring, by combining culture dependent and independent techniques.

Methods

Samples and Sequencing

A thermal spring sediment sample was collected from Tikitere (NZ13: 38°03'47"S, 176°21'39"E, pH 6.0 and 74°C) in Rotorua, New Zealand. Enrichments were obtained in a modified DSMZ 88 media (per liter): 1.30 g (NH₄)₂SO₄; 0.28g KH₂PO₄; 0.12g MgSO₄; 0.07g CaCl₂·6H₂O; 4 mg FeCl₃·6H₂O; 4.5 mg Na₂B₄O₇·10H₂O; 2.00g

H₂S₂O₃, supplemented with vitamins (Ferguson et al. 1983), 0.5g yeast extract and 0.5g trypticase. The pH was adjusted to 6.0 and incubated at 80°C under an atmosphere of N₂:CO₂ (80%:20%). Genomic DNA was extracted from the environmental sample using the Ultraclean Soil DNA isolation kit (MoBio Laboratories) and enrichment cultures using the DNA Tissue Kit (Qiagen) (K. Brileya, personal communication). Purified DNAs were sent to Oak Ridge National Laboratory for library preparation and metagenomic sequencing. DNA was sequenced on an Illumina MiSeq platform and generated 35 million paired-end reads (2x250bp) (Table 1). Using Trimmomatic, paired-end reads were trimmed for adapter sequences and quality (Bolger et al. 2014). The quality thresholds set discarded bases whose 4-base average fell below a Phred score of 20 and removed reads shorter than 50 bp.

Community Composition

The microbial compositions of the environmental and enrichment samples were compared by extracting the SSU rRNA reads. SortMeRNA v2.0 identified candidate SSU rRNA reads by comparing the reads against aligned SSU reference databases with an e-value threshold of 10⁻⁵ (Kopylova et al. 2012). The reads were then classified using SINA v1.2.11 and a minimal 70% identity threshold against the small subunit Ref NR 99 128 database (Pruesse et al. 2012).

Assembly and Binning

Reads from the three NZ13 datasets were concatenated, interleaved and co-assembled (Albertsen et al. 2013) *de novo* using IDBA v1.1.0 (Peng et al. 2012), which resulted in one set of 57,040 contigs. The three sets of reads were mapped back to the

contigs individually using Bowtie2 v2.2.5 and Samtools v1.2 (Li et al. 2009; Langmead et al. 2012). The assembly was binned using GroopM v0.3.3 differential binning program, which incorporated the assembly and read mapping data (Figure 14) (Imelfort et al. 2014). The contigs were binned using two different thresholds. The nanoarchaeotal contigs were binned using a 1,000 bp cutoff, while the korarchaeotal contigs were binned using a 1500 bp cutoff.

Refinement of nanoarchaeotal and korarchaeotal bins

Some contigs did not bin during the GroopM process despite using GroopM's unbinned contig recruitment tool. Unbinned contigs were classified using BLASTp v2.2.28 (Camacho et al. 2009). Unbinned contigs with nanoarchaeotal or korarchaeotal BLASTp results were added to their respective bins. Reads were again mapped back to each bin individually and re-assembled using IDBA (Langmead et al. 2012; Peng et al. 2012). The nanoarchaeotal and korarchaeotal assemblies were then individually binned, this time using MaxBin v1.4.5 (Wu et al. 2014). Bins were manually curated using BLASTp, Geneious and coverage data (Kearse et al. 2012). CheckM was used to assess bins for contamination and completion (Parks et al. 2015).

Genome annotation

The two draft genomic bins (Nanoarchaeota and Korarchaeota) were annotated using Prodigal, MALT, MEGAN5, KEGG, Infernal and HMMR3 software. The protein files, generated by Prodigal v2.6.2, were ran through MALT using default parameters, limiting search results to bit-scores greater than 50 (Hyatt et al. 2010; Huson et al. 2016). The BLASTp output from MALT was imported into MEGAN5 utilizing the KEGG

mapping software (Aoki-Kinoshita et al. 2007). Protein domains were searched using HMMR3 against the Pfam and TIGRfam databases using trusted cutoffs (Eddy 1998; Haft et al. 2003; Punta et al. 2012). Additionally, some proteins were manually uploaded to InterproScan 5 for further assessment (Jones et al. 2014). To search for RNA motifs, the DNA sequences were processed using Infernal against the Rfam database, once again using trusted cutoffs (Griffiths-Jones et al. 2003). For arCOG analysis, BLASTp was utilized to compare the protein sequences against the curated arCOG database using an e -value of 10^{-5} and keeping the top result for each protein sequence (Makarova et al. 2015b). Genome plots and genome similarity plots were generated using ClicO FS and finalized through the Circos software (Cheong et al. 2015; Krzywinski et al. 2009).

Genome comparisons

Available nanoarchaeotal and korarchaeotal genomes were used for comparisons against the New Zealand draft genomes. Two complete nanoarchaeotal genomes were available: *N. equitans* and *N. acidilobi* (Waters et al. 2003; Wurch et al. 2016). A third draft nanoarchaeotal genome, Nst1, is a close relative to *N. acidilobi* based on the SSU rRNA alignment (Podar et al. 2013). *Ca. K. cryptofilum* is the only complete korarchaeotal genome (Elkins et al. 2008). However, metagenomic sequence data was provided to our lab from the Hedlund lab (University of Nevada, Las Vegas) which contained korarchaeotal reads from a hot spring sediment sample in Little Hot Creek, California (Saw et al. 2015). The reads were processed in a similar manner as the New Zealand nanoarchaeotal and korarchaeotal genomes. All genomes were analyzed using the same processing analysis pipeline as the New Zealand genomes.

Results

Community Analysis

From the three metagenomes, a total of 46,000 SSU rRNA reads were classified (Figure 1). The enrichment cultures were enriched in crenarchaeotes. Nanoarchaeotal and korarchaeotal sequences were detected in all three samples.

Nanoarchaeota

The draft nanoarchaeotal genome (NCBI Biosample ID: SAMN05172240) is 532,337 bp and consists of 52 contigs with an average GC content of 32.6% (Table 2). Completeness, based on lineage specific marker genes, is estimated at 99% (Table 2). The draft genome encodes for 35 tRNAs throughout 18 contigs. Transfer RNAs (tRNA) aspartic acid, cysteine and glutamine were not detected. The large and small subunit rRNAs are on the same contig (Nnz13_1) separated by six protein coding genes and the 5S rRNA is on contig Nnz13_39 (Figure 2).

The nanoarchaeotal SSU rRNA gene was assembled individually, using only nanoarchaeotal SSU rRNA reads from NZ13en-R and NZ13en-P. The *de novo* SSU rRNA gene was aligned with a clone library sequence and they are 99.9% similar (Figure 15).

Genomic comparisons among the nanoarchaeotal genomes reveal a closer relationship between the YNP nanoarchaeotal genomes than to *N. equitans* (Figure 3; Table 9). The NZ13 nanoarchaeote and the YNP nanoarchaeotes SSU rRNA genes share an 88% identity, likely forming a distinct family within the terrestrial Nanoarchaeota (Table 7). *N. equitans* and the NZ13 nanoarchaeote share an 84% SSU rRNA identity. Synteny plots show a higher percentage of protein homology between *N. acidilobi*-NZ13

than between *N. equitans*-NZ13 (Figure 4).

The NZ13 nanoarchaeotal genome shares several genomic features with other nanoarchaeotes, such as a reduced genome, *cis*-spliced tRNAs and split genes (Table 3). However, there are differences among the common core of split genes. Glu-tRNA^{Gln} amidotransferase has a conserved split site within *N. equitans* and *N. acidilobi* (Waters et al. 2003; Podar et al. 2013; Wurch et al. 2016) while the gene is not split in the NZ13 nanoarchaeote (Figure 16). Though not all split genes are shared, glucosamine-fructose-6-phosphate aminotransferase (GFAT) is unique to the NZ13 nanoarchaeote (Table 3). If functional, GFAT can catalyze the reaction of fructose-6-phosphate and glutamine to produce glutamate.

Only two other genes, *trkA* and *trkH*, are unique to the NZ13 nanoarchaeotal genome. They are a low affinity potassium uptake transport system common in Archaea (Figure 5) (Kraegeloh et al. 2005). *TrkH* is a transmembrane protein responsible for potassium translocation and *trkA* is the cytoplasmic membrane receptor.

The NZ13 nanoarchaeotal genome share many metabolic features with the terrestrial nanoarchaeotal genomes, which are absent in *N. equitans* (Figure 3). The NZ13 nanoarchaeotal genome encodes for the gluconeogenesis and glycolysis pathways (Figure 5). Two gluconeogenic genes, glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase were not present in the draft genome. All other gluconeogenic genes are present, including those for the complete synthesis and breakdown of glycogen. In the glycolytic pathway 6-phosphofructokinase, NADP⁺ dependent nonphosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPN) and pyruvate kinase were not

identified.

Another shared physiology between the NZ13 nanoarchaeote and the terrestrial nanoarchaeotes are the presence of archaellum genes. All essential subunits for archaellum assembly and function have been identified in the genome (Figure 6). Additionally, the genes are not contained in an operon, but are fragmented throughout the genome.

A partial CRISPR-cas system was identified in the NZ13 nanoarchaeotal genome (Figure 5; Table 9). The NZ13 nanoarchaeotal genome encodes *cas1*, *cas4* and *cas6*.

Korarchaeota

The draft genome of the NZ13 korarchaeote (NCBI Biosample ID: SAMN05172504) is 1.375 Mbp and consists 148 contigs with an average GC content of 56.7% (Table 4). The genome is estimated to be 91% complete (Table 4). Only a partial 23S rRNA and complete 5S rRNA gene are located on the draft genome, while there are a full complement of tRNA genes (Figure 7).

A *de novo* assembly of the NZ13 korarchaeotal SSU rRNA gene is presented in Figure 17. A large portion of the SSU rRNA gene was assembled from the environmental SSU rRNA reads (NZ13). The assembled SSU rRNA sequence is 99.39% similar to a clone library sequence obtained previously (R. Davis, unpublished results) (Figure 18). The NZ13 korarchaeote SSU rRNA sequence shares a 91% nucleotide similarity to *Ca. K. cryptofilum* (Table 10). A synteny plot also shows a high level of protein homology between the NZ13 korarchaeotal and *Ca. K. cryptofilum* genomes (Figure 8).

The NZ13 korarchaeotal genome encodes over 100 transport genes (Table 11).

There are transporters for many cations, anions and ionic compounds. Two transport genes, the sodium/hydrogen antiporters, are not present in the other korarchaeotal genomes (Figure 9). However, most transporters are classified as ABC transporters for amino acids or peptides, with only one gene being classified as a sugar transporter.

The NZ13 korarchaeotal genome encodes an extensive set of amino acid pathways. Amino acid degradation pathways proceed through pyruvate or the partial TCA cycle to enter the central metabolism (Figure 9). Citrate synthase, aconitase and isocitrate dehydrogenase were not identified in the genome. Gluconeogenesis and glycolysis pathways proceed through the archaeal Embden-Meyerhof-Parnas pathway, however, a hexokinase was not identified in the genome. Furthermore, the pentose pathway proceeds through the ribulose monophosphate pathway for pyrimidine synthesis. Purines cannot be synthesized *de novo*, but purine salvage and interconversion pathways are present.

The NZ13 korarchaeotal genome contains an ATP synthase to utilize a proton motive force for generating ATP (Figure 9). Proton translocation can occur through NADH dehydrogenase. The electrons may then be transferred to a menaquinone and terminal electrons can be shuttled to a NiFe hydrogenase. Electrons could also flow through formate dehydrogenase to heterodisulfide reductase (Costa et al. 2010). The subunits of F₄₂₀ reducing hydrogenase are also present, but the genes for F₄₂₀ synthesis were not detected. Lastly, two genes are present for cytochrome *bd* oxidase subunit I.

Several coenzyme biosynthesis pathways are incomplete. For instance, the coenzyme pathway gene, phosphopantothenoylcysteine synthetase/decarboxylase, was not

identified. Menaquinone biosynthesis probably proceeds through the archaeal alternate mevalonate pathway but phosphomevalonate decarboxylase was not detected. While the pathway for riboflavin was not detected, there is a riboflavin transport gene. Other incomplete coenzyme pathways include those for pterin, lipoate and porphyrin.

Discussion

Pairing environmental and enrichment samples with differential coverage binning of metagenomic data allows for the recovery of low abundance, high quality microbial genomes (Albertsen et al. 2013). These methods bypass the arduous task of isolating an organism for sequencing, producing the addition of the nearly complete nanoarchaeotal and korarchaeotal genomes. The genomes demonstrate the conserved physiologies among terrestrial members of two basal archaeal lineages.

Aside from the genomes, the enrichment cultures in this study provide insights into another nanoarchaeotal-host system. In line with other known nanoarchaeotal hosts (Waters et al. 2003; Podar et al. 2013; Wurch et al. 2016), the NZ13 cultures were highly enriched for crenarchaeotal sequences. However, *N. equitans*, *N. acidilobi* and Nst1 utilize three host from different crenarchaeotal orders, therefore only a coculture or single cell sequencing could resolve the potential NZ13 crenarchaeotal host.

Previous studies have shown the archaellum genes are expressed in the YNP nanoarchaeotes, but their purpose remains uncertain (Podar et al. 2013; Wurch et al. 2016). The archaellum genes are typically located in a single operon (Jarrell et al. 2012), but they are fragmented in the nanoarchaeotal genomes thus far studied. Their suggested function would allow the nanoarchaeotes to move between hosts or have involvement in

the host attachment process. However, archaella are predicted to function using ATP (Streif et al. 2008), which the nanoarchaeotes must obtain from their host. Therefore, the use of archaella during detachment from their host would drain ATP reserves, effectively making the nanoarchaeotes non-motile. If the archaella are functional while attached to their host, they could potentially help the nanoarchaeotes and the host to move to a more nutrient rich location. Evidence for this symbiosis comes from the physiologies of the hosts. Archaella have been observed on the host of *N. equitans*, *Ignicoccus hospitalis* (Paper et al. 2007; Podar et al. 2008), so the loss of archaellum genes in *N. equitans* would be expected. Conversely, the terrestrial nanoarchaeotes have retained their archaellum genes while their host do not have archaella. For example, the host of Nst1 is predicted to have lost their archaellum genes (Podar et al. 2013), while the host of *N. acidilobi* is from a non-motile genus (Prokofeva et al. 2000; Mardanov et al. 2010; Wurch et al. 2016). The different coevolutionary paths, possibly dependent on host motility, may explain the terrestrial retention of their archaellum genes.

Another shared nanoarchaeotal terrestrial trait is carbohydrate metabolism. The NZ13 nanoarchaeotal genome does not encode for two glycolytic enzymes that are present in *N. acidilobi* and Nst1 (Podar et al. 2013; Wurch et al. 2016). These genes could be encoded in the completed gene, but phosphofructokinase is absent from all genomes (complete and draft), leaving the glycolytic pathway unable to proceed past fructose-6-phosphate (F6P). In the NZ13 nanoarchaeote, the breakdown of glycogen could proceed through F6P utilizing the split gene, GFAT, to produce glutamate. Glutamate is five times more prevalent than glutamine in the predicted NZ13

nanoarchaeotal proteome, a common occurrence in Archaea (Table 8) (Schmidt et al. 2012).

A common core of split genes is found within all nanoarchaeotal genomes (Table 3). The similarities of split genes suggest that the genome reduction process began before the terrestrial and marine lineages diverged. As there are split genes unique to each genome, it is likely that genome streamlining continued to occur after each lineage diverged. (Waters et al. 2003; Podar et al. 2013; Wurch et al. 2016).

One shared suite of genes between *N. equitans* and the NZ13 nanoarchaeotal genome are the CRISPRs. The three NZ13 nanoarchaeotal *cas* genes are associated with CRISPR type I-B, the same proteins found in *N. equitans* (Waters et al. 2003; Randau 2012; Makarova et al. 2015a). Although Munson-McGee (2015) postulated that *N. equitans* likely gained their CRISPR genes through horizontal transfer, rather than Nst1 (or *N. acidilobi*) losing their CRISPR system through genome reduction. The presence of a similar CRISPR system found in the NZ13 nanoarchaeote suggests the YNP nanoarchaeotes have likely lost their viral defense system.

Like the Nanoarchaeota, the terrestrial korarchaeotes have similar metabolisms (Figure 10). Only two transport genes differentiates the NZ13 korarchaeotal genome from *Ca. K. cryptofilum* or the LHC korarchaeote. In all three genomes, the abundance of peptide transporters and amino acid degradation pathways demonstrates the korarchaeotes are amino acid fermenters. Additionally, the only amino acids the korarchaeotal genomes cannot synthesize *de novo* are histidine and tryptophan.

Other incomplete pathways in the korarchaeotal genomes involve coenzymes and

a menaquinone. The *Ca. K. cryptofilum* coenzyme A synthesis pathway is missing one gene, phosphopantothienoylcysteine synthetase/decarboxylase and was not identified in the NZ13 korarchaeote (Elkins et al. 2008). With a large portion of the genomes categorized as having an unknown function, there is the possibility of an undiscovered enzyme that can complete the pathway or even an undiscovered pathway unique to Archaea. Similarly, a carboxy-lyase gene may complete the menaquinone biosynthesis pathway. If not, the abundance of transport genes within the korarchaeotal genomes may allow for efficient scavenging of nutrients.

Energy metabolism within the korarchaeotal genomes is also an area of uncertainty. Methanogen-like genes, such as the heterodisulfide reductase and F_{420} hydrogenase, have been found in non-methanogens, but their functional role has not been determined (Lee et al. 2017). Typical methanogen genes are not found in the korarchaeotal genomes, such as *mcrA* or pathways for coenzyme M, B and F_{420} synthesis. Moreover the presence of the cytochrome *bd* oxidases are not unusual in anaerobes (Giuffrè et al. 2014), but the korarchaeotal genomes only contain two copies of subunit I. If functional they could provide the korarchaeotes with oxidative stress protection. Biochemical studies of korarchaeotes or other non-methanogens are needed to fully understand early archaeal energy metabolism.

Both the NZ13 nanoarchaeote and NZ13 korarchaeote contain transport genes related to osmotic regulation. The NZ13 nanoarchaeote contains the *trkAH* genes that import potassium. The NZ13 korarchaeote contains a Na^+/H^+ antiporter, which studies have shown can also uptake potassium (Verkhovskaya et al. 1995; Southworth et al.

2001). In halotolerant organisms, internal potassium levels are maintained in high concentrations relative to external sodium levels (Martin et al. 1999). These potassium transport genes may provide salt tolerance to the NZ13 nanoarchaeotes and NZ13 korarchaeotes.

Recently metagenomic sequencing has revealed extensive microbial diversity, including many deep-branching archaeal lineages (Rinke et al. 2013; Spang et al. 2015; Castelle et al. 2015). Some of these lineages have relatively small genomes and lack core biosynthetic pathways, similar to the Nanoarchaeota and Korarchaeota (Baker et al. 2010; Castelle et al. 2015). As a result they may form symbiotic relationships or be efficient scavengers. With this in mind, isolation of symbiotic and uncultivated archaeal lineages, such as the Nanoarchaeota and Korarchaeota, are needed to better understand archaeal symbiosis, metabolism and evolution.

Table 1. Summary of raw data from the three metagenomes and co-assembly. NZ13 is the environmental sample. NZ13en-R and NZ13en-P are enrichment samples.

Sequence Data	NZ13	NZ13en-R	NZ13en-P
Number of Paired-end Reads	12,517,520	10,038,732	12,316,813
Total Base Pairs (Mbp)	5,158	4,496	5,570
Average Read Length (bp)	206	224	226
SSU rRNA Breakdown			
SSU rRNA Read Counts	19,088	11,878	15,034
Archaea SSU rRNA	69.2%	66.5%	99.7%
Bacteria SSU rRNA	29.6%	33.4%	0.2%
Eukarya SSU rRNA	1.2%	0.1%	0.0%
Assembly Data	Co-assembly		
Total Co-assembly Size (bp)*	137,989,742		
Total Number of Contigs*	57,040		
N50 (bp)*	2,716		
Longest Contig (bp)	107,757		

*Only contigs greater than 1kbp

Figure 1. Relative abundance of SSU rRNA trimmed read counts from NZ13, NZ13en-R and NZ13en-P. Only shown are Family level classification with higher than 1% relative abundance, all other reads are grouped into their respective domain. Reads were classified against the SILVA SSU Ref NR128 database.

Table 2. Comparison of general features of nanoarchaeotal genomes. Predicted proteins were generated using Prodigal (Hyatt et al. 2010). Completeness and contamination were assessed using CheckM (Parks et al. 2015).

Features	NZ13 Nanoarchaeota	<i>Nanopusillus acidilobi</i>	Nst1	<i>Nanoarchaeum equitans</i>
Genome Length (bp)	532,337	605,887	593,789	490,885
No. of Contigs	52	-	7	-
Longest Contig (bp)	88,440	-	192,298	-
G+C%	33%	24%	24%	32%
Predicted Proteins	647	665	654	583
rRNA	3	3	3	3
tRNA	35	43	41	43
Completeness	99%	100%	98%	100%
Contamination	0	0	0.93	0

Figure 2. NZ13 nanoarchaeotal genome plot. ORFs, GC% and RNAs were predicted by Prodigal and Rfam. BLASTp was used to generate similarity tiles. Bowtie2 and Samtools were used to assess coverage.

Figure 3. Relative abundance of arCOG categories from ORFs in nanoarchaeotal genomes. The arCOG categories A, B, W, Y and Z did not have any matches. Not shown are proteins that were labeled unclassified. Relative abundances of unclassified proteins are expressed as a percentage of ORFs: NZ13 Nanoarchaeota (10.41%), *N. acidilobi* (22.59%), Nst1 (25.46%) and *N. equitans* (24.45%).

Figure 4. NZ13 nanoarchaeotal BLASTp synteny plots. **A.** NZ13 nanoarchaeotal protein to protein comparison against *Nanopusillus acidilobi*. **B.** NZ13 nanoarchaeotal protein to protein comparison against *Nanoarchaeum equitans*. Connecting lines represent shared proteins between the genomes, the color represents the percent of amino acid identity.

Table 3. Overview of nanoarchaeotal similarities and differences: shared split proteins, *trans* and *cis*-spliced tRNA and genomic features.

Split proteins	NZ13 Nanoarchaeota	<i>Nanopusillus</i> <i>acidilobi</i> *	Nst1*	<i>Nanoarchaeum</i> <i>equitans</i> *
Reverse gyrase	Nnz13 13_3-3_10	Nps 2030-2705	Nst1 337-402	NEQ 318-434
Glu-tRNA ^{Gln} amidotransferase	Nnz13 0_76	Nps 2280-3415	Nst1 197-449	NEQ245-396
Predicted RNA-binding protein	Nnz13 2_37-1_56	Nps 30-3140	Nst1 176-251	NEQ 438-506
Archaeosine tRNA-guanine transglycosylase	Nnz13 11_6-1_37	Nps 1135-3235	Nst1 096-232	NEQ 124-305
RNA polymerase subunit B	Nnz13 0_36-0_35	Nps 545-550	Nst1 632-633	NEQ 156-173
Large helicase-related protein	Nnz13 0_74-1_43	Nps 745-3195	Nst1 172-239	NEQ 003-409
DNA polymerase I	Nnz13 25_5	Nps 2105	Nst1 417	NEQ 068-528
Topoisomerase I	Nnz13 2_35	Nps 45	Nst1 174	NEQ 045-324
P-loop ATPase-acetyltransferase fusion protein	Nnz13 3_11	Nps 2025	Nst1 401	NEQ 096-495
Alanyl-tRNA synthetase	Nnz13 10_7	Nps 290	Nst1 054	NEQ 211-547
Diphthamide synthase sub. DPH2	Nnz13 1_28-8_15	Nps 3285-2235	Nst1 222-440	NEQ226
Uncharacterized conserved protein (arCOG04253)	Nnz13 49_7	Nps 2410	Nst1 474-480	-
Glucosamine-fructose-6- phosphate aminotransferase	Nnz13 0_85-0_87	-	-	-
tRNA				
<i>cis</i> -spliced	Ile, Tyr, Met	Ile, Tyr	Ile, Tyr	Ile, Met, Trp, Tyr iMet, His, Lys, Gln, Glu (2)
<i>trans</i> -spliced	-	-	-	
Genome Features				
Gluconeogenesis-Glycolysis	+	+	+	-
CRISPR related genes	+	-	-	+
Polyamine biosynthesis	+	+	+	-
Archaeallum	+	+	+	-
ATP synthase	-	-	-	+ [†]
Glutamate dehydrogenase	-	-	-	+

*Annotations from NCBI, Accession numbers: *N. acidilobi* (CP010514), Nst1 (APJZ000000000), *N. equitans* (AE017199)

- absent

+ present

[†] Lacking several subunits

Figure 5. Reconstructed proposed metabolic chart of NZ13 Nanoarchaeota. Percentages for transcription, replication and translation are derived from arCOGs. The CRISPR genes missing here are present in *N. equitans*.

NZ13 Nanoarchaeota ***Nanopusillus acidilobi*** **Nst1** ***Sulfolobus acidocaldarius* DSM 639** ***Pyrococcus horikoshi* OT3**

Figure 6. Archaeallum gene locations and orientation in the terrestrial nanoarchaeotal genomes: NZ13 Nanoarchaeota, *N. acidilobi* and Nst1. *Sulfolobus* and *Pyrococcus* are shown to illustrate a typical archaeallum operon structure.

Table 4. General features table comparing three korarchaeotal genomes. Predicted proteins were generated using Prodigal (Hyatt et al. 2010). Completeness and contamination were assessed with CheckM (Parks et al. 2015).

Features	NZ13 Korarchaeota	<i>Candidatus</i> Korarchaeum cryptofilum	Little Hot Creek Korarchaeota
Base Pairs (bp)	1,374,210	1,590,757	1,358,156
No. of Contigs	148	1	405
Longest Contig (bp)	107,729	-	22,651
G+C%	56.7%	49.0%	47.6%
Predicted Proteins	1587	1683	1751
rRNA	5S; 23S†	5S ;16S ;23S	5S
tRNA	41	45	31
- <i>cis</i> -spliced	Met, Trp	Met, Tyr	Met, Ile
Completeness	90.9%	100%	88.7%
Contamination	0.93	2.8	5.14

† partial

Figure 7. NZ13 korarchaeotal genome map. ORFs, GC% and RNAs were predicted by Prodigal and Rfam. BLASTp was used to generate similarity tiles. Bowtie2 and Samtools were used to assess coverage.

NZ13 korarchaeote

Candidatus
Korarchaeum cryptofilum

Figure 8. NZ13 korarchaeotal BLASTp syntenic plots. NZ13 Korarchaeota protein to protein comparison against *Candidatus Korarchaeum cryptofilum*. Connecting lines represent shared proteins between the genomes, the color represents the percent of amino acid identity.

Figure 9. Reconstructed proposed NZ13 korarchaeotal metabolism chart. Amino acids are green, with tryptophan requiring input of indole to be synthesized. A red X denotes an incomplete pathway. Translation, replication and transcription are percentages from arCOGs. Menaquinone is abbreviated MK. Blue arrows indicate the transport gene found only in the NZ13 korarchaeote.

Figure 10. Relative abundance of arCOG categories from ORFs in korarchaeotal genomes. The arCOG categories A, B, W, Y and Z did not have any matches. Not shown are proteins that were labeled unclassified. Relative abundances of unclassified proteins are expressed as a percentage of ORFs: NZ13 Korarchaeota (8.04%), *Ca. K cryptofilum* (8.81%) and Little Hot Creek Korarchaeota (7.75%).

Chapter 3: Microbial Community Composition of Deep-sea Hydrothermal Vents from the Eastern Lau Spreading Center

Abstract

Archaea inhabiting hydrothermal vents occupy niches with low pH and high temperature and pressure. Previous studies have indicated that euryarchaeotal methanogens, *Thermococcales* and *Archaeoglobales* were some of the more abundant Archaea from deep-sea hydrothermal vents. In contrast to the above findings, recent data from deep-sea hydrothermal vents along the Eastern Lau Spreading Center (ELSC), a major vent system in the southwestern Pacific, indicates that Crenarchaeota were more abundant and diverse than previously thought. The metagenomes of ten sulfide samples were sequenced and SSU rRNA reads were classified to identify the microbial composition. With the exception of the Vai Lili sample, *Epsilonproteobacteria* were the most abundant phylum in every sample. Crenarchaeota were the most abundant archaeal phylum in only three of the ten samples, while the Korarchaeota were the most abundant archaeal phylum at Vai Lili. *Archaeoglobaceae* and *Thermococcaceae* were the most abundant euryarchaeotal families in several samples.

Introduction

Along the Eastern Lau Spreading Center (ELSC) and Valu Fa Ridge (VFR) the Pacific oceanic plate is subducting under the Indo-Australian plate, generating a spreading center with several hydrothermal vent fields (Martinez et al. 2007). At active deep-sea hydrothermal vents, Bacteria are the most abundant microbes, with *Epsilonproteobacteria* often being the dominant lineage (Reysenbach et al. 2000; Corre 2001; Flores et al. 2011; Flores et al. 2012a). While less abundant, the Archaea of deep-

sea hydrothermal vents represent a rich phylogenetic diversity (Auguet et al. 2010). Until recently, the Euryarchaeota phylum accounted for most of this diversity with members of *Archaeoglobales*, *Thermococcales* and DHVE2 (Nercessian et al. 2003; Flores et al. 2011; Takai et al. 2011; Flores et al. 2012a; Flores et al. 2012b). The Crenarchaeota were represented predominately by *Desulfurococcales* and *Thermoproteales*, though there are many uncultivated deep-sea hydrothermal vent lineages (Erauso et al. 1993; González et al. 1998; Huber et al. 2000; Takai et al. 2000; Jolivet et al. 2003; Reysenbach et al. 2006).

Hydrothermal fluid chemistry has been shown to influence the composition of microbial communities. For example, the presence of methanogens is dependent on high concentrations of H_2 (Flores et al. 2011). Flores et al. (2012a) showed that bacterial and archaeal community structure were significantly different when end member hydrothermal fluid was acidic ($pH < 3$) and had high concentrations of CO_2 (Flores et al. 2012). The culture-independent techniques used by Flores et al. (2011, 2012) at two separate hydrothermal locations revealed great diversity amongst crenarchaeotal sequences, with many unclassified and uncultured lineages. For example, the acidic hydrothermal fluid hosted a newly detected family for deep-sea vents, *Caldisphaeraceae*, a thermoacidophilic Crenarchaeota.

The purpose of this study was to determine the microbial community metagenomic composition of ten sulfide samples and to identify the samples that should be further explored for as yet uncultivated bacterial and archaeal genomes. The genomic information and metabolic potentials revealed by this study may help develop media for isolating uncultured microbes, but more importantly, has the potential to show the roles

these uncultivated microbes may have at deep-sea hydrothermal vents.

Methods

Sample collection and processing

The spreading center of the Lau back-arc basin is 397 km and is located between the Lau and Tonga Ridge (Ferrini et al. 2008). A cruise aboard *R/V Roger Revelle* in April-May 2015 visited the ELSC and VFR. Fifty samples were collected using the ROV *Jason II* from four vent fields: ABE, Tui Malila, Mariner and Vai Lili. Ten samples were selected for metagenomic analysis (see below) (Figure 11, Figure 19, Table 12). Each rock sample was placed in a biobox until the rock could be brought on deck for processing. On deck, the outer few millimeters of each sample was scraped off and homogenized. DNA was extracted using the Ultra Clean Soil DNA isolation kit (MoBio Laboratories) and stored in multiple cryovials at -80°C.

Sequence processing and analysis

SSU rRNA sequence data for 29 samples were provided by Gilberto Flores, CSU Northridge (Flores, unpublished data). Samples with a high percentage of archaeal reads were prioritized, including those with a high occurrence of recently proposed archaeal phyla, such as the Korarchaeota, Woesearchaeota, Pacearchaeota and Aigarchaeota.

DNA samples were sent to Oregon State University Center for Genome Research and sequenced using Illumina HiSeq 3000. A total of 1.2 billion paired-end reads (2 x150 bp) were generated (Table 5). Paired-end reads were trimmed for adapter sequences and quality using Trimmomatic (Bolger et al. 2014). The quality thresholds set discarded bases whose 4-base average fell below a Phred score of 20 and removed reads shorter

than 50 bp.

Community Composition

The microbial composition of the environmental metagenomes were analyzed by extracting the SSU rRNA reads using SortMeRNA v2.0 and an e-value threshold of 10^{-5} (Kopylova et al. 2012). The reads were then classified using SINA v1.2.11 and a minimal 70% identity threshold against the small subunit Ref NR 99 123 database (Pruesse et al. 2012).

Results

From the ten metagenomes, 5.6 million SSU rRNA reads were classified. Around 4.9 million bacterial SSU rRNA reads were identified (Figure 12), while a total of 171,970 archaeal SSU rRNA reads were detected (Figure 13).

Bacterial Composition

Epsilonproteobacteria accounted for most of the classified SSU rRNA read counts in all samples, except at Vai Lili where the *Gammaproteobacteria* read counts were the most abundant. *Epsilonproteobacteria* only accounted for the majority (>50%) of reads in five samples, all of the Mariner samples and Tui Malila-11. Furthermore, on average over 50% of the bacterial reads from each sample were classified to family level or below. At the family level, *Nautiliaceae* accounted for 24% of all read counts and was observed in all samples (Table 6). *Campylobacteraceae*, *Hydrogenothermaceae* and candidate division SR1 families accounted for 1.1% of total reads. Other candidate division bacterial lineages were identified such as Gracilibacteria (GN02) and Parcubacteria (OD1) which had relative abundances above 1% in several samples.

Archaeal Composition

The most abundant archaeal lineages overall, based on classified SSU rRNA read counts were from Euryarchaeota (Figure 13). However, many uncultivated deep-sea hydrothermal vent Archaea were represented throughout the samples. The Woesearchaeota were present in all samples. At ABE-3, several uncultivated phyla were present at about 1% archaeal abundances. These were the Marine Hydrothermal Vent Group, Marine Hydrothermal Vent Group 1, Bathyarchaeota (formally Miscellaneous Crenarchaeota Group) and Miscellaneous Euryarchaeota Group. Aenigmarchaeota were present in low abundance in all samples except Mariner-1. Nanoarchaeotal reads were detected in seven out of the ten samples and korarchaeotal sequences were detected in all samples (Table 5).

Discussion

In this study, metagenomic sequencing and SSU rRNA classification were used to characterize ten hydrothermal vent samples from four vent fields along the ELSC and VFR. Results were similar to previous microbial composition studies, which showed that *Epsilonproteobacteria* were the dominant lineage from the majority of hydrothermal vent systems (Hoek et al. 2003; Flores et al. 2011; Flores et al. 2012; Gulmann et al. 2015). In agreement with Flores et al. (2012), samples from the Mariner vent field were abundant in euryarchaeotal sequences, while at other vent locations, crenarchaeotal sequences were abundant. It is important to note that the short metagenomic reads (max. 150bp) are spread across any region of the SSU rRNA, potentially skewing classification efforts. This became apparent in the many sequences that could not be classified beyond the bacterial or archaeal domains, as well as the majority of *Nautiliaceae* reads that could not

be further classified. In addition, the comparisons in this study were made with read counts, without trying to approximate SSU rRNA gene copy numbers for each lineage. This is more problematic in bacterial lineages and may cause them to be overrepresented in bacterial to archaeal comparisons.

Bacterial Communities

The most abundant bacterial communities were from *Epsilonproteobacteria*. Similar to the Flores et al. (2012) study, *Helicobacteraceae*, a mesophilic lineage, were more abundant in the northern vent fields, while *Nautiliaceae*, a thermophilic family, were more abundant in the Mariner vent field. Flores et al. (2012) did observe an unexpected abundance of *Lebetimonas* sequences (Flores et al. 2011), whereas in this study, very few *Lebetimonas* sequences were classified. The discrepancy is attributed to the few *Nautiliaceae* reads that were classified beyond the family level.

This study also highlights certain vent samples that could be targeted for genome recovery of uncultured bacterial lineages. Candidate division SR1, Gracilibacteria (GN02) and Parcubacteria (OD1) have not been cultured. Through metagenomic and single cell sequencing, draft genomes are available for these bacterial lineages (Campbell et al. 2013; Kantor et al. 2013; Rinke et al. 2013), but none have been recovered from the ELSC. Additionally, candidate division SR1 varied in abundance at Mariner-1 and Mariner-2, both subsamples from the same sulfide. These samples would be good candidates for differential coverage assembly and binning to recover a high quality SR1 draft genome.

Archaeal Communities

In this study, specific samples were selected based on archaeal diversity. Thus

differences should be expected when compared to the microbial diversity study by Flores et al. (2012). The differences included higher abundances of *Archaeoglobaceae*, Korarchaeota and *Methanocaldococcaceae*, while there were lower abundances of *Thermofilaceae* and Nanoarchaeota.

Comparative differences between this study and Flores et al. (2012) were observed in many archaeal branches. In Flores et al. (2012), *Thermofilaceae* were prevalent in chimney communities in all sites, except Mariner. In the current samples, *Thermofilaceae* only accounted for a fraction of reads from ABE-1 and Tui Malila-10. Contrary to Flores et al. (2012), *Archaeoglobaceae* were a major part of the archaeal community in four of the ten sulfides. This is probably because of the known bias of the primers used by Flores et al. (2012) (Shakya et al. 2013). Two samples, Mariner-10 and Mariner-17, each accounted for greater than 60% of the archaeal reads. Nanoarchaeotal sequences accounted for only a small percentage of reads in seven of the sulfide samples (Table 5). Flores et al. (2012) showed several sulfide samples with relatively abundant nanoarchaeotal representation, but all were from the northern ELSC vent fields. A similar trend was observed for korarchaeotal sequences, which were detected in all ten samples. However, they were more abundant in northern vents fields, except for Via Lili-2. In 2005, fluid samples from Tui Malila had low concentrations of H₂ (Mottl et al. 2011). In previous studies, low H₂ concentration has correlated with a low presence of methanogens, so the high abundance of *Methanocaldococcaceae* sequences at Tui Malila-11 was unexpected (Flores et al. 2011; Flores et al. 2012a).

Crenarchaeotal and uncultured archaeal sequences were abundant in several

sulfide samples. Assembly and binning of the ten metagenomes could reconstruct the metabolism of several of these uncultured deep-sea microbes. The metabolic reconstruction of ten hydrothermal vent sulfides will be the largest deep-sea hydrothermal vent microbial comparative study to date. The data once fully analyzed will likely provide a more comprehensive picture of the microbial processes within the vent fields at ELSC-VFR.

Figure 11. Hydrothermal vent fields along ELSC and VFR. Map was generated using GeoMapApp (<http://www.geomapapp.org>)

Table 5. Raw sequence data, trimmed reads and SSU rRNA read counts by site. Listed are Nanoarchaeota and Korarchaeota read counts and percentage abundance per site.

	ABE-1	ABE-3	Mariner-1	Mariner-2	Vali Lili-2
Raw Paired Reads	412,295,856	437,906,048	294,978,076	444,287,522	310,302,926
Avg. Read (bp)	139.10	133.94	141.77	138.76	139.42
Total bp (Gbp)	43.5	40.4	35.5	49.1	34.0
Reads Paired	312,659,126	301,881,238	250,687,500	353,874,178	243,508,032
Reads Unpaired	99,636,730	136,024,810	44,290,576	90,413,344	66,794,894
Percentage Lost	24.17%	31.06%	15.01%	20.35%	21.53%
Total SSU rRNA Reads	449,275	501,224	1,315,104	611,241	179,841
Archaea SSU rRNA	33,619	7,048	13,176	11,116	4,486
Archaea (%)	7.48%	1.41%	1.00%	1.82%	2.49%
Nanoarchaeota (#/%)	17 / 0.05%	5 / 0.07%	0 / 0.0%	0 / 0.0%	3 / 0.07%
Korarchaeota (#/%)	3,305 / 9.83%	155 / 2.20%	3 / 0.02%	8 / 0.07%	1,220 / 27.0%
Bacteria SSU rRNA	392,451	281,336	1,300,099	569,538	169,913
Bacteria (%)	87.35%	56.13%	98.86%	93.18%	94.48%
Eukarya SSU rRNA	23,205	212,840	1,829	30,587	5,442
Eukarya (%)	5.16%	42.46%	0.14%	5.00%	3.03%
	Mariner-10	Mariner-17	Tui Malila-10	Tui Malila-11	Tui Malila-2
Raw Paired Reads	266,076,280	331,585,098	399,974,900	226,203,648	341,621,574
Avg. Read (bp)	138.38	134.94	134.29	133.79	134.50
Total bp (Gbp)	29.6	33.1	37.1	22.0	33.7
Reads Paired	214,104,898	245,155,634	276,069,690	164,640,234	250,390,060
Reads Unpaired	51,971,382	86,429,464	123,905,210	61,563,414	91,231,514
Percentage Lost	19.53%	26.07%	30.98%	27.22%	26.71%
Total SSU rRNA Reads	707,086	584,636	473,091	487,524	320,762
Archaea SSU rRNA	25,342	23,983	25,373	22,445	5,382
Archaea (%)	3.58%	4.10%	5.36%	4.60%	1.68%
Nanoarchaeota (#/%)	27 / 0.11%	5 / 0.02%	26 / 0.10%	32 / 0.14%	-
Korarchaeota (#/%)	2 / 0.01%	9 / 0.04%	602 / 2.37%	30 / 0.13%	100 / 1.86%
Bacteria SSU rRNA	681,368	560,396	394,930	256,033	304,961
Bacteria (%)	96.36	95.85%	83.48%	52.52%	95.07%
Eukarya SSU rRNA	376	257	52,788	209,046	10,419
Eukarya (%)	0.05%	0.04%	11.16%	42.88%	3.25%

Table 6. Summary of epsilonproteobacterial abundances. Percentages are based on total read counts of epsilonproteobacterial SSU rRNA per sample. SSU rRNA reads were identified with SortMeRNA and classified with SINA against the SILVA SSU Ref NR123 database.

	ABE-1	ABE-3	Tui Malila-10	Tui Malila-11	Tui Malila-2
Total bacterial read counts	392,451	281,336	394,930	256,033	304,961
Total ϵ-proteobacteria read counts	124,747	68,597	158,533	140,736	105,467
Epsilonproteobacteria	36.8%	34.8%	25.0%	17.0%	21.2%
Campylobacterales	4.2%	6.8%	6.3%	6.7%	6.7%
Campylobacteraceae	0.7%	1.0%	1.4%	0.9%	1.5%
<i>Arcobacter</i>	0.3%	1.2%	0.5%	0.2%	0.3%
<i>Campylobacter</i>	1.0%	2.2%	0.9%	0.2%	3.0%
<i>Sulfurospirillum</i>	0.2%	0.5%	0.2%	0.1%	0.6%
<i>Thiofractor</i>	0.0%	0.0%	0.0%	0.0%	0.0%
Helicobacteraceae	2.0%	3.9%	3.9%	4.3%	3.9%
<i>Helicobacter</i>	0.3%	0.4%	0.3%	0.4%	0.4%
<i>Sulfuricurvum</i>	0.3%	0.3%	0.7%	1.4%	0.2%
<i>Sulfurimonas</i>	0.5%	3.9%	3.7%	1.0%	9.6%
<i>Sulfurovum</i>	20.9%	32.0%	32.5%	43.5%	47.5%
uncultured	0.0%	0.0%	0.0%	0.0%	0.0%
Nautiliales					
Nautiliaceae	28.7%	10.0%	22.3%	22.8%	3.9%
<i>Caminibacter</i>	0.4%	0.1%	0.3%	0.3%	0.0%
<i>Lebetimonas</i>	0.0%	0.0%	0.0%	0.1%	0.0%
<i>Nautilia</i>	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Nitratifractor</i>	3.4%	2.6%	1.6%	1.1%	1.2%
<i>Nitratiruptor</i>	0.2%	0.2%	0.3%	0.1%	0.0%
<i>Thioreductor</i>	0.0%	0.0%	0.0%	0.0%	0.0%
uncultured	0.0%	0.0%	0.0%	0.0%	0.0%
Total bacterial read counts	1,300,099	569,538	681,368	560,396	169,913
Total ϵ-proteobacteria read counts	1,050,334	336,730	566,716	338,927	19,688
	Mariner-1	Mariner-2	Mariner-10	Mariner-17	Via Lili-2
Epsilonproteobacteria	8.6%	15.6%	32.7%	24.2%	24.2%
Campylobacterales	1.4%	7.0%	4.4%	2.6%	4.5%
Campylobacteraceae	0.3%	1.4%	1.6%	0.6%	0.5%
<i>Arcobacter</i>	0.1%	0.4%	0.2%	0.1%	0.2%
<i>Campylobacter</i>	0.0%	0.1%	1.1%	0.8%	1.8%
<i>Sulfurospirillum</i>	0.0%	0.4%	1.3%	0.5%	0.6%

<i>Thiofractor</i>	0.0%	0.1%	0.0%	0.0%	0.0%
Helicobacteraceae		10.5%	1.4%	3.1%	3.1%
<i>Helicobacter</i>	0.1%	0.2%	0.3%	0.3%	0.5%
<i>Sulfuricurvum</i>	0.0%	0.1%	0.1%	0.1%	0.1%
<i>Sulfurimonas</i>	9.2%	39.1%	4.8%	8.1%	4.6%
<i>Sulfurovum</i>	0.6%	13.2%	9.2%	21.2%	53.1%
uncultured	0.0%	0.0%	0.0%	0.0%	0.0%
Nautiliales					
Nautiliaceae	75.5%	10.8%	39.8%	36.2%	4.5%
<i>Caminibacter</i>	1.0%	0.1%	0.2%	0.3%	0.0%
<i>Lebetimonas</i>	0.0%	0.1%	0.1%	0.3%	0.0%
<i>Nautilia</i>	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Nitratifractor</i>	0.1%	1.0%	2.8%	1.6%	2.4%
<i>Nitratiruptor</i>	0.0%	0.0%	0.0%	0.0%	0.0%
<i>Thioreductor</i>	0.0%	0.0%	0.0%	0.0%	0.0%
uncultured	0.1%	0.0%	0.0%	0.0%	0.0%

Figure 12. Relative abundance of bacterial families from sulfide samples taken along the ELSC and VFR. Only bacterial families with greater than 1% abundance are shown. All other bacteria are grouped together under "Other Bacteria". SSU rRNA reads were identified with SortMeRNA and classified with SINA against the SILVA SSU Ref NR123 database.

Figure 13. Relative abundance of archaeal families from sulfide samples taken along the ELSC and VFR. Only archaeal families with greater than 1% abundance are shown. All other Archaea are grouped together under Other Archaea. SSU rRNA reads were identified with SortMeRNA and classified with SINA against the SILVA SSU Ref NR123 database.

Chapter 4: Conclusion

Metagenomics has become a revolutionary tool for microbial ecologists.

Advanced techniques, such as differential coverage, have led to the successful recovery of low abundant microbes. Metagenomics is also useful in profiling community composition without primer biases; potentially revealing a more accurate picture of the community diversity and identifying rare microbes missed using traditional cultivated independent and dependent methods.

In Chapter 2, differential coverage binning was used to recover two archaeal genomes from low abundant samples from a New Zealand hot spring. The recovered korarchaeotal and nanoarchaeotal genomes were the first representative genomes from New Zealand. Both archaeal genomes show extensive genomic synteny between their respective terrestrial lineages. The enrichment of crenarchaeotal sequences in the NZ13 cultures show a continued pattern of crenarchaeotes being the only known nanoarchaeotal host. The host metabolisms, in particular the presence or absence of archaeella, correlate with the retention or reduction of nanoarchaeotal archaeellum genes. Further suggesting the absence of archaeella in the New Zealand host. Though host physiology does not depict whether or not the nanoarchaeotes retain gluconeogenesis/glycolysis genes. The host of Nst1 is suspected to be an autotroph, while the host of *N. acidilobi* is a heterotroph. Seemingly unrelated to the host physiology, the NZ13 nanoarchaeote contains CRISPR type I-B genes, which are not present in the other representative terrestrial nanoarchaeotes. The marine nanoarchaeote, *Nanoarchaeum equitans*, contains the same CRISPR type I-B gene. This supports a previous conclusion, that the CRISPR system has been lost through genome reduction in the Yellowstone National Park (YNP)

nanoarchaeotes. The genome reduction process likely occurred after the YNP nanoarchaeotes and NZ13 nanoarchaeote lineages diverged (Randau 2012; Podar et al. 2013). The next step will be to identify the NZ13 nanoarchaeote host. So far all nanoarchaeotes isolated or sequenced have hosts from different crenarchaeotal orders (Huber et al. 2002; Podar et al. 2013; Wurch et al. 2016). More nanoarchaeote-host systems may help resolve the underlying mechanisms to the nanoarchaeotes attachment, genome reduction process, host specificity and nutrient transfer.

In addition, the NZ13 nanoarchaeote and NZ13 korarchaeote genomes possess osmotic regulation genes which are unique to the New Zealand genomes. The uptake of potassium may provide the organisms a higher salt tolerance. The osmotic regulation genes were the only differences among the three korarchaeotal genomes compared in this study. Major differences were observed between the terrestrial and marine Nanoarchaeota lineages, therefore a marine korarchaeotal genome will provide insights into the conserved korarchaeotal physiologies.

In Chapter 3, metagenomic sequencing and SSU rRNA identification tools were used to classify and compare ten microbial communities from ELSC-VFR. The community profiles showed *Epsilonproteobacteria* were the most abundant microbes at hydrothermal vents, with the exception of Vai Lili. The Euryarchaeota were the most abundant at the Mariner vent locations, while the Crenarchaeota were more abundant at ABE and Tui Malila. Korarchaeotal sequences were detected in relatively high abundance in five samples. A korarchaeotal genome should easily be recovered from the ELSC metagenomes and provide the first genome of a marine korarchaeote. In contrast,

the Nanoarchaeota were present in such low numbers, assembling a near complete genome may be difficult. Other genomes from uncultured members of both the bacterial and archaeal should be recoverable.

References:

- Albertsen, M., Hugenholtz, P., Skarshewski, A., Nielsen, K.L., Tyson, G.W. and Nielsen, P.H., 2013. Genome sequences of rare, uncultured bacteria obtained by differential coverage binning of multiple metagenomes. *Nature Biotechnology*, 31(6), pp.533–8.
- Aoki-Kinoshita, K.F. and Kanehisa, M., 2007. Gene annotation and pathway mapping in KEGG. *Methods In Molecular Biology*, 396, pp.71–91.
- Auchtung, T.A., Takacs-Vesbach, C.D. and Cavanaugh, C.M., 2006. 16S rRNA phylogenetic investigation of the candidate division “Korarchaeota.” *Applied and Environmental Microbiology*, 72(7), pp.5077–5082.
- Auguet, J.-C., Barberan, A. and Casamayor, E.O., 2010. Global ecological patterns in uncultured Archaea. *The ISME Journal*, 4(2), pp.182–90.
- Baker, B.J., Comolli, L.R., Dick, G.J., Hauser, L.J., Hyatt, D., Dill, B.D., Land, M.L., Verberkmoes, N.C., Hettich, R.L. and Banfield, J.F., 2010. Enigmatic, ultrasmall, uncultivated Archaea. *Proceedings of the National Academy of Sciences of the United States of America*, 107(19), pp.8806–11.
- Baker, B.J., Saw, J.H., Lind, A.E., Lazar, C.S., Hinrichs, K., Teske, A.P. and Ettema, T.J.G., 2016. Genomic inference of the metabolism of cosmopolitan subsurface Archaea, Hadesarchaea. *Nature Microbiology*, 1(3), p.16002.
- Barns, S.M., Delwiche, C.F., Palmer, J.D. and Pace, N.R., 1996. Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences. *Proceedings of the National Academy of Sciences of the United States of America*, 93(17), pp.9188–93.
- Barns, S.M., Fundyga, R.E., Jeffries, M.W. and Pace, N.R., 1994. Remarkable archaeal

diversity detected in a Yellowstone National Park hot spring environment.

Proceedings of the National Academy of Sciences of the United States of America, 91(5), pp.1609–1613.

Béjà, O., Aravind, L., Koonin, E. V., Suzuki, M.T., Hadd, A., Nguyen, L.P., Jovanovich, S.B., Gates, C.M., Feldman, R.A., Spudich, J.L., Spudich, E.N. and DeLong, E.F., 2000. Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. *Science*, 289(5486), pp.1902–6.

Bolger, A.M., Lohse, M. and Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 30(15), pp.2114–2120.

Bomar, L., Maltz, M., Colston, S. and Graf, J., 2011. Directed culturing of microorganisms using metatranscriptomics. *mBio*, 2(2), pp.1–8.

Brochier-Armanet, C., Boussau, B., Gribaldo, S. and Forterre, P., 2008. Mesophilic Crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. *Nature Reviews Microbiology*, 6(3), pp.245–252.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K. and Madden, T.L., 2009. BLAST+: architecture and applications. *BMC Bioinformatics*, 10(421), p.421.

Campbell, J.H., O'Donoghue, P., Campbell, A.G., Schwientek, P., Sczyrba, A., Woyke, T., Söll, D. and Podar, M., 2013. UGA is an additional glycine codon in uncultured SR1 bacteria from the human microbiota. *Proceedings of the National Academy of Sciences of the United States of America*, 110(14), pp.5540–5.

Casanueva, A., Galada, N., Baker, G.C., Grant, W.D., Heaphy, S., Jones, B., Yanhe, M.,

- Ventosa, A., Blamey, J. and Cowan, D. a., 2008. Nanoarchaeal 16S rRNA gene sequences are widely dispersed in hyperthermophilic and mesophilic halophilic environments. *Extremophiles*, 12(5), pp.651–656.
- Castelle, C.J., Wrighton, K.C., Thomas, B.C., Hug, L.A., Brown, C.T., Wilkins, M.J., Frischkorn, K.R., Tringe, S.G., Singh, A., Markillie, L.M., Taylor, R.C., Williams, K.H. and Banfield, J.F., 2015. Genomic expansion of domain Archaea highlights roles for organisms from new phyla in anaerobic carbon cycling. *Current Biology*, 25(6), pp.690–701.
- Cheong, W.H., Tan, Y.C., Yap, S.J. and Ng, K.P., 2015. ClicO FS: An interactive web-based service of Circos. *Bioinformatics*, 31(22), pp.3685–3687.
- Corre, E., 2001. ϵ -Proteobacterial diversity from a deep-sea hydrothermal vent on the Mid-Atlantic Ridge. *FEMS Microbiology Letters*, 205(2), pp.329–335.
- Costa, K.C., Wong, P.M., Wang, T.S., Lie, T.J., Dodsworth, J.A., Swanson, I., Burn, J.A., Hackett, M. and Leigh, J.A., 2010. Protein complexing in a methanogen suggests electron bifurcation and electron delivery from formate to heterodisulfide reductase. *Proceedings of the National Academy of Sciences of the United States of America*, 107(24), pp.11050–11055.
- Eddy, S.R., 1998. Profile hidden Markov models. *Bioinformatics*, 14(9), pp.755–63.
- Elkins, J.G., Podar, M., Graham, D.E., Makarova, K.S., Wolf, Y., Randau, L., Hedlund, B.P., Brochier-Armanet, C., Kunin, V., Anderson, I., Lapidus, A., Goltsman, E., Barry, K., Koonin, E. V, Hugenholtz, P., Kyrpides, N., Wanner, G., Richardson, P., Keller, M., et al., 2008. A korarchaeal genome reveals insights into the evolution of

- the Archaea. *Proceedings of the National Academy of Sciences of the United States of America*, 105(23), pp.8102–8107.
- Erauso, G., Reysenbach, A.-L., Godfroy, A., Meunier, J.-R., Crump, B., Partensky, F., Baross, J., Marteinsson, V., Barbier, G., Pace, N. and Prieur, D., 1993. *Pyrococcus abyssi* sp. nov., a new hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent. *Archives of Microbiology*, 160(5), pp.307–10.
- Ferguson, T.J. and Mah, R.A., 1983. Effect of H₂-CO₂ on methanogenesis from acetate or methanol in *Methanosarcina* spp. *Applied and Environmental Microbiology*, 46(2), pp.348–355.
- Ferrini, V.L., Tivey, M.K., Carbotte, S.M., Martinez, F. and Roman, C., 2008. Variable morphologic expression of volcanic, tectonic, and hydrothermal processes at six hydrothermal vent fields in the Lau back-arc basin. *Geochemistry, Geophysics, Geosystems*, 9(7).
- Flores, G.E., Campbell, J.H., Kirshtein, J.D., Meneghin, J., Podar, M., Steinberg, J.I., Seewald, J.S., Tivey, M.K., Voytek, M. a, Yang, Z.K. and Reysenbach, A.-L., 2011. Microbial community structure of hydrothermal deposits from geochemically different vent fields along the Mid-Atlantic Ridge. *Environmental Microbiology*, 13(8), pp.2158–71.
- Flores, G.E., Shakya, M., Meneghin, J., Yang, Z.K., Seewald, J.S., Geoff Wheat, C., Podar, M. and Reysenbach, A.-L., 2012a. Inter-field variability in the microbial communities of hydrothermal vent deposits from a back-arc basin. *Geobiology*, 10(4), pp.333–46.

- Flores, G.E., Wagner, I.D., Liu, Y. and Reysenbach, A.-L., 2012b. Distribution, abundance, and diversity patterns of the thermoacidophilic “deep-sea hydrothermal vent euryarchaeota 2”. *Frontiers in Microbiology*, 3(February), p.47.
- Fox, G.E., Magrum, L.J., Balch, W.E., Wolfe, R.S. and Woese, C.R., 1977. Classification of methanogenic bacteria by 16S ribosomal RNA characterization. *Proceedings of the National Academy of Sciences of the United States of America*, 74(10), pp.4537–41.
- Giovannoni, S.J., Britschgi, T.B., Moyer, C.L. and Field, K.G., 1990. Genetic diversity in Sargasso Sea bacterioplankton. *Nature*, 345(6270), pp.60–3.
- Giuffrè, A., Borisov, V.B., Arese, M., Sarti, P. and Forte, E., 2014. Cytochrome bd oxidase and bacterial tolerance to oxidative and nitrosative stress. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, 1837(7), pp.1178–1187.
- González, J.M., Masuchi, Y., Robb, F.T., Ammerman, J.W., Maeder, D.L., Yanagibayashi, M., Tamaoka, J. and Kato, C., 1998. *Pyrococcus horikoshii* sp. nov., a hyperthermophilic archaeon isolated from a hydrothermal vent at the Okinawa Trough. *Extremophiles*, 2(2), pp.123–130.
- Griffiths-Jones, S., Bateman, A., Marshall, M., Khanna, A. and Eddy, S.R., 2003. Rfam: An RNA family database. *Nucleic Acids Research*, 31(1), pp.439–441.
- Gulmann, L.K., Beaulieu, S.E., Shank, T.M., Ding, K., Seyfried, W.E. and Sievert, S.M., 2015. Bacterial diversity and successional patterns during biofilm formation on freshly exposed basalt surfaces at diffuse-flow deep-sea vents. *Frontiers in Microbiology*, 6(September), pp.1–16.

- Haft, D.H., Selengut, J.D. and White, O., 2003. The TIGRFAMs database of protein families. *Nucleic Acids Research*, 31(1), pp.371–3.
- Handelsman, J., Rondon, M.R., Brady, S.F., Clardy, J. and Goodman, R.M., 1998. Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. *Chemistry & Biology*, 5(10), pp.R245–R249.
- Hoek, J., Banta, A., Hubler, F. and Reysenbach, A.-L., 2003. Microbial diversity of a sulphide spire located in the Edmond deep-sea hydrothermal vent field on the Central Indian Ridge. *Geobiology*, 1(2), pp.119–127.
- Huber, H., Burggraf, S., Mayer, T., Wyszchony, I., Rachel, R. and Stetter, K.O., 2000. *Ignicoccus* gen. nov., a novel genus of hyperthermophilic, chemolithoautotrophic Archaea, represented by two new species, *Ignicoccus islandicus* sp. nov. and *Ignicoccus pacificus* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, 50(6), pp.2093–2100.
- Huber, H., Hohn, M.J., Rachel, R., Fuchs, T., Wimmer, V.C. and Stetter, K.O., 2002. A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. *Nature*, 417(6884), pp.63–7.
- Hug, L.A., Baker, B.J., Anantharaman, K., Brown, C.T., Probst, A.J., Castelle, C.J., Butterfield, C.N., Hernsdorf, A.W., Amano, Y., Ise, K., Suzuki, Y., Dudek, N., Relman, D.A., Finstad, K.M., Amundson, R., Thomas, B.C. and Banfield, J.F., 2016. A new view of the tree of life. *Nature Microbiology*, 1(5), p.16048.
- Huson, D.H., Beier, S., Flade, I., Górska, A., El-Hadidi, M., Mitra, S., Ruscheweyh, H.-J. and Tappu, R., 2016. MEGAN community edition-interactive exploration and

- analysis of large-scale microbiome sequencing data. *PLoS Computational Biology*, 12(6), p.e1004957.
- Hyatt, D., Chen, G.-L., LoCascio, P.F., Land, M.L., Larimer, F.W. and Hauser, L.J., 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics*, 11(1), p.119.
- Imelfort, M., Parks, D., Woodcroft, B.J., Dennis, P., Hugenholtz, P. and Tyson, G.W., 2014. GroopM: an automated tool for the recovery of population genomes from related metagenomes. *PeerJ*, 2, p.e603.
- Jahn, U., Gallenberger, M., Paper, W., Junglas, B., Eisenreich, W., Stetter, K.O., Rachel, R. and Huber, H., 2008. *Nanoarchaeum equitans* and *Ignicoccus hospitalis*: new insights into a unique, intimate association of two archaea. *Journal of Bacteriology*, 190(5), pp.1743–1750.
- Jarrell, K.F. and Albers, S.V., 2012. The archaellum: An old motility structure with a new name. *Trends in Microbiology*, 20(7), pp.307–312.
- Jolivet, E., L’Haridon, S., Corre, E., Forterre, P. and Prieur, D., 2003. *Thermococcus gammatolerans* sp. nov., a hyperthermophilic archaeon from a deep-sea hydrothermal vent that resists ionizing radiation. *International Journal of Systematic and Evolutionary Microbiology*, 53(Pt 3), pp.847–851.
- Jones, P., Binns, D., Chang, H.Y., Fraser, M., Li, W., McAnulla, C., McWilliam, H., Maslen, J., Mitchell, A., Nuka, G., Pesseat, S., Quinn, A.F., Sangrador-Vegas, A., Scheremetjew, M., Yong, S.Y., Lopez, R. and Hunter, S., 2014. InterProScan 5: genome-scale protein function classification. *Bioinformatics*, 30(9), pp.1236–1240.

- Kantor, R.S., Wrighton, K.C., Handley, K.M., Sharon, I., Hug, L. a., Castelle, C.J., Thomas, B.C. and Banfield, J.F., 2013. Small genomes and sparse metabolisms of sediment-associated bacteria from four candidate phyla. *mBio*, 4(5), pp.e00708-13.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. and Drummond, A., 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28(12), pp.1647–1649.
- Könneke, M., Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B. and Stahl, D. a, 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature*, 437(7058), pp.543–6.
- Kopylova, E., Noé, L. and Touzet, H., 2012. SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. *Bioinformatics*, 28(24), pp.3211–7.
- Kraegeloh, A., Amendt, B. and Kunte, H.J., 2005. Potassium transport in a halophilic member of the Bacteria domain: Identification and characterization of the K⁺ uptake systems TrkH and TrkI from *Halomonas elongata* DSM 2581T. *Journal of Bacteriology*, 187(3), pp.1036–1043.
- Krzywinski, M., Schein, J., Birol, I., Connors, J., Gascoyne, R., Horsman, D., Jones, S.J. and Marra, M.A., 2009. Circos: an information aesthetic for comparative genomics. *Genome research*, 19(9), pp.1639–45.
- Langmead, B. and Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. *Nature Methods*, 9(4), pp.357–359.

- Lee, S.H., Kim, M.-S., Kim, Y.J., Kim, T.W., Kang, S.G. and Lee, H.S., 2017. Transcriptomic profiling and its implications for the H₂ production of a non-methanogen deficient in the *frhAGB*-encoding hydrogenase. *Applied Microbiology and Biotechnology*, pp.1–8.
- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G. and Durbin, R., 2009. The sequence alignment/map format and SAMtools. *Bioinformatics*, 25(16), pp.2078–2079.
- Makarova, K.S., Wolf, Y.I., Alkhnbashi, O.S., Costa, F., Shah, S.A., Saunders, S.J., Barrangou, R., Brouns, S.J.J., Charpentier, E., Haft, D.H., Horvath, P., Moineau, S., Mojica, F.J.M., Terns, R.M., Terns, M.P., White, M.F., Yakunin, A.F., Garrett, R.A., van der Oost, J., et al., 2015a. An updated evolutionary classification of CRISPR-Cas systems. *Nature Reviews Microbiology*, 13(11), pp.722–736.
- Makarova, K.S., Wolf, Y.I. and Koonin, E. V., 2015b. Archaeal Clusters of Orthologous Genes (arCOGs): an update and application for analysis of shared features between *Thermococcales*, *Methanococcales*, and *Methanobacteriales*. *Life*, 5(1), pp.818–40.
- Mardanov, A. V., Svetlitchnyi, V.A., Beletsky, A. V., Prokofeva, M.I., Bonch-Osmolovskaya, E.A., Ravin, N. V. and Skryabin, K.G., 2010. The genome sequence of the crenarchaeon *Acidilobus saccharovorans* supports a new order, *Acidilobales*, and suggests an important ecological role in terrestrial acidic hot springs. *Applied and Environmental Microbiology*, 76(16), pp.5652–5657.
- Martin, D.D., Ciulla, R.A. and Roberts, M.F., 1999. Osmoadaptation in archaea. *Applied and Environmental Microbiology*, 65(5), pp.1815–1825.

- Martinez, F., Okino, K., Ohara, Y., Reysenbach, A.-L. and Goffredi, S., 2007. Back-Arc Basins. *Oceanography*, 20(1), pp.116–127.
- Miller-Coleman, R.L., Dodsworth, J. a., Ross, C. a., Shock, E.L., Williams, A.J., Hartnett, H.E., McDonald, A.I., Havig, J.R. and Hedlund, B.P., 2012. Korarchaeota diversity, biogeography, and abundance in Yellowstone and Great Basin hot springs and ecological niche modeling based on machine learning. *PLoS ONE*, 7(5).
- Mottl, M.J., Seewald, J.S., Wheat, C.G., Tivey, M.K., Michael, P.J., Proskurowski, G., McCollom, T.M., Reeves, E., Sharkey, J., You, C.-F., Chan, L.-H. and Pichler, T., 2011. Chemistry of hot springs along the Eastern Lau Spreading Center. *Geochimica et Cosmochimica Acta*, 75(4), pp.1013–1038.
- Nercessian, O., Reysenbach, A.-L., Prieur, D. and Jeanthon, C., 2003. Archaeal diversity associated with in situ samplers deployed on hydrothermal vents on the East Pacific Rise (13oN). *Environmental Microbiology*, 5(6), pp.492–502.
- Paper, W., Jahn, U., Hohn, M.J., Kronner, M., Näther, D.J., Burghardt, T., Rachel, R., Stetter, K.O. and Huber, H., 2007. *Ignicoccus hospitalis* sp. nov., the host of *Nanoarchaeum equitans*. *International Journal of Systematic and Evolutionary Microbiology*, 57(4), pp.803–808.
- Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P. and Tyson, G.W., 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Research*, 25(7), pp.1043–55.
- Peng, Y., Leung, H.C.M., Yiu, S.M. and Chin, F.Y.L., 2012. IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven

- depth. *Bioinformatics*, 28(11), pp.1420–8.
- Podar, M., Anderson, I., Makarova, K.S., Elkins, J.G., Ivanova, N., Wall, M.A., Lykidis, A., Mavromatis, K., Sun, H., Hudson, M.E., Chen, W., Deciu, C., Hutchison, D., Eads, J.R., Anderson, A., Fernandes, F., Szeto, E., Lapidus, A., Kyrpides, N.C., et al., 2008. A genomic analysis of the archaeal system *Ignicoccus hospitalis*-*Nanoarchaeum equitans*. *Genome Biology*, 9(11), p.R158.
- Podar, M., Makarova, K.S., Graham, D.E., Wolf, Y.I., Koonin, E. V and Reysenbach, A.-L., 2013. Insights into archaeal evolution and symbiosis from the genomes of a nanoarchaeon and its inferred crenarchaeal host from Obsidian Pool, Yellowstone National Park. *Biology Direct*, 8(1), p.9.
- Prokofeva, M.I., Miroshnichenko, M.L., Kostrikina, N.A., Chernyh, N.A., Kuznetsov, B.B., Tourova, T.P. and Bonch-Osmolovskaya, E.A., 2000. *Acidilobus aceticus* gen. nov., s.p. nov., a novel anaerobic thermoacidophilic archaeon from continental hot vents in Kamchatka. *International Journal of Systematic and Evolutionary Microbiology*, 50(6), pp.2001–2008.
- Pruesse, E., Peplies, J. and Glöckner, F.O., 2012. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics*, 28(14), pp.1823–9.
- Punta, M., Coggill, P.C., Eberhardt, R.Y., Mistry, J., Tate, J., Boursnell, C., Pang, N., Forslund, K., Ceric, G., Clements, J., Heger, A., Holm, L., Sonnhammer, E.L.L., Eddy, S.R., Bateman, A. and Finn, R.D., 2012. The Pfam protein families database. *Nucleic Acids Research*, 40(Database issue), pp.D290-301.

- Randau, L., 2012. RNA processing in the minimal organism *Nanoarchaeum equitans*. *Genome biology*, 13(7), p.R63.
- Randau, L., Münch, R., Hohn, M.J., Jahn, D. and Söll, D., 2005. *Nanoarchaeum equitans* creates functional tRNAs from separate genes for their 5'- and 3'-halves. *Nature*, 433(1999), pp.537–541.
- Reysenbach, A.-L., Liu, Y., Banta, A.B., Beveridge, T.J., Kirshtein, J.D., Schouten, S., Tivey, M.K., Von Damm, K.L. and Voytek, M. a, 2006. A ubiquitous thermoacidophilic archaeon from deep-sea hydrothermal vents. *Nature*, 442(7101), pp.444–7.
- Reysenbach, A.-L., Longnecker, K. and Kirshtein, J., 2000. Novel bacterial and archaeal lineages from an in situ growth chamber deployed at a Mid-Atlantic Ridge hydrothermal vent. *Applied and Environmental Microbiology*, 66(9), pp.3798–3806.
- Rinke, C., Schwientek, P., Sczyrba, A., Ivanova, N.N., Anderson, I.J., Cheng, J.-F., Darling, A.E., Malfatti, S., Swan, B.K., Gies, E. a, Dodsworth, J. a, Hedlund, B.P., Tsiamis, G., Sievert, S.M., Liu, W.-T., Eisen, J. a, Hallam, S.J., Kyrpides, N.C., Stepanauskas, R., et al., 2013. Insights into the phylogeny and coding potential of microbial dark matter. *Nature*, 499(7459), pp.431–437.
- Saw, J.H., Spang, A., Zaremba-Niedzwiedzka, K., Juzokaite, L., Dodsworth, J.A., Murugapiran, S.K., Colman, D.R., Takacs-Vesbach, C., Hedlund, B.P., Guy, L. and Ettema, T.J.G., 2015. Exploring microbial dark matter to resolve the deep archaeal ancestry of eukaryotes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370(1678), p.20140328.

- Schmidt, A., Rzanny, M., Schmidt, A., Hagen, M., Schütze, E. and Kothe, E., 2012. GC content-independent amino acid patterns in Bacteria and Archaea. *Journal of Basic Microbiology*, 52(2), pp.195–205.
- Shakya, M., Quince, C., Campbell, J.H., Yang, Z.K., Schadt, C.W. and Podar, M., 2013. Comparative metagenomic and rRNA microbial diversity characterization using archaeal and bacterial synthetic communities. *Environmental microbiology*, 15(6), pp.1882–99.
- Southworth, T.W., Guffanti, A.A., Moir, A. and Krulwich, T.A., 2001. GerN, an endospore germination protein of *Bacillus cereus*, is an Na⁺/H⁺-K⁺ antiporter. *Journal of Bacteriology*, 183(20), pp.5896–5903.
- Spang, A., Saw, J.H., Jørgensen, S.L., Zaremba-Niedzwiedzka, K., Martijn, J., Lind, A.E., van Eijk, R., Schleper, C., Guy, L. and Ettema, T.J.G., 2015. Complex archaea that bridge the gap between prokaryotes and eukaryotes. *Nature*, 521(7551), pp.173–179.
- Stein, J.L., Marsh, T.L., Wu, K.Y., Shizuya, H. and DeLong, E.F., 1996. Characterization of uncultivated prokaryotes: isolation and analysis of a 40-kilobase-pair genome fragment from a planktonic marine archaeon. *Journal of Bacteriology*, 178(3), pp.591–9.
- Stone, N.E., Fan, J.B., Willour, V., Pennacchio, L. a, Warrington, J. a, Hu, A., de la Chapelle, A., Lehesjoki, a E., Cox, D.R. and Myers, R.M., 1996. Construction of a 750-kb bacterial clone contig and restriction map in the region of human chromosome 21 containing the progressive myoclonus epilepsy gene. *Genome*

- Research*, 6(3), pp.218–25.
- Streif, S., Staudinger, W.F., Marwan, W. and Oesterhelt, D., 2008. Flagellar rotation in the archaeon *Halobacterium salinarum* depends on ATP. *Journal of Molecular Biology*, 384(1), pp.1–8.
- Takai, K. and Nakamura, K., 2011. Archaeal diversity and community development in deep-sea hydrothermal vents. *Current Opinion in Microbiology*, 14(3), pp.282–291.
- Takai, K., Sugai, A., Itoh, T. and Horikoshi, K., 2000. *Palaeococcus ferrophilus* gen. nov., sp. nov., a barophilic, hyperthermophilic archaeon from a deep-sea hydrothermal vent chimney. *International Journal of Systematic and Evolutionary Microbiology*, 50(2), pp.489–500.
- Torsvik, V., Goksøyr, J. and Daae, F.L., 1990. High diversity in DNA of soil bacteria. *Applied and Environmental Microbiology*, 56(3), pp.782–7.
- Tyson, G.W., Chapman, J., Hugenholtz, P., Allen, E.E., Ram, R.J., Richardson, P.M., Solovyev, V. V, Rubin, E.M., Rokhsar, D.S. and Banfield, J.F., 2004. Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature*, 428(6978), pp.37–43.
- Tyson, G.W., Lo, I., Baker, B.J., Allen, E.E., Hugenholtz, P. and Banfield, J.F., 2005. Genome-directed isolation of the key nitrogen fixer *Leptospirillum ferrodiazotrophum* sp. nov. from an acidophilic microbial community. *Applied and Environmental Microbiology*, 71(10), pp.6319–24.
- Verkhovskaya, M.L., Verkhovsky, M.I. and Wikström, M., 1995. A novel antiporter activity catalyzing sodium and potassium transport from right-side-out vesicles of *E.*

- coli*. *FEBS Letters*, 363(1–2), pp.46–8.
- Ward, D.M., Weller, R. and Bateson, M.M., 1990. 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. *Nature*, 345(6270), pp.63–5.
- Waters, E., Hohn, M.J., Ahel, I., Graham, D.E., Adams, M.D., Barnstead, M., Beeson, K.Y., Bibbs, L., Bolanos, R., Keller, M., Kretz, K., Lin, X., Mathur, E., Ni, J., Podar, M., Richardson, T., Sutton, G.G., Simon, M., Soll, D., et al., 2003. The genome of *Nanoarchaeum equitans*: insights into early archaeal evolution and derived parasitism. *Proceedings of the National Academy of Sciences of the United States of America*, 100(22), pp.12984–12988.
- Woese, C.R., Kandler, O. and Wheelis, M.L., 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Sciences*, 87(12), pp.4576–4579.
- Wu, Y.-W., Tang, Y.-H., Tringe, S.G., Simmons, B. a and Singer, S.W., 2014. MaxBin: an automated binning method to recover individual genomes from metagenomes using an expectation-maximization algorithm. *Microbiome*, 2(1), p.26.
- Wurch, L., Giannone, R.J., Belisle, B.S., Swift, C., Utturkar, S., Hettich, R.L., Reysenbach, A.-L. and Podar, M., 2016. Genomics-informed isolation and characterization of a symbiotic Nanoarchaeota system from a terrestrial geothermal environment. *Nature Communications*, 7, p.12115.

Appendix A: Supplemental information from the nanoarchaeotal and korarchaeotal genomic analysis

Figure 14. **A.** Overview of the shotgun sequencing process with an explanation of paired end sequencing. Paired reads are assembled into contigs through overlapping and stacking reads. Coverage is representative of genomic abundance. Various methods of binning help group the contigs in to bins. **B.** To use differential binning multiple, relatable samples are required. Paired end reads are combined for assembly. Reads are mapped back to the contigs individually to produce a multi-sample coverage profile used for binning. Differential coverage binning is still complemented with classical binning methods.

Figure 15. NZ13 nanoarchaeotal SSU rRNA sequence alignment. **(1)** The PCR sequence from an enrichment culture. **(2)** The *de novo* assembly of SSU rRNA from only NZ13en-R and NZ13en-P reads. **(3)** The error prone sequence generated during assembly.

Table 7. Distance identity map of nanoarchaeotal SSU rRNA sequences. NZ13en-R and NZ13 PCR are not 100% identical, due to a variable base pair change. See Figure 15.

	<i>N. equitans</i>	Nst1	<i>N. acidilobi</i>	NZ13en-R 16S rRNA	NZ13 enrichment
<i>N. equitans</i>		81.05%	81.12%	84.95%	83.48%
Nst1	81.05%		97.47%	89.02%	87.63%
<i>N. acidilobi</i>	81.12%	97.47%		89.17%	87.77%
NZ13en-R 16S rRNA	84.95%	89.02%	89.17%		99.92%
NZ13 enrichment	83.48%	87.63%	87.77%	99.92%

Figure 16. Protein alignment of Glu-tRNA^{Gln} amidotransferase. *N. equitans* (1, 5), Nst1 (2, 6) and *N. acidilobi* (3, 7) have a split gene, while NZ13 nanoarchaeote (4) is intact.

Table 8. Relative abundance of amino acids present in the NZ13 nanoarchaeote. Open reading frames were predicted using Prodigal and individual amino acids were calculated.

Relative abundance	Hits	Abbreviation	Amino Acids
4.0%	6746	A	Alanine
0.7%	1194	C	Cysteine
5.1%	8575	D	Aspartic Acid
8.4%	14077	E	Glutamic acid
4.9%	8278	F	Phenylalanine
5.7%	9505	G	Glycine
1.1%	1910	H	Histidine
11.2%	18829	I	Isoleucine
9.6%	16152	K	Lysine
10.0%	16690	L	Leucine
1.8%	3035	M	Methionine
5.0%	8359	N	Asparagine
3.2%	5420	P	Proline
1.5%	2560	Q	Glutamine
5.1%	8525	R	Arginine
6.1%	10190	S	Serine
3.7%	6217	T	Threonine
6.3%	10596	V	Valine
0.9%	1565	W	Tryptophan
5.5%	9247	Y	Tyrosine

Table 9. Nanoarchaeotal genomic comparison table. Contig numbers are listed in the column below each respective Nanoarchaeota. All hits had an e-value greater than 10^{-5} . Genes not detected in the genomes are denoted by ‘-’.

Protein	Gene Name	EC	Protein Domain	NZ13 Nano-archaeota	<i>Nanopusillus acidilobi</i> *	<i>Nanoarchaeum equitans</i> *
Glycogen						
Glycogen synthase		2.4.1.11	PF05693	0_16	75	-
Nucleotidyl transferase			PF00483	17_3	321	-
Glycogen debranching enzyme		3.2.1.18	PF03065	0_13	72	-
Glucan phosphorylase		2.4.1.1	PF00343	4_12	585	-
Phosphoglucose mutase	pgm	5.4.2.2	PF02878	22_7	326	-
Glycolysis - modified Embden-Meyerhoff pathway						
Glucokinase	glk	2.7.1.2	PF02685	-	-	-
Glucose-6-Phosphate isomerase	pgi	5.3.1.9	PF06560	0_96	483	-
6-phosphofructokinase	pfk	2.7.1.11	PF00365	-	-	-
Fructose biphosphate aldolase class I	pfk	4.1.2.13	PF00274	-	-	-
Fructose biphosphate aldolase class II	pfk	4.1.2.13	PF01116	-	-	-
Triosephosphate isomerase	tim	5.3.1.1	PF00121	112_1	469	-
Glyceraldehyde-3-phosphate dehydrogenase	gapn	1.2.1.9	PF00171	-	456	-
Glyceraldehyde-3-phosphate: ferredoxin oxidoreductase	gapor	1.2.7.6	PF02730	16_4 18_1 26_10	415 532 145	-
Phosphoglycerate mutase	pam	5.4.2.1	PF10143	1_9	648	-
Enolase	eno	4.2.1.11	PF00113	0_102	355	-
Phosphoenolpyruvate synthase	peps	2.7.9.2	PF01326	28_3	513	-
Pyruvate kinase	pk	2.7.1.40	PF00224	-	327	-
Pyruvate: phosphate dikinase	ppdk	2.7.9.1	TIGR01828	-	-	-
Pyruvate-ferredoxin oxidoreductase	por	1.2.7.1	PF01855	18_7 18_6	312 340	-
Acetyl_CoA synthetase (NDP forming)	acs	6.2.1.13	TIGR02717	19_11 19_12	503 504	-
Gluconeogenesis						
Fructose-1,6-bisphosphate class V	fbpase	3.1.3.11	PF01950	10_4	58	-

Glyceraldehyde 3-phosphate dehydrogenase	gapdh	1.2.1.12	PF02800	-	-	-
Phosphoglycerate kinase	pgk	2.7.2.3	PF00162	-	-	-
Energy Metabolism						
ATPase		3.6.3.6	PF00122	21_8 112_2	470, 244	-
Inorganic pyrophosphatase		3.6.1.1	PF00719	15_12	413	-
Cytochrome bd oxidase subunit I		1.10.3.-	PF01654	-	116	-
Transporter						
ABC transporter			PF00005	2_39	5	79
ABC transporter – ATPase			PF00005	33_3	659	312
ABC transporter ATP-binding			PF00005	7_15	487	440
ABC transporter			PF00005	19_6	280	-
ABC transporter			BLAST	107_1	270	-
ABC-2 type transporter			PF01061	33_4	486	-
K ⁺ transporter	trkA		PF02254	54_2	-	-
K ⁺ transporter	trkH		PF02386	54_3	-	-
Archaeum						
Archaeal flagellin	flab		PF01917	3_14	381	-
Archaeal flagellin	flab		PF01917	3_16	-	-
Archaeal flagellin	flab		PF01917	3_19	384	-
Archaeal flagellin	flag		PF01917	4_9	655	-
Archaeal flagellar protein F	flaf		BLAST	4_10	-	-
Archaeal flagellar protein D/E	flad/e		PF04659	22_2	183	-
Flagellar accessory protein FlaH	flah		BLAST	22_4	181	-
Archaeal flagellar protein FlaI	flai		BLAST	22_5	180	-
Archaeal flagella assembly protein J	flaj		PF00482	38_6	445	-
CRISPER						
CRISPR-associated protein Cas1 – subtype IB	cas1		PF01867	104_1	-	19
CRISPR-associated protein Cas2	cas2		PF09827	-	-	18
CRISPR-associated protein Cas3	cas3		TIGR01587	-	-	23
CRISPR-associated protein Cas4	cas4		PF01930	21_1	-	24

CRISPR-associated protein Cas5	cas5	PF09704	-	-	22
CRISPR-associated protein Cas6	cas6	PF01881	21_2	-	1
CRISPR-associated protein Cas7	cas7	PF05107	-	-	21
Fe-S assembly					
SufS	sufs	TIGR01979	3_12	495	-
SufA	sufa	TIGR01997	-	-	-
SufB	sufb	TIGR01980	7_16	385	138
SufC	sufc	BLAST	7_15	270	79
SufD	sufd	PF01458	7_17	271	-
Sec protein translocase					
SecY	secY	PF00344	4_5	575	176
Sec61	sec61	PF03911	1_31	-	-
SecF	secF	PF02355	26_3	295	458
SecD	secD	PF02355	0_98	-	459
Polyamine					
Pyruvoyl-dependent arginine decarboxylase		PF02675	0_22	115	-
Agmatinase		TIGR01230	0_33	90	232

*Annotations from NCBI, Accession numbers: *N. acidilobi* (CP010514), *N. equitans* (AE017199)

Figure 17. NZ13 korarchaeotal consensus sequence alignment of SSU rRNA from (1) NZ13, (2) NZ13en-R and (3) NZ13en-P.

Figure 18. Sequence alignment of SSU rRNA of *Ca. K. cryptofilum*, NZ13 Korarchaeota PCR sequence and NZ13 korarchaeotal metagenome consensus sequence.

Table 10. Distance identity map of korarchaeotal SSU rRNA sequences. NZ13 Korarchaeota PCR and NZ13 korarchaeotal metagenome are not 100% identical, due to many base pair changes. See *Figure 17*.

	<i>Candidatus</i> K. cryptofilum	NZ13 Korarchaeota enrichment	NZ13 Korarchaeota metagenome
<i>Candidatus</i> K. cryptofilum		91.39%	91.66%
NZ13 Korarchaeota enrichment	91.39%		99.39%
NZ13 Korarchaeota metagenome	91.66%	99.39%	

Table 11. Korarchaeotal genomic comparison table. Contig numbers are listed in the column below each respective korarchaeote. All hits had an evalule greater than 10^{-5} . Genes not present in the genomes are denoted by ‘-’.

Protein	Gene Name	EC	Protein Domain	NZ13 Kor-archaeota	<i>Candidatus</i> Korarchaeum cryptofilum*	Little Hot Creek
Glycogen						
Glycogen synthase		2.4.1.11	PF05693	-	-	-
Glycogen debranching enzyme		3.2.1.18	PF03065	-	-	-
Glucan phosphorylase		2.4.1.1	PF00343	-	-	-
Phosphoglucose mutase	Pgm	5.4.2.2	PF02878	3_22	1562	14949_1
Glycolysis - modified EM pathway						
Glucokinase	Glk	2.7.1.2	PF02685	-	-	
Glucose-6-Phosphate isomerase	Pgi	5.3.1.9	PF06560	9_16	1071	1123_8
6-phosphofructokinase	Pfk	2.7.1.56	PF00365	-	-	-
ATP-dependent phosphofructokinase	PfkB	2.7.1.11	PF00294	0_97, 63_8	1260, 376	825_18, 5083_4
Fructose bisphosphate aldolase class I	Fba	4.1.2.13	PF00274	-	-	-
Fructose bisphosphate aldolase class II	Fba	4.1.2.13	PF00596	0_36	1320	3725_1
Triosephosphate isomerase	Tim	5.3.1.1	PF00121	1_13	1532	4478_5
Glyceraldehyde-3-phosphate dehydrogenase	Gapn	1.2.1.9	PF00171	81_4	518	6772_1, 29715_2
Glyceraldehyde-3-phosphate: ferredoxin oxidoreductase	Gapor	1.2.7.6	PF02730	4_42, 38_1	1132	1955_3, 15344_2
Phosphoglycerate mutase	Pgam	5.4.2.1	PF10143	0_89	1268	825_10
Enolase	Eno	4.2.1.11	PF00113	5_14	1672	2240_2
Phosphoenolpyruvate synthase	Peps	2.7.9.2	PF01326	59_10	61	6462_1, 1636_3
Pyruvate kinase	Pk	2.7.1.40	PF00224	2_1, 92_5	155	2671_4
Pyruvate: phosphate dikinase	Ppdk	2.7.9.1	TIGR0182 8	38_5	961	
Pyruvate-ferredoxin oxidoreductase	Por	1.2.7.1	PF01855	47_3, 0_9, 0_41, 0_76	1280, 716, 1315, 1346, 75	5199_6, 737_3
Acetyl_CoA synthetase (NDP forming)		6.2.1.13	TIGR0271 7	47_2	31, 76, 204, 293, 1187	
Gluconeogenesis						
Fructose-1,6-bisphosphate class V	Fbpase	3.1.3.11	PF01950	1_12	1531	4478_6, 7484_1
Glyceraldehyde 3-	Gapdh	1.2.1.12	PF02800	54_2	238	3737_2

phosphate dehydrogenase

Phosphoglycerate kinase	Pgk	2.7.2.3	PF00162	3_25	1565	6779_1
Fructose 1,6 biphosphate	Fbp	3.11.3.11	PF00459	24_9	10	428_15

TCA cycle enzymes

Phosphoenolpyruvate carboxykinase (ATP)	Pepck	4.1.1.49	PF01293	33_3	328	1859_2
Citrate synthase	Cs	2.3.3.1	PF00285 TIGR0123	-	-	-
Pyruvate carboxylase	Pc	6.4.1.1	5	-	-	-
Malate dehydrogenase	Mdh	1.1.1.37	PF02615	44_3	611	3083_2
Malic enzyme		1.1.1.40	PF00390	10_15	997	-
Fumarase subunit A	FumA	4.2.1.2	PF05681	7_13	96	2810_2
Fumarase subunit B	FumB	4.2.1.2	PF05683	7_12	97	2810_3
Fumarase subunit C	FumC	4.2.1.2	PF00206	14_10, 1_20, 2_14	1414, 168	6731_1, 2137_5
Fumarate Reductase/ Succinate dehydrogenase	sdhD	1.3.5.1	PF01127	13_19	1220	1576_8
Fumarate Reductase/ Succinate dehydrogenase	sdhC	1.3.5.1	PF01127	13_20	1221	1576_9
Fumarate Reductase/ Succinate dehydrogenase	sdhA	1.3.5.1	PF01127	13_21	1219	1576_10
Fumarate Reductase/ Succinate dehydrogenase	FrdB	1.3.5.1	PF13085	13_18	1222	1576_7
Succinyl-CoA synthetase, alpha subunit	SucD	6.2.1.5	PF00549	1_58	1453	11011_1
Succinyl-CoA synthetase, beta subunit	SucC	6.2.1.5	PF00549	1_57	1454	11011_2
Aspartate aminotransferase	Aat	2.6.1.1	PF00155	0_99	329	7238_1
2-oxoacid:ferredoxin oxidoreductase, gamma subunit	KorC	1.2.7.3	PF01558 arCOG009	0_38	1316	5199_3
Ferredoxin 2-oxoacid:ferredoxin oxidoreductase, beta subunit	KofD	1.2.7.3	59	0_39	1317	5199_4
2-oxoacid:ferredoxin oxidoreductase, alpha subunit	KorB	1.2.7.3	PF02775	0_40	1318	7093_3
	KorA	1.2.7.3	PF01855	0_41	1319	7093_2

Pyruvate formate lyase

Pyruvate-formate lyase	PflD	2.3.1.54	TIGR0177 4	26_10	538	3985_3
Pyruvate-formate lyase- activating enzyme	PflA		TIGR0433 7	93_7	553	8379_4
Pyruvate-formate lyase- activating enzyme	PflA		TIGR0433 7	202_1	680	1514_1
Pyruvate-formate lyase- activating enzyme	PflA		TIGR0433 7	90_4	97	2612_1

Pyruvate-formate lyase-activating enzyme	PflA		TIGR0433 7	91_5	969	6514_5
Pyruvate-formate lyase-activating enzyme	PflA		TIGR0249 4	26_11	284	3985_4
Pyruvate-formate lyase-activating enzyme	PflA		TIGR0249 4	157_2	539	4645_2

Pentose Pathway – RuMP pathway

3-hexulose-6-phosphate isomerase	HxlB	5.3.1.27	TIGR0312 7	5_12	1674	2240_4
3-hexulose-6-phosphate synthase	Hps	4.1.2.43	PF00215	8_8	1361	4319_3
Ribose 5-phosphate isomerase	RpiA	5.3.1.6	PF06026	5_1	2	428_7
ribose-phosphate pyrophosphokinase	PrsA	2.7.6.1	PF00156	0_95	1262	825_16

Urea Cycle

Carbamate kinase	ArcC	2.7.2.2	PF00696	0_53	1303	1846_2
Ornithine carbamoyltransferase	ArgF	2.1.3.3	TIGR0065 8	0_52, 67_3, 8_34	1387, 522, 1304	1846_3, 2916_4, 4393_1
Argininosuccinate synthase	ArgG	6.3.1.5	arCOG001 12	2_15	169	6731_2
Argininosuccinate lyase	ArgH	4.3.2.1	TIGR0083 8	2_14	168	6731_1
Arginine deiminase	ArcA	3.5.3.6	arCOG031 08	67_5	524	2916_2

Energy Metabolism

ATPase

Archaeal/vacuolar-type H ⁺ -ATPase subunit A	NtpA	3.6.3.14	PF02874	0_1	-	-
Archaeal/vacuolar-type H ⁺ -ATPase subunit A	NtpA	3.6.3.14	PF02874	8_1	1354	3143_4
Archaeal/vacuolar-type H ⁺ -ATPase subunit B	NtpB	3.6.3.14	PF02874	8_2	1355	3143_5
Archaeal/vacuolar-type H ⁺ -ATPase subunit C	NtpC	3.6.3.14	arCOG024 59	8_4	1357	3143_7
Archaeal/vacuolar-type H ⁺ -ATPase subunit D	NtpD	3.6.3.14	PF01813	8_3	1356	3143_6
Archaeal/vacuolar-type H ⁺ -ATPase subunit E	NtpE	3.6.3.14	arCOG008 69	0_2	1353	3143_3
Archaeal/vacuolar-type H ⁺ -ATPase subunit H	NtpF	3.6.3.14	arCOG033 63	0_3	1352	3143_2
Archaeal/vacuolar-type H ⁺ -ATPase subunit I	NtpI	3.6.3.14	PF01496	8_6	1359	4319_1
Archaeal/vacuolar-type H ⁺ -ATPase subunit I	NtpI	3.6.3.14	PF01496	95_3	1122	2123_2
Archaeal/vacuolar-type H ⁺ -ATPase subunit I	NtpI	3.6.3.14	PF01496	72_6	-	-
Archaeal/vacuolar-type H ⁺ -ATPase subunit I	NtpI	3.6.3.14	PF01496	50_7	-	-

Archaeal/vacuolar-type Na ⁺ /H ⁺ -ATPase, subunit K	AtpK	3.6.3.14	arCOG024 55	8_5	1358	-
NADH dehydrogenase						
NADH dehydrogenase subunit A	NuoA	1.6.5.3	arCOG015 57	6_27	141	1485_5
NADH dehydrogenase subunit B	NuoB	1.6.5.3	PF01058	6_28	142	1485_4
NADH dehydrogenase subunit C	NuoC	1.6.5.3	PF00329	6_29	143	1485_3
NADH dehydrogenase subunit D	NuoD	1.6.5.3	PF00346	6_30	144	1485_2
NADH dehydrogenase subunit H	NuoH	1.6.5.3	PF00146	6_31	145	16741_2
NADH dehydrogenase subunit I	NuoI	1.6.5.3	arCOG015 43	6_32	146	16741_2
NADH dehydrogenase subunit J	NuoJ	1.6.5.3	arCOG046 54	6_33	147	16741_3
NADH dehydrogenase subunit 4L (K,kappa)	NuoK	1.6.5.3	arCOG030 73	6_34	148	1478_10
NADH:ubiquinone oxidoreductase subunit 4 (chain M)	NuoM	1.6.5.3	PF00361	6_35	149	1478_7
NADH dehydrogenase subunit L	NuoL	1.6.5.3	PF00361	6_36	150	1478_8
NADH dehydrogenase subunit L	NuoL	1.6.5.3	PF00361	92_1	151	-
NADH dehydrogenase subunit N	NuoN	1.6.5.3	PF00361	92_2	225	1478_9
Heterodisulfide Reductase						
Heterodisulfide reductase, subunit B	HdrB	1.8.98.1	TIGR0328 8	49_6	806	9841_3
Heterodisulfide reductase, subunit C	HdrC	1.8.98.1	arCOG009 64	49_5	805	9841_4
Heterodisulfide reductase, subunit A	HdrA	1.8.98.1	arCOG022 35	49_3	803	9012_1
Heterodisulfide reductase, subunit A	HdrA	1.8.98.1	arCOG022 35	4_17	1163	-
Heterodisulfide reductase, subunit A	HdrA	1.8.98.1	arCOG022 35	4_16	1164	-
Heterodisulfide reductase, subunit E, CoB-Com	HdrE	1.8.98.1	arCOG050 14	4_18	1162	-
F420-Reducing Hydrogenase						
Coenzyme F420-reducing hydrogenase, alpha subunit	FrhA		PF00374	33_6	332	4115_1
Coenzyme F420-reducing hydrogenase, gamma subunit	FrhG		PF01058	4_14	333	4813_4
Coenzyme F420-reducing hydrogenase, gamma	FrhG		PF01058	33_7	1166	-

subunit						
Coenzyme F420-reducing hydrogenase, delta subunit	FrhD		arCOG024 75	4_15	1165	-
Coenzyme F420-reducing hydrogenase, delta subunit	FrhD		arCOG024 75	49_4	804	-
Carbon-monoxide Dehydrogenase						
carbon-monoxide dehydrogenase, catalytic subunit	-		PF03063	49_1	801	9012_3
CO dehydrogenase maturation factor	CooC		PF01656	49_2	802	9012_2
Formate Dehydrogenase						
Protein involved in formate dehydrogenase formation	FdhE		arCOG056 34	4_7	1173	1107_7
formate dehydrogenase - subunit B	FdhB		BLAST	123_2	821	-
Menaquinone – alternate Mevalonate pathway						
Acetyl-CoA C-acetyltransferase	PaaJ	2.3.1.9	arCOG012 78	13_13	1227	1576_2
Hydroxymethylglutaryl-CoA synthase	PksG	2.3.3.10	PF08545	13_14	1226	1576_3
Hydroxymethylglutaryl-CoA reductase	HMG1	1.1.1.88	PF00368 arCOG010	203_3	585	9728_1
Mevalonate kinase	ERG12	2.7.1.36	28	35_3	1115	3841_6
Phosphomevalonate decarboxylase	Pmd	4.1.1.99	COG3407 arCOG008	-		
Isopentenyl-diphosphate	Ipk	2.7.4.26	60	5_25	1661	14454_2
Isopentenyl-diphosphate isomerase	Ipp	5.3.3.2	arCOG006 13	9_23	1064	1123_1
1,4-Dihydroxy-2-naphtoate prenyltransferase	MenA	2.5.1.74	arCOG004 80	70_5, 68_2	-	-
Demethylmenaquinone methyltransferase	UbiE/Me nG	2.1.1.163	arCOG043 48	-	835	3560_2
4-hydroxybenzoate polyprenyltransferase	UbiA		arCOG004 76	3_4, 65_5	507, 1544	675_9,
Hydrogenase						
Ni,Fe-hydrogenase I large subunit	HyaB		PF00374	4_13	1167	1107_2
Ni,Fe-hydrogenase maturation factor	HyaD		PF01750	4_12	1168	1107_6
Ni,Fe-hydrogenase maturation factor	HyaD		PF01750	33_5	331	4115_2
4Fe-4S ferredoxin iron-	HybA		TIGR0295	4_43	1131	1955_2

sulfur binding domain protein		1			
Zn finger protein					
HypA/HybF (possibly regulating hydrogenase expression)	HybF	PF01155	4_11	1169	1107_11
Hydrogenase maturation factor	HypC	PF01455	4_9	1171	1107_9
Hydrogenase maturation factor	HypD	PF01924	4_20	1160	9763_2
Hydrogenase maturation factor	HypE	PF00586	4_8	1172	1107_8
Hydrogenase maturation factor	HypE	PF00586	32_5	233	6319_1
Hydrogenase maturation factor	HypE	PF00586	12_24	1185	1675_7
Hydrogenase maturation factor	HypF	PF00708	4_19	1161	1107_12
Cytochrome					
cytochrome bd-type quinol oxidase subunit 1- like protein	CydA	PF01654	5_37	1649	5788_1
cytochrome bd ubiquinol oxidase subunit I	CydA	PF01654	5_36	1650	5788_2
Cytochrome b/b6 domain protein	QcrB	PF13631	13_24	1216	7179_1
Rieske Fe-S protein	QcrA	PF00355	13_23	1217	1576_12
Bacterioferritin (cytochrome b1)	Bfr	arCOG010 94	158_5	605	3301_2
Electron transfer proteins					
Electron transfer flavoprotein alpha/beta- subunit	FixA	PF01012	9_1	1085	9229_3
Electron transfer flavoprotein alpha/beta- subunit	FixA	PF01012	32_6	232	8913_3
Electron transfer flavoprotein alpha subunit	FixB	PF01012 PF00766	32_7	1086	8913_2
Electron transfer flavoprotein alpha subunit	FixB	PF01012 PF00766	119_1	231	9229_2
geranylgeranyl reductase	FixC	PF13450	1_25	212	2227_8
geranylgeranyl reductase	FixC	PF13450	12_14	59	6050_3
monooxygenase FAD- binding	FixC	PF13450	34_6	1195	6127_5
Dehydrogenase (flavoprotein)	FixC	PF13450	48_11	1419	10480_5
Dehydrogenase (flavoprotein)	FixC	PF13450	59_12	-	1636_1
Sec protein translocase					
SecY	secY	PF00344	-	1639	6621_5

Sec61	sec61	PF03911	0_65	1291	3038_8
SecF	secF	PF02355	-	-	-
SecD	secD	PF02355	-	-	-
S-layer proteins					
S-layer domain			0_59	1297	2461_2
S-layer-like protein			2_21	177	13474_2
S-layer-like protein			7_2	630	21019_2
S-layer-like protein			114_2	435	2461_2
S-layer protein, possibly associated with type IV pili like system			7_4	1022	17340_2
S-layer protein, possibly associated with type IV pili like system			10_24	1198	19576_1
S-layer protein, possibly associated with type IV pili like system			12_11	556	2858_1
S-layer protein, possibly associated with type IV pili like system			53_8	601	7280_3
S-layer protein, possibly associated with type IV pili like system			56_10	1006	820_10
S-layer protein, possibly associated with type IV pili like system			91_2	112	8379_1
S-layer protein, possibly associated with type IV pili like system			-	-	9439_2
S-layer protein, possibly associated with type IV pili like system			-	1392	9439_3
S-layer protein			57_5	-	3745_2
S-layer protein			160_3	630	4098_6
S-layer domain-like protein			12_21	113	4627_4
S-layer-like protein			139_2	1107	-
CRISPR					
CRISPR-associated protein Cas1	cas1	PF01867	-	439	-
CRISPR-associated protein Cas2	cas2	PF09827	-	441	27922_2
CRISPR-associated protein Cas3	cas3	TIGR01587	-	449	1843_8
CRISPR-associated protein Cas4	cas4	PF01930	259_2	442	6649_2
CRISPR-associated protein Cas5	cas5	PF09704	-	446	14137_4
CRISPR-associated protein Cas6	cas6	TIGR01877	7_28	81	2330_4

CRISPR-associated protein Cas7	cas7	PF05107	-	-	-
CRISPR-associated protein Cmr5	cmr5	PF09701	-	461	19543_2
CRISPR-associated protein Cmr3	cmr3	PF09700	-	459	-
Transporter					
ABC-type dipeptide/oligopeptide/nickel transport system, ATPase component	DppD	PF00005	0_47	1110	12421_2
ABC-type oligopeptide transport system, ATPase component	AppF	PF00005	0_48	1111	1514_8
ABC-type dipeptide/oligopeptide/nickel transport system, ATPase component	DppD	PF00005	0_58	1144	15269_2
ABC-type oligopeptide transport system, ATPase component	AppF	PF00005	1_47	1145	1669_13
Energy-coupling factor transporter ATP-binding protein EcfA2	EcfA2	PF00005	1_48	1156	16996_1
ABC-type multidrug transport system, ATPase component	CcmA	PF00005	12_10	1190	1742_4
ABC-type multidrug transport system, ATPase component	CcmA	PF00005	12_19	1199	1846_7
ABC-type antimicrobial peptide transport system, ATPase component	SalX	PF00005	16_13	1298	1846_8
ABC-type branched-chain amino acid transport system, ATPase component	LivG	PF00005	16_9	1308	2118_9
ABC-type branched-chain amino acid transport system, ATPase component	LivF	PF00005	17_22	1309	22872_3
Energy-coupling factor transporter ATP-binding protein EcfA2	EcfA2	PF00005	2_29	1443	2426_2
ABC-type Mn/Zn transport system, ATPase component	ZnuC	PF00005	204_1	186	2426_3
ABC-type multidrug transport system, ATPase component	CcmA	PF00005	204_2	425	2461_3
ABC-type nitrate/sulfonate/bicarbonate transport system,	TauB	PF00005	208_4	505	24788_2

ATPase component					
ABC-type oligopeptide transport system, ATPase component	AppF	PF00005	25_14	527	3558_8
ABC-type dipeptide/oligopeptide/nickel transport system, ATPase component	DppD	PF00005	27_15	528	3841_1
ABC-type multidrug transport system, ATPase component	CcmA	PF00005	27_16	563	3841_2
ABC-type multidrug transport system, ATPase component	CcmA	PF00005	342_1	671	4356_4
ABC-type multidrug transport system, ATPase component	CcmA	PF00005	342_2	726	4837_3
ABC-type nitrate/sulfonate/bicarbonate transport system, ATPase component	TauB	PF00005	35_8	780	6376_1
ABC-type multidrug transport system, ATPase component	CcmA	PF00005	35_9	832	6649_3
ABC-type branched-chain amino acid transport system, ATPase component	LivG	PF00005	38_4	840	7280_4
ABC-type branched-chain amino acid transport system, ATPase component	LivF	PF00005	4_24	841	7336_4
ABC-type branched-chain amino acid transport system, ATPase component	LivF	PF00005	42_8	85	7812_2
ABC-type branched-chain amino acid transport system, ATPase component	LivG	PF00005	60_9	86	7812_3
ABC-type branched-chain amino acid transport system, ATPase component	LivF	PF00005	61_3	866	9197_1
ABC-type branched-chain amino acid transport system, ATPase component	LivG	PF00005	7_23	867	9197_2
ABC-type sugar transport system, ATPase component	MalK	PF00005	7_24	918	9535_2
ABC-type phosphate transport system, ATPase	PstB	PF00005	75_8	962	9535_3

component					
ABC-type dipeptide/oligopeptide/nickel transport system, ATPase component	DppD	PF00005	80_4	976	19041_2
ABC-type oligopeptide transport system, ATPase component	AppF	PF00005	80_5	977	2362_4
ABC-type uncharacterized transport system, ATPase component	-	PF00005	40_9	1079	2725_1
Energy-coupling factor transporter ATP-binding protein EcfA2	EcfA2	PF00005	47_5	1442	4519_1
ABC-type uncharacterized transport system, ATPase component	-	PF00005	65_10	320	6096_1
Energy-coupling factor transporter ATP-binding protein EcfA2	EcfA2	PF00005	9_8	348	7238_3
Energy-coupling factor transporter ATP-binding protein EcfA2	EcfA2	PF00005	-	411	-
Energy-coupling factor transporter ATP-binding protein EcfA2	EcfA2	PF00005	-	698	-
copper--translocating P-type ATPase/heavy metal-(Cd/Co/Hg/Pb/Zn)	ZntA	PF00403	96_5	-	-
Amino acid transporter	PotE	PF00324	-	523	2916_3
ABC-type transport system, periplasmic component	DdpA	PF00496	26_1	1141	16523_1
ABC-type transport system, periplasmic component	DdpA	PF00496	-	531	2612_5
ABC-type transport system, periplasmic component	DdpA	PF00496	-	973	-
ABC-type amino acid transport/signal transduction system	HisJ	PF00497	-	305	2142_5
ABC-type dipeptide/oligopeptide/nickel transport system, permease component	DppB	PF00528	16_10	1108	16523_2
ABC-type dipeptide/oligopeptide/nickel transport system, permease component	DppC	PF00528	17_15	1109	16996_3
ABC-type	DppB	PF00528	17_17	1142	2142_2

dipeptide/oligopeptide/nickel transport system, permease component					
ABC-type amino acid transport system, permease component	HisM	PF00528	204_4	302	2142_4
ABC-type amino acid transport system, permease component	HisM	PF00528	35_10	304	24788_1
ABC-type dipeptide/oligopeptide/nickel transport system, permease component	DppB	PF00528	35_11	530	2612_6
ABC-type dipeptide/oligopeptide/nickel transport system, permease component	DppB	PF00528	-	974	-
ABC-type anion transport system, duplicated permease component	-	PF00528	-	781	-
ABC-type multidrug transport system, ATPase and permease component	MdlB	PF00664	-		26967_1
Cation transport ATPase	MgtA	PF00690	-	402	
Permease of the drug/metabolite transporter (DMT) superfamily	RhaT	PF00892	203_2	586	25176_2
Permease of the drug/metabolite transporter (DMT) superfamily	RhaT	PF00892	65_2	110	4073_7
Permease of the drug/metabolite transporter (DMT) superfamily	RhaT	PF00892	-	509	14121_4
Permease of the drug/metabolite transporter (DMT) superfamily	RhaT	PF00892	-	-	17340_1
Permease of the drug/metabolite transporter (DMT) superfamily	RhaT	PF00892	-	-	20484_1
ABC-type Mn ²⁺ /Zn ²⁺ transport system, permease component	ZnuB	PF00950	2_28	185	3558_7
Kef-type K ⁺ transport system, membrane component	KefB	PF00999	20_5	546	6925_1
Kef-type K ⁺ transport system, membrane component	KefB	PF00999	20_6	-	-

ABC-type multidrug transport system, permease component	-	PF01061	12_20	1189	15269_1
ABC-type multidrug transport system, permease component	-	PF01061	16_11	424	26249_2
ABC-type multidrug transport system, permease component	-	PF01061	16_12	564	-
ABC-type multidrug transport system, permease component	-	PF01061	25_13	-	-
ABC-type metal ion transport system, periplasmic component/surface adhesin	LraI	PF01297	2_30	187	3558_9
ABC-type transport system, periplasmic component/surface lipoprotein	Med	PF01456	83_5	321	27098_1
Mg ²⁺ and Co ²⁺ transporter	CorA	PF01544	-	771	
Predicted Co/Zn/Cd cation transporter	MMT1	PF01545	161_4	557	12748_1
Predicted Co/Zn/Cd cation transporter	MMT1	PF01545	91_1	-	19576_2
Permease, similar to cation transporter	MgtE2	PF01769	21_4	925	1742_9
Permease, similar to cation transporter	MgtE2	PF01769	-	-	4067_5
ABC-type Co ²⁺ transport system, permease component	CbiM	PF01891	4_21	1159	4356_1
Multiple antibiotic transporter	MarC	PF01914	-	583	-
Predicted Fe ²⁺ /Mn ²⁺ transporter, VIT1/CCC1 family	CCC1	PF01988	-	1675	2240_5
Fe ²⁺ transport system protein B	FeoB	PF07670	19_12	1617	17285_1
Predicted divalent heavy-metal cations transporter	-	PF02535	27_2	223	1478_4
ABC-type transport system, periplasmic component/surface lipoprotein	Med	PF02608	9_11	1076	1123_13
Uncharacterized ABC-type transport system, permease component	-	PF02653	0_49	1077	1846_5
ABC-type uncharacterized transport system, permease	-	PF02653	0_50	1078	1846_6

component					
Branched-chain amino acid ABC-type transport system, permease component	LivH	PF02653	18_1	1306	19041_1
ABC-type branched-chain amino acid transport system, permease component	LivM	PF02653	18_2	1307	20703_1
Uncharacterized ABC-type transport system, permease component	-	PF02653	27_13	318	20703_2
ABC-type uncharacterized transport system, permease component	-	PF02653	27_14	319	2426_4
Branched-chain amino acid ABC-type transport system, permease component	LivH	PF02653	7_20	842	2426_5
ABC-type branched-chain amino acid transport system, permease component	LivM	PF02653	7_21	843	2725_2
ABC-type branched-chain amino acid transport system, permease component	LivM	PF02653	80_6	868	2725_3
Branched-chain amino acid ABC-type transport system, permease component	LivH	PF02653	80_7	869	6376_2
Branched-chain amino acid ABC-type transport system, permease component	LivH	PF02653	9_10	88	6376_3
ABC-type branched-chain amino acid transport system, permease component	LivM	PF02653	9_9	89	9197_3
Branched-chain amino acid ABC-type transport system, permease component	LivH	PF02653	-	-	9197_4
Branched-chain amino acid ABC-type transport system, permease component	LivH	PF02653	-	-	9362_3
ABC-type antimicrobial peptide transport system, permease component	SalY	PF02687	0_60	1296	2461_1
Predicted Fe ²⁺ /Mn ²⁺ transporter, VIT1/CCC1	CCC1	PF01988	-	194	4596_2

family					
Na ⁺ /H ⁺ antiporter NhaD or related arsenite permease	ArsB	PF03600	0_32	-	-
Na ⁺ /H ⁺ antiporter NhaD or related arsenite permease	ArsB	PF03600	20_11	-	-
Fe ²⁺ transport system protein A	FeoA	PF04023	-	1616	2345_2
ABC-type multidrug transport system, permease component	-	PF04123	172_3	1093	1132_7
ABC-type multidrug transport system, permease component	-	PF04123	-	-	2916_6
Tripartite tricarboxylate transporter (TTT) class transporter	-	PF04165	-	594	19512_2
Tripartite tricarboxylate transporter (TTT) class transporter	-	PF04165	-	-	7776_3
QueT transporter, involved in queuosine biosynthetic intermediate uptake	-	PF06177	-	1225	1576_4
Uncharacterized membrane protein, a putative transporter component	-	PF06197	-	292	1511_3
Uncharacterized membrane protein, a putative transporter component	-	PF06197	-	982	-
TRAP-type uncharacterized transport system, fused permease component	-	PF06808	-	685	-
ECF-type riboflavin transporter	-	PF07155	32_4	234	13621_2
ECF-type riboflavin transporter	-	PF07155	-	299	6319_2
ECF-type riboflavin transporter	-	PF07155	-	412	-
major facilitator superfamily MFS_1	-	PF07690	10_13	-	-
major facilitator superfamily MFS_1	-	PF07690	150_4	-	-
major facilitator superfamily MFS_1	-	PF07690	16_6	-	-
major facilitator superfamily MFS_1	-	PF07690	18_7	-	-
major facilitator superfamily MFS_1	-	PF07690	94_2	-	-

Thiamine-precursor transporter protein	ThiW	PF09512		911	3602_5
ABC-type phosphate transport system, periplasmic component	PstS	PF12849	25_4	861	8246_2
ABC-type dipeptide/oligopeptide/nickel transport system, permease component	DppC	PF12911, PF00528	204_3	1143	16996_2
ABC-type dipeptide/oligopeptide/nickel transport system, permease component	DppC	PF12911, PF00528	-	529	7812_1
ABC-type dipeptide/oligopeptide/nickel transport system, permease component	DppC	PF12911, PF00528	-	975	-
ABC-type nitrate/sulfonate/bicarbonate transport system, periplasmic component	TauA	PF12974	-	503	6514_1
TRAP-type uncharacterized transport system, periplasmic component	Imp	PF12974	-	684	-
ABC-type Co ²⁺ transport system, permease component	CbiM	PF13190	4_22	1158	4356_2
ABC-type Mn/Zn transport system, ATPase component	ZnuC	PF13304	-	724	-
ABC-type branched-chain amino acid transport system, periplasmic component	LivK	PF13458	0_51	1305	1846_4
ABC-type branched-chain amino acid transport system, periplasmic component	LivK	PF13458	27_12	839	2426_6
ABC-type branched-chain amino acid transport system, periplasmic component	LivK	PF13458	7_22	87	3560_6
ABC-type branched-chain amino acid transport system, periplasmic component	LivK	PF13458	80_8	870	9362_2
Amino acid transporter	PotE	PF13520	18_4	1098	2362_1
Amino acid transporter	PotE	PF13520	46_2	336	2927_1
Amino acid transporter	PotE	PF13520	69_3	343	4212_4
Amino acid transporter	PotE	PF13520	86_1	436	4813_1
Amino acid transporter	PotE	PF13520	86_2	695	-

Amino acid transporter	PotE	PF13520	86_3	845	-
ABC-type molybdate transport system, periplasmic component	ModA	PF13531	208_2	920	12421_3
ABC-type sulfate transport system, permease component	CysU	arCOG001 64	208_3	919	1742_5
ABC-type phosphate transport system, permease component	PstC	arCOG001 67	38_2	964	1514_6
ABC-type phosphate transport system, permease component	PstA	arCOG001 68	38_3	963	1514_7
ABC-type nitrate/sulfonate/bicarbonate transport system, permease component	TauC	arCOG001 69	-	504	-
ABC-type molybdate transport system, periplasmic component	ModA	arCOG002 19	-	-	1742_6
ABC-type polar amino acid transport system, ATPase component	GlnQ	arCOG009 23	17_16	303	2142_3
ABC-type multidrug transport system, permease component	-	arCOG014 65		672	4837_2
Energy-coupling factor transporter transmembrane protein	EcfT	arCOG022 50	40_10	1444	13621_3
Energy-coupling factor transporter transmembrane protein	EcfT	arCOG022 50	65_8	298	7238_2
Energy-coupling factor transporter transmembrane protein	EcfT	arCOG022 50	-	349	7336_3
Energy-coupling factor transporter transmembrane protein	EcfT	arCOG022 50	-	410	-
Mn ²⁺ and Fe ²⁺ transporter of the NRAMP family	MntH	arCOG041 18	-	959	5150_6
Mn ²⁺ and Fe ²⁺ transporter of the NRAMP family	MntH	arCOG041 18	-	-	6462_3
ABC-2 family transporter protein	-	arCOG081 48	-	727	-

*Annotations from NCBI, Accession number: *Ca. K. cryptofilum* (CP000968)

Appendix B: Sulfide sample details from the Eastern Lau Spreading Center

Figure 19. Photographs of samples used for the metagenomic study. Chimney sample ABE-1 on the seafloor (A) and onboard (B). Flange sample ABE-2 on the seafloor (C) and onboard (D). Flange sample Mariner-1 and Mariner-2 on the seafloor, with a pulled back view (E) and up close (G). Mariner-1 and Mariner-2 (F, H) are from the same rock. Mariner-1 (F) was subsampled from the white biofilm and Mariner-2 (H) was subsampled from the orange biofilm. Flange sample Vai Lili-2 on the seafloor (I) and onboard (J). Chimney sample Mariner-10 on the seafloor (K) and onboard (L). Chimney sample Mariner-17 on the seafloor (M) and onboard (N). Chimney sample Tui Malila-2 on the seafloor (O) and onboard (P). Chimney sample Tui Malila-10 on the seafloor (Q) and onboard (R). Flange sample Tui Malila-11 on the seafloor (S) and onboard (T).

Table 12. Collection summary of sulfides used for the metagenomes.

Sample	ID	Sub-Type	Date Collected	Latitude (S)	Longitude (W)
J2-815-16-R1	ABE-3	Flange	4/27/2015	20 45.954715	176 11.594939
J2-815-1-R1	ABE-1	Chimney	4/27/2015	20 45.661689	176 11.458083
J2-816-3-R1	Mariner-1 Mariner-2	Flange	4/29/2015	20 10.810572	176 36.069006
J2-817-10-R2	Vai Lili-2	Flange	4/30/2015	22 12.942024	176 36.516612
J2-818-10-R1	Mariner-10	Chimney	5/8/2015	22 10.820502	176 36.100164
J2-818-8-R1	Mariner-17	Chimney	5/8/2015	22 10.806366	176 36.090282
J2-819-10-R1	Tui Malila-2	Chimney	5/10/2015	21 59.433203	176 34.152799
J2-819-6-R1	Tui Malila-10	Chimney	5/9/2015	21 59.265642	176 34.079022
J2-819-7-R2	Tui Malila-11	Flange	5/9/2015	21 59.363164	176 34.093517