Diversity of methanogenic archaea in the natural subsurface sediments revealed by culture-independent genomic approaches

by

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Dedicated to Yanagawa Sensei who brought me from the deep hole of depression and truly understood how hard I have tried to be a better researcher

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ABSTRACT

Methane (CH_4) is considered a potent greenhouse gas, playing a major role in controlling atmospheric climate. Biochemical pathways of the formation and consumption of methane involve diverse groups of bacteria and archaea, which are responsible for the decomposition of organic substances under certain anoxic and favorable thermodynamic conditions. Methanogenic archaea are known to produce a large amount of biogenic methane, accounting for more than half of total atmospheric methane emissions. Although methanogenic archaea are taxonomically diverse, they have been thought to be members of the archaea limited to the phylum Euryarchaeota. However, recent advances in environmental omics revealed the phylogenetic diversity of novel microbial lineages, including putative methanogenic archaea beside the Euryarchaeota, which have not been previously placed in the traditional tree of life. This study aimed to verify the key players in methane production, either well-known archaeal members or yet-identified lineages, in estuarine sediments of the Onga River, northern Kyushu, and in peat soils collected from the Bogatsuru wetland. Estuary sites and wetlands are considered the largest source of atmospheric methane released from the entire ocean and terrestrial ecosystem, respectively. Furthermore, they were recognized to be ecological niches for diverse microorganisms, particularly, methane producers. Based on an analysis of the microbial communities using 16S rRNA gene sequencing, and molecular cloning of the functional gene, *mcrA*, a marker gene for methanogenesis, members of Methanosarcinales were only detected in estuarine sediment samples based on the 16S rRNA gene analysis, while the taxonomic classification of the functional *mcrA* genes showed the existence of diverse archaeal lineages, particularly hydrogenotrophic Methanomicrobiales. Furthermore, yet-uncultivated putative methanogenic archaea such as Verstraetearchaeota, and Methanofastidiosales were also detected, indicating the importance of hydrogen-dependent methane generation from methanol, methylated amines, and dimethyl sulfide. These results suggested that methane production in estuary might be conducted by more phylogenetically widespread archaea than previously assumed. For peat soils, methanogenic archaea belonging to the order Methanomicrobiales, Methanosarcinales,

Methanobacteriales, and Methanomassiliicoccales were detected in anoxic peat soils, suggesting the potential for methane production in the Bogatsuru wetland. Interestingly, the "Candidatus Bathyarchaeota", archaea with vast metabolic capabilities and widespread in anoxic environments, was highly observed in subsurface peat soils (up to 96% of the archaeal community) based on microbial gene quantification by the quantitative real-time PCR. Furthermore, this study discovered partial fragments of the *mcrA* gene, belonging to the Bathyarchaeota, based on the functional gene search from an unassembled metagenome of the peat soil, as well as the closely related group that was significantly different from the previously identified *mcrA*-containing methanogenic archaea. Methane-metabolizing archaea that possess *mcrA* have been thought to be restricted to only the phylum Euryarchaeota. Our metagenome-assembled genomes (MAGs) analysis provides gene-based evidence of the diversity of divergent *mcrA*-containing archaea outside of the Euryarchaeota, which was reported only once in 2015 from genome reconstruction of formation water in coal-bed methane reservoirs. Also, these results firstly demonstrated that Bathyarchaeota that possess the *mcrA* gene and its potential in methane production could be observed in a natural wetland ecosystem. These findings emphasize the importance of discovering archaea members outside of the Euryarchaeota lineage that may have significant functions in the methane and biogeochemical cycle in natural wetlands. The discovery of novel Mcr and Mcr-like genes provides a great discussion and interpretation of the origin and evolutionary history of the *mcrA* and Mcr complex. Further studies should focus on the entire recovery of microbial genomes from methanogenic sources and the effort on cultivation under laboratory conditions, since no representative of Bathyarchaeota and Verstraetearchaeota, has been successfully cultivated in a single cell isolate, neither from terrestrial nor marine sediments. The physiology of these putative methanogenic archaea is largely unknown and still unclear which members of them could be involved in methane cycling.

CHAPTER I

GENERAL INTRODUCTION AND OBJECTIVES

Methane (CH_4) is the second most important greenhouse gas next to carbon dioxide (CO_2). It is recognized as a potent contributor to global warming by controlling the temperature of the Earth's troposphere. An increase in atmospheric methane has been observed since 1983 (Dlugokencky *et al.*, 1994) until the present time (Figure 1.1). Its strong increase in concentration has raised a major challenge to meet the international treaty goals. For instance, the Paris Agreement aims to limit the rise in average global temperature below 2°C above pre-industrial levels. At COP26 (2020), over 100 parties have committed to reducing methane emissions by 30% from 2020 levels by 2030. Sources of atmospheric methane emissions have been categorized into two major types, biogenic and thermogenic methane, based on methane carbon isotopic characteristics (Schwietzke *et al.*, 2016). The methane carbon isotopic signature indicates that methane produced from biological sources, which generate from microbial processes, contributes a larger number of atmospheric methane than that of thermogenic sources (Figure 1.2). Biogenic methane can be emitted from various both land-based and aquatic sources. The average global methane emission is estimated to be 592 Tg yr^{-1} and approximately one-third of the emissions are derived from natural wetlands. These ecosystems are known as the largest natural source of the atmospheric methane budget (Jackson *et al.*, 2020). In addition to natural wetlands, estuarine environments have been certified as biological sources that can emit methane into the atmosphere (Park *et al.*, 1969; Reeburg *et al.*, 1969). Although estuaries cover only 0.4% of the total ocean area, they are thought to be a major source of methane emissions, accounting for about 75% of the methane released from the entire ocean (Bange *et al.*, 1994). Collectively, these natural habitats likely represent the significant contributors to global atmospheric methane that strongly influence carbon sequestration and potent greenhouse gas dynamics.

Biogenic methane is the terminal product of methanogenesis, the ancient anaerobic biochemical pathway that plays a crucial role in the methane and carbon cycle so far. Methanogenesis involves the conversion of organic polymers into simple methanogenic substrates, which are further used for producing methane by a specific group of microorganisms called “methanogenic archaea” or “methanogen”. Despite archaea comprising as many as 29 phyla, methanogenesis is exclusively found in methanogenic archaea belonging to the archaea phylum Euryarchaeota. Most methanogenic archaea reported in the previous research is derived from the recovery of the small-subunit ribosomal RNA (rRNA) sequences data from environmental samples and the identification of ribosomal RNA sequences has relied on the amplification of microbial DNA. To amplify target environmental DNA, the synthesized small strands of DNA (primer) that are designed to match all known target sequences are basically used based on polymerase chain reactions (PCR). Therefore, only the microbial DNA matching the sequence of primers is amplified, leading to the limitation of primer coverage if the target gene sequences are strongly divergent from the primers. Recently, the revolution of cultivation-independent genomic approaches, such as metagenomics and single-cell genomics, have enabled genomic information from microbes without biases from primer selection and coverage. Because cultivation-independent genomic studies can capture all microbial DNA from complex environmental samples, including many previously unidentified archaeal genomes and several putative methanogenic archaea beside the Euryarchaeota. For instance, the candidate phyla ‘Bathyarchaeota’ and ‘Verstraetearchaeota’, recovering from environmental metagenomic reconstruction, have been found to encode genes necessary for the methanogenesis pathway. This finding indicates the more widespread methanogenesis than previously assumed and suggests the existence of unidentified methanogenic archaea in nature.

In most studies, the production of methane through microbial methanogenesis is determined by targeting functional genes corresponding to the presence of methanogenic communities. Cultivation-independent techniques, such as next-generation sequencing (16S rRNA gene-based) and phylogenetic construction, have been widely used to directly investigate microbial diversity and reveal distinct bacterial and archaeal lineages with high efficiency in various environmental samples. In addition, the functional *mcrA* gene, encoding the alpha subunit of methyl-coenzyme M reductase (Mcr), is frequently used as a genetic marker to detect Mcr-containing methanogenic archaea owing to the consistency with 16S rRNA gene tree topologies. Moreover, genome-resolved metagenomics is a powerful tool that can be used to identify entire microbial communities (at species or strain levels) and characterize their metabolic capabilities which usually be limited by primer-based amplification.

As more than a half of emitted greenhouse gas methane is exclusively produced from methanogenic archaea through methanogenesis pathways and many unidentified archaea, including putative methanogens, are likely present in nature, this research attempts to identify methanogenic archaea communities in anaerobic environments, either well-known members or unrecognized lineage groups. The study of methanogenic communities here will provide our understanding of important contributors to atmospheric methane from biogenic sources which might be used to aid climate change prediction and the potential consequence of global warming.

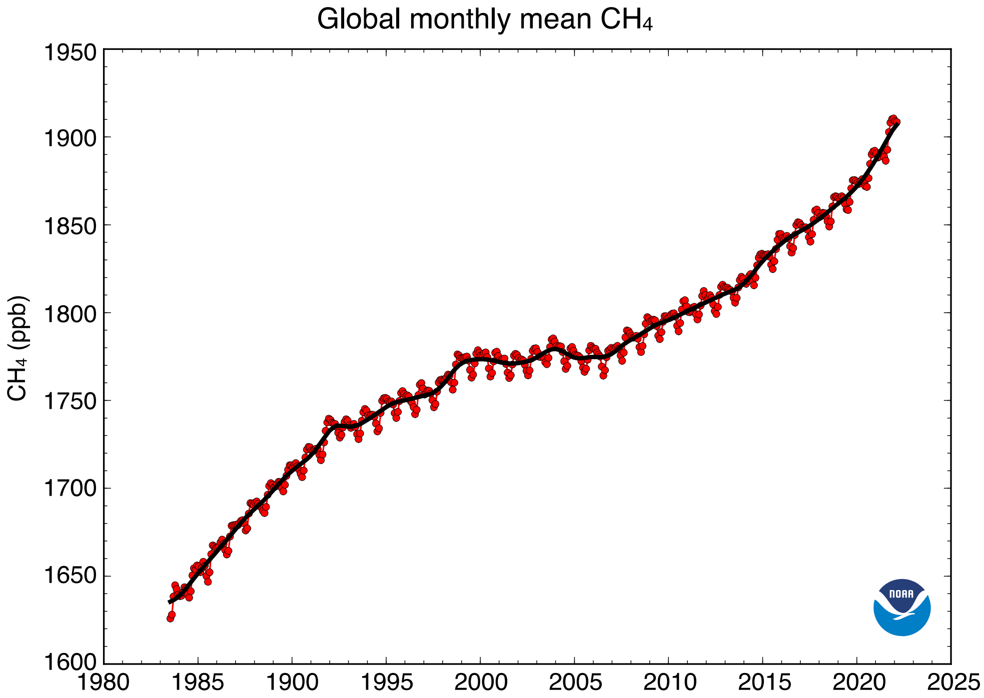

Figure 1.1 Graphical long-term trend in atmospheric methane measured since 1983 (Dlugokencky, NOAA/GML [gml.noaa.gov/ccgg/trends_ch4/]). The red line and circle are globally averaged monthly mean values. The black line indicates the trend.

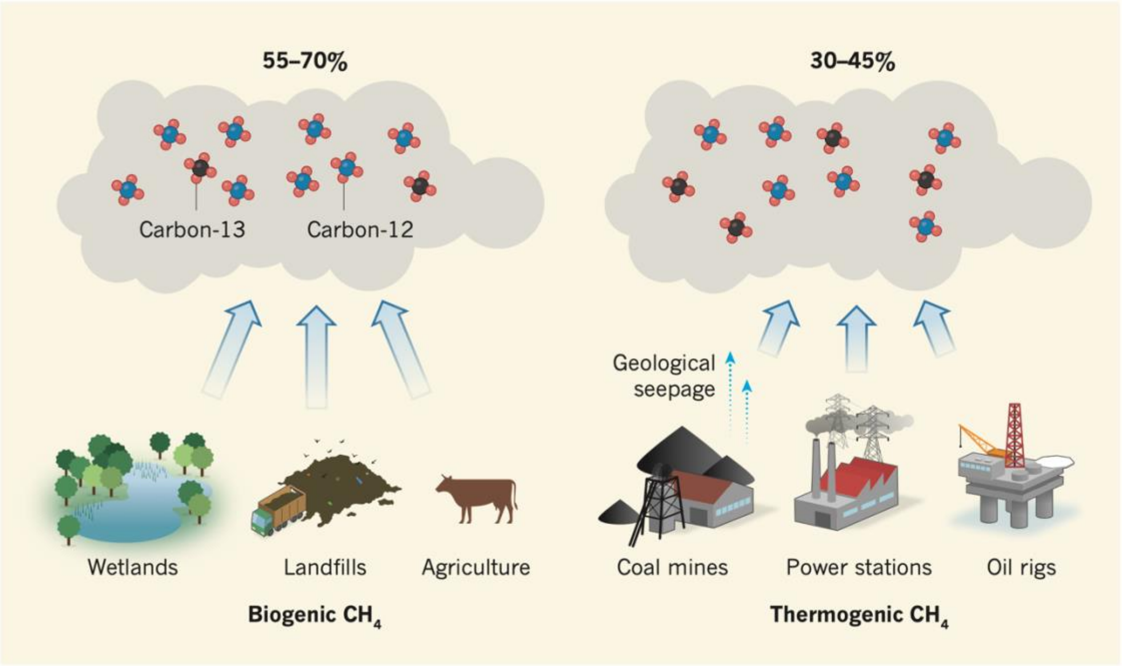

Figure 1.2 Contributors to atmospheric methane (Biogenic and Thermogenic) based on carbon isotopic signatures. The percentages shown were calculated and are rounded to the nearest 1%. The ratio of carbon-12 and carbon-13 depicted in the clouds is illustrative, and does not precisely reflect experimental data.

To clarify the research directions, the outline of the thesis and specific objectives are described below:

Chapter 1

The overview of greenhouse gas rising, and global warming issues are strongly stated. The important natural sources of atmospheric methane emissions include terrestrial wetlands and marine sediments are statistically shown. The majority of produced methane from methanogenic archaea through methanogenesis pathways is shown to not be limited to only the archaea phylum Euryarchaeota, as revealed by microbial genomic reconstruction from environmental samples. Cultivation-independent approaches have enabled several novel putative methanogens, promising the possibility of discovery of yet-to-be-identified methanogenic archaea in nature.

Chapter 2

Types of methanogenic biochemical reactions by methanogens and methanotrophy (consumption) are introduced. The expansion of archaeal diversity and the discovery of novel methanogens based on the innovation of metagenomics and single-cell genomic approaches are described. The biochemistry of methane metabolisms, a key enzyme for methanogenesis, and sets of other genes and enzymes necessary for catalyzing the whole reactions are explained step by step. The culture-independent techniques used in the present study are introduced with an illustrative explanation.

Chapter 3

Based on the large important natural sources of biogenic methane emissions from marine environments, the highest biodiversity and biological production, sediment samples from the estuarine environment (Onga River) were selected for studying microbial community structures targeting the methanogenic archaea to understand their phylogenetic diversity and functional roles involved in methane production.

Chapter 4

Based on the biggest important source of biogenic methane released from the terrestrial environments and unstudied habitat, which are expected to be the ecological niches for diverse microorganisms, peat soil samples from the Bogatsuru wetland were selected for investigating key methanogenic archaea in order to elucidate their phylogenetic distribution and metabolic functions on methane production.

Chapter 5

The analysis of microbial communities detected in two natural habitats based on taxonomic marker genes for bacteria and archaea classification showed a high proportion of archaea phylum Bathyarchaeota in the peat soils. Also, members of this archaeal phylum are known to contain some of the few putative methanogenic archaea lineages from outside the Euryarchaeota. This further emphasizes the use of metagenomic studies and computational models to assess the actual microbial diversity and describe metabolic functions of Bathyarchaeota (in peat soil), in particular, the potential role for methane production, which was described in this chapter.

Chapter 6

Preliminary results of methane production potential from peat biomass were discussed based on anaerobic batch incubation of peat biomass and digested sludge.

Chapter 7

The key findings from this research are summarized. The remaining challenges of research and the recommendations for further studies were mentioned.

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CHAPTER II

LITERATURE REVIEW

2.1 Bioconversion of organic polymers to methane

The metabolism of biogenic methane is generally controlled by groups of bacteria and archaea through anaerobic biochemical reactions (Figure 2.1). In methanogenic habitats, the organic polymers are initially degraded and subsequently fermented by anaerobic bacteria and fungi into simple sugars, amino acids, fatty acids, and alcohols. These products are further converted by syntrophic bacteria (when the hydrogen concentration is kept below 10 Pa by methanogenic archaea or acetogenic bacteria) to acetate, H_2 and CO_2, and methylated compounds which are sole precursors for methanogenesis. The final step of methane production is performed via either hydrogenotrophic (CO_2 and H_2), acetolactic (acetate), or methylotrophic (methyl compound) methanogenesis. Therefore, on the basis of trophic interactions, methanogens are typically categorized into three groups based on substrate colonization by methanogens (see methanogenic reaction below) (Drake *et al*., 2009; Conrad, 2020). However, it has been proposed that nearly all produced methane can be oxidized through the anaerobic oxidation of methane (AOM) pathway by anaerobic methane-oxidizing archaea (ANME) (Lyu *et al*., 2018). These ANMEs are thought to use a reverse methanogenesis pathway transferring electrons to the bacterial consortium (e.g., sulfate- and nitrate-reducing bacteria) or by reducing inorganic ions (e.g., Fe^{3+} and Mn^{4+}) (McGlynn, 2017; Timmers *et al*., 2017). In addition to the anaerobic process, some methanotrophic bacteria also consume methane by using different enzymatic pathways, catalyzing by the methane monooxygenase (MMO) under aerobic conditions (Dedysh and Knief, 2018).

Types of methanogenic reactions

1) Hydrogenotrophic $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$
2) Methylotrophic (methanol) $4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$
3) Acetoclastic $CH_3COOH \rightarrow CH_4 + CO_2$

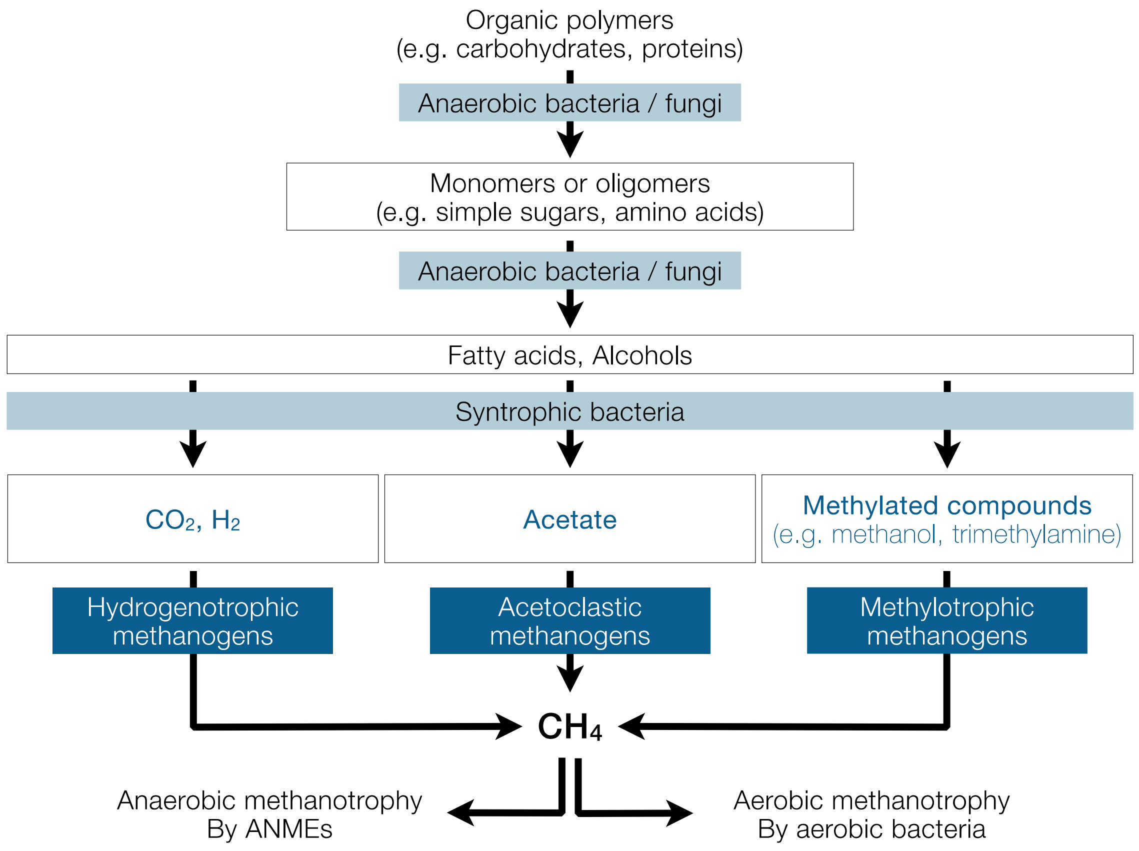

Figure 2.1 An overview of the multistep bioconversion process of complex organic polymers decomposition linked to the production and consumption of methane before releasing into the atmosphere (modified from Drake *et al*., 2009 and Evans *et al*., 2019).

2.2 Archaea: The third branch of life

In 1977, Archaea have been first discovered as a new cellular life form on Earth before being placed as a third domain besides Bacteria and Eukarya in the phylogenetic tree of life in the following decade (Woese and Fox, 1977; Woese *et al*., 1990) relied on the development of ribosomal RNA comparative and phylogenetic analyses. All members of archaea have primarily been thought to be extremophiles, living in only extreme environments (e.g., high temperature, high salt, low or high pH, and no oxygen). However, since the PCR amplification has been developed to characterize the composition of the marine archaeal community (Fuhrman *et al*., 1992; DeLong, 1992), it realized that those archaea were also able to reside in non-extreme conditions (i.e., mesophilic). The development in microbial DNA amplification and sequencing led to the acquisition of large numbers of the 16S rRNA gene sequences directly from environmental samples, which expanded our view that Archaea are distributed in diverse possible ecological niches (Pace *et al.*, 2009). The 16S rRNA gene survey (a marker gene that is generally conserved in all known organisms and ideally used for microbial taxonomic identification) has resulted in the discovery of uncultured microbial diversity (referred to as 'microbial dark matter'; Rinke *et al.*, 2013), which is often unseen and unable to cultivate in the laboratory (Rappe and Giovannoni, 2003; Pace, 1997; Baker and Dick, 2013). In the past decade, cultivation-independent genomic approaches, such as high-throughput sequencing technologies, metagenomics, and bioinformatics have enabled the reconstruction of microbial genomes directly from nature. More importantly, genome-resolved metagenomics has led to the discovery of novel taxonomic lineages, in addition to those that had been recovered through the 16S rRNA-based studies (see Figure 2.2 for the expanding of archaeal diversity).

Since archaea have been grouped into the tree of life until the beginning of the 2000s, only the Euryarchaeota and Crenarchaeota were known as archaeal phyla. In the following decade, several new archaeal phyla were proposed including Korarchaeota, Nanoarchaeota, and Thaumarchaeota (Elkins *et al.*, 2008; Huber *et al.*, 2002; Brocjier-Armanet *et al.*, 2008). The Aigarchaeota was later proposed (Nunoura *et al.*, 2011) and grouped into a superphylum, called "TACK", together with Thaumarchaeota, Crenarchaeota, and Korarchaeota (Guy *et al.*, 2011). To date, TACK archaea include additional phyla: Geoarchaeota (Kozubal *et al.*, 2013; Guy *et al.*, 2014), Bathyarchaeota (Evans *et al.*, 2015; He *et al.*, 2016; Lazar *et al.*, 2016), and Verstraetearchaeota (Vanwonterghem *et al.*, 2016). Moreover, the "DPANN" superphylum, as well as the "Asgard" superphylum, have also been proposed. DPANN refers to the Diapherotrites, Parvarhaeota, Aenigmarchaeota, Nanohaloarchaeota, and Nanoarchaeota (Castelle *et al.*, 2015; Rinke *et al.*, 2013). At the present, Asgard archaea comprise five described phyla: Lokiarchaeota, Thorarchaeota, Odinarchaeota, Heimdallarchaeota, and Helarchaeota. A list of proposed archaeal phyla (including recently assigned) is shown in Table 2.1.

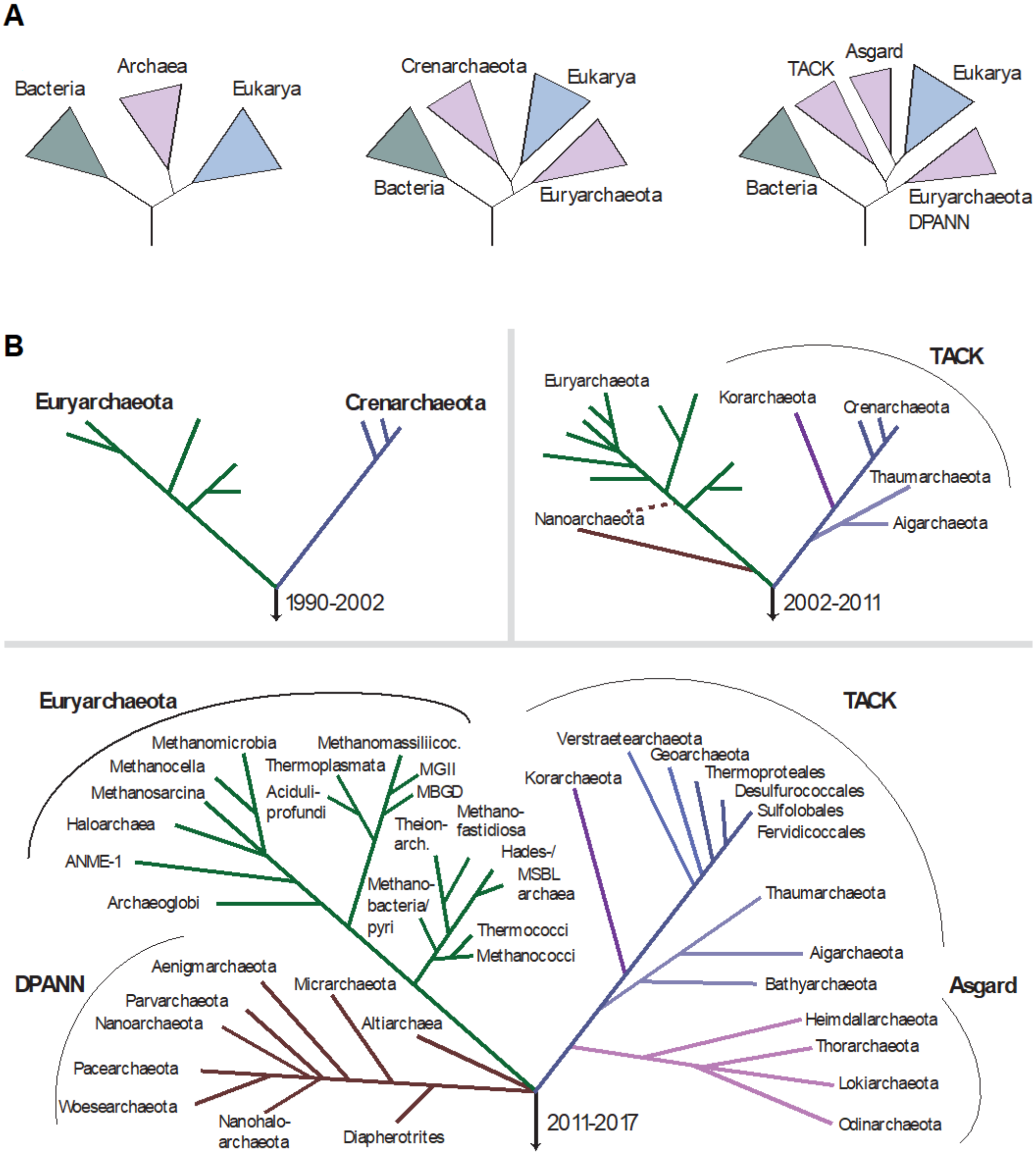

Figure 2.2 The expanding archaeal diversity proposing since the development of sequencing technologies and the innovation of metagenomics and single-cell genomic approaches (Spang *et al.*, 2017). (A) The relationship of Archaea, Bacteria, and Eukarya. (B) Timeline of archaeal tree contribution and expansion.

Table 2.1 List of currently proposed archaeal phyla (Baker *et al*., 2020)

Superphylum	**Phylum**	**Previous name**	**References**
	Euryarchaeota		Woese *et al*., 1990
	Hydrothermarchaeota	MBG-E	Jungbluth *et al*., 2017
Asgard	Lokiarchaeota	MBG-B & DSAG	Spang *et al*., 2015
	Thorarchaeota		Lazar *et al*., 2017
	Odinarchaeota		Zaremba-Niedzwiedzka *et al*., 2017
	Heimdallarchaeota	AAG & MHVG	Zaremba-Niedzwiedzka *et al*., 2017
	Helarchaeota		Seitz *et al*., 2019
DPANN	Micrarchaeota	ARMAN-1 & -2	Castelle *et al*., 2015; Baker *et al*., 2010
	Parvarchaeota	ARMAN-4 & -5	Castelle *et al*., 2015; Baker *et al*., 2010
	Pacearchaeota	DHVEG-6	Castelle *et al*., 2015
	Aenigmarchaeota	DSEG	Rinke *et al*., 2013
	Diapherotites	pMC2A384	Rinke *et al*., 2013
	Woesearchaeota		Castelle *et al*., 2015
	Altiarchaeota	SM1	Probst *et al*., 2018
	Nanoarchaeota		Probst *et al*., 2018; Huber *et al*., 2002
	Nanohaloarchaeota		Rinke *et al*., 2013; Narasingarao *et al*., 2012
	Huberarchaeota		Probst *et a*l., 2018
TACK	Korarchaeota		Barn *et al*., 1996
	Verstraetearchaeota	TMCG	Jungbluth *et al*., 2017; Vanwonterghem *et al*., 2016
	Nezhaarchaeota		Wang *et al*., 2019
	Crenarchaeota		Woese *et al*., 1990
	Aigarchaeota	HWCG-1	Martijn and Ettema, 2013
	Thaumarchaeota	MG-I, MBG-A	Brochier-Armanet *et al*., 2008
	Bathyarchaeota	MCG	Meng *et al*., 2014
	Geothermarchaeota	THSCG	Jungbluth *et al*., 2017
	Geoarchaeota	NAG1	Kozubal *et al*., 2013
	Marsarchaeota	NAG2	Jay *et al*., 2018

2.3 Archaeal methane metabolisms

Methanogenesis is the terminal step in the decomposition of biomass in anaerobic environments. This biochemical pathway is an important component of the global carbon cycles, producing a huge amount of potent greenhouse gas, methane (CH_4), even most of which is oxidized to CO_2 by anaerobic methanotrophic microbes and aerobic methanotrophic bacteria. Methanogenesis is the unique metabolism of methane-producing microbes, commonly called methanogens. It has been proposed that more than 200 genes are required for methanogenesis and its associated pathways for catalyzing and completing the reactions (Kaster *et al.*, 2011). Although CH_4 is generally produced by three major pathways: reduction of CO_2, acetate cleavage, and disproportion of methanol or methyl compounds, they all end with common reactions, the conversion of a methyl group to CH_4 and recycling of coenzymes (CoM and CoB) (Figure 2.3; Shima and Thauer, 2005). This rate-liming and final step of methanogenesis are catalyzed by the methyl-coenzyme M reductase (Mcr, EC 2.8.4.1) complex, making Mcr the key enzyme in the methanogenesis pathway (Thauer, 1998). Specifically, the Mcr alpha subunit (McrA) has been analyzed and was found to be largely consistent with the 16S rRNA gene profile, making *mcrA* a marker gene and phylogenetic tool for investigating methane-metabolizing archaea (Luton *et al.*, 2002; Friedrich *et al.*, 2005).

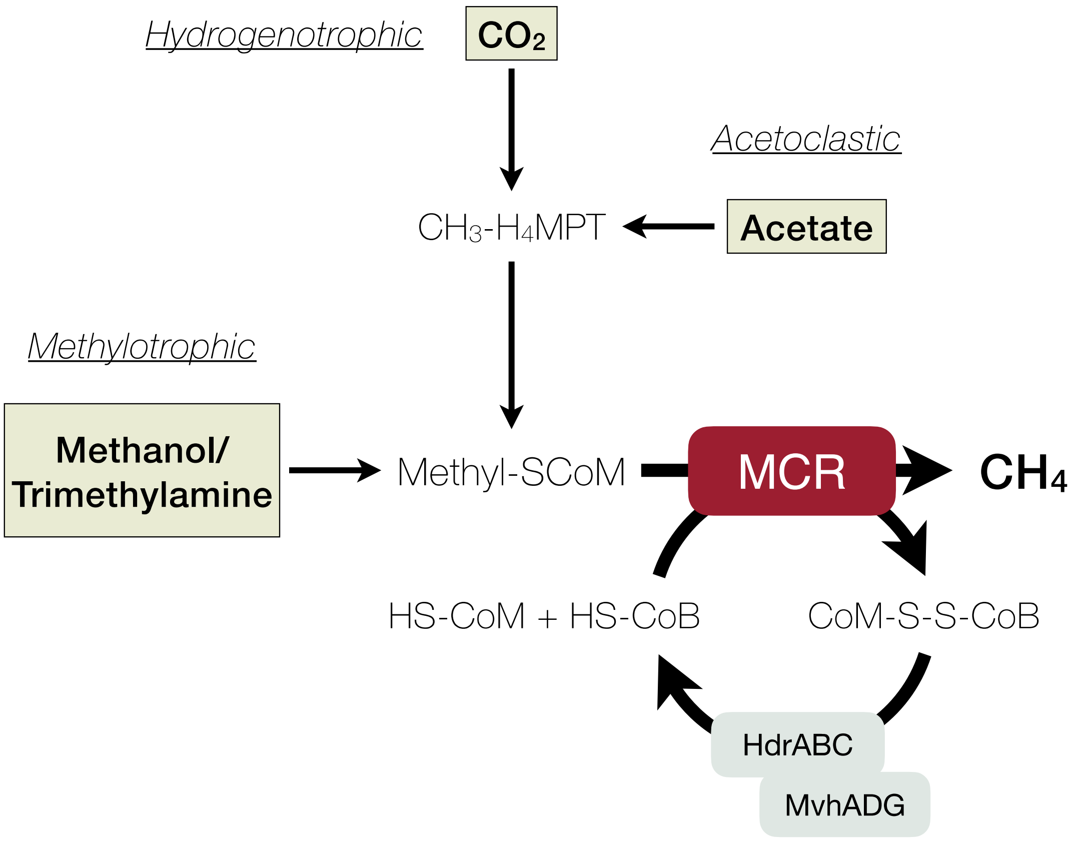

Figure 2.3 The conversion of a methyl group to CH_4 and the recycling of coenzymes (CoM and CoB) by the methyl-coenzyme M reductase (Mcr).

The reduction of CO_2 with H_2 to CH_4 in methanogenic archaea shared the first five steps (steps 1-5 in Figure 2.4) of the archaeal version of the Wood-Ljungdahl pathway (Berg *et al*., 2011). The final two steps involved energy conservation (step 6 in Figure 2.4) and electron bifurcation (step 7 in Figure 2.4) (Thauer *et al*., 2008). In the energy-conserving step, a methyl group (-CH_3) from methyltetrahydromethanopterin (H_4MPT) is transferred to coenzyme M (HS-CoM; also known as 2-thioethanesulphonate), which is involves membrane-associated protein complex, Na+-translocating methyl-H_4MPT-coenzyme M methyltransferase (MtrA-H), which resulted in the formation of methyl-CoM and generate sodium ion motive force used for driving the phosphorylation of ADP by ATPase. The electron-bifurcating step involves the reduction of methyl-CoM by Mcr to CH_4 coupled with the oxidation of CoM with coenzyme B (HS-CoB; also known as 7-thioheptanoyl-o-phospho-L-threonine) to form heterodisulfide (CoM-S-S-CoB). This heterodisulfide can be subsequently recycled to HS-CoM and HS-CoB catalyzed by the cytosolic hydrogenase-heterodisulfide reductase complex (MvhADG-HdrABC). The MvhADG-HdrABC proceeds the coupling of ferredoxin and CoM-S-S-CoB reduction with H_2 (step 8 in Figure 2.4), in which the reduced ferredoxin is further required for the reduction of CO_2 to formylmethanofuran (step 1 in Figure 2.4). The reduced ferredoxin can also be generated by the function of membrane-bound [NiFe]-hydrogenase (Eha), driven by sodium ion translocating (step 9 in Figure 2.4). The full reactions involved in CO_2 reduction to CH_4 with the standard Gibb's free energy ($\Delta G°'$) which generally be used to describe the favorability of redox reactions are shown below. The standard Gibb's free energy ($\Delta G°'$) is calculated from the standard free energies of formation at 25°C with H_2, CO_2, and CH_4 in the gaseous state at 10^5 Pa, H_2O in the liquid state, pH at 7.0, and all other compounds at 1 molar activity (Thauer *et al*., 2008).

Reaction	**$\Delta G°'$ (kJ/mol)**
$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	**-131**
$CO_2 + MFR + Fd_{red}^{2-} + 2H^+ \leftrightharpoons CHO\text{-}MFR + Fd_{ox} + H_2O$	0
$CHO\text{-}MFR + H_4MPT \leftrightharpoons CHO\text{-}H_4MPT + MFR$	-5
$CHO\text{-}H_4MPT + H^+ \leftrightharpoons CH{\equiv}H_4MPT^+ + H_2O$	-5
$CH{\equiv}H_4MPT^+ + F_{420}H_2 \leftrightharpoons CH_2{=}H_4MPT + F_{420} + H^+$	+6
$CH_2{=}H_4MPT + F_{420}H_2 \leftrightharpoons CH_3\text{-}H_4MPT + F_{420}$	-6
$CH_3\text{-}H_4MPT + HS\text{-}CoM \leftrightharpoons CH_3\text{-}S\text{-}CoM + H_4MPT$	-30
$2Na^+$ (inside) $\leftrightharpoons$ Na^+ (outside)	+30
$CH_3\text{-}S\text{-}CoM + HS\text{-}CoB \leftrightharpoons CH_4 + CoM\text{-}S\text{-}S\text{-}CoB$	-30
$2H_2 + CoM\text{-}S\text{-}S\text{-}CoB + Fd_{ox} \leftrightharpoons HS\text{-}CoM + HS\text{-}CoB + Fd_{red}^{2-} + 2H^+$	-39
$H_2 + F_{420} \leftrightharpoons F_{420}H_2$	-11
$H_2 + Fd_{ox} \leftrightharpoons Fd_{red}^{2-} + 2H^+$	+16

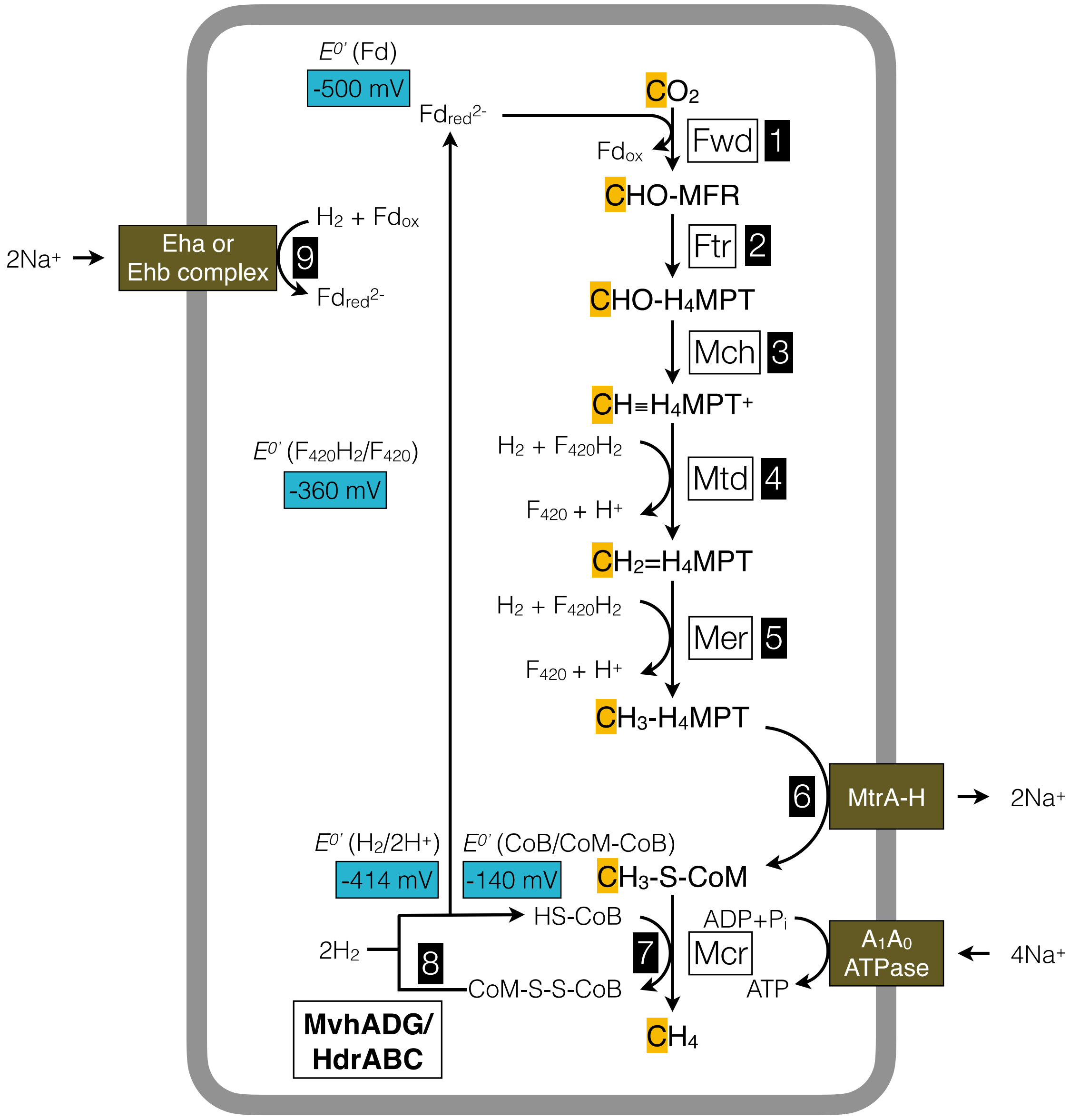

Figure 2. 4 Energy conservation in hydrogenotrophic methanogens. The reaction numbers correspond to the step numbers discussed in the main text. All standard redox potentials involved in the reactions are listed in the boxes. The C1 units are highlighted in yellow. MFR, methanofuran; Fd, ferredoxin; H_4MPT, tetrahydromethanopterin; HS-CoB, coenzyme B; HS-CoM, coenzyme M; Eha and Ehb, energy-converting hydrogenase A and hydrogenase B.

On the other hand, acetoclastic methanogens split acetate into CH_4 and CO_2 either by AMP-forming acetyl-CoA synthetase (Acs) in *Methanothrix* or acetate kinase (Ack) and phosphoacetyltransferase (Pta) in *Methanosarcina* (Figure 2.5). Acetate is converted to methyl-H_4MPT (step 1 in Figure 2.5) by a function of the carbon monoxide dehydrogenase/acetyl-CoA synthase (Codh-Acs) complex (Welte *et al*., 2014). Then, a methyl group from methyl-H_4MPT is transferred to methyl-S-CoM by a methyltransferase (Mtr), forcing the sodium ion to translocate across the cell membrane and generating CoM-S-S-CoB by Mcr complex (steps 2-3 in Figure 2.5). The generated CoM-S-S-CoB is further reduced by a cytochrome-containing membrane-bound heterodisulfide reductase (HdrDE) complex (step 4 in Figure 2.5). In contrast to cytosolic heterodisulfide reductase (MvhADG-HdrABC), HdrDE reduces CoM-S-S-CoB to HS-CoM by using electrons from the methanophenazine (MP) rather than H_2 as a reducing equivalent couple with proton translocation (Welander *et al*., 2005). For the energy conservation step, two models of acetoclastic methanogens, *Methanosarcina mazei* and *Methanosarcina acetivorans* have been reported to use different mechanisms (Lessner *et al*., 2009). In *M. mazei*, ferredoxin oxidation is coupled to the formation of H_2 by an energy-conserving hydrogenase (Ech). The Vho hydrogenase oxidizes H_2, transferring electrons to the methanophenazine and generating proton. In *M. acetivorans*, oxidation of ferredoxin is coupled to the reduction of the methanophenazine by Na^+-translocating ferredoxin oxidoreductase (MA-Rnf). The full reactions involved in the conversion of acetate to CH_4 are shown below.

Reaction	**Δ*G*°' (kJ/mol)**
$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$ (sum of all below reactions)	**-36**
$CH_3COO^- + ATP \rightarrow CH_3CO_2PO_3^{2-} + ADP$	
$CH_3CO_2PO_3^{2-} + HS\text{-}CoA \rightarrow CH_3COSCoA + Pi$	
$CH_3COSCoA + H_4MPT + H_2O \rightarrow CH_3\text{-}H_4MPT + 2e\text{-} + 2H^+ + CO_2 + HS\text{-}CoA$	
$CH_3\text{-}H_4MPT + HS\text{-}CoM \rightarrow CH_3\text{-}S\text{-}CoM + H_4MPT$	
$CO_2 + H_2O \rightarrow HCO_3^- + H^+$	
$CH_3\text{-}S\text{-}CoM + HS\text{-}CoB \rightarrow CH_4 + CoM\text{-}S\text{-}S\text{-}CoB$	
$Pi + ADP + CoM\text{-}S\text{-}S\text{-}CoB + 2e^- + 3H^+ \rightarrow HS\text{-}CoM + HS\text{-}CoB + ATP + H_2O$	

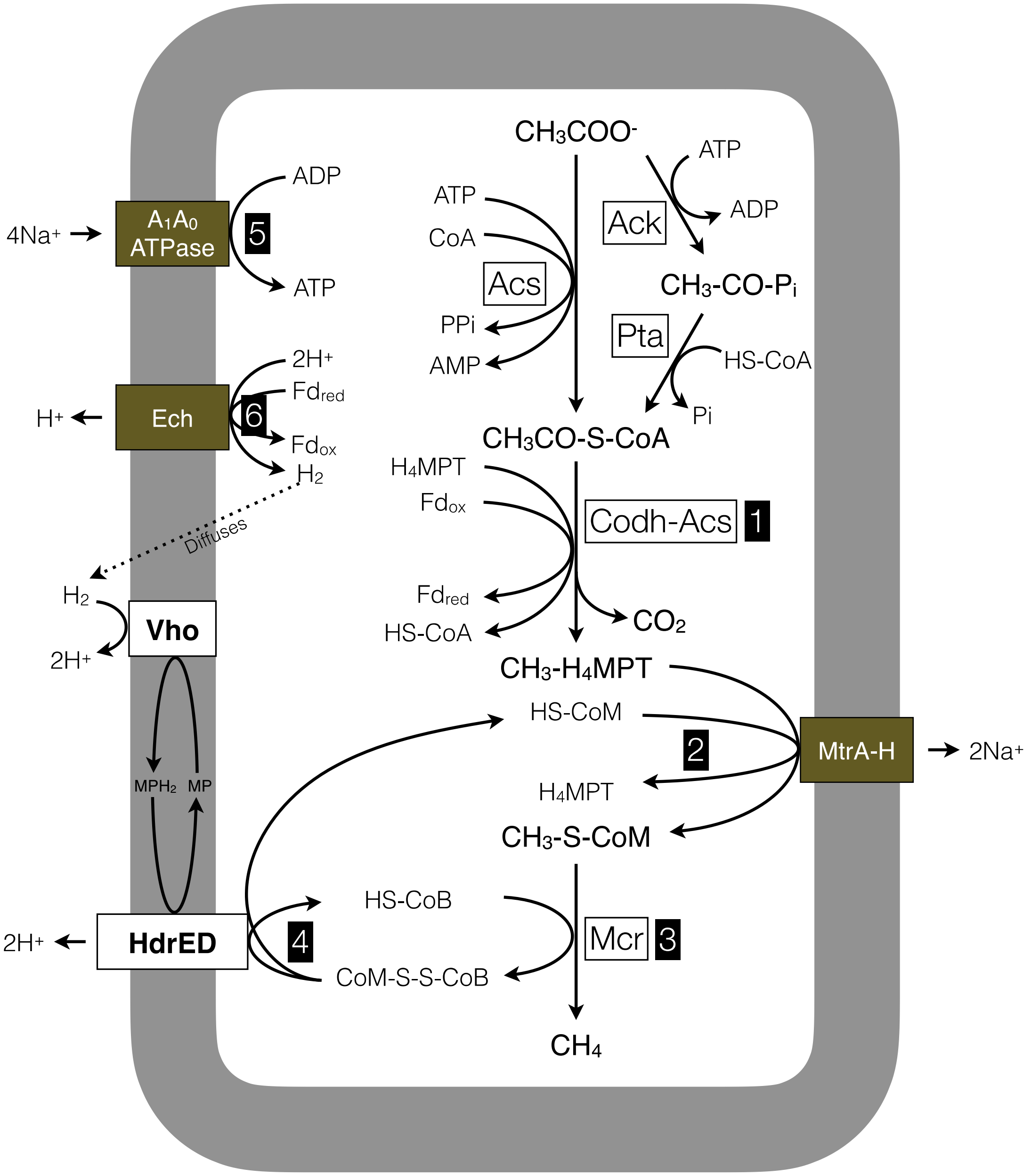

Figure 2.5 Energy conservation in acetoclastic methanogens. The conversion of acetate in *Methanosarcina* (through Ack pathway) and *Methanothrix* (through Acs pathway) leads to the CH_3-H_4MPT. CoA, coenzyme A; H_4MPT, tetrahydromethanopterin; Fd_{ox}/Fd_{red}, oxidized and reduced ferredoxin; HS-CoB, coenzyme B; HS-CoM, coenzyme M; MP/MPH_2, oxidized and reduced methanophenazine.

Last, methylotrophic methanogens are able to use methanol and methyl compounds to form CH_4. In this pathway, one methyl group of the substrate is reduced to CO_2 providing reducing equivalents for the reduction of three additional methyl groups to CH_4 (see reactions 1-2 below). The methyl group from methyl compounds is transferred to CoM by a methyltransferase (Mta) to form methyl-CoM (Figure 2.6), which is further reduced by Mcr to CH_4. In the oxidative direction, $F420_{red}$ and Fd_{red} are generated for use in the reductive direction of the pathway. Hydrogen cycling can be utilized from the oxidation of $F420_{red}$ and Fd_{red} by the Ech and Frh hydrogenase, respectively. The H_2 generated inside the cell diffuses across the cell membrane to the active site of methanophenazine-reducing hydrogenase (Vht), where it is oxidized and shuttles electrons to the methanophenazine (MP) to complete the cycle. In addition, the variation of the usual methylotrophic methanogenesis pathway is observed in the *Methanosphaera stadtmanae*. *M. stadtmanae* has been reported to use H_2 dependently for the reduction of methyl-CoM to CH_4 (Fricke *et al.*, 2006). Recently, this H_2-dependent methylotrophic has been observed in Methanomassiliicoccales, Methanonatroarchaeia, and Ca. Methanofastidiosa (Nobu *et al.*, 2016; Sorokin *et al.*, 2017; Lang *et al.*, 2015; Borrel *et al.*, 2014). These recently discovered archaea are proposed to reduce methanol, methyl amines, and methyl sulfide to CH_4 depending on the use of H_2 as an electron donor. The reactions involved in the conversion of methyl compounds to methane are shown below.

Reaction	**$\Delta G^{\circ\prime}$ (kJ/mol)**
1) $R\text{-}CH_3 + 2H_2O \rightarrow RH + CO_2 + 6e^- + 6H^+$	
2) $3R\text{-}CH_3 + 6e^- + 6H^+ \rightarrow 3CH_4 + 3RH$	
3) $4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$	**-105**
4) $4CH_3\text{-}NH_2 + 2H_2O \rightarrow 3CH4 + CO_2 + 4NH_3$	**-75**
5) $2(CH_3)_2\text{-}S + 2H_2O \rightarrow 3CH4 + CO_2 + 2H_2S$	**-49**

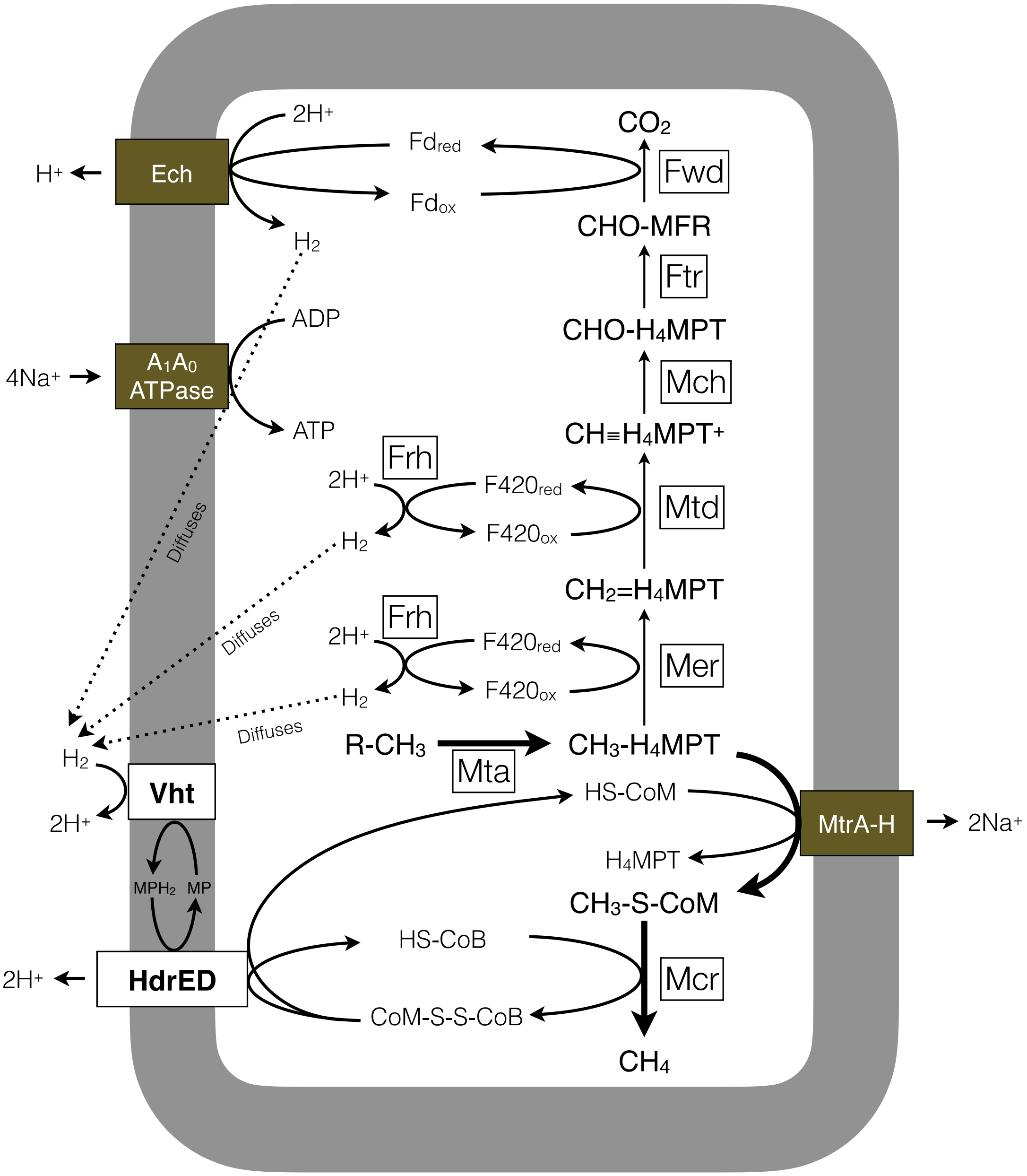

Figure 2.6 Energy conservation in methylotrophic methanogens. The R groups include -OH (methanol), $-NH_2$ (methylamine), -SH (methanethiol). Other methyl compounds include dimethylamine, trimethylamine, and dimethyl sulfide. Fd_{ox}/Fd_{red}, oxidized and reduced ferredoxin; MP/MPH_2, oxidized and reduced methanophenazine; Mta, methyltransferase; Vht, F420 non-reducing hydrogenase.

In addition to methanogenic archaea, anaerobic methanotrophic archaea (ANME) use the Mcr complex to oxidize CH_4 in the reverse direction of the methanogenesis pathway (possibly via reverse CO_2 reduction) (Figure 2.7). These anaerobic methanotrophs phylogenetically belong to euryarchaeotal methanogenic lineages, for instance, ANME-1 (also known as Ca. Methanophagales) and ANME-2 and -3, which belong to the Methanosarcinales. The electrons generated by the oxidation of CH_4 are predicted to transfer to terminal electron acceptors, such as iron and manganese (Ettwig *et al.*, 2016; Beal *et al.*, 2009), nitrate (Haroon *et al.*, 2013), and sulfate-reducing bacteria (Wegener *et al.*, 2015; McGlynn *et al.*, 2015) (Figure 2.7). However, some anaerobic methanotrophs have been suggested to reduce sulfate by a dissimilatory sulfate reduction without syntrophic bacterial partners (Milucka *et al.*, 2012). The Rnf and Fpo complexes have been proposed to possess sodium ion and proton transportation, respectively, for energy conservation in ANME (Wang *et al.*, 2014; Meyerdierks *et al.*, 2010).

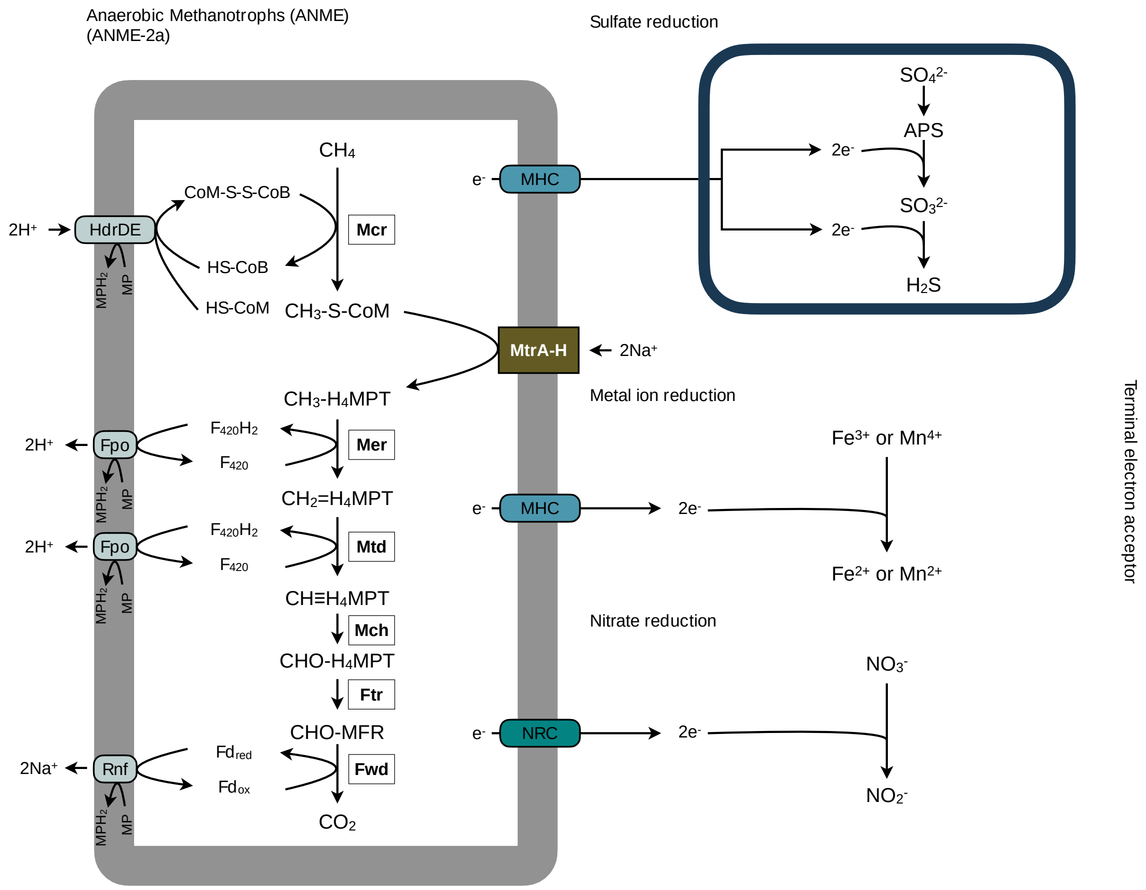

Figure 2.7 Proposed methanotrophic pathway in ANME-2d in the presence of terminal electron acceptors (sulfate-reducer, metal ions, and nitrate). MP/MPH$_2$, oxidized and reduced methanophenazine; Fpo, F$_{420}$H$_2$ dehydrogenase; Rnf, Na$^+$-translocating ferredoxin oxidoreductase; MHC, multi-haem C-type cytochrome; NRC, nitrate reductase complex.

Contrary to anaerobic respiration, methanotrophic bacteria can utilize methane as a sole energy source by a catalysis function of the methane monooxygenase (MMO) enzyme (Hanson and Hanson, 1996), which has two forms, a membrane-bound or particulate (pMMO) and a soluble form (sMMO). Methanotrophic bacteria can be found in a broad range of habitats where methane and oxygen are available (Knief, 2015). To date, members of isolated methanotrophs that possess MMO (Table 2.2) belong to the bacterial phyla Proteobacteria and Verrucomicrobia (Dedysh and Knief, 2018). Proteobacterial methanotrophs are affiliated with the classes Gammaproteobacteria (type I methanotroph) and Alphaproteobacteria (type II methanotroph) while members of the Verrucomicrobia phylum were only discovered by a limited number of isolates (Op den Camp *et al*., 2009) including genus *Methyacidiphilum* and *Methylacidimicrobium*.

Table 2.2 Aerobic methanotrophic bacteria that were described by pure cultures

Genus	**Species**	**References**
Class Gammaproteobacteria, family Methylococcaceae		
Methylococcus	*M. capsulatus*	Bowman, 2015a
	M. thermophilus	
Methylomonas	*M. methanica*	Bowman, 2016b
	M. aurantiaca	
	M.fodinarum	
	M. koyamae	
	M. scandinavica	
	M. lenta	
	M. paludism	
	M. denitrificans	
Methylobacter	*M. luteus*	Collins *et al*., 2017
	M. marinus	
	M. whittenburyi	
	M. tundripaludum	

	M. psychrophilus	
	M. modestohalophilus	
Methylomicrobium	*M. agile*	Kalyuzhnaya, 2016a
	M. album	
	M. alcaliphilum	
	M. japanense	
	M. kenyense	
	M. pelagicum	
	M. buryatense	
Methylosarcina	*M. fibrate*	Kalyuzhnaya, 2016b
	M. quisquiliarum	
	M. lacus	
Methylocaldum	*M. gracile*	Takeuchi, 2016
	M. marinum	
	M. szegediense	
	M. tepidum	
Methylogaea	*M. oryzae*	Tarlera, 2016
Methylosoma	*M. difficile*	Schink and Rahalkar, 2016
Methyloparacoccus	*M. murrellii*	Hoefman *et al*., 2014
Methyloglobulus	*M. morosus*	Schink and Deutzmann, 2016
Methyloprofundus	*M. sedimenti*	Tavormina, 2016
Methylomarinum	*M. vadi*	Hirayama, 2016a
Methylovulum	*M. miyakonense*	Iguchi *et al*., 2016
	M. psychrotolerans	Oshkin *et al*., 2016
Methylomagnum	*M. ishizawai*	Khalifa *et al*., 2015
Methylosphaera	*M. hansonii*	Bowmann, 2015b
Class Gammaproteobacteria, family Methylothermaceae		
Methylothermus	*M. thermalis*	Hirayama, 2016c

	M. subterraneus	
Methylohalobius	*M. crimeensis*	Dunfield, 2016
Methylomarinovum	*M. caldicuralii*	Hirayama, 2016d
Class Alphaproteobacteria, family Methylocystaceae		
Methylosinus	*M. sporium*	Bowmann, 2015d
	M. trichosporium	
Methylocystis	*M. parvus*	Bowman, 2015c; Belova *et al*., 2013
	M. echinoides	
	M. heyeri	
	M. hirsute	
	M. rosea	
	M. bryophila	
Class Alphaproteobacteria, family Beijerinckiaceae		
Methylocella	*M. palustris*	Dedysh and Dunfield, 2016b
	M. silvestris	
	M. tundrae	
Methylocapsa	*M. acidiphilia*	Dedysh, 2016
	M. aurea	
	M. palsarum	
Methyloferula	*M. stellata*	Dedysh and Dunfield, 2016c
Phylum Verrucomicrobia		
Methylacidiphilum	*M. infernorum*	Dunfield *et al*., 2007; Pol *et al*., 2007; Islam *et al*., 2008; Op den Camp *et al*., 2009
	M. fumarolicum	
	M. kamchatkense	
Methylacidimicrobium	*M. fagopyrum*	van Teeseling *et al*., 2014
	M. cyclopophantes	
	M. tartarophylax	

2.4 Methane and short-chain alkane metabolizing archaea

Methanogenic archaea have long been thought to affiliate with only eight orders within the phylum Euryarchaeota (Evans *et al.*, 2019) including Methanobacteriales, Methanopyrales, Methanococcales, Methanomicrobiales, Methanonatronarchaeales, Methanosarcinales, Methanocellales, and Methanomassiliicoccales. These archaeal lineages can produce CH_4 as a final product through methanogenesis pathways relied on the methyl-coenzyme M reductase (Mcr). However, some archaeal lineages of Euryarchaeota are able to oxidize CH_4 to CO_2 by reverse methanogenesis (Hallam *et al.*, 2004), dependent on the syntrophic partners, for example, sulfate and nitrate reducers (Orphan *et al.*, 2001; Haroon *et al.*, 2013). These lineages are recognized as anaerobic methane oxidizers (ANME), comprising at least three distinct clusters, called ANME-1, ANME-2, and ANME-3 (Knittel *et al.*, 2009). The development of cultivation-independent genomics approaches and computational tools have uncovered novel uncultivated archaeal lineages, including Ca. Methanofastidiosa (formerly known as WSA2/Arc1), Verstraetearchaeota (formerly known as TMCG), and Bathyarchaeota (formerly known as MCG). They were found to contain Mcr and Mcr-like genes, which could not be observed by traditional PCR-based amplification with common primers (Figure 2.8). Bathyarchaeota was the first instance of archaea outside of the Euryarchaeota that encode the Mcr complex (Evans *et al.*, 2015). Although they have been formerly proposed to be methylotrophic methanogens, recent interpretation with the divergent archaeal Mcr-containing lineages proposed that Mcr in Bathyarchaeota likely activate alkane oxidation instead of methane metabolisms (Vanwonterghem *et al.*, 2016; Laso-Perez *et al.*, 2016; Evans *et al.*, 2015; Hua *et al.*, 2019). The second non-euryarchaeotal archaea that contain the Mcr complex, now so-called Verstraetearchaeota, was also reported from the same research group as Bathyarchaeota (Vanwonterghem *et al.*, 2016). Members of Verstraetearchaeota were recovered from cellulose-degrading anaerobic digesters and found to use methylated compounds dependent on H_2. Ca. Methanofastidiosa were recovered from the wastewater treatment digester. Their genomic contents revealed that they possess methanogenesis through methylated thiol reduction with H_2 (Nobu et al., 2016). In addition, two Ca. Syntrophoarchaeum strains (Ca. S. Caldarius and Ca. S. Butanivorans) have recently been enriched from hydrothermal sediments. Their genomic constructions showed that they contain genes encoding enzymes similar to methyl-coenzyme M reductase (Laso-Perez *et al*., 2016), but rather use for activating butane toward butyl-CoM instead of methane. These two archaea are dependent on the sulfate-reducing bacterium, similar to what has been seen in ANME and sulfate-reducing syntrophs. It has also been suggested that Mcr-containing Bathyarchaeota may possess the same metabolism (Laso-Perez *et al*., 2016), which will require further experimental investigation for confirmation.

Interestingly, Bathyarchaeota, Verstraetearchaeota, and Ca. Syntrophoarchaeum, are likely to be the first representative putative methanogenic lineages outside the Euryarchaeota that may be involved in CH_4 metabolisms, the ancient biochemical pathways that are so far thought to limited in only the Euryarchaeotal clade. To date, the Mcr-containing metagenomic-assembled metagenomes (MAGs) obtained from various environments belonged to the Archaeoglobi, Hadesarchaeota, Nezhaarchaeota, and Korarchaeota (Wang *et al*., 2019). Furthermore, the genome reconstruction from hydrothermal deep-sea sediments showed the presence of Mcr-like containing archaea belonging to the Asgard superphylum, Helarchaeota (Seitz *et al*., 2019) (Figure 2.9). Phylogenomic analyses suggested that they form monophyletic closely to Mcr-containing Bathyarchaeota and are likely to oxidize short-chain alkane (via Mcr) rather than methane. Collectively, the recent studies revealed genes for the methyl-coenzyme M reductase (Mcr) expanding beyond the identified methanogenic/methanotrophic archaeal lineages and their metabolic potentials may not limit only to methane. Therefore, there is a need for further studies that will enhance our understanding of methane/alkane metabolizing archaea and their metabolic capabilities wider than predicted only by genomic information. Nonetheless, these findings expanded our view on archaeal diversity, particularly potential methanogenic groups, as well as their metabolic functions associated with carbon and nutrient cycles. The available genomic data and phylogenetic tree infer that putative methanogenic archaea are larger than our previous interpretation and still needed to be discovered either via complete genome construction or culture-based investigation. It also emphasizes the importance of the discovery of new members to obtain a clearer view of microbial ecology, metabolic interdependencies, and the evolutionary history of life.

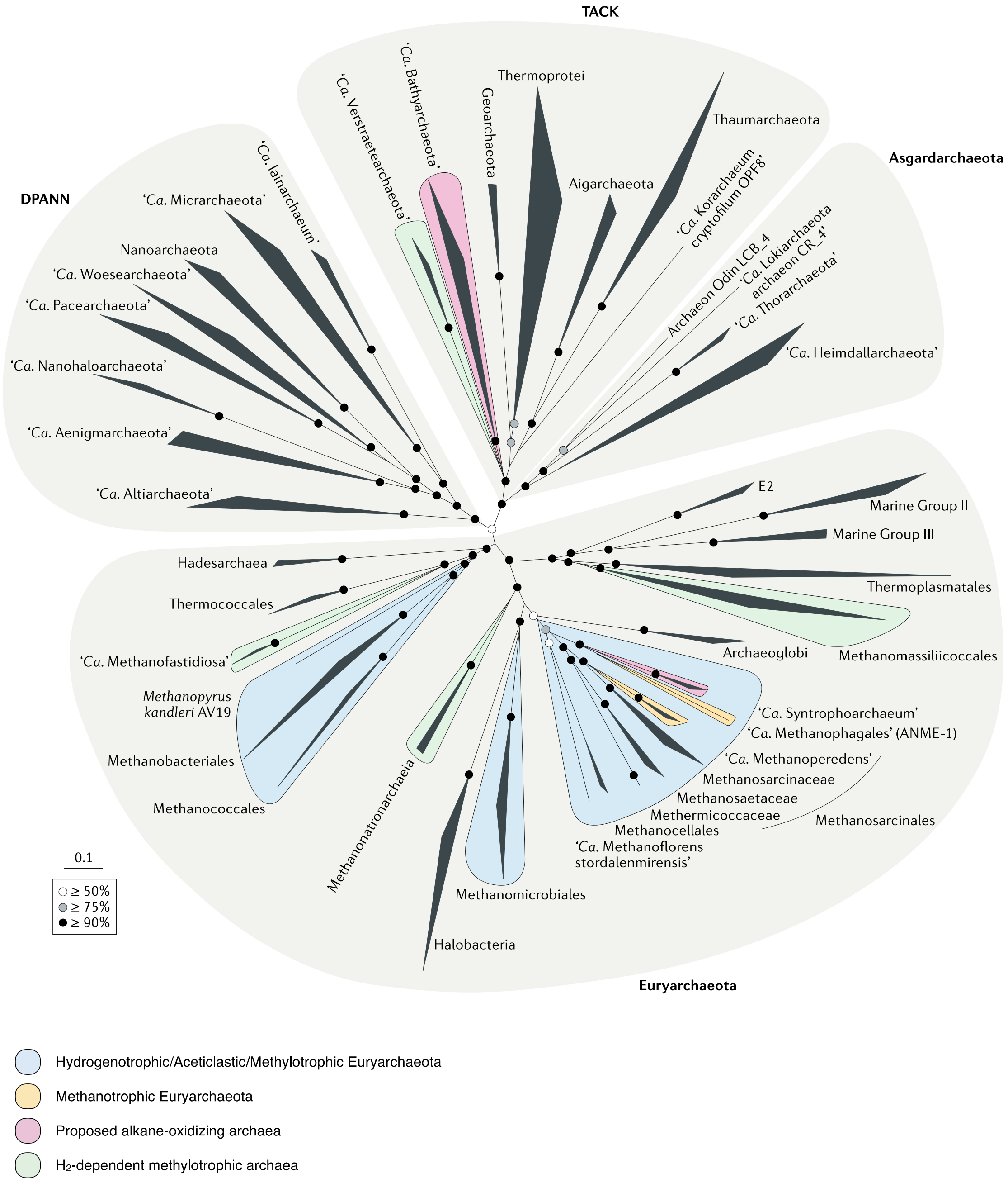

Figure 2.8 Archaeal genome tree containing Mcr and Mcr-like genes within Euryarchaeota phylum and expanding within TACK superphylum. Lineages that harbor organisms with Mcr and Mcr-like genes are highlighted in four colors indicated in the figure.

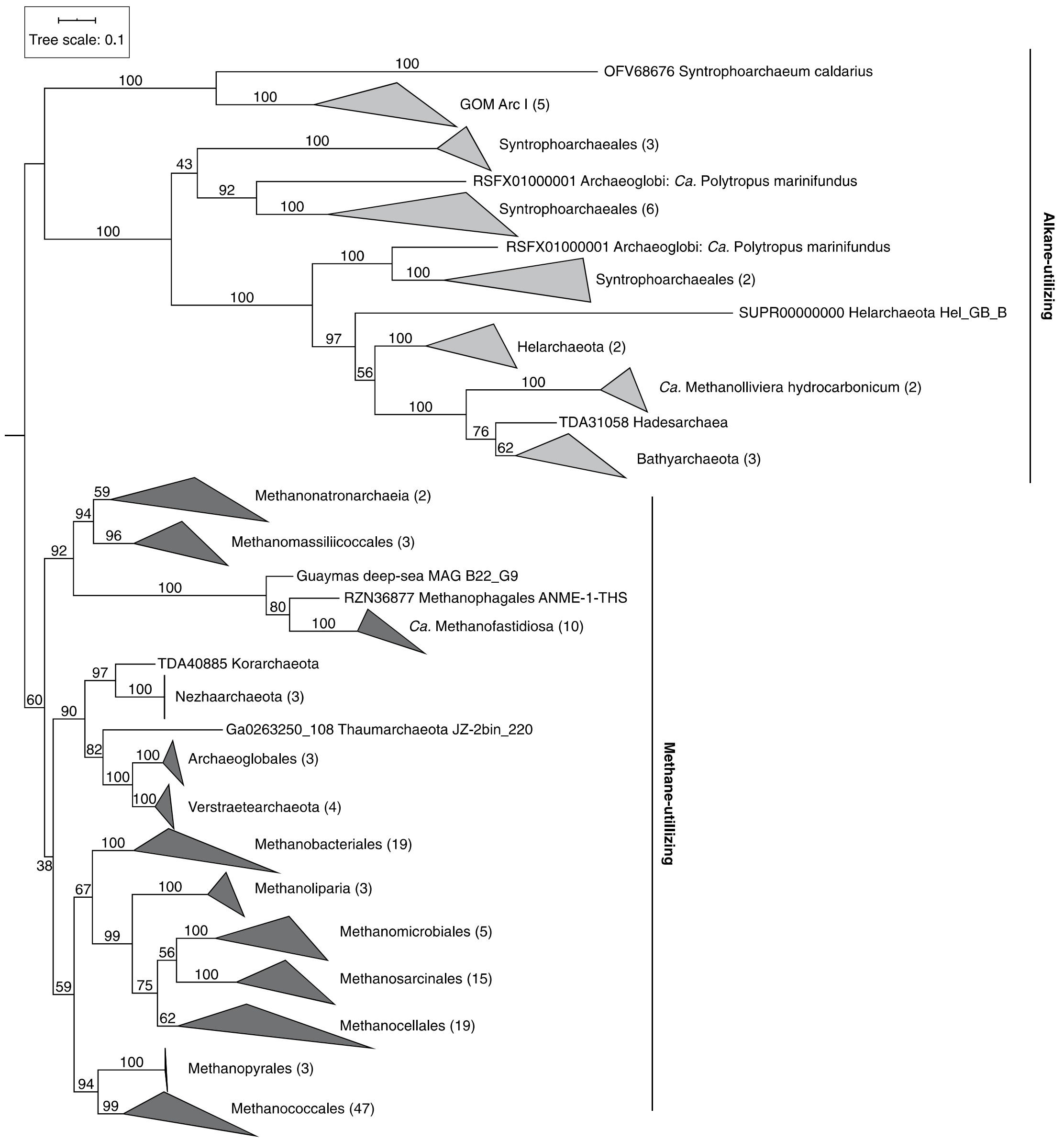

Figure 2.9 Phylogenetic tree depicts the diversity of Mcr-containing archaea and their metabolic traits (Baker *et al*., 2020), in which Bathyarchaeota, Helarchaeota, Hadesarchaea, and Syntrophoarchaea were proposed to belong to an alkane-metabolizing group.

2.5 Microbiome analysis

The development in DNA sequencing technologies has brought our accessibility to investigate the composition and complex microbial communities that inhabit various environments. So far, different methods for studying microbial communities, including marker gene targeting and metagenomic sequencing, can provide detail at low-to-high levels of microbial complexity. Marker gene amplification and sequencing (i.e., 16S rRNA gene for bacteria and archaea) are a well-known and friendly-used method for obtaining an overview of the microbial community (Figure 2.10; Tringe and Rubin, 2005). This technique uses primers that are specific to the gene of interest in a sample, for instance, the 16S rRNA gene (Figure 2.11) with a combination of conserved, variable, and hypervariable regions (Yarza *et al*., 2014) to systematic classify bacteria and archaea. Then, the taxa classification at different ranks (phylum to genus) is calculated based on threshold sequence identity (Table 2.3) against the validly published database. However, primers could not be designed to match all possible DNA sequences (Eloe-Fadrosh *et al*., 2016), causing bias during the amplification step for harboring microbial genetic contents from samples. The method of DNA extraction from specimen and PCR conditions can also be a source of bias and error. Although well-optimized primer selection can reduce the effects of amplification biases, an assessment of taxonomic resolution and coverage capacity of the target community still be required (Walters *et al*., 2011). Despite these technical constraints, marker gene sequencing (i.e., 16S rRNA gene) is a common practice widely applied with diverse sample types for microbiome study at the beginning step (Okuda *et al*., 2012; Langille *et al*., 2013).

Introducing new sequencing technologies, such as metagenomics (i.e., shotgun metagenomics; Quince *et al*., 2017) and single-cell genome, combined with computational analyses can fully recover genomic content of bacteria and archaea that are undetectable through marker gene amplification, including uncultivated diversity. These methods capture the entire genomic DNA of organisms present within a given sample, providing genomic information and taxonomic depth to species or strain level (Scholz *et al*., 2016) and sufficient starting material for other downstream applications, such as microbial gene/enzyme catalogs, metabolic functions, evolutionary aspects or even assembly of whole microbial genomes from short sequence reads (Mukherjee *et al*., 2017). However, there are some biases and limitations that are possibly caused during the preparation and analysis steps (Table 2.4; Knight *et al*., 2018). In metagenomic workflow (Figure 2.12; Lasken *et al*., 2014), all genomic DNA can be directly extracted from the environmental microbiome. The mixture of extracted DNA fragments is sequenced for generating sequencing libraries and assembled the reads into continuous longer sequences (contigs). These contigs can be further grouped (binning) into partial to whole genomes of individual microorganisms that are classified against already sequenced genomes databases.

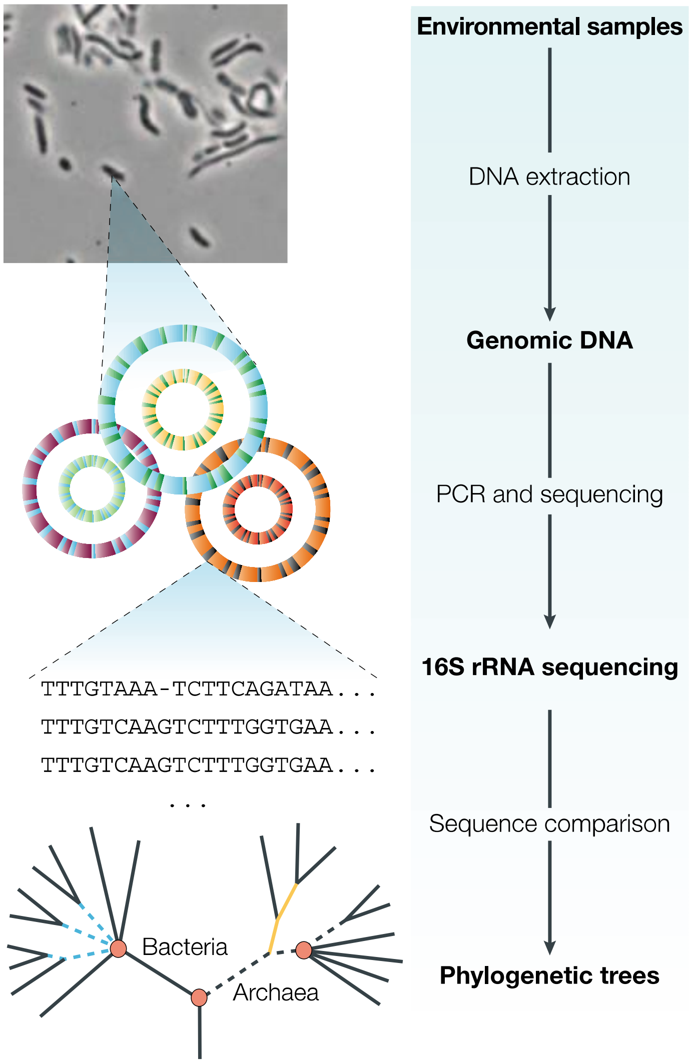

Figure 2.10 16S ribosomal RNA analysis of microbial communities. DNA is directly extracted from environmental samples and the 16S rRNA genes of microbes presented in the samples are then amplified by PCR. The PCR products are cloned and sequenced, producing rRNA signatures for the microorganisms. Phylogenetic of microbes is classified by comparing these signature sequences with the 16S rRNA gene databases, providing an overview of community structure.

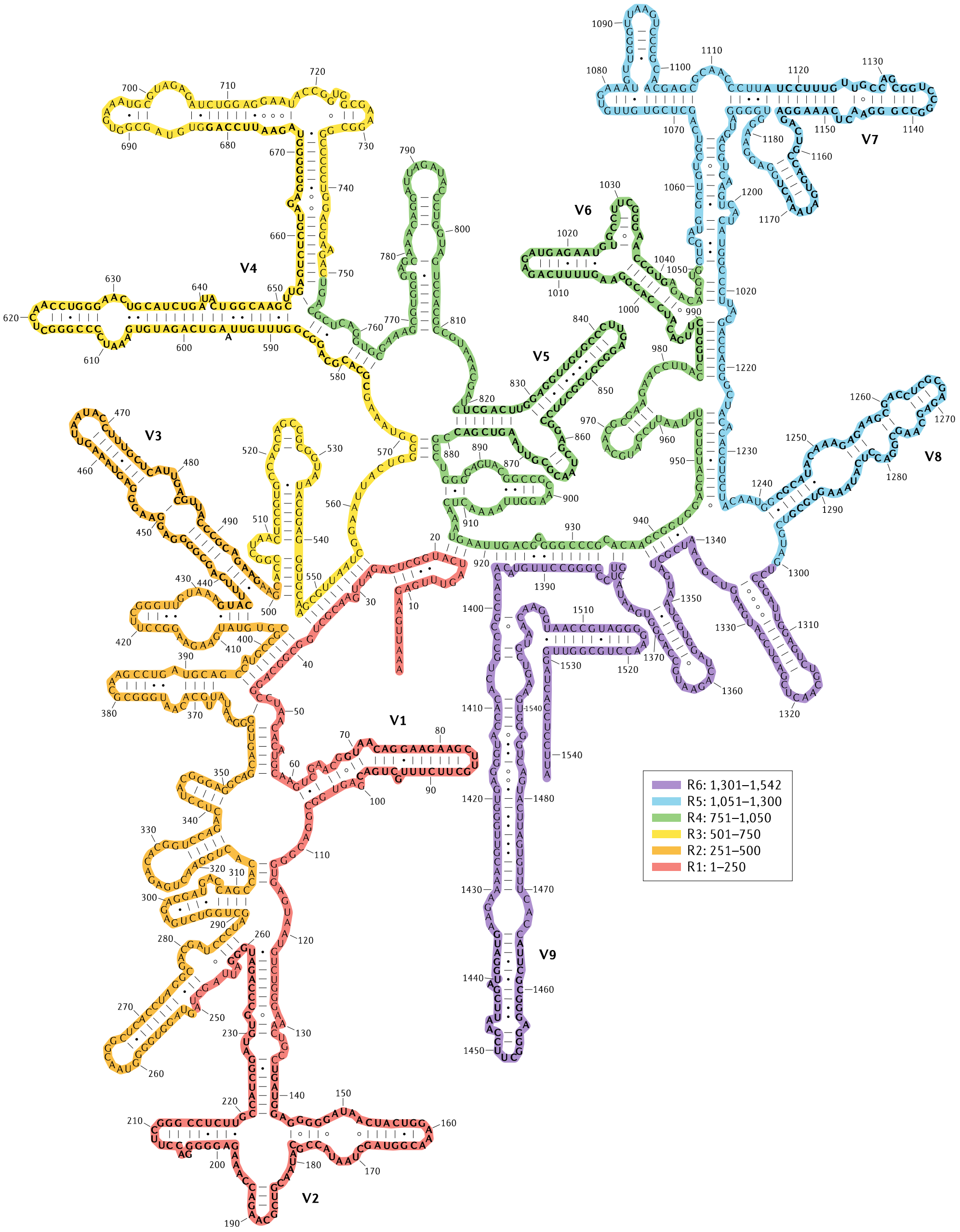

Figure 2.11 Variable regions of the 16S ribosomal RNA. In red, fragment R1 including regions V1 and V2; in orange, fragment R2 including region V3; in yellow, fragment R3 including region V4; in green, fragment R4 including regions V5 and V6; in blue, fragment R5 including regions V7 and V8; and in purple, fragment R6 including region V9.

Table 2.3 Taxonomic threshold of bacteria and archaea classification (Yarza *et al*., 2014)

	Genus	Family	Order	Class	Phylum
Number of taxa	568	201	85	39	23
Sequence identity CUTOFF	94.5%	86.5%	82.0%	78.5%	75.0%

Table 2.4 Pros and cons of metagenome analysis for evaluating microbial community

Pros	**Cons**
Directly infer the relative abundance of microbial functional genes, microbial taxonomic, and phylogenetic identity to species level	Relative expensive, complex sample preparation and analysis
No PCR-related biases	Deep sequencing depths are required relative to other methods
Assembly of microbial genomes and annotate of novel gene families	Microbial genomes tend to be inaccurate owing to assembly artefacts

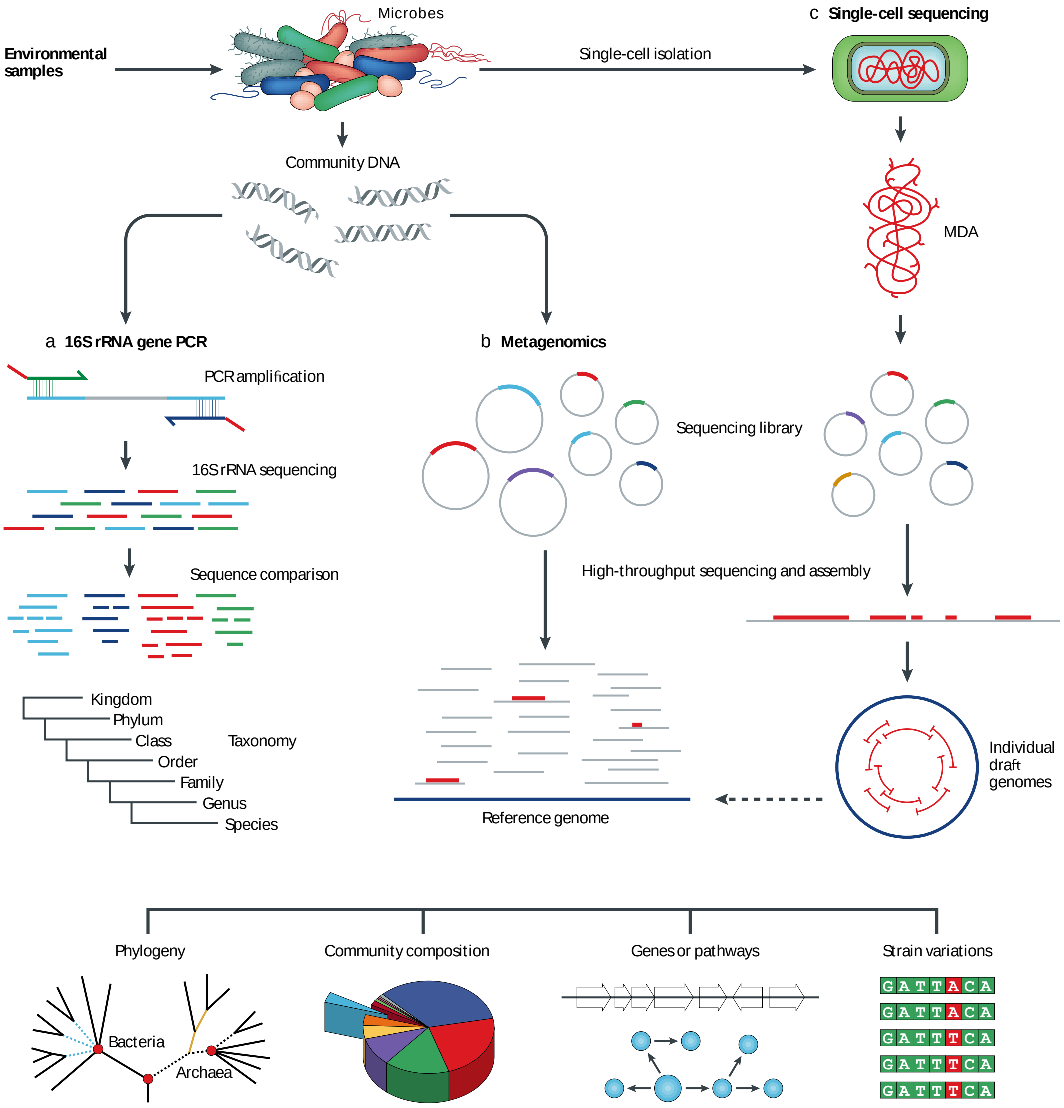

Figure 2.12 The workflow of methods used to investigate the genomics of uncultivated microbes for downstream applications (phylogenetic analyses, community structure, genes and enzymes, and strain variation). (a) PCR amplification of the 16S rRNA gene. (b) Metagenomic sequencing. (c) Sigle-cell genome sequencing.

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CHAPTER III

ESTUARY (ONGA RIVER): WHERE THE WATER MEETS THE SEA

3.1 Introduction

Since the largest methane storage is sitting under the seafloor (Kvenvolden, 1988; Yanagawa *et al*., 2012); thus, the release of methane from this habitat to the hydrosphere and atmosphere, should be wisely monitored. However, its contribution is relatively small because of suppression by anaerobic methane oxidation reactions in sediments. In this reaction, anaerobic methane-oxidizing archaea and sulfate-reducing bacteria syntrophically oxidize methane to carbon dioxide (Borowski *et al*., 1996; Knittel and Boetius, 2009). It has been estimated that anaerobic methane oxidation consumes 90% of the methane released from marine sediments (Valentine and Reeburgh, 2000). In the sulfate-containing environment, methanogenic archaea compete with sulfate-reducing bacteria for hydrogen and acetate, and sulfate-reducing bacteria tended to dominate due to their higher substrate affinity (King, 1984). Therefore, in many sulfate-bearing environments, such as shallow sediments, methanogenic archaea, and traces of methane typically to be undetected.

Estuary and coastal areas are distinctive environments where biological production is enhanced, and methane production is also active depending on the supply of organic matter. Likewise, these environments are susceptible to eutrophication because of the direct effects of organic matter and nutrients contained in domestic wastewater (Egger *et al*., 2016). In addition, the brackish water of the estuary is located at the bottom of the river where large amounts of suspended solids are accumulated (Figure 3.1). It is estimated that 95% of suspended sediment is deposited at the exit of an estuary where water flow is slow. These suspended sediments compose clay minerals and organic matter, and the organic portion undergoes aerobic degradation by microorganisms, which reduces dissolved oxygen in the environment. Moreover, sulfate reduction and methanogenesis proceed in the final stage of organic decomposition. For these reasons, although estuaries cover only a few percent of the total ocean area, they are thought to be a major source of methane emissions released from the entire ocean (Bange *et al*., 1994).

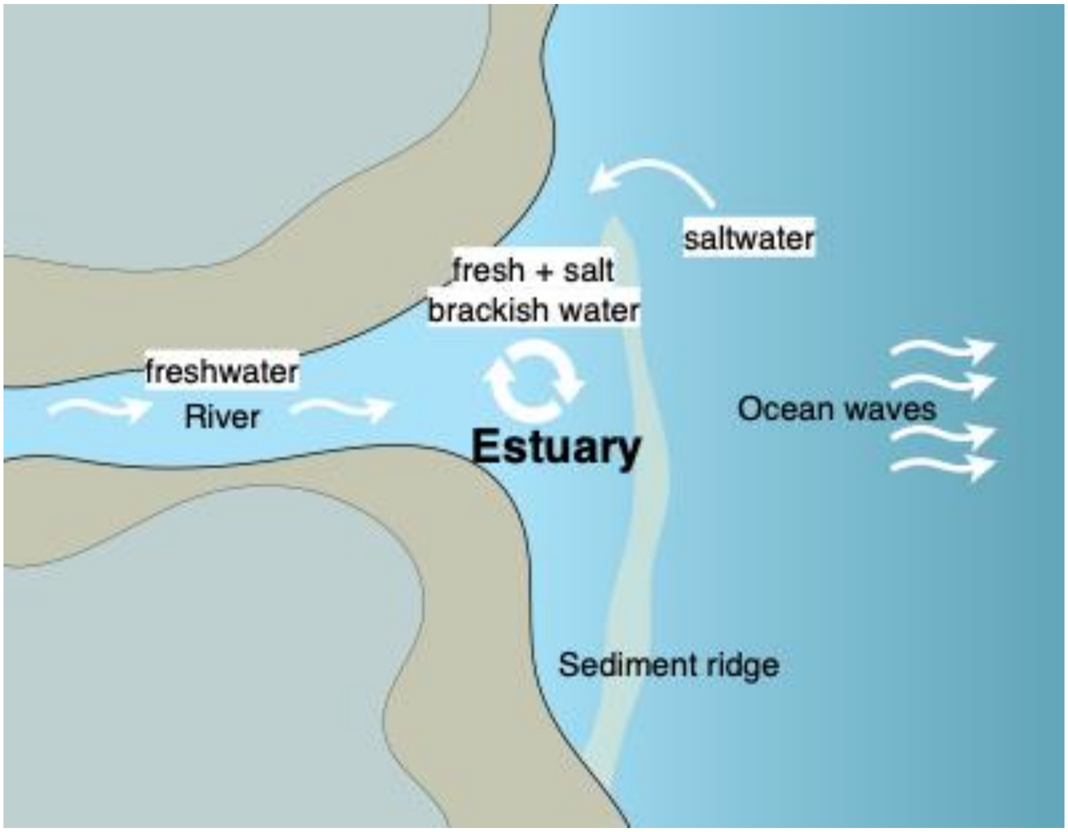

Figure 3.1 The illustration depicts how estuaries are formed. The land elevation toward the sea must be low that is how will be a gentle slope that will allow the river to meet the sea. The ocean water continuously hits the coastline along the way bringing as well as washing away a lot of sediment. Due to the continuous hitting of waves the sediment churns and turns into a sediment ridge. As a result, a small water pool of water gets separated from the ocean called an estuary connected both to the freshwater river and to a small stream from the ocean for getting its saltwater supply.

In recent years, next-generation sequencing (NGS) has been widely applied in the field of environmental microbiology to rapidly acquire large numbers of genomic sequences. NGS allows us to explore microbial communities and reveal complicated interactions among microbial populations with small amounts of samples (Figure 3.2).

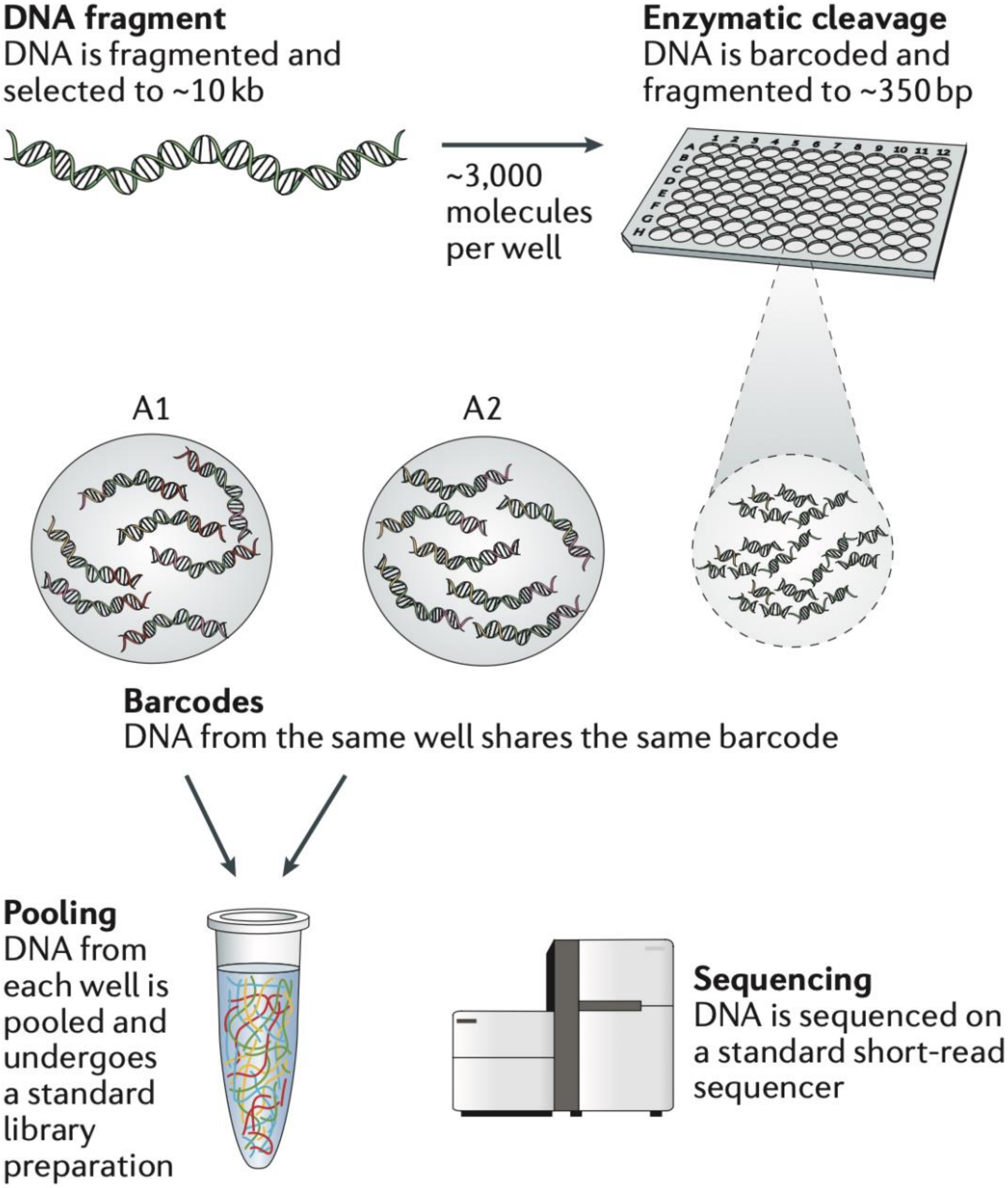

Figure 3.2 Synthetic long-read sequencing (Illumina technology). Genomic DNA templates are fragmented to 8-10 kb pieces. They are then partitioned into a microtitre plate such that there are around 3,000 templates in a single well. Within the plate, each fragment is sheared to around 350 bp and barcoded with a single barcode per well. The DNA can then be pooled and sent through standard short-read pipelines (Goodwin *et al.*, 2016).

Although NGS-based analysis targets the 16s rRNA gene, other genes with highly conserved nucleotide sequences that are generally consistent with the molecular phylogenetic tree based on the 16s rRNA gene can also be useful in determining biological classification. To detect methane-producing archaea, the *mcrA* gene, which encodes the alpha subunit of the methyl-coenzyme M reductase (Mcr) and catalyzes the last step of methanogenesis, is frequently used in molecular genomic analysis. Furthermore, the construction of gene libraries by PCR cloning, focusing on specific genes, is still a useful tool for exploring microbial delineation. The molecular cloning approach is shown in Figure 3.3.

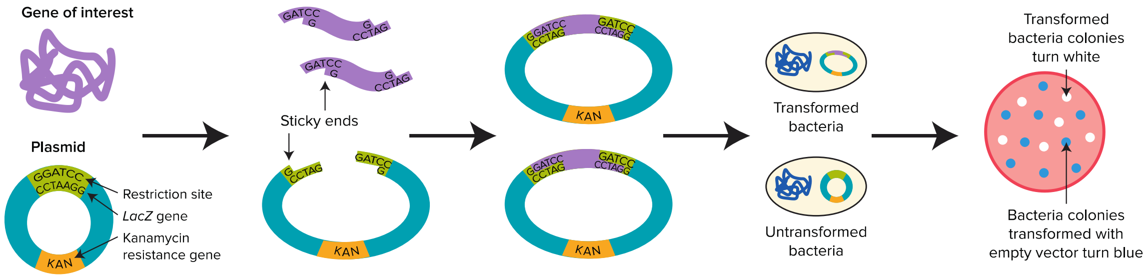

Figure 3.3 Schematic representation of a typical blue-white screening procedure. Blue-white screening of bacterial colonies involves cloning of gene inserted into a plasmid vector with an antibiotic resistance (ampicillin was used in this study instead of kanamycin) and LacZ reporter gene. The ligation of the insert into the multiple cloning site of the vector inactivates the LacZ gene. The transformation of competent E. coli with the ligated mixture in the presence of X-gal in culture media results in the formation of blue and white colonies.

This chapter focuses on the methanogenic archaea in a brackish environment by comparing the 16S rRNA gene amplicon analysis using next-generation sequencing with the functional target gene using cloning methods.

3.2 Materials and experimental methods

3.2.1 Sample collection and preparation

The Onga river is the most important source of freshwater in the Hibikinada Sea. The annual flow rate is about 944 × 10^6 m^3. On August 16, 2018, surface sediments were collected using a cylindrical corer (Wildco, Yulee, FL, USA) from the estuary of the Onga River (see Appendix A for sampling site). The on-site temperature and salinity were 24.9°C and 1.2%, respectively. Sediment samples were brought to the laboratory and were separated at every 1 cm (0-1, 1-2, 2-3, 3-4). Samples for molecular analysis were kept at -80°C until further processing.

3.2.2 Quantification of microbial gene abundance

To estimate the microbial biomass, gene quantification was performed by real-time quantitative PCR system qTOWER^3 G touch (Analytik Jena AG, Germany). Briefly, microbial DNA in each sediment sample was extracted using the DNeasy PowerSoil Kit (Qiagen) according to the manufacturer’s recommendations. Prokaryotic 16S rRNA gene and archaeal 16S rRNA gene were quantified using universal primer-probe sets (Univ340F, Univ806R, Univ516F probe) and archaea-specific primer-probe sets (Arc349F, Arc806R, Arc516F probe), respectively (Takai and Horikoshi, 2000). The amplification conditions were 50 cycles of denaturation at 98°C for 10 s, annealing at 50°C (universal 16S rRNA gene) or 52°C (archaeal 16S rRNA gene) for 45 s, and an extension at 72°C for 30 s. In addition, the *mcrA* gene, a functional key gene for methanogenesis, was quantified systematically based on intercalating fluorescent using SYBR Green I. The amplification was performed by using mcrA-specific primers (ME3MF and ME2’r) (Nunoura *et al.*, 2008) and Mighty Amp for Real-Time (TaKaRa Bio, Inc., Otsu, Japan). The amplification conditions were 40 cycles of denaturation for 40 s at 94°C, annealing at 52°C for 30 s, and an extension at 68°C for 60 s. All qPCR assays were conducted in triplicate. Details of primer-probe sets and amplification protocols are summarized in Appendix B and C, respectively.

3.2.3 Microbial community structure analysis based on 16S rRNA gene and functional *mcrA* gene

To determine the microbial community composition based on the 16S rRNA gene, prokaryotic-target PCR amplification was performed from extracted microbial DNA using universal primers (515F/806R) (Caporasp *et al.*, 2011) and Mighty Amp DNA Pol (TaKaRa Bio, Inc., Otsu, Japan). Amplification was performed in a 30-µL reaction volume with an initial denaturation at 98 °C for 2 min, 40 cycles of denaturation at 98 °C for 30 s, annealing at 55 °C for 30 s, elongation at 68 °C for 30 s, and final elongation at 68 °C for 5 min (Appendix C). The PCR products were purified and processed using Agencourt AMPure XP beads (Beckman Coulter) and a Nextrera XT Index Kit (Illumina) and sequenced using the next-generation sequencer Miseq (Illumina, Inc.). Microbial phylotype composition was analyzed using QIIME2 (Bolyen *et al.*, 2019), in which representative sequences were assigned at different taxonomic levels based on the SILVA 128 database comparison.

The *mcrA* gene was amplified to construct the PCR clone library from the extracted DNA using a specific primer set and reaction condition as described in real-time quantitative PCR (section 3.2.2). Amplified PCR products were ligated into vector pTA2 (see Appendix D for vector information) using a TA cloning kit (TOYOBO, Japan), and the ligation products were used to transform competent Escherichia coli DH5 alpha cells. The LB agar supplemented with ampicillin and DMF X-gal were used for blue-white colony screening of transformed cells. The colonies of transformed cells were used as DNA templates directly for PCR amplification using M13-20 and M13 reverse primers (TOYOBO, Japan). Amplification was performed in a 10-µL reaction volume with an initial denaturation at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 20 s, annealing at 53 °C for 45 s, elongation at 72 °C for 45 s, and final elongation at 72 °C for 5 min (Appendix C). The molecular phylogenetic tree was constructed using ARB software (Ludwig et al., 2004). Molecular phylogeny and compositional ratio were determined by referring to known sequences in the database at the 95% dissimilatory cut-off levels.

3.3 Results and discussion

3.3.1 Microbial gene abundance

Microbial gene numbers were quantified by real-time quantitative PCR with specific primer-probe sets. Prokaryotic 16S rRNA genes were detected at about 4.23×10^9 to 13.4×10^9 genes g^{-1} sediment (Figure 3.4a). The number of genes tended to increase slightly with depth. On the other hand, the archaeal gene numbers ranged from 5.0×10^7 to 9.6×10^7 genes g^{-1} sediment, and no particular change was observed with depth. The highest archaeal gene abundance ratio was found at a depth of 3-4 cm. The ratio of archaeal 16S rRNA genes to prokaryotic 16S rRNA genes was 0.6-1.3%, indicating archaea were low in abundance at all sedimental depths. These values are consistent with a relative abundance of archaea distributed in other estuarine ecosystems around the world (Liu *et al*., 2018).

3.3.2 Microbial community structures

The composition of the microbial community in each sediment was determined by 16S rRNA gene amplicon sequencing. It should be prior noted that the 16S rRNA gene sequencing lacks the representation of actual microbial abundance in the samples owing to the limitation of PCR-based amplification coverage. Therefore, the proportion of microorganisms obtained in this study is discussed on a basis of total microbial sequence reads detected by sequencing methods.

In the estuary sediments, a total of 12,171 reads were obtained with an average length of 464 bp. Bacterial communities dominated over 98.5%, and archaea accounted for less than 1.4% (Figure 3.4b) of total microbial sequences. In comparison, this archaeal ratio was consistent with the result obtained from qPCR (0.6-1.3%). Microbial community classification at the phylum level showed that Bacteroidetes and Proteobacteria were dominant among the bacterial sequences (Figure 3.4c). The dominant members in the phylum Proteobacteria were Gammaproteobacteria and Deltaproteobacteria. The Methanomicrobia, methanogenic archaea belonging to the Euryarchaeota, were detected with a proportion of 0.4-0.7%. Looking at deeper taxonomic levels, these archaea were taxonomically classified into the family Methanosaetaceae in the order Methanosarcinales. In addition, *Ca*. Woesearchaeota (formerly called DEVEG-6) was detected at the top layer of sediment with a ratio of 1% of microbial sequences (Figure 3.4c, ②), as they were shown in other oxygen-depleted environments (Liu *et al.*, 2018). In addition to archaea communities, members of sulfate-reducing bacteria (in the class Deltaproteobacteria) including Desulfobacterales, Desulfuromonadales, and Desulfarculales were dominant throughout the sediment core. These results suggested that next-generation sequencing has proved to be a useful tool used for a comparison of the microbial community among multiple samples at a glance. The rare microbial species (i.e., *Ca*. Woesearchaeota) that are yet cultivated in the laboratory were also detected based on the amplicon sequencing method.

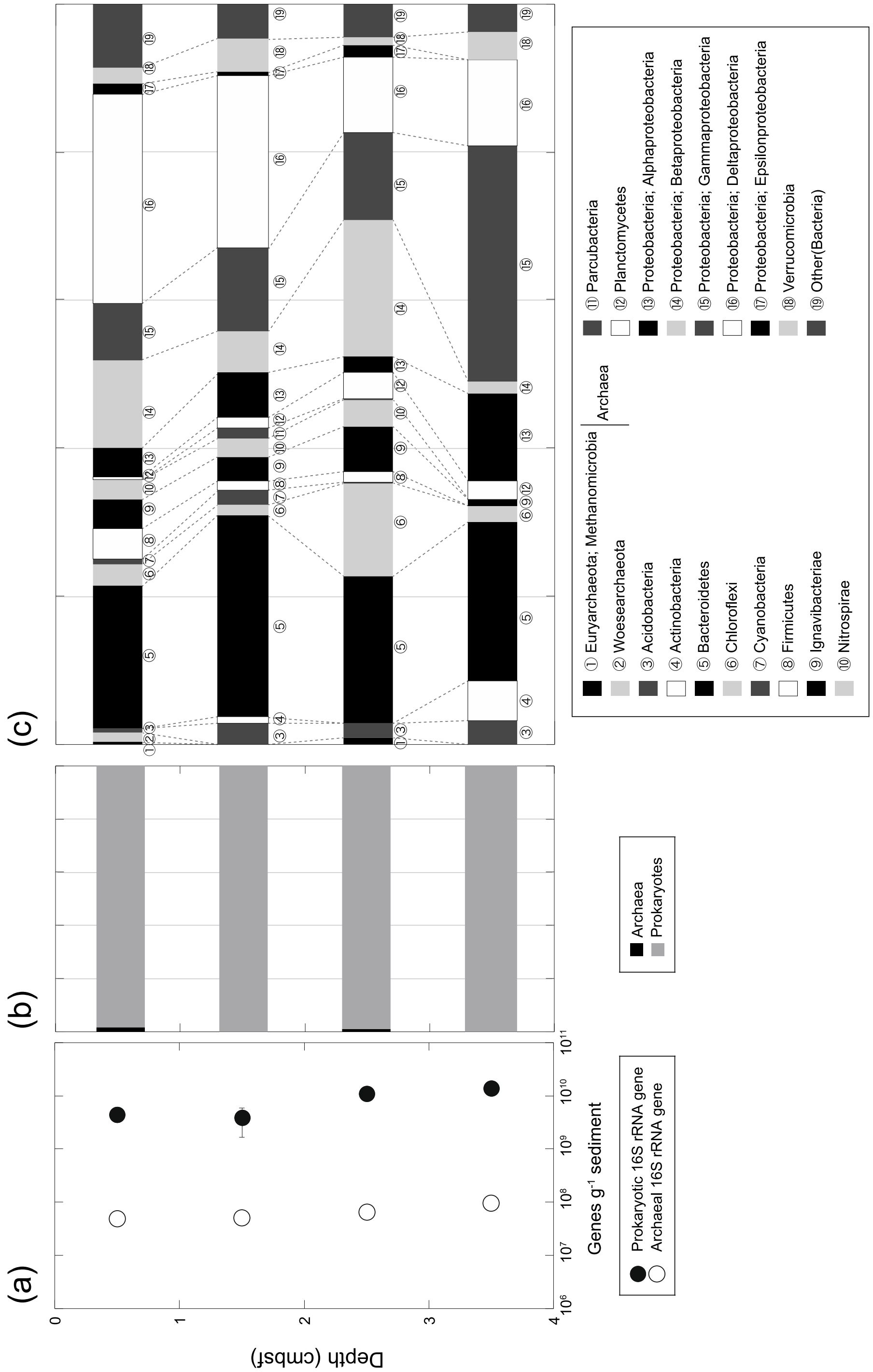

Figure 3.4 (a) Number of microbial 16S rRNA genes in the sediments quantified by qPCR and plotted logarithmically in the depth direction. (b) Microbial community composition at the domain level based on 16S rRNA gene amplicon sequencing analysis. (c) Microbial community composition at the phylum and class level. Microbial taxa are indicated in the circled number.

3.3.3 Phylogenetic diversity based on *mcrA* gene

The *mcrA* gene was quantified using a quantitative real-time PCR. The result showed that the *mcrA* gene was detected in as many as 2.4-6.6 × 10^7 genes per gram of the surface sediments (at depth of 0-3 cm) (Figure 3.5a). This observation indicated that large amounts of the *mcrA* gene, comparable with archaeal 16S rRNA gene abundance (logarithmic range of 10^7), were distributed in the surface layer of the sediment, which is easily exposed to oxygen. In fact, in organic-rich sediments aerobic bacteria actively consume molecular oxygen, resulting in the formation of anaerobic environments and the production of methane as the final product of organic matter decomposition.

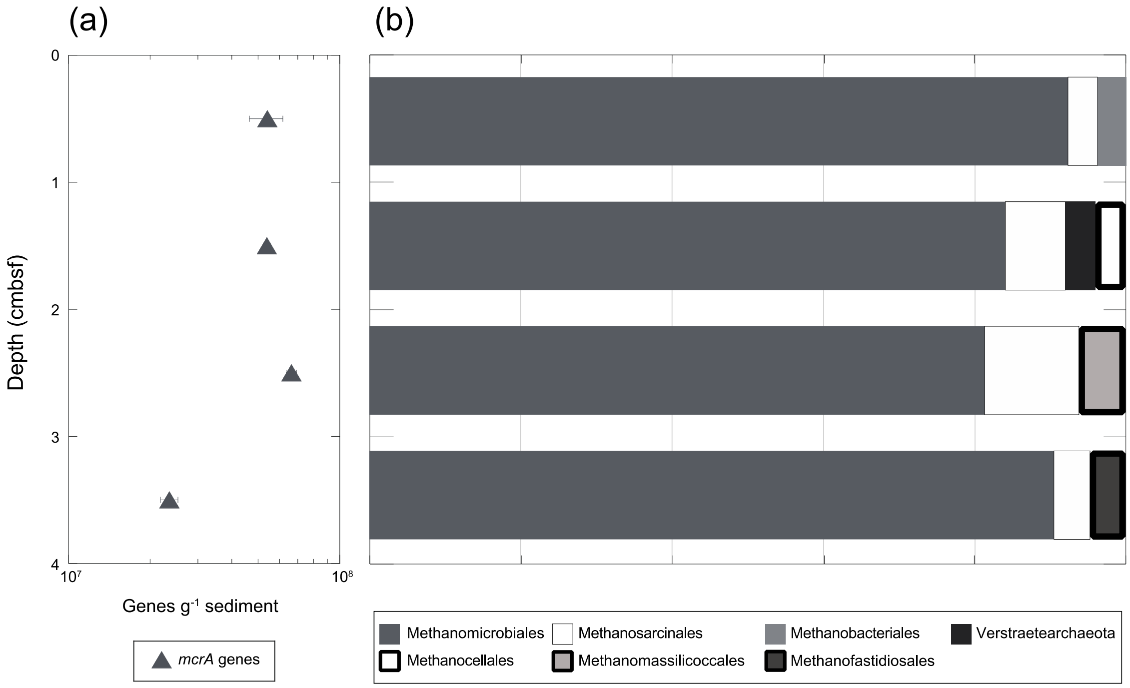

Figure 3.5 (a) Vertical distribution of the *mcrA* gene abundance in sediments. The number of genes g^{-1} sediment was quantified by qPCR and plotted logarithmically in the depth direction. (b) Phylogenetic composition of the *mcrA* gene classified based on molecular cloning method.

The nucleotide sequences of the amplified *mcrA* gene were further determined by the molecular cloning method. Phylogenetic classification of the *mcrA* gene sequences obtained in this study is shown in Figure 3.5b, and the molecular phylogenetic composition on the phylogenetic tree is shown in Figure 3.6. At all depths of sediment, most of the obtained *mcrA* genes were classified into the order Methanomicrobiales, accounting for 81.3-92.3% of the total obtained sequences (33 cloned sequences). Groups of Methanosarcinales were the second most abundant, accounting for 3.8-12.5%. The order Methanosarcinales contained genes closely related to the family Methanosaetaceae, which were also detected by the 16S rRNA gene amplicon at 3.8%. At a depth of 0-1 cm, uncultured methanogenic archaea, order Methanocellales and phylum Verstraetearchaeota, were presented at about 4% of the total sequences. In addition, 6.2% of Methanomassiliicoccales and 4.7% of Methanofastidiosales were detected at a depth of 2-3 cm and 3-4 cm, respectively. This result suggests the existence of a diverse methanogenic archaea community, in addition to those that were recovered by 16S rRNA gene amplicon analysis. It should be noted that the gene sequence ONG01_mcrA08 obtained from the top sediments showed high homology to the limited sequences. A closely related sequence was reported from estuarine sediments in China and Germany (accession number JX942636, JN684176) with about 92.1% homology. The other sequences showed remarkably low homology (less than 82%) and formed their own cluster (Estuary Group on the phylogenetic tree in Figure 3.4). Therefore, the Estuary Group belonging to the family Methanosaetaceae in the phylogenetic tree shown in Figure 3.4, was considered to belong to a different family. For the *mcrA* gene sequence, the average homology 88.9% at a genus level and was 83.5% at a family level (Steinberg and Regan, 2008). Therefore, the Estuary Group likely represents a distinct lineage at a family level.

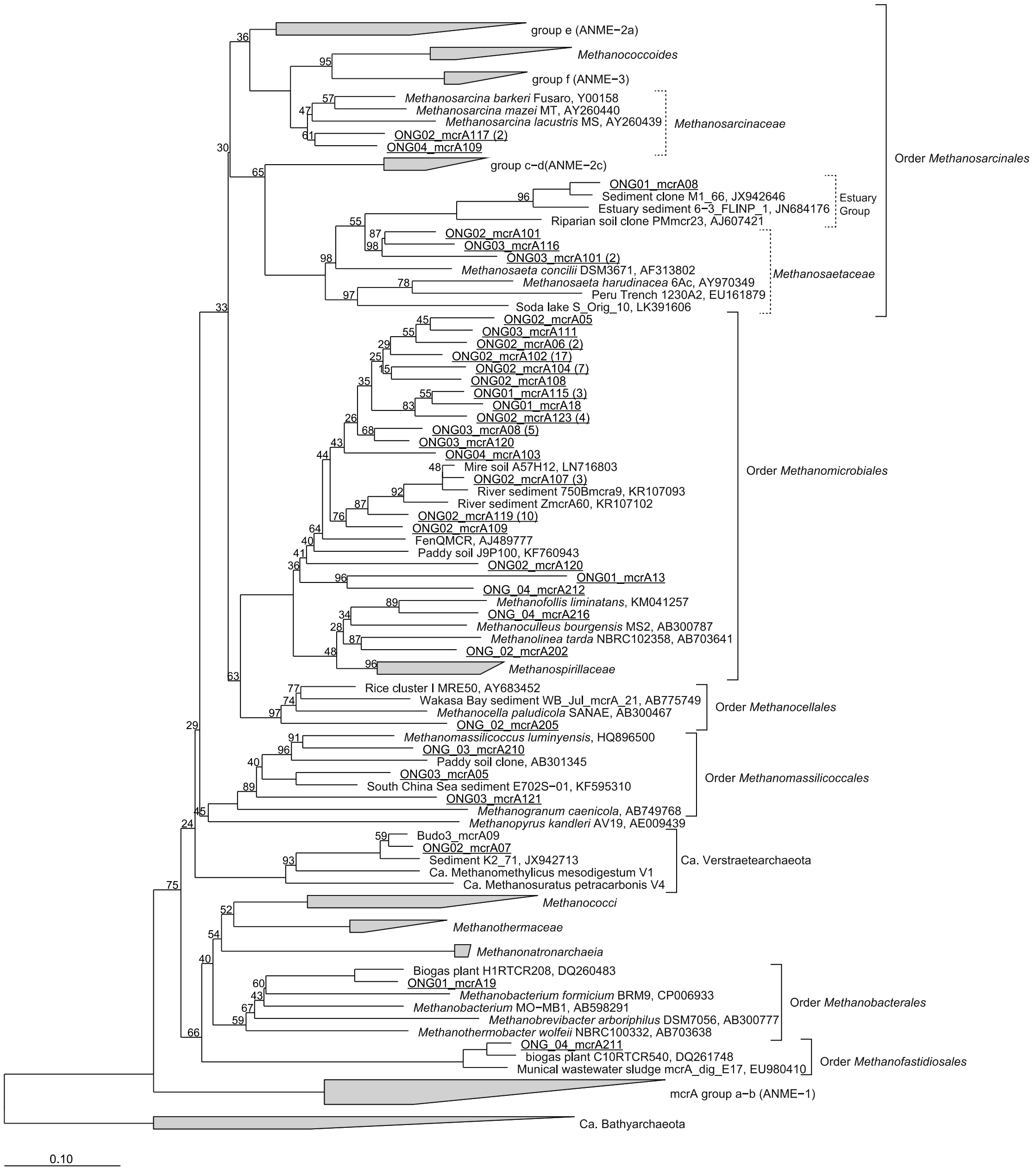

Figure 3.6 Phylogenetic tree of the *mcrA* gene sequences detected in sediments. The sequences obtained in this study are shown in the bolded underline covering 6 orders and one putative phyla. Scale bar indicates the number of substitutions per site.

3.3.4 Rethinking of methane cycle in brackish water sediment

In this study, the family Methanosaetaceae was detected based on a 16S rRNA gene amplicon while the mcrA gene cloning analysis recovered more diverse sequences spanning 7 phylum- or order-level lineages such as Methanomicrobiales, Methanosarcinales, Methanocellales, Methanomassiliicoccales, Methanobacterales, Methanofastidiosales, and *Ca*. Verstraetearchaeota. This discrepancy is possibly due to the coverage capacity of the 16S rRNA gene-specific PCR primers for the known methanogenic archaea sequences in the study site. Members of the Methanomicrobiales were dominant based on the mcrA gene cloning and most of them are known to be hydrogenotrophic methanogen, which produces methane from H_2 and CO_2 (Liu and Whitman, 2008). The second dominant group was Methanosarcinales, a methanogenic lineage that was widely detected in major sources of methane emissions such as rice paddies, permafrost, and peat bogs (Oren, 2014). They are capable of acetoclastic methanogenesis, which is thought to be an important factor in increasing atmospheric methane. Members of Methanosarcinales are also known to use other substrates, including CO_2/H_2, formic acid, and methyl compounds (methanol and methylamine). The family Methanosaetaceae, which were detected based on the 16S rRNA gene, also belongs to the order Methanosarcinales, but its methanogenic substrate is limited to acetic acid. The *mcrA* genes from *Ca*. Verstraetearchaeota (previously called the Terrestrial Miscellaneous Crenarchaeota Group, TMCG) and Methanocellales, were found at a depth of 1-2 cm. All the isolated species of Methanocellales are known to reduce CO_2 with H_2 (Sakai *et al*., 2014). *Ca*. Verstraetearchaeota is frequently detected in anaerobic environments with high methane flux. Recently, the metagenomic analysis revealed that Verstraetearchaeota has the potential to produce methane from methanol, methanethiol, and methylamine (Vanwonterghem *et al*., 2016). The Methanomassiliicoccales, methane-producing archaea in the class Thermoplasmata (Iino *et al*., 2013), were detected at a depth of 2-3 cm. They have been found in animal intestines, wetlands, and sewage treatment plants. Methanomassiliicoccales are known to generate methane from methanol, methylamine, and dimethyl sulfide by using H_2 as an electron donor dependently (Lang *et al*., 2015). The order Methanofastidiosales, a lineage previously referred to as WSA2 or Arc I (Chouari *et al*., 2005), was presented at a depth of 3-4 cm. This group has been reported in freshwater, marine sediments, contaminated groundwater, and bioreactors. Recently, the metagenomic analysis showed that they reduced methylated thiol for methane generation since none of the essential genes in CO_2

reduction and acetoclastic methanogenesis pathway were detected (Nobu *et al*., 2016). These results suggested that surface sediments in brackish water could be a site for methanogenesis.

Typically, it has long been known that sulfate-reducing bacteria and methanogens compete for the same substrate in sulfate-containing environments, such as the seafloor. Although acetic acid and hydrogen are known to be competing substrates, sulfate-reducing bacteria can utilize them at lower concentrations than methanogenic archaea according to thermodynamic reasons. Thus, methanogenesis is less likely to proceed in sulfate-reduced zones (Lueders *et al*., 2001). Nonetheless, several studies have shown that methane can be detected in surface sediments of sulfate-reducing zones (Dale *et al*., 2008; Knap *et al*., 2008; Sela-Adler *et al*., 2017). This scenario is usually interpreted as an increase in methanogenesis where common substrates do not use by sulfate-reducing bacteria, such as methanol, methylamine, methanethiol, and dimethyl sulfide (Oremland and Polcin, 1982). Since the present study site is a sulfate-rich environment supplied by seawater, the methanogenic archaea detected in the samples were likely to utilize non-competitive substrates. In fact, small numbers of methanogenic archaea with the ability to use methyl compounds have been detected based on the *mcrA* gene analysis. The methyl-dependent methanogenic archaea can be divided into two groups based on the presence of cytochromes. Methanogens with cytochromes such as Methanosarcinales, and methanogens without cytochromes, but depend on hydrogen such as Methanomassiliicoccales (Thauer *et al*., 2008). In recent years, the latter type has been reported, the Ca. Bathyarchaeota, and is thought to use hydrogen to produce methane from methanol, methylamine, and methyl sulfide (Evans *et al*., 2015). Collectively, methanogenic archaea recovered from the brackish water in this study, such as Methanomassiliicoccales, Verstraetearchaeota, and Methanofastidiosales, were proposed to exhibit similar hydrogen-dependent metabolism, supplying methane to the surface sediment through methylotrophic methanogenesis.

In the marine environment, anaerobic methane oxidation (AOM) is a well-known biodegradation process using sulfate as electron acceptors. However, neither the 16S rRNA gene of the anaerobic methane-oxidizing archaea nor the *mcrA* gene, which is responsible for this reaction, was detected in this study. Anaerobic methane oxidation may be less likely to proceed in a brackish water environment where the dilution of seawater by freshwater inflow is variable. If methane suppression by anaerobic methane oxidation does not occur, the methane produced in the surface sediments will directly escape into the upper water and the atmosphere. Alternatively, coupling reactions of anaerobic methane oxidation and denitrification from river sediments and intertidal zones have been reported in several works (Shen *et al.*, 2019; Wang *et al.*, 2019).

In recent years, next-generation sequencing has become mainstream in environmental microbiology applicable for elucidating microbial community composition. This approach has made it possible to detect small numbers of microorganisms in environmental samples. However, when targeting rare communities, less than a few percent, multiple approaches should be combined for verification. For instance, new approaches have been developed and used for searching functional genes in the pool of nucleotide sequences obtained by metagenomic analysis, which can be used for constructing genomes (Speth and Orphan, 2018). For methanogenic archaea detection, PCR amplification targeting the *mcrA* gene is still an effective method, as applied in this study and previous research (Wilkins *et al.*, 2015). Remarkably, PCR-dependent methods are unable to recover some targets as a result of primer biases. Since the discovery of methanogenic archaea besides Euryarchaeota, i.e., Ca. Bathyarchaeota and Ca. Verstraetearchaeota, a selection of primers for detecting potential methanogenic archaea communities must be reconsidered. The present study showed that a wide variety of methanogenic archaea strains were recovered by using frequently used primers, and the mcrA gene-dependent techniques could also be used to clarify methanogenic phylogeny.

3.4 Conclusions

This study analyzed the microbial community in brackish water sediments. Next-generation sequencing of the 16S rRNA gene and classical molecular cloning targeting the functional *mcrA* gene were used to examine methanogenic microbes. The 16S rRNA gene amplicon sequencing could detect methanogenic archaea belonged to the Methanosaetaceae, whereas the mcrA gene cloning technique recovered more diverse methanogenic sequences, including a new candidate family-level lineage that had never been reported in brackish water. Although 16S rRNA gene-based sequencing is often regarded as the definitive method for microbial ecology analysis, this study suggested that diversity assessment of relatively small populations could be made more reliable by combining it with conventional methods based on functional genes.

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CHAPTER IV

WETLAND (BOGATSURU): THE LARGEST SOURCE OF METHANE

4.1 Introduction

The average global CH_4 emission is estimated to be 592 Tg yr^{-1} (Figure 4.1). Further, as approximately one-third of the emissions are derived from natural wetlands, these environments are known as the largest natural source of the atmospheric CH_4 budget (Jackson *et al*., 2020). Also, natural wetlands are considered important terrestrial carbon reservoirs that may respond significantly to the global biogeochemical cycle and nutrient fluxes (Gorham, 1991). Biogenic CH_4 released from wetland is generally derived from anaerobic fermentation of organic matter and subsequent methanogenesis under thermodynamically favorable conditions for anaerobic bacteria and methane-producing archaea (Liu and Whitman, 2008).

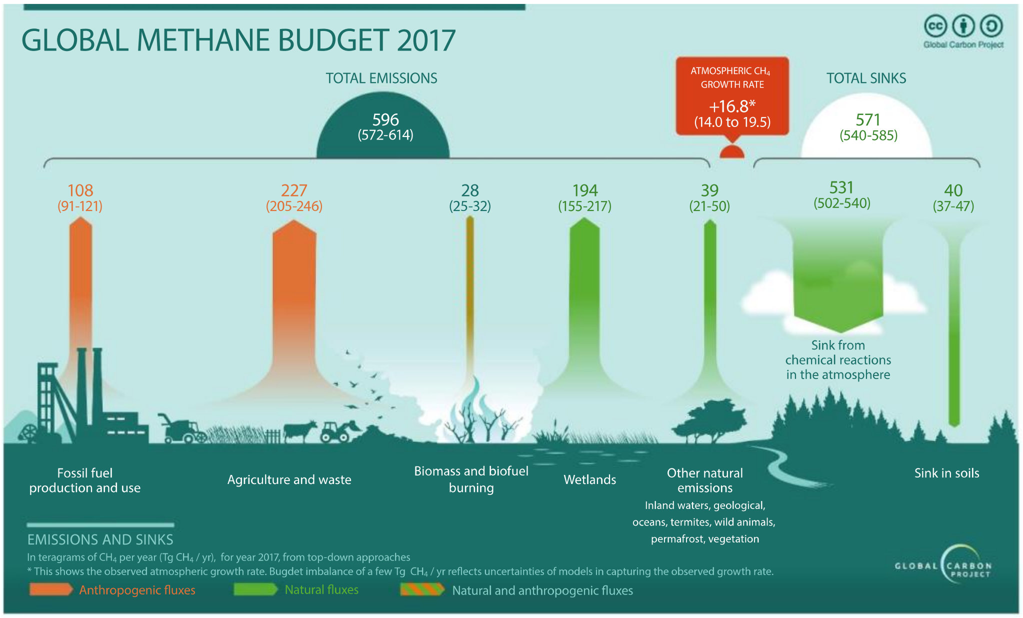

Figure 4.1 The global methane budget for year 2017 based on top-down method for natural sources sinks (green), anthropogenic sources (orange), and mixed natural and anthropogenic sources (hatch orange-green for ‘biomass and biofuel burning’).

To date, cultivated methanogenic archaea are thought to be restricted to only eight orders within the phylum Euryarchaeota (Lyu *et al*., 2018), including Methanomicrobiales, Methanonatronarchaeales, Methanosarcinales, Methanobacteriales, Methanopyrales, Methanococcales, and Methanomassiliicoccales. Among these archaeal lineages, only five groups (Methanobacteriales, Methanomicrobiales, Methanocellales, Methanosarcinales, and Methanomassiliicoccales) are commonly found in peatlands (one of the wetland types) (Bräuer *et al*., 2020).

Since methanogenic community and physiological ecology have been well studied in the Northern peatlands, the important source of atmospheric methane and a large reservoir of terrestrial carbon (Basiliko *et al*., 2003; Galand *et al*., 2003; Yavitt *et al*., 2012; Abdalla *et al*., 2016), this study, thus, focused on the methanogenic community in the peat soils of the Bogatsuru mire in Oita Prefecture (Kyushu, Japan), in which microbial community has never been investigated. Accumulation of peat material in the wetlands induces a transition from low to high moor, corresponding to the shift from nutrient-rich to nutrient-poor conditions. The intermediate moor harbors nutritionally diverse environments, which is expected to enable the formation of ecological niches for diverse microorganisms. Therefore, based on 16S rRNA gene amplicon sequencing and functional gene targeting, this study attempted to identify key methanogenic archaea in the acidic wetland, either well-established methanogenic archaea or novel lineages, to uncover their phylogenetic distribution and metabolic functions.

4.2 Materials and experimental methods

4.2.1 Sampling site and sample collection

On 21 July 2020, peat soil samples were vertically collected from the Bogatsuru mire (33°05'47.3'' N, 131°15'35.2'' E) using a Tomas type peat sampler (Nose Tekkosho, Okayama, Japan) at three depths from the surface (10, 45, and 90 cm) to cover aerobic and anaerobic representatives, which were designated as BO10, BO45, and BO90, respectively (see Appendix E for sample collection). The abundant species of vegetation at the sampling site were Phragmites australis (Cav.) Trin. ex Steud., Moliniopsis japonica (Hackel) Hayata, Juncus effusus L. var. decipiens Buchen, Sphagnum fimbriatum Wils. and S. palustre L. The atmospheric temperature at the time of sampling was 26 °C. Water on the surface of the peatland was collected using a sterile syringe to perform an on-site measurement of the pH and oxidation-reduction potential (ORP) with a portable meter (D-52, Horiba, Kyoto. Japan). For soil CH_4 gas analysis, 2 g of soil sample was fixed on-site with mixed solutions containing 0.5 mL of 10% benzalkonium chloride and 9.5 mL of saturated sodium chloride solution in 20-mL vials sealed with headspace caps (Agilent, USA). All samples were transferred to the laboratory on the same day. Samples for chemical and molecular biological analyses were stored at 4 °C and -80 °C, respectively, until further processing.

4.2.2 Soil gas analysis and water chemical composition

To quantify CH_4 potentially produced from peat soils, 1 mL of the headspace was analyzed using a gas chromatograph equipped with a MICROPACKED ST column (Shinwa Chemical Industries, Kyoto, Japan) and flame ionization detection (FID). The temperatures of the column, injector, and detector were 80, 100, and 300 °C, respectively. The standard curve of CH_4 (Appendix G) was constructed between a peak area (RT 2.5 min) and CH_4 concentration (0, 1, 10, and 100 ppm). The detection limit was 1 ppm. For water geochemistry, water samples were filtered using a 0.2 μm single-use cellulose acetate membrane filter (Sartorius Stedim Biotech, Germany) prior to quantification of ion concentration. Cations (Ca^{2+}, Fe^{3+}, K^+, Mg^{2+}, and Na^+) were analyzed using ICP-AES (ICPE-9800 Shimadzu, Kyoto, Japan), and anions (F^-, Cl^-, NO_3^-, SO_4^{2-}) were analyzed using an ion chromatograph (DX-120, Thermo Scientific Dionex). Acetate was analyzed using ion chromatography (ICS-2100, Thermo Scientific Dionex).

4.2.3 Microbial community structure analysis based on the 16S rRNA gene

The prokaryotic DNA of peat soil samples (BO10, B045, and BO90) was extracted using the DNeasy PowerSoil Kit (Qiagen), according to the manufacturer's instructions. During extraction, microbial cells were mechanically disrupted using a µT-01 bead crusher (TAITEC, Kashigaya, Japan). All extracted DNA samples were quantified using Qubit dsDNA HS Assay Kit (Invitrogen, USA) and kept at -80 °C until further analysis. Purified genomic DNA was used to construct a PCR library of the hypervariable V3-V4 region of the 16S rRNA gene using primers Bakt_341F and Bakt_805R, which were designed by Herlemann *et al*. (2011) and evaluated the taxonomic coverage by Klindworth *et al*. (2013), and Mighty Amp DNA Pol (TaKaRa Bio, Inc., Otsu, Japan). Amplification was performed in a 30-µL reaction volume with an initial denaturation at 98 °C for 2 min, 40 cycles of denaturation at 98 °C for 30 s, annealing at 55 °C for 30 s, elongation at 68 °C for 30 s, and final elongation at 68 °C for 5 min (Appendix C). The PCR products were purified and processed using Agencourt AMPure XP beads (Beckman Coulter) and a Nextrera XT Index Kit (Illumina), respectively. Sequencing was performed on the Illumina MiSeq platform. Microbiome analyses, including quality filtering, sequence trimming, operational taxonomic unit (OTU) clustering (97% cut-off), and taxonomic assigning, were processed using QIIME2 2018.2 (Bolyen *et al*., 2019). The Bathyarchaeotal sequences obtained from PCR amplicon sequencing were aligned with known representative sequences of Bathyarchaeota from previously reported genomic databases. A phylogenetic tree was constructed using the Maximum Likelihood method by RAxML 8.0 with the GTR GAMMA model in the ARB software (Ludwig *et al*., 2004). Bootstrap values were computed using 1,000 replicates.

4.2.4 Quantification of microbial 16S rRNA gene abundance

Prokaryotic 16S rRNA gene abundance was quantified using the Taqman probe-based qPCR method with the universal primer-probe set (Uni340F/Uni806R/Uni516F probe), the archaeal-specific primer-probe set (Arch349F/Arch806R/Arch516F probe), and the innuDry qPCR MasterMix Probe (Analytik Jena AG, Germany). The amplification was conducted in a 20-µL reaction volume with an initial denaturation at 98 °C for 2 min, 50 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C (for universal 16S rRNA gene) or 52 °C (for archaeal 16S rRNA gene) for 45 s, and elongation at 72 °C for 30 s (Appendix C). The targeted *mcrA* genes (marker genes for methanogenesis) were amplified using a specific primer set (ME3MF/ME2'R) and MightyAmp for Real-Time PCR (TaKaRa Bio, Inc., Otsu, Japan) under the following amplification conditions: initial denaturation at 94 °C for 2 min, 40 cycles of denaturation at 94 °C for 40 s, annealing at 52 °C for 30 s, and elongation at 68 °C for 1 min (Appendix C). In addition to methanogenic archaea, bacterial methanotroph was also quantified by using primers specific to the methane monooxygenase gene (Appendix D) with Mighty Amp DNA Pol (TaKaRa Bio, Inc., Otsu, Japan) under the following amplification conditions: initial denaturation at 98 °C for 2 min, 30 cycles of denaturation at 98 °C for 30 s, annealing at 56 °C for 30 s, elongation at 68 °C for 30 s, and final elongation at 68°C for 5 min, or with KOD FX Neo (TOYOBO, Japan) under the following amplification conditions: initial denaturation at 94 °C for 2 min, 40 cycles of denaturation at 94 °C for 40 s, annealing at 52 °C for 30 s, elongation at 68 °C for 1 min, and final elongation at 68°C for 7 min. The Bathyarchaeotal 16S rRNA gene fragments were amplified using MightyAmp for Real-Time (TaKaRa Bio, Inc., Otsu, Japan) and modified primers (MCG410F'/MCG528R'; Kubo *et al*., 2012) under the following 2-step amplification conditions (Lloyd et al., 2011): initial denaturation at 95 °C for 5 min, 40 cycles of denaturation at 95 °C for 10 s, and combined annealing and elongation at 60 °C for 45 s (Appendix C). All targeted gene abundances were quantified in triplicate using a real-time PCR system qTOWER^3 G Touch (Analytik Jena AG, Germany). Non-specific amplification of the targeted genes was confirmed by gel electrophoresis of the PCR product and melting curve (for the *mcrA* gene). Details of the primers (and primer design) and probes used for qPCR are provided in Appendix B.

4.2.5 Molecular phylogeny of *mcrA* gene

PCR amplification of the *mcrA* genes was conducted using a specific primer set and reaction condition, as previously described for qPCR (section 4.2.4) with a KOD FX Neo (TOYOBO, Japan). Amplified PCR products were ligated into vector pTA2 using a TA cloning kit (TOYOBO, Japan), and the ligation products were used to transform competent Escherichia coli DH5 alpha cells. The LB agar supplemented with ampicillin and DMF X-gal were used for blue-white colony screening of transformed cells. The transformed cells were used as DNA templates directly for PCR amplification using M13-20 and M13 reverse primers (TOYOBO, Japan). Amplification was performed in a 10-µL reaction volume with an initial denaturation at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 20 s, annealing at 53 °C for 45 s, elongation at 72 °C for 45 s, and final elongation at 72 °C for 5 min (Appendix C). The obtained *mcrA* gene sequences were assigned to the phylotype at a 95% sequence identity cut-off. Molecular phylogenetic trees of the *mcrA* gene were constructed using the neighbor-joining method in ARB software (Ludwig *et al*., 2004). Bootstrap values were computed using 1,000 replicates.

4.3 Results and discussion

4.3.1 Surface water chemistry

Surface water collected at the sampling site was used to determine the ORP, pH, and ion content. Based on the pH value, the peat soil was mild acidic (pH 6.45). Further, based on the samples collected during the rainy season (a period from June to July with a high average precipitation of 250-300 mm, according to the Japan Meteorological Agency) and the value obtained from previous field measurements (pH 4.78) on 28 November 2019, soil pH may be slightly higher than expected owing to the dilution effect of rainfall. The surface water redox potential was 204 mV, suggesting oxidizing conditions at the soil surface. Ion composition analysis revealed that sulfate (307 µM), nitrate (200 µM), and Ca^{2+} (238 µM) were major ions found in the surface water. Other minor cations and anions detected in the surface water are listed in Table 4.1. Calcium and the other 3 base cations (Na^{+}, Mg^{2+}, and K^{+}) are important components that generally form most cation groups found in peat surface water (Bourbonniere et al., 2009). The level of calcium ions in our study was slightly higher than those of maximum concentration from peat bogs in Canada and northern USA (170 µM), northern and central Europe (125 µM), and from the subtropical peatlands in central China (73 µM). The concentrations of other base cations in our analysis were in the range determined in surface water from northern hemisphere bogs (Bourbonniere *et al*., 2009). The variations of major cations are probably dependent on the mineral rock fragments and ion exchange capacity of Sphagnum plants (Verry, 1975; Sjors and Gunnarsson, 2002). The presence of nitrate in surface peat water is possibly due to microbial nitrification under elevated surface water temperature and higher pH (Freeman *et al.*, 1993; Whitfield *et al.*, 2010). In comparison, nitrate concentration in surface water from Bogatsuru was relatively higher than those reported from northern and central Europe peat bogs (maximum concentration at 39 µM) (Bourbonniere *et al.*, 2009). However, the contribution and variation of nitrate content in the present study still need further investigation whether biological relevant or anthropogenic disturbances. It should be noted that this research has been interpreted only from the surface water chemistry profile (at a time point), the analysis of the physicochemical characteristic of subsurface waters would elucidate more environmental features clearer than the present limited information.

Table 4.1 Water chemistry characteristic

ORP (mV)	Acetate (µM)	K^+ (µM)	Na^+ (µM)	Ca^{2+} (µM)	Mg^{2+} (µM)	Fe^{3+} (µM)	F^- (µM)	Cl^- (µM)	NO_3^- (µM)	SO_4^{2-} (µM)
204	5.4	17	137	238	31	3	4	59	200	307

4.3.2. Microbial community in the surface layer (BO10)

The microbial community composition of the peat soils was analyzed using the 16S rRNA gene amplicon and the next-generation sequencing platform. It should be prior noted that the 16S rRNA gene sequencing could not capture all microorganisms in the samples due to the limitation of PCR amplification and sequencing (e.g., method of DNA extraction and purification, primer selection, and error from sequencing technology) (Schloss *et al.*, 2011). In this study, a total of 31,794 microbial sequences were obtained from peat soil samples (8,237 reads from BO10, 10,702 reads from BO45, and 12,855 reads from BO90). The taxonomic classification and relative abundance of the microorganisms are summarized in Figure 4.2. At the domain level, bacterial sequences were dominant at all depths. Taxonomic classification at the phylum level revealed that members of the phyla, Proteobacteria, Acidobacteriota, Planctomycetota, and Cyanobacteria, were dominant in the surface peat soil (Figure 4.2B).

Water analysis revealed that nitrate content (200 µM) was higher when compared to the analytical range of surface peat waters (0.3-39 µM) in northern peatland (Bourbonniere *et al.*, 2009), this may indicate the availability of nitrogen-transforming reactions in the Bogatsuru habitat. Microbial nitrogen-transformation pathways (e.g., nitrogen fixation, nitrification, and denitrification) involve diverse groups of microorganisms. Based on the microbial community profile and taxonomic classification, several groups of bacteria associated with nitrogen fixation and transformation were identified in this study. Taxonomic characterization at lower levels showed that bacterial sequences of the orders Rhizobiales and Planctomycetales were detected in the highest proportion in the surface peat soil (BO10). These bacterial groups have been reported to play a functional role in nitrogen cycling. Rhizobiales (Bradyrhizobium spp.) are nitrogen-fixing bacteria that generally live symbiotically with plant legumes (Kuypers *et al.*, 2018). Some members of Planctomycetota can oxidize ammonium anaerobically using nitrite as an electron acceptor (Fuerst, 2005; Fuerst and Sagulenko, 2011). Cyanobacteria, which were detected in only BO10, have also been shown to assimilate nitrogen for growth through nitrogenase catalysis (Berman-Frank *et al.*, 2003; Bothe *et al.*, 2010).

4.3.3 Microbial community in the middle and deep layers (BO45 and BO90)

Based on the results of soil gas analysis, CH_4 could only be detected in BO45 with a concentration of 0.27±0.14 mM. Referring to the 16S rRNA gene amplicon sequencing, archaeal sequences were detected in BO45 and BO90 (Figure 4.2A). The community compositions of the peat soil at depths of 45 and 90 cm were slightly different from those of surface samples, mainly Anaerolineales, Nitrospirales, Syntrophobacteriales, and the candidate order GIF9, which were detected in a high proportion (>500 sequences read counts) compared to the surface soil. Bacteria from the genus Nitrospira (belong to phylum Nitrospirota) were recently discovered to perform complete ammonia oxidation (comammox) (Daims *et al.*, 2015; van Kessel *et al.*, 2015). Further, diverse clades of comammox Nitrospira have been detected in the sediment along estuarine tidal flat wetlands (Sun *et al.*, 2020). Nitrospira members were dominant in a low dissolved oxygen reactor of the wastewater treatment system (Roots *et al.*, 2019) and were proven to be able to oxidize formate using nitrate as an electron acceptor under anoxic conditions (Koch *et al.*, 2015).

Other major bacterial sequences detected in the subsurface peats were affiliated with the phylum Chloroflexota (Figure 4.2B). Members of Chloroflexota have been identified in sediments and are suggested to be involved in the subsurface carbon cycle (Blazejak and Schippers, 2010; Kadnikov *et al.*, 2012). The metabolic lifestyles of Chloroflexota in sediments retrieved from genomic analyses include sugar and amino acid degradation, acetate utilization, and nitrate respiration and nitrification (Hug *et al.*, 2013).

The sequences of well-known methanogenic archaea in the orders Methanomicrobiales and Methanosarcinales were detected at a depth of 45 cm. Thermoplasmatales were also detected in the subsurface peats (BO45 and BO90). In addition, Ca. Bathyarchaeota sequences were highly observed in BO45 and BO90 compared to those other archaeal sequences. Other archaeal groups, including Diapherotrites, Hadesarchaea, Thaumarchaeota, and Woesearchaeota, were also detected in the subsurface peat soils based on taxonomic ranking characterization (Figure 4.3)

4.3.4 Microbial gene abundance

The distribution of microbial gene numbers along the vertical soil depth (Table 4.2) was quantified by qPCR using specific primer sets. Prokaryotic 16S rRNA gene numbers ranged from 2.56×10^8 to 8.73×10^8 genes g^{-1} peat. The archaeal 16S rRNA gene was lower than that of the prokaryotes at all soil depths, ranging from 2.81×10^6 to 2.58×10^7 genes g^{-1} peat. The abundance of archaeal genes was highest in the middle depth layer. Further, the ratio of archaeal 16S rRNA genes to prokaryotic 16S rRNA genes ranged from 0.4% to 2.9%, suggesting the low abundance of archaea at all depths.

The abundance of the *mcrA* gene was interpreted based on qPCR data analysis (melting curve comparison) and gel-electrophoresis confirmation (Appendix H), and *mcrA* genes were detected in only subsurface soils (45 and 90 cm) with the highest copy number of 3.91×10^6 genes g^{-1} peat at a depth of 45 cm. The *mcrA* gene numbers were higher than those previously observed in the subsurface peats (ranging from 10^4-10^5 genes g^{-1} peat) of wetlands in Hokkaido (Akiyama *et al.*, 2011). If we assume that archaea and methanogens carry one copy of the 16S rRNA and *mcrA* genes, respectively (Kembel *et al.*, 2012; Louca *et al*., 2018), Mcr-containing archaea in this study may have accounted for approximately 15% (calculated based on qPCR gene quantification) of the archaeal sequences at a depth of 45 cm. Furthermore, the high copy number of the *mcrA* gene corresponded with the detection of CH_4 at a depth of 45 cm from soil gas analysis, suggesting the production potential of CH_4 from methanogenic archaea. For bacterial methanotrophs, none of the *pmoA* gene sequences were detected based on PCR amplification with all primer pairs and gel electrophoresis confirmation.

Ca. Bathyarcheota 16S rRNA genes were detected at depths of 45 and 90 cm using the modified primers developed in this study (Appendix I), with copy numbers of 4.45×10^6 and 4.59×10^6 genes g^{-1} peat, respectively. If we assume that the copy number of the 16S rRNA gene of Ca. Bathyarchaeota is equal to 1, the ratio of Ca. Bathyarchaeota to archaea in BO45 and BO90 was 18% and 97%, respectively. This pointed to the distribution of Ca. Bathyarchaeota in the archaeal community in Bogatsuru wetland.

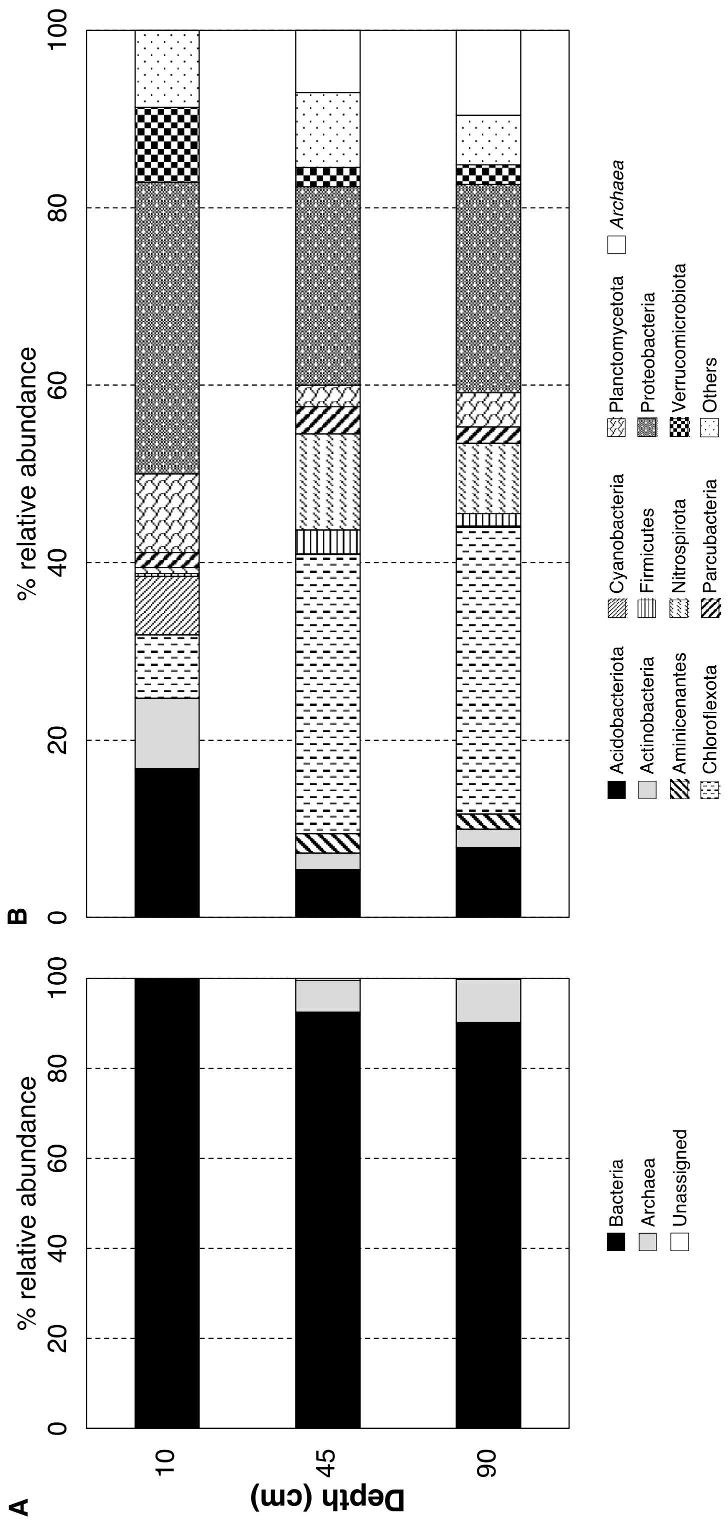

Figure 4.2 Microbial community compositions of the peat soils based on 16S rRNA gene amplicon analysis using next-generation sequencing. (A) Domain level. (B) Microbial diversity at the phylum level.

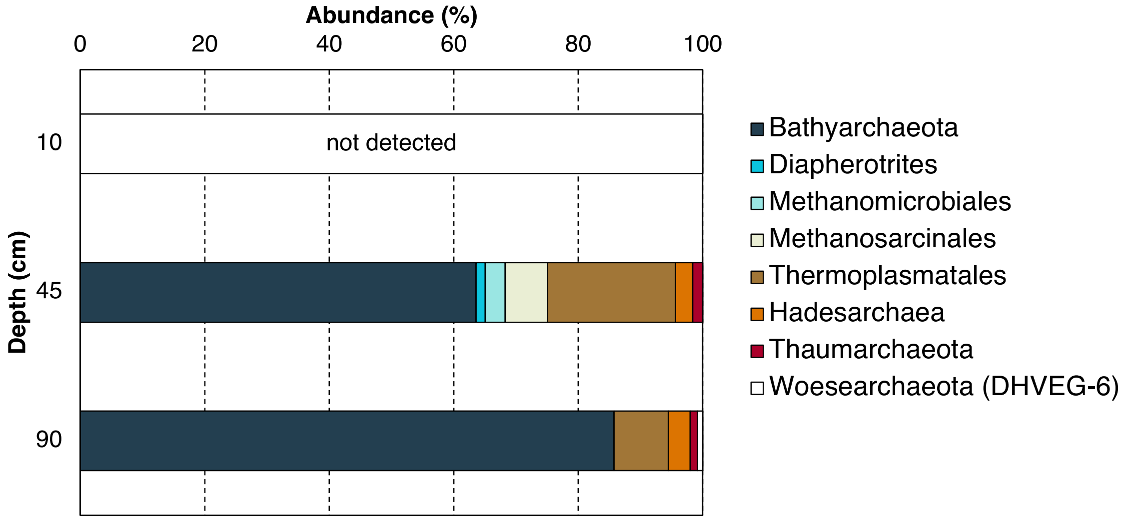

Figure 4.3 Archaeal community structures of the peat soils based on the 16S rRNA gene amplicon sequencing. None of archaeal sequences were detected at a depth of 10 cm.

Table 4.2 Microbial sequence count based on 16S rRNA gene amplicon sequencing and microbial gene abundance in the peat soils determined by qPCR.

Sample ID	Prokaryotic 16S rRNA (genes g^{-1} peat)	Archaeal 16S rRNA (genes g^{-1} peat)	*mcrA* (genes g^{-1} peat)	Bathyarchaeotal 16S rRNA (genes g^{-1} peat)
BO10	$7.21 \pm 1.62 \times 10^8$	$2.81 \pm 1.92 \times 10^6$	Not detected	Not detected
BO45	$8.73 \pm 6.07 \times 10^8$	$2.58 \pm 0.22 \times 10^7$	$3.91 \pm 1.82 \times 10^6$	$4.59 \pm 1.07 \times 10^6$
BO90	$2.56 \pm 3.10 \times 10^8$	$4.64 \pm 1.11 \times 10^6$	$6.36 \pm 2.10 \times 10^5$	$4.45 \pm 0.99 \times 10^6$

4.3.5 Phylogenetic composition of the *mcrA* gene

The phylogenetic diversity of *mcrA* was determined based on molecular cloning. The taxonomic classification of the mcrA sequences is shown in Figure 4.4A while the phylogenetic distribution is shown in Figure 4.4B. A total of 23 and 22 clones were obtained in the *mcrA* clone library of BO45 and BO90 samples, respectively. Based on the results, most of the mcrA nucleotide sequences obtained in the BO45 library were phylogenetically classified into Methanomicrobiales, which accounted for about 78% of the total *mcrA* clone sequences. Methanobateriaceae *mcrA* was dominant in the BO90, constituting about 59% of the total sequences. Methanogenic lineage in Methanosaetaceae was detected as a minority group at depths of 45 and 90 cm. This observation corresponded with previous records where Methanomicrobiales were found to be dominant in the wetland in Hokkaido, followed by a small proportion of Methanosaetaceae (Narihiro *et al.*, 2011). Methanomassiliicoccales accounted for 9% of the total *mcrA* clones in the deepest peat soil (BO90).

4.3.6 Phylogenetic composition and metabolic potential of Ca. Bathyarchaeota

Ca. Bathyarchaeota sequences obtained from the 16S rRNA gene amplicon analysis were aligned and affiliated with the phylogenetic tree of archaea (Figure 4.5). Based on phylogenetic analysis, the Ca. Bathyarchaeota detected in this study affiliated with various subgroups (Subgroup-5a, 5b, 5bb, 7, 9, 13, 17, and 18; Zhou *et al.*, 2018), indicating the diversity of this archaeal lineage in the terrestrial wetland ecosystem. Ca. Bathyarchaeota sequences have been previously detected in more than half of the archaeal populations in various peatlands (Rooney-Varga *et al.*, 2007; Hawkins *et al.*, 2014; Xiang *et al.*, 2017). Nonetheless, their ecological functions in peatland ecosystems are yet to be confirmed. Based on physiological and metagenomic characterizations, member of Ca. Bathyarchaeota possesses diverse trophic and metabolic potential including methanogenesis and/or anaerobic methane oxidation. They have been reported to utilize proteins, aromatic compounds, plant-derived carbohydrates, and lignin (Lloyd *et al.*, 2013; Meng *et al.*, 2014; Lazar *et al.*, 2016; Yu *et al.*, 2018) based on functional gene prediction and enrichment demonstration. However, the Ca. Bathyarchaeota sequences retrieved in this study form a divergence branch from the identified methane-metabolizing group BA1 (subgroup-3) and BA2 (subgroup-8) that have been proposed to encode methyl coenzyme M reductase (Evans *et al.*, 2015). Notably, the Ca. Bathyarchaeota showed a high proportion in archaea by qPCR quantification, this positively encourages the need for further studies. The future effort should focus on the characterization of metabolic capability, particularly the confirmation of Mcr-containing Ca. Bathyarchaeota.

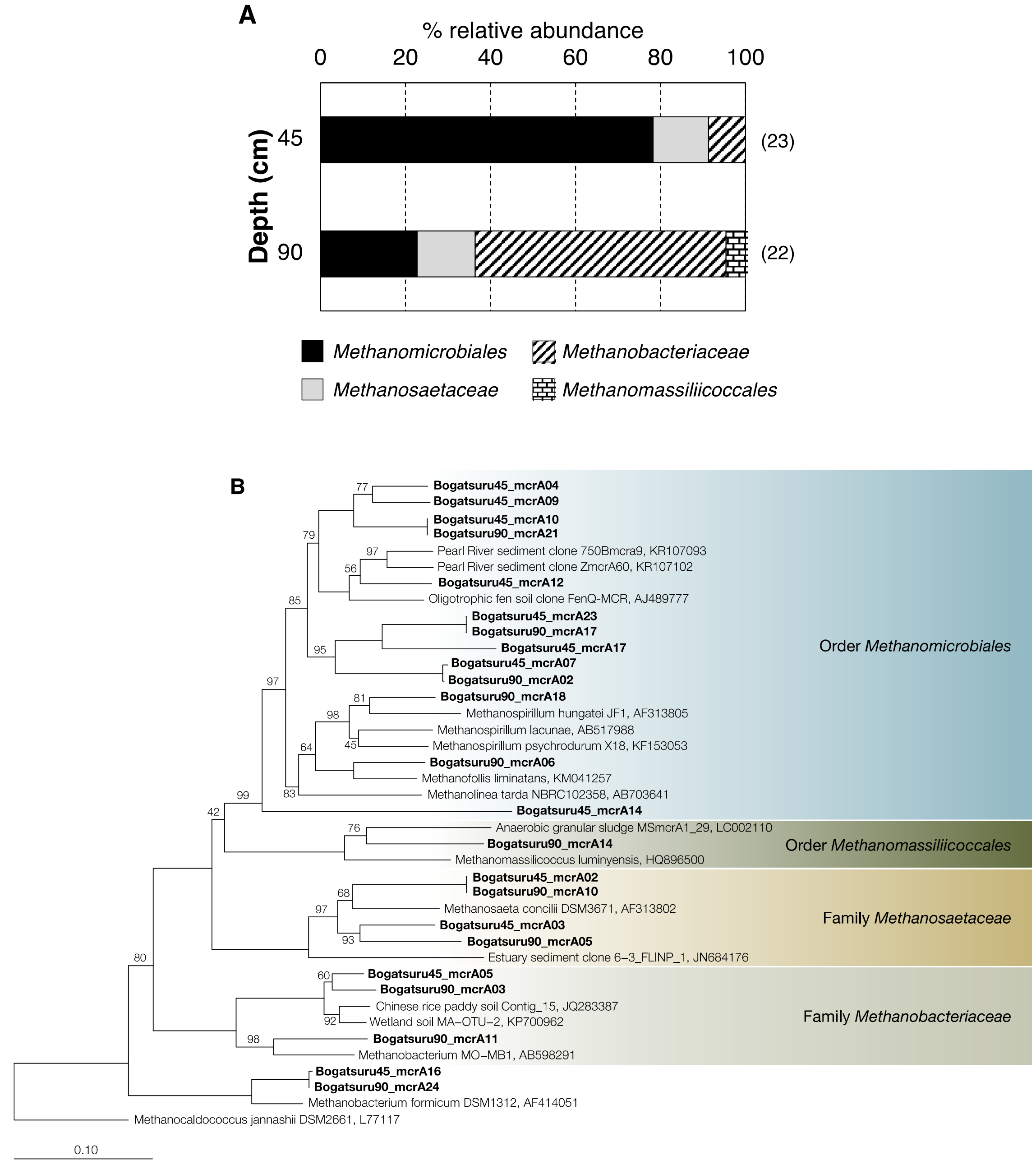

Figure 4.4 (A) Relative abundance of the *mcrA* phylotypes. Number of *mcrA* clones are indicate in bracket. (B) Molecular phylogenetic tree of the *mcrA* gene sequences detected in the peat soils constructed by the neighbor-joining method. Bootstrap values were computed with 1,000 replicates. The sequences obtained in this study are indicated in bold characters covering 2 orders and 2 families of Mcr-containing methanogenic archaea. Scale bar indicates the number of substitutions per site.

4.3.7 Methanogenic potential and biogeochemical interaction in wetland soils

The *mcrA* gene phylotype revealed that members of Methanomicrobiales, which are well-known hydrogenotrophic methanogens and generally reduce CO_2 to methane with H_2 and/or formate as the electron donor, were mainly detected in the present study. They have been found in diverse anaerobic natural habitats, such as freshwater and marine sediments, rice paddies, animal digestive tracts, and wetlands (Jabłoński *et al.*, 2015). The known Methanomicrobiales representatives that have been successfully isolated from peat include *Methanosphaerula palustris* E1-9c (Cadillo-Quiroz *et al.*, 2008; Cadillo-Quiroz *et al.*, 2009) and *Methanoregular boonei* 6A8 (Bräuer *et al.*, 2006). The minor groups of methanogenic archaea present in peat soils were Methanobacteriaceae (a member of Methanobacteriales) and Methanoseataceae (a member of Methanosarcinales). Methanobacteriaceae also perform CO_2 reduction coupled with H_2 oxidation for methanogenesis. However, Methanoseataceae are thought to be acetate utilizers, with acetate cleaved to form methane and carbon dioxide as terminal products through acetoclastic methanogenesis. A culture representative of Methanobacteriales and Methanosarcinales is Methanobacterium paludism SWAN1 (Cadillo-Quiroz *et al.*, 2014) and *Methanothrix thermoacetophila* PT (Kamagata *et al.*, 1992), respectively. The order Methanomassiliicoccales (also known as Rice cluster III), which was detected in the deepest soil in the present study, is known to be an obligate methylotroph that produces methane from the reduction of methanol, methyl sulfide, and methylated amines (Lang *et al.*, 2015, Paul *et al.*, 2012) with H_2. The representative of this order was first isolated from human feces and called *Methanomassiliicoccus luminyensis* B10 (Dridi *et al.*, 2012). Thermoplasmatales, which are members of the class Thermoplasmata, similar to Methanomassiliicoccales, were also detected based on 16S rRNA gene amplicon sequencing.

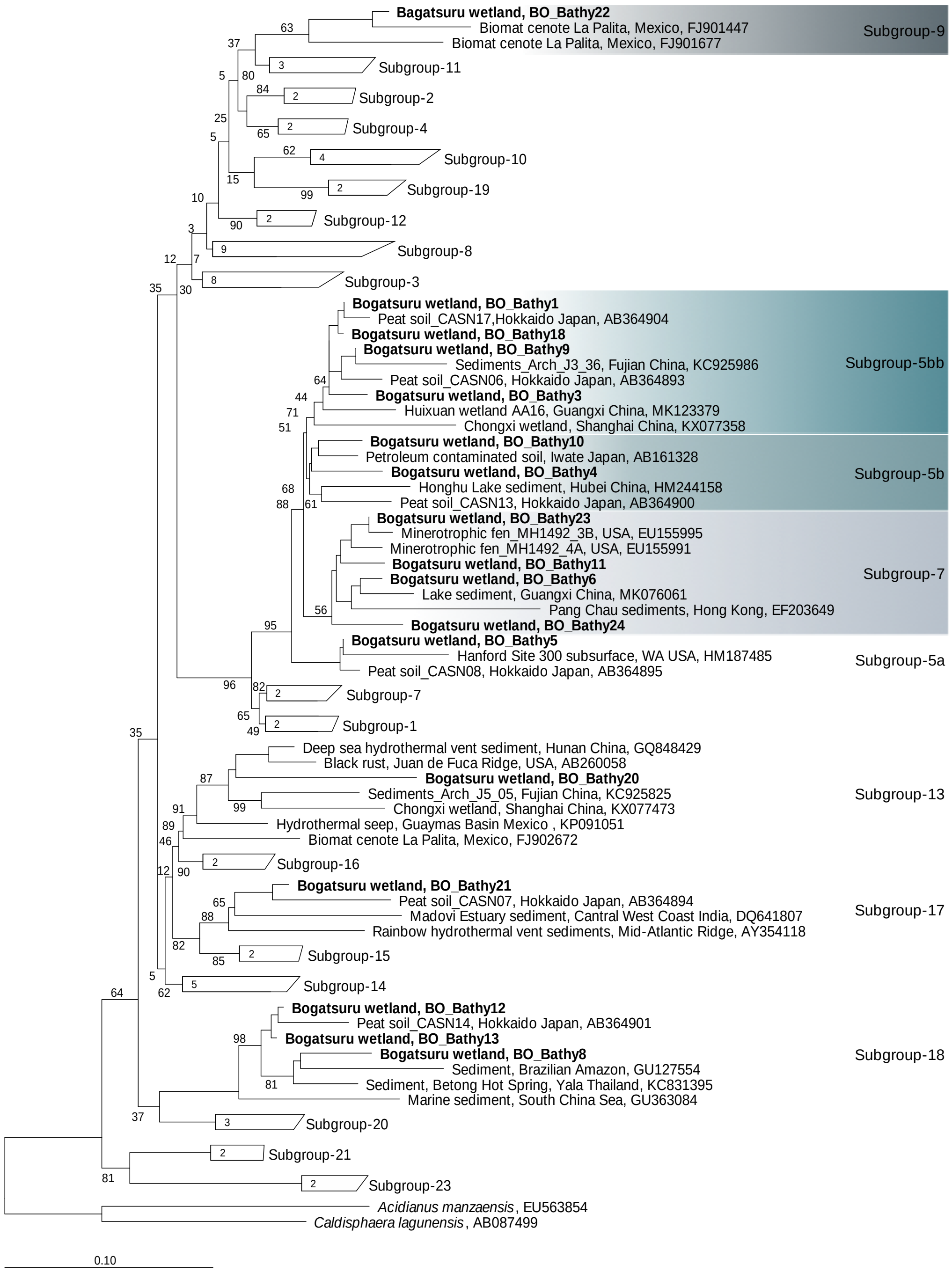

Figure 4.5 Molecular phylogenetic tree of the Ca. Bathyarchaeotal 16S rRNA gene sequences detected in the peat soils constructed by Maximum Likelihood method. Bootstrap values were computed with 1,000 replicates. The sequences obtained in this study are indicated in bold characters spanning in the Ca. Bathyarchaeota subgroups. The name of Ca. Bathyarchaeota subgroups were based on Zhou *et al*, 2018. Scale bar indicates the number of substitutions per site.

The detection of methanogenic archaea and a functional gene for methanogenesis in this study suggested the potential of methane production from peat soils utilization, either via hydrogenotrophic (CO_2/H_2) or acetoclastic (acetate) methanogenesis. In contrast, the net methane emissions from marine sediments and terrestrial environments can be neutralized by anaerobic methanotrophic archaea (ANME) via anaerobic oxidation of methane prior to escape into the atmosphere (Knittel and Boetius, 2009). Based on the 16S rRNA gene reads, none of ANME were detected in this study. Nonetheless, another group of microbes can utilize methane in the presence of oxygen, namely the aerobic methanotrophic bacteria (Dedysh and Knief, 2018). These organisms use methane monooxygenase (MMO) to convert methane to methanol. In fact, sequences of aerobic methanotrophic bacteria of the phylum Verrucomicrobiota (genus *Methylacidiphilum*) were detected at subsurface peats (BO45 and BO90). Genomic analyses have shown that the representative strains of *Methylacidiphilum* possess the monooxygenase as observed in methanotrophs in the phylum Proteobacteria, which demonstrates the capability to oxidize methane (Dunfield *et al*., 2007; Op den Camp *et al.*, 2009).

In anaerobic environments, methanogenic archaea compete with sulfate-reducing bacteria for available common substrates (Muyzer and Stams, 2008). Therefore, the presence of sulfate in such an environment is a key factor in trophic competition. In the present study, sulfate was detected based on the geochemistry of surface water (Table 4.1). The results of 16S rRNA gene amplicon sequencing revealed that the sulfate-reducing bacteria, Desulfobacca, affiliated with class Deltaproteobacteria, were dominant in anoxic subsurface peats (BO45 and BO90), as depicted by the constitution of relative high ratio of Deltaproteobacteria sequences (Appendix J). Calculation based on total Deltaproteobacteria sequence reads, *Desulfobacca* accounted for about 46% and 49% in BO45 and BO90, respectively. Bacterial isolates belonging to the genus *Desulfobacca* have been isolated from granular sludge (Göker *et al*., 2011). Further, the physiological analysis showed that they utilize acetate as the sole carbon source and sulfate as an electron acceptor. The competition between sulfate reducers and acetoclastic methanogens for acetate utilization may occur in the Bogatsuru wetland, as *Desulfobacca* and Methanoseataceae were detected in the present study.

Collectively, combined environmental omics approaches have been extensively proven as an advantageous strategy for identifying unknown microbial diversity, mainly in the context of exploring key players in biogeochemical processes. As methanogenic archaea in peatlands are difficult to culture due to, for example, their specific optimal growth requirements, potential syntrophic bacterial partners, environmental conditions, and generation time (Wolfe, 2011; Khelaifia *et al.*, 2013; Narihiro and Kamagata, 2013; Carson *et al.*, 2019), the recovery of genomic data may reveal taxonomic profiles and imply their functional properties. Further challenges should focus on the cultivation and isolation of uncultured methanogens and microbial syntrophs that will likely guide our understanding and provide important information on microbial physiology and their functions that could provide feedback regarding the global methane and carbon cycle.

4.4 Conclusions

In this study, cultivation-independent molecular analyses based on the 16S rRNA gene amplicon and functional *mcrA* gene were used to evaluate key microbial groups and their potential capabilities involving metabolic methane production. Members of the well-known methanogenic archaea were detected, which corresponded with the detection of the *mcrA* gene in anoxic subsurface peats. Members of Ca. Bathyarchaeota, the yet uncultivated archaea that are thought to play an important role in biogeochemical cycles, was highly detected in this study. Thus, such findings prompted further development of culturing innovations (culture-based experiments) and complete genomic characterization, which will be useful for providing comprehensive metabolic insights into Ca. Bathyarchaeota.

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CHAPTER V

BOGATSURU METAGENOME

5.1 Introduction

According to the 16S rRNA gene-based sequencing cannot capture all microbial sequences and is unable to visualize the actual microbial community in the sample owing to the amplification biases, advance metagenomic sequencing techniques, thus, have been adopted into microbiology study to address those technical limitations. Notably, metagenome-assembled genomes (MAGs) and single-amplified genomes (SAGs) obtained from the metagenomic and single-cell genomes, respectively, can effectively be used to identify entire complex microbial communities (at species or strain levels). For the metagenomic workflow (Figure 5.1), the genetic material (DNA) from a mixed community of microorganisms is isolated and mixed together as a pool of DNA from all the community members. Isolated DNA fragments are then multiplied by amplification and sequenced (sometimes called 'read'). Based on the sequencing technique (i.e., shotgun sequencing) for short reads, the sequenced DNA is assembled into long continuous sequences (called 'contig') and subsequently grouped (binning) into a single microbial genome (called 'genomic bin'). Then, genomic bins are classified and annotated for identifying genes and enzymes present in the sample as well as inferring metabolic capability. These developed technologies have enabled genomic data and revealed the metabolic potential of previously unidentified archaeal lineages (Table 5.1). For instance, they have been identified as important players in carbon and nitrogen cycles, as they are capable of methanogenesis and anaerobic methane oxidation, ammonia oxidation as well as the Wood-Ljungdahl (WL) carbon fixation pathway (Pester *et al*., 2011; Stahl *et al*., 2012; Offre *et al*., 2013). As archaeal diversity is known to dominate by uncultured groups (Figure 5.2; Lewis *et al*., 2020), accessing the genomic information of these undescribed lineages directly from nature without the need for cultivation likely provides an important new outlook on the diversity and ecological function of the domain Archaea.

The analysis of microbial communities detected in wetland peat soils (Chapter IV) based on taxonomic marker genes for bacteria and archaea classification showed a high proportion of archaea phylum Bathyarchaeota in the peat soils. Also, members of this archaeal phylum are known to contain some of the few putative methanogenic archaea lineages from outside the Euryarchaeota. Therefore, wetland peat soil was selected for further in-depth studies by using metagenomic platforms and computational models to confirm the actual microbial diversity, which was observed in amplicon sequencing (Chapter IV), and describe metabolic functions of Bathyarchaeota (in peat soil), in particular, the potential role for methane production.

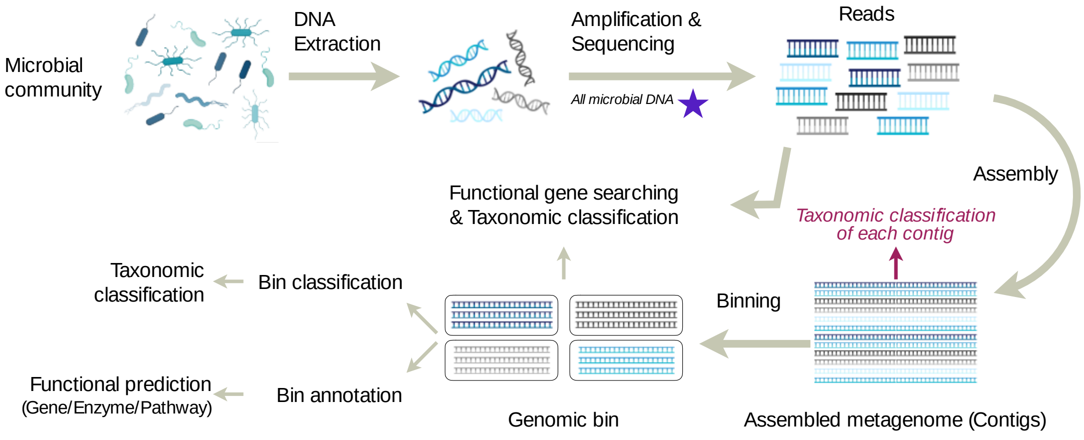

Figure 5.1 Genome-resolved metagenomic workflow for accessing genomic information of unculture microorganisms and metabolic pathways prediction. Microbial DNA is extracted from mixed microorganisms for amplification and sequencing. The sequence reads are assembled back into partial- or complete microbial genome for interpreting genomic features and functions.

Table 5.1 Candidate new phyla revealed by using MAGs or SAGs (Jiao *et al*., 2021)

Candidate phylum	**MAG or SAG**	**Environmental description**	**References**
Aenigmarchaeota (DSEG)	SAG	Homestake Mine	Rinke *et al*., 2013
Aigarchaeota (pSL4; HWCG-I)	MAG, SAG	Geothermal water stream from a subsurface mine in Japan	Takami *et al*., 2012; Rinke *et al*., 2013
Bathyarchaeota (MCG)	SAG	Marine sediment	Lloyd *et al*., 2013
Diapherotrites (pMC2A384)	SAG	Homestake Mine	Rinke *et al*., 2013
Geoarchaeota	MAG	Acidic iron mats in Yellowstone National Park	Kozubal *et al*., 2013
Heimdallarchaeota	MAG	Marine sediments (Loki's Castle and Aarhus Bay)	Zaremba-Niedzwiedzka *et al*., 2017
Korarchaeota	MAG	Obsidian Pool, Yellowstone National Park	Hugenholtz *et al*., 1998
Lokiarchaeota	MAG	Arctic Mid-Ocean Ridge	Spang *et al*., 2015
Nanoarchaeota	MAG	Submarine hot vent	Huber *et al*., 2002
Nanohaloarchaeota	MAG, SAG	Ponds of Bras del Port salterns, Spain	Ghai *et al*., 2011
Nezhaarchaeota	MAG	Jinze Hot Spring, Yunnan, China	Wang *et al*., 2019
Odinarchaeota	MAG	Hot spring (Yellowstone National Park and Radiata Pool)	Zaremba-Niedzwiedzka *et al*., 2017
Pacearchaeota	MAG	Aquifer adjacent to the Colorado River	Castelle *et al*., 2015
Parvarchaeota (ARMAN)	MAG, SAG	A drift of Richmond Mine, Northern California	Rinke *et al*., 2013
Thorarchaeota	MAG	White Oak River estuary sediments	Seitz *et al*., 2016
UAP1-3	MAG	Assembled from public metagenomes	Parks *et al*., 2017
Verstraetearchaeota	MAG	Cellulose-degrading anaerobic digesters	Vanwonterghem *et al*., 2016
Woesearchaeota	MAG	Aquifer adjacent to the Colorado River	Castelle *et al*., 2015

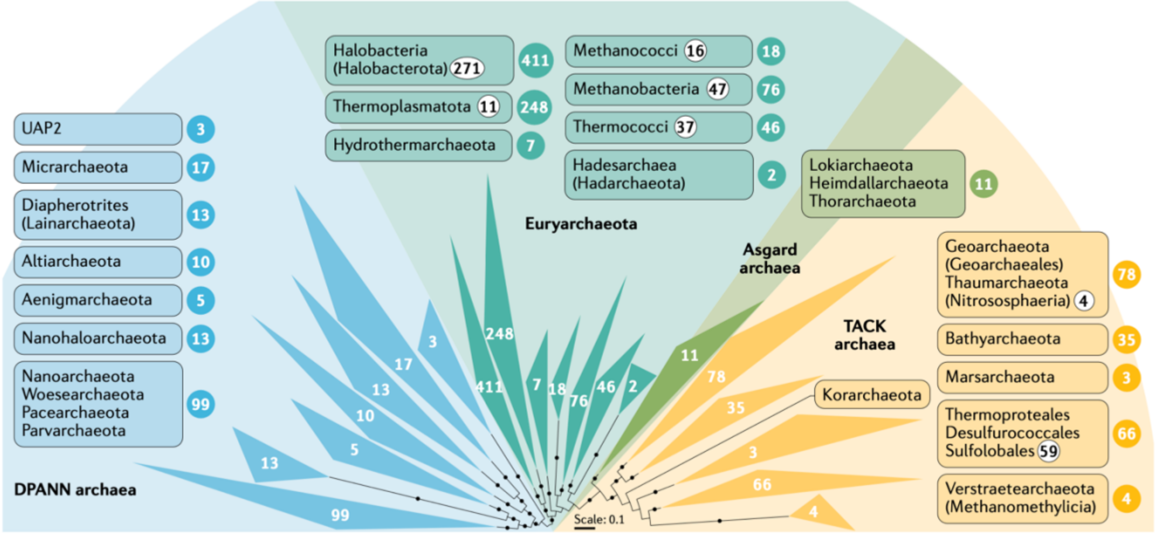

Figure 5.2 A phylogenetic species tree for Archaea, inferred from concatenated alignments of a minimum of 5 out of the total 15 ribosomal proteins per species, encoded by 1,166 archaeal genomes that were obtained from the Genome Taxonomy Database (Parks *et al*., 2018). Numbers in white font in coloured circles are the number of individual taxa in each collapsed clade and are also used to connect corresponding taxa names to clades. Numbers in black font in white ellipses next to taxa names indicate the total number of species-level cultured isolates described for those taxa, based on the number of species type strains assigned to each clade that are present in the BacDive database (last accessed 6 April 2020). Taxa without numbers have no culture isolates recorded in BacDive (Reimer *et al*., 2018).

5.2 Materials and experimental methods

5.2.1 DNA preparation and sequencing

The extracted prokaryotic DNA of the peat soil sample (BO45), as previously described in section 4.2.3, was quantified using Qubit dsDNA HS Assay Kit (Invitrogen, USA). The peat soil sample (BO45) was selected for metagenomic analysis based on the previous quantitative microbial gene analysis, in which Bathyarchaeotal 16S rRNA gene sequences were highest. DNA library was constructed using the QIAseq FX DNA Library

Kit (Qiagen), according to the manufacturer's instructions. The libraries were sequenced on an Illumina Miseq sequencing platform (2 × 300 paired-end).

5.2.2 De novo assembly and binning of metagenomic sequences

The raw shotgun sequencing metagenomic reads were trimmed using Trimmomatic v0.36 (Bolger *et al*., 2014) and were checked for quality using FastQC v0.11.9 (Andrew, 2010). The small-subunit rRNA (SSU rRNA) gene in the trimmed metagenome reads was assessed to profile microbial community structure using phyloFlash v3.4 (Gruber-Vodicka *et al*., 2020), in which the SSU Ref NR99 database from the SILVA release 138 used as the reference for the taxonomic assignment (Quast *et al*., 2013). Metagenomic reads were processed by the MetaWRAP pipeline (Uritskiy *et al*., 2018), in which the qualified pair-end reads were assembled into contigs using either metaSPAdes v3.15.3 (Nurk *et al*., 2017) or MegaHit (Li *et al*., 2016). Contigs were automatically binned using either MaxBin2 v2.2.4 (Wu *et al*., 2016), metaBAT2 (Kang *et al*., 2019) or CONCOCT (Alneberg *et al*., 2014). The quality of genomic bins was assessed using CheckM v1.0.18 (Parks *et al*., 2015). Also, an assembly was taxonomically classified with the Kraken (Wood *et al*., 2014) module, providing community taxonomy at the assembled level (Appendix K).

5.2.3 McrA gene identification using GraftM

Reads of the *mcrA* gene in the unassembled metagenome sequence were searched and classified using GraftM v0.14.0 (Boyd *et al*., 2018) with the *mcrA*-specific GraftM package (gpkg) as the reference database (downloaded from https://data.ace.uq.edu.au/public/graftm/7/).

5.2.4 Genome annotation

Obtained genomes were annotated using Prokka v1.14.5 (Seemann, 2014), RASTtk v1.073 (Brettin *et al*., 2015), and DRAM v0.1.0 (Shaffer *et al*., 2020) frameworks, with default parameters. Some of the predicted coding sequences (CDS) and functional annotations were manually inspected owing to the annotations curated by more than one source database.

5.3 Results and discussion

5.3.1 Microbial abundance and overall community structures of peat soil metagenome

Taxonomic affiliation of SSU rRNA reads was mapped based on the SILVA database and classified using phyloFlash. Within the domain Archaea, Bathyarchaeota were highly presented, accounting for about 46% of the total archaeal sequences (Figure 5.3). This observation was consistent with the previous assessments of microbial community structure based on the 16S rRNA gene amplicon sequencing (section 4.3.3). Archaea in the superphylum DPANN, particularly Woesearchaeales and Aenigmarchaeota, were detected in a minority, accounting for about 8% and 6% of the total archaeal sequences, respectively. Members of Methanomassiliicoccales, Methanosarcinales, Methanomicrobiales, and Thermoplasmata, were also detected among archaeal diversities. In addition to Archaea, bacterial members belonging to the Anaerolineales (class of the phylum Chloroflexi) were the most dominant group, which account for about 7% of the total bacterial sequences. Other bacterial phyla (relative abundance ordering) include Thermodesulfovibrionia, Desulfobaccales, GIF9, Sva0485, and GIF3. Notably, because as diverse as 158 identified bacterial taxa, which show a relative abundance of less than 2%, an affiliation of “Others” is thus represented in a high proportion (Figure 5.3).

Co-occurrence of proposed alkane-oxidizing archaea (Helarchaeota), which contain MCR-like complex, and sulfate-reducing bacteria (Desulfobacterota), was recently reported (Zhao *et al.*, 2021). They have been suggested to engage syntrophic relationships in subseafloor sediments for hydrocarbon cycling. Furthermore, the phylogenetic tree of mcrA sequences of Helarchaeota forms the divergent cluster, with the monophyletic cluster together with a homolog of Hadesarchaea, Bathyarchaeota, and Ca. Methanoliparia (Zhao *et al.*, 2021). Bathyarchaeota, which have also been deduced as the short-chain-alkane-oxidizing archaea (Borrel *et al.*, 2019), may involve in hydrocarbon transformation in the wetland environment, as their sequences were detected together with the sulfate-reducing bacteria. However, the syntrophic relationship between anaerobic methane-oxidizing archaea and sulfate-reducing bacteria is known to be complex interaction, requiring further experimental investigation.

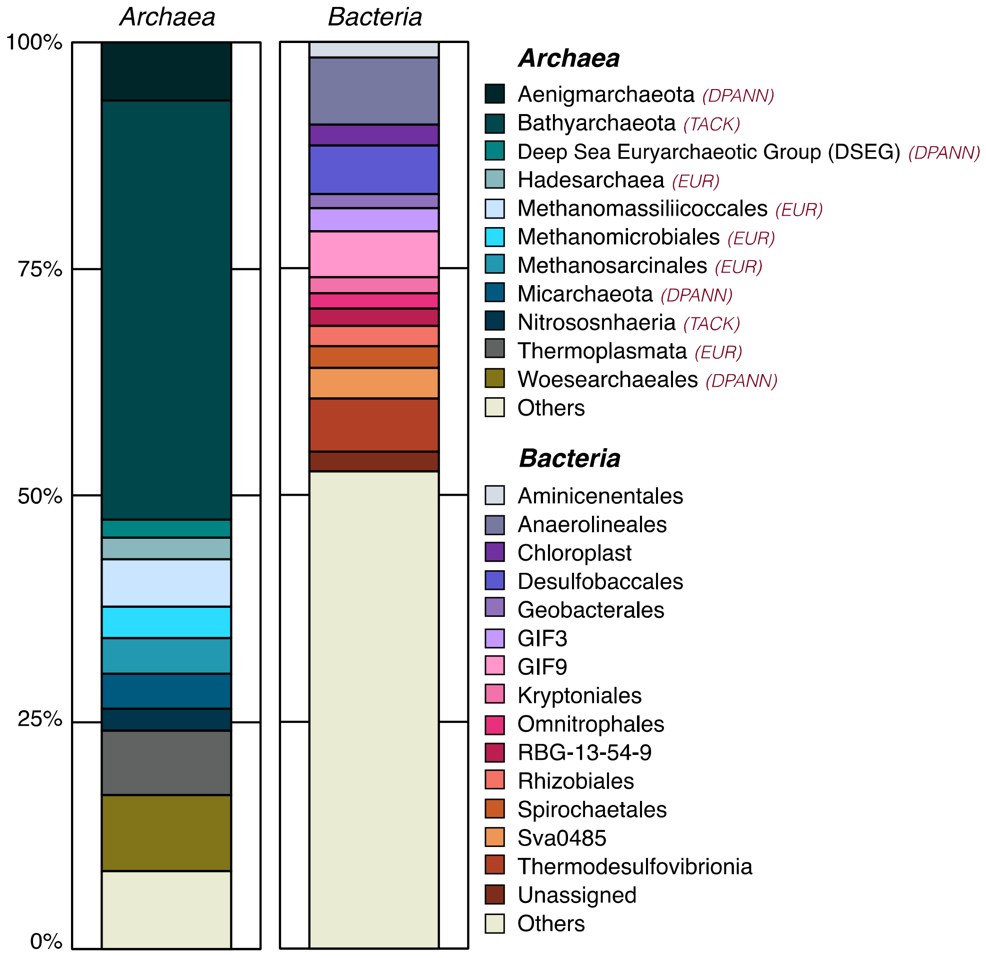

Figure 5.3 Microbial community composition based on a reconstruction of full-length SSU rRNA sequences from unassembled reads in the metagenomes mapped to the SILVA database (132 release) and classified using phyloFlash v3.4. Eukaryotes reads are not included. Taxa less than 2% relative abundance was grouped into 'Others'. Sequences that could not be assigned to a taxonomic group were affiliated to 'Unassigned'.

5.3.2 Genetic feature and metabolic reconstruction of Bathyarchaeota

De novo assembly of metagenomic reads and binning revealed three archaeal genomic bins, in which Bathyarchaeota were taxonomically classified within the bin (Table 5.2). Metabolic reconstruction revealed that Bathyarchaeota bins (Refined bins 12, 13, and

16) identified in the present study contain genetic materials for their energy metabolism, in particular, methane formation through acetate and/or CO_2 reduction (Table 5.3 and Figure 5.4). They possess genes for methanogenesis from CO_2 and H_2, including formylmethanofuran dehydrogenase (Fwd/Fmd), and methylene-H_4MPT dehydrogenase (Mtd). Genes for acetoclastic methanogenesis including acetate kinase (Ack) and the carbon monoxide dehydrogenase/acetyl-CoA synthase (Codh-Acs) complex, were detected in genomic bins. In addition, Bathyarchaeota bins also contain genes (HdrABC and MvhADG) encoding for a heterodisulfide reductase-F_{420} non-reducing hydrogenase electron bifurcating complex, which is required for the cycling of coenzyme M (CoM) and coenzyme B (CoB) with H_2, and reduction of ferredoxin (Fd). It should be noted that the key gene for methanogenesis (*mcrA*) was not identified in the Bathyarchaeota bins. However, based on a functional gene searching from an unassembled metagenome (GraftM), partial fragments of the *mcrA* gene could be recovered. Taxonomic assignment of methyl coenzyme M reductase alpha subunit (*mcrA*) indicated the presence of methane-metabolizing archaea belonged to the Bathyarchaeota and the closely related group that divert from the well-known methanogens (Figure 5.5). Furthermore, these *mcrA* sequences were likely matched to *mcrA* sequences of Bathyarchaeota (BA1 and BA2) from previous research (Appendix L). This provides another gene-based evidence of the putative *mcrA*-containing Bathyarchaeota since first published previously (Evans *et al*., 2015). Additionally, the proton-translocating ATPase (Atp) was also present in the genomic bin, suggesting that they may generate energy forcing by the H^+ gradient

Table 5.2 Overview of genomic bins that Bathyarchaeota contigs were identified

Bin ID	Completeness	Contamination	Strain heterogeneity
Refined 12	32.60%	5.61%	14.29%
Refined 13	43.23%	4.37%	33.33%
Refined 16	21.41%	0%	0%

Table 5.3 Summary of annotated genes and protein products that matched the Bathyarchaeota contigs (Appendix J) based on using RAST and DRAM annotations.

RAST annotation										
	Contigs						position	Bathy hit	Product	Gene
Refined_12	NODE	610	length	4414	cov	4.692819	3	/	heterodisulfide reductase, subunit A/methylviologen reducing hydrogenase, subunit delta;rbs	HdrA/MvhD
	NODE	1264	length	3441	cov	5.701418	3	/	Methylene tetrahydromethanopterin dehydrogenase (EC 1.5.99.9);rbs	Mtd
	NODE	1572	length	3208	cov	6.31589	5		Nickel-dependent hydrogenase, large subunit;rbs	
	NODE	1680	length	3121	cov	5.493151	3		V-type ATP synthase subunit K (EC 3.6.3.14);rbs	
	NODE	1760	length	3073	cov	5.401259	4		Acetyl-CoA synthetase (ADP-forming) alpha and beta chains, putative;rbs	
	NODE	1811	length	3051	cov	4.013351	5		Heterodisulfide reductase, cytochrome reductase subunit;rbs	
	NODE	3898	length	2382	cov	5.111302	1	/	CoB--CoM heterodisulfide reductase subunit A (EC 1.8.98.1);rbs	HdrA
	NODE	4386	length	2289	cov	5.337511	2		CoB--CoM heterodisulfide reductase subunit B (EC 1.8.98.1);rbs	
	NODE	5710	length	2086	cov	5.701625	1		V-type ATP synthase subunit A (EC 3.6.3.14);rbs	
	NODE	5813	length	2075	cov	5.510891	1		CO dehydrogenase/acetyl-CoA synthase subunit alpha, CO dehydrogenase subcomplex (EC 1.2.99.2);rbs	
	NODE	8143	length	1842	cov	3.385562	1	/	CoB--CoM heterodisulfide reductase subunit A (EC 1.8.98.1);rbs	HdrA
Refined_13	NODE	5219	length	2154	cov	6.345879	1		CoB--CoM heterodisulfide reductase subunit A (EC 1.8.98.1);rbs_motif	
	NODE	4383	length	2290	cov	4.509172	2		F420-dependent methylenetetrahydromethanopterin dehydrogenase (EC 1.5.99.9);rbs_motif	
	NODE	4383	length	2290	cov	4.509172	1		Formylmethanofuran--tetrahydromethanopterin N-formyltransferase (EC 2.3.1.101);rbs_motif	
	NODE	4320	length	2301	cov	3.743989	2		Acetyl-CoA synthase corrinoid iron-sulfur protein, large subunit;rbs_motif	
	NODE	4282	length	2308	cov	3.948069	1		Acetyl-coenzyme A synthetase (EC 6.2.1.1);rbs_motif	
	NODE	387	length	5315	cov	4.78403	5	/	Acetyl-CoA synthetase (ADP-forming) alpha and beta chains, putative;rbs_motif	Acs
	NODE	3493	length	2475	cov	4.132645	1		Acetyl-CoA synthetase (ADP-forming) alpha and beta chains, putative;rbs_motif	
	NODE	2869	length	2643	cov	5.651468	3		Formylmethanofuran dehydrogenase subunit A (EC 1.2.99.5);rbs_motif	
	NODE	2869	length	2643	cov	5.651468	1		N(5),N(10)-methenyltetrahydromethanopterin cyclohydrolase (EC 3.5.4.27);rbs_motif	
	NODE	2564	length	2733	cov	4.551531	1	/	CoB--CoM heterodisulfide reductase subunit A (EC 1.8.98.1);rbs_motif	HdrA
	NODE	2564	length	2733	cov	4.551531	2		CoB--CoM heterodisulfide reductase subunit D (EC 1.8.98.1);rbs_motif	
	NODE	1918	length	2996	cov	6.961238	2		CoB--CoM heterodisulfide reductase subunit A (EC 1.8.98.1);rbs_motif	
	NODE	1907	length	3002	cov	7.894469	2		CoB--CoM heterodisulfide reductase subunit A (EC 1.8.98.1);rbs_motif	
	NODE	1907	length	3002	cov	7.894469	3		CoB--CoM heterodisulfide reductase subunit D (EC 1.8.98.1);rbs_motif	
	NODE	11437	length	1632	cov	2.660748	1		CO dehydrogenase accessory protein CooC (nickel insertion);rbs_motif	
Refined_16	NODE	4323	length	2300	cov	5.616927	2		Acetyl-CoA synthetase (ADP-forming) alpha and beta chains, putative;rbs_motif	
	NODE	2841	length	2650	cov	4.264355	1		Acetyl-coenzyme A synthetase (EC 6.2.1.1);rbs_motif	
	NODE	5107	length	2171	cov	2.729679	1	/	CO dehydrogenase/acetyl-CoA synthase subunit alpha, CO dehydrogenase subcomplex (EC 1.2.99.2);rbs_motif	CODH/ACS
	NODE	415	length	5139	cov	3.900865	5		CoB--CoM heterodisulfide reductase subunit A (EC 1.8.98.1);rbs_motif	
	NODE	415	length	5139	cov	3.900865	4		CoB--CoM heterodisulfide reductase subunit B (EC 1.8.98.1);rbs_motif	
	NODE	415	length	5139	cov	3.900865	3		CoB--CoM heterodisulfide reductase subunit C (EC 1.8.98.1);rbs_motif	
	NODE	2801	length	2661	cov	2.930161	3	/	Heterodisulfide reductase, cytochrome reductase subunit;rbs_motif	HdrDE

Table 5.3 (continue)

DRAM annotation										
	Contigs						**position**	**Bathy_hit**	**Product**	**Gene**
	NODE	610	length	4414	cov	4.692819	3	/	F420-non-reducing hydrogenase iron-sulfur subunit [EC:1.12.99.- 1.8.98.5 1.8.98.6]	MvhDG
Refined_12	NODE	610	length	4414	cov	4.692819	4	/	heterodisulfide reductase subunit A2 [EC:1.8.7.3 1.8.98.4 1.8.98.5 1.8.98.6]	HdrA
	NODE	610	length	4414	cov	4.692819	2	/	heterodisulfide reductase subunit C2 [EC:1.8.7.3 1.8.98.4 1.8.98.5 1.8.98.6]	HdrC
	NODE	1062	length	3643	cov	5.646042	5	/	membrane-bound hydrogenase subunit alpha [EC:1.12.7.2]	MbhL
	NODE	1062	length	3643	cov	5.646042	4		membrane-bound hydrogenase subunit beta [EC:1.12.7.2]	
	NODE	1062	length	3643	cov	5.646042	3	/	membrane-bound hydrogenase subunit mbhJ [EC:1.12.7.2]	MbhJ
	NODE	1552	length	3224	cov	4.137267	3		heterodisulfide reductase subunit D [EC:1.8.98.1]	
	NODE	1596	length	3191	cov	3.602679	7	/	formylmethanofuran dehydrogenase subunit E [EC:1.2.7.12]	FwdE
	NODE	1680	length	3121	cov	5.493151	2	/	V/A-type H+/Na+-transporting ATPase subunit C	AtpC
	NODE	1680	length	3121	cov	5.493151	4	/	V/A-type H+/Na+-transporting ATPase subunit I	AtpI
	NODE	1680	length	3121	cov	5.493151	3		V/A-type H+/Na+-transporting ATPase subunit K	
	NODE	1861	length	3023	cov	3.79717	1	/	7,8-dihydro-6-hydroxymethylpterin dimethyltransferase [EC:2.1.1.-]	MPT synthesis
Refined_13	NODE	2684	length	2695	cov	5.079167	1		5,6,7,8-tetrahydromethanopterin hydro-lyase [EC:4.2.1.147]	
	NODE	2717	length	2686	cov	8.736222	3	/	7,8-dihydro-6-hydroxymethylpterin dimethyltransferase [EC:2.1.1.-]	MPT synthesis
	NODE	668	length	4294	cov	4.23331	3		trimethylamine---corrinoid protein Co-methyltransferase [EC:2.1.1.250]	
	NODE	1907	length	3002	cov	7.894469	3		F420-non-reducing hydrogenase iron-sulfur subunit [EC:1.12.99.- 1.8.98.5 1.8.98.6]	
	NODE	1907	length	3002	cov	7.894469	2		heterodisulfide reductase subunit A2 [EC:1.8.7.3 1.8.98.4 1.8.98.5 1.8.98.6]	
	NODE	1941	length	2986	cov	3.934835	3		trimethylamine---corrinoid protein Co-methyltransferase [EC:2.1.1.250]	
	NODE	2564	length	2733	cov	4.551531	3	/	F420-non-reducing hydrogenase iron-sulfur subunit [EC:1.12.99.- 1.8.98.5 1.8.98.6]	MvhDG
	NODE	2869	length	2643	cov	5.651468	3		formylmethanofuran dehydrogenase subunit A [EC:1.2.7.12]	
	NODE	2869	length	2643	cov	5.651468	2	/	formylmethanofuran dehydrogenase subunit C [EC:1.2.7.12]	FwdC
	NODE	2869	length	2643	cov	5.651468	1		methenyltetrahydromethanopterin cyclohydrolase [EC:3.5.4.27]	

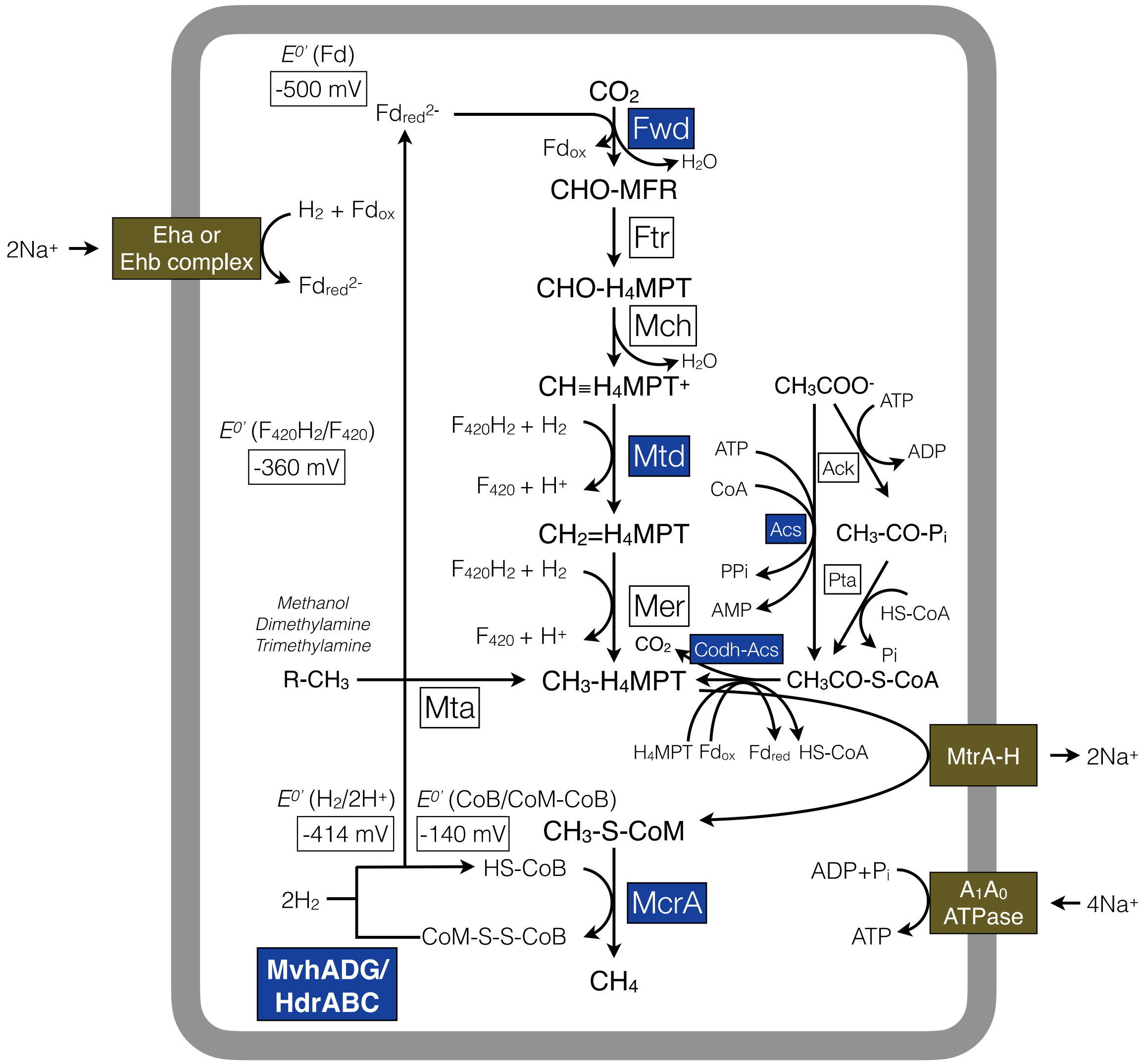

Figure 5.4 Overview of pathways reconstructed in Bathyarchaeota. Genes related to the methane metabolisms via CO_2 reduction (archaeal Wood-Ljungdahl), acetate fermentation, and electron bifurcation found in Bathyarchaeota peat soil metagenome are highlighted in blue.

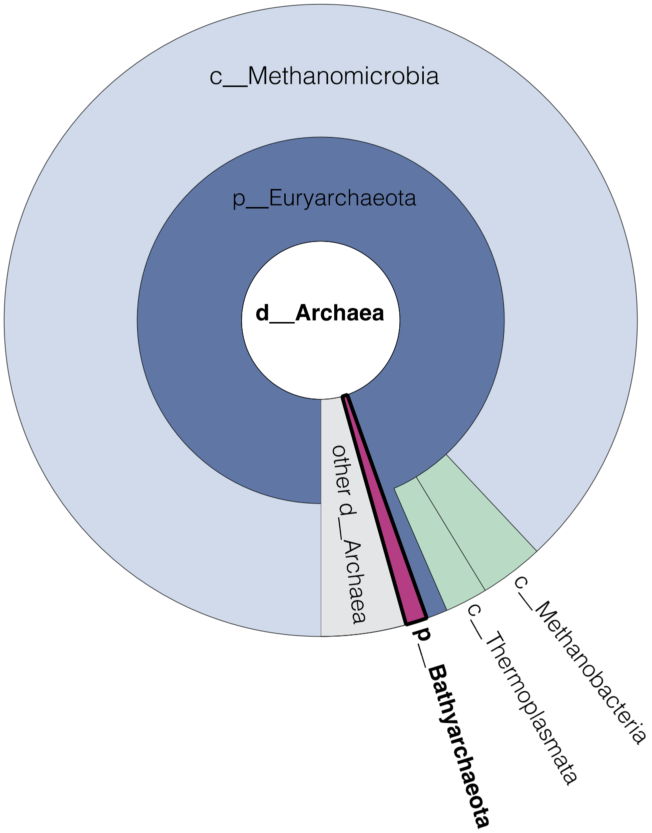

Figure 5.5 Taxonomic classification of methyl coenzyme M reductase alpha subunit (*mcrA*) found in the peat soil unassembled metagenome analyzed by GraftM. Taxonomic levels were assigned as d (domain), p (phylum), and c (class).

5.4 Conclusions

This work reveals the partial fragments of the mcrA gene, a key gene for methanogenesis, by using a functional gene search against metagenomic sequences, suggesting the methane production potential of the Bathyarchaeota in the wetland ecosystem. Genes associated with methane formation through CO_2/H_2 and/or acetate, and energy conservation, were also present in the Bathyarchaeota genomic bins. Nonetheless, owing to the metagenome-assembled genomes in this study could not entirely recover the *mcrA* gene of Bathyarchaeota, further genome sampling still be needed for the comprehensive conclusion. Furthermore, its metabolic capability on methane or alkane metabolism, as a recently published discussion, remains to be confirmed.

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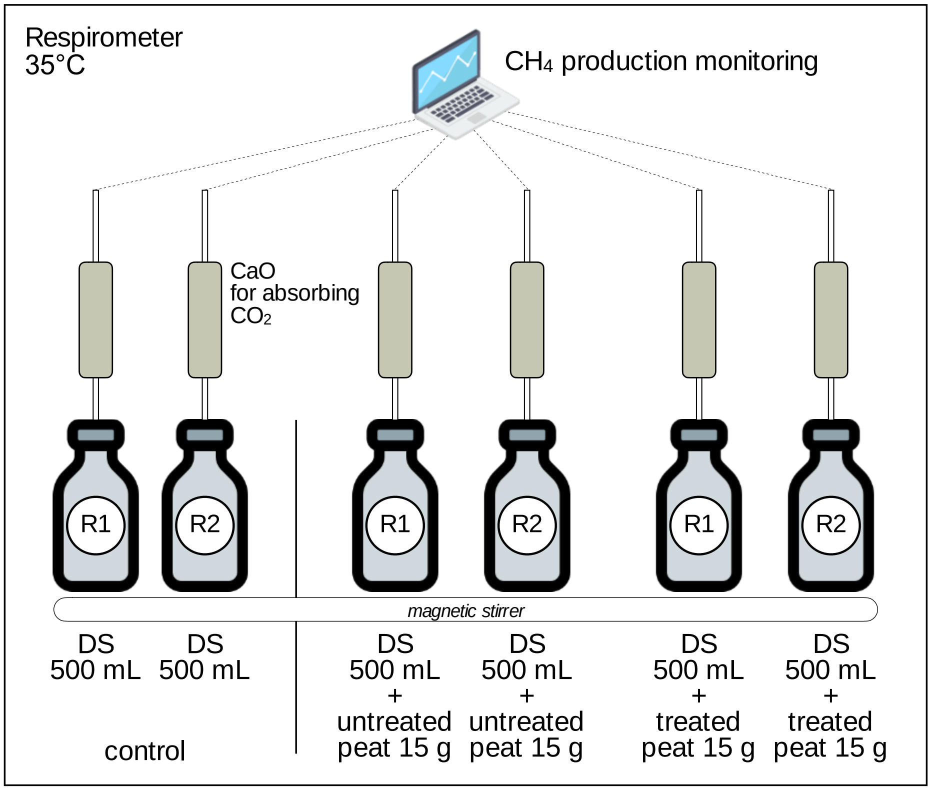
CHAPTER VI

BIOGENIC METHANE PRODUCTION FROM PEAT BIOMASS

To examine the production potentials of methane from peat biomass, a dried commercial peat moss (PROTOLEAF, US) with pH 3.57 was used as a methanogenic substrate. A dried sample was treated with $Ca(OH)_2$ solution (pH 12) in order to neutralize peat material and eliminate unwanted debris. Untreated and treated peats were dried at 45°C for 2 days, manually pulverized, and filtered by a 2-mm sieve before use. The digested sludge (DS) collected from the Hiagari municipal wastewater treatment plant (Kitakyushu) with pH 7.5 was used as a source of microorganisms. For the incubation experiment, peat was incubated with digested sludge at 35°C under anaerobic conditions for 29 days. The experiment was conducted in duplicate. Digested sludge was used as a control. The cumulative CH_4 was monitored automatically using a respirometer. An experimental scheme is shown below.



The results showed that CH_4 could be produced when using peat biomass (untreated and treated) as a methanogenic substrate, suggesting that intermediate biodegradable organics of peat could be decomposed to CH_4 by methanogenic communities in the digested sludge. CH_4 production efficiency was obtained at 29.4 mL-CH_4/g-VS from treated peat and at 20 mL-CH_4/g-VS from untreated peat. CH_4 production efficiency of treated peat was slightly higher than that of untreated peat possibly as a result of alkaline pretreatment of biomass recalcitrance prior to incubation for enhancing the digestibility. Considering the effects of ecological disturbance of peatland, changes in peat soil pH (from low to high) may alter the accessibility of recalcitrant biomass to anaerobic microbial decomposition and increase CH_4 emissions as well as the overall greenhouse gas balance.

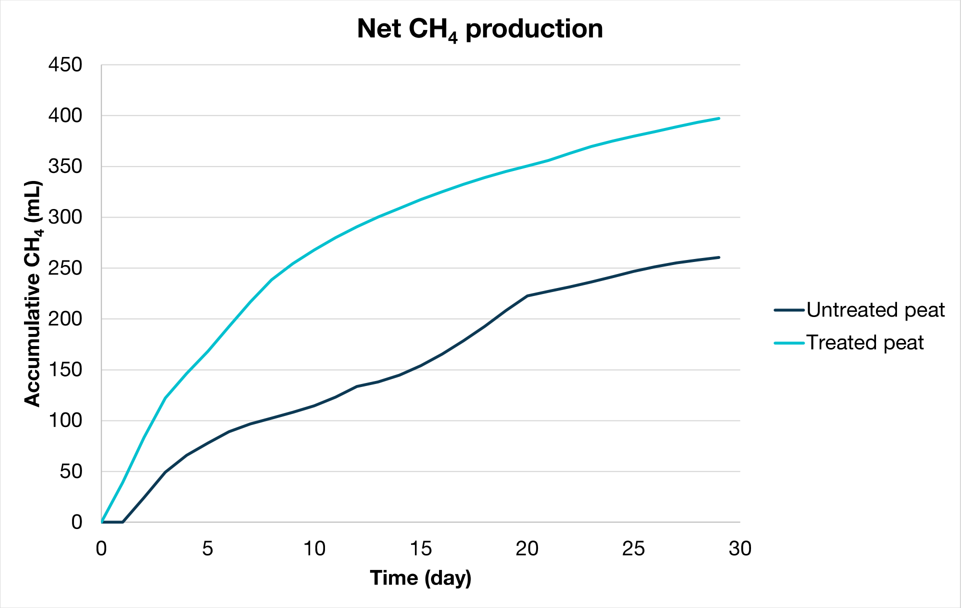


Figure 6.1 CH_4 production from peat incubation using digested sludge as a source of microorganisms. Peat biomass with alkaline pretreatment was used for comparing the digestibility and CH_4 gas production efficiency. Accumulative CH_4 was recorded automatically using a respirometer for 29 days.

CHAPTER VII

CONCLUSIONS AND FUTURE PERSTPECTIVES

This study focused on the characterization of microbial communities in aquatic and terrestrial subsurface sediments, as they are the largest natural source of greenhouse gas methane. We identified methanogenic archaea, a key player of biological methane producer as well as explored their phylogenetic distribution and metabolic potential. Next-generation sequencing of the 16S rRNA gene (marker gene for microbial identity), the classical molecular cloning of targeting the *mcrA* gene as well as metagenomic sequencing, were used to analyze methanogenic microbes.

In the brackish water sediments, the Methanosaetaceae was only detected based on the 16S rRNA gene amplicon, while 8 phyla, including some new phylogenic candidates that have been reported in brackish water, were identified based on the functional *mcrA* gene analysis. This suggests that methanogenesis in estuary sediments could be conducted by diverse methanogenic archaea either from cultivated or uncultivated lineages.

In the wetland peat soils, we observed the dominance of the archaea phylum Bathyarchaeota based on the 16S rRNA gene sequences and quantitative amplification. These uncultivated archaea are thought to play a crucial role in the biogeochemical cycle. Further, we discovered fragments of the *mcrA* gene, a key gene in the methane metabolism pathway, based on the metagenomic approaches. Our analysis suggests that these *mcrA* genes belonged to the Bathyarchaeota and the closely related group that divert from the traditional well-known methanogenic archaea. Methane-metabolizing archaea that possess *mcrA* have been thought to be restricted to only the phylum Euryarchaeota. Our metagenome-assembled genomes (MAGs) analysis provides another gene-based evidence of the diversity of divergent *mcrA*-containing archaea outside of the Euryarchaeota, reported only once in 2015 from a microbial metagenomic reconstruction. It should also be noted that Bathyarchaeota that possess genes necessary for methane production could be observed in natural wetlands since they were first observed in coal-bed reservoirs. The existence of putative methane producers in wetland environments suggests the possibility of the discovery of new methanogenic members in other environmental niches facilitating methane production. Also, this ground-breaking discovery is an important consequence of our interpretation of the origin and evolutionary history of the *mcrA* and Mcr complex.

Nonetheless, our metagenome-assembled genomes (MAGs) could not entirely recover *mcrA*-containing Bathyarchaeota (as we obtain only short amino acid sequences for the *mcrA*), probably because of the MAGs completeness and recovery of genomic content from the samples. Also, up to date, no representative of Bathyarchaeota has been successfully cultivated in a single isolate, neither from terrestrial nor marine sediment and therefore, their physiology is largely unknown. Therefore, additional genome sampling and in vitro cultivation of Bathyarchaeota representatives as well as other key targets for cultivation (Table 7.1) will be required to validate their metabolic hypotheses and phylogenetic distribution of the divergent *mcrA*-containing archaea for future study. Successful cultivation will greatly reveal comprehensive key metabolic processes that have been identified only in omics studies. In addition, studying using pure cultures will likely provide insight into cell features and complete detail of metabolic capability (i.e., methanogenesis), which will possibly observe under experimental studies to confirm certain functions in nature. More importantly, obtaining such pure cultures can be practically used for other downstream applications to perform the replicate experiments under controlled circumstances and to enhance reproducibility for generating statistical confidence. Increasing taxonomical sampling and providing cell biology information will serve as the first full characterization of key target microorganisms for cultivation attempts. This creates a meaningful discussion and hypothesis conclusion and a great impact on microbial ecological studies, promoting the exploration efforts to generate further strong evidence for scientific interpretation.

Table 7.1 Key targets for cultivation of Archaea (Lewis *et al*., 2020)

Target microorganisms	**Common environments**	**Superphylum or phylum**	**Reasons they are interest for cultivation**
Anaerobic methanotroph	Sediments	Euryarchaeota	(Bhattarai *et al*., 2019).
Bathyarchaeota	Sediments	TACK	They are a group of globally widespread metabolic generalists that are abundant in anoxic environments. They contain some of the few known putative methanogenic archaea lineages from outside the Euryarchaeota (Zhou *et al*., 2018)
Verstraetearchaeota	Sediments	TACK	Some of the few known putative methanogenic archaea from outside the Euryarchaeota belong to this phylum (Vanwonterghem *et al*., 2016).
Candidate phyla Heimdallarchaeota, Helarchaeota, Lokiarchaeota, Odinarchaeota, and Thorarchaeota	Marine sediments and hydrothermal vents	ASGARD	These archaea belonging to the Asgard superphylum are important for understanding the origin of eukaryotes. The Heimdallarchaeota are currently the best-supported sister linage of eukaryotes, and are therefore the most important target for cultivation. Some lineages are also abundant in some marine sediments (Zaremba-Niedzwiedzka *et al*., 2017).
DPANN archaea	Assorted	DPANN	They are a major archaeal group, currently thought to consist of at least 12 different phyla, with 6 cultured representatives across the entire group. They typically have small cell and genome sizes, limited metabolic capabilities and are likely to be symbionts or parasites of other microorganisms (Dombrowski *et al*., 2019)
Marine Group II, III, and IV archaea	Marine	Euryarchaeota	Marine Group II are abundant in some marine environments and are

			thought to be important for the degradation of organic carbon (Zhang *et al*., 2015). Marine Groups III and IV are abundant and widespread in some marine environments, and there are currently no cultured representatives for any of these clades (Haro-Moreno *et al*., 2017).
Water column B Thaumarchaeota	Marine	Thaumarchaeota	They have a key role in biogeochemistry by participating in carbon and nitrogen cycling in the deeper layers of oceans (Reji *et al*., 2019).

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Zaremba-Niedzwiedzka, K., Caceres, E. F., Saw, J. H., Bäckström, D., Juzokaite, L., Vancaester, E., et al. (2017). Asgard archaea illuminate the origin of eukaryotic cellular complexity. Nature, **541**: 353-358.

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APPENDICES

APPENDIX A

Sampling site for estuary sediments

APPENDIX B

Details of primers and probes used in this study (section 3.2.2 and section 4.2.4)

Target gene	Assay	Primer/Probe	Sequence (5’-3’)	Reference
Prokaryotic 16S rRNA gene	qPCR	Uni340F	CCTACGGGRBGCASCAG	Takai and Horikoshi, 2000
		Uni806R	GGACTACNNGGGTATCTAAT	
		Uni516F (Taqman probe)	TGYCAGCMGCCGCGGTAAHACVNRS	
Archaeal 16S rRNA gene	qPCR	Arch349F	GYGCASCAGKCGMGAAW	Takai and Horikoshi, 2000
		Arch806R	GGACTACVSGGGTATCTAAT	
		Arch516F (Taqman probe)	TGYCAGCCGCCGCGGTAAHACCVGC	
Prokaryotic 16S rRNA gene	NGS	Bakt_341F	CCTACGGGNGGCWGCAG	Herlemann *et al.*, 2010
		Bakt_805R	GACTACHVGGGTATCTAATCC	
Prokaryotic 16S rRNA gene	NGS	515F	GTGCCAGCMGCCGCGGTAA	Caporasp *et al.*, 2011
		806R	GGACTACHVGGGTWTCTAAT	
McrA gene	qPCR/Cloning	ME3MF	ATGTCNGGTGGHGTMGGSTTYAC	Nunoura *et al.*, 2008
		ME2’R	TCATBGCRTAGTTDGGRTAGT	
Bathyarchaeotal 16S rRNA gene	qPCR	MCG410F	WCCGCTGAGGDYGGCTTTT	Modified from Kubo *et al.*, 2012
		MCG528R	CTCRGRGRGCTGGTATTACCG	
Cloned vector	colony PCR	M13-20	GTAAAACGACGGCCAGTG	TOYOBO, Japan
		M13 Reverse	GGAAACAGCTATGACCATG	

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., et al. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci USA, **108**: 4516.

Herlemann, D. P. R., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J. J., & Andersson, A. F. (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J, **5**: 1571-1579.

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APPENDIX C

PCR amplification mixture according to the list in Appendix B

Reaction volume (total)	**20µL**	**Reaction volume (total)**	**20µL**
Sterilized water	7.5	Sterilized water	7.5
innuDry qPCR MasterMix Probe	10	innuDry qPCR MasterMix Probe	10
Univ 340F	0.4	Arc 349F	0.4
Univ 806R	0.4	Arc 806R	0.4
Taqman probe (Univ 516F)	1.2	Taqman probe (Arc 516F)	1.2
DNA template	0.5	DNA template	0.5

Reaction volume (total)	**30µL**	**Reaction volume (total)**	**30µL**
Sterilized water	10	Sterilized water	10
2X Mighty Amp Buffer Ver.3	15	2X Mighty Amp Buffer Ver.3	15
10X Additive for High Specificity	3	10X Additive for High Specificity	3
Bakt_341F	0.6	515F	0.6
Bakt_805R	0.6	806R	0.6
Mighty Amp DNA polymerase	0.3	Mighty Amp DNA polymerase	0.3
DNA template	0.5	DNA template	0.5

Reaction volume (total)	**20µL**	**Reaction volume (total)**	**20µL**
Sterilized water	3.9	Sterilized water	8.7
2X PCR Buffer for KOD FX Neo	20	Mighty Amp for Real Time	10
2mM dNTPs	4	MCG 410F	0.4
ME3MF	0.6	MCG 528R	0.4
ME2’R	0.6	DNA template	0.5
KOD FX Neo (1U/µL)	0.4		
DNA template	0.5		

Reaction volume (total)	**10µL**
Sterilized water	5
EmeraldAmp PCR Master Mix (2X)	5
M13-20	0.5
M13 Reverse	0.5
Colony	

APPENDIX D

Detail of primer pairs and amplification condition for bacterial methanotroph

Target gene	Assay	Primer	Sequence (5'-3')	Reference
Methanotrophs	PCR	pmoA189F	GGNGACTGGGACTTCTGG	Holmes *et al.*, 1995
		pmoA682R	GAASGCNGAGAAGAASGC	
	PCR	pmoA189F	GGNGACTGGGACTTCTGG	Holmes *et al.*, 1995; Costello & Lidstrom, 1999
		mb661R	CCGGMGCAACGTCYTTACC	
	PCR	pmoA189F	GGNGACTGGGACTTCTGG	Holmes *et al.*, 1995; Bourne *et al.*, 2001
		pmoA650R	ACGTCCTTACCGAAGGT	

Reaction volume (total)	**20µL**	**Reaction volume (total)**	**20µL**
Sterilized water	3.8	Sterilized water	3.4-3.9
2X Mighty Amp Buffer Ver.3	10	2X PCR Buffer for KOD FX Neo	10
10X Additive for High Specificity	2	2nM dNTPs	4
Forward primer (F)	0.6	Forward primer (F)	0.6
Reverse primer (R)	0.6	Reverse primer (R)	0.6
Mighty Amp DNA polymerase	0.4	KOD FX Neo (1U/µL)	0.4
DNA template	1	DNA template	0.5-1

Bourne, D. G., McDonald, I. R., & Murrell, J. C. (2001). Comparison of pmoA PCR primer sets as tools for investigating methanotroph diversity in three Danish soils. Appl Environ Microbiol, **67**: 3802-3809.

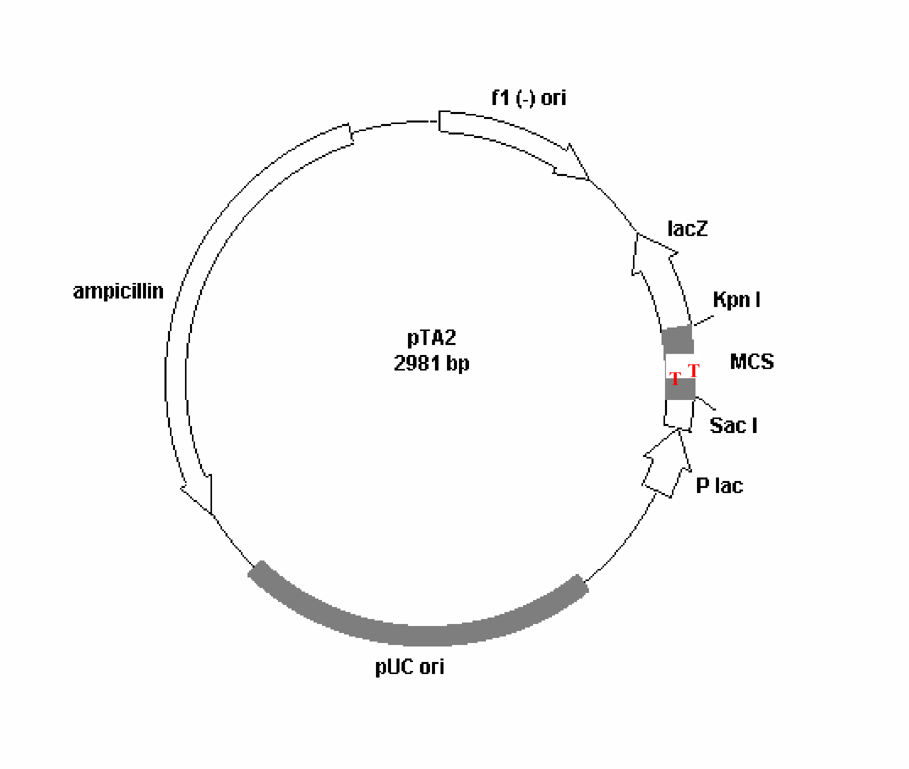
Costello, A. M., & Lidstrom, M. E. (1999). Molecular Characterization of Functional and Phylogenetic Genes from Natural Populations of Methanotrophs in Lake Sediments. Appl Environ Microbiol, **65**: 5066-5074.

Holmes, A. J., Costello, A., Lidstrom, M. E., & Murrell, J. C. (1995). Evidence that particulate methane monooxygenase and ammonia monooxygenase may be evolutionarily related. FEMS Microbiol Lett, **132**: 203-208.

APPENDIX E

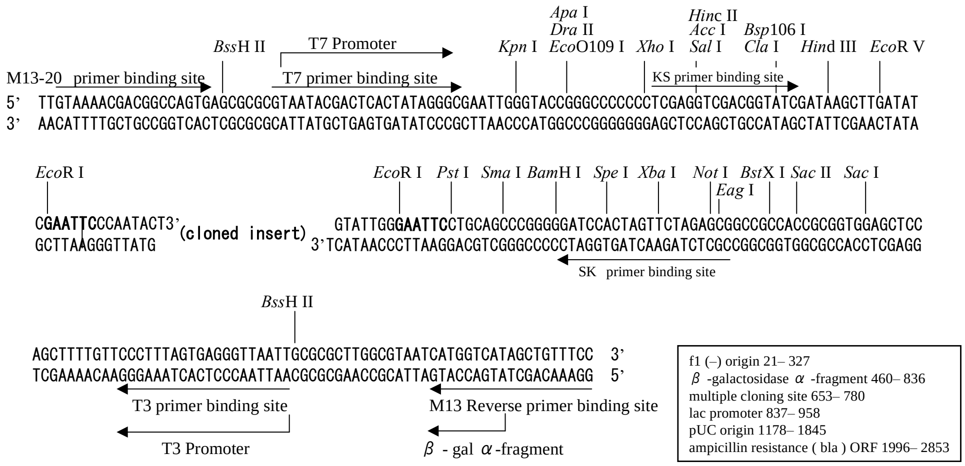
Vector information

1. Map of pTA2 Vector



*The vector generates blue *E. coli* colonies on X-gal/(IPTG) plates in case of no inserts.

2. DNA sequence around the multi-cloning site of pTA2 Vector.



f1 (–) origin 21– 327
β -galactosidase α -fragment 460– 836
multiple cloning site 653– 780
lac promoter 837– 958
pUC origin 1178– 1845
ampicillin resistance (bla) ORF 1996– 2853

APPENDIX F

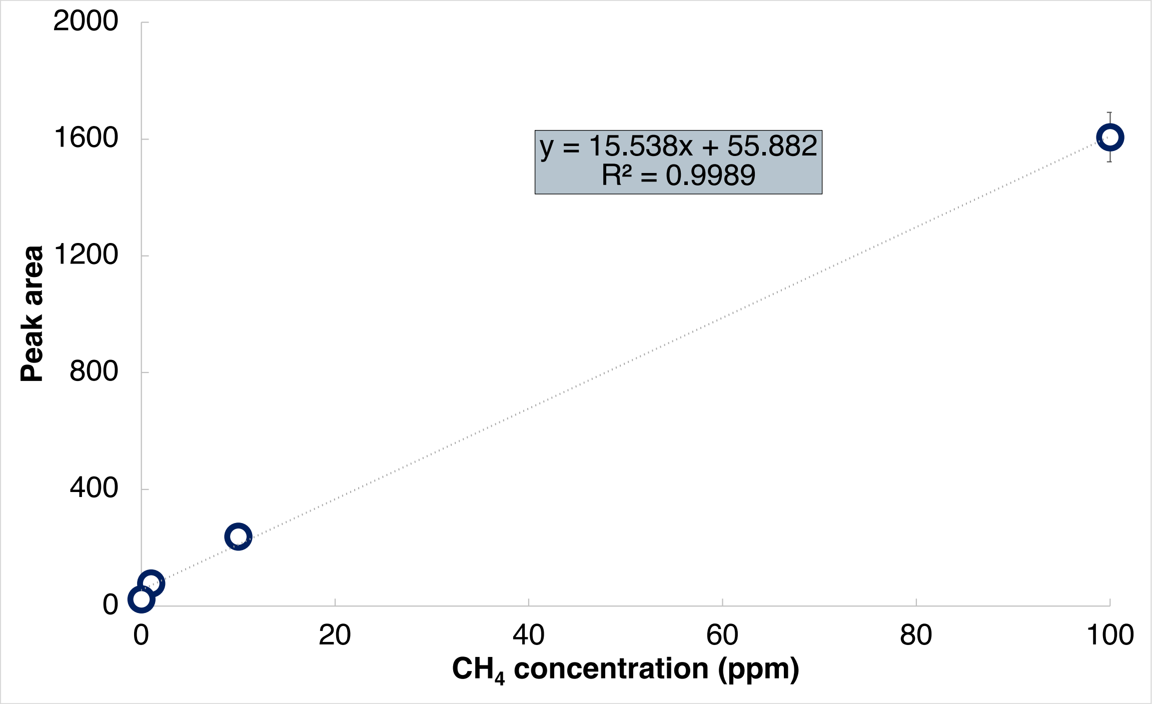
Collection of peat soil samples and surface water from the Bogatsuru mire
July 21, 2020

Surface water

Peat soil

APPENDIX G

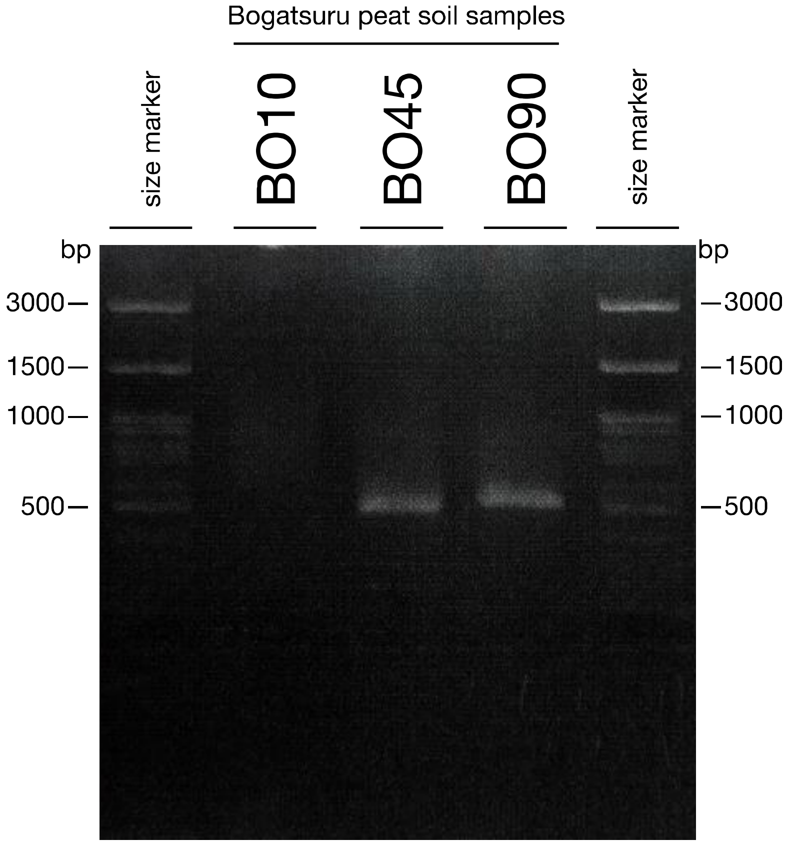
Standard curve of methane from the headspace analysis.



To quantify CH_4 potentially produced from peat soils, 1 mL of the headspace was analyzed using a gas chromatograph equipped with a MICROPACKED ST column (Shinwa Chemical Industries, Kyoto, Japan) and flame ionization detection (FID). The temperatures of the column, injector, and detector were 80, 100, and 300 °C, respectively. The standard curve of CH_4 (Appendix G) was constructed between a peak area (RT 2.5 min) and CH_4 concentration (0, 1, 10, and 100 ppm).

APPENDIX H

Result of mcrA gene amplification from Bogatsuru peat soil samples for qPCR analysis. A molecular marker (GeneDirex, USA) is indicated at both sides of the sample lanes. The gel was made using 2% TAE agarose. PCR products (~ 500 bp) were loaded with Novel Juice (GeneDirex, USA) staining. None of mcrA gene product was obtained at a depth of 10 cm (BO10).

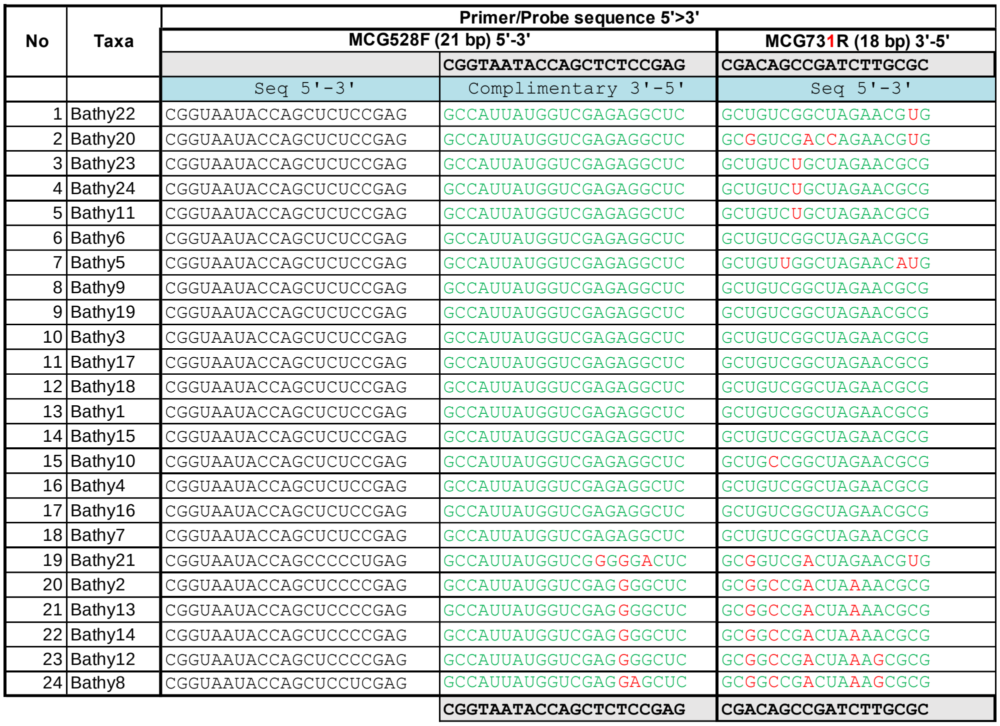


APPENDIX I

Optimization of primers for detection of the Bathyarchaeotal 16S rRNA gene

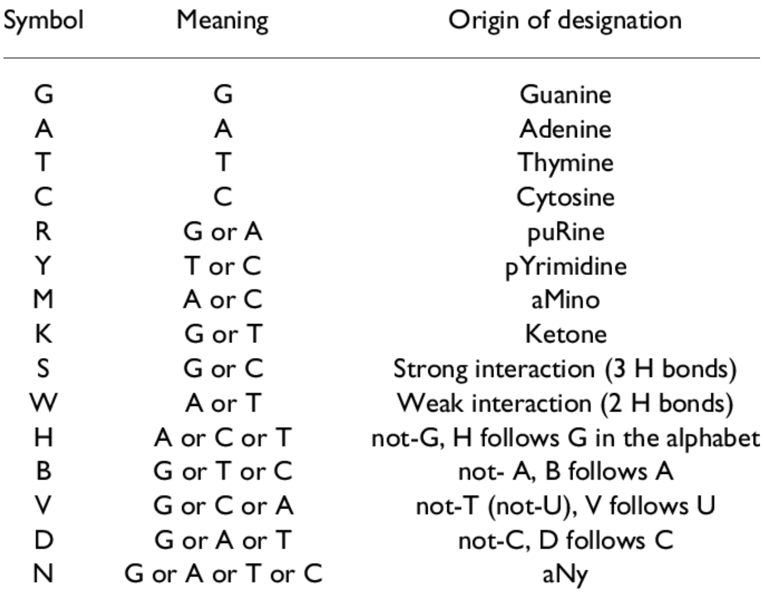
All 24 Bathyarchaeotal sequences obtained from the 16S rRNA gene amplicon sequencing were aligned with primers, which were previously designed (Kubo *et al*., 2012). After alignment, mismatched nucleotides were identified for optimization of degenerated primers. Degenerated primers were investigated for efficiency before further use. IUPAC nucleotide codes were used for degenerated primer sequences.

No	Taxa	Primer/Probe sequence 5'>3'		
		MCG410F (19 bp) 5'-3'		MCG528R (21 bp) 3'-5'
			TCCGCTGAGGATGGCTTTT	**GCCATTATGGTCGAGAGGCTC**
		Seq 5'-3'	Complimentary 3'-5'	Seq 5'-3'
1	Bathy22	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCUCCGAG
2	Bathy20	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCUCCGAG
3	Bathy23	UCCGCUGAGGAAGGCUUUU	AGGCGACUCCUUCCGAAAA	CGGUAAUACCAGCUCUCCGAG
4	Bathy24	ACCGCUGAGGUUGGCUUUU	UGGCGACUCCAACCGAAAA	CGGUAAUACCAGCUCUCCGAG
5	Bathy11	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCUCCGAG
6	Bathy6	UCCGCUGAGGGUGGCUUUU	AGGCGACUCCCACCGAAAA	CGGUAAUACCAGCUCUCCGAG
7	Bathy5	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCUCCGAG
8	Bathy9	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCUCCGAG
9	Bathy19	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCUCCGAG
10	Bathy3	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCUCCGAG
11	Bathy17	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCUCCGAG
12	Bathy18	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCUCCGAG
13	Bathy1	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCUCCGAG
14	Bathy15	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCUCCGAG
15	Bathy10	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCUCCGAG
16	Bathy4	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCUCCGAG
17	Bathy16	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCUCCGAG
18	Bathy7	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCUCCGAG
19	Bathy21	UCCGAUGAGGAUGGCUUUU	AGGCUACUCCUACCGAAAA	CGGUAAUACCAGCCCCUGAG
20	Bathy2	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCCCCGAG
21	Bathy13	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCCCCGAG
22	Bathy14	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCCCCGAG
23	Bathy12	UCCGCUGAGGAUGGCUUUU	AGGCGACUCCUACCGAAAA	CGGUAAUACCAGCUCCCCGAG
24	Bathy8	UCCGCUGAGGGCGGCUUUU	AGGCGACUCCCGCCGAAAA	CGGUAAUACCAGCUCCUCGAG
			TCCGCTGAGGATGGCTTTT	**GCCATTATGGTCGAGAGGCTC**



No	Taxa	Primer/Probe sequence 5'>3'		
		MCG528F (21 bp) 5'-3'		MCG731R (18 bp) 3'-5'
			CGGTAATACCAGCTCTCCGAG	CGACAGCCGATCTTGCGC
		Seq 5'-3'	Complimentary 3'-5'	Seq 5'-3'
1	Bathy22	CGGUAAUACCAGCUCUCCGAG	GCCAUUAUGGUCGAGAGGCUC	GCUGUCGGCUAGAACGUG
2	Bathy20	CGGUAAUACCAGCUCUCCGAG	GCCAUUAUGGUCGAGAGGCUC	GCGGUCGACCAGAACGUG
3	Bathy23	CGGUAAUACCAGCUCUCCGAG	GCCAUUAUGGUCGAGAGGCUC	GCUGUCUGCUAGAACGCG
4	Bathy24	CGGUAAUACCAGCUCUCCGAG	GCCAUUAUGGUCGAGAGGCUC	GCUGUCUGCUAGAACGCG
5	Bathy11	CGGUAAUACCAGCUCUCCGAG	GCCAUUAUGGUCGAGAGGCUC	GCUGUCUGCUAGAACGCG
6	Bathy6	CGGUAAUACCAGCUCUCCGAG	GCCAUUAUGGUCGAGAGGCUC	GCUGUCGGCUAGAACGCG
7	Bathy5	CGGUAAUACCAGCUCUCCGAG	GCCAUUAUGGUCGAGAGGCUC	GCUGUUGGCUAGAACAUG
8	Bathy9	CGGUAAUACCAGCUCUCCGAG	GCCAUUAUGGUCGAGAGGCUC	GCUGUCGGCUAGAACGCG
9	Bathy19	CGGUAAUACCAGCUCUCCGAG	GCCAUUAUGGUCGAGAGGCUC	GCUGUCGGCUAGAACGCG
10	Bathy3	CGGUAAUACCAGCUCUCCGAG	GCCAUUAUGGUCGAGAGGCUC	GCUGUCGGCUAGAACGCG
11	Bathy17	CGGUAAUACCAGCUCUCCGAG	GCCAUUAUGGUCGAGAGGCUC	GCUGUCGGCUAGAACGCG
12	Bathy18	CGGUAAUACCAGCUCUCCGAG	GCCAUUAUGGUCGAGAGGCUC	GCUGUCGGCUAGAACGCG
13	Bathy1	CGGUAAUACCAGCUCUCCGAG	GCCAUUAUGGUCGAGAGGCUC	GCUGUCGGCUAGAACGCG
14	Bathy15	CGGUAAUACCAGCUCUCCGAG	GCCAUUAUGGUCGAGAGGCUC	GCUGUCGGCUAGAACGCG
15	Bathy10	CGGUAAUACCAGCUCUCCGAG	GCCAUUAUGGUCGAGAGGCUC	GCUGCCGGCUAGAACGCG
16	Bathy4	CGGUAAUACCAGCUCUCCGAG	GCCAUUAUGGUCGAGAGGCUC	GCUGUCGGCUAGAACGCG
17	Bathy16	CGGUAAUACCAGCUCUCCGAG	GCCAUUAUGGUCGAGAGGCUC	GCUGUCGGCUAGAACGCG
18	Bathy7	CGGUAAUACCAGCUCUCCGAG	GCCAUUAUGGUCGAGAGGCUC	GCUGUCGGCUAGAACGCG
19	Bathy21	CGGUAAUACCAGCCCCCUGAG	GCCAUUAUGGUCGGGGGACUC	GCGGUCGACUAGAACGUG
20	Bathy2	CGGUAAUACCAGCUCCCCGAG	GCCAUUAUGGUCGAGGGGCUC	GCGGCCGACUAAAACGCG
21	Bathy13	CGGUAAUACCAGCUCCCCGAG	GCCAUUAUGGUCGAGGGGCUC	GCGGCCGACUAAAACGCG
22	Bathy14	CGGUAAUACCAGCUCCCCGAG	GCCAUUAUGGUCGAGGGGCUC	GCGGCCGACUAAAACGCG
23	Bathy12	CGGUAAUACCAGCUCCCCGAG	GCCAUUAUGGUCGAGGGGCUC	GCGGCCGACUAAAGCGCG
24	Bathy8	CGGUAAUACCAGCUCCUCGAG	GCCAUUAUGGUCGAGGAGCUC	GCGGCCGACUAAAGCGCG
			CGGTAATACCAGCTCTCCGAG	CGACAGCCGATCTTGCGC

IUPAC nomenclature for nucleic acids



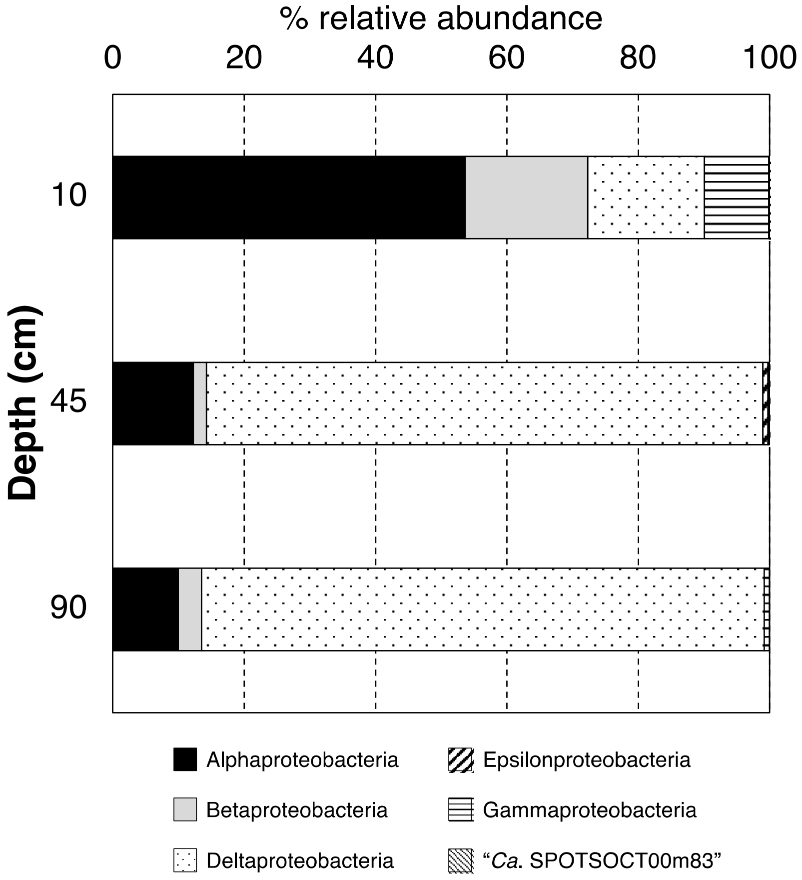
Symbol	Meaning	Origin of designation
G	G	Guanine
A	A	Adenine
T	T	Thymine
C	C	Cytosine
R	G or A	puRine
Y	T or C	pYrimidine
M	A or C	aMino
K	G or T	Ketone
S	G or C	Strong interaction (3 H bonds)
W	A or T	Weak interaction (2 H bonds)
H	A or C or T	not-G, H follows G in the alphabet
B	G or T or C	not- A, B follows A
V	G or C or A	not-T (not-U), V follows U
D	G or A or T	not-C, D follows C
N	G or A or T or C	aNy

Summary of degenerated primers used in this study

Primer	Reference Sequence 5'-3'	Degenerated Sequence 5'-3'
MCG410F	TCCGCTGAGGATGGCTTTT	WCCGCTGAGGDYGGCTTTT
MCG528R	CTCGGAGAGCTGGTATTACCG	CTCRGRGRGCTGGTATTACCG
MCG528F	CGGTAATACCAGCTCTCCGAG	CGGTAATACCAGCYCYYYGAG
MCG731R	CGCGTTCTAGCCGACAGC	CRYGYTYTRGYMRRCMGC

APPENDIX J

Relative abundance ratio of the class-level groups of Proteobacteria in the peat soils.



APPENDIX K

Taxonomic classification at an assembled level (contigs). Each contig was identified by Kraken module (only Refined bin 12, 13, and 16 that Bathyarchaeota were identified)

Classification of Refined bin 12

Contigs							Lineage name
NODE	258	length	6434	cov	5.017871	1	
NODE	258	length	6434	cov	5.017871	2	Candidatus Bathyarchaeota (phylum)
NODE	258	length	6434	cov	5.017871	3	Candidatus Bathyarchaeota (phylum)
NODE	258	length	6434	cov	5.017871	4	Candidatus Bathyarchaeota (phylum)
NODE	258	length	6434	cov	5.017871	5	Candidatus Bathyarchaeota (phylum)
NODE	386	length	5331	cov	4.58188	1	Candidatus Bathyarchaeota (phylum)
NODE	386	length	5331	cov	4.58188	2	Candidatus Bathyarchaeota (phylum)
NODE	386	length	5331	cov	4.58188	3	Candidatus Bathyarchaeota (phylum)
NODE	386	length	5331	cov	4.58188	4	Candidatus Bathyarchaeota (phylum)
NODE	386	length	5331	cov	4.58188	5	Candidatus Bathyarchaeota (phylum)
NODE	386	length	5331	cov	4.58188	6	Candidatus Bathyarchaeota (phylum)
NODE	386	length	5331	cov	4.58188	7	Candidatus Bathyarchaeota (phylum)
NODE	386	length	5331	cov	4.58188	8	Candidatus Bathyarchaeota (phylum)
NODE	405	length	5173	cov	5.322392	1	
NODE	405	length	5173	cov	5.322392	2	Candidatus Bathyarchaeota (phylum)
NODE	405	length	5173	cov	5.322392	3	Candidatus Bathyarchaeota (phylum)
NODE	405	length	5173	cov	5.322392	4	Candidatus Bathyarchaeota (phylum)
NODE	405	length	5173	cov	5.322392	5	Candidatus Bathyarchaeota (phylum)
NODE	405	length	5173	cov	5.322392	6	Candidatus Bathyarchaeota (phylum)
NODE	438	length	5046	cov	8.728912	1	
NODE	438	length	5046	cov	8.728912	2	Candidatus Bathyarchaeota (phylum)
NODE	438	length	5046	cov	8.728912	3	Candidatus Bathyarchaeota (phylum)
NODE	438	length	5046	cov	8.728912	4	Candidatus Bathyarchaeota (phylum)
NODE	438	length	5046	cov	8.728912	5	Candidatus Bathyarchaeota (phylum)
NODE	438	length	5046	cov	8.728912	6	
NODE	438	length	5046	cov	8.728912	7	Candidatus Lokiarchaeota (phylum)
NODE	438	length	5046	cov	8.728912	8	Candidatus Bathyarchaeota (phylum)
NODE	517	length	4728	cov	4.063985	1	
NODE	517	length	4728	cov	4.063985	2	Candidatus Bathyarchaeota (phylum)
NODE	517	length	4728	cov	4.063985	3	Candidatus Bathyarchaeota (phylum)
NODE	517	length	4728	cov	4.063985	4	Candidatus Bathyarchaeota (phylum)
NODE	517	length	4728	cov	4.063985	5	Candidatus Bathyarchaeota (phylum)
NODE	609	length	4418	cov	3.388265	1	Candidatus Bathyarchaeota (phylum)
NODE	609	length	4418	cov	3.388265	2	Candidatus Bathyarchaeota (phylum)
NODE	609	length	4418	cov	3.388265	3	Candidatus Bathyarchaeota (phylum)
NODE	609	length	4418	cov	3.388265	4	Candidatus Bathyarchaeota (phylum)
NODE	609	length	4418	cov	3.388265	5	Candidatus Bathyarchaeota (phylum)
NODE	609	length	4418	cov	3.388265	6	Candidatus Bathyarchaeota (phylum)
NODE	610	length	4414	cov	4.692819	1	Candidatus Bathyarchaeota (phylum)
NODE	610	length	4414	cov	4.692819	2	Candidatus Bathyarchaeota (phylum)
NODE	610	length	4414	cov	4.692819	3	Candidatus Bathyarchaeota (phylum)
NODE	610	length	4414	cov	4.692819	4	Candidatus Bathyarchaeota (phylum)
NODE	815	length	4004	cov	4.187896	1	Candidatus Bathyarchaeota (phylum)
NODE	815	length	4004	cov	4.187896	2	Candidatus Bathyarchaeota (phylum)

NODE	815	length	4004	cov	4.187896	3	
NODE	815	length	4004	cov	4.187896	4	Candidatus Bathyarchaeota (phylum)
NODE	815	length	4004	cov	4.187896	5	
NODE	815	length	4004	cov	4.187896	6	Candidatus Bathyarchaeota (phylum)
NODE	815	length	4004	cov	4.187896	7	Candidatus Bathyarchaeota (phylum)
NODE	821	length	3992	cov	4.680975	1	Candidatus Bathyarchaeota (phylum)
NODE	821	length	3992	cov	4.680975	2	Candidatus Bathyarchaeota (phylum)
NODE	821	length	3992	cov	4.680975	3	Candidatus Bathyarchaeota (phylum)
NODE	821	length	3992	cov	4.680975	4	Candidatus Bathyarchaeota (phylum)
NODE	821	length	3992	cov	4.680975	5	Candidatus Bathyarchaeota (phylum)
NODE	821	length	3992	cov	4.680975	6	Candidatus Bathyarchaeota (phylum)
NODE	978	length	3748	cov	3.897103	1	Candidatus Bathyarchaeota (phylum)
NODE	978	length	3748	cov	3.897103	2	Candidatus Bathyarchaeota (phylum)
NODE	978	length	3748	cov	3.897103	3	
NODE	978	length	3748	cov	3.897103	4	
NODE	978	length	3748	cov	3.897103	5	Candidatus Bathyarchaeota (phylum)
NODE	1020	length	3688	cov	6.055877	1	Candidatus Bathyarchaeota (phylum)
NODE	1020	length	3688	cov	6.055877	2	Candidatus Bathyarchaeota (phylum)
NODE	1020	length	3688	cov	6.055877	3	Candidatus Bathyarchaeota (phylum)
NODE	1020	length	3688	cov	6.055877	4	
NODE	1034	length	3679	cov	3.541115	1	Candidatus Bathyarchaeota (phylum)
NODE	1034	length	3679	cov	3.541115	2	Candidatus Bathyarchaeota (phylum)
NODE	1034	length	3679	cov	3.541115	3	Candidatus Bathyarchaeota (phylum)
NODE	1034	length	3679	cov	3.541115	4	Candidatus Bathyarchaeota (phylum)
NODE	1046	length	3666	cov	5.878981	1	Candidatus Bathyarchaeota (phylum)
NODE	1046	length	3666	cov	5.878981	2	Candidatus Bathyarchaeota (phylum)
NODE	1046	length	3666	cov	5.878981	3	Candidatus Bathyarchaeota (phylum)
NODE	1046	length	3666	cov	5.878981	4	Candidatus Bathyarchaeota (phylum)
NODE	1046	length	3666	cov	5.878981	5	Candidatus Bathyarchaeota (phylum)
NODE	1062	length	3643	cov	5.646042	1	
NODE	1062	length	3643	cov	5.646042	2	Candidatus Bathyarchaeota (phylum)
NODE	1062	length	3643	cov	5.646042	3	Candidatus Bathyarchaeota (phylum)
NODE	1062	length	3643	cov	5.646042	4	
NODE	1062	length	3643	cov	5.646042	5	Candidatus Bathyarchaeota (phylum)
NODE	1062	length	3643	cov	5.646042	6	Candidatus Bathyarchaeota (phylum)
NODE	1102	length	3587	cov	4.278313	1	Candidatus Bathyarchaeota (phylum)
NODE	1102	length	3587	cov	4.278313	2	Candidatus Bathyarchaeota (phylum)
NODE	1102	length	3587	cov	4.278313	3	Candidatus Bathyarchaeota (phylum)
NODE	1102	length	3587	cov	4.278313	4	Candidatus Bathyarchaeota (phylum)
NODE	1102	length	3587	cov	4.278313	5	Candidatus Bathyarchaeota (phylum)
NODE	1102	length	3587	cov	4.278313	6	
NODE	1102	length	3587	cov	4.278313	7	Candidatus Bathyarchaeota (phylum)
NODE	1125	length	3566	cov	4.921105	1	Candidatus Bathyarchaeota (phylum)
NODE	1125	length	3566	cov	4.921105	2	Candidatus Bathyarchaeota (phylum)
NODE	1209	length	3486	cov	3.801516	1	Candidatus Bathyarchaeota (phylum)
NODE	1209	length	3486	cov	3.801516	2	Candidatus Bathyarchaeota (phylum)
NODE	1209	length	3486	cov	3.801516	3	Candidatus Bathyarchaeota (phylum)
NODE	1209	length	3486	cov	3.801516	4	
NODE	1209	length	3486	cov	3.801516	5	Candidatus Bathyarchaeota (phylum)
NODE	1209	length	3486	cov	3.801516	6	Candidatus Bathyarchaeota (phylum)
NODE	1209	length	3486	cov	3.801516	7	Candidatus Bathyarchaeota (phylum)
NODE	1264	length	3441	cov	5.701418	1	Candidatus Bathyarchaeota (phylum)
NODE	1264	length	3441	cov	5.701418	2	Candidatus Bathyarchaeota (phylum)
NODE	1264	length	3441	cov	5.701418	3	Candidatus Bathyarchaeota (phylum)
NODE	1264	length	3441	cov	5.701418	4	Candidatus Bathyarchaeota (phylum)
NODE	1264	length	3441	cov	5.701418	5	Candidatus Bathyarchaeota (phylum)

NODE	1305	length	3412	cov	4.467977	1	Candidatus Bathyarchaeota (phylum)
NODE	1305	length	3412	cov	4.467977	2	Candidatus Bathyarchaeota (phylum)
NODE	1305	length	3412	cov	4.467977	3	Candidatus Bathyarchaeota (phylum)
NODE	1346	length	3378	cov	5.063196	1	Candidatus Bathyarchaeota (phylum)
NODE	1346	length	3378	cov	5.063196	2	Candidatus Bathyarchaeota (phylum)
NODE	1348	length	3377	cov	8.399759	1	Candidatus Bathyarchaeota (phylum)
NODE	1348	length	3377	cov	8.399759	2	Candidatus Bathyarchaeota (phylum)
NODE	1348	length	3377	cov	8.399759	3	
NODE	1348	length	3377	cov	8.399759	4	
NODE	1373	length	3356	cov	4.339594	1	Chloroflexi (phylum)
NODE	1373	length	3356	cov	4.339594	2	
NODE	1373	length	3356	cov	4.339594	3	Candidatus Bathyarchaeota (phylum)
NODE	1373	length	3356	cov	4.339594	4	
NODE	1416	length	3323	cov	4.324969	1	Crenarchaeota (phylum)
NODE	1416	length	3323	cov	4.324969	2	
NODE	1416	length	3323	cov	4.324969	3	Candidatus Bathyarchaeota (phylum)
NODE	1416	length	3323	cov	4.324969	4	
NODE	1416	length	3323	cov	4.324969	5	Candidatus Bathyarchaeota (phylum)
NODE	1416	length	3323	cov	4.324969	6	
NODE	1434	length	3307	cov	6.551353	1	Candidatus Bathyarchaeota (phylum)
NODE	1434	length	3307	cov	6.551353	2	Candidatus Bathyarchaeota (phylum)
NODE	1434	length	3307	cov	6.551353	3	Candidatus Bathyarchaeota (phylum)
NODE	1434	length	3307	cov	6.551353	4	Candidatus Bathyarchaeota (phylum)
NODE	1434	length	3307	cov	6.551353	5	Candidatus Bathyarchaeota (phylum)
NODE	1472	length	3271	cov	4.804726	1	
NODE	1552	length	3224	cov	4.137267	1	Chloroflexi (phylum)
NODE	1552	length	3224	cov	4.137267	2	Candidatus Thorarchaeota (phylum)
NODE	1552	length	3224	cov	4.137267	3	
NODE	1552	length	3224	cov	4.137267	4	Candidatus Helarchaeota (phylum)
NODE	1552	length	3224	cov	4.137267	5	
NODE	1572	length	3208	cov	6.31589	1	Candidatus Bathyarchaeota (phylum)
NODE	1572	length	3208	cov	6.31589	2	Candidatus Bathyarchaeota (phylum)
NODE	1572	length	3208	cov	6.31589	3	Candidatus Bathyarchaeota (phylum)
NODE	1572	length	3208	cov	6.31589	4	Candidatus Bathyarchaeota (phylum)
NODE	1572	length	3208	cov	6.31589	5	Candidatus Bathyarchaeota (phylum)
NODE	1572	length	3208	cov	6.31589	6	
NODE	1596	length	3191	cov	3.602679	1	
NODE	1596	length	3191	cov	3.602679	2	Candidatus Bathyarchaeota (phylum)
NODE	1596	length	3191	cov	3.602679	3	Candidatus Bathyarchaeota (phylum)
NODE	1596	length	3191	cov	3.602679	4	Candidatus Bathyarchaeota (phylum)
NODE	1596	length	3191	cov	3.602679	5	
NODE	1596	length	3191	cov	3.602679	6	Candidatus Bathyarchaeota (phylum)
NODE	1596	length	3191	cov	3.602679	7	Candidatus Bathyarchaeota (phylum)
NODE	1596	length	3191	cov	3.602679	8	Candidatus Bathyarchaeota (phylum)
NODE	1624	length	3169	cov	3.56808	1	Candidatus Bathyarchaeota (phylum)
NODE	1624	length	3169	cov	3.56808	2	Candidatus Bathyarchaeota (phylum)
NODE	1624	length	3169	cov	3.56808	3	Candidatus Bathyarchaeota (phylum)
NODE	1624	length	3169	cov	3.56808	4	Candidatus Bathyarchaeota (phylum)
NODE	1680	length	3121	cov	5.493151	1	Candidatus Bathyarchaeota (phylum)
NODE	1680	length	3121	cov	5.493151	2	Candidatus Bathyarchaeota (phylum)
NODE	1680	length	3121	cov	5.493151	3	
NODE	1680	length	3121	cov	5.493151	4	Candidatus Bathyarchaeota (phylum)
NODE	1749	length	3081	cov	4.11996	1	Candidatus Bathyarchaeota (phylum)
NODE	1749	length	3081	cov	4.11996	2	Candidatus Bathyarchaeota (phylum)
NODE	1749	length	3081	cov	4.11996	3	Candidatus Bathyarchaeota (phylum)
NODE	1760	length	3073	cov	5.401259	1	delta/epsilon subdivisions (subphylum)

NODE	1760	length	3073	cov	5.401259	2	
NODE	1760	length	3073	cov	5.401259	3	Chloroflexi (phylum)
NODE	1760	length	3073	cov	5.401259	4	
NODE	1768	length	3069	cov	4.092236	1	Candidatus Bathyarchaeota (phylum)
NODE	1768	length	3069	cov	4.092236	2	Candidatus Bathyarchaeota (phylum)
NODE	1811	length	3051	cov	4.013351	1	Candidatus Bathyarchaeota (phylum)
NODE	1811	length	3051	cov	4.013351	2	
NODE	1811	length	3051	cov	4.013351	3	
NODE	1811	length	3051	cov	4.013351	4	Candidatus Bathyarchaeota (phylum)
NODE	1811	length	3051	cov	4.013351	5	
NODE	1861	length	3023	cov	3.79717	1	Candidatus Bathyarchaeota (phylum)
NODE	1861	length	3023	cov	3.79717	2	Candidatus Bathyarchaeota (phylum)
NODE	1952	length	2979	cov	4.349179	1	
NODE	1952	length	2979	cov	4.349179	2	
NODE	1952	length	2979	cov	4.349179	3	Candidatus Bathyarchaeota (phylum)
NODE	1952	length	2979	cov	4.349179	4	Candidatus Bathyarchaeota (phylum)
NODE	1952	length	2979	cov	4.349179	5	
NODE	1954	length	2978	cov	3.805679	1	Candidatus Bathyarchaeota (phylum)
NODE	1954	length	2978	cov	3.805679	2	
NODE	1954	length	2978	cov	3.805679	3	Candidatus Bathyarchaeota (phylum)
NODE	1954	length	2978	cov	3.805679	4	
NODE	1973	length	2962	cov	5.596835	1	Candidatus Bathyarchaeota (phylum)
NODE	1973	length	2962	cov	5.596835	2	Candidatus Bathyarchaeota (phylum)
NODE	1973	length	2962	cov	5.596835	3	Candidatus Bathyarchaeota (phylum)
NODE	1990	length	2957	cov	6.421778	1	Candidatus Bathyarchaeota (phylum)
NODE	1990	length	2957	cov	6.421778	2	Candidatus Bathyarchaeota (phylum)
NODE	1990	length	2957	cov	6.421778	3	Candidatus Bathyarchaeota (phylum)
NODE	1990	length	2957	cov	6.421778	4	Candidatus Bathyarchaeota (phylum)
NODE	1993	length	2955	cov	5.730345	1	Candidatus Bathyarchaeota (phylum)
NODE	1993	length	2955	cov	5.730345	2	Candidatus Bathyarchaeota (phylum)
NODE	2007	length	2949	cov	5.969592	1	Candidatus Bathyarchaeota (phylum)
NODE	2007	length	2949	cov	5.969592	2	Candidatus Bathyarchaeota (phylum)
NODE	2007	length	2949	cov	5.969592	3	Candidatus Bathyarchaeota (phylum)
NODE	2015	length	2946	cov	4.711519	1	Thaumarchaeota (phylum)
NODE	2015	length	2946	cov	4.711519	2	Candidatus Bathyarchaeota (phylum)
NODE	2015	length	2946	cov	4.711519	3	
NODE	2015	length	2946	cov	4.711519	4	
NODE	2043	length	2935	cov	4.712153	1	
NODE	2043	length	2935	cov	4.712153	2	Candidatus Bathyarchaeota (phylum)
NODE	2043	length	2935	cov	4.712153	3	
NODE	2043	length	2935	cov	4.712153	4	Candidatus Korarchaeota (phylum)
NODE	2068	length	2927	cov	5.420961	1	Candidatus Bathyarchaeota (phylum)
NODE	2068	length	2927	cov	5.420961	2	Candidatus Bathyarchaeota (phylum)
NODE	2068	length	2927	cov	5.420961	3	Candidatus Bathyarchaeota (phylum)
NODE	2068	length	2927	cov	5.420961	4	
NODE	2068	length	2927	cov	5.420961	5	
NODE	2090	length	2921	cov	4.348569	1	Candidatus Bathyarchaeota (phylum)
NODE	2090	length	2921	cov	4.348569	2	
NODE	2116	length	2914	cov	4.642882	1	Candidatus Bathyarchaeota (phylum)
NODE	2116	length	2914	cov	4.642882	2	Candidatus Bathyarchaeota (phylum)
NODE	2116	length	2914	cov	4.642882	3	Candidatus Bathyarchaeota (phylum)
NODE	2116	length	2914	cov	4.642882	4	Candidatus Bathyarchaeota (phylum)
NODE	2116	length	2914	cov	4.642882	5	Candidatus Bathyarchaeota (phylum)
NODE	2147	length	2903	cov	5.007725	1	Candidatus Bathyarchaeota (phylum)
NODE	2147	length	2903	cov	5.007725	2	Candidatus Bathyarchaeota (phylum)
NODE	2147	length	2903	cov	5.007725	3	Candidatus Bathyarchaeota (phylum)

NODE	2147	length	2903	cov	5.007725	4	Candidatus Bathyarchaeota (phylum)
NODE	2147	length	2903	cov	5.007725	5	Candidatus Bathyarchaeota (phylum)
NODE	2152	length	2901	cov	4.708011	1	Candidatus Bathyarchaeota (phylum)
NODE	2152	length	2901	cov	4.708011	2	Candidatus Bathyarchaeota (phylum)
NODE	2152	length	2901	cov	4.708011	3	Candidatus Bathyarchaeota (phylum)
NODE	2152	length	2901	cov	4.708011	4	
NODE	2179	length	2888	cov	5.14437	1	
NODE	2179	length	2888	cov	5.14437	2	Candidatus Bathyarchaeota (phylum)
NODE	2179	length	2888	cov	5.14437	3	Candidatus Bathyarchaeota (phylum)
NODE	2179	length	2888	cov	5.14437	4	Candidatus Bathyarchaeota (phylum)
NODE	2234	length	2862	cov	5.084432	1	Candidatus Bathyarchaeota (phylum)
NODE	2234	length	2862	cov	5.084432	2	Candidatus Bathyarchaeota (phylum)
NODE	2234	length	2862	cov	5.084432	3	Candidatus Bathyarchaeota (phylum)
NODE	2234	length	2862	cov	5.084432	4	Candidatus Bathyarchaeota (phylum)
NODE	2234	length	2862	cov	5.084432	5	Candidatus Bathyarchaeota (phylum)
NODE	2241	length	2860	cov	5.06025	1	unclassified Acidobacteria (no rank)
NODE	2241	length	2860	cov	5.06025	2	Candidatus Bathyarchaeota (phylum)
NODE	2241	length	2860	cov	5.06025	3	Candidatus Bathyarchaeota (phylum)
NODE	2241	length	2860	cov	5.06025	4	Candidatus Bathyarchaeota (phylum)
NODE	2241	length	2860	cov	5.06025	5	Thaumarchaeota (phylum)
NODE	2256	length	2853	cov	5.883488	1	
NODE	2256	length	2853	cov	5.883488	2	Candidatus Bathyarchaeota (phylum)
NODE	2256	length	2853	cov	5.883488	3	
NODE	2256	length	2853	cov	5.883488	4	
NODE	2256	length	2853	cov	5.883488	5	
NODE	2273	length	2847	cov	5.28904	1	Candidatus Bathyarchaeota (phylum)
NODE	2273	length	2847	cov	5.28904	2	
NODE	2273	length	2847	cov	5.28904	3	
NODE	2318	length	2827	cov	4.90873	1	Candidatus Bathyarchaeota (phylum)
NODE	2318	length	2827	cov	4.90873	2	
NODE	2318	length	2827	cov	4.90873	3	Candidatus Bathyarchaeota (phylum)
NODE	2319	length	2827	cov	4.485931	1	
NODE	2319	length	2827	cov	4.485931	2	
NODE	2319	length	2827	cov	4.485931	3	Candidatus Bathyarchaeota (phylum)
NODE	2462	length	2770	cov	4.805157	1	Candidatus Bathyarchaeota (phylum)
NODE	2462	length	2770	cov	4.805157	2	
NODE	2462	length	2770	cov	4.805157	3	Candidatus Bathyarchaeota (phylum)
NODE	2462	length	2770	cov	4.805157	4	
NODE	2485	length	2761	cov	5.660015	1	Candidatus Bathyarchaeota (phylum)
NODE	2499	length	2757	cov	4.522206	1	Candidatus Bathyarchaeota (phylum)
NODE	2499	length	2757	cov	4.522206	2	Candidatus Bathyarchaeota (phylum)
NODE	2499	length	2757	cov	4.522206	3	Candidatus Bathyarchaeota (phylum)
NODE	2499	length	2757	cov	4.522206	4	Candidatus Bathyarchaeota (phylum)
NODE	2537	length	2743	cov	3.859375	1	
NODE	2537	length	2743	cov	3.859375	2	Candidatus Bathyarchaeota (phylum)
NODE	2537	length	2743	cov	3.859375	3	Candidatus Bathyarchaeota (phylum)
NODE	2537	length	2743	cov	3.859375	4	
NODE	2558	length	2734	cov	5.379619	1	
NODE	2558	length	2734	cov	5.379619	2	Candidatus Bathyarchaeota (phylum)
NODE	2577	length	2729	cov	3.587883	1	Candidatus Bathyarchaeota (phylum)
NODE	2577	length	2729	cov	3.587883	2	Candidatus Bathyarchaeota (phylum)
NODE	2596	length	2723	cov	4.634933	1	Candidatus Bathyarchaeota (phylum)
NODE	2596	length	2723	cov	4.634933	2	Candidatus Bathyarchaeota (phylum)
NODE	2596	length	2723	cov	4.634933	3	Candidatus Bathyarchaeota (phylum)
NODE	2596	length	2723	cov	4.634933	4	
NODE	2670	length	2698	cov	4.034052	1	Candidatus Bathyarchaeota (phylum)

NODE	2670	length	2698	cov	4.034052	2	Candidatus Bathyarchaeota (phylum)
NODE	2670	length	2698	cov	4.034052	3	Candidatus Bathyarchaeota (phylum)
NODE	2707	length	2689	cov	5.542141	1	
NODE	2707	length	2689	cov	5.542141	2	Candidatus Bathyarchaeota (phylum)
NODE	2707	length	2689	cov	5.542141	3	Candidatus Bathyarchaeota (phylum)
NODE	2713	length	2688	cov	5.312571	1	
NODE	2713	length	2688	cov	5.312571	2	Candidatus Bathyarchaeota (phylum)
NODE	2713	length	2688	cov	5.312571	3	Candidatus Bathyarchaeota (phylum)
NODE	2737	length	2681	cov	3.909368	1	Candidatus Bathyarchaeota (phylum)
NODE	2737	length	2681	cov	3.909368	2	Candidatus Bathyarchaeota (phylum)
NODE	2737	length	2681	cov	3.909368	3	Candidatus Bathyarchaeota (phylum)
NODE	2739	length	2680	cov	5.321524	1	Candidatus Bathyarchaeota (phylum)
NODE	2739	length	2680	cov	5.321524	2	Candidatus Bathyarchaeota (phylum)
NODE	2739	length	2680	cov	5.321524	3	Candidatus Bathyarchaeota (phylum)
NODE	2739	length	2680	cov	5.321524	4	Candidatus Bathyarchaeota (phylum)
NODE	2793	length	2664	cov	4.553852	1	Candidatus Bathyarchaeota (phylum)
NODE	2793	length	2664	cov	4.553852	2	
NODE	2793	length	2664	cov	4.553852	3	
NODE	2850	length	2648	cov	2.822985	1	
NODE	2850	length	2648	cov	2.822985	2	Candidatus Bathyarchaeota (phylum)
NODE	2850	length	2648	cov	2.822985	3	
NODE	2850	length	2648	cov	2.822985	4	Candidatus Bathyarchaeota (phylum)
NODE	2850	length	2648	cov	2.822985	5	Candidatus Bathyarchaeota (phylum)
NODE	2850	length	2648	cov	2.822985	6	Candidatus Bathyarchaeota (phylum)
NODE	2878	length	2639	cov	7.430341	1	
NODE	2878	length	2639	cov	7.430341	2	Candidatus Bathyarchaeota (phylum)
NODE	2878	length	2639	cov	7.430341	3	Candidatus Bathyarchaeota (phylum)
NODE	2878	length	2639	cov	7.430341	4	Candidatus Bathyarchaeota (phylum)
NODE	2882	length	2638	cov	4.557878	1	
NODE	2882	length	2638	cov	4.557878	2	Candidatus Bathyarchaeota (phylum)
NODE	2882	length	2638	cov	4.557878	3	Candidatus Bathyarchaeota (phylum)
NODE	2888	length	2636	cov	5.18055	1	Candidatus Bathyarchaeota (phylum)
NODE	2888	length	2636	cov	5.18055	2	Candidatus Bathyarchaeota (phylum)
NODE	2888	length	2636	cov	5.18055	3	Candidatus Bathyarchaeota (phylum)
NODE	2888	length	2636	cov	5.18055	4	Candidatus Bathyarchaeota (phylum)
NODE	2888	length	2636	cov	5.18055	5	Candidatus Bathyarchaeota (phylum)
NODE	2921	length	2626	cov	5.005056	1	Candidatus Bathyarchaeota (phylum)
NODE	2921	length	2626	cov	5.005056	2	
NODE	2921	length	2626	cov	5.005056	3	Candidatus Bathyarchaeota (phylum)
NODE	2921	length	2626	cov	5.005056	4	Candidatus Bathyarchaeota (phylum)
NODE	2967	length	2615	cov	4.375781	1	Candidatus Bathyarchaeota (phylum)
NODE	2967	length	2615	cov	4.375781	2	Candidatus Bathyarchaeota (phylum)
NODE	2967	length	2615	cov	4.375781	3	Candidatus Bathyarchaeota (phylum)
NODE	2967	length	2615	cov	4.375781	4	
NODE	3052	length	2589	cov	3.462904	1	Candidatus Bathyarchaeota (phylum)
NODE	3052	length	2589	cov	3.462904	2	Candidatus Bathyarchaeota (phylum)
NODE	3052	length	2589	cov	3.462904	3	Candidatus Bathyarchaeota (phylum)
NODE	3052	length	2589	cov	3.462904	4	Candidatus Bathyarchaeota (phylum)
NODE	3060	length	2587	cov	4.506714	1	Candidatus Bathyarchaeota (phylum)
NODE	3060	length	2587	cov	4.506714	2	Candidatus Bathyarchaeota (phylum)
NODE	3060	length	2587	cov	4.506714	3	Candidatus Bathyarchaeota (phylum)
NODE	3084	length	2578	cov	4.405073	1	Stenosarchaea group (clade)
NODE	3084	length	2578	cov	4.405073	2	
NODE	3084	length	2578	cov	4.405073	3	Candidatus Bathyarchaeota (phylum)
NODE	3084	length	2578	cov	4.405073	4	Betaproteobacteria (class)
NODE	3166	length	2559	cov	3.648962	1	Candidatus Bathyarchaeota (phylum)

NODE	3166	length	2559	cov	3.648962	2	Candidatus Bathyarchaeota (phylum)
NODE	3166	length	2559	cov	3.648962	3	Candidatus Bathyarchaeota (phylum)
NODE	3180	length	2554	cov	4.428571	1	Candidatus Bathyarchaeota (phylum)
NODE	3180	length	2554	cov	4.428571	2	Candidatus Bathyarchaeota (phylum)
NODE	3180	length	2554	cov	4.428571	3	Candidatus Bathyarchaeota (phylum)
NODE	3281	length	2527	cov	4.714401	1	Candidatus Bathyarchaeota (phylum)
NODE	3281	length	2527	cov	4.714401	2	Candidatus Bathyarchaeota (phylum)
NODE	3281	length	2527	cov	4.714401	3	Candidatus Bathyarchaeota (phylum)
NODE	3281	length	2527	cov	4.714401	4	Candidatus Bathyarchaeota (phylum)
NODE	3285	length	2526	cov	4.095103	1	Candidatus Bathyarchaeota (phylum)
NODE	3285	length	2526	cov	4.095103	2	Candidatus Bathyarchaeota (phylum)
NODE	3285	length	2526	cov	4.095103	3	Candidatus Bathyarchaeota (phylum)
NODE	3285	length	2526	cov	4.095103	4	Candidatus Bathyarchaeota (phylum)
NODE	3308	length	2521	cov	4.281022	1	Candidatus Bathyarchaeota (phylum)
NODE	3308	length	2521	cov	4.281022	2	Candidatus Bathyarchaeota (phylum)
NODE	3308	length	2521	cov	4.281022	3	Candidatus Korarchaeota (phylum)
NODE	3308	length	2521	cov	4.281022	4	Crenarchaeota (phylum)
NODE	3316	length	2519	cov	3.397727	1	
NODE	3316	length	2519	cov	3.397727	2	Candidatus Bathyarchaeota (phylum)
NODE	3328	length	2515	cov	6.205285	1	Candidatus Bathyarchaeota (phylum)
NODE	3328	length	2515	cov	6.205285	2	
NODE	3328	length	2515	cov	6.205285	3	Candidatus Bathyarchaeota (phylum)
NODE	3328	length	2515	cov	6.205285	4	Candidatus Bathyarchaeota (phylum)
NODE	3336	length	2513	cov	4.460537	1	Candidatus Bathyarchaeota (phylum)
NODE	3336	length	2513	cov	4.460537	2	Candidatus Bathyarchaeota (phylum)
NODE	3336	length	2513	cov	4.460537	3	Candidatus Bathyarchaeota (phylum)
NODE	3372	length	2505	cov	3.937143	1	Candidatus Bathyarchaeota (phylum)
NODE	3372	length	2505	cov	3.937143	2	Candidatus Bathyarchaeota (phylum)
NODE	3379	length	2503	cov	4.837827	1	Candidatus Bathyarchaeota (phylum)
NODE	3379	length	2503	cov	4.837827	2	
NODE	3379	length	2503	cov	4.837827	3	
NODE	3413	length	2494	cov	3.575236	1	
NODE	3413	length	2494	cov	3.575236	2	
NODE	3413	length	2494	cov	3.575236	3	
NODE	3471	length	2479	cov	4.262789	1	Candidatus Bathyarchaeota (phylum)
NODE	3471	length	2479	cov	4.262789	2	Candidatus Bathyarchaeota (phylum)
NODE	3471	length	2479	cov	4.262789	3	Candidatus Bathyarchaeota (phylum)
NODE	3471	length	2479	cov	4.262789	4	Candidatus Bathyarchaeota (phylum)
NODE	3496	length	2474	cov	4.714758	1	
NODE	3496	length	2474	cov	4.714758	2	Candidatus Bathyarchaeota (phylum)
NODE	3496	length	2474	cov	4.714758	3	
NODE	3496	length	2474	cov	4.714758	4	Candidatus Bathyarchaeota (phylum)
NODE	3576	length	2451	cov	4.886895	1	Candidatus Bathyarchaeota (phylum)
NODE	3576	length	2451	cov	4.886895	2	Candidatus Bathyarchaeota (phylum)
NODE	3582	length	2450	cov	4.287683	1	Candidatus Bathyarchaeota (phylum)
NODE	3582	length	2450	cov	4.287683	2	Candidatus Bathyarchaeota (phylum)
NODE	3611	length	2443	cov	4.708961	1	
NODE	3611	length	2443	cov	4.708961	2	Methanomada group (clade)
NODE	3611	length	2443	cov	4.708961	3	
NODE	3611	length	2443	cov	4.708961	4	Candidatus Bathyarchaeota (phylum)
NODE	3611	length	2443	cov	4.708961	5	
NODE	3614	length	2443	cov	4.270519	1	Candidatus Bathyarchaeota (phylum)
NODE	3614	length	2443	cov	4.270519	2	Candidatus Bathyarchaeota (phylum)
NODE	3614	length	2443	cov	4.270519	3	Candidatus Bathyarchaeota (phylum)
NODE	3648	length	2438	cov	4.094838	1	
NODE	3648	length	2438	cov	4.094838	2	Candidatus Bathyarchaeota (phylum)

NODE	3648	length	2438	cov	4.094838	3	
NODE	3648	length	2438	cov	4.094838	4	Candidatus Bathyarchaeota (phylum)
NODE	3697	length	2428	cov	3.556258	1	Candidatus Bathyarchaeota (phylum)
NODE	3697	length	2428	cov	3.556258	2	Candidatus Bathyarchaeota (phylum)
NODE	3792	length	2409	cov	3.190739	1	Candidatus Bathyarchaeota (phylum)
NODE	3792	length	2409	cov	3.190739	2	Candidatus Bathyarchaeota (phylum)
NODE	3792	length	2409	cov	3.190739	3	Candidatus Bathyarchaeota (phylum)
NODE	3792	length	2409	cov	3.190739	4	Chloroflexi (phylum)
NODE	3794	length	2408	cov	4.39354	1	Candidatus Bathyarchaeota (phylum)
NODE	3794	length	2408	cov	4.39354	2	Candidatus Bathyarchaeota (phylum)
NODE	3794	length	2408	cov	4.39354	3	Candidatus Bathyarchaeota (phylum)
NODE	3823	length	2401	cov	4.936061	1	Candidatus Bathyarchaeota (phylum)
NODE	3823	length	2401	cov	4.936061	2	Candidatus Bathyarchaeota (phylum)
NODE	3823	length	2401	cov	4.936061	3	
NODE	3823	length	2401	cov	4.936061	4	Candidatus Bathyarchaeota (phylum)
NODE	3898	length	2382	cov	5.111302	1	Candidatus Bathyarchaeota (phylum)
NODE	3898	length	2382	cov	5.111302	2	Candidatus Bathyarchaeota (phylum)
NODE	3999	length	2363	cov	2.92721	1	uncultured archaeon (species)
NODE	3999	length	2363	cov	2.92721	2	Chloroflexi (phylum)
NODE	4112	length	2341	cov	5.276465	1	Candidatus Bathyarchaeota (phylum)
NODE	4112	length	2341	cov	5.276465	2	Candidatus Bathyarchaeota (phylum)
NODE	4112	length	2341	cov	5.276465	3	Candidatus Bathyarchaeota (phylum)
NODE	4122	length	2339	cov	5.078371	1	Candidatus Bathyarchaeota (phylum)
NODE	4122	length	2339	cov	5.078371	2	Candidatus Bathyarchaeota (phylum)
NODE	4122	length	2339	cov	5.078371	3	Candidatus Bathyarchaeota (phylum)
NODE	4130	length	2338	cov	3.291721	1	Candidatus Bathyarchaeota (phylum)
NODE	4130	length	2338	cov	3.291721	2	Candidatus Bathyarchaeota (phylum)
NODE	4130	length	2338	cov	3.291721	3	Candidatus Bathyarchaeota (phylum)
NODE	4226	length	2320	cov	5.802649	1	Candidatus Bathyarchaeota (phylum)
NODE	4226	length	2320	cov	5.802649	2	Candidatus Bathyarchaeota (phylum)
NODE	4236	length	2318	cov	5.954485	1	Candidatus Bathyarchaeota (phylum)
NODE	4236	length	2318	cov	5.954485	2	
NODE	4236	length	2318	cov	5.954485	3	Candidatus Bathyarchaeota (phylum)
NODE	4236	length	2318	cov	5.954485	4	Candidatus Bathyarchaeota (phylum)
NODE	4268	length	2312	cov	4.582189	1	Candidatus Bathyarchaeota (phylum)
NODE	4268	length	2312	cov	4.582189	2	Candidatus Bathyarchaeota (phylum)
NODE	4268	length	2312	cov	4.582189	3	Candidatus Bathyarchaeota (phylum)
NODE	4386	length	2289	cov	5.337511	1	Bacteria candidate phyla (clade)
NODE	4386	length	2289	cov	5.337511	2	Crenarchaeota (phylum)
NODE	4397	length	2288	cov	2.904165	1	archaeon RBG_16_50_20 (species)
NODE	4397	length	2288	cov	2.904165	2	unclassified Acidobacteria (no rank)
NODE	4397	length	2288	cov	2.904165	3	
NODE	4398	length	2288	cov	2.742051	1	Candidatus Bathyarchaeota (phylum)
NODE	4398	length	2288	cov	2.742051	2	Candidatus Bathyarchaeota (phylum)
NODE	4398	length	2288	cov	2.742051	3	Candidatus Bathyarchaeota (phylum)
NODE	4398	length	2288	cov	2.742051	4	Candidatus Bathyarchaeota (phylum)
NODE	4434	length	2283	cov	3.561939	1	Candidatus Bathyarchaeota (phylum)
NODE	4434	length	2283	cov	3.561939	2	Candidatus Bathyarchaeota (phylum)
NODE	4434	length	2283	cov	3.561939	3	Candidatus Heimdallarchaeota (phylum)
NODE	4478	length	2275	cov	3.144144	1	Hadesarchaea (class)
NODE	4478	length	2275	cov	3.144144	2	
NODE	4478	length	2275	cov	3.144144	3	
NODE	4512	length	2269	cov	4.557814	1	Candidatus Bathyarchaeota (phylum)
NODE	4512	length	2269	cov	4.557814	2	Candidatus Bathyarchaeota (phylum)
NODE	4551	length	2262	cov	2.847304	1	
NODE	4551	length	2262	cov	2.847304	2	Candidatus Bathyarchaeota (phylum)

NODE	4551	length	2262	cov	2.847304	3	Candidatus Bathyarchaeota (phylum)
NODE	4664	length	2242	cov	6.778235	1	Candidatus Bathyarchaeota (phylum)
NODE	4664	length	2242	cov	6.778235	2	Candidatus Bathyarchaeota (phylum)
NODE	4664	length	2242	cov	6.778235	3	
NODE	4664	length	2242	cov	6.778235	4	Candidatus Bathyarchaeota (phylum)
NODE	4667	length	2242	cov	4.418839	1	Candidatus Bathyarchaeota (phylum)
NODE	4667	length	2242	cov	4.418839	2	Candidatus Bathyarchaeota (phylum)
NODE	4667	length	2242	cov	4.418839	3	Candidatus Bathyarchaeota (phylum)
NODE	4667	length	2242	cov	4.418839	4	Candidatus Bathyarchaeota (phylum)
NODE	4720	length	2231	cov	4.633272	1	Candidatus Bathyarchaeota (phylum)
NODE	4720	length	2231	cov	4.633272	2	
NODE	4720	length	2231	cov	4.633272	3	Candidatus Bathyarchaeota (phylum)
NODE	4723	length	2231	cov	3.846048	1	Candidatus Bathyarchaeota (phylum)
NODE	4723	length	2231	cov	3.846048	2	
NODE	4723	length	2231	cov	3.846048	3	
NODE	4751	length	2227	cov	3.081031	1	delta/epsilon subdivisions (subphylum)
NODE	4751	length	2227	cov	3.081031	2	
NODE	4751	length	2227	cov	3.081031	3	
NODE	4751	length	2227	cov	3.081031	4	
NODE	4751	length	2227	cov	3.081031	5	Candidatus Bathyarchaeota (phylum)
NODE	4869	length	2208	cov	2.984208	1	Candidatus Bathyarchaeota (phylum)
NODE	4869	length	2208	cov	2.984208	2	Candidatus Bathyarchaeota (phylum)
NODE	4869	length	2208	cov	2.984208	3	Candidatus Bathyarchaeota (phylum)
NODE	4869	length	2208	cov	2.984208	4	Candidatus Bathyarchaeota (phylum)
NODE	4933	length	2200	cov	3.455478	1	Candidatus Bathyarchaeota (phylum)
NODE	4933	length	2200	cov	3.455478	2	Candidatus Bathyarchaeota (phylum)
NODE	4937	length	2199	cov	4.057836	1	Candidatus Bathyarchaeota (phylum)
NODE	4937	length	2199	cov	4.057836	2	Candidatus Bathyarchaeota (phylum)
NODE	4973	length	2193	cov	3.515903	1	Candidatus Bathyarchaeota (phylum)
NODE	4973	length	2193	cov	3.515903	2	
NODE	4973	length	2193	cov	3.515903	3	Candidatus Bathyarchaeota (phylum)
NODE	4975	length	2192	cov	5.345344	1	Candidatus Bathyarchaeota (phylum)
NODE	4975	length	2192	cov	5.345344	2	Candidatus Bathyarchaeota (phylum)
NODE	4975	length	2192	cov	5.345344	3	
NODE	5036	length	2182	cov	4.538787	1	Candidatus Bathyarchaeota (phylum)
NODE	5036	length	2182	cov	4.538787	2	Candidatus Bathyarchaeota (phylum)
NODE	5036	length	2182	cov	4.538787	3	Candidatus Bathyarchaeota (phylum)
NODE	5036	length	2182	cov	4.538787	4	Candidatus Bathyarchaeota (phylum)
NODE	5072	length	2176	cov	3.987742	1	Candidatus Bathyarchaeota (phylum)
NODE	5072	length	2176	cov	3.987742	2	Candidatus Bathyarchaeota (phylum)
NODE	5072	length	2176	cov	3.987742	3	Candidatus Bathyarchaeota (phylum)
NODE	5079	length	2175	cov	5.225472	1	Candidatus Marsarchaeota (phylum)
NODE	5079	length	2175	cov	5.225472	2	Candidatus Bathyarchaeota (phylum)
NODE	5079	length	2175	cov	5.225472	3	Candidatus Bathyarchaeota (phylum)
NODE	5197	length	2157	cov	4.934348	1	
NODE	5197	length	2157	cov	4.934348	2	Candidatus Bathyarchaeota (phylum)
NODE	5197	length	2157	cov	4.934348	3	Candidatus Bathyarchaeota (phylum)
NODE	5290	length	2145	cov	2.172249	1	Candidatus Bathyarchaeota (phylum)
NODE	5290	length	2145	cov	2.172249	2	Candidatus Bathyarchaeota (phylum)
NODE	5290	length	2145	cov	2.172249	3	
NODE	5301	length	2143	cov	4.699713	1	Candidatus Bathyarchaeota (phylum)
NODE	5301	length	2143	cov	4.699713	2	Candidatus Bathyarchaeota (phylum)
NODE	5301	length	2143	cov	4.699713	3	Candidatus Bathyarchaeota (phylum)
NODE	5302	length	2143	cov	3.914751	1	Candidatus Bathyarchaeota (phylum)
NODE	5302	length	2143	cov	3.914751	2	
NODE	5388	length	2131	cov	4.456647	1	Candidatus Bathyarchaeota (phylum)

NODE	5388	length	2131	cov	4.456647	2	Candidatus Bathyarchaeota (phylum)
NODE	5388	length	2131	cov	4.456647	3	
NODE	5388	length	2131	cov	4.456647	4	
NODE	5401	length	2130	cov	3.594699	1	Candidatus Bathyarchaeota (phylum)
NODE	5401	length	2130	cov	3.594699	2	Candidatus Bathyarchaeota (phylum)
NODE	5463	length	2121	cov	5.484027	1	Candidatus Bathyarchaeota (phylum)
NODE	5482	length	2119	cov	4.252422	1	Candidatus Bathyarchaeota (phylum)
NODE	5482	length	2119	cov	4.252422	2	Candidatus Bathyarchaeota (phylum)
NODE	5482	length	2119	cov	4.252422	3	Candidatus Bathyarchaeota (phylum)
NODE	5489	length	2118	cov	3.043626	1	Candidatus Bathyarchaeota (phylum)
NODE	5489	length	2118	cov	3.043626	2	Candidatus Bathyarchaeota (phylum)
NODE	5556	length	2109	cov	4.556962	1	
NODE	5556	length	2109	cov	4.556962	2	Candidatus Bathyarchaeota (phylum)
NODE	5556	length	2109	cov	4.556962	3	Candidatus Bathyarchaeota (phylum)
NODE	5556	length	2109	cov	4.556962	4	Candidatus Bathyarchaeota (phylum)
NODE	5556	length	2109	cov	4.556962	5	Candidatus Bathyarchaeota (phylum)
NODE	5582	length	2106	cov	3.812287	1	Actinobacteria (phylum)
NODE	5582	length	2106	cov	3.812287	2	
NODE	5582	length	2106	cov	3.812287	3	
NODE	5582	length	2106	cov	3.812287	4	Candidatus Bathyarchaeota (phylum)
NODE	5582	length	2106	cov	3.812287	5	Candidatus Bathyarchaeota (phylum)
NODE	5621	length	2099	cov	4.814579	1	Candidatus Bathyarchaeota (phylum)
NODE	5621	length	2099	cov	4.814579	2	Candidatus Bathyarchaeota (phylum)
NODE	5621	length	2099	cov	4.814579	3	
NODE	5639	length	2097	cov	3.366797	1	Candidatus Bathyarchaeota (phylum)
NODE	5639	length	2097	cov	3.366797	2	Candidatus Bathyarchaeota (phylum)
NODE	5646	length	2096	cov	3.053895	1	Candidatus Bathyarchaeota (phylum)
NODE	5646	length	2096	cov	3.053895	2	Candidatus Bathyarchaeota (phylum)
NODE	5659	length	2094	cov	3.113291	1	Candidatus Bathyarchaeota (phylum)
NODE	5659	length	2094	cov	3.113291	2	Candidatus Bathyarchaeota (phylum)
NODE	5659	length	2094	cov	3.113291	3	Candidatus Bathyarchaeota (phylum)
NODE	5659	length	2094	cov	3.113291	4	
NODE	5705	length	2087	cov	5.366634	1	Candidatus Bathyarchaeota (phylum)
NODE	5705	length	2087	cov	5.366634	2	Candidatus Bathyarchaeota (phylum)
NODE	5705	length	2087	cov	5.366634	3	Candidatus Bathyarchaeota (phylum)
NODE	5705	length	2087	cov	5.366634	4	Candidatus Bathyarchaeota (phylum)
NODE	5710	length	2086	cov	5.701625	1	
NODE	5710	length	2086	cov	5.701625	2	
NODE	5782	length	2079	cov	3.084486	1	Candidatus Bathyarchaeota (phylum)
NODE	5782	length	2079	cov	3.084486	2	Candidatus Bathyarchaeota (phylum)
NODE	5782	length	2079	cov	3.084486	3	Candidatus Bathyarchaeota (phylum)
NODE	5787	length	2078	cov	4.871478	1	
NODE	5787	length	2078	cov	4.871478	2	Candidatus Bathyarchaeota (phylum)
NODE	5787	length	2078	cov	4.871478	3	Candidatus Bathyarchaeota (phylum)
NODE	5813	length	2075	cov	5.510891	1	
NODE	5813	length	2075	cov	5.510891	2	
NODE	5840	length	2072	cov	3.10709	1	Candidatus Bathyarchaeota (phylum)
NODE	5840	length	2072	cov	3.10709	2	Candidatus Bathyarchaeota (phylum)
NODE	5840	length	2072	cov	3.10709	3	Candidatus Bathyarchaeota (phylum)
NODE	5844	length	2071	cov	5.885913	1	Candidatus Bathyarchaeota (phylum)
NODE	5844	length	2071	cov	5.885913	2	Candidatus Bathyarchaeota (phylum)
NODE	5881	length	2067	cov	2.996521	1	Candidatus Bathyarchaeota (phylum)
NODE	5881	length	2067	cov	2.996521	2	Candidatus Bathyarchaeota (phylum)
NODE	5881	length	2067	cov	2.996521	3	Candidatus Bathyarchaeota (phylum)
NODE	5881	length	2067	cov	2.996521	4	Candidatus Bathyarchaeota (phylum)
NODE	5897	length	2064	cov	5.987556	1	Candidatus Bathyarchaeota (phylum)

NODE	5932	length	2062	cov	3.218734	1	Candidatus Bathyarchaeota (phylum)
NODE	5932	length	2062	cov	3.218734	2	Candidatus Bathyarchaeota (phylum)
NODE	5938	length	2061	cov	3.950648	1	Candidatus Bathyarchaeota (phylum)
NODE	5938	length	2061	cov	3.950648	2	
NODE	5943	length	2061	cov	3.427717	1	Candidatus Bathyarchaeota (phylum)
NODE	5943	length	2061	cov	3.427717	2	Candidatus Bathyarchaeota (phylum)
NODE	5973	length	2057	cov	4.025974	1	Candidatus Bathyarchaeota (phylum)
NODE	5973	length	2057	cov	4.025974	2	Candidatus Bathyarchaeota (phylum)
NODE	5997	length	2053	cov	4.855355	1	Candidatus Bathyarchaeota (phylum)
NODE	6063	length	2041	cov	5.178751	1	Candidatus Bathyarchaeota (phylum)
NODE	6063	length	2041	cov	5.178751	2	Candidatus Bathyarchaeota (phylum)
NODE	6063	length	2041	cov	5.178751	3	Candidatus Bathyarchaeota (phylum)
NODE	6063	length	2041	cov	5.178751	4	Candidatus Bathyarchaeota (phylum)
NODE	6073	length	2040	cov	5.948111	1	Candidatus Bathyarchaeota (phylum)
NODE	6073	length	2040	cov	5.948111	2	Candidatus Bathyarchaeota (phylum)
NODE	6073	length	2040	cov	5.948111	3	Candidatus Bathyarchaeota (phylum)
NODE	6153	length	2031	cov	5.121457	1	Candidatus Bathyarchaeota (phylum)
NODE	6153	length	2031	cov	5.121457	2	Theionarchaea (class)
NODE	6236	length	2021	cov	4.584435	1	Bacteria candidate phyla (clade)
NODE	6236	length	2021	cov	4.584435	2	unclassified Acidobacteria (no rank)
NODE	6236	length	2021	cov	4.584435	3	
NODE	6260	length	2019	cov	2.860489	1	Candidatus Bathyarchaeota (phylum)
NODE	6260	length	2019	cov	2.860489	2	Candidatus Bathyarchaeota (phylum)
NODE	6279	length	2016	cov	3.4513	1	
NODE	6279	length	2016	cov	3.4513	2	
NODE	6279	length	2016	cov	3.4513	3	
NODE	6339	length	2010	cov	3.952941	1	Candidatus Bathyarchaeota (phylum)
NODE	6339	length	2010	cov	3.952941	2	Candidatus Bathyarchaeota (phylum)
NODE	6339	length	2010	cov	3.952941	3	
NODE	6342	length	2010	cov	3.325831	1	Candidatus Bathyarchaeota (phylum)
NODE	6342	length	2010	cov	3.325831	2	Candidatus Bathyarchaeota (phylum)
NODE	6404	length	2004	cov	3.23961	1	Candidatus Bathyarchaeota (phylum)
NODE	6404	length	2004	cov	3.23961	2	
NODE	6467	length	1997	cov	6.995881	1	Candidatus Bathyarchaeota (phylum)
NODE	6467	length	1997	cov	6.995881	2	Candidatus Bathyarchaeota (phylum)
NODE	6494	length	1995	cov	4.757216	1	
NODE	6494	length	1995	cov	4.757216	2	Candidatus Bathyarchaeota (phylum)
NODE	6494	length	1995	cov	4.757216	3	Candidatus Bathyarchaeota (phylum)
NODE	6494	length	1995	cov	4.757216	4	Candidatus Bathyarchaeota (phylum)
NODE	6541	length	1991	cov	4.331095	1	Candidatus Bathyarchaeota (phylum)
NODE	6541	length	1991	cov	4.331095	2	Candidatus Bathyarchaeota (phylum)
NODE	6572	length	1988	cov	3.527677	1	Candidatus Bathyarchaeota (phylum)
NODE	6572	length	1988	cov	3.527677	2	Candidatus Bathyarchaeota (phylum)
NODE	6572	length	1988	cov	3.527677	3	Candidatus Bathyarchaeota (phylum)
NODE	6572	length	1988	cov	3.527677	4	
NODE	6609	length	1984	cov	3.007258	1	Candidatus Bathyarchaeota (phylum)
NODE	6609	length	1984	cov	3.007258	2	Candidatus Bathyarchaeota (phylum)
NODE	6609	length	1984	cov	3.007258	3	Candidatus Bathyarchaeota (phylum)
NODE	6609	length	1984	cov	3.007258	4	Candidatus Bathyarchaeota (phylum)
NODE	6646	length	1980	cov	4.343377	1	Candidatus Bathyarchaeota (phylum)
NODE	6646	length	1980	cov	4.343377	2	Candidatus Bathyarchaeota (phylum)
NODE	6711	length	1974	cov	3.809797	1	Candidatus Bathyarchaeota (phylum)
NODE	6711	length	1974	cov	3.809797	2	
NODE	6731	length	1972	cov	3.252478	1	Candidatus Bathyarchaeota (phylum)
NODE	6731	length	1972	cov	3.252478	2	Candidatus Bathyarchaeota (phylum)
NODE	6823	length	1961	cov	4.714061	1	Candidatus Bathyarchaeota (phylum)

NODE	6823	length	1961	cov	4.714061	2	Candidatus Bathyarchaeota (phylum)
NODE	6824	length	1961	cov	4.121196	1	
NODE	6824	length	1961	cov	4.121196	2	Candidatus Bathyarchaeota (phylum)
NODE	6824	length	1961	cov	4.121196	3	Candidatus Bathyarchaeota (phylum)
NODE	6824	length	1961	cov	4.121196	4	Candidatus Bathyarchaeota (phylum)
NODE	6886	length	1954	cov	3.586098	1	
NODE	6886	length	1954	cov	3.586098	2	
NODE	6886	length	1954	cov	3.586098	3	Candidatus Bathyarchaeota (phylum)
NODE	6886	length	1954	cov	3.586098	4	Candidatus Bathyarchaeota (phylum)
NODE	6886	length	1954	cov	3.586098	5	Candidatus Bathyarchaeota (phylum)
NODE	6886	length	1954	cov	3.586098	6	Candidatus Bathyarchaeota (phylum)
NODE	6893	length	1953	cov	4.219705	1	
NODE	6893	length	1953	cov	4.219705	2	Candidatus Bathyarchaeota (phylum)
NODE	6893	length	1953	cov	4.219705	3	Candidatus Bathyarchaeota (phylum)
NODE	6919	length	1950	cov	4.862269	1	Candidatus Bathyarchaeota (phylum)
NODE	6919	length	1950	cov	4.862269	2	
NODE	6919	length	1950	cov	4.862269	3	Candidatus Bathyarchaeota (phylum)
NODE	6954	length	1947	cov	4.286998	1	Candidatus Bathyarchaeota (phylum)
NODE	6954	length	1947	cov	4.286998	2	Candidatus Bathyarchaeota (phylum)
NODE	6954	length	1947	cov	4.286998	3	Candidatus Bathyarchaeota (phylum)
NODE	7029	length	1939	cov	4.264862	1	
NODE	7029	length	1939	cov	4.264862	2	Candidatus Bathyarchaeota (phylum)
NODE	7029	length	1939	cov	4.264862	3	Candidatus Bathyarchaeota (phylum)
NODE	7029	length	1939	cov	4.264862	4	Candidatus Bathyarchaeota (phylum)
NODE	7250	length	1918	cov	4.37037	1	Candidatus Bathyarchaeota (phylum)
NODE	7250	length	1918	cov	4.37037	2	Candidatus Bathyarchaeota (phylum)
NODE	7250	length	1918	cov	4.37037	3	Candidatus Bathyarchaeota (phylum)
NODE	7250	length	1918	cov	4.37037	4	Candidatus Bathyarchaeota (phylum)
NODE	7252	length	1918	cov	4.228663	1	Candidatus Bathyarchaeota (phylum)
NODE	7252	length	1918	cov	4.228663	2	Candidatus Bathyarchaeota (phylum)
NODE	7252	length	1918	cov	4.228663	3	Candidatus Bathyarchaeota (phylum)
NODE	7292	length	1914	cov	4.301237	1	Candidatus Bathyarchaeota (phylum)
NODE	7292	length	1914	cov	4.301237	2	Candidatus Bathyarchaeota (phylum)
NODE	7292	length	1914	cov	4.301237	3	
NODE	7331	length	1911	cov	3.624461	1	
NODE	7331	length	1911	cov	3.624461	2	Firmicutes (phylum)
NODE	7336	length	1910	cov	4.967655	1	Candidatus Bathyarchaeota (phylum)
NODE	7336	length	1910	cov	4.967655	2	Candidatus Bathyarchaeota (phylum)
NODE	7336	length	1910	cov	4.967655	3	
NODE	7624	length	1884	cov	6.255331	1	Candidatus Bathyarchaeota (phylum)
NODE	7624	length	1884	cov	6.255331	2	
NODE	7624	length	1884	cov	6.255331	3	
NODE	7647	length	1882	cov	5.211823	1	Candidatus Bathyarchaeota (phylum)
NODE	7647	length	1882	cov	5.211823	2	
NODE	7667	length	1880	cov	3.539178	1	Candidatus Bathyarchaeota (phylum)
NODE	7667	length	1880	cov	3.539178	2	Candidatus Bathyarchaeota (phylum)
NODE	7667	length	1880	cov	3.539178	3	Candidatus Bathyarchaeota (phylum)
NODE	7694	length	1877	cov	6.498902	1	
NODE	7694	length	1877	cov	6.498902	2	Candidatus Bathyarchaeota (phylum)
NODE	7694	length	1877	cov	6.498902	3	
NODE	7705	length	1877	cov	2.232711	1	Candidatus Bathyarchaeota (phylum)
NODE	7705	length	1877	cov	2.232711	2	Candidatus Bathyarchaeota (phylum)
NODE	7705	length	1877	cov	2.232711	3	Candidatus Bathyarchaeota (phylum)
NODE	7720	length	1875	cov	4.068132	1	Candidatus Bathyarchaeota (phylum)
NODE	7720	length	1875	cov	4.068132	2	Candidatus Bathyarchaeota (phylum)
NODE	7720	length	1875	cov	4.068132	3	Candidatus Bathyarchaeota (phylum)

NODE	7749	length	1873	cov	3.877338	1	Candidatus Bathyarchaeota (phylum)
NODE	7749	length	1873	cov	3.877338	2	Candidatus Bathyarchaeota (phylum)
NODE	7749	length	1873	cov	3.877338	3	Candidatus Bathyarchaeota (phylum)
NODE	7749	length	1873	cov	3.877338	4	Candidatus Bathyarchaeota (phylum)
NODE	7756	length	1872	cov	4.865162	1	Candidatus Bathyarchaeota (phylum)
NODE	7756	length	1872	cov	4.865162	2	
NODE	7756	length	1872	cov	4.865162	3	Crenarchaeota (phylum)
NODE	7825	length	1867	cov	3.986203	1	
NODE	7825	length	1867	cov	3.986203	2	Candidatus Bathyarchaeota (phylum)
NODE	7870	length	1864	cov	2.323936	1	Candidatus Bathyarchaeota (phylum)
NODE	7870	length	1864	cov	2.323936	2	Candidatus Bathyarchaeota (phylum)
NODE	7870	length	1864	cov	2.323936	3	Candidatus Bathyarchaeota (phylum)
NODE	7871	length	1863	cov	6.531527	1	Hadesarchaea (class)
NODE	7871	length	1863	cov	6.531527	2	
NODE	7871	length	1863	cov	6.531527	3	
NODE	7871	length	1863	cov	6.531527	4	Candidatus Bathyarchaeota (phylum)
NODE	7888	length	1862	cov	3.732706	1	Candidatus Bathyarchaeota (phylum)
NODE	7888	length	1862	cov	3.732706	2	Candidatus Bathyarchaeota (phylum)
NODE	7888	length	1862	cov	3.732706	3	
NODE	7999	length	1852	cov	2.970506	1	
NODE	7999	length	1852	cov	2.970506	2	
NODE	8002	length	1851	cov	5.44265	1	
NODE	8002	length	1851	cov	5.44265	2	archaeon RBG_16_50_20 (species)
NODE	8002	length	1851	cov	5.44265	3	archaeon RBG_16_50_20 (species)
NODE	8030	length	1850	cov	3.500836	1	Candidatus Bathyarchaeota (phylum)
NODE	8030	length	1850	cov	3.500836	2	Candidatus Bathyarchaeota (phylum)
NODE	8083	length	1846	cov	4.786711	1	Candidatus Bathyarchaeota (phylum)
NODE	8083	length	1846	cov	4.786711	2	Candidatus Bathyarchaeota (phylum)
NODE	8083	length	1846	cov	4.786711	3	Candidatus Bathyarchaeota (phylum)
NODE	8086	length	1846	cov	4.151312	1	
NODE	8086	length	1846	cov	4.151312	2	
NODE	8086	length	1846	cov	4.151312	3	Candidatus Bathyarchaeota (phylum)
NODE	8086	length	1846	cov	4.151312	4	Candidatus Bathyarchaeota (phylum)
NODE	8089	length	1846	cov	3.514238	1	
NODE	8089	length	1846	cov	3.514238	2	Candidatus Bathyarchaeota (phylum)
NODE	8089	length	1846	cov	3.514238	3	Candidatus Bathyarchaeota (phylum)
NODE	8143	length	1842	cov	3.385562	1	Candidatus Bathyarchaeota (phylum)
NODE	8196	length	1837	cov	4.053872	1	Candidatus Bathyarchaeota (phylum)
NODE	8196	length	1837	cov	4.053872	2	Candidatus Bathyarchaeota (phylum)
NODE	8233	length	1834	cov	4.477797	1	
NODE	8233	length	1834	cov	4.477797	2	
NODE	8260	length	1832	cov	5.2009	1	
NODE	8260	length	1832	cov	5.2009	2	Candidatus Bathyarchaeota (phylum)
NODE	8260	length	1832	cov	5.2009	3	
NODE	8280	length	1831	cov	2.375563	1	
NODE	8280	length	1831	cov	2.375563	2	
NODE	8288	length	1830	cov	3.12169	1	Candidatus Bathyarchaeota (phylum)
NODE	8288	length	1830	cov	3.12169	2	Candidatus Bathyarchaeota (phylum)
NODE	8292	length	1829	cov	4.983089	1	Candidatus Bathyarchaeota (phylum)
NODE	8292	length	1829	cov	4.983089	2	Candidatus Bathyarchaeota (phylum)
NODE	8292	length	1829	cov	4.983089	3	Candidatus Bathyarchaeota (phylum)
NODE	8321	length	1827	cov	3.584086	1	Candidatus Bathyarchaeota (phylum)
NODE	8321	length	1827	cov	3.584086	2	Candidatus Bathyarchaeota (phylum)
NODE	8330	length	1826	cov	3.572558	1	
NODE	8330	length	1826	cov	3.572558	2	Candidatus Bathyarchaeota (phylum)
NODE	8330	length	1826	cov	3.572558	3	Candidatus Bathyarchaeota (phylum)

NODE	8347	length	1824	cov	6.616167	1	Candidatus Bathyarchaeota (phylum)
NODE	8347	length	1824	cov	6.616167	2	
NODE	8347	length	1824	cov	6.616167	3	
NODE	8351	length	1824	cov	3.920294	1	Candidatus Bathyarchaeota (phylum)
NODE	8351	length	1824	cov	3.920294	2	Candidatus Bathyarchaeota (phylum)
NODE	8351	length	1824	cov	3.920294	3	Candidatus Bathyarchaeota (phylum)
NODE	8352	length	1824	cov	3.67948	1	Candidatus Bathyarchaeota (phylum)
NODE	8352	length	1824	cov	3.67948	2	Candidatus Bathyarchaeota (phylum)
NODE	8404	length	1820	cov	6.010198	1	Candidatus Bathyarchaeota (phylum)
NODE	8404	length	1820	cov	6.010198	2	Candidatus Bathyarchaeota (phylum)
NODE	8404	length	1820	cov	6.010198	3	Candidatus Bathyarchaeota (phylum)
NODE	8404	length	1820	cov	6.010198	4	Candidatus Bathyarchaeota (phylum)
NODE	8448	length	1817	cov	6.210556	1	Candidatus Bathyarchaeota (phylum)
NODE	8448	length	1817	cov	6.210556	2	Candidatus Bathyarchaeota (phylum)
NODE	8481	length	1815	cov	4.167045	1	Candidatus Bathyarchaeota (phylum)
NODE	8542	length	1811	cov	3.170273	1	Bacteroidetes/Chlorobi group (clade)
NODE	8542	length	1811	cov	3.170273	2	Candidatus Bathyarchaeota (phylum)
NODE	8542	length	1811	cov	3.170273	3	Candidatus Bathyarchaeota (phylum)
NODE	8542	length	1811	cov	3.170273	4	Candidatus Bathyarchaeota (phylum)
NODE	8554	length	1810	cov	3.08433	1	Candidatus Bathyarchaeota (phylum)
NODE	8554	length	1810	cov	3.08433	2	
NODE	8643	length	1804	cov	2.513436	1	Candidatus Bathyarchaeota (phylum)
NODE	8643	length	1804	cov	2.513436	2	Candidatus Bathyarchaeota (phylum)
NODE	8643	length	1804	cov	2.513436	3	Candidatus Bathyarchaeota (phylum)
NODE	8648	length	1803	cov	4.522883	1	Candidatus Bathyarchaeota (phylum)
NODE	8648	length	1803	cov	4.522883	2	Candidatus Bathyarchaeota (phylum)
NODE	8648	length	1803	cov	4.522883	3	
NODE	8648	length	1803	cov	4.522883	4	
NODE	8696	length	1800	cov	2.54212	1	Candidatus Bathyarchaeota (phylum)
NODE	8696	length	1800	cov	2.54212	2	Candidatus Bathyarchaeota (phylum)
NODE	8696	length	1800	cov	2.54212	3	Candidatus Bathyarchaeota (phylum)
NODE	8711	length	1799	cov	2.688073	1	Candidatus Bathyarchaeota (phylum)
NODE	8711	length	1799	cov	2.688073	2	Candidatus Bathyarchaeota (phylum)
NODE	8711	length	1799	cov	2.688073	3	Candidatus Bathyarchaeota (phylum)
NODE	8713	length	1798	cov	7.051635	1	Candidatus Bathyarchaeota (phylum)
NODE	8884	length	1787	cov	3.210739	1	Candidatus Bathyarchaeota (phylum)
NODE	8884	length	1787	cov	3.210739	2	Candidatus Bathyarchaeota (phylum)
NODE	8884	length	1787	cov	3.210739	3	
NODE	8939	length	1782	cov	5.906775	1	
NODE	8939	length	1782	cov	5.906775	2	
NODE	8986	length	1779	cov	3.950116	1	Candidatus Bathyarchaeota (phylum)
NODE	8986	length	1779	cov	3.950116	2	
NODE	8986	length	1779	cov	3.950116	3	
NODE	9014	length	1777	cov	3.46748	1	Candidatus Bathyarchaeota (phylum)
NODE	9014	length	1777	cov	3.46748	2	Firmicutes (phylum)
NODE	9014	length	1777	cov	3.46748	3	Candidatus Bathyarchaeota (phylum)
NODE	9052	length	1774	cov	6.168121	1	
NODE	9052	length	1774	cov	6.168121	2	Candidatus Bathyarchaeota (phylum)
NODE	9052	length	1774	cov	6.168121	3	Candidatus Bathyarchaeota (phylum)
NODE	9133	length	1769	cov	4.172112	1	Candidatus Bathyarchaeota (phylum)
NODE	9133	length	1769	cov	4.172112	2	Candidatus Bathyarchaeota (phylum)
NODE	9146	length	1768	cov	5.339171	1	Candidatus Bathyarchaeota (phylum)
NODE	9146	length	1768	cov	5.339171	2	Candidatus Bathyarchaeota (phylum)
NODE	9146	length	1768	cov	5.339171	3	
NODE	9151	length	1768	cov	2.931115	1	Candidatus Bathyarchaeota (phylum)
NODE	9200	length	1765	cov	1.822222	1	Firmicutes (phylum)

NODE	9200	length	1765	cov	1.822222	2	Candidatus Bathyarchaeota (phylum)
NODE	9508	length	1745	cov	2.692308	1	Candidatus Bathyarchaeota (phylum)
NODE	9508	length	1745	cov	2.692308	2	Candidatus Bathyarchaeota (phylum)
NODE	9548	length	1742	cov	3.25489	1	Candidatus Bathyarchaeota (phylum)
NODE	9548	length	1742	cov	3.25489	2	
NODE	9572	length	1740	cov	2.477745	1	Candidatus Bathyarchaeota (phylum)
NODE	9572	length	1740	cov	2.477745	2	Candidatus Bathyarchaeota (phylum)
NODE	9572	length	1740	cov	2.477745	3	Candidatus Bathyarchaeota (phylum)
NODE	9572	length	1740	cov	2.477745	4	
NODE	9634	length	1736	cov	2.450327	1	
NODE	9634	length	1736	cov	2.450327	2	Candidatus Bathyarchaeota (phylum)
NODE	9671	length	1733	cov	2.924911	1	Candidatus Bathyarchaeota (phylum)
NODE	9671	length	1733	cov	2.924911	2	Candidatus Bathyarchaeota (phylum)
NODE	9671	length	1733	cov	2.924911	3	Bacteria candidate phyla (clade)
NODE	9780	length	1725	cov	5.104192	1	
NODE	9780	length	1725	cov	5.104192	2	Crenarchaeota (phylum)
NODE	9791	length	1725	cov	2.802395	1	Candidatus Bathyarchaeota (phylum)
NODE	9791	length	1725	cov	2.802395	2	Candidatus Bathyarchaeota (phylum)
NODE	9791	length	1725	cov	2.802395	3	Candidatus Bathyarchaeota (phylum)
NODE	9815	length	1723	cov	2.979616	1	Candidatus Bathyarchaeota (phylum)
NODE	9815	length	1723	cov	2.979616	2	
NODE	9815	length	1723	cov	2.979616	3	Candidatus Bathyarchaeota (phylum)
NODE	9941	length	1715	cov	3.101807	1	Candidatus Bathyarchaeota (phylum)
NODE	9941	length	1715	cov	3.101807	2	
NODE	9941	length	1715	cov	3.101807	3	
NODE	9956	length	1714	cov	4.21097	1	Candidatus Bathyarchaeota (phylum)
NODE	9956	length	1714	cov	4.21097	2	Candidatus Bathyarchaeota (phylum)
NODE	9961	length	1714	cov	3.69138	1	
NODE	9961	length	1714	cov	3.69138	2	Candidatus Bathyarchaeota (phylum)
NODE	9964	length	1714	cov	3.168776	1	Candidatus Bathyarchaeota (phylum)
NODE	9964	length	1714	cov	3.168776	2	Candidatus Bathyarchaeota (phylum)
NODE	9965	length	1714	cov	3.006631	1	
NODE	9965	length	1714	cov	3.006631	2	Candidatus Bathyarchaeota (phylum)
NODE	9965	length	1714	cov	3.006631	3	Candidatus Bathyarchaeota (phylum)
NODE	9989	length	1712	cov	3.733856	1	Candidatus Bathyarchaeota (phylum)
NODE	9989	length	1712	cov	3.733856	2	Candidatus Bathyarchaeota (phylum)
NODE	10047	length	1709	cov	4.033857	1	Candidatus Bathyarchaeota (phylum)
NODE	10088	length	1707	cov	3.251211	1	Candidatus Bathyarchaeota (phylum)
NODE	10113	length	1705	cov	4.96303	1	Candidatus Bathyarchaeota (phylum)
NODE	10113	length	1705	cov	4.96303	2	Candidatus Bathyarchaeota (phylum)
NODE	10113	length	1705	cov	4.96303	3	Candidatus Bathyarchaeota (phylum)
NODE	10227	length	1697	cov	4.099269	1	
NODE	10227	length	1697	cov	4.099269	2	
NODE	10227	length	1697	cov	4.099269	3	
NODE	10227	length	1697	cov	4.099269	4	
NODE	10227	length	1697	cov	4.099269	5	
NODE	10234	length	1697	cov	3.461023	1	Candidatus Bathyarchaeota (phylum)
NODE	10234	length	1697	cov	3.461023	2	
NODE	10234	length	1697	cov	3.461023	3	
NODE	10240	length	1697	cov	2.686358	1	Candidatus Bathyarchaeota (phylum)
NODE	10240	length	1697	cov	2.686358	2	archaeon RBG_16_50_20 (species)
NODE	10341	length	1691	cov	3.576406	1	Candidatus Bathyarchaeota (phylum)
NODE	10341	length	1691	cov	3.576406	2	Candidatus Bathyarchaeota (phylum)
NODE	10341	length	1691	cov	3.576406	3	Candidatus Bathyarchaeota (phylum)
NODE	10353	length	1690	cov	4.395719	1	Candidatus Bathyarchaeota (phylum)
NODE	10353	length	1690	cov	4.395719	2	Candidatus Bathyarchaeota (phylum)

NODE	10353	length	1690	cov	4.395719	3	Candidatus Bathyarchaeota (phylum)
NODE	10387	length	1688	cov	4.851806	1	Candidatus Bathyarchaeota (phylum)
NODE	10387	length	1688	cov	4.851806	2	Candidatus Bathyarchaeota (phylum)
NODE	10387	length	1688	cov	4.851806	3	
NODE	10393	length	1688	cov	4.507042	1	Candidatus Bathyarchaeota (phylum)
NODE	10393	length	1688	cov	4.507042	2	
NODE	10394	length	1688	cov	4.377832	1	Candidatus Bathyarchaeota (phylum)
NODE	10394	length	1688	cov	4.377832	2	Candidatus Bathyarchaeota (phylum)
NODE	10446	length	1685	cov	4.911656	1	
NODE	10446	length	1685	cov	4.911656	2	Candidatus Bathyarchaeota (phylum)
NODE	10446	length	1685	cov	4.911656	3	Candidatus Bathyarchaeota (phylum)
NODE	10448	length	1685	cov	4.58773	1	Candidatus Bathyarchaeota (phylum)
NODE	10448	length	1685	cov	4.58773	2	Candidatus Bathyarchaeota (phylum)
NODE	10448	length	1685	cov	4.58773	3	
NODE	10473	length	1684	cov	4.834868	1	Bacteroidetes/Chlorobi group (clade)
NODE	10473	length	1684	cov	4.834868	2	Candidatus Bathyarchaeota (phylum)
NODE	10473	length	1684	cov	4.834868	3	Candidatus Bathyarchaeota (phylum)
NODE	10489	length	1683	cov	5.095209	1	Candidatus Bathyarchaeota (phylum)
NODE	10504	length	1683	cov	2.821253	1	Candidatus Bathyarchaeota (phylum)
NODE	10504	length	1683	cov	2.821253	2	Candidatus Bathyarchaeota (phylum)
NODE	10655	length	1673	cov	6.341162	1	Candidatus Bathyarchaeota (phylum)
NODE	10655	length	1673	cov	6.341162	2	
NODE	10671	length	1673	cov	2.600742	1	Candidatus Bathyarchaeota (phylum)
NODE	10671	length	1673	cov	2.600742	2	Candidatus Bathyarchaeota (phylum)
NODE	10671	length	1673	cov	2.600742	3	Candidatus Bathyarchaeota (phylum)
NODE	10678	length	1672	cov	3.926407	1	Candidatus Bathyarchaeota (phylum)
NODE	10678	length	1672	cov	3.926407	2	Candidatus Bathyarchaeota (phylum)
NODE	10718	length	1670	cov	3.86192	1	Candidatus Bathyarchaeota (phylum)
NODE	10718	length	1670	cov	3.86192	2	Candidatus Bathyarchaeota (phylum)
NODE	10718	length	1670	cov	3.86192	3	
NODE	10752	length	1668	cov	6.262864	1	Candidatus Bathyarchaeota (phylum)
NODE	10752	length	1668	cov	6.262864	2	Candidatus Bathyarchaeota (phylum)
NODE	10752	length	1668	cov	6.262864	3	Candidatus Bathyarchaeota (phylum)
NODE	10785	length	1666	cov	5.834264	1	Candidatus Bathyarchaeota (phylum)
NODE	10785	length	1666	cov	5.834264	2	Candidatus Bathyarchaeota (phylum)
NODE	10813	length	1665	cov	3.39441	1	Candidatus Bathyarchaeota (phylum)
NODE	10813	length	1665	cov	3.39441	2	Candidatus Bathyarchaeota (phylum)
NODE	10907	length	1659	cov	2.344763	1	Firmicutes (phylum)
NODE	10907	length	1659	cov	2.344763	2	Thermococci (class)
NODE	10907	length	1659	cov	2.344763	3	
NODE	10923	length	1658	cov	3.572052	1	Candidatus Bathyarchaeota (phylum)
NODE	10923	length	1658	cov	3.572052	2	Candidatus Bathyarchaeota (phylum)
NODE	10930	length	1657	cov	5.428839	1	Thaumarchaeota (phylum)
NODE	10930	length	1657	cov	5.428839	2	Candidatus Bathyarchaeota (phylum)
NODE	10930	length	1657	cov	5.428839	3	Candidatus Bathyarchaeota (phylum)
NODE	10991	length	1654	cov	4.157598	1	Candidatus Bathyarchaeota (phylum)
NODE	11013	length	1653	cov	4.168961	1	Candidatus Bathyarchaeota (phylum)
NODE	11013	length	1653	cov	4.168961	2	Candidatus Bathyarchaeota (phylum)
NODE	11022	length	1653	cov	3.266583	1	Candidatus Bathyarchaeota (phylum)
NODE	11022	length	1653	cov	3.266583	2	Candidatus Bathyarchaeota (phylum)
NODE	11069	length	1650	cov	3.67837	1	Candidatus Bathyarchaeota (phylum)
NODE	11069	length	1650	cov	3.67837	2	Candidatus Bathyarchaeota (phylum)
NODE	11075	length	1650	cov	3.342947	1	Candidatus Bathyarchaeota (phylum)
NODE	11075	length	1650	cov	3.342947	2	
NODE	11106	length	1648	cov	4.883239	1	
NODE	11106	length	1648	cov	4.883239	2	Candidatus Bathyarchaeota (phylum)

NODE	11106	length	1648	cov	4.883239	3	
NODE	11120	length	1648	cov	2.06968	1	
NODE	11120	length	1648	cov	2.06968	2	Candidatus Bathyarchaeota (phylum)
NODE	11134	length	1647	cov	3.595477	1	
NODE	11134	length	1647	cov	3.595477	2	
NODE	11167	length	1645	cov	4.173585	1	Candidatus Bathyarchaeota (phylum)
NODE	11167	length	1645	cov	4.173585	2	Candidatus Bathyarchaeota (phylum)
NODE	11167	length	1645	cov	4.173585	3	Candidatus Bathyarchaeota (phylum)
NODE	11167	length	1645	cov	4.173585	4	Candidatus Bathyarchaeota (phylum)
NODE	11167	length	1645	cov	4.173585	5	Candidatus Bathyarchaeota (phylum)
NODE	11202	length	1644	cov	2.548143	1	
NODE	11202	length	1644	cov	2.548143	2	Candidatus Bathyarchaeota (phylum)
NODE	11220	length	1643	cov	3.103275	1	Candidatus Bathyarchaeota (phylum)
NODE	11220	length	1643	cov	3.103275	2	Candidatus Bathyarchaeota (phylum)
NODE	11272	length	1640	cov	7.080757	1	
NODE	11272	length	1640	cov	7.080757	2	Candidatus Korarchaeota (phylum)
NODE	11285	length	1639	cov	4.853535	1	Candidatus Bathyarchaeota (phylum)
NODE	11285	length	1639	cov	4.853535	2	
NODE	11349	length	1636	cov	3.860215	1	
NODE	11349	length	1636	cov	3.860215	2	Candidatus Bathyarchaeota (phylum)
NODE	11349	length	1636	cov	3.860215	3	Candidatus Bathyarchaeota (phylum)
NODE	11371	length	1635	cov	3.508861	1	
NODE	11371	length	1635	cov	3.508861	2	
NODE	11371	length	1635	cov	3.508861	3	
NODE	11385	length	1634	cov	4.543382	1	
NODE	11385	length	1634	cov	4.543382	2	Candidatus Bathyarchaeota (phylum)
NODE	11385	length	1634	cov	4.543382	3	Candidatus Bathyarchaeota (phylum)
NODE	11481	length	1630	cov	1.994286	1	Candidatus Bathyarchaeota (phylum)
NODE	11481	length	1630	cov	1.994286	2	Candidatus Bathyarchaeota (phylum)
NODE	11481	length	1630	cov	1.994286	3	Candidatus Bathyarchaeota (phylum)
NODE	11503	length	1629	cov	2.393901	1	Candidatus Bathyarchaeota (phylum)
NODE	11503	length	1629	cov	2.393901	2	
NODE	11503	length	1629	cov	2.393901	3	
NODE	11503	length	1629	cov	2.393901	4	
NODE	11517	length	1628	cov	3.441831	1	Candidatus Bathyarchaeota (phylum)
NODE	11517	length	1628	cov	3.441831	2	
NODE	11584	length	1624	cov	2.808158	1	Candidatus Bathyarchaeota (phylum)
NODE	11584	length	1624	cov	2.808158	2	Candidatus Bathyarchaeota (phylum)
NODE	11584	length	1624	cov	2.808158	3	Chloroflexi (phylum)
NODE	11655	length	1621	cov	2.837803	1	Candidatus Bathyarchaeota (phylum)
NODE	11655	length	1621	cov	2.837803	2	Candidatus Bathyarchaeota (phylum)
NODE	11657	length	1621	cov	1.816092	1	Candidatus Bathyarchaeota (phylum)
NODE	11657	length	1621	cov	1.816092	2	Candidatus Bathyarchaeota (phylum)
NODE	11660	length	1620	cov	5.410224	1	Candidatus Bathyarchaeota (phylum)
NODE	11660	length	1620	cov	5.410224	2	Candidatus Bathyarchaeota (phylum)
NODE	11680	length	1619	cov	6.457801	1	
NODE	11719	length	1617	cov	3.565941	1	Candidatus Bathyarchaeota (phylum)
NODE	11719	length	1617	cov	3.565941	2	Candidatus Bathyarchaeota (phylum)
NODE	11720	length	1617	cov	3.524968	1	Candidatus Bathyarchaeota (phylum)
NODE	11720	length	1617	cov	3.524968	2	Candidatus Bathyarchaeota (phylum)
NODE	11720	length	1617	cov	3.524968	3	Candidatus Bathyarchaeota (phylum)
NODE	11737	length	1616	cov	6.876361	1	
NODE	11737	length	1616	cov	6.876361	2	Candidatus Bathyarchaeota (phylum)
NODE	11942	length	1607	cov	2.533505	1	
NODE	11942	length	1607	cov	2.533505	2	Crenarchaeota (phylum)
NODE	11947	length	1606	cov	11.132173	1	Candidatus Bathyarchaeota (phylum)

NODE	11947	length	1606	cov	11.132173	2	Candidatus Bathyarchaeota (phylum)
NODE	11947	length	1606	cov	11.132173	3	Candidatus Bathyarchaeota (phylum)
NODE	11948	length	1606	cov	7.018053	1	
NODE	11955	length	1606	cov	4.117988	1	Candidatus Bathyarchaeota (phylum)
NODE	12083	length	1600	cov	4.252427	1	
NODE	12083	length	1600	cov	4.252427	2	
NODE	12100	length	1599	cov	5.803756	1	Candidatus Bathyarchaeota (phylum)
NODE	12100	length	1599	cov	5.803756	2	
NODE	12135	length	1598	cov	3.944913	1	Candidatus Bathyarchaeota (phylum)
NODE	12135	length	1598	cov	3.944913	2	Candidatus Bathyarchaeota (phylum)
NODE	12135	length	1598	cov	3.944913	3	Candidatus Bathyarchaeota (phylum)
NODE	12222	length	1593	cov	5.716515	1	Candidatus Bathyarchaeota (phylum)
NODE	12222	length	1593	cov	5.716515	2	Candidatus Bathyarchaeota (phylum)
NODE	12222	length	1593	cov	5.716515	3	Candidatus Bathyarchaeota (phylum)
NODE	12265	length	1591	cov	3.837891	1	Candidatus Bathyarchaeota (phylum)
NODE	12265	length	1591	cov	3.837891	2	Candidatus Bathyarchaeota (phylum)
NODE	12265	length	1591	cov	3.837891	3	Candidatus Bathyarchaeota (phylum)
NODE	12360	length	1586	cov	5.772698	1	Candidatus Bathyarchaeota (phylum)
NODE	12360	length	1586	cov	5.772698	2	
NODE	12360	length	1586	cov	5.772698	3	
NODE	12360	length	1586	cov	5.772698	4	Candidatus Bathyarchaeota (phylum)
NODE	12597	length	1575	cov	3.841447	1	Candidatus Bathyarchaeota (phylum)
NODE	12597	length	1575	cov	3.841447	2	
NODE	12607	length	1575	cov	2.865132	1	Candidatus Bathyarchaeota (phylum)
NODE	12607	length	1575	cov	2.865132	2	
NODE	12622	length	1574	cov	3.919026	1	Candidatus Bathyarchaeota (phylum)
NODE	12794	length	1566	cov	3.170748	1	Candidatus Bathyarchaeota (phylum)
NODE	12804	length	1565	cov	6.256291	1	Candidatus Bathyarchaeota (phylum)
NODE	12804	length	1565	cov	6.256291	2	Candidatus Bathyarchaeota (phylum)
NODE	12826	length	1565	cov	3.030464	1	
NODE	12826	length	1565	cov	3.030464	2	
NODE	12826	length	1565	cov	3.030464	3	
NODE	12844	length	1564	cov	2.91385	1	
NODE	12844	length	1564	cov	2.91385	2	
NODE	12844	length	1564	cov	2.91385	3	
NODE	12948	length	1559	cov	3.445479	1	Candidatus Bathyarchaeota (phylum)
NODE	12948	length	1559	cov	3.445479	2	Candidatus Bathyarchaeota (phylum)
NODE	12948	length	1559	cov	3.445479	3	Candidatus Bathyarchaeota (phylum)
NODE	12948	length	1559	cov	3.445479	4	Candidatus Bathyarchaeota (phylum)
NODE	13032	length	1555	cov	3.461333	1	Candidatus Bathyarchaeota (phylum)
NODE	13032	length	1555	cov	3.461333	2	Candidatus Bathyarchaeota (phylum)
NODE	13054	length	1554	cov	3.837225	1	
NODE	13054	length	1554	cov	3.837225	2	
NODE	13054	length	1554	cov	3.837225	3	
NODE	13131	length	1550	cov	4.50301	1	Candidatus Bathyarchaeota (phylum)
NODE	13131	length	1550	cov	4.50301	2	Candidatus Bathyarchaeota (phylum)
NODE	13163	length	1549	cov	2.923695	1	Candidatus Bathyarchaeota (phylum)
NODE	13163	length	1549	cov	2.923695	2	Candidatus Bathyarchaeota (phylum)
NODE	13163	length	1549	cov	2.923695	3	Candidatus Bathyarchaeota (phylum)
NODE	13258	length	1545	cov	2.818792	1	
NODE	13258	length	1545	cov	2.818792	2	Candidatus Bathyarchaeota (phylum)
NODE	13258	length	1545	cov	2.818792	3	
NODE	13258	length	1545	cov	2.818792	4	Candidatus Bathyarchaeota (phylum)
NODE	13391	length	1538	cov	4.523938	1	Candidatus Bathyarchaeota (phylum)
NODE	13391	length	1538	cov	4.523938	2	Candidatus Bathyarchaeota (phylum)
NODE	13391	length	1538	cov	4.523938	3	Candidatus Bathyarchaeota (phylum)

NODE	13474	length	1535	cov	3.999324	1	Candidatus Bathyarchaeota (phylum)
NODE	13474	length	1535	cov	3.999324	2	
NODE	13583	length	1531	cov	2.174119	1	Bacteria candidate phyla (clade)
NODE	13583	length	1531	cov	2.174119	2	
NODE	13588	length	1530	cov	5.149153	1	Candidatus Bathyarchaeota (phylum)
NODE	13588	length	1530	cov	5.149153	2	Candidatus Bathyarchaeota (phylum)
NODE	13713	length	1526	cov	2.292318	1	Candidatus Bathyarchaeota (phylum)
NODE	13713	length	1526	cov	2.292318	2	
NODE	13713	length	1526	cov	2.292318	3	
NODE	13776	length	1523	cov	3.282016	1	Candidatus Bathyarchaeota (phylum)
NODE	13780	length	1523	cov	3.017711	1	Candidatus Bathyarchaeota (phylum)
NODE	13780	length	1523	cov	3.017711	2	Candidatus Bathyarchaeota (phylum)
NODE	13780	length	1523	cov	3.017711	3	Candidatus Bathyarchaeota (phylum)
NODE	13781	length	1523	cov	3.014305	1	Candidatus Bathyarchaeota (phylum)
NODE	13781	length	1523	cov	3.014305	2	Candidatus Bathyarchaeota (phylum)
NODE	13781	length	1523	cov	3.014305	3	Candidatus Bathyarchaeota (phylum)
NODE	13792	length	1522	cov	5.860941	1	Candidatus Bathyarchaeota (phylum)
NODE	13792	length	1522	cov	5.860941	2	Candidatus Bathyarchaeota (phylum)
NODE	13792	length	1522	cov	5.860941	3	
NODE	13852	length	1520	cov	5.050512	1	Candidatus Bathyarchaeota (phylum)
NODE	13852	length	1520	cov	5.050512	2	Candidatus Bathyarchaeota (phylum)
NODE	13940	length	1517	cov	3.859097	1	
NODE	13940	length	1517	cov	3.859097	2	
NODE	13987	length	1515	cov	5.620548	1	Candidatus Bathyarchaeota (phylum)
NODE	13987	length	1515	cov	5.620548	2	Candidatus Bathyarchaeota (phylum)
NODE	14043	length	1513	cov	3.902606	1	Candidatus Bathyarchaeota (phylum)
NODE	14043	length	1513	cov	3.902606	2	
NODE	14074	length	1512	cov	3.276596	1	Bacteroidetes/Chlorobi group (clade)
NODE	14074	length	1512	cov	3.276596	2	Candidatus Bathyarchaeota (phylum)
NODE	14111	length	1510	cov	5.63299	1	Candidatus Bathyarchaeota (phylum)
NODE	14111	length	1510	cov	5.63299	2	
NODE	14160	length	1509	cov	3.515131	1	Candidatus Bathyarchaeota (phylum)
NODE	14234	length	1506	cov	3.47071	1	Candidatus Bathyarchaeota (phylum)
NODE	14243	length	1506	cov	2.313577	1	
NODE	14243	length	1506	cov	2.313577	2	Candidatus Bathyarchaeota (phylum)
NODE	14243	length	1506	cov	2.313577	3	
NODE	14293	length	1504	cov	3.823326	1	
NODE	14293	length	1504	cov	3.823326	2	Thermodesulfobacteria (class)
NODE	14293	length	1504	cov	3.823326	3	
NODE	14351	length	1502	cov	3.573601	1	Candidatus Bathyarchaeota (phylum)
NODE	14351	length	1502	cov	3.573601	2	Candidatus Bathyarchaeota (phylum)
NODE	14373	length	1501	cov	3.492393	1	Candidatus Bathyarchaeota (phylum)
NODE	14375	length	1501	cov	3.378976	1	Candidatus Bathyarchaeota (phylum)
NODE	14375	length	1501	cov	3.378976	2	
NODE	14375	length	1501	cov	3.378976	3	Candidatus Bathyarchaeota (phylum)

Classification of Refined bin 13

Contigs							**Lineage name**
NODE	204	length	7278	cov	7.711062	1	
NODE	204	length	7278	cov	7.711062	2	Candidatus Bathyarchaeota (phylum)
NODE	204	length	7278	cov	7.711062	3	Candidatus Bathyarchaeota (phylum)
NODE	204	length	7278	cov	7.711062	4	Candidatus Bathyarchaeota (phylum)
NODE	204	length	7278	cov	7.711062	5	archaeon (species)
NODE	204	length	7278	cov	7.711062	6	archaeon (species)

NODE	204	length	7278	cov	7.711062	7	
NODE	204	length	7278	cov	7.711062	8	
NODE	271	length	6217	cov	5.722817	1	
NODE	271	length	6217	cov	5.722817	2	Bacteria candidate phyla (clade)
NODE	271	length	6217	cov	5.722817	3	
NODE	271	length	6217	cov	5.722817	4	archaeon (species)
NODE	271	length	6217	cov	5.722817	5	archaeon (species)
NODE	271	length	6217	cov	5.722817	6	archaeon RBG_16_50_20 (species)
NODE	305	length	5906	cov	6.559221	1	archaeon RBG_16_50_20 (species)
NODE	305	length	5906	cov	6.559221	2	
NODE	305	length	5906	cov	6.559221	3	archaeon RBG_16_50_20 (species)
NODE	305	length	5906	cov	6.559221	4	archaeon RBG_16_50_20 (species)
NODE	305	length	5906	cov	6.559221	5	Candidatus Bathyarchaeota (phylum)
NODE	307	length	5885	cov	5.440137	1	archaeon RBG_16_50_20 (species)
NODE	307	length	5885	cov	5.440137	2	archaeon RBG_16_50_20 (species)
NODE	307	length	5885	cov	5.440137	3	
NODE	307	length	5885	cov	5.440137	4	
NODE	307	length	5885	cov	5.440137	5	Thaumarchaeota (phylum)
NODE	307	length	5885	cov	5.440137	6	Candidatus Bathyarchaeota (phylum)
NODE	307	length	5885	cov	5.440137	7	
NODE	387	length	5315	cov	4.78403	1	bacterium (species)
NODE	387	length	5315	cov	4.78403	2	Candidatus Bathyarchaeota (phylum)
NODE	387	length	5315	cov	4.78403	3	Candidatus Bathyarchaeota (phylum)
NODE	387	length	5315	cov	4.78403	4	
NODE	387	length	5315	cov	4.78403	5	Candidatus Bathyarchaeota (phylum)
NODE	387	length	5315	cov	4.78403	6	archaeon RBG_16_50_20 (species)
NODE	387	length	5315	cov	4.78403	7	
NODE	387	length	5315	cov	4.78403	8	archaeon RBG_16_50_20 (species)
NODE	392	length	5286	cov	7.817817	1	
NODE	392	length	5286	cov	7.817817	2	archaeon (species)
NODE	392	length	5286	cov	7.817817	3	Thaumarchaeota (phylum)
NODE	392	length	5286	cov	7.817817	4	Candidatus Bathyarchaeota (phylum)
NODE	392	length	5286	cov	7.817817	5	Candidatus Bathyarchaeota (phylum)
NODE	392	length	5286	cov	7.817817	6	
NODE	417	length	5137	cov	5.324085	1	
NODE	417	length	5137	cov	5.324085	2	Thaumarchaeota (phylum)
NODE	417	length	5137	cov	5.324085	3	Thaumarchaeota (phylum)
NODE	417	length	5137	cov	5.324085	4	
NODE	417	length	5137	cov	5.324085	5	
NODE	417	length	5137	cov	5.324085	6	Candidatus Bathyarchaeota (phylum)
NODE	417	length	5137	cov	5.324085	7	Candidatus Bathyarchaeota (phylum)
NODE	417	length	5137	cov	5.324085	8	
NODE	455	length	4961	cov	5.482878	1	
NODE	455	length	4961	cov	5.482878	2	archaeon (species)
NODE	455	length	4961	cov	5.482878	3	archaeon (species)
NODE	455	length	4961	cov	5.482878	4	archaeon (species)
NODE	455	length	4961	cov	5.482878	5	archaeon (species)
NODE	455	length	4961	cov	5.482878	6	archaeon (species)
NODE	455	length	4961	cov	5.482878	7	
NODE	524	length	4704	cov	6.293612	1	Candidatus Bathyarchaeota (phylum)
NODE	524	length	4704	cov	6.293612	2	
NODE	524	length	4704	cov	6.293612	3	bacterium (species)
NODE	524	length	4704	cov	6.293612	4	archaeon (species)
NODE	524	length	4704	cov	6.293612	5	Candidatus Bathyarchaeota (phylum)
NODE	539	length	4664	cov	4.381428	1	
NODE	539	length	4664	cov	4.381428	2	

NODE	539	length	4664	cov	4.381428	3	bacterium (species)
NODE	570	length	4538	cov	6.534464	1	
NODE	570	length	4538	cov	6.534464	2	archaeon RBG_16_50_20 (species)
NODE	570	length	4538	cov	6.534464	3	Candidatus Bathyarchaeota (phylum)
NODE	570	length	4538	cov	6.534464	4	
NODE	570	length	4538	cov	6.534464	5	
NODE	570	length	4538	cov	6.534464	6	
NODE	570	length	4538	cov	6.534464	7	bacterium (species)
NODE	570	length	4538	cov	6.534464	8	Candidatus Korarchaeota (phylum)
NODE	587	length	4478	cov	5.457834	1	Candidatus Bathyarchaeota (phylum)
NODE	587	length	4478	cov	5.457834	2	
NODE	587	length	4478	cov	5.457834	3	archaeon (species)
NODE	587	length	4478	cov	5.457834	4	archaeon (species)
NODE	587	length	4478	cov	5.457834	5	archaeon (species)
NODE	587	length	4478	cov	5.457834	6	archaeon (species)
NODE	587	length	4478	cov	5.457834	7	archaeon RBG_16_50_20 (species)
NODE	626	length	4371	cov	4.286376	1	Candidatus Bathyarchaeota (phylum)
NODE	626	length	4371	cov	4.286376	2	Candidatus Bathyarchaeota (phylum)
NODE	626	length	4371	cov	4.286376	3	
NODE	626	length	4371	cov	4.286376	4	
NODE	626	length	4371	cov	4.286376	5	
NODE	626	length	4371	cov	4.286376	6	archaeon (species)
NODE	627	length	4371	cov	3.506487	1	archaeon RBG_16_50_20 (species)
NODE	627	length	4371	cov	3.506487	2	
NODE	627	length	4371	cov	3.506487	3	Candidatus Bathyarchaeota (phylum)
NODE	627	length	4371	cov	3.506487	4	
NODE	663	length	4303	cov	5.15113	1	
NODE	663	length	4303	cov	5.15113	2	
NODE	663	length	4303	cov	5.15113	3	
NODE	663	length	4303	cov	5.15113	4	bacterium (species)
NODE	666	length	4299	cov	5.854383	1	Candidatus Bathyarchaeota (phylum)
NODE	666	length	4299	cov	5.854383	2	archaeon RBG_16_50_20 (species)
NODE	666	length	4299	cov	5.854383	3	archaeon RBG_16_50_20 (species)
NODE	666	length	4299	cov	5.854383	4	unclassified Euryarchaeota (no rank)
NODE	666	length	4299	cov	5.854383	5	Diaforarchaea group (clade)
NODE	668	length	4294	cov	4.23331	1	
NODE	668	length	4294	cov	4.23331	2	
NODE	668	length	4294	cov	4.23331	3	
NODE	668	length	4294	cov	4.23331	4	
NODE	668	length	4294	cov	4.23331	5	archaeon RBG_16_50_20 (species)
NODE	680	length	4265	cov	4.834442	1	
NODE	680	length	4265	cov	4.834442	2	
NODE	680	length	4265	cov	4.834442	3	
NODE	680	length	4265	cov	4.834442	4	archaeon (species)
NODE	691	length	4237	cov	4.884744	1	
NODE	691	length	4237	cov	4.884744	2	
NODE	691	length	4237	cov	4.884744	3	Candidatus Bathyarchaeota (phylum)
NODE	691	length	4237	cov	4.884744	4	
NODE	691	length	4237	cov	4.884744	5	Candidatus Bathyarchaeota (phylum)
NODE	691	length	4237	cov	4.884744	6	
NODE	691	length	4237	cov	4.884744	7	Candidatus Bathyarchaeota (phylum)
NODE	691	length	4237	cov	4.884744	8	
NODE	817	length	4000	cov	6.206591	1	archaeon RBG_16_50_20 (species)
NODE	817	length	4000	cov	6.206591	2	Candidatus Bathyarchaeota (phylum)
NODE	817	length	4000	cov	6.206591	3	archaeon RBG_16_50_20 (species)
NODE	817	length	4000	cov	6.206591	4	

NODE	817	length	4000	cov	6.206591	5	
NODE	848	length	3935	cov	4.199227	1	
NODE	848	length	3935	cov	4.199227	2	
NODE	848	length	3935	cov	4.199227	3	bacterium (species)
NODE	848	length	3935	cov	4.199227	4	bacterium (species)
NODE	848	length	3935	cov	4.199227	5	archaeon RBG_16_50_20 (species)
NODE	848	length	3935	cov	4.199227	6	Candidatus Thorarchaeota (phylum)
NODE	865	length	3905	cov	6.199481	1	archaeon RBG_16_50_20 (species)
NODE	865	length	3905	cov	6.199481	2	
NODE	865	length	3905	cov	6.199481	3	archaeon RBG_16_50_20 (species)
NODE	865	length	3905	cov	6.199481	4	archaeon RBG_16_50_20 (species)
NODE	865	length	3905	cov	6.199481	5	unclassified Euryarchaeota (no rank)
NODE	885	length	3879	cov	5.687238	1	unclassified Euryarchaeota (no rank)
NODE	885	length	3879	cov	5.687238	2	Diaforarchaea group (clade)
NODE	885	length	3879	cov	5.687238	3	
NODE	893	length	3868	cov	5.661159	1	
NODE	893	length	3868	cov	5.661159	2	
NODE	893	length	3868	cov	5.661159	3	
NODE	893	length	3868	cov	5.661159	4	Candidatus Bathyarchaeota (phylum)
NODE	902	length	3858	cov	3.882198	1	
NODE	902	length	3858	cov	3.882198	2	
NODE	902	length	3858	cov	3.882198	3	
NODE	902	length	3858	cov	3.882198	4	
NODE	902	length	3858	cov	3.882198	5	
NODE	902	length	3858	cov	3.882198	6	archaeon (species)
NODE	927	length	3826	cov	6.006895	1	archaeon (species)
NODE	927	length	3826	cov	6.006895	2	archaeon (species)
NODE	927	length	3826	cov	6.006895	3	archaeon (species)
NODE	927	length	3826	cov	6.006895	4	archaeon (species)
NODE	927	length	3826	cov	6.006895	5	archaeon (species)
NODE	927	length	3826	cov	6.006895	6	archaeon (species)
NODE	927	length	3826	cov	6.006895	7	unclassified Euryarchaeota (no rank)
NODE	940	length	3818	cov	4.424395	1	
NODE	940	length	3818	cov	4.424395	2	unclassified Euryarchaeota (no rank)
NODE	940	length	3818	cov	4.424395	3	bacterium (species)
NODE	1031	length	3679	cov	6.521799	1	
NODE	1031	length	3679	cov	6.521799	2	
NODE	1031	length	3679	cov	6.521799	3	Candidatus Bathyarchaeota (phylum)
NODE	1031	length	3679	cov	6.521799	4	
NODE	1031	length	3679	cov	6.521799	5	
NODE	1047	length	3663	cov	5.669069	1	
NODE	1047	length	3663	cov	5.669069	2	
NODE	1047	length	3663	cov	5.669069	3	archaeon (species)
NODE	1054	length	3658	cov	3.907299	1	archaeon (species)
NODE	1054	length	3658	cov	3.907299	2	
NODE	1054	length	3658	cov	3.907299	3	archaeon (species)
NODE	1074	length	3621	cov	5.525799	1	archaeon (species)
NODE	1074	length	3621	cov	5.525799	2	
NODE	1074	length	3621	cov	5.525799	3	archaeon RBG_16_50_20 (species)
NODE	1074	length	3621	cov	5.525799	4	
NODE	1074	length	3621	cov	5.525799	5	archaeon RBG_16_50_20 (species)
NODE	1128	length	3564	cov	5.058136	1	
NODE	1128	length	3564	cov	5.058136	2	Candidatus Parvarchaeota (phylum)
NODE	1128	length	3564	cov	5.058136	3	Candidatus Verstraetearchaeota (phylum)
NODE	1128	length	3564	cov	5.058136	4	archaeon RBG_16_50_20 (species)
NODE	1128	length	3564	cov	5.058136	5	Candidatus Geothermarchaeota (phylum)

NODE	1148	length	3546	cov	4.596104	1	
NODE	1148	length	3546	cov	4.596104	2	
NODE	1148	length	3546	cov	4.596104	3	
NODE	1148	length	3546	cov	4.596104	4	bacterium (species)
NODE	1148	length	3546	cov	4.596104	5	archaeon (species)
NODE	1151	length	3542	cov	7.159163	1	archaeon (species)
NODE	1151	length	3542	cov	7.159163	2	archaeon (species)
NODE	1151	length	3542	cov	7.159163	3	archaeon (species)
NODE	1151	length	3542	cov	7.159163	4	archaeon (species)
NODE	1156	length	3537	cov	5.485928	1	archaeon (species)
NODE	1156	length	3537	cov	5.485928	2	archaeon (species)
NODE	1156	length	3537	cov	5.485928	3	archaeon (species)
NODE	1156	length	3537	cov	5.485928	4	archaeon (species)
NODE	1156	length	3537	cov	5.485928	5	archaeon RBG_16_50_20 (species)
NODE	1267	length	3439	cov	7.059397	1	archaeon RBG_16_50_20 (species)
NODE	1267	length	3439	cov	7.059397	2	
NODE	1267	length	3439	cov	7.059397	3	unclassified Euryarchaeota (no rank)
NODE	1294	length	3420	cov	4.9263	1	unclassified Euryarchaeota (no rank)
NODE	1294	length	3420	cov	4.9263	2	
NODE	1294	length	3420	cov	4.9263	3	unclassified Euryarchaeota (no rank)
NODE	1294	length	3420	cov	4.9263	4	
NODE	1378	length	3352	cov	5.373066	1	
NODE	1378	length	3352	cov	5.373066	2	
NODE	1378	length	3352	cov	5.373066	3	bacterium (species)
NODE	1407	length	3329	cov	3.818571	1	archaeon (species)
NODE	1407	length	3329	cov	3.818571	2	Thaumarchaeota (phylum)
NODE	1424	length	3314	cov	3.559681	1	Candidatus Bathyarchaeota (phylum)
NODE	1424	length	3314	cov	3.559681	2	bacterium (species)
NODE	1424	length	3314	cov	3.559681	3	Candidatus Bathyarchaeota (phylum)
NODE	1424	length	3314	cov	3.559681	4	Candidatus Bathyarchaeota (phylum)
NODE	1424	length	3314	cov	3.559681	5	Candidatus Bathyarchaeota (phylum)
NODE	1474	length	3270	cov	4.419907	1	bacterium (species)
NODE	1474	length	3270	cov	4.419907	2	
NODE	1474	length	3270	cov	4.419907	3	
NODE	1474	length	3270	cov	4.419907	4	
NODE	1474	length	3270	cov	4.419907	5	
NODE	1489	length	3262	cov	4.427191	1	
NODE	1489	length	3262	cov	4.427191	2	
NODE	1489	length	3262	cov	4.427191	3	
NODE	1489	length	3262	cov	4.427191	4	
NODE	1517	length	3244	cov	5.720602	1	archaeon (species)
NODE	1517	length	3244	cov	5.720602	2	
NODE	1541	length	3231	cov	5.528338	1	Candidatus Bathyarchaeota (phylum)
NODE	1541	length	3231	cov	5.528338	2	archaeon (species)
NODE	1541	length	3231	cov	5.528338	3	archaeon (species)
NODE	1541	length	3231	cov	5.528338	4	Chloroflexi (phylum)
NODE	1555	length	3222	cov	3.976003	1	
NODE	1555	length	3222	cov	3.976003	2	
NODE	1555	length	3222	cov	3.976003	3	Candidatus Korarchaeota (phylum)
NODE	1555	length	3222	cov	3.976003	4	Thaumarchaeota (phylum)
NODE	1555	length	3222	cov	3.976003	5	Thaumarchaeota (phylum)
NODE	1555	length	3222	cov	3.976003	6	archaeon RBG_16_50_20 (species)
NODE	1555	length	3222	cov	3.976003	7	unclassified Euryarchaeota (no rank)
NODE	1577	length	3202	cov	5.668573	1	
NODE	1577	length	3202	cov	5.668573	2	
NODE	1577	length	3202	cov	5.668573	3	

NODE	1592	length	3192	cov	5.607268	1	
NODE	1592	length	3192	cov	5.607268	2	
NODE	1592	length	3192	cov	5.607268	3	archaeon RBG_16_50_20 (species)
NODE	1592	length	3192	cov	5.607268	4	
NODE	1595	length	3191	cov	4.810268	1	Candidatus Bathyarchaeota (phylum)
NODE	1595	length	3191	cov	4.810268	2	
NODE	1595	length	3191	cov	4.810268	3	
NODE	1595	length	3191	cov	4.810268	4	Candidatus Bathyarchaeota (phylum)
NODE	1631	length	3165	cov	6.063666	1	
NODE	1631	length	3165	cov	6.063666	2	
NODE	1631	length	3165	cov	6.063666	3	
NODE	1666	length	3130	cov	6.72065	1	archaeon (species)
NODE	1666	length	3130	cov	6.72065	2	archaeon (species)
NODE	1666	length	3130	cov	6.72065	3	unclassified Euryarchaeota (no rank)
NODE	1675	length	3125	cov	5.002932	1	unclassified Euryarchaeota (no rank)
NODE	1675	length	3125	cov	5.002932	2	
NODE	1675	length	3125	cov	5.002932	3	
NODE	1715	length	3104	cov	4.104624	1	archaeon RBG_16_50_20 (species)
NODE	1715	length	3104	cov	4.104624	2	archaeon RBG_16_50_20 (species)
NODE	1715	length	3104	cov	4.104624	3	archaeon RBG_16_50_20 (species)
NODE	1715	length	3104	cov	4.104624	4	Candidatus Marsarchaeota (phylum)
NODE	1742	length	3085	cov	4.475908	1	
NODE	1742	length	3085	cov	4.475908	2	Candidatus Bathyarchaeota (phylum)
NODE	1742	length	3085	cov	4.475908	3	archaeon RBG_16_50_20 (species)
NODE	1742	length	3085	cov	4.475908	4	
NODE	1826	length	3042	cov	4.072648	1	Thaumarchaeota (phylum)
NODE	1826	length	3042	cov	4.072648	2	
NODE	1826	length	3042	cov	4.072648	3	
NODE	1857	length	3025	cov	5.293939	1	
NODE	1857	length	3025	cov	5.293939	2	unclassified Euryarchaeota (no rank)
NODE	1857	length	3025	cov	5.293939	3	unclassified Euryarchaeota (no rank)
NODE	1857	length	3025	cov	5.293939	4	
NODE	1907	length	3002	cov	7.894469	1	
NODE	1907	length	3002	cov	7.894469	2	Thaumarchaeota (phylum)
NODE	1907	length	3002	cov	7.894469	3	Thaumarchaeota (phylum)
NODE	1907	length	3002	cov	7.894469	4	
NODE	1918	length	2996	cov	6.961238	1	
NODE	1918	length	2996	cov	6.961238	2	
NODE	1918	length	2996	cov	6.961238	3	
NODE	1918	length	2996	cov	6.961238	4	Candidatus Bathyarchaeota (phylum)
NODE	1941	length	2986	cov	3.934835	1	Candidatus Bathyarchaeota (phylum)
NODE	1941	length	2986	cov	3.934835	2	
NODE	1941	length	2986	cov	3.934835	3	
NODE	1968	length	2969	cov	6.491078	1	
NODE	1968	length	2969	cov	6.491078	2	bacterium (species)
NODE	1968	length	2969	cov	6.491078	3	
NODE	1972	length	2963	cov	7.480399	1	Candidatus Bathyarchaeota (phylum)
NODE	1972	length	2963	cov	7.480399	2	unclassified Euryarchaeota (no rank)
NODE	1972	length	2963	cov	7.480399	3	
NODE	1972	length	2963	cov	7.480399	4	
NODE	1972	length	2963	cov	7.480399	5	Thaumarchaeota (phylum)
NODE	1986	length	2959	cov	3.832989	1	Thaumarchaeota (phylum)
NODE	1986	length	2959	cov	3.832989	2	Thaumarchaeota (phylum)
NODE	1986	length	2959	cov	3.832989	3	
NODE	2012	length	2947	cov	6.341978	1	
NODE	2012	length	2947	cov	6.341978	2	

NODE	2012	length	2947	cov	6.341978	3	Candidatus Bathyarchaeota (phylum)
NODE	2118	length	2914	cov	2.835957	1	archaeon RBG_16_50_20 (species)
NODE	2118	length	2914	cov	2.835957	2	
NODE	2118	length	2914	cov	2.835957	3	
NODE	2118	length	2914	cov	2.835957	4	
NODE	2118	length	2914	cov	2.835957	5	
NODE	2127	length	2908	cov	4.59972	1	archaeon RBG_16_50_20 (species)
NODE	2127	length	2908	cov	4.59972	2	Thaumarchaeota (phylum)
NODE	2127	length	2908	cov	4.59972	3	
NODE	2127	length	2908	cov	4.59972	4	
NODE	2194	length	2880	cov	7.165664	1	
NODE	2194	length	2880	cov	7.165664	2	
NODE	2194	length	2880	cov	7.165664	3	
NODE	2194	length	2880	cov	7.165664	4	Candidatus Bathyarchaeota (phylum)
NODE	2196	length	2880	cov	5.303009	1	Candidatus Bathyarchaeota (phylum)
NODE	2196	length	2880	cov	5.303009	2	
NODE	2196	length	2880	cov	5.303009	3	
NODE	2196	length	2880	cov	5.303009	4	
NODE	2196	length	2880	cov	5.303009	5	Candidatus Bathyarchaeota (phylum)
NODE	2350	length	2817	cov	6.385952	1	archaeon RBG_16_50_20 (species)
NODE	2350	length	2817	cov	6.385952	2	archaeon RBG_16_50_20 (species)
NODE	2350	length	2817	cov	6.385952	3	
NODE	2350	length	2817	cov	6.385952	4	Crenarchaeota (phylum)
NODE	2371	length	2807	cov	3.938227	1	Firmicutes (phylum)
NODE	2371	length	2807	cov	3.938227	2	
NODE	2371	length	2807	cov	3.938227	3	
NODE	2371	length	2807	cov	3.938227	4	
NODE	2492	length	2760	cov	4.131608	1	
NODE	2492	length	2760	cov	4.131608	2	Chloroflexi (phylum)
NODE	2492	length	2760	cov	4.131608	3	Chloroflexi (phylum)
NODE	2564	length	2733	cov	4.551531	1	Candidatus Bathyarchaeota (phylum)
NODE	2564	length	2733	cov	4.551531	2	
NODE	2564	length	2733	cov	4.551531	3	Candidatus Bathyarchaeota (phylum)
NODE	2564	length	2733	cov	4.551531	4	
NODE	2618	length	2715	cov	4.663534	1	Thaumarchaeota (phylum)
NODE	2618	length	2715	cov	4.663534	2	
NODE	2618	length	2715	cov	4.663534	3	Archaeoglobi (class)
NODE	2618	length	2715	cov	4.663534	4	Thaumarchaeota (phylum)
NODE	2618	length	2715	cov	4.663534	5	
NODE	2624	length	2714	cov	6.188793	1	Candidatus Bathyarchaeota (phylum)
NODE	2624	length	2714	cov	6.188793	2	Candidatus Bathyarchaeota (phylum)
NODE	2624	length	2714	cov	6.188793	3	
NODE	2624	length	2714	cov	6.188793	4	
NODE	2624	length	2714	cov	6.188793	5	
NODE	2624	length	2714	cov	6.188793	6	
NODE	2624	length	2714	cov	6.188793	7	
NODE	2624	length	2714	cov	6.188793	8	bacterium (species)
NODE	2625	length	2714	cov	5.252351	1	archaeon RBG_16_50_20 (species)
NODE	2625	length	2714	cov	5.252351	2	archaeon RBG_16_50_20 (species)
NODE	2625	length	2714	cov	5.252351	3	archaeon (species)
NODE	2625	length	2714	cov	5.252351	4	Candidatus Verstraetearchaeota (phylum)
NODE	2635	length	2711	cov	5.681476	1	Thaumarchaeota (phylum)
NODE	2635	length	2711	cov	5.681476	2	Thaumarchaeota (phylum)
NODE	2635	length	2711	cov	5.681476	3	
NODE	2661	length	2703	cov	2.963746	1	
NODE	2661	length	2703	cov	2.963746	2	Candidatus Bathyarchaeota (phylum)

NODE	2684	length	2695	cov	5.079167	1	
NODE	2684	length	2695	cov	5.079167	2	Candidatus Bathyarchaeota (phylum)
NODE	2684	length	2695	cov	5.079167	3	
NODE	2696	length	2693	cov	4.322593	1	Thaumarchaeota (phylum)
NODE	2696	length	2693	cov	4.322593	2	Thaumarchaeota (phylum)
NODE	2717	length	2686	cov	8.736222	1	archaeon (species)
NODE	2717	length	2686	cov	8.736222	2	archaeon (species)
NODE	2717	length	2686	cov	8.736222	3	Candidatus Bathyarchaeota (phylum)
NODE	2719	length	2686	cov	5.686431	1	
NODE	2719	length	2686	cov	5.686431	2	bacterium (species)
NODE	2719	length	2686	cov	5.686431	3	
NODE	2752	length	2677	cov	3.477117	1	
NODE	2752	length	2677	cov	3.477117	2	Bacteria candidate phyla (clade)
NODE	2752	length	2677	cov	3.477117	3	Diaforarchaea group (clade)
NODE	2752	length	2677	cov	3.477117	4	
NODE	2798	length	2662	cov	4.070196	1	
NODE	2798	length	2662	cov	4.070196	2	
NODE	2869	length	2643	cov	5.651468	1	
NODE	2869	length	2643	cov	5.651468	2	Candidatus Bathyarchaeota (phylum)
NODE	2869	length	2643	cov	5.651468	3	bacterium (species)
NODE	2920	length	2626	cov	5.625827	1	
NODE	2920	length	2626	cov	5.625827	2	archaeon (species)
NODE	2982	length	2611	cov	3.5223	1	archaeon (species)
NODE	2982	length	2611	cov	3.5223	2	archaeon (species)
NODE	2982	length	2611	cov	3.5223	3	archaeon (species)
NODE	2982	length	2611	cov	3.5223	4	archaeon (species)
NODE	2982	length	2611	cov	3.5223	5	
NODE	3009	length	2601	cov	7.152789	1	
NODE	3009	length	2601	cov	7.152789	2	Candidatus Bathyarchaeota (phylum)
NODE	3009	length	2601	cov	7.152789	3	archaeon (species)
NODE	3129	length	2566	cov	5.671446	1	archaeon (species)
NODE	3129	length	2566	cov	5.671446	2	
NODE	3129	length	2566	cov	5.671446	3	
NODE	3130	length	2566	cov	4.342095	1	Candidatus Bathyarchaeota (phylum)
NODE	3130	length	2566	cov	4.342095	2	
NODE	3130	length	2566	cov	4.342095	3	Candidatus Bathyarchaeota (phylum)
NODE	3130	length	2566	cov	4.342095	4	
NODE	3130	length	2566	cov	4.342095	5	archaeon RBG_16_50_20 (species)
NODE	3133	length	2565	cov	5.578088	1	archaeon RBG_16_50_20 (species)
NODE	3202	length	2548	cov	4.315283	1	archaeon RBG_16_50_20 (species)
NODE	3202	length	2548	cov	4.315283	2	
NODE	3202	length	2548	cov	4.315283	3	
NODE	3202	length	2548	cov	4.315283	4	archaeon (species)
NODE	3207	length	2547	cov	4.422953	1	archaeon (species)
NODE	3207	length	2547	cov	4.422953	2	archaeon (species)
NODE	3207	length	2547	cov	4.422953	3	
NODE	3224	length	2541	cov	5.135961	1	bacterium (species)
NODE	3224	length	2541	cov	5.135961	2	bacterium (species)
NODE	3224	length	2541	cov	5.135961	3	
NODE	3224	length	2541	cov	5.135961	4	archaeon RBG_16_50_20 (species)
NODE	3252	length	2533	cov	7.496368	1	
NODE	3252	length	2533	cov	7.496368	2	bacterium (species)
NODE	3252	length	2533	cov	7.496368	3	
NODE	3259	length	2531	cov	5.014136	1	
NODE	3259	length	2531	cov	5.014136	2	
NODE	3259	length	2531	cov	5.014136	3	archaeon RBG_16_50_20 (species)

NODE	3259	length	2531	cov	5.014136	4	Candidatus Bathyarchaeota (phylum)
NODE	3266	length	2530	cov	3.694545	1	archaeon RBG_16_50_20 (species)
NODE	3266	length	2530	cov	3.694545	2	
NODE	3266	length	2530	cov	3.694545	3	
NODE	3287	length	2526	cov	3.691218	1	
NODE	3287	length	2526	cov	3.691218	2	
NODE	3287	length	2526	cov	3.691218	3	archaeon RBG_16_50_20 (species)
NODE	3337	length	2513	cov	4.190399	1	
NODE	3337	length	2513	cov	4.190399	2	
NODE	3337	length	2513	cov	4.190399	3	
NODE	3337	length	2513	cov	4.190399	4	
NODE	3337	length	2513	cov	4.190399	5	
NODE	3346	length	2511	cov	5.506515	1	
NODE	3346	length	2511	cov	5.506515	2	
NODE	3346	length	2511	cov	5.506515	3	
NODE	3354	length	2510	cov	4.434623	1	Candidatus Bathyarchaeota (phylum)
NODE	3354	length	2510	cov	4.434623	2	
NODE	3354	length	2510	cov	4.434623	3	Chloroflexi (phylum)
NODE	3354	length	2510	cov	4.434623	4	
NODE	3381	length	2503	cov	4.472631	1	
NODE	3381	length	2503	cov	4.472631	2	
NODE	3381	length	2503	cov	4.472631	3	Chloroflexi (phylum)
NODE	3389	length	2502	cov	4.727013	1	Candidatus Bathyarchaeota (phylum)
NODE	3389	length	2502	cov	4.727013	2	archaeon RBG_16_50_20 (species)
NODE	3389	length	2502	cov	4.727013	3	
NODE	3476	length	2478	cov	6.457697	1	archaeon (species)
NODE	3476	length	2478	cov	6.457697	2	Candidatus Bathyarchaeota (phylum)
NODE	3476	length	2478	cov	6.457697	3	
NODE	3476	length	2478	cov	6.457697	4	
NODE	3493	length	2475	cov	4.132645	1	bacterium (species)
NODE	3493	length	2475	cov	4.132645	2	archaeon RBG_16_50_20 (species)
NODE	3509	length	2469	cov	6.840099	1	
NODE	3509	length	2469	cov	6.840099	2	Candidatus Bathyarchaeota (phylum)
NODE	3523	length	2466	cov	4.947325	1	
NODE	3523	length	2466	cov	4.947325	2	Candidatus Bathyarchaeota (phylum)
NODE	3523	length	2466	cov	4.947325	3	
NODE	3545	length	2459	cov	6.033694	1	archaeon RBG_16_50_20 (species)
NODE	3545	length	2459	cov	6.033694	2	Candidatus Bathyarchaeota (phylum)
NODE	3545	length	2459	cov	6.033694	3	
NODE	3545	length	2459	cov	6.033694	4	Candidatus Bathyarchaeota (phylum)
NODE	3554	length	2457	cov	5.590341	1	Candidatus Bathyarchaeota (phylum)
NODE	3554	length	2457	cov	5.590341	2	Candidatus Bathyarchaeota (phylum)
NODE	3554	length	2457	cov	5.590341	3	archaeon (species)
NODE	3570	length	2453	cov	5.802335	1	archaeon (species)
NODE	3570	length	2453	cov	5.802335	2	archaeon (species)
NODE	3570	length	2453	cov	5.802335	3	bacterium (species)
NODE	3760	length	2417	cov	4.163421	1	bacterium (species)
NODE	3760	length	2417	cov	4.163421	2	archaeon (species)
NODE	3760	length	2417	cov	4.163421	3	Bacteria candidate phyla (clade)
NODE	3761	length	2416	cov	4.870817	1	Bacteria candidate phyla (clade)
NODE	3761	length	2416	cov	4.870817	2	archaeon (species)
NODE	3761	length	2416	cov	4.870817	3	archaeon (species)
NODE	3798	length	2407	cov	5.171344	1	
NODE	3912	length	2379	cov	6.54432	1	archaeon RBG_16_50_20 (species)
NODE	3912	length	2379	cov	6.54432	2	
NODE	3912	length	2379	cov	6.54432	3	

NODE	3912	length	2379	cov	6.54432	4	Candidatus Bathyarchaeota (phylum)
NODE	3981	length	2366	cov	4.353094	1	Chloroflexi (phylum)
NODE	3981	length	2366	cov	4.353094	2	Candidatus Bathyarchaeota (phylum)
NODE	3981	length	2366	cov	4.353094	3	
NODE	3990	length	2364	cov	5.009095	1	
NODE	3990	length	2364	cov	5.009095	2	
NODE	4028	length	2357	cov	4.152911	1	archaeon RBG_16_50_20 (species)
NODE	4028	length	2357	cov	4.152911	2	
NODE	4028	length	2357	cov	4.152911	3	
NODE	4029	length	2357	cov	2.823632	1	
NODE	4029	length	2357	cov	2.823632	2	
NODE	4029	length	2357	cov	2.823632	3	
NODE	4029	length	2357	cov	2.823632	4	
NODE	4078	length	2347	cov	3.302356	1	Thaumarchaeota (phylum)
NODE	4078	length	2347	cov	3.302356	2	Crenarchaeota (phylum)
NODE	4078	length	2347	cov	3.302356	3	
NODE	4078	length	2347	cov	3.302356	4	
NODE	4165	length	2330	cov	4.741978	1	
NODE	4165	length	2330	cov	4.741978	2	
NODE	4165	length	2330	cov	4.741978	3	Methanomada group (clade)
NODE	4165	length	2330	cov	4.741978	4	
NODE	4173	length	2328	cov	2.908491	1	
NODE	4173	length	2328	cov	2.908491	2	Bacteria candidate phyla (clade)
NODE	4173	length	2328	cov	2.908491	3	Chloroflexi (phylum)
NODE	4183	length	2326	cov	5.162924	1	archaeon (species)
NODE	4212	length	2323	cov	3.960758	1	archaeon (species)
NODE	4212	length	2323	cov	3.960758	2	archaeon (species)
NODE	4212	length	2323	cov	3.960758	3	
NODE	4282	length	2308	cov	3.948069	1	archaeon (species)
NODE	4285	length	2307	cov	4.690941	1	Thaumarchaeota (phylum)
NODE	4285	length	2307	cov	4.690941	2	Thaumarchaeota (phylum)
NODE	4285	length	2307	cov	4.690941	3	Thaumarchaeota (phylum)
NODE	4285	length	2307	cov	4.690941	4	
NODE	4285	length	2307	cov	4.690941	5	Candidatus Bathyarchaeota (phylum)
NODE	4320	length	2301	cov	3.743989	1	Candidatus Verstraetearchaeota (phylum)
NODE	4320	length	2301	cov	3.743989	2	archaeon (species)
NODE	4367	length	2293	cov	3.810098	1	
NODE	4367	length	2293	cov	3.810098	2	
NODE	4383	length	2290	cov	4.509172	1	archaeon (species)
NODE	4383	length	2290	cov	4.509172	2	archaeon (species)
NODE	4383	length	2290	cov	4.509172	3	
NODE	4387	length	2289	cov	4.162041	1	
NODE	4387	length	2289	cov	4.162041	2	
NODE	4387	length	2289	cov	4.162041	3	Chloroflexi (phylum)
NODE	4532	length	2265	cov	5.142986	1	
NODE	4532	length	2265	cov	5.142986	2	archaeon RBG_16_50_20 (species)
NODE	4610	length	2253	cov	4.132848	1	archaeon RBG_16_50_20 (species)
NODE	4610	length	2253	cov	4.132848	2	Candidatus Bathyarchaeota (phylum)
NODE	4610	length	2253	cov	4.132848	3	
NODE	4644	length	2246	cov	5.828845	1	archaeon (species)
NODE	4644	length	2246	cov	5.828845	2	archaeon (species)
NODE	4644	length	2246	cov	5.828845	3	
NODE	4644	length	2246	cov	5.828845	4	bacterium (species)
NODE	4739	length	2229	cov	3.646274	1	Actinobacteria (phylum)
NODE	4739	length	2229	cov	3.646274	2	
NODE	4807	length	2218	cov	4.979196	1	

NODE	4807	length	2218	cov	4.979196	2	
NODE	4807	length	2218	cov	4.979196	3	Thaumarchaeota (phylum)
NODE	4904	length	2204	cov	3	1	Chloroflexi (phylum)
NODE	4906	length	2203	cov	6.441341	1	archaeon RBG_16_50_20 (species)
NODE	4906	length	2203	cov	6.441341	2	Candidatus Marsarchaeota (phylum)
NODE	4906	length	2203	cov	6.441341	3	
NODE	4910	length	2203	cov	5.136872	1	
NODE	4910	length	2203	cov	5.136872	2	
NODE	4910	length	2203	cov	5.136872	3	delta/epsilon subdivisions (subphylum)
NODE	4918	length	2203	cov	2.94041	1	bacterium (species)
NODE	4918	length	2203	cov	2.94041	2	
NODE	4918	length	2203	cov	2.94041	3	
NODE	4936	length	2199	cov	4.073694	1	
NODE	4936	length	2199	cov	4.073694	2	archaeon (species)
NODE	4943	length	2198	cov	3.65609	1	archaeon (species)
NODE	4943	length	2198	cov	3.65609	2	
NODE	4961	length	2194	cov	6.222066	1	archaeon RBG_16_50_20 (species)
NODE	4961	length	2194	cov	6.222066	2	
NODE	4961	length	2194	cov	6.222066	3	
NODE	4961	length	2194	cov	6.222066	4	bacterium (species)
NODE	4994	length	2189	cov	4.93627	1	bacterium (species)
NODE	4994	length	2189	cov	4.93627	2	
NODE	4994	length	2189	cov	4.93627	3	bacterium (species)
NODE	4994	length	2189	cov	4.93627	4	
NODE	5043	length	2180	cov	6.163765	1	
NODE	5043	length	2180	cov	6.163765	2	Candidatus Bathyarchaeota (phylum)
NODE	5113	length	2170	cov	4.56974	1	Thaumarchaeota (phylum)
NODE	5113	length	2170	cov	4.56974	2	bacterium (species)
NODE	5120	length	2169	cov	4.485336	1	
NODE	5120	length	2169	cov	4.485336	2	
NODE	5175	length	2161	cov	4.184236	1	
NODE	5175	length	2161	cov	4.184236	2	unclassified Acidobacteria (no rank)
NODE	5176	length	2161	cov	3.753086	1	Candidatus Bathyarchaeota (phylum)
NODE	5219	length	2154	cov	6.345879	1	archaeon (species)
NODE	5219	length	2154	cov	6.345879	2	
NODE	5219	length	2154	cov	6.345879	3	
NODE	5219	length	2154	cov	6.345879	4	
NODE	5267	length	2148	cov	3.931677	1	Candidatus Bathyarchaeota (phylum)
NODE	5267	length	2148	cov	3.931677	2	
NODE	5323	length	2140	cov	3.968825	1	Candidatus Bathyarchaeota (phylum)
NODE	5323	length	2140	cov	3.968825	2	Candidatus Bathyarchaeota (phylum)
NODE	5323	length	2140	cov	3.968825	3	unclassified Euryarchaeota (no rank)
NODE	5444	length	2123	cov	5.283366	1	Diaforarchaea group (clade)
NODE	5444	length	2123	cov	5.283366	2	archaeon (species)
NODE	5446	length	2123	cov	4.70793	1	archaeon (species)
NODE	5446	length	2123	cov	4.70793	2	archaeon (species)
NODE	5446	length	2123	cov	4.70793	3	archaeon (species)
NODE	5446	length	2123	cov	4.70793	4	archaeon (species)
NODE	5504	length	2116	cov	3.893741	1	
NODE	5504	length	2116	cov	3.893741	2	
NODE	5525	length	2113	cov	5.199708	1	
NODE	5525	length	2113	cov	5.199708	2	archaeon (species)
NODE	5541	length	2111	cov	4.578794	1	archaeon (species)
NODE	5541	length	2111	cov	4.578794	2	archaeon (species)
NODE	5541	length	2111	cov	4.578794	3	
NODE	5566	length	2107	cov	5.408869	1	Chloroflexi (phylum)

NODE	5566	length	2107	cov	5.408869	2	
NODE	5599	length	2103	cov	3.862305	1	
NODE	5633	length	2098	cov	3.150269	1	Chloroflexi (phylum)
NODE	5633	length	2098	cov	3.150269	2	Chloroflexi (phylum)
NODE	5633	length	2098	cov	3.150269	3	
NODE	5633	length	2098	cov	3.150269	4	Candidatus Bathyarchaeota (phylum)
NODE	5698	length	2088	cov	4.447614	1	Candidatus Bathyarchaeota (phylum)
NODE	5698	length	2088	cov	4.447614	2	Candidatus Bathyarchaeota (phylum)
NODE	5698	length	2088	cov	4.447614	3	
NODE	5698	length	2088	cov	4.447614	4	Candidatus Bathyarchaeota (phylum)
NODE	5713	length	2086	cov	4.858198	1	
NODE	5713	length	2086	cov	4.858198	2	Chloroflexi (phylum)
NODE	5764	length	2081	cov	3.727048	1	
NODE	5764	length	2081	cov	3.727048	2	
NODE	5764	length	2081	cov	3.727048	3	
NODE	5764	length	2081	cov	3.727048	4	
NODE	5769	length	2080	cov	4.304691	1	archaeon (species)
NODE	5769	length	2080	cov	4.304691	2	archaeon (species)
NODE	5769	length	2080	cov	4.304691	3	archaeon (species)
NODE	5769	length	2080	cov	4.304691	4	archaeon (species)
NODE	5799	length	2077	cov	4.706231	1	archaeon (species)
NODE	5799	length	2077	cov	4.706231	2	
NODE	5843	length	2071	cov	6.631944	1	
NODE	5843	length	2071	cov	6.631944	2	Candidatus Bathyarchaeota (phylum)
NODE	5850	length	2071	cov	2.681052	1	Candidatus Bathyarchaeota (phylum)
NODE	5850	length	2071	cov	2.681052	2	
NODE	5883	length	2066	cov	5.79811	1	Thaumarchaeota (phylum)
NODE	5883	length	2066	cov	5.79811	2	
NODE	5883	length	2066	cov	5.79811	3	Candidatus Bathyarchaeota (phylum)
NODE	5883	length	2066	cov	5.79811	4	Diaforarchaea group (clade)
NODE	5914	length	2063	cov	5.618028	1	
NODE	5914	length	2063	cov	5.618028	2	
NODE	5934	length	2061	cov	6.001994	1	archaeon RBG_16_50_20 (species)
NODE	5934	length	2061	cov	6.001994	2	archaeon RBG_16_50_20 (species)
NODE	5934	length	2061	cov	6.001994	3	
NODE	5939	length	2061	cov	3.942173	1	
NODE	5939	length	2061	cov	3.942173	2	
NODE	5939	length	2061	cov	3.942173	3	Candidatus Bathyarchaeota (phylum)
NODE	5947	length	2060	cov	5.226434	1	archaeon (species)
NODE	5947	length	2060	cov	5.226434	2	Candidatus Bathyarchaeota (phylum)
NODE	5947	length	2060	cov	5.226434	3	
NODE	5947	length	2060	cov	5.226434	4	archaeon (species)
NODE	6162	length	2029	cov	7.222898	1	archaeon (species)
NODE	6162	length	2029	cov	7.222898	2	
NODE	6229	length	2022	cov	3.6182	1	
NODE	6229	length	2022	cov	3.6182	2	Chloroflexi (phylum)
NODE	6229	length	2022	cov	3.6182	3	archaeon (species)
NODE	6269	length	2017	cov	4.966871	1	archaeon (species)
NODE	6269	length	2017	cov	4.966871	2	Candidatus Bathyarchaeota (phylum)
NODE	6293	length	2014	cov	6.61511	1	
NODE	6293	length	2014	cov	6.61511	2	
NODE	6410	length	2003	cov	3.688912	1	archaeon RBG_16_50_20 (species)
NODE	6410	length	2003	cov	3.688912	2	
NODE	6410	length	2003	cov	3.688912	3	Candidatus Bathyarchaeota (phylum)
NODE	6410	length	2003	cov	3.688912	4	Chloroflexi (phylum)
NODE	6413	length	2003	cov	3.505647	1	

NODE	6413	length	2003	cov	3.505647	2	archaeon (species)
NODE	6413	length	2003	cov	3.505647	3	Bacteroidetes/Chlorobi group (clade)
NODE	6428	length	2002	cov	2.840267	1	Candidatus Bathyarchaeota (phylum)
NODE	6428	length	2002	cov	2.840267	2	
NODE	6488	length	1996	cov	3.958784	1	
NODE	6488	length	1996	cov	3.958784	2	archaeon RBG_16_50_20 (species)
NODE	6496	length	1995	cov	4.003608	1	
NODE	6496	length	1995	cov	4.003608	2	
NODE	6542	length	1991	cov	4.23812	1	
NODE	6542	length	1991	cov	4.23812	2	
NODE	6546	length	1990	cov	5.381395	1	archaeon (species)
NODE	6546	length	1990	cov	5.381395	2	Thaumarchaeota (phylum)
NODE	6581	length	1987	cov	3.534679	1	Thaumarchaeota (phylum)
NODE	6581	length	1987	cov	3.534679	2	Candidatus Thorarchaeota (phylum)
NODE	6581	length	1987	cov	3.534679	3	
NODE	6613	length	1983	cov	5.099066	1	
NODE	6613	length	1983	cov	5.099066	2	archaeon RBG_16_50_20 (species)
NODE	6680	length	1977	cov	3.074922	1	
NODE	6680	length	1977	cov	3.074922	2	Candidatus Bathyarchaeota (phylum)
NODE	6680	length	1977	cov	3.074922	3	
NODE	6709	length	1974	cov	6.293903	1	Candidatus Bathyarchaeota (phylum)
NODE	6709	length	1974	cov	6.293903	2	
NODE	6709	length	1974	cov	6.293903	3	Thaumarchaeota (phylum)
NODE	6863	length	1958	cov	2.987388	1	
NODE	6863	length	1958	cov	2.987388	2	
NODE	6865	length	1958	cov	2.147136	1	
NODE	6865	length	1958	cov	2.147136	2	archaeon RBG_16_50_20 (species)
NODE	6874	length	1956	cov	4.37191	1	
NODE	6874	length	1956	cov	4.37191	2	
NODE	6874	length	1956	cov	4.37191	3	bacterium (species)
NODE	6917	length	1951	cov	3.353376	1	Candidatus Bathyarchaeota (phylum)
NODE	6917	length	1951	cov	3.353376	2	
NODE	6931	length	1949	cov	5.318902	1	
NODE	6931	length	1949	cov	5.318902	2	
NODE	6931	length	1949	cov	5.318902	3	
NODE	6931	length	1949	cov	5.318902	4	
NODE	6993	length	1943	cov	4.78178	1	Candidatus Bathyarchaeota (phylum)
NODE	6993	length	1943	cov	4.78178	2	Candidatus Bathyarchaeota (phylum)
NODE	6993	length	1943	cov	4.78178	3	
NODE	6993	length	1943	cov	4.78178	4	archaeon RBG_16_50_20 (species)
NODE	7073	length	1934	cov	5.728047	1	archaeon RBG_16_50_20 (species)
NODE	7073	length	1934	cov	5.728047	2	
NODE	7073	length	1934	cov	5.728047	3	Candidatus Bathyarchaeota (phylum)
NODE	7073	length	1934	cov	5.728047	4	Thaumarchaeota (phylum)
NODE	7104	length	1931	cov	4.910448	1	
NODE	7104	length	1931	cov	4.910448	2	
NODE	7105	length	1931	cov	4.712154	1	
NODE	7105	length	1931	cov	4.712154	2	
NODE	7128	length	1928	cov	5.722904	1	
NODE	7128	length	1928	cov	5.722904	2	archaeon (species)
NODE	7347	length	1909	cov	4.854908	1	archaeon (species)
NODE	7347	length	1909	cov	4.854908	2	archaeon (species)
NODE	7347	length	1909	cov	4.854908	3	archaeon RBG_16_50_20 (species)
NODE	7347	length	1909	cov	4.854908	4	
NODE	7358	length	1908	cov	5.368591	1	Candidatus Bathyarchaeota (phylum)
NODE	7358	length	1908	cov	5.368591	2	archaeon (species)

NODE	7358	length	1908	cov	5.368591	3	Diaforarchaea group (clade)
NODE	7450	length	1900	cov	3.543631	1	
NODE	7450	length	1900	cov	3.543631	2	archaeon (species)
NODE	7470	length	1899	cov	2.868221	1	archaeon (species)
NODE	7470	length	1899	cov	2.868221	2	archaeon (species)
NODE	7470	length	1899	cov	2.868221	3	
NODE	7571	length	1889	cov	5.013086	1	Thaumarchaeota (phylum)
NODE	7571	length	1889	cov	5.013086	2	
NODE	7571	length	1889	cov	5.013086	3	archaeon (species)
NODE	7597	length	1887	cov	3.590066	1	archaeon (species)
NODE	7597	length	1887	cov	3.590066	2	archaeon (species)
NODE	7597	length	1887	cov	3.590066	3	
NODE	7664	length	1880	cov	3.754521	1	archaeon (species)
NODE	7666	length	1880	cov	3.579178	1	archaeon RBG_16_50_20 (species)
NODE	7666	length	1880	cov	3.579178	2	archaeon (species)
NODE	7666	length	1880	cov	3.579178	3	
NODE	7687	length	1878	cov	3.58859	1	Thaumarchaeota (phylum)
NODE	7687	length	1878	cov	3.58859	2	Thaumarchaeota (phylum)
NODE	7687	length	1878	cov	3.58859	3	Candidatus Bathyarchaeota (phylum)
NODE	7690	length	1878	cov	3.114098	1	
NODE	7690	length	1878	cov	3.114098	2	
NODE	7690	length	1878	cov	3.114098	3	
NODE	7690	length	1878	cov	3.114098	4	Chloroflexi (phylum)
NODE	7765	length	1872	cov	3.274629	1	archaeon (species)
NODE	7765	length	1872	cov	3.274629	2	
NODE	7813	length	1868	cov	4.294539	1	
NODE	7813	length	1868	cov	4.294539	2	
NODE	7813	length	1868	cov	4.294539	3	
NODE	7817	length	1868	cov	3.433536	1	
NODE	7817	length	1868	cov	3.433536	2	unclassified Spirochaetes (no rank)
NODE	7817	length	1868	cov	3.433536	3	Candidatus Bathyarchaeota (phylum)
NODE	7822	length	1867	cov	9.204194	1	Candidatus Bathyarchaeota (phylum)
NODE	7822	length	1867	cov	9.204194	2	
NODE	7868	length	1864	cov	3.07518	1	
NODE	7868	length	1864	cov	3.07518	2	
NODE	7868	length	1864	cov	3.07518	3	bacterium (species)
NODE	7901	length	1861	cov	3.835548	1	Crenarchaeota (phylum)
NODE	7901	length	1861	cov	3.835548	2	unclassified Euryarchaeota (no rank)
NODE	7928	length	1859	cov	4.073725	1	
NODE	7928	length	1859	cov	4.073725	2	
NODE	7928	length	1859	cov	4.073725	3	Crenarchaeota (phylum)
NODE	7958	length	1856	cov	4.694059	1	
NODE	7958	length	1856	cov	4.694059	2	
NODE	7986	length	1853	cov	5.442158	1	
NODE	7986	length	1853	cov	5.442158	2	
NODE	7986	length	1853	cov	5.442158	3	
NODE	7986	length	1853	cov	5.442158	4	Diaforarchaea group (clade)
NODE	8022	length	1850	cov	4.235097	1	Candidatus Bathyarchaeota (phylum)
NODE	8022	length	1850	cov	4.235097	2	
NODE	8022	length	1850	cov	4.235097	3	
NODE	8261	length	1832	cov	4.405177	1	
NODE	8261	length	1832	cov	4.405177	2	Hadesarchaea (class)
NODE	8322	length	1827	cov	2.78386	1	
NODE	8322	length	1827	cov	2.78386	2	archaeon (species)
NODE	8386	length	1821	cov	6.473386	1	archaeon (species)
NODE	8386	length	1821	cov	6.473386	2	

NODE	8395	length	1821	cov	3.499434	1	
NODE	8467	length	1816	cov	3.616127	1	
NODE	8467	length	1816	cov	3.616127	2	
NODE	8467	length	1816	cov	3.616127	3	Chloroflexi (phylum)
NODE	8525	length	1812	cov	4.944223	1	
NODE	8525	length	1812	cov	4.944223	2	Candidatus Bathyarchaeota (phylum)
NODE	8618	length	1805	cov	4.492571	1	
NODE	8618	length	1805	cov	4.492571	2	
NODE	8743	length	1797	cov	5.2124	1	
NODE	8743	length	1797	cov	5.2124	2	
NODE	8764	length	1796	cov	4.126939	1	archaeon RBG_16_50_20 (species)
NODE	8778	length	1795	cov	7.431034	1	
NODE	8778	length	1795	cov	7.431034	2	archaeon (species)
NODE	8797	length	1794	cov	4.384704	1	archaeon (species)
NODE	8797	length	1794	cov	4.384704	2	
NODE	8837	length	1790	cov	5.54928	1	Chloroflexi (phylum)
NODE	8886	length	1787	cov	2.937644	1	
NODE	8886	length	1787	cov	2.937644	2	Candidatus Bathyarchaeota (phylum)
NODE	8886	length	1787	cov	2.937644	3	archaeon RBG_16_50_20 (species)
NODE	8927	length	1783	cov	4.161458	1	
NODE	8927	length	1783	cov	4.161458	2	Thaumarchaeota (phylum)
NODE	8967	length	1780	cov	4.612174	1	bacterium (species)
NODE	8967	length	1780	cov	4.612174	2	
NODE	8972	length	1780	cov	3.669565	1	Candidatus Bathyarchaeota (phylum)
NODE	8972	length	1780	cov	3.669565	2	Candidatus Bathyarchaeota (phylum)
NODE	8973	length	1780	cov	3.598841	1	
NODE	8983	length	1779	cov	6.546404	1	
NODE	8983	length	1779	cov	6.546404	2	
NODE	8983	length	1779	cov	6.546404	3	bacterium (species)
NODE	9053	length	1774	cov	5.964514	1	
NODE	9053	length	1774	cov	5.964514	2	
NODE	9127	length	1770	cov	2.446064	1	
NODE	9129	length	1769	cov	5.453909	1	archaeon RBG_16_50_20 (species)
NODE	9129	length	1769	cov	5.453909	2	Stenosarchaea group (clade)
NODE	9197	length	1765	cov	3.502339	1	
NODE	9197	length	1765	cov	3.502339	2	Planctomycetes (phylum)
NODE	9197	length	1765	cov	3.502339	3	archaeon HR06 (species)
NODE	9232	length	1762	cov	4.380785	1	Chloroflexi (phylum)
NODE	9232	length	1762	cov	4.380785	2	
NODE	9232	length	1762	cov	4.380785	3	Diaforarchaea group (clade)
NODE	9253	length	1761	cov	4.297186	1	Diaforarchaea group (clade)
NODE	9253	length	1761	cov	4.297186	2	
NODE	9290	length	1758	cov	4.729888	1	Candidatus Bathyarchaeota (phylum)
NODE	9290	length	1758	cov	4.729888	2	Diaforarchaea group (clade)
NODE	9310	length	1757	cov	4.063455	1	
NODE	9310	length	1757	cov	4.063455	2	Candidatus Bathyarchaeota (phylum)
NODE	9310	length	1757	cov	4.063455	3	archaeon RBG_16_50_20 (species)
NODE	9378	length	1753	cov	7.808009	1	Crenarchaeota (phylum)
NODE	9378	length	1753	cov	7.808009	2	Candidatus Bathyarchaeota (phylum)
NODE	9427	length	1750	cov	5.292035	1	
NODE	9427	length	1750	cov	5.292035	2	Candidatus Bathyarchaeota (phylum)
NODE	9427	length	1750	cov	5.292035	3	
NODE	9427	length	1750	cov	5.292035	4	Crenarchaeota (phylum)
NODE	9438	length	1750	cov	3.228909	1	
NODE	9438	length	1750	cov	3.228909	2	
NODE	9438	length	1750	cov	3.228909	3	Candidatus Bathyarchaeota (phylum)

NODE	9472	length	1748	cov	2.405789	1	Candidatus Bathyarchaeota (phylum)
NODE	9472	length	1748	cov	2.405789	2	
NODE	9519	length	1744	cov	4.172291	1	Bacteria candidate phyla (clade)
NODE	9519	length	1744	cov	4.172291	2	
NODE	9519	length	1744	cov	4.172291	3	
NODE	9519	length	1744	cov	4.172291	4	archaeon (species)
NODE	9584	length	1739	cov	3.980998	1	archaeon (species)
NODE	9584	length	1739	cov	3.980998	2	archaeon (species)
NODE	9584	length	1739	cov	3.980998	3	
NODE	9591	length	1739	cov	2.501188	1	
NODE	9591	length	1739	cov	2.501188	2	bacterium (species)
NODE	9591	length	1739	cov	2.501188	3	Bacteria candidate phyla (clade)
NODE	9591	length	1739	cov	2.501188	4	archaeon (species)
NODE	9594	length	1738	cov	5.632204	1	archaeon (species)
NODE	9594	length	1738	cov	5.632204	2	
NODE	9594	length	1738	cov	5.632204	3	archaeon (species)
NODE	9650	length	1734	cov	4.037522	1	archaeon (species)
NODE	9650	length	1734	cov	4.037522	2	archaeon (species)
NODE	9672	length	1732	cov	7.178295	1	
NODE	9672	length	1732	cov	7.178295	2	Candidatus Marsarchaeota (phylum)
NODE	9678	length	1732	cov	3.962433	1	Candidatus Marsarchaeota (phylum)
NODE	9678	length	1732	cov	3.962433	2	
NODE	9707	length	1730	cov	4.309254	1	
NODE	9707	length	1730	cov	4.309254	2	
NODE	9797	length	1724	cov	4.680647	1	
NODE	9797	length	1724	cov	4.680647	2	
NODE	9797	length	1724	cov	4.680647	3	
NODE	9829	length	1722	cov	3.453509	1	bacterium (species)
NODE	9829	length	1722	cov	3.453509	2	bacterium (species)
NODE	9829	length	1722	cov	3.453509	3	
NODE	9846	length	1721	cov	3.34994	1	archaeon RBG_16_50_20 (species)
NODE	9846	length	1721	cov	3.34994	2	archaeon (species)
NODE	9866	length	1719	cov	5.626202	1	archaeon (species)
NODE	9866	length	1719	cov	5.626202	2	archaeon (species)
NODE	9866	length	1719	cov	5.626202	3	Thaumarchaeota (phylum)
NODE	10163	length	1702	cov	2.844566	1	Candidatus Bathyarchaeota (phylum)
NODE	10200	length	1700	cov	2.920365	1	
NODE	10200	length	1700	cov	2.920365	2	
NODE	10209	length	1699	cov	4.298054	1	Armatimonadetes (phylum)
NODE	10209	length	1699	cov	4.298054	2	Chloroflexi (phylum)
NODE	10209	length	1699	cov	4.298054	3	
NODE	10250	length	1696	cov	3.338208	1	
NODE	10250	length	1696	cov	3.338208	2	
NODE	10261	length	1695	cov	4.570122	1	
NODE	10261	length	1695	cov	4.570122	2	
NODE	10392	length	1688	cov	4.513166	1	
NODE	10392	length	1688	cov	4.513166	2	
NODE	10392	length	1688	cov	4.513166	3	
NODE	10411	length	1687	cov	5.980392	1	
NODE	10411	length	1687	cov	5.980392	2	
NODE	10429	length	1686	cov	6.722869	1	Candidatus Bathyarchaeota (phylum)
NODE	10429	length	1686	cov	6.722869	2	
NODE	10429	length	1686	cov	6.722869	3	archaeon (species)
NODE	10443	length	1685	cov	6.193865	1	
NODE	10443	length	1685	cov	6.193865	2	
NODE	10443	length	1685	cov	6.193865	3	

NODE	10582	length	1677	cov	4.475339	1	Thaumarchaeota (phylum)
NODE	10582	length	1677	cov	4.475339	2	archaeon RBG_16_50_20 (species)
NODE	10652	length	1674	cov	2.110562	1	archaeon RBG_16_50_20 (species)
NODE	10652	length	1674	cov	2.110562	2	
NODE	10654	length	1673	cov	6.896168	1	Candidatus Bathyarchaeota (phylum)
NODE	10654	length	1673	cov	6.896168	2	Candidatus Bathyarchaeota (phylum)
NODE	10654	length	1673	cov	6.896168	3	Candidatus Bathyarchaeota (phylum)
NODE	10856	length	1662	cov	3.528936	1	Candidatus Bathyarchaeota (phylum)
NODE	10856	length	1662	cov	3.528936	2	Candidatus Bathyarchaeota (phylum)
NODE	10883	length	1660	cov	5.671028	1	
NODE	10883	length	1660	cov	5.671028	2	archaeon RBG_16_50_20 (species)
NODE	10912	length	1658	cov	4.716781	1	
NODE	11089	length	1649	cov	3.900878	1	
NODE	11089	length	1649	cov	3.900878	2	unclassified Euryarchaeota (no rank)
NODE	11104	length	1648	cov	5.195857	1	
NODE	11104	length	1648	cov	5.195857	2	
NODE	11104	length	1648	cov	5.195857	3	
NODE	11146	length	1646	cov	7.673162	1	archaeon (species)
NODE	11146	length	1646	cov	7.673162	2	archaeon (species)
NODE	11146	length	1646	cov	7.673162	3	archaeon (species)
NODE	11324	length	1637	cov	6.189633	1	
NODE	11324	length	1637	cov	6.189633	2	archaeon (species)
NODE	11324	length	1637	cov	6.189633	3	
NODE	11341	length	1636	cov	6.277672	1	
NODE	11341	length	1636	cov	6.277672	2	Candidatus Bathyarchaeota (phylum)
NODE	11341	length	1636	cov	6.277672	3	Candidatus Bathyarchaeota (phylum)
NODE	11341	length	1636	cov	6.277672	4	
NODE	11377	length	1635	cov	2.996835	1	unclassified Euryarchaeota (no rank)
NODE	11377	length	1635	cov	2.996835	2	Candidatus Bathyarchaeota (phylum)
NODE	11390	length	1634	cov	3.351488	1	Candidatus Bathyarchaeota (phylum)
NODE	11390	length	1634	cov	3.351488	2	Candidatus Bathyarchaeota (phylum)
NODE	11421	length	1632	cov	5.051363	1	
NODE	11421	length	1632	cov	5.051363	2	
NODE	11437	length	1632	cov	2.660748	1	
NODE	11437	length	1632	cov	2.660748	2	
NODE	11437	length	1632	cov	2.660748	3	archaeon (species)
NODE	11443	length	1631	cov	4.236041	1	archaeon (species)
NODE	11443	length	1631	cov	4.236041	2	
NODE	11606	length	1622	cov	7.470964	1	archaeon RBG_16_50_20 (species)
NODE	11606	length	1622	cov	7.470964	2	
NODE	11631	length	1622	cov	2.900447	1	
NODE	11631	length	1622	cov	2.900447	2	unclassified Acidobacteria (no rank)
NODE	11681	length	1619	cov	5.655371	1	unclassified Euryarchaeota (no rank)
NODE	11681	length	1619	cov	5.655371	2	
NODE	11681	length	1619	cov	5.655371	3	
NODE	11795	length	1613	cov	6.515404	1	Candidatus Bathyarchaeota (phylum)
NODE	11795	length	1613	cov	6.515404	2	Candidatus Bathyarchaeota (phylum)
NODE	11912	length	1608	cov	3.71539	1	Candidatus Bathyarchaeota (phylum)
NODE	11912	length	1608	cov	3.71539	2	
NODE	11946	length	1607	cov	2.291237	1	
NODE	11946	length	1607	cov	2.291237	2	Diaforarchaea group (clade)
NODE	11946	length	1607	cov	2.291237	3	
NODE	12184	length	1595	cov	8.106494	1	Candidatus Bathyarchaeota (phylum)
NODE	12184	length	1595	cov	8.106494	2	Candidatus Bathyarchaeota (phylum)
NODE	12184	length	1595	cov	8.106494	3	
NODE	12184	length	1595	cov	8.106494	4	

NODE	12224	length	1593	cov	5.548114	1	
NODE	12230	length	1593	cov	4.804941	1	archaeon RBG_16_50_20 (species)
NODE	12230	length	1593	cov	4.804941	2	
NODE	12230	length	1593	cov	4.804941	3	Candidatus Bathyarchaeota (phylum)
NODE	12286	length	1590	cov	6.035831	1	Candidatus Bathyarchaeota (phylum)
NODE	12286	length	1590	cov	6.035831	2	
NODE	12286	length	1590	cov	6.035831	3	
NODE	12304	length	1589	cov	4.710561	1	
NODE	12304	length	1589	cov	4.710561	2	
NODE	12307	length	1589	cov	4.301825	1	
NODE	12307	length	1589	cov	4.301825	2	
NODE	12307	length	1589	cov	4.301825	3	Candidatus Bathyarchaeota (phylum)
NODE	12308	length	1589	cov	4.048892	1	Thaumarchaeota (phylum)
NODE	12325	length	1588	cov	4.141553	1	Thaumarchaeota (phylum)
NODE	12325	length	1588	cov	4.141553	2	
NODE	12796	length	1566	cov	2.98544	1	Candidatus Bathyarchaeota (phylum)
NODE	12796	length	1566	cov	2.98544	2	Candidatus Bathyarchaeota (phylum)
NODE	12873	length	1562	cov	5.123424	1	
NODE	12873	length	1562	cov	5.123424	2	archaeon RBG_16_50_20 (species)
NODE	12880	length	1562	cov	3.566689	1	unclassified Acidobacteria (no rank)
NODE	12880	length	1562	cov	3.566689	2	Candidatus Bathyarchaeota (phylum)
NODE	12966	length	1558	cov	3.300067	1	Thaumarchaeota (phylum)
NODE	12966	length	1558	cov	3.300067	2	archaeon RBG_16_50_20 (species)
NODE	12966	length	1558	cov	3.300067	3	Candidatus Bathyarchaeota (phylum)
NODE	13065	length	1554	cov	2.333556	1	Candidatus Bathyarchaeota (phylum)
NODE	13065	length	1554	cov	2.333556	2	Bacteria candidate phyla (clade)
NODE	13075	length	1553	cov	3.76235	1	Chloroflexi (phylum)
NODE	13075	length	1553	cov	3.76235	2	
NODE	13095	length	1552	cov	3.175017	1	
NODE	13095	length	1552	cov	3.175017	2	archaeon (species)
NODE	13129	length	1550	cov	5.1699	1	archaeon (species)
NODE	13129	length	1550	cov	5.1699	2	
NODE	13129	length	1550	cov	5.1699	3	
NODE	13252	length	1545	cov	3.916779	1	Chloroflexi (phylum)
NODE	13252	length	1545	cov	3.916779	2	
NODE	13332	length	1541	cov	6.453567	1	
NODE	13332	length	1541	cov	6.453567	2	bacterium (species)
NODE	13342	length	1541	cov	3.353297	1	
NODE	13342	length	1541	cov	3.353297	2	
NODE	13471	length	1535	cov	4.417568	1	
NODE	13471	length	1535	cov	4.417568	2	
NODE	13486	length	1534	cov	4.233266	1	Thaumarchaeota (phylum)
NODE	13486	length	1534	cov	4.233266	2	
NODE	13635	length	1529	cov	3.541384	1	bacterium (species)
NODE	13635	length	1529	cov	3.541384	2	Candidatus Bathyarchaeota (phylum)
NODE	13721	length	1525	cov	4.646939	1	
NODE	13721	length	1525	cov	4.646939	2	
NODE	13721	length	1525	cov	4.646939	3	Firmicutes (phylum)
NODE	13721	length	1525	cov	4.646939	4	Candidatus Thorarchaeota (phylum)
NODE	13724	length	1525	cov	4.240136	1	archaeon (species)
NODE	13771	length	1523	cov	3.858992	1	archaeon (species)
NODE	13771	length	1523	cov	3.858992	2	Candidatus Lokiarchaeota (phylum)
NODE	13839	length	1521	cov	2.991132	1	archaeon RBG_16_50_20 (species)
NODE	13839	length	1521	cov	2.991132	2	Chloroflexi (phylum)
NODE	14030	length	1514	cov	2.197395	1	archaeon RBG_16_50_20 (species)
NODE	14030	length	1514	cov	2.197395	2	archaeon (species)

NODE	14097	length	1511	cov	3.197115	1	archaeon (species)
NODE	14097	length	1511	cov	3.197115	2	
NODE	14231	length	1506	cov	3.829083	1	
NODE	14231	length	1506	cov	3.829083	2	
NODE	14299	length	1504	cov	2.327122	1	
NODE	14299	length	1504	cov	2.327122	2	Candidatus Bathyarchaeota (phylum)
NODE	14299	length	1504	cov	2.327122	3	
NODE	14317	length	1503	cov	3.61395	1	Candidatus Bathyarchaeota (phylum)
NODE	14317	length	1503	cov	3.61395	2	Candidatus Bathyarchaeota (phylum)
NODE	14411	length	1500	cov	2.687889	1	
NODE	14411	length	1500	cov	2.687889	2	
NODE	14411	length	1500	cov	2.687889	3	

Classification of Refined bin 16

Contigs							**Lineage name**
NODE	316	length	5827	cov	4.927755	1	
NODE	316	length	5827	cov	4.927755	2	
NODE	316	length	5827	cov	4.927755	3	Candidatus Bathyarchaeota (phylum)
NODE	316	length	5827	cov	4.927755	4	Candidatus Bathyarchaeota (phylum)
NODE	316	length	5827	cov	4.927755	5	archaeon RBG_16_50_20 (species)
NODE	316	length	5827	cov	4.927755	6	archaeon RBG_16_50_20 (species)
NODE	316	length	5827	cov	4.927755	7	archaeon RBG_16_50_20 (species)
NODE	399	length	5233	cov	4.6236	1	Candidatus Bathyarchaeota (phylum)
NODE	399	length	5233	cov	4.6236	2	archaeon RBG_16_50_20 (species)
NODE	399	length	5233	cov	4.6236	3	archaeon RBG_16_50_20 (species)
NODE	399	length	5233	cov	4.6236	4	archaeon RBG_16_50_20 (species)
NODE	399	length	5233	cov	4.6236	5	archaeon RBG_16_50_20 (species)
NODE	399	length	5233	cov	4.6236	6	archaeon RBG_16_50_20 (species)
NODE	399	length	5233	cov	4.6236	7	archaeon RBG_16_50_20 (species)
NODE	399	length	5233	cov	4.6236	8	archaeon RBG_16_50_20 (species)
NODE	415	length	5139	cov	3.900865	1	
NODE	415	length	5139	cov	3.900865	2	Chloroflexi (phylum)
NODE	415	length	5139	cov	3.900865	3	
NODE	415	length	5139	cov	3.900865	4	
NODE	415	length	5139	cov	3.900865	5	
NODE	415	length	5139	cov	3.900865	6	
NODE	496	length	4813	cov	3.869903	1	Candidatus Bathyarchaeota (phylum)
NODE	496	length	4813	cov	3.869903	2	
NODE	496	length	4813	cov	3.869903	3	
NODE	496	length	4813	cov	3.869903	4	
NODE	520	length	4713	cov	4.25526	1	archaeon RBG_16_50_20 (species)
NODE	520	length	4713	cov	4.25526	2	archaeon RBG_16_50_20 (species)
NODE	520	length	4713	cov	4.25526	3	
NODE	520	length	4713	cov	4.25526	4	archaeon RBG_16_50_20 (species)
NODE	520	length	4713	cov	4.25526	5	archaeon RBG_16_50_20 (species)
NODE	520	length	4713	cov	4.25526	6	archaeon RBG_16_50_20 (species)
NODE	547	length	4610	cov	6.43798	1	archaeon (species)
NODE	547	length	4610	cov	6.43798	2	archaeon (species)
NODE	547	length	4610	cov	6.43798	3	
NODE	547	length	4610	cov	6.43798	4	
NODE	547	length	4610	cov	6.43798	5	Candidatus Bathyarchaeota (phylum)
NODE	563	length	4555	cov	3.075111	1	
NODE	563	length	4555	cov	3.075111	2	
NODE	563	length	4555	cov	3.075111	3	

NODE	563	length	4555	cov	3.075111	4	Candidatus Marsarchaeota (phylum)
NODE	641	length	4338	cov	4.697408	1	archaeon RBG_16_50_20 (species)
NODE	641	length	4338	cov	4.697408	2	archaeon RBG_16_50_20 (species)
NODE	641	length	4338	cov	4.697408	3	archaeon RBG_16_50_20 (species)
NODE	641	length	4338	cov	4.697408	4	archaeon RBG_16_50_20 (species)
NODE	641	length	4338	cov	4.697408	5	archaeon RBG_16_50_20 (species)
NODE	641	length	4338	cov	4.697408	6	archaeon RBG_16_50_20 (species)
NODE	641	length	4338	cov	4.697408	7	archaeon RBG_16_50_20 (species)
NODE	681	length	4265	cov	3.720665	1	Thaumarchaeota (phylum)
NODE	681	length	4265	cov	3.720665	2	
NODE	681	length	4265	cov	3.720665	3	
NODE	681	length	4265	cov	3.720665	4	archaeon RBG_16_50_20 (species)
NODE	681	length	4265	cov	3.720665	5	archaeon RBG_16_50_20 (species)
NODE	681	length	4265	cov	3.720665	6	archaeon RBG_16_50_20 (species)
NODE	692	length	4231	cov	3.48683	1	archaeon RBG_16_50_20 (species)
NODE	692	length	4231	cov	3.48683	2	
NODE	692	length	4231	cov	3.48683	3	archaeon (species)
NODE	692	length	4231	cov	3.48683	4	
NODE	692	length	4231	cov	3.48683	5	
NODE	703	length	4213	cov	3.43771	1	
NODE	703	length	4213	cov	3.43771	2	
NODE	703	length	4213	cov	3.43771	3	
NODE	703	length	4213	cov	3.43771	4	bacterium (species)
NODE	703	length	4213	cov	3.43771	5	
NODE	703	length	4213	cov	3.43771	6	
NODE	851	length	3932	cov	4.123291	1	archaeon RBG_16_50_20 (species)
NODE	851	length	3932	cov	4.123291	2	archaeon RBG_16_50_20 (species)
NODE	851	length	3932	cov	4.123291	3	archaeon RBG_16_50_20 (species)
NODE	854	length	3923	cov	3.408997	1	archaeon RBG_16_50_20 (species)
NODE	854	length	3923	cov	3.408997	2	archaeon RBG_16_50_20 (species)
NODE	854	length	3923	cov	3.408997	3	archaeon RBG_16_50_20 (species)
NODE	854	length	3923	cov	3.408997	4	archaeon RBG_16_50_20 (species)
NODE	857	length	3913	cov	4.17859	1	archaeon RBG_16_50_20 (species)
NODE	857	length	3913	cov	4.17859	2	
NODE	857	length	3913	cov	4.17859	3	archaeon RBG_16_50_20 (species)
NODE	857	length	3913	cov	4.17859	4	archaeon RBG_16_50_20 (species)
NODE	857	length	3913	cov	4.17859	5	archaeon RBG_16_50_20 (species)
NODE	922	length	3832	cov	4.284617	1	
NODE	922	length	3832	cov	4.284617	2	
NODE	922	length	3832	cov	4.284617	3	
NODE	922	length	3832	cov	4.284617	4	
NODE	931	length	3822	cov	4.70507	1	
NODE	931	length	3822	cov	4.70507	2	
NODE	931	length	3822	cov	4.70507	3	
NODE	931	length	3822	cov	4.70507	4	Candidatus Bathyarchaeota (phylum)
NODE	931	length	3822	cov	4.70507	5	archaeon RBG_16_50_20 (species)
NODE	931	length	3822	cov	4.70507	6	
NODE	931	length	3822	cov	4.70507	7	archaeon RBG_16_50_20 (species)
NODE	931	length	3822	cov	4.70507	8	archaeon RBG_16_50_20 (species)
NODE	931	length	3822	cov	4.70507	9	archaeon RBG_16_50_20 (species)
NODE	945	length	3809	cov	3.204848	1	
NODE	945	length	3809	cov	3.204848	2	
NODE	945	length	3809	cov	3.204848	3	Hadesarchaea (class)
NODE	1107	length	3583	cov	3.483277	1	archaeon RBG_16_50_20 (species)
NODE	1107	length	3583	cov	3.483277	2	archaeon RBG_16_50_20 (species)
NODE	1107	length	3583	cov	3.483277	3	archaeon RBG_16_50_20 (species)

NODE	1119	length	3572	cov	5.117998	1	
NODE	1119	length	3572	cov	5.117998	2	
NODE	1119	length	3572	cov	5.117998	3	
NODE	1119	length	3572	cov	5.117998	4	Candidatus Bathyarchaeota (phylum)
NODE	1119	length	3572	cov	5.117998	5	
NODE	1163	length	3529	cov	3.795912	1	archaeon RBG_16_50_20 (species)
NODE	1163	length	3529	cov	3.795912	2	archaeon RBG_16_50_20 (species)
NODE	1163	length	3529	cov	3.795912	3	archaeon RBG_16_50_20 (species)
NODE	1163	length	3529	cov	3.795912	4	archaeon (species)
NODE	1163	length	3529	cov	3.795912	5	archaeon RBG_16_50_20 (species)
NODE	1248	length	3456	cov	4.235225	1	
NODE	1248	length	3456	cov	4.235225	2	Candidatus Bathyarchaeota (phylum)
NODE	1248	length	3456	cov	4.235225	3	
NODE	1248	length	3456	cov	4.235225	4	
NODE	1258	length	3447	cov	3.523585	1	Candidatus Bathyarchaeota (phylum)
NODE	1258	length	3447	cov	3.523585	2	
NODE	1258	length	3447	cov	3.523585	3	
NODE	1258	length	3447	cov	3.523585	4	Candidatus Bathyarchaeota (phylum)
NODE	1451	length	3293	cov	4.670167	1	archaeon RBG_16_50_20 (species)
NODE	1451	length	3293	cov	4.670167	2	archaeon RBG_16_50_20 (species)
NODE	1490	length	3262	cov	3.822264	1	
NODE	1490	length	3262	cov	3.822264	2	archaeon RBG_16_50_20 (species)
NODE	1490	length	3262	cov	3.822264	3	archaeon RBG_16_50_20 (species)
NODE	1490	length	3262	cov	3.822264	4	archaeon RBG_16_50_20 (species)
NODE	1490	length	3262	cov	3.822264	5	archaeon RBG_16_50_20 (species)
NODE	1505	length	3252	cov	4.268689	1	delta/epsilon subdivisions (subphylum)
NODE	1505	length	3252	cov	4.268689	2	archaeon RBG_16_50_20 (species)
NODE	1505	length	3252	cov	4.268689	3	archaeon RBG_16_50_20 (species)
NODE	1505	length	3252	cov	4.268689	4	archaeon RBG_16_50_20 (species)
NODE	1505	length	3252	cov	4.268689	5	
NODE	1633	length	3164	cov	4.333226	1	
NODE	1633	length	3164	cov	4.333226	2	Thaumarchaeota (phylum)
NODE	1633	length	3164	cov	4.333226	3	Thaumarchaeota (phylum)
NODE	1802	length	3054	cov	3.700567	1	
NODE	1802	length	3054	cov	3.700567	2	Candidatus Bathyarchaeota (phylum)
NODE	1802	length	3054	cov	3.700567	3	
NODE	1802	length	3054	cov	3.700567	4	Candidatus Bathyarchaeota (phylum)
NODE	1832	length	3038	cov	4.569896	1	
NODE	1832	length	3038	cov	4.569896	2	
NODE	1832	length	3038	cov	4.569896	3	
NODE	1832	length	3038	cov	4.569896	4	Thaumarchaeota (phylum)
NODE	1832	length	3038	cov	4.569896	5	Candidatus Bathyarchaeota (phylum)
NODE	1925	length	2993	cov	4.230769	1	
NODE	1925	length	2993	cov	4.230769	2	
NODE	1925	length	2993	cov	4.230769	3	Thaumarchaeota (phylum)
NODE	1938	length	2988	cov	2.731674	1	
NODE	1938	length	2988	cov	2.731674	2	
NODE	1938	length	2988	cov	2.731674	3	
NODE	1938	length	2988	cov	2.731674	4	
NODE	1944	length	2984	cov	4.937863	1	archaeon RBG_16_50_20 (species)
NODE	1944	length	2984	cov	4.937863	2	archaeon RBG_16_50_20 (species)
NODE	1944	length	2984	cov	4.937863	3	archaeon RBG_16_50_20 (species)
NODE	1944	length	2984	cov	4.937863	4	
NODE	2010	length	2948	cov	6.854822	1	
NODE	2010	length	2948	cov	6.854822	2	
NODE	2010	length	2948	cov	6.854822	3	

NODE	2087	length	2922	cov	4.031392	1	
NODE	2087	length	2922	cov	4.031392	2	
NODE	2087	length	2922	cov	4.031392	3	
NODE	2087	length	2922	cov	4.031392	4	
NODE	2138	length	2905	cov	4.445263	1	archaeon RBG_16_50_20 (species)
NODE	2138	length	2905	cov	4.445263	2	
NODE	2138	length	2905	cov	4.445263	3	archaeon RBG_16_50_20 (species)
NODE	2138	length	2905	cov	4.445263	4	archaeon RBG_16_50_20 (species)
NODE	2138	length	2905	cov	4.445263	5	
NODE	2158	length	2898	cov	5.132606	1	
NODE	2158	length	2898	cov	5.132606	2	archaeon RBG_16_50_20 (species)
NODE	2158	length	2898	cov	5.132606	3	archaeon (species)
NODE	2259	length	2853	cov	4.120086	1	archaeon RBG_16_50_20 (species)
NODE	2259	length	2853	cov	4.120086	2	archaeon RBG_16_50_20 (species)
NODE	2259	length	2853	cov	4.120086	3	archaeon RBG_16_50_20 (species)
NODE	2362	length	2811	cov	4.523585	1	
NODE	2362	length	2811	cov	4.523585	2	archaeon RBG_16_50_20 (species)
NODE	2362	length	2811	cov	4.523585	3	archaeon RBG_16_50_20 (species)
NODE	2389	length	2797	cov	2.944566	1	
NODE	2389	length	2797	cov	2.944566	2	unclassified Euryarchaeota (no rank)
NODE	2389	length	2797	cov	2.944566	3	
NODE	2389	length	2797	cov	2.944566	4	archaeon RBG_16_50_20 (species)
NODE	2394	length	2794	cov	3.386272	1	Thaumarchaeota (phylum)
NODE	2394	length	2794	cov	3.386272	2	Thaumarchaeota (phylum)
NODE	2394	length	2794	cov	3.386272	3	Thaumarchaeota (phylum)
NODE	2394	length	2794	cov	3.386272	4	
NODE	2430	length	2782	cov	3.348735	1	
NODE	2430	length	2782	cov	3.348735	2	
NODE	2430	length	2782	cov	3.348735	3	
NODE	2484	length	2762	cov	2.903953	1	Candidatus Bathyarchaeota (phylum)
NODE	2484	length	2762	cov	2.903953	2	Candidatus Bathyarchaeota (phylum)
NODE	2484	length	2762	cov	2.903953	3	
NODE	2543	length	2742	cov	4.248604	1	
NODE	2543	length	2742	cov	4.248604	2	
NODE	2543	length	2742	cov	4.248604	3	Thaumarchaeota (phylum)
NODE	2543	length	2742	cov	4.248604	4	
NODE	2562	length	2734	cov	2.385965	1	Chloroflexi (phylum)
NODE	2562	length	2734	cov	2.385965	2	Thaumarchaeota (phylum)
NODE	2562	length	2734	cov	2.385965	3	Thaumarchaeota (phylum)
NODE	2653	length	2705	cov	5.06717	1	Chloroflexi (phylum)
NODE	2653	length	2705	cov	5.06717	2	Thaumarchaeota (phylum)
NODE	2653	length	2705	cov	5.06717	3	Thaumarchaeota (phylum)
NODE	2653	length	2705	cov	5.06717	4	Thaumarchaeota (phylum)
NODE	2653	length	2705	cov	5.06717	5	
NODE	2653	length	2705	cov	5.06717	6	
NODE	2770	length	2672	cov	4.245319	1	archaeon RBG_16_50_20 (species)
NODE	2770	length	2672	cov	4.245319	2	
NODE	2770	length	2672	cov	4.245319	3	archaeon RBG_16_50_20 (species)
NODE	2770	length	2672	cov	4.245319	4	archaeon RBG_16_50_20 (species)
NODE	2770	length	2672	cov	4.245319	5	archaeon RBG_16_50_20 (species)
NODE	2801	length	2661	cov	2.930161	1	Candidatus Bathyarchaeota (phylum)
NODE	2801	length	2661	cov	2.930161	2	Candidatus Bathyarchaeota (phylum)
NODE	2801	length	2661	cov	2.930161	3	Candidatus Bathyarchaeota (phylum)
NODE	2841	length	2650	cov	4.264355	1	Candidatus Verstraetearchaeota (phylum)
NODE	2841	length	2650	cov	4.264355	2	
NODE	2841	length	2650	cov	4.264355	3	

NODE	2872	length	2642	cov	4.669115	1	archaeon RBG_16_50_20 (species)
NODE	2872	length	2642	cov	4.669115	2	Candidatus Bathyarchaeota (phylum)
NODE	2872	length	2642	cov	4.669115	3	Candidatus Bathyarchaeota (phylum)
NODE	2872	length	2642	cov	4.669115	4	
NODE	2950	length	2620	cov	5.047563	1	
NODE	2950	length	2620	cov	5.047563	2	
NODE	2950	length	2620	cov	5.047563	3	
NODE	2950	length	2620	cov	5.047563	4	Chloroflexi (phylum)
NODE	2989	length	2609	cov	3.646437	1	archaeon RBG_16_50_20 (species)
NODE	2989	length	2609	cov	3.646437	2	archaeon RBG_16_50_20 (species)
NODE	3099	length	2573	cov	4.223193	1	Candidatus Bathyarchaeota (phylum)
NODE	3099	length	2573	cov	4.223193	2	Candidatus Bathyarchaeota (phylum)
NODE	3099	length	2573	cov	4.223193	3	Bacteria candidate phyla (clade)
NODE	3099	length	2573	cov	4.223193	4	
NODE	3158	length	2560	cov	3.751697	1	archaeon RBG_16_50_20 (species)
NODE	3158	length	2560	cov	3.751697	2	
NODE	3158	length	2560	cov	3.751697	3	
NODE	3198	length	2549	cov	5.301925	1	Candidatus Bathyarchaeota (phylum)
NODE	3198	length	2549	cov	5.301925	2	
NODE	3199	length	2549	cov	4.013633	1	archaeon RBG_16_50_20 (species)
NODE	3199	length	2549	cov	4.013633	2	Thaumarchaeota (phylum)
NODE	3199	length	2549	cov	4.013633	3	
NODE	3277	length	2528	cov	4.435503	1	
NODE	3277	length	2528	cov	4.435503	2	Crenarchaeota (phylum)
NODE	3277	length	2528	cov	4.435503	3	Thaumarchaeota (phylum)
NODE	3313	length	2520	cov	4.608519	1	
NODE	3313	length	2520	cov	4.608519	2	archaeon RBG_16_50_20 (species)
NODE	3313	length	2520	cov	4.608519	3	archaeon RBG_16_50_20 (species)
NODE	3313	length	2520	cov	4.608519	4	archaeon RBG_16_50_20 (species)
NODE	3387	length	2502	cov	5.70045	1	bacterium (species)
NODE	3387	length	2502	cov	5.70045	2	
NODE	3393	length	2501	cov	4.833197	1	archaeon RBG_16_50_20 (species)
NODE	3393	length	2501	cov	4.833197	2	archaeon RBG_16_50_20 (species)
NODE	3393	length	2501	cov	4.833197	3	archaeon RBG_16_50_20 (species)
NODE	3401	length	2497	cov	4.453317	1	
NODE	3401	length	2497	cov	4.453317	2	
NODE	3401	length	2497	cov	4.453317	3	archaeon RBG_16_50_20 (species)
NODE	3442	length	2488	cov	3.328401	1	
NODE	3442	length	2488	cov	3.328401	2	
NODE	3442	length	2488	cov	3.328401	3	archaeon RBG_16_50_20 (species)
NODE	3442	length	2488	cov	3.328401	4	
NODE	3453	length	2484	cov	7.512557	1	
NODE	3453	length	2484	cov	7.512557	2	unclassified Euryarchaeota (no rank)
NODE	3453	length	2484	cov	7.512557	3	Candidatus Bathyarchaeota (phylum)
NODE	3504	length	2471	cov	5.015315	1	Thaumarchaeota (phylum)
NODE	3504	length	2471	cov	5.015315	2	Thaumarchaeota (phylum)
NODE	3504	length	2471	cov	5.015315	3	
NODE	3504	length	2471	cov	5.015315	4	Candidatus Bathyarchaeota (phylum)
NODE	3504	length	2471	cov	5.015315	5	
NODE	3512	length	2469	cov	2.983016	1	Chloroflexi (phylum)
NODE	3512	length	2469	cov	2.983016	2	
NODE	3512	length	2469	cov	2.983016	3	
NODE	3540	length	2460	cov	6.729314	1	Thaumarchaeota (phylum)
NODE	3540	length	2460	cov	6.729314	2	
NODE	3618	length	2443	cov	3.41541	1	
NODE	3618	length	2443	cov	3.41541	2	archaeon RBG_16_50_20 (species)

NODE	3618	length	2443	cov	3.41541	3	archaeon RBG_16_50_20 (species)
NODE	3668	length	2433	cov	3.6291	1	archaeon RBG_16_50_20 (species)
NODE	3668	length	2433	cov	3.6291	2	archaeon RBG_16_50_20 (species)
NODE	3668	length	2433	cov	3.6291	3	
NODE	3668	length	2433	cov	3.6291	4	
NODE	3668	length	2433	cov	3.6291	5	
NODE	3717	length	2425	cov	5.055274	1	archaeon RBG_16_50_20 (species)
NODE	3717	length	2425	cov	5.055274	2	archaeon RBG_16_50_20 (species)
NODE	3717	length	2425	cov	5.055274	3	archaeon RBG_16_50_20 (species)
NODE	3759	length	2417	cov	4.257409	1	
NODE	3759	length	2417	cov	4.257409	2	archaeon RBG_16_50_20 (species)
NODE	3759	length	2417	cov	4.257409	3	archaeon RBG_16_50_20 (species)
NODE	3759	length	2417	cov	4.257409	4	archaeon RBG_16_50_20 (species)
NODE	3759	length	2417	cov	4.257409	5	
NODE	3774	length	2412	cov	5.020789	1	
NODE	3774	length	2412	cov	5.020789	2	
NODE	3774	length	2412	cov	5.020789	3	
NODE	3774	length	2412	cov	5.020789	4	Thaumarchaeota (phylum)
NODE	3774	length	2412	cov	5.020789	5	
NODE	3816	length	2403	cov	6.332624	1	archaeon RBG_16_50_20 (species)
NODE	3816	length	2403	cov	6.332624	2	archaeon RBG_16_50_20 (species)
NODE	3816	length	2403	cov	6.332624	3	archaeon RBG_16_50_20 (species)
NODE	3886	length	2386	cov	4.974689	1	Chloroflexi (phylum)
NODE	3886	length	2386	cov	4.974689	2	
NODE	3886	length	2386	cov	4.974689	3	
NODE	3914	length	2379	cov	3.943632	1	
NODE	3914	length	2379	cov	3.943632	2	Candidatus Bathyarchaeota (phylum)
NODE	4003	length	2362	cov	3.913741	1	archaeon RBG_16_50_20 (species)
NODE	4003	length	2362	cov	3.913741	2	archaeon RBG_16_50_20 (species)
NODE	4003	length	2362	cov	3.913741	3	archaeon RBG_16_50_20 (species)
NODE	4016	length	2360	cov	3.589154	1	archaeon RBG_16_50_20 (species)
NODE	4016	length	2360	cov	3.589154	2	
NODE	4022	length	2358	cov	4.568823	1	archaeon RBG_16_50_20 (species)
NODE	4064	length	2349	cov	5.037053	1	
NODE	4064	length	2349	cov	5.037053	2	
NODE	4064	length	2349	cov	5.037053	3	
NODE	4081	length	2346	cov	4.361414	1	
NODE	4081	length	2346	cov	4.361414	2	archaeon RBG_16_50_20 (species)
NODE	4081	length	2346	cov	4.361414	3	Thaumarchaeota (phylum)
NODE	4081	length	2346	cov	4.361414	4	
NODE	4086	length	2346	cov	3.49498	1	
NODE	4086	length	2346	cov	3.49498	2	archaeon RBG_16_50_20 (species)
NODE	4120	length	2339	cov	5.268827	1	archaeon RBG_16_50_20 (species)
NODE	4120	length	2339	cov	5.268827	2	archaeon RBG_16_50_20 (species)
NODE	4136	length	2337	cov	3.123576	1	Candidatus Bathyarchaeota (phylum)
NODE	4136	length	2337	cov	3.123576	2	
NODE	4210	length	2323	cov	4.491182	1	Verrucomicrobia (phylum)
NODE	4210	length	2323	cov	4.491182	2	Thaumarchaeota (phylum)
NODE	4210	length	2323	cov	4.491182	3	
NODE	4238	length	2318	cov	3.406098	1	Thaumarchaeota (phylum)
NODE	4238	length	2318	cov	3.406098	2	bacterium (species)
NODE	4253	length	2315	cov	5.565044	1	
NODE	4253	length	2315	cov	5.565044	2	archaeon (species)
NODE	4292	length	2306	cov	4.712572	1	
NODE	4292	length	2306	cov	4.712572	2	
NODE	4292	length	2306	cov	4.712572	3	

NODE	4312	length	2303	cov	3.667705	1	
NODE	4312	length	2303	cov	3.667705	2	archaeon RBG_16_50_20 (species)
NODE	4312	length	2303	cov	3.667705	3	archaeon RBG_16_50_20 (species)
NODE	4313	length	2302	cov	5.067201	1	archaeon RBG_16_50_20 (species)
NODE	4313	length	2302	cov	5.067201	2	archaeon RBG_16_50_20 (species)
NODE	4323	length	2300	cov	5.616927	1	unclassified Euryarchaeota (no rank)
NODE	4323	length	2300	cov	5.616927	2	archaeon (species)
NODE	4323	length	2300	cov	5.616927	3	archaeon (species)
NODE	4582	length	2257	cov	4.725704	1	bacterium (species)
NODE	4582	length	2257	cov	4.725704	2	Candidatus Bathyarchaeota (phylum)
NODE	4582	length	2257	cov	4.725704	3	
NODE	4608	length	2253	cov	4.758872	1	Candidatus Bathyarchaeota (phylum)
NODE	4608	length	2253	cov	4.758872	2	
NODE	4608	length	2253	cov	4.758872	3	Thaumarchaeota (phylum)
NODE	4674	length	2241	cov	3.5	1	
NODE	4674	length	2241	cov	3.5	2	archaeon RBG_16_50_20 (species)
NODE	4674	length	2241	cov	3.5	3	
NODE	4851	length	2210	cov	7.02181	1	
NODE	4851	length	2210	cov	7.02181	2	
NODE	4851	length	2210	cov	7.02181	3	
NODE	4916	length	2203	cov	3.991155	1	unclassified Euryarchaeota (no rank)
NODE	4917	length	2203	cov	3.816108	1	archaeon RBG_16_50_20 (species)
NODE	4917	length	2203	cov	3.816108	2	archaeon RBG_16_50_20 (species)
NODE	4917	length	2203	cov	3.816108	3	archaeon RBG_16_50_20 (species)
NODE	5022	length	2184	cov	3.963833	1	
NODE	5022	length	2184	cov	3.963833	2	Candidatus Bathyarchaeota (phylum)
NODE	5022	length	2184	cov	3.963833	3	archaeon RBG_16_50_20 (species)
NODE	5029	length	2183	cov	2.744831	1	
NODE	5029	length	2183	cov	2.744831	2	
NODE	5046	length	2180	cov	3.275294	1	Candidatus Bathyarchaeota (phylum)
NODE	5046	length	2180	cov	3.275294	2	Candidatus Bathyarchaeota (phylum)
NODE	5046	length	2180	cov	3.275294	3	Chloroflexi (phylum)
NODE	5107	length	2171	cov	2.729679	1	Candidatus Bathyarchaeota (phylum)
NODE	5144	length	2166	cov	2.6054	1	Thaumarchaeota (phylum)
NODE	5144	length	2166	cov	2.6054	2	Thaumarchaeota (phylum)
NODE	5213	length	2156	cov	2.783436	1	archaeon RBG_16_50_20 (species)
NODE	5238	length	2152	cov	4.429661	1	Candidatus Bathyarchaeota (phylum)
NODE	5238	length	2152	cov	4.429661	2	
NODE	5238	length	2152	cov	4.429661	3	Chloroflexi (phylum)
NODE	5243	length	2151	cov	5.093034	1	Zetaproteobacteria (class)
NODE	5243	length	2151	cov	5.093034	2	archaeon RBG_16_50_20 (species)
NODE	5243	length	2151	cov	5.093034	3	archaeon RBG_16_50_20 (species)
NODE	5253	length	2150	cov	6.447733	1	
NODE	5253	length	2150	cov	6.447733	2	Crenarchaeota (phylum)
NODE	5253	length	2150	cov	6.447733	3	Candidatus Bathyarchaeota (phylum)
NODE	5269	length	2148	cov	3.07119	1	Candidatus Bathyarchaeota (phylum)
NODE	5269	length	2148	cov	3.07119	2	Candidatus Bathyarchaeota (phylum)
NODE	5280	length	2146	cov	3.458154	1	
NODE	5280	length	2146	cov	3.458154	2	
NODE	5286	length	2145	cov	4.058373	1	
NODE	5286	length	2145	cov	4.058373	2	archaeon RBG_16_50_20 (species)
NODE	5298	length	2144	cov	3.775491	1	archaeon RBG_16_50_20 (species)
NODE	5298	length	2144	cov	3.775491	2	
NODE	5321	length	2140	cov	4.752038	1	Thaumarchaeota (phylum)
NODE	5321	length	2140	cov	4.752038	2	
NODE	5321	length	2140	cov	4.752038	3	archaeon RBG_16_50_20 (species)

NODE	5360	length	2135	cov	2.903846	1	Crenarchaeota (phylum)
NODE	5360	length	2135	cov	2.903846	2	
NODE	5365	length	2134	cov	4.440596	1	
NODE	5365	length	2134	cov	4.440596	2	Candidatus Bathyarchaeota (phylum)
NODE	5365	length	2134	cov	4.440596	3	Candidatus Bathyarchaeota (phylum)
NODE	5365	length	2134	cov	4.440596	4	Candidatus Bathyarchaeota (phylum)
NODE	5440	length	2124	cov	3.798453	1	
NODE	5440	length	2124	cov	3.798453	2	
NODE	5440	length	2124	cov	3.798453	3	
NODE	5448	length	2123	cov	4.516441	1	Candidatus Bathyarchaeota (phylum)
NODE	5465	length	2121	cov	4.933204	1	archaeon RBG_16_50_20 (species)
NODE	5474	length	2120	cov	4.355448	1	archaeon RBG_16_50_20 (species)
NODE	5474	length	2120	cov	4.355448	2	archaeon RBG_16_50_20 (species)
NODE	5474	length	2120	cov	4.355448	3	Candidatus Bathyarchaeota (phylum)
NODE	5491	length	2117	cov	5.390398	1	
NODE	5491	length	2117	cov	5.390398	2	
NODE	5598	length	2103	cov	3.90625	1	
NODE	5598	length	2103	cov	3.90625	2	
NODE	5598	length	2103	cov	3.90625	3	
NODE	5598	length	2103	cov	3.90625	4	
NODE	5631	length	2098	cov	4.295644	1	archaeon RBG_16_50_20 (species)
NODE	5631	length	2098	cov	4.295644	2	
NODE	5631	length	2098	cov	4.295644	3	Chloroflexi (phylum)
NODE	5675	length	2092	cov	5.316642	1	Candidatus Bathyarchaeota (phylum)
NODE	5675	length	2092	cov	5.316642	2	Candidatus Bathyarchaeota (phylum)
NODE	5790	length	2078	cov	3.772121	1	archaeon RBG_16_50_20 (species)
NODE	5790	length	2078	cov	3.772121	2	archaeon RBG_16_50_20 (species)
NODE	5845	length	2071	cov	4.797123	1	Thaumarchaeota (phylum)
NODE	5845	length	2071	cov	4.797123	2	archaeon RBG_16_50_20 (species)
NODE	5870	length	2068	cov	4.028813	1	archaeon RBG_16_50_20 (species)
NODE	5870	length	2068	cov	4.028813	2	
NODE	5887	length	2066	cov	3.544505	1	archaeon RBG_16_50_20 (species)
NODE	5887	length	2066	cov	3.544505	2	archaeon RBG_16_50_20 (species)
NODE	5901	length	2064	cov	4.273768	1	Candidatus Bathyarchaeota (phylum)
NODE	5901	length	2064	cov	4.273768	2	archaeon (species)
NODE	5901	length	2064	cov	4.273768	3	Candidatus Bathyarchaeota (phylum)
NODE	5906	length	2064	cov	3.524639	1	
NODE	5906	length	2064	cov	3.524639	2	
NODE	5906	length	2064	cov	3.524639	3	
NODE	5906	length	2064	cov	3.524639	4	Candidatus Bathyarchaeota (phylum)
NODE	5907	length	2064	cov	3.422598	1	Thaumarchaeota (phylum)
NODE	5907	length	2064	cov	3.422598	2	
NODE	5954	length	2059	cov	5.133234	1	archaeon HR01 (species)
NODE	5954	length	2059	cov	5.133234	2	Thaumarchaeota (phylum)
NODE	5954	length	2059	cov	5.133234	3	
NODE	5969	length	2058	cov	2.648527	1	archaeon RBG_16_50_20 (species)
NODE	5969	length	2058	cov	2.648527	2	archaeon RBG_16_50_20 (species)
NODE	5969	length	2058	cov	2.648527	3	archaeon RBG_16_50_20 (species)
NODE	5976	length	2057	cov	3.237263	1	archaeon RBG_16_50_20 (species)
NODE	5976	length	2057	cov	3.237263	2	archaeon RBG_16_50_20 (species)
NODE	5983	length	2056	cov	4.137931	1	archaeon RBG_16_50_20 (species)
NODE	5983	length	2056	cov	4.137931	2	archaeon RBG_16_50_20 (species)
NODE	5983	length	2056	cov	4.137931	3	archaeon RBG_16_50_20 (species)
NODE	6017	length	2050	cov	3.745865	1	
NODE	6017	length	2050	cov	3.745865	2	
NODE	6017	length	2050	cov	3.745865	3	

NODE	6048	length	2044	cov	4.318753	1	archaeon RBG_16_50_20 (species)
NODE	6048	length	2044	cov	4.318753	2	archaeon RBG_16_50_20 (species)
NODE	6097	length	2038	cov	3.17297	1	archaeon RBG_16_50_20 (species)
NODE	6097	length	2038	cov	3.17297	2	archaeon RBG_16_50_20 (species)
NODE	6097	length	2038	cov	3.17297	3	
NODE	6097	length	2038	cov	3.17297	4	archaeon RBG_16_50_20 (species)
NODE	6146	length	2032	cov	5.948407	1	
NODE	6146	length	2032	cov	5.948407	2	
NODE	6155	length	2031	cov	4.084514	1	archaeon RBG_16_50_20 (species)
NODE	6155	length	2031	cov	4.084514	2	
NODE	6286	length	2015	cov	3.207143	1	archaeon RBG_16_50_20 (species)
NODE	6286	length	2015	cov	3.207143	2	archaeon RBG_16_50_20 (species)
NODE	6286	length	2015	cov	3.207143	3	archaeon RBG_16_50_20 (species)
NODE	6286	length	2015	cov	3.207143	4	
NODE	6340	length	2010	cov	3.810742	1	archaeon RBG_16_50_20 (species)
NODE	6340	length	2010	cov	3.810742	2	archaeon RBG_16_50_20 (species)
NODE	6445	length	2000	cov	3.121337	1	Candidatus Bathyarchaeota (phylum)
NODE	6445	length	2000	cov	3.121337	2	
NODE	6445	length	2000	cov	3.121337	3	
NODE	6487	length	1996	cov	4.054096	1	archaeon RBG_16_50_20 (species)
NODE	6487	length	1996	cov	4.054096	2	archaeon RBG_16_50_20 (species)
NODE	6487	length	1996	cov	4.054096	3	
NODE	6487	length	1996	cov	4.054096	4	archaeon RBG_16_50_20 (species)
NODE	6523	length	1993	cov	4.430857	1	archaeon RBG_16_50_20 (species)
NODE	6523	length	1993	cov	4.430857	2	archaeon RBG_16_50_20 (species)
NODE	6523	length	1993	cov	4.430857	3	archaeon RBG_16_50_20 (species)
NODE	6608	length	1984	cov	3.011405	1	delta/epsilon subdivisions (subphylum)
NODE	6608	length	1984	cov	3.011405	2	archaeon (species)
NODE	6608	length	1984	cov	3.011405	3	unclassified Euryarchaeota (no rank)
NODE	6612	length	1983	cov	5.379149	1	
NODE	6612	length	1983	cov	5.379149	2	unclassified Euryarchaeota (no rank)
NODE	6612	length	1983	cov	5.379149	3	
NODE	6629	length	1981	cov	5.358775	1	
NODE	6629	length	1981	cov	5.358775	2	
NODE	6629	length	1981	cov	5.358775	3	
NODE	6634	length	1981	cov	3.95379	1	Thaumarchaeota (phylum)
NODE	6634	length	1981	cov	3.95379	2	Candidatus Abyssubacteria (phylum)
NODE	6634	length	1981	cov	3.95379	3	Candidatus Bathyarchaeota (phylum)
NODE	6634	length	1981	cov	3.95379	4	bacterium (species)
NODE	6694	length	1976	cov	3.522644	1	unclassified Euryarchaeota (no rank)
NODE	6694	length	1976	cov	3.522644	2	archaeon (species)
NODE	6694	length	1976	cov	3.522644	3	Candidatus Bathyarchaeota (phylum)
NODE	6708	length	1975	cov	3.402083	1	archaeon RBG_16_50_20 (species)
NODE	6708	length	1975	cov	3.402083	2	
NODE	6748	length	1970	cov	4.617232	1	
NODE	6748	length	1970	cov	4.617232	2	
NODE	6750	length	1970	cov	4.029243	1	
NODE	6750	length	1970	cov	4.029243	2	
NODE	6768	length	1968	cov	4.989545	1	archaeon RBG_16_50_20 (species)
NODE	6787	length	1966	cov	5.421769	1	
NODE	6787	length	1966	cov	5.421769	2	
NODE	6787	length	1966	cov	5.421769	3	Bacteria candidate phyla (clade)
NODE	6792	length	1966	cov	4.210361	1	
NODE	6792	length	1966	cov	4.210361	2	
NODE	6807	length	1964	cov	2.953903	1	
NODE	6807	length	1964	cov	2.953903	2	

NODE	6807	length	1964	cov	2.953903	3	
NODE	6816	length	1962	cov	4.424227	1	
NODE	6816	length	1962	cov	4.424227	2	Chloroflexi (phylum)
NODE	6816	length	1962	cov	4.424227	3	
NODE	7267	length	1917	cov	4.044039	1	Candidatus Marsarchaeota (phylum)
NODE	7267	length	1917	cov	4.044039	2	
NODE	7267	length	1917	cov	4.044039	3	
NODE	7287	length	1915	cov	2.637097	1	bacterium (species)
NODE	7287	length	1915	cov	2.637097	2	archaeon RBG_16_50_20 (species)
NODE	7328	length	1911	cov	4.087823	1	archaeon RBG_16_50_20 (species)
NODE	7328	length	1911	cov	4.087823	2	archaeon RBG_16_50_20 (species)
NODE	7353	length	1909	cov	3.914779	1	archaeon RBG_16_50_20 (species)
NODE	7353	length	1909	cov	3.914779	2	
NODE	7364	length	1908	cov	3.666487	1	Candidatus Marsarchaeota (phylum)
NODE	7364	length	1908	cov	3.666487	2	Candidatus Bathyarchaeota (phylum)
NODE	7364	length	1908	cov	3.666487	3	
NODE	7364	length	1908	cov	3.666487	4	
NODE	7392	length	1905	cov	4.542703	1	
NODE	7392	length	1905	cov	4.542703	2	
NODE	7395	length	1905	cov	3.153514	1	
NODE	7444	length	1900	cov	4.502981	1	
NODE	7444	length	1900	cov	4.502981	2	archaeon RBG_16_50_20 (species)
NODE	7444	length	1900	cov	4.502981	3	
NODE	7446	length	1900	cov	4.21084	1	
NODE	7446	length	1900	cov	4.21084	2	
NODE	7446	length	1900	cov	4.21084	3	
NODE	7456	length	1900	cov	2.971274	1	
NODE	7456	length	1900	cov	2.971274	2	archaeon RBG_16_50_20 (species)
NODE	7484	length	1897	cov	4.314332	1	Thaumarchaeota (phylum)
NODE	7484	length	1897	cov	4.314332	2	Thaumarchaeota (phylum)
NODE	7484	length	1897	cov	4.314332	3	
NODE	7487	length	1897	cov	3.918024	1	archaeon RBG_16_50_20 (species)
NODE	7487	length	1897	cov	3.918024	2	archaeon RBG_16_50_20 (species)
NODE	7487	length	1897	cov	3.918024	3	
NODE	7506	length	1895	cov	5.610326	1	
NODE	7506	length	1895	cov	5.610326	2	
NODE	7506	length	1895	cov	5.610326	3	archaeon RBG_16_50_20 (species)
NODE	7510	length	1895	cov	4.380978	1	Thaumarchaeota (phylum)
NODE	7513	length	1895	cov	4.041304	1	archaeon RBG_16_50_20 (species)
NODE	7513	length	1895	cov	4.041304	2	archaeon RBG_16_50_20 (species)
NODE	7513	length	1895	cov	4.041304	3	
NODE	7534	length	1893	cov	3.982046	1	
NODE	7534	length	1893	cov	3.982046	2	
NODE	7543	length	1892	cov	3.097986	1	archaeon RBG_16_50_20 (species)
NODE	7543	length	1892	cov	3.097986	2	archaeon RBG_16_50_20 (species)
NODE	7543	length	1892	cov	3.097986	3	
NODE	7577	length	1889	cov	2.941112	1	
NODE	7577	length	1889	cov	2.941112	2	
NODE	7577	length	1889	cov	2.941112	3	Thaumarchaeota (phylum)
NODE	7696	length	1877	cov	4.879254	1	
NODE	7696	length	1877	cov	4.879254	2	unclassified Nitrospirae (no rank)
NODE	7696	length	1877	cov	4.879254	3	Candidatus Bathyarchaeota (phylum)
NODE	7809	length	1868	cov	5.517926	1	
NODE	7809	length	1868	cov	5.517926	2	
NODE	7854	length	1865	cov	2.910497	1	archaeon RBG_16_50_20 (species)
NODE	7854	length	1865	cov	2.910497	2	

NODE	7854	length	1865	cov	2.910497	3	
NODE	7854	length	1865	cov	2.910497	4	Candidatus Altiarchaeota (phylum)
NODE	7854	length	1865	cov	2.910497	5	Chloroflexi (phylum)
NODE	7864	length	1864	cov	3.621891	1	
NODE	7864	length	1864	cov	3.621891	2	archaeon RBG_16_50_20 (species)
NODE	7864	length	1864	cov	3.621891	3	
NODE	7981	length	1854	cov	3.466926	1	
NODE	7981	length	1854	cov	3.466926	2	archaeon RBG_16_50_20 (species)
NODE	7981	length	1854	cov	3.466926	3	Candidatus Bathyarchaeota (phylum)
NODE	7981	length	1854	cov	3.466926	4	
NODE	8009	length	1851	cov	3.291203	1	Thermococci (class)
NODE	8009	length	1851	cov	3.291203	2	
NODE	8072	length	1847	cov	4.603795	1	archaeon RBG_16_50_20 (species)
NODE	8155	length	1840	cov	4.792717	1	
NODE	8155	length	1840	cov	4.792717	2	Thaumarchaeota (phylum)
NODE	8157	length	1840	cov	4.396639	1	archaeon RBG_16_50_20 (species)
NODE	8157	length	1840	cov	4.396639	2	archaeon RBG_16_50_20 (species)
NODE	8157	length	1840	cov	4.396639	3	archaeon RBG_16_50_20 (species)
NODE	8204	length	1836	cov	6.998316	1	archaeon RBG_16_50_20 (species)
NODE	8204	length	1836	cov	6.998316	2	
NODE	8231	length	1834	cov	6.459809	1	
NODE	8231	length	1834	cov	6.459809	2	
NODE	8231	length	1834	cov	6.459809	3	Crenarchaeota (phylum)
NODE	8245	length	1833	cov	4.81946	1	
NODE	8245	length	1833	cov	4.81946	2	
NODE	8245	length	1833	cov	4.81946	3	
NODE	8275	length	1831	cov	4.074887	1	
NODE	8275	length	1831	cov	4.074887	2	
NODE	8350	length	1824	cov	4.161108	1	archaeon RBG_16_50_20 (species)
NODE	8350	length	1824	cov	4.161108	2	archaeon RBG_16_50_20 (species)
NODE	8350	length	1824	cov	4.161108	3	archaeon RBG_16_50_20 (species)
NODE	8372	length	1822	cov	4.096208	1	archaeon RBG_16_50_20 (species)
NODE	8372	length	1822	cov	4.096208	2	archaeon RBG_16_50_20 (species)
NODE	8372	length	1822	cov	4.096208	3	archaeon RBG_16_50_20 (species)
NODE	8372	length	1822	cov	4.096208	4	archaeon RBG_16_50_20 (species)
NODE	8499	length	1814	cov	4.101762	1	
NODE	8499	length	1814	cov	4.101762	2	
NODE	8499	length	1814	cov	4.101762	3	
NODE	8831	length	1791	cov	4.210253	1	
NODE	8831	length	1791	cov	4.210253	2	archaeon RBG_16_50_20 (species)
NODE	8831	length	1791	cov	4.210253	3	Candidatus Bathyarchaeota (phylum)
NODE	8831	length	1791	cov	4.210253	4	
NODE	8874	length	1787	cov	4.840069	1	archaeon RBG_16_50_20 (species)
NODE	8955	length	1781	cov	4.200463	1	
NODE	8955	length	1781	cov	4.200463	2	Thaumarchaeota (phylum)
NODE	8966	length	1780	cov	5.449275	1	archaeon RBG_16_50_20 (species)
NODE	8966	length	1780	cov	5.449275	2	archaeon RBG_16_50_20 (species)
NODE	8995	length	1778	cov	5.3935	1	Thaumarchaeota (phylum)
NODE	8995	length	1778	cov	5.3935	2	
NODE	8995	length	1778	cov	5.3935	3	
NODE	8997	length	1778	cov	4.432385	1	
NODE	8997	length	1778	cov	4.432385	2	
NODE	8997	length	1778	cov	4.432385	3	
NODE	8997	length	1778	cov	4.432385	4	Armatimonadetes (phylum)
NODE	9047	length	1775	cov	2.688372	1	
NODE	9100	length	1772	cov	3.075713	1	archaeon RBG_16_50_20 (species)

NODE	9100	length	1772	cov	3.075713	2	archaeon RBG_16_50_20 (species)
NODE	9100	length	1772	cov	3.075713	3	archaeon RBG_16_50_20 (species)
NODE	9185	length	1765	cov	4.860234	1	archaeon RBG_16_50_20 (species)
NODE	9185	length	1765	cov	4.860234	2	Candidatus Verstraetearchaeota (phylum)
NODE	9185	length	1765	cov	4.860234	3	Candidatus Bathyarchaeota (phylum)
NODE	9257	length	1761	cov	3.524619	1	
NODE	9257	length	1761	cov	3.524619	2	
NODE	9257	length	1761	cov	3.524619	3	Candidatus Bathyarchaeota (phylum)
NODE	9272	length	1760	cov	3.093842	1	
NODE	9272	length	1760	cov	3.093842	2	
NODE	9382	length	1753	cov	4.667845	1	Thaumarchaeota (phylum)
NODE	9405	length	1752	cov	3.085445	1	archaeon RBG_16_50_20 (species)
NODE	9492	length	1746	cov	3.729154	1	
NODE	9492	length	1746	cov	3.729154	2	Chloroflexi (phylum)
NODE	9616	length	1737	cov	3.520214	1	archaeon RBG_16_50_20 (species)
NODE	9616	length	1737	cov	3.520214	2	archaeon RBG_16_50_20 (species)
NODE	9706	length	1730	cov	4.395821	1	
NODE	9706	length	1730	cov	4.395821	2	Chloroflexi (phylum)
NODE	9749	length	1727	cov	4.363636	1	
NODE	9749	length	1727	cov	4.363636	2	
NODE	9783	length	1725	cov	3.968862	1	
NODE	9783	length	1725	cov	3.968862	2	
NODE	9811	length	1723	cov	4.083333	1	
NODE	9811	length	1723	cov	4.083333	2	
NODE	9811	length	1723	cov	4.083333	3	
NODE	9811	length	1723	cov	4.083333	4	
NODE	9813	length	1723	cov	3.908273	1	archaeon RBG_16_50_20 (species)
NODE	9813	length	1723	cov	3.908273	2	archaeon RBG_16_50_20 (species)
NODE	9896	length	1718	cov	2.555021	1	Candidatus Bathyarchaeota (phylum)
NODE	10104	length	1706	cov	3.377347	1	
NODE	10104	length	1706	cov	3.377347	2	
NODE	10141	length	1704	cov	3.349909	1	
NODE	10141	length	1704	cov	3.349909	2	archaeon RBG_16_50_20 (species)
NODE	10222	length	1698	cov	2.869142	1	archaeon RBG_16_50_20 (species)
NODE	10222	length	1698	cov	2.869142	2	archaeon RBG_16_50_20 (species)
NODE	10222	length	1698	cov	2.869142	3	archaeon RBG_16_50_20 (species)
NODE	10266	length	1695	cov	3.781707	1	Candidatus Bathyarchaeota (phylum)
NODE	10266	length	1695	cov	3.781707	2	
NODE	10307	length	1693	cov	3.476801	1	archaeon RBG_16_50_20 (species)
NODE	10307	length	1693	cov	3.476801	2	archaeon RBG_16_50_20 (species)
NODE	10354	length	1690	cov	4.185321	1	Candidatus Bathyarchaeota (phylum)
NODE	10354	length	1690	cov	4.185321	2	
NODE	10354	length	1690	cov	4.185321	3	
NODE	10375	length	1689	cov	3.722766	1	Candidatus Bathyarchaeota (phylum)
NODE	10375	length	1689	cov	3.722766	2	
NODE	10375	length	1689	cov	3.722766	3	
NODE	10375	length	1689	cov	3.722766	4	
NODE	10460	length	1685	cov	2.667485	1	archaeon RBG_16_50_20 (species)
NODE	10460	length	1685	cov	2.667485	2	Candidatus Marsarchaeota (phylum)
NODE	10521	length	1681	cov	4.155597	1	Thaumarchaeota (phylum)
NODE	10521	length	1681	cov	4.155597	2	bacterium (species)
NODE	10586	length	1677	cov	3.798397	1	archaeon RBG_16_50_20 (species)
NODE	10586	length	1677	cov	3.798397	2	archaeon RBG_16_50_20 (species)
NODE	10586	length	1677	cov	3.798397	3	
NODE	10601	length	1676	cov	4.188155	1	
NODE	10601	length	1676	cov	4.188155	2	Candidatus Bathyarchaeota (phylum)

NODE	10601	length	1676	cov	4.188155	3	
NODE	10709	length	1671	cov	2.792698	1	
NODE	10826	length	1664	cov	3.366687	1	
NODE	10826	length	1664	cov	3.366687	2	Chloroflexi (phylum)
NODE	10884	length	1660	cov	4.183178	1	Thaumarchaeota (phylum)
NODE	10884	length	1660	cov	4.183178	2	unclassified Nitrospirae (no rank)
NODE	10886	length	1660	cov	3.306542	1	archaeon RBG_16_50_20 (species)
NODE	10886	length	1660	cov	3.306542	2	
NODE	10917	length	1658	cov	4.202745	1	
NODE	10917	length	1658	cov	4.202745	2	
NODE	10917	length	1658	cov	4.202745	3	
NODE	10995	length	1654	cov	3.679174	1	archaeon RBG_16_50_20 (species)
NODE	10995	length	1654	cov	3.679174	2	
NODE	11008	length	1653	cov	5.54005	1	archaeon RBG_16_50_20 (species)
NODE	11008	length	1653	cov	5.54005	2	archaeon RBG_16_50_20 (species)
NODE	11137	length	1647	cov	3.405151	1	
NODE	11137	length	1647	cov	3.405151	2	archaeon RBG_16_50_20 (species)
NODE	11153	length	1646	cov	4.288498	1	Candidatus Bathyarchaeota (phylum)
NODE	11153	length	1646	cov	4.288498	2	
NODE	11153	length	1646	cov	4.288498	3	
NODE	11193	length	1644	cov	3.753304	1	unclassified Euryarchaeota (no rank)
NODE	11193	length	1644	cov	3.753304	2	Thaumarchaeota (phylum)
NODE	11254	length	1641	cov	4.368852	1	Thaumarchaeota (phylum)
NODE	11254	length	1641	cov	4.368852	2	Thaumarchaeota (phylum)
NODE	11254	length	1641	cov	4.368852	3	
NODE	11263	length	1641	cov	3.141866	1	archaeon RBG_16_50_20 (species)
NODE	11263	length	1641	cov	3.141866	2	
NODE	11583	length	1624	cov	2.865519	1	
NODE	11583	length	1624	cov	2.865519	2	archaeon RBG_16_50_20 (species)
NODE	11583	length	1624	cov	2.865519	3	archaeon RBG_16_50_20 (species)
NODE	11583	length	1624	cov	2.865519	4	
NODE	11714	length	1617	cov	3.829065	1	archaeon RBG_16_50_20 (species)
NODE	11714	length	1617	cov	3.829065	2	archaeon RBG_16_50_20 (species)
NODE	11802	length	1613	cov	4.738768	1	archaeon RBG_16_50_20 (species)
NODE	11802	length	1613	cov	4.738768	2	archaeon RBG_16_50_20 (species)
NODE	11802	length	1613	cov	4.738768	3	archaeon RBG_16_50_20 (species)
NODE	11803	length	1613	cov	4.709884	1	
NODE	11803	length	1613	cov	4.709884	2	Candidatus Bathyarchaeota (phylum)
NODE	11812	length	1613	cov	3.092426	1	archaeon RBG_16_50_20 (species)
NODE	11923	length	1607	cov	5.901418	1	archaeon RBG_16_50_20 (species)
NODE	11923	length	1607	cov	5.901418	2	archaeon RBG_16_50_20 (species)
NODE	11931	length	1607	cov	4.009021	1	
NODE	11931	length	1607	cov	4.009021	2	Candidatus Bathyarchaeota (phylum)
NODE	11941	length	1607	cov	2.746134	1	
NODE	11941	length	1607	cov	2.746134	2	Candidatus Bathyarchaeota (phylum)
NODE	11941	length	1607	cov	2.746134	3	
NODE	11962	length	1606	cov	3.469375	1	Chloroflexi (phylum)
NODE	11962	length	1606	cov	3.469375	2	
NODE	12018	length	1603	cov	4.26615	1	archaeon RBG_16_50_20 (species)
NODE	12018	length	1603	cov	4.26615	2	archaeon RBG_16_50_20 (species)
NODE	12018	length	1603	cov	4.26615	3	archaeon RBG_16_50_20 (species)
NODE	12140	length	1598	cov	3.175632	1	
NODE	12140	length	1598	cov	3.175632	2	
NODE	12140	length	1598	cov	3.175632	3	
NODE	12299	length	1590	cov	1.957003	1	
NODE	12299	length	1590	cov	1.957003	2	Candidatus Bathyarchaeota (phylum)

NODE	12618	length	1574	cov	5.038183	1	unclassified Euryarchaeota (no rank)
NODE	12618	length	1574	cov	5.038183	2	unclassified Euryarchaeota (no rank)
NODE	12618	length	1574	cov	5.038183	3	
NODE	12640	length	1573	cov	4.22859	1	
NODE	12640	length	1573	cov	4.22859	2	Candidatus Bathyarchaeota (phylum)
NODE	12640	length	1573	cov	4.22859	3	
NODE	12786	length	1566	cov	4.484447	1	
NODE	12786	length	1566	cov	4.484447	2	
NODE	12895	length	1561	cov	4.172643	1	archaeon RBG_16_50_20 (species)
NODE	12895	length	1561	cov	4.172643	2	
NODE	12984	length	1557	cov	4.147803	1	
NODE	12984	length	1557	cov	4.147803	2	Candidatus Bathyarchaeota (phylum)
NODE	12984	length	1557	cov	4.147803	3	
NODE	12984	length	1557	cov	4.147803	4	
NODE	13068	length	1553	cov	5.236315	1	
NODE	13068	length	1553	cov	5.236315	2	archaeon RBG_16_50_20 (species)
NODE	13178	length	1548	cov	3.691226	1	archaeon RBG_16_50_20 (species)
NODE	13178	length	1548	cov	3.691226	2	archaeon RBG_16_50_20 (species)
NODE	13406	length	1538	cov	2.982468	1	
NODE	13538	length	1532	cov	4.457684	1	
NODE	13627	length	1529	cov	4.054274	1	
NODE	13627	length	1529	cov	4.054274	2	archaeon RBG_16_50_20 (species)
NODE	13627	length	1529	cov	4.054274	3	
NODE	14115	length	1510	cov	4.580756	1	
NODE	14115	length	1510	cov	4.580756	2	Candidatus Bathyarchaeota (phylum)
NODE	14183	length	1508	cov	3.194081	1	Candidatus Bathyarchaeota (phylum)
NODE	14183	length	1508	cov	3.194081	2	Thaumarchaeota (phylum)
NODE	14183	length	1508	cov	3.194081	3	

APPENDIX L

Alignment of mcrA gene sequences obtained from the unassembled metagenome of peat soil (BO45) with the mcrA gene sequences of Bathyarchaeota BA1 (ALK27805.1) and BA2 (ALK27806.1) genomes. Partial sequences of mcrA of BO45 were likely matched to the known reference sequences, as indicated by colors.