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Comparison of microbial communities associated with three Atlantic ultramafic hydrothermal systems

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Abstract :

The distribution of *Archaea* and methanogenic, methanotrophic and sulfate-reducing communities in three Atlantic ultramafic-hosted hydrothermal systems (Rainbow, Ashadze, Lost City) was compared using 16S rRNA gene and functional gene (*mcrA*, *pmoA* and *dsrA*) clone libraries. The overall archaeal community was diverse and heterogeneously distributed between the hydrothermal sites and the types of samples analyzed (seawater, hydrothermal fluid, chimney and sediment). The Lost City hydrothermal field, characterized by high alkaline warm fluids (pH>11; *T*<95 °C), harbored a singular archaeal diversity mostly composed of unaffiliated *Methanosarcinales*. The archaeal communities associated with the recently discovered Ashadze 1 site, one of the deepest active hydrothermal fields known (4100 m depth), showed significant differences between the two different vents analyzed and were characterized by putative extreme halophiles. Sequences related to the rarely detected *Nanoarchaeota* phylum and *Methanopyrales* order were also retrieved from the Rainbow and Ashadze hydrothermal fluids. However, the methanogenic *Methanococcales* was the most widely distributed hyper/thermophilic archaeal group among the hot and acidic ultramafic-hosted hydrothermal system environments. Most of the lineages detected are linked to methane and hydrogen cycling, suggesting that in ultramafic-hosted hydrothermal systems, large methanogenic and methanotrophic communities could be fuelled by hydrothermal fluids highly enriched in methane and hydrogen.

Keywords : *Archaea* ; hydrothermal vent ; Mid-Atlantic Ridge ; 16S rRNA gene ; Sediment ; ultramafic

Introduction

Deep-sea hydrothermal environments are characterized by intense physico-chemical gradients providing a large range of habitats for chemolithoautotrophic microorganisms (Kelley *et al.*, 2002). Most of the studies of microbial diversity associated with deep-sea hydrothermal environments have mainly investigated basaltic-hosted hydrothermal systems (Kelley *et al.*, 2002). However, a few studies showed that ultramafic-hosted hydrothermal systems contained specific microbial communities (Brazelton *et al.*, 2006; Perner *et al.*, 2007; Voordeckers *et al.*, 2008; Flores *et al.*, 2011). To date, only three ultramafic sites were fully described on the Mid-Atlantic Ridge (Rainbow, Lost City and Logatchev), and were characterized by high concentrations of methane and hydrogen, in contrast with basaltichosted hydrothermal systems (Kelley *et al.*, 2001; Charlou *et al.*, 2002; Schmidt *et al.*, 2007). Moreover, Ashadze, a novel hydrothermal site, was recently reported on the Mid-Atlantic Ridge (MAR) (Bel'tenev *et al.*, 2005; Charlou *et al.*, 2007; Fouquet *et al.*, 2007; Mozgova *et al.*, 2008; Bassez *et al.*, 2009; Charlou *et al.*, 2010). This part of the MAR is characterized by rock compositions indicating that anomalously enriched mantle domains are involved in the melting region (Dosso *et al.*, 1993), and also by numerous outcrops of serpentinized mantlederived rocks (Bougault *et al.*, 1993; Cannat *et al.*, 1997). However, these ultramafic systems expelling fluids characterized by moderate to high temperatures are also probably linked to magmatic heating processes (Allen & Seyfried, 2004). Ultramafic hydrothermal fluids are

highly enriched in abiogenic methane and hydrogen as a result of serpentinization reactions between the ultramafic rocks and seawater (Holm & Charlou, 2001; Charlou *et al.*, 2002; Allen & Seyfried, 2004), and could therefore supply twice as much chemical energy as basaltic-hosted hydrothermal systems (McCollom, 2007). Hence, most of the prokaryotes found at these sites seemed to be related to methane and hydrogen cycling (Boetius, 2005; Perner *et al.*, 2007; Voordeckers *et al.*, 2008; Flores *et al.*, 2011).

A large number of microbial communities from hydrothermal environments could be fuelled by inorganic compounds (Amend & Shock, 2001). Although these microbial communities occupy both aerobic and anaerobic habitats, anaerobic hyper/thermophilic *Archaea* are reported to be usually associated with the hottest parts of these environments (Kelley *et al.*, 2002; Schrenk *et al.*, 2003; Takai *et al.*, 2004a), some of which could be entrained by hydrothermal fluids from subsurface ecosystems (Deming & Baross, 1993; Holden *et al.*, 1998; Summit & Baross, 1998). Moreover, it was suggested that *Archaea* could encompass up to 33-50% of the total microbial community in deep-sea hydrothermal environments (Harmsen *et al.*, 1997; Nercessian *et al.*, 2003).

Although an increasing number of thermophilic prokaryotes are cultivated from hydrothermal environments (Huber *et al.*, 2002; Miroshnichenko & Bonch-Osmolovskaya, 2006; Reysenbach *et al.*, 2006; Wagner & Wiegel, 2008; Slobodkina *et al.*, 2009), molecular phylogenetic approaches have revealed several new uncultivated lineages (Takai & Horikoshi, 1999; Nercessian, 2003; Kormas *et al.*, 2006; Moussard *et al.*, 2006a). Metagenomic approaches and functional gene analyses have also contributed to the characterization of metabolic and physiological properties of these communities (Nercessian *et al.*, 2005; Moussard *et al.*, 2006b; Moussard *et al.*, 2006c). However, to our knowledge, rRNA-based molecular approaches have seldom been used to compare the microbial diversity from multiple different hydrothermal sites (López-García *et al.*, 2003a; López-García *et al.*, 2003b; Voordeckers *et al.*, 2008; Flores *et al.*, 2011).

In the present study, we characterized the molecular genetic diversity, using 16S rRNA gene and functional genes of methanogens, methanotrophs and sulfate-reducers, associated with

three ultramafic-hosted hydrothermal sites: Rainbow, Lost City and Ashadze. As these hydrothermal fluids are highly enriched in methane and hydrogen, these environments could harbour specific prokaryotic communities possibly associated with potential subsurface chemolithoautotrophic ecosystems. Hence, the aim of this study was to compare the microbial communities of these ultramafic-hosted hydrothermal sites using molecular genetic methods, in order to correlate their phylogeny with ecological niches.

Materials and methods

Site location and sampling techniques

Fluid, chimney and sediment samples were collected during the scientific cruises EXOMAR (2005), SERPENTINE (2007) and MoMARDREAM-Naut (2007) conducted with the R. V. "*L'Atalante*" and "*Pourquoi pas ?*" and using the ROV "*Victor 6000*" and DSV "*Nautile*". The three hydrothermal fields explored, Rainbow (36°13'N; 33°54'W; ~ 2300 m depth), Lost City (30°07'N; 42°07'W; ~ 750 m depth) and Ashadze 1 (12°58'N; 44°51'W; ~ 4090 m depth) were all located along the Mid-Atlantic Ridge (MAR), although Lost City and Ashadze were further from the axis (Fig. 1a).

Fluid samples from Rainbow, Lost City and Ashadze were collected respectively from the "*thermitière*" chimney (36°13'76''N; 33°54'16''W; 2294 m depth, Fig. 1e), from a flange near the EXOMAR 11 Marker (30°07'43'' N; 42°07'16''W; 748 m depth, Fig. 1c), and from two chimneys in the SE2 area at Ashadze 1 site (12°58'33''N; 44°51'78''W; 4097 m depth, Fig. 1f). Chimney samples were also retrieved from the two chimneys in the SE2 area of Ashadze 1 site where the fluids were previously collected. The sediment samples were retrieved from the Rainbow site close to the active hydrothermal area (36°13'76''N; 33°54'04''W; 2287 m depth, Fig. 1d) and from the immediate periphery of the Lost City site (30°07'57'' N; 42°07'05''W; 752 m depth, Fig. 1b).

In order to describe the microbial communities from the surrounding seawater, the water column from the Rainbow site (36°13'76''N; 33°54'06''W; 2291 m depth) was also sampled.

All fluid samples were collected using titanium syringes and analyzed as described elsewhere (Charlou *et al.*, 2002). On board, the fluid samples were immediately removed aseptically from the titanium syringes and stored at −80°C for molecular genetic analyses. On board the sediment cores (~ 20 cm in length, 5 cm diameter) collected from the Rainbow site, using a push-core device operated by the arm of the DSV "*Nautile*", were sectioned in three equal samples and were designated as top, middle and bottom. The sediment surface sample from the Lost City site was collected using PSDE system (Fig. 1b, Kato C., unpublished). The chimney fragments were collected in biobox and sediment samples were stored aseptically at −80°C for molecular genetic analyses.

DNA extractions and PCR amplification

To avoid contaminations, all manipulations were carried out in a PCR cabinet (Biocap™ RNA/DNA, erlab®), using Biopur® 1.5 mL Safe-Lock micro test tubes (Eppendorf™), Rnase/Dnase Free Water (MP Biomedicals™) and UV-treated (>60 min) plasticware and pipettes.

DNA extractions from fluids were performed from 50 mL of fluid left to thaw on ice prior centrifugation (15000 g for 60 min). Supernatant was carefully discarded and DNA was extracted from the pellet, following a modified FastDNA® Spin Kit for Soil (Bio101 Systems, MP Biomedicals™) protocol (Webster *et al.*, 2003; Roussel *et al.*, 2009a). The DNA extractions from sediments and chimney fragments were also performed using the modified FastDNA® Spin Kit for Soil as described elsewhere (Roussel *et al.*, 2009a).

All amplifications were performed using a "GeneAmp PCR system" 9700® (Applied Biosystems™). All PCR mixtures (50 µL) contained 5 µL of DNA template, 1X Taq DNA polymerase buffer (MP Biomedicals™), 1 µL of dNTP (10 mM of each dATP, dCTP, dGTP and dTTP), 10 µM of each primer and 0.5 µL of Taq DNA polymerase (MP Biomedicals™). Negative controls were also carried out with DNA extractions performed without any sample. For all controls, no PCR products were detected. Inhibition of PCR amplification by soluble

contaminants in the DNA extracts was also tested as described elsewhere (Juniper *et al.*, 2001).

Archaeal 16S rRNA gene amplification was conducted by nested PCR with combination of primers A8f (5'-CGG TTG ATC CTG CCG GA-3') and A1492r (5'-GGC TAC CTT GTT ACG ACT T-3') in the first round (Teske *et al.*, 2002; Lepage *et al.*, 2004), and with A344f (5'-AYG GGG YGC ASC AGG SG-3') and A915r (5'-GTG CTC CCC CGC CAA TTC CT-3') in the second round (Stahl & Amann, 1991; Sørensen *et al*., 2004). PCR cycles for the first round (A8f/A1492r), and for the second round (A344f/A915r) were as previously described (Roussel *et al.*, 2009a). To minimize PCR bias, five independent PCR products from the first round were pooled and purified (QIAquick PCR purification Kit; Qiagen™) and used as template for the second round. This nested PCR was necessary to obtain visible PCR products on a 0.8% (w/v) agarose gel stained with ethidium bromide.

A portion of the *mcrA* gene was amplified using the ME primers (Hales *et al.*, 1996) with the following reaction conditions as described elsewhere (Roussel *et al.*, 2009a). A fragment of the *pmoA* gene was amplified using the pmoA189-mb661 primer couple (Holmes *et al.*, 1995; Costello & Lidstrom, 1999) with the following reaction conditions: 1 cycle of 4 min at 92°C, 35 cycles of 1 min at 92°C, 1.5 min at 55°C and 1 min at 72°C, and 1 cycle of 9 min at 72°C. A portion of the *dsrA* gene was amplified using the DSR1F+ and DSR-R primers (Kondo *et al.*, 2004) with the following reaction conditions: 1 cycle of 5 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at 54°C and 2.5 min at 72°C, and 1 cycle of 8 min at 72°C. For all functional genes, two rounds with the previous reaction conditions were required to obtain visible amplification products. An aliquot (5 µL) of three pooled PCR products of the primary amplification was used as template for the second amplification round.

CM-DGGE analysis

In order to obtain the general archaeal 16S rRNA gene diversity associated with the hydrothermal environment and to compare it with the seawater diversity, a preliminary CM-DGGE analysis was performed as described elsewhere (Roussel *et al.*, 2009b).

After amplification of the nested PCR products, using two different fluorescent reverse labelled (Cy3 or Cy5) primers from total DNA from either a hydrothermal sample or seawater, these were pooled and loaded into the same lane. Archaeal 16S rRNA gene amplification was performed with primers Saf-PARCH 519r, labelled with either Cy3 (hydrothermal samples) or Cy5 (seawater), following touchdown PCR protocol as previously described (Nicol *et al.*, 2003). All manipulations were performed in the dark. The PCR products were analyzed by DGGE using a DCode Universal Mutation Detection System® (BioRad™) on a 1 mm thick (16 × 16 cm) 8% (w/v) polyacrylamide gel (acrylamide/bisacrylamide, 40%, 37.5:1, BioRad™) with a denaturant gradient between 30 and 70% prepared with 1 × TAE buffer (pH 8, 40 mM Tris Base, 20 mM acetic acid, 1 mM EDTA, MP Biomedicals™) and poured with a "Gradient maker" (Hoefer SG30®). Electrophoresis was carried in 1 × TAE buffer at 60°C for 330 min at 200 V (initially at 80 V for 10 min). The gel was scanned using a Phospho fluorimager Typhoon 9400® (Amersham Biosciences™).

Cloning and sequencing

Fourteen 16S rRNA gene, one *dsrA* gene, four *mcrA* gene, and eight *pmoA* gene clone libraries were constructed. To minimize PCR bias (Polz & Cavanaugh, 1998), five independent PCR products were pooled, purified (QIAquick PCR purification Kit; Qiagen™), and cloned into *Escherichia coli* (XL10-Gold; Stratagene™) using the pGEM-T Easy vector system I (Promega™) following the manufacturer's instructions. Positive transformants were screened by PCR amplification of the insert using the vector-specific M13 primers. Plasmid extraction, purification and sequencing of the insert were carried out by the sequencing Ouest-Genepole platform® of Roscoff Marine laboratory (France).

Phylogenetic analysis and statistical analyses

Chimeras (Cole *et al.*, 2003) were excluded from the clone libraries and a total of 759 sequences (including those from the 16S rRNA gene and functional genes) were used for further phylogenetical analysis. The phylogenetic placement was carried out using NCBI

BLAST search program within GenBank (http://www.ncbi.nlm.nih.gov/blast) (Altschul *et al.*, 1990). The 16S rRNA gene sequences (~553 bases) were then edited in the BioEdit 7.0.5.3 program (Hall, 1999) and aligned using CLUSTALW (Thompson *et al.*, 1994). The phylogenetic trees were constructed by the PHYLO_WIN program (http:// pbil.univ-lyon1.fr/) (Galtier *et al.*, 1996) with Neighbour-Joining method (Saitou & Nei, 1987) and Jukes and Cantor correction. The nonchimeric *mcrA* (~0.76 kb), *pmoA* (~0.51 kb) and *dsrA* (~0.22 kb) sequences were translated into amino acids using BioEdit and then aligned using CLUSTALW, and the PHYLO_WIN program with Neighbour-Joining algorithm, and PAM distance (Dayhoff *et al.*, 1978) was then used for phylogenetic tree construction. For the entire phylogenetic reconstruction, the robustness of inferred topology was tested by bootstrap resampling (1000), values over 50% are shown on the trees. The richness from the clone libraries was estimated, with the rarefaction curves at 99%, 97% and 95% sequence identity levels, using the DOTUR program (Schloss & Handelsman, 2005). Operational taxonomic units (OTUs), using a 95% or 97% sequence similarity, were generated with the SON program (Schloss & Handelsman, 2006), and the percentage of coverage (*Cx*) of the clone libraries was calculated by Good's method (Good, 1953) as described by Singleton and colleagues (Singleton *et al.*, 2001). Statistical estimators, the significance of population differentiation among clone libraries (F_{ST}) (Martin, 2002), and the exact tests of population genetic differentiation (Raymond & Rousset, 1995), were calculated using Arlequin 3.11 (Excoffier *et al.*, 2005).

Nucleotide sequence accession numbers

The sequences are available from GenBank database under the following accession numbers and names: 16S rRNA gene (FN650174 to FN650288), *mcrA* gene (FN650315 to FN650322), *dsrA* (FN650289 to FN650291) and *pmoA* (FN650292 to FN650314).

Results

Site description

A total of 15 samples encompassing fluids, chimney fragments and sediments, were retrieved from three Atlantic ultramafic-hosted hydrothermal sites: Rainbow, Lost City and Ashadze (Fig. 1). The dilution of the hydrothermal fluid sample was estimated according to pH measurements. Overall, the three sites had much higher hydrogen (<16 mM) and abiogenic methane (<2.5 mM) concentrations than the MAR basaltic-hosted hydrothermal sites (Charlou *et al.*, 2010).

All the hydrothermal fluid samples from the Rainbow site were retrieved from the “thermitière” chimney group (Fig. 1e), except the “PP27 swarm” sample which was obtained in close proximity to a shrimp swarm on the side of the PP27 chimney. The “thermitière” chimney group was composed of both diffuse and black smoker venting. The Rainbow sediment samples were retrieved nearby the hydrothermal chimneys and were predominantly made of pelagic sediment (98% calcite) with a small amount of hematite, indicating a small hydrothermal contribution (Fig. 1d). For this study, the maximum temperature measured at Rainbow was 324°C, and the less diluted hydrothermal fluid analyzed had a pH of 3.40 (Fig. 2b), and high concentrations of hydrogen (>10 mM), carbon dioxide (17 mM), iron (>17 mM) and methane (>1mM) (Charlou *et al.*, 2010).

The Lost City fluid samples were obtained from one of the hottest venting areas of this site, which was located above a flange (Fig. 1c). To date, Lost City is a unique off-axis hydrothermal site expulsing fluids with a high pH (~ 11), as opposed to the other known ultramafic environments that are acidic (Rainbow, Ashadze pH = ~ 3). The maximum temperature recorded at Lost City (93°C) was lower than for Rainbow and Ashadze. The less diluted hydrothermal fluid analyzed had a pH of 11.75 (Fig. 2a), and high concentrations of hydrogen (>7 mM) and methane (0.9 mM).

Ashadze, a hydrothermal field that was recently explored for the first time during the French-Russian Serpentine cruise (Fouquet *et al.*, 2008), is one of the deepest active black smoker

fields discovered so far (4100 m depth). Ashadze is characterized by an ultramafic rock environment (Charlou *et al.*, 2007; Fouquet *et al.*, 2007; Fouquet *et al.*, 2008). Several groups of active one to two meter high chimneys were observed at Ashadze 1 site. The fluid and chimney fragments were obtained from two different active chimneys in a unique group near the SE-2 marker (Fig. 1f). For this study, the maximum temperature measured at Ashadze was 353°C. The less diluted hydrothermal fluid analyzed had a pH of 4.02 (Fig. 2b), and high concentrations of hydrogen (>10 mM), carbon dioxide (>2.5 mM), iron (7.3 mM) and methane (>0.80 mM).

Archaeal 16S rRNA gene analyses

CM-DGGE. All the 16S rRNA gene PCR products from all the samples were screened by Co-Migration DGGE (CM-DGGE) prior cloning, in order to estimate the archaeal phylogenetic diversity of each hydrothermal sample and to compare it directly with the seawater diversity (Fig. 2a). Band pattern intensities from all Lost City samples, and from the less diluted hydrothermal fluids, were weaker than for all the other samples, suggesting a lower biomass and/or high concentration of PCR inhibitors (Fig. 2a). The archaeal seawater CM-DGGE band pattern was different from all the hydrothermal fluid and chimney band patterns (Fig. 2a), suggesting low levels of seawater contamination. The band patterns from hydrothermal samples were mostly composed of DGGE fragments with higher melting points, a probable consequence of higher GC content of the 16S rRNA gene. The high-GC content of these 16S rRNA gene sequences indicates that the *Archaea* could be hyper/thermophiles (Kimura *et al.*, 2006), as also suggested by the several putative hyper/thermophilic lineages detected in the clone libraries from hydrothermal fluids and chimneys (*Archaeoglobales*, *Methanococcales*, *Thermococcales*, *Methanopyrales*, *Desulfurococcales*, *Nanoarchaeota*, DHVE; Fig. 2b).

Clone libraries. After technical optimization and removal of soluble PCR inhibitors and in order to amplify sufficient PCR product for cloning, archaeal amplifiable DNA from all samples was retrieved by nested PCR. However, no sufficient amplified PCR product was

295 obtained for cloning from the less diluted fluid samples (pH 11.75) and from the chimney
296 samples from Lost City. Fourteen different 16S rRNA gene clone libraries were constructed,
297 representing a total of 610 sequences. The coverage values for the 16S rRNA gene clone
298 libraries ranged from 68 to 97%, based on a 97% sequence similarity level (Fig. 2b). On the
299 whole, rarefaction curves were asymptotic for all clone libraries, based on a 95% sequence
300 similarity level, confirming sufficient sampling effort (Fig. S1).
301 The overall archaeal diversity analyzed was similar to previous studies (e.g. Brazelton *et al.*,
302 2006; Flores *et al.*, 2011) and very heterogeneously distributed between the sites (Lost City,
303 Rainbow, and Ashadze) and between types of samples (seawater, hydrothermal fluid,
304 chimney and sediment). The number of OTUs per clone library ranged from five to nineteen,
305 based on a 95% genus level of phylotype differentiation (Schloss & Handelsman, 2004), and
306 the Shannon-Wiener index of diversity ranged between 0.63 and 3.04 (Fig. 2b). The archaeal
307 diversity indices of all the samples were in the same range, except for the fluid associated
308 with Lost City which, as previously described (Schrenk *et al.*, 2004), displayed the lowest
309 detectable diversity (Fig. 2a and 2b). On average, the hydrothermal samples contained six
310 different lineages, except for Lost City (Fig. 2a and 2b), which is also in agreement with most
311 published studies on hydrothermal environments (e.g. Takai *et al.*, 2001; Nercessian *et al.*,
312 2003; Schrenk *et al.*, 2003; Schrenk *et al.*, 2004; Takai *et al.*, 2004b; Kormas *et al.*, 2006;
313 Page *et al.*, 2008; Nunoura *et al.*, 2010). All the 16S rRNA gene sequences obtained from
314 the clone libraries were assigned to 95 OTUs, based on a 95% sequence similarity level,
315 forming a total of 21 different phylogenetic lineages (Fig. 2b, 3a and 3b). On the whole, 16S
316 rRNA gene sequences were related to *Euryarchaeota* (51%), *Crenarchaeota* (48%) and
317 *Nanoarchaeota* (1%). The 16S rRNA gene clone libraries obtained from hydrothermal
318 samples (fluid and chimney) were dominated by sequences related to *Euryarchaeota* (69%),
319 whereas sequences related to *Crenarchaeota* were a majority in the sediment (92%) and
320 seawater samples (70%) (Fig. 2b). Seven of the fifteen *Euryarchaeota* lineages detected had
321 at least one known cultured representative, and six of these seven had known thermophilic
322 *Archaea* (*Halobacteriales*, DHVE2, *Archaeoglobales, Methanococcales*, *Thermococcales*,

Methanopyrales). Although three of the five *Crenarchaeota* lineages detected had at least one cultured representative, only one was known to be thermopilic (*Desulfurococcales*). Moreover, Marine Group I (MG-I) *Archaea* had the highest intra-lineage diversity representing 25 OTUs based on a 95% genus level of phylotype differentiation.

Functional gene clone libraries

Diversity of mcrA gene. The operon coding for the MCR-I, which includes McrA subunit, is found in all known methanogens (Reeve *et al.*, 1997). Four *mcrA* clone libraries were obtained from sediment, fluid and chimney samples from Ashadze and Rainbow sites. Although detected by previous studies (Kelley *et al.*, 2005), no *mcrA* gene sequences were detected from Lost City samples. The diversity of the four *mcrA* libraries was limited to sequences related to the H_2/CO_2 methanogens *Methanopyrales* and *Methanococcales* orders (Fig. 4a), congruently with the 16S rRNA gene clone libraries (Fig. 3a). *mcrA* gene sequences affiliated to *Methanopyrales* were only detected at Rainbow. Moreover, the *mcrA* gene sequences from Rainbow and Ashadze matched the two groups of uncultured methanogenic *Archaea* previously retrieved from Rainbow (Nercessian *et al.*, 2005).

Diversity of dsrA gene. Sequences coding for the *dsrA* gene were only retrieved from Ashadze chimney 1 (Fig. 4b). *dsrA* gene sequences were previously detected in chimney samples from Lost City (Gerasimchuk *et al.*, 2010), and in sediments from Rainbow (Nercessian *et al.*, 2005). All *dsrA* gene sequences detected from Ashadze site were all related to sequences from marine sediments and East-Pacific Rise hydrothermal vents, as a probable consequence of a lack of *dsrA* gene sequences from the MAR in the databases. *dsrA* gene sequences were mainly affiliated to sequences from the *Desulfobulbaceae* family (Fig. 4b).

Diversity of pmoA gene. The *pmoA* gene was the most widespread functional gene detected, as a PCR amplification was obtained on eight out of the fifteen samples tested (Fig. 2b and 4c). The phylogeny of the *pmoA* gene is usually poorly resolved, the bacterial *pmoA* gene being distantly related to the ammonia monooxygenase subunit A (*amoA*) (Holmes *et al.*,

1995; Nicol & Schleper, 2006), as revealed by incongruence between tree topologies performed with different phylogenetic methods. However, two groups of *pmoA* sequences from Rainbow fluids and Ashadze chimney samples clustered (cluster *pmoA* 1 and cluster P-A) with sequences related to thermophilic methylotrophs (Inagaki *et al.*, 2003; Hirayama *et al.*, 2007)(Fig. 4c). Moreover, *pmoA* gene sequences from sediments from Rainbow grouped into two major clusters (cluster *pmoA* 2 and *pmoA* 3). Sequences from *cluster pmoA 2* did not have any closely related sequences (Fig. 4c).

Community structures and distribution analyses

Although the seawater and the Rainbow sediment CM-DGGE band patterns were quite similar (Fig. 2a), all the sediment clone library community structures were indistinguishable from the combined communities and significantly different ($P < 0.001$) from the seawater (Fig. 2b). Insignificant F_{ST} and P tests ($P < 0.001$), based on an analysis at a 97% sequence similarity level, suggested that community structures from all the Rainbow hydrothermal fluids and Ashadze chimney 1 clone libraries were similar and indistinguishable from the combined communities (Fig. 2b). However, although the archaeal community structures from all the Rainbow hydrothermal fluids were also from similar lineage distributions, all the Ashadze chimney and fluid samples had significantly different population structures ($P < 0.001$; Fig. 2a and 2b). The archaeal diversity of all the other clone libraries was also significantly different from the seawater clone library ($P < 0.001$), showing that the hydrothermal vent archaeal communities are probably adapted to their environment. According to pH measurements, the archaeal diversity in the hydrothermal fluids was always the most reduced in the less diluted fluids (Fig. 2b). Moreover, a correlation was also observed between *Methanococcales* ($P < 0.001$) and *Thermococcales* ($P < 0.05$) lineages and the Rainbow fluids. Correlations were also shown between MG-I lineage and the hydrothermal sediments ($P < 0.01$), and between the unaffiliated *Methanosarcinales* cluster and the Lost City fluids ($P < 0.0001$).

Discussion

High diversity of putative chemolithoautotrophs

Overall, the analysis of the phylogenetic data showed a specific distribution of different putative metabolic processes over the different MAR ultramafic-hosted hydrothermal environments that were mainly dominated by putative chemolithoautotrophs.

Putative ammonia-oxidizing Crenarchaeota. Marine Group I (MG-I) was the most ubiquitous lineage found in the MAR ultramafic-hosted hydrothermal environments, as sequences related to the MG-I *Archaea* were detected in the majority of clone libraries (93%). Interestingly, the archaeal community structure of the seawater clone library was dominated by sequences related to MG-I (41%) and Marine Group II (48%), but was significantly different from all the other clone libraries ($P < 0.001$). Congruently, Takai and colleagues showed that the highest proportion of MG-I members in a hydrothermal environment from the Central Indian Ridge, was found in the seawater adjacent to the hydrothermal emissions (Takai *et al.*, 2004c). MG-I sequences also dominated the sediment 16S rRNA gene clone libraries (≥ 86%), as commonly found in marine surface sediments (Inagaki *et al.*, 2001; Teske & Sorensen, 2008; Roussel *et al.*, 2009b). The highest diversity indices were also observed in these sediment samples, as a consequence of the very high intra-phylum diversity observed within the MG-I. However, all the MG-I 16S rRNA gene sequences from the sediment clustered in phylogenetic groups different from the seawater MG-I, suggesting that specific MG-I communities could be associated with sedimentary environmental conditions (Roussel *et al.*, 2009b). Moreover, the G + C content of all the MG-I sequences ranged between 48% to 52%, in opposition to the high-GC content of the 16S rRNA gene sequences from hydrothermal fluids or chimneys, thus supporting the hypothesis that these MG-I *Archaea* are probably adapted to the cooler ecological niches of the hydrothermal environments (Kimura *et al.*, 2006; Ehrhardt *et al.*, 2007). Several studies also showed that specific phylogenetic groups of MG-I *Archaea* appear to be endemic to basaltic crust

environments (Ehrhardt *et al.*, 2007; Mason *et al.*, 2007; Mason *et al.*, 2009). Some of these specific subclades of MG-I *Archaea* could therefore be adapted to various environments as they were also detected in aerobic and anaerobic basalt enrichment cultures and sediment slurries (Mason *et al.*, 2007; Webster *et al.*, 2010). Hence, as the MG-I *Archaea* were widespread in our hydrothermal fluid and chimney clone libraries, their presence could be the result of the mixing of ambient seawater with cool niche water in the rocks of the hydrothermal system.

MG-1 *Archaea*, regularly described as aerobic autotrophic ammonia oxidizers (Francis *et al.*, 2005; Konneke *et al.*, 2005; Hallam *et al.*, 2006), are commonly found in seawater and marine sediments, forming several phylogenetic clusters with several cultured relatives (e.g. Preston *et al.*, 1996; Konneke *et al.*, 2005). Moreover, based on the analysis of the first sequenced genome of a cultured relative (*Cenarchaeum symbiosum*), the MG-1 were proposed as a novel archaeal phylum named *Thaumarchaeota* (Brochier-Armanet *et al.*, 2008). Interestingly, moderate thermophilic ammonia-oxidizing crenarchaeotes were recently isolated from hot springs (de la Torre *et al.*, 2008; Hatzenpichler *et al.*, 2008) and may also play a major role in the nitrogen cycle in these environments (Zhang *et al.*, 2008; Wang *et al.*, 2009). High ammonium concentration and removal rates were previously measured from a Pacific hydrothermal system (Lam *et al.*, 2008), and a thermophilic origin for anaerobic ammonium oxidation was also suggested (Canfield *et al.*, 2006). Hence, according to their widespread dissemination in hydrothermal systems, as shown in several studies, and due to the high mixing processes occurring in these dynamic systems (e.g. Takai *et al.*, 2004c), MG-I may also play a role in ammonium oxidation in hydrothermal systems as previously suggested for marine basalts (Mason *et al.*, 2007; Mason *et al.*, 2009).

Putative hydrogen-oxidizing chemolithoautotrophs. All the ultramafic hydrothermal fluids from Rainbow, Ashadze and Lost city were highly enriched in abiogenic methane and hydrogen as a result of serpentinization reactions between ultramafic rocks and seawater (Holm & Charlou, 2001; Charlou *et al.*, 2002; Allen & Seyfried, 2004). Moreover, McCollom showed

that ultramafic-hosted hydrothermal systems could theoretically provide twice as much chemical energy as comparable basaltic-hosted systems (McCollom, 2007). More than half of the archaeal lineages detected from Rainbow, Ashadze and Lost City were related to known cultured species, two thirds of which were involved in hydrogen or methane cycling processes, supporting the theory that these ecosystems could be mainly fuelled by hydrothermal fluids highly enriched in hydrogen and methane.

Methanogenesis was the most common putative hydrogen-oxidizing metabolism detected among ultramafic hydrothermal fluids and chimney samples. Indeed, among all archaeal lineages in the hydrothermal samples, *Methanococcales* was the most widespread, as it was detected in a majority of the clone libraries (78%) obtained from hydrothermal fluids or chimney samples. Interestingly, all the sequences related to *Methanococcales* from Rainbow and Ashadze grouped with sequences previously detected at Rainbow (Nercessian *et al.*, 2005), suggesting that these could be long-term stabilized population in these chemically slow evolving environments (Charlou *et al.*, 2002). *Methanococcales Archaea* are strictly anaerobic autotrophic methanogens, using hydrogen and carbon dioxide or formate as energy sources (Whitman *et al.*, 2001). Strains affiliated to *Methanocaldococcus infernus* were successfully cultured from hydrothermal chimney samples from Rainbow and Ashadze (Jeanthon C. and L'Haridon S. personal communication, respectively). Moreover, the methanogenic potential of *Methanococcales* at Rainbow and Ashadze was also confirmed by the detection of *mcrA* genes related to *Methanothermococcus thermolithotrophicus* (> 97% similarity) and *Methanocaldococcus infernus* (> 97%). Although hyperthermophilic or thermophilic members of the *Methanococcales* are commonly cultured and detected with molecular tools from marine hydrothermal vent systems (e.g. Kelley *et al.*, 2002; Nercessian *et al.*, 2003; Schrenk *et al.*, 2003; Takai *et al.*, 2004a; Perner *et al.*, 2007; Page *et al.*, 2008), Schrenk and colleagues reported that *Methanococcales* encompassed a low proportion (<5%) of the hydrothermal prokaryotic communities associated with the walls of a sulphide chimney, whereas on different hydrothermal sites Takai and colleagues reported proportions

up to 76.5% (Schrenk *et al.*, 2003; Takai *et al.*, 2004a). These differences could be linked to environmental factors such as the high hydrogen production from these ultramafic systems, which fuel these communities.

Moreover, putative hyperthermopilic methanogens were also represented by *Methanopyrales*. *Methanopyrales* were rarely detected on the MAR by molecular methods (Flores *et al.*, 2011), probably as a consequence of technical biases or of the restricted number of microbial studies of the MAR, though the first isolated member originates from a hydrothermal system north of Iceland (Kurr *et al.*, 1991). Sequences related to *Methanopyrales* were rarely detected elsewhere (Nercessian, 2003; Takai *et al.*, 2004a; Ehrhardt *et al.*, 2007; Page *et al.*, 2008). However, in this study, eighteen sequences related to *Methanopyrus kandleri* (>96% similarity) were detected from Rainbow and Ashadze fluids. Interestingly, Takai and colleagues also reported recently an isolate related to *Methanopyrus kandleri* capable of methanogenesis with H_2/CO_2 under elevated hydrostatic pressures and at 122°C (Takai *et al.*, 2008). As *mcrA* gene sequences related to *Methanopyrus kandleri* (88% similarity) were also detected at Rainbow and as strains affiliated to *Methanopyrales* were successfully cultured from Rainbow and Ashadze (Jeanthon and L'Haridon personal communication, respectively), the *Methanopyrales* detected were probably capable of methanogenesis, thus supporting the hypothesis that these sites may harbour large methanogenic communities.

Archaeoglobales, another putative hydrogen-oxidizing archaeal lineage, was also found to be widespread among hydrothermal samples from Rainbow and Ashadze. 16S rRNA gene sequences closely related (> 96% similarity) to members of genus *Archaeoglobus*, *Geoglobus* and *Ferroglobus*, were retrieved from Rainbow and Ashadze. Interestingly, a new dissimilatory Fe(III)-reducing *Archaeoglobaceae* was also isolated from Ashadze and reported as growing autotrophically on hydrogen (Slobodkina *et al.*, 2009). As several *Archaeoglobales* are also iron-cycling *Archaea* (e.g. Kashefi *et al.*, 2002), the high concentrations of iron (> 3 mM) released by acidic ultramafic-hosted hydrothermal environments could possibly fuel specific members of these *Archaeoglobaceae* communities.

Putative sulphur-cycling and methane-oxidizing communities. Members of the *Archaeoglobales* lineage also belong to the hyperthermophilic sulfate-reducing *Archaea* (Miroshnichenko & Bonch-Osmolovskaya, 2006). Interestingly, putative sulphur-cycling *Archaea* related to *Thermococcales* and *Archaeoglobales* lineages were detected in more than half of the clone libraries (56%) obtained from hydrothermal samples (fluid or chimney) and were always detected together, suggesting they could require similar environmental conditions. However, contrary to all Rainbow hydrothermal fluid samples, *Thermococcales* and *Archaeoglobales* at Ashadze were only detected from the Ashadze chimney 1 samples, suggesting that all hydrothermal vents from Ashadze site did not share optimal conditions for putative sulphur-cycling microorganisms. Interestingly, the first obligate piezophilic hyperthermophilic microorganism, *Pyrococcus* CH1, was also recently isolated from the Ashadze chimney 1 (Zeng *et al.*, 2009). Members of the *Thermococcales* order are mainly characterized as thermophilic to hyperthermophilic anaerobic heterotrophs that ferment peptides and sugars, and their growth can also be stimulated by sulphur reduction (Miroshnichenko & Bonch-Osmolovskaya, 2006; Zeng *et al.*, 2009). However, some members of the *Thermococcales* were also able to grow on acetate-utilising Fe(III) (Summit & Baross, 2001) or capable of lithotrophic growth on carbon monoxide coupled with hydrogen production (Sokolova *et al.*, 2004), thus matching the environmental conditions of ultramafic-hosted hydrothermal systems. It has also been suggested that *Thermococcales* and hyper/thermophilic members of the *Methanococcales* order could inhabit sub-seafloor ecosystems (Summit & Baross, 1998; Summit & Baross, 2001; Kelley *et al.*, 2002; Takai *et al.*, 2004a), and could be part of a hydrogen-driven subsurface lithoautotrophic microbial ecosystem (Nealson *et al.*, 2005).

Within methane cycling communities associated with Rainbow, putative methanotrophic ANME-2 sequences were detected, suggesting occurrence of anaerobic methane oxidation communities associated with anoxic habitats below 90°C (Kallmeyer & Boetius, 2004). Interestingly, *dsrA* gene sequences detected at Ashadze clustered with sequences

previously detected in methane-rich hydrothermal systems and related to the *Desulfobulbaceae* family (Teske *et al.*, 2002; Nercessian *et al.*, 2005), indicating that these putative sulfate-reducing bacteria could be linked to these specific environmental conditions. Besides, some members of *Desulfobulbaceae* can live syntrophically with ANME-3 members (Niemann *et al.*, 2006). However, although 16S rRNA gene sequences related to ANME-2 *Archaea* were detected, no ANME-3 *Archaea* were found. Nevertheless, the most widespread functional gene (*pmoA*) detected in ultramafic-hosted hydrothermal environments remained related to methanotrophic communities as a probable consequence of the high methane concentration prevailing in these ultramafic-hosted hydrothermal systems. Methanotrophic bacteria were also previously detected in *Bathymodiolus* species and among the gill chamber of *Rimicaris exoculata* at the Rainbow hydrothermal field (Duperron *et al.*, 2006; Zbinden *et al.*, 2008), suggesting that these symbionts could also be present in seawater. However, no known sequences related to symbionts were detected in the seawater, as a possible consequence of low cell concentrations or of a technical bias. Moreover, the phylogenetic distribution of the *pmoA* gene was related to the habitat, suggesting that different methanotrophic communities were specifically adapted to different ecological niches (e.g. sediments and fluid/seawater mixing zones).

Specific distribution and ecological niches

The different environmental conditions (temperature, pH, hydrostatic pressure, metabolic substrates) at the different MAR ultramafic-hosted hydrothermal sites generate diverse microbial ecological niches (hydrothermal fluid, chimney, sediment, and seawater) that seem to strongly select for specific communities.

Site specific phylotypes. Although molecular techniques (PCR and cloning) used to build clone libraries are known to be inherently biased (Suzuki & Giovannoni, 1996; von Wintzingerode *et al.*, 1997; Polz & Cavanaugh, 1998; Nocker *et al.*, 2007), we assumed that the biases were equal for all samples as they were analyzed under the same strict conditions (storage, DNA extraction, PCR amplification, cloning, sequencing) (von Wintzingerode *et al.*,

1997). However, comparisons of population structures from other studies using different experimental conditions remain unreliable. For example, archaeal diversity from Rainbow chimneys as described by Flores and colleagues (2011) was much higher than that described by Voordeckers and colleagues (2008), suggesting either spatial and temporal heterogeneity or a technical bias. Interestingly, archaeal diversity observed from the Rainbow fluids in the present study was similar to that from the chimneys analysed using pyrosequencing reported by Flores and colleagues (2011). The present study also shows that some communities seemed to be site-specific and specifically adapted to different ecological niches (e.g. sediments and fluid/seawater mixing zones).

Sequences related to *Nanoarchaeota*, for example, were only detected in the Rainbow hydrothermal system, showing that the nanoarchaeal habitat extends to at least one of the deep hot marine hydrothermal systems of the MAR. The recently discovered novel *Nanoarchaeota* phylum has shown a wide distribution in high temperature ecosystems (Hohn *et al.*, 2002; Huber *et al.*, 2002), and may represent pioneering communities in deep-sea hydrothermal vents (McCliment *et al.*, 2006). *Nanoarchaeota* could also represent a fast-evolving euryarchaeal lineage related to *Thermococcales* (Brochier *et al.*, 2005). Moreover, the nano-sized *Nanoarchaeota* were previously described to have a symbiotic relationship with *Ignicoccus hospitalis*, a member of the *Desulfurococcales* order isolated from the Kolbeinsey Ridge, north of Iceland (Paper *et al.*, 2007). Interestingly, 16S rRNA gene sequences with 94% similarity to the hyperthermophilic chemolithoautotrophic suphur and hydrogen-utilizing *Ignicoccus hospitalis*, were also retrieved exclusively from the same Rainbow hydrothermal fluids, suggesting that a similar symbiotic relationship could also occur between the *Nanoarchaeota* and specific *Desulfurococcales* from Rainbow. Differences between the composition of archaeal communities associated with the two hydrothermal chimneys from Ashadze could be probably linked to environmental factors as the Ashadze chimney 3 has a higher copper concentration than chimney 1 (Charlou JL., Donval JP. and Konn C., unpublished). Moreover, 16S rRNA gene sequences related to

565 *Halobacteriales* were only detected from Ashadze, which is to date the deepest known
566 hydrothermal site. The highest similarity with a cultured relative was *Natronomonas*
567 *pharaonis* (98%), an extremely halo-alkaliphilic archaeon. The occurrence of halotolerant
568 prokaryotes in hydrothermal environments, growing at higher NaCl concentrations than most
569 marine microorganisms, was previously reported (Takai *et al.*, 2001). Due to phase
570 separation it is admitted that venting of a condensed vapor phase with low salinity will
571 generate a high salinity phase at depth. This phase may be venting later or be trapped in the
572 subsurface environments. In addition some authors have suggested a double diffusive
573 hydrothermal system where brines are trapped in the deepest part of the system and
574 exchange only heat with the upper convective system (Bischoff & Rosenbauer, 1989;
575 Fouquet *et al.*, 1993). If partially cooled, this deep high salinity reservoir may constitute an
576 extensive location for halotolerant prokaryotes. Hence, it was also suggested that these
577 communities could be associated with a sub-vent ecosystem, as well as with hydrothermal
578 chimneys (Kaye & Baross, 2000; Takai *et al.*, 2001).
579 As previously described, the off-axis Lost City hydrothermal system is remarkable by its
580 geological, geochemical and biological settings (Kelley *et al.*, 2005). The archaeal diversity
581 associated with hot and very alkaline Lost City hydrothermal fluid was limited to unaffiliated
582 *Methanosarcinales* and to MG-I sequences. The detected unaffiliated *Methanosarcinales*
583 sequences matched the Lost City *Methanosarcinales* cluster (99% similarity) described by
584 Schrenk and colleagues (Schrenk *et al.*, 2004), suggesting that these *Archaea* were involved
585 in methane cycling processes (Schrenk *et al.*, 2004; Boetius, 2005). However, no *mcrA* gene
586 sequences were detected at Lost City, as a likely consequence of low cell densities in the
587 Lost City fluids (Brazelton *et al.*, 2006). Members of the Lost City uncultured
588 *Methanosarcinales* cluster are probably endemic communities associated to cooler (<95°C)
589 and very alkaline habitats as they were not detected from any other hydrothermal sites. The
590 occurrence of molecular genetic evidences in hot and very alkaline fluids also suggests that
591 the Lost City *Methanosarcinales* have physiological potentials beyond the capacities of any
592 known cultured isolates (Mesbah & Wiegel, 2008).

593 *Specific ecological niches.* To summarize, besides some specific *Archaea* that seemed
594 endemic to some hydrothermal sites, the distribution of archaeal phylotypes and putative
595 metabolic processes was linked to different microbial niches (seawater, sediments,
596 macrofaunal communities, hydrothermal chimneys and fluids, Fig. 5). The cold and
597 oxygenated seawater (< 10°C) overlaying the hydrothermal systems probably represented
598 one of the largest microbial niches, and was characterized only by marine group lineages,
599 some of which could be aerobic ammonia oxidizers. These psychrophilic seawater
600 communities surrounding hydrothermal vents are most likely to benefit from high ammonium
601 inputs from the chemolithotrophic primary producer associated with the hydrothermal
602 structures. Another large ecological niche is probably the cold (< 10°C) and porous
603 sediments surrounding the hydrothermal systems, which may represent a stable environment
604 and suitable substrate for selection of specific seawater phylotypes (MG-I) and for
605 colonization by specific psychrophilic unaffiliated *Euryarchaeota* and methylotrophic bacteria.
606 The specific sedimentary microbial communities could be fuelled by the products from
607 organic matter degradation, but also by methane seepage from these ultramafic systems. In
608 contrast, the warm (~15°C) and relatively unstable mixing zones colonized by macrofaunal
609 communities were probably the most metabolically active microbial niche benefiting from
610 oxidized seawater compounds and from reduced compounds from the hydrothermal system.
611 Mixing zones between the adjacent ecological niches also occur as a result of steep physico-
612 chemical gradients characterizing these dynamic hydrothermal environments, therefore
613 resulting in exchanging microbial communities. Mesophilic to thermophilic methane-oxidizing
614 bacteria could dominate the moderate oxic habitats in the mixing environment as revealed by
615 the *pmoA* gene analyses. The detection of ANME-2 members suggests that moderate
616 thermophilic (<90°C) anaerobic methanotrophs could occur in probably restricted anoxic
617 habitats, as a consequence of the very steep oxygen and temperature gradients.
618 Methanotrophic archaeal communities fuelled by hydrogen and carbon dioxide could
619 probably dominate the more chemically reduced zones of this niche, which is closer to the
620 hydrothermal chimney. The thermophilic communities composed of *Methanococcales*,

Methanopyrales, *Thermococcales*, *Archaeoglobales*, and *Desulfurococcales* were in all likelihood harboured by the hydrothermal chimneys and could mainly be composed of hydrogen-oxidizing members. Although hydrothermal fluids from ultramafic systems such as Rainbow do not have significant levels of hydrocarbons from biogenic origin, methanogenesis could still be the dominant archaeal metabolic process, as the high abiogenic methane concentration may mask the biogenic methane. Moreover, the hyper/thermophilic methanogen *Methanococcales* order and the *Thermoccocales* order could be typical members of the hot anaerobic microbial ecosystem that could extend below the Rainbow hydrothermal system seafloor.

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References

Allen DE & Seyfried WE (2004) Serpentinization and heat generation: Constraints from Lost City and Rainbow hydrothermal systems. *Geochim Cosmochim Acta* **68**: 1347-1354.

Altschul SF, Gish W, Miller W, Myers EW & Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* **215**: 403-410.

Amend JP & Shock EL (2001) Energetics of overall metabolic reactions of thermophilic and hyperthermophilic *Archaea* and *Bacteria*. *FEMS Microbiol Rev* **25**: 175-243.

Bassez MP, Takano Y & Ohkouchi N (2009) Organic analysis of peridotite rocks from the Ashadze and Logatchev hydrothermal sites. *Int J Mol Sci* **10**: 2986-2998.

650 Bel'tenev V, Ivanov V, Shagin A, Sergeyev M, Rozhdestvenskaya I, Shilov V, Debretzova I,
651 Cherkashev G, Samovarov M & Poroshina I (2005) New hydrothermal sites at 13ºN, Mid-
652 Atlantic Ridge. *InterRidge News* **14**: 14-16.

653 Bischoff JL & Rosenbauer RJ (1989) Salinity variations in submarine hydrothermal systems
654 by layered double-diffusive convection. *J Geol* **97**: 613-623.

655 Boetius A (2005) Lost City Life. *Science* **307**: 1420-1422.

656 Bougault H, Charlou JL, Fouquet Y*,* Needham HD, Vaslet N, Appriou P, Jean-Baptiste P,
657 Rona PA, Dmitriev L & Silantiev S (1993) Fast and slow-spreading ridges - Structure and
658 hydrothermal activity, ultramafic topographic highs, and CH_4 output. *J Geophys Res* **98**:
659 9643-9651.

660 Brazelton WJ, Schrenk MO, Kelley DS & Baross JA (2006) Methane- and sulfur-metabolizing
661 microbial communities dominate the Lost City hydrothermal field ecosystem. *Appl Environ*
662 *Microbiol* **72**: 6257-6270.

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

665 Brochier C, Gribaldo S, Zivanovic Y, Confalonieri F & Forterre P (2005) Nanoarchaea:
666 representatives of a novel archaeal phylum or a fast-evolving euryarchaeal lineage related to
667 *Thermococcales*? *Genome Biol* **6**: R42.

668 Canfield DE, Rosing MT & Bjerrum C (2006) Early anaerobic metabolisms. *Philos Trans R*
669 *Soc Lond B Biol Sci* **361**: 1819-1836.

670 Cannat M, Lagabrielle Y, Bougault H, Casey J, deCoutures N, Dmitriev L & Fouquet Y (1997)
671 Ultramafic and gabbroic exposures at the Mid-Atlantic Ridge: geological mapping in the 15
672 degrees N region. *Tectonophysics* **279**: 193-213.

673 Charlou JL, Donval JP, Fouquet Y, Jean-Baptiste P & Holm N (2002) Geochemistry of high
674 H_2 and CH_4 vent fluids issuing from ultramafic rocks at the Rainbow hydrothermal field (36
675 degrees 14 ' N, MAR). *Chem Geol* **191**: 345-359.

676 Charlou JL, Donval JP, Konn C, Ondréas H, Fouquet Y, Jean-Baptiste P & Fourré E (2010)
677 High production and fluxes of H_2 and CH_4 and evidence of abiotic hydrocarbon synthesis by
678 serpentinization in ultramafic-hosted hydrothermal systems on the Mid-Atlantic Ridge.
679 *Diversity of Hydrothermal Systems on Slow-spreading Ocean Ridges, Vol. 188* (Rona P,
680 Devey C, Dyment J & Murton B, eds.), pp. 265-296. American Geophysical Union,
681 Washington DC.

682 Charlou JL, Donval JP, Konn C*,* Birot D, Sudarikov S, Jean-Baptiste P, Fouquet Y & the
683 Scientific Party of the SERPENTINE cruise*.* (2007) High hydrogen and abiotic hydrocarbons
684 from new ultramafic hydrothermal sites between 12°N and 15°N on the Mid Atlantic Ridge-
685 Results of the Serpentine cruise (March 2007). *Eos Trans AGU* **88**, Fall Meet Suppl, Abstract
686 T51F-04.

687 Cole JR, Chai B, Marsh TL*, et al.* (2003) The Ribosomal Database Project (RDP-II):
688 previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy.
689 *Nucleic Acids Res* **31**: 442-443.

690 Costello AM & Lidstrom ME (1999) Molecular characterization of functional and phylogenetic
691 genes from natural populations of methanotrophs in lake sediments. *Appl Environ Microbiol*
692 **65**: 5066-5074.

693 Dayhoff MO, Schwartz RM & Orcutt BC (1978) A model of evolutionary change in proteins.
694 *Atlas of Protein Sequences and Structure, Vol. 5* (Dayhoff MO, ed.), pp. 345-352. National
695 Biomedical Research Foundation, Washington DC.

de la Torre JR, Walker CB, Ingalls AE, Konneke M & Stahl DA (2008) Cultivation of a thermophilic ammonia oxidizing archaeon synthesizing crenarchaeol. *Environ Microbiol* **10**: 810-818.

Deming JW & Baross JA (1993) Deep-sea smokers: windows to a subsurface biosphere? *Geochim Cosmochim Acta.* **57**: 3219-3230.

Dosso L, Bougault H & Joron JL (1993) Geochemical morphology of the North Mid-Atlantic Ridge, 10-Degrees-24-Degrees-N - Trace Element-Isotope Complementarity. *Earth Planet Sci Lett* **120**: 443-462.

Duperron S, Bergin C, Zielinski F*,* Pernthaler A, Dando P, McKiness ZP, DeChaine E, Cavanaugh CM & Dubilier N*.* (2006) A dual symbiosis shared by two mussel species, *Bathymodiolus azoricus* and *Bathymodiolus puteoserpentis* (Bivalvia: Mytilidae), from hydrothermal vents along the northern Mid-Atlantic Ridge. *Environ Microbiol* **8**: 1441-1447.

Ehrhardt CJ, Haymon RM, Lamontagne MG & Holden PA (2007) Evidence for hydrothermal *Archaea* within the basaltic flanks of the East Pacific Rise. *Environ Microbiol* **9**: 900-912.

Excoffier L, Laval G & Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evol Bioinform Online* **1**: 47-50.

Flores GE, Campbell JH, Kirshtein JD*, et al.* (2011) Microbial community structure of hydrothermal deposits from geochemically different vent fields along the Mid-Atlantic Ridge. *Environ Microbiol*. doi:10.1111/j.1462-2920.2011.02463.x

Fouquet Y, Vonstackelberg U, Charlou JL, Erzinger J, Herzig PM, Muhe R & Wiedicke M (1993) Metallogenesis in Back-Arc Environments - the Lau Basin Example. *Econ Geol* **88**: 2154-2181.

Fouquet Y, Cherkashov G, Charlou JL, Ondreas H, Cannat M, Bortnikov N, Silantiev S, Etoubleau J & the Scientific Party of the SERPENTINE cruise*.* (2007) Diversity of ultramafic hosted hydrothermal deposits on the Mid Atlantic Ridge: first submersible studies on Ashadze, Logatchev 2 and Krasnov vent fields during the Serpentine cruise. *Eos Trans AGU* **88**, Fall Meet Suppl, Abstract T51F-03.

Fouquet Y, Cherkashov G, Charlou JL*, et al.* (2008) Serpentine cruise - ultramafic hosted hydrothermal deposits on the Mid-Atlantic Ridge: First submersible studies on Ashadze 1 and 2, Logatchev 2 and Krasnov vent fields. *InterRidge News* **17**: 16-41.

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

Galtier N, Gouy M & Gautier C (1996) SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. *Comput Appl Biosci* **12**: 543-548.

Gerasimchuk A, Shatalov A, Novikov A*, et al.* (2010) The search for sulfate-reducing bacteria in mat samples from the lost city hydrothermal field by molecular cloning. *Microbiology* **79**: 96-105**.**

Good IJ (1953) The Population frequencies of species and the estimation of population parameters. *Biometrika* **40**: 237-264.

Hales BA, Edwards C, Ritchie DA, Hall G, Pickup RW & Saunders JR (1996) Isolation and identification of methanogen-specific DNA from blanket bog peat by PCR amplification and sequence analysis. *Appl Environ Microbiol* **62**: 668-675.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**: 95-98.

741 Hallam SJ, Mincer TJ, Schleper C, Preston CM, Roberts K, Richardson PM & DeLong EF
742 (2006) Pathways of carbon assimilation and ammonia oxidation suggested by environmental
743 genomic analyses of marine Crenarchaeota. *PLoS Biol* **4**: 520-536.

744 Harmsen HJM, Prieur D & Jeanthon C (1997) Distribution of microorganisms in deep-sea
745 hydrothermal vent chimneys investigated by whole-cell hybridization and enrichment culture
746 of thermophilic subpopulations. *Appl Environ Microbiol* **63**: 2876-2883.

747 Hatzenpichler R, Lebecleva EV, Spieck E, Stoecker K, Richter A, Daims H & Wagner M
748 (2008) A moderately thermophilic ammonia-oxidizing crenarchaeote from a hot spring. *Proc*
749 *Natl Acad Sci U S A* **105**: 2134-2139.

750 Hirayama H, Sunamura M, Takai K*, et al.* (2007) Culture-dependent and -independent
751 characterization of microbial communities associated with a shallow submarine hydrothermal
752 system occurring within a coral reef off Taketomi Island, Japan. *Appl Environ Microbiol* **73**:
753 7642-7656.

754 Hohn MJ, Hedlund BP & Huber H (2002) Detection of 16S rDNA sequences representing the
755 novel phylum "Nanoarchaeota": Indication for a wide distribution in high temperature
756 biotopes. *Syst Appl Microbiol* **25**: 551-554.

757 Holden JF, Summit M & Baross JA (1998) Thermophilic and hyperthermophilic
758 microorganisms in 3-30 degrees C hydrothermal fluids following a deep-sea volcanic
759 eruption. *FEMS Microbiol Ecol* **25**: 33-41.

760 Holm NG & Charlou JL (2001) Initial indications of abiotic formation of hydrocarbons in
761 Rainbow ultramafic hydrothermal system, Mid-Atlantic Ridge. *Earth Planet Sci Lett* **191**: 1-8.

762 Holmes AJ, Costello A, Lidstrom ME & Murrell JC (1995) Evidence that particulate methane
763 monooxygenase and ammonia monooxygenase may be evolutionarily Related. *FEMS*
764 *Microbiol Lett* **132**: 203-208.

765 Huber H, Hohn MJ, Rachel R, Fuchs T, Wimmer VC & Stetter KO (2002) A new phylum of
766 *Archaea* represented by a nanosized hyperthermophilic symbiont. *Nature* **417**: 63-67.

767 Inagaki F, Takai K, Komatsu T, Kanamatsu T, Fujioka K & Horikoshi K (2001) Archaeology of
768 *Archaea*: geomicrobiological record of Pleistocene thermal events concealed in a deep-sea
769 subseafloor environment. *Extremophiles* **5**: 385-392.

770 Inagaki F, Takai K, Hirayama H, Yamato Y, Nealson KH & Horikoshi K (2003) Distribution
771 and phylogenetic diversity of the subsurface microbial community in a Japanese epithermal
772 gold mine. *Extremophiles* **7**: 307-317.

773 Juniper SK, Cambon MA, Lesongeur F & Barbier G (2001) Extraction and purification of DNA
774 from organic rich subsurface sediments (ODP Leg 169S). *Mar Geol* **174**: 241-247.

775 Kallmeyer J & Boetius A (2004) Effects of temperature and pressure on sulfate reduction and
776 anaerobic oxidation of methane in hydrothermal sediments of Guaymas Basin. *Appl Environ*
777 *Microbiol* **70**: 1231-1233.

778 Kashefi K, Tor JM, Holmes DE, Gaw Van Praagh CV, Reysenbach AL & Lovley DR (2002)
779 *Geoglobus ahangari* gen. nov., sp. nov., a novel hyperthermophilic archaeon capable of
780 oxidizing organic acids and growing autotrophically on hydrogen with Fe(III) serving as the
781 sole electron acceptor. *Int J Syst Evol Microbiol* **52**: 719-728.

782 Kaye JZ & Baross JA (2000) High incidence of halotolerant bacteria in Pacific hydrothermal-
783 vent and pelagic environments. *FEMS Microbiol Ecol* **32**: 249-260.

784 Kelley DS, Baross JA & Delaney JR (2002) Volcanoes, fluids, and life at mid-ocean ridge
785 spreading centers. *Annu Rev Earth Planet Sci* **30**: 385-491.

786 Kelley DS, Karson JA, Blackman DK*, et al.* (2001) An off-axis hydrothermal vent field near
787 the Mid-Atlantic Ridge at 30[deg] N. *Nature* **412**: 145-149.

788 Kelley DS, Karson JA, Fruh-Green GL*, et al.* (2005) A serpentinite-hosted ecosystem: the
789 Lost City hydrothermal field. *Science* **307**: 1428-1434.

790 Kimura H, Sugihara M, Kato K & Hanada S (2006) Selective phylogenetic analysis targeted
791 at 16S rRNA genes of thermophiles and hyperthermophiles in deep-subsurface geothermal
792 Environments. *Appl Environ Microbiol* **72**: 21-27.

793 Kondo R, Nedwell DB, Purdy KJ & Silva SD (2004) Detection and enumeration of sulphate-
794 reducing bacteria in estuarine sediments by competitive PCR. *Geomicrobiol J* **21**: 145-157.

795 Konneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB & Stahl DA (2005)
796 Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* **437**: 543-546.

797 Kormas KA, Tivey MK, Von Damm K & Teske A (2006) Bacterial and archaeal phylotypes
798 associated with distinct mineralogical layers of a white smoker spire from a deep-sea
799 hydrothermal vent site (9 degrees N, East Pacific Rise). *Environ Microbiol* **8**: 909-920.

800 Kurr M, Huber R, Konig H*, et al.* (1991) *Methanopyrus kandleri*, Gen and sp-nov represents a
801 novel group of hyperthermophilic methanogens, Growing at 110°C. *Arch Microbiol* **156**: 239-
802 247.

803 Lam P, Cowen JP, Popp BN & Jones RD (2008) Microbial ammonia oxidation and enhanced
804 nitrogen cycling in the Endeavour hydrothermal plume. *Geochim Cosmochim Acta* **72**: 2268-
805 2286.

806 Lepage E, Marguet E, Geslin C, Matte-Tailliez O, Zillig W, Forterre P & Tailliez P (2004)
807 Molecular diversity of new Thermococcales isolates from a single area of hydrothermal deep-
808 sea vents as revealed by randomly amplified polymorphic DNA fingerprinting and 16S rRNA
809 gene sequence analysis. *Appl Environ Microbiol* **70**: 1277-1286.

810 López-García P, Philippe H, Gail F & Moreira D (2003a) Autochthonous eukaryotic diversity
811 in hydrothermal sediment and experimental microcolonizers at the Mid-Atlantic Ridge. *Proc*
812 *Natl Acad Sci U S A* **100**: 697-702.

813 López-García P, Duperron S, Philippot P, Foriel J, Susini J & Moreira D (2003b) Bacterial
814 diversity in hydrothermal sediment and epsilonproteobacterial dominance in experimental
815 microcolonizers at the Mid-Atlantic Ridge. *Environ Microbiol* **5**: 961-976.

816 Martin AP (2002) Phylogenetic approaches for describing and comparing the diversity of
817 microbial communities. *Appl Environ Microbiol* **68**: 3673-3682.

818 Mason OU, Di Meo-Savoie CA, Van Nostrand JD, Zhou JZ, Fisk MR & Giovannoni SJ (2009)
819 Prokaryotic diversity, distribution, and insights into their role in biogeochemical cycling in
820 marine basalts. *ISME J* **3**: 231-242.

821 Mason OU, Stingl U, Wilhelm LJ, Moeseneder MM, Di Meo-Savoie CA, Fisk MR &
822 Giovannoni SJ (2007) The phylogeny of endolithic microbes associated with marine basalts.
823 *Environ Microbiol* **9**: 2539-2550.

824 McCliment EA, Voglesonger KM, O'Day PA, Dunn EE, Holloway JR & Cary SC (2006)
825 Colonization of nascent, deep-sea hydrothermal vents by a novel Archaeal and
826 Nanoarchaeal assemblage. *Environ Microbiol* **8**: 114-125.

827 McCollom TM (2007) Geochemical constraints on sources of metabolic energy for
828 chemolithoautotrophy in ultramafic-hosted deep-sea hydrothermal systems. *Astrobiology* **7**:
829 933-950.

830 Mesbah NM & Wiegel J (2008) Life at extreme limits: the anaerobic halophilic
831 alkalithermophiles. *Ann N Y Acad Sci* **1125**: 44-57.

832 Miroshnichenko ML & Bonch-Osmolovskaya EA (2006) Recent developments in the
833 thermophilic microbiology of deepsea hydrothermal vents. *Extremophiles* **10**: 85-96.

834 Moussard H, Corre E, Cambon-Bonavita MA, Fouquet Y & Jeanthon C (2006a) Novel
835 uncultured *Epsilon-Proteobacteria* dominate a filamentous sulphur mat from the 13°N
836 hydrothermal vent field, East Pacific Rise. *FEMS Microbiol Ecol* **58**: 449-463.

837 Moussard H, Moreira D, Cambon-Bonavita MA, López-García P & Jeanthon C (2006b)
838 Uncultured *Archaea* in a hydrothermal microbial assemblage: phylogenetic diversity and
839 characterization of a genome fragment from a euryarchaeote. *FEMS Microbiol Ecol* **57**: 452-
840 469.

841 Moussard H, Henneke G, Moreira D, Jouffe V, López-García P & Jeanthon C (2006c)
842 Thermophilic lifestyle for an uncultured archaeon from hydrothermal vents: Evidence from
843 environmental genomics. *Appl Environ Microbiol* **72**: 2268-2271.

844 Mozgova NN, Trubkin NV, Borodaev YS, Cherkashev GA, Stepanova TV, Semkova TA &
845 Uspenskaya TY (2008) Mineralogy of massive sulfides from the Ashadze hydrothermal field,
846 13°N, Mid-Atlantic Ridge. *Can Mineral* **46**: 545-567.

847 Nealson KH, Inagaki F & Takai K (2005) Hydrogen-driven subsurface lithoautotrophic
848 microbial ecosystems (SLiMEs): do they exist and why should we care? *Trends Microbio* **13**:
849 405-410.

850 Nercessian O, Reysenbach AL, Prieur D & Jeanthon C (2003) Archaeal diversity associated
851 with in situ samplers deployed on hydrothermal vents on the East Pacific Rise (13 °N).
852 *Environ Microbiol* **5**: 492-502.

853 Nercessian O, Bienvenu N, Moreira D, Prieur D & Jeanthon C (2005) Diversity of functional
854 genes of methanogens, methanotrophs and sulfate reducers in deep-sea hydrothermal
855 environments. *Environ Microbiol* **7**: 118-132.

856 Nicol GW & Schleper C (2006) Ammonia-oxidising Crenarchaeota: important players in the
857 nitrogen cycle? *Trends Microbiol* **14**: 207-212.

858 Nicol GW, Glover LA & Prosser JI (2003) The impact of grassland management on archaeal
859 community structure in upland pasture rhizosphere soil. *Environ Microbiol* **5**: 152-162.

860 Niemann H, Losekann T, de Beer D*, et al.* (2006) Novel microbial communities of the
861 Haakon Mosby mud volcano and their role as a methane sink. *Nature* **443**: 854-858.

862 Nocker A, Burr M & Camper AK (2007) Genotypic microbial community profiling: A critical
863 technical review. *Microb Ecol* **54**: 276-289.

864 Nunoura T, Oida H, Nakaseama M*, et al.* (2010) Archaeal diversity and distribution along
865 thermal and geochemical gradients in hydrothermal sediments at the Yonaguni Knoll IV
866 hydrothermal field in the southern Okinawa Trough. *Appl Environ Microbiol* **76**: 1198-1211.

867 Page A, Tivey MK, Stakes DS & Reysenbach AL (2008) Temporal and spatial archaeal
868 colonization of hydrothermal vent deposits. *Environ Microbiol* **10**: 874-884.

869 Paper W, Jahn U, Hohn MJ*, et al.* (2007) *Ignicoccus hospitalis* sp nov, the host of
870 '*Nanoarchaeum equitans*'. *Int J Syst Evol Microbiol* **57**: 803-808.

871 Perner M, Kuever J, Seifert R*, et al.* (2007) The influence of ultramafic rocks on microbial
872 communities at the Logatchev hydrothermal field, located 15 degrees N on the Mid-Atlantic
873 Ridge. *FEMS Microbiol Ecol* **61**: 97-109.

874 Polz MF & Cavanaugh CM (1998) Bias in template-to-product ratios in multitemplate PCR.
875 *Appl Environ Microbiol* **64**: 3724-3730.

876 Preston CM, Wu KY, Molinski TF & DeLong EF (1996) A psychrophilic crenarchaeon inhabits
877 a marine sponge: *Cenarchaeum symbiosum* gen nov, sp, nov. *Proc Natl Acad Sci U S A* **93**:
878 6241-6246.

879 Raymond M & Rousset F (1995) An exact test for population differentiation. *Evolution* **49**:
880 1280-1283.

881 Reeve JN, Nolling J, Morgan RM & Smith DR (1997) Methanogenesis: genes, genomes, and
882 who's on first? *J Bacteriol* **179**: 5975-5986.

883 Reysenbach AL, Liu YT, Banta AB*,* Beveridge TJ, Kirshtein JD, Schouten S, Tivey MK, Von
884 Damm KL & Voytek MA (2006) A ubiquitous thermoacidophilic archaeon from deep-sea
885 hydrothermal vents. *Nature* **442**: 444-447.

886 Roussel EG, Sauvadet A-L, Allard J, Chaduteau C, Richard P, Cambon Bonavita M-A &
887 Chaumillon E (2009a) Archaeal methane cycling communities associated with gassy
888 subsurface sediments of Marennes-Oléron Bay (France). *Geomicrobiol J* **26**: 31-43.

889 Roussel EG, Sauvadet AL, Chaduteau C, Fouquet Y, Charlou JL, Prieur D & Cambon
890 Bonavita MA (2009b) Archaeal communities associated with shallow to deep subseafloor
891 sediments of the New Caledonia Basin. *Environ Microbiol* **11**: 2446-2462.

892 Saitou N & Nei M (1987) The neighbor-joining method: a new method for reconstructing
893 phylogenetic trees. *Mol Biol Evol* **4**: 406-425.

894 Schloss PD & Handelsman J (2004) Status of the microbial census. *Microbiol Mol Biol Rev*
895 **68**: 686-691.

896 Schloss PD & Handelsman J (2005) Introducing DOTUR, a computer program for defining
897 operational taxonomic units and estimating species richness. *Appl Environ Microbiol* **71**:
898 1501-1506.

899 Schloss PD & Handelsman J (2006) Introducing SONS, a tool for operational taxonomic unit-
900 based comparisons of microbial community memberships and structures. *Appl Environ*
901 *Microbiol* **72**: 6773-6779.

902 Schmidt K, Koschinsky A, Garbe-Schonberg D, de Carvalho LM & Seifert R (2007)
903 Geochemistry of hydrothermal fluids from the ultramafic-hosted Logatchev hydrothermal
904 field, 15 degrees N on the Mid-Atlantic Ridge: Temporal and spatial investigation. *Chem Geol*
905 **242**: 1-21.

906 Schrenk MO, Kelley DS, Delaney JR & Baross JA (2003) Incidence and diversity of
907 microorganisms within the walls of an active deep-sea sulfide chimney. *Appl Environ*
908 *Microbiol* **69**: 3580-3592.

909 Schrenk MO, Kelley DS, Bolton SA & Baross JA (2004) Low archaeal diversity linked to
910 subseafloor geochemical processes at the Lost City Hydrothermal Field, Mid-Atlantic Ridge.
911 *Environ Microbiol* **6**: 1086-1095.

912 Singleton DR, Furlong MA, Rathbun SL & Whitman WB (2001) Quantitative comparisons of
913 16S rRNA gene sequence libraries from environmental samples. *Appl Environ Microbiol* **67**:
914 4374-4376.

915 Slobodkina GB, Kolganova TV, Querellou J, Bonch-Osmolovskaya EA & Slobodkin AI (2009)
916 *Geoglobus acetivorans* sp. nov., an iron(III)-reducing archaeon from a deep-sea
917 hydrothermal vent. *Int J Syst Evol Microbiol* **59**: 2880-2883.

918 Sokolova TG, Jeanthon C, Kostrikina NA, Chernyh NA, Lebedinsky AV, Stackebrandt E &
919 Bonch-Osmolovskaya EA (2004) The first evidence of anaerobic CO oxidation coupled with
920 H_2 production by a hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent.
921 *Extremophiles* **8**: 317-323.

922 Summit M & Baross JA (1998) Thermophilic subseafloor microorganisms from the 1996
923 north Gorda Ridge eruption. *Deep Sea Res Part 2 Top Stud Oceanogr* **45**: 2751-2766.

924 Summit M & Baross JA (2001) A novel microbial habitat in the mid-ocean ridge subseafloor.
925 *Proc Natl Acad Sci U S A* **98**: 2158-2163.

926 Suzuki MT & Giovannoni SJ (1996) Bias caused by template annealing in the amplification of
927 mixtures of 16S rRNA genes by PCR. *Appl Environ Microbiol* **62**: 625-630.

928 Takai K & Horikoshi K (1999) Genetic diversity of *Archaea* in deep-sea hydrothermal vent
929 environments. *Genetics* **152**: 1285-1297.

930 Takai K, Nakamura K, Toki T, Tsunogai U, Miyazaki M, Miyazaki J, Hirayama H, Nakagawa
931 S, Nunoura T & Horikoshi K (2008) Cell proliferation at 122°C and isotopically heavy CH_4
932 production by a hyperthermophilic methanogen under high-pressure cultivation. *Proc Natl*
933 *Acad Sci U S A* **105**:10949-10954

934 Takai K, Komatsu T, Inagaki F & Horikoshi K (2001) Distribution of *Archaea* in a black
935 smoker chimney structure. *Appl Environ Microbiol* **67**: 3618-3629.

936 Takai K, Gamo T, Tsunogai U, Nakayama N, Hirayama H, Nealson KH & Horikoshi K
937 (2004a) Geochemical and microbiological evidence for a hydrogen-based, hyperthermophilic
938 subsurface lithoautotrophic microbial ecosystem (HyperSLiME) beneath an active deep-sea
939 hydrothermal field. *Extremophiles* **8**: 269-282.

940 Takai K, Inagaki F, Teske A*, et al.* (2004b) Subseaflloor microbial ecosysteme associated
941 with physical, geochimical and hydrogeologic variations in th mid Okinawa through
942 hydrothermal systems. *IODP Proposal 601 full* 1-29.

943 Takai K, Oida H, Suzuki Y*,* Hirayama H, Nakagawa S, Nunoura T, Inagaki F, Nealson KH *&*
944 Horikoshi K (2004c) Spatial distribution of marine crenarchaeota group I in the vicinity of
945 deep-sea hydrothermal systems. *Appl Environ Microbiol* **70**: 2404-2413.

946 Teske A & Sorensen KB (2008) Uncultured *Archaea* in deep marine subsurface sediments:
947 have we caught them all? *ISME J* **2**: 3-18.

948 Teske A, Hinrichs KU, Edgcomb V*,* de Vera Gomez A, Kysela D, Sylva SP, Sogin ML *&*
949 Jannasch HW (2002) Microbial diversity of hydrothermal sediments in the Guaymas Basin:
950 evidence for anaerobic methanotrophic communities. *Appl Environ Microbiol* **68**: 1994-2007.

951 Thompson JD, Higgins DG & Gibson TJ (1994) CLUSTAL W: improving the sensitivity of
952 progressive multiple sequence alignment through sequence weighting, position-specific gap
953 penalties and weight matrix choice. *Nucleic Acids Res* **22**: 4673-4680.

954 von Wintzingerode F, Gobel UB & Stackebrandt E (1997) Determination of microbial diversity
955 in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiol Rev* **21**:
956 213-229.

957 Voordeckers JW, Do MH, Hugler M, Ko V, Sievert SM & Vetriani C (2008) Culture dependent
958 and independent analyses of 16S rRNA and ATP citrate lyase genes: a comparison of
959 microbial communities from different black smoker chimneys on the Mid-Atlantic Ridge.
960 *Extremophiles* **12**: 627-640.

961 Wagner ID & Wiegel J (2008) Diversity of thermophilic anaerobes. *Ann N Y Acad Sci* **1125**:
962 1-43.

963 Wang SF, Xiao X, Jiang LJ, Peng XT, Zhou HY, Meng J & Wang FP (2009) Diversity and
964 abundance of ammonia-oxidizing *Archaea* in hydrothermal vent chimneys of the Juan de
965 Fuca Ridge. *Appl Environ Microbiol* **75**: 4216-4220.

966 Webster G, Newberry CJ, Fry JC & Weightman AJ (2003) Assessment of bacterial
967 community structure in the deep sub-seafloor biosphere by 16S rDNA-based techniques: a
968 cautionary tale. *J Microbiol Methods* **55**: 155-164.

969 Webster G, Rinna J, Roussel EG, Fry JC, Weightman AJ & Parkes RJ (2010) Prokaryotic
970 functional diversity in different biogeochemical depth zones in tidal sediments of the Severn
971 Estuary, UK, revealed by stable-isotope probing. *FEMS Microbiol Ecol* **72**: 179-197.

972 Whitman WB, Boone DR & Koga Y (2001) Bergey's Manual of Systematic Bacteriology. *The*
973 *Archaea and The Deeply Branching and Phototrophic Bacteria, Vol. 1* (Boone DR,
974 Castenholz RW & Garrity G, eds.), pp. 236-246. Springer, Berlin & Heidelberg.

975 Zbinden M, Shillito B, Le Bris N*, et al.* (2008) New insigths on the metabolic diversity among
976 the epibiotic microbial communitiy of the hydrothermal shrimp *Rimicaris exoculata*. *J Exp Mar*
977 *Bio Ecol* **359**: 131-140.

978 Zeng X, Birrien JL, Fouquet Y*, et al.* (2009) *Pyrococcus* CH1, an obligate piezophilic
979 hyperthermophile: extending the upper pressure-temperature limits for life. *ISME J* **3**: 873-
980 876.

981 Zhang CL, Ye Q, Huang ZY*, et al.* (2008) Global occurrence of archaeal *amoA* genes in
982 terrestrial hot springs. *Appl Environ Microbiol* **74**: 6417-6426.

983

984 Captions

985 **Fig. 1.** (a) Location map of Atlantic ultramafic-hosted hydrothermal sites mentioned in this
986 study. (b) Photograph of the sediment sampling at Lost City hydrothermal field. (c)
987 Photograph of the fluid sampling using titanium syringe at Lost City hydrothermal field. (d).
988 Photograph of the sediment sampling using push-core devices at Rainbow hydrothermal
989 field. (e). Photograph of the fluid sampling using titanium syringe at Rainbow hydrothermal
990 field. (f). Photograph of temperature measurements at Ashadze 1 hydrothermal field.

991 **Fig. 2.** (a) Co-migration denaturant gradient gel electrophoresis (CM-DGGE) analysis of
992 archaeal 16S rRNA genes from seawater (blue) compared to Rainbow, Ashadze and Lost
993 City hydrothermal environments (red). The white arrows indicate the position of faint DGGE
994 bands. PCR products were amplified with the Saf-PARCH 519r*Cy5 (blue) or Saf-PARCH
995 519r*Cy3 (red) primer set and electrophoresis was performed using a gradient of 30–70%
996 denaturant. (b) Distribution of the archaeal phylogenetic communities based on 16S rRNA
997 gene from three ultramafic-hosted hydrothermal sites. The phylogenetic affiliation of each
998 clone sequence was determined by similarity analysis. For each phylogenetic affiliation, the
999 average G + C content of the detected 16S rRNA gene sequences is shown in brackets. The
1000 relative abundance of each phylotype was calculated and represented in a column diagram.
1001 Cx indicates coverage percentage for each clone library. OTU indicates the number of
1002 operational taxonomic units (95%) for each clone library. SW indicates the Shannon-Wiener
1003 index of diversity. *dsr*, *pmo* and *mcr*, respectively indicate positive amplification of the
1004 functional genes. ND: not determined. The asterisks indicate groups of clone libraries with
1005 insignificant ($P < 0.001$) differences between all the diversity indices (F_{ST} and the exact test

method). ANME-2: *an*aerobic *me*thane oxidizers, DHVE2: Deep-sea Hydrothermal Vent *Euryarchaeota*, MBG-D: Marine Benthic Group D, MBG-A: Marine Benthic Group A, MG-1 (II, III, IV): Marine Group 1 (II, III, IV), MBG-E: Marine Benthic Group E, UHE-1: Unaffiliated Hydrothermal *Euryarchaeota*.

Fig. 3. (a) Phylogenetic tree representing the *Euryarchaeota* 16S rRNA gene sequences. Each phylotype is represented by one sequence with ≥97% similarity grouping. The tree was constructed using the Neighbor-Joining method with Jukes and Cantor correction. Bootstrap values <50% are not shown. Circles symbolize Ashadze clone libraries. Triangles symbolize Rainbow clone libraries. Squares symbolize Lost City clone libraries. Underlined sequences: seawater clone library. ANME: *an*aerobic *me*thane oxidizers, DHVE: Deep-sea Hydrothermal Vent *Euryarchaeota*, MBG-D: Marine Benthic Group D, MBG-E: Marine Benthic Group E, SAGMEG: South African Gold Mine Euryarchaeotic Group, UHE-1: Unaffiliated Hydrothermal *Euryarchaeota*. (b) Phylogenetic tree representing the *Crenarchaeota* 16S rRNA gene sequences. Each phylotype is represented by one sequence with ≥97% similarity grouping. The tree was constructed using the Neighbor-Joining method with Jukes and Cantor correction. Bootstrap values <50% are not shown. Circles symbolize Ashadze clone libraries. Triangles symbolize Rainbow clone libraries. Squares symbolize Lost City clone libraries. Underlined sequences: seawater clone library. MCG: Miscellaneous Crenarchaeotal Group, MBG-B: Marine Benthic Group B, MBG-A: Marine Benthic Group A.

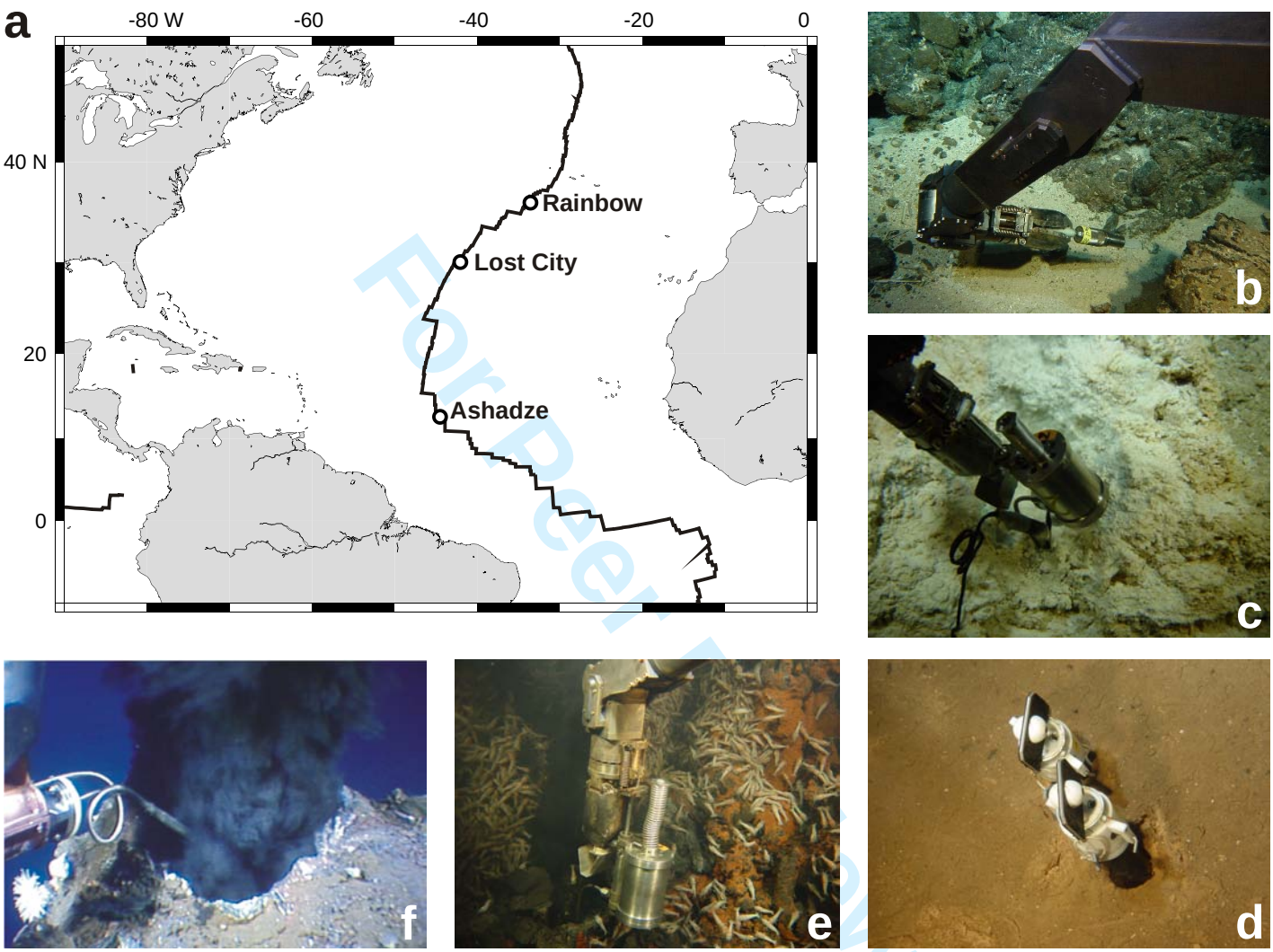
Fig. 4. Phylogenetic trees based on translated partial amino acid sequences of functional genes (*mcrA*, *dsrA*, *pmoA*). The trees were constructed using the Neighbor-Joining method using PAM distance (Dayhoff *et al.*, 1978). Bootstrap values <50% are not shown. Circles symbolize Ashadze clone libraries. Triangles symbolize Rainbow clone libraries. Squares symbolize Lost City clone libraries. (a) *mcrA* gene. (b) *dsrA* gene. (c) *pmoA* gene.

Fig. 5. Hypothetical model (not to scale) of microbial ecological niches in acidic Atlantic ultramafic-hosted hydrothermal systems (Rainbow, Ashadze). Each ecological niche was described by its average temperature, potential electron donors and acceptors metabolized

by the microbial communities described in this study, and the distribution of these microbial communities. OM: Organic matter. *Lineage only detected from Ashadze.

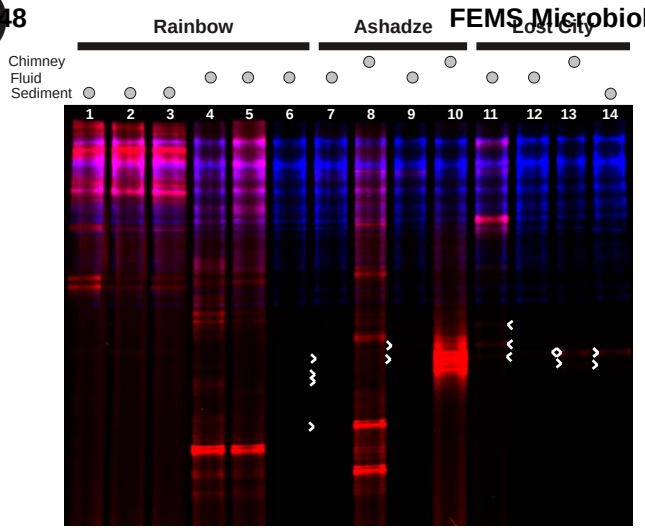
Supplementary material

Fig. S1. Rarefaction curves for the 16S rRNA gene clone libraries from the Fairway and New Caledonia Basin sites (Schloss and Handelsman, 2005). The sequence identity levels are represented in brackets.



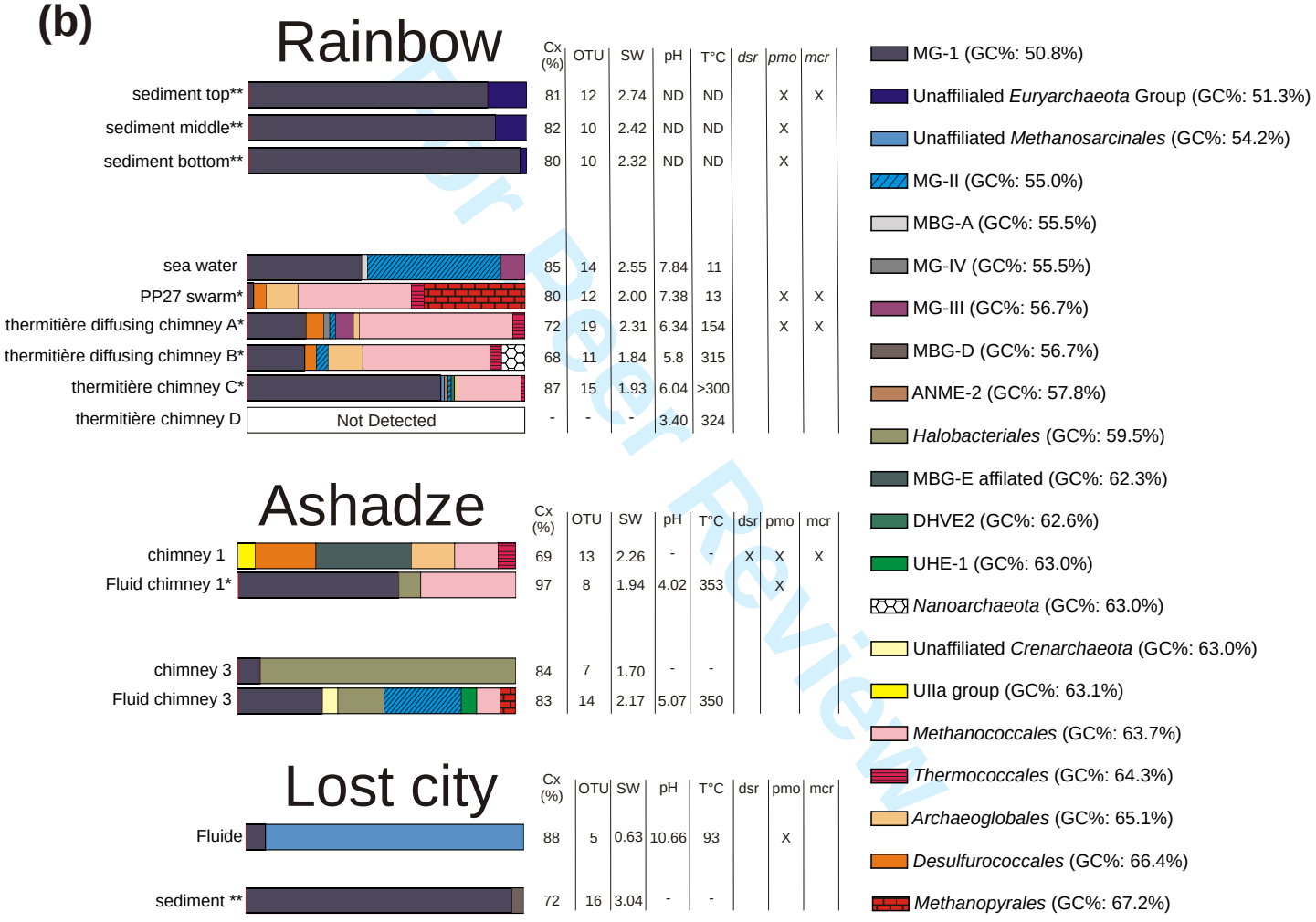
For Peer Review

(a)



1 Rainbow, sediment top + Seawater
2 Rainbow, sediment middle + Seawater
3 Rainbow, sediment bottom + Seawater
4 Rainbow, PP27 swarm + Seawater
5 Rainbow, thermitière diffusing chimney A + Seawater
6 Rainbow, thermitière diffusing chimney B + Seawater
7 Ashadze, Fluid chimney 1 + Seawater
8 Ashadze, chimney 1 + Seawater
9 Ashadze, Fluid chimney 3 + Seawater
10 Ashadze, chimney 3 + Seawater
11 Lost City, Fluid (pH 10.66) + Seawater
12 Lost City, Fluid (pH 11.75) + Seawater
13 Lost City, chimney + Seawater
14 Lost City, sediment + Seawater

(b)



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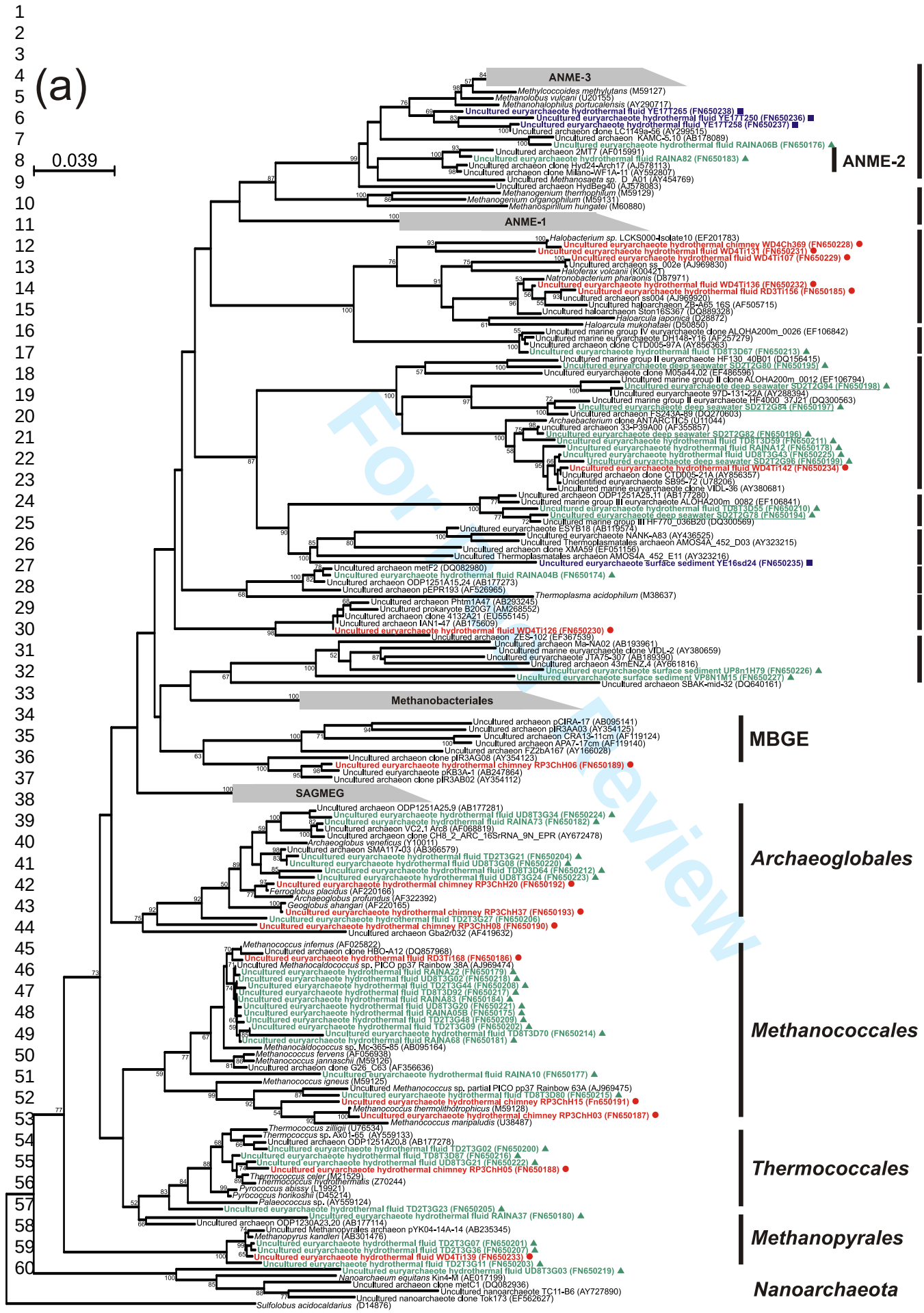

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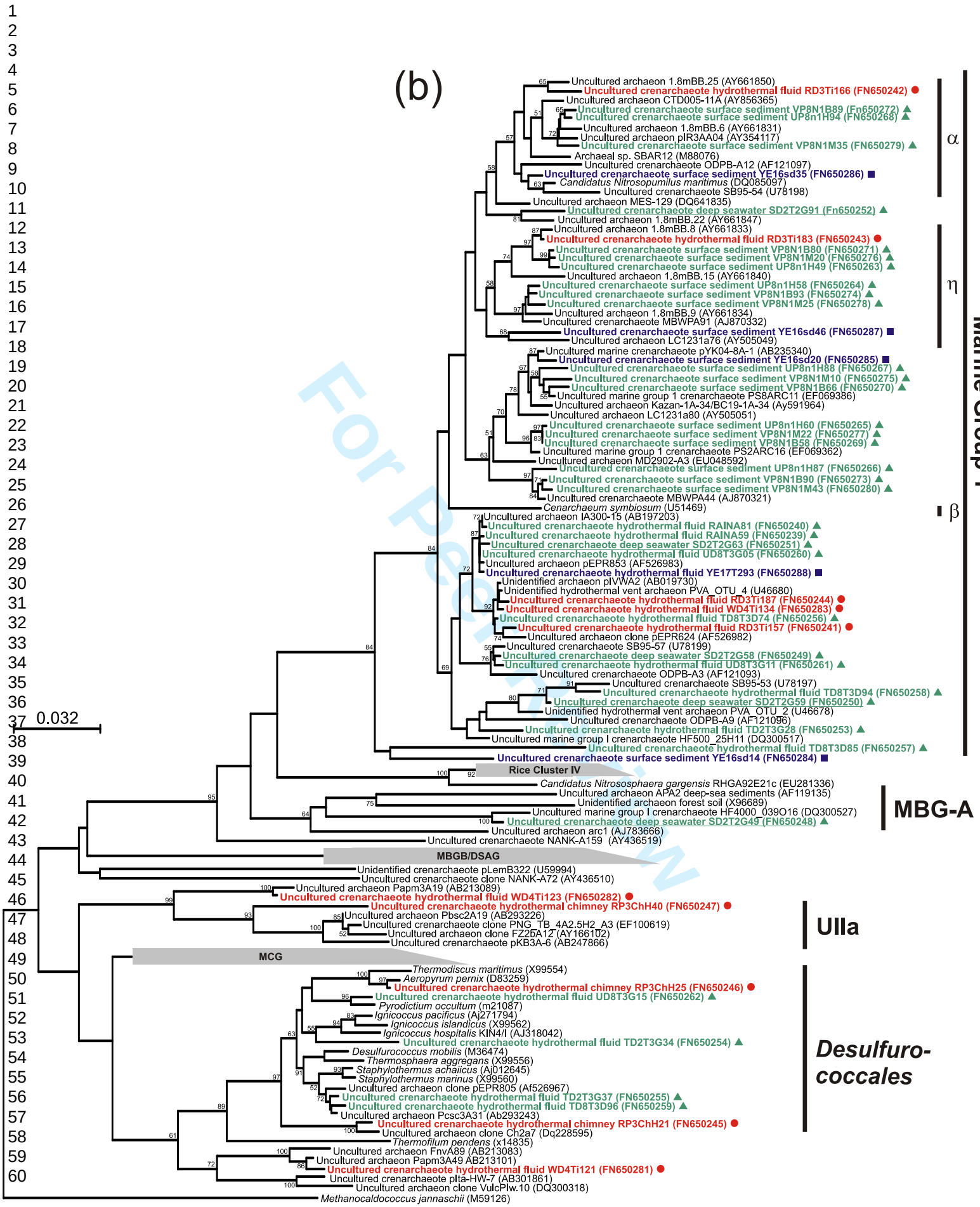

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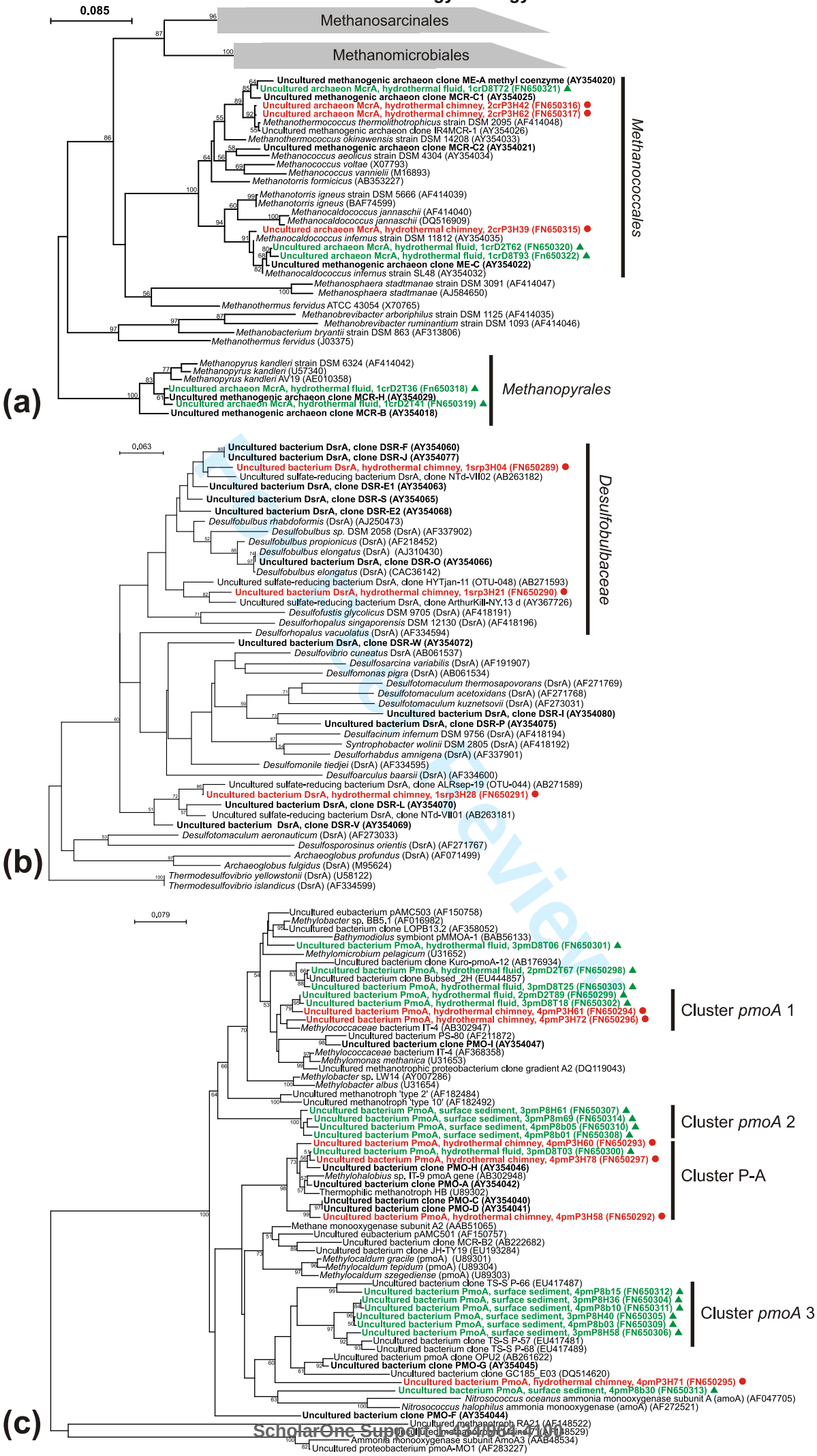

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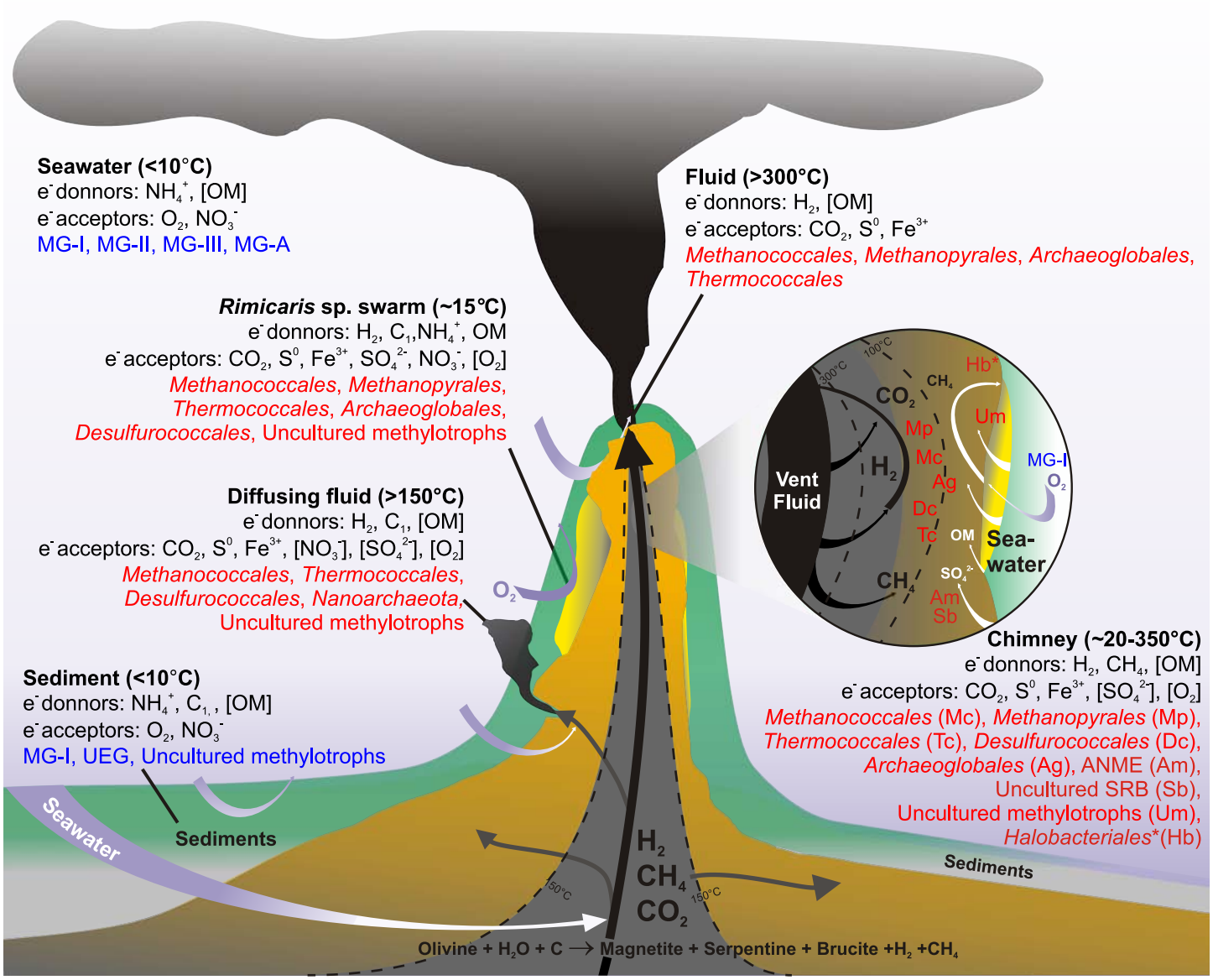
Seawater (<10°C)
e⁻ donnors: NH4+, [OM]
e⁻ acceptors: O2, NO3⁻
MG-I, MG-II, MG-III, MG-A
Fluid (>300°C)
e⁻ donnors: H2, [OM]
e⁻ acceptors: CO2, S0, Fe3+
Methanococcales, Methanopyrales, Archaeoglobales, Thermococcales
Rimicaris sp. swarm (~15°C)
e⁻ donnors: H2, C1,NH4+, OM
e⁻ acceptors: CO2, S0, Fe3+, SO4 2-, NO3⁻, [O2]
Methanococcales, Methanopyrales, Thermococcales, Archaeoglobales, Desulfurococcales, Uncultured methylotrophs
Diffusing fluid (>150°C)
e⁻ donnors: H2, C1, [OM]
e⁻ acceptors: CO2, S0, Fe3+, [NO3⁻], [SO4 2-], [O2]
Methanococcales, Thermococcales, Desulfurococcales, Nanoarchaeota, Uncultured methylotrophs
Sediment (<10°C)
e⁻ donnors: NH4+, C1, , [OM]
e⁻ acceptors: O2, NO3⁻
MG-I, UEG, Uncultured methylotrophs
Chimney (~20-350°C)
e⁻ donnors: H2, CH4, [OM]
e⁻ acceptors: CO2, S0, Fe3+, [SO4 2-], [O2]
Methanococcales (Mc), Methanopyrales (Mp), Thermococcales (Tc), Desulfurococcales (Dc), Archaeoglobales (Ag), ANME (Am), Uncultured SRB (Sb), Uncultured methylotrophs (Um), Halobacteriales*(Hb)
Vent Fluid
Sea-water
OM
Seawater
Sediments
Sediments
H2
CH4
CO2
Olivine + H2O + C → Magnetite + Serpentine + Brucite +H2 +CH4
Macrofaunal communities
Anaerobic Hydrogen-oxidizing communities
Aerobic methane-oxidizing communities
Psycrophilic communities
Mesophilic to hyperthermophilic communities
[Variable concentration]

Supplementary material

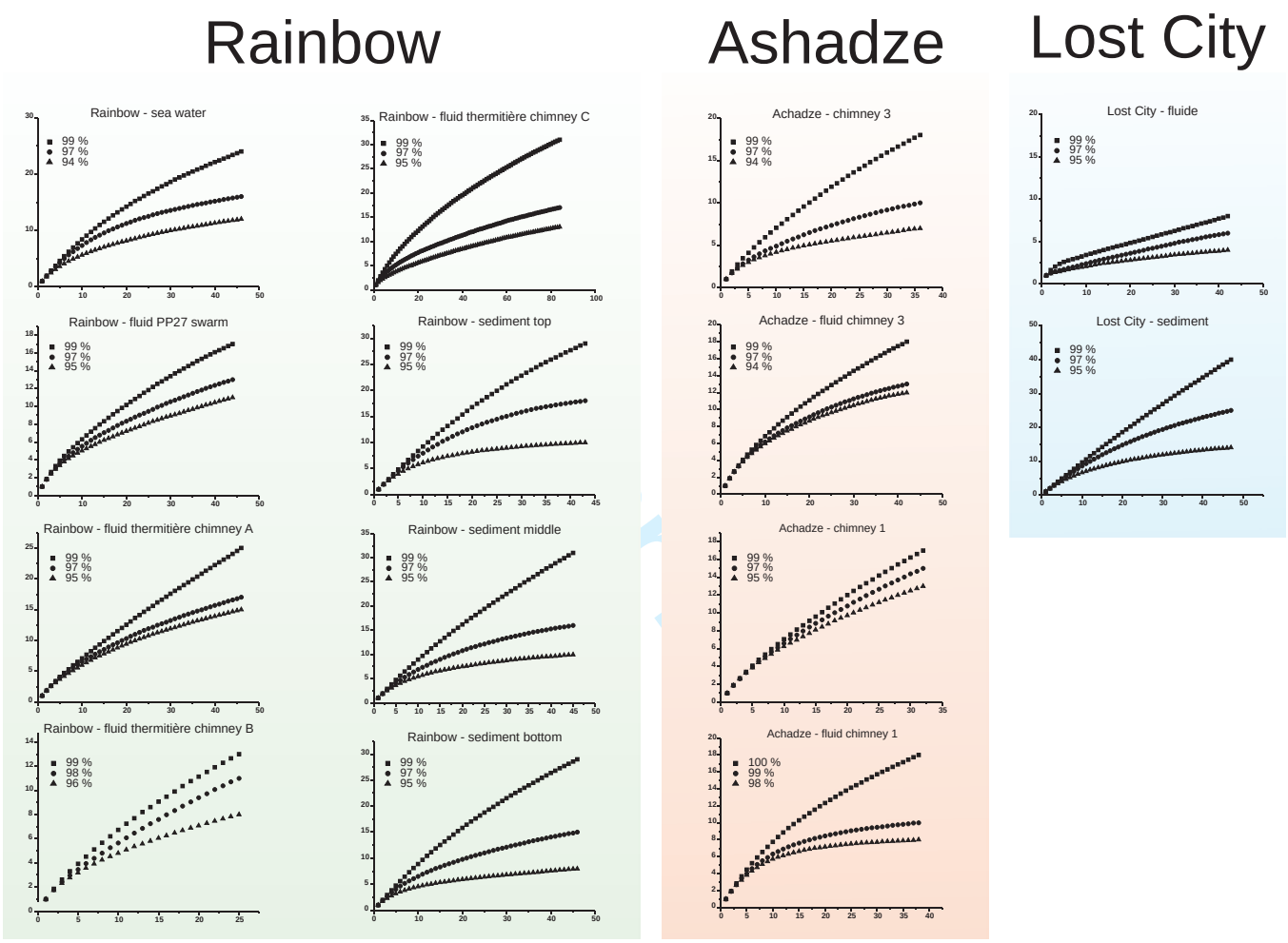

Fig. S1. Rarefaction curves for the 16S rRNA gene clone libraries from the Fairway and New Caledonia Basin sites (Schloss and Handelsman, 2005). The sequence identity levels are represented in brackets.