A Serpentinite-Hosted Ecosystem: The Lost City Hydrothermal Field

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Supporting Online Material

Materials and Methods

Bathymetric Analyses using the Autonomous Benthic Explorer (ABE): (Dana Yoerger, Al Bradley, Mike Jakuba, Woods Hole Oceanographic Institution)

The 17 missions flown by ABE in 2003 produced a bathymetric map of the Lost City Hydrothermal Field that is resolved at the meter scale. All bathymetric data were collected using a SM2000 sonar mounted on the vehicle in either a down-looking or forward-looking mode. This configuration allowed optimal imaging of the relatively smooth, flat-topped surface of the massif as well as the steep cliffs and pinnacles. Typically ABE flew at an altitude of 50-100 m off the bottom, with survey lines commonly flown at 100 m spacing to provide significant overlap.

Digital Imaging: (Deborah Kelley, Mitch Elend, University of Washington, Jeff Karson, Duke University)

Digital images used in this paper were obtained using deep-sea digital cameras made by DeepSea Power & Light (DSPL), San Diego, CA (DigiSeacam model). They

were kindly provided by D. J. Fornari, Woods Hole Oceanographic Institution. The cameras were developed jointly by D.J. Fornari and M. Olsson at DSPL with funding from the National Science Foundation. Each image is 3.3 Mpixel in size, taken with a ®Nikon 995 camera mounted in a DSPL 6000 meter-rated housing.

Carbonate Chemical and Petrological Analyses: (Kris Ludwig, University of Washington)

Carbonate samples were analyzed for mineralogical composition using powder x-ray diffraction (XRD) at the University of Washington (UW) and at ETH-Zurich. Polished thin sections were analyzed using a Nikon Eclipse E600 petrographic microscope. Concentrations of major cations (Na, K, Mg, Ca) in the carbonate samples were measured at the Pacific Marine Environmental Laboratories of the National Oceanographic and Atmospheric Administration (PMEL-NOAA) in Washington. Sub-samples of digested carbonate were diluted 100-fold by mass and analyzed using a Dionex DX500 ion chromatograph with 2 sigma, 2% precision. The carbonates were analyzed for trace element concentrations at the UW. All samples were hand-crushed to a fine-grained powder using an agate mortar and pestle. Approximately 75 mg sub-samples of each sample were homogenized using a fair-split method. These were digested using a three day digestion procedure modified from Murray and Leinen (*2*) and described in Morford and Emerson (*3*) and analyzed for trace metal concentrations using a Perkin Elmer Elan 5000 Ar-gas inductively coupled plasma-mass spectrometer (ICP-MS) in peak-hopping mode with an AS-90 autosampler. A standard curve technique was used for all analyses,

with a $CaCO_3$ matrix-matched curve. Sr concentrations were analyzed using a Perkin Elmer 5000 flame atomic absorption (AA) spectrophotometer at the UW.

Analyses of Hydrothermal Vent Fluids and Water Column Samples: (Dave Butterfield, Marvin Lilley, Kevin, Roe, Eric Olson, Giora Proskurowski, Ben Larson, University of Washington)

Vent fluid samples were collected from the submersible Alvin in titanium major and evacuated "gas-tight" samplers. Temperatures were measured with the Alvin high-temperature probe before and/or after sampling. In addition, fluid samples were collected from Alvin using the Hydrothermal Fluid and Particle Sampler (HFPS). The HFPS pumps vent fluid through a titanium intake nozzle and measures the temperature of the fluid just inside the nozzle and at a second point inside the titanium/teflon fluid line near the sample containers. Temperature is recorded at 1Hz and the average inlet temperature is calculated for the period during which the sample is taken. This allows us to know with precision the temperature of the water collected. Sample containers are either collapsible plastic bags (with valves) within rigid housings, or PVC piston samplers with teflon spring seals. A small inlet orifice and Teflon check valves prevent the samples from leaking out or being drawn out of the containers. The HFPS was configured to collect 14 discrete fluid samples of up to 800 ml volume each, with the option of filtering 8 of those samples. In addition, 9 separate filters were used to concentrate particles from fluids of known temperature by in-situ filtration, allowing us to collect and analyze minerals or microbes by a number of techniques.

Upon arrival on deck, sample valves were closed and samples stored under

refrigeration until processed. Fluid samples were analyzed on board ship for pH, alkalinity, hydrogen sulfide, dissolved silica, ammonia, and major elements. Subsamples were taken directly from the collapsible bags into syringes without exposure to air for shipboard analysis of methane and hydrogen by gas chromatography. Gas-extracted water from gas-tight samplers (acidified with sulfamic acid) was analyzed for major elements on shore. Major, minor, and trace elements were analyzed at Pacific Marine Environmental Laboratory and the University of Washington. Analytical methods for major and minor elements are described in Butterfield et al., (*4,5*).

The total dissolved gas content of the fluids collected with gas tight titanium bottles was determined manometrically aboard ship using a vacuum extraction line. Aliquots of the extracted total gas were packaged in glass ampoules and returned to several shore-based laboratories for compositional and isotopic analyses. Compositional analysis of major gases was done gas chromatographically on a porous polymer column temperature programmed from –50°C to 125ºC. Typically, 7 or 8 components were quantified. Hydrogen and methane were the dominant gases in these samples with hydrogen commonly exceeding 70%. Accuracy is estimated to be at about 5%, with measurement precision of the larger components at 2-3%. Additional injections were done on a carbon molecular sieve column with temperature programmed from 125°C to 300ºC to allow determination of the C_2 to C_4 saturated (and select unsaturated) hydrocarbons. These trace component measurements have accuracy and precision estimates of 10%.

Water column samples collected with the conductivity-temperature-depth package were subsampled into syringes in a bubble-free manner. High-purity helium was added as headspace and dissolved hydrogen and methane were allowed to equilibrate into the gas

phase. Aliquots of the headspace were then injected into a gas chromatograph equipped with a molecular sieve column and detected with either a Valco Pulsed Discharge Detector (PDD) or a Flame Ionization Detector (FID). Detection limits were at or better than 1 nanomolar with precision of replicate sampling/equilibration/analyses being on the order of 10-20% at these very low levels. Accuracy of standard dilutions (prepared from commercially available ppm level standards) is estimated to be 5-10%.

***Stable Isotope Analyses of Carbonates and Hydrothermal Vent Fluids*:** (Gretchen Früh-Green, Stefano Bernasconi, and Giora Proskurowski)

Carbonate Samples

More than 50 samples of carbonates from the vent structures and fissure fillings and representative samples from vein and bulk carbonate in the basement rocks were selected from all sites sampled in 2000 and 2003 within and around the LCHF. Mineralogies were determined prior to analyses by X-ray diffraction and textures were examined in thin sections with the petrographic microscope. Carbon and oxygen isotope ratios were determined by reaction at 90°C with 100% phosphoric acid on an automated carbonate device connected to a *VG-PRISM* mass spectrometer and calibrated with NBS19, NBS18 and NBS20. Before isotopic analysis, all carbonate samples were roasted in vacuum at 200°C for 2 hours to avoid liberation of contaminants derived from the reaction of organic matter in the carbonate matrix with phosphoric acid. After roasting, all samples were run in duplicate to test reproducibility. Selected sample gases were scanned for purity and no anomalous masses were observed. The roasting treatment did not affect the original isotopic composition of the carbonate. This procedure was tested by roasting

pure, in-house calcite and aragonite standards, as well as mixtures of brucite and in-house carbonate standard, and measuring their isotope composition. The results are in the conventional δ-notation (in ‰) with reference to the Vienna Pee Dee Belemnite standard (VPDB). Analytical reproducibility based on repeated standards is better than ±0.1‰.

The stable isotope compositions of the carbonates in vent and fissure-filling samples lie in a range from -7 to +13‰ in $\delta^{13}C$ and from -7 to +5‰ (VPDB) in $\delta^{18}O$. More than 70% of the samples have $\delta^{13}C$ values close to average bulk marine compositions (0 ± 2‰ VPDB) but have ^{18}O-enriched compositions of >2‰ VPDB. Discrete carbonate veins and bulk carbonate in the basement have $\delta^{13}C$ values of -6 to +3.5‰ and $\delta^{18}O$ values down to -19‰. Large differences occur within single samples and at different locations, but the greatest variations are in vent samples with abundant brucite. Significant differences in composition were detected on a cm-scale within individual samples, and profiles taken across samples from different areas show varying trends. This variability reflects heterogeneities in mineralogies, precipitation temperatures, microbial activity and isotopic compositions of the fluids during precipitation and growth of the structures.

Fluid Samples

Oxygen isotope analyses of the vent fluids were determined by CO_2-equilibration at 25°C with an automated device connected to a *Micromass-OPTIMA,* calibrated with SMOW and SLAP standards. The results are reported in the conventional ‰ notation relative to the Vienna Standard Mean Ocean Water standard (VSMOW).

Sulfur isotope analyses were carried out on 21 selected samples obtained in titanium major samplers or using the HTFP. Approximately 30 ml of water was fixed with

cadmium acetate to immediately precipitate sulfide and was then sealed in a glass vial. The precipitated sulfide was separated by centrifugation. The supernatant containing dissolved sulfate was then transferred to a separate vial and barium sulfate was precipitated quantitatively by the addition of a saturated barium chloride solution. The precipitated barium sulfate was collected by centrifugation, washed and dried. Sulfur isotope compositions were measured on the *Micromass*-OPTIMA mass spectrometer coupled in continuous flow with a Carlo Erba elemental analyzer. Data are reported in the conventional δ–notation relative to the Vienna-Canyon Diablo Troilite (VCDT) standard. The system was calibrated using the international standards IAEA-S1 ($\delta^{34}S$ = -0.3‰) and IAEA-S2 ($\delta^{34}S$ = +22.7‰). The mean $\delta^{34}S$ value obtained for the international standard NBS127 was 21.2‰. Analytical reproducibility of the measurements was ±0.3‰.

Stable carbon isotopic analyses of methane were performed at the Stable Isotope Laboratory at the University of Washington, School of Oceanography. Methane was isolated from the non-condensable gas fraction by sorption onto silica gel at liquid nitrogen temperatures. Subsequent to isolation in a quartz tube with 300 mg of CuO, the methane sample was oxidized to carbon dioxide and water by heating to 900°C for one hour. The resultant CO_2 was isolated and collected for measurement on a Finnigan 251 dual-inlet mass spectrometer, with a measurement error of ±0.01‰. Analysis of blanks and an internal methane standard yields a total analytical error of ±0.3‰.

Carbon isotopes in DIC

Because specific sample aliquots for carbon isotopes in DIC were not collected, carbon isotope compositions of dissolved inorganic carbon (DIC) were determined on residual DIC in solution in the same aliquots of water as those used for the sulfur isotope

analyses. For determination of the concentrations and isotopic compositions of the residual DIC in solution, an aliquot of 2 milliliters was immediately drawn with a syringe upon opening of the sealed glass vials. This was injected in an evacuated vial containing 300 microliters of concentrated phosphoric acid. The liberated CO_2 was purified by cryogenic distillation and the amount was determined manometrically by expanding the gas in a calibrated volume. The gas was then sealed in a glass tube for later analysis by conventional dual inlet mass spectrometry. The extraction procedure was calibrated using solutions of sodium bicarbonate of known isotopic composition.

Because the pH of the vent fluids is high (9 to 11), the addition of cadmium acetate also resulted in precipitation of Cd-carbonate and Cd-sulfide. Fractionation factors predict that carbonate is 1-2‰ enriched in ^{13}C relative to DIC and, therefore, our measured DIC $\delta^{13}C$ values must be considered minimum estimates of the total DIC at Lost City. In spite of the limitations with sampling pure end member fluids and analysis, the measured compositions of -8 to +3‰ VPDB are consistent with the range of $\delta^{13}C$ values measured in the vent carbonates. We consider the measured $\delta^{13}C$ DIC values of the residual DIC in solution reported here to be a valid representation of the actual variability of the system. It should be noted that after careful re-evaluation of the experimental conditions and results, we consider that the range of DIC compositions reported in our earlier abstract are most likely erroneous (*6*). The values reported there were calculated on the basis of mass balance and included the isotopic composition of Cd-carbonate, which particularly in samples with extremely high pH, may have been contaminated by adsorption of atmospheric CO_2 during sample processing.

Microbiological Studies: **(**John Baross, Matthew Schrenk, William Brazelton, University of Washington)

Carbonate Samples

Samples were collected from the LCHF during cruises AT03-6 (2000) and AT07-34 (2003) aboard the *R/V Atlantis* using the *DSV Alvin*. Parallel sub-samples of material were partitioned for biological, petrological, and geochemical analyses. Hydrothermal fluids were fixed in a 2% formalin solution and stored at 4°C. Rock samples were frozen at -80°C or fixed in 4% paraformaldehyde in phosphate buffered saline and stored in 70% ethanol at -20°C.

Microscopy and Cell Enumeration

Microbial cells extracted from the carbonate structures using previously described methods (*7, 8*), and from fixed hydrothermal fluid samples were captured on 0.22-μm-pore-size black polycarbonate filters (*9*). Individual filters were cut into four, approximately equivalent sections and used for enumeration by epifluorescence microscopy. Total cell populations were quantified using the DNA stain DAPI (*10*). Fluorescence in situ hybridization (FISH) was performed with the Cy3-labeled oligonucleotides EUB338 and ARC915 (Qiagen-Operon)(*9*), according to the method of Glöckner, et al. (*9*). FISH with probe LCMS860 was performed under similar hybridization conditions using solutions containing 30% formamide (*12*). All epifluorescence and light microscopic observations were made using a Nikon Eclipse E600 POL epifluorescence microscope equipped with the appropriate fluorescence filter sets (DAPI: 360/400/460, ex/di/em, Cy3: 545/575/610). Digital images were obtained

using a Roper Scientific digital CCD camera and processed in Adobe Photoshop version 6.0 (Adobe).

DNA Extraction and PCR Amplification

Extraction and purification of nucleic acids (*8*) and PCR amplification of the 16S rRNA gene were performed as previously described (*13*) using the universal primers ARC-8f (5'-TCCGGTTGATCCTGCC-3') and ARC-1492R (5'GGCTACCTTGTTACGACTT-3') for Archaea and BAC-8F (5'-AGRGTTTGATCCTGGCTCAG-3') and BAC-1492R(5'-CGGCTACCTTGTTACGACTT-3') for Eubacteria. The PCR-amplified DNA was re-conditioned according to the protocol of Thompson et al. (*14*) and cloned using the TOPO-TA cloning kit (Invitrogen) according to the manufacturer's instructions. Clones were PCR-amplified with vector-specific primers and screened by RFLP as previously described (*8*). Representative ribotypes were selected for partial sequencing and in several cases full-length sequences of the clones were obtained.

Clone libraries were prepared from environmental genomic DNA with degenerate chimeric primers amplifying the alpha subunit of methyl coenzyme-M reductase (*mcr*A). (M13F/ME1: TGTAAAACGACGGCCAGTGGCMATGCARATHGGWATGTC and M13R/ME2: CAGGAAACAGCTATGACCTCATHGCRTAGTTDGGRTAGT, OPERON BIOTECHNOLOGIES, ALAMEDA, CA) (*15,16*).

Carbonate rock samples 3869-1443, 3869-1404, 3869-1446 (all from Marker C), 3876-1133 and 3880-1557 (both carbonate veins) were tested for the presence of sulfate-reducing bacteria by PCR amplification with the dissimilatory sulfite-reductase primers

DSR1F (ACSCACTGGAAGCACG) and DSR1F (ACSCACTGGAAGCACG) and DSR4R (GTGTAGCAGTTACCGCA) (Operon Biotechnologies, Alameda, CA) (*17*). All attempts to amplify DSR from these samples failed; DNA extracted from a culture of *Desulfovibrio vulgaris* was utilized as a positive control.

TRFLP primers for Archaea 16S rRNA: ARC21F (6-FAM-TTCYGGTTGATCCYGCCRGA) and ARC922R (YCCGGCGTTGANTCCAATT) (Glen Research, Sterling, VA) where Y indicates the pyrimidine analog P, R indicates the purine analog K, and N is an equal mixture of the two analogs.

Phylogenetic Analysis

Nearly complete 16S rDNA sequences obtained from this study were generally aligned using the Ribosomal Database Project II (RDP II: http://rdp.cme.msu.edu/html) Sequence Aligner program (*18*) and manually aligned to reference sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov/Genbank) in BioEdit version 5.0.9 (*19*). Approximately >1300 nucleotide bases were used in phylogenetic analyses, with only homologous position used in comparisons. Alignments were checked for chimeric sequences using the CHIMERA_CHECK program of RDP II. Sequence similarities were calculated using BioEdit. The Phylip version 3.6 software package (obtained from J. Felsenstein, University of Washington, Seattle, WA) was used to construct distance (NEIGHBOR and FITCH) and maximum likelihood (DNAML) trees, which resulted in congruent topologies. Confidence estimates for tree topology were obtained through bootstrap analysis (SEQBOOT) using 100 replicates.

GenBank Accession Numbers are as follows: *Methanolobus tindarius,* M59135; *Methanolobus vulcani*, U20155, *Methanohalophilus mahii*, M59133; *Methanococcoides methylutens*, M59127; LC1022a-1, AY299515; LC1149a-56, AY299515; Eel-36a2H11, AF354136; *Methanosarcina barkeri*, M59144; *Methanosarcina thermophila*, M59140; *Methanohalobium evestigatum*, U20149; SB-17a1d3, AF354142; *Methanothrix thermophila*, AB071701; *Methanospirillum hungatei*, M60880; *Methanomicrobium mobile*, M59142; LC1133a-9, AY760632; SB-17a1A11, AF354126; *Thermoplasma acidophilum*, M38637; pMC2A33, AB019738; *Methanobacterium bryantii*, M59124; *Methanocaldococcus jannaschii*, M59126; *Methanococcus voltae*, M59290; *Thermococcus celer*, M21529; *Archaeoglobus fulgidus*, X05567; pIVWA108, AB019726; ODPB-A2, AF121092; LC1231a-51, AY505046; *Cenarchaeum symbiosum*, AF083071; *Pyrodictium occultum*, M21087; *Desulfurococcus mobilis*, M36474; *Sulfolobus acidocaldarius*, D14053

Isotopic and quantitative analyses of total organic carbon in carbonate samples (Sean Sylva and John Hayes, Woods Hole Oceanographic Institution)

Concentrations and Isotopic compositions of total organic carbon at presented in Table S1. Samples were dried at 105°C and ground to a powder. The homogenized material (30 – 40 mg) was weighed into cleaned, silver capsules. To release CO_2 from carbonate minerals without addition of large amounts of water and consequent loss of soluble organic material, the unsealed capsules were placed in an evacuated chamber and exposed for four days to vapor in equilibrium with concentrated HCl. Complete removal of carbonate was verified by the addition of 40 mL of 2*N* HCl directly to the silver

capsules. The capsules and residues were dried at 60°C and transferred to cleaned, tin capsules (*n. b.*, water was removed only by evaporation and a residue of $CaCl_2$ was allowed to remain with the sample). The tin capsules containing the organic material were burned in a Fisons Elemental Analyzer at 1000°C. The resulting CO_2 was purified, quantified, and trapped on an automated vacuum line and sealed into 6-mm Pyrex tubes, then analyzed by conventional, dual-inlet isotope-ratio mass spectrometry.

Structural and isotopic analyses of lipid biomarkers (Alex Bradley and Roger Summons, Massachusetts Institute of Technology)

Extraction

Samples of frozen carbonate chimney material (15 – 25 g wet weight, solids + ice) were freeze-dried for 24 hours in precombusted (550°C, 8 hrs) glass jars, then crushed to a fine powder in a solvent-washed (methanol, dichlormethane, hexane) rock mill. Lipids were extracted three times with approximately 20 mL of a mixture of 3:1 dichlor-omethane:methanol by sonication for 30 min at room temperature. Extracts were collected in centrifuge tubes and centrifuged at 2000 rpm for 15 min to remove fine particles of carbonate. Extracts were then transferred to a new precombusted glass vial and blown to dryness under N_2. This total lipid extract (TLE) was filtered through pre-combusted glass-fiber filters and blown to dryness a second time to obtain a TLE weight.

Separation

1. Liquid chromatography: Total lipid extracts were applied to a 10-cm silica gel column. Columns were sequentially eluted with 1.4 column-volumes of hexane (alkane

fraction), 2.5 column volumes of dichloromethane (aromatic fraction) and 4 column volumes of 7:3 v/v dichloromethane-methanol (polar fraction).

2. Thin-layer chromatography: Total lipid extracts were separated by cold acetone precipitation into polar-lipid and neutral-lipid fractions. Neutral-lipid fractions were applied to Silica 60 (Merck 20 × 20 cm, 250 μm) preparative TLC plates and eluted twice to 12 cm with dichloromethane and once to 15 cm with hexane. Following elution, plates sprayed with rhodamine and fraction boundaries were visualized under ultraviolet light and identified using coeluted standards. Silica was scraped from the plate extracted with a Bligh-Dyer solution of methanol-chloroform-water (10:5:4 v/v), which was then blown to dryness under N_2. Portions of the plates were charred with 50% ethanolic sulfuric acid and heated overnight at 100°C to retain a permanent record of the separation.

Transesterification

Polar lipid fractions were transesterified by mild alkaline methanolysis (*23*) in which they were suspended in methanol-toluene (1:1 v/v) to which was added 0.2 *N* methanolic KOH. This mixture was incubated for 15 min. at 37°C, then neutralized with acetic acid and extracted with hexane-chloroform (4:1 v/v). The resulting fatty acid methyl esters (FAME) were analyzed by GC-MS.

Analysis

1. Gas Chromatography-Mass Spectrometry. Aliquots (10% or 20%) of total lipid extracts were analyzed by GCMS using an HP 6890 GC fitted with split/splitless injector

and a Varian WCOT fused silica capillary column (60 m × 0.32 mm; 0.25 μm film thickness) attached to an Agilent 5973 Mass Selective Detector. Prior to injection, samples were blown to dryness under N_2 and derivatized with 10 μl of N,O-bis(trimethylsilyl)-acetaminde (BSTFA) and 10 μl pyridine (60°C, 1 hr).

2. Isotope-ratio monitorinig GCMS: Aliquots of TLEs or sample fractions were analyzed on a ThermoFinnigan trace GC with Varian WCOT fused silica capillary column (60 m × 0.32 mm; 0.25 μm film thickness) attached to a ThermoFinnigan Deltaplus XL isotope ratio mass spectrometer via a combustion interface at 1000°C. All analyzed components were corrected for carbon in BSTFA derivatives.

Macrofaunal Analyses: (Timothy Shank, Kate Buckman, Woods Hole Oceanographic Institution) DNA was extracted using the DNeasy Tissue Kit (Qiagen, Valencia, CA). Mitochondrial DNA (MtDNA) was amplified with the use of universal COI primers (HCO-2198 and LCO-1490) for amphipods (*24)*. Polymerase chain reaction (PCR) products were sequenced directly using ABI 3730xl sequencer. In all cases, both forward and reverse DNA strands were sequenced and aligned. LCHF amphipods were compared with amphipod sequences from Atlantic localities (T. Shank, unpublished data). Sequence divergence estimates (uncorrected for multiple hits) were determined via Paup 4.0 and should be considered preliminary. Taxonomic specialists consulted include J. Martin (Crustacea); J. Blake, (Polychaetea); W. Newman and Paulo Young (Barnacles).

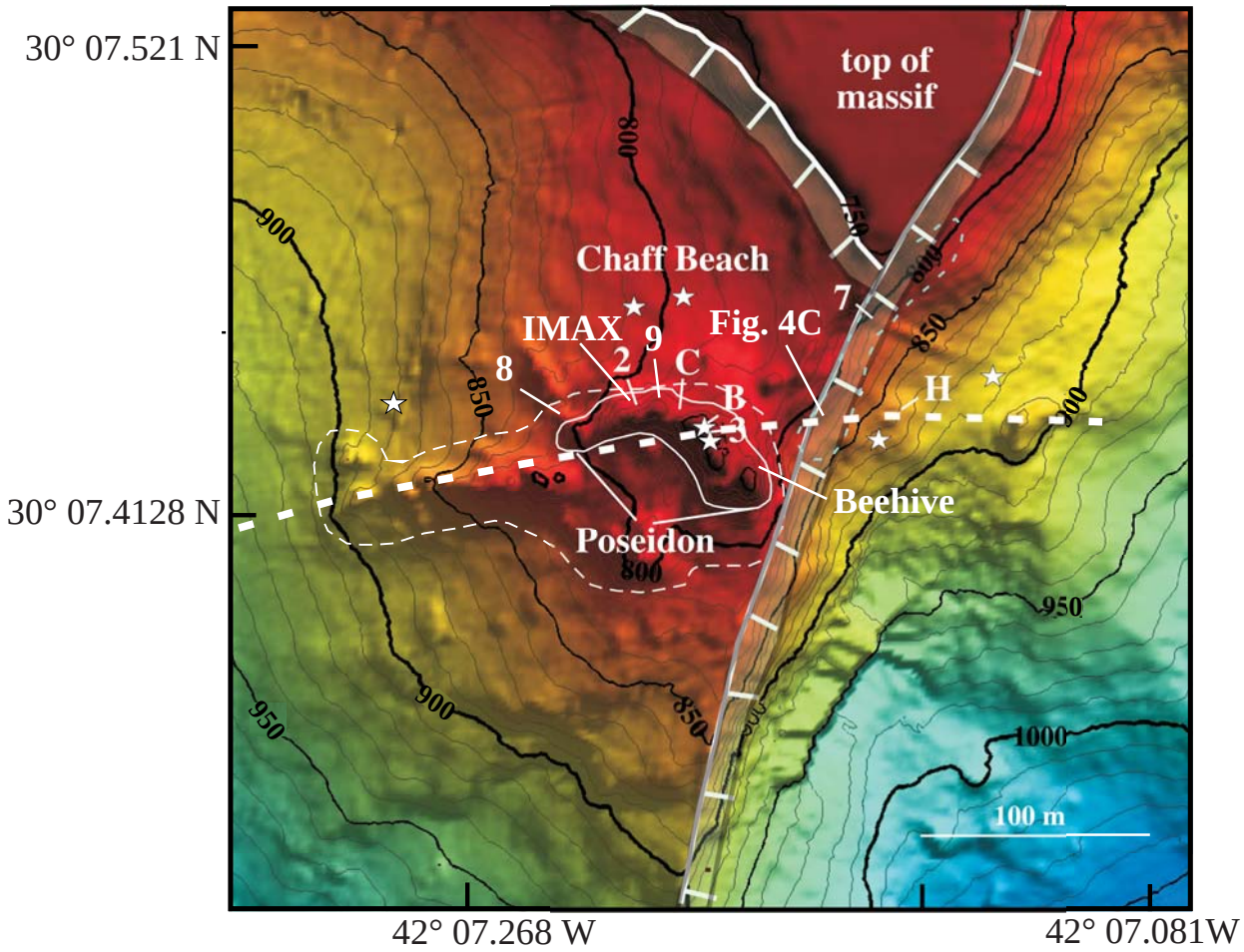

Fig. S1 Shaded bathymetric map based on ABE data of the Lost City Hydrothermal Field and adjacent terrane. This image shows the locations of important areas within the field, which are denoted by markers deployed during the field investigations. The Atlantis Massif at shallow crustal levels is underlain by variably serpentinized material, but seismic interpretations indicate that fresh upper mantle peridotite is only < 300-500 m below the seafloor (1). The LCHF field rests on a triangular-shaped, structural bench situated near the intersection of several, relatively large, steeply dipping zones near the central summit of the massif. An ~ 50 m thick zone of intensely deformed rocks near the summit of the massif are believed to represent the surface of a long-lived detachment fault that exposed the mantle and lower crustal rock sequences that make up the massif. The field is bounded to the north by a small basin, nicknamed Chaff Beach, and to the south by the Atlantis Fracture Zone. The ~020-trending cliffs to the east (hatched lines are on down-dropped sides of the faults) mark the surface of a steep normal fault that cuts gabbroic and serpentinite rocks. Fluids are weeping actively from many of these steep cliffs (Marker 7 and H). Stars indicate the location of vertical hydrocasts for water column analyses. Numbers and letters denote location of markers placed for repeat visitation of vent sites.

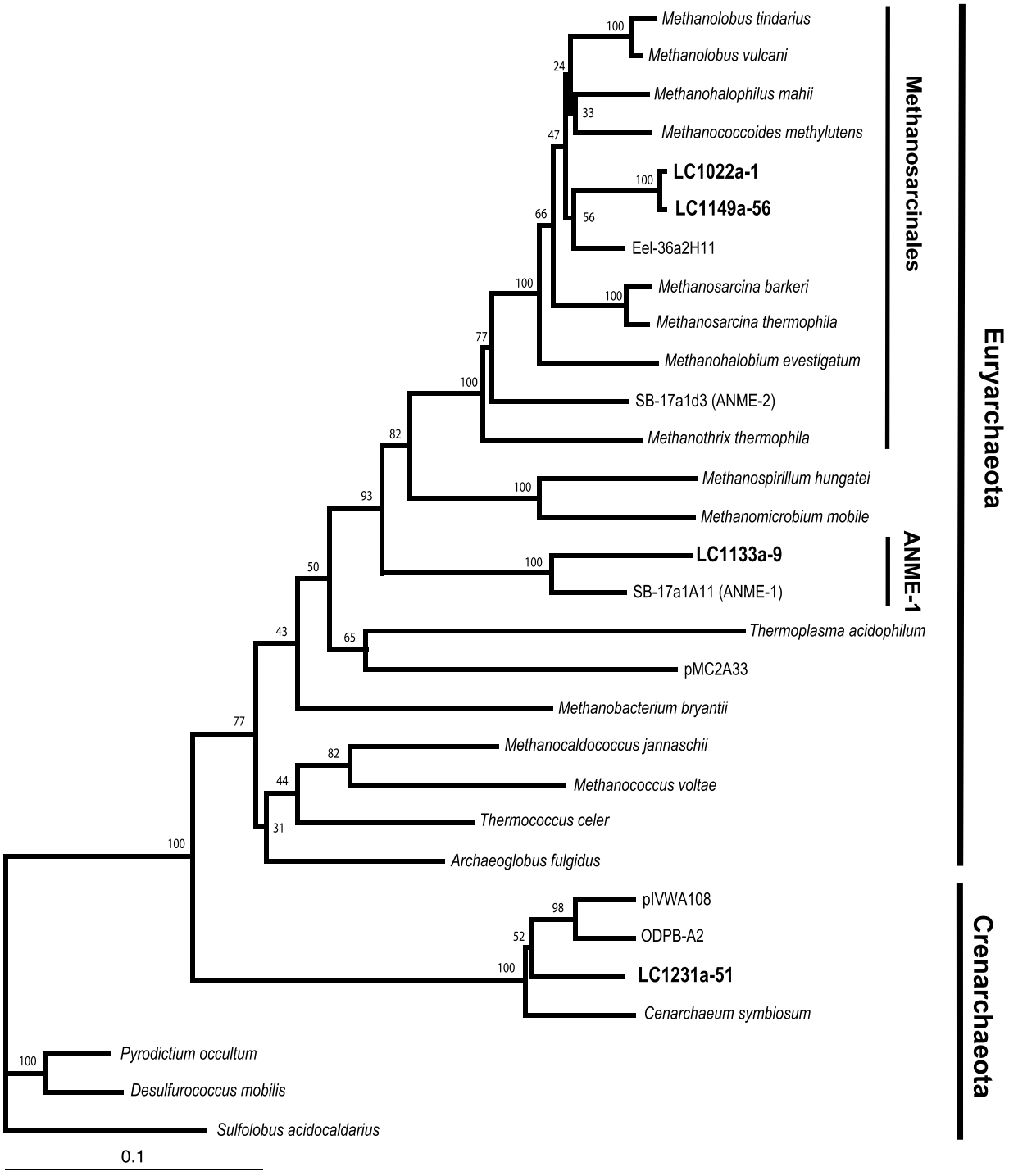
Methanolobus tindarius
Methanolobus vulcani
Methanohalophilus mahii
Methanococcoides methylutens
LC1022a-1
LC1149a-56
Eel-36a2H11
Methanosarcina barkeri
Methanosarcina thermophila
Methanohalobium evestigatum
SB-17a1d3 (ANME-2)
Methanothrix thermophila
Methanospirillum hungatei
Methanomicrobium mobile
LC1133a-9
SB-17a1A11 (ANME-1)
Thermoplasma acidophilum
pMC2A33
Methanobacterium bryantii
Methanocaldococcus jannaschii
Methanococcus voltae
Thermococcus celer
Archaeoglobus fulgidus
pIVWA108
ODPB-A2
LC1231a-51
Cenarchaeum symbiosum
Pyrodictium occultum
Desulfurococcus mobilis
Sulfolobus acidocaldarius
0.1
Methanosarcinales
ANME-1
Euryarchaeota
Crenarchaeota

Figure S2. Neighbour-joining tree representing the phylogenetic diversity of nearly complete sequence archaeal 16S rDNA clones isolated from carbonate samples relative to published sequences. The sequence alignment was generated with ClustalX (*20*) and edited with SeaView (*21*). The PHYLIP version 3.62 software package (J. Felsenstein, University of Washington, Seattle, WA, USA) was used to construct a distance (NEIGHBOR and FITCH) tree, using the sequences for *Sulfolobus acidocaldarius* as the outgroup. Confidence estimates for tree topology were obtained through bootstrap analysis (SEQBOOT) using 100 replicates.

Table S1. Concentrations and Isotopic Compositions of Total Organic Carbon

Dive	Time	Location	TOC	
			%[a]	δ^{13}C ‰
3871	1147	8	0.13	-11.3
3871	1147	8	0.10	-11.1
3871	1319	C	0.60	
3871	1319	C	0.33	-18.4
3875	1244	9	0.10	-16.1
3875	1409	Beehive	0.29	-18.4
3876	1427	Beehive	0.06	-18.7
3878	stowaway	Poseidon	0.08	-6.7
3879	1216	H	0.05	-11.7
3879	1500	2	0.07	-4.6
3879	1605	7	0.28	-3.1
3881	1225	H	0.36	

a. Reported concentrations are minima.

Movie S1. This structure, called the Nature pinnacle, rises 30 m above the seafloor and is actively venting fluids at temperatures up to 62°C. It is located on the eastern side of the field on a down-dropped bench (H on Fig. S1). This video was taken from the submersible Alvin looking towards the north, and is shown at 4 times actual collection rates. The line on Marker H, shown at the summit of the tower, is 1-m in length.

Movie S2. Strands of filamentous bacteria are abundant in areas of diffuse flow at Lost City. This video was taken with Alvin near the top of one of the edifices on Poseidon. The red dots in the first portion of the video are lasers spaced 10 cm apart. Venting temperatures measured around these zones were 60-70°C. Small, overhanging flanges of carbonate commonly trap pools of buoyantly rising hydrothermal fluids, providing excellent habitats for organisms. The red material in the second portion of the clip has not yet been identified, but we suspect that it is biological in origin.

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