

1 **Detection of putatively thermophilic anaerobic methanotrophs (ANMEs) in diffuse**  
2 **hydrothermal vent fluids**

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11 **SUPPLEMENTAL MATERIAL**

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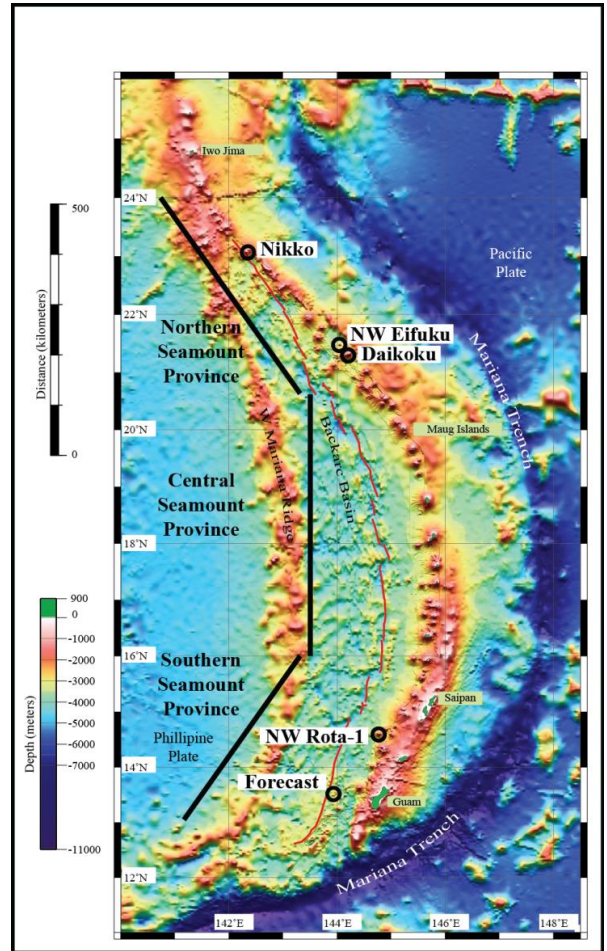
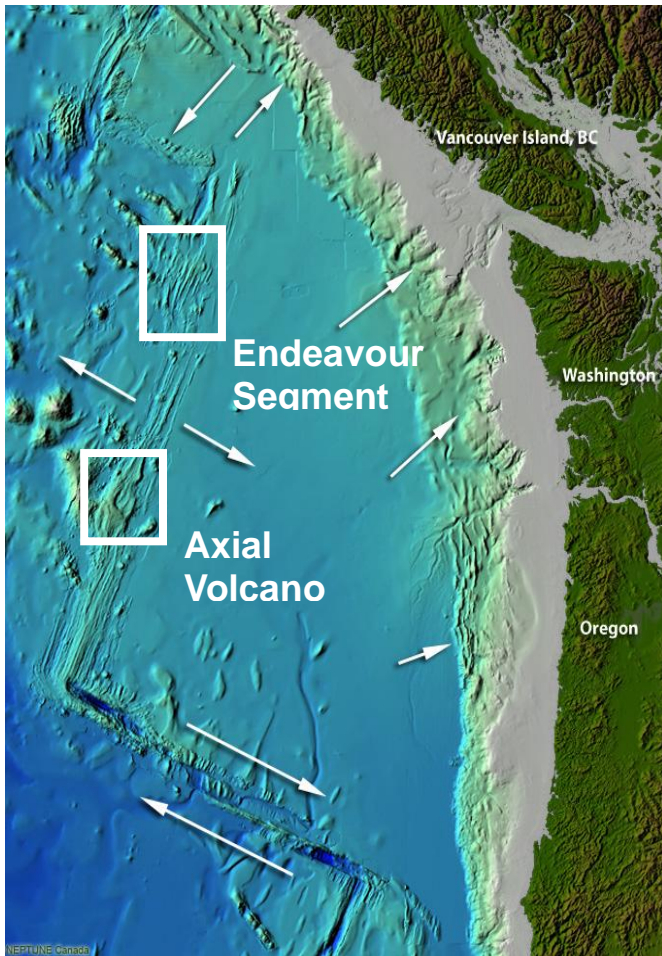
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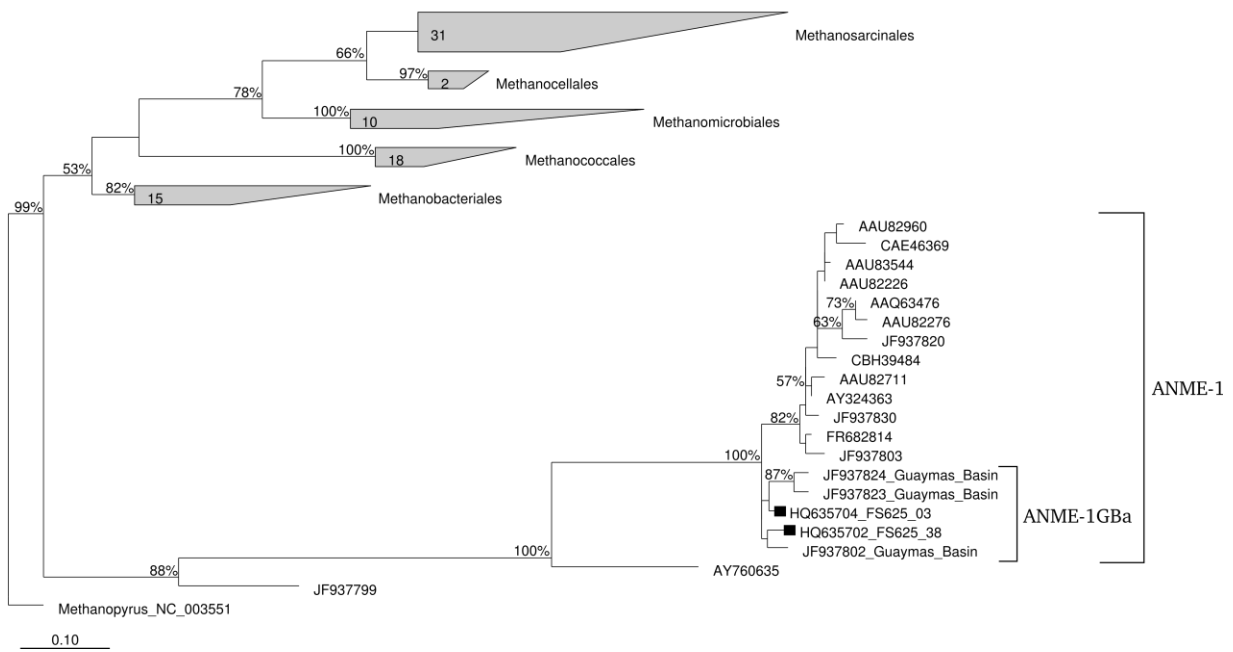
21 **Figure S1.** (Left) Locations of the Endeavour Segment and Axial Volcano hydrothermal vent sites  
22 on the Juan de Fuca Ridge in the northeastern Pacific Ocean (map courtesy of NEPTUNE Canada,  
23 NOAA, and NASA). Reprinted from (1). (Right) Map of the Mariana Arc and five volcanoes where  
24 vent fluids were collected (Map courtesy of R. Embley and S. Merle, NOAA/PMEL.). Reprinted  
25 from (2).



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28 **Figure S2.** Dendrogram showing the phylogenetic position of the deduced amino acid sequences of  
 29 *mcrA* genes retrieved from the FS625 sample (GenBank: HQ635702 and HQ635704) (1) among  
 30 other McrA sequences. The tree was constructed in the ARB software package (3) using maximum-  
 31 likelihood (PHYML) algorithm and non-parametric bootstrap analysis. The percent bootstrap values  
 32 are based on 100 replicates and are indicated at the nodes with  $\geq 50\%$  bootstrap support. Bar=10%  
 33 estimated sequence divergence.



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37 **Table S1.** Description of all diffuse vent fluid samples screened with newly designed primer pair  
 38 targeting ANME-1 16S rRNA genes

Sample	Site	Vent	Year	T <sub>max</sub> (°C)	T <sub>avg</sub> (°C)	Vol. (ml)	PCR result
A1-Sx10	Axial	Marker 33 dome	2007	18.4	14.7	2568	-
A1-Sx12	Axial	The Spot near Vixen	2007	30.8	30	3556	-
A1-Sx13	Axial	Bag City @ market	2007	13.7	13.2	3153	-
A1-Sx15	Axial	Cloud Pit	2007	6.5	6.8	3005	-
A2-Sx10	Axial	Shepherd	2007	27.9	25	3049	-
A2-Sx13	Axial	T&S @ Chasm	2007	78.8	75.9	2808	-
A3-Sx10	Axial	Zen Garden	2007	25.7	23.7	3403	-
A3-Sx11	Axial	Zen Garden	2007	7.7	7.2	2903	-
A3-Sx13	Axial	Forum cold	2007	6.1	5.7	2046	-
A4-Sx11	Axial	Marker 113	2007	31.1	30.7	2600	-
A4-SX12	Axial	Marker 113	2007	31.5	31.3	2617	-
A5-Sx15	Axial	Gollum @ Ashes	2007	22.3	21.7	2554	-
A6-Sx15	Axial	Notdeadyet @ Cobb	2007	33.6	24.3	2547	-
FS600	Axial	Gollum	2008	23.3	20.5	4550	-
FS601	Axial	Marshmallow	2008	88	84.9	2026	-
FS608	Axial	Marker 33	2008	20.4	19.7	4048	-
FS609	Axial	Cloud	2008	6.9	6.7	2027	-
FS610	Axial	Near Vixen	2008	24.1	23.5	1268	-
FS611	Axial	Bag City	2008	11.4	11.2	1612	+
FS612	Axial	Marker 113	2008	24.8	23	4001	-

FS616	Axial	Village	2008	18.5	15.4	2002	-
FS617	Axial	Hermosa	2008	34.4	32.9	632	-
FS619	Axial	9m	2008	35.9	29.7	2001	-
FS624	Endeavour	Salut LT	2008	49.9	33.7	2007	-
FS625	Endeavour	Easter Island	2008	22.1	17.8	2015	+
FS627	Endeavour	S&M LT, east side	2008	52.4	43.4	4010	-
FS630	Endeavour	Lobo LT	2008	28.7	22.7	2105	-
FS632	Endeavour	Hulk LT	2008	33.7	24.8	4030	-
FS634	Endeavour	Grotto LT	2008	19.8	18.1	1999	-
FS635	Endeavour	Cathedral LT	2008	24.6	22	1500	-
FS639	Endeavour	Cauldron LT	2008	45.3	38.8	1500	-
FS640	Endeavour	Cuchalainn LT	2008	293.4	31.5	1496	-
FS642	Endeavour	background: 1750-1500m	2008	2.6	2.4	1500	-
FS724	Endeavour	Fairy Castle	2009	31.9	28.9	2527	-
FS725	Endeavour	Boardwalk	2009	18.2	16.4	1070	+
FS643	Endeavour	Boardwalk LT	2008	23.2	21.1	1500	-
FS645	Endeavour	Godzilla LT	2008	35.1	29	3964	-
FS431	Forecast	Snail Scrum	2006	16.0	6.0	2050	-
FS432	Forecast	Homer Vent	2006	19.0	6.5	2015	-
FS445	NW Rota- 1	Brimstone	2006	27.9	19.7	3004	-
FS446	NW Rota- 1	Iceberg	2006	53.8	48.0	3007	-
FS447	NW Rota- 1	Sandy Saddle	2006	35.6	29.0	1960	-
FS448	NW Rota- 1	Fault Shrimp	2006	25.9	25.0	3010	+
FS449	NW Rota- 1	Scarp Top	2006	17.6	15.1	2000	-
A1-Sx10	Axial	Marker 33 dome	2007	18.4	14.7	2568	-

A1-Sx12	Axial	The Spot near Vixen	2007	30.8	30	3556	-
A1-Sx13	Axial	Bag City @ market	2007	13.7	13.2	3153	-
A1-Sx15	Axial	Cloud Pit	2007	6.5	6.8	3005	-
A2-Sx10	Axial	Shepherd	2007	27.9	25	3049	-
A2-Sx13	Axial	T&S @ Chasm	2007	78.8	75.9	2808	-

39 Note. The samples were screened for ANME-1 phylotypes using ANME-1-25(F) – ARCH-915(R)  
40 (4) primer pair.

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42 **Table S2.** Archaeal isolates with unreported temperature optima that have a 16S rRNA gene G+C  
 43 content ( $P_{GC}$ ) above 60 mol% and are not affiliated with known thermophilic genera

Organism	Accession in RDP (in GenBank)	Culmination of taxonomic lineage in GenBank document	Isolation source	Reference	$P_{GC}$ , mol%
haloarchaeon_str. T1.6	S000433079 (AJ270240)	Halobacteriales	solution-mined brine	5	60.1
halophilic archaeon MK206-1	S002949589 (AB638840)	Halobacteriales	no data	6	60.3
haloarchaeon str. T4.2	S000433070 (AJ270231)	Halobacteriales	solution-mined brine	5	60.8
Halobacteriales_archaeon YIM 93590	S002447619 (JF449412)	Halobacteriales	no data	7	63.6

44 Notes: The table is based on analysis of the 16S rRNA genes of archaeal isolates available from the  
 45 RDP database (Release 10, Update 28, Jan 12, 2012; 2202 sequences with length >1200 nt).

46 **Table S3.** G+C content of 16S rRNA gene sequences ( $P_{GC}$ ) representing ANME microorganisms  
 47 that were detected in or enriched from various hydrothermal-associated environments with  
 48 indication of corresponding *in situ* or incubation temperatures.

Accession number	Silva taxonomy	Our Taxonomy (only in case of ANME-1 cluster) <sup>1</sup>	Taxonomy in original article	$P_{GC}$	<i>In situ</i> or incubation temperature	Ref.
AF419638	ANME-2c	-	ANME-2c	55.3	2°C to 57°C	8
AF419647	ANME-2c	-	ANME-2c	55.4	nd	8
AF419650	ANME-2c	-	ANME-2c	54.8	nd	8
AF419644	ANME-2c	-	ANME-2c	55.3	2°C to 57°C	8
AF419624	ANME-1a	ANME-1a	ANME-1a	56.0	2°C to 74°C	8
AF419625	ANME-1a	ANME-1a	ANME-1a	56.4	2°C to 74°C	8
AF419626	ANME-1a	ANME-1AT	ANME-1a	57.5	2°C to 74°C	8
AF419649	ANME-1a	ANME-1AT	ANME-1a	57.6	nd	8
AF419652	na <sup>2</sup>	ANME-1AT	ANME-1a	57.1	nd	8
AF419654	ANME-1a	ANME-1AT	ANME-1a	57.1	nd	8
AF419627	ANME-1	ANME-1AT	ANME-1a	58.3	2°C to 74°C	8
AF419630	ANME-1	ANME-1GBa	ANME-1b	63.1	2°C to 74°C	8
AF419655	ANME-1	ANME-1GBa	ANME-1b	62.8	nd	8
AF419631	ANME-1	ANME-1GBa	ANME-1b	62.7	2°C to 74°C	8
AF419632	ANME-1	ANME-1GBa	ANME-1b	62.5	2°C to 74°C	8
AF419638	ANME-2c	-	ANME-2c	55.3	2°C to 57°C	8
AY299516	ANME-3	-	ANME-3	52.8	20°C to 100°C	9
AY299515	ANME-3	-	ANME-3	53.0	20°C to 100°C	9
AY760632	ANME-1b	ANME-1a	ANME-1a	54.1	7°C to 60°C	9
DQ270605	Unclassified	ANME-1a	ANME-1a	53.2	7°C to 60°C	9
AB252423	ANME-2c	-	ANME-2c	55.0	>10°C	10
AB252424	ANME-2ab	-	ANME-2c	55.5	>10°C	10
AB260054	ANME-2ab	-	ANME-2b	56.8	64°C	11
AB260055	ANME-2ab	-	ANME-2b	56.9	64°C	11
AB301860	ANME-1a	ANME-1a	ANME-1a	55.8	52°C	12
AM268264	Unclassified	ANME-1a	ANME-1	56.9	45 °C	13
AM418591	Unclassified	-	ANME-3	56.1	55°C to 90°C	14
AM418592	Unclassified	-	ANME-3	56.5	55°C to 90°C	14
AM418593	Unclassified	-	ANME-2	56.3	50°C to 70°C	14
AM418594	Unclassified	-	ANME-2	56.7	50°C to 70°C	14
EF644781	ANME-2ab	-	ANME-2	56.0	nd	15
EF644784	ANME-2ab	-	ANME-2	56.7	nd	15
FR682489	ANME-1a	ANME-1a	ANME-1-Guaymas I	56.4	50°C	16
FR682490	ANME-1	ANME-1AT	ANME-1-Guaymas II	56.6	50°C	16
FR682491	ANME-1	ANME-1AT	ANME-1-Guaymas II	56.9	50°C	16
JF937764	Unclassified	-	ANME-2c	54.1	30°C to 35°C	17



JF937743	Unclassified	-	ANME-2ab	54.5	30°C to 35°C	17
JF937751	Unclassified	ANME-1AT	ANME-1a	56.2	15°C to 20°C	17
JF937770	Unclassified	ANME-1AT	ANME-1a	55.3	60°C to 95°C	17
JF937719	Unclassified	ANME-1GBa	ANME-1 Guaymas	64.0	60°C to 95°C	17
JF937715	Unclassified	ANME-1GBa	ANME-1 Guaymas	63.8	60°C to 95°C	17
JF937746	Unclassified	ANME-1GBa	ANME-1 Guaymas	64.3	15°C to 20°C	17
JF937791	Unclassified	ANME-1GBa	ANME-1 Guaymas	64.6	60°C to 95°C	17
JF937755	Unclassified	ANME-1GBa	ANME-1 Guaymas	63.5	15°C to 20°C	17
JN919590	Unclassified	nd <sup>3</sup>	ANME-1a	57.6	52°C	18
JN925693	Unclassified	nd	ANME-1a	57.5	52°C	18
JN922003	Unclassified	nd	ANME-1a	58.4	52°C	18
JN916619 <sup>4</sup>	Unclassified	nd	ANME-1a	61.5	52°C	18
JN917740	Unclassified	ANME-1a	ANME-1a	56.5	52°C	18
JN920217	Unclassified	ANME-1AT	ANME-1a	56.7	52°C	18
JN923285	Unclassified	ANME-1AT	ANME-1a	56.8	52°C	18
JN923760	Unclassified	ANME-1AT	ANME-1a	56.6	52°C	18
JN919387	Unclassified	ANME-1AT	ANME-1a	57.7	52°C	18
JN916455	Unclassified	ANME-1AT	ANME-1a	57.4	52°C	18
JN928779	Unclassified	ANME-1a	ANME-1a	56.3	52°C	18
JN924050	Unclassified	ANME-1a	ANME-1a	55.1	52°C	18
JN918036	Unclassified	ANME-1a	ANME-1a	56.7	52°C	18
JN915944	Unclassified	nd	ANME-1a	56.9	52°C	18
JN934186	Unclassified	nd	ANME-1a	56.4	52°C	18
JN918739	Unclassified	nd	ANME-1a	57.7	52°C	18
JN928776	Unclassified	ANME-1a	ANME-1c	57.3	52°C	18
JN932428	Unclassified	ANME-1a	ANME-1c	57.0	52°C	18
JN921789	Unclassified	ANME-1a	ANME-1c	56.2	52°C	18
JN928790	Unclassified	ANME-1a	ANME-1c	57.3	52°C	18
JN919646	Unclassified	ANME-1a	ANME-1c	57.7	52°C	18
JN929819	Unclassified	ANME-1a	ANME-1c	58.2	52°C	18
JN928810	Unclassified	ANME-1a	ANME-1c	57.4	52°C	18
JN925024	Unclassified	ANME-1a	ANME-1c	57.8	52°C	18
JN933005	Unclassified	ANME-1a	ANME-1c	57.3	52°C	18
JN915819	Unclassified	ANME-1a	ANME-1c	56.5	52°C	18
JN929815	Unclassified	ANME-1a	ANME-1c	57.8	52°C	18
JN920932	Unclassified	ANME-1a	ANME-1c	58.4	52°C	18
JN920961	Unclassified	ANME-1a	ANME-1c	58.9	52°C	18
JN928769	Unclassified	ANME-1a	ANME-1c	57.4	52°C	18
JN927216	Unclassified	ANME-1a	ANME-1c	57.8	52°C	18
JN926589	Unclassified	ANME-1a	ANME-1c	56.6	52°C	18

49 <sup>1</sup> Taxonomic position of sequences was determined by constructing a phylogenetic tree in the ARB  
50 software package (3) using maximum-likelihood (PHYML) algorithm.

51 <sup>2</sup> na – not applicable (no such sequence in Silva database)

52 <sup>3</sup> nd - not determined (sequences represent separate phylogenetic lineages)

53 <sup>4</sup>This is the only sequence beyond the ANME-1GBa group that has  $P_{GC}$  above 60%. However, it is  
54 short (413 bp) and may be chimeric (55% Pintail Quality according to Silva database).

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