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Supplement of

Niche differentiation of ammonia and nitrite oxidizers along a salinity gradient from the Pearl River estuary to the South China Sea

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Table S1. Abundances of the archaeal and *β*-proteobacterial *amo*A genes and *Nitrospira* and *Nitrospina* 16S rRNA genes in the PRE.

Station	Water Depth (m)	Sampling Depth (m)	Archaeal *amo*A (copies L^{-1})				*β*-proteobacterial *amo*A (copies L^{-1})				*Nitrospira* 16S rRNA (copies L^{-1})				*Nitrospina* 16S rRNA (copies L^{-1})			
			FL[a]	SD	PA[b]	SD	FL[a]	SD	PA[b]	SD	FL[a]	SD	PA[b]	SD	FL[a]	SD	PA[b]	SD
P1	8.9	1	0		1501	40	0		1338	799	1533	446	33025	525	909	747	4537	691
		7	0		1248	57	228	56	528	17	776	76	53287	1086	121	6	3902	179
P2	9.8	1	NS[c]				NS[c]				NS[c]				NS[c]			
		7	2768	27	4462	1243	526	6	7441	562	29374	5945	328697	15430	4851	447	45997	2792
P3	10.2	1	NS[c]				NS[c]				NS[c]				NS[c]			
		8	2556	251	11321	85	298	14	8239	482	25360	1478	573425	12573	5103	850	85027	9576
P4	21.5	1	NS[c]				NS[c]				NS[c]				NS[c]			
		18	657	22	12080	516	308	49	34158	2469	9175	1541	2024263	198739	3068	441	380537	14232
P5	22.5	1	4104	80	6535	30	961	58	5532	428	40070	3306	365741	18556	13556	1852	84860	3967
		19	3263	109	9162	327	672	70	12341	292	38076	5273	763345	93318	12186	1715	125427	10142
P6	18.8	1	3617	305	11219	241	488	71	4136	208	21516	2437	482519	13994	3763	250	56974	2244
		16	NS[c]				NS[c]				NS[c]				NS[c]			
P7	12	1	40742	2180	108877	4425	2012	245	14259	3443	69806	5991	735150	42882	7820	652	95572	4870
		10	10212	2234	8267	677	221	11	974	171	11393	3342	46220	3334	950	103	7540	399
P8	5	1	2614	47	64350	3095	150	40	1024	72	16111	1427	70874	15050	3368	539	7479	625
		3.5	2904	272	49549	4515	201	117	1228	92	26179	3334	83656	10345	5913	668	11065	1838
P9	8	1	20355	1102	35409	2540	70		553	127	3536	475	40278	3435	8309	1501	75342	10147
		6	10081	442	681539	25091	0		1392	64	0		47889	10565	769	139	296757	20048
P10	12.9	1	72002	20991	25516	4630	0		121		506	128	1663	946	1814	98	2033	214
		11	113345	4922	185761	31978	298	71	165	29	63	23	973	292	2340	256	14241	2378
P11	14.2	1	14384	2520	775	81	0		172	118	43		495	135	412	151	51	

		12	130393	6302	137480	14835	0	224	14	154	101	2542	750	3400	315	12913	2894
P12	16	1	12087	1917	4724	984	0	0		68		0		6945	3360	362	
		14	302349	78106	240640	4899	0	1423	74	227	110	5294	807	6635	851	20858	530

1 [a], Free-living; [b], Particle-associated; [c], No sample

Table S2. Primer set sequences, PCR reaction mixtures and conditions for each gene.

Target gene	Primer	Sequence (5'-3')	PCR mixture	PCR conditions	References
β-proteobacterial *amo*A	amoA-34F	GCGGCRAAAATGCCGCCGGAAGCG	50 μL reaction mixture: Failsafe Premix F (Epicentre Biotechnologies, Madison, WI, U.S.A.) 25 μL, primers 0.5 μM, plantium Taq DNA polymerase (Invitrogen, Carlsbad, CA, U.S.A.) 1 U, DNA template 1 μL	95 °C for 2 min; hot start at 80 °C; and 25 x (95 °C for 30 s, 57 °C for 30 s, 73 °C for 3 min).	Kim et al., 2008; Hu et al., 2010
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC			
Archaeal *amo*A	Arch-amoAF	STAATGGTCTGGCTTAGACG	50 μL reaction mixture: Failsafe Premix F (Epicentre Biotechnologies) 25 μL, primers 0.5 μM, plantium Taq DNA polymerase (Invitrogen) 1 U, DNA template 1 μL	95 °C for 5 min; 30 x (94 °C for 45 s, 53 °C for 60 s, and 72 °C for 60 s); and 72 °C for 15 min.	Francis et al., 2005; Hu et al., 2010
	Arch-amoAR	GCGGCCATCCATCTGTATGT			
Nitrobacter and *Nitrococcus nxr*B	nxrBF706	AAGACCTAYTTCAACTGGTC	50 μL reaction mixture: Ex Taq DNA polymerase 0.25 μL (TaKaRa), 10×Buffer 5 μL, dNTP 4 μL, Mg^{2+} 4 μL, primers 1 μM, BSA (20 μg/uL) 0.125 μL, DNA template 2 μL	95 °C for 5 min; 35 x (95 °C for 40 s, 56 °C 30 s, 72 °C 30 s), 72 °C for 10 min.	Koch, 2009
	nxrBR1431	CGCTCCATCGGYGGAACMAC			
*Nitrospira nxr*B	nxrB169F	TACATGTGGTGGAACA	25 μL reaction mixture: Platinum Taq DNA polymerase 0.1 μL (Invitrogen), 10×Buffer 2.5 μL, dNTP 2 μL, Mg^{2+} 4 μL, primers 1 μM, BSA (200 ng/μL) 2.5 μL, DNA template 1 μL	95 °C for 5 min; 35 x (95 °C for 40 s, 56.2 °C 40 s, 72 °C 90 s), 72 °C for 10 min.	Modified from Pester et al., 2013
	nxrB638R	CGGTTCTGGTCRATCA			
*Nitrospina nxr*B	nxrBNF	GGGCGACCAGATGGAAAC	25 μL reaction mixture: LA Taq DNA polymerase 0.25 μL (TaKaRa), 10×Buffer 2.5 μL, dNTP 1 μL, Mg^{2+} 5 μL, primers 1 μM, BSA (200 ng/μL) 2.5 μL, DNA template 1 μL	95 °C for 5 min; 35 x (95 °C for 40 s, 56.2 °C 40 s, 72 °C 90 s), 72 °C for 10 min.	This study
	nxrBNR	GGGCCGGACATAGAAAGG			

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Table S3. Primer pair sequences, qPCR mixtures and conditions for each gene.

Target gene	Primer	Sequence (5'-3')	PCR mixture	PCR conditions	Efficiency	Detection limits	References
β-proteobacterial *amo*A	amoA-1F	GGGGHTTYTACTGGTGGT	25 μL reaction mixture: SYBR® Premix Ex Taq™ (TakaRa, Dalian, China) 12.5 μL, BSA 5 μg, primers 0.4 μM, DNA template 1 μL	94 °C for 30 s; 45 × (94 °C for 15 s, 60 °C for 60 s, and 72 °C for 90 s).	96-104%	2 copies μl^{-1}	Rotthauwe et al., 1997; Hu et al., 2011; Mincer et al., 2007
	amoA-2R	CCCCTCKGSAAAGCCTTCTTC					
Archaeal *amo*A	Arch-amoAF	STAATGGTCTGGCTTAGACG	25 μL reaction mixture: SYBR® Premix Ex Taq™ (TakaRa) 12.5 μL, BSA 5 μg, primers 0.4 μM, DNA template 1 μL	95 °C for 30 s; 40 × (95 °C for 30 s, 53 °C for 60 s, and 72 °C for 45 s).	91-98%	3 copies μl^{-1}	Francis et al., 2005; Hu et al., 2011
	Arch-amoAR	GCGGCCATCCATCTGTATGT					
Archaeal *amo*A[a]	Arch-amoA-for	CTGAYTGGGCYTGGACATC	25 μL reaction mixture: SYBR® Premix Ex Taq™ (TakaRa) 12.5 μL, BSA 10 μg, primers 1 μM, DNA template 1 μL	95 °C for 30 s; 41 × (95 °C for 30 s, 58.5 °C for 40 s, and 72 °C for 30 s and 80°C for 25 s).	94-99%	2 copies μl^{-1}	Wuchter et al., 2006 Bergauer et al., 2013
	Arch-amoA-rev	TTCTTCTTTGTTGCCCAGTA					
Nitrospira 16S rRNA	Nspra-675f	GCGGTGAAATGCGTAGAKATCG	25 μL reaction mixture: SYBR® Premix Ex Taq™ (TakaRa) 12.5μL, BSA 15 μg, primers 0.2 μM, DNA template 1 μL	95 °C for 10 min; 45 × (94 °C for 30 s, 64 °C for 30 s, 72 °C for 60 s).	92-98%	2 copies μl^{-1}	Graham et al., 2007; Attard et al., 2010
	Nspra-746r	TCAGCGTCAGRWAYGTTCCAGAG					
Nitrospina 16S rRNA	NitSSU_130F	GGGTGAGTAACACGTGAATAA	25 μL reaction mixture: SYBR® Premix Ex Taq™ (TakaRa) 12.5μL, BSA 1 μg, primers 0.4 μM, DNA template 1 μL	94 °C for 15 min; 45 × (94 °C for 15 s, 57.5 °C for 15 s, 72 °C for 30 s, 77 °C for 1 s).	90-100%	3 copies μl^{-1}	Mincer et al., 2007
	NitSSU_282R	TCAGGCCGGCTAAMCA					

[a], The primer set was used in the samples from the lower reaches (sites P9–12) of the estuary.

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Table S4. Diversity indices of AOA and *β*-AOB *amo*A, *Nitrospira*, *Nitrospina*, and *Nitrobacter nxr*B genes based on 5% nucleic acid sequences cutoff.

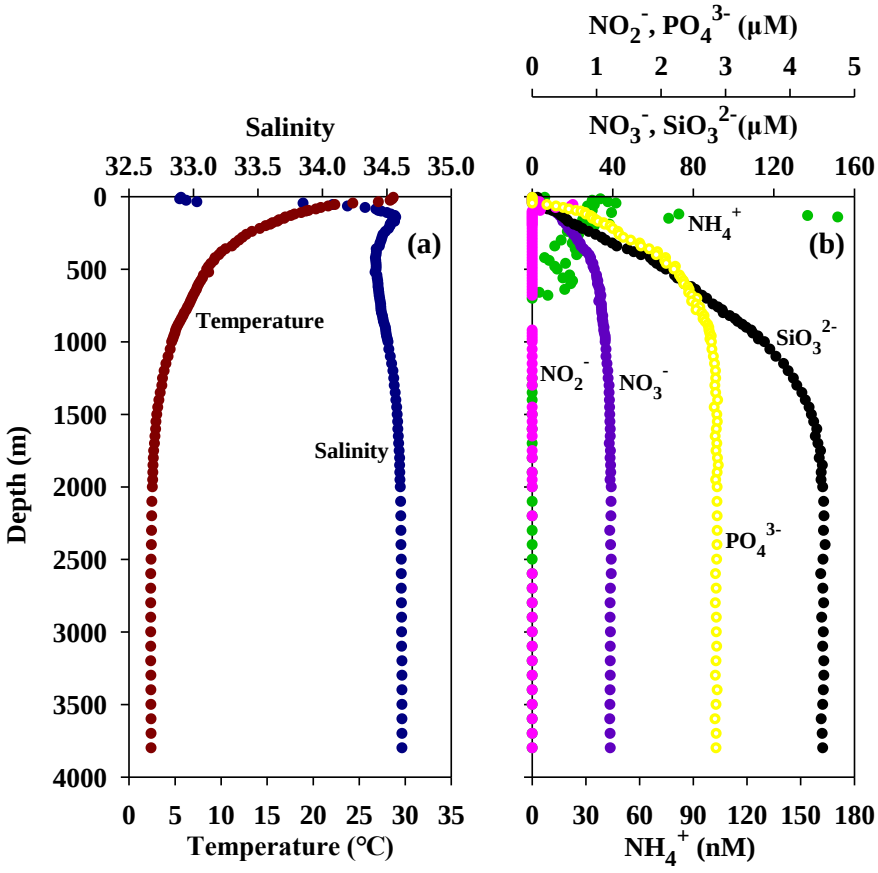
Genes	No. of Libraries	n	No. of OTUs	C (%)	H'	1/D	Chao1
AOA *amo*A (SCS)	4/4	392	60	0.94	3.04	10.64	49.46
AOA *amo*A (PRE)	4/4	127	23	0.90	2.13	4.87	42.5
β-AOB *amo*A (PRE)	2/4	26	3	0.96	0.43	1.28	3
*Nitrospira nxr*B (PRE)	4/4	345	29	0.96	1.79	3.57	42
*Nitrospina nxr*B (PRE & SCS)	4/8	185	12	0.98	1.79	4.83	12.75
*Nitrobacter nxr*B (PRE)	2/4	48	3	0.98	0.78	2.13	3

n, number of sequences; OTU, operational taxonomic unit; C, coverage; H', Shannon-Wiener Index; 1/D, Simpson's diversity Index; SCS, South China Sea; PRE, Pearl River estuary. Numbers before slash indicate successful libraries; numbers after slash indicate all amplified samples.

Table S5. *r* values for the relationship between gene abundances of nitrifiers and environmental parameters in the PRE.

Gene	Community	Water mass parameters			Substrate parameters			Parameters influencing substrate availability		
		Temperature (n = 20)	Salinity (n = 20)	SiO_3^{2-} (n = 20)	NH_4^+ (n = 15)	NO_2^- (n = 20)	NO_3^- (n = 20)	TSM (n = 19)	DO (n = 20)	pH (n = 20)
AOB *amo*A	**FL[a]**	0.302	−0.441	0.439	−0.108	0.527*	0.759**	−0.053	−0.425	−0.512*
	PA[b]	0.332	−0.474*	0.475*	−0.048	0.706**	0.464*	0.520*	−0.525*	−0.496*
	FL+PA	0.341	−0.471*	0.487*	−0.053	0.718**	0.491*	0.504*	−0.536*	−0.513*
AOA *amo*A	**FL[a]**	−0.754**	0.691**	−0.709**	−0.376	−0.461*	−0.728**	−0.203	0.412	0.585**
	PA[b]	−0.528*	0.539*	−0.524*	−0.407	−0.361	−0.486*	0.498*	0.348	0.434
	FL+PA	−0.717**	0.703**	−0.697**	−0.468	−0.470*	−0.673**	0.330	0.441	0.577**
***Nitrospira* 16S rRNA**	**FL[a]**	0.426	−0.580**	0.537*	−0.205	0.643**	0.772**	−0.099	−0.464*	−0.625**
	PA[b]	0.356	−0.474*	0.491*	−0.073	0.730**	0.518*	0.504*	−0.541*	−0.524*
	FL+PA	0.367	−0.475*	0.503*	−0.080	0.743**	0.539*	0.493*	−0.550*	−0.540*
***Nitrospina* 16S rRNA**	**FL[a]**	0.097	−0.167	0.158	−0.268	0.436	0.253	−0.315	−0.190	−0.230
	PA[b]	0.108	−0.134	0.162	−0.105	0.453*	0.173	0.822**	−0.276	−0.221
	FL+PA	0.111	−0.140	0.167	−0.115	0.468*	0.182	0.811**	−0.282	−0.229

[a], Free-living; [b], Particle-associated; *, $P < 0.05$; **, $P < 0.01$; TSM, Total suspended material; DO, Dissolved oxygen



1

2 **Figure S1.** Depth profiles of biogeochemical parameters at SEATS.

3

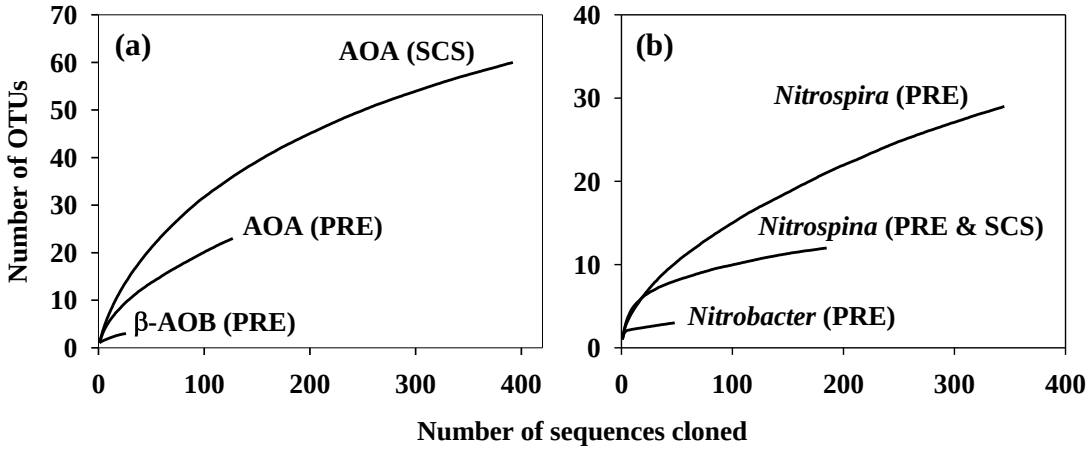


Figure S2. Rarefaction curves of (a) AOA and *β*-AOB *amo*A gene sequences and (b) *Nitrospira*, *Nitrospina*, and *Nitrobacter nxr*B gene sequences. The curves were generated at 95% DNA sequence identity.

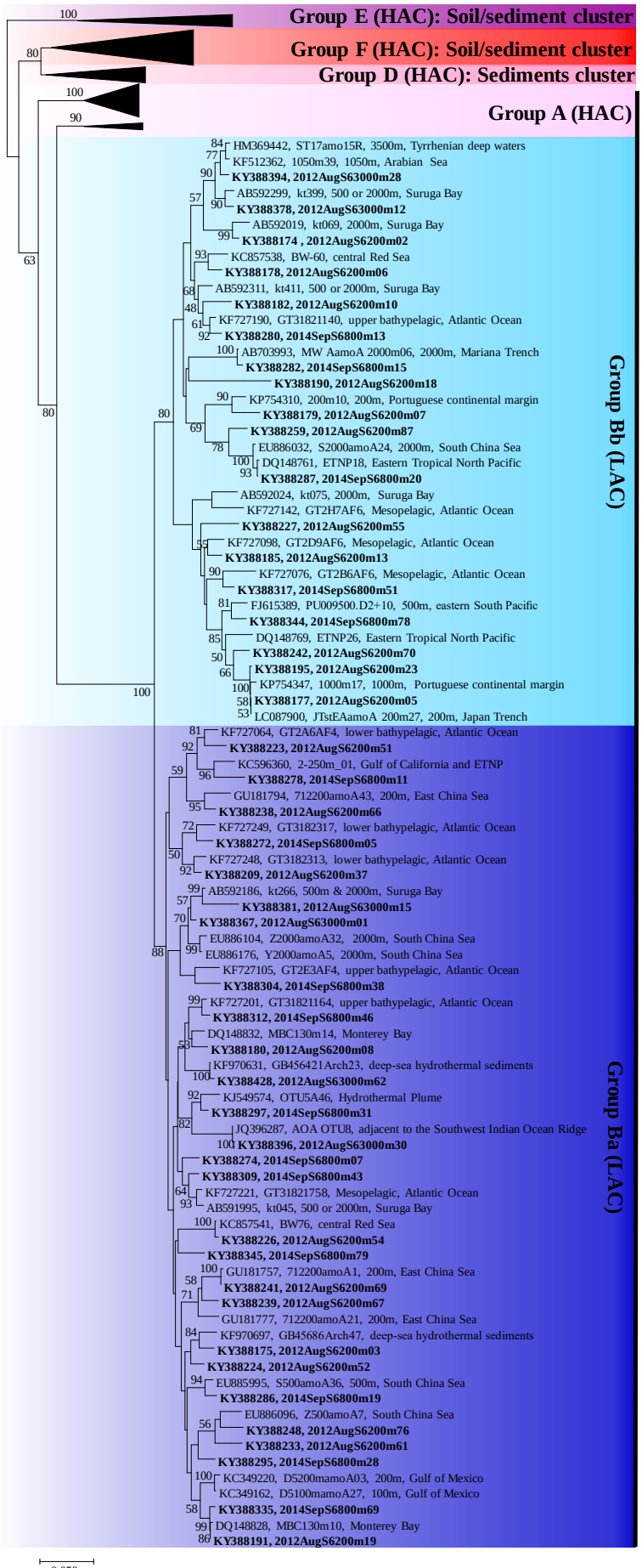

Group E (HAC): Soil/sediment cluster
Group F (HAC): Soil/sediment cluster
Group D (HAC): Sediments cluster
Group A (HAC)
Group Bb (LAC)
Group Ba (LAC)
Water column cluster
HM369442, ST17amo15R, 3500m, Tyrrhenian deep waters
KF512362, 1050m39, 1050m, Arabian Sea
KY388394, 2012AugS63000m28
AB592299, kt399, 500 or 2000m, Suruga Bay
KY388378, 2012AugS63000m12
AB592019, kt069, 2000m, Suruga Bay
KY388174 , 2012AugS6200m02
KC857538, BW-60, central Red Sea
KY388178, 2012AugS6200m06
AB592311, kt411, 500 or 2000m, Suruga Bay
KY388182, 2012AugS6200m10
KF727190, GT31821140, upper bathypelagic, Atlantic Ocean
KY388280, 2014SepS6800m13
AB703993, MW AamoA 2000m06, 2000m, Mariana Trench
KY388282, 2014SepS6800m15
KY388190, 2012AugS6200m18
KP754310, 200m10, 200m, Portuguese continental margin
KY388179, 2012AugS6200m07
KY388259, 2012AugS6200m87
EU886032, S2000amoA24, 2000m, South China Sea
DQ148761, ETNP18, Eastern Tropical North Pacific
KY388287, 2014SepS6800m20
AB592024, kt075, 2000m, Suruga Bay
KF727142, GT2H7AF6, Mesopelagic, Atlantic Ocean
KY388227, 2012AugS6200m55
KF727098, GT2D9AF6, Mesopelagic, Atlantic Ocean
KY388185, 2012AugS6200m13
KF727076, GT2B6AF6, Mesopelagic, Atlantic Ocean
KY388317, 2014SepS6800m51
FJ615389, PU009500.D2+10, 500m, eastern South Pacific
KY388344, 2014SepS6800m78
DQ148769, ETNP26, Eastern Tropical North Pacific
KY388242, 2012AugS6200m70
KY388195, 2012AugS6200m23
KP754347, 1000m17, 1000m, Portuguese continental margin
KY388177, 2012AugS6200m05
LC087900, JTstEAamoA 200m27, 200m, Japan Trench
KF727064, GT2A6AF4, lower bathypelagic, Atlantic Ocean
KY388223, 2012AugS6200m51
KC596360, 2-250m_01, Gulf of California and ETNP
KY388278, 2014SepS6800m11
GU181794, 712200amoA43, 200m, East China Sea
KY388238, 2012AugS6200m66
KF727249, GT3182317, lower bathypelagic, Atlantic Ocean
KY388272, 2014SepS6800m05
KF727248, GT3182313, lower bathypelagic, Atlantic Ocean
KY388209, 2012AugS6200m37
AB592186, kt266, 500m & 2000m, Suruga Bay
KY388381, 2012AugS63000m15
KY388367, 2012AugS63000m01
EU886104, Z2000amoA32, 2000m, South China Sea
EU886176, Y2000amoA5, 2000m, South China Sea
KF727105, GT2E3AF4, upper bathypelagic, Atlantic Ocean
KY388304, 2014SepS6800m38
KF727201, GT31821164, upper bathypelagic, Atlantic Ocean
KY388312, 2014SepS6800m46
DQ148832, MBC130m14, Monterey Bay
KY388180, 2012AugS6200m08
KF970631, GB456421Arch23, deep-sea hydrothermal sediments
KY388428, 2012AugS63000m62
KJ549574, OTU5A46, Hydrothermal Plume
KY388297, 2014SepS6800m31
JQ396287, AOA OTU8, adjacent to the Southwest Indian Ocean Ridge
KY388396, 2012AugS63000m30
KY388274, 2014SepS6800m07
KY388309, 2014SepS6800m43
KF727221, GT31821758, Mesopelagic, Atlantic Ocean
AB591995, kt045, 500 or 2000m, Suruga Bay
KC857541, BW76, central Red Sea
KY388226, 2012AugS6200m54
KY388345, 2014SepS6800m79
GU181757, 712200amoA1, 200m, East China Sea
KY388241, 2012AugS6200m69
KY388239, 2012AugS6200m67
GU181777, 712200amoA21, 200m, East China Sea
KF970697, GB45686Arch47, deep-sea hydrothermal sediments
KY388175, 2012AugS6200m03
KY388224, 2012AugS6200m52
EU885995, S500amoA36, 500m, South China Sea
KY388286, 2014SepS6800m19
EU886096, Z500amoA7, South China Sea
KY388248, 2012AugS6200m76
KY388233, 2012AugS6200m61
KY388295, 2014SepS6800m28
KC349220, D5200mamoA03, 200m, Gulf of Mexico
KC349162, D5100mamoA27, 100m, Gulf of Mexico
KY388335, 2014SepS6800m69
DQ148828, MBC130m10, Monterey Bay
KY388191, 2012AugS6200m19
0.050

Figure S3. Unrooted neighbor-joining (NJ) phylogenetic tree of the archaeal *amo*A gene sequences (expanded view for group Ba and Bb (LAC)). Clone sequences from this study are shown in bold and sequences sharing 95% DNA identity are grouped. Phylogenetic relationships were bootstrapped 1000 times, and bootstrap values greater than 50% are shown. The scale bar indicates 5% estimated sequence divergence.