
1 **Ecological functions of uncultured microorganisms in the cobalt-rich ferromanganese**
2 **crust of a seamount in the central Pacific are elucidated by fosmid sequencing**

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16 **Summary**

17 Cobalt-rich ferromanganese is an important seafloor mineral and is abundantly present in the
18 seamount crusts. Such crusts form potential hotspots for biogeochemical activity and
19 microbial diversity, yet our understanding of their microbial communities is lacking. In this
20 study, we used a cultivation-independent approach to recover genomic information and derive
21 ecological functions of the microbes in a sediment sample collected from the cobalt-rich
22 ferromanganese crust of a seamount region in the central Pacific. A total of 78 distinct clones
23 were obtained by fosmid library screening with a 16S rRNA based PCR method.
24 Proteobacteria and MGI Thaumarchaeota dominated the bacterial and archaeal 16S rRNA
25 gene sequence results in the microbial community. Nine fosmid clones were sequenced and
26 annotated. Numerous genes encoding proteins involved in metabolic functions and heavy
27 metal resistance were identified, suggesting alternative metabolic pathways and stress
28 responses that are essential for microbial survival in the cobalt-rich ferromanganese crust. In
29 addition, genes that participate in the synthesis of organic acids and exopolymers were
30 discovered. Reconstruction of the metabolic pathways revealed that the nitrogen cycle is an
31 important biogeochemical process in the cobalt-rich ferromanganese crust. In addition,
32 horizontal gene transfer (HGT) events have been observed, and most of them came from
33 bacteria, with some occurring in archaea and plants. Clone W4-93a, belonging to MGI
34 Thaumarchaeota, contained a region of gene synteny. Comparative analyses suggested that a
35 high frequency of HGT events as well as genomic divergence play important roles in the
36 microbial adaptation to the deep-sea environment.

37

38 **Key words:** seamount; cobalt-rich ferromanganese crust; metagenome; horizontal gene
39 transfer

40 **1 Introduction**

41 Seamounts are widespread and defined as topographic rises from the ocean floor with a
42 limited area across the summit, which is below sea level or emerges above the sea surface
43 only for short periods of time (Menard, 1964; Staudigel et al., 2010). Recently, 33,452
44 seamounts (elevation of > 1 000 m) were identified in global bathymetric datasets, most of
45 them (57.2%) located in the Pacific Ocean (Yesson et al., 2011). However, the number of
46 seamounts remains under debate due to the different definitions of what constitutes a
47 seamount as well as the variation in techniques used to count them (Hillier and Watts, 2007;
48 Iyer et al., 2012; Wessel et al., 2010). Although seamounts have a high degree of biodiversity,
49 harbour unique biological communities, display high levels of endemism, represent hotspots
50 of nutrient cycling and support commercial fisheries, fewer than 300 seamounts have been
51 thoroughly sampled, and the majority of these studies have focused on hydrothermal vents
52 (Clark et al., 2010; Duffy, 2008; Emerson and Moyer, 2010; Rowden et al., 2010; Schlacher et
53 al., 2010).

54 Deposits of cobalt-rich, oxidised ferromanganese in crusts that cover seamounts were
55 first discovered in 1980 (Craig et al., 1982; Ito et al., 2008; Muiños et al., 2013). These crusts
56 usually grow at very slow rates (1-10 μm per 10^3 years) and exist only on a few seamounts
57 (Fu et al., 2005; Koschinsky and Hein, 2003). Cobalt-rich ferromanganese crusts are a rich
58 source of metals such as ferromanganese oxide, cobalt, copper, nickel, platinum and other rare
59 earth elements (Fu et al., 2005; Fuyuan et al., 2008). Currently, our knowledge of biological
60 interactions at the cobalt-rich ferromanganese crusts is very limited, and more research is
61 needed into their resident microbial communities and their ecosystem functions to evaluate
62 the environmental impacts of future crust exploration and mining.

63 The role of biogenesis in cobalt-rich ferromanganese crust formation on seamounts
64 remains controversial and poorly understood. Previous results from a northern Pacific
65 seamount indicated that crust accretion is not a purely physicochemical process as it also
66 involves microbial processes (Verlaan, 1992). Microorganisms act as biological nuclei for the
67 formation of cobalt-rich crusts, suggesting that biomineralization is indispensable in the
68 mineral formation process (Wang and Müller, 2009). Scanning electron microscopy studies
69 suggest that biological processes are involved in the formation of the ferromanganese crusts
70 covering seamounts in the central Atlantic (Wang et al., 2011). Several studies of microbial
71 diversity in sediments from cobalt-rich ferromanganese crusts of central Pacific seamounts
72 have been performed using culture-independent approaches (Jiang et al., 2012; Liao et al.,
73 2011). Phylogenetic analyses of bacterial and archaeal 16S rRNA clone libraries have

74 revealed the predominance of *Proteobacteria* and marine archaeal group I (MGI), and it has
75 been suggested that members of this community may be involved in sulphur, nitrogen and
76 metal cycling in cobalt-rich ferromanganese crusts (Liao et al., 2011). Recently, nine novel
77 lipolytic enzymes were identified, suggesting that the microbial populations participate in
78 carbon degradation, calcium deposition and contribute to biomineralization (Jiang et al.,
79 2012).

80 Recent work revealed the microbial community structure and diversity of the microbes in
81 cobalt-rich ferromanganese crusts by using molecular and electron microscopy approaches.
82 However, the functional aspects of the microbes should not be overlooked. In this study, a
83 metagenomic library of deep-sea sediment collected from a cobalt-rich ferromanganese crust
84 region from a seamount was screened to describe the genome content and biological
85 properties of uncultivated microorganisms. In total, approximately 21,000 randomly selected
86 fosmid clones were subjected to PCR-based screening, 35 archaeal and 43 bacterial 16S
87 rRNA gene-containing clones were obtained, and nine fragments were sequenced and
88 analysed. To our knowledge, this is the first report of a metagenomic approach to identify the
89 role of microorganisms in the formation of cobalt-rich ferromanganese crusts of seamounts.

90

91 **2 Materials and Methods**

92 ***2.1 Sample collection, geochemical properties analysis and library construction***

93 A deep-sea sediment sample was collected from the skirt of a seamount located in the
94 cobalt-rich crust deposit region in the central Pacific Ocean. The sample collection method,
95 location, DNA extraction procedure and library construction information were described in
96 detail in an earlier publication (Jiang et al., 2012). The elemental composition of the sample
97 was determined by the Zhejiang Institute of Geology and Mineral Resources using various
98 methods, including the gravimetric method (for SiO₂), inductively coupled plasma-atomic
99 emission spectrometry (ICP-AES; for Al₂O₃, Fe₂O₃, CaO, MgO, TiO₂, MnO and P₂O₅),
100 atomic absorption spectrometry (AAS; for K₂O and Na₂O) and inductively coupled
101 plasma-mass spectrometry (ICP-MS; for Ni, Co, V, Cr, Cu, Zn, Cd, Pb, Mo and Ba).

102

103 ***2.2 16S rRNA gene screening***

104 Approximately 21,000 randomly selected fosmid clones were subjected to PCR-based
105 screening. The DNA of pooled fosmid clones was extracted using the Axygen Plasmid
106 Miniprep Kit (Axygen Biotechnology, Hangzhou, China). The fosmid DNA templates were
107 treated with the plasmid-safe ATP-dependent DNase (Epicentre Biotechnologies, Madison,

108 Wisconsin, USA) to remove the chromosomal DNA contamination of the host strain
109 (*Escherichia coli* EPI300). Primers Ar20F (5'-TTCCGGTTGATCCYGCCTGA-3') and
110 Arch958R (5'-TCCGGCGTTGAMTCCAATT-3') (DeLong, 1992) were used for archaeal
111 16S rRNA gene amplification. Primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and
112 23S1R (5'-GGGTTTCCCATTCGGAAATC-3') were used for the identification of the
113 bacterial 16S rRNA gene with the adjacent intergenic spacer region (ISR) (Garcia-Martinez et
114 al., 1996). Thirty-five cycles of amplification were carried out under the following conditions
115 for the archaeal 16S rRNA gene: denaturation at 94°C for 45 seconds, annealing at 55°C for
116 45 seconds and elongation at 72°C for 1 minute. Thirty-five cycles of amplification of
117 bacterial 16S rRNA gene were performed under the following conditions: denaturation at
118 94°C for 15 seconds, annealing at 50°C for 30 seconds and elongation at 72°C for 2 minutes
119 (Martín-Cuadrado et al., 2007). PCR fragments were extracted from the gel using the
120 AXYGEN gel extraction kit (AXYGEN, Hangzhou, China) and then cloned into the
121 pMD19-T vector (TAKARA, Dalian, China). The 16S rRNA gene fragments were sequenced
122 with the primers M13F/M13R (archaea) and 27F/1492R (bacteria). The closest relatives of
123 the 16S rRNA sequences were obtained from the NCBI GenBank database using blastn.
124 Evolutionary distances were calculated according to Kimura's two-parameter correction
125 method. Neighbour-joining trees were constructed with a bootstrap value of 500 using MEGA
126 version 5.0 (Tamura et al., 2011). The accession numbers of the 16S rRNA gene sequences are
127 JQ013299-JQ013333 (archaeal) and JQ013334-JQ013376 (bacterial).

128

129 **2.3 Fragments sequencing and analysis**

130 Two archaeal and seven bacterial fosmid clones were selected and their sequences were
131 determined by sequencing using a Roche 454 GS-FLX and Illumina/Solexa Genome Analyzer
132 II platforms (Tongji-SCBIT Biotechnology Co., Ltd). Gaps were closed with the help of
133 targeted PCR. The PCR products were sequenced by primer walking. Open reading frames
134 (ORFs) were predicted using MetaGeneMark (Zhu et al., 2010). Gene identification was
135 obtained by submitting the deduced protein sequences for ortholog/homolog searches in the
136 NCBI nr database, the Cluster of Orthologous Groups (COG) database (Tatusov et al., 2000)
137 and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa and Goto,
138 2000) using blastp. A threshold e-value of 1e-5 was used for all analyses. For phylogenetic
139 analysis of ORFs, a blastp search of protein sequences in the NCBI nr database was carried
140 out with default parameters, and the sequences for the best blast hits were retrieved from the
141 database. Neighbour-joining trees were constructed with a bootstrap value of 500 using the

142 Poisson model option in the MEGA 5.0 phylogenetic software package (Tamura et al., 2011).
143 The Genbank accessions of the fosmid sequences are JQ085817-JQ085825.

144

145 **3 Results**

146 **3.1 Elemental composition**

147 The elemental composition of the sediment sample SEAM02 is shown in Table 1. Compared
148 with offshore sediment (Zhao, 1988) and continental crust (Wedepohl, 1995), the
149 concentrations of Al₂O₃ and CaO were much lower in the sample, whereas those of Na₂O,
150 MnO, Ni, Co, Cu, Zn, Pb and Ba were higher. Specifically, Na₂O, MnO, Co, Cu and Ba,
151 reached concentrations of more than twice those found in the offshore sediment and
152 continental crust. Taken together, the sample SEAM02 was mainly composed of SiO₂
153 (54.07%), Al₂O₃ (8.35%), Na₂O (7.18%) and Fe₂O₃ (4.14%) and was rich in metals, including
154 Mn, Ni, Co, Cu, Zn, Pb and Ba.

155

156 **3.2 Microbial community composition**

157 A total of 35 archaeal 16S rRNA-containing clones were obtained from the fosmid library. All
158 of the archaeal clones belonged to Marine Group I (MGI) in the phylum Thaumarchaeota
159 except clone W5-61a, which was grouped into Marine Benthic Group A (MBGA) (Fig. 1a).
160 These sequences were most closely related to those from other deep-sea sediment
161 environments, including sediments from the Pacific nodule province (Xu et al., 2005), the
162 Weddell Sea of Antarctica (Gillan and Danis, 2007), the southern Mariana Trough (Kato et al.,
163 2009a; Kato et al., 2009b), the east Pacific rise (Ehrhardt et al., 2007; Li et al., 2008), the
164 Mediterranean cold seep (Heijs et al., 2007) and the Barents Sea cold seep (Losekann et al.,
165 2007). This result indicated that our clones represent members of a common and abundant
166 archaeal community in deep-sea sediments. In accordance with findings from previous
167 microbial diversity studies of deep-sea sediment with cobalt-rich crust deposits (Liao et al.,
168 2011), MGI was the dominant archaeal group. The sequence of clone W5-61a, the only clone
169 that affiliated with members of group MBGA, shared <90% identity with previously reported
170 clones, with the exception of clones aEPR13S208 (97.6%) and YS16As04 (92.4%) retrieved
171 from the East Pacific Rise (Li et al., 2008) and the Southern Mariana Trough (Kato et al.,
172 2009a), respectively. There are no cultivated species reported for MBGA members, and only a
173 single strain, *Nitrosopumilus maritimus* SCM1, within the MGI group has been isolated so far
174 (Könneke et al., 2005), leaving us with little insight into the genetic make-up of their genomes
175 and the physiological functions they encode.

176 A total of 43 bacterial fosmid clones were obtained that distributed over 7 bacterial
177 groups (Fig. 1b): Alphaproteobacteria (6 clones), Betaproteobacteria (1 clone),
178 Gammaproteobacteria (14 clones), Deltaproteobacteria (3 clones), Actinobacteria (11 clones),
179 Gemmatimonadetes (3 clones) and Chlorobi (1 clone). The other 4 clones (W4-21b, W4-50b,
180 W5-15b and W5-77b) could not be assigned to any taxonomic division. A total of 38 bacterial
181 clones showed high identities with uncultured clones from the deep-sea surface sediments of
182 the south Atlantic Ocean (Schauer et al., 2009), the seafloor lavas of the east Pacific rise (EPR)
183 and the Hawaiian basalts (Santelli et al., 2008), indicating these bacteria may be common to
184 these deep-sea environments. In addition, the 16S rRNA gene sequences of clones W4-21b,
185 W5-15b and W5-102b did not have matches with >90% identity to other sequences in the
186 database, suggesting they might represent novel taxa for the deep-sea environment, and they
187 may have unique adaptation to the cobalt-rich crust environment.

188

189 **3.3 Gene content of fosmid clones**

190 To obtain more genomic information on microbial adaptation to deep-sea sediments, we
191 sequenced two archaeal and seven bacterial genome fragments. The fosmid insert sizes ranged
192 from 23 to 45 kb, with G+C content ranging from 36% to 65%. A detailed description of DNA
193 insert sizes, ORF positions, predicted functions, closest relatives, and COG classification are
194 summarised in Table S1. In addition, inserts were subjected to gene annotation, revealing a
195 range of gene functions.

196

197 ***Archaeal fosmid clones***

198 To the best of our knowledge, clone W5-61a is the first genome fragment to be sequenced for
199 a MBGA member of the Thaumarchaeota. The fragment has a G+C content of 48.6%,
200 considerably higher than that of MGI Thaumarchaeota (approx. 34%) (Gilbert et al., 2011;
201 Walker et al., 2010). Clone W5-61a (32,142 bp) contains 25 ORFs, of which 23 of them could
202 be assigned with the COG classification system. Of these, 17 encode functions in basic
203 metabolic processes: leucine biosynthesis (ORFs 1-3), ion transport (ORFs 7-9), DNA
204 protection and repair (ORFs 13-14) and purine metabolism (ORFs 15-22). Most of the
205 predicted genes had the most significant blast hits to members of archaea, whereas ORF2,
206 ORF3 and ORF6 showed the highest identity to bacterial genes. ORF2 and ORF3 encoded the
207 large and small subunits of 3-isopropylmalate dehydratase, and phylogeny indicates that they
208 may have been transferred from Firmicutes via HGT (Fig. S1a,b). Both ORF6 and ORF11
209 were identified as encoding the pyrroloquinoline quinone biosynthesis protein C (PqqC);

210 however, only 53.4% of the amino acid residues were similar between them. The phylogenetic
211 tree was constructed based on bacterial and available archaeal original PqqC genes (Fig. S1c).
212 The two genes formed distinct phyletic lines with high bootstrap values towards the periphery
213 of the bacterial lineage, revealing that they may be of bacterial origin. ORF12 encodes a
214 PQQ-dependent alcohol dehydrogenase, which is rarely found in archaea. The function of
215 archaeal PQQ-dependent alcohol dehydrogenase is still unknown; however, bacterial alcohol
216 dehydrogenase had been proved to oxidize various alcohols for bioenergy generation (Adachi
217 et al., 2007). This gene might be important for the energy requirement of Archaea in deep sea
218 sediments.

219 Clone W4-93a (34,190 bp) was most closely related to *Candidatus Nitrosoarchaeum*
220 *limnia* within the marine group I Thaumarchaeota (> 95.8% 16S sequence identity). The G +
221 C content of this fragment was 36.3%, and a total of 49 ORFs were predicted. Deduced amino
222 sequences indicated that only 21 proteins (44.7%) could be assigned a physiological function
223 with the COG classification system, and 9 proteins (19.1%) did not show significant
224 similarity to any proteins in the NCBI nr database. Apart from these 9 predicted proteins that
225 had no significant relatives, the other ORFs were most closely related to known archaeal
226 genes found in the members of Thaumarchaeota (17 ORFs to *Candidatus Nitrosoarchaeum*
227 *limnia*, 16 ORFs to *Nitrosopumilus maritimus*, 4 ORFs to *Candidatus Cenarchaeum*
228 *symbiosum* and 2 ORFs to *Candidatus Nitrosoarchaeum koreensis*). Interestingly, ORF25 was
229 most closely related to the nitrogen regulatory protein P-II from *Thermococcus sibiricus*, a
230 hyperthermophilic member of the Euryarchaeota (Miroshnichenko et al., 2001) (Table S1).
231 Phylogenetic analysis also indicated that this gene may have been obtained from
232 euryarchaeotal species by HGT (Fig. S1d).

233 Gene organisation and synteny was determined by comparing clone W4-93a with two
234 MGI Thaumarchaeota fragments (Fig. 2), *Nitrosopumilus maritimus* SCM1 and clone 74A4
235 (Béjà et al., 2002). *N. maritimus* SCM1 isolated from seawater from the Seattle Aquarium was
236 the first member of the Thaumarchaeota to have been cultured (Könneke et al., 2005), and this
237 isolate was identified as a key chemolithoautotrophic ammonia-oxidiser in the marine
238 environment. Fosmid clone 74A4 was obtained from a surface sample in the Southern Ocean
239 (Béjà et al., 2002). The 16S rRNA gene sequence of clone W4-93a was > 95% identical to
240 that of *N. maritimus* SCM1 as well as clone 74A4. Comparative genomic analysis showed
241 that the gene content and arrangement in the three related MGI Thaumarchaeota genome
242 fragments were largely conserved at the 5'-end of the 16S-23S rRNA operon, but not at the
243 3'-end (Fig. 2). Upstream of the 16S-23S rRNA operon, a colinear region spanning 12 genes

244 (approx. 13 kbp), was shared by the three fragments. Downstream of the 16S-23S rRNA
245 genes, however, strain SCM1 harboured an approximately 26.5 kbp fragment, whereas both
246 clone W4-93a and 74A4 did not contain this region. A single gene (ORF48,
247 biotin--acetyl-CoA-carboxylase ligase) was common to the three fragments. Although clone
248 W4-93a lacked a genome fragment near the 16S-23S rRNA genes, it contained a nitrogen
249 regulatory protein P-II gene (ORF25) and a nitroreductase gene (ORF31) that participates in
250 nitrogen cycling.

251 ***Bacterial fosmid clones***

252 Clone W4-39b contained 28 ORFs and a 16S-23S-5S rRNA operon with two tRNA genes
253 (tRNA-Ala and tRNA-Ile). The 16S rRNA and 23S rRNA sequences identified this clone as
254 belonging to the Deltaproteobacteria. COG classification was successful in assigning
255 functions to most of the predicted proteins, including genes responsible for L-glutamate
256 synthesis (ORF7-8), glycerophospholipids metabolism (ORF10-11), *de novo* purine
257 biosynthesis (ORF18), substrate transport (ORF13, ORF15-16 and ORF23) and transposition
258 (ORF20-22). ORF20-22 encoded transposases that clustered with Alphaproteobacteria in a
259 monophyletic tree (Fig. S1f). ORF24-26 had a small number of close relatives, including the
260 genera *Erythrobacter*, *Novosphingobium*, *Sphingobium* and *Sphingomonas* of
261 Alphaproteobacteria and the genera *Gallionella*, *Methylotenera* and *Limnobacter* of
262 Betaproteobacteria (Fig. S1g-i). Intracellular ammonium is incorporated into carbon skeletons
263 via the glutamate / glutamine synthase pathway. Interestingly, ORF7 and ORF8, which
264 encoded small and large subunits of glutamate synthase, were most similar to those found in
265 Actinobacteria (Fig. S1e).

266 Clone W4-87b exhibited the highest 16S and 23S rRNA gene sequence identities to
267 *Nitrosospira multiformis* ATCC 25196 (97.8% and 97.1%, respectively), which is an
268 ammonia-oxidising bacterium (AOB) isolated from soil (Norton et al., 2008). Among the
269 predicted 18 ORFs, only seven ORFs were related to the Betaproteobacteria class. ORF4
270 showed a clearly Bacteroidetes origin (Fig. S1j). Clone W4-87b encoded a urease accessory
271 protein (UreD) involved in the activation of a urease that hydrolyses urea to ammonia.
272 Considering the phylogenetic analyses of the rRNA genes, as well as that of *ureD*, we
273 considered that clone W4-87b might have derived from an AOB similar to *N. multiformis*
274 which plays an important role in the nitrogen cycle. In addition, some oxidative stress
275 resistance genes, including thioredoxin (ORF3 and ORF13), superoxide dismutase (ORF9)
276 and universal stress protein A (ORF10) were found in clone W4-87b.

277 A similar situation was observed for clone W5-47b, which showed the highest 16S rRNA

278 sequence identity to *Ignavibacterium album* Mat9-16 (92.6%) belonging to the Chlorobi.
279 Chlorobi are obligate anaerobic photoautotrophic bacteria. Clone W5-47b contained several
280 antibiotic-synthesising and metabolic genes from other bacteria, as well as archaea, to survive
281 in the deep-sea environment. Examples of these were ORF1 (penicillin synthesis), ORF2-5
282 (pentose phosphate pathway), ORF10 (streptomycin biosynthesis), ORF17 and ORF24
283 (purine and pyridine metabolism) and ORF22 (poly- γ -glutamate synthesis).

284 The 16S and 23 rRNA genes found in clone W5-102b identified this fragment as being
285 derived from a member of the Actinobacteria. Most ORFs (54.8%) were predicted to be
286 hypothetical proteins, and 13 ORFs had no orthologs in the NCBI nr database. Some ORFs
287 might have been acquired by HGT, including ORF6 from Firmicutes (Fig S1k), ORF10 from
288 other bacteria (Fig. S1l), ORF18 from Euryarchaeota (Fig. S1m), ORF25 and ORF30 from
289 Chloroflexi and ORF26 from Bacteroidia.

290 Tree topology with a high bootstrap value (100%) revealed that clone W5-51b fell within
291 a cluster composed of Gemmatimonadetes members, and formed an independent clade (Fig.
292 1b). However, this clone was distinguishable from the known Gemmatimonadetes species
293 based on the low (< 85%) (Table S1) identities of its 16S rRNA gene and other functional
294 genes. Upstream of the 5S-23S-16S rRNA genes, clone W5-51b fragments were partially
295 conserved, and most ORFs (10/13, 76.9%) exhibited the highest sequence identities with
296 genes found in *Gemmatimonas aurantiaca*. Downstream of the rRNA gene cluster, ORFs
297 were similar to those of other Gemmatimonadetes members, and most of them were more
298 closely related to Proteobacteria. Phylogenetic analysis revealed that ORF17 and ORF18-19
299 might have been acquired by HGT from Alphaproteobacteria and Gammaproteobacteria,
300 respectively (Fig. S1n-o). Our data suggest that clone W5-15b presented a new lineage in the
301 phylum Gemmatimonadetes.

302 Clones W4-21b and W5-15b belonged to unknown taxonomic groups within the
303 phylogenetic tree (Fig. 1) and showed very low 16S rRNA sequence identity with known
304 bacterial species (< 78%, Table S1). Some important functional genes were detected in these
305 two clones. In clone W4-21b, ORF22 encoded a SpoIID/LytB domain-containing protein that
306 is involved in sporulation (Lopez-Diaz et al., 1986); ORF24 encodes a heavy metal resistance
307 protein (CzcD) that is able to mediate metal efflux and so enhance the cell's resistance to e.g.,
308 cobalt, zinc and cadmium (Nies, 1992); and ORF15 (RadC), ORF16 (RecJ), ORF29 (DNA
309 polymerase) and ORF30 (LexA repressor) were related to DNA repair. Sporulation, heavy
310 metal resistance and DNA repair are important mechanisms of environmental stress resistance
311 in microbes, indicating that clone W4-21b belonged to a spore-forming bacterium adapted the

312 deep-sea cobalt-rich ferromanganese crust environment. In clone W5-15b, ORF1
313 (adenylate/guanylate cyclase), ORF3 (CspE), ORF6 (PspC), ORF9 (PhoH), ORF22
314 (glutathione S-transferase) and ORF26 (RecB) were recognized as the stress response
315 regulating genes, and ORF32 encoded a poly- γ -glutamate synthesis protein. Poly- γ -glutamate
316 is a natural polymer synthesised by gram-positive bacteria. It allows bacteria to survive at
317 high salt concentrations and may also act as a virulence factor or a storage element for carbon
318 and nitrogen precursors or as an energy source (Candela and Fouet, 2006). The above genes
319 reveal that clone W5-15b was derived from a gram-positive bacterium and was able to resist
320 various stresses, including low temperature, high osmotic pressure and low nutrient
321 availability. In addition, potential HGT of ORF22 from plant was identified (Fig. S1q).

322

323 **4 Discussion**

324 Recently, we published the first assessment of the bacterial and archaeal diversity in the
325 sediment collected from a cobalt-rich ferromanganese crust (Liao et al., 2011). Proteobacteria
326 and MGI Thaumarchaeota dominated the bacterial and archaeal communities, respectively. In
327 addition, the microbial diversity inside nodules and in the surrounding sediments collected
328 from a cobalt-rich ferromanganese crust were compared (Wu et al., 2013). Here, we focused
329 on the ecological functions of these microbial communities with a special emphasis on their
330 adaptive ability and survival in cobalt-rich ferromanganese crusts.

331 Manganese, cobalt, copper and nickel are the most important metal elements available in
332 cobalt-rich ferromanganese crust. The content of Co + Cu + Ni is one of the significant
333 evaluation indicators of the crust. Our study revealed that the concentrations of Mn, Ni, Co
334 and Cu in the sediment from station SEAM02 were more than twice those found in offshore
335 sediments or in continental crust (Table 1), whereas they were at much lower concentrations
336 than those in the cobalt-rich ferromanganese crust (data not shown). Some oxidative stress
337 resistance genes, including thioredoxin, superoxide dismutase and PqqC, were detected in our
338 fosmid library. A gene encoding a cobalt-zinc-cadmium resistance protein (ORF24 in clone
339 W4-21b) was also found, suggesting that some microbes that inhabit in the sediment adapt to
340 heavy metal toxicity (Supplementary Table 1). Recent works demonstrate that cobalt-rich
341 ferromanganese crusts are formed by biologically driven processes involving microbes (Wang
342 et al., 2009; Wang and Müller, 2009), and that microorganisms are responsible for the bulk of
343 Mn oxide formation (Tebo et al., 2004). Some microorganisms produce high amounts of
344 organic acids or exopolymers to aggregate the metal granules (Gadd, 2007; Guibaud et al.,
345 2009), and the surfaces of the budding and sheathed bacteria are surrounded by Mn oxide,

346 which forms a shell and protects the microbes from heavy metal invasion (Ghiorse, 1984;
347 Santelli et al., 2011). Many genes participating in the synthesis of organic acids and
348 exopolymers were discovered, such as isopropylmalate synthase, poly-hydroxyalkanoic acid
349 synthase and polyglutamate synthase.

350 The large abundance of phosphorus (Pi) and barium (Ba) in the sediment indicated that
351 the seamount cobalt-rich ferromanganese crust region possessed a high level of biological
352 productivity, which may directly support a rich diversity of microbes as well as benthos on the
353 seafloor. Oceanic Pi is usually enriched by marine organisms and settles into the sediment
354 (Delaney, 1998). The concentration of P₂O₅ in the sediment from station SEAM02 was 0.22%
355 higher than that from offshore sediment and continental crust (Table 1). Some genes involved
356 in the P cycle were found in the fosmid library, and the ratio of those genes to the functional
357 genes was 12.6% (22 / 174). Sedimentary Ba has been used as a proxy for the reconstruction
358 of past oceanic productivity (Schenau and De Lange, 2001). The concentration of Ba in the
359 sediment was 4.2 and 3.1 times than that in the offshore sediment and the continental crust,
360 respectively.

361 In a deep-sea environment, the electron acceptors are reduced in a sequential order based
362 on the free energy yield: O₂, NO₃⁻ and NO₂⁻, Mn and Fe, SO₄²⁻, and CO₂ (Wright et al., 2012).
363 The nitrogen cycle plays a key role not only in energy metabolism but also in the element
364 cycle, which affects the Mn and Fe oxidation and even mineralisation process. Ammonia
365 oxidation is a key process in marine nitrogen cycling and is executed by several microbial
366 groups, including aerobic chemoautotrophic archaea (ammonia-oxidising archaea, AOA) and
367 bacteria (ammonia-oxidising bacteria, AOB) (Beman et al., 2012; Li and Gu, 2013).
368 *Nitrosopumilus maritimus* SCM1 was the first discovered isolate of MGI Thaumarchaeota and
369 the first archaeal strain observed to undergo ammonia oxidation (Könneke et al., 2005). This
370 strain grows chemoautotrophically by oxidising ammonia aerobically and assimilating carbon
371 through the 3-hydroxypropionate/4-hydroxybutyrate pathway (Walker et al., 2010), indicating
372 that marine MGI Thaumarchaeota may be important to the nitrogen and carbon cycles in
373 ecosystem. In our study, MGI Thaumarchaeota was the most abundant archaea in the deep-sea
374 sediment (Fig. 1). Ammonia monooxygenase (AMO) is a key enzyme in the ammonia
375 oxidation process. An *amo* gene, having 98.0% amino acid sequence similarity with that from
376 *N. maritimus* SCM1, was observed in fosmid-end sequences (data not shown). Clone W4-93a
377 contained a 3-hydroxybutyryl-CoA dehydrogenase gene (ORF11), which is involved in the
378 3-hydroxypropionate/4-hydroxybutyrate cycle for autotrophic carbon fixation. Clone W4-87b,
379 a potential chemoautotrophic AOB (97.8% 16S rRNA gene identity with *Nitrosospira*

380 *multiformis*), was annotated. Although the *amo* gene was not observed in clone W4-87b, an
381 UreD gene (ORF4), which participates in the hydrolysis of urea to ammonia, was detected.
382 The enzyme could provide a substrate for ammonia oxidation. In addition, clone W4-93a
383 contained putative genes for a nitrogen regulatory protein (ORF25) and a nitroreductase
384 (ORF31). It is noteworthy that the combined microbial community and functional genes
385 imply a nitrogen cycle was an important biogeochemical process in the deep-sea sediment
386 from the seamount cobalt-rich ferromanganese crust region.

387 Our results not only support previous observations showing a relatively high abundance
388 of Proteobacteria and MGI Thaumarchaeota in the microbial community (Liao et al., 2011)
389 but also reveal the genomic and biological properties of the microbes in the deep-sea sediment
390 from seamount cobalt-rich ferromanganese deposit region. In total, 78 clones containing
391 archaeal and bacterial 16S rRNA genes were screened from 21000 clones in the metagenome
392 library; Proteobacteria (55.8%) and MGI Thaumarchaeota (97.1%) dominated in the bacterial
393 and archaeal communities, respectively. The continued analysis of microbial genomic data
394 indicated that numerous genes are involved in metabolism, which is the most abundant gene
395 group (44.0% in genes assigned to the COG classification system) (Fig. 3). Interestingly, a
396 significant proportion of genes (8.2%) were related to DNA transport and metabolism. The
397 plankton and microbe from the upper layers of seawater sink to the seafloor and become an
398 energy resource there. The total DNA sinking to the seafloor was estimated to 1.26×10^7
399 metric tons year⁻¹, and up to 0.45 gigatons of extracellular DNA is present in the top 10
400 centimetres of deep-sea sediments (Dell'Anno and Danovaro, 2005). Recent research has
401 found the DNA-eating ability of *Escherichia coli* during long-term survival (Finkel and Kolter,
402 2001; Finkel, 2006). Considering most of the planktons and microbes from the upper layers of
403 seawater are not able to survive in the deep-sea environment, the release of their DNA into the
404 sediment might be used as an important nutrient for indigenous microbes. Several
405 antibiotic-synthesising genes (penicillin and streptomycin) were also detected in the
406 metagenome library. Antibiotics can inhibit or kill some microbes, as well as benthos, and
407 help the microbes to occupy an ecological niche. Therefore, it is essential for microbes to
408 have alternative metabolic pathways to survive in the deep-sea environment.

409 Genomic divergence and HGT played important roles in the microbial adaption to the
410 heavy metal rich and cold deep-sea environment. Previous comparative genomic studies of
411 uncultivated marine planktonic archaea from different oceanic regions revealed significant
412 genomic divergences, regardless of the 16S rRNA gene sequence variation (Béjà et al., 2002;
413 Martin-Cuadrado et al., 2008). In our study, the comparative genomic analysis of clone

414 W4-93a, *N. maritimus* SCM1 and fosmid clone 74A4 suggested that considerable genome
415 divergence exists at the genus level (95.2% 16S rRNA gene sequence identity) between
416 sedimentary and planktonic lineages (Fig. 2). Although the 16S rRNA gene sequences of
417 fosmid W4-87b and *N. multiformis* ATCC 25196 showed a high identity (97.8%), their G+C
418 content (44.7% and 53.9%) and genome synteny surrounding the rRNA operon (data not
419 shown) were surprisingly different. Clone W5-51b also showed different genome synteny with
420 *Gemmatimonas aurantiaca* T-27^T, which is the only isolate in class Gemmatimonadetes
421 (Zhang et al., 2003). These differences may have been caused by genome evolution during
422 adaption to different habitats. Many HGT events have been observed in genomes, and the
423 HGT rate was 11.4% (23 / 201) among the seven fragments of known phylogenetic lineages
424 (W5-61a, W4-93a, W4-39b, W4-87b, W5-47b, W5-102b and W5-51b). However, 17.9% (36 /
425 201) of the predicted genes have no significant relatives, and the origin of some cannot be
426 determined due to a lack of sufficient information in the database. Taken together, the 11.4%
427 HGT rate should be most likely an underestimation. Most HGTs were from bacteria to
428 bacteria, with a few possibly were from bacteria to Thaumarchaeota, from Euryarchaeota to
429 Thaumarchaeota, from Euryarchaeota to bacteria, and even from eukarya to bacteria (Fig. S1).
430 Most of the genes acquired through HGT were involved in metabolism, including carbon and
431 energy metabolism (isopropylmalate dehydratase, pyrroloquinoline quinone biosynthesis
432 protein C, glutamate synthase, cytochrome c family protein, MIP family channel protein and
433 alcohol dehydrogenase) and nitrogen metabolism (nitrogen regulatory protein and urease
434 accessory protein UreD).

435 In conclusion, element concentrations in the sediment from the seamount cobalt-rich
436 ferromanganese crust region are different from those in other marine or terrestrial
437 environments. The large abundances of heavy metals (Mn, Ni, Co, Cu), P and Ba in the
438 sediment from station SEAM02 implied a unique microbial community with high biodiversity.
439 Microbes inhabiting the cobalt-rich ferromanganese crust region not only adapt to high
440 amounts of heavy metal but also might participate in the biomineralization process, as
441 observed at the gene level. Alternative metabolic pathways and a variety of stress genes are
442 essential for microbial survival in the deep-sea environment. Genomic divergence and HGT
443 may played important roles in the microbial adaption to the deep-sea environment. Some
444 microbes, which come from the upper seawater, might obtain a series of new features and
445 adapt to this harsh environment via high frequency HGT events. The information gathered via
446 the rRNA-gene based PCR screening method provided insight only into the genomic regions
447 directly adjacent to rRNA operons. However, this is the first metagenomic study of deep-sea

448 sediment from the cobalt-rich ferromanganese crust region, giving us some insights into the
449 genetic and functional information about uncultured microorganisms in the cobalt-rich
450 ferromanganese crust region.

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452

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454 **References**

455 Adachi O, Ano Y, Toyama H, Matsushita K (2007). Biooxidation with PQQ- and
456 FAD-dependent dehydrogenases. *Modern Biooxidation*. Wiley-VCH Verlag GmbH & Co.
457 KGaA. pp 1-41.

458

459 Béjà O, Koonin EV, Aravind L, Taylor LT, Seitz H, Stein JL *et al* (2002). Comparative
460 genomic analysis of archaeal genotypic variants in a single population and in two different
461 oceanic provinces. *Applied and Environmental Microbiology* **68**: 335-345.

462

463 Beman JM, Popp BN, Alford SE (2012). *Quantification of ammonia oxidation rates and*
464 *ammonia-oxidizing archaea and bacteria at high resolution in the Gulf of California and*
465 *eastern tropical North Pacific Ocean*, vol. 57. American Society of Limnology and
466 Oceanography: Waco, TX, ETATS-UNIS.

467

468 Candela T, Fouet A (2006). Poly-gamma-glutamate in bacteria. *Molecular Microbiology* **60**:
469 1091-1098.

470

471 Clark MR, Rowden AA, Schlacher T, Williams A, Consalvey M, Stocks KI *et al* (2010). The
472 ecology of seamounts: structure, function, and human impacts. *Ann Rev Mar Sci* **2**: 253-278.

473

474 Craig JD, Andrews JE, Meylan MA (1982). Ferromanganese deposits in the Hawaiian
475 Archipelago. *Marine Geology* **45**: 127-157.

476

477 Delaney ML (1998). Phosphorus accumulation in marine sediments and the oceanic
478 phosphorus cycle. *Global Biogeochemical Cycles* **12**: 563-572.

479

480 Dell'Anno A, Danovaro R (2005). Extracellular DNA plays a key role in deep-sea ecosystem
481 functioning. *Science* **309**: 2179.

482

483 DeLong EF (1992). Archaea in coastal marine environments. *Proceedings of the National*
484 *Academy of Sciences of the United States of America* **89**: 5685-5689.

485

486 Duffy EJ (2008). "Seamount". *CenSeam: a Global Census of Marine Life on Seamounts*
487 *(Content Partner) and National Oceanic and Atmospheric Administration (Content source)*.
488 Environmental Information Coalition, National Council for Science and the Environment:
489 Washington D.C.

490
491 Ehrhardt CJ, Haymon RM, Lamontagne MG, Holden PA (2007). Evidence for hydrothermal
492 Archaea within the basaltic flanks of the East Pacific Rise. *Environmental Microbiology* **9**:
493 900-912.
494
495 Emerson D, Moyer CL (2010). Microbiology of seamounts: common patterns observed in
496 community structure. *Oceanography* **23**: 148-163.
497
498 Finkel SE, Kolter R (2001). DNA as a nutrient: novel role for bacterial competence gene
499 homologs. *Journal of Bacteriology* **183**: 6288-6293.
500
501 Finkel SE (2006). Long-term survival during stationary phase: evolution and the GASP
502 phenotype. *Nat Rev Microbiol* **4**: 113-120.
503
504 Fu Y, Peng J, Qu W, Hu R, Shi X, Du A (2005). Os isotopic compositions of a cobalt-rich
505 ferromanganese crust profile in Central Pacific. *ChinSciBull* **50**: 2106-2112.
506
507 Fuyuan Z, Weiyan Z, Kechao ZHU, Shuitu GAO, Haisheng Z, Xiaoyu Z *et al* (2008).
508 Distribution characteristics of cobalt-rich ferromanganese crust resources on submarine
509 seamounts in the Western Pacific. *Acta Geologica Sinica - English Edition* **82**: 796-803.
510
511 Gadd GM (2007). Geomycology: biogeochemical transformations of rocks, minerals, metals
512 and radionuclides by fungi, bioweathering and bioremediation. *Mycological Research* **111**:
513 3-49.
514
515 Garcia-Martinez J, Martinez-Murcia A, Anton AI, Rodriguez-Valera F (1996). Comparison of
516 the small 16S to 23S intergenic spacer region (ISR) of the rRNA operons of some Escherichia
517 coli strains of the ECOR collection and E. coli K-12. *Journal of Bacteriology* **178**:
518 6374-6377.
519
520 Ghiorse WC (1984). Biology of iron- and manganese-depositing bacteria. *Annu Rev*
521 *Microbiol* **38**: 515-550.
522
523 Gilbert J, Blainey PC, Mosier AC, Potanina A, Francis CA, Quake SR (2011). Genome of a
524 low-salinity ammonia-oxidizing archaeon determined by single-cell and metagenomic
525 analysis. *PLoS ONE* **6**: e16626.
526
527 Gillan DC, Danis B (2007). The archaeobacterial communities in Antarctic bathypelagic
528 sediments. *Deep Sea Research Part II: Topical Studies in Oceanography* **54**: 1682-1690.
529
530 Guibaud G, van Hullebusch E, Bordas F, d'Abzac P, Joussein E (2009). Sorption of Cd(II) and
531 Pb(II) by exopolymeric substances (EPS) extracted from activated sludges and pure bacterial
532 strains: Modeling of the metal/ligand ratio effect and role of the mineral fraction. *Bioresource*
533 *Technology* **100**: 2959-2968.

534
535 Heijs SK, Haese RR, Wielen PWJJ, Forney LJ, Elsas JD (2007). Use of 16S rRNA gene based
536 clone libraries to assess microbial communities potentially involved in anaerobic methane
537 oxidation in a Mediterranean cold seep. *Microbial Ecology* **53**: 384-398.
538
539 Hillier JK, Watts AB (2007). Global distribution of seamounts from ship-track bathymetry
540 data. *Geophysical Research Letters* **34**: L13304.
541
542 Ito M, Tsunekawa M, Yamaguchi E, Sekimura K, Kashiwaya K, Hori K *et al* (2008).
543 Estimation of degree of liberation in a coarse crushed product of cobalt-rich ferromanganese
544 crust/nodules and its gravity separation. *International Journal of Mineral Processing* **87**:
545 100-105.
546
547 Iyer SD, M. MC, Das P, Kalangutkar NG (2012). Seamounts - characteristics, formation,
548 mineral deposits and biodiversity. *Geologica Acta* **10**: 295-308.
549
550 Jiang X, Xu X, Huo Y, Wu Y, Zhu X, Zhang X *et al* (2012). Identification and characterization
551 of novel esterases from a deep-sea sediment metagenome. *Archives of Microbiology* **194**:
552 207-214.
553
554 Könneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl DA (2005).
555 Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* **437**: 543-546.
556
557 Kanehisa M, Goto S (2000). KEGG: kyoto encyclopedia of genes and genomes. *Nucleic
558 Acids Research* **28**: 27-30.
559
560 Kato S, Kobayashi C, Kakegawa T, Yamagishi A (2009a). Microbial communities in
561 iron-silica-rich microbial mats at deep-sea hydrothermal fields of the Southern Mariana
562 Trough. *Environmental Microbiology* **11**: 2094-2111.
563
564 Kato S, Yanagawa K, Sunamura M, Takano Y, Ishibashi J-i, Kakegawa T *et al* (2009b).
565 Abundance of Zetaproteobacteria within crustal fluids in back-arc hydrothermal fields of the
566 Southern Mariana Trough. *Environmental Microbiology* **11**: 3210-3222.
567
568 Koschinsky A, Hein JR (2003). Uptake of elements from seawater by ferromanganese crusts:
569 solid-phase associations and seawater speciation. *Marine Geology* **198**: 331-351.
570
571 Li M, Gu J-D (2013). Community structure and transcript responses of anammox bacteria,
572 AOA, and AOB in mangrove sediment microcosms amended with ammonium and nitrite.
573 *Applied Microbiology and Biotechnology*: 1-16.
574
575 Li Y, Li F, Zhang X, Qin S, Zeng Z, Dang H *et al* (2008). Vertical distribution of bacterial and
576 archaeal communities along discrete layers of a deep-sea cold sediment sample at the East
577 Pacific Rise (~13°N). *Extremophiles* **12**: 573-585.

578
579 Liao L, Xu X-W, Jiang X-W, Wang C-S, Zhang D-S, Ni J-Y *et al* (2011). Microbial diversity
580 in deep-sea sediment from the cobalt-rich crust deposit region in the Pacific Ocean. *FEMS*
581 *Microbiology Ecology* **78**: 565-585.
582
583 Lopez-Diaz I, Clarke S, Mandelstam J (1986). spoIID Operon of *Bacillus subtilis*: cloning and
584 sequence. *Journal of General Microbiology* **132**: 341-354.
585
586 Losekann T, Knittel K, Nadalig T, Fuchs B, Niemann H, Boetius A *et al* (2007). Diversity and
587 abundance of aerobic and anaerobic methane oxidizers at the Haakon Mosby Mud Volcano,
588 Barents Sea. *Applied and Environmental Microbiology* **73**: 3348-3362.
589
590 Martín-Cuadrado A-B, López-García P, Alba J-C, Moreira D, Monticelli L, Strittmatter A *et al*
591 (2007). Metagenomics of the deep Mediterranean, a warm bathypelagic habitat. *PLoS ONE* **2**:
592 e914.
593
594 Martín-Cuadrado A-B, Rodríguez-Valera F, Moreira D, Alba JC, Ivars-Martínez E, Henn MR
595 *et al* (2008). Hindsight in the relative abundance, metabolic potential and genome dynamics
596 of uncultivated marine archaea from comparative metagenomic analyses of bathypelagic
597 plankton of different oceanic regions. *The ISME Journal* **2**: 865-886.
598
599 Menard HW (1964). *Marine Geology of the Pacific*. McGraw-Hill: New York.
600
601 Miroshnichenko M, Hippe H, Stackebrandt E, Kostrikina N, Chernyh N, Jeanthon C *et al*
602 (2001). Isolation and characterization of *Thermococcus sibiricus* sp. nov. from a Western
603 Siberia high-temperature oil reservoir. *Extremophiles* **5**: 85-91.
604
605 Muiños SB, Hein JR, Frank M, Monteiro JH, Gaspar L, Conrad T *et al* (2013). Deep-sea
606 Fe-Mn crusts from the Northeast Atlantic Ocean: composition and resource considerations.
607 *Marine Georesources & Geotechnology* **31**: 40-70.
608
609 Nies DH (1992). Resistance to cadmium, cobalt, zinc, and nickel in microbes. *Plasmid* **27**:
610 17-28.
611
612 Norton JM, Klotz MG, Stein LY, Arp DJ, Bottomley PJ, Chain PSG *et al* (2008). Complete
613 genome sequence of *Nitrosospira multiformis*, an ammonia-oxidizing bacterium from the soil
614 environment. *Applied and Environmental Microbiology* **74**: 3559-3572.
615
616 Rowden AA, Dower JF, Schlacher TA, Consalvey M, Clark MR (2010). Paradigms in
617 seamount ecology: fact, fiction and future. *Marine Ecology* **31**: 226-241.
618
619 Santelli CM, Orcutt BN, Banning E, Bach W, Moyer CL, Sogin ML *et al* (2008). Abundance
620 and diversity of microbial life in ocean crust. *Nature* **453**: 653-656.
621

622 Santelli CM, Webb SM, Dohnalkova AC, Hansel CM (2011). Diversity of Mn oxides
623 produced by Mn(II)-oxidizing fungi. *Geochimica et Cosmochimica Acta* **75**: 2762-2776.
624

625 Schauer R, Bienhold C, Ramette A, Harder J (2009). Bacterial diversity and biogeography in
626 deep-sea surface sediments of the South Atlantic Ocean. *The ISME Journal* **4**: 159-170.
627

628 Schenau SJ, De Lange GJ (2001). Phosphorus regeneration vs. burial in sediments of the
629 Arabian Sea. *Marine Chemistry* **75**: 201-217.
630

631 Schlacher TA, Rowden AA, Dower JF, Consalvey M (2010). Seamount science scales
632 undersea mountains: new research and outlook. *Marine Ecology* **31**: 1-13.
633

634 Staudigel H, Koppers AAP, Plank TA, Hanan BB (2010). Seamounts in the subduction factory.
635 *Oceanography* **23**: 176-181.
636

637 Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011). MEGA5: Molecular
638 Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and
639 Maximum Parsimony Methods. *Molecular Biology and Evolution* **28**: 2731-2739.
640

641 Tatusov RL, Galperin MY, Natale DA, Koonin EV (2000). The COG database: a tool for
642 genome-scale analysis of protein functions and evolution. *Nucleic Acids Research* **28**: 33-36.
643

644 Tebo BM, Bargar JR, Clement BG, Dick GJ, Murray KJ, Parker D *et al* (2004). Biogenic
645 manganese oxides: properties and mechanisms of formation. *Annual Review of Earth and*
646 *Planetary Sciences* **32**: 287-328.
647

648 Verlaan PA (1992). Benthic recruitment and manganese crust formation on seamounts. *Marine*
649 *Biology* **113**: 171-174.
650

651 Walker CB, de la Torre JR, Klotz MG, Urakawa H, Pinel N, Arp DJ *et al* (2010).
652 *Nitrosopumilus maritimus* genome reveals unique mechanisms for nitrification and
653 autotrophy in globally distributed marine crenarchaea. *Proceedings of the National Academy*
654 *of Sciences of the United States of America* **107**: 8818-8823.
655

656 Wang X-H, Schlossmacher U, Natalio F, Schroder HC, Wolf SE, Tremel W *et al* (2009).
657 Evidence for biogenic processes during formation of ferromanganese crusts from the Pacific
658 Ocean: implications of biologically induced mineralization. *Micron* **40**: 526-535.
659

660 Wang X, Müller WEG (2009). Marine biominerals: perspectives and challenges for
661 polymetallic nodules and crusts. *Trends in Biotechnology* **27**: 375-383.
662

663 Wang X, Wiens M, Schröder H, Schloßmacher U, Müller WG (2011). Molecular
664 biomineralization: toward an understanding of the biogenic origin of polymetallic nodules,
665 seamount crusts, and hydrothermal vents. In: Müller WEG (ed). *Molecular Biomineralization*.

666 Springer Berlin Heidelberg. pp 77-110.
667
668 Wedepohl KH (1995). The composition of the continental crust. *Geochimica et*
669 *Cosmochimica Acta* **59**: 1217-1232.
670
671 Wessel P, Sandwell DT, S.-S. K (2010). The global seamount census. *Oceanography* **23**:
672 115-129.
673
674 Wright JJ, Konwar KM, Hallam SJ (2012). Microbial ecology of expanding oxygen minimum
675 zones. *Nat Rev Microbiol* **10**: 381-394.
676
677 Wu Y-H, Liao L, Wang C-S, Ma W-L, Meng F-X, Wu M *et al* (2013). A comparison of
678 microbial communities in deep-sea polymetallic nodules and the surrounding sediments in the
679 Pacific Ocean. *Deep Sea Research Part I: Oceanographic Research Papers* **79**: 40-49.
680
681 Xu M, Wang P, Wang F, Xiao X (2005). Microbial diversity at a deep-sea station of the
682 Pacific nodule province. *Biodiversity and Conservation* **14**: 3363-3380.
683
684 Yesson C, Clark MR, Taylor ML, Rogers AD (2011). The global distribution of seamounts
685 based on 30 arc seconds bathymetry data. *Deep Sea Research Part I: Oceanographic*
686 *Research Papers* **58**: 442-453.
687
688 Zhang H, Sekiguchi Y, Hanada S, Hugenholtz P, Kim H, Kamagata Y *et al* (2003).
689 *Gemmatimonas aurantiaca* gen. nov., sp. nov., a Gram-negative, aerobic,
690 polyphosphate-accumulating micro-organism, the first cultured representative of the new
691 bacterial phylum Gemmatimonadetes phyl. nov. *International Journal of Systematic and*
692 *Evolutionary Microbiology* **53**: 1155-1163.
693
694 Zhao Q (1988). *Ocean geochemistry*. the Geological Publishing House: Beijing.
695
696 Zhu W, Lomsadze A, Borodovsky M (2010). Ab initio gene identification in metagenomic
697 sequences. *Nucleic Acids Research* **38**: e132-e132.
698

699 Table 1. Geochemical properties of the sediment sample SEAM02, offshore sediment and
 700 continental crust

	SEAM02	Offshore Sediment*	Continental Crust†
SiO ₂ /%	54.07	54.43	61.50
Al ₂ O ₃ /%	8.35	12.03	15.10
Fe ₂ O ₃ /%	4.14	4.59	6.28
CaO /%	0.92	10.05	5.50
MgO /%	2.58	1.84	3.70
K ₂ O /%	2.10	1.98	2.40
Na ₂ O /%	7.18	2.24	3.20
TiO ₂ /%	0.48	0.57	0.68
MnO /%	0.33	0.12	0.10
P ₂ O ₅ /%	0.22	0.12	0.18
Ni /μg g-1	74	27	56
Co /μg g-1	52	14	24
V /μg g-1	94	-	98
Cr /μg g-1	57	43	126
Cu /μg g-1	134	20	25
Zn /μg g-1	83	72	65
Cd /μg g-1	0.09	-	-
Pb /μg g-1	35	28	14.8
Mo /μg g-1	4.4	-	-
Ba /μg g-1	1821	431	584

701 *Data of the East China Sea from Zhao, 1988;

702 †Data from Wedepohl, 1995.

703

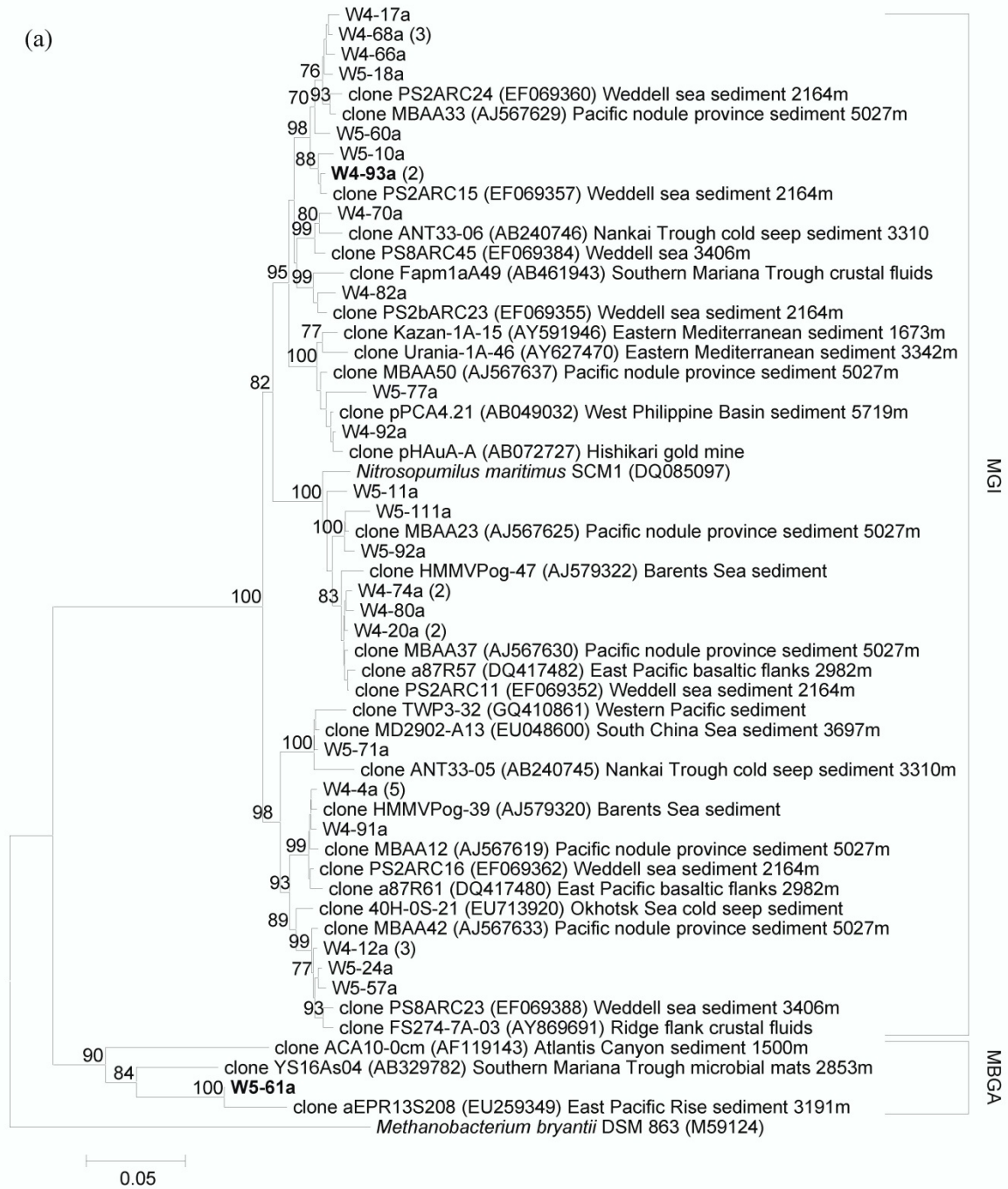
704 Fig. 1. Neighbour-joining trees of archaeal (a) and bacterial (b) 16S rRNA genes amplified
705 from the metagenomic library SEAM02. Numbers in parentheses represent the total number
706 of clones with the same 16S rRNA sequence. The environments where the relatives were
707 obtained from are given after their NCBI accession numbers. Numbers at nodes correspond to
708 bootstrap values based on 500 replicates, and the values less than 70% were omitted. Bar,
709 0.05 substitutions per nucleotide position.

710

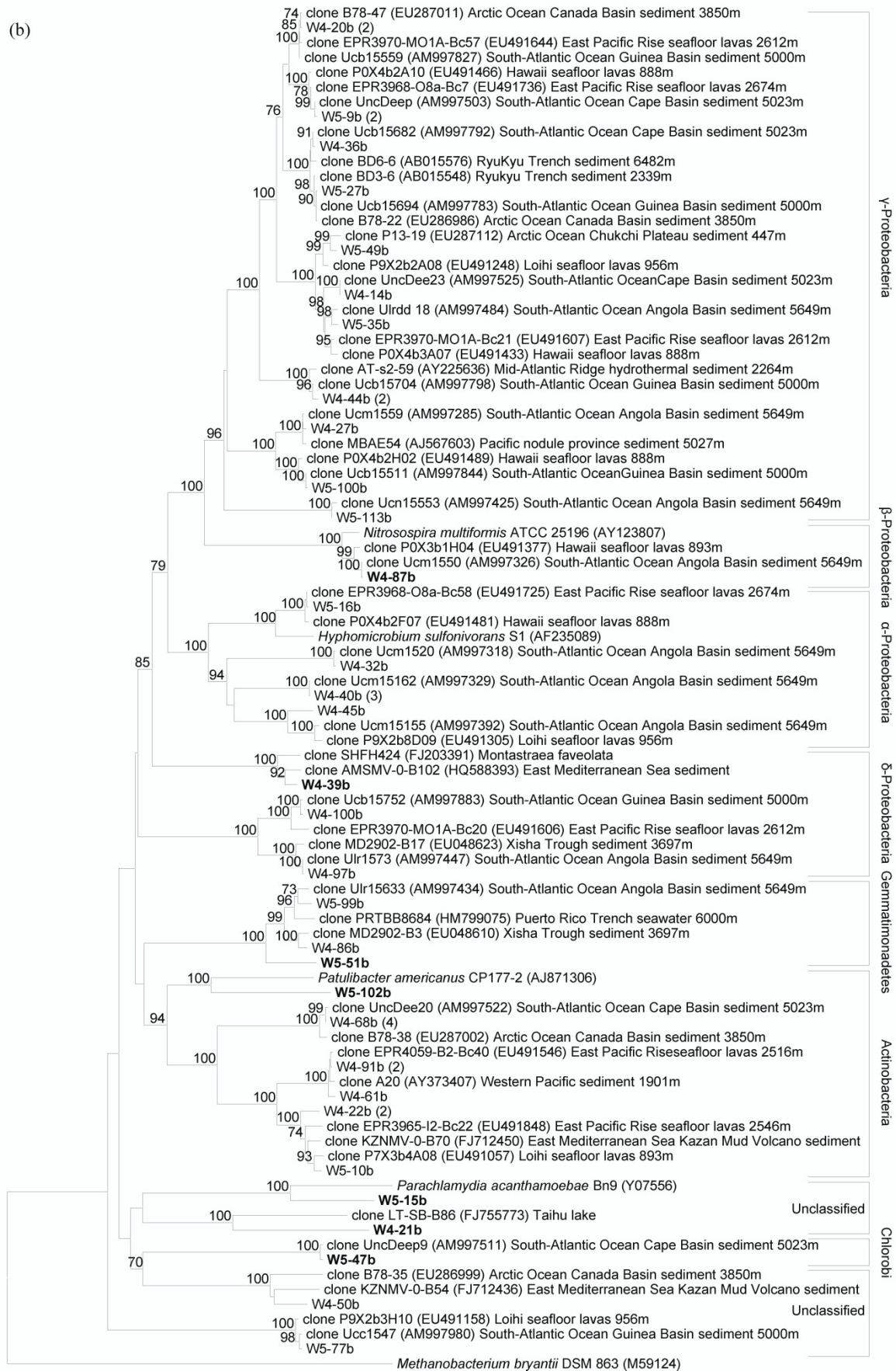
711 Fig. 2. Gene maps of fosmid clone W4-93a, *Nitrosopumilus maritimus* SCM1 and fosmid
712 74A4. The rRNA genes were used as an alignment point. ORFs: 1, ATP-dependent DNA
713 ligase; 3, hypothetical protein; 4, fructose-1,6-bisphosphatase; 5, translation elongation factor
714 EF-1 alpha; 6, ribosomal protein S10; 7, RNA polymerase Rbp10; 8, C2H2 Zn finger protein;
715 9, hypothetical protein; 11, rossmann fold nucleotide-binding protein; 12,
716 3-hydroxybutyryl-CoA dehydrogenase; 13, hypothetical protein; 14, HIT superfamily
717 hydrolase; 15, DnaJ class molecular chaperone; 18, TPR repeat-containing protein; 23,
718 glutamate-1-semialdehyde aminotransferase; 30, hypothetical protein; 34, peptide methionine
719 sulfoxide reductase; 36, alpha/beta hydrolase; 37, AbrB family transcription regulator; 38,
720 hypothetical protein; 39, poly(R)-hydroxyalkanoic acid synthase subunit PhaC; 40,
721 hypothetical protein; 41, hypothetical protein; 44, transcription factor TFIIB cyclin-related
722 protein; 46, TPR repeat-containing protein; 48, biotin--acetyl-CoA-carboxylase ligase.

723

724 Fig. 3. Functional classification of genes of the nine fosmids according to COG classification
725 system. Blue, the COG corresponding to the “cellular processes and signalling”; green -
726 “information storage and processing”; red - “metabolism”; orange - “poorly characterised”.

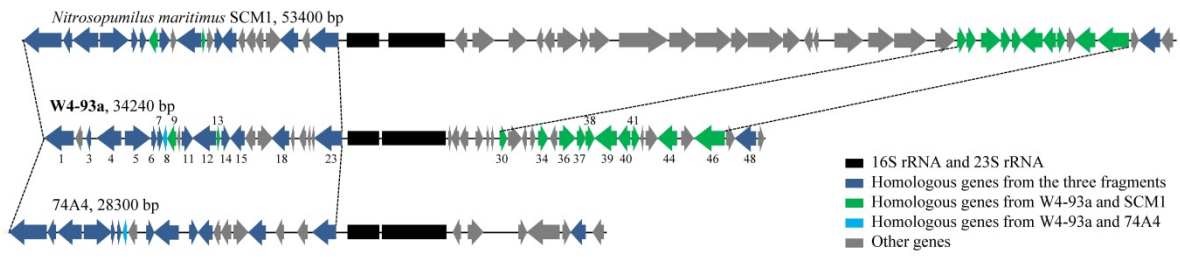


(b)



0.05

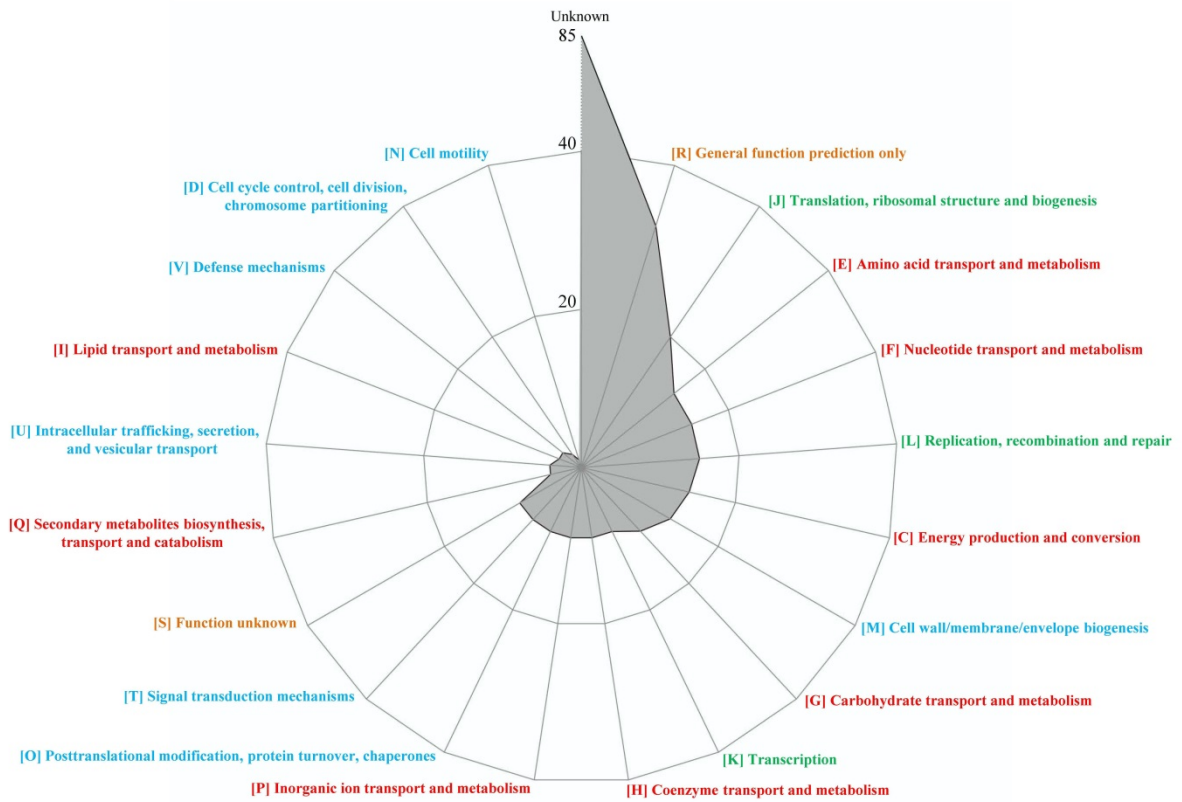
730 Fig. 2.



731

732

733 Fig. 3.



734