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Different *Planctomycetes* Diversity Patterns in Latitudinal Surface Seawater of the Open Sea and in Sediment

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The 16S rRNA gene approach was applied to investigate the diversity of *Planctomycetes* in latitudinal surface seawater of the Western Pacific Ocean. The results revealed that the *Pirellula-Rhodopirellula-Blastopirellula* clade dominated the *Planctomycetes* community at all surface seawater sites while the minority genera *Gemmata* and *Planctomyces* were only found at sites H5 and H2 respectively. Although the clone frequency of the PRB clade seemed stable (between 83.3% and 94.1%) for all surface seawater sites, the retrieved *Pirellula-Rhodopirellula-Blastopirellula* clade presented unexpected diversity. Interestingly, low latitude seawater appeared to have higher diversity than mid-latitudes. ∫-LIBSHUFF software analysis revealed significantly different diversity patterns between in latitudinal surface seawater and in the sediment of South China Sea station M2896. Our data suggested that different hydrological and geographic features contributed to the shift of *Planctomycetes* diversity in marine environments. This is, to our knowledge, the first systematic assessment of *Planctomycetes* in latitudinal surface seawater of the open sea and the first comparison of diversity pattern between surface seawater and sediments and has broadened our understanding of *Planctomycetes* diversity in marine environments.

***Keywords*:** *Planctomycetes*, diversity, seawater, 16S rRNA gene

In recent decades, *Planctomycetes* has become one of the focused groups, for *Planctomycetes* is an unusual, Gram-negative, deep-branching group of *The Bacteria* and a microbial model to explore the evolutionary relationship between bacteria and eukaryotes in terms of their compartment and peptidoglycan-less characteristics (Konig *et al.*, 1984; Stachebrandt *et al.*, 1984; Fuerst, 1995; Lindsay *et al.*, 1997). Budding bacteria of the phylum *Planctomycetes* comprise *Panctomyces*, *Isosphaera*, *Gemmata*, and the *Pirellula-Rhodopirellula-Blastopirellula* clade (PRB clade) (Schlesner *et al.*, 2004). Recently, 4 uncultured anammox "*Candidatus*" genera "Anammoxoglobus", "Brocadia", "Kuenenia", and "Scalindua" have been defined (Schmid *et al.*, 2003; Kartal *et al.*, 2007). Previous work show that anammox bacteria can perform anaerobic ammonium oxidation and some genera perform C1 or sulfatase metabolism from oxygen minimum zones, which makes *Planctomycetes* an important group in natural ecosystems (Schmid *et al.*, 2003; Bauer *et al.*, 2004; Elshahed *et al.*, 2007).

Previous culture-independent 16S rRNA gene based surveys reveal the ubiquity of *Planctomycetes* in various marine environments such as marine sediment, the water column, marine snow, marine ice, etc (Miskin *et al.*, 1999; Freitag and Prosser, 2003; Brummer *et al.*, 2004; Ivanova and Dedysh, 2006). Recent investigation of *Planctomycetes* also shows that a gradient in phylotype diversity is found in the Black Sea (Kirkpatrick *et al.*, 2006). Although investigations of *Planctomycetes* in marine environments have increased, no report has focused on the systematic assessment of *Planctomycetes* diversity in gradient surface seawater latitudes of the open sea, or on the comparison of *Planctomycetes* diversity between surface seawater and sediment. Whether *Planctomycetes* diversity changes within a large latitude scale of open sea such as the Western Pacific Ocean, and whether *Planctomycetes* diversity significantly differs between in surface seawater and in sediment, are still unknown. Such investigations and comparison of *Planctomycetes* diversity will help us to interpret their potentially ecological role and adaptation in marine environments.

In this study, we investigated how the diversity and composition of *Planctomycetes* bacteria varied with surface seawater latitudes of the Western Pacific Ocean, and we also aimed to compare diversity patterns between in surface seawater and in the sediment.

Materials and Methods

Sample collection and DNA extraction

The seawater samples were collected from a series of latitudinal sites in the West Pacific Ocean (Fig. 1 and Table 1) in November 2005. Surface seawater (about 2 L) was filtered through 0.22 μm pore size, 47 mm diameter filters (Pall-Gelman, USA) with a vacuum of less than 0.03 MPa. The filters were immediately immersed in 1.8 ml lysis buffer (40 mM EDTA, 50 mM Tris-HCl, 0.75 M sucrose, pH 8.0). In order to account for the total abundance of bacterium and viruses, surface seawater samples were also collected with Niskin bottles and fixed with glutaraldehyde (final concen-

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tration 0.5%) for 15 min and then were stored for later flow cytometric analysis. All samples were frozen at -20°C until analysis.

DNA extraction was performed as described previously (Brummer *et al.*, 2004). Seawater DNA was further purified using 1% (w/v) low-melting-point agarose and a TaKaRa Agarose Gel DNA Purification Kit (TaKaRa, Japan) based on the manufacturer's instructions. Total abundance of bacterium and viruses were determined using flow cytometric analysis (Jiao *et al*., 2002).

16S rRNA gene PCR amplifications

Seawater DNAs were used as a template to amplify the 16S rRNA gene using the primer set Pla-46-F/1392-R: Pla-46-F; GACTTGCATGCCTAATCC and 1392-R; ACGGGC GGTGTGTAC, which covered almost the full length of the 16S rRNA gene and often used to explore the *Planctomycetes* community in various environments (Chouari *et al*., 2003; Freitag and Prosser, 2003; Buckley *et al*., 2006; Elshahed *et al*., 2007). 16S rRNA gene PCR amplifications using primer set Pla-46-F/1392-R were carried out as previously reported (Freitag and Prosser, 2003). Meanwhile, a newly designed primer set: Pla-46-F/pla-1097R; GGTTTCGCTCGTTANGG (Shu and Jiao, unpublished data) was used to supplement the diversity at site H5. Amplification with Pla-46-F/Pla-1097-R was carried out as follows: A total volume of 50 μl containing 10 nmol dNTP, 15 nmol buffer plus Mg^{2+}, 15 pmol each primer, 100 ng of template DNA and 5 U of LA *Taq* DNA polymerase (TaKaRa) was used. Thermal cycling was carried out for 4 min at 95°C, followed by 34 cycles of 60 sec at 95°C, 70 sec at 52°C, and 8 min at 72°C. A negative control was included in each PCR reaction.

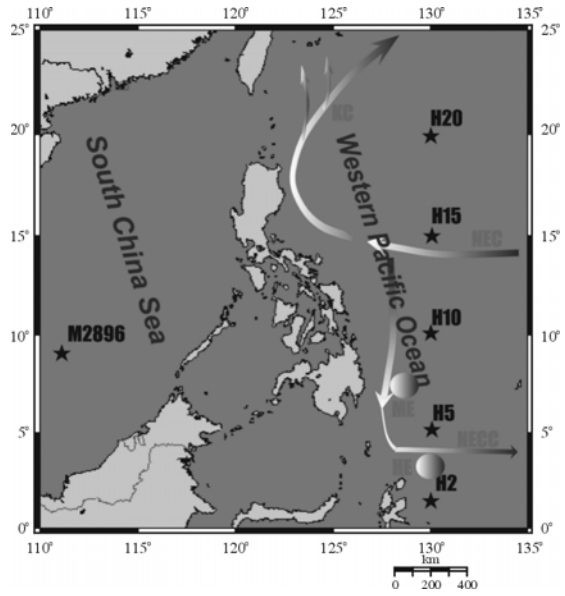

Fig. 1. The sampling sites used in this study. KC, Kuroshio Current; NEC, North Equatorial Current; NECC, North Equatorial Counter Current; MC, Mindanao Current; HE, Halmahera Eddy; ME, Mindanao Eddy.

16S rRNA gene cloning, RFLP analysis, and sequencing

All PCR products amplified from community DNA were purified using a TaKaRa Agarose Gel DNA Purification Kit. The cleaned fragments were introduced to a pMD18-T Vector (TaKaRa, Japan) based on the manufacturer's instructions. The ligations were transformed into supercompetent *E. coli* cells and clone libraries were constructed. Clones with appropriately sized inserts were chosen randomly from each clone library, and fragments of the *Planctomycetes* 16S rRNA gene were re-amplified from these clones. To screen the clones for grouping into similar clone types and subsequent sequence analysis, clones containing the 16S rRNA gene were subjected to RFLP analysis using two restriction enzymes (*Rsa*I and *Hae*III, TaKaRa, Japan) which recognize a 4-bp restriction site. The restriction enzyme reaction products were electrophoresed in a 2% agarose gel in 1× TAE. Clones were discriminated according to their RFLP patterns. Representatives of all RFLP pattern clones were chosen for sequencing. The sequencing was performed on an ABI 3730 automated sequencer (Sangon, China).

16S rRNA gene sequence analysis

Diversity of *Planctomycetes* was evaluated using sequence similarity of 97% as the definition of operational taxonomic units (OTUs). To statistically analyze the size and quality of the clone libraries, non-parametric coverage and phylotype richness estimators were calculated using DOTUR software (http://www.plantpath.wisc.edu/fac/joh/dotur.html). Sequences were subjected to BLAST and the Ribosomal Database Project online to determine the level of similarity with other *Planctomycetes* 16S rRNA gene sequences. The presence of chimeras was determined using the CHIMERA_CHECK program. Partial 16S rRNA gene sequences were aligned and

Table 1. Information of sampling sites and clone libraries

Samples	Location	Water depth (m)	Temp (°C)	Total bacterium (cell/ml)	Total viruses (cell/ml)	Primer sets	Total clone number	Number of *Planctomycetes* -related clones	Number of *Planctomycetes* OTUs
H2	2°N, 130°E	>5000	31.7	3.26E+05	7.22E+06	I	48	47	7
H5	5°N, 130°E	>5000	31.6	4.87E+05	7.86E+06	I, II	107	97	22
H10	10°N, 130°E	>5000	31.5	5.47E+05	5.64E+06	I	62	60	4
H15	15°N, 130°E	>5000	31.4	6.01E+05	6.96E+06	I	55	55	4
H20	20°N, 130°E	>5000	29.3	1.17E+06	5.91E+06	I	42	41	3

* Primer sets include I, Pla-46-F/1392-R; II, Pla-46-F/Pla-1097-R.

compiled using the BioEdit v7.0.5 Program and CLUSTAL X 1.83 software packages. Bootstrapping of the neighbor-joining tree was carried out with 100 replicates using the Phylip package v3.63 (http://evolution.genetics.washington.edu/phylip.html) (Lim and Zhang, 1999). In order to assess the branch support given using the neighbor-joining method, tree topology was also calculated using the MrBayes program (http://morphbank.ebc.uu.se/mrbayes) (Ronquist and Huelsenbeck, 2003). To determine the significance of differences between clone libraries of environmental 16S rRNA gene sequences, selective sequences were analyzed using ∫-LIBSHUFF software (http://whitman.myweb.uga.edu/libshuff.html) (Schloss *et al.*, 2004).

Nucleotide sequence accession numbers

The partial clone sequences determined in this study have been deposited in the GenBank database under accession no. EU127924 to EU127958, EU140628 to EU140743 and EU169222.

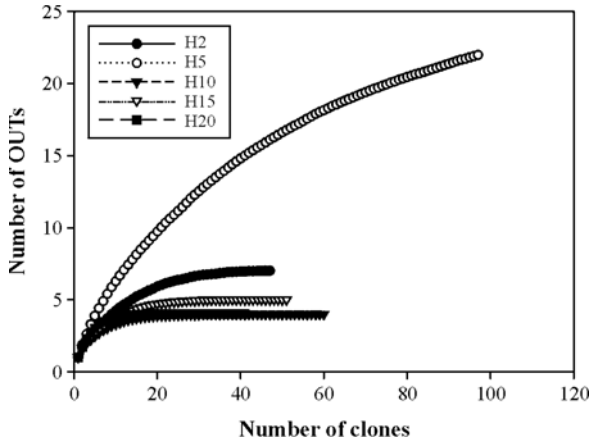

Fig. 2. Rarefaction analyses for clone libraries in surface seawater.

Results

Out of the all surface seawater analyzed sequences, a total of 314 clones from clone libraries generated using primer sets Pla-46-F/1392-R or Pla-46-F/Pla-1097-R (Table 1) were obtained, and 227 clones were selected after RFLP analysis. Representatives of these RFLP pattern clones were chosen for sequencing. After the exclusion of chimeric sequences (n=5) and non-*Planctomycetes* sequences (n=14), a total of 39 *Planctomycetes* OTUs, which covered the PRB clade, *Gemmata*, *Planctomyces,* and unidentified *Planctomycetes*, were identified using a sequence similarity of 97% as the OTU. The bacterial clone library constructed from surface seawater at sites H2, H5, H10, H15, and H20 consisted of 7, 22, 4, 4, and 3 OTUs, respectively. The highest diversity of *Planctomycetes* was found at sites H5 and H2 (Table 1). Rarefaction analysis using the DOTUR program showed that the *Planctomycetes* diversity observed in Western Pacific Ocean surface seawater is nearly exhaustive (Fig. 2).

Considering all seawater sites together, 47.7% unique sequences were found to be ≤97% similar to environmental and cultured 16S rDNA sequences from the databases, and 24.3% sequences were ≤95% similarity. 92.2% clustered with the uncultured PRB clade, 2.7% with *Planctomyces*, 1.8% with *Gemmata*, and 3.3% with an unidentified group. As Fig. 3 shows, the majority of sequences were PRB clade related *Planctomycetes* and the percentages of PRB clade seemed stable at the 5 latitudinal seawater samples. The highest percentage of the PRB clade *Planctomycetes* was found at site H5 with 94.1% and the lowest, with 83.3%, at site H20. Sites H2 and H5 showed higher diversity than other seawater sites, as supported by more OTUs. Other genera of *Planctomycetes* such as *Planctomyces* and *Gemmata* were only found at sites H2 and H5 respectively (Fig. 3).

In order to analyze further the retrieved sequences and to compare the different *Planctomycetes* diversity patterns between in surface seawater and in the sediment, a phylo-

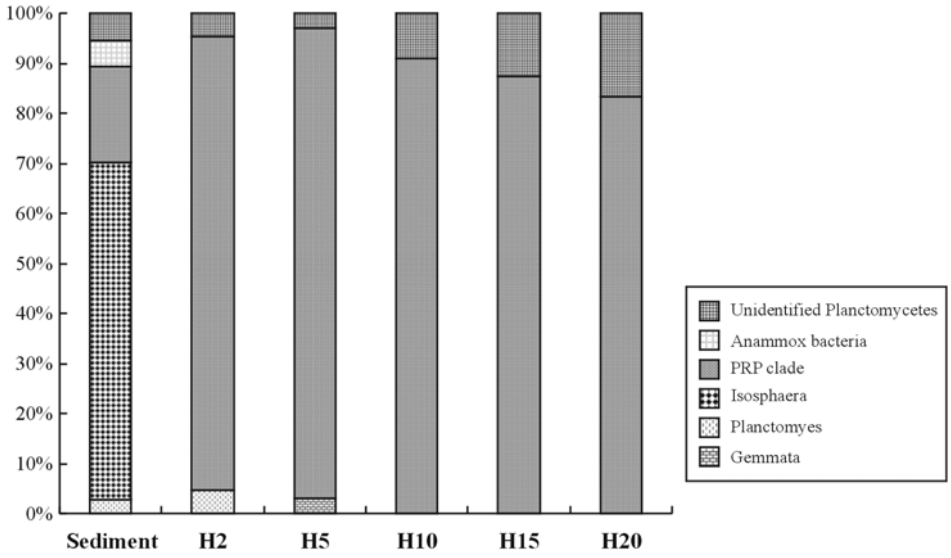

Fig. 3. Composition and diversity of *Planctomycetes* for SCS sediment and Western Pacific Ocean surface seawater.

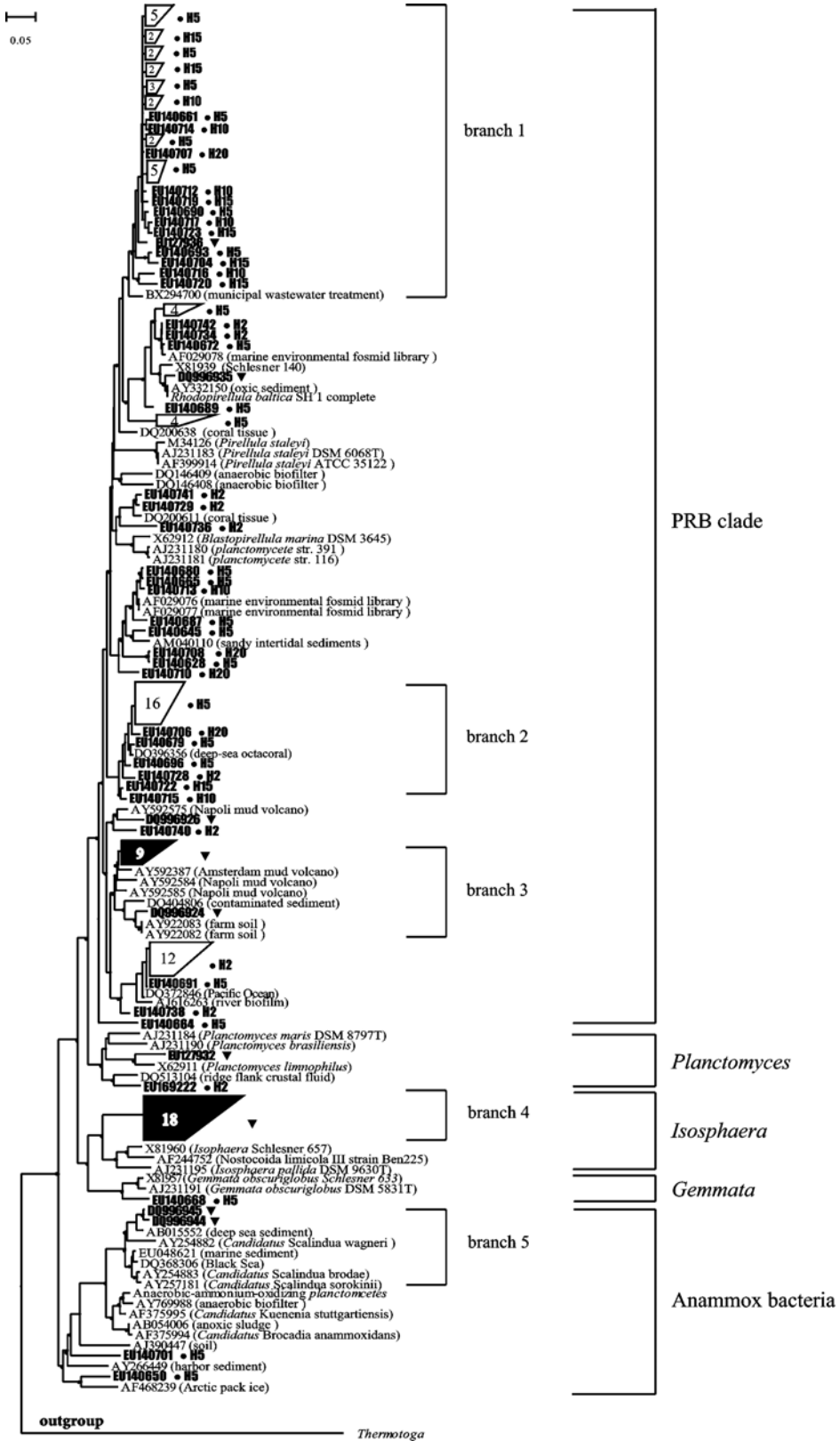

Fig. 4. The tree was constructed using neighbor-joining methods (Phylip software), and tree topology was also calculated using the MrBayes program. The root was determined using the 16S rRNA gene sequences of *Themotoga* as an outgroup reference. The black dots on the nodes represent neighbor-joining probability values >50%. In the tree, (●) represents sediment sequence (▼) represents surface seawater sequence. Scale bar=5% nucleotide sequence divergence.

genetic tree was constructed with representative seawater *Planctomycetes* sequences, sediment *Planctomycetes* sequences retrieved from one SCS (South China Sea) site M2896 (DQ996916 to DQ996974, EU127924 to EU127958) (Fig. 4). For surface seawater samples, the majority of sequences belonged to the PRB clade. The minority of sequences, EU 169222 and EU140668, were related to genera *Planctomyces* and *Gemmata,* respectively. Meanwhile, two sequences (EU 140701 and EU140650) generated from the site H5, were distantly related to anammox sequences. According to the topology of the phylogenetic tree, branch 1 and 2 contained sequences generated from nearly all seawater sites, showing the most widely distributed groups in surface seawater. The majority of seawater branches contained sequences retrieved from site H5, showing the site H5 to have the highest diversity in surface seawater. For the SCS sediment, branches 3, 4, and 5 were quite independent of the surface seawater groups (Fig. 4).

Discussion

Planctomycetes have been recognized as an important microbial group and molecular surveys aimed at *Planctomycetes* have become increasingly common. However, no work has focused on the surface seawater of oligotrophic open seas, one of the most important marine environments. Heretofore, members of the *Planctomycetes* group have been detected very rarely or at low levels in marine environments (Freitag and Prosser, 2003; Brummer *et al.*, 2004; Buckley *et al.*, 2006). In the present study, the first systematic large-scale assessment using culture-independent 16S rRNA gene approach provided good phylogenetic diversity information: PRB clade, *Gemmata* and *Planctomyces*-related *Planctomycetes* were present in latitudinal surface seawater. PRB clade-related *Planctomycetes* appeared to be a significant member of the clone libraries of Western Pacific Ocean surface seawater. Such dominance is also reported in a wastewater treatment plant (32%) (Chouari *et al.*, 2003) and in freshwater (64%) (Brummer *et al.*, 2004). Furthermore, the majority of PRB clade sequences exhibited high divergence with available sequences in the NCBI database (50.1% of PRB clade sequences exhibited ≤97% and 35.9% of PRB clade sequences exhibited ≤95% sequence similarity with the closest sequences in the NCBI database, respectively) and exhibited a low sequence similarity with each other, indicating an unexpectedly high diversity within the PRB clade.

The dominant PRB clade was present in all seawater clone libraries and the clone frequency of the PRB clade seemed to be stable (between 83.3% and 94.1%), while a minority of the genera *Gemmata* and *Planctomyces* was found only at sites H5 and H2, respectively. Meanwhile, by numbers of OTUs, sites H2 and H5 appeared to show higher diversity than the other sites, indicating that the surface seawater at low latitudes is a more ideal habitat for *Planctomycetes* than mid latitudes. Results of ∫-LIBSHUFF analysis also revealed that the phylogenetic composition at site H2 significantly differed from the other seawater sites and H5 significantly differed from sites H2, H10, and H15 (Table 2). The seawater region surveyed spans the equatorial North Pacific to the subtropical North Pacific with 18 latitudinal units. The Western Pacific Ocean is characterized by a complex hydrological background, since four prominent currents including the North Equatorial Current, the North Equatorial Counter Current, the Kuroshio Current and the Mindanao Current are mixed; and two major eddies, the Halmahera Eddy and the Mindanao Eddy, occur nearby (Fine *et al.*, 1994; Zhou *et al.*, 2006) (Fig. 1). With the North Equatorial Counter Current and the variable Halmahera Eddy, the surface water could exchange its organic matter with other water masses and even with the sediment (Huang and Wang, 2001). The surface seawater with its relatively high organic matter may contribute to high diversity at low latitudes.

Unlike diversity patterns in surface seawater, *Isosphaera* dominated the sediment *Planctomycetes* community; other genera such as anammox-related sequences and *Planctomyces* were also found in the sediment (Fig. 3). The *Planctomycetes* community in the sediment appeared to have higher diversity than in the surface seawater when using phylogenetic and BLASTn analysis. Additionally, results of the ∫-LIBSHUFF software analysis supported the significant difference of *Planctomycetes* diversity between in the sediment and at each surface seawater site (Table 2). The different geographic characteristics possibly contributed to the different pattern, since the sediment is characterized by high organic carbon (Yang *et al.*, 1998), high water pressure and low temperature availability, while the surface seawater is characterized by plenty of sun light, high temperature, high dissolved oxygen, and complicated currents. The relationship between the diversity of *Planctomycetes* and environmental factors seems complicated. Previous authors report that polluted and eutrophic environments may be associated with an increase in *Planctomycetes* diversity while anammox-related bacteria are strictly anoxic inhabitants. It is suggested that high organic carbon and oxygen may be two of the most important envi-

Table 2. *P* values of different clone libraries using ∫-LIBSHUFF software analysis

X	Y					
	H2	H5	H10	H15	H20	Sediment
H2	-	<0.001	<0.001	<0.001	<0.001	<0.001
H5	<0.001	-	<0.001	<0.001	0.0067	<0.001
H10	<0.001	<0.001	-	0.067	0.0016	<0.001
H15	<0.001	<0.001	0.078	-	0.0012	<0.001
H20	<0.001	0.0434	0.062	<0.001	-	<0.001
Sediment	<0.001	<0.001	<0.001	<0.001	<0.001	-

ronmental factors which have an impact on the *Planctomycetes* diversity (Staley *et al.*, 1973; Fuerst *et al.*, 1995; Morris *et al.*, 2006; Tadonleke, 2007). Even though the exact adaptive strategy of *Planctomycetes* remains unknown, such an investigation and comparison of *Planctomycetes* diversity in different hydrological and geographic environments will enhance our understanding of the potentially ecological role of *Planctomycetes* in marine environments.

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References

Bauer, M., T. Lombardot, H. Teeling, N.L. Ward, R. Amann, and F. Glockner. 2004. Archaea-like genes for C-1-transfer enzymes in *Planctomycetes*: Phylogenetic implications of their unexpected presence in this phylum. *J. Mol. Evol.* 59, 571-586.

Brummer, I.H.M., A.D.M. Felske, and I. Wagner-Dobler. 2004. Diversity and seasonal changes of uncultured *Planctomycetales* in river biofilms. *Appl. Environ. Microbiol.* 70, 5094-5101.

Buckley, D.H., V. Huangyutitham, T.A. Nelson, A. Rumberger, and J.E. Thies. 2006. Diversity of *Planctomycetes* in soil in relation to soil history and environmental heterogeneity. *Appl. Environ. Microbiol.* 72, 4522-4531.

Chouari, R., D. Le Paslier, P. Daegelen, P. Ginestet, J. Weissenbach, and A. Sghir. 2003. Molecular evidence for novel *Planctomycete* diversity in a municipal wastewater treatment plant. *Appl. Environ. Microbiol.* 69, 7354-7363.

Elshahed, M.S., N.H. Youssef, Q.W. Luo, F.Z. Najar, B.A. Roe, T.M. Sisk, S.I. Buhring, K.U. Hinrichs, and L.R. Krumholz. 2007. Phylogenetic and meta-bolic diversity of *Planctomycetes* from anaerobic, sulfide- and sulfur-rich Zodletone Spring, Oklahoma. *Appl. Environ. Microbiol.* 73, 4707-4716.

Fine, R.A., R. Lukas, F.M. Bingham, M.J. Warner, and R.H. Gammon. 1994. The western equatorial Pacific: A water mass crossroads. *J. Geophys. Res*. 99, 25063-25080.

Freitag, T.E. and J.I. Prosser. 2003. Community structure of ammonia-oxidizing bacteria within anoxic marine sediments. *Appl. Environ. Microbiol.* 69, 1359-1371.

Fuerst, J.A. 1995. The *planctomycetes*: emerging models for microbial ecology, evolution and cell biology. *Microbiology* 141, 1493-1506.

Huang, R.X. and Q. Wang. 2001. Interior communication from the subtropical to the tropical oceans. *J. Phys. Oceanogr.* 31, 3538-3550.

Ivanova, A.O. and S.N. Dedysh. 2006. High abundance of *Planctomycetes* in anoxic layers of a Sphagnum peat bog. *Microbiology* 75, 716-719.

Jiao, N.Z., Y.H. Yang, H. Koshigawa and M. Watanabe. 2002. Influence of hydrographic conditions on picoplankton distribution in the East China Sea, a marginal sea of the Northwest Pacific. *Aquat. Microb. Ecol.* 30, 37-48.

Kartal, B., J. Rattray, L.A. Van Niftrik, J. Van De Vossenberg, M.C. Schmid, R.I. Webb, S. Schouten, J.A. Fuerst, J.S.S. Damste, M.S.M. Jetten, and M. Strous. 2007. *Candidatus* "Anammoxoglobus propionicus" a new propionate oxidizing species of anaerobic ammonium oxidizing bacteria. *Syst. Appl. Microbiol.* 30, 39-49.

Kirkpatrick, J., B. Oakley, C. Fuchsman, S. Srinivasan, J.T. Staley, and J.W. Murray. 2006. Diversity and distribution of *Planctomycetes* and related bacteria in the suboxic zone of the Black Sea. *Appl. Environ. Microbiol.* 72, 3079-3083.

Konig, E., H. Schlesner, and P. Hirsch. 1984. Cell-wall studies on budding bacteria of the *Planctomycetes pasteuria* group and on a *Prosthecomicrobium* sp.. *Arch. Microbiol.* 138, 200-205.

Lim, A. and L.X. Zhang. 1999. WebPHYLIP: a web interface to PHYLIP. *Bioinformatics* 15, 1068-1069.

Lindsay, M.R., R.I. Webb, and J.A. Fuerst. 1997. Pirellulosomes: A new type of membrane-bounded cell compartment in *Planctomycete* bacteria of the genus Pirellula. *Microbiology* 143, 739-748.

Miskin, I.P., P. Farrimond, and I.M. Head. 1999. Identification of novel bacterial lineages as active members of microbial populations in a freshwater sediment using a rapid RNA extraction procedure and RT-PCR. *Microbiology* 145, 1977-1987.

Morris, R.M., K. Longnecker, and S.J. Giovannoni. 2006. *Pirellula* and OM43 are among the dominant lineages identified in an Oregon coast diatom bloom. *Appl. Environ. Microbiol.* 8, 1361-1370.

Ronquist, F. and J.P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572-1574.

Schlesner, H., C. Rensmann, B.J. Tindall, D. Gade, R. Rabus, S. Pfeiffer, and P. Hirsch. 2004. Taxonomic heterogeneity within the *Planctomycetales* as derived by DNA-DNA hybridization, description of *Rhodopirellula baltica* gen. nov., sp. nov., transfer of *Pirellula marina* to the genus *Blastopirellula* gen. nov. as *Blastopirellula marina* comb. nov. and emended description of the genus *Pirellula*. *Int. J. Syst. Evol. Microbiol.* 54, 1567-1580.

Schloss P.D., B.R. Larget, and J. Handelsman. 2004. Integration of microbial ecology and statistics: a test to compare gene libraries. *Appl. Environ. Microbiol.* 70, 5485-5492.

Schmid, M., K. Walsh, R. Webb, W.I.C. Rijpstra, K. Van De Pas-Schoonen, M.J. Verbruggen, T. Hill, B. Moffett, J. Fuerst, S. Schouten, J.S.S. Damste, J. Harris, P. Shaw, M. Jetten, and M. Strous. 2003. *Candidatus* "Scalindua brodae", sp. nov., *Candidatus* "Scalindua wagneri", sp. nov., two new species of anaerobic ammonium oxidizing bacteria. *Syst. Appl. Microbiol.* 26, 529-538.

Stachebrandt, E., W. Ludwig, W. Schubert, F. Klink, H. Schlesner, T. Roggentin, and P. Hirsch. 1984. Molecular genetic evidence for early evolutionary origin of budding pepetidoglycan-less eubacteria. *Nature* 307, 735-737.

Staley, J.T. 1973. Budding bacteria of the Pasteuria-Blastobacter group. *Can. J. Microbiol*. 19, 609-614.

Tadonleke, R.D. 2007. Strong coupling between natural *Planctomycetes* and changes in the quality of dissolved organic matter in freshwater samples. *FEMS Microbiol. Ecol.* 59, 543-555.

Yang, G.P., X.L. Liu, and J.W. Zhang. 1998. Distribution of dibenzothiophene in the sediments of the South China Sea. *Environ. Pollut.* 101, 405-414.

Zhou, H., J.P. Jian, P.F. Guo, M.C. Shi, Z.H. Liu, and Y.F. Li. 2006. A study on an anticyclonic eddy east of Mindanao observed by an Argo profiling float. *J. Trop. Oceano.* 25, 8-14.