The diversity of Planctomycetes in glacier sediment, and freshwater and marine sediments in Glacier Bay, Alaska

Victoria Campbell

Abstract

The phylogenetic diversity of Planctomycetes within sediment of a marine, freshwater and glacier ice environment was compared as a means to observe how time and environmental factors determine the distribution and composition of microbial communities. Sediment was collected from Bartlett Cove, Blackwater pond and from a piece of glacier ice in March 2008 in Glacier Bay, Alaska. Clone libraries were created using primers that specifically target the 16s rDNA of the Planctomycetes-Verrucomicrobia-Chlamydiae-Lentisphaera superphylum. The Shannon-Weaver index for diversity of the glacier ice sediment (1.03) was found to be less than the diversity found in both the pond (3.50) and marine sediment (3.59). This provides an illustration of how microbial communities become more diverse with time. The three sequence libraries did not overlap except for one pond sequence that was found in a cluster of glacier ice sequences, suggesting that the microbial communities are being influenced from separate sources.

Introduction

Both spatial distribution and environmental characteristics have an impact on the presence of the bacterial communities that inhabit an environment. Similar species may be found in two environments that are near in proximity but differ in characteristics, such as the chemistry or temperature; but similar species may also be found in similar environments that are great distances apart from one another. Bacteria that inhabit a variety of diverse geographic regions are referred to as 'cosmopolitan,' and those bacteria that are only found in specific regions, such as hot springs, are considered to be endemic (Staley and Gosink 1999). However, this distinction is difficult to demonstrate with bacteria because they may survive as long as their environmental requirements are met (Fenchel 2003) and be widely dispersed. Possible sources of dispersal of microorganisms include wind, water and animals. Some of the bacterial species that are transported to the exposed environment may not be able to survive, however it is more likely for several species to survive if the environment has been exposed for a long time.

Planctomycetes have been found in a variety of diverse environments, including freshwater, marine, soils and even in animal intestines (Fuerst 2004). They can live in both anaerobic and aerobic environments and those genera found living aerobically are thought to be heterotrophic, while those found living anaerobically are chemoautotrophic (Fuerst 2004). Carbohydrate fermentation and sulfur reduction are possible metabolic activities used by heterotrophic Planctomycetes genera that were dis-

covered in anaerobic environments; however it is unknown whether certain genera are obligate or facultative in their environment (Elshahed et al. 2007). Only four genera of chemoheterotrophic bacteria have been isolated in pure culture: *Pirellula, Planctomyces, Gemmata* and *Isosphaera* (Fuerst 2004), yet new genera of these bacteria are continuously being identified. Recently, the anaerobic ammonium oxidation reaction in which NO_2 and NH_4 react to form N_2 was discovered and some bacteria found to specialize in this reaction are certain genus of Planctomycetes. Previous research on aerobic Planctomycetes suggests that certain genera are found in either freshwater or marine environments, but not both. However, more recent research has shown that some genera can survive in both. For example, the genera *Pirellula* has been found in both freshwater and marine environments (Elshahed et al. 2007, Kirkpatrick et al. 2006).

Glacier Bay is a recently deglaciated estuarine fjord in southeast Alaska, site of Glacier Bay National Park. Glacier Bay is spatially very heterogeneous and diverse and glacier soils, freshwater pond and marine sediments are found in close proximity to one another. Glaciers are constantly retreating each year and all the glacial melt is contributing to the local watershed, including freshwater ponds, streams, or hyporheic flow, which together serve as transport corridor to the marine environments (Fig. 1). As the water flows from one system to the next, microorganisms and particles are continually being added to the water various sources. Investigating and contrasting the diversity from a newly exposed area by glaciers and an area which has been exposed for a long period of time will foster our understanding of the source regions for bacterial colonization. In Glacier Bay, there are several different environments that are close in proximity. This study compares the phylogenetic diversity of Planctomycetes within the glacier soil, freshwater pond sediment and marine sediment.

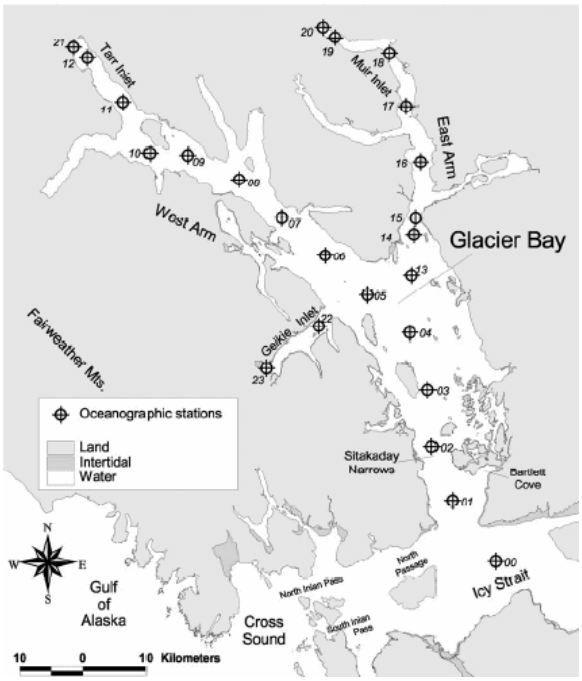

Figure 2: Map of Glacier Bay, Alaska, identifying the three sampling locations (Hooge and Hooge 2002).

Methods

Field Work

Samples were collected during March 19th – 22nd in Glacier Bay, Alaska, aboard the research vessel *Thomas G. Thompson.* The freshwater sediment was collected from Blackwater pond (58°27'4.95"N, 135°53'5.67"W) (Fig. 2), using a 2 m long piece of PVC piping (6.5 cm diameter) which was pushed into the sediment. This sampling site was chosen in order to remove the risk of saltwater contamination so that a clear distinction between the Planctomycetale communities in both freshwater and marine environments could be later analyzed. A Soutar core was used to collect marine sediment in Bartlett Cove (58° 27' N, 135° 55' W). These sampling sites were chosen based on the similar time frame when the sediment was deposited or uncovered after the glacier retreated. Glacier soil was collected by melting pieces of glacier ice that contained trapped sediments from Margerie

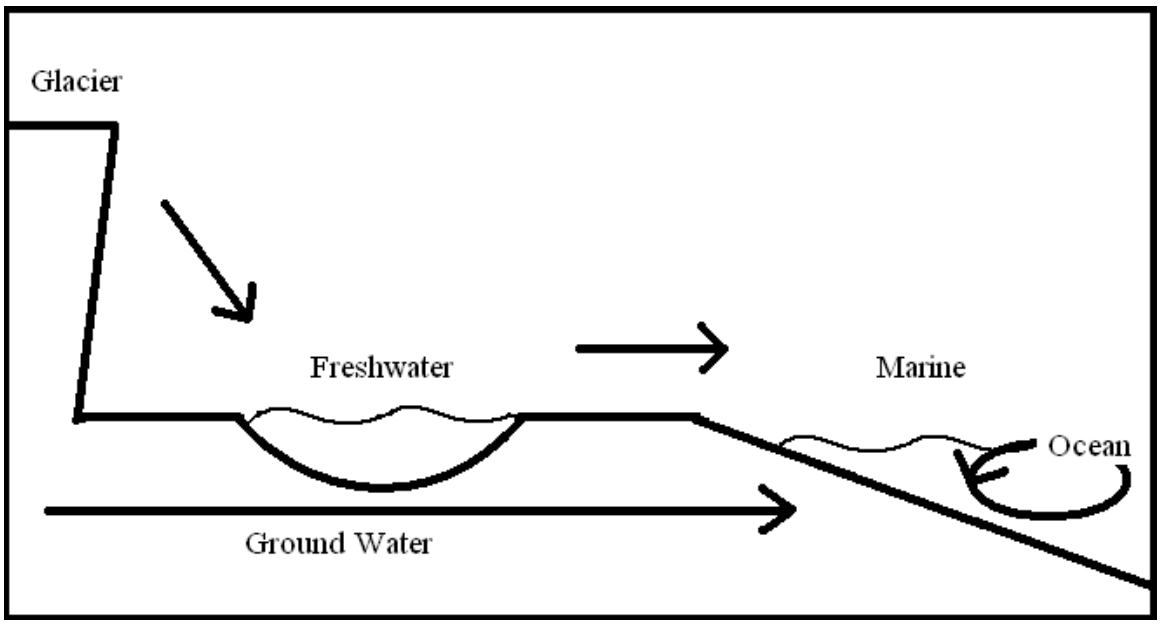

Figure 1: A diagram showing possible water transportation routes that could aid in the dispersal of bacteria. Each arrow shows the possible direction in which an environment could influence another environment.

glacier at the head of Tarr Inlet. A subsample of the melt water was filtered onto a sterilized fiberglass filter pad. This sample contains sediment directly from the glacier and although it is unknown, it could have been covered for quite a long time. One sample was taken near the entrance of Glacier Bay to allow a comparison site where exposure from glacier differs in time by ˜120 years (Engstrom et al. 2000). All samples were kept frozen at -80° C until lab analysis.

Lab Analysis

Lab analyses were conducted in the Staley lab at the University of Washington in Seattle, WA. Sediment was thawed and DNA was extracted by using the Sediment DNA Extraction Protocol by Kerkhof et al. (2002), with alterations made by the Staley lab. The DNA was cleaned with Qiagen QiaQuick kit after each extraction and at times with a PEG cleanup protocol serving as a second cleanup method under conditions where the Qiagen QiaQuick kit was unsuccessful. Since every extraction using the Kerkhof protocol was still unsuccessful after cleaning, DNA was extracted successfully using PowerSoil DNA Isolation kit by Mo Bio.

First eubacterial primers 27f and 1492r were used to ensure the presence of bacteria, then Planctomycetes-specific primers 58f (5-GGCATGGATTAGGCATGC-3) and 926r (5-CCACCGCTTGTGTGAGCCCC-3), which target the 16S rDNA gene and amplify both aerobic and anaerobic genera, were used for partial gene sequencing (Kirkpatrick et al. 2006). PCR Thermocyclers were used to amplify the DNA. A nested PCR was done on the glacier ice sediment sample by first using the eubacterial primers and running them through PCR, then Planctomycetes-specific primers 58f and 926r were added to the PCR product and run through PCR again. This was done in order to make sure there was enough amplified DNA in the sample for the next PCR that involved the Planctomycetes primers to be successful. Ligations were completed for each sample using TOPO TA Cloning Kit (with pCR2.1-TOPO vector) and were sent to the UW High Throughput Genomics (www.htseq.org) laboratory in

Seattle for transformation and sequencing.

The sequence data was loaded into Sequencher in order to clean the sequences by trimming the vectors and removing sequences with less than 500 base pairs (bp) or that had poor reads in the chromatograms. The clean sequences were loaded into GreenGenes to be aligned. The align sequences were then added into the ARB tree library using maximum parsimony. A percent identity similarity matrix was created using only regions that were present in all sequences. A lane mask was used to filter hyper variable regions; in the glacier ice sediment (GIS) sample 546 base pairs were used to make the matrix, 517 bp in the Blackwater pond (Pond) sample, and 612 bp in the Bartlett Cove (BC) sample. The similarity matrix was then converted to a distance matrix.

Using DOTUR, the distance matrix was used to call OTU's at the 97 percent similarity cutoff. The OTU's were then used to create neighbor joining trees and rarefaction curves.

Subsamples of water collected from the pond and the glacier ice were tested for both salinity using a refractometer and pH values using pH strips.

Results

The diversities of Planctomycetes found in Bartlett Cove and Blackwater pond were about four times greater than the diversity found in the glacier ice sediment. The Shannon-Weaver indices for all three sampling locations also indicated that the diversity difference between Bartlett Cove and Blackwater pond was very small, but that their diversities were much greater than the diversity found in the glacier ice sediment (Fig. 3). The rarefaction curves were steep, indicating that they were not fully sampled (Fig. 4). Chao estimates created from DOTUR gave an estimate of the predicted diversity found in the samples (Fig. 5); 30% of the diversity was represented in the sample collected in Bartlett Cove, 63% in Blackwater pond, and 41% in the glacier ice sediment. Although there was no difference in diversity by the Shannon-Weaver index, there was a significant difference between the compositions of the microbial communities in Blackwater pond and Bartlett Cove detected by webLIBSHUFF ($p = 0.001$).

There is essentially no overlap between all three libraries, with only the exception of one Blackwater pond sequence found in a cluster of glacier ice sediment sequences. In the Planctomycetes tree, each sampling location seemed to be different from one another with sequences from Bartlett Cove matching primarily with Pirellula and Blackwater pond sequences matching with Isosphaera and Planctomyces (Fig. 6). In the non-Planctomycetes tree the cultured species that best matched the sample sequences were Lentisphaera, Chlamydiae, and Verrucomicrobia (Fig. 7). However in both of the trees, the majority of clusters did not significantly match with any cultured or uncultured species in the database.

The pH of the pond water measured around 4.5 and the glacier ice pH measured around 5.3. The salinities of both samples were low, about 0.0 psu for the glacier ice and 1.5 psu for the pond.

Discussion

Multiple bacterial species may be transported to a new environment if it has been exposed for a longer period of time. Some of the bacteria that are transported to the exposed environment may not be able to survive, however it is more likely for several species to survive if the environment has been exposed for a long time. The glacier ice sediment has a much smaller diversity than those of the pond and marine sediment, which was expected since the sediment could have been trapped within the glacier ice for thousands of years. It was hypothesized that a source of bacteria to the pond and marine environments was coming from glacier melt, but since there was no

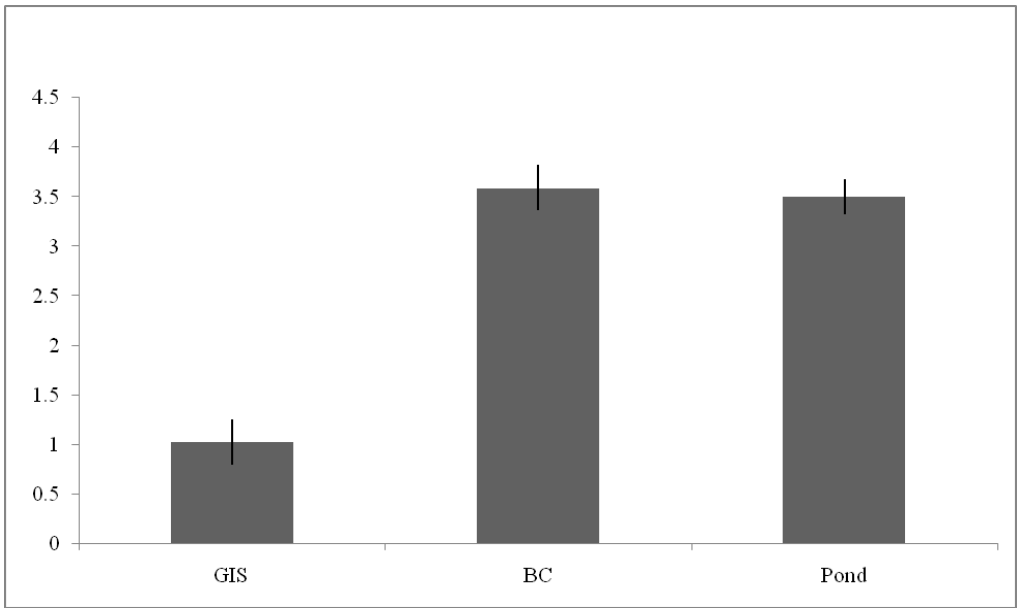

Figure 3: The Shannon-Weaver indices for the glacier ice sediment (GIS), Bartlett Cove (BC) and Blackwater pond (Pond). The diversity in both BC and Pond is much greater than the diversity found in the glacier ice sediment.

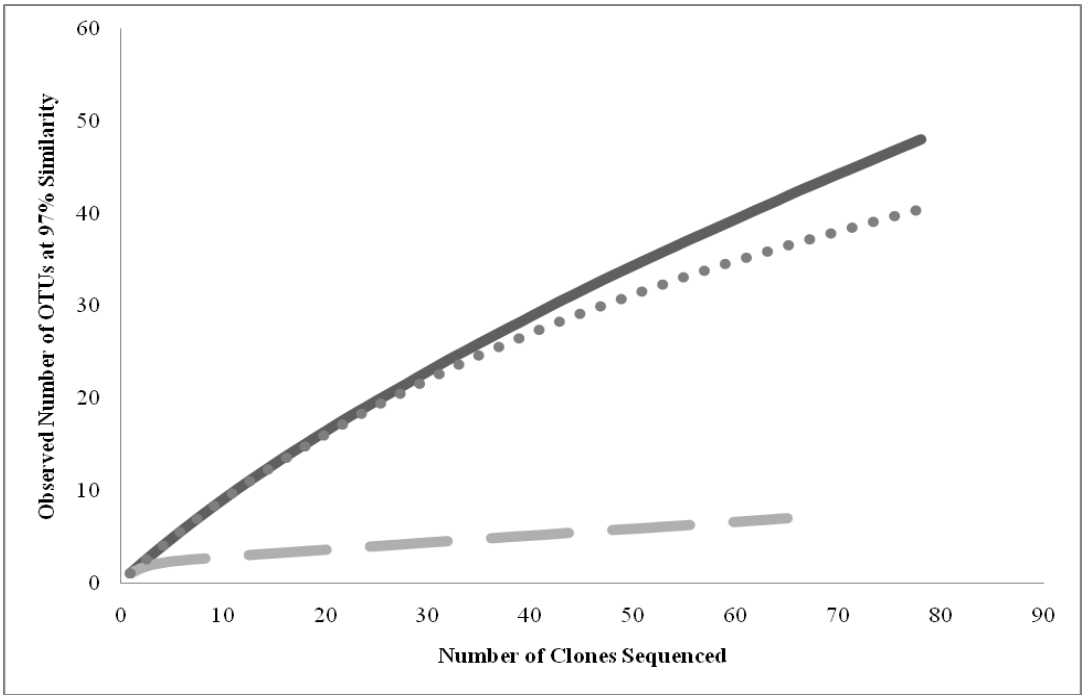

Figure 4: Rarefaction curves of all three sampling locations. The solid line represents Bartlett Cove, the dotted line represents Blackwater pond, and the dashed line represents glacier ice sediment. The curves for the pond and Bartlett Cove samples are steep, indicating that these locations were under sampled.

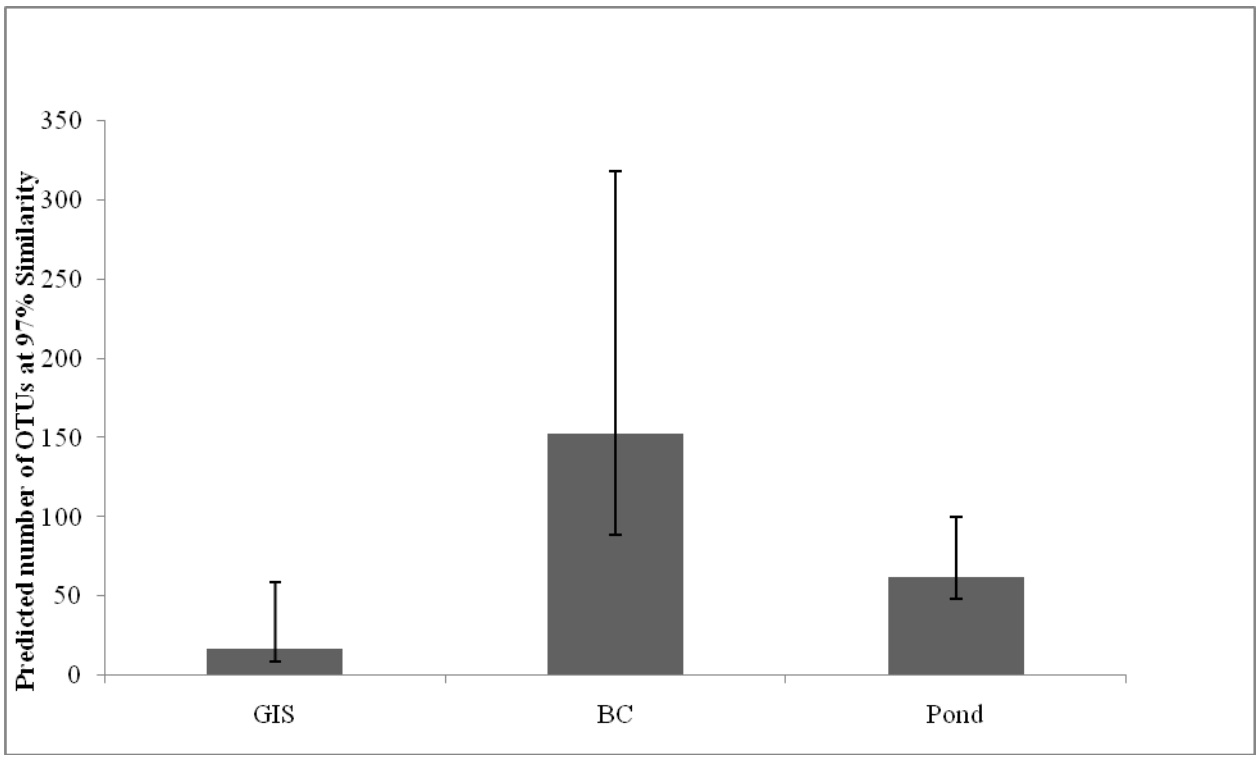

Figure 5: The Chao estimates for the predicted diversity of the glacier ice sediment (GIS), Bartlett Cove (BC) and Blackwater pond (Pond). The predicted number of OTUs at the 97% similarity cutoff, and its error bar, is greatest in BC, suggesting that only 30% of the diversity was actually observed from the samples.

significant overlap between the three locations, the microbial compositions of the pond and the marine sediment must be influenced from other sources.

Only one sequence from the glacier ice sediment is considered to be a Planctomycetes, although it is considerable distance from the nearest cultured reference Pirellula. Given that large clusters of these sequences were found to be unknown non-Planctomycetes, this lone sequence may not be viable information. The larger clusters are most closely matched to a Lentisphaera, but again there is a large distance between them. This could be evidence of a different, unknown species.

It is known that in areas of southeast Alaska, acidic black-water ponds are formed when the forest becomes waterlogged (Milner et al. 1997). Given its location and name, Blackwater pond was probably formed this way. An article by Engstrom et al. (2000), studied the chemistry of more than 30 lakes in Glacier Bay and found that as the age of the lakes increase, their chemical composition changes remarkably within the first 100 years, due to rapid succession in the surrounding vegetation. This change in the chemical composition can alter the biological aspects of the lake, including the microbial community. According to the webLIBSHUFF results, there was a significant difference between the composition of the microbial communities sampled from the pond and Bartlett Cove. The rapid change in the chemistry of the pond may explain why there was such a difference.

Sequences did not overlap, however given previous research and the fact that the Chao estimates revealed that the locations were under sampled, it is possible that genera can be found living in both freshwater and marine environments.

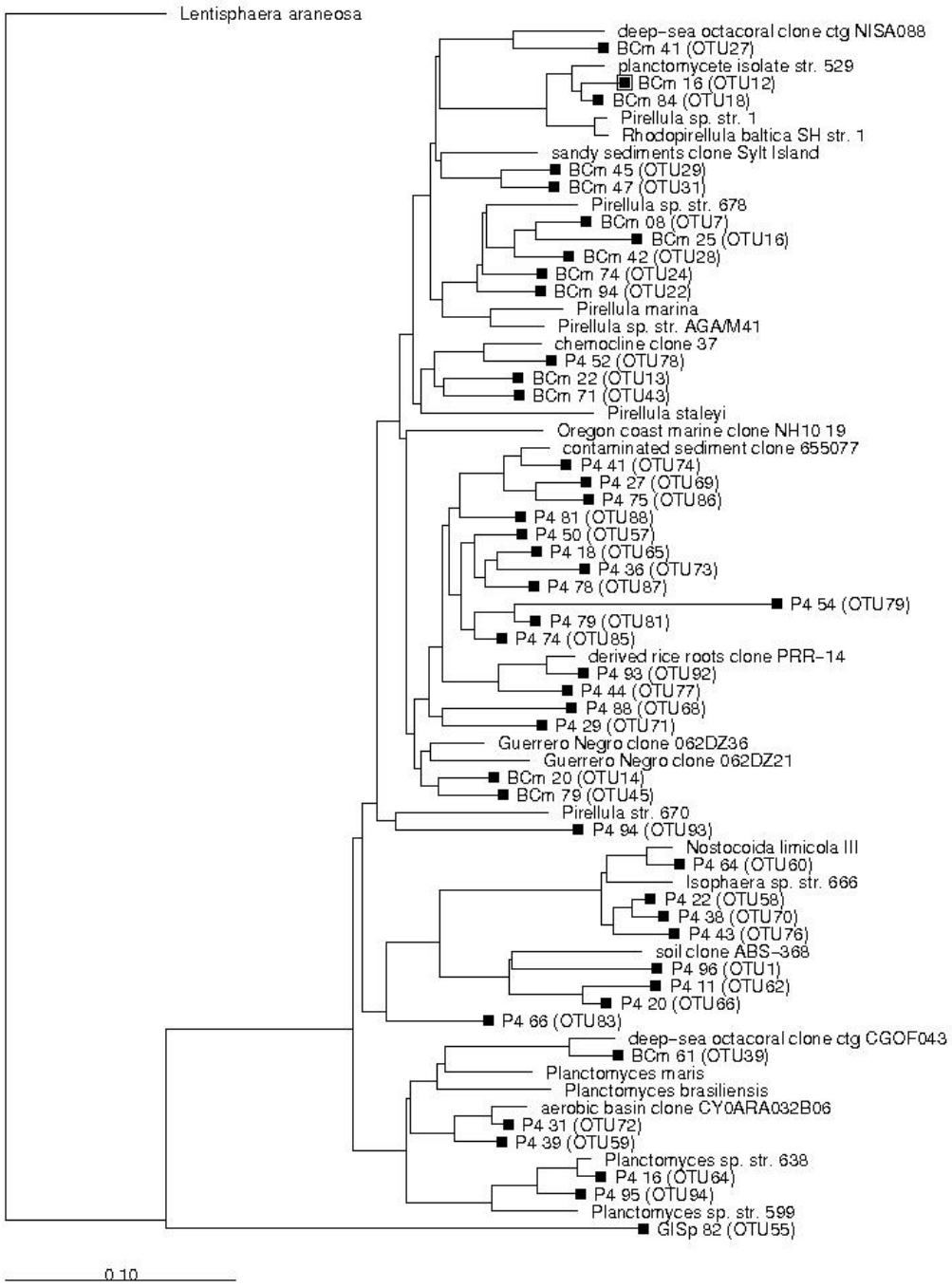

Figure 6: Tree containing sequences that matched with Planctomycetes, the three genera that matched sequences in the database were Pirellula, Isophaera, and Planctomyces.

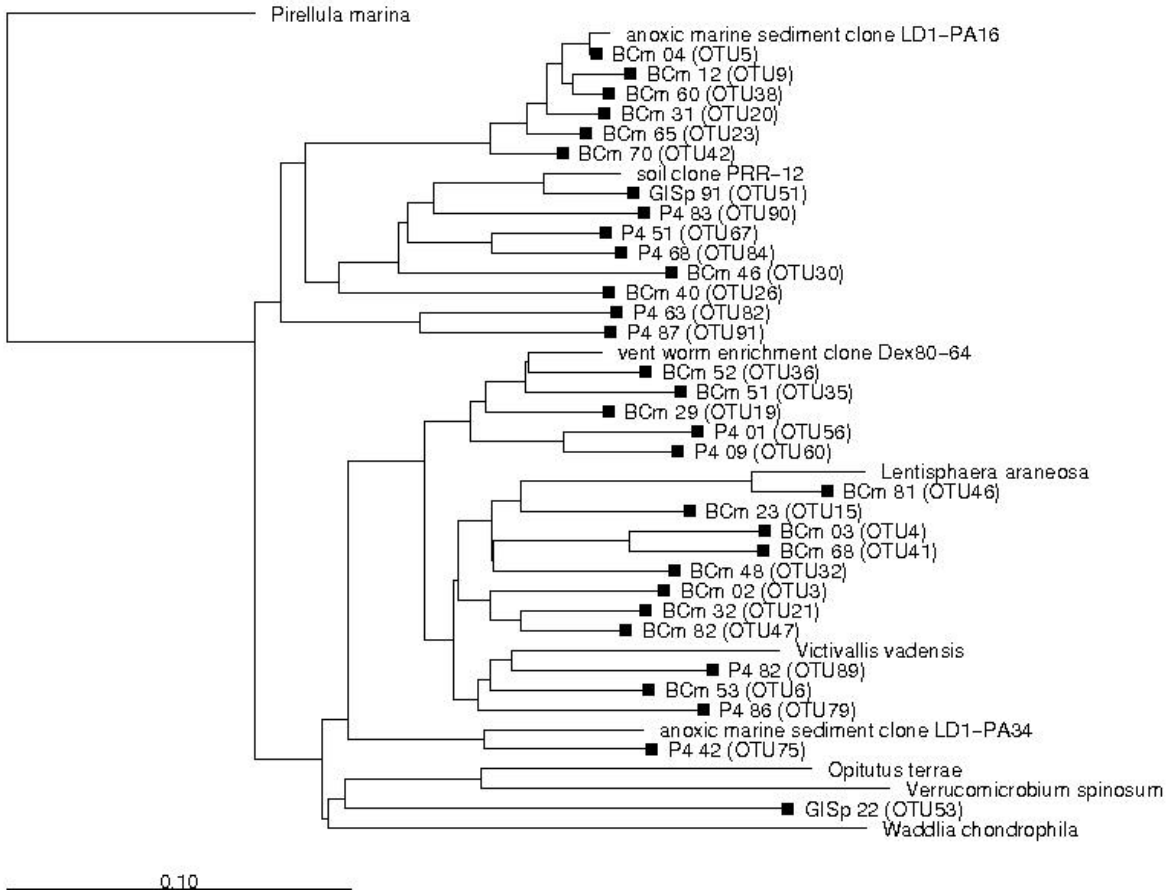

Figure 7: Tree containing sequences that matched the non-Planctomycetes sister phyla Lentisphaera, Verrucomicrobia, and Chlamydiae.

A fair share of the sequences matched with non-Planctomycetes groups such as Verrucomicrobia, Lentisphaerae and Chlamydiae, which are referred to as the sister groups of Planctomycetes since they consistently group together (Wagner and Horn 2006). The primers used in this study seemed to hit only the groups within this superphylum. The remaining sequences were found in to be Planctomycetes, the majority is similar to Planctomyces, Pirellula and Isosphaera.

Acknowledgments

Thanks to the School of Oceanography and the University of Washington for funding this project, and to Carmela Llaneta for contributing part of her own budget money to help fund this project. Thanks to the crew of the *Thomas G. Thompson,* Kathy Newell, and the Glacier Bay National Parks Service members for making this project happen.

A special thanks to John Kirkpatrick and Eric Collins for guiding me from start to finish, and to Dr. James Staley and his lab for providing a work area in their lab. Lastly, thanks to all my classmates and instructors for all their support and encouragement.

References

Elshahed, M. S. 2007. Phylogenetic and metabolic diversity of Planctomycetes from anaerobic, sulfide- and sulfur-rich Zodletone Spring, Oklahoma. Appl. Environ. Microbiol. **73:** 4707 -4716.

Engstrom, D. R., et al. 2000. Chemical and biological trends during lake evolution in recently deglaciated terrain. Nature. **408:** 161-166.

Fenchel, T. 2003. Biogeography for Bacteria. Science. **301:** 925-926.

Fuerst, J. 2004. Planctomycetes: A Phylum of Emerging Interest for Microbial Evolution and Ecology. World Federation for Culture Collections Newsletter. **38:** 1-11.

Hooge, P.N. and Hooge, E.R. 2002. Fjord Oceanographic Processes in Glacier Bay, Alaska. USGS-Alaska Science Center.

Kirkpatrick, J., et al. 2006. Diversity and distribution of Planctomycetes and related bacteria in the suboxic zone of the Black Sea. Appl. Environ. Microbiol. **72:** 3079-3083.

Milner, A. M., et al. 1997. Freshwaters of Alaska: Ecological Syntheses. Springer.

Staley, J. T., and J. J. Gosink. 1999. Poles apart: biodiversity and biogeography of sea ice bacteria. Annu. Rev. Microbiol. **53:** 189-215.

Staley, J. T., et al. 2007. Microbial Life, 2nd ed. Sinauer Associates, Inc.

Wagner, M. and M. Horn. 2006. The Planctomycetes, Verrucomicrobia, Chlamydiae and sister phyla comprise a superphylum with biotechnological and medical relevance. Current Opinion in Biotechnology. **17:** 241-249.