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Basin-scale seasonal changes in marine free-living bacterioplankton community in the Ofunato Bay

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Running title: Seasonal changes of metagenome profiles in the Ofunato Bay

ABSTRACT

The Ofunato Bay in the northeastern Pacific Ocean area of Japan possesses the highest biodiversity of marine organisms in the world and has attracted much attention due to its economic and environmental importance. We report here a shotgun metagenomic analysis of the year-round variation in free-living bacterioplankton collected across the entire length of the bay. Phylogenetic differences among spring, summer, autumn and winter bacterioplankton suggested that members of Proteobacteria tended to decrease at high water temperatures and increase at low temperatures. It was revealed that *Candidatus Pelagibacter* varied seasonally, reaching as much as 60% of all sequences at the genus level in the surface waters during winter. This increase was more evident in the deeper waters, where they reached up to 75%. The relative abundance of *Planktomarina* also rose during winter and fell during summer. A significant component of the winter bacterioplankton community was Archaea (mainly represented by *Nitrosopumilus*), as their relative abundance was very low during spring and summer but high during winter. In contrast, Actinobacteria and Cyanobacteria appeared to be higher in abundance during high-temperature periods. It was also revealed that Bacteroidetes constituted a significant component of the summer bacterioplankton community, being the second largest bacterial phylum detected in the Ofunato Bay. Its members, notably *Polaribacter* and *Flavobacterium*, were found to be high in abundance during spring and summer, particularly in the surface waters. Principal component analysis and hierarchical clustering analyses showed that the bacterial communities in the Ofunato Bay changed seasonally, likely caused by the levels of organic matter, which would be deeply mixed with surface runoff in the winter.

Keywords

Free-living bacteria

Ofunato Bay

Seasonal variation

Shotgun metagenomics

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1. Introduction

Marine bacterioplankton are free-living to particle-associated natural assemblages mediating organic matter transformations and the cycling of nutrients in the oceans (Lovejoy et al., 1996; Crump et al., 1999; Simon et al., 2002). While significant taxonomic and functional differences have been reported between free-living and particle-associated bacteria in aquatic systems (Whitmire et al., 2009; Mohit et al., 2014), differences in their specific activity and total concentration in aquatic systems seem to be related to physical environments and the chemical properties of the water, including trophic status and phytoplankton blooms (Simon 1987; Ghiglione et al., 2007). It is, however, well established that free-living bacteria are more abundant than their particle-associated counterparts in diverse marine and freshwater ecosystems (Grossart et al., 1998; Ghiglione et al., 2007; Mohit et al., 2014), reaching more than 90% of the total bacterial population (Simon, 1987; Unanue et al., 1992), indicating their considerable input to aquatic environments. Their contribution to the total bacterial activity has also been reported to be higher in oligotrophic and mesotrophic pelagic marine systems and slightly lower in eutrophic and estuarine systems compared to particle-associated bacteria, with a relatively higher activity of free-living fraction during the day and opposite for the particle-associated fraction and vice versa during the night (Simon et al., 2002; Ghiglione et al., 2007). Using conventional molecular approaches that utilized a limited number of clones and, very recently, high-throughput next generation sequencing (NGS) technology, many studies have revealed the significance of particle-associated bacteria in relation to the nutrient status of aquatic systems and environmental conditions (DeLong et al., 1993; Acinas et al., 1999; Kellogg and Deming, 2009; Ghai et al., 2012; Crespo et al., 2013; Mohit et al., 2014; Sakami et al., 2016), but very few studies have addressed free-living bacteria in relation to their seasonal variations and environmental functions. Considering the higher abundance of

free-living microorganisms in the aquatic environment, it is important to understand their diversity and dynamic changes in the ecosystem.

NGS technology has evolved as a tool of choice to elucidate the taxonomic status and functions of free-living microorganisms in rivers, lakes, coastal zones, estuaries and the deep sea. While utilizing the unprecedented sequencing depth of the technology, 16S rRNA gene and shotgun metagenomic surveys have demonstrated genetic diversity and functional differences among different environments. However, little has been reported on the novel genetic diversity within the communities of temperate coastal bays, which are defined as bodies of water partially surrounded by land and connected to the ocean via a mouth. In view of this situation, we investigated the free-living microbial communities in the Ofunato Bay, a coastal bay on the northeastern coast of Japan (also known as the *Sanriku Rias Coast*). This area is located in a typical temperate region, possessing the highest biodiversity of marine organisms in the world (Crist et al., 2010). The Ofunato Bay in Iwate Prefecture possesses a complex physical structure and is famous for its complicated coastal line (Okaichi, 2004). Chlorophyll *a* (chl-*a*) concentrations have been reported to be high in the bay during spring (JAXA, 2004), which has attracted buoy-and-rope type scallop and oyster culture facilities (Komatsu et al., 2012). Considering its economic and environmental importance, the Ofunato Bay has received much attention, and there has been continuous monitoring of its seawater quality parameters by organizations such as the Iwate Fisheries Technology Center and Japan Aerospace Exploration Agency (JAXA).

We previously reported the major taxonomic profiles of free-living microbial communities at three sampling stations in the Ofunato Bay (Reza et al., 2018). In line with these investigations, we initiated an effort to monitor the Ofunato Bay using a shotgun metagenomic approach to provide a comprehensive understanding of the seasonal variation in free-living microorganisms.

2. Materials and methods

2.1. Sampling sites

The study area is in the Ofunato Bay, Iwate Prefecture, Japan. To comprehensively cover the total length of the bay, we selected three locations for sample collection as described in Reza et al. (2017): KSt. 1 (141.73457E, 39.063370N; avg. depth 10.3 m), KSt. 2 (141.73245E, 39.044612N; avg. depth 25.3 m) and KSt. 3 (141.72820E, 39.019030N; avg. depth 38.5 m), where KSt. 1 and 3 were located at the northeast (viz. inner part) and southwest (viz. bay entrance) parts of the Bay, respectively, while KSt. 2 was located in the center of the bay and near the oyster culture facilities.

2.2. Sample collection and processing

Seawater collection and processing were performed according to Reza et al. (2018). Briefly, seawater sampling was carried out every month (usually during the first week of the month) for 1 year from January 07, 2015, to December 03, 2015. Samples were collected from two depths: 1 m and 8 m at KSt. 1 and 1 m and 10 m at KSt. 2 and 3. No specific permits were required for sampling, and the study did not involve any endangered or protected species. Collections were made using a vertical water sampler for metagenomic analyses as well as nutrient and chl-*a* measurements, where an ~8 L seawater sample was first prefiltered through a 100 µm filter to remove debris and then transported to the laboratory at the Sanriku Coastal Education and Research Center, Kitasato University School of Marine Biosciences, Ofunato, under refrigerated conditions (~4°C) for processing. Corresponding seawater quality parameters, including temperature, salinity and dissolved oxygen (DO), were measured on site using an AAQ-RINKO-Profilier AAQ176 (JFE Advantec Co. Ltd., Hyogo, Japan). Nutrient and chl-*a* measurements and seawater filtration were performed as in our previous paper (Reza et al., under review).

2.3. Determination of cyanobacterial and heterotrophic bacterial abundance

The numerical abundance of heterotrophic bacteria and cyanobacteria was determined using seawater samples preserved with glutaraldehyde (final concentration, 1%) by epifluorescence microscopy. Heterotrophic bacteria were stained with 4,6-diamidino-2-phenylindole (DAPI) and filtered through 0.2- μ m black Nuclepore filters. Cyanobacteria were filtered through another 0.2- μ m Nuclepore filter. The samples were subjected to UV excitation for heterotrophic bacteria counting and green excitation for cyanobacteria counting.

2.4. DNA extraction and shotgun metagenomic sequencing

DNA extraction and shotgun metagenomic sequencing were performed according to Reza et al. (2018). Briefly, genomic DNA was extracted from the 0.2- μ m filters using the PowerWater® DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's recommendations and stored at -20°C until use. The DNA concentration was measured with a Qubit dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA) and read with a Qubit Fluorometer 2.0 (Invitrogen). The DNA aliquots were used for metagenomic shotgun sequencing using an Illumina MiSeq platform and a MiSeq Reagent Kit v3 (600 cycles) (Illumina Inc., San Diego, CA) after treatment with the NexteraXT DNA Sample Preparation Kit (Illumina Inc.).

2.5. Sequence processing and analyses

Sequence processing was performed according to Reza et al. (2018). Briefly, the acquired Illumina paired reads for each library were first joined by overlapping the forward and reverse reads of the same DNA fragment (paired-end sequences) using the software FLASH (Magoc and Salzberg, 2011) with the default parameters (overlap minimum, 10 nt; maximum

allowed ratio between the number of mismatched base pairs and the overlap length, 0.25). These whole-genome shotgun (WGS) reads have been deposited into the DDBJ Sequence Read Archive under the accession number DRA005744. Quality filtering of these WGS reads was then performed by removing the reads of <50 bp, and they were quality trimmed to Phred 20 using Genomics Workbench (CLCbio, Cambridge, MA). BLASTn was performed locally on a server at the Sagami-hara campus of the Kitasato University School of Marine Biosciences, Kanagawa Prefecture, using the quality- and size-filtered sequences against the NCBI-nt reference database. Taxonomic analysis up to the species level was then performed using MEGAN ver 5.10.3 (Huson et al., 2007) after parsing the BLAST output. Comparative analysis in MEGAN was also performed after normalizing the counts according to the recommendations of the authors (Huson et al., 2007). The least common ancestor (LCA) assignment algorithm had the following parameters: min support = 1; min score = 50; top percent = 10 and maximum expected $1.0E^{-10}$.

2.6. Statistical analyses

A hierarchical cluster dendrogram based on common genera found across the surface (1 m) and deep (8 or 10 m) seawaters and principal component analyses (PCA) based on the relative abundance of bacterial genera obtained from the shotgun metagenomic data from three sampling stations at the Ofunato Bay were carried out using R software (R Development Core Team, 2013). The number of differentially abundant taxa among different months of the year was estimated, and these values were subjected to a t-test ($p < 0.05$).

3. Results and discussion

3.1. Seasonal changes in the environmental setting of the Ofunato Bay

The Ofunato Bay possesses a calm environment throughout its 7 km length and 2.5 km width. In addition, the breakwater concrete structure, built in 1967 as a protective edifice from tsunami, caused slight stagnation of the water inside the bay (Miyazawa and Hayakawa, 1994; Yamada et al., 2017). Based on the temperature profile in the Ofunato Bay, we used WGS data from 4 months, viz., May (as spring), August (as summer), November (as autumn) and March (as winter). In the Ofunato Bay, winter is usually delayed due to the late arrival of the cold *Oyashio Current* from the north, and in our study, we found the seawater temperature in March 2015 to be lowest, ranging from 4.6 to 6.4°C at the three sampling stations. Basic water quality parameters at all three sampling stations, including temperature, salinity, pH, DO and photon flux density (PFD), showed that the water column of the Ofunato Bay was well mixed during spring and summer (Supplementary Table S1) but stratified during winter and autumn. The recorded average temperature from the surface to the bottom at the Ofunato Bay during the one-year period from January to December 2015 was found to be lowest in March (4.8°C) and highest in September (22.4°C). The bay was found to be well oxygenated throughout the year, with slightly lower DO content in the deeper waters during autumn. The average water salinity, on the other hand, ranged from 20.7 to 33.9 throughout the sampling period, with the lowest value, 20.7, found in surface seawaters at station KSt. 2 during December and the highest value, 33.9, in deep seawaters (10 m) during the same period. The sudden decrease in surface water salinity was caused by heavy rainfall before the sampling date, and we observed little fluctuation in the deep water. A similar occurrence of low salinity (<30.0) in the surface water of the Ofunato Bay has also been reported by Yamada et al. (2017).

Seawater of the Ofunato Bay was also found to be well mineralized, with persistent alkaline pH values ranging from 7.8 to 8.3 (Ghai et al., 2012; Yamada et al., 2017). With additional nutrient inputs from the cultivated oysters and a varying amount of fluxes of chemical elements, the concentrations of biologically available nitrogen and phosphorus are considerably high in the bay (Hayakawa et al., 2001). It was observed that the soluble forms of nitrogen and phosphorus fluctuated throughout the year at both water depths in the Ofunato Bay. Phosphate concentrations were remarkably reduced during late spring and early summer, which were counterbalanced by high concentrations of ammonia during those periods at all three sampling stations. Seasonal variations in silicate concentrations were also similar to those of phosphate, except that remarkably higher concentrations were observed for surface waters compared to deeper waters. Differences in the nitrate concentration between surface and deep waters were also recorded in the Ofunato Bay. These results, together with the observed strong negative correlation between salinity and nitrate ($r = -0.75323$) and silicate ($r = -0.85072$), suggest that nitrate and silicate concentrations were strongly affected by surface runoff (Yamada et al., 2017).

Chl-*a* concentrations provide proxy information on the abundance of phytoplankton and the amount of primary production (Boyer et al., 2009). In the Ofunato Bay we observed seasonal differences in its concentrations that were similar to those of biologically available nitrogen and phosphorus (Supplementary Table S2). Strong seasonal variation was confirmed at three sampling stations, with relatively lower chl-*a* concentrations during late autumn through early winter (from 0.37 to 3.27 $\mu\text{g/L}$) and the highest concentrations during spring (20.31 $\mu\text{g/L}$). We also observed a positive correlation between water pH and chl-*a* concentration ($r = 0.0799$). Hayakawa et al. (2001) reported that oysters cultivated in the surface waters of the Ofunato Bay caused high biodeposition during March and September and corresponded to elevated chl-*a* concentrations. These reports, together with our

measurements of nutrient concentrations at three sampling stations in the bay, suggest a mesotrophic to eutrophic state of the bay.

3.2. Seasonal changes in heterotrophic bacterial and cyanobacterial abundance in the Ofunato Bay

The numerical abundance of heterotrophic bacteria was very stable throughout the year, ranging from 0.4×10^6 to 2.4×10^6 cells/mL, as estimated by direct counting under a fluorescence microscope (Supplementary Table S2). We observed a slight tendency toward a lower abundance in the deeper waters compared to the surface waters. A similar abundance was also reported by Yamada et al. (2017). This observation regarding the heterotrophic bacterial abundance, however, contrasts with the concentrations of chl-*a* recorded in the Ofunato Bay. Estimation of cyanobacterial abundance, on the other hand, showed the lowest concentration of 0.68×10^3 during early spring and the highest concentration of 2.6×10^3 during late spring. These results are similar to those reported for the central and western Pacific by Jiao and Ni (1997), and available reports suggest that there is a close association between cyanobacterial and heterotrophic bacterial contents (Stewart et al., 2006).

3.3. Seasonal changes in the free-living bacterial community of the Ofunato Bay

Seasonal changes in WGS sequence reads were analyzed using BLASTn and MEGAN for the closest phylogenetic match in different seasons at KSt. 1, 2 and 3.

During the colder months of March and May, the number of bacterial genera was consistently low in the upper and lower water depths at all three sampling stations, showing a significant rise of 1.2 to 1.5 times as many genera in the warmer months of August and November (Table 1). The number of species was twice as high in August and November than in March and May. Most bacterial genera were shared among the months of the year, while

several others, including *Synechococcus* sp. CC9902 (class Cyanobacteria), *Synechococcus* sp. RCC37 (class Cyanobacteria) and *Ca. Actinomarina minuta* (class Actinobacteria), were unique to the warmer months (Supplementary Table S3). Changes were most evident for Alphaproteobacteria and Gammaproteobacteria where they together constituted more than half of the total bacterial population in the Ofunato Bay. Similarly, changes in Gammaproteobacteria were also conspicuous, with a significantly higher increase in relative abundance during the warmer months of the year.

The most abundant WGS sequence reads were assigned to Gammaproteobacteria and included *Alteromonas macleodii*, *Buchnera aphidicola*, SAR86 cluster, *Glaciecola psychrophila*, *G. nitratireducens*, *Colwellia psychrerythraea* and *Ca. Ruthia magnifica*. With some exceptions, in the deeper waters of KSt. 1 and 3, the widely distributed marine Gammaproteobacteria *Alteromonas macleodii* was dominant throughout the year, with a sharp rise during the month of November. *Alteromonas macleodii* plays an important role in biogeochemical cycles, including nitrogen and dissolved organic carbon cycling in the marine environment (McCarren et al., 2010). Their rise in abundance during November is related to the nutrient availability of phosphate and silicate in the Ofunato Bay (Shi et al., 2012). *Buchnera aphidicola*, the obligate intracellular bacteria of aphid insects, was the second most abundant Gammaproteobacteria after *A. macleodii*. They are mainly associated with most phloem sap-feeding aphids (Fukatsu and Ishikawa, 1992), suggesting their entrance into the environment from the neighboring woods of the Ofunato Bay. Together with *B. aphidicola*, some other intracellular symbiotic bacteria of aphids were detected at low abundance, including *Regiella insecticola* and *Serratia symbiotica* (data not shown).

The SAR86 cluster was found to be the next most abundant Gammaproteobacteria in the Ofunato Bay (data not shown). These are aerobic chemoheterotrophs and widely abundant at the ocean surface (Dupont et al., 2012). In the present study, the SAR86 cluster was present

throughout the year but increased in abundance during colder months of the year in the surface waters at the three sampling stations in the Ofunato Bay, whereas in deeper waters, their abundance showed a sharp increase during the warmer months at KSt. 2. This indicates that their abundance is dependent on water mixing/overturning and thermal stratification (Treusch et al., 2009), as the onset of thermal stratification in the bay has been reported to commence from the month of June through October (Yamada et al., 2017). Psychrophilic bacteria of the genus *Glaciecola* were also present throughout the year in a minor quantity except for *G. psychrophila*, which showed a sudden increase during May in surface and deep waters across all three sampling stations. This sharp increase in *G. psychrophila* was probably related to the spring phytoplankton bloom in the Ofunato Bay (von Scheibner et al., 2014).

Results from the comparative analysis also suggested that the members of Actinobacteria and Cyanobacteria appeared to increase in abundance during high-temperature periods. It was revealed that *Ca. Pelagibacter* varied seasonally, reaching 60% of all sequences at the genus level during winter (Fig. 1). This increase was more evident in the deeper waters, where they reached up to 75% of all sequences at the genus level. In addition, in the case of *Planktomarina*, their relative abundance rose during winter and fell during summer. Similar seasonal variation in relative abundance among members of Alphaproteobacteria have also been reported in the English Channel (Gilbert et al., 2011) and Sargasso Sea (Carlson et al., 2009), where ecotype variation associated with peaks in winter or subsequent deep-mixing events has been observed. Another winter bacterioplankton taxon in the Ofunato Bay was *Ca. Actinomarina* (Fig. 1), which possesses rhodopsins and is capable of performing photoheterotrophic metabolism. They also showed peaks in winter in the Ofunato Bay.

In contrast, a significant component of the summer bacterioplankton was Bacteroidetes, which was also the second largest bacterial phylum detected in the Ofunato Bay. Its members

Polaribacter and *Flavobacterium* were found to be high in abundance during spring and summer, particularly in the surface waters (Fig. 1). *Polaribacter* is a psychrophilic, proteorhodopsin-containing bacterial genus that has also been reported to be only detected in summer along the Antarctic Peninsula (Grzymski et al., 2012). Since its members are non-motile rods, they were thought to have drifted to the Ofunato Bay by the *Oyashio Current*. A minor component of the Ofunato Bay metagenome, *Ca. Puniceispirillum*, on the other hand, tended to decrease at low water temperatures and increase during high-temperature periods (Fig. 1).

3.4. Seasonal changes in the archaeal community of the Ofunato Bay

A significant component of the winter bacterioplankton community was Archaea, as their relative abundances were very low during spring and summer but high during winter. *Nitrosopumilus*, an ammonia-oxidizing crenarchaeon of Marine Group I Archaea, showed higher abundance in the deep waters (i.e., 10 m), with significantly higher peaks during winter. Similar observations have been reported by Murray et al. (1998). Other archaeal genera, including methanogenic *Methanococcus*, *Methanobrevibacter*, and *Methanocaldococcus*, were minor components in the Ofunato Bay archaeal community and did not show any particular relationship with environmental factors (Fig. 1 and Supplementary Table S1).

3.5. Seasonal changes in the eukaryotic community of the Ofunato Bay

Although their abundance was relatively low in the Ofunato Bay metagenome, eukaryotic members, including *Bathycoccus*, *Ostreococcus* and *Micromonas*, were found to show peaks during the winter season. These three photosynthetic picoplankton genera formed the majority (~85%) of the eukaryotic community in the Ofunato Bay, with *Bathycoccus* alone

constituting the major part. They are considered to be the major producers in oceanic food webs, although these members have previously been reported to form blooms in temperate waters, suggesting a linkage with phytoplankton blooms in the Ofunato Bay (Yamada et al., 2017).

3.6. Diversity pattern in the free-living bacterial community of the Ofunato Bay

We performed cluster analysis on the datasets of the relative abundances of metagenomes (Supplementary Figure S1), in which the data were analyzed by the hierarchical clustering method. It was revealed that the datasets formed six different clusters, with autumn and winter metagenomes grouped together, while spring and summer metagenomes were separated, indicating a relatively stable taxonomic composition during colder periods and slightly different compositions during the warmer periods of the year. Indeed, several winter bacterial genera, including *Ca. Pelagibacter* and *Planktomarina*, dominated the bacterioplankton community during winter. Summer bacterial genera, such as *Flavobacterium* and *Ca. Actinomarina*, though composing a minor portion of the metagenomes, dominated during summer. Interestingly, the winter bacterial genus *Polaribacter* was found to dominate in the spring and summer communities (Collins et al., 2010). They, together with various minor components of the Ofunato Bay metagenomes, showed shifts in the community composition throughout the year primarily due to the changes in temperature and other water quality parameters. Although the metagenomic datasets for surface waters were separated from those from deeper waters from July to October, we did not find any depth-related differences from November through February (Fig. 2). A clear indication of thermal stratification has been reported in the Ofunato Bay (Murakami et al., 2013; Furudoi et al., 2014). We, however, observed a slight difference in the metagenomic community in the surface waters of KSt. 3, which is situated near the mouth

of the bay (Fig. 2). This is likely attributed to differences in the distribution pattern of several dominant bacterioplankton, such as *Ca. Pelagibacter*, *Polaribacter* and *Planktomarina*, and a higher influence of the Pacific Ocean near the mouth of the bay. Principal component analysis (PCA) using the same datasets (Fig. 3) also showed that the bacterial communities in the Ofunato Bay changed seasonally. Although it is too early to generalize such seasonal patterns in the bacterial community structure in the Ofunato Bay, we predict that the organic matter levels, deep mixing in the winter and surface runoff could be responsible for the shifting bacterioplankton community composition.

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Legends to Figures

Fig. 1. Shotgun metagenomic sequence reads assigned to specific genera, with Archaea, bacteria and eukaryotic genome sequence representation based on MEGAN analysis. Reads assigned to (a) Archaea, (b) bacteria and (c) eukaryotes were categorized by genera based on January to December 2015 shotgun metagenomic libraries. These are genera based on normalized hits, where only those genera that had >1% hits are shown for ease of data representation.

Fig. 2. Grouping of shotgun metagenomic sequence reads from surface (1 m) and deeper (8 or 10 m) seawaters at three sampling stations of the Ofunato Bay in accordance with months based on hierarchal clustering analysis. Data employed were only from 0.2- μ m size filters targeting the free-living fractions from samples collected during January to December 2015.

Fig. 3. Principal component analyses (PCAs) based on relative abundance of bacterial genera obtained from shotgun metagenomic data. Panel 'a' shows that several datasets from KSt. 3 derived from 1 m water depth were clustered (dotted parabola), while Panel 'b' shows that datasets derived from spring, summer and winter were clustered together (parabola filled with green, red and blue, respectively). Data employed was only from 0.2- μ m size filters targeting the free-living fractions from samples collected during January to December.

Table 1.

Number of bacterial genera and species occurring in different months at the two depths from the three samplings stations of the Ofunato Bay.

Sampling station	Water depth	No. of genera											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
KSt. 1	1 m	509	455	491	485	427	456	494	615	645	619	558	513
		(964)	(848)	(921)	(941)	(742)	(776)	(872)	(1218)	(1245)	(1284)	(1211)	(1004)
	8 m	484	574	473	492	447	403	584	578	548	568	569	443
		(924)	(1158)	(893)	(969)	(839)	(677)	(1124)	(1083)	(1079)	(1163)	(1173)	(872)
KSt. 2	1 m	496	440	454	467	382	414	495	618	569	498	487	449
		(920)	(817)	(823)	(901)	(626)	(706)	(899)	(1245)	(1121)	(972)	(975)	(859)
	10 m	605	447	559	516	421	406	551	565	575	510	546	513
		(1270)	(829)	(1165)	(985)	(762)	(704)	(1020)	(1159)	(1150)	(1012)	(1082)	(1017)
KSt. 3	1 m	600	482	540	558	518	450	552	685	571	585	572	529
		(1265)	(939)	(1064)	(1154)	(1016)	(804)	(1073)	(1477)	(1125)	(1198)	(1217)	(1017)
	10 m	455	400	470	519	424	378	491	524	570	537	495	447
		(859)	(736)	(883)	(1077)	(759)	(635)	(865)	(978)	(1182)	(1101)	(965)	(850)

*Values in the parentheses indicate number of species corresponding to the genera for each WGS data

Figure 2

Figure 3