이학석사학위 논문

Illumina Miseq을 이용한 한국 연안의 진핵생물 다양성에 대한 메타지놈 분석

Metagenomic analysis of Eukaryote diversity in coastal areas of South Korea revealed by Illumina Miseq

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ABSTRACT

Metagenomic analysis of Eukaryote diversity in coastal areas of South Korea revealed by Illumina Miseq

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Metagenomic analysis was carried out on the multiple taxa of eukaryotes, for the first time in Korea. Each samples were obtained from fourteen sites of Jeju island and the seas surrounding the Korean peninsula (East Sea, East China Sea, Yellow Sea) during August 2015. To more focus on Metazoa, total fourteen samples were washed through with a 63㎛ mesh sieve, then amplified based on 18S rDNA V4 hypervariable region. High-throughput sequencing of PCR amplicons was performed using the Illumina MiSeq. Paired-ends sequences, ranged from 25,637-38,463 reads per sample, revealed a mean of unique 230 OTUs (operational taxonomic units, 97% similarity). PoHang, had the highest number of unique OTUs, which showed 334 OTUs, while GoChang had the lowest unique OTUs, which had 49 OTUs. A total of 319 OTUs were identified into the species level by BLAST against SILVA database. For Metazoa, 103 species of 101 genera belonging to 75 families in 10 phyla were represented. Among these phyla, Annelida, Arthropoda, Mollusca, Nematoda and Platyhelminthes were dominated, account for 92.23% of the total. Of these, several undescribed taxa were detected at family-level identification; Nematoda 100% (6 families), Annelida 31.6% (6 families), Arthropoda 30.8% (4 families), Mollusca 20% (4 families), Platyhelminthes 100% (10 families) compared with integrated three DBs (MABIK, KOMBIS, MRBR). Also, relatedness community structure of eukaryotes and habitat were analyzed by beta-diversity using PCoA (Principle Coordinate Analysis) plot and UPGMA (Unweighted Pair Group Method with Arithmetic mean) tree. As a result, composition of eukaryotes community is likely to be largely affected by the habitat, however, the same habitat not always guarantee the same community structure.

Key words: Metagenomics, Eukaryote, Illumina Mi-seq, High-throughput technology, 18s rDNA V4, biodiversity

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Introduction

Eukaryotes, especially invertebrates are known for represent over 97% of the animal kingdom (Buchsbaum 2013). These invertebrates exist in every ecosystem on the earth. Their astronomical numbers, ubiquity, and diversity are known for playing significant roles in ecological process.

According to reports on CoML (Census of Marine Life), researching network of global marine life diversity and distribution, South Korea topped with 32.3 species per 1 km^2 among the National and Regional Implementation Committee (Costello *et al*. 2010). These data officially demonstrate the high abundance of potential marine life resources in South Korea, which need to be discovered. However, there are still lack of knowledge about how many marine creatures exist, due to lack of taxonomic expertise and poor infrastructure. In addition, traditional morphological way is not good enough to estimate diversity of life, and many groups and species still remain undescribed.

Also, some invertebrates (e.g. nematode, copepod) are represented by good indicators of climate change due to sensitivity of their response to environmental change. Moreover, composition of eukaryotic community and their diversity are important to setting an effective strategies for restoration and monitoring programs. The Korean peninsula basically belongs to the temperate zone, however, according to the distribution of biota, Jeju island and southern regions of peninsula, somehow, seem to be influenced by subtropical climate. Several studies suggested climatic regime shifts of North Pacific Ocean, which are likely to have profound effects to marine ecosystem (Zhang *et al.* 2000; Kang & Kim 2002; Tian *et al.* 2006). Not only climate change but also habitat loss and invasive organism of anthropogenic effects should be checked through routine biomonitoring.

Along with increased demand for high-throughput technology, metagenomic studies on micro organisms have attracted intensive research interest during recent decade. Since the term 'metagenomics' suggested in 1998, studies of metagenomics drastically increased through this high technology. However, previous studies on metagenomics have mainly focused on non-eukaryotic organism (usually bacteria) and based on 16S rDNA, while eukaryote-targeted approach has relatively short history (Table 1). However, recent study, it is also applicable for metazoan such as; phytoplankton (Cuvelier *et al.* 2010; Worden *et al.* 2012; Faria *et al.* 2014; Boophathi *et al.* 2015), nematodes (Porazinska *et al.* 2009; Porazinska *et al.* 2010a; Porazinska *et al.* 2010b), copepods (Hirai *et al.* 2015; Shoemaker & Moisander 2015), larvae of

Table 1. Summary of major history in metagenomics study

Year	Authors	Summary
1998	Handelsman *et al.*	First use of the term 'metagenomics'
2004	Venter C.	Global Ocean Sampling Expedition (GOS) project has begun
2006	Edwards *et al.*	Early studies on mining microbial diversity using 16S rDNA
2010	Chariton *et al.*	First metagenomic analysis of eukaryotes on the estuarine sediments using 18S rDNA
2010b	Porzanska *et al.*	Evaluate the utility of metageomics studies to nematodes in Field (18S rDNA)
2014	Faria *et al.*	First metagenomic analysis of eukaryotic marine phytoplankton in Jeju, South Korea (18S rDNA)
2015	Boophathi *et al.*	Eukaryotic phytoplankton diversity using pyrosequencing from ECS (East China Sea) near Jeju, South Korea (18S rDNA)

macroinvertebrates (Carew *et al.* 2013) or targeting micro-invertebrate of multiple taxa (Chariton *et al.* 2010; Bik *et al.* 2012). These studies of metagenomic approach of metazoan demonstrate the feasibility of high-throughput technology for even species level in metazoan.

Here we present the first metagenomic analyses of fourteen sites samples from Jeju Island and three seas surrounding the Korean peininsula (East Sea, East China Sea, Yellow Sea). PCR amplicons of 18S rDNA V4 hypervariable region were sequenced using Illumina miseq, for unraveling integrated view of eukaryotes community composition. The present study aimed to 1) assess the utility of high-throughput sequencing as tool for unraveling undescribed species and 2) compare the structure of eukaryotes among samples.

Materials and Methods

Sample collection

Samples were obtained from intertidal zones in fourteen sites of South Korea, Dangjin, Gunsan, Gochang, Mokpo, Jindo (West), Jindo (East), Yeosu, Tongyong, Busan, Pohang, Uljin, Yangyang, Jeju and Seoguipo (Table 2, Fig. 1, Fig. 2), 6-9 August 2015. Materials were washed through a 63-㎛-mesh sieve and preserved in ice box for DNA work. After sieving, meiofauna was extracted from the samples using the Ludox HS40 protocol (Burgess 2001).

DNA extraction, PCR and Illumina Mi-seq

Total DNA was extracted using Power Soil® DNA Isolation Kit (Mo Bio, Carlsbad, CA USA), following the manufacturer's instructions. For amplification of 18S rDNA partial V4 hypervariable region, F512 with Illumina forward overhang adapter sequence and R978 with Illumina reverse overhang sequence primer set were used (Table 3). Reactions of total 20 ㎕ volume consisted of 0.1 ug of environmental DNA template, 5 ㎕ of 5X color Go Taq buffer (Promega, Madison City, USA), 1 ㎕ of dNTP mixture (10 mM), 1ul of each primer (10 uM), 0.3 ㎕ of Go Taq DNA polymerase (Promega, Madison City, USA).

PCR conditions for amplification were an initial denaturation at 94℃ for 3 min, following 35 cycles of 94℃ for 45 sec, annealing for 45 sec at 50℃, extension at 72℃ for 90 sec, and final extension at 72℃ 10 min. PCR products were confirmed through visualisation on a 1.5% agarose gel and purified with the QIAquick® PCR Purification Kit (QIAGEN, Hilden, Germany). Illumina Mi-seq of PCR amplicons was performed using a commercial service at Macrogen (Seoul, Korea).

Data analysis

Paired-end reads sequences gernerated from the Illumina Mi-seq were processed using Qiime (Quantitative Insights Into Microbial Ecology) 1.9.1v pipeline (Caporaso *et al.* 2010). All reads were checked and trimmed for quality (maximum homopolymers=6, minimum length=200bp). After initial filtering, OTUs (Operational Taxonomic Units) were clustered with cutoff values of 97% similarity through Usearch (http://www.drive5.com/usearch/). Each read was searched by UCLUST against SILVA 108 database (http://arb-silva.de/, Oct 2015). In order to minimize error, singleton reads were removed for strict filtering.

Both alpha and beta diversity were analyzed as described in the Qiime software manual. Alpha diversity indices (Good’s coverage (Good 1953), Shannon-Weaver diversity index (H') (Shannon & Weaver 1948), Evenness index (E)) and rarefaction curves (observed species) were performed for comparison of OTUs richness. For beta diversity, Principal coordinate analysis (PCoA (Gower 1966)) and UPGMA (unweighted pair group method with arithmetic mean) were constructed by unweighted (no abundance information) pairwise UniFrac (Lozupone & Knight 2005) distance matrix.

Sequences which classified into 'bacteria' kingdom were excluded from subsequent diversity analyses.

Table 2. Collection sites information of samples used in this study

No	Collection site	Latitude (N)	Longitude (E)	Date	ㄴsediment type
1	Janggohang, Seokmun-myeon, Dangjin-si, Chungcheongnam-do	37°02’1.37’’N	126°45’14.8’’E	2015.08.06	Fine Mud
2	Naeheung-dong, Gunsan-si, Jeollabuk-do	36°00’14.1’’N	126°44’2.36’’E	2015.08.06	Fine Mud
3	Sangam-ri, Buan-myeon, Gochang-gun, Jeollabuk-do	35°58’56.8’’N	126°38’24.58’’E	2015.08.06	Fine Mud
4	Hakgyo-ri Aphae-eup, Shinan-gun, Jeollanam-do	34°51’44.9’’N	126°19’9.47’’E	2015.08.06	Fine Mud
5	Sanwol-ri Jindo-eup, Jindo-gun Jeollanamdo	34°31’5.68‘‘N	126°13’27‘‘E	2015.08.06	Fine Mud
6	Geumgap-ri Uishin-myeon Jindo-gun, Jeollanam-do	34°23’44.9‘‘N	126°15’47.2‘‘E	2015.08.06	Fine Sand
7	Manseoung-ri Manheung-dong Yeosu-si Jeollanamdo	34°46’29.0‘‘N	127°44’42.3‘‘E	2015.08.07	Rocky/Sandy
8	Wonpyeong-gil Yongnam-meon Tongyong-si Gyeongsangnam-do	34°54’37.3‘‘N	128°26’34.6‘‘E	2015.08.07	Fine Mud
9	Chungsapo-ro Haewundae-gu Busan	35°09’41.1‘‘N	129°11’38.8‘‘E	2015.08.07	Rocky/Sandy
10	Yonghan-ri Heunghae-eup Buk-gu Pohang-si Gyeongsangbuk-do	36°05’6.68‘‘N	129°33’10.7‘‘E	2015.08.07	Rocky/Sandy
11	Bongpyeong Beach Jukbyeon-meon Uljin-gun Gyeongsangbuk-do	37°02’28.7‘‘N	129°24’53.6‘‘E	2015.08.08	Rocky/Sandy
12	Namae-ri Hyunnam-myeon Yangyang-gun Gyeongsangbuk-do	37°56’23.6‘‘N	128°47’19.5‘‘E	2015.08.08	Rocky/Sandy
13	Dongil-ri Daejung-eup Seoguipo-si Jeju	33°13’57.8‘‘N	126°14’12.9‘‘E	2015.08.09	Fine Mud
14	Ioteu Beach Dori-ro Jeju-si Jeju	33°29’53.6‘‘N	126°27’10.3‘‘E	2015.08.09	Rocky/Sandy

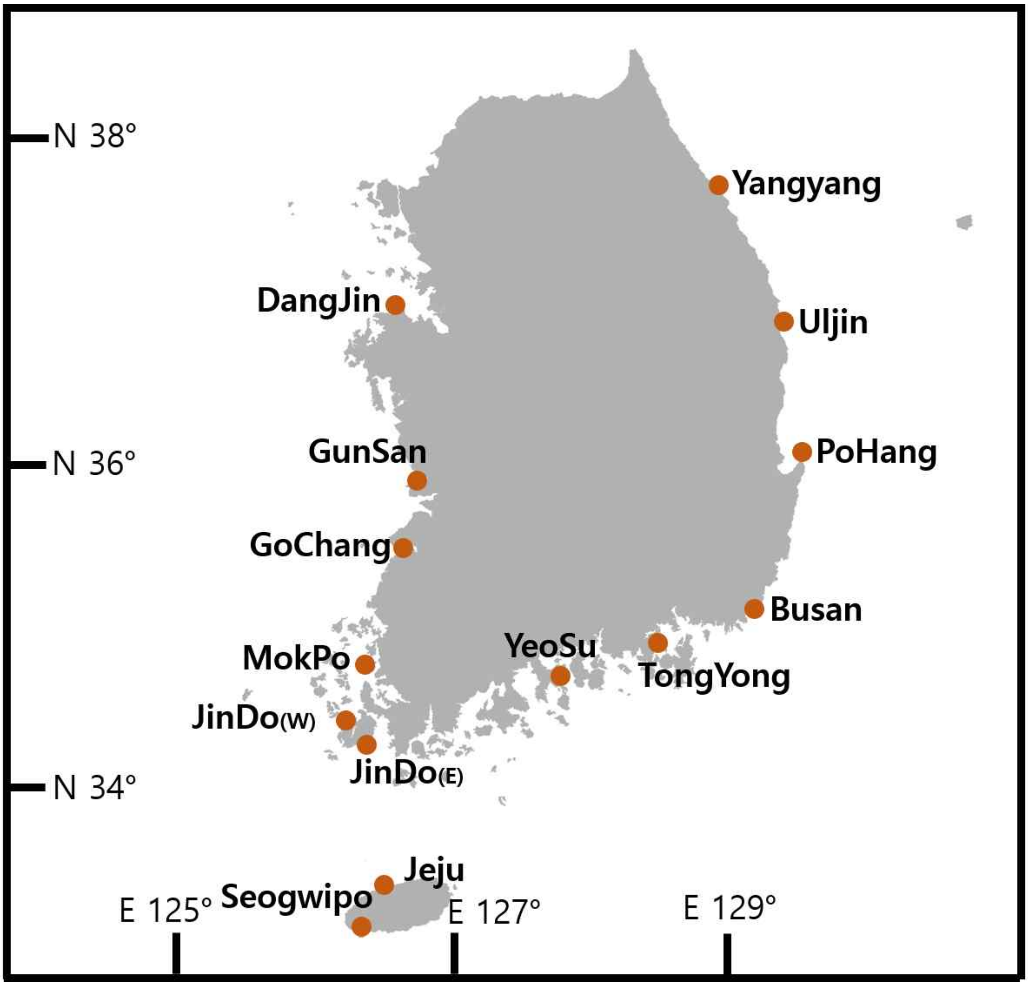

Fig. 1 Collection sites of samples used in this study

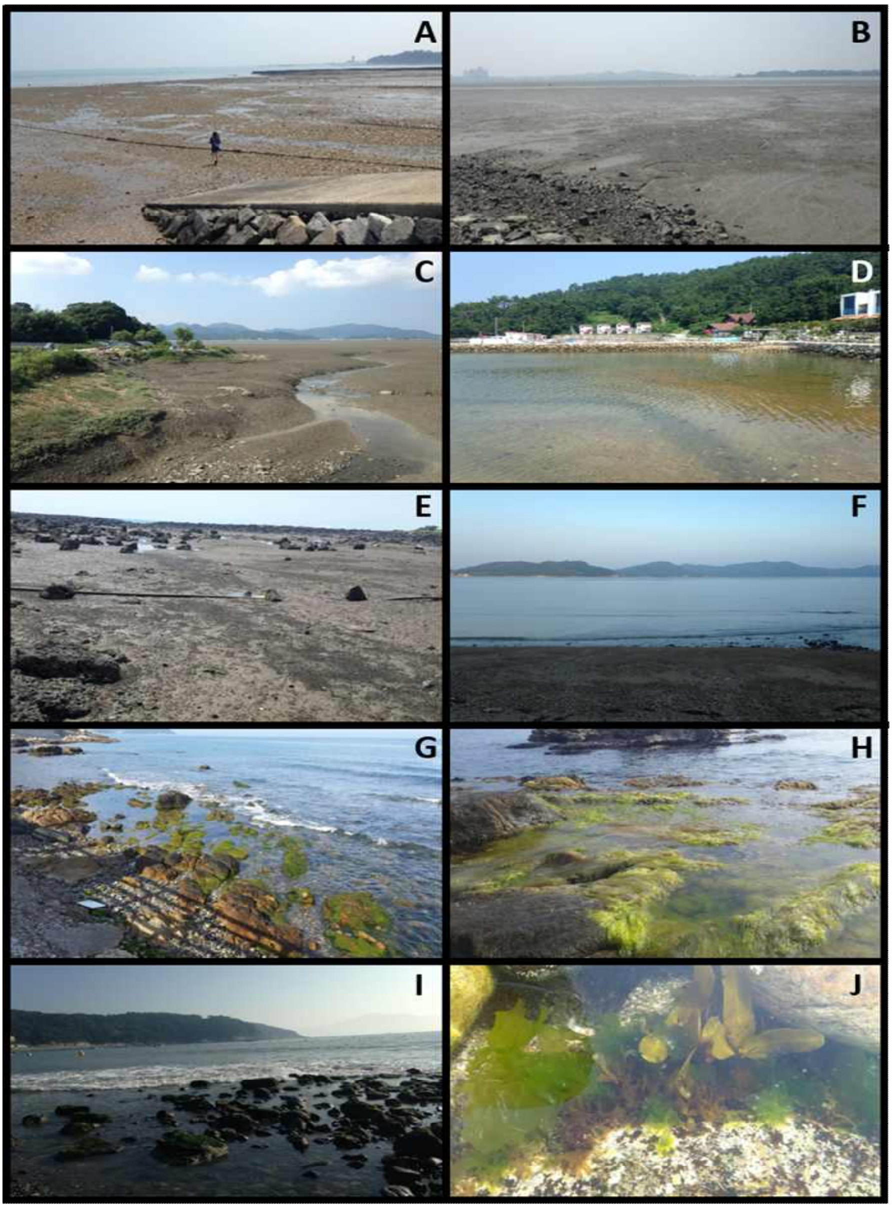

Fig. 2 Habitats of samples A. Flat mud in Dangjin B. Muddy beach in Gunsan C. Flat mud in Gochang D. Muddy beach in Tongyong E. Flat mud in Seogui F, Sandy Beach in Jindo (East) G. Rocky shore in Busan H. Rocyshore with seaweeds, Uljin I. Rochy shore with seaweeds in Yeosu J. Close-up of fresh seaweeds on a rocky shore, Uljin

Table 3. 18S ribosomal DNA primer used in this study

Primer		Reference
D512	ATT CCA GCT CGA ATA GCG	Zimmermann, 2011
D978	GAC AGG ACT ACG ATG GTA TCT AAT C	
Miseq adapter forward	TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG	
Miseq adapter reverse	GTC TCG TGG GCT CGG AGA TGT GTA TAA GA	

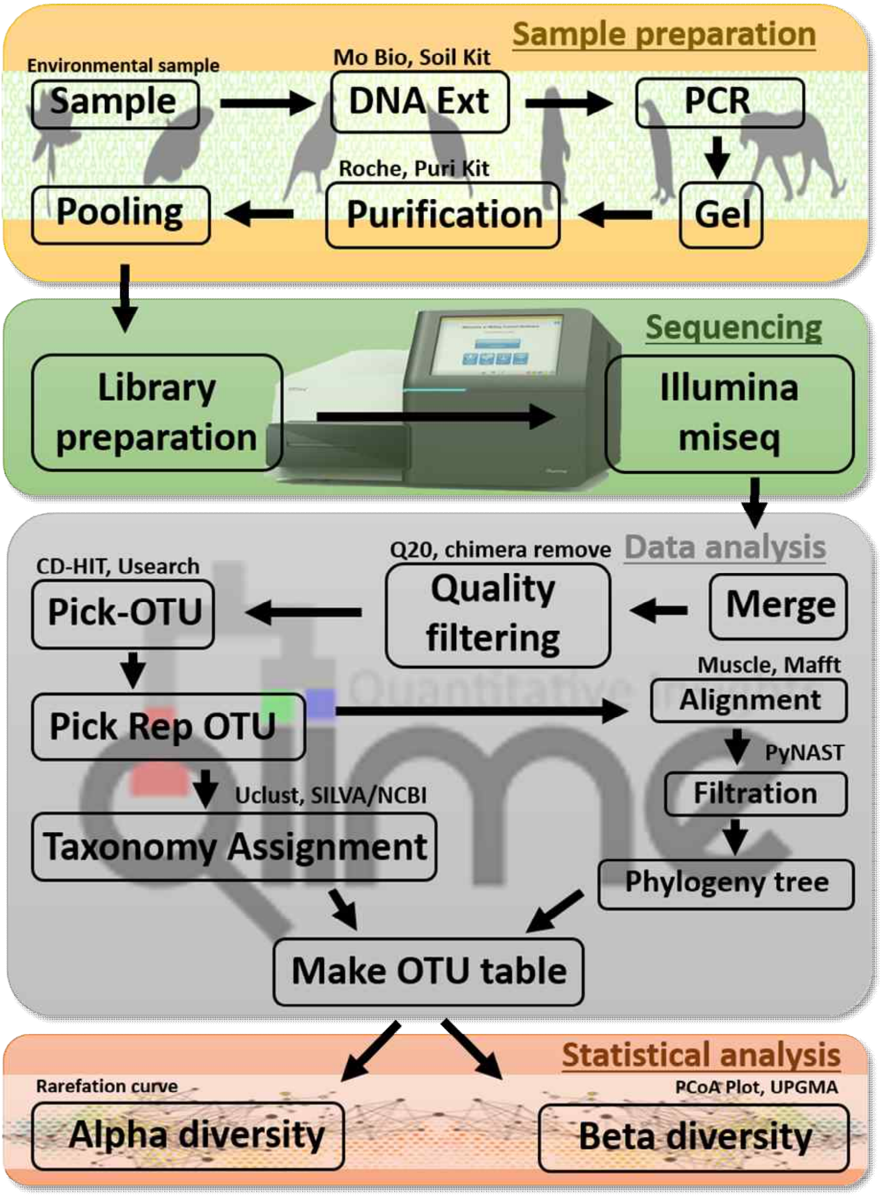

Fig. 3 Processing pipeline from collection of environmental sample through data analysis

Results

Alpha-diversity analysis

A total of 1,854,290 raw data reads were generated from Illumina Miseq, 436,154 high-quality reads were remained after trimming and filtering, with a ranged from 25,637-38,463 reads per sample.

All rarefaction curves (Fig 4.) of ‘Observed species richneess’ likely reached near-saturation. As ‘Observed species richness’ number usually consistent with unique OTUs number, in this view, PH, which had 334 OTUs, the highest number of species richness, while the GC sample had the lowest unique OTUs, which had 49 unique OTUs. According to the rarefaction curves, the second highest number of unique OTUs, was YY with 321 OTUs, closely followed by SG and UJ, both are 314 OTUs; and JDW, TY, BS, JDE, JJ, GS, DJ, MP, YS and GC at 308, 297, 247, 236, 190, 178, 175, 170, 87 and 49 unique OTUs respectively.

Good’s coverage is the percentage of the total species that represented in a sample, allowing us to evaluate the completeness of sampling. The Good’s coverage was high with an average of 99.84%.

Shannon-Weaver diversity index and Eveness index were ranged from 5.08 (TY) to 0.58 (GC) and 0.62 (TY) to 0.11 (GC) respectively. TY had the highest Shannon-Weaver diversity index with 5.08(H')-0.62(E)

followed by MP, UJ, SG, JDW, YY, GS, JJ, DJ, JDE, BS, MP, YS, GC at 5.06 (H')-0.61(E), 4.98(H')-0.61(E), 4.82(H')-0.59(E), 4.59 (H')-0.57(E), 4.38(H')-0.53(E), 4.13(H')-0.56(E), 3.87(H')-0.52(E), 3.71 (H')-0.50(E), 3.66(H')-0.47(E), 3.61(H')-0.46(E), 2.36(H')-0.32(E), 1.86 (H')-0.29(E), 0.58(H')-0.11(E), respectively (Table 4).

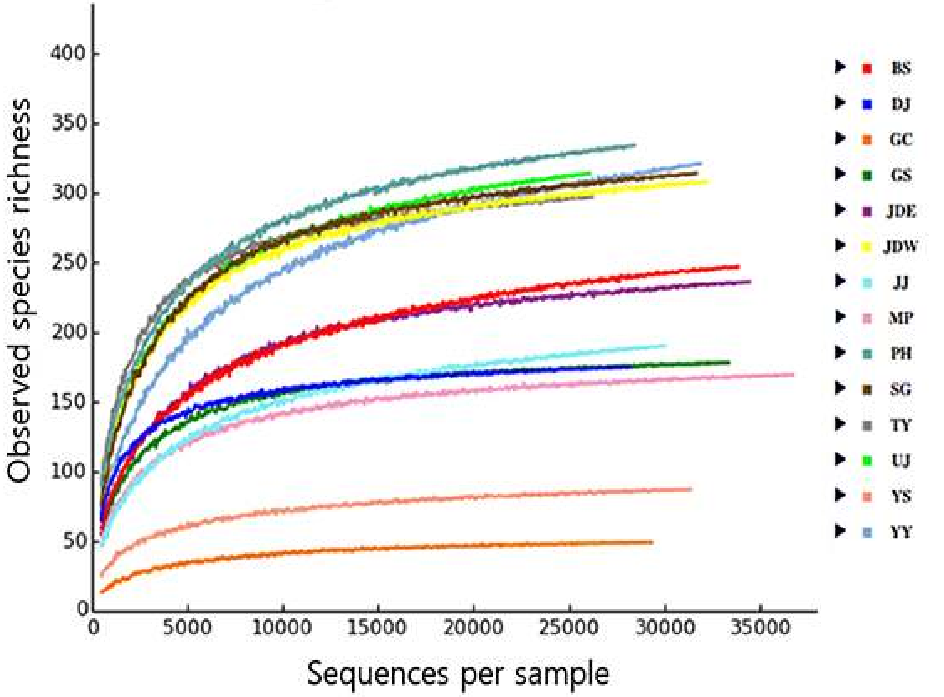

Fig. 4 Rarefaction curves comparing observed species richness between fourteen samples

Table 4. Number of unique OTUs, Good’s coverage, Shannon diversity index(H') and Evenness idex (*E*) obtained from fourteen samples

	No. unique OTUs	**Good’s Coverage(%)**	**Shannon diversity index (H')**	**Evenness (*E*) (Shannon Equitability)**
DJ	175	99.91	3.71	0.50
GC	49	99.96	0.58	0.11
GS	178	99.91	4.13	0.56
JDW	308	99.79	4.59	0.57
TY	297	99.85	5.08	0.62
JDE	236	99.81	3.66	0.47
SG	314	99.81	4.82	0.59
MP	170	99.88	2.36	0.32
JJ	190	99.86	3.87	0.52
YS	87	99.93	1.86	0.29
PH	334	99.76	5.06	0.61
UJ	314	99.75	4.98	0.61
YY	321	99.70	4.38	0.53
BS	247	99.77	3.61	0.46

Beta-diversity analysis

Community diversity of eukaryotes using 18S rDNA partial V4 region in South Korea and JeJu Island coasts were analyzed by PCoA (Principal coordinate analysis) plot and UPGMA (unweighted pair group method with arithmetic mean) tree.

PCoA plot was performed for visualizing similarities and clustering patterns of data(Fig. 5). Similarities and dissimilarities were determined by unweighted (an assessment of community composition) parewise UniFrac distance values. The two axes of the PCoA analysis indicated 24.97% and 11.85% of the total variance of the eukaryotes communities, respectively. Before analysis, samples were divided into two large groups based on characteristic of habitats; eastern-type group (blue circle), and western-type group (red rectangle). The habitat characteristic of eastern-type group was sandy shore and sublittoral rocks with epiphytic algaes, whereas western-type group was usually flat mud and sandy without rocks and algaes (JDE only). According to PCoA plot, datasets formed separate clusters represented that the composition of species among the fourteen sites divided into four different groups; 1) eastern-type group 2) major western-type group 3) minor western-type group and 4) JDE only. The largest major clustered group in the PCoA plot was 1) eastern-type group comprised of PH, YY, UJ, BS, JJ and YS. Four sites (BS, PH, UJ, YY) are located in east coast of South Korea, JJ is located in North-West of Jeju island

and YS located in south coast of South Korea. The second-largest group was 2) major western-type group, comprised of JDW, DJ, GC, GS and TY which are all located in west coast of Korea except TY. TY is located in south coast of Korea. The 3) minor western-type group, including MP and SG are situated in south-west coast and Jeju island each. Although their habitats were similar with 2) major western-type group, which were also flat mud, however, the plot shows that these two groups were clearly separated with each other. 4) JDE was the only sample that did not included any groups, which located in South coast of Korea.

To further compare the composition of the community, we subjected these 18S rDNA gene sequences to UPGMA tree (Fig. 6). The UPGMA tree results were consistent with PCoA plot results. Two Major groups, 1) eastern-type group and 2) major western-type group, and one 3) minor western-type group were clearly distinguished, and 4) JDE grouped in a clade with 3) minor western-type group (SG, MP). Generally, UPGMA tree shows the relationship among samples that are more closely related are closer together. In this study, however, UPGMA tree indicates weak relationship between geological distances and clustering pattern.

Figure 7 was created to give a location information of beta-diversity data. Yellow circles represent fourteen sampling sites. Eastern-type group and western-type group were surrounded by brown and green dotted line, respectively. In western-type group, 3) minor western-group and 4) JDE are marked with asterisk. The clear differences are shown within western-type groups.

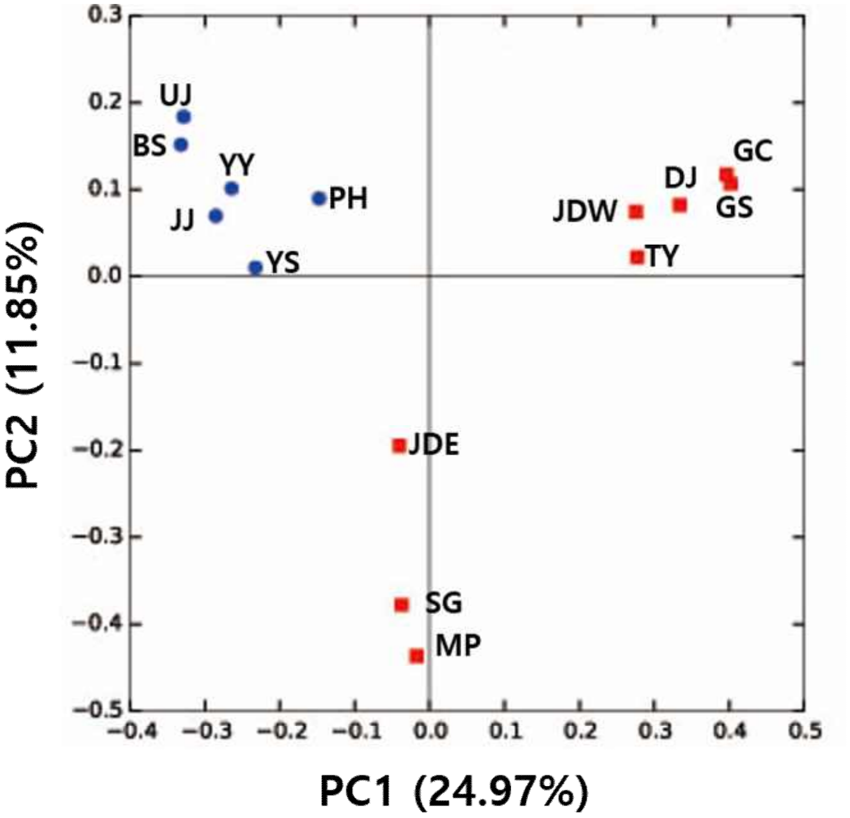

Fig. 5 Principle Coordinate Analysis (PCoA) plot comparing compositional relatedness among samples (Blue circle represents eastern-type group, Red rectangle represents western-type group)

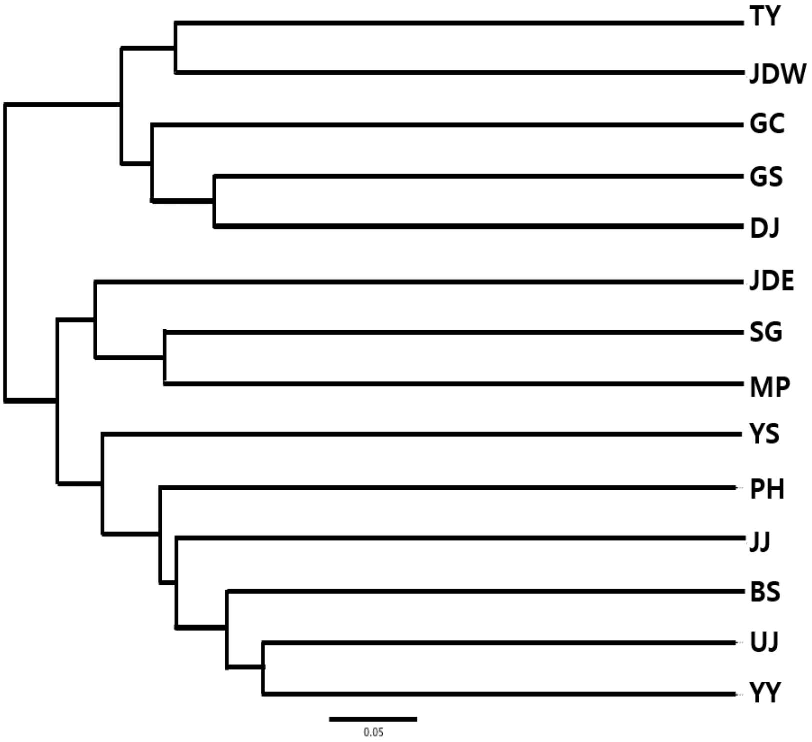

Fig. 6 Unweighted Pair Group Method with Arithmetic mean (UPGMA) tree constructed from fourteen samples. Below scale bar indicates 0.05% sequence divergence

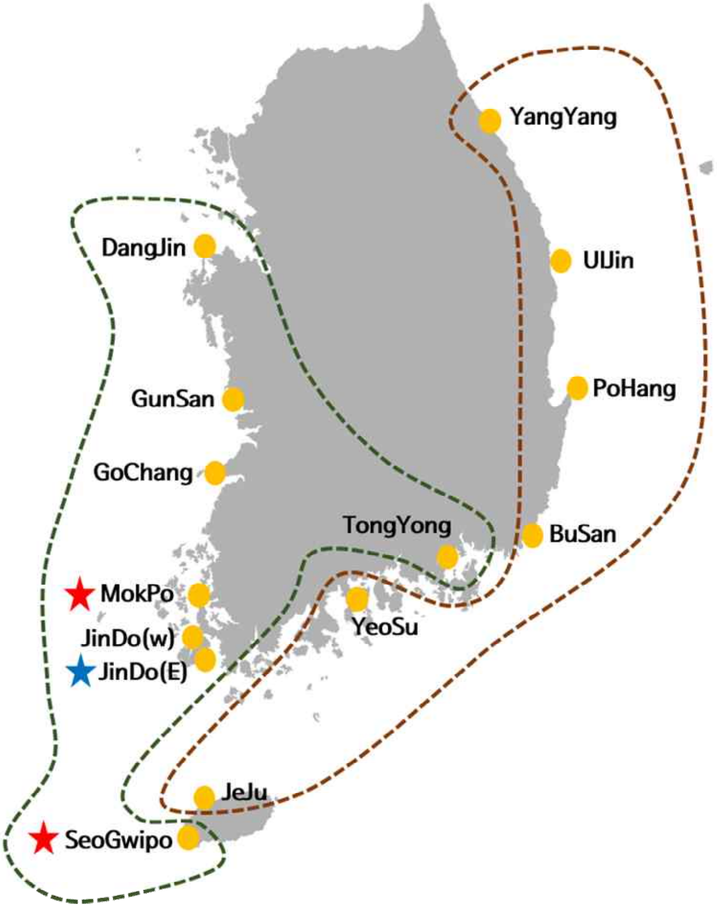

Fig. 7 Distribution of sampling sites. Eastern-type group and western-type group surrounded by brown and green dotted line, respectively. Minor clustered groups were marked with asterisk

Composition of eukaryotes phyla

The Figure 8 represents the proportion of kingdom (Metazoa, Protist, Fungi, Algae, Plant and unclassified eukarytoes) among fourteen samples.

The graph reveals that major western-type group (DJ, GC, GS, JDW and TY were 49%, 95%, 68%, 27%, and 40%, respectively), had greater percentage of metazoan in phyla than eastern-type group (JJ, YS, PH, UJ, YY and BS were 2%, 2%, 21%, 22%, 3%, and 0%, respectively). Maximum proportion of metazoan in eastern-type group (22%, UJ) were lower than minimum proportion of metazoan in western-type group (27%, JDW). Reversely, proportion of Protist and Algae was higher in eastern-type group than western-type group (Fig 9-15). The median percentage of Protist/Algae in eastern-type group were 42%/35.17% (range, 18-80%/5-66% Protist/Algae), whereas western-type group shows 18%/11.2% (range, 1-47%/4-30% Protist/Algae). Since the materials were washed through 63-㎛-mesh sieve during the sampling, fungi, small-sized protist, and unicellular algae could have passed through the sieve, which might have led to a decrease.

JDE, which was located in the middle of PCoA plot had similar proportion of both Metazoa and Protist. In addition, minor of western-type group (SG, MP) showed considerable occupation of unclassified eukaryotes. About 74% of MP and 42% of SG fell into a category of “unclassified eukaryotes”.

The number of OTUs from different taxa are shown in Table 5 For metazoan, Annelida was the most common, followed by Arthropoda, Mollusca, Nematoda, Platyhelminthes. For protist, Stramenopile was the most frequent phyla, followed by Ciliophora, Cerozoa, Apicomplexa, Myzozoa. For fungi Ascomycota, Chytridiomycota, Microsporidia were the common.

A huge data of meio- and micro- faunal phyla were simplified (Figs. 9-15), to inform a better understand of eukaroytes profile in phylum level based on locality. The number of unique OTUs from each sites are presented in the middle of circle graph, inner circle reveals the proportion of phyla and outer circle shows the proportion of phylum-level. Reads number of top five phylum from each phyla has been transformed by using [log (abundance +1)] in order to normalize frequency and homoscedasticity (Clarke 1993).

Interestingly, *Ulva pertusa* of green algae and *Labyrinthuloides miuta* of protist were detected from all fourteen sites. *Ulva pertusa*, makes a severe massive blooms of the green tide seasonally in Yellow Sea, is known to distributed along the entire coast of Korea (Bae 2001), which is consistent with previous study.

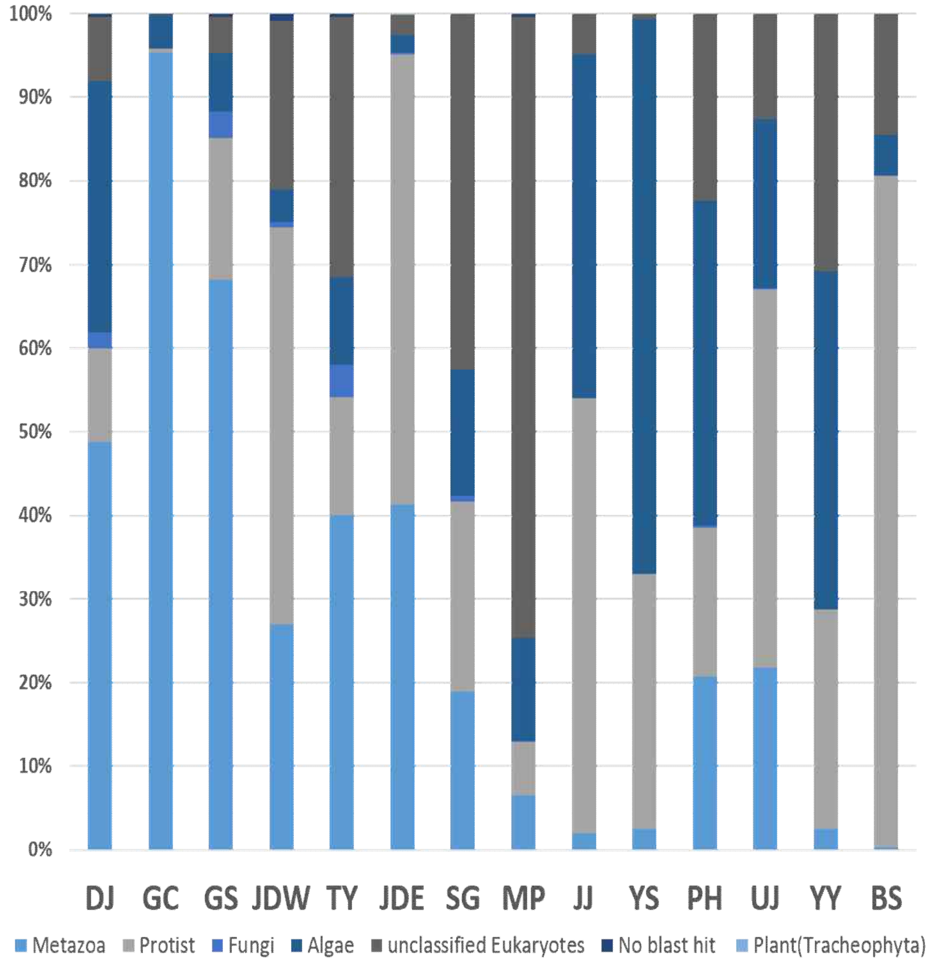

Fig. 8 Proportions of taxonomic assignments from obtained samples

Table 5. Operational Taxonomic Units (OTUs) reads number and proportion of each phylum from samples

Phylum	DJ		GC		GS		JDW		TY		JDE		SG		MP		JJ		YS		PH		UJ		YY		BS	
	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%
Api-complexa	33	0.119	51	0.174	21	0.063	102	0.318	479	1.868	113	0.327	56	0.177	242	0.629	-	-	-	-	163	0.573	-	-	23	0.072	-	-
Amoe-bozoa	26	0.093	-	-	6	0.018	8	0.025	22	0.086	5	0.014	37	0.117	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Apusozoa	-	-	-	-	-	-	-	-	48	0.187	9	0.026	-	-	5	0.013	-	-	-	-	-	-	-	-	1	0.003	-	-
Centro heliozoa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	0.025	1	0.003
Cercozoa	17	0.061	30	0.102	16	0.048	72	0.225	337	1.315	42	0.122	178	0.562	145	0.377	-	-	-	-	12	0.042	-	-	7	0.022	-	-
Choa-nozoa	23	0.083	-	-	-	-	32	0.100	16	0.062	4	0.012	2	0.006	-	-	2	0.007	-	-	18	0.063	5	0.019	49	0.154	-	-
Ciliophora	-	-	8	0.027	1419	4.256	538	1.678	5	0.020	15682	45.429	31	0.098	196	0.510	4536	15.089	317	1.009	490	1.723	31	0.119	335	1.050	734	2.164
Myzozoa	46	0.165	-	-	8	0.024	-	-	25	0.098	-	-	-	-	3	0.008	-	-	-	-	22	0.077	-	-	-	-	-	-
Strame-nopiles	2864	10.289	46	0.157	4185	12.551	14455	45.075	2513	9.802	2740	7.937	6900	21.771	1736	4.513	11115	36.975	9277	29.524	4374	15.384	11769	45.082	7930	24.847	26497	78.114
unclassified Protist	104	0.374	-	-	22	0.066	55	0.172	156	0.608	16	0.046	1	0.003	149	0.387	-	-	-	-	13	0.046	1	0.004	4	0.013	12	0.035
Asco-mycota	390	1.401	1	0.003	814	2.441	146	0.455	787	3.070	22	0.064	107	0.338	52	0.135	-	-	-	-	19	0.067	-	-	4	0.013	-	-
Basidio-mycota	-	-	-	-	-	-	16	0.050	-	-	-	-	27	0.085	-	-	-	-	-	-	20	0.070	-	-	-	-	-	-

Phylum	DJ		GC		GS		JDW		TY		JDE		SG		MP		JJ		YS		PH		UJ		YY		BS	
	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%
Chytridio-mycota	144	0.517	-	-	59	0.177	36	0.112	154	0.601	22	0.064	66	0.208	14	0.036	-	-	-	-	-	-	27	0.103	7	0.022	-	-
Micro-sporidia	7	0.025	-	-	65	0.195	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Zygo-mycota	4	0.014	-	-	38	0.114	-	-	38	0.148	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
unclassified Fungi	2	0.007	-	-	47	0.141	-	-	28	0.109	21	0.061	20	0.063	-	-	-	-	-	-	4	0.014	-	-	11	0.034	-	-
Annelida	12362	44.410	27820	94.777	4444	13.328	8266	25.776	9617	37.512	11100	32.155	4753	14.997	197	0.512	541	1.800	99	0.315	1980	6.964	3197	12.246	674	2.112	92	0.271
Arthropoda	548	1.969	152	0.518	9085	27.246	69	0.215	6	0.023	566	1.640	18	0.057	1987	5.166	4	0.013	-	-	30	0.106	-	-	-	-	-	-
Brachio-poda	10	0.036	18	0.061	-	-	13	0.041	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cephalo-rhyncha	25	0.090	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chordata	65	0.234	4	0.014	11	0.033	12	0.037	13	0.051	5	0.014	1	0.003	51	0.133	2	0.007	84	0.267	-	-	-	-	-	-	-	-
Mollusca	262	0.941	14	0.048	25	0.075	244	0.761	33	0.129	1971	5.710	1114	3.515	109	0.283	64	0.213	602	1.916	3728	13.112	2498	9.569	81	0.254	18	0.053
Nematoda	78	0.280	-	-	9	0.027	15	0.047	-	-	28	0.081	20	0.063	74	0.192	-	-	-	-	8	0.028	1	0.004	1	0.003	-	-
Platyhe-lminthes	195	0.701	-	-	14	0.042	-	-	598	2.333	508	1.472	65	0.205	53	0.138	1	0.003	-	-	155	0.545	-	-	17	0.053	1	0.003

Phylum	DJ		GC		GS		JDW		TY		JDE		SG		MP		JJ		YS		PH		UJ		YY		BS	
	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%	#Reads of OTU	%
Porifera	-	-	-	-	-	-	10	0.031	7	0.027	-	-	9	0.028	-	-	-	-	-	-	-	-	-	-	36	0.113	-	-
Rotifera	28	0.101	-	-	9149	27.438	1	0.003	1	0.004	65	0.188	-	-	-	-	-	-	-	-	-	-	-	-	2	0.006	-	-
unclassified Metazoa	18	0.065	-	-	10	0.030	-	-	-	-	-	-	28	0.088	25	0.065	-	-	-	-	-	-	-	-	-	-	-	-
Rhodophyta	46	0.165	-	-	-	-	7	0.022	60	0.234	71	0.206	1	0.003	-	-	3	0.010	-	-	356	1.252	94	0.360	11	0.034	52	0.153
Chlorophyta	8304	29.832	1133	3.860	2301	6.901	1248	3.892	2624	10.235	628	1.819	4766	15.038	4738	12.318	12335	41.033	20856	66.374	10699	37.630	5192	19.888	12921	40.486	1631	4.808
Charophyta	-	-	-	-	4	0.012	-	-	-	-	23	0.067	-	-	-	-	-	-	-	-	-	-	5	0.019	-	-	-	-
Tracheophyta	-	-	-	-	-	-	-	-	-	-	21	0.061	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
unclassified Eukaryotes	2135	7.670	31	0.106	1492	4.475	6469	20.172	7971	31.092	858	2.486	13494	42.576	28571	74.282	1458	4.850	187	0.595	6341	22.302	3286	12.587	9793	30.685	4883	14.395
No blast hit	100	0.359	45	0.153	100	0.300	255	0.795	99	0.386	-	-	-	-	116	0.302	-	-	-	-	-	-	-	-	-	-	-	-
total	27836	100	29353	100	33344	100	32069	100	25637	100	34520	100	31694	100	38463	100	30061	100	31422	100	28432	100	26106	100	31915	100	33921	

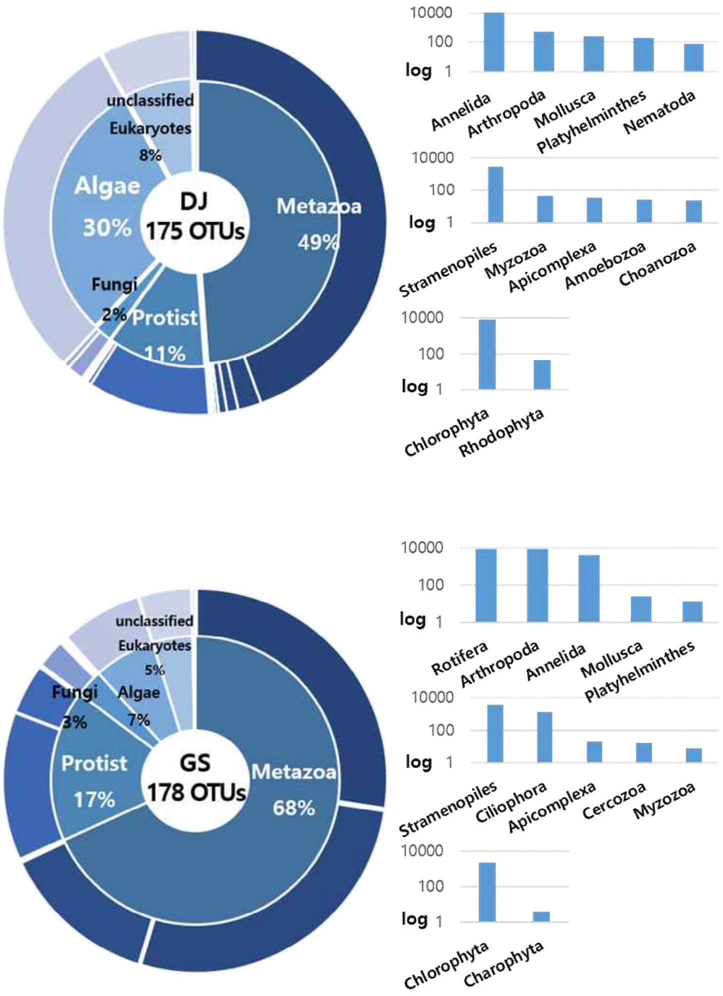

Fig. 9 Rings represent the abundance of DangJin and GunSan (inner ring; kingdom, outer ring; phylum) with number of OTUs. OTU abundance of top five phylum from top three kingdom were transformed by using [log (abundance +1)] for homoscedasticity.

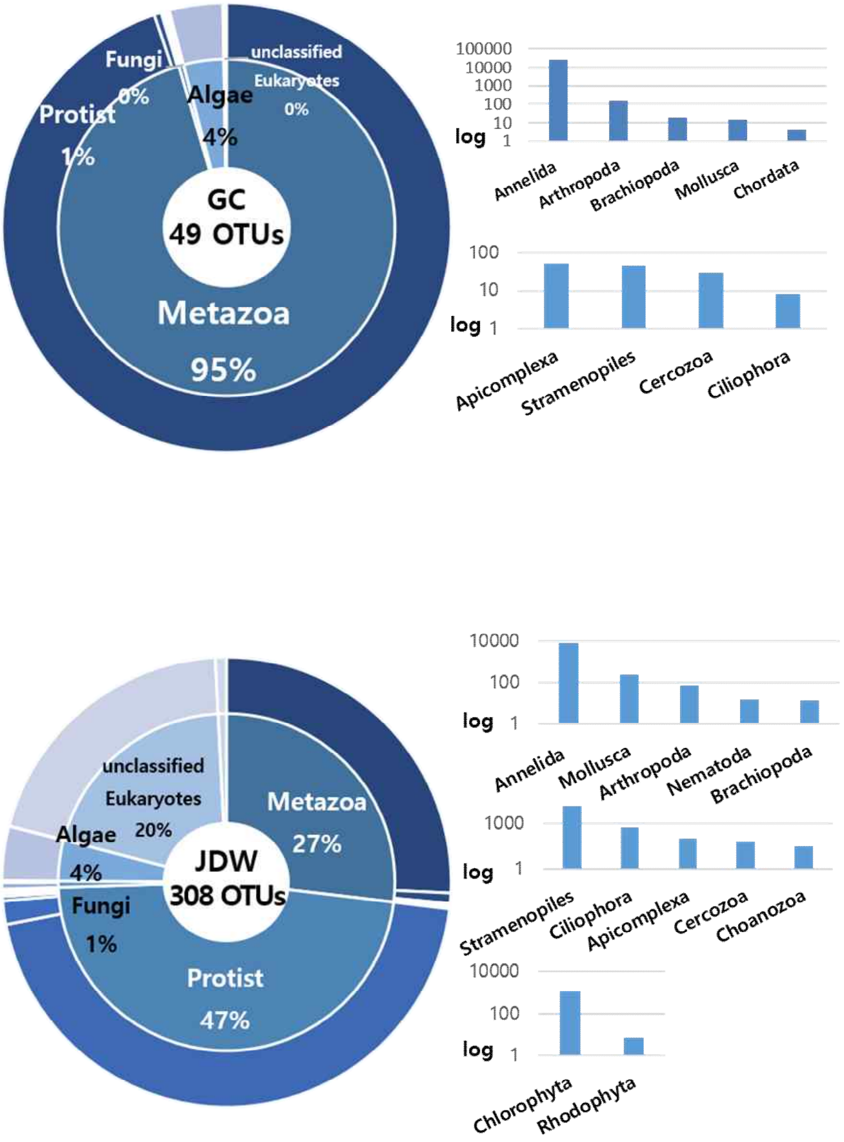

Fig. 10 Rings represent the abundance of GoChang and JinDo (West) (inner ring; kingdom, outer ring; phylum) with number of OTUs. OTU abundance of top five phylum from top three kingdom were transformed by using [log (abundance +1)] for homoscedasticity.

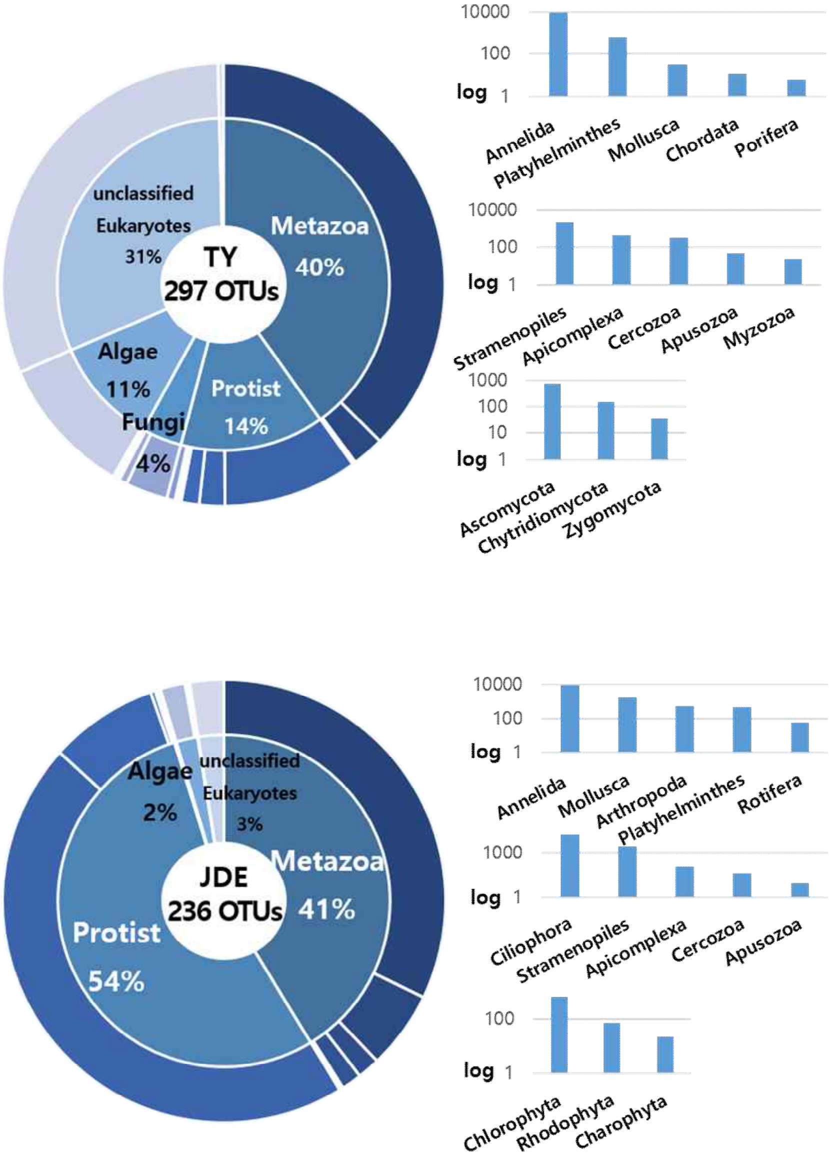

Fig. 11 Rings represent the abundance of TongYong and JinDo (East) (inner ring; kingdom, outer ring; phylum) with number of OTUs. OTU abundance of top five phylum from top three kingdom were transformed by using [log (abundance +1)] for homoscedasticity.

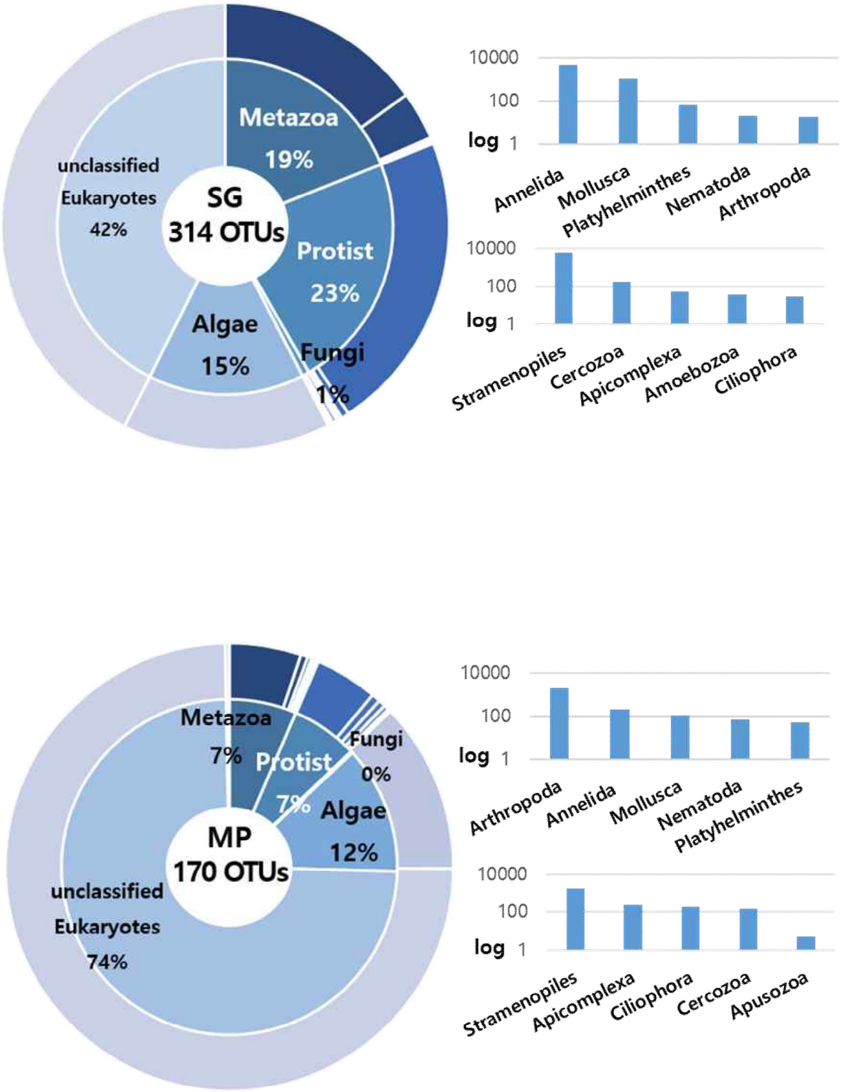

Fig. 12 Rings represent the abundance of SeoGuipo and Mokpo (inner ring; kingdom, outer ring; phylum) with number of OTUs. OTU abundance of top five phylum from top three kingdom were transformed by using [log (abundance +1)] for homoscedasticity.

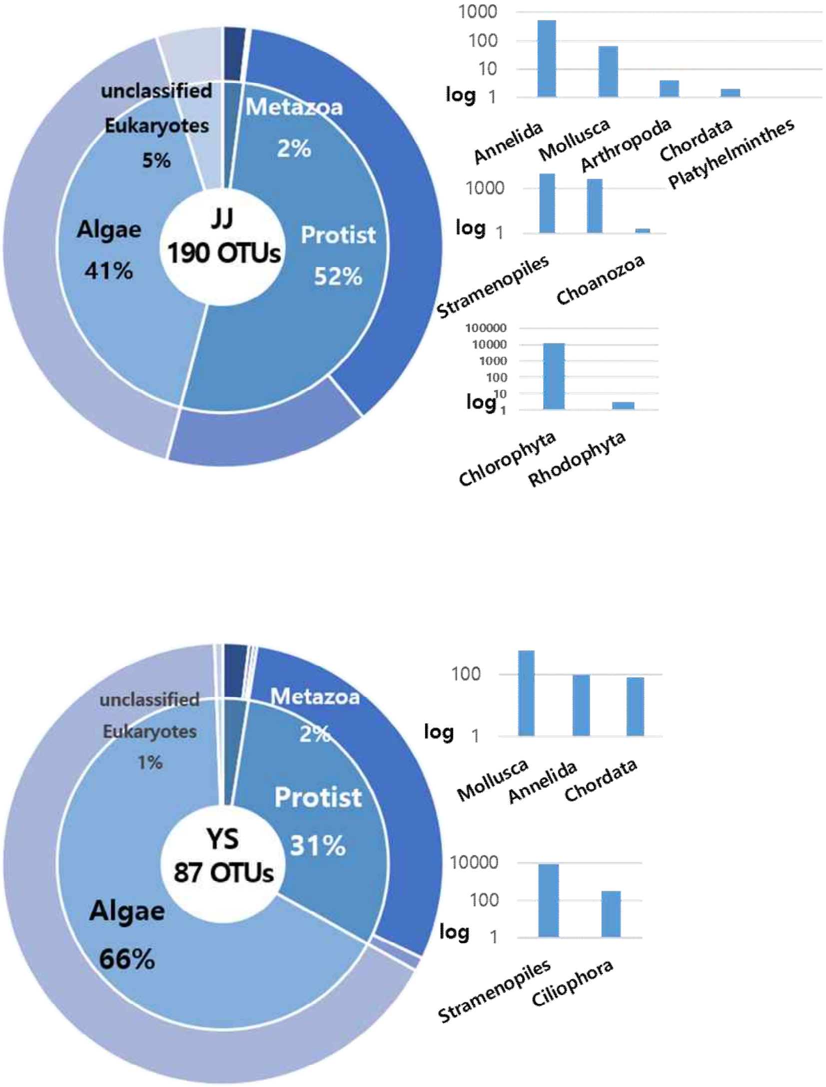

Fig. 13 Rings represent the abundance of JeJu and YeoSu (inner ring; kingdom, outer ring; phylum) with number of OTUs. OTU abundance of top five phylum from top three kingdom were transformed by using [log (abundance +1)] for homoscedasticity.

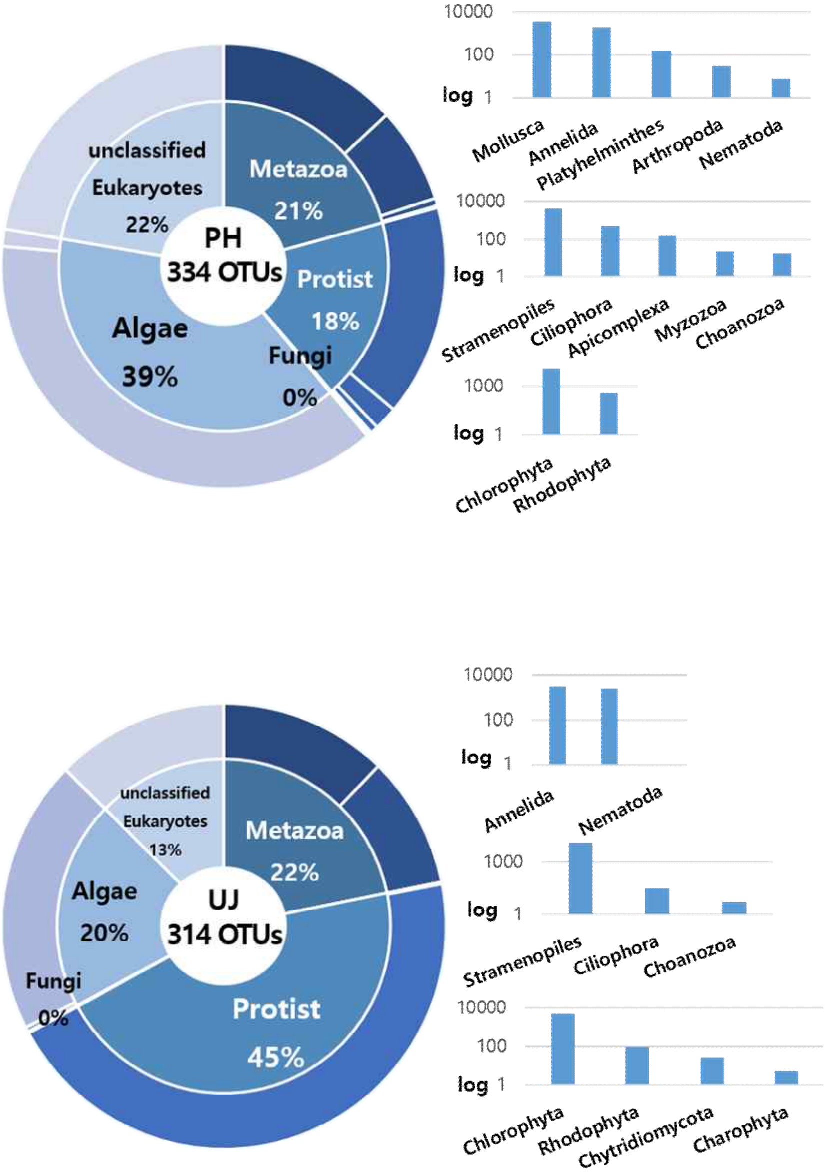

Fig. 14 Rings represent the abundance of PoHang and UlJin (inner ring; kingdom, outer ring; phylum) with number of OTUs. OTU abundance of top five phylum from top three kingdom were transformed by using [log (abundance +1)] for homoscedasticity.

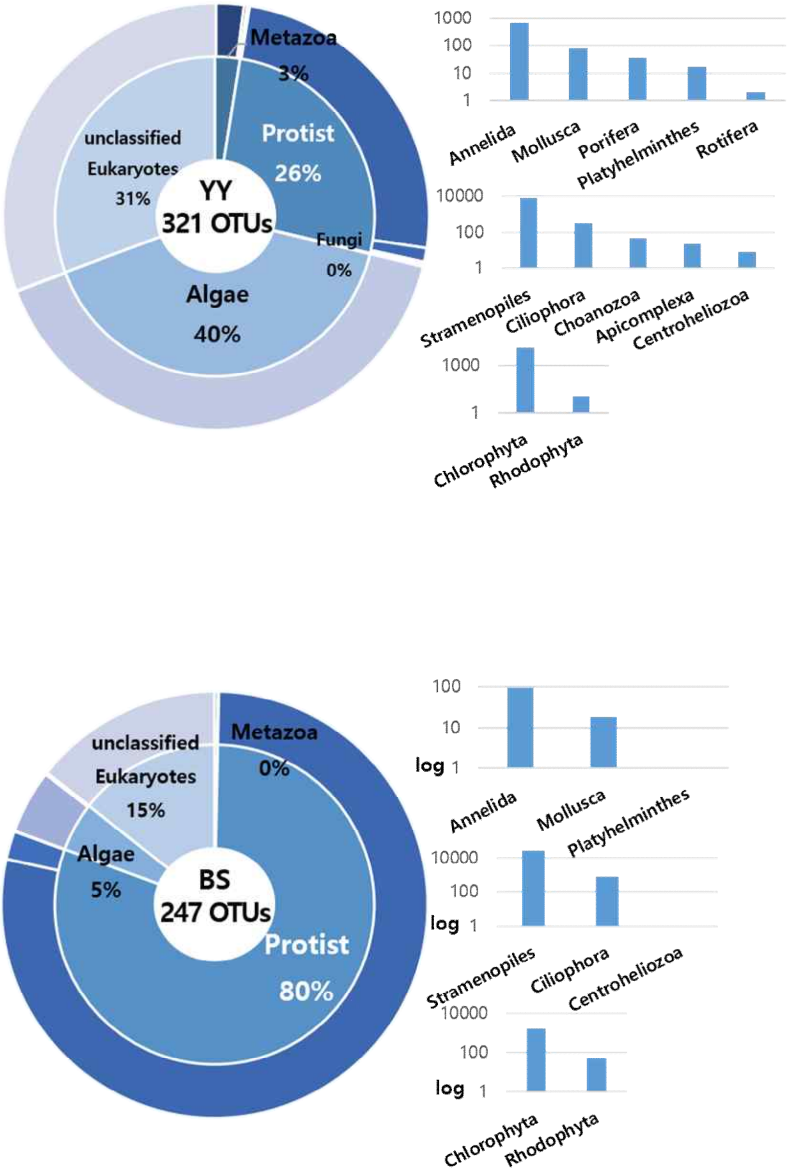

Fig. 15 Rings represent the abundance of YangYang and BuSan (inner ring; kingdom, outer ring; phylum) with number of OTUs. OTU abundance of top five phylum from top three kingdom were transformed by using [log (abundance +1)] for homoscedasticity.

Metazoa

In Total, 112 unique of 97% similarity OTUs were observed in metazoan of fourteen samples; 103 species, 101 genus, 75 family, 40 order, 14 class, and 10 phylum were identified among them. Among the 10 (Annelida, Arthropoda, Brachiopoda, Chordata, Cephalorhyndna, Mollusca, Nematoda, Platyhelminthes, Porifera, Rotifera) of three phylums (Brachiopoda, Chordata, Cephalorhydna) contained only one species, respectively. Top five abundant metazoan phyla (Annelida, Arthropoda, Mollusca, Nematoda, Platyhelminthes) occupied 92.233% in terms of total number of species. The phylum Annelida, which was the most diverse and abundant group within our datasets, showed 36 species, 35 genus, 19 family, 9 order and 2 class.

The most common species, which detected simultaneously at more than 10 different region of fourteen sites, were *Notomastus tenuis* (Annelida), *Cirratulus spectabilis* (Annelida), *Conuber melanostoma* (Mollusca). Conversely, 43 out of 103 species (41.75%, *Grania variochaeta*, *Haplotaxis* cf. *gordioides*, *Propappus volki*, *Doliodrilus chinensis, Limnodrilus hoffmeisteri, Protodrilus purpureus, Ceratocephale loveni, Abarenicola affinis, Arenicola brasiliensis, Maldane sarsi, Paraonis* sp., *Candacia truncata, Argestigens* sp., *Itunella muelleri*, *Aphotopontius mammillatus*, *Heterocypris* sp., *Leptocythere* sp., *Echinoderes lanceolatus*, *Laternula creccina*, *Cardita* *leana*, *Macoma balthica*, *Musculus discors*, *Lymnaea* sp., *Tricolia variabilis*, *Chromadorita tentabundum*, *Pareurystomina* sp., *Halalaimus* sp., *Oxystomina* sp., *Halichoanolaimus* sp., *Macrostomum tuba*, *Microdalyellia rossi*, *Arrawaria* sp., *Gyratrix hermaphroditus*, *Polycystis naegelii*, *Dendrocoelopsis lactea*, *Romankenkius libidinosus*, *Spheciospongia vesparium*, *Collotheca campanulata*, *Sinantherina socialis*) were the only species that existed in just one site.

Table 6. Number of OTUs obtained from fourteen samples with taxonomic rank

Phylum	Class	Order	Family	Genus	Species	Number of OTUs													
						DJ	GC	GS	JDW	TY	JDE	SG	MP	JJ	YS	PH	UJ	YY	BS
Annelida	Clitellata	Acanthobdellida			unclassified Acanthobdellida	-	-	-	-	96	-	-	-	-	-	-	-	-	-
Annelida	Clitellata	Haplotaxida	Enchytraeidae	Grania	*Grania variochaeta*	-	200	-	-	-	-	-	-	-	-	-	-	-	-
Annelida	Clitellata	Haplotaxida	Enchytraeidae	Haplotaxis	*Haplotaxis* cf. *gordioides*	-	-	-	-	-	-	8	-	-	-	-	-	-	-
Annelida	Clitellata	Haplotaxida	Enchytraeidae	Marionina	*Marionina coatesae*	-	-	-	-	-	194	-	-	13	-	15	-	64	-
Annelida	Clitellata	Haplotaxida	Enchytraeidae	Mesenchytraeus	*Mesenchytraeus solifugus*	-	18	-	-	6692	-	10	1	-	1	-	-	-	-
Annelida	Clitellata	Haplotaxida	Naididae	Stylaria	*Stylaria* sp.	-	-	8	1	42	-	41	13	3	-	167	-	-	-
Annelida	Clitellata	Haplotaxida	Propappidae	Propappus	*Propappus volki*	-	-	-	-	-	-	-	-	-	-	-	7	-	-
Annelida	Clitellata	Haplotaxida	Tubificidae	Doliodrilus	*Doliodrilus chinensis*	-	-	19	-	-	-	-	-	-	-	-	-	-	-
Annelida	Clitellata	Haplotaxida	Tubificidae	Limnodrilus	*Limnodrilus hoffmeisteri*	-	-	-	-	-	-	15	-	-	-	-	-	-	-
Annelida	Clitellata	Haplotaxida	Tubificidae	Monopylephorus	*Monopylephorus rubroniveus*	-	-	-	47	-	-	-	-	-	-	473	-	1	-
Annelida	Clitellata	Haplotaxida	Tubificidae	Olavius	*Olavius algarvensis*	-	23	327	126	2508	4	1945	114	7	-	212	-	-	-
Annelida	Clitellata	Haplotaxida	Tubificidae	Tubifex	*Tubifex tubifex*	-	-	-	1	9	-	42	-	-	-	3	-	-	-
Annelida	Polychaeta	Canalipalpata	Protodrilidae	Protodrilus	*Protodrilus purpureus*	-	-	-	-	-	-	-	-	-	-	19	-	-	-
Annelida	Polychaeta	Canalipalpata	Saccocirridae	Saccocirrus	*Saccocirrus papillocercus*	-	-	-	-	-	-	-	-	-	-	69	2688	1	-
Annelida	Polychaeta	Canalipalpata	Sabellidae	Amphicorina	*Amphicorina mobilis*	-	-	-	-	-	-	-	-	-	-	-	135	-	90
Annelida	Polychaeta	Phyllodocida	Nereididae	Ceratocephale	*Ceratocephale loveni*	-	-	-	-	-	-	-	-	-	-	1003	-	-	-
Annelida	Polychaeta	Phyllodocida	Nereididae	Ceratonereis	*Ceratonereis erythraeenis*	-	-	-	11	-	49	-	-	-	-	-	-	-	-
Annelida	Polychaeta	Phyllodocida	Nereididae	Platynereis	*Platynereis dumerilii*	-	5	-	-	238	2	-	-	4	-	3	-	-	-
Annelida	Polychaeta	Phyllodocida	Phyllodocidae	Eteone	*Eteone longa*	-	-	-	-	-	-	-	6	-	-	1	-	-	-
Annelida	Polychaeta	Capitellida	Arenicolidae	Abarenicola	*Abarenicola affinis*	-	-	-	2	-	-	-	-	-	-	-	-	4	-
Annelida	Polychaeta	Capitellida	Arenicolidae	Arenicola	*Arenicola brasiliensis*	-	-	-	-	-	-	-	-	502	-	-	-	-	-
Annelida	Polychaeta	Capitellida	Capitellidae	Capitella	*Capitella* sp.	118	19	29	98	5	321	8	43	-	-	-	-	22	-
Annelida	Polychaeta	Capitellida	Capitellidae	Dasybranchus	*Dasybranchus caducus*	1	3	3	9	25	-	-	-	-	2	-	-	-	-
Annelida	Polychaeta	Capitellida	Capitellidae	Notomastus	*Notomastus tenuis*	11950	27551	160	7544	-	5	1	2	2	2	1	-	-	-
Annelida	Polychaeta	Capitellida	Maldanidae	Maldane	*Maldane sarsi*	-	-	-	-	-	-	-	-	-	-	-	-	4	-
Annelida	Polychaeta	Opheliida	Opheliidae	Euzonus	*Euzonus ezoensis*	293	1	-	1	-	8619	-	-	-	-	-	2	1	-

Phylum	Class	Order	Family	Genus	Species	Number of OTUs													
						DJ	GC	GS	JDW	TY	JDE	SG	MP	JJ	YS	PH	UJ	YY	BS
Annelida	Polychaeta	Orbiniida	Paraonidae	Paraonis	*Paraonis* sp.	-	-	-	-	-	-	-	-	-	-	9	-	-	-
Annelida	Polychaeta		Potamodrilidae	Potamodrilus	*Potamodrilus fluviatilis*	-	-	-	2	-	-	-	-	-	-	-	10	-	-
Annelida	Polychaeta	Spionida	Cirratulidae	Cirratulus	*Cirratulus spectabilis*	-	-	-	402	2	190	2637	1	10	94	3	308	461	-
Annelida	Polychaeta	Spionida	Spionidae	Asetocalamyzas	*Asetocalamyzas laonicola*	-	-	-	2	-	-	-	17	-	-	-	-	-	-
Annelida	Polychaeta	Spionida	Spionidae	Aurospio	*Aurospio dibranchiata*	-	-	3847	-	-	157	-	-	-	-	-	35	1	-
Annelida	Polychaeta	Spionida	Spionidae	Boccardiella	*Boccardiella ligerica*	-	-	-	-	-	13	-	-	-	-	2	2	115	1
Annelida	Polychaeta	Spionida	Spionidae	Polydora	*Polydora giardi*	-	-	-	8	-	1546	46	-	-	-	-	-	-	-
Annelida	Polychaeta	Spionida	Spionidae	Prionospio	*Prionospio dubia*	-	-	-	-	-	-	-	-	-	-	-	10	-	-
Annelida	Polychaeta	Spionida	Spionidae	Prionospio	*Prionospio ehlersi*	-	-	51	-	-	-	-	-	-	-	-	-	-	-
Annelida	Polychaeta	Terebellida	Pectinariidae	Pectinaria	*Pectinaria gouldii*	-	-	-	5	-	-	-	-	-	-	-	-	-	-
Annelida	Polychaeta	Terebellida	Terebellidae	Polycirrus	*Polycirrus* sp.	-	-	-	7	-	-	-	-	-	-	-	-	-	-
Arthropoda	Arachnida	Oribatida	Trhypochthonioidea	unclassified Trhypochthonioidea		-	-	-	-	-	-	-	-	-	-	12	-	-	-
Arthropoda	Arachnida	Oribatida	Parhypochthonioidea	unclassified Parhypochthonioidea		-	-	-	-	-	-	-	-	-	-	11	-	-	-
Arthropoda	Maxillopoda	Calanoida	Acartiidae	Acartia	*Acartia pacifica*	54	-	2614	69	-	66	-	73	4	-	-	-	-	-
Arthropoda	Maxillopoda	Calanoida	Candaciidae	Candacia	*Candacia truncata*	-	-	56	-	-	-	-	-	-	-	-	-	-	-
Arthropoda	Maxillopoda	Calanoida	Centropagidae	Centropages	*Centropages typicus*	140	-	231	-	-	-	-	-	-	-	-	-	-	-
Arthropoda	Maxillopoda	Calanoida	Centropagidae	Sinocalanus	*Sinocalanus tenellus*	228	54	3886	-	-	-	-	1322	-	-	-	-	-	-
Arthropoda	Maxillopoda	Calanoida	Clausocalanidae	Clausocalanus	*Clausocalanus furcatus*	-	85	118	-	-	123	-	504	-	-	-	-	-	-
Arthropoda	Maxillopoda	Calanoida	Pontellidae	Labidocera	*Labidocera euchaeta*	75	-	2164	-	-	-	-	-	-	-	-	-	-	-
Arthropoda	Maxillopoda	Calanoida	Pontellidae	Pontella	*Pontella fera*	-	-	-	-	-	341	-	88	-	-	-	-	-	-
Arthropoda	Maxillopoda	Harpacticoida	Ameiridae	Argestigens	*Argestigens* sp.	-	6	-	-	-	-	-	-	-	-	-	-	-	-
Arthropoda	Maxillopoda	Harpacticoida	Canthocamptidae	Itunella	*Itunella muelleri*	-	-	12	-	-	-	-	-	-	-	-	-	-	-
Arthropoda	Maxillopoda	Siphonostomatoida	Dirivultidae	Aphotopontius	*Aphotopontius mammillatus*	-	-	-	-	-	-	10	-	-	-	-	-	-	-
Arthropoda	Ostracoda	Podocopida	Cyclocyprididae	Dolerocypria	*Dolerocypria* sp.	-	-	-	-	-	-	8	-	-	-	3	-	-	-
Arthropoda	Ostracoda	Podocopida	Cyprididae	Heterocypris	*Heterocypris* sp.	-	-	-	-	-	14	-	-	-	-	-	-	-	-
Arthropoda	Ostracoda	Podocopida	Cytheridae	Cythere	*Cythere* sp.	47	-	-	-	-	11	-	-	-	-	-	-	-	-
Arthropoda	Ostracoda	Podocopida	Leptocytheridae	Leptocythere	*Leptocythere* sp.	-	-	-	-	-	6	-	-	-	-	-	-	-	-
Arthropoda	Ostracoda	Podocopida	Loxoconchidae	Cytheromorpha	*Cytheromorpha* sp.	4	-	-	-	-	5	-	-	-	-	-	-	-	-

Phylum	Class	Order	Family	Genus	Species	Number of OTUs													
						DJ	GC	GS	JDW	TY	JDE	SG	MP	JJ	YS	PH	UJ	YY	BS
Arthropoda	Insecta	Diptera	Chironomoidea	unclassified	Chironomoidea	-	7	4	-	-	-	-	-	-	-	4	-	-	-
Arthropoda	Insecta	Blattaria	Blaberidae	unclassified	Blaberidae	-	-	-	-	6	-	-	-	-	-	-	-	-	-
Brachiopoda	Rhynchonellata	Rhynchonellida	Frieleiidae	Manithyris	*Manithyris rossi*	10	18	-	13	-	-	-	-	-	-	-	-	-	-
Chordata	Mammalia	Rodentia		unclassified	Rodentia	65	4	11	12	13	5	1	51	2	84	-	-	-	-
Cephalorhyncha	Kinorhyncha	Cyclorhagida	Echinoderidae	Echinoderes	*Echinoderes lanceolatus*	25	-	-	-	-	-	-	-	-	-	-	-	-	-
Mollusca	Bivalvia	Anomalodesmata	Laternulidae	Laternula	*Laternula anatina*	-	-	-	12	-	-	66	8	-	-	-	-	-	-
Mollusca	Bivalvia	Anomalodesmata	Laternulidae	Laternula	*Laternula creccina*	-	-	-	4	-	-	-	-	-	-	-	-	-	-
Mollusca	Bivalvia	Veneroida	Cardiidae	Parvicardium	*Parvicardium exiguum*	3	3	1	3	-	4	-	-	-	-	-	-	-	-
Mollusca	Bivalvia	Veneroida	Carditidae	Cardita	*Cardita leana*	-	-	-	-	-	7	-	-	-	-	-	-	-	-
Mollusca	Bivalvia	Veneroida	Tellinidae	Macoma	*Macoma balthica*	69	-	-	-	-	-	-	-	-	-	-	-	-	-
Mollusca	Bivalvia	Veneroida	Glauconomidae	Glauconome	*Glauconome virens*	-	-	24	47	1	163	2	91	17	214	-	-	-	-
Mollusca	Bivalvia	Veneroida	Veneridae	Ruditapes	*Ruditapes philippinarum*	-	-	-	-	-	-	-	-	1	18	-	-	-	-
Mollusca	Bivalvia	Mytiloida	Mytilidae	Musculus	*Musculus discors*	-	-	-	-	-	-	-	-	41	-	-	-	-	-
Mollusca	Bivalvia	Mytiloida	Mytilidae	Leiosolenus	*Leiosolenus lithurus*	-	-	-	-	-	6	-	-	-	115	-	-	-	-
Mollusca	Bivalvia	Ostreoida	Ostreidae	Ostrea	*Ostrea edulis*	-	-	-	7	-	-	-	-	-	83	-	-	-	-
Mollusca	Bivalvia	Pectinoida	Pectinidae	Pecten	*Pecten maximus*	1	-	-	-	-	-	-	-	-	-	-	79	-	-
Mollusca	Gastropoda	Architaenioglossa	Viviparidae	Viviparus	*Viviparus georgianus*	-	-	-	8	1	2	20	-	-	19	1963	210	3	1
Mollusca	Gastropoda	Littorinimorpha	Calyptraeidae	Crepidula	*Crepidula fornicata*	-	-	-	-	-	-	-	7	-	-	-	-	-	2
Mollusca	Gastropoda	Littorinimorpha	Naticidae	Conuber	*Conuber melanostoma*	186	11	-	163	31	1782	1021	3	2	147	1374	73	11	15
Mollusca	Gastropoda	Littorinimorpha	Truncatellidae	Truncatella	*Truncatella guerinii*	-	-	-	-	-	6	5	-	-	5	6	1	-	-
Mollusca	Gastropoda	Cephalaspidea	Philinoglossidae	Philinoglossa	*Philinoglossa* sp.	-	-	-	-	-	-	-	-	-	-	1	4	-	-
Mollusca	Gastropoda	Cephalaspidea	Aglajidae	Chelidonura	*Chelidonura* sp.	3	-	-	-	-	-	-	-	2	-	350	2	-	-
Mollusca	Gastropoda	Eupulmonata	Otinidae	Otina	*Otina* sp.	-	-	-	-	-	-	-	-	-	-	5	183	3	-
Mollusca	Gastropoda	Eupulmonata	Helicoidea	unclassified	Helicoidea	-	-	-	-	-	-	-	-	-	-	-	25	-	-
Mollusca	Gastropoda	Hygrophila	Lymnaeidae	Lymnaea	*Lymnaea* sp.	-	-	-	-	-	-	-	-	-	-	-	10	-	-
Mollusca	Gastropoda	Sacoglossa	Placobranchidae	Elysia	*Elysia* sp.	-	-	-	-	-	1	-	-	1	-	29	1897	64	-

Phylum	Class	Order	Family	Genus	Species	Number of OTUs													
						DJ	GC	GS	JDW	TY	JDE	SG	MP	JJ	YS	PH	UJ	YY	BS
Mollusca	Gastropoda	Archaeogastropoda	Haliotidae	Haliotis	*Haliotis tuberculata*	-	-	-	-	-	-	-	-	-	1	-	3	-	-
Mollusca	Gastropoda	Archaeogastropoda	Turbinidae	Tricolia	*Tricolia variabilis*	-	-	-	-	-	-	-	-	-	-	-	11	-	-
Nematoda	Chromadorea	Chromadorida	Chromadoridae	Chromadorita	*Chromadorita tentabundum*	-	-	-	-	-	16	-	-	-	-	-	-	-	-
Nematoda	Chromadorea	Chromadorida	Chromadoridae	Ptycholaimellus	*Ptycholaimellus* sp.	-	-	9	15	-	-	-	-	-	-	-	-	-	-
Nematoda	Enoplea	Enoplida	Anoplostomatidae	Anoplostoma	*Anoplostoma* sp	26	-	-	-	-	-	10	24	-	-	-	-	-	-
Nematoda	Enoplea	Enoplida	Enchelidiidae	Bathyeurystomina	*Bathyeurystomina* sp.	4	-	-	-	-	-	-	-	-	-	-	1	-	-
Nematoda	Enoplea	Enoplida	Enchelidiidae	Pareurystomina	*Pareurystomina* sp.	23	-	-	-	-	-	-	-	-	-	-	-	-	-
Nematoda	Enoplea	Enoplida	Oxystominidae	Halalaimus	*Halalaimus* sp.	-	-	-	-	-	-	-	38	-	-	-	-	-	-
Nematoda	Enoplea	Enoplida	Oxystominidae	Oxystomina	*Oxystomina* sp.	-	-	-	-	-	-	-	-	-	-	8	-	-	-
Nematoda	Enoplea	Enoplida	Tripyloididae	Bathylaimus	*Bathylaimus assimilis*	25	-	-	-	-	-	-	12	-	-	-	-	-	-
Nematoda	Chromadorea	Chromadorida	Selachinematidae	Halichoanolaimus	*Halichoanolaimus* sp.	-	-	-	-	-	-	4	-	-	-	-	-	-	-
Nematoda				unclassified	Nematoda	-	-	-	-	-	12	6	-	-	-	-	-	1	-
Platyhelminthes	Turbellaria	Macrostomida	Macrostomidae	Macrostomum	*Macrostomum tuba*	-	-	-	-	-	-	-	19	-	-	-	-	-	-
Platyhelminthes	Turbellaria	Dalyellioida	Dalyelliidae	Microdalyellia	*Microdalyellia rossi*	-	-	14	-	-	-	-	-	-	-	-	-	-	-
Platyhelminthes	Turbellaria	Dalyellioida	Pterastericolidae	Pterastericola	*Pterastericola australis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Platyhelminthes	Turbellaria	Kalyptorhynchia	Polycystididae	Arrawaria	*Arrawaria* sp.	-	-	-	-	-	-	19	-	-	-	-	-	-	-
Platyhelminthes	Turbellaria	Kalyptorhynchia	Polycystididae	Gyratrix	*Gyratrix hermaphroditus*	-	-	-	-	8	-	-	-	-	-	-	-	-	-
Platyhelminthes	Turbellaria	Kalyptorhynchia	Polycystididae	Polycystis	*Polycystis naegelii*	-	-	-	-	-	-	-	-	-	-	-	-	7	-
Platyhelminthes	Turbellaria	Kalyptorhynchia	Polycystididae	Stradorhynchus	*Stradorhynchus* sp.	20	-	-	-	-	-	46	34	-	-	3	-	-	-
Platyhelminthes	Turbellaria	Kalyptorhynchia	Karkinorhynchidae	Cheliplana	*Cheliplana* cf. *orthocirra*	163	-	-	-	557	446	-	-	1	-	-	-	-	-
Platyhelminthes	Turbellaria	Typhloplanoida	Promesostomidae	Promesostoma	*Promesostoma* sp.	-	-	-	-	33	8	-	-	-	-	-	-	-	-

Phylum	**Class**	**Order**	**Family**	**Genus**	**Species**	**Number of OTUs**													
						DJ	**GC**	**GS**	**JDW**	**TY**	**JDE**	**SG**	**MP**	**JJ**	**YS**	**PH**	**UJ**	**YY**	**BS**
Platyhelminthes	Turbellaria	Typhloplanoida	Solenopharyngidae	Trisaccopharynx	*Trisaccopharynx westbladi*	-	-	-	-	-	47	-	-	-	-	-	-	-	-
Platyhelminthes	Turbellaria	Proseriata	Monocelididae	Pseudmonocelis	*Pseudomonocelis ophiocephala*	12	-	-	-	-	-	-	-	-	-	-	-	10	1
Platyhelminthes	Turbellaria	Tricladida	Dendrocoelidae	Dendrocoelopsis	*Dendrocoelopsis lactea*	-	-	-	-	-	-	-	-	-	-	6	-	-	-
Platyhelminthes	Turbellaria	Tricladida	Dugesiidae	Romankenkius	*Romankenkius libidinosus*	-	-	-	-	-	-	-	-	-	-	146	-	-	-
Porifera	Demospongiae	Haplosclerida	Lubomirskiidae	Baikalospongia	*Baikalospongia intermedia*	-	-	-	10	7	-	9	-	-	-	-	-	-	-
Porifera	Demospongiae	Hadromerida	Spirastrellidae	Spheciospongia	*Spheciospongia vesparium*	-	-	-	-	-	-	-	-	-	-	-	-	36	-
Rotifera	Monogononta	Flosculariacea	Flosculariidae	Collotheca	*Collotheca campanulata*	-	-	22	-	-	-	-	-	-	-	-	-	-	-
Rotifera	Monogononta	Flosculariacea	Flosculariidae	Sinantherina	*Sinantherina socialis*	-	-	17	-	-	-	-	-	-	-	-	-	-	-
Rotifera	Monogononta	Ploimida	Lecanidae	Lecane	*Lecane bulla*	28	-	9132	1	1	65	-	-	-	-	-	-	2	-
				unclassified	Metazoan 1	-	-	10	-	-	-	-	-	-	-	-	-	-	-
				unclassified	Metazoan 2	18	-	-	-	-	-	28	25	-	-	-	-	-	-
Total number of OTUs						1229	188	18376	376	658	3143	1255	2299	71	686	3921	2509	137	19

Differences within the Western-type group

According to beta-diversity analysis, PCoA plot and UPGMA tree, SG/MP were clearly distinguished from major western-type group (DJ, GS, GC, JDW, TY) despite of similar habitat, flat mud. There were two large differences between two groups, one was the five species (*Gregarina blattarum*, *Ephelota gemmipara*, *Sorosphaera veronicae*, *Gomphonema micropus*, *Ochromonas danica*) belongs to all protists, that only existed in SG/MP. The other was, a large number of 'unclassified eukaryotes' in both sites (Table 7).

For JDE, which habitat was more likely to represent sandy shore rather than flat mud, shows quite different community structure; *Babesia poelea*, *Rhytidocystis cyamus*, *Monodinium* sp. *Frontonia didieri*, *Nematostelium ovatum*, *Ancyromonas micra* belong to protist, *Pinnularia viridiformis*, *Chaetoceros* sp. unclassified Ascomycota 1 belong to Fungi, *Marionina* coatesae, *Heterocypris* sp., *Cardita leana*, *Chromadorita tentabundum*, *Pterastericola australis*, *Trisaccopharynx westbladi* and landplant (*Zostera marina*) were detected at JDE only compared to the other 13 samples (Table 8).

Table 7. List of species obtained from MP. SG only compare to the others

Kingdom	Phylum	Class	Order	Family	Genus	Species
Protist	Apicomplexa	Conoidasida	Eugregarinida	Gregarinidae	Gregarina	*Gregarina blattarum*
Protist	Ciliophora	Phyllopharynge	Exogenida	Ephelotidae	Ephelota	*Ephelota gemmipara*
Protist	Cercozoa	Phytomyxea	Plasmodiophorida	Plasmodiophoridae	Sorosphaera	*Sorosphaera veronicae*
Protist	Bacillariophyta	Bacillariophyceae	Cymbellales	Gomphonemataceae	Gomphonema	*Gomphonema micropus*
Protist	Ochrophyta	Chrysophyceae	Chromulinales	Chromulinaceae	Ochromonas	*Ochromonas danica*

Table 8. List of species obtained from JDE only compare to the others

Kingdom	Phylum	Class	Order	Family	Genus	Species
Protist	Apicomplexa	Aconoidasida	Piroplasmida	Babesiidae	Babesia	*Babesia poelea*
Protist	Apicomplexa	Conoidasida	Agamococcidiorida	Rhytidocystidae	Rhytidocystis	*Rhytidocystis cyamus*
Protist	Ciliophora	Litostomatea	Haptorida	Didiniidae	Monodinium	*Monodinium* sp.
Protist	Ciliophora	Oligohymenophorea	Peniculida	Frontoniidae	Frontonia	*Frontonia didieri*
Protist	Amoebozoa	Protostelea	Protosteliales	Protosteliaceae	Nematostelium	*Nematostelium ovatum*
Protist	Apusozoa			Ancyromonadidae	Ancyromonas	*Ancyromonas micra*
Fungi	Ascomycota					unclassified Ascomycota
Metazoa	Annelida	Clitellata	Haplotaxida	Enchytraeidae	Marionina	*Marionina coatesae*
Metazoa	Arthropoda	Ostracoda	Podocopida	Cyprididae	Heterocypris	*Heterocypris* sp.
Metazoa	Mollusca	Bivalvia	Veneroida	Carditidae	Cardita	*Cardita leana*
Metazoa	Nematoda	Chromadorea	Chromadorida	Chromadoridae	Chromadorita	*Chromadorita tentabundum*
Metazoa	Platyhelminthes	Turbellaria	Dalyellioida	Pterastericolidae	Pterastericola	*Pterastericola australis*
Metazoa	Platyhelminthes	Turbellaria	Typhloplanoida	Solenopharyngidae	Trisaccopharynx	*Trisaccopharynx westbladi*
Rhodophyta	Rhodellophyceae	Glaucosphaerales	Glaucosphaeraceae	Glaucosphaera	Glaucosphaera vacuolata	
Plantae	Tracheophyta	Monocots	Alismatales	Zosteraceae	Zostera	*Zostera marina*
Protist	Bacillariophyta	Bacillariophyceae	Naviculales	Pinnulariaceae	Pinnularia	*Pinnularia viridiformis*
Protist	Bacillariophyta	Mediophyceae	Chaetocerotales	Chaetocerotaceae	Chaetoceros	*Chaetoceros* sp.

Discussion

Tool for unraveling undescribed species; comparison with previous study of top five Metazoa

Nematoda

The phylum Nematoda, consisting of about 25,000 described species, is one of the most diverse groups of animal kingdom, but it is often difficult to identify them accurately due to microscopic size.

Since Rho & Kim (2004a) described the first Draconematidae (*Tenuidraconema koreense*) from Korea, four unrecorded species (Rho & Kim 2004b; Lim & Chang 2006; Rho *et al*. 2006) and thirty-six new species (Rho & Kim 2005a;2005b;2005c; Rho & Kim 2006; Rho *et al*. 2007; Rho *et al*. 2010; Rho *et al*. 2011) were reported (Table 9). However, taxonomic study of Nematoda in Korea was highly focus on family Draconematidae (85%, 34 out of 40 species). In addition, draconematids are mostly collected from 50 m - 200 m water depth with relatively coarse sediments by SCUBA diving. In this study, six novel families were obtained from intertidal zone (usually fine mud); Chromadoridae, Anoplostomatidae, Enchelidiida, Oxystominidae, Tripyloidae, Selachinematidae which had not been reported yet.

In previous study of metagenomics on nematodes, the suitability of high-throughput sequencing for assessing soil nematode diversity has been demonstrated (Porazinska *et al*. 2010a; Porazinska *et al*. 2010b). For this reason, metagenomic approaches for discovering potential undescribed nematodes are seem to be quite useful as a preliminary study.

Table 9. Comparison with previous work on Nematoda

This study			previous study (Rho, H. S. personal communication)		
Chromadoridae (new)	*Chromadorita tentabundum* *Ptycholaimellus* sp.	2	Comesomatidae	*Cervonema pseudodeltensis* *Laimella socotris* *Laimella tongyeongensis*	4
Anoplostomatidae (new)	*Anoplostoma* sp.	1	Desmoscolecidae	*Desmoscolex cosmopolites* *Desmoscolex coreensis* *Desmoscolex pedunculus*	3
Enchelidiidae (new)	*Bathyeurystomina* sp. *Pareurystomina* sp.	2	Draconematidae	*Draconema hoonsooi* *Draconema japonicum* *Draconema youngeouni* *Dracogalerus koreanus* *Dracograllus jongmooni* *Dracograllus brevitubulus* *Dracograllus filipjevi* *Dracograllus gosanensis* *Dracograllus geomunensis* *Dracograllus jaewani* *Dracograllus sungjooni* *Dinetia orientalis* *Dinetia decraemerae* *Dinetia donghaeense* *Megadraconema cornutum* *Notochaetosoma namaeense* *Notochaetosoma jeodongense* *Notochaetosoma commensalis* *Paradraconema floridense* *Paradraconema jejuense* *Paradraconema tamraense* *Prochaetosoma dokdoense* *Prochaetosoma cracense* *Prochaetosoma saheungi* *Prochaetosoma sujungi* *Prochaetosoma supseomense* *Prochaetosoma youngdeokense* *Prochaetosoma beomseomense* *Prochaetosoma brevicaudatum* *Prochaetosoma byungilli* *Tenuidraconema koreense* *Tenuidraconema seongsanensis* *Tenuidraconema imwonensis*	33
Oxystominidae (new)	*Halalaimus* sp. *Oxystomina* sp.	2			
Tripyloidae (new)	*Bathylaimus assimilis*	1			
Selachinematidae (new)	*Halichoanolaimus* sp.	1			

Annelida

The phylum Annelida, generally divided into two classes; Clitellata (mostly freshwater), Polychaeta (mostly marine), approximately 9,000 species are known in worldwide and this study focused on marine taxon, Polychaeta. Although they have great abundance in diversity, polychaetes are easily broken and damaged during the process of sampling, making them difficult to morphologically identify. Historically, three species (*Diopatra neapolitana*, *Ceratocephale osawai*, *Sternaspis costata*) reported by Kamita & Sato (1941) were considered as a first record of Korean polychaetes. Since then, Paik (1989) reviewed 265 species, and about 300 polychaete species are reported from Korea so far. However, due to its similar morphology and limited information (by damage) of polychaetes, the number of polychaete species is unclear. Hence, there was a large difference between recorded species; 593 species were reported from the organization of three DBs (Marine Biodiversity Institute of korea (MABIK) DB, Korea Marine Biodiversity Information System (KOMBIS) DB, and Marine BioResources Bank (MRBR) DB), which is more than double from previous taxonomy reports (Table 10). By accumulating of well-designed high-throughput data, it can give us a new insight of estimating the true number of species.

In this study, four clitellates and fifteen polychates family were obtained.

Comparison to integrated DB, family Enchytraeidae, Propappidae, Tubificidae in clitellates and family Protodrilidae, Saccocirridae, Potamodrilidae in polychaetes were novel.

Table 10. Comparison with previous work on Annelida

This study				(integrated MABIK, KOMBIS, MRBR DBs)
Clitellata	Enchytraeidae	4	*Grania variochaeta* *Haplotaxis* cf. *gordioides* *Marionina coatesae* *Mesenchytraeus solifugus*	(new)
	Naididae	1	*Stylaria* sp.	1
	Propappidae	1	*Propappus volki*	(new)
	Tubificidae	5	*Doliodrilus chinensis* *Limnodrilus hoffmeisteri* *Monopylephorus rubroniveus* *Olavius algarvensis* *Tubifex tubifex*	(new)
Polychaeta	Protodrilidae	1	*Protodrilus purpureus*	(new)
	Saccocirridae	1	*Saccocirrus papillocercus*	(new)
	Sabellidae	1	*Amphicorina mobilis*	16
	Nereididae	3	*Ceratocephale loveni* *Ceratonereis erythraeenis* *Platynereis dumerilii*	14
	Phyllodocidae	1	*Eteone longa*	20
	Arenicolidae	2	*Abarenicola affinis* *Arenicola brasiliensis*	6
	Capitellidae	3	*Capitella* sp. *Dasybranchus caducus* *Notomastus tenuis*	6
	Maldanidae	1	*Maldane sarsi*	34
	Opheliidae	1	*Euzonus ezoensis*	13
	Paraonidae	1	*Paraonis* sp.	33
	Potamodrilidae	1	*Potamodrilus fluviatilis*	(new)
	Cirratulidae	1	*Cirratulus spectabilis*	10
	Spionidae	6	*Asetocalamyzas laonicola* *Aurospio dibranchiata* *Boccardiella ligerica* *Polydora giardi* *Prionospio dubia* *Prionospio ehlersi*	71
	Pectinariidae	1	*Pectinaria gouldii*	4
	Terebellidae	1	*Polycirrus* sp.	23

Arthropoda

The phylum Arthropoda is known as the largest and the most successful group of the animal kingdom. Eight families of Maxillopoda (Acartiidae, Candaciidae, Centropagidae, Pontellidae, Ameiridae, Canthocamptidae, Dirivultidae) and five families of Ostracoda (Cyclocyprididae, Cyprididae, Cytheridae, Leptocytheridae, Loxoconchidae) all belonging to Crustacea are detected in present study (Table 11). Of these, four species of four families (Dirivultidae, Cyclocyprididae, Cytheridae, Loxoconchidae) are expected to be candidate undescribed species in Korea. All detected species were belong to relatively small size (<20 mm) groups in crustacean, such as calanoids, harpacticoids, siphonostomatoids, and podocopids. However, Some dominant taxa were not obtained in this analysis; mysids, amphipods. A possible cause of these problems is the primer used in this study may not effective to these groups, therefore application of alternative 18S rDNA primer combination or other genetic region might be appropriate. As a good candidate, mitochondrial cytochrome c oxidase subunit I (COI) is one of the most common molecular marker for metazoan and shows high suitability in not only individual DNA barcoding but also targeted-metagenetic analysis of Arthropods (Hajibabaei *et al.* 2011; Yu *et al.* 2012; Carew *et al.* 2013; Hirai *et al.* 2015).

Table 11. Comparison with previous work on Arthropoda

This study				(integrated MABIK, KOMBIS, MRBR DBs)
Maxillopoda	Acartiidae	1	*Acartia pacifica*	32
	Candaciidae	1	*Candacia truncata*	11
	Centropagidae	2	*Centropages typicus* *Sinocalanus tenellus*	22
	Clausocalanidae	1	*Clausocalanus furcatus*	12
	Pontellidae	2	*Labidocera euchaeta* *Pontella fera*	21
	Ameiridae	1	*Argestigens* sp.	16
	Canthocamptidae	1	*Itunella muelleri*	9
	Dirivultidae	1	*Aphotopontius mammillatus*	(new)
Ostracoda	Cyclocyprididae	1	*Dolerocypria* sp.	(new)
	Cyprididae	1	*Heterocypris* sp.	6
	Cytheridae	1	*Cythere* sp.	(new)
	Leptocytheridae	1	*Leptocythere* sp.	1
	Loxoconchidae	1	*Cytheromorpha* sp.	(new)

Other metazoan phyla (Mollusca, Platyhelminthes)

The phylum Mollusca, which is extraordinarily varied, about more than 80,000 species were described. Nine families (Laternulidae, Cardiidae, Carditidae, Tellinidae, Glauconomidae, Veneridae, Mytilidae, Ostreidae, Pectinidae) belong to Bivalvia, and eleven families (Viviparidae, Calyptraeidae, Naticidae, Truncatellidae, Philinoglossidae, Aglajidae, Otinidae, Lymnaeidae, Placobranchidae, Haliotidae, Turbinidae) belong to Gastropoda were observed in this study. Of these, four families Philinoglossidae, Otinidae, Lymnaeidae, Placobranchidae were novel (Table 12). According to catalogue of molluscan fauna published by Min *et al*. 2004, 1,681 species were listed, and integrated DB had 2,213 species. Molluscs, especially bivalves, have long been considered as good bioindicators due to their feeding behavior, which tend to accumulate heavy metals (Liang *et al*. 2004; Wang *et al*. 2005). Several high-throughput studies used to characterize micro-organism communities from internal organs (stomach and gut; King *et al*. 2012) to external environmental condition (Wegner *et al*. 2013; Chauhan *et al*. 2014; Fernández *et al*. 2014).

Platyhelminthes, commonly known as flatworm, containing about 20,000 species. The study on Platyhelminthes is poorly studied in Korea, often difficult to identify due to its relatively simple morphology. There was no common family between this study and integrated DB. All detected families were novel; Macrostomidae, Dalyelliidae, Pterastericolidae, Polycystididae, Karkinorhynchidae, Promesostomidae, Solenopharyngidae, Monocelididae, Dendrocoelidae, Dugesiidae. These discordant data suggest that the group of Platyhelminthes need further careful study (Table 13).

Table 12. Comparison with previous work on Mollusca

This study				(integrated MABIK, KOMBIS, MRBR DBs)
Bivalvia	Laternulidae	2	*Laternula anatina* *Laternula creccina*	6
	Cardiidae	1	*Parvicardium exiguum*	22
	Carditidae	1	*Cardita leana*	14
	Tellinidae	1	*Macoma balthica*	46
	Glauconomidae	1	*Glauconome virens*	2
	Veneridae	1	*Ruditapes philippinarum*	75
	Mytilidae	2	*Musculus discors* *Leiosolenus lithurus*	59
	Ostreidae	1	*Ostrea edulis*	15
	Pectinidae	1	*Pecten maximus*	17
Gastropoda	Viviparidae	1	*Viviparus georgianus*	1
	Calyptraeidae	1	*Crepidula fornicata*	12
	Naticidae	1	*Conuber melanostoma*	50
	Truncatellidae	1	*Truncatella guerinii*	2
	Philinoglossidae	1	*Philinoglossa* sp.	(new)
	Aglajidae	1	*Chelidonura* sp.	3
	Otinidae	1	*Otina* sp.	(new)
	Lymnaeidae	1	*Lymnaea* sp.	(new)
	Placobranchidae	1	*Elysia* sp.	(new)
	Haliotidae	1	*Haliotis tuberculata*	12
	Turbinidae	1	*Tricolia variabilis*	25

Table 13. Comparison with previous work on Platyhelminthes

This study			(integrated MABIK, KOMBIS, MRBR DBs)	
Macrostomidae (new)	1	*Macrostomum tuba*	Anoplodiscidae	*Anoplodiscus spari*
Dalyelliidae (new)	1	*Microdalyellia rossi*	Anoplodiscidae	*Anoplodiscus tai*
Pterastericolidae (new)	1	*Pterastericola australis*	Capsalidae	*Benedenia sekii*
Polycystididae (new)	4	*Arrawaria* sp. *Gyratrix hermaphroditus* *Polycystis naegelii* *Stradorhynchus* sp.	Microcotylidae	*Bivagina tai*
Karkinorhynchidae (new)	1	*Cheliplana* cf. *orthocirra*	Diclidophoridae	*Choricotyle elongata*
Promesostomidae (new)	1	*Promesostoma* sp.	Udonellidae	*Udonella fugu*
Solenopharyngidae (new)	1	*Trisaccopharynx westbladi*	Notoplanidae	*Notocomplana humilis*
Monocelididae (new)	1	*Pseudomonocelis ophiocephala*		
Dendrocoelidae (new)	1	*Dendrocoelopsis lactea*		
Dugesiidae (new)	1	*Romankenkius libidinosus*		

Tool for unraveling undescribed species; comparison with previous study of top five Metazoa (continued)

We compared species list of top five metazoan (Nematoda, Annelida, Arthropoda, Mollusca, Platyhelminthes) phylum between the results of present study and organized three (MABIK, KOMBIS, MRBR) DBs. Only phylum Nematoda was treated individually, since its recent study has not yet been updated on the three DBs. Removing the duplicates were performed, however, information of synonymized species needs to be eliminated continuously to establish reliable database.

It is difficult to measuring the true number of evolutionary species and understanding their community structures in certain area. Not only limitation of method but also cascading error of misidentified taxonomy could cause a negative impact on true mass of biodiversity (Bortolus 2008). Likewise, there were big gap between species number of previous taxonomy reports (approximately 300 species) and three integrated DB (594 species) in phylum Annelida, which needs to be organized to reduce the gap. In other words, the classical method provides limited view of biodiversity and further study will be needed in a different way, such as constructing of metagenomic sequence library. In conclusion, metagenomics is one of potential keys that leads us to deeper understand of broad-scale marine biodiversity as a new tool.

It is ideal for use of 99% threshold similarity to identify species, however, could give rise to overestimate of OTUs, like artificial sequences. Previous study supported that 97% similarity threshold provides reliable resolution of eukaryotic 18S rDNA (Ďıez *et al.* 2001, Fierer *et al.* 2007, Porazinska *et al.* 2009) and reduces possible for artificial inflation (Kunin *et al.* 2010).

Metagenomics as tool for biomonitoring

More than 97% of species on Earth animals are invertebrate (Buchsbaum, 2013). Marine invertebrates, as they are widespread, with high diversity and varying sensitivity to environmental disturbance, they are useful for evaluating ecosystem health (Hodkinson *et al.* 2005). However, traditional taxonomic framework based on morphological characteristic is too laborious, costly and time-consuming to apply upon a routine biomonitoring. Metagenomics are expected to solve these problems and they also have advantages in environment-friendly process that need only few materials.

Certain biomass of marine invertebrates is useful for biomonitoring tools, such as ratio of Copepods/Nematodes (Raffaelli & Mason 1981, Warwick 1981), polychaetes, and also their deep relatedness of organic enrichment (Molluscs, Gray *et al.* 2002; Nematodes, Nillson and Rosenberg 2000) was suggested. Although, in metagenomic approach, the number of OTUs is not perfectly reflect the proportion of species, but somehow, positively related each other (Porazinska *et al.* 2009).

Although we focused on Metazoa, metagenomic analysis on phytoplankton using pyrosequencing had already performed several times in Korea as biomonitoring tool (Faria *et al.* 2014, Boophathi *et al.* 2015). For developing metagenomic analysis utility as biomonitoring tool, various conditions such as concentration of heavy metals, TOC (total organic carbon), pH concentration would be undertaken together.

Linking community patterns to habitat

Eastern-type group and western-type group differed in terms of physical habitat and relative composition of communities, suggesting that community pattern is more influenced by similarity of habitat rather than geological distance. Small invertebrate fauna are tend to be more exist in mud flat habitat (western-type group), while protists/algae covers a large proportion of the rocky-shore habitat (eastern-type group). Based on these results, different strategy would be needed for more close-up study.

The more interesting result is that all sequences from Eastern-type group clustered together, however, MP/SG (minor western-type group) was distinct from the clusters formed by major western-type group. We could not figure out the exact explanation based on datasets, since the large amount of sequences of MP/SG fell into ‘unclassified eukaryotes’. One of possible reason is that their local characteristic. MP is a city located on the South-west coast of Korea that served as a crucial port for international shipping and commercial transportation. As an industrial and port city, Such anthropogenic activities conditions, somehow, might effect to differences.

In conclusion, the composition of community is seemed to be largely affected by the habitat, however, it should be also noted that the same habitat does not guarantee the same community structure.

To get more reliable results, future high-throughput studies will additionally need to 1) survey more detailed sampling to clarify the variation of community structure across marine ecosystems. In addition 2) accuracy would be expected to increase with taxon-targeted way. Also 3) choice of primer sets may have strong influence on study, using other primer sets or 4) relatively long DNA fragments such as COI, LSU will be needed for cross-check.

Conclusion

Metagenomic analysis of multiple taxa on eukaryotes were tested, for the first time in Korea. Although our sample set was limited to fourteen sites, we could found meaningful taxa potentially represent novel families from top five Metazoa (Nematoda; 100% (6 families), Annelida; 31.6% (6 families), Arthropoda; 30.8% (4 families), Mollusca; 20% (4 families), Platyhelminthes; 100% (10 families)). This makes it possible to determine the priorities of research by revealing not only novel groups but also data-poor taxonomic groups. Moreover, it can also work as reference database to estimate true number of species by well-accumulating high-throughput sequencing data through further study. Alpha and beta diversity were analyzed to understand relationship between habitat and eukaryotes community structure. As a results, two major habitat groups were divided into four clustered group based on different community composition.

Two major conclusions can be drawn from this study: First, high-throughput analysis as tool of unraveling the undescribed species is feasible. Second, the community structure is more likely to be influenced by habitat rather than geological distance, however, the similar habitat does not always promising the similar community structure.

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국문 초록

여러 진핵 분류군을 대상으로 메타지놈 분석을 이용한 연구가 국내에서 처음으로 수행되었다. 각각의 샘플은 제주와 한반도를 둘러싼 동해, 서해 및 남해 14지역에서 2015년 6월 사이 채집되었다. 14 샘플은 모두 63-㎛ 시브로 걸러졌으며, 그 후 18S rDNA V4 유전자 부위를 증폭 시 Illumina Miseq을 이용하여 시퀀싱이 진행되었다. Paired-end 기법으로 읽혀진 시퀀스는 샘플 당 25,637-38,463 리드수로, 평균 230 개의 unique OTU (97% 유사도 이용)가 발견되었다. 가장 많은 unique OTU를 보유한 지역은 포항으로 334 개 였고, 가장 적은 지역은 고창으로 49 개 였다. 총 319 개의 OTU는 SILVA 데이터베이스를 기준으로 BLAST를 이용하여 종 수준까지 동정되었다. 동물 문에서는 103 종 101 속 75 과에 속하는 10 개의 문이 탐지되었으며, 탐지된 동물 문 중 환형동물 문, 절지동물 문, 연체동물 문, 선형동물 문, 편형동물 문에 해당되는 시퀀스가 전체에서 92.23%를 차지했다. 이 중 세 기관(MABIK, KOMBIS, MRBR)의 통합된 데이터베이스와 비교하였을 때, 과(family) 수준에서 다음과 같은 미기록 분류군이 탐지됐다. 선충동물 문 6 과 (100%) 환형동물 문 6 과 (31.6%), 절지동물 문 4 과 (30.8%), 연체동물 문 4 과 (20%), 편형동물 문 10 과 (100%)로 미기록 종의 가능성을 제시하였다. 또한, 진핵생물의 군집구조와 서식지의 상관관계를 보기 위한 베타-다양성이 PCoA plot과 UPGMA tree을 통해 분석되었다. 그 결과, 서식지가 군집구조에 큰 영향을 미치지만, 서식지가 같다고 해서 반드시 같은 군집구조를 갖는 건 아닌 것으로 나타났다.

주요어: 메타지놈 분석, 해양 연안 무척추동물, Illumina Miseq, 차세대 염기서열 기술, 18s rDNA V4, 생물다양성

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