

Illumina MiSeq Analysis and Comparison of Freshwater Microalgal Communities on Ulleungdo and Dokdo Islands

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Abstract

Ulleungdo and Dokdo are volcanic islands with an oceanic climate located off the eastern coast of South Korea. In the present study, we used barcoded Illumina MiSeq to analyze eukaryotic microalgal genera collected from Seonginbong, the highest peak on Ulleungdo, and from groundwater sites on Dongdo and Seodo Islands, which are part of Dokdo. Species richness was significantly greater in the Seonginbong samples than in the Dongdo and Seodo samples, with 834 operational taxonomic units (OTUs) identified from Seonginbong compared with 203 OTUs and 182 OTUs from Dongdo and Seodo, respectively. Taxonomic composition analysis was also used to identify the dominant microalgal phyla at each of the three sites, with Chlorophyta (green algae) the most abundant phyla on Seonginbong and Dongdo, and Bacillariophyta (diatoms) the most abundant on Seodo. These findings suggest that differences in the abundances of Chlorophyta and Bacillariophyta species in the Seonginbong, Dongdo, and Seodo samples are due to variations in species richness and freshwater resources at each sampling location. To the best of our knowledge, this is the first report to detail freshwater microalgal communities on Ulleungdo and Dokdo. As such, the number of species identified in the Seonginbong, Dongdo, and Seodo samples might be an indicator of the ecological differences among these sites and varying characteristics of their microbial communities. Information regarding the microalgal communities also provides a basis for understanding the ecological interactions between microalgae species and other eukaryotic microorganisms.

Key words: amplicon sequencing, Dokdo Island, microalgal community, MiSeq system, Ulleungdo Island

Introduction

Ulleungdo and Dokdo, located to the east of the Korean peninsula, are volcanic islands formed by the lava flows resulting from volcanic activity. Ulleungdo consists of one main island, with Seonginbong as its highest peak, and several small islets. Dokdo comprises two major islets, Dongdo and Seodo, and several exposed rocks (Sohn 1995; Kim et al. 2013). Ulleungdo and Dokdo share an oceanic climate due to the influence of warm and cold currents (Chang et al. 2002; Lee et al. 2010), although average annual precipitation is higher on Ulleungdo (1574 mm) than on Dokdo (660 mm). Annual average temperatures of both islands range from 12°C to 14°C (Chang et al. 2002; Lee et al. 2010). These islands are characterized by steep slopes

that facilitate significant surface runoff when it rains, and it is thereby difficult for rainwater to collect on the surface. Indeed, volcanic islands formed from the lava are often characterized by a water-deficient environment. However, Ulleungdo and Dokdo have springs or small streams that originate from the groundwater to create an environment wherein fresh surface water is available (Sohn 1995; Chang et al. 2002).

The uneven distribution of freshwater sources influences the overall vegetation community and its successional processes. Ulleungdo, due to its relatively high precipitation, has greater vegetation species richness, with 487 vascular plants species and 104 woody plant species, than Dokdo, with 46 vascular plant species and eight woody plant species (Shin et al. 2004; Kim et al. 2007; Park et al. 2010), indicating that Ulleungdo is at

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a more advanced successional stage than Dokdo (Kim et al. 2007; Park et al. 2010; Jung et al. 2014). These patterns also extend to the microbial ecosystems, meaning that the different environments of Ulleungdo and Dokdo affect their microbial communities (Busse et al. 2006; Han et al. 2007; Djukic et al. 2010; Merilä et al. 2010). However, previous studies on the microbial communities on these islands have focused on the fungal and bacterial complements thereof (Kim et al. 2014; Nam et al. 2015), and little is known about the microalgal constituent. The discovery of new microalgal species is important in terms of the use of the algal biomass as a biological resource under different environmental conditions (Krustok et al. 2015).

Microalgae participate in carbon, nitrogen, and phosphorus cycles (Lehman 1980; Berner 1992; Vitousek et al. 2002) and, as photosynthetic organisms, are key producers and pioneers across a range of ecosystems (Booth 1941; Jackson 1971; Bellinzoni et al. 2003). In early successional stages, microalgae are the predominant production group, facilitating the subsequent arrival of herbaceous and woody plants, which can grow in the fertilized environment created by the microalgae (Booth 1941; Jackson 1971; Bellinzoni et al. 2003). The microalgal group promotes successional vegetation processes and allows for the emergence of predators and pathogenic microbes. The former mainly comprises zooplankton such as nematodes and arthropods (Havens and DeCosta 1987; Canovas et al. 1996; Mayer et al. 1997), while the latter causes disease in plants and animals and inhibits the biodegradation capacity of microbes (Littler and Littler 1998; Chen et al. 2014).

Interactions between microalgae and their abiotic and biotic environments drive the evolution of the microalgal community. Species dominance depends on environmental conditions, such as inorganic nutrient composition, water temperature, and light (Prowse and Talling 1958; Goldman and Shapiro 1973; Porter 1977). In particular, microalgae composition is dominated by large-cell and needle-type algae, which are difficult to prey. Because the microalgal community supports the ecosystem and serves the producer-consumer relationship, analysis of this community can improve our understanding of the local environment, elemental recycling (carbon, nitrogen, and phosphorus), and micro-ecosystem relationships between producer and consumer trophic levels (Berner 1992; Vitousek et al. 2002; Cardinale et al. 2011). However, microalgal community research based solely on the culturing faces certain limitations, particularly the difficulty in identifying and analyzing unculturable microorganisms (Handelsman 2004; Streit and Schmitz 2004). Consequently, amplicon sequencing analysis using Illumina MiSeq can be a powerful tool for the investigation of unculturable microorganisms in their natural environment

(Knight 2000; Handelsman 2004; Streit and Schmitz 2004; Schloss and Handelsman 2005).

Previous studies have yet to analyze the microalgal communities in the freshwater ecosystems on Ulleungdo (Seonginbong) and Dokdo (Dongdo and Seodo). This study investigated eukaryotic microalgal communities on these islands by taking freshwater samples from groundwater and tributary streams for the Illumina MiSeq analysis. Illumina MiSeq allows a large amount of sequencing information to be processed in a short time, and taxonomic analyses can then be conducted based on this information (Handelsman 2004; Streit and Schmitz 2004; Buée et al. 2009; Shokralla et al. 2012). In this study, microalgal species richness and diversity were characterized using taxonomic analysis, revealing that the composition of these communities varied by region, from phylum to species units.

Experimental

Materials and Methods

Collection of samples. Freshwater samples were collected from freshwater sources on Seonginbong (37° 30' 05.9" N 130° 52' 04.9" E) in Buk-myeon, Ulleung-gun, Gyeongsangbuk-do, South Korea, and on Dongdo (37° 14' 21.0" N 131° 52' 10.4" E) and Seodo Islands (37° 14' 31.5" N 131° 51' 51.6" E) in Dokdo-ri, Ulleung-gun, Gyeongsangbuk-do, South Korea (Supplemental Fig. S1). Seonginbong is the highest peak on Ulleungdo, and tributaries flow from here to freshwater sources. Freshwater sources are rare on Dokdo because of smaller volumes of groundwater, with only one groundwater source each on Dongdo and Seodo. Freshwater resources were harvested by collecting 100 ml from the water surfaces at each site on October 3, 2018. The collected samples were shipped to Macrogen Co., Ltd. on October 3, 2018, using the Same Day Express Courier Service and analyzed while maintained at room temperature.

DNA extraction and MiSeq system analysis. MiSeq system analysis (Macrogen, Seoul, South Korea) involved amplicon sequencing of whole DNA, with DNA extracted by the PowerSoil® DNA Isolation Kit (Cat. No. 12888, MO BIO) according to the manufacturer's protocol (Claassen et al. 2013). Extracted DNA was amplified with PCR to assess the 18S region for identifying eukaryotic microorganisms. Each sequenced sample was prepared according to the Illumina 18S MiSeq System Library protocols (Vo and Jedlicka 2014). DNA quantification and quality measurements were conducted using PicoGreen and Nanodrop. The 18S rRNA genes were amplified using 18S V4 primers (Stoeck et al. 2010; Luddington et al. 2012; Tragin et al. 2018). The amplicon PCR forward primer

sequence was TAREuk454FWD1 (5'-CCAGCA(G-C)C(C-T)GCGGTAATTCC-3'), and the amplicon PCR reverse primer sequence was TAREukREV3 (5'-ACT-TTCGTTCTTGAT(C-T)(A-G)A-3') (Stoeck et al. 2010). Input gDNA was amplified using targeted DNA fragments (18S V4 primers size, 420 bp), and subsequent limited-cycle amplification was conducted to add multiplexing indices and Illumina sequencing adapters (Meyer and Kircher 2010). The final products were normalized and pooled using PicoGreen, and the sizes of the libraries were verified using the TapeStation DNA D1000 ScreenTape system (Agilent). The Illumina MiSeq data was analyzed on the MiSeq™ platform (Illumina, San Diego, USA; Kozich et al. 2013).

Taxonomic identification analysis. After sequencing, the Illumina MiSeq data were demultiplexed using the index sequence, and a FASTQ file was generated for each sample. The adapter sequence was removed using SeqPurge (Sturm et al. 2016), and error correction was performed on the overlapping areas of the two readings, with low-quality barcode sequences (read length <400 bp or average quality value <25) trimmed and filtered out. All raw Illumina MiSeq reads were identified using a BLASTN search of the NCBI database based on their barcode sequences (Zhang et al. 2000). If the results could not be taxonomically classified into a sublevel, unclassified (uc) was added to the end of the name. Operational taxonomic units (OTUs) were analyzed using CD-HIT at a 97% sequence similarity threshold (Unno et al. 2010; Li et al. 2012; Chen et al. 2013). The mothur platform was used to calculate rarefaction curves and diversity indices (Shannon, Simpson, and Chao1; Heck et al. 1975; Schloss et al. 2009). Beta diversity, which refers to sample diversity information among samples in a comparison group, was obtained based on weighted UniFrac distances. A UPGMA tree was used to visualize the flexibility between samples (FigTree, <http://tree.bio.ed.ac.uk/software/figtree/>) and demonstrate relationships among the three sites.

Results and Discussion

Sequencing results analysis. Table I presents the total number of reads and OTUs obtained from the three study sites. A total of 580 853 reads were sequenced from Seonginbong, with 290 919 validated reads remaining after preprocessing. The mean read length was 408.1 bp, and the maximum read length was 418 bp. A total of 534 141 reads were sequenced from Dongdo, with 289 610 validated reads remaining after preprocessing. The mean read length was 416.7 bp, and the maximum read length was 418 bp. A total of 469 920 reads were sequenced from Seodo, and the number of validated reads after preprocessing was 275 387. The mean read

Table I
Illumina MiSeq results for the operational taxonomic units (OTUs) and statistical analysis.

| | Seonginbong | Dongdo | Seodo |
|-----------------------------|-------------|---------|---------|
| Total reads | 580 853 | 534 141 | 469 920 |
| Validated reads | 290 919 | 289 610 | 275 387 |
| Mean read length (bp) | 408.1 | 416.7 | 412.54 |
| Maximum read length (bp) | 418 | 418 | 408 |
| Number of OTUs ¹ | 834 | 203 | 182 |
| Chao1 ² | 834 | 203.75 | 182 |
| Shannon ³ | 6.722 | 2.038 | 5.118 |
| Simpson ⁴ | 0.9655 | 0.5569 | 0.9174 |
| Goods Coverage ⁵ | 1 | 0.9999 | 1 |

¹OTUs: Operational taxonomic units

²Chao1: Species richness estimation

³Shannon: Shannon diversity index (>0, higher is more diverse)

⁴Simpson: Simpson diversity index (0–1, 1 = most simple)

⁵Goods Coverage: 1 – (number of singleton OTUs/number of sequences); 1 = 100% coverage

length was 412.54 bp, and the maximum read length was 418 bp. As seen in Table I, the Seonginbong sample contained the highest number of OTUs with 834 units, while the Dongdo and Seodo samples contained fewer OTUs at 203 and 182 units, respectively.

The species richness of the samples is represented by rarefaction curves in Fig. 1, while the Chao1 species richness, the Shannon diversity index, and the Simpson diversity index are summarized in Table I (Heck et al. 1975; Schloss et al. 2009). The Seonginbong sample had the greatest species richness for all indicators (Chao1: 934; Shannon: 6.7222; Simpson: 0.9655), while Dongdo and Seodo had similar results to one another for Chao1 (203.75 and 182, respectively). However, Seodo had Shannon and Simpson index scores (5.118 and 0.9174, respectively) that were similar to those at Seonginbong (6.722 and 0.9655, respectively), and much higher than those of Dongdo (2.038 and 0.5569, respectively). Based on the OTU and species richness results, the diversity of the eukaryotic microbial composition on Seonginbong appeared to be greater than on Dongdo and Seodo (Fig. 1 and Table I). These results confirmed differences in species diversity among Seonginbong, Dongdo, and Seodo.

Analysis of the eukaryotic microbial communities on Seonginbong, Dongdo, and Seodo. After a BLASTN search of the NCBI database, the validated reads in Table I were assigned to a eukaryotic microbial taxonomic group (Table II; Niu et al. 2010). When a BLASTN search generated a specific scientific name with regards to phylum, class, order, family, genus, or species, the OTU was labeled as classified (c); if not, it was labeled as unclassified (uc). Table II summarizes the number of classified and unclassified OTUs

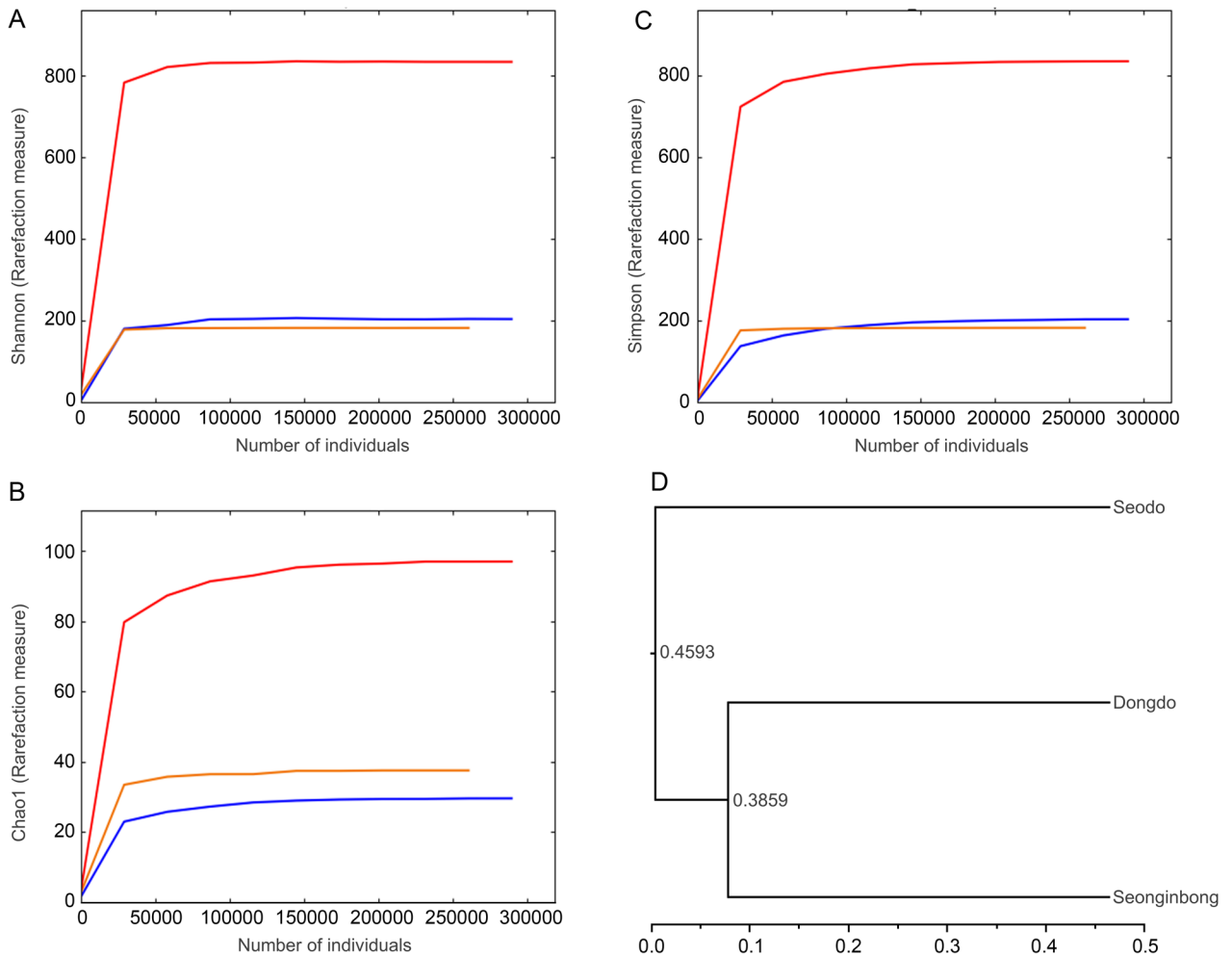


Fig. 1. Rarefaction curves for operational taxonomic units (OTUs) from the Seonginbong, Dongdo, and Seodo samples. (a) Shannon, (b) Simpson, and (c) Chao1 indexes. (d) UPGMA tree based on the community structures of Seonginbong, Dongdo, and Seodo. Seonginbong (red line), Dongdo (blue line), and Seodo (orange line).

from phylum to species for the Seonginbong, Dongdo, and Seodo samples. For the Seonginbong, Dongdo, and Seodo samples, 165 646, 30 911, and 164 678 reads were classified, and 125 273, 258 699, and 110 709 reads were unclassified at the phylum level, respectively. At

the class level, 128 160, 23 011, and 144 662 reads were classified, and 162 759, 266 599, and 130 725 reads were unclassified for the Seonginbong Dongdo and Seodo regions respectively. In addition 99 964, 22 791, and 123 329 reads, respectively, were classified at the order

Table II
Number of eukaryotic microalgal taxa observed in the Seonginbong, Dongdo, and Seodo samples.

| | Seonginbong | | Dongdo | | Seodo | |
|---------|----------------|-----------------|----------------|-----------------|----------------|-----------------|
| | c ¹ | uc ² | c ¹ | uc ² | c ¹ | uc ² |
| Phylum | 165 646 | 125 273 | 30 911 | 258 699 | 164 678 | 110 709 |
| Class | 128 160 | 162 759 | 23 011 | 266 599 | 144 662 | 130 725 |
| Order | 99 964 | 190 955 | 22 791 | 266 819 | 123 329 | 152 058 |
| Family | 96 751 | 194 168 | 22 751 | 266 859 | 120 206 | 155 181 |
| Genus | 92 628 | 198 291 | 22 707 | 266 903 | 110 674 | 164 713 |
| Species | 84 154 | 206 765 | 17 930 | 271 680 | 97 541 | 177 846 |

¹Number of sequencing reads with a scientific name for the taxon (classified, *c*)

²Number of sequencing reads either unclassified into a sublevel or classified as an unknown name for the taxon (unclassified, *uc*)

level. Similarly, 96 751, 22 751, and 120 206 reads were classified at the family level, and 92 628, 22 707, and 110 674 reads were classified at the genus level. Only 84 154, 17 930, and 97 541 sequences were classified at the species level. The number of validated reads was lower than the number of total reads because of the lack of information on unculturable microorganisms in the NCBI database. Therefore, the total reads and validated reads were both utilized for microorganism classification from the phylum to species level. Total reads and validated reads at the species level could be classified using information about their taxonomic levels, such as phylum, class, order, family, and genus.

The taxonomic compositions of the eukaryotic microbial communities on Seonginbong, Dongdo, and Seodo were then analyzed. It was found that the communities contained a combination of 17 phyla: Xanthophyceae, Streptophyta, Rotifera, Porifera, Platyhelminthes, Nematoda, Eustigmatophyceae, Chytridiomycota, Chordata, Chlorophyta, Blastocladiomycota, Basidiomycota, Bacillariophyta, Ascomycota, Arthropoda, Apicomplexa, and Annelida (Fig. 2). The communities were dominated by the microalgal phyla Chlorophyta and Bacillariophyta, although their combined relative abundance was significantly higher in the Dongdo and Seodo samples (93.52% and 91.77%, respectively) than in the Seonginbong sample (31.02%). Differences in population densities were more profound in the Seonginbong sample than in the Dongdo and Seodo samples (Fig. 2). This analysis of differences in the community composition could contribute significantly to our understanding of the microbial ecosystems at each site (Wegley et al. 2007; Rodriguez-Brito et al. 2010; Fierer et al. 2012). Microbial community compositions already reported suggest a need for further research on the eukaryotic microorganisms in each

region (Knight 2000; Chiao 2004; Schloss and Handelsman 2005). In this regard, amplicon sequencing using Illumina MiSeq is a powerful tool for the identification of unculturable microalgae. More important, MiSeq system analysis can also generate useful information on new species in the natural environments of Ulleungdo and Dokdo that could be helpful in studying unculturable eukaryotic microorganisms.

Comparison of the microalgal communities on Seonginbong, Dongdo, and Seodo. We compared the structures of the microalgal communities on Seonginbong, Dongdo, and Seodo by constructing phylogenetic trees (Fig. 1) using UPGMA analysis with eukaryotic microorganisms. The taxonomic compositions of Seonginbong, Dongdo, and Seodo were analyzed from the phylum to species level. Overall, it was found that Seonginbong was more closely related to Dongdo than Seodo. At the phylum level, the microalgal communities of Seonginbong, Dongdo, and Seodo exhibited differences in their taxonomic compositions despite being dominated by two phyla: Chlorophyta (Round 1963) and Bacillariophyta (Fig. 2; Kaczmarzka et al. 2007). The relative abundance of Chlorophyta was very high on Dongdo (93.52%), while Bacillariophyta was dominant on Seodo (89.13%). On Seonginbong, the relative abundance of Chlorophyta was higher than that of Bacillariophyta (Chytridiomycota 39.43%, Chlorophyta 27.1%, and Bacillariophyta 4.31%).

At the class level, five distinct microalgal classes (Bacillariophyceae, Coscinodiscophyceae, Chlorophyceae, Trebouxiophyceae, and Ulvophyceae) were detected in the overall sample (Fig. 3), with the dominant groups in each region differing: Seonginbong, Chlorophyceae; Dongdo, Trebouxiophyceae; and Seodo, Bacillariophyceae. In particular, the relative abundance of Bacillariophyceae was higher in Seodo (88.24%) than

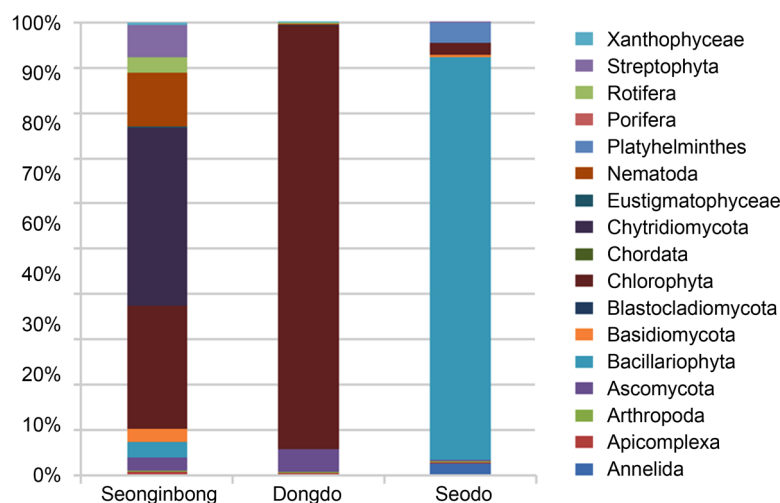


Fig. 2. Taxonomic composition of the eukaryotic microbial phyla on Seonginbong, Dongdo, and Seodo.

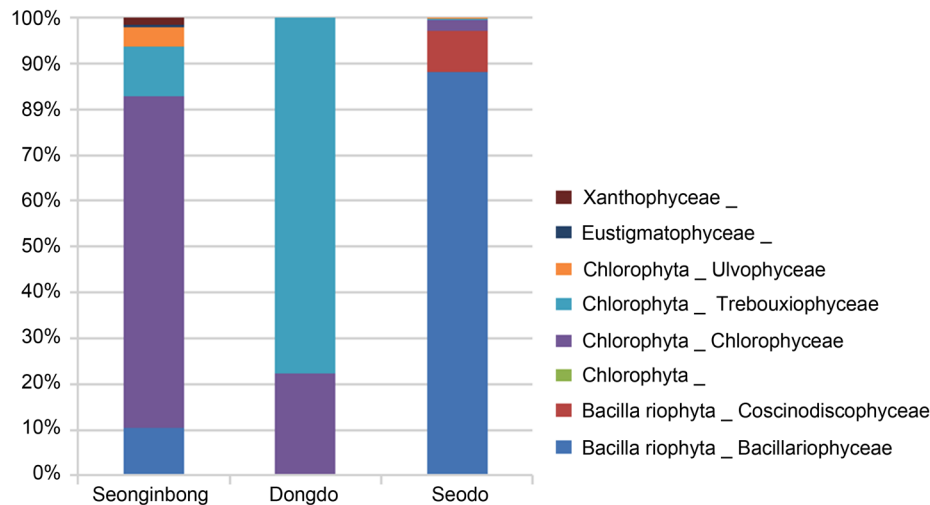


Fig. 3. Taxonomic composition of the microalgal classes on Seonginbong, Dongdo, and Seodo.

in Seonginbong (10.56%) or Dongdo (0%). The Coscinodiscophyceae was only present on Seodo (8.86%). In addition, two or three green algae classes were present at the study sites, including Chlorophyceae (73.39%), Trebouxiophyceae (11.17%), and Ulvophyceae (4.18%) on Seonginbong; Chlorophyceae (22.22%) and Trebouxiophyceae (77.67%) on Dongdo; and Chlorophyceae (2.28%), Trebouxiophyceae (0.49%), and Ulvophyceae (0.13%) on Seodo.

A total of 30 families were detected in each region. Seventeen families had identified scientific names, and nine had a relative abundance of at least 1%. These families are summarized in Table III. On Seonginbong, three diatom families (*Bacillariaceae*, *Pinnulariaceae*, and *Stauroneidaceae*) and eight green algae families (*Characiochloridaceae*, *Chlamydomonadaceae*, *Chlorococcaceae*, *Scenedesmaceae*, *Coccomyxaceae*, *Chlorellaceae*, and *Ctenocladaceae*) were identified, with the most dominant being *Chlorococcaceae* (1.53%), and two unclassified green algae families (*Chlorophyta*, *Chlorophyceae*, *Chlamydomonadales*: 3.47%; *Chlorophyta*, *Chlorophyceae*, *Sphaeropleales*: 2.53%). Conversely, only one diatom or green algae family was dominant in Dongdo and Seodo. One diatom family (*Diadesmidaceae*) and three green algae families (*Chlamydomonadaceae*, *Chlorococcaceae*, and *Chlorellaceae*) were present on Dongdo, with the most dominant being *Chlorellaceae* (64.91%), distantly followed by *Chlorococcaceae* (18.46%). Conversely, Seodo had nine diatom families (*Achnanthaceae*, *Bacillariaceae*, *Amphipleuraceae*, *Diadesmidaceae*, *Naviculaceae*, *Sellaphoraceae*, *Catenulaceae*, and *Stephanopyxidaceae*) and two green algae families (*Scenedesmaceae* and *Chlorellaceae*). The dominant family on Seodo was an unclassified diatom family (21.62%), distantly followed by three other diatom families (*Bacillariaceae*: 3.26%, *Sellaphoraceae*: 3.12%, and *Stephanopyxidaceae*: 3.14%) with relative

abundances of at least 3%. Four families were found to be unique to a specific area: *Stauroneidaceae* on Seonginbong and *Achnanthaceae*, *Sellaphoraceae*, and *Stephanopyxidaceae* on Seodo. In summary, although Dongdo and Seodo are proximally located, the species composition on Seodo differs from that on Seonginbong and Dongdo; these two regions exhibit greater similarity to one another than either does to Seodo.

A total of 50 microalgal genera were detected, with 37 identified by scientific name. Fourteen genera had a relative abundance of at least 1% (Table ???). Three diatom genera (*Nitzschia*, *Pinnularia*, and *Amphora*) known to produce toxins were identified on Seonginbong (*Pinnularia*, 0.09%) and Seodo (*Nitzschia*, 3.26%; *Amphora*, 0.12%). For the diatom genera with a relative abundance of at least 1%, genera were uniquely distributed in each region; however, microalgal genera were found at all three sites. In particular, the microalgal taxonomic compositions of Seonginbong and Dongdo were more similar to one another than either was to Seodo. There were six dominant genera (*Stauroneis*, 1.16%; *Chlorococcum*, 1.53%; *Chlorosarcinopsis*, 1.29%; *Bracteacoccus*, 1.89%), and two unclassified microalgal genera (1.48% and 1.47%) present on Seonginbong. On Dongdo, unclassified microalgal genera (63.78%), *Chlorococcum* (18.46%), and *Pseudochlorella* (1.13%) dominated. Six diatom genera were dominant on Seodo (*Achnanthidium*, 20.76%; *Achnanthes*, 1.54%; *Nitzschia*, 3.26%; *Diadesmis*, 2.15%; *Sellaphora*, 3.12%; *Stephanopyxis*, 3.14%). These findings indicate that microalgal genera are widely distributed across all three regions, whereas diatom genera are restricted to specific areas. Of note, microalgal taxonomic composition showed that the Seonginbong and Dongdo communities were closely related at the genus level.

For species-level analyses, the microalgal species identified from the Seonginbong, Dongdo, and Seodo

Table III
Relative abundance of eukaryotic microalgal families in the Seonginbong, Dongdo, and Seodo samples.

| Taxonomy | | | | Seonginbong | | Dongdo | | Seodo | |
|-----------------|---------------------|---------------------|----------------------|----------------|-----------------|----------------|-----------------|----------------|-----------------|
| Phylum | Class | Order | Family | % ¹ | Fr ² | % ¹ | Fr ² | % ¹ | Fr ² |
| Bacillariophyta | Bacillariophyceae | – | – | 0.00 | 0 | 0.00 | 0 | 21.62 | 59 531 |
| Bacillariophyta | Bacillariophyceae | – | Achnantheaceae | 0.00 | 0 | 0.00 | 0 | 1.54 | 4 239 |
| Bacillariophyta | Bacillariophyceae | – | Bacillariaceae | 0.15 | 423 | 0.00 | 0 | 3.26 | 8 974 |
| Bacillariophyta | Bacillariophyceae | Naviculales | Amphipleuraceae | 0.00 | 0 | 0.00 | 0 | 0.23 | 645 |
| Bacillariophyta | Bacillariophyceae | Naviculales | Diadesmidaceae | 0.00 | 0 | 0.00 | 7 | 2.38 | 6 551 |
| Bacillariophyta | Bacillariophyceae | Naviculales | Naviculaceae | 0.00 | 0 | 0.00 | 0 | 0.29 | 802 |
| Bacillariophyta | Bacillariophyceae | Naviculales | Pinnulariaceae | 0.09 | 256 | 0.00 | 0 | 0.00 | 0 |
| Bacillariophyta | Bacillariophyceae | Naviculales | Sellaphoraceae | 0.00 | 0 | 0.00 | 0 | 3.12 | 8 579 |
| Bacillariophyta | Bacillariophyceae | Naviculales | Stauroneidaceae | 1.16 | 3 379 | 0.00 | 0 | 0.00 | 0 |
| Bacillariophyta | Bacillariophyceae | Thalassiosiphysales | Catenulaceae | 0.00 | 0 | 0.00 | 0 | 0.12 | 329 |
| Bacillariophyta | Coscinodiscophyceae | Melosirales | Stephanopyxidaceae | 0.00 | 0 | 0.00 | 0 | 3.14 | 8 660 |
| Bacillariophyta | Coscinodiscophyceae | Paraliales | – | 0.00 | 0 | 0.00 | 0 | 0.13 | 354 |
| Chlorophyta | – | – | – | 0.00 | 0 | 0.02 | 65 | 0.00 | 0 |
| Chlorophyta | – | Chlorodendrales | – | 0.00 | 0 | 0.01 | 21 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | – | – | 0.03 | 91 | 0.00 | 13 | 0.62 | 1 708 |
| Chlorophyta | Chlorophyceae | Chlamydomonadales | – | 2.53 | 7 368 | 0.09 | 271 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | Chlamydomonadales | Characiochloridaceae | 0.34 | 1 002 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | Chlamydomonadales | Chlamydomonadaceae | 0.38 | 1 096 | 0.02 | 48 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | Chlamydomonadales | Chlorococcaceae | 1.53 | 4 437 | 18.46 | 53 463 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | Chlorosarcinales | – | 1.29 | 3 761 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | Sphaeropleales | – | 3.47 | 10 096 | 0.00 | 2 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | Sphaeropleales | Scenedesmaceae | 0.08 | 243 | 0.00 | 0 | 0.22 | 597 |
| Chlorophyta | Trebouxiophyceae | – | – | 0.56 | 1 624 | 0.00 | 0 | 0.12 | 329 |
| Chlorophyta | Trebouxiophyceae | – | Coccomyxaceae | 0.01 | 23 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Trebouxiophyceae | Chlorellales | – | 0.00 | 0 | 0.00 | 7 | 0.00 | 0 |
| Chlorophyta | Trebouxiophyceae | Chlorellales | Chlorellaceae | 0.58 | 1 689 | 64.91 | 187 999 | 0.06 | 162 |
| Chlorophyta | Trebouxiophyceae | Ctenocladales | Ctenocladaceae | 0.21 | 604 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Trebouxiophyceae | Microthamniales | – | 0.11 | 323 | 0.02 | 48 | 0.00 | 0 |
| Chlorophyta | Ulvophyceae | Ulotrichales | – | 0.55 | 1 605 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Ulvophyceae | Ulvales | – | 0.00 | 0 | 0.00 | 0 | 0.05 | 136 |

The microalgal families detected in at least one of the three samples are shown. Unclassified taxonomic names (phylum, class, order, and family) are replaced with a dash (–)

¹Relative abundance

²Frequency of microalgae detected at each sampling site

samples were organized in a phylogenetic tree (Fig. 4). For groups without a scientific name at the genus level (Fig. 3), names were only added to those with scientific names at the species level (Fig. 4). Phylum and class boundaries were identified for the microalgal species based on species-level sequencing analysis for Seonginbong, Dongdo, and Seodo. In Fig. 4, the boundary between Bacillariophyta and Chlorophyta is marked with a yellow box, and boundaries between the classes belonging to each phylum are marked with purple boxes (Metting 1996). Among the microalgal groups, some of the Chlorophyceae belonged to Trebouxiophyceae from class via phylum (Tables III and IV). At the

species level, dominant species were identified on each island, to include six species on Seonginbong, two species on Dongdo, and six species on Seodo; these are marked by boxes in Fig. 4 (Seonginbong, red; Dongdo, blue; Seodo, green). Of the species shown on the phylogenetic tree, some have been associated with shellfish toxins (Falconer 2012) frequently found on Seodo. In particular, *Nitzschia* sp. (Bates et al. 1989; Martin et al. 1990), known to be associated with shellfish toxins, was one of the dominant species on Seodo.

We organized the three microalgal communities from the phylum to species levels to analyze the taxonomic compositions of the three study sites. The approximate

Table IV
Relative abundance of eukaryotic microalgal genera in the Seonginbong, Dongdo, and Seodo samples.

| Taxonomy | | | | Seonginbong | | Dongdo | | Seodo | | |
|-----------------|---------------------|---------------------|----------------------|------------------------|----------------|-----------------|----------------|-----------------|----------------|-----------------|
| Phylum | Class | Order | Family | Genus | % ¹ | Fr ² | % ¹ | Fr ² | % ¹ | Fr ² |
| Bacillariophyta | Bacillariophyceae | - | - | - | 0.00 | 0 | 0.00 | 0 | 0.86 | 2 363 |
| Bacillariophyta | Bacillariophyceae | - | - | <i>Achnanthyidium</i> | 0.00 | 0 | 0.00 | 0 | 20.76 | 57 168 |
| Bacillariophyta | Bacillariophyceae | - | Achnanthaceae | <i>Achnanthes</i> | 0.00 | 0 | 0.00 | 0 | 1.54 | 4 239 |
| Bacillariophyta | Bacillariophyceae | - | Bacillariaceae | <i>Hantzschia</i> | 0.15 | 423 | 0.00 | 0 | 0.00 | 0 |
| Bacillariophyta | Bacillariophyceae | - | Bacillariaceae | <i>Nitzschia</i> | 0.00 | 0 | 0.00 | 0 | 3.26 | 8 974 |
| Bacillariophyta | Bacillariophyceae | Naviculales | Amphipleuraceae | - | 0.00 | 0 | 0.00 | 0 | 0.23 | 645 |
| Bacillariophyta | Bacillariophyceae | Naviculales | Diadesmidaceae | <i>Diadesmis</i> | 0.00 | 0 | 0.00 | 0 | 2.15 | 5 922 |
| Bacillariophyta | Bacillariophyceae | Naviculales | Diadesmidaceae | <i>Luticola</i> | 0.00 | 0 | 0.00 | 7 | 0.23 | 629 |
| Bacillariophyta | Bacillariophyceae | Naviculales | Naviculaceae | <i>Navicula</i> | 0.00 | 0 | 0.00 | 0 | 0.29 | 802 |
| Bacillariophyta | Bacillariophyceae | Naviculales | Pinnulariaceae | <i>Pinnularia</i> | 0.09 | 256 | 0.00 | 0 | 0.00 | 0 |
| Bacillariophyta | Bacillariophyceae | Naviculales | Sellaphoraceae | <i>Sellaphora</i> | 0.00 | 0 | 0.00 | 0 | 3.12 | 8 579 |
| Bacillariophyta | Bacillariophyceae | Naviculales | Stauroneidaceae | <i>Stauroneis</i> | 1.16 | 3 379 | 0.00 | 0 | 0.00 | 0 |
| Bacillariophyta | Bacillariophyceae | Thalassiosiphysales | Catenulaceae | <i>Amphora</i> | 0.00 | 0 | 0.00 | 0 | 0.12 | 329 |
| Bacillariophyta | Coscinodiscophyceae | Melosirales | Stephanopyxidaceae | <i>Stephanopyxis</i> | 0.00 | 0 | 0.00 | 0 | 3.14 | 8 660 |
| Bacillariophyta | Coscinodiscophyceae | Paraliales | - | <i>Paralia</i> | 0.00 | 0 | 0.00 | 0 | 0.13 | 354 |
| Chlorophyta | - | - | - | - | 0.00 | 0 | 0.02 | 65 | 0.00 | 0 |
| Chlorophyta | - | Chlorodendrales | - | - | 0.00 | 0 | 0.01 | 21 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | - | - | - | 0.03 | 91 | 0.00 | 13 | 0.62 | 1 708 |
| Chlorophyta | Chlorophyceae | Chlamydomonadales | - | - | 1.48 | 4 308 | 0.09 | 249 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | Chlamydomonadales | - | <i>Actinochloris</i> | 0.00 | 0 | 0.01 | 22 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | Chlamydomonadales | - | <i>Ettlia</i> | 0.62 | 1 793 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | Chlamydomonadales | - | <i>Spongiochloris</i> | 0.44 | 1 267 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | Chlamydomonadales | Charactochloridaceae | <i>Charactochloris</i> | 0.34 | 1 002 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | Chlamydomonadales | Chlamydomonadaceae | - | 0.02 | 50 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | Chlamydomonadales | Chlamydomonadaceae | <i>Chlamydomonas</i> | 0.35 | 1 026 | 0.00 | 0 | 0.00 | 0 |

The microalgal genera detected in at least one of the three samples are shown. Unclassified taxonomic names (phylum, class, order, family, and genus) are replaced with a dash (-)

¹Relative abundance

²Frequency of microalgae detected at each sampling site

Table IV
Continued.

| Taxonomy | | | | Seonginbong | | Dongdo | | Seodo | | |
|-------------|------------------|-------------------|--------------------|--------------------------|----------------|-----------------|----------------|-----------------|----------------|-----------------|
| Phylum | Class | Order | Family | Genus | % ¹ | Fr ² | % ¹ | Fr ² | % ¹ | Fr ² |
| Chlorophyta | Chlorophyceae | Chlamydomonadales | Chlamydomonadaceae | <i>Chloromonas</i> | 0.01 | 20 | 0.02 | 48 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | Chlamydomonadales | Chlorococcaceae | <i>Chlorococcum</i> | 1.53 | 4 437 | 18.46 | 53 463 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | Chlorosarcinales | - | <i>Chlorosarcinopsis</i> | 1.29 | 3 761 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | Sphaeropleales | - | - | 1.47 | 4 271 | 0.00 | 2 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | Sphaeropleales | - | <i>Bracteacoccus</i> | 1.89 | 5 490 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | Sphaeropleales | - | <i>Dictyochloris</i> | 0.12 | 335 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Chlorophyceae | Sphaeropleales | Scenedesmaceae | <i>Coelastrella</i> | 0.02 | 64 | 0.00 | 0 | 0.22 | 597 |
| Chlorophyta | Chlorophyceae | Sphaeropleales | Scenedesmaceae | <i>Desmodesmus</i> | 0.06 | 179 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Trebouxiophyceae | - | - | - | 0.04 | 102 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Trebouxiophyceae | - | - | <i>Myrmecia</i> | 0.51 | 1 487 | 0.00 | 0 | 0.12 | 329 |
| Chlorophyta | Trebouxiophyceae | - | - | <i>Watanabea</i> | 0.01 | 35 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Trebouxiophyceae | - | Coccomyxaceae | <i>Coccomyxa</i> | 0.01 | 23 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Trebouxiophyceae | Chlorellales | - | - | 0.00 | 0 | 0.00 | 7 | 0.00 | 0 |
| Chlorophyta | Trebouxiophyceae | Chlorellales | Chlorellaceae | - | 0.06 | 182 | 63.78 | 184 700 | 0.06 | 162 |
| Chlorophyta | Trebouxiophyceae | Chlorellales | Chlorellaceae | <i>Auxenochlorella</i> | 0.34 | 999 | 0.00 | 2 | 0.00 | 0 |
| Chlorophyta | Trebouxiophyceae | Chlorellales | Chlorellaceae | <i>Chlorella</i> | 0.04 | 102 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Trebouxiophyceae | Chlorellales | Chlorellaceae | <i>Heveochlorella</i> | 0.00 | 0 | 0.00 | 12 | 0.00 | 0 |
| Chlorophyta | Trebouxiophyceae | Chlorellales | Chlorellaceae | <i>Lobosphaera</i> | 0.00 | 14 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Trebouxiophyceae | Chlorellales | Chlorellaceae | <i>Pseudochlorella</i> | 0.13 | 392 | 1.13 | 3 285 | 0.00 | 0 |
| Chlorophyta | Trebouxiophyceae | Ctenocladales | Ctenocladaceae | <i>Leptosira</i> | 0.21 | 604 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Trebouxiophyceae | Microthamniales | - | - | 0.09 | 272 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Trebouxiophyceae | Microthamniales | - | <i>Dictyochloropsis</i> | 0.02 | 51 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Trebouxiophyceae | Microthamniales | - | <i>Stichococcus</i> | 0.00 | 0 | 0.02 | 48 | 0.00 | 0 |
| Chlorophyta | Ulvophyceae | Ulotrichales | - | - | 0.55 | 1 605 | 0.00 | 0 | 0.00 | 0 |
| Chlorophyta | Ulvophyceae | Ulvales | - | - | 0.00 | 0 | 0.00 | 0 | 0.05 | 136 |

The microalgal genera detected in at least one of the three samples are shown. Unclassified taxonomic names (phylum, class, order, family, and genus) are replaced with a dash (-)

¹Relative abundance

²Frequency of microalgae detected at each sampling site

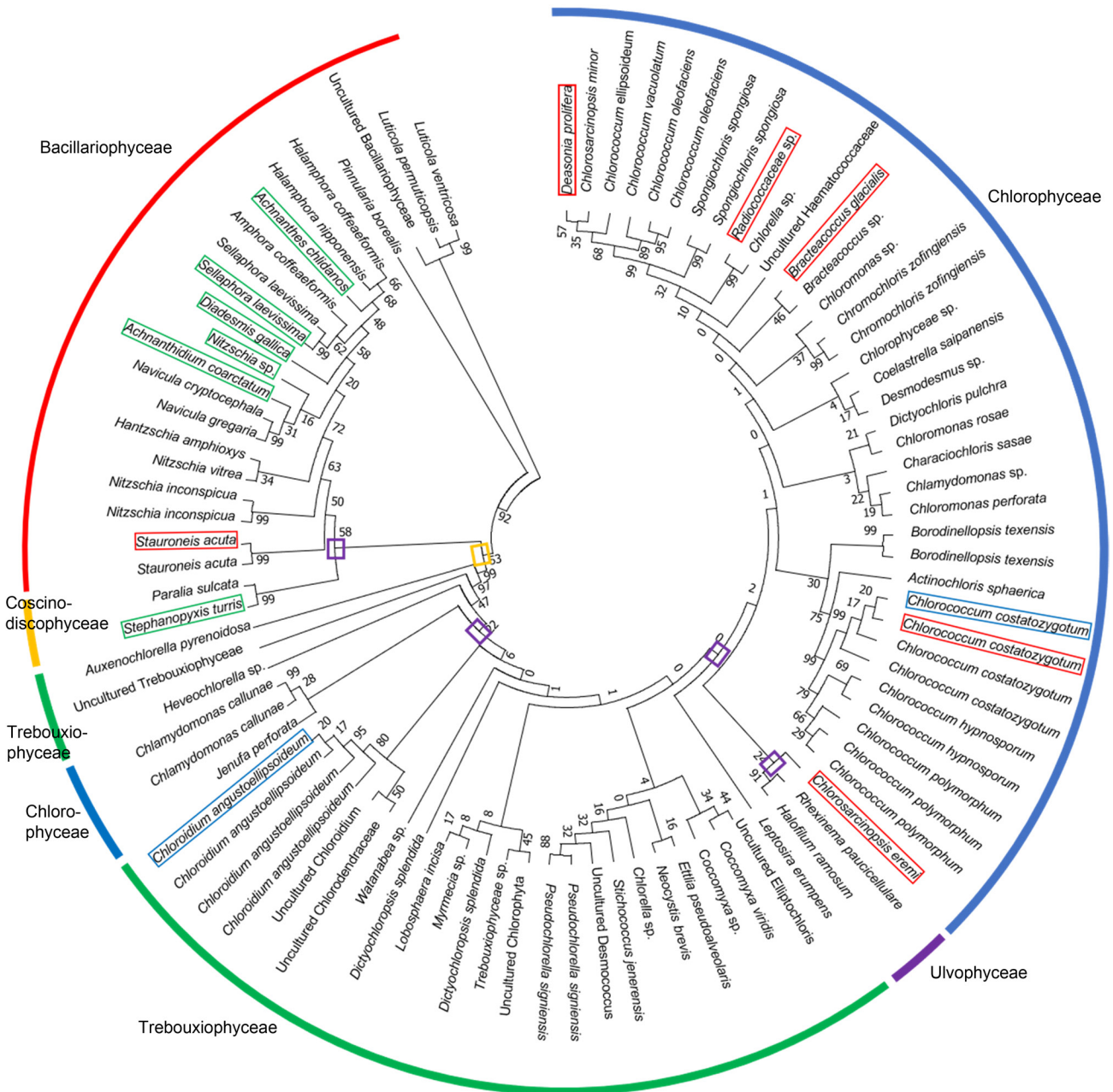


Fig. 4. Molecular phylogenetic analysis using a maximum likelihood (ML) tree. The boundary between phyla is marked with a yellow box, and the boundaries between classes are marked with purple boxes. Five classes are displayed about the species names in the phylogenetic tree. The dominant species in each sample is marked with a colored box (Seonginbong: red; Dongdo: blue; Seodo: green). The class of each group is presented at the edge (Bacillariophyceae; red, Coscinodiscophyceae; yellow, Chlorophyceae; blue, Trebouxiophyceae; green and Ulvophyceae; purple).

amount of available sunlight was highest at the Seonginbong sampling site and lowest at the Seodo site (Supplementary Fig. S1), and the relative abundance of diatoms strongly correlated with sunlight availability (Hudon and Bourget 1983; Post et al. 1984; Lange et al. 2011). Our results and those from previous studies indicate that further research on the relationship between light and microalgal community composition is required. Research also suggests that microalgal community composition is influenced by natural enemies or disease (Hudon and Bourget 1983; Post et al. 1984; Lange et al.

2011). In accordance with these findings, we observed differences in natural compositions among Seonginbong, Dongdo, and Seodo; the microalgal group was dominant on Seodo. At the phylum level, Seonginbong was characterized by zooplankton and pathogenic fungal groups (Fig. 2). At the class level, the microalgal group was dominated by Chlorophyceae on Seonginbong and Trebouxiophyceae (particularly *Chlorellaceae*) on Dongdo (Fig. 3). Trebouxiophyceae, which contains a family of small-celled organisms (*Chlorellaceae*), are relatively vulnerable to predators compared to other

classes, and results of the present study suggest that the presence of consumers (zooplankton and pathogenic fungi) affects the dominance of Chlorophyceae on Seonginbong and Dongdo to a greater extent than on Seodo (Fig. 2 and 3; Johnson and Agrawal 2003; Sarma et al. 2003; Yoshida et al. 2004; Pradeep et al. 2015).

Previous studies also indicate that microalgae can affect the external environment. A previous report found that the *Trebouxia* genus of the Trebouxiophyceae class forms a symbiotic association with lichen, fungi, and algae and is directly involved in changes to the terrestrial environment (Ahmadjian, 1988; Piercey-Normore 2006). The results of this study indicated that Trebouxiophyceae was not accurately detected at the phylum level, although a greater presence of Trebouxiophyceae at the class level was found on Seonginbong than on Dongdo and Seodo, as evidenced by the identification of microalgal communities via eukaryotic microbial communities (Table III and IV). This suggests that the microalgal group on Seonginbong engages in a symbiotic relationship with the fungi group, unlike on Dongdo and Seodo, and that this relationship directly impacts the Seonginbong natural environment. Previous studies have found that microalgae secrete a range of substances that influence their natural environment, including fungal toxins and predators (Havens and DeCosta 1987; Canovas et al. 1996; Mayer et al. 1997; Falconer 2012). The genera *Nitzschia* (Bates et al. 1989; Martin et al. 1990), *Amphora* (Daniel et al. 1980), and *Paralia* (Sar et al. 2012) are reported to be closely associated with shellfish toxins on Seodo (Falconer 2012; Sar et al. 2012) that can be harmful to human health when ingested orally. Although they only account for a small fraction of the detected microalgal community, it is nonetheless necessary to monitor their toxin-producing abilities and biological resources. Our findings indicate that microalgae are influenced both by environmental factors and the surrounding microbial community and that characteristics of the microbial community are influenced by the natural environment.

Conclusion

The present study analyzed the overall species richness and taxonomic compositions of the microalgal communities of Ulleungdo (Seonginbong) and Dokdo (Dongdo and Seodo). Amplicon sequencing analysis was performed using Illumina MiSeq, and microbiological OTUs from Seonginbong (834), Dongdo (203), and Seodo (182) were identified. Three indicators (Chao1, Shannon, and Simpson) were used to analyze species richness, and it was found that the species richness of Seonginbong was higher than those of Dongdo and Seodo. Classified reads were used for taxonomic analy-

sis, with the communities exhibiting differences in their composition from the phylum to species levels. In the Seonginbong sample, several other eukaryotic microorganisms were present in the community in addition to microalgae, while microalgae (Chlorophyta) and diatoms (Bacillariophyta) were found to be extremely dominant on Dongdo and Seodo, respectively. Analyses of the relative abundances of the different communities added details to information regarding the differences in species richness between the three regions. We obtained information on microalgae on Seonginbong, Dongdo, and Seodo via MiSeq tools; however, MiSeq analysis does have some limitations with regards to dependence on existing taxonomies in screening and identifying microalgal species. Despite these experimental limitations, MiSeq analysis provided in-depth information on the microalgal communities of Ulleungdo and Dokdo.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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