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ORIGINAL PAPER

Environmental Factors Associated with the Eukaryotic Microbial Community and Microalgal Groups in the Mountain Marshes of South Korea

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Abstract

The diversity indices of eukaryotic microalgal groups in the Jeonglyeongchi, Waegok, and Wangdeungjae marshes of Mount Jiri, Korea, were measured using Illumina MiSeq and culture-based analyses. Waegok marsh had the highest species richness, with a Chao1 value of 828.00, and the highest levels of species diversity, with Shannon and Simpson index values of 6.36 and 0.94, respectively, while Wangdeungjae marsh had the lowest values at 2.97 and 0.75, respectively. The predominant species in all communities were *Phagocata sibirica* (Jeonglyeongchi, 68.64%), *Aedes albopictus* (Waegok, 34.77%), *Chaetonotus* cf. (Waegok, 24.43%), *Eimeria* sp. (Wangdeungjae, 26.17%), and *Eumonhystera* cf. (Wangdeungjae, 22.27%). Relative abundances of the microalgal groups Bacillariophyta (diatoms) and Chlorophyta (green algae) in each marsh were respectively: Jeonglyeongchi 1.38% and 0.49%, Waegok 7.0% and 0.3%, and Wangdeungjae 10.41% and 4.72%. Illumina MiSeq analyses revealed 34 types of diatoms and 13 types of green algae. Only one diatom (*Nitzschia dissipata*) and five green algae (*Neochloris* sp., *Chlamydomonas* sp., *Chlorococcum* sp., *Chlorella vulgaris*, *Scenedesmus* sp.) were identified by a culture-based analysis. Thus, Illumina MiSeq analysis can be considered an efficient tool for analyzing microbial communities. Overall, our results described the environmental factors associated with geographically isolated mountain marshes and their respective microbial and microalgal communities.

K e y w o r d s: environmental sample, Illumina MiSeq, Mount Jiri marshes, microbial community, microalgal community

Introduction

Mount Jiri (hereafter referred to as Jiri) is located at the southern tip of the Sobaek Mountain ranges in the southern part of the Korean peninsula. It covers a vast area, spanning five cities, and it is the second-highest mountain (1915 m) in South Korea, with slopes of 28°–30° (Kim and Jung 2018). Jiri presents annual average temperature of 13°C and an average annual precipitation of 1,350–1,510 mm, with 69% of the rainfall concentrated between June and September (Kim and Jung 2018). Mountain streams and high marshes have developed depending on groundwater and rainfall. Such freshwater ecosystems may be geographically isolated due to weathering and erosion (Wieringa 1964; Kim and Jung 2018). Jiri has well-developed mountain marshes that can be separated and isolated by the mountain ranges or originated from separate water sources (Wieringa 1964; Kim and Jung 2018). Here, we studied three mountain marshes – Jeonglyeongchi, Waegok, and Wangdeungjae – and their different environmental factors associated with their respective microbial and microalgal communities.

Jiri's high marshes characteristics have been influenced by topography and soil properties (Yang 2008; Kim et al. 2010). In particular, the soil of Jiri's high

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marshes presents high water retention and poor permeability, allowing fresh water to flow into the wetlands (Yang 2008; Kim et al. 2010). Because of the low soil permeability, sediments around Mount Jiri tend to build up, influencing the development of soil layers (Yang 2008; Kim et al. 2010). Thus, soil in Mount Jiri is characterized by organic layers and deep O and A soil horizons (Anderson 1988; Bormann et al. 1995; Huggett 1998; Hartemink et al. 2020). The soil supports a thriving vegetation, along with peat deposits (Anderson 1988; Bormann et al. 1995; Huggett 1998). Some microorganisms can use the peat as an energy source, leading to the formation of a unique type of microbial community (Williams and Yavitt 2003; Dobrovol'skaya et al. 2012). This microbial community contains decomposers that can degrade cellulose and/or lignin as well as consumers that utilize the resulting degradation products (Berg and McClaugherty 2003; Berg and Laskowski 2005; Stone et al. 2020), including organic carbon sources, nitrogen, phosphorus, and trace elements (Jewell 1971; Garber 1984; Canfield et al. 2020; Zhang et al. 2020). In addition, microalgal groups consume nitrogen and phosphorus (Di Termini et al. 2011) and are involved in cycling these elements through photosynthesis (McGlathery et al. 2004). Microalgal groups can act as producers (of oxygen), consumers (of organic carbon sources), and decomposers (of cellulose and lignin, using them as energy sources) (Schoenberg et al. 1984; Perez-Garcia et al. 2011; Blifernez-Klassen et al. 2012). Therefore, microalgal groups can play a variety of ecological roles and potentially affect the diversity of the microbial community (Schoenberg et al. 1984; Perez-Garcia et al. 2011; Blifernez-Klassen et al. 2012).

Each of the Jiri marshes possesses unique characteristics, making them attractive sites for the comparative analyses of physicochemical factors and microbial communities (Yang 2008; Kim and Jung 2018). In this study, we investigated three mountain marsh sites by analyzing the microbial community DNA of eukaryotic microalgal groups and other microorganisms based on the amplification of the 18S rRNA gene. In addition, the geographic isolation between the mountain marshes was tested to identify the environmental factors affecting microbial and microalgal communities in the marshes.

Experimental

Materials and Methods

Collection of samples. Samples were collected from Jeonglyeongchi marsh (35°21'52.5"N 127°31'25.5"E, Deokdong-ri, Sannae-myeon, Namwon-si, Jeollabuk-do, South Korea), Waegok marsh (35°22'57.0"N 127°46'49.7"E, Yupyeong-ri, Samjang-myeon, Sancheong-gun, Gyeongsangnam-do, South Korea), and Wangdeungjae marsh (35°23'21.8"N 127°47'19.0"E, Yupyeong-ri, Samjang-myeon, Sancheong-gun, Gyeongsangnam-do, South Korea) (Fig. 1) in July 2019, at ten different locations within each marsh. Each sample consisted of 500 ml of freshwater. Samples were transported to the laboratory, then shipped to Macrogen Co., Ltd. using the same-day express courier service. All analyses were performed at room temperature. All living materials were immediately examined and then fixed in 5% formalin for permanent preservation and detailed identification (Kim and Jung 2018).

Physicochemical analysis. Temperature, pH, electrical conductivity (EC), salinity, dissolved oxygen (DO), and nephelometric turbidity of the samples were measured on-site using a multiparameter instrument (U-50 Multiparameter Water Quality Meter, HORIBA, Kyoto, Japan). A water test kit (HUMAS, Daejeon, South Korea) was used to measure total nitrogen (TN) and total phosphorus (TP) in each sample.

Microbial community analysis. Illumina MiSeq analyses of the microbial communities were performed by the Macrogen (Macrogen, Seoul, South Korea, https://dna.macrogen.com/kor/), as described previously (Yun et al. 2019). DNA for Illumina MiSeq sequencing was extracted from the samples according to the manufacturer's protocol of the PowerSoil® DNA Isolation Kit (Cat. No. 12888, MO BIO) (Claassen et al. 2013). PicoGreen and Nanodrop were used for quantification and quality measurements of the extracted DNA. Extracted DNA samples were amplified by PCR according to the Illumina 18S Metagenomic Sequencing Library protocols (Vo and Jedlicka 2014). The 18S V4 primer set was used to amplify the 18S rRNA regions (Stoeck et al. 2010). TAReuk454FWD1 (forward primer, 5'-CCAGCA(G/C)C(C/T)GCGGTAATTCC-3') and TAReukREV3 (reverse primer, 5'-ACTTTCGTTCTTGAT(C/T)(A/G)A-3') were used as the 18S V4 primer set (Stoeck et al. 2010). A subsequent limited-cycle amplification was conducted for the addition of multiplexing indices and Illumina sequencing adapters (Meyer and Kircher 2010). The target DNA fragment size of PCR amplification is approximately 420 bp; the final DNA fragments were pooled and normalized using PicoGreen. TapeStation DNA and D1000 ScreenTape system (Agilent) was used to verify the library size. The sequencing data results were analyzed using the MiSeq™ platform (Illumina, San Diego, USA) (Kozich et al. 2013).

Taxonomic identification and phylogenetic analysis. The raw sequencing data were demultiplexed using the index sequence, and a FASTQ file was generated for each sample (Yun et al. 2019). The adapter sequence was removed using SeqPurge, and the sequencing error correction was performed on the overlapping areas of the

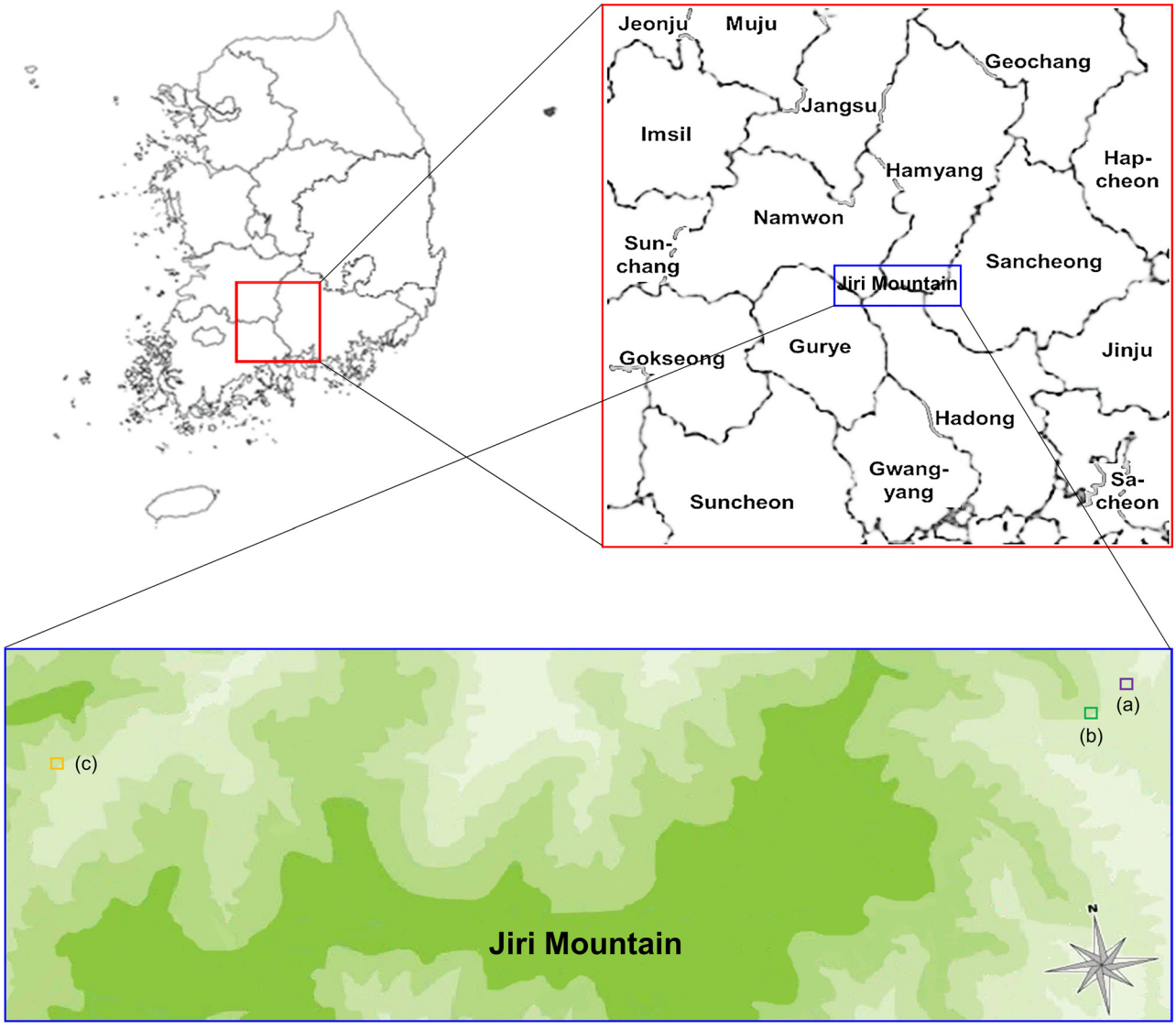


Fig. 1. Location of sampling sites at three mountain marshes. Red box: location of Mountain Jiri, covering five cities in the southern part of the Korean peninsula. Blue box: location of Mountain Jiri and sampling sites marked with small boxes.

a) Purple box, Wangdeungjae marsh, 35°23'21.8"N 127°47'19.0"E. b) Green box, Waegok marsh, 35°22'57.0"N 127°46'49.7"E. c) Orange box, Jeonglyeongchi marsh, 35°21'52.5"N 127°31'25.5"E.

correct reads (Sturm et al. 2016). Low-quality sequences of barcode sequences were trimmed and filtered (standard: 400 bp < read length or 25 < average quality value). The trimmed and filtered sequencing data were identified using a BLASTN search from the NCBI database, based on their barcode sequences (Zhang et al. 2000). For the unclassified results, "–" was marked to the end of the name for each sublevel. Each operational taxonomic unit (OTU) was analyzed based on the CD-HIT at a 97% sequence similarity level (Li et al. 2012). The rarefaction curves and the diversity indicators (Shannon, Simpson, and Chao1) were calculated using the Mothur platform (Heck Jr et al. 1975; Schloss et al. 2009). Based on the weighted UniFrac distance, Beta diversity (sample diversity information of the comparison group) was calculated and used to visualize the relationship between the samples using the UPGMA tree (FigTree, http://tree.bio.ed.ac.uk/software/figtree/). Phylogenetic analysis was performed using the software package MEGA version 7.0 (Kumar et al. 2008; Kumar et al. 2016). The identified sequencing data groups were aligned using ClustalW and incorporated in MEGA 7.0 (Kumar et al. 2008; Kumar et al. 2016). The best-fit nucleotide substitution model was selected based on the Bayesian information criterion (Schwarz 1978). The maximum likelihood (ML) phylogenetic tree was built according to the best-fit nucleotide substitution model (Felsenstein 1985).

Culture-based analysis of microalgal groups. To culture microalgae, 1 ml of each sample was inoculated into 100 ml of culture medium in a 250 ml flask (Rippka et al. 1979; Bolch and Blackburn 1996). Four types of culture media were used: Blue Green-11 (BG11)

medium, Optimum *Haematococcus* Medium (OHM), Bold Basal medium (BB), and Diatom Medium (DM) (Agrawal and Sarma 1982; Bolch and Blackburn 1996; Fábregas et al. 2000; Safonova et al. 2007). The cultures were grown under constant shaking (VS-202D orbital shaker, Vision Scientific, Bucheon, South Korea) and exposed to light in an illuminated incubation room (light: dark cycle of 16:8 h, fluorescent lamp, approximately 55 µmol photons) set at 25°C. Microalgae were cultivated for two weeks, and the resulting cultures were spread on agar plates and incubated until algal colonies formed. Then, the latter would be transferred aseptically to fresh medium (Stanier et al. 1971). The number of colonies that formed on the first set of plats was counted, and data were analyzed as described in the next section. An optical microscope (Nikon Eclipse E100 Biological Microscope, Tokyo, Japan) was used for morphological identification and the 18S V4 region of selected cultures was amplified and sequenced for molecular identification (Stoeck et al. 2010).

Statistical analysis. We compared individual data points using the Student's *t*-test. A p-value of < 0.05 was considered statistically significant. All data were subjected to one-way analysis of variance (ANOVA). All statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS). All the experiments were performed at least in triplicate, and all the traditional microbiological data are expressed as mean ± standard deviation (SD) (n = 3).

Results

Environmental factors and species diversity estimates. The physicochemical characteristics of Jeonglyeongchi, Waegok, and Wangdeungjae marshes are summarized in Table I. The registered average temperatures in Jeonglyeongchi, Waegok, and Wangdeungjae were 12.75°C, 16.55°C, and 22.93°C, respectively. The pH values of all marshes were between pH 6 and 7 – pH 6.95 at Jeonglyeongchi, pH 6.84 at Waegok, and pH 6.48 at Wangdeungjae. The EC values at Jeonglyeongchi and Waegok were 32 and 36 µS/cm, respectively, and significantly lower than 96 µS/cm registered at Wangdeungjae. The marshes differed by approximately 3 mg/l in DO, as its values at Jeonglyeongchi, Waegok, and Wangdeungjae were 10.51, 7.98, and 4.71 mg/l, respectively. The turbidity at Waegok averaged 42.30 nephelometric turbidity units (NTU), which was considerably higher than those at Jeonglyeongchi (2.51 NTU) and Wangdeungjae (6.26 NTU). The TP levels at Jeonglyeongchi and Waegok were 1.57 ± 0.16 and 0.94 ± 0.01 mg/l, respec-

Table I
Physicochemical measurements, sequencing results, and ecological diversity analysis of Mount Jiri marsh samples.

x		Jeonglyeongchi	Waegok	Wangdeungjae
Physico-chemical factors	Temperature (°C)	12.75	16.55	22.93
	pH	6.95	6.84	6.48
	EC (µS/cm)	32	36	96
	Salinity (ppt)	0.0	0.0	0.0
	DO (mg/l)	10.51	7.98	4.71
	Turbidity (NTU)	2.51	42.30	6.26
	TN (mg/l)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	TP (mg/l)	1.57 ± 0.16	0.94 ± 0.01	0.00 ± 0.00
Sequencing results	Total reads	122,953	113,853	121,392
	Validated reads	98,159	80,099	22,249
	Mean read length (bp)	406.28	402.63	401.70
	Maximum read length (bp)	419	407	407
	Number of OTUs[a]	243	828	64
Diversity indicators	Chao1[b]	243.00	828.00	64.00
	Shannon[c]	4.84	6.36	2.97
	Simpson[d]	0.91	0.94	0.75
	Goods Coverage[e]	1.00	1.00	1.00

[a] – OTUs: Operational Taxonomic Units
[b] – Chao1: species richness estimation, a count of the species present
[c] – Shannon: Shannon diversity index (> 0, higher is more diverse)
[d] – Simpson: Simpson diversity index (0 – 1, 1 = most diverse)
[e] – Goods Coverage: number of singleton OTUs/number of sequences (1 = 100% coverage)

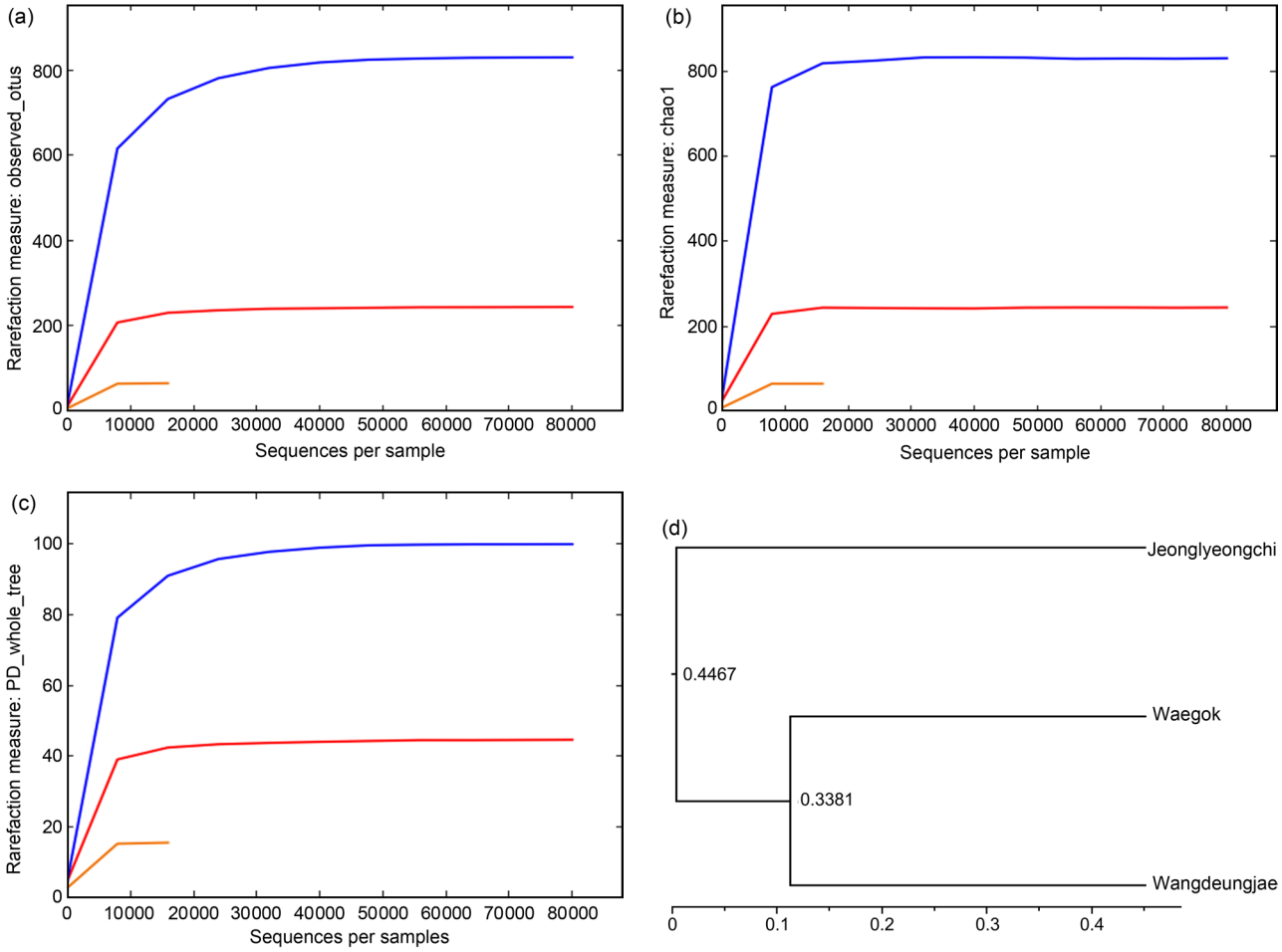


Fig. 2. Rarefaction curves for OTUs representing the eukaryotic microbial communities associated with the marsh samples. The OTUs were analyzed using the cluster database that was set at high identity, with the tolerance (CD-HIT) program set at a 97% sequence similarity. The Mothur platform was used to calculate the rarefaction curves and diversity indices.

a) OTUs. b) Chao1 estimator. c) Whole tree (Waegok, red curve; Jeonglyeongchi, blue curve; Wangdeungjae, orange curve). d) UPGMA tree illustrating the relationships based on weighted UniFrac distances between the eukaryotic microbial communities associated with Jeonglyeongchi, Waegok, and Wangdeungjae marshes.

tively, and undetectable in Wangdeungjae. The salinity and TN levels in all the marshes were below the detection limits. Overall, Jeonglyeongchi and Waegok have shown to have similar physicochemical characteristics.

The analysis of Illumina MiSeq results and taxonomic identifications based on the NCBI database are summarized in supplementary Table SI. The GenBank accession numbers (PRJNA694792) for the microbial community in South Korean Mount Jiri marshes were accepted. In terms of the number of validated reads and their ratio to phylogenetics, Jeonglyeongchi (ratio = 79.83 %) had the highest number and ratio of validated reads, followed by Waegok (ratio = 70.35 %), and Wangdeungjae (ratio = 18.33 %). The mean and maximum read lengths for each marsh were as follows: Jeonglyeongchi, 406.28 and 419 bp; Waegok, 402.63 and 407 bp; and Wangdeungjae, 401.70 and 407 bp. Using a 3% sequence cutoff value, OTUs totaled 243 for Jeonglyeongchi, 828 for Waegok, and 64 for Wangdeungjae. The high numbers of OTUs at Jeonglyeongchi and Waegok have indirectly confirmed the high diversity of the habitats, especially at Waegok.

We measured the species' richness using the Chao1 estimator, which counts the number of species within a community without considering their abundance levels. Shannon and Simpson's diversity indices measured the species' diversity, both of which account for the evenness of species distribution and their abundance (the number of individuals per species). The Chao1, Shannon, and Simpson index values for Waegok were 828.00, 6.36, and 0.94, respectively, which were remarkably higher than the corresponding Wangdeungjae values of 64.00, 2.97, and 0.75, respectively (Fig. 2). The whole tree was obtained by adding up all the branch lengths of a phylogenetic tree to measure diversity based on Waegok, Jeonglyeongchi, and Wangdeungjae (Fig. 2c). The relationships between sites based on the weighted UniFrac distances were generated from our sequence data. Fig. 2d shows that Waegok and Wangdeungjae were the marshes with the most similarity in

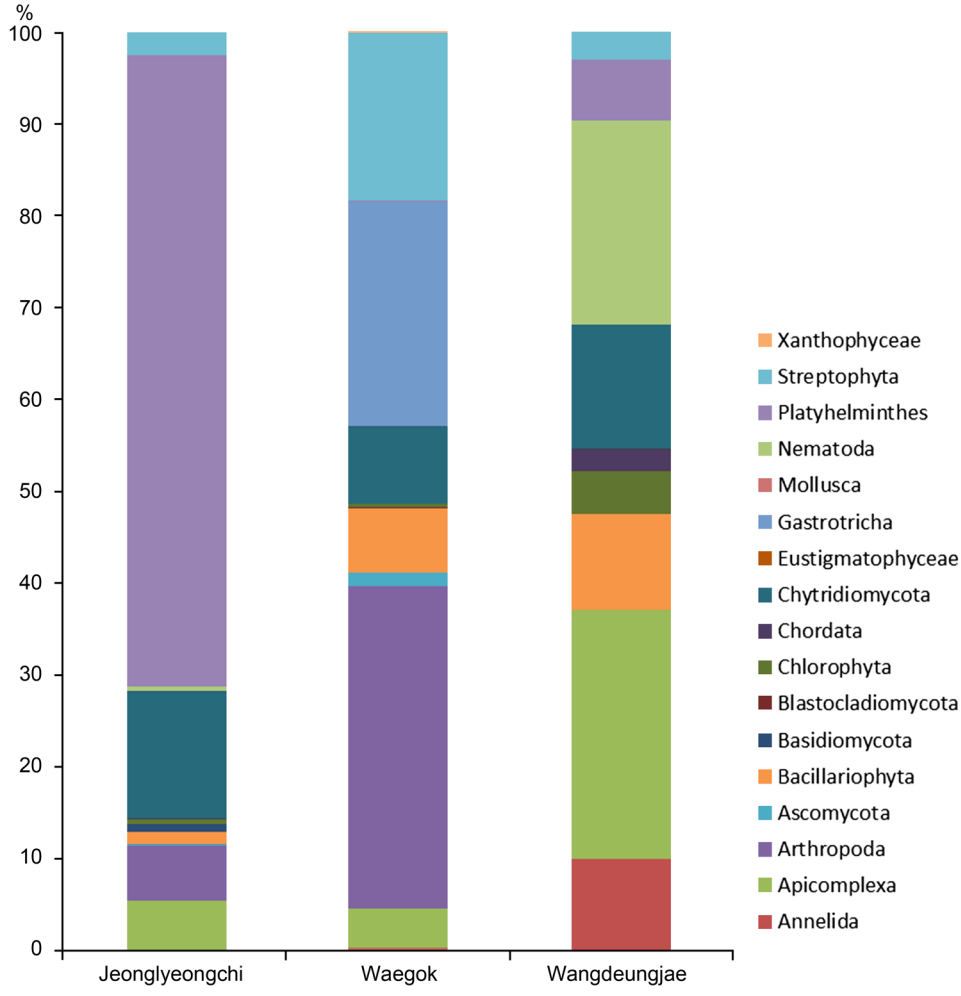


Fig. 3. Taxonomic composition of microalgal and other microbial phyla found in Jeonglyeongchi, Waegok, and Wangdeungjae marsh samples.

eukaryotic communities. Waegok is characterized by moderate environmental conditions and had the highest species richness and diversity among the three sites.

Structure of microbial community and microalgal composition. The taxonomic composition of the eukaryotic microbial communities was analyzed at the phylum level (Fig. 3). Seventeen phyla were detected in the three marshes (Fig. 3), 11 of which were present in Jeonglyeongchi (Table II). Only Chytridiomycota (13.95%) and Platyhelminthes (68.71%) were present at abundance levels greater than 10%. The highest number of phyla was detected in Waegok (15 phyla) (Table II). Of these, Arthropoda (35.01%), Gastrotricha (24.43%), and Streptophyta (18.30%) were present at levels greater than 10%. Nine phyla were detected at Wangdeungjae (Table II), of which Apicomplexa (27.10%), Bacillariophyta (10.41%), Chytridiomycota (13.47%), and Nematoda (22.27%) were present at abundance levels greater than 10%. Phylum distribution was not biased toward a specific phylum. However, Jeonglyeongchi was dominated by phylum Platyhelminthes (among 11 phyla), whereas three-four phyla dominate Waegok and Wangdeungjae. Among the three marshes, Waegok presented the most diverse eukaryotic community.

We found 123 species of unclassified taxonomic names in the three marshes. Table II and supplementary Table SI summarize the relative abundance levels of species in Jeonglyeongchi (33 species), Waegok (96 species), and Wangdeungjae (21 species). The following species were present at abundance levels greater than 5%: Jeonglyeongchi, four species (*Eimeriidae environmental*, *Hygrobates norvegicus*, *Rhizoclosmatium globosum*, and *Phagocata sibirica*); Waegok, three species (*Aedes albopictus*, *Chaetonotus* cf., and *Stipa narynica*); Wangdeungjae, six species (*Dero* sp. *Eimeria* sp., *Aulacoseira* sp., *Chytriomyces* sp., *Eumonhystera* cf., and *Stenostomum* sp.). The phylogenetic relationships between all species comprising the marsh communities were visualized using the ML tree analysis (Fig. 4a) (Schwarz 1978; Felsenstein 1985; Kumar et al. 2008; Kumar et al. 2016). Samples from Waegok had the highest species richness and diversity, with 96 species representing 78.04% of the total species present in all communities.

Table II
Relative abundance of species in the Jeonglyeongchi, Waegok, and Wangdeungjae samples.

Taxonomy					Relative abundance (%)		
Phylum	Class	Order	Family	Species	Jeonglyeongchi	Waegok	Wangdeungjae
Annelida	–	Haplotaxida	Enchytraeidae	*Mesenchytraeus pelicensis*	0	0.05	0
Annelida	–	Haplotaxida	Naididae	*Dero* sp.	0	0.17	9.98
Apicomplexa	–	–	–	*Apicomplexan Acarus*	0	0.01	0
Apicomplexa	–	–	Sphaerocystidae	*Paraschneideria metamorphosa*	0	0.19	0
Apicomplexa	Coccidia	Eucoccidiorida	Cryptosporidiidae	*Cryptosporidiidae environmental*	0	3.09	0.93
Apicomplexa	Coccidia	Eucoccidiorida	Eimeriidae	*Eimeriidae environmental*	5.43	1.11	0
Apicomplexa	Coccidia	Eucoccidiorida	Eimeriidae	*Eimeria* sp.	0	0	26.17
Arthropoda	–	Cyclopoida	Cyclopidae	*Paracyclops chiltoni*	0	0.16	0
Arthropoda	Arachnida	–	Anystidae	*Anystis* sp.	0	0.03	0
Arthropoda	Arachnida	–	Hygrobatidae	*Hygrobates norvegicus*	5.73	0	0
Arthropoda	Insecta	Diptera	Chironomidae	*Micropsectra* sp.	0.31	0	0
Arthropoda	Insecta	Diptera	Chironomidae	*Monodiamesa* sp.	0	0.05	0
Arthropoda	Insecta	Diptera	Culicidae	*Aedes albopictus*	0	34.77	0
Ascomycota	–	–	–	Uncultured ascomycete	0	0.04	0
Ascomycota	Saccharomycetes	Saccharomycetales	Debaryomycetaceae	[Candida] schatavii	0.07	0	0
Ascomycota	Sordariomycetes	–	–	*Leptosporella* sp.	0	0.11	0
Ascomycota	Sordariomycetes	Chaetosphaeriales	Chaetosphaeriaceae	*Thozetella pandanicola*	0	1.24	0
Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	*Diaporthe amygdali*	0	0.02	0
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium oxysporum*	0	0.05	0
Ascomycota	Sordariomycetes	Xylariales	–	*Discosia querci*	0	0.01	0
Bacillariophyta	Bacillariophyceae	–	–	*Achnanthidium daonense*	0	0.04	0
Bacillariophyta	Bacillariophyceae	–	–	*Achnanthidium digitatum*	0	0.11	0
Bacillariophyta	Bacillariophyceae	–	–	*Achnanthidium minutissimum*	0	0.25	0
Bacillariophyta	Bacillariophyceae	–	–	*Achnanthidium straubianum*	0	0.12	0
Bacillariophyta	Bacillariophyceae	–	Bacillariaceae	*Nitzschia acidoclinata*	0	0.04	0
Bacillariophyta	Bacillariophyceae	–	Bacillariaceae	*Nitzschia dissipata*	0	0.18	0
Bacillariophyta	Bacillariophyceae	–	Cymbellaceae	*Cymbella aspera*	0	1.45	0
Bacillariophyta	Bacillariophyceae	–	Cymbellaceae	*Cymbopleura naviculiformis*	0	0.82	0
Bacillariophyta	Bacillariophyceae	–	Cymbellaceae	*Placoneis elginensis*	0	0.09	0
Bacillariophyta	Bacillariophyceae	–	Gomphonemataceae	*Gomphonema affine*	0.57	0.36	0
Bacillariophyta	Bacillariophyceae	–	Gomphonemataceae	*Gomphonema* cf.	0	0.18	0

Table II. Continued.

Taxonomy					Relative abundance (%)		
Phylum	Class	Order	Family	Species	Jeonglyeongchi	Waegok	Wangdeungjae
Bacillariophyta	Bacillariophyceae	Eunotiales	Eunotiaceae	*Eunotia* sp.	0.24	0.14	0.81
Bacillariophyta	Bacillariophyceae	Naviculales	–	*Humidophila australis*	0	0.03	0
Bacillariophyta	Bacillariophyceae	Naviculales	–	Uncultured *Halamphora*	0	0.03	0
Bacillariophyta	Bacillariophyceae	Naviculales	Amphipleuraceae	*Halamphora* sp.	0	0.11	0
Bacillariophyta	Bacillariophyceae	Naviculales	Naviculaceae	*Pinnunavis* sp.	0	0.18	0
Bacillariophyta	Bacillariophyceae	Naviculales	Naviculaceae	*Navicula* sp.	0	0.04	0
Bacillariophyta	Bacillariophyceae	Naviculales	Neidiaceae	*Neidium hitchcockii*	0	0.01	0
Bacillariophyta	Bacillariophyceae	Naviculales	Neidiaceae	*Neidium* sp.	0	0.11	0
Bacillariophyta	Bacillariophyceae	Naviculales	Pinnulariaceae	*Pinnularia* cf.	0	0.11	0
Bacillariophyta	Bacillariophyceae	Naviculales	Pinnulariaceae	*Pinnularia microstauron*	0	0.51	0
Bacillariophyta	Bacillariophyceae	Naviculales	Pinnulariaceae	*Pinnularia subgibba*	0.34	0	0
Bacillariophyta	Bacillariophyceae	Naviculales	Pinnulariaceae	*Pinnularia viridiformis*	0	0.04	0
Bacillariophyta	Bacillariophyceae	Naviculales	Sellaphoraceae	*Sellaphora* cf.	0	0.01	0
Bacillariophyta	Bacillariophyceae	Naviculales	Sellaphoraceae	*Sellaphora pupula*	0	0.04	0
Bacillariophyta	Bacillariophyceae	Surirellales	–	*Surirella brebissonii*	0	0.75	0
Bacillariophyta	Bacillariophyceae	Surirellales	–	*Surirella* cf.	0	0.08	0
Bacillariophyta	Bacillariophyceae	Surirellales	–	*Surirella* sp.	0	0.09	0
Bacillariophyta	Bacillariophyceae	Thalassiophysales	Catenulaceae	*Amphora copulata*	0	0.21	0
Bacillariophyta	Coscinodiscophyceae	–	Aulacoseiraceae	*Aulacoseira alpigena*	0	0.12	1.83
Bacillariophyta	Coscinodiscophyceae	–	Aulacoseiraceae	*Aulacoseira* sp.	0	0	7.77
Bacillariophyta	Coscinodiscophyceae	Chaetocerotales	Chaetocerotaceae	Uncultured Chaetoceros	0	0.02	0
Bacillariophyta	Fragilariophyceae	Fragilariales	Fragilariaceae	*Fragilaria vaucheriae*	0	0.29	0
Bacillariophyta	Fragilariophyceae	Tabellariales	Tabellariaceae	*Tabellaria flocculosa*	0.23	0.44	0
Basidiomycota	Agaricomycetes	Agaricales	–	*Inocybe spuria*	0	0.01	0
Basidiomycota	Agaricomycetes	Polyporales	–	*Fibroporia gossypium*	0.11	0.08	0
Basidiomycota	Tremellomycetes	–	–	*Holtermanniella nyarrowii*	0.04	0	0
Basidiomycota	Tremellomycetes	Cystofilobasidiales	Cystofilobasidiaceae	*Cystofilobasidium macerans*	0.31	0.01	0
Basidiomycota	Tremellomycetes	Filobasidiales	–	*Solicoccozyma terricola*	0.21	0	0
Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	*Filobasidium magnum*	0.15	0	0
Basidiomycota	Tremellomycetes	Tremellales	–	*Cryptococcus carnescens*	0.02	0	0
Basidiomycota	Tremellomycetes	Tremellales	–	*Papiliotrema flavescens*	0.03		0 0

Table II. Continued.

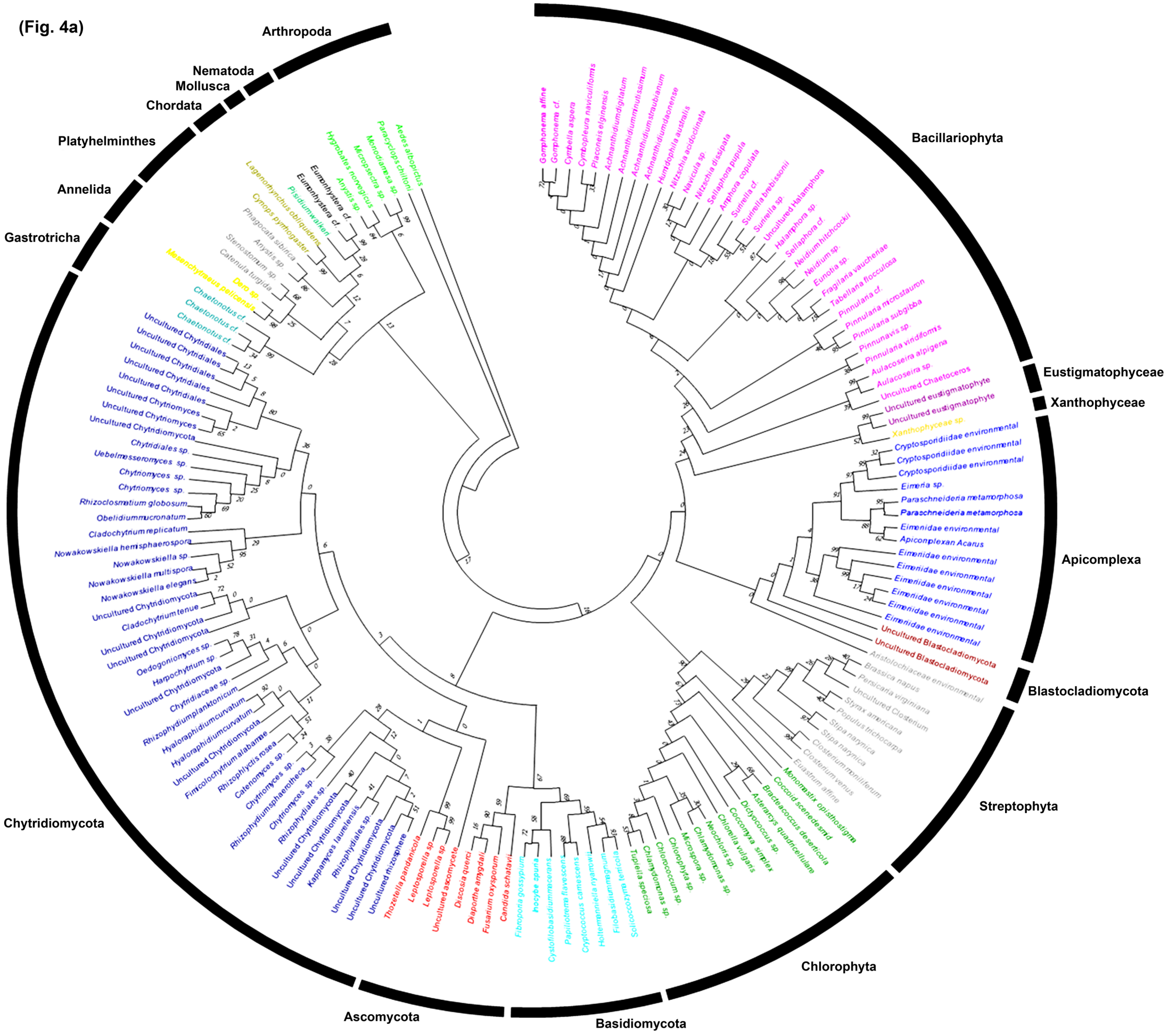
Taxonomy					Relative abundance (%)		
Phylum	Class	Order	Family	Species	Jeonglyeongchi	Waegok	Wangdeungjae
Blastocladiomycota	–	–	–	Uncultured Blastocladiomycota	0	0.11	0
Chlorophyta	–	–	–	Chlorophyta sp.	0	0.01	0
Chlorophyta	Chlorophyceae	–	Microsporaceae	*Microspora* sp.	0	0	1.24
Chlorophyta	Chlorophyceae	Chlamydomonadales	Chlamydomonadaceae	*Chlamydomonas* sp.	0	0.05	1.24
Chlorophyta	Chlorophyceae	Chlamydomonadales	Chlorococcaceae	*Chlorococcum* sp.	0	0.04	0
Chlorophyta	Chlorophyceae	Sphaeropleales	–	*Dictyococcus* sp.	0	0.08	0
Chlorophyta	Chlorophyceae	Sphaeropleales	–	*Bracteacoccus deserticola*	0	0	0.09
Chlorophyta	Chlorophyceae	Sphaeropleales	Neochloridaceae	*Neochloris* sp.	0.22	0	0
Chlorophyta	Chlorophyceae	Sphaeropleales	Scenedesmaceae	*Scenedesmus* sp.	0	0	1.71
Chlorophyta	Chlorophyceae	Sphaeropleales	Scenedesmaceae	*Asterarcys quadricellulare*	0	0.02	0
Chlorophyta	Trebouxiophyceae	–	Coccomyxaceae	*Coccomyxa simplex*	0.15	0.01	0
Chlorophyta	Trebouxiophyceae	Chlorellales	Chlorellaceae	*Chlorella vulgaris*	0	0.01	0.44
Chlorophyta	Ulvophyceae	Ulotrichales	–	*Tupiella speciosa*	0.12	0.03	0
Chlorophyta	prasinophytes	–	–	*Monomastix opisthostigma*	0	0.05	0
Chordata	Amphibia	Caudata	Salamandridae	*Cynops pyrrhogaster*	0.05	0	0
Chordata	Mammalia	Cetacea	Delphinidae	*Lagenorhynchus obliquidens*	0	0	2.45
Chytridiomycota	–	–	–	Uncultured Chytridiomycota	1.31	1.09	0.44
Chytridiomycota	–	–	–	Uncultured rhizosphere	0	0.01	0
Chytridiomycota	Chytridiomycetes	–	–	*Catenomyces* sp.	0	0.08	0
Chytridiomycota	Chytridiomycetes	–	–	*Rhizophlyctis rosea*	0	0	1.58
Chytridiomycota	Chytridiomycetes	Chytridiales	–	*Chytridiales* sp.	0	0.41	0
Chytridiomycota	Chytridiomycetes	Chytridiales	–	Uncultured Chytridiales	0	0.46	0
Chytridiomycota	Chytridiomycetes	Chytridiales	–	Uncultured Chytriomyces	0	0.23	0
Chytridiomycota	Chytridiomycetes	Chytridiales	–	*Chytriomyces* sp.	0.12	0.09	10.94
Chytridiomycota	Chytridiomycetes	Chytridiales	–	*Obelidium mucronatum*	2.62	0.58	0
Chytridiomycota	Chytridiomycetes	Chytridiales	–	*Rhizoclosmatium globosum*	9.07	0	0
Chytridiomycota	Chytridiomycetes	Chytridiales	Chytridiaceae	*Chytridiaceae* sp.	0	0.11	0
Chytridiomycota	Chytridiomycetes	Cladochytriales	–	*Nowakowskiella elegans*	0	0.02	0
Chytridiomycota	Chytridiomycetes	Cladochytriales	–	*Nowakowskiella hemisphaerospora*	0.43	0.17	0
Chytridiomycota	Chytridiomycetes	Cladochytriales	–	*Nowakowskiella multispora*	0	1.61	0
Chytridiomycota	Chytridiomycetes	Cladochytriales	–	*Nowakowskiella* sp.	0	0.16	0

Table II. Continued.

Taxonomy					Relative abundance (%)		
Phylum	Class	Order	Family	Species	Jeonglyeongchi	Waegok	Wangdeungjae
Chytridiomycota	Chytridiomycetes	Cladochytriales	Cladochytriaceae	*Cladochytrium replicatum*	0	0.02	0
Chytridiomycota	Chytridiomycetes	Cladochytriales	Cladochytriaceae	*Cladochytrium tenue*	0	0.01	0
Chytridiomycota	Chytridiomycetes	Rhizophydiales	–	*Rhizophydiales* sp.	0	0.08	0
Chytridiomycota	Chytridiomycetes	Rhizophydiales	–	*Uebelmesseromyces* sp.	0	0.94	0
Chytridiomycota	Chytridiomycetes	Rhizophydiales	Kappamycetaceae	*Kappamyces laurelensis*	0.13	0.01	0
Chytridiomycota	Chytridiomycetes	Rhizophydiales	Rhizophydiaceae	*Rhizophydium planktonicum*	0	0	0.51
Chytridiomycota	Chytridiomycetes	Rhizophydiales	Rhizophydiaceae	*Rhizophydium sphaerotheca*	0	2.14	0
Chytridiomycota	Chytridiomycetes	Spizellomycetales	–	*Fimicolochytrium alabamae*	0.18	0.15	0
Chytridiomycota	Monoblepharidomycetes	Monoblepharidales	–	*Hyaloraphidium curvatum*	0.09	0.02	0
Chytridiomycota	Monoblepharidomycetes	Monoblepharidales	Harpochytriaceae	*Harpochytrium* sp.	0	0.06	0
Chytridiomycota	Monoblepharidomycetes	Monoblepharidales	Oedogoniomycetaceae	*Oedogoniomyces* sp.	0	0.01	0
Eustigmatophyceae	–	–	–	Uncultured eustigmatophyte	0	0.04	0
Gastrotricha	–	Chaetonotida	Chaetonotidae	*Chaetonotus* cf.	0	24.43	0
Mollusca	Bivalvia	Veneroida	Sphaeriidae	*Pisidium walkeri*	0	0.03	0
Nematoda	Chromadorea	Monhysterida	Monhysteridae	*Eumonhystera* cf.	0.46	0	22.27
Platyhelminthes	–	Catenulida	Catenulidae	*Catenula turgida*	0.07	0	0
Platyhelminthes	–	Catenulida	Stenostomidae	*Stenostomum* sp.	0	0.06	6.62
Platyhelminthes	–	Rhabdocoela	Typhloplanidae	*Phaenocora* sp.	0	0.02	0
Platyhelminthes	–	Tricladida	Planariidae	*Phagocata sibirica*	68.64	0	0
Streptophyta	–	Brassicales	Brassicaceae	*Brassica napus*	0	0	0.34
Streptophyta	–	Caryophyllales	Polygonaceae	*Persicaria virginiana*	1.96	0.01	0
Streptophyta	–	Ericales	Styracaceae	*Styrax americana*	0	0.04	0
Streptophyta	–	Malpighiales	Salicaceae	*Populus trichocarpa*	0	2.84	0
Streptophyta	–	Piperales	–	*Aristolochiaceae environmental*	0.16	0	0
Streptophyta	Liliopsida	Poales	Poaceae	*Stipa narynica*	0	15.14	0
Streptophyta	Zygnemophyceae	Desmidiales	–	Uncultured Closterium	0.43	0.06	0
Streptophyta	Zygnemophyceae	Desmidiales	Closteriaceae	*Closterium moniliferum*	0	0.21	0
Streptophyta	Zygnemophyceae	Desmidiales	Closteriaceae	*Closterium venus*	0	0	0.62
Streptophyta	Zygnemophyceae	Desmidiales	Desmidiaceae	*Euastrum affine*	0	0	2.02
Xanthophyceae	–	–	–	*Xanthophyceae* sp.	0	0.05	0

The microbial species detected in at least one of the three samples are shown. Unclassified taxonomic names (phylum, class, order, family, and species) are replaced using underlining (–)

(Fig. 4a)


Arthropoda
Nematoda
Mollusca
Chordata
Platyhelminthes
Annelida
Gastrotricha
Aedes albopictus
Paracyclops chiltoni
Monodiamesa sp.
Micropsectra sp.
Hyrgobates norvegicus
Anystis sp.
Eumonhystera cf.
Eumonhystera cf.
Plesidumwalkeri
Cynops pyrrhogaster
Lagenorhynchus obliquidens
Phagocata sibirica
Anystis sp.
Stenostomum sp.
Catenula turgida
Mesenchytraeus pelicensis
Daro sp.
Chaetonotus cf.
Chaetonotus cf.
Chaetonotus cf.
Uncultured Chytridiales
Uncultured Chytridiales
Uncultured Chytridiales
Uncultured Chytridiales
Uncultured Chytridiales
Uncultured Chytriomyces
Uncultured Chytriomyces
Uncultured Chytridiomycota
Chytridiales sp.
Uebelmesseromyces sp.
Chytriomyces sp.
Chytriomyces sp.
Rhizoclosmatium globosum
Obelidiummucronatum
Cladochytrium replicatum
Nowakowskiella hemisphaerospora
Nowakowskiella sp.
Nowakowskiella multispora
Nowakowskiella elegans
Uncultured Chytridiomycota
Cladochytrium tenue
Uncultured Chytridiomycota
Uncultured Chytridiomycota
Oedogoniomyces sp.
Harpochytrium sp.
Uncultured Chytridiomycota
Chytridiaceae sp.
Rhizophydiumplanktonicum
Rhizophydiumcurvatum
Hyaloraphidiumcurvatum
Hyaloraphidiumcurvatum
Uncultured Chytridiomycota
Fimicolochytrium alabamae
Rhizophlyctis rosea
Catenomyces sp.
Chytriomyces sp.
Rhizophydiumsphaerotheca
Chytriomyces sp.
Rhizophydiales sp.
Uncultured Chytridiomycota
Uncultured Chytridiomycota
Kappamyces laurelensis
Rhizophydiales sp.
Uncultured Chytridiomycota
Uncultured Chytridiomycota
Uncultured rhizosphere
Thozetella pandanicola
Leptosporella sp.
Leptosporella sp.
Uncultured ascomycete
Discosia quercí
Diaporthe amygdali
Fusarium oxysporum
Candida schatavi
Fibroporia gossypium
Inocybe spuna
Cystofilobasidiummicerans
Papiliotrema flavescens
Cryptococcus carnescens
Holtermanniella nyarrowii
Filobasidiummagnum
Solicoccozyma terricola
Tupiella speciosa
Chlamydomonas sp.
Chlorococcum sp
Chlorophyta sp.
Microspora sp.
Chlamydomonas sp.
Neochloris sp.
Chlorella vulgaris
Coccomyxa simplex
Dictyococcus sp
Asterarcys quadricellulare
Bracteacoccus deserticola
Coccoid scenedesmd
Monomastix opisthostigma
Elaastrum affine
Closterium venus
Closterium moniliferum
Stipa narynica
Stipa narynica
Populus trichocarpa
Styrax americana
Uncultured Closterium
Brassica napus
Persicaria virginiana
Aristolochiaceae environmental
Uncultured Blastocladiomycota
Uncultured Blastocladiomycota
Eimeridae environmental
Eimeridae environmental
Eimeridae environmental
Eimeridae environmental
Eimeridae environmental
Eimeridae environmental
Eimenidae environmental
Apiconplexan Acarus
Eimenidae environmental
Paraschneideria metamorphosa
Paraschneideria metamorphosa
Eimeria sp.
Cryptosporidiidae environmental
Cryptosporidiidae environmental
Cryptosporidiidae environmental
Xanthophyceae sp.
Uncultured eustigmatophyte
Uncultured eustigmatophyte
Uncultured Chaetoceros
Aulacoseira sp.
Aulacoseira alpigena
Pinnunavis sp.
Pinnularia viridiformis
Pinnularia subgibba
Pinnularia microstauron
Pinnularia cf.
Tabellaria flocculosa
Fragilaria vaucheriae
Eunotia sp.
Neidium sp.
Neidium hitchcockii
Sellaphora cf.
Halamphora sp.
Uncultured Halamphora
Sunrella sp.
Sunrella brebissonii
Sunrella cf.
Amphora copulata
Sellaphora pupula
Sellaphora dissipata
Mitzschia sp.
Navicula sp.
Nitzschia acidoclinata
Humidophila australis
Achnanthidiumstraubianum
Achnanthidiumminutissimum
Achnanthidiumdigitatum
Achnanthidiumdaonense
Placoneis elginensis
Cymbopleura naviculiforms
Cymbella aspera
Gomphonema cf.
Gomphonema affine
Bacillariophyta
Eustigmatophyceae
Xanthophyceae
Apicomplexa
Blastocladiomycota
Streptophyta
Chlorophyta
Basidiomycota
Ascomycota
Chytridiomycota

(Fig. 4b)

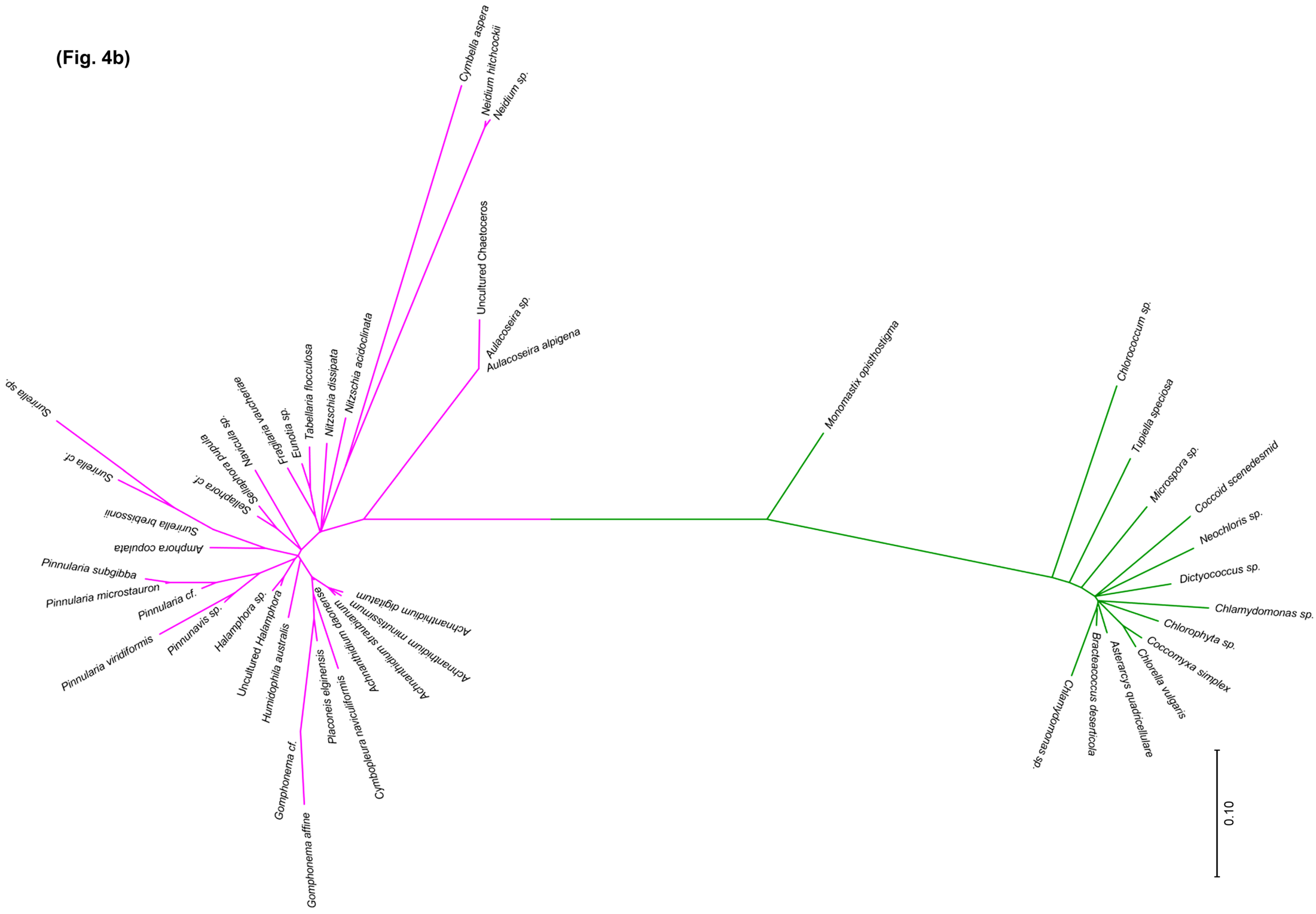


Fig. 4. Molecular phylogenetic analysis by the maximum likelihood (ML) tree.
Numbers at the nodes indicate bootstrap probabilities (> 50 v%)
of the ML analyses (1,000 replicates).

a) Phylogenetic relationship between all species identified using a BLASTN search within the NCBI database. Seventeen phyla corresponded to the species names listed in the phylogenetic tree.
b) Phylogenetic distances between the identified microalgal species (pink branch, Bacillariophyta; green branch, Chlorophyta).

Microalgal groups represented 6.29% in Jeonglyeongchi (1.38% Bacillariophyta, 0.49% Chlorophyta, 0.00% Eustigmatophyceae, 2.55% Streptophyta, and 0.00% Xanthophyceae); 25.69% in Waegok (7.00% Bacillariophyta, 0.30% Chlorophyta, 0.04% Eustigmatophyceae, 18.30% Streptophyta, and 0.05% Xanthophyceae); and 18.11% in Wangdeungjae (10.41% Bacillariophyta, 4.72% Chlorophyta, 0.00% Eustigmatophyceae, 2.98% Streptophyta, and 0.00% Xanthophyceae) (Fig. 3). The mountain marsh microalgae were composed of 34 Bacillariophyta species, 13 Chlorophyta species, one Eustigmatophyceae species, 10 Streptophyta species, and one Xanthophyceae species (Table II): Jeonglyeongchi contained seven species (four Bacillariophyta and three Chlorophyta), Waegok contained 41 species (32 Bacillariophyta and nine Chlorophyta), and Wangdeungjae contained eight species (three Bacillariophyta and five Chlorophyta). The microalgae in Wangdeungjae were eight times more abundant than those at Jeonglyeongchi, although both marshes shared similar numbers of species (eight and seven, respectively). The phylogenetic distances between the identified microalgal species are represented in Fig. 4b. Waegok, which comprised the highest eukaryotic species richness and diversity, also presented the highest number and abundance of microalgal species. Therefore, the diversity of microalgal groups can be related to the diversity and composition of other groups and species in the eukaryotic microbial communities.

Screening of culturable microalgal species. Microalgae were screened and isolated in four media (BG11, OHM, BB, DM) (Table III, Fig. 5 and supplementary Fig. S1). Although sequencing data identified 34 species of diatom (Bacillariophyta) and 13 species of green algae (Chlorophyta) (Table II), only one species of diatom and five species of green algae were isolated from the four media (Table III). Only *Neochloris* sp. was isolated in all four media inoculated with samples from Jeonglyeongchi. Four species (*Nitzschia dissipata*, *Chlamydomonas* sp., *Chlorococcum* sp., and *Chlorella vulgaris*) were isolated on BG11, BB, and DM from the samples from Waegok, whereas two species were isolated on OHM (*Nitzschia dissipata* and *Chlorococcum* sp.), and *Chlamydomonas* sp. and *Scenedesmus* sp. were isolated from all media inoculated with samples from Wangdeungjae (Fig. 5). Overall, while 47 microalgal species were detected via Illumina MiSeq analysis, only six species (12.77 %) were able to be isolated from cultures.

Discussion

Physicochemical characteristics of Jiri marsh sites. Each marsh presents distinctive environmental characteristics. Jeonglyeongchi marsh had the lowest temperatures registered and the highest DO and TP concentrations (Table I), whereas the temperature at Wangdeungjae marsh (above 20°C) was suitable for the cultivation of microorganisms. The latter marsh also recorded the lowest DO and TP concentrations (Tanner 2007). These mesophilic conditions can promote higher levels of microbial activity compared to low temperatures (Tanner 2007). This increased level of metaolic activity can then change the consumption and overall concentrations of DO and TP (Amon and Benner 1996; Levantesi et al. 2002). In addition, pH and EC, which depend on ion concentrations, vary due to metabolites produced during degradation (Kwabiah et al. 2001; Berg and Laskowski 2005; Rousk et al. 2010). These results support the idea that temperature plays a major role as an environmental factor in all the studied marshes (Witkamp and Frank 1970; Tanner 2007; Kukharenko et al. 2010).

Moreover, Illumina MiSeq analyses were used to characterize the diversity of the microbial communities in the three sites. The sequence analysis revealed a Goods Coverage value of 1.00, which means that our sequencing efforts were 100% effective. Waegok marsh had the highest number of OTUs and diversity index values (Chao1, Shannon, Simpson). By associating the physicochemical characteristics of each site with the corresponding diversity results, we can conclude that the moderate environmental conditions in Waegok marsh, in contrast to the relatively extreme conditions in Jeonglyeongchi and Wangdeungjae, provided a more suitable ecosystem for the microbial community (Zhou et al. 2002; Curtis and Sloan 2004; Roesch et al. 2007). Our research suggests that environmental conditions can determine the degree of diversity of the microbial community, resulting from various adaptation processes. The environmental conditions at each site were influenced by the geographic isolation between the mountain marshes.

Ecological differences and relationships among mountain marsh sites in Jiri mountain. According to the UPGMA tree, which analyzed the relationship between the microbial communities of the investigated mountain marsh sites, it can be concluded that the microbial communities of Weagok and Wangdeungjae, which are geographically close (Fig. 1), presented a higher similarity than the microbial communities of Jeonglyeongchi (Fig. 2). In addition, the physicochemical factors of Jeonglyeongchi were different from those of Weagok and Wangdeungjae (Table I). In Jeonglyeongchi, the measured values for temperature (12.75°C), EC (32 μS/cm), and turbidity (2.51 NTU) were the lowest recorded, whereas higher values were observed for pH (6.95), DO (10.51 mg/l), and TP (1.57 ± 0.16 mg/l). Given these facts, it was possible to explain that the microbial community of Jeonglyeongchi was distinctive

Table III
Illumina MiSeq (M) and culture-based analyses of microalgae from Jeonglyeongchi, Waegok, and Wangdeungjae marsh samples.

Species	Accession number	Jeonglyeongchi		Waegok		Wangdeungjae	
		M	CB	M	CB	M	CB
Achnanthidium daonense	KJ658413	–	–	+	–	–	–
Achnanthidium digitatum	KX946582	–	–	+	–	–	–
Achnanthidium minutissimum	MH358459	–	–	+	–	–	–
Achnanthidium straubianum	KY863467	–	–	+	–	–	–
Nitzschia acidoclinata	KT072971	–	–	+	–	–	–
Nitzschia dissipata	AJ867018	–	–	+	+	–	–
Cymbella aspera	KJ011615	–	–	+	–	–	–
Cymbopleura naviculiformis	AM501997	–	–	+	–	–	–
Placoneis elginensis	AM501953	–	–	+	–	–	–
Gomphonema affine	MN197879	+	–	+	–	–	–
Gomphonema cf.	AM502005	–	–	+	–	–	–
Eunotia sp.	KJ961696	+	–	+	–	+	–
Humidophila australis	KM116120	–	–	+	–	–	–
Uncultured Halamphora	MK656307	–	–	+	–	–	–
Halamphora sp.	MG027261	–	–	+	–	–	–
Pinnunavis sp.	KJ961669	–	–	+	–	–	–
Navicula sp.	MK177604	–	–	+	–	–	–
Neidium hitchcockii	KU674393	–	–	+	–	–	–
Neidium sp.	KU674445	–	–	+	–	–	–
Pinnularia cf.	JN418569	–	–	+	–	–	–
Pinnularia microstauron	AM501981	–	–	+	–	–	–
Pinnularia subgibba	KT072984	+	–	–	–	–	–
Pinnularia viridiformis	AM501985	–	–	+	–	–	–
Sellaphora cf.	EF151967	–	–	+	–	–	–
Sellaphora pupula	AJ544653	–	–	+	–	–	–
Surirella brebissonii	KX120739	–	–	+	–	–	–
Surirella cf.	KX120782	–	–	+	–	–	–
Surirella sp.	KX120781	–	–	+	–	–	–
Amphora copulata	MG027291	–	–	+	–	–	–
Aulacoseira alpigena	AY569578	–	–	+	–	+	–
Aulacoseira sp.	AY569587	–	–	–	–	+	–
Uncultured Chaetoceros	MH023058	–	–	+	–	–	–
Fragilaria vaucheriae	AM497736	–	–	+	–	–	–
Tabellaria flocculosa	MH356258	+	–	+	–	–	–
Chlorophyta sp.	MK929233	–	–	+	–	–	–
Microspora sp.	AF387160	–	–	–	–	+	–
Chlamydomonas sp.	MH683856	–	–	+	+	+	+
Chlorococcum sp.	MK954470	–	–	+	+	–	–
Dictyococcus sp.	HM852440	–	–	+	–	–	–
Bracteacoccus deserticola	JQ259938	–	–	–	–	+	–
Neochloris sp.	AB917132	+	+	–	–	–	–
Scenedesmus sp.	MH010849	–	–	–	–	+	+
Asterarcys quadricellulare	MN179327	–	–	+	–	–	–
Coccomyxa simplex	MH196858	+	–	+	–	–	–
Chlorella vulgaris	MK652782	–	–	+	+	+	–
Tupiella speciosa	MF000567	+	–	+	–	–	–
Monomastix opisthostigma	FN562445	–	–	+	–	–	–

+ – detected; – – undetected

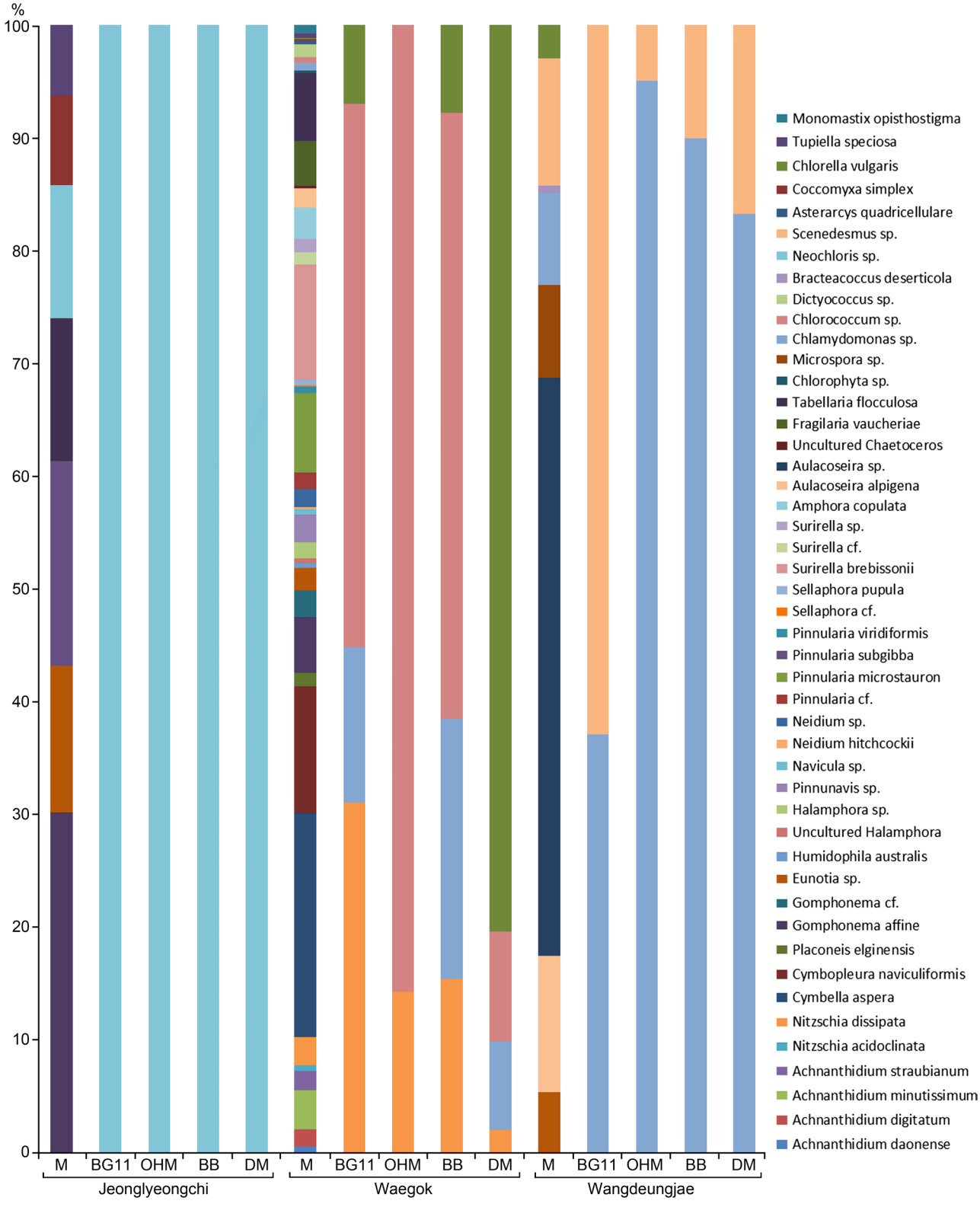


Fig. 5. Composition of microalgal species grown in each culture medium and identified using the Illumina MiSeq analysis (M). Four culture media were used: Blue Green-11 (BG11) medium, Optimum *Haematococus* Medium (OHM), Bold Basal medium (BB), and Diatom Medium (DM).

from other sites, and this was due to the variable inter-marsh physicochemical factors. However, when comparing the differences between microbial communities through the number of OTUs and diversity indicators (Chao1, Shannon, Simpson), these values showed high similarity between Jeonglyeongchi and Waegok and less to Wangdeungjae (Table I). This fact contradicted the relationship between mountain marshes based

on physicochemical factors. This disparity could be resolved through the composition of the microbial community (supplementary Table SII). While 68.71% of the microbial community in Jeonglyeongchi was dominated by one species belonging to Platyhelminthes, the microbial community of Weagok and Wangdeungjae was composed of several species belonging to 3–4 phyla (Table II). Thus, it is believed that the similarity between microbial communities does not depend on diversity indicators (Miller et al. 2020; Wen et al. 2020). Nonetheless, we support that the comparison between microbial communities should be accompanied by a composition comparison factor (Shi et al. 2020). The composition of microbial communities is thought to be influenced by physicochemical factors, and this way, both studies are complementary (Sun et al. 2020). Thus, the microbial community of mountain marshes, separated due to the topographic features of Mount Jiri, needs diverse research approaches study of physicochemical factors and diversity indicators to understand their microbial community fully.

Taxonomic composition of phyla at mountain marsh sites. The phyla comprising the microbial communities of the three marsh sites is shown in Fig. 3. In addition, the taxonomic compositions from phyla to respective species levels are summarized in Table II. The most abundant phyla (present in more than 10% of the microbiome's taxonomic) included Apicomplexa, Arthropoda, Bacillariophyta, Chytridiomycota, Gastrotricha, Nematoda, Platyhelminthes, and Streptophyta (Fig. 3). Each phylum plays a particular ecological role, either as a producer, decomposer, or consumer. For example, many species of Apicomplexa are parasitic to aquatic animals (Bolland et al. 2020; Laghzaoui et al. 2020). Arthropoda includes animal species such as insects that consume a variety of materials, from living biomass (e.g., algae) to organic carbon sources (e.g., plant byproducts) (Shayanmehr et al. 2020; Sperfeld et al. 2020). Bacillariophyta is composed of autotrophic, photosynthetic organisms such as microalgae that are easily observed in aquatic ecosystems (Al-Handal et al. 2020; Stancheva et al. 2020). Chytridiomycota is a phylum of fungi that includes zoosporic fungal species, which function as heterotrophs in aquatic environments (Jeronimo and Amorim Pires-Zottarelli 2020; McKindles et al. 2020). Gastrotricha comprises various zooplankton species, including predators that feed on phytoplankton (Bosco et al. 2020), whereas Nematoda combines parasitic species and species that consume and decompose organic matter (Jeong et al. 2020; Netherlands et al. 2020). The phylum Platyhelminthes includes species that consume organic matter attached to the bottom and surface, and feed on algae and other microorganisms and plant byproducts (Geraerts et al. 2020; Schadt et al. 2021). Species belonging to Streptophyta include autotrophs capable of photosynthesis (Stamenković et al. 2020; Williamson and Carter 2020). Based on these characteristics, Bacillariophyta and Streptophyta are considered producers (Pushkareva et al. 2016; Shnyukova and Zolotareva 2017); multicellular Arthropoda, Nematoda, and Platyhelminthes and unicellular Chytridiomycota are considered decomposers that decompose and consume organic materials (Berg and McClaugherty 2003; Berg and Laskowski 2005; Gessner et al. 2007; Gulis et al. 2019); and predators (Gastrotricha) and parasites (Apicomplexa) are considered consumers (Norén et al. 1999; Todaro et al. 2006). Most of the major taxa constituting the microbial community of the marshes are decomposers, and their composition differed by region. Jeonglyeongchi comprises more Chytridiomycota and Platyhelminthes, whereas Arthropoda is mostly seen in Waegok, and Chytridiomycota and Nematoda in Wangdeungjae. Among these phyla, only Chytridiomycota exceeded 5% abundance in all investigated regions (Fig. 3). Chytridiomycota is considered a decomposer that can parasitize microalgae (Ibelings et al. 2004; Gessner et al. 2007; Scholz et al. 2014; Gulis et al. 2019). Several species of Chytridiomycota are also parasitic on microalgal populations, thus affecting their growth (Ibelings et al. 2004; Scholz et al. 2014). This parasitic capacity of Chytridiomycota suggested that it may influence the community composition of Bacillariophyta and Chlorophyta in Jiri marshes. Finally, the predatory activity of Gastrotricha (a consumer) suggests that this group may be involved in the predominance of Streptophyta (a producer) by inhibiting the population growth of other microalgae (Todaro et al. 2006).

Our analysis reveals that each major phylum is represented by specific species. The major phyla at Jeonglyeongchi marsh, Chytridiomycota and Platyhelminthes, were represented by *Rhizoclosmatium globosum* and *Phagocata sibirica*, respectively. The major phyla at Waegok marsh, Arthropoda, Gastrotricha, and Streptophyta, were represented by *Aedes albopictus*, *Chaetonotus* cf., and *Stipa narynica*, respectively. The major phyla of Wangdeungjae marsh, Apicomplexa, Bacillariophyta, Chytridiomycota, and Nematoda, were represented by *Eimeria* sp., *Aulacoseira* sp., *Chytriomyces* sp., and *Eumonhystera* cf., respectively. The relative abundances of the predominant species ranged from 65.02% to 100.00%. Bacillariophyta and Chytridiomycota were least likely to be dominated by specific species. Furthermore, Bacillariophyta (34 species) and Chytridiomycota (26 species) were the largest phyla, representing 27.64% and 21.14%, respectively, of a total of 123 detected species. These results suggested that Bacillariophyta and Chytridiomycota were strongly associated with the species richness and diversity of microbial communities in mountain marshes.

Of all the microorganisms recorded in the three studied marshes, producers (Bacillariophyta and Streptophyta) accounted for less than 30% of the total abundance. Because producers were not a significant fraction of the community, consumers were probably dependent on externally derived organic materials (Lu and Wu 1998). For example, Platyhelminthes, a dominant consumer in Jeonglyeongchi, is likely dependent on externally derived organic materials (Roca et al. 1992; Lu and Wu 1998). Although producers were not abundant, their diversity may have had a significant impact on the diversity of the microbial community (Worm et al. 2002; Hillebrand et al. 2007; Cardinale et al. 2011). Bacillariophyta (with the most significant number of species, 34) and Streptophyta (with the fourth-largest number of species, 10) accounted for 35.77% of the total species. The producer group accounted for 17.65–39.58% of the species in the region (17.65% in Jeonglyeongchi, 39.58% in Waegok, and 28.57% in Wangdeungjae). These results discriminated the distribution of species relative to the abundance of the producer group (Hillebrand et al. 2007; Cardinale et al. 2011). Thus, the diversity of producers is highly important in determining the diversity of the local microbial community.

Comparison of marsh sites using culture-based and Illumina MiSeq analyses. We have cultured and identified one-four microalgal species from each marsh site using several types of media (Fig. 5 and supplementary Fig. S1). The following species were isolated and identified: *Neochloris* sp. at Jeonglyeongchi; *Nitzschia dissipata*, *Chlamydomonas* sp., *Chlorococcum* sp., and *Chlorella vulgaris* at Waegok; and *Chlamydomonas* sp. and *Scenedesmus* sp. at Wangdeungjae. Although the species were distributed disproportionately in each medium, only one species tended to be dominant among the few that grew (supplementary Fig. S1). A single species dominated in the BG11 and DM medium but not in the OHM and BB medium (supplementary Fig. S1). We were able to isolate representatives of Bacillariophyta and Chlorophyta, but not Streptophyta, in the culture media (Table III, Fig. 5 and supplementary Fig. 1). Isolated species included *Neochloris* sp., *Nitzschia dissipata*, *Chlamydomonas* sp., *Chlorococcum* sp., *Chlorella vulgaris,* and *Scenedesmus* sp. Only one species, *Nitzschia dissipata,* belonged to Bacillariophyta. The relative abundances of isolated species varied depending on the medium used (Fig. 5 and supplementary Fig. S1) (DiGiulio et al. 2008). It is known that only certain species can be cultivated and their growth depends on the composition of the medium chosen (Harrison and Davis 1979). It suggests that culture-based methods are not suitable for detecting multiple microalgal species, a severe limitation in determining community compositions (Alain and Querellou 2009). Furthermore, the inability to purely isolate 100% of all microbial species present using existing culture techniques and media means that the identification of unculturable microbes is limited. Therefore, microalgal community research based solely on culture analysis is limited because of the difficulty in identifying unculturable microorganisms (Handelsman 2004; Shokralla et al. 2012; Bodor et al. 2020). In contrast to culture-based methods, Illumina MiSeq can effectively analyze the microbial community structure of environmental samples, including the identification and analysis of unculturable microorganisms. Illumina MiSeq analysis overcomes the limitations of the culture-based analysis, providing a more accurate representation of the diversity of the microbial community.

Characteristics of microalgae in the marshes of Jiri. Most microalgae in aquatic environments with water flow are attached to surfaces (Benito 2020; Plante et al. 2021). Typically, attached algae are dominated by diatoms, including Bacillariophyta and some green algae, including Chlorophyta (Yun et al. 2019; Benito 2020; Plante et al. 2021). Therefore, in an environment with water flow, the floating algae are relatively less abundant (Yun et al. 2019; Prazukin et al. 2020). In an aquatic environment where water flow is weak, floating algae dominate, with its species' composition often determined by environmental factors (Mashwani 2020). The microalgae present in the Jiri marshes were mainly composed of Bacillariophyta and Streptophyta (Ali et al. 2019; Garduño-Solórzano et al. 2020). While it is known that Chlorophyta tends to dominate in other aquatic environments (Amorim and Moura 2021), our results suggest that environmental differences determined the dominant microalgal groups.

Furthermore, to better understand the differences between these regional microalgal groups, a more comprehensive set of environmental factors should be investigated using a multidisciplinary rather than a fragmentary approach (Paquette et al. 2020; Sutherland et al. 2020). Our study provides information on the microbial communities and microalgal groups present in the Jiri marshes. Furthermore, our results suggest that it is important to analyze the taxonomic composition of the microalgae present in mountain marshes.

Conclusion

The highest levels of species richness and diversity among the three Jiri high marshes were found in the Waegok marsh, which may be due to the environment's physicochemical characteristics. Analysis of community composition revealed that species' abundance was concentrated in the decomposer group, whereas species' diversity was based in the producer group. Moreover, the consumer group was related to the producer group.

Based on these results, we suggest that producers do not support the entire microbial community, but they determine phylogenetic diversity. Illumina MiSeq analysis overcame the inherent limitations of the culture-based analysis, i.e., incomplete or biased results. Our analyses provide a clear association between the environmental conditions of three mountain marshes and the properties of their respective microbial and microalgal communities. Further research on the roles and interactions between microbial and microalgal communities should be investigated along with their environmental impacts. The data generated in this study can be used to identify mountain areas based on their microalgal communities and help understand the role of environmental factors in their geography.

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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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