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Phylogenetic Analysis of Harmful Algal Bloom (HAB)-Causing Dinoflagellates Along the Korean Coasts, Based on SSU rRNA Gene

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Abstract Twenty-three cultures of harmful algal bloom (HAB)-causing dinoflagellates were isolated from the coastal waters of Korea. For each of the 14 morphospecies, the nuclear-encoded small subunit (SSU) rDNA was analyzed to determine the phylogenetic relatedness of the species. Despite temporal and spatial isolation, 3–4 clonal cultures of *Alexandrium catenella*, *Cochlodinium polykrikoides*, and *Gymnodinium catenatum* had 100% identical SSU rDNA sequences. In contrast, heterogeneities in the SSU rDNA sequences were observed in *Akashiwo sanguinea* and *Lingulodinium polyedrum* strains. Extreme sequence polymorphism was shown within the SSU rRNA genes of an *Al. tamarense* clonal culture. A homology search in GenBank revealed that 11 dinoflagellate species were located in clusters corresponding to their morphological classification. The SSU rDNA sequences of *C. polykrikoides*, *Gyrodinium instriatum*, and *Pheopolykrikos hartmannii*, which were determined for the first time in this study, showed the following phylogenetic relationships: *C. polykrikoides* formed an independent branch separated from other dinoflagellates; *Gyr. instriatum* was placed in a monophyletic group with *Gyr. dorsum* and *Gyr. uncatenum*; and *Ph. hartmanii*, which forms a distinct two-celled pseudocolony, belonged to *Gymnodinium sensu* Hansen and Moestrup.

Key words: SSU rDNA, dinoflagellate, harmful algal bloom, phylogeny

Microalgae are unicellular protists that live in diverse habitats and have a variety of trophic behaviors and feeding strategies. They play important ecological roles in marine aquatic habitats as primary producers and some of them produce commercially useful pigments with antioxidant properties [22]. Among them, some dinoflagellates produce toxins that threaten human health, or form large harmful algal blooms (HABs) that cause considerable economic losses [4, 15].

HABs were rare along the Korean coasts until the 1970s. During the 1980s and 1990s, their frequency and persistency levels gradually increased, and in the last decade, long stretches of Korean coastline have been threatened by a variety of harmful or toxic algal species, leading to large economic losses for local fishery industries and development of technology for the mitigation of HABs [5, 24]. The conventional HAB monitoring program has involved in identifying and counting algal species in field samples, mapping their spatial and temporal distribution, and correlating this information with physical and chemical properties of the surrounding waters. However, the fine-

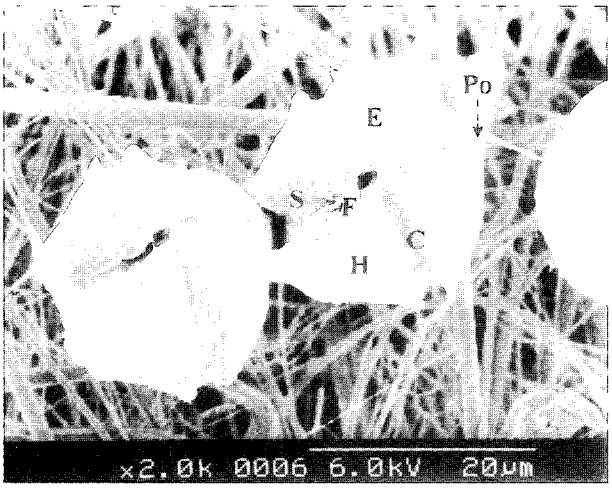

Fig. 1. The ventral view of a dinoflagellate cell (*Alexandrium famarense*) showing morphology.
Po, apical pore plate; E, epitheca; C, cingulum; S, sulcus; F, flagella; H, hypotheca.

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scale morphological features of HAB-causing species are difficult to distinguish at the species level under a light microscope (Fig. 1) [42].

Ribosomal RNA (rRNA) or its coding gene (rDNA) has been used extensively to infer the phylogenetic relationships of diverse organisms [8, 41]. Small subunit (SSU) rDNA contains not only highly conserved regions, but also divergent regions that may be useful for making a discrimination of closely related organisms [50]. In the past decade, considerable work has been done in the genetic characterization of dinoflagellate species, and their rDNA sequences have been used for phylogenetic studies [10, 35, 37]. In this study, we analyzed the SSU rDNA sequences of 23 dinoflagellate isolates, which included 14 species known to be HAB-causing along the Korean coast [24, 26], collected from Korean coastal waters. Our goals were to provide the genetic information of these species and to determine their phylogenetic relationships.

MATERIALS AND METHODS

Cultures and Growth Conditions

Twenty-three clonal cultures of dinoflagellates known to be HAB-causing along the Korean coast were obtained; their sources are listed in Table 1. *Akashiwo sanguinea* Gsakor11, *Alexandrium catenella* Axsp-K01, Axsp-K03, AxCt-K01, *Alexandrium* sp. AspAxp03, and *Prorocentrum minimum* PmiPrMu21 were obtained from culture collection of algae at the Korea Ocean Research and Development Institute. *Cochlodinium polykrikoide* CP0, CP-PP4, and CP-PP6 were obtained from the culture collection of algae at National Fisheries Research and Development Institute. The rest were obtained from the Department of Aquaculture, Pukyung National University, Korea. Algal cultures were maintained in SW II medium (72 mg KNO_3, 4.5 mg KH_2PO_4, 10.5 mg Na_2-glycerophosphate, 0.5 mg Fe-EDTA, 0.3 μg Vitamin B_{12}, 1 mg Biotin, 0.1 μg Thiamine, 1 g Tris, 30 ml soil extract in 970 ml of sea water, pH 7.85) [21] at 20°C and 50–100 μmol photons·m^{-2}·s^{-1} of cool white light under a 14L:10D photoperiod. At monthly intervals, a small portion of each culture was transferred to another test tube or a 250-ml flask containing a fresh medium.

SSU rDNA Analyses

Genomic DNA was extracted from late-exponential phase cells using an Aqua pure Genomic DNA isolation kit (Bio-Rad), according to the manufacturer's protocol (with RNase H addition). The SSU rDNAs (*ca.* 1,790 nucleotides)

Table 1. List of dinoflagellate strains examined in this study and GenBank Accession Numbers for their nuclear SSU rDNA sequences.

Species	Strain Code[1]	Sampling date	Sampling site	Remark[2]	GenBank Acc. No.
Akashiwo sanguinea	GSW0207	Jul 2002	Gosung	Bloom former	AY421770
Akashiwo sanguinea	Gsakor11	Sep 2000	Geoje	Bloom former	AY421771
Alexandrium catenella	Axsp-K01	Apr 1998	Jinhae Bay	PST producer	AY421772
Alexandrium catenella	Axsp-K03	Apr 1998	Geoje	PST producer	AY421773
Alexandrium catenella	AxCt-K01	Apr 1994	Jinhae Bay	PST producer	AY421774
Alexandrium catenella	JHW9706	Jun 1997	Jinhae Bay	PST producer	AY421775
Alexandrium fraterculus	JHW0108-1	Aug 2001	Jinhae Bay	Bloom former	AY421776
Alexandrium tamarense	JHW0004-12	Apr 2000	Jinhae Bay	PST producer	AY421777
Alexandrium sp.	AspAxp03	Apr 2000	Geoje	Bloom former	AY421778
Cochlodinium polykrikoides	BSW0109	Sep 2001	Busan	Harmful species	AY421779
Cochlodinium polykrikoides	CP0	Sep 1997	Yeosu	Harmful species	AY421780
Cochlodinium polykrikoides	CP-PP4	Sep 2000	Geoje	Harmful species	AY421781
Cochlodinium polykrikoides	CP-PP6	Sep 2000	Yeosu	Harmful species	AY421782
Gymnodinium catenatum	JHW9910	Oct 1999	Jinhae Bay	PST producer	AY421783
Gymnodinium catenatum	DC99A44	Aug 1998	Deukryang Bay	PST producer	AY421784
Gymnodinium catenatum	GCSW962	Sep 1996	Jinhae Bay	PST producer	AY421785
Gyrodinium instriatum	JHW0007	Jul 2000	Jinhae Bay	Bloom former	AY421786
Heterocapsa triquetra	GSW0206-2	Jun 2002	Gosung	Bloom former	AY421787
Lingulodinium polyedrum	DRW0208	Aug 2001	Deukryang Bay	YTXs producer	AY421788
Pheopolykrikos hartmannii	JHC0203	Mar 2001	Jinhae Bay	Bloom former	AY421789
Prorocentrum minimum	PmiPrMu21	Jul 1999	Geoje	VST producer	AY421790
Protoceratium reticulatum	JHW0007-6	Jul 2000	Jinhae Bay	YTXs producer	AY421791
Scrippsiella trochoidea	GSW9808	Aug 1998	Gunsan	Bloom former	AY421792

[1]Strain name was usually designated as a mnemonics for the sampling site, origin, and date (GS: Gosung; W: water column origin; C: sediment cyst origin; 0207, Jul 2002) with a serial number.

[2]See the text for the literatures cited; PST: paralytic shellfish toxin; VST: venerupin shellfish toxin; YST: yessotoxin.

Table 2. Primers used for PCR amplification and sequencing of SSU rDNA.

Primer[1]	Sequence (5'→3')	Source
18S-0009f[2]	GATCCTGCCAGTAGTCATAT	This study
18S-0302f[3]	AGTTTCTGACCTATCAG	This study
18S-0437r[3]	GCGCCTGCTGCCTTCCTTA	This study
18S-0613f[3]	GCGGTTAAAAAGCTCGTAGT	This study
18S-0897f[3]	AGAGGTGAAATTCTTGGAT	This study
18S-1179f[3]	CTTAATTTGACTCAACACG	This study
18S-1435f[3]	AACAGGTCTGTGATGCCCTT	This study
18S-1797r[2]	GATCCTTCYGCAGGTTCACCTAC	This study

[1]Primer nomenclature corresponds to *Prorocentrum micans* SSU rDNA position [18]; "f" represents forward and "r" reverse.
[2]Primer set for PCR amplification and sequencing.
[3]Primers for sequencing.

were amplified by PCR with the universal eukaryotic primer set (18S-0009f 5'-GAT CCT GCC AGT AGT CAT AT-3' and 18S-1797r 5'-GAT CCT TCY GCA GGT TCA CCT AC-3') using a DNA Thermal Cycler (Perkin Elmer). PCR reactions were performed in 50 µl of 1×Ex *Taq*™ buffer, 2.5 mM dNTPs, 100 pmol of the primer set, 100 ng of template DNA, and 0.5 units of Takara Ex *Taq*™ (Takara, Japan). The optimal cycling conditions were an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min, with a final elongation at 72°C for 7 min. The fresh PCR product was purified with a QIAquick™ PCR Purification Kit (Qiagen), and the cycle sequencing reaction was performed using an ABI PRISM BigDye™ Terminator v3.0 Cycle Sequencing Kit (Applied Biosystems, Perkin Elmer). The sequencing was run on an ABI 3100 Genetic Analyzer (Applied Biosystems) with PCR primers and conserved internal primers (Table 2). The fresh PCR product of *Alexandrium tamarense* JHW0004-12, which contained many polymorphic sites, including deletions was ligated and transformed using pGEM®-T Vector Systems (Promega), according to the manufacturer's instructions. After color selection, white *E. coli* colonies were cultured, and the plasmid DNAs were extracted using the Wizard® Plus SV Minipreps DNA Purification System (Promega). Finally, eight plasmid DNAs were subjected to the cycle sequencing reaction, as described above. The SSU rDNA sequences of the dinoflagellate strains analyzed in this study were submitted to the GenBank database under accession numbers of AY421770 to AY421792 (Table 1).

Phylogenetic Analyses

The sequences were aligned using Clustal W [46] and edited manually. The SSU rDNAs, excluding primer regions, were used for the phylogenic analyses. Ambiguously aligned regions were removed prior to performing the analyses. Sequence polymorphisms were treated as "n". A maximum-likelihood (ML) tree was constructed with representatives of each dinoflagellate species available in GenBank, and using *Noctiluca scintillans* as an outgroup. Ambiguously aligned taxa and highly divergent taxa were not included in the final phylogenetic analyses. When the sequence homology for a given species was 100%, only one representative sequence was included to minimize calculation time. The ML tree was constructed with a General Time Reversible model using invariant sites and a gamma distribution (GTR+I+G) by PAUP* 4.0b8 [43] using the following likelihood settings: base frequencies of A=0.24485, C=0.20752, G=0.21871, T=0.32892; base substitution rates of AC=1.081900, AG=3.343160, AT=1.168710, CG=0.558320, CT=5.082800, GT=1.00000; the assumed proportion of invariable sites=0.160021; and the gamma shape parameter=0.239081. Heuristic searches with random sequence addition and tree bisection-reconnection (TBR) branch rearrangements were carried out with 100 replicates. Bootstrap values of the ML tree were substituted with Bayesian posterior probabilities (PP) that are roughly equivalent to the ML bootstrap [19, 20]. A Markov Chain Monte Carlo (MCMC) from a random starting tree was initiated in the Bayesian inference (BI; MrBayes 3.0b4) [19] using a GTR+I+G model and was run for 1,000,000 generations. After convergence, a consensus tree was made using the MCMC trees.

Results and Discussion

Phylogenetic Relationships Among Major Dinoflagellate Clades

Twenty-three dinoflagellate strains, which included 14 species associated with harmful or toxic algal blooms, were isolated from the coastal waters of Korea. The SSU rDNA sequences of these dinoflagellates were analyzed to reveal their phylogenetic relationships. PCR amplification of the SSU rDNAs using the universal primer set consistently yielded a single PCR band of *ca.* 1.8 kb (Table 1). The direct sequencing of the PCR products using the PCR primer set and internal sequencing primers successfully generated nearly complete SSU rDNA sequences for 22 strains. However, in the case of *Alexandrium tamarense* JHW0004-12, clean electropherograms were not obtained because of extensive sequence polymorphisms in the SSU rRNA genes (see below).

Twenty-five representative dinoflagellate sequences, including *Noctiluca scintillans* as an outgroup taxon, were obtained from GenBank and were subjected to phylogenetic analysis with the 14 species in this study. Tree topologies were slightly different according to the tree-making algorithms employed, such as the neighbor-joining (NJ), maximum-parsimony (MP), and maximum-likelihood (ML) methods (data not shown). Only the ML tree is presented here because this method, along with the GTR+I+G model,

were considered to reflect the true phylogeny (Fig. 2). Generally, the phylogenetic relationships among the major dinoflagellate clades were poorly resolved and did not have strong statistical supports for their branching order. However, some generalized conclusions were made as follows.

Dinoflagellate species belonging to the Order Gonyaulacales consistently formed a monophyletic branch supported by high posterior probability (PP) values in the phylogenetic tree. The Order Prorocentrales that have been divided into two clades (i.e., *Exuviella* and *Prorocentrum*) in other studies [33, 35], grouped together in the ML tree (Fig. 2). However, the PP value was too low to ascertain whether desmokont prorocentroids, which have two dissimilar flagella emerged from the anterior part of the cell, form a true monophyletic clade. In contrast, the Order Gymnodiniales, characterized by the lack of thecal plates, was polyphyletic and split into two clades.

Unarmored dinoflagellates with a horseshoe-shaped apical groove running in an anticlockwise direction (i.e., *Gymnodinium sensu* Hansen and Moestrup) [10] always grouped into a single clade with *Lepidodinium* and *Pheopolykrikos*. The genus *Lepidodinium*, which contains chlorophyll *b* as the major accessory pigment, emerged within the *Gymnodinium sensu stricto* clade that usually has a common dinoflagellate carotenoid, peridinin [38]. This result further supports the contention that pigmentation in dinoflagellates arose multiple times during evolution [35]. Gymnodinioids with apical grooves of other shapes and/or with different pigment compositions tended to branch near each other in the SSU

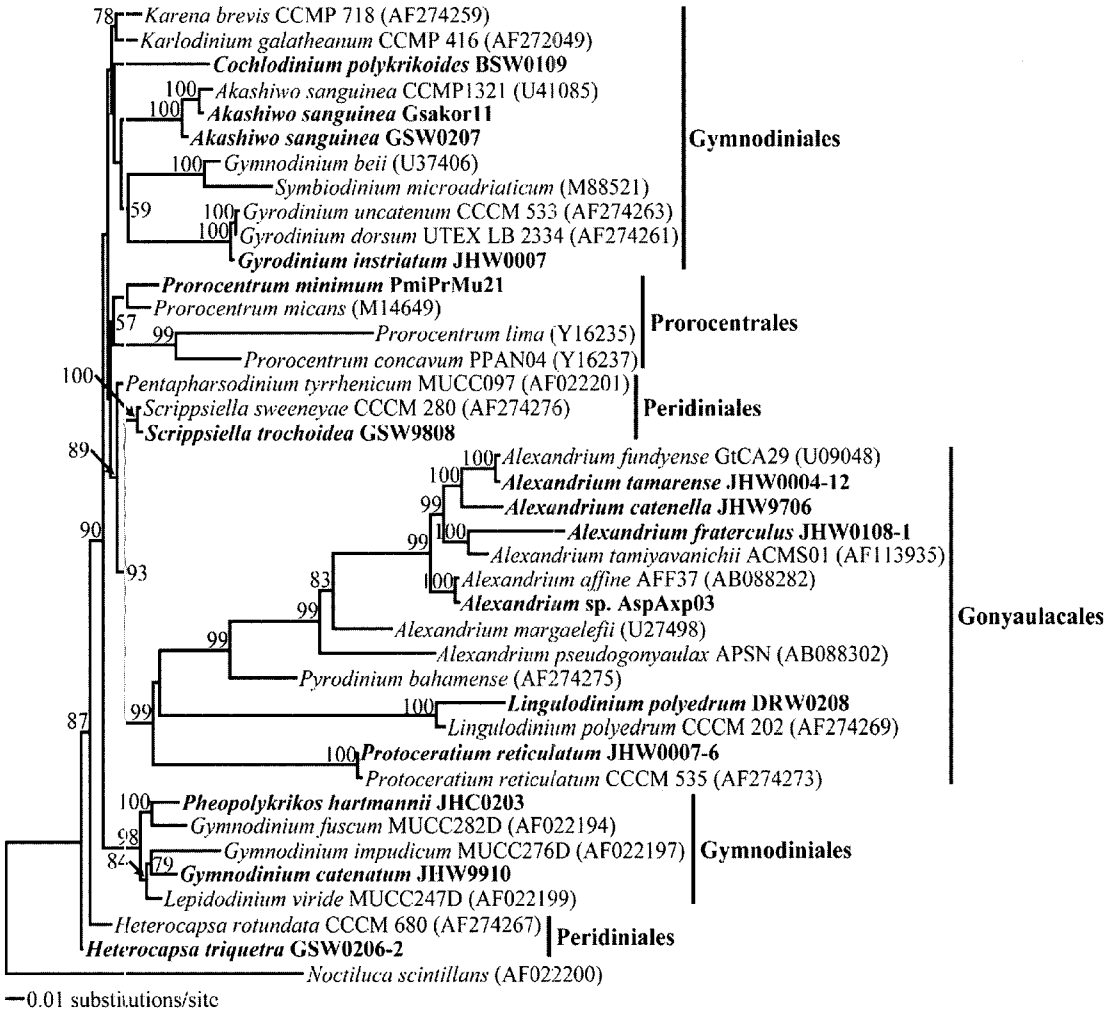

Fig. 2. The maximum-likelihood tree based on dinoflagellate SSU rDNA sequences.
Noctiluca scintillans was used as an outgroup taxon. The tree was constructed using PAUP* 4.0b8 with a GTR+I+G model with assumed proportion of invariable sites=0.160021 and gamma shape parameter=0.239081. Bootstrap values of the ML tree were substituted with Bayesian posterior probabilities (PP). Markov Chain Monte Carlo (MCMC) from a random starting tree was initiated in the Bayesian inference (MrBayes 3.0b4) using a GTR+I+G model and run for 1,000,000 generations. Only PP values above 50% were indicated at each of the branches. The sequences of dinoflagellate species with bold-faced letters were determined in this study. Species names of some unarmored dinoflagellates were corrected according to Daugbjerg *et al.* [10].

rDNA phylogeny. They were separated from the *Gymnodinium sensu stricto* clade, but the robustness of this clade was ambiguous in considering them to be monophyletic.

The Order Peridiniales, characterized by the canal plate in the epitheca, was polyphyletic with taxa sorting into three main clades: calcareous cyst-producing *Pentapharsodinium* and *Scrippsiella* were clearly separated from *Heterocapsa*. Apparently, the presence of the canal plate in the epitheca is insufficient to warrant grouping peridinioids in a monophyletic clade, and therefore, the definition of the Order Peridiniales will need to be revised.

Analysis of Fourteen Dinoflagellate Species

Two *Akashiwo sanguinea* (formally *Gym. sanguineum*) strains (Gsakor11, GSW0207) were isolated from the south coast of Korea (Table 1) and their SSU rDNA sequences were determined. In the phylogenetic tree, this dinoflagellate formed a distinct branch from other taxa (Fig. 2). A comparison of sequences from both strains revealed nucleotide substitutions and insertion/deletion (indel) events. The sequences of strains Gsakor11 and GSW0207 showed 99.27% and 98.49% identity with *Gym. sanguineum* CCMP1321, respectively. Despite temporal and spatial isolation, such intraspecific genetic heterogeneities are found to be interesting when compared to species with 100% identity, such as *Al. catenella*, *C. polykrikoides*, and *Gym. catenatum* (see below). Natural population of *Ak. sanguinea* have extensive variation in cell length, pigmentation, and the shape of the hypocone [42]. The high genetic heterogeneity of *Ak. sanguinea* with respect to its SSU rDNA appears to reflect this pleomorphism. However, a direct relationship between morphology and genetic data has not been recognized until now. A cosmopolitan species, *Ak. sanguinea*, has been associated with fish kills, although its toxicity level has not been determined [42]. Blooms of this dinoflagellate have been reported from Jinhae Bay, Korea [24].

Alexandrium catenella, *Al. fundyense*, and *Al. tamarense* have received considerable attention because of their worldwide distribution, taxonomic difficulty, and potent toxicity [44]. We isolated four *Al. catenella* strains (Axsp-K01, Axsp-K03, AxCt-K01, JHW9706), collected on different dates for temporal variation, from Geoje and Jinhae Bay (Table 1). Their SSU rDNA sequences showed 100% similarity without any polymorphism, in contrast to *Al. fundyense* and *Al. tamarense*, both of which had sequence heterogeneities [39, 40]. The lack of SSU rDNA heterogeneity in *Al. catenella* is interesting, because the LSU rDNA in a temperate Asian ribotype of this dinoflagellate was also reported to have this type of heterogeneity [40]. *Al. catenella* occurs along several parts of the Korean coasts [27], and is suspected to be the cause of annual paralytic shellfish poisoning (PSP) in Korea. In the phylogenetic tree, *Al. catenella* was separated from *Al. fundyense* and *Al. tamarense*, both of which are morphologically closely related to *Al. catenella* (Fig. 2). Kim *et al.* [27] reported on the fine-scale morphological differences in the sulcal plate of *Al. catenella* and *Al. tamarense*, and this appears to be a good characteristic for their taxonomic differentiation.

Al. fraterculus JHW0108-1, which forms a long chain, was isolated from Jinhae Bay (Table 1). This dinoflagellate is distributed worldwide, and Lee *et al.* [29] have reported its occurrence in Korea. The SSU rDNA sequence of JHW0108-1 was 100% identical to the sequences of SJW9709 (GenBank Acc. No.: AB088315) and DPW9709 (AB088290). *Al. fraterculus* has homogeneous morphology with *Al. tamiyavanichii*, except for the position of the anterior attachment pore along with the shape of the anterior sulcal plate [6], and the two species grouped together in the phylogenetic tree (Fig. 2). This dinoflagellate was suspected of forming the first *Alexandrium* bloom at Jinhae Bay in 1977 [9] and has been listed as a HAB-causing species in Korea [24].

The toxic *Al. tamarense* is found throughout Korean coastal waters [16, 27], and is one of the main HAB-causing species responsible for the annual PSP events in Korea [17, 23]. The first monospecific bloom of this species was reported at Jinhae Bay during the spring of 1997 [51]. For this study, *Al. tamarense* JHW0004-12 was isolated from Jinhae Bay (Table 1). This dinoflagellate clustered with *Al. catenella* and *Al. fundyense* at the terminus of the *Alexandrium* clade (Fig. 2). Direct sequencing of its SSU rDNA PCR product resulted in severe sequence noises, of which cloning and sequencing revealed that it was caused by two deletions in the middle of the rRNA gene. Sequence heterogeneities elsewhere had double electropherograms. In total, 34 polymorphic sites, including two deletions, were found in the SSU rRNA genes. These heterogeneities had been reported previously in the SSU and LSU rDNAs of *Al. fundyense* [39, 40]. Strain JHW0004-12 was 100% identical to *Al. fundyense* GtCA29 [39], except for the polymorphic sites. More surprisingly, the sequence polymorphisms of our strain were highly similar to those of *Al. fundyense* GtCA29 [39]. This result indicates that the two morphospecies are conspecific and, therefore, the presence or absence of the ventral pore is not a taxonomically fixed criterion [27].

The SSU rDNA sequence of an unidentified *Alexandrium* sp., AspAxp03, was similar to *Al. affine* AFF37, with 99.55% similarity and no indels. If this species designation is correct, the intraspecific genetic heterogeneity among *Al. affine* stains is interesting in comparison to *Al. catenella* and *Al. tamarense*. *Al. affine* has been reported to occur at Jinhae Bay, Korea [29] and also to cause red tides in Japan [14].

Cochlodinium polykrikoides is the most notorious HAB-causing species in Korea for its extensive annual blooms associated with massive fish kills along the coasts. In 1995, a *C. polykrikoides* bloom caused mass mortalities

of both wild and farmed fish, and caused an estimated economic loss of US $ 95.5 million [24]. *C. polykrikoides* has also been observed in Japan [52], and a *Cochlodinium* sp. bloom caused mortality of farmed salmon in Canada [49]. In this study, we have analyzed the SSU rDNA sequences of *C. polykrikoides* for the first time. Four temporally and spatially isolated strains (BSW0109, CP0, CP-PP4, and CP-PP6) (Table 1) were analyzed, but their SSU rDNA sequences were 100% identical. This species appeared on a phylogenetic branch distinct from other dinoflagellate taxa, and did not have any apparent sister relationships (Fig. 2). Recently, attempts have been made to find genetic markers that are appropriate for tracing the biogeographical distribution and dispersal of noxious HAB species [2, 40]. However, given the conservative nature of SSU rDNA, genetic determination might be difficult for *C. polykrikoides* strains that are spatially and temporally diverse. Analyses of other hypervariable genes, such as microsatellite DNA, may be required.

Three *Gymnodinium catenatum* strains (JHW9910, DC99A44, and GCSW962) were isolated from the south coast of Korea (Table 1). Their SSU rDNA sequences were 100% identical, and they were also homogeneous with *Gym. catenatum* MUCC273 (AF022193) despite their geographic separation. This dinoflagellate is a cosmopolitan species and well known as a PSP toxin producer [15]. In Korea, a resting cyst of this species was first found at Jinhae Bay in 1991 [25], and Kim and Shin [23] demonstrated its PSP toxin production. Phylogenetically, this dinoflagellate emerged within *Gymnodinium sensu stricto* [10] (Fig. 2).

Gyrodinium instriatum (JHW0007-2) was isolated from Jinhae Bay (Table 1). Its SSU rDNA sequence is presented here for the first time. This taxon formed an apparent monophyletic clade with *Gyr. dorsum* and *Gyr. uncatenum* (Fig. 2). The genus *Gyrodinium* had been defined previously to include individuals with a girdle displacement greater than one-fifth of the body length [28]. Recently, Hansen and Moestrup refined the definition of *Gyrodinium* as a heterotrophic species with an elliptical apical groove [10]. However, *Gyr. instriatum* is autotrophic and its major carotenoid is peridinin. Its apical groove is dorsoventrally elongated and loop-shaped (see [42]), which clearly differentiates it from other unarmored dinoflagellates. Although there is no genetic data for *Gyrodinium sensu* Hansen and Moestrup, the phylogenetic positions of *Gyr. dorsum, Gyr. instriatum*, and *Gyr. uncatenum* are sufficiently distinct to consider them as morphologically and genetically distinct taxa. In order to designate a new genus to contain these three species, it would be necessary to expand sampling to their type localities, and to conduct further ultrastructural characterizations. This species often causes HAB events in Japan [47].

The genus *Heterocapsa* is characterized by thin thecal plates with distinct body scales and has been listed as a HAB species in Korea [24]. The SSU rDNA sequence of *H. triquetra* (GSW0206-2), obtained from the south coast of Korea, showed 100% identity to *H. triquetra* MUCC285 (AF022198).

Lingulodinium polyedrum DRW0108 was isolated from Deukryang Bay, Korea and its SSU rDNA sequence was 97.02% similar to that of *L. polyedrum* CCCM202, and formed a deep branch in the phylogenetic tree (Fig. 2). The cosmopolitan *L. polyedrum* produces yessotoxin (YTX) and its derivative [11], and is suspected as the cause of shellfish contamination in the Adriatic Sea [48].

We analyzed the SSU rDNA sequence of *Pheopolykrikos hartmannii* for the first time. This species forms a characteristic two-celled pseudocolony. Mastuoka and Fukuyo [30] separated this dinoflagellate from the genus *Polykrikos*, and transferred it to the new genus *Pheopolykrikos*, based on its feeding strategy and cyst morphology. *Ph. hartmannii* is cosmopolitan and occurs frequently in tropical and temperate regions [42]. It is considered to be a HAB species in Korea [24]. Phylogenetically, *Ph. hartmannii* JHC0203 was placed within the *Gymnodinium sensu stricto* clade as a sister taxon to *Gym. fuscum* (Fig. 2). Morphologically, it has a horseshoe-shaped apical groove running in a counterclockwise direction [30], which implies that the shape of the apical groove in unarmored dinoflagellates is a synapomorphic characteristic.

We established a clonal culture of *Protoceratium reticulatum* JDW0007-6 from Jinhae Bay (Table 1). This species is characterized by heavily reticulated thecal plates and the production of a distinct resting cyst known as *Operculodinium centrocarpum*. It was recently shown to produce YTX and its analog [36], which cause shellfish contamination. The SSU rDNA sequence of the strain showed moderately high similarity (99.71% including two indels) to *Prot. reticulatum* CCCM 535, and formed a long branch among the gonyaulacoids (Fig. 2). This species produced a red tide in South Africa [34].

Several species of the genus *Prorocentrum* have been implicated in diarrhetic shellfish poisoning (DSP) [13]. For example, *Pror. minimum* was responsible for a venerupin shellfish poising (VSP) that resulted in the deaths of 114 people in Japan in 1942 [3]. The planktonic *Pror. minimum* is a bloom-forming dinoflagellate in Korea [24]. *Pror. minimum* PmiPrMu21 (Table 1) showed 100% identity with *Pror. minimum* PmS1 (Y16238), except for one indel. In the phylogenetic tree, this species grouped with *Pror. micans* (*Prorocentrum* group) and had a sister relationship with *Pror. lima* along with *Pror. concavum* of the *Exuviaella* group [33] (Fig. 2).

Scrippsiella trochoidea is a cosmopolitan dinoflagellate that produces an ovoid calcareous cyst. This species was reported to have formed a red tide in Korea [24]. For our study, *S. trochoidea* GSW9808 was isolated from Gunsan, Korea (Table 1), and its SSU rDNA showed 100% identity with *S. trochoidea* CCCM 602 (AF274277). In the phylogenetic tree, this species was closely affiliated with

other *Scrippsiella* species, and formed a sister group with *Pentapharsodinium tyrrhenicum*, which also produced a calcareous cyst [31] (Fig. 2).

In this study, we have reported on the SSU rDNA sequence data of several HAB-causing dinoflagellates that frequently occur in Korean coastal waters. Some of these are presented here for the first time. These sequences will be useful for identifying dinoflagellate species with taxonomic ambiguities, and also for resolving the phylogenetic and evolutionary lineages of major taxonomic groups. Recent efforts were made to understand the dinoflagellate phylogeny using the molecular sequence data of various subcellular organelles (i.e., nucleus, mitochondrium, plastid) which have encouraged the design of oligonucleotide probes or primer sets with high specificity and sensitivity for making a simple, rapid, and accurate detection of the target microorganisms [1, 7, 45, 53]. Our molecular data will also contribute to the base of data that facilitates the study of algal biodiversity [12, 32].

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