



Research Article

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Comparison of the spatial-temporal distributions of the heterotrophic dinoflagellates *Gyrodinium dominans*, *G. jinhaense*, and *G. moestrupii* in Korean coastal waters

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Heterotrophic dinoflagellates *Gyrodinium* spp. are one of the major grazers of phytoplankton in many coastal waters. *Gyrodinium dominans*, *G. jinhaense*, and *G. moestrupii* have similar morphologies but different edible prey species. To explore the variations in the ecological niches of these three species, we investigated their spatial-temporal distributions in Korean waters. Because of the high similarity in morphology among these three *Gyrodinium* species, we used real-time polymerase chain reactions to quantify their abundance in water samples that were seasonally collected from 28 stations along the Korean Peninsula from April 2015 to October 2018. Cells of *G. dominans* were found at all sampling stations, *G. jinhaense* at 26 stations, and *G. moestrupii* at 22 stations, indicating that all three species were widely distributed in Korea. Furthermore, all three species displayed strong seasonal distributions. The largest numbers of the stations where *G. dominans* and *G. jinhaense* cells were present were found during the summer (26 and 23 stations, respectively), but that for *G. moestrupii* was found in the autumn (15 stations). The abundance of *G. dominans* was positively correlated with that of *G. jinhaense*, but not with that of *G. moestrupii*. The highest abundances of *G. dominans* (202.5 cells mL⁻¹) and *G. jinhaense* (20.2 cells mL⁻¹) were much greater than that of *G. moestrupii* (1.2 cells mL⁻¹). The highest abundances of *G. dominans* and *G. jinhaense* were found in July, whereas that of *G. moestrupii* was found in March. The abundances of *G. dominans* and *G. jinhaense*, but not *G. moestrupii*, were positively correlated with water temperature. Therefore, the spatial-temporal distributions of *G. dominans* and *G. jinhaense* were closer than those of *G. moestrupii* and *G. dominans* or *G. jinhaense*. This differs from results based on the relative differences in ribosomal DNA sequences and the types of edible prey reported in the literature. Thus, the variations in spatial-temporal distributions and prey species of these three *Gyrodinium* species suggest that they may have different ecological niches in Korean coastal waters.

Key Words: Chl-*a*; harmful algal bloom; protist; qPCR; red tide; specific primer



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INTRODUCTION

Dinoflagellates are ubiquitous and major components of marine ecosystems (Smayda 1997, Jeong 1999, Baker 2003, Sherr and Sherr 2007, Taylor et al. 2008, Stern et al. 2010, Lee et al. 2020). They possess all three trophic modes: autotrophy, heterotrophy, and mixotrophy (Menden-Deuer et al. 2005, Burkholder et al. 2008, Jeong et al. 2010, 2021, Hansen 2011, Stoecker et al. 2017). In marine planktonic food webs, heterotrophic dinoflagellates are predators of heterotrophic bacteria, cyanobacteria, microalgae, and eggs and early naupliar stages of metazoans (Hansen 1991, Strom and Buskey 1993, Buskey et al. 1994, Jeong 1994, Jeong and Latz 1994, Kiørboe and Titelman 1998, Jeong et al. 2008, 2010, Kang et al. 2020), and also prey for diverse metazoans (Gifford and Dagg 1991, Xu et al. 2017, Traboni et al. 2020). Furthermore, heterotrophic dinoflagellates impose a considerable grazing impact on populations of microalgae, causing red tides or harmful algal blooms (Johnson et al. 2003, Menden-Deuer et al. 2005, Jeong et al. 2015, Lim et al. 2017). Therefore, understanding their spatial-temporal distributions is a critical concern for scientists and aquaculture farmers.

The genus *Gyrodinium* is one of the major dinoflagellate groups and is widespread in the world's oceans (Larsen 1996, Daugbjerg et al. 2000, Takano and Horiguchi 2004, Sherr and Sherr 2007, Kilias et al. 2013). Among the species in this genus, heterotrophic species such as *Gyrodinium dominans* and *Gyrodinium moestrupii* are known to feed on diverse algal species and sometimes control prey populations (Nakamura et al. 1995a, 1995b, Kim and Jeong 2004, Potvin et al. 2013, Yoo et al. 2013a, 2013b, Lee et al. 2014, Kang et al. 2018, 2020, Jang et al. 2019). Recently, *Gyrodinium jinhaense*, isolated from Korean waters, was newly described by Jang et al. (2019). The size of *G. jinhaense* (equivalent spherical diameter = 10.2 µm) is smaller than that of *G. dominans* (11.6 µm) or *G. moestrupii* (16.0 µm) (Jang et al. 2019, Kang et al. 2020). Furthermore, among the 20 prey species tested, *G. jinhaense* was able to feed on 15 prey species, fewer than that of *G. dominans* (19 species) and *G. moestrupii* (18 species) (Kang et al. 2020). Moreover, these three *Gyrodinium*

rodinium species respond differently to the chlorophyte *Dunaliella salina* and the phototrophic dinoflagellates *Prorocentrum cordatum* and *Scrippsiella acuminata* (Kang et al. 2020). Thus, these *Gyrodinium* species may have different spatial and temporal distributions and ecological niches. However, there have been no study on distributions of these three *Gyrodinium* species yet.

Although the equivalent spherical diameters of *G. dominans*, *G. jinhaense*, and *G. moestrupii* differ from one another, the ranges of their cell length and width overlap (Jang et al. 2019). Furthermore, the overall morphologies of these three *Gyrodinium* species in fixed samples are similar (Hansen and Daugbjerg 2004, Yoon et al. 2012, Jang et al. 2019). Therefore, to distinguish one from the others, molecular techniques are needed, as in other studies which investigate dinoflagellate species which are morphologically similar (Smith et al. 2016, Lee et al. 2017, 2019, 2020, Kang et al. 2019, Jang and Jeong 2020). To investigate the spatial and temporal distributions of *G. dominans*, *G. jinhaense*, and *G. moestrupii*, we quantified their abundances in the waters of 28 stations along the Korean Peninsula from 2015 to 2018, after developing species-specific primer and probe sets. Furthermore, to explore the effects of major environmental factors on species distributions, we measured water temperature, salinity, and chlorophyll-a in the water samples. The results of the present study provide a basis for better understanding the ecophysiology of *G. dominans*, *G. jinhaense*, and *G. moestrupii*, as well as the evolution of the genus.

MATERIALS AND METHODS

Cultures of three *Gyrodinium* species

To explore the distributions of *G. dominans*, *G. jinhaense*, and *G. moestrupii*, quantitative real-time polymerase chain reaction (qPCR) analysis was performed using clonal cultures of the three *Gyrodinium* species (Table 1): *G. dominans* (GDJK1907), originally isolated from Jeongok Harbor, Korea in July 2019; *G. jinhaense* (GSJH1710), originally isolated from Jinhae Bay, Korea,

Table 1. Strain, location of collection (LC), collection date, water temperature (T, °C), and salinity (S) of *Gyrodinium dominans* (GDJK1907), *Gyrodinium jinhaense* (GSJH1710), and *Gyrodinium moestrupii* (GMSMK0910), isolated from the coastal waters off Korea

Species	Strain	LC	Date	T	S
<i>Gyrodinium dominans</i>	GDJK1907	Jeongok harbor, Korea	Jul 2019	25.2	31.9
<i>Gyrodinium jinhaense</i>	GSJH1710	Jinhae Bay, Korea	Oct 2017	21.6	32.5
<i>Gyrodinium moestrupii</i>	GMSMK0910	Saemangeum, Korea	Oct 2009	21.2	31.0

in October 2017; and *G. moestrupii* (GMSMK0910), originally isolated from Saemangeum, Korea, in October 2009 (Yoon et al. 2012, Jang et al. 2019, Kang et al. 2020).

To maintain *G. dominans*, a clonal culture was provided with cells of the phototrophic dinoflagellate *Amphidinium carterae* (SIO PY-1) as prey. A 250-mL polycarbonate (PC) bottle containing the culture was placed on a plankton wheel rotating at 0.9 rpm (0.00017 g). The bottle was incubated at 20°C and 20 $\mu\text{E m}^{-2} \text{s}^{-1}$ illumination on a 14-h light : 10-h dark cycle. To maintain *G. jinhaense*, a clonal culture was provided with heat-killed cells of the chlorophyte *D. salina* (DSJH1710) as prey and a 250-mL tissue culture flask (Falcon, Heidelberg, Germany) containing the culture was placed on a shelf and incubated at 20°C under the same light conditions as described above. To maintain *G. moestrupii*, a clonal culture was provided with cells of the phototrophic dinoflagellate *Alexandrium minutum* (CCMP113) as prey and a 250-mL PC bottle containing the culture was placed on a plankton wheel rotating at 0.9 rpm (0.00017 g) and incubated at 20°C under the light conditions as described above.

Species-specific primer and probe design, and specificity analysis

To develop the species-specific primer and probe set of *G. dominans*, the internal transcribed spacer ribosomal DNA sequence was obtained (Table 2). Then, sequences of *G. dominans* were aligned with sequences of other *Gyrodinium* species and related dinoflagellates, which were available from GenBank, using the program MEGA

ver. 4 (Tamura et al. 2007). Manual searches of the alignments were conducted to identify unique sequences for developing the *G. dominans*-specific qPCR assay. The sequences for the primer-probe set were selected from the regions that were conserved in *G. dominans* but allowed for discrimination with other dinoflagellates. Primers and probe sequences of each species were analyzed using Primer 3 (Whitehead Institute and Howard Hughes Medical Institute, MD) and Oligo Calc: Oligonucleotide Properties Calculator (Kibbe 2007) software to determine the optimal melting temperature and secondary structure. Subsequently, primers and probes were synthesized by Bioneer (Daejeon, Korea). Each probe was dual labeled with the fluorescent dyes FAM and BHQ1 (Bioneer) at the 5' and 3' ends, respectively (Table 2). The development of the species-specific primer and probe sets of *G. jinhaense* or *G. moestrupii* were also conducted using the methods described above.

To validate the specificity of the specific primer and probe set for each of these three *Gyrodinium* species, a specificity analysis was conducted using DNA extracts of other *Gyrodinium* species, related species, and their prey species. Then, the following steps, modified from Lee et al. (2018), were conducted using the primers and probe for each target species: 1 μL of the DNA template, 0.2 μM of the specific primers (forward and reverse), 0.15 μM of the specific probe (final concentration), and 5 μL of the qPCR Bio Probe Separate-ROX (Genepole, Gwangmyeong, Korea) were combined, and deionized sterilized water (DDW) was added to each sample, resulting in a total final volume of 10 μL . The qPCR assay was performed

Table 2. Oligonucleotide primers used to amplify the internal transcribed spacer (ITS1, 5.8S, ITS2) regions of ribosomal DNA and the species-specific primers and TaqMan probes used to quantify the abundance of *Gyrodinium dominans* (Gdo), *Gyrodinium jinhaense* (Gjin), and *Gyrodinium moestrupii* (Gmoe), using qPCR

Target species	Analysis	Primer name	Direction	Primer region	Primer sequence (5'-3')	Reference
Gdo, Gjin, Gmoe	PCR	Euk1209F	Forward	SSU-ITS	GGGCATCACAGACCTG	Giovannoni et al. (1988)
Gdo, Gjin, Gmoe	PCR	ITSR2	Reverse	ITS	TCCCTGTTCATTCGCCATTAC	Litaker et al. (2003)
Gdo	qPCR	Gdominans_F	Forward	ITS	GAGCTTCATGCCCTTGATGCATC	This study
	qPCR	Gdominans_R	Reverse	ITS	AAGCTCCGATGAAAGCAGAG	This study
	qPCR	Gdominans_P	Probe	ITS	[FAM] GCGAGCAACTAACAGAAC-GCCTTCTG [BHQ1]	This study
Gjin	qPCR	Gjinhaense_IF	Forward	ITS	TGTCTATTCCCTGCCCTCATGC	This study
	qPCR	Gjinhaense_IR	Reverse	ITS	TTGTAAGGAAAGCTCACTGCAC	This study
	qPCR	Gjinhaense_IP	Probe	ITS	[FAM] AGGAGCCTGTGGTGCCCCT-GATG [BHQ1]	This study
Gmoe	qPCR	Gmoestrupii_F	Forward	ITS	ATTCAATGCAGTGAGCTTCG	This study
	qPCR	Gmoestrupii_R	Reverse	ITS	AGGCACCGACGAGTACGA	This study
	qPCR	Gmoestrupii_P	Probe	ITS	[FAM] CTTGCGAGCATCTAACAGAAC-GCCTT [BHQ1]	This study

ITS, internal transcribed spacer; qPCR, quantitative real-time polymerase chain reaction; SSU, small subunit.

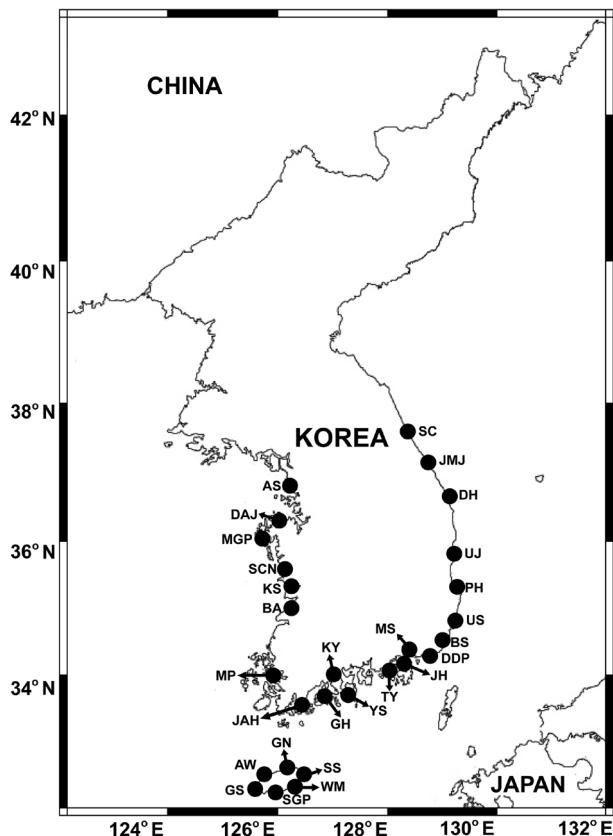


Fig. 1. Map showing the 28 sampling stations during the study period. SC, Sokcho; JMJ, Jumunjin; DH, Donghae; UJ, Uljin; PH, Pohang; US, Ulsan; BS, Busan; DDP, Dadaepo; MS, Masan; JH, Jinhae; TY, Tongyeong; YS, Yeosu; KY, Kwangyang; GH, Goheung; JAH, Jangheung; AS, Ansan; DAJ, Dangjin; MGP, Mageompo; SCN, Seocheon; KS, Kunsan; BA, Buan; MP, Mokpo; AW, Aewol; GS, Gosan; SGP, Seogwipo; WM, Wimi; SS, Seongsan; GN, Gimnyeong.

using Rotor-Gene Q (Qiagen, Hilden, Germany) under the following thermal cycling conditions: 3 min at 95°C, followed by 45 cycles of 10 s at 95°C, and 40 s at 58°C.

Generation of the standard curve

Standard curves for determining the abundance of each of the three *Gyrodinium* species were obtained using DNA extracted from the exponential growth phase culture of each species. DNA was extracted from 10,000 cells each of *G. dominans*, *G. jinhaense*, and *G. moestrupii*, and the final elution volume of 100 µL was extracted using an AccuPrep Genomic DNA extraction kit (Bioneer) according to the manufacturer's instructions. The extracted DNA of *G. dominans*, *G. jinhaense*, and *G. moestrupii* was then serially diluted by adding predetermined volumes of DDW to 1.5-mL tubes to prepare five different DNA concentrations, targeting 100, 10, 1, 0.1, and 0.01%

DNA concentrations of the originally extracted DNA. The extracted and diluted DNA samples were then stored at -20°C in a freezer and qPCR amplification was conducted within a day. qPCR amplification was used to construct a standard curve using the aforementioned methods and conditions detailed in the specificity analysis section.

Collection of field samples

The field samples for this study were obtained from our previous studies (Lee et al. 2018, 2019, Jang and Jeong 2020). These samples were collected from April 2015 to October 2018 from the surface waters located in the 28 stations of the East, West, and South Seas, as well as on Jeju Island, Korea (Fig. 1). Surface water samples from each station were collected using a clean bucket, and the physical and chemical properties were obtained using these samples.

Quantification of the abundance of three *Gyrodinium* species

qPCR analysis was performed to determine the distribution of the three *Gyrodinium* species in Korean waters. First, cells in 50–300 mL of each water sample collected from each station at each time interval were collected by filtering through a 25-mm GF/C filter (Whatman Inc., Clifton, NJ, USA). The filter was loosely rolled, placed into a 1.5-mL tube, and frozen at -20°C until it was transported to the laboratory. Subsequently, DNA was extracted from the collected cells that were captured on the filters using an AccuPrep Genomic DNA extraction kit (Bioneer) according to the manufacturer's instructions. Extracted DNA was stored at -20°C until qPCR was performed. In addition, to ensure accuracy of the results, DNA from each sample was amplified four times. Samples using DDW as the template were used as non-template controls, samples using only DDW as the template and reaction mix were used as negative controls, and DNA used to construct the standard curve was used as a positive and standard control. The conditions used for qPCR and quantification of the abundances of the three *Gyrodinium* species in the field samples were similar to those listed in the specificity analysis section.

Data analysis

Statistical analyses were performed using the statistical software package SPSS ver. 25.0 (IBM Corp., Armonk, NY, USA). Pearson's correlation analysis was used to de-

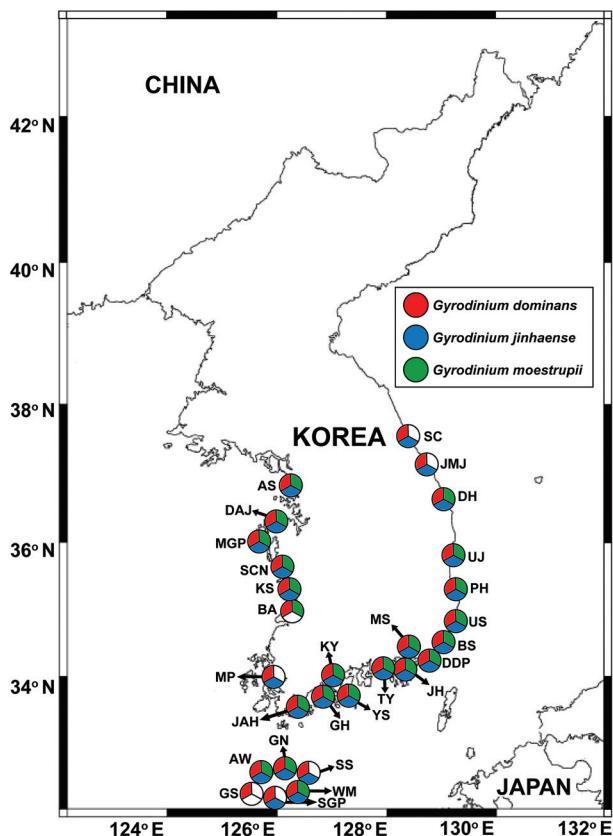


Fig. 2. Map of the sampling stations, indicating the presence and absence of *Gyrodinium dominans*, *G. jinhaense*, and *G. moestrupii* during the study period. The red, blue, and green circular sectors indicate the stations at which *G. dominans*, *G. jinhaense*, and *G. moestrupii* cells were detected, respectively, and the white circular sector indicates the stations at which *Gyrodinium* cells were not detected. SC, Sokcho; JMJ, Jumunjin; DH, Donghae; UJ, Uljin; PH, Pohang; US, Ulsan; BS, Busan; DDP, Dadaepo; MS, Masan; JH, Jinhae; TY, Tongyeong; YS, Yeosu; KY, Kwangyang; GH, Goheung; JAH, Jangheung; AS, Ansan; DAJ, Dangjin; MGP, Mageompo; SCN, Seocheon; KS, Kunsan; BA, Buan; MP, Mokpo; AW, Aewol; GS, Gosan; SGP, Seogwipo; WM, Wimi; SS, Seongsan; GN, Gimnyeong.

termine any one-to-one relationships between the cell abundances of the three *Gyrodinium* species and environmental factors.

RESULTS

Spatial and temporal distributions of three *Gyrodinium* species in Korean waters

We successfully developed specific primer and probe sets for *G. dominans*, *G. jinhaense*, and *G. moestrupii* (Table 2). Using these sets, we quantified the abundance of each species in the water samples collected from the 28

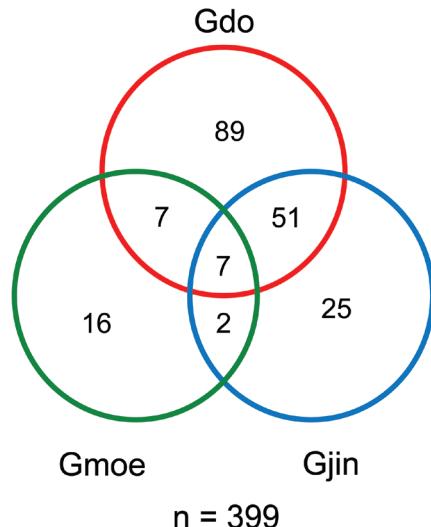


Fig. 3. Venn diagram of the presence of *Gyrodinium dominans* (Gdo), *G. jinhaense* (Gjin), and *G. moestrupii* (Gmoe) in the water samples collected from the 28 stations during the present study ($n = 399$). Absence of all three species in 128 samples.

stations from April 2015 to October 2018 and investigated the specific spatial and temporal distribution of each *Gyrodinium* species. In general, all three species had nationwide distributions in Korean coastal waters (Fig. 2).

During the study period, *G. dominans* was found at all 28 sampling stations, *G. jinhaense* was found at 26 stations, and *G. moestrupii* was found at 22 stations (Fig. 2). All three *Gyrodinium* species were found at 21 stations, *G. dominans* and *G. jinhaense* without *G. moestrupii* were found together at five stations, and *G. dominans* and *G. moestrupii* without *G. jinhaense* were found together at one station. *G. dominans* was found alone at one station.

Among the 399 samples collected during the study period, all three *Gyrodinium* species were found in seven samples, *G. dominans* and *G. jinhaense* without *G. moestrupii* were found together in 51 samples, *G. dominans* and *G. moestrupii* without *G. jinhaense* were found together in seven samples, and *G. jinhaense* and *G. moestrupii* without *G. dominans* were found together in two samples (Fig. 3). However, *G. dominans* alone was found in 89 samples, *G. jinhaense* alone in 25 samples, and *G. moestrupii* alone in 16 samples.

Cells of *G. dominans* were detected at 26 stations in summer, 20 stations in spring, 22 stations in autumn, and 12 stations in winter (Fig. 4, Supplementary Table S1, Fig. S1). The highest abundance of *G. dominans* ($202.5 \text{ cells mL}^{-1}$) was found in Jinhae Bay (South Sea) in July 2017, where the water temperature and water salinity were 23.8°C and 28.3, respectively.

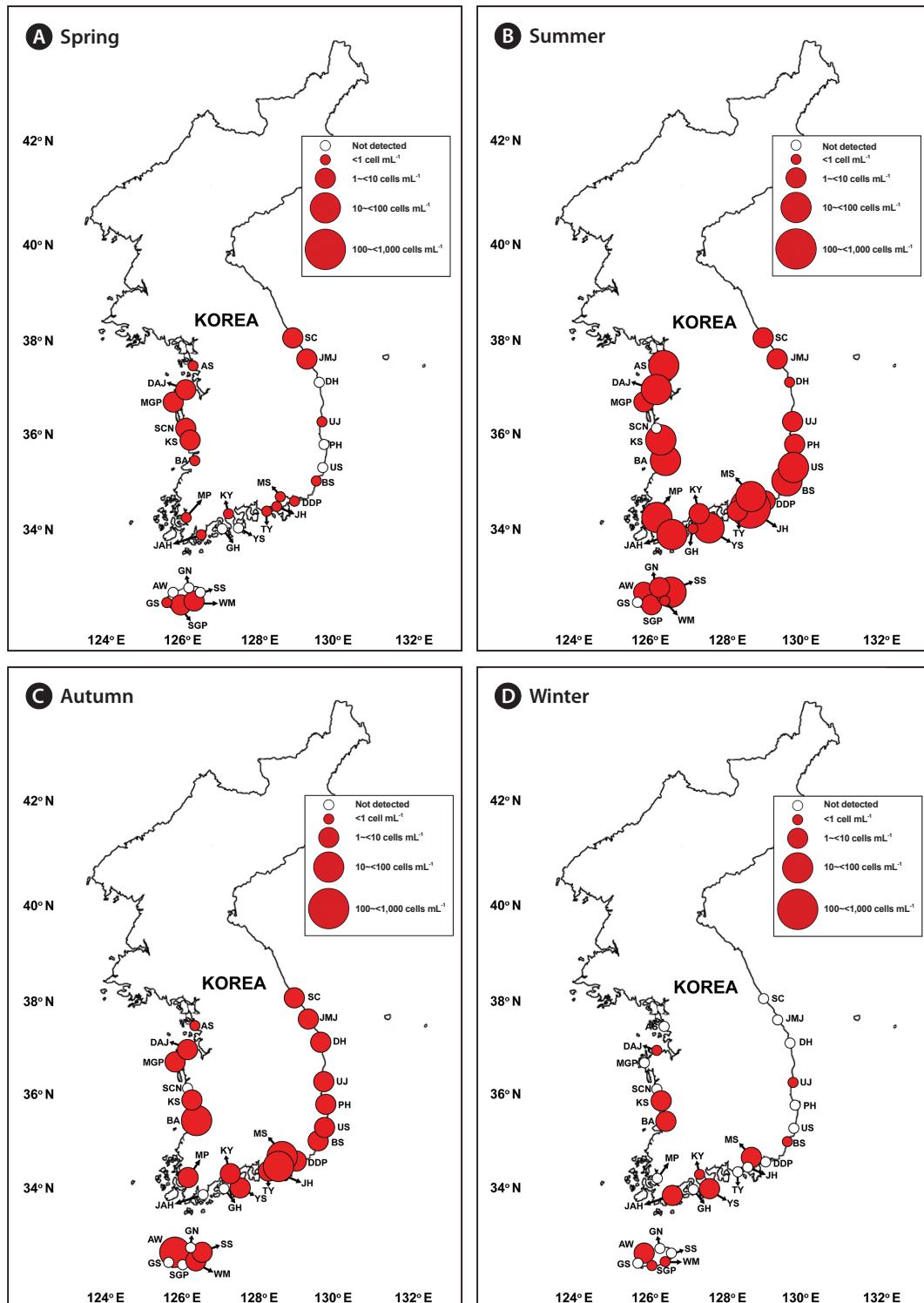


Fig. 4. Map of the sampling stations, indicating the presence and absence of *Gyrodinium dominans* in spring (March or April) (A), summer (June or July) (B), autumn (September or October) (C), and winter (December or January) (D) from 2015 to 2018. The red closed circles indicate the stations at which *G. dominans* cells were detected, whereas the black open circles indicate the stations at which *G. dominans* cells were not detected. The scale of the circles indicates the abundance of *G. dominans* cells (cells mL^{-1}). SC, Sokcho; JMJ, Jumunjin; DH, Donghae; UJ, Uljin; PH, Pohang; US, Ulsan; BS, Busan; DDP, Dadaepo; MS, Masan; JH, Jinhae; TY, Tongyeong; YS, Yeosu; KY, Kwangyang; GH, Goheung; JAH, Jangheung; AS, Ansan; DAJ, Dangjin; MGP, Mageompo; SCN, Seocheon; KS, Kunsan; BA, Buan; MP, Mokpo; AW, Aewol; GS, Gosan; SGP, Seogwipo; WM, Wimi; SS, Seongsan; GN, Gimnyeong.

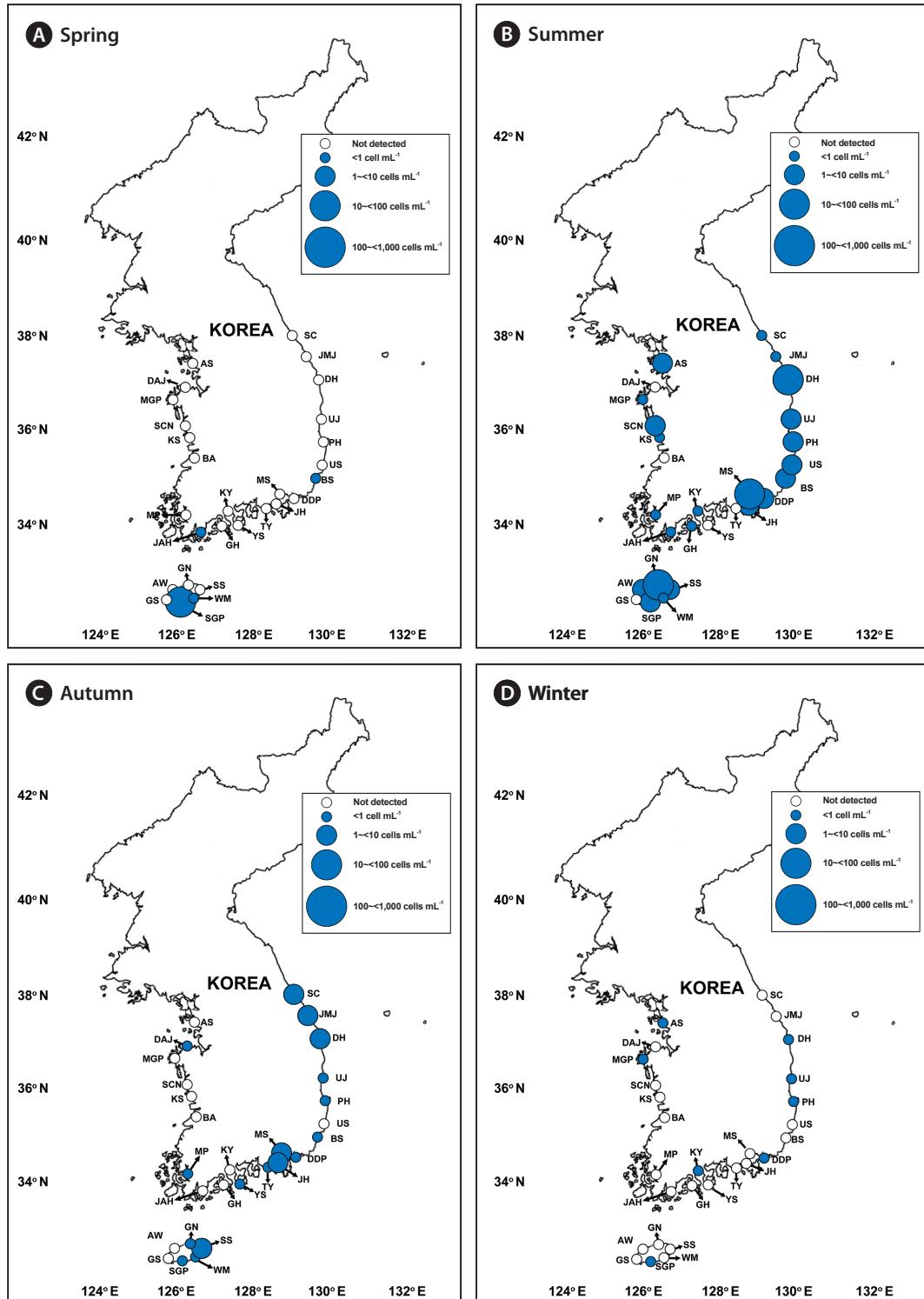


Fig. 5. Map of the sampling stations, indicating the presence and absence of *Gyrodinium jinhaense* in spring (March or April) (A), summer (June or July) (B), autumn (September or October) (C), and winter (December or January) (D) from 2015 to 2018. The blue closed circles indicate the stations at which *G. jinhaense* cells were detected, whereas the black open circles indicate the stations at which *G. jinhaense* cells were not detected. The scale of the circles indicates the abundance of *G. jinhaense* cells (cells mL⁻¹). SC, Sokcho; JMJ, Jumunjin; DH, Donghae; UJ, Uljin; PH, Pohang; US, Ulsan; BS, Busan; DDP, Dadaepo; MS, Masan; JH, Jinhae; TY, Tongyeong; YS, Yeosu; KY, Kwangyang; GH, Goheung; JAH, Jangheung; AS, Ansan; DAJ, Dangjin; MGP, Mageompo; SCN, Seocheon; KS, Kunsan; BA, Buan; MP, Mokpo; AW, Aewol; GS, Gosan; SGP, Seogwipo; WM, Wimi; SS, Seongsan; GN, Gimnyeong.

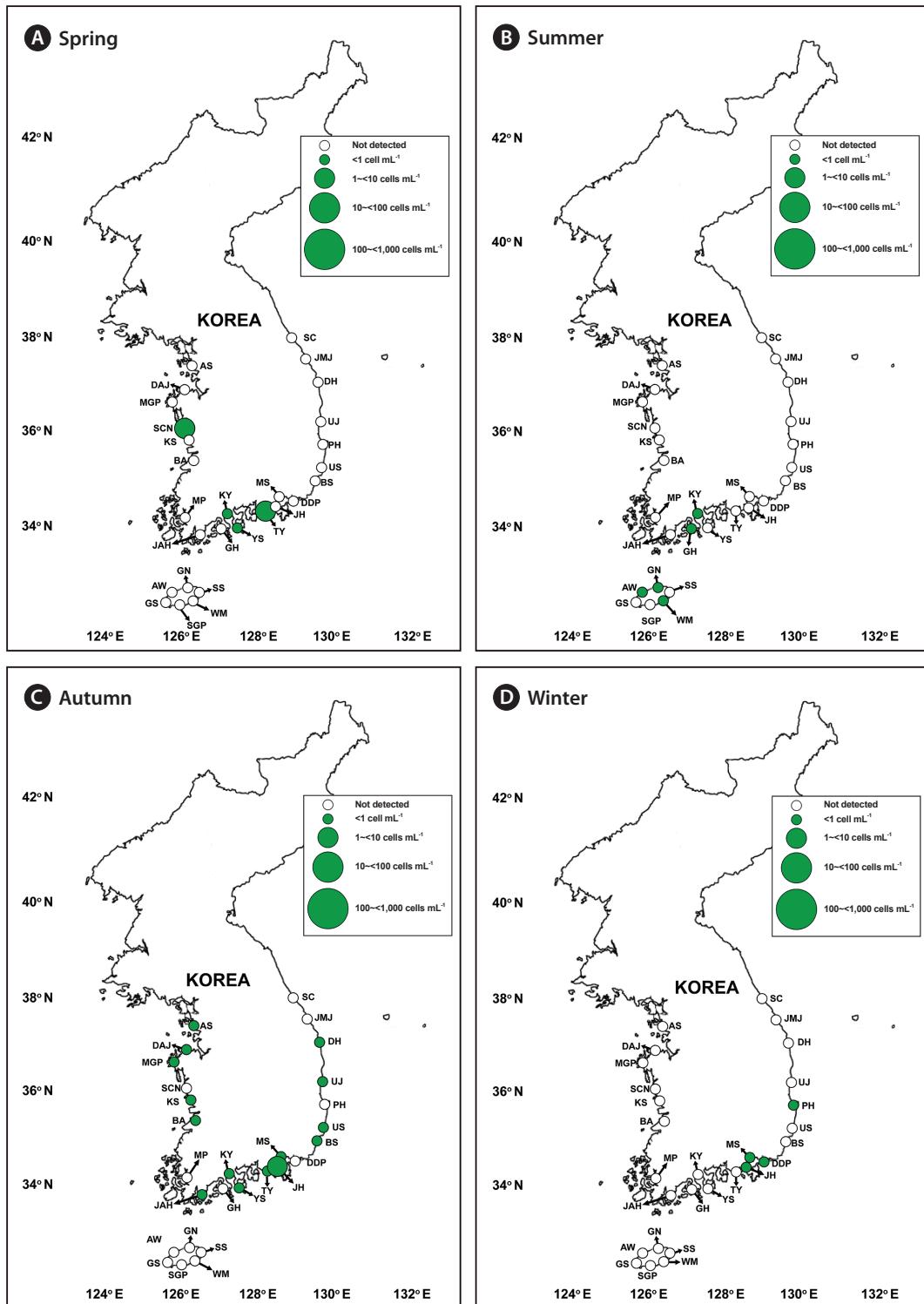


Fig. 6. Map of the sampling stations, indicating the presence and absence of *Gyrodinium moestrupii* in spring (March or April) (A), summer (June or July) (B), autumn (September or October) (C), and winter (December or January) (D) from 2015 to 2018. The green closed circles indicate the stations at which *G. moestrupii* cells were detected, whereas the black open circles indicate the stations at which *G. moestrupii* cells were not detected. The scale of the circles indicates the abundance of *G. moestrupii* cells (cells mL^{-1}). SC, Sokcho; JMJ, Jumunjin; DH, Donghae; UJ, Uljin; PH, Pohang; US, Ulsan; BS, Busan; DDP, Dadaepo; MS, Masan; JH, Jinhae; TY, Tongyeong; YS, Yeosu; KY, Kwangyang; GH, Goheung; JAH, Jangheung; AS, Ansan; DAJ, Dangjin; MGP, Mageompo; SCN, Seocheon; KS, Kunsan; BA, Buan; MP, Mokpo; AW, Aewol; GS, Gosan; SGP, Seogwipo; WM, Wimi; SS, Seongsan; GN, Gimnyeong.

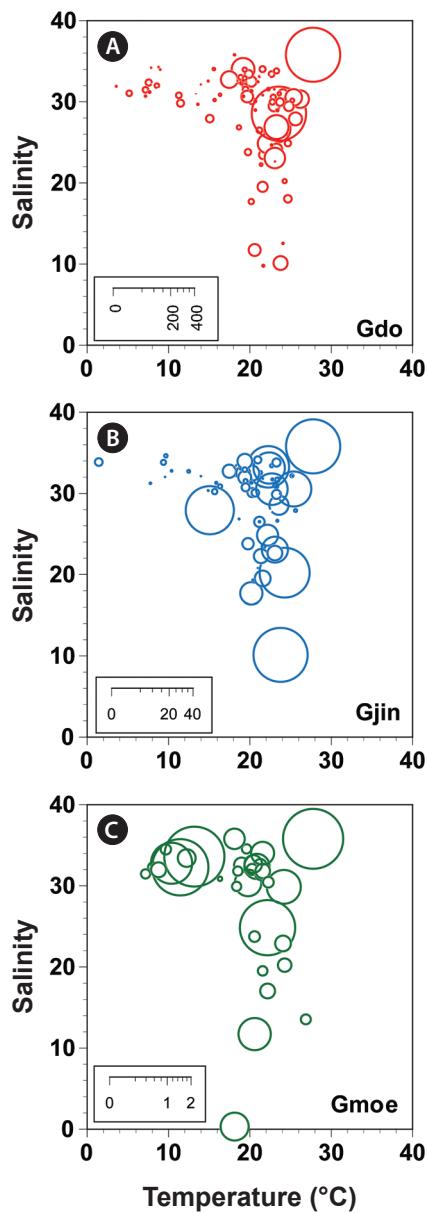


Fig. 7. Abundances (cells mL^{-1}) of *Gyrodinium dominans* (Gdo) (A), *Gyrodinium jinhaense* (Gjin) (B), and *Gyrodinium moestrupii* (Gmoe) (C), as a function of water temperature ($^{\circ}\text{C}$) and salinity at all stations from 2015 to 2018. The scale of the circles in the inset represents the abundance of each species (cells mL^{-1}).

Table 3. Ranges of the measured abiotic environmental factors during the study period and when *Gyrodinium dominans*, *Gyrodinium jinhaense*, and *Gyrodinium moestrupii* were detected in Korean coastal waters from April 2015 to October 2018

Species	Temperature ($^{\circ}\text{C}$)	Salinity
In this study period	0.2–28.0	0.1–35.6
<i>Gyrodinium dominans</i>	3.8–26.4	9.6–35.6
<i>Gyrodinium jinhaense</i>	1.7–25.8	9.9–34.4
<i>Gyrodinium moestrupii</i>	7.4–27.1	0.1–35.6

Cells of *G. jinhaense* were detected at 23 stations in the summer, four stations in the spring, 17 stations in the autumn, and eight stations in the winter (Fig. 5, Supplementary Table S2, Fig. S1). Cells of *G. jinhaense* were not detected in the waters of Buan (West Sea) and Gosan (Jeju Island) (Fig. 2). The highest abundance of *G. jinhaense* ($20.2 \text{ cells mL}^{-1}$) was found in the waters off Masan (South Sea) in July 2016, where the water temperature and water salinity were 24.0°C and 9.9, respectively.

Cells of *G. moestrupii* were detected at 15 stations in autumn, four stations in the spring, five stations in the summer, and four stations in the winter (Fig. 6, Supplementary Table S3, Fig. S1). Cells of *G. moestrupii* were not detected in the waters of Sokcho, Jumunjin (East Sea), Mokpo (West Sea), Gosan, Seogwipo, and Seongsan (Jeju Island) (Fig. 2). The highest abundance of *G. moestrupii* ($1.2 \text{ cells mL}^{-1}$) was found in the waters off Tongyeong (South Sea) in March 2016, where the water temperature and water salinity were 13.4°C and 33.4, respectively.

Hydrographic properties during the study period

The range of water temperature in which each *Gyrodinium* species was found in the Korean coastal waters during the study period was $3.8\text{--}26.4^{\circ}\text{C}$ for *G. dominans*, $1.7\text{--}25.8^{\circ}\text{C}$ for *G. jinhaense*, and $7.4\text{--}27.1^{\circ}\text{C}$ for *G. moestrupii* (Table 3, Fig. 7). Furthermore, the range of salinity in which each *Gyrodinium* species was found was 9.6–35.6 for *G. dominans*, 9.9–34.4 for *G. jinhaense*, and 0.1–35.6 for *G. moestrupii* (Table 3, Fig. 7).

Relationships between *Gyrodinium* species abundance and environmental factors

The abundance of *G. dominans* was positively correlated with that of *G. jinhaense* ($p < 0.001$, $r = 0.195$), but not with that of *G. moestrupii* (Table 4). The abundance of *G. dominans* was significantly and positively correlated with Chl-a concentration ($p < 0.001$, $r = 0.548$) and water temperature ($p = 0.001$, $r = 0.167$), but negatively correlated with water salinity ($p = 0.011$, $r = -0.128$) (Table 4).

The abundance of *G. jinhaense* was significantly and positively correlated with water temperature ($p = 0.001$, $r = 0.161$) and negatively correlated with water salinity ($p < 0.001$, $r = -0.244$) (Table 4). However, there were no significant correlations between the abundance of *G. jinhaense* and Chl-a concentration (Table 4).

The abundance of *G. moestrupii* was significantly and negatively correlated with water salinity ($p = 0.042$, $r = -0.102$). However, there were no significant correlations

between the abundance of *G. moestrupii* and water temperature or Chl-*a* concentration (Table 4).

DISCUSSION

Heterotrophic dinoflagellates *Gyrodinium* spp. are one of the major grazers on phytoplankton in many coastal waters (Kim and Jeong 2004, Jeong et al. 2011, 2014, Yoo et al. 2013b, Anderson and Menden-Deuer 2017, Kang et al. 2020). For many years, *G. dominans* was representative of small heterotrophic *Gyrodinium* species before the small heterotrophic *G. moestrupii* and *G. jinhaense* were described in 2012 and 2019, respectively (Yoon et al. 2012, Jang et al. 2019); thus, some small heterotrophic *Gyrodinium* species in the water samples may be *G. jinhaense* or *G. moestrupii*. The prey of one of *G. dominans*, *G. jinhaense*, and *G. moestrupii* differs from that of the other species (Yoo et al. 2013b, Kang et al. 2020). Furthermore, the growth and ingestion rates of one of these three *Gyrodinium* species on target prey species also differ from those of the other *Gyrodinium* species (Kim and Jeong 2004, Yoo et al. 2013b, Kang et al. 2020). To assess the ecological roles of each of these *Gyrodinium* species in marine planktonic food webs, the abundance of each species should be quantified. The three heterotrophic dinoflagellates studied here have similar morphology, and, thus, it is difficult to distinguish them from each other in live and fixed samples under light microscopy (Fig. 8); thus, molecular methods are needed. In the present study, we successfully developed a species-specific primer and probe set for each species and quantified their abundance using qPCR. These primer and probe sets can be used to quantify the abundance of each species in other regions to better understand the wider ecological roles of *G. dominans*, *G. jinhaense*, and *G. moestrupii*.

During the study period, all three *Gyrodinium* species were found at 21 stations and in seven samples, *G. dominans* and *G. jinhaense* were found together at five stations and in 51 samples, and *G. dominans* and *G. moestrupii* were found together at one station and in seven samples. Cells of *G. dominans* were found at all 28 sampling stations and in 154 samples (39% of 399 samples), *G. jinhaense* cells were found at 26 stations and in 85 samples (21%), and *G. moestrupii* cells were found at 22 stations and in 32 samples (8%). These results support our contention that the abundance of each small heterotrophic *Gyrodinium* species should be quantified by establishing primer and probe sets and using the qPCR method. If all small heterotrophic *Gyrodinium* species are assumed to

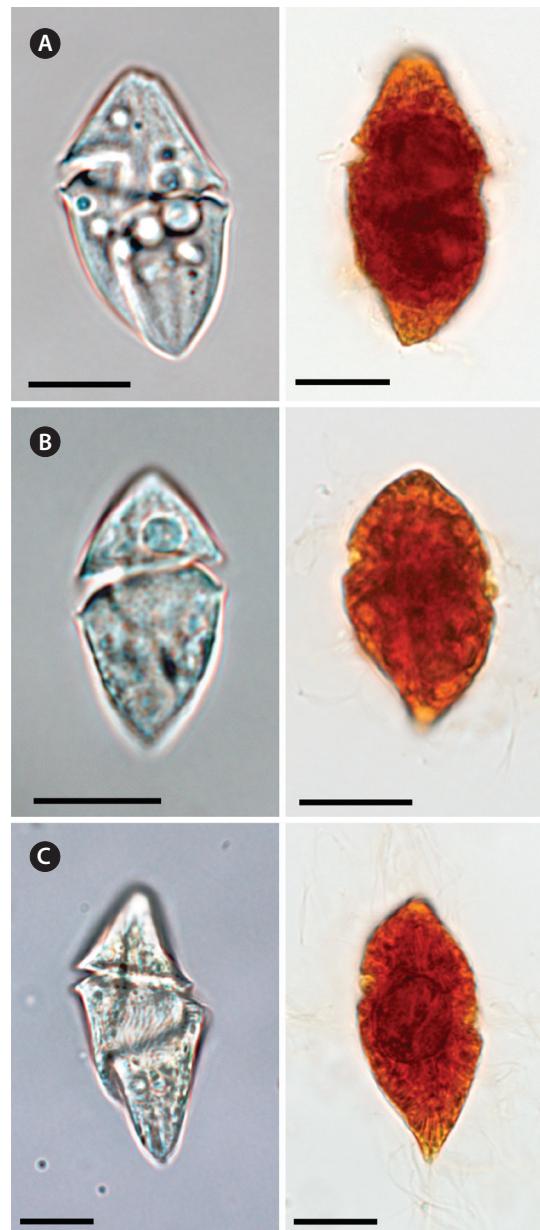


Fig. 8. Light micrographs of *Gyrodinium dominans* (A), *G. jinhaense* (B), and *G. moestrupii* (C). Living cells (left) and cells fixed in 4% Lugol's solution (right). Scale bars represent: A–C, 10 µm.

Table 4. Pearson's correlation coefficients for the relationships between the abundances (cells mL^{-1}) of *Gyrodinium dominans* (Gdo), *Gyrodinium jinhaense* (Gjin), and *Gyrodinium moestrupii* (Gmoe) and environmental factors during the study period

Species	Gdo	Gjin	Gmoe	Chl- <i>a</i>	T	S
Gdo	-	0.195**	0.053	0.548**	0.167**	-0.128*
Gjin	0.195**	-	0.038	0.052	0.161**	-0.244**
Gmoe	0.053	0.038	-	-0.008	0.022	-0.102*

Chl-*a*, chlorophyll-*a*; T, temperature (°C); S, salinity.

* $p < 0.05$, ** $p < 0.001$, $n = 399$.

be *G. dominans*, the abundance of *G. dominans* and its grazing impact on prey populations may be overestimated. However, because *G. dominans* did predominate over *G. jinhaense* or *G. moestrupii* in the number of stations where each species was found in Korean waters, assuming that all small heterotrophic *Gyrodinium* species are *G. dominans* may produce smaller errors than assuming that all small heterotrophic *Gyrodinium* species are *G. jinhaense* or *G. moestrupii* in situations where only light microscopy without qPCR, or only old data, is available for quantification. In particular, in the spring, *G. dominans* was found at 20 stations, but *G. jinhaense* and *G. moestrupii* were only found at four stations. This indicates that the scope for error that all small heterotrophic *Gyrodinium* species are not *G. dominans* is small in the spring samples.

The highest abundances of *G. dominans* and *G. jinhaense* were found in Jinhae Bay and Masan Bay in July, respectively, whereas that of *G. moestrupii* was found in its highest abundances in the waters off Tongyeong in March. Jinhae Bay, Masan Bay, and Tongyeong are located in the South Sea of Korea. The water temperatures when *G. dominans* and *G. jinhaense* exhibited their highest abundances were very similar (23.8 and 24.0°C, respectively); however, these water temperature were much greater than those where *G. moestrupii* displayed its highest abundance (13.4°C). Furthermore, the range of water temperature in which *G. dominans* was found (3.8–26.4°C) was similar to that of *G. jinhaense* (1.7–25.8°C), but somewhat differed from that of *G. moestrupii* (7.4–27.1°C). Thus, *G. dominans* and *G. jinhaense* may have similar responses to water temperature, which differ from the response of *G. moestrupii*. The salinities where *G. dominans* (28.3), *G. jinhaense* (9.9), and *G. moestrupii* (33.4) had the highest abundances were very different from one another. Therefore, *G. dominans*, *G. jinhaense*, and *G. moestrupii* may have unique salinity preferences that differ from that of the other species.

The highest abundance of *G. dominans* (202.5 cells mL⁻¹) was much greater than that of *G. jinhaense* (20.2 cells mL⁻¹) or *G. moestrupii* (1.2 cells mL⁻¹). The maximum growth rate of *G. dominans* on its optimal prey, *Prorocentrum donghaiense* (1.62 d⁻¹), was comparable to that of *G. moestrupii* on its optimal prey, *A. minutum* (1.60 d⁻¹), but much higher than that of *G. jinhaense* on its optimal prey, *D. salina* (0.655 d⁻¹) (Yoo et al. 2013b, Kang et al. 2020, You et al. 2020). The abundance of *G. dominans* was significantly correlated with Chl-a concentration, whereas that of *G. jinhaense* or *G. moestrupii* was not correlated with Chl-a concentration. The Chl-a concentration when the

highest abundance of each species was found during the study period was 127.0, 6.8, and 0.9 ng Chl-a mL⁻¹ for *G. dominans*, *G. jinhaense*, and *G. moestrupii*, respectively. Assuming that the ratio of carbon to Chl-a was 40 (Peterson and Festa 1984) and all Chl-a were attributed to *P. donghaiense*, using the equation of You et al. (2020), the calculated growth rate of *G. dominans* on *P. donghaiense* at 127 ng Chl-a mL⁻¹ (5,080 ng C mL⁻¹) would be 1.09 d⁻¹. Similarly, if all Chl-a belonged to *D. salina*, using the equation of Kang et al. (2020), the calculated growth rate of *G. jinhaense* on *D. salina* at 6.8 ng Chl-a mL⁻¹ (270 ng C mL⁻¹) would be 0.20 d⁻¹. In the same manner, if all Chl-a belonged to *A. minutum*, using the equation of Yoo et al. (2013b), the calculated growth rate of *G. moestrupii* on *A. minutum* at 0.9 ng Chl-a mL⁻¹ (36 ng C mL⁻¹) would be 1.30 d⁻¹. The results of these calculations suggest that when each of these two *Gyrodinium* species were in their highest abundances, *G. dominans* may have had enough preferred prey to support its growth, whereas *G. moestrupii* may not have had sufficient amounts of prey.

There were smaller differences in the ribosomal DNA sequences and prey species preferences between *G. dominans* and *G. moestrupii* than those between *G. dominans* and *G. jinhaense* (Kang et al. 2020). However, the spatial-temporal distributions of *G. dominans* and *G. jinhaense* in Korean waters identified during the present study were closer than those of *G. dominans* and *G. moestrupii*. Therefore, differences in ribosomal DNA sequences and also the kind of the edible prey species among these *Gyrodinium* species may not affect spatial-temporal distributions. Physical-chemical properties such as water temperature and salinity and biological properties such as competition and predation may more strongly influence the differences in the distributions of these *Gyrodinium* species.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary Table S1. The abundance (cells mL⁻¹) of *Gyrodinium dominans* at each station from April 2015 to October 2018, quantified using qPCR (<https://e-algae.org>).

Supplementary Table S2. The abundance (cells mL⁻¹) of *Gyrodinium jinhaense* at each station from April 2015 to October 2018, quantified using qPCR (<https://e-algae.org>).

Supplementary Table S3. The abundance (cells mL⁻¹) of *Gyrodinium moestrupii* at each station from April 2015 to October 2018, quantified using qPCR (<https://e-algae.org>).

Supplementary Fig. S1. Map of the sampling stations, indicating the abundances of *Gyrodinium dominans*, *G. jinhaense*, and *G. moestrupii* in spring (March or April) (A), summer (June or July) (B), autumn (September or October) (C), and winter (December or January) (D) from 2015 to 2018 (<https://e-algae.org>).

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