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Occurrence of Sand-dwelling and Epiphytic Dinoflagellates Including Potentially Toxic Species along the Coast of Jeju Island, Korea

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ABSTRACT

Marine benthic (sand-dwelling and epiphytic) dinoflagellates consists of known potentially toxic species which are harmful to marine organisms as well as human by consumption of sea food, alerting scientists, aquaculture industry and government in mostly tropical and subtropical region. Occurrence and distribution of these species are less studied from temperate region. Occurrence and distribution of benthic dinoflagellates in the intertidal zone along the coasts of Jeju Island, Korea was determined by monthly collection of sand sediment and macroalgal samples from eight sampling locations from March 2011 to February 2012. Identification and taxonomic observations were made of the benthic dinoflagellate samples using light and epifluorescence microscopy. Thirty-seven dinoflagellate taxa belong to eighteen genera were identified. Among them, twenty six were found only in sand sediment, seven in macroalgal samples and four in both sand and macroalgal samples. Of the 37 species, nine were potentially toxic. The most frequently occurring species was *Amphidinium carterae*, followed by *A. operculatum*, *Coolia malayensis* and *Ostreopsis ovata*, occurred at all sampling stations. *Gambierdiscus yasumotoi* and *Gambierdiscus* sp. were rarely occurred. Monthly variations in number of occurring benthic dinoflagellates showed that higher number of species could be found during early of spring to early summer (March to June) with moderate to high salinity (26-34 psu) and low to moderate temperature (12-20°C). Epiphytic dinoflagellates did not show specific preference for macroalgal species as host. Potentially toxic species were present throughout the year, however, varied within the stations. These results suggest that diversified benthic dinoflagellates including several potentially toxic species occurring in Jeju Island, which may indicate a potential risk of toxicity in the marine ecosystem.

Key words: Benthic dinoflagellates, epiphytic, intertidal zone, Jeju Island, sand-dwelling, toxic

INTRODUCTION

Marine benthic dinoflagellates are unicellular organisms that have received a great attraction due to their importance as primary producers in the marine ecosystem and also due to the problems encountered with potentially harmful species which causes economic losses (particularly in the aquaculture, recreation and tourism industries) by killing fish/shellfish through toxicity and human health problems (e.g., Paralytic Shellfish Poisoning, PSP; Neurotoxic Shellfish Poisoning, NSP; azaspiracid poisoning AZP, Diarrheic Shellfish Poisoning, DSP and Ciguatera Fish Poisoning, CFP) in subtropical to tropical coasts (Lehane and Lewis, 2000; Godhe *et al.*, 2002; Hallegraeff, 2002;

Glibert *et al.*, 2005). Globally, one million people may be affected by ciguatera annually (Ramsdell *et al.*, 2005), with estimated economic impact of ciguatera in the United States to be \$21.19 million year^{-1} on average (Anderson *et al.*, 2000). Benthic epiphytic dinoflagellates are found associated with sea grasses, macro algae, dead corals and sediments in tropical and subtropical regions of the Pacific Ocean, Indian Ocean and the Caribbean (Fukuyo, 1981; Bomber *et al.*, 1985; Morton and Faust, 1997; Pearce *et al.*, 2001; Hernandez-Becerril and Almazan-Becerril, 2004; Parsons and Preskitt, 2007). However, some species also live in temperate regions (Hurbungs *et al.*, 2002; Selina and Levchenko, 2011). The occurrence of epiphytic and benthic dinoflagellates in temperate waters has been reported as an evidence of increasing water temperature (Rhodes, 2011; Jeong *et al.*, 2012a, b). Many of the benthic dinoflagellates are abundant in intertidal substrata and their contributions to benthic and shallow marine ecosystems may be significant. Several species of the benthic dinoflagellate genera such as *Ostreopsis, Coolia, Prorocentrum* and *Amphidinium* are known to be potentially toxic (Fukuyo, 1981; Mohammad-Noor *et al.*, 2007). Therefore, the study of their occurrence and potential toxic reactions in intertidal waters is important.

Jeju Island belongs to temperate region based on classification by air temperature. Tsushima Warm Current (TWC) is one of the factors affecting on the shifting ecosystem of the Jeju Island from temperate to subtropical environment (Pang *et al.*, 1996). However, some tropical fishes and invertebrates have been found during the last decade mainly by acceleration of global warming. Previously, only planktonic toxic dinoflagellates were recorded from Korean temperate waters but potentially toxic benthic sand dwelling and epiphytic species have not been well documented. Information on diversity and distribution patterns for benthic toxic/non toxic dinoflagellates in Jeju Island is limited. Till date no toxic event caused by marine benthic dinoflagellates is reported from this Island. Recently, (Kim *et al.*, 2011; Jeong *et al.*, 2012a, b; Kang *et al.*, 2013; Lim *et al.*, 2013; Shah *et al.*, 2013) reported the presence of some benthic epiphytic dinoflagellates in Jeju Island. In-depth information on occurrence, seasonal distribution and diversity of benthic dinoflagellate population from this Island are yet to be achieved. The aim of this study was to document the biodiversity and occurrence of benthic sand dwelling and epiphytic as well as potentially toxic dinoflagellates from the intertidal zone of coastal waters of Jeju Island.

MATERIALS AND METHODS

Study sites and sample collection: This study was carried out from March 2011 to February 2012 in the intertidal zone along the coasts of Jeju Island, Korea. Monthly sampling was carried out at eight designated stations (beaches) (Fig. 1) and 480 samples of sand sediment and macroalgae were collected during the lowest low tide. A brief description of the characteristics, latitudes and longitudes of the sampling stations is presented in Table 1. Sand sediment samples

Table 1: Summary of sampling stations in the intertidal zone along the coasts of Jeju Island, Korea

Locations	Station No.	Latitude	Longitude	Characteristics of sampling stations
Se-hwa	1	33°31'29.86"N	126°51'40.50"E	Sandy beach with fine white sand and volcanic rocks
Kim-nyung	2	33°33'29.64"N	126°45'35.23"E	Sandy beach comprising white, very fine sand and rocky shore
Ham-duk	3	33°32'32.94"N	126°40'12.27"E	White sandy beach with fine white sand
Hyup-jae	4	33°23'38.88"N	126°14'23.02"E	Large sandy beach with volcanic rocks
Hamo	5	33°12'39.86"N	126°15'38.23"E	Very small beach with coarse black and white sand with volcanic rocks
Hwa-sun	6	33°14'22.38"N	126°19'55.67"E	Small beach with coarse black and white sand with volcanic rocks
Pyosun	7	33°19'34.73"N	126°50'29.30"E	Large sandy beach with fine white sand
Sinyang	8	33°31'29.86"N	126°51'40.50"E	Sandy beach with fine white sand and volcanic rocks

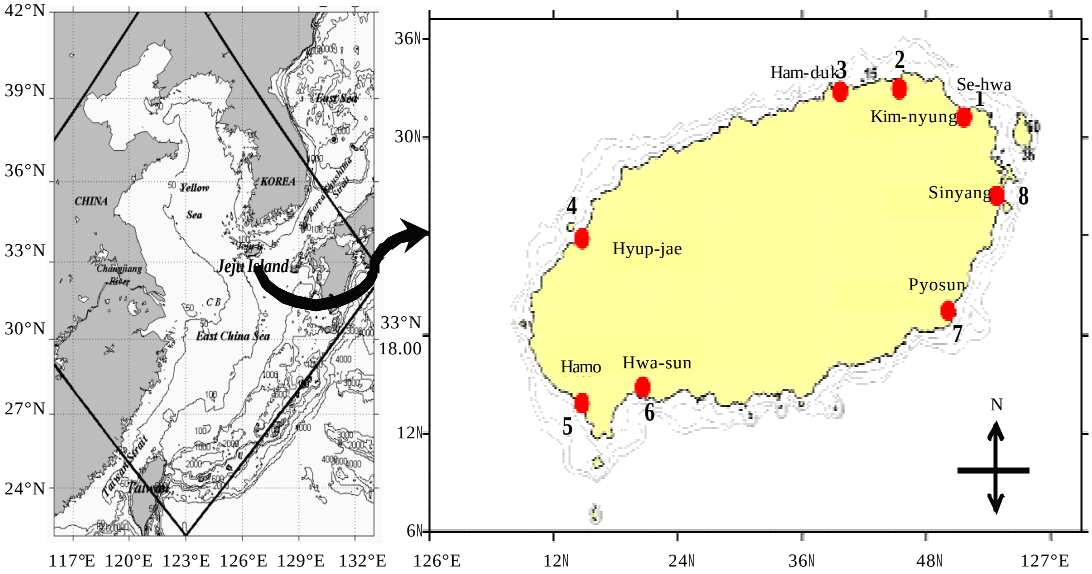

Fig. 1: Jeju Island, Korea and locations of eight sampling stations along the coasts of Jeju Island

(50-150 g wet weight) were collected from depths of 3-4 cm on sandy beaches using a plastic tube sample corer. The samples were transferred to plastic bottles with ambient seawater. In the laboratory, the sediment samples were vigorously shaken for 1 min and the material was passed through 200 and 100 µm mesh sieves to remove large particles and finally passed through a 20 µm mesh sieve. The material retained by the sieve was resuspended in sterile filtered seawater in a petri dish for live cell examination and isolation (modification of a method of Selina and Hoppenrath (2008). To survey epiphytic dinoflagellates, we collected 20-100 g wet weight macroalgae (Rhodophyta, Phaeophyta and Chlorophyta) on the intertidal zone of the sampling stations by hand picking and placing them into separate plastic ziploc bags with ambient seawater. Epiphytic dinoflagellates were separated from the macroalgae by vigorous shaking of the macroalgae into a plastic container with 200 mL of fresh filtered seawater for 1 min to dislodge the attached dinoflagellate cells and then we followed the procedure described above to extract the dinoflagellates from the sand sediment.

Identification of dinoflagellates and macroalgae: Freshly-collected living dinoflagellates were isolated by micropipette-washing method, placed on slide glass covered by cover slip and morphometric features were observed under transmitted light with bright field and phase contrast at 400 magnification and photographed using a microscope (Axioplan 2, Carl Zeiss, Oberkochen, Germany) equipped with a digital camera (Axiocam ERc5s, Carl Zeiss). Both the dorsal and ventral sides of each dinoflagellate were examined. Cell size and some morphometric measurements were obtained from micrographs using Carl Zeiss ZEN Lite software. Thecal plate patterns for armored dinoflagellates were identified using Calcofluor White M2R (Fritz and Triemer, 1985). The Calcofluor stained cells were examined using an epifluorescence (violate excitation ca 430 nm, blue emission ca 490 nm) microscope (Axioplan 2, Carl Zeiss) equipped with digital camera (Axiocam ICm1, Carl Zeiss). Unarmored dinoflagellates were identified based on morphological features such as body contour and proportion, cingulum displacement, sulcus extension and direction on epitheca, presence and location of specific organelles. Dinoflagellates were identified using previously published schemes (Fukuyo, 1981; Faust, 1996; Steidinger and Tangen, 1996; Hoppenrath, 2000; Hansen *et al*., 2001; Faust and Gulledge, 2002; Murray and Patterson, 2002; Hoppenrath *et al*., 2007; Mohammad-Noor *et al*., 2007; Al-Yamani and Saburova, 2010; Shah *et al*., 2013). Identification was also confirmed by molecular analysis (genomic DNA extraction, LSU rDNA D1D3 region sequenced and phylogenetic analysis) (unpublished data). Macroalgae were identified using appropriate keys (Lee and Kang, 2001; Lee, 2008) and cataloged.

Measurements of hydrological parameters: Hydrological variables such as water temperature (°C) and salinity (psu) were estimated every month during sample collection in the water column of intertidal zone of all sampling stations with a temperature-salinity meter (YSI 35; Yellow Spring Instrument, Ohio, USA).

RESULTS AND DISCUSSION

Occurrence of sand dwelling and epiphytic dinoflagellates: A total of thirty seven benthic dinoflagellate species, belonging to eighteen genera: *Amphidienella*, *Amphidiniopsis*, *Amphidinium*, *Cabra*, *Coolia*, *Durinskia*, *Gambierdiscus*, *Gymnodinium*, *Gyrodinium*, *Heterocapsa*, *Katodinium*, *Ostreopsis*, *Oxyrrhis*, *Polykrikos*, *Prorocentrum*, *Testudodinium*, *Thecadinium* and *Togula*, were identified from the intertidal zone of Jeju Island, Korea (Table 2). The number of identified species is comparatively higher than previously published literature; (Fukuyo, 1981) (11 species), Mohammad-Noor *et al.* (2007) (9 genera and 24 species), Parsons and Preskitt (2007) (26 species), Kim *et al.* (2011) (5 genera, 5 species), Selina and Levchenko (2011) (8 genera, 13 species, including 5 potentially toxic species). This may indicate that intertidal zone of Jeju Island's coastal areas contain diversified benthic dinoflagellate flora.

In present study, majority of the benthic dinoflagellate species (twenty six) were found to be associated with sand sediments. Among the identified species, seven were associated with macroalgal samples and four found in both sand and macroalgal samples. *Coolia*, *Gambierdiscus* and *Ostreopsis* were exclusively found on macrophyte. *Amphidinium* and *Prorocentrum* were found on macroalgae as well as sand sediment. The number of sand-dwelling dinoflagellates in the present study was higher than those reported by Faust (1996, 2009) but lower compared to the studies by Murray (2003) and Saburova *et al*. (2009). The difference in species number of sand-dwelling dinoflagellates is attributed to several factors such as the variation or the uniqueness of the sampling station, period and type of sediment. Sampling strategies (collection methods, seasonal distribution of species, sampling duration etc.) could also be responsible for the variation in the number of species identified (Hoppenrath and Elbrachter, 1998). Presence of greater number of sand dwelling benthic dinoflagellates compared to the epiphytic assemblage may indicate that intertidal zone of Jeju Island's coasts are serving as potential nursery or seed-bank for dinoflagellates. In addition, sand sediments of the sampling stations of Jeju Island may provide ideal habitat where benthic dinoflagellates divide among sand grains proliferate in nutrient enriched benthic environment which was also observed by Fenchel (1988). It is also known that dinoflagellates seek shelter from meiofauna predators in the interstitial spaces between sand grains (Faust, 1995). Furthermore, high cell density of sand dwelling dinoflagellates causing the discoloration of sand has been reported (Hoppenrath *et al*., 2007). Therefore, this indicated that marine sand-dwelling dinoflagellates are capable of forming bloom.

Among the identified genera, the most dominant and diverse genus was *Amphidinium* belonging 10 species followed by genus *Prorocentrum* (6 species), *Amphidiniopsis* and *Togula* genera comprised of three species each and *Durinskia*, *Gambierdiscus* and *Thecadinium* genera two species each. However, *Amphidienella*, *Cabra*, *Coolia*, *Gymnodinium*, *Gyrodinium*,

Table 2: List of benthic dinoflagellates and their occurrences in intertidal zone along the coast of Jeju Island from March 2011 to February 2012

	Sampling stations								
Taxa	1	2	3	4	5	6	7	8	Substrate
Amphidienella sp.	-	-	+	+	-	-	-	-	Sand
Amphidiniopsis rotundata Hoppenrath and Selina	-	-	+	+	-	-	-	-	Sand
Amphidiniopsis swedmarkii (Balech) Dodge	+	++	++	-	-	-	+	+	Sand
Amphidinium carterae Hulbert	++	++	+	++	+++	+++	++	+	Sand/macroalgae
Amphidinium gibbosum (Maranda et Shimizu) Flø Jørgensen et Murray	-	++	+	+	-	+	-	+	Sand
Amphidinium herdmanii Kofoid et Swezy	++	-	++	+	-	-	-	+	Sand
Amphidinium incoloratum Campbell	-	-	+	-	-	-	+	-	Sand
Amphidinium massartii Biecheler	+	++	+	-	-	-	-	-	Sand
Amphidinium mootonorum Murray et Patterson	+	+	++	+	-	-	-	+	Sand
Amphidinium operculatum Claparede et Lachmann	+	++	+	+	++	+++	+	++	Sand/macroalgae
Amphidinium scissum Kofoid et Swezy	+	++	++	+	-	-	+	-	Sand
Amphidinium steinii Lemmermann	-	+	+	-	++	-	+	-	Sand
Amphidinium trulla Murray, Rhodes et Flø Jørgensen	-	-	-	-	+	-	-	-	Sand
Cabra matta Murray and Patterson	+	++	++	+	-	-	+	-	Sand
Coolia malayensis Leaw, Lim., et Usup	-	-	+	+	++	-	+	-	Macroalgae
Durinskia baltica (Levander) Carty et Cox	+	+	+	+	-	-	-	-	Sand
Durinskia capensis Pienaar, Sakai et Horiguchi	-	-	-	+	-	-	+	-	Sand
Gambierdiscus yasumotoi Holmes	-	-	-	-	-	*	-	-	Macroalgae
Gambierdiscus sp.	-	-	*	-	-	-	-	-	Macroalgae
Gymnodinium venator Flø Jørgensen et Murray	+	+	-	+	-	-	-	+	Sand
Gyrodinium instriatum Freudenthal et Lee	+	+++	++	++	-	-	-	+	Sand
Heterocapsa psammophila Tamura, Iwataki et Horiguchi	+	+	+	+	-	+	++	+	Sand
Katodinium asymmetricum (Massart) Loeblich	+	-	-	+	-	-	+	-	Sand
Ostreopsis ovata Fukuyo	+	++	++	+++	+	++	+	+	Macroalgae
Oxyrrhis marina Dujardin	+	-	-	-	++	-	+	-	Sand
Polykrikos lebourae Herdman	++	-	+	+	-	-	+	-	Sand
Prorocentrum clipeus Hoppenrath	-	-	++	++	-	-	-	-	Sand
Prorocentrum concavum Fukuyo	++	++	++	++	+	-	-	+	Macroalgae
Prorocentrum emarginatum Fukuyo	++	-	++	-	+	-	-	-	Macroalgae
Prorocentrum fukuyoi Murray et Nagahama	++	+	+++	++	+	-	+	+	Sand/macroalgae
Prorocentrum lima (Ehrenberg) Dodge	+++	-	++	+	-	-	-	-	Macroalgae
Prorocentrum rhathymum Loeblich III, Sherley, et Schmidt	++	-	-	-	++	+++	-	-	Sand/macroalgae
Testudodinium corrugatum (Larsen and Patterson) Horiguchi, Tamura, et Yamaguchi	+	-	++	+	+	-	-	++	Sand
Thecadinium kofoidii (Herdman) Schiller	+	+++	++	++	-	+	++	+	Sand
Thecadinium yashimaense Yoshimatsu, Toriumi, et Dodge	++	-	+	+	-	-	+	-	Sand
Togula britanica (Herdman) Flø Jørgensen, Murray et Daugbjerg	+	+	+	+	-	-	-	+	Sand
Togula jolla Flø Jørgensen Murray et Daugbjerg	-	+	-	+	-	-	-	+	Sand
Total = 37	25	19	29	27	12	08	17	16	

-: Specie is absent, *Rarely occurred, +: Sporadically occurred, ++: Commonly occurred, +++: Frequently occurred

Heterocapsa, *Katodinium*, *Ostreopsis*, *Oxyrrhis*, *Polykrikos* and *Testudodinium* were monospecific. *Amphidinium carterae*, *A. operculatum*, *Coolia malayensis*, *Ostreopsis ovata* occurred frequently at most sampling stations. *Heterocapsa psammophila*, *Thecadinium koifoidii* occurred sporadically to commonly at most stations except at st. 5 and *P. fukuyoi* occurred at most stations except 6. Additionally, *P. concavum* was also reported at most stations except st. 6 and 7 (Table 2). *Gambierdiscus yasumotoi* and *Gambierdiscus* sp., were recorded rarely and found only at st. 6 and 3, respectively.

The water temperature exhibited a predictable seasonal fluctuation by a minimum of 11.7°C in March 2011 (spring) and a maximum of 26.8°C in July 2011 (summer), with an average of 18.2°C (Fig. 2a). During this study period, average water temperature at each station ranged from 17.8-19.0°C. Salinity of the coastal seawaters in intertidal zone ranged from 22.6-35.9 psu, with an average of 32.6 psu. For all the sampling stations, the lowest salinity of 22.6 psu was recorded in August 2011 (summer) and the highest salinity of 35.9 psu in February 2012 (winter). The average salinity throughout the sampling period at each station varied from 30.8-33.6 psu (Fig. 2b). The seasonal dynamincs patterns of hydrological parameters along the coasts of Jeju Island in the present study agreed with the previous study (Lee *et al.*, 2009).

Monthly variations in number of occurring benthic dinoflagellates during March 2011 to February 2012 at different sampling stations in intertidal zone along the coasts of Jeju Island showed that highest number of benthic dinoflagellate species could be found from the early of

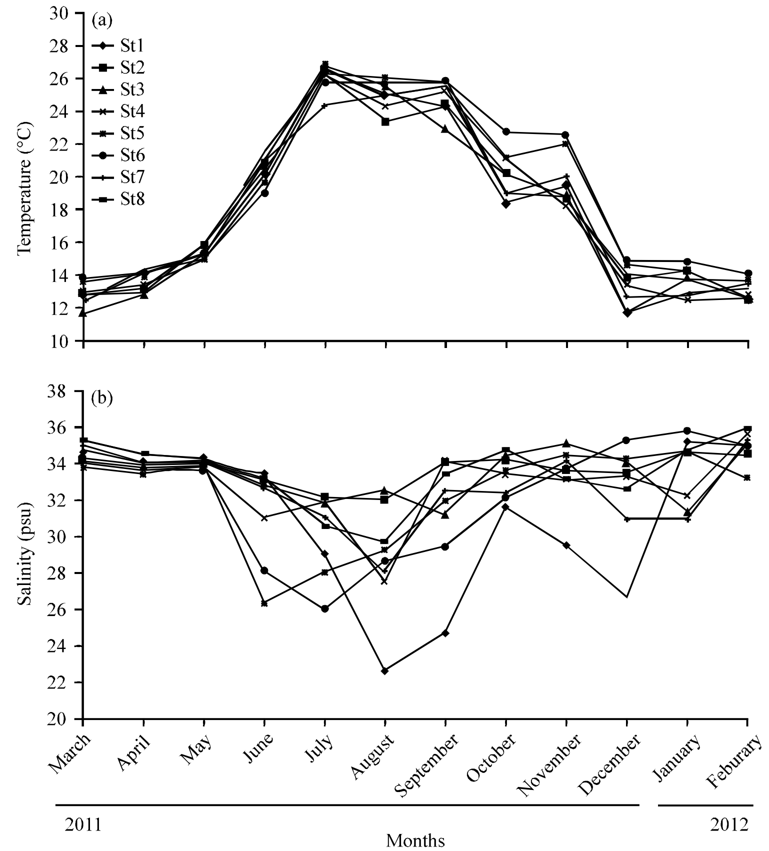

Fig. 2(a-b): Monthly variations in (a) water temperature and (b) salinity in the intertidal zone from March 2011 to February 2012 at different sampling stations of Jeju Island, Korea

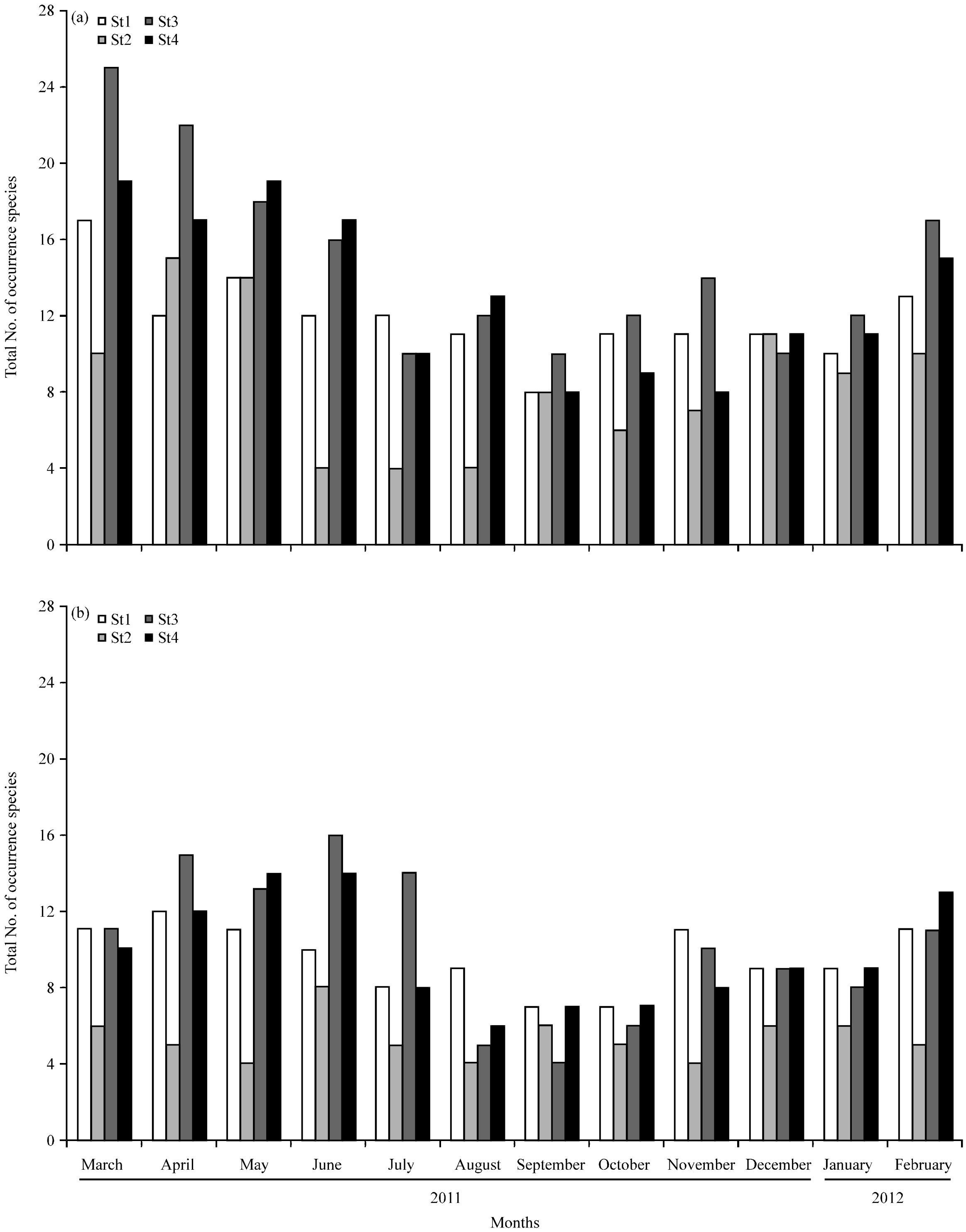

Fig. 3(a-b): Monthly variations in numbers of occurrence benthic dinoflagellate species at sampling stations (a) st. 1-4 and (b) st. 5-8 along the coasts of Jeju Island, Korea

spring to early summer (March-June) with moderate to high salinity (26-34 psu) and low to moderate temperature (12-20°C) (Fig. 3a and b). Among the benthic dinioflagellates identified

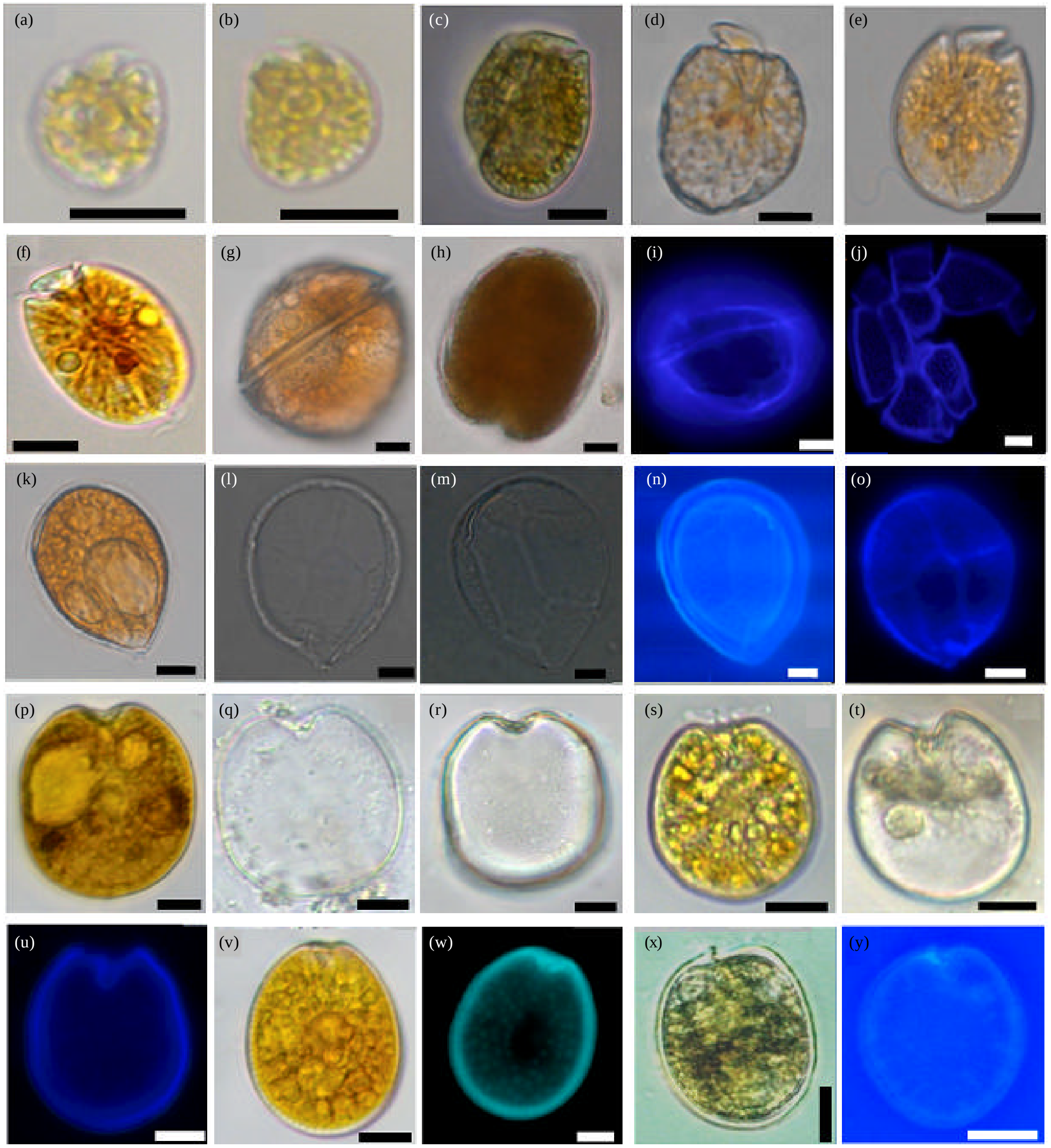

Fig. 4(a-y): Photographs of the potentially toxic benthic dinoflagellates found during March 2011 to February 2012 in the intertidal zone in Jeju Island (a-b) *Amphidinium carterae*, (c-d) *A. gibbosum*, (e-f) *A. operculatum*, (g-j) *Gambierdiscus yasumotoi*, (k-o) *Ostreopsis ovata*, (p-r) *Prorocentrum concavum*, (s-u) *P. emarginatum*, (v-w) *P. lima*, (x-y) *P. rhathymum*, (i, j, n, o, u, w, y) Epifluorescence. Scale bar = 10 µm for all photos

from this study, highest No. 25 of benthic dinoflagellates was observed in st. 3 during March. Comparatively lower No. (Avg. 5) of benthic dinoflagellates was recorded at st. 6 during the study period. During the months of July, August, September and October, lower number of benthic dinoflagellate species could be found than the rest of the months of study period at all stations. The presence of lower number of benthic dinoflagellates during the month from summer to autumn (July-October) with lower salinity at all sampling stations might be due to the heavy rainfall and flows of freshwater from the Changjiang River. Among the sampling stations, st. 1, 3, 4 and 7 showed higher species number occurrence while it was lower at st. 6 over the study period. This variation of species occurrence based on sampling stations could be due to the characteristics of sampling locations.

Epiphytic dinoflagellates and association with macroalage: A total of 21 macroalgal species (9 Rhodopyta, 8 Phaeophyta and 4 Chlorophyta) were identified as associated species with epiphytic dinoflagellate in the study area (Table 3). *Amphidinium*, *Coolia*, *Gambierdiscus*, *Ostreopsis* and *Prorcentrum* were recorded as epiphytic.

Amphidium carterae and *A. operulatum* were observed on the thalli of all chlorophytes (*Cladophora wrightiana*, *Codium fragile*, *Enteromorpha linza* and *Ulva pertusa*), Rhodophytes (*Galaxaura apiculata*, *Gracilaria vermiculophylla*) and Phaeophytes (*Dictyopteris divaricata* and *Sargassum thunbergii*). In fact, *A. carterae* and *A. operulatum* were the only dinoflagellate species,

Table 3: Distribution of epiphytic dinoflagellates on different macroalgae from March 2011 to February 2012 in intertidal zone along the coasts of Jeju Island, Korea

	Epiphytic dinoflagellates										
Macroalgae	AC	AO	CM	GY	G sp.	OO	PC	PE	PF	PL	PR
Rhodophyta											
Amphiora beauvoisii	-	-	-	+	+	+	+	-	-	+	-
Chondrus canaliculatus	-	+	-	-	-	+	+	-	-	-	-
Coralina pilulifera	-	+	-	-	-	+	-	+	-	+	-
Galaxaura apiculata	+	+	-	-	-	+	+	+	-	-	-
Gelidium amansii	+	-	+	-	-	+	-	+	-	+	-
Gracilaria vermiculophylla	+	+	-	-	-	+	-	-	-	-	-
Hypnea charoides	-	+	-	-	-	+	-	+	+	+	-
Plocamium telfairiae	-	+	+	-	-	+	+	-	+	-	-
Jania adhaerens	+	-	+	-	+	+	+	+	-	+	-
Phaeophyta											
Dictyopteris divaricata	+	+	+	-	-	+	-	-	-	+	+
Ecklonia cava	+	+	-	-	-	-	-	-	-	-	-
Hizikia fusiformis	-	-	+	-	-	+	+	+	-	-	-
Halopteris filicina	-	+	-	-	-	+	-	-	+	+	-
Sargassum micracanthum	-	-	+	-	-	+	+	-	-	-	+
Sargassum confusum	-	+	-	-	-	+	+	+	+	-	-
Sargassum siliquastrum	-	-	+	-	+	+	+	-	+	+	-
Sargassum thunbergii	+	+	+	-	-	+	-	-	+	-	+
Chlorophyta											
Cladophora wrightiana	+	+	-	-	-	+	-	-	-	-	-
Codium fragile	+	+	-	-	-	-	-	-	-	-	-
Enteromorpha linza	+	+	-	-	-	-	-	-	+	+	-
Ulva pertusa	+	+	-	-	-	+	-	-	+	-	-

-: Species is absent, +: Species is present, AC: *Amphidinium carterae,* AO: *Amphidinium operculatum,* CM: *Coolia malayensis,* GY: *Gambierdiscus yasumotoi,* G sp.: *Gambierdiscus* sp., OO: *Ostreopsis ovata,* PC: *Prorocentrum concavum,* PE: *Prorocentrum ermarginatum,* PF: *Prorocentrum fukuyoi,* PL: *Prorocentrum lima,* PR: *Prorocentrum rhathymum* isolated from *Ecklonia cava* and *Codium fragile* (Table 3). Kim *et al.* (2011) observed *Amphidinium* spp. with seven macroalgal species (*Cladophora wrightiana, Sargassum* sp., *Dictyopteris divaricata, Chordaria flagelliformis, Padina arborescens* and *Martensia* sp.) From these seven macroalgal species, *Chordaria flagelliformis, Padina arborescens* were not found during this study. *C. malayensis* was found on Rhodophytes (*Plocamium telfairiae, Jania adhaerens*) and Phaeophytes (*Hizikia fusiformis, Sargassum siliquastrum* and *Sargassum siliquastrum)* but absent from Chlorophytes. Kim *et al.* (2011) identified *Coolia* spp., from seven different species of macroalgae which disagree with the present study. *Gambierdiscus* was absent from chlorophytes. *G. yasumotoi* only found on Rhodophytes (*Amphiora beauvoisii*) but *Gambierdiscus* sp. was associated with Rhodophytes (*Amphiora beauvoisii* and *Jania adhaerens*) and Phaeophyte (*Sargassum siliquastrum*). *Gambierdiscus* spp., were attached all the macroalgae, except *Codium fragile* and *Sargassum siliquastrum*, collected by Kim *et al.* (2011). *G. toxicus* was found on Rhodophyte *Jania* in a Gambier Island reef (Yasumoto *et al.*, 1980). Depending on the geographic region, *G. toxicus* has been shown to prefer different macroalgal host species and has been found with more than 50 algal genera (Carlson and Tindall, 1985; Cruz-Rivera and Villareal, 2006). *O. ovata* was present on almost all the macroalgae species with some expectations. A result that 18 of the 21 macroalgae were preferred by *O. ovata* is generally agreed with Kim *et al.* (2011) who observed *Ostreopsis* spp. on 21 of the 24 macroalgal species but host preference of this species was completely different from the observation by Parsons and Preskitt (2007) and Vila *et al.* (2001). *P. concavum, P. emarginatum* and *P. rhathymum* were absent from all of Chlorophytes. *P. rhathymum* was also absent from all of Rhodophytes. Macroalgae, *Chondrus canaliculatis* and *Ecklonia cava* were not preferred by *Prorocentrum. Ulva pertusa* was preferred by all species of *Prorocentrum* except *P. fukuyoi* (Table 3). Kim *et al.* (2011) found *Prorocentrum* spp. from eight macroalgal species (*Ulva pertusa, Ecklonia cava, Sargassum* sp., *Dictyopteris divaricata, Chordaria flagelliformis, Padina arborescens, Martensia* sp., *Gelidium amansii, Corallina* sp.) which were partially similar with the present study. Bomber *et al.* (1985) found *P. lima* to be the most abundant species on the green algae *Halimeda opuntia, Penicillus capitatus* and *Avrainvillea nigricans* among 16 species of Chlorophyta, Phaeophyta and Rhodophyta and on the seagrass *Thalassia testudinum.*

Generally, no macroalgae species appeared to be an overall best or worst host for specific epiphytic dinoflagellates. Additionally, preferences were not consistent among the dinoflagellates. Substrate preference of epiphytic dinoflagellates species may be related with species interactions of the epiphytes with physical and biological environment. Dinoflagellates of epiphytic assemblages on macrophytes are more diverse and abundant than those in the water column and in sediment (Vila *et al.*, 2001; Faust, 2004) and also the macroalgal substrate not significant with respect to association with dinoflagellate species (Vila *et al.*, 2001). Epiphytic dinoflagellates have been shown to have distinct substrate preferences (Heil *et al.*, 1998; Heimann *et al.*, 2009). The present study further highlights the claim and corroborates findings of previous researchers that the presence of epiphytic dinoflagellate genera is indeed affected by the variant of macroagal species in different geographical locations.

Distribution of potentially toxic benthic dinoflagellates: Nine potentially toxic/harmful benthic dinoflagellates such as *Amphidinium carterae, A. gibbosum, A. operculatum, Gambierdiscus yasumotoi, Ostreopsis ovata, Prorocentrum concavum, P. emarginatum, P. lima* and *P. rhathymum* were identified in this study (Table 4) and morphological features are illustrated in

Table 4: Distribution of potentially toxic/harmful benthic dinoflagellates at eight sampling stations from March 2011 to February 2012 along the coasts of Jeju Island, Korea

	Months							
	2011							
Taxa	March	April	May	June	July	August	Toxicity and references	Figure
Amphidinium carterae	St 1-8	St 2, 4-7	St 3, 4, 6	St1-4, 6	St1, 4, 6-8	St 1-6	Ichthyotoxins and hemolytic substances, Ciguatera (Baig *et al.*, 2006)	Fig. 4a-b
A. gibbosum	St 3, 8	St 2, 8	-	-	-	St 2, 8	Cytotoxic metabolites (Maranda and Shimizu, 1996)	Fig. 4c-d
A. operculatum	St 6, 8	St 1, 4	St 2, 5	St 3, 5, 6	St 2, 4, 7	St 1, 2	Haemolytic and amphidinols (Yasumoto *et al.*, 1987)	Fig. 4e-f
Gambierdiscus yasumotoi	-	-		St 6	-	-	Produces maitotoxin (Holmes *et al.*, 1998)	Fig. 4g-j
Ostreopsis ovate	St 7	St 4, 6	St 1, 3, 5-7	St 1-8	St 1-4, 6	St 3-5	Produce a toxic butanol-soluble compound (Elbrachter and Faust, 2002)	Fig. 4k-o
Prorocentrum concavum	St 2	St 1, 4	St 2-5	St 3	St 2	St 1, 4	Okadaic acid (Hu *et al.*, 1993) and ichthyotoxin (Yasumoto *et al.*, 1987)	Fig. 4p-r
P. emarginatum	St 3	St 3	St 1, 3	St 5	St 1	-	Haemolytic and fibroblast activity (Turquet, 1997)	Fig. 4s-u
P. lima	St 1, 3	St 3	St 1, 4	St 3	St 1	-	Okadaic acid, dinophysitoxin-2 (Hu *et al.*, 1993)	Fig. 4v-w
P. rhathymum	-	St 6	St 5	St 1	St 5	-	Hemolytic activity (Nakajima *et al.*, 1981)	Fig. 4x-y

	Months							
	2011				2012			
Taxa	September	October	November	December	January	February	Toxicity and references	Figure
A. carterae	St 1, 5	St 8	St 1, 4, 5	St 2, 4, 5, 6	St 2, 4-6	St 4, 5, 7, 8	Ichthyotoxins and hemolytic substances, Ciguatera (Baig *et al.*, 2006)	Fig. 4a-b
A. gibbosum	-	-	St 2, 6, 7	St 8	St 8	St 2, 4, 6, 8	Cytotoxic metabolites (Maranda and Shimizu, 1996)	Fig. 4c-d
A. operculatum	St 1, 3	St 3, 6	St 8	St 8	St 6, 8	St 6	Haemolytic and amphidinols (Yasumoto *et al.*, 1987)	Fig. 4e-f
Gambierdiscus yasumotoi	-	-	-	-	-	-	Produces maitotoxin (Holmes *et al.*, 1998)	Fig. 4g-j
O. ovate	St 3-7	St 4-6	St 5, 6, 8	St 4	St 8	St 1, 4	Produce a toxic butanol-soluble compound (Elbrachter and Faust, 2002)	Fig. 4k-o
P. concavum	St 2-5	St 3	St 1-4	St 1	St 4	St 8	Okadaic acid (Hu *et al.*, 1993) and ichthyotoxin (Yasumoto *et al.*, 1987)	Fig. 4p-r
P. emarginatum	St 1	-	St 1, 5	St 3, 5	St 1, 3	St 3	Haemolytic and fibroblast activity (Turquet, 1997)	Fig. 4s-u
P. lima	-	St 3	St 3, 4	St 3, 4	St 1, 4	St 3, 4	Okadaic acid, dinophysitoxin-2 (Hu *et al.*, 1993)	Fig. 4v-w
P. rhathymum	St 5, 6	St 1, 6	St 5	St 5, 6	-	St 6	Hemolytic activity (Nakajima *et al.*, 1981)	Fig. 4x-y

Fig. 4. Morphological observations on benthic dinoflagellates including the potentially toxic species from this study generally agreed with taxonomic descriptions from previous studies in Jeju Island (Shah *et al*., 2013) and other geographical locations. The toxic genera were also the most dominant genera, that is, *Amphidinium* and *Ostreopsis* were present at most of the sampling stations. Species belong to the genus *Amphidium* were present throughout the year, however, varied within the stations. *Amphidinium carterae* was domimant in March while *A. operculatum* was dominant in June and July. *A. gibbosum* was present for seven months during the sampling period. Although, most of the potentially toxic species were recorded throughout the year, *Gambierdiscus yasumotoi* was only found at st. 6 in June 2011, which might indicate that *Gambierdiscus* was not common benthic dinoflagellate in the intertidal zone in Jeju Island. *O. ovata* was present throughout the year and especially recorded at all stations in June. *P. concavum* was found to be distributed dominantly in May and September at st. 2~5. *P. emarginatum* was common in May, November, December and January. *P. lima* found most of the months during the sampling period, while *P. rhathymum* was found commonly in September, October and December at the sampling stations. *P. lima, P. rhathymum, A. carterae, G. yasumotoi, O. ovata* are responsible for producing a variety of toxins such as maitotoxins, saxitoxins or okadaic acid and also suspected of contributing to Ciguatera Fish Poisioning (CFP) along with *Gambierdiscus toxicus* world-wide (Faust, 2009). Changes of occurrence of macroalgae and expansion of macroalgae into Jeju marine habitats could be a primary vector for the invasion of their epiphytic microalgal communities into coastal waters of Jeju Island. In addition, the cysts from ballast water of commercial ships from other regions could be another possible reason for the introduction of new toxic species. Occurrence of potentially toxic benthic dinoflagellates in the waters of Jeju Island could be viewed as a threat to seafood safety. Furthermore, there is a necessity for the expansion of the regulatory monitoring for marine biotoxins to additional marine edible organisms, such as fishes and cephalopods.

CONCLUSION

The results of this study indicate that, high species diversity characterized the assemblage of benthic sand dwelling and epiphytic dinoflagellates in the intertidal zone along the coasts of Jeju Island. *Amphidinium* was the main dominant genera while *G. yasumotoi* and *Gambierdiscus* sp., were recorded rarely. The number of occurring benthic dinoflagellates was higher during early of spring to early summer (March-June). Epiphytic dinoflagellates did not show specific preference for macroalgae species as host. Several potentially toxigenic dinoflagellate species are present in marine benthic environments of Jeju Island. Occurrences of subtropical/tropical benthic dinoflagellates in Jeju Island may point towards the changing climate from temperate to subtropical. The presence of known tropical toxic benthic dinoflagellates may indicate that there might have risk of potential toxicity by benthic dinoflagellates in Jeju Island.

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