DOI: 10.31398/tpjf/31.1.2023-0093

RESEARCH ARTICLE

Morphology, Phylogeny and Discovery of the Antioxidant Potential of a Marine Dinoflagellate *Karlodinium ballantinum* (Kareniaceae, Dinophyceae) Isolated from Subic Bay, Zambales, Central Luzon, Philippines

Garry A. Benico[1]*, Julienne Alrisse V. Flores[1], Samantha Patricia Esteban[1], Jerwin R. Undan[1], Koyo Kuwata[2], Mitsunori Iwataki[2]

[1] *Central Luzon State University, Science City of Munoz, Nueva Ecija 3120 Philippines*
[2] *Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo 113-8657, Japan*

ABSTRACT

Morphological characteristics, phylogenetic analysis, and antioxidant activity of a marine unarmored dinoflagellate *Karlodinium ballantinum* were examined using a culture established from Subic Bay, Zambales in November 2022. Detailed examinations were based on light microscopy and molecular phylogeny inferred from internal transcribed spacer (ITS) and LSU rDNA sequences. Cells of *K. ballantinum* were small and ellipsoid, possessing a straight ASC, large central nucleus, accumulation bodies, and unequally distributed chloroplasts with internal pyrenoids. Cell size ranged from 7.2–13.7 µm in length and 5.4–9.0 µm in width. Phylogenetic analyses suggested that *K. ballantinum* has a close affinity to *K. gentienii* and *K. zhouanum*. Upscaling the culture into 20 L, the final cell density of the culture reached approximately 177.5×10^{6} cells/mL, which produced 0.1 g of algal biomass. The antioxidant activity of *K. ballantinum,* as screened using the DPPH assay, showed a 23.39% antioxidative property. This result displayed the potential of *K. ballantinum* to be beneficial microalgae despite being known as a causative agent of HABs. The present study is the first record of *K. ballantinum* in Subic Bay, Zambales, and serves as the first report of the antioxidant activity of a species under the genus *Karlodinium*.

*Corresponding Author: *gabenico@clsu.edu.ph*
Received: *December 1, 2023*
Accepted: *May 13, 2024*

Keywords: *fisheries, red tide, taxonomy, harmful algal blooms, antioxidant activity, kareniacean dinoflagellate*

1. INTRODUCTION

The small naked dinoflagellate *Karlodinium* is a well-known genus in the family Kareniaceae with reports of occurrence in several coastal and oceanic waters worldwide (Bachvaroff et al., 2009; Benico et al., 2020; Bergholtz et al., 2006; de Salas et al., 2005, 2008; Escobar-Morales & Hernández-Becerril, 2015; Fabro & Almandoz, 2021; Garcés et al., 2006; Luo et al., 2018; Nézan et al., 2014; Siano et al., 2009; Yang et al., 2000). Among species of *Karlodinium*, *K. veneficum*, *K. australe*, *K. digitatum*, and *K. decipiens* were reported to form devastating algal blooms that resulted in major fishery damages in countries such as Japan, the USA, and Malaysia (Garcés et al., 2006; Lim et al., 2014; Place et al., 2012; Sakamoto et al., 2021; Yñiguez et al., 2021). Because of its notoriety for forming harmful red tides, regional reports of *Karlodinium* species with species identification are important in understanding their distribution and species composition focusing on distinguishing harmful and non-harmful species.

Karlodinium is an unarmored dinoflagellate having a straight apical structure complex, a ventral pore, and chloroplasts containing internal lenticular pyrenoids and fucoxanthin or fucoxanthin derivatives as main accessory pigments (Benico et al., 2020; Daugbjerg et al., 2000). Characters such as the shape and location of the nucleus, cell shape and size, and the presence or absence of the ventral pore are critically observed to morphologically distinguish species of *Karlodinium*. With 16 currently taxonomically accepted species, this genus has the greatest number of species in the family Kareniaceae, with *K. azanzae* and *K. elegans* as the most recent additions (Benico et al. 2020; Cen et al. 2021).

Despite being known as a harmful species, many dinoflagellates were investigated for their potential industrial, pharmaceutical, and biotechnological applications. For example, *Alexandrium catenella,* which is a toxic dinoflagellate that is known for causing paralytic shellfish poisoning (PSP), has been reported to produce alexandrolide. These allelopathic molecules produced by *A. catenella* possess selective properties of growth inhibition against species of diatoms (Satake et al., 2019). Moreover, it can be used as an agricultural additive for developing biological pesticides in controlling aphids, scale insects, thrips, tetranychids, etc. such as cotton bollworms, bridging worms, beet armyworms, and other pests with chewing mouthparts (Casanova et al., 2023). Furthermore, *Lingulodinium polyedra*, a harmful dinoflagellate known for producing yessotoxins which can cause symptoms similar to those exhibited by PSP toxins, has been reported to generate valuable secondary metabolites against *Vibrio vulnificus* and *Staphylococcus aureus*, making it an excellent source of biologically active compounds for pharmaceutical applications ((Quijano-Scheggia et al., 2016).

Because *Karlodinium* species possess a rich pool of fucoxanthin as its primary carotenoid, they can also be harnessed as one of the important bioresources (Benico et al., 2020). Over the years, fucoxanthin has been recognized for its outstanding potential and significant benefits to human health. It has been shown to possess a range of important biological properties, including antioxidant properties (Kim & Pangestuti, 2011). In fact, the free radical scavenging activity of fucoxanthin is more remarkable than β-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin (Nomura et al., 1997) (Foo et al., 2020). To date, the majority of fucoxanthin used in industrial manufacturing comes from seaweeds. However, several studies have proven that selected marine microalgae species can also be a promising source of fucoxanthin that can be harnessed for industrial applications. For example, a species of marine microalgae, *Phaeodactylum tricornutum*, was reported to be a viable source of fucoxanthin due to its high volumetric productivity under specific growth conditions (Pereira et al., 2021).

In the present study, the species identity of the cultured *Karlodinium* was characterized using morpho-molecular analyses. The antioxidant activity of a species of *Karlodinium* isolated from Subic Bay, Zambales, was determined using a DPPH assay.

2. MATERIALS AND METHODS

2.1 Culture and observation

Water samples were collected using a 5 µm and 20 µm plankton nets at two sampling locations on the coast of Subic Bay, Zambales (14°8'60.25" N, 120°28'18.91" E; 14°87'54.52" N, 120°23'21.25" E) in Nov. 2022 (Figure 1). A culture of unarmored dinoflagellate *K. ballantinum* (strain GBSUB96) was successfully isolated from the seawater collected at Site 2, located at Subic Bay Fish Port. Sea surface temperature and salinity were recorded at 30.5°C and 30 ppt, respectively. A single cell of *K. ballantinum* was isolated using a capillary Pasteur pipette under an Olympus CKX31 (Olympus, Tokyo, Japan) inverted light microscope and was cultivated in sterile filtered seawater with a salinity of 30 ppt and enriched with a half-strength IMK medium (Wako, Tokyo, Japan). Cultures were subcultured every two weeks in the Algal Diversity and Bioresources Laboratory of the Central Luzon State University (CLSU-ADB), Science City of Muñoz, Nueva Ecija, with a room temperature of 23 ± 2°C and photoperiodic condition of light: dark cycle = 12:12 h under 30 µE m^{-2} s^{-1} light illumination

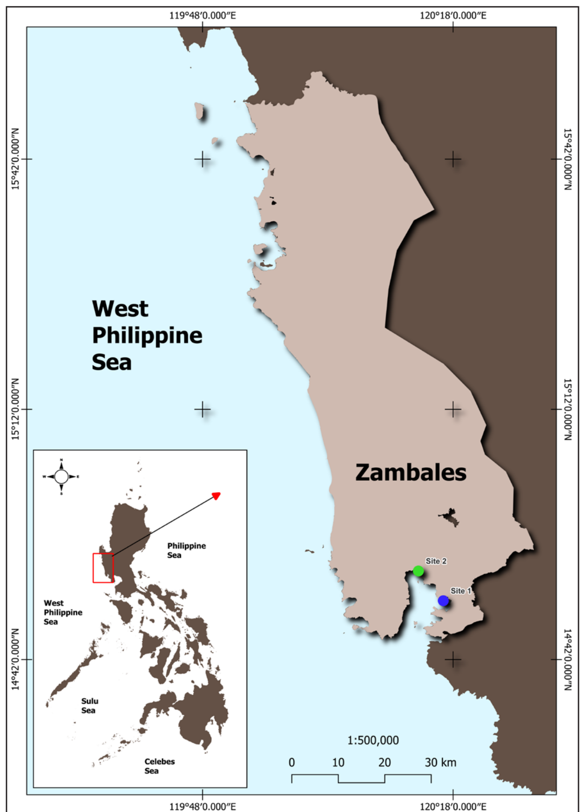

Figure 1. Sampling sites. Site 1 (blue dot) is located at Subic Bay Freeport Zone; Site 2 (green dot) is located at Subic Bay Fish Port.

in preparation for morphological observation and molecular analyses. The GBSUB96 strain was also routinely upscaled to a final volume of 20 L in sterile glass bottles for the antioxidant test.

External cellular morphology of the laboratory-established culture of *K. ballantinum* was observed using an Olympus BX60 (Olympus, Tokyo, Japan), a compound microscope with DIC optics, and fluorescence applications. Micrographs were documented using Canon EOS M200 (Canon, Tokyo, Japan) mirrorless digital camera and cell size was measured from at least 30 living cells. For fluorescence microscopy, cells were stained with 4'6-diamidino-2-phenylindole (DAPI) and observed under UV excitation.

2.2 Molecular analyses

Genomic DNAs from the exponentially growing culture of *K. ballantinum* that have been in culture for two weeks were extracted using a Qiagen DNEasy Plant Mini-Kit following the manufacturer's protocol (Valencia, CA, USA).

PCR amplification was performed to amplify nuclear-encoded rDNA ITS1-5.8S-ITS2 region and LSU rDNA region (D1–D5) using oligonucleotide primer pairs SR10 and 9R (Takahashi et al., 2014). Ex Taq was utilized as the DNA polymerase to run the PCRs. For each Ex Taq-polymerase, the final volume of the PCR mix was 10 µL – comprising of 6.15 µL of sterile distilled water, 1 µL of 10× Taq DNA polymerase buffer, 0.8 µL of deoxynucleotide triphosphates (dNTPs) mixture, 0.5 µL each of forward and reverse primer (5 pmol $µL^{-1}$), 0.05 µL of Taq DNA polymerase solution, and 1 µL of DNA template. Several thermal conditions for PCR were applied, and the most successful condition was as follows: an initial denaturation takes place at a temperature of 94°C for 1 min; followed by 40 cycles of 3 steps, 94°C for 20 s, 54°C for 30 s, and 72°C for 2 min and 30 s, with a final extension step of 72°C for 7 min. The cycling sequencing was carried out using GeneAmp PCR System 9700. The PCR products were subjected to gel electrophoresis for 25 min at 80 V on 1.5% agarose gel comprising 1 µL of SYBR Safe DNA gel stain per 10 mL of agarose gel. This was visualized using a gel imaging device.

The PCR product was purified using a Qiagen QIAquick Gel Purification Kit (Bothell, Washington, United States) following the manufacturer's protocol. The purified amplicon was outsourced to Eurofins Genomics, Tokyo, Japan, for DNA sequencing.

2.3 Phylogenetic analysis

The generated sequence was assembled into contigs using the Staden package software –Gap4 and Pregap (Staden et al., 2000). Homologous sequences of related species were acquired from GenBank to establish a phylogenetic tree. Contigs were imported and aligned with other kareniacean sequences using Clustal X v2.1 and were manually refined and adjusted with the help of BioEdit Sequence Alignment Editor v7.2.5 (Benico & Azanza, 2022; Takahashi et al., 2019). For the outgroup, three *Gyrodinium* species were used for analyses. MEGA v.7 was utilized to determine the most appropriate substitution model and carry out the Maximum-Likelihood (ML) and Neighbor-Joining analysis with 500 and 1000 bootstraps, respectively (Tamura et al., 2013)The best-fitting substitution and rate heterogeneity model of Tamura-Nei (TN93) was selected for maximum likelihood (ML) and neighbor-joining (NJ) analysis with a gamma distribution (G = 0.35) and the proportion of invariable sites (I = 0.17) for LSU and a **gamma distribution (G = 0.40) for ITS.**

2.4 DPPH radical scavenging assay

Cell harvest was performed using a sterile filter set with 3 µm membrane filter paper (diameter = 142 mm). Algal biomass was promptly filtered from the seawater with the help of a rotary vane vacuum pump. The final cell densities of the extracted cells were quantified using a Sedgewick-rafter counting chamber. Algal biomass of *K. ballantinum* was sent to Saint Mary's University – Center for Natural Sciences, located at Bayombong, Nueva Vizcaya, for methanolic crude extraction and DPPH radical scavenging assay.

The procedure for extracting crude material was adapted from the procedure described by (Arguelles et al., 2017) with minor modifications. Overnight, methanol was mixed with the algal biomass (20 mL of methanol: 1 g of algal biomass). The extract was filtered by means of a glass funnel and Whatman No. 1 filter paper. The resultant extract was concentrated to dryness in a rotary evaporator under decreased pressure (at 40°C) until a crude extract was obtained. The methanol extracts were stored at 4°C.

For the DPPH radical scavenging assay, the concentrated extract was utilized to generate a stock solution, and an aliquot was taken to prepare a 1000 ppm dilution, as well as a 1000 ppm dilution of catechin to serve as a control (1 mg/mL). In a separate plastic cuvette, 1 mL of the prepared stock solution was mixed

with 4 mL of the 0.1 mM DPPH solution. There were three separate reactions carried out. After preparation, the mixtures were left to incubate in the dark at 37°C for 30 min. Utilizing a UV VIS spectrophotometer (Biobase BK-UV1000, China), absorbance values were recorded at 517 nm. The reaction mixture's ability to scavenge free radicals was measured by its decrease in absorbance. The following formula was used to determine DPPH radical scavenging capacity:

Radical Scavenging Effect (%) = [A control – A sample) / A control] × 100

The absorbance of the DPPH without the test sample is denoted by A control, while the absorbance of the DPPH, including the test sample (sample), is denoted by A sample.

3. RESULTS

3.1 Morphology

Cells of laboratory established culture of *K. ballantinum* from Subic Bay, measuring 7.2–13.7 µm long (mean 11.2 ± 1.3, n = 30) and 5.4–9.0 µm wide (mean 7.4 ± 1.0, n = 30), were ellipsoid and smaller in size when compared to original *K. ballantinum* (Figure 2). The shape of the apical structure complex (ASC) was straight and extended briefly through the dorsal surface of the episode (Figure 2, A and B). The ventral pore was not observed in the cultured cells, but each cell showed 5–10 chloroplasts distributed unequally in the hypocone and epicone together with the accumulation bodies (Figure 2, C and D). The nucleus of *K. ballantinum* located centrally, was observed to be large, occupying much of the cure 2ell (Figure 2, E–G). The chloroplasts were highly pigmented with individual internal pyrenoids (Figure 2, D and H).

3.2 Molecular phylogeny

Phylogenetic trees based on LSU rDNA region and ITS region were constructed by ML and NJ. The aligned matrix of LSU rDNA nucleotide sequences of kareniacean species consisted of 79 taxa and yielded 969 bps, including gaps, of which 597 sites (61.6%) were constant, 363 sites (37.5%) were variable, and 288 sites (29.7%) were parsimony informative. Its average base compositions were A = 0.193, C = 0.254, G = 0.248, and T = 0.305. The aligned matrix of ITS region nucleotide sequences of kareniacean species consisted of 51 taxa and yielded 478 bps, including gaps, of which 184 sites (39.3%) were constant, 292 sites (61.1%) were variable, and 272 sites (56.9%) were parsimony informative. Its average base compositions were A = 0.220, C = 0.221, G = 0.254, and T = 0.305. The ML trees inferred from LSU and ITS rDNA are shown in Figures 3 and 4, respectively. Bootstrap support values derived from ML and NJ analyses were provided in each node.

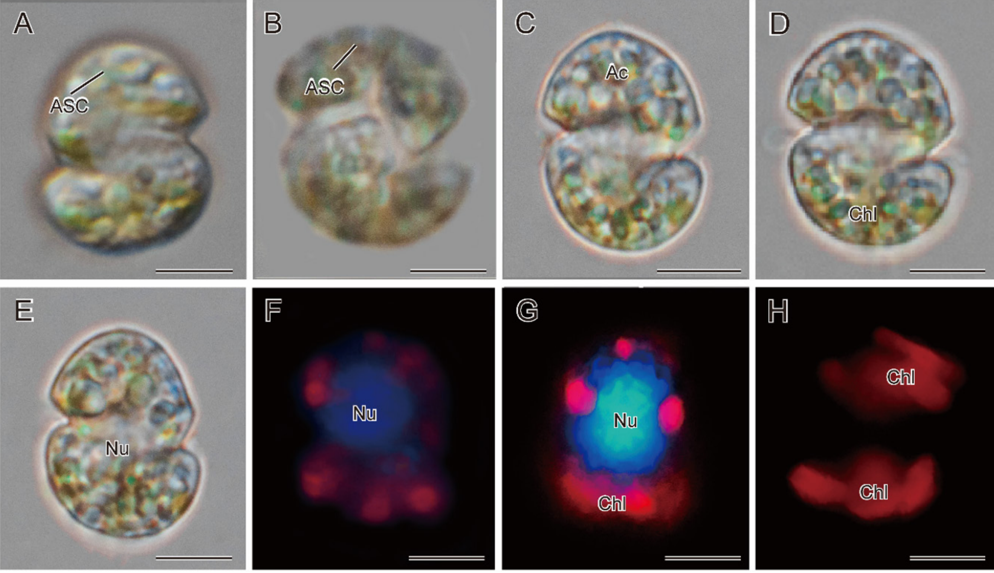

Figure 2. LM and fluorescence microscopy of *Karlodinium ballantinum*. Scale bar = 10 µm. (A) Surface focus from the ventral view showing the general shape of the cell and the straight apical structure complex (ASC). (B) Surface focus showing the ASC that extends briefly through the dorsal surface of the epicone. (C, D) Deeper focus showing the accumulation bodies (Ac) and highly pigmented chloroplasts (Chl). (E) Deeper focus from the ventral view showing the large nucleus at the center. (F, G, H) Deeper view of epifluorescence micrograph of the cell stained by DAPI (4',6-diamidino-2-phenylindole), showing the location and shape of the nucleus (Nu) and the chloroplasts (Chl).

In the ML tree inferred from LSU rDNA, *Karlodinium* species were separated into three major subclades (Figure 3). Clade 1 was composed of *K. ballantinum*, *K. gentienii*, *K. zhouanum*, *Karlodinium* sp., *K. corrugatum*, *K. elegans*, *K. conicum*, *K. micrum*, and *K. veneficum* (ML/NJ Bootstrap values = 78/87%). Clade 2 consisted of *K. digitatum*, *K. armiger*, *K. azanzae*, and *K. australe* (ML/NJ BS values = 100/99%). Clades 1 and 2 formed a robust clade (ML/NJ BS values = 91/93%). Clade 3 was composed of *K. decipiens* (ML/NJ BS values = 100/99%) and was closely related to the *Takayama* clade (ML/NJ BS values = 100/98%) with high support (ML/NJ BS values = 98/70%).

In the ML tree inferred ITS region, *Karlodinium* species were separated also into three subclades (Figure 4). Clade 1 was composed of *K. ballantinum*, *K. gentienii*, *K. zhouanum*, and *K. veneficum* (ML/NJ BS values = 93/94%). Clade 2 consisted of *K. digitatum*, *K. armiger*, *K. azanzae*, and *K. australe* (ML/NJ BS values = 98/100%). However, unlike in the ML tree inferred from the LSU region, Clades 1 and 2 in the ML tree inferred from the ITS region formed a poor clade (ML/NJ BS values = 66/59%). Clade 3 was composed of *K. decipiens* (ML/NJ BS values = 99/99%) and was closely related to the *Takayama* clade with high support (ML/NJ BS values = 97/88%).

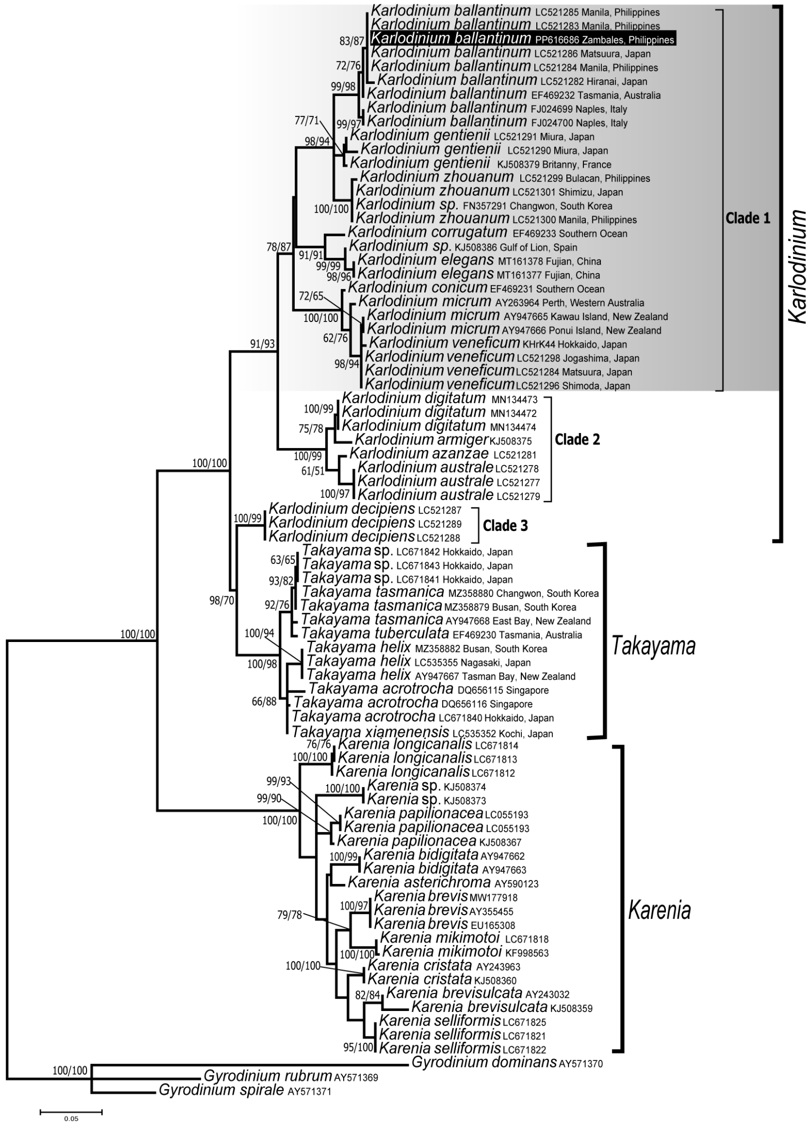

Figure 3. Maximum likelihood (ML) phylogeny of Kareniaceae inferred from LSU rDNA (D1–D5 regions, 969 bp in alignment profile). Bootstrap support (BS) values of maximum likelihood (left) and neighbor joining (right) are shown (DNA sequences analyzed in this study are highlighted in black boxes)

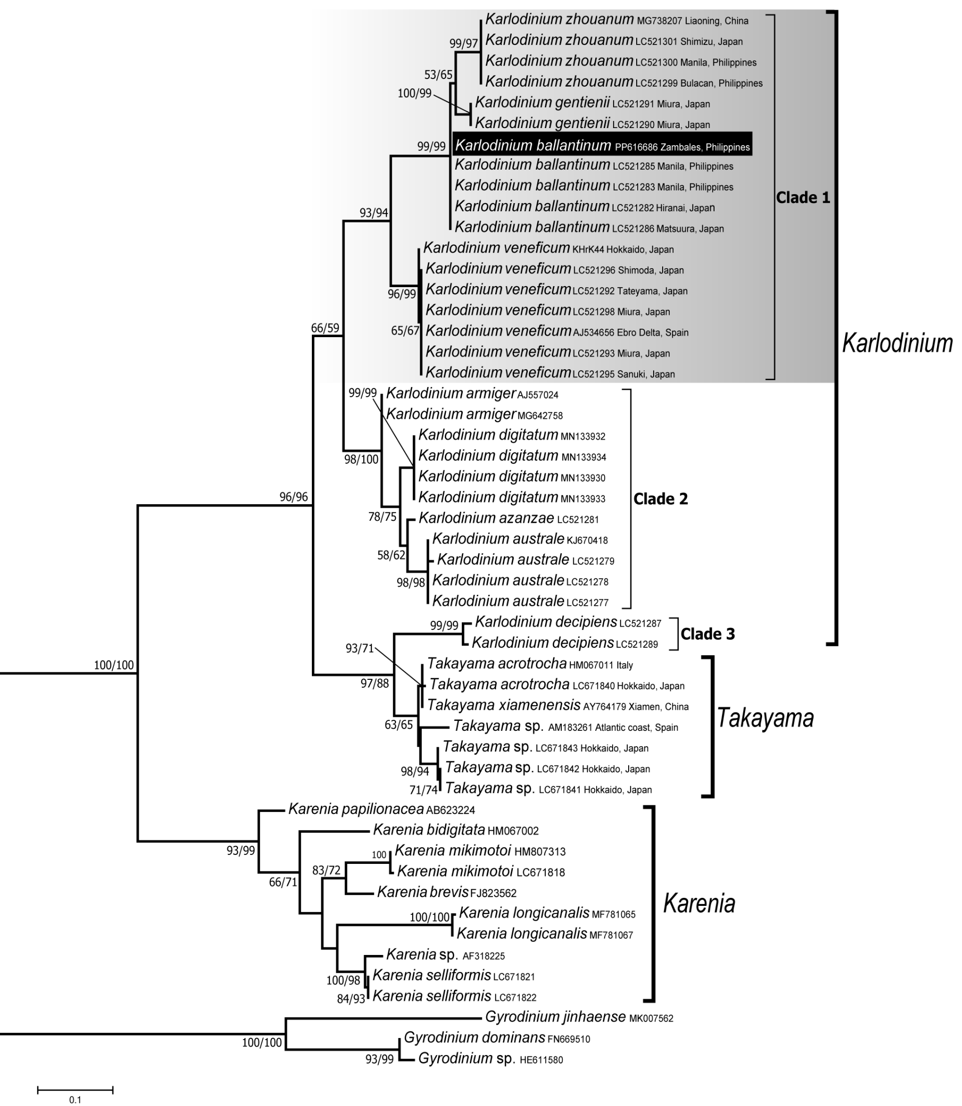

Figure 4. Maximum likelihood (ML) phylogeny of Kareniaceae inferred from ITS region (478 bp in alignment profile). Bootstrap support (BS) values of maximum likelihood (left) and neighbor-joining (right) are shown (DNA sequences analyzed in this study are highlighted in black boxes).

The ML phylogenetic tree inferred from ITS and LSU rDNA (D1–D5 regions) showed that the Philippine strain (GBSUB96) and the isolates of *K. ballantinum* from Japan, Australia, Italy, and Manila Bay, Philippines branched together within the *Karlodinium* clade. Comparing its nucleotide sequence with the nucleotide sequence of the Philippine strains of *K. ballantinum* isolated from Manila Bay, 936 out of 969 sites (96.6%) in LSU and 418 out of 478 (87.4%) in ITS were constant, and 12 out of 969 sites (1.2%) in LSU and 17 out of 478 sites (3.6%) in ITS were variable. Strain GBSUB96 being positioned together with other strains of *K. ballantinum* in a well-supported monophyletic clade (ML/NJ BS values = 99/98% in LSU, ML/NJ BS values = 99/99% in ITS) indicated that these strains descend from a common ancestor. Furthermore, as shown in the tree, this species is closely related to *K. gentienii* and *K. zhouanum*.

3.3 Antioxidant profile

The cells obtained from harvesting the 20-L culture of *K. ballantinum* dinoflagellates were 0.1 gram of algal biomass. The final density of the mass-cultured *K. ballantinum* (20 L) was quantified to be 177.52×10^6.

The methanolic extract of *K. ballantinum* showed 23.39% radical scavenging activity (Table 1). This implies that the generated % RSA of *K. ballantinum* was relatively low when compared to catechin, which served as the control.

4. DISCUSSION

The observed morphological characteristics and phylogenetic trees constructed from LSU and ITS rDNA clearly revealed the species identity of *K. ballantinum* isolated from Subic Bay, Zambales. Molecular analyses placed the strain in a well-supported clade of *K. ballantinum*, which grouped together with similar species from other geographic regions.

Species of *Karlodinium* were widely reported in various geographic regions but *K. ballantinum* was not frequently reported like other species, such as *K. veneficum* as shown in Figure 5 (Guiry and Guiry 2023; (Yang et al., 2020). This species was first isolated by (de Salas et al., 2008) in Mercury Passage, Tasmania, Australia (strain EF469232) in 2006. The morphological characters observed in the cells of *K. ballantinum* isolated from Subic Bay, Zambales under light microscopy were consistent with the morphological description by de Salas et al. (2008). This was especially true with regard to the cultured cells' cell size, cell shape, placement of the nucleus, presence or absence of ventral pore, number of chloroplasts, and description of apical groove. Furthermore, it was also consistent with the morphological description by (Siano et al., 2009) who isolated strains of *K. ballantinum* in the Gulf of Naples, Italy for the first time (strain FJ024699 and FJ024700).

In the Philippines, the first record of *K. ballantinum* was reported in a study of (Benico et al., 2020). In 2018 and 2019, the species of *K. ballantinum* was first isolated in Manila Bay, Manila, Philippines (strain LC521283, LC521284, LC521285). The isolated strains of *K. ballantinum* in Manila Bay were reported to be also consistent with the original description of de Salas et al. (2008), with an additional report of multiple detections of ventral pore observed in most of their cultured cells. In this study, the strain GBSUB96 is the second report of *K. ballantinum* in the coastal

Table 1. Radical Scavenging Activity (% RSA) of the methanolic extract of *Karlodinium ballantinum* (PP616686).

Sample	Absorbance Reading			Mean Absorbance	% RSA
	R1	R2	R3		
1	0.683	0.689	0.672	0.681	23.39%
Catechin	0.099	0.100	0.101	0.100	88.75%

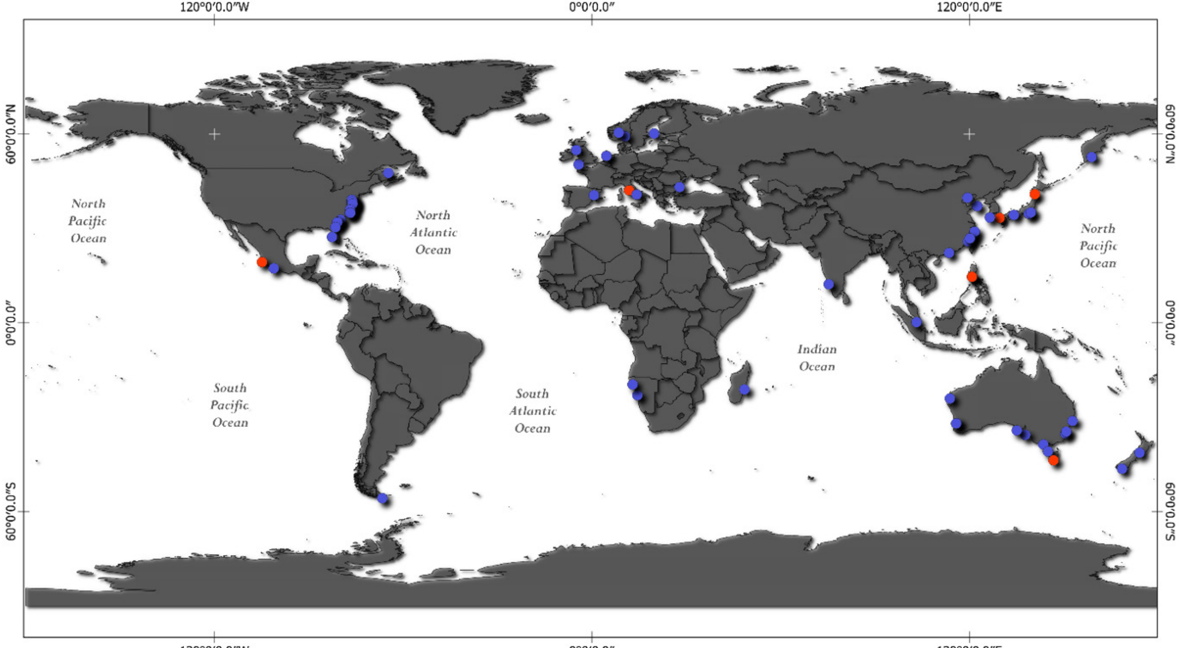

Figure 5. Global distribution of *K. ballantinum* and *K. veneficum* based on reports listed in AlgaeBase (Guiry and Guiry 2023). The two species of *Karlodinium* are marked by different colors of dots: red for *K. ballantinum* and blue for *K. veneficum*.

waters of the Philippines. The presence of this species in Subic Bay, as well as in other nearby countries such as Japan, may indicate its widespread occurrence in Asian Pacific waters (Benico et al. 2020).

Certainly, the presence of *K. ballantinum* in coastal waters of Asian countries, as well as in Australia, Mexico, and Europe, indicates its widespread occurrence across the world. One possible reason for the occurrence of *K. ballantinum* in the coastal waters of the Philippines is the ocean currents. Because ITS and LSU rDNA sequences of *K. ballantinum* from Asia, Australia and Europe are highly similar, it might be possible that oceanic currents transported the population of this species to different regions. Another possible reason is "port-hopping," which is a term used to describe how marine species are transferred into a new environment through vessels (Donelan et al. 2022). In order to increase vessels stability, ships put saltwater into specialized ballast water tanks located within the cargo holds of their ships. The ballast water was allowed to be released once the vessel had arrived at its destination port, which made it possible for many introduced species to be released into the new environment (Ricciardia 2016).

Regarding its antioxidant activity, the generated % RSA of *K. ballantinum* was relatively low when compared to catechin, which served as the control in the procedure as well as with other species of microalgae. In the study of (Arguelles et al., 2017), which served as the reference material for the protocol of crude extraction used in this study, the assessment of the antioxidant activity of *Desmodesmus* sp. led to a conclusion that the antioxidant activity of algal extracts may possibly increase when its concentration also increases. Furthermore, the most important parameters that determine the composition of biomass are the amount of light, the nutrients in the culture medium, the salinity of the water, and the source of nitrogen. In the study of Vega et al. (2021), studies on the antioxidant activity of *Navicula incerta* involved the optimization of various factors which resulted in major improvements to pigment production and the density of algal biomass, allowing for maximum efficiency to be achieved, which could then be utilized in a variety of industrial sectors. Still, the antioxidant activity of the cultured *K. ballantinum* was comparatively low with other species of microalgae, such as *Spirulina platensis*, commercially available powdered algae associated with powerful antioxidant properties (Anbarasan et al. 2011). However, although at a low amount, this study served as the first report of the antioxidant activity of species under the genus *Karlodinium*. This showed the potential of *K. ballantinum*, although a causative agent of HABs, as a source of important biological compounds that can be tapped for various industrial applications. Furthermore, this study confirms the significant expansion of *K. ballantinum* across Asia and increases our knowledge and understanding of this organism. Assessing the antioxidant potential of other kareniacean dinoflagellates, aside from *K. ballantinum*, in future studies may be an outstanding contribution to the field of biomedicine and pharmacotherapy, which may be explored for further development and production of biological compounds derived from microalgae.

5. CONCLUSION

The present study documented the first record of *Karlodinium ballantinum* in Subic Bay. This was the second successful isolation of this species in Philippine coastal waters after it was reported in Manila Bay in 2019. Together with other strains of *K. ballantinum*, it formed a strong monophyletic clade.

Furthermore, this study concluded that *K. ballantinum* possesses radical scavenging activity, although at a low amount. This was the first report of the antioxidant activity of a species under the genus *Karlodinium*.

ACKNOWLEDGMENT

The authors are grateful for the funding support from the DOST-JSPS project "Distribution and Diversity of Harmful Kareniacean Dinoflagellates from Philippine Coastal Waters." The study was also funded by the Department of Science and Technology–Science Education Institute (DOST-SEI). We also wish to acknowledge the laboratory and fieldwork staff who assisted in the study's conduct.

AUTHOR CONTRIBUTIONS

Benico GA: Conceptualization, Writing-Original draft preparation; Methodology, Reviewing and Editing. **Flores JAV:** Sampling, Data curation, Writing-Original draft preparation; Methodology. **Esteban SP**: Sampling, Methodology. **Undan JR:** Writing-Reviewing and Editing. **Kuwata K:** Writing-Reviewing and Editing. **Iwataki M:** Writing-Reviewing and Editing.

CONFLICTS OF INTEREST

To the best of our knowledge, no conflict of interest exists.

ETHICS STATEMENT

No animal or human studies were carried out by the authors.

REFERENCES

Anbarasan V, Kumar K, Kumar P, Venkatachalam T. 2011. *In vitro* Evaluation of Antioxidant Activity of Blue Green Algae *Spirulina platensis*. Int J Pharm Sci Res. 2(10):2616–2618.

Arguelles ED, Laurena AC, Martinez-Goss MR, Monsalud RG. 2017. Antibacterial Activity, Total Phenolic Content and Antioxidant Capacity of a Green Microalga *Desmodesmus* sp. (U-AU2) from Los Baños, Laguna (Philippines). JNS. 16(2):1–13.

Bachvaroff TR, Adolf JE, Place AR. 2009. Strain Variation in *Karlodinium veneficum* (Dinophyceae): Toxin Profiles, Pigments, And Growth Characteristics. J Phycol. 45(1):137–153. https://doi.org/10.1111/j.1529-8817.2008.00629.x

Benico G, Azanza R. 2022. Characterization of *Alexandrium tamutum* (Dinophyceae) Isolated from Philippine Waters, with the Rare Detection of Paralytic Shellfish Toxin. Philipp J Syst Biol. 15(1):1–13. https://doi.org/10.26757/pjsb2021a15009

Benico G, Takahashi K, Lum WM, Yñiguez AT, Iwataki M. 2020. The Harmful Unarmored Dinoflagellate *Karlodinium* in Japan and Philippines, with Reference to Ultrastructure and Micropredation of *Karlodinium azanzae* sp. nov. (Kareniaceae, Dinophyceae). J Phycol. 56(5):1264–1282. https://doi.org/10.1111/jpy.13030.

Bergholtz T, Daugbjerg N, Moestrup Ø. 2006. On the Identity of *Karlodinium veneficum* and Description of *Karlodinium armiger* sp. nov. (Dinophyceae), Based on Light and Electron Microscopy, Nuclear-Encoded LSU rDNA, and Pigment Composition. J Phycol. 42(1):170–193. https://doi.org/10.1111/j.1529-8817.2006.00172.x

Casanova LM, Macrae A, De Souza JE, Junior AN, Vermelho AB. 2023. The Potential of Allelochemicals from Microalgae for Biopesticides. Plants. 12(9):1896. https://doi.org/1 0.3390/plants12091896

Cen J, Wang J, Huang L, Lin, Ding G, Qi, Lü S. 2021. *Karlodinium elegans* sp. nov. (Gymnodiniales, Dinophyceae), a Novel Species Isolated from the East China Sea in a Dinoflagellate Bloom. J Oceanol Limnol. 39(1):242–258. https://doi.org/10.1007/s00343-020-0221-4

Daugbjerg NG, Larsen J, Moestrup Ø. 2000. Phylogeny of some of the Major Genera of Dinoflagellates Based on Ultrastructure and Partial LSU rDNA Sequence Data, Including the Erection of Three New Genera of Unarmoured Dinoflagellates. Phycologia. 39:302–317. https://doi.org/10.2216/i0031-8884-39-4-302.1

de Salas MF, Bolch CJS, Hallegraeff GM. 2005. *Karlodinium australe* sp. nov. (Gymnodiniales, Dinophyceae), A New Potentially Ichthyotoxic Unarmoured Dinoflagellate from Lagoonal Habitats of South-Eastern Australia. Phycologia. 44(6):640–650. https://doi.org/10.2216/0031-8884(2005)44[640:KASNGD]2.0.CO;2

de Salas MF, Laza-Martínez A, Hallegraeff GM. 2008. Novel Unarmored Dinoflagellates from the Toxigenic Family Kareniaceae (Gymnodiniales): Five New Species of *Karlodinium* and One New *Takayama* from the Australian Sector of the Southern Ocean. J Phycol. 44(1):241–257. https://doi.org/10.1111/j.1529-8817.2007.00458.x

Donelan SC, Miller AW, Muirhead JR, Ruiz GM. 2022. Marine Species Introduction via Reproduction and its Response to Ship Transit Routes. Front Ecol Environ. 20(10):581–588. https://doi.org/10.1002/fee.2551

Escobar-Morales S, Hernández-Becerril DU. 2015. Free-Living Marine Planktonic Unarmoured Dinoflagellates from the Gulf of Mexico and the Mexican Pacific. Bot Mar. 58(1):9–22. https://doi.org/10.1515/bot-2014-0049

Fabro E, Almandoz GO. 2021. Field Observations on Rare or Overlooked Dinoflagellates from the

Argentine Sea. Bol Soc Argent Bot. 56(2):123–140. https://doi.org/10.31055/1851.2 372.v56.n2.31421

Foo SC, Khong NMH, Yusof FM. 2020. Physicochemical, Microstructure and Antioxidant Properties Of Microalgae- Derived Fucoxanthin Rich Microcapsules. Algal Res. 51:102061. https://doi.org//10.1016/j.algal.2020.102061

Garcés E, Fernandez M, Penna A, Lenning KV, Gutierrez A, Camp J, Zapata M. 2006. Characterization of New Mediterranean *Karlodinium* spp. (Dinophyceae) Strains using Morphological, Molecular, Chemical, and Physiological Methodologies. J Phycol. 42(5):1096–1112. https://doi.org/10.1111/j.1529-8817.2006.00270.x

Guiry MD, Guiry GM. 2023. AlgaeBase. Galway (IE): National University of Ireland; [accessed 2023 Sept 23]. https://www.algaebase.org.

Kawami H, Iwataki M, Matsuoka K. 2006. A New Diplopsalid Species *Oblea acanthocysta* sp. nov. (Peridiniales, Dinophyceae). Plankton Benthos Res. 1(4):183–190. https://doi.org//10.3800/pbr.1.183

Kim S-K, Pangestuti, R. 2011. Biological Activities and Potential Health Benefits of Fucoxanthin Derived from Marine Brown Algae. In: Kim S-K, editor. Advances in Food and Nutrition Research. Vol. 64. Elsevier. pp. 111–128. https://doi.org/10.1016/B978-0-12-387669-0.00009-0

Lim HC, Leaw CP, Tan TH, Kon NF, Yek LH, Hii KS, Teng ST, Razali RM, Usup G, Iwataki M, Lim PT. 2014. A Bloom of *Karlodinium australe* (Gymnodiniales, Dinophyceae) Associated with Mass Mortality of Cage-Cultured Fishes in West Johor Strait, Malaysia. Harmful Algae. 40:51–62. https://doi.org//10.1016/j.hal.2014.10.005

Luo Z, Wang L, Chan L, Lu S, Gu H. 2018. *Karlodinium zhouanum*, a New Dinoflagellate Species from China, and Molecular Phylogeny of *Karenia digitata* and *Karenia longicanalis* (Gymnodiniales, Dinophyceae). Phycologia. 57(4):401–412. https://doi.org/10.2216/17-106.1

Nézan E, Siano R, Boulben S, Six C, Bilien G, Chèze K, Duval K, Le Panse S, Quéré J, Chomérat N. 2014. Genetic Diversity of the Harmful Family Kareniaceae (Gymnodiniales, Dinophyceae) in France, with the Description of *Karlodinium gentienii* sp. nov.: A New Potentially Toxic Dinoflagellate. Harmful Algae. 40:75–91. https://doi.org//10.1016/j.ha l.2014.10.006

Nomura T, Kikuchi M, Kubodera A, Kawakami Y. 1997. Proton-Donative Antioxidant Activity of Fueoxanthin with 1,1-Diphenyl-2-Picrylhydrazyl (DPPH). Int J Biochem Mol. 42(2):361–370. https://doi.org//10.1080/15216549700202761

Pereira H, S´a M, Maia I, Rodrigues A, Teles I, Wijffels RH, Navalho J, Barbosa M. 2021. Fucoxanthin Production from *Tisochrysis lutea* and *Phaeodactylum tricornutum* at Industrial Scale. Algal Res. 56:102322. https://doi.org//10.1016/j.algal.2021.10232 2

Place AR, Bowers HA, Bachvaroff TR, Adolf JE, Deeds JR, Sheng J. 2012. *Karlodinium veneficum*–the Little Dinoflagellate with a Big Bite. Harmful Algae. 14:179–195. https://doi.org//10.1016/j.hal.2011.10.021

Quijano-Scheggia S, Barajas-Gonzalez M, Lim HC, Leaw CP, Olivos-Ortiz A, Gaviño-Rodriguez J, Pérez JB, Bates SS. 2016. The Inhibitory Effect of a Non-yessotoxin-producing Dinoflagellate, *Lingulodinium polyedrum* (Stein) Dodge, Towards *Vibrio vulnificus* and *Staphylococcus aureus*. Rev Biol Trop (Int J Trop Biol). 64(2):805–816. https://doi.org/10.15517/rbt.v64i2.19320

Ricciardia A. 2016. Tracking marine alien species by ship movements. PNAS. 113(20):5470–5471. https://doi.org/10.1073/pnas.1605152113

Sakamoto S, Lim WA, Lu D, Dai X, Orlova T. 2021. Harmful Algal Blooms and Associated Fisheries Damage in East Asia: Current Status and Trends in China, Japan, Korea and Russia. Harmful Algae. 102:101787. https://doi.org//10.1016/j.hal.2020.101787

Satake M, Honma D, Watanabe R, Oshima Y. 2019. Alexandrolide, a Diatom Growth Inhibitor

Isolated from the Dinoflagellate *Alexandrium catenella*. Tetrahedron Lett. 60(19):1341–1344. https://doi.org//10.1016/j.tetlet.2019.04.019

Siano R, Kooistra W, Montresor M, Zingone A. 2009. Unarmoured and Thin-Walled Dinoflagellates from the Gulf of Naples, with the Description of *Woloszynskia cincta* sp. nov. (Dinophyceae, Suessiales). Phycologia .48(1):44–65. https://doi.org/10.2216/08-61.1

Staden R, Beal KF, Bonfield JK. 2000. The Staden Package, 1998. Methods Mol Biol. 132:115–130. https://doi.org/10.1385/1-59259-192-2:115

Takahashi K, Benico G, Lum WM, Iwataki M. 2019. *Gertia stigmatica* gen. et sp. nov. (Kareniaceae, Dinophyceae), a New Marine Unarmored Dinoflagellate Possessing the Peridinin-Type Chloroplast with an Eyespot. Protist. 170(5):125680. https://doi.org/10.1016/j.protis.2019.125680

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evo. 30(12):2725–2729. https://doi.org/10.1093/molbev/mst197

Vega R, López J, Elías J, Cruz S, Diaz A, Perez L, Moroyoqui F, Zepeda R, Flores J, Sanchez C. 2021. Optimization of Growing Conditions For Pigments Production From Microalga *Navicula incerta* Using Response Surface Methodology And Its Antioxidant Capacity. Saudi J Biol Sci. 28(2):1401–1416. https://doi.org/10.1016/j.sjbs.2020.11.076

Yang ZB, Takayama H, Matsuoka K, Hodgkiss IJ. 2000. *Karenia digitata* sp. nov. (Gymnodiniales, Dinophyceae), A New Harmful Algal Bloom Species from the Coastal Waters of West Japan and Hong Kong. Phycologia. 39(6):463–470. https://doi.org/10.2 216/i0031-8884-39-6-463.1

Yang H, Hu Z, Shang L, Deng Y, Tang YZ. 2020. A Strain of the Toxic Dinoflagellate *Karlodinium veneficum* Isolated from the East China Sea is an Omnivorous Phagotroph. Harmful Algae. 93:101775. https://doi.org/10.1016/j.hal.2020.101775

Yñiguez AT, Lim PT, Leaw CP, Jipanin SJ, Iwataki M, Benico G, Azanza RV. 2021. Over 30 Years of HABs in the Philippines and Malaysia: What have we learned?. Harmful Algae. 102:101776. https://doi.org//10.1016/j.hal.2020.101776

© 2024 The authors. Published by the National Fisheries Research and Development Institute. This is an open access article distributed under the CC BY-NC 4.0 license.