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Alexandrium pacificum (Group IV) (Dinophyceae) in tropical Asia: Evidence from morphology, phylogeny, and toxicity of Philippine strains

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Abstract

Species belonging to the toxic dinoflagellate *Alexandrium tamarense* complex can cause Paralytic Shellfish Poisoning, have a global distribution, but their occurrence in the tropics has never been established. In this study, the morphology, phylogeny, and toxicity of *A. pacificum* (Group IV) were examined using cultures established from two different geographic locations in the Philippines. Our analyses were based on light microscopy, confocal laser scanning microscopy, high-performance liquid chromatography (HPLC), and molecular phylogeny inferred from LSU and SSU rDNA. Cellular and thecal plate morphology of the Philippine strains showed characters typical of the species complex such as an isodiametric cell shape, chain formation (maximum of two cells), absence of ventral pore, isodiametric posterior sulcal plate, although these characters were not enough to assign a species designation. Phylogenetic analyses however revealed the well-supported placement of the Philippine strains in the *A. pacificum* (Group VI) and thus confirm their species identification. Paralytic shellfish toxins (PSTs) were detected in our two tested cultures, with N-sulfocarbamoyl C1/C2 as the most abundant toxin component and toxicity level ranging from 32.70–38.18 fmol of STX eq cell^{-1}. The present study confirmed the occurrence of *A. pacificum* (Group IV) in the tropics.

Keywords: *Alexandrium*, dinoflagellate, harmful algal blooms, paralytic shellfish poisoning, thecal plates

INTRODUCTION

Many marine dinoflagellates produce toxins that can contaminate fish and shellfish, posing serious health risks when these are ingested by animals, including marine mammals, birds, and even humans. Species belonging to *Alexandrium* Halim, *Gymnodinium* F.Stein, and *Pyrodinium* L.Plate are among these toxigenic dinoflagellates capable of producing highly potent neurotoxins causing Paralytic Shellfish Poisoning (PSP) (Hall et al. 1990). Of the three genera, *Alexandrium* is the most speciose with 33 known morphologically and/or molecularly established species and thus includes the largest number of toxic species (Mertens et al. 2020; Guiry & Guiry 2026). Out of the 33 *Alexandrium* species, 14 species are known to be toxic: *A. affine* (H.Inoue & Y.Fukuyo) Balech, *A. andersonii* Balech, *A. australiense* Sh.Murray, *A. balechii* (Steidinger) Balech, *A. catenella* (Whedon & Kofoid) Balech, *A. fragae* S.Branco & M.Menezes, *A. hiranoi* T.Kita & Y.Fukuyo, *A. minutum* Halim, *A. monilatum* (J.F.Howell) Balech, *A. ostenfeldii* (Paulsen) Balech & Tangen, *A. pacificum* R.W.Litaker, *A. pseudogonyaulax* (Biecheler) Horiguchi ex K.Yuki & Y.Fukuyo, *A. tamiyavanichii* Balech, and *A. taylorii* Balech (Lundholm et al. 2009).

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Alexandrium tamarense complex, which traditionally encompasses morphospecies *A. tamarense*, *A. catenella,* and *A. fundyense* Balech, is the most widely distributed group with reports of occurrence in all continents (Lilly et al. 2007; Ho et al. 2012; Litaker et al. 2018). Their distribution, however, appears to be limited in temperate regions with restricted range in warm- to cold-temperate coastal waters across the globe (Bolch & de Salas 2007; Lilly et al. 2007; Gu et al. 2013). The presence of the *A. tamarense* complex species in tropical waters, particularly in Southeast Asia, remains uncertain as the morphospecies *A. tamarense* previously reported from Malaysia, Thailand, and Vietnam either lacked the molecular data or did not demonstrate phylogenetic attribution to this species complex (Fukuyo et al. 1988; Usup et al. 2002; Nguyen-Ngoc 2004; Leaw et al. 2005). Several molecular phylogeographic studies have shown that the species complex is separated into five distinct ribotypes, which does not reflect the traditional morphospecies circumscription (Scholin et al. 1994; Lilly et al. 2007; Orr et al. 2011; Miranda et al. 2012; John et al. 2014a; Wang et al. 2014). Because of the large genetic distances among ribotypes, several naming schemes (i.e., geographic signature, group number) have been proposed over the years, including recognizing each ribotype as cryptic species (Scholin et al. 1994; Lilly et al. 2007; Orr et al. 2011; Miranda et al. 2012; John et al. 2014a; Wang et al. 2014; Fraga et al. 2015). The taxonomic nomenclature changes of the species complex particularly the dispute on naming the Group I (John et al. 2014b; Fraga et al. 2015) was finally adjudicated by the International Code of Nomenclature for algae, fungi, and plants (ICN) and the species complex now comprises *A. catenella* (Group I), *A. mediterraneum* U.John (Group II), *A. tamarense* (Group III), *A. pacificum* (Group IV), and *A. australiense* (Group V) (Prud'homme van Reine 2017; Litaker et al. 2018).

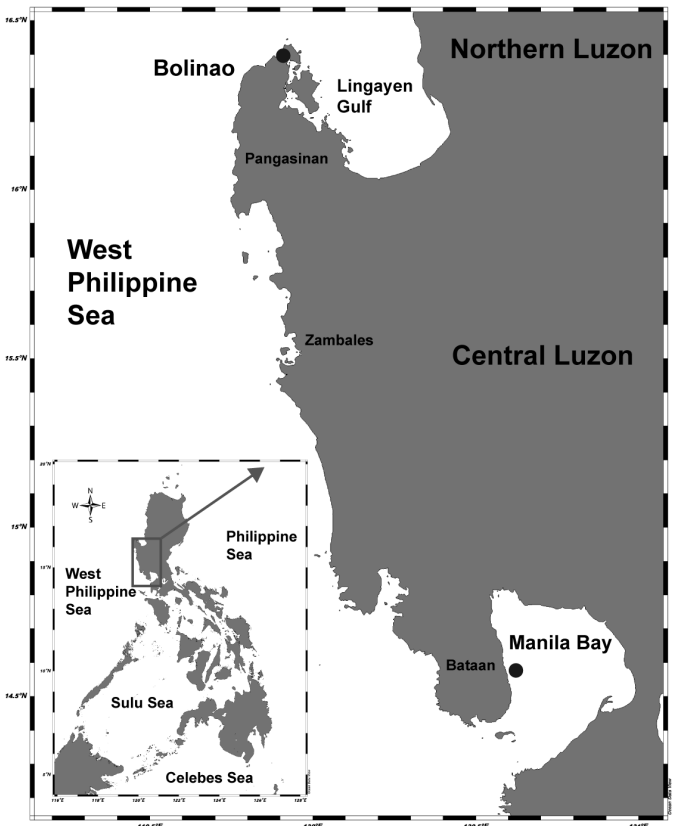

Figure 1. Sampling locations (blue dots) of *Alexandrium pacificum* R.W.Litaker in Manila Bay and Bolinao, Pangasinan. Inset (red rectangle) showing the location of Manila Bay and Bolinao, Pangasinan in the Philippines.

The Philippines has been constantly affected by harmful algal blooms (HABs), in particular blooms of *Pyrodinium bahamense* L.Plate in many coastal areas of the country, causing shellfish toxicities (Azanza & Taylor 2000; Fukuyo et al. 2012; Furuya et al. 2018; Yñiguez et al. 2012, 2021; Lum et al. 2022; Malto et al. 2022; Azanza et al. 2017, 2024; Borja et al. 2019; San Diego-McGlone et al. 2024). However, *Alexandrium* has recently been gaining attention due to its recurrent blooms in fish farming sites in the northwestern Philippines with direct and indirect effects on public health and economy (Azanza & Benico 2013; Subong et al. 2017; Yñiguez et al. 2021; Benico & Azanza 2021). To date, the species diversity of *Alexandrium* in the country remains underreported due to the difficulty of identifying wild specimens and the lack of an established culture to carry out detailed morphological and molecular analyses. Only six species have been recorded so far in the Philippines, namely *A. affine*, *A. leei* Balech, *A. minutum*, *A. tamiyavanichii*, *A. fraterculu*s (Balech) Balech and *A. tamutum* (Balech et al. 1995; Montojo et al. 2003; Onda et al. 2013; Benico et al. 2021). In this study, we report the occurrence of *A. pacificum* in tropical Asia for the first time, by providing information on the morphology, toxin profile, and phylogeny of Philippine isolates as examined by light microscopy, confocal laser scanning microscopy, HPLC, and molecular phylogeny inferred from LSU and SSU rDNA regions. This study therefore provides significant data and information showing the potential greater diversity of toxic species in the Philippines and adds to the resolution on the global distribution and diversity of *Alexandrium* species.

MATERIALS AND METHODS

Culture and observation

Four monoclonal cultures of *Alexandrium pacificum* examined in this study were established by single-cell isolation from water samples collected in Anda (April 2010), Bolinao (March 2011 and January 2013), Pangasinan, Northwestern Philippines, and Manila Bay (April 2013) (Fig. 1). Temperature and salinity ranged 31–32°C and 29–33 PSU, respectively. Isolated cells were cultivated in sterile seawater with salinity 33, enriched with a half-strength F medium (Guillard & Ryther 1962), and incubated at room with a temperature of 24 ± 2°C, irradiance of 150 µmoles photons m^{-2} $s^{-1}$and a 12:12 h light:dark cycle.

Cells were observed under a Zeiss Axioskop 2 (Carl Zeiss, Göttingen, Germany) equipped with a digital camera Zeiss Axiocam HRc (Carl Zeiss, Göttingen, Germany). For the observation of thecal plates, cells were stained with 1% calcofluor white (Fritz & Triemer 1985) and visualized under a confocal laser scanning microscope (CLSM 710, Carl Zeiss, Germany). using a 405 nm excitation laser and detecting emission at 425–475 nm with Images were captured by stack scans, which were then modeled into 3D images using Zen 10 software (Carl Zeiss, Germany). To view the nucleus, cells were stained with SYBR Green (Sigma Aldrich, St. Louis, MO, USA) and observed at 520 nm with the same microscope. Chloroplasts were detected using their autofluorescence captured in the far-red spectra. Cell measurements were done on micrographs using Image J software.

Molecular analyses

Genomic DNA was extracted using the Qiagen DNeasy Plant Mini-Kit (Valencia, CA, USA) following the manufacturer's protocol with some modifications. In brief, cells were pelleted by centrifugation at 2,600× *g* for 15 min and resuspended in 400 µL AP1 buffer, then three to four sterile silica beads were added. They were then homogenized by vortexing for 2–3 min. The liquid phase was transferred to a new tube, and extraction was continued as prescribed by the kit. Extracted materials were quantified by spectrophometry (Nanodrop) and kept at -20°C until further use. The universal primer pairs 4616F (5′-AACCTGGTTGATCCTGCCAG-3′) and 4618R (5′-GATCCTTCTGCAGGTTCACCTAC-3′), and DinFi (5′-GCATATAAGTAMGYGGWGG-3′) and DinRi (5′- CCGTGTTTCAAGACGGGTC-3′) were used to amplify the SSU rDNA and LSU rDNA sequences, respectively, following the conditions described by Logares et al. (2007) with slight modifications. An annealing temperature of 55°C instead of 60°C was used in amplifying the ~1.5 kb product for the SSU rDNA fragments. All PCR reactions were done in 25 µL reaction mixture with 0.26 U Titanium *Taq* DNA polymerase, 0.13 µM dNTPs, 1× *Taq* buffer (Clonetech, USA), with at least 50 ng of template. Amplicons were purified using QIAquick Gel Purification Kit (Qiagen Genomics, Bothell, WA, USA) and were then sent to 1st Base (Selangor, Malaysia) for sequencing.

Phylogenetic analyses

Generated sequences were used to search the GenBank for related *Alexandrium* sequences using BLASTn (Altschul et al. 1990) and similar sequences (i.e., identical sequences after trimming the terminal regions) were compiled and presented as

Table 1. LSU rDNA sequence divergence (%, upper diagonal) of *Alexandrium tamarense* complex based on Kimura-2 Parameter DNA model . Diagonal element represents the intraspecific sequence divergence of each species. The n value indicates the total number of comparisons made for each analysis.

	A. pacificum (Philippines)	*A. australiense*	*A. catenella*	*A. mediteraneum*	*A. pacificum*	*A. tamarense*
A.pacificum (Philippines)	0	(0.078± 0.00) 0.078 n=16	(0.131±0.01) 0.123–0.143 n=104	(0.131±0.001) 0.130–0.133 n=20	(0.026±0.001) 0.025–0.029 n=76	(0.90±0.00) 0.090 n=16
A. australiense		0	(0.118±0.01) 0.110–0.129 n=104	(0.124±0.001) 0.124–0.127 n=20	(0.070±0.001) 0.068–0.073 n=76	(0.89±0.00) 0.89 n=16
A. catenella			(0.011±0.01) 0.00–0.027 n=351	(0.081±0.01) 0.073–0.093 n=130	(0.137±0.005) 0.127–0.150 n=494	(0.073±0.01) 0.066–0.083 n=104
A. mediteraneum				0.001±0.001 0.00–0.002 n=15	(0.139±0.002) 0.134–0.147 n=95	0.076±0.01 0.075–0.078 n=20
A. pacificum					(0.02±0.002) 0.00–0.006 n=190	0.091±0.002 0.087–0.096 n=76
A. tamarense						0

a single sequence. A matrix of multiple sequences comprising 83 taxa for LSU and 61 for SSU were aligned using MAFFT v7.110 (Katoh & Standley 2013) and manually refined using BioEdit Sequence Alignment Editor v7.2.5 (Hall 1999). The computation of pairwise distance divergence among LSU rDNA sequences was carried out with Kimura 2-Parameter Model (K2P) distances using MEGA v.11 (Tamura et al. 2021). Maximum-likelihood (ML) analysis was performed with PhyML (Guindon et al. 2010) running a bootstrap with 1,000 replicates. The best fitting substitution model for the ML tree, as selected by the Smart Model Selection (SMS) program (Lefort et al. 2017) was general time reversible (GTR) with gamma distribution (G = 0.28) for LSU rDNA, and GTR + G (0.640) plus proportion of invariable sites (I = 0.462) for SSU rDNA. Bayesian inference (BI) was computed via MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) using a Metropolis-coupled Markov chain Monte Carlo run for 10 million generations, with sampling every 100 iterations. The best-fit substitution model for the BI tree, selected by jModelTest 2.1.10 (Diego et al. 2012), was GTR + G (0.627) for LSU rDNA, and GTR + G (0.643) + I (0.459) for SSU rDNA. All trees were rooted with *Pyrodinium bahamense* (AB970721 for LSU rDNA, KX377203 for SSU rDNA) and *Lingulodinium polyedra* (EF613357, AF274269) as the outgroups.

Toxin analyses

Fifty mL of exponentially growing cultures of *A. pacificum* (ATC4BOLRVA and ATMBRVA) as representatives of isolates from Manila Bay and Bolinao were filtered onto GF/F glass fiber filters (Whatman, Maidstone, UK). Prior to filtration, cell densities of each culture were determined using a Sedgwick-Rafter cell counting chamber. Collected cells were resuspended in 0.1 N acetic acid and were sonicated on ice at 60 MHz. Homogenized samples were decanted and the resulting supernatant was filtered through 0.45 µm single-use syringe filters.

Paralytic shellfish toxins (PSTs) were analyzed using high performance liquid chromatography-fluorescence detection (HPLC-FD) by adapting the pre-chromatographic oxidation method as described in Lawrence & Ménard (1991). The HPLC system (Shimadzu, Kyoto, Japan) was equipped with SPD-535 UV/Vis detector in series with an RF-535 Fluorescence detector set at wavelengths 330 nm (excitation) and 400 nm (emission). Periodate analysis was conducted for the detection STX, NEO, and GTX1/4, while peroxide analysis was carried out for the detection of STX and GTX2/3. The quantities of N-sulfocarbamoyl toxins were derived from the difference in the gonyautoxin concentration before and after acid hydrolysis. Sample extracts were subsequently reacted with 1.00 M sodium hydroxide and 10% hydrogen peroxide in 10:1 ratio before adding concentrated glacial acetic acid after 2 min. Toxin standards purchased from the Certified Reference Materials Program, National Research Council of Canada, were similarly prepared. The mixture was then eluted into a C18 reversed-phase column with 1.0 M ammonium formate (pH 6.0) and 5% acetonitrile (ACN) at a flow rate of 0.8 mL per min. The retention times used for the toxins were as follows: for periodate oxidation STX ~12 min, NEO ~7 min, GTX-1/4 ~8 min and for peroxide oxidation STX ~12 min and GTX-2/3 ~8 min.

RESULTS

Light and confocal laser scanning microscopy

Cultured cells of *A. pacificum* examined in this study possessed features typical of species of the *Alexandrium tamarense* complex. Cells were isodiametric and pentagonal, measuring 21.7–25.8 µm (mean 24.2 ± 2.8 µm, n = 30) in length and 20.3–24.7 µm (mean 21.6 ± 3.2 µm, n = 30) in width. The episome was hemispherical or conical, often with a noticeable shoulder, while the hyposome was hemispherical or trapezoidal with left lobe being larger than the right (Fig. 2A–C). Cells were solitary but could also form chains in pairs without flattening of the cell shape (Fig. 2A–C). There were numerous chloroplasts, multi-lobed when viewed in 3D but typically radially arranged throughout the cell (Fig. 2D, E). The nucleus was located at the center and curved dorsally (Fig. 2E).

The thecal plates, as viewed via CLSM, showed tabulation typical of *Alexandrium*: Po, 4′, 6″, 6c, 8s, 5‴, 2⁗. The first apical plate (1′) was pentagonal and contacted the

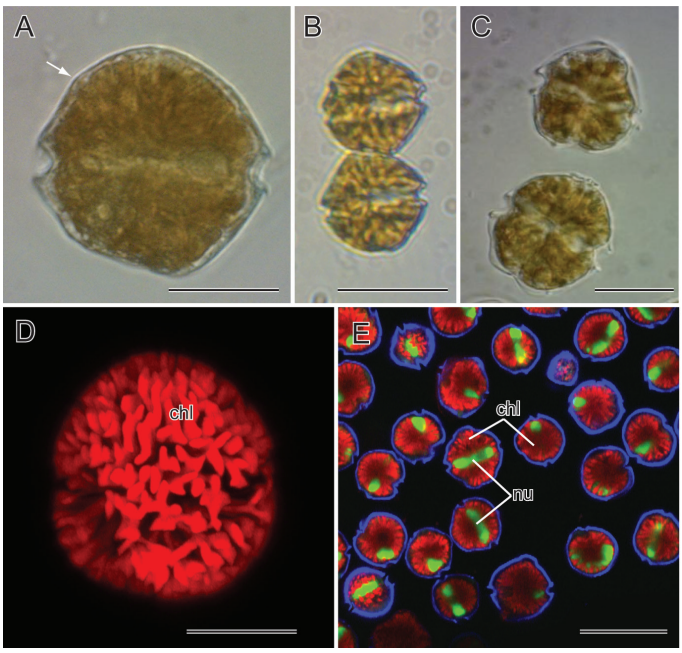

Figure 2. Light microscopy and confocal laser scanning microscopy of *Alexandrium pacificum* (ATC4RVA). (A) Ventral view at deep focus showing isodiametric cell shape and a shoulder at the epicone (arrow). (B) Cells in chain. (C) Cells from the field showing thick thecal outline. (D, E) FM showing multi-lobed chloroplast (chl) and U-shaped, equatorially located nucleus (nu). Scale bars = 10 µm (A–D), and 50 µm (E).

apical pore complex (APC) directly (Fig. 3A, B, H). The third apical plate (3′) was asymmetrical. No ventral pore was observed in plate 1′ of both cultured and wild specimens (Fig. 3A, B, D). The sixth precingular plate (6″) was pentagonal and almost equal in height and width. The APC appeared triangular or rectangular and tapered towards the posterior end (Fig. 3B, C). This plate possessed a fish-hooked slit and strongly developed callous and canopy (Fig. 3C). The anterior attachment pore, when present, was located to the right of the apical pore (Fig. 3C). The anterior sulcal plate was undivided, and did not intrude into the episome (Fig. 3A). The posterior sulcal plate (sp) was isodiametric and the second antapical plate had a longer transversal axis than the longitudinal axis ("type B" sensu Balech 1995), (Fig. 3F, G). The posterior attachment pore, if present, was prominent and located near the right margin of the plate (sp; Fig. 3G). Cells from the field possessed thick cingular and sulcal lists (Fig. 3G). There were no cysts observed in culture.

Phylogenetic analyses

BI analyses for LSU and SSU rDNA sequence-based phylogenies both showed the monophyly of the genus *Alexandrium* (Bayesian posterior probabilities/ML bootstrap support, 0.98/61% for LSU rDNA and 0.99/88% for SSU rDNA) (Figs. 4 and 5). In both trees, *Alexandrium tamarense* complex was a well-supported clade (1.00/99% for LSU rDNA; 1:00/99% for SSU rDNA) composed of five species,

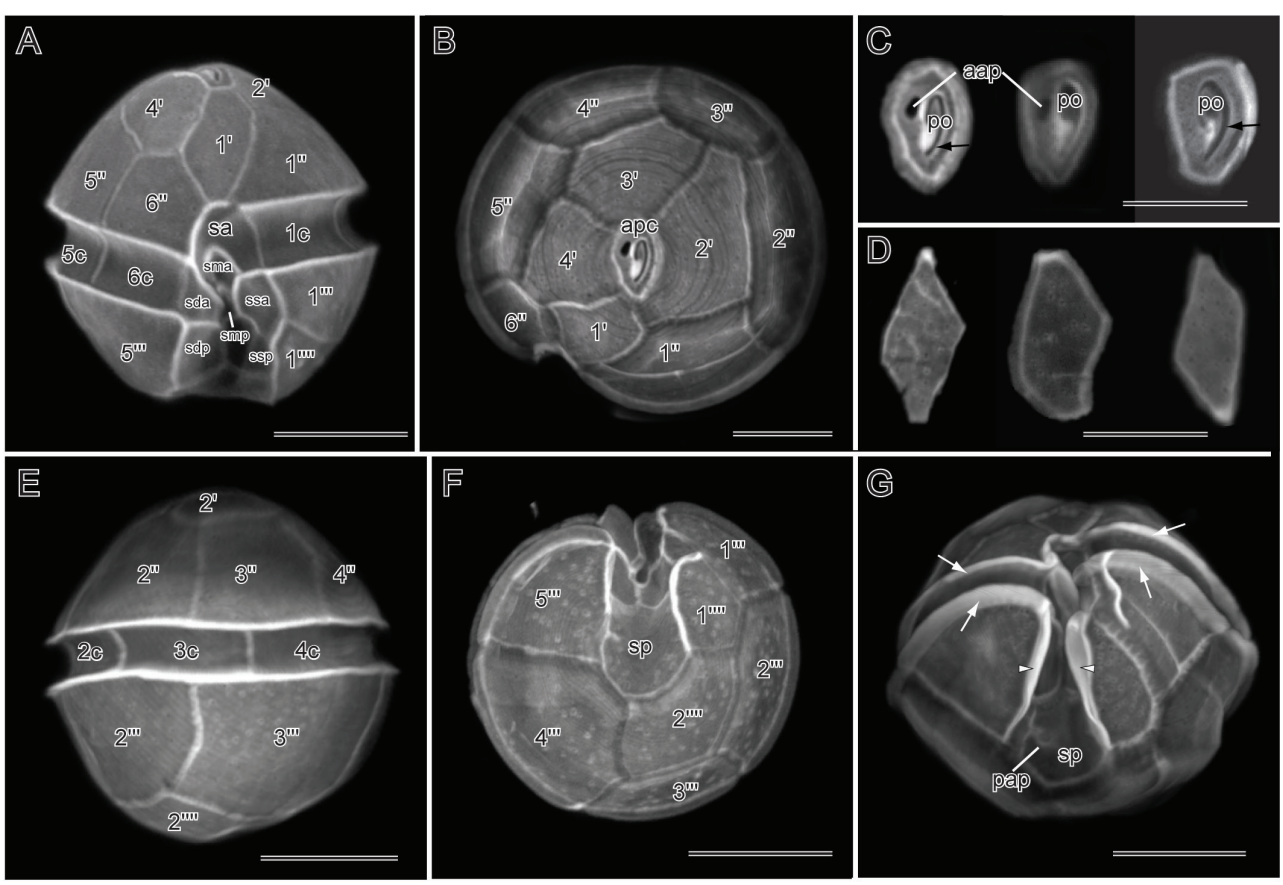

Fig. 3. Confocal laser scanning microscopy of *Alexandrium pacificum* (ATC4RVA) thecal plates. (a) Ventral view. (b) Apical view. (c) Apical pore complex showing anterior attachment pore (aap) and apical pore (po). (d) Plate 1′ without ventral pore. (e) Dorsal view. (f) Antapical view. (g) Ventro-antapical view showing well-developed sulcal list (arrowheads) and cingular lists (arrows). Note the presence of posterior apical pore (pap) in plate sp. Apical pore plate (apc), apical plates (1′–4′), precingular plates (1″–6″), cingular plates (C1–C6), sulcal plates (sa, sma, sda, ssa, sdp, ssp, sp), postcingular plates (1‴–5‴), and antapical plates (1⁗ and 2⁗). Scale bars = 10 µm (A, B, E–G), and 2 µm (C, D).

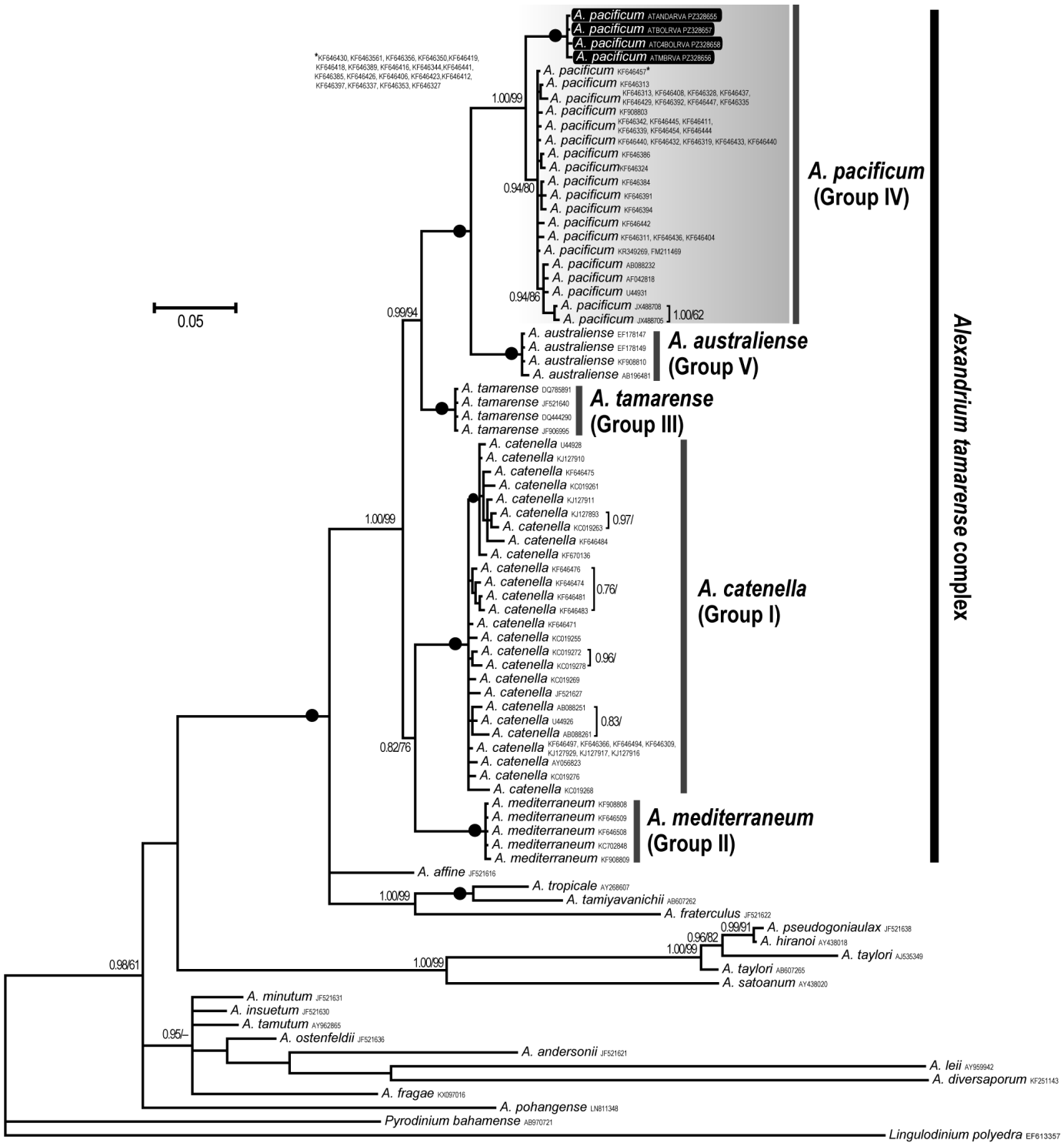

Figure 4. Bayesian inference (BI) phylogeny of *Alexandrium* species inferred from LSU rDNA (728 bp) based on GTR + G model. Posterior probabilities (PP, ≥0.7) of BI and bootstrap support (BS, ≥50%) values of maximum likelihood (ML) were indicated. Black dots on nodes indicate maximum supports for both BI and ML (PP/BS = 1.00/100). DNA sequences analyzed in this study are highlighted in black box.

namely *A. catenella* (Group I) (1.00/100%; 1.00/100%), *A. mediterraneum* (1.00/100%; 1.00/100%), *A. tamarense* (1.00/100%; 1.00/98%), *A. pacificum* (Group IV) (1.00/99%; 1:00/98%) and *A. australiense* (Group V) (1.00/100%; 1.00/100%). BI analysis further showed the strong statistical support (1.00/99%) for the inclusion of Philippine strains of *A. pacificum* with other sequences of *A. pacificum* (Group IV). The four LSU rDNA sequences were almost identical, only variable at two positions. The LSU rDNA sequences of the Philippine strains showed 0.025–0.029% sequence divergence based on K2P distance to the *A. pacificum* (Group IV) with a strong support (1.00/99%) for their separation (Table 1). Similarly, the BI tree inferred from SSU sequences supported the inclusion of Philippine strains in *A. pacificum* (Group IV) clade with strong support (1.00/98). Unlike the LSU, however, a sequence from Spain (AJ535392) was separated from the Philippine strains and the rest of the *A. pacificum* (Group IV) sequences forming three subgroups in *A. pacificum* (Group IV).

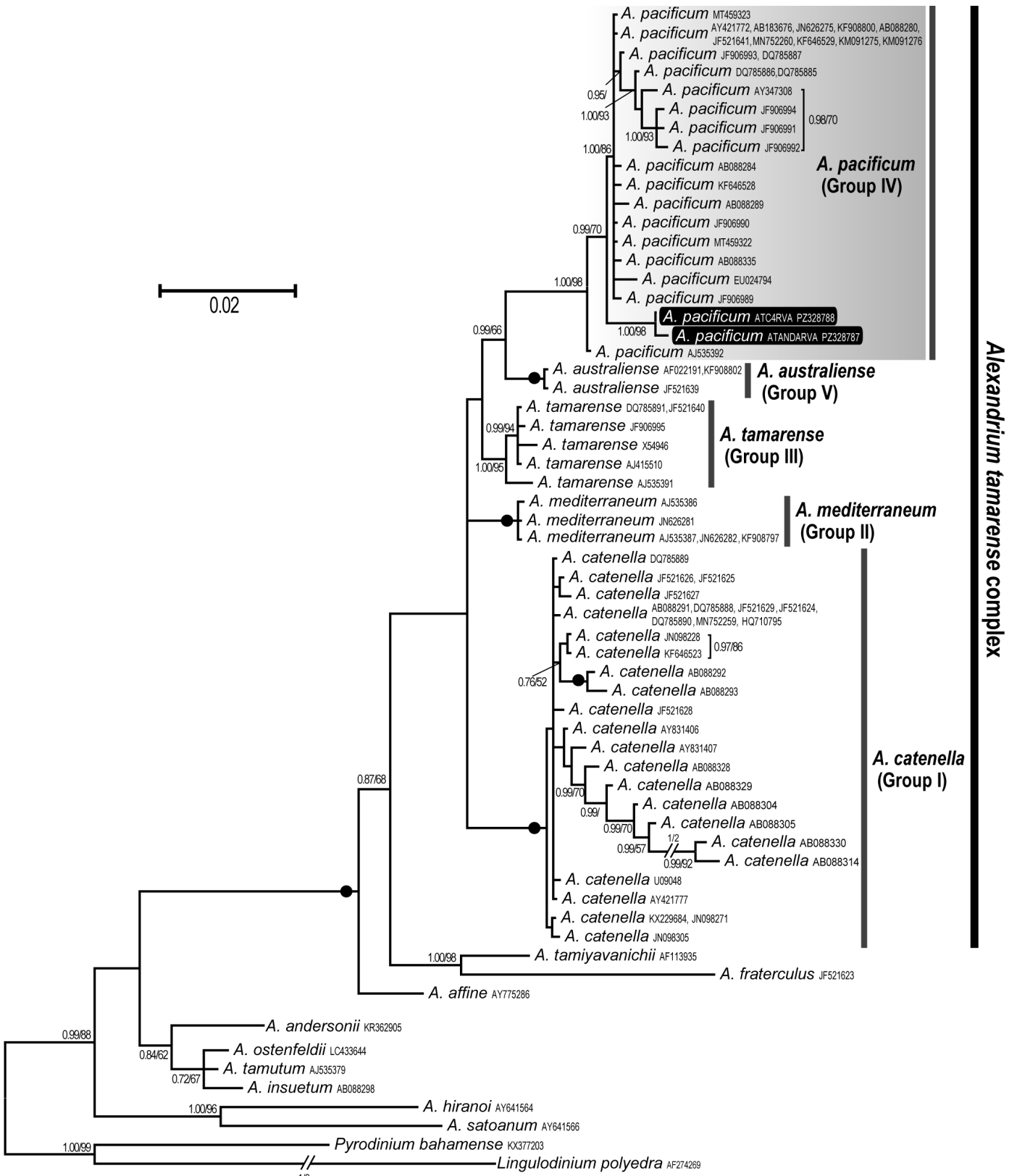

Figure 5. Bayesian inference (BI) phylogeny of *Alexandrium* species inferred from SSU rDNA (1,685 bp) based on GTR + I + G model. Posterior probabilities (PP, ≥0.7) of BI and bootstrap support (BS, ≥50%) values of maximum likelihood (ML) were indicated. Black dots on nodes indicate maximum supports for both BI and ML (PP/BS = 1.00/100). DNA sequences analyzed in this study are highlighted in black box.

Toxin profile and content

PSTs were detected in two culture strains of *A. pacificum* (ATC4BOL and ATMBRVA) (Fig. 6). Toxins from the two strains were dominated by N-sulfocarbamoyl toxin (C1/C2), which represented 80.02–88.15% of the total toxin content on an average mol percentage basis, gonyautoxin (GTX 2/3) with 11.31%–19.53%, and traces of neosaxitoxin (NEO), GTX 1/4, C3/C4. The total cell toxin content ranged from 32.70–38.18 fmol of STX eq cell^{-1}.

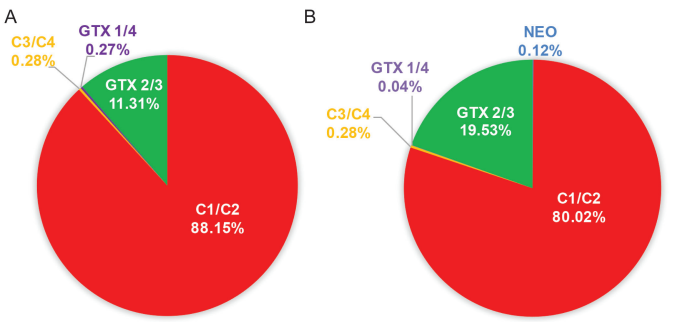

Figure 6. Paralytic shellfish toxin composition (mol %) of Philippine *Alexandrium pacificum* isolates. (A) ATC4RVA. (B) ATMBRVA.

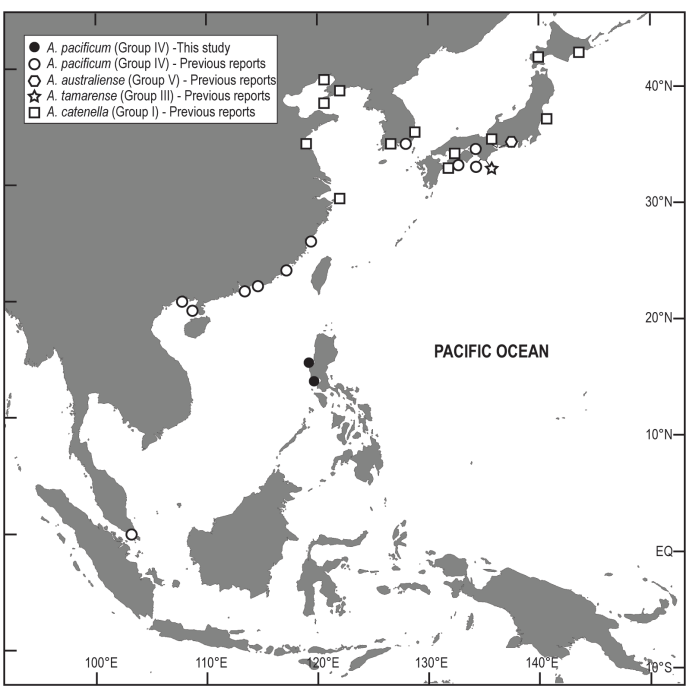

Figure 7. Occurrences of *Alexandrium tamarense* complex in the Asia Pacific. Closed circles are records in this study and open circles, squares, star and hexagon are records in previous studies (Scholin et al. 1994, Adachi et al. 1996, Cho & Lee 2001, Kim et al. 2002, Tanabe & Sako 2006, Murray et al. 2012, Gu et al. 2013, Zou et al. 2014, Shi et al. 2017 and Onishi et al. 2020).

DISCUSSION

Occurrences of the *A. tamarense* species *complex* mainly by *A. catenella* (Group I) and *A. pacificum* (Group IV) in Asia have been reported only from China, Japan, and South Korea (Fig. 7) (Adachi et al. 1996; Cho & Lee 2001; Kim et al. 2002; Tanabe & Sako 2006; Gu et al. 2013; Zou et al. 2014; Shi et al. 2017; Onishi et al. 2021). A few occurrences of *A. tamarense* (Group III) and *A. australiense* (Group V) in Japan and *A. pacificum* (KF646503) from Singapore were also previously reported, but it remains unsettled if they were either natural or human-mediated introduction (i.e., ballast water transfer) from other regional populations (Scholin et al. 1994; Zou et al. 2004; Bolch & de Salas 2007; Murray et al. 2012). Here, we report the first observed occurrence *A. pacificum* (Group IV) in the Philippines in a tropical region, further expanding its known geographic distribution. Although there were earlier reports of the morphospecies *A. tamarense* in Malaysia, Thailand, and Vietnam, their molecular identification did not corroborate morphological characterization. For example, isolates from Vietnam and Malaysia were initially identified as *A. tamarense* based on morphological similarity, but re-evaluation using phylogenetic placement revealed their affinity to *A. affine* (Usup et al. 2002; Nguyen-Ngoc 2004; Leaw et al. 2005). One strain from Thailand that had been reported as a tropical *A. tamarense* (CU15) was eventually re-classified as *A. tropicale* after reexamination of its thecal plate morphology and analysis of its molecular sequence (Lilly et al. 2007). Hence, the identified strains in this study provide evidence for the occurrence of this species of the *A. tamarense* species complex in the tropics, with strong support from its morphological, molecular, and toxin characteristics.

Alexandrium pacificum (Group IV) has been established mainly based on their molecular distances because morphological characters traditionally used to delineate species were overlapping among the species within the *A. tamarense* complex (John et al. 2014a). Many of the *A. pacificum* (Group IV) strains were formerly reported as morphospecies *A. catenella,* which were identified based on the morphological description provided by Balech (1995). In his description, *A. catenella* is mainly distinguished from *A. tamarense* and *A. fundyense* by its antero-posteriorly flattened cell and the lack of a ventral pore in the first apical plate. Based on our morphological examination, the Philippine strains had an isodiametric cell shape, and lacked ventral pore in plate 1′, which are characters intermediate of morphospecies *A. catenella* and *A. tamarense*. This demonstrates and affirms the difficulty of utilizing traditional morphological characteristics in identifying species of the *A. tamarense* complex, which has also previously been reported to have a high phenotypic plasticity (Kim et al. 2002; Wang et al. 2008; Orlova et al. 2007; John et al. 2014a)

Phylogenetic analyses inferred from LSU and SSU rDNA identified the Philippine strains as *A. pacificum* (Group IV) by forming a well-supported clade including the sequence of the strain (KF908803 and KF908800) used for molecular diagnosis (John et al. 2014a). Interestingly, the Philippine strains formed a distinct subclade sister to *A. pacificum,* which is mostly comprised of sequences from temperate Asia, further indicating a possible separate lineage and endemism in tropical east Asia. Similar results were obtained using SSU rDNA phylogeny, although a non-Asian strain from Spain (AJ535392) diverged from the Philippine and temperate Asian strains. The sampling locations of our strains (i.e., Bolinao-Anda Channel and Manila Bay) are situated in the northwestern Philippines facing the South China Sea (SCS), and are possibly influenced by the large-scale SCS gyre (Morton & Blackmore 2001; Wang et al. 2006), which may explain the affinity of the Philippine strains to the other temperate Asian group of *A. pacificum*. The relatively lower genetic distance of the Philippine strains from other *A. pacificum* strains, as compared to their distances from the other clades, possibly indicates that their divergence has just occurred recently.

Consistent with previous reports from other *A. pacificum* strains, PSTs were detected in the two tested Philippine cultures with cell toxin quota ranging from 32.70–38.18 fmol of STX eq cell^{-1} (Table 2). The toxicity of the Philippine strains was relatively higher than those reported in most strains of *A. pacificum* except for strains CAWD44 and CAWD121 from New Zealand (150.20–328.05 fmol of STX eq cell^{-1}), strain ATTL01 from France (44.3 fmol of STX eq cell^{-1}) and strain

Table 2. Paralytic shellfish toxin (PST) profile and toxicity of *Alexandrium pacificum* (Group IV).

Strains	Country	Toxin component	Toxin content (fmole/ cell)	Reference
ATC4RVA	Philippines	C1/C2, C3/C4, GTX1/4, GTX2/3	32.7	This study
ATMBRVA	Philippines	C1/C2, C3/C4, GTX1/4, GTX2/3, NEO	38.18	This study
AN G4-4	Algeria	GTX6, C2, GTX5, NEO	3.8	Hadjadji et al. 2020
AND6-2	Algeria	C1, GTX1, GTX5, GTX6, NEO	30.82	Hadjadji et al. 2020
ACPP09	Australia	GTX6, C1/C2, GTX1/4, GTX 2/3, GTX5, NEO	N.D.	Hallegraeff et al. 1991
ACSH02	Australia	C1/C2, GTX1/4, GTX5, GTX6	12.1	Murray et al. 2011
ACCC01	Australia	C1/C2, C3/C4, GTX1,4, GTX6	14.7	Murray et al. 2011
ACTRA02	Australia	C1/C2,GTX1/4,	3.5	Murray et al. 2011
ATMJ02	China	C1/C2, Neo	19.2	Zou et al. 2014
ATXM08	China	GTX 1/4, GTX2/3, dcGTX2/3, NEO, STX	10.76	Zou et al. 2014
ADH01	China	C1/C2, GTX1/4, GTX2,3, dcGTX2/3	4.91	Zou et al. 2014
ADH02	China	C1/C2, dcGTX2/3	0.46	Zou et al. 2014
ADH05	China	C1/C2, GTX1/4	3.27	Zou et al. 2014
ADH18	China	C1/C2, GTX2/3 dcGTX2/3	4.26	Zou et al. 2014
HK9301	China	C1/C2, GTX1/4,	6.58	Zou et al. 2014
ATHKQ	China	C1/C2, GTX1/4, B1,STX	0.91	Zou et al. 2014
ATGX02	China	GTX1/4, dcGTX2/3, dcSTX, STX	4.03	Zou et al. 2014
ATDY04	China	C1/C2, GTX1/4, dcGTX2/3, NEO	6.34	Zou et al. 2014
ATTL01	France	C1/C2, C3/C4, GTX1/4, GTX5	44.3	Lilly et al. 2002
ATTL01	France	C1/C2, C3/C4, GTX1/4, GTX5	5.3	Lilly et al. 2002
Ac91-2	Japan	C1/C2, GTX1/4, GTX3, C4, Neo	11.7	Yoshida et al. 2001
Acko2	Japan	C1/C2, C3/C4, GTX1/4, GTX3, GTX5,	11.9	Yoshida et al. 2001
Acko5	Japan	C1/C2, GTX1, GTX3, GTX5,	19.4	Yoshida et al. 2001
Acy7	Japan	C1/C2, C3/C4, GTX1/4, GTX3, Neo	16.8	Yoshida et al. 2001
Acy8	Japan	C1/C2, C3/C4, GTX1/4, GTX2/3, Neo	16.8	Yoshida et al. 2001
OF101	Japan	C1/C2, GTX3, GTX4, GTX5, NEO, STX	24.7	Yoshida et al. 2001
OF102	Japan	C1/C2, GTX3, GTX5, NEO, STX	58.5	Yoshida et al. 2001
TN11	Japan	C1/C2, C4, GTX3, GTX1/4, GTX5, NEO, STX	12.6	Yoshida et al. 2001
TN22	Japan	C1/C2, GTX1/4, C4 GTX3, GTX5, STX	17.4	Yoshida et al. 2001
CAWD121	New Zealand	C3/C4, C1/C2, GTX1/4,	328.05	Mackenzie et al. 2004
CAWD44	New Zealand	C1/C2, C3/C4, GTX1/4, GTX2/3, NEO	150.2	Mackenzie et al. 2004
Various strains	South Korea	C1/C2, GTX 1/4, GTX 2/3, NEO	0.040–5.716	Cho and Lee 2002

OF2 from Japan (58.5 fmol of STX eq cell^{-1}) (Yoshida et al. 2001; Lilly et al. 2002; Mackenzie et al. 2004) (Table 2). The toxin profile of the Philippine strains was dominated by high proportion of N-sulfocarbomoyl C1/C2. Similarly, C1/C2 have been the major PST component in many strains of *A. pacificum* from Japan, Korea, China, Australia, New Zealand, and France (Hallegraeff et al. 1991; Cho & Lee 2001; Yoshida et al. 2001; Lilly et al. 2002; Mackenzie et al. 2004; Murray et al. 2012). In some strains, however, other derivatives dominated such as those in strains ACPP09 and ACPP02 from Australia with a high proportion of GTX 6 (Hallegraeff 1991), and strain CAWD121 from New Zealand with C3/C4 (Mackenzie et al. 2004). Toxin profiling of the tropical *A. pacificum* from both Bolinao and Manila Bay, therefore, showed their similarity mostly to temperate strains in terms of dominant toxin analogues. Notably, although the toxin content was generally higher than most strains of *A. pacificum*, several studies have demonstrated that toxicity level can be variable even among different strains, species, clades depending on local environmental conditions such as nutrient availability, temperature, and salinity (Siu et al. 1997; Hamasaki et al. 2001; Laabir et al. 2011) or the presence of certain associated bacterial communities (Uribe & Espejo 2003).

CONCLUSIONS

The present study reports the occurrence of *A. pacificum* not only in the Philippines but also in tropical Southeast Asia, as confirmed by morpho-molecular examination. Our toxin data also showed the dominance of N-sulfocarbamoyl toxin (C1/C2), similar to most temperate strains of *A. pacificum* in *A. tamarense* complex. This paper thus serves as the first verified record of this highly toxic dinoflagellate not only in

the Philippines but also in Southeast Asia. Awareness on the presence of this species could drive further work to understand their ecology, bloom dynamics, and distribution, which remains relatively limited compared to the other tropical PSP-causing dinoflagellates such as *Pyrodinium bahamense* and *A. minutum*.

CONTRIBUTION STATEMENT

Garry A. Benico: Wrote the original draft of the manuscript, Sample collection and establishment of culture strains, Acquisition and analysis of morphological, molecular and toxicity data. **Deo Florence L. Onda**: Analysis of molecular data, Provided research materials, Revision of the article. **Mitsunori Iwataki**: Revision of the article, Supervision, Provided research materials. **Rhodora V. Azanza**: Conceptualized the research, Funding acquisition, Supervision, Review of final manuscript, Provided research materials.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests nor personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY

The manuscript has no associated data.

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ETHICAL APPROVAL

No animal testing was performed during this study.

REFERENCES

Adachi, M., Sako, Y. & Ishida, Y., 1996. Analysis of *Alexandrium* (Dinophyceae) species using sequences of the 5.8S ribosomal DNA and internal transcribed spacer regions. J. Phycol. 32, 424–432.

Altschul, S.F., Gish, W., Miller, W., Myers, E. W. & Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410.

Azanza, R.V. & Taylor, M.F.J.R., 2001. Are *Pyrodinium* blooms in the Southeast Asian region recurring and spreading? A view at the end of the millennium. Ambio 30, 356–64.

Azanza, R.V., Yñiguez, A.T., Onda, D.F., Benico, G.A., Lim, P.T.; Leaw, C.P. & Iwataki, M. 2024. Expansion of toxic algal blooms in coastal and marine areas in the Philippines and Malaysia: Is it climate change related? Sustainability 16, 1–17.

Azanza, R., & Benico, G., 2013. Toxic *Alexandrium* blooms in fish farming sites in Bolinao, Pangasinan. J. Environ. Sci. Manag. Special Issue 1, 44–49.

Balech, E., 1995. The Genus *Alexandrium* Halim (Dinoflagellata). Sherkin Island Marine Station, Sherkin Island, Co. Cork, Ireland, 151 pp.

Benico, G. & Azanza, R.V., 2021. Characterization of *Alexandrium tamutum* (Dinophyceae) isolated from Philippine waters, with the rare detection of paralytic shellfish toxin. Philipp. J. Syst. Biol. 15, 1–13.

Bolch, C.J.S. & de Salas, M.F., 2007. A review of the molecular evidence for ballast water introduction of the toxic dinoflagellates *Gymnodinium catenatum* and the *Alexandrium* "tamarensis complex" to Australasia. Harmful Algae 6, 465–485.

Borja, V.M., Furio, E.F., Gatdula, N.C. & Iwataki, M., 2019. Occurrence of harmful algal blooms caused by various phytoplankton species for the last three decades in Manila Bay, Philippines. Philipp. J. Natur. Sci. 24: 80–90.

Cho, E.S. & Lee, H.J., 2001. Thecal plates, toxin content and growth of five clones of *Alexandrium tamarense* (Dinophyceae) isolated from Jinhae Bay, Korea. Phycologia 40, 435–439.

Diego, D., Guillermo, L.T., Ramn, D. & David, P., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9, 772.

Katoh, K. & Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.

Fraga, S., Sampedro, N., Larsen, J., Moestrup, Ø. & Calado, A.J., 2015. Arguments against the proposal 2302 by John and al. to reject the name *Gonyaulax catenella* (*Alexandrium catenella*). Taxon 64, 634–635.

Fritz, L. & Triemer, R.E., 1985 A rapid technique utilizing Calcofluor White M2R for the visualization of dinoflagellate thecal plates. J. Phycol. 21, 662–664.

Fukuyo, Y., Pholpunthin, P. & Yoshida, K., 1988. *Protogonyaulax* (Dinophyceae) in the Gulf of Thailand. Bull. Plankton Soc. Japan 35, 9–20.

Fukuyo, Y., Kodama, M., Omura, T., Furuya, K., Furio, E., Cayme, M., Lim, P. T., Ha, D. V., Kotaki, Y., Matsuoka, K., Iwataki, M., Sriwoon, R. & Lirdwitayaprasit, T., 2011. Ecology and oceanography of harmful marine microalgae. In: Nishida, S., Fortes, M.D., Miyazaki, N. (Eds.), Coastal Marine Science in Southeast Asia. Terra Pub, Tokyo, pp. 23–48.

Furuya, K., Iwataki, M., Lim, P.T., Lu, S., Leaw, C.P., Azanza, R. V., Kim, H.G. & Fukuyo, Y., 2018. Overview of harmful algal blooms in Asia. In: Glibert, P.M., Berdalet, E., Burford, M.A., Pitcher, G.C., Zhou, M. (Eds.), Global Ecology and Oceanography of Harmful Algal Blooms. Springer, pp. 289–308.

Gu, H., Zeng, N., Liu, T., Yang, W., Mu, A. & Krock, B., 2013. Morphology, toxicity, and phylogeny of *Alexandrium* (Dinophyceae) species along the coast of China. Harmful Algae 27, 68–81.

Guillard, R.R.L. & Ryther, J.H., 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. Canadian J.

Microbiol. 8, 229–239.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321.

Guiry, M.D. & Guiry, G.M., 2021. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org (last accessed on 18 April 2021).

Hall, S., Strichartz, G., Moczydlowski, E., Ravindran, A. & Reichardt, P.B. 1990. The saxitoxins. Sources, chemistry, and pharmacology. In: Hall, S., Strichartz, G. (Eds.), Marine Toxins: Sources, Chemistry and Pharmacology. American Chemical Society, Washington, DC, pp. 29–65.

Hall, T., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids 41, 95–98.

Hallegraeff, G., Bolch, C., Blackburn, S. & Oshima, Y., 1991. Species of the toxigenic dinoflagellate genus *Alexandrium* in southeastern Australian waters. Bot. Mar. 34, 575–588.

Hamasaki, K., Horie, M., Tokimitsu, S., Toda, T. & Taguchi, S., 2001. Variability in toxicity of the dinoflagellate *Alexandrium tamarense* isolated from Hiroshima Bay, western Japan, as a reflection of changing environmental conditions. J. Plankton Res. 23, 271–278.

Ho, K.C., Lee, T.C.H., Kwok, O.T. & Lee, F.W.F., 2012. Phylogenetic analysis on a strain of *Alexandrium tamarense* collected from Antarctic Ocean. Harmful Algae 15, 100–108.

John, U., Litaker, R., Montresor, M., Murray, S., Brosnahan, M. & Anderson, D., 2014a. Formal revision of the *Alexandrium tamarense* species complex (Dinophyceae) taxonomy: The introduction of five species with emphasis on molecular-based (rDNA) classification. Protist 165, 779–804.

John, U., Litaker, W., Montresor, M., Murray, S., Brosnahan, M. & Anderson, D., 2014b. Proposal to reject the name *Gonyaulax catenella* (*Alexandrium catenella*) (Dinophyceae). Taxon 63, 932–933.

Katoh, K. & Standley, D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30: 772–80.

Kim, K. Y., Yoshida, M., Fukuyo, Y. & Kim, C., 2002. Morphological observation of *Alexandrium tamarense* (Lebour) Balech, *A. catenella* (Whedon et Kofoid) Balech and one related morphotype (Dinophyceae) in Korea. Algae 17, 11–19.

Laabir, M., Jauzein, C., Genovesi, B., Masseret, E., Grzebyk, D., Perrin, Y. & Cecchi, P., 2011. Influence of temperature, salinity and irradiance on the growth and cell yield of the harmful red tide dinoflagellate *Alexandrium catenella* colonizing Mediterranean waters. J. Plankton Res. 33, 1550–1563.

Lawrence, J.F. & Ménard, C., 1991. Liquid chromatography determination of paralytic shellfish poisons in shellfish after prechromatographic oxidation. J. Assn. Off. Anal. Chem. 74, 1006–1012.

Leaw, C.P, Lim, P.T, Ng, B., Cheah, M., Ahamad, A. & Usup, G., 2005. Phylogenetic analysis of *Alexandrium* species and *Pyrodinium bahamense* based on theca morphology and nuclear ribosomal gene sequence. Phycologia 44, 550–565.

Lefort, V., Longueville, J. & Gascuel, O., 2017. SMS: Smart model selection in PhyML. Mol. Biol. Evol. 34, 2422–2424.

Lilly, E.L., Halanych, K.M. & Anderson, D.M., 2007. Species boundaries and global biogeography of the *Alexandrium tamarense* complex (Dinophyceae). J. Phycol. 43, 1329–1338.

Lilly, E., Kulis, D., Gentien, P. & Anderson, D., 2002. Paralytic shellfish poisoning toxins in France linked to a human-introduced strain of *Alexandrium catenella* from the western Pacific: evidence from DNA and toxin analysis. J. Plankton Res. 24, 443–452.

Litaker, R.W., Fraga, S., Montresor, M., Brosnahan, M., Anderson, D.M., Hoppenrath, M., Murray, S., Wolny, J., John, U., Sampedro, N., Larsen, J. & Calado, A.J., 2018. A practical guide to new nomenclature for species within the "*Alexandrium tamarense species complex*". Harmful Algae News 61, 13–15.

Logares, R., Shalchian-Tabrizi, K., Boltovskoy, A. & Rengefors, K., 2007. Extensive dinoflagellate phylogenies indicate infrequent marine–freshwater transitions. Mol. Phylogenet. Evol. 45, 887–903.

Lum, W.M., Lim, H.C., Lau, W.L.S., Law, I.K., Teng, S.T., Benico, G., Leong, S.C.Y., Takahashi, K., Gu, H., Lirdwitayaprasit, T., Leaw, C.P., Lim, P.T., Iwataki, M., 2022. Description of two new species *Chattonella tenuiplastida* sp. nov. and *Chattonella malayana* sp. nov. (Raphidophyceae) from South China Sea, with a report of wild fish mortality. Harmful Algae 118, doi:10.1016/j.hal.2022.102322.

Lundholm, N., Churro, C., Fraga, S., Hoppenrath, M., Iwataki, M., Larsen, J., Mertens, K., Moestrup, Ø. & Zingone, A. (Eds.) (2009 onwards). IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae. Accessed at https://www.marinespecies.org/hab on 2022-09-20. doi:10.14284/362

Malto, Z.B.L., Benico, G.A., Batucan, J.D., Dela Cruz, J., Romero, M.L.J., Azanza, R.V., Salvador-Reyes, L.A., 2022**.** Global mass spectrometric analysis reveals chemical diversity of secondary metabolites and 44-methylgambierone production in Philippine *Gambierdiscus* strains. Front. Mar. Sci. 8, 1–17.

Mackenzie, L., de Salas, M., Adamson, J. & Beuzenberg, V., 2004. The dinoflagellate genus *Alexandrium* (Halim) in New Zealand coastal waters: comparative morphology, toxicity and molecular genetics. Harmful Algae 3, 71–92.

Mertens, K.N., Adachi, M., Anderson, D.M., Band-Schmidt, C.J., Bravo, I., Brosnahan, M.L., Bolch, C.J.S. et al. 2020. Morphological and phylogenetic data do not support the split of *Alexandrium* into four genera. Harmful Algae 98, 101902.

Miranda, L.N., Zhuang, Y., Zhang, H. & Lin, S., 2012. Phylogenetic analysis guided by intragenomic SSU rDNA polymorphism refines classification of "*Alexandrium tamarense*" species complex. Harmful Algae 16, 35–48.

Montojo, U.M., Sakamoto, S., Furio, E.F., Gatdula, N.C., Borja, V.M., Formeloza, M.A., Yoshida, M., Fukuyo, Y., Ogata,

T. & Kodama, M., 2003. Occurrence of three species of toxic dinoflagellates in Manila Bay, Philippines. In: The First Joint Seminar on Coastal Oceanography. Chiang Mai, Thailand, pp. 19.

Morton, B. & Blackmore, G., 2001. South China Sea. Mar. Pollut. Bull. 42, 1236–1263.

Murray, S.A., Wiese, M., Neilan, B.A., Orr, R.J.S., de Salas, M., Brett, S. & Hallegraeff, G., 2012. A reinvestigation of saxitoxin production and sxtA in the "non-toxic" *Alexandrium tamarense* Group V clade. Harmful Algae 18, 96–104.

Nguyen-Ngoc, L., 2004. An autecological study of the potentially toxic dinoflagellate *Alexandrium affine* isolated from Vietnamese waters. Harmful Algae 3, 117–129.

Onda, D., Benico, G., Sulit, A., Lorenzo, P., Azanza, R. & Lluisma A., 2013. Morphological and molecular characterization of some HAB-forming dinoflagellates from Philippine waters. Philipp. Sci. Letters 6, 97–106.

Onishi, Y., Tuji, A., Yamaguchi, A. & Imai, I., 2021. Distribution of growth-inhibiting bacteria against the toxic dinoflagellate *Alexandrium catenella* (Group I) in Akkeshi-Ko Estuary and Akkeshi Bay, Hokkaido, Japan. Appl. Sci. 11, 1–22.

Orlova, T.Y., Selina, M.S., Lilly, E.L., Kulis, D.M. & Anderson, D.M., 2007. Morphogenetic and toxin composition variability of *Alexandrium tamarense* (Dinophyceae) from the east coast of Russia. Phycologia 46, 534–548.

Orr, R.J.S., Stüken, A., Rundberget, T., Eikrem, W. & Jakobsen, K.S., 2011. Improved phylogenetic resolution of toxic and non-toxic *Alexandrium* strains using a concatenated rDNA approach. Harmful Algae 10, 676–688.

Prud'homme van Reine, W.F., 2017. Report of the nomenclature committee for algae:15. Taxon 66, 191–192.

Ronquist, F. & Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

San Diego-McGlone, M.L., Yñiguez, A.T., Benico, G., Lum, W.M., Hii, K.S., Leong, S.C.Y., Leaw, C.P., Iwataki, M. & Lim, P.T., 2024. Fish kills related to harmful algal bloom events in Southeast Asia. Sustainability 16, 10521.

Scholin, C.A., Herzog, M., Sogin, M. & Anderson, D.M., 1994. Identification of group- and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). II. Sequence analysis of a fragment of the LSU rRNA gene. J. Phycol. 30, 999–1011.

Shin, H.H., Li, Z., Kim, E.S., Park, J.W. & Lim, W.A. 2017. Which species, *Alexandrium catenella* (Group I) or *A. pacificum* (Group IV), is really responsible for past paralytic shellfish poisoning outbreaks in Jinhae-Masan Bay, Korea? Harmful Algae 68, 31–39.

Siu, G., Young, M. & Chan, D., 1997. Environmental and nutritional factors which regulate population dynamics and toxin production in the dinoflagellate *Alexandrium catenella*. Hydrobiologia 352, 117–140.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S., 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.

Tanabe, S.H. & Sako, Y., 2006. Genetic differentiation in the marine dinoflagellates *Alexandrium tamarense* and *Alexandrium catenella* based on DNA-DNA hybridization. Plankton Benthos Res. 1, 138–146.

Uribe, P. & Espejo, R.T., 2003. Effect of associated bacteria on the growth and toxicity of *Alexandrium catenella*. Appl. Environ. Microbiol. 69, 659–662.

Usup, G., Leaw, C.P, Ahmad, A. & Lim, P.T., 2002. *Alexandrium* (Dinophyceae) species in Malaysian waters. Harmful Algae 1, 265–275.

Wang, G.H., Chen, D.K. & Su, J.L., 2006. Generation and life cycle of the dipole in the South China Sea summer circulation. J. Geophys. Res. 111, C06002.

Wang, L., Zhuang, Y., Zhang, H., Lin, X. & Lin, S., 2014. DNA barcoding species in *Alexandrium tamarense* complex using ITS and proposing designation of five species. Harmful Algae 31, 100–113.

Wang, S., Tang, D., He, F., Fukuyo, Y. & Azanza, R.V., 2008. Occurrences of harmful algal blooms (HABs) associated with ocean environments in the South China Sea. Hydrobiologia 596, 79–93.

Yñiguez, A.T., Lim, P.T., Leaw, C.P., Jipanin, S.J., Iwataki, M., Benico, G. & Azanza, R.V., 2021. Over 30 years of HABs in the Philippines and Malaysia: What have we learned? Harmful Algae 102, 101776.

Yñiguez, A.T., Cayetano, A., Villanoy, C.L., Alabia, I., Fernandez, I., Palermo, J.D., Benico, G.A., Siringan, F.P. & Azanza, R.V., 2012. Investigating the roles of intrinsic and extrinsic factors in the blooms of *Pyrodinium bahamense* var. *compressum* using an individual-based model. Procedia Environ. Sci. 13, 1462–1476.

Yoshida, T., Sako, Y. & Uchida, A., 2001. Geographic differences in paralytic shellfish poisoning toxin profiles among Japanese populations of *Alexandrium tamarense* and *A. catenella* (Dinophyceae). Phycol. Res. 49, 13–21.

Zou, C., Ye, R.M., Zheng, J.W., Luo, Z.H., Gu, H.F., Yang, W.D., Li, H.Y. & Lie J.S., 2014. Molecular phylogeny and PSP toxin profile of the *Alexandrium tamarense* species complex along the coast of China. Mar. Pollut. Bull. 89, 209–219.