

Research Article

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Diversity and distribution of species of the planktonic dinoflagellate genus *Alexandrium* (Dinophyta) from the tropical and subtropical Mexican Pacific Ocean

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Abstract: Species of the dinoflagellate genus *Alexandrium* are marine and planktonic forms, widely distributed, and some are recognized to form harmful algal blooms and to produce saxitoxins causing Paralytic Shellfish Poisoning in humans, and other toxins. We studied the species composition of *Alexandrium* in tropical and subtropical coastal areas of the Mexican Pacific: from the southern Gulf of California to the Gulf of Tehuantepec. Eleven *Alexandrium* species were identified, described, and illustrated using light microscopy, and occasionally scanning electron microscopy for certain

species. Additionally, the genetic characterization of seven strains and four species, was performed, using sequences of the D1/D2 LSU rDNA and ITS regions. We identified the species (morphospecies): *Alexandrium affine*, *A. gaarderae*, *A. globosum*, *A. leei*, *A. margalefi*, *A. minutum*, *A. monilatum*, *A. pseudogonyaulax*, *A. tamarensis*, *A. tamiyavanichii*, and *A. tropicale*. Of these, *A. affine*, *A. leei*, *A. minutum*, *A. monilatum*, *A. pseudogonyaulax*, *A. tamarensis* and *A. tamiyavanichii* have been widely recognized as harmful algae. *Alexandrium gaarderae*, *A. globosum* and *A. tropicale* are new records for the Mexican Pacific. This is the first morphological documentation of *A. pseudogonyaulax*. Future studies of the genus might increase its species richness if more cultures are established and metabarcoding approach is used.

Keywords: dinoflagellates; molecular phylogenies; morphology; phytoplankton; taxonomy

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1 Introduction

Dinoflagellates are a major and important protist taxonomic group in aquatic environments, and especially in the marine phytoplankton. This group contains a large number of harmful and toxin-producing species. Among the toxigenic genera, *Alexandrium* Halim comprises an increasing interest group of marine and planktonic species, which are spread all over the world (Anderson et al. 2012; Klemm et al. 2022). The study of *Alexandrium* species gained importance because some can produce saxitoxin and other potent toxins such as spirolides and goniodomins, which can cause different syndromes such as Paralytic Shellfish Poisoning (PSP) in humans, due to contaminated seafood consumption (Abdullah et al. 2023; Anderson et al. 2012; Gu et al. 2013; Liu et al. 2021; Tillmann et al. 2020).

The genus *Alexandrium* currently contains 44 names, with 33 species accepted (Guiry et al. 2023), 18 of which are considered as harmful (Escalera 2023). The morphology of the cells has been the basis for species identification (morphospecies) considering “traditional”, general morphological characters, such as growth form (solitary or chain-forming species), cell shape (cell outline) and size (cell length and width), and others more specifically related to the theca conformation such as shape and position of the Po plate, shape and position of pores in Po and sulcal plates, displacement of the first apical plate (1’), shape and size of the sixth precingular plate (6’), presence or absence of the ventral pore and its shape and size, shape of the anterior (Sa) and the posterior (Sp) sulcal plates (Balech 1995; Steidinger and Tangen 1997). Some of these morphological characters may be variable among few species (Gu et al. 2013; Lim et al. 2007). About half of *Alexandrium* species may produce cyst stages (Menezes et al. 2018; Tang et al. 2021; Wang et al. 2022).

Molecular techniques have also become decisive tools to recognize species of the genus, and it is widely recommended to use both approaches, morphological and molecular ones to reveal the true diversity of the genus. The recognition of “cryptic” and “pseudocryptic” species and the erection of many new species have been possible by combining these two approaches. Recently, molecular phylogenies showed the polyphyletic origin of *Alexandrium* and its possible resolution by subdividing this genus into four genera (Gómez and Artigas 2019); however, this proposal has not been formally accepted and the phylogenetic classification has not yet been resolved (Mertens et al. 2020).

In the Mexican Pacific there are few investigations devoted to this genus and some have no or limited photographic evidence, illustrations or descriptions, and therefore some records are considered doubtful. Species of the genus *Alexandrium* from the subtropical Mexican Pacific have been described and illustrated since 1995, when Licea et al. (1995) studied *Alexandrium catenella* and *A. monilatum* from the Gulf of California. Later, Band-Schmidt et al. (2003a, 2003b) recognized *Alexandrium affine* and *A. margalefi* from Bahía Concepción, within the Gulf of California, following molecular tools. Okolodkov and Gárate-Lizárraga (2006) listed dinoflagellate species found in the Mexican Pacific, including 16 species of *Alexandrium*, whereas Hernández-Becerril et al. (2007) listed some species of the genus related to Harmful Algal Blooms (HAB) and toxin production from the Mexican Pacific.

Additionally, *A. tamiyavanichii* and *A. fraterculus* were found and studied from coasts of the tropical Mexican Pacific (Esqueda-Lara and Hernández-Becerril 2010; Meave del

Castillo et al. 2012a, respectively), Maciel-Baltazar (2015) reported the presence of two fully identified species of *Alexandrium*: *A. monilatum* and *A. tamiyavanichii* and three other incompletely identified species (*A. cf. catenella*, *A. cf. minutum*, and *A. cf. tamarensis*) from coastal lagoons in Chiapas (the most southern part of the Mexican Pacific), Morquecho-Escamilla et al. (2016) documented the morphology of the species *Alexandrium margalefi* and *A. tamiyavanichii*, and *Alexandrium ostenfeldii* was recognized in western coasts off Baja California (Almazán-Becerril et al. 2016). Torres-Ariño et al. (2019) prepared a list of algae from the south eastern Mexican Pacific, including 10 species of the genus *Alexandrium*, whereas Hernández-Becerril et al. (2021) described and illustrated eight species of *Alexandrium* from the central Mexican Pacific.

Most probably, the first published report of an *Alexandrium* bloom and its effect on human health in the Mexican Pacific, was made by Saldate-Castañeda et al. (1991) who reported several intoxicated persons for shellfish consumption (PSP), associated with “red tides” caused by *Gymnodinium catenatum* and *Alexandrium (Gonyaulax) catenella*. Later, Cortés-Lara (2005) reported a bloom caused by *Alexandrium* sp. in Bahía Banderas (central Mexican Pacific), with densities reaching 3.176×10^6 cells L^{-1} , although her illustrations showed specimens with no details, more similar to *Scrippsiella acuminata*, and Meave del Castillo et al. (2012b) reported a bloom of *Alexandrium cf. tamarensis* from coasts of the southern part of the Mexican Pacific, whereas Hernández-Becerril et al. (2018) determined the abundance and distribution of the toxic species *Alexandrium tamiyavanichii* in the central Mexican Pacific, following the quantitative PCR method, finding very low densities, reaching to a maximum of 25 cells L^{-1} . Meave del Castillo and Reséndiz-Zamudio (2018) compiled the algal blooms occurring in coasts of Guerrero for 15 years (2000–2015), including blooms of the species *A. catenella*, *A. monilatum*, *A. ostenfeldii* and *A. tamarensis*, with cell densities of 5×10^3 cells L^{-1} , and more recently, *A. catenella* and *A. monilatum* were cited in the Mexican Pacific, within the most recent HAB revision, indicating a high frequency of HAB events of *Alexandrium* species (Rodríguez-Palacio et al. 2019).

This study attempts to give a contemporary account of the species of *Alexandrium* found in coasts of the tropical and subtropical Mexican Pacific (including locations within the Gulf of California and the Gulf of Tehuantepec), using morphological characters mostly observed by light microscopy (LM), and occasionally by scanning electron microscopy (SEM), and additionally following molecular phylogenies from sequences obtained from few cultured species.

2 Materials and methods

Coastal samples from various locations along the tropical to subtropical Mexican Pacific (from sites of the Gulf of California to the Gulf of Tehuantepec; Figure 1) were collected using bottles and/or phytoplankton nets (20- μm mesh) and fixed (Lugol's solution or formaldehyde 4 %). Most net collections were made by vertical hauls (maximum 15 m depth). A few other samples obtained with bottles were kept alive for posterior isolations for culturing in the laboratory (Figure 1). Some strains were also obtained by germination of cysts that were isolated from surface marine sediment samples (Figure 1).

Samples kept alive were observed with an Olympus LX50 light microscope (4 \times , 10 \times , 40 \times) to establish unialgal cultures. Single cell and/or chains isolations by micropipette were conducted according to Matsuoaka and Fukuyo (2000). Part of the cultures were deposited in the Marine Dinoflagellate Collection (CODIMAR, for its acronym in Spanish) at CIBNOR in La Paz, B.C.S., México (www.cibnor.mx/colecciones/codimar/icodimar.php) and other parts at the Laboratorio de Biotoxinas Marinas ICML UNAM, Mazatlán, Sinaloa, México. Cultures were isolated and are re-inoculated every 28 days in f/2 + Se (Guillard 1975) or GSe media (Blackburn et al. 2001) depending on the species. All media were prepared with filtered natural seawater (GF/F ~0.7 μm , nylon 0.45 and 0.22 μm), with salinity values from 32 to 34. Isolation conditions are 20 ± 0.3 °C, a 12/12 h dark/light cycle under an irradiance of 60 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ (Band-Schmidt 2007; Morquecho et al. 2014).

Net samples were analyzed with an AXIOSTAR plus Zeiss and a LM Olympus (20X, 40X and 100X). Sodium hypochlorite was added to some samples to make the theca transparent and observe the thecal plates. Additionally, epifluorescence microscopy was used to visualize relevant features of the vegetative cells, a solution of 1 % of calcofluor (Andersen

2010) was added to subsamples of the cultures to help to reveal such characteristics. A Nikon Eclipse TS100 microscope with a mercury bulb was used to carry out this technique. Some subsamples or specimens were rinsed with Milli-Q water, mounted onto aluminum stubs, air-dried, coated with gold, and then studied by SEM (JEOL JSM6360LV). For other subsamples, dehydration by an alcohol series (30 %, 50 %, 79 %, 90 % and 100 %) and then a hexamethyldisilane treatment were followed. Terminology for *Alexandrium* follows recommendation by Balech (1995) and Steidinger and Tangen (1997).

For molecular analysis, DNA was extracted using dinoflagellate liquid cultures, which were filtered with a sterile 20- μm mini mesh, supernatant was discarded, and the cellular pellet was processed with FastDNA SPIN Kit for Soil (Catalog # 6560–200; MP Biomedicals, Solon OH, USA). Total genomic DNA isolated from dinoflagellates was used as template for PCR amplification of rDNA D1-D2 LSU region using D1R (5'-ACCCGCTGAATTTAAGCATA-3') and D2C (5'-CCTTGGTCCGTGTTCAAGA-3') primers (Scholin et al. 1994), and the internal transcribed spacer ITS1-5.8S-ITS2 region of the rDNA using the ITSa (5'-CCT CGT AAC AAG GHT CCG TAG GT-3') and ITSb (5'-CAG ATG CTT AAR TTC AGC RGG-3') primers (Adachi et al. 1996). Thermocycler conditions with D1R and D2C were as follows: initial denaturation at 95 °C for 1 min, followed by 35 cycles of 95 °C for 30 s, 45 °C for 30 s, and 72 °C for 1 min 45 s, with a final extension period at 72 °C for 5 min. Thermocycler conditions with ITSa and ITSb were as follows: initial denaturation at 94 °C for 3.5 min followed by 35 cycles at 94 °C for 50 s, 47 °C for 60 s, and 72 °C for 80 s, with a final extension period at 72 °C for 10 min. PCR amplification products were electrophoretically resolved in agarose 1 % (w/v) gel. The thermocycler used was Veriti™ Thermal Cycler, Applied Biosystems™, PN4375786, Singapore. The amplification products were purified and Sanger sequenced (Macrogen, Seoul, Korea), both forward and reverse strands.

The sequences were edited with Bioedit (Hall 1999) or Sequencher (Gene Codes Corporation, Ann Arbor, MI, USA) programs, and aligned

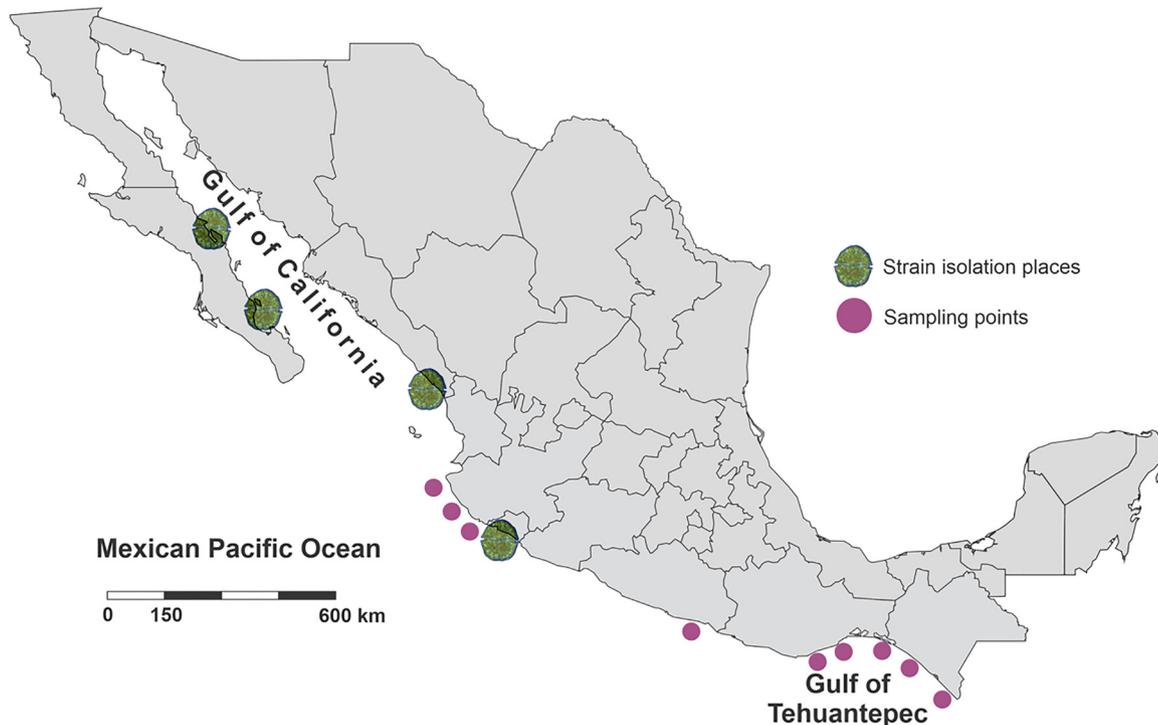


Figure 1: Map with the sampling points where species of *Alexandrium* were found and the sites from which the established strains were isolated.

with Clustal W (Thompson et al. 1994). The LSU rDNA alignment consisted of seven new and 48 GenBank sequences and ITS alignment consisted of eight and 38 GenBank sequences. The species *Fragilidium subglobosum* (Stosch) Loeblich III, *Gonyaulax spinifera* (Claparède et Lachmann) Diesing and *S. acuminata* (Ehrenberg) Kretschmann, Elbrächter, Zinssmeister, S. Soehner, Kirsch, Kusber et Gottschling were used as the outgroups. MEGA 7.0 (Kumar et al. 2016) was used to calculate genetic distances. The best evolutionary model for phylogenetic analyses selected with the JModelTest 2.1.10 program (Dariba et al. 2012) was GTR + G + I for both data sets. Phylogenies of LSU and ITS datasets were inferred using maximum likelihood (ML) and Bayesian inference (BI). The ML analyses were conducted using RAxML v8.0.X (Stamatakis 2014) set as follows: a rapid bootstrap analysis and search for the best scoring ML tree in one single program run with 1000 bootstrap replicates under the GTR + G + I substitution model. BI was performed for individual datasets with MrBayes v3.2.1 (Ronquist et al. 2012) using the Metropolis coupled Markov chain Monte Carlo under the GTR + G + I model. For the matrix, two million generations of two independent runs were performed with four chains and sampling trees every 100 generations. The burn-in period was identified graphically by tracking the likelihood at each generation to determine whether they reached a plateau. Twenty-five percent of the saved trees were removed, and the remaining trees were used to calculate Bayesian posterior probabilities (BPPs).

3 Results

3.1 Descriptions of *Alexandrium* species identified

Eleven species (morphospecies) of *Alexandrium* were identified and are described and illustrated here. Morphological and morphometric characters and toxicity of the species found are given in Table 1, whereas the distribution of the species in this work is discussed below.

3.1.1 *Alexandrium affine* (Inoue et Fukuyo) Balech (Figures 2–8)

References: Balech 1995, 55, pl. XIII, figs 20–36; Usup et al. 2002, 267, figs 2 A–D; Gu et al. 2013, 72, figs 2 A–D; Lassus et al. 2016, 42, pl. 2 A–E; Kim et al. 2017, 428, figs 1 A–L.

Synonyms = *Protogonyaulax affine* Inouye et Fukuyo

Chain-forming species, with chains of 3–8 cells observed (Figures 2 and 3). Individual cells of medium-size, slightly longer than wide (Figures 2 and 3). The epitheca is domed to conical, cingulum excavated and the hypotheca is concave (Figure 4). The first apical plate (1') is rhomboidal, in contact with Po, and with a small ventral pore on its right margin (Figures 5 and 6). The apical complex pore is conspicuous, with a large connecting pore and a relatively small foramen, both aligned in the same axis (Figures 6 and

7). The posterior sulcal plate (Sp) has a small pore and the two anterior ends are projected (Figure 8).

Measurements: 30–43 µm length (L), 33–43 µm width (W) (Table 1).

Toxicity: this is a species that produces saxitoxins at very low concentrations, so they do not represent a risk of poisoning (Kim et al. 2023).

Distribution: in the southern part of the Gulf of California.

3.1.2 *Alexandrium gaarderae* Nguyen-Ngoc et Larsen (Figures 9–11)

References: Nguyen and Larsen 2004, 90, pl. 6, figs 1–8; Menezes et al. 2018, 4.

Synonyms = *Goniodoma concavum* Gaarder, *Gonyaulax concava* (Gaarder) Balech, *Alexandrium concavum* (Gaarder) Balech.

Reference: Balech 1995, 60, pl. XVII, figs 24–29.

A very characteristic species with solitary and large cells, pentagonal in shape (Figure 9). Epitheca irregularly conical with somewhat sinuous margins, hypotheca more rounded, flattened at the posterior margin, wide and excavated cingulum, and wide and deep sulcus (Figures 9 and 10). Apical pore (Po) large and conspicuous (Figure 11).

Measurements: 58–68 µm L, 48–55 µm W (Table 1).

Toxicity: this species is not considered to be toxic (Long et al. 2021).

Distribution: in the Gulf of Tehuantepec.

3.1.3 *Alexandrium globosum* Nguyen-Ngoc et Larsen (Figures 12–19)

References: Nguyen and Larsen 2004, 93, pl. 7, figs 1–8; Alvarado et al. 2015, 3, figs 2 a–c.

Solitary cells, ovoid, slightly depressed longitudinally, epitheca and hypotheca about the same proportion (Figure 12), wide and excavated cingulum (Figures 13 and 14), smooth theca (Figures 14–17) with scattered pores; the nucleus is central, to the left side (Figure 12). The first apical plate (1') is not in connection with Po, is asymmetrically pentagonal and has a small ventral pore between this plate and 4' (Figures 13 and 15), whereas 2' and 4' are large plates, and plate 6'' is rhomboid (Figure 15). Po is elongate and more triangular (Figure 17). The posterior sulcal plate (Sp) is oblique pentagonal, longer than wide, with no apparent pore (Figure 18). Some particular plates of the epitheca, cingulum and sulcus were also observed (Figure 19).

Measurements: 35–42 µm L, 40–45 µm W (Table 1).

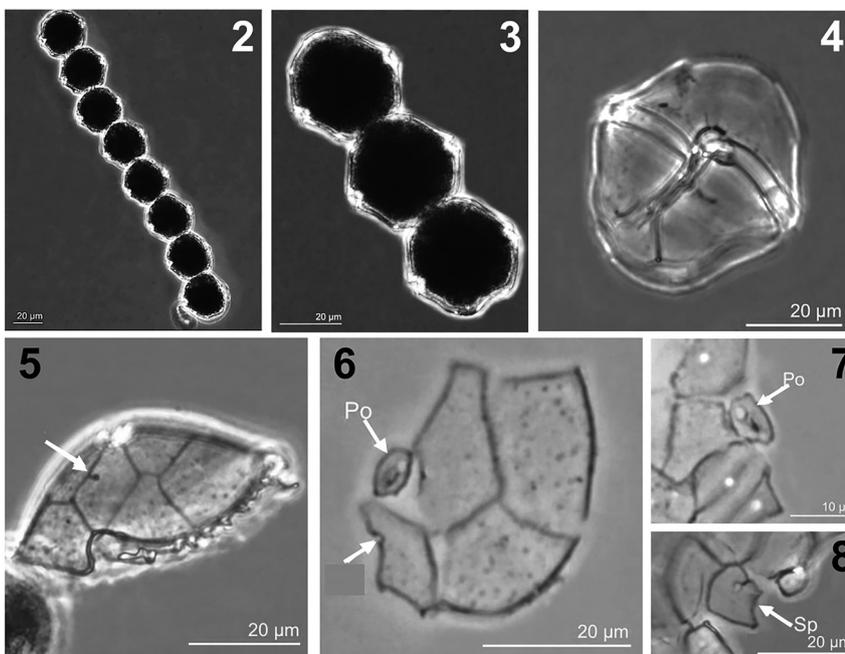
Toxicity: this species is not considered to be toxic (Long et al. 2021).

Distribution: in the Gulf of Tehuantepec.

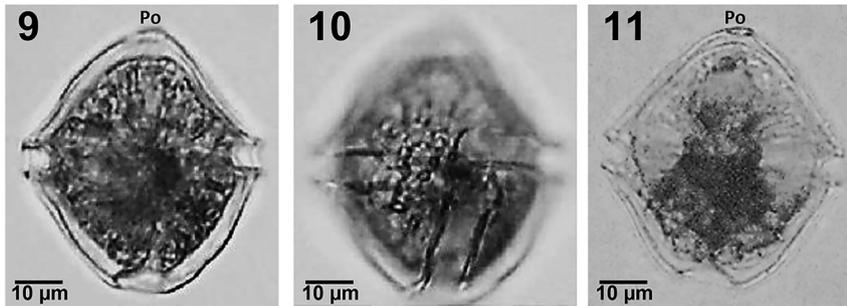
Table 1: Comparative morphological and morphometric characteristics of the *Alexandrium* species described and illustrated in this work.

Species	Shape	Size (µm)	Solitary/chain	Known toxicity	Plates description	Po
<i>A. affine</i>	Rounded to oblong	30–43 L 33–43 W	Chains of 3–8 cells	Potentially toxic (PSP)	1' rhomboidal, in contact with Po. Sa nearly quadrangular, with pore	Small foramen, large connecting pore
<i>A. gaarderae</i>	Pentagonal	58–68 L 48–55 W	Solitary	Not toxic	1' rhomboidal, connected with Po, small pore	Large and conspicuous
<i>A. globosum</i>	Ovoid, slightly depressed longitudinally	35–42 L 40–45 W	Solitary	Not toxic	1' pentagonal, not in connection with Po, with pore. 6'' is rhomboid	Narrow and elongate comma
<i>A. leei</i>	Ovoid to spherical	50–58 L 49–55 W	Solitary	Potentially ichthyotoxic	1' rhomboidal, with pore. 6'' small and pentagonal	Large comma and no visible connecting pore
<i>A. margalefii</i>	Subspherical	26–36 L 26–35 W	Solitary	Not toxic	1' quadrilateral, not connected with Po. Sp with oblique anterior border	Large comma and no visible connecting pore
<i>A. minutum</i>	Oval to spherical	22–26 L 20–24 W	Solitary	Potentially toxic (PSP)	1' rhomboidal, connected with Po, small pore	With a comma shape
<i>A. monilatum</i>	Cells strongly depressed, longer than wide.	38–46 L 45–63 W	Medium chains (8 cells per chain)	Potentially toxic (goniodomins)	1' rhomboid, not connected with Po. Sp rhomboid, with large pore	Evident apical pore. Large structure, comma-shaped
<i>A. pseudogonyaulax</i>	Ovoid to pentagonal, slightly depressed longitudinally	35–42 L 40–45 W	Solitary	Potentially toxic (goniodomins)	1' pentagonal, not connected with Po, strong posterior notch, large pore. 6'' quadrangular	Elongate, classical comma shape
<i>A. tamarensis</i>	Irregularly pentagonal	26–35 L 28–36 W	Solitary, also in couples	Potentially toxic (PSP)	1' rhomboid, connected with Po, ventral pore. 6'' small, pentagonal. Sp wide, pentagonal, with pore	Elongate, more rectangular and comma-shaped
<i>A. tamiyavanichii</i>	Nearly spherical. Non-pronounced shoulders	35–42 L 40–45 W	Chains up to 64 cells	Toxic (PSP)	1' rhomboid, in connection with Po, with ventral pore	Large, comma-shaped, connecting pore
<i>A. tropicale</i>	Rounded, longer than wide. Non-pronounced shoulders	28–38 L 22–33 W	Solitary, chain with 2 cells	Not toxic	1' rhomboid, little connection with Po, with ventral pore. Sp irregular with no pore	With evident foramen

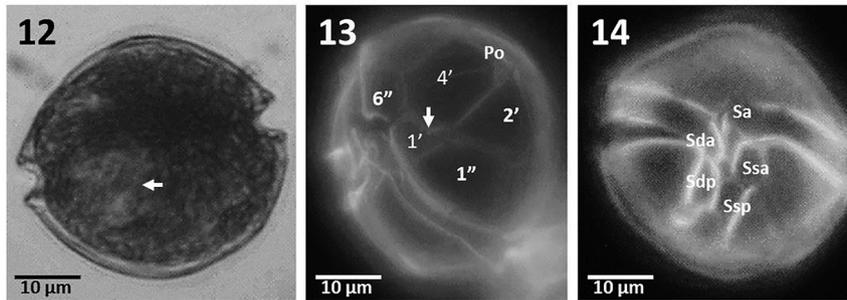
L, length; W, width; PSP, paralytic shellfish poisoning.



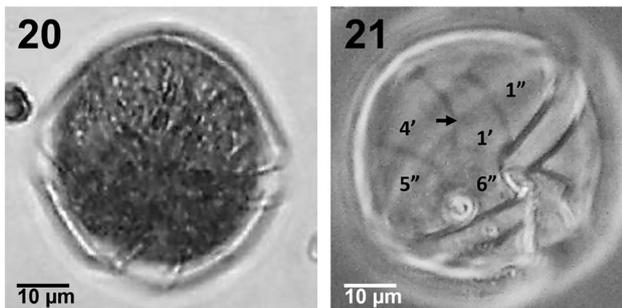
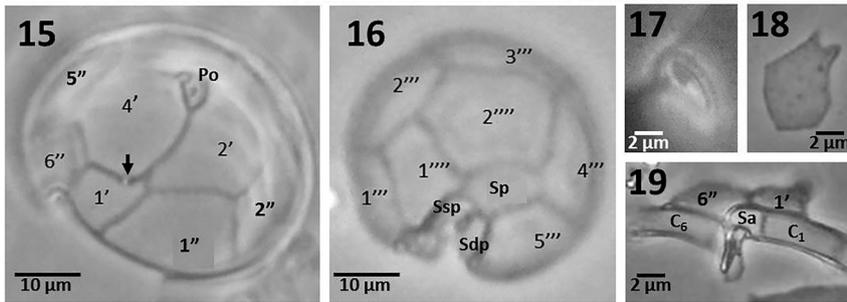
Figures 2–8: *Alexandrium affine*, LM. (2, 3) Chains of 8 and 3 cells, respectively. (4) An empty cell showing only the theca in ventral view. (5) Epitheca in ventral view showing the ventral pore (arrow) in the first apical plate (1'). (6) Epitheca with Po and 1' showing the ventral pore (arrow). (7, 8) Po and posterior sulcal plate (Sp) (with a connecting pore), respectively.



Figures 9–11: *Alexandrium garderae*, LM. (9, 10) Two different focal planes of a solitary cell in ventral view, showing the cell outline, cingulum and sulcus. (11) A cell in dorsal view.



Figures 12–19: *Alexandrium globosum*, LM. (12) Cell outline, with the central nucleus arrowed. (13, 14) Two different cells in ventro-lateral and ventral views, respectively, showing some plates of the epitheca and the sulcus. (15) Epitheca with plate tabulation, arrow indicates the location of the ventral pore in the first apical plate (1'). (16) Hypotheca showing plate tabulation. (17) Po plate. (18) Posterior sulcal plate (Sp). (19) Detail of some precingular, cingular and sulcal plates.



Figures 20–21: *Alexandrium leei*, LM. (20) Recently fixed cell in ventral view. (21) An empty cell in ventral view showing plate tabulation, arrow indicates the ventral pore in the first apical plate (1').

3.1.4 *Alexandrium leei* Balech (Figures 20 and 21)

References: Balech 1995, 35, pl. IV, figs 20–28, pl. V, figs 1–13; Usup et al. 2002, 268, figs 3 A–D; Nguyen and Larsen 2004, 97, pl. 10, figs 1–7; Tang et al. 2007, 545, figs 1 a–I; Gu et al. 2013, 75, figs 3 F–M; Lassus et al. 2016, 47, pl. 6 A–D.

Solitary and relatively large cells with a somewhat ovoid to spherical shape, slightly asymmetric. Epitheca conical convex, slightly shorter than epitheca, with non-prominent apical pore (Figure 20). Wide cingulum and sulcus (Figure 21). Chloroplasts radiating from the center (Figure 20). First apical plate (1') rhomboidal, fairly wide and elongate, with a small ventral pore (Figure 21), sixth precingular plate (6'') relatively small and pentagonal (Figure 21).

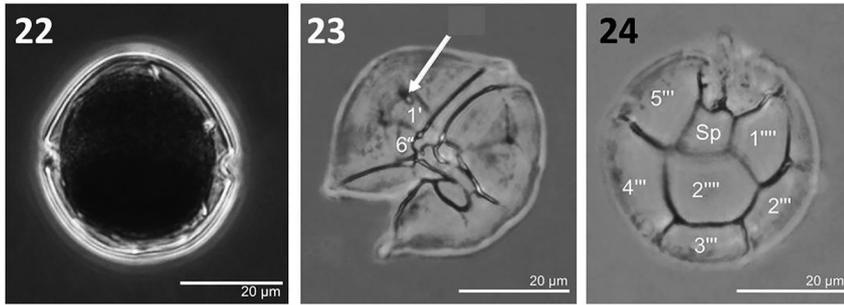
Measurements: 50–58 µm L, 49–55 µm W (Table 1).

Toxicity: this species is considered to be potentially ichthyotoxic (Tang et al. 2007).

Distribution: in the Gulf of Tehuantepec.

3.1.5 *Alexandrium margalefii* Balech (Figures 22–24)

References: Hallegraeff et al. 1991, 580, figs 10–16; Balech 1994, 220, figs 12–15; Balech 1995, 78, pl. XVI, figs 19–32; Hallegraeff et al. 2010, 186, figs 4.18F, 4.19D.



Figures 22–24: *Alexandrium margalefii*, LM. (22) General outline of a cell. (23) An empty cell in ventral view showing 1' and 6'' and the ventral pore (arrow) in the first apical plate (1'). (24) Hypotheca with plate tabulation.

Solitary cells of medium-size, subspherical shaped, longer than wide (Figure 22). The epitheca is convex (Figure 23), wide and excavated cingulum, and the hypotheca is convex (Figure 24). The first apical plate (1') is not connected with Po, is quadrilateral and has a small ventral pore on its upper margin (Figure 23). The posterior sulcal plate (Sp) has an oblique anterior border.

Measurements: 26–36 μm L, 26–35 μm W (Table 1).

Toxicity: this species is not considered to be toxic (Long et al. 2021).

Distribution: in the middle of the Gulf of California.

3.1.6 *Alexandrium minutum* Halim (Figures 25–27)

References: Halim 1960, 101, figs I a-j; Balech 1989, 207, figs 1–26; Hallegraeff et al. 1991, 577, figs 2–9; Balech 1995, 24, pl. I, figs 1–50; Usup et al. 2002, 269, figs 4 A–D; Nguyen and Larsen 2004, 99, pl. 11, figs 1–6; Lim et al. 2007, 17, figs 3 A–K; Fabro et al. 2017, p. 1211, figs 1 A–H.

Solitary and small cells, oval to spherical in shape (Figures 25 and 26). Epitheca rounded, slightly shorter than epitheca, Po non-prominent (Figure 25). Cingulum wide and excavated, sulcus narrow (Figure 25). First apical plate (1') rhomboidal, in contact with Po, and with a small ventral pore on its right margin, 2' larger than the other apical plates (Figure 27). Po with a comma shape (Figure 27).

Measurements: 22–26 μm L, 20–24 μm W (Table 1).

Toxicity: this species is toxic and associated to saxitoxin production (Zingone et al. 2021).

Distribution: in the Gulf of Tehuantepec.

3.1.7 *Alexandrium monilatum* (Howell) Balech (Figures 28–37)

References: Balech 1995, 67, pl. XV, figs 1–16; Faust and Guldledge 2002, pl. 4 fig. 1; Taylor et al. 2003, 406, fig. 15.16; Lassus et al. 2016, 50; Hernández-Becerril et al. 2021, 70, 71.

Chain-forming species (Figures 28–30), with medium to long chains (up to 8 cells per chain), cells strongly depressed, longer than wide, with an evident apical pore (Figures 31 and 32). Convex epitheca, wide and excavated cingulum and concave hypotheca (Figure 31). The first apical plate (1') is not in connection with Po (Figures 32–34), which is a large structure, with a comma-shape and a large connecting pore (Figures 34 and 36). The posterior sulcal plate (Sp) is rhomboid and has a large pore (Figures 35 and 37).

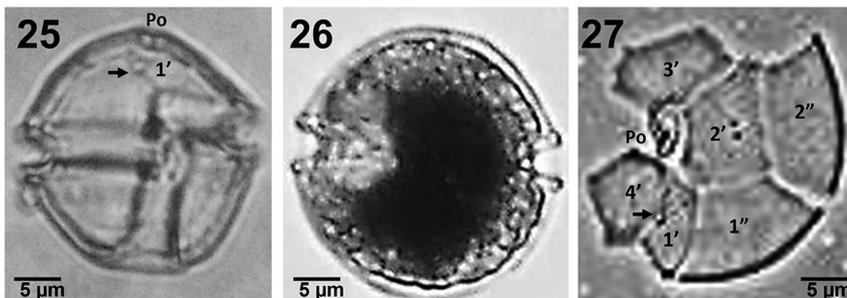
Measurements: 38–46 μm L, 45–63 μm W (Table 1).

Toxicity: this species produces gonoidomin A, toxin that causes hemolysis to erythrocytes in mammalian species, including humans (May et al. 2010).

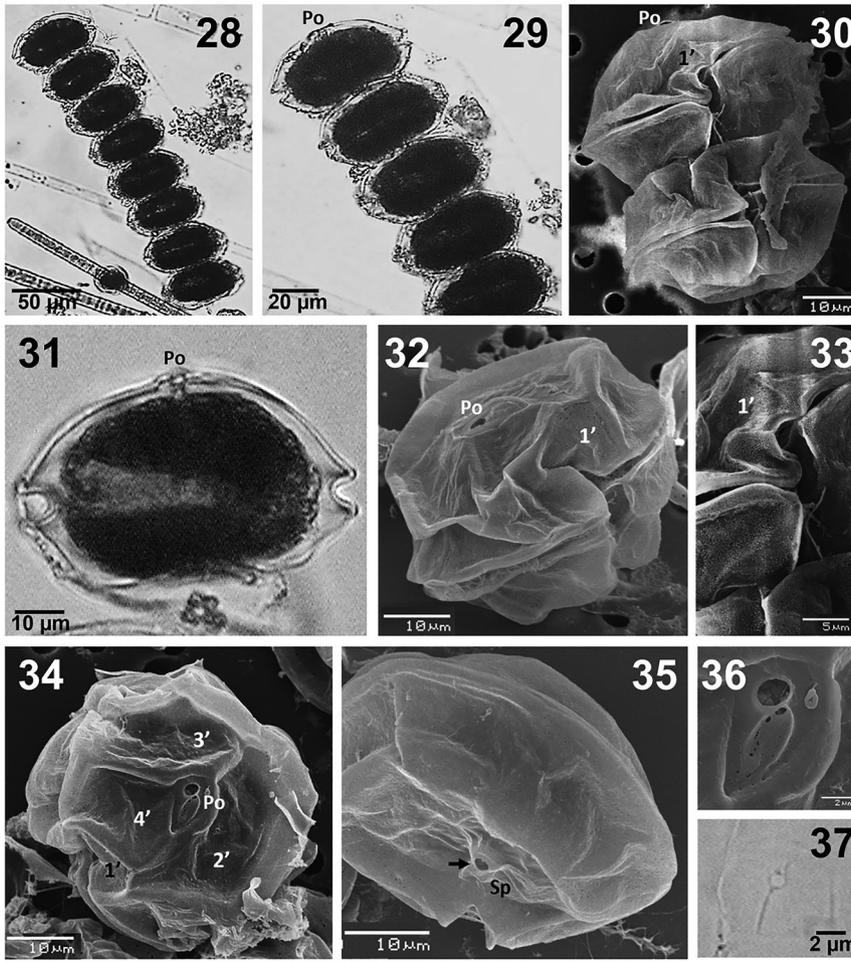
Distribution: in the Gulf of Tehuantepec.

3.1.8 *Alexandrium pseudogonyaulax* (Biecheler) Horiguchi ex Yuki et Fukuyo (Figures 38–43)

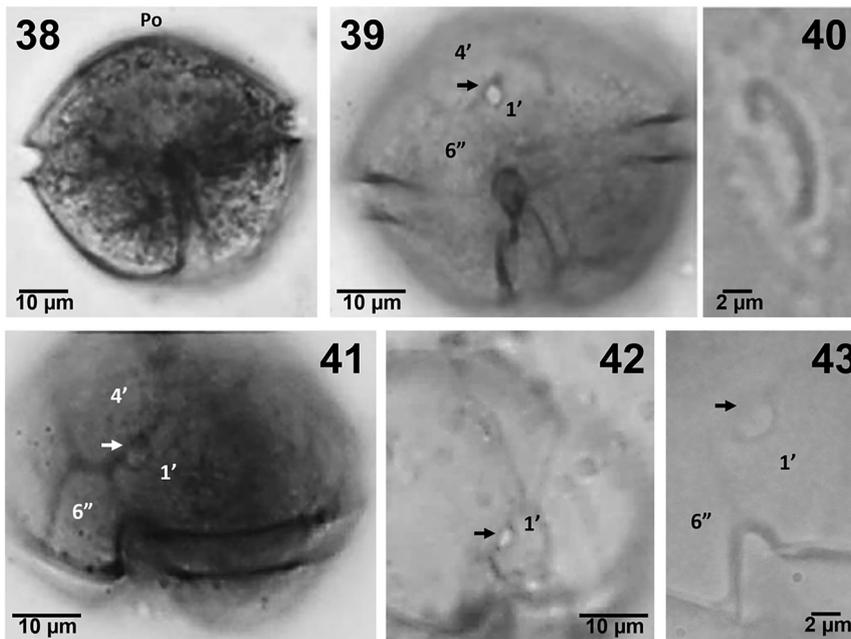
References: Balech 1995, 73, pl. XVI, figs 1–18; Nguyen and Larsen 2004, 102, pl. 13, figs 1–3; Gu et al. 2013, 75, figs 4 A–D;



Figures 25–27: *Alexandrium minutum*, LM. (25) Empty cell in ventral view with the first apical plate (1'), ventral pore (arrow) and Po. (26) Outline of a cell. (27) Epitheca with plate tabulation and ventral pore (arrow).



Figures 28–37: *Alexandrium monilatum*, LM and SEM. (28, 29) A long chain (8 cells) and detail of that chain, respectively, LM. (30) Pair of cells in ventral view, SEM. (31) General outline of a cell, LM. (32) Cell in ventral view showing Po and 1', SEM. (33) Detail of the cingulum and sulcus, showing the first apical plate (1'), SEM. (34) Apical view with plate tabulation, SEM. (35) Hypotheca showing the posterior sulcal plate (Sp) and its connecting pore (arrow), SEM. (36) Po plate with the conjunction pore and foramen, SEM. (37) Posterior sulcal plate showing the connection pore, LM.



Figures 38–43: *Alexandrium pseudogonyaulax*, LM. (38) Cell in ventral view. (39) Empty cell in ventral view, showing 1', 4', 6'' and the large ventral pore (arrow). (40) Detail of Po with the foramen. (41–43) Epitheca in ventral view showing 1', 4', 6'', and ventral pore (arrow).

Lassus et al. 2016, 53, pl. 9 A–D; Hernández-Becerril et al. 2021, 70, 71.

Solitary cells of medium to relatively large size, ovoid to pentagonal and slightly depressed longitudinally (Figure 38), epitheca slightly domed and shorter than hypotheca (Figures 38 and 39), which has an antapical concavity, cingulum wide and excavated (Figure 41), and sulcus wide and deep (Figures 38 and 39). First apical plate (1') is not connected with Po (Figure 39) and has a pentagonal shape (Figures 39, 41, and 42) and a strong posterior indentation (notch). A large ventral pore appears between this plate and 4' (Figures 39, 41–43), whereas 6'' is quadrangular. Po is elongate with a classical comma shape (Figure 40).

Measurements: 35–42 µm L, 40–45 µm W (Table 1).

Toxicity: this species produces goniodomins A and B (Krock et al. 2018).

Distribution: in the Gulf of Tehuantepec.

3.1.9 *Alexandrium tamarense* (Lebour) Balech (Figures 44–52)

References: Hallegraeff et al. 1991, 582, figs 33–42; Balech 1995, 38, pl. VI, figs 1–40, pl. VII, figs 1–9; Usup et al. 2002, 270,

figs 5 A–E; Nguyen and Larsen 2004, 104, pl. 14, figs 1–5; Gu et al. 2013, 75, figs 4 E–I; Fabro et al. 2017, p. 1210, figs 1 A–M.

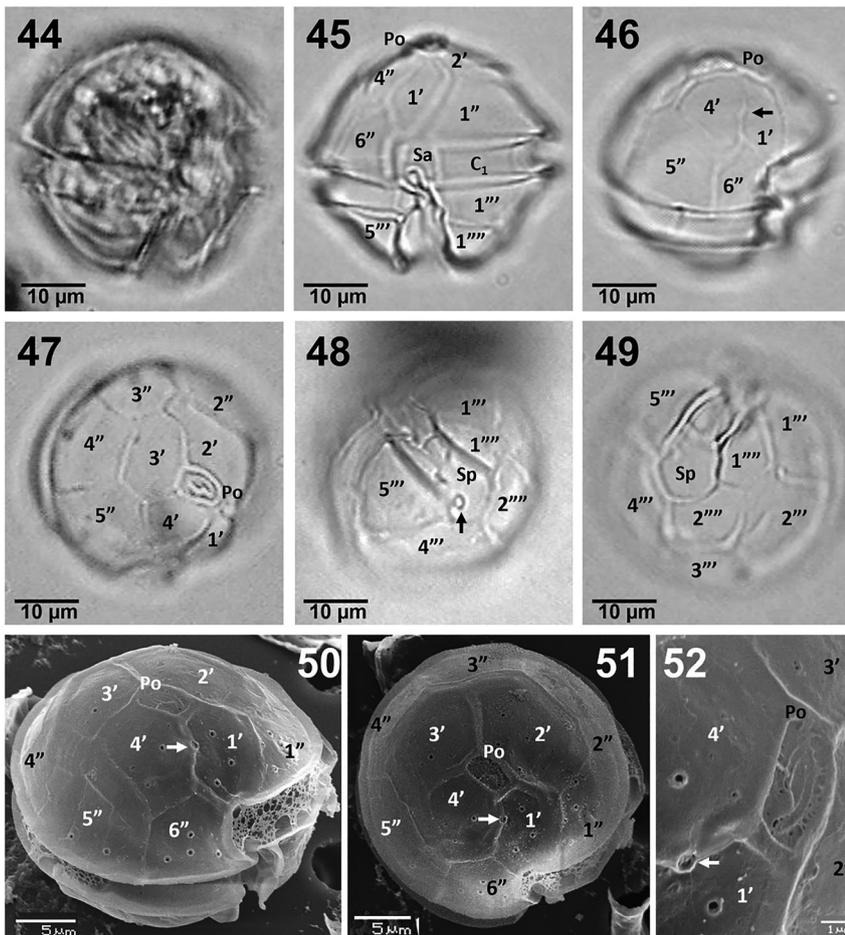
Cells usually solitary, but cells in couples were also found. Cells relatively small, irregularly pentagonal in shape, with the epitheca slightly larger than the hypotheca (Figure 44), with not pronounced shoulders (Figure 45), wide and excavated cingulum (Figure 46), and central nucleus (Figure 44). The first apical plate (1') is connected with Po (Figures 45, 47, 50–52), widely rhomboid in shape (Figures 45 and 51), with a ventral pore (Figures 50–52), whereas 2', 3' and 4' are larger plates (Figure 51), and plate 6'' is relatively small and pentagonal (Figures 46 and 50). Po is elongate, more rectangular and comma-shaped, with no pore (Figures 50–52). The posterior sulcal plate (Sp) is wide and pentagonal, with a conspicuous pore (Figures 48 and 49).

Measurements: 26–35 µm L, 28–36 µm W (Table 1).

Toxicity: this species may produce toxins associated to PSP (Lugliè et al. 2017).

Distribution: in the southern Mexican Pacific, including the Gulf of Tehuantepec.

Remarks: All morphological characters examined led us to identify this species as *A. tamarense*. However, the fact that species of the “*A. tamarense* complex” cannot be



Figures 44–52: *Alexandrium tamarense*, LM and SEM. (44) Cell in ventral view, LM. (45) Empty cell in ventral view with plate tabulation, LM. (46) Detail of the epitheca with some plates and ventral pore (arrow), LM. (47) Apical view, with plate tabulation, LM. (48, 49) Hypotheca with plate tabulation, including the posterior sulcal plate (Sp) and its pore (arrow), LM. (50) Epitheca with plate tabulation and the ventral pore (arrow), SEM. (51) Epitheca with plate tabulation, SEM. (52) Po with some plates surrounding it, and the ventral pore (arrow), SEM.

distinguished based on morphology alone and that molecular assays are required for reliable identification (Litaker et al. 2018), puts us in a difficult situation, and it is recommended to consider *Alexandrium pacificum* Litaker as the prevalent species of that complex in the Pacific Ocean. See Discussion.

3.1.10 *Alexandrium tamiyavanichii* Balech (Figures 53–66)

References: Balech 1994, 217, figs 1–6; Balech 1995, 57, pl. XIII, figs 1–19; Usup et al. 2002, 271, figs 6 A–H; Nguyen and Larsen 2004, 106, pl. 15, figs 1–5; Lim et al. 2007, 15, figs 2 A–J; Menezes et al. 2018, 5, figs 2 G–K; Hernández-Becerril et al. 2021, 72, 73.

This species forms medium to large chains with more than 64 cells (Figure 53). Cells are nearly spherical (Figure 53), with non-pronounced shoulders (Figure 54), epitheca and hypotheca about the same proportion (Figure 55), wide and excavated cingulum (Figures 56 and 59), and not prominent apical pore (Figures 62 and 63). Reduced cingular lists and more developed sulcal lists, with their margins reaching until the antapical end, resembling

short spines (Figure 59). Plate 1' is in connection with Po, and is widely rhomboid, with a ventral pore (Figure 58). Po is large, comma-shaped, with a large connecting pore (Figures 62 and 63). The anterior sulcal plate (Sa) is composed by two parts, the most anterior is trapezoid and the posterior similar to others of the genus (Figures 55, 57, 65, and 66), with a conspicuous notch, whereas the posterior sulcal plate (Sp) is irregularly hexagonal and has a large attachment pore with a fine furrow running from its right margin (Figures 60, 61, and 64).

Measurements: 35–42 μm L, 40–45 μm W (Table 1).

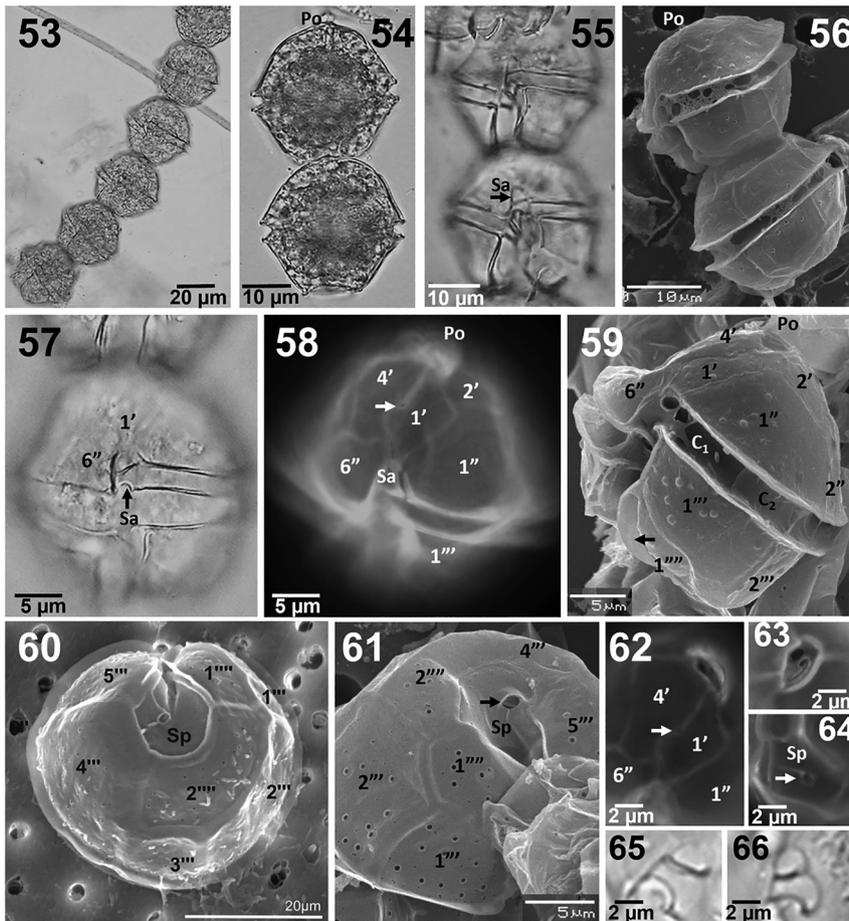
Toxicity: this species produces toxins associated to PSP (Xu et al. 2021).

Distribution: in the southern Gulf of California and the central Mexican Pacific.

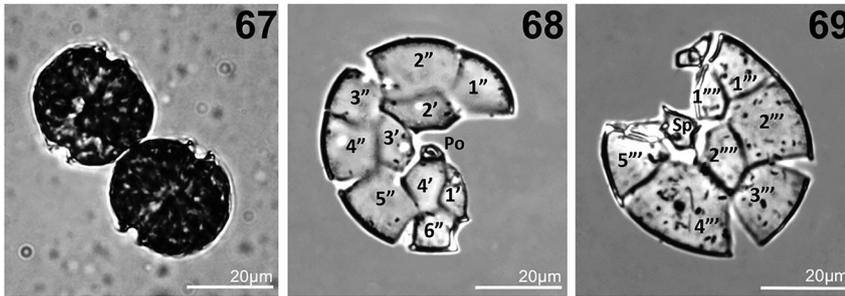
3.1.11 *Alexandrium tropicale* Balech (Figures 67–69)

Reference: Balech 1995, 46, pl. IX, figs 11–27.

Species either solitary or cells paired. Cells are rounded, small, longer than wide, with non-pronounced shoulders, excavated cingulum, not prominent apical pore, and convex



Figures 53–66: *Alexandrium tamiyavanichii*, LM and SEM. (53) Chain of 6 cells, LM. (54) Detail of two cells with cellular content of a chain, LM. (55) Cells in ventral view showing the anterior sulcal plate (Sa), LM. (56) Two cells slightly twisted in a chain, SEM. (57) Cell in ventral view showing plates of the ventral area, LM. (58) Empty cell in ventral view showing plate tabulation, the ventral pore is arrowed, LM. (59) Epitheca in ventro-lateral view with plate tabulation, the left sulcal list is arrowed, SEM. (60, 61) Hypotheca with plate tabulation and pore at the posterior sulcal plate (Sp), SEM. (62, 63) Po and plates around it; the ventral pore is arrowed, LM. (64) Posterior sulcal plate (Sp) with pore (arrow), LM. (65, 66) Anterior sulcal plate, LM.



Figures 67–69: *Alexandrium tropicale*, LM. (67) Pair of cells. (68, 69) Epitheca and hypotheca with plate tabulation.

hypotheca (Figure 67). Plate 1' has a very little connection with Po, and is rhomboid, larger than wide, with a ventral pore (Figure 68). Po has a large foramen (Figure 68). The posterior sulcal plate (Sp) is irregular and has no pore (Figure 69).

Measurements: 28–38 μm L, 22–33 μm W (Table 1).

Toxicity: this species is not considered to be toxic (Long et al. 2021).

Distribution: in the central Mexican Pacific.

3.2 Molecular phylogeny of Mexican Pacific *Alexandrium* species

Only sequences obtained from strains housed in CODIMAR were considered for the phylogenetic analysis: *A. affine* (AAMV-1, 2, 3, 4, from Bahía Mazatlán), *A. margalefii* (AMCQ-1 from Bahía Concepción), *A. tamiyavanichii* (AYPV-1, from Bahía de La Paz) and *A. tropicale* (ATUV-1, from Jalisco) (Figure 1, Table 2). Seven sequences were obtained for the D1-D2 LSU rDNA, and six sequences for the ITS rDNA

(Table 2). Both topologies of the trees following the Maximum likelihood and Bayesian inference were generally consistent, thus, only the maximum likelihood trees are shown (Figures 70 and 71).

The trees showed that *A. affine*, *A. margalefii*, *A. tropicale* and *A. tamiyavanichii* strains from the Mexican tropical Pacific clustered together with the corresponding species reported from various marine regions worldwide, retrieved from the NCBI, with high and moderate values of bootstrap and Bayesian posterior probabilities for LSU (Figure 70) and for *A. affine*, *A. margalefii* and *A. tropicale* for ITS (Figure 71). In the LSU rDNA tree, *A. tamiyavanichii* clustered close to the presumably closely related species *A. cohorticula* (Figure 70), whereas the ITS tree showed *A. tropicale* close to *A. fraterculus*, but with moderate support values (Figure 71).

The Jukes-Cantor genetic distance matrix calculated based on LSU and ITS rDNA showed the values of intraspecific distance of *A. affine*, *A. margalefii*, *A. tamiyavanichii* and *A. tropicale* compared with strains of these species from other regions of the world, and with other species of

Table 2: General information and GenBank accession numbers for *Alexandrium* strains from the Mexican Pacific studied in this work.

Species	CODIMAR code	GenBank accession number		Collection date	Isolation date	Culture media and incubation temperature	Locality	Coordinates
		LSU rDNA (D1/D2)	ITS rDNA (ITS1/5.8S/ITS2)					
<i>A. affine</i>	AAMV-1	OQ676503	OQ676843	16/06/13	17/06/13	f/2 + Se, 20 °C	BAMAZ	23°09'48.30"N 106°26'15.90"W
	AAMV-2	OQ676504	OQ676844	24/02/20	03/03/20	GSe, 20 °C		23°11'2.7"N 106°25'34.5"W
	AAMV-3	OQ676505	OQ676845	24/02/20	03/03/20	GSe, 20 °C		23°11'2.7"N 106°25'34.5"W
	AAMV-4	OQ676506	OQ676846	24/02/20	03/03/20	GSe, 20 °C		23°11'2.7"N 106°25'34.5"W
<i>A. margalefii</i>	AMCQ-1	OQ676523	OQ676882	15/04/00	17/04/00	GSe, 20 °C	BACO	26°35'46.30"N 111°44'20.66"W
<i>A. tamiyavanichii</i>	AYPV-1	OQ676527	–	09/2003	09/2003	GSe, 20 °C	BAPAZ	24°17'12.66"N 110°20'31.92"W
<i>A. tropicale</i>	ATUV-1	OQ676586	OQ676883	20/08/18	21/08/18	GSe, 25 °C	BACUA	19°13'46.00" N 104°43'51.00"W

BAMAZ, Bahía de Mazatlán, Sinaloa; BACO, Bahía Concepción, Baja California Sur; BAPAZ, Bahía de La Paz, Baja California Sur; BACUA, Bahía de Cuastecomates, Jalisco; –, no information.

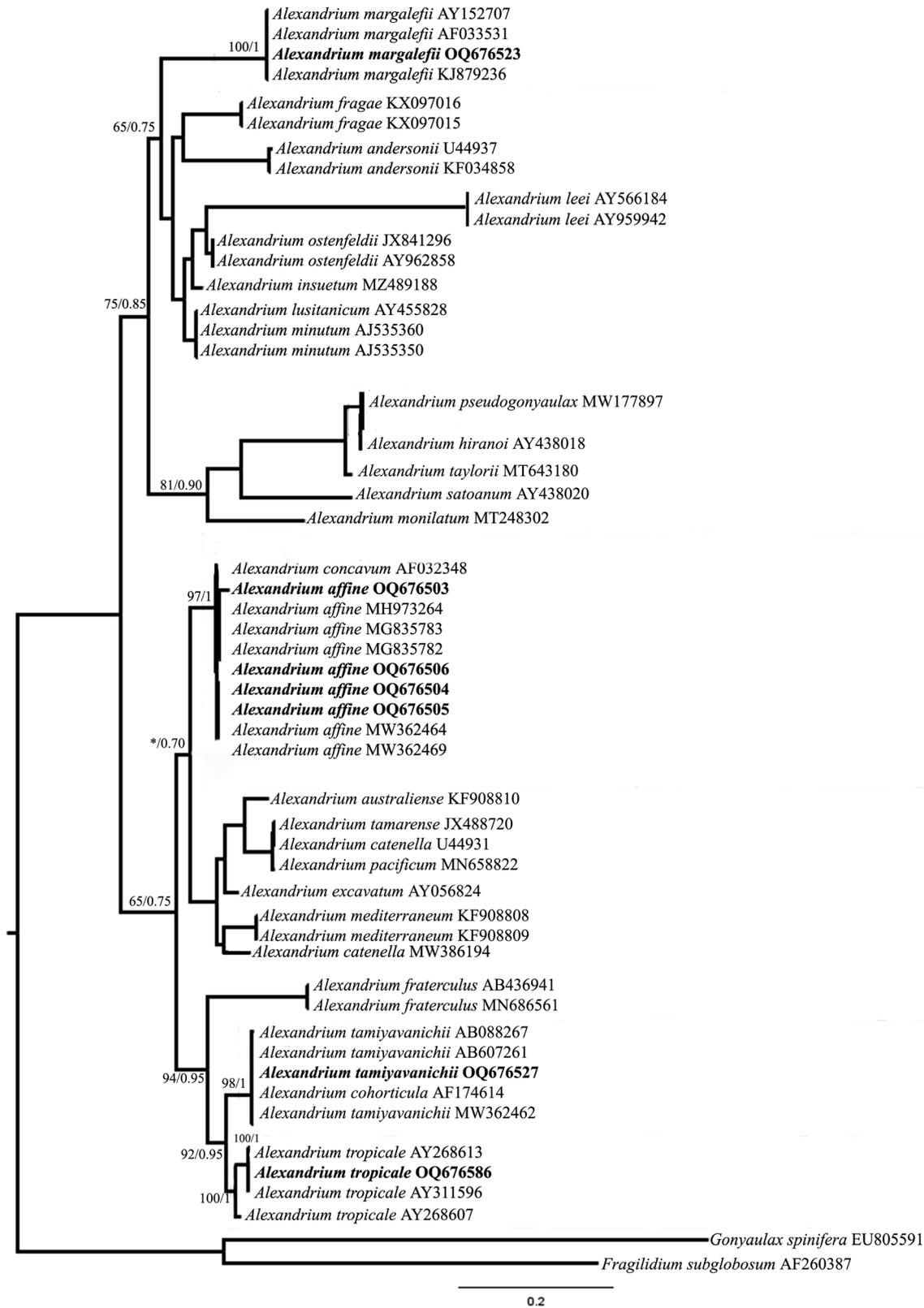


Figure 70: Maximum-likelihood (ML) tree inferred from D1-D2 LSU rDNA sequences of *Alexandrium*. ML bootstrap and Bayesian posterior probabilities values are shown at branches. Bold letters indicate newly generated sequences in this study. Bootstrap values <50 and posterior probabilities <0.50 are not shown.

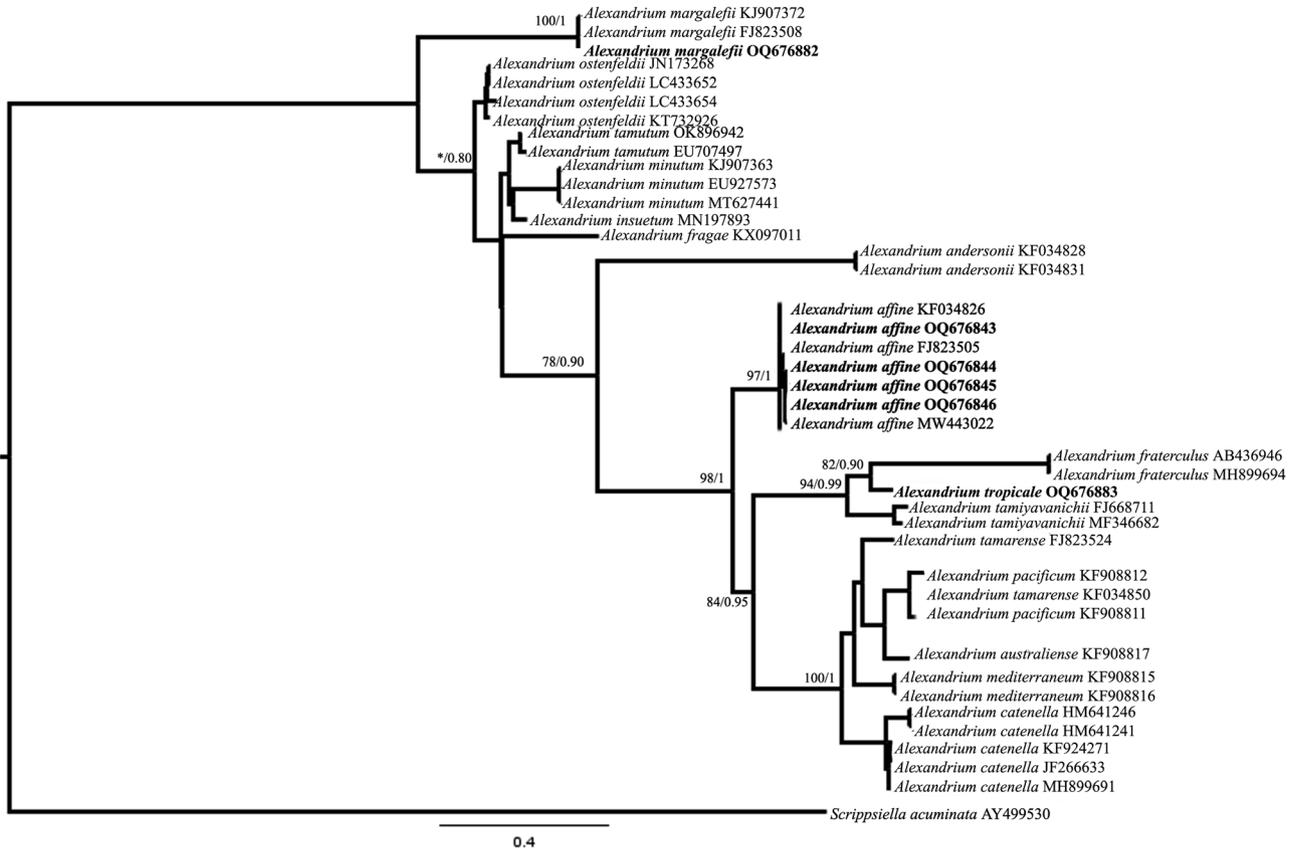


Figure 71: Maximum-likelihood (ML) tree inferred from ITS sequences of *Alexandrium*. ML bootstrap and Bayesian posterior probabilities values are shown at branches. Bold letters indicate newly generated sequences in this study. Bootstrap values <50 and posterior probabilities <0.50 are not shown.

Alexandrium found in previous studies (Table 3). These responses were concordant with values in the literature.

4 Discussion

4.1 Diversity

Identifying species of *Alexandrium* provides an important and relevant basis to continuing to monitor species and for

detailed studies of distribution and ecology. We now have many references, reports and lists of species of the genus (some recording blooms and effects on human health) from the Mexican Pacific, although most references lack proper support data, illustrations, descriptions and a reliable basis for assessing their importance and impact (Hernández-Becerril et al. 2007; Torres-Ariño et al. 2019).

In addition to the traditional morphological characters used to identify species: growth form, cell shape and size, shape and position of the pore plate (Po), shape and position

Table 3: Jukes-Cantor genetic distance matrix calculated based on LSU and ITS rDNA sequences for *Alexandrium affine*, *A. margalefii*, *A. tamiyavanichii* and *A. tropicale* in this study compared with strains of these species from other regions of the world, and with other species of *Alexandrium* found in the literature.

Mexican strains	Strains from other regions of the world								Other species of <i>Alexandrium</i>	
	<i>A. affine</i>		<i>A. margalefii</i>		<i>A. tamiyavanichii</i>		<i>A. tropicale</i>		LSU	ITS
	LSU	ITS	LSU	ITS	LSU	ITS	LSU	ITS		
<i>A. affine</i>	0.000–0.004	0.000–0.010	–	–	–	–	–	–	0.070–0.30	0.239–0.470
<i>A. margalefii</i>	–	–	0.000–0.002	0.0002	–	–	–	–	0.131–0.310	0.269–0.543
<i>A. tamiyavanichii</i>	–	–	–	–	0.000–0.002	–	–	–	0.115–0.307	–
<i>A. tropicale</i>	–	–	–	–	–	–	0.000–0.018	–	0.040–0.29	0.153–0.505

of pores in the Po, features of first apical plate (1'), shape and size of sixth precingular plate (6'), presence, position and size of ventral pore, shape of anterior and posterior sulcal plates (Sa, Sp) (Balech 1995; Steidinger and Tangen 1997), there are other more specific characters such as the shape of the third apical plate (3') and the second postcingular plate (2'''), presence of the posterior attachment pore (located at the posterior sulcal plate, Sp), and presence of sulcal lists, which aid recognition of species. Of course, there are many associated problems in species identification, especially if only LM is available or if fixed samples are damaged. Some species are very similar to others and particular methods such as addition of sodium hypochlorite to the samples to make the theca more visible for thecal dissections, and/or using calcofluor in combination with epifluorescence facilities should be used to observe those tiny and delicate morphological characters (e.g., pore plate, first apical plate, anterior and posterior sulcal plates, etc.). Relevant morphological, morphometric and toxicity information for the species dealt with in this study is provided in Table 1.

Only recently, some studies have confirmed the presence of species of the genus such as *Alexandrium compressum*, *A. fraterculus*, *A. margalefii*, *A. monilatum*, *A. ostenfeldii*, *A. tamarensense* and *A. tamiyavanichii* (Esqueda-Lara and Hernández-Becerril 2010; Hernández-Becerril et al. 2021; Licea et al. 1995; Meave del Castillo et al. 2012a; Morquecho-Escamilla et al. 2016). *Alexandrium fraterculus* illustrated by Meave del Castillo et al. (2012a) is a misidentification, and their specimens appear more likely to be *A. gaarderae*, as described and illustrated here.

In this paper, 11 species (morphospecies) were found and identified following morphological characters observed mostly by LM. New records are: *A. gaarderae*, *A. globosum* and *A. tropicale*, which had not been previously found nor listed (Hernández-Becerril et al. 2021; Okolodkov and Gárate-Lizárraga 2006; Torres-Ariño et al. 2019), although *A. globosum* has been previously detected on the Pacific coasts of Central America (Alvarado et al. 2015). The current paper also includes the first detailed morphological documentation of *A. pseudogonyaulax* from the study area. Seven species included in this study may be considered as harmful: *A. affine*, *A. leei*, *A. minutum*, *A. monilatum*, *A. pseudogonyaulax*, *A. tamarensense* and *A. tamiyavanichii* (Escalera 2023).

Molecular approaches have become powerful and decisive tools to study diversity, taxonomy, phylogeny, ecology and biogeography of problematic or enigmatic species assemblages, such as *Alexandrium* species. Combining these approaches with “traditional” microscopical studies is widely recommended when using morphological characters alone is insufficient. The molecular evidence shown here (the phylogenetic trees, Figures 70 and 71) from the 13

sequences obtained (7 of LSU rDNA and 6 of ITS rDNA), from 4 species (*A. affine*, *A. margalefii*, *A. tamiyavanichii* and *A. tropicale*) supports their identification based on morphological characters.

Additionally, metabarcoding (V9 region, SSU rDNA; Hernández-Becerril, unpublished data) information has indicated the unambiguous recognition of the following species: *A. affine*, *A. andersonii*, *A. fraterculus*, *A. hiranoi*, *A. leei*, *A. monilatum*, *A. ostenfeldii*, *A. tamiyavanichii*, and *A. taylorii*, whereas there are two ill-defined taxa: *A. ostenfeldii/minutum/tamutum/insuetum* and *A. pseudogonyaulax/A. hiranoi*. These results will be reported elsewhere.

Considering the results of this study as well as the literature, we can now list 17 *Alexandrium* species (mainly morphospecies, recognized by microscopy) found in the Mexican Pacific, with their synonyms for at least two species (Table 4).

4.2 Harmful blooms and distribution

During the course of this investigation, no harmful bloom caused by *Alexandrium* species was detected. However, heavy “red tides” were reported in Oaxaca’s coasts, in the current study area, caused by *Alexandrium* cf. *tamarensense*, a potentially toxic species, with densities reaching 56.6×10^6 cells L^{-1} , with negative tests of toxicity (Meave del Castillo et al. 2012b), and the presence, abundance and distribution of *A. tamiyavanichii*, another toxic species, was

Table 4: List of *Alexandrium* species identified (with their synonyms) in the Mexican Pacific.

<i>Alexandrium affine</i> (Inoue et Fukuyo) Balech ^a
<i>Alexandrium catenella</i> (Whedon et Kofoid) Balech [this includes <i>Alexandrium acatenella</i> (Whedon et Kofoid) Balech]
<i>Alexandrium cohorticula</i> (Balech) Balech
<i>Alexandrium compressum</i> (Fukuyo, Yoshida et Inoue) Balech
<i>Alexandrium fraterculus</i> (Balech) Balech
<i>Alexandrium gaarderae</i> Nguyen-Ngoc et Larsen ^b
<i>Alexandrium globosum</i> Nguyen-Ngoc et Larsen ^b
<i>Alexandrium kutnerae</i> (Balech) Balech
<i>Alexandrium leei</i> Balech ^a
<i>Alexandrium margalefii</i> Balech ^a
<i>Alexandrium minutum</i> Halim ^a
<i>Alexandrium monilatum</i> (Howell) Balech ^a
<i>Alexandrium ostenfeldii</i> (Paulsen) Balech et Tangen [this includes <i>Alexandrium peruvianum</i> (Balech et Mendiola) Balech et Tangen]
<i>Alexandrium pseudogonyaulax</i> (Biecheler) Horiguchi ex Yuki et Fukuyo ^a
<i>Alexandrium tamarensense</i> (Lebour) Balech ^a
<i>Alexandrium tamiyavanichii</i> Balech ^a
<i>Alexandrium tropicale</i> Balech ^b

^aIndicates species studied here; ^bindicates new records for the Mexican Pacific.

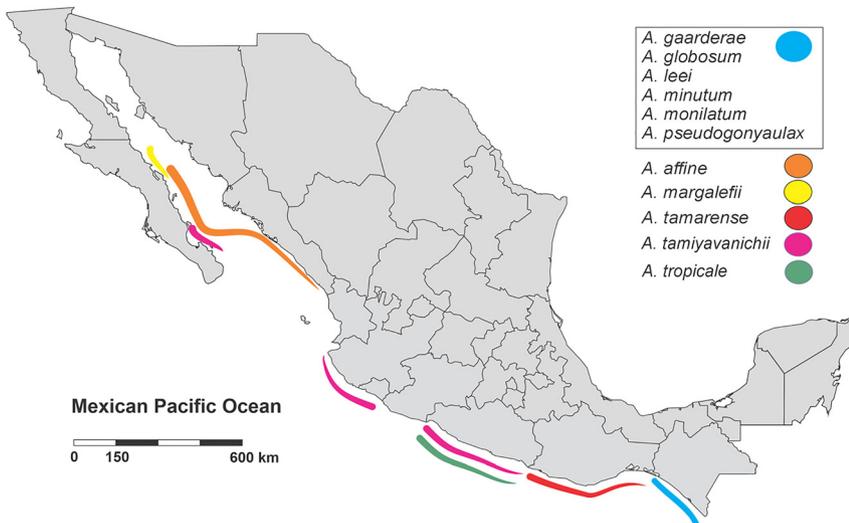


Figure 72: Distribution map of *Alexandrium* species from this study in the Mexican Pacific.

assessed in the central Mexican Pacific, following the quantitative PCR technique (Hernández-Becerril et al. 2018).

In areas close to the current study area, but to the south, the occurrence of *A. monilatum* was recorded, in low densities, in coasts of El Salvador, associated with sea turtle mortalities (Amaya et al. 2018), whereas harmful blooms of *A. monilatum* have been recorded in the Costa Rican Pacific (Vargas-Montero et al. 2008). The species *A. tamarensis* produced various HAB events in the Colombian Pacific, with high densities (up to 7.5×10^6 cells L^{-1}), which apparently produced no toxins or mortality (García-Hansen et al. 2004).

The recorded species in this study, *Alexandrium globosum*, *A. leei*, *A. monilatum*, *A. tamiyavanichii* and *A. tropicale* may be considered as more tropical (warm water) forms (Balech 1995), with a limited distribution toward northern areas in the Mexican Pacific, whereas the rest of the species studied here are more widely distributed: *A. affine*, *A. gaarderae*, *A. margalefii*, *A. minutum*, *A. pseudogonyaulax*, *A. tamarensis* (Figure 72).

4.3 Taxonomy

We have followed the “classic” morphological and taxonomic approach, considering that *Alexandrium* is a diverse but single genus, despite the proposals of splitting into four genera, including the resurrection of two genera, *Gessnarium* and *Protogonyaulax*, and the erection of a new one, *Episemicolon* (Gómez and Artigas 2019). Various reasons were provided in a recent paper by Mertens et al. (2020) to reject those proposals, based on the following points: (1) the proposed taxa were not based on monophyletic groups,

(2) the morphological characterizations, which “do not convincingly support taxa delimitations”, and (3) the lack of diagnostic traits (i.e., autapomorphies). These authors (Mertens et al. 2020) suggest that a “more phylogenetically accurate revision can and should wait until more complete evidence becomes available” and they call for “the need for integrative taxonomy”.

We already mentioned the difficult case of species identification based on morphology, as in *A. tamarensis*, described above, as part of the “*A. tamarensis* complex”. Litaker et al. (2018) wrote that these species “cannot be distinguished based on morphology” and that “molecular assays are required for reliable identification”. We also posed the possibility that our specimens assigned to *A. tamarensis*, can be considered as *A. pacificum*, as the prevalent species of that complex in the Pacific Ocean. We would need to establish cultures and obtain molecular sequences or apply the metabarcoding approach to define if we are right. For the moment, we maintain our identification as *A. tamarensis*, following the morphological and morphometric information available (Table 1).

The species *Alexandrium cohorticula* and *A. tamiyavanichii* are remarkably similar in morphology, in such a way that Balech (1995) mentioned their high similarity and questioned the separation of the two species in terms of morphology. Additionally, molecular sequences are identical in most molecular phylogenies (Anderson et al. 2012; Shikata et al. 2020), including this study (Figure 70). This evidence strongly suggests that the species should be considered conspecific, with the priority given to *A. cohorticula*, as it was described before *A. tamiyavanichii*. However, we consider it too preliminary to make a formal taxonomic proposal, as more investigations based on material from the original localities should be made.

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Research ethics: This research followed the procedures in accordance with national laws and complied with all the ethical standards.

Author contributions: DUHB contributed to conception, design, analysis of samples by light microscopy and scanning electron microscopy, taxonomic interpretation of the species of *Alexandrium* and writing. JGPV contributed to sampling, conceptual, writing and graphics design. RAR contributed to conception, design and execution of the experiment. EMB studied several field samples by light microscopy and identified various species. LM contributed to the molecular characterization (from DNA extraction to obtaining genetic sequences) and morphometrical identification of the CODIMAR strains. KEL studied field samples, identified some species and checked different drafts. SABC studied field samples and identified species. NQG analyzed molecular sequences and performed the phylogenetic trees.

Competing interests: The authors declare that they have no conflicts of interest regarding this article.

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