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POSGRADO EN CIENCIAS BIOLÓGICAS Y DE LA SALUD

TESIS

"Diversidad y relaciones filogenéticas de dinoflagelados atecados (Dinoflagellata) en Bahía de Acapulco, Guerrero, México"

QUE PARA OBTENER EL GRADO DE DOCTOR EN CIENCIAS BIOLÓGICAS Y DE LA SALUD

PRESENTA

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Veritas in scientia, virtus in labore.

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Declaración de originalidad

El que suscribe Alexis de Jesús Escarcega Bata, alumno del Doctorado en Ciencias Biológicas y de la Salud, con número de matrícula 2212801264, de la Universidad Autónoma Metropolitana, Unidad Iztapalapa, y autor de la tesis titulada: “Diversidad y relaciones filogenéticas de dinoflagelados atecados (Gymnodiniales: Dinophyceae) en Bahía de Acapulco, Guerrero, México”.

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Alexis de Jesús Escarcega Bata

Contenido

1. RESUMEN

Los dinoflagelados comúnmente llamados atecados o desnudos, conforman un extenso grupo de organismo, principalmente marinos, sin embargo, el orden que agrupa el mayor número de especies es el de los Gymnodiniales, el cuál actualmente está constituido por 11 familias, 63 géneros y 641 especies taxonómicamente válidas. La importancia del estudio de estos organismos se centra principalmente en el potencial que presentan para la formación de extensos florecimientos algales, así mismo, algunas especies pueden llegar a producir potentes toxinas. En el Pacifico mexicano, los estudios enfocados en este orden siguen siendo limitados, dado que actualmente solo se tiene reporte de 103 especies para este litoral mexicano, lo cual constituye un reducido porcentaje de la diversidad en comparación con el total de especies actualmente conocidas alrededor del mundo. El presente trabajo está constituido por diferentes capítulos, cada uno de ellos enfocado en el estudio de dinoflagelados Gymnodiniales. El capítulo I aborda de manera general, la diversidad actual de dinoflagelados Gymnodiniales en el Pacifico mexicano, mediante una revisión bibliográfica desde 1943 hasta 2022, donde cada registro fue confirmado mediante micrografías ópticas y de electrónica de barrido. En el capítulo II, se presenta el primer reporte del género *Grammatodinium* para la Bahía de Acapulco, México, mediante una identificación morfológica y molecular, así como la descripción de las condiciones ambientales bajo las cuales estuvo presente dicha especie, adicionalmente, se presentan datos moleculares que indican la presencia de una nueva especie. En el capítulo III, se reporta el florecimiento generado por la especie *Karenia longicanalis* en la Bahía de Acapulco, la cual fue identificada mediante un análisis morfológico y molecular, adicionalmente, se presenta la distribución global de la especie. Finalmente, en el capítulo IV, se presenta un análisis de la variación genética de la especie *Karenia selliformis*, mediante el análisis de secuencias de los marcadores moleculares 28S del ADNr y de las espaciadoras transcritas internas (ITS1 + 5.8S + ITS2), asimismo, se describe la distribución global de la especie y su potencial para la formación de florecimientos algales en el Pacífico mexicano.

Palabras clave: Gymnodiniales, Pacífico mexicano, Florecimientos algales, morfología, marcadores moleculares, filogenia, variación genética.

2. ABSTRACT

Dinoflagellates, commonly known as athecates or unarmored, comprise a large group of organisms, primarily marine. However, the order that groups the largest number of species is Gymnodiniales, which currently consists of 11 families, 63 genera, and 641 taxonomically valid species. The importance of studying these organisms lies primarily in their potential for the formation of extensive algal blooms. Likewise, some species can produce potent toxins. In the Mexican Pacific, studies focused on this order remain limited, given that currently only 103 species have been reported for this Mexican coast, which constitutes a small percentage of diversity compared to the total number of species currently known worldwide. This work is composed of different chapters, each of them focusing on the study of Gymnodiniales dinoflagellates. Chapter I generally addresses the current diversity of Gymnodiniales dinoflagellates in the Mexican Pacific, through a bibliographic review from 1943 to 2022, where each record was confirmed by optical and scanning electron micrographs. In Chapter II, the first report of the genus *Grammatodinium* for Acapulco Bay, Mexico, is presented through a morphological and molecular identification, as well as the description of the environmental conditions under which said species was present, additionally, molecular data are presented that indicate the presence of a new species. In Chapter III, the bloom generated by the species *Karenia longicanalis* in Acapulco Bay is reported, which was identified through a morphological and molecular analysis, additionally, the global distribution of the species is presented. Finally, in chapter IV, an analysis of the genetic variation of the species *Karenia selliformis* is presented, through the analysis of sequences of the molecular markers 28S of the rDNA and the internal transcribed spacers (ITS1 + 5.8S + ITS2), also, the global distribution of the species and its potential for the formation of algal blooms in the Mexican Pacific are described.

Keywords: Gymnodiniales, Mexican Pacific, algal blooms, morphology, molecular markers, phylogeny, genetic variation.

3. Introducción general

El fitoplancton constituye una comunidad algal muy diversa e importante en el ambiente marino, debido a su alta productividad primaria, siendo responsable del 1% de la biomasa fotosintética del planeta (Simon et al., 2009). Dentro de esta comunidad se encuentran diferentes clases tales como: Bacillariophyceae Haeckel, Coccolithophyceae Rothmaler, Cyanophyceae Schaffner, Chlorophyceae Wille, Dinophyceae Pascher, Raphidophyceae Chadefaud ex P.C. Silva, entre otras (Guiry y Guiry, 2025). Sin embargo, las clases Bacillariophyceae y Dinophyceae son las más diversas y abundantes en el ambiente marino (Meave del Castillo et al., 2012; Simon et al., 2009), siendo la primera la que agrupa el mayor número de especies (Guiry y Guiry, 2025). Por otro lado, la clase Dinophyceae (dinoflagelados) es el segundo grupo más abundante en el ambiente marino, con un número estimado de 2,500 especies vivientes, repartidas en seis clases, 16 órdenes, 65 familias y 259 géneros, aunque de este total, solo se han listado 1,555 especies de vida libre (Taylor et al., 2008; Gómez, 2012; Guiry y Guiry, 2025).

Los dinoflagelados son un grupo de organismos generalmente solitarios, móviles y unicelulares, aunque algunas especies llegan a desarrollar formas muy atractivas y ornamentadas (apéndices notables como: cuernos, aletas y espinas), con diferencias morfológicas notables (Fensome et al., 1993). La identificación morfológica tradicional de estos organismos se ha basado, principalmente, en las características presentes en su pared celular. Con base en este criterio, Lindemann (1928) clasificó a los dinoflagelados en dos grupos: Thecales o dinoflagelados tecados (aquellos que poseen celulosa en las placas corticales) y Athecales dinoflagelados atecados (aquellos que carecen de placas de celulosa).

Los dinoflagelados atecados se caracterizan, principalmente, por tener una pared celular muy frágil, compuesta por vesículas delgadas y aplanadas (Fensome et al., 1993). En la actualidad, aún pondera la identificación de estos organismos se realiza a través de la observación de ciertos caracteres morfológicos, como criterio preponderante, tales como: ubicación, forma y desplazamiento del cingulum y sulcus, ausencia o presencia de estrías, así como de una estructura única denomina surco apical. Sin embargo, en el caso de ciertos órdenes y familias, estos caracteres no son suficientes ni apropiados para poder discriminar a las especies (Fensome et al., 1993; Daugbjerg et al., 2000; Gómez, 2011), ya que la morfología como criterio de identificación por sí solo, es subjetivo, pues no sólo depende de la expresión genética en función a las variables ambientales preponderantes en los sitios en que estas especies se distribuyen, sino que, la observación y documentación de ciertos caracteres morfológicos diagnósticos, no siempre es posible para todas las especies.

Como consecuencia y a la luz de la evidencia molecular, los dinoflagelados atecados no son considerados un grupo natural (monofilético). Este grupo comprende varios ordenes, siendo los Gymnodiniales Apstein el más importante, debido al número de especies y al potencial que tienen algunas para formar grandes florecimientos algales nocivos (Gómez, 2011, Band-Schmidt et al., 2011; Guiry y Guiry, 2025). Actualmente, el orden Gymnodiniales cuenta con 641 especies, las cuales están distribuidas en 11 familias y 63 géneros (*Gymnodinium* Stein representa al género tipo del orden) (Escarcega-Bata et al., 2023; Guiry y Guiry, 2025). De este modo, el estudio de Gymnodiniales presenta grandes limitaciones, una de ellas y la principal, es la baja resistencia ante fijadores convencionales (formaldehído), por lo que la identificación a nivel específico suele ser complicada y, en algunos casos, imposible (Gárate-Lizárraga, 2020), generando que los taxónomos realicen identificaciones y descripciones erróneas, con la consecuente subestimación o sobreestimación de la diversidad real de este grupo (Reñé et al., 2015; Escarcega-Bata et al., 2023). Por otro lado, existen diversas técnicas que permiten una mejor aproximación en la identificación de estas especies, tales como la microscopia electrónica de barrido y microscopia de transmisión, aunque estas siguen siendo metodologías convencionales. A raíz de esta problemática, el objetivo del presente estudio fue realizar un análisis integral sobre la diversidad y las relaciones filogenéticas de dinoflagelados Gymnodiniales presentes en la Bahía de Acapulco, Guerrero, México.

3.1 Sistemática filogenética de dinoflagelados Gymnodiniales

Los sistemas de clasificación actuales de los dinoflagelados, han unificado a los taxones basándose en caracteres morfológicos que se consideraban diagnósticos para cada grupo. En dinoflagelados tecados, un criterio de clasificación se ha basado en la tabulación de la teca (pared celular), sin embargo, en dinoflagelados atecados, dicho criterio es subjetivo pues, al carecer de una pared rígida, como es el caso en las especies del orden Gymnodiniales, es complicado observar dicha tabulación complicando su identificación mediante caracteres morfológicos únicos (Daugbjerg et al., 2000). En consecuencia, el uso de marcadores moleculares en Gymnodiniales ha tomado relevancia, permitiendo la generación de hipótesis filogenéticas más robustas y, por ende, una mejor aproximación de las relaciones evolutivas que guardan las diferentes especies.

Particularmente, en el orden Gymnodiniales, la sistemática filogenética ha desempeñado un papel importante, permitiendo cambios en todos los niveles jerárquicos, tales como el establecimiento de nuevas familias, géneros y especies, así como el reacomodo de taxones previamente establecidos en otros grupos taxonómicos (Escarcega-Bata et al., 2023; Gómez, 2011, 2014). Tal es el caso del trabajo de Daugbjerg et al., (2000), quienes analizaron y compararon secuencias del gen 28S del ADNr de especies del género *Gymnodinium* Stein. Con base en la evidencia obtenida, erigieron tres nuevos géneros con las consecuentes combinaciones taxonómicas (*Akashiwo* Hansen & Moestrup, *Karenia* Hansen & Moestrup y *Karlodinium* Larsen), permitiendo ampliar la diversidad de la familia Gymnodiniacea Lankester. En estudios como los de Reñe et al., (2015) y Escarcega-Bata et al., (2021), se presentan topologías extensas de los marcadores 18S y 28S del ADNr, mostrando las relaciones que guardan cada una de las familias que conforman el orden Gymnodiniales; adicionalmente, se proponen nuevas familias para los géneros monoespecíficos *Akashiwo* y *Levanderina* Moestrup, Hakanen, Hansen, Daugbjerg & Ellegaard junto con *Margalefidinium* Gómez, Richlen & Anderson, así como el establecimiento de una nueva familia para el género *Balechina* Loeblich & Loeblich. Sin embargo, en la actualidad el reconocimiento de estas nuevas familias no ha sido posible, debido a la discrepancia por parte de los especialistas, en donde se solicita de manera imperativa, el carácter morfológico o ultraestructural presente en estos géneros, para que de esta forma pueda validarse una nueva familia. Estas posturas han generado una de las mayores limitantes en la clasificación de estos organismos.

Actualmente, el orden Gymnodiniales está conformado por 11 familias y 636 géneros (Guiry y Guiry, 2025), sin embargo, la taxonomía del grupo se encuentra en constante cambio debido a la incorporación y redefinición de nuevos géneros y especies. Las familias con el mayor número de especies son Gymnodiniaceae, Gyrodiniaceae Moestrup & Calado, Kareniaceae Bergholtz, Daugbjerg, Moestrup & Fernández-Tejedor, y Warnowiaceae Lindemann. Adicionalmente, estos grupos presentan una gran importancia ecológica, debido a su potencial como formadoras de florecimientos algales nocivos (FAN) (Escarcega-Bata et al., 2023; Guiry y Guiry, 2025). La familia Gymnodiniaceae es la más representativa dentro del orden Gymnodiniales, debido al número de géneros (24) y especies (356) que agrupa (Escarcega-Bata et al., 2023; Guiry y Guiry, 2025). Recientemente, se han descrito y redefinido nuevas especies como; *Margalefindinium catenatum*, *M. citron*, *M. flavum*, *M. fulvescens* y *M. polykrikoides*, las cuales anteriormente se ubicaban dentro del género *Cochlodinium* Schütt; este nuevo establecimiento se dio a partir de secuencias de ADNr del gen 28S (Gómez et al., 2017). Sin embargo, dicha propuesta no contemplo su correcta posición dentro del orden Gymnodiniales, generando un estatus provisional e incorrecto en la familia Gymnodiniacea que debe ser evaluado. En Escarcega-Bata et al., (2021), se sugiere que en realidad este género constituye una nueva familia. Esta deficiente interpretación de los datos filogenéticos obtenidos por los diversos autores, ha generado sesgos en la ubicación de los diferentes linajes en Gymnodiniales, por lo que es necesario abordar de manera detallada la filogenia de aquellas especies de interés.

La familia Kareniaceae aloja seis géneros y 37 especies, no obstante, en los últimos años el estudio de este grupo ha tomado gran relevancia, debido al potencial tóxico y nocivo de algunas de sus especies, así como de su capacidad para formar extensos florecimientos (Brand et al., 2012; Guiry y Guiry, 2025, Iwataki, 2025). En la última década se han erigido nuevos géneros como *Shimiella* Ok, Jeong, Lee & Noh y *Gertia* Takahashi, Benico, Wai Mun Lum & Iwataki, ambos géneros fueron establecidos a partir de descripciones morfológicas e identificaciones moleculares, con diversos marcadores (18S, ITS1, 5.8S, ITS2 y 28S) (Ok et al., 2021; Takahashi et al., 2019). Sin embargo, la posición actual de dichos géneros dentro de la familia Kareniaceae es incierta, debido nuevamente, a la escasa interpretación de los datos moleculares obtenidos. Por otro lado, géneros como *Karenia* Gert Hansen & Moestrup y *Karlodinium* Larsen, también han sufrido constantes cambios, relacionados al establecimiento de nuevas especies (e.g. *Karenia hui* Lu, Cen, Wang & Moestrup; *Karlodinium azanzae* Benico & Iwataki; *Karl. elegans* Cen, Lu & Wang; *Karl. zhouanum* Luo & Gu), así como la reclasificación de algunas otras (e.g. *Karenia umbella* Salas, Bolch & Hallegraeff; *K. digitata* Yang, Takayama, Matsuoka & Hodgkiss; *Karlodinium decipiens* Salas & Laza-Martinez; *Karl. jejuense* Li & Shin). El aumento en la diversidad de estos géneros, solo ha sido posible con la integración de herramientas moleculares, sin embargo, es necesario un análisis genético de todas aquellas especies que conforman complejos crípticos (caso específico del género *Karenia*) (Arana-García et al., 2025; Benico et al., 2020; Cen et al., 2021, 2024; Luo et al., 2018; Yao et al., 2024; Wang et al., 2018).

La familia Ceratoperidiniaceae Loeblich III agrupa cuatro géneros y nueve especies, por lo que es considerada una familia poco representada dentro del orden Gymnodiniales, sin embargo, se han descrito nuevos géneros como *Torquentidium* Shin, Li, Lee & Matsuoka y nuevas especies como *Kirithra asteri* Boutrup, Tillmann, Daugbjerg & Moestrup y *Kirithra sigma* Hu, Li, Shin & Tang (Boutrup et al., 2017; Hu et al., 2020; Shin et al., 2019), así mismo, se han redefinido los géneros *Ceratoperidinium* y *Pseliodinium*, ambos mediante el análisis de secuencias del gen 28S (D1-D2) del ADNr (Gómez, 2018). El aumento en el número de representantes de esta familia ha sido posible mediante el uso de marcadores nucleares (18S y 28S) y plastidiales (*rbc*L), permitiendo tener una mejor aproximación de la diversidad real de la familia alrededor del mundo. A su vez, dentro del orden Gymnodiniales existen géneros que, en la actualidad, no han podido ser asignados a una familia, como el caso de *Balechina* Loeblich & Loeblich III, *Cucumeridinium* Gómez, López-García, Takayama & Moreira, *Dissodinium* Klebs, *Grammatodinium* Li & Shin, *Lebouridinium* Gómez, Takayam, Moreira & López y *Levanderina*, por lo que el uso de marcadores alternativos (mitocondriales y plastidiales) resultarían útiles para la redefinición de estos géneros, así como un amplio criterio para la definición de nuevas especies por parte de los especialistas, en donde no solo se prepondere a la morfología como carácter unificador (Gómez et al., 2015, 2016, 2021; Moestrup et al., 2014).

En la actualidad, el uso de los diferentes marcadores moleculares empleados para la identificación de dinoflagelados marinos, ha presentado diversas limitantes, esto derivado tanto de la biología de los diferentes ordenes que conforman a la clase Dinophyceae, el reducido nivel de polimorfismo del marcador, así como el criterio sesgado de los especialistas, en donde se sigue preponderando a la morfología como el criterio decisivo en la clasificación de estos organismos sobre la evidencia genética. A partir de estas limitantes, es necesario explorar nuevos marcadores que permitan una mejor inferencia filogenética y el consecuente establecimiento de sistemas de clasificación más robustos (estables), ya que en la actualidad no existen marcadores específicos para este conjunto de organismos. Adicionalmente, es necesario un mayor enfoque en aquellos organismos con un tipo de nutrición heterótrofa (Delgado-Villar et al., 2021), en donde el establecimiento de cultivos monoclonales para estos dinoflagelados es uno de los mayores retos en la actualidad. Por otro lado, no basta con la identificación de especies a través de la obtención de secuencias, ya que, adicionalmente, es necesario la aplicación de la sistemática filogenética para poder establecer las relaciones evolutivas que guardan los diversos linajes, revelando así, la diversidad real de este grupo de organismos y un mejor entendimiento de los procesos responsables de dicha diversidad (Escarcega-Bata et al., 2021; Gómez, 2011).

4. PREGUNTAS DE INVESTIGACIÓN

- ¿El establecimiento de cultivos monoclonales permitirá una mejor aproximación al estudio de las especies atecadas, mediante una descripción morfológica fina y caracterización molecular?

- ¿Dicha caracterización morfológica y molecular generará cambios en la diversidad actual de dinoflagelados atecados?

- ¿Cuáles variables abióticas favorecer la presencia o ausencia de dinoflagelados atecados en el sitio?

- ¿La integración de la evidencia total (morfológica, molecular y datos ecológicos) permitirá establecer las relaciones filogenéticas entre las especies de dinoflagelados atecados en Bahía de Acapulco?

5. HIPÓTESIS

Si el uso de caracteres morfológicos y moleculares permiten conocer la diversidad real de dinoflagelados atecados en la Bahía de Acapulco, entonces será posible describir sus relaciones filogenéticas y establecer los consecuentes cambios a la taxonomía del grupo.

6. OBJETIVOS

6.1 General

Describir la diversidad y relaciones filogenéticas de los dinoflagelados atecados en la Bahía de Acapulco, Guerrero, México.

6.2 Específicos

- Describir la morfología de las especies de dinoflagelados atecados en Bahía de Acapulco.
- Obtener secuencias moleculares de ADNr con dos marcadores moleculares para las morfoespecies de dinoflagelados atecados en Bahía de Acapulco.
- Caracterizar las condiciones abióticas que favorecen la presencia de especies de dinoflagelados atecados.
- Construir hipótesis filogenéticas a partir de secuencias con ADNr.
- Describir las relaciones filogenéticas integrando la evidencia total.

7. Capítulo 1

Diversidad de dinoflagelados atecados del orden Gymnodiniales (Dinophyceae), con énfasis en aquellos formadores de florecimientos algales nocivos en las costas del Pacífico Mexicano.

Diversity of athecate dinoflagellates of the order Gymnodiniales (Dinophyceae), with emphasis on those that form harmful algal blooms on the Mexican Pacific coast.

RESUMEN

El orden Gymnodiniales está compuesto por 11 familias, 63 géneros y 641 especies taxonómicamente válidas. Estos dinoflagelados se caracterizan por presentar una pared celular muy frágil. Además, algunas especies pueden llegar a producir potentes toxinas e incluso tienen la capacidad para formar grandes florecimientos. Los estudios enfocados en conocer la diversidad de este grupo, en el caso de las costas del Pacífico Mexicano, siguen siendo limitados, a pesar de la importancia económica, social y ecológica de estos microorganismos. Por lo tanto, el objetivo de este estudio fue realizar un listado florístico a partir de una revisión bibliográfica, que integre únicamente a los registros respaldados por micrografías, para conocer la diversidad de dinoflagelados atecados del orden Gymnodiniales presentes en el Pacífico Mexicano, con énfasis en aquellos formadores de florecimientos algales nocivos. La búsqueda de información se realizó en publicaciones (artículos y capítulos de libros), desde el primer registro (1943) hasta la actualidad (noviembre 2022). Cada registro fue confirmado únicamente mediante la existencia de micrografías ópticas y de electrónicas de barrido. El Pacífico Mexicano está conformado por 11 estados costeros, donde actualmente se tiene reportada la presencia de 10 familias, 31 géneros y 103 especies (16% del total de las registradas a nivel mundial) para el orden Gymnodiniales. De las 11 familias que conforman a este orden, la familia Gymnodiniaceae agrupó 29% del total de especies registradas para el Pacífico Mexicano, donde los géneros *Gymnodinium* y *Gyrodinium* presentaron el mayor número de especies. La evidencia presentada en este estudio demostró que existe una baja diversidad para este orden en el Pacífico Mexicano, por lo que es necesario implementar un mayor esfuerzo de muestreo, además de incursionar en diferentes herramientas moleculares que permitan obtener una mejor aproximación a la diversidad alfa en las costas mexicanas.

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Artículo de investigación

Diversidad de dinoflagelados atecados del orden Gymnodiniales (Dinophyceae), con énfasis en aquellos formadores de florecimientos algales nocivos en las costas del Pacífico Mexicano

Diversity of athecate dinoflagellates of the order Gymnodiniales (Dinophyceae), with emphasis on those that form harmful algal blooms on the Mexican Pacific coast

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Resumen:

Antecedentes y Objetivos: El orden Gymnodiniales está compuesto por 11 familias, 63 géneros y 641 especies taxonómicamente válidas. Estos dinoflagelados se caracterizan por presentar una pared celular muy frágil. Además, algunas especies pueden llegar a producir potentes toxinas e incluso tienen la capacidad para formar grandes florecimientos. Los estudios enfocados en conocer la diversidad de este grupo, en el caso de las costas del Pacífico Mexicano, siguen siendo limitados, a pesar de la importancia económica, social y ecológica de estos microorganismos. Por lo tanto, el objetivo de este estudio fue realizar un listado florístico a partir de una revisión bibliográfica, que integre únicamente a los registros respaldados por micrografías, para conocer la diversidad de dinoflagelados atecados del orden Gymnodiniales presentes en el Pacífico Mexicano, con énfasis en aquellos formadores de florecimientos algales nocivos.

Métodos: La búsqueda de información se realizó en publicaciones (artículos y capítulos de libros), desde el primer registro (1943) hasta la actualidad (noviembre 2022). Cada registro fue confirmado únicamente mediante la existencia de micrografías ópticas y de electrónicas de barrido.

Resultados clave: El Pacífico Mexicano está conformado por 11 estados costeros, donde actualmente se tiene reportada la presencia de 10 familias, 31 géneros y 103 especies (16% del total de las registradas a nivel mundial) para el orden Gymnodiniales. De las 11 familias que conforman a este orden, la familia Gymnodiniaceae agrupó 29% del total de especies registradas para el Pacífico Mexicano, donde los géneros *Gymnodinium* y *Gyrodinium* presentaron el mayor número de especies.

Conclusiones: La evidencia presentada en este estudio demostró que existe una baja diversidad para este orden en el Pacífico Mexicano, por lo que es necesario implementar un mayor esfuerzo de muestreo, además de incursionar en diferentes herramientas moleculares que permitan obtener una mejor aproximación a la diversidad alfa en las costas mexicanas.

Palabras clave: FAN, Gymnodiniaceae, *Gymnodinium*, *Gyrodinium*, herramientas moleculares.

Abstract:

Background and Aims: The order Gymnodiniales is composed of 11 families, 63 genera and 641 taxonomically valid species. These dinoflagellates are characterized by having a very fragile cell wall. In addition, some species can produce phycotoxins and form dense blooms. In the case of the Mexican Pacific coast, studies focused on knowing the diversity of this group are still limited, despite the economic, social, and ecological importance of these microorganisms. Therefore, the objective of this study was to make a floristic list from a bibliographical review, integrating only the records supported by micrographs, to know the diversity of athecate dinoflagellates of the order Gymnodiniales present in the Mexican Pacific Ocean, with emphasis on those that form harmful algal blooms.

Methods: The search for information was carried out in publications (articles and book chapters), from the first record (1943) to the present (November 2022). Each record was confirmed only by the existence of optical and scanning electron micrographs.

Key results: The Mexican Pacific is made up of 11 coastal states, where the presence of 10 families, 31 genera and 103 species (which represents 16% of the total number of species recorded worldwide) is currently reported for the order Gymnodiniales. Of the 11 families that make up this order, the Gymnodiniaceae family grouped 29% of the total number of species recorded for the Mexican Pacific, where the genera *Gymnodinium* and *Gyrodinium* presented the largest number of species.

Conclusions: The evidence presented in this study showed that there is a low diversity for this order in the Mexican Pacific, so it is necessary to implement a greater sampling effort, in addition to venturing into different molecular tools that allow obtaining a better approximation of the diversity in the Mexican coasts.

Key words: Gymnodiniaceae, *Gymnodinium*, *Gyrodinium*, HAB, molecular tools.

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Introducción

Los dinoflagelados constituyen, después de las diatomeas, el segundo grupo más diverso y abundante dentro del fitoplancton marino. Actualmente, se registran aproximadamente 2500 especies vivas, divididas en cinco clases, 16 órdenes, 65 familias y 259 géneros (Gómez, 2012; Guiry y Guiry, 2022). La identificación morfológica tradicional de estos organismos se basa en las características de su pared celular. Con base en esto, Lindemann (1928) clasificó a los dinoflagelados en dos grupos: Thecales (dinoflagelados tecados) y Athecales (dinoflagelados atecados o desnudos). Los dinoflagelados atecados (Fig. 1) se caracterizan principalmente por tener una pared celular muy frágil compuesta por vesículas delgadas y aplanadas. Los criterios para la identificación de las diferentes especies de Gymnodiniales, hasta principios del año 2000, solían basarse en la ubicación, forma y desplazamiento del *cingulum* y *sulcus*, pero estos caracteres no eran suficientes ni apropiados para poder discriminar entre los diferentes géneros (Fensome et al., 1993; Daugbjerg et al., 2000).

En la actualidad, el estudio de los dinoflagelados atecados sigue teniendo limitaciones. Una de ellas, siendo la principal, es la poca resistencia que estos organismos tienen frente a los fijadores (formaldehído), por lo que se vuelve aún más compleja la identificación a nivel de especie (Gárate-Lizárraga, 2020). Esto ha generado un problema que con el tiempo llevó a los taxónomos a realizar identificaciones o descripciones incorrectas de las diferentes especies atecadas (Escarcega-Bata et al., 2022). A pesar de los esfuerzos de identificación a través de múltiples técnicas de microscopía, no ha sido posible conocer la diversidad real del grupo, debido a que estos organismos presentan un ciclo de vida complejo y una plasticidad fenotípica alta (Reñé et al., 2015; Gárate-Lizárraga, 2020; Escarcega-Bata et al., 2021). A partir de la década de 1990, se comenzaron a abordar nuevas técnicas de identificación en dinoflagelados mediante la extracción y amplificación de ADN ribosómico, pero esto solo era posible con especies disponibles en cultivos monoclonales (Hinnebusch et al., 1981). Un problema derivado de esta metodología es que muchas de las especies atecadas presentan una alimentación de tipo mixotrófica y heterótrofa, lo que complica la obtención de cultivos de dinoflagelados (Saunders et al., 1997; López-García et al., 2001). Como resultado de este problema, Bolch (2001) propuso un enfoque para la identificación molecular de especies mediante la amplificación por PCR de genes del ADNr en quistes y células individuales (Single cell-PCR), lo que permitió la obtención de secuencias para especies que no son muy abundantes en el medio marino.

Por otro lado, la integración de herramientas moleculares en la identificación de dinoflagelados del orden Gymnodiniales ha permitido realizar cambios en la sistemática del grupo en todos los niveles jerárquicos. Tal es el caso del trabajo de Daugbjerg et al. (2000), quienes analizaron secuencias del gen 28s del ADNr de especies de los géneros *Gymnodinium* Stein y *Gyrodinium* Kofoid & Swezy, comparando secuencias, y erigiendo tres nuevos géneros (*Akashiwo* Hansen & Moestrup, *Karenia* Hansen & Moestrup y *Karlodinium* Larsen). De los resultados obtenidos observaron que el carácter morfológico que permitía diferenciar entre géneros era el complejo de la estructura o surco apical. Sin embargo, en el estudio de Gómez et al. (2011), la filogenia molecular de géneros como *Gymnodinium* da como resultado grupos polifiléticos, lo que complica aún más su clasificación y da paso a nuevos análisis exploratorios con marcadores moleculares más específicos. Otros estudios moleculares, como los de Hu et al. (2018) y Hyeon et al. (2020), han permitido redefinir especies como *Margalefidinium fulvescens* (Iwataki, Kawami & Matsuoka) Gómez, Richlen & Anderson (=*Cochlodinium fulvescens*) y *Torquentidium flavescens* (Kofoid & Swezy) Shin, Li & Matzuoka (=*Gyrodinium flavescens*). Asimismo, estudios como los de Takahashi et al. (2019) y Ok et al. (2020) han logrado describir nueva diversidad a nivel específico y genérico.

En el caso de la costa del Pacífico Mexicano (PM) (Fig. 2), la diversidad de dinoflagelados atecados se conoce exclusivamente con base en su morfología (Licea et al., 1995; Esqueda-Lara y Hernández-Becerril, 2010; Meave del Castillo et al., 2012; Almazán-Becerril et al., 2016; Gárate-Lizárraga, 2020; Escarcega-Bata et al., 2022). Adicionalmente, el único estudio de evaluación molecular en especies atecadas es el realizado por Escarcega-Bata et al. (2021), donde se detectó mediante marcadores moleculares (genes 18S y 28S del ADNr), un problema en la clasificación actual de varias familias del orden Gymnodiniales, a partir de muestras colectadas en Bahía Todos Santos, Baja California. El

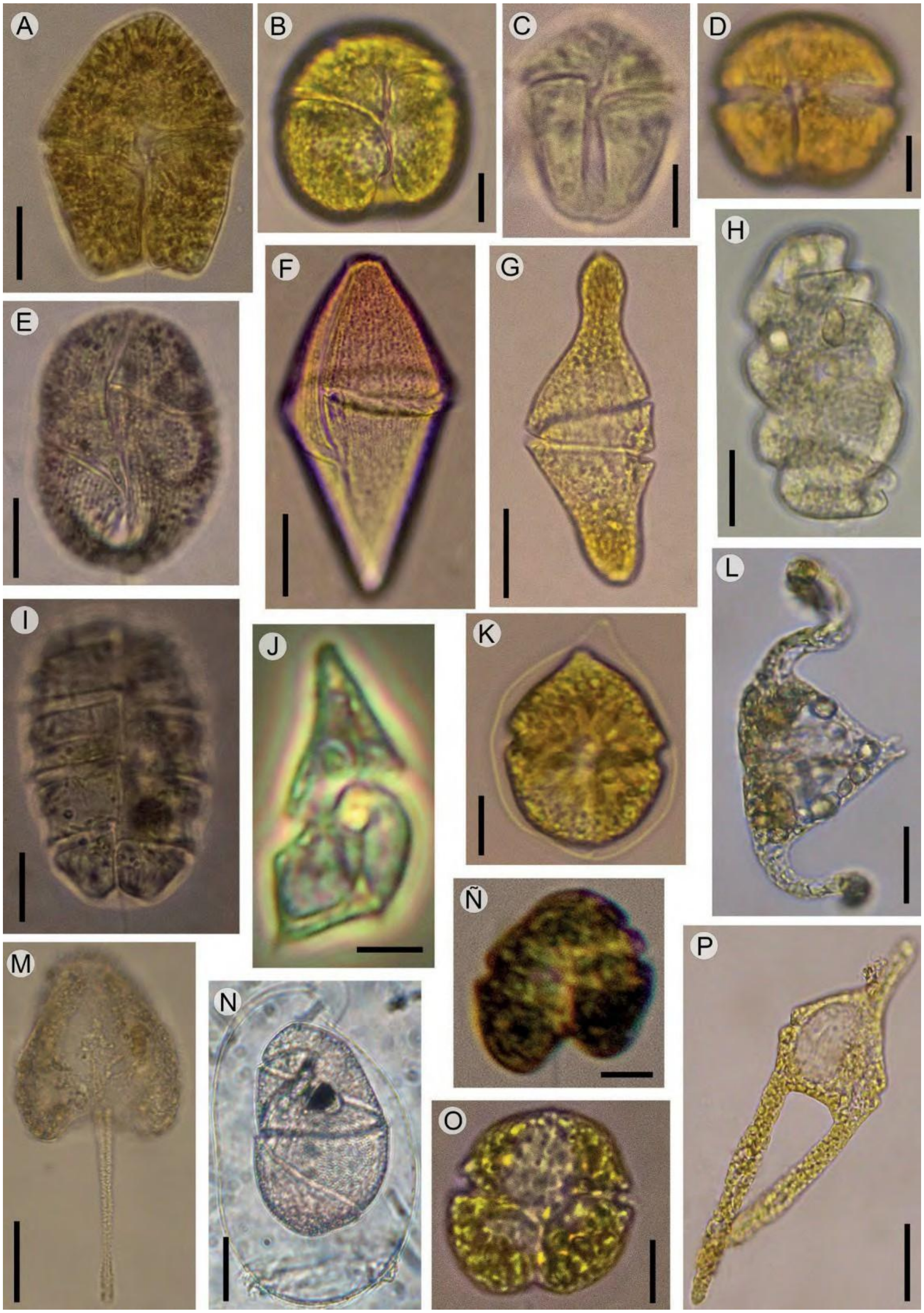

Figura 1: Especies de dinoflagelados atecados del orden Gymnodiniales, registradas para las costas del Pacífico Mexicano: A. *Akashiwo sanguinea* (Hirasaka) Hansen & Moestrup; B. *Gymnodinium incisum* Kofoid & Swezy; C. *Gymnodinium* sp.; D. *Gymnodinium impudicum* (Fraga & Bravo) Hansen & Moestrup; E. *Gyrodinium dorsum* Kofoid & Swezy; F. *Gyrodinium corallinum* Kofoid & Swezy; G. *Pseliodinium fusus* (Schütt) Gómez; H. *Warnowia juno* (Schütt) Schiller; I. *Polykrikos kofoidii* Chatton; J. *Dicroerisma* sp; K. *Kirithra sigma* Hu, Li, Shin & Tang; L. *Gynogonadinium aequatoriale* Gómez; M. *Greuetodinium cylindricum* (Greuet) Loeblich III; N. *Warnowia maxima* (Kofoid & Swezy) Lindemann; Ñ. *Karenia* sp.; O. *Takayama helix* Salas, Bolch, Botes & Hallegraeff; P. *Ceratoperidinium margalefii* Loeblich III. Escalas B, D, J, Ñ, O = 10 µm; C, H, K, L, P = 15 µm; A, E, G, I = 20 µm; F, M = 30 µm; N = 60 µm. Las figuras A-G, I, K, Ñ, O fueron registradas para la Bahía de Todos Santos, Baja California y las figuras H, J, L, M, P se registraron para la Bahía de Acapulco, Guerrero.

objetivo de este estudio fue realizar un listado florístico a partir de una revisión bibliográfica, que integre únicamente a los registros respaldados por micrografías, para conocer la diversidad de dinoflagelados atecados del orden Gymnodiniales distribuidos a lo largo del PM, con énfasis en aquellos formadores de florecimientos algales nocivos.

Materiales y Métodos

El Pacífico Mexicano (Fig. 2) cuenta con una extensión litoral aproximada de 4120 km de longitud. De acuerdo con los criterios climáticos-oceanográficos regionales reconocidos por De la Lanza (1991), el PM se encuentra dividido en diferentes regiones. Para este estudio se consideraron solo cuatro (Fig. 2).

La búsqueda de información se realizó en publicaciones (artículos y capítulos de libros), desde el primer registro (1943) hasta la actualidad (noviembre 2022). Se consultaron 44 citas (Apéndice), donde cada uno de los registros presentados se confirmaron únicamente mediante la existencia de micrografías ópticas y de electrónica de barrido. A partir de este criterio, se efectuó una revisión bibliográfica para cada una de las regiones del PM, con el objetivo de conocer la diversidad de dinoflagelados Gymnodiniales. Con los datos obtenidos (registros) se generó un análisis gráfico (curva de acumulación) (Fig. 3), empleando hojas de cálculo (Excel v. 2210), con la finalidad de conocer el número de registros reportados por década (1981-2022). Se elaboró un listado taxonómico actualizado del orden Gymnodiniales (Cuadro 1), para ubicar el total de especies reportadas en el PM. La validación de cada especie fue confirmada en la base de datos AlgaeBase (Guiry y Guiry, 2022).

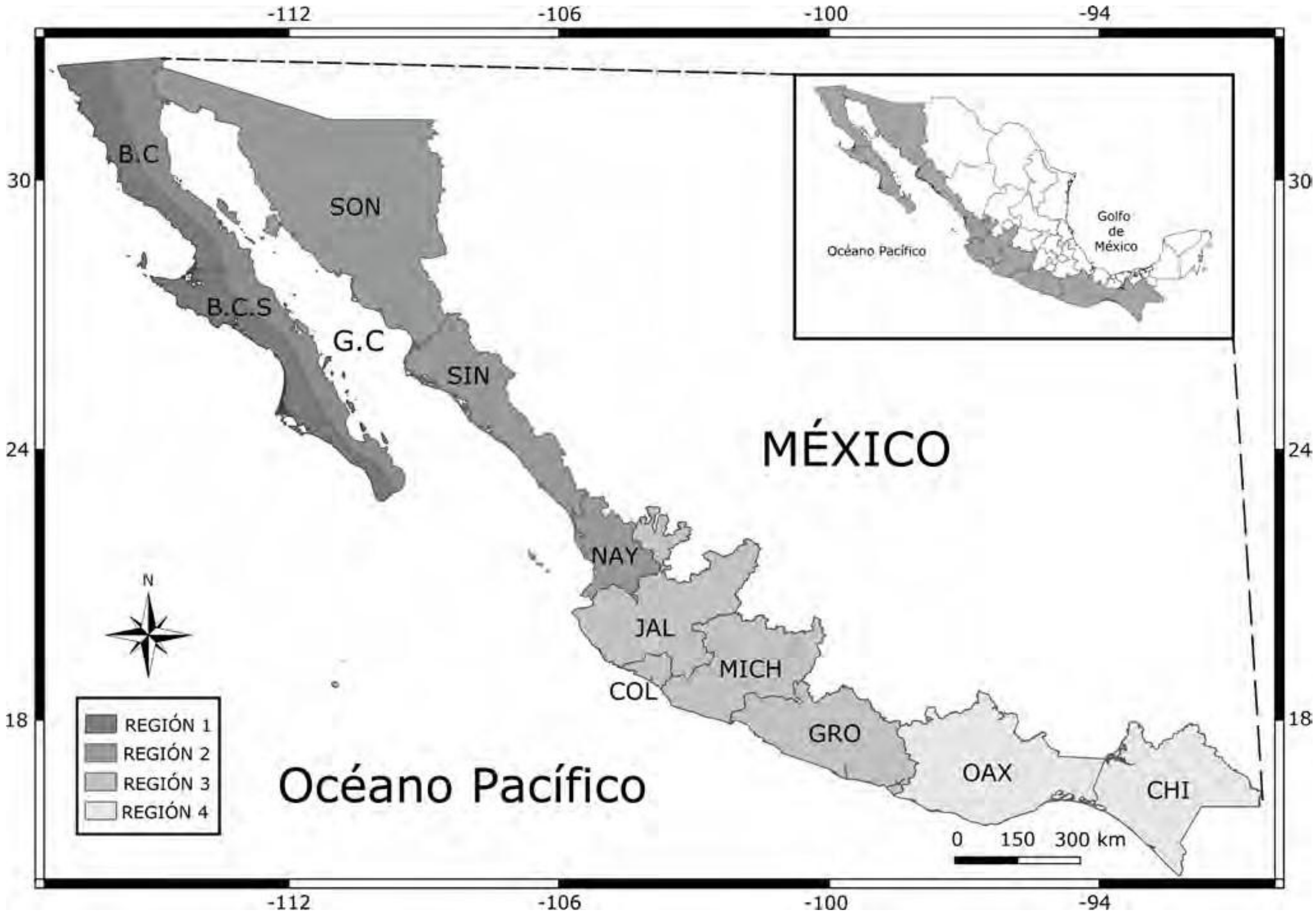

Figura 2: Litoral costero del Pacífico Mexicano seccionado en cuatro regiones de acuerdo con De la Lanza (1991). Región 1: Península de Baja California (costa oeste); Región 2: Península de Baja California (costa oriental), Sonora, Sinaloa y Nayarit; Región 3: Jalisco, Colima, Michoacán y Guerrero; Región 4: Golfo de Tehuantepec (Oaxaca y Chiapas).

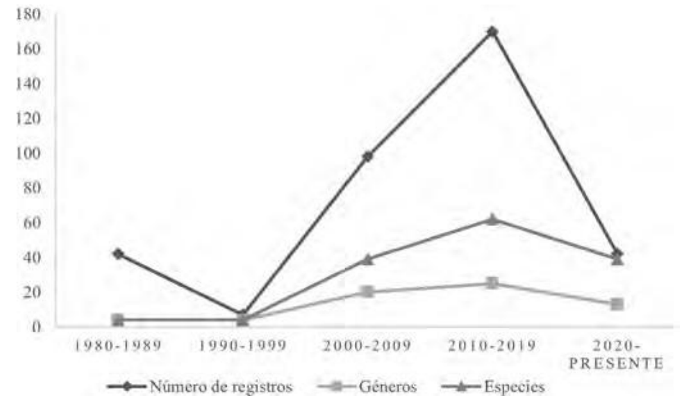

Figura 3: Curva acumulativa de registro, de géneros y especies de Gymnodiniales en el Pacífico Mexicano, considerando solo especies actualmente válidas según Guiry y Guiry (2022). Los datos obtenidos se cuentan en intervalos de 10 años desde 1980 al presente (noviembre 2022).

Resultados y Discusión

Diversidad

De las 792 especies de dinoflagelados atecados pertenecientes a la clase Dinophyceae, cerca de 81% se ubican dentro del orden Gymnodiniales. Este orden agrupa 11 familias, 63 géneros (*Gymnodinium* representa al género tipo del orden Gymnodiniales) y 641 especies (Cuadro 1) (Guiry y Guiry, 2022). Las familias con el mayor número de especies y de importancia ecológica, debido a su potencial para la formación de florecimientos algales nocivos (FAN), son: Gymnodiniaceae, Gyrodiniaceae, Kareniaceae, y Warnowiaceae. La familia Gymnodiniaceae agrupa 24 géneros (38% del total, con 400 especies), siendo *Gymnodinium* el más diverso. Este género fue erigido por Stein (1878) y hasta la actualidad se han descrito 297 especies. Algunas especies de esta familia, como *Akashiwo sanguinea* (Hirasaka) Hansen & Moestrup, *Gymnodinium catenatum* W.H. Graham (especie productora de saxitoxinas), *Margalefidinium catenatum* (Okamura) Gómez, Richlen & Anderson, *Margalefidinium polykrikoides* (Margalef) Gómez, Richlen & Anderson y *Polykrikos hartamannii* Zimmermann, son formadores de grandes FAN (Cortés-Altamirano, 1987, 2002; Alonso-Rodríguez et al., 2004; Band-Schmidt et al., 2006; Cortés-Lara et al., 2004, 2012, 2017; Gárate-Lizárraga et al., 2004, 2016; Gárate-Lizárraga, 2014c; Hansen, 2021; Guiry y Guiry, 2022). Por otro lado, la familia Gyrodiniaceae,

Cuadro 1: Clasificación actual de dinoflagelados del orden Gymnodiniales, de acuerdo con el esquema taxonómico de Guiry y Guiry (2022). El número de especies para cada género se indica en paréntesis. Los números en negrita indican el número de especies reportadas para el Pacífico Mexicano.

Clase Dinophyceae
Orden Gymnodiniales
Familia Actiniscaceae
Actiniscus Ehrenberg (6) (**1**)
Diaster Meunier (1)
Foliactiniscus Dumitrica (1)
Familia Amphitholaceae
Monaster Schütt (1) (**1**)
Familia Brachidiniaceae
Brachydinium Taylor (4) (**1**)
Gynogonadinium Gómez (1) (**1**)
Microceratium Sournia (1)
Pavillardia Kofoid & Swezy (1)
Familia Ceratoperidiniaceae
Ceratoperidinium Margalef ex Loeblich III (1) (**1**)
Kirithra Boutrup, Tillmann, Daugbjerg & Moestrup (2) (**1**)
Pseliodinium Sournia (2) (**1**)
Torquentidium Shin, Li, Lee & Matsuoka (4) (**4**)
Familia Chytriodiniaceae
Chytriodinium Chatton (3) (**1**)
Myxodinium Cachon, Cachon & Bouquaheux (1)
Schizochytriodinium Elbrächter (1)
Syltodinium Drebes (2)
Familia Dicroerismataceae
Dicroerisma Taylor Cattell (1) (**1**)
Familia Gymnodiniaceae
Akashiwo Hansen & Moestrup (1) (**1**)
Ankistrodinium Matsuoka & Bujak (2) (**1**)
Apicophorus Sparmann, Leander & Hoppenrath (3)
Barrufeta Sampedro & Fraga (2)
Bindiferia Borchhardt, Chomérat, Murray & Hoppenrath (2)
Cochlodinium Schütt (29) (**4**)
Dinogymnium Evitt, Clarke & Verdier (7)
Gymnocystodinium Baumeister (1)
Gymnodinium Stein (297) (**18**)
Gyrodiniellum Kang, Jeong & Moestrup (1)
Imbatodinium Vozzhennikova (5)
Lepidodinium Watanabe, Suda, Inouye, Sawaguchi & Chihara (2) (**2**)
Margalefidinium Gómez, Richlen & Anderson (5) (**3**)
Moestrupia Hansen & Daugbjerg (1)

Cuadro 1: Continuación.

Nusuttodinium Takano & Horiguchi (21)
Paragymnodinium Kang, Jeong, Moestrup & Shin (5)
Pheopolykrikos Chatton (1)
Plectodinium Biecheler (1)
Schillingia Schiller (1)
Spiniferodinium Horiguchi & Chihara (2)
Spirodinium Schütt (3)
Testudodinium Horiguchi, Tamura, Katsumata & Yamaguchi (4)
Togula Jorgensen, Murray & Daugbjerg (3)
Wangodinium Luo, Hu, Tang & Gu (1)
Familia Gyrodiniaceae
Gyrodinium Kofoid & Swezy (105) (**26**)
Familia Kareniaceae
Asterodinium Sournia (2) (**2**)
Gertia Takahashi, Benico, Lum & Iwataki (1)
Karenia Hansen & Moestrup (10) (**7**)
Karlodinium Larsen (16) (**3**)
Shimiella Ok, Jeong, Lee & Noh (1)
Takayama Salas, Bolch, Botes & Hallegraeff (7) (**3**)
Familia Polykrikaceae
Polykrikos Bütschli (9) (**3**)
Familia Warnowiaceae
Erythropsidinium Silva (9) (**1**)
Greuetodinium Loeblich III (1) (**1**)
Nematodinium Kofoid & Swezy (5) (**3**)
Nematopsides Greuet (2)
Pouchetia Schütt (1)
Proterythropsis Kofoid & Swezy (1)
Protopsis Kofoid & Swezy (5)
Warnowia Lindemann (25) (**5**)
Incertae sedis
Balechina Loeblich & Loeblich III (1) (**1**)
Cucumeridinium Gómez, López-García, Takayama & Moreira (2) (**1**)
Dissodinium Klebs (2)
Grammatodinium Li & Shin (1)
Lebouridinium Gómez, Takayama, Moreira & López (1) (**1**)
Levanderina Moestrup, Hakanen, Hansen, Daugbjerg & Ellegaard (1) (**1**)

recientemente erigida por Moestrup y Calado (2018), agrupa únicamente al género *Gyrodinium*. Actualmente se tienen descritas 105 especies, pero ninguna ha sido reportada como formadora de florecimientos. La familia Kareniaceae aloja seis géneros y un total de 37 especies. Los géneros con el mayor número de especies y de importancia ecológica son: *Karenia* (género tipo de la familia Kareniaceae), *Karlodinium* y *Takayama* Salas, Bolch, Botes & Hallegraeff. *Karenia* concentra diez especies, de las cuales nueve forman FAN (con excepción de *K. astericroma* Salas, Bolch & Hallegraeff). *Karlodinium* agrupa 16 especies, de las cuales solo seis son formadoras de FAN (*K. armiger* Bergholtz, Daugbjerg & Moestrup, *K. conicum* Salas, *K. corsicum* (Paulmier, Berland, Billard & Nezan) Siano & Zingone, *K. digitatum* (Yang, Takayama, Matsuoka & Hodkiss) Gu, Chan & Lu, *K. gentienii* Nézan, Chomérat & Siano y *K. veneficum* (Ballantine) Larsen) (Hansen, 2021). *Takayama* presenta siete especies, de las cuales solo *T. cladochroma* (Larsen) Salas, Bolch & Hallegraeff es nociva. Finalmente, la familia Warnowiaceae, descrita por Lindemann en 1928, aloja 8 géneros y 49 especies. El género tipo para esta familia y con el mayor número de especies es *Warnowia* Lindemann, con un total de 25 especies descritas; ninguna de ellas ha sido reportada como formadora de FAN.

Diversidad de Gymnodiniales en México

La diversidad actual de dinoflagelados atecados del orden Gymnodiniales para el PM es de 103 especies (Apéndice), lo que representa 16% del total de especies actualmente válidas para este orden. En total se obtuvo información correspondiente a 189 registros de dinoflagelados atecados (Cuadro 2) distribuidos a lo largo del litoral del PM. A continuación, se describe la flora reportada para cada una de las regiones del PM.

Región 1

Esta región comprende la costa oeste de la Península de Baja California. Los sitios donde se ha reportado la presencia de dinoflagelados Gymnodiniales son: Bahía Todos Santos (BTS), Baja California y el sistema lagunar Magdalena-Almejas, Baja California Sur. La diversidad de Gymnodiniales para esta región estuvo distribuida en seis familias, 13 géneros y 42 especies (Apéndice, Cuadro 2). La BTS se ubica en el municipio Ensenada, Baja California, y en ella se ha reportado la presencia de 39 especies (Almazán-Becerril et al., 2016; Escarcega-Bata et al., 2021, 2022), algunas de ellas for-

Cuadro 2: Número de familias, géneros y especies de dinoflagelados atecados, registrados en cada región y estado del Pacífico mexicano.

Región	Estado	Familias	Géneros	Especies
1-2	Baja California (costa este y oeste)	5	14	39
1-2	Baja California Sur (costa este y oeste)	9	25	51
2	Sonora	1	4	5
	Sinaloa	1	4	5
	Nayarit	1	2	2
	Jalisco	1	1	1
3	Colima	3	7	9
	Michoacán	2	2	2
4	Guerrero	9	21	45
	Oaxaca	3	5	5
	Chiapas	6	14	18

madoras de florecimientos (*Akashiwo sanguinea*, *Karenia selliformis* Haywood, Steidinger & MacKenzie, *Levanderina fissa* (Levander) Moestrup, Hakanen, Hansen, Daugbjerg & Ellegaard, *Lepidodinium chlorophorum* (Elbrächter & Schnepf) Hansen, Botes & Salas, *L. viride* Watanabe, Suda, Inouye, Sawaguchi & Chihara, *Margalefidinium fulvescens* y *Takayama tasmanica* Salas, Bolch & Hallegraeff). Las especies formadoras de FAN reportadas para el sistema lagunar Magdalena-Almejas, Baja California Sur, han sido *Akashiwo sanguinea*, *Gymnodinium catenatum* y *Margalefidinium polykrikoides* (Gárate-Lizárraga, 2005; Gárate-Lizárraga et al., 2007).

Región 2

Esta región comprende la costa oriental de la Península de Baja California, hacia el litoral costero de Sonora, Sinaloa y Nayarit (hasta los límites de Bahía de Banderas). La diversidad de Gymnodiniales para la región estuvo distribuida en nueve familias, 23 géneros y 52 especies (Apéndice, Cuadro 2). El mayor número de especies (51) ha sido reportado para la Bahía de La Paz, Baja California Sur, donde se ha registrado la formación de florecimientos algales de especies como: *Akashiwo sanguinea*, *Gymnodinium catenatum, Margalefidinium polykrikoides, M. fulvescens, Polykrikos hartamanni* y *Levanderina fissa* (Garate-Lizárraga et al., 2004, 2009a, 2011a; Band-Schmidt et al., 2011; Gárate-Lizárraga, 2014c, 2020). Para la costa de Sonora, solo hay reportes de cuatro especies formadoras de florecimientos (*A. sanguinea*, *G. catenatum*, *Levanderina fissa* y *M. polykrikoides*). Sin embargo, solo *G. catenatum* ha formado FAN en esta zona (Alonso-Rodríguez et al., 2004). Para la costa de Sinaloa se tiene registro de seis especies de Gymnodiniales, pero solo *A. sanguinea*, *G. catenatum*, *M. fulvescens* y *M. polykrikoides* han formado FAN. En el caso particular de la Bahía de Mazatlán, hay investigaciones durante varias décadas sobre la formación de grandes florecimientos de la especie *G. catenatum* (Cortés-Altamirano, 1987, 2002; Cortés-Altamirano y Licea-Durán, 1999, 2004; Alonso-Rodríguez et al., 2008; Morquecho-Escamilla y Alonso-Rodríguez, 2008). En cuanto al litoral costero de Nayarit, solo hay registro de tres especies de Gymnodiniales: *A. sanguinea, G. catenatum* y *Margalefidinium catenatum.* En el caso de estas dos últimas especies, ambas han formado grandes florecimientos en Bahía de Banderas (Cortés-Lara, 2002; Cortés-Lara et al., 2004, 2012, 2015, 2017).

Región 3

Esta región corresponde al Pacífico Tropical Mexicano (PTM) y representa aproximadamente 40% del PM. Varios autores indican que el PTM inicia en punta de Cabo Corrientes, Jalisco, y se extiende hasta los márgenes del Golfo de Tehuantepec (De la Lanza, 1991; Espinoza-Ávalos, 1993; Serviere-Zaragoza et al., 1993; Hernández-Becerril et al., 2003). La diversidad de dinoflagelados Gymnodiniales reportada para la región estuvo distribuida en nueve familias, 23 géneros y 47 especies (Apéndice, Cuadro 2). La Bahía de Acapulco, Guerrero, fue el sitio con el mayor número de especies para este orden. Meave del Castillo et al. (2012) reportaron la presencia de 32 especies distribuidas dentro y fuera de la Bahía. Por otro lado, Gárate-Lizárraga et al. (2009b, 2011b, 2013, 2016) han registrado florecimientos de las especies *A. sanguinea*, *G. catenatum*, *Karenia* sp., *Levanderina fissa*, *Margalefidinium polykrikoides* y *Polykrikos* sp. para este mismo sitio. Para la costa de Guerrero, Escobar-Morales y Hernández-Becerril (2015) solo registraron 11 especies. De acuerdo con nuestros resultados, las especies formadoras de FAN, *Gymnodinium catenatum*, *Karenia bicuneiformis* Botes, Sym & Pitcher, *K. mikimotoi* (Miyake & Kominami ex

Oda) Hansen & Moestrup, *K. selliformis*, *Levanderina fissa* y *Margalefidinium polykrikoides*, tienen un amplio rango de distribución a lo largo del litoral costero de esta región.

Región 4

Esta región corresponde al Golfo de Tehuantepec (De la Lanza, 1991), que comprende los estados de Chiapas y parte del litoral costero de Oaxaca. La diversidad de dinoflagelados del orden Gymnodiniales para la región estuvo distribuida en seis familias, 14 géneros y 19 especies (Apéndice, Cuadro 2). Maciel-Baltazar y Hernández-Becerril (2013) reportaron 17 especies para las costas de Chiapas, mientras que Maciel-Baltazar (2015) registraron cinco especies tóxicas (*G. catenatum*, *K. bicuneiformis* Botes, Sym & Pitcher, *K. brevisulcata* (Chang) Hansen & Moestrup, *K. papilionacea* Haywood & Steidinger y *K. selliformis* Haywood, Steidinger & MacKenzie), también para las costas de Chiapas. En el caso de las costas de Oaxaca, existen reportes de FAN de *Margalefidinium polykrikoides* (Santiago-Morales, 2016). De acuerdo con nuestros resultados, las especies formadoras de FAN *A. sanguinea*, *G. catenatum* y *L. fissa* se encuentran distribuidas a lo largo de esta región.

Curva de acumulación de especies

El primer registro del orden Gymnodiniales para el Pacífico Mexicano fue *Gymnodinium catenatum* reportado para el Golfo de California en 1943. Sin embargo, fue hasta 1980 cuando un mayor número de registros para este orden comenzó a conocerse. En la figura 3 se presenta por década (a partir de los 80´s, ya que no hay registros desde 1943 hasta 1980) el conocimiento acumulado de registros, géneros y especies (actualmente taxonómicamente válidas en Guiry y Guiry, 2022) de dinoflagelados del orden Gymnodiniales reportados para las costas del Pacífico Mexicano. Durante la primera década de 1980-1989, hubo un total de 42 registros, pero solo se reportó la presencia de cuatro géneros y cuatro especies, esto a partir del trabajo realizado por Gárate-Lizárraga et al. (2007). Para la siguiente década (1990-1999), el número de registros disminuyó considerablemente, Hernández-Becerril y Bravo-Sierra (2004) reportaron cinco registros (4 géneros y 4 especies) para diferentes localidades del PM. Como se observa en la figura 3, a partir de la década de 2000-2009, se observa un considerable incremento en el número de registros (98), géneros (20) y especies (39). Este aumento se debe principalmente a la contribución de los trabajos realizados por Gárate-Lizárraga et al. (2009a), Maciel-Baltazar y Hernández-Becerril (2013), Escobar-Morales y Hernández-Becerril (2015) y Gárate-Lizárraga (2020) para diferentes localidades del PM. Sin embargo, el máximo número de registros (171) se observa en la década de 2010-2019, donde se reportó la presencia de 25 géneros y 63 especies. Finalmente, para el periodo comprendido de 2020-2022 solo hubo 65 registros, 15 géneros y 41 especies, principalmente para la costa de la Península de Baja California (Gárate-Lizárraga, 2020; Escarcega-Bata et al., 2021, 2022).

En conclusión, con base en la revisión bibliográfica a partir de registros fotográficos, el conocimiento taxonómico actual de dinoflagelados Gymnodiniales para el Pacífico Mexicano (10 familias, 31 géneros y 103 especies) resulta mínimo en comparación con el total de especies registradas y descritas alrededor del mundo. Esto sugiere que es necesario implementar un mayor esfuerzo de muestreo e incursionar en diferentes técnicas de identificación (PCR de una célula, secuenciación masiva, etc.), para poder actualizar la diversidad de dinoflagelados Gymnodiniales a lo largo del litoral del Pacifico Mexicano.

Contribución de autores

AEB y AS concibieron y diseñaron el estudio. AEB recopiló la información, la analizó e integro la base de datos y resultados. AS y MEZR administraron los proyectos y se encargaron de la adquisición de fondos. AEB, MLNR, MCRT, KMD, MEZR y AS contribuyeron a la redacción, discusión, revisión y aprobación del manuscrito final.

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Apéndice: Listado florístico y distribución por estado de las familias, géneros y especies del orden Gymnodiniales en el Pacífico Mexicano. BC, Baja California; BCS, Baja California Sur; SON, Sonora; SIN, Sinaloa; NAY, Nayarit; JAL, Jalisco; COL, Colima; MICH, Michoacán; GRO, Guerrero; OAX, Oaxaca; CHIS, Chiapas. La letra "X" indica que el registro fue reportado en la región y cuenta con respaldo fotográfico. El asterisco (*) indica que el registro fue reportado para la costa este de la Península de Baja California. 1=Alonso-Rodríguez (2003), 2=Alonso-Rodríguez et al. (2004), 3=Alonso-Rodríguez et al. (2008), 4=Acosta-Chamorro et al. (2016), 5=Almazán-Becerril et al. (2016), 6=Cortés-Altamirano (1987), 7=Cortés-Altamirano (2002), 8=Cortés-Altamirano y Licea-Durán (1999), 9=Cortés-Lara (2002), 10=Cortés-Lara et al. (2004), 11=Cortés-Lara et al. (2012), 12=Cortés-Lara et al. (2017), 13=Escobar-Morales y Hernández-Becerril (2015), 14=Escarcega-Bata et al. (2021), 15=Escarcega-Bata et al. (2022), 16=Figueroa-Torres y Zepeda-Esquivel (2001), 17=Gárate-Lizárraga et al. (2004), 18=Gárate-Lizárraga (2005), 19=Gárate-Lizárraga et al. (2007), 20=Gárate-Lizárraga y Verdugo-Díaz (2007), 21=Gárate-Lizárraga et al. (2010a), 22=Gárate-Lizárraga et al. (2010b), 23=Garate-Lizárraga et al. (2011a), 24=Gárate-Lizárraga (2012a), 25=Gárate-Lizárraga (2012b), 26=Garate-Lizárraga et al. (2013), 27=Gárate-Lizárraga (2014a), 28=Gárate-Lizárraga (2014b), 29=Gárate-Lizárraga (2014c), 30=Gárate-Lizárraga et al. (2016), 31=Gárate-Lizárraga et al. (2019), 32=Gárate-Lizárraga (2020), 33=Hernández-Becerril y Bravo-Sierra (2004), 34=Hernández-Becerril et al. (2007), 35=Herrera-Silveira (1999), 36=Licea et al. (1995), 37=López-Cortés et al. (2014), 38=Maciel-Baltazar y Hernández-Becerril (2013), 39=Maciel-Baltazar (2015), 40=Meave del Castillo et al. (2012), 41=Morales-Blake et al. (2001), 42=Morquecho-Escamilla y Alonso-Rodríguez (2008), 43=Santiago-Morales (2016).

Taxones	REGIÓN 1-2		RE 2				REGIÓN 3			REGIÓN 4		Referencias
	BC	BCS	SON	SIN	NAY	JAL	COL	MICH	GRO	OAX	CHIS	
Actiniscaceae												
1. *Actiniscus pentasterias* (Ehrenberg) Ehrenberg		*								X	X	24, 28, 32, 33
Amphitholaceae												
2. *Monaster rete* Schütt		*							X			28, 32, 33, 40
Brachidiniaceae												
3. *Brachidinium capitatum* Taylor		X*									X	24, 32, 33, 38
4. *Gynogonadinium aequatoriale* Gómez									X		X	39, 40
Ceratoperidiniaceae												
5. *Ceratoperidinium margalefii* Loeblich III									X			40
6. *Kirithra sigma* Hu, Deng, Iwataki, Luo, Wang, Sun, Zhao, Gu, Shin & Tang	X											15
7. *Pseliodinium fusus* (Schütt) Gómez	X	X*							X		X	13, 14, 15, 19, 21, 24, 28, 32, 3
8. *Torquentidium convolutum* (Kofoid & Swezy) Shin, Li, Lee & Matsuoka	X	*							X			5, 23, 40
9. *T. flavescens* (Kofoid & Swezy) Shin, Zhun & Matsuoka	X											14, 15
10. *T. helix* (Pouchet) Shin, Li, Lee & Matsuoka		*									X	23, 32, 38
11. *T. pirum* (Schütt) Shin, Li, Lee & Matsuoka		*										28, 32
Chytriodiniaceae												
12. *Chytriodinium affine* (Dogiel) Chatton									X			40
Gymnodiniaceae												
13. *Akashiwo sanguinea* (Hirasaka) Hansen & Moestrup	X	X*	X	X	X		X		X	X	X	1, 2, 5, 6, 7, 13 14, 15, 18, 1 , 28, 29, 30, 32, 35, 38
14. *Ankistrodinium semilunatum* (Herdman) Hoppenrath, Murray, Sparmann & Leander		*										29
15. *Cochlodinium faurei* Kofoid & Swezy		*										27
16. *C. pulchellum* Lebour		*							X	X	X	13, 26, 38

Apéndice: continuación.

Taxones	**REGIÓN 1-2**		**REGIÓN 2**			**REGIÓN 3**				**REGIÓN 4**		**Referencias**
	BC	BCS	SON	SIN	NAY	JAL	COL	MICH	GRO	OAX	CHIS	
17. *C. virescens* Kofoid & Swezy		*										25
18. *C. rosaceum* Kofoid & Swezy		*										27
19. *Gymnodinium allophron* Larsen									X			40
20. *G. auratum* Kofoid & Swezy	X											15
21. *G. aureolum* (Hulburt) Hansen									X			40
22. *G. aureum* Kofoid & Swezy									X			40
23. *G. catenatum* H.W.Graham		X*	X	X	X	X	X	X	X		X	2, 4, 6, 13, 19, 24, 26, 28, 30, 32, 34, 38, 39, 40
24. *G. contractum* Kofoid & Swezy	X											15
25. *G. gelbum* Kofoid		*										28
26. *G. grammaticum* (Pouchet) Kofoid & Swezy									X			40
27. *G. heterostriatum* Kofoid & Swezy	X											15
28. *G. impudicum* (Fraga & Bravo) Hansen & Moestrup	X											15
29. *G. incisum* Kofoid & Swezy	X											15
30. *G. inusitatum* Gu, Liu, Vale & Luo	X	*										15, 32
31. *G. incoloratum* Conrad & Kufferath			X									8
32. *G. multiestriatum* Kofoid & Swezy	X											15
33. *G. pyrenoidosum* Horiguchi & Chihara	X											15
34. *G. translucens* Kofoid & Swezy									X			40
35. *G. ravenescens* Kofoid & Swezy	X											15, 40
36. *G. sphaericum* Kofoid & Swezy	X											15, 40
37. *G. venator* Jørgensen & Murray		*										31
38. *Lepidodinium chlorophorum* (Elbrächter & Schnepf) Hansen, Botes & Salas	X	*										15, 28
39. *L. viride* Watanabe, Suda, Inouye, Sawaguchi & Chihara	X	*										14
40. *Margalefidinium catenatum* (Okamura) Gómez, Richlen & Anderson					X							9, 10
41. *M. fulvescens* (Iwataki, Kawami & Matsuoka) Gómez, Richlen & Anderson	X	*		X					X			5, 14, 15, 24,27,28 32, 40, 42
42. *M. polykrikoides* (Margalef) Gómez, Richlen & Anderson		X*	X	X			X		X	X	X	2, 13, 16, 1 , 18, 24, 27, 2 , 30, 32, 34, 35, 38, 40, 41, 43
Gyrodiniaceae												
43. *Gyrodinium acutum* (Schütt) Kofoid & Swezy		*							X			28, 40
44. *G. bepo* Schütt	X											15
45. *G. britannicum* Kofoid & Swezy	X								X			15, 40

Apéndice: continuación.

Taxones	REGIÓN 1-2		REGIÓN 2			REGIÓN 3				REGIÓN 4		Referencias
	BC	BCS	SON	SIN	NAY	JAL	COL	MICH	GRO	OAX	CHIS	
46. *G. capsulatum* Kofoid & Swezy	X											15
47. *G. cochlea* Lebour									X			13
48. *G. corallinum* Kofoid & Swezy	X											15
49. *G. dominans* Hulbert	X	*										15, 24
50. *G. dorsum* Kofoid & Swezy	X											15
51. *G. fissoides* Elbrachter		*										24
52. *G. foliaceum* Kofoid & Swezy									X			40
53. *G. fusiforme* Kofoid & Swezy	X	*										15, 24
54. *G. fusus* (Meunier) Akselman									X			40
55. *G. glaebum* Hulburt									X			13
56. *G. grave* (Meunier) Kofoid & Swezy	X											15
57. *G. lacryma* (Meunier) Kofoid & Swezy	X	*										15, 28, 29, 32
58. *G. maculatum* Kofoid & Swezy	X											15
59. *G. metum* Hulburt									X			13
60. *G. obtusum* Kofoid & Swezy	X											15
61. *G. ochraceum* Kofoid & Swezy									X			40
62. *G. parvulum* (Schütt) Kofoid & Swezy									X			40
63. *G. pepo* (Schütt) Kofoid & Swezy		*							X			32, 40
64. *G. pingue* (Schütt) Kofoid & Swezy	X											15
65. *G. rubrum* (Kofoid & Swezy) Takano & Horiguichi		*										28, 32
66. *G. spirale* (Bergh) Kofoid & Swezy	X	X*					X					5, 13, 15, 18, 19, 24, 28, 32, 36, 40
67. *G. submarinum* Kofoid & Swezy		*										24
68. *G. virgatum* Kofoid & Swezy	X											15
Kareniaceae												
69. *Asterodinium gracile* Sournia		*									X	20, 32, 38
70. *A. spinosum* Sournia								X				33
71. *Karenia asterichroma* Salas, Bolch & Hallegraeff									X		X	38, 40
72. *K. bicuneiformis* Botes, Sym & Pitcher		*					X		X		X	13, 28, 32, 38, 39, 40
73. *K. brevis* (Davis) Hansen & Moestrup		*							X			32, 40
74. *K. brevisulcata* (Chang) Hansen & Moestrup									X		X	39, 40
75. *K. mikimotoi* (Miyake & Kominami ex Oda) Hansen & Moestrup		*					X		X	X		13, 30, 38, 40
76. *K. papilionacea* Haywood & Steidinger									X		X	38, 39, 40
77. *K. selliformis* Haywood, Steidinger & MacKenzie	X						X		X		X	5, 13, 39, 40
78. *Karlodinium ballantinum* Salas									X			13
79. *K. australe* Salas, Bolch & Hallegraeff	X											15

Apéndice: continuación.

Taxones	REGIÓN 1-2		REGIÓN 2			REGIÓN 3				REGIÓN 4		Referencias
	BC	BCS	SON	SIN	NAY	JAL	COL	MICH	GRO	OAX	CHIS	
80. *K. veneficum* (Ballantine) Larsen									X			13
81. *Takayama cladochroma* (Larsen) Salas, Bolch & Hallegraeff									X			40
82. *T. helix* Salas, Bolch & Hallegraeff	X											15
83. *T. pulchella* Salas, Bolch & Hallegraeff		*										32
84. *T. tasmanica* Salas, Bolch & Hallegraeff	X	*										14, 28
Polykrikaceae												
85. *Polykrikos hartmannii* Zimmermann		*									X	13, 24, 28, 30, 32, 38
86. *P. kofoidii* Chatton	X								X			5, 15, 40
87. *P. schwartzii* Bütschli		*										24
Warnowiaceae												
88. *Erythropsidinium agile* (Hertwig) Silva		*							X			22, 29, 32, 40
89. *Greuetodinium cylindricum* (Greuet) Loeblich III									X			40
90. *Nematodinium armatum* (Dogiel) Kofoid & Swezy		*										24
91. *N. torpedo* Kofoid & Swezy		*							X			24, 40
92. *Nematopsides vigilans* (Marshall) Greuet		*										24
93. *Warnowia juno* (Schütt) Schiller									X			40
94. *W. polyphemus* (Pouchet) Schiller		*										24
95. *W. pouchetii* (Kofoid & Swezy) Schiller		*										24
96. *W. maxima* (Kofoid & Swezy) Lindemann									X			40
97. *W. violescens* (Kofoid & Swezy) Lindemann		*										24
Incertae sedis												
98. *Balechina glacilis* (Bergh) Gómez, Artigas & Gast	X	*										5, 13, 14, 15, 28, 29, 32
99. *Dissodinium pseudolunula* Swift ex Elbrächter & Drebes		*										32
100. *Cucumeridinium coeruleum* (Dogiel) Gomez, López-García, Takayama & Moreira		*					X		X		X	13, 24, 28, 29, 32, 38
101. *Cucumeridinium cucumis* (Schütt) Gómez, López-García, Takayama & Moreira		*										32
102. *Lebouridinium glaucum* (Lebour) Gómez, Takayam, Moreira & López-García		*							X			13, 24
103. *Levanderina fissa* (Levander) Moestrup, Hakanen, Hansen, Daugbjerg & Ellegaard	X	*	X	X			X		X		X	1, 2, 13, 15, 24, 26, 29, 32, 38

8. Capítulo 2

Primer reporte de *Grammatodinium* (Dinophyceae) para la costa del Pacífico Americano Oriental: confirmación morfológica, molecular y ecológica

First record of *Grammatodinium* (Dinophyceae) for the American Eastern Pacific coast: Morphological, molecular and ecological confirmation

RESUMEN

Grammatodinium Li & Shin es un género monoespecífico descrito en la zona de la bahía de Tongyeong en Corea. En este estudio, describimos por primera vez su presencia en la costa del Pacífico oriental americano, particularmente en la bahía de Acapulco, México, utilizando datos morfológicos, moleculares y ambientales. Las secuencias generadas en este estudio con SSU y LSU formaron un grupo monofilético con otras secuencias de GenBank pertenecientes a *Gr*. *tongyeonginum*, la única especie conocida del género; sin embargo, los valores de distancia genética entre esta especie y nuestros especímenes (8.5% SSU; 2.8% LSU) fueron equivalentes o incluso mayores que los reportados en otros géneros de dinoflagelados. Nuestra filogenia mostró claramente la relación de *Grammatodinium* con las familias Pyrocystaceae y Gonyaulacaceae. En nuestros especímenes, las células aparecieron individualmente y en colonias de hasta 16 células, observadas principalmente durante la época seca, por lo que podrían confundirse con *Gymnodinium catenatum*, un dinoflagelado común en Acapulco con el que pueden coexistir y compartir su apariencia general. Sin embargo, se diferencian claramente por la presencia de surcos longitudinales en todo el cuerpo y una coloración verde amarillenta, ambos ausentes en *Gymnodinium catenatum*. Si bien nuestra evidencia sugiere firmemente la presencia de una nueva especie para la región, se requieren estudios morfológicos más detallados para confirmar esta afirmación.

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First record of *Grammatodinium* (Dinophyceae) for the American Eastern Pacific coast: Morphological, molecular and ecological confirmation

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Abstract

Grammatodinium Li & Shin is a monospecific genus described from the Tongyeong Bay area in Korea. In the current study, we describe its presence in the American Eastern Pacific coast for the first time, particularly in Acapulco Bay, Mexico, using morphological, molecular and environmental data. Sequences generated in this study with SSU and LSU formed a monophyletic group with other sequences from GenBank belonging to *Gr. tongyeonginum*, the only species known for the genus; however, genetic distance values between this species and our specimens (8.5% SSU; 2.8% LSU) were equivalent or even greater than those reported in other genera of dinoflagellates. Our phylogeny clearly showed the relationship of *Grammatodinium* with the families Pyrocystaceae and Gonyaulacaceae. In our specimens, cells appeared individually and in colonies of up to 16 cells, which were observed mainly during the dry season, so they could be confused with *Gymnodinium catenatum*, a common dinoflagellate in Acapulco with which they can coexist and share their general appearance, but they are clearly differentiated by the presence of longitudinal furrows throughout the body and a yellowish-green coloration, both absent in *Gymnodinium catenatum*. Although our evidence strongly suggests the presence of a new species for the region, more detailed morphological examinations are needed to confirm this statement.

Keywords: LSU; Phylogeny; Phytoplankton; Single cell PCR; SSU

Introduction

Dinoflagellates constitute the second-most important group within marine phytoplankton due to their high diversity and abundance (mostly in tropical environments). Currently, this group contains approximately 2,500 living species, distributed in 259 genera (Gómez, 2012; Guiry and Guiry, 2022). The traditional morphological identification of these organisms has been based mainly on the characteristics of their cell wall. Currently, dinoflagellates are classified in two non-natural groups: thecate and a thecate, these latter mainly characterized by having a very fragile cell wall composed of thin and flattened vesicles (Fensome et al., 1993).

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Most of the athecate species (635 species) belong to the order Gymnodiniales Apstein, whose type genus is *Gymnodnium* Stein (Guiry and Guiry, 2022). However, its current classification continues to be subject to important taxonomic changes, since in phylogenetic studies carried out mainly with rDNA, the polyphyletic nature of the genus and the order Gymnodiniales is clear (Daugbjerg et al., 2000; Gómez et al., 2011; Escarcega-Bata et al., 2021).

Recently, the germination of resistance cysts collected in surface sediments from the coastal area of Tongyeong, Korea, gave rise to the description of a new genus and species *Gramatodinium tongyeonginum* Li & Shin, which morphologically seems to correspond to an athecate dinoflagellate; however, it is genetically closer to the order Gonyaulacales (Li et al., 2017). Morphological studies in combination with molecular tools from multiple markers, show that the order Gonyaulacales currently consists of 736 living species (Guiry and Guiry, 2022), which makes it the second most diverse order within the Dinophyceae class. However, taxonomic difficulties persist with the identification of these species, which has led to the suggestion of new characters (cyst shape, use of genes encoding for phycotoxins, etc.) (Mertens et al., 2020). Since its circumscription, *Grammatodinium* Li & Shin has not been recorded again, much less outside from its original distribution range (Li et al., 2017). In Acapulco Bay Mexico, which constitutes the most diverse region with the occurrence of approximately 70 % of the known species in the Mexican Pacific (Meave et al., 2012), an organism forming long cell chains was observed, similar at first sight to *Gymnodinium catenatum* Graham, however, its morphological, molecular, and ecological data confirm its position within the genus *Grammatodinium*, therefore, in this study we present the first record of the genus in the American Eastern Pacific.

Material and methods

Study area

Acapulco Bay is located on the coast of the state of Guerrero (16º 47^{0} 00^{00}- 16º 52^{0} 00^{00} N, 99º 50^{0} 52^{00} – 99º 56^{0} 00^{00} W) in the southern portion of the Mexican Pacific (MP). Phytoplankton was sampled at four sites (Fig. 1): Station (St.) Muelle (16º 50^{0} 29.10^{00} N, 99º 54^{0} 05.16^{00} W, 10 m maximum depth), St. Centro (16º 50^{0} 59.45^{00} N, 99º52^{0} 30.96^{00} W, 30 m maximum depth), St. Naval (16º 50^{0} 18.33^{00} N, 99º 51^{0} 27.16^{00} W, 13 m maximum depth) and St. Sinfonía (16º 50^{0} 23.81^{00} N, 99º 55^{0} 20.03^{00} W, 31 m maximum depth).

Collection of phytoplankton material

The phytoplankton collections were realized during the years 2010, 2011, 2014, 2017 and 2018 (covering different seasons and months: March, April, September and November) using a boat with an outboard motor. Samples were collected *in vivo* by anchoring the boat at the sampling stations. Seawater was obtained with a Van Dorn bottle at a depth of 1 to 5 m and then transferred to a 10*L* plastic bottle filled, and kept in a cooler without ice to protect the organisms from the light and heat.

Sample processing

Each collected sample was filtered with a reverse filtration system, an aliquot of 100 ml was taken for observation in a Leica DM750 (Germany) brightfield microscope at low magnification (10x) and without coverslips. Cells of interest were isolated using the reduced micropipette technique. In May 2017, a chain of two cells was isolated and in March 2018, a solitary cell with an unarmored appearance was also found, with some resemblance to *Gymnodinium catenatum*, both isolates were rinsed 3 or four times with local filtered seawater; the isolated cells were deposited in 0.2 Ll PCR tubes and frozen at −20º C for their subsequent molecular analysis. Micrographs were taken with a Canon Powershot sx600 camera (Taiwan).

Morphological analysis

Thirty cells taken at random were videotaped *in vivo* with the software Video to Picture V5. Videos were transformed into photographs to obtain measurements for qualitative morphology: life form, cell shape, total length (TL), epicone length (eL), hypocone length (hL), maximum width (Mw) evaluated at the base of epicone near to the cingulum, transdiameter (Tr), cell thickness, length and width of the nucleus, cingulum width and displacement, type and color of chloroplasts, nucleus shape and presence longitudinal furrows and red pigment accumulation bodies. In addition, the following ratios were calculated: total length (TL) of the cell divided by the transdiameter (Tr) (TL/Tr) and epicone length divided by the hypocone length (eL/hL). All the characters evaluated in this work were compared with the material from Korea (Li et al., 2017). The quantitative characters omitted (eL, hL, Tr, el/hL and TL/TR) by Li et al. (2017) in the original description, were measured directly from the photographs shown in their manuscript, taking the scale bar indicated by the authors as a reference.

PCR and phylogenetic analysis

Single-cell PCR was performed directly, following the protocol described by Hernández-Rosas et al. (2018). The ribosomal SSU marker was amplified using the primer pairs SR4-F and SR9-R (Matsuoka et al., 2006). The LSU was amplified using the primer pairs D1R-F and D2C-R (Scholin et al., 1994). These markers were selected because of the number of sequences of Gonyaulacales available in GenBank with which to compare our specimens. PCR

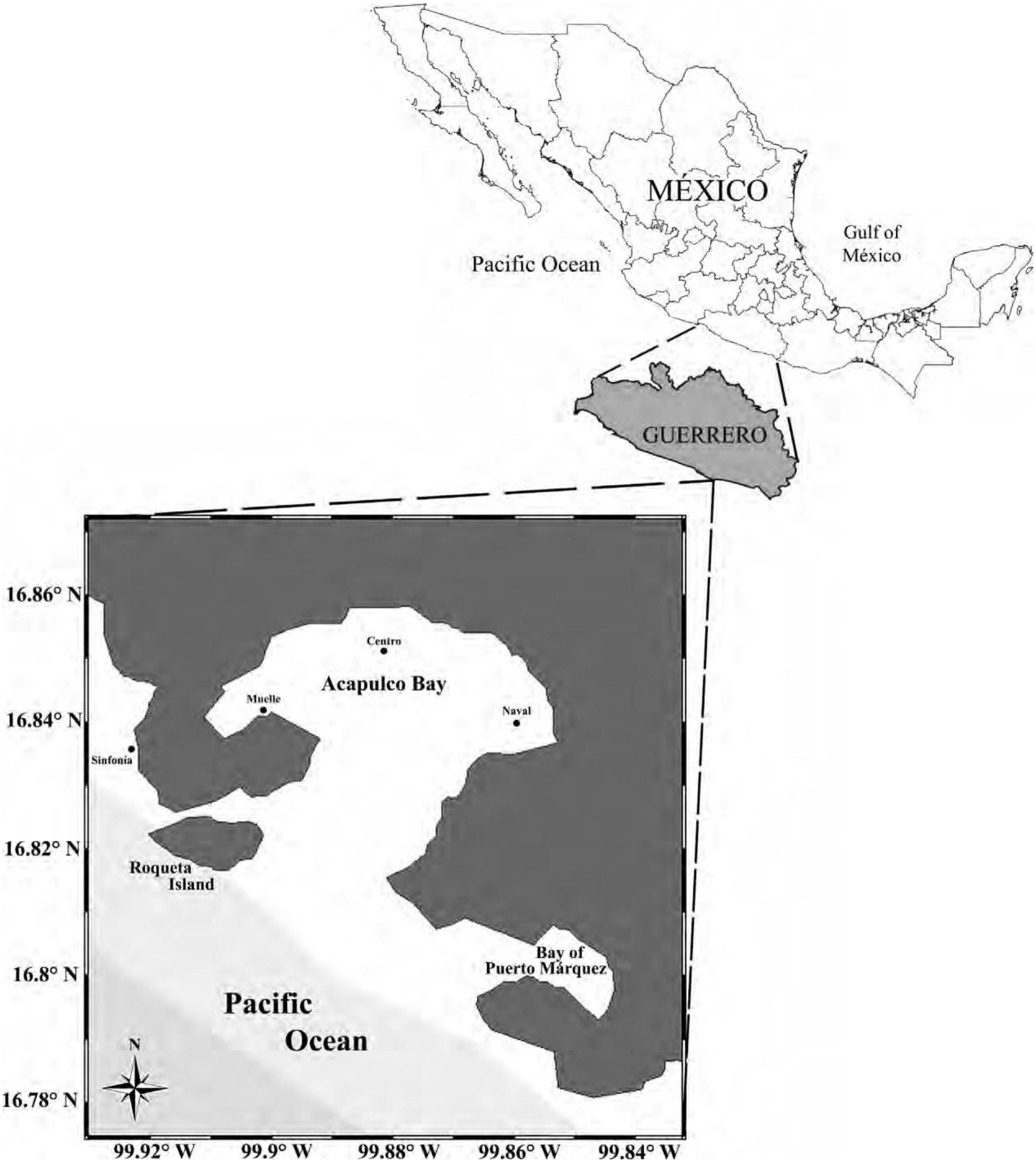

Fig. 1. Map of the study area with sampling stations, inside and outside Acapulco Bay. The black dots show the collection stations.

amplification was confirmed by electrophoresis with 1 % agarose gel for 20 min at 90 V, the staining was performed with GelRed® (Biotium). Visualization was by a gel documentation system with UV light. PCR products were purified using ExoSAP-IT (USB, Cleveland, Ohio, USA) and sequenced commercially in Macrogen Inc. (Seoul, Korea). The same set of primers was used for sequencing.

Sequences were assembled and edited using the program Sequencher® 5.4.5. The final alignment including sequences of other species of Gonyaulacales from GenBank, was performed using Bioedit (Hall, 1999). *Syndinium turbo* Chatton and *Hematodinium perezii* Chatton & Poisson were used as outgroups for both markers. These species were selected as they belong to different orders. We analyzed the SSU and LSU data sets both separately and then concatenated to give a combined SSU-LSU data set. In the combined SSU-LSU data set, taxa for which SSU or LSU sequence data were unavailable, therefore they were treated as missing data. Phylogenetic analyses using maximum likelihood (ML) and Bayesian inference (BI) were performed separately, partitioning the data into codon positions. The selected evolutionary model was JC (Jukes Cantor), determined based on the Maximum Likelihood ratio test implemented by TOPALi version 2 software (Milne et al., 2009). ML analysis was performed using RAxML software (Stamatakis, 2006). Support for each branch was obtained from 1000 bootstrap replications. BI analysis was performed using MrBayes 3.2.2 (Ronquist et al., 2012). Four chains of Markov chain Monte Carlo were used, starting with a random tree and sampling the data every 500 generations for 5×10^6 generations, 25 % of the trees were discarded as burn-in. Pairwise distance values (p distances) were calculated using the software Mega version 5 (Tamura et al., 2011).

Measurement of environmental parameters and temporal series

Simultaneously with phytoplankton collections, the environmental parameters of water (temperature, salinity, and dissolved oxygen) were evaluated with multiparameter probes (YSI-556 MPS and YSI-550A), calibrated every day according to the manufacturer's recommendation. In addition, the transparency of the water column was measured with a marine Secchi disc (completely white). In several collections, the nutrient concentration was also evaluated for orthophosphates, ammonium, nitrites, nitrates and silicates. The determination of nutrients was carried out in situ filtering 1*L* water through GF/F filters of 0.7 µm of pore, freezing it until processed at the laboratory by the following techniques: ascorbic acid-molybdate for orthophosphates (Murphy and Riley 1962), indophenol blue for ammonium (Solórzano, 1969), reduction by Cd-Cu columns for nitrates plus nitrites (Strickland and Parsons 1972), and p-silicomolybdic acid for silicates (Schwartz, 1942). The time series were elaborated with the Ocean Data View software version 5.1.7 (Schlitzer, 2019), using data obtained from temperature, salinity, and dissolved oxygen, only for the years where *Grammatodinium* sp. was observed.

Results

Morphology

Grammatodinium sp. (Fig. 2A-J).

Description: Solitary cells (Fig. 2B- D and G) or forming chains from 2 to 16 cells (the apex of the cell is attached halfway to the cingulum of adjacent cell) (Fig. 2A and E-F), some cells are round, usually with elongated ellipsoidal shape (Fig. 2B-D and G); total length: 39.7–65.7 µm ($\bar{x}$ = 50.8, *n* = 30); transdiameter: 17.9–34.8 µm (*x* = 26, *n* = 30); ratio TL/Tr = 1.6–2.4; Ratio eL/hL = 1.0–2.0. Epicone longer than hypocone (Fig. 2B-C and G; Table 1); flattened apex (Fig. 2B, D and E) and antapex slightly bilobed, sometimes asymmetric with the right lobe more prominent and sometimes slightly directed out of body (Fig. 2A-C and G). *Cingulum* narrow and slightly excavated, descending, displaced its width by 2.3–2.6 times (Fig. 2B and G; Table 1). *Sulcus* narrow, more evident and wider in the hypocone, crossing the organism from the epicone to the antapex (Fig. 2C and G). Multiple spherical-shape chloroplasts, with yellow-green coloration (Fig. 2C and E). Elliptical nucleus, located from the middle portion of epicone and up to the cingulum (Fig. 2B-C and E-G). Some cells with prominent red bodies (assimilation bodies) in the middle and lower portions of hypocone (Fig. 2A). Cell wall with evident longitudinal furrows that run throughout the organism (Fig. 2A and E-F).

Phylogenetic analysis

The genetic sequences were obtained from organisms collected in May 2017 (Fig. 2A) and February 2018 (Fig. 2G) respectively. The SSU alignment was of 393 base pairs (bp) in length, the LSU alignment of 412 bp, and the combined SSU-LSU alignment of 805 bp. In the combined SSU-LSU analysis (Fig. 3) and in both single analyses (SSU, Supplementary Fig. 1; LSU, Supplementary Fig. 2), ML and BI trees did not differ, and our samples were always placed in a monophyletic group together with *Grammatodinium tongyeonginum*. However, there were slight differences in the obtained phylogenies, mainly in the relationships at the family level, depending on the markers used, due to the sample number and species included in each analysis (Fig. 3; Supplementary Figs. 1 and 2).

The topologies resulting from all markers used (combined SSU-LSU, SSU, and LSU) showed that our specimens were placed within the order Gonyaulacales in a different family from those known until now (Fig. 3; Supplementary Figs. 1 and 2). With the combined SSU-LSU, our sequences formed a monophyletic group with the maximum phylogenetic support (BI = 1.0 %, ML = 100 %), which in turn formed a sister clade from the clade integrated by the family Pyrocystaceae (Schütt) Lemmermann, with a high phylogenetic support (BI = 1.0 %, ML = 98 %); together they formed a major and well supported clade (BI = 0.92 %, ML = 88 %) with the family Gonyaulacaceae Lindemann (Fig. 3). With SSU, our sequence also formed a monophyletic group, with the maximum phylogenetic support (BI = 1.0 %, ML = 100 %), which in turn was resolved, with a low phylogenetic support (BI = 0.85, ML = 56 %), as a sister group of the family Gonyaulacaceae; together they formed a major clade with the family Ceratiaceae Kofoid, also with high phylogenetic support (Supplementary Fig. 1). With LSU, our sequences besides forming a monophyletic group with the maximum phylogenetic support (BI = 1.0 %, ML = 100 %), were also resolved as a sister clade, also with the maximum phylogenetic support (BI = 1.0 %, ML = 100 %), of the clade integrated by the family Pyrocystaceae (Supplementary Fig. 2).

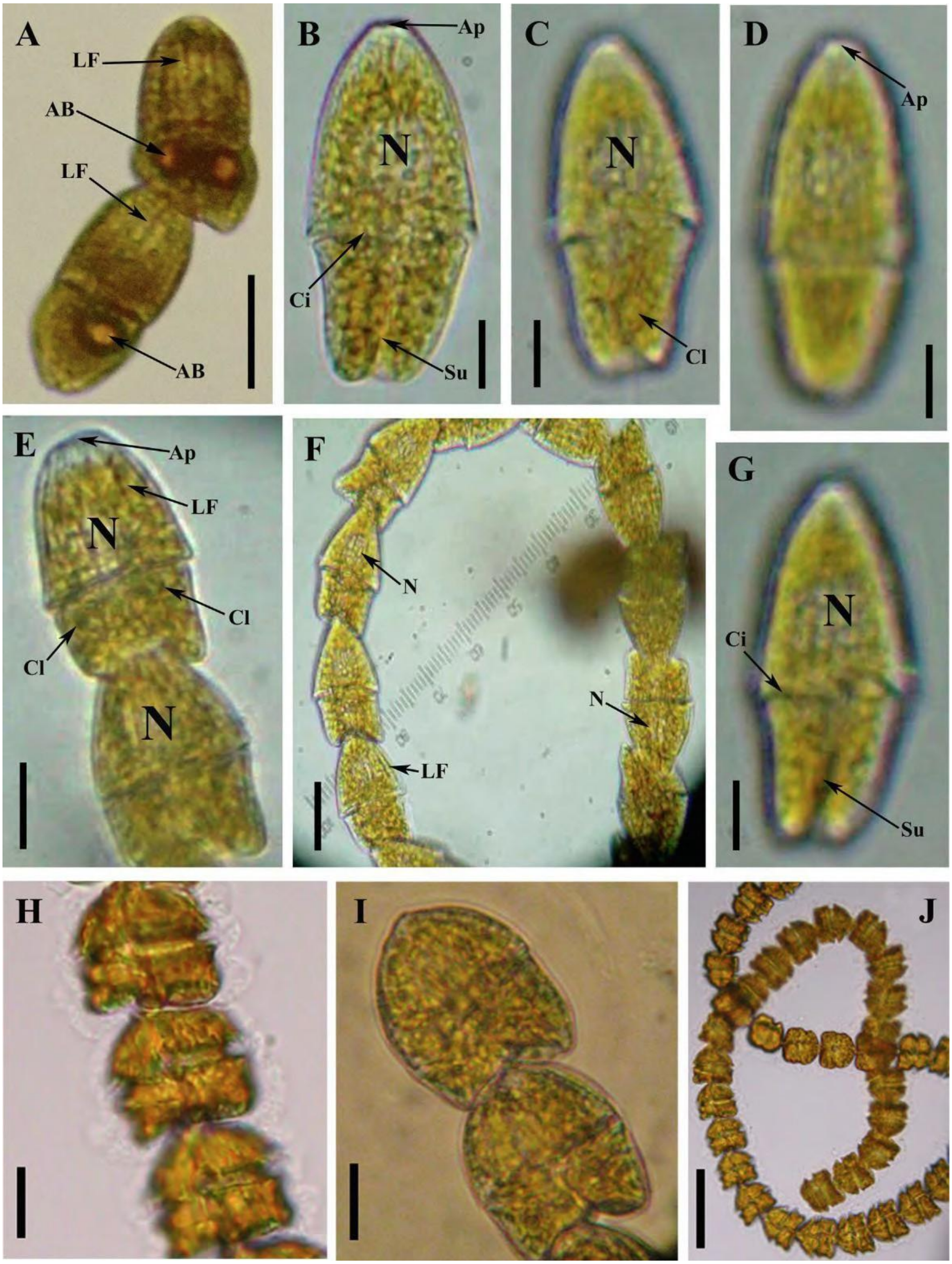

Fig. 2. A-J. Micrographs of living cells of *Grammatodinium* sp. and *Gymnodinium* catenatum collected in Acapulco Bay, Mexico. Fig. 2A-G *Grammatodinium* sp; (Fig. 2A) Two cells in chain (sequenced for LSU ARNr), in dorsal and lateral views; (Fig. 2B) Solitary cell in dorsal view; (Fig. 2C,G) Solitary cell in ventral view; (Fig. 2D) Solitary cell in lateral view; (Fig. 2E) Two cells in chain (sequenced for SSU ARNr), in dorsal view; (Fig. 2F) Chain of 12 cells showing the bilobated hypocone and the elongated nuclei in the epicone. Fig. 2H-J *Gymnodinium catenatum*; (Fig. 2H) Chain of cells in ventral view; (Fig. 2I) Chain of cells in dorsal view; (Fig. 2J). Chain of thirty-two cells in dorsal and ventral view. Apex (Ap), Cingulum (Ci), Sulcus (Su), Nucleus (N), Chloroplast (Cl), Longitudinal Furrows (LF), Assimilation Bodies (AB). Scale Bar: Figs. B-D, G, I = 10 μm; Figs. A, E = 20 μm; Fig. F, H = 25 μm; Fig. J = 100 μm.

Table 1. Morphological and morphometric data of *Grammatodinium* sp. from Acapulco Bay, Mexico and *Grammatodinium tongyeonginum* Z.Li *et* H.H.Shin from the coastal area of Tongyeong, Korea, including data from *Gymnodinium catenatum* Graham collected in Acapulco. TL = total length; eL = epicone length; hL = hypocone length; eL/hL = ratio epicone length and hypocone length; Mw: maximum width; Tr = Width at transdiameter level; TL/Tr = Proportion between the total length and the width at the transdiameter level.

	Life-form	Cell-shape	Chloroplasts	Acummulation bodies	TL (μm)	eL (μm)	hL (μm)	eL/hL	Mw (μM)	Tr (μm)	TL/Tr
Grammatodinium sp.											
Present study n = 30	Solitary or chain of up to 16 cells	Rounded or elongated, ellipsoidal with flattened apex. Antapex slightly bilobate	Many rod-shaped, green-yellowish color	Present (red color) or absent	39.7–65.7 $\bar{x}$ = 50.8 ± 45.7	23.4–40.3 $\bar{x}$ = 33.9 ± 3.5	14.9–26.7 $\bar{x}$ = 19.9 ± 3.03	1.03–2.05 $\bar{x}$ = 1.6 ± 0.28	21.7–37.6 $\bar{x}$ = 30 ±4.6	17.9–34.8 $\bar{x}$ = 26 ± 4.3	1.6–2.4 $\bar{x}$ = 2.0 ± 0.21
Grammatodinium tongyeonginum											
Cultures Korea (Li et al. 2017) n = 40	Solitary cells or chain of up to 8 cells	Elongated, ellipsoidal with flattened apex. Antapex flattened to slightly or deeply bilobate	Many rod-shaped, green-yellowish color	Present (red color) or absent	24.6–40.9 $\bar{x}$ = 32 (55.5–87)	29.3–50* $\bar{x}$ = 36.6*	22.5–38.5* $\bar{x}$ = 27.3*	1.1–1.5 * $\bar{x}$ = 1.4*	16.4–27.3 $\bar{x}$ = 22 (36–46)	30.2–41.6* $\bar{x}$ = 35.8*	1.6–2.1* $\bar{x}$ = 1.8*
Gymnodinium catenatum											
Organisms collected in Acapulco in November 2010, n = 10	Solitary or chain of up to 64 cells	Biconical, flattening in chain	Many rounded (yellow -brown)	Absent	22.5–51.4 $\bar{x}$ = 36.6 ±7	18.3–31.2 $\bar{x}$ = 23.6	15.7–33.5 $\bar{x}$ = 22.2	0.6–1.6 $\bar{x}$ = 1.1	30.3–45.4 $\bar{x}$ = 39.4	14.3–32.1 $\bar{x}$ = 23.9 ± 4.69	1.0–2.4 $\bar{x}$ = 1.6 ± 0.29

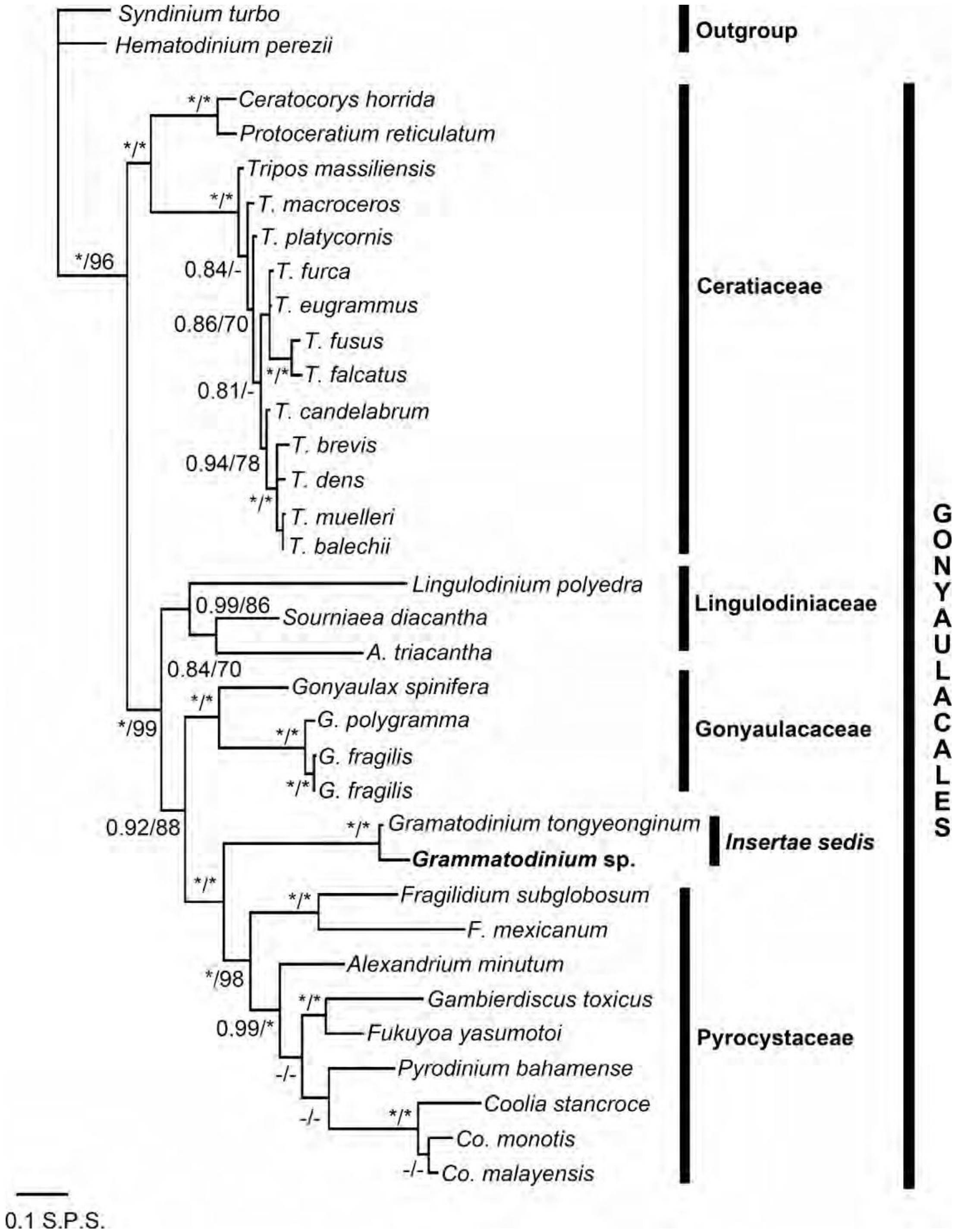

Fig. 3. Bayesian inference topology based on combined SSU-LSU sequences data. GenBank accession numbers and collection sites are found in Supplementary Figs. 1 and 2. BI (left) followed by ML bootstrap values (right) on branches. Asterisks indicate full support (ML = 100 %, BI = 1. 0 %), hyphens indicate values below 70 %. Sequences generated in this study are in boldface. S.P.S. = substitutions per site.

For SSU, the genetic distance between our specimens and *Grammatodinium tongyeonginum* from Korea was 8.5 %, while the genetic distance between these both species and species of other families within Gonyaulacales, ranged from a minimum of 25.1 % (with *Gonyaulax spinifera* Claparéde & Lachmann (Gonyaulacaceae) to 35.8 % (with *Coolia malayensis* Leaw, Lim & Usup) (Pyrocystaceae). For LSU, the genetic distance between our specimens and *Gra. tongyeonginum* was 2.8 %, while the genetic distance between these specimens and species of other families within Gonyaulacales, ranged from a minimum of 27.3 % (with *Tripos furca* (Ehrenberg) Gómez) (Ceratiaceae) to 56.7 % (with *Coolia monotis* Meunier) (Pyrocystaceae). In the analysis with the marker SSU, *Grammatodinium* was phylogenetically more closely related to species of the family Gonyaulacaceae; however, this phylogenetic hypothesis was not supported as the bootstrap values were very low (BI = 0.85 %, ML = 56 %).

Environmental parameters

Grammatodinium sp. was seen in Acapulco since April 2010, although it was then identified as *Gymnodinium* sp. (Meave et al., 2012); it was not until its molecular sequencing was obtained that it could be correctly identified. This dinoflagellate occurs during the dry season (November to May), covering both the warm and cold dry seasons, with the water temperature range (1 to 10 m) from 23.5 to 28.5 ºC (Fig. 4A; Table 2) and the highest average values in the warm dry season (27.5–27.6 ºC). The average salinity was found above 34.2, with the highest values in November 2010 (Fig. 4B; Table 2). In relation to the concentration of O_2, the average values were >5.8 mg/L, with the lowest values (2.6 mg/L) during the warm dry season (Fig. 4C; Table 2). The average depth of the Secchi disk (4.3 m; Table 2) indicates a well-lit water column up to 10 m.

Regarding nutrients in general, phosphates were found below 1 μM, with the lowest values in March 2014 (Table 3), while for ammonium, the average values were close to 2 μM (Table 3), with the highest values in March 2014, when *Grammatodinium* sp. was more abundant. The average values of nitrites plus nitrates were above 2 μM (Table 3), with the lowest values (0.2 μM) in the warm dry season. Silicate values were low, <0.8 μM, and again with the lowest values in March 2014 (Table 3).

Discussion

From the morphological, molecular and environmental data obtained in the present study, it was possible to confirm the presence of *Grammatodinium* sp. in Acapulco Bay, Mexico. This genus represents a new record not only for the study area, but also for the entire Eastern Pacific coast, because until now it had only been reported for the Tongyeong Bay, Korea.

Morphologically, our specimens exhibit the diagnostic characters that define it as a species of *Grammatodinium*, such as the cell shape and the presence of the longitudinal furrows evident in their cover. Other features such as the ability to form chains, the flattened apex, the epicone longer than the hypocone, the general shape of the nucleus and its position in the epicone, the presence of spherical yellowish-green color chloroplasts and the presence of red bodies of assimilation; all of these characters are consistent with the type species *Gr. tongyeonginum*. However, there were also morphological differences between the Acapulco material with respect to that from Korea, Li et al. (2017) that indicate that the longest chains found were 8 cells, while in the Acapulco material chains made up of 16 cells were common; this could be due to geographic variation, it should be noted that our collected specimens were found in their vegetative form and not the cysts form. The Acapulco specimens were larger (both in total length (TL) and maximum width (Mw), see Table 1), both the interval and the mean value from both measurements were completely separated, TL: 39.7–65.7 μm, x = 50.8 μm vs 24.6– 40.9 μm, x^{-} = 32 μm; Mw: 21.7– 37.6 μm, x = 30 μm vs 16.4– 27.3, x = 22 μm. Nevertheless, the measure of the width of the cell at the transdiameter level was lower in our specimens (17.9–34.8 μm vs 30.2–41.6 μm; Table 1).

Direct measurement of the photographs published by Li et al. (2017), showed a much higher value than the one indicated in the diagnosis of the species (TL = 55.5–87 μm), perhaps because such elongated cells were quite rare in the population from Korea and were not considered in the 40 randomly measured cells to indicate the values in the description. The difference in the shape of the nucleus may be due to an imprecise observation in the material from Acapulco Bay, since the specimens were only observed *in vivo* at 10x without coverslips to avoid cell deformation.

On the other hand, our specimens presented several morphological similarities (Table 1) with *Gymnodinium catenatum* (Fig. 2H-J), another species of dinoflagellate with which it can co-occur in the region, however, remarkable differences were also observed between them. Although both species form chains that can be twisted themselves and the cell connections are quite similar; *G. catenatum* has larger dimensions (maximum width), the length of the epicone and hypocone are more similar, the displacement of the cingulum is smaller, and the nucleus is bigger. However, the main differences to avoid confusion between them are that, *G. catenatum* does not present longitudinal grooves and its color is reddish brown. In *Grammatodinium* species, the grooves are evident even when viewed at low magnification (10x) and the cells are yellowish green in color. Another important difference is that *G. catenatum* can form chains of up to 128 cells, while in our specimens chains never exceeded 16 cells.

Phylogenetic analysis. In addition to morphological evidence, our molecular data also provided a strong support for the recognition and confirmation of the presence of *Grammatodinium* sp. in the Mexican Pacific coasts, and also suggested the possibility of a new species within the genus until undescribed; additionally, there is a clear phylogenetic relationship of the genus with the order Gonyaulacales.

In the phylogenetic analyses with both markers and the combined SSU-LSU, the phylogenetic relationships of *Grammatodinium* within the order Gonyaulacales were not the same. With SSU, *Grammatodinium* was phylogenetically more closely related to species of the family

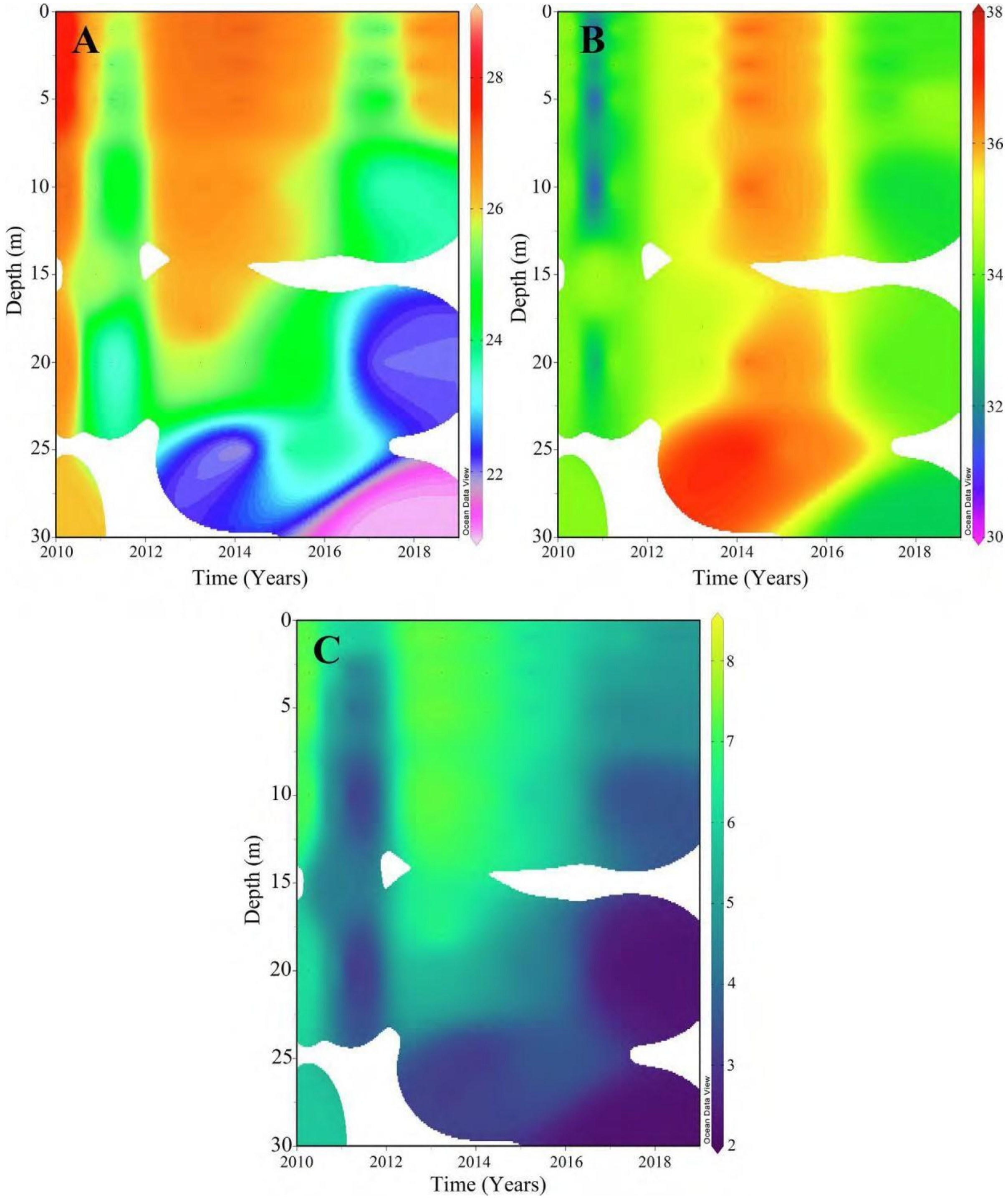

Fig. 4. A-C. Time series (years 2011, 2013–2015, 2017–2018) of environmental data for Acapulco Bay, Mexico. A: Temperature time series (ºC). B: Salinity time series. C: Dissolved Oxygen time series (mg/L).

Gonyaulacaceae; however, this phylogenetic hypothesis was not supported since values were very low (BI = 0.85 %, ML = 56 %). On the other hand, in the LSU analyses and the combined SSU-LSU, that included a greater number of taxa and base pairs to compare, *Grammatodinium* was phylogenetically closer to family Pyrocystaceae; this hypothesis was robust and supported with the maximum phylogenetic support (BI = 1.0 %, ML = 100 %), unlike that shown with SSU. Species of Gonyaulacales are characterized by being thecate, with the exception of *Pyrocytis* Wyville-Thompson which motile cells produced within the cyst can be thecate or athecate

Table 2. Environmental data in Acapulco Bay, Mexico, during the presence of *Grammatodinium* sp. Values correspond to the interval and the averages of the data evaluated at 1, 3, 5 and 10 m depth, in the sampling sites: Naval, Centro and Muelle, inside the bay, and Sinfonía in the adjacent coastal area.

Date	Season	Temperature (ºC)	Salinity	O_2 (mg L^{-1})	Secchi (m)
2010 monthly sampling (February to December)					
10/04/2010	Warm- dry	26.6– 28.5 x = 27.6 ± 0.5	34.2– 34.3 x = 34.3 ± 0.5	6.3– 7.9 x = 7.3 ± 0.5	4.3– 6 x = 5.1
19/11/2010	Cold-dry	24.9– 27.2 x = 26.1 ± 0.5	30– 37 x = 31.5 ± 2	2.6– 8 x = 5.6 ± 1.7	4– 4.5 x = 4.1
2011 (January, February, March, May, June, August, and October)					
28/03/2011	Cold-dry	23.5– 26.2 x = 25.1 ± 0.9	34.2– 34.4 x = 34.3 ± 0.06	2.6– 6.7 x = 4.2 ± 1.4	7
2012 (June and October), 2013 (March, June, and October), 2014 (March, April and November)					
27/03/2014	Cold-dry	25.9– 27.84 x = 27.1 ± 0.51	36.7– 36.8 x = 36.7 ± 0.3	6.4– 7.6 x = 7.2 ± 0.4	4.5– 7.5 x = 6.3
2015(March, April, June, and November), 2016 (March, April, and November), 2017 (February, May, and November)					
13/05/2017 (material sequenced SSU)	Warm-dry	26.6– 28.2 x = 27.5 ± 0.5	34.4– 34.6 x = 34.5 ± 0.04	5.7– 7.4 x = 6.8 ± 0.4	2.8
2018 (February, April, and November)					
24/02/2018) (material sequenced LSU)	Cold-dry	26.2– 26.6 x = 26.4	33.5– 34 x = 33.7	4– 4.5 x = 4.2	6
General average	Dry season (Cold and warm)	23.5– 28.5 x = 26.6 ± 0.6	30– 37.2 x = 34.2 ± 0.6	2.6– 8 x = 5.8 ± 0.9	2.8– 7.5 x = 4.3

Table 3. Nutrient concentrations in Acapulco Bay during the presence of *Grammatodinium* sp. The values correspond to the interval and the averages of the data evaluated at 1, 3, 5 and 10 m depth, in the sampling sites: Naval, Centro and Muelle, inside the bay, and Sinfonía in the adjacent coastal area.

Date	NH_4 (mM)	PO_4 (mM)	$NO_2 + NO_3$ (mM)	SiO_2 (mM)
10/04/2010	0.32– 3.4 x = 1.7 ± 1.09	0.38– 2.8 x = 1.6 ± 0.7	0.2– 5.05 x = 1.5 ± 1.5	0.32– 3.2 x = 0.9 ± 0.95
19/11/2010	0.29– 0.9 x = 0.54 ± 0.2	0.61– 3.1 x = 1.54 ± 0.8	0.45– 10.05 x = 2.3 ± 2.8	0.14– 0.78 x = 0.47 ± 0.2
28/03/2011	0.85– 2.8 x = 1.4 ± 0.53	0.0001– 0.87 x = 0.47 ± 0.3	1.42– 15.31 x = 5.2 ± 4.74	0.44– 2.5 x = 1.5 ± 0.7
27/03/2014	0.49– 6.65 x = 4.3 ± 1.6	0.04– 0.15 x = 0.7 ± 0.02	0.12– 4.33* x = 1.57 ± 1.22	0.03– 0.15 x = 0.08 ± 0.4
General average	0.29– 6.65 x = 2.0 ± 0.9	0.0001– 3.1 x = 1.1 ± 0.5	0.2– 15.31 x = 2.6 ± 2.5	0.03– 3.2 x = 0.74 ± 0.6

* Only Nitrate (NO_3) values were measured.

(Fensome et al., 1993); the main stage of the life cycle in this genus, is a large vegetative cyst with different shapes (spheroidal, semilunar or fusiform), and a continuous unornamented wall. According to our molecular phylogeny (Fig. 3 and Supplementary Fig. 2), *Grammatodinium* is closely related to the Pyrocystaceae family as sister clades. Alternatively, in the phylogeny shown by Gómez et al. (2015), *Balechina* and *Cucumeridinium* Gómez, López-García, Takayama & Moreira (both athecate and *incertae sedis*) are also closely related genera to Gonyaulacales species. Still, more studies need to be carried out to reevaluate the morphological characters in Gonyalacales and be able to accommodate organisms which life cycles exhibit athecate phases, currently not described for any known species in the order. With the present evidence, we can establish that the species of *Grammatodinium* are closely related to Pyrocystaceae, and its basal position also suggests that this genus could belong to a new family.

Interspecific genetic distance values also supported the phylogenetic hypothesis described above for LSU and the combined SSU-LSU. The genetic distance values found between *Grammatodinium* sp. and *Gr. tongyeonginum* (8.5 % SSU and 2.8 % LSU), were equivalent, and even higher, to the interspecific distance values previously reported by other authors among species of the genus *Gambierdiscus* in SSU and LSU, 2.8– 4.0 % and 1.8– 2.5 %, respectively (Nishimura et al., 2013).

Environmental data. According to our data, the presence and abundance of *Grammatodinium* sp. correspond to a tropical environment, since the site temperature during the different sampling periods ranged from 23.5 ºC to 28.5 ºC (Fig. 4A), with a salinity range of 30–37.2 (Fig. 4B); however, *Grammatodinium* sp. is expected to be present during the dry season, when temperature and salinity are adequate for its growth and development. It should be noted that *Gr. tongyeonginum* was cultivated at 20 ºC from cysts obtained out of sediments of the coastal zone on Tongyeong Bay, Korea on December 18, 2013, when the water temperature was close to 10 ºC and the salinity was 33.3. Tongyeong Bay is located further north than Acapulco Bay, in a temperate latitude (34º 47.15^{0} N, 128º 22.77^{0} E) and throughout the year the water temperature ranges from 9 to 26 ºC. From July to September (rainy season) salinities are <32 and low nutrient concentration is from March to September ($PO_4 < 0.2$, $NH_4 < 1.0$, $NO_3 < 2.0$, $NO_2 < 0.2$), and high concentration from October to February (PO_4) > 4.0, $NH_4 > 4$, $NO_3 > 2.0$ and $NO_2 > 0.2$) (Lee et al., 2011). In this way, *Grammatodinium tongyeonginum* could be a species adapted to live in colder waters. A temperature of 10 ºC is presumably low for its reproduction. This is possibly the reason it was originally found only in a cyst form.

Finally, based on our morphological, molecular and environmental data, we can speculate that this new record could be a not yet described species within *Grammatodinium*. However, the photographic record that we have, limits us to describe it as a new species since it is not possible to clearly show all the diagnostic characteristics; therefore, it is necessary to increase the sampling efforts in order to obtain a greater amount of biomass, and with it, give way to better microscopy (Scanning Electron Microscopy) studies that allow us to evaluate these characters in detail and establish a new species. Likewise, it is important to continue with the study of phytoplanktonic organisms in Acapulco Bay, since our results also show that there is still a great underestimated diversity in the region that must be described by an integration of molecular, morphological, ecological and physiological data to allow a better understanding of the biology of these organisms. This will allow the generation of more solid knowledge and will also contribute to create more stable classification systems.

Data availability

All raw sequence data generated during this study and the metadata are deposited in the NCBI under number MN193415-MN193416.

Contributions

All the authors have actively contributed to this study and have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Ethics declarations

Competing interests: The authors declare no competing interests.

CRediT authorship contribution statement

Alexis Escarcega-Bata: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Supervision. **María Eugenia Zamudio-Resendiz:** Methodology, Validation, Investigation, Resources, Writing – original draft, Funding acquisition. **Adriana Hernández-Rosas:** Methodology, Investigation. **María Luisa Núñez Resendiz:** Validation, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Kurt M. Dreckmann:** Validation, Visualization. **Abel Sentíes:** Validation, Resources, Data curation, Visualization, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejop.2022.125942.

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9. Capítulo 3

Evidencia morfo-molecular y ambiental de la ocurrencia de *Karenia longicanalis* (Dinophyceae: Kareniaceae) como formadora de florecimientos en el Océano Pacífico Oriental

Morpho-molecular and environmental evidence of the ocurrence of *Karenia longicanalis* (Dinophyceae: Kareniaceae) as a bloom former in the Eastern Pacific Ocean

RESUMEN

Karenia longicanalis, un dinoflagelado atecado, fue descrito por primera vez durante una floración en el puerto de Victoria (Hong Kong, China). Este estudio confirma la presencia de *K. longicanalis* como formador de floraciones en el Océano Pacífico oriental. Los especímenes fueron colectados en marzo de 2019 en tres estaciones de muestreo en la Bahía de Acapulco, México. La temperatura del agua, la salinidad, el oxígeno disuelto y el pH se midieron in situ en el momento de la recolección de las muestras. La morfología celular fue examinada por microscopía óptica y electrónica de barrido. Un análisis molecular basado en la amplificación de la región de la subunidad grande (LSU) del ADNr reveló que las secuencias LSU formaron un grupo monofilético con otras secuencias de GenBank pertenecientes a *K. longicanalis*. La filogenia resultante demuestra que *Karenia* está estrechamente relacionada con *Asterodinium*, *Gertia* y *Shimiella*. La morfología de los especímenes fue consistente con descripciones previas.

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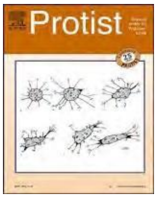

Morpho-molecular and environmental evidence of the ocurrence of *Karenia longicanalis* (Dinophyceae: Kareniaceae) as a bloom former in the Eastern Pacific Ocean

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ABSTRACT

Karenia longicanalis, an athecate dinoflagellate, was first described during a bloom in Victoria Harbour (Hong Kong, China). This study confirms the presence of *K. longicanalis* as a bloom former in the eastern Pacific Ocean. Specimens were collected in March 2019 at three sampling stations in Acapulco Bay, Mexico. Water temperature, salinity, dissolved oxygen, and pH were measured in situ at the time of sample collection. Cell morphology was examined by optical and scanning electron microscopy. A molecular analysis based on the amplification of the large subunit (LSU) rDNA region revealed that the LSU sequences formed a monophyletic group with other GenBank sequences belonging to *K. longicanalis*. The resulting phylogeny demonstrates that *Karenia* is closely related to *Asterodinium*, *Gertia*, and *Shimiella*. The morphology of the specimens was consistent with previous descriptions.

1. Introduction

Harmful algal blooms (HABs), which are characterized by the rapid proliferation of planktonic, toxin-producing algal species, pose risks to aquatic organisms and human health (Band-Schmidt et al., 2011). The family Kareniaceae Bergholtz, Daugbjerg, Moestrup & Fernández-Tejedor consists of six genera comprising 37 athecate dinoflagellate species that are widely distributed across temperate and tropical waters worldwide (Bergholtz et al., 2005; Guiry and Guiry, 2023). Many species of this family are potentially harmful, making Kareniaceae one of the most ecologically important families within the Gymnodiniales order due to the socioeconomic and public health risks generated by its members. Kareniaceae species are morphologically characterized by the presence of an apical groove (i.e., acrobase), which varies in shape and arrangement among genera, and the presence of numerous chloroplasts, with fucoxanthin and its derivatives constituting the primary pigments (Bergholtz et al., 2005; Benico et al., 2019; Daugbjerg et al., 2000; De Salas et al., 2003; Ok et al., 2020; Takahashi et al., 2019).

Currently, the Kareniaceae family includes 16 harmful toxin-producing species (Iwataki, 2023) comprising three genera: *Karenia* Hansen & Moestrup (9 out of 10 species are toxic), *Karlodinium* Larsen (6 out of 16 species are toxic), and *Takayama* Salas, Bolch, Botes & Hallegraeff (1 out of 7 species is toxic; Guiry and Guiry, 2023). The primary toxins reported for these species include Brevetoxins, gymnocins (A and B), gymnodimins, hemolysins, polyunsaturated fatty acids, and sterols (Arzul et al., 1995; Brand et al., 2012; Fowler et al., 2015; Holland et al., 2012; Mooney et al., 2007; Satake et al., 2002, 2005). These compounds are ichthyotoxic due to their ability to alter membrane permeability, which damages gill epithelia and consequently results in death due to suffocation (Bergholtz et al., 2005).

Karenia brevis (Davis) Hansen & Moestrup, *Karenia brevisulcata* (Chang) Hansen & Moestrup, *Karenia concordia* Chang & Ryan, *Karenia cristata* Botes, Sym & Pitcher, *Karenia mikimotoi* (Miyake & Kominami ex Oda) Hansen & Moestrup, and *Karenia selliformis* Haywood, Steidinger & MacKenzie are among the main HAB-forming species and have been widely reported along the coasts of Asia (Okaichi, 2004; Yang et al., 2000), Europe (Davidson et al., 2009; Orlova et al., 2022), South Africa (Botes et al., 2003), Australia (Chang, 1999; Chang et al., 2008; Wear

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Abbreviations: BI, Bayesian inference; HABs, harmful algal blooms; LSU, large subunit; ML, maximum likelihood; SEM, scanning electron microscopy.

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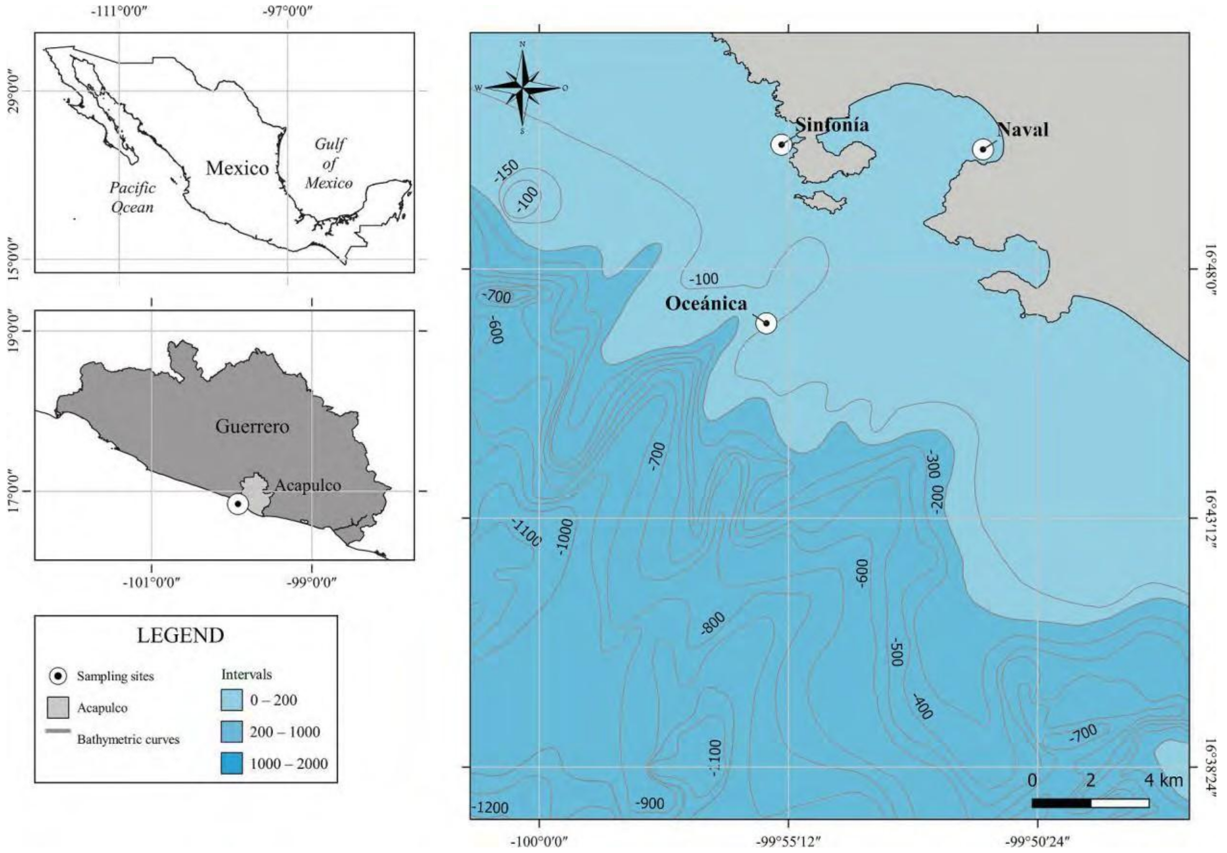

Fig. 1. Map of the study area with sampling stations inside and outside Acapulco Bay. The black dots show the sampling sites.

Table 1

Large subunit (LSU) sequences used for phylogenetic analysis, including geographic information and GenBank accession numbers. The hyphen (-) indicates no collection record. The GenBank accession numbers in boldface correspond to sequences generated in this study. The analysis included a total of 20 species.

Taxa	Distribution data	GenBank accession number
Hematodinium perezi Chatton & Poisson	Gulf of Mexico	JQ815886
Syndinium turbo Chatton	–	KF359488
Takayama tasmanica Salas, Bolch & Hallegraeff	Todos Santos Bay (Mexico)	OL439700
Takayama tasmanica	Todos Santos Bay (Mexico)	OL439701
Takayama acrotrocha (Larsen) Salas, Bolch & Hallegraeff	Todos Santos Bay (Mexico	OL439715
Takayama tuberculata Salas	Southern Ocean	EF469230
Takayama helix Salas, Bolch, Bore & Hallegraeff	North West Bay (Australia)	AY284950
Karlodinium jujuense Li & Shin	Northern East China Sea	KX519410
Karlodinium zhouanum Luo & Guo	–	MK848615
Karlodinium ballantinum Salas	Manila Bay (Philippines)	LC521284
Karlodinium armiger Bergholtz, Daugbjerg & Moestrup	Catalan coast	KP790218
Karlodinium gentienii Ne´zan, Chom´erat & Siano	Brest Bay (France)	KJ508378
Karenia mikimotoi (Oda) Hansen & Moestrup	New Caledonia	KJ508365
Karenia cristata Botes, Sym & Pitcher	South African coast	AY243963
Karenia brevis (Davis) Hansen & Moestrup	Texas, Corpus Christi (United States)	AY355455
Karenia brevisulcata (Chang) Hansen & Moestrup	Concarneau Bay (France)	KJ508359
Karenia selliformis Haywood, Steidinger & Mackenzie	China	KY580786
Karenia longicanalis Yang, Hodgkiss & Hansen	Acapulco Bay (Mexico)	**OQ547171**
Karenia longicanalis	Acapulco Bay (Mexico)	**OQ547172**
Karenia longicanalis	Acapulco Bay (Mexico)	**OQ547173**
Karenia longicanalis	China Sea	KY287670
Karenia longicanalis	China Sea	KY216192
Karenia longicanalis	China Sea	KY216194
Karenia longicanalis	China Sea	KY216193
Karenia longicanalis	South China Sea	MG737366
Karenia longicanalis	South China Sea	MG737367
Karenia longicanalis	Khalaktyrsky Beach (Russia)	MZ465594
Karenia longicanalis	Tasman Bay (New Zealand)	AY947664
Karenia longicanalis	Saint Pierre and Miquelon (Eastern North America)	KJ508372
Karenia longicanalis	Douarnenez Bay (France)	KJ508372
Karenia longicanalis	Catalan coast (Nortwestern Mediterranean Sea)	KP790217
Shimiella gracilenta Ok, Jeong, Lee & Noh	Jinhae Bay (Korea)	MN965778
Gertia stigmatica Takahashi, Benico, Wai Mun Lum & Iwataki	Sagami Bay (Japan)	LC490696
Asterodinium gracile Sournia	Imari Bay (Japan)	LC438754

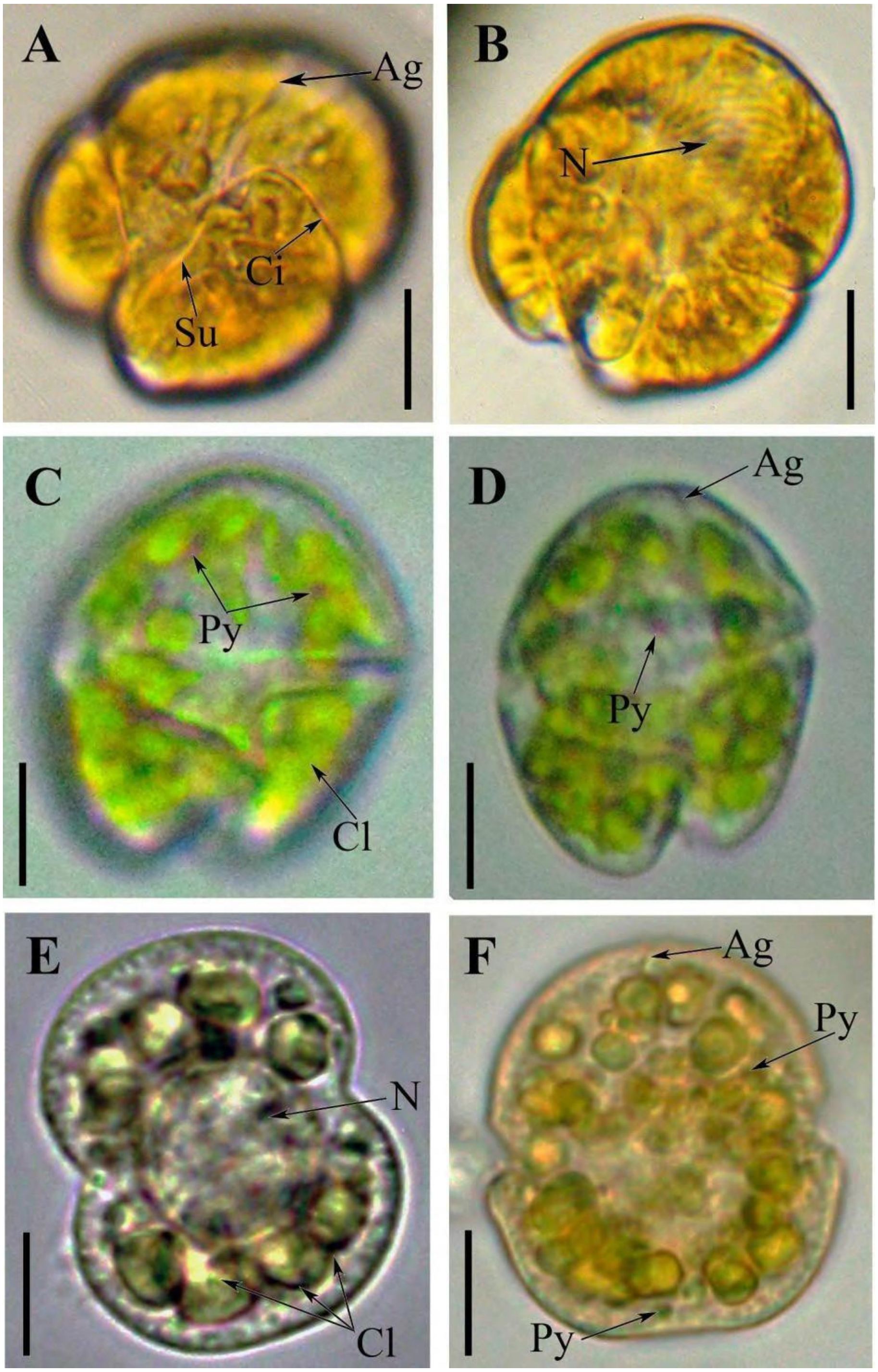

Fig. 2. A ¡ F. Light micrographs of *Karenia longicanalis*. Cells in ventral (A, C − D) and dorsal (B, E − F) views. Apical groove (Ag), Cingulum (Ci), Chloroplast (Cl), Nucleus (N), Pyrenoids (Py), and Sulcus (Su). Scale Bar = 10 μm.

and Gardner, 2001;), and America (Steidinger et al., 2008). Blooms of these species have induced massive mortality events in fishes, invertebrates, and marine mammals, in addition to causing neurotoxic shellfish poisoning and respiratory problems in humans (Bergholtz et al., 2005; Brand et al., 2012; Hansen, 2023; Orlova et al., 2022; Ramsdell, 2008).

Karenia longicanalis Yang, Hodgkiss & Hansen was first described by Yang et al. (2001) from a massive bloom in Victoria Harbour (Hong Kong, China), with no harmful effects attributed to this event. Since then, *K. longicanalis* has been reported off the coasts of France, Japan, Russia, and eastern North America (Saint Pierre and Miquelon) (Nézan et al., 2014; Omura et al., 2012; Orlova et al., 2022). Except for a small bloom in Russia in 2020, no *K. longicanalis* blooms have been reported in any of these locations (Orlova et al., 2022). In the Mexican Pacific, seven *Karenia* species have been reported, with *K. bicuneiformis* Botes, Sym & Pitcher, *K. selliformis*, and *K. mikimotoi* being the most widely distributed species (Escarcega-Bata et al., 2023b). Within this region, *K. mikimotoi* HABs associated with fish mortality have been reported from the Gulf of California to the state of Guerrero (Acosta-Chamorro et al., 2016; Escarcega-Bata et al., 2023b; Gárate-Lizárraga et al., 2016a, b; González-Chan et al., 2007).

Acapulco Bay, which is located in the state of Guerrero, is of great

Table 2
Morphological comparison of *Karenia longicanalis* in the published literature. Only the features observed in *K. longicanalis* cells and those in this study were compared. nd: no data.

Morphological features	*K. longicanalis* [a]	*K. longicanalis* [b]	*K. longicanalis* [c]	*K. longicanalis* [d]	*K. longicanalis* [e]
Cell shape	ovate, slightly dorso-ventrally flattened	lightly dorso-ventrally flattened	ovate, slightly dorso-ventrally flattened	slightly dorso-ventrally flattened	ovoid
Cell length (μm)	18–30.3	17.5–35	29–42	21.70–40	27–34
Cell width (μm)	12.7–25.5	10–22.5	21–32	15.6–34.5	18–22
Length-width ratio	1.12–1.32	1.23	1.03–1.53	1.02–1.54	nd
Thickness (μm)	10–17	slightly flattened	4/5 of cell width	nd	nd
Epicone shape	hemispherical	hemispherical	conical or hemispherical	conical	dome-shaped with broadly rounded apex
Hypocone shape	asymmetric	hemispherical to slightly truncated	truncated	asymmetric	asymmetric
Apical groove	linear, shallow, and narrow, extending ¼ down the epicone	linear, long, and thick, extending about 2/3 down the dorsal side	linear, wide, and shallow, extending about half-way down dorsal side	straight and very long, extending down the ventral and dorsal sides of the epicone	Straight and narrow, extending over the apex to about half-way down the dorsal epicone.
Nucleus	round, central	round, central	round, central	round to ellipsoidal, anterior to subcentral	large, ellipsoidal, and central
Chloroplast	Round and peripheral, as many as 30	round, as many as 30	multilobed, flattened, peripheral, up to 20	lobed, as many as 20	irregularly shaped and arranged peripherally
Pyrenoid	present	present	present	nd	nd

[a] This study; [b]Yang et al., 2001; [c]De Salas et al., 2004b; [d]Wang et al., 2018; [e]Al-Yamani and Saburova, 2019.

biological importance given the numerous phytoplankton species within the bay (Escarcega-Bata et al. 2023a, b; Meave-del Castillo et al., 2012). Indeed, the plankton community within the bay comprises 70 % of the known plankton species in Mexico, 52 of which are HAB formers. Dinoflagellates are particularly important within the bay because they include 29 potentially toxic species (Meave-del Castillo et al., 2012; Meave-del Castillo and Zamudio-Resendiz, 2018). Although multiple HAB-forming plankton species have been identified in Acapulco Bay, information on the diversity of toxic species remains incomplete.

Given the importance of Acapulco Bay, continual efforts are needed to monitor bloom formation and identify new bloom formers. In March 2019, specimens were collected that resembled *K. longicanalis*. Although this species was reported in the area by Meave-del Castillo and Zamudio-Resendiz (2018), no morphological or molecular support was provided in that study to confirm its identity. Additionally, the specimen abundance in that study suggested the species had proliferated inside and outside the bay. Thus, the aim of the present study was to identify the specimens collected in March 2019 in Acapulco Bay, which resembled *K. longicanalis*, through morphological and molecular analyses. Taking into consideration environmental data of water temperature, salinity, dissolved oxygen, and pH, we present robust evidence to confirm the presence of *K. longicanalis* as a bloom former in the eastern Pacific Ocean.

2. Materials and methods

2.1. Study area

Acapulco Bay is found in the southern portion of the tropical Mexican Pacific (16° 47′ to 16° 51′ 40″ N, 99° 50′ 52″ to 99° 56′ W; Fig. 1). The depths of this semicircular bay range from ~50 m near its entrance to 10–30 m within the bay.

2.2. Sampling

Biological samples were collected at three stations (Fig. 1): Naval (16° 50′ 18.33″ N, 99° 51′ 27.16″ W), Sinfonia (16° 50′ 23.81″ N, 99° 55′ 20.03″ W), and Oceanica (16° 46′ 57.24″ N, 99° 55′ 37.56″ W). In March 2019, samples were collected at depths of 1, 3, 5, 10, 18, 20, 30, and 50 m in Van Dorn bottles (3 L) and transferred to amber bottles (450 ml). The samples were fixed in situ with Lugol's iodide acid or glutaraldehyde. Additionally, two live samples per station were collected by vertical trawls (3 and 5 m), transferred to plastic bottles (500 ml), and stored in a cooler to protect them from light and heat until laboratory analysis.

2.3. Microscopy

A 250-μl aliquot of each sample was observed without a coverslip under a Leica DM750 brightfield optical microscope (Leica Microsystems GmbH, Wetzlar, Germany) at low magnification (10x). Cells of interest were isolated using the reduced micropipette technique described by Escarcega-Bata et al. (2021). The micrographs were taken using a Powershot sx600 camera (Canon Inc., Tokyo, Japan). Multiple planes were photographed to capture as many morphological characteristics as possible. Cell density was evaluated with the Utermöhl (1958) method using a 50-ml column and an AE31 brightfield inverted microscope (Motic, Kowloon City, Hong Kong). We also employed a JMS 5900LV (JEOL, Tokyo, Japan) scanning electron microscope (10 Kv accelerating voltage) to analyze samples, which were prepared following the critical point drying method (Boltovskoy, 1995).

2.4. Single-cell and sequencing

We followed the single-cell PCR protocol described by Hernández-Rosas et al. (2018), with minor modifications (Escarcega-Bata et al., 2021). The ribosomal LSU marker was amplified using the primer pairs D1R-F (ACCCGCTGAATTTAAGCATA) and D3B-R (TCGGAGGGAACCAGCTACTA; Hansen et al., 2000; Scholin et al., 1994). The LSU marker was selected because of the number of sequences available for the family Kareniaceae in GenBank, which we used to compare our results. PCR amplification was confirmed by electrophoresis using 1 % agarose gel (20 min at 90 V). Staining was performed with GelRed® (Biotium, Fremont, CA, USA). Visualization was conducted with a gel documentation system with UV light. The PCR products were stored at −80 °C and sent to Macrogen, Inc. (Seoul, Korea) for purification and sequencing. The rDNA sequences were determined for only one strand using the forward primer.

2.5. Sequence alignments and phylogenetic analyses

We used BioEdit v. 7.0.5 (Hall, 1999) to align the sequences obtained in this study with those of species of the family Kareniaceae from GenBank (Table 1). *Syndinium turbo* Chatton and *Hematodinium perezii*

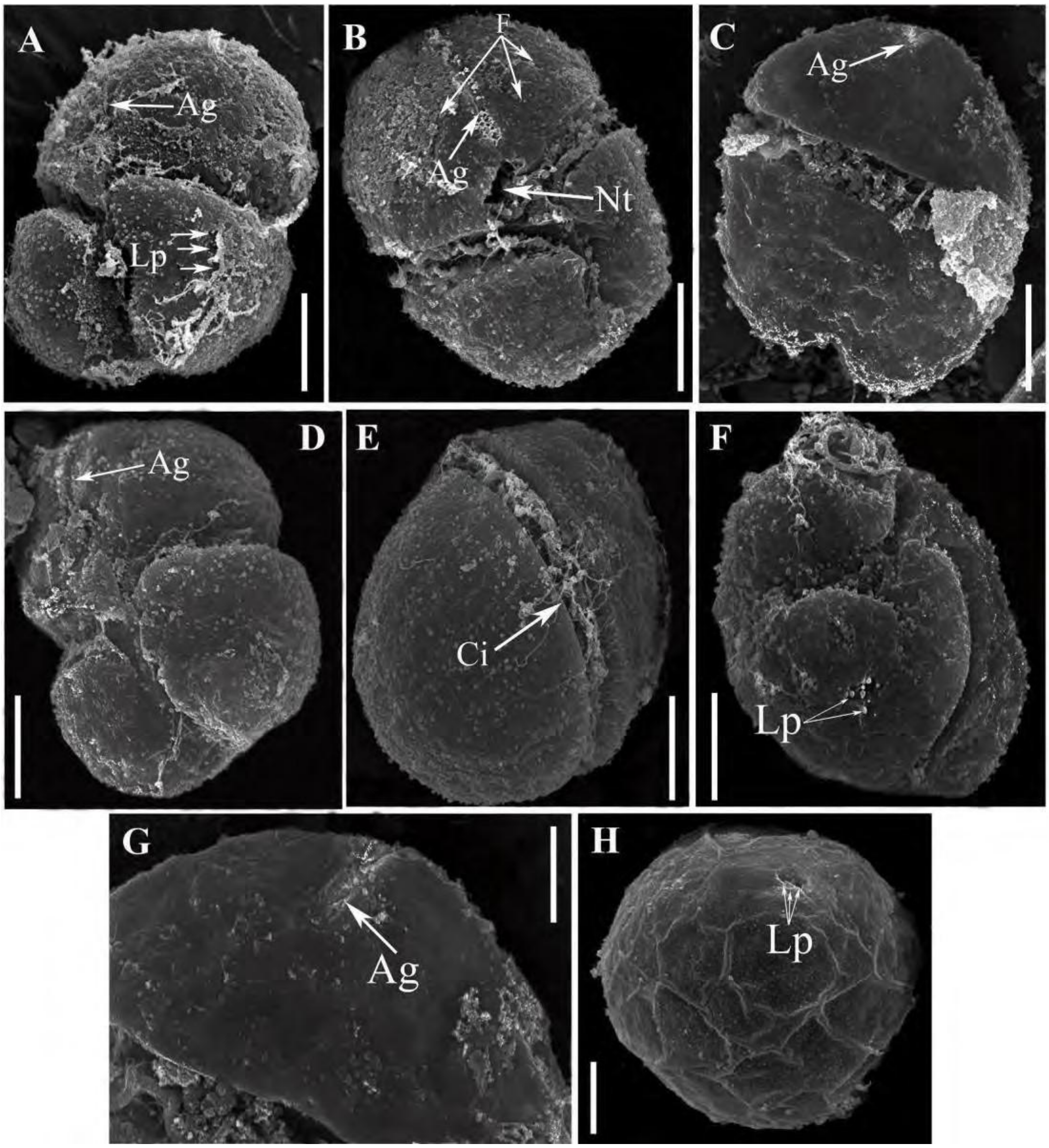

Fig. 3. A ¡ H. Scanning electron micrographs of *Karenia longicanalis*. Cells in ventral (A − B, D), dorsal (C, E), apical (G), and antapical (F, H) views. Apical groove (Ag), Furrows (F), Lateral pores (Lp), and Notch (Nt). Scale Bar: Figs. A − F = 10 μm; Figs. G − H = 5 μm.

Chatton & Poisson comprised the outgroup. We performed maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses. The ML analysis was conducted in RAxML (Stamatakis, 2006), with support for each branch determined from 1000 bootstrap replications. The BI analysis was conducted in TOPALi v. 2 (Milne et al., 2009). The evolutionary model (GTR + G) was selected based on the ML ratio test. Four parallel Markov chain Monte Carlo simulations were conducted. Each began with a random tree, and the data were sampled every 500 generations for 10×10^6 generations, discarding the first 25 % of the trees as the burn-in. Pairwise distance values (p-distances) and Jukes-Cantor corrected distances were calculated in Mega v. 5 (Tamura et al., 2011).

2.6. *Environmental conditions and distribution*

During sampling, water temperature, salinity, dissolved oxygen, and pH were measured with a YSI-556 MPS multiparameter probe (Yellow Springs Instruments, OH, USA), which was calibrated each day following the protocol of the manufacturer. We also created a map in Ocean Data View v. 5.1.7 (Schlitzer, 2019) to visualize the global distribution of *K. longicanalis* based on geographical data from the literature.

3. Results

3.1. *Morphology*

Karenia longicanalis Yang, Hodgkiss & Hansen, 2001 (Figs. 2A–F; 3A–H; Table 2).

References: Al-Yamani and Saburova (2019), p. 116, Plate 62; De Salas et al., (2004b), p. 169–170 Figs. 2–11); Nézan et al., (2014), p. 79 Fig. 1D); Omura et al., (2012), p. 73 Fig. a–f); Wang et al., (2018), p. 2205–2207, Fig. 1a–f; 2a–g; 3a–c; 4a–e; Yang et al., (2001), p. 69 Figs. 3–9.

Description: Cells are solitary, ovate, slightly dorso-ventrally flattened (Figs. 2A-B; 3C), 18–30.3 μm (*mean* 23.9 ± 2.74, *n* = 45) long, and 12.7–25.5 μm (*mean* 19.3 ± 2.43, *n* = 45) wide. The epicone is slightly smaller than the hypocone (Figs. 2C; 3C), rounded on the ventral side, and slightly conical on the dorsal side, with few visible furrows (Figs. 2C–D; 3B). The hypocone is asymmetrical, with the right lobe bigger and slightly more rounded than the left lobe (Figs. 2F; 3B–C),

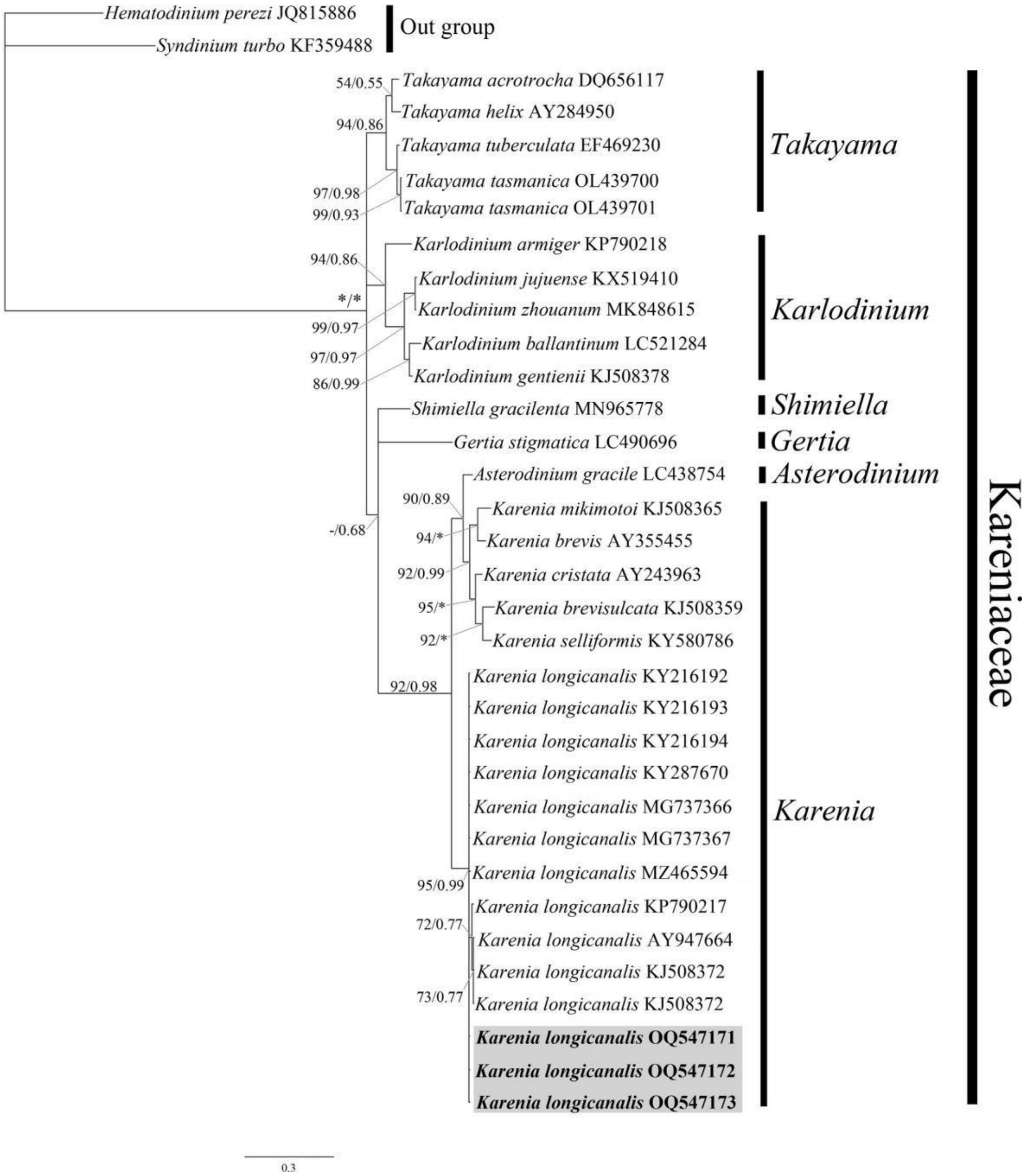

Fig. 4. Bayesian inference topology based on LSU rDNA (772 bp) sequence data from Kareniaceae (Dinophyceae) species. Bayesian inference (BI; right) followed by maximum likelihood (ML) bootstrap values (left) on branches. Asterisks indicate full support (ML = 100 %, BI = 1. 00). Hyphens indicate values below 50 %. Sequences generated in this study are in boldface. SPS: substitutions per site.

which exhibits lateral pores on its ventral side (Fig. 3A, F, H). The median cingulum is narrow, deep, and descending at its anterior right end (Figs. 2A; 3A-B). The sulcus is narrow and deep from the antapex to the intercingulate region. This canal extends to ¼ of the epicone, where it forms a notch (Figs. 2A; 3B). The apical groove is observed in both ventral and dorsal views. Ventrally, the apical grove is straight, narrow, and few deep, extending from the apex to the margin of the sulcus, where the notch is located (Figs. 2A, D; 3B, D). Dorsally, the apical groove is also few deep and narrow, extending to ¼ of the epicone (Fig. 3C, G). The nucleus is large, spherical, located in the central portion of the cell, and clearly visible in the dorsal view (Fig. 2B, E). Cells have ~ 30 chloroplasts (Fig. 2C, D–E) that vary in color from brown to green (Fig. 2A–D) and contain pyrenoids.

3.2. Phylogeny

The final LSU alignment (772 bp) consisted of 34 sequences, three of which were obtained for *K. longicanalis* for the first time in Mexican waters (Table 1). The topologies obtained with the ML and BI approaches did not differ significantly; thus, only the BI results are shown with additional ML values (Fig. 4).

The phylogenetic analysis of the LSU data set (maximum phylogenetic support ML = 100 %, BI = 1.00) revealed three major clades (Fig. 4). Clade I grouped the sequences of the genus *Takayama*, with high phylogenetic support (ML = 94 %, BI = 0.86). Clade II grouped the sequences of the genus *Karlodinium*, with high phylogenetic support (ML = 94 %, BI = 0.97). Clade III was composed of three sister subclades, with low phylogenetic support (ML = 50 %, BI = 0.68). These three

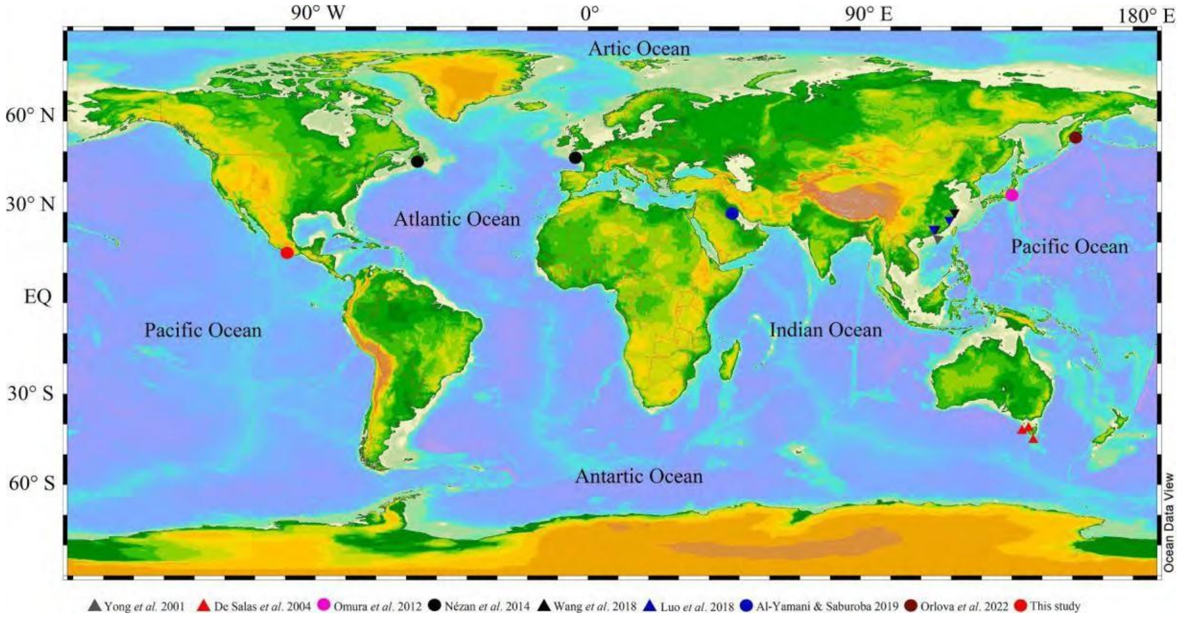

Fig. 5. Known geographical distribution of *Karenia longicanalis* in coastal waters worldwide. The citations below the map correspond to each record of the species.

subclades grouped sequences from *Shimiella*, *Gertia*, *Asterodinium*, and *Karenia*. In the *Karenia* subclade, the sequences generated in this study (accession numbers: OQ547171–OQ547173) were grouped with those of the remaining *Karenia* species available in GenBank, with high phylogenetic support (ML = 92 %, BI = 0.98). These formed an independent clade with *K. longicanalis* sequences, with high phylogenetic support (ML = 95 %, BI = 0.99).

The genetic distances found between *K. longicanalis* sequences and species of the Kareniaceae family ranged from minimum values of 7.2 % (*p*-distance) and 7.9 % (corrected distance) for *Asterodinium gracile* to maximum values of 30 % (*p*-distance) and 50 % (corrected distance) for *Gertia stigmatica* (Table 3). The distance values within *K. longicanalis* were not included because the variation between them was either null or 0.001 % in most cases.

3.3. Environmental conditions and abundance

A *K. longicanalis* bloom occurred in March 2019 during the dry season in Acapulco Bay. The temperature and salinity of the water column (1 to 50 m) ranged from 23.43 to 27.08 °C (average of 26.44 °C) and 34.62 to 34.77 among stations (Table 4). Sinfonia station exhibited the highest average salinity (34.74; Table 4). The average dissolved oxygen concentration was 6.74 mg/L, with the lowest value (4.03 mg/L) recorded at Oceanica station (Table 4). The pH values ranged from 7.41 to 8.94 (average of 8.33; Table 4).

The abundance of *K. longicanalis* ranged from 200 to 526 x 10^3 cells/L among sampling stations. The highest abundance values were recorded in the nearshore Sinfonia station at 3 and 5 m (Table 4). Within the bay at Naval station, the lowest abundance of this species (200 cells/L) was observed at 10 m depth, while the highest abundance (2.9 x 10^3 cells/L) was recorded at 5 m depth (Table 4). At Oceanica station, the abundance of *K. longicanalis* was lower than those of the other two stations. The highest (1.3 x 10^3 cells/L) and lowest (850 cells/L) abundance values at Oceanica station were recorded at 5 and 1 m depth, respectively (Table 4).

4. Discussion

From the morphological, molecular, and ecological characterization of the specimens collected in March 2019 in Acapulco Bay, Mexico, we confirmed the presence of *K. longicanalis* in the eastern Pacific and the potential of this species to form blooms.

4.1. Morphology

The morphological description of the *K. longicanalis* specimens in this study agreed with the original description of Yang et al. (2001) and with the descriptions of similar organisms from the coasts of Australia, Kuwait (e.g., *K. umbella*), and China (Al-Yamani & Saburoba, 2019; De Salas et al., 2004a; Wang et al., 2018;) with regard to characteristics such as cell shape and size (Al-Yamani and Saburoba, 2019; Wang et al., 2018; Yang et al., 2001). However, the cell size of our *K. longicanalis* specimens differed slightly from what has been reported for specimens from the Tasmanian coast (De Salas et al., 2004b).

No differences were present between our specimens and those of previous studies concerning the shape and displacement of the apical groove. It should be noted that the presence of longitudinal grooves in the epicone (barely visible) and some pores in the hypocone were also reported by De Salas et al., (2004b) and Wang et al. (2018). Additionally, we observed some chloroplasts (~30) in the cell periphery, which also agrees with the description provided by Yang et al. (2001) but not with the descriptions of De Salas et al., (2004b) or Wang et al. (2018), who reported specimens with ~ 20 chloroplasts. The variation in cell color from dark green to brown observed in this study was also reported by Yang et al. (2001), who collected samples from a *K. longicanalis* bloom in Victoria Harbour (Hong Kong, China). Although the shape of the nucleus varied from rounded to ellipsoidal in the cells in this study, its central position did not vary. In addition, we noted the presence of pyrenoids.

Karenia longicanalis has been reported along the coasts of France, Japan, Russia, and Saint Pierre and Miquelon (Omura et al., 2012; Nézan et al., 2014; Orlova et al., 2022; Wang et al., 2018). However, these studies only included light micrographs and lacked complete descriptions of this species. Therefore, it was not possible to morphologically compare our specimens with those of earlier reports. Meave-del Castillo and Zamudio-Resendiz (2018) reported a bloom of *K. longicanalis* in Acapulco Bay. As with other previous studies, this study was based on light micrographs and lacked detailed descriptions of the morphotype. It also did not include molecular identification techniques or information on ecological variables associated with the bloom, which prohibited the validation of that record.

Table 3
Genetic divergence (percentage) between *Takayama*, *Karlodinium*, *Karenia*, *Shimiella*, *Gertia*, and *Asterodinium* sequences. Uncorrected distances are shown below the diagonal, and Jukes Cantor distance values are located above the diagonal. GenBank accession numbers are shown below each taxon. The sequence in boldface was generated in this study.

Species	**1**	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. *Karenia longicanalis* OQ547171	–	37.7	37.7	38.5	34.3	40.3	40.3	39.4	42.1	41.2	10.8	9.4	10.3	12.4	12.4	37.7	50.1	7.9
2. *Takayama tasmanica* OL439700	25.1	–	3.5	0.038	6.6	22.8	22.8	22.8	19.07	21.5	41.2	40.3	41.2	43.1	42.2	22.1	39.4	36.8
3. *Takayama acrotrocha* DQ656117	25.1	3.4	–	4	4.4	22.8	22.8	22.8	21.5	22.8	39.4	38.5	41.2	42.1	41.2	22.1	40.3	35.1
4. *Takayama tuberculata* EF469230	25.1	0.04	3.8	–	6.1	23.4	23.4	23.4	19.6	22.1	42.1	39.4	42.17	44.06	41.2	22.8	40.3	37.1
5. *Takayama helix* AY284950	23.6	6.1	4.2	5.7	–	24.1	24.1	24.1	20.9	24.1	37.7	34.3	37.7	43.1	39.5	22.1	37.7	30.5
6. *Karlodinium jejuense* KX519410	26.2	17.5	17.5	17.9	18.3	–	0	7.9	15.06	4.4	43.1	45.03	43.1	45.03	47	22.8	36.02	40.3
7. *Karlodinium zhouanum* MK848615	26.2	17.5	17.5	17.9	18.3	0	–	7.9	15.06	4.4	43.1	45.03	43.1	45.03	47	22.8	36.02	40.3
8. *Karlodinium ballantinum* LC521284	25.9	17.1	17.9	17.5	18.6	7.2	7.2	–	18.4	5.2	37.7	39.4	36.8	41.2	41.3	21.5	36.8	36
9. *Karlodinium armiger* KP790218	27.0	15.2	16.7	15.6	16.3	12.5	12.5	14.8	–	14.5	49.05	44.06	45.03	49.05	49	23.4	39.4	41.2
10. *Karlodinium gentienii* KJ508378	26.6	16.7	17.5	17.1	18.3	4.2	4.2	4.9	12.2	–	41.25	43.1	39.4	44.06	45	20.2	37.7	39.4
11. *Karenia mikimotoi* KJ508365	9.5	26.6	25.9	27	25.1	27.4	27.4	25.1	29.7	26.6	–	7.07	44.06	10.8	9.4	42.1	49.05	9.4
12. *Karenia cristata* AY243963	8.4	26.2	25.5	25.9	23.6	28.1	28.1	25.9	27.8	27.4	6.5	–	43.1	6.1	5.7	38.5	54.4	8.4
13. Karenia brevis AY355455	9.1	26.2	26.6	27	25.1	27.3	27.4	24.7	28.1	26.6	5.3	7.2	7.07	11.8	10.8	42.1	49.05	7.07
14. *Karenia brevisulcata* KJ508359	10.6	27.4	27	27.8	27.4	28.1	28.1	26.6	29.7	27.8	9.5	5.7	10.3	–	6.6	42.1	55.6	11.3
15. *Karenia selliformis* KY580786	10.6	27	26.6	26.6	25.9	28.9	28.9	26.6	29.7	28.1	8.4	5.3	9.5	6.1	–	42.1	50.1	11.3
16. *Shimiella gracilenta* MN965778	25.1	17.1	17.1	17.5	17.1	17.5	17.5	16.7	17.9	16.0	27	25.5	27	27	27	–	35.1	36
17. *Gertia stigmatica* LC490696	30.0	25.9	26.2	26.2	25.1	24.3	24.3	24.7	25.9	25.1	29.7	31.6	29.7	31.9	30	24	–	45
18. *Asterodinium gracile* LC438754	7.2	24.7	24	25.1	21.7	26.2	26.2	24.3	26.6	25.6	8.4	7.6	6.5	9.9	9.9	24.3	28.1	–

4.2. Phylogenetic analysis

When combined, the partial LSU sequences obtained in this study (OQ547171–OQ547173) and the sequences obtained from specimens collected in the China Sea (type region), Australia, France, Japan, Russia, and Saint Pierre and Miquelon formed a monophyletic group, revealing that all sequences correspond to *K. longicanlis*. Thus, it was possible to establish the record of *K. longicanalis* in the Mexican Pacific. Additionally, *Karenia* was found to be closely related to *Asterodinium*, *Gertia*, and *Shimiella*, which agrees with the phylogenic classifications presented by Benico et al. (2019), Ok et al. (2020), and Takahashi et al. (2020).

Although many dinoflagellate studies include molecular evidence, phylogenetic relationships between organisms are often not discussed (Escarcega-Bata et al., 2021). Benico et al. (2019), show a phylogeny with LSU in which most of the representatives of the Kareniaceae family are included. From their results it can be observed that Takayama and *Karlodinium* present a close relationship between them and, in turn, they are resolved as the sister group of the clade integrated by *Asterodinium* and *Karenia*, as sister genera; *Gertia* and *Shimiella* were not included. Ok et al. (2020), in their phylogeny of the Kareniaceae family with the LSU, show a similar hypothesis where *Karlodinium* and *Takayama* shown a closer relationship between them and were resolved, in turn, as the sister group of the clade conformed by *Karenia*, *Gertia*, and *Shimiella*; the genus *Asterodinium* was not included. Takahashi et al. (2020), in their concatenated analysis (with SSU, ITS and LSU), show the same relationship between *Karenia* and *Gertia*, which were resolved, in turn, as the sister group of the clade conformed by *Karlodinium* and *Takayama*, both closely related to each other. Our results, in agreement, showed a similar hypothesis to those previously described, although the authors do not discuss these relationships, it is possible to appreciate them in their analyses shown. Therefore, from our analysis, we can establish that *Asterodinium* is phylogenetically closer to *Karenia*, and that in turn, they form the sister group of *Gertia* and *Shimiella*. On the other hand, both

Table 4

Environmental data and *Karenia longicanalis* abundance during the bloom in Acapulco Bay, Mexico. The values correspond to the data evaluated at 1, 3, 5, 10, 18, 20, 30, and 50 m depth in the sampling sites of Naval (inside the bay), Sinfonia (adjacent coastal area), and Oceanica (adjacent coastal area).

Station	Coordinates	Sampling depth (m)	Temperature (°C)	O^2 (mg L^{-1})	Salinity (psu)	pH	Abundance (cells L^{-1})
Naval	16° 50′ 18.33″ N99° 51′ 27.16″ W	1	27.08	7.65	34.74	8.89	950
		3	27.07	7.63	34.74	8.90	1.2 x 10^3
		5	27.00	7.66	34.72	8.89	2.9 x 10^3
		10	26.76	7.94	34.72	8.92	200
		18	26.68	7.08	34.74	8.94	n.d
Sinfonia	16° 50′ 23.81″ N99° 55′ 20.03″ W	1	26.34	6.79	34.74	7.41	1.4 x 10^3
		3	26.32	6.80	34.73	7.55	526 x 10^3
		5	26.28	6.79	34.74	7.60	196 x 10^3
		10	25.94	6.52	34.74	7.70	n.d
		20	25.25	4.87	34.77	8.32	n.d
Oceanica	16° 46′ 57.24″ N99° 55′ 37.56″ W	1	27.08	6.97	34.64	8.25	850
		3	27.08	6.98	34.64	8.27	1.1 x 10^3
		5	27.08	7.00	34.64	8.30	1.3 x 10^3
		10	27.07	6.99	34.64	8.33	0
		20	26.60	6.89	34.62	8.50	n.d
		30	26.50	6.09	34.63	8.51	0
		50	23.43	4.03	34.74	8.49	0

n.d = means no data.

Table 5

References for each *Karenia longicanalis* record in coastal waters worldwide. The geographic coordinates are included for each the collection site.

Reference	Location	Geographical coordinates
Yang et al. (2001)	Victoria Harbour, Hong-Kong	nd
De Salas et al., (2004b)	Georges Bay, Australia	41° 19′ 39″ S, 148° 15′ 14″ E
	Hastings Bay, Australia	43° 25′ 57″ S, 146° 56′ 01″ E
	Mudunna, Australia	42° 56′ 51″ S, 147° 50′ 43″ E
	Port Arthur, Australia	42° 12′ 30″ S, 145° 56′ 41″ E
	River Derwent, Australia	42° 56′ 50″ S, 147° 23′ 08″ E
	Taranna, Australia	42° 02′ 50″ S, 147° 51′ 40″ E
	Triabunna, Australia	42° 30″ 44″ S, 147° 54′ 42″ E
Omura et al. (2012)	Japan	nd
Nézan et al. (2014)	France	48° 9′ 47″ N, 4° 23′ 31″ W
	Saint Pierre and Miquelon	46° 56′ 53″ N, 56° 27′ 04″ W
Luo et al. (2018)	Fuzhou, East China Sea	22° 17.37′ N, 114° 09.67′ E
Wang et al. (2018)	Dongtou Island, China	27° 51′ 29″ N, 121° 09′ 09″ E
	Dongyu Island, China	22° 33′ 31″ N, 114° 32′ 22″ E
	Victoria Harbour, Hong-Kong	22° 27′ 14″ N, 114° 12′ 50″ E
	Nanji Island, China	27° 27′ 22″ N, 121° 06′ 01″ E
Al-Yamani and Saburoba (2019)	Arabian Gulf, Kuwait	nd
Orlova et al. (2022)	Kamchatka, Russia	nd
This study	Acapulco Bay, Mexico	16° 50′ 24″ N, 99° 55′ 20″ W

nd: no data.

Takayama and *Karlodinium* constitute sister genera, resolved, in turn, into a basal position in the family. Considering that species with toxic potential have been recorded within these genera (Iwataki, 2023), we would expect them to be closely related to *Karenia*, which contains the largest number of toxic species in the family (Guiry and Guiry, 2023), as have been recorded for other genera of dinoflagellates where toxicity is a character that sustains a clade (Gárate-Lizárraga et al., 2012; Meave-del Castillo et al., 2012; Zamudio-Resendiz et al., 2022). However, as we have already discussed, *Karenia* is not closely related to *Karlodinium* or *Takayama*, which suggests that the toxicity in this group is not a synapomorphic character, but has occurred independently at different evolutionary moments, but this condition must be studied in detail.

4.3. Environmental conditions, abundance, and biogeography

Our results revealed that *K. longicanalis* (Fig. 5; Table 5) is likely a cosmopolitan species. Massive blooms of this species were reported in Victoria Harbour, Hong Kong, and Kamchatka, Russia; however, no environmental data were reported for those blooms (Orlova et al., 2022; Yang et al., 2001;), which prohibits comparisons with the results of this study.

In the laboratory, *K. longicanalis* was cultivated by De Salas et al., (2004b) at 17 °C and by Luo et al. (2018) and Wang et al. (2018) at 20 °C (salinity of 30). In our sampling sites, temperature and salinity ranged from 23.43 to 27.08 °C and 34.64 to 34.74, respectively. These values are considerably higher than those of previous culturing experiments. Our results provide a first approximation of the natural environmental conditions that favor the development of *K. longicanalis* blooms. Since strains can show local adaptation, it is necessary to culture *K. longicanalis* cells under these conditions to confirm if the warm water temperatures of the Mexican Pacific favor their growth and development.

The abundance of *K. longicanalis* recorded in the three sampling stations in this study during the bloom ranged from 200 to 526 x 10^3 cells/L. These values are higher compared to those reported by Yang et al. (2001) in Victoria Harbour (1.0 x 10^5 to 1.8 x 10^5 cells/L) and Orlova et al. (2022) in Kamchatka, Russia (105 x 10^3 cells/L). The blooms in Acapulco Bay and Victoria Harbour did not harm marine fauna or flora, nor were any human health problems reported. In contrast, the massive bloom in Kamchatka resulted in the death of fish and marine invertebrates; however, this event was associated with the abundant presence of *K. selliformis* and not *K. longicanalis* (Orlova et al., 2022).

Seasonal conditions could have favored the *K. longicanalis* bloom in the Mexican Pacific. During spring (sampling period), this region is influenced by the cold waters of the California Current that promote the formation of a mixed layer (Acosta-Chamorro et al., 2016; Sosa-Avalos et al., 2006). This allows athecate species, such as *K. longicanalis*, to remain in surface waters with favorable light and nutrient conditions that support phytoplankton growth and proliferation. Our results also allow us to extend the known distribution range of *K. longicanalis*, which previously encompassed subtropical and temperate zones, to include tropical waters. By confirming the presence of this species in the eastern Pacific, *K. longicanalis* should be considered a cosmopolitan species.

5. Conclusions

Acapulco Bay is an important site for studying blooms, given the high diversity of marine microalgae and numerous blooms that have been recorded throughout the history of the bay. The *K. longicanalis* bloom recorded in March 2019 had no negative consequences for the biota, economy, or people of Acapulco Bay. However, continual monitoring employing fine and molecular microscopy techniques is needed to identify the warning signs and early stages of blooms in Acapulco Bay. Furthermore, the abiotic factors contributing to bloom formation must be understood to untangle the root causes of these events. This knowledge will complement the information generated by biological and ecological studies of bloom-forming species.

CRediT authorship contribution statement

Alexis Escarcega-Bata: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Conceptualization. **María Luisa Núñez Resendiz:** Writing – original draft, Validation, Resources, Methodology, Investigation, Funding acquisition. **María Eugenia Zamudio-Resendiz:** Writing – original draft, Validation, Resources, Methodology, Investigation, Funding acquisition. **Kurt M. Dreckmann:** Visualization, Validation, review & editing. **Estefany Cuevas Sánchez:** Writing – review & editing, Validation. **Abel Sentíes:** Visualization, Validation, Resources, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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10. Capítulo 4

Análisis de la variación genética de *Karenia selliformis* (Dinophyceae: Kareniaceae) y su potencial para la formación de florecimientos en el Pacífico mexicano

Analysis of genetic variation in *Karenia selliformis* (Dinophyceae: Kareniaceae) and its bloom-forming potential in the Mexican Pacific

RESUMEN

Karenia selliformis es un dinoflagelado tóxico y atecado que se encuentra en todo el mundo. Esta especie puede formar extensas floraciones de algas nocivas que se han asociado con la mortalidad masiva de la fauna marina. El 24 de agosto de 2020, se registró una floración de dinoflagelados en la Bahía de Todos Santos, México. El análisis morfológico y molecular de las muestras recolectadas de la floración en la Bahía de Todos Santos en el presente estudio confirmó que la floración se debió a *K. selliformis*. Sin embargo, esta floración de *K. selliformis* no afectó negativamente la vida marina ni la salud pública. Se analizaron un total de 44 secuencias genéticas (620 pb) de la subunidad grande y 37 secuencias genéticas (570 pb) del espaciador del transcriptor interno (ITS1 + 5.8s + ITS2) del ADNr. En la LSU, 15 sitios fueron informativos y 605 sitios fueron invariantes. De estos 15 sitios polimórficos, se identificaron 8 ribotipos, con una diversidad haplotípica (Hd) de 0,647 y una diversidad de nucleótidos (π) de 0,00558. Con el espaciador de transcripción interna, 21 sitios resultaron informativos y 549 sitios resultaron invariantes. De estos 21 sitios polimórficos, se identificaron 5 ribotipos, con una diversidad haplotípica (Hd) de 0,611 y una diversidad de nucleótidos (π) de 0,01330. Además, durante la floración se registraron una temperatura de 23,68 °C, valores de clorofila a >10 mg·m–3 y una densidad celular de 200–900 células·L–1. Los resultados del presente estudio confirman la presencia de variabilidad genética dentro del complejo críptico de *K. selliformis,* lo que indica que se necesitan estudios más profundos sobre su variación genética para resolver los problemas taxonómicos asociados con la clasificación de las especies de fitoplancton.

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Analysis of genetic variation in *Karenia selliformis* (Dinophyceae: Kareniaceae) and its bloom-forming potential in the Mexican Pacific

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ABSTRACT

Karenia selliformis is a toxic, athecate dinoflagellate that is found worldwide. This species can form extensive harmful algal blooms that have been associated with the mass mortality of marine fauna. On 24 August 2020, a dinoflagellate bloom was recorded in Todos Santos Bay, Mexico. The morphological and molecular analysis of samples collected from the bloom in Todos Santos Bay in the present study confirmed that the bloom was due to *K. selliformis*. However, this *K. selliformis* bloom did not negatively affect marine life or public health. A total of 44 genetic sequences (620 bp) of the large subunit and 37 genetic sequences (570 bp) of Internal Transcriber Spacer (ITS1 + 5.8s + ITS2) of rDNA were analyzed. In the LSU, 15 sites were informative and 605 sites were invariant. Of these 15 polymorphic sites, 8 ribotypes were identified, with a haplotypic diversity (*Hd*) of 0.647 and a nucleotide diversity (π) of 0.00558. With the Internal Transcribed Spacer, 21 sites were informative and 549 sites were invariant. Of these 21 polymorphic sites, 5 ribotypes were identified, with a haplotypic diversity (*Hd*) of 0.611 and a nucleotide diversity (π) of 0.01330. In addition, a temperature of 23.68 °C, chlorophyll *a* values of >10 mg·m^{-3}, and cell density values of 200–900 cell·L^{-1} were recorded during the bloom. The results of the present study confirm the presence of genetic variability within the *K. selliformis* cryptic complex, indicating that more in-depth studies on its genetic variation are needed to resolve the taxonomic problems associated with classifying phytoplankton species.

Key words: Pacific Ocean**,** Genetic variation, Cryptic complex, Dinoflagellates, *Karenia,* Harmful algal bloom, LSU

1 Introduction

The Kareniaceae family Bergholtz, Daugbjerg, Moestrup & Fernández-Tejedor is made up of six genera: *Asterodinium* Sournia; *Gertia* Takahashi, Benico, Lum & Iwataki; *Shimiella* Ok, Jeong, Lee & Noh; *Karlodinium* Larsen; *Karenia* Hansen & Moestrup; and *Takayama* Salas, Bolch, Botes & Hallegraeff (Guiry & Guiry, 2025). *Karenia* species are morphologically characterized by the presence of a straight apical groove, which extends from the ventral to the dorsal portion of the epicone. Additionally, *Karenia* species contain numerous chloroplasts containing fucoxanthin and fucoxanthin derivatives (Daujberg et al., 2000). *Karenia selliformis* Haywood, Steidinger & MacKenzie differs morphologically from other species of the genus due to the elongated shape and horizontal position of the nucleus, which is located in the hypocone. However, the recently described *Karenia hui* Lu, Cen, Wang et Moestrup also presents this character (Cen et al., 2024), as well as the presence of numerous chloroplasts (46 to 105) and a sulcal intrusion in the epicone, which is open in *K. selliformis*, *Karenia asterichroma* Salas, Bolch & Hallegraeff, *Karenia brevis* Gert Hansen & Moestrup, *Karenia papilionacea* Haywood & Steidinger, and *K. hui* (Haywood et al., 2004; Iwataki et al., 2022; Cen et al., 2024). Importantly, these characters can be used to identify species during algal blooms (Iwataki et al., 2022).

Among the genera of the Kareniaceae family, *Karenia* is the most harmful genus (9 of 11 species are harmful), whereas *Karlodinium* (6 of 16 species) and *Takayama* (1 of 7 species) are less harmful (Hansen et al., 2021; Escarcega-Bata et al., 2023, Cen et al., 2024). *Karenia* species produce various toxins, including brevetoxins, gymnocins (A and B), gymnodimins, hemolysins, polyunsaturated fatty acids, and sterols (Chang et al., 2006; Steidinger et al., 2008; Brandt et al., 2012; Fowler et al., 2015). *Karenia selliformis* has been responsible for blooms associated with the mass mortality of organisms in sites around the world such as New Zealand (Haywood et al., 2004), Mexico (Haywood et al., 2007), Tunisia (Munday et al., 2004; Boudriga 2023), Kuwai (Heil et al., 2001), Chile (Guillou et al., 2002), Japan (Iwataki et al., 2022), Russia (Orlova et al., 2022), and the United States (Wolny et al., 2024).

On the Mexican Pacific coasts, only one bloom of *K. longicanalis* has been reported, which occurred in Acapulco Bay in 2019 (Escarcega-Bata et al., 2024). There have been no other reports of blooms of potentially harmful *Karenia* species on the Mexican coast. Although the presence of *K. selliformis* has been recorded several times in the area (Meave del Castillo et al., 2012; Escobar-Morales & Hernández Becerril, 2015; Maciel-Baltazar, 2015; Almazán-Becerril et al., 2016; Escarcega-Bata et al., 2023), no molecular information, abundance data, or detailed morphological analyses have been reported for this species in the Mexican Pacific. However, in August 2020, a bloom was recorded in Bahía Todos Santos, Mexico. Given its importance and the potential impact of its presence on other organisms, human health, and the economy, the objective of the present study was to analyse the genetic variation of *K. selliformis* around the world and its potential for bloom formation in the eastern Pacific.

2 Materials and methods

2.1 Site description and sample collection

Bahía Todos Santos (BTS) is located on the northwest coast of the Baja California peninsula, Mexico (31°40–56' N, 116°36–50' W; Fig. 1). The semi-enclosed BTS spans an area of ~180 km^2, exhibits and average depth of less than 50 m, and contains a submarine canyon (400 m depth) in the southwest area.

On 24 August, 2020, phytoplankton sampling was carried out in BTS at two sites (Fig. 1): Station 1 (31°48'21.9" N, 116°36'58.9" W) and Station 2 (31°50'28.9" N, 116°37'3" W). Biological material was collected via vertical casts with a phytoplankton net (20-µm mesh) at each sampling station. The samples were concentrated, placed in 250-mL plastic containers, and stored in a cooler for subsequent analysis in the laboratory. Additionally, samples were collected at depths of 1 m and 20 m in Niskin bottles (5 L) and transferred to amber bottles (250-mL). The Niskin samples were fixed in situ with 1% Lugol's acid and analyzed under an inverted microscope to determine absolute abundance.

2.2 Microscopy

Prior to morphological analysis, each sample was filtered through a sieve (130-µm mesh) to remove zooplankton and other organisms. A 500-µL aliquot of each sample was observed without a coverslip under a DM750 brightfield optical microscope (Leica Microsystems GmbH, Wetzlar, Germany) at low magnification (10×). A DMLB epifluorescence microscope (Leica Microsystems) was also used (excitation at 480–490 nm and emission at 520–540 nm). Cells of interest were isolated using the reduced micropipette technique described by Escarcega-Bata et al. (2021). Micrographs were taken with an EOS Rebel T6 camera (Canon Inc., Tokyo, Japan). Multiple cell planes were observed to identify morphological characters, including the shape and size of the epicone and hypocone, position and displacement of the cingulum and sulcus, shape and size of the apical groove, number of chloroplasts, and position and size of the nucleus. Cell abundance was assessed with the Utermöhl method (1958) using a 50-mL column and a DM IRB brightfield inverted microscope (Leica Microsystems).

2.3 Single-cell PCR and sequencing

The single-cell PCR protocol described by Hernández-Rosas et al. (2018) was followed, with minor modifications (Escarcega-Bata et al., 2021). The ribosomal marker LSU (Large Subunit) was amplified using primer pairs D1R-F (5'-ACCCGCTGAATTTAAGCATA-3') and D3B-R (TCGGAGGGAACCAGCTACTA) (Scholin et al., 1994; Hansen et al., 2000). PCR amplification was confirmed by electrophoresis using a 1.5% agarose gel (20 min at 90 V). Staining was performed with ethidium bromide. Gels were visualized using a UV light documentation system. PCR products were stored at –80 °C and sent to Retrogen Inc. (San Diego, USA) for purification and sequencing. The LSU sequences for both chains were determined using the referenced primers.

2.4 Sequence alignments and phylogenetic analyses

Sequences were aligned in BioEdit v. 7.0.5 (Hall, 1999) with those of a selected group of *Karenia* species obtained from GenBank of the National Center for Biotechnology Information. *Syndinium turbo* Chatton and *Hematodinium perezii* Chatton & Poisson formed the outgroup. Phylogenetic analyses were performed using Maximum Likelihood (ML) and Bayesian Inference (BI) for the LSU and Internal Transcribed Spacer (ITS) markers. The ML analysis was performed in RAxML (Stamatakis, 2006). Support for each branch was determined from 1000 bootstrap replicates. The BI analysis was performed in MrBayes v. 3.2.2 (Ronquist et al., 2012). The evolutionary model GTR + G (general time reversible + gamma distribution) was selected based on the Maximum Likelihood ratio test, using the jModeltest software (Posada, 2008). Four Markov chain Monte Carlo chains were used, starting with a random tree and sampling every 1,000 iterations for 1×10^8 generations, discarding the first 25% of trees (chain warm-up). Pairwise distance values (p-distances), Jukes-Cantor + Gamma (LSU) and Kimura 2 (ITS) corrected distances were calculated in Mega v. 11 (Tamura et al., 2011).

2.5 Genetic variation, genetic diversity, structure and differentiation

The measures of genetic variation and diversity were estimated, and polymorphic sites were identified from a genetic matrix that included the complete set of LSU sequences using DNASP v. 6.12.01 (Librado & Rozas, 2017). To evaluate the genetic structure of *K. selliformis*, gene flow measurements were obtained from the rates of the number of migrants (Nm) estimated under different models, and the fixation index (FST); both parameters were obtained using DNASP v. 6.12.01 (Librado & Rozas, 2017).

2.6 Ribotype analysis

An analysis of the sequences grouped by ribotype was performed in DNASP v. 6.12.01 (Librado & Rozas, 2017). To evaluate the relationships of the ribotypes of the large subunit and ITS (ITS1 + 5.8s + ITS2), a statistical parsimony network was constructed using Pop Art v. 4.8.4 (Leigh & Bryant, 2015).

2.7 Processing of satellite-derived imagery

To analyze sea surface temperature (SST) and the chlorophyll *a* (Chl *a*) concentration, a data set of satellite-derived images (1-km spatial resolution) was constructed for the west coast of North America (16–44° N, 140–100° W). The data were available in daily, 5-day, 15-day, and monthly composites. To enhance coverage and reliability, the daily products were merged from multiple satellite sensors. The Chl *a* data were obtained from the following sensors: CZCS, OCTS, SeaWiFS, MODIS-Terra/Aqua, MERIS, VIIRS-SNPP, OLCI-A/B, VIIRS-JPSS1, and SGLI. The SST data were merged from the MODIS-Terra/Aqua and

VIIRS sensors. The dataset (HDF4 format) contained numerical values, and the full-resolution images employed Lambert equal-area projection mapping. The SST values were linearly scaled, while the Chl *a* values were logarithmically scaled. To collect oceanographic data during each sampling date, an RBR CTD was deployed that measured temperature, conductivity, photosynthetically active radiation (PAR) (spectrometer, Ocean Optics, Orlando, USA), and fluorescence (fluorescence sensor, Turner Designs, San Jose, USA). Finally, based on geographic data obtained from the literature, we created a distribution map to visualize the known global distribution of *K. selliformis* in Ocean Data View v. 5.1.7 (Schlitzer, 2019).

3 Results

3.1 Morphology

Karenia selliformis Haywood, Steidinger & MacKenzie (Haywood et al., 2004) (Fig. 2A–F).

References: Al-Kandari et al. (2009; p. 157, Plate 7A–D); Al-Kandari et al. (2025; Fig. 3A–L); Al-Yamani & Saburova (2019; p. 113, plate 61); Cen et al. (2024; Figs. 1B1–B3; 2B1–B2); Haywood et al. (2004; p. 172, Fig. 4a–j); Iwataki et al. (2022; Figs. 2A–P; 3A–C); Lassus et al. (2016; p. 101, Plate 32C); Mardones et al. (2020; Fig. 3A–G); Orlova et al. (2022; Fig. 8A–O).

Description: solitary and athecate cells, discoid, dorsoventrally flattened (Figs. 2A–C), 34–40 µm (*mean* =36.7 ± 2.11, *n* = 30) in length, and 30–39 µm (*mean* = 34.3 ±2.8, *n* = 45) in width. Epicone roundish, smaller than the hypocone (Fig. 2A–B, E). Hypocone excavated in the center and bilobed (Fig. 2D-F); the right lobe extends more than the left. The cingulum is slightly pre-median, narrow, and deep (Fig. 2E–F). Apical groove straight, descending by 1/5 of the cell length, narrow, and deep. Chloroplasts numerous, peripheral, green to yellow in color (Fig. 2A-B), with pyrenoids (Fig. 2E). Big nucleus, spherical, located in the central portion of the hypocone, clearly visible in dorsal and ventral views (Fig. 2B-C, E-F).

3.2 Phylogeny

Three partial LSU rDNA sequences were obtained for *K. selliformis*. The final LSU alignment (620 bp) included 65 sequences. Phylogenetic reconstruction from the LSU dataset (with maximum phylogenetic support; ML/BI = 100/1) was performed with sequences of *Karenia* species (Fig. 3). In the resulting topology, sequences of *K. selliformis* formed a monophyletic clade with good phylogenetic support (ML/BI = 85/0.90). In turn, seven subclades were recognized within this clade. The first subclade, with high phylogenetic support (ML/BI = 90/0.92), grouped sequences from Japan (LC671821-LC671839) and Russia (MZ465595-MZ465598). The second subclade, with high phylogenetic support (ML/BI = 89/0.91), grouped sequences from Japan (LC671820), China (MT754561, OR543976, PP130347-PP130350, KY580784-KY580788) and Tunisia (AF318225). The third subclade, with maximum phylogenetic support (ML/BI = 100/1), grouped two sequences from Chile (MN203220-MN203221). Subclades four, five and six were made up of sequences from Chile (AF218247), New Zealand (U92250) and Kuwait (PP951879). Finally, subclade seven consisted of sequences from the Mexico generated in this study (PQ319544-PQ319546).

The final ITS alignment (570 bp) included 39 sequences. Phylogenetic reconstruction from the ITS (ITS1 + 5.8S + ITS2) dataset (with maximum phylogenetic support; ML/BI = 100/1) was performed with sequences of *K. selliformis* (Fig. 4). In the resulting topology, sequences of *K. selliformis* formed a monophyletic clade. The resulting topology consisted of two large clades. Clade one with maximum phylogenetic support (ML/BI= 100/1), grouped sequences from Russia (MZ465599-MZ465602) and Japan (LC671821-LC671839). Clade two with good phylogenetic support (ML/BI = 82/0.85) was divided into two subclades. Subclade one with good phylogenetic support (ML/BI= 80/0.82), grouped sequences only from Chile (AF318247, MT804573 and MT808180). Subclade two with high phylogenetic support (ML/BI= 95/1) was separated into two clades. Clade I with maximum phylogenetic support (ML/BI= 100/1) grouped sequences from Thailand (MT160343) and New Zealand (HM067008 and HM807324). Clade II with maximum phylogenetic support (ML/BI= 100/1) grouped sequences from Tunisia (AF318225), Japan (LC671820) and China (OR604560-OR604565, PP126483 and OR543987).

The uncorrected genetic distances (*p*-distances) obtained in the present study for LSU, ranged from 0.16 to 2.10%, and the corrected distances (from the best model) ranged from 0.17 to 2.14% (Table 1). The uncorrected genetic distances (p-distances) obtained in the present study for ITS (ITS1 + 5.8S + ITS2) ranged from 0.52 to 2.80%, and the corrected distances (from the best model) ranged from 0.53 to 2.87% (Table 2).

3.3 Genetic variation and diversity

Intraspecific analyses revealed that the LSU dataset of *K. selliformis* consisted of 44 sequences (620 bp); 15 sites were informative (Table 3), and 605 sites were invariant, representing 2.42% of the variation for that gene. Eight ribotypes (R1–R8; Table 5) were defined from the 15 polymorphic sites. The genetic diversity of the large subunit in *K. selliformis* indicated 15 segregating sites (S), with a haplotypic diversity (Hd) of 0.647 and nucleotide diversity (π) of 0.00558. The intraspecific analysis of the ITS (ITS1 + 5.8s + ITS2) data set of *K. selliformis*, consisted of 37 sequences (570 bp); 21 sites were informative (Table 4), and 549 sites were invariant, representing 3.68% of the variation for that gene.

Five ribotypes (R1–R5; Table 5) were defined from the 21 polymorphic sites. The genetic diversity of the ITS (ITS1 + 5.8s + ITS2) in *K. selliformis* indicated 21 segregating sites (S), with a haplotypic diversity (Hd) of 0.611 and nucleotide diversity (π) of 0.01330.

3.4 Ribotype analysis

The statistical parsimony network for LSU revealed the presence of eight interconnected ribotypes: R1–R8 (Fig. 5). The ribotype that grouped the largest number of sequences was R1 (n = 23), followed by R3 (n = 13) and R4 (n = 3). The remaining ribotypes (R2, R5, R6, R7 and R8) were unique, as they grouped only one sequence. The statistical parsimony network for the ITS (ITS1 + 5.8s + ITS2) revealed the presence of four interconnected ribotypes: R1–R5 (Fig. 6). The ribotype that grouped the largest number of sequences was R1 (n = 21), followed by R3 (n = 10), R2 (n = 3), R4 (n = 2), and R5 (n = 1).

The ribotype network LSU revealed a structure composed of different levels of mutational divergence. The lowest divergence was identified between ribotypes R5 and R6, which are separated by one mutational step. In contrast, the greatest differentiation was observed between ribotypes R1 and R8, with a separation of 10 mutational steps. In this sense, ribotypes R1 and R2, as well as R7 and R8, presented moderate differentiation, with three mutational steps between each pair of ribotypes. Between ribotypes R4 and R5, two mutational steps were identified, with the presence of an unsampled or absent intermediate ribotype, which acts as a transition between R4 and R5. Similarly, between R3 and R5, a distance of two mutational steps was present, and between these steps, another unsampled or absent ribotype was observed as a transition between R3 and R5, as well as between R1 and R5. In total, the network suggests the existence of three unsampled or absent ribotypes, the third being the one that connecting to the R7 ribotype. The structure of the network built from the ITS region showed the formation of two ribogroups: the first one formed by the ribotypes R1 and R2, and the second one composed of the ribotypes R4 and R5. A distance of 12 mutational steps was observed between these ribogroups, with the presence of three unsampled or absent ribotypes acting as a transition between both ribogroups. One of these non-sampled ribotypes gave way to the R3 ribotype, which occupies an intermediate position between both ribogroups. Within each ribogroup, a distance of three mutational steps was observed between the ribotypes R1 and R2 as well as between R4 and R5.

3.5 Structure and differentiation

From the information provided by the LSU haplotypes and under the model of Nei (1973), a calculated migration rate (Nm) of 0.06 and a Gst value of 0.79595 were obtained. Based on the model of Nei (1982), the migration rates calculated from nucleotide sequence data were: Nm = 0.06, DeltaSt = 0.00424, and GammaSt = 0.80026. Based on the model of Lynch and Crease (1990), with the Jukes and Cantor correction, the Nm value was 0.13, and the Nst value was 0.65348. Based on the model of Hudson et al. (1992), the Nm value was 0.13, and the FST value was 0.65496. Based on the information provided by the ITS haplotypes (ITS1 + 5.8s + ITS2) and under the model of Nei (1973), a calculated migration rate (Nm) of 0.02 and a Gst value of 0.91751 were obtained. Based on the model of Nei (1982), the migration rates calculated from nucleotide sequence data were: Nm = 0.03, DeltaSt = 0.01231, and GammaSt = 0.90620. Based on the model of Lynch and Crease (1990), with the Jukes and Cantor correction, the Nm value was 0.01, and the Nst value was 0.97646. Based on the model of Hudson et al. (1992), the Nm value was 0.01, and the FST value was 0.97650.

3.6 Sea surface temperature (SST) and the surface chlorophyll a (Chl a) concentration

The satellite data corresponding to each field sampling date (i.e., when in situ water samples were collected) represent the average of three consecutive days centered on the sampling date. The study periods considered in the present study were June, July, August, September, October, and November 2020 and March 2021. Averages were calculated for both SST and Chl *a* variables. A one-way analysis of variance (ANOVA) was conducted for each variable, and significant differences were found between sampling dates. In particular, SST was significantly different across the analyzed periods (p-value < 0.05). The Bonferroni multiple comparison test confirmed statistically significant differences in all pairwise comparisons ($p < 2e^{-16}$ in most cases), indicating that SST varied significantly between periods. These findings were supported by the mean plot with confidence intervals, which shows the notable temporal variation in SST. The results suggest that SST underwent significant changes during the evaluated periods. Importantly, August stood out among the study periods, given that it exhibited an average SST of 23.68 °C (Fig. 5), which was significantly higher than those of the other periods.

The ANOVA also revealed significant differences in Chl *a* between sampling dates, and the Bonferroni test showed that almost all group comparisons were statistically significant. Specifically, the mean Chl *a* concentration in March was much higher than those of the other periods (12.94 $mg \cdot m^{-3}$). In contrast, the mean Chl *a* concentration in June showed the least difference from those of the other periods and was most similar to the mean Chl *a* concentration in August (3.26 $mg \cdot m^{-3}$). The mean Chl *a* concentration in August was significantly different from that of July (5.54 $mg \cdot m^{-3}$) when a dense *Lingulaulax polyedra* (Stein) Head, Mertens & Fensome bloom was present in BTS. Additionally, *Karenia* abundance values in August were 200 cells·L^{-1} and 900 cells·L^{-1} at 2 m and 15 m, respectively. In the satellite images, Chl *a* values >10 mg·m^{-3} were observed at the surface near station 2, where *Karenia* abundance values of 290 cell·L^{-1} were recorded at the surface.

4 Discussion

4.1 Morphology

Although the *Karenia* specimens collected in this study from BTS presented some of the morphological variations that have been documented in previous studies (Iwataki et al., 2022), its shape and size coincide with those of *K. selliformis*. In our specimens, the epicone was rounded and small relative to the hypocone, which is consistent with previous descriptions of the species (Haywood et al., 2004; Escobar-Morales y Hernández-Becerril, 2015; Orlova et al., 2020; Iwataki et al., 2022; Cen et al., 2024; Wonly et al., 2024). The specimens collected from BTS were larger (34–40 µm) compared to those reported by Haywood et al. (2004) in the original description (20–30 µm; New Zealand) and to those reported by Escobar-Morales and Hernández-Becerril (2015) in the corresponding record from the Mexican Pacific (22–24 µm; coast of Colima). However, the cell dimensions of the specimens in the present study coincide with those reported by Iwataki et al. (2022) (35.3–43.6 µm; Hokkaido, Japan).

Another relevant character was the presence of small and numerous chloroplasts, which are a distinctive feature of *K. selliformis* (Haywood et al., 2004; Mardones et al. 2020; Iwataki et al., 2022). It should be noted that the specimens collected from BTS showed numerous parietal chloroplasts containing pyrenoids, similar to those described by Orlova et al. (2020) and Cen et al. (2024). The presence or absence of chloroplasts is a useful criterion for differentiating species during blooms, despite the considerable variability in shape and number, given the reports of 18 to 105 chloroplasts per cell and even reports of cells without chloroplasts (Iwataki et al., 2022). Nonetheless, a detailed analysis of chloroplast shape and size is needed to accurately compare this trait across species.

The nucleus of *K. selliformis* is known to be variable in shape, with reports of reniform, oval, elliptical, oblong, and elongated morphologies (Haywood et al., 2004; Escobar-Morales and Hernández-Becerril, 2015; Orlova et al., 2020; Mardones et al., 2020; Iwataki et al., 2022; Cen et al., 2024; Wonly et al., 2024). In the present study, the nucleus was slightly elongated and spherical, but always located in the hypocone. The morphological differences between the previous reports of *K. selliformis* from the coast of Colima and BTS may be due to differences in environmental conditions between sites and the influence of the fixatives used to prepare the specimens, which can result in morphological changes.

4.2 Phylogeny

The data set analyzed from partial sequences of the LSU revealed different molecular entities (i.e., ribotypes; Table 5) distributed among different localities around the world. It is important to note that each of these entities exhibited divergence at the intraspecific level (Table 1), which is consistent with the data presented by Mardones et al. (2020). In addition, the topologies reported by Iwataki et al. (2022) and Orlova et al. (2022) were consistent with the one generated in this study; however, in both studies, not all available sequences were considered, thus the results presented by these authors do not represent the current genetic variability of *K. selliformis*. Cen et al. (2024) provided *K. selliformis* sequences from the coast of China, however, the data used to construct the LSU topology were limited, as the clades obtained in that study were considered groups (two in total), as presented by Iwataki et al. (2022) (two in total). Additionally, in both studies, sequences were excluded as part of these groups, which underestimated the genetic variability of this species. Recently, in the study by Al-Kandari et al. (2025), a single *K. selliformis* sequence was presented for the waters of Kuwait (PP951879), however, this corresponded to a new ribotype, wich is closely related to the New Zealand ribotype (Figs. 3, 5). A better approximation of this genetic variability was presented by Mardones et al. (2020); these authors pointed out the presence of at least three different phylotypes, although they suggested that greater efforts were needed to detect additional isolates from other places, including Kuwait and Mexico. A greater number of sequences from different regions of the world (i.e., China, Chile, Japan, Kuwait, Mexico [this study], New Zealand, Russia and Tunisia) were integrated in the present study, allowing us to detect at least eight ribotypes.

The data set analyzed from partial sequences of the ITS (ITS1 + 5.8S + ITS2) also revealed different ribotypes (Table 5). It is important to note that each of these entities presented greater divergence at the intraspecific level (Table 2), compared to LSU. Mardones et al. (2020) indicated that this marker presented at least three phylotypes, however, these authors only used sequences from Chile, New Zealand, and Tunisia, thus the data of that study revealed low genetic variability. Orlova et al. (2022) reported two phylotypes, with sequences added for the coast of Russia; however, the arrangement of the phylotypes was not adequate, as the authors also considered sequences from Chile (AF318247 and MT808180) within the phylotype I, despite this being an independent phylotype. On the other hand, in the topology presented by Iwataki et al. (2022) and Cen et al. (2024) the phylotypes or ribotypes were treated as groups (two in total), however, there was no detailed discussion of this grouping, leaving aside the variability observed in this topology. Although this study did not generate sequences for ITS, data provided from sequences generated in previous studies support the existence of a cryptic complex within *K. selliformis,* suggesting the presence of a greater number of genetic entities around the world; however, isolates from other sampling sites are needed to evaluate this possibility, given that current reports are based on morphological descriptions.

4.3 Genetic variation and diversity

The genetic diversity analysis employing both the LSU and ITS markers supported the presence of genetically independent entities within *K. selliformis*. Although our analysis only included 44 genetic sequences with LSU and 37 with ITS, it was possible to detect high interpopulation genetic variation within the species. This variation was observed through the formation of several genetic groups in the statistical parsimony network. With LSU, the eight ribotypes obtained formed at least four ribogroups of which those represented by ribotypes R1, R2, and R8 were the most notable in terms of the number of connections and unsampled ribotypes that separated them, and the evident genetic distance between them (0.48% and 1.61%, respectively). On the other hand, with ITS, the five resulting ribotypes revealed at least three ribogroups of which, the most representative were R1, R4, and R5, which reflected the large genetic distance between them (0.52% and 2.63%, respectively). In addition, the total coefficient of interpopulation genetic differentiation obtained with the two calculated indices (LSU: Gst = 0.79595 and Fst = 0.65496; ITS: Gst = 0.91751 and Fst = 0.97650) revealed a value close to 1 between ribogroups with both markers, which supports total structure between them (Excoffier & Lischer, 2010). Thus, no genetic connection was found between these populations, which was also supported by the number of migrants calculated for each index under different models (LSU: Nm =0.06–0.13; ITS: 0.01–0.03). These results were also consistent with those from our phylogenetic analysis, in wich the ribogroups defined in the ribotype networks also formed an independent phylogenetic group, with good well phylogenetic support.

Mardones et al. (2020) suggested that *K. selliformis*, as it is currently understood, could constitute a cryptic complex that requires further study. The authors based this conclusion on the analysis of *K. selliformis* sequences from Chile, with sequencies from China, New Zealand, and Tunisia obtained from GenBank. The evidence provided by our genetic diversity analyses with two markers, which also included sequences from Mexico, supports that *K. selliformis*, which is currently understood to exhibit a worldwide distribution, is actually composed of a set of differentiated genetic entities that are morphologically indistinguishable based on the current evidence. This conclusion agrees with that of Mardones et al. (2020). Thus, *K. selliformis* constitutes an authentic complex of cryptic species. Moreover, although an analysis of genetic diversity is lacking, high interspecific differentiation was observed consistently in its phylogeny.

With both markers, the interspecific genetic distance values were higher between some ribogroups such as ribotypes R1 (which grouped sequences from Japan and Russia) and R3 (which grouped sequences from China Japan and Tunisia). With LSU, the relationships among the remaining ribotypes and the major ribogroups suggest that the R4 ribogroup, which currently corresponds to Mexico and is supported by the calculated interspecific genetic distance values (0.32–1.30%), actually corresponds to an independent species that should be described as such. Unfortunately, in the present study, we do not have sufficient evidence to be able to definitively support this idea. Despite having established the necessary morphological characterization, we have not yet identified a set of characters that allows us to establish that the genetic group from Mexico is distinct from those of Chile, China, Japan, Kuwait, New Zealand, Russia and Tunisia (Guillou et al., 2002; Haywood et al., 2004; Mardones et al., 2020; Iwataki et al., 2022; Orlova et al., 2022; Cen et al., 2024; Al-Kandari et al., 2025).

The ecological or biogeographic factors that may have resulted in the genetic isolation observed between the different populations of *K. selliformis* must be evaluated in detail in future studies. At present, the only apparent barrier between these populations is ecological, with one group distributed in temperate zones and another group distributed in tropical zones. However, among the *K. selliformis* populations of Chile, it is possible to detect three genetically independent groups, which correspond to the same region (with LSU). Thus, the mechanisms of genetic isolation cannot be established from the current evidence. Nonetheless, the high number of variable sites in LSU (620 bp analyzed) and ITS (570 bp analyzed), and the high number of sampled and unsampled ribotypes indicate that the process that gave rise to the genetic separation between the groups was not geologically recent, given that the high number of ribotypes and variable sites are indicators of stable populations that underwent divergence many millions of years ago (Rogers & Harpending, 1992; Grant & Bowen, 1998).

The evidence so far suggests that species discrimination within this complex must be conducted through geometric and numerical morphometry or even using geographic and ecological data. Based on the results of this study and those of Mardones et al. (2020), cryptic genetic variants are responsible for the worldwide distribution of *K. selliformis*. Thus, detailed studies of populations are needed that focus on the toxic potential and environmental temperatures associated with this species.

4.4 Harmful algal blooms (HABs) of Karenia selliformis in the Pacific Ocean

Karenia selliformis is present in different areas of the Atlantic, Indian, and Pacific Oceans (Fig. 8; Table 6); however, there are few records of this species associated with harmful algal blooms (HABs) (Mardones et al., 2020; Iwataki et al., 2022; Orlova et al., 2022). In the Pacific Ocean, extensive blooms of *K. selliformis* have been reported that were associated with the mortality of invertebrate and vertebrate organisms (Uribe & Ruiz, 2001; Mardones et al., 2020; Iwataki et al., 2022; Orlova et al., 2022).

On the western Pacific coast, Orlova et al. (2022) reported an extensive bloom along the coast of Kamchatka, Russia, which occurred in September and October 2020. During this event, the temperature ranged from 9–13 °C, and the maximum cell density and biomass values were 622 cell·mL^{-1} and >100 mg·m^{-3}, respectively. In the same portion of the Pacific Ocean, Iwataki et al. (2022) reported an extensive bloom along the coast of Hokkaido, Japan, during September 2021. The authors registered SST values of 8.1–19 °C and cell density values of 100–10,560 cell·mL^{-1}. Both blooms were associated with the presence of cold waters; however, a higher cell density was associated with the bloom in Hokkaido. It is important to note that the resistance and migration of R1 (LSU and ITS's; Table 5), which was present in the 2020 Kamchatka bloom, towards the coast of Hokkaido was likely due to the ability of *K. selliformis* to exhibit a zygotic stage in its life cycle (Mardones et al., 2020).

In the Eastern Pacific Ocean, several blooms of K. *selliformis* have been reported, particularly along the coast of Chile (Uribe & Ruiz, 2001). An extensive bloom event was reported in April 1999 in Magallanes fjords, which resulted in invertebrate and fish mortality. The temperature reported during the bloom in southern Chile was 13.5 °C, with cell density values of 3,000–43,000 cell·L^{-1}. During the austral summer of 2018, a bloom of *K. selliformis* was reported in southern Chile by Mardones et al. (2020), which was also responsible for the mortality of marine fauna. However, the cultivated strains of *K. selliformis* did not produce gymnodimines and instead produced a compound related to brevetoxin; the authors reported that the optimal growth temperature in culture was 18 °C (salinity of 30). Finally, the genetic analyses of that study identified at least three ribotypes with the LSU marker (R2, R7-R8) and one ribotype in ITS (R2) for the coast of Chile (Table 5). This region is of ecological interest for future studies of *K. selliformis* due to the potential of this species to form blooms and produce toxins.

In the Mexican Pacific, the wide distribution of *K. selliformis* spans the southern coast of the Gulf of Tehuantepec to the northwest coast of the Baja California peninsula (Maciel-Baltazar, 2015; Almazán-Becerril et al., 2016; Escarcega-Bata et al., 2023). In BTS, only one record of *K. selliformis* has been published (Almazán-Becerril et al., 2016), with no other previous reports of blooms generated by this species in BTS or the Mexican Pacific. However, in August 2020, a *K. selliformis* bloom (200–900 cell·L^{-1}) occurred within BTS. Moreover, based on temperature (23.68 °C) and Chl *a* (>10 mg·m^{-3}) data (Fig. 7), this bloom is the first in the Pacific Ocean to be associated with warm waters. However, in September 1999 in Kuwait Bay in the Arabian Sea, a bloom of this species (6 x 10^6 cell·L^{-1}) occurred that was associated with temperatures ranging from 26.9–28.6 °C, thus *K. selliformis* proliferate in warm waters (Heil et al., 2001). According to Smayda & Reynolds (2001), blooms are controlled by multiple biological, chemical, and physical processes, with temperature being a central factor determining the potential for HAB formation. Notably, this event did not cause any apparent damage to marine life. It is important to note, however, that field monitoring during and after the August 2020 bloom was limited due to the COVID-19 pandemic. As a result, potential sublethal or delayed ecological effects could not be fully assessed, nor were toxin analyses conducted at the time.

Although HABs have traditionally been defined based on high cell concentrations and the presence of toxins, multiple studies have shown that certain species can cause significant ecological and health impacts even at low densities. For example, *Dinophysis* spp., for instance, has been documented to induce shellfish toxicity events with abundances as low as 100–200 cells L^{-1}. (Panton & Puerdie, 2022; Velasco-Senovilla et al., 2023), wich have often resulted in harvest closures, even in the absence of large-scale blooms (Mardones et al., 2020; Garcia-Mendoza et al., 2014). Similarly, in our study, *Karenia selliformis* did not reach high cell densities, yet its persistent presence and genetic diversity suggest that it may have a harmful potential. Unlike other taxa, the effects of *K. selliformis* appear to be mediated by compounds that have not yet been fully characterized, such as long-chain polyunsaturated fatty acids (PUFAs) and other cytotoxic substances (Chang et al., 2006; Brand et al., 2012).

This concern is particularly relevant in coastal regions such, as Bahía de Todos Santos, Baja California, where the aquaculture industry is rapidly expanding. In this area, lipophilic toxins, including gymnodimine and other cyclic imines—have been detected in mussels even in the absence of evident bloom events or high cell concentrations, wich are likely associated with genera such as *Dinophysis* (García-Mendoza et al., 2014, Shultz et al., 2019). Importantly, *K. selliformis* has shown high cytotoxic potential and has been linked to fish mortality in other regions, despite variable or moderate cell abundances (Mardones et al., 2020). Altogether, this evidence underscores the importance of recognizing that a harmful algal bloom does not always require high cell abundances, measured toxin concentrations, or observable impacts to be of concern. In the case of *K. selliformis*, its persistence, genetic diversity, and the toxic potential reported in similar environments justify its consideration as a potentially harmful species. Continued monitoring is warranted, especially in regions undergoing aquaculture development, where even low-density events may pose a latent but relevant risk to marine life and human health.

5 Conclusions

Very few studies have evaluated the genetic variation of phytoplankton species. Given that phytoplankton are unicellular organisms with limited potential for genetic expression and, consequently, morphological differentiation, studies that evaluate the genetic variation of phytoplankton studies are needed to understand their evolutionary history, the processes involved in their origin, and their phylogenetic relationships. The results of this study support the phylogenetic independence of the different cryptic entities of the complex *Karenia selliformis*. Given that there are many taxonomic problems associated with classifying phytoplankton species, analyses are needed to elucidate these important relationships. Following the study of Zamudio-Resendiz et al. (2022), the present study is the second to contribute an intrapopulation analysis based on morphological and molecular data for a phytoplankton species in Mexican waters.

Additionally, the persistent presence of *K. selliformis* and the genetic diversity revealed in this study highlight the relevance of monitoring this species, even in the absence of observable ecological impacts or toxin measurements. Considering its documented capacity to form blooms in other regions and the expansion of aquaculture along the Mexican Pacific coast, it is important to consider its potential as an emerging harmful species. This study not only advances our understanding of its genetic diversity, but also underscores the need to integrate molecular, ecological, and monitoring approaches to better assess and manage the possible impacts of cryptic phytoplankton taxa in coastal environments

CRediT authorship contribution statement

Alexis Escarcega-Bata: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Conceptualization. **Mary Carmen Ruíz-de la Torre:** Writing – original draft, Validation, Investigation, Funding acquisition. **Estefany Cuevas Sanchez:** Data curation, Writing – review & editing, Methodology, Validation. **Jovanny Arana-Garcia:** Writing – review & editing, Validation. **María Luisa Núñez Resendiz:** Writing – review & editing, Validation, Investigation. **Beatriz Martin Atienza:** Data curation, Methodology, Validation. **Abel Sentíes:** Writing – review & editing, Visualization, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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TABLES

Table 1. Genetic divergence (percentage) between *Karenia selliformis* sequences of LSU. Uncorrected distances are shown below the diagonal, and Jukes-Cantor + Gamma distance values are located above the diagonal. Ribotypes are shown in parentheses. Letters highlighted in bold indicate data generated in this study.

	1	2	3	**4**	5	6	7	8
1. *Karenia selliformis* (R1)	-	0.49	0.81	1.14	0.81	0.97	1.14	1.62
2. *K. selliformis* (R2)	0.48	-	1.30	1.30	0.97	1.14	1.64	2.14
3. *K. selliformis* (R3)	0.80	1.29	-	0.65	0.32	0.48	0.65	0.81
4. *K. selliformis* (R4)	1.13	1.29	0.64	-	0.32	0.48	0.65	1.14
5. *K. selliformis* (R5)	0.80	0.96	0.32	0.32	-	0.17	0.65	1.14
6. *K. selliformis* (R6)	0.96	1.13	0.48	0.48	0.16	-	0.81	1.30
7. *K. selliformis* (R7)	1.13	1.61	0.64	0.64	0.64	0.80	-	0.50
8. *K. selliformis* (R8)	1.61	2.10	0.80	1.13	1.13	1.29	0.48	-

Table 2. Genetic divergence (percentage) between *Karenia selliformis* sequences of Internal Transcriber Spacer (ITS1 + 5.8s + ITS2). Uncorrected distances are shown below the diagonal, and Kimura 2 distance values are located above the diagonal. Ribotypes are shown in parentheses.

	1	2	3	4	5
1. *Karenia selliformis* (R1)	-	2.50	2.50	0.53	2.69
2. *K. selliformis* (R2)	2.45	-	1.06	2.68	1.24
3. *K. selliformis* (R3)	2.42	1.05	-	2.69	0.53
4. *K. selliformis* (R4)	0.52	2.63	2.63	-	2.87
5. *K. selliformis* (R5)	2.63	1.22	0.52	2.80	-

Table 3. Proportion of variable sites in *Karenia selliformis* rDNA sequences for LSU haplotypes. The dot indicates the same base as in the R1 ribotype sequence. The ribotype in bold indicates the one generated in this study.

Ribotype	11	129	267	448	477	523	531	541	548	554	562	584	597	610	616
R1	T	T	C	G	C	G	T	T	C	T	T	C	T	G	A
R2	.	.	.	.	.	.	.	.	T	C	C	.	.	.	.
R3	.	C	.	.	T	A	C	.	.	.	.	.	.	.	G
R4	.	C	.	.	T	.	C	.	.	.	C	T	C	.	G
R5	.	C	.	.	T	.	C	.	.	.	C	.	.	.	G
R6	.	C	.	.	T	.	C	.	.	.	C	.	.	T	G
R7	C	C	.	.	T	.	C	C	.	.	.	T	.	.	G
R8	C	C	T	A	T	A	C	C	.	.	.	T	C	.	G

Table 4. Proportion of variable sites in *Karenia selliformis* rDNA sequences for Internal Transcriber Spacer (ITS1 + 5.8s + ITS2) haplotypes. The dot indicates the same base as in the R1 ribotype sequence.

Ribotype	17	54	55	59	71	145	147	165	177	192	206	209	411	413	427	458	461	471	498	526	545
R1	C	T	T	G	T	T	T	T	C	T	G	G	C	T	C	C	T	G	G	C	T
R2	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
R3	A	.	.	A	C	.	G	C	A	.	A	A	.	C	A	T	C	A	A	.	C
R4	A	.	C	A	C	.	G	C	.	C	A	A	.	C	A	.	.	A	A	T	C
R5	A	.	C	A	C	G	G	C	.	C	A	A	T	C	A	.	.	A	A	.	C

Table 5. GenBank accession numbers associated with each of the resulting ribotypes.

RiboType	GenBank accession number (LSU)
R1	LC671821-839, MZ465595-598
R2	AF318247
R3	MT754561, OR543976, PP130347-350, AF318225, KY580784-88, LC671820
R4	**PQ319544-546**
R5	U92250
R6	PP951879
R7	MN203221
R8	MN203220
RiboType	GenBank accession number (ITS1 + 5.8s + ITS2)
R1	MZ465599-602, LC671821-839
R2	AF318247, MT804573, MT808180
R3	AF318225, LC671820, OR604560-565, PP126483, OR543987
R4	HM067008 HM807324
R5	MT160343

Table 6. References for each *Karenia selliformis* record in coastal waters worldwide. GenBank accession numbers are included and water temperature for each site.

Country	Locality	Reference	Temperature (°C)	GenBank accession number	
				LSU	ITS
Mexico	Bahía Todos Santos, Baja California	This study	23.68	PQ319544-46	-
		Almazán-Becerril et al., 2016	-	-	-
	Bahía de Acapulco, Guerrero	Meave del Castillo et al., 2012	19-31	-	-
	Costa de Chiapas	Maciel-Baltazar, 2015	-	-	-
	Costa de Colima	Escobar-Morales & Hernández-Becerril. 2015	-	-	-
	Gulf of Mexico	Anderson et al., 2021	-	-	-
		Steidininger et al., 2009	-	-	-
Japan	Aomori	Iwataki et al., 2022	23	LC671820	LC671820
	Hokkaido		9.8-17.6	LC671821-839	LC671821-839
Russia	Kamchatka	Orlova et al., 2022	9-13	MZ465595-98	MZ485599-602
Chile	Magellanic Fjords, Southern Chile	Uribe & Ruiz, 2001	13.5	-	-
	Canal Costa	Guillou et al., 2002	-	AF318247	AF318247
	Patagonian Fjjords	Mardones et al., 2020	15*	MN203220-21	MT808180, MT804573
China	Tolo Harbour	Cen et al., 2024	20*	MT754561, OR543976	OR527560-565
	Baguang Bay			PP130350	OR543987
	Beibu Gulf			PP130347-349	PP126483
New Zealand	Foveanux strait, South Island	Al-Kandari et al., 2011	19*	-	HM807324
		Haywood et al., 2004	19*	U92250	-
		Henrichs et al., 2011	19*	-	HM067008
		Stirling, 2001	-	-	-
		Miles et al., 2000	-	-	-
Kuwait	Kuwait Bay	Al-Kandari et al., 2009	-	-	-
		Al-Kandari et al., 2025	23.5*	PP951879	-
		Al-Yamani & Saburova, 2019	-	-	-

		Heil et al., 2001	26.9-28.6	-	-
Tunisia	Bay of Gabes	Guillou et al., 2002	20*	AF318225	AF318225
	Sfax, Gulf of Gabes	Medhioub et al., 2009	17-21*	-	-
	Gulf of Gabes	Feki et al., 2013	-	-	
	Harbour of Sfax	Boudriga et al., 2023	27-30	-	-
Saudi Arabia	Thuwal, Central Red Sea	Prabowo & Agusti, 2020	23.06–32.60	-	
USA	Delmarva Peninsula	Wolny et al., 2024	15.5-20.8	-	-
Thailand	Gulf of Thailand	Fu et al., 2021	29.6-30.8	-	MT160343

* Growth temperature in culture.

Figure legends

Figure 1. Study area with sampling sites (indicated with Arabic numbers) in Bahía Todos Santos, Mexico.

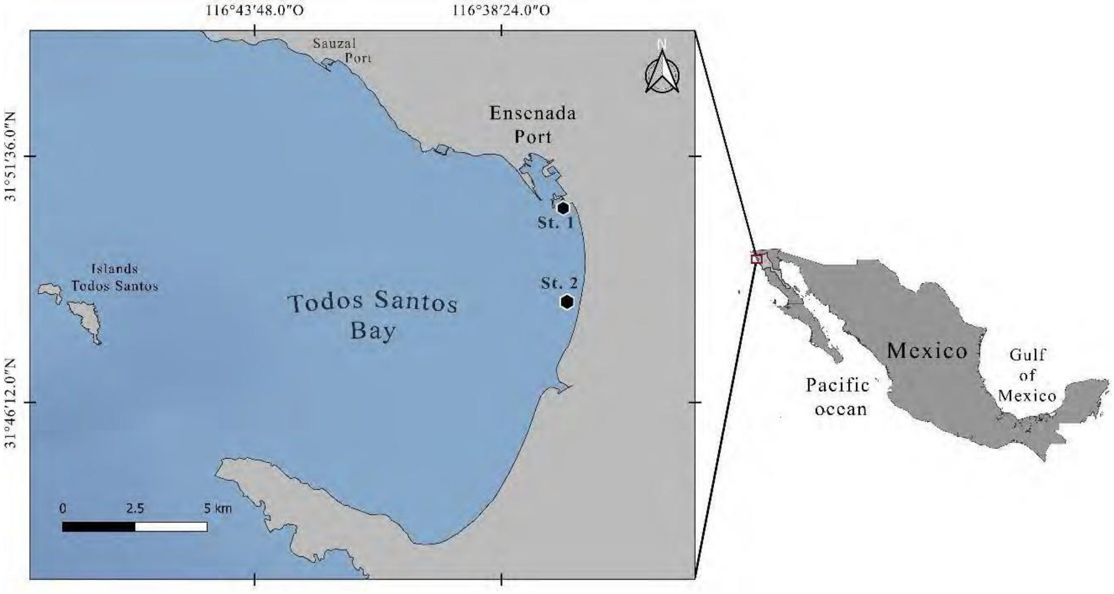


Figure 2. Light and fluorescence micrographs of *Karenia selliformis* during bloom of 2020 in Bahía Todos Santos, Mexico. Cells in dorsal (A, C, E-F) ventral (B) and lateral (D) views. Apical groove (Ag), Cingulum (Ci), Chloroplast (Cl), Nucleus (N), Pyrenoids (Py). Scale Bar: 10 µm.

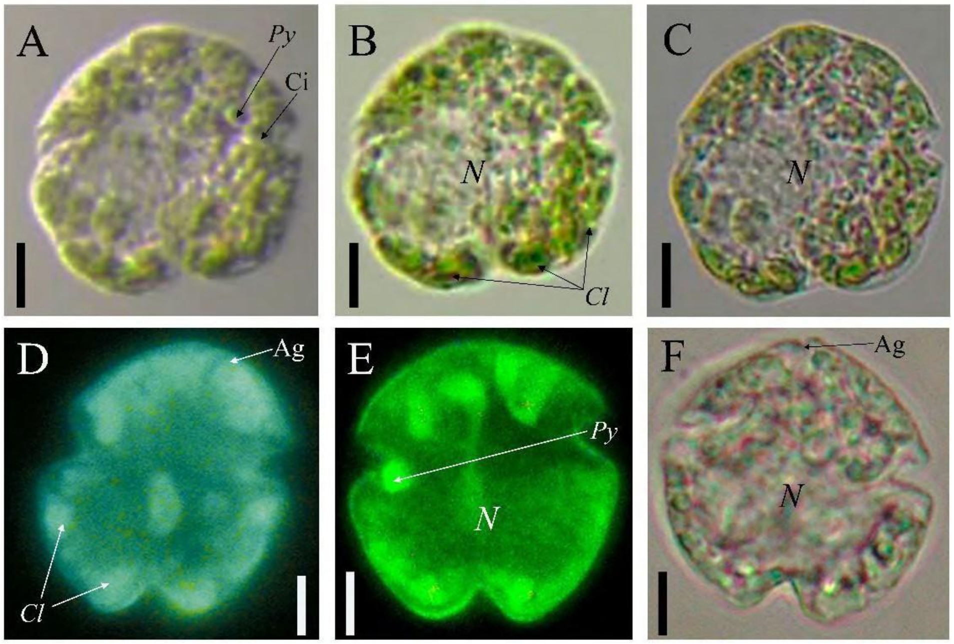


Figure 3. Bayesian inference topology and maximum likelihood based on LSU rDNA (620 pb) sequences data from species of genus *Karenia* (Dinophyceae). BI (right) followed by ML bootstrap values (left) on branches. Asterisks indicate full support (ML/BI= 100/1). Sequences generated in this study are in boldface. S.P.S. = substitutions per site.

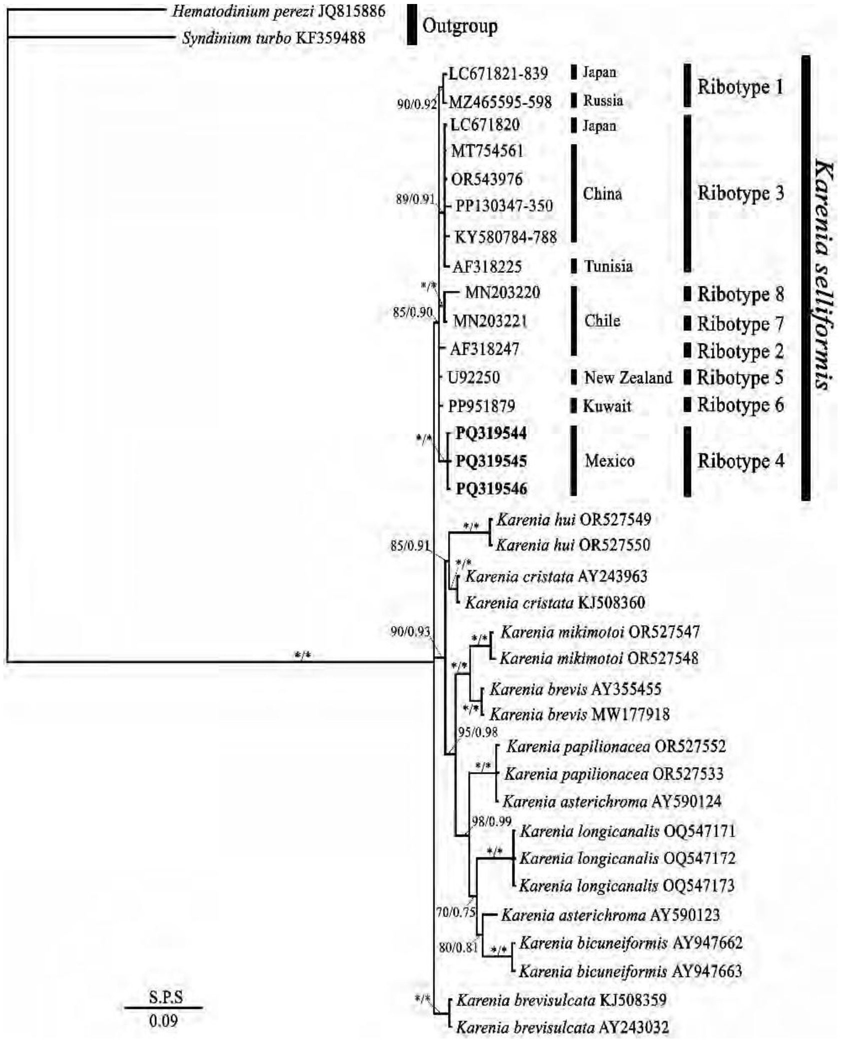


Figure 4. Bayesian inference topology and maximum likelihood based on Internal Transcriber Spacer (ITS1 + 5.8s + ITS2) (570 pb) sequences data from *Karenia selliformis* (Dinophyceae). BI (right) followed by ML bootstrap values (left) on branches. Asterisks indicate full support (ML/BI= 100/1). S.P.S. = substitutions per site.

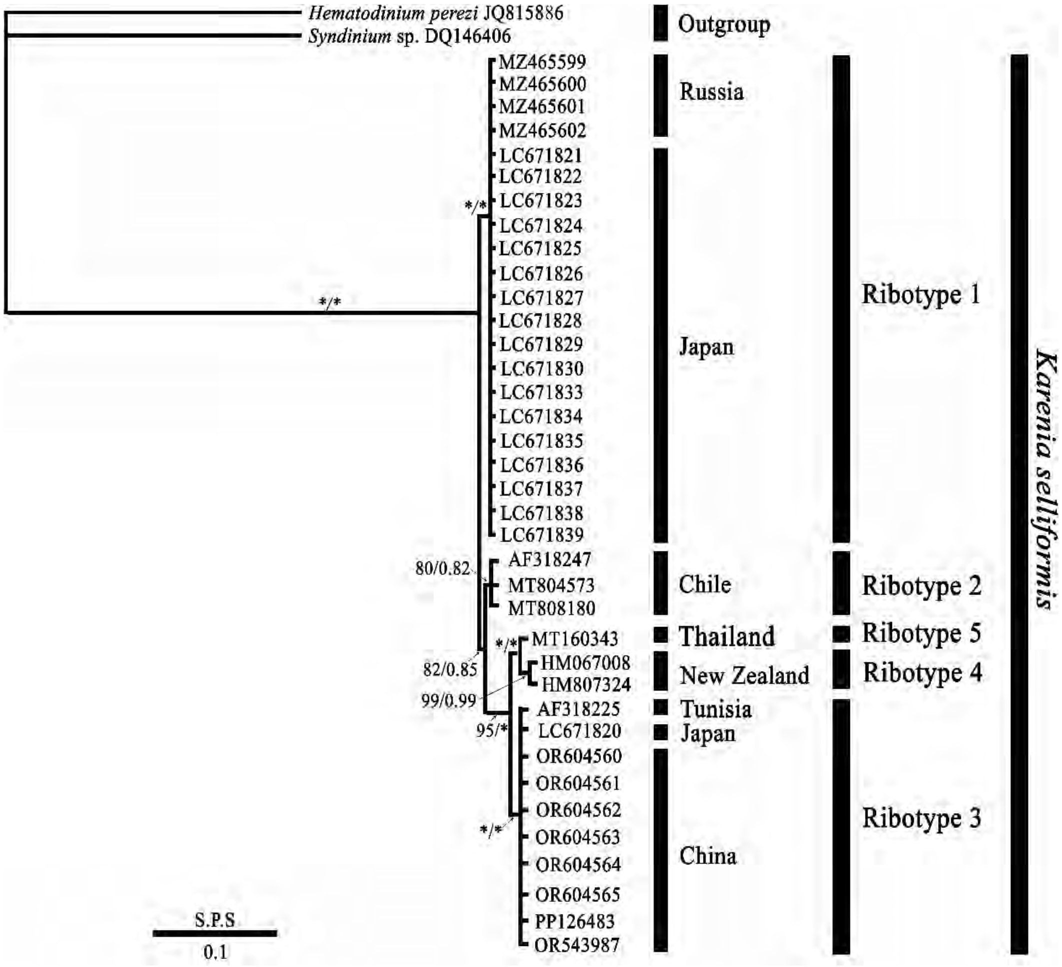


Figure 5. Statistical parsimony network of the *Karenia selliformis* large subunit. Lines represent parsimonious connections between haplotypes as a probability greater than 95%. Crossed bars represent the number of mutational steps between haplotypes. Each circle represents a ribotype and its size corresponds to its frequency. Filled circles represent a missing or unsampled ribotype; n= number of sequences belonging to each ribotype.

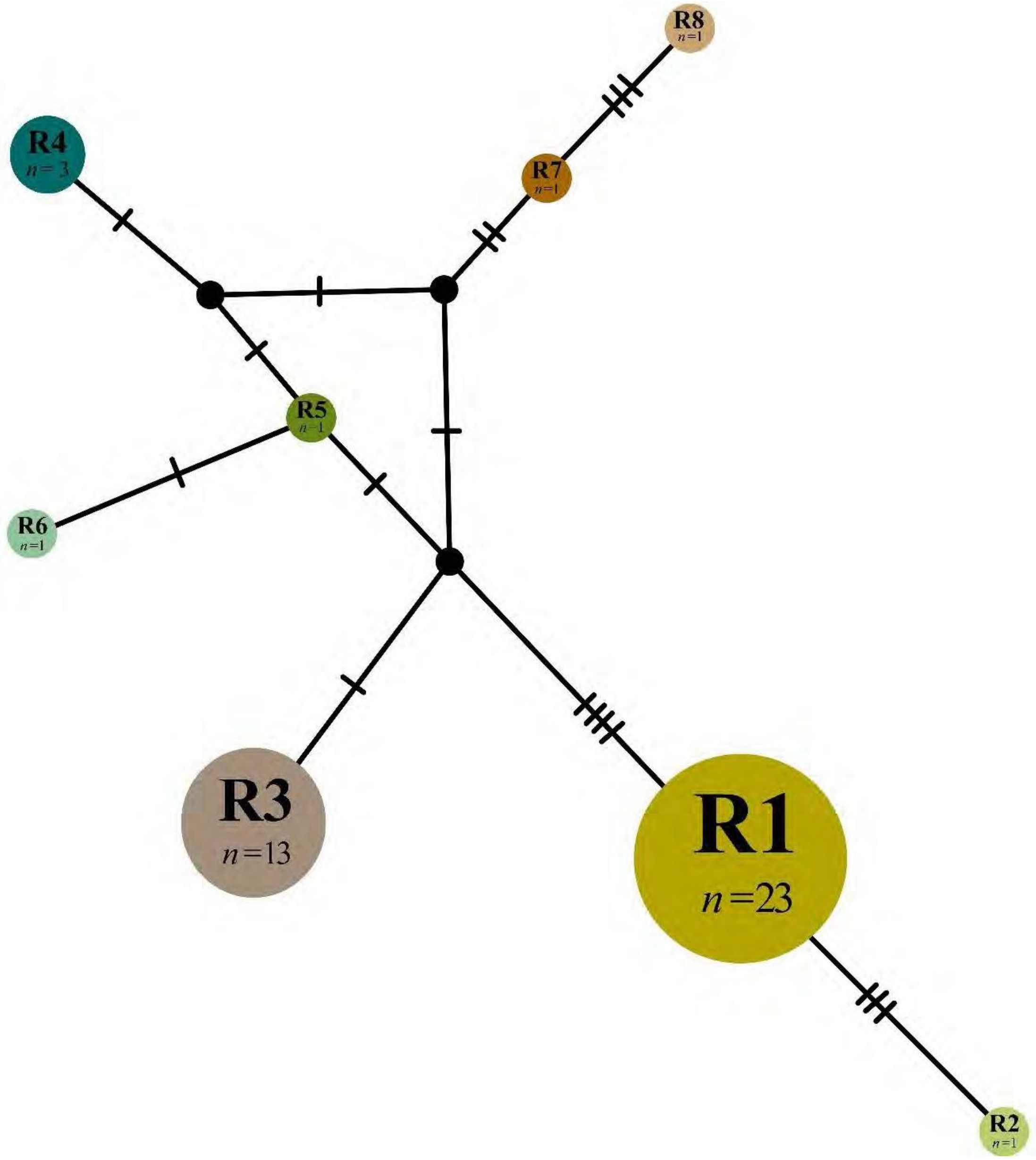


Figure 6. Statistical parsimony network of the *Karenia selliformis* Internal Transcriber Spacer (ITS1 + 5.8s + ITS2). Lines represent parsimonious connections between haplotypes as a probability greater than 95%. Crossed bars represent the number of mutational steps between haplotypes. Each circle represents a ribotype and its size corresponds to its frequency. Filled circles represent a missing or unsampled ribotype; n= number of sequences belonging to each ribotype.

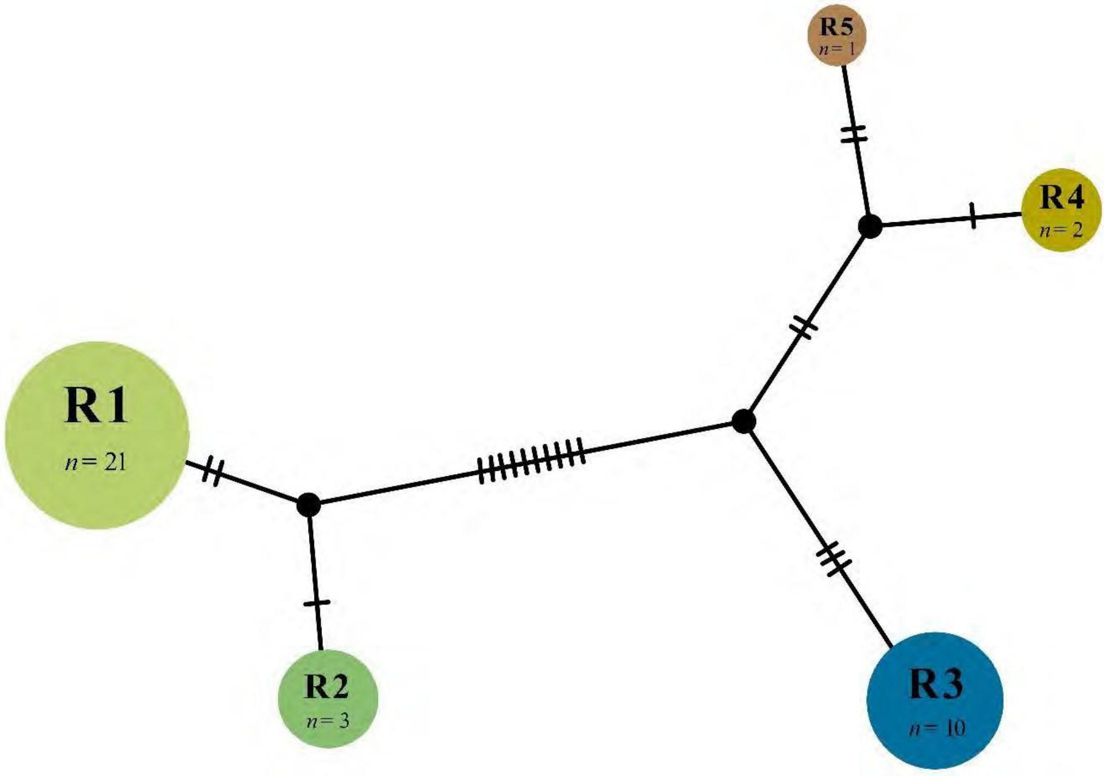


Figure 7. Satellite-derived images of sea surface temperature (◦C) (A) and chlorophyll *a* (mg·m–3) (B) of Todos Santos Bay, Mexico on August, 2020.

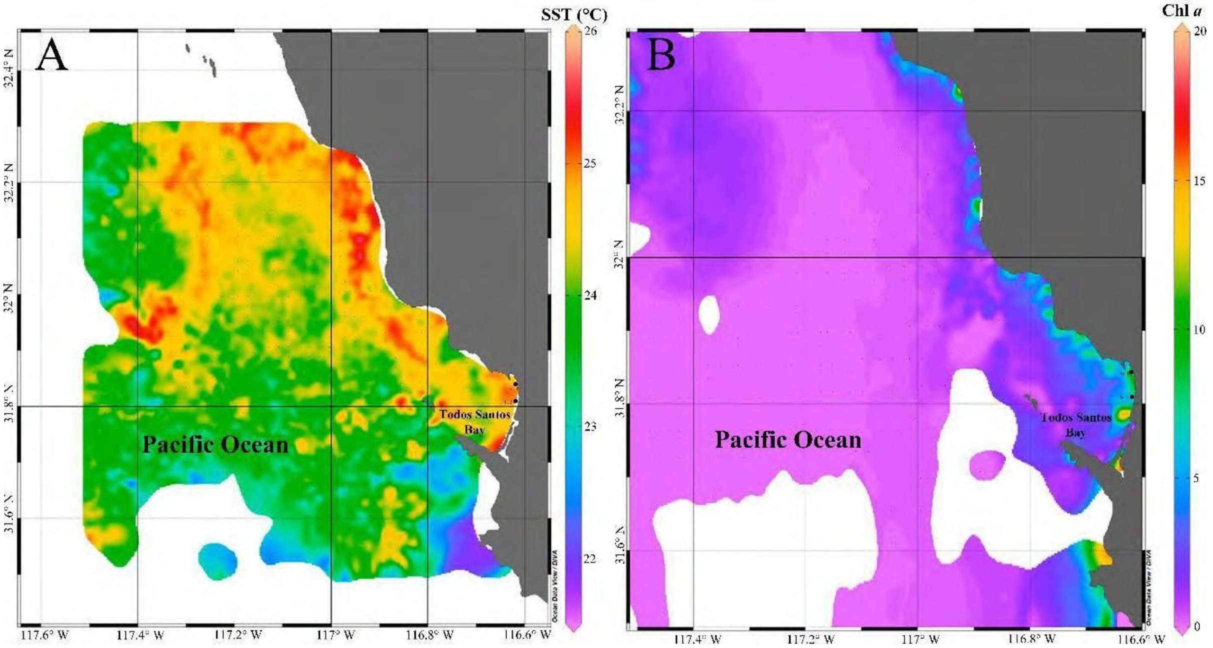
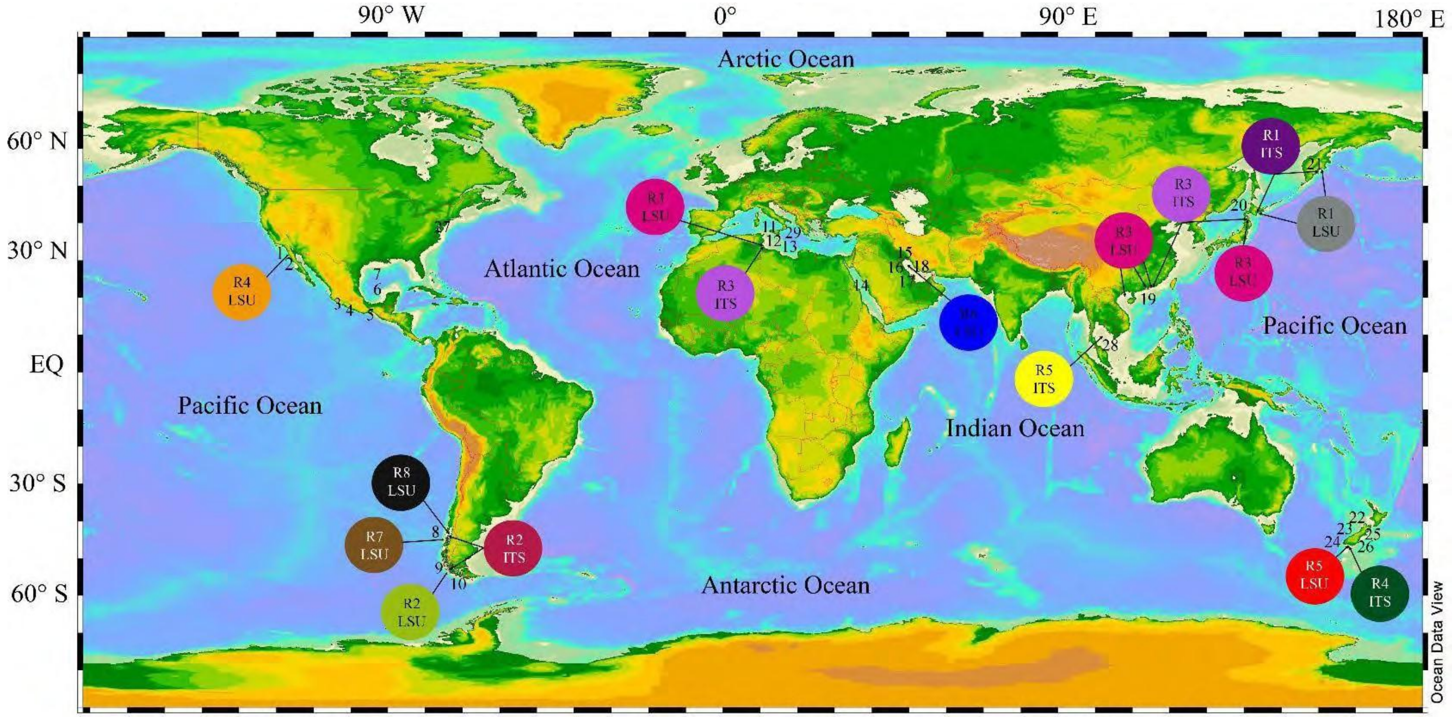


Figure 8. Known geographical distribution of *Karenia selliformis*, in coastal waters around the world. The circles indicate the ribotypes for LSU and ITS found. The citations below the map correspond to each record of the species.



1.- This study 2.- Almazán-Becerril et al., 216 3.- Escobar-Morales and Hernández-Becerril, 2015 4.- Meave del Castillo et al., 2012 5.- Maciel-Baltazar, 2015 6.- Steidininger et al., 2009 7.- Anderson et al., 2021 8.- Maradones et al., 2020 9.- Guillou et al., 2002 10.- Uribe & Ruiz, 2001 11.- Guillou et al. 2002 12.- Medhioub et al., 2009 13.- Feki et al., 2013 14.- Prabowo & Agusti, 2020 15.- Al-Kandari et al., 2009 16.- Al-Kandari et al., 2025 17.- Al-Yamani & Saburova, 2019 18.- Heil et al., 2001 19.- Cen et al., 2024 20.- Iwataki et al., 2022 21.- Orlova et al., 2022 22.- Haywood et al., 2004 23.- Stirling, 2001 24.- Miles et al., 2000 25.- Henrichs et al., 2011 26.- Al-Kandari et al., 2011 27.- Wonly et al., 2024 28.- Fu et al., 2021 29.- Boudriga et al., 2023

11. CONCLUSIONES GENERALES

La diversidad actual de dinoflagelados Gymnodiniales en el Pacífico mexicano es baja, ya que solo el 16% del total de las especies registradas a nivel mundial, se encuentran reportadas para los estados costeros de este litoral mexicano.

El uso de marcadores del ADN ribosomal (18S y 28S), resultan esenciales en la identificación de dinoflagelados Gymnodiniales, sin embargo, el bajo nivel de polimorfismo en cada uno de ellos representa una limitante en la generación de aproximaciones filogenéticas más robustas dentro del grupo.

Las espaciadoras transcritas internas (ITS1 e ITS2), del ADNr, presentan un nivel resolutivo eficiente en la identificación molecular a nivel intraespecífico, sin embargo, su uso se encuentra limitado solo a algunos géneros, como en el caso de la especie *Karenia selliformis*.

Los análisis de la variación genética permiten reconocer y distinguir especies cripticas. En el caso particular de la especie *Karenia selliformis*, la baja plasticidad fenotípica ha llevado a una identificación sesgada por parte de los especialistas, en donde los caracteres morfológicos que exhibe, han preponderado sobre otras metodologías de identificación. Los distintos ribotipos reportados a nivel mundial, indican una alta variabilidad intraespecífica dentro de la especie, por lo que se confirma el complejo críptico *K. selliformis*.

El reporte de especies potencialmente formadoras de florecimientos algales, brindan una mejor aproximación en la implementación de estrategias que permitan prevenir riesgos sanitarios, socioeconómicos y protección a la biodiversidad marina.

La medición de parámetros ambientales como: temperatura, salinidad y oxígeno disuelto, permiten conocer la estructura, dinámica y función de la comunidad fitoplanctonica, adicionalmente, es posible comprender las condiciones ambientales que pueden afectar a los procesos ecológicos en el ambiente marino, especialmente relacionados con los eventos de florecimientos algales nocivos.

12. PERSPECTIVAS

Debido a la subestimación de la diversidad de Gymnodiniales en el Pacífico mexicano, es necesario incursionar en diferentes metodologías de colecta e identificación, asimismo, el conjunto de estas herramientas permitirá contar una taxonomía integrativa.

Debido al bajo nivel de polimorfismo de los marcadores mitocondriales empleados en la actualidad, es necesario explorar nuevos marcadores moleculares que permitan tener una mejor aproximación filogenética.

Estudios futuros deben enfocarse en aquellas especies tóxicas emergentes, en la relación de las condiciones ambientales con la presencia de FAN en zonas de interés socioeconómico y en el desarrollo de modelos predictivos para este tipo de eventos.

Es necesario aumentar los esfuerzos de muestreo, ya que el desarrollo de estudios sistemáticos permitirá establecer alertas tempranas y protocolos de respuesta ante la abundancia de especies Gymnodiniales con potencial tóxico.

El mayor reto en el estudio de dinoflagelados Gymnodiniales, es la consolidación entre la ciencia y la comunidad local, sin embargo, la estrecha relación entre ambas partes permitirá fortalecerá la vinculación, así como la toma de decisiones.

13. REFERENCIAS

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14. TRABAJOS DERIVADOS DE LA TESIS DOCTORAL

14.1 Presentaciones en congresos

- VI Congreso Nacional de la SOMEFAN 2022. **Escarcega Bata, A**, Nuñez Resendiz, M. L, Dreckmann, K., Sentíes, A y Zamudio-Reséndiz, M. E. “Biodiversidad de dinoflagelados atecados del orden Gymnodiniales (Dinophyceae) en las costas del Pacifico Mexicano". 03 al 07 de octubre, 2022. Ensenada, Baja California, México.
- VI Congreso Nacional de la SOMEFAN 2022. **Escarcega Bata, A**, Ruiz-de la Torre, M. C., Nuñez Resendiz, M. L, Dreckmann, K y Sentíes, A. “Nuevos registros de dos especies del género *Alexandrium* para la Bahía de Todos Santos, México: *A. pseudogonyaulax* y *A. margalefii*". 03 al 07 de octubre, 2022. Ensenada, Baja California, México.
- 1er encuentro de alumnas y alumnos del Doctorado en Ciencias Biológicas y de la Salud. **Escarcega Bata, A,** Nuñez Resendiz, M. L, Dreckmann, K., Sentíes, A. "Diversidad y relaciones filogenéticas de dinoflagelados atecados (Dinoflagellata), en Bahía de Acapulco, Guerrero, México. 03 de julio de 2023. Ciudad de México, México.
- Marco del Foro Cambio Climático, Universidad Autónoma Metropolitana. **Escarcega-Bata, A.,** Zamudio-Resendiz, M. E., Núñez Resendiz, M. L., Dreckmann, K y Sentíes, A. Florecimientos Algales Nocivos en México: Abundancia y persistencia relacionada con el cambio climático global”. 21 de septiembre de 2023.
- 1er simposio de Hidrobiología de la XIX Semana de Hidrobiología. Universidad Autónoma Metropolitana-Iztapalapa. **Escarcega Bata, A,** Nuñez Resendiz, M. L, Dreckmann, K., Sentíes, A. “Evidencia morfológica, molecular y ambiental de la presencia de *Karenia longicanalis* (Dinophyceae: Kareniaceae) como formadora de floraciones en el Océano Pacífico Oriental”. 25 al 29 de septiembre del 2023. Ciudad de México, México.
- 8vo SIMPOSIO RECORECOS 2024. Universidad Veracruzan. **Escarcega Bata, A,** Nuñez Resendiz, M. L, Dreckmann, K., Sentíes, A. "Ocurrencia del dinoflagelado

parasito *Amoebophyra* sp., en células de *Margalefidinium fulvescens*, presentes en Bahía de Todos Santos, México". 06 al 09 de mayo de 2024. Veracruz, México.

14.2 Publicaciones

- **Escarcega-Bata, A**., Núñez Resendiz, M. L., Ruiz-de la Torre, M. C., Dreckmann, K. M., Zamudio-Resendiz, M. E., Sentíes, A., 2023. Diversidad de dinoflagelados atecados del orden Gymnodiniales (Dinophyceae), con énfasis en aquellos formadores de florecimientos algales nocivos en las costas del Pacífico Mexicano. Ac. Bot. Mex. 130, e2126 https://doi.org/10.21829/abm130.2023.2126

- **Escarcega-Bata, A.,** Zamudio-Resendiz, M.E., Hernandez-Rosas, A., Núñez Resendiz, M. L., Dreckmann, K.M., Sentíes, A., 2023. First record of *Grammatodinium* (Dinophyceae) for the American Eastern Pacific coast: Morphological, molecular and ecological confirmation. European Journal of Protistology. 87, 125942 https://doi.org/10.1016/j. ejop.2022.125942

- **Escarcega-Bata, A.,** Núñez Resendiz, M. L., Zamudio-Resendiz, M. E., Dreckmann, K. M., Sánchez, E. C., Sentíes, A., 2024. Morpho-molecular and environmental evidence of the ocurrence of *Karenia longicanalis* (Dinophyceae: Kareniaceae) as a bloom former in the Eastern Pacific Ocean. Protist 175(2), 126022. https://doi.org/10.1016/j.protis.2024.126022

Casa abierta al tiempo
UNIVERSIDAD AUTÓNOMA METROPOLITANA

ACTA DE DISERTACIÓN PÚBLICA

No. 00163
Matrícula: 2212801264

Diversidad y relaciones filogenéticas de dinoflagelados atecados (Dinoflagellata) en Bahía de Acapulco, Guerrero, México.

En la Ciudad de México, se presentaron a las 12:30 horas del día 25 del mes de julio del año 2025 en la Unidad Iztapalapa de la Universidad Autónoma Metropolitana, los suscritos miembros del jurado:

DR. ABEL SENTIES GRANADOS
DRA. MARIA LUISA NUÑEZ RESENDIZ
DR. IGNACIO LEYVA VALENCIA
DR. JOSE ANTOLIN AKE CASTILLO
DR. KURT MARTIN DRECKMANN ESTAY

Bajo la Presidencia del primero y con carácter de Secretario el último, se reunieron a la presentación de la Disertación Pública cuya denominación aparece al margen, para la obtención del grado de:

DOCTOR EN CIENCIAS BIOLÓGICAS Y DE LA SALUD

DE: ALEXIS DE JESUS ESCARCEGA BATA

y de acuerdo con el artículo 78 fracción IV del Reglamento de Estudios Superiores de la Universidad Autónoma Metropolitana, los miembros del jurado resolvieron:

APROBAR

Acto continuo, el presidente del jurado comunicó al interesado el resultado de la evaluación y, en caso aprobatorio, le fue tomada la protesta.

ALEXIS DE JESUS ESCARCEGA BATA
ALUMNO

REVISÓ

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DIRECTORA DE SISTEMAS ESCOLARES

DIRECTOR DE LA DIVISIÓN DE CBS

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