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“Taxonomía y filogenia de dinoflagelados planctónicos mixótrofos y heterótrofos en la Bahía de Acapulco, México”

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1. Resumen

Los dinoflagelados mixótrofos y heterótrofos han recibido históricamente menor atención que sus contrapartes autótrofas. Las dificultades de su estudio residen en la biología de los organismos, el problema en el establecimiento de cultivos y obtención de secuencias genéticas. El aporte de nueva información morfológica y molecular permitirá tener un entendimiento más claro de la filogenia de los dinoflagelados. En este estudio se abordan las especies mixótrofas y heterótrofas bajo un enfoque integrativo de morfología y filogenia molecular que permita construir una clasificación más robusta al interior del grupo. Se tomaron muestras en cuatro sitios dentro y fuera de la Bahía de Acapulco en 2023, 2024 y 2025, tomando muestras con botella van Dorn y con una red de plancton. Los resultados se presentan en cuatro capítulos. En el primero, se crea el complejo *Peridiniella sensu stricto* para acomodar a dinoflagelados con tres placas intercalares anteriores de igual tamaño, equidistantes del complejo del poro apical y con dos placas intercalares posteriores. En el segundo, se confirma la presencia de dos especies de *Polykrikos* (*P. hartmannii* y *P. kofoidii*) en la Bahía de Acapulco. En el tercero, se describe la evolución de caracteres morfológicos en los miembros de la familia Polykrikaceae y se establece *P. erythrogranulatus* sp. nov. En el cuarto, se describe *Dodgeum nauticum* gen. et sp. nov., un dinoflagelado atecado heterótrofo con posición taxonómica incierta a nivel orden. Los resultados anteriores, evidencian la necesidad de continuar con el estudio de los dinoflagelados marinos, con el fin de lograr un mejor entendimiento de sus relaciones filogenéticas y su evolución.

Palabras clave: dinoflagelados, filogenia, fitoplancton, Pacífico mexicano, taxonomía.

2. Abstract

Mixotrophic and heterotrophic dinoflagellates have historically received less attention than their autotrophic counterparts. Difficulties in their study lie in the nature of the organisms, complication in culture establishment and genetic sequencing. Providing new morphological and molecular information will allow a clearer understanding of dinoflagellate phylogeny. In this study, mixotrophic and heterotrophic species are approached, from a morphological and molecular phylogeny integrative standpoint that will help construct a more robust classification within the group. Samples were taken at four sites inside and outside Acapulco Bay with a van Dorn water bottle and a plankton net. Results are presented in four chapters. In the first, the species complex *Peridiniella sensu stricto* is created to accommodate dinoflagellates with three equal-sized anterior intercalary plates equidistant from the apical pore complex and with two posterior intercalary plates. In the second, the presence of two *Polykrikos* species (*P. hartmannii* and *P. kofoidii*) in Acapulco Bay is confirmed. In the third, the morphological character evolution of members of the family Polykrikaceae is described and *P. erythrogranulatus* sp. nov. is established. Finally, in the fourth chapter, we describe an athecate, heterotrophic dinoflagellate *Dodgeum nauticum* gen. et sp. nov. These aforementioned results demonstrate the necessity of continuing studies on marine dinoflagellates to gain a better understanding of their phylogenetic relationships and their evolution.

Keywords: dinoflagellates, Mexican Pacific, phylogeny, phytoplankton, taxonomy.

3. Introducción

Los dinoflagelados son microorganismos eucariontes de gran importancia en los ecosistemas acuáticos, debido a su papel general como productores primarios y depredadores (Dodge & Lee, 2000). Son el segundo grupo con mayor riqueza específica en el plancton marino (Taylor & Pollinger, 1987). Se clasifican en el supergrupo TSAR (Telonemida, Stramenopila, Alveolata, Rhizaria) (Burki *et al.*, 2019), dentro del grupo Alveolata, junto con los Ciliophora y Apicomplexa (Adl *et al.* 2018), con quienes comparten la presencia de alvéolos corticales en la pared celular formando una estructura compleja denominada Amfiesma (Adl *et al.*, 2018; Kwok *et al.*, 2023; Guiry & Guiry, 2025). Se caracterizan por poseer dos flagelos heterocontos en algún estadio de su ciclo biológico, así como un núcleo denominado dinocarion, que posee pocas histonas, cromatina permanentemente condensada (Rizzo & Noodén, 1974; Lin, 2011; Marinov & Lynch, 2016) y un gran número de genes y cromosomas (LaJeunesse *et al.*, 2005; Gornik *et al.*, 2019). Asimismo, los dinoflagelados pueden presentar celulosa dentro de los alveolos corticales, característica que, en el pasado, permitió su diferenciación convencional en dos grandes grupos, tecados y atecados, aunque el anfiesma es compartida entre todos los dinoflagelados (Hoppenrath, 2017).

Los dinoflagelados atecados, carecen de celulosa en los alvéolos corticales o está presente en cantidades muy pequeñas, por lo que son más flexibles y a la vez más frágiles. Para su identificación, históricamente, se recurrió a caracteres morfológicos y ultraestructurales, como la posición del núcleo, presencia y forma del surco apical, posición, desplazamiento y número de giros del cingulum (Steidinger & Tangen, 1996). Este grupo se encuentra conformado por las clases Ellobiophyceae, Noctilucophyceae, Oxyrrhinophyceae, Perkinsea y Syndiniophyceae. Dentro de la clase Dinophyceae, está representada por los órdenes Gymnodiniales, Amphidinales, Noctilucales, entre otros, de los cuales los Gymnodiniales son

el grupo con mayor riqueza de especies (Orr *et al.*, 2012; Cavalier-Smith, 2018; Guiry & Guiry, 2025).

Las formas tecadas depositan celulosa en estos alvéolos (sacos anfiesmales) formando placas externas, que les confieren rigidez. Tanto el número como forma y posición de las mismas sirven como carácter taxonómico importante en la determinación de especies (Fensome *et al.*, 1993; Steidinger & Tangen, 1996; Kwok *et al.*, 2023). Dentro de la clase Dinophyceae, este grupo se encuentra representado por los órdenes Dinophysiales, Gonyaulacales, Peridinales y Prorocentrales (Guiry & Guiry, 2025). Recientemente, tanto para las formas atecadas como las tecadas se ha implementado el uso de marcadores moleculares como una herramienta para determinar su posición taxonómica y resolver problemáticas en torno a sus relaciones filogenéticas (Daugbjerg *et al.*, 2000; Hoppenrath, 2017).

Gran parte de los dinoflagelados obtuvieron capacidad fotosintética a partir de procesos de endosimbiosis con diversos grupos de algas (Waller & Korený, 2017). Sin embargo, en todos los órdenes de dinoflagelados, existen especies heterótrofas o mixótrofas. Su alimentación puede basarse en el consumo de partículas orgánicas (osmotrofia), bacterias y pequeños flagelados (pinocitosis, fagocitosis), así como el consumo de organismos más grandes por medio del *pallium* (extensión pseudopodial). También se han reportado numerosos casos de cleptoplastia, comensalismo, simbiosis, así como ecto y endoparasitismo (Schnepf & Elbrächter, 1992). Aproximadamente, la mitad de los dinoflagelados son heterótrofos o mixótrofos con diferentes grados de autotrofia (Hacket *et al.*, 2004; Gómez, 2012; Hansen & Tillmann, 2020).

La Bahía de Acapulco presenta una gran diversidad y riqueza de especies del fitoplancton, debido a la heterogeneidad de condiciones ambientales. Hasta la fecha, se han documentado 4,135 especies de dinoflagelados a nivel mundial (Guiry & Guiry, 2025), 347 especies de

dinoflagelados en Acapulco (Meave-del Castillo *et al.*, 2012) y 103 especies de dinoflagelados atecados en el Pacífico mexicano con evidencia fotográfica, sin contar taxones infraespecíficos (Escarcega-Bata *et al.*, 2023), muy pocas confirmadas molecularmente (Escarcega-Bata *et al.*, 2021; Núñez-Resendiz *et al.*, 2025). En el presente estudio, organizado en cuatro capítulos, se pretende abordar los aspectos taxonómicos y filogenéticos del grupo y ampliar el catálogo de especies en la Bahía de Acapulco.

4. Objetivos

4.1 Objetivo general

Caracterizar taxonómica y filogenéticamente a especies planctónicas heterótrofas y mixótrofas de dinoflagelados en la Bahía de Acapulco, Pacífico mexicano.

4.2 Objetivos específicos

- Establecer cultivos de especies heterótrofas y mixótrofas para realizar técnicas de microscopía electrónica y secuenciación.
- Identificar morfológica, molecular y ultraestructuralmente a las especies heterótrofas y mixótrofas de dinoflagelados.
- Construir hipótesis filogenéticas que permitan esclarecer las relaciones evolutivas de los organismos en función de la evidencia molecular obtenida a partir de la secuenciación de organismos.

5. Preguntas de Investigación

¿Existirán nuevos registros o especies para la Bahía de Acapulco y para el Pacífico mexicano?

¿A través de la integración de la investigación morfológica y molecular será posible resolver problemas filogenéticos y taxonómicos dentro del grupo?

6. Hipótesis

La integración de los aspectos morfológicos y moleculares de las especies de dinoflagelados heterótrofos y mixótrofos, registrados en la Bahía de Acapulco, permitirá una caracterización más robusta, lo que derivará en un sistema de clasificación más estable y la posible descripción de nuevos registros o taxones que aumentará la riqueza de especies en la región.

7. Material y Métodos

7.1 Área de estudio

La Bahía de Acapulco posee una forma semicircular, localizada en la costa del estado de Guerrero, perteneciente a la región del Pacífico Tropical Mexicano (PTM) (De-la Lanza 1991). Presenta un clima tropical cálido, con lluvias en verano, con una temperatura media anual de 27.9 °C y una precipitación media anual de 1240.2 mm (INEGI, 1997). Presenta tres temporadas del año distintas, lluvias: junio a septiembre), secas frías: octubre-enero y secas cálidas: febrero-mayo, que determinan la presencia y dominancia de los organismos.

Dada su forma de anfiteatro, por las montañas que la rodean, la bahía se alimenta de aproximadamente 30 ríos temporales y permanentes que desembocan en diferentes puntos a lo largo de la costa. Muchos de ellos llevan aguas residuales que contribuyen a la contaminación del agua (Sampedro-Rosas, 2014). Los vientos y corrientes oceánicas provenientes del oeste empujan el agua hacia el interior de la bahía, saliendo nuevamente a mar abierto en Punta Bruja. La Corriente Costera Mexicana forma la principal influencia de agua durante la temporada de secas-cálidas, mientras que la Corriente de California empuja agua fría desde el

norte durante la temporada de secas-frías (Limoges *et al.*, 2010; Meave-del Castillo *et al.* 2012).

7.2 Muestreo

Se seleccionaron cuatro estaciones dentro y fuera de la bahía de Acapulco: Naval ($16^{\circ}50'07.5''\text{N}$, $99^{\circ}51'37.2''\text{O}$), Muelle ($16^{\circ}50'24.4''\text{N}$, $99^{\circ}54'00.2''\text{O}$), Sinfonía ($16^{\circ}50'25.8''\text{N}$, $99^{\circ}55'15.1''\text{O}$) y Oceánica ($16^{\circ}45'28.7''\text{N}$, $99^{\circ}53'56.4''\text{O}$). Durante 2023, 2024 y 2025, a bordo de una lancha con motor fuera de borda se recolectaron muestras de agua con botella Van Dorn a 3 y 5 m de profundidad, hasta llenar un botellón de 10 L en cada estación. Adicionalmente, se realizó un arrastre horizontal durante cinco minutos con red de plancton con apertura de malla de 20 μm .

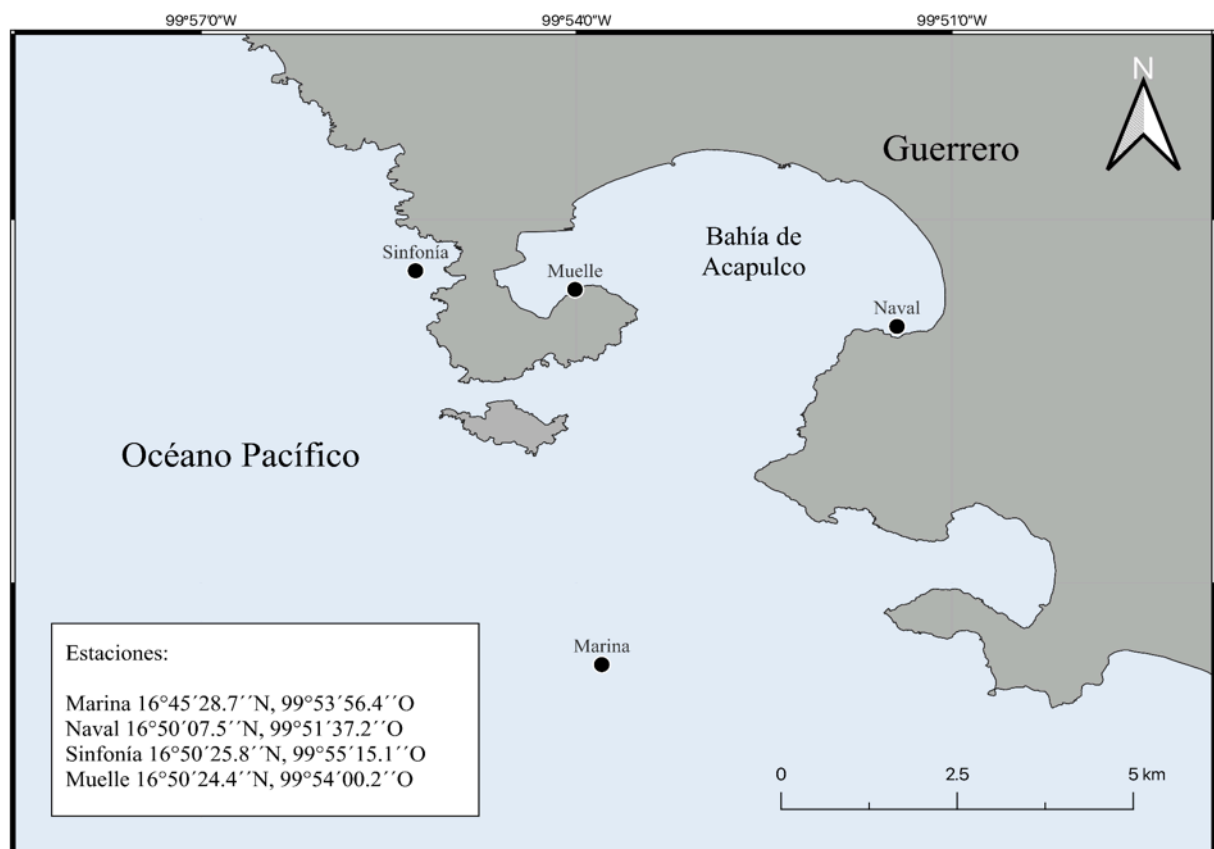


Fig. 1. Estaciones de muestreo dentro y fuera de la bahía de Acapulco en 2023–2025.

7.3 Procesamiento de muestras en campo

Las muestras de botella se concentraron por filtración inversa. Estas muestras y aquellas colectadas con red, se observaron con un microscopio óptico y se aislaron células de los dinoflagelados usando una micropipeta de vidrio reducida. Las células fueron transferidas a un portaobjetos con agua del sitio filtrada. Se fotografiaron las células con un microscopio óptico Reichert Microstar IV (Vienna, Austria), Leica DMLB (Vienna, Austria) y Olympus BX50 (Tokio, Japón). Posteriormente, se procesaron para ser observadas en microscopio electrónico de transmisión, barrido o para su secuenciación.

7.4 Cultivos

Para establecer policultivos se tomó una alícuota de las muestras concentradas y se realizó un pre-filtrado con malla de 100 μm para remover los organismos zooplanctónicos y mantener los organismos fitoplanctónicos. Posteriormente se añadió agua filtrada, medio F2 o medio GSe enriquecido con vitaminas del complejo B:B1 (tiamina), B6 (piridoxina) y B12 (hidroxicobalamina) con una salinidad de 35, un pH de 8.1. Para cultivos monoclonales se depositaron las células de interés en cajas de cultivo de 96 pozos y en tubos Corning de 15 ml con agua filtrada, medio F2 o medio GSe.

Los cultivos fueron depositados en una incubadora y mantenidos a una temperatura de 27 °C con un fotoperíodo 12:12 luz/oscuridad.

7.5 PCR de una sola célula

Se realizó una amplificación de ADN por PCR de una sola célula aislada y de los cultivos siguiendo el protocolo establecido por Hernández-Rosas *et al.* (2018) modificado por

Escarcega-Bata *et al.* (2021), utilizando la siguiente combinación de cebadores específicos para organismos eucariontes (Tabla 1).

Tabla 1. Cebadores específicos de los genes LSU, SSU e ITS1 del ADN ribosomal.

Gen	Primer Forward	Primer Reverse
LSU	D1R-F 5'-ACCCGCTGAATTTAAGCATA-3'	D2C-R 5'-CCTTGGTCCGTGTTTCAAGA-3'
SSU	SR4-F 5'-AGGGCAAGTCTGGTGCCAG-3'	SR7-R 5'-TCCTTGGGCAAATGCTTTCGC-3'
ITS	ITS2-F 5'-TACGTCCCTGCCCTTTGTAC-3'	ITS2-R 5'-TCCCTGTTCATTCGCCATTAC-3'

7.6 Análisis filogenéticos

Se realizó una búsqueda con la herramienta BLAST para obtener secuencias homólogas del gen LSU y SSU. Se descargaron las secuencias y se alinearon con el programa Bioedit usando el algoritmo Clustal W. Con el programa MEGA X (Kumar *et al.*, 1994) se calcularon las distancias genéticas entre las especies, así como entre los géneros. Posteriormente se realizaron análisis de Máxima Verosimilitud e Inferencia Bayesiana, utilizando los programas TOPALi (Milne *et al.*, 2008), RaxML y Mr. Bayes (Huelsenbeck & Ronquist, 2001).

7.7 Microscopía electrónica de barrido

Para la observación en microscopio electrónico de barrido, se siguió el protocolo descrito por Reñé *et al.* (2014) con modificaciones menores. Las células aisladas se fijaron con una solución de glutaraldehído de Karnovsky al 5% hasta alcanzar una concentración final de 1% y se postfijaron en tetróxido de osmio al 0.01% en agua Mili-Q. Posteriormente, se

deshidrataron en una serie de seis pasos de etanol de 15 min: 30%, 50%, 70%, 80%, 90% y 96%, con dos lavados en alcohol absoluto de 20 min. Las células deshidratadas en alcohol absoluto se secaron a punto crítico y se cubrieron con oro. Finalmente, se montaron en portamuestras de vidrio para realizar una observación en microscopio electrónico de barrido JEOL JSM-7600F a voltaje de aceleración de 5 kV en alto vacío (Distancia de trabajo: 1.5–25mm).

8. Resultados

Dada la naturaleza tan diversa de los resultados obtenidos, la presente idónea comunicación de resultados se conforma de cuatro capítulos separados sobre distintos dinoflagelados, en los cuales se tratan, de manera particular, su problemática y como esta fue abordada mediante la implementación de técnicas moleculares, morfológicas y de microscopía fina. En el capítulo I y III se incorporaron muestras provenientes de Yucatán y Campeche, respectivamente, para complementar la información perteneciente al área de estudio.

El capítulo I aborda el género *Peridiniella*, un género complejo de dinoflagelados tecados. Se discute su filogenia y delimitación basado en caracteres morfológicos respaldado por información molecular.

El capítulo II trata sobre dos especies de *Polykrikos* encontradas en la Bahía de Acapulco, el respaldo fotográfico y su identificación.

El capítulo III se enfoca en la confirmación morfológica y molecular de *Polykrikos* en la Bahía de Acapulco y el Golfo de México, con una enmienda de la familia y la evolución de caracteres del grupo.

El capítulo IV trata sobre el descubrimiento, descripción morfológica y posición filogenética de un nuevo género y especie de dinoflagelados heterótrofos.

9. Capítulo 1

Morphological and molecular assessment of *Peridiniella* (Dinophyceae): a polyphyletic genus

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Resumen

Peridiniella danica fue descrita como *Glenodinium danicum* en 1907, pero las descripciones fueron conflictivas, hasta que Okolodkov y Dodge enmendaron la descripción en 1995. Comparte una forma esférica y tabulación de la epiteca con otros miembros del género *Peridiniella*. Actualmente, se considera un género de posición incierta dentro del orden Peridinales. Se encontraron células de *P. danica* en la Bahía de Acapulco, México en abril de 2024. Se obtuvieron imágenes de microscopía de luz y secuencias parciales del gen LSU. La única especie del género que cuenta con una secuencia genética en el GenBank, *P. catenata*, tiene tres placas intercalares anteriores de distinto tamaño, con una placa 2a más lejos del complejo del poro apical (CPA). *P. catenata* y *P. globosa* tienen tres placas intercalares anteriores a la derecha del CPA en vista ventral, mientras que *P. sphaeroidea* y *P. danica* poseen tres placas del mismo tamaño, equidistantes del CPA. La filogenia molecular y la morfología de placas sugiere que *Peridiniella* es un género polifilético, mientras que la distancia genética entre nuestras secuencias y otros miembros del género para el gen LSU es alta (>7%); por lo tanto, proponemos la creación de *Peridiniella sensu stricto* para la especie tipo, *P. sphaeroidea*, y *P. danica*, subrayando la necesidad de evaluar a *P. catenata* y *P. globosa*.

Morphological and molecular assessment of *Peridiniella* (Dinophyceae): a polyphyletic genus

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ABSTRACT

Peridiniella danica was first described as *Glenodinium danicum* by Paulsen in 1907, but descriptions were conflicting until Okolodkov and Dodge amended the description in 1995. It shares a spherical shape and epithecal tabulation with other members of the genus *Peridiniella*. Currently, it is regarded as a genus of uncertain placement in the order Peridiniales. Cells of *P. danica* and *P. sphaeroidea* were found in coastal waters of Mexico. We obtained light microscopy and SEM images and LSU sequences (D1–D2 regions) of the cells. *Peridiniella globosa* and the only other identified species of the genus with a genetic sequence in GenBank, *P. catenata*, have three different-sized anterior intercalary plates, with plate 2a disconnected from the apical plates and a small x plate preceding the second intercalary plate dorsally. They possess a single posterior intercalary plate. *Peridiniella danica* and the type species *P. sphaeroidea* have three equal-sized anterior intercalary plates equidistant from the APC and two posterior intercalary plates. Molecular phylogeny and plate morphology suggest *Peridiniella* is polyphyletic, and the genetic distance between our sequences with members of *Peridiniella* for the D1–D2 regions of LSU is high (>10%). Therefore, we propose the creation of *Peridiniella sensu stricto* for the type species, *P. sphaeroidea* and *P. danica*, highlighting the need for a re-evaluation of *P. catenata* and *P. globosa*.

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INTRODUCTION

Glenodinium danicum Paulsen (1907) was described based on material from Skagen, Denmark, using a drawing of the cell shape, cingulum and sulcus outline, without naming a type specimen. Descriptions were conflicting until Okolodkov & Dodge (1995) amended the description using data from plate tabulation and morphology, and created the new combination *Peridiniella danica* (Paulsen) Okolodkov & Dodge and placed it in the family Cladopyxidaceae of the order Gonyaulacales. It shares a general plate tabulation with other members of *Peridiniella* (Okolodkov & Dodge, 1995), which itself has had various taxonomic problems. Currently, the genus consists of four taxonomic accepted species (*P. sphaeroidea* Kofoid & J.R. Michener, *P. catenata* (Levander) Balech, *P. globosa* (P.J.L. Dangeard) Okolodkov and *P. danica*), with *P. sphaeroidea* being the type species. Species of the genus inhabit a wide range of environmental conditions. *Peridiniella sphaeroidea* is found in tropical waters, while *P. catenata* is known to live associated with Arctic sea ice (Okolodkov, 1999). *Peridiniella danica* has been reported mostly in European seas (Guiry & Guiry, 2025), but also in Argentina (Fabro & Almandoz, 2021) and Mexican Pacific (Okolodkov & Gárate-Lizárraga, 2006).

Most reassignments to this genus have acknowledged the dubious monophyly of the group. Dodge (1987) stressed that

P. catenata and *P. sphaeroidea* fit in no other genus described at the time, while Okolodkov & Dodge (1995) noted *P. danica* may have a different number of cingular and sulcal plates than *P. sphaeroidea*. Dangeard (1927) described *P. globosa* from French Guiana based on a drawing of the cell showing the plates without naming them. Fabro & Almandoz (2021) later found *P. globosa* and provided a tabulation pattern, highlighting the smaller 2a plate. Dodge (1987) considered *P. globosa* and *P. catenata* to have a small intercalary antapical plate between the first three postcingular plates and the antapical plate, whereas Okolodkov & Dodge (1995) considered *P. danica* to have two antapical plates.

Carbonell-Moore *et al.* (2022) noted that *Peridiniella* has a gonyaulacalean tabulation of the epitheca and a peridinean tabulation of the hypotheca. Thus, no set of morphological characters has been found to help assign this genus to a certain position. Some other genera of uncertain taxonomic position, such as *Amphidoma* F. Stein, *Cladopyxis* F. Stein and the more recently described *Azadinium* Elbrächter & Tillmann (Tillmann *et al.*, 2009) and *Fensomea setacea* Tillmann & Gottschling (Gottschling *et al.*, 2021) have similar thecal morphologies.

Molecular studies have been limited as well: only three sequences of *Peridiniella* are in GenBank, one corresponding to *P. catenata* and another two as unidentified species. In

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molecular analyses, some authors obtained potential relationships of *Peridiniella* with the prorocentralean dinoflagellates (Gottschling *et al.*, 2021). Until now, its generic position is regarded as uncertain within the order Peridiniales (Guiry & Guiry, 2025). Therefore, a revision of these taxa using molecular phylogenetic techniques for elucidating relationships is clearly needed.

In this study, we obtained the first sequence of *Peridiniella danica* and compared thecal morphology of all members of the genus.

MATERIAL AND METHODS

Sampling

Four sites in Acapulco Bay, Mexican Pacific (Fig. S1), were selected for sampling, on 14–16 April 2025: Offshore, 16° 45.45' N 99°53.94' W; Naval, 16°50.12' N, 99°51.51' W; Symphony, 16°50.43' N, 99°55.25' W; Dock, 16°50.40' N, 99° 54.00' W.

At each site, a horizontal 20 µm mesh plankton net tow was taken, and the samples were fixed in 1% glutaraldehyde dissolved in seawater. Water was collected using a Van Dorn bottle, at 1 m, 3 m and 10 m depths. Salinity and temperature were recorded with a hand refractometer and a mercury thermometer at each depth. Organisms were later concentrated using inverse filtration equipment, with a 4 µm pore size cellulose membrane (Millipore, Darmstadt, Germany). Individual cells were transferred to clean filtered seawater with a glass micropipette.

Phytoplankton samples, in which a few cells of *P. sphaeroidea* were found, were taken in December 2011 with a net in the 30 cm surface layer along the northern coast of the Yucatán Peninsula, southern Gulf of Mexico, 10 km offshore the fishing settlement Dzilam de Bravo (21° 29'00.5"N 88°53'30.8"W). Some cells of the species were also observed in net samples offshore the state of Tabasco.

Microscopy

LIGHT MICROSCOPY

Fixed cells were observed with a Leica DMLB compound epifluorescence microscope (Wetzlar, Germany), equipped with achromatic objectives, a 24 MP Nikon Z6 (Tokyo, Japan) and 3.1 MP AmScope MU-H300s digital cameras. The fluorochrome Calcofluor White M2R at 1% was added without washing to stain thecal plates. The DAPI filter cube arrangement was 352–405 nm excitation and 417–477 nm emission was used. Additionally, a Zeiss Axio Observer (Jena, Germany) confocal microscope, with a 40x/1.30 apochromatic objective was used to obtain higher resolution images for reconstruction of the thecal plates.

SCANNING ELECTRON MICROSCOPY

Samples from the northern coast of the Yucatán Peninsula were fixed with 4% formaldehyde dissolved in seawater and washed in distilled water. Afterwards, dinoflagellate cells were dehydrated in an ethanol series of increasing concentration (30, 50, 70, 90 and 100% – two times in the latter), 30 min per step, in

15 ml glass tubes, without centrifugation. The samples were mounted directly onto aluminium stubs with a micropipette, dried at room temperature, and coated by high-resolution sputtering (Polaron SC7640, Quorum Technologies, Newhaven, SXE, UK), with Au-Pd. Observations were made and micrographs taken with a JEOL JSM-7600 F SEM at 5 kV, at a working distance of 15 mm, in the Laboratorio Nacional para el Estudio de Nano y Biomateriales of the Departamento de Física Aplicada del Centro de Investigación y Estudios Avanzados of the Instituto Politécnico Nacional (CINVESTAV-IPN), Unidad Mérida, Yucatán, Mexico.

Molecular analyses

SINGLE-CELL PCR

Individual live cells from fresh natural samples were transferred to clean 0.2 ml PCR tubes and kept stored at –20°C. PCR reactions were carried out using the Promega GoTaq® G2 colourless master mix (Madison, WI, USA) with the primers D1R-F and D2C-R (Scholin *et al.*, 1994) for the partial nuclear-encoded LSU rDNA gene (D1–D2 domains). Conditions were: denaturation at 98°C for 3 min, 35 cycles at 95°C for 30 s, 52°C for 30 s and 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products were sent to the Laboratorio de Secuenciación Genómica, Pabellón de la Biodiversidad, Instituto de Biología, Universidad Nacional Autónoma de México for commercial sequencing using the PCR primers. The sequences obtained in this study were deposited in the GenBank with the accession numbers: PV729985 and PV729986.

PHYLOGENY

Consensus sequences were assembled and edited with Sequencher® v5.4.6 (Gene Codes Corporation, Ann Arbor, MI, U.S.A.). BLAST analysis was performed for a preliminary taxonomic identification. Similar sequences were downloaded from GenBank, including sequences from various dinoflagellate orders (Peridiniales, Gonyaulacales and Prorocentrales) (Table S1) and aligned in Bioedit (Hall, 1999) using Clustal W generating a 1,016 bp alignment. *Perkinsus marinus* (Mackin, H.M. Owen & Collier) Levine and *Amoebophrya* Koeppen were selected as an outgroup. Evolutionary model selection was performed in TOPALi® v2 (Milne *et al.*, 2009) using the model selection tool. Bayesian Inference (BI) analyses were performed in MrBayes v3.2 (Ronquist *et al.*, 2012) using GTR+I +G as an evolutionary model. The data matrix was run for 6×10^6 generations, with six Monte Carlo Markov chains (two hot chains and four cold chains) starting with a random tree, sampling every 500 generations and discarding 25% as burn-in. Maximum Likelihood (ML) analysis was executed with the PhyML program in TOPALi using a TIM+G evolutionary model and 1,000 non-parametric bootstrap replicates (Felsenstein, 1985). Uncorrected *p*-distance were performed in MEGA 11 (Tamura *et al.*, 2021).

RESULTS

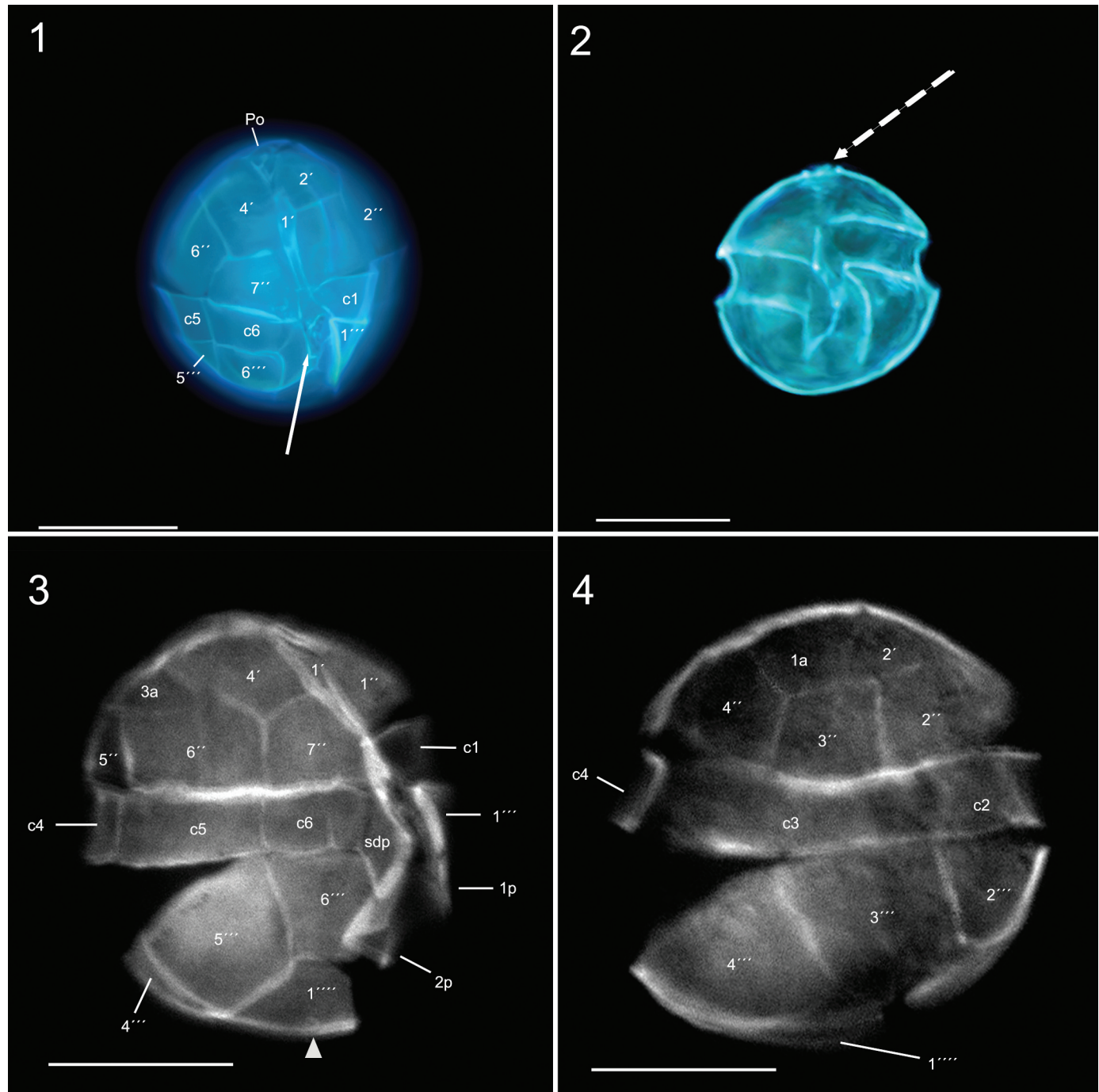
From the collected samples, we obtained light microscopy, and SEM images, as well as genetic sequences from

individuals of *Peridiniella*. The results showed *P. danica* and *P. sphaeroidea* should be considered members of *Peridiniella sensu stricto*, while *P. globosa* and *P. catenata* should be re-evaluated to determine their correct taxonomy.

Morphology

The samples from Acapulco Bay were a heterotrophic, thecate dinoflagellate with motile, solitary cells, found in subtropical

waters (19–24°C). The cell outlines were subspherical, slightly dorsoventrally compressed, colourless, 19–25 µm long and 17–22 µm wide (Fig. 1). Epitheca slightly conical, smaller than the hypotheca. Hypotheca is rounded. The cingulum displaced about its own width (Fig. 2), and the cingulum, sulcus, APC and the 1' plate have narrow lists. The thecal formula is Po, x, 4', 3a, 7'', 6c, 5s, 6''', 2p, 1'''' (Figs 1, 3, 4, 9–11). The 1' plate is long and narrow, touching the APC and running down towards the sulcus (Figs 1, 3), similar to



Figs 1–4. *Peridiniella danica*. Epifluorescence images of *P. danica* stained with Calcofluor White M2R. Plate numbering shown. Scale bar = 10 µm.

Fig. 1. Ventral view of the cell. Sulcal plates (arrow), with plates numbered.

Fig. 2. Overall shape of the cell; an excavated cingulum is evident, APC (dotted arrow).

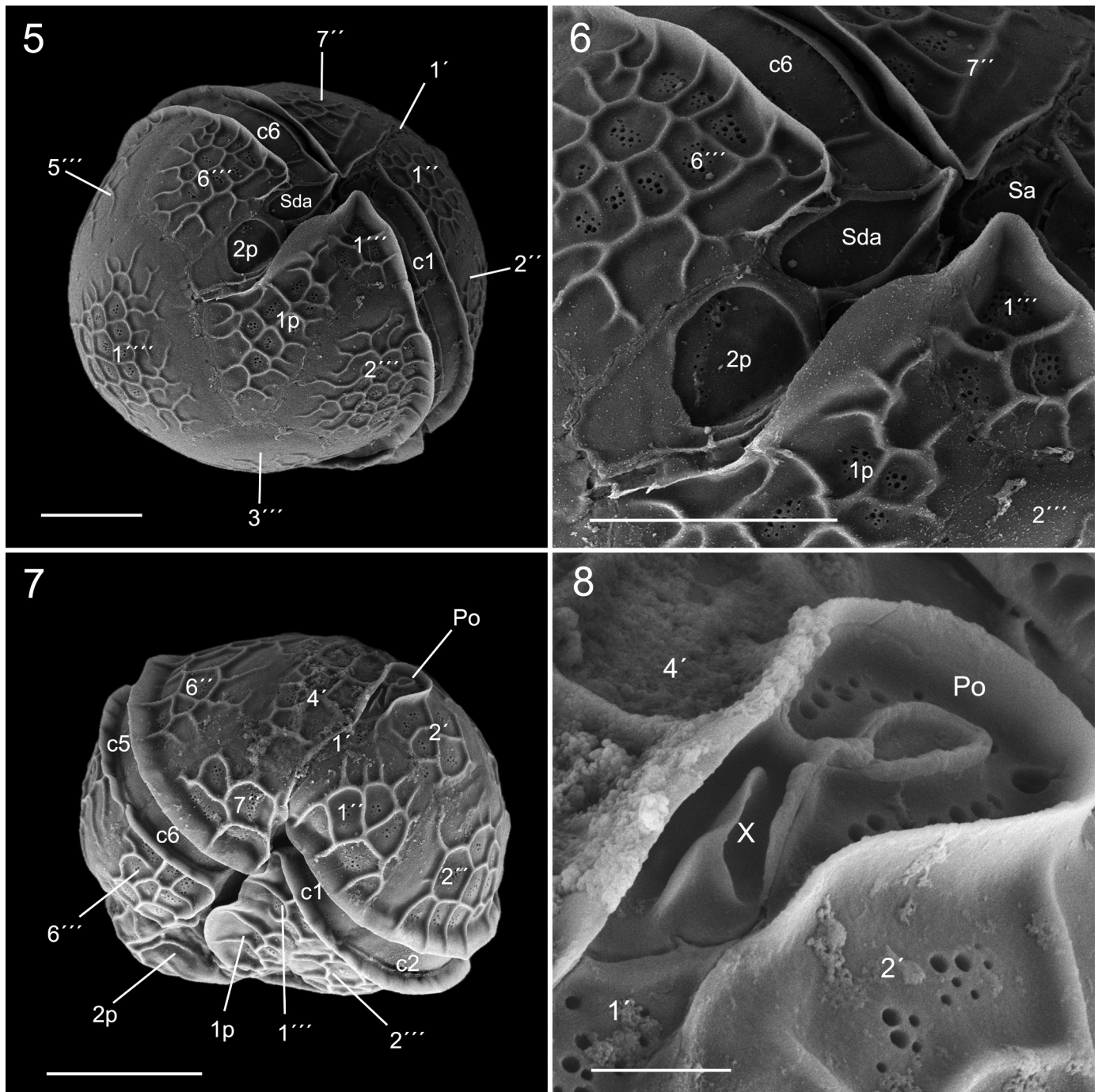
Fig. 3. Confocal image of the right side, plates numbered. Group of pores (arrowhead).

Fig. 4. Confocal image of the left side, The photo was taken from inside the theca.

P. sphaeroidea (Kofoid & Michener, 1911). The anterior intercalary plates are equal in size (Figs 3, 4, 11). Plate 1''' partially overlaps the sulcus (Figs 1–3). Plate 1'''' is wide, sometimes with a group of pores (Fig. 3). The cells fit the description of *Peridiniella danica* by Okolodkov & Dodge (1995), but we increase the number of posterior intercalary and sulcal plates considering epifluorescence and confocal images taken in this study. Plates are smooth and display

rows of pores along their margins, only visible in SEM (Fabro & Almandoz, 2021).

Our sample from Yucatán, Mexico was a thecate dinoflagellate with motile, solitary cells found in 19–24°C seawater. Cells were subspherical, 30–52 µm long and 34–50 µm wide ($n = 4$; Fig. 5). The epitheca was rounded, slightly conical and smaller than the hypotheca. Cingulum displacement was equal to its own width (Figs 5–7). Cingulum, sulcus, APC and the 1'



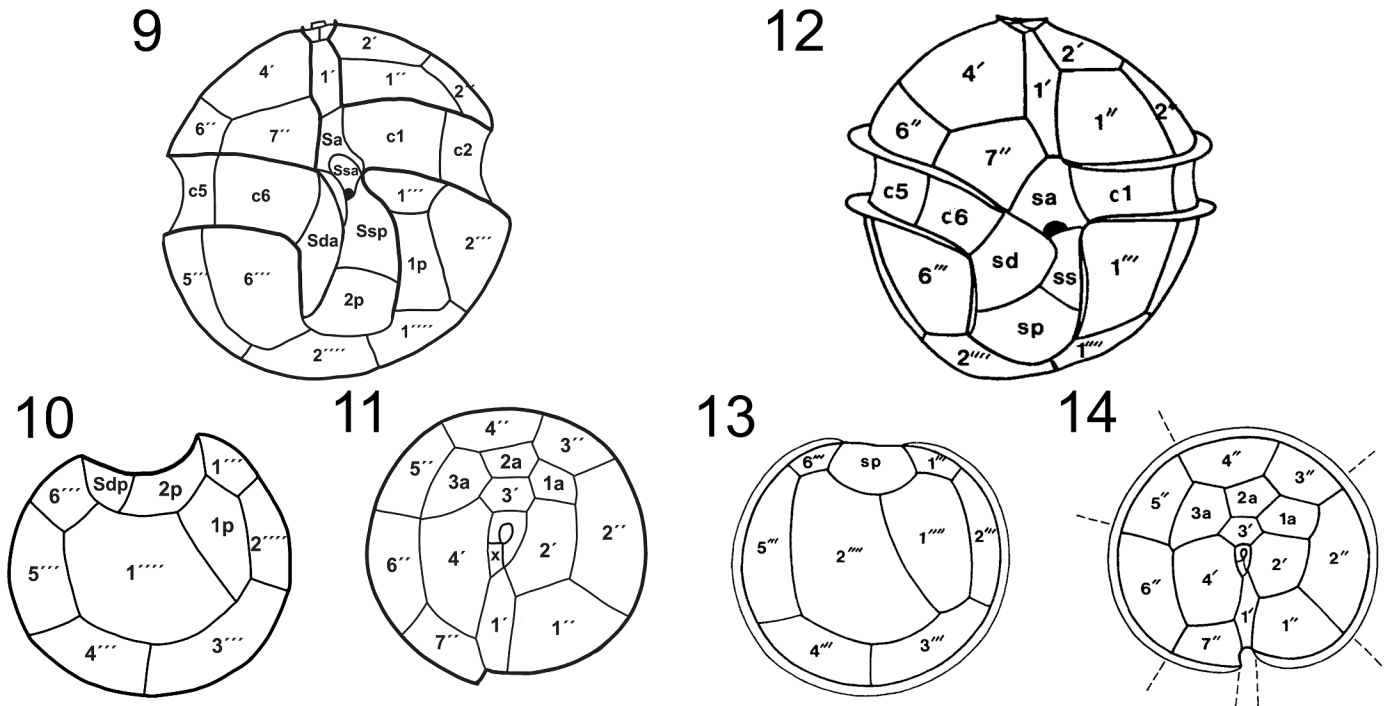
Figs 5–8. *Peridiniella sphaeroidea*. SEM micrographs of the cells. Scale bar = 10 µm unless stated otherwise.

Fig. 5. Ventral-antapical view.

Fig. 6. A fragment of theca showing the sulcal area and adjacent plates. Sa = anterior sulcal plate, Sda = right anterior sulcal plate.

Fig. 7. Ventral-apical view of a cell displaying plate reticulation and a partially obscured sulcus. Po = pore plate.

Fig. 8. The apical pore complex and adjacent plates with numerous pores. Scale bar = 1 µm.



Figs 9–14. Line drawings of thecal tabulation of *Peridiniella danica* from this study (Figs 9–11) and Okolodkov & Dodge (1995) (Figs 12–14). Sa = anterior sulcal plate; Sda = right anterior sulcal; Ssa = left anterior sulcal; Ssp = left posterior sulcal.

Fig. 9. Ventral view. Sulcal area with six plates.

Fig. 10. Antapical view with a single antapical plate.

Fig. 11. Apical view showing a pentagonal anterior intercalary plate (2a), and the small canal plate in the APC (x).

Fig. 12. Ventral view. Sulcal area with four plates

Fig. 13. Antapical view with two antapical plates.

Fig. 14. Apical view of the pentagonal variation in the anterior intercalary plate (2a).

plate had narrow lists. The thecal formula is Po, x, 4', 3a, 7'', 6c, 5s, 6''', 2p, 1'''' (Figs 5–8). The 1' plate was long and narrow, touching the APC and running down towards the sulcus (Figs 5–7). The anterior intercalary plates were equal in size. Plate 1''' partially overlaps the sulcus (Figs 6–7). These cells fit the description of *P. sphaeroidea* provided by Dodge (1987). However, we see a difference in the number of sulcal and posterior intercalary plates, as the most posterior sulcal plate should be considered a posterior intercalary plate; therefore, we emend the description of the species and increase the number of plates in the thecal formula. Thecal plates display a reticulate pattern in SEM and groups of pores in between the ridges (Figs 6, 8).

Phylogenetic analyses

Both BI and ML analyses displayed similar topologies in our phylogenetic analyses (Fig. 15). Five clades of dinoflagellates can be distinguished. Clade I is composed of representatives from various groups, such as Peridiniales (*Preperidinium* sp. and *Protoperidinium claudicans* (Paulsen) Balech, *Prorocentrales* (*Plagiodinium*), *Tovelliales* (*Jadwigia* and *Tovellia*), *Suessiales* (*Nottbeckia ochracea* (Levander) Gert Hansen, Daugbjerg & Moestrup), *Gymnodiniales* (*Grammatodinium tongyeonginum* Z. Li & H.H. Shin), and taxa of uncertain placement (*Fensomea setacea* Tillmann & Gottschling), many of these relationships were only supported by BI analysis. Clade II, unsupported, is formed by *Apicoporus*

spp., *Azadinium* spp., *Adenoides eludens* (Herdman) Balech, *Pseudadenoides kofoidii* (Herdman) F. Gomez, R. Onuma, Artigas & Horiguchi and *Peridiniella* spp. *Peridiniella catenata* formed a supported clade (BI = 1.00, ML = 83%) with the other two unnamed sequences of the genus. *Peridiniella catenata*, the two sequences of *Peridiniella* sp., *Adenoides* and *Pseudadenoides* formed a clade with low support. Clade IV is composed of Peridiniales (*Heterocapsa* spp., *Glenodinium* spp.) and including our sequences of *Peridiniella danica*, *Thoracosphaerales* (*Scrippsiella donghaiensis* H. Gu), *Amphidoma* spp. and *Prorocentrum* spp. Clade V is composed of gonyaulacalean dinoflagellates, with good BI support.

All sequences of *Heterocapsa* spp. formed a single clade with maximum support. A subclade containing *Peridiniella danica* together with *Amphidoma languida* Tillmann, Salas & Elbrächter, *Scrippsiella donghaiensis* and *Prorocentrum* spp. is poorly supported (BI = 0.91). The two sequences obtained in this study cluster together in a single clade with moderate support (BI = 0.97, ML = 80%). *p*-distances also confirmed the differences between the sequenced *Peridiniella* samples (Table S2).

DISCUSSION

From morphological and molecular evidence, we confirm *Peridiniella* is at present a polyphyletic genus. We conclude that *P. sphaeroidea* and *P. danica* should belong to *Peridiniella sensu stricto* based on morphological similarity, while

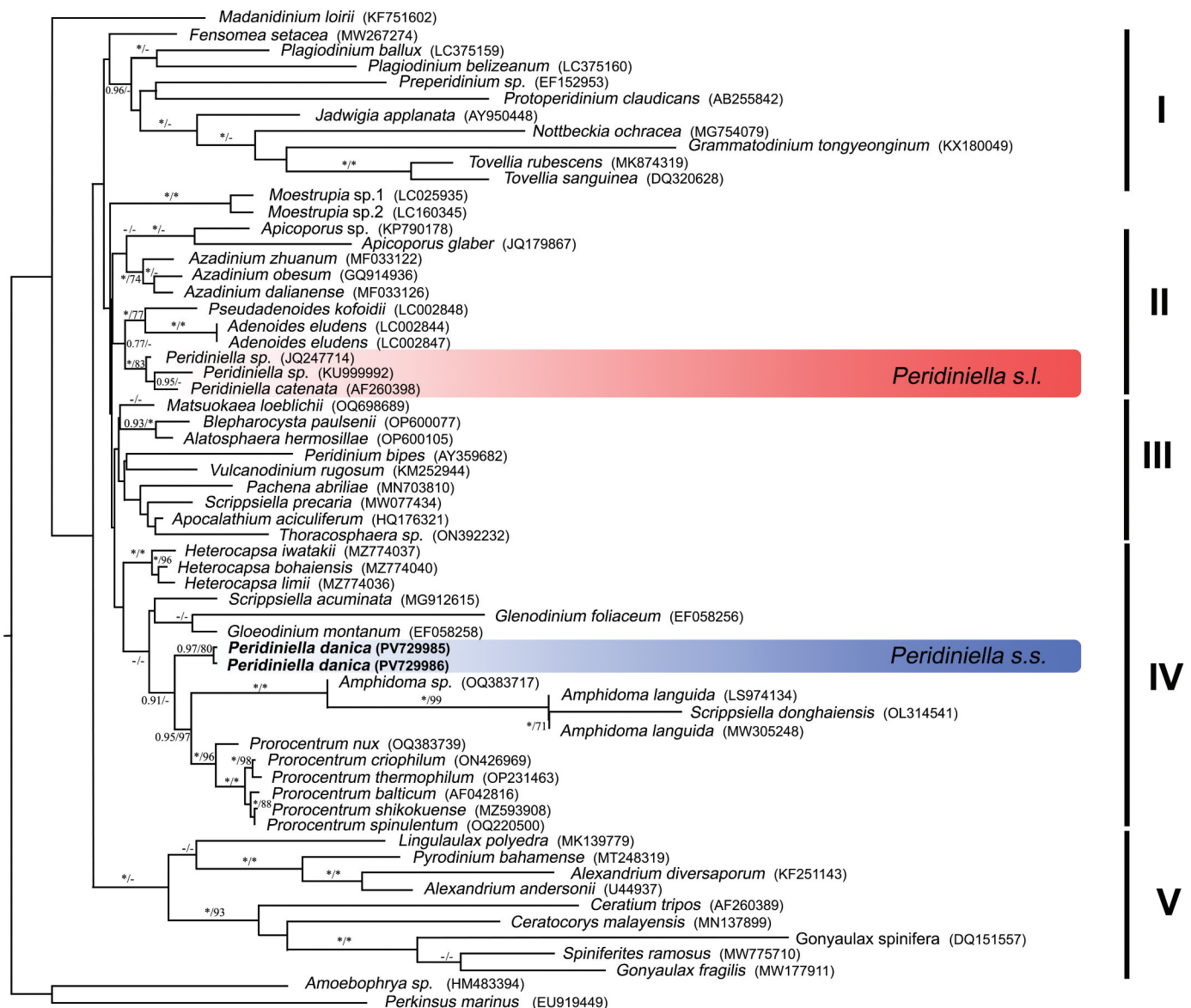


Fig. 15. Maximum likelihood phylogeny inferred from partial LSU rDNA sequences of dinoflagellates in the orders Peridiniales, Gonyaulacales and Prorocentrales. Outgroup used is *Perkinsus marinus* and *Amoebophrya*. Asterisks indicate maximum phylogenetic support of the branch (BI = 1.00/ML = 100%). Hyphens indicate low support values (BI < 0.90 and ML < 70%). Sequences of *Peridiniella danica* obtained in this study are shown in bold.

P. catenata and possibly *P. globosa* should be re-evaluated to assess their taxonomic position.

The cells of *P. danica* found in this study fit well with the redescription provided by Okolodkov & Dodge (1995) in which the epithecal plate arrangement and the cell shape are identical to our specimens. However, we provide new information regarding sulcal and posterior intercalary plates. The suture between plate 1''' and plate 1p is almost invisible in epifluorescence microscopy. The second posterior intercalary plate is located beneath the sulcal plates but is not part of the sulcus, as noted by Carbonell-Moore *et al.* (2022). Even though we did not find the variation in the 2a plate, our specimen and *P. danica* are undoubtedly the same taxonomic entity.

The genus *Peridiniella* was described originally as a dinoflagellate with the formula Po, 4', 3a, 7'', 6c?, 6s?, 6''', 1p, 1'''''. *Peridiniella danica* shares an overall

spherical cell shape and epithecal and cingular plate number with *P. catenata*, *P. sphaeroidea* and *P. globosa*. Anterior intercalary plates are similar sized and equidistant from the APC in *P. danica* and *P. sphaeroidea*, which differs from the bigger 3a plate and the smaller 2a plate in *P. catenata* and *P. globosa*, respectively. *Peridiniella catenata* and *P. globosa* also have a small x plate in front of the second intercalary plate, closer to the APC. All *Peridiniella* species exhibit a small first posterior intercalary plate shifted upwards from the only antapical plate.

Peridiniella sphaeroidea and *P. danica* also share the same plate tabulation (Table 1) and morphology. However, they should not be considered the same species, since *P. sphaeroidea* is larger (30–52 µm long; Dodge, 1987; Kofoid & Michener, 1911), has narrower lists and a continuous reticulation pattern in the thecal plates, whereas *P. danica* is smaller (19–24 µm long),

Table 1. The Kofoidian tabulation of the known *Peridiniella* species and selected genera or dinoflagellate groups (Chomérat & Billien, 2014; Dodge, 1987; Gottschling et al., 2021; Hoppenrath et al., 2017; Kuwata et al., 2024; Mertens et al. 2021; Okolodkov, 1999; Okolodkov & Dodge, 1995; Tillmann et al., 2011). Numbers are number of plates. ? – lacking confirmation. Numbers in parentheses indicate an alternate interpretation (Gómez et al., 2015). †- fossil taxa.

Species or genus	Apical	Anterior intercalary	Precingular	Cingular	Sulcal	Postcingular	Posterior intercalary	Antapical
<i>P. danica</i>	4	3	7	6	5	6	2	1
<i>P. catenata</i>	4	3 + x	7	6	7	6	1	1
<i>P. sphaeroidea</i>	4	3	7	6?	5	6	2	1
<i>P. globosa</i>	4	3 + x	7	?	?	6	1?	1
<i>Adenoides</i>	5 (4)	0	6 (7)	0 (1)	3 (1)	5	3 (2)	1
<i>Pseudadenoides</i>	4	0	0	6	4 (5)	5	5 (3)	1 (2)
<i>Fensomea</i>	3	4	7	7	5	6	0	2
<i>Micracanthodinium</i>	4	4	7	7	4	6	0	2
<i>Cladopyxis</i> †	4	3	7	7	2	6	0	2
<i>Cladopyxidium</i> †	4	4	7	7	6	6	0	2
<i>Amphidoma</i>	3–4	2–3	6	6	4	6	2	1
<i>Azadinium</i>	4	3	6			6	0?	2
<i>Madanidinium</i>	2	1	7	5	2	5	0	1
<i>Oxytoxum</i>	4	1	6	5	4?	5	0	1
<i>Prorocentrum</i>	7–8	2						

dorsoventrally flattened, with wider lists and with a smoother plate surface.

The sequences of our samples of *P. danica* and available sequences of *Peridiniella* spp. did not form a single clade and were distantly related. The unidentified *Peridiniella* sp. sequences formed an unsupported clade with *Adenoides eludens*, with which it shares the presence of posterior intercalary plates, and with *Pseudadenoides kofoidii*, which has an equal number of apical and antapical plates (Gomez et al., 2015; Hoppenrath et al., 2017) (Table 1). In our analyses, both sequences of *P. danica* formed an unsupported clade together with a sequence of *Scrippsiella donghaiensis*, a sequence of *Amphidoma* sp., and *Amphidoma languida*, a species with a long, narrow apical plate 1', six cingular and six postcingular plates, and a slightly smaller, long 1'''' plate. These are also in the same clade as *Prorocentrum*.

This closeness of *Peridiniella catenata* and *Peridiniella* sp. to *Adenoides* and *Pseudadenoides* in our analysis (although not supported) corresponds to the results of Gottschling et al. (2021) which included partial sequences of the SSU and LSU genes. Even when certain affinity is seen, morphological data and the ML analysis do not support a definitive taxonomic position for *Peridiniella catenata* and the undescribed sequences of *Peridiniella* sp. Based on our analysis, both sequences of the unidentified *Peridiniella* sp. and *P. catenata* grouped together in a single branch with some support (BI = 1.00, ML = 83%). Sequences of *Peridiniella* could belong to an undescribed species, to *P. globosa*, or to undescribed genera. Without information on their morphology description of these taxa cannot be done.

Our molecular phylogeny and morphological differences supports the polyphyletic nature of *Peridiniella* as presently circumscribed. Therefore, we propose *P. sphaeroidea* and *P. danica* should be considered *Peridiniella sensu stricto*, which should be restricted to dinoflagellates with a spherical body, a long 1' plate, three equal-sized anterior intercalary plates, equidistant from the APC, five sulcal plates, two posterior intercalary plates and one antapical plate.

Since the original description, no type specimens have been named for *Peridiniella sphaeroidea* and *P. danica*, therefore, making *Peridiniella sphaeroidea*, *Glenodinium danicum* and *Peridiniella danica nomen illeg.* To solve this problem, we hereby designate the drawings in Figs 211–217 and Fig. 2. provided by Balech (1979) and Paulsen (1907) as the lectotype for *P. sphaeroidea* and *P. danica*, respectively.

Peridiniella catenata and *P. globosa* should retain the name *Peridiniella* until a phylogenetic analysis is carried out using sequences of all known members of the genus.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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10. Capítulo 2

Revisión del estatus de *Polykrikos* (Dinophyceae: Polykrikaceae) en la Bahía de Acapulco, Pacífico mexicano

Estatus: Publicado en la revista Acta Botanica Mexicana

Resumen

Polykrikos es un género de dinoflagelados planctónicos de gran interés debido a sus sinapomorfias: la formación de pseudocolonias y presencia de complejos nematocisto-teniocisto. La presencia de *P. kofoidii* se registró por primera vez en el Pacífico mexicano en 2006 y en la Bahía de Acapulco en 2012, mientras que *P. hartmannii* fue registrado en el Pacífico mexicano en 2006, ambos identificados a partir de evidencia morfológica. Se recolectaron muestras de fitoplancton en cuatro sitios dentro y fuera de la Bahía de Acapulco entre julio 2022 y marzo 2025 (una vez por año), y se observaron células utilizando microscopía de luz. Se obtuvieron imágenes de microscopía de luz de dos morfotipos de *Polykrikos*, los cuales fueron identificados con literatura especializada. La observación morfológica detallada permitió confirmar la presencia de *P. hartmannii* y *P. kofoidii*. Además, se corrige la identidad del individuo en la literatura publicada donde se le registra, añadiendo su referencia fotográfica. Tanto *Polykrikos hartmannii* como *P. kofoidii* se encuentran de manera recurrente en la Bahía de Acapulco y en la porción tropical del Pacífico mexicano.



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Revisión del estatus de *Polykrikos* (Dinophyceae: Polykrikaceae) en la Bahía de Acapulco, Pacífico mexicano

Revision of the status of *Polykrikos* (Dinophyceae: Polykrikaceae) in Acapulco Bay, Mexican Pacific

Uriel T. Ruíz-García¹ , María Eugenia Zamudio-Resendiz² , Yuri B. Okolodkov³ , María Luisa Nuñez-Resendiz² 

Resumen:

Antecedentes y Objetivos: *Polykrikos* es un género de dinoflagelados planctónicos de gran interés debido a sus sinapomorfías: la formación de pseudocolonias y presencia de complejos nematocisto-teniocisto. La presencia de *P. kofoidii* se registró por primera vez en el Pacífico mexicano en 2006 y en la Bahía de Acapulco en 2012, mientras que *P. hartmannii* fue registrado en el Pacífico mexicano en 2006, ambos identificados a partir de evidencia morfológica.

Métodos: Se recolectaron muestras de fitoplancton en cuatro sitios dentro y fuera de la Bahía de Acapulco entre julio 2022 y marzo 2025 (una vez por año), y se observaron células utilizando microscopía de luz.

Resultados clave: Se obtuvieron imágenes de microscopía de luz de dos morfotipos de *Polykrikos*, los cuales fueron identificados con literatura especializada. La observación morfológica detallada permitió confirmar la presencia de *P. hartmannii* y *P. kofoidii*. Además, se corrige la identidad del individuo en la literatura publicada donde se le registra, añadiendo su referencia fotográfica.

Conclusiones: Tanto *Polykrikos hartmannii* como *P. kofoidii* se encuentran de manera recurrente en la Bahía de Acapulco y en la porción tropical del Pacífico mexicano.

Palabras clave: dinoflagelados, distribución, identificación, taxonomía.

Abstract:

Background and Aims: *Polykrikos* is a genus of planktonic dinoflagellates of great interest due to its synapomorphies: a pseudocolony-forming habit and the presence of a nematocyst-taeniocyst complex. *Polykrikos kofoidii* was first recorded for the Mexican Pacific in 2006 and for Acapulco Bay in 2012, while *P. hartmannii* was recorded for the Mexican Pacific in 2006, based on morphological evidence.

Methods: Phytoplankton samples were collected in four sampling stations inside and outside Acapulco Bay between July 2022 and March 2025 (once per year), and the cells were observed using light microscopy.

Key results: Light microscopy images of two distinct *Polykrikos* morphotypes were obtained and identified using specialized literature. Detailed morphological observation allowed us to confirm the presence of both *P. hartmannii* and *P. kofoidii*, with an amendment to the identity of previous records and the addition of photographic evidence.

Conclusions: In Acapulco Bay and the tropical portion of the Mexican Pacific, both *P. hartmannii* and *P. kofoidii* are found recurrently.

Key words: dinoflagellates, distribution, identification, taxonomy.

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Introducción

El género *Polykrikos* Bütschli 1873 se caracteriza por diversas sinapomorfías, como la formación de pseudocolonias, la presencia de complejos nematocisto-teniocisto para la captura de presas y un surco apical en forma de lazo cerrado. Las especies descritas hasta el presente han tenido diversos problemas de delimitación, por lo que Hoppenrath y Leander (2007) realizaron una enmienda de las descripciones originales para facilitar su reconocimiento y robustecer el conocimiento actual de la riqueza de especies del género. Actualmente, se reconocen nueve especies de *Polykrikos* aceptadas (Guiry y Guiry, 2025). Algunas llegan a presentar cierta plasticidad fenotípica, lo que ha dificultado el reconocimiento de sus rasgos diagnósticos y, por lo tanto, su identificación.

Este género es considerado de gran importancia a nivel mundial, debido a los registros previos de florecimientos nocivos causados por *P. hartmannii* (Sabanci et al., 2025), algunos con ictiotoxicidad, tal como lo registraron Tang et al. (2013) cerca de Nueva York, EUA. Se reportaron florecimientos de *Polykrikos* en las aguas costeras del Pacífico mexicano de los estados de Sinaloa (Ibarguen-Zamudio, 2006), Chiapas (Maciel-Baltazar y Hernández-Becerril, 2013), Guerrero (Gárate-Lizárraga et al., 2011), y en el Golfo de California (Gárate-Lizárraga et al., 2009; Gárate-Lizárraga, 2014). Tanto *P. hartmannii* como *P. kofoidii* se han registrado previamente en el Pacífico mexicano (Okolodkov y Gárate-Lizárraga, 2006; Maciel-Baltazar y Hernández-Becerril, 2013; Escobar-Morales y Hernández-Becerril, 2015; Torres-Ariño et al., 2019).

En la Bahía de Acapulco, únicamente *Polykrikos kofoidii* Chatton ha sido registrada (Meave-del Castillo et al., 2012), caracterizada por formar pseudocolonias de cuatro zooides y dos núcleos, con estrías finas en el hipocono del último zooide. Sin embargo, durante un muestreo reciente dos morfoespecies de *Polykrikos* fueron recolectadas en la Bahía de Acapulco. Por lo tanto, los objetivos de este trabajo son aclarar la identidad de ambas y corregir el registro previo.

Materiales y Métodos

Se seleccionaron cuatro puntos dentro y fuera de la Bahía de Acapulco (Fig. 1). Marina (16°45'28.7"N, 99°53'56.4"W),

Naval (16°50'07.5"N, 99°51'37.2"W), Sinfonía 16°50'25.8"N, 99°55'15.1"W) y Muelle (16°50'24.4"N, 99°54'00.2"W). Se realizaron cuatro muestreos entre julio de 2022 y marzo de 2025 (un muestreo por año), utilizando una botella Van Dorn (General Oceanics, Florida, EUA) y concentrando las muestras con un equipo de filtración inversa. Asimismo, durante cada muestreo se realizó un arrastre horizontal en la capa superficial de agua, de 0-30 cm, durante 5 min con red planctónica de 20 µm de apertura de malla. Se fotografiaron pseudocolonias de *Polykrikos*, usando microscopios ópticos Olympus BX50 (Tokio, Japón) de campo claro y Reichert Microstar IV de contraste de interferencia diferencial (Leica, Vienna, Austria), ambos equipados con objetivos acromáticos y una cámara digital Nikon Z6 de 24 megapíxeles (Tokio, Japón) acoplada. Algunas fotografías fueron tomadas con un apilamiento de enfoques en Helicon Focus (Kharkiv, Ucrania) para tener mayor cantidad de estructuras enfocadas en una misma imagen.

Los morfotipos observados fueron comparados con aquellos presentados por Meave-del Castillo et al. (2012) y fueron identificados a partir de la comparación de descripciones morfológicas contenidas en literatura especializada (Matsuoka et al., 2000; Hoppenrath y Leander, 2007; Hoppenrath et al., 2010).

Resultados

Se encontró un morfotipo de *Polykrikos* que coincidió con la fotografía aportada en el estudio de Meave-del Castillo et al. (2012: Fig. 97), identificada entonces como *P. kofoidii*. Adicionalmente, en abril de 2023 y marzo de 2025, se halló otro individuo, que coincidió con las características diagnósticas descritas por Tillmann y Hoppenrath (2013) de *P. kofoidii*. A continuación, se presenta una corrección de la identidad para las dos especies presentes en la Bahía de Acapulco (Fig. 1): *P. hartmannii* (previamente registrada como *P. kofoidii*) y *P. kofoidii*.

Polykrikos hartmannii W.M.Zimmermann, Zeitschrift für Botanik 23: 419-442. 1930. Figs. 2A-C.

TIPO: ITALIA. Golfo de Nápoles, información sobre profundidad y distancia no disponible, 1930, W. M. Zimmermann (holotipo: INA 1996).



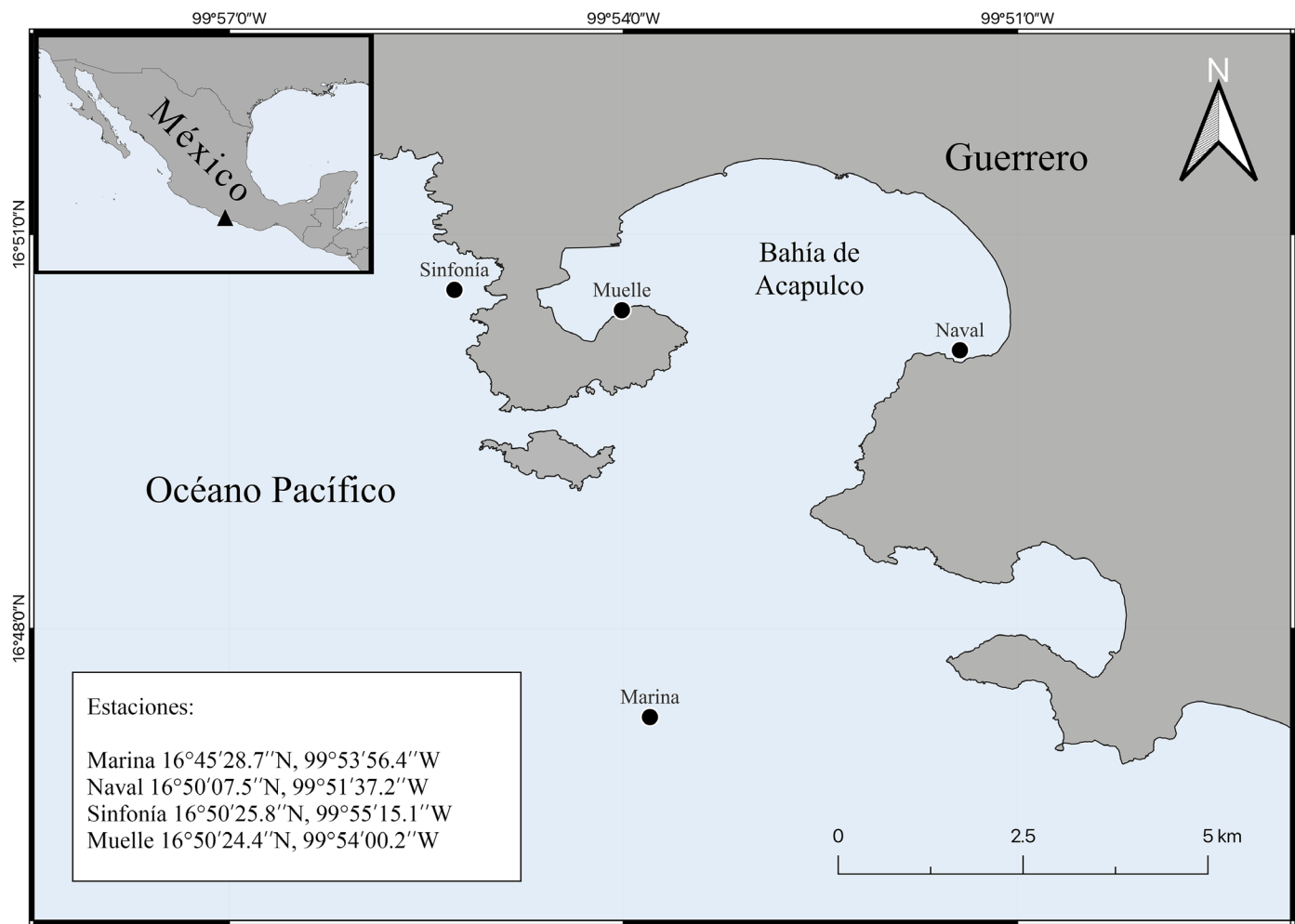


Figura 1: Estaciones de muestreo dentro y fuera de la Bahía de Acapulco, México, entre 2022 y 2025.

Células formando pseudocolonias pardas, 62 μm de largo, 42 μm de ancho, formada por dos zooides, sin torsión a lo largo del eje longitudinal, y dos núcleos grandes (25 μm de diámetro), centrales; cingulum completo, profundamente excavado, descendente, desplazado aproximadamente una a dos veces su mismo diámetro; sulcus recto, descendiendo hasta conectar con el sulcus contiguo; epicono del primer zooide (anterior) e hipocono del segundo zooide (posterior) redondeados; división entre zooides escalonada; cloroplastos numerosos y aproximadamente 6 complejos nematocisto-teniocisto, distribuidos en todo el citoplasma.

Polykrikos kofoidii Chatton, Archives de zoologie expérimentale et générale 54: 157-194. 1914. Figs. 2D-F.

TIPO: PACIFIC OCEAN. UNITED STATES OF AMERICA. California, San Diego, Pacific Ocean (Iconotipo: *Kofoid*, 1907, p. 292, fig. en texto (como *Polykrikos schwartzii*)).

Células formando pseudocolonias incoloras, 100 μm de largo, 58 μm de ancho, formadas por cuatro zooides, con ligera torsión del cuerpo hacia la izquierda y dos núcleos grandes (33 μm de diámetro), subcentrales; cingulum completo, profundamente excavado, descendente, desplazado dos veces su mismo diámetro; cingulum de cada zooide desplazado una vez su propio diámetro; sulcus recto, descendiendo hasta conectar con el sulcus contiguo; epicono del primer zooide redondeado; hipocono del último zooide ligeramente bilobulado, con finas estrías; aproximadamente ocho complejos nematocisto-

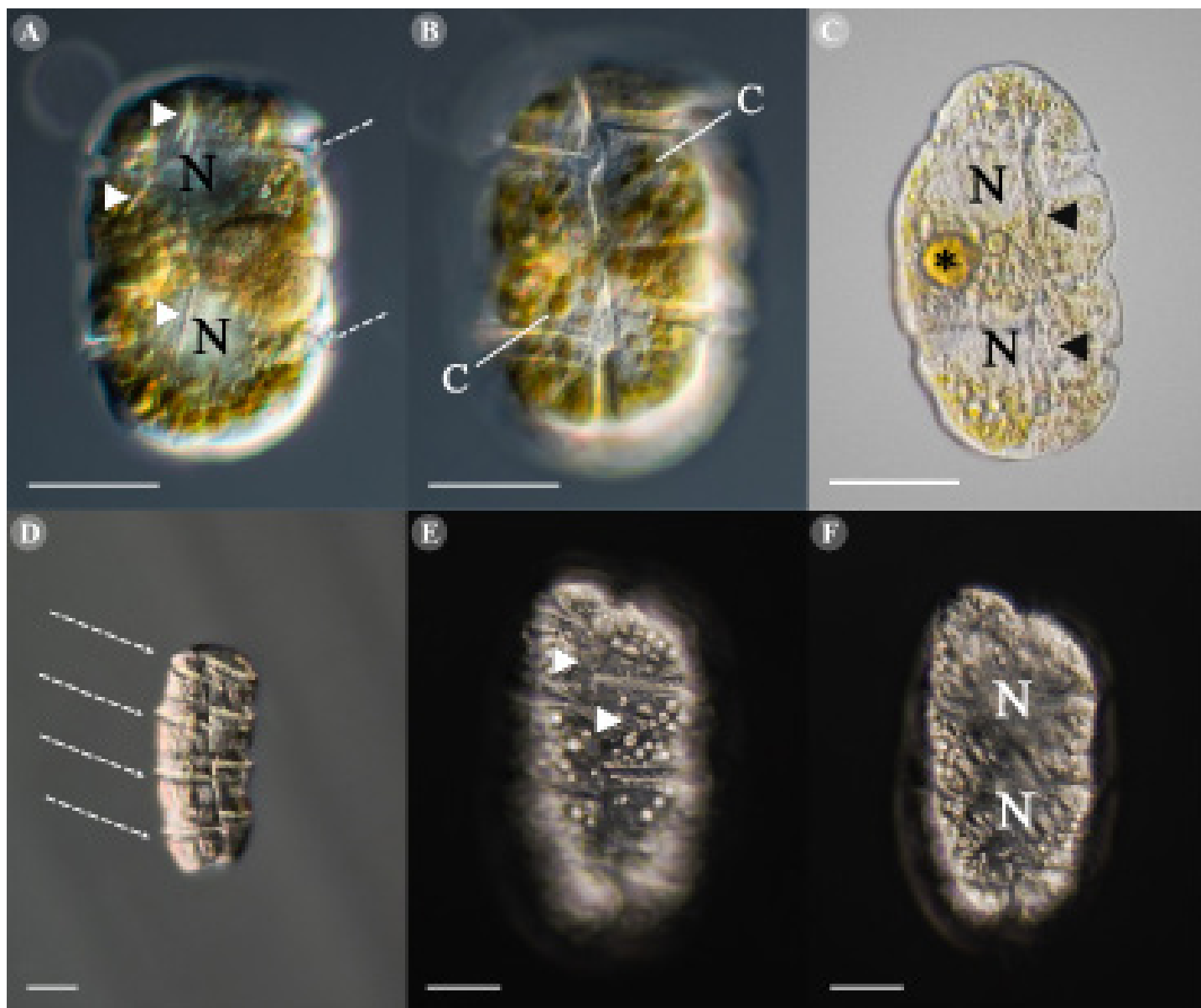


Figura 2: *Polykrikos hartmannii* W.M.Zimmermann y *P. kofoidii* Chatton encontrados en la Bahía de Acapulco, México. A. *Polykrikos hartmannii*, encontrada en marzo de 2023, enfoque a la mitad de la pseudocolonia. Pseudocolonia recta de 2 zooides (flechas punteadas), se observan 2 núcleos (N) y complejos nematocisto-teniocisto (flechas); B. *Polykrikos hartmannii*, encontrada en julio de 2022 en vista ventral, enfoque en la superficie celular, mostrando numerosos cloroplastos (C); C. vista lateral, se observan 2 zooides y 2 núcleos (N), numerosos cloroplastos, una vacuola de alimento (asterisco), así como complejos nematocisto-teniocisto (flechas); D. pseudocolonia de *P. kofoidii*, encontrada en abril de 2024, mostrando 4 zooides (flechas punteadas) y ligera torsión hacia la izquierda; E. enfoque a la mitad de la pseudocolonia, mostrando numerosos complejos nematocisto-teniocisto (flechas); F. enfoque en la superficie de la pseudocolonia, se observan 2 núcleos (N) y numerosas gotas de aceite en la superficie celular. Escala: 50 µm.

teniocisto, distribuidos en todo el citoplasma, numerosas gotas de aceite en la capa superficial de las células.

Nota taxonómica: la especie identificada como *P. kofoidii* en el estudio de Meave-del Castillo et al. (2012) corresponde,

en realidad, con *P. hartmannii* W.M. Zimmermann. El individuo observado posee las características morfológicas propias de *P. hartmannii*: dos zooides y dos núcleos, cloroplastos numerosos, mientras que el presente estudio representa el primer registro fotográfico de *P. kofoidii* para la Bahía de Acapulco.

Discusión

Polykrikos hartmannii y *P. kofoidii* poseen dos núcleos, así como numerosos complejos nematocisto-teniocisto (Fig. 2A, E), lo que pudo llevar a una confusión y la consecuente identificación errónea en el estudio de Meave-del Castillo et al. (2012). Sin embargo, las pseudocolonias de *P. hartmannii* se componen de dos zooides únicamente. Además, poseen numerosos cloroplastos (Fig. 2B), a diferencia de *P. kofoidii*, donde las pseudocolonias están conformadas por cuatro zooides (Fig. 2D), excepto durante la formación de gametos. Aquí se forman ocho zooides y se dividen en cuatro pseudocolonias de dos zooides, pero carentes de cloroplastos y únicamente con un núcleo (Hoppenrath y Leander, 2007). *Polykrikos schwartzii* Bütschli, reportado sin ilustración por Meave-del Castillo et al. (2012) de la Bahía de Acapulco, no se observó en nuestras muestras. Se confirma también la sinapomorfía establecida para todas las especies de *Polykrikos*: la presencia de un surco apical en forma de lazo cerrado y numerosos complejos nematocisto-teniocisto en el citoplasma (Fig. 2A-E).

Con lo anterior, concluimos que en la Bahía de Acapulco están presentes tanto *P. hartmannii* como *P. kofoidii*, cuyos registros se sustentan a partir de las descripciones morfológicas que proporcionamos para ambas especies, así como el registro fotográfico. Dado que *P. hartmannii* y *P. kofoidii* fueron encontrados en años y temporadas climáticas distintas, probablemente son residentes frecuentes de la Bahía.

Contribución de autores

URG y MZR concibieron y diseñaron el estudio. URG realizó el análisis y escribió el manuscrito con la ayuda de MNR y YBO. MZR y YBO realizaron los análisis morfológicos. Todos los autores contribuyeron a la discusión, revisión y aprobación del manuscrito final.

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11. Capítulo 3

Evolution of the family Polykrikaceae (Gymnodiniales) in Mexico: an inclusion of

Pseudocochlodinium and the description of *P. erythrogranulatus* sp. nov.

Estatus: En revisión en la revista Ecology and Evolution

Resumen

Polykrikos se caracteriza por la formación de pseudocolonias multinucleadas, un complejo estructural apical en forma de coma y la presencia de complejos nematocisto-teniocisto; considerados como sinapomorfias estables dentro del grupo. Existen nueve especies descritas, todas con secuencias genéticas en la base de datos GenBank, sin embargo, su historia evolutiva y los caracteres que delimitan las especies permanecen poco entendidos. Específicamente, únicamente tres especies han sido documentadas en México, generalmente sin evidencia fotográfica y genética. Dos especies producen florecimientos algales nocivos, *P. hartmannii* y *P. geminatus*, lo que resalta la necesidad de información ecológica y taxonómica actualizada. Este estudio informa sobre la diversidad y las relaciones evolutivas dentro de *Polykrikos*, con un énfasis en especies encontradas en aguas Mexicanas. La colecta fue llevada a cabo en la Bahía de Acapulco (Pacífico Mexicano) y la Laguna de Términos (Golfo de México), combinando análisis morfológicos y moleculares, específicamente microscopía óptica, distancia genética, filogenia molecular usando secuencias parciales de los genes SSU y LSU. Se confirmaron morfológicamente cuatro especies: *P. hartmannii*, *P. schwartzii*, *Pseudocochlodinium profundisulcus* y la nueva especie propuesta *P. erythrogranulatus* sp. nov., una especie heterótrofa distinguida por característicos gránulos de pigmentos rojos distribuidos en la superficie celular y un complejo estructural apical en forma de bucle cerrado; con la confirmación molecular de *P. kofoidii*. Nuestros análisis confirman que *Polykrikos* es un grupo monofilético, y dejan ver la afinidad de *Gymnodinium catenatum*, *G. fuscum* y *Pheopolykrikos beuchampii*. Enmendamos la diagnosis y la circunscripción de la familia para acomodar a especies sin la organización pseudocolonial y consideramos a *Pseudocochlodinium profundisulcus* un miembro, haciendo a *P. geminatus* un sinónimo heterotípico, adicionalmente sugerimos una historia evolutiva probable para el grupo considerando la información actual de la morfología, filogenia molecular y la ultraestructura de las especies. Los datos de distribución indican que las especies de *Polykrikos* son más prevalentes en las temporadas secas, en aguas cálidas con salinidades altas. Estos hallazgos expanden la diversidad actual de *Polykrikos* en México y proveen nuevas perspectivas de su historia evolutiva.

Evolution of the family Polykrikaceae (Gymnodiniales) in Mexico: an inclusion of *Pseudocochlodinium* and the description of *P. erythrogranulatus* sp. nov.

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Abstract

Polykrikos is characterised by the formation of multinucleated pseudocolonies, a comma or horseshoe-shaped apical structure complex and the presence of nematocyst-taeniocyst complexes, considered as stable synapomorphies within the group. There are nine currently described species, all of which have genetic sequences uploaded to the GenBank database, however, their evolutionary history and delimitation characters remain poorly understood. Particularly, only three species have been documented in Mexico, often without photographic or genetic evidence. Two species are known to cause harmful algal blooms, *P. hartmannii* and *P. geminatus*, highlighting the need for updated taxonomic and ecological information. This study reports on the diversity and evolutionary relationships within *Polykrikos*, with an emphasis on species found in Mexican waters. Sampling was conducted in Acapulco Bay (Mexican Pacific), and Laguna de Términos (Gulf of México), combining morphological and molecular analyses, namely light microscopy, genetic distances and molecular phylogeny using SSU and LSU partial sequences. Four species were morphologically confirmed: *P. hartmannii*, *P. schwartzii*, *Pseudocochlodinium profundisulcus*, and the newly described *P. erythrogranulatus* sp. nov., a heterotrophic species distinguished by unique red pigment granules distributed throughout the cell surface and a closed loop Apical Structure Complex, with the molecular confirmation of *P. kofoidii*. Our analyses confirm *Polykrikos* as a monophyletic group, and hint at the close affinity of *Gymnodinium catenatum*, *G. fuscum* and *Pheopolykrikos beuchampii*. We emend the diagnosis and circumscription of the family to accommodate species without the pseudocolonial organization, and consider *Ps. profundisulcus* a member, making *P. geminatus* a heterotypic synonym, and suggest a probable evolutionary history of the group considering known information of the morphology, molecular phylogeny and ultrastructure of the species. Distribution data indicate *Polykrikos* species are more prevalent during dry seasons in warm, saline waters. These findings expand the known diversity of *Polykrikos* in Mexico and provide new insights into their evolutionary history.

Introduction

The genus *Polykrikos* Chatton is a group of athecate dinoflagellates in the order Gymnodiniales, characterised by the formation of pseudocolonies and the presence of nematocyst-taeniocyst complexes. (Takyama, 1985; Hoppenrath et al., 2007a). Currently, the genus consists of nine species (Guiry & Guiry, 2025), two of which are regarded as uncertain (*P. barnegatensis* Martin & *P. grassei* Lecal-Schlauder). Species recognition has been based on the size, the number of zooids in a pseudocolony, number of nuclei, presence of chloroplasts, cingular displacement and lateral compression (Leander & Hoppenrath, 2007b; Matsuoka et al., 2009; Hoppenrath et al., 2010).

Polykrikos appears to have a cosmopolitan distribution given the few records scattered throughout the globe, however, the distribution of each species remains incomplete. In México, particularly in the Mexican Pacific, only three species have been previously recorded, *P. hartmannii*, *P. kofoidii*, and *P. schwartzii*. Of these, only *P. hartmannii* and *P. kofoidii* records have provided photographic evidence (Meave-del Castillo et al., 2012; Gárate-Lizarraga, 2014; Escobar-Morales & Hernandez-Becerril, 2015; Ruiz-García et al., 2025), records of *P. schwartzii* have not provided photographs (Okolodkov & Gárate-Lizárraga, 2006, Torres-Ariño et al., 2019). In the southern Gulf of Mexico, no species has been recorded, except for the Yucatan peninsula, where *P. kofoidii* was reported in 2010 and 2011 without an image (Merino-Virgilio et al., 2013).

Two species of *Polykrikos* are known to cause harmful algal blooms, *P. hartmannii*, which has caused HABs in the Eastern Mediterranean (Sabanci et al., 2025), East coast of the U.S.A. (Tang et al., 2013) and Baja California, Mexico (Gárate-lizarraga et al. 2009; Gárate-Lizárraga et al. 2011); and *P. geminatus* (syn. *Pseudocochlodinium profundisulcus*), which has caused recurrent blooms in Pearl River Estuary, China (Dong et al., 2020), therefore, it is important to have up-to-date knowledge of the group.

Despite the group's uniqueness, there are few studies regarding their evolutionary aspects, although some studies focus on species delimitation and relationships (Nagai et al., 2002; Matsuoka et al., 2009; Tang et al., 2013; Reñé et al., 2014; Hu et al., 2021). Hoppenrath & Leander (2007a) revised *Polykrikos* and the character evolution; however, new information has been obtained since. Hoppenrath & Leander (2007b) described the

photosynthetic *P. herdmaniae*, a sister clade of *P. lebourae*. In 2013, Qiu *et al.* transferred *Cochlodinium geminatum* to *Polykrikos* based on the presence of nematocyst-taeniocyst complexes. This species was later erected as the new taxon *Pseudocochlodinium profundisulcus* (Hu *et al.*, 2021), based primarily on molecular phylogeny, genetic distance and cyst morphology; it is currently considered as a genus of uncertain placement inside the order Gymnodiniales. At the moment, both *P. geminatus* and *Ps. profundisulcus* remain independent entities in the AlgaeBase (Guiry & Guiry, 2025). Reñé *et al.* (2014) described *Polykrikos tanit*, a new, photosynthetic species with two zooids and two nuclei; a stepped joint between the zooids, a sulcus descending at an angle and a “tentacle” in the ventral area.

In the global database for genetic sequences (GenBank), most species described in the genus already have rDNA sequences uploaded (*P. geminatus* (F.Schütt) D.X.Qiu & Senjie Lin, *P. hartmannii* W.M.Zimmermann, *P. herdmaniae* Hoppenrath & Leander, *P. kofoidii* Chatton, *P. lebourae* E.C.Herdman, *P. schwartzii* Bütschli & *P. tanit* Reñé).

The description of the family Polykrikidae under the International Code for Zoological Nomenclature by Kofoid & Swezy (1921) considered the formation of permanent “colonies” of two, four or eight individuals, the presence of nematocysts and the absence of chloroplasts as diagnostic characteristics. Fensome *et al.* (1993) made Polykrikidae and other names for the family invalid, and established only the multinucleated, pseudocolonial cell in the diagnosis. It is now recognized the existence of more species without the synapomorphic characters of the genus and the family, but clearly belonging to the same group. Such is the case of the photosynthetic species (*P. hartmannii*, *P. tanit* & *P. herdmaniae*), some that do not form pseudocolonies (*P. geminatus*) and others that present a single cell life stage (*P. hartmannii*). Therefore, the group is in need of a clear delimitation and a revision of its taxonomic problems.

In this study, we describe the presence of five *Polykrikos* species along the Mexican coastline, from which one constitutes a new morphological record and a molecular confirmation. We describe a new species and reconstruct the probable evolution of the group based on molecular phylogeny, morphology and known ultrastructure.

Materials & methods

Sampling

In April 2023 and March 2025, three sampling stations were selected for sampling in Acapulco Bay, Mexican Pacific. (Fig.1). At each site, a horizontal net tow was performed with a 20 µm mesh size plankton net.

In May 2024, four stations were selected in Laguna de Términos, Gulf of Mexico. At each station, temperature and salinity were measured with a mercury thermometer and a hand refractometer (Atago Co. Ltd., Tokyo, Japan), respectively. Water was collected with a van Dorn bottle (General Oceanics, Florida, EE.UU.), filling up a two-gallon tank. Samples were concentrated using an inverse filtration system with a 4 µm pore sized membrane (Millipore, Merck KGaA, Darmstadt, Germany).

Light microscopy

Samples were viewed with a Reichert, Microstar IV microscope (Leica, Vienna, Austria) equipped with a DIC system, achromatic objectives and coupled with a 24 MP camera (Nikon, Tokyo, Japan).

Molecular Analyses

Single-cell PCR

Cells of *P. kofoidii* described in Ruíz-García *et al.* (2025) were transferred to clean 0.2 ml PCR tubes and kept stored at -20°C. PCR reactions were carried out using the Promega GoTaq® G2 colourless master mix (Madison, Wisconsin, USA) and the eukaryote primers D1R-F, D2C-R, 28-1483-R (Scholin *et al.*, 1994; Daugbjerg *et al.*, 2000) for the partial LSU rDNA (D1-D2 domains), and SR4-F, SR7-R for the partial SSU rDNA (D4–D7 domains). Amplification was performed in a BioRad T100 Thermal cycler. Conditions for the amplification of the LSU partial sequence were those described by Hernandez-Rosas *et al.* (2018), modified by Escarcega-Bata *et al.* (2023), denaturation at 98°C for 3 min, 35 cycles at 95°C for 30s, 52°C for 30s and 72°C for 1 min, with a final extension at 72°C for 10 min. For the SSU partial sequence, the conditions were: denaturation at 95°C for 3 min, 32 cycles at 95°C for 1 min, 56°C for 1 min and 72°C for 4 min, with a final extension at 72°C for 10 min. Amplification was confirmed through electrophoresis in 1.5% agarose gel stained with Biotium GelRed® (San Francisco, California, USA) for 40 min at 90 V and visualised in a UV photodocumentation system. Samples were sent to the Laboratorio de Secuenciación

Genómica, Pabellón de la Biodiversidad, Instituto de Biología, Universidad Nacional Autónoma de México for commercial sequencing. Obtained sequences were uploaded to the GenBank database under the accession numbers PX249749–PX249754.

Phylogeny

Consensus sequences were generated with Bioedit (Hall, 1999). A Blast analysis was performed for a preliminary taxonomic identification. Similar sequences were downloaded from GenBank (Supplementary Information) and aligned in Bioedit (Hall, 1999) using the Clustal W algorithm generating data matrices for the SSU and LSU genes, including sequences from closely related Gymnodiniales reported previously (Hoppenrath & Leander, 2007; Tang et al., 2013; Hu et al., 2021); *Apocalathium aciculiferum* (Lemmermann) Craveiro, Daugbjerg, Moestrup & Calado was selected as outgroup. A concatenated data matrix of both genes was constructed. Evolutionary model selection was performed in TOPALi ® V2 (Milne et al., 2009) using the model selection tool. Bayesian Inference (BI) analyses were performed in Mr. Bayes v3.2 (Ronquist et al., 2012) using GTR+I+G (General Time Reversible + Invariable sites + Gamma distribution) as an evolutionary model. The data matrix was run for 6×10^6 generations, with 6 Monte Carlo Markov chains (2 hot chains and 4 cold chains) starting with a random tree, sampling every 500 generations and discarding 30% of outliers. The data matrix was imported into Mega 11 (Tamura et al., 2021), in which an uncorrected p-distance analysis for all *Polykrikos* species was performed using 1,000 bootstrap replicates.

Results

The family Polykrikaceae is emended to fit current knowledge of the group. In Acapulco Bay, three species of *Polykrikos* were found (*P. erythrogranulatus* sp. nov., *P. hartmannii*, *P. schwartzii*), with the molecular confirmation of the presence of *P. kofoidii*; meanwhile, in Laguna de Términos, only *Pseudocochlodinium profundisulcus* was recorded.

Taxonomy

Polykrikaceae (Kofoid & Swezy) emend Ruíz-García, Balboa-Calvillo

Emended diagnosis: Gymnodinium-like cells. Solitary, forming chains or pseudocolonies. Nematocyst-taeniocyst complexes present. Cingulum a descending left spiral of approximately 1–1.2 turns around the cell. Ocellus and piston lacking. Chloroplast present or absent. APC an incomplete or complete loop around the apex. Marine, planktonic or benthic.

Type genus: *Polykrikos* Bütschli

***Polykrikos* (Bütschli) emend Ruíz-García, Gutiérrez-Mendieta, Balboa-Calvillo**

Emended diagnosis: Cells forming pseudocolonies of two, four, eight or up to sixteen zooids. Nematocyst-taeniocyst complexes present. Chloroplasts present or absent.

Type species: *Polykrikos schwartzii* Bütschli

***Polykrikos erythrogranulatus* Ruíz-García, sp. nov. (Figs 2-7)**

DIAGNOSIS: Cells forming pseudocolonies 68–73 µm long (mean= 66.4 µm, s.d.= 7.4 µm), 37–47 µm wide (mean= 43 µm, s.d.= 3.9 µm), consisting of two zooids with two nuclei, sometimes with a stepped joint between the zooids. The ASC is a closed loop. The cingulum descends about its own width and goes around the cell about 1.1 times, making the sulcus descend at an angle. Numerous red pigment granules underlying the cell wall. Last zooid with faint striae.

HOLOTYPE: Figs 2-7.

TYPE LOCALITY: Naval station (16°50.12' N, 99°51.51' W), Acapulco Bay, México.

ETYMOLOGY: The word *erythrogranulatus* refers to the distinctive red pigment granules present on the surface.

Description: Barrel-shaped pseudocolonies consisting of two zooids and two nuclei at the center of each zooid. The cingulum is complete, going around the cell approximately 1.1 times. Sulcus descending at an angle. The ASC is a comma-shaped closed loop.

Pseudocolonies display a characteristic red colour due to several pigment granules distributed throughout the cell wall. No chloroplasts were observed. The most posterior zooid has faint striae. Ingested prey were common, mainly *Prorocentrum*. Several pseudocolonies were found in the Naval station, during April 2023 and March 2025. The water temperature was 25°C, with a salinity of 30. Cell division by means of elongation, and formation of a chain of

four zooids and two nuclei (Fig. 3), with a posterior separation leaving a pseudocolony of two zooids and one nucleus.

***Polykrikos hartmannii* W.M.Zimmermann. (Figs 12-15)**

Cells were always found forming barrel-shaped pseudocolonies of two zooids with numerous chloroplasts and nematocyst-taeniocysts complexes. Pseudocolonies were 60–70 μm long and 38–42 μm wide (n=6). No engulfed prey were observed.

Several cells were found in Diamond beach, in April 2023 and March 2025, with a salinity of 35 and 33, and a surface temperature of 27°C and 25°C respectively.

***Polykrikos schwartzii* Bütschli. (Figs 16-21)**

Only pseudocolonies with 8 zooids were observed. Pseudocolonies were 90 μm long, 43 μm wide (n=4) at the widest point, and displayed four nuclei to the left side and numerous nematocyst-taeniocyst complexes to the right side. Observed pseudocolonies in the last stages of asexual division showed torsion to the left and a slightly smaller first zooid. *P. schwartzii* was found in Diamond beach and Naval station in April 2025. Surface temperature was 25°C and salinity of 33.

***Pseudocochlodinium profundisulcus* Zhangxi Hu, N.Xu, H.Gu, M.Iwataki, T.Takahashi, Y.Z.Tang & K.Matsuoka (syn= *Polykrikos geminatus* (F.Schütt) D.X.Qiu & Senjie Lin). (Figs 8-11)**

The observed cells were approximately 36 μm long, 30 μm wide, had a descending cingulum going around the cell 1.5 times, displaced about half the length of the total cell height, a centrally located nucleus, several chloroplasts and numerous nematocysts distributed throughout the cytoplasm and trichocysts in the cell wall. We did not observe two-zooid pseudocolonies. Cells were found at the center of Laguna de Términos, in the station “Center” (E12: 18°38'3.38" N 91°35'24.63" W). Surface temperature was 30.7°C, with a salinity of 35.6.

Molecular phylogeny

A 1755 bp and a 763 bp database matrix was built for partial sequences of the SSU and LSU genes correspondingly (domains 4-7, domains D1-D3). A Concatenated data matrix of 2517 bp was made.

In the BI analysis (Fig. 22), *Gymnodinium fuscum* (Ehrenberg) F.Stein and *G. catenatum* H.W.Graham were grouped together in the clade of *Polykrikos* and *Pheopolykrikos* Chatton with low support (BI=0.56). *Ph. beuchampii* displays some affinity to the clade of *Polykrikos*, however its position was not resolved.

All sequences of current members of *Polykrikos* were grouped together in a monophyletic clade with maximal support (BI=1.00). *Ps. profundisulcus* was placed basally to the rest of the species. *P. tanit* and *P. hartmannii* diverge next. *P. herdmaniae* and *P. lebourae* formed a single clade with maximum support, as did *P. schwartzii* and *P. kofoidii*.

As for the ML analysis (Fig. 23), the sequences of *Pheopolykrikos beuchampii* were retrieved far from the clade of *Polykrikos*, and with a low bootstrap value. The closest relatives to the genus *Polykrikos* are *G. catenatum* and *G. fuscum*, clustered in a sister clade, albeit with low support (ML<50%). *P. geminatus* is displayed in a basal position to the rest of the genus, followed by *P. tanit* and *P. hartmannii*. *P. lebourae* and *P. herdmaniae* are grouped as sister taxa, as well as *P. kofoidii* and *P. schwartzii*, both with high support values (ML= 93%, 100%), but the clade of *P. kofoidii* and *P. schwartzii* displays many subclades composed of mixed sequences from both taxa. The sequences at the genus level are nested within this clade.

Low bootstrap values outside the *Polykrikos* clade in the ML analysis are explained by the lack of an exhaustive sampling; however, we do not consider this an issue, given the high posterior probability (PP) values in the BI analysis, and the distant relationship of *Polykrikos* to the rest of the genera inside the order Gymnodiniales.

Distance analysis

Genetic distances (uncorrected/corrected) between Polykrikoid species using partial sequences of the SSU gene, ranged from 1.95%/2.01% between *P. tanit* and *P. hartmannii* to 8.01%/9.04% between *Ph. beuchampii* and *P. lebourae*. For the partial sequences of the LSU gene, the distances ranged from 7.4% between *P. kofoidii* and *P. schwartzii* to 32% between *Ph. beuchampii* and *P. lebourae*.

As for the distance among the species, values for the SSU sequences are low (0%-1.56%). Distances using partial LSU sequences range between 0% and 8.49% (Table 3).

Discussion

P. erythrogranulatus is a distinct species of *Polykrikos*.

The organism found in this study displays a set of morphological differences from all known members of the Genus, which warrants its recognition as a distinct species. The primary differential traits of *P. erythrogranulatus* are the red pigment granules, absent in any other species, a closed, comma-shaped ASC, which differs from the closed loop of *P. hartmannii*, *P. lebourae* and *P. herdmaniae*, the faint striae in the hypocone currently restricted to *P. kofoidii*, but which generally is composed of four zooids and lacks pigment granules. While a full molecular assessment of this species is necessary to establish a definitive phylogenetic position within the *Polykrikos* clade, the combination of these traits make it a probable lineage between *P. hartmannii* and the clade of *P. kofoidii* and *P. schwartzii*, where photosynthesis was lost, but zooid doubling had not occurred (Fig. 24).

Emendment of the family Polykrikaceae and the genus *Polykrikos*.

The clade of *Gymnodinium fuscum* and *G. catenatum* are closely related to the clade of *Polykrikos* and *Pheopolykrikos*, as noted previously by (Hoppenrath et al. 2007a, Hoppenrath et al. 2007b). Kofoid & Swezy (1921) consider the permanent pseudocolonial nature of the cells and the presence of nematocyst-taeniocyst complexes, meanwhile Fensome et al. (1993) considered multinucleated, pseudocolonial cells. Nevertheless, up until that point, there were no described Gymnodiniales pseudocolonial species with a unicellular life stage, nor photosynthetic species. We consider the presence of nematocysts as the most stable synapomorphy of the family and therefore propose an emendment to the family diagnosis. We restrict the pseudocolonial organisation as the synapomorphy of the type genus *Polykrikos*. The emendment of the family changes the current circumscription to include *Ps. profundisulcus* as a member, making *P. geminatum* a heterotypic synonym.

Position of *Ph. beuchampii* with respect to *Polykrikos*.

Although *Ph. beuchampii* belongs to a distinct genus placed in the family Gymnodiaceae, there is no doubt that *Pheopolykrikos* and *Polykrikos* share many important characteristics. The formation of pseudocolonies, (nematocyst-taeniocyst complexes), a lasso-shaped ASC, etc. (Matsuoka et al. 2009). Although the ML analysis did not show close affinity for the clade of *Polykrikos*, the BI tree hints at the possibility of *Ph. beuchampii* belonging to the Family Polykrikaceae. There is a need for more genetic sequences to better sample intermediate branches and resolve relationships with more confidence.

Evolution of Polykrikoid morphology as supported by phylogenetic data

In 2007, Hoppentath & Leander constructed a hypothesis of Polykrikoid character evolution, in which *G. fuscum* is the closest relative of *Polykrikos*. Either zooid doubling and a loss of Photosynthesis occurred to the *Pheopolykrikos* clade, or a zooid and nuclear doubling occurred to the clade of *P. kofoidii* and gave rise to *Polykrikos schwartzii*. New molecular and morphological information can support both *G. catenatum* and *G. fuscum* as the closest relatives of *Polykrikos*, showing predisposition to form chains (Fig. 24). The picture of character evolution is more complete, backed up by molecular analyses of SSU, LSU and concatenated sequences (Figs. 22, 23) not available when the group was first studied.

It is still unclear the relationship of *Ph. beuchampii* to the Polykrikaceae, but our analyses support the idea that convergent evolution led to the pseudocolonial nature of both clades. *Ps. profundisulcus* already displays the presence of nematocysts. *P. hartmannii* and *P. tanit* are the first step in the formation of pseudocolonies; probably after an event of nuclear doubling with an incomplete cytokinesis. Over time, the single-celled life stage was lost, since the rest of the species have no single-celled stage. A zooid doubling gave rise to the clade containing *P. herdmaniae*, *P. lebourae*, *P. kofoidii* and *P. schwartzii*, with *P. kofoidii* showing sometimes 8 zooids, but normally only four. Another zooid doubling may have given rise to the clade of *P. lebourae* and *P. herdmaniae*, with an independent event doubling zooid and nuclei number in *P. schwartzii*.

The chloroplasts present in *P. lebourae* are ultrastructurally different from typical peridinin containing plastids of closely related taxa (*Gymnodinium fuscum*, and autotrophic *Polykrikos* species), in that they appear to lack a stigma, have only two enveloping membranes, thylakoids in stacks of two and central pyrenoids without thylakoids. This supports the hypothesis that photosynthesis was lost during Polykrikoid evolution, in the subclade containing *P. erythrogranulatus*, *P. herdmaniae*, *P. lebourae*, *P. kofoidii*, and *P. schwartzii*, with a posterior endosymbiotic event giving *P. herdmaniae* photosynthetic capacity, as suggested by Hoppenrath & Leander (2007b).

Distribution

Polykrikos appears to have a certain affinity for the dry season. In Laguna de Términos, Gulf of México, *Polykrikos geminatus* was found in May 2024, which corresponds to the dry season, matching descriptions of the same species in PRe, China. HAB events caused by *P. geminatus* have occurred occasionally between October and November (Shen et. al. 2012). It has also been reported from the Black and Mediterranean seas, and Australia.

In Acapulco Bay, cells have been found between December and May, when surface temperature is still high, with a salinity of at least 30.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Supplementary information

Supplementary Table S1 Genetic distance between different *Polykrikos* species based on partial sequences of the SSU rDNA gene.

Supplementary Table S2 Genetic distance between different *Polykrikos* species based on partial sequences of the LSU rDNA gene.

Supplementary Table S3 Genetic distance inside the different *Polykrikos* species based on partial sequences of the SSU and LSU rDNA genes.

Supplementary Table S4 Sequences used in this study

Author contributions

U.T. Ruíz-García: Original concept, methodology, morphological analysis, original draft, review and editing. F.J. Gutierrez-Mendieta: Validation, resources, funding acquisition, review and editing. A.K. Balboa-Calvillo: Validation, morphological analysis, review and editing. M.L. Nunez-Resendiz: Validation, resources, analysis of molecular data, funding acquisition, review and editing, supervision.

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Tables

Table 1. Morphological comparison of currently recognized Polykrikos species. ?- lacking confirmation, N/A- Not applicable

Figures

Fig. 1 Sampling stations in Acapulco Bay and Laguna de Términos, Mexico.

Figs 2-7. *Polykrikos erythrogranulatus* sp. nov. found in march 2023. 2. Dorsal view of the pseudocolony with an ingested *Prorocentrum* (P). 3. Dividing pseudocolony of 4 zooids. 4. Ventral-apical view. Lasso- shaped ASC (arrows). 5. Focus on the surface of a Pseudocolony showing the sulci descending at an angle (s). 6. Mid focus of the pseudocolony showing an ingested *Prorocentrum* (p), nuclei (N), probable furrows (arrowheads). 7. Subsuperficial focus of a pseudocolony showing the two nuclei (N), and food vacuoles (fv).

Figs 8-11. *Polykrikos geminatus* found in Laguna de Términos. 8. Side view; trichocysts (arrowhead). 9. Mid portion focus. Nucleus (N) 10. Right-ventral side focus. Nematocyst-taeniocyst complexes (arrowheads), chloroplasts (c) 11. Focus on the right-apical side. ASC (Arrow). Scale bar: 25 μ m

Figs 12-15. *Polykrikos hartmannii*. 12. Pseudocolony found in April 2023. 13. Pseudocolony found in March 2025. Mid focus showing nematocyst-taeniocyst complexes (arrowhead) and numerous chloroplasts (c). 14. Focus on the apical portion. ASC (arrow). 15. Mid focus showing the two nuclei (N). Scale bar: 20 μ m

Figs 16-21. Pseudocolony of *Polykrikos schwartzii* in the late stages of division. 16. Ventral view showing fused sulci. 17. Dorsal view showing a nematocyst-taeniocyst complex (arrowhead). 18. Mid focus of the pseudocolony. Trichocysts (arrows), nematocysts (arrowheads). 19. Midfocus showing the nuclei before full karyocinesis (N). 20. Ventral view displaying a lasso-shaped ASC (arrow) and eight cinguli (narrow arrowheads). 21. A barrel-shaped colony. Cinguli (narrow arrowheads), nematocysts (arrowheads).

Fig. 22. Bayesian inference phylogenetic tree inferred from concatenated partial sequences of the SSU+LSU genes of nuclear encoded rDNA.

Fig. 23. Maximum likelihood phylogenetic tree inferred from concatenated partial sequences of the SSU+LSU genes of nuclear encoded rDNA.

Fig 24. Polykrikoid evolution as supported by molecular data. Modified from Hoppenrath & Leander (2007a, 2007b), Reñé et al. (2013), and Hu et al. (2021). Nematocyst-taeniocyst complexes are drawn in red. Uncertain taxa (*P. barnegatensis* & *P. grassei*) are not included.

Supplementary Material

Supplementary Table S1 Genetic distance between different *Polykrikos* species based on partial sequences of the SSU rDNA gene. Uncorrected distance below the diagonal, and corrected distances (in Mega 11) using the Kimura 2 parameter model above the diagonal.

Taxon	<i>Ph.</i> <i>beuchampii</i>	<i>P.</i> <i>geminatus</i>	<i>P. tanit</i>	<i>P.</i> <i>hartmannii</i>	<i>P. kofoidii</i>	<i>P.</i> <i>schwartzii</i>	<i>P.</i> <i>herdmaniae</i>	<i>P. lebourae</i>
<i>Ph.</i> <i>beuchampii</i>	-	5.37%	5.05%	5.11%	6.16%	6.20%	7.45%	9.04%
<i>P. geminatus</i>	4.97%	-	2.48%	3.24%	5.0%	5.53%	5.46%	7.13%
<i>P. tanit</i>	4.69%	2.39%	-	2.01%	4.01%	4.64%	4.86%	5.87%
<i>P.</i> <i>hartmannii</i>	4.75%	3.08%	1.95%	-	4.26%	4.79%	5.84%	7.55%
<i>P. kofoidii</i>	5.65%	4.65%	3.79%	3.95%	-	3.31%	5.49%	7.03%
<i>P. schwartzii</i>	5.7%	5.11%	4.35%	4.43%	3.16%	-	5.46%	7.94%
<i>P.</i> <i>herdmaniae</i>	6.71	5.02%	4.53%	5.33%	5.06%	5.86%	-	4.01%
<i>P. lebourae</i>	8.01%	6.44%	5.42%	6.78%	6.36%	7.10%	3.79%	-

Supplementary Table S2 Genetic distance between different *Polykrikos* species based on partial sequences of the LSU rDNA gene. Uncorrected distance below the diagonal, and corrected distances (in Mega 11) using the Kimura 2 parameter model above the diagonal.

Taxon	<i>Ph.</i> <i>beuchampii</i>	<i>P.</i> <i>geminatus</i>	<i>P. tanit</i>	<i>P.</i> <i>hartmannii</i>	<i>P. kofoidii</i>	<i>P.</i> <i>schwartzii</i>	<i>P.</i> <i>herdmaniae</i>	<i>P. lebourae</i>
<i>Ph.</i> <i>beuchampii</i>	-							
<i>P. geminatus</i>	24.49%	-						
<i>P. tanit</i>	21.57%	13.66%	-					
<i>P.</i> <i>hartmannii</i>	20.77%	10.91%	8.88%	-				

<i>P. kofoidii</i>	27.05%	22.81%	19.60%	18.10%	-			
<i>P. schwartzii</i>	29.38%	23.07%	20.64%	18.96%	7.4%	-		
<i>P. herdmaniae</i>	31.44%	22.23%	23.48%	21.0%	25.53%	25.40%	-	
<i>P. lebourae</i>	32.70%	22.19%	25.97%	20.16%	27.47%	27.48%	13.51%	-

Supplementary Table S3 Genetic distance inside the different *Polykrikos* species based on partial sequences of the SSU and LSU rDNA genes.

Species	Distance within	
	SSU Gene	LSU Gene
<i>Ph. beuchampii</i>	0.46%	0%
<i>P. geminatus</i>	0.71%	4.93%
<i>P. tanit</i>	0%	0.13%
<i>P. hartmannii</i>	0.12%	0.08%
<i>P. kofoidii</i>	0.93%	3.05%
<i>P. schwartzii</i>	0.09%	8.49%
<i>P. herdmaniae</i>	0.42%	5.62%
<i>P. lebourae</i>	1.56%	5.62%

Supplementary Table S4 Sequences used in this study

Taxon	Accession number	Gene and region	Isolate or strain	Sampling locality
<i>Apocalathium</i>	KF446621	SSU	KOT2011	-
<i>aciculiferum</i>	EF417313	SSU	PABR3	-
(Lemmermann)	EF417315	SSU	PASP11	-
Craveiro, Daugbjerg,	HQ176321	LSU	K-0998	Italy
Moestrup & Calado	AY970652	LSU	PAER-1	-
<i>Barrufeta bravensis</i>	MH732694	SSU	DAG8	Damai Beach,
N.Sampedro &	KP790179	LSU		Malaysia
S.Fraga				Catalan Coast, Spain
<i>Cochlodinium</i>	OR921494	SSU + ITS1	Cpu-Q14	Canada
<i>pulchellum</i>				
Lebour				
<i>Cochlodinium</i>	KY468923	LSU	FG10_clone4	Sao Sebastiao
<i>strangulatum</i>				Channel,
(F.Schütt) F.Schütt				Brazil
<i>Gymnodinium</i>	FN647677	LSU	VG0743	Algeria
<i>catenatum</i>	MT659673	LSU	QZ10	Quanzhou, Fujian,
H.W.Graham				China
<i>Gymnodinium</i>	DQ837534	SSU	KDAAD	Darwin, Australia
<i>dorsalisulcum</i>	LC054930	SSU	HG154	-
(Hulburt,	MW177915	LSU	CAWD225	Roberton Island, New
J.J.A.McLaughlin &	DQ336190	LSU	SM28	Zealand
Zahl)	MH732682	LSU	TI009	Broome, Australia
Sh.Murray, Salas &				Sanya, South China
Hallegraeff				Sea, China
<i>Gymnodinium</i>	MK405489	SSU+ITS+LSU	GeoM*864	Germany
<i>fuscum</i>	EU025011	SSU	LO228-109	-
(Ehrenberg) F.Stein	AF022194	SSU	MUCC282D	-
	AF200676	LSU	CCMP1677	-
	MK405489	SSU+ITS+LSU	GeoM*864	Germany
<i>Gymnodinium</i>	MH732686	LSU	TI0251	Corsica, France
<i>impudicum</i>				

(S.Fraga & I.Bravo) Gert Hansen & Moestrup				
<i>Gymnodinium</i>	AB265968	LSU	-	Obama Bay, Japan
<i>microreticulatum</i>	OR778352	LSU	BGERL268	Beihai, China
C.J.S.Bolch & G.M.Hallegraeff				
<i>Gymnodinium</i> sp.	HG005135	SSU+ITS+LSU	GSSW10	-
F. Stein				
<i>Katodinium</i>	ON015058	SSU	ExtE	Maade Siel, Germany
<i>asymmetricum</i>	ON015071	LSU		
(Massart) A.R.Loeblich				
<i>Lepidodinium</i>	EF010974	LSU	LCDE01	River Derwent,
<i>Chlorophorum</i>	MT850080	LSU		Tasmania, Australia
(Elbrächter & Schnepf) Gert Hansen Botes & Salas				
<i>Lepidodinium viride</i>	AF022199	SSU	RCC6910	Kervel, France
M.Watanabe, S.Suda, Inouye T.Sawaguchi & Chihara				
<i>Margalefidinium</i>	PP801156	SSU	-	China
<i>polykrikoides</i>	AY421780	SSU	CP0	-
(Margalef) F.Gómez, Richlen & D.M.Anderson	MK685214	LSU	CP1	-
<i>Pheopolykrikos</i>	DQ371294	SSU	1	-
<i>beuchampii</i>	DQ371295	SSU	2	-
Chatton	EF616463	LSU	PBDB	Dianas Basin, Tasmania, Australia

<i>Polykrikos</i> <i>geminatus</i> (F.Schütt) D.X.Qiu & Senjie Lin	MF445297	SSU	KX-25	Zhuhai, China
	MT946914	SSU	SZ19	-
	MW788712	SSU	-	-
	MW811436	SSU	G1115	China
	MW811437	SSU	G2	China
	MF445291	LSU	JX-25	Zhuhai, China
	KF878934	LSU	CG	-
	KT389895	LSU	-	South China Sea,
	OK667934	LSU	1	China
	ON392371	LSU	RZ-2	-
	OR855909	LSU	LMBE-CYST-S hh	Yellow Sea, China South Korea
<i>Polykrikos</i> <i>hartmannii</i> W.M.Zimmermann	AY421789	SSU	JHC0203	-
	KC814183	SSU	FR4	Forge River, New York,
	LC482678	SSU	-	USA
	HQ834210	LSU	FR4	Japan
	ON392363	LSU	SGW-1	Forge River, New York,
	OP482176	LSU	kky25	USA
	OQ383732	LSU	BS7-E9	Yellow Sea, China
	MK610718	LSU	PHJZB1705	-
	FJ947045	LSU	-	- China -
<i>Polykrikos</i> <i>herdmaniae</i> Hoppenrath & Leander	KP790164	SSU	AR420	Catalan Coast, Spain
	KP790165	SSU	AR427	Catalan Coast, Spain
	DQ822481	SSU	-	-
	DQ975470	SSU	-	-
	KP790225	LSU	AR418	Catalan Coast, Spain

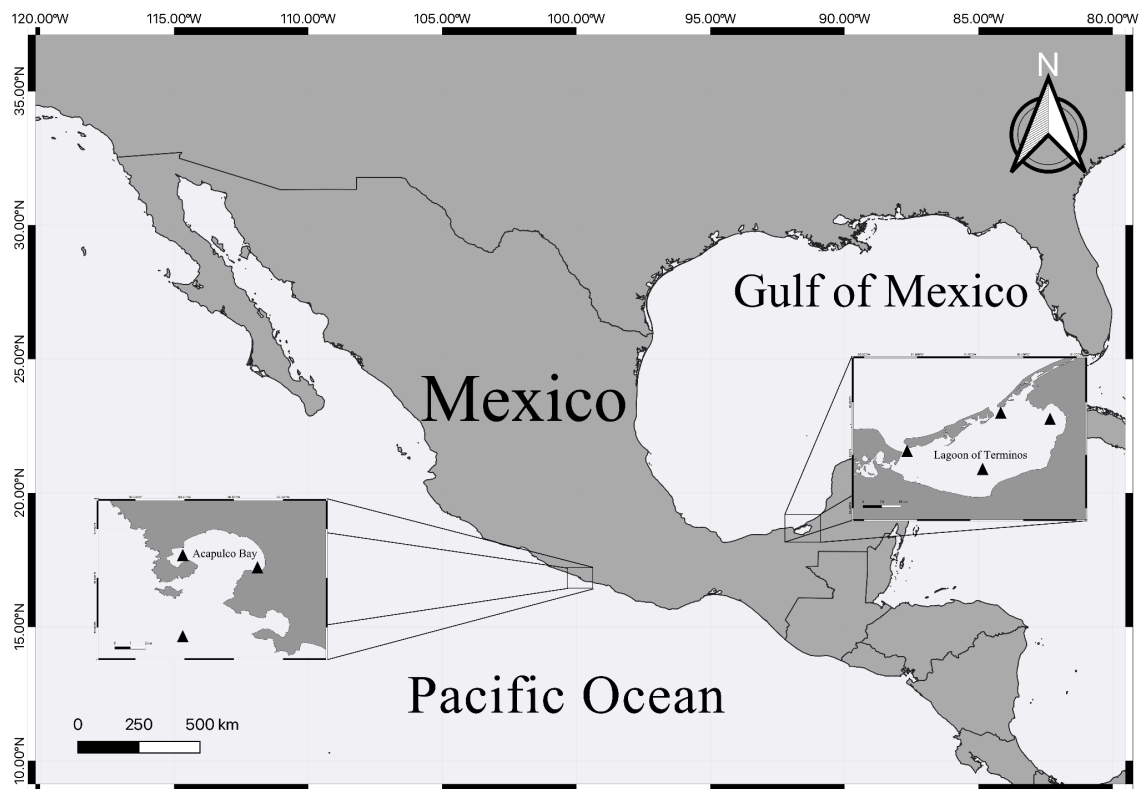
<i>Polykrikos kofoidii</i> Chatton	OP604725	SSU	Pk-FC2	-
	DQ371291	SSU	1	-
	DQ371292	SSU	2	-
	AB466292	SSU	070216-6	Omura Bay, Nagasaki,
	AB466294	SSU	2	Japan
	PP905715	SSU	PolQI	Hiroshima Bay, Japan
	PX249749	SSU	RG1	Canada
	PX249750	SSU	RG2	Acapulco Bay, México
	PX249751	SSU	RG3	Acapulco Bay, México
	AB466284	LSU	070216-6	Acapulco Bay, México
	FJ947043	LSU	-	Omura Bay, Nagasaki,
	ON392367	LSU	RZ-3	Japan
	KP790226	LSU	AR2	-
	PX249752	LSU	RG1	Yellow Sea, China
	PX249753	LSU	RG2	Catalan Coast, Spain
	PX249754	LSU	RG3	Acapulco Bay, México
				Acapulco Bay, México
				Acapulco Bay, México
<i>Polykrikos lebouriae</i> E.C.Herdman	DQ371293	SSU	-	-
	DQ975471	SSU	-	-
	DQ975472	SSU	-	-
	KT371436	LSU	PLSH2	-
	KT371438	LSU	PIDH1	-
	FJ947044	LSU	-	-
<i>Polykrikos sp.</i> Chatton	OM617931	SSU	Korea 2	-
	OM617934	SSU	Korea 5	-
	OM617936	SSU	Korea 7	-
<i>Polykrikos schwartzii</i> Bütschli	AB466286	SSU	-	Inokushi Bay, Oita,
	AB466287	SSU	-	Japan
	AB466288	SSU	031003-16	Omura Bay, Nagasaki,
	AB466289	SSU	070215-2	Japan
	AB466281	LSU	-	Nagasaki, Japan
	AB466282	LSU	070215-2	Omura Bay, Nagasaki,
	EF192408	LSU	PSHK00	Japan
	EF192409	LSU	PSSH00	Inokushi Bay, Oita,
	EF205013	LSU	-	Japan

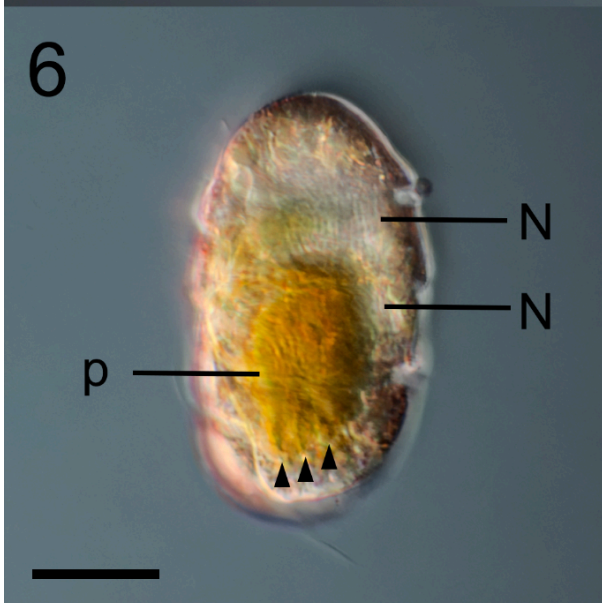
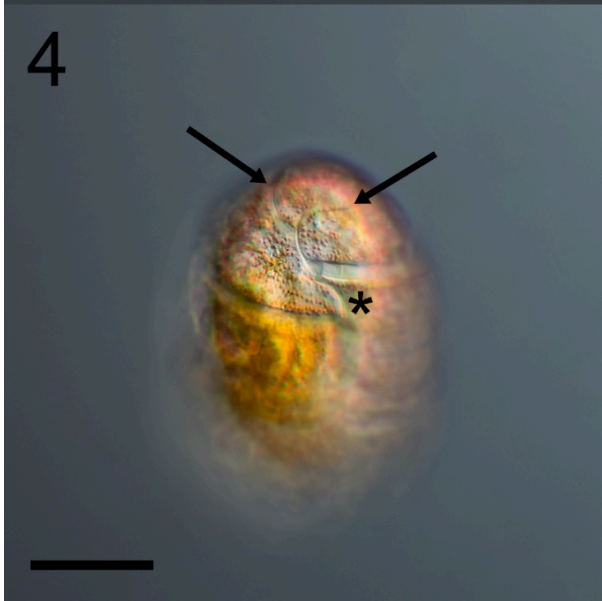
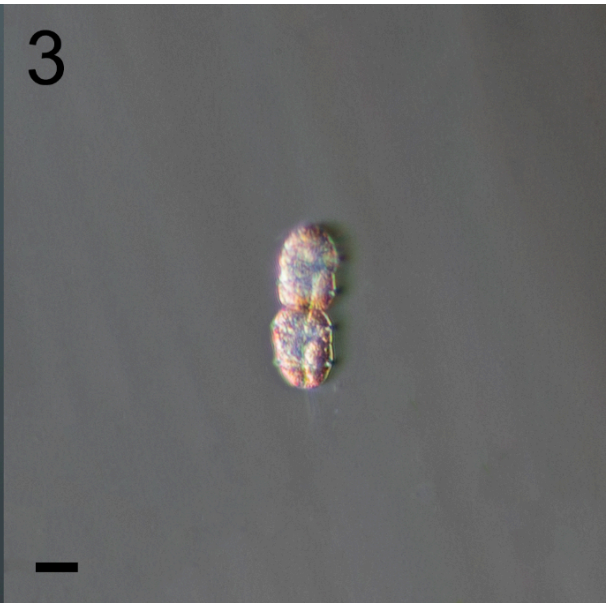
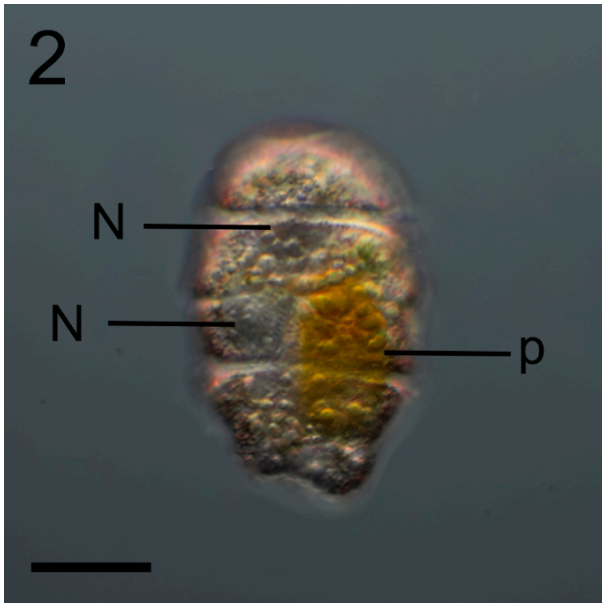
				Omura Bay, Nagasaki, Japan - - Denmark
<i>Polykrikos tanit</i>	KF806598	SSU	1	Arenys Harbour,
Reñé	KF806599	SSU	2	Catalonia, Spain
	KF806600	LSU	3	Arenys Harbour,
	KF806601	LSU	4	Catalonia, Spain
	KF806602	LSU	5	Vilanova Harbour, Catalonia, Spain Arenys Harbour, Catalonia, Spain Barcelona, Catalonia, Spain
<i>Stoeckeria</i>	HG005132	SSU+ITS+LSU	SSSC09	-
H.J.Jeong, J.S.Kim, J.Y.Park, J.H.Kim, S.Kim, I.Lee, S.H.Lee, J.H.Ha & W.H.Yih	FN557541	SSU+ITS+LSU	SSMS0806	Masan, South Korea
<i>Wangodinium</i>	MH732687	SSU	G27	Xiamen, East China
<i>sinense</i>	MH732688	SSU	GBH03	Sea, China
Z.Luo, Zhangxi Hu,	MH732681	LSU	GBH03	Beihai, South China
Yingzhong Tang	OL699923	LSU	WsLomme01	Sea, China
& H.F.Gu				Beihai, South China Sea, China Busan, South Korea
<i>Warnowia</i>	KY980035	SSU	NY13S_167	-
Er.Lindemann	KY980245	SSU	BH46_156	-
	KY980382	SSU	BH65_109	-

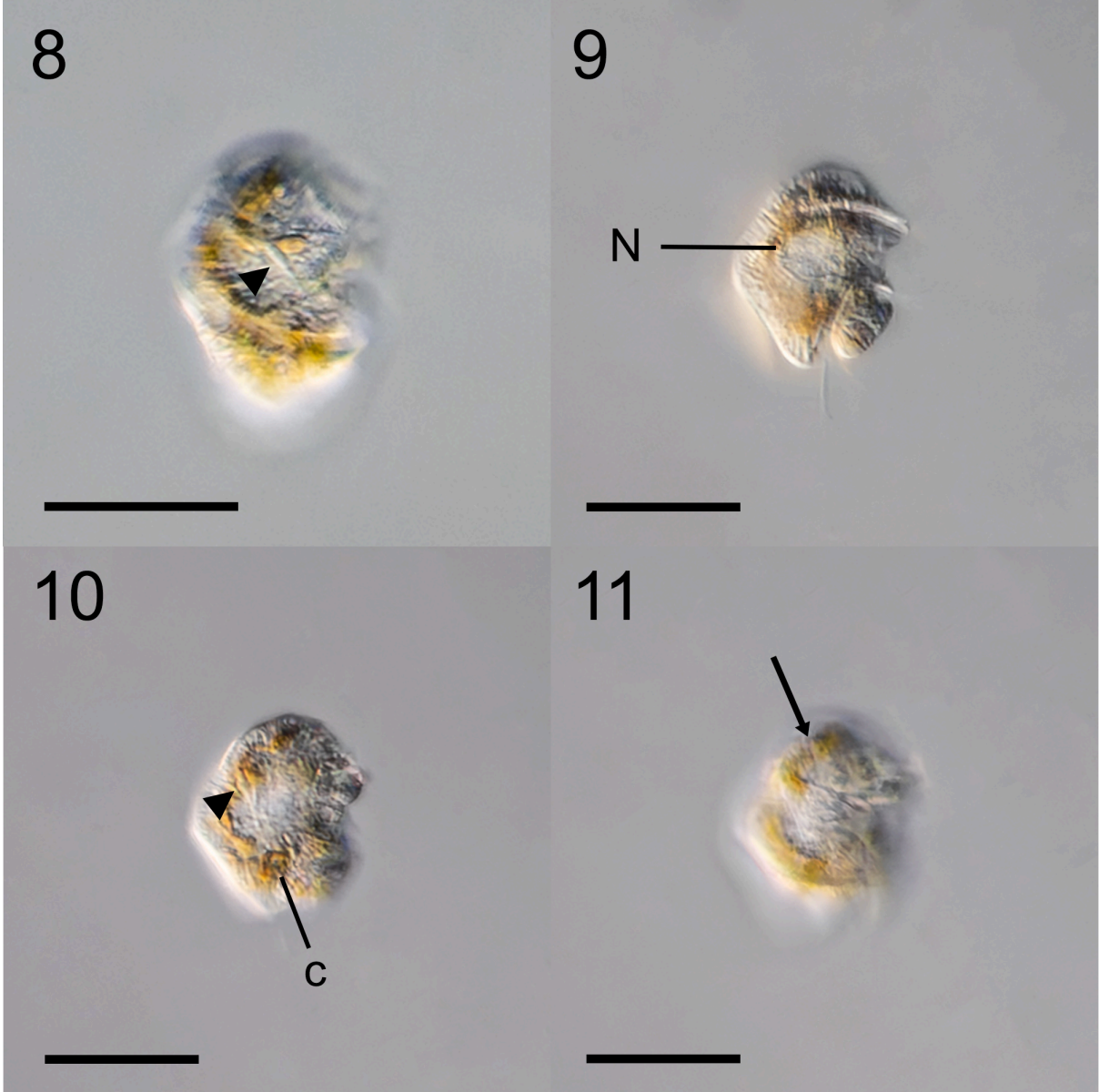
	<i>Ph. beauchampii</i> a	<i>Ps. profundisulcus</i> b,c	<i>P. tanit</i> d	<i>P. hartmannii</i> e, f, this study	<i>P. erythrogranulatus</i> This study	<i>P. kofoidii</i> g, h	<i>P. schwartzii</i> g, h	<i>P. herdmaniae</i> i	<i>P. lebourae</i>
Length	100-120 µm	20-29 µm ^a 27-39 µm ^b	46-76 µm	32-62 µm	64 µm	65-120 µm	80-180 µm		
Width	60-75 µm	20.2- 30.6 µm ^a 25-37 µm ^b	26-50 µm	39-53 µm	43µm	35-60 µm	40-75 µm		
# of zooids or cells in vegetative state	4	2 in chain	2	2	2	2, 4 or 8	8 or 16	8	8
# of nuclei	4	1 per cell	2	2	2	2 or 4	4 or 8		
Stepped joint	No	N/A	Yes	No	Yes	No	No	No	No
Plastids	Yes	Yes	Yes	Yes	No	No	No	Yes	No
ASC shape	?	Open comma	Open comma	Closed loop	Closed loop	Open comma	Closed loop	Closed loop	Closed loop
Nematocysts	?	Yes	Yes, putatively	Yes	Yes	Yes	Yes	Yes	Yes
Cyst shape	?	Oval, with papules	?	Spherical, with erect spines	?				
Habitat		Planktonic	Planktoni	Planktonic	Planktonic	Planktoni		Benthic	Benthic

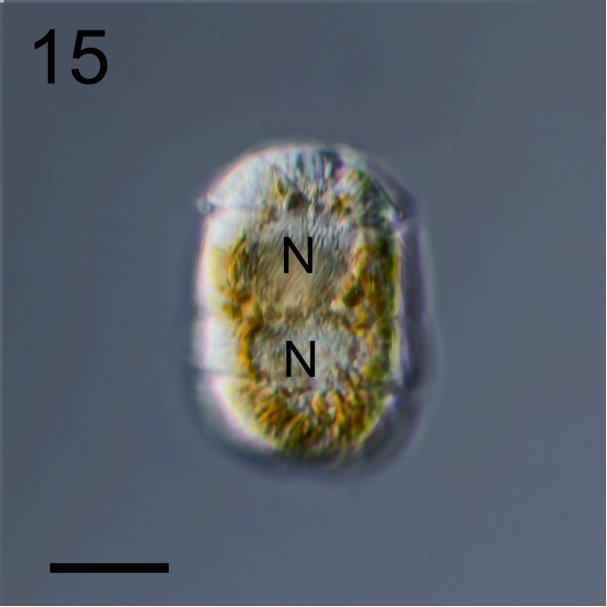
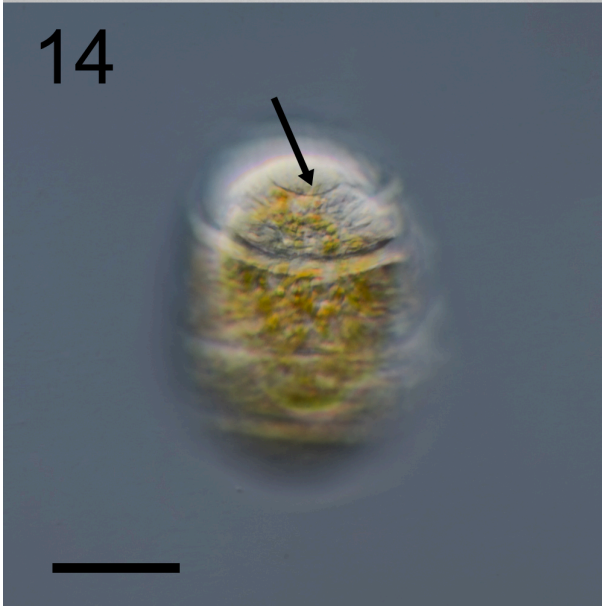
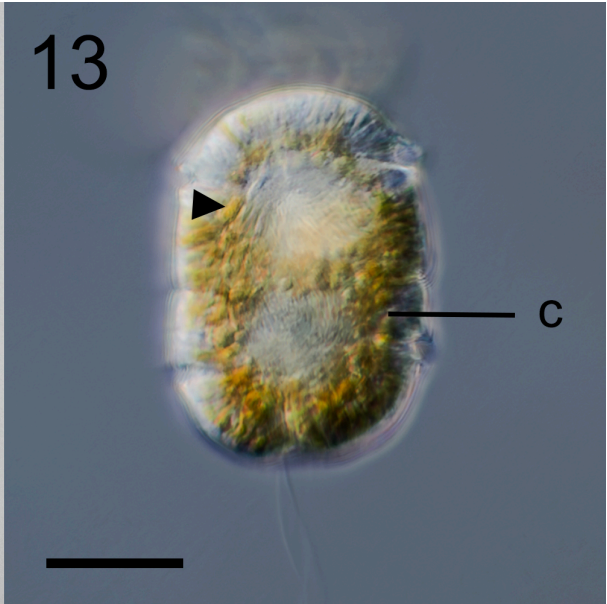
			c			c			
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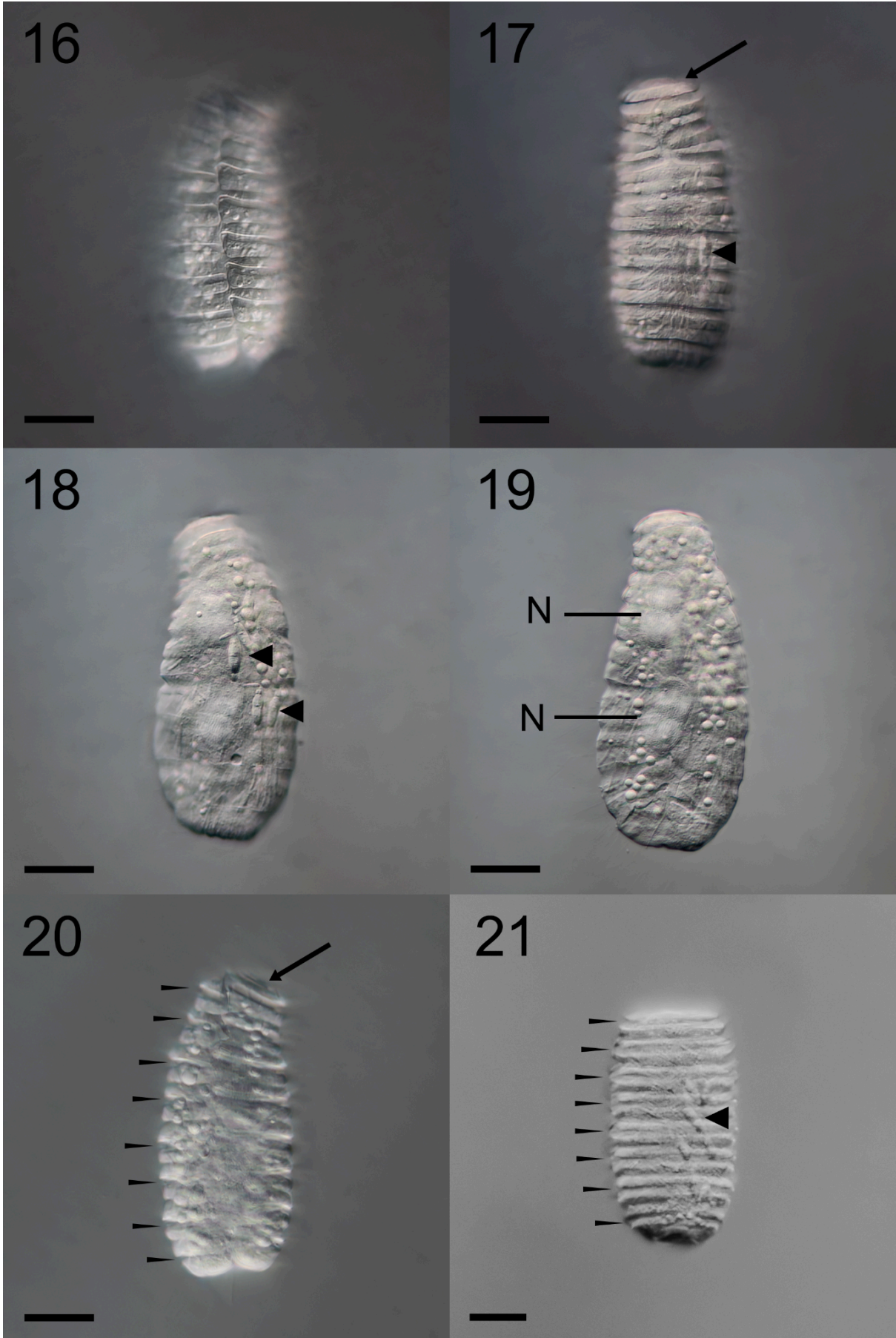
a. Chatton 1933
 b. Qiu *et al.* 2013
 c. Hu *et al.* 2021
 d. Reñé *et al.* 2014
 e. Tang *et al.* 2013
 f. Ruíz-García *et al.* 2013
 g. Nagai *et al.* 2002
 h. Kofoid & Swezy 1921

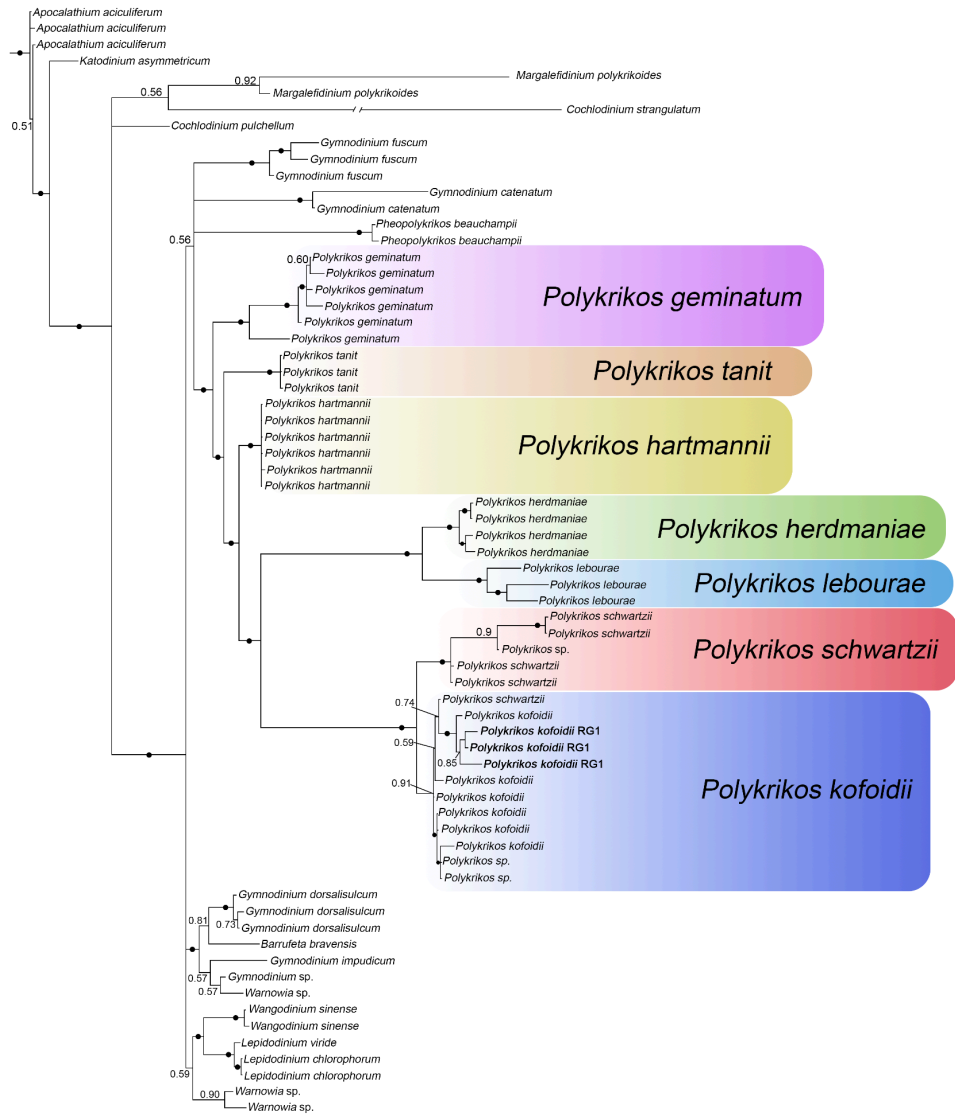




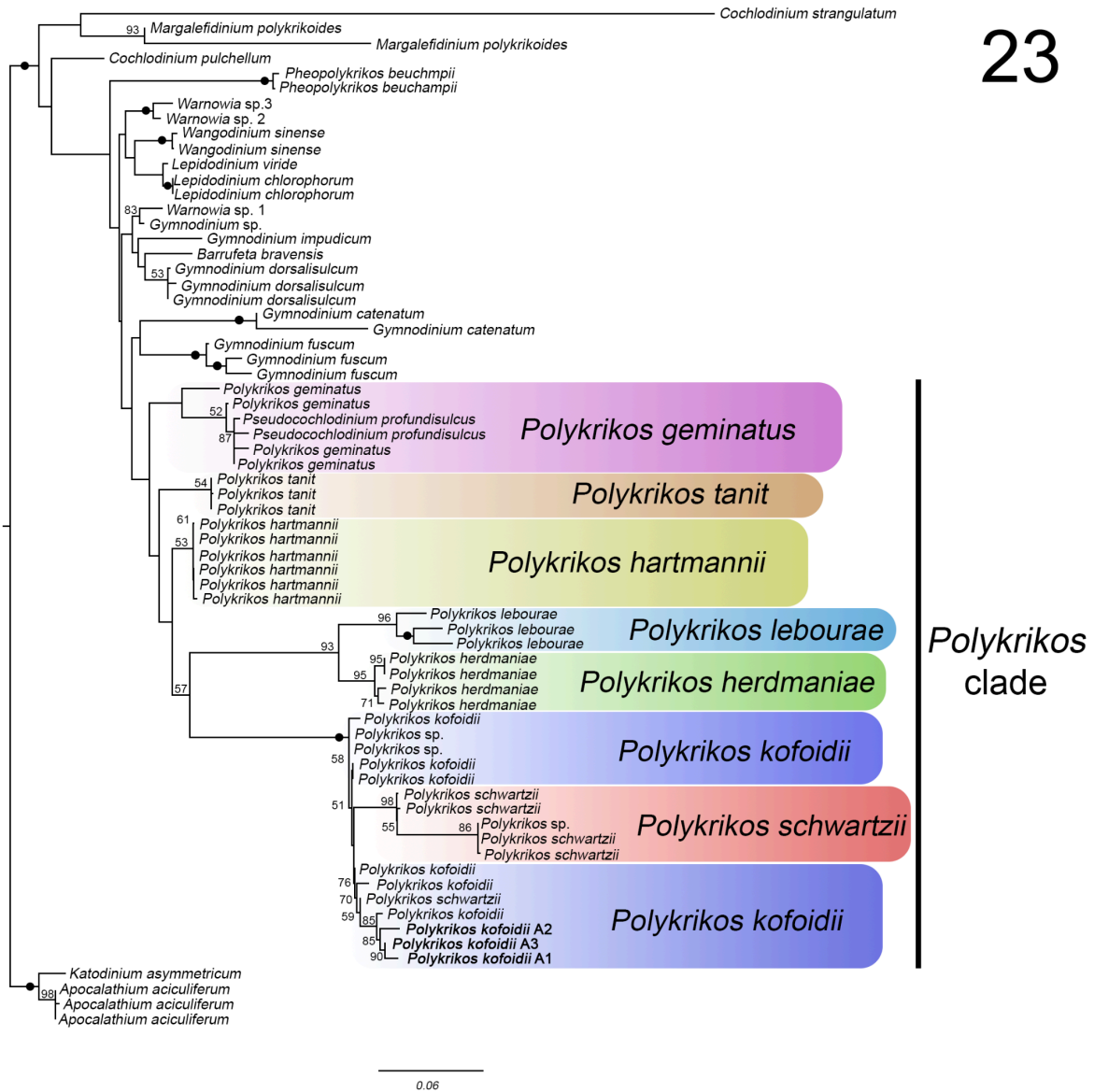


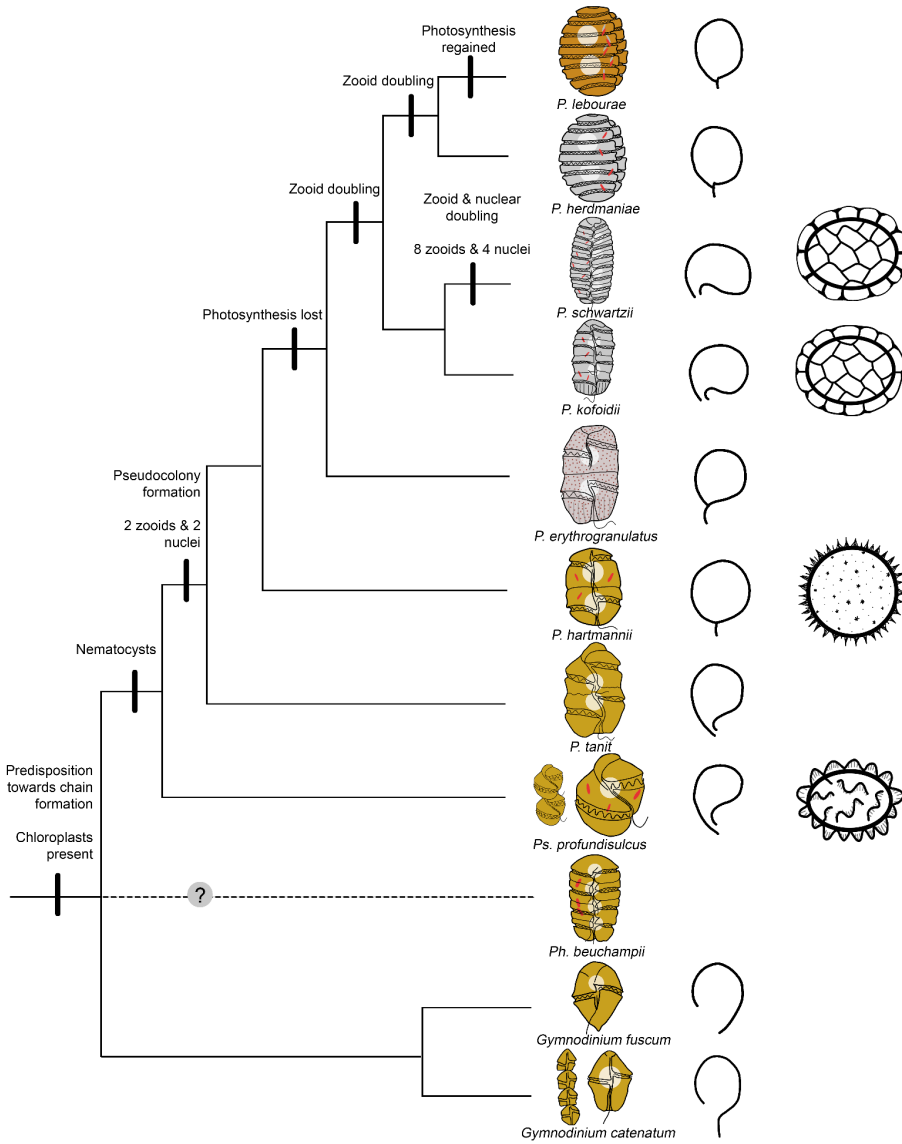






Polykrikos
clade





Polykrikos

Polykrikaceae

12. Capítulo 4

***Dodgeum nauticum* (Dinophyceae) gen. et sp. nov.: morphology and phylogeny of a group with a basal position among core dinoflagellates.**

Estatus: Aceptado en la revista Botanica Marina

Resumen

Históricamente, los dinoflagelados se han dividido en atecados y tecados, con ausencia o presencia de celulosa en los alvéolos corticales, respectivamente. La reconstrucción filogenética en los clados profundos ha sido compleja debido tanto a la falta de representación de especies en el GenBank con las cuales comparar, como al poder computacional y la falta de resolución de los marcadores moleculares actuales. Los dinoflagelados atecados han sido menos estudiados respecto de las especies tecadas, dada su fragilidad estructural y la complicación de preservarlos para su traslado a laboratorio. En la bahía de Acapulco, Guerrero se colectaron muestras en cuatro sitios en febrero de 2024 y se estableció un policultivo de dinoflagelados atecados. Se describe a *Dodgeum nauticum*, un dinoflagelado atecado, incoloro, de 9-11 μm de largo y 7-9 μm de ancho; redondeado en corte transversal, reniforme en vista lateral; con un cingulum descendente, desplazado aproximadamente la mitad de la longitud total de la célula; sulcus recto, corto; surco apical ascendente en la parte central, pasando por la porción izquierda del ápice y descendiendo de manera contraria a las manecillas del reloj en la parte dorsal. La comparación morfológica y molecular permitió determinar que los individuos cultivados no presentan correspondencia con otro grupo conocido de dinoflagelados. La filogenia molecular revela una relación lejana con clados basales o derivados de los dinoflagelados. *Dodgeum nauticum* constituye un clado taxonómicamente independiente dentro de Dinophyceae, con posición incierta desde la categoría de orden.

Research Article

Uriel T. Ruíz-García*, María Luisa Núñez Resendiz, Yuri B. Okolodkov and
María Eugenia Zamudio Resendiz

Dodgeum nauticum (Dinophyceae) gen. et sp. nov.: morphology and molecular phylogeny of a group with a basal position among dinoflagellates

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Keywords: dinoflagellate; LSU; Mexican Pacific; new diversity; SSU

Abstract: Classification of athecate dinoflagellates has historically relied on phenotypic characteristics, yet molecular phylogenetics has revealed the group to be polyphyletic, highlighting the lack of information on species richness and genetic sequences. The apical groove has been found to be an adequate character to draw group boundaries. Here, we describe *Dodgeum nauticum* gen. et sp. nov., a novel heterotrophic athecate dinoflagellate isolated from Acapulco Bay, Mexican Pacific. Morphological analysis revealed a small (9–11 µm long and 7–9 µm wide), chloroplast-lacking species with an excavated cingulum that descends three girdle widths and a distinctive apical groove that spirals counterclockwise along the dorsal cell surface. Morphological observation revealed our specimens do not display any similarities to known groups of dinoflagellates. Molecular phylogenetic analyses of combined SSU and LSU rDNA sequences position *Dodgeum* as a group without clear affiliations within the Dinophyceae. The lack of identifiable features linking *Dodgeum* to existing groups, together with its genetic distinctiveness, supports its status as an independent genus with uncertain taxonomic position at the class level. This study highlights the gaps in our knowledge of dinoflagellate systematics, and underscores the importance of integrating morphological and molecular approaches in revealing hidden dinoflagellate diversity and solving phylogenetic problems.

1 Introduction

Dinoflagellates have been historically divided into armoured or thecate and unarmoured or athecate dinoflagellates based on the presence of cellulose inside the cortical alveoli (Kwok et al. 2023; Steidinger and Tangen 1996). Athecate dinoflagellates had been classified based on morphological characteristics, such as the number of cingulum turns around the cell, chloroplast type, nucleus position and surface details (Hoppenrath 2017; Kofoid and Swezy 1921).

The use of genetic markers has revealed that classification based on the presence or absence of cellulose is non-natural because athecate dinoflagellates are a paraphyletic group in comparison to thecate forms (Janouškovec et al. 2017). Consequently, the most common genera of athecate dinoflagellates, *Amphidinium* Claparède et Lachmann, *Cochlodinium* F.Schütt, *Gymnodinium* F.Stein and *Gyrodinium* Kofoid et Swezy, were found to be polyphyletic, and family, genus and species complexes were formed to reflect the need for re-evaluating these groups to clarify their boundaries (Borchhardt et al. 2021; Daugbjerg et al. 2000; Jørgensen et al. 2004; Sparmann et al. 2008).

Since the advent of molecular phylogeny techniques, the group has undergone major revisions. Many species classified inside traditional genera (Daugbjerg et al. 2000) have been transferred to others, and new species of taxonomic uncertainty have been described (Borchhardt et al. 2021; Hoppenrath 2017; Mun-Lum et al. 2019; Watanabe et al. 2014). Such is the case of *Moestrupia oblonga* (J.Larsen et D.J.Patterson) Gert Hansen et Daugbjerg, previously classified as *Gyrodinium oblongum* J.Larsen et D.J.Patterson, based solely on the cingular displacement; *Akashiwo sanguinea* (K.Hirasaka) Gert Hansen et Moestrup (syn. *Gymnodinium sanguineum* K.Hirasaka, now in an uncertain taxonomic position; *Lepidodinium* Watanabe, Suda, I.Inouye, Sawaguchi et Chihara from a clade

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of the gymnodinial dinoflagellates with chlorophyte derived chloroplasts; and many others.

Most groups of athecate dinoflagellates possess an apical structure complex (ASC), except for *Amphidinium*, *Togula* Sh.Murray et Daugbjerg, *Psammosa* N.Okamoto, A.Horák et P.J.Keeling and other small groups that do not resolve as a monophyletic clade, which may point to convergent evolution or an ancestral trait (Janouškovec et al. 2017). The combined use of genetic markers and ultrastructural studies have established the apical groove structure and shape as a stable morphological character to draw generic and family level boundaries (Daugbjerg et al. 2000; Hoppenrath 2017). For example, members of the family Ceratoperidiniaceae have a closed circular loop ASC (Reñé et al. 2013), *Ankistrodinium* Hoppenrath, Sh.Murray, Sparmann et Leander has a hook-shaped ASC (Hoppenrath 2017), while *Gyrodinium* has a bisected oval ASC (Hansen and Daugbjerg 2004).

The use of molecular phylogeny tools has aided in the reconstruction of dinoflagellate evolution; however, the phylogenetic reconstruction using ribosomal genes (SSU, LSU, ITS) is far from complete (Hackett et al. 2004). The phylogenetic relationships in the most basal clades or suprageneric level of dinoflagellates has not been fully clarified (Daugbjerg et al. 2000; Okamoto et al. 2012; Saunders et al. 1997; Yamada et al. 2013), leaving many taxa as groups of taxonomic uncertainty (Guiry and Guiry 2025). This could be potentially solved by adding more information to the reconstruction, such as the phylotranscriptomic approach by Janouškovec et al. (2017) and combining morphological and molecular data (Daugbjerg et al. 2000; Hoppenrath 2017).

Given the gaps in dinoflagellate taxonomy and phylogeny knowledge, a need for new information regarding this group is clear. Even if many species have been thoroughly studied, more emphasis has been put on autotrophic thecate species, especially if they are potentially toxic or harmful non-toxic. This is due to the ease in collecting specimens, greater success rate in establishing cultures, and cells retaining their shape after fixation following standard procedures (Murray et al. 2004; Onuma 2015). This is not the case for heterotrophic athecate species, whose fragile structure does not always allow them to be sampled with a plankton net, transferring from the field to the laboratory or applying common fixation protocols (Menden-Deuer et al. 2001), while heterotrophic or mixotrophic species do not grow easily in culture (García-Portela et al. 2023).

Until 2012, in Acapulco Bay, 347 dinoflagellates species had been reported (Meave-del Castillo et al. 2012). This high species richness may be undercalculated, due to the lack of morphological confirmation to back up the records.

Escarcega-Bata et al. (2023) described a total species richness of 103 athecate dinoflagellate species in the Mexican Pacific backed up by photographic evidence. There is an overall lack of genetic sequences of dinoflagellates from Mexican waters, with an even bigger lack for phylopecies as described by Nuñez-Resendiz et al. (2024).

Given the above, in this study we describe *Dodgeum nauticum* gen. et sp. nov. from Acapulco Bay, Mexican Pacific, a dinoflagellate new to science, that does not belong to any previously known group of dinoflagellates. Morphology and molecular phylogeny suggest it represents a basal clade within the Dinophyceae.

2 Materials and methods

Four sites in Acapulco Bay, tropical Mexican Pacific (Figure 1) were selected for sampling during February 2024 and 2025. At each site, water was collected using a Van Dorn bottle at 1, 3 and 10 m depths. Salinity and temperature were recorded with an Atago hand refractometer and a mercury thermometer, respectively, at each depth. Seawater was later concentrated using inverse-flow filtration equipment with a Merck 4- μ m pore size membrane (Millipore, Merck KGaA, Darmstadt, Germany).

An aliquot of the previously concentrated seawater was transferred to Corning 15-ml tubes filled with clean filtered seawater. Cultures were kept in a chamber with a 13-W bulb ($18 \mu\text{mol m}^{-2} \text{s}^{-1}$) and a 12:12 h photoperiod.

Live cells were observed with a Reichert Microstar IV compound microscope (Wetzlar, Germany) equipped with 10 $\times$ /0.25, 40 $\times$ /0.65, 100 $\times$ /1.25 achromatic objectives, differential interference contrast, and a coupled 24-MP Nikon Z6 (Tokyo, Japan) digital camera.

Epifluorescence examination was done by adding a small drop of 1 % Calcofluor White M2R to an aliquot and observing directly in a Leica DMLB microscope equipped with 60 $\times$ /0.75 and 100 $\times$ /1.25 fluorite and achromatic objectives, and a DAPI filter cube.

Individual cells were transferred to clean 0.2-ml PCR tubes and kept stored at -20°C . PCR was carried out using the Promega GoTaq[®] G2 colourless master mix (Madison, WI, USA) and the eukaryote primers SR4-F, SR7-R for the partial SSU rDNA (D4–D7 domains) and D1R-F, 28-1483-R (Daugbjerg et al. 2000; Scholin et al. 1994) for the partial LSU rDNA (D1–D3 domains). Amplification was performed in a T100 Thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). For the SSU partial sequence, the conditions were as follows: denaturation at 95°C for 3 min, 32 cycles at 95°C for 1 min, 56°C for 1 min and 72°C for 4 min, with a final extension at 72°C for 10 min. For the LSU partial sequence,

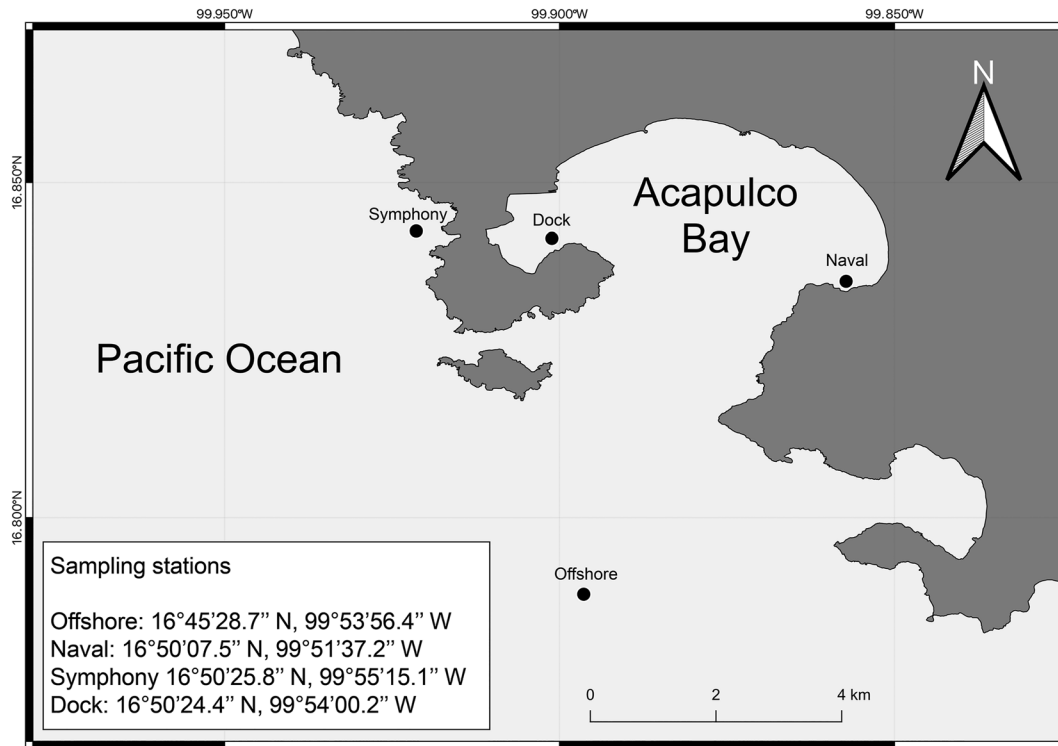


Figure 1: Sampling stations in Acapulco Bay and adjacent waters, Mexican Pacific.



the conditions were denaturation at 98 °C for 3 min, 35 cycles at 95 °C for 30 s, 52 °C for 30 s and 72 °C for 1 min, with a final extension at 72 °C for 10 min. Amplification was confirmed through electrophoresis in 1.5 % agarose gel stained with Biotium GelRed® (San Francisco, CA, USA) for 40 min at 90 V and visualised in a UV photodocumentation system.

Samples were sent to the Laboratorio de Secuenciación Genómica, Pabellón de la Biodiversidad, Instituto de Biología, Universidad Nacional Autónoma de México for commercial sequencing, and later uploaded to GenBank under the accession numbers PX111704–PX111705 and PX120405–PX120407.

Consensus sequences were generated with Sequencher® (GeneCodes Corporation, Ann Arbor, MI, USA). A BLAST analysis (Altschul et al. 1990) was performed for a preliminary taxonomic identification against NCBI's "core nucleotide database" (core_nt). Given the low identity percentages, sequences from various dinoflagellate taxa were downloaded from GenBank (Supplementary Table S1) and aligned using the MAFFT sequence alignment server, with the G-ins-i strategy, generating three different data matrices, one for the SSU gene, one for the LSU gene, and a combined data set: SSU + LSU. We included SSU gene sequences of representatives from all classes of the phylum Dinoflagellata; however, there are few LSU gene sequences of

members of other classes, therefore, the matrix was significantly smaller. For all data matrices, the ciliate *Paramecium bursaria* Ehrenberg (Ciliophora: Parameciidae), and the parasitic apicomplexan *Toxoplasma gondii* (Nicolle et Manceaux) Nicolle et Manceaux (Apicomplexa: Sarcocystidae) were used as outgroups.

Bayesian Inference (BI) analyses were performed in Mr. Bayes v3.2 (Ronquist et al. 2012) using GTR + I + G (General Time Reversible + Invariable sites + Gamma distribution) as evolutionary model, with partitioned data to allow for different evolutionary rates to apply to each gene and 8 gamma categories. The data set was analysed using three runs, for 6×10^6 generations, with six Monte Carlo Markov chains, a temperature setting of 0.20, sampling every 500 generations and a burn-in step of 30 %.

Maximum likelihood (ML) analyses were performed in the Phylogeny.fr platform using the PhyML program, with a minimum of SH-like and Chi2-based approximate likelihood-ratio test (aLRT), a GTR + I + G model of evolution, and eight substitution rate categories.

The SSU and LSU gene data sets were imported into Mega 11; the taxa were arranged into class level groups. An uncorrected mean *p*-distance analysis between the groups, and a mean within group distance analysis were performed using 1,000 bootstrap replicates in Mega 11 (Tamura et al. 2021).

3 Results

Water from the Naval site was 25 °C, with a salinity of 30. From this site, one culture was successfully established, and maintained in filtered seawater without any additional culture medium. Attempts to establish monoclonal cultures have been unsuccessful. The polycultures contain a number of different species, including colourless filamentous bacteria and autotrophic dinoflagellates, such as *Amphidinium*.

3.1 *Dodgeum* U.Ruíz-García, M.Núñez-Resendiz et Okolodkov gen. nov. (Figure 2)

Description: Athecate dinoflagellate without chloroplasts; epicone round in cross-section, slightly broader than the hypocone which is kidney shaped in cross-section; cingulum deeply excavated that descends three girdle widths; apical groove runs straight from the upper circular border to the left side of the apex and descends counterclockwise in the dorsal part of the cell.

Etymology: This genus is named after Prof. John D. Dodge on the occasion of his 90th birthday (born 6 August 1935).

Type species: *Dodgeum nauticum* U.Ruíz-García, M.Núñez-Resendiz et Okolodkov sp. nov. (Figure 2).

3.2 *Dodgeum nauticum* U. Ruíz-García, M.Núñez-Resendiz et Okolodkov sp. nov. (Figure 3)

Description: Athecate dinoflagellate without chloroplasts; 9–11 µm long and 7–9 µm wide. Epicone round in cross-section, slightly broader than the hypocone, which is kidney-

shaped in cross-section. Cingulum deeply excavated, descending three girdle widths; its diameter about one fifth of the total cell length. The apical groove runs straight from the upper circular border to the left side of the apex and descends counterclockwise in the dorsal part of the cell to about half the cell length.

Type locality: Naval station (16°50'07.5" N, 99°51'37.2" W), Acapulco Bay, Mexico.

Holotype: A tube with fixed cells has been deposited at the Herbarium of the Universidad Autónoma Metropolitana–Iztapalapa (UAMIZ).

Isotype: An aliquot of the culture is deposited at the FitoProt Herbarium (PHYCOP).

Etymology: The specific epithet (*nauticum* = of ships, of sailors) refers to the Naval station where it was found.

Observations: Cells very small, 9–11 µm long, 7–9 µm wide, kidney-shaped in lateral view and oval in ventral view. Cingulum wide, significantly displaced, about one half of the total cell length. Sulcus short, running straight down. Both flagella are very thick in comparison to the cell. Left side of the cell beneath the start of the cingulum protruding more than the left side beneath the end of the cingulum. The apical groove extends from the cingulum to a side of the apex, forming a distinct notch on the left side and descending in a spiral fashion approximately one third of the cell length. Observed cells were colourless, without evident chloroplasts, probably phagotrophic or osmotrophic given the

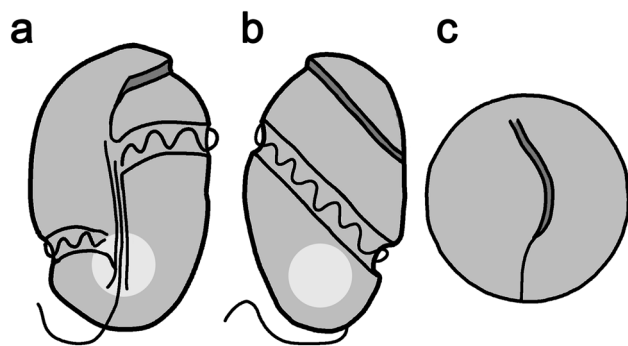


Figure 2: Line drawings of *Dodgeum nauticum*. (a) Cell in ventral view. (b) Cell in left side view. (c) Cell in apical view.

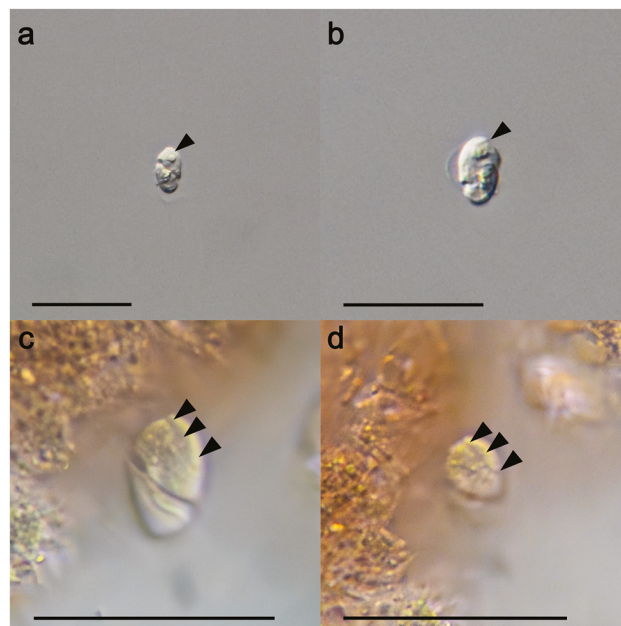


Figure 3: *Dodgeum nauticum* under the light microscope. (a) Cell in lateral view. (b) Cell in ventral view. (c) Cell in lateral view, focus on the surface. (d) Cell in lateral-apical view, focus on the apex. Arrowheads show the apical groove. Scale bars = 20 µm.

small bacteria in the culture. Several accumulation bodies or food vacuoles are visible, distributed throughout the cytoplasm, as well as refractive granules visible when light polarisation techniques are applied.

Habitat: Marine, planktonic.

3.3 Molecular analysis

The SSU + LSU combined data set had a total length of 2,439 bp. The SSU gene data set had an extension of 1,681 bp, and the LSU gene data set was 751 bp long. Both analyses, ML

and Bayesian Inference have similar topologies, so only the Bayesian tree with ML bootstrap values is shown.

In the combined (SSU + LSU) analysis (Figure 4), the class Perkinsea had robust support (BI = 0.90, ML = 98 %), however, it did not include *Psammosa*. The classes Syndiniophyceae and Noctilucopephyceae were rendered in multiple groups. Our sequences of *Dodgeum* were grouped in the clade of the Dinophyceae. The relationships within the class could not be resolved. The classes Oxyrrhinophyceae and the Ellobiophyceae displayed long branch attraction (LBA) in both analyses, therefore, their branch lengths were reduced by 50 %.

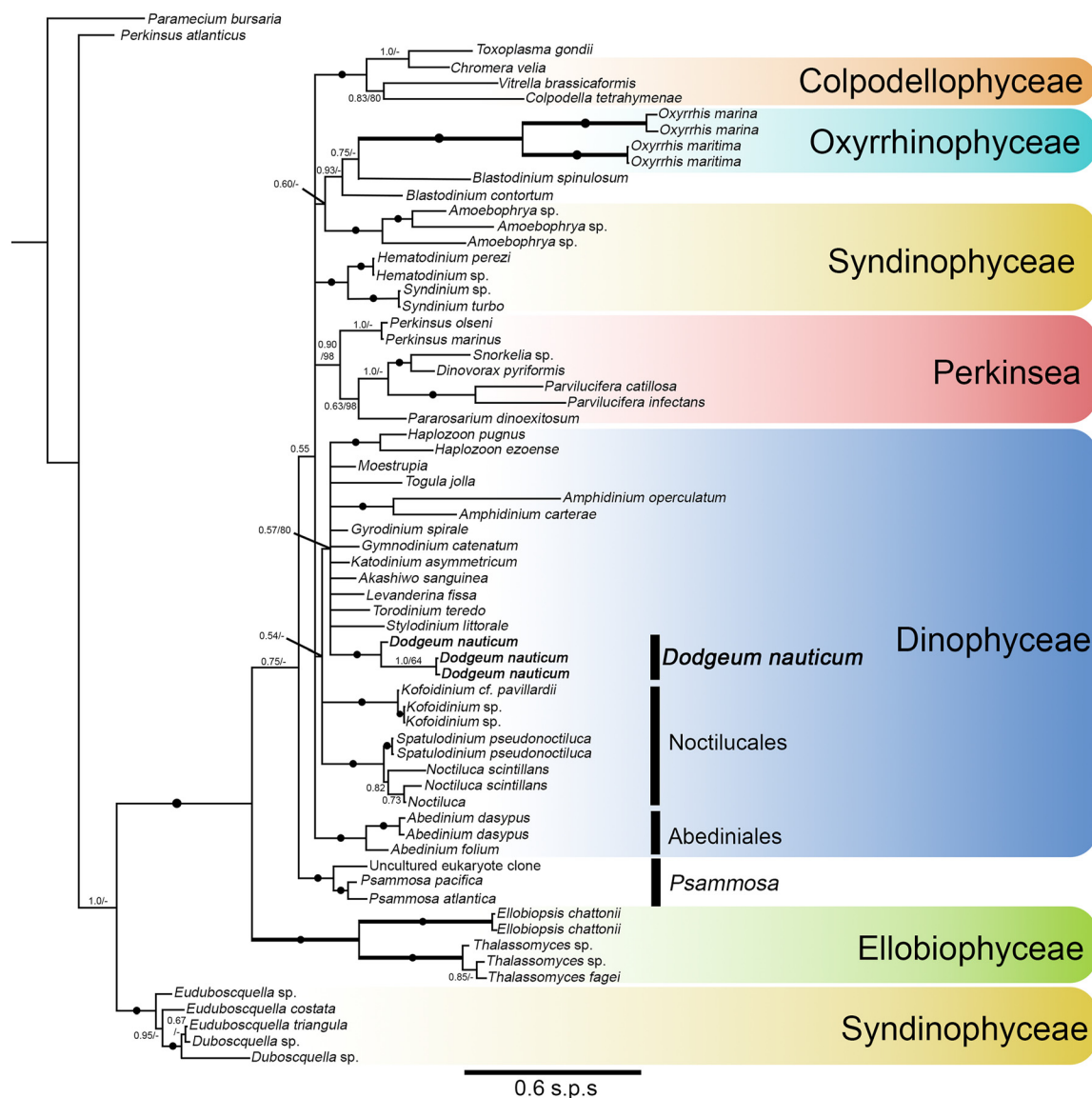


Figure 4: Bayesian inference (BI) hypothesis obtained from SSU + LSU rDNA concatenated sequences of representatives from the classes within the phylum Dinoflagellata. Dots indicate full phylogenetic support in both analyses (BI > 0.98, ML > 98 %). Only values for BI > 0.70, and ML > 70 % are displayed.

In the SSU analysis (Supplementary Figure S1), our sequences of *Dodgeum* are clustered within the Perkinsea (BI = 0.60, $M < 70\%$). The class is monophyletic, but with low support (BI = 0.62, $ML < 70\%$). Longer runs and higher burn-ins did not increase the support values. The classes Oxyrrhinophyceae and Ellobiophyceae displayed LBA. Members of the classes Noctilucopephyceae and Syndiniophyceae appeared in two distinct groups.

In the LSU analysis (Supplementary Figure S2), a clade containing the Syndiniophyceae, Perkinsea and Dinophyceae had moderate support (BI = 0.87, $ML = 82\%$). The Oxyrrhinophyceae and *Blastodinium* Chatton were grouped together, albeit with LBA. The two sequences of the Syndiniophyceae had maximal support, the Perkinsea had low support (BI = 0.65, $ML < 70\%$), meanwhile the Dinophyceae had maximal support. Our sequences of *Dodgeum* were grouped inside the Dinophyceae, in a subclade containing both *Dodgeum* and *Amphidinium* (BI = 0.96, $ML = 93\%$). The rest of the members of the Dinophyceae were grouped in a separate subclade (BI = 0.99, $ML = 87\%$).

Genetic distances using the SSU gene (Table 1) showed *Dodgeum* displays the highest difference with the class Ellobiophyceae (34.81 %) and the lowest with the Dinophyceae (14.83 %). This distance is higher than the in-group variation within the Dinophyceae (11.16 %; Table 2). Genetic distances using partial LSU sequences spanning the D1-D3 regions (Table 3) showed *Dodgeum* displayed the lowest distance to the Perkinsea (36.51 %) and Dinophyceae (42.05 %). These distances are slightly higher than the in-group variation (Table 4) for the Perkinsea (36.72 %) and Dinophyceae (41.39 %).

4 Discussion

Based on both morphological and molecular evidence, we establish *Dodgeum* as a new genus of unarmoured

heterotrophic dinoflagellates with uncertain taxonomic position at the order level.

Athecate dinoflagellates comprise the classes Ellobiophyceae, Noctilucopephyceae, Oxyrrhinophyceae, Perkinsea and Syndiniophyceae and, within the class Dinophyceae, the orders Akashiwales, Amphidinales, Amphilotales, Gymnodinales, Suessiales, and Tovelliales (Adl et al. 2019; Guiry and Guiry 2025). While Cooney et al. (2020, 2024) have provided a robust phylogeny and treated the orders Noctilucales and Abedinales as members of the Dinophyceae, AlgaeBase has not recognized this change. After an exhaustive search for morphologically similar taxa, no group was found that shares the set of characteristics of *Dodgeum*, such as the shape of the apical groove (Figures 2c, 3c and d), the size and displacement of the cingulum (Figure 3b and c), and a lack of visible striae or chloroplasts (Figure 3a–c). Many parasitic or free-living dinoflagellates, whose life cycles have not been thoroughly studied, may have a similar external morphology (Dodge and Lee 2000); however, the culture was established in February 2024, and the dinoflagellates have been maintained under the same conditions, which suggests the cells photographed are the vegetative cells and not gametes or an alternative life-cycle stage.

Similar looking taxa include some species of *Gyrodinium*, *Gymnodinium*, *Moestrupia* and other genera. Our specimens, however, lack the typical apical groove structure and a striated surface characteristic of *Gyrodinium*. *Gymnodinium* and allied genera present a lasso or loop-shaped apical groove and are generally larger. Dodge and Lee (2000) present a dinoflagellate with a similar morphology in the ventral area, identified as *Crepidodinium* J.Lom et Lawler; however, it is a genus of mixotrophic, ectocommensal dinoflagellates up to 100 μm long, while *Dodgeum* is strictly heterotrophic and never exceeds 11 μm in length (Figure 3).

Species previously classified within these genera were also examined, yet they did not present any morphological similarities. *Moestrupia oblonga*, a species previously

Table 1: p -distance values using partial SSU sequences between the different groups of the superclass Dinoflagellata and *Dodgeum*.

Class	Core Dinophyceae (%)	Ellobiophyceae (%)	Noctilucales (%)	Oxyrrhinophyceae (%)	Perkinsea (%)	Syndiniophyceae (%)	<i>Dodgeum</i>
Core Dinophyceae	–						
Ellobiophyceae	25.15	–					
Noctilucales	12.55	24.33	–				
Oxyrrhinophyceae	29.71	33.15	29.38	–			
Perkinsea	15.09	24.51	14.97	30.60	–		
Syndiniophyceae	15.64	25.28	15.21	30.55	16.64	–	
<i>Dodgeum</i>	14.83	34.81	16.24	34.33	22.36	21.63	–

Table 2: Within-group mean distances using partial SSU sequences from the different groups of the Dinoflagellata and *Dodgeum*.

Group	Mean distance (%)
Core Dinophyceae	11.16
Ellobiophyceae	14.38
Noctilucales	9.40
Oxyrrhinophyceae	15.20
Perkinsea	13.48
Syndiniophyceae	14.96
<i>Dodgeum</i>	0.56

Table 4: Within-group mean distances using partial LSU sequences from the different classes of the Dinoflagellata and *Dodgeum*.

Group	Mean distance (%)
Core Dinophyceae	41.39
Ellobiophyceae	–
Noctilucopephyceae	45.21
Oxyrrhinophyceae	44.71
Perkinsea	36.72
Syndiniophyceae	49.76
<i>Dodgeum</i>	16.94

classified inside *Gyrodinium*, is probably one of the earliest lineages of free-living dinophyceans, a relationship found since its re-description by Hansen and Daugbjerg (2011). It possesses a displaced cingulum, typical chloroplasts, and an apical groove running to the left side of the apex and going half a circle around it (Hansen and Daugbjerg 2011; Prabowo et al. 2015). This apical groove shape resembles that of *Dodgeum*; however, the apical groove of our specimens is long, descending in the dorsal part of the cell (Figures 2c and 3c). *Dodgeum* is smaller and colorless. It lacks the ventral flange and pyrenoid (Figures 2a, 3a and b).

Attempts to prepare cells for scanning electron microscope observation were unsuccessful due to the delicate nature of the cell wall. The fixation step with glutaraldehyde, osmium tetroxide, or a combination of the two, often made the cells burst. The use of Calcofluor White M2R and epifluorescence microscopy revealed an absence of cellulose in the cell wall. Therefore, we consider our cells to be an athecate dinoflagellate.

The concatenated sequences of *Dodgeum* clustered together with maximum support in all analyses (Figure 4, Supplementary Figures S1 and S2). Our SSU + LSU concatenated phylogenetic hypothesis (Figure 4) shows the clade corresponding to *Dodgeum* to be inserted within the Dinophyceae, in a clade excluding the Noctilucales and

Abediniales, but the relationship to core members could not be established. This may be due to the short SSU sequences from *Dodgeum*. The distinctiveness of the Noctilucales and the Abediniales, both nested within the Dinophyceae was confirmed, although the monophyly of Noctiluca, Spatulodinium and Kofoidinium could not be resolved.

The SSU analysis (Supplementary Figure S1) is fairly well-resolved, and groups *Dodgeum* with the Perkinsea, but the support is very low (BI = 0.60, ML < 70), therefore, we consider this relationship poorly supported. The short sequences may not provide enough informative sites for a good comparison. The close relationship of the Syndinians and Dinophyceae compared to other groups of basal dinokaryotes (Cooney et al. 2022; Hoppenrath and Leander 2010) was also observed. The LSU analysis (Supplementary Figure S2) shows high support values for the class level grouping, as well as for the relationship between *Dodgeum* and *Amphidinium*, but with a moderate LBA and a paraphyletic assemblage for *Dodgeum*.

The genetic distance between groups using the SSU partial sequences (Table 1) shows *Dodgeum* displayed lower differences with members of the core Dinophyceae and Noctilucales compared with other groups. The results using the LSU gene (Table 3) show *Dodgeum* displays lower distance values with the Perkinsea (36.51 %), core Dinophyceae

Table 3: *p*-distance values using partial LSU sequences between the different classes of the superclass Dinoflagellata and *Dodgeum*.

Class	Dinophyceae (%)	Ellobiophyceae (%)	Noctilucales (%)	Oxyrrhinophyceae (%)	Perkinsea (%)	Syndiniophyceae (%)	<i>Dodgeum</i>
Core Dinophyceae	–						
Ellobiophyceae	49.06	–					
Noctilucales	41.65	45.78	–				
Oxyrrhinophyceae	60.69	61.99	60.90	–			
Perkinsea	45.22	48.91	42.11	60.13	–		
Syndiniophyceae	51.28	51.19	48.46	61.81	48.42	–	
<i>Dodgeum</i>	42.05	52.50	42.27	59.21	36.51	50.33	–

(42.05 %) and Noctilucales (42.27 %). Our results agree in general with the taxonomical position of Noctiluroid dinoflagellates proposed by Cooney et al. (2020, 2024), we believe *Dodgeum* should be classified as a member of the class Dinophyceae, but remaining a group with an uncertain affiliation at the order level. Revision of the known groups is far from completion, but the description of unknown extant lineages providing genetic sequences will help create a more complete picture of the dinoflagellate tree.

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13. Conclusiones

En el presente estudio, se establecieron exitosamente cultivos de dinoflagelados heterótrofos y mixótrofos. Además, se obtuvieron secuencias parciales del gen SSU para tres especies (*Amphidinium* sp., *Dodgeum nauticum* y *Polykrikos kofoidii*) y secuencias parciales del gen LSU de trece especies (*Amphidinium* sp., *Apicoporus parvidiaboli*, *Cucumeridinium* sp., *D. nauticum*, *Gyrodinium* sp.1, *Gyrodinium* sp.2, *Grammatodinium* sp., *Levanderina fissa*, *Peridiniella danica*, *P. kofoidii*, *Proterothropsis* sp. y *Warnowia* sp.).

En el primer capítulo se creó el complejo *Peridiniella sensu stricto* para especies de dinoflagelados con dos placas intercalares anteriores de igual tamaño, dos placas intercalares posteriores y una placa antapical. Se identificaron morfológica, ultraestructural y molecularmente dos especies en costas mexicanas, una de ellas encontrada en la Bahía de Acapulco. El enfoque integrativo permitió la solución puntual de la problemática taxonómica al interior del género y la propuesta de una clasificación más estable, aunque se abre la puerta a nuevas investigaciones que aporten secuencias de especies sin caracterización filogenética.

En el segundo capítulo se identificaron morfológicamente dos especies del género *Polykrikos* (*P. hartmannii* y *P. kofoidii*) en la Bahía de Acapulco, una de ellas por primera vez, aportando evidencia fotográfica. Se constata que la microscopía óptica, una herramienta tradicional, sigue siendo de gran utilidad para el estudio e identificación de especies. La corrección de los registros históricos en la región cumplen con el objetivo de un sistema de clasificación más preciso.

En el tercer capítulo se confirma morfológicamente la presencia de cuatro especies del género *Polykrikos* y molecularmente de *Polykrikos kofoidii* en la Bahía de Acapulco, se describe una nueva especie y se enmienda la diagnosis de la familia y el género tipo. Además, se propone una probable evolución de caracteres al interior de la familia Polykrikaceae. La integración de

múltiples enfoques permitió reestructurar la familia, lo cual provee una clasificación más estable. La propuesta de una nueva especie aumenta la riqueza de especies en la Bahía de Acapulco y el Pacífico Mexicano.

En el cuarto capítulo se caracterizó morfológica y molecularmente una nueva especie de dinoflagelado atecado heterótrofo con posición taxonómica incierta. Se estableció exitosamente un cultivo, con lo que se cumple con el primer objetivo; además se incrementa la riqueza de especies en la región. Finalmente se aportan datos moleculares de un linaje antiguo de la clase Dinophyceae que tiene el potencial de esclarecer las relaciones al nivel de clados profundos.

Con lo anterior, se confirma la existencia de nuevos registros en la Bahía de Acapulco, incluyendo nuevos registros para el Pacífico Mexicano. Se logró, de manera muy puntual, resolver algunos problemas taxonómicos y filogenéticos dentro del grupo, ampliando y profundizando el conocimiento del grupo en la región y a nivel mundial.

14. Perspectivas

A pesar de la solución de problemáticas específicas al interior del grupo de los dinoflagelados, tanto la caracterización de las especies, como la resolución de su posición taxonómica y filogenética sigue enfrentando numerosos retos:

- La falta de evidencia fotográfica de numerosos taxa descritos previo a la fotomicrografía vuelve subjetiva la interpretación de los iconotipos. Para resolver esto sugerimos incrementar los esfuerzos de muestreo, presentando información fotográfica de las especies encontradas para evitar sesgos en la interpretación.
- La ausencia de secuencias moleculares en el GenBank dificulta la comparación y el muestreo exhaustivo de los distintos grupos de dinoflagelados. Se necesita aportar

mayor número de secuencias genéticas al GenBank y datos proteómicos, transcriptómicos y genómicos; sobre todo de especies sin datos moleculares.

- El uso actual de marcadores moleculares universales para el estudio de este grupo no siempre permite la resolución a niveles taxonómicos inferiores. Es necesario explorar métodos como el establecimiento de marcadores moleculares específicos para el grupo, con mayor capacidad de resolución a los distintos niveles.
- Finalmente, la interpretación de los resultados filogenéticos en la mayoría de los artículos publicados es superflua, por lo que interpretar a mayor profundidad los resultados filogenéticos es de especial importancia para comenzar a resolver problemáticas más amplias al interior del grupo.

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16. Anexos

Anexo 1. Participación en foros académicos nacionales e internacionales, seminarios y cursos como ponente.

8vo simposio RECORECOS, Veracruz, Veracruz:

- Cartel: “Estado del Conocimiento Sobre la Familia Warnowiaceae (Dinoflagellata)”

XXIV Reunión nacional de la Sociedad Mexicana de Planctología A.C. y la XVII International Meeting of the Mexican Society of Planktology, Hermosillo, Sonora.

- Curso impartido: “Nociones básicas de microscopía y fotografía enfocada al estudio del plancton”
- Ponencia oral: “Uso de técnicas de microscopía óptica para la identificación del fitoplancton”

Dinophyte Seminars, Ifremer, Francia:

- Seminario: “Recent progress and emerging tools in athecate dinoflagellate classification and phylogeny”

1ª Reunión Nacional de Protistología y Ficología, C.U., CDMX:

- Ponencia oral: “Confirmación morfológica y molecular de *Apicoporus parvidiaboli* (Dinophyceae: Gymnodiniaceae) en el Pacífico mexicano”

X Congreso Mexicano de Ficología, Mérida, Yucatán:

- Ponencia oral: “*Dodgeum nauticum* gen. et sp. nov. (Dinophyceae), un grupo de dinoflagelados heterótrofos filogenéticamente antiguos”
- Cartel: "Nuevos registros de dinoflagelados atecados (Dinophyceae) para el Pacífico Tropical Mexicano"



Casa abierta al tiempo

UNIVERSIDAD AUTÓNOMA METROPOLITANA

ACTA DE EXAMEN DE GRADO

No. 00247

Matrícula: 2233804747

Taxonomía y filogenia de dinoflagelados mixótrofos y heterótrofos en la Bahía de Acapulco, México.

En la Ciudad de México, se presentaron a las 10:00 horas del día 17 del mes de diciembre del año 2025 en la Unidad Iztapalapa de la Universidad Autónoma Metropolitana, los suscritos miembros del jurado:

DR. YURI B. OKOLODKOV
DRA. ITZEL SIGALA REGALADO
DR. JOSE ANTOLIN AKE CASTILLO
DR. ALEXIS DE JESUS ESCARCEGA BATA

Bajo la Presidencia del primero y con carácter de Secretario el último, se reunieron para proceder al Examen de Grado cuya denominación aparece al margen, para la obtención del grado de:

MAESTRO EN BIOLOGÍA

DE: URIEL TENATIC RUIZ GARCIA

y de acuerdo con el artículo 78 fracción III 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.

REVISÓ

MTRA. ROSALIA SERRANO DE LA PAZ
DIRECTORA DE SISTEMAS ESCOLARES

DIRECTORA DE LA DIVISIÓN DE CBS

DRA. EDITH ARENAS RIOS

PRESIDENTE

DR. YURI B. OKOLODKOV

VOCAL

DRA. ITZEL SIGALA REGALADO

VOCAL

DR. JOSE ANTOLIN AKE CASTILLO

SECRETARIO

DR. ALEXIS DE JESUS ESCARCEGA BATA