Research Article

Ana E. Ramos-Santiago, Christine J. Band-Schmidt*, Ignacio Leyva-Valencia*, Leyberth J. Fernández-Herrera, Erick J. Núñez-Vázquez and Yuri B. Okolodkov

Gambierdiscus carpenteri (Dinophyceae) from Bahía de La Paz, Gulf of California: morphology, genetic affinities, and mouse toxicity

https://doi.org/10.1515/bot-2024-0017
Received March 18, 2024; accepted May 29, 2024;
published online July 8, 2024

Abstract: *Gambierdiscus* is a marine benthic dinoflagellate genus that currently contains 19 species; some of them are toxigenic, producing ciguatoxins, maitotoxins, and other toxic compounds. The objective of this study was to document the morphological and molecular identification (ITS, 5.8S and 28S of the rDNA) of two strains of *Gambierdiscus* from La Gaviota Island, Gulf of California, Mexico, and a toxicity test. The shape of the 2′ plate varied between hatchet-shaped and rectangular, that complicated the differentiation between *G. carpenteri* and *G. toxicus*. Molecular markers of the three rDNA regions allowed confirmation of the taxonomic identity of *G. carpenteri*, separating this species from other congeners with high phylogenetic affinity, such as *G. excentricus*, *G. toxicus* and *G. caribaeus*. Studies of the morphological taxonomy of *G. carpenteri* are scarce; therefore, due to the similarity between species, the combination of morphological and molecular tools is recommended for the identification of species, such as *G. carpenteri*, *G. excentricus*, *G. toxicus* and *G. caribaeus*. The mouse bioassay showed that the examined isolate was toxic, and it is a potential etiology of ciguatera fish poisoning cases in the region. This study provides the first reliable report and ribosomal sequences of *G. carpenteri* for the Gulf of California, as well as data on mouse bioassay toxicity.

Keywords: *Gambierdiscus carpenteri*; Gulf of California; morphological taxonomy; molecular identification; toxicity

***Corresponding authors: Christine J. Band-Schmidt**, Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, Av. Instituto Politécnico Nacional s/n, Col. Playa Palo de Santa Rita, La Paz, Baja California Sur 23079, Mexico, E-mail: cbands@ipn.mx; and **Ignacio Leyva-Valencia**, Consejo Nacional de Humanidades, Ciencias y Tecnologías, Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, Av. Instituto Politécnico Nacional s/n, Col. Playa Palo de Santa Rita, La Paz, Baja California Sur 23079, Mexico, E-mail: ileyvav@ipn.mx. https://orcid.org/0000-0001-9904-1512

Ana E. Ramos-Santiago, Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, Av. Instituto Politécnico Nacional s/n, Col. Playa Palo de Santa Rita, La Paz, Baja California Sur 23079, Mexico, E-mail: aramoss2100@alumno.ipn.mx. https://orcid.org/0009-0000-3652-3565

Leyberth J. Fernández-Herrera and Erick J. Núñez-Vázquez, Centro de Investigaciones Biológicas del Noroeste, Laboratorio de Toxinas Marinas y Aminoácidos, La Paz, Baja California Sur 23096, Mexico, E-mail: leyberthf@gmail.com (L.J. Fernández-Herrera), enunez04@cibnor.mx (E.J. Núñez-Vázquez). https://orcid.org/0000-0001-6174-5303 (L.J. Fernández-Herrera). https://orcid.org/0000-0003-1257-731X (E.J. Núñez-Vázquez)

Yuri B. Okolodkov, Universidad Veracruzana, Mar Mediterráneo 314, Fracc. Costa Verde, Boca del Río, Veracruz 94294, Mexico

1 Introduction

Gambierdiscus R. Adachi *et* Y. Fukuyo 1979 (Dinophyceae: Gonyaulacales) is a *sensu stricto* epibenthic group of thecate dinoflagellates. It is found living on a variety of substrata, such as macroalgae (e.g., *Dictyota* spp., *Padina* sp., *Phyllospora* sp., *Halimeda* spp. and others), seagrasses (e.g., *Thalassia testudinum* Banks *ex* König), sandy substrata, as well as live and dead corals (Kohli et al. 2014; Larsson et al. 2018; Litaker et al. 2009; Murray et al. 2014; Xu et al. 2016).

Currently, *Gambierdiscus* comprises 19 taxonomically accepted species (Guiry and Guiry 2024), with a pantropical distribution (Litaker et al. 2009). *Gambierdiscus carpenteri* Litaker, M.A. Faust, W.C. Holland, Vandersea *et* P.A. Tester has its type locality in South Water Cay, Belize, and it has been hypothesized that it has a wide geographic distribution since, under laboratory conditions, it shows a high tolerance to changes in temperature, salinity, and irradiance (Litaker et al. 2009, 2017; Xu et al. 2016). *Gambierdiscus carpenteri* has been reported in the Mexican Caribbean (Litaker et al. 2009), Florida (Rains and Parsons 2015; Xu et al. 2016), North Carolina, USA, Mariana Islands, Guam Island, Fiji Islands

(Litaker et al. 2009), Australia (Kohli et al. 2014; Larsson et al. 2018; Sparrow et al. 2017), Bolinao, Pangasinan, Philippines (Vacarizas et al. 2018), and in Ly Son Island, Vietnam (Nguyen-Ngoc et al. 2023).

Gambierdiscus species have large cells with a trans-diameter ranging from 31 to 140 µm (Hoppenrath et al. 2014). The cells are asymmetric, anteroposteriorly (lenticular) or laterally compressed (globular) and rounded in apical and antapical view. The cingulum is ascending with a curved end that can be seen in ventral view. Live cells present numerous green to brown chloroplasts (Hoppenrath et al. 2014). Following the Kofoidean system, the plate formula is Po, 3′, 0a, 7″, 6c, 6–8s, 5‴, 1p, 2⁗ (Hoppenrath et al. 2023).

The taxonomic diagnostic characters are the shape and size of the plates: 2′ (hatchet or quadrangular shape), 4″ (symmetrical or asymmetrical) and 2⁗ with a dorsal end terminating in a straight or oblique point (Litaker et al. 2009; Wang et al. 2022). The shape and size of the apical pore plate (Po) varies (Hoppenrath et al. 2014). Identification to species level requires a combination of molecular and morphological techniques, due to high morphological similarity between species (e.g., *G. toxicus* R. Adachi *et* Y. Fukuyo, *G. excentricus* S. Fraga and *G. carpenteri*).

Information about toxicity and toxin profiles in *G. carpenteri* is scarce (Litaker et al. 2009). Strains from Australia have been reported to produce maitotoxin-3 (MTX-3) and related compounds in cells grown at 18 and 27 °C (Kohli et al. 2014; Larsson et al. 2018). Strains from the Caribbean Sea and the Gulf of Mexico contain low concentrations of ciguatoxins (CTXs; 0–1.4 fg eq cell^{-1}) (Litaker et al. 2017). In the mouse bioassay (MBA), Australian strains at a dose of 2.4 mg kg^{-1} caused a decreased respiratory rate and respiratory paralysis (Kohli et al. 2014). This species also caused intermediate hemolytic activity in human erythrocytes (Holland et al. 2013).

2 Materials and methods

2.1 Strains and culture conditions

Two strains (GCARBAPAZ-1, GCARBAPAZ-3) of *Gambierdiscus* were obtained from La Gaviota Island (24°17′24.7″ N, 110°19′52.6″W), Bahía de La Paz, Gulf of California. Strains were isolated in April 2019 by L.J. Fernández-Herrera. Cells were separated by capillary pipettes in drops of modified GSe medium (Blackburn et al. 2001; Bustillos-Guzmán et al. 2015), and a progressive scale-up was performed until reaching 20–25 ml volumes. Monoclonal cultures were maintained for several months in modified K media (de Vera et al. 2018) at a salinity of 34, temperature of 24 °C ± 1 °C, with continuous illumination of 150 µmol m^{-2} s^{-1}, and a 12 h light:12 h dark cycle in flat-bottom 50-ml tubes.

2.2 Light microscopy

Cellular characteristics, such as cell shape, size, and the shape, arrangement, and size of thecal plates, were obtained using a light microscope (LM): an inverted microscope (Axio Vert.A1, Oberkochen, Carl Zeiss, Germany) and an epifluorescence compound microscope (Axio Scope.A.1, Carl Zeiss, Oberkochen, Germany) equipped with an Axiocam 506 color 6-megapixel digital camera. Staining was performed with Calcofluor White M2R 0.2 % (Fritz and Triemer 1985) and Trypan Blue 0.2 % (Taylor 1978). Dissection of the theca was conducted to observe the position and shape of the thecal plates.

2.3 Scanning electron microscopy

To prepare samples for observation in a scanning electron microscope (SEM), protocols of different authors were used to process thecate dinoflagellate cells (Gómez-Lizárraga et al. 2019). Cells were fixed in glutaraldehyde (4 %), and organic matter was removed with hydrogen peroxide stock solution (37 %). Further, samples were left for 5 days in hydrogen peroxide to remove excess mucilage and, once the thecae were clean, they were centrifuged (Thermo Scientific™ Sorvall™ Legend™ XTR) at 800*g* at 22–24 °C for 2 min with cold sterile distilled water. Samples were dehydrated gradually in ethanol (EtOH) of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 %. At each dehydration step, cells were resuspended gently for approximately 1 min and centrifuged at 800*g* at 4 °C for 5 min. Samples were dried with the addition of 200 µl of HMDS (hexamethyldisilazane) and mounted on aluminum sample holders.

Samples were processed at the SEM Academic Service of the Instituto de Ciencias del Mar y Limnología (ICMyL), of the Universidad Nacional Autónoma de México (UNAM). Samples were coated with gold (standard thickness of 20 nm) in an ionizer (Ion Sputter JEOL-JFC-1100, Japan) for 5 min, voltage of 10–20 kV, working distance 20 mm, and examined in a JEOL JMS-6360-LV type SEM, equipped with secondary electron and backscattered electron detectors.

2.4 DNA extraction, amplification, and sequencing

DNA extraction was carried out using the kit Quick-and™ Miniprep plus universal (Zymo Research, Irvine, CA, USA). For polymerase chain reaction (PCR), a mixture of 4 µl Mmix 5X, 14.8 µl of milli-Q H_2O, 2 µl of each primer and 2 µl of DNA was added to each sample. Primers for the amplification of the 5.8S, 28S and ITS regions of rDNA published by Adachi et al. (1996) for the 28S region and Hosoi-Tanabe et al. (2006) for the ITS region. All PCR reactions were performed using an iCycler PCR System (Bio-Rad Laboratories, CA, USA). The amplification program for the 28S (gen D1-D2) and 5.8S regions of the rDNA consisted of an initial denaturation at a 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 52 °C for 1 min and 72 °C for 2 min, with a final extension at 72 °C for 7 min. The amplification program for the ITS region consisted of a denaturation at 94 °C for 230 s, 35 cycles of 94 °C for 50 s, 47 °C for 1 min and a 72 °C for 80 s, and a final extension of 72 °C for 10 min. PCR samples were purified using the Quick-DNA™ Miniprep kit (Zymo Research). The purified PCR products were sent for sequencing to the Molecular Cloning Laboratories (MCLAB, San Francisco, CA, USA).

2.5 Sequence alignment and phylogenetic analyses

The sequences were edited with the program Sequencher v.4.1.4 to obtain a consensus sequence (*Forward* + *Reverse*). BLAST analyses of the consensus sequences were performed with the GenBank databases, and sequences were selected for the reconstruction of the phylogenetic trees. Sequences were aligned with the software MEGA 10.0.5 using the MUSCLE alignment algorithm. For the phylogenetic analysis, the models that best described the nucleotide substitution rates were selected using the software JModel Test v2.1.10. The Mega 10.0.5 software was used for the maximum parsimony (MP) and maximum likelihood (ML) algorithms and to compare the phylogenetic reconstruction Mr. Bayes v. 3.2.7a for the Bayesian inference (BI) algorithm. Tree construction was performed with a bootstrap of 1,000 replicates for ML, and 3×10^6 generations for BI, sampling every 100 generations.

2.6 Toxin extraction

A batch culture of *G. carpenteri* (GCARBAPAZ-3) was established in 2.8-l Fernbach flasks (Pyrex®, England) with 1 l of culture media. During stationary growth phase with a cell density of 48 ± 2 cells ml^{-1}, cells were harvested on glass fiber filters (GF/F) of 0.7 µm pore size and 47 mm diameter (Whatman™, UK). Filters were stored at −20 °C until extraction (Chinain et al. 2010).

For toxin extraction, the GF/F filter was transferred to a 14-ml high clarity round polypropylene bottom sterile test tube (Falcon®), vigorously macerated with a glass rod in absolute MeOH. Volumes of 2 ml with 10×10^3 cells ml^{-1} of the methanolic extract were recovered by centrifugation at 1,200*g* at 10 °C for 10 min (Eppendorf® 5702 R). This step was repeated three times. All crude extracts were collected and filtered with Acrodisc® Syringe Filters (Pall Life Sciences GxF Glass 25 mm diameter HPLC certified 0.45 µm pore size) and dried at 40 °C in a rotavapor (IKA® RV05). A solvent partition was obtained from the resuspended extract with 25 ml of dichloromethane and transferred to a separation funnel of 250 ml and washed twice with 12.5 ml MeOH: H_2O (60:40). The dichloromethane phase recovered the ciguatoxins (CTXs-like) and the MeOH: H_2O phase contained maitotoxins (MTXs-like) (Satake et al. 1993). The phases were stored at −20 °C in glass vials until the MBA was performed.

2.7 Toxicity assay

Toxicity tests were performed using the MBA model for CTX-like and MTX-like activity in *G. carpenteri*; dichloromethane and MeOH: H_2O phases were evaporated and resuspended in 1 % Tween 60 and 0.9 % sterile saline solution. For intraperitoneal (i.p.) toxicity aliquots of 500 µl were injected into male mice with a weight between 18 and 20 g (strain CD-1 Harlan Laboratories, Mexico), in groups of two animals. The control group was injected with Tween solution. Clinical signs were observed for 24 h (FAO 2005, Holmes and Lewis 1994, Satake et al. 1993), and results were reported in mouse units (MU) according to Lewis (1995). One MU is defined as the i.p. LD_{50} dose for a 20-g mouse. This methodology was carried out in accordance with the NOM-062-ZOO-1999 (SAGARPA 1999) and following the recommendations of Hedrich (2012).

The acute toxicity tests in the mouse model were carried out based on the Official Mexican Standard NOM-242-SSA1-2009 (https://www.dof.gob.mx/normasOficiales/4295/salud2a/salud2a.htm) of the Ministry of Health (official method in Mexico for the analysis of ciguatoxins) and in accordance with the Official Mexican Standard NOM-062-ZOO-1999 (https://www.gob.mx/cms/uploads/attachment/file/203498/NOM-062-ZOO-1999_220801.pdf) of the Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food;

Technical Specifications for Production, Care and Use of Laboratory Animals (Bioterium certified by SAGARPA no. B000.02.03.02.01.1155/08).

3 Results

3.1 Morphological observations

Gambierdiscus carpenteri Kibler, Litaker, M.A. Faust, W.C. Holland, Vandersea *et* P.A. Tester 2009

Sampling locality: La Gaviota Island, Bahía de La Paz, Gulf of California, Mexico.

Vegetative cell size: Cells in apical view presented a length of 84.39 ± 6.18 µm and a width (transdiameter) of 87.03 ± 8.81 µm (n = 48). The cell length in anteroposterior view was 55.87 ± 6.82 µm (n = 10). The cell width in anteroposterior view was 85.48 ± 8.49 µm (n = 8).

Description: Solitary cells, with active movement, are attached by a mucilage filament that allows their attachment to the bottom of culture tubes (Figure 1A–C). Anteroposteriorly compressed cells, oval-shaped (lens-shaped = lenticular) in apical and antapical views (Figure 1D–G). Numerous brown-green chloroplasts are present. Cingulum narrow, median, deeply excavated (cavozone), ascending, and displaced twice the cingulum width, with no offset of its ends (Figure 1H–J). Resting oval shape cysts, with a thickened wall, colorless theca, where the protoplasm is concentrated in the center of the cell (Figure 1K). Temporary cysts with one membrane, oval-shaped, dark brown (Figure 1L).

The apical plate 1′ is hexagonal, connected to the apical pore plate (Po) and to the plates 2′, 3′, 1″, 2″, 6″ and 7″ (Figures 2A–D, F–L, 3A and 4F). The apical 2′ is the largest of the epitheca, long, rectangular, connected to plates 1′, 2′, the apical pore plate (Po), and the precingular plates 2″, 3″ and 4″ (Figure 2A–L). The Po is oval, surrounded by pores and fishhook-shaped (Figures 3B and 4C). The Po plate measured 9.51 ± 1.46 µm in length and 6 ± 1.25 µm in width (n = 10). Aberrant cells were observed where plate 2′ appeared to have three divisions; such an arrangement of apical plates is not consistent with what is currently reported for the genus *Gambierdiscus* (Figure 4A). The 2″, 3″ and 4″ precingular plates are elongated (Figures 2A–L, 4A–B and 5A). Plate 4″ is quadrangular and asymmetrical (Figure 2C). Plate 2⁗ is asymmetrical and connected to plates 2‴, 3‴, 4‴, 1⁗ (Figures 3C–I, 4D and 5B–F). Thecal surface has a high density of rather homogeneously distributed medium-sized and smaller pores (Figure 5G–H). The average diameter of the medium-sized pores is 0.41 ± 0.04 µm (n = 14), and that of the smaller pores is 0.10 ± 0.02 µm (n = 10).

Plate formula: Po, 3′, 7″, 6c?, 6–7s?, 5‴, 1p, 2⁗ (Hoppenrath et al. 2014; Litaker et al. 2009).

3.2 Molecular phylogenetic analyses

The phylogenetic trees generated for 5.8S, ITS, and 28S rDNA, with the sequences of GCARBAPAZ-1 (accession numbers: OR389496, OR389494, OR389492) and GCARBAPAZ-3 (accession numbers: OR389497, OR389495, OR389493) were included in the *G. carpenteri* clade. Therefore, both strains were identified as *G. carpenteri*, with bootstrap values of 99–100 with MP and MV, and posterior probability values of 0.98–1 for IB.

The phylogenetic tree sequences obtained from the 5.8S region formed a subclade with bootstrap supports above 98 %, which grouped the two sequences of this study with a sequence of *G. carpenteri* from the Mariana Islands (Figure 6). Phylogenetic reconstruction conducted with sequences of the 28S rDNA region showed a clear separation between *G. carpenteri*, *G. toxicus* and *G. excentricus* (Figure 7), in spite of the fact that these three species share similar morphologies. Sequences of the ITS region for *Gambierdiscus* are scarce, and molecular information does not exist for all currently accepted taxa; however, the sequences analyzed in this study formed a clade of sequences identified as *G. carpenteri* from Guam Island and Vietnam with a *bootstrap* support of 95/90 and posterior probability of 0.87 (Figure 8).

3.3 Toxicity assay

In the mouse bioassay, mice showed typical clinical signs for CTX-like and MTX-like activities. The clinical signs for CTX-like activity included: hypoactivity, hind limb paralysis, piloerection, diarrhea (1/2 mice), lachrymation, dyspnea, ataxia, tremor, wobbly upright gait, jumping, terminal convulsions, with tail arching and death from respiratory failure. In the clinical signs for MTX-like activity, mice showed hypoactivity, piloerection, progressive and severe paralysis from hind extending to fore limbs, gasping and mild convulsions preceding death.

The *Gambierdiscus carpenteri* strain from the Gulf of California showed CTX-like and MTX-like activities. The LD_{50} of extracts with CTX-like was 5.99 mg kg^{-1}. For the MTX-like extracts, the LD_{50} was equivalent to 0.06 mg kg^{-1}. Total extracts from both phases are equivalent to 1,400 cells per MU.

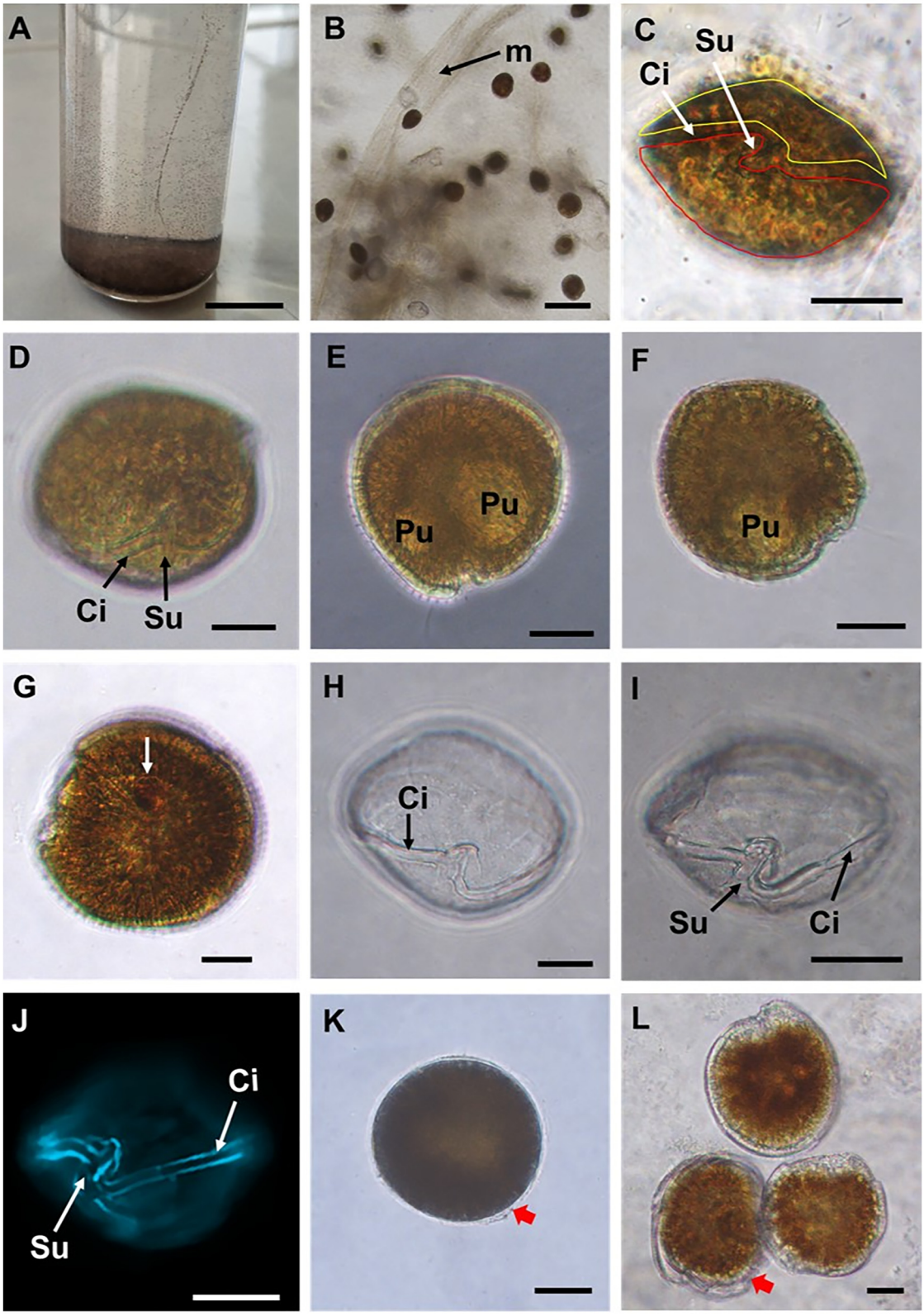

Figure 1: Light micrographs of *Gambierdiscus carpenteri*. Strain GCARBAPAZ-1: (D, F–H, K, L); strain GCARBAPAZ-3: (A–C, E, I, J). (A) Culture in K medium. (B) Mobile cells joined by mucilage (m). (C) Ventral view, epitheca (yellow contour), hypotheca (red contour), cingulum (Ci) and sulcus (Su). (D, H and I) Apical-ventral view (H and I – empty thecae). (E and F) Antapical view; pusules (Pu) are shown. (G) Apical view; white arrow – eyespot. (J) Ventro-lateral view, cingulum displacement shown. (K) Temporal cyst with a thin cell wall. (L) Non-motile cells with a thick cell wall, showing the process of encystment (formation of resting cysts). Red arrows show the cell wall. (B–D, F–I, K and L): in bright field; (E): in phase contrast; (J) in epifluorescence regime. Scale bars: 2.5 cm in (A); 160 µm in (B); 25 µm in (C, E, I and K); 20 µm in (J, D, F–G and L).

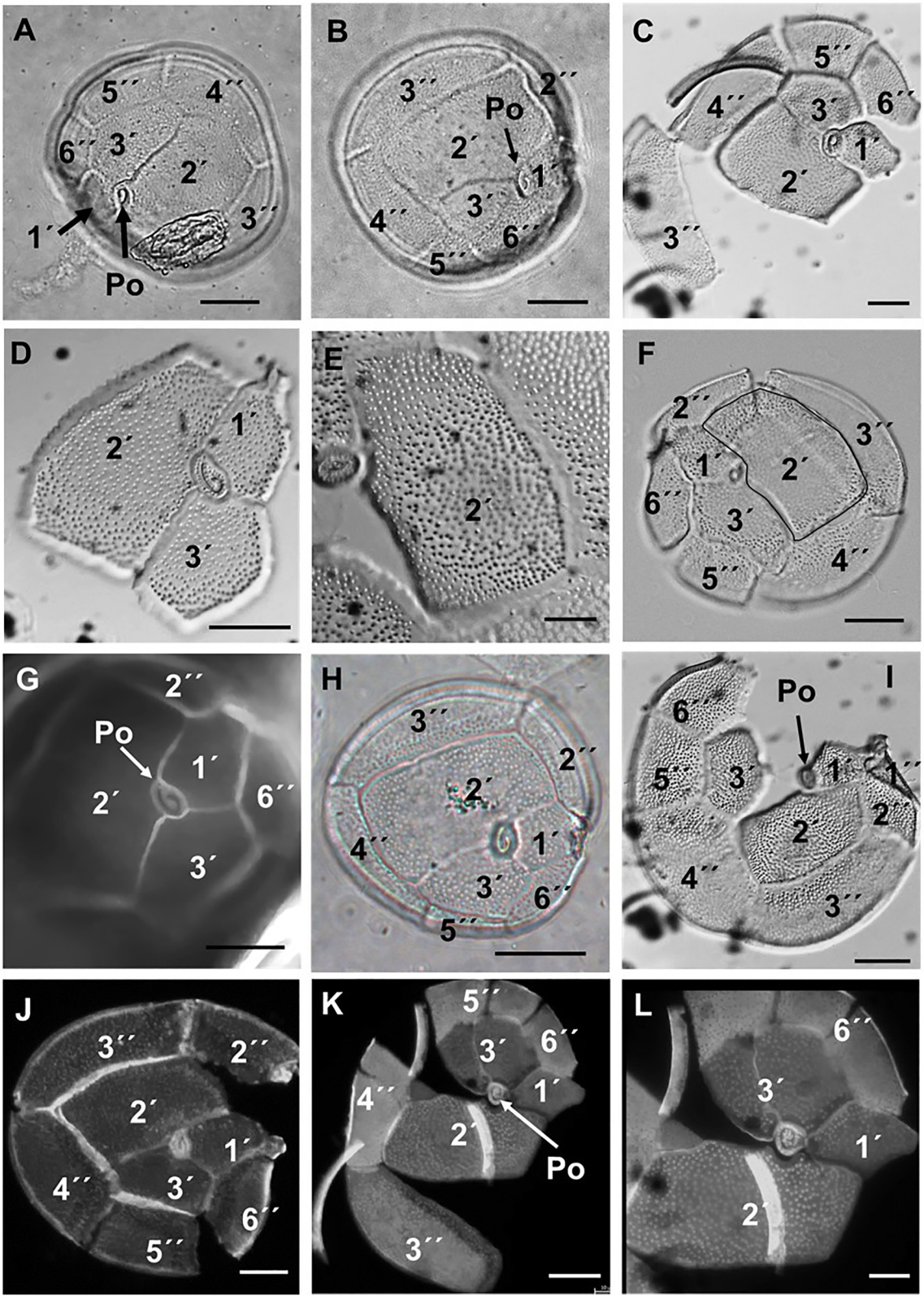

Figure 2: Light micrographs of *Gambierdiscus carpenteri* from the Gulf of California, thecae and their fragments. Strain GCARBAPAZ-1: (A and B); strain GCARBAPAZ-3: [C–L (C, F, I, K and L – thecae from inside)]. (A–D, F–L) Apical view, apical (′), precingular (″) and the pore (Po) plates. (E) The 2′ plate, hatchet-shaped. Po – the apical pore plate. (A–F, H and I): in bright field; (G and J–L): in epifluorescence regime. Scale bars: 20 µm in (A, B, C, F, G and K); 10 µm in (D, E, I, J and L); 30 µm in (H).

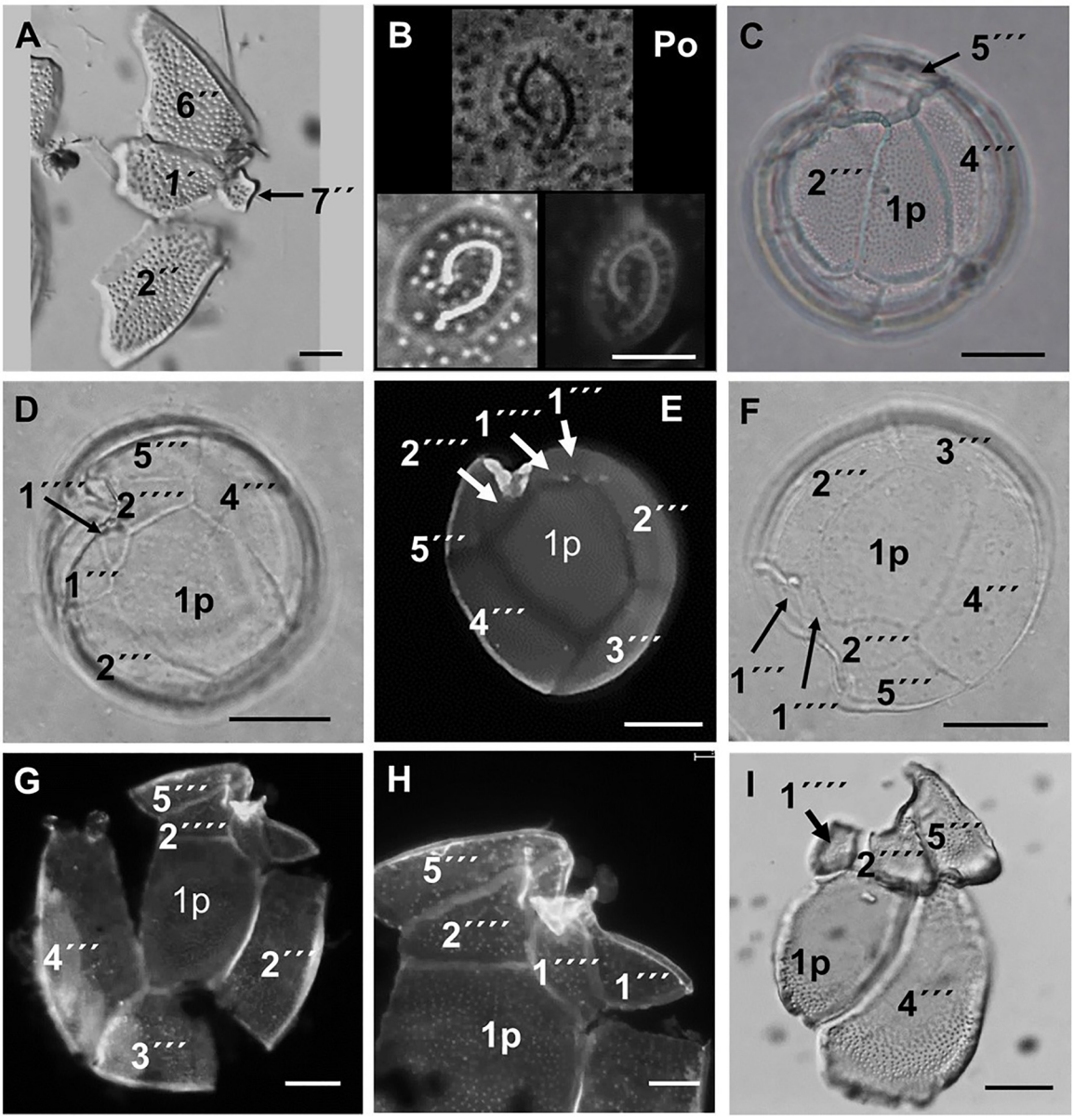

Figure 3: Light micrographs of *Gambierdiscus carpenteri*, thecae and their fragments. Strain GCARBAPAZ-1: (C and I); strain GCARBAPAZ-3: [A, B, D and E-H (A, C, D and I – thecae from inside)]. (A) Apical-ventral view. (B) The hook-shaped apical pore plate (Po). (C) Antapical view, postcingular plates (‴). (D) Ventro-antapical view, 1‴, 5‴, 1⁗ and 2⁗ plates. (E, G and H) Antapical view, postcingular (‴), antapical (⁗) and posterior intercalary (1p) plates. (A, C, D, F and I): in bright field; (E, G and H): in epifluorescence regime. Scale bars: 10 µm in (A and H); 5 µm in (B); 25 µm in (C, D and E); 30 µm in (F); 20 µm in (G and I).

4 Discussion and conclusions

In this study, plate 2′ was almost rectangular, with a small protrusion toward the apical pore complex, being morphologically similar to *G. toxicus*, and initially ruling out *G. carpenteri* and *G. caribaeus* (Litaker et al. 2009). However, in strains of *G. carpenteri* from Australia, this plate was observed to be hatchet-shaped or rectangular (Kohli et al. 2014). This differed from the descriptions in Litaker et al. (2009) and Wang et al. (2022) for strains from Belize. The 2′ plate observed in the strains GCARBAPAZ-1 and GCARBAPAZ-3 is consistent with those in Kohli et al. (2014) for the strain from Australia, indicating that in *G. carpenteri* both morphologies can be present in plate 2´ (rectangular or hatchet-shaped).

Cell size of *G. carpenteri* in this study averaged 88.2 µm long and 84.7 µm wide in apical view (transdiameters). Although the species exhibits variable cell size, the examined cells are consistent with what has been reported, with cell sizes from 66 to 92 µm long and 65 to 85 µm wide

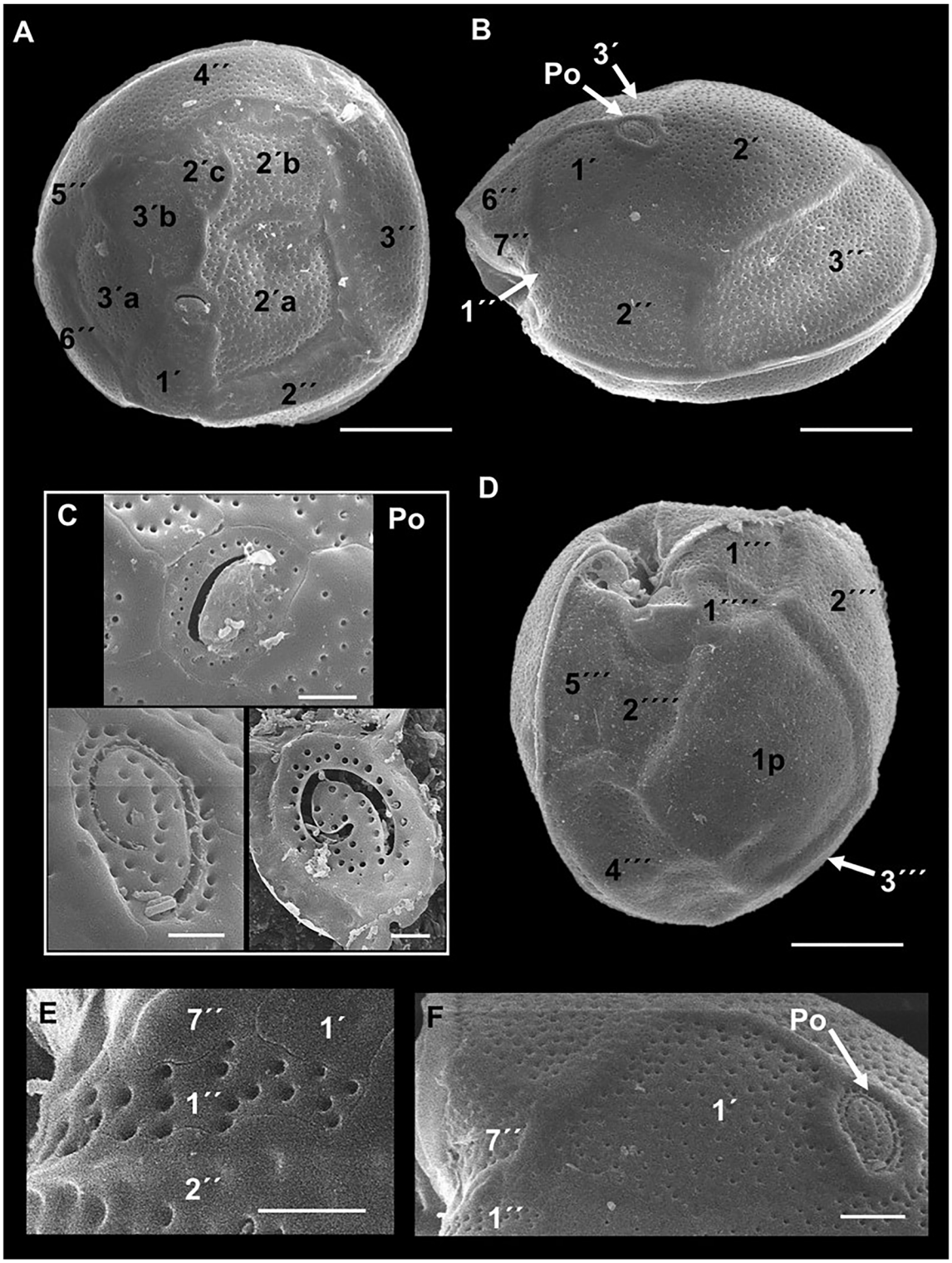

Figure 4: Scanning electron micrographs of *Gambierdiscus carpenteri*, strain GCARBAPAZ-1, from La Gaviota Island, Bahía de La Paz, Gulf of California. (A) Cell in apical view: the apical (′) and precingular (″) plates; and the 2′ plate is split into three plates (a–c), and plate 3′ is split into two plates (a and b). (B) Apical-left-side view, showing precingular plates and the apical pore plate (Po). (C) The apical pore plate (Po); variation in the number and arrangement of thecal pores is shown. (D) Antapical-right-side view, postcingular (‴), antapical (⁗) and posterior intercalary (1p) plates. (E and F) Apical view, a fragment of the epitheca. Scale bars: 20 µm in (A, B and D); 2 µm in (C); 5 µm in (E); 2.5 µm in (F).

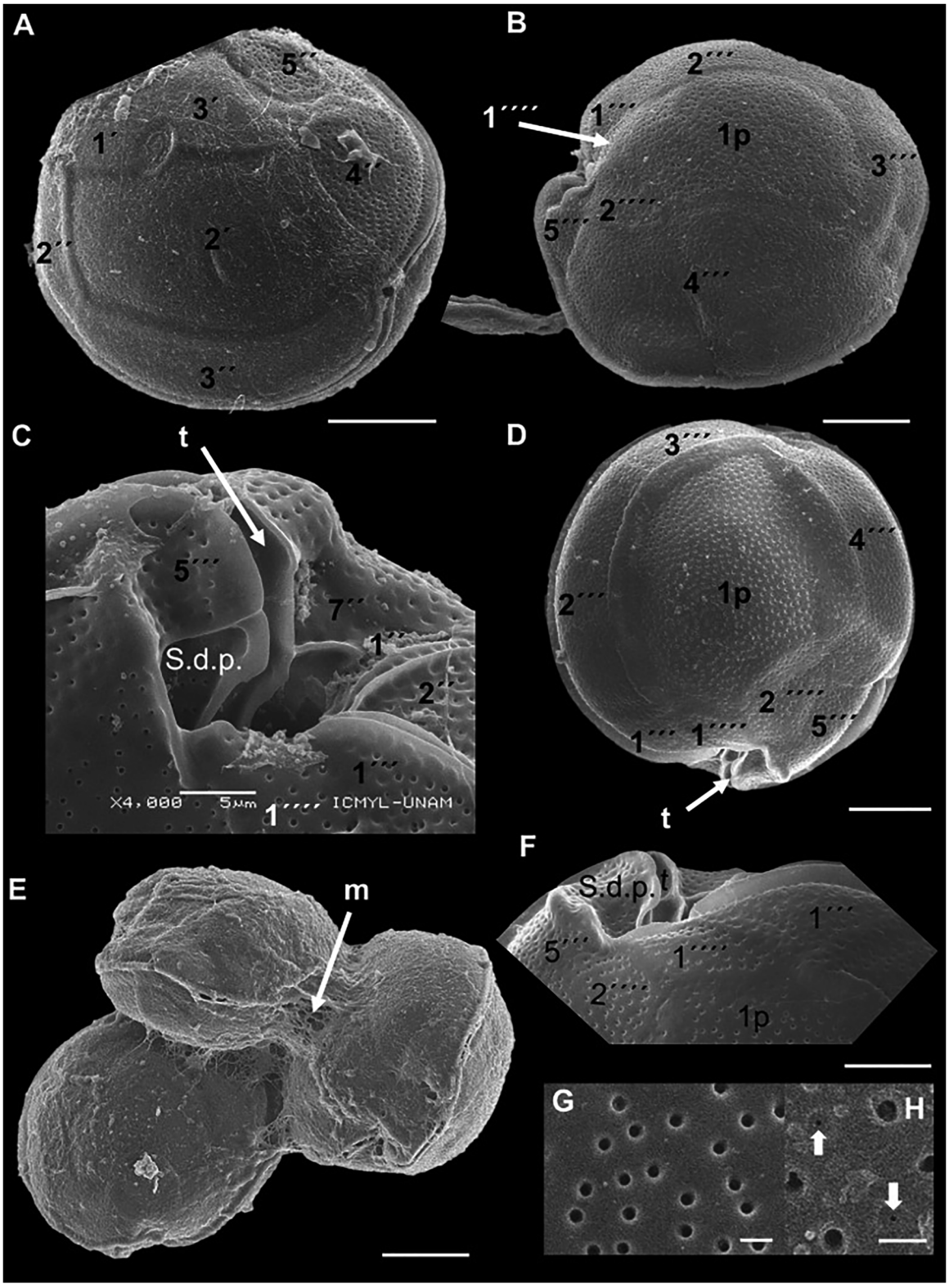

Figure 5: Scanning electron micrographs of *Gambierdiscus carpenteri*, strain GCARBAPAZ-3, from La Gaviota Island, Bahía de La Paz, Gulf of California. (A) Apical view. (B, D and F) Antapical view, postcingular (‴), antapical (⁗) and posterior intercalary (1p) plates. (C) Antapical-ventral view, transitional plate (t), sulcal right posterior plate (S.d.p.), precingular (″), postcingular (‴) and antapical (⁗) plates. (E) Cells bound by mucilage (m). (G and H) Fragments of thecal surface, white arrows indicate the smaller pores. Scale bars: 20 µm in (A, B and D); 10 µm in (F); 30 µm in (E); 1 µm in (G and H).

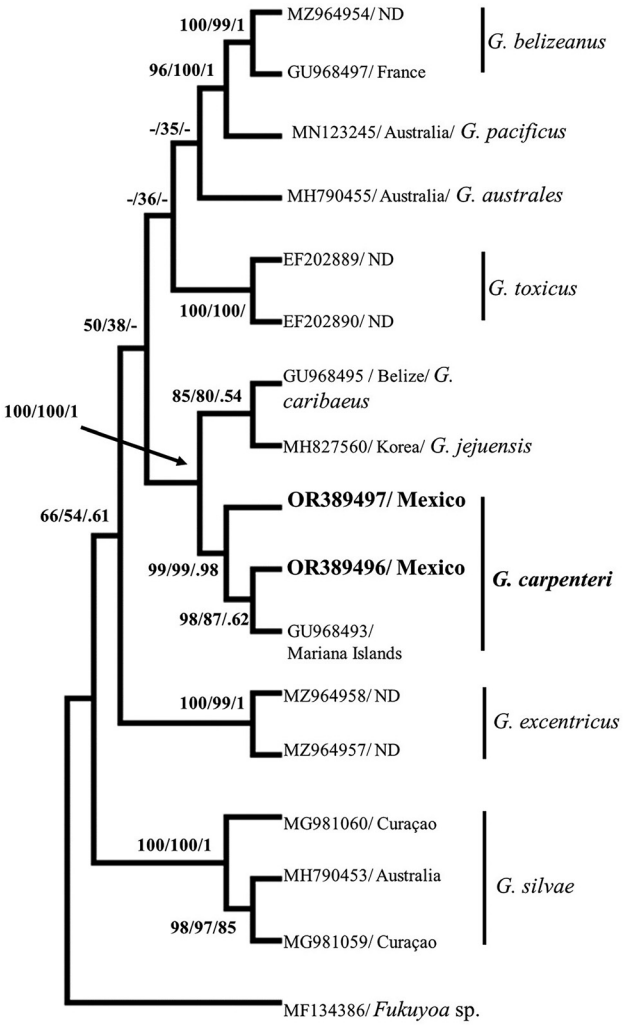

Figure 6: Phylogenetic tree of the 5.8S rDNA region of species of *Gambierdiscus*. The analysis was derived using the maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) methods. At the nodes the bootstrap percentage (MP/ML) and posterior probability (BI) are shown. The analysis included 17 sequences; dataset based on 712 pb. **OR389496** = GCARBAPAZ-1, **OR389497** = GCARBAPAZ-3, ND = no data.

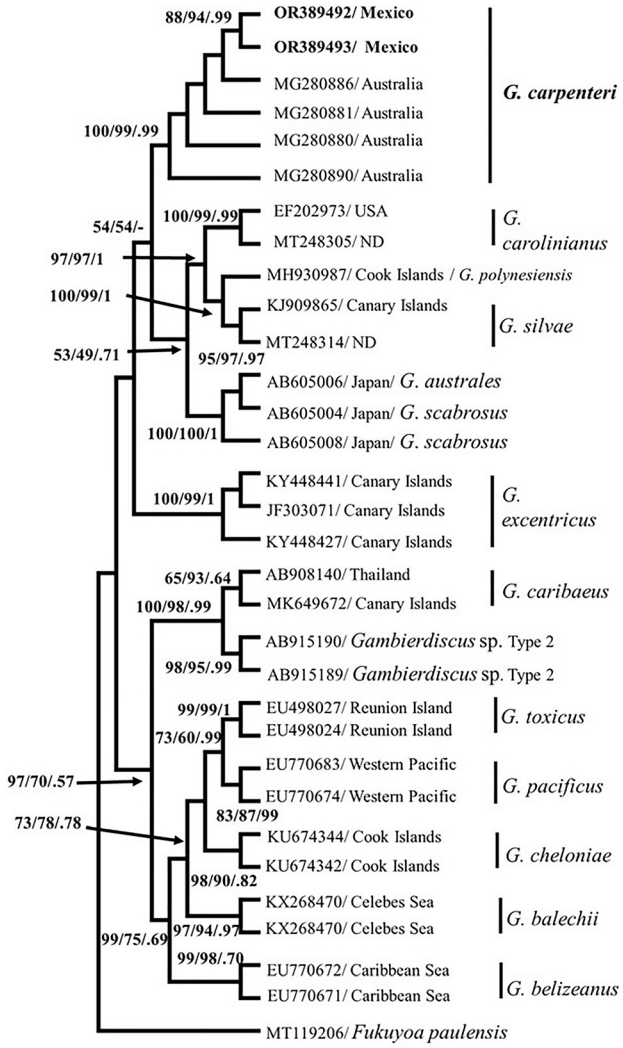

Figure 7: Phylogenetic tree of the 28S rDNA (D1–D3 region) of species of *Gambierdiscus*. The analysis was derived using the maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) methods. Bootstrap values (MP/ML) and posterior probability (BI) of each method are shown at the nodes. The analysis included 32 sequences, with a total of 358 pb. **OR389492** = GCARBAPAZ-1, **OR389493** = GCARBAPAZ-3, ND = no data.

(Hoppenrath et al. 2014; Kohli et al. 2014; Litaker et al. 2009; Vacarizas et al. 2018). The Po sizes in this study (9.51 µm long and 6 µm wide) agree with those reported in the literature for *G. carpenteri* (Litaker et al. 2009). Position and connection of the diagnostic plates 2′ and 4″ correspond to those reported for *G. carpenteri* (Kohli et al. 2014; Litaker et al. 2009; Wang et al. 2022). Among the known *Gambierdiscus* species, two types of thecal pores differing in size have been reported so far in *G. carpenteri*, *G. australes*, *G. carolinianus*, *G. pacificus* and *G. caribaeus* (Hoppenrath et al. 2023). This study confirms the presence of the larger and smaller pores in *G. carpenteri* (Figure 5H); however, this feature cannot be considered as discriminatory, because of scarcity of information about the theca ornamentation in other species of the genus.

In culture, temporary cysts, which are a part of the life cycle of species of *Gambierdiscus*, were observed. These cysts can take from three days to five months to germinate when conditions are suitable (Assunção and Portillo 2018). This study also documented cysts with the morphology of vegetative cells, without flagella, absence of motility, a thick wall, some with a reddish body, and the cytoplasm concentrated in the center of the cell. The above also was documented for *Gambierdiscus balechii* (Fraga et al. 2016).

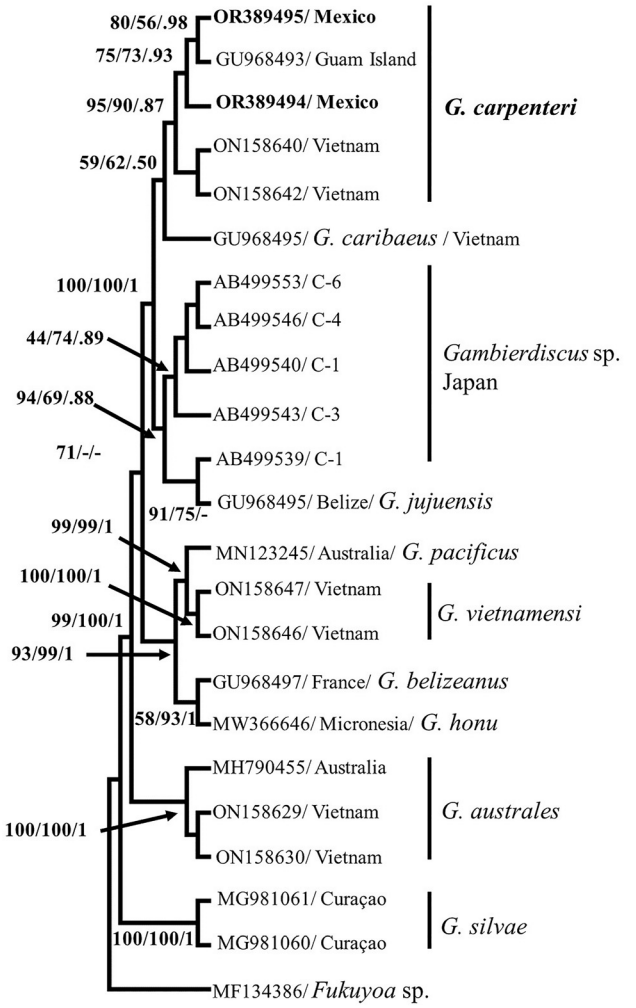

Figure 8: Phylogenetic tree of the ITS region of the rDNA of species of *Gambierdiscus*. At the nodes, the bootstrap percentage (MP/ML) and posterior probability (BI) are shown. The analysis included 23 sequences, with a total of 283 pb. **OR389494** = GCARBAPAZ-1, **OR389495** = GCARBAPAZ-3.

The species similar to *G. carpenteri*, both morphologically and in terms of the genetic distances based on the LSU sequences D8–D10, are *G. excentricus*, *G. toxicus* and *G. caribaeus* (Rodríguez et al. 2017; Table 1). The D8–D10 (28S) region of the LSU has been reported to be sufficient for the identification of the genus *Gambierdiscus* (Vacarizas et al. 2018); however, this region does not separate *G. excentricus* from *G. carpenteri*. This contrasts with the D1–D3 region, which separates species with a high phylogenetic affinity, such as *G. carpenteri*, *G. excentricus*, *G. toxicus* and *G. caribaeus* (Ott et al. 2022; this study; Wang et al. 2022). For example, the genetic distance between *G. carpenteri* and closely related species is higher with the D1–D3 gene (0.052) than with the D8–D10 region (0.005) (Fraga and Rodríguez 2014).

The taxa included in the phylogenetic analyses were distinct; this was due to the limited information available for each rDNA region, especially in the 5.8S and ITS regions of the rDNA. This makes it difficult to perform a concatenated analysis of the sequences of *G. carpenteri*, even with sequences of three markers, because there are no sequences for some species or the sequences in the databases do not come from publications and present inconsistencies in the identification in the database. It has been widely discussed whether the ITS/5.8S, D1–D3, D8–D10 LSU or SSU ribosomal genes alone are sufficient to distinguish between dinoflagellate species. In this study, the three phylogenies showed a pattern where the sequences obtained cluster with the sequences of *G. carpenteri* that have been previously published. In addition, the combination of the D1–D3 (28S) and ITS/5.8S phylogenies in other studies were able to resolve 97 % of the dinoflagellate species examined, thus classifying them as a rapid and reliable method for identification and description of *Gambierdiscus* species (Ott et al. 2022; Wang et al. 2022). In this study and others, the D1–D3 gene produces coherent terminal groupings of all species described within the genus *Gambierdiscus*, the same groupings being obtained if multigene phylogenies are performed (Kohli et al. 2014; Ott et al. 2022; Wang et al. 2022). It was demonstrated that there may be interspecific divergences in some species of *Gambierdiscus*, such as *G. carpenteri*, because the sequences may contain variants with insertions and deletions that are sometimes reflected in subclades within the main clade (see Ott et al. 2022).

In the Mexican Caribbean Sea, *G. carpenteri* has been previously reported in Cancún (Litaker et al. 2009), Isla Contoy, Puerto Morelos (Almazán-Becerril et al. 2020), and *G.* cf. *carpenteri* in Isla San José, Gulf of California (Morquecho-Escamilla et al. 2017). Studies of the morphological taxonomy of *G. carpenteri* are rare. To our knowledge, only five studies include such analysis (Kohli et al. 2014; Litaker et al. 2009; Nguyen-Ngoc et al. 2023; Vacarizas et al. 2018; Wang et al. 2022). Therefore, it was necessary to integrate morphological and molecular tools (5.8S, 28S and ITS rDNA gene sequences) to corroborate that both strains from the La Gaviota Island, Gulf of California are *G. carpenteri*.

Several toxicity assays have been performed in strains of *G. carpenteri* (Table 2) that have provided different results depending on the assay and the strain tested. The *G. carpenteri* strain tested in this study had a higher toxicity using MBA, than strains from Australia. Kohli et al. (2014) and Larsson et al. (2018) reported that the toxicity in MBA is not sensitive to CTX-like or MTX-like. By LC-MS/MS no known analogs of CTXs characterized in dinoflagellates were found. In tropical strains of *G. carpenteri* isolated from Australia, only analogs of MTXs were reported; however,

Table 1: Morphological comparison of *Gambierdiscus* species.

Cell shape		*G. carpenteri* Lens-shaped/lenticular[a,f]	*G. excentricus* Lens-shaped/lenticular[d]	*G. toxicus* Lens-shaped/lenticular[a]	*G. caribaeus* Round or lens-shaped[a,f]
Cell	Depth	75–110[a,f,g]	84–115[b,d,e]	93–103[a,f]	51–98[a,f]
size	Width	66–86[a,c,f,g]	69–110[b,d,e]	78–85[a,f]	47–95[a,f]
Po	Length	8.1[a]	8–10[b,d]	8–10[a,f]	6–11[a,f]
	Width	4.9[a]	ND	6.3[a]	5.3[a]
	Shaped	Oval, fishhook-shaped[a,f,g]	Fishhook-shaped[b]	Oval, short-shank, fishhook-shaped[a,f]	Oval, short-shank, fishhook-shaped[a,f]
2′		Hatchet-shaped or rectangular[a,b,c,g]	Rectangular-shaped[b,d]	Moderately hatchet- shaped[a,f]	Rectangular to hatchet-shaped[f]
4″		Asymmetric[a,c,f]	ND	ND	Symmetric[a,f]
6″		Protrudes ventrally[a]	ND	ND	Does not protrude ventrally[a,f]
1p		Wide, asymmetric, large[a]	Narrow, width variable[b,d]	Large and wide[f]. Dorsal end pointed[a]	Shorter and differently shaped from *G. carpenteri*[a,f]
Thecal surface		Numerous pores and small very shallow depressions between pores	Numerous pores[b,d,e]	Smooth with numerous pores[a]	Smooth with numerous pores and small depressions between pores[f]

Dimensions are given in µm. ND, no data. [a]Litaker et al. (2009), [b]Fraga et al. (2011), [c]Kohli et al. (2014), [d]Nascimento et al. (2015), [e]Hoppenrath et al. (2014), [f]Hoppenrath et al. (2023), [g]this study.

temperate strains did not produce MTX-3 (Larsson et al. 2018). Polyether toxic compounds that have been detected by LC-MS/MS in *G. carpenteri* were 2,33-dihidroxy-PCTX3C and 44-methylgambierone (previously reported as MTX-3, Chinain et al. 2010).

Toxicity in *G. carpenteri* strains varies according to their geographic origin. *Gambierdiscus carpenteri* isolated from Hawaii presented 1.4 ± 0.6 fg CTX3C eq cell^{-1} and 6.3 ± 1.9 fg MTX eq cell^{-1} (Pisapia et al. 2017). Similarly, Litaker et al. (2017) reported five toxic strains of *G. carpenteri* isolated from the Great Caribbean with a toxicity ranging from 0.3 to 1.4 fg CTX3C eq cell^{-1}. In contrast, strains of *G. carpenteri* isolated from the Philippines had a higher toxicity. Vacarizas et al. (2018) reported a toxicity of 7.48 ± 0.49 pg Pbtx-3 eq cell^{-1} by the use of receptor binding assays, and Malto et al. (2022) reported 12.36 ± 4.38 pg Pbtx-3 eq cell^{-1} using a radioligand receptor-binding assay. Not much is known about the effect of environmental conditions on the toxin content in *Gambierdiscus* species, although *G. carpenteri* has a wide range of tolerance to environmental conditions, on which toxin production depends (Vacarizas et al. 2018).

The toxicity range of *G. carpenteri* is similar to the toxicity reported for *G. australes*, *G. balechii*, *G. carolineanus* and *G. belizeanus* from the North Atlantic, Caribbean Sea, Gulf of Mexico, and Pacific, showing a similar CTX-like activity, suggesting comparable levels of toxins produced (Díaz-Asencio et al. 2019; Litaker et al. 2017; Pisapia et al. 2017). These results suggest that the toxicity documented in *G. carpenteri* is closely related to the diversity of the geographical environments in which it occurs and could be a possible fingerprint of CTX and MTX production.

In the state of Baja California Sur, Mexico, 240 cases of ciguatera fish poisoning (CFP) were reported from 1984 to 2011 (Nuñez-Vázquez et al. 2019). Four human intoxications were associated with the consumption of Lutjanidae and Serranidae fish species (Nuñez-Vázquez et al. 1998, 2019). This study provides the first sequences of *G. carpenteri* for the Gulf of California, and the MBA showed that the isolate examined exhibits CTX-like and MTX-like activities. Therefore, *G. carpenteri* is a possible agent that may be contributing to CFP cases in the region; however, further confirmatory studies are required through various analytical techniques to confirm the presence of toxins as well as to determine their chemical structure. It is important to monitor this species from various substrata and benthic environments and to evaluate the possible conditions in which it can proliferate, with the aim of protecting human and animal health. The global distribution and the wide range of environmental adaptability of *G. carpenteri* indicate the need for future studies after the first confirmed record and possible toxicity of this species in the Gulf of California.

Acknowledgments: We thank L.E. Gómez-Lizárraga for the scanning electron microscopy service of the UNAM, MSc. D.I. Rojas-Posadas for her advice on molecular techniques, A. Evhelin-Mendez of University of Tennessee (Knoxville, TN, USA) and M.M. Gowing (Seattle, WA, USA) for the language edition support.

Table 2: Toxicity bioassays performed on *Gambierdiscus carpenteri* strains.

Strains	Origin	Assays	LC-MS/MS or UPLC-MS/MS	References
NCMA 1654, GT4, Pat HI jar 5 gam 3, WBHR 21, ETB Exp 24 gam 1 and Jamaica Algae 2 gam 1	Guam, Belize, Hawaii, Flower Gardens, Dry Tortugas y Ocho Ríos	Intermediate hemolytic activity in human erythrocytes	–	Holland et al. (2013)
–	New South Wales, Australia	MBA, 2.4 mg kg^{-1}	Not detected	Kohli et al. (2014)
CAWD 237	New South Wales, Australia	–	Not detected	Munday et al. (2017)
Bill Aruba Gam 15, GT4, Jamaica Algae 2, Gam 1, Mexico Algae 2, Gam 1, WBHR21	Aruba, Belize, Jamaica in Caribbean, Mexico, and Gulf of Mexico	CBA-N2a – 0.3 to 1.4 fg CTX3C eq $cell^{-1}$	–	Litaker et al. (2017)
NHA-19, -20	Nuku Hiva Island, Marquesas Archipelago	CBA-N2a – not detected	–	Darius et al. (2018)
UTSHI2C4, UTSHI6C3, UTSHI6A1 and UTSHI6D2	Heron Island Lagoon, Australia	Ca2+ influx SH-SY5, cell FLIPR assay with MTX- type activity	Maitotoxin-3 (MTX-3)	Larsson et al. (2018)
GAM1BOL_080513	Bolinao, Pangasinan, Philippines	7.48 ± 0.49 pg PbTx- 3 eq $cell^{-1}$	–	Vacarizas et al. (2018)
Gam1BOL080513	Bolinao, Pangasinan	RBA 12.36 ± 4.38 pg PbTx-3 eq $cell^{-1}$	44-methylgambierone	Malto et al. (2022)
GCARBAPAZ-3	La Gaviota Island, Bahía de La Paz, Gulf of California	MBA, CTXs-like and MTX-like activities	–	This study

Research ethics: The acute toxicity tests in the mouse model were carried out based on the Official Mexican Standard NOM-242-SSA1-2009 (https://www.dof.gob.mx/normasOficiales/4295/salud2a/salud2a.htm) of the Ministry of Health (official method in Mexico for the analysis of ciguatoxins) and in accordance with the Official Mexican Standard NOM-062-ZOO-1999 (https://www.gob.mx/cms/uploads/attachment/file/203498/NOM-062-ZOO-1999_220801.pdf) of the Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food; Technical Specifications for Production, Care and Use of Laboratory Animals (Bioterium certified by SAGARPA no. B000.02.03.02.01.1155/08).

Author contributions: A.E. Ramos-Santiago: conceptualization; research; data curation; formal analysis; writing – original draft preparation; writing – review & editing. C.J. Band-Schmidt: conceptualization; funding acquisition; methodology; supervision; writing – review & editing. I. Leyva-Valencia: conceptualization; methodology; supervision; review & editing. L.J. Fernández-Herrera: data curation, methodology; review & editing. E.J. Núñez-Vázquez: methodology; supervision; funding; review & editing. Y.B. Okolodkov: methodology; writing – review & editing; supervision. The authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: The authors state no conflict of interest.

Research funding: This study was financed with the projects CONAHCyT A1-S-14968, CONAHCyT PRONAII, PRONACES SSyS 319104 and the institutional projects SIP-2024-0486 and SIP-2024-0544. A.E.R.S. was recipient to the scholarship 1151607 from CONAHCyT and B210365 from BEIFI. C.J.B.S. is a COFAA and BEIFI recipient.

Data availability: DNA sequences, GenBank accessions: Strain GCARBAPAZ-1: OR389496 (5.8S), OR389494 (ITS) and OR389492 (28S). Strain GCARBAPAZ-3: OR389497 (5.8S), OR389495 (ITS) and OR389493 (28S).

References

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

Almazán-Becerril, A., Delgado-Pech, B., Núñez-Vázquez, E., Escalante-Abusto, M., Irola-Sansores, E.D., Arredondo-Chávez, A.T., Peniche-Pérez, J.C., Caballero-Vázquez, A., Uitzil-Castañeda, D.A., Méndez-Torres, J., et al. (2020). *Presencia de ciguatoxinas en la especie invasora pez león (Pterois volitans) y en otros peces arrecifales en dos áreas naturales protegidas de Quintana Roo: Arrecife de Puerto Morelos e Isla Contoy*. Comisión Nacional para el Conocimiento y Uso de la

Biodiversidad, Ciudad de México, Mexico, Available at: https://www.gbif.org/dataset/1a903bc5-bedf-4414-809c-7439843dc336.

Assunção, P. and Portillo, E. (2018). Cysts, germination and other findings in *Gambierdiscus* spp, Available at: http://mimarjvm1.jvmhost.net/mimar/opencms/cptss/proyectos_europeos/mimar/es/inicio/index.html.

Blackburn, S.I., Bolch, C.J.S., Haskard, K.A., and Hallegraeff, G.M. (2001). Reproductive compatibility among four global populations of the toxic dinoflagellate *Gymnodinium catenatum* (Dinophyceae). *Phycologia* 40: 78–87.

Bustillos-Guzmán, J.J., Band-Schmidt, C.J., Durán-Riveroll, L.M., Hernández-Sandoval, F.E., López-Cortés, D.J., Núñez-Vázquez, E.J., Cembella, A., and Krock, B. (2015). Paralytic toxin profile of the marine dinoflagellate *Gymnodinium catenatum* Graham from the Mexican Pacific as revealed by LC-MS/MS. Food Addit. Contam. A 32: 381–394, https://doi.org/10.1080/19440049.2014.1000978.

Chinain, M., Darius, H.T., Ung, A., Cruchet, P., Wang, Z., Ponton, D., Laurent, D., and Pauillac, S. (2010). Growth and toxin production in the ciguatera-causing dinoflagellate *Gambierdiscus polynesiensis* (Dinophyceae) in culture. *Toxicon* 56: 739–750.

Darius, H.T., Roué, M., Sibat, M., Viallon, J., Gatti, C.M.I., Vandersea, M.W., Tester, P.A., Litaker, R.W., Amzil, Z., Hess, P., et al. (2018). Toxicological investigations on the sea urchin *Tripneustes gratilla* (Toxopneustidae, Echinoid) from Anaho Bay (Nuku Hiva, French Polynesia): evidence for the presence of pacific ciguatoxins. *Mar. Drugs* 16: 122.

de Vera, C.R., Díaz-Crespín, G., Hernández-Daranas, A., Montalvão-Looga, S., Lillsunde, K.E., Tammela, P., Perälä, M., Hongisto, V., Virtanen, J., Rischer, H., et al. (2018). Marine microalgae: promising source for new bioactive compounds. *Mar. Drugs* 16: 317.

Díaz-Asencio, L., Clausing, R.J., Vandersea, M., Chamero-Lago, D., Gómez-Batista, M., Hernández-Albernas, J.I., Chomérat, N., Rojas-Abrahantes, G., Litaker, R.W., Tester, P., et al (2019). Ciguatoxin occurrence in food-web components of a Cuban coral reef ecosystem: risk-assessment implications. Toxins 11: 722.

FAO (2005). Marine biotoxins. In: *Food and nutrition paper 80*. Organization of the United Nations, Rome, Italy, p. 278.

Fraga, S. and Rodríguez, F. (2014). Genus *Gambierdiscus* in the Canary Islands (NE Atlantic Ocean) with description of *Gambierdiscus silvae* sp. nov., a new potentially toxic epiphytic benthic dinoflagellate. *Protist* 165: 839–853.

Fraga, S., Rodríguez, F., Caillaud, A., Diogène, J., Raho, N., and Zapata, M. (2011). *Gambierdiscus excentricus* sp. nov. (Dinophyceae), a benthic toxic dinoflagellate from the canary islands (NE Atlantic Ocean). *Harmful Algae* 11: 10–22.

Fraga, S., Rodríguez, F., Riobó, P., and Bravo, I. (2016). *Gambierdiscus balechii* sp. nov. (Dinophyceae), a new benthic toxic dinoflagellate from the Celebes Sea (SW Pacific Ocean). *Harmful Algae* 58: 93–105.

Fritz, L. and Triemer, R.E. (1985). A rapid simple technique utilizing calcofluor white M2R for the visualization of dinoflagellates thecal plates. *J. Phycol.* 21: 662–664.

Gómez-Lizárraga, L.E., Durán-Riveroll, L.M., and Pérez-López, H.I. (2019). Scanning electron microscopy preparation method for *Coolia* sp., a delicate dinoflagellate. *Microsc. Microanal.* 25: 1180–1181.

Guiry, M.D. and Guiry, G.M. (2024). AlgaeBase. World-Wide electronic publication, national university of Ireland, Galway, Available at: https://www.algaebase.org.

Hedrich, H. (2012). *The laboratory mouse*, 2nd ed. Academic Press, Elsevier, Oxford, UK.

Holland, W.C., Litaker, R.W., Tomas, C.R., Kibler, S.R., Place, A.R., Davenport, E.D., and Tester, P.A. (2013). Differences in the toxicity of six *Gambierdiscus* (Dinophyceae) species measured using an *in vitro* human erythrocyte lysis assay. *Toxicon* 65: 15–33.

Holmes, M.J. and Lewis, R. (1994). Purification and characterization of large and small maitotoxins from cultures *Gambierdiscus toxicus*. *Nat. Toxins* 2: 64–72.

Hoppenrath, M., Murray, S.A., Chomérat, N., and Horiguchi, T. (2014). *Marine benthic dinoflagellates – unveiling their worldwide biodiversity. Kleine Senckenberg-Reihe*, Vol. 54. Schweizerbart'sche Verlagsbuchhandlung (Nägele u. Obermiller), Stuttgart, Germany, p. 276.

Hoppenrath, M., Chomérat, N., Horiguchi, T., Murray, S.A., and Rhodes, L. (2023). *Marine benthic dinoflagellates – their relevance for science and society*, 2nd ed. Senckenberg Gesellschaft für Naturforschung, Germany, p. 375.

Hosoi-Tanabe, S., Otake, I., and Sako, Y. (2006). Phylogenetic analysis of noxious red tide flagellates *Chattonella antiqua*, *C. marina*, *C. ovata*, and *C. verruculosa* (Raphidophyceae) based on the rRNA gene family. Fish. Sci. 72: 1200–1208.

Kohli, G.S., Murray, S.A., Neilan, B.A., Rhodes, L.L., Harwood, D.T., Smith, K.F., Meyer, L., Capper, A., Brett, S., and Hallegraeff, G.M. (2014). High abundance of the potentially maitotoxic dinoflagellate *Gambierdiscus carpenteri* in temperate waters of New South Wales, Australia. *Harmful Algae* 39: 134–145.

Larsson, M., Laczka, O., Harwood, D., Lewis, R., Himaya, S., Murray, S., and Doblin, M. (2018). Toxicology of *Gambierdiscus* spp. (Dinophyceae) from tropical and temperate Australian waters. *Mar. Drugs* 16: 7.

Lewis, R.L. (1995) Detection of ciguatoxins and related benthic dinoflagellate toxins: *in vivo* and *in vitro* methods. In: Hallegraeff, G.M., Anderson, D.M., Cembella, A.D., and Enevoldsen, H.O. (Eds.). *Manual on harmful marine microalgae*. UNESCO-IOC, Paris, France, pp. 135–161.

Litaker, R.W., Vandersea, M.W., Faust, M.A., Kibler, S.R., Chinain, M., Holmes, M.J., Holland, W.C., and Tester, P.A. (2009). Taxonomy of *Gambierdiscus* including four new species, *Gambierdiscus caribaeus*, *Gambierdiscus carolinianus*, *Gambierdiscus carpenteri* and *Gambierdiscus ruetzleri* (Gonyaulacales, Dinophyceae). *Phycologia* 48: 344–390.

Litaker, R.W., Holland, W.C., Hardison, D.R., Pisapia, F., Hess, P., Kibler, S.R., and Tester, P.A. (2017). Ciguatoxicity of *Gambierdiscus* and *Fukuyoa* species from the Caribbean and Gulf of Mexico. *PLoS One* 12: e0185776.

Malto, Z.B.L., Benico, G.A., Batucan, J.D., De la Cruz, J., Romero, M.L.J., Azanza, R.V., and Salvador-Reyes, L.A. (2022). Global mass spectrometric analysis reveals chemical diversity of secondary metabolites and 44-Methylgambierone production in Philippine *Gambierdiscus* strains. *Front. Mar. Sci.* 8: 767024.

Morquecho-Escamilla, L., Reyes-Salinas, A., Okolodkov, Y.B., Gárate-Lizárraga, I., Mazariegos-Villarreal, A., and Galicia-García, C. (2017) Aislamiento y caracterización taxonómica de dinoflagelados epífitos en Bahía de La Paz e Isla San José, Golfo de California. Abstract book. In: *IV Congreso de la SOMEFAN y II Reunión ALEAN, October 23–27, 2017, Sociedad Mexicana de Florecimientos Algales Nocivos*. Cancún, Quintana Roo, Mexico.

Munday, R., Murray, S., Rhodes, L., Larsson, M., and Harwood, D. (2017). Ciguatoxins and maitotoxins in extracts of sixteen *Gambierdiscus* isolates and one *Fukuyoa* isolate from the South Pacific and their

toxicity to mice by intraperitoneal and oral administration. *Mar. Drugs* 15: 208.
Murray, S., Momigliano, P., Heimann, K., and Blair, D. (2014). Molecular phylogenetics and morphology of *Gambierdiscus yasumotoi* from tropical eastern Australia. *Harmful Algae* 39: 242–252.
Nascimento, S.M., Melo, G., Salgueiro, F., Diniz, B., dos, S., and Fraga, S. (2015). Morphology of Gambierdiscus excentricus (Dinophyceae) with emphasis on sulcal plates. Phycologia 54: 628–639.
Nguyen-Ngoc, L., Larsen, J., Doan-Nhu, H., Nguyen, X., Chomérat, N., Lundholm, N., Phan-Tan, L., Dao, H.V., Nguyen, N., Nguyen, H., et al. (2023). *Gambierdiscus* (Gonyaulacales, Dinophyceae) diversity in Vietnamese waters with description of *G. vietnamensis* sp. nov. *J. Phycol.* 59: 496–517.
Núñez-Vázquez, E.J., Sierra-Beltrán, A.P., Cruz-Villacorta, A.A., and Ochoa, J.L. (1998). Ciguatoxins on Serranidae and Lutjanidae fish of Baja California Sur, Mexico. *Toxicon* 36: 1224.
Núñez-Vázquez, E.J., Almazán-Becerril, A., López-Cortés, D.J., Heredia-Tapia, A., Hernández-Sandoval, F.E., Band-Schmidt, C.J., Bustillos-Guzmán, J.J., Gárate-Lizárraga, I., García-Mendoza, E., Salinas-Zavala, C.A., et al. (2019). Ciguatera in Mexico (1984–2013). *Mar. Drugs* 17: 13.
Ott, B.M., Litaker, R.W., Holland, W.C., and Delwiche, C.F. (2022). Using RDNA sequences to define dinoflagellate species. *PLoS One* 17: e0264143.
Pisapia, F., Holland, W.C., Hardison, D.R., Litaker, R.W., Fraga, S., Nishimura, T., Adachi, M., Nguyen-Ngoc, L., Séchet, V., Amzil, Z., et al. (2017). Toxicity screening of 13 *Gambierdiscus* strains using neuro-2a and erythrocyte lysis bioassays. *Harmful Algae* 63: 173–183.
Rains, L.K. and Parsons, M.L. (2015). *Gambierdiscus* species exhibit different epiphytic behaviors toward a variety of macroalgal hosts. *Harmful Algae* 49: 29–39.
Rodríguez, F., Fraga, S., Ramilo, I., Rial, P., Figueroa, R.I., Riobó, P., and Bravo, I. (2017). Canary Islands (NE Atlantic) as a biodiversity 'hotspot' of *Gambierdiscus*: implications for future trends of ciguatera in the area. *Harmful Algae* 67: 131–143.
SAGARPA (1999). NOM-062ZOO-1999: technical specification for production, care and use of laboratory animals, Available at: https://www.fmvz.unam.mx/fmvz/principal/archivos/062ZOO.PDF.
Satake, M., Murata, M., and Yasumoto, T. (1993). The structure of CTX3C, a ciguatoxin congener isolated from cultured *Gambierdiscus toxicus*. *Tetrahedron Lett.* 34: 1975–1978.
Sparrow, L., Momigliano, P., Russ, G.R., and Heimann, K. (2017). Effects of temperature, salinity, and composition of the dinoflagellate assemblage on the growth of *Gambierdiscus carpenteri* isolated from the great barrier reef. *Harmful Algae* 65: 52–60.
Taylor, F.J.R. (1978) Dinoflagellates (Chapter 6. Identification problems. Some specific preparations). In: Sournia, A. (Ed.), *Phytoplankton manual. Monographs on oceanographic methodology*, Vol. 6. UNESCO, Paris, France, pp. 143–147.
Vacarizas, J., Benico, G., Austero, N., and Azanza, R. (2018). Taxonomy and toxin production of *Gambierdiscus carpenteri* (Dinophyceae) in a tropical marine ecosystem: the first record from the Philippines. *Mar. Pollut. Bull.* 137: 430–443.
Wang, D.-Z., Xin, Y.-H., and Wang, M.-H. (2022). *Gambierdiscus* and its associated toxins: a minireview. *Toxins* 14: 485.
Xu, Y., Richlen, M.L., Liefer, J.D., Robertson, A., Kulis, D., Smith, T.B., Parsons, M.L., and Anderson, D.M. (2016). Influence of environmental variables on *Gambierdiscus* spp. (Dinophyceae) growth and distribution. *PLoS One* 11: e0153197.

Bionotes

Ana E. Ramos-Santiago
Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, Av. Instituto Politécnico Nacional s/n, Col. Playa Palo de Santa Rita, La Paz, Baja California Sur 23079, Mexico
aramoss2100@alumno.ipn.mx
https://orcid.org/0009-0000-3652-3565

Ana E. Ramos-Santiago is currently a PhD student in Marine Sciences at IPN-CICIMAR, La Paz, Mexico. Her main research interests are epibenthic marine dinoflagellates, sampling techniques, cultivation, and their identification by the use of microscopy and molecular tools. Currently, she is dedicated to understanding the autecology of epibenthic dinoflagellates species from Bahía de La Paz, Mexico.

Christine J. Band-Schmidt
Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, Av. Instituto Politécnico Nacional s/n, Col. Playa Palo de Santa Rita, La Paz, Baja California Sur 23079, Mexico
cbands@ipn.mx
https://orcid.org/0000-0002-8251-9820

Christine J. Band-Schmidt is a researcher at IPN-CICIMAR where she is leader of the research team studying harmful marine algae. She is a founder member of the Mexican Society for the Study of Harmful Algae (SOMEFAN, A.C.) and the CONAHCyT network of harmful algal blooms (RedFAN). Her research has been focused mainly on the autecology of harmful algal species in the Gulf of California with particular interest to understand the interactions between phytoplankton species and bacteria-phytoplankton interactions.

Ignacio Leyva-Valencia
Consejo Nacional de Humanidades, Ciencias y Tecnologías, Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, Av. Instituto Politécnico Nacional s/n, Col. Playa Palo de Santa Rita, La Paz, Baja California Sur 23079, Mexico
ileyvav@ipn.mx
https://orcid.org/0000-0001-9904-1512

Ignacio Leyva-Valencia is a CONAHCyT researcher, affiliated to IPN-CICIMAR. His main research is focused on the isolation and identification of toxigenic dinoflagellate species by the use of microscopy and molecular biology tools. He also conducts experimental work to determine the cytotoxic and antibacterial activity of dinoflagellate extracts, as well as their effect on bivalve mollusks. He is a member of the Council Board of the Mexican Society for the Study of Harmful Algal Blooms (SOMEFAN, A.C.).

Leyberth J. Fernández-Herrera
Centro de Investigaciones Biológicas del Noroeste, Laboratorio de Toxinas Marinas y Aminoácidos, La Paz, Baja California Sur 23096, Mexico
leyberthf@gmail.com
https://orcid.org/0000-0001-6174-5303

Leyberth J. Fernández-Herrera is currently a postdoctoral researcher at the CIBNOR. He has studied the chronic effect of marine neurotoxins in murine models, and has also contributed to the chemical ecology interactions between harmful phytoplankton species and their physiological responses, such as cellular stress, apoptosis and toxin content. His current research focuses on evaluating the effects of toxic microalgae on aquatic organisms, studying the toxic-genomic effects of neurotoxins on commercially important fish species.

Erick J. Núñez-Vázquez
Centro de Investigaciones Biológicas del Noroeste, Laboratorio de Toxinas Marinas y Aminoácidos, La Paz, Baja California Sur 23096, Mexico
enunez04@cibnor.mx
https://orcid.org/0000-0003-1257-731X

Erick J. Núñez-Vázquez is a marine toxicologist. He is responsible for the analysis of marine toxins at CIBNOR and a member of the Mexican SCFFP of the Codex Alimentarius coordinated by COFEPRIS-SSA before FAO-WHO. Since 2021, he has been a member of the IPHAB of the IOC-UNESCO in the working group of ciguatera. His research has focused on the identification, effects and understanding the mechanisms of action of marine toxins from a biochemical and biomedical perspective.