LIMNOLOGY and OCEANOGRAPHY LETTERS

ASLO

Open Access

Limnology and Oceanography Letters 11, 2026, e70160

doi: 10.1002/lol2.70160

LETTER

Emerging occurrence of *Gambierdiscus carolinianus* in the Azores: The northernmost Atlantic record

Miguel Barbosa [1,2] **Catarina Churro** [1,3] **Inês Gomes** [4] **Sergei Danchenko** [1] **Maria Filomena Caeiro** [5,6] **Pedro Afonso** [4] **Ana Amorim** [2,6*] **Pedro Reis Costa** [1,7]

[1]IPMA—Portuguese Institute for the Sea and Atmosphere, Lisbon, Portugal; [2]MARE—Marine and Environmental Sciences Centre/ARNET—Aquatic Research Network, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal; [3]CIIMAR—Blue Biotechnology, Environment and Health, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Matosinhos, Portugal; [4]Institute of Marine Sciences—Okeanos, University of the Azores and Instituto do Mar—IMAR, Horta, Portugal; [5]Centre for Ecology, Evolution and Environmental Changes (CE3C) & CHANGE—Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal; [6]Departamento de Biologia, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal; [7]Centre of Marine Sciences (CCMAR/CIMAR LA), University of Algarve, Faro, Portugal

Scientific Significance Statement

In the past 20 yr, ciguatera poisoning, once considered limited to tropical and subtropical regions, has been reported in new areas, including the northeast Atlantic archipelagos of Madeira (Portugal) and the Canary Islands (Spain). The present study investigated benthic microalgae in the temperate Azores archipelago (> 39°N), following a marine heatwave that produced sea surface temperature anomalies exceeding 2°C and coincided with a mass mortality event of wild groupers caused by Viral Nervous Necrosis (VNN). Detailed investigation of the microalgal community resulted in the detection of *Gambierdiscus*. Morphological and phylogenetic studies allowed the identification of *G. carolinianus* with higher affinity with West Atlantic populations, than with populations from Madeira or the Canary Islands within the same biogeographical region. This suggests possible connectivity between the two regions driven by oceanographic processes.

Abstract

The dinoflagellate genus *Gambierdiscus* was detected for the first time in the Azores Archipelago, representing its northernmost record to date (> 39°N). Two strains were isolated from macroalgal substrates on Corvo and Faial Islands during the summer of 2024, coinciding with a marine heatwave in which sea-surface temperature anomalies exceeded +2°C. Morphological and molecular analyses identified both isolates as *Gambierdiscus carolinianus*, extending further North the known range of this genus in the Atlantic and complementing previous records from other Macaronesian archipelagos. The Azorean isolate from Corvo clustered more closely with western Atlantic populations than with Madeira or the Canary Islands strains, suggesting long-range connectivity potentially driven by oceanographic processes. Although no ciguatera poisoning (CP) cases have been reported in the Azores, the occurrence of *Gambierdiscus* highlights the need for sustained monitoring and

*Correspondence: aaferreira@ciencias.ulisboa.pt

Associate editor: Jeffrey W. Krause

ecological assessments to evaluate the risk of CP emergence in this remote temperate archipelago under shifting environmental conditions.

The dinoflagellate genus *Gambierdiscus* is particularly relevant due to its association with ciguatera poisoning (CP), which is one of the most common nonbacterial illnesses related to the consumption of coral reef fish toxified with ciguatoxins (CTX) in tropical and subtropical regions. Over the past few decades, CP has been reported in areas where it was previously unreported, such as the NE Atlantic archipelagos of Madeira and the Canary Islands (Rodríguez et al. 2017; Costa et al. 2023). While the expansion of CP is evident from reported human poisoning cases, the geographic spread of the causative toxin-producing dinoflagellates is harder to detect, as it is constrained by limited routine monitoring, the logistical challenges of sampling benthic substrates across regions, and the difficulty of accurately identifying and characterizing these taxa (Gouveia et al. 2026).

Over four decades ago, the geographic distribution of the causative dinoflagellates of the genera *Gambierdiscus* and *Fukuyoa* was considered confined to endemic areas in the Pacific and Indian oceans and the Caribbean, between 35°N and 35°S (Adachi and Fukuyo 1979; Taylor 1979; Gillespie et al. 1985; Chinain et al. 2021). Expansion to new areas is supported by increasing reports from subtropical and temperate regions, including the Mediterranean Sea (Aligizaki and Nikolaidis 2008; Tudó et al. 2020b), and the NE Atlantic Macaronesian region, including Selvagens, Madeira (Reverté et al. 2018; Godinho et al. 2023) and the Canary Islands, where high species diversity occurs (Fraga et al. 2011; Fraga and Rodríguez 2014; Fernández-Zabala et al. 2022). Additionally, *Gambierdiscus* DNA was recently detected in the northern Iberian Atlantic (Mengs et al. 2024). In 2015, Silva and co-authors presented evidence for the presence of ciguatoxins in marine invertebrates from the Azores, the northernmost archipelago of Macaronesia (Silva et al. 2015). However, the presence of *Gambierdiscus* remained unreported.

In the summer of 2024, exceptionally high sea surface temperatures (SST) were recorded in this northern Archipelago of the Macaronesia. On August 25, SST reached a maximum daily value of 27.3°C, the highest value recorded at least since 1941. Positive SST anomalies exceeded +2°C across the region, peaking at +2.7°C, which made it the hottest summer in the region in the last 80 yr (IPMA 2024). In the following month, an unusual mortality event involving dusky grouper (*Epinephelus marginatus*) was observed, with affected individuals showing abnormal behavior and external lesions, potentially linked to thermal stress (Gomes et al. 2025). Nevertheless, other hypotheses were considered and, given the limited knowledge of benthic harmful algal bloom (BHAB) species assemblages in the Azores, for the first time in the archipelago, seawater and macroalgal samples were collected from impacted habitats specifically targeting BHABs. At the time of sampling, the only published record of BHAB species in the archipelago was based on phytoplankton surveys conducted along the coast of S. Miguel Island, which reported the presence of *Ostreopsis* cf *ovata*, *O.* cf. *siamensis* and *O. heptagona* (Silva et al. 2010).

The 2024 sampling campaign led to the first detection of *Gambierdiscus* in the Azores Archipelago, with morphological and molecular analyses identifying the isolates as *G. carolinianus*. This record extends the known northern limit of the genus in the Atlantic Ocean beyond 39°N and provides new insights in the understanding of *Gambierdiscus* biogeography.

Materials and methods

Study area

The Azores Archipelago is the most remote island group in the North Atlantic Ocean, located between 36°55′–39°43′N and 24°46′–31°16′W. The archipelago lies approximately 1500 km west of mainland Portugal and about 2000 km east of North America. The islands are distributed along a WNW–ESE axis on the triple junction of the Eurasian, African, and North American tectonic plates.

The Azores region holds significant scientific interest due to its critical role in North Atlantic circulation and biological enrichment across the Mid- and North Atlantic. Oceanographically, the area is an oceanic confluence zone shaped by major North Atlantic circulation systems (Caldeira and Reis 2017). To the west, the Gulf Stream, originating in the Gulf of Mexico and flowing northeast along the eastern coast of the United States, splits near the Grand Banks into the North Atlantic Current (NAC) and the Azores Current (AzC). The NAC flows north of the archipelago, transporting warm, saline waters poleward, while the AzC moves eastward south of the islands, delineating the northern boundary of the subtropical gyre. The interaction between these currents generates a pronounced mesoscale eddy field, characterized by cyclonic and anticyclonic eddies propagating around the islands.

Sampling and culture conditions

Five macroalgal samples targeting dominant macroalgae species were collected by scuba diving in September 2024 off Corvo Island, Western Group (39°40′N, 31°06′W), and Faial Island, Central Group (38°34′N, 28°42′W), at 15 and 25 m depth, respectively (Fig. 1). At the time of sampling, seawater temperature and salinity were 23.2°C and 36 PSU at Corvo, and 23.7°C and 36 PSU at Faial, respectively (IPMA 2024). After collection, the epiphytic assemblage was recovered by vigorously shaking the blades inside a zip-lock plastic bag with local seawater and subsequently sieving the water through a 20 μm nylon sieve and retrieving the retained cells.

In the laboratory, 14 single cells were isolated by micropipetting under an inverted light microscope (Zeiss Axioscope,

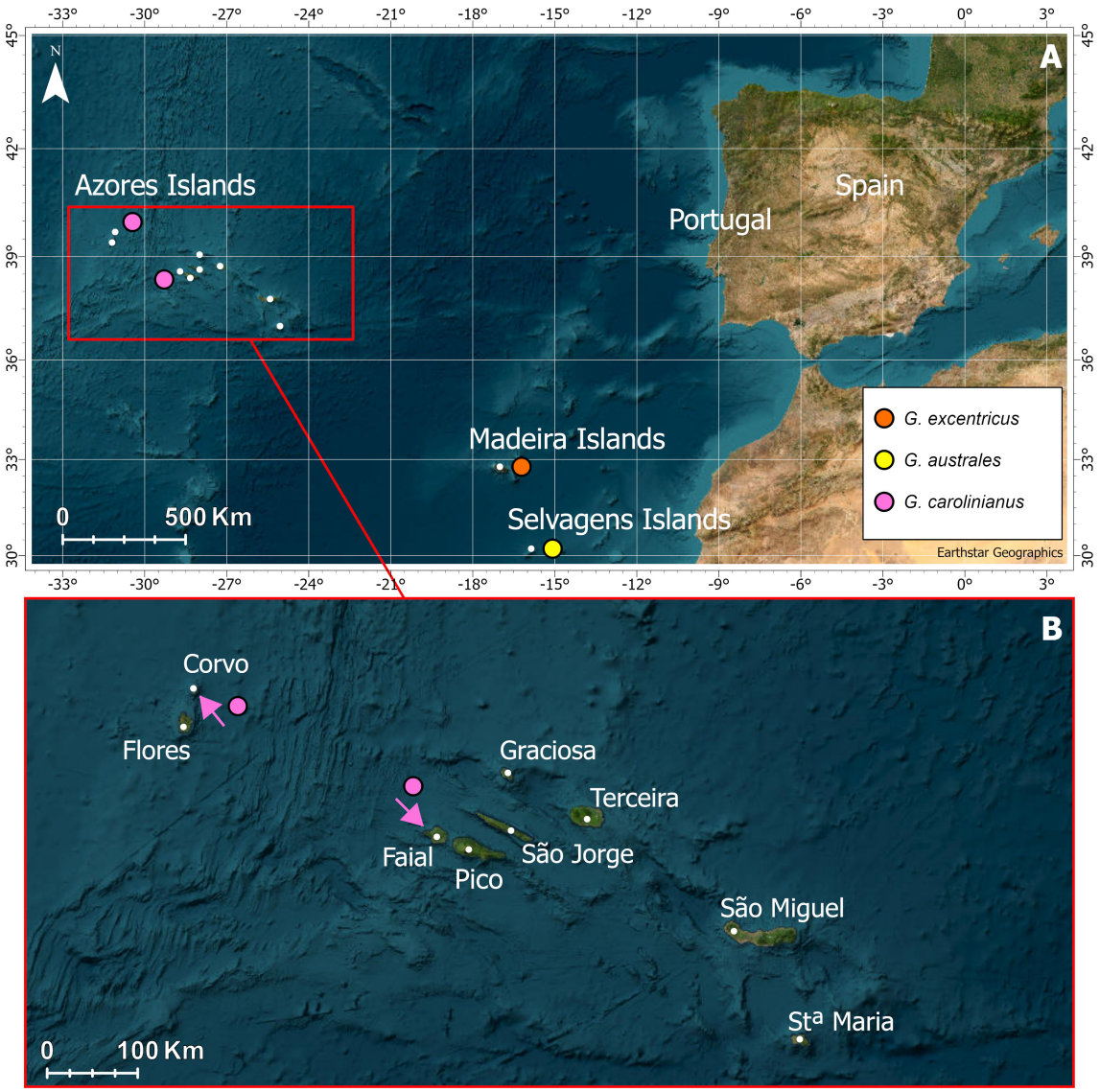

Fig. 1. The Azores Archipelago and geographic reports of *Gambierdiscus* species in the Portuguese Atlantic Islands (**A**), and detailed distribution in the Azores archipelago (**B**) (Portugal, NE Atlantic).

Germany), from which two clonal strains were successfully established in culture and further characterized, namely IPMA-GBAZ01 and IPMA-GBAZ02 (Supporting Information Table S1). The strains were grown in a vented T-flask (50 cm^2) (SARSTEDT, Germany) containing 25 mL of modified K medium prepared without adding tris-base, silicate solution, and copper solution (Keller and Guillard 1985). The cells were incubated at 24°C under a 12 : 12 h light–dark cycle, with a photon flux density of approximately 100 $\mu mol\ m^{-2}\ s^{-1}$ (Fitoclima 600PL, Aralab, Portugal).

For growth rate analysis, each strain was monitored in three replicate cultures. Sub-samples of 3 mL were collected every 5 d over a 25-d period. Growth rates were calculated using cell counts obtained between day 5 and day 15 after subculturing. Growth rates (divisions per day, k) were calculated according to (Wood et al. 2005):

$$k\left(\text{div d}^{-1}\right) = \frac{log_2\left(\frac{N_t}{N_0}\right)}{\Delta t} \quad (1)$$

where N_0 is the population size at the beginning of the time interval, N_t the population size at the end of the time interval, and Δt the length of the time interval.

Morphological analysis

Morphological observations were conducted by light microscopy (LM) and scanning electron microscopy (SEM), using the strain IPMA-GBAZ01. LM observations were

performed with a Leica DMi8 inverted microscope (Leica Microsystems CMS GmbH, Wetzlar, Germany), equipped with differential interference contrast optics and fluorescence at 400× magnification. Digital images were captured using a Leica DFC7000 T camera (Leica Microsystems CMS GmbH, Wetzlar, Germany). Thecal plates were visualized by staining with Calcofluor White (Fluka Analytical, Sigma-Aldrich, Germany). Thecal plate nomenclature followed the Kofoidian tabulation system, as applied to *Gambierdiscus* by Fraga et al. (2011), where thecal plates are grouped into positional series, providing a standardized framework for taxonomic identification. Morphometric measurements included dorso-ventral depth (DV), width (W), antero-posterior length (L), apical pore plate dimensions, and specific plate ratios following Bravo et al. (2019) (Table 1, Fig. 2, Supporting Information Table S2). Measurements were performed using Leica Application Suite X imaging software (LAS X; Leica Microsystems, Wetzlar, Germany). All morphometric measurements are available on Zenodo (Barbosa et al. 2026a).

Considering SEM analysis, sample preparation followed a protocol previously optimized for *Gambierdiscus* cells. A 1.5 mL subsample of culture was fixed overnight at 4°C in 1% glutaraldehyde. Samples were centrifuged at 2700*g* for 3 min (Eppendorf 5810, New York, USA), and the supernatant was discarded. The cell pellet was washed in sterile seawater followed by five centrifugation-resuspension steps in sterilized demineralized water. Final pellets (0.1–0.2 mL) were air-dried overnight on carbon adhesive SEM stubs. Images were acquired using a Hitachi TM4000 SEM (Hitachi High-Tech Corporation, Tokyo, Japan) under standard vacuum conditions. Metal coating was not applied, as the contrast of dried cellulose thecas was found to be sufficient for SEM imaging. For surface imaging, the SE (surface electrons) detector at 5 kV (Mode 3) was used; for general cell morphology, the BSE (back-scattering electrons) or mix of SE and BSE detectors typically at 10 kV (Mode 3) was employed.

DNA extraction and amplification

For DNA extraction, 5 mL of culture (exponential phase) were centrifuged at 2700*g* for 5 min (Eppendorf 5810, New York, USA). The pellet was stored at −20°C until extraction. Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. PCR amplification of the D8–D10 region of the LSU rRNA gene was performed with FD8/RB primers (Chinain et al. 1999) and the 2× Xpert Fast Hotstart DNA Polymerase Master Mix (GRiSP, Portugal) in 20 μL reactions. Cycling conditions: initial denaturation step at 95°C for 3 min; 35 cycles at 95°C for 15 s, 46°C for 15 s, 72°C for 1 min; final extension at 72°C for 3 min (Bioer TC-24/H thermal cycler, Bioer Technology Co. Ltd, China). Amplicons were size-verified by agarose gel electrophoresis and purified using AMPure XP Beads (Beckman Coulter, USA). Sequencing was performed by a commercial provider (StabVida, Portugal).

Phylogenetic analysis

Sequences were manually edited using BioEdit v7.7.1 (Hall 1999) and compared with *Gambierdiscus* sequences available in NCBI GenBank. The sequences obtained in this study were deposited in GenBank (Supporting Information Table S1). For phylogenetic analysis of the D8–D10 LSU rDNA region, isolates IPMA-GBAZ01 and IPMA-GBAZ02 were aligned with 40 *Gambierdiscus* reference sequences, including sequences for each described species whenever available and all available *G. carolinianus* sequences covering this region. *Fukuyoa paulensis* was used as outgroup.

A multi sequence alignment (MSA) performed with the MAFT algorithm (Katoh et al. 2017) was subjected to a phylogenetic analysis in MEGA12 (Kumar et al. 2024). The MSA was manually trimmed to equal sequence length and

Table 1. Morphological characteristics of *Gambierdiscus carolinianus* from the Azores Islands determined using epifluorescence microscopy with calcofluor staining. Data include mean value ± standard deviation and range.

G. carolinianus IPMA-GBAZ01		Mean ± SD	Range
Dorso-ventral axis (depth) (μm)		74.38 ± 6.93	57.33–86.88 ($N = 69$)
Width (μm)		81.77 ± 6.58	66.62–95.30 ($N = 69$)
Antero-posterior axis (length) (μm)		49.81 ± 5.39	38.9–49.05 ($N = 12$)
Ratio DV:W		0.91 ± 0.06	0.71–1.06 ($N = 69$)
Apical pore plate	Length (μm)	9.09 ± 0.65	7.47–10.9 ($N = 26$)
	Width (μm)	6.18 ± 0.98	4.35–9.45 ($N = 26$)
	L : W	1.5 ± 0.98	1.09–2.02 ($N = 26$)
2′′′′ plate	Length (μm)	49.4 ± 5.57	30.49–57.15 ($N = 31$)
	Width (μm)	37.62 ± 0.98	21.69–44.03 ($N = 31$)
	L : W (R3)	1.32 ± 0.09	1.19–1.61 ($N = 31$)
Ratio	2′/1″ : 2′/3″ (R1)	0.54 ± 0.08	0.34–0.66 ($N = 24$)

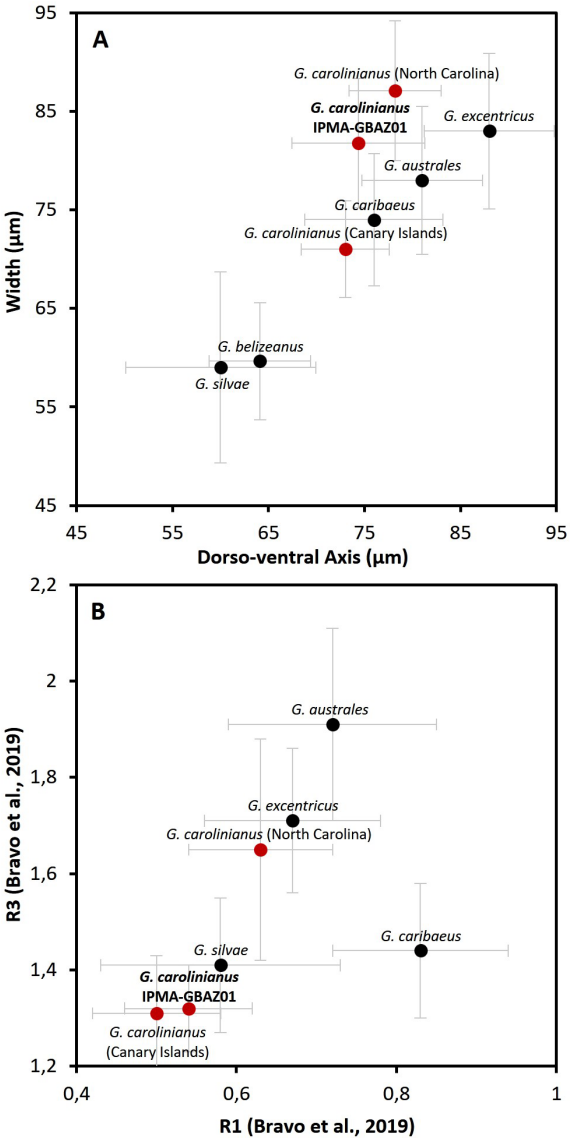

Fig. 2. Morphometry of *Gambierdiscus carolinianus* IPMA-GBAZ01 in comparison with *Gambierdiscus* species occurring in Macaronesia (Bravo et al. 2019; Tudó et al. 2020a) and with the original description of *G. carolinianus* (Litaker et al. 2009). (**A**) Comparison of dorsoventral axis (depth) vs. width values (mean ± SD). (**B**) Comparison of R1 (2′/1″ : 2′/3″) and R3 (2‴Plate L : W) ratios (mean ± SD) (Bravo et al. 2019).

elimination of internal gaps involving more than 50% of the sequences, yielding a final MSA with 782 positions.

The phylogenetic tree was reconstructed using the Maximum Likelihood (ML) method. The Jukes-Cantor model of nucleotide substitutions was applied (Jukes and Cantor 1969). Bootstrap support was assessed with 3000 replicates. Gaps and missing data were included in the analysis by using all sites. The final alignment used for phylogenetic analysis was deposited in Zenodo (Barbosa et al. 2026b).

Results

Specimens of the genus *Gambierdiscus* were found in samples from Corvo and Faial islands (Fig. 1). The strain IPMA-GBAZ01 was isolated from samples of the macroalga *Zonaria tournefortii* (Phaeophyceae), collected in Corvo Island. The strain IPMA-GBAZ02 was isolated from a mixed algal community collected in Faial Island, composed of *Asparagopsis taxiformis* (Rhodophyta) and *Rugulopteryx okamurae* (Phaeophyceae), two non-native species in the Azores (Supporting Information Table S1). Other common co-occurring benthic dinoflagellate taxa were also observed in the assemblages, including representatives of the genera *Prorocentrum, Ostreopsis, Coolia*, and *Amphidinium*. Under studied culture conditions, strains IPMA-GBAZ01 and IPMA-GBAZ02 showed growth rates of 0.20 ± 0.04 and 0.18 ± 0.03 div d^{-1} ($n = 3$), respectively (Supporting Information Table S1).

Morphology

The cells of strain IPMA-GBAZ01 investigated in this study displayed the characteristic lenticular morphology of the genus *Gambierdiscus*, with pronounced anteroposterior compression. In apical view, the cells were rounded to slightly oblong, while in lateral view they exhibited a symmetrical and flattened profile. The cell surface appeared smooth under light microscopy (LM), and the arrangement of thecal plates and pores was consistent with the typical *Gambierdiscus* pattern. Morphometric analysis (Table 1, Fig. 2) revealed that the cells had an average dorso-ventral depth of 74.38 ± 6.93 µm (range: 57.33–86.88 µm; $n = 69$), a width of 81.77 ± 6.58 µm (range: 66.62–95.30 µm; $n = 69$), and an antero-posterior length of 49.81 ± 5.39 µm (range: 38.9–49.05 µm; $n = 12$). The depth-to-width ratio averaged 0.91 ± 0.06 (range: 0.71–1.06), indicating a shape slightly wider than deep.

The thecal plate tabulation of the strain IPMA-GBAZ01 had the characteristic plate formula of *Gambierdiscus*: Po, 4′, 6″, 6c, ?s, 5‴, 1p, 2⁗. The second apical plate (2′) was the largest of the apical series and hatchet-shaped (Fig. 3A). Within the precingular series (1″–6″), pate 2″ was notably the largest. The 6″ plate was small and positioned near the sulcal area. In antapical view (Fig. 3B), five postcingular plates (1‴–5‴) and the large antapical plate 2⁗ were evident. The Sp plate and 5‴ formed the posterior boundary of the sulcal region. In ventral view (Fig. 3C), sulcal plates Sa, Sda, Sdp, Ssp and Ssa were visible, forming a deep sulcal cavity. The elliptical apical pore complex (Fig. 3D) had a fishhook-shaped slit and was surrounded by Plates 2′, 3′ and 4′.

Phylogeny

The Maximum Likelihood (ML) phylogenetic tree inferred from the D8–D10 region of the LSU rRNA gene encompassed all recognized species of *Gambierdiscus* with available sequences of the target region, including the sequences

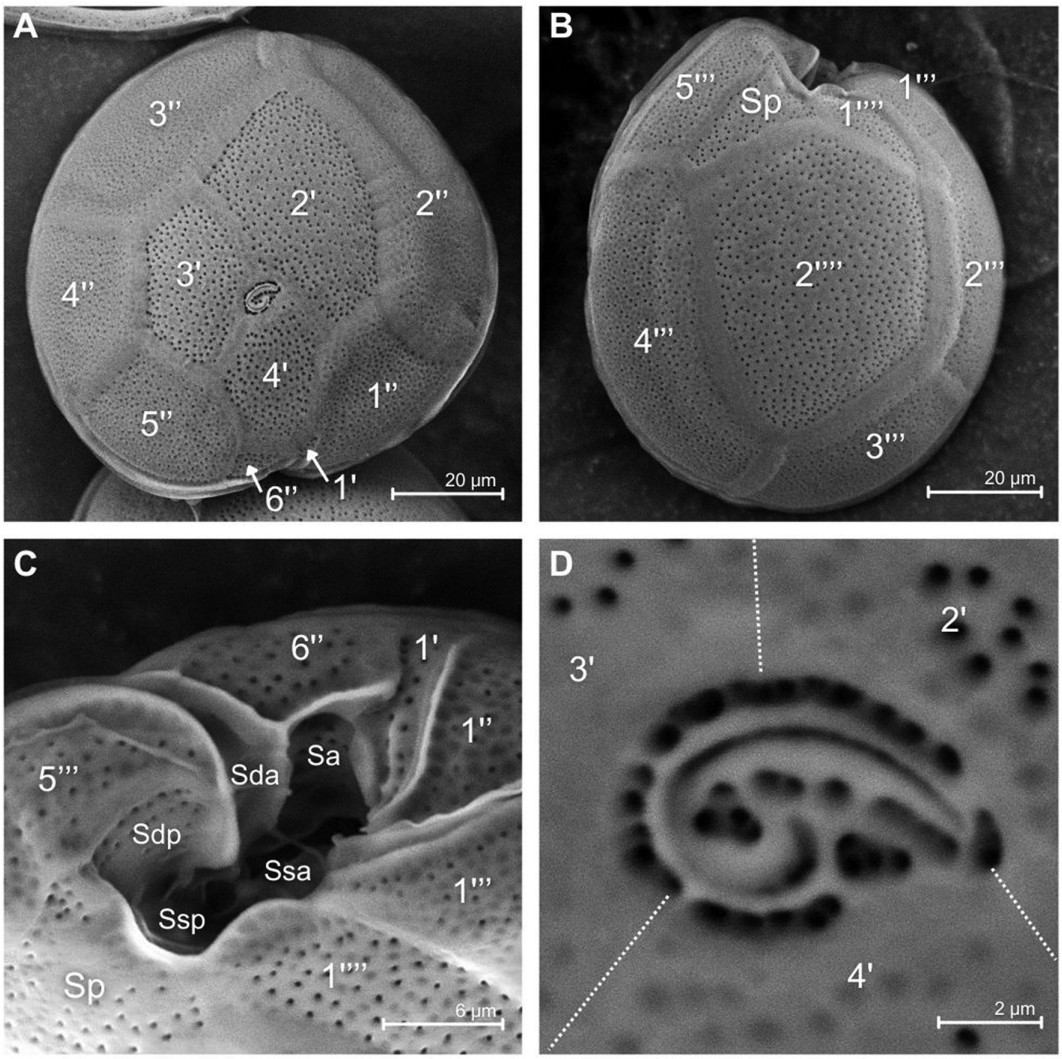

Fig. 3. Scanning electron microscopy (SEM) images of *Gambierdiscus carolinianus* (strain IPMA-GBAZ01). (**A**) Apical view, (**B**) Antapical view, (**C**) Ventral view, (**D**) Po Plate.

generated in this study (Fig. 4). Bootstrap support values for the main nodes exceeded 65%, reinforcing the robustness of the inferred relationships. Our isolates clustered strongly (BS = 100) in the *G. carolinianus* cluster (*Gambierdiscus* clade III), showing no affinity with the *Gambierdiscus* clades I and II, as well as with *Fukuyoa paulensis* (outgroup).

The *G. carolinianus* clade appeared mainly divided into two well-supported subclades, as the isolate IPMA-GBAZ02 groups apart. One subclade (A) groups strains from tropical to subtropical regions (approximately 20–28°N): Bahamas, Mexico, Canary Islands. The other subclade (B) clusters the Azorean isolate IPMA-GBAZ01 obtained in this study, and the other strains, most of them from temperate and subtropical waters at higher latitudes (approximately 32–39°N): North Carolina (USA) and the Bermuda Islands. However, the strain BZ08066 from Belize (17°N), also clusters in this sub-clade. The genetic distances between all these isolates ranged from 0% to 2% (data not shown).

Discussion

Cell morphology

The morphology of the studied strains fitted the original description of *G. carolinianus* (Litaker et al. 2009), with an average depth of 78.2 ± 4.8 μm, a width of 87.1 ± 7.1 μm, and a similar depth-to-width ratio (0.90) (Supporting Information Table S2). The deviations in length and plate ratios could reflect variability typically observed in cultured cells.

Considering the *Gambierdiscus* species previously reported from Macaronesia (Bravo et al. 2019; Tudó et al. 2020a), several key morphometric parameters overlap among *G. carolinianus*, *G. australes*, *G. caribaeus*, and *G. excentricus*

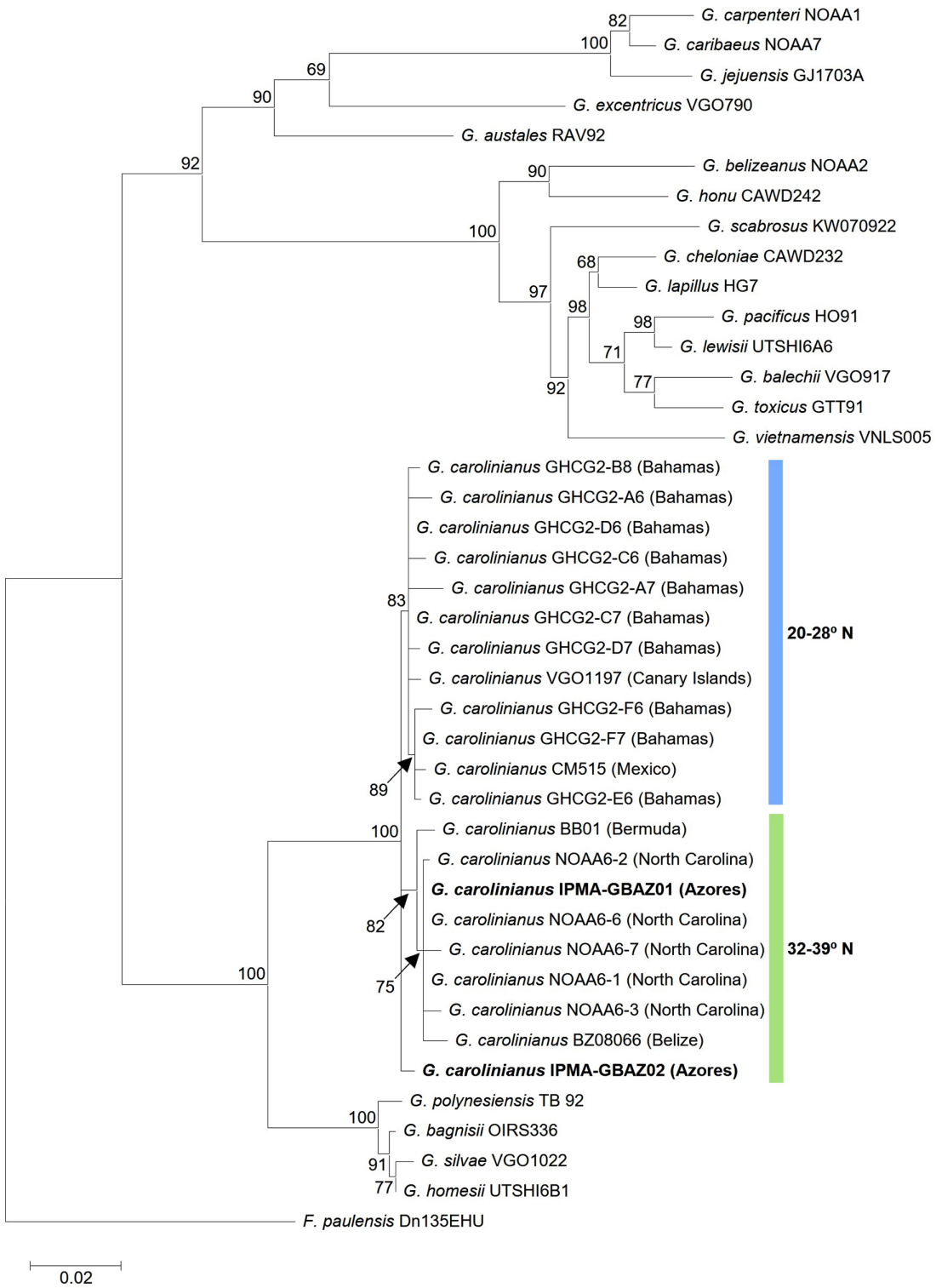

Fig. 4. Phylogenetic tree of *Gambierdiscus*, including *G. carolinianus* (IPMA-GBAZ01 and IPMA-GBAZ02) inferred from D8-D10 LSU rDNA sequences, using the Maximum Likelihood method and Jukes-Cantor model of nucleotide substitutions. The tree with the highest log likelihood (−4485.18) is shown. Numbers on the nodes represent bootstrap values, and only values above 65% are shown. Sequences obtained in this study are in bold. The sequences used to construct the phylogenetic tree are presented with the strain code and isolation region. Scale bar refers to nucleotide substitutions per site. *G. carolinianus* BZ08066 represents an exception to the latitudinal interval associated with subclade B lineage.

(Fig. 2). Discrimination among species was only possible when the different parameters were considered in combination. In addition, *G. carolinianus* has high intraspecific morphological variability (Fig. 3), a pattern that is expected within the genus and has been previously reported (Litaker et al. 2009). These observations underscore the limitations of morphology alone for reliable species-level identification. As demonstrated by Litaker et al. (2009), subtle morphological distinctions often require complementary molecular methods to achieve reliable taxonomic resolution.

Molecular identification and phylogenetic analysis

The phylogenetic analysis of the *Gambierdiscus* confirmed a well-supported topology, consistent with previously published phylogenies (Fig. 4) (Godinho et al. 2023; Kretzschmar et al. 2019; Nguyen-Ngoc et al. 2023). *Gambierdiscus carolinianus* segregates with strong support from a sister clade, also highly supported, clustering *G. polynesiensis, G. holmesii, G. silvae*, and the recently described *G. bagnisii* (Murray et al. 2025). Both clades group in a highly supported main clade, reflecting their close phylogenetic relationship.

Within the *G. carolinianus* cluster, the Azorean isolates did not group with the available Canary Islands sequence despite geographic proximity (Fig. 4). Instead, the isolate IPMA-GBAZ01 showed closer affinity with the isolates from North Carolina. This pattern is potentially related to the connectivity offered by the Gulf Stream and the North Atlantic Current, which are known to transport warm, nutrient-rich waters and associated biota from the western Atlantic toward the northeastern Atlantic (Etter and Bower 2015; Mccarthy et al. 2017). Additionally, the Azores Current, which flows east-southeast south of the Azores, and the broader North Atlantic Subtropical Gyre, which bathes both the Azores and Bermuda, may facilitate long-distance dispersal and connectivity between these archipelagos (Sala et al. 2016). In contrast, the Canary Islands are more influenced by the southward-flowing Canary Current and the North African upwelling regime, which could limit genetic exchange with northern populations despite relatively similar environmental conditions across the Macaronesian islands.

On the other hand, shipping routes have been recognized as potential vectors for the dispersal of harmful algal bloom (HAB) species, including benthic dinoflagellates, through ballast water discharge and biofouling (Chainho et al. 2015; Wells et al. 2020). Furthermore, recent studies have shown the capacity of plastics and other floating debris to act as colonization substrates for harmful microalgae, which could further enhance their dispersal across oceanic basins (Leite et al. 2022; Pires et al. 2025). These vectors, combined with oceanographic features, could offer a possible explanation for the presence of *G. carolinianus* in the Azores islands.

Northern expansion of *Gambierdiscus*

From a different perspective, the presence of *Gambierdiscus* in the Azores reported in this study represents a northward extension of its known distribution within the Macaronesian region and the North Atlantic Ocean. It aligns with recent reports of the expansion to temperate and subtropical Atlantic regions of CF and the associated causative species (Tester et al. 2020; Chinain et al. 2021). *Gambierdiscus* species have been documented in the Canary Islands (Fraga et al. 2011; Rodríguez et al. 2017), the Selvagens Islands (Godinho et al. 2023), Madeira (Kaufmann and Böhm-Beck 2013; Hoppenrath et al. 2019; Silva et al. 2022), the Balearic Islands in the western Mediterranean (Tudó et al. 2020a), and Morocco (Ennaffah and Chaira 2015). In the Selvagens Islands, only *G. australes* was identified, with genetic differentiation among strains suggesting localized evolutionary processes (Godinho et al. 2023), whereas in Madeira only *G. excentricus* was reported (Hoppenrath et al. 2019). By contrast, the Canary Islands host a diverse assemblage of species, including *G. australes*, *G. belizeanus*, *G. caribaeus*, *G. carolinianus*, *G. excentricus*, and *G. silvae* (Rodríguez et al. 2017; Tudó et al. 2020a). Notably, *G. carolinianus* has also been reported from several higher latitude western Atlantic locations, including Bermuda, North Carolina, and the Flower Garden Banks (Litaker et al. 2010), suggesting that this species may be particularly well suited to persist across a broader thermal range than many tropical congeners.

The unusually high sea surface temperatures recorded during summer 2024, with August SST anomalies reaching 2.7°C (IPMA 2024), may have facilitated the detection of *G. carolinianus* in the Azores, but are unlikely to fully explain its occurrence at such high latitudes. Because this study is based on a single sampling campaign conducted in September 2024, during relatively warm-water conditions, it cannot determine whether *G. carolinianus* populations are established year-round or whether they are able to persist through winter temperatures in the region. This is particularly relevant given that regional SST typically ranges from 15 to 16°C in winter to 24–25°C in summer, approaching the lower growth limits reported for *G. carolinianus* in previous culture-based experiments (Tester et al. 2020; Xu et al. 2016). Therefore, this occurrence of *G. carolinianus* in a temperate archipelago should be interpreted with caution until seasonal sampling and culture-based experiments determine its local temperature optima and tolerance limits.

Further work is needed to determine whether *G. carolinianus* in the Azores reflects a recent introduction, facilitated by ocean warming or human-mediated transport, or a previously undetected component of regional benthic dinoflagellate assemblages. Its detection during the first targeted BHAB survey in the archipelago is noteworthy and highlights the urgent need for systematic assessment of BHAB diversity, distribution and potential toxicity in the region. Future seasonal surveys, including winter sampling, together with controlled growth experiments using the Azorean isolates, will be necessary to assess whether this species can persist under local

temperature regimes and whether populations are established in the archipelago. Reports of ciguatoxins in starfish collected on São Miguel Island (Azores) suggest that *Gambierdiscus* spp. have been present at least since 2013 (Silva et al. 2015). Regarding the dusky grouper mortality event in Azores, which triggered the present investigation, it was not related to any contamination by dinoflagellate biotoxins. The cause was ultimately attributed to a viral pathogen (Gomes et al. 2025).

Conclusion

This study provides the first record of the genus *Gambierdiscus* in the Azores Archipelago, expanding its known distribution in the North Atlantic to latitudes above 39°N. Our results show one Azorean isolate identified as *G. carolinianus* exhibit morphological and genetic features consistent with West Atlantic populations of this species. Pending further investigation, *G. carolinianus* should be considered cryptogenic in the Azores.

Occurrences of *Gambierdiscus* and reports of ciguatera poisoning (CP) in Portugal have so far been restricted to Madeira and the southern Selvagens Islands. The detection of *Gambierdiscus* in the Azores therefore warrants further investigation into local population dynamics, substrate preferences, toxin profiles, and potential CP transmission pathways. These findings highlight the need for implementing monitoring and risk assessment programs to address the potential public health implications of *Gambierdiscus*' presence in the Azores archipelago.

Author Contributions

Miguel Barbosa: Conceptualization, Methodology, Investigation, Writing—original draft. Catarina Churro: Conceptualization, Methodology, Investigation, Writing—Review and Editing. Inês Gomes: Investigation, Writing—Review and Editing. Sergei Danchenko: Methodology, Investigation. Maria Filomena Caeiro: Methodology, Investigation, Writing—Review and Editing. Pedro Afonso: Investigation, Supervision, Writing—Review and Editing. Ana Amorim: Conceptualization, Supervision, Writing—Review and Editing. Pedro Reis Costa: Conceptualization, Supervision, Writing—Review and Editing.

Acknowledgments

This study was supported by the European Food Safety Authority (EFSA), under the EuroCigua II project (GP/EFSA/KNOW/2022/03). Additional support was provided by Portuguese national funds from FCT—Foundation for Science and Technology, through projects UIDB/04326/2020 (https://doi.org/10.54499/UIDB/04326/2020), LA/P/0101/2020 (https://doi.org/10.54499/LA/P/0101/2020), CIIMAR (UIDB/04423/2020 and UIDP/04423/2020), UID/4292/2025 awarded to Centro de Ciências do Mar e do Ambiente (MARE), LA/P/0069/2020 (https://doi.org/10.54499/LA/P/0069/2020) granted to the Associate Laboratory ARNET, UIDB/00329/2020 awarded to CE3C—Centre for Ecology, Evolution and Environmental Changes & CHANGE—Global Change and Sustainability Institute, and UIDB/05634/2025 and UIDP/05634/2025. This work was also supported by the Azorean Regional Government under the MoniCO Program—Azores Coastal Resources and Environmental Monitoring Program (SRMCT/DRP), and by the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 862428 (MISSION ATLANTIC). This work further benefited from support provided by the "Pacto da Bioeconomia Azul" (Project No. C644915664-00000026), within the WP5 Algae Vertical, funded by the Next Generation EU European Fund and the Portuguese Recovery and Resilience Plan (PRR), under the scope of the incentive line "Agendas for Business Innovation" through the funding scheme C5—Capitalization and Business Innovation. IG was supported by an FCT individual contract (CEECINST/00024/2021), and PA was supported by the MoniCO Program—Azores Coastal Resources and Environmental Monitoring Program.

Conflicts of Interest

None declared.

Data Availability Statement

Data and metadata are available at https://doi.org/10.5281/zenodo.18316252 and https://doi.org/10.5281/zenodo.20646015.

References

Adachi, R., and Y. Fukuyo. 1979. "The Thecal Structure of a Marine Toxic Dinoflagellate *Gambierdiscus toxicus* Gen. Et Sp. Nov. Collected in a Ciguatera-Endemic Area." *Nippon Suisan Gakkaishi* 45: 67–71. https://doi.org/10.2331/suisan.45.67.

Aligizaki, K., and G. Nikolaidis. 2008. "Morphological Identification of Two Tropical Dinoflagellates of the Genera *Gambierdiscus* and *Sinophysis* in the Mediterranean Sea." *Journal of Biological Research-Thessaloniki* 9: 75–82.

Barbosa, M., C. Churro, I. Gomes, et al. 2026a. *Emerging Occurrence of Ciguatera-Causing Gambierdiscus in the Azores: Morphometric Data of Gambierdiscus carolinianus From the Azores*. Zenodo. https://doi.org/10.5281/zenodo.18316252.

Barbosa, M., C. Churro, I. Gomes, et al. 2026b. *Emerging Occurrence of Ciguatera-Causing Gambierdiscus in the Azores: D8–D10 LSU rDNA Sequence Alignments of Gambierdiscus carolinianus and Related Species*. Zenodo. https://doi.org/10.5281/zenodo.20646015.

Bravo, I., F. Rodriguez, I. Ramilo, P. Rial, and S. Fraga. 2019. "Ciguatera-Causing Dinoflagellate *Gambierdiscus* Spp. (Dinophyceae) in a Subtropical Region of North Atlantic Ocean (Canary Islands): Morphological Characterization and Biogeography." *Toxins* 11: 423. https://doi.org/10.3390/TOXINS11070423.

Caldeira, R. M. A., and J. C. Reis. 2017. "The Azores Confluence Zone." *Frontiers in Marine Science* 4: 202943. https://doi.org/10.3389/FMARS.2017.00037/BIBTEX.

Chainho, P., A. Fernandes, A. Amorim, et al. 2015. "Non-indigenous Species in Portuguese Coastal Areas, Coastal Lagoons, Estuaries and Islands." *Estuarine, Coastal and Shelf Science* 167: 199–211. https://doi.org/10.1016/j.ecss.2015.06.019.

Chinain, M., M. A. Faust, and S. Pauillac. 1999. "Morphology and Molecular Analyses of Three Toxic Species of *Gambierdiscus* (Dinophyceae): *G. pacificus*, Sp. Nov., *G. australes*, Sp. Nov., and *G. polynesiensis*, Sp. Nov." *Journal of Phycology* 35: 1282–1296. https://doi.org/10.1046/j.1529-8817.1999.3561282.x.

Chinain, M., C. M. I. Gatti, H. T. Darius, J. P. Quod, and P. A. Tester. 2021. "Ciguatera Poisonings: A Global Review of Occurrences and Trends." *Harmful Algae* 102, no. September 2020: 101873. https://doi.org/10.1016/j.hal.2020.101873.

Costa, P. R., C. Churro, S. M. Rodrigues, et al. 2023. "A 15-Year Retrospective Review of Ciguatera in the Madeira Islands (North-East Atlantic, Portugal)." *Toxins (Basel)* 15, no. 11: 630. https://doi.org/10.3390/toxins15110630.

Ennaffah, B., and K. Chaira. 2015. "First Report of *Gambierdiscus* in Moroccan Atlantic Waters." *Harmful Algae News* 20: 50. http://doi.org/10.5281/zenodo.5110030.

Etter, R. J., and A. S. Bower. 2015. "Dispersal and Population Connectivity in the Deep North Atlantic Estimated From Physical Transport Processes." *Deep Sea Research Part I, Oceanographic Research Papers* 104: 159–172. https://doi.org/10.1016/j.dsr.2015.06.009.

Fernández-Zabala, J., A. Amorim, F. Tuya, R. Herrera, and E. Soler-Onís. 2022. "Playing Hide and Seek: Distribution With Depth of Potentially Harmful Epibenthic Dinoflagellates of Southern EL HIERRO Island, Canary Islands (Ne Atlantic)." *Harmful Algae* 117: 102271. https://doi.org/10.1016/j.hal.2022.102271.

Fraga, S., and F. Rodríguez. 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. https://doi.org/10.1016/j.protis.2014.09.003.

Fraga, S., F. Rodríguez, A. Caillaud, J. Diogène, N. Raho, and M. Zapata. 2011. "*Gambierdiscus excentricus* Sp. Nov. (Dinophyceae), a Benthic Toxic Dinoflagellate From the Canary Islands (NE Atlantic Ocean)." *Harmful Algae* 11: 10–22. https://doi.org/10.1016/j.hal.2011.06.013.

Gillespie, N. C., M. J. Holmes, J. B. Burke, and J. Doley. 1985. "Distribution and Periodicity of *Gambierdiscus toxicus* in Queensland, Australia." In *Toxic Dinoflagellates*, edited by D. M. Anderson, A. W. White, and D. G. Baden, 183–188. Oxford: Elsevier.

Godinho, L., L. Soliño, C. Churro, et al. 2023. "Distribution, Identification and Cytotoxicity of *Gambierdiscus* (Dinophyceae) in the Atlantic Selvagens Islands (Madeira, Portugal): A Ciguatera Gateway to Europe." *European Journal of Phycology* 58: 156–168. https://doi.org/10.1080/09670262.2022.2086710.

Gomes, I., A. Novoa-Pabon, M. Abbadi, et al. 2025. "Marine Heatwave Associated Betanodavirus Outbreak in Wild Groupers of the Azores." *Frontiers in Marine Science* 12. https://doi.org/10.3389/fmars.2025.1712250.

Gouveia, N., V. Timóteo, S. C. Ferreira, P. R. Costa, and R. Câmara. 2026. "Emerging Ciguatera Fish Poisoning in the Archipelago of Madeira (Portugal): Epidemiology and Clinical Profile." *Food Research International* 231: 118731. https://doi.org/10.1016/j.foodres.2026.118731.

Hall, T. A. 1999. "BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT." *Nucleic Acids Symposium Series* 41: 95–98. https://www.academia.edu/download/29520866/1999hall1.pdf.

Hoppenrath, M., A. L. Kretzschmar, M. J. Kaufmann, and S. A. Murray. 2019. "Morphological and Molecular Phylogenetic Identification and Record Verification of *Gambierdiscus excentricus* (Dinophyceae) From Madeira Island (NE Atlantic Ocean)." *Marine Biodiversity Records* 12, no. 1: 1–9. https://doi.org/10.1186/S41200-019-0175-4.

IPMA. 2024. Boletim Climatológico, Agosto 2024, Região Autónoma dos Açores. https://www.ipma.pt/resources.www/docs/im.publicacoes/edicoes.online/20240928/otpQzlqWOgqEhDYLtkiO/cli_20240801_20240831_pcl_mm_az_pt.pdf.

IPMA – Portuguese Institute for Sea and Atmosphere. 2024. "WWW Document". https://www.ipma.pt/en/maritima/sst/.

Jukes, T. H., and C. R. Cantor. 1969. "Evolution of Protein Molecules." In *Mammalian Protein Metabolism*, edited by H. N. Munro, 21–132. New York: Academic Press.

Katoh, K., J. Rozewicki, and K. D. Yamada. 2017. "MAFFT Online Service: Multiple Sequence Alignment, Interactive Sequence Choice and Visualization." *Briefings in Bioinformatics* 20, no. 4: 1160–1166. https://doi.org/10.1093/bib/bbx108.

Kaufmann, M., and M. Böhm-Beck. 2013. "*Gambierdiscus* and Related Benthic Dinoflagellates From Madeira Archipelago (NE Atlantic)." *Harmful Algae News* , no. 47: 18–19. https://doi.org/10.5281/zenodo.5159137.

Keller, M. D., and R. R. L. Guillard. 1985. "Factors Significant to Marine Dinoflagellate Culture." In *Toxic Dinofagellates*, edited by D. M. Anderson, A. W. White, and D. G. Baden, 113–116. New York: Elsevier.

Kretzschmar, A. L., M. E. Larsson, M. Hoppenrath, M. A. Doblin, and S. A. Murray. 2019. "Characterisation of Two Toxic *Gambierdiscus* Spp. (Gonyaulacales, Dinophyceae) From the Great Barrier Reef (Australia): *G. lewisii* Sp. Nov. and *G. holmesii* Sp. Nov." *Protist* 170: 125699. https://doi.org/10.1016/j.protis.2019.125699.

Kumar, S., G. Stecher, M. Suleski, M. Sanderford, S. Sharma, and K. Tamura. 2024. "MEGA12: Molecular Evolutionary Genetic Analysis Version 12 for Adaptive and Green Computing." *Molecular Biology and Evolution* 41, no. 12. https://doi.org/10.1093/molbev/msae263.

Leite, I. P., A. Menegotto, P. C. Lana, and L. L. M. Júnior. 2022. "A New Look at the Potential Role of Marine Plastic

Debris as a Global Vector of Toxic Benthic Algae." *Science of the Total Environment* 838: 156262. https://doi.org/10.1016/j.scitotenv.2022.156262.

Litaker, R. W., M. W. Vandersea, M. A. Faust, et al. 2009. "Taxonomy of *Gambierdiscus* Including Four New Species, *Gambierdiscus caribaeus*, *Gambierdiscus carolinianus*, *Gambierdiscus carpenteri* and *Gambierdiscus ruetzleri* (Gonyaulacales, Dinophyceae)." *Phycologia* 48: 344–390. https://doi.org/10.2216/07-15.1.

Litaker, R. W., M. W. Vandersea, M. A. Faust, et al. 2010. "Global Distribution of Ciguatera Causing Dinoflagellates in the Genus *Gambierdiscus*." *Toxicon* 56: 711–730. https://doi.org/10.1016/j.toxicon.2010.05.017.

Mccarthy, G., D. Smeed, S. Cunningham, and C. Roberts. 2017. *Atlantic Meridional Overturning Circulation (AMOC)*. MCCIP Science Review. 15–21. https://doi.org/10.14465/2017.arc10.002-atl.

Mengs, G., R. F. Stern, J. L. Clarke, M. Faith, and L. K. Medlin. 2024. "Mapping Selected Emergent Marine Toxin-Producing Organisms Using Historical Samples With Two Methods (Biosensors and Real-Time PCR): A Comparison of Resolution." *Applied Microbiology* 4, no. 1: 312–328. https://doi.org/10.3390/applmicrobiol4010021.

Murray, S. A., A. Verma, M. Hoppenrath, et al. 2025. "High Ciguatoxin-Producing Gambierdiscus Clade (Gonyaulacales, Dinophyceae) as a Source of Toxins Causing Ciguatera Poisoning." *Science of the Total Environment* 994: 179990. https://doi.org/10.1016/j.scitotenv.2025.179990.

Nguyen-Ngoc, L., J. Larsen, H. Doan-Nhu, et al. 2023. "*Gambierdiscus* (Gonyaulacales, Dinophyceae) Diversity in Vietnamese Waters With Description of *G. vietnamensis* Sp. Nov." *Journal of Phycology* 59: 496–517. https://doi.org/10.1111/jpy.13326.

Pires, E., M. C. S. Kutz, A. B. Mendes, I. C. Riegel-Vidotti, and L. L. Mafra. 2025. "Toxic Plastisphere: How the Characteristics of Plastic Particles Can Affect Colonization of Harmful Microalgae and Adsorption of Phycotoxins." *Journal of Hazardous Materials* 486: 15. https://doi.org/10.1016/j.jhazmat.2024.137019.

Reverté, L., A. Toldrà, K. B. Andree, et al. 2018. "Assessment of Cytotoxicity in Ten Strains of *Gambierdiscus australes* From Macaronesian Islands by Neuro-2a Cell-Based Assays." *Journal of Applied Phycology* 30, no. 4: 2447–2461. https://doi.org/10.1007/S10811-018-1456-8.

Rodríguez, F., S. Fraga, I. Ramilo, et al. 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. https://doi.org/10.1016/j.hal.2017.06.009.

Sala, I., C. S. Harrison, and R. M. A. Caldeira. 2016. "The Role of the Azores Archipelago in Capturing and Retaining Incoming Particles." *Journal of Marine Systems* 154: 146–156. https://doi.org/10.1016/J.JMARSYS.2015.10.001.

Silva, A., V. Brotas, E. Orive, and A. Neto. 2010. "First Records of *Ostreopsis heptagona*, *O.* cf. *Siamensis* and *O.* cf. *Ovata*—In the Azores Archipelago, Portugal." *Harmful Algae News* 42: 6–7. https://doi.org/10.5281/zenodo.7562147.

Silva, M., I. Rodriguez, A. Barreiro, et al. 2015. "First Report of Ciguatoxins in Two Starfish Species: *Ophidiaster ophidianus* and *Marthasterias glacialis*." *Toxins* 7: 3740–3757. https://doi.org/10.3390/toxins7093740.

Silva, T., A. Amorim, A. Sousa, and M. Kaufmann. 2022. "Benthic Harmful Algal Blooms Occurrence in the South Coast of Madeira Island, Portugal, From 2018 to 2020." In *Proceedings of the 19th International Conference on Harmful Algae, La Paz, B.C.S., Mexico*, edited by C. J. Band-Schmidt and C. F. Rodríguez-Gómez, 138–143. International Society for the Study of Harmful Algal Blooms. https://doi.org/10.5281/zenodo.7035055.

Taylor, F. J. R. 1979. "The Description of the Benthic Dinoflagellate Associated With Maitotoxin and Ciguatoxin, Including Observations on Hawaiian Material." In *Toxic Dinoflagellate Blooms*, edited by D. L. Taylor and H. H. Seliger, 71–77. New York: Elsevier Scientific.

Tester, P. A., R. W. Litaker, and E. Berdalet. 2020. "Climate Change and Harmful Benthic Microalgae." *Harmful Algae* 91: 101655. https://doi.org/10.1016/j.hal.2019.101655.

Tudó, À., G. Gaiani, M. R. Varela, et al. 2020a. "Further Advance of *Gambierdiscus* Species in the Canary Islands, With the First Report of *Gambierdiscus belizeanus*." *Toxins* 12, no. 11: 692. https://doi.org/10.3390/toxins12110692.

Tudó, À., A. Toldrà, M. Rey, et al. 2020b. "*Gambierdiscus* and *Fukuyoa* as Potential Indicators of Ciguatera Risk in the Balearic Islands." *Harmful Algae* 99: 101913. https://doi.org/10.1016/J.HAL.2020.101913.

Wells, M. L., B. Karlson, A. Wulff, et al. 2020. "Future HAB Science: Directions and Challenges in a Changing Climate." *Harmful Algae* 91: 101632. https://doi.org/10.1016/j.hal.2019.101632.

Wood, A. M., R. C. Everroad, and L. M. Wingard. 2005. "Measuring Growth Rates in Microalgal Cultures." In *Algal Culturing Techniques*, edited by R. A. Andersen, 269–286. Elsevier Academic Press.

Xu, Y., M. L. Richlen, J. D. Liefer, et al. 2016. "Influence of Environmental Variables on *Gambierdiscus* Spp. (Dinophyceae) Growth and Distribution." *PLoS One* 11: e0153197. https://doi.org/10.1371/journal.pone.0153197.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Submitted 22 January 2026
Revised 19 June 2026
Accepted 09 July 2026