

First report of *Gambierdiscus* in the Western Mediterranean Sea (Balearic Islands)

Gambierdiscus (Dinophyceae) species are benthic dinoflagellates living in marine littoral zones of circumtropical areas and have recently been described in temperate waters [1]. Some species are producers of potent neurotoxins: ciguatoxins (CTXs) and maitotoxins (MTXs). Ciguatoxins are linked to Ciguatera Fish Poisoning (CFP). Ciguatera used to be restricted to tropical and subtropical areas, but since the last decade, it appears to be expanding to more temperate latitudes. For example, outbreaks of ciguatera have been reported in the Canary Islands and Madeira (eastern Atlantic Ocean), where several species of the genus *Gambierdiscus* have been identified [2].

In the Mediterranean Sea, no thorough evidence of cases of ciguatera exist. The only reports of CTX-like toxins in fish, which are not confirmed, were based on the Cigua-Check Fish Poison Test kit (Oceanit, Hawaii), a method that has proved to be unreliable [3]. Nonetheless, *Gambierdiscus* species have been reported in the last decade in Crete and Cyprus (eastern Mediterranean Sea) [4-6]. One species of *Fukuyoa* (*F. paulensis*), a genus that includes species previously included in the genus *Gambierdiscus*, was reported in the Balearic Islands in 2015 [7]. Little is known about diversity, distribution and toxicity of *Gambierdiscus* spp. in the

Mediterranean Sea. The present study confirms the presence of *G. australes* in the two Balearic Islands of Majorca and Minorca, and this constitutes the first report of *Gambierdiscus* genus in the western Mediterranean Sea.

In this study, microalgal samples were collected from macroalgae and rocky substrates in 19 stations in Majorca and Minorca in September 2017. Water temperatures ranged from 24 to 27 °C and salinity from 36.2 to 38.0. In the laboratory, samples were observed under the microscope and individual cells were isolated with micropipettes to establish cultures for morphological and molecular analysis. Calcofluor white stain was used for morphological identification. Cells were observed with a compound microscope equipped with epifluorescence at 630X (Leica DMLB). The *Gambierdiscus* cells observed were anterior-posteriorly compressed. Morphology of the epitheca and the hypotheca is shown in Fig. 1 in which the plate terminology employed follows Fraga and collaborators [8]. The epitheca has a rectangular-shaped 2' apical plate and the P₀ plate is ventrally oriented; the hypotheca has a narrow 2''' plate equivalent to 1p plate in Chinain [9]. The cell surface is smooth. The cell length and width of 62 individuals were measured. Length ranged from 60.9 to 92.3 µm (mean of 75.6 µm) and width

ranged from 64.1 to 90.8 µm (mean of 78.6 µm). The original description [9] described a length range of 76-93 µm and a cell width of 65-84 µm. Further morphological analysis will be performed using electron microscopy.

To facilitate molecular identification to species level, DNA was extracted from individual or a few clonal cells using the Arcturus™ PicoPure™ DNA Extraction Kit (Applied Biosystems, CA, USA). Afterwards, the domain D8-D10 of the LSU rRNA gene was amplified by a Polymerase Chain Reaction (PCR) using the pair of primers FD8 and RB [9], and products were sequenced. The D8-D10 sequences obtained in this study were deposited in GenBank under accession numbers: MG708117- MG708130. DNA sequence analysis of amplified rDNA fragments confirmed that all *Gambierdiscus* spp. corresponded to *G. australes*, which was in accordance with the morphological identification. *G. australes* was present in 10 out of the 19 sampling stations in Majorca and Minorca (Fig. 2), indicating that this species is well established at different locations around the coasts of both islands. It will be important to evaluate the temporal distribution of this species.

The first report of *G. australes* was in the Australes archipelago (French Polynesia). This species is also widely distributed in areas such as New Zealand and the Canary Islands, but it had not been reported yet in the Mediterranean Sea. Some studies mentioned that the spatial expansion of *Gambierdiscus* and CFP may be related to the increase of temperatures caused by climate change

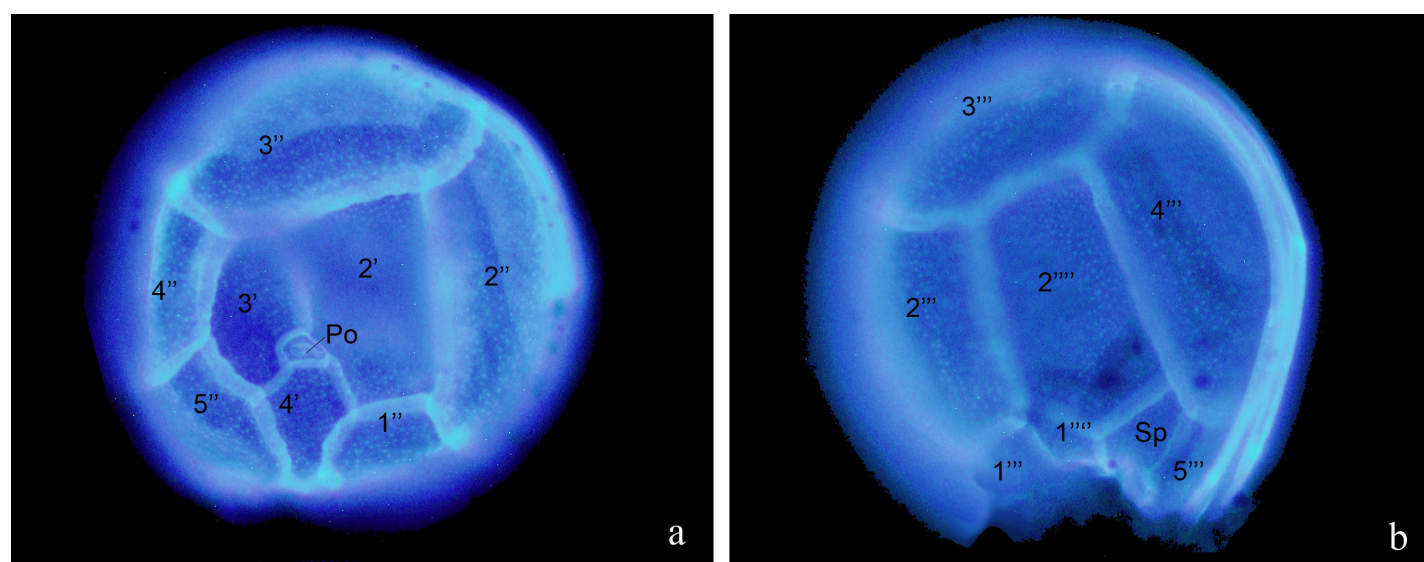


Fig. 1. Epitheca (a) and hypotheca (b) of *Gambierdiscus australes* cells stained with Calcofluor White.

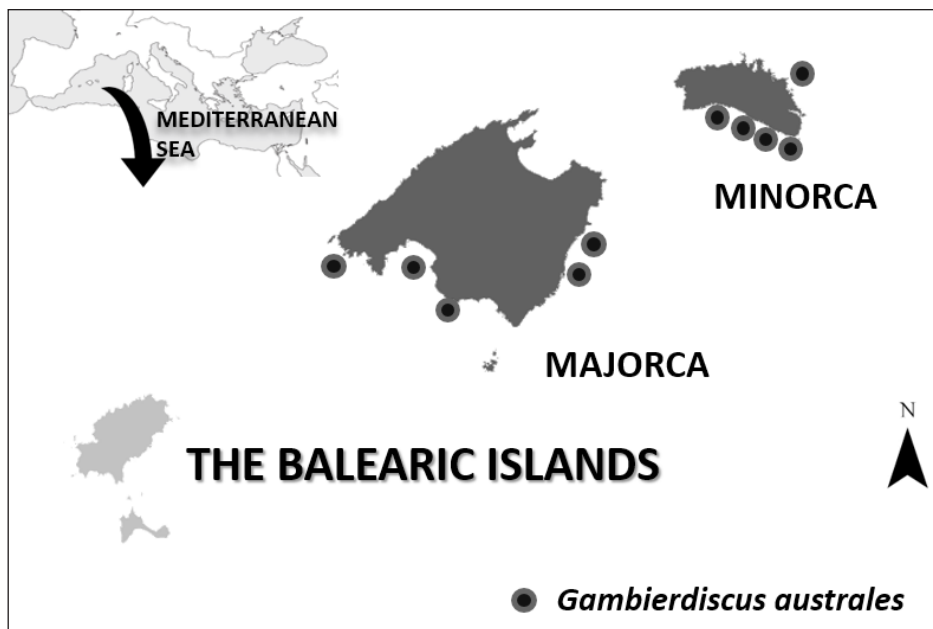


Fig. 2. Locations where *Gambierdiscus australes* was recorded in the Balearic Islands (39° 30'N, 3° 00' E), Spain.

[10]. The Mediterranean Sea, which is a semi-enclosed sea, seems to be one of the regions strongly affected by the rising of temperatures, and this makes this region more suitable for tropical species [11]. A recent study describes a high diversity of *Gambierdiscus* species in the Canary Islands which would suggest that this genus is not a recently introduced taxon in that area, although climate change may contribute to increase the populations density [2].

It will be important to understand the origin of *Gambierdiscus* in the Mediterranean and the effect that climate change may have on *Gambierdiscus* populations. Moreover, it will be neces-

sary to study whether the Balearic Islands could be a new spot of ciguatera.

Improving our knowledge about diversity and toxicity of these benthic dinoflagellates will provide a better characterization of health risks taking into consideration climate change trends.

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Forthcoming Events



ICES-IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD)

The next ICES-IOC WGHABD, hosted by Margarita Fernandez-Tejedor at IRTA, Sant Carles de la Rapita, Tarragona, Spain, will meet from the 23rd – 26th April (inclusive) to work on the following terms of reference:

- A. Deliver National Reports on harmful algal events and bloom dynamics for the year 2017.
- B. Review progress and summary of fish killing algae activities underway during the reporting period 2017–2020.
- C. Updating of the ICES-PICES-IOC Harmful Algal Event Database (HAE-DAT).
- D. Global HAB Status Report for the North Atlantic area: report using data and products generated from HAE-DAT and supplementary time series data as appropriate.
- E. New findings in harmful algal bloom dynamics.
- F. HABs and the EU Marine Strategy Framework Directive (MSFD). Approaches in Europe to including HABs in the assessment of the Good Environmental Status for the EU Marine Strategy Framework Directive.
- G. New results about how physical, chemical and biological interactions control the dynamics of selected harmful micro-algae.
- H. Ciguatera Fish Poisoning (CFP) in the ICES area: review new developments in methodology to research the issue, modelling efforts, risk assessments to protect human health, initiatives in other bodies (IPHAB, PICES etc.).