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Gambierdiscus excentricus sp. nov. (Dinophyceae), a benthic toxic dinoflagellate from the Canary Islands (NE Atlantic Ocean)

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ABSTRACT

A new benthic toxic dinoflagellate is described from the Canary Islands, Spain. *Gambierdiscus excentricus* sp. nov. was isolated from seaweeds growing in tidal ponds and was observed in winter and summer. Its morphology was studied by means of light microscopy (LM) and scanning electron microscopy (SEM); *G. excentricus* is a lenticular species having a Po plate ventrally displaced in relation to other species of the genus *Gambierdiscus*. Phylogenetic trees from large subunit (LSU) of ribosomal RNA gene sequences displayed a topology confirming that *G. excentricus* clustered in its' own group, separated from the rest of *Gambierdiscus* species and with *Gambierdiscus australes* as its closest relative. Pigment composition studied from *G. excentricus* cultures, included peridinin, as the major carotenoid, chlorophyll *a* and the accessory chlorophylls c_1 and c_2. The Neuroblastoma cell-based assays for ciguatoxins (CTX) and maitotoxin (MTX) confirmed *G. excentricus* as a CTX- and MTX-like compounds producer. The finding of a toxic species of *Gambierdiscus* in the Canary Islands may explain the recent reported cases of ciguatera in the area.

1. Introduction

Ciguatera fish poisoning (CFP) is a food-borne disease widespread in tropical and sub-tropical marine areas affecting mainly the Caribbean Sea, Polynesia and other areas in the Pacific, Indian Ocean (Lewis, 2006) although it has been also recently reported in the Canary Islands (Spain), a temperate area (Pérez-Arellano et al., 2005) and in Madeira (Gouveia et al., 2010; Otero et al., 2010). CFP occurs after consumption of fish contaminated with ciguatoxins (CTXs) (Alfonso et al., 2005) but presence of additional toxins has been also proposed and cannot be discarded (Anderson and Lobel, 1987). Marine benthic dinoflagellate of the genus *Gambierdiscus* Adachi et Fukuyo (Adachi and Fukuyo, 1979; Yasumoto et al., 1977) are responsible for the production of CTXs further transmitted through the food web among reef fishes (Alfonso et al., 2005). The same genus may also produce other toxins i.e. maitotoxins (MTXs), gambierol and gambieric acid. MTXs have been found in the viscera of herbivorous fish but are unlikely to produce human illness due to their low capacity for bioaccumulation in fish tissue and low oral potency (Alfonso et al., 2005).

The genus *Gambierdiscus* had been considered monospecific for fifteen years with *Gambierdiscus toxicus* Adachi & Fukuyo (Adachi and Fukuyo, 1979) as the only described species, a thecate gonyaulacoid dinoflagellate anteroposteriorly compressed with lenticular shape. The original plate formula was defined as Po, 3′, 0a, 7″, 6c, 8s, 6‴, 1p, 1⁗ (Adachi and Fukuyo, 1979). *Gambierdiscus belizeanus* Faust (Faust, 1995) was the second species of the genus and it is easily distinguished from *G. toxicus* in having an ornamented theca and some differences in relation to the shapes of plates. The third species being described was *Gambierdiscus yasumotoi* Holmes (Holmes, 1998), a species very different from the others in being globular instead of discoid. Later, the diversity of the genus was found to be much higher than expected and recently seven new species have been added to the genus (Chinain et al., 1999; Litaker et al., 2009) based on morphology and on genetics which helped to find semicryptic species (Litaker et al., 2009; Richlen et al., 2008). Genetic sequences enabled even to find that the original description of *G. toxicus* was based on more than one species making it necessary to describe a new epitype of the species (Litaker et al., 2009).

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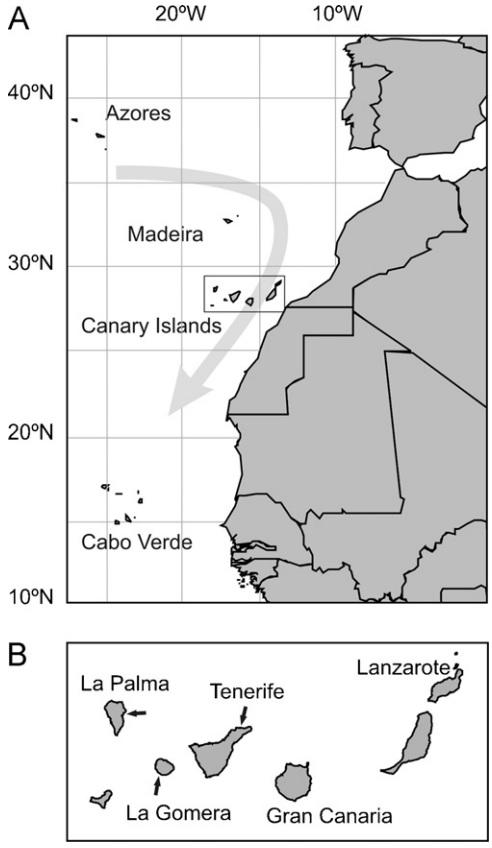

Fig. 1. (a) Map of the East Atlantic archipelagos. (b) Map of the Canary Islands showing the localities where *Gambierdiscus excentricus* was found.

The Canary Islands archipelago (Fig. 1) is bathed by the Canary Current which is the eastern boundary current of the subtropical North Atlantic gyre. The area is characterized by low biomass and very oligotrophic waters where nutrients are depleted in summer (Cianca et al., 2007; Neuer et al., 2007). In this paper we describe *Gambierdiscus excentricus*, a new toxic dinoflagellate found in the Canary Islands coasts and report the presence of *Gambierdiscus* cf. *polynesiensis* in the same area. In addition to the taxonomic description of *G. excentricus*, production of toxins was examined.

2. Materials and methods

2.1. Source of specimens and culture conditions

Samples were collected at several locations in the Canary Islands' archipelago in the NE Atlantic Ocean (Fig. 1): (1) Punta Hidalgo, a rocky shore on the north coast of Tenerife (28°34′N, 16°19′W) on March 28th, 2004; (2) Charca del Conde, La Gomera (28°05′N, 17°20′W) on November 15, 2005; and (3) Playa Las Cabras, La Palma (28°29′N, 17°49′W) on March 13, 2010. Samples of small mixed seaweeds and turf in grooves were collected from tidal pools on the rocks during low tide or from drifting seaweeds very near the coast and placed in plastic bottles and shaken. Afterwards, the gross particles were removed and the remaining seawater was used for cell isolation. Isolation was carried out by a capillary pipette with the aid of a ZEISS Invertoscop D microscope (Carl Zeiss AG, Germany). Isolated cells were incubated in 96 microwells plates in half strength K medium without silicates (Keller et al., 1987) made with seawater from Ría de Vigo (NW Spain) with a salinity adjusted to 34 psu and incubated at 25 °C and a photon irradiance of about 90 μmol m^{-2} s^{-1} of PAR measured with a QSL-100 irradiameter (Biospherical Instruments Inc., San Diego, CA, USA) and at a 14:10 L:D photoperiod. The cultures were transferred to 100 mL Erlenmeyer flasks and to 50 mL polystyrene tissue culture flasks. The cultured strains VGO790, VGO791 and VGO792 were from Tenerife Island and VGO1035 from La Palma Island and all were deposited at the Culture Collection of Microalgae (CCVIEO) of the Instituto Español de Oceanografía in Vigo.

2.2. Light microscopy

Light microscopy observations were carried out under a Leica DMLA light microscope (Leica Microsystems GmbH, Wetzlar, Germany) with phase contrast, differential interference contrast and epifluorescence with an UV lamp. The cultured cells were observed alive or fixed with formalin. For plate pattern identification the cells were stained with Fluorescent Brightener 28 (Sigma–Aldrich, St. Louis, MO, USA) following a modified technique (Fritz and Triemer, 1985). Other cells were dissected, squashing the cells by gently pressing the cover slip over them occasionally with the aid of sodium hypochlorite. Microphotographs were taken with a Canon EOS D60 (Canon Inc., Tokyo, Japan) digital camera. When the depth of field was not enough for the whole object, several pictures were taken at a series of different foci and were then merged using Adobe Photoshop (Adobe Systems Incorporated, San Jose, CA, USA). Cell size was measured by bright field LM on living cells on calibrated digital photographs. Cells stained with Fluorescent Brightener 28 (Sigma–Aldrich, St. Louis, MO, USA) were also observed with a Leica TCS SP5 confocal microscope with UV light (Leica Microsystems GmbH, Wetzlar, Germany) at the CACTI facilities (Universidade de Vigo, Spain). The nucleus was stained using SYBR Green (Molecular Probes, Eugene, OR, USA) following a modified method (Figueroa et al., 2010) as follows: a 10 mL aliquot of culture was fixed with 0.5% paraformaldehyde for 10 min and washed in PBS pH7.0 (Sigma–Aldrich, St. Louis, USA) by centrifugation at $1200 \times g$ during 10 min. Chlorophyll was extracted by resuspending the pellet in 5 mL of cold methanol and then storing the suspension overnight in the refrigerator. The cells were then washed twice in PBS (pH 7.0) as described above and the pellet was stained with a 1:200 solution of SYBR green in PBS 0.01 M (pH7.4) and observed in a Leica DM LA epifluorescence microscope (Leica Microsystems GmbH, Wetzlar, Germany) with blue excitation and photographed with a Canon EOS D60 (Canon Inc., Tokyo, Japan) digital camera. The autofluorescence of the chloroplasts was photographed with a Canon EOS 5D Mark II (Canon Inc., Tokyo, Japan) digital camera.

2.3. Scanning electron microscopy

Five millilitre of exponentially growing cultures were fixed with glutaraldehide (GTA) at a final concentration of 4%. After 2 h at room temperature, they were rinsed three times with distilled water and dehydrated in a series of 30, 50, 75, 95 and 100% EtOH and 100% hexamethyldisilazane (HMDS). After being air dried overnight, they were coated with gold with a K550 X sputter coater (Emitech Ltd., Ashford, Kent, UK) and observed with a Phillips XL30 scanning electron microscope (FEI Company, Hillsboro, OR, USA).

2.4. Nomenclature

In this study, a modified Kofoid tabulation system (Kofoid, 1909) as described in Besada et al. (1982) was followed to name the plates therefore allowing comparisons with other genera. The main differences are: in the epitheca, we considered as the first apical plate (1′) what most of the authors consider as first precingular plate (1″) and in the hypotheca, second antapical plate (2‴) instead of 1p, and sulcal posterior (S.p.) instead of second antapical