Harmful Algae 67 (2017) 131–143

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

"Canary Islands (NE Atlantic) as a biodiversity 'hotspot' of *Gambierdiscus*: Implications for future trends of ciguatera in the area"

Francisco Rodríguez[a,*], Santiago Fraga[a], Isabel Ramilo[a], Pilar Rial[a],
Rosa Isabel Figueroa[a,b], Pilar Riobó[c], Isabel Bravo[a]

[a] *Centro Oceanográfico de Vigo, Instituto Español de Oceanografía (IEO), Subida a Radio Faro 50, 36390 Vigo, Spain*
[b] *Aquatic Ecology, Biology Building, Lund University, 22362 Lund, Sweden*
[c] *Instituto de Investigaciones Marinas, CSIC, Eduardo Cabello, 6, 36208 Vigo, Spain*

ARTICLE INFO

Article history:
Received 11 April 2017
Received in revised form 27 June 2017
Accepted 29 June 2017
Available online xxx

Keywords:
Benthic dinoflagellates
Canary Islands
Ciguatera
Gambierdiscus
LSUrRNA
SSUrRNA

ABSTRACT

In the present study the geographical distribution, abundance and composition of *Gambierdiscus* was described over a 600 km longitudinal scale in the Canary Islands. Samples for cell counts, isolation and identification of *Gambierdiscus* were obtained from five islands (El Hierro, Tenerife, Gran Canaria, Fuerteventura and Lanzarote). Average densities of *Gambierdiscus* spp. between 0 and 2200 cells g^{-1} blot dry weight of macrophyte were recorded. Morphological (light microscopy and SEM techniques) and molecular analyses (LSU and SSU rDNA sequencing of cultures and single cells from the field) of *Gambierdiscus* was performed. Five *Gambierdiscus* species (*G. australes, G. caribaeus, G. carolinianus, G. excentricus* and *G. silvae*), together with a new putative species (*Gambierdiscus* ribotype 3) were identified. These results suggest that some cases of CFP in the region could be associated with the accumulation of ciguatoxins in the marine food web acquired from local populations of *Gambierdiscus*. This unexpected high diversity of *Gambierdiscus* species in an area which a priori is not under risk of ciguatera, hints at an ancient settlement of *Gambierdiscus* populations, likely favored by warmer climate conditions in the Miocene Epoch (when oldest current Canary Islands were created), in contrast with cooler present ones. Currently, warming trends associated with climate change could contribute to extend favorable environmental conditions in the area for *Gambierdiscus* growth especially during winter months.

1. Introduction

Ciguatera fish poisoning (CFP) is one of the most relevant seafood-borne illnesses worldwide (Litaker et al., 2010). It is caused typically by the ingestion of warm water fish species claimed to bioaccumulate lipophilic ciguatoxins (CTXs) in the food web (Randall, 1958). These compounds and other related biotoxins (maitotoxins, MTXs) are produced by benthic dinoflagellates of the genus *Gambierdiscus* (reviewed in Parsons et al., 2012).

CFP is endemic across the tropical and subtropical Pacific, Indian Ocean and the Caribbean Sea (Chinain et al., 2010; Litaker et al., 2009; Tester et al., 2013). Rarely fatal, the most common CFP symptoms include gastrointestinal (nausea, vomiting, diarrhoea), neurological (numbness in extremities, weakness, muscle pain) and cardiovascular disorders (Alexander et al., 2010). These disturbances usually abate within a few days or weeks, although they may persist for considerable longer periods (months or years) in subsequent CFP relapses.

The latitudinal distribution of CFP, formerly considered tropical disease, has apparently migrated toward temperate areas in recent decades (Bravo et al., 2015; Toda et al., 2012).

Gambierdiscus species were reported from non-tropical seas only recently (Aligizaki and Nikolaidis, 2008; Aligizaki et al., 2008; Fraga et al., 2011; Nishimura et al., 2013). In the last decade, several outbreaks of ciguatera affecting ~100 people have been confirmed in the Canary Islands (Central Northeast Atlantic), following the consumption of local fishes (Boada et al., 2010; Bravo et al., 2015; Peréz-Arellano et al., 2005). Currently, ciguatera constitutes a major alimentary concern in this temperate area where it has become a notifiable disease since 2015.

There is an urgent need to investigate the genus *Gambierdiscus* in the Canary Islands as a first step towards defining potentially CFP

---

[*] Corresponding author.
E-mail addresses: francisco.rodriguez@vi.ieo.es (F. Rodríguez), santi.fraga@vi.ieo.es (S. Fraga), isabel.ramilo@vi.ieo.es (I. Ramilo), pilar.rial@vi.ieo.es (P. Rial), rosa.figueroa@vi.ieo.es (
.I. Figueroa), pilarriobo@iim.csic.es (P. Riobó), isabel.bravo@vi.ieo.es (I. Bravo).

http://dx.doi.org/10.1016/j.hal.2017.06.009

risk locations in the area. Defining the relationship between toxic cells with the detection of ciguatoxic fishes, allows a forward projection of health risks based on climate change trends. There is discussion about the apparent spreading of the distribution of *Gambierdiscus* as a direct consequence of increasing temperatures derived from climate change. Benthic dinoflagellates are understudied (Hoppenrath et al., 2016) but are now being sampled and studied in new locations. Frequent first reports in the current literature give the impression of range extensions for Gambierdiscus.

To now, three *Gambierdiscus* species (*G. australes, G. excentricus* and *G. silvae*) have been reported in the Canary Islands. Among them *G. excentricus* and *G. silvae* were described as new species (Fraga and Rodríguez, 2014; Fraga et al., 2011) from opportunistic samplings in La Palma, La Gomera, Tenerife and Gran Canaria Islands. This is an unexpected diversity for the genus *Gambierdiscus* given that, *a priori* and based on seawater temperatures, these islands are out of the typical range of distribution (Berdalet et al., 2017; GEOHAB, 2012; Litaker et al., 2010; Parsons et al., 2012).

While fossilized cysts of *Gambierdiscus* have not been reported to our knowledge, it can be guessed from paleoclimate records that warmer environmental conditions like those from Miocene onwards ($\sim$23 Ma, the approximate time when current Madeira and Canary Islands were created), would have favored the thriving of warm water species. It must be mentioned that in the early Miocene the closure of the Tethys Sea interrupted the flow of tropical seawater through a circumequatorial current (Scotese, 1991; Stille et al., 1996), and the closure of circumtropical seaways was effective after the formation of the Isthmus of Panama around 3 Ma (Schmidt, 2007). Coincident with this closure, a global cooling started in the transition from Pliocene to Pleistocene and a significant intensification of the Northern Hemisphere glaciation occurred, intensely in the mid and high latitudes (Dowsett et al., 1996).

These events led to the separation of the Tropical Indo-Pacific Ocean from the Atlantic Ocean and the subsequent environmental changes (Haug and Tiedemann, 1998; Lunt et al., 2008) caused vicariance in many groups of organisms. These organisms display patterns of genetic differentiation related to geography as do the benthic dinoflagellate genus *Ostreopsis* (Penna et al., 2010), also found in tropical coastal macrophytes (Kooistra et al., 1992).

The aim of the present study was to describe the geographical distribution, species abundance and composition of *Gambierdiscus* over a broad longitudinal scale in the Canary Islands. Despite the current mild climate conditions over the whole archipelago, the western islands (e.g. El Hierro) are more humid in their northern areas due to the influence of trade winds. These islands are also relatively younger than the oriental ones and have steeper topography. In contrast, the eastern islands (such as Fuerteventura and Lanzarote), have smoother relief and semiarid conditions are dominate, due to their older origin and close proximity to the Moroccan upwelling front. Based on these observations and our results, the origin and fate of *Gambierdiscus* populations in the Canary Islands are discussed.

2. Materials and methods

2.1. Field sampling

Macrophyte samples were taken from tidal ponds during low tide of by snorkeling up to a maximum depth of 3 m. Each sample was placed in a plastic bottle and shaken to detach epiphytes. Afterwards, the gross materials were removed through a 300 μm opening nylon mesh and the remaining seawater was filtered again on a 20 μm nylon mesh to concentrate the samples. Aliquots were taken of live samples for isolation of specimens in the laboratory and *in situ* fixed with Lugol's acid solution and formaldehyde for identification and enumeration in the laboratory.

In one location (Puerto de La Estaca, El Hierro, Station “S”, Table 1), samples were only obtained using the screen sampling method described by Tester et al. (2014) because no macrophytes were found in that area. These samples were only used for further culture isolation and species identification (molecular analyses) but quantitative data were not obtained. Briefly, it consisted in a 9 × 15 cm rectangular piece of fiberglass, attached to a fishing line and suspended in the water column within 30 cm of the seabed using a weight and a plastic bottle partially filled with seawater as subsurface float. After deployment at low tide, the screens were allowed to passively sample for 48 h before being retrieved. The screens were transferred underwater to a jar and processed in the same way as macrophyte samples.

Seawater temperature was measured by means of a CastAway CTD (YSI, USA) or by an alcohol thermometer.

2.2. Sampling strategies

Two sampling strategies were followed in order to study the spatial and temporal variations of *Gambierdiscus* distribution.

2.2.1. Spatial study

A total of 128 macrophyte samples were collected in 31 stations by snorkeling or directly by foot in tide pools, all of them in rocky shores and beaches of five islands (El Hierro, Tenerife, Gran Canaria, Fuerteventura and Lanzarote), during September–October 2015 (Fig. 1, Table 1). The sampling stations were visited only during low tide and samples were taken from high level tide pools to upper infralitoral zone (accessible by foot or simple snorkeling at <3 m depth). From 2 to 8 samples of different macrophyte species were collected from each station trying to gather the most representative material from each sampling location. When

Table 1
Locations of sampling stations in the Canary Islands.

Station	Island	Locality	Coordinates (DD)
S	El Hierro	La Estaca	–17.9035 27.7846
1	El Hierro	Charco manso	–17.9238 27.8492
2	El Hierro	Tacorón	–18.0266 27.6703
3	El Hierro	Puerto Naos	–17.9926 27.6398
4	El Hierro	Orchilla	–18.1333 27.7044
5	Tenerife	Los Silos	–16.8143 28.3809
6	Tenerife	Punta Teno	–16.9201 28.3416
7	Tenerife	Güimar	–16.3636 28.3353
8	Tenerife	Pta.del Hidalgo	–16.3265 28.5681
9	Tenerife	Las Américas	–16.7355 28.0613
10	Tenerife	Pta. Blanca	–16.8272 28.2152
11	Tenerife	Tajao	–16.4710 28.1085
12	Gran Canaria	Playa del Hombre	–15.3746 27.9981
13	Gran Canaria	Playa de la Laja	–15.4186 28.0637
14	Gran Canaria	Charcas de Agaete	–15.7111 28.1066
15	Gran Canaria	Caleta de Arriba	–15.6495 28.1651
16	Gran Canaria	El Puertillo	–15.5373 28.1511
17	Fuerteventura	Aguas verdes	–14.0945 28.4852
18	Fuerteventura	Puerto Lajas	–13.8362 28.5349
19	Fuerteventura	El Cotillo	–14.0172 28.6938
20	Fuerteventura	Las Playitas	–13.9838 28.2283
21	Fuerteventura	Pto. Jandía	–14.3777 28.0502
22	Fuerteventura	La Pared	–14.2245 28.2180
23	Fuerteventura	Costa Calma	–14.1971 28.1734
24	Fuerteventura	Castillo del Fuste	–13.8614 28.3860
25	Lanzarote	Arrecife	–13.5347 28.9566
26	Lanzarote	Charco del Palo	–13.4520 29.0807
27	Lanzarote	Caletón Blanco	–13.4418 29.2171
28	Lanzarote	Famara	–13.5636 29.1178
29	Lanzarote	Caleta Caballo	–13.6397 29.1176
30	Lanzarote	Playa Blanca	–13.8462 28.8605
31	Lanzarote	El Golfo	–13.8319 28.9836

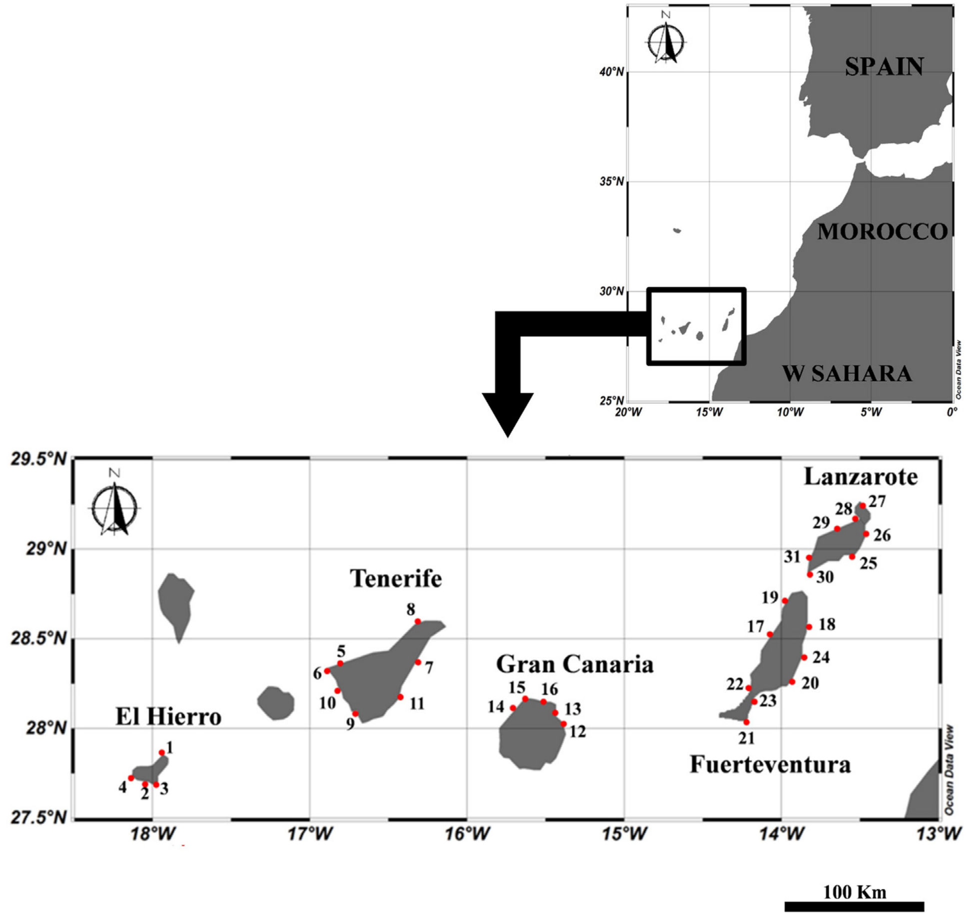

Fig. 1. Map of the East Atlantic region including the Canary Islands. Sampling localities (n = 31) are indicated. Station numbers and its identification are provided in Table 1.

possible each sample contained one specific macrophyte, however many times there were a mix of seaweeds.

In addition, opportunistic samples were collected in winter: one sample from 11 stations in La Graciosa (Chinijo archipelago, north Lanzarote) in March 2015, and 10 samples from Caleta de Arriba (Gran Canaria) in December 2015 (Fig. 1, Table 1).

2.2.2. Temporal study

During one year-round (December 2014–November 2015) samples were collected once a month at intertidal ponds mainly at three locations: Punta del Hidalgo, Playa de Las Américas and Alcalá, following the same procedures described for the spatial study.

2.3. Cell isolation and cultures of Gambierdiscus spp.

Isolation of live *Gambierdiscus* cells was carried out by a capillary pipette with the aid of a Zeiss Invertoscop D microscope (Carl Zeiss AG, Germany) and isolated cells were incubated in 96 microwell plates in half strength K medium (Keller et al., 1987) made with seawater from Ría de Vigo, Spain, with a salinity adjusted to 32 and incubated at 25 °C and under an irradiance of about 90 μmol quanta $m^{-2}\,s^{-1}$ of PAR measured with a QSL-100 irradiameter (Biospherical Instruments Inc. San Diego, CA, USA), at a 14:10 L:D photoperiod. Cultures of *Gambierdiscus* (n = 68; Table 1) were obtained and deposited at the Culture Collection of Harmful Microalgae (CCVIEO) of the Instituto Español de Oceanografía in Vigo.

2.4. Cell enumeration and light microscopy

Formaldehyde fixed epiphyte samples were stained with the fluorescent dye Calcofluor White M2R (Fritz and Triemer, 1985) to stain the dinoflagellate thecae, and were counted under an Axiovert 125 epifluorescence inverted microscope (Carl Zeiss AG, Germany) with UV excitation and a UV filter set. Quantitative data were obtained for *Gambierdiscus* species and qualitative analyses of the accompanying dominant dinoflagellates were recorded too. *Gambierdiscus* abundance was expressed as cells per gram blot dry weight of host macrophyte (abbreviated as cells g^{-1} in the results section). For this purpose, weight measures were taken on macrophytes after being blot-dried (draining overnight over soft laboratory paper).

2.5. Sample preparation for SEM

Exponentially growing cultures (5 mL) were fixed with glutaraldehyde at a final concentration of 2%. After two hours at room temperature, they were rinsed three times with distilled water and dehydrated in a series of 30, 50, 75, 95 and 100% EtOH followed by Hexamethyldisilazane. 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 FEI Quanta 200 scanning electron microscopes (FEI Company, Hillsboro, OR, USA).

2.6. DNA extraction

Samples from cultures and single cells of *Gambierdiscus* were analyzed as follows. Exponentially growing cultures (1.5 mL) were harvested by centrifugation (13,000 rpm, 2 min) using an Eppendorf 5424R centrifuge (Eppendorf AG, New York, USA), the cellular pellets were rinsed in 1 mL distilled water, centrifuged again and the supernatant discarded. Samples were frozen overnight at −20 °C and DNA extraction was done either using a modified Chelex procedure as described in Fraga and Rodríguez (2014) or the following CTAB protocol adapted from Doyle and Doyle (1987). Briefly, cellular pellets were mixed with 700 μL of CTAB buffer solution premixed with 3 μL of mercaptoethanol and 30 μL of proteinase K. Samples were thawed three times in N_2 and incubated (65 °C, 1 h) with agitation. Tubes were left at room temperature during 10 min and 700 μL of chlorophorm:isoamyl alcohol (24:1) were added. 1.5 vol of 96% ethanol and 0.1 vol. of sodium acetate 3 M, pH 5.2 were added and samples kept overnight at −20 °C. After a centrifugation step (13,000 rpm, 15 min, 4 °C), pellets were rinsed with cold 70% ethanol, centrifuged again in the same conditions and dried at room temperature. DNA samples were eluted in TE buffer (25 μL), quantified and checked for its purity in a Nanodrop Lite spectrophotometer (Thermo Scientific, Waltham, MA, USA), and stored at −20 °C until further processing. In the case of single cells of *Gambierdiscus*, these were picked up with a micropipette (both alive or Lugol's fixed individuals from field samples), carefully washed in three distilled water droplets, and stored overnight at −80 °C in 200 μL microtubes. Prior to direct PCR on these single cells, samples were heated at 94 °C during 1 min in the thermal cycler.

2.7. PCR amplification and DNA sequencing

The D1–D3 and D8–D10 regions of the LSUrRNA as well as partial SSUrRNA genes were amplified using the pairs of primers D1R/LSUB, FD8/RB and EUKA/EUKB, respectively (Chinain et al., 1999; Litaker et al., 2003; Scholin et al., 1994). Amplification reaction mixtures (25 μL) contained 1.25 mM $MgCl_2$, 0.25 pmol of each primer, 2.4 mM of dNTPs, 0.25 units Taq DNA polymerase (New England Biolabs, MA, USA), and 1–2 μL from the DNA extracts. The DNA was amplified in a Surecycler 8800 thermocycler as follows: 4 min denaturing at 94 °C, followed by 30 cycles of 30 s denaturing at 94 °C, 1 min annealing at 54 °C and 2 min elongation at 72 °C, with an elongation step of 10 min at 72 °C. A 10 μL aliquot of each PCR reaction was checked by agarose gel electrophoresis (1.5% TAE, 80 V) and GelRed^{TM} nucleic acid gel staining (Biotium, Hayward, CA, USA). The PCR products were purified with ExoSAP-IT (USB Corporation, OH, USA). Purified DNA was sequenced using the Big Dye Terminator v3.1 Reaction Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and migrated in an AB 3130 sequencer (Applied Biosystems) at the CACTI sequencing facilities (Universidade de Vigo, Spain) and the LightRun^{TM} sequencing service (GATC Biotech AG, Germany). The amplified LSUrRNA and partial SSUrRNA gene sequences obtained in this study were deposited in GenBank (for accesion numbers see Table S1 in the online version at DOI: 10.1016/j.hal.2017.06.009, Figs. 5 and 6).

2.8. Phylogenetic analyses

Sequences were inspected and aligned using MEGA 7 (Kumar et al., 2016). Net average genetic distances ($dA = dXY - (dX + dY)/2$), where dXY is the average distance between groups X and Y, and dX and dY are the mean within-group distances (Nei, 1987), were calculated between *Gambierdiscus* clades for the original alignments using MEGA 7. It must be remembered that an important

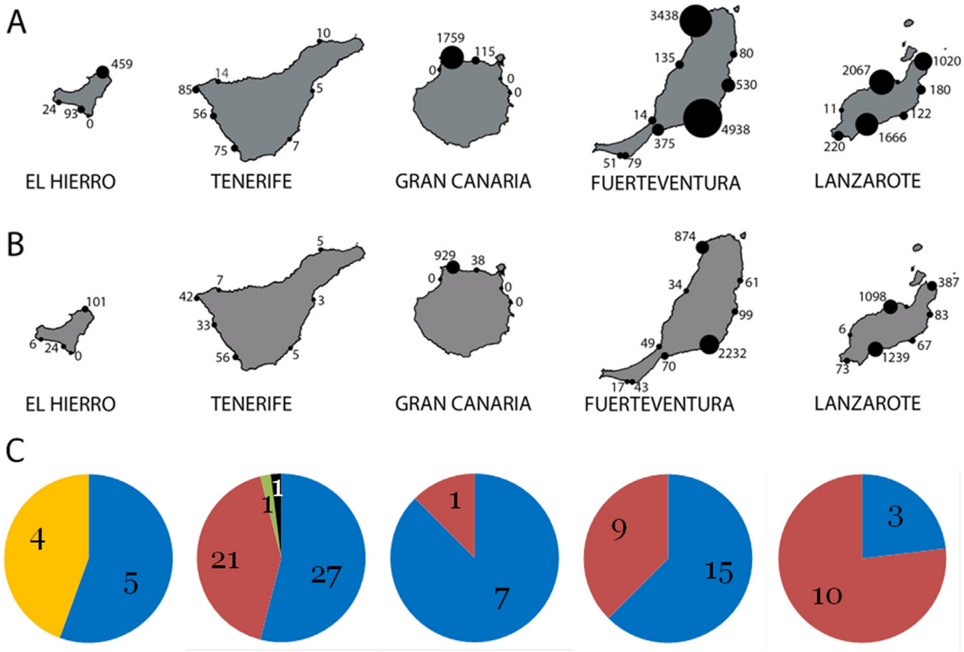

Fig. 2. A) Maximum abundance of *Gambierdiscus* spp. (cells g^{-1} blot dry weight of macrophyte) in the five Canary Islands sampled during the study. B) Average abundance of *Gambierdiscus* spp. (cells g^{-1} blot dry weight macrophyte) and, C) number of sequences retrieved for individual *Gambierdiscus* species from molecular analyses in each island. Species are indicated by the following colors: *G. caribaeus* (yellow), *G. australes* (blue), *G. carolinianus* (green), *G. silvae* (black), *G. excentricus* (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

assumption behind the Nei genetic distance method is that the divergence rates among species are equivalent. D1-D3 and D8–D10 LSUrRNA and SSUrRNA alignments included 1169 and 902 and positions, respectively. Phylogenetic model selection (ML) was performed on MEGA 7. A K2 + G model was selected in D8–D10 LSU phylogeny (gamma shape parameter $\gamma = 0.50$), while K2 + *I* + G ($I = 0.23$, $\gamma = 0.51$) was selected in D1-D3 LSU phylogeny. Several sequences from genus *Fukuyoa* were used to root the trees. The phylogenetic relationships were also determined using Bayesian phylogenetic inference and in this case the substitution models were obtained by sampling across the entire general time reversible (GTR) model space following the procedure described in Mr. Bayes v3.2 manual. Bayesian trees were performed with Mr. Bayes v3.2 (Huelsenbeck and Ronquist, 2001) and the program parameters were statefreqpr = dirichlet (1,1,1,1), nst = mixed, rates = gamma. The phylogenetic analyses involved two parallel analyses, each with four chains. Starting trees for each chain were selected randomly using the default values for the Mr. Bayes program. The corresponding number of unique site patterns for D1-D3 and D8–D10 LSU alignments were 739 and 310, respectively. The number of generations used in these analyses was 1,000,000. Posterior probabilities were calculated from every 100th tree sampled after log-likelihood stabilization ("burn-in" phase). Maximum Likelihood phylogenetic analyses were conducted in MEGA 7. Bootstrap values were estimated from 1000 replicates. Overall topologies by ML and Bayesian inference method were very similar. The phylogenetic trees were represented using the ML method with bootstrap values and posterior probabilities from the Bayesian inference.

3. Results

3.1. *Gambierdiscus* abundance and distribution

Cells of *Gambierdiscus* species were present in all Canary Islands sampled during the spatial study. However, remarkable differences were detected among the different islands both in relation to cell concentrations and to species composition. The two easternmost islands situated closest to African coast (Lanzarote and Fuerteventura) showed the maximum cell concentrations and higher mean values of *Gambierdiscus* spp. than the central and westernmost islands (Fig. 2A, B). So, the maximum values were recorded in eastern islands of Fuerteventura and Lanzarote with 4938 and 2067 cells g^{-1} alga respectively. Whereas in the western islands of El Hierro and Tenerife, maxima were of 459 and 85 cells g^{-1} macrophyte, respectively (Fig. 2A). In the geographically central island of Gran Canary, maximum concentration of 1759 cells g^{-1} alga was observed. Nevertheless, the distribution of *Gambierdiscus* spp. showed a high heterogeneity that can be drawn from the data in the islands with the highest concentrations (Gran Canaria, Fuerteventura and Lanzarote). That heterogeneity occurred both inter stations but also within stations, finding a high variability among stations as well as among samples from the same station (Fig. 3). Thus, concentrations fluctuated from 4938 cells g^{-1} to 377 cells g^{-1} alga in Las Playitas (Fuerteventura), and no *Gambierdiscus* cells were found in samples from other stations in the same island as for example Castillo del Fuste (20 km northwards). Similar variations occurred in other islands (Figs. 2 and 3).

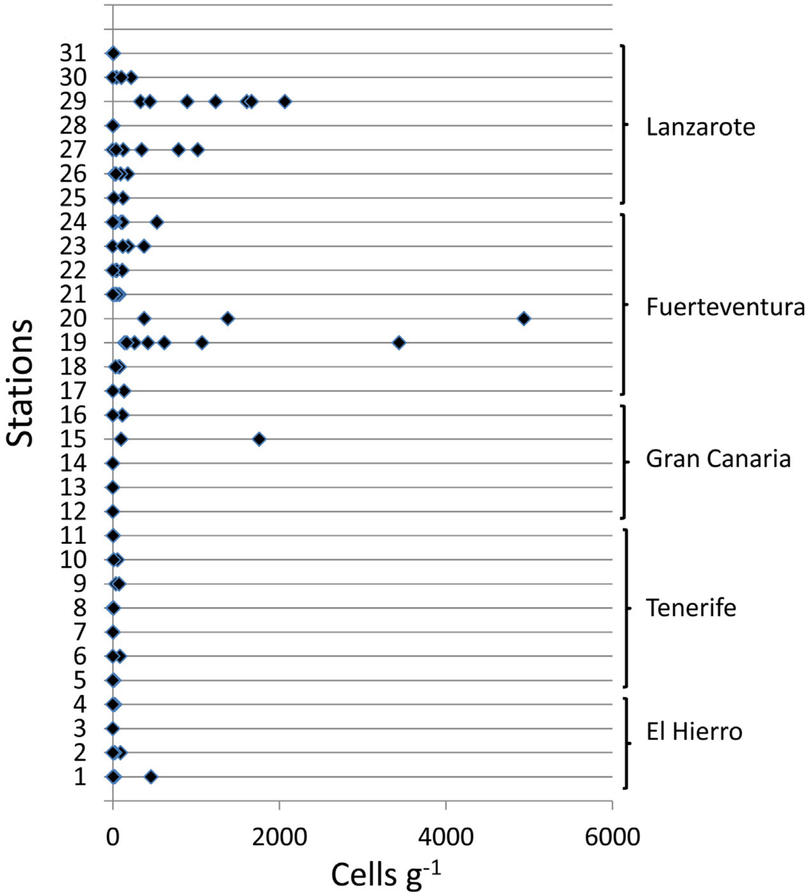

Fig. 3. Abundance of *Gambierdiscus* spp. (cells g^{-1} blot dry weight of macrophyte) in the different sampling areas on the five Canary Islands considered in this study.

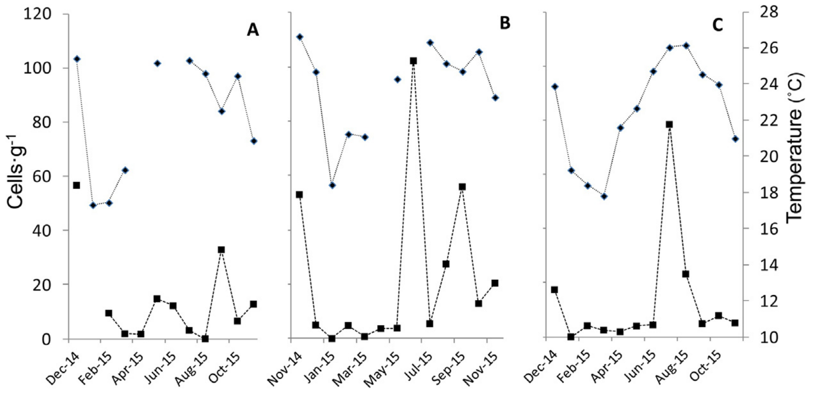

Fig. 4. Abundance of *Gambierdiscus* spp. (cells g^{-1} blot dry weight of macrophyte) in the temporal study at Tenerife, from three stations (North: Punta del Hidalgo, South: Las Américas, and West: Alcalá).

Other common dinoflagellate genera observed were: *Prorocentrum* (in 111 samples), *Coolia* (in 98 samples), *Ostreopsis* (in 99 samples), and others like *Sinophysis* and *Vulcanodinium*. Massive *Ostreopsis* spp. blooms were detected in several samples from Caleta Caballo (Lanzarote) with up to 28,117 and 151,499 cells g^{-1} alga.

In the temporal study at Tenerife, data from three stations (North: Punta del Hidalgo, South: Las Américas, and West: Alcalá) revealed maximum densities between 60 and 100 cells g^{-1}, usually recorded in the second half of the year (May-September). Although the low cell concentrations detected in this island do not allow a certain result about temporal distributions of *Gambierdiscus*, an association of cell peaks with the highest water temperature was observed. So, the cell peaks were associated to temperatures higher than 20 °C (Fig. 4). In opportunistic samplings during winter, *Gambierdiscus* was only observed in two samples (with a maximum of 28 cells g^{-1}) out of 11 in La Graciosa in December 2015, whereas it was not detected in Gran Canaria in March in the same station where 1700 cells g^{-1} alga had been found during spatial study (September 2015).

3.2. Molecular characterization of Gambierdiscus *from the Canary Islands*

Molecular analyses on strains and single cell isolates (LSU & SSUrRNA sequencing) allowed identifying five *Gambierdiscus* species, namely *G. australes*, *G. caribaeus*, *G. carolinianus*, *G. excentricus* and *G. silvae* (Fig. 7). Estimates of relative proportions between *Gambierdiscus* spp. are qualitative and based only on the number of ribosomal sequences retrieved from cultures and single cell isolates. The geographical distribution of species and their relative proportions are detailed in Fig. 2C. From these results, minoritary species such as *G. caribaeus, G. carolinianus* and *G. silvae* were only found in the westernmost sampled islands (El Hierro and Tenerife) although *G. silvae* had been observed previously in Gran Canaria where is the type locality of the species (Fraga and Rodríguez, 2014). The dominant species, *G. australes* and *G. excentricus* were the only taxa detected in the other islands (Gran Canaria, Fuerteventura and Lanzarote) (Fig. 2C). *G. excentricus* appeared to increase its relative proportion against *G. australes* from Gran Canaria to Fuerteventura and Lanzarote Islands (Fig. 2C).

G. australes and *G. excentricus* were the dominant species in the Canary Islands, accounting for ~95% (58 and 40 sequences, respectively) of the 111 sequences obtained in this study. The other *Gambierdiscus* spp. only contributed 6 sequences [*G. caribaeus* (n = 4) and *G. carolinianus/G. silvae* (n = 1 each)].

The phylogenetic analyses based on D1/D3 and D8/D10 regions of LSUrRNA are shown in Figs. 5 and 6, respectively. In the D8/D10 phylogeny it was noteworthy the high average net genetic distance (0.014) between the sequence from the strain VGO1258 (Punta del Hidalgo, Tenerife), and the rest of those in the *G. australes* clade (within distance: 0.002, n = 26). We labeled strain VGO1258 as *Gambierdiscus* ribotype 3 based on the fact that its genetic distance relative to the *G. australes* clade was similar or surpassed the boundaries (< 0.010) between pairs of close *Gambierdiscus* species such as *G. pacificus/G. toxicus* and *G. carpenteri/G. caribaeus*.

SSUrRNA sequences identification is detailed in Table S1 in the online version at DOI: 10.1016/j.hal.2017.06.009. SSU phylogenetic tree was not elaborated given the different lengths for these SSUrRNA sequences (258–906 nt) and their specific identification was based on their best match against individual *Gambierdiscus* species in the GenBank database. Two *G. excentricus* [Table S1 in the online version at DOI: 10.1016/j.hal.2017.06.009, 100% match with Acc. No. KP290887 (*G. excentricus* UNR8, Brazil, Nascimento et al. (2015)) and four *G. australes* [Table S1 in the online version at DOI: 10.1016/j.hal.2017.06.009, 0–3 mismatches with Acc. Nos. EF202971 and EF202986 (*G. australes* RAV92_2 and RAV92_3_8, North Carolina, (USA), Litaker et al. (2009)).

4. Discussion

4.1. Canary Islands as a biodiversity "hotspot" of Gambierdiscus

Since its formal description, most studies on *Gambierdiscus* Adachi and Fukuyo (1979) have focused on CFP endemic areas, mainly in Caribbean and Pacific locations. It is not a surprise that most of species were described from these areas, the only exceptions being *G. carolinianus* from continental shelf of North Carolina, NW Atlantic (Litaker et al., 2009), *G. excentricus* and *G. silvae* from Canary Islands (Fraga and Rodríguez, 2014; Fraga et al., 2011), *G. scabrosus* from Japanese waters (Nishimura et al., 2014), and G. *balechii* from Indonesia (Fraga et al., 2016).

Despite being originally described in the Pacific (Gambier Islands, French Polynesia), the genus *Gambierdiscus* was first observed (named as *Goniodoma* sp.) in Central East Atlantic, Cape Verde Islands, (Sousa e Silva, 1956) ~1500 km southwest of Canary Islands. In recent years, three species, *G. australes, G. excentricus* and *G. silvae* were detected in that archipelago (Fraga and Rodríguez, 2014), the former one being known previously only from the Pacific.

Though is common to detect several species of *Gambierdiscus* in the same area (GEOHAB, 2012; Tester et al., 2013), Canary Islands represents a "biodiversity hotspot" with the finding of at least five species after the reports of *G. caribaeus* and *G. carolinianus* in this study. Such specific diversity was rather surprising given the small relative geographical extension of Canary Islands. Furthermore, a

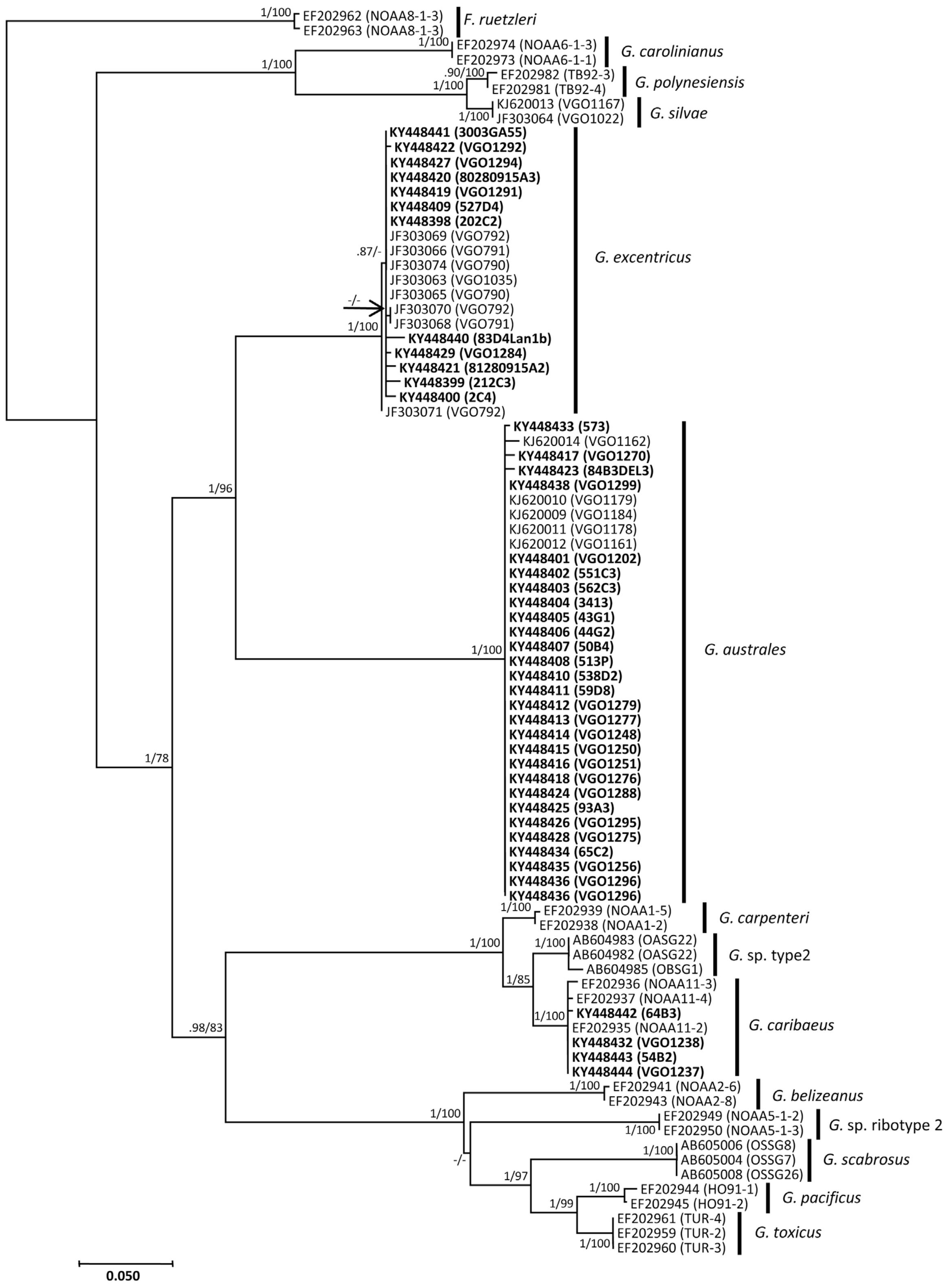

Fig. 5. LSUrRNA phylogeny (D1–D3 region) showing the relationships between *Gambierdiscus* sequences obtained in the present study and other *Gambierdiscus* species/phylotypes. Geographical origins of the sequences are detailed in Table S1. New sequences from this study are in bold. Internal nodes supports are posterior probabilities (Bayesian analyses) and bootstrap values (Maximum Likelihood). Hyphens indicate bootstrap values <60.

new *Gambierdiscus* ribotype 3, sister to *G. australes* was also retrieved in Tenerife, but the establishment of new cultures would be needed to confirm its taxonomic entity as a new species. For the sake of comparison, 6 species/ribotypes have been observed until date in the whole Caribbean Sea (Litaker et al., 2010; Tester et al., 2013), excluding *F. ruetzleri* from the sister genus *Fukuyoa* (Gómez et al., 2015), which was originally described as *G. ruetzleri* (Litaker et al., 2009).

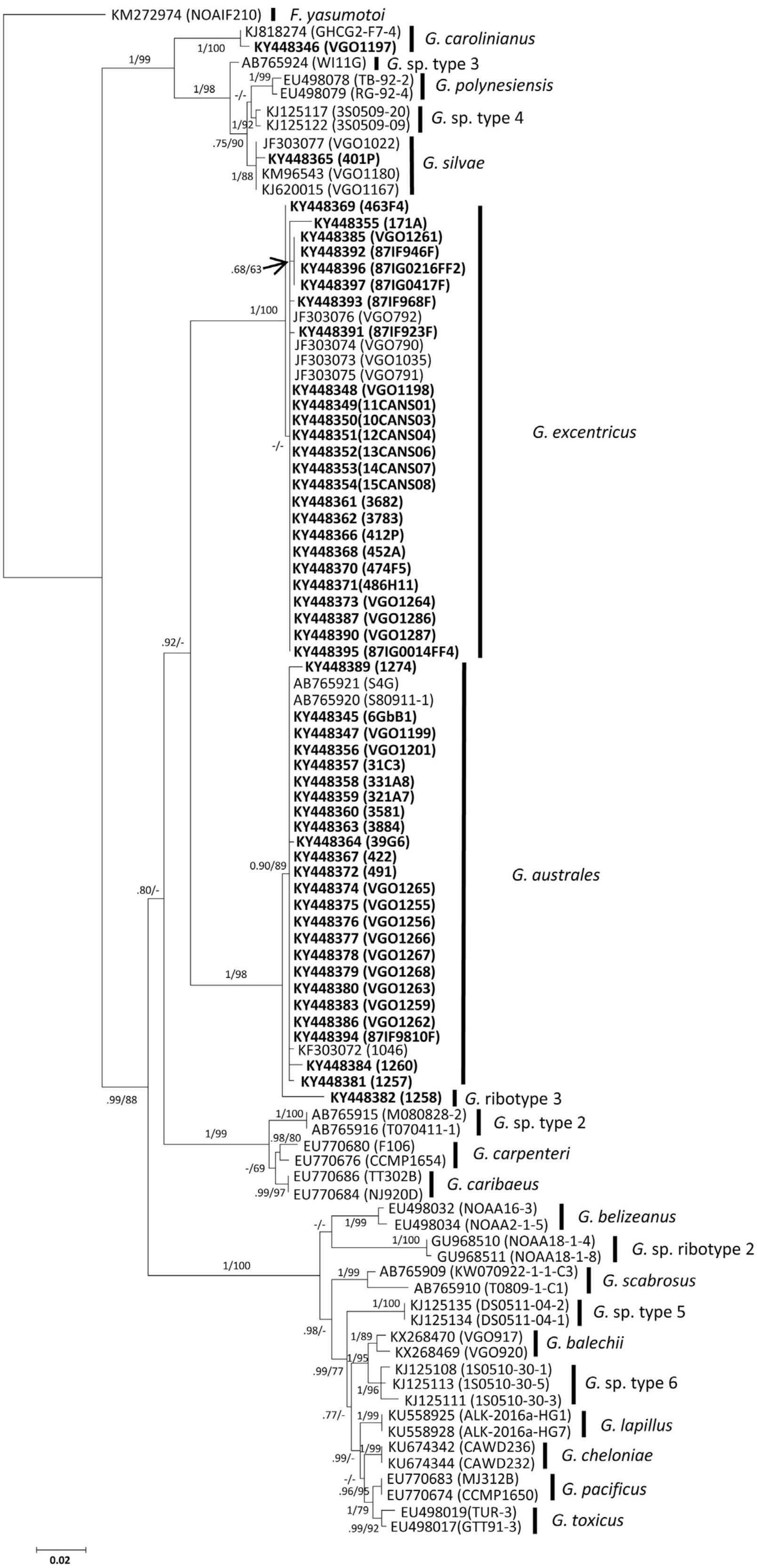

Fig. 6. LSUrRNA phylogeny (D8–D10 region) showing the relationships between *Gambierdiscus* sequences obtained in the present study and other *Gambierdiscus* species/phylotypes. Geographical origins of the sequences are detailed in Table S1. New sequences from this study are in bold. Internal nodes supports are posterior probabilities (Bayesian analyses) and bootstrap values (Maximum Likelihood). Hyphens indicate bootstrap values <60.

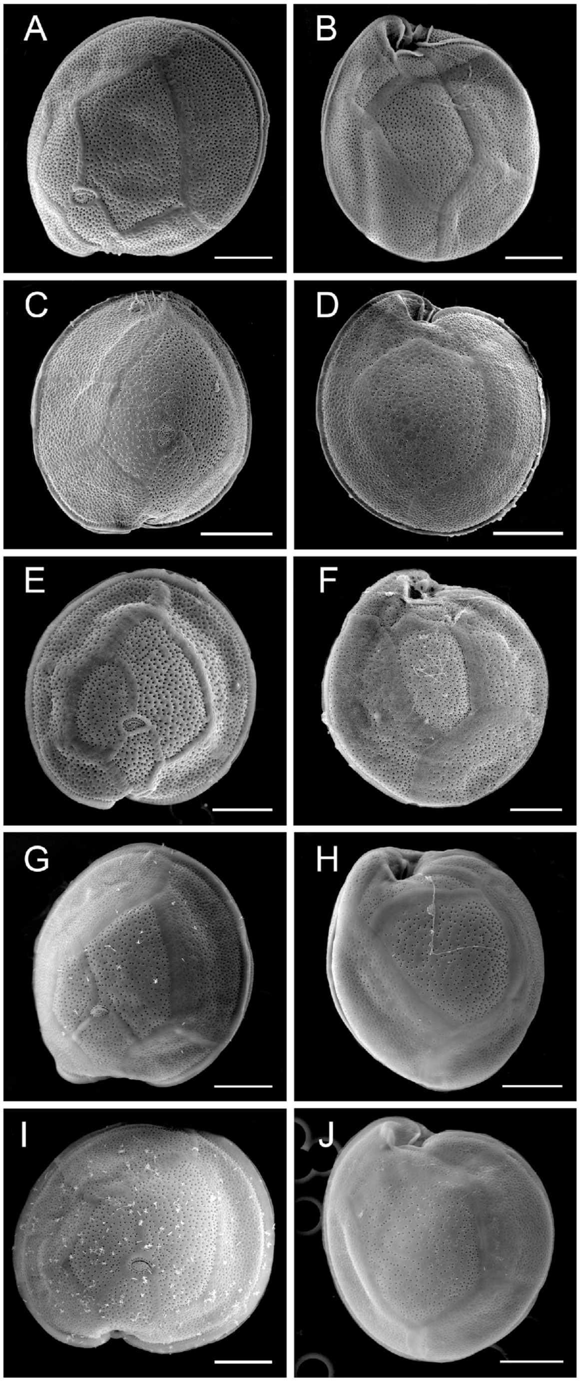

Fig. 7. SEM images from *Gambierdiscus* species found in the Canary Islands.

4.2. Benthic communities and sampling of Gambierdiscus

Macrophyte populations were not examined into taxonomical detail on the course of this study. Therefore, a putative relationship between *Gambierdiscus* spp. abundance and macroalgal assemblages could not be ascertained. However, in the studied area, diverse macroalgal communities can be found in the marine eulittoral zone. These appeared dominated in most cases by red and brown taxa. Nevertheless, cyanobacterial and green algae assemblages can be also abundant in some occasions. In this sense, some shifts in the distribution and importance of non-native members of these groups associated with recent trends of warming in surface seawater temperatures have been already noticed (Sangil et al., 2012), and the interactions in benthic habitats between macroalgae and phytobenthic communities should be explored to determine their potential role in the spatial and temporal distribution of *Gambierdiscus* spp.

The range of average densities of *Gambierdiscus* spp. in the present study (0–2,200 cells g^{-1}), match and even surpass values common in CFP endemic areas in the Atlantic and the Pacific (Litaker et al., 2010; Parsons et al., 2012), although these cannot be directly compared due to the methodological differences among studies (cells g^{-1} blot dry *vs* wet weight macrophyte). For example, Litaker et al. (2010) reported >85% average *Gambierdiscus* density estimates in both areas to be <1000 cells g^{-1} wet weight, and only <10% were in the range 1000–10,000. In the Canary Islands a recent bloom of *Gambierdiscus,* identified as *G. caribaeus* on the basis of morphological characteristics (SEM and light microscopy), has been reported in El Hierro Island (Soler-Onís et al., 2017). Samples were collected in the harbor of La Restinga, from cnidarian colonies at 2 m depth, and attained even higher abundances than in our survey (up to 10^4 cells g^{-1} wet weight macrophyte). As above mentioned, care must be taken regarding the comparisons of *Gambierdiscus* abundance from different studies due to the problems inherent to the harvesting and quantitation of these organisms in benthic and seawater samples (Tester et al., 2014). In the present work macrophytes were blot-dried as mentioned also by Lobel et al. (1988). This procedure was chosen in order to eliminate as much as possible the variable seawater volume retained by each macrophyte. In turn, most of previous studies express *Gambierdiscus* abundance by wet weight of macrophyte, with no particular details on that methodological aspect. As far as we know, there is not yet consensus on a well-defined methodology to quantify epiphytic dinoflagellates, which would be advisable to facilitate comparisons between different studies.

4.3. CFP and Gambierdiscus *distribution in the Canary Islands*

Despite the wide geographical distribution of *Gambierdiscus*, particularly in tropical and subtropical latitudes (35°N–35°S), CFP had not been reported in the East Atlantic until 2004 when 5 people became intoxicated in Canary Islands (Peréz-Arellano et al., 2005). Later, it was confirmed that ciguatoxic fish (amberjack) was purchased at a supermarket but captured off the northern coast of the Canary Islands (Boada et al., 2010), near the Selvagens Islands (Portugal). In the Canary Islands, a total of 108 people have been affected by CFP after consumption of local fish during the period from 2008 to 2016 according to the Canary Islands Health Service (Canary-Islands-Health-Service). Amberjack (*Seriola* spp.) has been responsible of two out of three intoxications, whereas dusky grouper (*Ephinephelus marginatus*), comb grouper (*Mycteroperca fusca*) and bluefish (*Pomatomus saltatrix*) have been involved in other official cases registered by Canary Islands Health Service. CFP outbreaks and the identification of CTXs have also been reported in Selvagens Islands (Portugal), which are at 165 km North of the Canary Islands, from 2007 to 2009 (Gouveia et al., 2009; Otero et al., 2010). Because ciguatera can be difficult to diagnose when it is not acute, previous cases not reported before the first diagnosis in 2004 in Macaronesia cannot be discounted

As mentioned above, ciguatoxic fish in the Canary Islands include a number of species, mostly large migratory ones (amberjack: *Seriola* spp.). Nonetheless, sedentary species like dusky and comb groupers (*Epinephelus* sp. *Mycteroperca* sp.), which typically inhabit rocky reefs from shallow to deeper waters (150–300 m), have been responsible for most recent CFP outbreaks from 2012 onwards. This observation, coupled to our density estimates of *Gambierdiscus* spp. in Canary Islands, strongly support the idea that CFP in the region could be (at least in some cases) associated with the accumulation of CTXs in the marine food web acquired from local populations of *Gambierdiscus*.

Since CTX analyses are only available from neuro-2a assays in terms of CTX-3 equivalents (Bravo et al., 2015), incorporate new evidence to substantiate the link between local populations of *Gambierdiscus* spp. and CFP in the Canary Islands, it is crucial to examine their toxic profiles using LC-HRMS analyses in both algae and ciguatoxin-containing fish. The available data on CTXs activity on predatory fish samples in the Canary Islands have been summarized by these authors, including the results of cytotoxic assays (neuro-2a) on 711 fishes between September 2011 and December 2014. In their work, Bravo et al. (2015) discarded any relationship between positive CTXs results and seasonal temperature during sampling (warmer or colder months). Approximately 10% of neuro-2a analyses were positive, especially in the case of samples from sport fishing (9 out of 25). Their conclusions were that there exists double the risk of catching a toxic fish in eastern islands (Lanzarote and Fuerteventura), than in western ones.

These conclusions by Bravo et al. (2015) are in agreement with the higher *Gambierdiscus* densities measured in our study in eastern islands, where Fuerteventura registered the average and maximum abundance in the spatial sampling. The abundance of *G. excentricus*, a highly toxic species (Pisapia et al., 2017) is also higher in the East than in the West. As detailed in the introduction, eastern islands display more arid conditions but also geological characteristics in their shoreline that could help to explain a relative higher abundance of *Gambierdiscus* spp. Lanzarote and Fuerteventura share a large shelf, especially wide in southern Fuerteventura, that provides a shallow well-lit substrate for the growth of benthic flora/fauna communities (plankton, fish, seaweeds, invertebrates), in comparison with a narrow shelf and steep shoreline along most western islands. Correspondingly, with current knowledge from CTXs assays on predatory fish and *Gambierdiscus* spp. populations, eastern islands seem to display a higher potential for ciguatoxic fish and risk of future CFP outbreaks.

4.4. Origin of Gambierdiscus in the Canary Islands

The presence of the genus *Gambierdiscus* in the Canary Islands could be derived either from an ancient settlement or a recent colonization. CFP outbreaks, only confirmed during the last decade, could be argued to support the recent colonization. Nevertheless, based on the results gathered from the present study (high biodiversity and widespread occurrence of *Gambierdiscus*), it is more likely that Canary Islands *Gambierdiscus* come from an ancient origin. This hypothesis is also supported by favorable warmer conditions in the Macaronesian region evoked by palaeoclimatological and fossil data (Fernández-Palacios et al., 2011). Before the closing of Tethys Sea, the east to west circum-equatorial global marine current during the Neogene kept the Macaronesia biogeographical area with a tropical climate (Fernández-Palacios et al., 2011), and these conditions could have sustained *Gambierdiscus* populations all along the region. Besides the continuous cooling since the Miocene, it is believed that many invertebrate species survived isolated in caves of the Canary Islands, being considered relicts of Tethyan origin (Iliffe et al., 1984). The islands of Macaronesia have numerous endemisms which have been considered relicts of a Tertiary flora since early studies of the 19th century (Kondraskov et al., 2015, and references therein), although this hypothesis is now questioned based on fossil data and molecular phylogenies of representative species of the Macaronesian laurisilva (Kondraskov et al., 2015). Although most of the potential relicts are recent taxa, there is no doubt that the typical land ecosystems of the Canary Islands (such as the laurisilva, the Pinus forest and the thermophilous scrubland), already existed in the Miocene–Pliocene (Anderson et al., 2009). In a similar way, we suggest that the current species of *Gambierdiscus* found in the Canary Islands could have been present in the area at least since the Neogene. The presence of warm water faunal species in Mio-Pliocene in sediments of the oldest Canary Islands, like reefal madrepores such as the genus *Siderastraea*, indicates that climate conditions then were similar to present-day conditions in the Caribbean Sea (Meco et al., 2007), and an appropriate environment for *Gambierdiscus*.

4.5. Climate change and future trends of ciguatera in Canary Islands

The effects of climate change and its influence on recent CFP detection in Canary Islands are a matter of debate. Indeed, Macaronesian archipelagos (Azores, Madeira and Canary Islands), are experiencing climate warming trends during the last decades as summarized by the Institute for European Environmental Policy (Sauter et al., 2013).

The Canary Islands are located in the eastern margin of the North Atlantic subtropical gyre (warm oligotrophic waters), in the coastal transition zone of the Canary Current Upwelling System (colder and productive waters). Thereby, a thermal gradient of about 0.5 °C is observed between warm western and cold eastern islands (Navarro-Pérez and Barton, 2001), gradually strengthened towards the African coast. (Bakun, 1990) hypothesized that as a consequence of the global climate change, an increase in upwelling favorable winds in the eastern boundary current systems could be expected. This trend has been proved in the Canary current system (Cropper et al., 2014; Sydeman et al., 2014) and hence a decrease in sea surface temperature could be expected with the increase of upwelling of cold subsurface waters. Nevertheless, as 90% of the total of the Earth's energy increase is in the oceans (Church et al., 2011), their warming can be stronger than the upwelling related cooling with important biological consequences (McGowan et al., 1998). An increase of 0.32 °C/decade on the upper 600 m between the Eastern island of Lanzarote and the African coast was reported (Tel et al., 2016). An ongoing tropicalization process has been reported in the Canary Islands, including fish populations, seaweed and invertebrates (Bravo et al., 2015; Brito, 2008; Brito et al., 2005; Sangil et al., 2012). Accordingly, the warming of land and oceanic temperatures has also been stressed by several authors (Luque et al., 2014; Tel et al., 2016), demonstrating an acceleration since the mid-seventies, Independently of climate warming, seawater temperatures in subtropical latitudes like the Canary Islands are adequate for the growth of *Gambierdiscus* and other warm benthic dinoflagellates found elsewhere in our study (*Ostreopsis, Coolia, Prorocentrum, Sinophysis*, etc). Nevertheless, during winter months seawater stays below 20 °C, whereas available culture studies indicate that the optimal range of growth for *Gambierdiscus* spp. is ∼24–31 °C (Kibler et al., 2012; Xu et al., 2016; Yoshimatsu et al., 2014), with lower and higher limits of 15–21° C and 31–34° C depending on the studies. In the case of *Gambierdiscus* species found in the Canary Islands, data are available for *G. australes*, *G. caribaeus*, *G. carolinianus* and *G. silvae* (Kibler et al., 2012; Tawong et al., 2016; Xu et al., 2016), with maximum growth rates between 25–31 °C, but intraspecific variability among strains can be within the same range (e.g. *G. caribaeus*; Tawong et al., 2016). Therefore, the expected temperature rise associated with climate change could likely contribute to extend favorable environmental conditions particularly during winter months, when seawater stays below 20 °C. It is observed in the Pacific Ocean, where a good epidemiological dataset exists, that ciguatera is more prevalent where minimum sea surface temperatures are 24 °C or above and maximum incidence rates are found when average sea surface temperatures are 28–29 °C (Llewellyn, 2010). The Canary Islands are below that lower limit set for the Pacific and the fact that at least five species of *Gambierdiscus* were identified in these islands could be considered an unexpected finding.

In our opinion, it cannot be discarded that ciguatera intoxications were overlooked prior to the first official ciguatera poisonings in the Canary Islands. The reasons could be that CFP outbreaks were ignored in the past given that their symptomatology, at least in mild intoxications, could be associated with common digestive disorders. It was not in vain that locals have asserted us that in some cases, doctors from endemic areas for CFP (such as the Caribbean region), familiar with the symptoms, have identified the clinical syndrome as ciguatera.

The socioeconomic impact of ciguatera on fisheries activity and public health in Canary Islands requires further efforts to implement a faster analytical response to detect CTXs in fish samples, and multidisciplinary research to depict the distribution, species composition, life cycle, ecology and toxicity of *Gambierdiscus* spp., as well as to track the fate of CTXs in coastal marine ecosystems, from small invertebrates to herbivorous and large predatory fish consumed in the Canary Islands.

5. Conclusions

The present study stated for the first time that *Gambierdiscus* spp. are a widespread, diverse and likely autochthonous component of the benthic microalgal communities in the Canary Islands. The large diversity detected suggests an ancient settlement and diversification of this genus in the area and not the result of a recent introduction. Furthermore, their relative high abundances, similar or even higher to those recorded in CFP endemic areas, support the possibility that toxic fish and ciguatera outbreaks in the region is due to local *Gambierdiscus* populations. The higher risk of catching fish with CTXs in the oriental islands of Lanzarote and Fuerteventura stated in the literature, agrees with the highest relative abundances of *Gambierdiscus* recorded in our study. In addition, *G. excentricus* (the largest species and the one with highest toxin contents detected up to now in the area), was usually the dominant species in the present study. As a consequence of the tropicalization trends in the Canary Islands due to the global climate change, a potential increase of CFP in this archipelago is likely to occur in the future.

Acknowledgements

We thank Pedro Pascual-Alayón for all the help during the duration of CICAN project in sampling and organization and Gustavo González for his help during sampling plan. We thank one anonymous reviewer and especially Dr. Patricia A. Tester for their careful reading of our manuscript and their many insightful comments and suggestions. This work was funded by Spanish project CICAN (CGL2013-40671-R). This is a contribution of Unidad Asociada “Microalgas Nocivas” IEO-CSIC.[CG]

References

Adachi, R., Fukuyo, Y., 1979. The thecal structure of the marine toxic dinoflagellate Gambierdiscus toxicus gen et. sp. nov. collected in a ciguatera endemic area. Bull. Jpn. Soc. Sci. Fish. 45, 67–71.

Alexander, J., Benford, D., Boobis, A., Ceccatelli, S., Cravedi, J.-P., Di Domenico, A., Doerge, D., Dogliotti, E., Edler, L., Farmer, P., Filipič, M., Fink-Gremmels, J., Fürst, P., Guerin, T., Knutsen, H., Machala, M., Mutti, A., Schlatter, J., van Leeuwen, R., 2010. EFSA panel on contaminants in the food chain; scientific opinion on marine biotoxins in shellfish –emerging toxins: ciguatoxin group. EFSA J. 2010, 1627–1665.

Aligizaki, K., Nikolaidis, G., 2008. Morphological identification of two tropical dinoflagellates of the genera Gambierdiscus and Sinophysis in the Mediterranean. Sea. J. Biol. Res. Thessaloniki 9, 75–82.

Aligizaki, K., Nikolaidis, G., Fraga, S., 2008. Is *Gambierdiscus* expanding to newareas? Harmful Algae News 36, 6–7.

Anderson, C., Channing, A., Zamuner, A., 2009. Life, death and fossilization on Gran Canaria –implications for Macaronesian biogeography and molecular dating. J. Biogeogr. 36, 2189–2201.

Bakun, A., 1990. Global climate change and intensification of coastal ocean upwelling. Science 247, 198–201.

Berdalet, E., Tester, P., Chinain, M., Fraga, S., Lemée, R., Litaker, W., Penna, A., Usup, G., Vila, M., Zingone, A., 2017. Harmful algal blooms in benthicsystems: recent progress and future research. Oceanography 30, 36–45.

Boada, L., Zumbado, M., Luzardo, O., Almeida-González, M., Plakas, S., Granade, H., Abraham, A., Jester, E., Dickey, R., 2010. Ciguatera fish poisoning on the West Africa Coast: an emerging risk in the Canary Islands (Spain). Toxicon 56, 1516–1519.

Bravo, J., Suárez, F., Ramírez, A., Acosta, F., 2015. Ciguatera, an emerging human poisoning in Europe. J. Aquacult. Mar. Biol. 3 (1), 00053.

Brito, A., Falcón, J., Herrera, R., 2005. Sobre la tropicalización reciente de la ictiofauna litoral de las islas Canarias y su relación con los cambios ambientales y actividades antrópicas. Vieraea 33, 515–525.

Brito, A., 2008. Influencia del calentamiento global sobre la biodiversidad marina de las Islas Canarias. In: Afonso-Carrillo, J. (Ed.), Naturaleza amenazada por los cambios en el clima. Actas III Semana Científica Telesforo Bravo. IEHC, Puerto de la Cruz, pp. 141–161.

Canary-Islands-Health-Service, 2016. Summary of records from SVEICC (Sistema de Vigilancia Epidemiológica de la Intoxicación por Ciguatera en Canarias) during 2008–2015 period. .

Chinain, M., Faust, M.A., Pauillac, S., 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. J. Phycol. 35 (6), 1282–1296.

Chinain, M., Darius, H., Ung, A., Fouc, M., Revel, T., Cruchet, P., Pauillac, S., Laurent, D., 2010. Ciguatera risk management in French Polynesia: the case study of Raivavae Island (Australes Archipelago). , pp. 674–690.

Church, J., White, N., Konikow, L., Domingues, C., ogley, C.J., Rignot, E., Gregory, J., van den Broeke, M., Monaghan, A., Velicogna, I., 2011. Revisiting the Earth's sea-level and energy budgets from 1961 to 2008. Geophys. Res. Lett. 38, L18601.

Cropper, T., Hanna, E., Bigg, G., 2014. Spatial and temporal seasonal trends in coastal upwelling off Northwest Africa,1981–2012. Deep-Sea Res. I 86, 94–111.

Dowsett, H., Barron, J., Poore, R., 1996. Middle Pliocene sea surface temperatures: a global reconstruction. Mar. Micropaleontol. 27, 13–25.

Doyle, J., Doyle, J., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

Fernández-Palacios, J., de Nascimento, L., Rüdiger, O., Delgado, J., García-del-Rey, E., Arévalo, J., Whittaker, R., 2011. A reconstruction of Palaeo-Macaronesia, with particular reference to the long-term biogeography of the Atlantic island laurel forests. J. Biogeogr. 38, 226–246.

Fraga, S., 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 (6), 839–853.

Fraga, S., Rodríguez, F., Caillaud, A., Diogène, J., Raho, N., 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., 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., Triemer, R.E., 1985. A rapid simple technique utilizing calcofluor white M2R for the visualization of dinoflagellate thecal plates. J. Phycol. 662–664.

Gómez, F., Qiu, D., Lopes, R., Lin, S., 2015. Fukuyoa paulensis gen. et sp. nov., a new genus for the globular species of the dinoflagellate Gambierdiscus (Dinophyceae). Fukuyoa paulensis gen. et sp. nov., a new genus for the globular species of the dinoflagellate Gambierdiscus (Dinophyceae). PLoS One 10 (4), e0119676.

GEOHAB, 2012. Global Ecology and Oceanography of HarmfulAlgal Blooms, In: E. Berdalet, P.T., A. Zingone (Eds.) , GEOHAB Core Research Project: HABs in Benthic Systems, Paris and Newark, p. 64.

Gouveia, N., Delgado, J., Gouveia, N., Vale, P., 2009. Primeiro registro da ocorrência de episoódios do tipo ciguatérico no arquipélago da Madeira. In: al, al, C.e (Eds.), Fitoplancton Tóxico E Biotoxinas X Reuniao Berica. IPIMAR, Lisbon, Portugal, pp. 152–157.

Haug, G., Tiedemann, R., 1998. Effect of the formation of the Isthmus of Panama on Atlantic Ocean thermohaline circulation. Nature 393, 673–676.

Hoppenrath, M., Shauna, A., Chomérat, N., Horiguchi, T., 2016. Marine Benthic Dinoflagellates—Unveiling Their Worldwide Biodiversity. Schweizerbart Science Publishers, Sttutgart (Germany).

Huelsenbeck, J.P., Ronquist, F., 2001. MR. Bayes: bayesian inference of phylogenetic trees. Bioinformatics 17 (8), 754–755.

Iliffe, T., Wilkens, H., Parzefall, J., Williams, D., 1984. Marine lava cave fauna: composition, biogeography and origins. Science 225, 309–311.

Kibler, S., Litaker, R., Holland, W., Vandersea, M., Tester, P., 2012. Growth of eight Gambierdiscus (Dinophyceae) species: effects of temperature, salinity and irradiance. Harmful Algae 19, 1–14.

Kondraskov, P., Schütz, N., Schüßler, C., de Sequeira, M., Guerra, A., Caujapé-Castells, J., Ruth Jaén-Molina4 Marrero-Rodríguez, A., Koch, M., Linder, P., Kovar-Eder, J., Thiv, M., 2015. Biogeography of mediterranean hotspot biodiversity: re-evaluating the 'Tertiary relict' hypothesis of macaronesian laurel forests. PLoS One 10, e0132091.

Kooistra, W., Stam, W., Olsen, J., van den Hoek, C., 1992. Biogeography of Cladophoropsis membranacea (Chlorophyta) based on comparisons of nuclear rDNA ITS sequences. J. Phycol. 28, 660–668.

Kumar, S., Stecher, G., Tamura, K., 2016. Mol. Biol. Evol. 33, 1870–1874.

Litaker, R., Vandersea, M., Kibler, S., Reece, K., Stokes, N., Steidinger, K., Millie, D., Bendis, B., Pigg, R., Tester, P., 2003. Identification of P*fiesteria piscicida* (Dinophyceae) and P*fiesteria*-like organisms using internal transcribed spacer-specific PCR assays. J. Phycol. 39, 754–761.

Litaker, R., Vandersea, M., Faust, M., Kibler, S., Chinain, M., Holmes, M., Holland, W., Tester, P., 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., Vandersea, M., Faust, M., Kibler, S., Nau, A., Holland, W., Chinain, M., Holmes, M., Tester, P., 2010. Global distribution of ciguatera causing dinoflagellates in the genus Gambierdiscus. Toxicon 56, 711–730.

Llewellyn, L., 2010. Revisiting the association between sea surface temperature and the epidemiology of fish poisoning in the South Pacific: reassessing the link between ciguatera and climate change. Toxicon 56, 691–697.

Lobel, P.S., Anderson, D.M., Durand-Clement, M., 1988. Assessment of ciguatera dinoflagellate populations: sample variability and algal substrate selection. Biol. Bull. 175, 94–101.

Lunt, D., Valdes, P., Haywood, A., Rutt, I., 2008. Closure of the Panama Seaway during the Pliocene: implications for climate and Northern Hemisphere glaciation. Clim. Dyn. 30, 1–18.

Luque, A., Martín, J., Dorta, P., Mayer, P., 2014. Temperature trends on gran canaria (Canary islands): an example of global warming over the subtropical northeastern atlantic. ACS 4, 20–28.

McGowan, J., Cayan, D., Dorman, L., 1998. Climate-ocean variability and ecosystem r esponse in the Northeast Pacific. Science 281, 210–217.

Meco, J., Scaillet, S., Guillou, H., Lomoschitz, A., Carracedo, J., Ballester, J., Betancort, J., Cilleros, A., 2007. Evidence for long-term uplift on the Canary Islands from emergent Mio–Pliocene littoral deposits. Global Planet. Change 57, 222–234.

Nascimento, S., Melo, G., Salgueiro, F., dos Santos, B., Fraga, S., 2015. Morphology of *Gambierdiscus excentricus* (Dinophyceae) with emphasis on sulcal plates. Phycologia 54, 628–639.

Navarro-Pérez, E., Barton, E., 2001. Seasonal and interannual variability of the Canary Current. Sci. Mar. 65, 205–213.

Nei, M., 1987. Genetic distance and molecular phylogeny. In: Ryman, R., Utter, F. (Eds.), Population Genetics and Fishery Management. University of Washington Press, Seattle, WA, pp. 193–223.

Nishimura, T., Sato, S., Tawong, W., Sakanari, H., Uehara, K., Shah, M., Suda, S., Yasumoto, T., Taira, Y., Yamaguchi, H., Adachi, M., 2013. Genetic diversity and distribution of the ciguatera causing dinoflagellate *Gambierdiscus* spp. (Dinophyceae) in coastal areas of Japan. PLoS One 8, e60882.

Otero, P., Pérez, S., Alfonso, A., Vale, C., Rodríguez, P., Gouveia, N., Gouveia, N., Delgado, J., Vale, P., Hirama, M., Ishihara, Y., Molgó, J., Botana, L., 2010. First toxin profile of ciguateric fish in Madeira Archipelago (Europe). Anal. Chem. 82 (14), 6032–6039.

Parsons, M., Aligizaki, K., Dechraoui Bottein, M.-Y., Fraga, S., Morton, S., Penna, A., Rhodes, L., 2012. *Gambierdiscus* and *Ostreopsis* Reassessment of the state of knowledge of their taxonomy, geography, ecophysiology, and toxicology. Harmful Algae 14, 107–129.

Penna, A., Fraga, S., Battocchi, C., Casabianca, S., Giacobbe, M., Riobó, P., Vernesi, C., 2010. A phylogeographical study of the toxic benthic dinoflagellate genus *Ostreopsis* Schmidt. J. Biogeogr. 37, 830–841.

Peréz-Arellano, J.L., Luzardo, O.P., Brito, A.P., Hernández Cabrera, M., Zumbado, M., Carranza, C., Angel Moreno, A., Boada, L.D., 2005. Ciguatera fish poisoning, Canary Islands. Emerg. Infect. Dis. 11 (12), 1981–1982.

Pisapia, F., Holland, W., Hardison, D., Litaker, R., Fraga, S., Nishimura, T., Adachi, M., Nguyen-Ngoc, L., Séchet, V., Amzil, Z., Herrenknecht, C., Hess, P., 2017. Toxicity screening of 13 *Gambierdiscus* strains using neuro-2a and erythrocyte lysis bioassaysToxicity screening of 13 Gambierdiscus strains using neuro-2a and erythrocyte lysis bioassays. Harmful Algae 63, 173–183.

Randall, J., 1958. A review of ciguatera, tropical fish poisoning, with a tentative explanation of its cause. Bull. Mar. Sci. Gulf. Caribb. 8, 236–267.

Sangil, C., Sansón, M., Afonso-Carrillo, J., Herrera, R., Rodríguez, A., Martín-García, L., Díaz-Villa, L., 2012. Changes in subtidal assemblages in a scenario of warming: proliferations of ephemeral benthic algae in the Canary Islands (eastern Atlantic Ocean). Mar. Environ. Res. 77, 120–128.

Sauter, R., ten Brink, P., Withana, S., Mazza, L., Pondichie, F., Clinton, J., Lopes, A., Bego, K., 2013. Impacts of climate change on all European islands, A report by the Institute for European Environmental Policy (IEEP) for the Greens/EFA of the European Parliament. Final Report. Brussels. .

Schmidt, D., 2007. The closure history of the Central American seaway: evidence from isotopes and fossils to models and molecules. In: Williams, M., Haywood, A., Greory, F., Schmidt, D. (Eds.), Deep-time Perspectives on Climate C Hange: Marrying the Signal from Computer Models and Biological Proxies. The Geological Society, pp. 429–444.

Scholin, C.A., Herzog, M., Sogin, M., Anderson, D.M., 1994. Identification of group and strain specific genetic markers for globally distributed Alexandrium (Dinophyceae). II. Sequence analysis of a fragment of the LSU rRNA gene. J. Phycol. 30, 999–1011.

Scotese, C., 1991. Jurassic and cretaceous plate tectonic reconstructions. Palaeogeogr. Palaeoclimatol. Palaeoecol. 87, 493–501.

Sousa e Silva, E., 1956. Contribution a L'étude du microplancton de Dakar et des régions maritimes voisines. Bulletin de L'I.F.A.N. XVIII, série A. T. XVIII, sér. A, no. 2, 335–371.

Stille, P., Steinmann, N., Riggs, S., 1996. Nd isotope evidence for the evolution of the paleocurrents in the Atlantic and Tethys Oceans during the past 180 Ma. Earth Planet. Sci. Lett. 144, 9–19.

Sydeman, W., García-Reyes, M., Schoeman, D., Rykaczewski, R., Thompson, S., Black, B., Bograd, S., 2014. Climate change and wind intensification in coastal upwelling ecosystems. Science 345, 77–80.

Tel, E., Balbin, R., Cabanas, J., Garcia, M., García-Martínez, M., González-Pola, C., Lavín, A., López-Jurado, J., Rodríguez, C., Villarreal, M., Sánchez-Leal, R., Vargas-Yáñez, M., Vélez-Belchí, P., 2016. IEOOS: the spanish institute of oceanography observing system. Ocean Sci. 12, 345–353.

Tester, P., Vandersea, M., Buckel, C., Kibler, S., Holland, W., Davenport, E., Clark, R., Edwards, K., Taylor, J., Vander Pluym, J., Hickerson, E., Litaker, R., 2013. Gambierdiscus (Dinophyceae) species diversity in the Flower Garden Banks National Marine Sanctuary, Northern Gulf of Mexico, USA. , pp. 1–9.

Tester, P.A., Kibler, S.R., Holland, W.C., Usup, G., Vandersea, M.W., Leaw, C.P., Teen, L. P., Larsen, J., Mohammad-Noor, N., Faust, M.A., Litaker, R.W., 2014. Sampling harmful benthic dinoflagellates: comparison of artificial and natural substrate methods. Harmful Algae 39, 8–25.

Toda, M., Uneyama, C., Toyofuku, H., Morikawa, K., 2012. Trends of food poisonings caused by natural toxins in Japan, 1989–2011. Shokuhin eiseigaku zasshi. J. Food Hyg. Soc. Jap. 53 (2), 105–120.

Xu, Y., Richlen, M., Liefer, J., Robertson, A., Kulis, D., Smith, T., Parsons, M., Anderson, D., 2016. Influence of environmental variables on *Gambierdiscus* spp. (Dinophyceae) growth and distribution. influence of environmental variables on Gambierdiscus spp. (Dinophyceae) growth and distribution. PLoS One 11 (4), e0153197.

Yoshimatsu, T., Yamaguchi, H., Iwamoto, H., Nishimura, T., Adachi, M., 2014. Effects of temperature: salinity and their interaction on growth of Japanese *Gambierdiscus* spp. (Dinophyceae). Harmful Algae 35, 29–37.