

Morphological identification of two tropical dinoflagellates of the genera *Gambierdiscus* and *Sinophysis* in the Mediterranean Sea

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Gambierdiscus cells were found on macroalgae samples (*Padina pavonica*, *Corallina elongata*, *Jania* sp. and *Cystoseira* sp.) collected from 11 sites in Crete Island in summer and autumn months of the years 2003, 2004, 2005 and 2007. The presence of the genus *Gambierdiscus* in Crete since 2003 constituted the earliest record of this genus in the Mediterranean Sea. The epiphytic dinoflagellate community comprised the genera *Gambierdiscus*, *Ostreopsis*, *Coolia*, *Prorocentrum*, *Amphidinium* and the non-photosynthetic benthic species *Sinophysis canaliculata*. The last species was also recorded for the first time in the Mediterranean Sea in the present study. Furthermore, diatoms of the genera *Coscinodiscus*, *Licmophora* and *Striatella* were observed as well. *Sinophysis canaliculata* cells fitted in the earlier descriptions of the species bearing the characteristic hole on the left lateral hypothecal plate. *Gambierdiscus* cells from both field samples and cultures were round to ellipsoid in shape (dorsoventral diameter/transdiameter ratio, DV/W: 1.02 ± 0.06) and anterior-posteriorly compressed ($AP < 45 \mu\text{m}$), while field specimens (DV: $57.12\text{--}76.16 \mu\text{m}$, W: $52.36\text{--}78.54 \mu\text{m}$) were found to be smaller than those grown in culture conditions (DV: $69.02\text{--}84.49 \mu\text{m}$, W: $69.02\text{--}85.68 \mu\text{m}$). Specific discriminative characteristics of *Gambierdiscus* specimens examined in this study, such as the thecal surface pattern and the posterior plate (1p), fitted well with those of the “*G. toxicus* type”; nevertheless, our specimens were not assigned to a species due to inconsistencies revealed lately regarding the identity of the type species.

Key words: *Gambierdiscus*, ciguatera, Mediterranean, benthic dinoflagellates, *Sinophysis canaliculata*.

INTRODUCTION

The distribution of *Gambierdiscus* species has been considered restricted in circumtropical areas in latitudes between 35°N and 35°S (Lehane & Lewis, 2000; Lewis, 2001). *Gambierdiscus* species are usually found on macrophyte, detritus, sand and coral reef surfaces along with other dinoflagellate species of the genera *Ostreopsis*, *Coolia*, *Prorocentrum* and *Amphidinium* (Fukuyo, 1981; Besada *et al.*, 1982; Faust, 1995; Mohammad-Noor *et al.*, 2007). *Gambierdiscus toxicus* has been proven to be the primary causative agent of

ciguatera fish poisoning (Yasumoto *et al.*, 1977), while several *Gambierdiscus* strains have been found to be toxic (Durand-Clement, 1986; Tosteson *et al.*, 1989; Bustillos-Gusmán & Diogène, 1998; Chinain *et al.*, 1999a).

Ciguatera is the most frequently reported marine toxin-derived disease worldwide resulting in illness for more than 50,000 people each year and also affecting fish industry with consequent economical losses (Glaziou & Legrand, 1994; Lewis, 2001). Symptoms of this syndrome include gastrointestinal, neurological and cardiovascular disturbances (Lewis, 2001).

Suspected ciguatera poisonings were reported in the Mediterranean Sea (Israel coast) after the con-

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sumption of rabbitfish (*Siganus luridus*) in 1971-1972 (Herzberg, 1973) and saupe (*Sarpa salpa*) in 1988 (Spanier *et al.*, 1989), but they were not associated with *Gambierdiscus* species. However, the latter report was questioned by Chevalloné (1990), who suggested the possibility of misinterpretation of this case with “*Caulerpa* poisoning”. Recently, two cases of another type of ichthyosarcotoxism, ichthyallyeinotoxism, caused after the consumption of *Sarpa salpa* in the Western Mediterranean Sea, were described and distinguished from ciguatera poisoning based on the typical clinical signs (hallucinations and nightmares for a few hours) and expressions (De Haro & Pommier, 2006). The causative agents of ichthyallyeinotoxism are still unknown, although representatives of the family Caulerpaceae have been suspected (Chevalloné, 1990). So far, the northernmost documented record of ciguatera incidents coinciding with the presence of *Gambierdiscus* sp. (Fraga *et al.*, 2004) concerns the Canary Islands (Pérez-Arellano *et al.*, 2005).

Reports on potentially toxic epiphytic dinoflagellates of the genera *Ostreopsis*, *Coolia* and *Prorocentrum* in the Mediterranean Sea have been increasing during the last decade (Vila *et al.*, 2001; Penna *et al.*, 2005; Turki, 2005; Aligizaki & Nikolaidis, 2006a, b; Ismael & Halim, 2006; Monti *et al.*, 2007). This fact represents the global trend of harmful algal blooms increase in frequency, intensity and geographic distribution (Hallegraeff *et al.*, 2003).

The recent (in 2007) and unexpected detection of *Gambierdiscus* cells in the Mediterranean Sea (Crete Island) (Aligizaki *et al.*, 2008) triggered off the examination of archival material collected from the same area. Thus, in this paper, the presence since 2003 of the ciguatera-causing tropical genus *Gambierdiscus* in the Mediterranean Sea is documented. Furthermore, morphological details of *Gambierdiscus* sp. and *Sinophysis canaliculata* are presented.

MATERIALS AND METHODS

Sampling

Samples of the macroalgae *Padina pavonica*, *Corallina elongata*, *Jania* sp. and *Cystoseira* sp. were collected in depths up to 1 m from 11 sampling sites (s1-s11) along the coasts of Crete Island (Fig. 1) in September 2003, 2004, August 2005 and September-October 2007 in order to investigate the presence of potentially toxic benthic dinoflagellates. Sea surface water temperature and salinity were measured with portable instruments (LF1700, WTW Germany) simultaneously

with sample collection only in the samplings of September and October 2007.

Macroalgae samples, which were collected in PVC bottles containing 200-250 ml of surface seawater, were vigorously shaken for approximately 1 min to detach the epiphytic cells. In 2007 samplings, the macrophytes were subsequently removed and weighed (fresh weight of macrophytes in grams). The seawater containing the suspended epiphytic cells was preserved in Lugol's iodine solution, while a small fraction of this sample was kept alive for cell isolation and culture establishment. Upon arrival to the lab, the suspended material was passed through 250 and 100 μm mesh size sieves to remove large particles and, subsequently, it was examined for the presence of benthic dinoflagellates. Abundance of epiphytic dinoflagellates was determined by counting under an inverted microscope according to Utermöhl's sedimentation method (Utermöhl, 1958) and expressed in cells g^{-1} fresh weight of macrophyte (fwm). In 2003, 2004 and 2005, the material was used only for qualitative investigation of epiphytic dinoflagellates; therefore cell density was not determined.

Isolation and culture conditions

For culture establishment, cells were obtained from the seawater sample containing the live epiphytic dinoflagellates from the sampling site s1 (West Crete) in 2007. Single cells were isolated by micropipetting, cleaned by sequential transfers through a small quantity of medium and, finally, each cell was placed in a 5 ml Petri dish containing 4 ml of sterile L1 medium (Andersen, 2005). Clonal cultures of *Gambierdiscus* species (KC81ALCCAUTH) were grown in L1 medium at a temperature of $24 \pm 0.5^\circ\text{C}$, photoperiod 14:10 hrs light:dark and illumination of $80 \mu\text{moles m}^{-2} \text{s}^{-1}$.

Gambierdiscus identification

Identification was based on light (LM) and electron microscopy of preserved field material and cells from the established clonal cultures. Morphometric features were observed under epifluorescence microscopy (Olympus IX71) using fluorescent brightener 28 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) (Fritz & Triemer, 1985), while for detailed observations scanning electron microscopy (SEM) was employed. For SEM preparation, samples were filtered on GF/C glass microfibre filters (diameter 47 mm, pore size 1.2 μm , Whatman), washed with distilled water, dehy-

drated through a graded ethanol series, critical point dried with CO₂ (Critical Point Dryer, Balzers CPD 030), filter-mounted to a stub (Standard JEOL stubs made of Cu and Zn, 32 mm) and coated with gold; samples were observed under a JEOL JSN 6400.

RESULTS AND DISCUSSION

General

The detection of *Gambierdiscus* cells in samples collected from Crete Island in September and October 2007 (Aligizaki *et al.*, 2008) prompted us to re-examine thoroughly larger volumes of qualitative samples collected in 2003, 2004 and 2005 from other stations on Crete Island (Fig. 1) in order to investigate the occurrence of *Gambierdiscus* in these samples; this re-examination revealed the presence of the genus *Gambierdiscus* in the Mediterranean Sea since 2003 (Table 1, Fig. 1).

In the epiphytic community, where *Gambierdiscus* sp. was detected, *Ostreopsis*, *Prorocentrum*, *Amphidinium* species and also *S. canaliculata* were found (Table 1) along with diatom species of the genera *Coscinodiscus*, *Licmophora* and *Striatella*. *Gambierdiscus* sp. and *S. canaliculata* constitute new records for the Mediterranean Sea, while *Ostreopsis*, *Prorocentrum* and *Amphidinium* species are currently widespread in both the western (Vila *et al.*, 2001; Penna *et al.*, 2005; Turki, 2005; Monti *et al.*, 2007) and eastern Mediterranean coastal waters (Aligizaki & Nikolaidis, 2006a, b; Ismael & Halim, 2006).

Sinophysis canaliculata and also *S. microcephala* are considered tropical species (Mohammad-Noor *et al.*, 2007), while other representatives of the genus have been found in the North Sea and the Sea of Japan (Hoppenrath, 2000; Selina & Hoppenrath, 2004). *Sinophysis canaliculata* has been previously reported only from the Indian Ocean Islands (Quod *et al.*, 1999), Malaysian waters (Mohammad-Noor *et al.*, 2007) and the Mexican Pacific (Okolodkov & Gárate-Lizárraga, 2006).

In September and October 2007, when *Gambierdiscus* sp. was detected, the epiphytic dinoflagellate community was dominated by *C. monotis* at abundance levels up to 670 cells g⁻¹ fwm (maximum abundance found on *Cystoseira* spp. at s1) followed by *Ostreopsis* spp. (*O. ovata*, *O. cf. siamensis*, *Ostreopsis* sp.), the epiphytic cell density of which did not exceed 268 cells g⁻¹ fwm (maximum abundance found on *Cystoseira* spp. at s1). At the same station and substrate, *Prorocentrum* spp. (*P. borbonicum*, *P. lima*, *P. emarginatum*, *P. rhathymum*, *Prorocentrum* sp.) and *Amphi-*

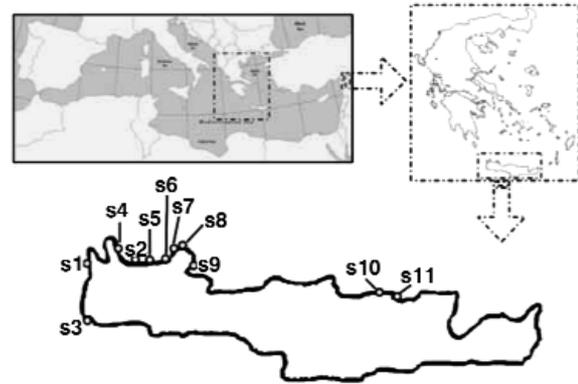


FIG. 1. Maps showing the location of sampling stations on Crete Island (Greece).

dinium spp. were recorded in maximum abundance levels of 53 and 14 cells g⁻¹ fwm, respectively, while *Gambierdiscus* sp. and *S. canaliculata* were found in lower cell densities (9.01 cells g⁻¹ fwm, for each one). During that time, sea surface water temperature ranged between 23.8 °C (October) and 26.1 °C (September) while salinity was 38.5 psu in the same period. It should be noted that no temporal or spatial pattern can be deduced by the present data. The abundance levels of epiphytic dinoflagellate genera *Ostreopsis*, *Coolia*, *Prorocentrum* and *Amphidinium* recorded in our samples are relatively low compared with other studies in Greek coastal waters (Aligizaki & Nikolaidis, 2006a) and other Mediterranean areas (Vila *et al.*, 2001), while *Gambierdiscus* sp. abundance, which can be compared only to other areas outside the Mediterranean Sea, was also very low. *Gambierdiscus* spp. were detected in abundance levels up to 75,793 cells g⁻¹ fwm in the Caribbean Sea (Carlson *et al.*, 1984) and 60,463 cells g⁻¹ fwm in Mayotte Island (SW Indian Ocean) (Turquet *et al.*, 2001), while relatively lower levels (10,880 cells g⁻¹ fwm) have been recorded in Tahiti (French Polynesia) (Chinain *et al.*, 1999a) and Hawaii Island (1,148 cells g⁻¹ fwm) (Parsons & Preskitt, 2007).

Cell morphology

Gambierdiscus sp.

Gambierdiscus cells were round to ellipsoid in shape (Fig. 2A-C) with an obvious Po (Fig. 2B, D) and anterior-posteriorly compressed (AP < 45 μm, Fig. 2E) with numerous golden-brown chloroplasts (Fig. 2A). Cells from field samples differed in dimensions from those grown in cultures, with the latter being larger. The dorsoventral diameter (DV) in field cells ranged

from 57.12 to 76.16 μm and the transdiameter (width, W) from 52.36 to 78.54 μm , while DV and W for cultured cells was measured between 69.02–84.49 μm and 69.02–85.68 μm , respectively. Despite the difference in size, both cultured and field cells displayed the same DV/W ratio of 1.02 ± 0.06 .

The thecal surface was smooth with numerous evenly distributed pores (Fig. 2B–E). The plate formula was Po, 3', 7'', 6c, (?), 5''', 1p and 2'''. The apical pore plate (Po) was ellipsoid, with a fish hook-shaped pore, situated towards the center of the epitheca (Fig. 2B, D); the first apical plate (1') was irregularly heptagonal (Fig. 2B) in contact with Po, 2', 3', 2'', 6'' and the minuscule precingular plates 1'' and 7'' (Fig. 2B, E). The second apical plate (2') was the

largest of the apicals, while 3'' and 4'' were the largest precingular (Fig. 2B). In the hypotheca, 1p was broad and large, occupying almost half of the hypothecal width. Plates 2''' and 4''' were irregularly elongated and the largest postcingular, while 1'''' and 2'''' were the smallest hypothecal plates (Fig. 2C).

The specimens presented in this study fit well to “*G. toxicus* type” mainly based on conservative features such as fine surface thecal morphology and 1p shape (Richlen *et al.*, 2008); however, they are not assigned to a certain species because the taxonomy of the genus *Gambierdiscus* is presently ambiguous. Six *Gambierdiscus* species have been described so far (Adachi & Fukuyo, 1979; Faust, 1995; Holmes, 1998; Chinain *et al.*, 1999b), while, recently, it was ques-

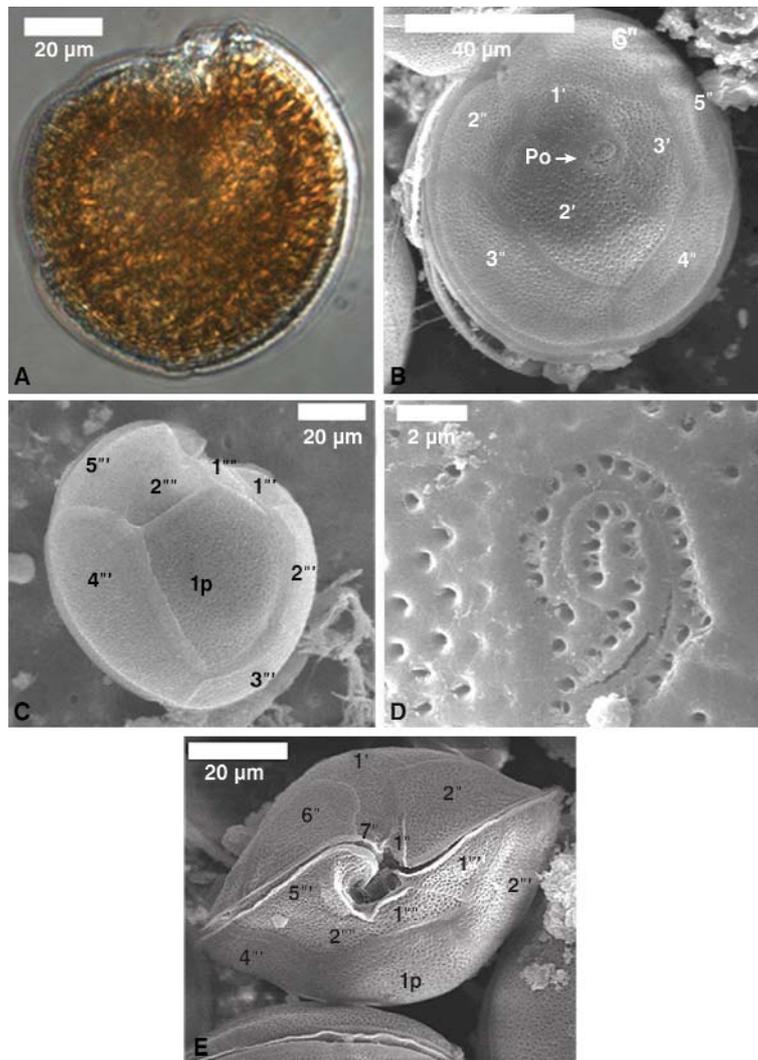


FIG. 2. *Gambierdiscus* sp. cells isolated (KC81ALCCAUTH) from Crete Island in LM (A) and SEM (B–E). A. Live cell. B. Apical view showing epithecal plates. C. Antapical view showing hypothecal plates. D. Close view of Po. E. Sulcal view showing the minuscule 1'' and 7'' precingular plates.

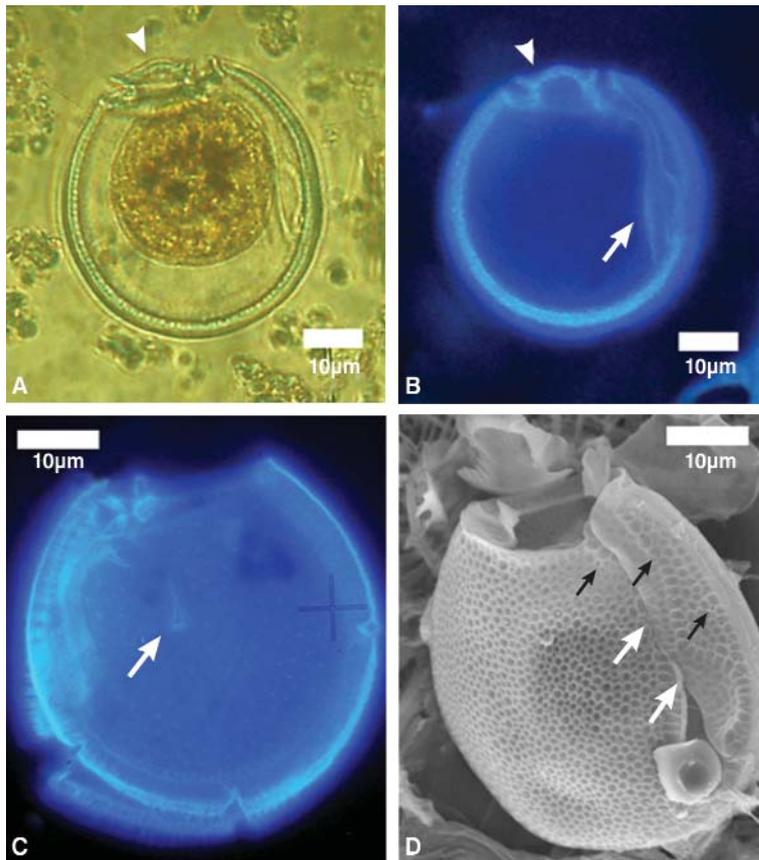


FIG. 3. *Sinophysis canaliculata* from Crete Island in LM (A-C) and SEM (D). A-B. Right lateral cell side (stained with fluorescent brightener in B) showing the small epitheca (arrowheads) and the sulcus (arrow). C. Left lateral cell side showing the characteristic thecal cut (arrow). D. SEM image showing the sulcus (white arrows) and the areolated thecal surface perforated with pores (black arrows).

tioned whether the description of the type species (*G. toxicus*) included multiple species (Tester *et al.*, 2006). Additional molecular and morphological data (Richlen *et al.*, 2008) suggested that *G. toxicus* is not a single cosmopolitan species but a species complex instead. Furthermore, the same authors also proposed that the presence of cryptic species could explain the high variability between *G. toxicus* strains toxicity and the patchiness of ciguatera disease.

Sinophysis canaliculata

The cells of the non-photosynthetic benthic dinoflagellate *S. canaliculata* detected during this study (Fig. 3A-D) conformed to the original description of the species (Quod *et al.*, 1999). Cells were round to slightly ovoid in shape, with a length range of 47.25-50.95 µm, a width range of 42.30-46.92 µm and a very small epitheca (Fig. 3A-B). The thecal surface was areolated and perforated with pores (Fig. 3D) with the characteristic thecal cut (hole) observed (Fig. 3C) in the left lateral hypothecal plate and a long, narrow sulcus in the right lateral plate (Fig. 3B, D).

Concluding remarks

The detection of tropical representatives of the genera *Gambierdiscus* and *Sinophysis* in the Mediterranean Sea raises issues concerning the geographical expansion of tropical species. Their record could indirectly indicate the consequences of climate change in this area, a suggestion which is in accordance with discussions about the “tropicalization” of the Mediterranean Sea (Bianchi, 2007) and the possibility of geographical expansion of tropical microalgae, such as *Gambierdiscus*, due to climate change (Fraga, 2007). On the other hand, the increasing records of benthic dinoflagellates in the Mediterranean Sea during the last years might be a result of intense research in this field. The data presented in this study cannot indicate the way or the specific point in time that *Gambierdiscus* has invaded the Mediterranean Sea; however, it could be suggested that the gradual increase in sea surface water temperature (Salat & Pascual, 2002) facilitated the acclimation and settlement of such a tropical species in the Mediterranean Sea, as it is the case for other marine organisms (e.g. mollusca, fish, macroalgae) (Occhipinti-Ambrogi, 2007).

The presence of *Gambierdiscus* sp. in the Mediterranean Sea may imply the onset of ciguatera disease in this area. This fact constitutes a novel and serious hazard for human health and sea-related activities demanding further research. In this context and with the aim to clarify the origin of *Gambierdiscus* strains, clonal cultures have been established and advanced morphological, toxicological and molecular analyses are underway.

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