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First report of *Gambierdiscus caribaeus* and *G. carpenteri* (Dinophyceae) from Nha Trang Bay, South Central Vietnam

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Original Article

Abstract

The present paper report two species of epibenthic dinoflagellates *viz., Gambierdiscus caribaeus* and *G. carpenteri* from Nha Trang Bay, South Central Vietnam for the first time as a result of experiments with artificial substrates. Detailed descriptions of these two species with illustrations by light and scanning electron micrographs were provided. Both *G. caribaeus* and *G. carpenteri* recorded during the study had morphological features similar to the original descriptions of these species. The apical plate 2' of them is large, rectangular and symmetrical. Plate 3'' and 3''' of *G. caribaeus* are slightly symmetrical, compared with asymmetrical 3'' and 3''' plates in *G. carpenteri*. Plates 2'''' of *G. caribaeus* seem to be shorter in comparison with that of *G. carpenteri*. The plate formula: Po, 4', 6'', ?c, ?s, 5''' and 2''''.

***Keywords**: Artificial substrate, epibenthic dinoflagellate, Gambierdiscus, Vietnam*

Introduction

Gambierdiscus species produce toxins that cause ciguatera fish poisoning (CFP), the common illness associated with fish consumption in tropical areas. The most harmful species of *Gambierdiscus* are epibenthic dinoflagellates that can be found on sandy bottoms, detrital aggregates, seagrasses, seaweeds and cyanobacteria (Ballantia *et al.*, 1985; Faust, 1995a; Richlen and Lobel, 2011; Rains and Parsons, 2015). Few researchers have used artificial substrate to collect dinoflagellate cells (Caire *et al.*, 1985; Kibler *et al.*, 2010; Tan *et al.*, 2013; Tester *et al.*, 2014). Until now, *G. caribaeus* and *G. carpenteri* have only been reported from Caribbean including Florida, Texas, Belize, Tahiti and Palau in the Pacific Ocean (Litaker *et al.*, 2009; 2010; Tester *et al.*, 2013). Recently, *G. caribaeus* has also been reported from Jeju Island, Korea, North Pacific Ocean (Jeong *et al.*, 2012) and the Gulf of Thailand (Tawong *et al.*, 2014). *G. carpenteri* was found in the temperate waters of New South Wales, Australia (Kohli *et al.*, 2014). In Vietnamese waters, *Gambierdiscus* species was found on sampled macroalgae and seagrasses (Chu, 2002; Nguyen and Larsen, 2004; Ho and Nguyen, 2009; Ho *et al.*, 2011). In the present study, we describe *G. caribaeus* and *G. carpenteri* and document their morphology with light microscopy (LM) including epifluorescence microscopy and scanning electron microscope (SEM) images. This is the first report of *G. caribaeus* and *G. carpenteri* from Vietnam.

Material and methods

Epibenthic dinoflagellate from five locations were collected on monthly intervals from 3–4 m depths by scuba diving in Nha Trang Bay (Fig. 1), during June to November 2014. Water temperature ranged from 25°C to 29°C and salinity varied from 32 psu to 33 psu.

The artificial substrate used in this study for collecting epibenthic dinoflagellate consisted of pieces of black fiberglass screen cut into rectangles measuring 10 cm x 15 cm (Fig. 2). Each screen was attached to filament (15 cm) and suspended in the water column within 30 cm of the seabed using a bottom weight and subsurface float (Fig. 3). After the screens were placed at

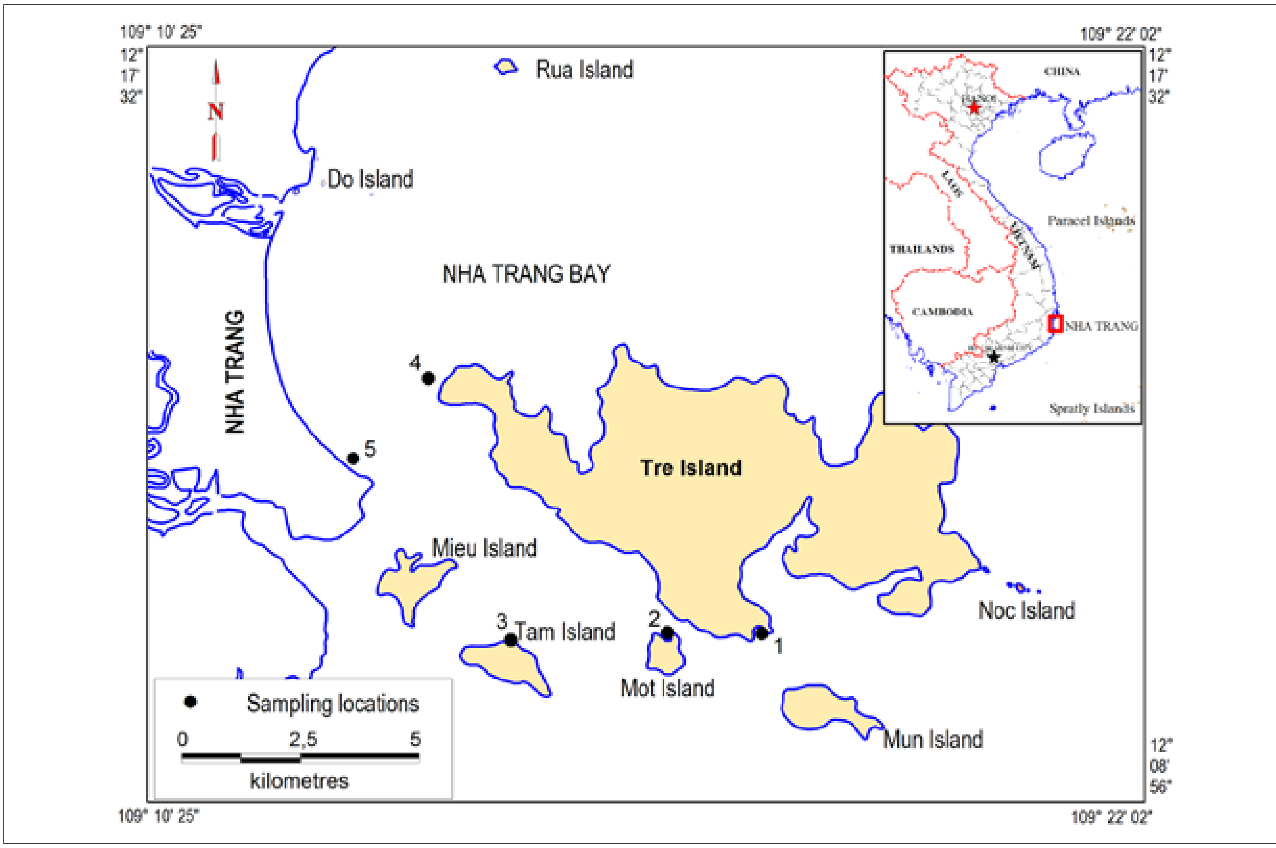
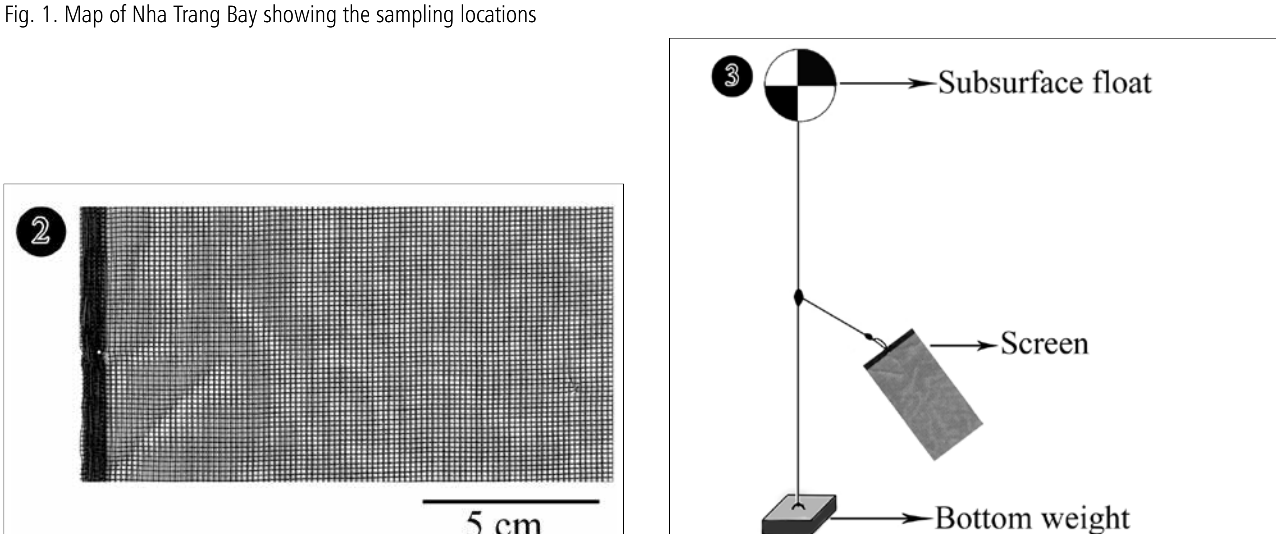


Fig. 1. Map of Nha Trang Bay showing the sampling locations



Figs. 2-3. Artificial substratum (screen window) and sampling methods. Fig. 2: Photograph of fiberglass screen window used as an artificial substratum for collecting epibenthic dinoflagellate. Fig. 3: Schematic drawing showing the fixing assembly for screen window with subsurface float and bottom weight.

sampling locations for 24 h, each screen was carefully collected by hand and placed into a plastic container and transported to the laboratory for further processing.

In the laboratory, the containers holding the screens were vigorously shaken with seawater collected from the same site to dislodge the epibenthic microalgae. All material was poured through stacked sieves of 250, 125, 64, 32 and 20 μm. Epibenthic microalgae on the 64, 32 and 20 μm sieves were observed by a Leica MZ 12 stereo microscope. Cells of *Gambierdiscus* were sought and isolated by a pipette.

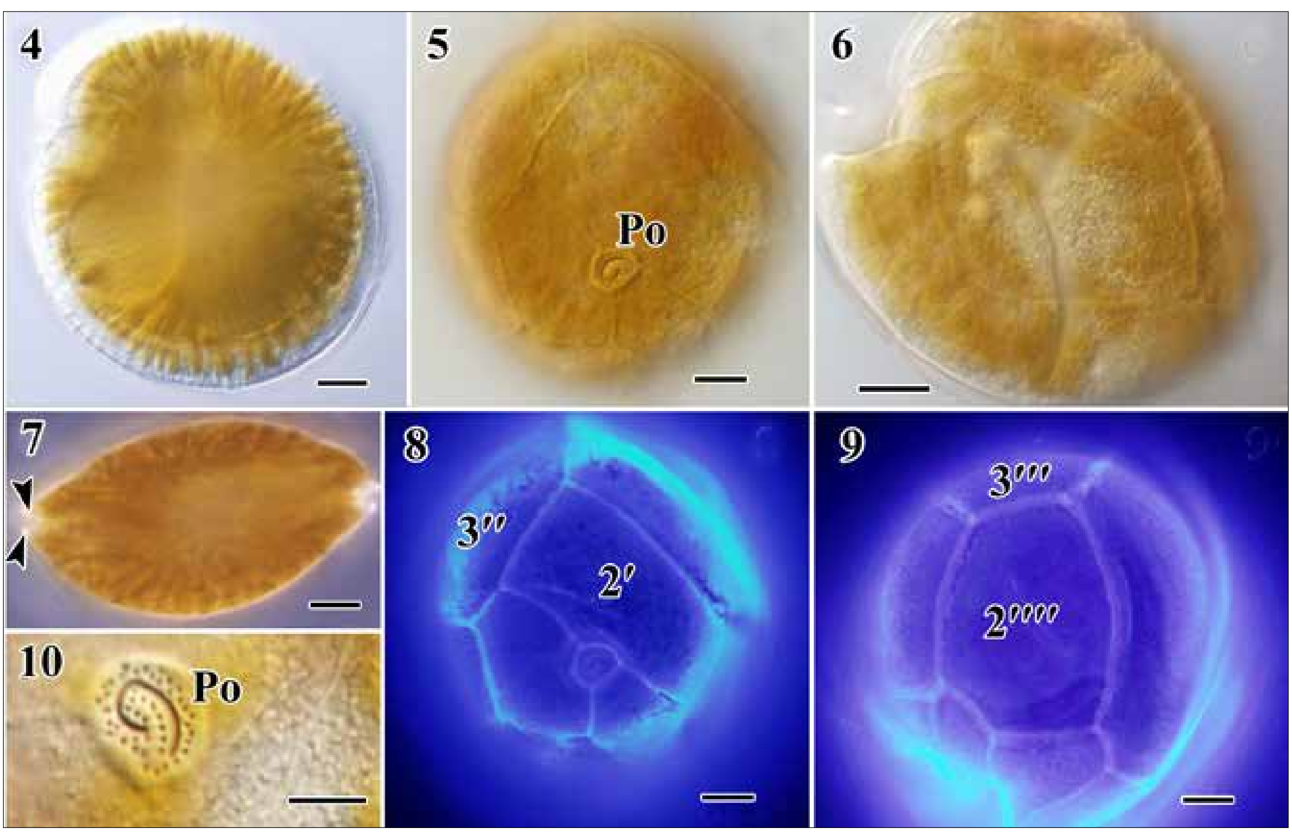
The wild cells of *Gambierdiscus* spp. were observed alive or fixed in formaldehyde (0.4%) by light microscopy (LM). Cell dimensions of specimens of each species were determined under the LM using an ocular micrometer. Cell length (distance from apex to antapex), width (transdiameter), and depth (distance from ventral to dorsal side) were measured. Thecal plate patterns were observed after staining with Calcofluor White M2R (Fritz and Triemer, 1985) by using a Leica LDMB microscope equipped with phase and differential interference contrasts. Photographs were taken with a digital camera (Olympus DP-71). For scanning electron microscopy (SEM), fixed specimens (wild cells) were isolated and placed on 5 μm carbon membrane in a filter-holder (Millipore), rinsed three times with distilled water and dehydrated through an ethanol series of 15, 30, 50, 70, 90, and 99.99%, then air dried. The filter was mounted on an aluminium stub with carbon tape and coated with gold in a vacuum sputter coater. Specimens were observed using a JEOL JSM-5410 LV scanning electron microscope.

Results and discussion

Systematics

Class: Dinophyceae
Order: Gonyaulacales
Family: Goniodomataceae
Genus: *Gambierdiscus*

The morphological characteristics of the two epibenthic dinoflagellate *Gambierdiscus caribaeus* and *G. carpenteri*



Figs. 4–10. Light micrographs of *Gambierdiscus caribaeus*. Figs.4, 5, 7, 8 & 10: same cell. Figs. 4–7: Cells with golden-brown chloroplasts. Figs. 5 & 8: Apical view showing the epithecal plates with nearly rectangular 2' plate and Po plate with a fishhook-shaped centrally located and the symmetrical 3" plate. Fig. 7: Dorsal view showing the cingulum (arrowheads) and convex valves. Figs 6 & 9: Antapical view showing the hypothecal plates with long and broad 2"" plate and the symmetrical 3"' plate. Fig. 10: The Po plate with a fishhook-shaped opening with many round pores. All scale bars: 10 μm, except Fig. 10: 5 μm

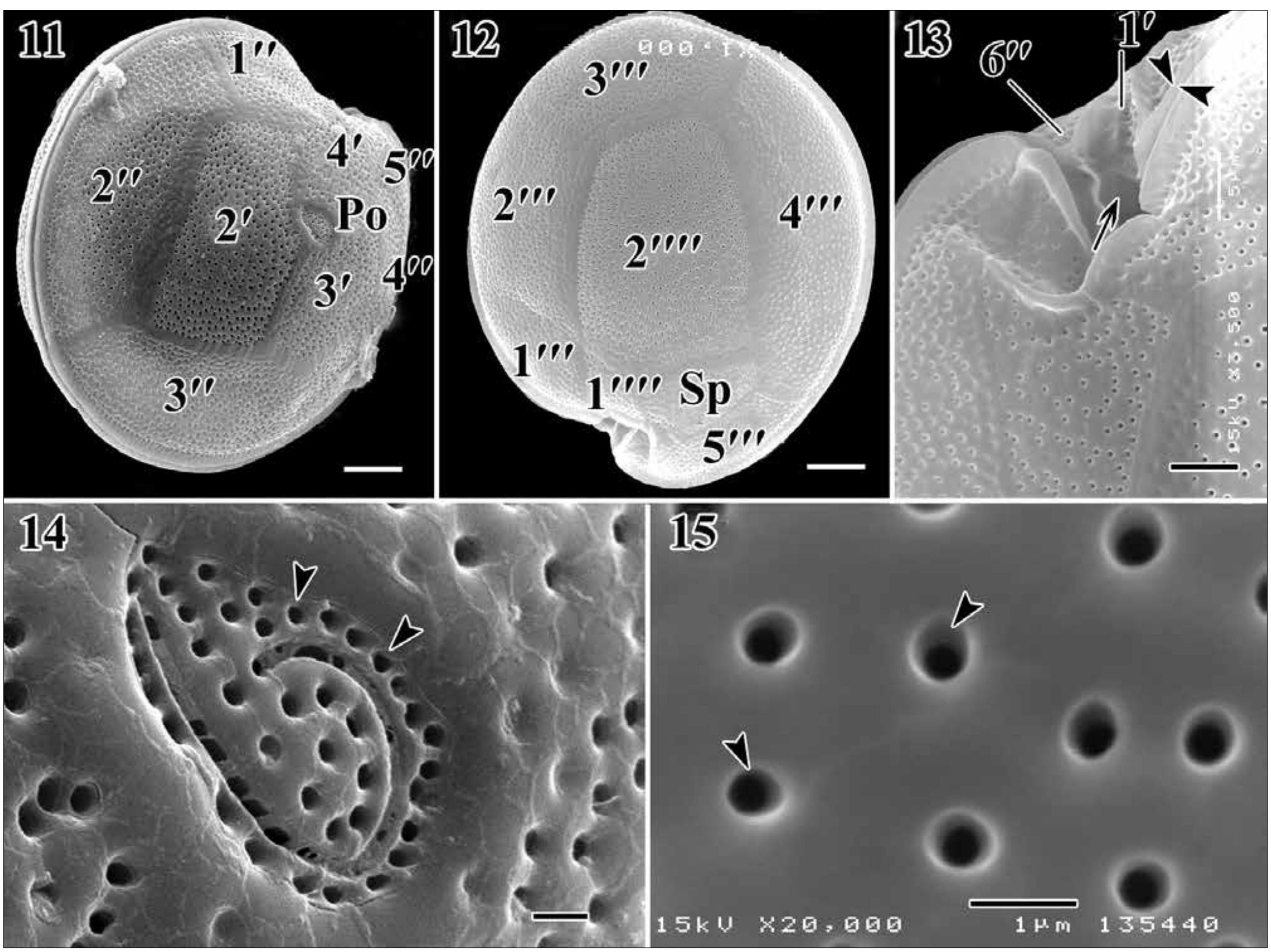
described in this study were determined using light microscopy and scanning electron microscopy (SEM). Species identifications were entirely based on cell size shape, architecture of thecal plates and cell surface morphology.

Gambierdiscus caribaeus Vandersea, Litaker, Faust, Kibler, Holland and Tester, 2009 (Figs. 4–15).

References: Litaker *et al*., 2009: Figs. 12–21, Jeong *et al*., 2012: Figs. 1–27, Hoppenrath *et al*., 2014: Figs. 41A–F.

Taxonomic remarks: Cells of *Gambierdiscus caribaeus* have golden-brown chloroplasts (Figs. 4–7 & 10). Cells are rounded in apical, antapex view. The cell size ranges from 40–42 μm long, 78–82 μm wide and 80–83 μm in depth. Cell surface is slightly thick and smooth with numerous round pores predominantly 0.35–0.40 μm in diameter (Figs. 11–15), both epitheca and hypotheca are convex (Fig. 7). The plate formula: Po, 4', 6", ?c, ?s, 5''' and 2''''.

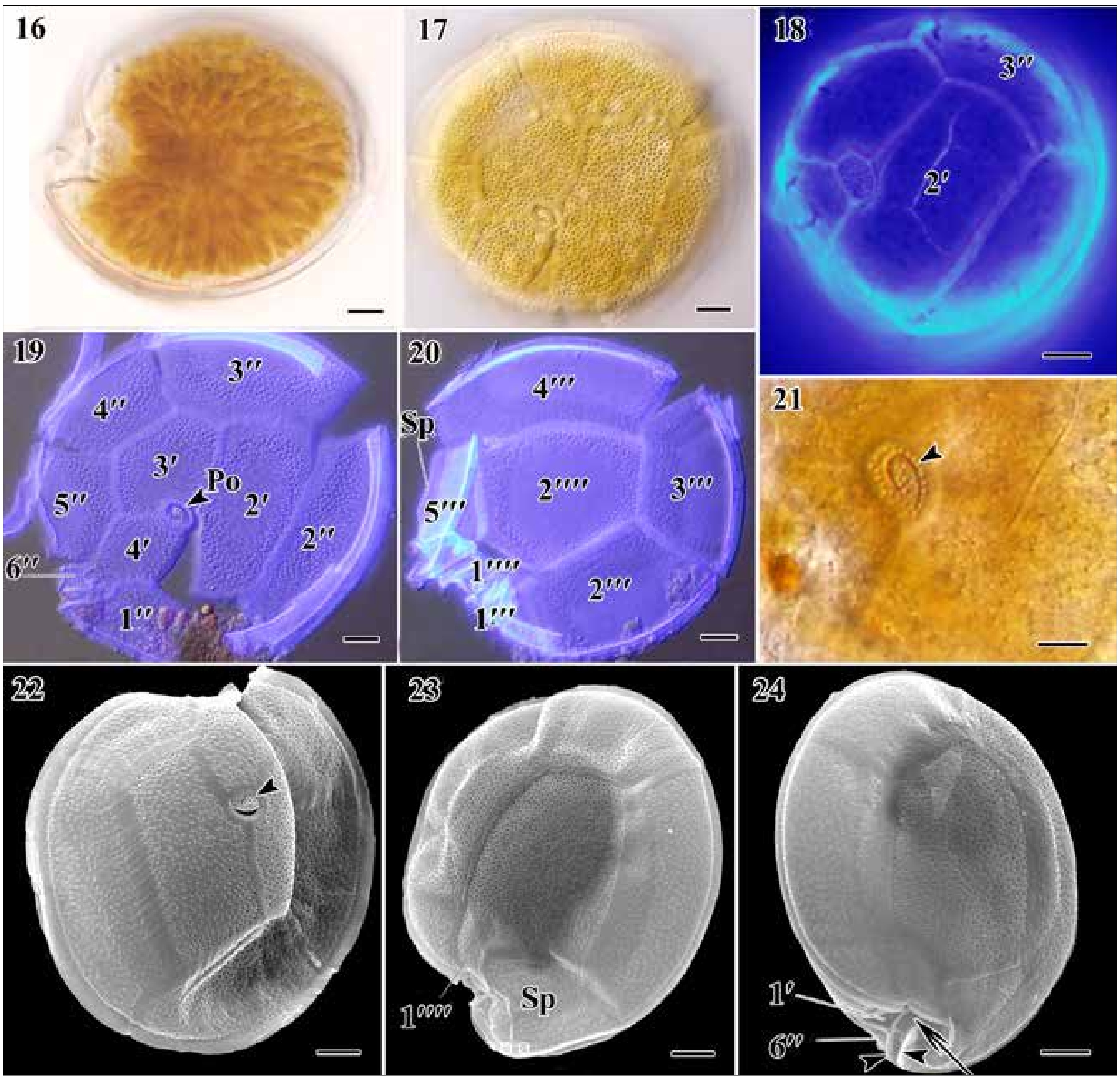
The epitheca of *G. caribaeus* consists of eleven plates: an apical pore plate (Po), four apical plates (4') and six precingular plates (6") (Figs. 5, 8 & 11). The Po plate is oval, 7–9 μm long, 6–7 μm wide, with a fishhook-shaped surrounded by 40 round pores whose diameter is 0.3 μm and it is located in the centre of the epitheca (Figs. 5, 10, 11 & 14). Plate 1' is the smallest of the apical plates (Fig. 13), it was not visible in apical view. The apical plate 2' is nearly rectangular, symmetrical and is the largest of the epithecal plate series, ranging in size from 40–44 μm long and 22–25 μm wide. Plate 3' is five-sided (Figs. 5, 8 & 11). Plate 4' is small and located in the ventral side. Plate 1" is five-sided and contacts with plates 1', 2', 4' and 2". Plate 2" is four-sided and elongated. Plate 3" is four-sided, slightly symmetrical and situated in the dorsal. Plates 4" and 5" are quadrangular and nearly equal in size. Plate



Figs: 11–15. SEM micrographs of *Gambierdiscus caribaeus*. Fig. 11: Apical-dorsal view showing the epithecal plates. Fig. 12: Antapical-ventral view showing the hypothecal plates with broad 2'''' plate and the symmetrical 3''' plate. Fig. 13: Antapical-ventral view showing the narrow cingulum (arrowheads) and the sulcus hole (arrow). Fig. 14: The apical pore plate Po with round pores (arrowheads) and a large fishhook-shaped opening. Fig. 15: Smooth thecal surface with many round pores (arrowheads). Scale bars: Figs. 11–12: 10 μm; Fig. 13: 5 μm; Figs 14–15: 1 μm

6'' is very small (Fig. 13). The cingulum is equatorial, deep and narrow. The cingulum extends into the sulcus, the sulcus has a small and deep opening (Fig. 13).

The hypotheca contains seven plates: five postcingular plates (5'''), and two antapical plates (2'''') in addition to S.p. plate (Figs. 6, 9 & 12). Plate 1''' is the smallest in the series of the hypothecal plates and triangular. Plate 2''' is trapezoidal and symmetrical. Plate 3''' is four-sided, slightly symmetrical and positioned in the dorsal. Plate 4''' is the largest of the postcingular plates, four-sided and elongated. Plate 5''' is small and narrow. Plate 1'''' is small, four-sided. It lies adjacent to plates 1''', 2''', 2'''' and S.p. (Fig. 12). The plate 2'''' is broad, pentagonal and wider towards the ventral side. It contacts with plates 2''', 3''', 4''', 1'''' and S.p. The 2'''' plate ranges from 45–50 μm long and 27–32 μm wide (Figs. 6, 9 & 12).



Figs: 16–24. Light and SEM micrographs of *Gambierdiscus carpenteri*. Figs. 16–17 & 19–20: same cell. Figs. 18 & 21: same cell. Figs. 16–17 & 20: Cell with golden-brown chloroplasts. Figs. 17–19 & 22: Apical view showing the epithecal plates with rectangular 2' plate, the Po centrally located (arrowhead) and the asymmetrical 3'' plate. Figs. 20 & 23: Antapical view showing the hypothecal plates with broad 2'''' plate and the asymmetrical 3''' plate. Fig. 21: Apical pore plate Po (arrowhead). Fig. 24: Antapical-ventral view showing the epithecal plates (plates 1' and 6'') hypothecal plates, narrow cingulum (arrowheads) and sulcus hole (arrow). All scale bars: 10 μm, except Fig. 21: 5 μm

Distribution and habitat: *G. caribaeus* was attached to a few macroalgae (Litaker *et al*., 2009; Jeong *et al*., 2012). This species was first recorded in Carrie Bow Cay, Belize, central America. It was widely distributed in the Caribbean Sea, including Florida, Texas, America, and the Gulf of Mexico (Litaker *et al*., 2009, 2010; Tester *et al*., 2013). Recently, it has been reported in Jeju Island, Korea and the Gulf of Thailand (Jeong *et al*., 2012; Tawong *et al*., 2014). In the present study, populations of *G. caribaeus* occurred at water temperatures ranging from 25°C to 29°C and salinity of 33 psu. It coexisted with other epibenthic dinoflagellates such as *Prorocentrum* spp., *Ostreopsis* spp. and *Coolia* spp. This is the first record of this species in Vietnam.

Gambierdiscus carpenteri Kibler, Litaker, Faust, Holland, Vandersea a Tester, 2009 (Figs. 16–24).

References: Litaker *et al*., 2009: Figs. 36–42, Kohli *et al*., 2014: Figs. 4A–L, 5A–G.

Taxonomic remarks: Cells of *Gambierdiscus carpenteri* have golden-brown chloroplasts (Figs. 16–17). Cells are round to ellipsoid in apical, antapex view. Cells range in size from 38–42 μm long, 80–84 μm wide, 80–83 μm in depth. The thecal surface is thick, smooth and perforated by numerous pores (Figs. 17, 19 & 20) and both valves are convex (Fig. 24). The plate formula: Po, 4', 6'', ?c, ?s, 5''' and 2''''.

The epitheca of *G. carpenteri* is composed of eleven plates: an apical pore plate (Po), four apical plates (4') and six precingular plates (6''). The apical pore plate Po is ellipsoid with a fishhook-shaped opening and it is situated in the center of the epitheca (Figs. 17, 19 & 22). The size of the Po ranges from 9–11 μm long, surrounded by many round pores (Fig. 21). The apical plate 1' is tiny (Fig. 24), whereas the apical plates 2' is large, rectangular and symmetrical, ranging in size from 42–45 μm long and 22–25 μm wide (Figs. 17, 18 & 22). Plate 3' is five-sided. Plate 4' is smaller than plate 3' and located in the ventral side. Plate 2'' is four-sided and elongated. Plate 3'' is situated in the dorsal, five-sided and asymmetrical (Figs. 17–19). Plates 1'', 4'' and 5'' are quadrangular and nearly equal in size (Fig. 19). The cingulum of this species is also deep and narrow, it is displaced to the sulcus, the sulcus has a small and deep opening (Fig. 24).

The hypotheca of *G. carpenteri* is made up of seven plates: five postcingular plates (5''') and two antapical plates (2'''') in addition S.p. plate (Figs. 20 & 23). Plate 1''' is triangular, whereas plate 5''' is elongated and narrow (Figs. 20, 23 & 24). Plates 2''', 3''' and 4''' are very large. Plate 2''' is positioned in the left lateral and trapezoidal. Plate 3''' is four-sided, asymmetrical and wider towards the plate 2''' (Fig. 20). Plate 4''', the largest in the series of the postcingular plates, is elongated and situated the right side of the hypotheca (Figs. 20 & 23). The antapical plate 1'''' is small, pointed, four-sided and located adjacent to plates 1''', 2''', 2'''' and S.p. (Fig. 23). Plate 2'''' is long, pentagonal and broader at the ventral side. It is connected with plates 1'''', 2''', 3''', 4''' and S.p. The 2'''' plate of *G. carpenteri* is larger than plate 2'''' of *G. caribaeus*. Size ranges from 50–55 μm long and 30–35 μm wide (Figs. 20, 23 & 24).

Distribution and habitat: *Gambierdiscus carpenteri* was first found in South Water Cay, Belize, Caribbean Sea, it was also found in Guam, Mariana Islands, Pacific Ocean. It was associated with macroalgae (Litaker *et al*., 2009, 2010; Tester *et al*., 2013). Lately, it has also been reported in temperate waters of New South Wales, Australia (Kohli *et al*., 2014). *G. carpenteri* shared its habitat with species of *Prorocentrum*, *Ostreopsis* and *Coolia*. This is the first report of *G. carpenteri* in Vietnam and Southeast Asia.

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