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RESEARCH NOTE

First report of the benthic dinoflagellate, *Gambierdiscus belizeanus* (Gonyaulacales: Dinophyceae) for the east coast of Sabah, Malaysian Borneo

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SUMMARY

Species of the genus *Gambierdiscus* Adachi & Fukuyo, in particular *G. toxicus* Adachi & Fukuyo are known producers of neurotoxins associated with ciguatera fish poisoning (CFP). In this study live samples were collected from seaweed beds of the east coast of Sabah, Malaysian Borneo and a strain of *Gambierdiscus* was isolated and cultured. Examination of the thecal fine morphology was undertaken using light, epifluorescence, and scanning electron microscopy. Observed morphological features and their associated morphometric information enabled identification to *Gambierdiscus belizeanus* Faust. This represents the first report for the occurrence of *G. belizeanus* in the Asia Pacific region.

Key words: Asia Pacific, benthic dinoflagellate, *Gambierdiscus belizeanus*, Malaysia, morphology.

Gambierdiscus Adachi & Fukuyo is the only genus of Gonyaulacales confirmed to be responsible for ciguatera fish poisoning (CFP) (Adachi & Fukuyo 1979). Historically CFP has not been associated with Southeast Asia (Sadovy 1997). In Malaysia, only one poisoning case owing to the consumption of red snapper, *Lutjanus bohar*, has been reported (Sabah Fisheries Department). No CFP has ever been reported in Peninsular Malaysia. However, since the 1980s CFP cases in Hong Kong have risen owing to an increase in the reef live fish trade (Sadovy 1997). Studies attempting to understand the triggers of CFP have been undertaken (e.g. Lewis 1986; Kohler & Kohler 1992; Chateau-Degat *et al*. 2005; Litaker *et al*. 2009) but none to date has produced compelling evidence of the ability to predict occurrences of CFP. Under the international Hazard Analysis and Critical Control Points (HACCP), ciguatera toxins are one of the items that must be tested for in fish intended for export.

Several *Gambierdiscus* morphospecies have been detected in samplings in Malaysian waters although, as yet, none have been tested for toxin production. Sampling was carried out in Kota Kinabalu (Sabah), Redang Island (Kelantan), Port Dickson (Negeri Sembilan), and Langkawi Island (Kedah) (Fig. 1). Seaweed, coral fragments, seagrasses, and sand were collected and placed, underwater, into separate plastic bags. Samples were brought back to the laboratory without preservation. In the laboratory, samples were shaken vigorously to dislodge attached dinoflagellate cells. The suspension was then passed through a 120 μm and 20 μm mesh sieves. Material retained by the 20 μm mesh sieve was resuspended in sterile filtered seawater for cell isolation. Cells were isolated singly for culture using a very finely-drawn Pasteur pipette. A clonal culture, named GdSA03, was established in ES-DK medium and maintained at 26°C under a 14:10 h LD (light : dark) photoperiod. Initial identification was done under a light microscope (LM) and an epifluorescence microscope. For LM, cells were stained with Lugol's solution. For epifluorescent observation, cells were stained with 1% Calcofluor White stock solution (Fluka) and viewed under an Olympus IX51 epifluorescence microscope (Olympus, Melville, USA) with a UV filter (Lim *et al*. 2005). Morphological observation was

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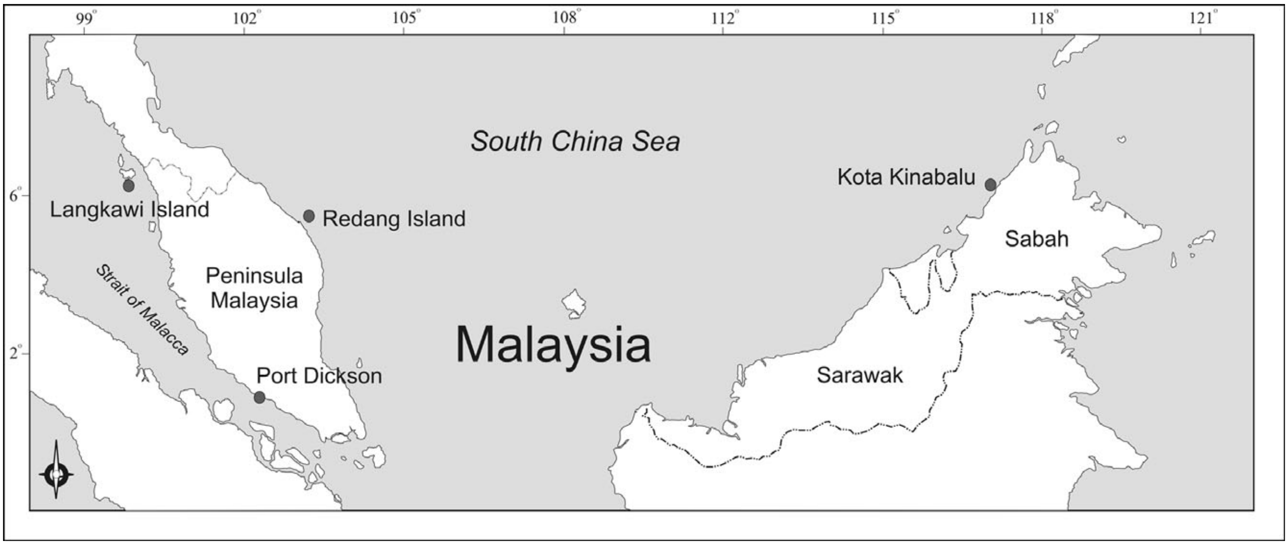

Fig. 1. Map of Malaysia showing the collection sites.

Table 1. Morphological measurements of *Gambierdiscus* GdSA03 from Kota Kinabalu, Sabah with comparison to other closely related *Gambierdiscus* species. Number of cells observed, $n = 30$ cells. Values in parentheses are the mean sizes

Morphological characters		GdSA03	*G. belizeanus**	*G. australes**	*G. pacificus**
Cell dimension (μm)	Depth (D)	58–64 (61)	61–65 (63)	81–91 (86)	65–75 (70)
	Width W)	53–59 (56)	55–61 (58)	73–81 (77)	59–67 (63)
	D:W	1.08	1.03	1.14	1.09
Peripheral apical platelet, Po (μm)	Length (L)	4.7–6.5 (5.6)	5.0–5.8 (5.38)	6.3–7.9 (7.1)	4.9–5.5 (5.2)
	Width (W)	3.0–4.4 (3.7)	3.5–4.3 (3.9)	5.7–6.5 (6.1)	3.7–4.5 (4.1)
	L:W	1.54	1.38	1.16	1.27
	Numbers of peripheral pores	29–31 (30)	13–25 (19)	27–35 (31)	28–32 (30)
Posterior intercalary plate, 1p (μm)	Length (L)	27.0–32.2 (29.6)	19.7–24.3 (22)	50.9–57.1 (54)	32.5–39.5 (36)
	Width (W)	15.3–19.5 (17.4)	9.9–14.1 (12)	24.3–29.7 (27)	10.5–17.5 (14)
	L:W	1.71	1.83	2.00	2.57
Ratio of 2′/2″ to 2′/4″ suture length		0.65 ± 0.15	0.64 ± 0.14	0.69 ± 0.09	0.36 ± 0.10

*Litaker *et al.* (2009).

undertaken using a JEOL JSM-6390LA scanning electron microscope (SEM) (JEOL, Japan). Cells were examined for several morphological characteristics according to Litaker *et al.* (2009). Thirty cells were then randomly selected from SEM observation and on these morphological measurements were performed.

Cells of GdSA03 are 61 ± 3.3 μm in depth (dorsoventral diameter) and 56 ± 2.7 μm in width ($n = 30$), with the depth-to-width ratio of 1.08 (Table 1). Cells are orbicular in apical view under LM (Fig. 2a, b), and anterio-posteriorly compressed under SEM observation. Cell surface is heavily areolated with small pores (Fig. 2c). Intercalary bands are striated (Fig. 2d,f), and the cingulum lists are supported by ridges. The sulcus is short and covered by sulcus lists. GdSA03 displayed the typical *Gambierdiscus* plate formula of Po, 3′, 7″, 1p, 5‴, 2⁗ (Fig. 2b). Plate 1″ and 7″ in the epitheca are small and hardly visible in apical view (Fig. 2d), and immersed into the ascending cingulum (Fig. 2c). The 3′ plate is pentagonal (Fig. 2d), and the 2‴ and 4‴ plates occupy most of the width of the hypotheca (Fig. 2e,f). 1p plate is pentagonal, narrow and relatively small (Fig. 2e,f), 29.6 ± 2.6 μm in length and 17.4 ± 2.1 μm in width, with a L:W ratio of 1.7 (±0.21) (see Table 1). The apical pore complex (APC) is suppressed (Fig. 2g). The peripheral apical platelet (Po) that hosts the comma-shaped apical pore (ap), is surrounded by an average of 30 (±1.3) pores (Fig. 2g), each 5.6 ± 3.7 μm in length and 3.7 ± 0.7 μm in width, with a L : W ratio of 1.54 (±0.22) (Table 1). The sulcal region is comprised of six sulcal plates, with S.d.a. forming the upper sulcal chamber, and S.d.p., S.s.p., S.s.a., and S.m. forming the lower sulcal chamber, separated by the t plate (Fig. 2h). The transverse flagellum pore is located in the upper chamber above the S.s.a. (Fig. 2h).

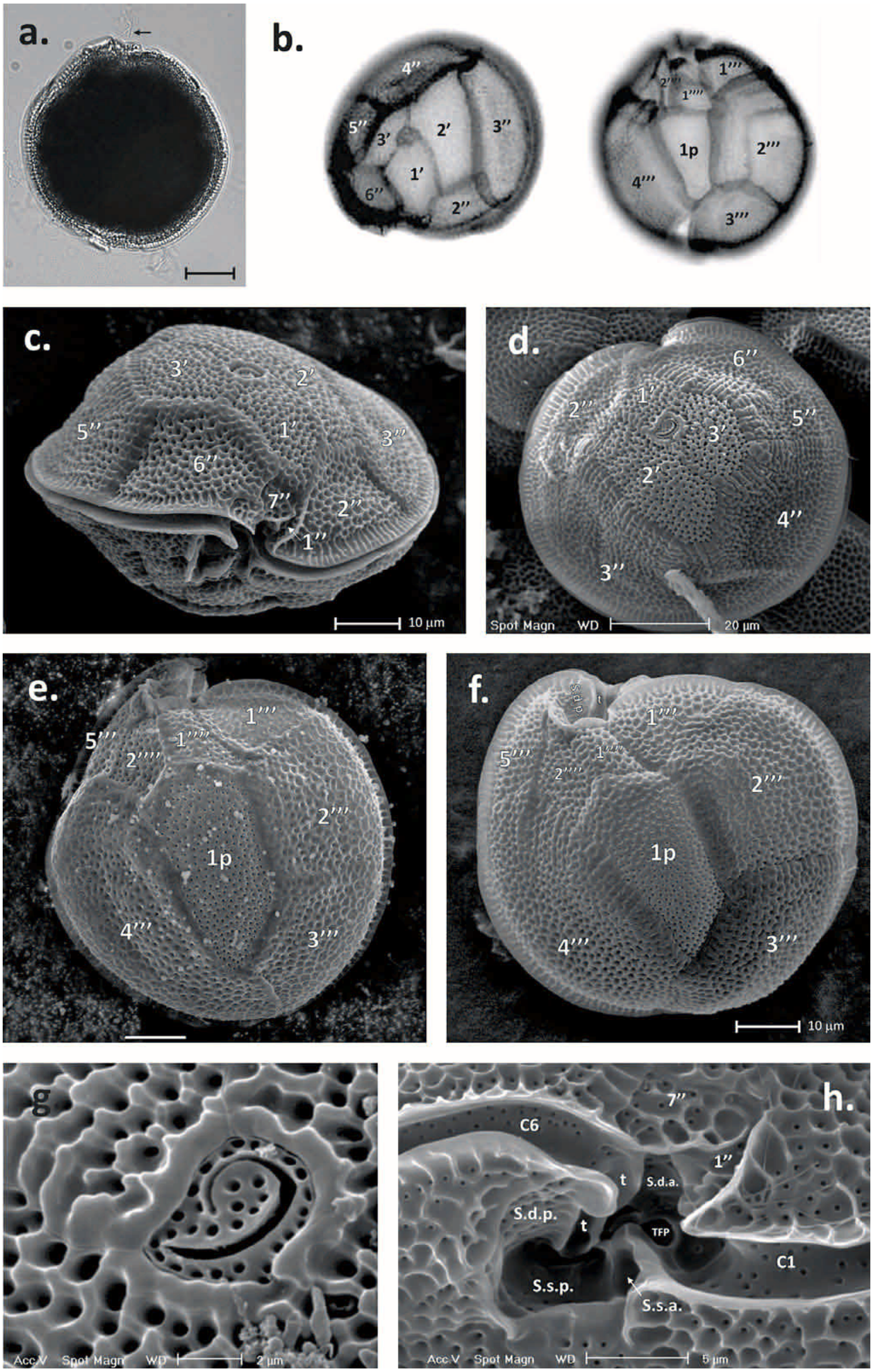

Fig. 2. *Gambierdiscus belizeanus*, GdSA03. (a) Light microscopy (LM), Lugol's stained cell showing the short longitudinal flagellum (arrow); (b) epifluorescent micrographs of divided cells showing both epi- and hypotheca; (c) ventral view of cell, showing first precingular plate (1″) and trapezoid 7″ immersed into the ascending cingulum, and the deep sulcus. Note the striation of the intercalary bands, and the ridged margin of cingulum list (d). Apical view of cell; (e–f) antapical view of cell, 1p is small and narrow; (g) close view of the apical pore complex (APC); (h) close view of the sulcal region, showing the S.d.a., t, S.d.p., S.s.p and S.s.a. plates.

GdSA03 possess the heavily areolated surface that is a unique feature of *G. belizeanus* Faust (Faust 1995; Holmes 1998; Chinain *et al.* 1999; Litaker *et al.* 2009). Additionally, the cell size is also in the range of *G. belizeanus* (Litaker *et al.* 2009).

The peripheral apical platelet (Po), the apical pore (ap), and the number of pores of the Po have been used in *Gambierdiscus* taxonomy as diagnostic features (Adachi & Fukuyo 1979; Chinain *et al.* 1999). Po in *Gambierdiscus* species range from ellipsoid in most of the species, broadly ellipsoid in *G. australes* Faust & Chinain, elongate ellipsoid in *G. yasumotoi* Holmes, triangular in *G. polynesiensis* Chinain & Faust, and four-sided in *G. pacificus* Chinain & Faust (Adachi & Fukuyo 1979; Faust 1995; Holmes 1998; Chinain *et al.* 1999; Litaker *et al.* 2009). GdSA03 has ellipsoid Po. The number of pores around Po estimated in this study (30 pores on average) is closer to that of *G. australes* and *G. pacificus*, and not in agreement with the number of pores observed in *G. belizeanus* by Litaker *et al.* (2009), but in agreement with the original description of Faust (1995) of the type from the Caribbean. Nonetheless, the dimensions of Po observed were consistent with the description of *G. belizeanus* in Litaker *et al.* (2009) (Table 1). It remains unclear whether the number of pores in Po is a reliable diagnostic feature.

Litaker *et al.* (2009) proposed the size and shape of the posterior intercalary plate, 1p, as the the most useful diagnostic morphological character with which to distinguish between *Gambierdiscus* species. The shape of 1p plate varies from broad, long, narrow and pentagonal (Adachi & Fukuyo 1979; Holmes 1998; Chinain *et al.* 1999). *Gambierdiscus* GdSA03 has a short, narrow, pentagonal 1p plate in accordance with that described for *G. belizeanus* and *G. pacificus*. The length-to-width ratio of the 1p of GdSA03 is consistent with that of *G. belizeanus* (Litaker *et al.* 2009), although overall 1p is slightly smaller. Additionally, symmetry of the 2′ plate and the ratio of the 2′/2″ to 2/4″ suture lengths has been proposed as informative in delimiting anterioposteriorly compressed species (Litaker *et al.* 2009). In this study, the strain examined had a ratio of 0.65 ± 0.15 (Table 1), which is consistent with *G. belizeanus* (Litaker *et al.* 2009). Despite these minor variations we are confident that *Gambierdiscus* GdSA03 is *Gambierdiscus belizeanus*.

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