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Molecular characterization and morpho-taxonomy of *Gambierdiscus caribaeus* Vandersea, Litaker, M.A.Faust, Kibler, W.C.Holland & P.A.Tester (Dinophyceae) from Mauritius Island, South-West Indian Ocean

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Molecular characterization and morpho-taxonomy of *Gambierdiscus caribaeus* Vandersea, Litaker, M.A.Faust, Kibler, W.C.Holland & P.A.Tester (Dinophyceae) from Mauritius Island, South-West Indian Ocean

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ABSTRACT

Species of the marine benthic dinoflagellate genus *Gambierdiscus* Adachi & Fukuyo are morphologically similar, and DNA based sequence analyses are necessary to complement morphological species determination. *Gambierdiscus* species may produce ciguatoxins, the causative agents of human illness ciguatera poisoning (CP). In this study, morphological descriptions, based on laboratory cultures, using light and scanning electron microscopy and DNA based characterisation of one strain (ISOG4) isolated from Petite Rivière Noire, Mauritius are provided. Our morphological observations coupled with DNA based sequence analyses indicate that the Mauritian strain is *Gambierdiscus caribaeus* Vandersea, Litaker, M.A.Faust, Kibler, W.C.Holland & P.A.Tester. Our strain bears close similarity (LSU 99% identity) to *Gambierdiscus caribaeus* (GenBank[KY448444]) isolated from Canary Islands, Spain, and phylogenetic results depict it as sister to *G. jejuensis* S.H.Jang & H.J.Jeong. *Gambierdiscus caribaeus* (ISOG4) is herein a first report from Mauritius Island and the Mascarene region.

KEY WORDS
Mauritius,
benthic dinoflagellates,
ciguatera poisoning,
morphology,
phylogeny,
new record.

RÉSUMÉ

Caractérisation moléculaire et morpho-taxonomie de Gambierdiscus caribaeus *Vandersea, Litaker, M.A.Faust, Kibler, W.C.Holland & P.A.Tester (Dinophyceae) de l'île Maurice, sud-ouest de l'océan Indien.* Les espèces du genre de dinoflagellés benthiques marins *Gambierdiscus* Adachi & Fukuyo sont morphologiquement similaires, et des analyses de séquences basées sur l'ADN sont nécessaires pour compléter la détermination morphologique des espèces. Les espèces de *Gambierdiscus* peuvent produire des ciguatoxines, agents responsables de la maladie humaine qu'est la ciguatera (CP). Dans cette étude, des descriptions morphologiques, basées sur des cultures en laboratoire, réalisées par microscopie optique et électronique à balayage, et une caractérisation basée sur l'ADN d'une souche (ISOG4) isolée de Petite Rivière Noire, Maurice sont fournies. Nos observations morphologiques couplées aux analyses de séquences basées sur l'ADN indiquent que la souche mauricienne est *Gambierdiscus caribaeus* Vandersea, Litaker, M.A.Faust, Kibler, W.C.Holland & P.A.Tester. Notre souche est très similaire (LSU 99 %) à *Gambierdiscus caribaeus* (GenBank[KY448444]) isolé des îles Canaries, Espagne, et les résultats phylogénétiques la décrivent comme sœur de *G. jejuensis* S.H.Jang & H.J.Jeong. *Gambierdiscus caribaeus* (ISOG4) est ici signalé pour la première fois à l'île Maurice et dans la région des Mascareignes.

MOTS CLÉS
Maurice,
dinoflagellés benthiques,
ciguatera,
morphologie,
phylogénie,
signalement nouveau.

INTRODUCTION

The order Gonyaulacales Taylor contains the family Pyrocystaceae (Schütt) Lemmermann and incorporates the genus *Gambierdiscus* Adachi & Fukuyo. So far, the genus *Gambierdiscus* comprises 18 species (Adachi & Fukuyo 1979; Faust 1995; Chinain *et al.* 1999; Litaker *et al.* 2009; Fraga *et al.* 2011; Fraga & Rodríguez 2014; Nishimura *et al.* 2014; Fraga *et al.* 2016; Smith *et al.* 2016; Kretzschmar *et al.* 2017; Rhodes *et al.* 2017; Jang *et al.* 2018; Nakada *et al.* 2018; Kretzschmar *et al.* 2019). This genus was initially described as a monotypic genus represented only by *Gambierdiscus toxicus* Adachi & Fukuyo from Gambier Islands, French Polynesia (Adachi & Fukuyo 1979). The original plate formula of *G. toxicus* was defined as Po, 3', 0a, 7'', 6c, 8s, 6''', 1p, 1''''. A second and third *Gambierdiscus* species were subsequently described, namely *G. belizeanus* M.A.Faust (Faust 1995) and *G. yasumotoi* M.J.Holmes (Holmes 1998). The latter differs morphologically from *Gambierdiscus* by its globular shape, and together with *G. ruetzleri* M.A.Faust, Litaker, Vandersea, Kibler, W.C.Holland & P.A.Tester (Litaker *et al.* 2009), has now been transferred to the genus *Fukuyoa* F.Gómez, D.X.Qiu, R.M.Lopes & Senjie Lin and is represented by *F. paulensis* F.Gómez, D.X.Qiu, R.M.Lopes & Senjie Lin (Gómez *et al.* 2015), as type species.

During the past few decades, species from the *Gambierdiscus* lineage (*Fukuyoa* and *Gambierdiscus* genera) have frequently been reported from tropical and subtropical regions (Tester *et al.* 2018). Nevertheless, observations from temperate regions are increasing (Aligizaki & Nikolaidis 2008; Nishimura *et al.* 2013; Kohli *et al.* 2014; Jang *et al.* 2018; Larsson *et al.* 2018; Chinain *et al.* 2020) and may also be as a result of climate change (Chinain *et al.* 2019). Early investigations suggested the toxins leading to ciguatera poisoning (CP) are of benthic dinoflagellate origin and subsequently was ascribed to *Gambierdiscus toxicus* (Parsons *et al.* 2011 and references therein). The interest for studying *Gambierdiscus* species is that some produce ciguatoxins (CTXs), the causative agents of human illness CP, following consumption of contaminated marine fish (Rongo & van Woesik 2011; Skinner *et al.* 2011; Robertson *et al.* 2014; Laza-Martínez *et al.* 2016; Friedman *et al.* 2017; Tamele *et al.* 2019).

Situated within a subtropical ciguatera endemic area of the Indian Ocean, Mauritius is also recognised as the island for the first reported case of CP in Western Indian Ocean (Quod *et al.* 1995; Lewis 2001; Hamilton *et al.* 2002). In 2011, the first recurrence case of CP was reported in two of four patients, after eleven months, following an initial CP in Mauritius linked with consumption of *Variola louti* Forsskål, 1775 (lunartail grouper) (Glaizal *et al.* 2011). However, the low reported incidence rate of CP from Mauritius may be the result of regulatory measures preventing the importation and sale of some toxic fish, while on a regional level may be attributed to food preference for pelagic fishes (Quod *et al.* 1995; Quod & Turquet 1996; Hamilton *et al.* 2002).

Past work, based solely on morphological identification, reported the presence of *Gambierdiscus toxicus* from Mauritius (Hansen *et al.* 2001; Hurbungs *et al.* 2001). Over the past decades, monitoring for potentially harmful benthic dinoflagellates in the island of Mauritius, using macroalgae as substrate, has been conducted. The published survey studies, conducted during 1998-1997 and 2013-2017, did not reveal an increase in *Gambierdiscus* spp. cell density over the years (Hurbungs *et al.* 2001; Neermul *et al.* 2021). Nevertheless, based on additional morphological investigations supported with DNA sequences analyses, Litaker *et al.* (2009) suggested that the original description of *G. toxicus* was based on more than one species. This is reflected in several recent studies revealing a higher diverse assemblage of *Gambierdiscus* species (Munir *et al.* 2011; Tester *et al.* 2013; Rodríguez *et al.* 2017; Bravo *et al.* 2019).

In this study, three sampling locations along the west coast of Mauritius were surveyed, primarily focusing on morphological and molecular characterization of species of *Gambierdiscus*.

MATERIAL AND METHODS

Study area and sample collections

The sampling locations were namely, Petite Rivière Noire (20°23'45"S, 57°21'55"E), Wolmar (20°18'39"S, 57°21'56"E) and Trou aux Biches (20°2'24"S, 57°32'25"E), and sampled in September 2015 (Fig. 1). Macrophyte samples were collected between 0.5 and 1.0 m depth and included macroalgae and seagrass. Macroalgae and seagrass were not identified to species; dominant representatives included genera of *Turbinaria* sp., *Padina* sp., *Dictyopteris* sp., *Syringodium* sp. and *Halodule* sp.

At the time of collection, sea surface temperature ranged between 26°C to 33°C and salinity ranged from 38 to 40 ppt. Macrophyte samples were collected and placed in 800 ml wide-mouthed plastic jars. They were filled with local seawater approximately to one third of plastic jar and kept in dark ambient temperature, until transfer and processing in the laboratory. Samples were vigorously shaken for approximately one minute to detach the dinoflagellate cells. The samples were then size fractionated by passing through 300 and 125 µm mesh sieves to remove large particles, and finally filtered through 20 µm pore size nylon mesh. The filtrates were examined for the presence of cells representing species from the *Gambierdiscus* lineage. Cells were isolated from field samples with modified Pasteur pipettes and sequentially cleaned by transferring through a minimum of three drops filter-sterilised sea water, under a compound microscope, to ensure a single cell was present. Each cell was added to separate well in a 24 well plate that was half-filled with filter-sterilised medium (modified K media; Holland *et al.* 2013). The culture plate was sealed with paraffin film to minimise evaporation and incubated at 26°C, and an irradiance of 50 µmol photons m^{-2} s^{-1} in a 14h:10h of light/dark cycle, without suspension. Cell growth was monitored every 2-4 days, under an inverted microscope. When sufficient cell density was achieved, following several division cycles, surviving cells were transferred to 125 ml polycarbonate Erlenmeyer flasks containing 80-100 ml, half strength culture medium. The cultures were sequentially transferred to full strength medium and maintained at 26°C, an irradiance of 100 µmol photons m^{-2} s^{-1} in a 14h:10h of light/dark cycle, without suspension. One isolate (ISOG4) obtained from *Padina* sp. representing a species of *Gambierdiscus* was successfully cultured and maintained.

In this study, the modified Kofoidian nomenclature system (Kofoid 1909) as described by Besada *et al.* (1982) was used for naming the plates; thereby allowing comparisons with other genera of Gonyaulacales (Fraga *et al.* 2011; Nishimura *et al.* 2014). The tabulation applied here is APC, 4', 6", 6c, 6-7 s, 5"', 2"".

Microscopy

Light microscopy observations were carried out under a Leica DM1000 light microscope. In addition, determination of the shape and position of the nucleus was achieved by fixing 1 ml exponential cultures with 3.7% formaldehyde (15 minutes). Cell size was estimated from length and width data of 30 cells for each strain, determined at ×400 magnification. For scanning electron microscopy, 1 ml from a mid-exponential growth phase culture of the strain was centrifuged. Discarding the supernatant, the pellet was rinsed three times with 1 X PBS pH 7.4, and subsequently fixed with 4% buffered formalin at room temperature. The samples were then filtered through polycarbonate membrane filters (pore size 5 µm, Nuclepore), washed with distilled water for two hours, dehydrated in a series of graded ethanol concentrations (30%, 50%, 70%, 96%, 99%) and critical point dried with CO_2 (BAL-TEC, CPD 030 critical point dryer). The filters were subsequently stub mounted, coated with platinum-palladium in a high-resolution fine coater (JFC-2300HR, JEOL, Japan). Micrographs were taken using a JEOL JSM-6335F field emission scanning electron microscope (JEOL, Japan).

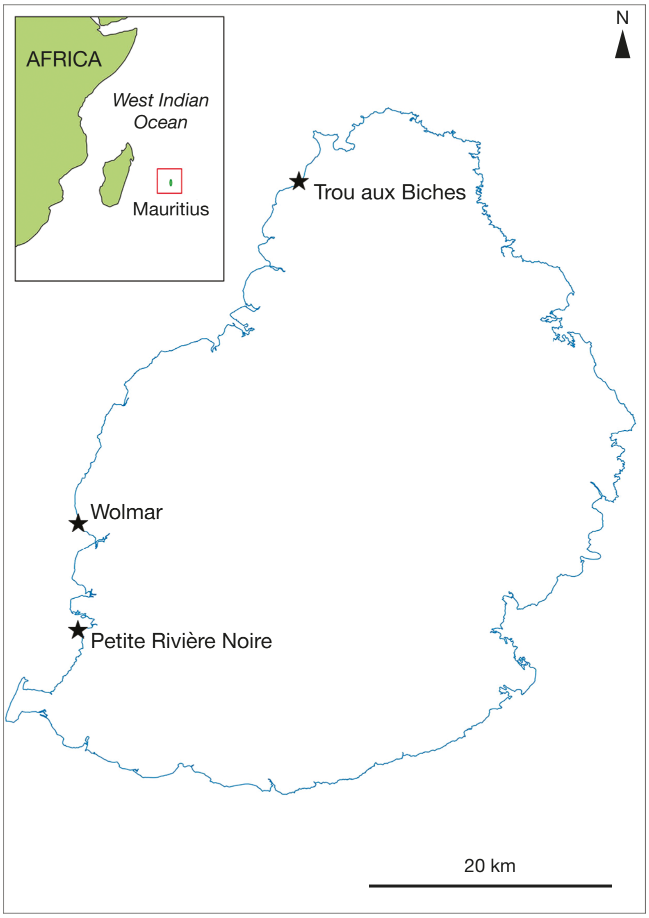

Fig. 1. — Map showing the three locations where samples were collected from Mauritius.

Nuclear DNA extraction and PCR amplification

DNA extraction and PCR amplifications of the LSU region was conducted as follows: 5-10 ml of clonal cultures was centrifuged and genomic DNA was extracted from the cell pellet using the 10% Chelex® 100 (Richlen & Barber 2005). The polymerase chain reaction (PCR) primers used in this study were obtained from Inqaba (South Africa). PCR amplification for 25 µl reactions were performed

TABLE 1. — Comparisons based on scanning microscopy (SEM) analyses of cell and thecal plate dimensions (mean in parenthesis) between strains of *Gambierdiscus caribaeus* Vandersea, Litaker, M.A.Faust, Kibler, W.C.Holland & P.A.Tester.

	Mauritius	**Belize**	**Thailand**	**Hainan Island**
Reference	This study	Litaker *et al*. 2009	Tawong *et al*. 2015	Zhang *et al*. 2016
Shape of cells	Compressed	Compressed	Compressed	Compressed
Surface	Smooth	Smooth	Smooth	Smooth
Thecal plates pores	Round	Round to oval	Round	Round to oval
Length (L, µm)	43.64-64.57 (50.9 ± 9.2) n = 4	–	(44.1)	(53.9)
Depth (D, µm)	45.18-75.45 (64.5 ± 8.3) n = 15	(77)	(64.4)	(87.6)
Width (W, µm)	51.43-86.36 (68.8 ± 11.9) n = 18	(79)	(62.9)	(84.7)
D:W ratio	0.72-1.18 (1.0 ± 0.1) n = 15	(1)	(1.0)	(1.0)
L:W ratio	0.57-1.20 (0.8 ± 0.3) n = 4	(0.73)	(0.73)	–
Shape of apical pore plate (Po)	Elliptical, fishhook	Elliptical, large fishhook	Elliptical, fishhook	Elliptical, fishhook
Position of Po	Centrally located	–	–	Centrally located
Length (L, µm) of Po	6.50-8.98 (8.1 ± 1.0) n = 8	(8.3)	(8.8)	(8.4)
Width (W, µm) of Po	4.00-6.33 (5.0 ± 0.7) n = 8	(5.3)	(6.7)	(5.5)
L:W ratio of Po	1.37-2.02 (1.6 ± 0.2) n = 8	(1.59)	(1.35)	(1.5)
Shape of Po pores	Round and oval	–	–	Round
Number of internal pores in Po	26-40 (33.4 ± 5.4) n = 10	37	(37)	(31)
Diameter of pores in Po (µm)	0.29-0.52 (0.40 ± 0.07) n = 40	0.34	(0.31)	(0.45)
Shape of 4' (equivalent 1' plate)	Hexagonal	Hexagonal	–	Hexagonal
Number of pores in 4' (equivalent 1' plate)	72-105 (91.6 ± 11.1) n = 8	–	–	(98)
Shape of 2' plate symmetry	Rectangular	Rectangular	Rectangular	Rectangular
Shape of 2'''' (equivalent 1p plate)	Pentagonal and broad	Pentagonal and broad	Pentagonal and broad	Pentagonal
Length (L, µm) of 2'''' (equivalent 1p plate)	37.4-48.81 (40.6 ± 4.7) n = 5	(43)	(37.8)	(51.0)
Width (W, µm) of 2'''' (equivalent 1p plate)	31.85-41.73 (35.1 ± 4.0) n = 5	(34)	(29.4)	(35.0)
L: W ratio of 2'''' (equivalent 1p plate)	1.05-1.21 (1.2 ± 0.1) n = 5	(1.26)	(1.28)	(1.5)
Ratio of width of the 2'''' (equivalent 1p plate) relative to cell width	37.89-48.68% (45.6 ± 5.2%) n = 4	–	–	(42.8%)

using OneTaq® 2X Master Mix (New England Biolabs, United Kingdom), following manufacturers' instructions with primer pair D1R-F & D2C-R for the LSU region (Scholin *et al.* 1994). The PCR reactions were carried in a thermocycler (Applied Biosystems, California, United States) and amplification profile for the LSU region was also set as initial denaturation step at 95°C for five minutes, followed by 35 cycles at 95°C for one minute; annealing at 53°C for 30 seconds; extension at 72°C for two minutes, with a final extension at 72°C for seven minutes. DNA sequencing were performed by the same primers as described above by Inqaba (South Africa). The LSU sequence of *Gambierdiscus* isolate ISOG4 obtained in this study was deposited in Genbank (GenBank[OK043676]).

PHYLOGENETIC ANALYSES

LSU (D1-D2) sequence reads from this study were manually checked and edited using BioEdit v7.2.5 software (Hall 1999). Phylogenetic inference of our sequences was preceded by BLAST searches on the GenBank database to assign putative identity and assist in taxon sampling for further analyses. Multiple sequence alignments of sequence reads together with other similar sequences downloaded from GenBank were performed in the Geneious Prime 2020.1.1 (Biomatters Ltd., Auckland, New Zealand) (Kearse *et al.* 2012) using Muscle 3.8.425. The optimal model of nucleotide evolution was established in MEGA X software (Kumar *et al.* 2018). The best-fitting DNA substitution model fitting our dataset for the LSU region (TN93 + G + I, G = 2.5) was used to construct the Maximum likelihood (ML)

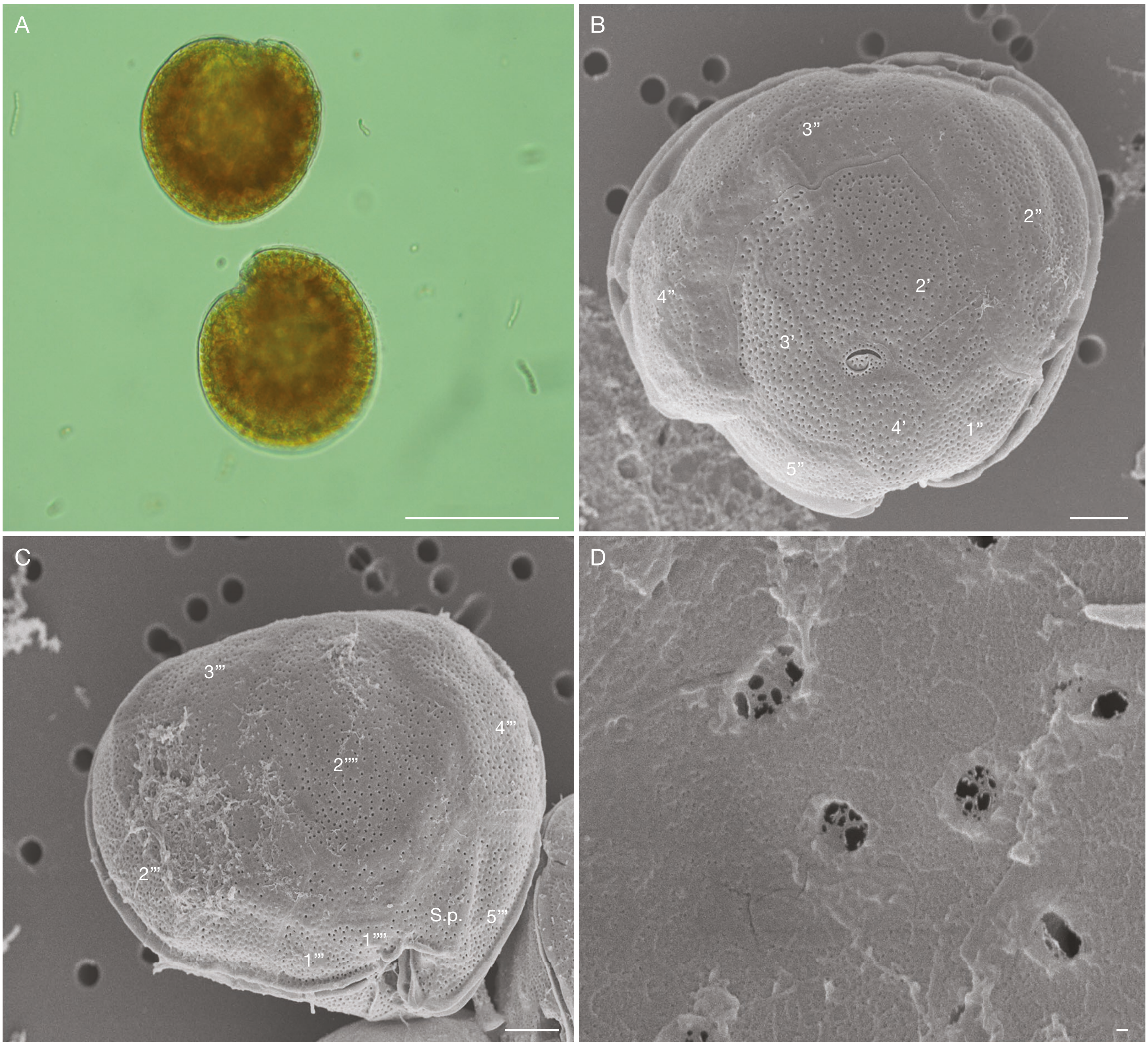

Fig. 2. — Light and scanning electron micrographs of *Gambierdiscus caribaeus* Vandersea, Litaker, M.A.Faust, Kibler, W.C.Holland & P.A.Tester (ISOG4): **A**, light micrograph of *Gambierdiscus caribaeus* Mauritian strain; **B**, scanning electron micrograph of apical view; **C**, scanning electron micrograph of antapical view; **D**, scanning electron micrograph of inside top view of thecal plate pores. Scale bars: A, 50 µm; B, C, 10 µm; D, 100 nm.

phylogenetic trees. The optimal ML tree search was conducted using 1000 bootstrap replicates. Bayesian inference (BI) were performed in Geneious Prime platform and conducted via Mr Bayes 3.2.6 plugin (Huelsenbeck & Ronquist 2001) using the evolutionary model (general time reversible with gamma-distributed rate variation across sites and a proportion of invariable sites, GTR + I + G). Markov Chain Monte Carlo procedure was based on two independent trials with four chains each. Each chain was run for 2 000 000 generations and trees were sampled every 1000 trees. A 50% majority-rule consensus tree was drawn from the last 1000 trees. All final spilt frequencies were < 0.01. The phylogenetic tree was represented using the ML results and bootstrap values from the analysis from ML, and posterior probability values from the bayesian analysis.

RESULTS

Morphology of Mauritian strain of *Gambierdiscus caribaeus*

The *Gambierdiscus* cell (strain ISOG4) isolated from Mauritius Island was observed using LM and SEM. The Mauritian strain was initially identified as *Gambierdiscus caribaeus* based on the observed morphological characteristics. Morphological details of the tested strain and those of other strains of *G. caribaeus* reported by others are summarised in Table 1.

The Mauritian strain was photosynthetic with golden granular chloroplasts, round to slightly ellipsoid in apical view, compressed in dorsal-ventral view and lenticular in the lateral view (Figs 2A-C; 3C). The length, depth and width of

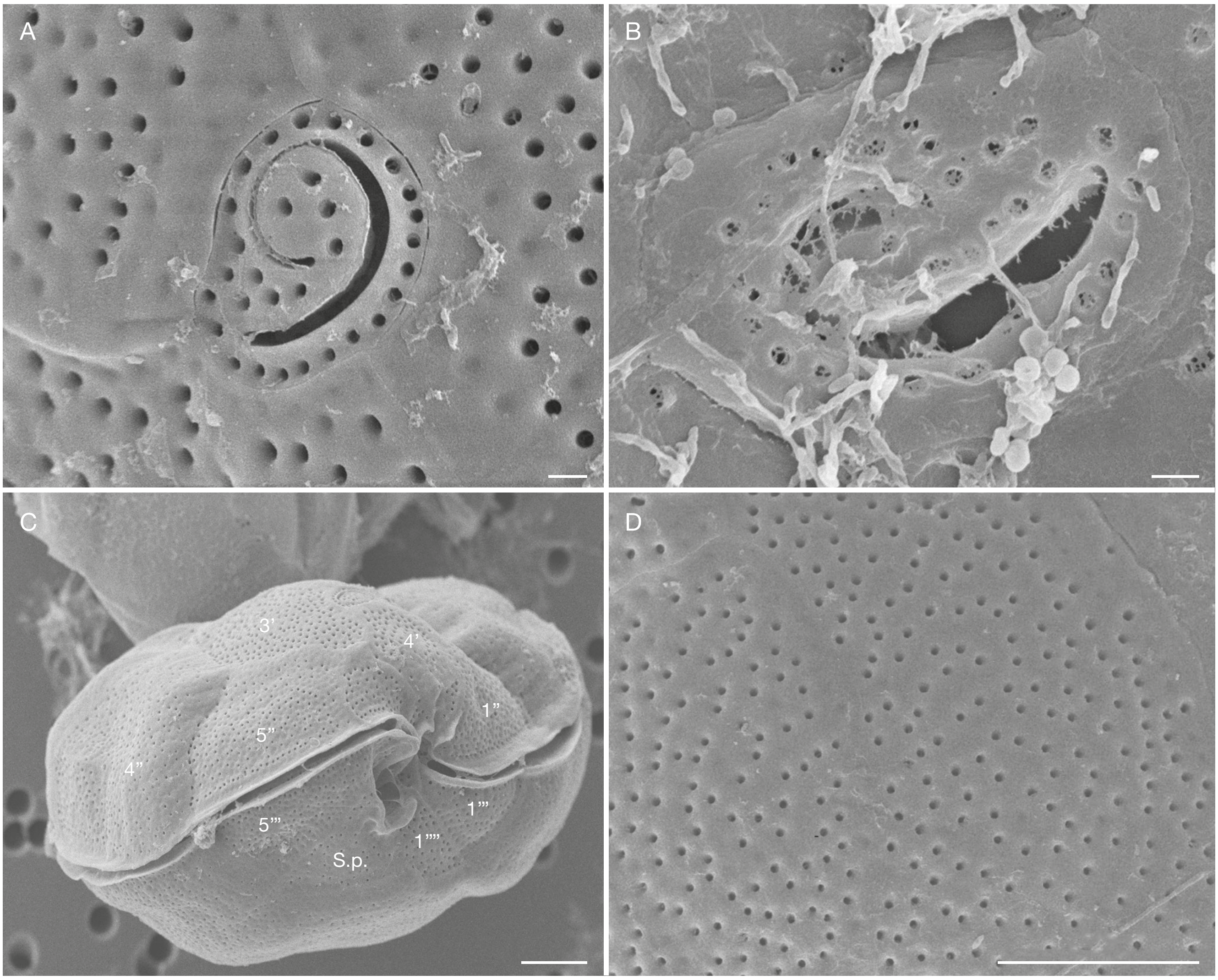

Fig. 3. — Scanning electron micrographs of *Gambierdiscus caribaeus* Vandersea, Litaker, M.A.Faust, Kibler, W.C.Holland & P.A.Tester (ISOG4): **A**, elliptical Po plate with pores and a large fishhook-shaped opening; **B**, inside top view of broken cell showing the elliptical Po plate with pores and a large fishhook shaped opening; **C**, ventral view showing the gradually ascend and then sharp descend of the right part of the cingulum near the sulcus; **D**, thecal pores on the smooth cell surface. Scale bars: A, B, 1 µm; C, D, 10 µm.

SEM treated cells were 43.64-64.57 µm, 45.18-75.45 µm and 51.43-86.36 µm. The ratios of length to width and depth to width were 0.57-1.20 and 0.72-1.18, respectively (Table 1). The epitheca and hypotheca surface were smooth and covered with many more or less round pores (Fig. 2B-C).

The Po plate touched three apical plates: 2', 3' and 4' (Fig. 2B). The rectangular 2' plate was the largest apical plate. The number of pores in 4' plate varied from 72 to105 pores. The 4" plate was quadrangular and the 5" was pentagonal. The apical pore plate was elliptical, and the apical pore was fishhook-shaped (Fig. 3A, B). Close to the cingulum edge the observed precingular plates were smooth (Fig. 3C). The level of the right side and left side of the cingulum was nearly the same. Nevertheless, in the direction of the sulcus, the right part of the cingulum gradually ascended and then sharply descended near the sulcus.

The hypotheca consisted of eight plates: S.p., 5''' and 2''''. The quadrangular 2''' and 4''' plates were the largest of the postcingular plates. Also, the 1''', 3''' and 5''' plates were quadrangular. The 2'''' was broad, pentagonal, 37.4-48.81 µm long and 31.85-41.73 µm wide. The ratio of 2'''' plate length to width was 1.05-1.21 (Table 1). The 2'''' plate contacts the postcingular plates 1''', 2''', 3''' and 4''' and the quadrangular hypothecal plates S.p. and 1''''.

Phylogenetics of Mauritian strain of *Gambierdiscus caribaeus*

The final LSU data matrix (841 bp in length) consisted of the *Gambierdiscus* strain isolated during this study, 47 *Gambierdiscus* strains and seven *Fukuyoa* strains. When properly aligned the DNA sequences of the Mauritian strain was similar to *G. caribaeus* LSU identifiers, namely EF202934 (Belize strain), EF202929 (Belize strain), KY448444 (Canary Islands strain), MN999499 (Bahía Concha strain), KY448432 (Canary Islands strain), KY448442 (Canary Islands strain), KR230005 (Hainan Island strain), KR230004 (Hainan Island

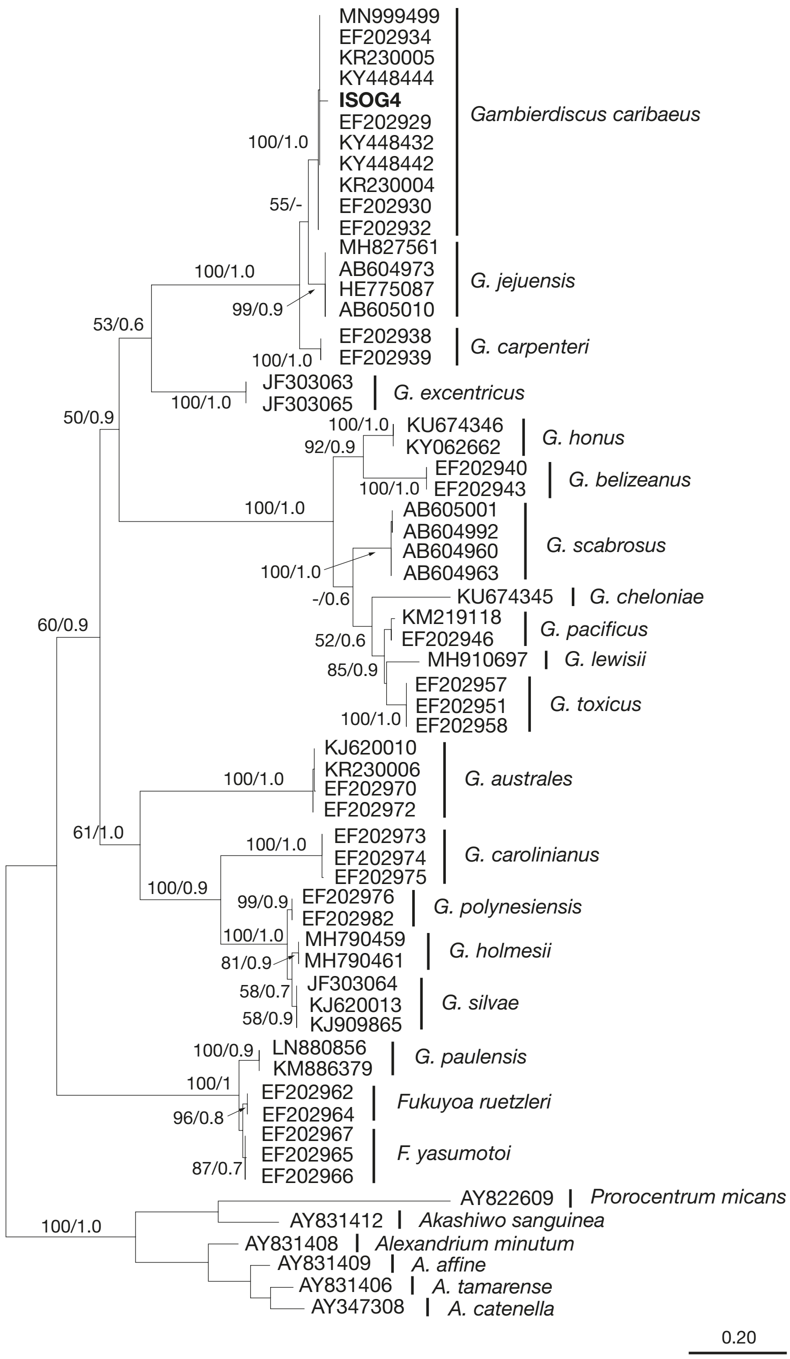

Fig. 4. — Phylogenetic analysis of the *Gambierdiscus caribaeus* Vandersea, Litaker, M.A.Faust, Kibler, W.C.Holland & P.A.Tester (ISOG4), showing alignment of D1-D2 LSU rDNA sequences using Bayesian inference and Maximum likelihood analyses. Values at nodes represent Bayesian posterior probability support and Bootstrap support. **-** represents unsupported value. Scale bar is substitution per site.

strain), EF202932 (Republic of Palau strain) and EF202930 (Mataiva, Tahiti strain), differing by 2 to 11 bp (similarities from 99.2 to 99.7%). However, wider nucleotide differences from 40 to 56 bases, were observed relative to *G. jejuensis* represented with Korean strain (HE775087) together with Japan strains AB605010 and AB604973 (similarities from 89.7 to 92.2%).

Bayesian inference (BI) and Maximum likelihood (ML) based on LSU sequences generated similar trees. DNA based phylogeny in Figure 4 shows that the Mauritian isolate is part of a clade containing *G. caribaeus* strains. The *Gambierdiscus caribaeus* clade was closely related to the clade consisting of *G. jejuensis* (strains HE775087, AB605010 and AB604973) and to the clade *G. carpenteri* Kibler, Litaker, M.A.Faust, W.C.Holland, Vandersea & P.A.Tester (strains EF202938 and EF202939). *Gambierdiscus caribaeus*, *G. jejuensis* and *G. carpenteri* grouped together with maximal support (Bayesian posterior probabilities: 1.00; ML bootstrap support

Table 2. — Evolutionary divergence shown as the number of base substitutions per site from averaging over all sequence pairs between *G. caribaeus* Vandersea, Litaker, M.A.Faust, Kibler, W.C.Holland & P.A.Tester and its closest sister clades are shown below the diagonal and standard error estimate(s) are shown above the diagonal. **0.01** and – represent within the clade divergences.

	G. caribaeus	*G. jejuensis*	*G. carpenteri*
G. caribaeus	**0.01**	0.009	0.012
G. jejuensis	0.056	–	0.013
G. carpenteri	0.087	0.094	–

values: 100), and they were a sister to *G. excentricus* S.Fraga (strains JF303063 and JF303065). *Gambierdiscus caribaeus* (11 seqs.) showed a rate of intraspecific evolutionary divergence at 0.01 while for *G. jejuensis* (3 seqs.) and *G. carpenteri* (2 seqs.) values were not obtained. The highest interspecific divergence was between *G. jejuensis* and *G. carpenteri* (0.094) and the lowest interspecific divergence was between *G. caribaeus* and *G. jejuensis* (0.056) (Table 2).

DISCUSSION

The Mauritian *Gambierdiscus* strain (ISOG4) isolated from Petite Rivière Noire shared general morphologies with the genus *Gambierdiscus* (Adachi & Fukuyo 1979; Faust 1995; Chinain *et al.* 1999; Litaker *et al.* 2009). The phenotypic traits of *Gambierdiscus* species consist of lenticular shapes (in the ventral view), anteroposteriorly highly compressed, an apical pore plate (Po) with a large fishhook-opening located (centrally or slightly displaced toward the ventral position), ascending cingular displacement, 2"" plate (1p plate equivalent), with 2' apical plate as the largest among the apical plates (Gómez *et al.* 2015; Jang *et al.* 2018).

Gambierdiscus caribaeus was described from Carrie Bow Key by Litaker *et al.* (2009). Cell size and thecal morphological characters used as major diagnostic features for species delineation, showed that the Mauritian strain was within the range reported in the original description of this species (Litaker *et al.* 2009) as well as with the subsequent descriptions by Tawong *et al.* (2015) and Zhang *et al.* (2016), but smaller than the recent record of *G. caribaeus* from Canary Islands (Tudó *et al.* 2020). The cell surface of *G. caribaeus* was smooth in agreement with the original description (Litaker *et al.* 2009). The epitheca and hypotheca surface were covered with round to sparse oval pores; similar to strains from Belize and Hainan Island (Litaker *et al.* 2009; Zhang *et al.* 2016) but differ from those of Thailand (Tawong *et al.* 2015) with presence of round pores. In the hypotheca the broad and pentagonal 2"" plate (1p plate equivalent) was proposed as a morphological trait by Litaker *et al.* (2009), to which the Mauritian strain was assigned – based on the L/W ratio (Table 1). Similarly, 2' plate was rectangular and agreed with previous records. The observed Po plate morphology and the calculated mean L to W ratio in this work is consistent with the original description of Litaker *et al.* (2009) and study reported by Zhang *et al.* (2016). In this study, the observed number of Po pores fell within the ranges described from Belize, Thailand and Hainan Island. This is in agreement with Zhang *et al.* (2016) who suggested that Po pores counts may not a reliable diagnostic feature due to the intra-specific variability among strains. Recently, Jang *et al.* (2018) described *Gambierdiscus jejuensis*, from the temperate waters of Jeju Island, which is morphologically similar to *G. caribaeus*. The authors reported on the central positioning of the nucleus in *Gambierdiscus jejuensis* as a possible reliable diagnostic feature for differentiating to *G. caribaeus* and related *Gambierdiscus* spp., where the nucleus is positioned in the hypotheca. The Mauritian strain was also observed with some aberrant forms, such as globular cells and with protruding plates (Appendix 1).

The phylogenetic analysis based of LSU sequence supported the results of the morphological study and further confirms that our strain isolated is *G. caribaeus*. Maximum likelihood analysis and Bayesian inference based on the LSU (D1-D2) region showed that *Gambierdiscus caribaeus* was clearly separated with high ML and posterior probability (BI) values, consistent with previous studies (Jang *et al.* 2018; Tudó *et al.* 2020). In this study, the *Gambierdiscus caribaeus* clade consisted of the Mauritian strain, the strains from the type locality (Belize) as well as strains from the South China Seas, eastern North Atlantic Ocean, and southern Pacific Ocean. Recently, based on D8-D10 phylogeny, Tudó *et al.* (2020) and Arteaga-Sogamoso *et al.* (2021) confirmed the presence of *Gambierdiscus caribaeus* from Canary Islands and Colombia, respectively. As a sister clade *Gambierdiscus jejuensis* and *G. carpenteri* were also well supported. *Gambierdiscus jejuensis* clade also included Korean strain GCJJ1 (HE775087) assigned to *G. caribaeus* (Jin *et al.* 2012) and *G. caribaeus* I clade (Zhang *et al.* 2016), together with two unidentified *Gambierdiscus* strains from Ryukyu Islands, Japan (Shah *et al.* 2013). The low genetic distance calculated between G. *caribaeus* and *G. jejuensis* was in agreement with Fraga & Rodríguez (2014) who found lower or similar values between closely related *Gambierdiscus* species in their D1-D3 and D8-D10 alignments.

To the best of our knowledge, this is the first strain of this species from Mauritius Island that has been characterised morphologically as well as through DNA sequence analyses. It is noteworthy that the ISOG4 was isolated from a mixed assemblage of dinoflagellates, such as *Ostreopsis* spp., *Prorocentrum* spp. and *Coolia* spp. The findings will contribute to broaden the knowledge on biogeographic distribution and morphology of this marine benthic dinoflagellate.

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APPENDIX

Appendix 1. — Scanning electron micrographs of *Gambierdiscus caribaeus* Vandersea, Litaker, M.A.Faust, Kibler, W.C.Holland & P.A.Tester (ISOG4) with aberrant forms: **A**, ventral view of globular shape cell; **B**, apical view showing cell with protruding plates. Scale bars: 10 μm.