

***Gambierdiscus* (Gonyaulacales, Dinophyceae) diversity in Vietnamese waters with description of *G. vietnamensis* sp. nov**

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Abstract :

Viet Nam has a coastline of 3,200 km with thousands of islands providing diverse habitats for benthic harmful algal species including species of *Gambierdiscus*. Some of these species produce ciguatera toxins, which may accumulate in large carnivore fish potentially posing major threats to public health. This study reports five species of *Gambierdiscus* from Vietnamese waters, notably *G. australes*, *G. caribaeus*, *G. carpenteri*, *G. pacificus*, and *G. vietnamensis* sp. nov. All species are identified morphologically by LM and SEM, and identifications are supported by molecular analyses of nuclear rDNA (D1–D3 and D8–D10 domains of LSU, SSU, and ITS1-5.8S-ITS2 region) based on cultured material collected during 2010–2021. Statistical analyses of morphometric measurements may be used to differentiate some species if a sufficiently large number of cells are examined. *Gambierdiscus vietnamensis* sp. nov. is morphologically similar to other strongly reticulated species, such as *G. belizeanus* and possibly *G. pacificus*; the latter species is morphologically indistinguishable from *G. vietnamensis* sp. nov., but they are genetically distinct, and molecular analysis is deemed necessary for proper identification of the new species. This study also revealed that strains denoted *G. pacificus* from Hainan Island (China) should be included in *G. vietnamensis* sp. nov.

Keywords : bHABs *Gambierdiscus vietnamensis* sp. nov., nuclear rDNA, LSU, SSU morphology, phylogenetic analysis, Viet Nam

Abbreviations:

bHABs, benthic Harmful Algal Blooms; **CDI**, Con Dao Islands; **CFP**, Ciguatera Fish

Poisoning; **DV**, Dorso-ventral depth; **LSI**, Ly Son Island; **NTB**, Nha Trang Bay; **PQI**, Phu

Quoc Islands; **W**, width. **APC**, Apical Pore Complex; **Sa**, anterior sulcal plate; **Sda**, right anterior lateral plate; **Sdp**, right posterior lateral plate; **Sp**, posterior sulcal plate; **Ssa**, left anterior lateral plate; **Ssp**, left posterior lateral plate

INTRODUCTION

The genus *Gambierdiscus* was first described by Adachi and Fukuyo (1979) with the type species *Gambierdiscus toxicus* from Gambier Island, French Polynesia, but without designating a holotype. Litaker et al. (2009), however, designated a lectotype (Adachi & Fukuyo 1979, figure 1) and an epitype (Chinain et al., 1999, figure 1). All *Gambierdiscus* species are phototrophic, lenticular in ventral view, more or less compressed antero-posteriorly, and asymmetrical rounded in apical and antapical views. There are different interpretations of how to apply the Kofoidian tabulation. Some authors indicated Po 3', 0a, 7'', 6c, 8s?, 5(6)''', 1p, 1–2'''' (Adachi & Fukuyo 1979, Chinain et al., 1999, Faust 1995, Litaker et al., 2009), while Besada et al. (1982) and Fraga et al. (2011) indicated Po, 0a, 4', 7'', 6c, 8s, 5(6)''', 0–1p, 1–2'''' and discussed in detail why they applied the latter interpretation, which is also used here.

Species of *Gambierdiscus* have an oval-polygonal apical pore plate with a characteristic fishhook-shaped pore. The cingulum is narrow with the distal end looping upwards, slightly ascending and clearly separating the epitheca and the hypotheca. The sulcus is broad, is deeply excavated, and does not extend to the antapex. The second apical plate (2') is among the largest plates on the epitheca together with the 2''–4'' plates. The 1' and 6'' plates are very small and usually difficult to observe by light microscopy (LM). The second antapical plate (2''') is situated centrally on the hypotheca and similar in size to the 2''–4'' plates. The shape and dimensions of the pore plate, the 2' plate, and the 2'''' plate may be used to distinguish some species morphologically.

Eighteen species of *Gambierdiscus* have now been described from tropical to subtropical waters of the Caribbean Sea, the Atlantic Ocean (Fraga & Rodriguez 2014, Fraga et al., 2011, Hoppenrath et al., 2019, Litaker et al., 2009, Tudó et al., 2018), the Hawaiian Islands, French Polynesia, Australia, the South China Sea in the Pacific Ocean (Adachi & Fukuyo 1979, Chinain et al., 1999, Dai et al., 2017, Faust 1995, Fraga et al., 2016, Jang et al.,

2018, Kretzschmar et al., 2019, Larsson et al., 2018, Nishimura et al., 2014, Rhodes et al., 2017, Smith et al., 2016, Tawong et al., 2016, Xu et al., 2014, Zhang et al., 2016), and Réunion Island in the Indian Ocean (Habibi et al., 2021, Saburova et al., 2013). The highest species diversity is found in the Pacific Ocean (15 species), while 7 and 4 species have been reported from the Atlantic and Indian Oceans, respectively. *G. carpenteri* was found in southeastern Australia in water temperatures of 16.5–17°C (Kohli et al., 2014).

Some species of *Gambierdiscus* produce toxins, which may cause ciguateric fish poisoning (CFP), an illness now recognized as a public health threat in many tropical and subtropical coastal areas (Friedman et al., 2017). CPF has been reported from Viet Nam in 2014 and 2016 (Ha et al., 2018) after consumption of red snapper (*Lutjanus bohar*). Four species of *Gambierdiscus* have been reported from Vietnamese waters previously: *G. belizeanus*, *G. pacificus*, *G. polynesiensis*, and *G. toxicus* (Ho-Van et al., 2010, Larsen & Nguyen 2004), but none of these records was supported by phylogenetic analyses. The present study describes five species from Vietnamese waters including a new species, *G. vietnamensis* sp. nov., and the identification of all species is supported by genetic analyses.

MATERIALS AND METHODS

Sampling, isolation, and culture

Sampling of benthic dinoflagellates were conducted in the dry season from May to August in 2006 and 2010 and from 2016 to 2021 in the coastal waters and islands of Viet Nam (Figure S1 in the Supporting Information and Table 1). Macroalgae such as *Padina* spp., *Sargassum* spp. (brown algae), and *Acanthophora spicifera* (red algae) were collected at 2–3 m depths during low tide and were brought to the laboratory in cooled containers. Processing of the samples and isolation were as described in Nguyen-Ngoc et al. (2021). Cultures were isolated and maintained in enriched K-medium (Keller et al., 1987) in a Versatile Environmental Test Chamber (MLR 351, Sanyo Electric Co. Ltd, Osaka, Japan) at a temperature of 26°C, light

intensity of $15 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$, and light:dark cycle of 12:12. Twenty-seven strains of *Gambierdiscus* were successfully established (Table 1). Type specimens were deposited in C and VMO; herbarium acronyms follow Thiers (2023, Index herbariorum online).

Observation

Cultures in exponential growth phase were collected and fixed in 2% glutaraldehyde or 4% neutral formaldehyde (final concentrations) for LM and SEM observations. LM was performed using an Olympus BX-53 microscope equipped with phase contrast and differential interference contrast optics and epifluorescence attachment (violet excitation *c.* 430 nm, blue emission *c.* 490 nm) (Olympus, Tokyo, Japan). A digital camera DP74 (Olympus, Japan) was used for micro-photography. Calcofluor White M2R (Fritz & Triemer 1985) or iodine-chloralhydrate (Taylor et al., 2003) were used to visualize and facilitate examination of plate patterns. A modified Kofoidian terminology was applied for naming the thecal plates (Besada et al., 1982, Fraga & Rodriguez 2014). Morphological measurements of at least 30 cells from each culture strain were carried out by LM using imaging software (Standard cellSens ver. 3.1.1, Olympus, Tokyo, Japan)

For SEM, a drop of the preserved sample was placed on a $5 \mu\text{m}$ pore size polycarbonate membrane filter, rinsed in distilled water to remove salt, dehydrated through an ethanol series (15, 30, 50, 70, 90, 95, and 100%), and critical point dried using critical point dryer (K850, Quorum, East Sussex, UK) (Nguyen-Ngoc et al., 2021). The polycarbonate filters were then mounted on aluminium stubs with double-sticky carbon tape and coated with platinum using an ion sputter (E-1020 Hitachi High-Technologies Corporation, Tokyo, Japan). Stubs were scanned on the Field Emission Scanning Electron Microscope S-4800 (Hitachi High-Technologies Corporation, Tokyo, Japan).

GraphPad Prism Ver.6 (GraphPad, San Diego, USA) was applied for box plots of biometrics. SPSS Ver. 20 (IBM, USA) was used for one way-ANOVA, and *post hoc* Tukey test for significant differences of dimensions at $P \leq 0.05$.

DNA extraction, PCR amplification and sequencing

About 20–30 cells of each strain were isolated by the micropipette method under a dissecting microscope (MZ12, Leica, Germany). Cells were transferred to a 0.2 mL PCR tube, washed three times with sterile-filtered seawater, followed by deionized water to remove salt; then 30 μL of 10% Chelex 100 was added, and the samples were stored in a deep freezer (-80°C) (Richlen & Barber 2005). DNA extraction of the cells was performed by exposing the samples to a thermal shock (94°C for 10 minutes), and afterwards samples were kept at 4°C until PCR steps proceeded.

The regions selected for PCR amplification were ITS1-5.8S-ITS2, the D1–D3 and D8–D10 domains of LSU, and SSU of the nuclear rDNA. Extracted DNA was used as a template to amplify rDNA sequences of each region in 25 μL reactions. Reactions consisted of a final concentration of 1 pM forward and reverse primer, 2 μL DNA, 12.5 μL 2x OneTag® Master Mix (New England Biolabs, Ipswich, MA, USA), and 10.5 μL nuclease-free water. PCR was performed in an Applied Biosystems 2720 thermocycler (Applied Biosystems, Foster City, CA, USA). The nuclear ITS region was amplified using the primers ITSA and ITSB (Sato et al., 2011) using 35 cycles and an annealing temperature of 47°C for 30 s. For the SSU and D1–D3 of LSU, the primers and methods followed Litaker et al. (2009). The D8–D10 of LSU was amplified using forward primer FD8 and reverse primer RB (Chinain et al., 1999) using 30 cycles and an annealing temperature of 55°C . The internal primers GLD8_421F and GLD8_677R (Nishimura et al., 2013) were used for sequencing. Sanger sequencing was conducted by 1ST BASE (Selangor, Malaysia) from both directions. All PCR reactions were repeated two to four times independently, sequenced and pooled in a

final consensus sequence. The consensus sequences were assembled using Clone Manager 9 (Sci-Ed, Cary, NC, USA).

Phylogenetic analysis

Independent analyses were applied to the four datasets: SSU rDNA, ITS1-5.8S-ITS2, LSU D1–D3 and LSU D8–D10. Sequences of *Gambierdiscus* spp. were retrieved from GenBank (www.ncbi.nlm.nih.gov) (Tables S1, S2, S3 in the Supporting Information). The SSU rDNA data set included one newly generated sequence obtained in this study and 37 other sequences of *Gambierdiscus* (Table S4 in the Supporting Information). The ITS1-5.8S-ITS2 dataset comprised 22 newly generated sequences aligned with 12 other sequences of *Gambierdiscus* (Table S1). The LSU D1–D3 dataset included 16 newly generated sequences and 40 other sequences of *Gambierdiscus* (Table S2), and LSU D8–D10 included 25 newly generated sequences and 45 other sequences of *Gambierdiscus* (Table S3). For all dataset, the sequences were aligned using the MAFFT algorithm with a selection of the q ins-i strategy (Katoh & Standley 2013). Sequence lengths of SSU rDNA, ITS1-5.8S-ITS2, D1–D3/ LSU and D8–D10/ LSU were 1,572, 511, 930 and 785 bp, respectively. *Alexandrium minutum* AY831408 (6476 bp) was used as outgroup in all datasets. The jModelTest v. 2.1.10 (Darriba et al., 2012) and the corrected Akaike Information Criterion were used to identify the best fitting substitution model. Maximum Likelihood (ML) analyses were performed using RAxML v. 8.1 (Stamatakis, 2014) with model parameters fixed according to values determined and bootstrap values of the ML tree estimated with 1,000 replications. Bayesian Inference (BI) analyses were performed in MrBayes v. 3.2.2 (Ronquist et al., 2012) using the same model as in the ML analyses. Two parallel runs with four chains each (three heated and one cold) were performed for 3 million generations, sampling a tree every 100 generations. A consensus tree based on two different trees (acquired from the two methods) was constructed by Dendro Scope software, v. 3.2.10 (Huson & Scornavacca 2012). Nucleotide differences and sequence

divergence between *Gambierdiscus vietnamensis* sp. nov. and other species of the genus were processed by MEGA v. 11 (Tamura et al., 2021).

RESULTS

Five species of *Gambierdiscus* were found in Vietnamese waters, *G. australes*, *G. caribaeus*, *G. carpenteri*, *G. pacificus*, and a previously undescribed species, formally described here as *G. vietnamensis* sp. nov. In the following descriptions, the terms “length, L” indicates the apical-antapical length, “width, W” indicates the trans-diameter, and “depth, DV” indicates the dorso-ventral depth.

G. belizeanus and *G. polynesiensis* were not included in the taxonomic account as genetic analyses were unsuccessful; the data in the morphological and biometric analysis (Tables S5–S6 in the Supporting Information) are based on Vietnamese material tentatively identified as *Gambierdiscus* cf. *polynesiensis* (Figure S2A–G in the Supporting Information) and *Gambierdiscus* cf. *belizeanus* (Figure S3A–J in the Supporting Information).

Morphological description of species observed in Vietnamese waters

1. *Gambierdiscus australes* Chinain et M.A. Faust 1999, Figure 1A–L

The illustrations are based on observations of five strains (Table 1) as well as wild material from Ly Son Island. Morphological biometric was summarized in Tables S1 and S2.

Molecular data. Acquired for ITS rDNA and D1–D3 and D8–D10 of LSU rDNA. See Tables S1–S3.

Occurrence and distribution. *Gambierdiscus australes* was found in Nha Trang Bay, Khanh Hoa Province and Ly Son Island, Quang Ngai Province (Table 1). It is presumably cosmopolitan in subtropical and tropical waters (Chinain et al., 1999, Litaker et al., 2009, Munir et al., 2011, Tudó et al., 2018).

Toxicity. Strains from French Polynesia were shown to produce P-CTX3C (RAV-92, MUR-6, MUR-14, and TB-1) (Chinain et al., 1999, Chinain et al., 2010). Tudó et al. (2020) reported a CFP-like toxin in *G. australes* from the Canary Islands, and MTX5 was detected in strains from the Mediterranean Sea (Estevez et al., 2021). The toxicity of the Vietnamese strains has not been tested.

2. *Gambierdiscus caribaeus* Vandersea, Litaker, M.A. Faust, Kibler, W.C. Holland et P.A. Tester 2009, Figure 2A–G

The illustrations are based on observations of two strains (Table 1) as well as wild material from Phu Quoc Island (in Kien Giang Province) and Ly Son Island (in Quang Ngai Province) in Viet Nam. Morphological biometric was summarized in Tables S5–S6.

Molecular data. Acquired for D8–D10 of LSU rDNA. See Table S3.

Occurrence and distribution. This species was found as epiphyte on *Padina* (brown algae) together with other benthic dinoflagellates such as *Coolia malayensis*, *Ostreopsis siamensis*, and *O. cf. ovata* in Phu Quoc and Con Dao Islands. In Viet Nam, it has previously been found in Nha Trang Bay (Ho and Bing 2019). It has been reported from Belize in the Caribbean Sea (Litaker et al., 2009), and it is globally distributed in warm temperate to tropical water (Litaker et al., 2010). It has later been reported from Rawa and Sibul Islands in Malaysia (Mustapa et al., 2019), Concha Bay in the Caribbean Sea, Columbia (Arteaga-Sogamoso et al., 2021), Canary Islands, Spain (Soler-Onís et al., 2016), Hainan Island, China (Zhang et al., 2016), and in Jeju Island, Korea (Jang et al., 2018, who observed this species at a water temperature of 14.5°C and a salinity of 33.8).

Toxicity. This species has been shown to be toxic to human erythrocytes (Holland et al., 2013). The toxicity of the Vietnamese strains has not been tested.

3. *Gambierdiscus carpenteri* Kibler, Litaker, M.A. Faust, W.C. Holland, Vandersea et P.A. Tester 2009, Figure 3A–G

The illustrations are based on observations of nine strains (Table 1) as well as wild material from Ly Son Island (Quang Ngai) and Nha Trang Bay (Khanh Hoa) in Viet Nam.

Morphological biometric was summarized in Tables S5–S6.

Molecular data. Acquired for ITS rDNA and D1–D3 and D8–D10 of LSU rDNA. See Tables S1–S3.

Occurrence and distribution. *G. carpenteri* was common in samples from Ly Son Island. It has been reported from Mariana Islands and Belize, Caribbean Sea (Litaker et al., 2009), Merimbula Lake and Tasman Sea, Australia (Kohli et al., 2014), and Republic of Kiribati (Xu et al., 2014).

Toxicity. *G. carpenteri* is toxic to human erythrocytes (Holland et al., 2013). The toxicity of the Vietnamese strains has not been tested.

4. *Gambierdiscus pacificus* Chinain et M.A. Faust 1999, Figure 4A–I, VNLS015

The illustrations based on observations of strains of VNLS015 (Table 1). Morphological biometric was summarized in Tables S5–S6.

Molecular data. Acquired for ITS rDNA and D1–D3 and D8–D10 of LSU rDNA. See Tables S1–S3.

Occurrence and distribution. This species was common Ly Son Island and has previously been found in Nha Trang Bay (Ho-Van et al., 2010). It has been reported from several places in the Pacific Ocean (Chinain et al., 1999; Litaker et al., 2009; Rhodes et al., 2014; Xu et al., 2014), Malaysia (Mohammad-Noor et al., 2005), and China (Zhang et al., 2016).

Toxicity. This species may produce ciguatoxin- and maitotoxins-like compounds (Chinain et al., 1999). The toxicity of the Vietnamese strains has not been tested.

5. *Gambierdiscus vietnamensis* sp. nov. Nguyen-Ngoc, J. Larsen, Chomérat, Lundholm, and Doan-Nhu, Figure 5A–I (LM), Figure 6A–J (SEM), and Figure 7A–D (line drawings)

Description: Cells were ellipsoid to round, anterior-posteriorly compressed (Figure 5A and B; Figure 6A, B, and F), and lenticular in both ventral and dorsal view (Figure 6A, B, and G). The cells possessed numerous yellow-brown rod-shaped chloroplasts (Figure 5B and C). They were 38.1–50.5 μm long ($n=70$), 49.3–69.8 μm wide ($n=120$), and 50.2–69.0 μm dorso-ventrally ($n=120$); DV:W was 0.9–1.1 ($n=120$).

Thecal plates were foveate to reticulate-foveate, with deep polygonal areolae in some parts, most of which had a pore at the bottom (Figure 6A, C, H, and I). The pre- and post-cingular plates formed cingular wings lined by elongated areolae with openings towards the cell periphery; these areolae had pores, which appeared as marginal pores along the cell periphery (Figure 5D, E, G, and I; Figure 6A, B, F, G, and H).

The epitheca was composed of 11 plates: an apical pore plate Po, 4 apical plates, and 6 pre-cingular plates (Figure 5D, E, F, and G). The pore plate was three (Figures 6C and 7D) or four sided (Figure 6D), 6.7 ± 0.5 μm long, 5.1 ± 0.7 μm wide; L:W ratio 1.3 ± 0.2 . The 2'-plate was the largest of the apical plates, hatchet shape (Figure 5D, E, and G; Figure 6A; Figure 7A), and 34.9 ± 1.7 μm long; the 1''-2' suture was 12.6 ± 2.0 μm long, the 2'-3'' suture was 17.3 ± 1.8 μm long, and 1''-2' / 2'-3'' suture ratio was 0.7 ± 0.1 . The small 1' plate had low rim-like sutures adjoining the 1'' and 6'' plates (Figure 6B, G, and J). The 3' and 4' plates were asymmetrical pentagonal and hexagonal, respectively (Figure 5D, E, and G; Figure 6A; Figure 7A). The precingular plates (") were surrounding apical plates, 2'' was symmetrical largest trapezoid shaped (Figures 5D, E, and G; Figure 6A; Figure 7A), 3'' was asymmetrical pentagonal (Figure 5D, E; Figure 7A) or symmetrical quadrangular (Figures 5G and 6A) shape, 4'' and 5'' were in the same size and shape (Figure 5D and E; Figure 6A; Figure 7A), and 6'' was smallest in precingular plate pattern (Figure 5D and E; Figure 6B, G, and J; Figure 7C).

The cingulum was narrow, deep like a chamber (Figures 6B and 7C), and composed of 6 plates (Figures 5F and 6E); the distal end of the cingulum bent upwards before ending in the sulcus, which was deep and did not reach the antapex (Figure 6B, G, and J). Five platelets such as Sa, Sdp, Sda, Ssa, and Ssp were found in the sulcus, while Sp-plate was out of the sulcus and contacting 4^{'''}, 5^{'''}, 2^{'''}, and 1^{'''} (Figure 6G and J; Figure 7B and C). The hypotheca was composed of 5 postcingular plates and 2 antapical plates (Figure 5H–I; Figure 6F–G; Figure 7B). The 1^{'''} was triangular (Figure 5I; Figure 6B, F, and G; Figure 7B–C) and the 2^{'''} and 3^{'''} were asymmetrical quadrangular and equal in size; the 4^{'''} was the largest of the hypotheca and trapezoid shape (Figure 6F–G; Figure 7B); and the 5^{'''} plate was narrow and elongated adjoining the 6c-plate (Figure 6G and J; Figure 7B). The shape of the 1^{'''} plate was asymmetrical rhomboid and the 2^{'''}-plate was narrow pentagonal wedged-shaped (Figure 5H–I; Figure 6F–G), $30.0 \pm 2.3 \mu\text{m}$ long and $17.2 \pm 1.2 \mu\text{m}$ wide, and the L/W ratio was 1.8 ± 0.2 . Genbank accession numbers of the new species were D1–D3 (ON158663) and D8–D10 (ON158620) regions of LSU rDNA, 18S (ON158627), and the ITS1-5.8S-ITS2 (ON158646) rDNA.

Holotype. SEM stub #VMO_202205

Isotypes. C-A-99704 (material of strain VNLS005 fixed in 4% formaldehyde)

Type locality. Ly Son Island, Quang Ngai Province, Viet Nam (15°23'22" N 109°08'01" E).

Etymology. The specific name “*vietnamensis*” refers to Viet Nam where it was commonly recorded.

Synonym. *Gambierdiscus pacificus* sensu Zhang et al., 2016, figures 1–14.

Distribution and habitat. The *G. vietnamensis* sp. nov. has been found commonly in dry season, April to August, around Ly Son Island (type locality), 15°23'22" N 109°08'01" E: strains VNLS005 and VNLS017; Phan Rang (Ninh Thuan Province), 11°37'05" N 109°09'30" E: strains VNPR010, VNPR011, and VNPR012; and Con Dao Island (Ba Ria – Vung Tau Province), 8°41'16" N 106°37'20" E: strain VNCD005. Zhang et al. (2016) found this species

in Hainan Island (Sanya, 18°12'33" N 109°29'27" E; Longshui, 18°18'38" N 109°58'37" E; and Wuzhizhou, 18°19'05"N 109°45'45" E) as *G. pacificus* II.

The species was epiphytic on *Padina* spp, *Sargassum* spp. (Phaeophyceae, Ochrophyta), and *Acanthophora spicifera* (Florideophyceae, Rhodophyta) in shallow waters, 2–4 m depth.

TOXICITY. Toxins and toxicity of strain VNLS005 has not been tested, but strain VNPR012 (referred to as *Gambierdiscus* sp. Viet Nam in Pisapia et al. (2017) has been reported to produce 40.8 ± 19.6 fg CTX3C eq cell⁻¹ and 70.0 ± 19.6 pg MTX eq · cell⁻¹.

Biometric analyses

Statistical analysis showed that the seven species of *Gambierdiscus* reported in this study may be divided into 2 size groups with significant differences in mean group values of the cell dimensions (DV, W, and L; full statistical information is in Table S6).

- 1) A group of large species (means of DV=78.5 ± 7.3, W = 79.4 ± 9.0, and L = 58.5 ± 5.7), *G. australes*, *G. caribaeus*, *G. carpenteri* (Figure S4A–B in the Supporting Information)
- 2) A group of small species (means of DV= 60.0 ± 4.8, W = 60.0 ± 5.2, and L = 42.5 ± 3.5) including *G. cf. polynesiensis*, *G. cf. belizeanus*, *G. pacificus*, and *G. vietnamensis*.

Within the group of large species, the cell dimensions were not significantly different except for L, which may be used to distinguish *G. caribaeus* and *G. carpenteri* (Table 2, Table S5, Figure S4C). *Gambierdiscus caribaeus* may also be distinguished from both *G. australes* and *G. carpenteri* by the shapes of the 2'- and 2''' plates, while *G. australes* and *G. carpenteri* can be distinguished only after genetic analysis (Tables S5, Figure S4E and F).

Phylogenetic analyses – Gambierdiscus vietnamensis sp. nov.

The phylogenetic analyses of the SSU rDNA, ITS1-5.8S-ITS2, LSU D1–D3 and LSU D8–D10 rDNA regions using BI and ML showed in all analyses that strains of *Gambierdiscus vietnamensis* sp. nov. formed a highly supported monophyletic clade comprising the holotype

strain VNLS005 (bootstrap value of >96 in all ML analyses; 1.00 in all BI analyses) (Figures 8, 9 and 10; Figures S5, S6, and S7 in the Supporting Information), except in the SSU tree where only strain VNLS017 was included. In the analyses inferred from the LSU D8–D10 region, the clade comprised the strains 1S1C5 and 1S1G7 from Hainan, China, denoted “*Gambierdiscus pacificus* II” in Zhang et al. (2016); accession numbers KR230000, KR229998; Figure 10). Sequence divergences (p-values and number of different bases) between *G. vietnamensis* sp. nov. (strain VNLS005) and the two strains from Hainan denoted *G. pacificus* II was very low, 0.3% (2 bp). For comparison, the p-values and number of different bases among the Vietnamese strains of *G. vietnamensis* sp. nov. were 0–0.6% and 0–4 bp, respectively.

In all analyses, *Gambierdiscus vietnamensis* sp. nov. was found as sister to a major clade comprising the taxa: *G. toxicus*, *G. lewisii*, *G. pacificus*, *G. balechii*, *G. lapillus*, and *G. cheloniae* (Figures 8, 9, and 10; Figures S5, S6, and S7), depending on availability of sequences for the genetic regions. However, an additional taxon, *G. belizeanus*, was found within the sister clade of *G. vietnamensis* in the ITS analyses (Figure 8) but basal to the sister clade and *G. vietnamensis* sp. nov. in the remaining analyses (Figures 9 and 10, and Figure S5). The clade comprising both *G. vietnamensis* sp. nov. and its sister clade was well-supported by bootstrap values of >92 in all ML analyses and posterior probabilities > 0.97 in BI inferences, except for D1–D3 where support was lower (Figures 8, 9 and 10; Figures S5 and S6).

The sequence divergence (p-distances and number of base pair differences) between *Gambierdiscus vietnamensis* sp. nov. (VNLS005) and the sister taxa ranged from 0.109 and 78 bp (*G. pacificus*, VNLS002) to 0.133 and 94 bp (*G. lewisii*) in LSU D1–D3 (Table 3). In D8–D10, the sequence divergence values between *G. vietnamensis* sp. nov. and the species in the sister clade ranged from 0.041 (31 bp) (*G. cheloniae* and *G. pacificus*) to 0.048 (36 bp; *G. lapillus*; Table 4, Figure S7). In the ITS rDNA region, the sequence divergence between *G.*

vietnamensis sp. nov (VNNT011, ON158648) and *G. pacificus* (CAWD213, MN123245) was lowest, 0.173 (54 bp; Table 5), and highest between *G. vietnamensis* sp. nov and *G. belizeanus* 0.376 (96 bp; Table 5). Finally, in SSU, the sequence divergence was lowest between *G. vietnamensis* sp. nov and *G. lewisii* (0.025 and 38bp) and highest between *G. vietnamensis* sp. nov and *G. toxicus* (0.036 and 54 bp; Table S7 in the Supporting Information).

Phylogenetic analyses – other Gambierdiscus taxa

In all phylogenetic analyses, the Vietnamese strains of *Gambierdiscus carpenteri*, *G. pacificus*, *G. australes*, and *G. caribaeus* grouped with previously sequenced strains of the respective taxa (Figures 8, 9 and 10; Figure S5).

In the LSU and SSU regions, *Gambierdiscus carpenteri* strains formed a well-supported monophyletic clade, with *G. jejuensis* and *G. caribaeus* as the closest related taxa (Figures 9 and 10; Figure S5). In the D1–D3 analyses, the Vietnamese strains of *G. carpenteri* formed a highly supported subclade separate from the strains from Australia and USA (Figure 9) because of a 60bp deletion in the Vietnamese strains whereas in D8–D10, the Vietnamese strains formed a subclade together with a strain from the type locality in Belize (NOAA12, EU498039, Litaker et al., 2009) separate from two Australian strains and a second strain (EU498038) from the type locality (Figure 10), but these subdivisions were not supported in D8–D10. Phylogenetic analyses of ITS rDNA revealed that *G. carpenteri*, similar to the LSU and SSU analyses, formed a monophyletic clade with *G. jejuensis* and *G. caribaeus* as the closest related taxa (Figure 8). For unknown reasons, one strain (strain NOAA1) of *G. carpenteri* from Guam (GU968493) did not cluster with the other *G. carpenteri* strains, although the same strain clustered within the *G. carpenteri* clades in the analyses of 18S and D1–D3.

In all analyses, *Gambierdiscus pacificus* formed a monophyletic clade with *G. lewisii* and *G. toxicus* as closest sister taxa, and in all regions, the genetic distances between taxa were relatively small (Figures 8, 9, and 10; Figure S5; and Tables 3–5). In the D8–D10 analyses, the *G. pacificus* clade comprised three Vietnamese strains (VNLS001, VNLS002, and VNLS015), the holotype from the Tuamoto Archipelago in the Pacific Ocean (EU498013, strain HO91) as well as other strains from the Pacific Ocean (Figure 10). In the D8–D10 region, no nucleotide differences were found among any of the strains of *G. pacificus*, including the Vietnamese, and the genetic distances between *G. pacificus* and *G. lewisii* were very low, 0.3% (2 bp; Table 4). In the D1–D3 analyses, the highly supported *G. pacificus* clade comprised strains from Viet Nam, Australia, and French Polynesia (Figure 9); in the ITS analyses, strains from Cook Island and French Polynesia were clustering among the Vietnamese strains, and the SSU analyses comprised strains from French Polynesia, Micronesia, and Kiribati, all indicating a wide distribution of *G. pacificus* in the warm Pacific. *Gambierdiscus australes* formed a highly supported monophyletic clade in all sequenced regions of the rDNA comprising strains from Canary Islands, French Polynesia, China, Australia, Hawaii, Florida, and Japan as well as Viet Nam. In both LSU analyses, strains from the type locality in Australia (EU498070/72/73, strain RAV-92) were found in the same clade (Figures 9 and 10).

The Vietnamese strains of *Gambierdiscus caribaeus* clustered in a monophyletic clade together with strains from Thailand, Florida, French Polynesia, China, and Grand Cayman Island in the D8–D10 region (Figure 10). The Vietnamese strains, the strain from Grand Cayman Island, Caribbean (strain NOAA20, EU498065) and a strain from Thailand (strain TF9G, AB908138) formed a weakly supported subclade. The other rDNA regions of the *G. caribaeus* genome were not sequenced.

DISCUSSION

Gambierdiscus vietnamensis sp. nov.

Phylogenetic inferences of the four genetic markers, the SSU, ITS1-5.8S-ITS2, LSU D1–D3 and LSU D8–D10 rDNA regions, supported the establishment of the new species *Gambierdiscus vietnamensis* nov. sp., in the D8–D10 analyses embracing the ribotype previously denoted “*G. pacificus* II” (Zhang et al., 2016). Our phylogenetic analyses of D8–D10 thus support previous indications suggesting that *G. pacificus* II could represent a novel species (Kretzschmar et al., 2019). A D1–D3 sequence of the “*G. pacificus* II” strain 1S1G7 (KT382828) differed in 120 bp from all other *Gambierdiscus* species in our analysis and was considered doubtful due to the high level of variation (of 160 bp; Kretzschmar et al., 2019). It was therefore not included in our LSU D1–D3 analyses.

Gambierdiscus vietnamensis sp. nov. has strongly reticulated thecal plates, and in this feature it is similar to several previously described species of *Gambierdiscus*, notably *G. balechii*, *G. belizeanus*, *G. cheloniae*, *G. honu*, *G. lewisii*, *G. lapillus*, *G. scabrosus*, and possibly *G. pacificus* (Chinain et al., 1999, Faust 1995, Fraga et al., 2016, Smith et al., 2016, Kretzschmar et al., 2017, Kretzschmar et al., 2019; Nishimura et al., 2014, Rhodes et al., 2017). All species have short, narrow 2^{'''}-plates and hatchet-shaped 2'-plates (Figure 7E–J) except *G. scabrosus* (Figure 7L) which has a rectangular 2'-plate; *G. lewisii* (Figure 7K) is considerably smaller than the other species (Kretzschmar et al., 2019). Kretzschmar et al. (2019) discussed the differences between these species in detail, and based on their observations and the previous descriptions, particularly by Fraga et al. (2016), Kohli et al. (2014), and Kretzschmar et al. (2017), they concluded that the intra-specific variation in plate pattern is considerable and recommend that a multiple approach including molecular data is applied in identification of these species. *Gambierdiscus vietnamensis* is morphologically very similar to *G. cf. belizeanus* and *G. pacificus* (Table S5) and molecular analyses are necessary for identification of the new species.

Species morphology and comparisons with previous descriptions of remaining species

Gambierdiscus australes. The Vietnamese material (Figure 1A–L) was in agreement with the original description of this species with regard to the average cell dimensions, which were well within the ranges given by Chinain et al. (1999) although the largest cell width observed in the present study was 95.4 μm versus 84 μm reported by Chinain et al. (1999). The size ranges of the present material were similar to what was observed in material from the Canary Islands, Spain (Bravo et al., 2019), while the New Zealand isolates of this species were considerably smaller than isolates from other regions (Table S1; Rhodes et al., 2014). The phylogenetic analyses also support the identification of the Vietnamese strains, as they clustered together with all other *G. australes* strains and most importantly with strains from the type locality (Figures 9 and 10). The species referred to *G. australes* by Munir et al. (2011) had a rather wide 2nd plate. The L/W ratio was not indicated by Munir et al. (2011) and molecular data were not provided, but from their illustrations (i.e., figure 6D, which incidentally is the same image as in figure 4E but turned 90°), it was estimated that the L/W ratio is about 1.3 which is considerably lower than in other species referred to *G. australes* (Table S5). We suppose that this species is misidentified and perhaps should be referred to *G. caribaeus*.

Gambierdiscus caribaeus. This species was described from Carrie Bow Key, Belize by Litaker et al. (2009). The Vietnamese material (Figure 2A–G) was in good agreement with the original description with regard to the average cell dimensions. However, the lowest cell width observed here was considerably lower (50 μm) than reported by Litaker et al. (2009; 70 μm). Small cells were also reported from Thailand (Tawong et al., 2016) and from the Canary Islands, Spain (Bravo et al., 2019), and *G. caribaeus* appears highly variable in size (Table S5).

Gambierdiscus carpenteri. This species was described from South Water Key, Belize by Litaker et al. (2009). The present observations (Figure 3A–G) are in good agreement with the original description regarding overall size and cell dimensions, and identification supported by the phylogenetic analyses, clustering in D8–D10 LSU analyses with a strain from the type locality (Figure 10). The size seems to be highly variable observed in the present study and as reported by Litaker et al. (2009); thus Kohli et al. (2014) reported cell widths (trans-diameter) of 86–110 μm in *G. carpenteri* from New South Wales in Australia, and the species identification was supported by molecular analysis.

Gambierdiscus carpenteri and *G. caribaeus* are phylogenetically closely related (Figures 8, 9, 10, and Figure S5) and morphologically different mainly by the shape of the 3''-plate (4''-plate in Litaker et al., 2009). It is symmetrical in *G. caribaeus* and asymmetrical in *G. carpenter* (Litaker et al., 2019, figures 12, 18, 36, and 42). This feature was also found in Vietnamese materials (Figures 2D and 3D). Litaker et al. (2009) also reported the presence of a dorsal rostrum and a short groove in the 2' plate in some cells, but none of these features was observed in the present material nor in Australian isolates (Kohli et al., 2014).

Gambierdiscus pacificus. This species was created by Chinain et al. (1999). However, there appear to be some discrepancies between the description of this species, and the scanty illustrations provided. It was described as having smooth thecal plates although the SEM images (l.c., figures 11–12) clearly show areolated thecal plates. The cell dimensions indicated in the description are also not consistent with the sizes, which may be measured on the illustrations. The DV depth is about 63 μm and the cell width about 56 μm in l.c., figure 11 as opposed to a 70 μm (range 67–77 μm) and 63 μm (range 60–67 μm), respectively, indicated in the description (l.c., table 1). Furthermore, the L/W ratio of the 2''' (1p) plate was given as 2.59 (l.c., table 1) but appeared to be about 2 (Chinain et al., 1999, figure 12).

The cell sizes of the Vietnamese material (Figure 4A–I) were slightly smaller than reported in the original description but in accordance with subsequent descriptions (Litaker et al., 2009, Mohammed-Noor et al., 2005, Rhodes et al., 2014; Table S5). The Vietnamese strains clustered in the D8–D10 LSU analyses together with the holotype strain (Figure 10), and in all phylogenetic analyses together with available sequences from above-mentioned studies (Figures 8, 9, and 10). The shape of the 2' plate was highly variable, from nearly rectangular to hatchet-shaped in the Vietnamese strains and consistent with the observations by other authors (Table S5). Also, the shape and L/W ratio of the 2''' plate was quite variable in the Vietnamese material, but most authors have reported a short and narrow plate about twice as long as wide (Table S5). The taxonomic value of the feature is discussed further below under *G. vietnamensis*.

Species identification and biometrics

Several species of *Gambierdiscus* share rather similar, in some cases subtle, morphological features, and identification by microscopy is difficult and often not possible without molecular analyses (Litaker et al., 2009, Parsons et al., 2012). It appears that no one single character can be used to differentiate the species. Litaker et al. (2009), Mustapa et al. (2019), and Zhang et al. (2016) applied twelve, thirteen, and twenty characters, respectively, in efforts to identify the different species of *Gambierdiscus* by their morphological features. In the present study, eleven characters were applied in attempts to distinguish the species of *Gambierdiscus* found in Vietnamese waters (Table S1). The studies by Fraga et al. (2016) and Bravo et al. (2019) as well as the present study (Tables S5 and S6) have demonstrated considerable variation and overlap in the overall dimensions of the cells as well as in the dimensions of the 2' and 2''' plates, the shape of which has been used in previous attempts to distinguish the species morphologically (Litaker et al., 2009).

Because of this variability, most species cannot be identified by their morphological features when only few cells are observed, which is often the case in natural samples. However, the biometric analyses of the Vietnamese species show that when a larger number of cells are observed (>30 cells in this study) the mean values of the biometric measurements of several species are significantly different in statistical tests (Figure S4A–F, Table S6). This means that it may be possible to identify at least some species in LM when cultures are available, but molecular analyses are still needed in order to differentiate between e.g., *Gambierdiscus vietnamensis*/*G. pacificus* or *G. australes*/*G. carpenteri* (Table 2). The present observations are based on a limited number of species, and the taxonomic value of these observations needs to be studied by further and detailed morphological examination and morphometric measurements of the reported species from other geographical areas and studies of more different species are also needed.

CONCLUSIONS

The present study revealed five different species of *Gambierdiscus* including a previously undescribed species, *G. vietnamensis*, from Vietnamese waters. Identifications were based on morphological analyses and the identification of five species was supported also by phylogenetic analyses. The species previously assigned to *G. pacificus* clade II was shown to belong to *G. vietnamensis* sp. nov. Biometric measurements showed that cell dimensions were significantly different in some species when a large number of cells were examined (>30 cells) and these species may be identified by their morphological features in cultures. However, delineation of *G. vietnamensis*/*G. pacificus* and *G. australes*/*G. carpenter* needs to be supported by molecular analyses. *Gambierdiscus belizeanus*, *G. polynesiensis*, and *G. toxicus* have been reported from Vietnamese waters previously (Ho-Van et al., 2010, Larsen & Nguyen 2004), but none of these records were supported by genetic data and further studies of the diversity of Vietnamese waters are needed to reveal of validity of these observations.

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Research concept and design: LN-N, JL, HD-N; sampling, isolation and maintenance of cultures: LN-N, LP-T, HD-N, CVT; microscope analyses (LM and SEM), morphometric measurements and preparation of illustrations: LN-N, LP-T, DNH; molecular analyses: LN-N, HD-N, VN-X, NC, N-LN, NL, H-VD, H-HN; preparation of manuscript: LN-N, JL, HD-N, VN-X, NC, NL

REFERENCES

- Adachi, R. & Fukuyo, Y. (1979). The thecal structure of a marine toxic dinoflagellate *Gambierdiscus toxicus* gen. et sp. nov. collected in a ciguatera-endemic area. *Nippon Suisan Gakkaishi*, 45, 67–71.
- Arteaga-Sogamoso, E., Rodríguez, F., & Mancera-Pineda, J. E. (2021). Morphological and molecular characterization of *Gambierdiscus caribaeus* (Dinophyceae), with a confirmation of its occurrence in the Colombian Caribbean Tayrona National Natural Park. *Botanica Marina*, 64, 149–159.

- Besada, E. G., Loeblich, L. A., & Loeblich, I. A. R. (1982). Observations on tropical, benthic dinoflagellates from ciguatera-endemic areas: *Coolia*, *Gambierdiscus*, and *Ostreopsis*. *Bulletin of Marine Science*, 32, 723–35.
- Bravo, I., Rodriguez, F., Ramilo, I., Rial, P., & Fraga, S. (2019). Ciguatera-causing dinoflagellate *Gambierdiscus* spp. (Dinophyceae) in a subtropical region of North Atlantic Ocean (Canary Islands): Morphological characterization and biogeography. *Toxins*, 11, 423.
- Chinain, M., Darius, H. T., Ung, A., Cruchet, P., Wang, Z., Ponton, D., Laurent, D., & Pauillac, S. (2010). Growth and toxin production in the ciguatera-causing dinoflagellate *Gambierdiscus polynesiensis* (Dinophyceae) in culture. *Toxicon*, 56(5), 739–750.
- 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. *Journal of Phycology*, 35, 1282–1296.
- Dai, X., Mak, Y. L., Lu, C. K., Mei, H. H., Wu, J. J., Lee, W. H., Chan, L. L., Lim, P. T., Mustapa, N. I., Lim, H. C., Wolf, M., Li, D., Luo, Z., Gu, H., Leaw, C. P., & Lu, D. (2017). Taxonomic assignment of the benthic toxigenic dinoflagellate *Gambierdiscus* sp. type 6 as *Gambierdiscus balechii* (Dinophyceae), including its distribution and ciguatoxicity. *Harmful Algae*, 67, 107–118.
- Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9, 772.
- Estevez, P., Castro, D., Leao-Martins, J. M., Sibat, M., Tudo, A., Dickey, R., Diogene, J., Hess, P., & Gago-Martinez, A. (2021). Toxicity Screening of a *Gambierdiscus australes* Strain from the Western Mediterranean Sea and Identification of a Novel Maitotoxin Analogue. *Marine Drugs*, 19.
- Faust, M. A. (1995). Observation of sand-dwelling toxic dinoflagellates (dinophyceae) from widely differing sites, including two new species. *Journal of Phycology*, 31, 996–1003.

- Fraga, S. & Rodriguez, 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, 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. *Journal of Phycology*, 21, 662–664.
- Ha, D. V., Uesugi, A., Uchida, H., Ky, P. X., Minh, D. Q., Watanabe, R., Matsushima, R., Oikawa, H., Nagai, S., Iwataki, M., Fukuyo, Y., & Suzuki, T. (2018). Identification of causative ciguatoxins in red snappers *Lutjanus bohar* implicated in ciguatera fish poisonings in Vietnam. *Toxins*, 10, 420.
- Habibi, N., Uddin, S., Bottein, M. D., & Faizuddin, M. (2021). Ciguatera in the Indian Ocean with special insights on the Arabian Sea and adjacent gulf and seas: A Review. *Toxins*, 13(8), 525.
- Ho, T. V. & Bing, H. Y. N. (2019). First report of *Gambierdiscus caribaeus* and *G. carpenteri* (Dinophyceae) from Nha Trang Bay, South Central Vietnam. *Marine Biological Association of India*, 60, 5–11.
- Holland, C. W., Kibler, R. S., Litaker, W. R., Place, R. A., Davenport, D. E., & Tester, A. P. (2013). Differences in the toxicity of six *Gambierdiscus* (Dinophyceae) species measured using an in vitro human erythrocyte lysis assay. *Toxicon*, 65, 15–33.
- Hoppenrath, M., Kretzschmar, A. L., Kaufmann, M. J., & Murray, S. A. (2019). Morphological and molecular phylogenetic identification and record verification of

Gambierdiscus excentricus (Dinophyceae) from Madeira Island (NE Atlantic Ocean).

Marine Biodiversity Records, 12.

Ho-Van, T., Nguyen-Ngoc, L. & Doan-Nhu, H. (2010). *Benthic dinoflagellates in Vietnamese*

Waters, Joint Stock Printing Company of Natural Science and Technology, Viet Nam

Academy of Science and Technology, 1–138 pp. (in Vietnamese).

Huson, D. H. & Scornavacca, C. (2012). Dendroscope 3: An interactive tool for rooted

phylogenetic trees and networks. *Systematic Biology*, 61, 1061–1067.

Jang, S. H., Jeong, H. J. & Yoo, Y. D. (2018). *Gambierdiscus jejuensis* sp. nov., an epiphytic

dinoflagellate from the waters of Jeju Island, Korea, effect of temperature on the

growth, and its global distribution. *Harmful Algae*, 80, 149157.

Katoh, K. & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software

Version 7: Improvements in performance and usability. *Molecular Biology and*

Evolution, 30, 772–780.

Keller, M. D., Selvin, R. C., Claus, W., Guillard, R. R. L. (1987). Media for the culture of

oceanic ultraphytoplankton. *Journal of Phycology*, 23(4), 633–638

Kohli, G. S., Murray, S. A., Neilan, B. A., Rhodes, L. L., Harwood, D. T., Smith, K. F.,

Meyer, L., Capper, A., Brett, S., & Hallegraeff, G. M. (2014). High abundance of the

potentially maitotoxic dinoflagellate *Gambierdiscus carpenteri* in temperate waters of

New South Wales, Australia. *Harmful Algae*, 39, 134–145.

Kretzschmar, A. L., Larsson, M. E., Hoppenrath, M., Doblin, M. A., & Murray, S. A. (2019).

Characterisation of Two Toxic *Gambierdiscus* spp. (Gonyaulacales, Dinophyceae) from

the Great Barrier Reef (Australia): *G. lewisii* sp. nov. and *G. holmesii* sp. nov. *Protist*,

170(6), 125699.

Kretzschmar, A. L., Verma, A., Harwood, T., Hoppenrath, M., & Murray, S. (2017).

Characterization of *Gambierdiscus lapillus* sp. nov. (Gonyaulacales, Dinophyceae): A

new toxic dinoflagellate from the Great Barrier Reef (Australia). *Journal of Phycology*, 53, 283–297.

Larsen, J., & Nguyen, N. L. (2004). Potentially toxic microalgae of Vietnamese waters. *Opera Botanica*, 140, 5–216).

Larsson, M. E., Laczka, O. F., Tim Harwood, T. D., Lewis, R. J., Himaya, S. W. A., Murray, S. A., & Doblin, M. A. (2018). Toxicology of *Gambierdiscus* spp. (dinophyceae) from tropical and temperate Australian waters. *Marine Drugs*, 16, 1–19.

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

Mohammad-Noor, N., Daugbjerg, N., Moestrup, Ø., & Anton, A. (2005). Marine epibenthic dinoflagellates from Malaysia - a study of live cultures and preserved samples based on light and scanning electron microscopy. *Nordic Journal of Botany*, 24, 629–690.

Munir, S., Siddiqui, P. J. A., & Morton, S. L. (2011). The occurrence of the ciguatera fish poisoning producing dinoflagellate genus *Gambierdiscus* in Pakistan waters. *Algae*, 26, 317–325.

Mustapa, N. I., Yong, H. L., Lee, L. K., Lim, Z. F., Lim, H. C., Teng, S. T., Luo, Z., Gu, H., Leaw, C. P., & Lim, P. T. (2019). Growth and epiphytic behavior of three *Gambierdiscus* species (Dinophyceae) associated with various macroalgal substrates. *Harmful Algae*, 89, 101671.

- Accepted Article
- Nguyen-Ngoc, L., Doan-Nhu, H., Larsen, J., Phan-Tan, L., Nguyen, X. V., Lundholm, N., Van Chu, T., & Huynh-Thi, D. N. (2021). Morphological and genetic analyses of *Ostreopsis* (Dinophyceae, Gonyaulacales, Ostreopsidaceae) species from Vietnamese waters with a re-description of the type species, *O. siamensis*. *Journal of Phycology*, 57, 1059–1083.
- Nishimura, T., Sato, S., Tawong, W., Sakanari, H., Uehara, K., Shah, M. 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.
- Nishimura, T., Sato, S., Tawong, W., Sakanari, H., Yamaguchi, H., & Adachi, M. (2014). Morphology of *Gambierdiscus scabrosus* sp. nov. (Gonyaulacales): A new epiphytic toxic dinoflagellate from coastal areas of Japan. *Journal of Phycology*, 50, 506–514.
- Parsons, M. L., Aligizaki, K., Bottein, M.-Y. D., Fraga, S., Morton, S. L., 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.
- Pisapia, F., Holland, W. C., Hardison, D. R., Litaker, R. W., 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 bioassays. *Harmful Algae*, 63, 173–183.
- Rhodes, L., Harwood, T., Smith, K., Argyle, P., & Munday, R. (2014). Production of ciguatoxin and maitotoxin by strains of *Gambierdiscus australes*, *G. pacificus* and *G. polynesiensis* (Dinophyceae) isolated from Rarotonga, Cook Islands. *Harmful Algae*, 39, 185–190.
- Rhodes, L., Smith, K. F., Murray, S., Harwood, D. T., Trnski, T., & Munday, R. (2017). The Epiphytic Genus *Gambierdiscus* (Dinophyceae) in the Kermadec Islands and Zealandia

Regions of the Southwestern Pacific and the Associated Risk of Ciguatera Fish Poisoning. *Marine Drugs*, 15.

- Rhodes, L., Smith, K. F., Verma, A., Curley, B. G., Harwood, D. T., Murray, S., Kohli, G. S., Solomona, D., Rongo, T., Munday, R., & Murray, A. S. (2017). A new species of *Gambierdiscus* (Dinophyceae) from the south-west Pacific: *Gambierdiscus honu* sp. nov., *Harmful Algae*, 65, 61–70.
- Richlen, M. L. & Barber, P. H. (2005). A technique for the rapid extraction of microalgal DNA from single live and preserved cells. *Molecular Ecology Notes*, 5, 688–691.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and model choice across a large model space. *Systematic Biology*, 61, 5395–42.
- Saburova, M., Polikarpov, I., & Al-Yamani, F. (2013). New records of the genus *Gambierdiscus* in marginal seas of the Indian Ocean. *Marine Biodiversity Records*, 6, e91.
- Sato, S., Nishimura, T., Uehara, K., Sakanari, H., Tawong, W., Hariganeya, N., Smith, K., Rhodes, L., Yasumoto, T., Taira, Y., Suda, S., Yamaguchi, H., & Adachi, M. (2011). Phylogeography of *Ostreopsis* along West Pacific Coast, with Special Reference to a Novel Clade from Japan. *PLoS ONE* 6, e27983.
- Smith, K. F., Rhodes, L., Verma, A., Curley, B. G., Harwood, D. T., Kohli, G. S., Solomona, D., Rongo, T., Munday, R., & Murray, S. A. (2016). A new *Gambierdiscus* species (Dinophyceae) from Rarotonga, Cook Islands: *Gambierdiscus cheloniae* sp. nov. *Harmful Algae*, 60, 45–56.
- Soler-Onís, E., Fernández-Zabala, J., Ojeda-Rodríguez, A., & Amorim, A. (2016). Bloom of *Gambierdiscus caribaeus* in the temperate-subtropical waters of El Hierro, Canary Islands (North East Atlantic). *Harmful Algae News*, 55, 14–17.

- Accepted Article
- Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30(9), 1312–1313.
- Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38(7), 3022–3027.
- Taylor, F. J. R., Fukuyo, Y., Larsen, J., & Hallegraeff, G. M. (2003). In Hallegraeff, G.M., Anderson, D.M. & Cembella, A.D (Eds.), *Manual on harmful marine microalgae* (pp. 389-432). UNESCO Publishing.
- Tawong, W., Yoshimatsu, T., Yamaguchi, H., & Adachi, M. (2016). Temperature and salinity effects and toxicity of *Gambierdiscus caribaeus* (Dinophyceae) from Thailand. *Phycologia*, 55(3), 274–278.
- Thiers, B. (2023). Index herbariorum. *New York, New York Botanical Garden*. <http://sciweb.nybg.org/science2/IndexHerbariorum.asp>. (Accessed January 2023).
- Tudó, A., Gaiani, G., Varela, M. R., Tsumuraya, T., Andree, K. B., Fernandez-Tejedor, M., Campas, M., & Diogene, J. (2020). Further advance of *Gambierdiscus* Species in the Canary Islands, with the First Report of *Gambierdiscus belizeanus*. *Toxins*, 12.
- Tudó, A., Toldrà, A., B., A. K., Rey, M., Fernández-Tejedor, M., Campàs, M., & Diogène, J. (2018). First report of *Gambierdiscus* in the Western Mediterranean Sea (Balearic Islands). *Harmful Algal News*, 59, 22–23.
- Xu, Y., Richlen, M. L., Morton, S. L., Mak, Y. L., Chan, L. L., Tekiau, A., & Anderson, D. M. (2014). Distribution, abundance and diversity of *Gambierdiscus* spp. from a ciguatera-endemic area in Marakei, Republic of Kiribati. *Harmful Algae*, 34, 56–68.
- Zhang, H., Wu, Z., Cen, J., Li, Y., Wang, H., & Lu, S. (2016). First report of three benthic dinoflagellates, *Gambierdiscus pacificus*, *G. australes* and *G. caribaeus* (Dinophyceae), from Hainan Island, South China Sea. *Phycological Research*, 64, 259–73.

TABLE 1 Geographical origin of *Gambierdiscus* strains isolated in Vietnamese waters in this study.

Taxa	Strain code	Sampling location, lat. /long.	Sampling Date (dd-mm-yyyy)	Macroalgal host
<i>Gambierdiscus australes</i>	VNLS007	Ly Son Island, 15°23'22" N 109°08'01" E	20-06-2021	<i>Pa, Sa</i>
<i>G. australes</i>	VNLS008	Ly Son Island, 15°23'22" N 109°08'01" E	20-06-2021	<i>Pa, Sa</i>
<i>G. australes</i>	VNLS012	Ly Son Island, 15°23'22" N 109°08'01" E	20-06-2021	<i>Pa, Sa, Ac</i>
<i>G. australes</i>	VNLS016	Ly Son Island, 15°23'22" N 109°08'01" E	20-06-2021	<i>Pa, Sa, Ac</i>
<i>G. australes</i>	VNNT012	Nha Trang Bay, 12°10'50" N 109°17'38" E	14-05-2019	<i>Pa, Sa, Ac</i>
<i>G. caribaeus</i>	VNPQ013	Phu Quoc Island, 10°22'58" N 103°51'00" E	06-06-2020	<i>Pa, Sa, Ac</i>
<i>G. caribaeus</i>	VNPQ014	Phu Quoc Island, 10°22'58" N 103°51'00" E	06-06-2020	<i>Pa, Sa</i>
<i>G. carpenteri</i>	VNLS004	Ly Son Island, 15°23'22" N 109°08'01" E	20-06-2021	<i>Pa, Sa</i>
<i>G. carpenteri</i>	VNLS006	Ly Son Island, 15°23'22" N 109°08'01" E	20-06-2021	<i>Pa, Sa</i>
<i>G. carpenteri</i>	VNLS009	Ly Son Island, 15°23'22" N 109°08'01" E	20-06-2021	<i>Pa, Sa</i>
<i>G. carpenteri</i>	VNLS010	Ly Son Island, 15°23'22" N 109°08'01" E	20-06-2021	<i>Pa, Sa, Ac</i>
<i>G. carpenteri</i>	VNLS011	Ly Son Island, 15°23'22" N 109°08'01" E	20-06-2021	<i>Pa, Sa, Ac</i>
<i>G. carpenteri</i>	VNLS013	Ly Son Island, 15°23'22" N 109°08'01" E	20-06-2021	<i>Pa, Sa, Ac</i>
<i>G. carpenteri</i>	VNLS014	Ly Son Island, 15°23'22" N 109°08'01" E	20-06-2021	<i>Pa, Sa, Ac</i>
<i>G. carpenteri</i>	VNPR014	Phan Rang, 11°37'05" N 109°09'30" E	25-06-2021	<i>Pa</i>
<i>G. carpenteri</i>	VNPR015	Phan Rang, 11°37'05" N 109°09'30" E	25-06-2021	<i>Pa</i>
<i>G. pacificus</i>	VNLS001	Ly Son Island, 15°23'22" N 109°08'01" E	20-06-2021	<i>Pa, Sa, Ac</i>
<i>G. pacificus</i>	VNLS002	Ly Son Island, 15°23'22" N 109°08'01" E	20-06-2021	<i>Pa, Sa</i>
<i>G. pacificus</i>	VNLS015	Ly Son Island, 15°23'22" N 109°08'01" E	20-06-2021	<i>Pa, Sa</i>
<i>G. vietnamensis</i>	VNCD007	Con Dao Islands, 8°41'16"N 106°37'20" E	03-07-2018	<i>Pa, Sa, Ac</i>
<i>G. vietnamensis</i>	VNCD008	Con Dao Islands, 8°41'16"N 106°37'20" E	12-07-2018	<i>Pa, Sa, Ac</i>
<i>G. vietnamensis</i>	VNLS005	Ly Son Island, 15°23'22" N 109°08'01" E	20-06-2021	<i>Pa, Sa, Ac</i>
<i>G. vietnamensis</i>	VNLS017	Ly Son Island, 15°23'22" N 109°08'01" E	02-05-2018	<i>Pa, Sa, Ac</i>
<i>G. vietnamensis</i>	VNNT011	Nha Trang Bay, 12°10'50" N 109°17'38" E	14-05-2019	<i>Pa, Sa</i>
<i>G. vietnamensis</i>	VNPR010	Phan Rang, 11°37'05" N 109°09'30" E	14-08-2016	<i>Pa, Sa, Ac</i>
<i>G. vietnamensis</i>	VNPR011	Phan Rang, 11°37'05" N 109°09'30" E	14-08-2016	<i>Pa, Sa, Ac</i>
<i>G. vietnamensis</i>	VNPR012	Phan Rang, 11°37'05" N 109°09'30" E	16-04-2010	<i>Pa, Sa, Ac</i>

Pa: *Padina* spp.; *Sa*: *Sargassum* spp., *Ac*: *Acanthophora spicifera*

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TABLE 2 The large species groups (light shadow) and small species group (dark shadow) differ significantly in mean values of the DV, W, and L dimensions. Within the groups some species may be distinguished by significant differences of the mean values of the morphometric ratios of the 2' and 2''' plates (see also Figures 2A–G, 3A–J, and S4A–F; Tables S5 and S6A–F). Note: *G. belizeanus* and *G. polynesiensis* were not included in the taxonomic account as genetic analyses were unsuccessful; the data in the biometric analysis are based on Vietnamese material tentatively identified as *G. cf. polynesiensis* and *G. cf. belizeanus*.

	Large species			Small species			
	<i>G. australes</i>	<i>G. caribaeus</i>	<i>G. carpenteri</i>	<i>G. cf. belizeanus</i>	<i>G. pacificus</i>	<i>G. cf. polynesiensis</i>	<i>G. vietnamensis</i>
<i>G. australes</i>	-	Differ in shapes of the 2'- and 2'''-plates	Genetic analysis needed				
<i>G. caribaeus</i>	Differ shapes of the 2'- and 2'''-plates	-	Differ shapes of the 2'- and 2'''-plates				
<i>G. carpenteri</i>	Genetic analysis needed	Differ shapes of the 2'- and 2'''-plates	-				
<i>G. cf. belizeanus</i>				-	Differ in shape of the 2'-plate	Differ in shape of the 2''''-plate	Differ in W, L, and shapes of the 2'- and 2'''-plates
<i>G. pacificus</i>				Differ in L, and shape of the 2'-plate	-	Differ in DV, W, and shapes of the 2'- and 2'''-plates	Genetic analysis needed
<i>G. cf. polynesiensis</i>				Differ in shape of the 2''''-plate	Differ in DV, W, and shapes of the 2'- and 2'''-plates	-	Differ in DV, W, and shapes of the 2'- and 2'''-plates
<i>G. vietnamensis</i>				Differ in W, L, and shapes of the 2'- and 2'''-plates	Genetic analysis needed	Differ in DV, W, and shapes of the 2'- and 2'''-plates	-

TABLE 3 Estimates of evolutionary divergences (p -distances, ranges) and number of different nucleotides (shaded cells) among *Gambierdiscus* species based on 930 bp of the D1–D3 rDNA region. The taxa included in the sister clade to *G. vietnamensis* is indicated by an *.

	<i>G. vietnamensis</i> ON158663 (VNLS005)	<i>G. toxicus</i> * EF202985	<i>G. lewisii</i> * MH910697	<i>G. pacificus</i> * ON158661 (VNLS002)	<i>G. pacificus</i> * EF202946	<i>G. scabrosus</i> AB640991	<i>G. belizeanus</i> EF202940
<i>G. vietnamensis</i> ON158663 (VNLS005)		89	94	78	79	107	142
<i>G. toxicus</i> * EF202985	0.125		65	43	42	114	128
<i>G. lewisii</i> * MH910697	0.133	0.081		50	50	131	144
<i>G. pacificus</i> * ON158661 (VNLS002)	0.109	0.053	0.062		5	110	128
<i>G. pacificus</i> * EF202946	0.110	0.052	0.062	0.006		109	125
<i>G. scabrosus</i> AB640991	0.153	0.150	0.176	0.144	0.142		134
<i>G. belizeanus</i> EF202940	0.231	0.199	0.228	0.199	0.194	0.209	

TABLE 4 Estimates of evolutionary divergences (p -distances, ranges) and number of different nucleotides (shading cells) between *Gambierdiscus* species based on based on 785 bp of D8/D10 rDNA region. The “*G. pacificus* II” (Zhang et al., 2016) is *Gambierdiscus vietnamensis*. The taxa included in the sister clade to *G. vietnamensis* is indicated by an *.

	<i>G.vietnamensis</i> ON158620 (VNLS005)	<i>G. pacificus</i> II KR230000 (1S1G7)	<i>G. cheloniae</i> * KU674344	<i>G. lapillus</i> * KU558826	<i>G. toxicus</i> * EU498017	<i>G. lewisii</i> * MH790462	<i>G. pacificus</i> * ON158618 (VNLS002)	<i>G. scabrosus</i> AB765912	<i>G. belizeanus</i> EU498028	<i>G. balechii</i> KX268469	<i>G. honu</i> KU674343
<i>G.vietnamensis</i> ON158620 (VNLS005)		2	31	36	35	33	31	36	53	33	52
<i>G. pacificus</i> II KR230000 (1S1G7)	0.003		29	34	33	31	29	34	51	31	50
<i>G. cheloniae</i> * KU674344	0.041	0.039		15	17	15	15	20	41	20	38
<i>G. lapillus</i> * KU558826	0.048	0.046	0.020		18	13	14	21	36	18	38
<i>G. toxicus</i> * EU498017	0.047	0.044	0.022	0.024		9	9	24	43	21	40
<i>G. lewisii</i> * MH790462	0.044	0.041	0.020	0.017	0.012		2	19	40	17	39
<i>G. pacificus</i> * VNLS002	0.041	0.039	0.020	0.018	0.012	0.003		16	39	15	38
<i>G. scabrosus</i> AB765912	0.048	0.045	0.026	0.028	0.032	0.025	0.021		38	23	40
<i>G. belizeanus</i> EU498028	0.073	0.070	0.056	0.048	0.058	0.054	0.053	0.051		43	34
<i>G. balechii</i> KX268469	0.044	0.041	0.026	0.024	0.028	0.022	0.020	0.030	0.058		42
<i>G. honu</i> KU674343	0.072	0.069	0.052	0.052	0.054	0.053	0.052	0.055	0.046	0.057	

TABLE 5 Estimates of evolutionary divergences (p -distances, ranges) and number of different nucleotides (shaded cells) between the four strains of *Gambierdiscus vietnamensis* sp. nov., *G. lewisii*, *G. belizeanus*, and *G. pacificus* (noted that VNLS015 – *G. pacificus* from Viet Nam was described in this study) based on 327 bp of ITS region. The taxa included in the sister clade to *G. vietnamensis* is indicated by an *.

	<i>G. vietnamensis</i> ON158646 (VNLS005)	<i>G. vietnamensis</i> ON158649 (VNPR012)	<i>G. vietnamensis</i> ON158648 (VNNT011)	<i>G. vietnamensis</i> ON158647 (VNLS017)	<i>G. lewisii</i> * MH790457	<i>G. belizeanus</i> * GU968497	<i>G. pacificus</i> * MN204475	<i>G. pacificus</i> * MN123245	<i>G. pacificus</i> * ON158645 (VNLS015)
<i>G. vietnamensis</i> ON158646 (VNLS005)		0	2	2	54	96	56	55	58
<i>G. vietnamensis</i> ON158649 (VNPR012)	0.000		2	2	54	96	56	55	58
<i>G. vietnamensis</i> ON158648 (VNNT011)	0.005	0.005		3	53	95	55	54	57
<i>G. vietnamensis</i> ON158647 (VNLS017)	0.005	0.005	0.008		54	95	56	55	58
<i>G. lewisii</i> * MH790457	0.196	0.196	0.191	0.196		78	13	12	13
<i>G. belizeanus</i> * GU968497	0.376	0.376	0.371	0.371	0.326		95	94	97
<i>G. pacificus</i> * MN204475	0.179	0.179	0.176	0.179	0.041	0.302		5	8
<i>G. pacificus</i> * MN123245	0.176	0.176	0.173	0.176	0.038	0.297	0.016		3
<i>G. pacificus</i> * ON158645 (VNLS015)	0.182	0.182	1.179	0.182	0.041	0.302	0.026	0.010	

Captions for figures

FIGURE 1 *Gambierdiscus australes*, B–D: wild materials, A and E–L: Cultured materials (strain VNLS016): - A (LM, DIC). Light micrographs; - B and D (LM, Epi-); - E–L (SEM): - B. Epithelial plate pattern showing the rectangular 2' plate; - C. A part of hypothecal plate pattern showing 2''' plate with curve lateral sides (see Figure 2K also); - D. Horizontal stripes visible of precingular plates (see also in Figures 1E, F, and K); - E and F. Epithelial plate patterns of the two different cells showing the slightly hatchet shaped of 2' plates; - G, H, and I. Detail of APC in different cells; - J. Thecal surface smooth with even round pores; - K. Antapical plate pattern show 2''', 6'' and 1' plates (asterisk) with low rim-like suture (arrows, also in Figure 2L); - L. The five sulcal platelets were counted in the sulcal area, note that Sa (asterik) was smallest platelet.

FIGURE 2 *Gambierdiscus caribaeus*, strain VNPQ013, VNPQ014: - A (LM, DIC). Cell in wide heart-shaped; - B and C (LM, Epi-). Apical plate pattern, 2' plate in rectangular shape (B) and antapical plate; - D–G (SEM): Cell in apical view showing plate pattern (D), detail of APC, antapical plate pattern (F) and details of sulcal plates and adjacent small plates (G).

FIGURE 3 *Gambierdiscus carpenteri*, strains of VNLS004, VNLS006, VNLS009, VNLS010, VNLS011, VNLS013, VNLS014, VNPR014, and VNPR015: - A (LM, DIC). Compressed and disc shaped cell; - B and C (LM, Epi-). Apical plate pattern (B) with slightly hatchet shaped of 2' (see Fig. 4D also) and antapical plates (C); - D – E (SEM): Plate patterns of epitheca (D) with large size of 2' and a detail of APC (E); - F–G. Plate pattern of hypotheca (with asymmetrical shape of 2''' (F) and sulcal plate pattern (G) showing platelets and adjacent plates of epitheca with narrow 1' plate (arrow head) having raised rim-like sutures (arrows, Figure 4F also).

FIGURE 4 *Gambierdiscus pacificus*: A (LM, DIC), B, C and D (LM, Epi-) from wild materials collected in Ly Son Island and E-I (SEM) from cultured materials, VNLS015: - A. Round-shaped cell with full chloroplast and granules; - B and C (LM, Epi-) showing the apical plate pattern (B) with hatchet shape of 2', small 6'', 1' (asterik), and antapical plate pattern (C) with narrow wedge-shape of 2''''; - D. The six cingular plates; - E and F, Apical and antapical view showing plate patterns; - G and H. Apical pore plate in three sided; - I. Sulcal platelet pattern associated with C6, C1, 6'', 1' (asterik), 5''', 1''', and 1'''.

FIGURE 5 (LM) *Gambierdiscus vietnamensis*, sp. nov, VNLS005: - A (DIC) and B (PC). Different round cells; - C (LM, Epi-). Cell shows numerous rod-shaped chloroplasts; D (Epi-) and E (DIC). Apical view showed plate pattern; F. Six cingular plates; - G (iodine-chloralhydrate stain). Antapical view showed plate patterns; - H (Epi-) and I (iodine-chloralhydrate stain). Antapical plate pattern showing the wedged shape of 2''''.

FIGURE 6 (SEM) *Gambierdiscus vietnamensis* sp. nov., VNLS005: - A and B. Apical view showed the plate patterns of the two different cells; - C and D. Pore plates of different cells showed oval-shaped (C) and three (four)-sided (D); - E. Inner view of antapical showed six cingular plates; - F and G. Plate pattern of antapical view with 2'''' wedged-shape; - H. Deeply reticulate-foveate thecal plates forming deep pentagonal depressions, note the thick ribs (arrows) present on the cingular list; - I. Deep foveated round thecal pores; - J. Five sulcal platelets were visible in associated with 1' (asterisk) had low rim-like sutures adjoining the 1'' and 6'' (arrows, see Figure 6P also) and Sp plate adjoining 4''', 5''', 2''', and 1''''.

FIGURE 7 Line drawings of nine *Gambierdiscus* species with narrow 2''''-plate: - A-D. *Gambierdiscus vietnamensis* sp. nov., VNLS005 (This study): - A. Apical view; - B. Antapical view; - C. Ventral view, the arrow points to the 1'-plate; - D. Apex showing Apical

Pore Complex (A.P.C.); Apical and antapical of: - E. *G. balechii* (redrawn from Fraga et al., 2016), - F. *G. belizeanus* (redrawn from Litaker et al., 2009), - G. *G. cheloniae* (redrawn from Smith et al., 2016), - H. *G. honu* (redrawn from Rhodes et al., 2017), - I. *G. lapillus* (redrawn from Kretzschmar et al., 2009), - J. *G. lewisii* (redrawn from Kretzschmar et al., 2019), - K. *G. pacificus* (redrawn from Litaker et al., 2009), - L. *G. scabrosus* (redrawn from Nishimura et al., 2014).

FIGURE 8 Phylogenetic tree of *Gambierdiscus* inferred from Bayesian inference and maximum likelihood. Data set based on 511 bp of ITS1-5.8S-ITS2 including gaps. Bootstrap values and posterior probability of each method were shown at each node: maximum likelihood (left); Bayesian inference (right); Bootstrap values < 50 are indicated with -; *: full support (Bootstrap values = 100, posterior probability = 1.0) in bold face, material from present study.

FIGURE 9 Phylogenetic tree of *Gambierdiscus* inferred from Bayesian inference and maximum likelihood. Data set based on 957 bp of D1–D3 LSU rDNA. Bootstrap values and posterior probability were shown at each node: maximum likelihood (above); Bayesian inference (below); Bootstrap values < 50 are indicated with; in bold face, material from present study.

FIGURE 10 Phylogenetic tree of *Gambierdiscus* inferred from Bayesian inference and maximum likelihood. Data set based on 782 bp of D8–D10 LSU rDNA. Bootstrap values and posterior probability were shown at each node: maximum likelihood (above); Bayesian inference (below); Bootstrap values < 50 are indicated with; in bold face, material from present study.

Captions for supplementary figures

FIGURE S1 Map shows the sampling sites (empty circles) of *Gambierdiscus* in South Central coast and Islands of Viet Nam.

FIGURE S2 *Gambierdiscus belizeanus*, wild materials. –A (LM, DIC), B–C (LM, Epi-) and D–G (SEM): - A, B, and D. Apical plate pattern shows the heavy areolation (reticulate-foveate) of theca, hatchet shape of 2' plate; - E. Detail of APC showing elliptical shape of apical plate; - C and F. Antapical plate pattern showing the wedge-shaped of 2''' ; - G. Details of sulcus and apart of thecal ornamentation shows a wide striated list (arrow heads, also in Figure 2A, B, D, G from precingular plates).

FIGURE S3 *Gambierdiscus polynesiensis*, cultured materials. - A (LM, DIC). A cell with two flagellates; - B (Epi). Apical plate pattern with hatchet shape of 2', which curve on the 2'–2'' suture; - C (LM, Epi-). Antapical plate pattern shows large pentagonal 2'''; - D (LM, Epi-). Pore plate is distinct triangular shaped; - F–G (SEM): Details of APC; - H (SEM). Antapical plate patterns; - G (SEM). Five platelets of sulcus can be seen in associated with 1', 6'', 5''', and 1'''' plates; - J (SEM). Smooth thecal round pores with sub-structure.

FIGURE S4 Box plots of morphological biometric variation of seven *Gambierdiscus* species in Vietnamese waters. Box diagrams show mean values (horizontal lines) and standard deviation bars. Different letters (a, b, c, and d) in each graph show significant differences.

FIGURE S5 Phylogenetic tree of *Gambierdiscus* inferred from Bayesian inference and maximum likelihood. Data set based on 1.572 bp of 18S including gaps. Bootstrap values and posterior probability of each method were shown at each node: maximum likelihood (left);

Bayesian inference (right); Bootstrap values < 50 are indicated with -; *: full support (Bootstrap values = 100, posterior probability = 1.0) in bold face, material from present study.

FIGURE S6 Phylogenetic tree of *Gambierdiscus*, within the clade of species closely related to *G. vietnamensis*, inferred from Bayesian inference and maximum likelihood. Data set based on 900 bp of D1–D3 LSU rDNA. Bootstrap values and posterior probability were shown at each node: maximum likelihood (above); Bayesian inference (below); Bootstrap values < 50 are indicated with (-); in bold face, material from present study.

FIGURE S7 Phylogenetic tree of *Gambierdiscus*, within the clade of species closely related to *G. vietnamensis*, inferred from Bayesian inference and maximum likelihood. Data set based on 780 bp of D8–D10 LSU rDNA. Bootstrap values and posterior probability were shown at each node: maximum likelihood (above); Bayesian inference (below); Bootstrap values < 50 are indicated with (-); in bold face, material from present study.

Captions for supplementary tables

TABLE S1 GenBank accession numbers of the ITS sequences used in the alignment.

Notes: Bold: samples collected in Viet Nam; -/-: as above; *: used as out-group; ** species was named as *Gambierdiscus caribaeus* in GenBank.

TABLE S2 GenBank accession numbers of the D1–D3 sequences used in the alignment.

Notes: Bold: samples collected in Viet Nam; -/-: as above; *: used as out-group.

TABLE S3 GenBank accession numbers of the LSU D8–D10 sequences used in the alignment. Notes: Bold: samples collected in Viet Nam; -/-: as above; *: used as out-group.

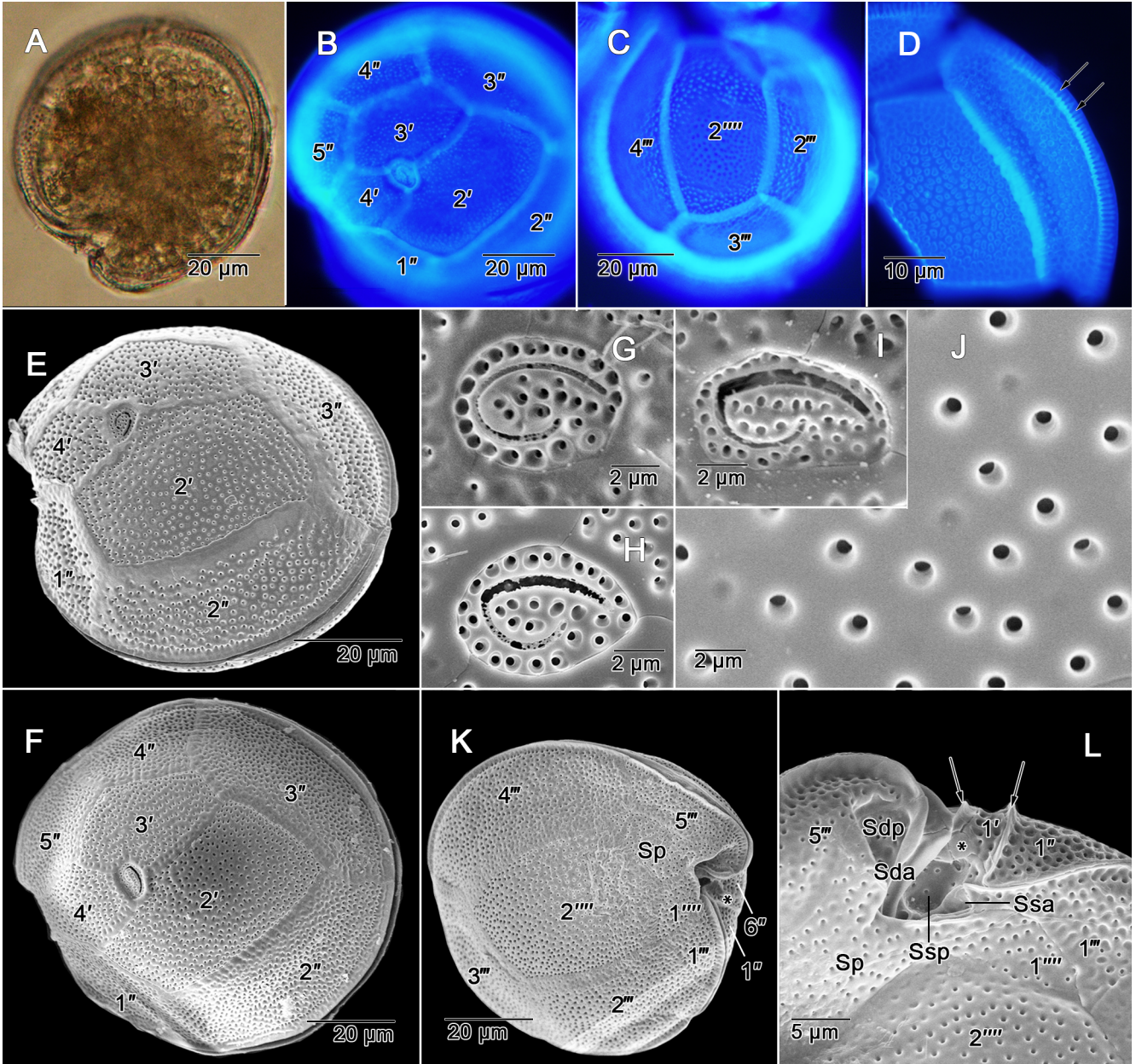
TABLE S4 GenBank accession numbers of the 18S sequences used in the alignment.

Notes: Bold: samples collected in Viet Nam; -/-: as above; *: used as out-group; ** species was named as *Gambierdiscus caribaeus* in Genbank.

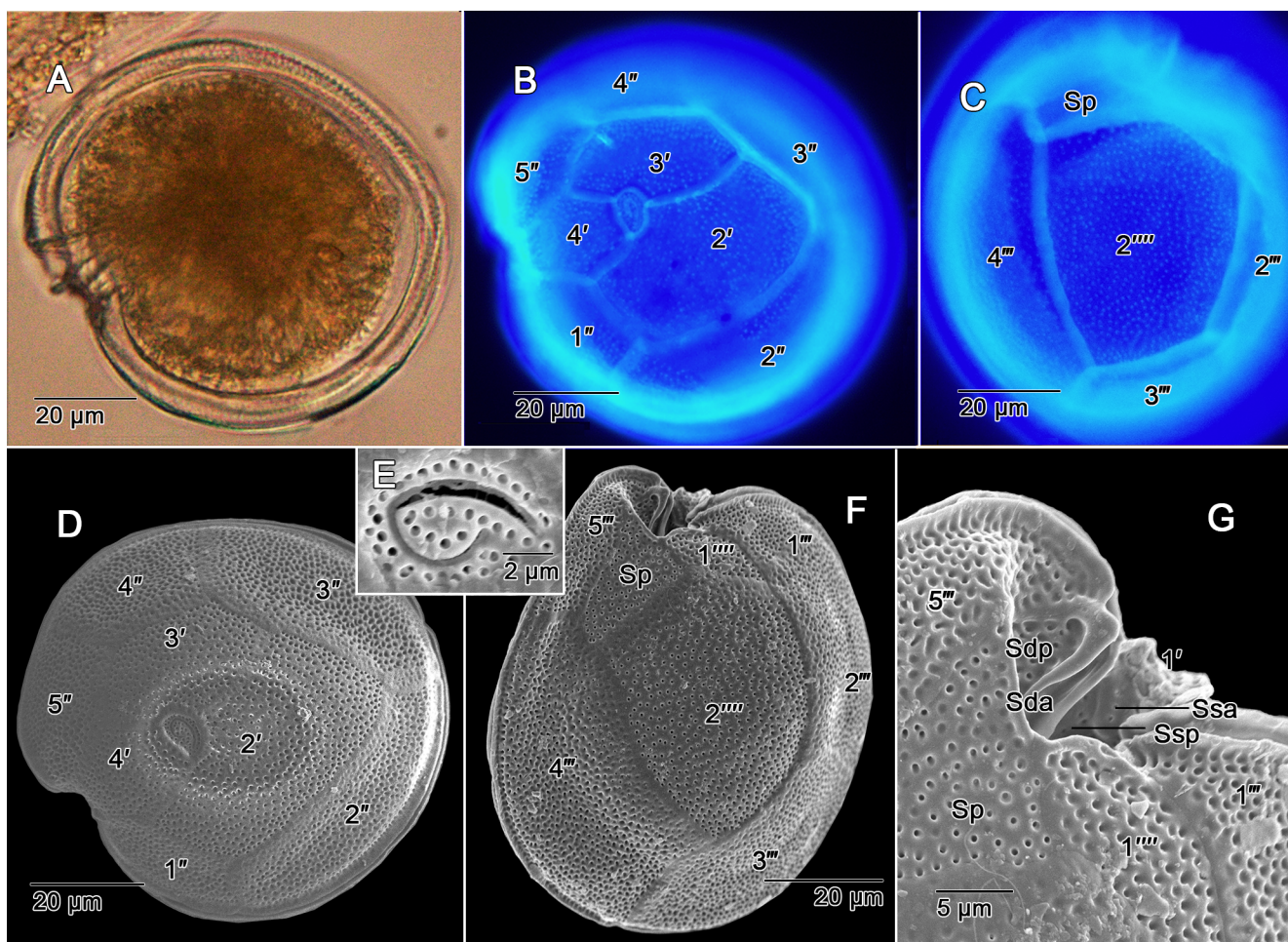
TABLE S5 A summary of morphological biometric of seven *Gambierdiscus* species from Vietnamese waters comparing with previous descriptions. Note: *G. belizeanus* and *G. polynesiensis* were not included in the taxonomic account as genetic analyses were unsuccessful; the data in the biometric analysis are based on Vietnamese material tentatively identified as *Gambierdiscus* cf. *polynesiensis* and *Gambierdiscus* cf. *belizeanus*, and data from the literatures.

TABLE S6 Pair comparison of dimension of seven *Gambierdiscus* species: using One way-ANOVA test, significant level at ≤ 0.05 . Note: *G. belizeanus* and *G. polynesiensis* were not included in the taxonomic account as genetic analyses were unsuccessful; the data in the biometric analysis are based on Vietnamese material tentatively identified as *Gambierdiscus* cf. *polynesiensis* and *Gambierdiscus* cf. *belizeanus*.

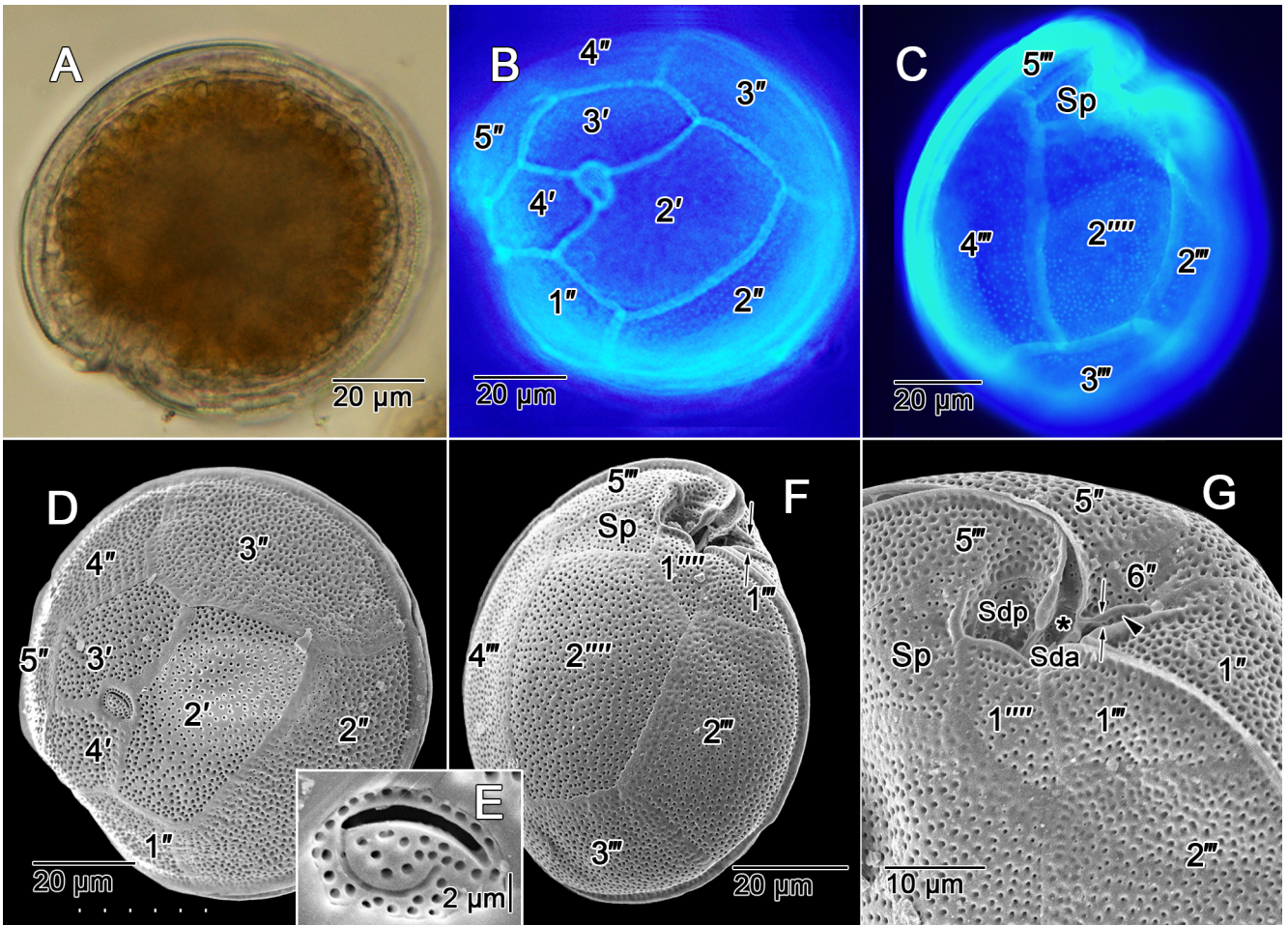
TABLE S7 Estimates of evolutionary divergence (*p*-distances, ranges) and number of different nucleotides (shading cells) between *Gambierdiscus* species based on 1572 bp of 18S region. The taxa included in the sister clade to *G. vietnamensis* is indicated by an *.



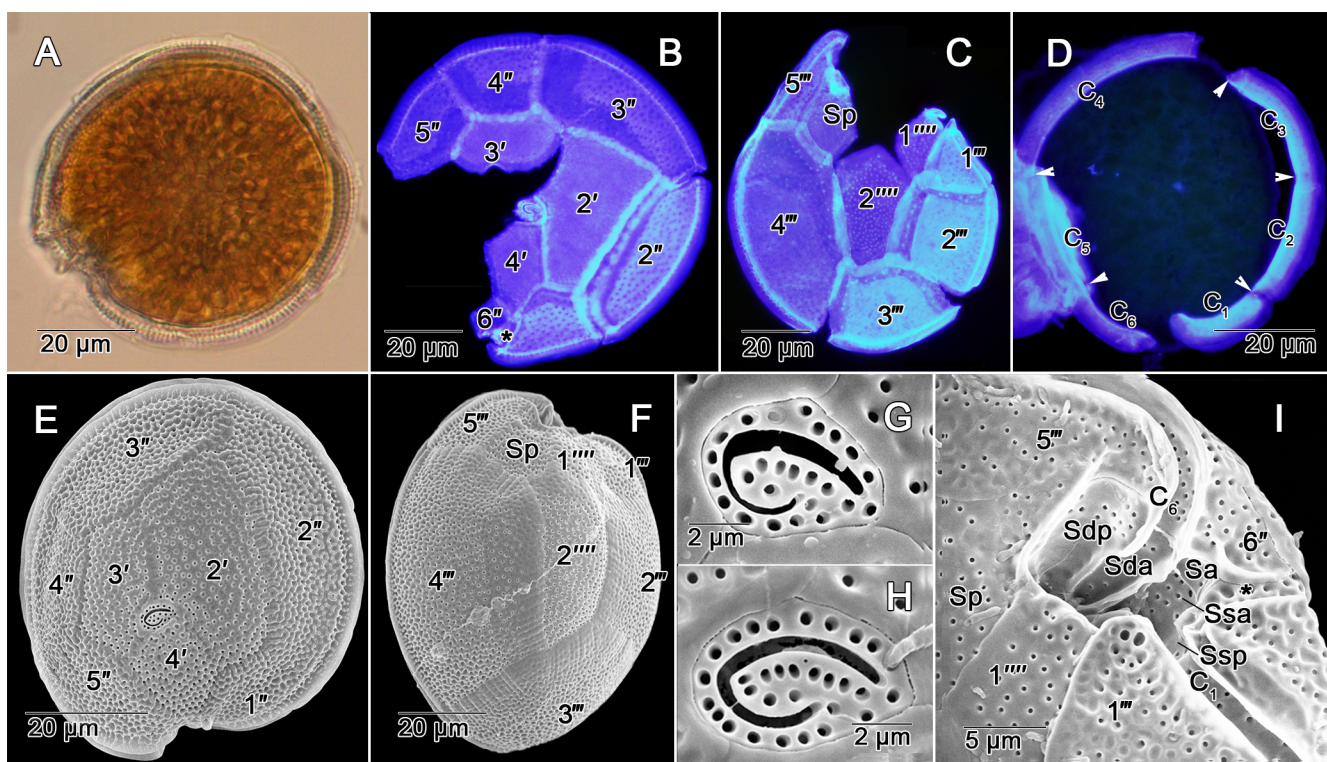
JPY_13326_Figure 1A-L_G australes-VNLS016.tif



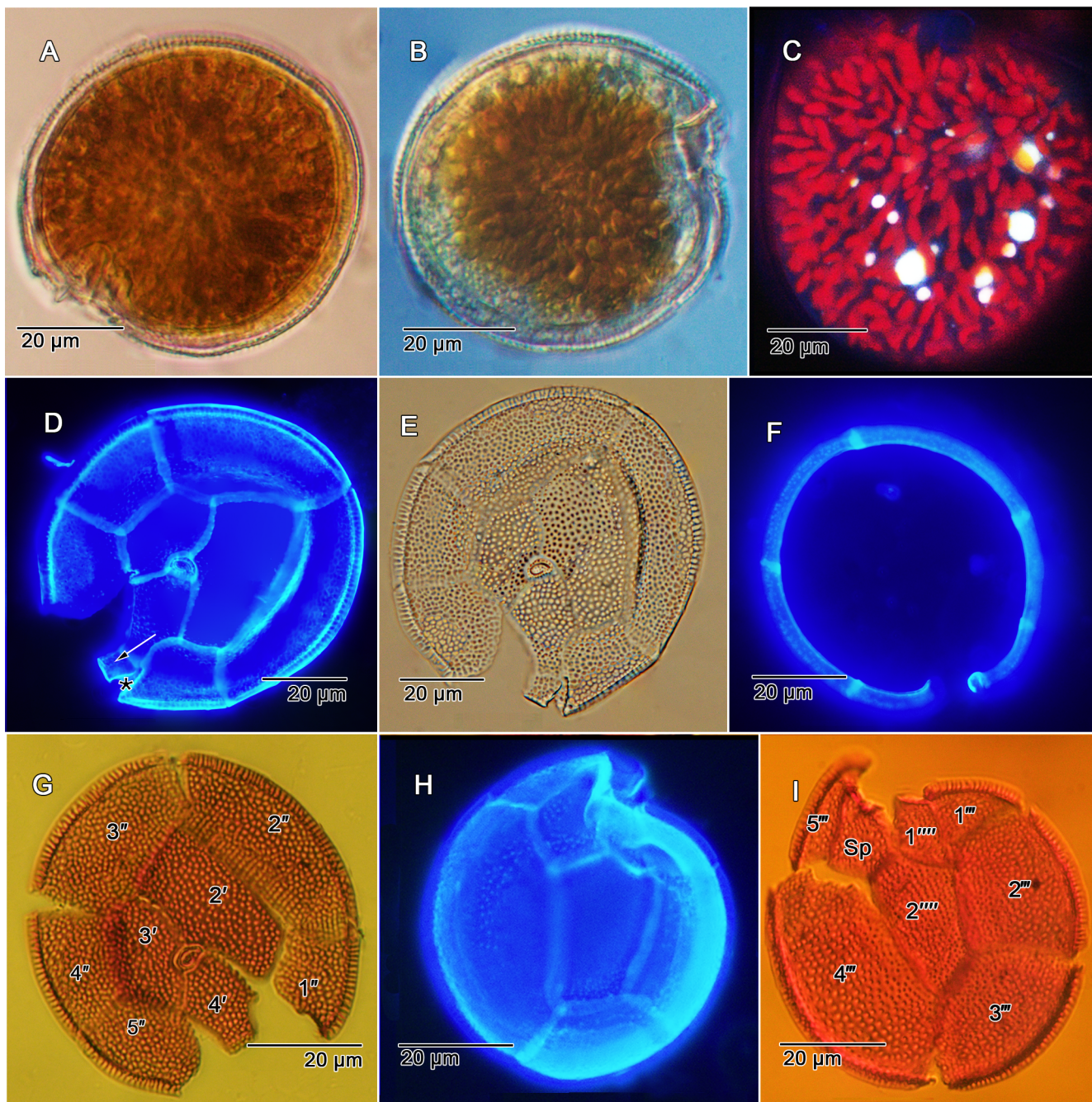
JPY_13326_Figure 2A-G_G caribaeus.tif



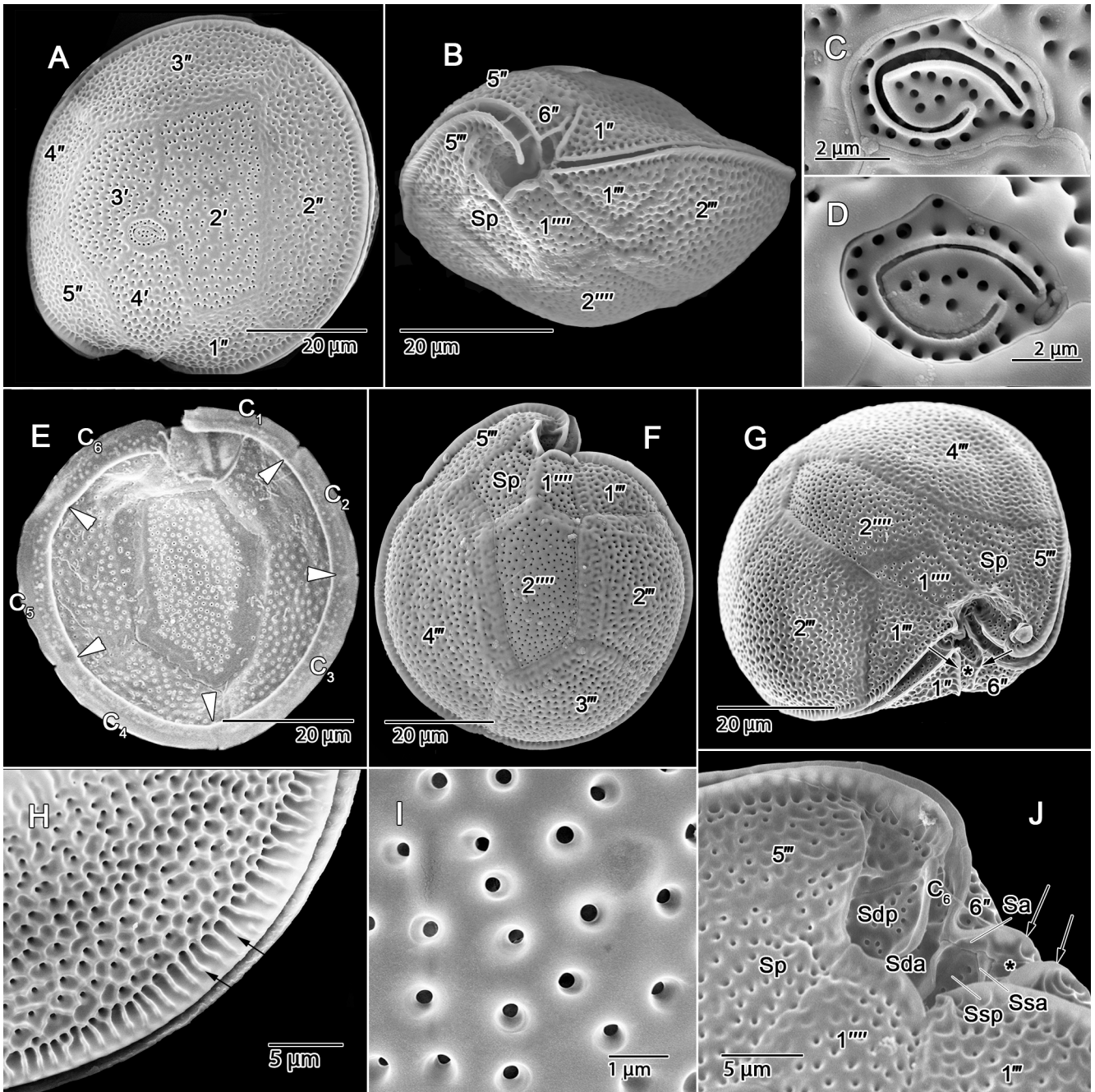
JPY_13326_Figure 3A-G_ G carpenteri.tif



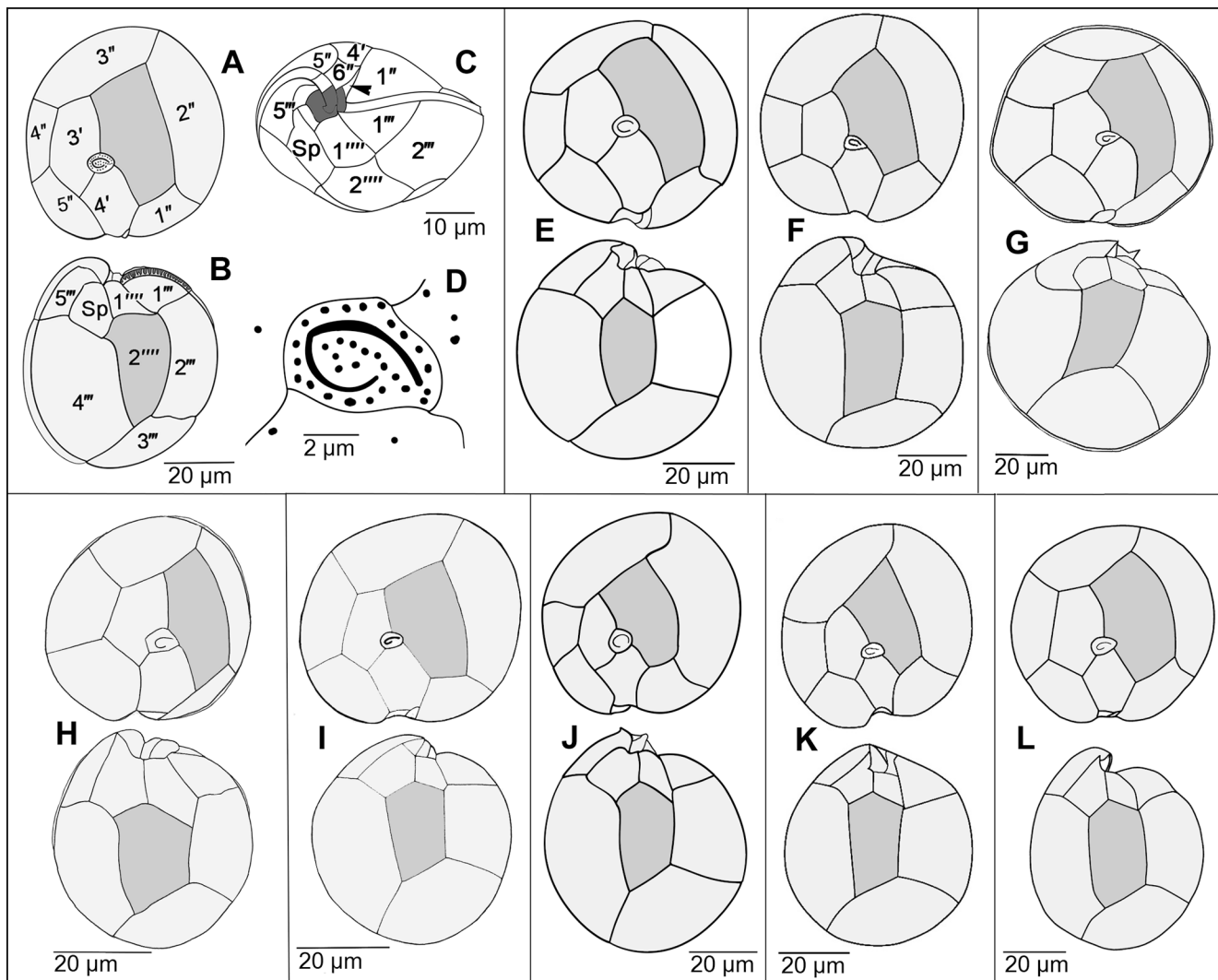
JPY_13326_Figure 4A-I_G pacificus-VNLS015.tif



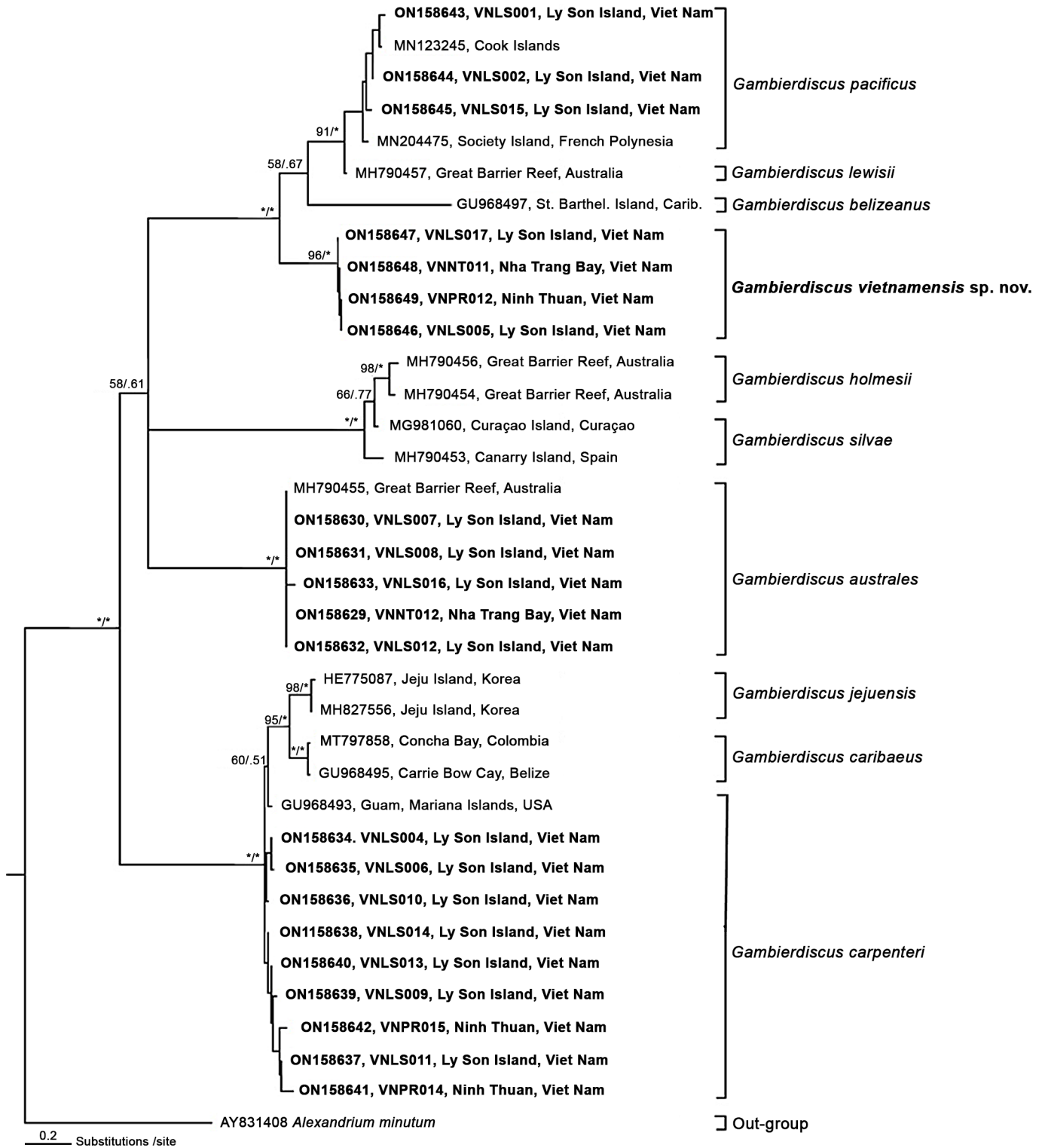
JPY_13326_Figure 5A-I_Gambierdiscus vietnamensis_VNLS005.tif



JPY_13326_Figure 6A-J_ G vietnamensis_VNLS005.tif



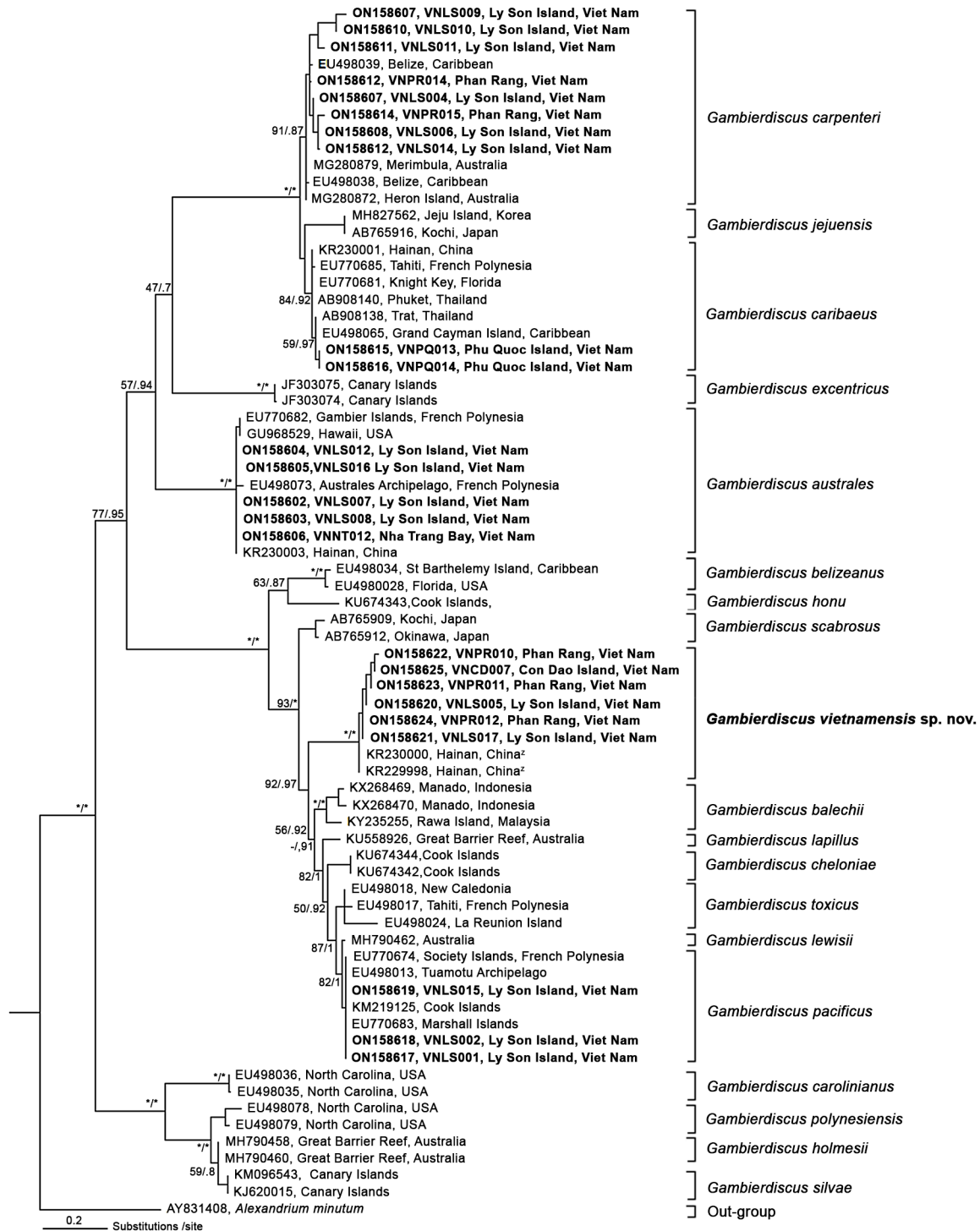
JPY_13326_Figure 7A-L_Line drawing 9 species.tif



JPY_13326_Figure 8 ITS.tif



JPY_13326_Figure 9_D1-D3.tif



JPY_13326_Figure 10_D8-D10.tif