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DINOFLAGELLATE CYSTS IN SURFACE SEDIMENTS AT VAN PHONG BAY, KHANH HOA PROVINCE, VIETNAM: DISTRIBUTION, ABUNDANCE AND POTENTIALLY HARMFUL ALGAL BLOOMS

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ABSTRACT

Studies on dinoflagellate cysts provide important knowledge on the ecology of dinoflagellates and harmful algal blooms (HABs). In this study, distribution and abundance of dinoflagellate cysts in surface sediments from 17 stations at Van Phong Bay were analyzed. There were 55 different types of cysts representing 3 orders and 18 genera, and 8 unidentified cyst types recorded. Peridiniales was the most diverse order with 29 cyst types, including 20 *Protoperidinium* cyst types. There were 10 cyst types of 7 potential toxic dinoflagellate species and 4 of bloom forming species found, indicating a potential risk of harmful algal blooms in Van Phong Bay. Number of cyst types and density ranged from 12 to 31 types and from 115 to 3,760 per gram of dry weight sediment, respectively. Cysts of *Leonella granifera* were dominant at stations in the mouth of the Bay, while *Scrippsiella trochoidea* cysts dominated at all stations. Shannon diversity index (H') was low, varying from 1.19 to 2.72. There were two distinct cyst assemblages identified with 40% Bray-Curtis similarity, assemblage I with 2 stations (VP09 and VP10), and assemblage II with the other stations.

Keywords: Dinoflagellate cyst, *Protoperidinium* cyst, potentially HABs, sediment, Van Phong Bay, Vietnam.

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INTRODUCTION

Van Phong Bay (VPB) is located in the northern part of Khanh Hoa Province, surrounded by districts Van Ninh and Ninh Hoa. It has an area of ca. 150,000 hectares, with 80,000 hectares of surface water. The inner part of the bay has depths less than 20 m, while it is slightly deeper (20–30 m) in the outer part, and the Co Co area is deeper than 34 m. Cua Be channel is connected to the open sea. Surface sediment in Van Phong Bay is dominated by sand, silt and clay, in which silt and clay cover the largest area (Pham Ba Trung et al., 2014). Recently, VPB has been having substantial development in coastal aquaculture, tourism, coastal construction and urban planning by the local government. The negative aspect of this development is the adverse impact on the environment that directly and indirectly cause blooms of phytoplankton that can harm the marine ecosystem and human health (Doan-Nhu et al., 2017).

Dinoflagellates are a major component of the phytoplankton and play an important role in the marine ecosystem. Many marine dinoflagellates include species that cause red tides, shellfish poisoning and other harmful events (Matsuoka et al., 2003). More than 80 species of these are known to have resting cyst stage as a part of their sexual cycle (Matsuoka & Fukuyo, 2000), often in response to harsh conditions (Mertens et al., 2012). Resting cysts can survive unfavorable environmental conditions and, therefore have an important role as a seeding source. Cysts sink to the seafloor in the same manner as fine particles (Dale, 1983) and have a mandatory resting period, after which they re-establish a motile population (Anderson, 1997; Pospelova et al., 2010). Linking these cysts to their respective motile stage is important for both biological and geological studies (Mertens et al., 2012). Cysts, therefore, significantly control the population dynamics of the planktonic stages of many dinoflagellates (Pitcher & Joyce, 2009).

Moreover, knowledge of the distribution and abundance of cysts can be very useful in ecological and monitoring investigations (Qi et al., 1996). Dinoflagellates cysts in marine sediments have been studied in many areas such as Woods Hole (Wall & Dale, 1968), Japan (Matsuoka, 1985, 1987), Australia (Bolch & Hallegraeff, 1990), the North Sea (Nehring, 1994), and the Gulf of Gemlik (Balkis et al., 2016). In Southeast Asia, research on the distribution of dinoflagellates cysts has been conducted in the Gulf of Thailand and East Coast of Peninsular Malaysia by Lirdwitayaprasit (1999). Twenty different types of cysts belonging to Goniolacoid, Tuberculodinioid, and Peridinioid were found. However, cysts of harmful species were not observed. In Manila Bay, dominance of *Pyrodinium* cysts was reported over the other 23 dinoflagellates cyst types (Azanza et al., 2004). In the offshore waters of the Bien Dong, intensive investigation has been done on Sunda Shelf and shelf of centre Vietnam (Kawamura, 2002). From this research, 45 organic-wall dinoflagellate cysts were found in the Bien Dong (Kawamura, 2002) and 35 were found on Sunda shelf (Kawamura, 2004). In Vietnam, knowledge of the composition and distribution of dinoflagellate cysts is limited. The first screening of dinoflagellate cysts was carried out in Cam Ranh Bay, Central Vietnam by Doan Nhu Hai & Nguyen Ngoc Lam (2002), where 25 cysts of different species were recorded and illustrated. Phan Tan Luom et al. (2017) described and illustrated in details 8 different types of cysts belonging to the genus *Protoperidinium* from seven stations in Phu Yen, Khanh Hoa and Ninh Thuan Provinces, South Central Vietnam.

The study of dinoflagellate cysts is important for understanding bloom mechanisms and possible oceanographic conditions associated with harmful algal blooms (HAB). In this paper, we presented the spatial distribution and abundance of dinoflagellate cysts in surface sediment of Van Phong Bay, Khanh Hoa Province, Vietnam.

MATERIALS AND METHODS

Sampling

Surface sediment samples were collected using a Petersen grab in June 2017 from 17 stations in Van Phong Bay, Khanh Hoa (Figure 1 and Table 1). The samples were stored in the dark at 4 °C for later study.

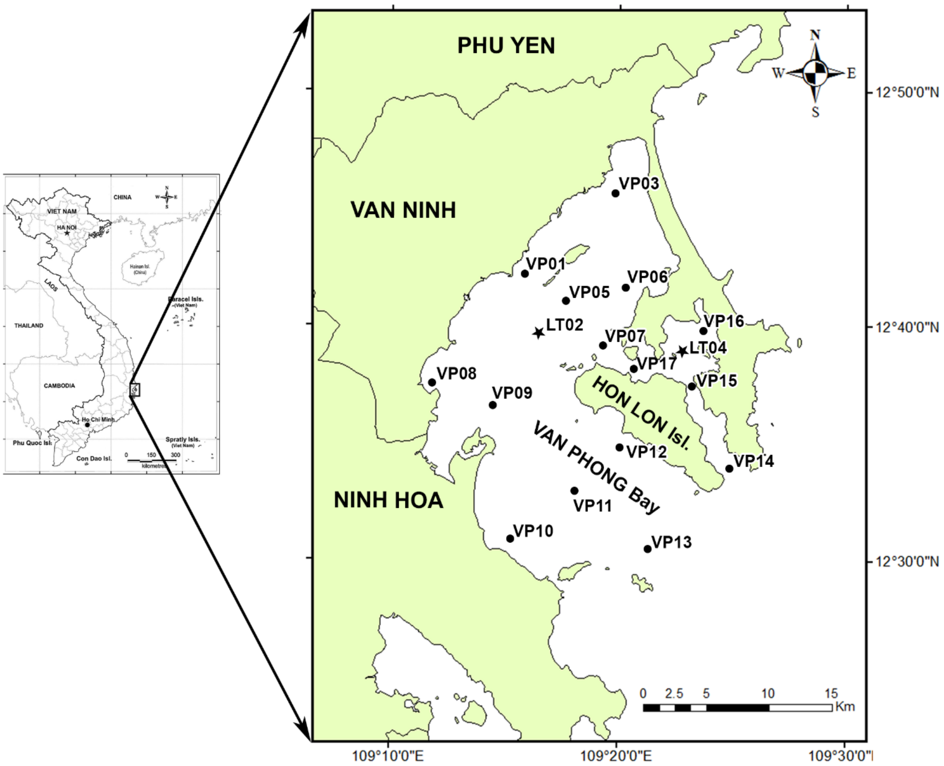

Figure 1. The map shows the 17 sampling stations in Van Phong Bay, Khanh Hoa

Table 1. Coordinates and water depths of sampling stations

Station	Longitude (E)	Latitude (N)	Water depth (m)
VP01	109.2680	12.7026	6.40
LT-02	109.2891	12.7257	7.00
VP03	109.3312	12.7584	7.00
LT-04	109.3266	12.7203	10.0
VP05	109.2969	12.6840	13.0
VP06	109.3396	12.6927	14.0
VP07	109.3231	12.6527	18.3
VP08	109.2010	12.6270	7.50
VP09	109.2449	12.6130	13.5
VP10	109.2570	12.5205	18.3
VP11	109.3027	12.5528	23.0
VP12	109.3357	12.5830	22.7
VP13	109.3545	12.5122	28.0
VP14	109.4135	12.5687	28.0
VP15	109.3878	12.6251	39.0
VP16	109.3950	12.6628	21.3
VP17	109.3454	12.6372	22.8

Analyses of samples

Wet sediment samples were divided into three subsamples with equal weight. One subsample was oven-dried at 70 °C for 24 hours to determine dry weight, two others were sieved for qualitative and quantitative analysis. Sediment processing generally followed the methods of Bolch & Hallegraeff (1990) and Matsuoka & Fukuyo (2000); approximately 4–5 g wet sediment were mixed with 0.2 µm filtered seawater and sonicated for 2 min using an Ultrasonic Processor (Name instrument, Nation) and then successively sieved through 125 and 20 µm metallic sieves (2 to 3 times) to remove coarse and fine materials. The samples retained on the 20 µm sieve were transferred into a plastic tube and suspended in 10 ml distilled water.

One-milliliter aliquots from subsample were counted in Sedgwick-Rafter counting chamber and a Leica LDMB microscope (Germany) at a magnification of 100x. Cyst abundance was calculated as cysts per gram dry weight sediment (cysts g^{-1} DW). Data analyses were performed using MS Excel 2010 and the PRIMER v6 package, in which the Shannon-Wiener index, hierarchical cluster and multidimensional scaling (MDS) were computed. Dinoflagellate cysts were identified based on published descriptions of Wall and Dale (1968), Matsuoka (1987), Bolch & Hallegraeff (1990), and Matsuoka & Fukuyo (2000).

RESULTS

There were 55 different types of dinoflagellate cysts belonging to 3 orders and 18 genera, and 8 unidentified cyst types recorded (Appendix). Peridiniales was the most diverse order with 29 cyst types, including 20 *Protoperidinium* species. Orders Gonyaulacales and Gymnodiniales had 18 and 8 cyst types, respectively (Tab. 2 and Fig. 2). Among 55 cyst types identified, 24 heterotrophic cyst types, which mainly consisted of *Protoperidinium* (20 cyst types), *Polykrikos* sp., *Diplopelta parva*, *Diplosalis* cf. *lebourae*, and *Zygabikodinium lenticulatum*, accounted for 43.64% of total dinoflagellate cyst assemblages recorded at most sampling sites. Meanwhile, there were 31 autotrophic cyst types (56.36%).

Table 2. Structure of the composition of dinoflagellate cysts in the surface sediment of Van Phong Bay

Order	Genus	Cyst types	Percentage (%)
Gonyaulacales	4	18	28.57
Gymnodiniales	5	8	12.70
Peridiniales	9	29	46.03
Unknown	0	8	12.70
Total	18	63	100.00

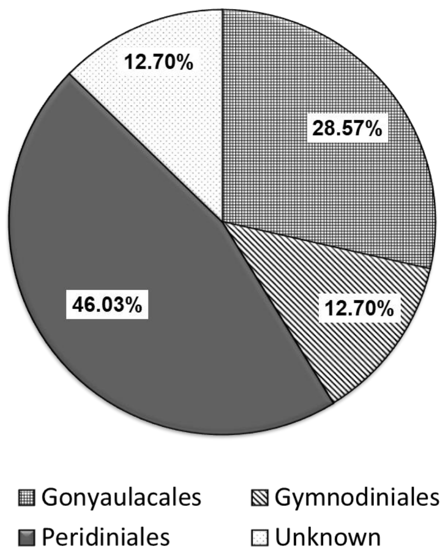

Figure 2. Percentage of different dinoflagellate cyst groups in the surface sediment of Van Phong Bay

The highest cyst abundance (3759 cysts g^{-1} DW) was recorded at station VP14 and the lowest (155 cysts g^{-1} DW) at station VP10 (Appendix and Fig. 3b). The number of cyst types were markedly higher (40–50 cyst types) at some innermost stations of the Bay (VP-01, VP-03, and VP-05), whereas the numbers were quite low (12–31 cyst types) at stations (VP-13 and VP-14) in the mouth of the Bay (Fig. 3a). Three cyst types, being *Leonella granifera*, *Scrippsiella trochoidea*, and unidentified brown dark round cysts, were commonly found at most stations (Fig. 4).

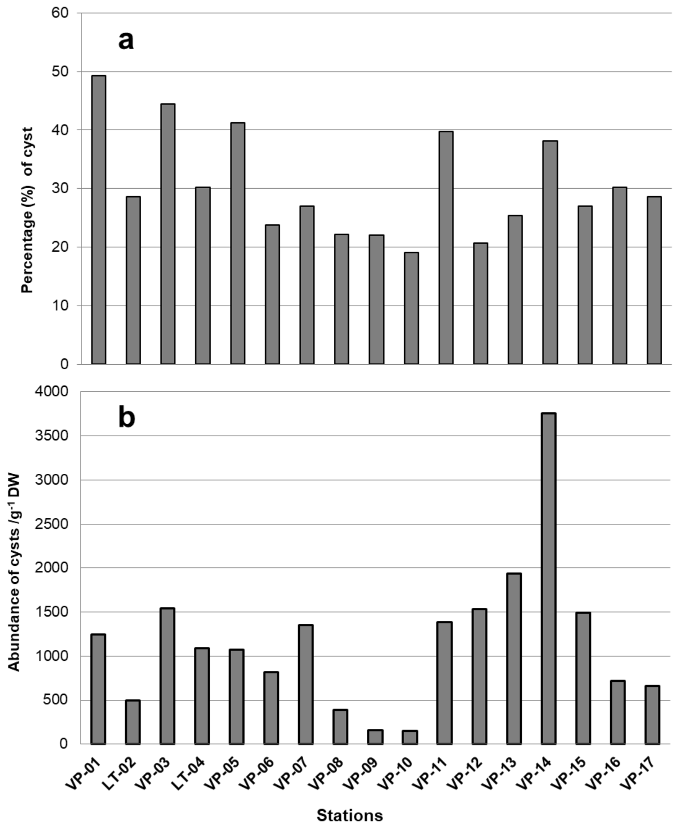

Figure 3a-b. Percentage of cysts composition (a) and abundance of cysts (g $^{-1}$ DW), (b) at each station in Van Phong Bay

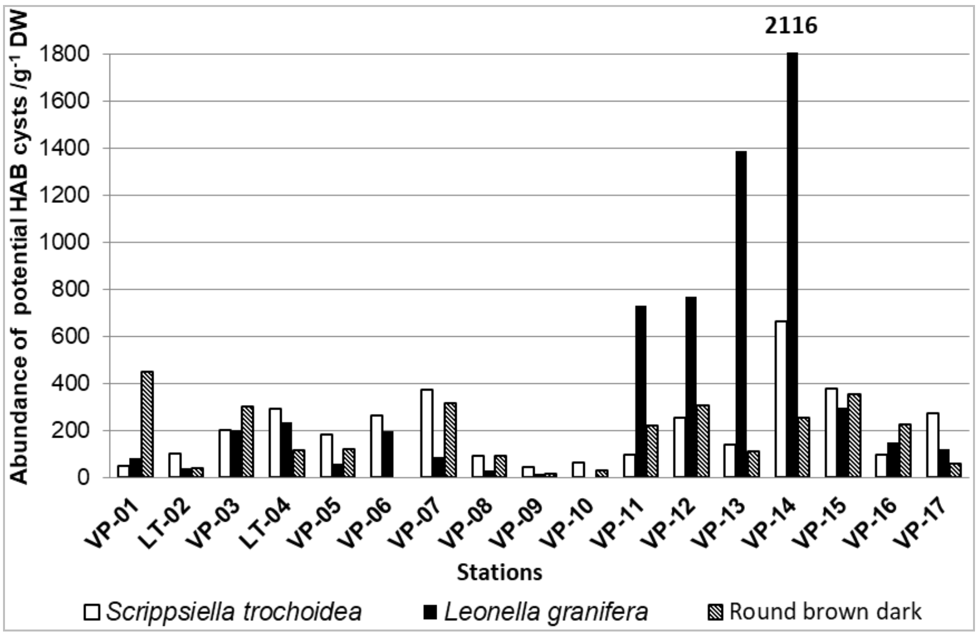

Figure 4. The abundance of potential bloom forming cysts at each station in Van Phong Bay

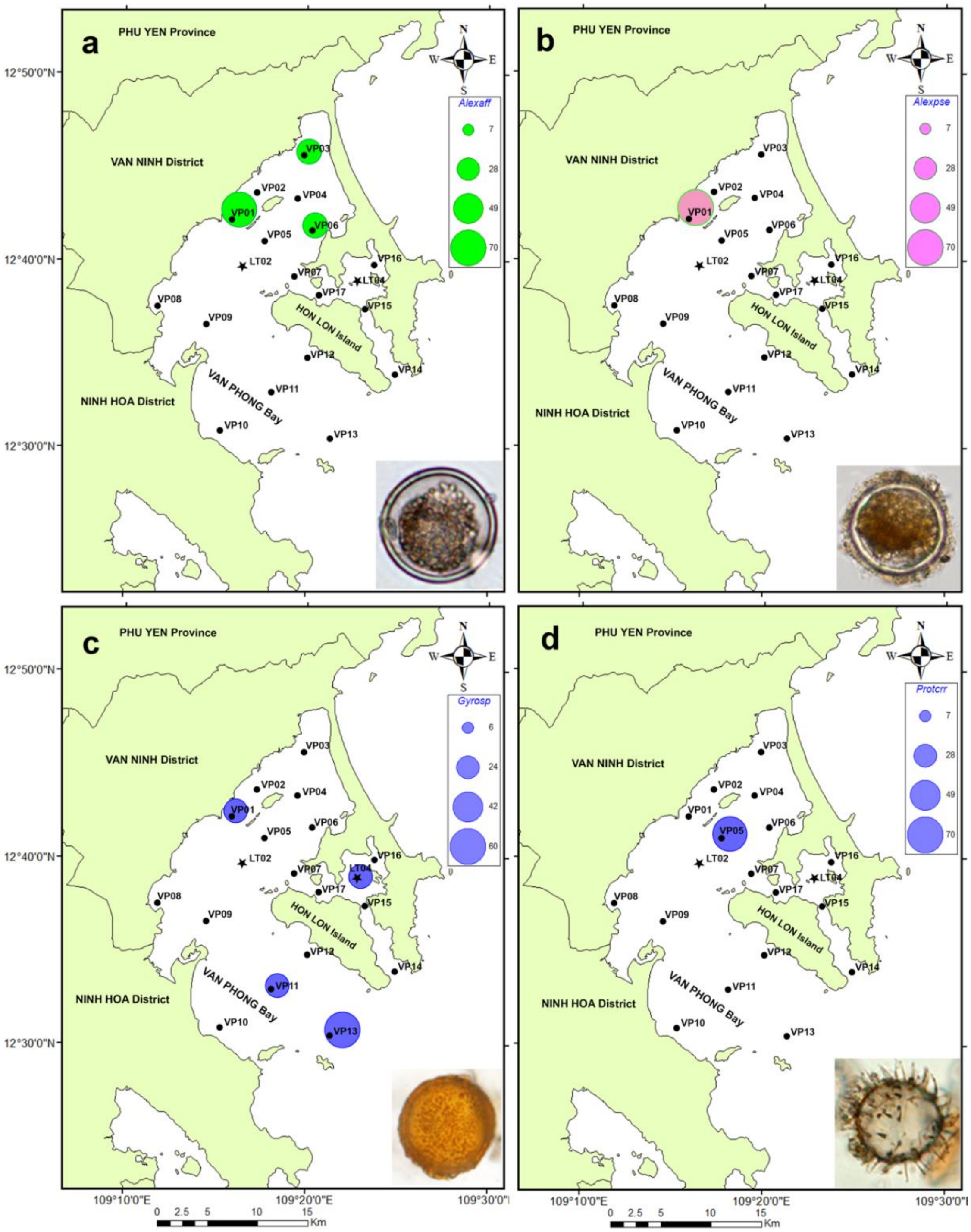

Figure 5a–5d. Distribution of potential HAB cysts recorded. (a) *Alexandrium affine*, (b) *A. pseudogonyaulax*, (c) *Gymnodinium* sp., (d) *Protoceratium reticulatum*

Among 55 identified cyst types, 10 cyst types of 7 potential HAB species were recorded in the study area, indicating a potential risk for outbreaks of harmful events associated with these in the future. *Lingulodinium polyedrum*, *Protoceratium reticulatum*, and *Gonyaulax spinifera* complex, are known to produce yessotoxin (YTXs). In addition, four potential forms recognized as paralytic shellfish poisoning (PSP) producer, comprising *Alexandrium affine*, *A. catenella/tamarense*, *A. pseudogonyaulax*, and *Gymnodinium catenatum*, were recorded at low numbers in the most stations (Fig. 5a–5d). Common bloom-forming species including *Scrippsiella trochoidea*, *Leonella granifera*, and *brown dark round* cysts were observed at high concentration (Fig. 4).

A cluster analysis of all stations revealed two different assemblages with 40% Bray-Curtis similarity within the Bay (Fig. 6a-b). Assemblage I included 2 stations (VP09 and VP10) and was separated from the main group (assemblage II) due to its low abundance and species richness. Cysts of *Scrippsiella trochoidea* dominated at these two stations. Shannon diversity index (H') was low in the study area, varying from 1.19 to 2.72.

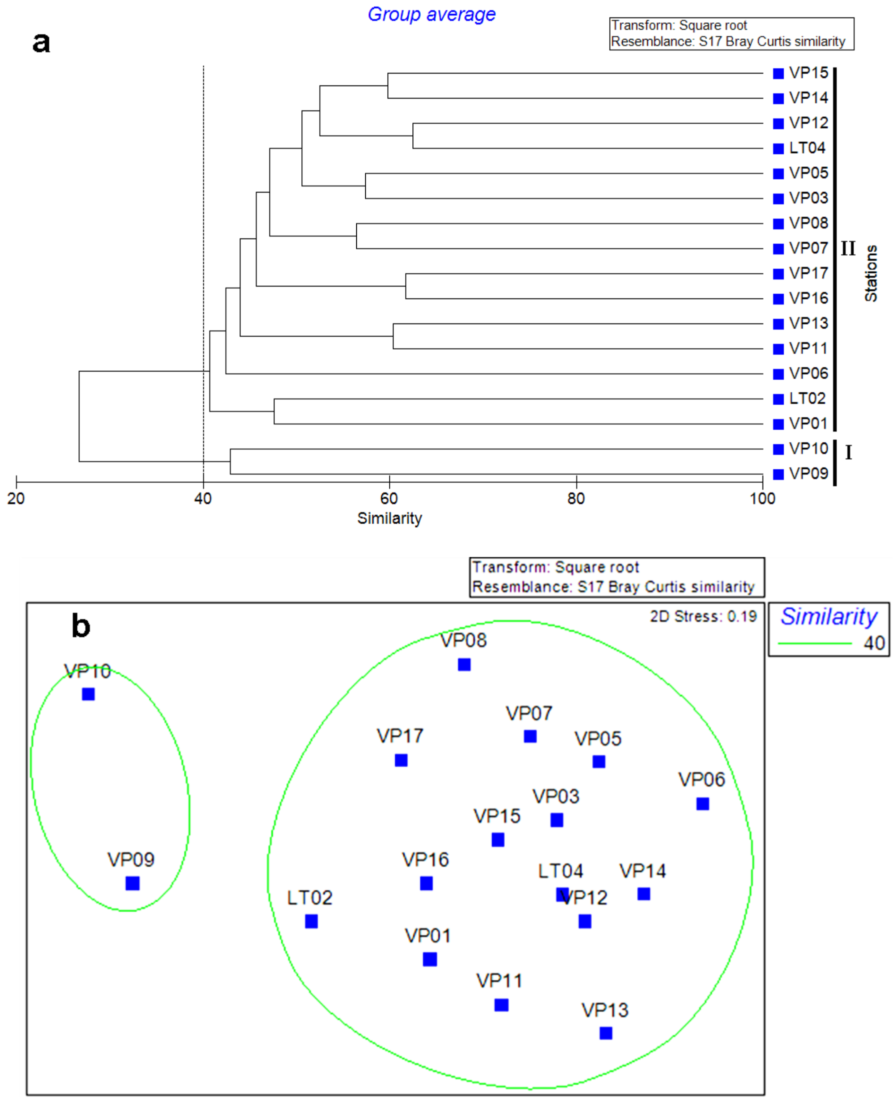

Figure 6a–6b. Similarity analysis for dinoflagellate cysts from 17 stations in Van Phong Bay. (a) hierarchical cluster, (b) MDS

DISCUSSION

This is the first study providing knowledge of the distribution and abundance of dinoflagellate cysts in Van Phong Bay, Vietnam. Sixty-three different cyst morphotypes were found in the studied area. Among them, there were 31 types of autotrophic and 24 types of heterotrophic species. The number of cyst types found in Van Phong Bay was higher than in Cam Ranh Bay: 25 cyst types (Doan Nhu Hai & Nguyen Ngoc Lam, 2002) and in the Gulf of Thailand and East Coast of Peninsular Malaysia: 20 cyst types (Lirdwitayaprasit, 1999). However, cysts of harmful species have not been observed in either of those studies.

Among the previous studies on dinoflagellate cysts on coastal waters, Van Phong Bay is the most diverse on cyst types (63) compared to the Gulf of Thailand: 18-30 (Lirdwitayaprasit, 1998; Srivilai et al., 2012), Malaysian coast: 11–25 (Furio et al., 2006), coastal waters of Indonesia: 5–35 (Matsuoka et al., 1999; Mizushima, 2007), and the Philippines; 23 (Azanza et al., 2004).

Cysts of *Protoperidinium* genus were the most diverse group with 20 cyst types recorded. The cysts of this genus were common and widespread, and they have also been found in other regions such as Woods Hole (Wall & Dale, 1968), Japan (Matsuoka, 1985, 1987; Matsuoka et al., 2003), Australia (Bolch & Hallegraeff, 1990), the Baltic Sea (Nehring, 1994), Gulf of Gemlik (Balkis et al., 2016), and China (Wang et al., 2003).

Leonella granifera cysts were found to be dominant at the mouth of Van Phong Bay. Only few species within this group were well studied, revealing their complex life cycle and distribution. An alternative life-cycle with the production of haploid vegetative calcareous cells as the dominant life-cycle stage was reported for the oceanic calcareous dinoflagellate such as and *Leonella granifera* (Meier et al., 2007) and *Thoracosphaera heimii* (Tangen et al., 1982). Van Phong Bay is open to a narrow shelf sea with just some 24 nautical miles to the shelf slope which explained the more abundance of *L. granifera* cysts at the mouth of the Bay. In this study we also found other calcareous cyst, *Calciodinellum* sp., with low numbers at few stations in the Bay. It was reported that a species of this genus, *Calciodinellum levantinum*, is a neritic species, forming calcareous cysts during the diploid stage (Meier et al., 2007).

In this study area, the cysts of some potentially toxic species, such as *Gymnodinium catenatum* cyst (PSP), were observed only at one station with low abundance. *Gymnodinium catenatum* is also a common bloom-forming species documented (Doblin et al., 1999; Band-Schmidt et al., 2010; Li et al., 2019), its bloom was reported in southern Tasmania (Anderson & Morel, 1979; Hallegraeff, 1995).

The cyst of *Diplopsalis* sp., *Leonella granifera*, *Scrippsiella trochoidea*, and brown dark round cysts were observed in most samples. Although *Scrippsiella trochoidea* is not a toxin-producing species, it is also harmful to the marine ecosystem and mariculture due to high oxygen consumption during the bloom. *Scrippsiella trochoidea* bloom was recorded in Daya Bay, China (Wang et al., 2003), the number of this species blooms has increased 15 times since 1998 in this bay (Li et al., 2019).

Although cyst abundance of potentially toxic dinoflagellate species was not high, toxin-production was an important issue that harmed the marine aquaculture industry. In particular in mariculture area, besides monitoring vegetative cells in upper water body, it was essential to include investigation on dinoflagellate cysts in the sediment. In addition, high density of potentially HABs cysts could be considered a potential ‘seedbed’ for production of motile cells that can initiate subsequent blooms. These data also suggested that additional cyst surveys should be conducted in Vietnamese waters and that the potential for blooms of toxic species might be widespread. Further taxonomic and biogeographic studies are needed to better understand the ecological dynamics of oceanic plankton in the Vietnamese waters.

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Appendix

Ord.	Cyst types	Stations																
		VP01	LT02	VP03	LT04	VP05	VP06	VP07	VP08	VP09	VP10	VP11	VP12	VP13	VP14	VP15	VP16	VP17
Order Gonyaulacales		8	2	6	6	9	4	5	1	3	3	6	2	2	6	2	6	6
1	*Alexandrium affine*	+		+		+	+											
2	*Alexandrium catenella/tamarense*									+								
3	*Alexandrium pseudogonyaulax*	+		+		+	+	+				+						+
4	*Alexandrium* sp.	+				+				+								+
5	*Gonyaulax scriipsae*	+			+	+	+				+	+			+		+	+
6	*Gonyaulax* sp.1											+			+	+	+	+
7	*Gonyaulax* sp.2	+															+	
8	*Gonyaulax* sp.3	+		+	+	+						+						+
9	*Gonyaulax* sp.4							+			+							
10	*Gonyaulax* sp.5			+	+		+	+				+	+	+	+		+	
11	*Gonyaulax* sp.6			+		+									+	+		
12	*Gonyaulax spinifera*				+	+					+	+						
13	*Gonyaulax spinifera*					+									+		+	
14	*Gonyaulax spinifera*				+					+								
15	*Gonyaulax spinifera*	+	+	+				+	+				+		+			
16	*Lingulodium polyedrum*	+			+			+						+			+	+
17	*Protoceratium reticulatum*					+												
18	*Protoceratium* sp.		+															
Percentages of Gonyaulacoid		12.70	3.17	9.52	9.52	14.29	6.35	7.94	1.59	4.76	4.76	9.52	3.17	3.17	9.52	3.17	9.52	9.52
Order Gymnodiniales		3	2	4	2	2	1	1	0	0	1	4	1	2	1	0	3	1
19	*Cochlodinium* sp.1	+										+		+				
20	*Cochlodinium* sp.2		+	+	+	+							+					
21	*Gyrodinium catenatum*																+	
22	*Gyrodinium* sp.	+		+	+							+		+				
23	*Pheopolykrikos hartmannii*			+		+						+			+		+	+
24	*Pheopolykrikos* sp.											+						
25	*Polykrikos* sp.	+	+	+			+	+									+	
26	*Pyrophacus steinii*										+							
Percentages of Gymnodiniales		4.76	3.17	6.35	3.17	3.17	1.59	1.59	0.00	0.00	1.59	6.35	1.59	3.17	1.59	0.00	4.76	1.59
Order Peridiniales		16	10	14	9	13	8	10	10	9	5	11	7	10	13	11	9	9
27	*Calciodinellum* sp.	+	+			+		+		+		+		+	+			
28	*Diplopelta parva*	+		+		+	+	+	+						+	+		
29	*Diplosalis* cf. *lebourae*	+	+		+	+				+		+				+		+

30	*Leonella granifera*	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+
31	*Oblea acanthocysta*							+	+								+	
32	*Preperidinium* cf. *meunieri*	+		+														
33	*Preperidinium* sp.			+														
34	*Protoperidinium avellana*							+						+	+		+	+
35	*P. conicoides*		+		+									+	+			
36	*P.conicum*			+					+				+					
37	*P. denticulatum*	+		+	3	+	+	+	+		+	+	+	+	+	+		+
38	*Protoperidinium* sp.1			+			+		+		+				+	+		
39	*Protoperidinium* sp.2	+	+	+	+	+				+	+	+			+	+	+	
40	*Protoperidinium* sp.3	+	+													+		
41	*Protoperidinium* sp.4	+	+	+	+	+		+	+	+			+		+		+	+
42	*Protoperidinium* sp.5									+								
43	*Protoperidinium* sp.6	+	+	+	+	+	+			+		+	+		+			+
44	*Protoperidinium* sp.7	+												+		+	+	
45	*Protoperidinium* sp.8																+	
46	*Protoperidinium* sp.9				+	+						+						
47	*Protoperidinium* sp.10			+														
48	*Protoperidinium* sp.11	+				+	+		+						+			
49	*Protoperidinium* sp.12	+						+				+		+				
50	*Protoperidinium* sp.13	+		+		+		+	+					+	+	+		+
51	*P. stellatum*																	
52	*P. subinerma*											+						
53	*P. thulensense*													+				
54	*Scrippsiella trochoidea*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
55	*Zygabikodinium lenticulatum*	+	+	+		+	+			+	+	+	+			+	+	+
Percentages of Peridiniales		25.40	15.87	22.22	14.29	20.63	12.70	15.87	15.87	14.29	7.94	17.46	11.11	15.87	20.63	17.46	14.29	14.29
Unidentified		4	4	4	2	2	2	1	3	2	3	4	3	2	4	4	1	2
56	Unidentified-1			+			+								+			
57	Unidentified-2			+										+				
58	Unidentified-3	+	+	+											+			
59	Unidentified-4	+								+		+	+		+	+		
60	Unidentified-5											+	+					
61	Unidentified-6		+			+			+		+	+				+		+
62	Unidentified-7	+	+		+				+		+					+		
63	Round brown dark	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Percentages of unidentified		6.35	6.35	6.35	3.17	3.17	3.17	1.59	4.76	3.17	4.76	6.35	4.76	3.17	6.35	6.35	1.59	3.17

Number of cysts/ stations	31	18	28	19	26	15	17	14	14	12	25	13	16	24	17	19	18
Percentages of cysts/ stations	49.21	28.57	44.44	30.16	41.27	23.81	26.98	22.22	22.22	19.05	39.68	20.63	25.40	38.10	26.98	30.16	28.57
Abundance cysts/ g^{-1} DW	1249.58	497.96	1545.08	1086.57	1069.94	817.96	1351.51	391.70	159.26	155.18	1388.69	1533.35	1938.52	3758.69	1491.00	719.75	661.89
Number of cyst Autotrophyic	11	5	9	7	10	5	6	4	4	1	9	4	6	9	4	6	6
Number of cyst Heterotrophyic	13	9	11	7	10	8	7	9	7	8	12	8	10	10	11	8	8
Abundance of cyst Autotrophyic	416.5	219.1	738.9	704.8	519.7	556.2	603.9	156.7	86.9	62.1	1,023.2	1,073.3	1,661.6	3,032.2	731.9	372.3	511.5
Abundance of cyst Heterotrophyic	316.56	199.18	436.65	234.93	427.98	229.03	431.33	141.01	43.43	31.04	146.18	102.22	138.47	379.03	379.53	124.09	90.26
Abundance of cyst Undificated	516	80	369	147	122	33	316	94	29	62	219	358	138	347	380	223	60
Percentege abundance of Autotrophic	33.33	44.00	47.83	64.86	48.57	68.00	44.68	40.00	54.55	40.00	73.68	70.00	85.71	80.67	49.09	51.72	77.27
Percentege abundance of Heterotrophic	25.33	40.00	28.26	21.62	40.00	28.00	31.91	36.00	27.27	20.00	10.53	6.67	7.14	10.08	25.45	17.24	13.64
Percentege abundance of Undificared	41.33	16.00	23.91	13.51	11.43	4.00	23.40	24.00	18.18	40.00	15.79	23.33	7.14	9.24	25.45	31.03	9.09