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RESEARCH PAPER

The First Recorded Bloom of Pyrodinium Bahamense Var Bahamense Plate in Yemeni Coastal Waters off Red Sea, near Al Hodeida City

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Abstract

As a part of an ongoing monitoring study of phytoplankton in Yemeni coastal waters, phytoplankton samples were collected from November 2012 to September 2013 at four sampling locations along the coast of Hodeida City, southern Red Sea. A bloom of toxic dinoflagellate *Pyrodinium bahamense* var. *bahamense* Plate was observed in August 2013. The population density of *P. bahamense* were ranged from 1.6×10^4 to 3.3×10^5 cell L^{-1} (accounting for 41.8 % of the overall phytoplankton community). This finding is the first observation of vegetative cells of this tropical species from the Red Sea. Water temperatures during the bloom was 32°C and salinity was 37 psu, indicating its tropical and subtropical nature. Among the phytoplankton species reported during this study, the red tide-forming species; *Trichodesmium erythraeum* (14.1%), *Protoperidinium quinquecorne* (6.3 %) and the known toxic species, *Dinophysis caudata* (1.4 %), and *D. acuminata* (1.00 %) were remarkable. It is apparent from our results that the toxic species do occur during many months of the year in the Coastal Waters of Yemen with high abundance observed in August followed by April 2013. Further studies are required to carry out the HAB monitoring for preventing the risk of HAB events that may outbreak in future at the Red Sea coast.

Keywords: *Pyrodinium bahamense***,** HAB, Red Sea, phytoplankton

Introduction

In recent years, Harmful algal blooms (HABs) have been of growing concern in the coastal waters of many parts of the world. They are increasingly becoming a global problem for fisheries, aquatic environment and the human health (Anderson, 1997). These bloom events can cause water discoloration and impact commercial and recreational interests in the affected region (Hallegraeft, 1993). Dinoflagellates and other phytoplankton are considered as food sources for fishery resources. When associated with harmful events, species identification of these organisms is critically important. The list of dinoflagellates causing harmful blooms and those capable of producing toxic compounds are incomplete and continues to grow (Steidinger and Tangen, 1996).

Pyrodinium bahamense was originally described from the Atlantic, specifically New Providence Island (Bahamas) by Plate (1906). Thereafter, Bohm (1931) observed *P. bahamense* samples from the Arabian Gulf and noted slight morphological differences such as shorter apical horn and antapical spine, and anterioposteriolly more compressed body and gave a new taxonomic status as forma, and named *P. bahamense* forma *compressa*. Later, Matzenauer (1933) described independently *Gonyaulax schilleri* Matzenauer from the Red Sea that is similar to the present *P. bahamese* var. *compressum*, its body was wider than longer, and observed four apical plates in the epitheca of this dinoflagellate. However, Schiller (1937) realized that *G. schilleri* is a junior synonym to *P. bahamese* f. *compressa*. Steidinger *et al*. (1980) recognized two varieties from this species (*P. bahamense*var. *Bahamense* Plate and *P. bahamense* var. *compressum* (Böhm) Steidinger, Tester & Taylor). Wall and Dale (1969) indicated that *P. bahamense* is generally confined to subtropical and tropical waters, occurs throughout the Indo-Pacific and the tropical Atlantic.

Pyrodinium bahamense is a very important member of paralytic shellfish toxin (PST)-producing marine dinoflagellates especially in tropical waters (Mertens *et al*., 2015). This species have caused more human illnesses and fatalities than any other PST producing dinoflagellates. The first confirmed toxic bloom of *P. bahamense* occurred in Papua New Guinea in 1972 (Maclean, 1989). Subsequently first incidences of toxic blooms of the species occurred in Brunei and Sabah, Malaysia in 1976, Manila Bay, the

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Philippines in 1983, Mindanao, the Philippines in 1983, Ambon, Indonesia in 1994, Palawan Island, the Philippines in 1998 and the Pacific coast of Guatemala in 1987 (Rosales-Loessener, 1989; Wiadnyana *et al*., 1996; Sombrito *et al*., 2004; Azanza *et al*., 2010; Usup *et al*., 2012).

The Red Sea is a narrow, linear oceanic basin separating northern Africa from the Arabian subcontinent, extending to about 30° N in the Gulf of Suez and the Gulf of Aqaba. The Red Sea terminates to the south at the straits of Bab al Mandeb at approximately 12.5° N. The Red Sea has the highest salinity (40–41‰ in the northern Red Sea, 36.5‰ in the far southern Red Sea) of any major oceanic basin, particularly tropical oceanic basins. Nutrient concentrations generally increase from north to south (Weikert, 1987), though Red Sea waters in general are considered highly deficient in major nutrients (nitrate, ammonium, phosphate, and silicate). The most nutrient-poor waters are in the northern Red Sea, while the southern Red Sea receives some nutrients from intermediate water inflow through the Gulf of Aden in the summer (Acker *et al*., 2008). Limited scientific literature exists describing the HABs in the Red Sea. Prior to date, only few harmful algal blooms had been documented in the Red Sea. Among them bloom of *Noctiluca scintillans* (Macartney) Kofoid & Swezy (Mohamed and Messad, 2007) and *Gonyaulax* sp. (Mohamed and Al-Shehri,2012) had been documented in the Red Sea off the southern coasts of Saudi Arabia – those events took place in 2004. Other bloom for *N. scintillans* was reported in 2009 from the coasts of Yemen by Alkershi and Menon (2011). In May 2010, a bloom of *Heterosigma akashiwo* (*Hada*) *Hada ex Hara & Chihara* was observed for the first time off Al Shouqyq region, making it the fourth HAB documented in Res Sea waters (Mohamed and Al-Shehri 2012). Thus, the aim of this study is to document the presence of *Pyrodinium bahamense* var. *bahamense*, to describe its bloom in Yemeni coastal waters of the Red Sea and provides a morphological description for this species. We also aimed to document other microalgae community present during this bloom and from this sparsely investigated region.

Material and Methods

Study Area and Sampling

The study was conducted at four sampling locations in the Yemeni coastal water of the Red Sea during the period of November 2012 to August 2013. Three stations located at the coast of Al Hodeida City were selected; station 1 (14° 47′ 07" N, 42° 56′ 46.31" E), station 2 (14° 49′ 09.11″ N, 42° 55′ 13.48" E) and station 3 (14° 51′ 15.18" N,42° 53′ 06.71" E) but the fourth station (14° 48′ 47.36" N, 42° 56′ 03.21" E), located inside Khor Al-Khateeb Lagoon (Figure 1). The lagoons are considered as potential habitats for the proliferation of epibenthic toxic species due to the absence of water movement and the stagnant environments. The sites depths were 1–3 m at different sampling locations and the sediment is sandy at all locations.

Phytoplankton Assemblage

For quantitative and qualitative analyses of phytoplankton, cell counts and composition, water samples from each of the above stations were collected monthly during the low tide-times in 2L capacity plastic containers and preserved using Lugol’s iodine solution and stored in the dark until analyzed. A settling and siphoning procedure was followed for counting phytoplankton cells and identification of genera and species, where possible. According this procedure, the samples were kept for 72 hours to allow complete settlement of phytoplankton cells. The clear surface layer was siphoned out, and the remaining sample was transferred into a measuring cylinder of 100 ml capacity and kept for another 72 hours to allow complete settlement of the microalgae. The clear surface layer was siphoned out, so as to concentrate the sample to 20 ml. By using Sedgwick-Rafter plankton counting chamber, phytoplankton cells were identified and counted at X200, X400 and X1000 with light microscope (Novex B-Range, Holland). The digital images were taken using Sony digital camera (DSC-W810 SONY CORP) connected to the microscope. Cells were enumerated and expressed as cells L^{-1}. The phytoplanktons were identified to their generic/specific level based on the available literature (Taylor, 1976; Dodge, 1982; Desikachary and Prema, 1987; Desikachary *et al*., 1987; Tomas, 1997.; Faust and Gulledge, 2002; Horner, 2002; Al-Kandari *et al*., 2009; Al-Yamani and Saburova, 2010; Al-Yamani and Saburova, 2011).Current name of algae species are controlled from algaebase.org website (Guiry and Guiry, 2016).

Water temperature and salinity were measured at each station during sample collection using a calibrated mercury thermometer and a standardized Portable Salinity Refractometer (Extech RF20) respectively.

Result

Pyrodinium bahamense was, for the first time, observed in survey samples in August 2013 in the Yemeni coastal waters of the Red Sea. Specimens of *P. bahamense* found near Yemeni coastal waters were only single (Fig. 2A). Cell shape was rounded with epitheca and hypotheca about equal in size and has more pronounced apical and antapical horns (Figure 2C). Cells range from 30 to 46 µm in length and 32–54 µm in trans diameter. An associated spine with the apical horn, and antapical spine were observed (Figure 2D). In general, all cells had morphological

Figure 1. Sampling stations from the Yemeni coastal waters off Red Sea, near Al Hodeida City.

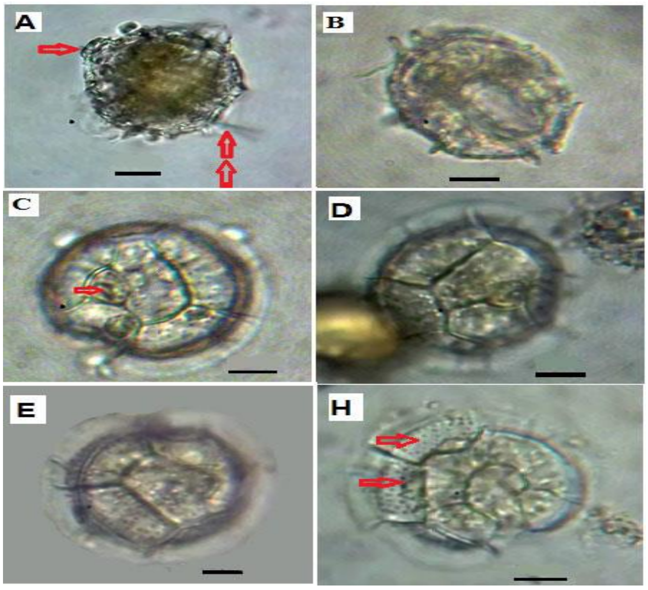

Figure 2. Light microscopy images of *Pyrodinium bahamense* var *bahamense* from the coastal waters of Al Hodieda City, Southern Red Sea, August 2013. (A) Single cell showing apical spine (arrow) and antapical spines (double-arrow) (B) Rounded cell with epitheca and hypotheca about equal in size; (C and D) apical view showing hypotheca arrangement and the apical attachment pore (arrow) (E) antapical view showing epitheca arrangement; (H) plates with pores aligned (arrow). (Scale bars: 10 µm for all images).

features corresponding to *P. bahamense* var. *bahamense*.

Water temperature recorded during the bloom of *P. bolmonense* var. *bahamense* in the study area was 32°C along the coast at all stations. The salinity during the bloom of this dinoflagellate species was 37 psu.

General Phytoplankton Composition

The population density of *Pyrodinium bahamense* var. *bahamense* at the surface water of the study area ranged from 1.6×10^4 to 3.3×10^5 cell L^{-1}, making a relative contribution to the total phytoplankton abundance of 41.8 %. This slightly harmful algal bloom phenomenon was verified only in two quantitative phytoplankton samples collected in late August 2013 at stations 2 and 3 (Figure 1). Station 2 had the highest cellular density 3.3×10^5 cell L^{-1}.

Along the coast of study during other sampling period, *P. bahamense* var. *bahamense* was absent, but other toxic and potentially toxic species were present in these monthly samples. Among them, *Dinophysis caudata* (1.4 %), and *D. acuminata* (1.0 %) were abundant specially during spring and summer months.

On the other hand, the red tide-forming species; *Trichodesmium erythraeum* (cyanobacteria) followed by the dinoflagellate species *Protoperidinium quinquecorne*, with relative contribution to the total phytoplankton abundance of 14.1 % and 6.3 % respectively, were the most abundant species among the four coastal sampling locations. *Prorocentrum micans*, and *Scrippsiella acuminata* were also abundant among dinoflagellates in the study area (Table 1). With the exception of *Lithodesmioides polymorphum*, *Achnanthes brevipes*, *Trieres chinensis* and *Rhizosolenia* spp. the diatom species were not dominant in Yemeni coastal waters off Red Sea (Table 1).

When *P.bahamense* var. *bahamense* was present and formed bloom in August 2013 in the study area, the abundance of other phytoplankton species were very low. The assemblage was clearly dominated by *P. bahamense* var. *bahamense* which reached up to 90% of the total microphytoplankton abundance. In general, the main dinoflagellates abundance and frequency consisted of *Protoperidinium quinquecorne*, *Prorocntrum micans* and *Protopredinium steinii*; the diatoms composition was mainly composed of *Lithodesmioides polymorphum*, *Cyclotella* sp, *Cocconeis* sp and *Diploneis* sp. Furthermore the cyanobacteria species, *Trichodesmium erythraeum* was also associated with *P. bahamense* bloom in the study area but in few number.

Discussion

This is the first report of a HAB of *Pyrodinium bahamense* var. *bahamense* from Yemeni coastal waters of the Red Sea. *P. bahamense* is a tropical-subtropical species, first described by Plate (1906) in samples from the Atlantic Ocean. Since its discovery, *P. bahamense* has been collected in the Indo-Pacific region and more tropical Atlantic waters (Rosales-Loessener *et al*. 1989, Badylak *et al*. 2004). Only two varieties of this species (*P. bahamense* var. *bahamense* and *P. bahamense* var. *compressum* have been recognized since 1980 (Steidinger *et al*., 1980; Badylak *et al*., 2004). The genetic differences between varieties have not been resolved and are still a point of contention (Balech, 1985). Vargas-Montero and Freer (2003) suggested that the morphological differences could be a response to environmental conditions and not genetically based. The morphological features and cell size of *Pyrodinium bahamense* from Yemeni coastal waters of the Red Sea are closely related to *P. bahamense* var. *bahamense*, as described by Steidinger *et al*. (1980), Steidinger and Tangen (1996), Badylak *et al*. (2004), Faust *et al*. (2005), Martınez-Lopez *et al*. (2007), Morquecho (2008) and Garate-Lizarraga and Gonzalez-Armas (2011). As these authors had documented, the specimens were less compressed antero-posteriorly than *P. bahamense* var. *compressum* and exhibit a prominent apical horn with a spine and prominent antapical spines with wide bases, as was the case in the samples of our study. Furthermore *P. bahamense* var *bahamense* observed in our study showed slight morphological differences with *P. bahamense* var *compressum* previously reported from the Red Sea. Cells of variety *compressum* of the Red Sea were wider than longer with more compressed body, and it had only ‘‘antapical spine. It was accepted that *P. bahamense* var *bahamense* was limited to the Atlantic (Steideinger *et al*. 1980). Later, many studies showed that *P. bahamense* var. *bahamense* occurred in the Gulf of Mexico and Mexican Caribbean (Cortes-Altamirano *et al*., 1996; Gomez-Aguirre and Licea, 1998; Herrera-Silveira, 1999). The first report of *P. bahamense* var. *bahamense* in the Pacific came from Osorio-Tafall (1942) off the Mexican coast. His

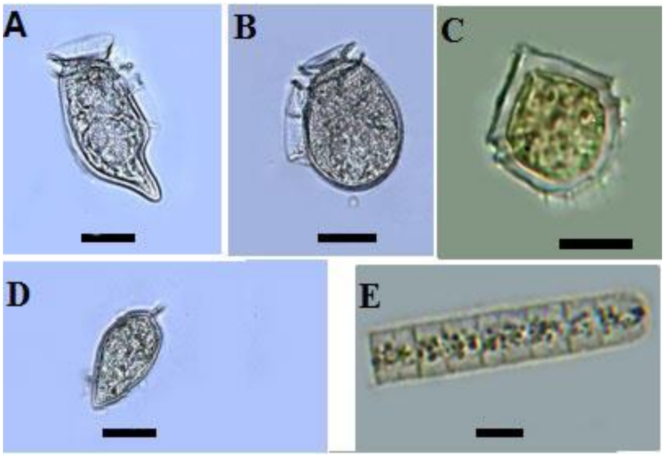

Figure 3. Light microscopy images of some harmful phytoplankton: (A) *Dinophysis caudata*; (B) *D. acuminata*; (C) *Protoperidinium quinquecorne;* (D) *Prorocentrum micans* and (E) *Trichodesmium erythraeum* from the coastal waters off Hodeida, Yemen, Southern Red Sea. (Scale Bars: A, B & D =20 μm; C = 10 μm; E=5 μm).

Table 1. List of phytoplankton species, with their cell counts L^{-1} recorded from the surface waters at different sampling locations off Yemen, Southern Red Sea

Taxon	Sampling locations			
	St1	St2	St3	St4
DIATOMS				
Achnanthes brevipes Agardh	4140	450	ND	ND
Amphora sp	20	100	ND	ND
Biddulphia bidulphiana (Smith) Boyer	ND	130	ND	ND
Campylodiscus sp	10	ND	10	ND
Ceratoneis closterium Ehrenberg	60	300	ND	ND
Chaetoceros curvisetus Cleve	ND	ND	ND	30
Chaetoceros sp	230	ND	ND	ND
Cocconeis sp	130	250	ND	20
Coscinodiscus marginatus Ehrenberg	10	ND	ND	ND
Coscinodiscus reniformis Castracane	20	ND	ND	20
Coscinodiscus sp	10	150	ND	ND
Cyclotella sp	ND	125	ND	ND
Diploneis sp	ND	72	ND	10
Grammatophora marina (Lyngbye) Kützing	40	150	ND	ND
Gyrosigma balticum (Ehrenberg) Rabenhorst	10	312	ND	10
Licmophora sp	ND	10	ND	ND
Lithodesmioides polymorphum Stosch	2930	1240	400	ND
Lyrella lyra (Ehrenberg) Karajeva	10	120	ND	ND
Melosira nummuloides Agardh	10	ND	ND	ND
Navicula directa (W.Smith) Ralfs	20	ND	ND	ND
Navicula sp	30	470	ND	ND
Odontella aurita (Lyngbye) C. Agardh	20	ND	ND	ND
Paralia sulcata (Ehrenberg) Cleve	40	ND	ND	ND
Parlibellus rhombicula (Hustedt) Witkowski	20	ND	ND	ND
Planktoniella sol (G.C.Wallich) Schütt	10	ND	ND	ND
Pleurosigma aestuarii (Brébisson ex Kützing) W. Smith	ND	20	100	ND
Rhizosolenia cochlea Brun	ND	14150	ND	ND
Rhizosolenia imbricata Brightwell	ND	50	ND	ND
Rhizosolenia setigera Brightwell	40	6000	ND	ND
Surirella fastuosa Ehrenberg	40	ND	ND	ND
Thalassionema frauenfeldii (Grunow) Hallegraeff	ND	300	ND	ND
Thalassiosira eccentrica (Ehrenberg) Cleve	220	50	ND	ND
Thalassiosira sp	170	150	ND	20
Triceratium sp	10	ND	10	ND
Trieres chinensis (Greville) Ashworth & Theriot	50	810	820	ND
Trieres mobiliensis (Bailey) Ashworth & Theriot	ND	300	ND	ND
Zygoceros rhombus Ehrenberg	90	20	ND	ND
DINOFLAGELLATES				
Dinophysis acuminata Claparède & Lachmann*	370	520	ND	83
Dinophysis acuta Ehrenberg*	ND	50	ND	ND
Dinophysis caudata Saville-Kent*	430	650	290	ND
Gonyaulax digitalis (Pouchet) Kofoid*	10	ND	ND	ND
Gyrodinium estuariale Hulburt	40	ND	ND	ND
Prorocentrum balticum (Lohmann) Loeblich*	30	ND	ND	280
Prorocentrum gracile Schütt	20	30	ND	ND
Prorocentrum micans Ehrenberg*	70	290	1840	10
Protoperidinium marielebourae (Paulsen) Balech	ND	130	ND	ND
Protoperidinium oceanicum (Vanhöffen) Balech	30	ND	ND	ND
Protoperidinium punctulatum (Paulsen) Balech	10	ND	ND	ND
Protoperidinium quinquecorne (Abé) Balech *	500	7695	ND	ND
Protoperidinium sp	20	50	ND	ND
Protoperidinium steinii (Jørgensen) Balech	120	333	20	110
Pyrodinium bahamense var. *bahamense* Plate*	ND	33125	16600	ND
Scrippsiella acuminata (Ehrenberg) Kretschmann *et al.*	ND	135	ND	ND
CYANOBACTERIA				
Trichodesmium erythraeum Ehrenberg ex Gomont*	16500	683	200	20

ND: Not detected.
* Potentially harmful species

report showed the forms corresponding to the two varieties proposed in the 1980s. More recently, *P. bahamense* var *bahamense* was observed in an extensive bloom off the Pacific coast of Costa Rica co-occurring along with variety *compressum* (Vargas-Montero and Freer 2003).

Specimens of *P. bahamense* var *bahamense* of our study occur as solitary cells in all samples. Morquecho (2008) reported that the specimens of same species found near Isla San Jose, Gulf of California, were mainly composed of single cell and few of them were paired cells. On the other hand, Badylak *et al*. (2004) did find four-cell chains in samples from Indian River Lagoon in Florida.

Although the high abundance of *Pyrodinium bahamense* var. *bahamense* (more than 10^5 cell L^{-1}) observed during the present study, there is no fish mortality was reported from Yemeni coastal water of the Red Sea. According to Landsberg *et al*. (2006), this species is not highly toxic because its toxin profile is mainly composed of dc-STX (1%), STX (26%), and B1 (73%). The amount of B1 analogues confers low toxicity to this variety. In fact, *P. bahamense* var. *bahamense* was known for not producing saxitoxin or at least not causing PSP intoxication (Steidinger *et al*., 1980). However, beginning in 2002, saxitoxin was detected in puffer fish harvested from the Indian River Lagoon (Florida, USA), which coincided with a *P. bahamense* var. *bahamense* bloom. Cultures established from these bloom waters demonstrated the ability to produce saxitoxin (Landsberg *et al*., 2006). Abbott *et al*. (2009) reported finding saxitoxin in seven other Florida estuaries, including Tampa Bay, where *P. bahamense* var. *bahamense* populations were examined by Steidinger *et al*. (1980).

Bloom of *P. bahamense* var. *Bahamense* in the present study was observed when the water temperature was 32°C. Previous studies had reported that the water temperatures in red tide areas of this species range from 27°C to 35°C (Maclean, 1989). Laboratory studies on cells were isolated from Malaysia (Usup, 1995) showed that the temperature limits for growth are 22–34°C, with optimum growth at 28°C. Phlips *et al*. (2006) reported that cells of *P. bahamense* var. *bahamense* appeared in the column of water when the water temperature reached 20°C and blooms of this species formed only when the temperature was 25°C or higher. Furthermore, the water temperature in the coastal waters of the Baja California Peninsula during the occurrences of *P. bahamense* var. *bahamense* was 24–31°C (Garate-Lizarraga and Gonzalez-Armas, 2011; Usup*et al*., 2012). All these data with our finding support the tropical and subtropicall origin of *P. bahamense* var. *bahamense* although its range could expand if seawater warming happens.

The salinity during *P. bahamense* var. *bahamense* bloom in Yemeni coastal waters was 37 psu. Wall and Dale (1969) reported that the optimum salinity for *P. bahamense* var. *bahamense* was 35 psu. On the other hand, most of the available studies indicate that *P. bahamense* is a tropical euryhaline species(Mclean, 1976; Azanza-Corrales and Hall, 1993). The high salinity requirement of *P. bahamense* was also evident from earlier attempts to culture isolated species. Although blooms only occurred at 20 psu or higher, *P. bahamense* var. *bahamense* in Florida has a salinity range of 10– 45 psu, (Phlips *et al*., 2006; Usup *et al*., 2012).

Among the known toxic dinoflagellates, the abundances of *Dinophysis caudata*, *D. acuminata* and the red tide-forming *Protoperidinium quinquecorne*, *Scrippsiella acuminata* and *Prorocentrum micans* were remarkable during this study period. The two species of *Dinophysis* recorded during this study are reported to be potential causative agents of DSP. The diarrhetic shellfish poisoning (DSP) caused mainly due to *Dinophysis* spp. has been reported to be the main toxin-related problem in several countries adjoining the Mediterranean Sea, from where mussel contamination due to okadaic acid and dinophysis toxins has been reported (Marasovic *et al*., 1998; Koukaras and Nikolaidis, 2004; Alkawri and Ramaiah, 2011).

In conclusion, our findings are the first report of the occurrence and description of the morphology of *P. bahamense* var. *bahamense* in the coastal waters off Red Sea. Although there is no fish mortality was reported in the study region during the present study, this organism and other potentially harmful algae, should be regularly monitored in Red Sea coastal waters in order to discover the factors triggering such harmful algal blooms which could affect all food web levels including the human level. In addition, a comparative genetic-taxonomic survey of *P. bahamense* var. *bahamense* must to be done for the coast of Yemen, as well as biological, ecological, and toxicological studies to clarify the potential impact of this dinoflagellate within the Yemeni waters.

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