IDENTIFICATION OF MARINE SAND-DWELLING DINOFLAGELLATES IN DINAWAN ISLAND, SABAH

Asilah Al-Has & Normawaty Mohammad-Noor

Borneo Marine Science Research Institute,
University Malaysia Sabah, Jalan UMS, 88400, Kota Kinabalu, Sabah, Malaysia.

ABSTRACT. *Identification of marine sand-dwelling species is important due to the toxin-producing abilities of some species that may harm human beings. In this study, sand samples were collected for 12 months at Dinawan Island, Sabah. Samples were identified using light microscopy (LM) and Scanning Electron Microscopy (SEM). Sixteen dinoflagellates species have been identified including 4 potential toxic species i.e. Prorocentrum arenarium, P. lima, P. rhatymum and Amphidinium carterae. Prorocentrum norrisianum is morphologically very similar to P. cassabicum. List of sand-dwelling dinoflagellates species especially toxic species provides important knowledge for monitoring and management of harmful algal bloom.*

KEYWORDS. Dinoflagellates, Dinawan Island, marine sand-dwelling, toxin

INTRODUCTION

Dinoflagellates are the second primary producer after diatoms in the marine food web. These organisms live in different habitats (water column, sand, seaweed and seagrasses) and can be found all over the world. In Sabah, Harmful Algal Bloom (HAB) is caused by *Pyrodinium bahamense* var. *compressum* since 1976 (Roy, 1977). Till today, none of the HAB problems is caused by the benthic dinoflagellates although there are several toxic benthic dinoflagellates recorded in this area. They are *Prorocentrum lima* (Mohammad Noor *et al.*, 2010) and *P. rhathymum* (Caillaud *et al.*, 2010). In Sabah coastal water, Mohammad-Noor *et al*. (2006) have described 24 species of epibenthic dinoflagellates collected mainly from seaweed, seagrasses and dead corals. Based on *Artemia* bioassays, 8 species have the potential to produce toxin. These species are *Coolia* sp., *Gambierdiscus pacificus, Ostreopsis labens, O. ovata, Prorocentrum arenarium, P.concavum, P. cf. faustiae* and *P. lima*.

The aim of this study is to identify the marine sand-dwelling dinoflagellates, collected in sand of Dinawan Island using light and electron microscopy. List of the species found in this area will add to the existing record on marine sand-dwelling dinoflagellates in Sabah coastal water and can be used for future reference in phytoplankton study.

MATERIAL AND METHOD

Study Area

Dinawan Island is located about 15 km to the southwest of Kota Kinabalu, Sabah (Figure 1). East and south areas of the island lined by sandy beaches while north and west are rocky shores. In the north of the island, there is a resort known as Borneo Dinawan Island Resort otherwise the island has no human residency. Locals from the mainland come to the island to fish.

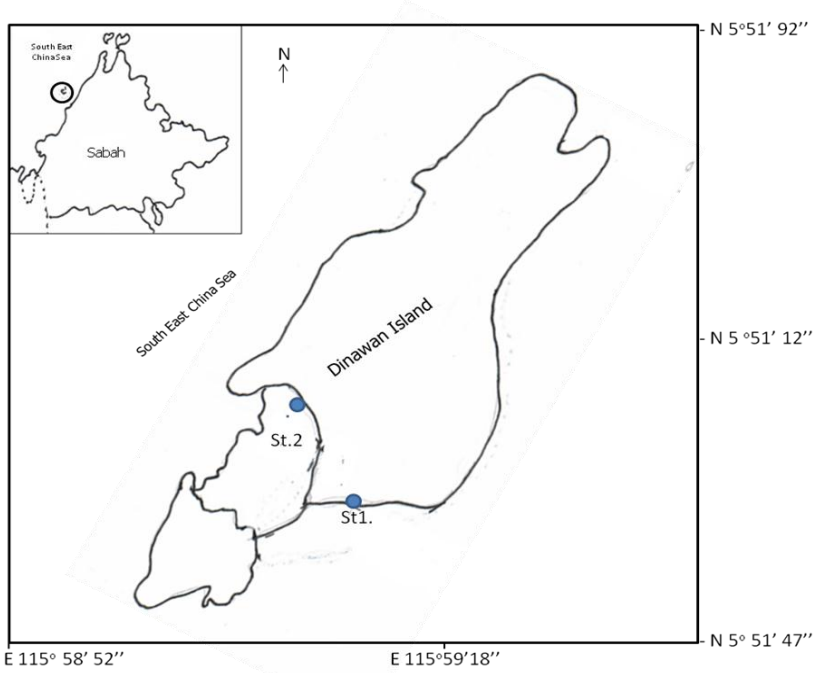

Figure 1. Sampling areas in Dinawan Island.

Sand Sample Collections

A total of 144 sand samples were collected from April 2009 to March 2010. Samples were collected every month at 2 sampling sites which are located in the beach (St.1) and lagoon (St.2) areas (Figure 1). Sand samples were collected by using PVC tubes with the length of 20 cm and diameter of 9 cm.

Extraction and Identification of Marine Sand-Dwelling Dinoflagellates

To extract the dinoflagellate cells from the sand, sand samples were mixed homogenously with distilled water and then filtered through different sieve sizes: 125 µm, 90 µm and 20 µm (modified from Selina and Hoppenrath, 2008). Samples between 20 m to 90 m were collected and preserved with Lugol-iodine solution. Besides the preserved samples, fresh samples were also collected. These samples were used to establish pure cultures by isolating single cell using micropippete into ESDK media. Both preserved and cultured cells were identified using light (LM) and scanning electron microscopy (SEM). For light microscopy, micrograph was obtained using Leica microscope equipped with a Nikon digital camera. Morphometric measurement was measured from the micrograph using NIV software. For scanning electron microscopy, cells were fixed with 2% gluteraldehydrate for 1 hour, filter-mounted on membrane cellulose and rinse with distilled water for 1 hour. Then the cells were dehydrated with series of ethanol (15,30,50,70,95 %) followed by t-butyl ethanol. The filter was freeze-dried and sputter-coated with platinum for 3 min at 45 mA before observation under JEOL scanning microscope 5610-Lv.

RESULTS AND DISCUSSIONS

Identification of Marine Sand-dwelling Dinoflagellates in Dinawan Island, Sabah

A total of 4 orders consisting of 16 species of marine sand-dwelling dinoflagellates were identified during the research period (Table 1, Figures 3). Previously Mohammad-Noor *et al.* (2007) have reported most of them occur in Sabah coastal area but in different substrates such as seaweed and sea grasses except from sand. The morphology of species found is in line with previous description elsewhere.

Table 1 List of dinoflagellates species identified and the occurences were defined as C-Common, F-Frequent and R-Rare at Dinawan Island. Some of the species have reported to produce toxin.

Order	Name	O	Toxic /Reference
Prorocentrales Lemmermann 1910	*Prorocentrum arenarium*	R	Okadaic acid (Ten-Hage *et al.*, 2000)
	Prorocentrum norrisianum	F	Unknown
	Prorocentrum lima	R	Okadaic acid (Hu *et al*,. 2010)
	Prorocentrum mexicanum	C	Unknown
	Prorocentrum rhatymum	R	Okadaic acid (Caillaud *et al*., 2010)
	Prorocentrum sp.1	R	Unknown
	Prorocentrum sp.2	C	Unknown
Gonyaulacales F.J.R. Taylor 1980	*Coolia malayensis*	F	Unknown
	Ostreopsis sp.1	F	Unknown
Gymnodiniales Apstein 1909	*Amphidinium carterae*	R	Hemolytic (Yasumoto *et al.*,1987)
	Amphidinium sp.1	R	Unknown
	Amphidinium sp.2	F	Unknown
	Gymnodinium sp.	R	Unknown
Peridiniales Haeckel 1894	*Bysmatrum caponii*	C	Unknown
	Peridinium quinquecorne	C	Unknown
	Heterocapsa sp.1	R	Unknown

* Unknown – No toxicity study have been reported.

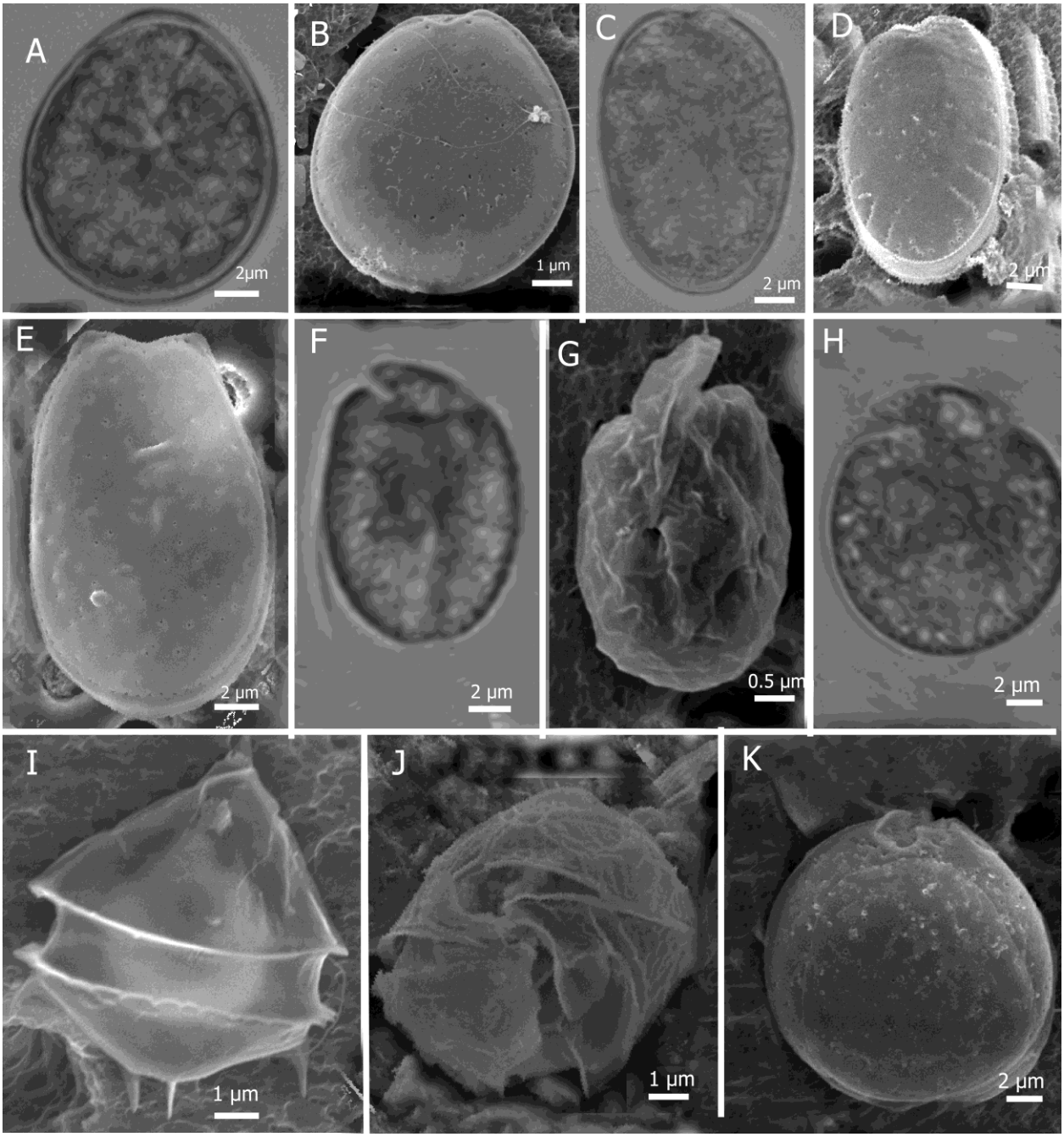

Figure 2. Micrographs of several species of marine sand-dwelling dinoflagellates identified in Dinawan Island. A and B. *Prorocentrum arenarium* A. LM. B. SEM. C and D. *Prorocentrum rhatymum* C. LM. D. SEM. E. SEM of *Prorocentrum lima* F. and G. *Amphidinium carterae* F. LM. G. SEM (cell shrank). H. LM of *Amphidinium* sp.1 I. SEM of *Peridinium quinquecorne* J. SEM of *Bysmatrum caponii* K. SEM of *Coolia malayensis.*

The number of marine sand dwelling dinoflagellates found in this study is less compared to the study by Saburova *et al.* (2009). They have reported 43 taxa of benthic dinoflagellates from different sediment types. The differences in number of marine sand dwelling

dinoflagellates reported is due to several factors including sampling period, different types of sediment and location of sampling area. Anderson and Kawachi (2005) have stated that not many of dinoflagellates species can be found in sediment compared to the water column.

Four species of the sand dwelling dinoflagellates identified are toxic species viz. *Prorocentrum arenarium, P. lima, P. rhathymum* and *Amphidinium carterae*. These three *Prorocentrum* species have been reported to produce okadaic acid (OA) that can cause diarrhetic shellfish poisoning (DSP) (Murakami *et al.,* 1982; Ten-Hage *et al.*, 2000 and Caillaud *et al.*, 2010). Consumption of shellfish contaminated by DSP toxins can cause illness to human such as diarrhea, nausea, abdominal cramps and vomiting (Dolah, 2000 and Madigan *et al.,* 2006). As toxin producer, *Prorocentrum* can also cause a problem to aquaculture industry. *Prorocentrum rhathymum* which is commonly found in the study areas has also been reported to cause mortality to oyster spat (Pearce *et al.,* 2006). *Amphidinium carterae* was found to be toxic to mice (Baig *et al*., 2006). High cell density of sand dwelling dinoflagellates causing the discoloration of sand has been reported (Herdman, 1924; Hoppenrath *et al.*, 2007). Therefore, this indicated that marine sand-dwelling dinoflagellates are capable of forming bloom.

Comparison between *Prorocentrum norrasianum* and *Prorocentrum cassabicum*

Taxonomy remarks: *Prorocentrum norrasianum* (Figure 3) is oval in shape and the size range from 20-25 µm. Pyrenoid located at the cell center and flagella area shifted towards the right side (Figure 3A). Scanning electron micrographs showed the detail morphology of *P. norrisianum* (Figures 3B-E). Periflagella area is in triangular shape (Figure 3B). The thecal surfaces are ornamented with fine pores (Figures 3C, E and D). Cell is posterior and anteriorly compressed (Figure 3D).

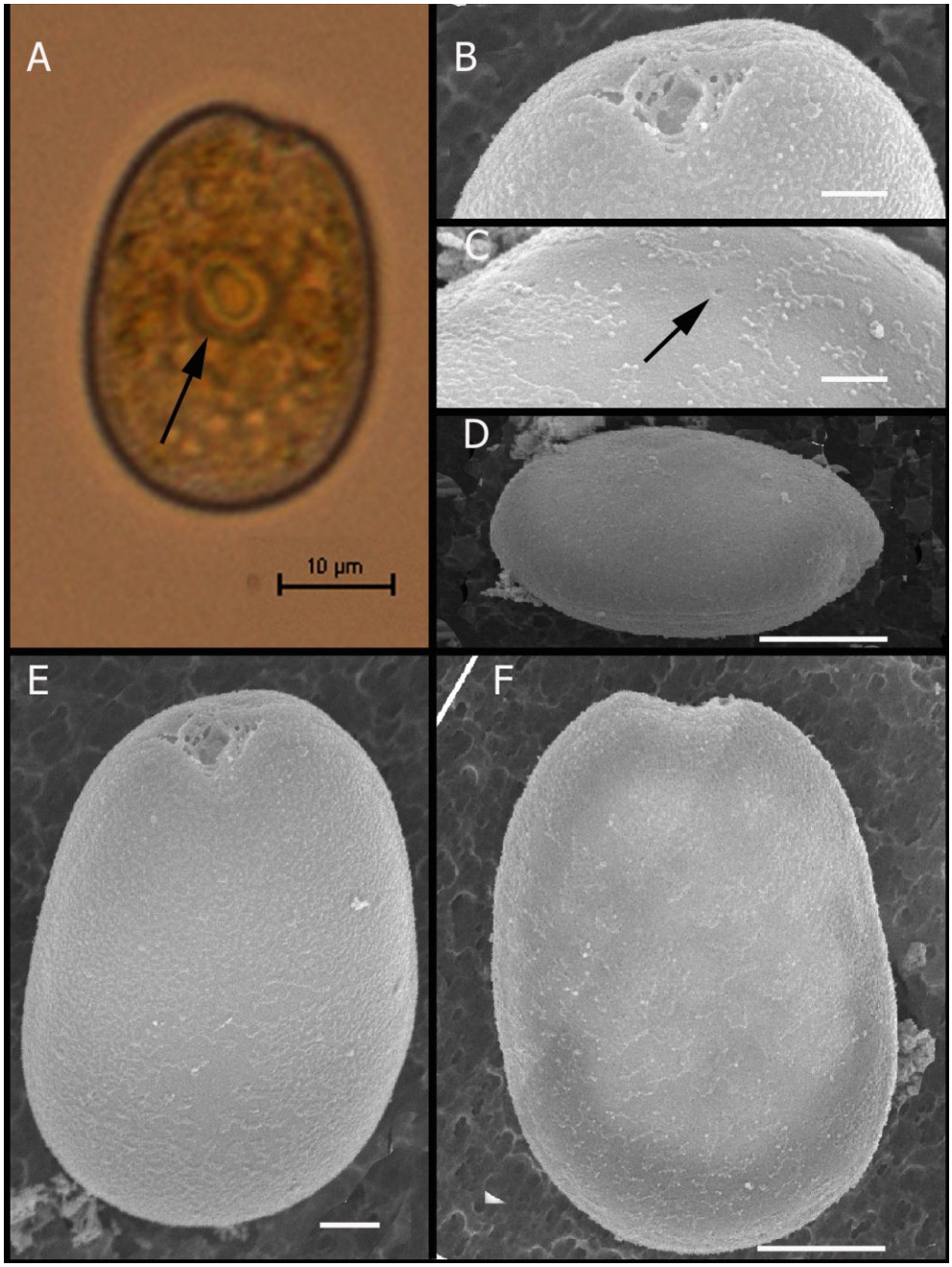

Figure 3. *Prorocentrum norrisianum.* A. LM showing centered pyrenoid (arrow). B. Periflagella area C. Fine pores on thecal surface (arrow). D. Side view E. Right valve. F. Left valve.

Study of marine sand-dwelling dinoflagellates including its taxonomy is still less and have caused many of the species to be poorly described, leading to confusion (Selina and Hoppenrath, 2008). In this study, the morphology of *Prorocentrum norrisianum* (Figure 3) isolated from Dinawan Island is in line well with the original description by Faust (1997). However when comparing its morphology with other *Prorocentrum* species, *P. norrisianum* is also very similar to *P. cassubicum* (*Exuviella cassubica*) (Dodge 1965). According to Faust

(1997), *P. norrisianum* differed from *P. cassubicum* in cell size where *P. norrisianum* is smaller than *P. cassubicum*. *Prorocentrum norrisianum* is 20-25µm in length and 13-16µm in width whereas *P. cassubicum* is 22-25µm in length and 16µm in width (Table 2). Faust (1997) also mentioned that the apical plate of *P. norrisianum* is vertical or curved while *P. cassubicum* is curved. The small differences between these two species suggested a close relationship between them. To further evaluate this matter, sample of *P. cassubicum* from type locality is needed for detailed morphology and DNA studies.

Table 2. Comparison of cell size and morphological features in *Prorocentrum norrisianum* and *Prorocentrum cassubicum*.

Characteristics	Faust, 1997 *P. norrisianum*	Dodge, 1965 *P. cassubicum*	This Study *P. norrisianum*
Cell size (µm) L=length, W=width	L: 20-25 W:13-16	L:22-25 W: 16	L:20 – 25 W: 11- 16
Cell shape	Oval with straight side	Oval	Oval
Marginal pores	Absent	Absent	Absent
Pores	Scattered	Scattered	Scattered
Pyrenoid	Present	No data	Present

Overall 16 species from 4 orders of marine sand-dwelling dinoflagellates were identified in Dinawan Island. Out of these, 4 species are potential toxic species viz. *P. lima, P. arenarium, P. rhathymum* and *Amphidinium caterae*. *Prorocentrum norrisianum* is similar with *P. cassubicum* based on morphology. However, sample of *P. cassubicum* from type locality is needed to confirm this matter. This study shows that toxic benthic dinoflagellates also occurred in sand. Therefore, monitoring of HAB species should take into account sand samples in addition to water samples alone.

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