

## COMMON GENUS OF BENTHIC DIATOMS ON THE SEAFLOOR OF THE SOUTHERN SOUTH CHINA SEA, PENINSULAR MALAYSIA - A PRELIMINARY SURVEY

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**Abstract:** Benthic diatoms are as important as the planktonic diatoms in the water column. Although benthic diatoms are quite well documented in most aquatic ecosystems, studies on their species composition on the offshore seafloor are very limited. Therefore, this study was conducted to preliminarily determine the diversity of benthic diatoms, which is the most ubiquitous group of the microphytobenthos (MPB) collected on the seafloor of the southern South China Sea along the Peninsular coastal waters during a research expedition in March 2019. Triplicate samples were collected from six stations (E1 to E6) and a total of 968 cells representing 17 species of benthic diatoms were identified and recorded. The highest species richness was recorded at E1, with 16 species, and the least number of species was found at E6, with nine species. The highest density was recorded at E3 with 30 cells cm<sup>-2</sup> and the lowest density was found at E6 with only three cells cm<sup>-2</sup>. *Coscinodiscus* sp. was the most abundant species. Although only preliminary, this study provides essential baseline data, especially due to limited information available on the diversity and richness of benthic diatom in the coastal waters of the South China Sea.

Keywords: Microphytobenthos, benthic diatoms, South China Sea, seafloor diatoms.

### Introduction

Benthic diatoms are diatom assemblages living on the seafloor sediment (Underwood *et al.*, 1998). These microorganisms are the most ubiquitous group of microphytobenthos (MPB) and are frequently reported with the highest community densities and species richness in coastal ecosystems. At high abundance and productivity, benthic diatoms form a thin, brownish mat, or carpet over the sediment surface, known as biofilm, which consists of a well-developed microbial community. Microphytobenthic biofilms are very important as a medium for changing nutrients across the sediment-water interface. They also stimulate and compete with most of the bacterial sediment process in the ocean, contributing significantly to the primary productivity within the sand, and muddy, soft habitats (Underwood, 2002; Desrosiers *et al.*, 2013; Miller *et al.*, 2018). Benthic diatoms are highly adaptable to different environments and, thus, can be found

in most substrates in the water. This adaptation occurred through the alteration of their cellular metabolism (Pongpan & Yuwadee, 2010). In addition, diatoms decrease the level of carbon dioxide in the air by converting it into sugar or fixing the carbon. Diatoms are also known to be the main producer in marine and aquatic environments, especially as a food source for benthic organisms which are heterotrophic (Lee, 1999; Miller *et al.*, 2018).

The South China Sea (SCS) is the most immense marginal sea in the Western Pacific (Wu *et al.*, 2013). It harbours the highest marine biodiversity on earth. SCS is an important source of pelagic propagules of fish and invertebrates. Although limited, several studies, such as Lu *et al.* (2006) and Lu *et al.* (2016), have reported on the dominant species of benthic diatom, namely *Thalassionema nitzschioides* and *Nitzschia bicapitata*, within the waters. The studies, however, were done on a more northern area of the southern South China Sea, where the

temperature is low. Meanwhile, in the centre of the South China Sea, *Thalassiosira oestrupii* is the most abundant, while *Hormophysa cuneiformis* is the most minor present. *Attiliosa nodulifera*, *Nitzschia marina*, *Rhizosolenia bergonii*, and *Azpeitia neocrenulata* have a most minor abundance in the north-easternmost and the southernmost parts but reach the highest values in the mid-north and the south of the South China Sea (Jiang *et al.*, 2004).

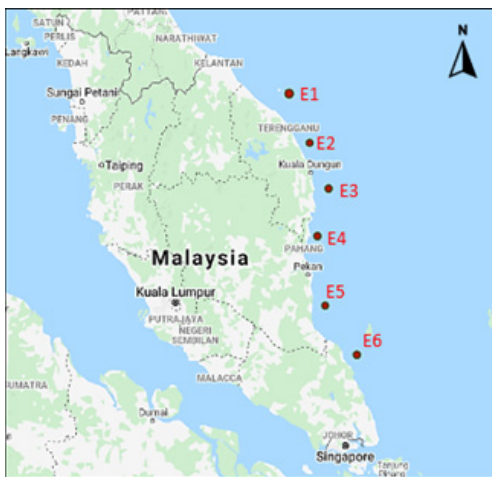
This study was conducted to preliminarily determine the diversity of benthic diatoms in different stations in the southern South China Sea due to the lack of such information reported from Malaysia. In this study, the identification of species diversity of benthic diatoms is reported from the sample collected from the seafloor of

the southern South China Sea within the coastal waters of Peninsular Malaysia, which will become one of the initial reports published from the area.

**Materials and Methods**

**Sampling Site**

The study was conducted within the coastal waters of the southern of South China Sea along Peninsular Malaysia. Samples were collected at six sampling stations: E1 (with a depth of 11.9 meters), E2 (with a depth of 24.8 meters), E3 (with a depth of 36.7 meters), E4 (with a depth of 38.0 meters), E5 (with a depth of 46.1 meters), and E6 (with a depth of 46.3 meters)



Station	Latitude	Longitude	Estimated Depth (m)
E1	102.7972	1.80128	11.9
E2	102.0183	2.8501	24.8
E3	101.2644	2.79549	36.7
E4	100.7351	3.24684	38
E5	100.6879	3.49099	46.1
E6	100.7969	3.57083	46.3

Figure 1: Location of the sampling stations namely E1, E2, E3, E4, E5, and E6 which located within the coastal waters of Peninsular Malaysia at the southern South China Sea. Also detailed next to the map, the latitude and longitude and the estimated depth of each station

(Figure 1).

**Samples Collection of One-off Sampling**

**Sediment Samples**

Sediment samples were collected from the six sampling stations using a Smith McIntyre grab (0.1 m<sup>2</sup>). The top 2 mm of sediment was obtained from the grab using a minicore with a

diameter of 3.7 cm (9.62 cm<sup>2</sup>). All samples were collected in a labelled 50 mL Falcon tube. For sample preservation, 5% of formalin was added to all sediment samples. Samples were stored in a fridge at 4°C prior to analysis.

### ***Extraction of Benthic Diatom Using Acid Wash Procedure***

Samples preserved in the 5% formalin were centrifuged three times at 2300 rpm for 15 minutes to separate the benthic diatom from the sediment (Underwood, 1995). After centrifugation, excess formalin was siphoned off and discarded. Distilled water was added to a depth of 0.5 cm in each tube, followed by the addition of potassium permanganate ( $\text{KMnO}_4$ ) to a depth of 0.5 cm. The fixed samples were left for 24 hours in a 4°C fridge. Afterwards, concentrated hydrochloric acid (HCl) was added to each sample, followed by placing them in the water bath at 80°C for one hour. Samples were centrifuged again at 2300 rpm for 15 minutes, and the supernatant was siphoned off and replaced by distilled water. This centrifugation process was repeated five times to ensure that the diatom samples were cleaned of organic material and microscopic debris for permanent mounting.

### ***Sedimentation Slides Preparation***

The sedimentation chamber method was used to prepare the slides. A cover slip was placed on the base of the sedimentation chamber by using a small amount of Vaseline gel. Then, the top of the chamber was lightly applied with the Vaseline gel to create an airtight seal. One mL of the sample was then added into the sedimentation chamber. This procedure was followed by adding one drop of Lugol's iodine solution into the samples to weigh and fix the diatom cells. This technique is important to allow cell sedimentation to happen in the sedimentation chamber (Bellinger & Sigee, 2010). The samples were left for two hours and immediately after that, the top of the chamber was slowly removed to separate it from the base,

leaving only the mix of samples and Lugol's iodine at the base.

### ***Diatoms Cells Enumeration and Identification***

Benthic diatom samples were identified under a compound light microscope (Leica ATC2000 Binocular Compound Microscope) to the lowest possible taxonomic level, at either genus or species level. The identification was carried out based on the morphology detailed by Tomas (1997), Salleh and Tajuddin (2006), and Redzuan (2012). The cell density of each identified diatom species was expressed in the unit of cells  $\text{cm}^{-2}$ .

### ***Statistical Analyses***

Spatial species diversity of benthic diatoms was analyzed according to stations and was investigated using evenness ( $E$ ) and Shannon Diversity Index ( $H'$ ). The indices were calculated using the Multi-Variate Statistical Package (MVSP) 3.1 software (Kovach, 1999). Data of  $n = 3$  of each species were pooled to carry out the mentioned diversity indices analyses.

## **Results and Discussion**

### ***Spatial Diversity of Benthic Diatoms***

A total of 968 diatom cells were recorded in this cruise survey. Overall, per centimetre squared ( $\text{cm}^2$ ) of the top sediment, the highest diatoms cells were recorded at E3 with a total of 30 cells  $\text{cm}^{-2}$ , followed by E5 with 27 cells per  $\text{cm}^2$ , E1 with 21 cells  $\text{cm}^{-2}$ , E2 (6 cells  $\text{cm}^{-2}$ ), E4 (4 cells  $\text{cm}^{-2}$ ) and finally, E6 with only three cells  $\text{cm}^{-2}$ . A total of 17 species of benthic diatoms were recorded. The species were categorized into 10 orders and 13 families (Table 1).

Table 1: List of genus or species, families, and orders of benthic diatoms recorded at the six stations of the study sites at southern of South China Sea

Order	Family	Species
Coscinodiscales	Coscinodiscaceae	<i>Coscinodiscus</i> sp.
Eunotiales	Eunotiaceae	<i>Eunotia</i> sp.
Bacillariales	Bacillariaceae	<i>Nitzschia</i> sp. <i>Nitzschia epithemoides</i>
Naviculales	Pleurosigmataceae	<i>Pleurosigma angulatum</i> .
	Amphipleuraceae	<i>Frustulia</i> sp.
Thalassiosiphysales	Catenulaceae	<i>Amphora</i> sp.
Tabellariales	Tabellariaceae	<i>Tabellaria</i> sp.
Fragilariales	Fragilariaceae	<i>Fragilaria</i> sp. <i>Synedra ulna</i> .
Naviculales	Naviculaceae	<i>Navicula</i> sp. A <i>Navicula</i> sp. B
	Pinnulariaceae	<i>Pinnularia</i> sp.
	Diploneidaceae	<i>Diploneis didyma</i>
Chaetocerotales	Chaetocerotaceae	<i>Chaetoceros curvisetus</i>
Triceratiales	Triceratiaceae	<i>Triceratium latius</i> <i>Triceratium favus</i>

Spatially, the E1 station displayed the highest number of species at 17 species, which is also concurrent with the highest  $H'$  and  $E$  values of 2.596 and 0.7887, respectively. At the same time, the lowest species number was at E6 (9 species) (Table 2). The lowest species number of E6 was also reflected by the station's low benthic diatoms diversity ( $H'$ : 2.053) and equitability ( $E$ : 0.7731) values (Table 2). The lowest values of diversity indices at E6 (46.3 m) are attributed to the low productivity due to the low penetration of sunlight towards the seafloor for benthic diatoms to undergo photosynthesis (Li *et al.*, 2022). Also, sunlight could be the limiting factor to species richness at the depth where light intensity is low.

In contrast to E6, the E1 with the shallowest depth (11.9 m) had the highest diversity indices ( $H'$ : 2.596,  $E$ : 0.7887) (Table 2). The high diversity and density of benthic diatoms at E1 are probably attributed to the high penetration

of sunlight. Cantonati *et al.* (2009) suggested that depth is also significantly associated with other parameters that have proven to control the benthic diatoms, such as the substrate type, turbidity, wave action, and water level fluctuation (Redzuan & Underwood, 2020) and temperature, which can change the effects of light on benthic algal communities. Therefore, on top of the intensity, other unknown limiting factors potentially also enhance such high diversity at E1. With regards to the fact that all stations, despite their varying depth, are of similar distance to the shore, nutrients are at a low chance of significantly affecting benthic diatom diversity in this study.

Interestingly, although E4 did not have the lowest number of species (11 species), both its  $H'$  and  $E$  displayed the lowest values of 1.002 and 0.2512, respectively. The low equitability in species diversity of benthic diatoms at E4 (Figure 2) was potentially responsible for

such findings at the station. However, the potential factors causing the finding were not investigated. It was observed that the trend of diversity and density of benthic diatoms between stations was not consistent. Other than depth, a study by Hildebrand *et al.* (2012) found that some benthic diatoms have specific extreme or optimum parameters, such as salinity, temperature and pH. Existing knowledge of

environmental controls and depth-distribution features is limited (Hansson, 1992) by the fact that benthic diatoms are much less studied than phytoplankton. Light and nutrients are among the most frequently reported environmental controls of benthic diatoms. Competition for light between the two main algal life forms in lakes (phytoplankton and benthic diatoms) has been demonstrated (Hansson, 1992).

Table 2: Spatial trends of number of diatoms species dan diversity at all the six stations

Spatial Trends	E1	E2	E3	E4	E5	E6
No. of species	16	10	14	11	14	9
Shannon Index (H')	2.596	2.146	2.424	1.002	2.158	2.053
Evenness (E')	0.7887	0.7772	0.7527	0.2512	0.577	0.7791

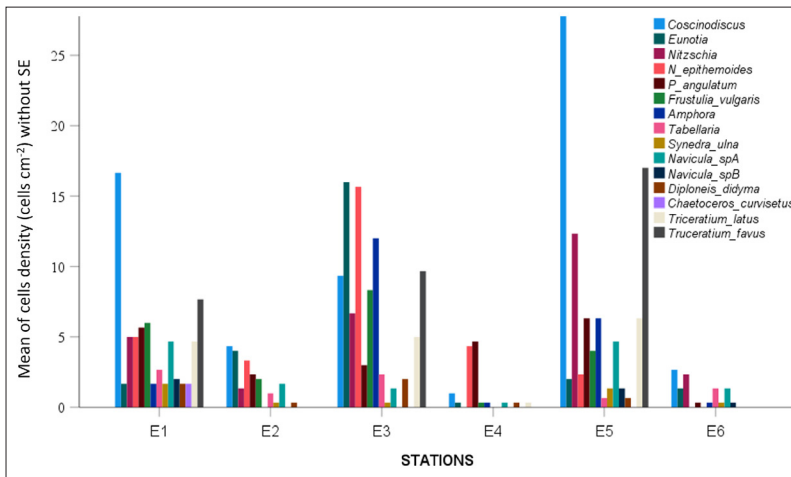


Figure 2: Mean of cell density of benthic diatoms on the seafloor at the six stations located in the Southern South China Sea. SE values were not included for clearer figure

***Coscinodiscus sp. as the Most Common Species***

*Cosconodiscus* sp. was the species with the highest total density being enumerated (188 cells cm<sup>-2</sup>) (Figure 3). This finding is in comparison with an earlier study by Olenina *et al.* (2006), who reported that *C. asteromphalus* was the most dominant species recorded on the

seafloor of the Baltic Sea. Genus *Coscinodiscus* has been frequently reported as a good indicator of rising water temperature. The distribution of *Coscinodiscus* spp. has been used in global warming monitoring through the digital imaging of their chlorophyll (Goessling *et al.*, 2016).

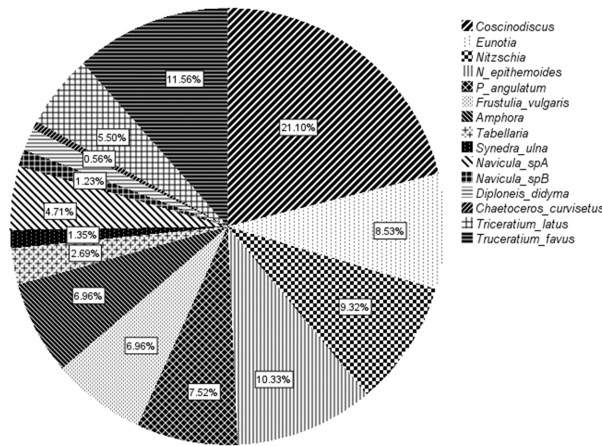


Figure 3: Percentages (%) of each benthic diatoms species in relation to total density

**Conclusion**

A total of 17 species, 10 orders, and 13 families of benthic diatoms have been documented in this survey. *Coscinodiscus* sp. was the most dominant species followed by a few other species. Depth possibly plays a vital role in controlling cell density and species diversity of benthic diatoms on the seafloor. Since this research vessel expedition was a one-off sampling survey, only limited data can be provided in this study. Therefore, this study can only display and discuss preliminary data that hopefully can still provide information for future studies. We believe that there are more species on the seafloor of the South China Sea than what was reported in this study and elsewhere.

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