MORPHOLOGICAL EXAMINATION OF THE *Thalassiosira* spp. IN TELUK BAHANG, PENANG

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ABSTRACT

This study describes the morphological structure of *Thalassiosira* spp. found in coastal water and intertidal area of Teluk Bahang, Penang. Examination of the samples collected from January 2015 to June 2016 showed that these species were the common genera in both benthic and surface seawater at Teluk Bahang. To obtain phytoplankton samples, five liters of seawater were filtered through the plankton net (35 µm). While for benthic samples, the samples were collected at sediment surface of 1 mm depth. Samples of *Thalassiosira* spp. were obtained from both phytoplankton and benthic samples. Scanning electron microscope (SEM) was used to study the morphology of *Thalassiosira* spp. Results from the SEM showed distinct differences in areolae pattern, sizes, position and number of processes among *Thalassiosira* spp. (*Thalassiosira nanolineata*, *Thalassiosira densannula*, and *Thalassiosira gravida*). *Thalassiosira densannula* and *Thalassiosira nanolineata* were found in both habitats, coastal and benthic area while *Thalassiosira gravida* was found only in coastal water. *Thalassiosira* spp. contribute approximately 15% of the total benthic community and approximately 35% of the total phytoplankton community.

KEY WORDS: *Thalassiosira* spp., coastal water, intertidal area, morphology, SEM

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INTRODUCTION

Diatoms belong to the class Bacillariophyceae of the phylum Bacillariophyta. They dominate the phytoplankton and microphytobenthos of the coastal marine environment. Various environmental conditions control the growth and behavior of their community structure in the pelagic and benthic environment. There are currently 174 taxonomically accepted species of *Thalassiosira* (Guiry & Guiry, 2014). The genus *Thalassiosira* Cleve was first established with the identification of *Thalassiosira nordenskioeldii* Cleve as the type, and it was characterized by its chain formation with central mucus filaments (Cleve, 1873). Scanning electron microscope (SEM) examination is more suitable for species identification compares to light microscope due to the difficulty in recognizing the fine line of *Thalassiosira* spp. The most important characteristics to identify *Thalassiosira* spp. are the position and number of fultoprotulae, rimoportulae, and occluded processes on the valve and mantle, the structure of girdle bands and the areolation pattern (Tomas, 1997). This study was done to document the morphological difference and provide fundamental information of *Thalassiosira* spp. in Teluk Bahang, Penang.

METHODS

Teluk Bahang is located in the northern Penang Island. Three stations (A, B, and C) were selected for this study (Figure 1). There were two stations (A and B) at the Pasir Pandak intertidal area where benthic samples were collected. At station A, the salinity ranged from 24 to 33, and in station B it was 5–33 throughout the year due to freshwater inflow. Station C was at the jetty where phytoplankton samples were collected. Its salinity was between 28 and 32. Tidal range in this area was 0.2 m to 1.0 m. These three stations were selected because it was located near the aquaculture farm where possibilities of nutrients flowing might cause changes in *Thalassiosira* spp. morphology.

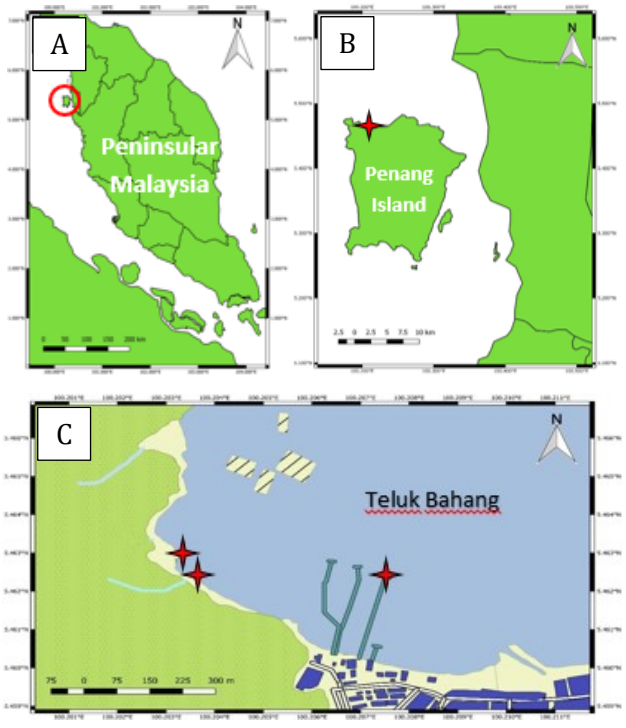

Figure 1. The map of Peninsular Malaysia showing Penang Island and Teluk Bahang. (A) Map of Peninsular Malaysia, the round symbol ⍰ indicates Penang Island; (B) map of Penang Island; and (C) map of Teluk Bahang, the red star symbols ✦ indicates the sampling sites.

The phytoplankton samples were collected with 35 µm mesh-size plankton net at the jetty. Five liters of water samples were filtered through the plankton net to acquire

the plankton samples (n = 3) while microphytobenthos samples (n = 3) were collected from surface layer of sediment, 5 mm depth. All the phytoplankton and microphytobenthos samples collected were stored in 50 ml centrifuge tube. The phytoplankton samples were preserved by adding a few drops of Lugols' solution, while the microphytobenthos samples were added with 20 ml filtered seawater before adding Lugols' solution to preserved the samples. Samples were shaken lightly after adding Lugols' solution.

Samples collected were treated with hydrogen peroxide to eliminate organic matter (modified from Taylor *et al.*, 2007). Approximately 5 ml of phytoplankton and benthic samples were transferred into a centrifuge tube and fill up with 5 ml of 10% hydrogen peroxide. The samples were left for 24 hours for organic matter digestion of the samples. The samples were rinsed with distilled water several times, after 24 hours, to obtain clean samples. The cleaned samples were stored in the refrigerator (4 °C). The samples were filtered using filtration set with isopore membrane filters (0.4 µm). The cleaned samples were viewed under SEM Carl Zeiss SMT (Germany), Leo Supra 50 VP Field Emission Scanning Electron Microscope. Clear SEM images of phytoplankton and microphytobenthos were used for identification. Identification of diatoms to species level was based on the general morphological description of phytoplankton (Marine Phytoplankton of the Western Pacific, 2012 and Algae of Australia: Phytoplankton of Temperate Coastal Waters, 2010).

RESULTS AND DISCUSSION

Thalassiosira is a common genus found in Teluk Bahang. *Thalassiosira nanolineata* and *Thalassiosira densannula* were found both in benthic and coastal samples. Only *Thalassiosira gravida* was found in coastal water.

Thalassiosira nanolineata (Figure 2) was first known as *Coscinodiscus nanolineatus* from the Philippine Islands (Mann, 1925). The diameter of the phytoplankton cell ranged from 32.7 to 43.2 µm while the microphytobenthos cell was a bit smaller, 15.9–26.8 µm. The areolae were arranged in straight parallel rows. Size of the areolae on the valve face was similar, 7–11 in 10 µm. The position labiates processes were separated into 2–3 striae. Two strutted processes (internal view) were located at the center of the cell, and a ring of strutted processes (short external tubes) was located near the marginal area. A labiate process was located between two marginal strutted processes.

Thalassiosira densannula (Figure 3) was first described in the Indian Ocean by Hasle & Fryxell (1977). Phytoplankton size ranged from 27.0 to 39.6 µm, and the size of microphytobenthos ranged from 23.1 to 31.3 µm. The areolae of this centric diatom were arranged in straight linear arrays, parallel to each other. Areolae in the middle of the cell were the same size as the areolae in near the margin (7–9 areolae in 10 µm). A ring of fultoportulae was present in the marginal area, each with double external tubes. There was rimoportula present near the margin area. The margin was narrow down with ribs corresponding to the marginal areolae. There were 6-7 striae in 10 µm, and the labiates were located in between of one striae.

Thalassiosira gravida (Figure 4) had a circular shape and was weakly silicified. There was a cluster of strutted processes in the center, and scattered strutted processes on the valve face. A single prominent labiate process located near the margin area. The diameter of phytoplankton ranged from 40.0 to 47.8 µm. This cell was not found in the benthic area. The areolae near the margin ranged from 18 to 21 areolae in 10 µm. Morphometric data of all *Thalassiosira* species were summarized in Table 1.

This *Thalassiosira* spp. are mainly found in marine waters. Due to many similar morphological features in *Thalassiosira* (Cleve) Hasle and *Coscinodiscus* (Ehrenberg) Hasle & Sims, it is easy to misidentify between these two genera. These two genera are large and distinctive diatoms. To identify the difference in morphological characteristics between the two genera, we used light and scanning electron microscopes. Besides 1–2 rimoportulae,

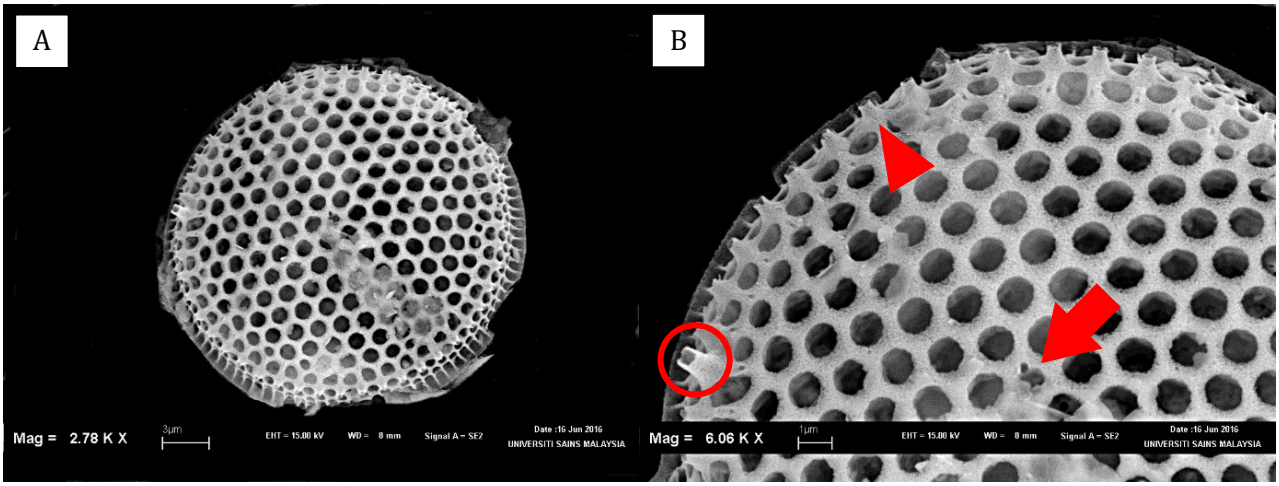

Figure 2. Micrographs of *Thalassiosira nanolineata* (A and B) observed under SEM. (A) The external valve view of *T. nanolineata,* (B) red arrow shows the external opening of central strutted processes, external view of marginal strutted (arrow head), and labiate processes (red circle).

Table 1. Morphometric data for *Thalassiosira* species

Species Name	Diameter (µm)		Areolae(10 µm)		Striae (10 µm)	Distance between labiates (striae)
	Phyto-plankton	MPB	Near center	Near margin		
Thalassiosira gravida Cleve, 1896	40.0– 47.8	-	-	18–21	-	-
Thalassiosira densannula Hasle & Fryxell, 1977	27.0– 39.6	23.1– 31.3	7–9*		6–7	1
Thalassiosira nanolineata (A.Mann) Hasle & Fryxell, 1977	32.7–43.2	15.9– 26.8	7–11*		9–11	2–3

*Note: Areolae size are the same at center and margin.

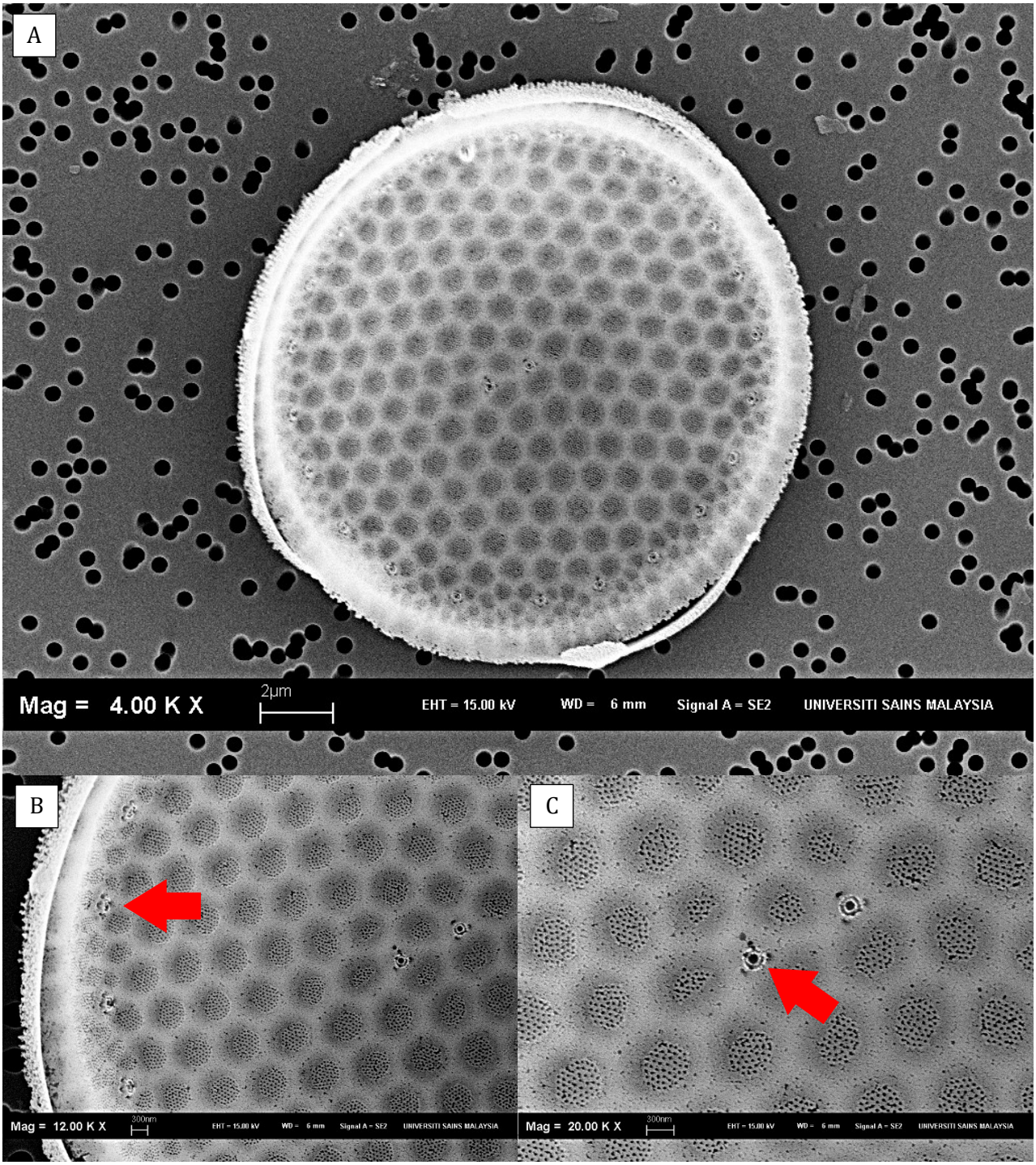

Figure 3. Micrographs of Thalassiosira *densannula* (A–C) observed under SEM. (A) The external valve view of *T. densannula*, (B) Red arrow showing the marginal fultoportulae, and (C) Red arrow showing the central fultoportula.

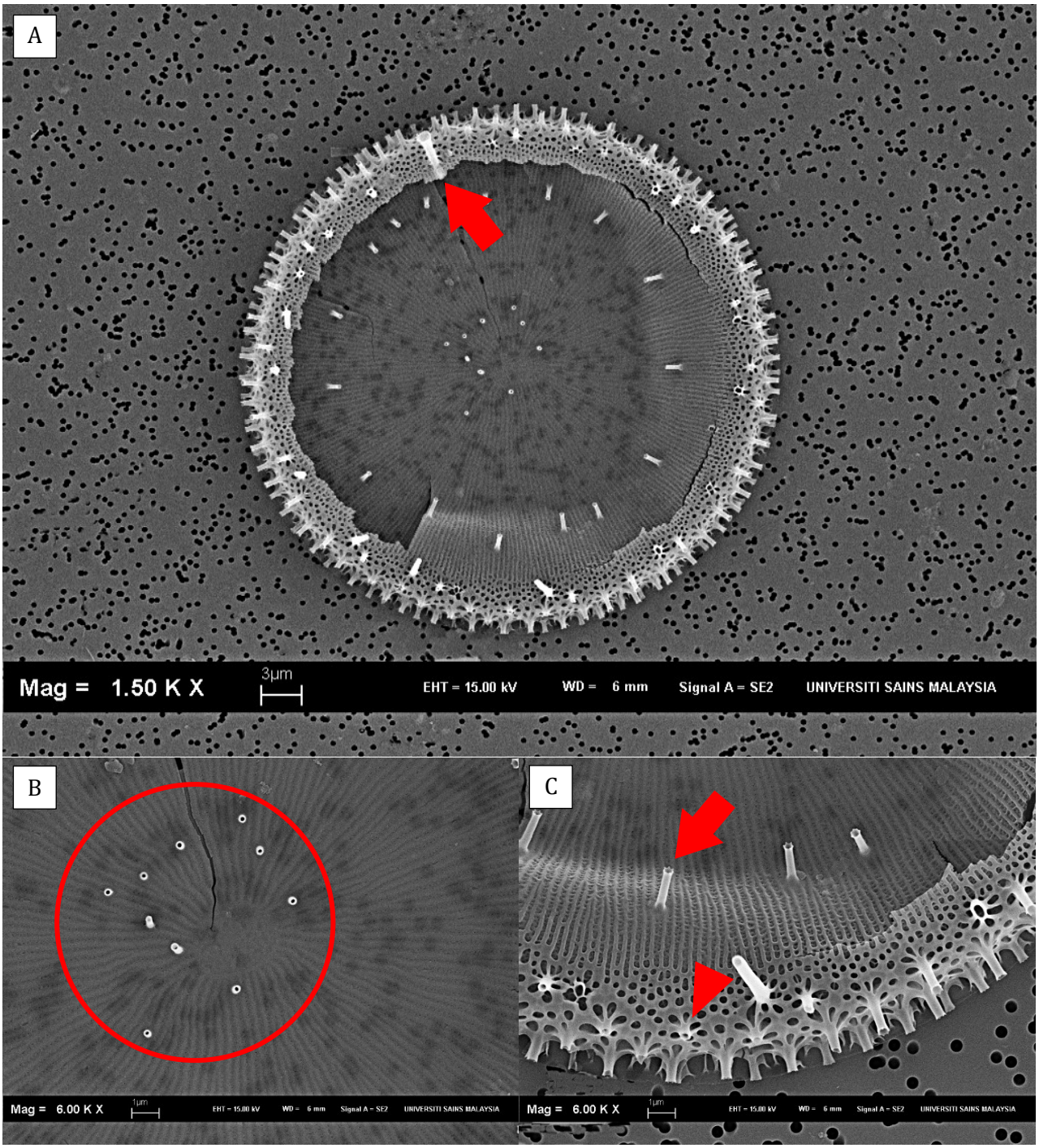

Figure 4. Micrographs of *Thalassiosira gravida* (A-C) observed under SEM. (A) The external valve view of *T. gravida* showing long tube of rimoportula (arrow), (B) Red circle shows central fultoportula, (C) Showing rimoportula (arrow) and marginal fultoportulae (arrowhead)

Thalassiosira species have many fultoportulae, and several species possess occluded processes. The cribra locate close to the internal valve face, and the foramens were distributed on the external valve face. In contrast, regarding *Coscinodicus* species, the cribra was located close to the external valve face, and the foramens were distributed on the internal valve face (Tomas, 1997). Without fultoportulae and occluded processes, *Coscinodiscus* species only have many rimoportulae.

Coscinodiscus subglobosus Cleve & Grunow might look identical to *Thalassiosira gravida*, and *Coscinodiscus subglobosus* has been misinterpreted as resting spore of *Thalassiosira gravid* (Tomas, 1997). This species has frequently been reported worldwide, in the Antarctic, and appears in mucilage colonies (Fryxell, 1989). *Thalassiosira densannula* has been recorded in China from Zianjiang off the Guandong coast (Li *et al.*, 2013).

Thalassiosira nanolineata is a warm-water species based on its distribution around the Philippine Islands, the Gulf of Mexico and Brazilian waters (Hasle & Fryxell, 1977) and of the Guandong coast (Li *et al*., 2013). Park & Lee (2010) was the first to report diatom species of *T. nanolineata* from the temperate region.

CONCLUSION

This study provides the first report of *Thalassiosira nanolineata, Thalassiosira densannula*, *Thalassiosira gravida* in Teluk Bahang coastal water and intertidal area. Since fine structure such as the strutted processes, and labiate processes cannot be observed by a light microscope, viewing by scanning electron microscope is essential to further analyze these striking features. In addition, detailed systematic studies are needed to confirm the presence of *Thalassiosira* species.

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