Morphological Observation of Common Pennate Diatoms (Bacillariophyceae) from Sarawak Estuarine Waters

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ABSTRACT

Common pennate diatoms in two estuaries of Kuching, Sarawak, Malaysia were documented based on samples collected from September 2008 till Febuary 2009. Plankton samples were subjected to acid wash before detailed morphological observations. All specimens were identified to species level under transmission and scanning electron microscopy (TEM & SEM). During the study period, seven genera of pennate diatoms were commonly found in the samples, they are *Amphiprora*, *Surirella*, *Delphineis, Navicula, Nitzschia, Cylindrotheca* and *Pleurosigma*. Identification of species was based on the raphe systems and poroid arrangement. A total of 16 species was documented with two species of *Amphiprora* (*A. gigantea* and *A. alata*), three species of *Surirella* (*S. fluminensis*, *S. fastuosa* and *S. norvegica*), two species of *Delphineis* (*Delphineis* and *D. kurtzii*), one species of *Navicula* (*N. distans*), four species of *Nitzschia* (*N. longissima, N. obtusa, N. panduriformis* and *N.* cf. *amphibia*), one species of *Cylindrotheca* (*Cylindrotheca closterium*) and three species of *Pleurosigma* (*P. angulatum*, *P. spencerii* and *P. normanii*) described. This study represents one of the few studies on marine and brackish diatoms in the Malaysian waters.

Keywords: TEM, SEM, pennate diatom, raphe, poroid

INTRODUCTION

Diatoms represent about 25% of the plant biomass in the world (Round *et al.*, 1990). These microscopic unicellular microalgae are important biomass and oxygen producers that can be found in all aquatic ecosystems (Werner, 1977). Various morphological characters have been used for the classification of diatoms.

The frustules of pennate diatoms are usually elongate and bilaterally symmetrical in valve view. The pennate forms has a narrow axial area thickened at each end (polar nodule), and the central area usually having a median thickening (central nodule). There are many features present on the surface of the frustule for identification to species level. These features are only well resolved under electron microscopic observation. In the axial of the valve of most pennate diatoms, a slit (raphe) runs from one polar nodule to the other, or a hyaline median line which give superficial appearance (pseudoraphe). The raphe seems to be associated with the movement of many pennate diatoms (Round *et al.*, 1990). The pore fields transmit mucilage pads to attach to another cell to form chains. Some of them also use the pads to attach to substrata (Hasle *et al.*, 1997). However, the canal raphe system of raphid diatom always consisting of raphe canal, fibula, interspace, central interspace and keel structure. A central interspace of raphid diatom usually indicates two raphe slit (central raphe and central nodules) (Anonymous, 1975).

In the Malaysian coastal waters, studies on marine and brackish phytoplankton remained very limited, particularly the diatom species. Study on diatom in the Malaysian waters with more than 100 species documented had been conducted by Shamsudin (1990). However, most of the diatom observed in that study was identified to only generic level due to lack of electron microscopic observation. In this study, diatom samples collected in two estuarine waters of Kuching, Sarawak were examined in detail under scanning and transmission electron microscopy. We report in this study a total of sixteen species from seven commonly found genera of pennate diatoms, *Amphiprora, Surirella, Delphineis, Navicula, Nitzschia, Cylindrotheca* and *Pleurosigma* in Samariang and Santubong estuaries.

MATERIALS AND METHODS

Acid Wash Methods

Plankton samples were subjected to acid wash method according to Hasle (1970) before light and electron microscopic observations. Around 20 mL of plankton samples were transferred into a 100 mL Erlenmeyer flask and treated with 20 mL of sulphuric acid H_2SO_4 (98%). Potassium permanganate ($KMnO_4$) then was added to the sample until sample turn to purple colour. Oxalic acid ($(COOH)_2.2H_2O$) was added to obtain clear solution. The sample was then rinsed with distilled water until the cell suspension become less acidic.

Species identification

For light microscopy (LM), acid-wash sample was evenly applied on a cover slip before mounting permanently onto the slide with Naphrax, a medium of high refractive index. The fine structure of silica wall was observed under magnifications of 400× and 1000×.

For TEM, a drop of acid-wash sample was transferred onto a copper grid and air dried. The sample was observed under JEOL JEM-1230 TEM (JEOL, Japan). The same procedure was applied to SEM, except that the acid-wash sample was transferred onto a 0.2 µm black polycarbonate membrane filter. The sample was then coated using gold-palladium under JEOL JFC-1600 Auto Fine Coater before observing under JEOL JSM-6390LA Analytical SEM (JEOL, Japan).

RESULTS AND DISCUSSION

A total of 25 species from the 16 genera of diatoms have been identified from the two estuaries (Hilaluddin *et al.*, 2010). Out of the sixteen genera, seven commonly found pennate diatoms from the genera of *Amphiprora*, *Surirella*, *Delphineis*, *Navicula*, *Nitzschia, Cylindrotheca* and *Pleurosigma* were documented by using electron microscopy in this study. Species was identified based on the raphe structure, poroid arrangement and the structure of fibulae and striae, including some specific features identified in difference species.

Amphiprora is commonly found in Sarawak estuary (Figure 1). Two species of *Amphiprora* i.e. *A. gigantea* and *A. alata* was identified in this study. They appear in a single cell and in a long chain with the frustule are constricted in the middle. According to Hendey (1964), some species are twisted into a figure eight pattern such as *A. alata*. Girdle structure has a numerous narrow bands and the girdle junction line with absent of sinuous (Hendey 1964). According to Hasle *et al.*, (1997), *A. alata* is tolerant of brackish waters but most other species of this genus prefer marine conditions. *Amphiprora gigantea* and *A. alata* have similar morphological appearance. However, the median line of *A. gigantea* is sigmoid while *A. alata* has straight line (Shamsudin, 1990). The morphometric measurement of the selected species of *Amphiprora* was listed in Table 1.

Surirella is a solitary cell with the valves that can be ovate, cuneate, reniform, elliptical or linear. Genus of *Surirella* is common in freshwater ecosystem but also found in estuarine water. Most *Surirella* species are benthic. In this study, three species of *Surirella* was identified, i.e. *S. fluminensis*, *S. fastuosa* and *S. norvegica* from the two locations (Figure 2, Table 2). The central space of this genus often called pseudoraphe. The structures of fine channels or canaliculi are running through the substance and connect the raphe with the internal cell contents (Shamsudin, 1990). This genus can be identified under LM with acid-cleaned materials. Each species was identified by having different raphe and canaliculi structure.

Delphineis usually occur in short or long chain and sometime in solitary, with valve in linear form or broadly elliptical to lanceolate (Figure 3). The genus is characterized with two small pores and one labiate process at the end of sternum, with a prominent clear area in the center of the valve. According to Andrews (1977), the transverse striae of the species in the genus *Delphineis* are generally parallel and aligned continuously across the axial area. Morphometric data of two species, i.e. *D. karstenii* and *D. surirelloides* are listed in Table 3.

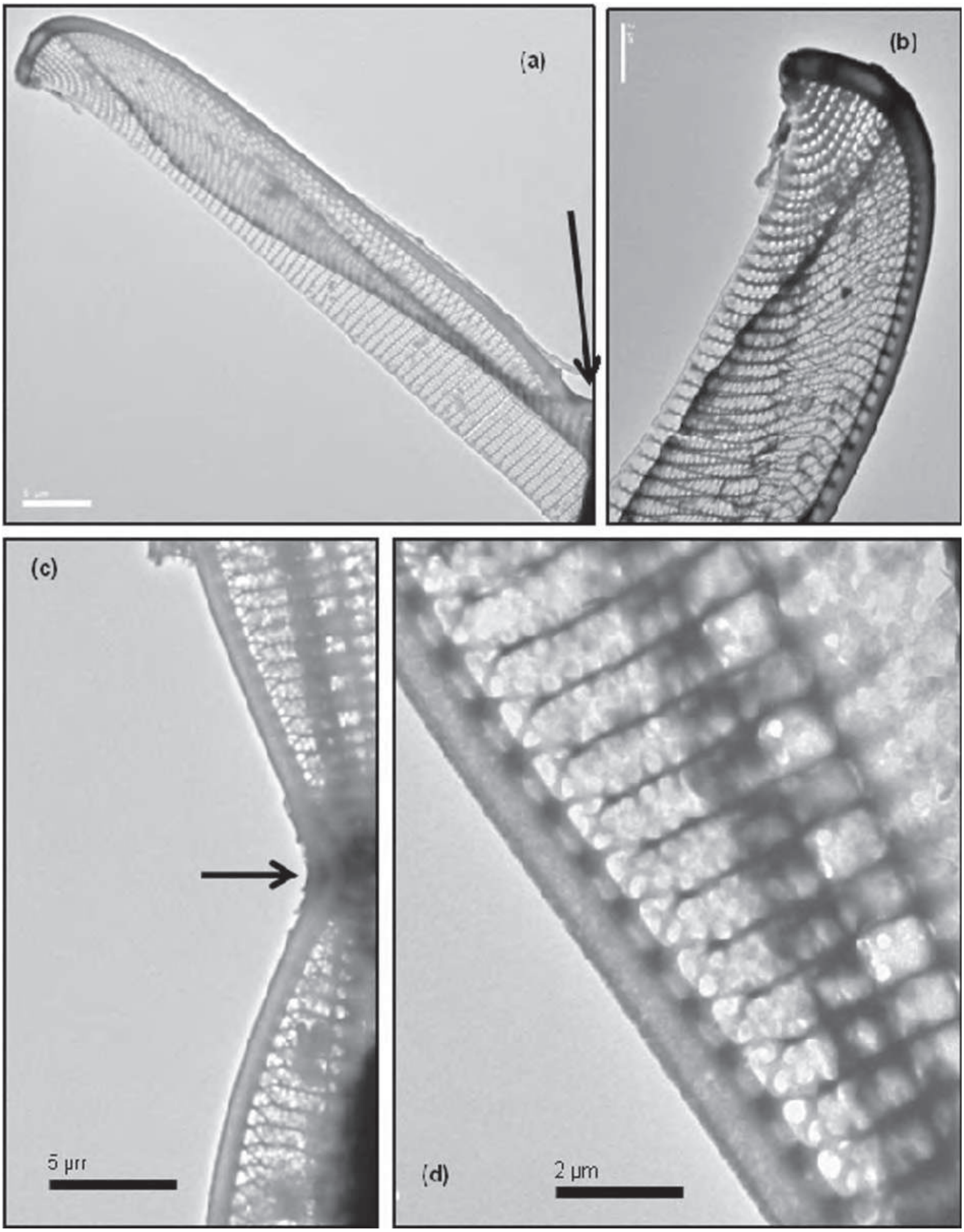

Figure 1. Micrographs of *Amphiprora* species. (a-b) *A. gigantea* observed under TEM. (a) Constricted structure in the middle of single valve can be observed (arrow), scale bar: 5 µm. (b) striae and puncta structure with narrow line, scale bar: 2µm. (c-d) *A. alata* observed under TEM. (c) Constricted structure in the middle of single valve (arrow), scale bar: 2µm. (d) Striae and puncta structure, scale bar: 2µm.

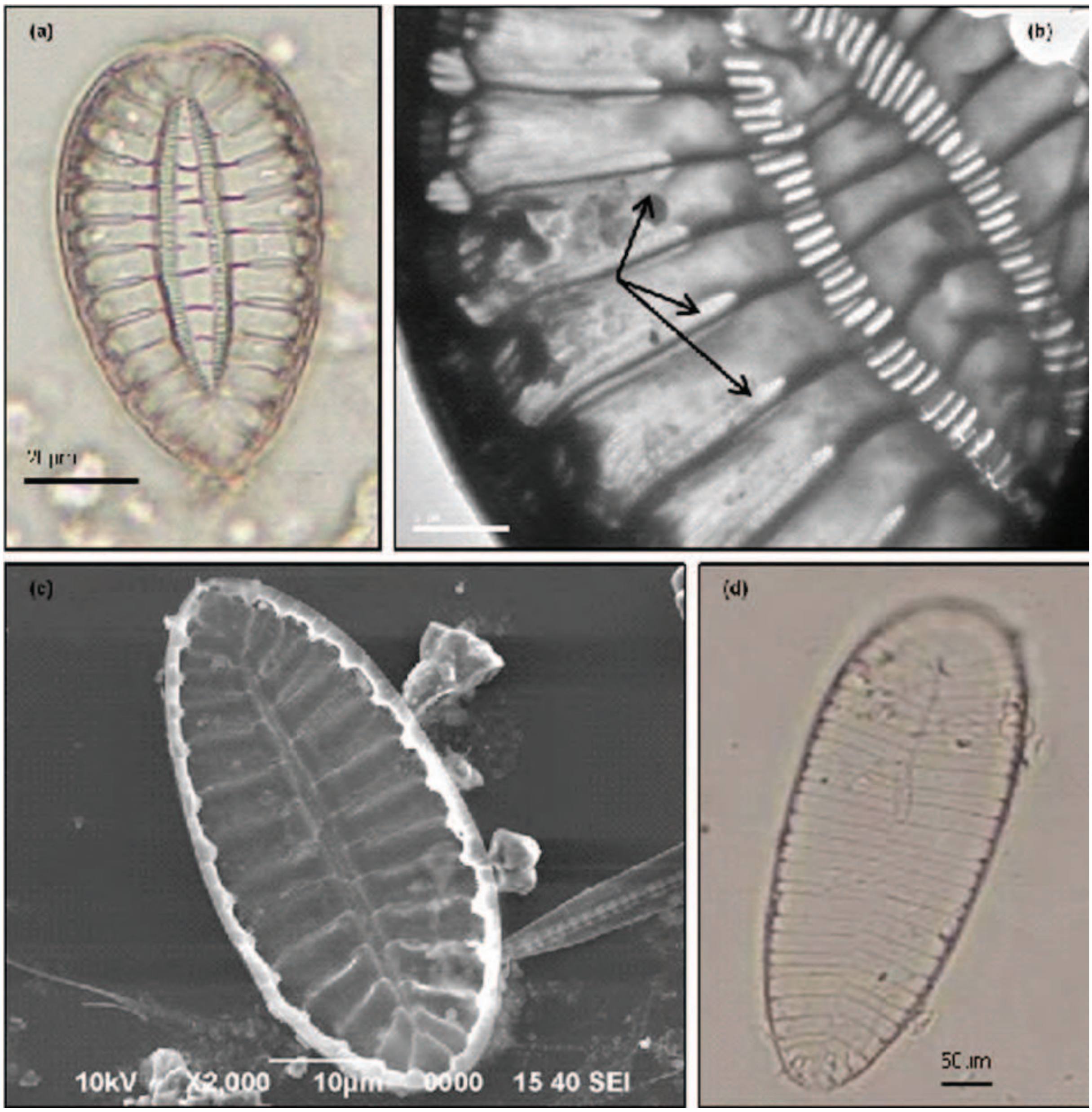

Figure 2. Micrographs of *Surirella* species. (a-b) *S. fluminensis*: (a) under LM, narrow median line of raphe structure is clearly observed which is different from other species, scale bar: 20 µm. (b) canaliculi structure which connect to raphe observed under TEM (arrow), scale bar: 5 µm. (c) Micrograph of *S. fastuosa* under SEM, lanceolate to ovate valve with long median line of raphe structure, scale bar: 10µm. (f) *S. norvegica* with keel are clearly observed on acid cleaned sample, scale bar: 50µm.

Most species of *Navicula* are solitary with boat-shaped cells and rectangular in girdle view. It has radiate striae with long aerolae structure (Figure 4, Table 4). The valves are symmetrical both apically and transapically, and may have rounded, with fine transapical striae and acute, or capitate ends. The striae of most species of this genus are parallel (Hasle *et al.*, 1997). *N. distans* is commonly observed in the two sampling locations.

Genus *Nitzschia* is the other genus of diatom that most commonly found in Malaysian water (Figure 5). Four species of *Nitzschia,* i.e. *N. longissima, N. obtusa, N. panduriformis* and *N.* cf. *amphibian* were recorded from two locations. Genus *Nitzschia* can be often found in form of chain or as free living cells. According to Hendey (1964), the keels are usually eccentric and can be central in some cases. The fibulae of *Nitzschia* may even extend across the valve and the raphe was usually observed near the proximal margin of the valve (Round *et al.* 1990). Round *et al.* (1990)

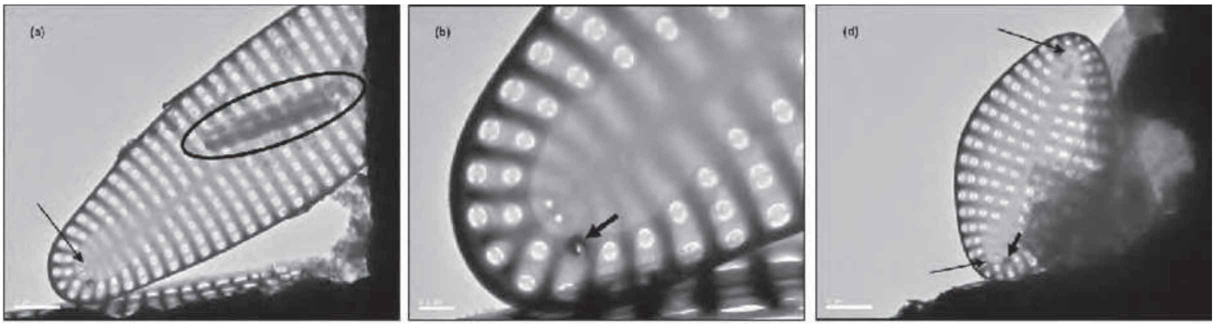

Figure 3. Micrograph of *Delphineis* species observed under TEM. (a-b) *D. karstenii*. (a) Prominent clear area at the center of valve (sternum) with two small pores (arrow) in single cell, circle: particle attached to cell frustules, scale bar: 2μm. (b) One labiate process at each valve apex (arrow), scale bar: 0.5μm. (d) *D. surirelloides* with two small pores and one labiate process (arrow) can be seen at each valve apex, scale bar: 2μm.

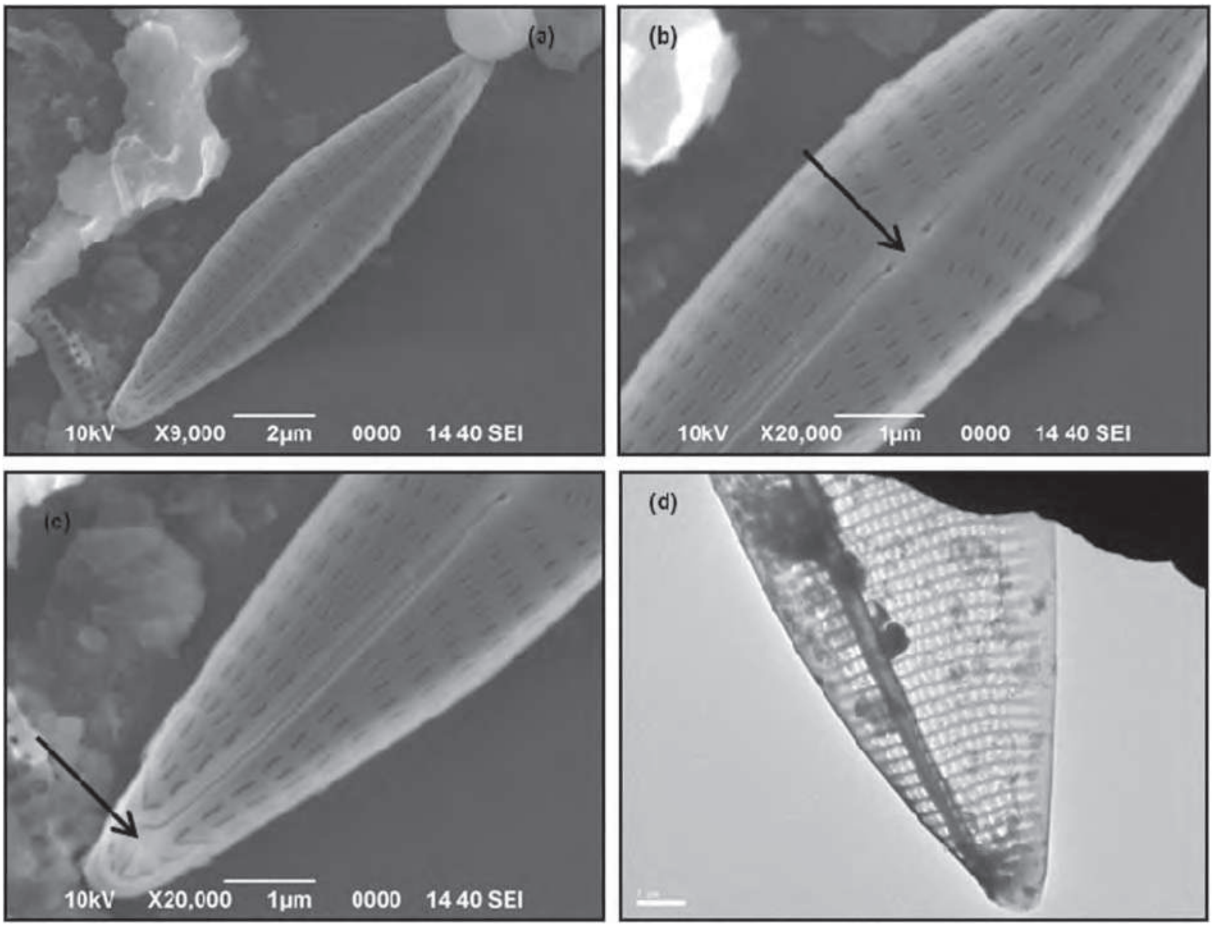

Figure 4. Micrograph of *Navicula* species. (a-c) Single valve of *N. distans* observed under SEM. (a) Radiate striae in single valve, scale bar: 2μm. (b) central nodule structure (arrow), scale bar: 2μm. (c) Apical end structure, scale bar: 1μm. (d) Girdle view observed under TEM, scale bar, 2μm.

have separated *Fragilariopsis, Cylindrotheca, Psammodictyon* and *Tryblionella* from *Nitzschia* group. The morphometric data of this genus was listed in Table 5.

All species of *Cylindrotheca* were characterized by having cylindrical frustules which is fusiform. They are needle-like with a swollen center. It will twist about the apical axis and rotating when in motion. The raphe system is traverse by a series of fibulae which join directly to the valve face. The most common species that has been identified in this genus is *Cylindrotheca closterium*

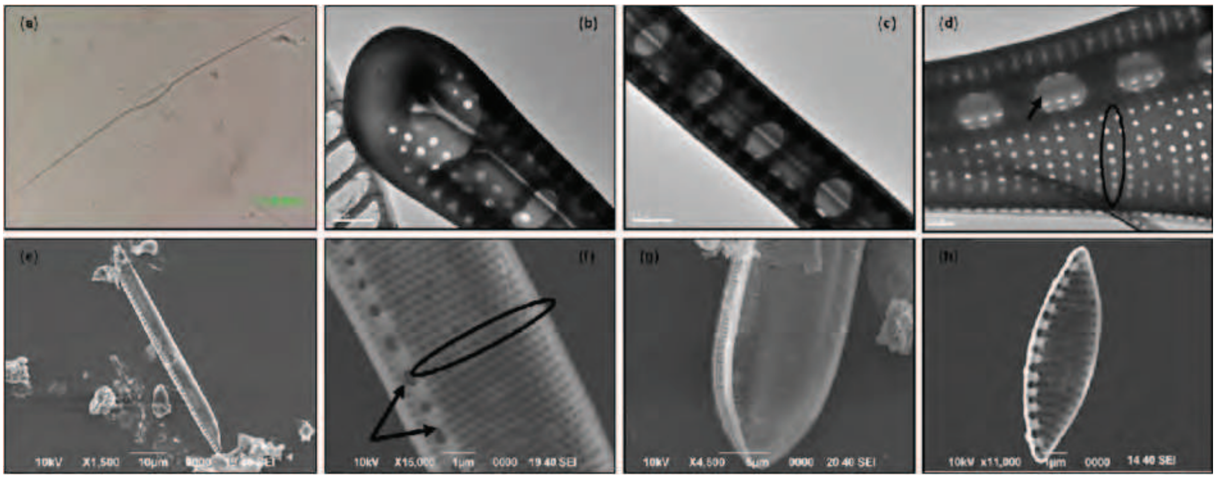

Figure 5. Micrographs of *Nitzschia* species. (a-d) *N. longissima*. (a) Acid cleaned sample under LM, scale bar: 50µm. (b-d) fibulae connected with silicified strips running parallel to the raphe slit under TEM, scale bar: 0.5µm. (d) Fibulae structure (arrow) and striae (circle), scale bar: 0.5µm. (e-f) Micrograph of *N. obtusa* under SEM. (e) Conoidal valve shape of cell, scale bar: 10µm. (f) External view of striae (circle) and fibulae structure (arrow), scale bar: 1µm. (g) Micrograph of *N. panduriformis* with S-shaped valves was identified from the Diatoms from the South China Sea, (h) *N.* cf. *amphibia* similar with micrograph in the Diatoms from the South China Sea which has lanceolate valve, scale bar: 1µm.

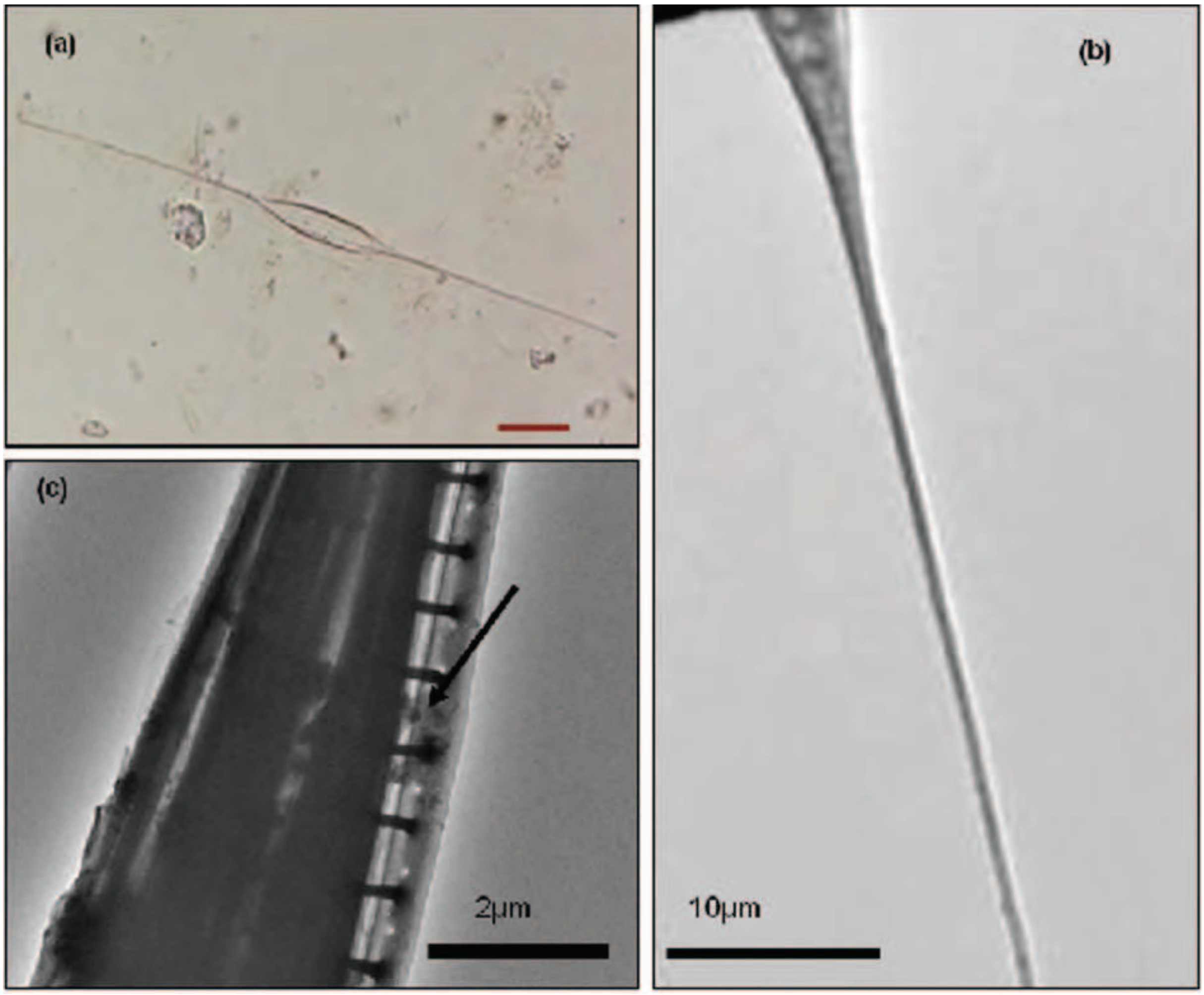

Figure 6. Micrograph of *Cylindrotheca closterium*. (a) Similar morphological apparent with *Nitzschia longissima* under LM, scale bar: 10µm. (b-c) Singular cell of *C. closterium* observe under TEM, (b) long projection of *C. closterium* differ from *N. longissima*, scale bar: 10µm. (c) Raphe slit running with fibulae (arrow), scale bar: 2µm.

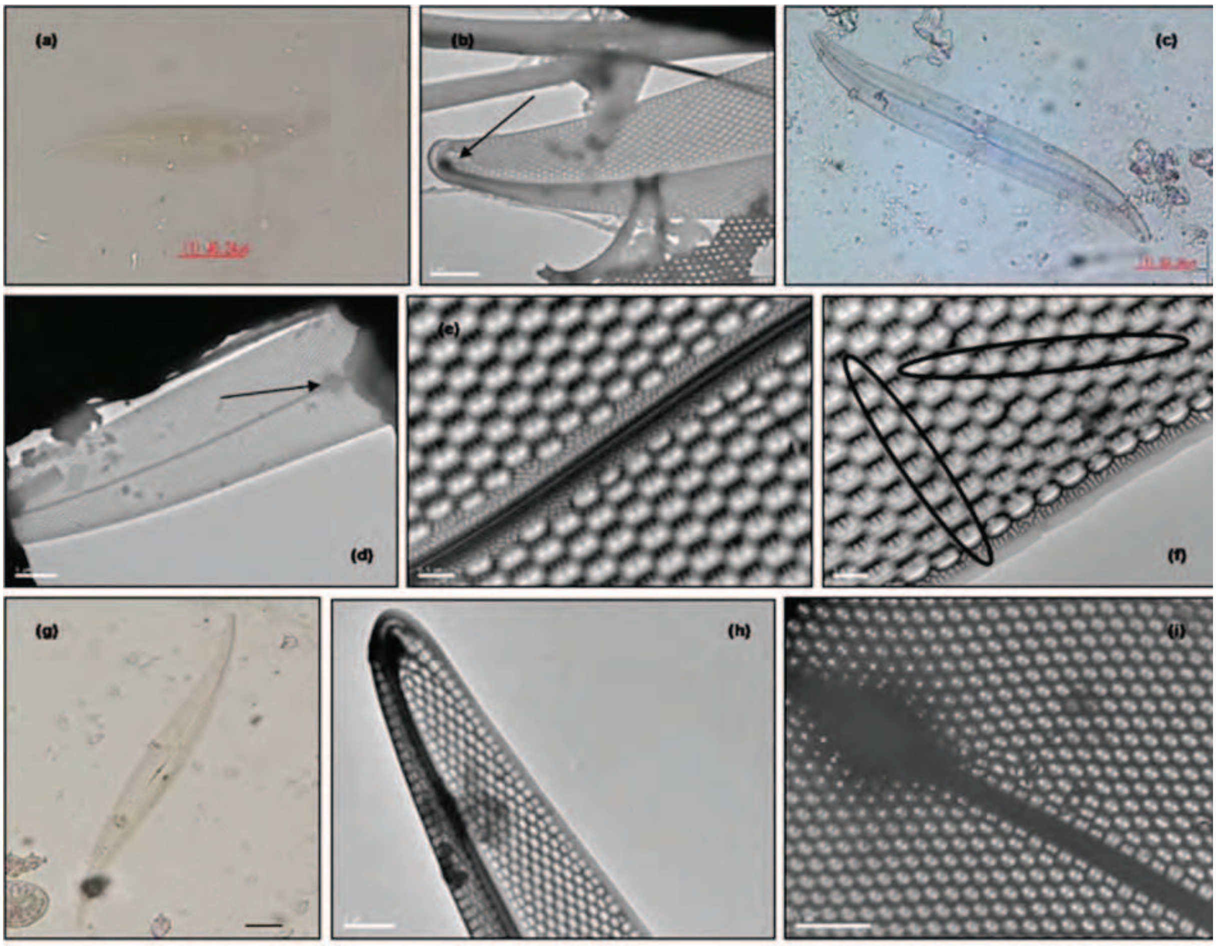

Figure 7. Micrograph of *Pleurosigma* species. (a-b) *P. angulatum*. (a) Sigmoid valve with raphe slit are clearly observed under LM, scale bar: 40.24µm. (b) raphe slit are slightly sigmoid with subacute end (arrow) observed under TEM, scale bar: 5µm. (c-f) *P. spencerii*. (c) Morphological apparent under LM, scale bar: 40.24µm. (d) Central nodule structure observed under TEM, scale bar: 5µm. (e) The central raphe is less sigmoid (TEM), scale bar: 0.5µm. (f) The striae structure clearly observed, transverse and oblique (circle) under TEM, scale bar: 0.5µm. (g-i) Valve striation of *P. normanii*. (g) LM, scale bar: 20µm. (h) subacute end observation under TEM, scale bar: 2µm. (i) Central nodule and valve striation clearly observed under TEM, scale bar: 2µm.

(Figure 6, Table 6). According to Round *et al.* (1990), this species was previously included in the genus *Nitzschia*. Reimann & Lewin (1964) transferred this species to *Cylindrotheca* based on the raphe structure (TEM) and the weakly silicified valves.

Three species of *Pleurosigma,* i.e *P. angulatum*, *P. spencerii* and *P. normanii* were found in this study (Figure 7, Table 7). Small populations of *Pleurosigma* commonly found in marine and brackish waters. *Pleurosigma* is solitary and slightly sigmoid especially near the tips. A central raphe also becomes sigmoidal near the ends of the valve. The raphe sternum is narrow and not centrally expanded (Hasle *et al.*, 1997). The striae structure are so fine that they can only be seen with LM on cleaned diatoms mounted in a medium of high refractive index. *Gyrosigma* have similar apparent with *Pleurosigma* by observation under LM. These two genera can only distinguish by striae system under electron microscopy. *Gyrosigma* was separated from *Pleurosigma* by having longitudinal and transverse striae with no oblique striae.

Based on Shamsudin (1990), 74 species of diatom had been reported from Sarawak waters. In coastal water of Sarawak, 16 pennate diatom species had been identified. Five genera was commonly found in these location were *Navicula, Pleurosigma, Nitzschia*, and *Surirella*. *Pleurosigma* sp. was the most commonly diatom reported with five species, followed by *Navicula, Nitzschia* and *Surirella* with two species identified in each genus. *Amphiprora* sp. was not reported

in this area.
The diatom densities were generally higher in the east coast of Peninsula Malaysia after the NE monsoon (SEAFDEC, 1999). In pennate diatom, *Pleurosigma* sp. was more common in the coastal areas of the Gulf of Thailand, while *Cylindrotheca closterium* were abundant in the offshore areas. *Pleurosigma* sp. had the highest relative abundance of up to 91.4% at some stations in the Upper Gulf (SEAFDEC, 1999). Others pennate diatoms species were reported for the first time in this area.

The taxonomy and ecology of the diatoms of northern South China Sea was reported by Dickman *et al.* (1999). *Pleurosigma, Navicula*, *Nitzschia* and *Surirella* were commonly found along the coast of Southern China. About 15 species of *Pleurosigma* was reported. *Nitzschia longissima* is one of *Nitzschia* species that most commonly observed from the South China Sea, however *N. obtusa* and *N. panduriformis* were less commonly observed in the samples.

Navicula directa was most commonly Navicula species observed from the Southern coast of China. According to Dickman *et al.* (1999). *Amphiprora alata* was observed along the coast of Fujian Province in China and in marine sediment cores collected from Hong Kong *Cylindrotheca closterium* is often found in the plankton, especially when storms produce strong currents of muddy water. *Delphineis surirella* was commonly encountered in samples from the South China Sea between the straits of Taiwan and Hong Kong (Dickman et al 1999).

The distributions and abundances of diatom species in water column are depending on environmental factors, such as currents, light, and nutrients. According to Pentecost (1984), diatoms are known to be the successful class inhabiting all types of habitats. Fine morphological structures studies of other diatom species from various locations currently on going to provide comprehensive documentation of diatom species in Malaysian waters.

Phytoplankton is important component in the coastal ecology. With the increased in anthropogenic activities and coastal eutrophication, phytoplankton will be affected not only in term of abundance but also composition. Precise identification of phytoplankton especially diatom species is important in monitoring the changes of coastal environments.

Table 1 Morphometric data of *Amphiprora* species (Shamsuddin, 1990) and species found in this study (in bracket).

Species	***A. gigantea***	***A. alata***
Puncta in 10 µm	13-15 (14)	- (12)
Striae in 10 µm	13-14 (14)	- (12)
Apical axis	~97 (~92)	60-160 (~106)
Transapical axis	- (18)	30-60 (25)
Median line	sigmoidal	straight

Table 2 Morphometric data of *Surirella* species (Shamsuddin, 1990) and species found in this study (in bracket).

Species	*S. fluminensis*	*S. fastuosa*	*S. norvegica*
Apical axis (µm)	44-56(53)	~65(52)	~260(236)
Transapical axis (µm)	28-36(32.6)	- (25)	~55 (67)
Valve shape	Ovate	Lanceolate to ovate	Ovate with round apical end
Canaliculi	~10 (13)	10-12 (14)	40-45 (~40)
Center valve	Narrow median line	Long median line	Long median line

Table 3 Morphometric data of *Delphineis* species (Hasle *et al.*, 1997) and species found in this study (in bracket).

Species	*D. karstenii*	*D. surirelloides*
Apical axis (µm)	27-86 (~30)	14-40 (~12)
Transapical axis (µm)	6-7 (7.25)	5.5-7.5 (5.8)
Striae in 10 µm	8-10 (14)	12-14 (14)
Areolae in 10 µm	- (17)	- (16)
Valve structure	Linear with rounded apices to slightly inflated in center, wide sternum	Linearly elliptical with broadly rounded apices
Sternum	Usually wide sternum	Variable width of sternum, widening slightly near apices

Table 4 Morphometric data of *Navicula* species (Hasle *et al.*, 1997) and species found in this study (in bracket).

Species		*N. distans*
Apical axis (µm)		70-130 (62.5)
Transapical axis (µm)		14-20(14.75)
Striae in 10 µm	**Tranverse**	5-6 (-)
	Longitudinal	- (-)
Striae structure		Radiate
Valve structure		Lanceolate

Table 5 Morphometric data of *Nitzschia* species (Hasle *et al.*, 1997), (Dickman *et al.*, n.d) with * symbol, and specimen found in this study (in bracket).

Species	***N. longissima***	***N. obtusa* ***	***N. panduriformis* ***	***N.* cf *amphibian* ***
Apical axis (µm)	125-450 (407)	- (63.8)	-	- (8.5)
Transapical axis (µm)	6-7 (8)	- (5.6)	-	- (2.7)
Fibulae in 10µm	6-14 (11)	- (9)	-	- (18)
Striae in 10µm	52-60 (48)	- (20)	-	- (14)
Striae structure	Transverse striae	Transverse striae	-	Transverse striae
Valve structure	Linear to lanceolate and tapering to very long projection	Long projection with eccentric end	Elips with constriction in the middle	Linear to lanceolate with rostrate end

Table 6 Morphometric data of *Cylindrotheca* species (Hasle *et al.*, 1997) and species found in this study (in bracket).

Species	***Cylindrotheca closterium***
Apical axis (µm)	30-400 (92.5-150)
Transapical axis (µm)	2.5-8 (5.3-5.8)
Fibulae in 10µm	10-12 (12)
Interstriae in 10µm	70-100 (-)

Table 7 Morphometric data of *Pleurosigma* species (Hasle *et al.*, 1997 and Shamsuddin, 1990) and species found in this study (in bracket).

Species		*P. angulatum*	*P. spencerii*	*P. normanii*
Apical axis (µm)		110-115 (150)	~120 (135)	90-220 (204)
Transapical axis (µm)		18-20 (24)	~10 (15.8)	28-36 (24)
Striae in 10 µm	**Tranverse**	18-21 (19)	18-22 (26)	19-22 (21)
	Oblique	18-21 (19)	18-22 (22)	16-19 (20)
Valve structure		Rhombus and lanceolate	Straight and lanceolate	Sigmoid and lanceolate
Apical end shape		Lanceolate	Rostrate	Subacute end

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REFERENCES

Andrew, G. W., 1977: Morphology and stratigraphic significance of *Delphineis*, as new marine diatom genus. *Nova Hedwigia*, Beih. **54**: 243-260.

Cheng, Z. D., Gao, Y. H. & Dickman, M. D. 1996. Colour Plates of the Diatoms.China Ocean Press, Beijing, China, 120pp.

Chua, T. E. & Chong , B. J. (1973). Plankton distribution in the straits of Malacca and its adjacent waters. *Special Symposium on Marine Science,* Dec. 1973, Hong Kong.

Cox, E. J. & Ross, R. (1981). The striae of pennate diatoms. *In* "Proceedings of the 6th Symposium on Recent and Fossil Diatoms" (R. Ross, ed), pp 267-278. Koeltz, Koenigstein.

Dickman, M. D., Hodgkiss, I. J. & Cheng, Z. D. (1999). The Diatoms of the South China Sea. Ecology and Biodiversity Department, The University of Hong Kong, Hong Kong. CD-ROM

Edna, G. & Jefferson, T. T. (Eds.), (2001). *Ecology of Harmful Algae.* Springer-Verlag, The Netherlands.

Harold, C. B., (1978). Introduction to the Algae: structure and reproduction, (2nd Edition). Prentice-Hall, Inc., New Jersey.

Hasle, G. R. & Syvertsen, E. E. 1996. Marine diatoms. In Tomas, C. R. [Ed.] *Identifying Marine Diatoms and Dinoflagellates*. Academic Press, San Diego, California, pp.5–385.

Hicks Y. A., Marshall D., Rosin P. L., Martin R. R., Mann D. G. & Droop S. J. M. (2006). A model of diatom shape and texture for analysis, synthesis and identification. *Springer-Verlag.* 17:297-307.

Hilaluddin, F., Leaw, C.P. & Lim, P. T. 2010. Fine structure of the diatoms *Thalassiosira* and *Coscinodiscus* (Bacillariophyceae): Ligh and electron microscopy observation. *Annals of*

Microscopy. 10:28-35.

Hustedt, F. (1930). Bacillariophyta (Diatomeae). In Pascher, A. [Ed.] *Die Su¨sswasser-Flora Mitteleuropas*, Heft 10. Gustav Fischer, Jena, Germany, 466 pp.

Kützing, F. T. (1844). *Die kieselschaligen Bacillarien oder Diatomeen.* Nordhausen. 152pp.

Lu, J., Chen, M. and Chen, Z. (2006). Distribution of Diatoms in the Water and Surface Sediments of Southern South China Sea. *Chinese Science Bulletin*. 51: 76-80.

Mann, D. G. 1981. A new species of sigmoid *Nitzschia* (Bacillariophyta). *Israel J. Bot.* 30:1 10.

Mann, D. G. 1984. An ontogenetic approach to diatom systematics. In Mann, D. G. [Ed.] *Proceedings of the 7th International Diatom Symposium*. O. Koeltz, Koenigstein, Germany, pp. 113–44.

Medlin, L. K. & Kaczmarska, I. 2004. Evolution of the diatoms: V. Morphological and cytological support for the major clades and a taxonomic revision. *Phycologia* 43:245–70.

Nobel web AB (2008). *The Transmission Electron Microscope*. Accessed on 23 Mac 2008, from http://nobelprize.org/educational_games/physics/microscopes/tem/index.html

Patrick, R. & Reimer, C. W. (1966-1975). *The diatoms of the United States.* 2:13.

Philip, S., (1993). *A Biology of Algae.* (2nd Edition). Wm. C. Brown Publishers.

Reid. G & Williams D. M. (2007). Some commentary on molecules and morphology, species and higher taxa in diatoms, with a note on the relationships of the genus *Cistula Cleve*.

Round F. E., Crawford R. M. & Mann D. G. (1990). *The Diatoms: Biology & Morphology of the Genera.* Published by Cambridge University Press, 1990.:27-28

Schütt, F. 1896. Bacillariales. In Engler, A. & Prantl, K. [Eds.] *Die Natrülichen Pflanzenfamilien*, Vol. 1. Wilhelm Engelmann, Leipzig, Germany, pp. 31–153.

Shamsudin L. (1990). *Diatom marine di Perairan Malaysia.* Dewan Bahasa dan Pustaka, Kuala Lumpur.

Simonsen, R. (1979). Fifth symposium on recent and fossil diatoms. Antwerp, 1978. Proceeding. *Nova Hedwigia, Beih.* 64:533 pp.

Werner, D. (Ed.) (1977). *The biology of diatoms.* Oxford. Blackwell. 498 pp.