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Pure Cultivation and Morphological Studies of Four *Chaetoceros* Taxa from the Coastal Waters of Pahang, Malaysia

(Pengkulturan Tulen dan Kajian Morfologi Empat Takson *Chaetoceros* dari Persisiran Pantai Pahang, Malaysia)

M.A.M. FUAD*, N. MOHAMMAD-NOOR, A.K.C. JALAL & B.Y. KAMARUZZAMAN

ABSTRACT

This study was conducted to study the morphological features of species under the genus Chaetoceros *isolated from the coastal waters of Pahang, Malaysia. The species were isolated, cultivated and viewed under light microscope (LM) and scanning electron microscope (SEM). Four taxa were successfully isolated and cultivated in pure culture, namely* Chaetoceros affinis *var.* affinis, Chaetoceros affinis *var.* willei, Chaetoceros anastomosans *and* Chaetoceros baculites. *Both varieties of* C. affinis *were considered as distinct taxa where both strains can be distinguished based on morphological characteristics*. C. affinis *var.* affinis *has a thick cell wall compared with* C. affinis *var.* willei. C. anastomosans *has special features including a silica bar on the intersection of setae and the production of mucous. The length of the aperture opening is a new record from this study. The new record obtained for* C. baculites *includes the size of the aperture, the terminal setae, spinal arrangement, the thinness of the cell wall and the location of the species in tropical waters. Detailed data on each species will be added to the taxonomic information for future studies.*

Keywords: Chaetoceros*; coastal water; LM; morphology; SEM*

ABSTRAK

Kajian ini dijalankan untuk mengkaji ciri morfologi spesies di bawah genus Chaetoceros *yang diasingkan dari pesisir pantai Pahang, Malaysia. Kesemua spesies dipencil, dikultur dan diperiksa melalui miskroskop cahaya (LM) dan mikroskop elektron imbasan (SEM). Empat takson telah berjaya dipencilkan dan dikulturkan kepada kultur tulen, iaitu* Chaetoceros affinis *var.* affinis, Chaetoceros affinis *var.* willei, Chaetoceros anastomosans *dan* Chaetoceros baculites. *Kedua-dua varieti* C. affinis *dikategorikan sebagai takson terdekat dengan kedua-dua strain boleh dibezakan berdasarkan ciri morfologi*. C. affinis *var.* affinis *mempunyai dinding sel yang tebal berbanding* C. affinis *var.* willei. C. anastomosans *mempunyai ciri-ciri istimewa iaitu bar silika di persilangan seta dan penghasilan mukus. Panjang bukaan merupakan rekod terbaru di dalam kajian ini. Rekod terbaru diperoleh bagi* C. baculites *yang merangkumi saiz bukaan, setae utama, susunan duri dan kenipisan dinding sel dan spesies ini dijumpai di kawasan tropika. Keperincian data untuk setiap spesies akan ditambah ke dalam informasi taksonomi untuk kajian masa hadapan.*

Kata kunci: Chaetoceros*; LM; morfologi; pesisiran pantai; SEM*

INTRODUCTION

The genus *Chaetoceros* is one of the largest cosmopolitan marine phytoplankton genera (Be´rard-Therriault et al. 1999) and among the most important genera in marine planktonic diatoms. The genus includes both neritic and oceanic species. There are no true freshwater species, but some species occur at very low estuarine salinities and in inland saline lakes (Jensen & Moestrup 1998). The genus *Chaetoceros* lives in coastal areas, producing high biomass comprising very long chains of cells and magnificent resting spores (Jensen & Moestrup 1998). Due to this, *Chaetoceros* has been listed as a major contributor to primary production in near-shore upwelling regions and coastal areas (Rines & Theriot 2003), where it contributes approximately 20-25% of the total marine primary production (Jensen & Moestrup 1998).

According to Simonsen (1974), the genus *Chaetoceros* belongs to the family Chaetoceraceae, which includes two other genera, *Bacteriastrum* and *Acanthoceras*, in the suborder Biddulphiineae, order Centrales and class Bacillariophyceae. However, according to Gran (1897), the genus *Chaetoceros* has been divided into two subgenera: *Phaeoceros* (which contains chloroplasts in the setae) and *Hyalochaete* (which does not contain chloroplasts in the setae). The genus *Chaetoceros* was first described by Ehrenberg in 1844 (Rines 1999). As many as 400 species have been described, although a significant proportion of them or half of the species are not valid (Hasle & Syvertsen 1997). Under nutrient-rich conditions, most *Chaetoceros* species reproduce rapidly and form long chains of thin-walled cells by the fusion of setae. As nutrient supplies are depleted, most species form thick-walled resting spores which sink to the sea floor, where they await favourable conditions to return (Itakura 2000).

Chaetoceros has bipolar valves and setae of a structure different from the valves. *Chaetoceros* forms chains very

often and only a few are solitary (Jensen & Moestrup 1998). There are various ways of connections in the chains such as the fusion of setae, the fusion of edge valves and setae, the holding of setae, the presence of prehensors, siliceous walls and the fusion of linking spines (Jensen & Moestrup 1998). Traditionally, identification at the species level has been based on morphological features observed by light microscopy which focused on the morphology of the colonies, shape and dimensions of cells, thickness and direction of setae, number and shape of chloroplasts, and presence and morphology of resting spores. However, some other features, which can mostly be seen by electron microscope, such as the fine structures of valves and setae and the location and number of rimoportulae, are now considered to be relevant in morphological studies (Jensen & Moestrup 1998).

Identification at the species level within the genus *Chaetoceros* is not an easy task and is mainly based on gross morphology investigated by light microscopy (LM). Some morphological characters of taxonomical value can only be detected in detail using scanning electron microscopy (SEM). This paper focuses on the pure cultivation of *Chaetoceros* taxa with morphology studies within the laboratory using LM and SEM.

Materials and Methods

Samples collection

The samples of microalgae were collected using 20 µm plankton net meshes along the coastal water. The samplings were randomly selected from locations covering Pantai Cherating to Tanjung Gemok starting in August 2011 until August 2012. The samples were then kept in a polyethylene bottle, covered with newspaper and kept in an ice chest. The samples were then transported to the laboratory for isolation.

Establishment of pure culture

In the laboratory, a single cell of the phytoplankton was isolated using the one-cell isolation technique (Mohammad-Noor 2012) under compound light microscope (Leica DME). The isolation was performed in a laminar flow using micropipette or glass pipette. The isolated cell was put into 24-well plates containing 1 mL of F/2 medium (Harrison & Berges 2005). The pH of the medium was adjusted from 7.2 to 8.2, the salinity was 28±1 ppt, the pressure was within 35 PSV (normal atmosphere) and temperature of 24±1°C. For light intensity, cold lights were used with the intensity of 4000-5000 Lux (Rika Partiwi et al. 2009). During cultivation, the air flow was given for 24 h using an air aerator and the light: dark cycle was 12:12 h. After one week of cultivation in the 24-well plates, the microalgae were transferred into a conical flask containing 25 mL F/2 medium. For up scaling, 250 mL conical flasks were used to cultivate the stock culture.

Identification process

Both light microscope (LM) and scanning electron microscope (SEM) were used for identification up to the species level. For LM, identification was performed under a compound light microscope (Leica DME) at a total magnification of 40, 100, 400 and 600×. For a detailed view of the morphology, the SEM was used. The method followed Mohammad-Noor (2012). Approximately 1 mL of life samples of respective strains was fixed with glutaraldehyde with the final concentration 2%. The fixation was conducted for 40 min. Next, the samples were collected by filtering the fixed life samples into Isopore Membrane Filters, 5.0 µm TMTP, which had been placed in a Swinnex Millipore filter holder. The samples were rinsed using distilled water for 1 h. For dehydration, acetone was chosen. The series of concentrations used were 30, 50, 70, 96 and 99%. The samples were soaked in each phase for 10 min except for the last phase, during which they were soaked for 30 min. After the dehydration process, the samples were dried using a critical point dryer (CPD) machine (model CPD 030 BAL-TEC). After that, the samples were mounted on the stub using double-sided tape. Next, the samples were coated with gold using a coating machine (model LEICA EM SCD 005). The samples were then examined using Zeiss Evo 50 at magnifications of 300 to 15000×. Pictures were captured using the program SmartSEM Version V05.

Results and Discussion

Taxonomy remarks

Four taxa of *Chaetoceros* from subgenus *Hyalochaete* Gran 1987 were identified: *Chaetoceros affinis* var. *affinis, Chaetoceros affinis* var. *willei, Chaetoceros anastomosans*, and *Chaetoceros baculites*.

Subgenus: *Hyalochaete* Gran 1897

The overall species identified are neritic but a few species are oceanic. In terms of setae, the cells usually have relatively thin intercalary setae with some species seen with thicker terminal setae. However, some species also possess thick intercalary setae. All of the setae do not contain chloroplasts. The chloroplasts inside the cells varied depending on the species. The labiates or rimoportula can be detected in terminal cells. Resting spores have also been known in many species.

Chaetoceros affinis var. *affinis* Lauder 1864

Previous descriptions Lauder (1864) p. 78, pl. 8, Figure 5; Evensen & Hasle (1975) p. 161, Figures 46-54; Rines & Hargraves (1988), p. 59, Figures 113-114; Hernandez-Becerrill (1996), p. 35, pl. 27-28; Jensen & Moestrup (1998), p. 20, Figures 30-43; Bérard-Therriault et al. (1999), p. 42, pl. 22, Figure g, pl. 23, Figures b,c; Horner (2002) p. 82; Shevchenko et al. (2006), Figures 20-24;

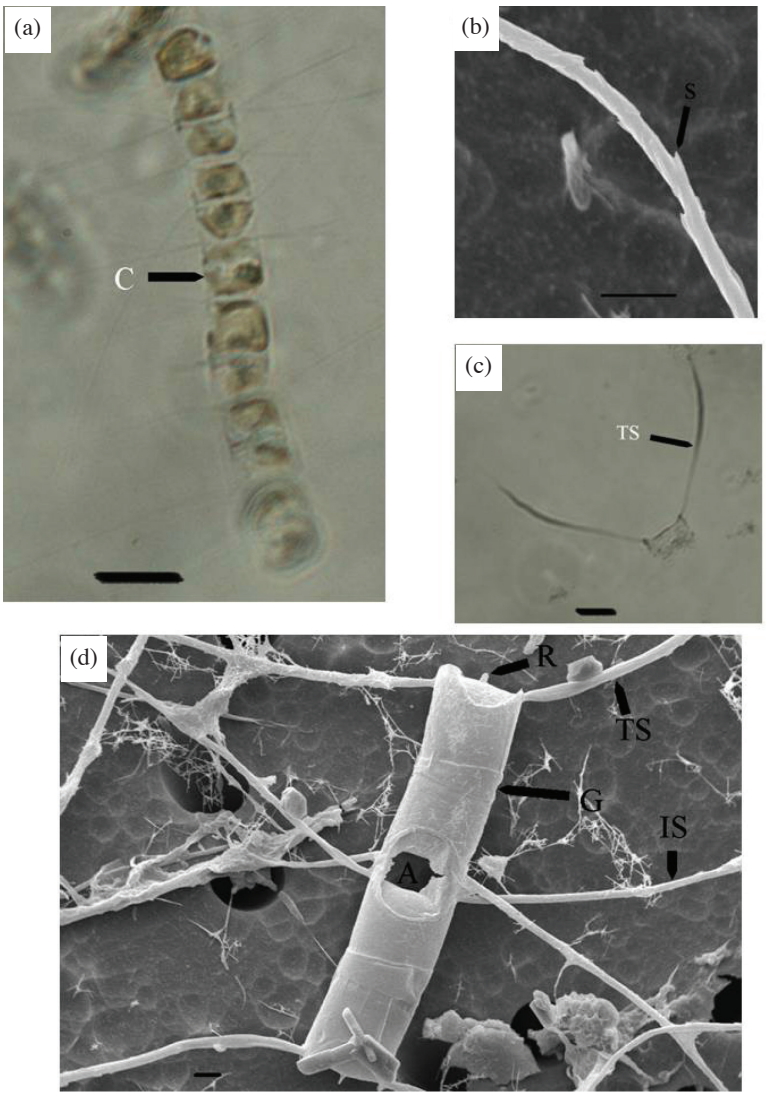

PLATE 1. *Chaetoceros affinis* var. *affinis* (a) Chain of cells with one large chloroplast (C) (Scale bar: 8 μm), (b) Spine (S) arrangements at setae (Scale bar: 2 μm), (c) Terminal setae (TS) under light microscopy (Scale bar: 8 μm) and (d) Whole cell body with aperture (A), intercalary setae (IS), girdle (G), terminal setae (TS) and rimoportula (R) (scale bar: 2 μm)

Sunesen et al. (2008), Figure 4A-C; Konno et al. (2010), Figures 3-9.

Morphological characteristics The cells form into medium to long chains (a) and have very thick cell walls. Inside the cell, one large chloroplast can be seen (a). The labiate or rimoportula appears at the centre of end cells and the cells in rectangular shape with a girdle (d). The setae are long and basal parts can be seen (d). The setae originated from the valve margin (d). Terminal setae are thicker compared with intercalary setae with a U or V-shape (c). However, the intercalary setae are moderately thick (d). At the setae, the spines are visible with a spiral arrangement (b).

Observation Brown colour appeared during cultivation. Not producing mucous.

Distribution This species was found in the South China Sea (Boonyapiwat 1998), Danish coastal waters (Jensen & Moestrup 1998), Peter Great Bay, Japan (Shevchenko et al. 2006), the Argentinian Sea, (Sunesen et al. 2008) and marine lakes (Konno et al. 2010).

CHAETOCEROS AFFINIS VAR. *WILLEI* LAUDER 1864

Previous descriptions Lauder (1864) p. 78, pl. 8, Figure 5; Cupp (1943), p. 125, Figure 78; Hendey (1964) p. 127, plate 18, Figure 3; Evensen & Hasle (1975) p. 161, Figures 46-54; Rines & Hargraves (1988), p. 59, Figures 113-114; Hernandez-Becerrill (1996), p. 35, pl. 27-28; Hasle & Syvertsen (1997) p. 216; Jensen & Moestrup (1998), p. 20, Figures 30-43; Bérard-Therriault et al. (1999), p. 42, pl. 22, Figure g, pl. 23, Figures b,c; Horner (2002) p. 82; Shevchenko et al. (2006), Figure 20-24; Sunesen et al.

TABLE 1. Comparison of morphological features of *Chaetoceros affinis* var. *affinis* with other studies

Characteristic	This Research	Previous research
Cell	Medium - to - long straight chains with thick cell walls	Medium - to - long straight chains with thick cell walls (Jensen & Moestrup 1998; Konno et al. 2010; Shevchenko et al. 2006; Sunesen et al. 2008)
	Labiate or rimoportula at centre of end cells	Rimoportula or labiate centrally located at terminal setae (end cells) (Jensen & Moestrup 1998; Konno et al. 2010; Shevchenko et al. 2006; Sunesen et al. 2008)
	Rectangular shape with a visible girdle	Rectangular shape with girdle (Jensen & Moestrup 1998; Shevchenko et al. 2006)
	One large chloroplast	One large chloroplast (Shevchenko et al. 2006; Sunesen et al. 2008)
Cell Length	19±0.5 µm	12-30 µm (Shevchenko et al. 2006)
Aperture	8±0.5 µm (Narrow)	Very narrow (Jensen & Moestrup 1998)
Apical axis	9±1 µm	9-30 µm (Jensen & Moestrup 1998) 10-30 µm (Shevchenko et al. 2006) 16-27 µm (Sunesen et al. 2008)
Setae	Terminal setae are very thick, long and have U or V - shaped	Terminal setae are thick, long and have U or V -shaped (Jensen & Moestrup 1998; Shevchenko et al. 2006; Sunesen et al. 2008; Konno et al. 2010)
	Intercalary setae are moderately thick and long	Intercalary setae can be seen, not very thick and long (Jensen & Moestrup 1998; Konno et al. 2010; Shevchenko et al. 2006; Sunesen et al. 2008)
Spines	Visible spines arranged in spiral	Visible, large spines arranged in spiral (Jensen & Moestrup 1998; Konno et al. 2010; Shevchenko et al. 2006; Sunesen et al. 2008)

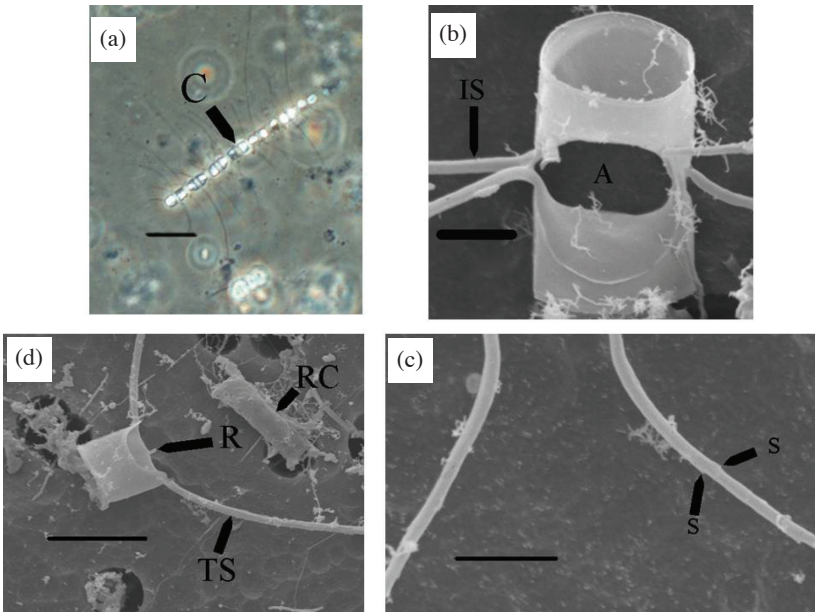

PLATE 2. *Chaetoceros affinis* var. *willei* (a). Chain of cells with chloroplasts (C) (Scale bar: 12 µm), (b) Terminal setae (TS) with view on rimoportula (R) and ruptured cell (RC) due to thin cell wall (Scale bar: 10 µm), (c) Setae with small spines (S) (Scale bar: 2 µm) and (d) Intercalary setae (IS) cross over point with valve and aperture (A) opening (scale bar: 2 µm)

(2008), Figure 4A-C; Konno et al. (2010), Figures 3-9; Asma & Saifullah (2010), Figure 2.

Morphological characteristics Most of the morphological characteristics are similar to the variety *affinis*. The cells sometimes tend to form medium to long, but mostly short, straight chains (a). The cells are delicate and fragile due to a thin cell wall (b, d). Terminal setae are slightly thicker compared with intercalary setae (b, d). One big chloroplast observed (a). Small spine arrange in spiral (c).

Observation High cell density of cell formed yellow colour. No mucous produced.

TABLE 2. Comparison of morphological features of *Chaetoceros affinis* var. *willei* with other studies

Characteristic	This research	Previous research
Cell	Medium - to - long straight chains. However, for this variation, the colonies sometimes short. Delicate and fragile due to thinness of the cell wall	Medium - to - long straight chains but sometimes short. Delicate and fragile (Asma & Saifullah 2010; Jensen & Moestrup 1998; Konno et al. 2010; Shevchenko et al. 2006; Sunesen et al. 2008)
	Labiate or rimoportula at centre of end cells	Rimoportula or labiates at centre of Terminal setae (Jensen & Moestrup 1998; Konno et al. 2010; Shevchenko et al. 2006; Sunesen et al. 2008)
	One chloroplast	One chloroplast (Jensen & Moestrup 1998; Shevchenko et al. 2006 & Sunesen et al. 2008)
Cell Length	12.5±0.5 μm	12-30 μm (Shevchenko et al. 2006)
Aperture	3.5±0.5 μm (Very Narrow)	Very narrow (Jensen & Moestrup 1998)
Apical axis	8±2 μm	9-30 μm (Jensen & Moestrup 1998) 10-30 μm (Shevchenko et al. 2006) 16-27 μm (Sunesen et al. 2008)
Setae	Intercalary setae usually long, stiff and moderately thick without basal parts. The terminal setae were U - shaped or V - shape. The thickness was the slightly thicker compared with intercalary setae	Intercalary setae usually long, stiff and moderately thick without basal parts. The terminal setae were U -shaped or V - shape and slightly thicker compared with intercalary setae (Asma & Saifullah 2010; Jensen & Moestrup 1998; Konno et al. 2010; Shevchenko et al. 2006; Sunesen et al. 2008)
Spines	Small spines arranged in a spiral	Spirally - arranged small spines (Jensen & Moestrup 1998; Konno et al. 2010; Shevchenko et al. 2006; Sunesen et al. 2008)

Distribution This species was found in western coast of North America (Cupp 1943), British coastal waters (Hendey 1964) and northwest Arabian Sea (Asma & Saifullah 2010).

CHAETOCEROS ANASTOMOSANS GRUNOW (GRAN)

Previous descriptions Grunow (1888) In Van Heurck (1880-1885), pl. 82, Figures 6-8; Jensen & Moestrup (1998), Figures 44-47; Herna´ ndez-Becerril & Granados (1998), p. 517, Figures 53, 54; Herna´ndez-Becerril & Aké-Castillo (2001), p. 57, Figures 1-6.

Morphological characteristics Long chain with more than three cells in one chain (c). The cells are rectangular in shape (a). The girdle can occupy up to 2/3 of the cells (a). Rimoportula detected centrally (e). The chains slightly curve with thick and stiff of cells and setae. The setae are long and stiff. The terminal setae form a U-shape (e). The intersection of the intercalary setae has a silica bar form to join the setae together (b). The spine appeared with a spiral arrangement (d). All of the chains are engulfed in a sheath of mucous. Inside the cells, two chloroplasts were observed (c).

Observation Milky brown colour can be seen during cultivation. Mucous produced.

Distribution This species can be found in the South China Sea (Boonyapiwat 1998), Danish coastal waters (Jensen & Moestrup 1998) and Peter Great Bay, Japan (Shevchenko et al. 2006).

CHAETOCEROS BACULITES MEUNIER

Plate 4: a-e

Previous descriptions Jensen & Moestrup (1998), Figure 48.

Morphological Characteristics The cells form a straight, narrow and fragile chain with 2-3 cells. The cells are delicate and fragile due to the thinness of the cells (d, e). The setae, originating from inside the valve edge with short basal parts, were thin and fragile. The terminal setae are long and thin (b) with the spine sparsely arranged (c). One chloroplast was observed inside the cells (a).

Observation Brown colour culture detected. No mucous appeared.

Distribution This species was found in Danish coastal waters (Jensen & Moestrup 1998)

Based on LM and SEM, two strains of *C. affinis* have been successfully isolated with several features distinguishing the two strains. *C. affinis* var. *affinis* is the strain where the major features of *C. affinis* still appeared. These major features consist of thick terminal setae where the shape is U or V-shaped, a thick cell wall with visible setae, the appearance of rimoportula and visible spines.

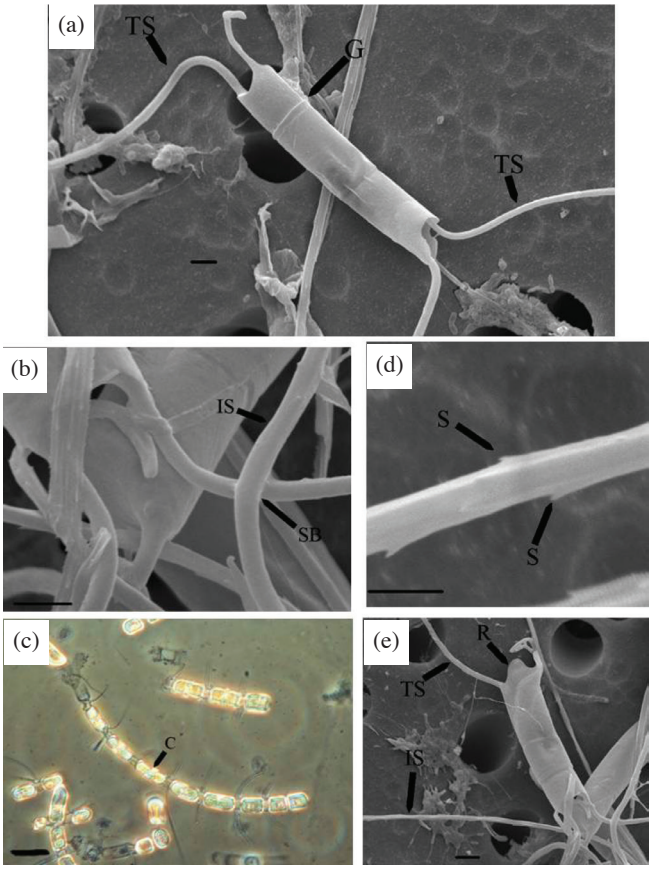

PLATE 3. *Chaetoceros anastomosans* (a) Whole cells with terminal setae (TS), girdle (G) and rectangular shape cell (Scale bar: 2 µm), (b) Intercalary setae (IS) with silica bar (SB) cross section (Scale bar: 2 µm), (c) Chain cells with two chloroplasts (C) (Scale bar: 25 µm), (d) Visible spine (S) arrangement (Scale bar: 1 µm) and (e) Cells with rimoportula (R), intercalary setae (IS) and terminal setae (TS) form U-shaped (scale bar: 2 µm)

TABLE 3. Comparison of morphological features of *Chaetoceros anastomosans* with other studies

Characteristic	This research	Previous research
Cell	Medium - to - long chains or slightly curved chains	Cells form medium - to - long straight or slightly curved chains (Jensen & Moestrup 1998; Shevchenko et al. 2006)
	Thick and stiff cells can be seen in rectangular shape with a girdle occupying up to 2/3 cells	Thick and stiff cells can be seen in rectangular shape with a girdle occupying up to 2/3 cells (Jensen & Moestrup 1998; Shevchenko et al. 2006)
	Rimoportula detected centrally	Rimoportula or labiates centrally (Shevchenko et al. 2006)
	The cells are engulfed with mucous, turning the media into mucous environment	Mucous appeared (Jensen & Moestrup 1998)
	Two chloroplasts detected inside the cells	Two chloroplasts (Jensen & Moestrup 1998; Shevchenko et al. 2006)
Cell Length	20±1 µm	10-20 µm (Shevchenko et al. 2006)
Aperture	9.2±0.5 µm	All references show no records
Apical axis	5±1 µm	6-20 µm (Jensen & Moestrup 1998) 10-16 µm (Shevchenko et al. 2006)
Setae	Intercalary setae were long and thin with long basal parts	Long and thin intercalary setae with long basal parts (Jensen & Moestrup 1998; Shevchenko et al. 2006)
	Setae or neighbouring cells connected by a silica bar	Setae connected with silica bar (Jensen & Moestrup 1998; Shevchenko et al. 2006)
	Terminal setae were long, thin and shape in U - shape curve	Terminal setae in U - shape curve with thin and long (Jensen & Moestrup 1998)
Spines	Visible spine arranged spirally	Spines spirally arranged (Shevchenko et al. 2006)

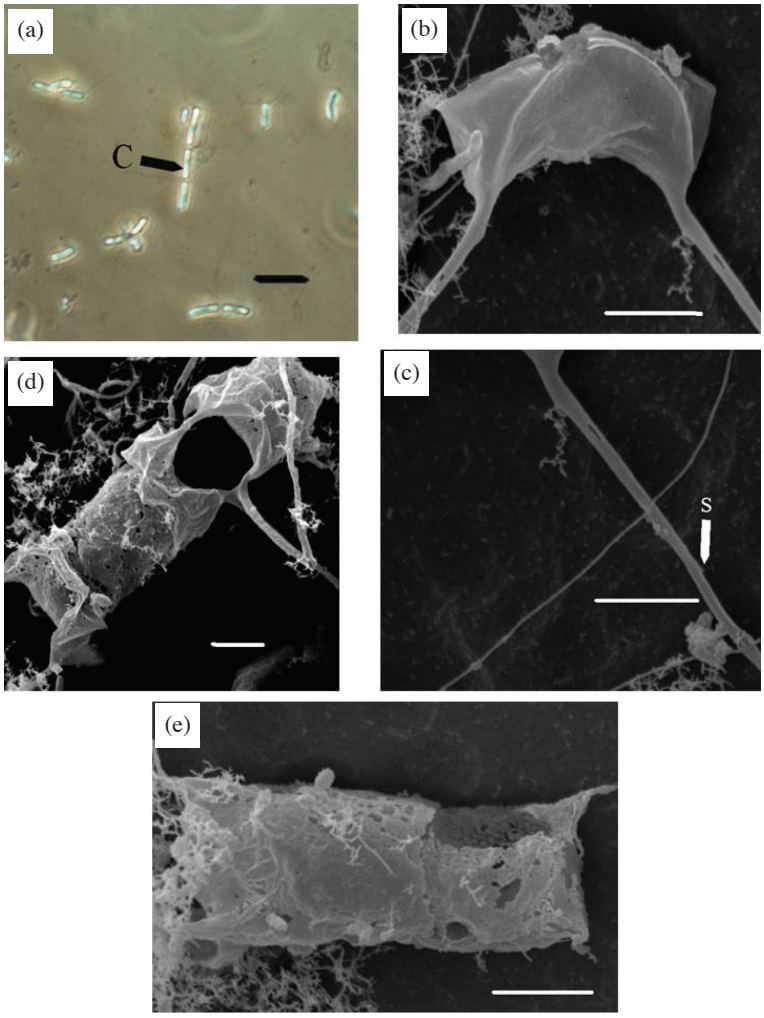

PLATE 4. *Chaetoceros baculites* (a) Light views on chloroplast (C) on the chain cells (Scale bar: 12 µm), (b) Terminal setae (Scale bar: 2 µm) (c), Spine (S) arrangement with inner skeleton (Scale bar: 2 µm), (d) Cells ruptured (Scale bar: 2 µm) and (e) Fragile cell (scale bar: 2 µm)

TABLE 4. Comparison of morphological features of *Chaetoceros baculites* with other studies

Characteristic	This research	Previous research
Cell	Straight, narrow and fragile chain cells. 2-3 cells in one chain. One chloroplast detected inside the cells. Delicate and fragile due to thinness of the cells	Cells united into straight, narrow and fragile chains. Numbers of chloroplasts are unknown (Jensen & Moestrup 1998)
Cell Length	8±0.5 µm	No records from previous study
Aperture	3.5±0.5 µm	No records from previous study
Apical axis	5±1 µm	No records from previous study
Setae	Originate from inside the valve edge with short basal parts. The setae were thin and fragile. Terminal setae are long and thin	Setae thin, fragile, and originate inside the valve edge with short basal parts. Terminal setae unknown (Jensen & Moestrup 1998)
Spines	Sparsely - arranged spine	No records from previous study

Unlike var. *affinis*, *C. affinis* var. *willei* has very delicate and fragile cell wall causing the cell to easily rupture during the SEM process. This is due to the thinness of the cell wall. Moreover, the spines have become much smaller due to this characteristic.

According to Jensen and Moestrup (1998), both strains could be identified as distinct taxa whereby both strains can be distinguished clearly based on morphological characteristics. In addition, both species are cosmopolitan and can be found almost in any marine waters of the world

(Shevchenko et al. 2006). During cultivation, var. *willei* formed a yellowish-brown culture, whereas var. *affinis* formed a pure brown culture. The genus *Chaetoceros* is a brown microalgae and the pigment fucoxanthin is the dominant pigment. Thus, a brown-coloured culture is common condition during cultivation. However, a slightly different colour can be observed due to a natural chemical or product stored inside the cells.

Chaetoceros affinis var. *affinis* has very distinct features that can be easily identified under SEM and light microscopy. Based on the available references, morphological characteristics such as rimoportula, chloroplast, setae, spinal arrangement, cell length and aperture opening are in agreement with other studies. However, for the apical axis, the length is shorter compared with other references. The average length in this study is approximately 8 µm with a longest length of 10 µm, whereas other references have recorded between 9 to 30 µm. The differences in the length at the apical axis may due to culture conditions.

For *C. affinis* var. *willei*, some features that can be seen such as rimoportula and chloroplast are consistent with other descriptions, associated with *C. affinis*. However, with the differences in thinness, this strain fits the description of var. *willei*. The cell length and aperture opening are acceptable for the taxonomical criteria of *C. affinis*. However, var. *willei* has a shorter apical axis length (8±2 μm) compared with other references, which have recorded lengths ranging from 9 to 30 µm. We can conclude that culture conditions influence the length of the apical axis in the same way they influence var. *affinis*.

Chaetoceros anastomosans has its own special features that distinguish this species from other *Chaetoceros* taxa. The appearance of a silica bar on the setae intersection and the production of mucous are the special criteria. The cells can be observed as medium to long chains with slightly curved cells and a very thick cell wall. Based on the results, this species has the longest cell length and largest aperture opening compared with other species identified in this study. The cell parts, setae and spinal arrangement are consistent with previous descriptions. The apical axis, which is 4 to 6 µm, is very short in length compared with species reported by Jensen and Moestrup (1998) (6-20 µm) and Shevchenko et al. (2006) (10-16 µm). This may be because most of the literature is based on species collected from temperate areas even though this species is also reported in tropical and warm environments (Shevchenko et al. 2006). Thus, the length of the aperture opening of *C. anastomosans* (9.2±0.5 µm) is a new record from this study and can be added to represent tropical and warm environments.

Chaetoceros baculites has so far been identified from Danish coastal waters (Jensen & Moestrup 1998). The morphological description of this species is still limited. In this study, new data obtained on the size of the aperture, the terminal setae and spinal arrangement will add to the available knowledge. This species is fragile and very delicate compared with *C. affinis* var. *willei* which has an aperture size of 3.5±0.5 µm. The terminal setae are long and thin with the thickness similar to intercalary setae. The seta is very thin with a spine arranged sparsely and only two to three cells can be seen in one chain. This is a new record of this species in tropical and warm waters.

CONCLUSION

In conclusion, four *Chaetoceros* taxa have been identified and cultivated in this study. Each species has its own morphological characteristics that distinguishes them from one to another. The morphology criteria for both varieties of *C. affinis* and *C. anastomosans* are consistent with previous studies. For *C. baculites*, this is the second record after the first record in the Danish coastal waters. Detailed data on the size of the aperture, the terminal setae and spinal arrangement of *C. baculites* will be added as a new description of this species.

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M.A.M. Fuad*
Institut Kesihatan Umum, Kementerian Kesihatan Malaysia
Jalan Bangsar, 50590 Kuala Lumpur
Malaysia

N. Mohammad-Noor, A.K.C. Jalal & B.Y. Kamaruzzaman
Kulliyyah of Science, International Islamic University Malaysia
Jalan Istana, Bandar Indera Mahkota
25200 Kuantan, Pahang Darul Makmur
Malaysia

*Corresponding author; email: crystal_fuad@yahoo.com.sg

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