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Observations of several cryptomonad flagellates from China Sea by scanning electron microscopy

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Abstract Three species and one variety of cryptomonad flagellates belonging to three genera were observed with the scanning electron microscope in sea water samples from Tolo Harbor (Hong Kong), Changjiang River Estuary and Xiamen Harbor. They are *Hemiselmis* sp. Novarino, *Plagioselmis prolonga* Butcher ex Novarino, Lucas & Morrall, *Plagioselmis prolonga* var. *nordica* Novarino, Lucas & Morrall and *Teleaulax acuta* (Butcher) Hill. The taxonomic characteristics, ecological habit and distribution of the above species are described and the LM and SEM photographs of the species are also presented. This is the first record of the genus *Hemiselmis* Parke in the China Sea, and the species *Plagioselmis prolonga* and *Teleaulax acuta* have records of producing blooms in the China Sea.
Key words China, cryptomonad, *Hemiselmis* Parke, *Hemiselmis* sp., marine, new record, *Plagioselmis prolonga*, *Plagioselmis prolonga* var. *nordica*, *Teleaulax acuta*.

Cryptomonad flagellates are widespread and abundant in the sea, especially in the pelagic environment (Haigh et al., 1992; Novarino, 2005), where their photosynthetic ability contributes significantly to primary productivity (Robinson et al., 1999). In addition, they are of great evolutionary importance owing to the presence of the nucleomorph (Lucas, 1970), and are environmentally important because some cryptomonads can form nuisance blooms in coastal areas (Andreoli et al., 1986; Dame et al., 2000). By virtue of their small size (mostly less than 20 μm), unambiguous cryptomonads identification generally cannot be accomplished by light microscopy. The taxonomic importance of cryptomonads in marine ecosystems and their contribution to marine primary productivity were largely ignored until the cryptomonads diversity in Adriatic Sea was investigated for the first time (Butcher, 1967). From his study, Butcher (1967) recognized 47 marine species from British coastal waters by light microscopy. Subsequently, with the recent applications of electron microscopic techniques in the study of cryptomonad flagellates, a large number of previously unknown cellular features have been revealed, many of which are important taxonomic characters within the cryptomonads (Klaveness, 1985; Hill, 1991a, b; Novarino & Lucas, 1993a; Clay et al., 1999; Novarino, 2003).

So far, the taxonomic studies of marine cryptomonads have been carried out only in a few countries (Butcher, 1967; Throndsen, 1976; Hill, 1991a; Novarino et al., 1997; Barlow & Kugrens, 2002; Novarino, 2003, 2005; Cerino & Zingone, 2006), and there was a first attempt at investigating cryptomonads diversity by Novarino (2005) using SEM. In China, only Hu et al. (2002) reported six species of the genus *Cryptomonas* Ehrenberg (Cryptophyceae) from China Sea, and the genus *Cryptomonas* has long been recognized as being exclusively freshwater. This situation resulted in the taxonomy of cryptomonad flagellates in China relatively understudied. This study reports several taxa from China Sea, the genera *Plagioselmis* Butcher emend. Novarino Lucas & Morrall, *Hemiselmis* Parke and *Teleaulax* Hill, and the species and/or varieties *Plagioselmis prolonga* Butcher ex Novarino, Lucas & Morrall, *P. prolonga* var. *nordica* Novarino, Lucas & Morrall, *Hemiselmis* sp. Novarino and *Teleaulax acuta* (Butcher) Hill. This is the first record of *Hemiselmis* Parke in Chinese water, and the only other report of *Plagioselmis prolonga* and *Teleaulax acuta* in China was in a marine environmental monitoring report of Hong Kong waters (Agriculture, Fisheries and Conservation Department of Hong Kong, 2006).

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1 Material and methods

Water samples were collected by the Environment Protection Department on May 8 and June 5, 2006 from Tolo Harbor, Hong Kong, China, by Xiaoli Xing on June 6, 2004, from Xiamen Harbor, Xiamen, China, and on May 20, 2005, from Changjiang (Yangtze) River Estuaries of East China Sea in China. Samples were prepared for examination using scanning electron microscopy (SEM). Fresh samples were fixed by 1% Lugol's solution and collected on 2 μm pore size nucleopore filters and rinsed with pure redistilled water for 10 minutes, then dehydrated in a series of 30%, 50%, 70% and 95% ethanol. The cells were submerged in each dilution of ethanol for 15 minutes. Each sample was then critical point dried with liquid CO_2. The dried samples were sputter-coated with gold for 25 seconds and examined with a LEO 1530 scanning electron microscope.

Cell count samples were condensed from 500 mL to a final volume of 10 mL by settlement for more than 48 h. Then 1 mL samples were used for cell counting. The samples were counted under a light microscope, Olympus BH-2, and the counting chamber for phytoplankton was used.

2 Results

1. Plagioselmis Butcher emend. Novarino, Lucas & Morrall in Cryptogamie Algologie 15: 90. 1994.

斜片藻属

Description: Cells with flagella inserted apically or subapically from a shallow anterior depression, the vestibulum; presence of the furrow; with a characteristically acute cell posterior (tail); and the presence of a non-plated periplast on the tail and the hexagonal plated periplast on the main portion of the cell body; marine and freshwaters.

Type: *Plagioselmis prolonga* Butcher ex Novarino, Lucas et Morrall.

Plagioselmis prolonga Butcher ex Novarino, Lucas & S. Morrall in Cryptogamie-Algol. 15: 90. 1994; Butcher in Fish. Invest. London, Ser. 4: 18. 1967; D. R. A. Hill in Ann. Bot. Fenn. 29: 165. 1992a; M. Kuylenstierna & B. Karlson in Botanica Mar. 37: 22. 1994.

伸长斜片藻 Figs. 1–9

Cells are 5–7 μm long and 3–3.7 μm wide. The cell anterior is rounded whereas the posterior end is acute (Figs. 1–4). Two slightly subequal flagella are inserted subapically into the right side of the vestibulum (which is a shallow anterior depression), the longer (dorsal) flagellum is approximately as long as the cell (Fig. 6). A relatively acute cell posterior- tail is present. A periplast composed of hexagonal plates on the main portion of the cell body and a non-plated periplast on the tail are present (Figs. 5–7). The tail is 1/5–1/3 of the cell length. Ventral furrow is present, and extends to about 1/2 of the cell length (Fig. 7), and a mid-ventral band extends from the tip of the tail to the base of the furrow (Figs. 8, 9).

The abundance of *Plagioselmis prolonga* reached 4.4×10^6 cells/L in our samples from Tolo Harbor, Hong Kong, on May 8, 2006, and decreased by 10^2 times, i.e. 3.6×10^4 cells/L on June 5, 2006.

Habitat: Marine.

Distribution: This species was observed from the samples of Tolo Harbor and Changjiang River Estuary. Previous records include the North Atlantic: British coastal waters (Butcher, 1967), Gulf of Saint-Laurent, Canada (Bérard-Therriault et al., 1999), Irish Sea and oyster basins south of La Rochelle, France (Novarino, 2005); Pacific Ocean: Salton Sea, California (Barlow & Kugrens, 2002); Baltic Sea (Hill, 1992a); Mediterranean Sea: Adriatic Sea (Po river delta lagoon) (Andreoli et al., 1986), Sea of Alboran and Barcelona coast (Novarino, 2005).

Plagioselmis prolonga var. **nordica** Novarino, Lucas & S. Morrall in Cryptogamie-Algol. 15: 90. 1994; G. Novarino et al. in J. Plankt. Res. 19: 1096. 1997; L. Bérard-Therriault et al. in Can. J. Fish. Aquat. Sci. 128: 249. 1999; G. Novarino in Nord. J. Bot. 11: 602. 1991a.

伸长斜片藻诺尔变种 Figs. 10, 11

The variety *nordica* is distinguished from the typical variety by the absence of a furrow on the

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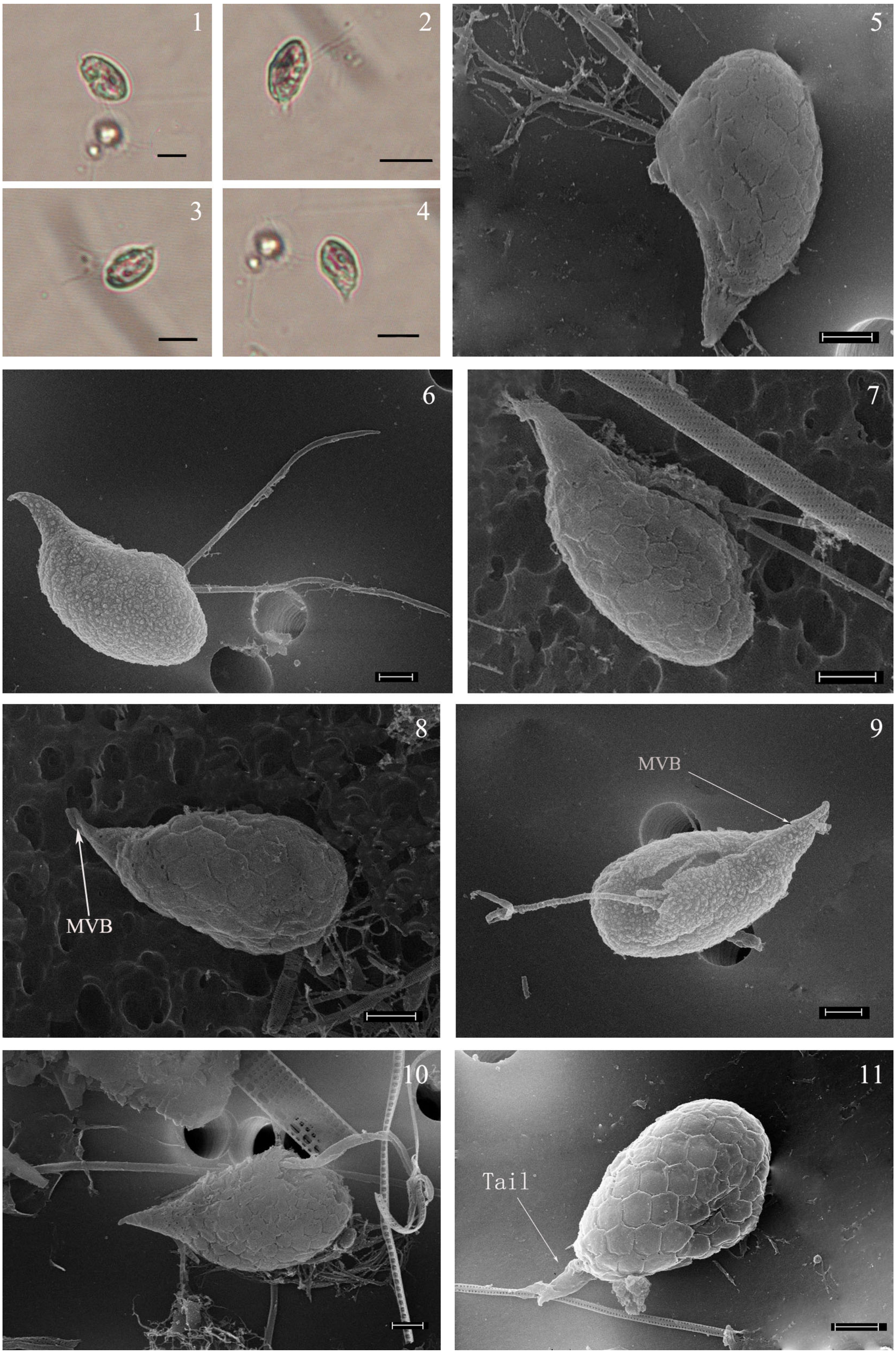
Figs. 1–11. **1–9.** The cell of *Plagioselmis prolonga* (Figs. 1–4 are LM and others are SEM). **1.** A cell from the field, dorsal view. **2.** A cell from the field, lateral view. **3, 4.** A cell from the field, dorsal-lateral view, and the prominent hyaline tail is visible. **5.** A cell from the field, lateral view, showing the rounded anterior end. **6.** A cell from the field, dorsal-lateral view, showing the unequal flagella. **7.** A cell from the field, ventral-lateral view. The furrow in ventral view is clearly visible, as is the hexagonal periplast plates on the main portion of the cell body, and the non-plated periplast on the posterior tail. **8.** A cell from the field, dorsal view. **9.** A cell from the field, dorsal view, showing the presence of mid-ventral band (MVB) (arrow) in the tail, extending to the base of the furrow. **10, 11.** The cell of *Plagioselmis prolonga* var. *nordica* in SEM. **10.** A cell from the field, ventral view. Note the absence of furrow. **11.** A cell from the field, dorsal view, showing the tail (arrow) and the hexagonal periplast plates in the main body of the cell.
Scale bars: 1–4=5 μm in LM; 5–9=1 μm in SEM; 10, 11=1 μm.


1
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MVB
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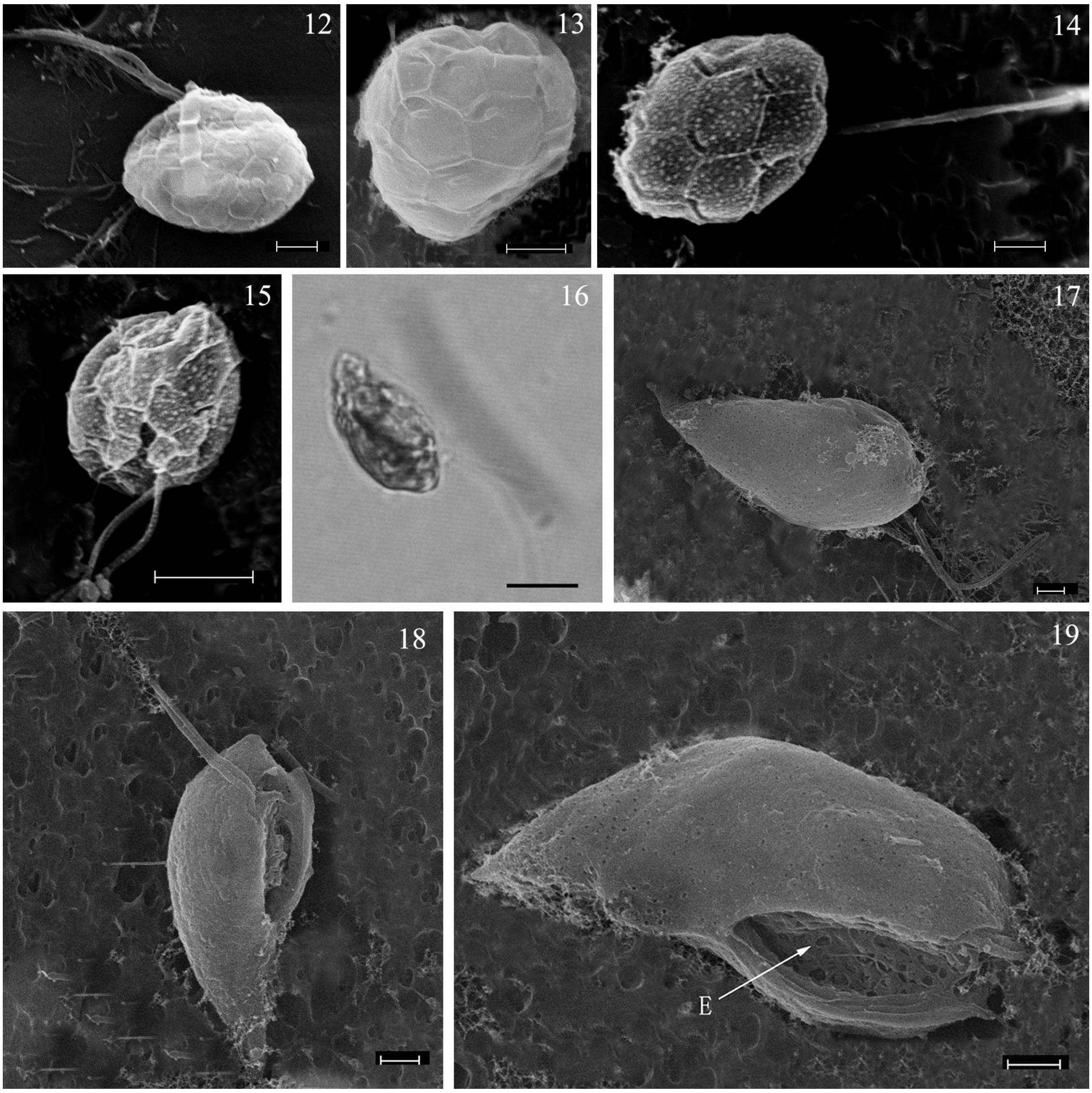
Tail



Figs. 12–19. **12–15.** The cell of *Hemiselmis* sp. in SEM. **12.** A cell from the field, dorsal view, showing the typical hexagonal plates. **13.** A cell from the field, dorsal view, showing the presence of the ridges between the periplast plates, probably the result of inadequate fixation. **14.** A cell from the field, dorsal view, showing the typical, larger and elongate hexagonal plates. **15.** A cell from the field, ventral view, showing the flagella inserted in the subapical position. **16–19.** The cell of *Teleaulax acuta*. (Fig. 16 is in LM and others are in SEM). **16.** A cell from the field, lateral-dorsal view, showing the rostrate anterior and a pointed posterior. **17.** A cell in dorsal view, showing the acute posterior end. **18.** A cell from the field, lateral-ventral view, showing the presence of rostrate anterior and the ventral furrow. **19.** A cell in ventral-lateral view, showing the presence of a long, deep furrow extending for about half of the cell length, and bearing numerous ejectosome pores (arrow).
Scale bars: 12–14, 17–19=1 μm; 15 = 2 μm; 16= 3 μm.

ventral cell surface (Figs. 10, 11).

Habitat: Marine.

Distribution: Our samples were collected from Tolo Harbor and Changjiang River Estuary. This species has been reported in the Mediterranean Sea: Adriatic Sea (Andreoli et al., 1986) and the North Atlantic: southern North Sea (Novarino et al., 1997) and St Lawrence estuary and gulf (Bérard-Therriault et al., 1999).

Note: Novarino (2005) raised the status of the variety *nordica* to a new species, *P. nordica*, mainly according to the features of cells with the point of the flagella insertion and absence of the ventral furrow. However, there is no clear difference between the variety *nordica* described here and *P. prolonga*, except absence of the ventral furrow. Hence, the variety *nordica* is still used here.

2. Hemiselmis Parke in Journal of the Marine Biological Association of the United Kingdom 28: 279. 1949.

半片藻属

Description: Cells with the rounded anterior and posterior end, dorsal-ventrally flattened, appearing reniform in lateral view; a furrow absent; periplast composed of the characteristic, larger hexagonal plates, and the sizes of the plate difference between species; marine and freshwaters.

Type: *Hemiselmis rufescens* Parke.

Hemiselmis sp. G. Novarino in Sci. Mar. 69 (1): 61. 2005.

半片藻 Figs. 12–15

Cells are 4.2–4.5 μm long and 3.2–3.4 μm wide, ovoidal in dorsal view, and the posterior and anterior ends of cells are rounded (Figs. 12, 14, 15). There are two almost equal flagella inserted in a median or subapical position (Figs. 12, 14, 15). It seems to possess a very short ventral furrow, possibly attributed to the absence of the hexagonal plates near the flagella, and no posterior tail is present (Fig. 15). The periplast is composed of the typical, larger and elongate hexagonal plates about 0.8–1.5 μm long (Figs. 12–15).

Habitat: Marine.

Distribution: Our samples were collected from Changjiang River Estuary.

Note: It could be asked if this cryptomonad should be assigned to a different genus because the feature of the periplast composed of hexagonal plates is also found in members of the genera *Plagioselmis* and *Rhinomonas* Hill, but the size of the hexagonal plate is different within the three genera. The hexagonal periplast plates of *Hemiselmis* are larger than those of *Plagioselmis*, and are elongate (Barlow & Kugrens, 2002). The cells of *Plagioselmis* have a characteristic tail with non-plate, but the cells of *Hemiselmis* have no tail and larger hexagonal plates are exhibited on all surfaces of the cells. The plates of *Rhinomonas* were also hexagonal but usually smaller than those of *Plagioselmis*. So, the typical features of the larger hexagonal periplast plates and no tail, as well as the bean-like or reniform shape of the cells, made it possible to distinguish *Hemiselmis* sp. from other cryptomonads.

3. Teleaulax Hill in Phycologia 30: 177. 1991a.

全沟藻属

Description: Cells with an acute posterior and a wide furrow on the ventral face reaching well into the median region of the cell; marine.

Type: *Teleaulax acuta* (Butcher) Hill = *Cryptomonas acuta* Butcher

Note: The genus *Teleaulax* was removed from the genus *Cryptomonas* by Hill (1991a) because the features of *Teleaulax* were different from the typical characteristics of *Cryptomonas*. Cells of the former have a long furrow and no plate-type periplast exists.

Teleaulax acuta (Butcher) D. R. A. Hill in Phycologia. 30 (2): 177. 1991a; D. R. A. Hill in Ann. Bot. Fenn. 29: 173, 174. 1992b; L. Bérard-Therriault et al. in Can. J. Fish. Aquat. Sci. 128: 250. 1999. ——*Cryptomonas acuta* G. Butcher in J. Mar. Biol. Assoc. UK. 31 (1): 188 1952; G. Novarino in Nord. J. Bot. 11: 602. 1991a; G. Novarino et al. in J. Plankt. Res. 19: 1094. 1997. Non *Cryptomonas acuta* sensu F. H. Chang in N. Z. J. Mar. Freshwater Res. 17: 291. 1983.

尖尾全沟藻 Figs. 16–19

Cells are 5.6–8.4 μm long and 2.8–3.7 μm wide, with a strongly rostrate anterior and a pointed posterior (Fig. 16). There are two equal flagella about 2/3 the cell length. They possess a characteristically acute posterior end (Fig. 17), and a long furrow that extends longitudinally from the vestibulum, extending roughly halfway along the cell length and bearing numerous ejectosome pores, usually four longitudinal rows of large ejectosomes (Fig. 19). The periplast does not have the contour of a plate-type periplast (Figs. 17–19). There are many small ejectosomes on the entire cell surface, including the posterior end (Figs. 17–19).

Habitat: Marine.

Distribution: Our samples were collected from Tolo Harbor and Changjiang River Estuary. This species has been previously found in the Mediterranean Sea: Adriatic Sea, port of Barcelona (Novarino, 2005) and the North Atlantic: Baltic Sea (Hill, 1992b), the southern North Sea (Novarino et al., 1997), and the St. Lawrence estuary and gulf (Bérard-Therriault et al., 1999), Victoria, Australia (Hill, 1991a).

3 Discussion

Because the samples in this investigation were collected in 2004, 2005, and 2006, and fixed with Lugol's solution in the field, the study of isolation and culture of the living cells, and the identity of pigment and the molecular phylogeny could not be done. Therefore, the identification in this paper was based mostly on the main features of the periplast revealed by SEM.

The shape of the periplast plates has been used extensively as a taxonomic feature at the generic level (Santore, 1984; Kugrens & Lee, 1987; Hill & Wetherbee, 1988, 1989, 1990; Hill, 1991a, b; Novarino, 1991a, b; Novarino & Lucas, 1993a, b, 1995; Novarino et al., 1994; Clay et al., 1999; Kugrens et al., 1999; Novarino, 2005; Cerino & Zingone, 2006). The particular feature of the genus *Plagioselmis* with the presence of a non-plated periplast on the tail and the hexagonal plated periplast on the main portion of the cell body make it possible to distinguish all members of this genus from other cryptomonads (Novarino, 2005; Cerino & Zingone, 2006). The diagnostic features of the genus *Plagioselmis* are shown in figures 5–11. The features of *P. prolonga* shown in figures 5–9 are consistent with the descriptions and features shown in other publications (Novarino et al., 1994, 1997; Novarino, 2003, 2005; Cerino & Zingone, 2006). The variety *nordica* is differentiated from *P. prolonga* with absence of the ventral furrow. Although figure 11 shows the dorsal view of *P. prolonga* var. *nordica*, the features in figure 11 are almost identical to those in figure 15 of Novarino et al. (1997).

The diagnostic features of the genus *Hemiselmis,* with the bean-like or reniform shape, the larger hexagonal plated periplast, even elongate (Barlow & Kugrens, 2002), and the presence of hexagonal periplast on the cell posterior, clearly set *Hemiselmis* apart from other cryptomonads. *Hemiselmis* sp. here shares the characteristics of strains by Novarino (2005), but the size of the hexagonal plates is different. Novarino's *Hemiselmis* sp. (see Fig. 6) has scales 0.4–0.65 μm in size, while figure 14 here shows a cell with larger size of hexagonal plates more than 1.0 μm. The cells of the former were 4.2–4.5 μm long and 3.2–3.4 μm wide, while the cells of the latter were about 4.1 μm long and 2.5 μm wide according to figure 6 (C, D). Meanwhile, *Hemiselmis* sp. here also had the similarities with *Hemiselmis* sp. by Cerino & Zingone (2006), but the size of hexagonal plates (about 0.8–1.5 μm, average more than 1.0 μm) of the former were also larger than those of the latter (about 0.5 μm). However, Novarino's *Hemiselmis* sp. (Fig 3: C, D) also had larger and elongate size of hexagonal plates (1.7–2.5 μm in size) (Novarino, 2005), and the cell shape was very similar to that of *Hemiselmis* sp. in this paper. Because our samples were fixed in field, and the identity of the other features such as the phycobilin pigment and presence of the refractive body were unknown, all mentioned made it difficult to identify them as the same species. In addition, the features revealed by SEM here could not be comparable with those eight new species of *Hemiselmis* in LM reported by Butcher (1967), so *Hemiselmis* sp. described here cannot be identified unambiguously as a new species instead of belonging to any of those eight by Butcher (1967).

The genus *Teleaulax* can be identified unambiguously due to its characteristically acute posterior end where the non-plated periplast was covered and the long furrow extended roughly halfway along the cell length in these samples (Figs. 12–15). The cells of *Teleaulax* superficially resemble those of *Plagioselmis* by their acute posterior end. However, the periplast covering the entire cell surface, including the posterior end, helps to identify them clearly as *Teleaulax*. The features shown in Figs. 12–15 were in accordance with other publications such as Novarino et al. (1997), Novarino (2005), and Cerino & Zingone (2006). The cells possess many small ejectosomes on the entire cell surface, including the posterior end. Kugrens et al. (1994) suggested that these ejectosomes on the surface are within the cell and are extruded once they are stressed.

So far, the systematics of the cryptomonads is based predominantly on ultrastructural features (Dean et al., 2002). Several molecular phylogenetic studies have been done by using nuclear SSU rDNA sequence data (Marin et al., 1998; Clay & Kugrens, 1999; Dean et al., 2002; Hoef-Emden et al., 2002). These studies showed that cryptomonads with plastids represent a monophyletic group. *Plagioselmis prolonga* was closely related to *Teleaulax* species, and form a clade together with *Geminigera cryophila* Taylor & Lee (Dean et al., 2002). *Hemiselmis* species form a monophyletic group within the one clade (including genus *Hemiselmis*, *Chroomonas* Hansgirg and *Komma* Hill) (Marin et al., 1998; Dean et al., 2002). The monophyletic evolution of cryptomonads showed that the three genera described here, previously identified mainly based on the ultrastructure features, are reliable. The molecular sequencing studies have provided a number of new phylogenetic information (Marin et al., 1998; Dean et al., 2002; Hoef-Emden et al., 2002). However, the molecular sequence data that currently exist in Genbank seem not to play a major role in cryptomonad identification (Novarino, 2003).

Plagioselmis prolonga and *Teleaulax acuta* are the most widespread and abundant cryptomonads in several investigated sea areas (Novarino et al., 1997; Barlow & Kugrens, 2002; Novarino, 2005; Cerino & Zingone, 2006). The species *Plagioselmis prolonga* was recorded throughout the year, and often reached high population densities (e.g., Cerino & Zingone, 2006). Its abundance reached 10^6 cells/L in our samples. However, *Hemiselmis* sp. was recorded only at certain periods of the year (Cerino & Zingone, 2006). On the other hand, *Plagioselmis prolonga* and *Teleaulax acuta* can cause blooms (Environmental Protection Department, Hong Kong, 2005), although they have not been found to produce toxins. The taxonomic identifications are necessary to any studies of such blooms, and the key taxonomic characteristics as defined here using SEM will be helpful to accurate taxonomy of future blooms.

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中国海区几种隐藻类鞭毛藻的扫描电镜观察

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摘要 报道了来自香港吐露港、中国长江口及厦门港的3个属的3种隐藻及1个变种, 即半片藻*Hemiselmis* sp. Novarino、伸长斜片藻*Plagioselmis prolonga* Butcher ex Novarino, Lucas & Morrall、伸长斜片藻北方变种*Plagioselmis prolonga* var. *nordica* Novarino, Lucas & Morrall、尖尾全沟藻*Teleaulax acuta* (Butcher) Hill, 并对每个种类的分类特征、生态分布进行描述, 同时提供每个种的光镜和扫描电镜照片。其中, 半片藻属*Hemiselmis* Parke是中国海区首次记录的属, 而伸长斜片藻*Plagioselmis prolonga*和尖尾全沟藻*Teleaulax acuta*可以引发赤潮。

关键词 中国; 隐藻; 半片藻属; 半片藻; 海洋; 新记录; 伸长斜片藻; 伸长斜片藻诺尔变种; 尖尾全沟藻