Dinoflagellates from Hainan Island:
Potential threat for transporting harmful algae from Hainan to Japan

Anqi Yin

Department of Ecosystem Studies
School of Environmental Science
The University of Shiga Prefecture

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ABSTRACT

Coastal areas in Hainan Island, China, are important for commercial fisheries, but few studies on harmful algal bloom (HAB) have been performed to date. In south part of Japan, large quantities of the seedlings for greater amberjack farming are imported from Hainan Island every year since 1988. In this study, the dinoflagellate species were identified and described from the plankton samples around Hainan Island to make a list of the species occurred including HAB species for providing against potential threat of HAB events. Additionally, phytoplankton communities between Hainan Island and the two bays in south part of Japan were determined from the plankton and the sediment samples using both microscopic and molecular analyses, to evaluate a possibility for artificial transportation of tropical HAB species to Japan.

A total of 37 dinoflagellate species in 11 genera from nine families were identified from 11 coastal stations around Hainan Island. Eight toxic and four red tide-forming species were found at most stations, except for Station (St.) 4. These species included the five genera of *Ceratium*, *Dinophysis*, *Lingulodinium*, *Prorocentrum*, and *Protoperidium*. *Ceratium furca*, a red tide-forming species, and *Prorocentrum rhathymum*, a ciguatera fish poisoning species, were occurred in large numbers at St. 7 and 8, respectively, both of which are near fish farms or fishing villages. *Prorocentrum lima* and *Dinophysis acuminata* are diarrhetic shellfish poisoning species and *Lingulodinium polyedrum* is a yessotoxin species, which appeared only at St. 5, near the location of a fish farm and seafood markets. These results suggest potential threats of harmful algal blooms around Hainan Island that could cause fish, shellfish, and human poisoning.

A total of 27 dinoflagellate species in eight genera from seven families were identified from the plankton samples in Kagoshima Bay and Uranouchi Bay, south Japan. Species from the Kareniaceae were found only in south Japan, while species from the Gonyaulacaceae, Peridiniaceae, and Heterocapsaceae in Hainan Island. Phytoplankton from the sediment samples in Hainan Island consists of 81 species from seven phyla. Whereas in Kagoshima Bay, it consists of 61 species from six phyla. Species from the Bacillariophyta were found most frequently in both Hainan Island and Kagoshima Bay. Species from the Cryptophyta and Phaeophyta were found only in Hainan Island, while species from the Haptophyta in Kagoshima Bay.

Thirteen dinoflagellate species including six harmful species were found in both Hainan Island and south Japan from the plankton samples, and most species were recorded from Japan recently, while 35 phytoplankton species including one harmful species were found in both Hainan Island and Kagoshima Bay from the sediment samples, being more than 50% of species which occurred in Kagoshima Bay, and red tide-forming species *Heterosigma carterae* recorded from Japan since 1994, after the importation of fish fry, indicated the invasion of tropical species, including harmful ones, from Hainan Island to Japan by the global warming through the current systems and/or by artificial transportation of fish fry may be plausible.

This study suggest that, Hainan Island potentially faces the danger of HABs, and the invasion of the tropical harmful species to Japan may be plausible.

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CHAPTER 1

GENERAL INTRODUCTION

Phytoplankton is the main producers of biomass and organic compounds in aquatic ecosystems and plays a key role at the base of the food chains in oceans and freshwaters (Daranas et al., 2001). The secondary metabolites produced by phytoplankton often have unusual biological activities, and their roles in aquatic ecosystems probably include chemical defense, allelopathy and inter-cellular communication (MacKenzie, 2008). One of these metabolites is the toxins involved in Harmful algal blooms (HABs) (Shimizu, 2003), having attracted attention in the past two decades, and the main producers of these compounds are dinoflagellates and cyanobacteria (Faulkner, 1996; Shimizu, 2003).

Dinoflagellates are an important part of the pelagic ecosystem (Hällfors, 2013), make a large component of primary producers, and are estimated to make up about 40% of the total species of marine phytoplankton (Simon et al. 2009). Over 2500 dinoflagellate species have been recognized worldwide (Hoppenrath et al., 2009), and about 76 of these species have been classified as harmful. The harmful species cause water discolorations known as red tides, and sometimes produce toxins that directly kill fish and shellfish or accumulate to lethal levels in higher-trophic level consumers in the food chain, including humans (Myat et al., 2012). In any aquatic ecosystem, there hardly exist an environmental condition not exploited by dinoflagellates (Smayda 2002).

1.1 HABs and dinoflagellates

The several damages of HABs are caused by the proliferation of planktonic cells forming high biomasses (Chikwililwa, 2014). Algal blooms, formed by dinoflagellates, thrive under high nutrient and low turbulent conditions (Chikwililwa, 2014). When dinoflagellates occur in high abundance and the water is discolored in various shades of reddish-brown, hence dinoflagellate blooms are called red tides (Hällfors, 2013). Particularly, dormant cysts of the red tide dinoflagellate species can survive in ocean sediments for years (Pfiester and Anderson, 1987), and germinate to produce HABs suddenly under the appropriate environmental conditions (Camacho et al., 2007).

According to the effect of HABs in the environment, HABs can be classified into three major groups (Hallegraeff, 1995):

1) HABs are historically formed in a particular region and mostly caused by non-toxic phytoplankton, resulting in red tides or brown tides, causing dissolved oxygen depletion in the waters from microbial activity during bloom decay, generating anoxic condition and therefore killing oxygen dependent organisms indiscriminately. The HABs caused by dinoflagellates of *Gonyaulax*, *Noctiluca* and *Scrippsiella* belong to this group (Hallegraeff, 1995).
2) HABs not producing toxins that can adversely affect humans but causing chemical and mechanical damage to fish gills, and can be more threatening to caged fish than wild fish which are able to avoid such dangerous cell concentrations. Dinoflagellate *Karenia mikimotoi* can produce two haemolytic glycolipids, monogalactosyl diacylglycerol and digalactosyl diacylglycerol, which damage fish gills and were responsible for fish kills in Japan and Norway (Parrish et al., 1998; Haywood et al., 2004).
3) HABs producing potent marine toxins cause both human illness and mass mortalities of fish and invertebrates (Garthwaite, 2000).

HABs are predominantly a coastal phenomenon, and their frequency, intensity and geographic range have increased since 1970s (Cembella et al., 2005; Camacho et al., 2007). The increased economic impact of HABs is probably associated with the increased consumption of seafood and the growth of coastal populations (Hallegraeff, 2003). HABs appeared to be stimulated by nutrient discharges in domestic, industrial and agricultural wastes (Lam and Ho, 1989), and several factors have been responsible for the HABs increase:

1) Eutrophication: The global increase of HABs is strongly linked to anthropogenic eutrophication caused by intensive agriculture, industrial activities and human waste (Shi, 2012). Increased global inputs of nutrients, such as phosphorus and nitrogen, have arisen from widespread use of agricultural fertilizers and sewage disposal to oceans (Pitois et al., 2001), and there are many evidences show that such increased inputs are significant factors in triggering phytoplankton production in fresh and marine waters (Anderson et al., 2002). Moreover, the increase in dissolved and particulate organic matter may favor many dinoflgellates (Hällfors, 2013).
2) Increased utilization of coastal waters for aquaculture: Aquaculture provides a sensitive

bioassay system for harmful algal species, and results in more reports of shellfish poisoning events (Hallegraeff, 1995). Finfish aquaculture in enclosed areas can be particularly sensitive to blooms of some dinoflagellate species, such as *Heterosigma carterae, Karenia. mikimoti* and *K. selliformis* (Shi, 2012).

3) Worldwide dispersion by artificial and natural factors: Through natural range expansion or assist by human, dinoflagellates have the capacity to invade new areas (Hallegraeff and Bolch, 1992; Smayda, 2002). Ship ballast water exchange transfer contaminated organisms in the fisheries stocks from one region to another, causing the invasion of dinoflagellates and their cysts into new habitat (Camacho et al., 2007). Hallegraeff and Bolch (1992) provided the first evidence that non-indigenous toxic microalgae were introduced to Australia via cargo vessel ballast water, causing widespread losses to a commercial shellfish industry. Bivalves and fish can harbor viable dinoflagellate cells and sometimes can also contain resistant resting cysts (Scarratt et al., 1993; Schwinghamer et al., 1994). Some tropical toxic dinoflagellates, such as *Pyrodinium bahamense,* which was initially found in the mangrove-fringed coastal waters of Atlantic and Indo-West Pacific, have extended their distribution to colder waters, and have been found as far south as Sydney and as far north as British waters (Hallegraeff, 1995).

1.2 Health effects of dinoflagellates

Human diseases associated with marine dinoflagellate toxins are well known, including paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), ciguatera fish poisoning (CFP), neurotoxic shellfish poisoning (NSP), and azaspiracid shellfish poisoning (AZP). The symptoms occur generally as a consequence of contaminated seafood consumption or direct exposure to HABs (Camacho et al., 2007). Particularly, seafood species can adapt to tolerate high levels of certain algal toxins. For example, softshell clams (*Myaarenaria*) from areas exposed to red tides are more resistant to PSP and can be accumulate toxins at greater rates than sensitive clams from unexposed areas, increasing the health risk to humans (Bricelj et al., 2005; Camacho et al., 2007).

In a global view, the consumption of shellfish is one of the most important pathways that dinoflagellate toxins affect humans (Hallegraeff, 2003). Table 1 summarized the dinoflagellate species, toxins and aquatic species that are generally associated with various types of human poisonings (Camacho et al., 2007). In addition, many other toxins have been isolated, but their effects and significance remain unknown (Daranas et al., 2001).

1.3 General features of Hainan Island

China has had productive ocean fisheries, harvesting 13.9 million tons of marine products in 2012, or 17.4% of marine harvests worldwide (FAO, 2014). Hainan Island, the southernmost part of China, is located in a tropical to subtropical climate region. This island faces the South China Sea, which comprises two-thirds of the sea area around China. This 2×10^6 km^2 marine area (Jin et al., 2009) contains more than 600 species of fish, more than 20 species of shrimp, 47 species of economically important shellfish, 45 species of sea cucumber, 72 species of crab, and 162 species of seaweed (Wu and Zhang, 1999). The broad range of marine products available from waters near Hainan Island demonstrates the high productivity of this coastal ecosystem. On the other hand, the demand for these seafood species now seems to have exceeded a sustainable level (Mai et al., 2012).

Around Hainan Island, fish and shrimp farming have rapidly developed in coastal areas during the last decade. For example, annual production of farmed *Tilapia* was 82,000 tons in 2002, and has increased to 121,000 tons in 2004 (Chen et al., 2007). The development of fish farms on land in coastal areas has resulted in pollutant and nutrient runoff from around those areas (Wang et al., 2010) and led to serious eutrophication of coastal waters, which is closely related to the frequency of HABs (Hallegraeff, 1993).

HABs have been increasing rapidly along the Chinese coast since the 1980s, and occur more frequently along the south coast than along the north coast (Yan et al., 2002). Most HABs occurring in the South China Sea have been comprised of dinoflagellates (Yan et al., 2002), and in the coastal areas around Hainan Island, 16 HAB events have been recorded between 1991 and 2009 (Wang et al., 2010).

Thus far, 148 HAB species have been found in China, of which 71 species were dinoflagellates. Among these 71 dinoflagellates species, 70 species have been found from South China Sea and 19 of them have caused HAB events in South China Sea (Yan et al., 2002). However, few studies on HAB species containing dinoflagellates around Hainan Island have been performed to date (Nie and

Wang, 1942).

1.4 Fishery resources from Hainan Island to Japan

Japan is one of the largest consumers of fishery products in the world, fish and shellfish are a staple part of the Japanese diet, and is due to the high consumer demand. Therefore, various fishery commodities were imported for aquaculture, including larvae and eggs (Wakabayashi, 1996).

Amberjack farming, including Japanese amberjack (*Seriola quinqueradiata*), greater amberjack (*Seriola dumerili*) and yellowtail amberjack (*Seriola lalandi*), have been one of the most important marine aquaculture in Japan (Wakabayashi, 1996; Ottolenghi et al., 2004). The greater amberjack is one of the most profitable species (Wakabayashi, 1996), and Kagoshima prefecture, which is located in the southernmost of the main island of Japan, has the largest greater amberjack farming area in Japan; account for about 67.3% of the total, which followed by Miyazaki (14.6%) and Kochi (7.4%) (Ottolenghi et al., 2004). Large quantities of the greater amberjack seedlings have been imported from Hainan Island every year since 1988, and more than ten million fries were introduced into fish farms in these area in 1993 (Wakabayashi, 1996; Ottolenghi et al., 2004).

1.5 Aims and objectives

This study was carried out to describe the occurrence of dinoflagellate species in the coastal areas around Hainan Island, and analysis for the invasion of tropical HAB species to Japan. The definitive purposes were summarized as follows:

1) The dinoflagellate species identified from the plankton samples were described to make a species list of dinoflagellates occurred around Hainan Island for potential threat of HAB events.
2) Phytoplankton community between Hainan Island and the two Japanese bays (Kagoshima Bay and Uranouchi Bay) were determined from the plankton and the sediment samples using both microscopic and molecular analyses, to evaluate a possibility for artificial transportation of tropical HAB species from Hainan Island to Japan.

CHAPTER 2

OCCURRENCE OF DINOFLAGELLATES IN THE COASTAL AREAS AROUND HAINAN ISLAND

2.1 Introduction

Phytoplankton is a primary producer in the marine food web that supports fish populations (Pauly and Christensen, 1995). In the coastal ecosystem, dinoflagellates in particular are one of the most important primary producers as food for fish, shrimp, and shellfish. However, some dinoflagellate species are harmful to other organisms, and should be carefully monitored (Myat et al., 2012). Over 2500 dinoflagellate species have been recognized worldwide (Hoppenrath et al., 2009), about 76 of which have been classified as harmful, these harmful species cause water discolorations known as red tides, and sometimes produce toxins that directly kill fish and shellfish or accumulate to lethal levels in higher-trophic level consumers in the food chain, including humans (Myat et al., 2012).

Around Hainan Island, as the rapid development of fish farms in the coastal areas, pollutant and nutrient runoff from those areas (Wang et al., 2010), and led to serious eutrophication of coastal waters, which is closely related to the frequency of HABs (Hallegraeff, 1993), and in the coastal areas around Hainan Island, 16 HAB events have been recorded between 1991 and 2009 (Wang et al., 2010), but unfortunately, the causative species haven’t listed.

In this chapter, we examined the occurrence of dinoflagellate species in several coastal regions around Hainan Island to provide a basis for future studies of the primary productivity of dinoflagellates and to clarify the mechanisms of development of HABs.

2.2 Materials and methods

2.2.1 Sampling sites

Eleven sampling sites were selected along the coastal regions around Hainan Island, all were chosen as shallow, about <5 m deep areas facing the South China Sea (Table 2 and Fig. 1). Phytoplankton samples were qualitatively collected with a 10-μm-mesh plankton net after prescreening with a 200-μm-mesh net. These samples were immediately transferred into 100-ml plastic bottles and preserved with neutral formalin at a final concentration of 1%.

2.2.2 Morphological taxonomy

Several morphological features including cell shape, position of nucleus, and pyrenoid were basically observed under a BX60F up-right microscope (OLYMPUS, Japan). For observation of the thecal plate, cells were stained with Calcofluor White M2R at a final concentration of 10 μg mls^{-1} (Fritz and Teiemer, 1985), analyzed under UV excitation using a BX60F fluorescence microscope. Photographs were captured with a charge-coupled device camera (OLYMPUS DP70; OLYMPUS, Japan) under 40× magnification. Dinoflagellate species were identified according to previous literatures (Chihara and Murano, 1997; Steidinger and Tangen, 1997; Hoppenrath et al., 2009; Omura et al., 2012).

2.2.3 Similarity of species occurrence

A Bray-Curtis similarity index of species occurrence was used as a clustering metric (Myat et al., 2012), and a tree was drawn using group average linkage to reveal clusters. Grouping of stations based on similarity indices for dinoflagellate species composition was carried out using data for species occurrence according to Clarke and Warwick (2001). An agglomerative hierarchical cluster analysis was carried out based on species occurrence (presence or absence) using the PRIMER v6® software package (PRIMER-E Ltd., UK).

2.3 Results

2.3.1 Distribution and species composition of dinoflagellates

A total of 37 dinoflagellate species in 11 genera from nine families were identified in samples from all of the stations in the present study (Table 3). These comprised seven species from the Prorocentraceae, three species from the Dinophysiaceae, two species from the Gonyaulacaceae, one species from the Pyrophacaceae, four species from the Ceratiaceae, two species from the Peridiniaceae, 13 species from the Protoperidiniaceae, one species from the Calciodinellaceae, and four species from the Heterocapsaceae.

The number of dinoflagellate species collected from each sampling site ranged from zero to 16 with an average of seven. Large numbers of dinoflagellate species, more than 11, were found at Sts. 5, 6, 8, and 9, which are located near fish farming systems or fishing villages. Sts. 1, 7, and 10 are located near seafood markets or shipping ports. The remaining four sites, Sts. 2, 3, 4, and 11, which are near natural ports or scenic spots, were relatively less affected by human activities and therefore eutrophication has not yet proceeded in these locations (Anderson et al., 2002).

Species from the Prorocentraceae were found most frequently at most of the sampling sites, except for Sts. 4 and 7, followed by species from the Protoperidiniaceae, Peridiniaceae, and Ceratiaceae (Table 3). Species from the Dinophysiaceae were found at just three sites, Sts. 5, 6, and 9, and species from the Gonyaulacaceae were identified at two sites, Sts. 5 and 6. Species from the Pyrophacaceae, Heterocapsaceae, and Calciodinellaceae families were found at three different sites, Sts. 6, 9, and 10. In particularly, harmful species were found at most sites except for St. 4, where no dinoflagellate species appeared. Only one and two harmful species appeared at Sts. 2 and 11, respectively (Table 3).

2.3.2 Description of each species

2.3.2.1 ***Prorocentrum emarginatum*** Fukuyo 1981 (Fig. 2-1)

Description: Cells are ovate to oblong (Fig. 2-1, b-d). The right valve having valve pores of two different sizes, the larger valve pores are arranged in radial rows, and the marginal pores are also present (Fig. 2-1, c). The periflagellar area on the right valve is deeply excavated, ending in a sharp narrow point with a rectangular structure (Fig. 2-1, b-d).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are broadly ovate to rotundate, 35-40 μm in length and 30-32 μm in width, possess a large kidney-shaped posterior nucleus. Both valves are concave in the center. The valve surface is smooth with both valves having valve pores of two different sizes, small (0.1 μm in diameter) and large (0.2 μm in diameter). These pores are situated in a deep depression with smooth margins and are uniformly round in shape. The larger valve pores, approximately 200 per valve, are arranged in radial rows, and spaced around the valve periphery. The marginal pores are also present. The centers of valves are void of pores. The periflagellar area is deeply excavated, ending in a sharp narrow point with a rectangular structure on the right valve that touches the intercalary band. The left valve is also deeply indented. The intercalary band is broad, transversely striate and sinuous (Fukuyo, 1981; Faust, 1990; Faust et al., 1999; Al-Yamani and Saburova, 2010; Hoppenrath et al., 2013).

Distribution: *P. emarginatum* is a benthic species, can be tychoplanktonic, with warm temperate to tropical coastal waters distribution (Steidinger and Tangen, 1996), reported from tropical Pacific coral reefs (Ryukyu Islands, Japan) (Fukuyo, 1981); Caribbean waters (Carlson, 1984); Belize (Faust et al., 1999); Reunion and Zanzibar Island (Hansen et al., 2001); North and Central Vietnam (Larsen and Nguyen, 2004), and this species was reported to be associated with macroalgae, seagrasses and dead corals in Malaysia (Mohammad-Noor et al., 2007).

Harmfulness: *P. emarginatum* is potentially toxic species (Hansen et al., 2001). Turquet (1997) found low haemolytic and fibroblast activity in crude extract of cells from the southwestern Indian Ocean. However, it was apparently nontoxic in Malaysia (Mohammad-Noor et al., 2007).

2.3.2.2 ***Prorocentrum hoffmannianum*** Faust 1990 (Fig. 2-2)

Synonym: *Exuviaella hoffmannianum* (Faust) McLachlan et Boalch 1997

Description: Cell is ovoid, broadest in the middle region, tapering slightly apically, containing golden-brown chloroplasts and a centrally located pyrenoid. The valve surface is deeply areolate. The periflagellar area is a wide triangle situated apically on the right valve (Fig. 2-2, b).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are ovoid, broadest in mid-region, tapering slightly apically, 45-55 μm in length and 40-45 μm in width, a centrally located pyrenoid and a posterior nucleus. Both valves are slightly concave in the center, and apically excavated. The valve surface is deeply areolate, approximately 650-700 areolae on each valve, and the areolate are dense, large, and round to oblong, 1-1.15 μm in diameter. Small round to ovoid pores are found within deep areolae, and these pores have smooth margins, are 1.0-1.5 μm in diameter, relatively large compared to other large pores in *Prorocentrum*, and many bear trichocyst pores. The periflagellar area is a broad triangle with a flared apical collar adjacent to the flagellar pore, and it lacks both valve spines and anterior spines. The left valve exhibits a flared and flattened curved apical collar that borders the periflagellar area. The intercalary band is smooth and appears as a flared ridge around the cell (Faust, 1990; Faust et al., 1999; Hoppenrath et al., 2013).

Distribution: *P. hoffmannianum* is a benthic species, can be tychoplanktonic, with warm temperate to tropical coastal waters distribution, reported from Belize (Faust, 1990), and this species was reported to be associated with floating detritus in tropical coastal regions of the Caribbean Sea (Faust, 1990), attached to macroalgae in the Belizean barrier reef ecosystem (Morton and Faust, 1997).

Harmfulness: *P. hoffmannianum* is considered toxic, producing fast-acting toxin (FAT) and okadaic acid (OA) (Aikman et al., 1993).

2.3.2.3 ***Prorocentrum lima*** (Ehrenberg) Stein 1878 (Fig. 2-3)

Basionym: *Cryptomonas lima* Ehrenberg 1860

Synonyms: *Exuviaella marina* Cienkowski 1881
Dinopyxis laevis Stein 1883
Exuviaella lima (Ehrenberg) Bütschli 1885
Exuviaella lima (Ehrenberg) Schütt 1896
Exuviaella laevis (Stein) Schroder 1900
Exuviaella cincta Schiller 1918
Exuviaella marina var. *lima* (Ehrenberg) Schiller 1931
Exuviaella ostenfeldi Schiller 1933
Exuviaella caspica Kiselev 1940
Prorocentrum marinum Dodge et Bibby 1973
Prorocentrum arenarium Faust 1994

Description: Cells are oblong to ovate, broad in the middle region, narrow at the anterior end (Fig. 2-3, b-d), containing a central pyrenoid and a large posterior nucleus (Fig. 2-3, d). The valve surface is covered with scattered pores, but the center is devoid of pores (Fig. 2-3, b), the right valve having valve pores of two different sizes (Fig. 2-3, c), and the marginal pores are present (Fig. 2-3, b). The periflagellar area is a wide triangle containing a curved apical collar (Fig. 2-3, d).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are oblong to ovate, broad in the middle region, narrow at the anterior end, 31-47 µm in length and 22-40 µm in width, a centrally located pyrenoid and a posterior nucleus. Both valves are concave. The valve surface is smooth, covered with scattered pores except in the central area, the right valve having valve pores of two different sizes, larger flagellar pores and smaller auxiliary pores, and marginal pores are present. The periflagellar area is a wide triangle containing a curved apical collar around the flagellar and apical pores and is void of valve spines or anterior spines. The hypnozygote is round and brown with a triple-layered wall (Dodge, 1975; Faust, 1990 and 1991; Steidinger and Tangen, 1996; Faust et al., 1999; Al-Yamani and Saburova, 2010; Hoppenrath et al., 2013).

Distribution: *P. lima* is a neritic and estuarine, benthic/epiphytic species (can be tychoplanktonic), with worldwide distribution, typically in temperate and tropical waters (Steidinger and Tangen, 1996; Faust et al., 1999), reported from North and Central Vietnam (Larsen and Nguyen, 2004); East China Sea and South China Sea (Lu, 2008); Japan, Indonesia and Philippines (Nagahama et al., 2011), and this species was reported to be attached to macroalgae, or was also observed swimming close to the bottom substrate, and was associated with coral reefs (Al-Yamani and Saburova, 2010), or can be found attached to floating detritus in mangrove habitats (Faust, 1991).

Harmfulness: *P. lima* is a toxic species known to produce a number of toxic substances: prorocentrolide (Torigoe et al., 1988); fast-acting toxin (FAT) (Tindall et al., 1989); and diarrhetic shellfish poisoning (DSP) toxins (Yasumoto et al., 1987) including okadaic acid (OA) (Murakami et al., 1982; Lee et al., 1989; Marr et al., 1992); dinophysistoxin-1 (DTX1) (Marr et al., 1992); dinophysistoxin-2 (DTX2) (Hu et al., 1993); and dinophysistoxin-4 (DTX4) (Hu et al., 1995).

2.3.2.4 ***Prorocentrum micans*** Ehrenberg 1834 (Fig. 2-4)

Synonyms: *Prorocentrum schilleri* Böhrn 1933

Prorocentrum levantinoides Bursa 1959

Prorocentrum pacificum Wood 1963

Description: Cells are tear-drop to heart shaped, generally rounded anteriorly, tapering posteriorly (Fig. 2-4, b-c). One convex side and one arched side, the convex arch profile is typically in the middle of the cell where is the broadest, and the winged apical spine is present (Fig. 2-4, c). Numerous tubular trichocyst pores are present in short rows arranged radially (Fig. 2-4, b).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are tear-drop to heart shaped, rounded anteriorly, tapering posteriorly, and broadest around the middle, 35-70 μm in length and 20-50 μm in width, with a length-width ratio usually less than two, strongly flattened with a well-developed winged apical spine. Numerous tubular trichocyst pores are present in short rows arranged radially. The periflagellar area is a relatively small, shallow, broad triangular depression situated apically on the right valve off-center (Dodge, 1975, 1982 and 1985; Taylor, 1980; Toriumi, 1980; Fukuyo et al., 1990; Steidinger and Tangen, 1996; Faust et al., 1999; Gul and Saifullah, 2011; Long et al., 2013).

Distribution: *P. micans* is a neritic and estuarine, planktonic species, but also found in oceanic environments, with cold temperate to tropical waters distribution (Steidinger and Tangen, 1996), reported from Japan (Chihara and Murano, 1997); Mexican Pacific Coast (Cohen-Fernandez et al., 2006); East China Sea and South China Sea (Lu, 2008); Taiwan (Shao et al., 2010); and New Zealand (Chang et al., 2012).

Harmfulness: Although *P. micans* is capable of forming extensive blooms, it is usually considered harmless (Taylor and Seliger, 1979; Anderson et al., 1985; Graneli et al., 1990). It may excrete substances that inhibit diatom growth, but apparently these substances do not enter the food chain or affect organisms at higher trophic levels (Uchida, 1977). Early reports on *P. micans* being a paralytic shellfish poison (PSP) producer (Pinto and Silva, 1956) are unconfirmed, and recent incidents involving shellfish mortality have been attributed to oxygen depletion (Lassus and Berthome, 1988).

2.3.2.5 ***Prorocentrum rhathymum*** Loeblich III, Sherley et Schmidt 1979 (Fig. 2-5)

Synonyms: *Prorocentrum maximum* Schiller 1937

Prorocentrum mexicanum Osorio-Tafall 1942

Description: Cells are asymmetric, ovate to oblong with straight sides (Fig. 2-5, b-f). The valve pores form the line patterns in the posterior part of the valve, and the center of the cell is devoid of pores (Fig. 2-5, b-c). The periflagellar area, located apically and off-center on the right valve, is a V-shaped, shallow depression, and the curved periflagellar collar is appear as an apical spine (Fig. 2-5, d-f).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are asymmetric, ovate to oblong with straight sides, 30-38 µm in length and 20-25 µm in width, a posterior nucleus. Both valves are escavated, have many large trichocyst pores radially arranged in furrowed depressions, and marginal pores are present. The centers of valves are devoid of pores. The periflagellar area, located apically and off-center on the right valve, is a relatively small, V-shaped, shallow depression. It houses a prominent curved periflagellar collar adjacent to the auxiliary pore. Opposite is a smaller periflagellar plate adjacent to the flagellar pore. The curved periflagellar collar is large and may appear as an apical spine. The intercalary band is broad and transversely striated (Fukuyo, 1981; Carlson, 1984; Faust, 1990; Al-Yamani and Saburova, 2010; Gul and Saifullah, 2011; Hoppenrath et al., 2013).

Distribution: *P. rhathymum* is a neritic and estuarine, benthic species, can be tychoplanktonic, with tropical and subtropical waters distribution (Steidinger and Tangen, 1996), reported from Cinnamon Bay, St. Johns, Virgin Islands (Loeblich et al., 1979); New Caledonia and Ryukyu Islands (Japan) (Fukuyo, 1981), and this species was reported to be associated with microalgae, dead coral and seagrasses in Malaysia (Mohammad-Noor et al., 2007).

Harmfulness: *P. rhathymum* may produce toxins with hemolytic activity (Nakajima et al. 1981), and also water-soluble fast-acting toxin (FAT) (Tindall et al. 1989).

2.3.2.6 ***Prorocentrum shikokuense*** Hada ex Balech 1975 (Fig. 2-6)

Basionym: *Prorocentrum shikokuensis* Hada 1975

Synonyms: *Prorocentrum dentatum* Stein 1883

Prorocentrum donghaiense Lu 2001

Description: Cells is elongated and asymmetric, narrowing toward the posterior ends, with a sunflower seed shaped. One side of the anterior end extends more than the other. The periflagellar area is slightly concave. The posterior end is generally rounded (Fig. 2-6, b).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are variable in shape, elongated and asymmetric, narrowing toward the posterior ends, with the shape being somewhat similar to that of a sunflower seed, 20-32 µm in length and 8-15 µm in width. Both valves have many knob-like spines spread densely over the surface, and trichocyst pores are mainly distributed around the peripheral margin of the cells, particularly around the antapex. The periflagellar area of the right valve is V-shaped and concave with an ear-shaped collar structure that varies in shape and size (Takano and Matsuoka, 2011).

Distribution: *P. shikokuense* is a planktonic species, with wide distribution in and around the East China Sea (Takano and Matsuoka, 2011), reported from Iwamatsu Bay (Japan) (Hada, 1975; Takano and Matsuoka, 2011); East China Sea and Japanese-Korean coastal waters (Yoo and Lee, 1986; Horiguchi, 1990; Park, 1991; Takano and Matsuoka, 2011); and Nagasaki (Japan) (Matsuoka et al., 2006).

Harmfulness: *P. shikokuense* has formed massive blooms in the Changjiang River mouth (Takano and Matsuoka, 2011).

2.3.2.7 ***Prorocentrum sigmoides*** Böhm 1933 (Fig. 2-7)

Description: Cells are elongated, in "S" shape, one convex side and one generally straight side, and tapering posteriorly (Fig. 2-7, b). Numerous trichocyst pores are present (Fig. 2-7, c). A sharp and tiny apical spine protruded from the anterior side (Fig. 2-7, d).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are elongated, sometimes in "S" shape, 60-85 µm in length and 20-30 µm in width. The surface perforated by trichocyst pores. Apical spine sharp and tiny, protruded from the anterior side, adjacent to the periflagellar area (Chihara and Murano, 1997; Gul and Saifullah, 2011).

Distribution: *P. sigmoides* is a planktonic species, with temperate to tropical waters distribution (Chihara and Murano, 1997), reported from South China Sea and Japan (Lu, 2008).

Harmfulness: *P. sigmoides* has never been reported to be a toxin producer, but it is a fish killer (Lu and Hodgkiss, 2004). It is capable of forming extensive blooms, which can consume dissolved oxygen and cause biota kills (Ajuzie and Houvenaghel, 2009).

2.3.2.8 ***Dinophysis acuminata*** Claparède et Lachmann 1859 (Fig. 2-8)

Synonyms: *Dinophysis ellipsoides* Kofoid 1907
Dinophysis boehmii Paulsen 1949
Dinophysis borealis Paulsen 1949
Dinophysis lachmannii Paulsen 1949

Dinophysis skagii Paulsen 1949

Dinophysis lachmanii Solum 1962

Description: Cells are almost oval in shape (Fig 2-8, b-c). The surface covered with prominent circular areolae, each with a pore (Fig 2-8, c). The epitheca is dorsoventrally reduced (Fig 2-8, c). The cingulum is bordered by two well-developed lists, anterior cingular list (ACL) and posterior cingular list (PCL) (Fig 2-8, b). The left sulcal list (LSL) extends beyond the midpoint of the cell (about 1/2 of cell length), and supported by three ribs (Fig 2-8, b). The posterior profile of hypotheca is rounded (Fig 2-8, b-c).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are almost oval or elliptical in shape, can vary from rotund to long and narrow in lateral view, 38-58 μm in length and 30-40 μm in dorso-ventral width, contain large chloroplasts and a posterior pyrenoid. The thick thecal plates are covered with prominent circular areolae, each with a pore. The epitheca is slightly convex and inclined ventrally. The cingulum is bordered by two well-developed lists, anterior cingular list (ACL) and posterior cingular list (PCL). The left sulcal list (LSL) extends beyond the midpoint of the cell (1/2 to 2/3 of cell length), and supported by three ribs (Fukuyo et al., 1990; Tomas, 1997; Faust and Gulledge, 2002; Hallegradff et al., 2003).

Distribution: *D. acuminata* is a neritic, planktonic species, with worldwide distribution, typically in cold and warm temperate waters (Steidinger and Tangen, 1996), most common and abundant in coastal waters of the northern Atlantic and Pacific Oceans, especially eutrophic areas (Taylor et al., 1995; Steidinger and Tangen, 1996), reported from Bohai Sea and East China Sea (Lu, 2008); and Japan (Nagai et al., 2011).

Harmfulness: *D. acuminata* is a toxic species that has been found to produce okadaic acid (OA) (Cembella, 1989; Lee et al., 1989) causing diarrhetic shellfish poisoning (DSP) (Kat, 1985). This species can cause shellfish toxicity at very low cell concentrations (as low as 200 cells/L) (Lassus et al., 1985).

2.3.2.9 ***Dinophysis caudata*** Saville-Kent 1881 (Fig. 2-9)

Synonyms: *Dinophysis homuncula* Stein 1883

Dinophysis diegensis Kofoid 1907

Description: Cells are large, long and fin sharped, with variation in the shape of the antapical horn, containing golden-brown chloroplasts, occur singly or paired cells (Fig. 2-9, b-c), and the paired cells dorsally joined at the widest point of the hypotheca (Fig. 2-9, c). The cingulum is narrow with two well-developed lists, anterior cingular list (ACL) and posterior cingular list (PCL), supported by ribs (Fig. 2-9, b-c). The long left sulcal list (LSL) extends to nearly half of the total cell length and supported by three ribs spaced equally apart (Fig. 2-9, b-c).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are large, long and irregularly subovate with a long ventral projection on the hypotheca, 70-110 μm in length and 37-50 μm in dorso-ventral width, contain large chloroplasts and a large posterior nucleus. This species occurs singly or paired cells, paired cells are common, dorsally joined at the widest point of the hypotheca. The thick thecal plates are heavily areolated, each areole with a pore. The cingulum is narrow with two well-developed lists, anterior cingular list (ACL) and posterior cingular list (PCL), supported by ribs. Both cingular lists are projected anteriorly, and the wide ACL forms a wide and deep funnel obscuring the epitheca. The long left sulcal list (LSL) extends to nearly half of the total cell length and supported by three ribs spaced equally apart. The right sulcal list (RSL) is also present. Both sulcal lists are often reticulated (Lebour, 1925; Dodge, 1982; Fukuyo et al., 1990; Taylor et al., 1995; Steidinger and Tangen, 1996).

Distribution: *D. caudata* is a neritic and estuarine, planktonic species, with worldwide distribution, typically in warm temperate to tropical waters, rarely in cold waters (Steidinger and Tangen, 1996), reported from Japan (Chihara and Murano, 1997); China (Lu, 2008); Hong Kong (Agriculture, Fisheries and Conservation Department, 2008); Taiwan (Shao et al., 2010); New Zealand (Chang et al., 2012); and Korea (Kim et al., 2012).

Harmfulness: *D. caudata* is a toxic species, produces okadaic acid (OA) that causing diarrhetic shellfish poisoning (DSP) toxic in humans or other mammals (Agriculture, Fisheries and Conservation Department, 2008).

2.3.2.10 ***Dinophysis rudgei*** Murray et Whitting 1899 (Fig. 2-10)

Synonyms: *Phalacroma rotundatum* Berg 1881

Dinophysis rotundata Paulsen 1908

Phalacroma Rudgei Paulsen 1908

Dinophysis rotundiformis Tai et Skogsberg 1934

Prodinophysis cf. *rotundata* Balech 1944

Description: Cell is almost egg shaped, the thecal plate heavily areolated, the left sulcal list (LSL) extends to nearly half of the total cell length and supported by three ribs, and the lateral outline of the hypotheca is longer than broad and evenly rounded nearly all around the body but the antero-dorsal portion (Fig. 2-10, b).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are biconvexed, with an especial bulge at the level of the middle of the body in ventral view, 55-65 µm in length and 50-59 µm in dorso-ventral width. The paired ventral epithecal plates are unequal, being arranged obliquely. The anterior cingular list (ACL) is folded, particularly distinctly. The obliquely truncated posterior end of the ventral area terminates just at or in front of the third rib of the left sulcal list (LSL). The lateral outline of the hypotheca is somewhat longer than broad and evenly rounded nearly all around the body but the antero-dorsal portion. The total length of the ventral hypothecal plates is about one-half of the dorso-ventral dimension of the epitheca (Abé, 1967).

Distribution: *D. rudgei* is a planktonic species, with subtropical and warm temperate waters distribution, recorded from Atlantic and Pacific Ocean; Mutsu Bay, Sagami Bay and the Inland Sea of Japan (Abé, 1967).

2.3.2.11 ***Gonyaulax polygramma*** Stein 1883 (Fig. 2-11)

Synonyms: *Protoperidinium pyrophyrum* Pouchet 1883

Gonyaulax schuettii Lemmermann 1899

Description: Cell is elongate, flattened apical spine, protruding spines at the surface of the cingulum, the thecal surface heavily areolated, ornamented with the conspicuous longitudinal ridges, and reticulations are present between the ridges (Fig. 2-11, b).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are elongate and pentagonal, 29-66 µm in length and 26-56 µm in dorso-ventral depth, contain chloroplasts and a posterior nucleus. Epitheca convex to angular, tapering to the apical horn, and exceeding hypotheca, girdle descending, displaced about

1.5 widths. Hypotheca symmetrical, rounded or truncate, may have a variable number of short antapical spines. Sulcus slightly excavated, widening to the posterior, and to the anterior running onto the epitheca. Longitudinal ridges ornament the thecal surface, and reticulations are present between the ridges. On mature cells, longitudinal ridges may be thick and spinulous (Dodge, 1982, Fukuyo et al., 1990, Hallegraeff, 1991, Steidinger and Tangen, 1996; Al-Kandari, et al., 2009).

Distribution: *G. polygramma* is a neritic and oceanic, planktonic species, with cold temperate to tropical waters distribution (Steidinger and Tangen, 1996), reported from Florida and South Africa (Dodge, 1982); Japan (Chihara and Murano, 1997); East China Sea and South China Sea (Lu, 2008); and Korea (Eissler et al., 2009).

Harmfulness: *G. polygramma* is a non-toxin producing species, but as a red tide species, it is associated with massive fish and invertebrate kills due to anoxia and high sulfide and ammonia levels resulting from cell decomposition (Hallegraeff, 1991; Koizumi et al., 1996).

2.3.2.12 ***Lingulodinium polyedrum*** (Stein) Dodge 1989 (Fig. 2-12)

Synonym: *Gonyaulax polyedra* Stein 1883

Description: Cells are roughly pentagonal and polyhedral shaped, no apical horn or antapical spines present (Fig. 2-12, c-e). The thecal plates are coarsely areolate, and the girdle wide and almost equatorial (Fig. 2-12, d-e). Descending cingulum without overhang (Fig. 2-12, d).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are angular, roughly pentagonal and polyhedral shaped, without antapical spines and apical horn, 40-54 µm in length and 37-53 µm in transdiameter width. Thecal plates are thick, well defined, coarsely areolate, and numerous large trichocyst pores are present within areolae. Distinct ridges are present along the plate sutures. The apical pore plate (Po) contains a raised inner elliptical ridge. The first apical plate (1') is long and narrow, comes in direct contact with the Po, and bears a ventral pore on its right side (Kofoid, 1911; Dodge, 1985; Lewis and Burton, 1988; Fukuyo et al., 1990; Steidinger and Tangen, 1996; Kim et al., 2005; Al-Kandari et al., 2009).

Distribution: *L. polyedrum* is a neritic, bioluminescent species, with warm temperate to tropical waters distribution (Steidinger and Tangen, 1996), reported from Japan (Chihara and Murano, 1997); East China Sea and South China Sea (Lu, 2008).

Harmfulness: *L. polyedrum* is a toxic species, produces yessotoxins (YTXs), which was demonstrated in mussels (Turabo et al., 1998; Paz et al., 2004).

2.3.2.13 ***Pyrophacus horologium*** Stein 1883 (Fig. 2-13)

Description: Cell compressed anteroposteriorly, occurs discoidal (Fig. 2-13, c), the apical pore area appears as the two slit-like (Fig. 2-13, d), and the numbers of the epitheca plate are fewer than the other *Pyrophacus* species (Fig. 2-13, e-f).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are biconvex, lens-shaped and compressed anteroposteriorly, occur discoidal, 32-125 μm in length and 35-136 μm in width. Thecal plate numbers are fewer than the other *Pyrophacus* species, and usually only a single posterior antapical plate. Cingulum narrow, equatorial (Steidinger and Tangen, 1996; Balkıs and Koray, 2001).

Distribution: *P. horologium* is a oceanic, neritic, estuarine species, with cold temperate to tropical waters distribution (Steidinger and Tangen, 1996), reported from Japan (Chihara and Murano, 1997); Mediterranean (Balkıs and Koray, 2001); China (Lu, 2008); and Taiwan (Shao et al., 2010).

2.3.2.14 ***Ceratium furca*** (Ehrenberg) Claparède et Lachmann 1859 (Fig. 2-14)

Basionym: *Peridinium furca* Ehrenberg 1835

Synonyms: *Ceratium furca* var. *berghii* Lemmermann 1899

Ceratium furca var. *eugrammum* (Ehrenberg) Schiller 1937

Description: Cells are large and long, with straight body. The epitheca tapering gradually into a rather long apical horn. Two unequal, parallel or slightly divergent antapical horns, the right shorter than the left. The widest point adjacent to the cingulum, and the thecal ornamented with surface ridges (Fig. 2-14, b-d).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are long, 155-260 μm in length and 30-42.5 μm in width. Epitheca tapers into a rather long apical horn. Hypotheca between the antapical horns markedly inclined towards the cingulum. Two robust antapical horns, parallel to each other or slightly divergent, parallel or slightly convergent, left one being about twice as long. Widest point adjacent to the cingulum (Dodge, 1982; Okolodkov, 2010).

Distribution: *C. furca* is a principally coastal species, but also found in estuarine and oceanic environments, with cold temperate to tropical waters distribution (Steidinger and Tangen, 1996), reported from Japan (Chihara and Murano, 1997); China (Lu, 2008); Taiwan (Shao et al., 2010); and New Zealand (Chang et al., 2012).

Harmfulness: *C. furca* is non-toxic, but it has the potentials to form massive blooms (Faust, 2000). Such blooms are capable of killing aquatic biota (Ajuzie and Houvenaghel, 2009).

2.3.2.15 ***Ceratium fusus*** (Ehrenberg) Dujardin 1841 (Fig. 2-15)

Basionym: *Peridinium fusus* Ehrenberg 1834

Synonym: *Ceratium seta* (Ehrenberg) Kofoid 1908

Description: Cell is long and spindle-shaped. The epitheca tapering gradually into a long apical horn. The hypotheca tapering into a long left antapical horn, which is slightly curved, and a reduced right antapical horn is present. The widest point adjacent to the cingulum (Fig. 2-15, b).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells spindle-shaped, long, 243-580 µm in length and 15-25 µm in width. Epitheca tapers continuously into a long apical horn and hypotheca tapers into a long left antapical horn. Apical and left antapical horns equal or subequal in length and slightly curved. A reduced right antapical horn may be present. Widest point adjacent to the cingulum (Dodge, 1982; Okolodkov, 2010).

Distribution: *C. fusus* is an oceanic to estuarine species, principally coastal, with cold temperate to tropical waters distribution (Steidinger and Tangen, 1996), reported from Japan (Chihara and Murano, 1997); China (Lu, 2008); and Taiwan (Shao et al., 2010).

Harmfulness: *C. fusus* is non-toxic (Taylor et al., 1995). However, it is a fish killer (Lu and Hodgkiss, 2004). It kills aquatic animals by depleting water oxygen content during high biomass blooms (Ajuzie and Houvenaghel, 2009).

2.3.2.16 ***Ceratium kofoidii*** Jörgensen 1911 (Fig. 2-16)

Description: Cells are large, with slender horns. The epitheca almost triangular, with well separated apical horn. Two slightly divergent antapical horns, the left being slightly longer. The widest point adjacent to the cingulum, and the theca weakly sculptured (Fig. 2-16, b-d).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are with straight sides and slender, delicate horns, 110-210 μm in length and 20-28 μm in width. Epitheca triangular, with well separated apical horn. Antapical horns short, the left being slightly longer. Widest point adjacent to the cingulum. Theca not sculptured or only weakly sculptured (Okolodkov, 2010).

Distribution: *C. kofoidii* is an oceanic species, with warm temperate to tropical waters distribution (Steidinger and Tangen, 1996), reported from Japan (Chihara and Murano, 1997); South Yellow Sea, East China Sea and South China Sea (Lu, 2008).

2.3.2.17 ***Ceratium tripos*** (Müller) Nitzsch 1817 (Fig. 2-17)

Basionym: *Cercaria tripos* Müller 1777

Description: Cell is large and long, and the subtriangular cell body broad as its long. The epitheca leading sharply into long straight horn, and the antapicals continuous with slightly flattened base, then sharply curved forward making an acute angle with apical horn. The widest point adjacent to the antapical horns (Fig. 2-17, b).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are long, 195-360 μm in length and 65-90 μm in width. Body of cell subtriangular, its length is equal to or slightly exceeds its width. Epitheca triangular leading sharply into a fairly long straight horn. Hypotheca slightly flattened at the posterior end. Apical horn straight, from short to long, positioned subcentrally, slightly inclined to the right. Antapical horns rather short, directed laterally at their bases and then bent anteriorly. Widest point adjacent to the antapical horns (Dodge, 1982; Al-Kandari, et al., 2009; Okolodkov, 2010).

Distribution: *C. tripos* is a coastal and oceanic species, with warm temperate to tropical waters distribution (Steidinger and Tangen, 1996), reported from Japan (Chihara and Murano, 1997); China (Lu, 2008); Taiwan (Shao et al., 2010); and New Zealand (Chang et al., 2012).

Harmfulness: *C. tripos* blooms can provoke both hypoxic and anoxic conditions (Taylor et al., 1995), events that deplete oxygen in water and cause biota kills (Ajuzie and Houvenaghel, 2009).

2.3.2.18 ***Durinskia capensis*** Pienaar, Sakai et Horiguchi 2007 (Fig. 2-18)

Description: Cells are almost rounded to ovoidal, the epitheca is slightly larger than the hypotheca, and the epitheca is almost hemispherical, the hypotheca is hemispherical to trapezoidal (Fig. 2-18, c-d). The cingulum is well excavated (Fig. 2-18, c).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are almost rounded to ovoidal in ventral view and dorsoventrally flattened, 15.6-28 µm in length and 16-26.6 µm in width. The epitheca is slightly larger than the hypotheca. The epitheca is hemispherical and the hypotheca is hemispherical to trapezoidal. The posterior of the cell is often flattened or notched by the distal end of the sulcus. The cingulum is well excavated, left-handed and is displaced by its width. The sulcus is conspicuous, wider toward the posterior, and reaches the antapex. The nucleus is typically dinokaryotic, ovoidal, and situated in the center or left side of the cell. The acetocarmine-stained cell has another small spherical nucleus at the right of the cell. The apical pore plate (Po) is small and circular in shape. The canal plate (x) is also small, and rectangular. The arrangement of the epitheca is asymmetrical. Variations in the shape of the 2a plate, which is pentagonal or hexagonal, have been noted. The arrangement of the hypotheca is almost symmetrical (Pienaar et al., 2007; Al-Yamani and Saburova, 2010).

Distribution: *D. capensis* is a planktonic species, often occurred in large numbers in tidal pools, resulting in very characteristic orange-red water, reported from supra littoral pools along the west coast of the Cape Peninsula, Republic of South Africa (Pienaar et al., 2007).

2.3.2.19 ***Peridinium quinquecorne*** Abé 1927 (Fig. 2-19)

Synonym: *Protoperidinium quinquecorne* (Abé) Balech 1974

Description: Cells are almost rhomboid, the epitheca is conical with a pointed apex, and the hypotheca is angular with four antapical spines (Fig. 2-19, c-g). The theca weakly sculptured (Fig. 2-19, c).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are rhomboid, 17.5-42.5 µm in length and 15-35 µm in width. Epitheca is conical with a pointed apex. Hypotheca is angular, 4 antapical spines variable in length. Intercalary plates 1a pentagonal and 2a heptagonal. Apical plate is a round chamber with a Po plate and X canal plate (Faust et al., 2005; Al-Yamani and Saburova, 2010; Aké-Castillo and Vázquez, 2011).

Distribution: *P. quinquecorne* is a benthic and planktonic species, with tropic waters distribution, reported from Japan, Philippines, Malaysia, Australia, Belize, South Africa (Horiguchi and Pienaar, 1991; Faust et al., 2005; Mohammad-Noor et al., 2007); and South China Sea (Lu, 2008).

Harmfulness: *P. quinquecorne* can cause anoxia and fish kills when it occurs in high abundance (Fukuyo et al., 1990).

2.3.2.20 ***Protoperidinium avellanum*** (Meunier) Balech 1974 (Fig. 2-20)

Basionym: *Properidinium avellana* Meunier 1919

Synonym: *Peridinium avellana* Lebour 1925

Description: Cells are roughly pentagonal shaped, no apical and antapical horns (Fig. 2-20, b-c), and the ventral area is strongly compressed bilaterally, forming a deep longitudinal furrow of subequal breadth (Fig. 2-20, b).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are biconical or globular, bulging along the girdle, without apical and antapical horns, 30-58 μm in width. The intercalary plate is rarely single but typically two or three in number. The ventral area is strongly compressed bilaterally, forming a deep longitudinal furrow of subequal breadth, and the typically symmetrical and exceptionally asymmetrical posterior sulcal plate lies in its hind end (Abé, 1981; Chihara and Murano, 1997).

Distribution: *P. avellanum* is a oceanic species, with temperate waters distribution (Chihara and Murano, 1997), reported from Japan (Chihara and Murano, 1997); and Baltic Sea (Hällfors, 2004).

2.3.2.21 ***Protoperidinium bipes*** (Paulsen) Balech 1974 (Fig. 2-21)

Basionym: *Glenodinium bipes* Paulsen 1904

Synonyms: *Peridinium miniscululm* Pavillard 1905

Minuscula bipes (Paulsen) Lebour 1925

Description: Cells are relatively small, with a triangular epitheca ending in an apical horn, and the hypotheca ending in two antapical horns (Fig. 2-21, b-c). The girdle is slightly righthanded, excavated and bordered by lists, and the sulcus wides posteriorly (Fig. 2-21, b).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are flattened dorsoventrally, have six precingular plates, 20-35 µm in length and 17-19 µm in width, contents are colourless, with the relatively small size, may cause to be overlooked. The triangular epitheca ending in a long apical horn and a shorter hypotheca ending in two fine, solid, antapical horns. The thecal plates are delicate. The first apical is of meta type and 2a is penta. In ventral view, the slides of the epitheca may be straight or concave and the hypotheca straight-sided with the posterior end flattened. The antapical spines are situated at the angle between the flattened antapex and the sides of the hypotheca and diverge outwards. The girdle is slightly righthanded, excavated and bordered by lists. The sulcus wides posteriorly (Dodge, 1982 and 1985; Chihara and Murano, 1997; Al-Kandari, et al., 2009).

Distribution: *P. bipes* is a neritic species (Al-Kandari, et al., 2009), with worldwide distribution (Chihara and Murano, 1997), reported from Japan (Yamaguchi et al., 2007); East China Sea (Lu, 2008); New Zealand (Chang et al., 2012); and Korea (Lee et al., 2014).

2.3.2.22 ***Protoperidinium claudicans*** (Paulsen) Balech 1974 (Fig. 2-22)

Basionym: *Peridinium claudicans* Paulsen 1907

Description: Cells are pyriform, with epitheca drawn into apical horn. The thecal plates are slightly reticulated, and the plate 1' is almost rhomboid, with nearly equal sides along the right margin and a longer proximal margin that distal one. The hypotheca is drawn into two high conical horns (Fig. 2-22, d-e).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are ortho-penta, pyriform, noticeably dorsoventrally compressed, with epitheca drawn into apical horn, 85-102.5 µm in length and 52.5-67.5 µm in width. Cingulum planozone, descending, with 1.5 cingulum width offset. Plate 1' rhomboid, with nearly equal sides along the right margin and a longer proximal margin that distal one. Plate 2a is about twice as wide as long. The hypothecal pore is absent. Hypotheca is drawn into two high conical horns terminated with two strong spines; frequently the left spine is directed backward and the right one upward-backward (Evagelopoulos 2002; Okolodkov, 2008).

Distribution: *P. claudicans* is a principally coastal and open water species, but also found in estuarine environment, with temperate to tropical waters distribution (Steidinger and Tangen, 1996), reported from Japan (Chihara and Murano, 1997); Yellow Sea, East China Sea and South China Sea (Lu, 2008); and New Zealand (Chang et al., 2012).

2.3.2.23 ***Protoperidinium divaricatum*** (Meunier) Parke et Dodge 1976 (Fig. 2-23)

Basionym: *Peridinium divaricatum* Meunier 1919

Description: Cell is quadrangular, no apical horn, and the plate 1' is rhomboid, symmetrical, with the proximal margins about twice as long as the distal ones (Fig. 2-23, b).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are ortho-hexa, quadrangular, with concave sides, with no apical horn, 63-83 μm in length and 46-75 μm in width. Cingulum deeply cavozone, descending, with about 0.5-1.0 cingulum width offset. Plate 1' is rhomboid, symmetrical, with the proximal margins about twice as long as the distal ones. Plate 2a is trapezoidal, almost as long as plate 4" or up to 1.5 times longer, with very long proximal and very short distal lateral margins, sometimes almost neutra or even neutra in plate 2a. The hypothecal pore is absent. Hypotheca has two low conical horns ending in strong, very divergent spines, the right horn being slightly larger. The left spine is directed downward-backward and the right one upward-backward (Okolodkov, 2008).

Distribution: *P. divaricatum* is a coastal species, with worldwide distribution (Chihara and Murano, 1997), reported from Japan (Chihara and Murano, 1997); East China Sea and South China Sea (Lu, 2008).

2.3.2.24 ***Protoperidinium excentricum*** (Paulsen) Balech 1974 (Fig. 2-24)

Basionym: *Peridinium excentricum* Paulsen 1907

Description: Cells are strongly compressed anteroposteriorly, apex-antapex axis is oblique, offset to the left and ventral (Fig. 2-24, e). The sulcus is deeply excavated and reaches beyond the centre of the hypotheca, the left projecting further than the right, and the cingulum is excavated, bordered by lists supported by spines (Fig. 2-24, f).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are discoid, strongly compressed anteroposteriorly, with the apical horn situated very close to the ventral side of the cell, 17.5-37 μm in length and 37.5-66.3 μm in width. Cingulum deeply cavozone, ascending, with about 0.5 cingulum width offset, with numerous longitudinal ribs. Plate 1' is rhomboid, symmetrical, narrow, as wide as the cingulum. Plate 2a is about four times larger than plate 1a. Hypotheca is slightly longer in its left part, sometimes bearing a small spine at the antapex (Dodge, 1982; Okolodkov, 2008).

Distribution: *P. excentricum* is a primarily estuarine and coastal species, with temperate to tropical waters distribution (Steidinger and Tangen, 1996), reported from Japan (Chihara and Murano, 1997); Yellow Sea (Lu, 2008); and New Zealand (Chang et al., 2012).

2.3.2.25 ***Protoperidinium latispinum*** (Mangin) Balech 1974 (Fig. 2-25)

Basionym: *Peridinium latispinum* Mangin 1926

Synonyms: *Peridinium africanoides* Dangeard 1927
Protoperidinium africanoides (Dangeard) Balech 1974

Description: Cells are pentagonal shaped, with a slight mid-ventral depression (Fig. 2-25, c-d). The epitheca is weakly convex conical, tapering distally to a short spical horn, and the hypotheca is obliquely truncated at its distal end between the two antapical spines (Fig. 2-25, c).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are pentagonal shape in ventral view and irregularly rhomboidal in side view, bulging more dorsally than ventrally and with a slight mid-ventral depression, 100-107 μm in length and 74-78 μm in width. The epitheca is weakly convex conical, tapering distally to a short spical horn. The girdle upward, with the divergence ends at 1/3-1 girdle width, flat with clear ribbed membrane. The hypotheca is obliquely truncated at its distal end between the two antapical spines. The ventral area is narrow, ending posteriorly in front of the center of hypotheca. The right sulcal list is represented by its anterior half lying along the median edge of the right ventral postcingular plate. The left sulcal list is, on the contrary, much strongly bulit, and merges posteriorly without interruption into the ventral sidewing of the left antapical spine. The thecal plate is covered with small polygonal meshes (Abé, 1981; Gómez; 2005).

Distribution: *P. latispinum* is a coastal species, with warm waters distribution (Chihara and Murano, 1997), reported from Japan (Chihara and Murano, 1997); Yellow Sea, East China Sea and South China Sea (Lu, 2008).

2.3.2.26 ***Protoperidinium marukawai*** (Abé) Balech 1974 (Fig. 2-26)

Synonym: *Peridinium marukawai* Abé 1936

Description: The epitheca drawn into apical horn, and the hypotheca is angular with two antapical spines (Fig. 2-26, b-c). The tetragonal middle intercalary plate is displaced slightly towards the left (Fig. 2-26, c-d). The cingulum is bordered by lists supported by spines (Fig. 2-26, b-c).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are 53-72 μm in length and 56-66 μm in width. Plate pattern is fairly constant so far as the materials are concerned. Tetragonal middle intercalary plate is displaced slightly towards the left. Cingular wall was established recently to consist of two minute and an elongated plates, the former lying at either end of the girdle and the latter covering the majority of it. Striated sutural zones between the cingular plates extend laterally nearly to the lateral extremities of the two ventral precingulars, the total length inclusive of the sutural zone is often misinterpreted as its ordinary length (Abé, 1981).

Distribution: *P. marukawai* is a neritic species, reported from North Japan (Chihara and Murano, 1997; Gómez, 2005).

2.3.2.27 ***Protoperidinium minutum*** (Kofoid) Loeblich III 1970 (Fig. 2-27)

Basionym: *Peridinium minutum* Kofoid 1907

Description: Cell is almost spherical shaped, with a conspicuous apical horn and no antapical extension, the cingulum was equatorial, and the thecal surface contained large pores that were sparsely distributed (Fig. 2-27, d).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are spherical in ventral view with a conspicuous apical horn and no antapical extension, 40-46.7 μm in diameter. Plate 1' was rhombic (ortho-type) and asymmetrical, did not connect with the cingulum. The anterior part of the sulcal anterior plate was narrow, extended between plates 1'' and 7'', and was pointed. The two anterior intercalary plates were hexagonal and subequal. The cingulum was equatorial with no displacement. The right sulcal plate connected with the cingulum plate and had a well-developed fin that covered the sulcal area. The left sulcal plate was long and formed a J-shaped curve. The posterior sulcal plate was pentagonal and almost symmetrical. The thecal surface contained large pores that were sparsely distributed (Yamaguchi et al., 2011).

Distribution: *P. minutum* is a coastal and open water species, with cold temperate to warm waters distribution (Chihara and Murano, 1997), reported from Japan (Chihara and Murano, 1997); Yellow

Sea, East China Sea and South China Sea (Lu, 2008); Taiwan (Shao et al., 2010); and New Zealand (Chang et al., 2012).

2.3.2.28 ***Protoperidinium obtusum*** (Karsten) Parke et Dodge 1976 (Fig. 2-28)

Basionym: *Peridinium divergens* var. *obtusum* Karsten 1906

Synonyms: *Peridinium obtusum* (Karsten) Fauré-Fremiet 1908

Peridinium leonis f. *matzenaueri* Schiller 1937

Description: Cell is quadrangular, with almost straight sides, no apical horn, but has a blunt apex. The epitheca covered by longitudinal striations, and the cingulum bordered by a list, supported by spines. The plate 1' is rhomboid, with the distal sides about 1.5 longer than the proximal ones (Fig. 2-28, d).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are orthoquadra, quadrangular, with almost straight sides, with no apical horn, 67.5-86 μm in length and 68-77 μm in width. Cingulum cavozone, descending, with 0.75-1.0 cingulum width offset, strongly inclined ventrally-antapically in relation to the longitudinal axis of the cell. Plate 1' is rhomboid, with the distal sides 1.5-2.0 longer than the proximal ones; distal sides are slightly or strongly concave toward the 1' plate anteriorly and straight or slightly convex posteriorly. Plate 2a is equal to plate 4'' in length. Precingular plates are characterized by a reticulation pattern tending to form longitudinal ridges. The hypothecal pore is absent. Hypotheca has two antapical low conical horns ending in strong spines (Okolodkov, 2008).

Distribution: *P. obtusum* is a coastal and oceanic species, with temperate to tropical waters distribution (Steidinger and Tangen, 1996), reported from Japan (Chihara and Murano, 1997); East China Sea and South China Sea (Lu, 2008).

2.3.2.29 ***Protoperidinium oceanicum*** (Vanhöffen) Balech 1974 (Fig. 2-29)

Basionym: *Peridinium oceanicum* Vanhöffen 1897

Synonyms: *Peridinium divergens* var. *oceanicum* Ostenfeld 1899

Peridinium murrayi Kofoid 1907

Protoperidinium murrayi (Kofoid) Hernández-Becerril 1991

Description: Cell is elongated, quadrangular body with slender apical horn (Fig. 2-29, d). The hypotheca has two antapical high, conical, slender horns ending in strong spines (Fig. 2-29, e).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are ortho-quadra, elongated, with quadrangular body, with more or less separated, slender apical horn, 100-130 µm in length and 68-77 µm in width. Cingulum planozone, descending, with about 1.0-2.0 cingulum width offset. Plate 1' is rhomboid, symmetrical. Plate 2a and plate 4'' are nearly equal in length. The hypothecal pore is absent. Hypotheca has two antapical high, conical, slender horns ending in strong spines (Evagelopoulos, 2002; Horner, 2002; Okolodkov, 2008).

Distribution: *P. oceanicum* is a coastal and oceanic species, with temperate to tropical waters distribution (Steidinger and Tangen, 1996), reported from Japan (Chihara and Murano, 1997); Yellow Sea, East China Sea and South China Sea (Lu, 2008); Taiwan (Shao et al., 2010); and New Zealand (Chang et al., 2012).

Harmfulness: *P. oceanicum* is a toxic species causing diarrhetic shellfish poisoning (DSP) (Camacho et al., 2007).

2.3.2.30 ***Protoperidinium pellucidum*** Bergh 1881 (Fig. 2-30)

Synonym: *Peridinium pellucidum* (Bergh) Schütt 1895

Description: Cells are pyriform (Fig. 2-30, e), or quadrangular (Fig. 2-30, f), with a short, not well-separated apex (Fig. 2-30, f). The cingulum slightly ascending, two antapical spines are slightly divergent, and the left sulcal list looks like a third spine (Fig. 2-30, e).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are para-hexa, pyriform or quadrangular, with a short, not well-separated apex, 40-59 µm in length and 35-46 µm in width. Cingulum planozone, ascending, with 0.5-0.9 cingulum width offset. Plate 1' with slightly concave proximal and distal margins; its suture contacting the 2'' plate is about twice as long as that contacting the 6'' plate. Plates 1a and 3a are pentagonal and are about half the size of plate 2a. Plate 2a is trapezoid, has long proximal and short distal lateral margins, and is 1.2-1.5 times longer than plate 4''. The hypothecal pore is present, and it is situated closer to the cingulum. Two antapical spines are slightly divergent, and two sulcal lists are noticeable between spines (Okolodkov, 2008).

Distribution: *P. pellucidum* is a mainly coastal species, with temperate to tropical waters distribution (Steidinger and Tangen, 1996), reported from Japan (Chihara and Murano, 1997); Bohai Sea, Yellow Sea, and East China Sea (Lu, 2008); and New Zealand (Chang et al., 2012).

Harmfulness: *P. pellucidum* is a toxic species causing diarrhetic shellfish poisoning (DSP) (Camacho et al., 2007).

2.3.2.31 ***Protoperidinium punctulatum*** (Paulsen) Balech 1974 (Fig. 2-31)

Basionym: *Peridinium punctulatum* Paulsen 1907

Synonym: *Peridinium subinerme* var. *punctulatum* (Paulsen) Schiller 1937

Description: Cell is pentagonal (Fig. 2-31, c). The epitheca has a conical form, and the cingulum is circular and excavated, whereas its lists are indistinct, and the 1' plate is ortho-type (Fig. 2-31, d). The height of the hypotheca is a little shorter than that of the epitheca, and the sulcus is deep and narrow and widens only a little at its lowest part, extends to the antapical surface of the hypotheca (Fig. 2-31, c). The ornamentation of the theca consists of small, robust spines (Fig. 2-31, d).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are pentagonal, 48-58 µm in length and 53-64 µm in width. The epitheca has a conical form. The cingulum is circular and excavated, whereas its lists are indistinct. The 1' plate is ortho-type, whereas the 2a plate is penta or hexa-type. The hypotheca is hemispherical with an antapical indentation formed by the sulcus; its height is similar or a little shorter than that of the epitheca. The sulcus is deep and narrow and widens only a little at its lowest part, forming a rounded lower margin; it extends to the antapical surface of the hypotheca. The ornamentation of the theca consists of small, robust spines (Evagelopoulos, 2002; Al-Kandari, et al., 2009).

Distribution: *P. punctulatum* is a coastal species, with worldwide distribution (Chihara and Murano, 1997), reported from Japan (Matsuoka et al., 2006); Yellow Sea and East China Sea (Lu, 2008); and New Zealand (Chang et al., 2012).

2.3.2.32 ***Protoperidinium pyriforme*** (Paulsen) Balech 1974 (Fig. 2-32)

Basionym: *Peridinium steinii* var. *pyriformis* Paulsen 1905

Synonym: *Peridinium pyriforme* Paulsen 1907

Description: Cells are pyriform, with a well-separated apical horn, the cingulum is bordered by lists supported by spines, and the hypotheca has two strong, long, widely winged antapical spines (Fig 2-32, b-c).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are meta-penta, pyriform, with a short, well-separated apical horn, 55-60 µm in length and 37.5-42 µm in width. Cingulum planozone, ascending, with 0.8-1.0 cingulum width offset. Plate 1' is asymmetrical, with the right distal side longest. Plate 2a is as long as plate 4'', slightly displaced to the left. The hypothecal pore is absent. Hypotheca has two strong, long, widely winged antapical spines (Okolodkov, 2008).

Distribution: *P. pyriforme* is a coastal species, with worldwide distribution (Chihara and Murano, 1997), reported from Japan (Chihara and Murano, 1997); South China Sea (Lu, 2008); and New Zealand (Chang et al., 2012).

2.3.2.33 ***Scrippsiella trochoidea*** (Stein) Balech ex Loeblich III 1965 (Fig. 2-33)

Basionym: *Glenodinium trochoideum* Stein 1883

Synonyms: *Glenodinium acuminatum* Jörgensen 1899
Peridinium faeroense Paulsen 1905
Peridinium trochoideum (Stein) Lemmermann 1910
Scrippsiella faeroensis (Paulsen) Balech et Soares 1966
Scrippsiella faeronese Dickensheets et Cox 1971
Calciodinellum faeroense (Paulsen) Havskum 1991

Description: Cell is small pear-shaped, containing yellow-brown chromatophores, the epitheca is conical with a raised apex, and the hypotheca is almost hemisperical with no antapical projections (Fig. 2-33, c).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are small pear-shaped, 16-36 µm in length and 20-23 µm in width, several yellow-brown chromatophores are present and a central nucleus. The girdle is wide, excavated, composed of six plates and surrounded by narrow lists. The epitheca is conical and has a short apical process. There are no antapical projections and the wide excavated sulcus does not indent the antapex. The hypotheca is round and does not have any projections. The theca has scattered poroids (Horner, 2002; Al-Kandari, et al., 2009).

Distribution: *S. trochoidea* is a neritic and estuarine species, with worldwide distribution (Steidinger and Tangen, 1996), reported from Japan (Gottschling et al., 2005); East China Sea and South China Sea (Lu, 2008); and New Zealand (Chang et al., 2012).

Harmfulness: *S. trochoidea* is not a toxin producer, but it has been associated with aquatic biota kills (Ajuzie and Houvenaghel, 2009).

2.3.2.34 ***Heterocapsa* Stein** 1883 (Fig. 2-34)

Description: Cells are almost spherical or ellipsoidal, relatively small, containing yellowish brown chloroplast, and the epitheca and hypotheca were hemispherical or conical and almost the same in the size, but included some variations (Fig. 2-34, a-d).

Remarks: The characteristics of this species are described in past studies as follows, which almost coincide with the above description: Cells are spherical or ellipsoidal, relatively small, 8.8-37.5 µm in length and 7.2-28 µm in width. The epitheca and hypotheca were hemispherical or conical and almost same in size, but included some variations. Possessed longitudinal and transverse flagella housed in sulcus and cingulum. A cingulum usually displaced about 1/2-1/3 of its own width. Autotrophic, possessing yellowish brown chloroplast periphery situated, and an eyespot lacked. A dinokaryotic nucleus and a pyrenoid (rarely two pyrenoids) surrounded by starch sheaths were present. The anterior part of thecal plates consisted of an apical pore plate (Po) and a canal plate (cp, or X plate), both of which were surrounded by five plates of the apical series. The Po plate was U-shaped and located at apical part of the cell. The cp plate was rather small and rhomboid, located in the opening of the Po plate, which slightly off-centered from ventral to right direction. In ventral view, posterior end of the first apical plate 1' stops in the middle of the epitheca, the anterior sulcal plate (as) deeply penetrated into the epitheca (Steidinger and Tangen, 1996; Iwataki, 2002).

Distribution: *Heterocapsa* Stein has been known as a worldwide distribution species, especially in the coastal waters (Iwataki, 2002). Among the species of this genus, *H. minima* and *H. triquetra* were get reports from East China Sea (Lu, 2008); *H. circularisquama* was first found at Uranouchi Bay (Japan) in 1988 (Matsuyama, 1999), and officially described as a new species by Horiguchi (1995), has been confirmed from more than coastal 20 areas along western Japan (Iwataki, 2002).

Harmfulness: *H. circularisquama* is the first species in the genus *Heterocapsa* that make harmful algal blooms (Iwataki, 2002), tremendous damage to bivalve aquaculture (Horiguchi, 1995; Matsuyama, 1999).

2.3.3 Harmful species

In total, eight toxic and four red tide-forming species were identified near Hainan Island (Tables 3 & 4). Toxic species included ciguatera fish poisoning (CFP) species, *Prorocentrum hoffmannianum* (Fig. 2-2), *P. lima* (Fig. 2-3), and *P. rhathymum* (Fig. 2-5); diarrhetic shellfish poisoning (DSP) species, *P. hoffmannianum*, *P. lima*, *Dinophysis acuminata* (Fig. 2-8), *D. caudata* (Fig. 2-9), *Protoperidium oceanicum*, (Fig. 2-29) and *Pro. pellucidum* (Fig. 2-30); and yessotoxin (YTX) species, *Lingulodinium polyedrum* (Fig. 2-12) (Kim et al., 2005; Camacho et al., 2007; Omura et al., 2012). Red tide-forming species identified included *P. micans* (Fig. 2-4), *P. sigmoides* (Fig. 2-7), *Ceratium furca* (Fig. 2-14), and *C. fusus* (Fig. 2-15) (Fraga et al., 1988; Myat et al., 2012).

Toxic species *P. lima*, *D. acuminata*, and *L. polyedrum* appeared at only St. 5, while *P. rhathymum* was found at most stations except for Sts. 4, 7, and 9. *P. rhathymum* was the only HAB species at St. 2, and occurred in large numbers at Sts. 1, 3, and 8. *Pro. pellucidum* occurred at five sites, Sts. 3, 5, 8, 9, and 10, and *Pro. oceanicum* appeared at just two, Sts. 1 and 5. *D. caudata* and *P. hoffmannianum* each appeared at only one site, either St. 9 or 8, respectively.

Among red tide-forming species, *P. micans* appeared at most stations except for Sts. 2, 3, and 4, which were near natural ports or scenic spots. *P. sigmoides*, *C. furca*, and *C. fusus* were found at Sts. 5 and 8, Sts. 1, 6, 7, 9, and 10, and Sts. 1, 6, 7, and 8, respectively, all of which are probably affected by human activities including fish farming, fishing villages, seafood markets, and shipping ports. In particularly, *C. furca* was occurred in large numbers at St. 7, which was near seafood markets and extensive fish farming.

2.3.4 Similarity of species among sampling sites

Cluster analysis of dinoflagellate species occurrence in samples from all stations revealed two clusters. One cluster included three sites (Sts. 2, 3, and 11) with 45% similarity in species composition. Because these sampling sites are near natural ports or scenic spots, they have been less affected by human activities. Therefore, eutrophication does not seem to have progressed much at these sites. The other cluster included seven sites (Sts. 1, 5, 6, 7, 8, 9, and 10) with 31.2% similarity in species composition. Fish farming, fishing villages, seafood markets, or shipping ports are common to these sites, which appear to be relatively strongly affected by human activities. Samples from one out-group, a scenic spot at St. 4, included no dinoflagellate species (Fig. 3).

2.4 Discussion

2.4.1 Species diversity

In this study, the southeastern coastal stations of Hainan Island (Sts. 6, 7, 8, and 9) showed higher dinoflagellate species diversity, possibly because the southeastern coast faces the open sea and is affected by multiple currents, including the South China Sea Offshore Current, the Hainan Coastal Current, and the Vietnam Coastal Current (Cai et al., 2007). These three currents shift with the southwestern monsoon in the summer and the northeastern monsoon in the winter, leading to high phytoplankton species diversity in these waters (Dai et al., 2007). On the other hand, there are many fish farms, fishing villages, and seafood markets near the sampling sites along the southeastern coast, which indicates that the species diversity of dinoflagellates may be also affected by human activity in these locations.

Tomczak and Godfrey (1994) summarized the circulation patterns of South China Sea as follows; the southwest monsoon pushes the shelf water northward so as to result in some compensatory southward movements over the deep basin in the eastern South Chia Sea, while the northeast monsoon reverses the flowing direction and a strong boundary current is thus developed along the Vietnam coast (Hu et al., 2000), which causes the active water exchange among Hainan Island, Hong Kong, and Vietnam. In Hong Kong, nine *Alexandrium* species and five *Karenia* species were found (Agriculture, Fisheries and Conservation Department, 2008), while 24 *Dinophysis* species were found in Vietnam (Le et al., 2012). Extreme differences from Hainan Island at present study might be the causes that sampling areas and season were limited (Sts. 1, 2 and 8 were only in summer, Sts. 3, 4 and 7 were only in winter), though seasons can be the factor to the dinoflagellate occurrence, such as *Alexandrium* speceis (Azanza and Benico, 2013).

2.4.2 Characteristics for the occurrence of dinoflagellates

Thirteen species of *Protoperidinium* were commonly found around Hainan Island, and their populations were especially diverse around the stations that were close to extensive fish farming or fishing villages. *Protoperidinium* is one of the most diverse and widespread genera among marine phytoplankton (Faust, 2002) that is distributed globally and often dominates coastal ecosystems (Evagelopoulos, 2002; Faust, 2002; Okolodkov, 2008; Taylor et al., 2008). It is nonphotosynthetic and often occurs in association with blooms of prey organisms such as diatoms and other dinoflagellates (Bralewska and Witek, 1995). *Protoperidinium* species also feed on bacteria and nanoflagellates (Sherr and Sherr, 2002; Naustvoll, 2000).

Cluster analysis using species similarity indices showed that one cluster with 31.2% species similarity comprised 37 species and included Sts. 1, 5, 6, 7, 8, 9, and 10 locations that were frequently exposed to anthropogenic disturbances such as terrestrial nutrient inputs from the mainland and construction of jetties and harbors for leisure that seem to lead dinoflagellate blooms (Garcés et al., 2000). The other group with 45% species similarity comprised only 4 species and included Sts. 2, 3, and 11, which were near natural ports or scenic spots. Relatively infrequent human activities in these areas, such as tourism or an occasional ship passing, seemed unlikely to be related to dinoflagellate blooms.

2.4.3 Potential threats of harmful dinoflagellates

Twelve harmful species, comprising of four red tide-forming species and eight toxic species, have been detected near Hainan Island, suggesting that the coastal areas around Hainan Island potentially face the danger of HABs.

Prorocentrum micans and *P. sigmoides* cause red water coloration and are responsible for the killing of oysters by oxygen depletion due to massive blooms (Pastoureaud et al., 2003; Lee et al., 2005). *Ceratium furca* has the possibility to cause oxygen depletion and also damage fish gills under the condition of high density (Morton et al., 2011). Bloom of *C. fusus* was thought to related to the death of invertebrate larvae (Taylor et al., 2004; Alkawri and Ramaiah, 2010), and cause extensive red tides in the waters around East Asian countries, such as China and Philippines (Baek et al., 2007). Large quantities of shellfish are consumed on Hainan Island, indicating a potentially high relative risk of poisoning by toxic dinoflagellates. Six DSP species (*P. hoffmannianum*, *P. lima*, *D. acuminate*, *D. caudata*, *Pro. oceanicum*, and *Pro. pellucidum*) were identified in this study, suggesting that DSP species in plankton and toxins in shellfish should be monitored in this region. Although quantitative analysis of species populations was not performed in the present study, the frequent occurrence of CFP species including *P. rhathymum* as the dominant species observed at St. 8 indicates that close attention must be paid to CFP events around Hainan Island.

CHAPTER 3

OCCURRENCE OF DINOFLAGELLATES IN THE KAGOSHIMA BAY AND URANOUCHI BAY, SOUTH JAPAN

3.1 Introduction

Both Kagoshima Bay (Kagoshima Prefecture) and Uranouchi Bay (Kochi Prefecture) are semi-enclosed embayment (Patel et al., 2000; Minowa et al., 2011), located on the south part of Japan. Kagoshima and Kochi Prefectures has been the largest and the third largest greater amberjack farming area in Japan, respectively, and large number of the cages for culturing greater amberjack have been widely distributed within the Kagoshima Bay and Uranouchi Bay (Ottolenghi et al., 2004). Large quantities of the greater amberjack seedlings have been imported from Hainan Island every year since 1988 (Wakabayashi, 1996; Ottolenghi et al., 2004), which might be cause the invasion of phytoplankton (Scarratt et al., 1993; Schwinghamer et al., 1994). On the other hand, semi-enclosed mariculture water is commonly subjected to recurrent eutrophic events due to poor exchange of water with the sea, heavy disturbance from the introduction of cultured fish or shellfish, and high nutrient and/or contaminant inputs either from marine sources or of autochthonous origin (Xu et al., 2010), which are closely related to the frequency of HABs (Hallegraeff, 1993).

These two areas in Japan are under the influence of the Kuroshio Current (Matsuyama et al., 2001; Bodergat et al., 2002). This current forms east of the Philippines around 13 °N and flows northward along the coast of Luzon as far as the east of Taiwan Island. Then, meanders along the shelf break of the East China Sea flow through the Tokara Strait (Feng et al., 2000; Hu et al., 2008). During this process, the Kuroshio flow path adapts to the western boundary topography and exhibits several changes in direction, resulting in the appearance of the South China Sea Branch of the Kuroshio (SCSBK), the Taiwan Warm Current (TWC), the Yellow Sea Warm Current (YSWC) and the Tsushima Warm Current (TSWC) (Liu et al., 2011). South China Sea Warm Current (SCSWC) is on the right side of the SCSBK flow route (Liu et al., 2011), had a close relationship with the SCSBK (Ye, 1994) and was even considered as an extension of the SCSBK on the continental shelf of the northern SCS (Wang et al., 2010). This current appears as an intermittent northeastward current along the shelf break of the northern South China Sea, causing the flow from the southeast of Hainan

Island to the southwest of Taiwan (Liu et al., 2011), and intrusion to the TWC. Then the branch of TWC intrudes into the Kuroshio (Hu et al., 2000; Liu et al., 2011). Therefore, the procession of these flows might be another opportunity for the invasion of organisms, including phytoplankton, from Hainan Island to Japan (Hallegraeff and Bolch, 1992; Smayda, 2002). In addition, in the past 100 years, the temperature of the coastal waters around Japan has risen by approximately 1 ℃ because of the global warming (Source: Japan Meteorological Agency), may led the procession of these flows more freely.

In this chapter, the dinoflagellate species occurred in Kagoshima Bay and Uranouchi Bay were determined and compared with the species occurred around Hainan Island, to discuss about a possibility for invasion of tropical HAB species from Hainan Island to Japan using the data from this study (see Chapter 2) and those from the historical records around the coastal areas in Japan.

3.2 Materials and methods

3.2.1 Sampling sites

Four pelagic sites and one coastal site were selected in Kagoshima Bay and Uranouchi Bay, south Japan, respectively, where the grate amberjack farming is important for local fishing industries, and its fries are all imported from Hainan Island (Table 2 and Fig. 4). Phytoplankton samples were qualitatively collected with a 5-μm-mesh plankton net. These samples were immediately transferred into 100-ml plastic bottles and preserved with neutral formalin at a final concentration of 1%.

3.2.2 Morphological taxonomy

The several morphological features including cell shape, position of nucleus, and pyrenoid were basically observed under a BX60F up right-microscope (OLYMPUS, Japan). For observation of the thecal plate, cells were stained with Calcofluor White M2R at a final concentration of 10 μg mls^{-1} (Fritz and Teiemer, 1985), analyzed under UV excitation using a BX60F fluorescence microscope. Photographs were captured with a charge-coupled device camera (OLYMPUS DP70; OLYMPUS, Japan) under 40× magnification. Dinoflagellate species were identified according to previous literatures (Chihara and Murano, 1997; Steidinger and Tangen, 1997; Hoppenrath et al., 2009; Omura et al., 2012).

3.2.3 Similarity of species occurrence

A Bray-Curtis similarity index of species occurrence was used as a clustering metric (Myat et al., 2012), and a tree was drawn using group average linkage to reveal clusters. Grouping of stations based on similarity indices for dinoflagellate species composition was carried out using data for species occurrence according to Clarke and Warwick (2001). An agglomerative hierarchical cluster analysis was carried out based on species occurrence (presence or absence) using the PRIMER v6® software package (PRIMER-E Ltd., UK).

3.2.4 A comparison between species occurred in this study and in the previous studies

The species newly recorded from either Kagoshima Bay or Uranouchi Bay and also from Hainan Island in this study would be possibly invasive species from Hainan, if no previous records were found from the literatures. Therefore, the species list in this study was compared from the list in the previous literatures, in order to check the historical occurrence of the species that could be found in the present study.

3.3 Results

3.3.1 Distribution and species composition of dinoflagellates

A total of 27 dinoflagellate species in eight genera from seven families were identified in samples from all of the stations in the present study (Table 5). These comprised five species from the Family Prorocentraceae, four species from the Dinophysiaceae, two species from the Kareniaceae, one species from the Pyrophacaceae, seven species from the Ceratiaceae, six species from the Protoperidiniaceae and two species from the Calciodinellaceae.

The number of dinoflagellate species collected from each sampling site ranged from six to 21.

The largest number of dinoflagellate species, 21, was found at St. C, followed by the species found at St. B, Sts. A and D, and these four sites were all located at Kagoshima Bay. St. E, the only site located at Uranouchi Bay, got the lowest number of dinoflagellate species, six.

Species from the Ceratiaceae were found most frequently at the sampling sites of Kagoshima Bay, followed by species from the Protoperidiniaceae, Prorocentraceae, and Dinophysiaceae. Species from the Kareniaceae were most frequently at Uranouchi Bay. Species from the Dinophysiaceae, Pyrophacaceae, and Protoperidiniaceae, were found at just Kagoshima Bay, while species from the Kareniaceae, and Calciodinellaceae, at Uranouchi Bay.

3.3.2 Harmful species

Harmful species were found at the all sites (Table 5). In total, four toxic and four red tide-forming species were identified from Kagoshima Bay and Uranouchi Bay (Tables 5 & 6). Toxic species included ciguatera fish poisoning (CFP) species, *Prorocentrum rhathymum*; and diarrhetic shellfish poisoning (DSP) species, *Dinophysis caudata*, *D. rotundata*, and *Protoperidium pellucidum* (Camacho et al., 2007). Red tide-forming species identified included *Prorocentrum sigmoides*, *Karenia mikimotoi*, *Ceratium furca*, and *C. fusus* (Fraga et al., 1988; Camacho et al., 2007; Myat et al., 2012).

Toxic species *P. rhathymum* and *D. rotundata* appeared at Sts. B and C, while *Dinophysis caudata* was found at Sts. B, C, and D, and *Pro. pellucidum* occurred at Sts. A, B and C. Among red tide-forming species, *P. sigmoides* was found at Sts. B, C and E, *K. mikimotoi* appeared at only St. E, and *C. furca* and *C. fusus* were found at four stations except for St. D and St. E, respectively. In particularly, *P. sigmoides* was occurred in large numbers at Sts. B and C, and *K. mikimotoi* was in large numbers at St. E.

3.3.3 Similarity of species among sampling sites

The dendrogram of species similarity showed the close relation among Sts. A, B, C and D at 54.65% similarity level (Fig. 5), and these stations are all located at Kagoshima Bay (Fig. 4). Only St. E, where showed small number of species occurrence (six species) and presence of four unique species from other stations, *Karenia digitata*, *K. mikimotoi*, *Scrippsiella spinifera*, and *S. trochoidea*, showed the distinct similarity from the other four sites (Fig. 5).

3.3.4 Similarity between south Japan and Hainan Island

Species from the Kareniaceae only appeared in south Japan, while species from the Gonyaulacaceae, Peridiniaceae, and Heterocapsaceae were found in Hainan Island. Thirteen dinoflagellate species from six families, including six harmful species, appeared in both south Japan and Hainan Island (Table 7). These comprised two species from the Prorocentraceae family, one species from the Dinophysiaceae, one species from the Pyrophacaceae, three species from the Ceratiaceae, five species from the Protoperidiniaceae and one species from the Calciodinellaceae. Cluster analysis in these six families between the two areas showed high similarity level (over 50%) except Prorocentraceae and Dinophysiaceae (Fig. 6a), and the similarity of the harmful species between the two areas was higher, 60%, than those of the total, 40.63% (Fig. 6b).

3.3.5 Species occurred in both south Japan and Hainan Island

In this study, 13 dinoflagellate species, *Prorocentrum rhathymum*, *P. sigmoides*, *Dinophysis caudata*, *Ceratium furca*, *C. fusus*, *C. tripos*, *Pyrophacus horologium*, *Protoperidinium excentricum*, *Pro. latispinum*, *Pro. obtusum*, *Pro. Pellucidum*, *Pro. Punctulatum*, and *Scrippsiella trochoidea* were occurred in both south Japan and Hainan Island (Table 7), all these species got the historical occurrence in Japan (Table 8).

Prorocentrum rhathymum was reported from the Ryukyu Islands (Fukuyo, 1981), but after that, it seems no records from Japan. In this study, we found only a few in Kagoshima Bay, while it was the most frequent species in Hainan Island.

Prorocentrum sigmoides, *Dinophysis caudata*, *Ceratium furca*, *C. fusus*, and *C. tripos* have been reported from the south part of Japan continuously since 2003 (National Research Institute of Fisheries and Environment of Inland Sea, 2001-2013). In the present study, although any quantitative analysis of populations was not performed, these five species were obviously abundant in south Japan, and also occurred with high frequency.

Pyrophacus horologium, *Protoperidinium excentricum*, *Pro. latispinum*, *Pro. obtusum*, and *Pro. punctulatum* were reported from Yatsushiro Sea in 2003 (National Research Institute of Fisheries and Environment of Inland Sea, 2001-2013), *Pro. pellucidum* from Okinawa in 2009 and 2010 (National Research Institute of Fisheries and Environment of Inland Sea, 2001-2013), and *Scrippsiella trochoidea* from Onagawa Bay in 1996 (Ishikawa and Taniguchi, 1996). These seven species were minorities in both south Japan and Hainan Island in this study.

3.4 Discussion

3.4.1 Environmental factor and high species diversity

A very important feature of Uranouchi Bay is that there are no other industries than marine aquaculture (De et al., 2009). On the other hand, environmental conditions in Kagoshima Bay have been affected by several activities including the stock farming industry of livestocks. In particular, volcanic ash often can fall into Kagoshima Bay from Mt. Sakurajima, which is an active volcano that rises from the water and divides the embayment into the northern and southern areas (Bodergat et al., 2002; Minowa et al., 2011), and a counterclockwise eddy current occurs in the widened central portion of the embayment (Bodergat et al., 2002). These activities might be responsible for the phytoplankton increase due to the increased nutrient supply (Langmann et al., 2010; Matear et al., 2013), which in turn may cause the higher dinoflagellate species diversity in Kagoshima Bay.

3.4.2 Potential threat for the invasion of species from Hainan Island by natural and artificial factors

Kagoshima Bay, Uranouchi Bay and Hainan Island, all the three areas emphasize on marine aquaculture, and thus have the serious eutrophication of waters, which is closely related to the frequency of HABs (Hallegraeff, 1993). On the other hand, as mentioned above, phytoplankton might expand to these two areas in south Japan from Hainan Island by the ocean currents and/or by the greater amberjack seedlings importation.

Therefore, these three factors, that are, eutrophicated environment, ocean currents relationship, and fish fry importation, might explain the high similarity of species composition between south Japan and Hainan Island (Fig. 6). In addition, 13 dinoflagellate species including six harmful species appeared in both south Japan and Hainan Island, suggesting the potential threat for the invasion of tropical HAB species from Hainan Island to Japan.

Although the historical occurrence of these 13 dinoflagellate species in Hainan Island is unknown, most of them recorded from Japan recently (since 2003, even 2009) (Table 8), indicated the invasion of tropical species, including harmful ones, from Hainan Island to Japan by the global warming through the current systems and/or by artificial transportation of fish fry may be plausible.

CHAPTER 4

PHYTOPLANKTON COMMUNITY FROM THE BOTTOM SEDIMENTS IN HAINAN ISLAND AND KAGOSHIMA BAY BASED ON METAGENOMIC ANALYSIS

4.1 Introduction

The microbial community including phytoplatokton has been studied based on microscopic analysis and culture-dependent method, contributing to the initial characterization of these microbial community such as morphological characteristics and the optimum growth condition of partial microorganisms (Pearce and Butler, 2002; Pearce et al., 2003). However, these methods limit the clarified knowledge about the microbial community because only a minuscule fraction, about 1%, of all bacteria in environment can be cultured and it is difficult to identify for a large portion of phytoplankton species based on morphological characteristics (Mitra, 2010; Pearman, 2012). Therefore, in the last decades, culture-independent methods using molecular biological technique such as Denaturing Gradient Gel Electrophoresis (DGGE), Fluorescence in situ hybridization (FISH), Restriction Fragment Length Polymorphism (RFLP), and 16S rDNA clone library, whose data is obtained directly from the entire microbial community of a certain environment without isolating and culturing and microscopic analysis, have provided the developed study, which could not be acquired only by culture-dependent technique and microscopic observation (Bowman et al., 1997, 2000; Brambilla et al., 2001; Díez et al., 2001; Pearce, 2003; Pearce et al., 2003). Especially, clone library method has been developed in order to know the microbial community and diversity, but this traditional genomic method cannot achieve the analysis of the comprehensive microbial diversity because of the limit of the analyzed number of targeted genomes. Recently, metagenomic analysis using high-throughout sequencing was developed as the technique that can fill the gap between these traditional molecular biological method and the true microbial community, their functions, cooperation and evolution in environments such as soil, water, ancient remains of animals, or the digestive system of animals and humans (Mitra, 2010; Kopylova, 2013).

Metagenomic analysis has been developed as a cutting-edge approach for the genomic study of microorganisms without the need for cultivation and isolation (Ogura et al., 2011). This study begin with obtaining a sample from a particular environment such as marine, soil, or the human biome, and then, extracting genetic material from ideally all of the organisms in the sample, which is similar to clone library method. In metagenomic analysis, data set can be obtained from more than 10 thousands sequenced amplicons of the extracted DNA as a collection to gain. For example, laboratory-cultured bacteria are, typically, induced to take up and replicate the fragments of the extracted DNA, creating a ‘library’ containing the genomes of all the bacteria and archaea found in the sampled environment (Mitra, 2010). Advances of this technique significantly have been promoting microbial diversity and ecological study without traditional culture-dependent methods with the cultivation and isolation requiring the laborious and much experimental time (Yan and Yu, 2011).

Resting cysts of phytoplankton including dinoflagellates are dormant stages resulting from sexual fusion of gametes, produced in response to certain physiochemical conditions, such as temperature and/or nutrient depletion (von Stosch, 1973; Godhe et al., 2001), may contribute to phytoplankton assemblages in the water column through germination (Shin et al., 2007), and play an important role in species dispersal, bloom initiation and termination (Anderson and Wall, l978; Anderson, 1998; Kotani et al., 2006; Shin et al., 2007). The cysts fall to the bottom and have mandatory dormancy periods of species-specific length, when germination of the cysts is impossible (Pfiester and Anderson, 1987), constitute a ‘seed bank’ for the region where they were present and they can remain alive in the bottom sediments for long period (Lewis et al., 1999). Many of the harmful species, i.e. toxin producers and species causing red tides, form cysts as a part of their life cycle (Matsuoka and Fukuyo, 1995). Therefore, cysts in the bottom sediments might reflect a history of species colonizing in a certain area and the whole community of phytoplankton (Shin et al., 2007), because a species found in the sediments does not necessarily occur in water column in a certain period. In this study, plankton samples were collected only in summer and winter, which might be partial knowledge of the community, so the information from the bottom sediments was necessary to clarify the whole phytoplankton community structures in the study sites.

In this chapter, a comparison of phytoplankton community structures from the bottom sediments between Hainan Island and Kagoshima Bay was carried out using Illumina sequencing, and discussed about a possibility for invasion of tropical HAB species from Hainan Island to Japan based on the data about more detailed microbial community.

4.2 Materials and methods

4.2.1 Sampling sites

The bottom sediments were collected by a grab sampler at St. 6 of Hainan Island in winter of 2013, and by an UWITEC sediment corer at St. A of Kagoshima Bay in winter of 2014 (Table 2, Fig. 1 & 4). These samples were immediately transferred into 200-ml plastic bottles and stored at 4 ℃ under dark until the analyses.

4.2.2 Metagenomic analysis

DNA were extracted from 1g of the sediment samples using an UltraClean® Soil DNA Isolation Kit (MO BIO Laboratories, Inc.) following the manufacturer's protocol.

A genomic region analyzed was *psb*A in chloroplast, in order to eliminate heterotrophs from analysis and focus on photoautotrophs. This procedure could be particularly beneficial in the ecological study of algae (Chafee, 2008). DNA was sheared ultrasonically to fragments, then TruSeq libraries were constructed for paired-end sequencing using the Illumina HiSeq and MiSeq platforms (Illumina, Inc.), and finally the sequences were output with numbers (Satinsky et al., 2014). Sequences were read at three times, and ran BLAST analysis with GenBank databases (Bench et al., 2007).

4.2.3 Similarity of species occurrence

Similarity levels between Hainan Island and Kagoshima Bay were determined by Bray-Curtis similarity index of species occurrence (Myat et al., 2012), using the PRIMER v6® software package (PRIMER-E Ltd., UK). The similarity indices were calculated in two ways, non-quantitative and quantitative analyses, i.e. based on presence or absence for each species or based on number of sequences for each species, respectively.

4.3 Results

4.3.1 Phytoplankton community of Hainan Island and Kagoshima Bay

Number of sequences with the homology of more than 95% of the Blast Hit identity values was ca. 135,152 sequences, accounted for 81.6% of total sequences obtained from Hainan Island, while 15,277 sequences, accounted for 34.8% from Kagoshima Bay (Table 9).

Phytoplankton in Hainan Island consists of 81 species from seven phyla, including 57 of Bacillariophyta, eight of Chlorophyta, seven of Cyanobacteria, six of Heterokonphyta, one of Cryptophyta, one of Dinoflagellate and one of Phaeophyta (Table 9, Fig. 7a). Whereas in Kagoshima Bay, it consists of 61 species from six phyla, including 28 of Bacillariophyta, 11 of Chlorophyta, seven of Cyanobacteria, five of Heterokonphyta, seven of Dinoflagellate, and three of Haptophyta (Table 9, Fig. 7b).

Species from the Bacillariophyta were found most frequently in both Hainan Island and Kagoshima Bay. Species from the Cryptophyta and Phaeophyta were found only in Hainan Island, while species from the Haptophyta only in Kagoshima Bay.

4.3.2 Harmful species

Four harmful species were found, including two toxic species and two red tide-forming species. Toxic species included paralytic shellfish poisoning (PSP) species, i.e. *Alexandrium catenella* and *A. tamarense* (Camacho et al., 2007). Red tide-forming species included *Heterocapsa rotundata* and *Heterosigma carterae* (Iwataki, 2008; Omura et al., 2012). Red tide-forming species *H. carterae* was Heterokonphyta and appeared in both two areas, while the other three were dinoflagellates and found only in Kagoshima Bay (Table 9).

4.3.3 Similarity between Hainan Island and Kagoshima Bay

Thirty-five species from five phyla, including 26 of Bacillariophyta, four of Chlorophyta, three of Cyanobacteria, one of Dinoflagellate, and one of Heterokonphyta, appeared in both two areas

(Table 10). Bacillariophyta was the most diverse phyla, and *Rhizosolenia setigera* was the most dominant species in both two areas (Table 9).

Similarity indices in nine classes and 17 orders that appeared in both areas between Hainan Island and Kagoshima Bay calculated using the non-quantitative analysis (Fig. 8, a - b), showed a high similarity level (>50%) except for Naviculales, which appeared 24 species of *Pinnularia* in Hainan Island while only three in Kagoshima Bay (Table 9, Fig. 8b). Similarity index for the harmful species between the two areas was 40%, being lower than those of total, 49% (Fig. 8c).

The quantitative similarity analyses (Fig. 9) showed extremely difference between the two areas with low similarity (<50%) except for Aulacoseirales, Anaulales and Cymatosirales, which were consisted of only one species (Table 9, Fig. 9b). Similarity of the harmful species between the two areas was only 0.3%, lower than those of total, 17% (Fig. 9c).

4.3.4 Species occurred in both Hainan Island and Kagoshima Bay

From the analysis, 35 phytoplankton species occurred in both Hainan Island and Kagoshima Bay (Table 10). Ten species from Bacillariophyta, i.e. *Psammodictyon constrictum*, *Navicula* sp., *Pinnularia subanglica*, *P. viridiformis*, *Stephanopyxis turris*, *Rhizosolenia setigera*, *Cyclotella meneghiniana*, *Asteroplanus karianus*, *Plagiostriata goreensis*, and *Pseudostaurosira brevistriata*, were abundant in Hainan Island with obviously larger number of sequences than those in Kagoshima Bay. Two species from Bacillariophyta, i.e. *Chaetoceros socialis* and *Skeletonema costatum*, were minorities in Hainan Island, while obviously abundant in Kagoshima Bay. Remaining 23 species were all minorities and occurred with similar frequency in both Hainan Island and Kagoshima Bay.

4.3.5 Comparison of dinoflagellate species occurrence between the plankton and sediment samples

In the sediment samples, only one species, *Peridinium foliaceum*, occurred at St. 6 in Hainan Island, while seven species, *Alexandrium tamarense*, *A. catenella*, *Heterocapsa rotundata*, *Peridinium foliaceum*, *Scrippsiella trochoidea*, *Polarella glacialis*, and *Protodinium simplex*, occurred at St. A in Kagoshima Bay (Table 9). These results showed extreme differences from the plankton samples. In the plankton samples, 16 species occurred at St. 6 in Hainan Island while 10 at St. A in Kagoshima Bay (Table 3 & 5), but the species which were present in the sediment samples were not in the phytoplankton samples in both Hainan Island and Kagoshima Bay, and vice versa (Table 3, 5 & 9). This might be related to just 1g sediments for using metagenomic analysis, which may not contain all the phytoplankton cells or cysts in the area.

4.4 Discussion

4.4.1 Species diversity in the sediment samples

In this study, Hainan Island showed higher phytoplankton species diversity, possibly because this area faces to the open sea, affected by multiple currents, and active water exchange with Hong Kong and Vietnam, which leading to the high phytoplankton species diversity (Dai et al., 2007; Hu et al., 2000), while Kagoshima Bay is semi-enclosed, only under the influence of the Kuroshio Current (Matsuyama et al., 2001; Minowa et al., 2011).

Only 34.8% of sequences from Kagoshima Bay were identified through the BLAST analysis. It might be explained from the low similarity level of species composition appeared in both Hainan Island and Kagoshima Bay by quantitative analysis, in spite of the high similarity by non-quantitative one. This may suggest that there are many unknown species, which could not be determined from the BLAST analysis, in Kagoshima Bay where the high diversity of dinoflagellates in plankton was shown.

4.4.2 Possibility of diatom blooms

Bacillariophyta was the most dominate community in both Hainan Island and Kagoshima Bay. Diatoms were considered to be the most widespread group of plants on the earth, being abundant not only in all waters but also in soil, on moist surfaces of rocks and plants (Guillard and Kilham, 1977). The contribution of diatoms to the world net primary production was estimated to be as high as 20 to 25% (Werner, 1977).

Rhizosolenia setigera from the Bacillariophyta was the most dominant species in both Hainan

Island and Kagoshima Bay. Blooms of *R. setigera* have been frequently recorded during low temperature seasons (spring, autumn, and winter), when mainly larger cells of *R. setigera* appear (Takano, 1990). A notable point is that *R. setigera* is one of the main species forming diatom blooms from winter through early spring in Ariake Sea in western Kyushu, Japan (Nagasaki et al., 2004).

Another notable species is *Skeletonema costatum*, which occurred in both Hainan Island and Kagoshima Bay in this study. In East China Sea, depending on the abundance of resting cysts in the sediments, active germinations occur over a wide range of environmental conditions, and the rapid division rates of vegetative cells (Yoder, 1979; Gallagher, 1982; Zhang et al., 2010). *Skeletonema* species appeared as a common planktonic species there during all seasons (Zhang et al., 2010), and formed bloom in the late spring and early summer (Wu et al., 2004). From 1986 to 1993, *Skeletonema* blooms accounted for 27% of the total algal abundance in the East China Sea (Hong, 2003). On the other hand, *S. costatum* was also shown to be the dominant species in Hiroshima Bay, Japan (Itakura et al., 1997). These suggest that the diatom blooms may occur during low temperature seasons in both areas, East China Sea and around the sea off the south part of Japan.

4.4.3 Possibility on colonization of harmful species

Information on algal cysts from the bottom sediments can allow us to know colonization of the algae in a certain area. Ten percent of ca. 2,000 dinoflagellate species has been known to produce resting cysts (Dale, 1983), and water temperature is an important factor that influencing the encystment and excystment of dinoflagellate cysts (Meier and Willems, 2003). The warm waters are adverse for the encystment of many dinoflagellate species (Dodge and Harland, 1991), such as *Protoperidinium* species may form cysts under cold waters (Dodge and Harland, 1991); the optimal temperature in terms of encystment was 21 ℃ for *Alexandrium tamarense*, whereas no cysts were produced above 25 ℃ (Anderson et al., 1984).

Cyst germination and the survival of germinated *Heterosigma carterae* cells are strongly controlled by temperature and are markedly activated when bottom water temperature exceeds 15 ℃ (Yamochi and Joh, 1986; Imai and Itakura, 1999; Shikata et al., 2007). Cyst germination of *Alexandrium catenella* was mostly frequent at 20 ℃, and the germination temperature window was 10 ℃ to 25 ℃. The germination time was prolonged with a decrease in incubation temperature

(Takeuchi, 1994). The optimal germination temperature of *Alexandrium tamarense* was 12.5 ℃, temperature for the germination ranged between 7.5 ℃ and 20 ℃, and the germination time was almost constant regardless of the incubation temperatures (Itakura and Yamaguchi, 2001).

H. carterae from the Heterokonphyta causes prodigious red tide blooms from the subpolar to the subtropical zones (Pratt, 1966; Honjo, 1993; Smayda, 1998; Shikata et al., 2008). *A. catenella* and *A. tamarense* are paralytic shellfish poisoning (PSP) species, and the PSP outbreaks are increasing in their intensity and geographic distribution in southwest Japan (Anraku, 1984; Yamamoto and Yamasaki, 1996; Yamaguchi et al., 1996; Itakura and Yamaguchi, 2001). In this study, *H. carterae* occurred in both Hainan Island and Kagoshima Bay, while *A. catenella* and *A. tamarense* occurred in Kagoshima Bay, suggesting that a part of the harmful species has been already colonized in both Hainan Island and Kagoshima Bay. This supports the idea from Chapter 3, i.e. potential danger on HABs in both areas.

4.4.4 Potential threat for the invasion of species from Hainan Island

As mentioned above, ocean currents systems, artificial fish fry transportation for the sake of fishery, and artificial eutrophication in the environment might explain the high similarity of dinoflagellate species composition from the plankton between Hainan Island and Kagoshima Bay. In addition, 35 phytoplankton species including one harmful species appeared from the bottom sediments in both Kagoshima Bay and Hainan Island, suggesting the potential threat for the invasion of species from Hainan Island to Japan by natural and artificial factors. Although the historical occurrence of most species is unclear in Hainan Island, more than 50% of species which occurred in Kagoshima Bay occurred in both two areas, and red tide-forming species *Heterosigma carterae* recorded from Japan since 1994 (Fukuyo et al., 2002). The great amount of fish fry of great amberjacks were artificially transported every year since 1988 (Wakabayashi, 1996; Ottolenghi et al., 2004). These results apparently support the conclusion of Chapter 3, in which the invasion of tropical species, including harmful ones, from Hainan Island to Japan by the global warming through the current systems and/or by artificial transportation of fish fry may be plausible.

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Table 1 Some harmful algal blooms caused by marine dinoflagellates and their effects (Camacho et al., 2007)

Effects	Causative dinoflagellates (examples)	Main toxins	Main aquatic species containing toxins
Harmless water discolorations (under exceptional conditions in sheltered bays, blooms can grow dense enough to cause indiscriminate kills of fish and invertebrates through oxygen depletion)	*Akashiwo sanguinea, Gonlyaylax polygramma, Noctiluca scinilans, Scrippsiella trochoidea*		
Produce potent toxins that if ingested by humans, cause gastrointestinal and neurological illnesses, such as:			
Paralytic shellfish poisoning (PSP)	*Alexandrium catenella; A. cohorticula; A. fundyense; A. fraterculus; A. leei; A. minutum; A. tamarense; A. andersonii; A. ostenfeldii; A. tamiyavanichii, Gymnodinium catenatum, Pyrodinium bahamense* var. *compressum*	Saxitoxins (STXs)	Clams, mussels, oysters, cockles, gastropods, scallops, whelks, lobsters, copepods, crabs, fish
Diarrhetic shellfish poisoning (DSP)	*Dinophysis acuta; D. caudate; D. fortii; D. norvegica; D. mitra; D. rotundata; D. sacculus; D. fortii; D. miles; D. norvegica, Prorocentrum lima; P. arenarium; P. belizeanum; P. cassubicum; P. concavum; P. faustiae; P. hoffmannianum; P. maculosum, Protoceratium reticulatum, Coolia* sp., *Protoperidium oceanicum; P. pellucidum, Phalacroma rotundatum*	Okadaic acid, dinophysis toxins (DTXs), yessotoxins (YTXs) and pectenotoxins (PTXs)	Mussels, scallops, clams, gastropods
Ciguatera fish poisoning (CFP)	*Gambierdiscus toxicus, Prorocentrum lima; P. concavum; P. hoffmannianum; P. mexicanum; P. rhathytum, Gymnodinium sangieneum, Gonyaulax polyedra, Ostreopsis lenticularis; O. siamensis*	Ciguatoxins (CTXs), maitotoxins (MTXs), palytoxin, gambierol	Fish, snail, shrimps, crabs
Neurotoxic shellfish poisoning (NSP)	*Karenia brevis; K. papilonacea; K. sellformis; K. bicuneiformis; K. Concordia, Gymnodinium breve*	Brevetoxins (PbTxs)	Oyster, clams, mussels, cockles, whelks
Azaspiracid shellfish poisoning (AZP)	*Protoperidinium crassipes*	Azaspiracids (AZAs)	Mussels, oyster
Non-toxic to humans but harmful to fish and invertebrates (especially in intensive aquaculture systems) by damaging or clogging their gills	*Karenia mikimotoi; K. brevisulkata, Karlodinium micrum*		

Table 2 Sampling sites, dates, and locations with latitude and longitude in parentheses

Station no.	Sampling location	Station character	Sampling date
Hainan Island			
1	Nangang, Haikou (20°3'30.55"N, 110°9'9.73"E)	Near ship transportation port	Aug 24, 2013
2	Zhenhai Harbor, Haikou (20°3'49.10"N, 110°11'38.69"E)	Natural port	Aug 24, 2013
3	New Harbor, Haikou (20°2'21.50"N, 110°18'52.29"E)	Natural port	Mar 3, 2013
4	Baishamen, Haikou (20°4'50.18"N, 110°20'3.40"E)	Scenic spot	Apr 4, 2014
5	Huxin Port, Wenchang (20°0'34.19"N, 110°56'4.60"E)	Fish farming Near seafood market	Aug 28, 2012 Apr 3, 2014
6	Lianfeng Port, Wanning (18°47'52.89"N, 110°31'49.79"E)	Fish farming Near seafood market	Feb 28, 2013 Apr 5, 2014 Aug 30, 2014
7	Wuchang Port, Wanning (18°46'14.39"N, 110°29'3.73"E)	Fish farming Near seafood market	Feb 27, 2013
8	Dazhou Island, Wanning (18°40'31.73"N, 110°28'31.53"E)	Being fish village	Aug 29, 2013
9	Hongsha Shipside, Sanya (18°14'15.58"N, 109°33'48.17"E)	Near seafood market	Aug 26, 2012 Feb 28, 2013 Aug 25, 2013 Aug 29, 2014
10	Basuo Port, Dongfang (19°6'54.00"N, 108°38'15.76"E)	Fish farming Near seafood market	Aug 25, 2013 Apr 1, 2014 Aug 29, 2014
11	Baimajing Bay, Danzhou (19°42'28.81"N, 109°12'41.71"E)	Natural port Scenic spot	Apr 1, 2014 Aug 29, 2014
Japan			

A	Kagoshima Bay, Kagoshima (31°30'58.64"N, 130°35'48.06"E)	Grate amberjack farming	Mar 3, 2014
B	Kagoshima Bay, Kagoshima (31°27'28.47"N, 130°37'19.13"E)	Grate amberjack farming	Jul 25, 2013 Mar 3, 2014
C	Kagoshima Bay, Kagoshima (31°24'6.72"N, 130°37'54.37"E)	Grate amberjack farming	Jul 25, 2013 Mar 3, 2014
D	Kagoshima Bay, Kagoshima (31°12'40.42"N, 130°43'2.15"E)	Grate amberjack farming	Mar 4, 2014
E	Uranouchi Bay, Kochi (33°26'28.38"N, 133°24'32.65"E)	Grate amberjack farming	Jun 21, 2014

Table 3 List of dinoflagellate species observed in the coastal areas around Hainan Island

No.	Species name	Station no.										
		1	2	3	4	5	6	7	8	9	10	11
	Family Prorocentraceae											
1	*Prorocentrum emarginatum*					+						
2	*P. hoffmannianum* ^{T}								+			
3	*P. lima* ^{T}					+						
4	*P. micans* ^{R}	+				+	+		+	+	+	+
5	*P. rhathymum* ^{T}	+	+	+		+	+		+		+	+
6	*P. shikokuense*	+		+								
7	*P. sigmoides* ^{R}					+			+			
	Family Dinophysiaceae											
8	*Dinophysis acuminata* ^{T}					+						
9	*D. caudata* ^{T}									+		
10	*D. rudgei*						+					
	Family Gonyaulacaceae											
11	*Gonyaulax polygramma*						+					
12	*Lingulodinium polyedrum* ^{T}					+						
	Family Pyrophacaceae											
13	*Pyrophacus horologium*									+		
	Family Ceratiaceae											
14	*Ceratium furca* ^{R}	+					+	+		+	+	
15	*C. fusus* ^{R}	+					+	+	+			
16	*C. kofoidii*							+		+		
17	*C. tripos*							+		+		
	Family Peridiniaceae											
18	*Durinskia capensis*					+			+			
19	*Peridinium quinquecorne*	+				+	+	+	+	+	+	
	Family Protoperidiniaceae											
20	*Protoperidinium avellanum*					+						
21	*P. bipes*						+			+		
22	*P. claudicans*					+	+					
23	*P. divaricatum*					+						
24	*P. excentricum*	+				+	+			+		
25	*P. latispinum*						+		+	+		
26	*P. marukawai*									+		
27	*P. minutum*								+			
28	*P. obtusum*								+			
29	*P. oceanicum* ^{T}	+				+						
30	*P. pellucidum* ^{T}			+		+			+	+	+	
31	*P. punctulatum*					+	+			+		
32	*P. pyriforme*									+		
	Family Calciodinellaceae											
33	*Scrippsiella trochoidea*										+	

	Family Heterocapsaceae	
34	*Heterocapsa* sp. 1	+
35	*Heterocapsa* sp. 2	+
36	*Heterocapsa* sp. 3	+
37	*Heterocapsa* sp. 4	+

+ Occurrence

^{T} Toxic species

^{R} Red tide-forming species

Table 4 Harmful dinoflagellates observed in the coastal areas around Hainan Island

Species name	Harmful characteristics
Prorocentrum hoffmannianum	Ciguatera fish poisoning, diarrhetic shellfish poisoning
P. lima	Ciguatera fish poisoning, diarrhetic shellfish poisoning
P. micans	Red tide
P. rhathymum	Ciguatera fish poisoning
P. sigmoides	Red tide
Dinophysis acuminata	Diarrhetic shellfish poisoning
D. caudata	Diarrhetic shellfish poisoning
Lingulodinium polyedrum	Yessotoxin
Ceratium furca	Red tide
C. fusus	Red tide
Protoperidium oceanicum	Diarrhetic shellfish poisoning
P. pellucidum	Diarrhetic shellfish poisoning

Table 5 List of dinoflagellate species observed in Kagoshima and Uranouchi Bays, south Japan

No.	Species name	Station no.				
		A	B	C	D	E
	Family Prorocentraceae					
1	*Prorocentrum compressum*	+	+	+		
2	*P. gracile*		+	+		
3	*P. rhathymum* ^{T*}		+	+		
4	*P. sigmoides* ^{R*}		+	+		+
5	*P. triestinum*	+		+		
	Family Dinophysiaceae					
6	*Dinophysis caudata* ^{T*}		+	+	+	
7	*D. parvula*		+	+		
8	*D. rotundata* ^{T}		+	+		
9	*Ornithocercus magnificus*				+	
	Family Kareniaceae					
10	*Karenia digitata*					+
11	*K. mikimotoi* ^{R}					+
	Family Pyrophacaceae					
12	*Pyrophacus horologium* *		+			
	Family Ceratiaceae					
13	*Ceratium boehmii*		+	+	+	
14	*C. candelabrum*	+		+	+	
15	*C. concilians*	+	+	+	+	
16	*C. furca* ^{R*}	+	+	+		+
17	*C. fusus* ^{R*}	+	+	+	+	
18	*C. trichoceros*		+	+	+	
19	*C. tripos* *	+	+	+	+	
	Family Protoperidiniaceae					
20	*Protoperidinium conicum*			+		
21	*P. excentricum* *			+		
22	*P. latispinum* *	+		+	+	
23	*P. obtusum* *			+		
24	*P. pellucidum* ^{T*}	+	+	+		
25	*P. punctulatum* *	+		+	+	
	Family Calciodinellaceae					
26	*Scrippsiella spinifera*					+
27	*S. trochoidea* *					+

+ Occurrence

^{T} Toxic species

^{R} Red tide-forming species

* Species appeared in both south Japan and Hainan Island

Table 6 Harmful dinoflagellates observed in Kagoshima and Uranouchi Bays, south Japan

Species name	Harmful characteristics	Appeared
Prorocentrum rhathymum	Ciguatera fish poisoning	Kagoshima
P. sigmoides	Red tide	Kagoshima and Uranouchi
Dinophysis caudata	Diarrhetic shellfish poisoning	Kagoshima
D. rotundata	Diarrhetic shellfish poisoning	Kagoshima
Karenia mikimotoi	Red tide	Uranouchi
Ceratium furca	Red tide	Kagoshima and Uranouchi
C. fusus	Red tide	Kagoshima
Protoperidium pellucidum	Diarrhetic shellfish poisoning	Kagoshima

Table 7 Dinoflagellates appeared in both south Japan and Hainan Island

No.	Species name	Stations															
		A	B	C	D	E	1	2	3	4	5	6	7	8	9	10	11
	Family Prorocentraceae																
1	*Prorocentrum rhathymum* T		+	+			+	+	+		+	+		+		+	+
2	*P. sigmoides* R		+	+		+					+			+			
	Family Dinophysiaceae																
3	*Dinophysis caudata* T		+	+	+						+						
	Family Pyrophacaceae																
4	*Pyrophacus horologium*		+												+		
	Family Ceratiaceae																
5	*Ceratium furca* R	+	+	+		+	+					+	+		+	+	
6	*C. fusus* R	+	+	+	+		+					+	+	+			
7	*C. tripos*	+	+	+	+								+		+		
	Family Protoperidiniaceae																
8	*Protoperidinium excentricum*			+			+				+	+			+		
9	*P. latispinum*	+		+	+							+		+	+		
10	*P. obtusum*			+										+			
11	*P. pellucidum* T	+	+	+					+		+			+	+	+	
12	*P. punctulatum*	+		+	+						+	+			+		
	Family Calciodinellaceae																
13	*Scrippsiella trochoidea*					+										+	

+ Occurrence

T Toxic species

R Red tide-forming species

Table 8 Historical occurrence of species which occurred in both south Japan and Hainan Island

Species name	Present occurrence in Japan	Historical occurrence in Japan
Prorocentrum rhathymum	Kagoshima Bay	Ryukyu Islands (1980s)
P. sigmoides	Kagoshima Bay Uranouchi Bay	Yatsushiro Sea (2003) Kii Channel (2005) Omura Bay (2005, 2011) Kagoshima Bay (2006, 2011, 2013) Hakata Bay (2009) Okinawa (2010)
Dinophysis caudata	Kagoshima Bay	Yatsushiro Sea (2003) Ago Bay (2011, 2013) Ariake Sea (2011, 2013) Japan Sea (2011) Seto Inland Sea (2011, 2013) Tanabe Bay (2011) Kii Channel (2013)
Pyrophacus horologium	Kagoshima Bay	Yatsushiro Sea (2003)
Ceratium furca	Kagoshima Bay Uranouchi Bay	Yatsushiro Sea (2003, 2011) Ariake Sea (2005, 2013) Tokyo Bay (2005, 2013) Ago Bay (2006) Kii Channel (2006, 2011) Okinawa (2007-2010) Sagami Bay (2007) Kagoshima Bay (2011)
C. fusus	Kagoshima Bay	Yatsushiro Sea (2003) Kagoshima Bay (2006) Okinawa (2007-2010) Seto Inland Sea (2013)
C. tripos	Kagoshima Bay	Yatsushiro Sea (2003) Okinawa (2007-2010)
Protoperidinium excentricum	Kagoshima Bay	Yatsushiro Sea (2003)
P. latispinum	Kagoshima Bay	Yatsushiro Sea (2003)
P. obtusum	Kagoshima Bay	Yatsushiro Sea (2003)
P. pellucidum	Kagoshima Bay	Okinawa (2009, 2010)
P. punctulatum	Kagoshima Bay	Yatsushiro Sea (2003)
Scrippsiella trochoidea	Uranouchi Bay	Onagawa Bay (1996)

Table 9 Phytoplankton species from the sediments of Hainan Island and Kagoshima Bay (based on the homology of more than 95% of the Blast Hit identity values)

No.	GenBank accession	Species name	Phylum	Class	Order	Number of sequences	
						Hainan Island	Kagoshima Bay
1	DQ473679.1	*Synechococcus* sp.1	Cyanobacteria	Cyanophyceae	Chroococcales	3	40
2	DQ473683.1	*Synechococcus* sp.2	Cyanobacteria	Cyanophyceae	Chroococcales	1	0
3	AF156980.1	*Synechococcus* sp.3	Cyanobacteria	Cyanophyceae	Chroococcales	16	0
4	AY995306.1	*Synechococcus* sp.4	Cyanobacteria	Cyanophyceae	Chroococcales	2	23
5	DQ473684.1	*Synechococcus* sp.5	Cyanobacteria	Cyanophyceae	Chroococcales	2	11
6	DQ473674.1	*Synechococcus* sp.6	Cyanobacteria	Cyanophyceae	Chroococcales	0	1197
7	AY176642.1	*Synechococcus* sp.7	Cyanobacteria	Cyanophyceae	Chroococcales	0	296
8	DQ473675.1	*Synechococcus* sp.8	Cyanobacteria	Cyanophyceae	Chroococcales	0	141
9	KC493138.1	*Synechococcus* sp.9	Cyanobacteria	Cyanophyceae	Chroococcales	0	6
10	U21331.1	*Anabaena* sp.	Cyanobacteria	Cyanophyceae	Nostocales	10	0
11	AY599029.1	*Prochlorococcus marinus*	Cyanobacteria	Cyanophyceae	Prochlorales	8	0
12	HQ828109.1	*Dunaliella tertiolecta*	Chlorophyta	Chlorophyceae	Chlamydomonadales	0	13
13	EU851956.1	*Micromonas pusilla*	Chlorophyta	Mamiellophyceae	Mamiellales	22	21
14	EU851964.1	*Mantoniella squamata*	Chlorophyta	Mamiellophyceae	Mamiellales	1	0
15	EU851970.1	*Ostreococcus* sp.1	Chlorophyta	Mamiellophyceae	Mamiellales	1	165
16	EU851961.1	*Ostreococcus* sp.2	Chlorophyta	Mamiellophyceae	Mamiellales	0	43
17	EU851965.1	*Micromonas pusilla*	Chlorophyta	Mamiellophyceae	Mamiellales	0	72
18	AY857618.1	*Tetraselmis marina*	Chlorophyta	Prasinophyceae	Chlorodendrales	48	7
19	AB561052.1	*Prasinococcus capsulatus*	Chlorophyta	Prasinophyceae	Prasinoccocales	1	0
20	AB561048.1	*Pseudoscourfieldia marina*	Chlorophyta	Prasinophyceae	Pseudoscourfieldiales	33	266
21	EU851957.1	*Pycnococcus provasolii*	Chlorophyta	Prasinophyceae	Pseudoscourfieldiales	0	29
22	JN542395.1	*Pyramimonas* sp.	Chlorophyta	Prasinophyceae		1	0
23	AB561051.1	*Prasinophyceae* sp.	Chlorophyta	Prasinophyceae		0	12
24	KC917288.1	*Chlorella* sp.	Chlorophyta	Trebouxiophyceae	Chlorellales	0	83
25	EU043045.1	*Auxenochlorella protothecoides*	Chlorophyta	Trebouxiophyceae	Chlorellales	0	1
26	AB561047.1	*Ulva arasakii*	Chlorophyta	Ulvophyceae	Ulvales	83	0
27	AB359447.1	*Alexandrium tamarense*	Dinoflagellate	Dinophyceae	Gonyaulacales	0	112
28	AB025590.1	*Alexandrium catenella*	Dinoflagellate	Dinophyceae	Gonyaulacales	0	4
29	AF206706.1	*Heterocapsa rotundata*	Dinoflagellate	Dinophyceae	Peridiniales	0	487
30	AY119764.1	*Peridinium foliaceum*	Dinoflagellate	Dinophyceae	Peridiniales	298	146
31	AF206710.1	*Scrippsiella trochoidea*	Dinoflagellate	Dinophyceae	Peridiniales	0	15
32	JN557868.1	*Polarella glacialis*	Dinoflagellate	Dinophyceae	Suessiales	0	34
33	JN557867.1	*Protodinium simplex*	Dinoflagellate	Dinophyceae	Suessiales	0	43
34	AB430734.1	*Cocconeis stauroneiformis*	Bacillariophyta	Bacillariophyceae	Achnanthales	9	0
35	AB430737.1	*Psammodictyon constrictum*	Bacillariophyta	Bacillariophyceae	Bacillariales	2597	25
36	AB430736.1	*Nitzschia dubiiformis*	Bacillariophyta	Bacillariophyceae	Bacillariales	31	0
37	AB430735.1	*Navicula* sp.	Bacillariophyta	Bacillariophyceae	Naviculales	4550	108
38	JN418735.1	*Mayamaea atomus*	Bacillariophyta	Bacillariophyceae	Naviculales	1	0
39	JN418728.1	*Caloneis silicula*	Bacillariophyta	Bacillariophyceae	Naviculales	0	11
40	JN418703.1	*Pinnularia* cf. *microstauron*	Bacillariophyta	Bacillariophyceae	Naviculales	6197	0

41	JN418733.1	*Pinnularia subanglica*	Bacillariophyta	Bacillariophyceae	Naviculales	3563	1
42	JN418714.1	*Pinnularia subcapitata*	Bacillariophyta	Bacillariophyceae	Naviculales	719	0
43	JN418709.1	*Pinnularia viridiformis*	Bacillariophyta	Bacillariophyceae	Naviculales	4873	52
44	JN418731.1	*Pinnularia neglectiformis*	Bacillariophyta	Bacillariophyceae	Naviculales	326	0
45	JN418708.1	*Pinnularia* cf. *altiplanensis*	Bacillariophyta	Bacillariophyceae	Naviculales	69	0
46	JN418737.1	*Pinnularia acrosphaeria*	Bacillariophyta	Bacillariophyceae	Naviculales	30	0
47	JN418719.1	*Pinnularia subcommutata*	Bacillariophyta	Bacillariophyceae	Naviculales	35	8
48	JN418706.1	*Pinnularia neomajor*	Bacillariophyta	Bacillariophyceae	Naviculales	76	0
49	JN418705.1	*Pinnularia borealis*	Bacillariophyta	Bacillariophyceae	Naviculales	19	0
50	JN418732.1	*Pinnularia acuminata*	Bacillariophyta	Bacillariophyceae	Naviculales	8	0
51	JN418729.1	*Pinnularia* cf. *isselana*	Bacillariophyta	Bacillariophyceae	Naviculales	1	0
52	JN418710.1	*Pinnularia borealis*	Bacillariophyta	Bacillariophyceae	Naviculales	1	0
53	JN418716.1	*Pinnularia* sp.1	Bacillariophyta	Bacillariophyceae	Naviculales	752	0
54	JN418712.1	*Pinnularia* sp.2	Bacillariophyta	Bacillariophyceae	Naviculales	489	0
55	JN418738.1	*Pinnularia* sp.3	Bacillariophyta	Bacillariophyceae	Naviculales	139	0
56	JN418721.1	*Pinnularia* sp.4	Bacillariophyta	Bacillariophyceae	Naviculales	112	0
57	JN418725.1	*Pinnularia* sp.5	Bacillariophyta	Bacillariophyceae	Naviculales	15	0
58	JN418717.1	*Pinnularia* sp.6	Bacillariophyta	Bacillariophyceae	Naviculales	36	0
59	JN418707.1	*Pinnularia* sp.7	Bacillariophyta	Bacillariophyceae	Naviculales	1	0
60	JN418718.1	*Pinnularia* sp.8	Bacillariophyta	Bacillariophyceae	Naviculales	14	0
61	JN418711.1	*Pinnularia* sp.9	Bacillariophyta	Bacillariophyceae	Naviculales	1	0
62	JN418713.1	*Pinnularia* sp.10	Bacillariophyta	Bacillariophyceae	Naviculales	1	0
63	JN418715.1	*Pinnularia* sp.11	Bacillariophyta	Bacillariophyceae	Naviculales	1	0
64	JN418734.1	*Sellaphora blackfordensis*	Bacillariophyta	Bacillariophyceae	Naviculales	465	6
65	AB430733.1	*Campylodiscus thuretii*	Bacillariophyta	Bacillariophyceae	Surirellales	23	6
66	JQ217358.1	*Chaetoceros socialis*	Bacillariophyta	Coscinodiscophyceae	Chaetocerotales	97	586
67	AB430706.1	*Chaetoceros radicans*	Bacillariophyta	Coscinodiscophyceae	Chaetocerotales	1	4
68	HQ710697.1	*Chaetoceros didymus*	Bacillariophyta	Coscinodiscophyceae	Chaetocerotales	0	10
69	AB430701.1	*Melosira dubia*	Bacillariophyta	Coscinodiscophyceae	Melosirales	134	15
70	AB430703.1	*Stephanopyxis turris*	Bacillariophyta	Coscinodiscophyceae	Melosirales	1297	243
71	AB430702.1	*Rhizosolenia setigera*	Bacillariophyta	Coscinodiscophyceae	Rhizosoleniales	48795	3796
72	AY119761.1	*Skeletonema costatum*	Bacillariophyta	Coscinodiscophyceae	Thalassiosirales	313	1690
73	AB430705.1	*Cyclotella meneghiniana*	Bacillariophyta	Coscinodiscophyceae	Thalassiosirales	10804	2900
74	JQ217360.1	*Discostella* sp.	Bacillariophyta	Coscinodiscophyceae	Thalassiosirales	12	1
75	JQ217365.1	*Stephanodiscus hantzschii*	Bacillariophyta	Coscinodiscophyceae	Thalassiosirales	24	9
76	JQ217366.1	*Stephanodiscus* sp.	Bacillariophyta	Coscinodiscophyceae	Thalassiosirales	6	0
77	JQ217364.1	*Cyclotella striata*	Bacillariophyta	Coscinodiscophyceae	Thalassiosirales	1	0
78	HQ710698.1	*Thalassiosira conferta*	Bacillariophyta	Coscinodiscophyceae	Thalassiosirales	307	867
79	JQ217368.1	*Thalassiosira punctigera*	Bacillariophyta	Coscinodiscophyceae	Thalassiosirales	3	140
80	AB430699.1	*Aulacoseira granulata*	Bacillariophyta	Coscinodiscophyceae	Aulacoseirales	28	18
81	AB430712.1	*Asteroplanus karianus*	Bacillariophyta	Fragilariophyceae	Fragilariales	13714	13
82	AB430724.1	*Plagiostriata goreensis*	Bacillariophyta	Fragilariophyceae	Fragilariales	17236	20
83	AB430728.1	*Pseudostaurosira brevistriata*	Bacillariophyta	Fragilariophyceae	Fragilariales	13852	75
84	AB430716.1	*Fragilaria bidens*	Bacillariophyta	Fragilariophyceae	Fragilariales	1200	0

85	HQ710696.1	*Asterionella glacialis*	Bacillariophyta	Fragilariophyceae	Fragilariales	35	4
86	AB430723.1	*Opephora* sp.	Bacillariophyta	Fragilariophyceae	Fragilariales	2	12
87	AB430720.1	*Nanofrustulum shiloi*	Bacillariophyta	Fragilariophyceae	Fragilariales	3	0
88	AB430721.1	*Rhaphoneis* sp.	Bacillariophyta	Fragilariophyceae	Rhaphoneidiales	166	0
89	AB430718.1	*Hyalosira delicatula*	Bacillariophyta	Fragilariophyceae	Striatellales	85	0
90	AB430708.1	*Eunotogramma laevis*	Bacillariophyta	Mediophyceae	Anaulales	984	520
91	AB430707.1	*Cymatosira cf. belgica*	Bacillariophyta	Mediophyceae	Cymatosirales	170	348
92	AB430715.1	*Dimeregramma minor*	Bacillariophyta	Mediophyceae	Triceratiales	13	0
93	GU325203.1	*Chromulina nebulosa*	Heterokonphyta	Chrysophyceae	Chromulinales	0	1
94	HQ710721.1	*Aureococcus anophagefferens*	Heterokonphyta	Pelagophyceae	Pelagomonadales	0	4
95	HQ710720.1	*Ankylochrysis lutea*	Heterokonphyta	Pelagophyceae	Sarcinochrysidales	1	0
96	HQ710726.1	*Sarcinochrysis* sp.1	Heterokonphyta	Pelagophyceae	Sarcinochrysidales	30	0
97	HQ710728.1	*Sarcinochrysis* sp.2	Heterokonphyta	Pelagophyceae	Sarcinochrysidales	1	0
98	AB561049.1	*Pterosperma cristatum*	Heterokonphyta	Pelagophyceae	Sarcinochrysidales	0	32
99	HQ710734.1	*Phaeomonas parva*	Heterokonphyta	Pinguiophyceae	Pinguiochrysidales	1	0
100	HQ710735.1	*Pinguiochrysis pyriformis*	Heterokonphyta	Pinguiophyceae	Pinguiochrysidales	2	0
101	U18090.1	*Heterosigma carterae*	Heterokonphyta	Raphidophyceae	Chattonellales	1	135
102	HQ710738.1	*Chattonella marina*	Heterokonphyta	Raphidophyceae	Chattonellales	0	194
103	HQ710692.1	*Aurearena cruciata*	Phaeophyta	Aurearenophyceae		47	0
104	AY141195.1	*Rhodomonas* sp.	Cryptophyta	Cryptophyceae	Pyrenomonadales	103	0
105	AY119752.1	*Emiliania huxleyi*	Haptophyta	Haptophyceae	Isochrysidales	0	1
106	AJ575572.1	*Chrysochromulina polylepis*	Haptophyta	Haptophyceae	Prymnesiales	0	42
107	JX660822.1	*Phaeocystis* sp.	Haptophyta	Haptophyceae	Phaeocystales	0	83
108	AY119753.1	*Isochrysis* sp.	Haptophyta	Haptophyceae	Isochrysidales	0	30

Table 10 Phytoplankton species appeared in both Hainan Island and Kagoshima Bay (based on the homology of more than 95% of the Blast Hit identity values)

No.	GenBank accession	Species name	Phylum	Class	Order	Sequences number	
						Hainan Island	Kagoshima Bay
1	DQ473679.1	*Synechococcus* sp.1	Cyanobacteria	Cyanophyceae	Chroococcales	3	40
2	AY995306.1	*Synechococcus* sp.4	Cyanobacteria	Cyanophyceae	Chroococcales	2	23
3	DQ473684.1	*Synechococcus* sp.5	Cyanobacteria	Cyanophyceae	Chroococcales	2	11
4	EU851956.1	*Micromonas pusilla*	Chlorophyta	Mamiellophyceae	Mamiellales	22	21
5	EU851970.1	*Ostreococcus* sp.1	Chlorophyta	Mamiellophyceae	Mamiellales	1	165
6	AY857618.1	*Tetraselmis marina*	Chlorophyta	Prasinophyceae	Chlorodendrales	48	7
7	AB561048.1	*Pseudoscourfieldia marina*	Chlorophyta	Prasinophyceae	Pseudoscourfieldiales	33	266
8	AY119764.1	*Peridinium foliaceum*	Dinoflagellate	Dinophyceae	Peridiniales	298	146
9	AB430737.1	*Psammodictyon constrictum*	Bacillariophyta	Bacillariophyceae	Bacillariales	2597	25
10	AB430735.1	*Navicula* sp.	Bacillariophyta	Bacillariophyceae	Naviculales	4550	108
11	JN418733.1	*Pinnularia subanglica*	Bacillariophyta	Bacillariophyceae	Naviculales	3563	1
12	JN418709.1	*Pinnularia viridiformis*	Bacillariophyta	Bacillariophyceae	Naviculales	4873	52
13	JN418719.1	*Pinnularia subcommutata*	Bacillariophyta	Bacillariophyceae	Naviculales	35	8
14	JN418734.1	*Sellaphora blackfordensis*	Bacillariophyta	Bacillariophyceae	Naviculales	465	6
15	AB430733.1	*Campylodiscus thuretii*	Bacillariophyta	Bacillariophyceae	Surirellales	23	6
16	JQ217358.1	*Chaetoceros socialis*	Bacillariophyta	Coscinodiscophyceae	Chaetocerotales	97	586
17	AB430706.1	*Chaetoceros radicans*	Bacillariophyta	Coscinodiscophyceae	Chaetocerotales	1	4
18	AB430701.1	*Melosira dubia*	Bacillariophyta	Coscinodiscophyceae	Melosirales	134	15
19	AB430703.1	*Stephanopyxis turris*	Bacillariophyta	Coscinodiscophyceae	Melosirales	1297	243
20	AB430702.1	*Rhizosolenia setigera*	Bacillariophyta	Coscinodiscophyceae	Rhizosoleniales	48795	3796
21	AY119761.1	*Skeletonema costatum*	Bacillariophyta	Coscinodiscophyceae	Thalassiosirales	313	1690
22	AB430705.1	*Cyclotella meneghiniana*	Bacillariophyta	Coscinodiscophyceae	Thalassiosirales	10804	2900
23	JQ217360.1	*Discostella* sp.	Bacillariophyta	Coscinodiscophyceae	Thalassiosirales	12	1
24	JQ217365.1	*Stephanodiscus hantzschii*	Bacillariophyta	Coscinodiscophyceae	Thalassiosirales	24	9
25	HQ710698.1	*Thalassiosira conferta*	Bacillariophyta	Coscinodiscophyceae	Thalassiosirales	307	867
26	JQ217368.1	*Thalassiosira punctigera*	Bacillariophyta	Coscinodiscophyceae	Thalassiosirales	3	140
27	AB430699.1	*Aulacoseira granulata*	Bacillariophyta	Coscinodiscophyceae	Aulacoseirales	28	18
28	AB430712.1	*Asteroplanus karianus*	Bacillariophyta	Fragilariophyceae	Fragilariales	13714	13
29	AB430724.1	*Plagiostriata goreensis*	Bacillariophyta	Fragilariophyceae	Fragilariales	17236	20
30	AB430728.1	*Pseudostaurosira brevistriata*	Bacillariophyta	Fragilariophyceae	Fragilariales	13852	75
31	HQ710696.1	*Asterionella glacialis*	Bacillariophyta	Fragilariophyceae	Fragilariales	35	4
32	AB430723.1	*Opephora* sp.	Bacillariophyta	Fragilariophyceae	Fragilariales	2	12
33	AB430708.1	*Eunotogramma laevis*	Bacillariophyta	Mediophyceae	Anaulales	984	520
34	AB430707.1	*Cymatosira cf. belgica*	Bacillariophyta	Mediophyceae	Cymatosirales	170	348
35	U18090.1	*Heterosigma carterae*	Heterokonphyta	Raphidophyceae	Chattonellales	1	135

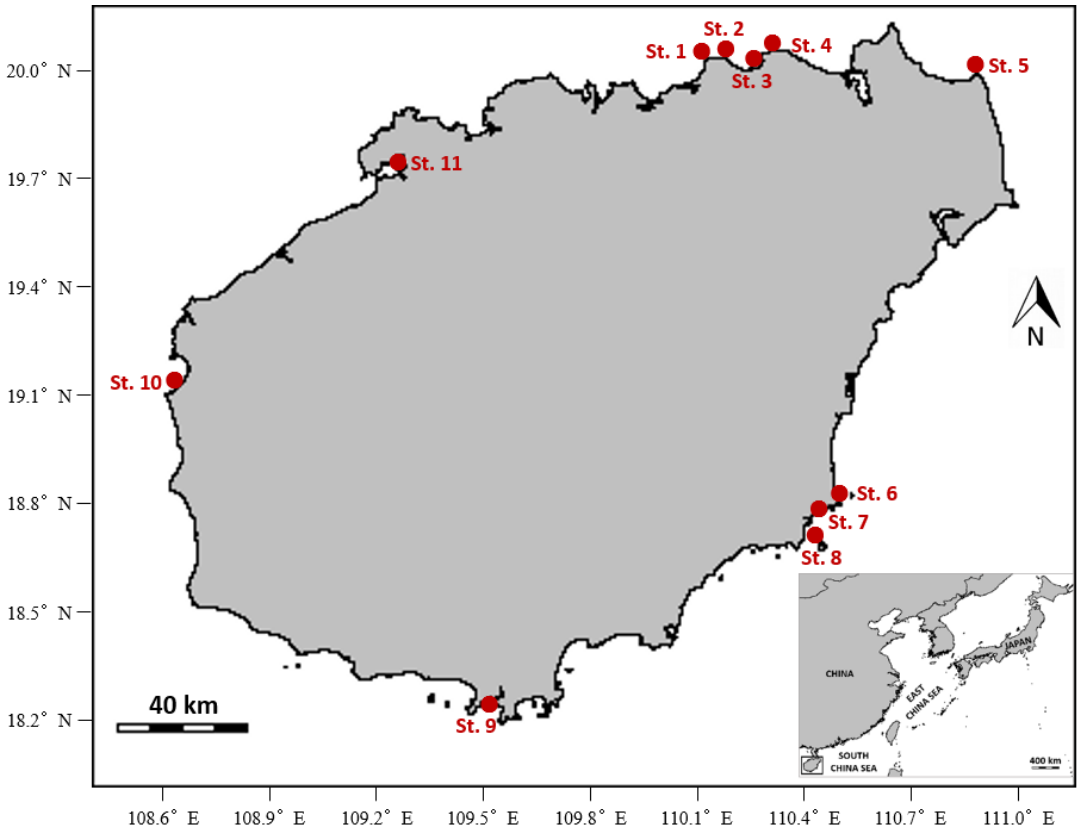

Fig. 1 Map of Hainan Island and locations of sampling sites along the coastal areas (●).

Fig. 2-1 *Prorocentrum emarginatum*

a - line drawing, right valve view. b-d - light micrographs, right valve views: c showing the larger valve pores are arranged in radial rows, and the marginal pores are present. b, c and d showing the periflagellar area is ending in a sharp narrow point with a rectangular structure (arrow). Scale bars = 10 µm.

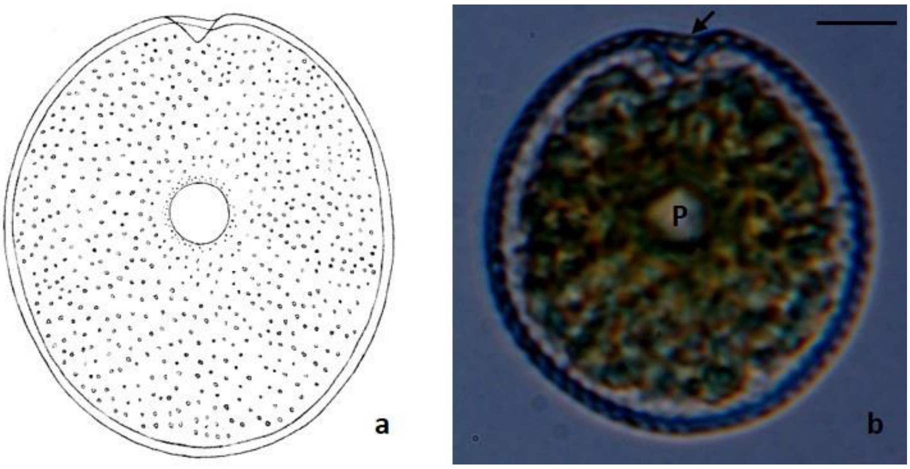

Fig. 2-2 *Prorocentrum hoffmannianum*

a - line drawing, right valve view. b - light micrograph, right valve view, showing pyrenoid (P) round and central; the valve surface is deeply areolate; the periflagellar area is a broad triangle (arrow). Scale bar = 10 µm.

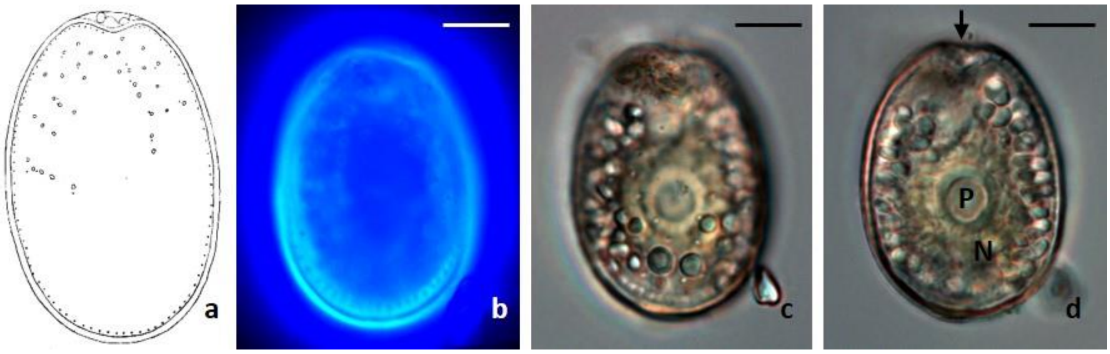

Fig. 2-3 *Prorocentrum lima*

a - line drawing, right valve view. b - epifluorescence micrograph, c-d - light micrographs. b-d - right valve views: b showing the marginal pores and the valve pores scattered on the valve surface. c showing larger flagellar pores and smaller auxiliary pores. d showing large distinct central pyrenoid (P), large posterior nucleus (N), and the periflagellar area is a wide triangle (arrow). Scale bars = 10 µm.

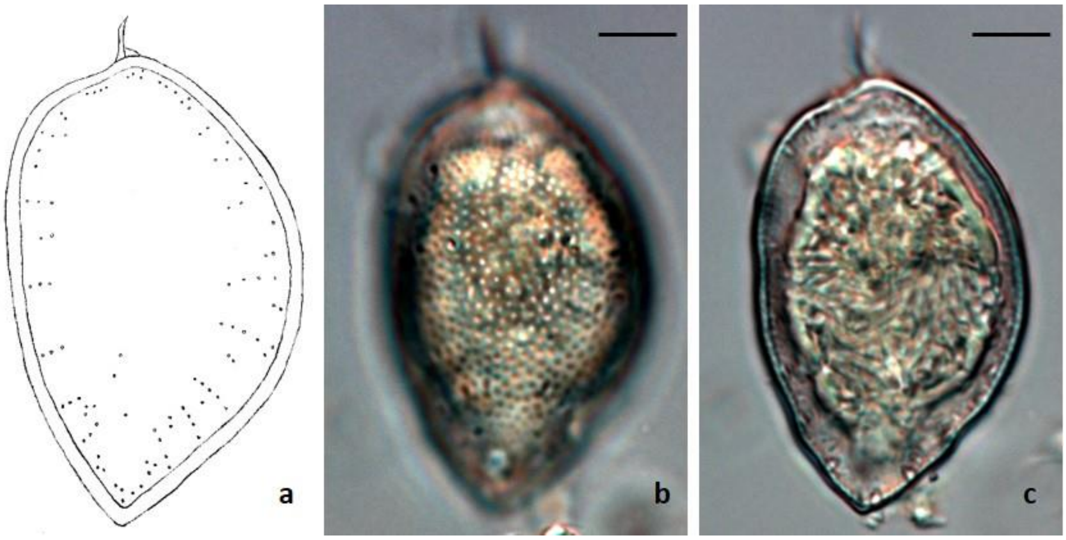

Fig. 2-4 *Prorocentrum micans*

a - line drawing, left valve view. b-c - light micrographs, left valve views: b showing numerous tubular trichocyst pores are present in short rows arranged radially. c showing rounded anterior end and a pointed posterior end; one convex side and one arched side, the convex arch profile is typically in the middle of the cell where the cell is the broadest; the winged apical spine is present. Scale bars = 10 µm.

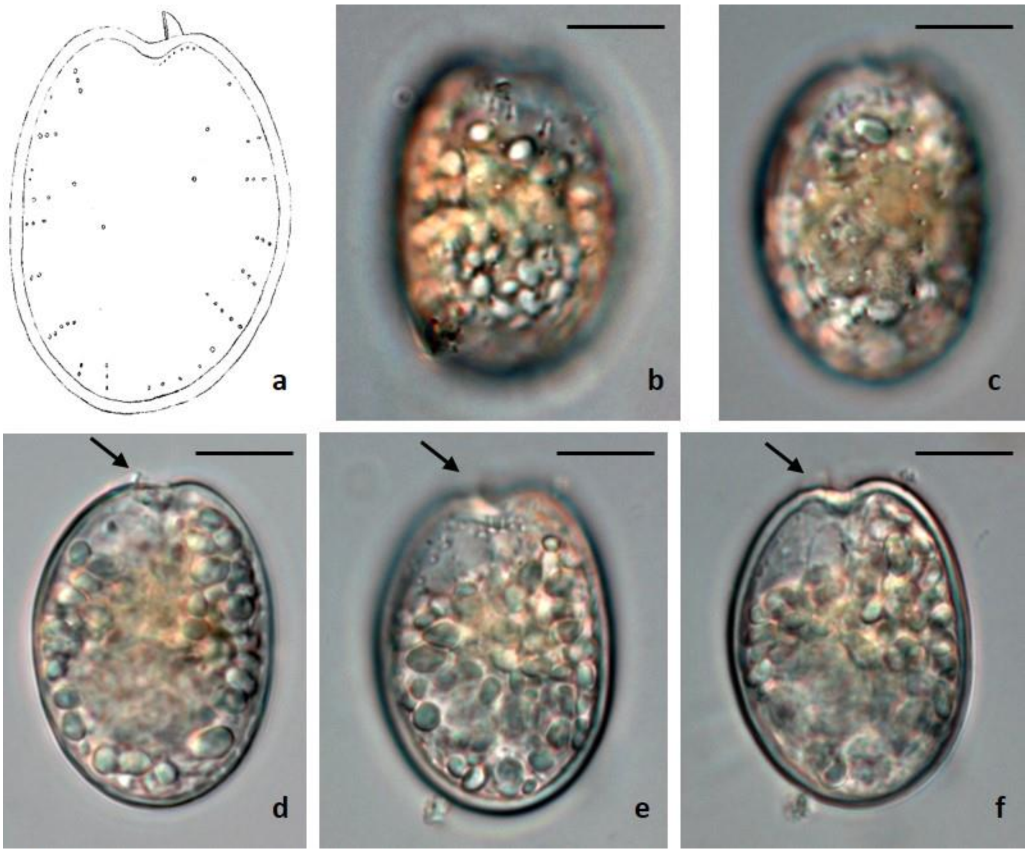

Fig. 2-5 *Prorocentrum rhathymum*

a - line drawing, left valve view. b-f - light micrographs. b - left valve view, c-f - right valve views: b and c showing the valve pores, which form line patterns in the posterior part of the valve. d, e and f showing the periflagellar area is V-shaped and shallow depression, and the curved periflagellar collar is appear as an apical spine (arrow). Scale bars = 10 µm.

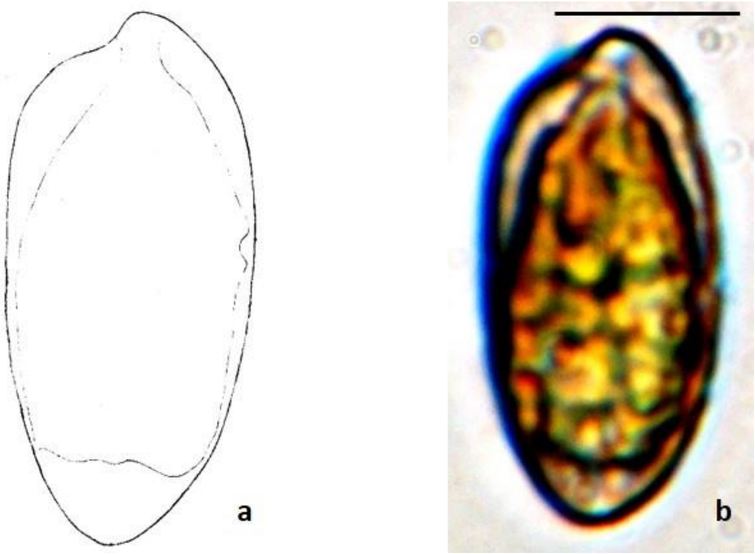

Fig. 2-6 *Prorocentrum shikokuense*

a - line drawing, right valve view. b - light micrograph, right valve view, showing elongated and asymmetric cell, narrowing toward the posterior ends. Scale bar = 10 µm.

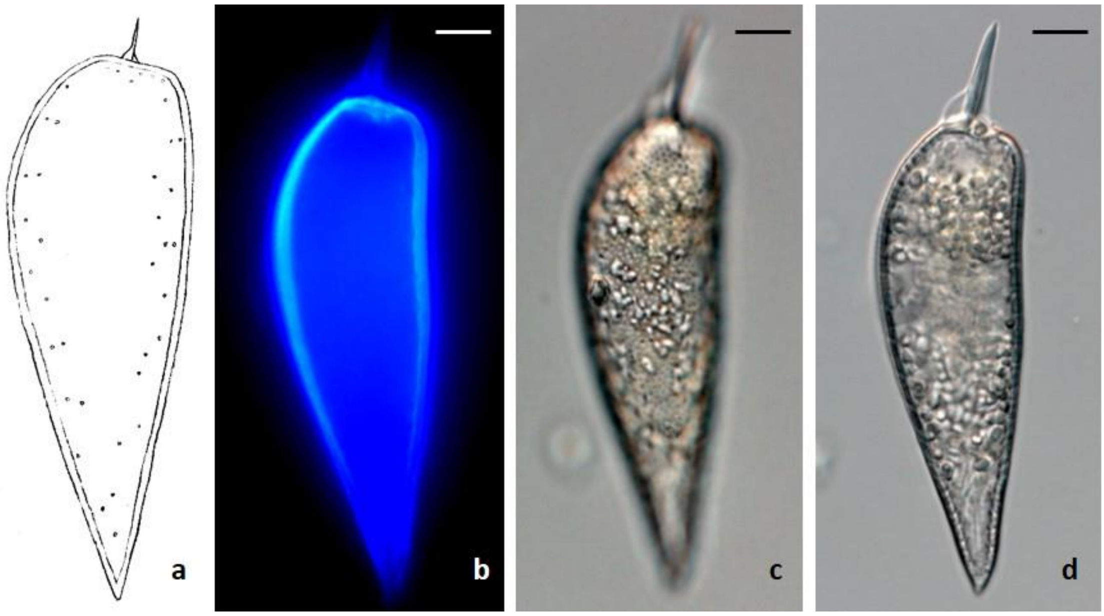

Fig. 2-7 *Prorocentrum sigmoides*

a - line drawing, left valve view. b - epifluorescent micrograph, c-d - light micrographs. b-d - left valve views: b showing elongated cell in "S" shape. c showing the cell surface perforated by trichocyst pores. d showing apical spine sharp and tiny, protruded from the anterior side. Scale bars = 10 µm.

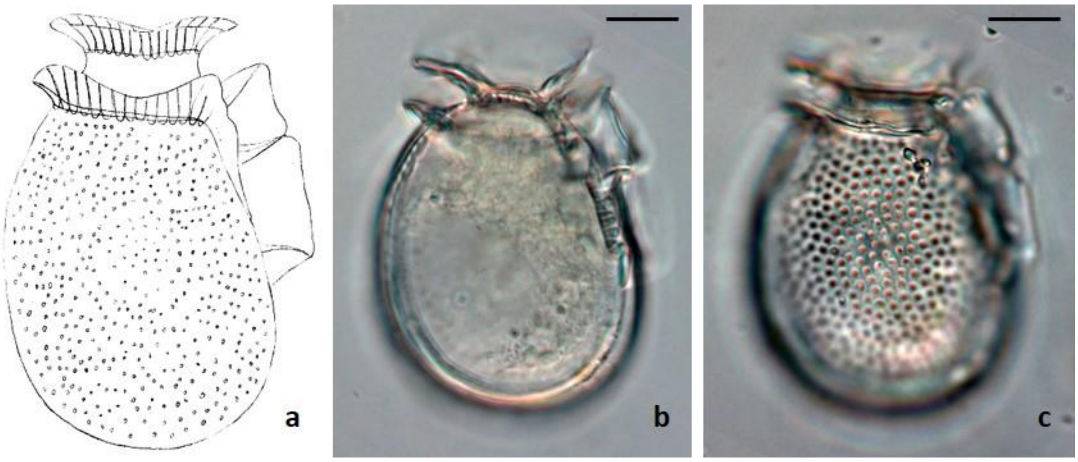

Fig. 2-8 *Dinophysis acuminata*

a - line drawing, lateral view. b-c - light micrographs, lateral views: b showing left sulcal list (LSL) is well developed, supported by 3 ribs and extends beyond the midpoint of the cell. c showing the thecal plate covered with prominent circular areolae, each with a pore. Scale bars = 10 μm.

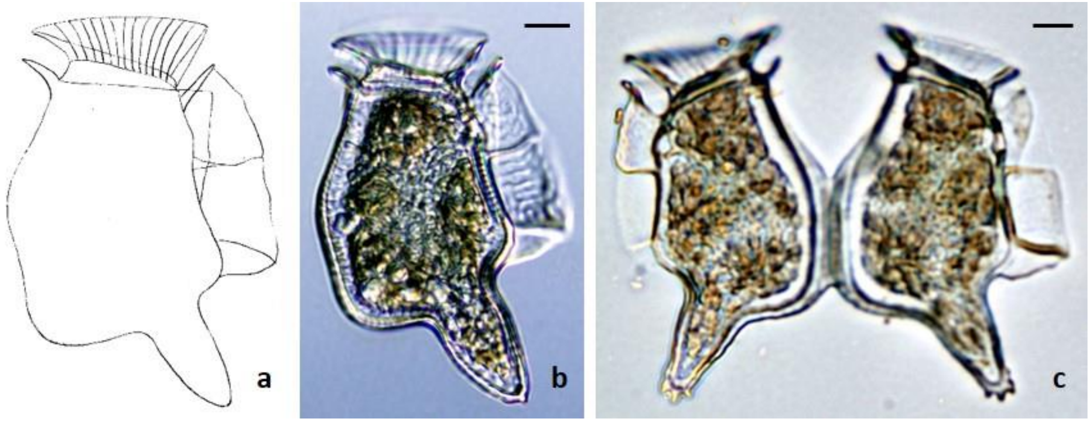

Fig. 2-9 *Dinophysis caudata*

a - line drawing, lateral view. b-c - light micrographs, lateral views: b showing the long left sulcal list (LSL) is supported by 3 ribs spaced equally apart and extends to nearly half of the total cell length. c showing the paired cells, dorsally joined at widest point of hypotheca. Scale bars = 10 μm.

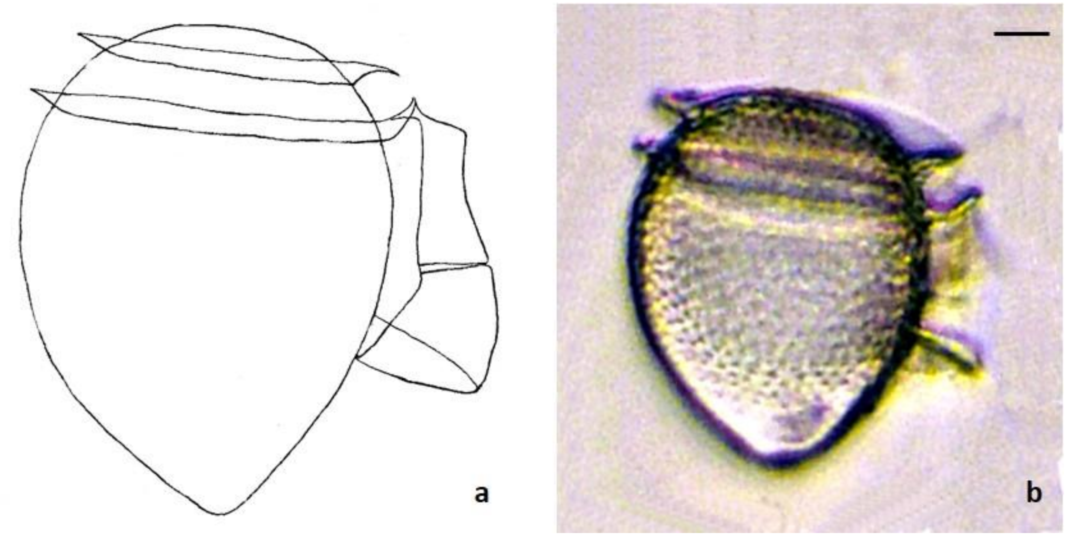

Fig. 2-10 *Dinophysis rudgei*

a - line drawing, lateral view. b - light micrograph, lateral view, showing the left sulcal list (LSL) with three supporting ribs, and the thecal plate heavily areolated. Scale bar = 10 µm.

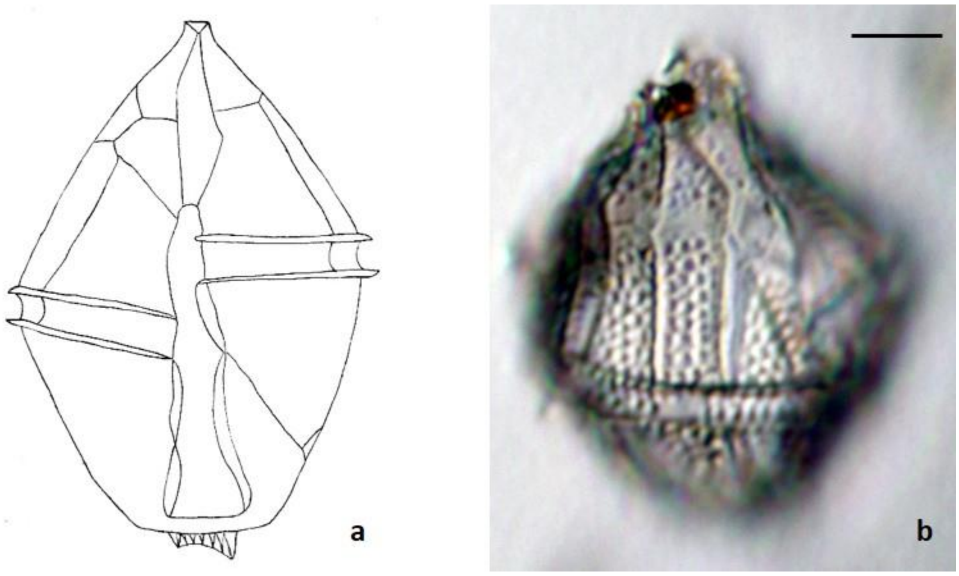

Fig. 2-11 *Gonyaulax polygramma*

a - line drawing, ventral view. b - light micrograph, side view, showing conspicuous longitudinal ridges. Scale bar = 10 µm.

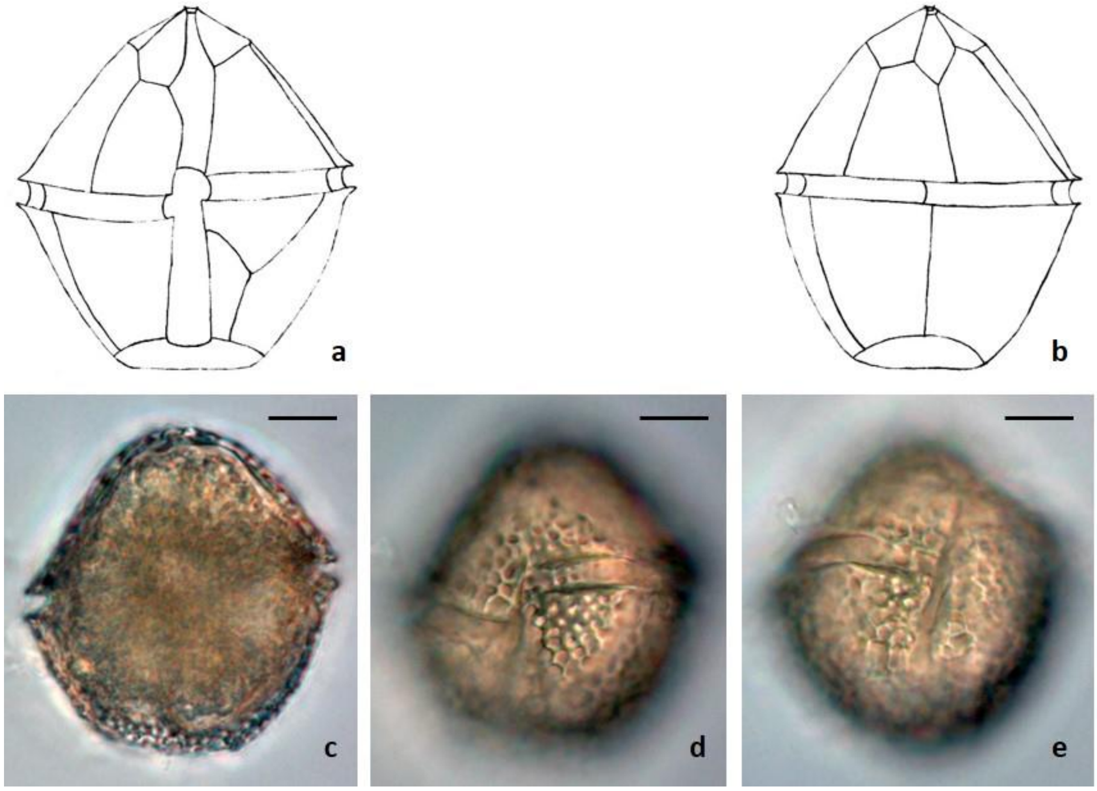

Fig. 2-12 *Lingulodinium polyedrum*

a-b - line drawing, a - ventral view; b - dorsal view. c-e - light micrographs. c - ventral view, d - sinistro-ventral view, e - postero-ventral view: c showing roughly pentagonal and polyhedral-shaped cell, without antapical spines and apical horn. d and e showing the thecal plates are coarsely areolate; girdle wide and almost equatorial. Scale bars = 10 μm.

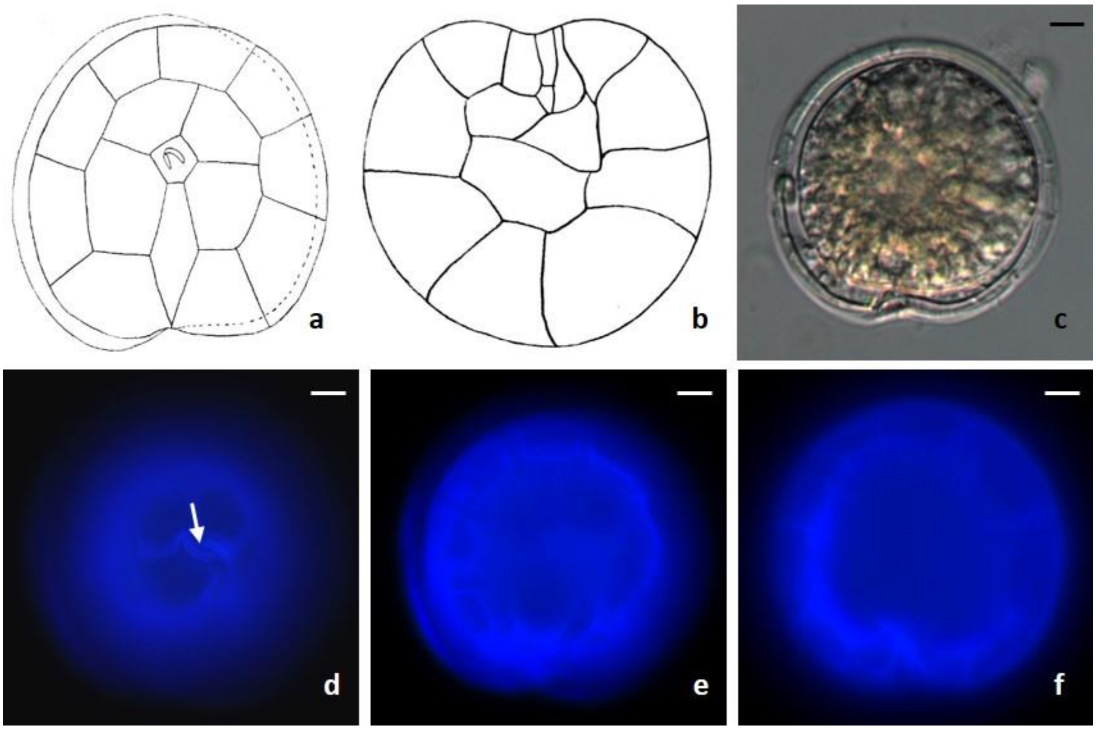

Fig. 2-13 *Pyrophacus horologium*

a-b - line drawing, a - apical view; b - antapical view. c - light micrograph, d-f - epifluorescent micrographs. c-f - apical views: c showing discoidal cell. d showing the apical pore area (arrow), where two slit-like. e and f showing epitheca plate pattern. Scale bars = 10 µm.

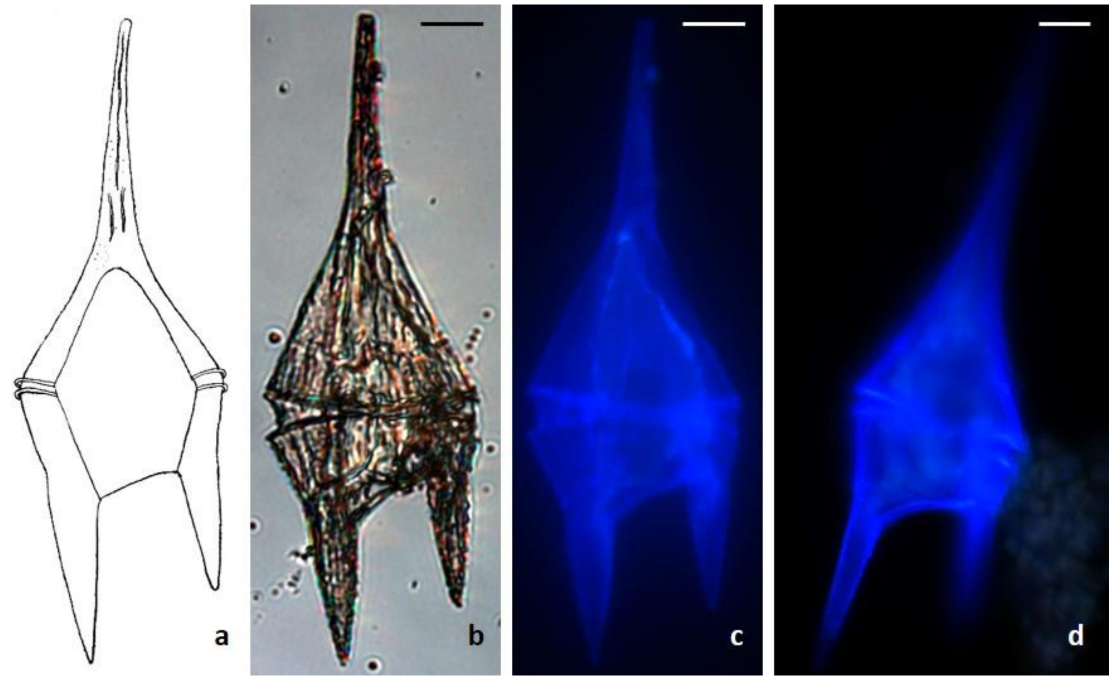

Fig. 2-14 *Ceratium furca*

a - line drawing, dorsal view. b - light micrograph, c-d - epifluorescent micrographs. b-d - dorsal views, showing large, straight body with cell being the widest either side of the girdle; two unequal, parallel or slightly divergent hypothecal horns, the right shorter than the left; epitheca tapering gradually into an apical horn; thecal plates thick and ornamented with surface ridges. Scale bars = 10 µm.

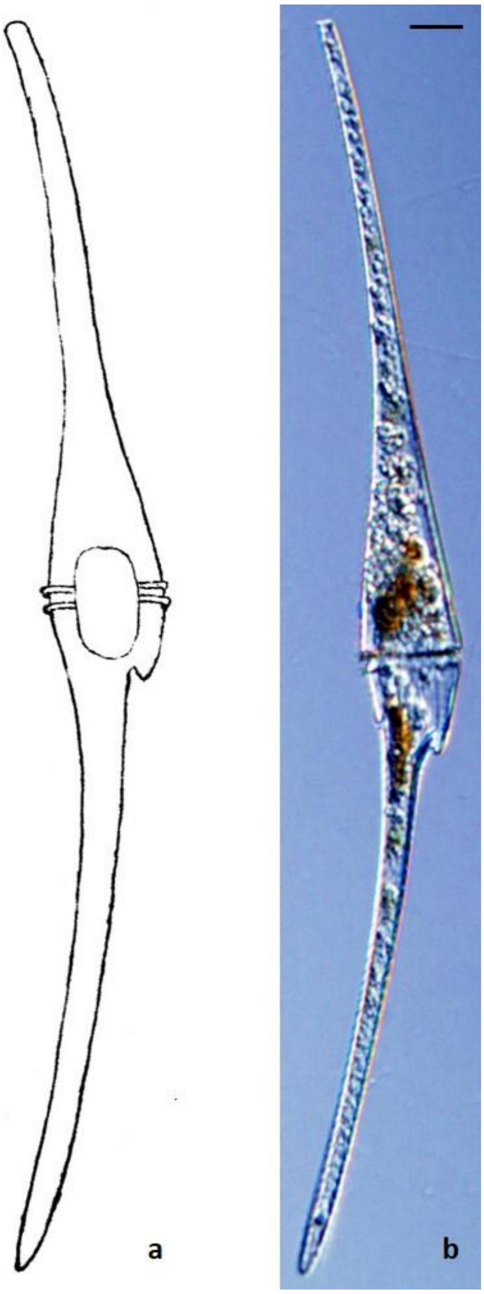

Fig. 2-15 *Ceratium fusus*

a - line drawing, dorsal view. b - light micrograph, dorsal view, showing epitheca tapers continuously into a long apical horn and hypotheca tapers into a long left antapical horn which is slightly curved, and a reduced right antapical horn is present. Scale bar = 10 μm.

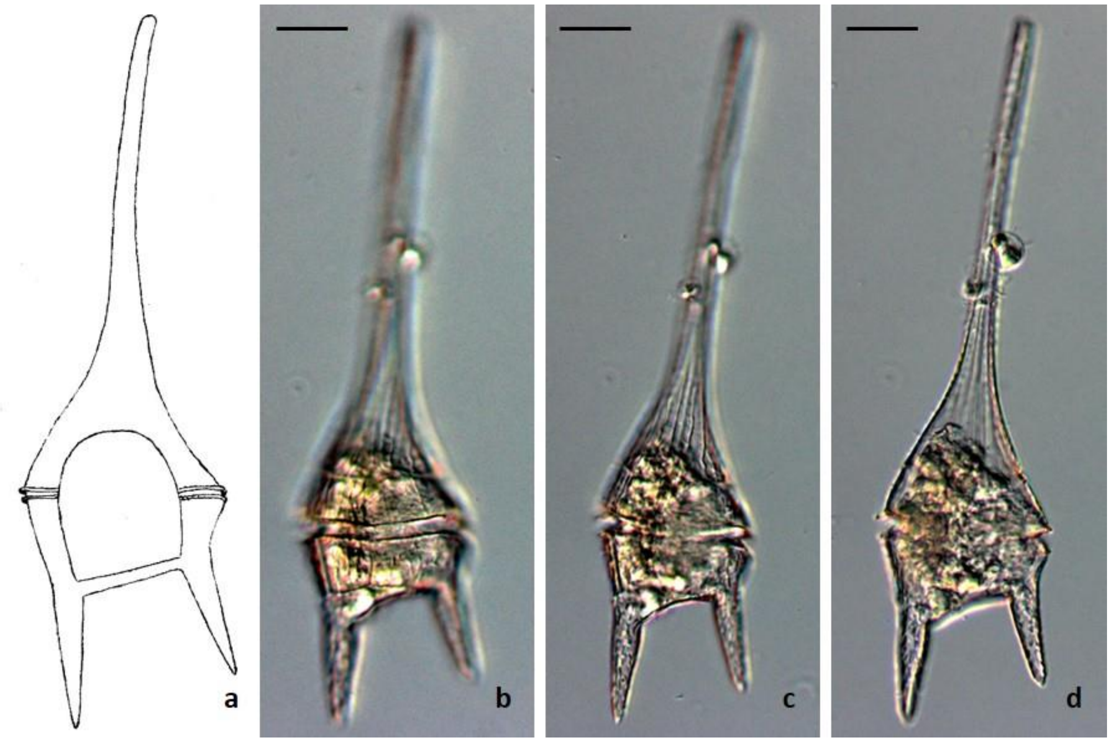

Fig. 2-16 *Ceratium kofoidii*

a - line drawing, dorsal view. b-d - light micrographs, dorsal views, showing epitheca triangular, with well separated apical horn; the left antapical horn being slightly longer; theca only weakly sculptured. Scale bars = 10 µm.

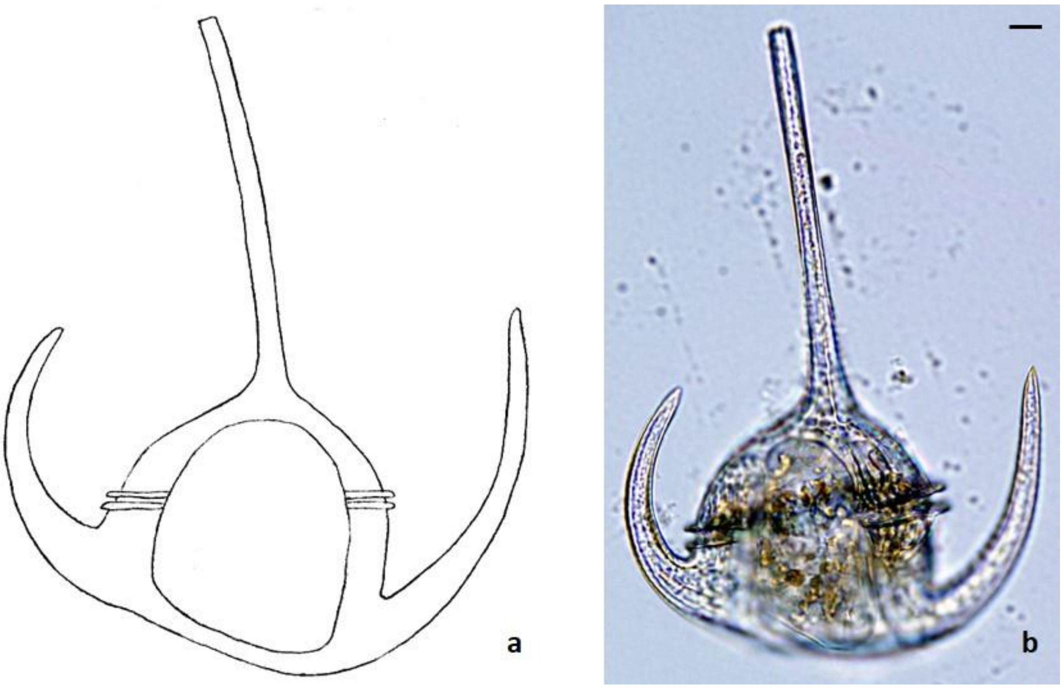

Fig. 2-17 *Ceratium tripos*

a - line drawing, dorsal view. b - light micrograph, dorsal view, showing cell body broad as its long; the epitheca leading sharply into long straight horn; the antapicals continuous with slightly flattened base, then sharply curved forward making an acute angle with apical horn. Scale bar = 10 μm.

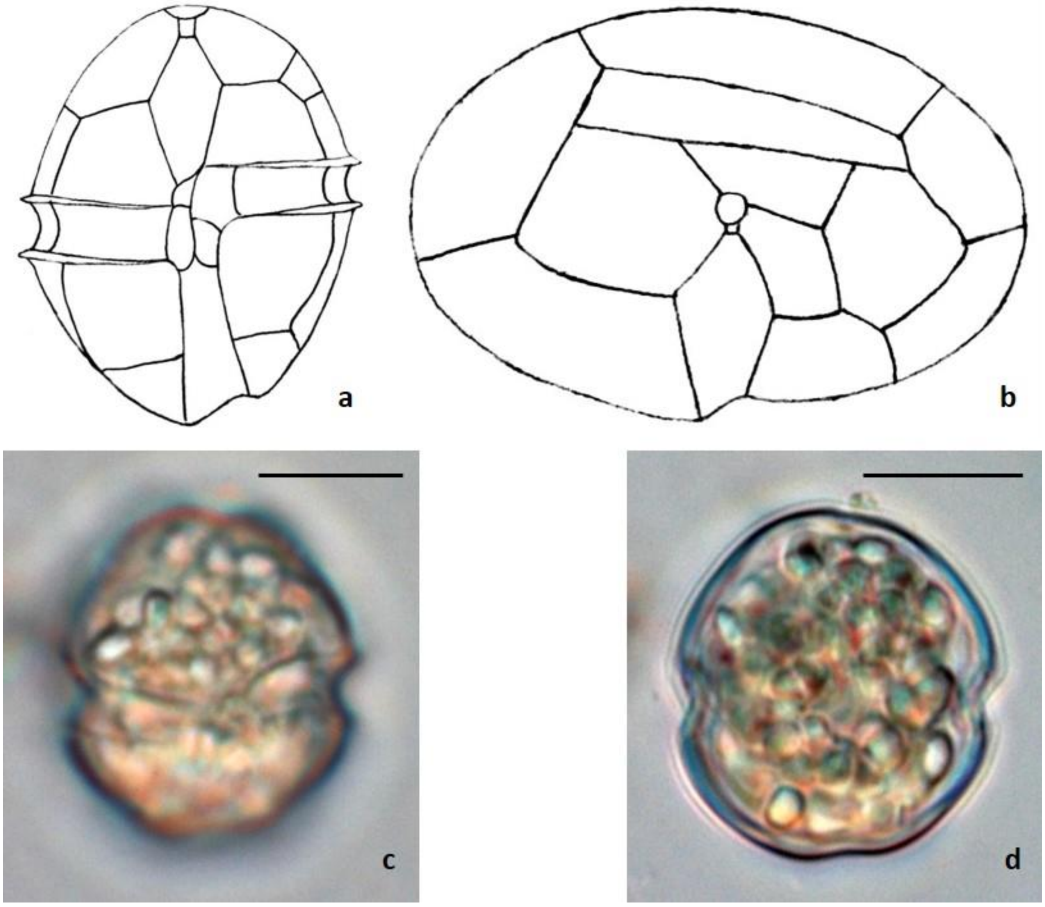

Fig. 2-18 *Durinskia capensis*

a-b - line drawing, a - ventral view; b - apical view. c-d - light micrographs. c - ventral view, d - dorsal view, showing the epitheca is slightly larger than the hypotheca, the epitheca is hemispherical and the hypotheca is hemispherical to trapezoidal; the cingulum is well excavated. Scale bars = 10 µm.

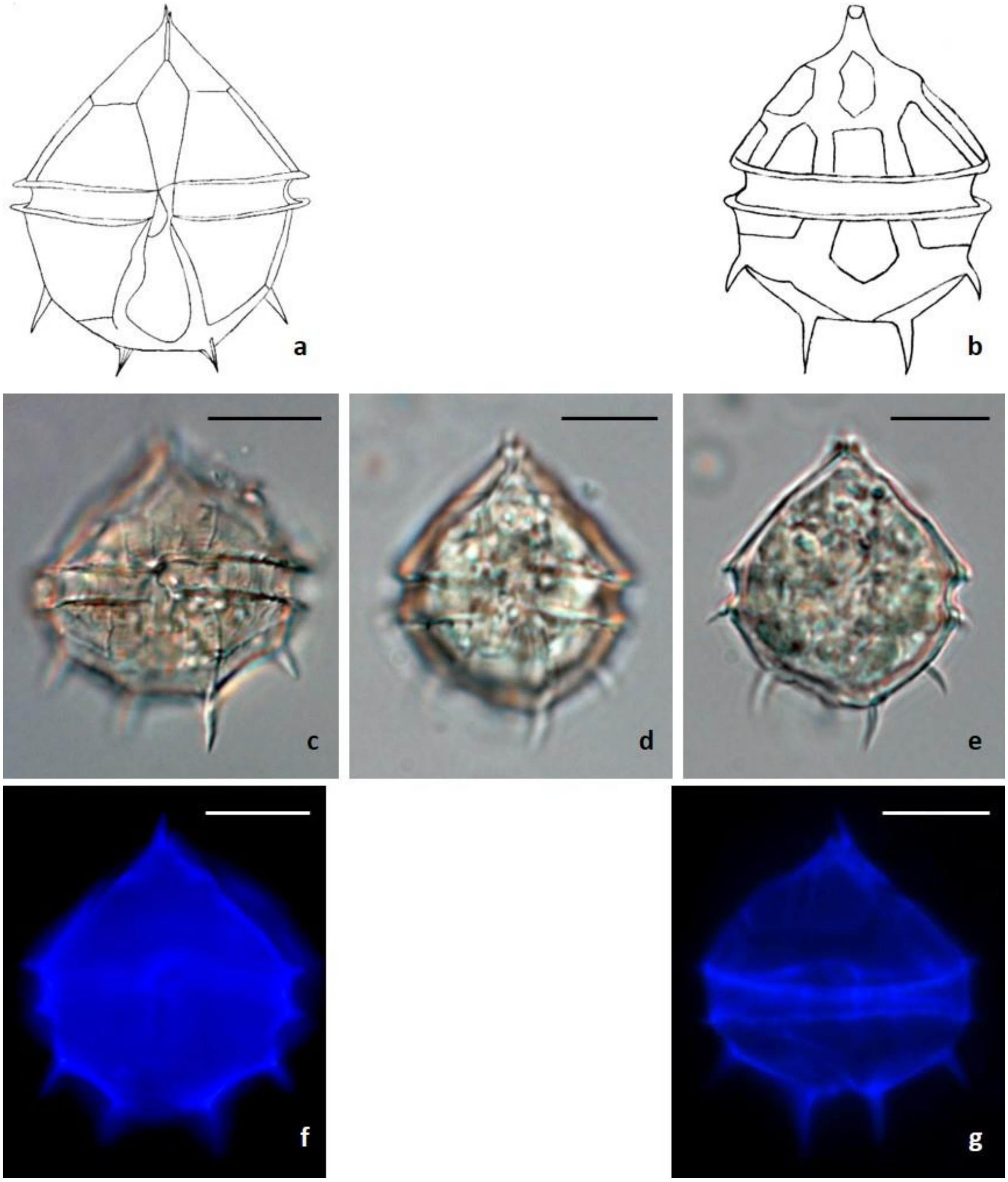

Fig. 2-19 *Peridinium quinquecorne*

a-b - line drawing, a - ventral view; b - dorsal view. c-e - light micrographs, f-g - epifluorescent micrographs. c-f - ventral views, g - dorsal view, showing epitheca is conical with a pointed apex, and hypotheca is angular with 4 antapical spines. Scale bars = 10 µm.

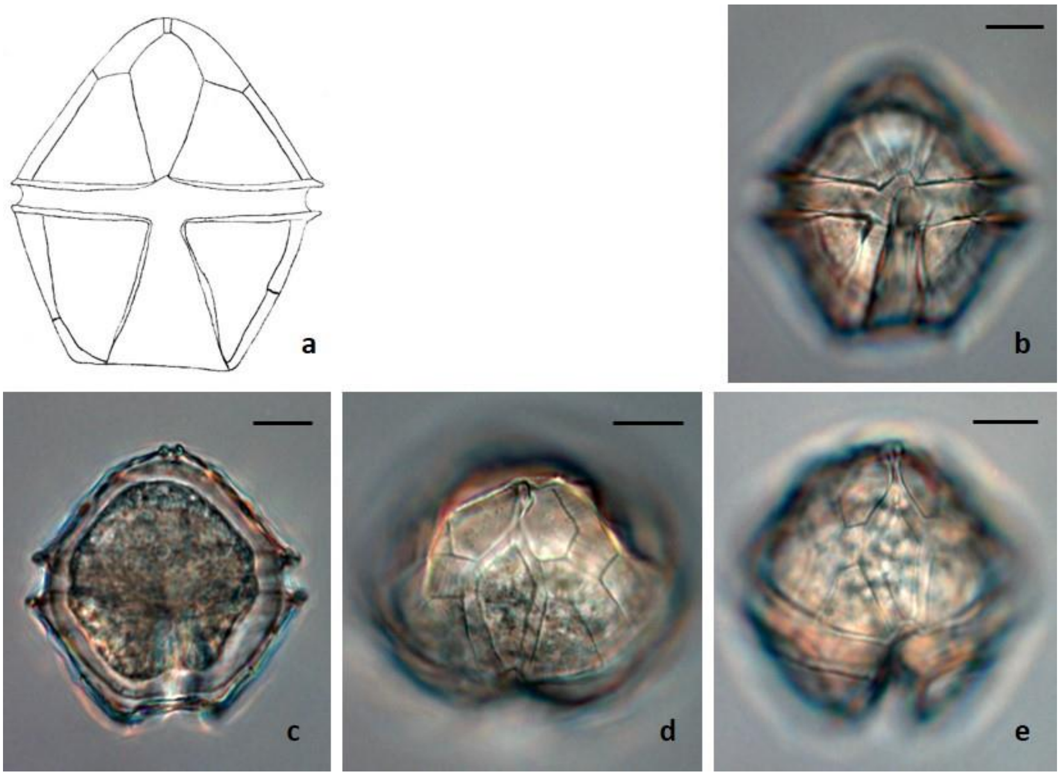

Fig. 2-20 *Protoperidinium avellanum*

a - line drawing, ventral view. b-e - light micrographs. b-c - ventral views, d - antero-ventral view, e - dextro-ventral view: b showing a deep longitudinal furrow of subequal breadth. c showing the cell without apical and antapical horns. d and e showing plate pattern. Scale bars = 10 µm.

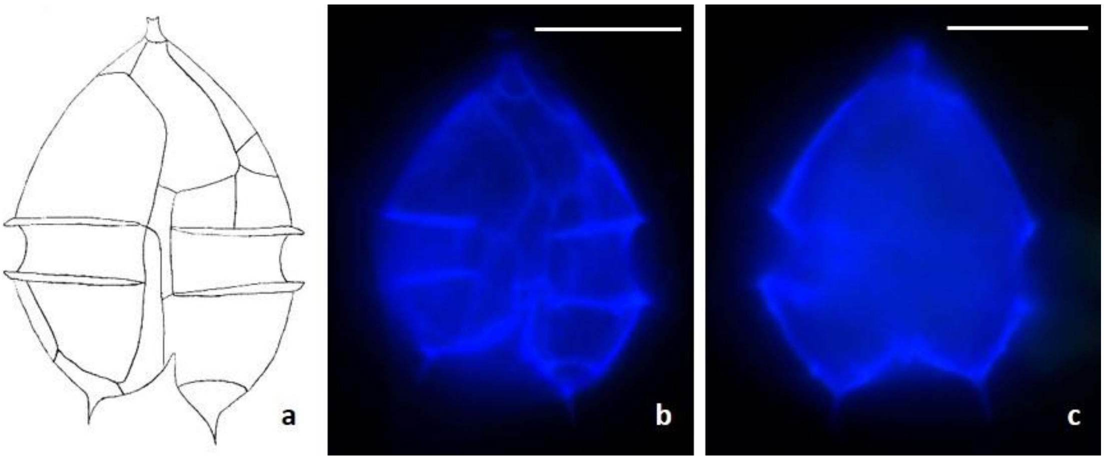

Fig. 2-21 *Protoperidinium bipes*

a - line drawing, ventral view. b-c - epifluorescent micrographs. b - ventral view, c - dorsal view, showing cell with a triangular epitheca ending in an apical horn, and the hypotheca ending in two antapical horns. Scale bars = 10 µm.

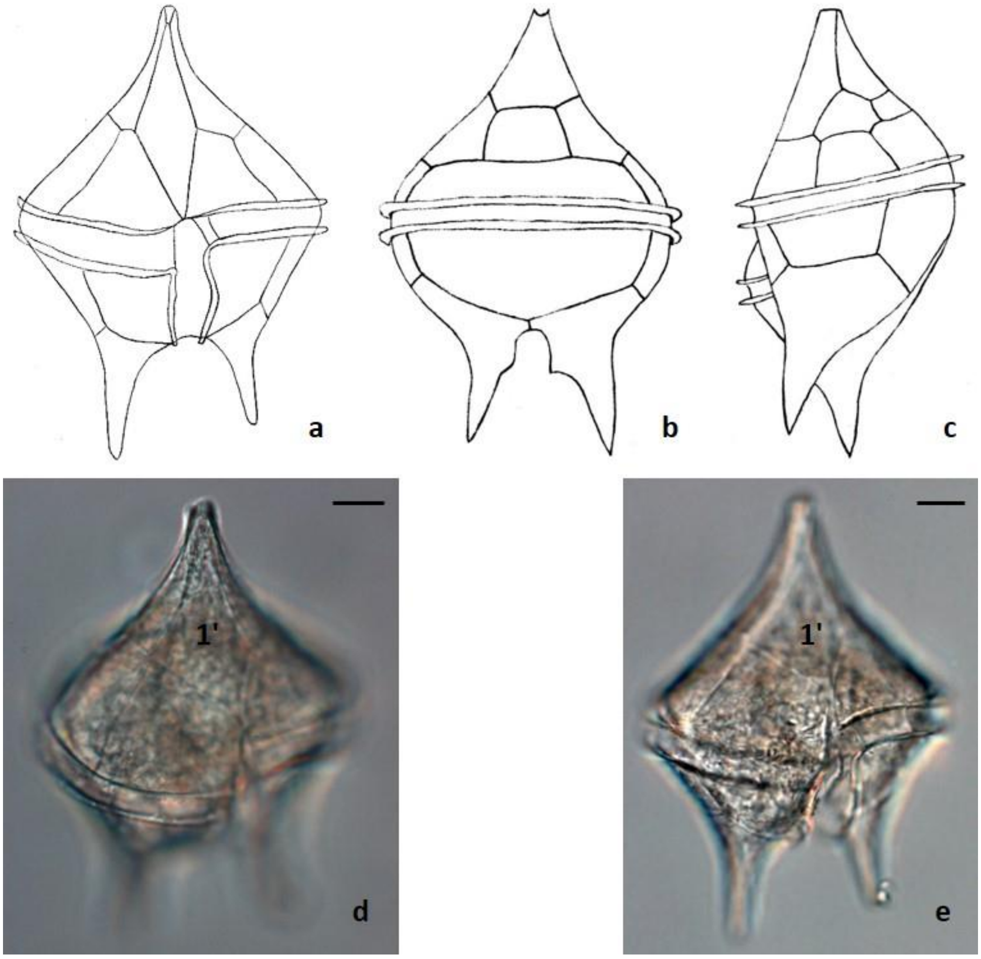

Fig. 2-22 *Protoperidinium claudicans*

a-c - line drawing, a - ventral view; b - dorsal view; c - lateral view. d-e - light micrographs, dextro-ventral views, showing the ortho shape of the 1' plate, and the thecal plates are slightly reticulated. Scale bars = 10 µm.

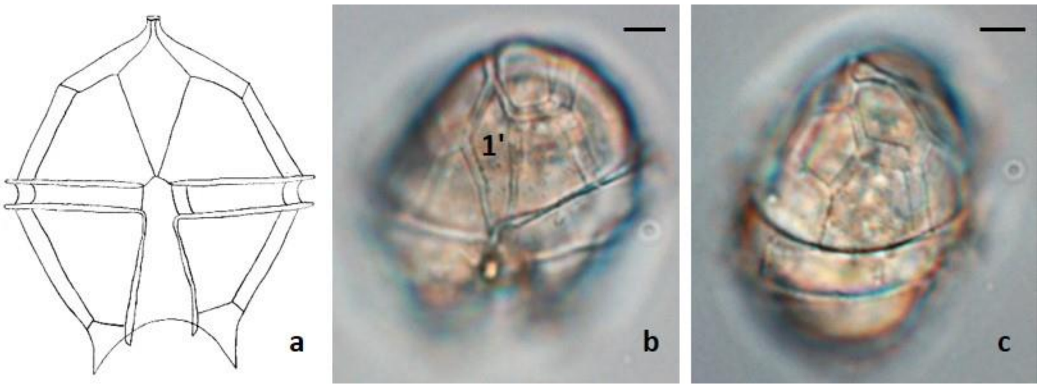

Fig. 2-23 *Protoperidinium divaricatum*

a - line drawing, ventral view. b-c - light micrographs. b - sinistro-ventral view, c - side view: b showing the Plate 1' is rhomboid, symmetrical, with the proximal margins about twice as long as the distal ones. Scale bars = 10 µm.

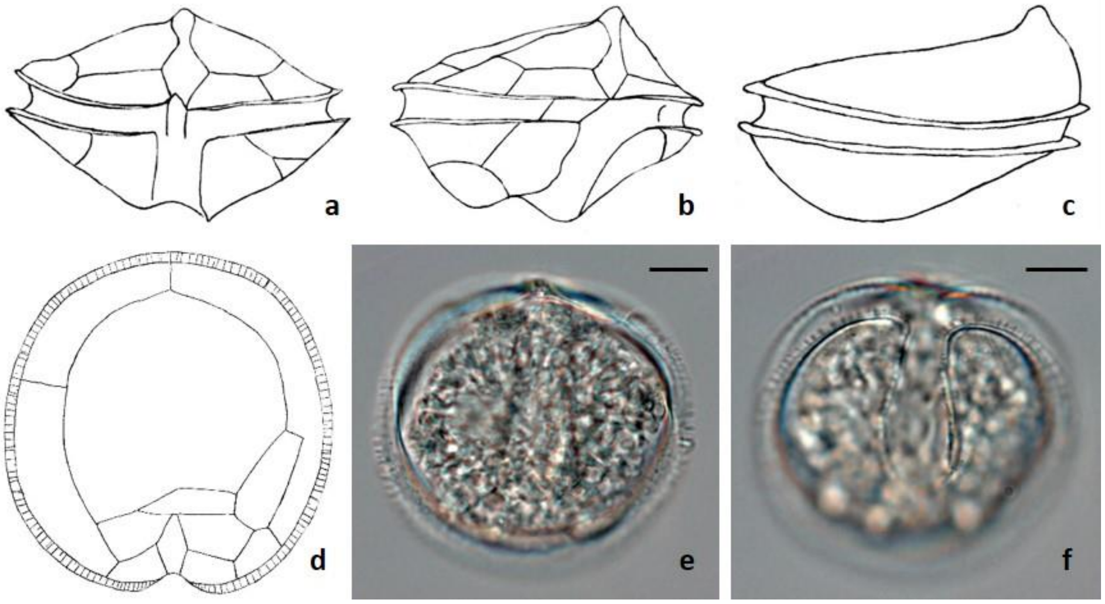

Fig. 2-24 *Protoperidinium excentricum*

a-d - line drawing, a - ventral view; b - dextro-ventral view; c - lateral view; d - antapical view. e-f - light micrographs. e - antapical view, f - postero-ventral view: e showing apex-antapex axis is oblique, offset to the left and ventral. f showing sulcus is deeply excavated and reaches beyond the centre of the hypotheca, the left projecting further than the right; cingulum is excavated, bordered by lists supported by spines. Scale bars = 10 µm.

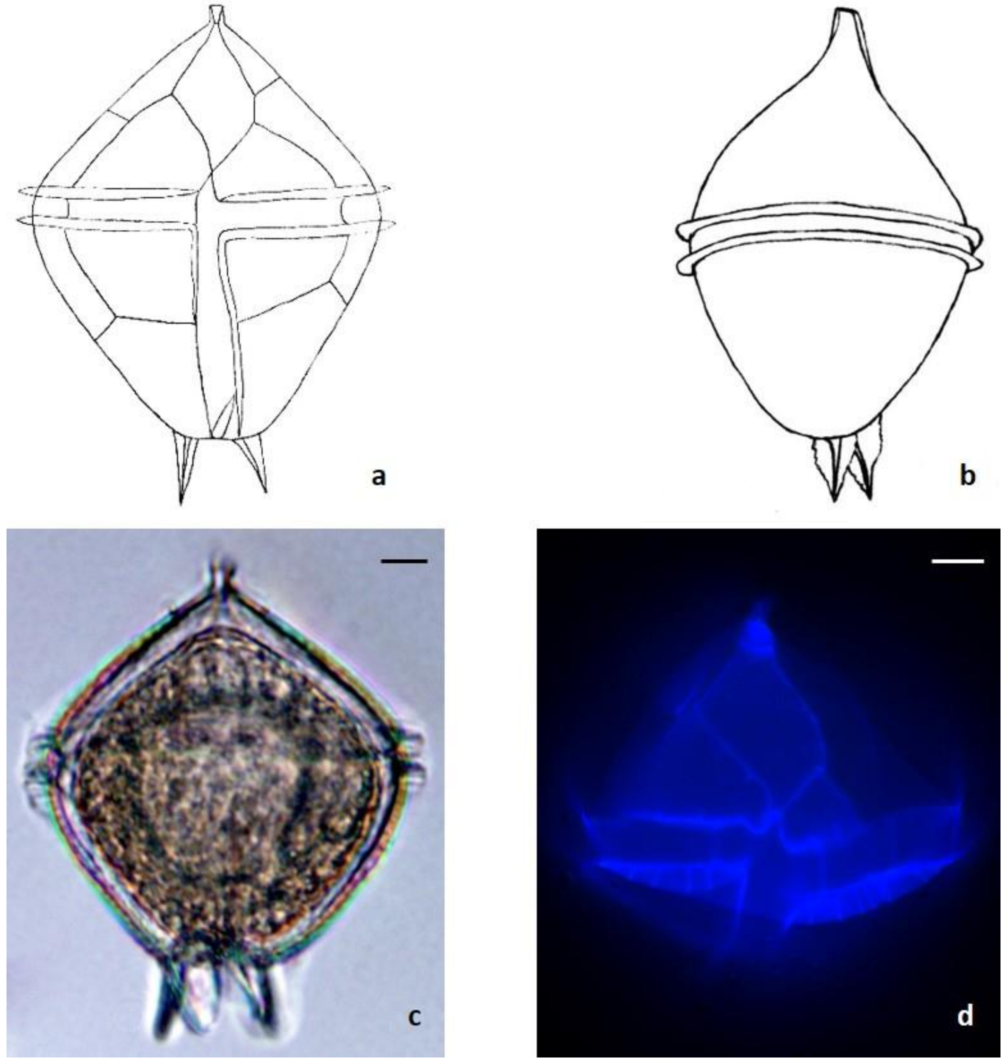

Fig. 2-25 *Protoperidinium latispinum*

a-b - line drawing, a - ventral view, b - lateral view. c - light micrograph, d - epifluorescent micrograph. c-d - ventral view: c showing the epitheca is weakly convex conical, tapering distally to a short spical horn; the hypotheca is obliquely truncated at its distal end between the two antapical spines. d showing thecal plate pattern. Scale bars = 10 µm.

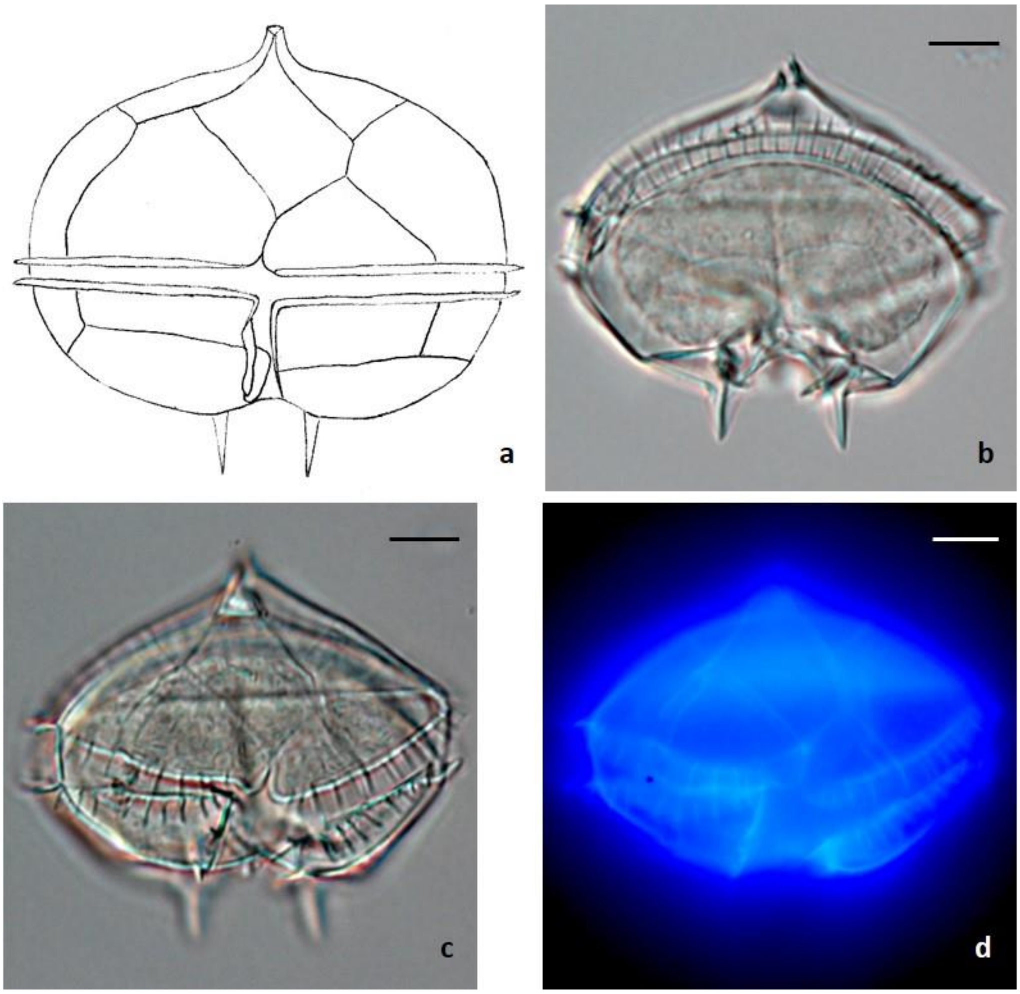

Fig. 2-26 *Protoperidinium marukawai*

a - line drawing, ventral view. b-c - light micrographs, d - epifluorescent micrograph. b - postero-dorsal view, c-d - ventral views: b showing cingulum is bordered by lists supported by spines. c and d showing tetragonal middle intercalary plate is displaced slightly towards the left; the apex and antapex are much displaced ventrally. Scale bars = 10 μm.

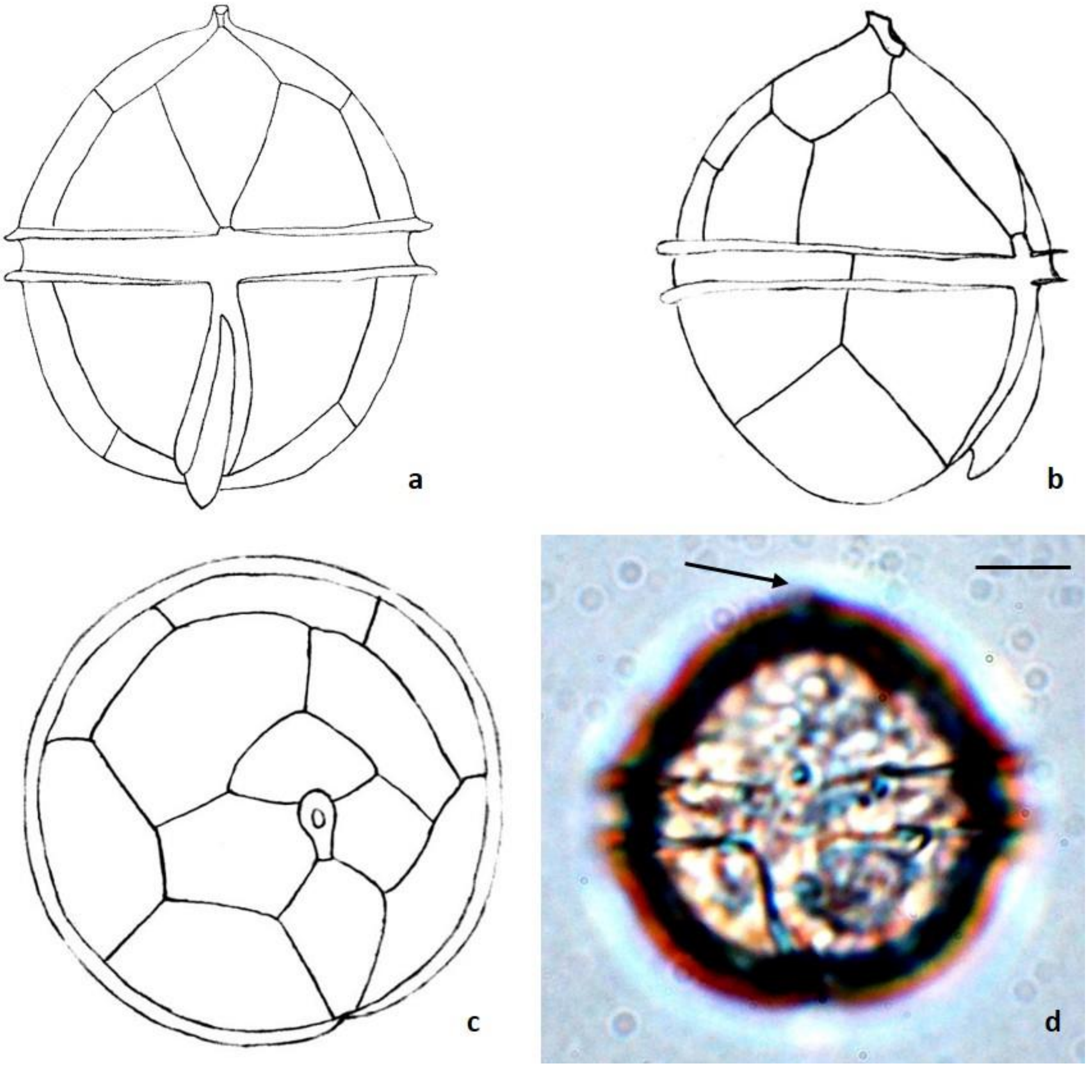

Fig. 2-27 *Protoperidinium minutum*

a-c - line drawing, a - ventral view; b - lateral view; c - apical view. d - light micrograph, sinistro-ventral view, showing a spherical cell with a apical horn (arrow) and no antapical extension; the thecal surface contained large pores. Scale bar = 10 μm.

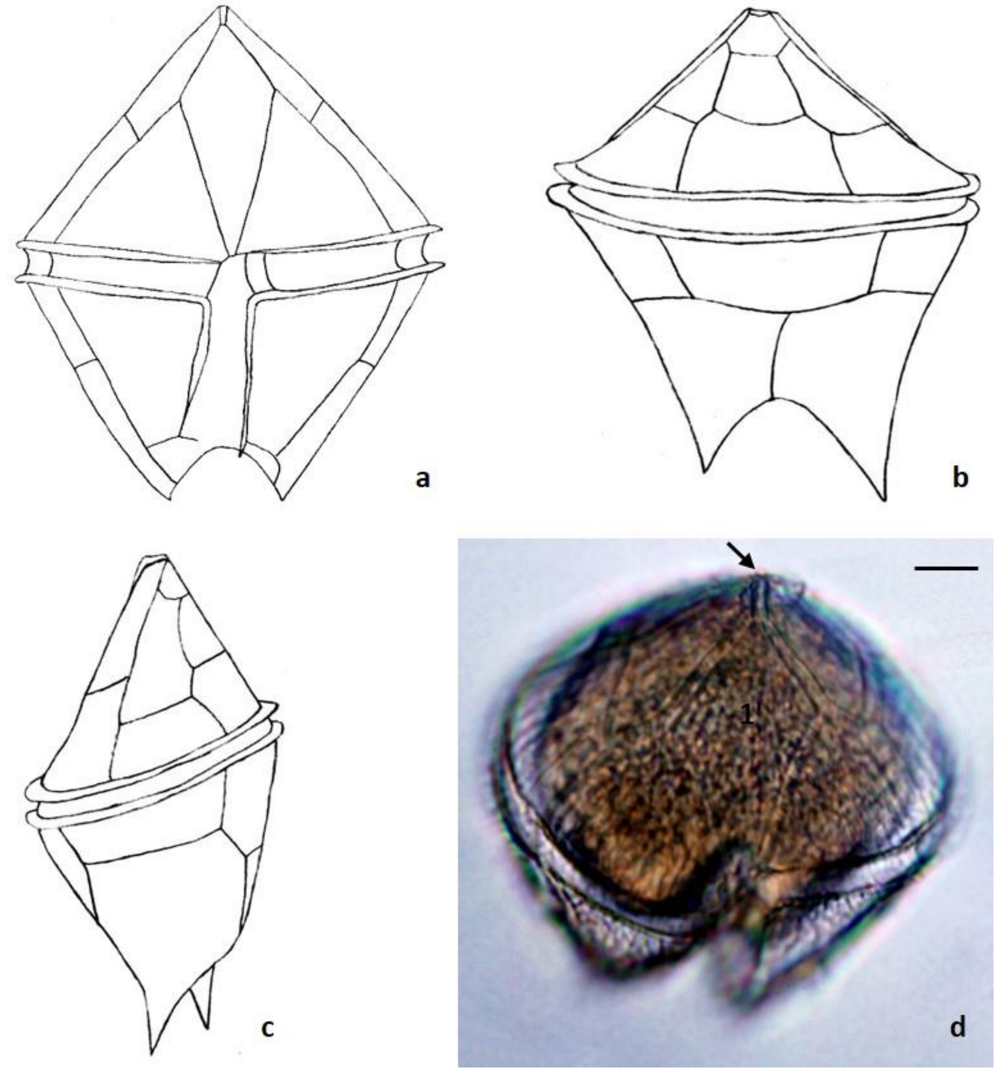

Fig. 2-28 *Protoperidinium obtusum*

a-c - line drawing, a - ventral view; b - dorsal view; c - lateral view. d - light micrograph, antero-ventral view, showing cell has a blunt apex (arrow); epitheca covered by longitudinal striations; cingulum bordered by a list, supported by spines; and plate 1' is rhomboid, with the distal sides about 1.5 longer than the proximal ones. Scale bar = 10 µm.

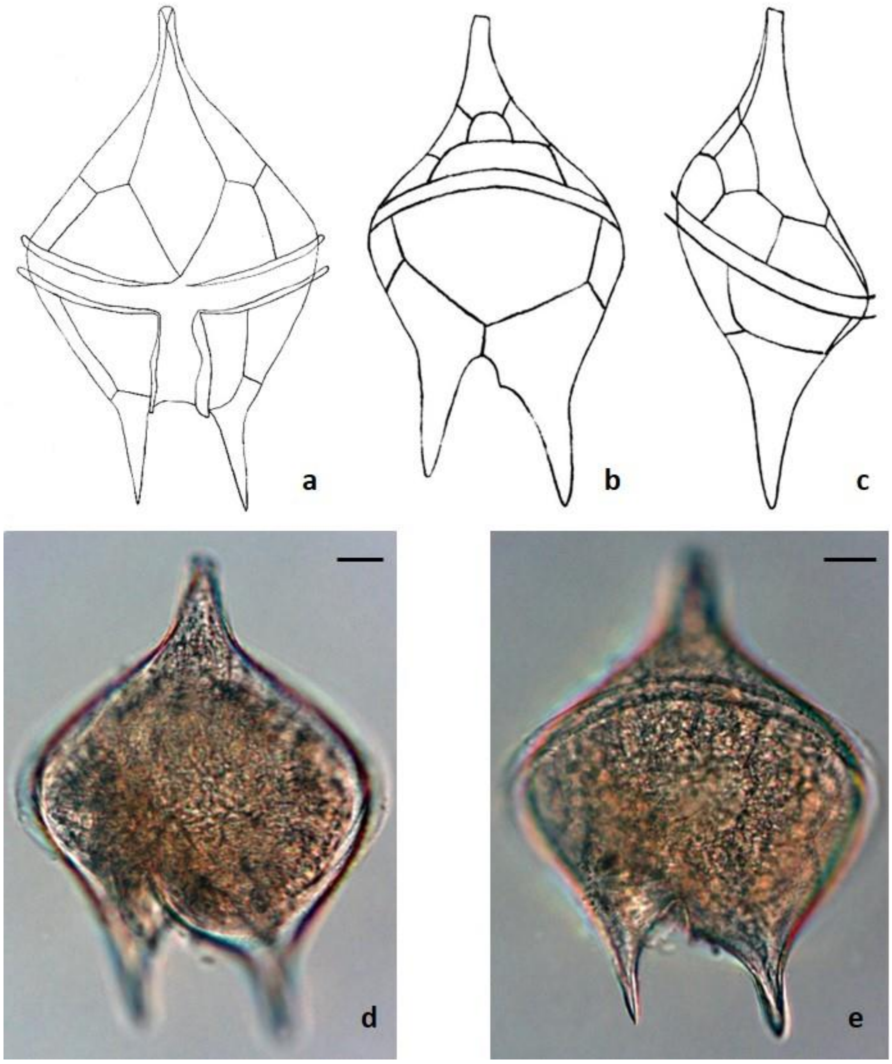

Fig. 2-29 *Protoperidinium oceanicum*

a-c - line drawing, a - ventral view; b - dorsal view; c - lateral view. d-e - light micrographs. d - sinistro-dorsal view, e - postero-dorsal view: d showing slender apical horn. e showing hypotheca has two antapical high, conical, slender horns ending in strong spines. Scale bars = 10 µm.

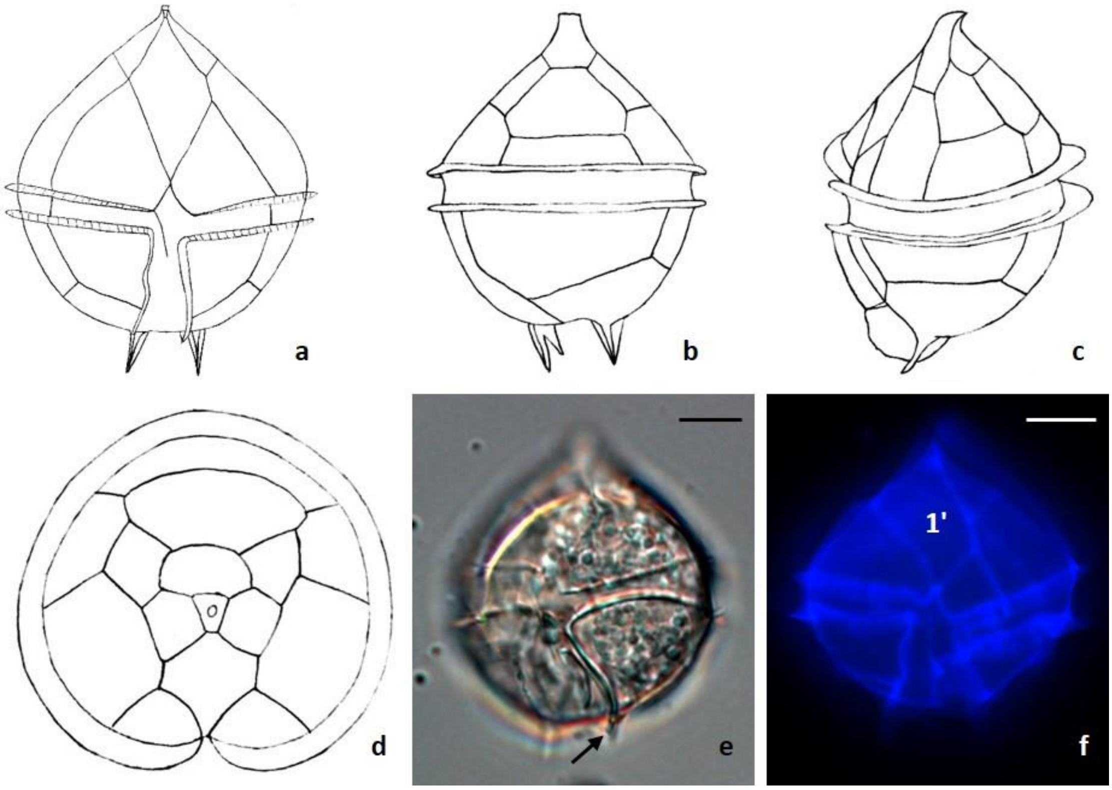

Fig. 2-30 *Protoperidinium pellucidum*

a-d - line drawing, a - ventral view; b - dorsal view; c - lateral view; d - apical view. e - light micrograph, f - epifluorescent micrograph. e - sinistro-ventral view, f - ventral view: e showing the slightly ascending cingulum, the antapcial spines, and the left sulcal list which looks like a third spine (arrow). f showing the ‘para’ 1' plate. Scale bars = 10 µm.

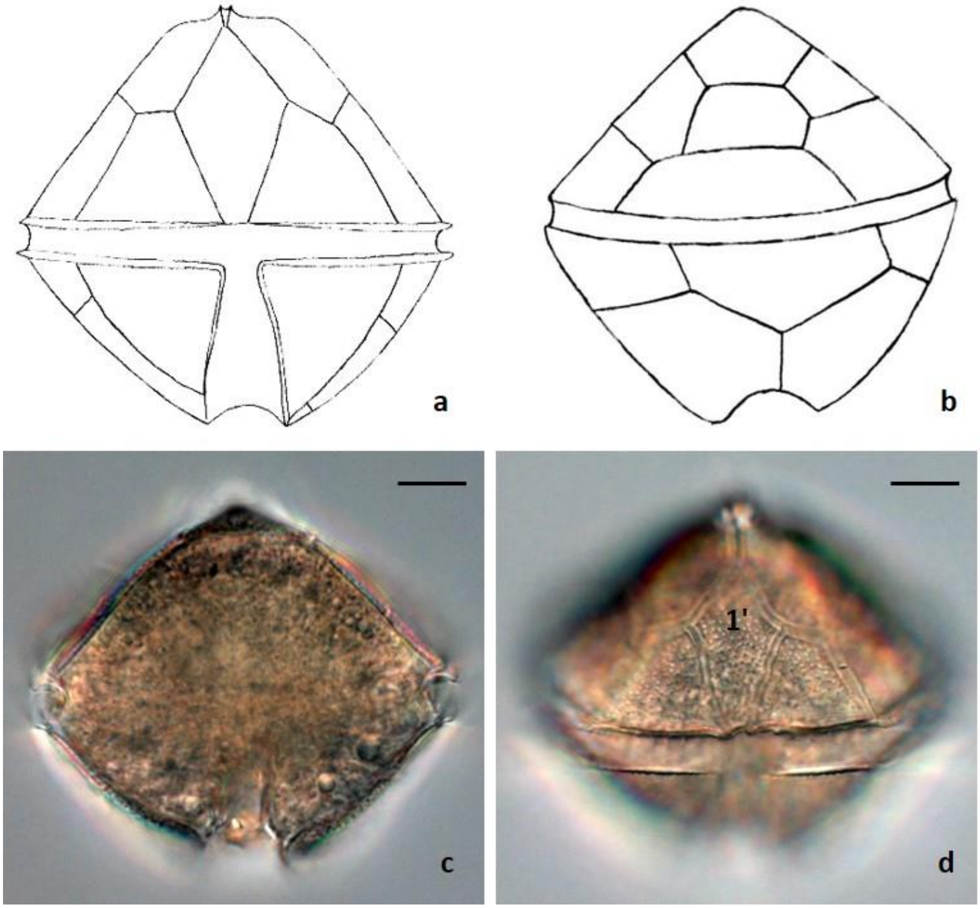

Fig. 2-31 *Protoperidinium punctulatum*

a-b - line drawing, a - ventral view; b - dorsal view. c-d - light micrographs. c - ventral view, d - antero-ventral view: c showing the cell outline; deep and narrow sulcus. d showing the ortho-shape of the 1' plate, and the theca with small, robust spines. Scale bars = 10 µm.

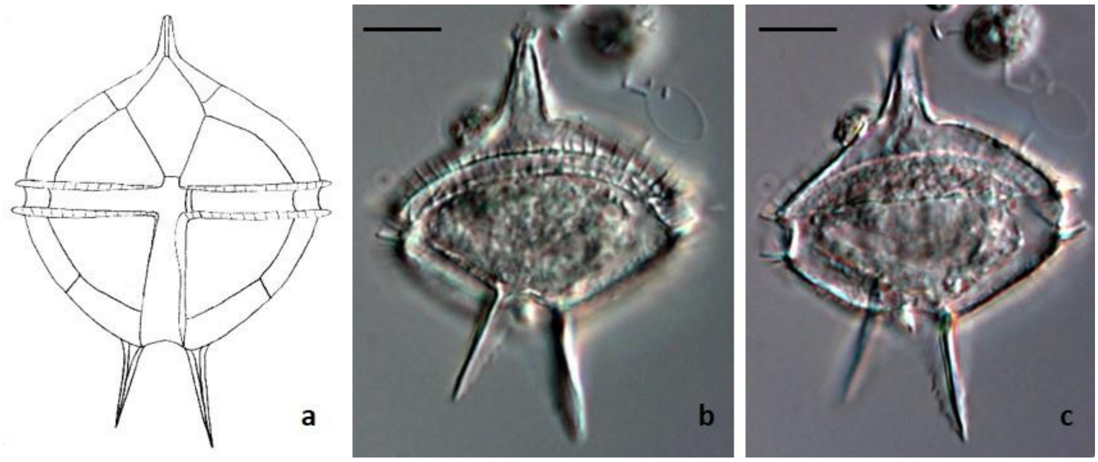

Fig. 2-32 *Protoperidinium pyriforme*

a - line drawing, ventral view. b-c - light micrographs, postero-dorsal views, showing well-separated apical horn; cingulum is bordered by lists supported by spines; hypotheca has two strong, long, widely winged antapical spines. Scale bars = 10 µm.

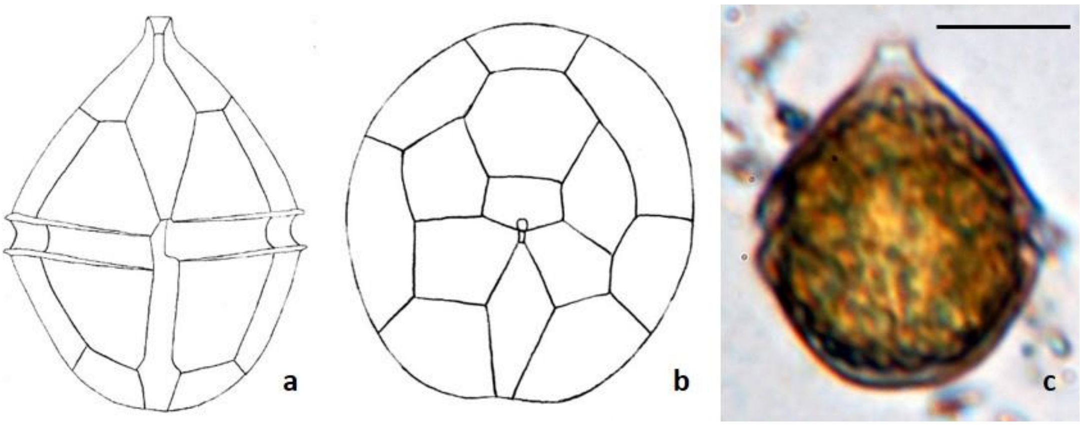

Fig. 2-33 *Scrippsiella trochoidea*

a-b - line drawing, a - ventral view; b - apical view. c - light micrograph, ventral view, showing small pear-shaped cell, yellow-brown chromatophores, epitheca is conical with a raised apex, and hypotheca hemisperical with no antapical projections. Scale bar = 10 μm.

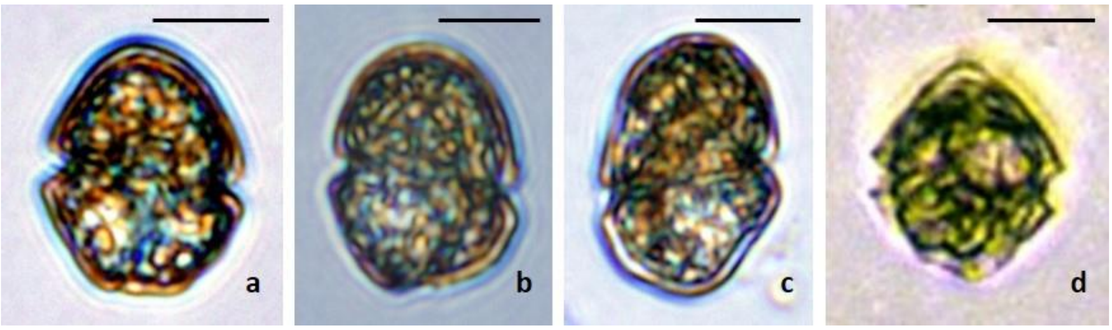

Fig. 2-34 *Heterocapsa* sp.

a-d - light micrographs, ventral views. Scale bars = 10 µm.

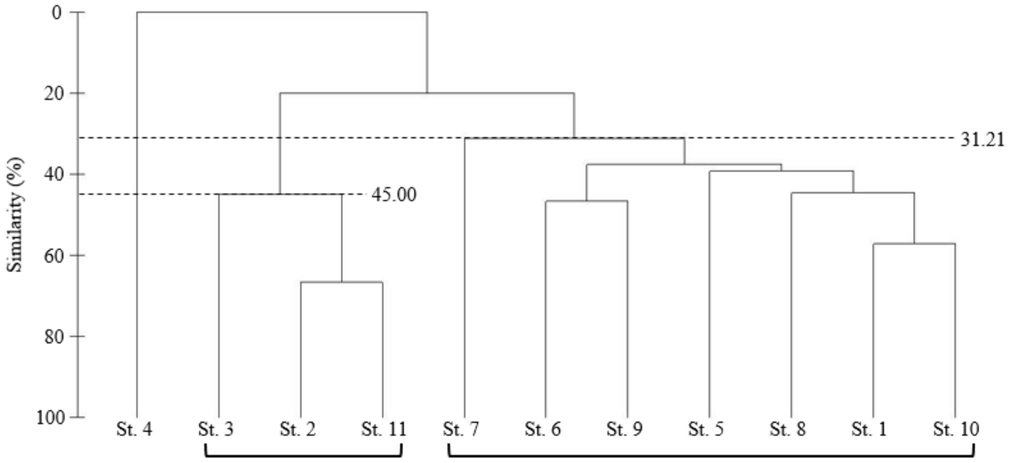

Fig. 3 Dendrogram of sampling stations (Hainan Island) based on clustering of dinoflagellate species composition similarity. Similarity levels are presented as percentages.

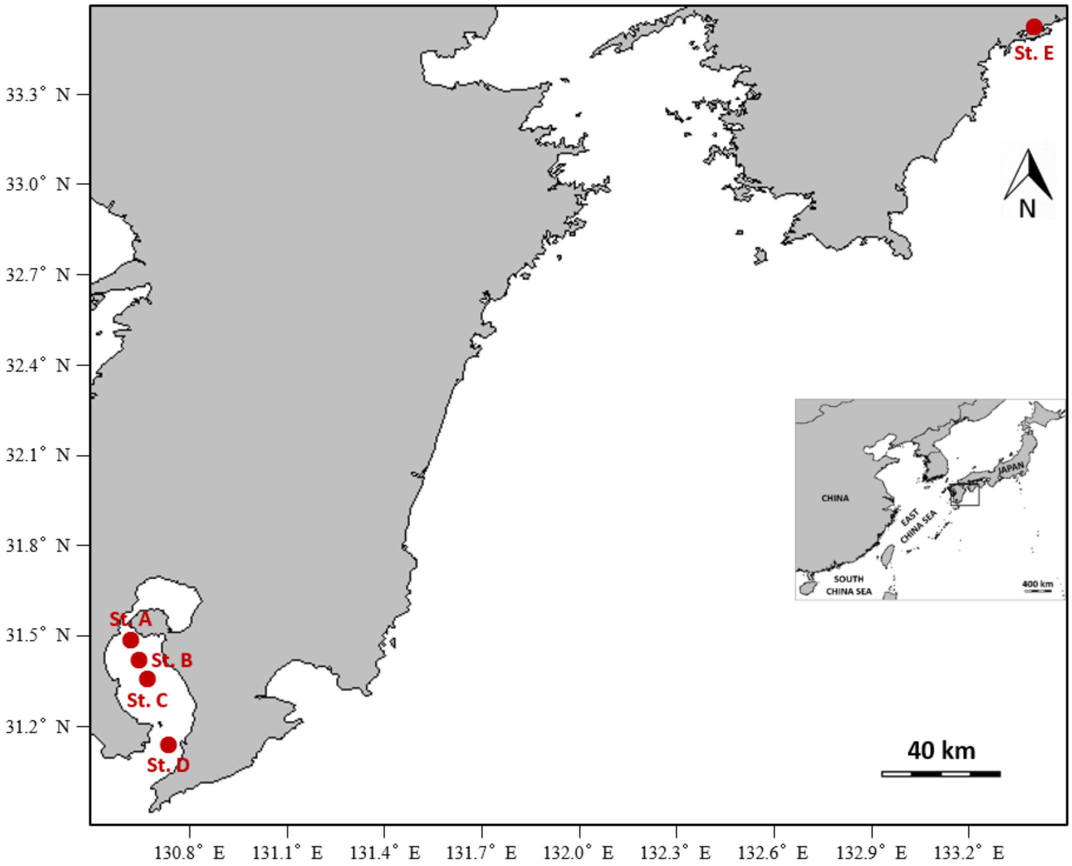

Fig. 4 Map of partial south Japan and locations of sampling sites at Kagoshima and Uranouchi Bays (●).

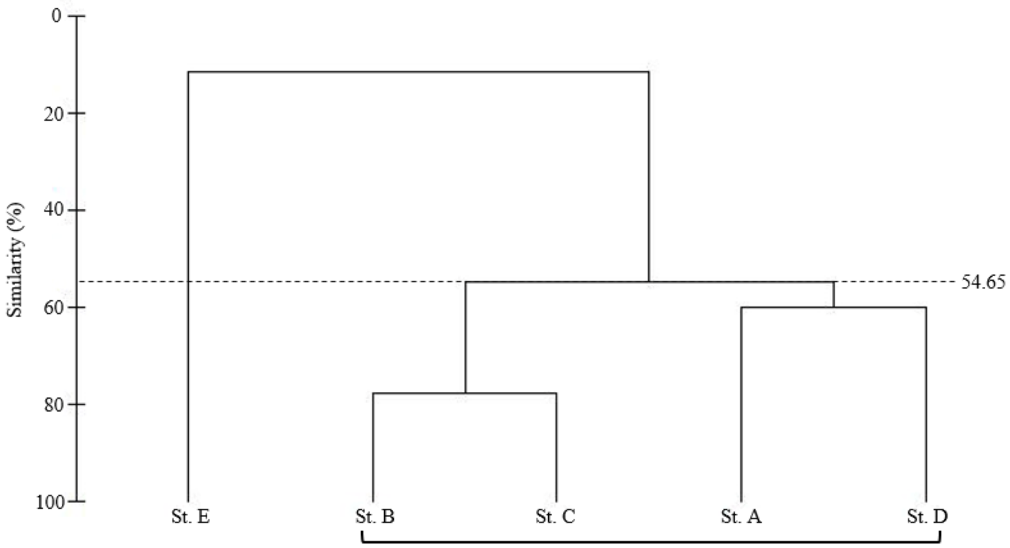

Fig. 5 Dendrogram of sampling stations (south Japan) based on clustering of dinoflagellate species composition similarity. Similarity level is presented as percentage.

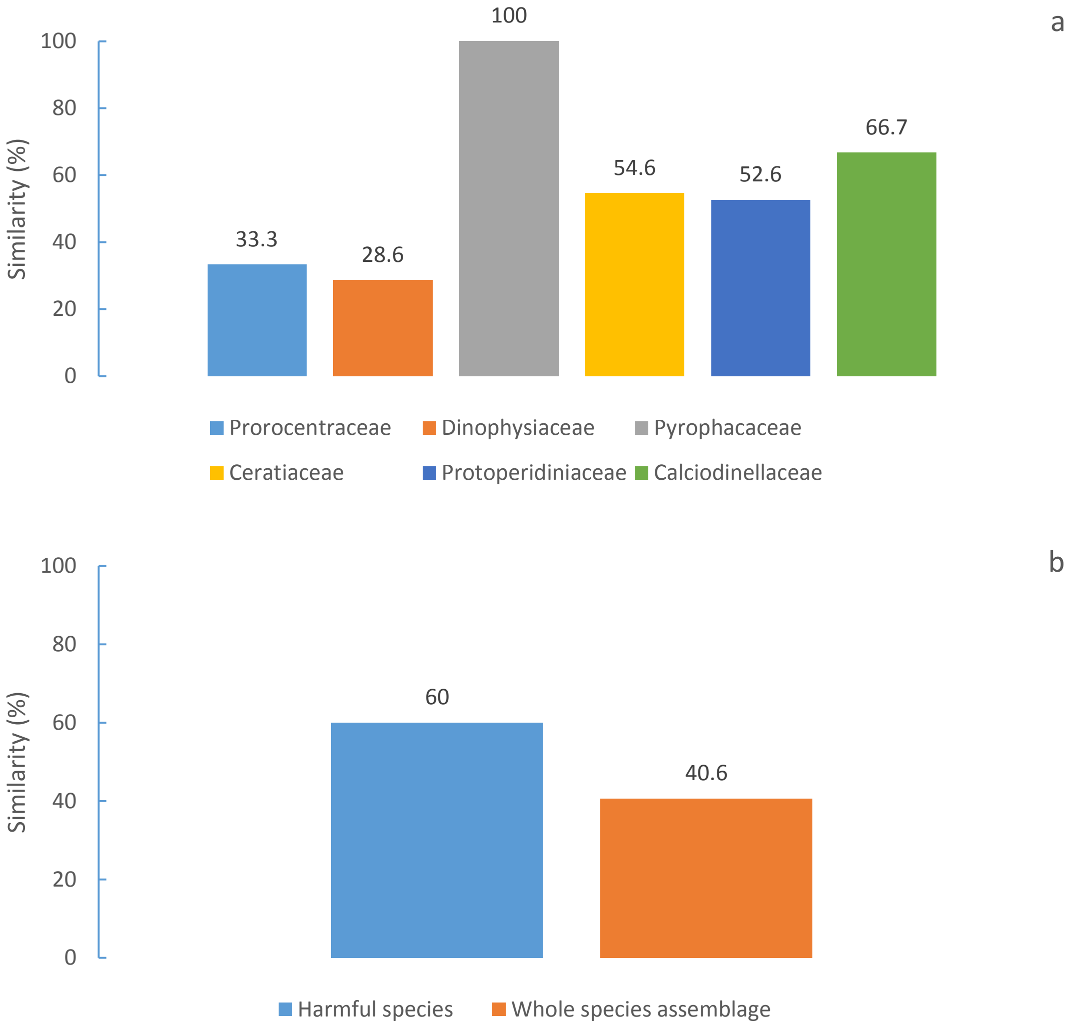

Fig. 6 Similarity between south Japan and Hainan Island on family level (a) and harmful species (b) (based on species occurrence, nonquantitatively).

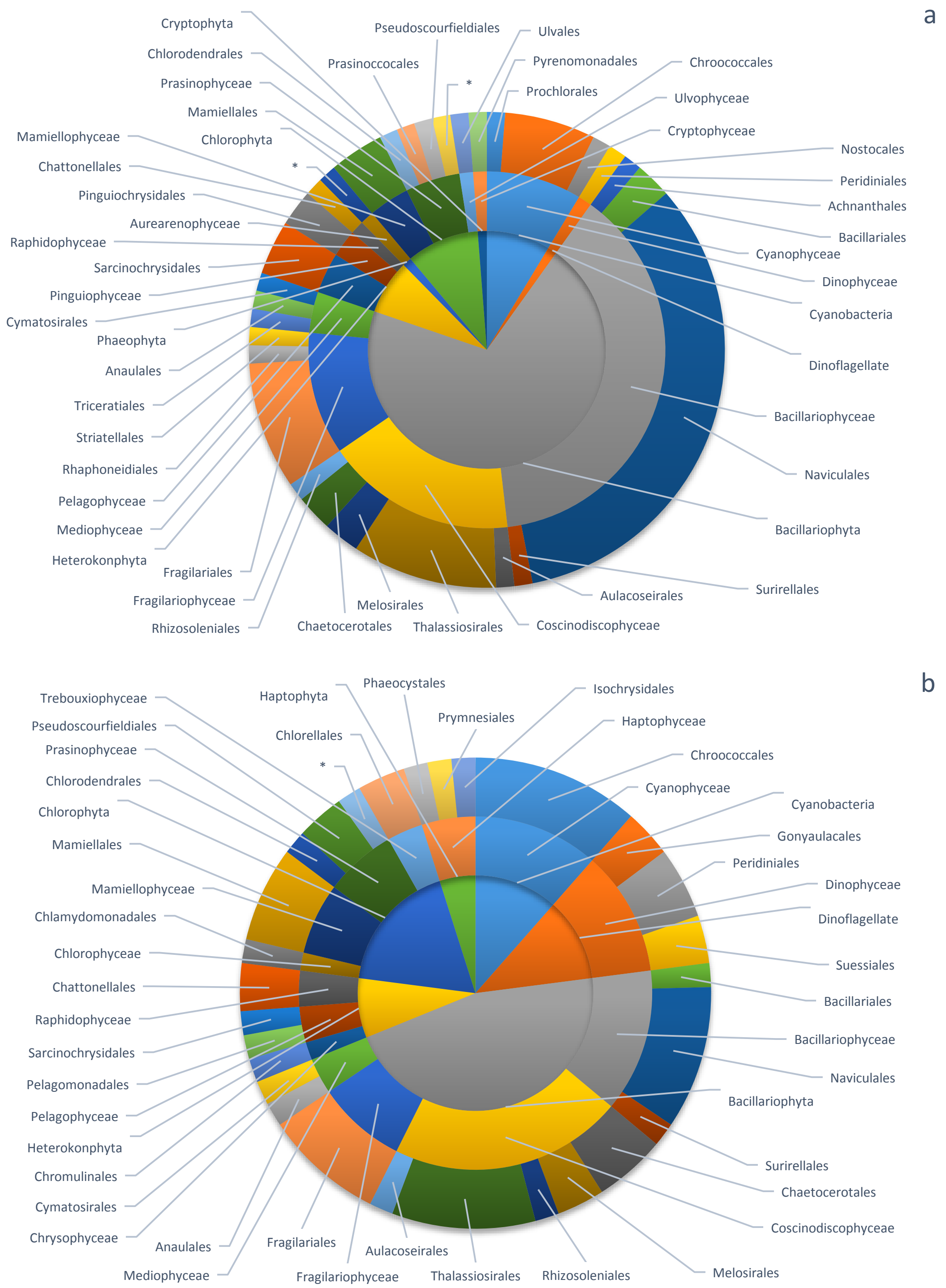

Fig. 7 Diversity richness of phytoplankton and phylogenetic distribution on phylum (inner circle), class (middle circle), and order level (outer circle) (based on over 95% homology) of Hainan Island (a) and Kagoshima Bay (b). '*' represents unclassified groups. No relationship among the same colors.

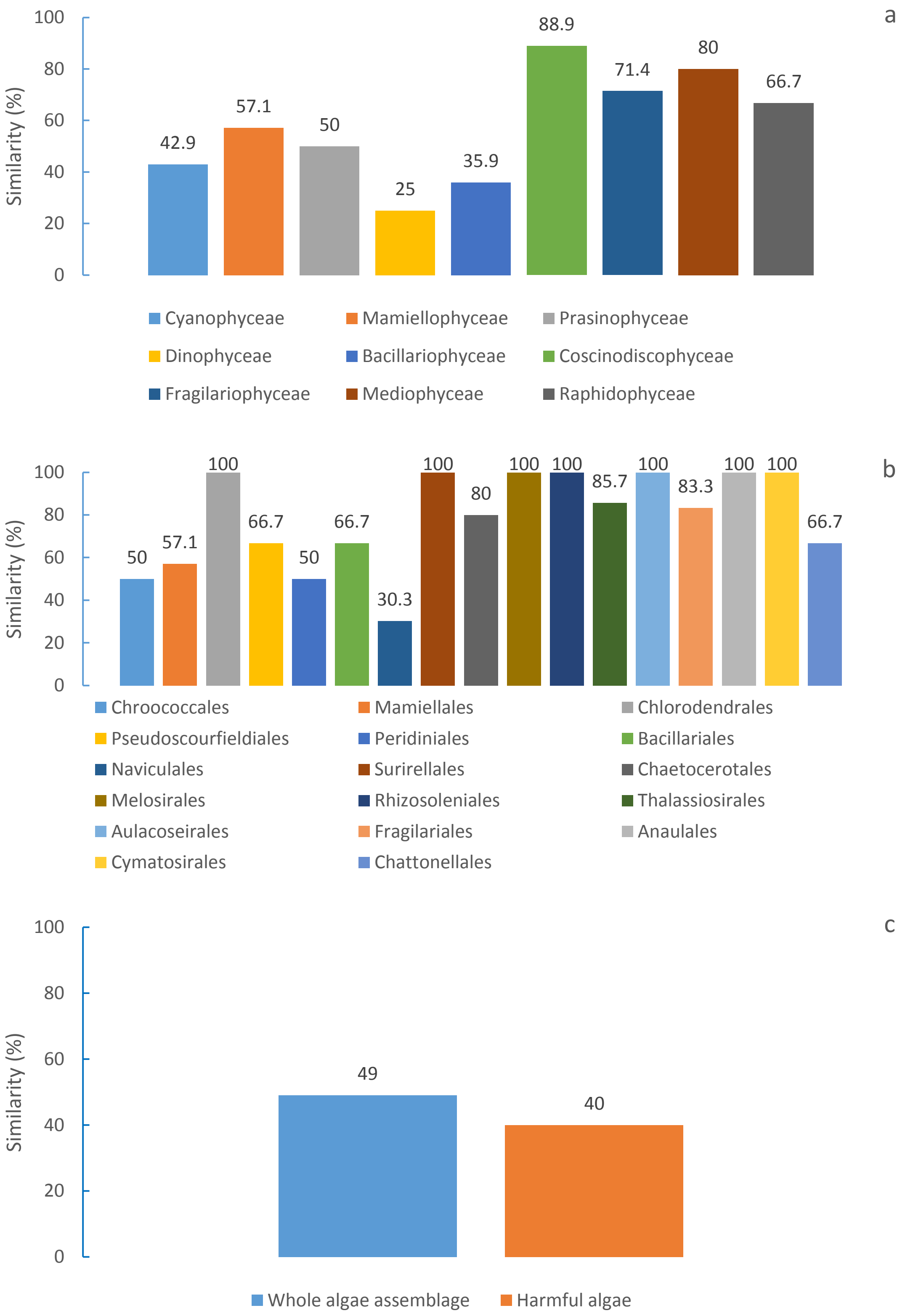

Fig. 8 Similarity between Hainan Island and Kagoshima Bay on class (a), order level (b) and Harmful algae (c) (based on species occurrence by metagenomic analysis in sediments, nonquantitatively).

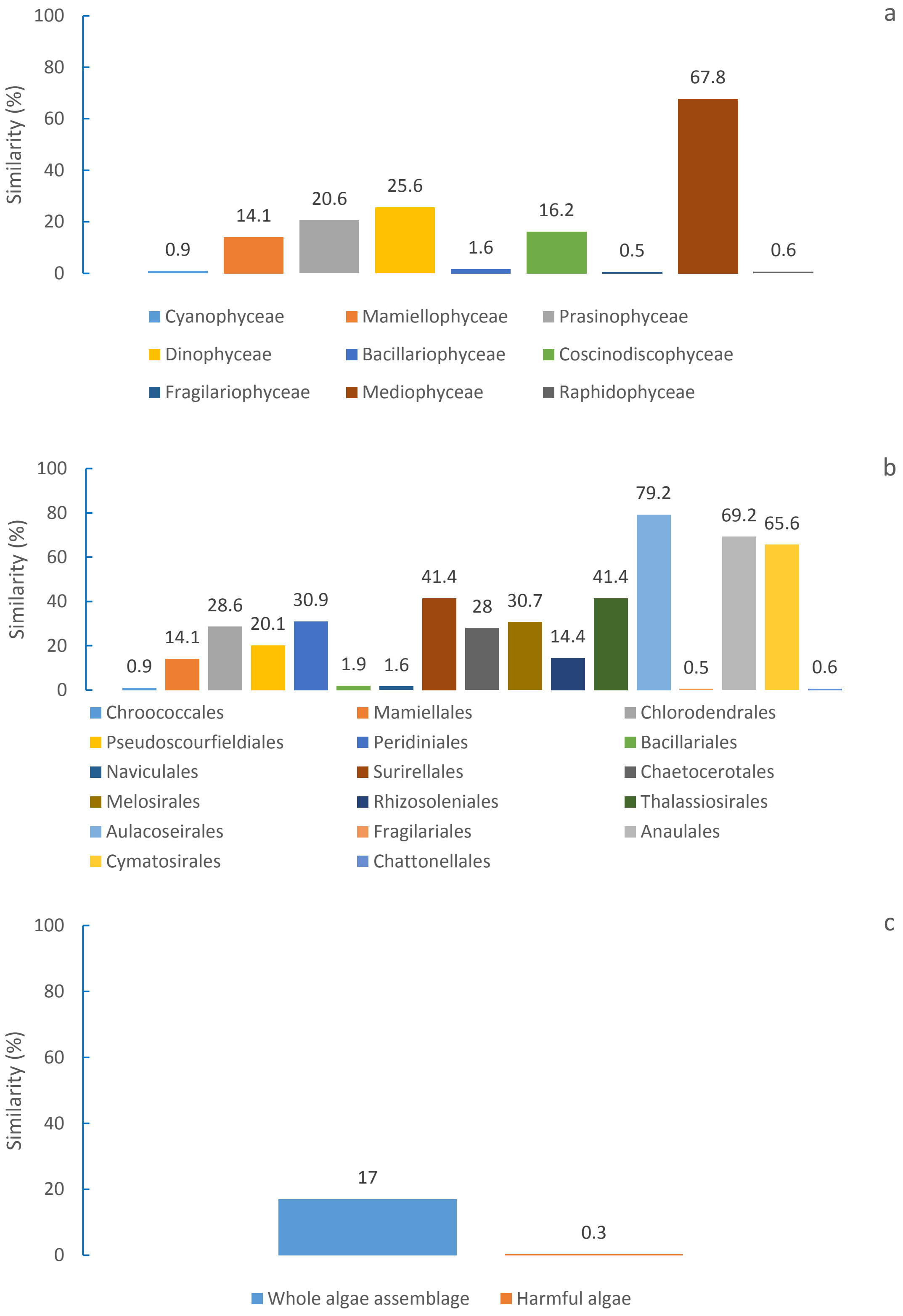

Fig. 9 Similarity between Hainan Island and Kagoshima Bay on class (a), order level (b) and Harmful algae (c) (based on species occurrence by metagenomic analysis in sediments, quantitatively).