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PLANKTONIC DINOFLAGELLATE GENUS *PROTOPERIDINIUM* BERGH 1881 IN VIETNAMESE WATERS

DISSERTATION SUMMARY

KHANH HOA – 2017

INTRODUCTION

Protoperidinium Bergh (1881) is one of the large genus of dinoflagellates, including heterotrophic species (Olseng et al. 2002) and widely distributed (Balech 1988). Species of *Protoperidinium* are important components of marine foodweb (Jakobsen and Hansen 1997). They could consume prey items as large or larger than themselves, and thus become competitors with mesozooplankton for food resources (Kjæret et al. 2000).

Most of the erlier studies of the genus *Protoperidinium* were on taxonomy. Among these, most literatures on identification of *Protoperidinium* were with line drawing illustration and incompleted species characteristics (e.g. Kofoid 1907; Lebour 1925; Paulsen 1931, Schiller 1935, 1937; Wood 1954). Several recent studies had used Scanning Electron Microscopy (SEM) to observe detail ornamentations of the surface of the plates (Dodge 1985, Taylor 1976, Yongshui & Jinming 1993). At the resent, molecular technique has provided an important tool in supporting classical morphological identification.

In Viet Nam, there are no recent studies solely on taxonomy and ecology of genus *Protoperidinium*, but mainly species composition were reported in different surveys of marine phytoplankton (e.g. Chu Van Thuoc et al., 1997; Boonyapiwat, 2001, Nguyen Tien Canh and Vu Minh Hao, 2001; Ho Van The and Nguyen Ngoc Lâm 2006, 2009).

With the gaps in recent taxonomy of *Protoperidinium* mentioned above, this present PhD research entitled "**Planktonic dinoflagellate genus *Protoperidinium* Bergh 1881 in Vietnamese waters"** aims to contribute to more complete description of the species *Protoperidinium* and species composition of marine dinoflagellate flora of Viet Nam, especially the genus *Protoperidinium*.

Objectives of the study:

Overal ojective:

Contribution to the research of dinoflagellate flora in Vietnamese waters.

Specific objectives:

- Contribute to detailed description and the completion of species composition of genus *Protoperidinium* in Vietnamese waters.
- Primary study on identification of dinoflagellate cysts, especially of *Protoperidinium* species.

Thesis content:

- Identification of dinoflagellate, the genus *Protoperidinium* Bergh 1881 based on morphological comparison.

- Temporal variation of species composition of *Protoperidinium* in Nha Trang Bay, Khanh Hoa province
- Morphology/identification of dinoflagellate cysts in sediment with emphasis on cysts of *Protoperidinium*.

Scientific and pratical significance:

Contribution on knowledge of species composition and geobiography of the genus *Protoperidinium* to plankton flora of Viet Nam.

CHAPTER 1. OVERVIEW

1.1. Introduction to genus *Protoperidinium*

Genus *Protoperidinium* includes species with varying sized (15-250 µm) and diversiform (spherical, pyriform to pentagonal) (Hoppenrath et al. 2009), and enveloped in the cellulose wall (theca) composed of a series of plates. The shape and arrangement of the plates as one of characteristics in the taxonomy (Balech 1974, 1980, 1988).

The identification of *Protoperidinium* species is based on the size, shape, presence of apical and/or antapical horns/spines, cingulum displacement, and particularly the pattern of the first apical (1') plate in ventral view and second intercalary plate (2a) in dorsal view of the epitheca. Position and shape of hypothecal pore in the first postcingular plate (1''') of sections *Paradivergentia* and *Pellucida*, as a stable taxonomic feature (Okolodkov 2008). Ornamentation of surface of the theca is one of characteristics also considered in the classification of *Protoperidinium*, the surface of plates is smooth or ornamented with reticulated, pores or reticulated with spines at the junctions (Evagelopouls 2002, Hoppenrath et al. 2009).

1.2. Morphology of resting cyst

Identification of dinodlagelate cysts based on a single morphological characteristic is not always reliable. Thus, general combination of several characteristics such as the type of archeopyle, morphology of ornaments, structure and color of wall, and paratabulation are indispensable (Matsuoka and Fukuyo 2000).

One of the main morphological characteristics used to identify dinoflagellate cyst is archeopyle type. They were divided into three types including: Saphopylic, Theropylic and Cryptopylic. Cryptopylic type subdivide into two types: Tremic and Chasmic (Masuoka and Fukuyo 2000).

1.3. Studies on *Protoperidinium* Bergh 1881 in the world.

1.3.1. Studies on classification

Most of the present *Protoperidinium* species had been formerly described as *Peridinium* Ehrenberg 1832 species. The importance of the morphology in identifying dinoflagellates was emphasized by Schütt (1895) and the genus *Peridinium* was divided by several workers into subgroups based on the cell outline and cingulum displacement (Chomérat and Couté 2008).

In 1902, Gran was first dividing genus *Peridinium* into two subgenera: *Protoperidinium* Bergh 1881 and *Euperidinium* Gran 1902, basing upon structures of antapical horns/spines and cingulum displacement.

Jörgensen (1912) divided *Peridinium* into two subgenera *Orthoperidinium* and *Metaperidinium,* using the shape of the first apical plate (1'). Subgenus *Orthoperidinium* was divided into three sections based on the shape of the second intercalary plate (2a) inlcuding *Tabulata, Conica* and *Oceanica*. Subgenus *Metaperidinium* was divided into four sections: *Pyriformia, Paraperidinium, Humilia* and *Divergens* (Graham 1942, Subrahmanyan 1971). Jörgensen (1912) erected the genus *Archaeperidinium* to incorporate species with only two apical intercalary plates and left those with three apical intercalary plates in the genus *Peridinium*.

Paulsen (1931) revised the system of Jörgensen (1912) and transferred genus *Archaeperidinum* to a subgenus of *Peridinium* (= *Protoperidinium*) with only two apical intercalary plates. This had been mentioned before by Lebour (1925) and some other scientists (Graham 1942, Subrahmanyan 1971).

Since the 1970s, studies on genus *Protoperidinium* Bergh 1881 were more or less focused by Balech (1974) and Abé (1981). Balech (1974) revived genus *Protoperidinium* Bergh (1881) by transferring heterotrophic marine species of *Peridinium* with four cingular (3c+t) and six sulcal (6s) plates to *Protoperidinium* and left *Peridinium* with autotrophic freshwater species, that have five or six cingular plates.

Yamaguchi et al. (2011) based on molecular phylogeny and morphological data was reinstated as a genus of Jörgensen 1912 from the section *Archaeperidinium*. This section was proposed by Taylor (1976) with *Archaeperidinium minutum* (Kofoid 1907) Jörgensen 1912 as type species.

One stable taxonomic feature is also very important, the hypothecal pore in the first postcingular (1'") plate. It was first decribed by Balech (1971) in *P. cruciferum.*

Previously, there were many studies on classification of order Peridiniales in many area around the world, in which genus *Peridinium* (*Protoperidinium*) was highly considered. There were very early research in North Sea by Lebour (1925), Antarctic (Balech 1947, 1958, 1973, 1976, 1988); Indian Ocean (e.g. Cleve 1900a, b; Böhm 1931; Matzenauer 1933; Silva 1956; Subrahamanyan 1971; Taylor 1976); Atlantic and Pacific e.g. Murray and Whitting (1899), Okamura (1906), Graham (1942), Balech

(1988), Abé (1927, 1936a, b, 1940, 1981), Böhm (1936), Konovalova (1998); Australia coastal (Wood 1954). However, most of these taxonomical literatures were with only line drawing illustration and incompleted characteristics of species.

1.3.2. Biology, ecology and distribution

Some studies in the laboratory and the observation of alive samples showed that *Protoperidinium* species consume their preys with a special mechanism, envelop the prey in a pseudopod, is called the "feeding the veil" or "pallium feeding". The digestion process occurs in this pseudopod (Jacobson and Anderson 1986, Gribble and Anderson 2006). Most *Protoperidinium* are very selective feeders, while few others may not be very selective in feeding and the growth rate is proportional to the variation of available diatoms and dinoflagellates (Gribble et al. 2007). Some other research study distribution of thecate and heterotrophic dinoflagellates in the different habitats (Jacobson 1987, Lessard and Murrell 1996, Kjaeret et al. 2000, and Nielsen Levinsen 2002), many species are discovered on a wide temperature range (Jacobson 1987).

In Southeast Asia waters, few studies were performed in the SEAFDEC suyveys on distribution, density and species composition of phytoplankton including *Protoperidinium* species (Boonyapiwat 1999a, b, 2000, 2001, 2005; Shamsudin et al. 1999a, b).

1.3.3. Studies on Dinoflagellates cysts

Dinoflagellates cysts in marine sediments have been studied in many area around the world: the US coast (Wall and Dale 1968, Merten et al. 2012), North Sea (Kawami et al. 2009), Japan (Matsuoka 1985, 1987; Matsuoka et al. 1982, 2003; Merten et al. 2015), Australia and New Zealand (Baldwin 1987), Tasmania (Bolch and Hallegraeff 1990), Korea (Li et al. 2015), China (Wang et al. 2004, Gu et al. 2015), South Africa (Joce et al. 2005), India (D'Silva et al. 2011, 2013), Baltic (Nehring 1994, 1997).

Cysts of *Protoperidinium* are diversified in morphology. Cysts taxonomy of *Protoperidinium* species was first proposed by Harland (1982). The author had emphasized the importance of alternating panels, which essentially corresponds to the opened shape "archeopyle" of *Protoperidinium* species.

Some later research combined with molecular phylogeny analysis, and cysts–theca relationship of *Protoperidinium* species have demonstrated and the detection of new species (Yamaguchi et al. 2011; Chomérat and Couté 2008; Kawami and Matsuoka 2009; Kawami et al. 2009; Liu et al. 2013; Matsuoka and Kawami 2013; Sarai et al. 2013; Mertens et al. 2012, 2013, 2015; Gu et al. 2015; Liu et al. 2015).

In Southeast Asia waters, some research on distribution of dinoflagellates cysts in surface sediment have performed in: Thailand, Eastern of Peninsular Malaysia, Brunei and Sabah, Philippine and Eastern of Indonesia (Lirdwitayaprasit 1999).

1.4. Study about *Protoperidinium* Bergh 1881 in Viet Nam

1.4.1. Studies on classification

The earliest research on phytoplankton in Vietnamese waters was done by Rose (1926), where 3 *Peridinium* species had listed, included *Peridinium divergens* Ehrenberg, *P. globulus* Stein and *P. oceanicum* Vanhöffen. Dawydoff (1936) also recorded the presence of *Peridinium* species.

Hoang Quoc Truong (1961) has preliminary compiled the studies about phytoplankton in Nha Trang Bay started from study of Rose (1926). In 1963, Hoang Quoc Truong had taken a survey of phytoplankton in Nha Trang Bay and reoported a lists of 14/15 *Peridinium* (=Protoperidinium) species. Shirota (1966) had studied phytoplankton in South of Viet Nam, and reported 13 *Peridinium* species with line drawing illutration and size but no description.

The studies on distribution, density and species composition of phytoplankton in Vietnamese waters during the SEAFDEC surveys were published by few authors. Nguyen Tien Canh and Vu Minh Hao (2001) reported 508 phytoplankton taxa, including 39 *Peridinium* (=*Protoperidinium*) species. Boonyapiwat (2001) also provided a list of 36 *Protoperidinium* species. Ton That Phap et al. (2001) had given a taxonomic key for 30 species and subspecies of *Protoperidinium* and 7 unidentified species in Tam Giang – Cau Hai lagoon. In 2009, Ton That Phap et al. had described and illustrated 30 species of *Protoperidinium* in Tam Giang – Cau Hai lagoon, Thua Thien Hue Province.

In more resent years, many cooperative research projects on phytoplankton in different waters of Viet Nam has been performed such as Viet Nam – Philippine Cooperation research (2007) in Paracel Islands; KC.09.03/06-10 Ninh Thuan, Binh Thuan waters; SAREC project (2006), Viet Nam – Germany (2009, 2010), CLIMEEViet (2009-2010), etc. However, only some common species of *Protoperidinium* has been recorded in phytoplankton lists (Nguyen Thi Mai Anh and Ho Van The 2001; Nguyen Ngoc Lam et al. 2002, 2006; Doan Nhu Hai and Nguyen Ngoc Lam 2008; Ho Van The and Nguyen Ngoc Lam 2005, 2006, 2009).

1.4.2. Study about dinoflagellates static cysts

In Viet Nam, until now, there was only one study on composition of dinoflagellates cysts by Doan Nhu Hai and Nguyen Ngoc Lam (2002) from sediment samples in Cam Ranh Bay. There were 25 different types of cysts has been recorded, in which 5 types of *Protoperidinium* cysts has been recorded and illustrated but no description.

CHAPTER 2. MATERIAL AND METHODS

2.1. Study subject

- Species composition of genus *Protoperidinium* Bergh 1881 in Vietnamese waters.

- Dinoflagellate cysts in sediment with emphasys on cysts of *Protoperidinium*.

2.2. Study sites and sampling periods

- Phytoplankton samples were collected in Vietnamese waters from different national and international cooretation project. Samples were stored in laboratory of the Derpartment of Marine phytoplankon, institute of Oceanography. There were about 100 samples were additional collected from Nha Trang bay, Ben Tre and Con Dao islands during this study.

- Sediment samples were collected at three location in the South Central Coast: Vung Ro bay, Phu Yen (5 samples), Nha Phu lagoon, Khanh Hoa (1 sample) and Ninh Thuan coastal waters (2 samples).

2.3. Methods

2.3.1. Sampling method

2.3.1.1. Phytoplankton samples

- Qualitative samples of phytoplankton were vertically haulled from near bottom to surface by using a 30 cm conical net with 20 µm mesh-size. Samples were fixed with formaldehyde to a final concentration of 4%.

- Quantitate samples of *Protoperidinium* were collected in Cua Be river estuary, in May/2012, by using Niskin bottle at both surface and near bottom layes. Sampling was at every 2 hours for 24 hours. Samples were fixed in neutral Lugol's solution

2.3.1.2. Sediment samples

- Sediment samples were collected by diving method, using plastic tubes (PVC) with 90 mm diameter to collect 20-30 surface sediment. All sediment cores were kept in vertical position, cool and dark condition until analysis.

2.3.2. Sample Analysis method

2.3.2.1. Identification of Protoperidinium *species*

- Observations of plate pattern were made using Calcofluor White M2R (Fritz & Triemer 1985). The samples were examined with an epifluorescence Leica LDMB microscope with phase contrast and differential interference contrast and fluorescent optics. A digital camera (Olympus DP-71) was used for photography.

- For examination under SEM. One drop of the sample was placed on a 5 µm carbon membrane filter, rinsed in distilled water to remove salt, dehydrated through an ethanol series 10, 30, 50, 70, 90 %, and the end with 99,99%, air-dried, the filter mounted on an aluminum stub with carbon tape, and finally coated with gold by E-

1045 (Hitachi, Nhật Bản). The stubs were examined on a Hitachi FM-SEM (Field Emission-Scanning Electron Microscope) model S4800 at National Institute of Hygiene and Epidemiology (NIHE.), Ha Noi, Viet Nam, VEGA3 TESCAN Optics Scanning Electron Microscope at the Leibniz Centre for Tropical Marine Ecology (ZMT), Bremen, German; and a JEOL 6510LV scanning electron microscope at the Center of Electron Microscopy of the Papanin's Institute for Biology of Inland Water, Russian Academy of Sciences (RAS).

Genus and subgenus of *Protoperidinium* were classified based on Balech (1974). Identification of *Protoperidinium* species was based on key literatures of Abé (1927, 1936a, b, 1940, 1981), Schiller (1935, 1937), Wood (1954), Subrahmanian (1971), Taylor (1976), Balech (1971, 1988), Steidinger and Tangen (1997), Okolodkov (2008) and Hoppenrath et al. (2009). The information on species were updated from Gómez (2005, 2012) and Guiry (in AlgaeBase, 2016).

2.3.2.2. Quantitative analysis of Protoperidinium

The *Protoperidinium* cells were counted by using Utermöhl method (1958). The steps were performed according to the description of a modified method in Larsen and Nguyen (2004).

2.3.2.3. Qualitative analysis of resting cysts

Cysts were separated from the sediment samples following method in Bolch (1997). Morphology of cysts were observed under a light microscope, particularly shape of archeopyle, color and ornamentation of the cyst wall.

Identification of the cysts was based on some key literatures including Wall and Dale (1968), Matsuoka (1987), Bolch and Hallegraeff (1990), Kawami and Matsuoka (2009), Liu et al. (2015) and Gu et al. (2015).

2.3.3. Data analysis

Pearson correlation (r) was analysed using software IBM SPSS Statistics Standard 20.0. The correlation coefficient r ranges from −1 to 1.

Sorensen index (S) was used for comparing the similarity species composition of *Protoperidinium* between research areas:

$$S = \frac{2c}{(a + b)}$$

Where: - a is the total of species in area A; - b is the total of species in area B; - c is the total of species shared by the area A and B.

The Sorensen index ranges from 0 to 1.

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Species composition of *Protoperidinium* in Vietnamese waters

3.1.1. Species list of genus Protoperidinium

A total 86 taxa belong to genus *Protoperidinium* were identified in Vietnamese waters, among them 72 species, 6 variety, 3 form and 5 unidentified species (sp.) including 3 subgenus: *Minusculum* (1 species), *Archaeperidinium* (9 species and 1 variety) and *Protoperidinium* (62 species, 5 variety, 3 form and 5 unidentified species) (Table. 3.1). Subgenus *Minusculum* is new record for marine phytoplankton of Viet Nam with one species new record for Asia Pacific (*Protoperidinium anomaloplaxum*).

Among them, one new species to science is *Protoperidinium larsenii* L. Phan-Tan, L. Nguyen-Ngoc, and H. Doan-Nhu 2016; 43 taxa (4 variety, 2 form) and 5 unidentified species are new record for dinoflagellate flora of Viet Nam (Table. 3.1). All the taxa found is presented in this thesis are described in detail and are illustrated with light microscope LM and scanning eletron microscope photographs. Among of 5 unidentified species, 2 species with plates 1' and 2a corresponding to ortho-penta types and 1 species with ortho-quadra types belong to section *Oceanica*, 1 species with ortho-quadra types belong to section *Conica* of *Orthoperidinium* group*;* and 1 species with para-hexa types belong to section *Paradivergentia* of *Metaperidinium* group.

Moreover, the morphology of "hypothecal pore" in the first postcingular plate (1''') was identified and described in detail of 11 taxa, of which 6 taxa were new recordes that possessed the hypothecal pore in the 1''' plate, including: *Protoperidinium gibberum, P. inclinatum, P. inflatiforme, P. nipponicum, P. solidicorne* var. *makronyx* and *Protoperidinium* sp. 5 (para-hexa) and 5 taxa were known possessed the hypothecal pore including: *P. longipes, P. ovum, P. pellucidum, P. schilleri* and *P. solidicorne*.

Table 3.1. Species composition of genus *Protoperidinium* in Vietnamese waters.

Ord.	Taxa	Ord.	Taxa
1	**Protoperidinium anomaloplaxum* (Bal. 1964) Balech 1974	44	**P. symmetricum* (Halim 1967) Balech 1974
2	**P. abei* (Paulsen 1930) Balech 1974	45	**P. thulesense* (Balech 1958) Balech 1973
3	**P. abei* var. *rotundata* (Abé 1936) Taylor 1976	46	**P. tohrui* (Abe 1981) Balech 1994
4	**P.* cf. *planiceps* (Abé 1981) Balech 1988	47	**Protoperidinium* sp. 4 (ortho-quadra)
5	**P. compressum* (Abé 1927) Balech 1974	48	**P. balechii* (Akselman 1972) Balech 1988
6	**P. excentricum* (Paulsen 1907) Balech 1974	49	**P. humile* (Schiller 1937) Balech 1974
7	**P. latum* Paulsen 1908	50	**P. nudum* (Meunier 1919) Balech 1974
8	**P. nux* (Schiller 1937) Balech 1974	51	**P. punctulatum* (Paulsen 1907) Balech 1974
9	**P. stellatum* (D. Wall 1968) Balech 1994	52	**P. angusticollum* Abe 1981
10	**P. thorianum* (Paulsen 1905) Balech 1973	53	**P. latispinum* (Mangin 1926) Balech 1974
11	**P. ventricum* (Abé 1927) Balech 1974	54	**P. quarnerense* (Schröder 1900) Balech 1974
12	**P. claudicans* (Paulsen 1907) Balech 1974	55	**P. steinii* (Jørgensen 1899) Balech 1974
13	**P. depressum* (Bailey 1854) Balech 1974	56	**P. yonedai* (Abé 1981) Balech 1994
14	**P. depressum* var. *claudicanoides* (Graham 1942) Taylor 1976	57	**P. globiferum* (Abé 1981) Balech 1994
15	**P. larsenii* L. Phan-Tan, L. Nguyen-Ngoc, & H. Doan-Nhu 2016	58	**P. globulus* (Stein 1883) Balech 1974

Ord.	Taxa	Ord.	Taxa
16	**P. murrayi* (Kofoid 1907) Hernández-Becerril 1991	59	**P. hamatum* Balech 1979
17	**P. oceanicum* (Vanhöffen 1897) Balech 1974	60	**P. majus* (P.-A. Dangeard 1927) Balech 1974
18	**P. oceanicum* (Vanhöffen 1897) var. *typica* Bhöm 1936	61	**P. simulum* (Paulsen 1931) Balech 1974
29	**P. oceanicum* f. *bisintercalares* Graham 1942	62	**P. sphaericum* (Murray & Whitting 1899) Balech 1974
20	**P. oceanicum* var. *tenellum* Graham 1942	63	**P. sphaeroides* (Dangeard 1927) Balech 1974
21	**P. paraoblongum* Sarai, Yamaguchi, Kawami & Matsuoka 2013	64	**P. acutipes* (P.-A. Dangeard 1927) Balech 1974
22	**P. quadrioblongum* Sarai,Yamaguchi, Kawami & Matsuoka 2013	65	**P. brochii* (Kofoid &Swezy 1921) Balech 1974
23	**P. venustum* (Matzenauer 1933) Balech 1974	66	**P. claudum* Balech 1994
24	**P. venustum* var. *facetum* Balech 1988	67	**P. crassipes* (Kofoid 1907) Balech 1974
25	**Protoperidinium* sp. 1 (ortho-penta)	68	**P. curtipes* f. *asymmetricum* Matzenauer1933
26	**Protoperidinium* sp. 2 (ortho-quadra)	69	**P. divergens* (Ehrenberg 1841) Balech 1974
27	**Protoperidinium* sp. 3 (ortho-penta)	70	**P. elegans* (Cleve 1900) Balech 1974
28	**P. achromaticum* (Levander 1902) Balech 1974	71	**P. fatulipes* (Kofoid 1907) Balech 1974
29	**P. biconicum* (P.-A. Dangeard 1927) Balech 1974	72	**P. grahamii* (Sournia1973) Balech 1994
30	**P. conicum* (Gran 1900) Balech 1974	73	**P. grande* (Kofoid 1907) Balech 1974
31	**P. conicum* f. *asamushii* Abe 1927	74	**P. remotum* (Karsten 1907) Balech 1974
32	**P. decollatum* (Balech 1971) Balech 1974	75	**P. subcrassipes* Balech 1988
33	**P. divaricatum* (Meunier 1919) Balech 1988	76	**P. inflatiforme* (Bohm 1936) Balech 1974
34	**P. expansum* Abé 1981	77	**P. solidicorne* (Mangin 1926) Balech 1974
35	**P. laciniosum* Balech 1994	78	**P. solidicorne* var. *makronyx* Schiller 1929
36	**P. latissimum* (Kofoid 1907) Balech 1974	79	**Protoperidinium* sp. 5 (para-hexa)
37	**P. leonis* (Pavillard 1916) Balech 1974	80	**P. gibberum* (Abé 1981) Balech 1994
38	**P. obtusum* (Karsten 1906) Parke & Dodge 1976	81	**P. inclinatum* (Balech 1964) Balech 1974
39	**P. pentagonum* (Gran 1902) Balech 1974	82	**P. longipes* Balech 1974
40	**P. persicum* Schiller 1937	83	**P. nipponicum* (Abe 1927) Balech 1974
41	**P. rhombiforme* (Abé 1981) Balech 1994	84	**P. ovum* (Schiller 1911) Balech 1974
42	**P. sinuosum* Lemmermann 1905	85	**P. pellucidum* Bergh 1881
43	**P. subinerme* (Paulsen 1904) Loeblich III 1970	86	**P. schilleri* (Paulsen 1931) Balech 1974

Note: * the taxa are new record for the dinoflagellate flora of Vietnamese waters.

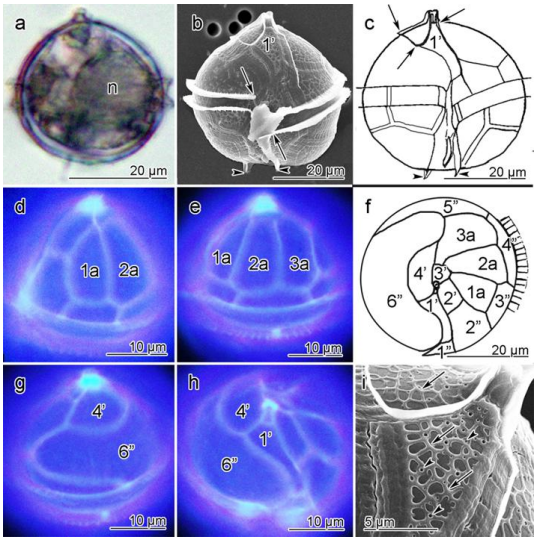
Illutration of a few represent *Protoperidinium* species

Subgenus *Minusculum* (Lebour 1925) Balech 1974

P. anomaloplaxum (Balech 1964) Balech 1974

Basionym: *Peridinium anomaloplaxum* Balech 1964.

References: Balech 1964: p. 28, pl. III (34-46); Balech 1988: p. 82, pl. 22, figs 9, 11-13; Jardim and Cardoso 2013: p. 634, Figs 1H-I.



***Protoperidinium anomaloplaxum*:** - a: cell in left lateral view with nucleus (n) in dorsal; - b and c: the narrow plate 1', ascending cingulum (arrows, fig. b), a right antapical spine and a left sulcal list (arrowheads), membrane of apical (arrows, fig. c); - d and e: intercalary plates; - f: epithecal plates; - g and h: pattern of plates 1', 4' and 6"; - i: reticulation (arrows) and pores (arrowheads). Figs a, d, e, g, and h (LM), b and i (SEM), and c and f (line drawing).

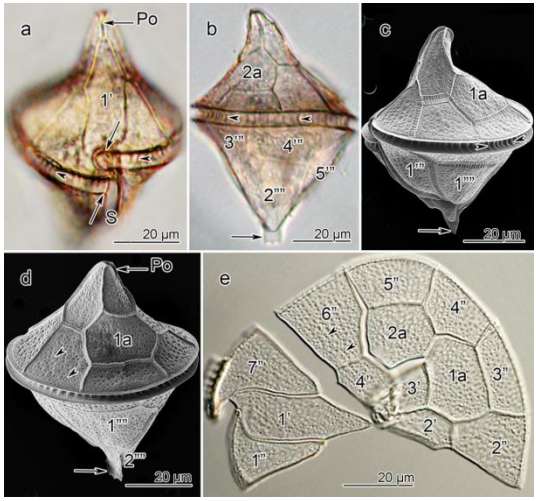
Subgenus *Archaeperidinium* (Jörgensen 1912) Balech 1974

***P. abei* (Paulsen 1931) Balech 1974**

Basionym:*Peridinium abei* Paulsen 1931.

Synonyms: *Peridinium biconicum* Abé 1927, (không phải *Peridinium biconicum* P.-A. Dangeard 1927).

References:Paulsen 1931: p. 73; Schiller 1935:p.138, figs 136a-h; Abé 1927: p. 416, figs 34A-H; Abé 1936a: p. 667, figs 52-61; Wood 1954: p. 229, fig. 91; Subrahmanyan 1971: p. 25, pl. 9, figs 1-8; Balech 1974: p. 54; Taylor 1976: p. 137, pl. 33, figs 363, 366, pl. 45, figs 521a-c; Okolodkov 2008: p. 105, pl. 1, figs 9-12.

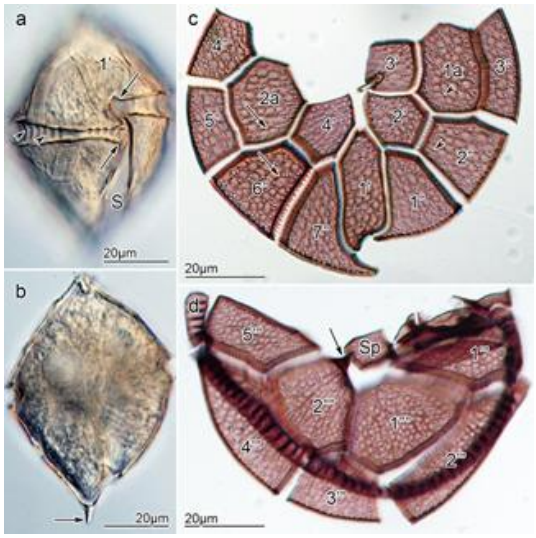


***Protoperidinium abei*:** - a: cell in ventral view with apical pore, descending cingulum (arrow), longitudinal ribs (arrowheads, also in figs b and c), and narrow sulcus (S); - b: narrow antapical spine (arrow, also in fig. d); - c: pointed antapical spine (arrow); - d: apical pore (Po), plate 1a, antapical spine and indentations and pores (arrowheads, also in fig. e); - e: epithecal plates. Figs a, b and e (LM), figs c and d (SEM).

***P. abei* var. *rotundata* (Abé 1936) Taylor 1976**

Basionym:*Peridinium abei* f. *rotundata* Abé 1936.

References: Abé 1936a: p. 667, figs 56-58; Taylor 1976: p. 137; Liu et al. 2015: p. 9, figs 49-55.

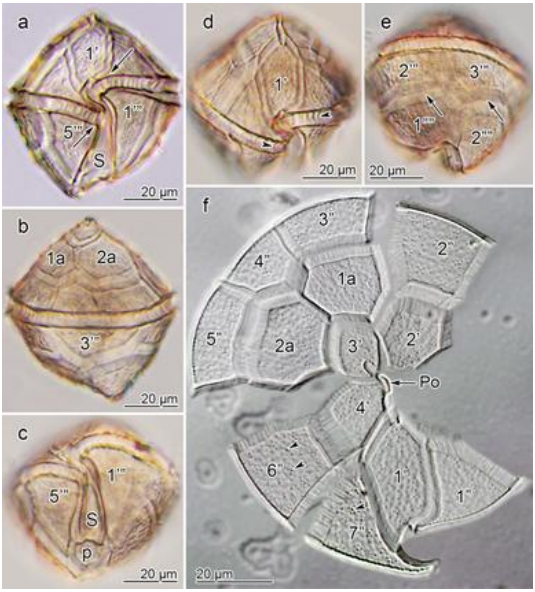


Protoperidinium abei* var. *rotundata: - a and b: cell in ventral view, descending cingulum (arrows) and longitudinal ribs (arrowheads), sulcus (S), right antapical spine (arrow, fig. b); - c: epithecal plates and ornamentations (arrow) and pores (arrowheads); - d: cingular plates, hypothecal plates, plate Sp and antapical spine on plate 2'''' (arrow). Figs a-d (LM).

P. cf. *planiceps* (Abé 1981) Balech 1988

Synonyms: *Peridinium thorianum* var. *planiceps* Abé 1981, *Protoperidinium* sp. aff. *P. thorianum* Balech 1988.

References: Abé 1981: p. 301, fig 40b (273-275); Balech, 1988: p. 84, pl. 20, figs 6-8; Gómez 2005: p. 202, tab. 1; Gómez 2012: p. 106, tab. 2.



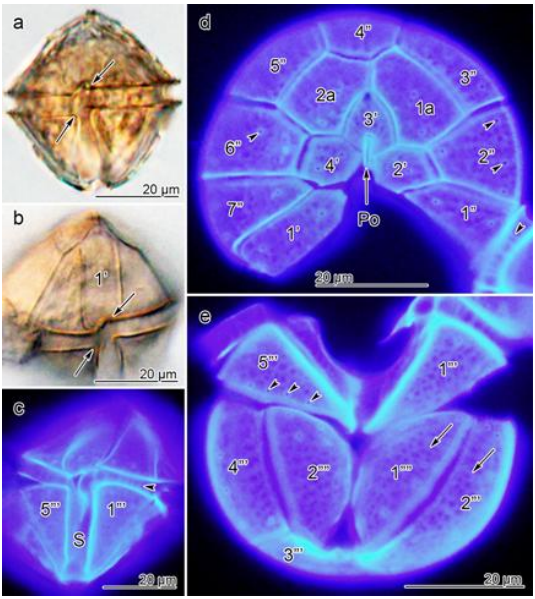
***Protoperidinium* cf. *planiceps*:** - a, c, and d: cell in ventral view, pattern of plate 1', descending cingulum (arrows), longitudinal ribs (arrowheads), sulcus (S), and plate Sp (p); - b and e: hypotheca in dorsal view with wide sutures (arrows); - f: epithecal plates with apical pote (Po), and ornamentations with pores (arrowheads). Figs a-f (LM).

Protoperidinium nux (Schiller 1937) Balech 1974

Basionym: *Peridinium nux* Schiller 1937.

Synonyms: *Peridinium levanderi* Abé 1927, *Peridinium aequilimbus* Abé 1981.

References: Abé 1927: p. 413, figs 32A-H, 1981: p. 302; Schiller 1937: p. 140, fig. 138; Subrahmanyan 1971: p. 27, figs 13-15; Balech 1974: p. 55.



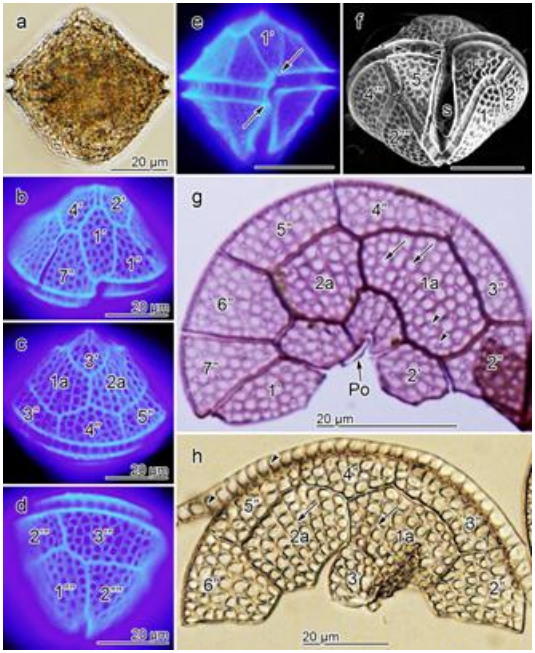
Protoperidinium nux: - a: cell is wide rhomboidal, convex sides, descending cingulum (arrows, figs a and b), and the right of cell slightly longer than the left one; - b: plate 1'; - c: sulcus (S), and longitudinal ribs (arrowheads); - d: epithecal plates, apical pore (Po), and large pore (arrowheads, figs d and e); - e: hypothecal plates, reticulations (arrows). Figs a-e (LM).

***P. thorianum* (Paulsen 1905) Balech 1973**

Basionym: *P. thorianum* Paulsen 1905.

Synonyms: *Properidinium thorianum* Meunier 1919.

References: Paulsen 1905: p. 3, figs 1a, b; Paulsen 1908: p. 62, fig. 81; Abé 1936a: p. 649, figs 1-15; Balech 1973: p. 347, pl. 1, figs 1-18; Steidinger and Tangen 1997: p. 546, pl. 53; Throndsen et al. 2007: p. 105; Okolodkov 2008: p. 107, pl. 2, figs 1-4; Hoppenrath et al. 2009: p. 148, figs 59m-o, q-s

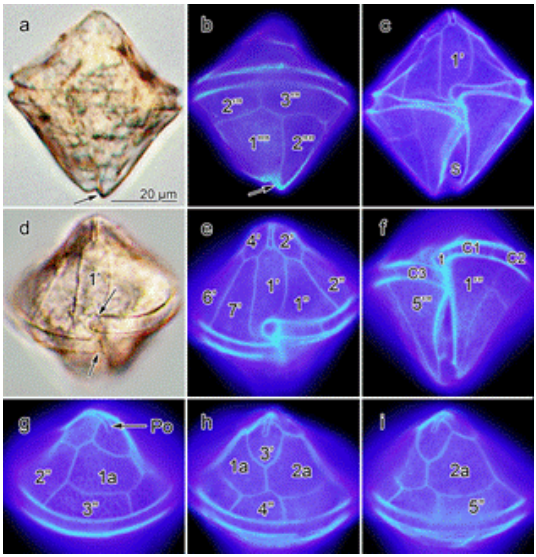


***Protoperidinium thorianum*:** - a: cell in dorsal view; - b: plate 1'; - c and d: pattern plates in dorsal view; - e: descending cingulum (arrows); - f: sulcus (S), the areolation of the thecal surface, cingular plates with longitudinal ribs (arrowheads, also in fig. h); - g and h: epithecal plates with reticulation (arrows), and small pore in junctions (arrowheads, fig. g), apical pore (Po) (fig. g). Figs a-e, g, and h (LM), f (SEM).

***P. ventricum* (Abé 1927) Balech 1974**

Basionym: *Peridinium ventricum* Abé 1927.

References: Abé 1927: p. 418, figs 35A-G, 1981: p. 302; Schiller 1937: p. 143, figs 144a-e; Wood 1954: p. 229, figs 93a-b; Subrahmanyan 1971: p. 25, pl. 8, figs 8-12; Balech 1974: p. 55; Taylor 1976: p. 137, pl. 45, figs 521a-c; Al-Kandari et al. 2009: p. 186, pl. 35 H-I, pl. 36 A-C.



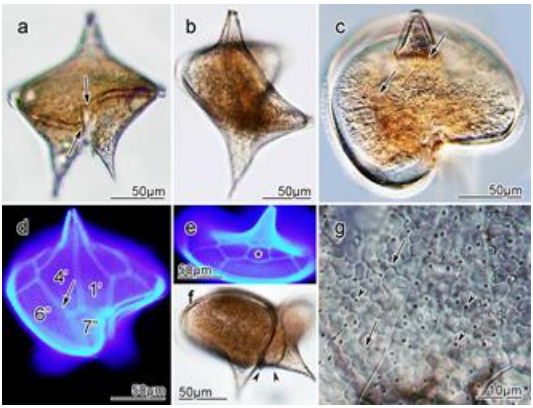
***Protoperidinium ventricum*:** - a and b: asymmetrical cell in dorsal view (arrow); - c-e: plate 1' and sulcus (S), descending cingulum (arrows); - f: cingular plates, plates 1''', 5''' and sulcal plate; - g-i: apical pore (Po) and epithecal plates in dorsal view. Figs a-i (LM). Scale bars in fig. a applies to figs a-i.

Subgenus *Protoperidinium* (Gran 1902) Balech 1974

***P. depressum* (Bailey 1854) Balech 1974**

Basionym: *Peridinium depressum* Bailey 1854.

References: Bailey 1854: p. 12, figs 33, 34; Balech 1974: p. 57; Steidinger and Tangen 1997: p. 538, pl. 52; Evagelopoulos 2002: p. 42, figs 2-3, 34; Okolodkov 2008: p. 118, pl. 6, figs 1-3; Al-Kandari et al. 2009: p. 180, pl. 25A-J; Hoppenrath et al. 2009: p. 151, figs 61d-h.

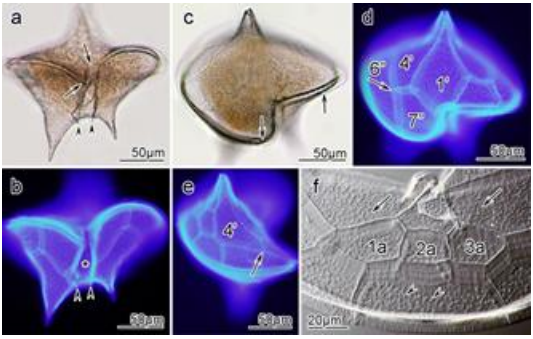


***Protoperidinium depressum*:** - a: cell with the descending cingulum (arrows); - b: cell in right lateral view showing the compressed theca and oblique cingulum; - c: the fibres of the protoplasm; - d: the 1' plate and the 6" plate connecting to both 7" and 4' with sutures that form an almost straight line (arrow); - e: the trapeziform 2a plate: - f: the deep sulcus, which becomes slightly wider posteriorly and with saw-edged sulcal lists (arrowheads): - g: surface of theca is reticulation with spiny junctions (arrows), and pores (arrowheads). Figs a-g (LM).

***P. depressum* var. *claudicanoides* (Graham 1942) Taylor 1976**

Basionym: *Peridinium claudicanoides* Graham 1942.

References: Graham 1942: p. 24, figs 29A-H; Taylor 1976: p. 161, pl. 45, fig 256.



***Protoperidinium depressum* var. *claudicanoides*:** - a and b: right antapical horn is larger than the left one, narrow cingulum is descending (arrows), the deep sulcus bears lists (arrowheads); - c: the left arc of the cingulum bending in the apical direction, whereas the right arc bends in the antapical direction (arrows); - d: the wide 1' plate, and the 6" plate connecting to the 7" and 4' with sutures that form an almost straight line (arrow, in Fig. e also); - e: cell in right lateral view showing compressed theca; - f: intercalary plates, reticulation of the theca with short spines at junctures (arrows), and pores (arrowheads). Figs a-f (LM).

***Protoperidinium larsenii* L. Phan-Tan, L. Nguyen-Ngoc, H. Doan-Nhu 2016**

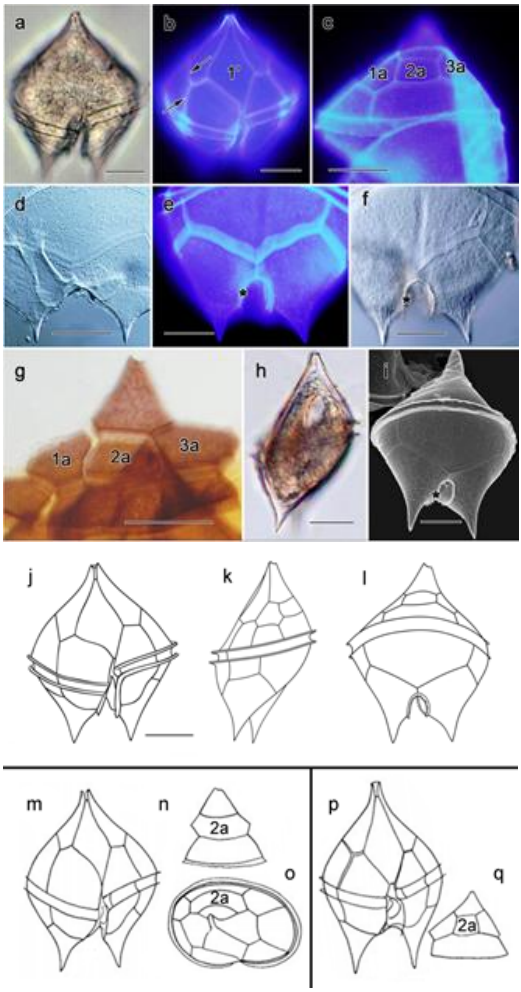
References: Balech 1951: p. 314, pl. 4, p. 317, pl. 5.

Mô tả: Medium-sized cells, pear-shaped body (figs a and j), length 77-87 µm, width 55-67 µm and depth 37-43 µm (Hình a, c, i, and j). The conical epitheca is drawn into short apical horn (figs a, b, i, and j). Plate 1' is asymmetrical rhomboid, expands

towards the right (figs b, e, and j). Plate 2a is trapezoidal (figs d, g, and l). The sutures of the 6" plate connecting to the 4' and 7'' plates form an almost straight line (figs b, e and j). The cingulum descending with about 1.8-2.0 girdle widths (figs a-b, e, and j). The hypotheca is drawn into two short pointed conical horns and almost parallel (figs a, h-j) and small distance between them (figs a, i-j). The sulcus is deeply incised (figs a, f, and h-i). Area sulcus is bordered by a continuous saw-edged membrane (Hình f, h, and i). The thecal plates have fine reticulations with short tiny spines at the junctions of the reticulations, and pores (figs g and i).

Ecology and Distribution: *P. larsenii* was commonly found in the coastal waters of Rach Gia, both high and low tides, at 10° 0'26N, 104°56'20E. Water temperature and salinity were 29 oC and 26 psu respectively.

Discussion: About fifty cells of the new species were observed from net samples showing that P. larsenii is a distinctive species but it can be confused with *Peridiniium claudicans* Paulsen 1907 (in Balech 1951). Both species have ortho-quadra plate arrangement (figs j, l, p-q).



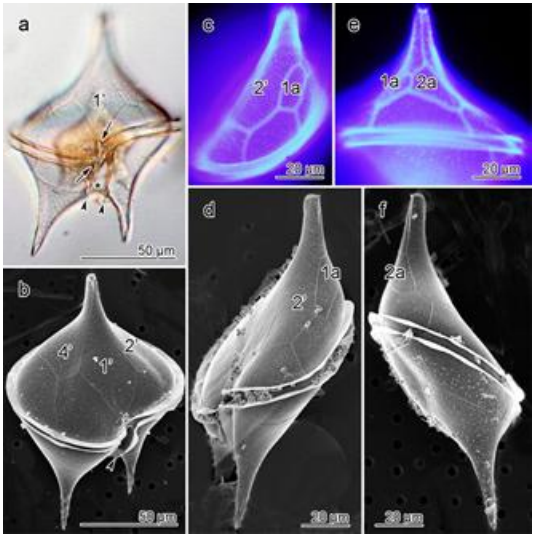
Figs a-i. *Protoperidinium larsenii*: - a and b: plate 1' is "ortho", descending cingulum, and the 6" plate with the straight sutures connecting to both 4' and 7" plates (arrows); - c and g: intercalary plates; - d-f: hypotheca in dorsal view, surface of plates with numerous pores (fig. d), saw-edged membrane posteriorly surrounding the sulcal area (asteriks in Figs e and f, in Fig. i also) and fainly reticulation of theca (fig. f, in fig. i also); - h: cell in left lateral view. Figs a-h (LM), fig. i (SEM). All Scale bars = 20 µm.

Figs j-l: *Protoperidinium larsenii*: line drawing in ventral view, cell in ventral view (fig. j), cell in left lateral view (fig. k) and dorsal view (fig. l). **Figs m-q:** line drawing of *Peridinium latidorsale* (Dangeard 1927) Balech 1951 (figs m-o) and *Peridinium claudicans* Paulsen 1907 (fig. p and q) (redrawing from Balech 1951), in ventral view (m and p), plate 2a (n and q), and apical view (o); both species have the angled sutures of 6" plate connecting to the 7" and 4' plates (arrows). Figs a-h (LM), fig. j (SEM), figs j-k (drawing by Photoshop CS6). All Scale bars = 20 µm.

***P. oceanicum* f. *bisintercalares* Graham 1942**

Basionym: *Peridinium oceanicum* f. *bisintercalares* Graham 1942.

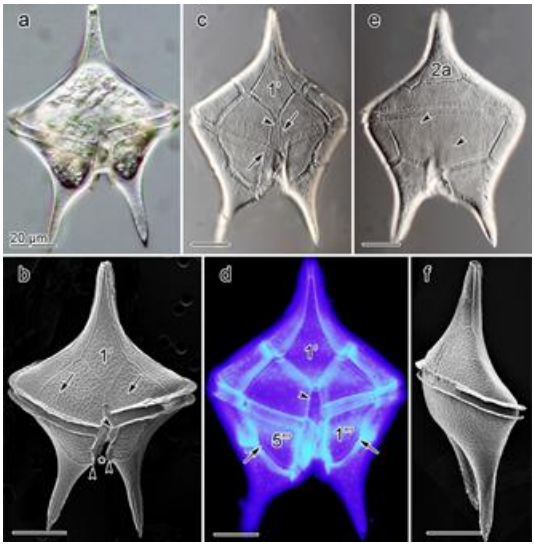
References: Graham 1942: p. 26, fig. 35A, B.



***Protoperidinium oceanicum* f. *bisintercalares*:** - a: descending cingulum (arrows), sulcus is deep, wide and slightly oblique (asterisk) with two sulcal lists (arrowheads, arrow in fig. b also); - b: plate 1' and cingular lists; - c and d: pattern plate 2' and 1a plate is quadrangular; - e: plate 1a is pentagonal, and 2a plate; - f: plate 2a and oblique cingulum. Figs a, c and e (LM), figs b, d and f (SEM).

***P. venustum* var. *facetum* Balech 1988**

References: Balech 1988: p. 188, pl. 84, figs 13-16; Ton That Phap et al. 2009: p. 78, figs 1.198(a-b).



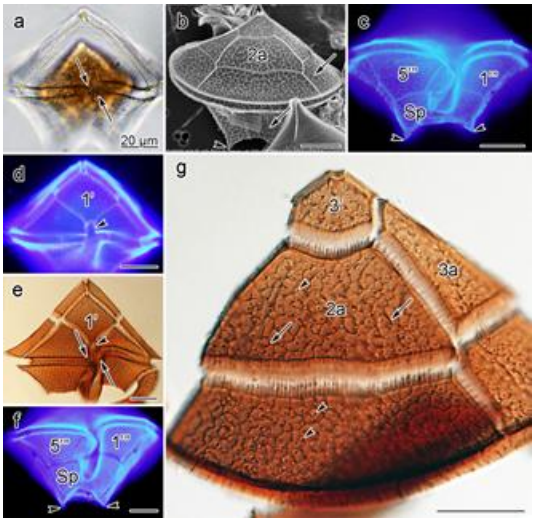
Protoperidinium venustum* var. *facetum: - a: outline of cell ; - b: plate 1', the deep sulcus (asterisk) with two lists continuous with the cingular lists (arrowheads), reticulation of theca with spine; - c: plate Sa (arrowhead, in fig. d also) and descending cingular (arrows); - d: showing the shape of the 1''' and 5''' plates with a brace-shape (arrows); - e: the trapezoidal 2a plate and thecal surface with pores (arrowheads); - f: cell in right lateral view showing dorso-ventrally compressed cell and oblique cingulum. Figs a, c-d (LM), figs b and f (SEM). All Scale bars = 20 µm.

***P. latissimum* (Kofoid 1907) Balech 1974**

Basionym: *Peridinium latissimum* Kofoid 1907.

Synonyms: *Peridinium pentagonoides* Balech 1949, *Peridinium pentagonum* var. *depressum* Abé 1927, *Protoperidinium pentagonoides* Balech 1949.

References: Kofoid 1907: p. 175, pl. 5, figs 31, 32; Abé 1927: p. 409, fig. 29; Balech 1974: p. 67; Taylor 1976:140, pl. 33, fig. 360; Balech 1988: p.116, pl. 27, figs 7-9, pl. 28, figs 1-2; Gómez 2012: p. 110, tab. 2; Guiry 2016.



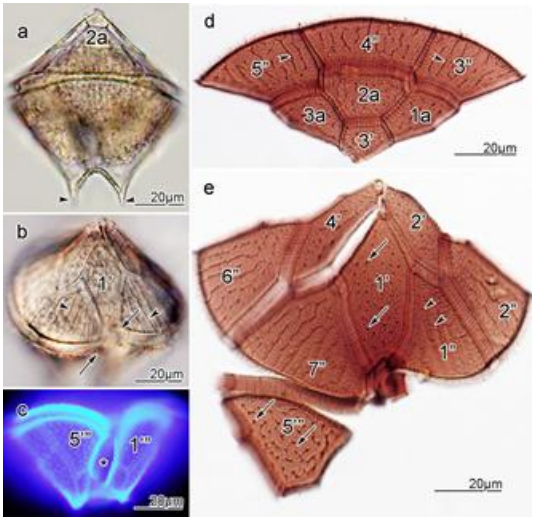
***Protoperidinium latissimum*:** a-d: cell with plate 1' is "para"-type. - a and d: plate 1' is "para"-type, descending cingulum (arrows); - b: plate 2a is "hexa"-type, two antapical horns ending spines (arrowhead, in fig. c also), surface of theca with reticulations (arrow, in fig. g also); - c: plate Sp; - d: plates 1' para-type and Sa (arrowhead); - e-g: cell with plate 1' "meta"-type. - e: plate 1' "meta"-type, descending cingulum and curve on the left (arrows), plate Sp (arrowhead); - f: plate Sp, two antapical horns ending spines (arrowheads); - g: plate 2a and numerous pores (arrowhead) and reticulation of theca with spiny junctions (arrows). Figs a, c-g (LM), fig. b (SEM). All scale bars = 20 µm.

***P. leonis* (Pavillard 1916) Balech 1974**

Basionym: *Peridinium leonis* Pavillard 1916

Synonym: *Peridinium saltans* Pavillard 1915

References: Pavillard 1916: p. 32, fig. 6; Lebour 1925: p. 112, pl. 21, figs 1a-d; Matzenauer 1933: p. 456, figs 29a-c; Wood 1954: p. 251, figs 148a-c; Abé 1981: p. 384, fig. 58 (398-404); Steidinger and Tangen 1997: p. 540, fig. 52; Throndsen et al. 2007: p. 98

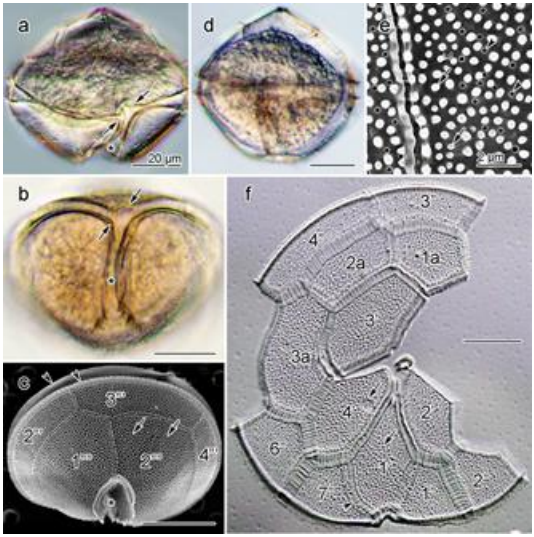


***Protoperidinium leonis*:** - a: cell with two antapical horns ending in strong spines (arrowheads), - b: plate 1', cingulum lightly descending (arrow), precingular plates are ornamented with undulating longitudinal ribs (arrowhead, in fig. d also); - c: the deep sulcus, inclined (asterisk); - d and e: showing plate pattern of epitheca plates, reticulation of theca with spiny junctions (arrows), and pores (arrowheads). Figs a-e (LM).

***P. punctulatum* (Paulsen 1907) Balech 1974**

Basionym: *Peridinium punctulatum* Paulsen 1907

References: Paulsen 1907: p.19, fig. 28; Paulsen 1908: p. 61, figs 79a-h; Schiller 1937: p. 245, figs 245a, b; Halim 1967: p. 746, pl. 9, fig. 128; Balech 1974: p. 58; Abé 1981: p. 352, fig. 49 (326-330); Hansen and Larsen 1992: p. 126, figs 4.75a-e; Steidinger and Tangen 1997: p. 545, pl. 51; Evagelopoulos 2002: p. 44, figs 12-14, 38-40; Hoppenrath et al. 2009: p. 162, fig. 65m; Al-Kandari et al. 2009: p. 184, pl. 32B-I.

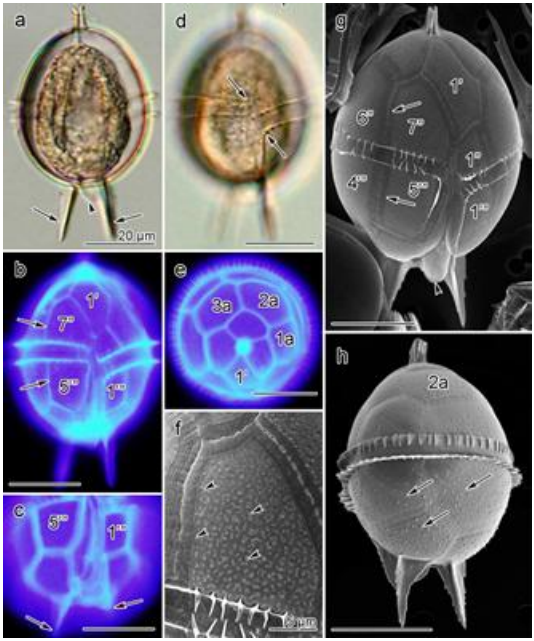


***Protoperidinium punctulatum*:** - a and b: descending cingulum (arrow), deep sulcus, narrow, oblique and extending to antapex (asterisk, also in Fig. c); - c: narrow cingular lists (arrowhaeds), the surface of the theca is bearing numerous punctuates (arrows, also in Fig. f) and a low membrane posteriorly surrounding the sulcal area; - d: showing outline of cell; - e: a part of theca numerous punctuates (arrow) and pores (arrowheads); - f: the 1' plate is asymmetrical (arrowhead). Figs a, b, d and f (LM), figs c and e (SEM). All scale bars = 20 µm.

***P. angusticollum* Abé 1981**

Basionym: *Peridinium angusticollis* Abé 1981

References: Abé 1981: p. 204, fig. 16 (95-99), Balech 1994: p. 63; Gómez 2005: p. 200, tab. 1; Gómez 2012: p. 109, tab. 2.



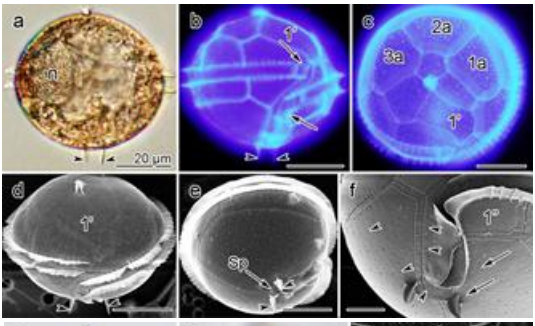
***Protoperidinium angusticollum*:** - a, b, d and g: cell in dorsal view. - a: narrow apical horn, long antapical spines (arrows) and list of Sd plate (arrowhead). - d: ascending cingulum. - b and g: plate 1', sutures between the plates 6" and 7" with 4"' and 5"' (arrows); - c: left antapical spine directed upward in comparison with the right one (arrow); - e: epithecal plates; - f: a part of theca with numerous indentations (arrowhead); - h: spiny at junctions of reticulation (arrow), antapical spines bears saw-edged membrane. Figs a-e (LM), figs f-h (SEM). Scale bar in fig. a is applies to figs b-e, g and h.

***P. quarnerense* (Schröder 1900) Balech 1974**

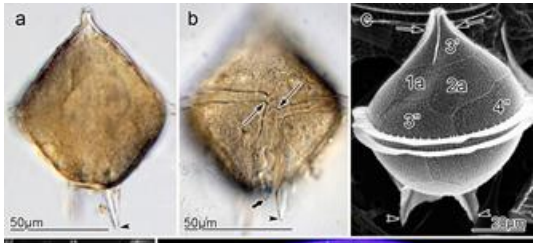
Basionym: *Peridinium globulus* var. *quarnerense* Schröder 1900.

Synonym: *Peridinium globulus* Stein 1883 (pl. 9, fig. 8), *Peridinium quarnerense* (Schröder 1900) Broch 1910.

Reference: Stein 1883: pl. 9, fig. 8, Balech 1974: p. 61; Abé 1940: p. 30, figs 4-7; Abé 1981: p. 196, fig. 14 (78-84);



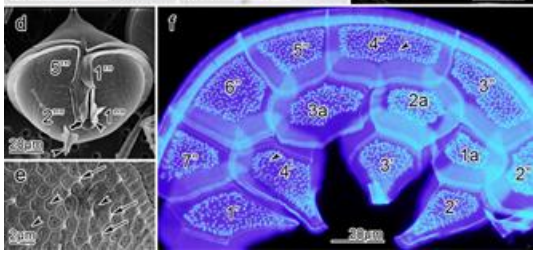
***Protoperidinium quarnerense*:** - a, b and d: cell in ventral view. - a: nucleus (n) on the right side, membrane surround apical pore and two antapical spines (arrowheads, figs a, b, d and e). - b: ascending cingulumand with overhang (arrows).- d: plate 1' and antapical spines (arrowheads); - c: epithecal plates; - e: plate Sp, membrances of spine (arrowheads); - f: list of Sd plate, plate Sp is V-shape, surface of theca with pores (arrowheads) and litle small pores (arrows). Figs a-c (LM), figs d-f (SEM). All scale bars = 20 µm.



***P. yonedai* (Abé 1981) Balech 1994**

Basionym: *Peridinium yonedai* Abé 1981

References: Abé 1981: p. 206, fig. 17 (103-109); Balech 1994: p. 64, 1999: p. 169, figs 20-24.

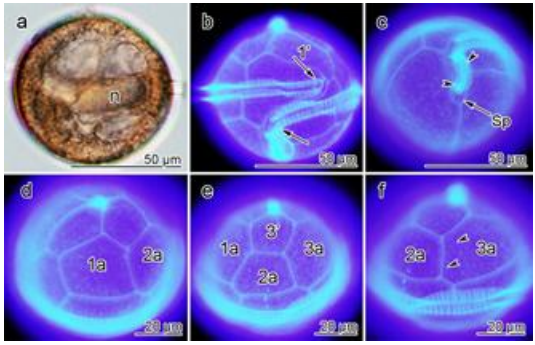


***Protoperidinium yonedai*:** - a, b and d: cell in ventral view, ascending (arrows), two asymmetrical antapical spine and bears saw-edged membrane and lists of Sd plate (thick arrow); - c: cell in dorsal view showing plates and lists of apical horn (arrows); - e: a part of theca is reticulated with spiny at junctions (arrows) and with a central pore (arrowheads); - f: epithecal plates and numerous potes (arrows). Figs a, b and f (LM), figs c-e (SEM).

***P. globiferum* (Abé 1981) Balech 1994**

Basionym: *Peridinium globifera* Abé 1981

References: Abé 1940: p.29, figs 2, 3; Abé 1981: p.195, fig.13a (72-77); Balech 1994: p.64;



***Protoperidinium globiferum*:** - a: cell with nucleus (n), - b: plate 1', ascending cingulum with overhang (arrow), sulcus of curve, - c: posterior sulcus with lists (arrow), and plate Sp is V-shape; - c-f: showing pattern plates 1a, 2a and 3a, numerous dots (spins or pores, arrowheads). Figs a-f (LM).

3.2. Species composition of resting cysts belong to Protoperidinoid from the costal waters of South Central

3.2.1. Cyst composition

There were 8 different types of cysts belonging to the genus *Protoperidinium* from three locations in the coastal waters of South Central Viet Nam. All types of cysts were first described, and discussed in its details (table. 3.2). Cysts of *P. latissimum*, *P. conicum*, *P.* cf. *shanghaiense* and *Protoperidinium* sp. 3, those have saphopylic-type of archeopyle that corresponded to the 2a plate. Cysts of *P. parthenopes* and *Protoperidinium* sp. 1 (*Islandinium brevispinosum*) have archeopyle of saphopylic-type including three apical paraplates.

More cyst types were found in this study comparing to previous study in Cam Ranh bay (Doan Nhu Hai and Nguyen Ngoc Lam 2002), where 25 different types of dinofladellate cysts were recorded, with 5 types of cysts belong to Protoperidinoid including: *Protoperidinium conicoides*, *P.* cf. *avellana/thorianum*, *P.* cf. *minutum*, *P.* cf. *leonis* and *P. oblongum.* Lirdwitayaprasit (1999) found 4 types of cysts belong to Protoperidinoid (*Protoperidinium pentagonum*, *Protoperidinium* sp. 1, *Protoperidinium* sp. 2 and *Protoperidinium* sp. 3) in sediment from 48 stations in the Gulf of Thailand and East coast of Peninsular Malaysia () . The results of the present study showed that composition of cysts belong to Protoperidinoid in the costal waters of South Central Viet Nam relatively diverse, although the number of analyzed samples is limited.

Table. 3.2. List of cysts belong to *Protoperidinium* were found in the surface sediments in the costal waters of South Central: VR = Vung Ro; NP = Nha Phu; NTh = Ninh Thuan.

Ord.	Species name	Stations						
		VR1	VR2	VR3	VR4	VR5	NP	NTh1
1	*P. abei* var. *rotundata* (Abé 1936) Taylor 1967	+						+
2	*P.* cf. *shanghaiense* Gu, Liu & Mertens 201							+
3	*P. conicum* (Gran, 1902) Balech 1974			+				
4	*P. latissimum* (Kofoid, 1907) Balech 1974							+
5	*P. parthenopes* Zingone & Montresor 1988	+	+					+
6	*Protoperidinium* sp.1		+					
7	*Protoperidinium* sp.2			+				+
8	*Protoperidinium* sp.3						+	

Illustration of few represent resting cysts belong to *Protoperidinium*

- Cyst of *Protoperidinium parthenopes* Zingone & Montresor 1988

References: Kawami & Matsuoka 2009: pl. 1, figs. 1–8, Text-Figure 1; Liu et al. 2013: p. 9, figs 54, 55; Zonneveld & Pospelova 2015.

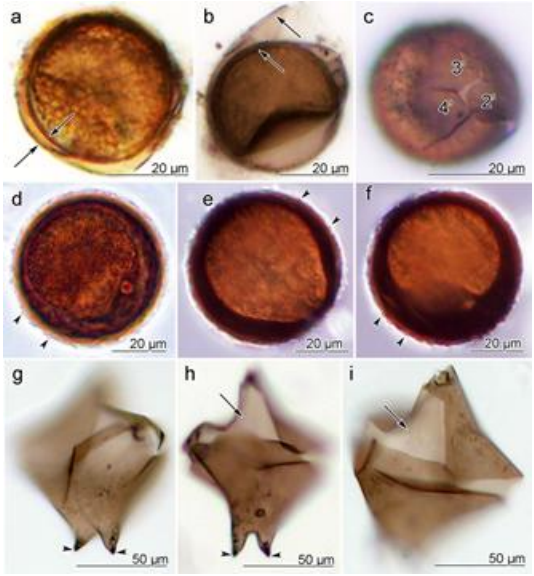
- Cyst of *Protoperidinium abei* var. *rotundata* (Abé) Taylor 1967

Cyst species (Paleontological name): *Brigantedinium cariacoense* (Wall) Lentin and Williams 1993

References: Liu et al. 2015: p. 9, fig. 56

- Cyst of *Protoperidinium latissimum* (Kofoid 1907) Balech

References: Gu et al. 2015: p. 55, figs 64, 65; Matsuoka & Fukuyo 2000: pl. 19, pl. 21, figs 10a, b;

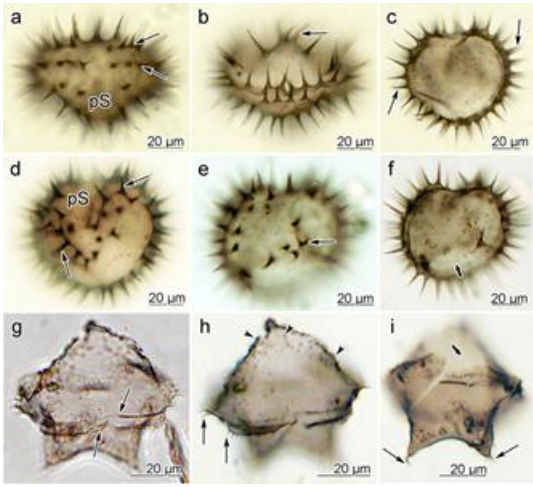


Cyst of *Protoperidinium parthenopes*: - a and b: cyst with double layerd cell wall (arrows), - c: cyst in apical view with sphaerical, the archeopyle formed by three apical plates (2'-4'); **- d-f: cyst of *Protoperidinium abei* var. *rotundata***: cyst is sphaerical, dark brown, surface is granulate (arrowheads); - **g-i: cyst of *Protoperidinium latissimum***: - g: cyst in ventral view and h & i: cyst in dorsal view with two antapical horns (arrowheads), shape of archeopyle (arrow). Figs a-i (LM).

- Cyst of *Protoperidinium conicum* (Gran) Balech 1974

Cyst species : *Selenopemphix quanta* (Bradford 1975) Mat. 1985.

- Cyst of *Protoperidinium* cf. *shanghaiense* Gu, Liu & Mertens 2015



- a-f: cysts of *Protoperidinium conicum*: - a: cyst in ventral view with parallel rows of spines along the paracingulum (arrows), parasulcus (pS) without spines, - b: cyst in dorsal view with three apical spines, - c: cyst in antapical view is heart in shaped with strong spines (arrows), - d: antapical spines (arrows), - e and f: apical spines (arrow) and archeopyle (thick arrow)**; - g-h: cyst of *Protoperidinium* cf. *shanghaiense*:** - g: circular paracingulum (arrows), - h: surface of cyst with small spines (arrowheads), margins of paracingumlum with numerous spines (arrows), - i: archeopyle is corresponded to the 2a plate (thick arrow), two antapical horn ending with spine (arrow). Figs a-i (LM).

3.3. Distribution of *Protoperidinium* species in Vietnamese waters

3.3.1. Spatial distribution

The South Central of Viet Nam hold the most diversity with 83 species and subspecies, following by the Gulf of Tonkin/North Central Viet Nam (54 species and subspecies), South East Viet Nam (51 species and subspecies), South West Viet Nam (46 species and subspecies), and Paracel Islands (31 species and subspecies). Three main group of species are characterized including cosmopolitan distribution, wide distribution, and narrow distribution.

Cosmopolitant distributed species include *Protoperidinium excentricum, P. thorianum, P. claudicans, P. depressum, P. oceanicum, P. conicum, P. leonis, P. obtusum, P. pentagonum, P. subinerme, P. quarnerense, P. steinii, P. crassipes, P. divergens, P. elegans, P. fatulipes, P. grande, P. longipes*, and *P. pellucidum.*

Widely distributed species include those found in most of Viet Nam coastal waters: *P. acutipes, P. conicum, P. crassipes, P. curtipes* f. *asymmetricum, P. divergens, P. globiferum, P. inclinatum, P. majus, P. nipponicum, P. oceanicum var. tenellum, P. ovum, P. pellucidum, P. quarnerense, P. sphaericum, and P. steinii.*

Narrowly distributed species include those only found in one or some certain research sites: *Protoperidinium* cf. *planiceps, P. decollatum, P. fatulipes, P. grande, P. hamatum, P. larsenii, P. longipes, P. oceanicum, P. oceanicum* f. *bisintercalares*, *Protoperidinium* sp. 1 ortho-penta, *Protoperidinium* sp. 3 ortho-penta, *Protoperidinium* sp. 4 ortho-quadra, *Protoperidinium* sp. 5 para-hexa, and *Protoperidinium* sp. 2 ortho-quadra.

Similarities among *Protoperidinium* species composition in research locations were analyzed using Sorensen index (S) (table 3.4). Results showed that there were high similarities among the species in North Central, South Central, South East, and South West Viet Nam (S ranges from 0.68 to 0.78). Comparison of species composition between the Paracel Islands and South Central, South West indicated relatively low species similarity with S index of 0.47 and 0.49, respectively.

3.3.2. Temporal distribution

3.3.2.1. Temporal variation of Protoperidinium *species composition in Nha Trang Bay, Khanh Hoa.*

A total 67 taxa were identified in genus *Protopreridinium*. Many species found almost year round including *Protoperidinium conicum, P. divergens, P. oceanicum* var. *tenellum, P. venustum* var. *facetum, P. abei, P. depressum* var. *claudicanoides, P. quarnerense, P. pellucidum, P. solidicorne, P. steinii,* and *P. yonedai.*

Species found only once in Nha Trang Bay in different months include *P. expansum*, *P. nudum* and *P. sphaericum* (January); *P. angusticollum*, *P. hamatum* và *P. ventricum* (March); *P. oceanicum* var. *typica* and *P. sinuosum* (April); *P. paraoblongum* (May); *Protoperidinium* sp. 4 (para-hexa), *P. claudum*, *P. laciniosum*, *P. persicum*, *P. remotum* and *P. stellatum* (August), and *P. inflatiforme* (October). However, some of these species are found frequently in other regions, i.e., *P. paraoblongum* appeared in Mekong Delta outfall from March to June, 2015 and in Ninh Thuan, Binh Thuan from July to October; *P. inflatiforme* appeared Do Son, Hai Phong in January, September, October, December, in Nghe An (November), Binh Thuan (October), Con Son Islands (July), Mekong Delta outfall (May and June) and Kien Giang (April).

3.3.2.2. Day and night variation in cell density

- Some environmental characteristics at anchored station:

At anchored station (sampling every 2-hour during 24h) at Cua Be estuary in May, 2012 showed that temperature, salinity were not varied much between day and night time but significantly different between surface and bottom layers (Figs 3.1 and 3.2).

There was significant difference in seawater temperature between surface and bottom layers from 12am to 20pm. The highest water level was at 12:00 (12.5m depth) and decrease to 10m at 18:00. During this time, there are significant difference in temperature between surface and bottom layers. Surface temperature is always higher than bottom temperature at all day (Fig. 3.1). However, water temperature between day and night are not significantly different. Temperature variated from 27.3 to 29.5^{o}C during the day and from 27.8 to 29.0^{o}C at night.

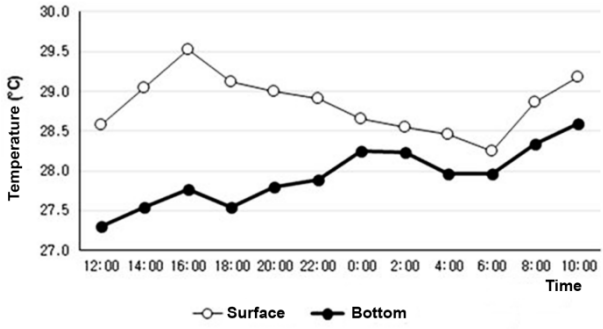


Figure 3.1. Day and night variation in temperature at surface and bottom layers at anchored station, Cua Be estuary.

There was also significant difference in salinity between surface and bottom layers from 12:00 to 20:00. Salinity at the bottom layer was much varied through day and night (33,3-33,6‰) while that at the surface was always lower and much varied (Figs 3.2).

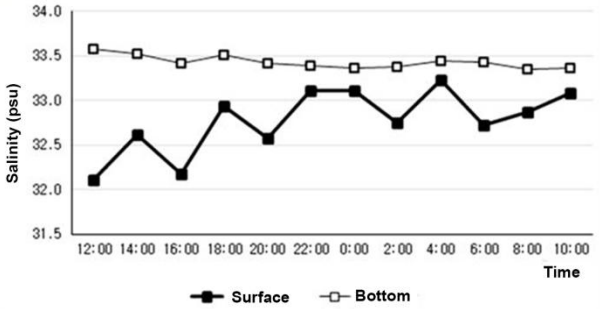


Figure 3.2. Day and night variation in salinity at surface and bottom layers at anchored station, Cua Be estuary.

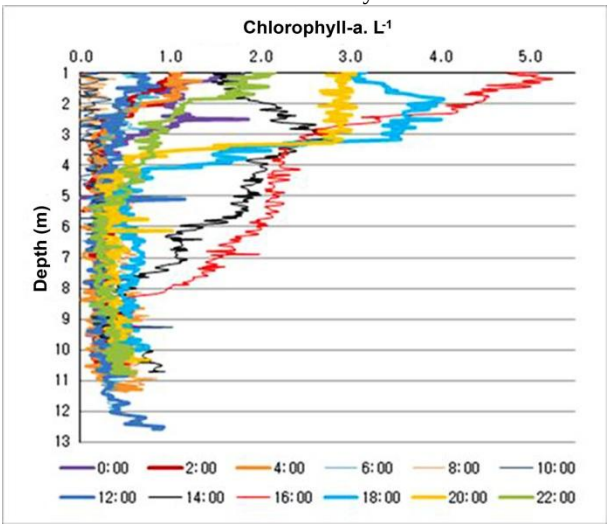


Figure 3.3. Day and night vertical distribution of chlorophyll-a at anchored station, Cua Be estuary.

Chlorophyll-a concentration were high at the surface and greatly decreased with depth in most sampling times. Chlorophyll-a concentration at the surface were distributed from 0-8m from 14:00-16:00 and later becoming narrower (0-4m). The peak of chlorophyll-a concentration is at 16:00 (more than 5.0 µg.L^{-1}) at the surface and tend to decrease from 18:00 to 20:00 (Fig. 3.3). Overall, chlorophyll concentration tend to decrease with depth, only less varied from 8:00 to 10:00.

- Day and night variation in *cell density at Cua Be estuary station.*

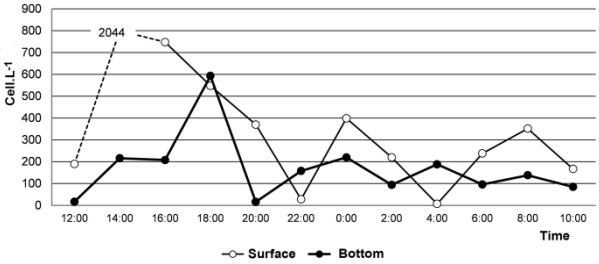


Figure 3.4. Cell density day-night fluctuation Cua Be river estuary stations

Continuous data of cell density of *Protoperidinium* during 24h indicated that these organisms were at the surface in most of time (Fig. 3.4). During some time points at night (18:00, 22:00, and 4:00), cell density at the bottom are higher than that at the surface. As these species are heterotrophic, they tend to gather at the surface, where there are other autotrophic algae. Chlorophyll-a were highly distributed at the surface (Fig. 3.3) may explain more this distribution of *Protoperidinium*.

Table 3.5. Pearson correlation (r) between cell density and each environmental factor.

		Density	Depth	Temperature	Salinity	Fluorescence
Density	r	1	-0,340	0,390	-0,424*	0,285
	p		0,104	0,059	0,039	0,177
	N	24	24	24	24	24

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

However, it should be noted that picophytoplankton contributing greatly to the chlorophyll concentration are not consumed by *Protoperidinium* (Gribble et al. 2007). At 14:00, *Protoperidinium* density increase remarkably (2044 cells/l) dominated by *P. pellucidum*. At this time, environmental factors such as temperature, salinity were not significant difference through out the water column while chlorophyll-a concentration was relatively high at the surface. Other factor may influence vertical distribution of phytoplankton at the estuarine is the tidal current.

- *Correlation between environmental factors and cell density of* Protopridinium*:*

Data in Table 3.5 show that there is no correlation ($p>0.05$) between cell density and depth ($p=0.104$), temperature ($p=0.059$), and chlorophyll ($p=0.177$), which mean that r correlation has no significance or there is no correlation between cell density and depth, temperature, chlorophyll. Only salinity show negative correlation with *Protoperidinium* cell density ($r = -0.424$, $p=0.039<0.05$, $N=24$). This leads to a

hypothesis that *Protoperidinium* population at Cua Be river estuary is influenced by those species which favor to lower salinity.

Correlation analysis between cell density of *Protoperidinium* at Cua Be and environmental factors are similar with previous researches in the same region. Nguyen Ngoc Lam et al. (2002) study of phytoplankton at Cua Be, with high sampling frequency (every week in 12 months) also found that there is no or low correlation between temperature, salinity and biology factors (bacillariophyta and dinophyta). Ho Van The and Nguyen Ngoc Lam (2005) research on dinoflagellates in Nha Trang Bay and Cua Be also indicated that correlations between dinoflagellates distribution and environmental factor are not significant. However, there was low correlation between temperature and biomass while salinity was correlated with both biomass and density of dinoflagellates.

CONCLUSIONS AND PETITIONS

CONCLUSIONS

1. The thesis provided an extensive and detailed data on morphology for species identification of 86 taxa of *Protoperidinium*. Genus *Protoperidinium* in Vietnamese waters were including 3 subgenus (*Minusculum, Archaeperidinium, Protoperidinium*), divided into 3 groups with 8 sections.

- The thesis has contributed: a species new to science, *Protoperidinium larsenii* L. Phan-Tan, L. Nguyen-Ngoc, and H. Doan-Nhu 2016 belong to subgenus *Protoperidinium, Orthoperidinium* group, *Oceanica* section; 48 taxa as new records for dinoflagellate flora of Viet Nam; one subgenus *Minusculum as* new record for marine phytoplankton of Viet Nam with one species as new record for Asia Pacific (*Protoperidinium anomaloplaxum*).
- Position and shape of the hypothecal pore in the 1''' plate were observed and described in detail for 11 species. Among them, 6 taxa were newly reported that possessed the hypothecal pore in the 1''' plate, including: *Protoperidinium gibberum, P. inclinatum, P. inflatiforme, P. nipponicum, P. solidicorne* var. *makronyx* and *Protoperidinium* sp. 5 (para-hexa).

2. A total 46 different types of cysts were recorded in sediments sampled at 8 stations from coastal South Central, of which 8 types belong to the genus *Protoperidinium* species were firstly described, illutrated and discussed in detail for dinoflagellate flora of Viet Nam.

3. Characteristics of distribution of species composition and cell density of *Protoperidinium* varied with space and time in 5 research areas in Vietnamese waters were analyzed and compared, and showed that:

- South Central of Viet Nam hold the most diversity with 83 species and subspecies, following by the Gulf of Tonkin-North Central (54), South East (51), South West (46), and Paracel Islands (31).
- Similarities of species compostition of *Protoperidinium* in coastal waters from Gulft of Tonkin to South West are high with Sorensen index ranges from 0.68 % to 0.78%. Species compostition similarity of coastal waters the Paracel Islands are low (47% to 54%).
- Qualitative analysis of species composition of *Protoperidinium* in 10 year data in Nha Trang Bay, Khanh Hoa showed that there was strong variation among the months of the year; 11 species were found throughout the year; and 16 other species occured only one time in the year.
- Correlation analysis between cell density of *Protoperidinium* and environmental factors at Cua Be river estuary shoed only salinity negatively correlated with *Protoperidinium* cell density ($r = -0.424$, $p=0.039<0.05$, $N=24$).

PETITIONS

- Continue with further research on biology and ecology of dinoflagelate cysts.
- More research is needed on morphology of *Protoperidinium* with the support of scanning electron microscopy (SEM) and molecular biology.

NEW CONTRIBUTIONS OF THIS DISSERTATION

1. The thesis has been provided an extensive and detailed data on morphology for species identification for *Protoperidinium* species in Vietnamese waters.

2. Described a species new to science, *Protoperidinium larsenii* L. Phan-Tan, L. Nguyen-Ngoc et H. Doan-Nhu 2016, published in the Nordic Journal of Botany.

3. Additional discovered 48 taxa for dinoflagellate and phytoplankton floras of Viet Nam; recorded one new subgenus *Minusculum* for marine phytoplankton of Viet Nam with one species as new record for Asia Pacific (*Protoperidinium anomaloplaxum*).

4. A total 46 different types of cysts were recorded in sediments sampled at 8 stations from three locations: Vung Ro bay, Nha Phu lagoon and Ninh Thuan coastal waters, of which the morphology of 8 types belong to the genus *Protoperidinium* species were described and illustrated including *Protoperidinium abei* var. *rotundata*, *P.* cf. *shanghaiense*, *P. conicum*, *P. latissimum*, *P. parthenopes*, *Protoperidinium* sp. 1, *Protoperidinium* sp. 2 and *Protoperidinium* sp. 3.

PUBLICATIONS

Published in countries specialized journals and others

1. Phan Tấn Lượm, Nguyễn Ngọc Lâm and Đoàn Như Hải (2016). Phân loại học phân chi *Archaeperidinium* thuộc chi *Protoperidinium* (Dinophyceae) ở vùng biển Việt Nam. Tap chí Sinh học. DOI: 10.15625/0866-7160/v38n1.7596, ISSN 0866-7160, 38(1): 39-52.
2. Phan Tấn Lượm, Nguyễn Ngọc Lâm, Đoàn Như Hải (2017). Bào tử nghỉ của một số loài thuộc chi *Protoperidinium* trong trầm tích ven bờ Phú Yên, Khánh Hòa and Ninh Thuận. Tạp chí Sinh học. DOI: 10.15625/0866-7160/v39n1.8403, ISSN 0866-7160, 39(1)
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Published in international specialized journals, the list of SCI

1. **Phan-Tan, L.**, L. Nguyen-Ngoc, H. Doan-Nhu (2016). Species diversity of sections *conica* and *tabulata* in the genus *Protoperidinium* (Dinophyceae) from tropical waters of the South China Sea. Nova Hedwigia, 103(3-4), 515-545. DOI: 10.1127/nova_hedwigia/2016/0369. ISSN 0029-5035.
2. **Phan-Tan, L.**, L. Nguyen-Ngoc, H. Doan-Nhu, Robin Raine and Jacob Larsen (2016). Species diversity of the dinoflagellate genus *Protoperidinium* section *Oceanica* (Dinophyceae, Peridiniales) in Vietnamese waters, with description of a new species - *P. larsenii sp. nov.* Nordic Journal of Botany. DOI: 10.1111/njb.01230. ISSN 0107-055X. Online: 12/9/2016.