METHODS FOR MODERN DINOFLAGELLATE CYST STUDIES

K. MATSUOKA*, Y. FUKUYO**, AND D. M. ANDERSON***
*Department of Geology, Nagasaki University, 1-14 Bunkyo-Machi, Nagasaki 852, Japan; **Department of Fisheries, University of Tokyo, 1-1-1 Yayoi-Cho, Bunkyo-Ku, Tokyo 113, Japan; ***Biology Department, Woods Hole Oceanographic Inst., Woods Hole, MA 02543

INTRODUCTION

The purpose of this workshop is to provide an introduction to modern dinoflagellate cyst studies. For supporting information, the reader is referred to several detailed reviews by Wall and Dale [59], Dale [11], and Fukuyo and Matsuoka [19] (in Japanese). This manual is prepared in a course format consisting of eight sections: Introduction, Sampling, Fixation and preservation, Cleaning and concentration, Isolation of single cysts, Cyst culture, and Identification. A useful key for identifying modern dinoflagellate cysts based on shape is provided, as is a list of all known cyst-producing, living dinoflagellate species.

Definitions

The term "cyst" is used to describe a non-motile cell which lacks flagella and an ability to swim. Two types of cysts are found in dinoflagellate life cycles - the temporary cyst and the resting cyst. Definitions given by Dale [11] are repeated here, with some modifications:

Temporary Cyst: This resting cell is formed asexually when motile cells experience unfavorable conditions. When conditions become favorable again, temporary cysts quickly re-establish their ability to swim, allowing the organism to withstand temporarily adverse environments. No internal morphological changes are observed during cyst formation: cell contents round off inside a smooth and transparent wall, and the theca is quickly shed by a process called ecdysis. The stage is mainly observed in laboratory cultures, but Kita et al. [23] recorded the temporary cyst of Goniodoma pseudogonyaulax (= Triadinium pseudogonyaulax) in a rock pool and documented an important role for this stage in red tides caused by that species. Other equivalent names given to this stage are "pellicle" or "ecdysal" cysts.

Resting Cyst: This stage is occasionally formed in cultures and routinely occurs in natural plankton populations, often towards the end of a bloom. Resting cyst formation is a sexual process which may be completed within a few days under bloom conditions, after which cysts quickly become less conspicuous in the plankton and finally sink to accumulate on the bottom. This, together with the fact that only a few percent of the motile cells produce cysts probably accounts for the paucity of cyst observations in plankton records. Many cysts seem to require a mandatory resting period (6 weeks to 5 months depending on species) before they will re-establish motile populations under favorable conditions. Recent study, however, suggests that some species may not need this maturation period. Under favorable conditions, cysts can remain viable in sediments for at least 6 years. In this workshop, we use the term "cyst" to mean "resting cyst" or hypnozygote.

Significance of Cysts in Dinoflagellate Red Tides

More than 60 marine and 15 freshwater species of modern dinoflagellates are known to produce cysts (Table I). Of these species, more than

RED TIDES: BIOLOGY, ENVIRONMENTAL SCIENCE, AND TOXICOLOGY
Okaichi, Anderson, and Nemoto, Editors

Table I Dinoflagellates producing a resting cyst

MARINE SPECIES	Reference
Gymnodiniales	
Cochlodinium sp.	Fukuyo and Matsuoka (1983), Matsuoka (1985a, 1987a)
Gymnodinium breve	Walker (1982)
Gymnodinium catenatum	Anderson et al. (in press), Matsuoka (1987a)
Gyrodinium instriatum	Wall and Dale (1968), Fukuyo and Matsuoka (1983)
Gyrodinium resplendens	Dale (1983)
Gyrodinium uncatenum	Tyler et al. (1982)
Pheopolykrikos hartmannii	Fukuyo and Matsuoka (1983), Matsuoka and Fukuyo (1986), Matsuoka (1985a)
Polykrikos kofoidii	Morey-Gains and Ruse (1980), Fukuyo and Matsuoka (1983), Matsuoka (1985a)
Polykrikos schwartzii	Wall and Dale (1968a), Matsuoka (1985a)
Gonyaulacales	
Gonyaulax digitalis	Wall and Dale (1968a)
Gonyaulax polyedra	Wall and Dale (1968a), Kobayashi et al. (1981)
Gonyaulax scrippsae	Wall and Dale (1968a), Matsuoka (1982a)
Gonyaulax spinifera	Wall and Dale (1968a)
Gonyaulax cf. spinifera	Dale (1983)
Gonyaulax verior	Matsuoka et al. (in press)
Gonyaulax sp.	Dobell and Taylor (1981)
Protoceratium reticulatum	Wall and Dale (1968a)
Alexandrium monilatum	Walker and Steidinger (1979)
Protogonyaulax affinis	Fukuyo et al. (1985)
Protogonyaulax catenella	Yoshimatsu (1981), Fukuyo (1985)
Protogpnyaulax globosa	Dale (1977b)
Protogonyaulax leei	Fukuyo (unpublished data)
Protogonyaulax perviana	Fukuyo et al. (unpublished data)
Protogonyaulax tamarensis	Dale (1977b), Fukuyo (1985)
Protogonyaulax sp. OMR	Matsuoka (1987a)
Helgolandium subglobosum	von Stosch (1969b)
Pyrodinium bahamense	
var. bahamense	Wall and Dale (1969)
var. compressum	Steidinger et al. (1980)
Triadinium pseudogonyaulax	Kita et al. (1985)
Pyrophacus horologium	Wall and Dale (1971)
Pyrophacus steinii	
var. steinii	Matsuoka (1985b)
var. vancampoae	Wall and Dale (1971)
Peridiniales	
Scrippsiella trochoidea	Wall et al. (1970)
Scrippsiella sweeniae	Wall and Dale (1968b)
Ensiculifera cf. mexicana	Wall and Dale (1968b)
Ensiculifera sp.	Matsuoka and Kobayashi (unpublished data)
Cachonina hallii	von Stosch (1969a)

Table I cont.

Hetrocapsa triquetra	Braarud and Pappas (1951)
Peridinium faeroense	Dale (1977a, 1978)
Peridinium hangoei	Iwasaki (1969)
Protoperidinium avellana	Wall and Dale (1968a), Matsuoka (1984a) Lewis et al. (1984)
Protoperidinium claudicans	Wall and Dale (1968a)
Protoperidinium compressum	Wall and Dale (1968a)
Protoperidinium conicoides	Wall and Dale (1968a)
Protoperidinium conicum	Wall and Dale (1968), Fukuyo (1980) Kobayashi and Matsuoka (1984)
Protoperidinium denticulatum	Wall and Dale (1968a)
Protoperidinium divaricatum	Matsuoka et al. (1983)
Protoperidinium excentricum	Wall and Dale (1968a), Lewis et al. (1984)
Protoperidinium latissinum	Wall and Dale (1968a)
Protoperidinium leonis	Wall and Dale (1968a)
Protoperidinium minutum	Wall and Dale (1968a), Fukuyo et al. (1977)
Protoperidinium nudum	Wall and Dale (1968a)
Protoperidinium oblongum	Wall and Dale (1968a)
Protoperidinium pentagonum	Wall and Dale (1968a), Matsuoka (1982) Lewis et al. (1984)
Protoperidinium punctulatum	Wall and Dale (1968a)
Protoperidinium subinerme	Wall and Dale (1968a)
Protoperidinium thorianum	Lewis et al. (1984)
Protoperidinium cf.divergens	Dale (1983)
Protoperidinium sp.	Dale (1983)
Diplopelta parva	Matsuoka (in press)
Diplopsalis lenticula	Wall and Dale (1968a), Matsuoka (in press)
Diplopsalis lebourae	Matsuoka (in press)
Diplopsalopis orbicularis	Wall and Dale (1968a), Matsuoka (in press)
Gotoius abei	Matsuoka (in press)
Zygabikodinium lenticulatum	Wall and Dale (1968a), Matsuoka (in press)
FRESHWATER SPECIES	
Gymnodiniales	
Cystodinium bataviense (=Dinococcus oedogonii)	Pfiester and Lynch (1980)
Gymnodinium dodgei	Sarma and Shyam (1974)
Gymnodinium fungiforme	Spero and Moree (1978)
Gymnodinium pseudopalustre	von Stosch (1973)
Woloszynskia apiculata	von Stosch (1973)
Woloszynskia tylota	Bibby and Dodge (1972)
Gonyaulacales	
Ceratium carolianum	Wall and Evitt (1975)
Ceratium cornutum	Wall and Evitt (1975)
Ceratium hirundinella	Wall and Evitt (1975), Chapmann et al. (1981, 1982)
Ceratium horridum	von Stosch (1972)

Table I cont.

Peridiniales	
Peridinium cinctum	
forma ovoplanum	Pfiester (1975)
forma westii	Eren (1969)
Peridinium cunningtonii	Sako et al. (1984)
Peridinium gatunense	Pfiester (1977)
Peridinium inconspicuum	Pfiester et al. (1984), Wall and Dale (in Wall et al. 1973)
Peridinium limbatum	Evitt and Wall (1968), Pfiester and Skvarla (1980)
Peridinium lubiniensiforme	Dilwald (1937)
Peridinium penardii	Sako et al. (1987)
Peridinium volzii	Pfiester and Skvarla (1979)
Peridinium willei	Pfiester (1976)
Peridinium wisconsinense	Wall and Dale (1968a)

16 have been known to cause red tides and four are toxic. Most modern cysts are spherical, ellipsoidal to peridinioid, with or without spine-like ornaments, and range from 20-80 μm in size. As the cysts lack flagella, they float for a short period and sink to the sediments after encystment. During dormancy, cysts may be resuspended and transported in much the same way as fine silt or mud particles are.

Physiologically there are two different cyst forms observed in surface sediments: living cysts and empty cyst walls. The living cyst with fresh protoplasm can germinate under favorable conditions. The empty cyst is the wall remaining after excystment, often with a distinct opening called an archeopyle. The cyst wall of modern dinoflagellates can be calcareous or organic (sporopollenin). As the organic sporopollenin wall is highly resistant to chemical and biological attack, some cysts are not affected by harsh palynological processing with strong acids and oxidants.

SAMPLING

Planning for Sampling

Two methods have been commonly adopted for collecting surface sediments - corers and sediment traps. The sampling method should be selected according to the purpose of the cyst study. If the objective is to know when and how many cysts of a certain species are produced, sediment traps are useful. If it is to document the presence of different species or the change in a dinoflagellate community, the coring method is preferable.

Core Sampling

The core sampler is used for collecting bottom sediments. In order to get surface sediments, which include many fresh living cysts, gravity corers such as the Phleger corer (Phleger bottom sampler) and piston corers are more desirable than other bottom samplers such as dredges or grab buckets which often lose the light fluffy material at the sediment surface. A light-weight core sampler (TFO gravity corer [19]) or its equivalent can be deployed from small boats and thus is useful in the investigation of near-shore and inner bay areas.

Coring procedures are very simple. The corer is deployed from the boat and retrieved with care to avoid losing sediment. Immediately on retrieval, the top and bottom of the clear core tubing must be capped with plastic covers or some other material to prevent water leakage. The intact core can then be stored in the cold and dark until needed. When core-samplers are not available or do not work in coarser sediments, Smith McIntyre or Ekman Berg type grab samples can be used. Small 10-15 cm tubes can be inserted in the bulk sample to collect subsamples. SCUBA diving is also a useful method for obtaining undisturbed bottom cores.

Sediment Traps

Sediment traps are used to catch sinking cysts before they settle to the sea or lake floor. There are no widely-accepted procedures or designs for sediment traps, as various configurations will collect different quantities of material. Furthermore, material resuspended from the bottom and collected in a trap can complicate the interpretation of sedimentation data. Quantitative analysis of sediment trap data is thus not recommended. It is, however, possible to learn a great deal at a qualitative level about the timing and relative magnitude of cyst formation as a component of dinoflagellate population dynamics. For these purposes, sediment traps

need not be elaborate or expensive. A small trap consisting of a 2-liter wide-mouth polyethylene bottle attached to a line between a surface buoy and a bottom weight will provide useful information. A vertical series of these bottles can even provide some indication of the extent of resuspension.

SEDIMENT PROCESSING

Two different processing methods have been adopted for cleaning and concentrating cysts from bulk sediment - a sieving technique that uses no chemicals and a palynological technique that use harsh chemicals. The choice of technique depends on the purpose of the study. When living cysts are needed (e.g. for germination, identification, or enumeration), the sieving technique is used. When a general survey of the cyst assemblage is desired, palynological processing can be used. One should recognize that the latter process will destroy certain fragile or non-resistant cysts that would be observed with the non-destructive sieving technique.

Sieving Procedure (Fig. 1)

1. Prepare a series of sieve of various mesh-sizes, with 250 μm being the upper sieve, 125 μm in the middle and 20 μm being the lowest sieve.

2. Mark the core tube at 2 cm intervals from the bottom.

3. Remove the plastic cover from the core tube and pipette the overlying seawater into a 50 ml vial.

4. Remove the cap from the bottom of the core tube.

5. Remove the bottom mud layer in the tube slowly by blowing at the upper end of the tube until the upper surface of the mud reaches the 2 cm mark. Sometimes it is just as easy to push the sediment out of the tube from below, using a stopper cut to be slightly smaller than the inside diameter of the core tube.

6. Put the remaining sediment into the 50 ml vial used in Step 3.

7. Rinse the inner surface of the tube with filtered seawater. Pour the rinse water into the 50 ml vial.

8. Mix all contents in the vial and pour onto the upper sieve. Rinse the vial with a small amount of filtered seawater if needed. Note that it is also possible to use an ultrasonic probe or bath to disaggregate sediment before sieving [59].

9. Wash the sediment on the upper sieve carefully with filtered seawater. The cysts and fine particles will pass through the 250 μm and 125 μm sieves and accumulate on the 20 μm sieve.

10. Transfer all the residue on the 20 μm sieve to a petri-dish.

11. Separate the cysts from the residue by squirting filtered seawater from the washing bottle. The water is injected at one side of the petri-dish in such a way that the residue is surrounded by the swirling motion of water. Cysts and other light-weight particles will be suspended in the circulating water while heavy sand particles remain at the bottom in the center of the petri-dish.

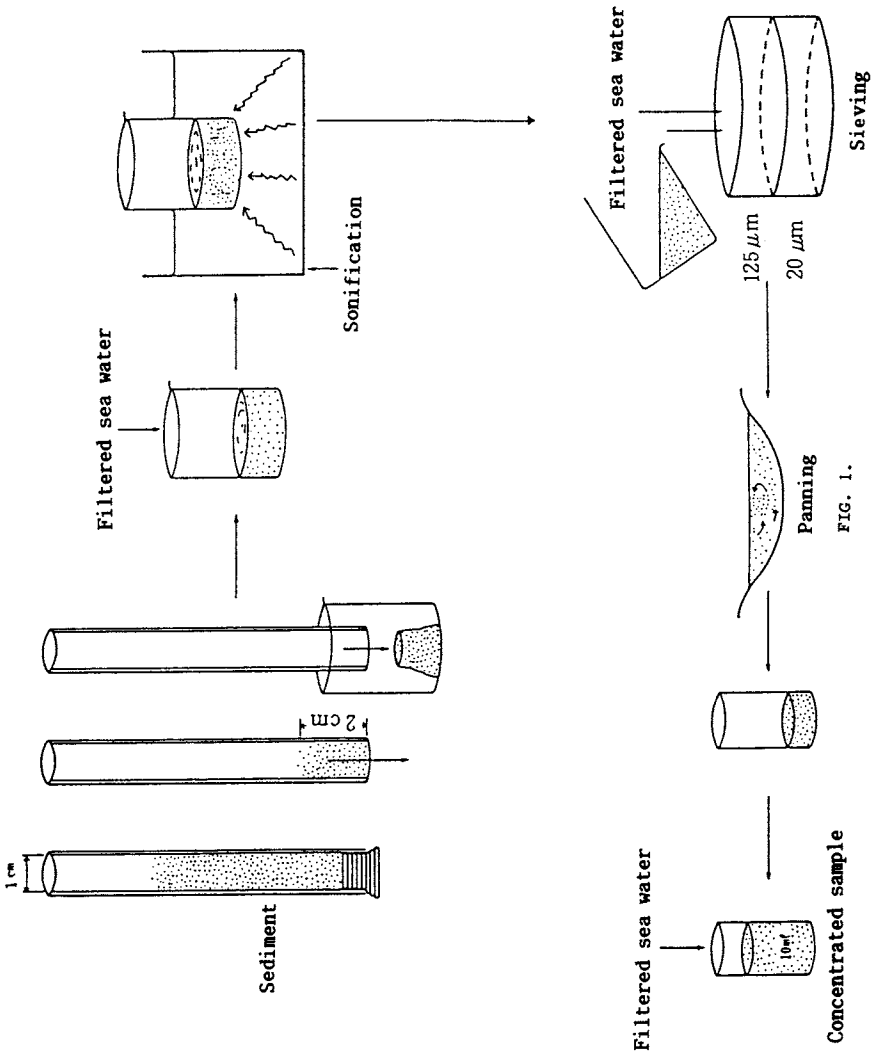
1 cm
2 cm
Sediment
Filtered sea water
Sonification
Filtered sea water
125 μm
20 μm
Sieving
Panning
Filtered sea water
10 ml
Concentrated sample

FIG. 1.

11a. Alternatively, transfer all residue on the 20 μm sieve to a watch glass. Make a water-eddy in the watch glass for separating cysts and light-weight particles from heavier sand grains (panning in the geological sense). After this process, cysts and other fine particles are concentrated at the center of the watch glass.

12. Gently pour the supernatant water with cysts suspended back onto the 20 μm sieve. The heavy sand particles are left on the petri-dish.

12a. Suck up the cysts and light-weight particles and transfer them into a test tube with a syringe. Then use a squeeze bottle to back-wash the material off the sieve. Minimize the amount of water used in order to achieve the highest concentration factor (10 ml total is useful).

13. Repeat the washing, if needed.

Palynological Technique

The technique introduced here is based on standard palynological processing. It uses several dangerous chemicals and therefore should be performed with adequate safety precautions.

Procedure

1. Put 1 ml of the original material into a 15 ml polyethylene centrifuge tube.

2. Wash with distilled water several times to remove salt.

3. Add 5% hydrochloric acid to remove calcium carbonate from calcareous nannoplankton, foraminifera and others. The calcareous cyst wall and ornaments such as on Scrippsiella trochoidea and Ensiculifera sp. are also removed at this time, but the inner organic phragma will remain.

4. Wash with distilled water.

5. Add 1% potassium hydroxide solution and warm to 70°C in a water bath for 3 mins. At higher temperature and with longer exposures, the relatively thin cyst phragma of Protoperidinium and Protogonyaulax sometimes disappear.

6. Wash with distilled water.

7. Add concentrated (25-30%) hydrofluoric acid to the tube for removing silicate materials such as sand, diatoms, silicoflagellates and others. Warm in the water bath at 70°C for 2-3 hrs. As the hydrofluoric acid is very dangerous and toxic, this processing should be conducted in a fume hood with rubber or vinyl gloves. The residue solution containing hydrofluoric acid should be neutralized with calcium carbonate.

8. Wash with distilled water.

9. When cellulose substances such as plant tissue are abundant in the sample, acetylation may be useful to remove it. The acetylation procedure is as follows:

   a. Add glacial acetic acid (CH_3COOH) to the tube.

b. After removing this chemical, add Erdtman's solution (9 parts of acetic anhydride [$(CH_3CO)_2O$] and one part of concentrated sulfuric acid [H_2SO_4]) to the tube and warm it to 70°C for 15 mins.

c. Remove the Erdtman's solution and add glacial acetic acid to the tube again.

d. Wash with distilled water.

10. Prepare a series of sieves of various mesh-sizes with 250 μm being the upper sieve, 125 μm in the middle and 20 μm at the bottom.

11. Pour all the residue onto the upper sieve. The cysts and other organic particles such as spores and pollen grains will pass through the 250 μm and 125 μm sieves and accumulate on the 20 μm sieve.

12. Wash the material on the 20 μm sieve into a vial for a final volume of 10 ml.

CYST ISOLATION

Isolation is an important technique because it must be carried out before the inoculation step in establishing cultures. It is also needed in morphological studies.

Procedure

1. A small needle (insect pin) attached to the tip of a glass tube is used to clean the background around the cysts on a slide. A Pasteur pipette drawn out under flame to give a capillary tip (50-100 um dia.) at one end and connected to a 60 cm long silicone tube at the wide end is used for micropipetting cells.

2. Take out a small amount (about 0.5 ml) of the sieved cyst sample and put it onto a large counting chamber slide. Spread the sample evenly on the slide by adding filtered seawater to 1 ml.

3. Search under the microscope for the desired cysts. Once a cyst is located, push away interfering material around the cyst with the small needle and use the Pasteur pipette and tube to suck up the cyst.

4. Transfer the cyst into culture medium. For morphological studies, the cyst is introduced into a droplet of water on a regular slide.

CYST CULTURE

Three types of culture chambers are commonly used - a glass tube, individual wells in tissue culture plates, or Palmer-Maloney slides as described in [59]. In attempts to establish axenic cultures using culture tubes, "f/10" is useful as a medium. This medium is made by adding 1 ml of the medium "f" [21] to 10 ml of sterile, filtered seawater. For Palmer-Maloney slides and tissue culture plates, sterile filtered seawater can be used without any added nutrients.

To use tissue culture plates (Corning Cell Wells No. 25820), the procedure is as follows:

1. Pipette 1 ml of sterile filtered seawater into each of the 24 wells of a tissue culture plate.

2. Inoculate one cyst into each well, cover the plate, and seal the chamber with vinyl tape to prevent evaporation.

3. Incubate the chamber at a constant temperature between 15-30°C (depending on species) with approximately 150 uE · $m^{-2}sec^{-1}$ illumination.

4. Observe the cysts daily for 2 or 3 weeks after inoculation using an inverted microscope.

5. When a cell germinates and has divided into more than 5 cells, micropipette one cell and observe morphological features under the microscope. Observation of the morphology of the empty cyst is also useful.

CYST IDENTIFICATION

The important morphological features used in identification of cysts are the shape of the cyst body and its ornaments, wall structure and color, paratabulation, and the type of archeopyle or exit opening. The archeopyle is very useful in classifying the genus and family to which cysts belong. As the opening is not visible before excystment, it is not possible to use this characteristic for identification of living cysts. Furthermore, in comparison with the morphology of motile cells of dinoflagellates, cysts are usually relatively simple, mostly spherical to peridinioid. As a result, identification of cysts based on a single morphological character is not always reliable, and other characters listed above must be examined. A diagram of archeopyle types is presented in Figure 2 and examples of species with each type are given in Table II. A key based on cyst shape is given in Table III, and a key based on shape and archeopyle type is given in Table IV.

General Character of Gymnodinalian Cysts

Shape: mostly spherical to ovoidal and sometimes ellipsoidal, with or without spinate ornaments.

Cyst wall: organic and brownish color; mostly a single layer and sometimes two layers.

Archeopyle type: cryptophylic; chasmic (slit) or tremic (hole) type [30].

Cysts of Gymnodinialean species causing red tides: Gymnodinium breve, Gymnodinium instriatum, Pheopolykrikos hartmannii, Polykrikos kofoidii, Polykrikos schwartzii.

Cysts of toxic Gymnodinialean species: Gymnodinium breve (= Ptychodiscus brevis), Gymnodinium catenatum.

General Character of Gonyaulacacean Cysts

Cyst shape: basically spherical to ellipsoidal and rarely discoidal, often without process-like ornaments.

Cyst wall: organic, colorless and sometimes transparent; usually two layers, rarely single.

Archeopyle type: mostly saphopylic; precingular, but sometimes epicystal, hypicystal or combination type.

Cysts of Gonyaulacacean species causing red tides: Gonyaulax polyedra, Gonyaulax spinifera, Gonyaulax verior, Protoceratium reticulatum, Protogonyaulax affinis, Protogonyaulax peruviana, Triadinium pseudogonyaulax.

Cysts of toxic Gonyaulacacean species: Protogonyaulax catenella, Protogonyaulax tararensis, Pyrodinium bahamense var. bahamense, Pyrodinium bahamense var. compressum

General Character of Peridiniacean Cysts

Cyst shape: mainly spherical, ellipsoidal, peridinioid, and rarely discoidal, mainly without process-like ornaments.

Cyst wall: mainly organic and brownish in color; sometimes calcareous; mainly a single layer and rarely two layers.

Archeopyle type: mainly saphopylic; intercalary type, and sometimes theropylic; apical, intercalary, epicystal and combination types.

Cysts of Peridiniacean species causing red tides: Scrippsiella trochoidea, Cachonina hallii, Heterocapsa triquetra, Peridinium hangoei, Peridinium cunningtonii.

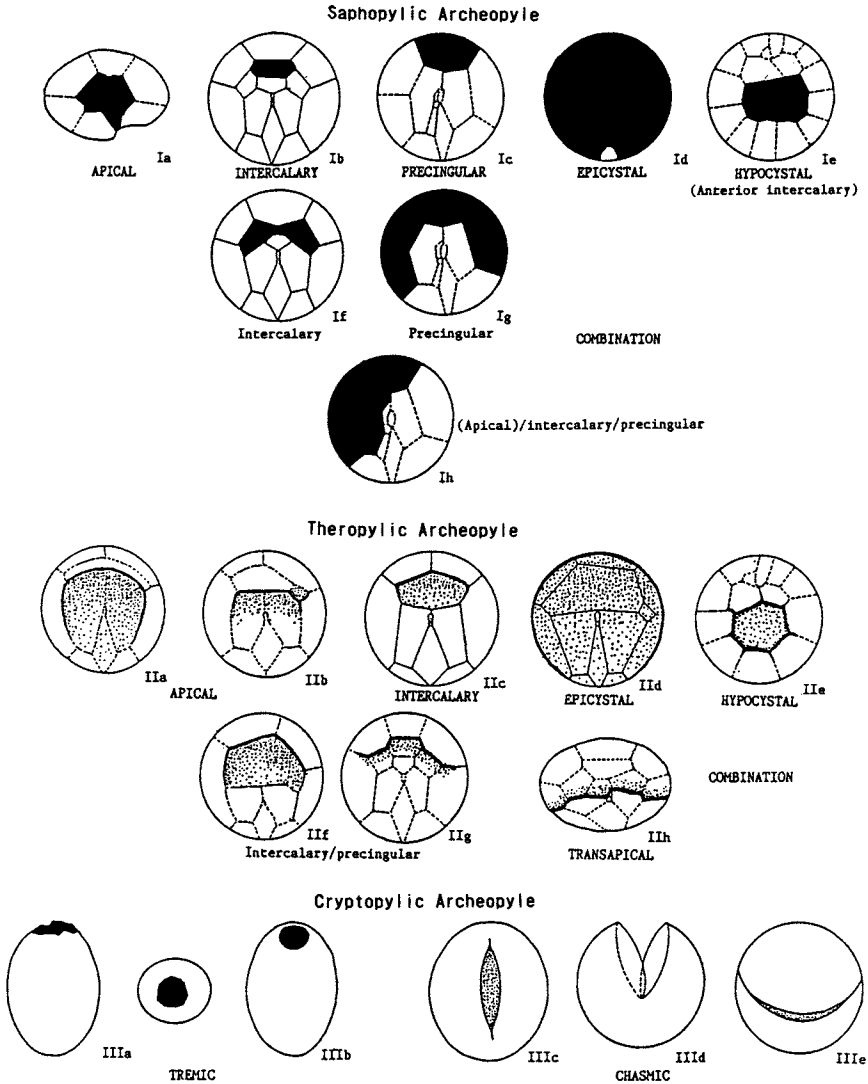
Saphopylic Archeopyle
Ia
APICAL
Ib
INTERCALARY
Ic
PRECINGULAR
Id
EPICYSTAL
Ie
HYPOCYSTAL
(Anterior intercalary)
If
Intercalary
Ig
Precingular
COMBINATION
(Apical)/intercalary/precingular
Ih
Theropylic Archeopyle
IIa
APICAL
IIb
IIc
INTERCALARY
IId
EPICYSTAL
IIe
HYPOCYSTAL
IIf
IIg
Intercalary/precingular
IIh
TRANSAPICAL
COMBINATION
Cryptopylic Archeopyle
IIIa
TREMIC
IIIb
IIIc
IIId
CHASMIC
IIIe

FIG. 2

Table II ARCHEOPYLE TYPES IN MODERN DINOFLAGELLATE CYSTS

Ia~Ih Saphopylic type	
BASIC FORM	
Ia Apical Archeopyle	Ceratium hirundinella, Ceratium cornutum(?) Ceratium carolianum(?)
Ib Intercalary archeopyle	Brigantedinium☆, Lejeunecysta☆, Leipokatium☆ Selenopemphix☆, Stelladinium☆, Trinovantedinium☆, Xandarodinium☆, Protoperidinium latissinum, Peridinium ponticum
Ic Precingular archeopyle	Ataxiodinium☆, Impagidinium☆, Nematosphaeridium☆, Operculodinium☆, Spiniferites☆, Tectatodinium☆
Id Epicystal Archeopyle	Polysphaeridium☆
Ie Hypocystal Arcehopyle	Tuberculodinium☆
COMBINATION FORM	
If Intercalary Archeopyle	Protoperidinium (Archaeperidinium)
Ig Precingular Archeopyle	Lingulodinium☆
Ih Apical+Intercaraly+Precingular Archeopyle	Lingulodinium☆
IIa~IIh Theropylic type	
BASIC FORM	
IIa,b Apical Archeopyle	Diplopelta parva, Diplopsalis lenticula, Peridinium wisconsinense, Gonyaulax verior(?) Protogonyaulax catenella(?), Protogonyaulax tamarensis(?), Protogonyaulax leei, Protogonyaulax perviana
IIc Intercalary Archeopyle	Diplopsalis lebourae
IId Epicystal Archeopyle	Zygabikodinium lenticulatum, Dubridinium cavatum☆, Dubridinium cassiculum☆, Dubridinium ulstrum☆, Dubridinium polygonum☆, Helgolandinium subglobosum, Fragilidium heterolobum(?)
IIe Hypocystal Archeopyle	Pyrophacus horologium (?)
COMBINATIOM FORM	
IIf,g Intercalary Archeopyle	Gotius abei, Scrippsiella trochoidea Scrippsiella sweeniae, Ensiculifera cf. mexicana
Apical(?)+Intercalary Archeopyle	Diplopsalopsis orbicularis
IIh Transapical Archeopyle	Peridinium limbatum
IIIa~IIIe Cryptopylic Archeopyle	
IIIa,b Chasmic Archeopyle	Cochlodinium sp., Gymnodinium catenatum, Gymnodinium pseudopalustre, Pheopolykrikos hartmannii
IIIc,d,e Tremic Archeopyle	Polykrikos schwartzii, Polykrikos kofoidii Gyrodinium instriatum

☆:name for cyst-based taxa.

Table. III Key to modern dinoflagellate cysts based on shape

1 Cordate in dorso-ventral view ----- Protoperidinium oblongum
----- Protoperidinium claudicans (Votadinium*)

1 Peridinioid in dorso-ventral view and compressed antero-posteriorly
----- Protoperidinium conicum
----- Protoperidinium nudum
----- Protoperidinium subinerme (Selenopemphix*)

1 Roundly polygonal with hollow processes distally closed
----- Protoperidinium divaricatum (Xandarodinium*)

1 Subspherical to ovoidal with well-developed parasuture
----- Gonyaulacaceae (Impagidinium*)

1 Subspherical endophragm (inner body) with membranous periphragm
----- Gonyaulax spinifera complex (Ataxiodinium*)

1 Discoidal with many short barrel-shaped processes
----- Pyrophacus steinii (Tuberculodinium*)

1 Ellipsoidal with coarsely reticulate ornaments on surface
----- Polykrikos schwartzii

1 Ellipsoidal with shelf-like or hollow processes
----- Polykrikos kofoidii

1 Ovoidal and transparent phragma, sometimes with mucilaginous material
----- Gyrodinium instriatum
----- Gonyaulax verior

1 Ellipsoidal and transparent phragma, sometimes with mucilaginous material
----- Protogonyaulax catenella
----- Protogonyaulax tamarensis

1 Spherical and transparent phragma, sometimes with mucilaginous material
----- Protogonyaulax leei
----- Protogonyaulax perviana
----- Triadinium pseudogoniaulax
----- Diplopsalopsis orbicularis

1 Spherical to ovoidal ---------- 2
1 Spherical to ovoidal with processes densly distributed ------ 3
1 Peridinioid (pentagonal to stellar) in dorso-ventral view --- 4
1 Spherical to ellipsoidal with well developed paratutures and processes ---------- 5

2 Ovoidal with two phragma well adpressed (simple precingular archeopyle)
----- Gonyaulax spinifera complex (Tectatodinium*)

2 Subsphaerical with two phragma well adpressed (combination precingular archeopyle)
----- Gonyaulax spinifera complex (Bitectatodinium*)

Table IIIcont.

2 Spherical to subspherical with a single brouwn layer (Intercalary archeopyle) ----- Protoperidinium avellana
----- Protoperidinium denticulatum
----- Protoperidinium punctulatum
----- Gotoius abei
(Brigantedinium*)

2 Subspherical, antero-posteriolly compressed with paracingular and parasulcus ----- Zygabikodinium lenticulatum
(Dubridinium*)

2 Spherical with finely reticulate surface
----- Gymnodinium catenatum

3 Evexate to bulbous processes closed distally
----- Gonyaulax polyedra
(Lingulodinium*)

3 Slender capitate processes closed distally
----- Protoceratium reticulatum
(Operculodinium*)

3 Short denticulate to patulate processes
----- Pyrodinium bahamense
(Polysphaeridinium*)

3 Calcareous wall with acuminate to conical processes
----- Scrippsiella trochoidea

3 Brownish cyst wall with long acuminate processes
----- Protoperidinium minutum
----- Diplopelta parva

3 Brownish cyst wall with acuminate processes striated proximally
----- Pheopolykrikos hartmannii

4 Long spines developed on each corner of peridinioid shape
----- Protoperidinium compressum
(Stelladinium*)

4 Parasutural and intratabular short spines
----- Protoperidinium pentagonum
(Trinovantedinium*)

4 A single apical and two antapical horns well developed
----- Protoperidinium leonis
----- Protoperidinium latissinum
(Lejeunecysta*)

5 Parasutural furcate processes
----- Gonyaulax spinifera complex
----- Gonyaulax scrippsae
----- Gonyaulax sp.
(Spiniferites*)

5 Parasutural furcate processes connected with ectophragm
----- Gonyaulax spinifera complex
(Nematosphaeridinium*)

*: name for cyst based taxa.

Table IV

Key to modern dinoflagellate cysts based on shape and archeopyle type

1 Sahophylic archeopyle ------ 2
1 Theropylic archeopyle ------ 11
1 Cryptophylic archeopyle ---- 14
2 Epicystal archeopyle ----- Polysphaeridium
2 Hypocystal archeopyle ----- Tuberculodinium
2 Intercalary archeopyle ----- 3
2 Precingular archeopyle ----- 7
2 Combination archeopyle ----- 10
3 Cordate in dorso-ventral view ---------- Votadinium
3 Spehrical and brownish autophragm without ornament ----- Brigantedinium
3 Peridinioid with dome-like epicyst and two antapical horns ----- 6
3 Brownish wall with several spines ---------- 4
3 Peridinioid with a single apical and two antapical horns-------- 5
4 A single apical, two antapical and a few cingular spines --- Stelladinium
4 Roundly hexagonal, antero-posteriorly compressed ---------- Selenopemphix
4 Subcircular to ellptical in dorso-ventral view with hollow spines closed distally ---------- Xandarodinium
5 Transparent wall with intratabular and parasutural spines - Trinovantedinium
5 Epicyst triangular in dorso-ventral view with distinct apical and antapical horns ---------- Lejeunecysta
6 Two small antapical horns ---------- Leipokatium
6 A single broad antapical boss ------- Selenopemphix
7 Various ornaments and processes ----- 8
7 No ornament ----- 9
8 Parasutural furcate processes and coasely reticulate ectophragm ---------- Nematosphaeropsis
8 Furcate gonal and/or processes ----- Spiniferites
8 Slender capitate processes --------- Operculodinium
9 Subspherical with thick spongy cyst wall ----- Tectatodinium
9 Spherical to ovoidal with distinct paratabulation indicated by parasutural septa ---------- Impagidinium
9 Subspherical endophragm covered with membranous periphragm ---------- Ataxiodinium
10 Spherical, without ornament and with operculum corresponding two precingular paraplates ----- Bitectatodinium
10 Spherical with bullbous processes and opercula corresponding four precingular and/or anterior intercalary and apical paraplates ---------- Lingulodinium
11 Spherical with brownish wall and acuminate processes, and apical archeopyle ---------- Diplopelta parva*
11 Spherical with brownish wall and apical archeopyle ---------- Diplopsalis lenticula*
11 Spherical with brownish wall and a simple intercalary archeopyel ---------- Diplopsalis lebourae*
11 Subspherical to lenticular with epicystal archeopyle ----- 12
11 Combination archopyle ---------- 13

TableIV cont.

12 Brounish wall with distinct paracingulum --- Dubridinium
Zygabikodinium lenticulatum*
12 Thin and smooth transparent wall ---------- Helgolandinium sunglobosum*
---------- Fragilidium heterolobum(?)*
13 Subspherical with opercula comprising apical and anterior intercalary paraplates ---------- Diplolsalopsis orbicularis*
13 Subspherical to lenticular, with opercula consisting of two anterior intercalary paraplates ---------- Gotoius abei*
13 Ovoidal with calcareous wall and ornaments ----- Scrippsiella trochoidea*
13 Transapical archoepyle ---------- Peridinium limbatum*
14 Tremic archeopyle (hole type) ----- Polykrikos schwartzii*
---------- Gyrodinium instriatum*
14 Chasmic archeopyle (slit type) ----- Pheopolikrikos hartmannii*
---------- Gymnodinium catenatum*
---------- Gymnodinium pseudopalustre*

*: Species name for motile form.

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