

THE UNIQUE, MICRORETICULATE CYST OF THE NAKED DINOFLAGELLATE *GYMNODINIUM CATENATUM*¹

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ABSTRACT

Gymnodinium catenatum Graham is an unarmored dinoflagellate responsible for episodes of paralytic shellfish poisoning. This species forms a resting cyst that is unique in several ways. The outer surface of the spherical, brownish cyst is microreticulate and composed of hundreds of 1–3 μm polygons. In several regions, these polygons are smaller, more uniform in shape, and oriented in distinct bands that define morphological features. These features on the cyst reflect the cingulum, sulcus, flagellar pore complex, and acrobase of the motile stage precursor to the cyst. The archeopyle is irregularly but extensively developed. Its margin is generally smooth and extends almost completely around the circumference of the cyst, though not consistently in the plane of the equator. The cyst wall is resistant to acetolysis and standard palynological preparation techniques. *Gymnodinium catenatum* Graham is emended to include the details of the cyst stage.

The significance of this cyst is that it is the first described cyst of a naked dinoflagellate that bears oriented surface ornamentation reflecting features of the motile dinoflagellate. Its microreticulate surface ornamentation is unique to dinocysts, naked or armored, living or fossilized. Resistance of the cyst wall to harsh processing techniques suggests the presence of sporopollenin-like material commonly associated with cysts of armored dinoflagellates. From an ecological standpoint, the existence of a *G. catenatum* cyst has important implications with respect to species bloom dynamics and geographic distribution. In addition, the distinct differences between this cyst and those of the armored saxitoxin-producing gonyaulacoid species argues against a proposed evolutionary linkage.

Key index words: cyst; dinoflagellate; *Gymnodinium catenatum*; microreticulation; paralytic shellfish poisoning; PSP; saxitoxin; toxicity

The naked dinoflagellate *Gymnodinium catenatum* Graham is the subject of active research in several

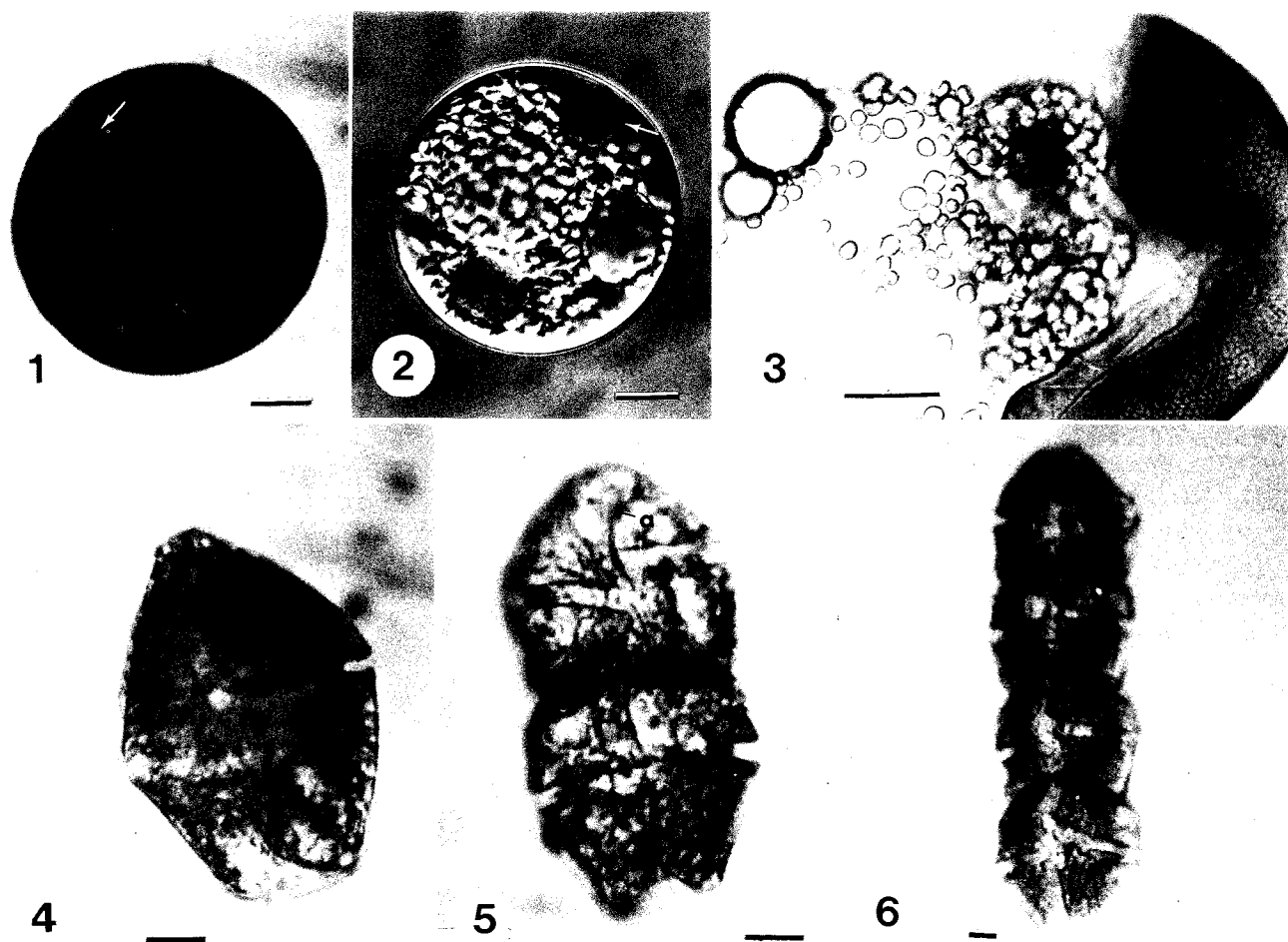
countries because of its recent association with outbreaks of paralytic shellfish poisoning (PSP). Although the organism was first described over forty years ago (Graham 1943), episodes of shellfish toxicity have been linked to this species only within the last decade. The first such outbreaks were in 1976 in Spain (Estrada et al. 1984) and in 1979 in Mazatlán, Mexico, where a bloom affected 200 km of coastline in the Gulf of California and caused three human fatalities (Morey-Gaines 1982, Mee et al. 1986). More recently, shellfish toxicity coincided with *G. catenatum* blooms in Spain in 1985 and 1986 (unpubl. data) and in Tasmania (Hallegraeff and Sumner 1986). The historical absence of similar toxic events in these regions has fueled speculation that the species may have been introduced via boat traffic, shellfish seeding, or other human activities.

Analysis of the toxin composition of shellfish from these outbreaks revealed the presence of several neurotoxins from the saxitoxin family (Luthy 1979, Morey-Gaines 1982, Hallegraeff and Sumner 1986, Mee et al. 1986, J. Sullivan, pers. comm.). Since these toxins are the same as those produced by certain armored dinoflagellates in the "tamarensis" complex (Taylor 1975), and since *G. catenatum* forms chains and has other morphological features similar to *Gonyaulax catenella* (= *Protogonyaulax catenella* = *Alexandrium catenella*) from this complex, it has been suggested that *G. catenatum* may be evolutionarily linked to the armored forms, despite its generic separation on the basis of cell wall type (Morey-Gaines 1982, Steidinger and Baden 1984).

We describe here the existence of a resting cyst for *G. catenatum*. This report is important not only because such a life history stage can have a major influence on the ecology and geographic distribution of this toxic species, but also because it provides yet another morphological feature which can be used to evaluate proposed evolutionary linkages between this naked dinoflagellate and armored species. There is also considerable paleontological interest in this cyst because it is from a naked dinoflagellate and

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FIGS. 1-6. Light micrographs of the cyst and motile stages of *Gymnodinium catenatum*. All scale bars = 10 μm . FIG. 1. Live cyst within brown cyst wall. Note dark (red pigment) spot or accumulation body (arrow). FIG. 2. Cyst protoplast; note accumulation body (arrow) and dinokaryotic nucleus (lower left). FIG. 3. Ruptured cyst showing wall reticulations (phase optics). FIG. 4. Excysted germling; note characteristically squared-off apex. FIG. 5. Two-cell stage, showing acrobase (a) and girdle displacement. FIG. 6. Four-cell stage.

has a highly resistant, presumably fossilizable wall and a unique morphology which includes a brown color, hundreds of small surface reticulations, and reflected markings from the unarmored motile stage precursor.

MATERIALS AND METHODS

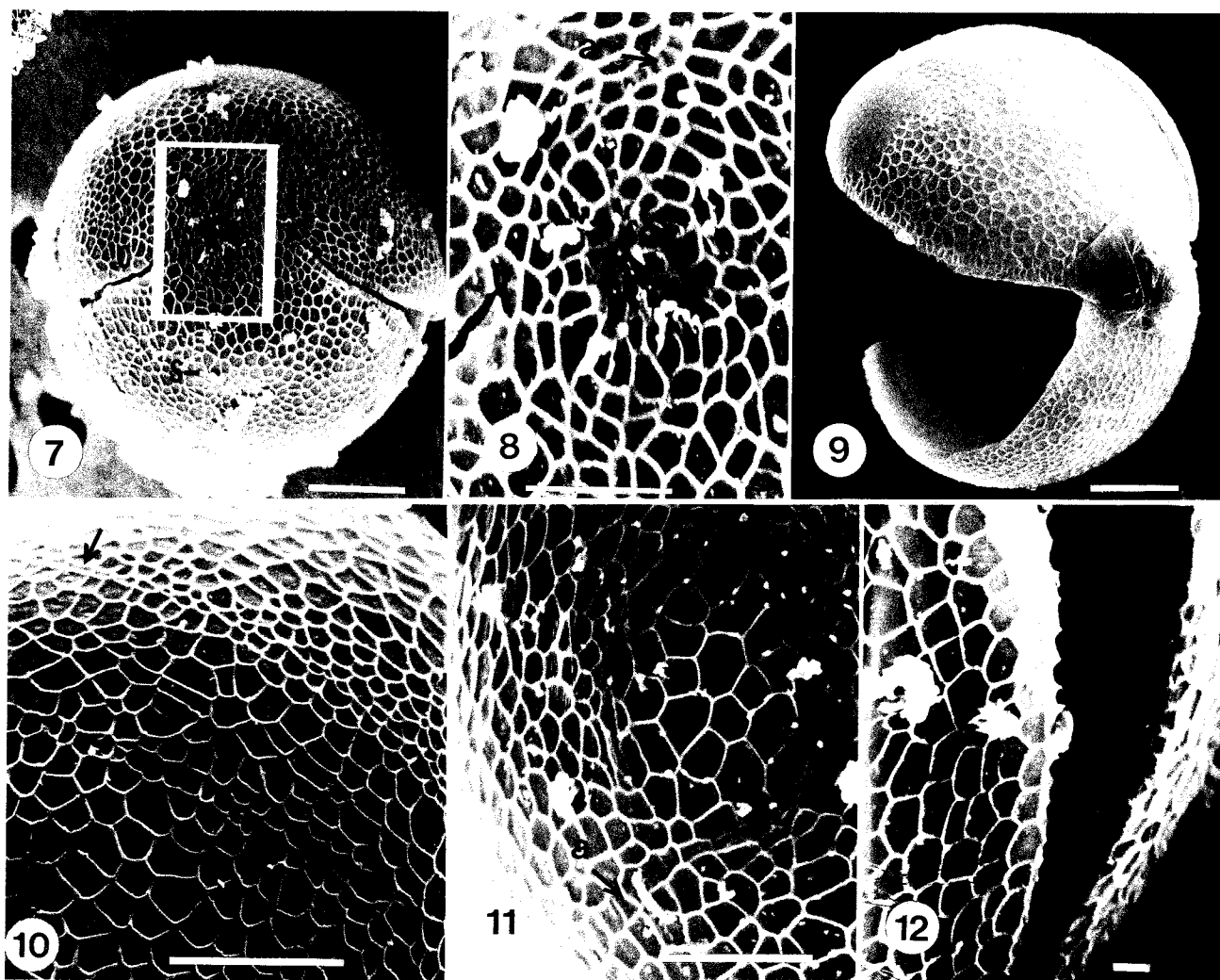
Cyst formation. The cysts used in this study were formed during laboratory incubation of a plankton sample collected during a *G. catenatum* bloom in Ria de Vigo, Spain. The naturally-occurring cells were collected in November 1985 and October 1986 in plankton net tows (42°14' N, 8°50' W) which were then diluted five-fold with filtered seawater enriched with f/2 levels of vitamins, metals and EDTA (Guillard and Ryther 1962). The diluted samples were incubated for several weeks at 18° C on a 14:10 h L:D cycle. Cysts formed during this process were removed with other settled material and stored in the dark at 4° C until needed.

Cyst germination. Subsamples from the stored cyst/detritus sample were disaggregated with an ultrasonic probe and sieved to retain the 20-80 μm size fraction. Cysts were then isolated by micropipette and placed in individual wells of tissue culture plates in 150 μL of k medium (Keller and Guillard 1985) at 20° C on a 14:10 h L:D cycle (100 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). All measurements of cysts and emerging cells were made with a stage micrometer.

Fixation and scanning electron microscopy. Fifty to 100 cysts were individually isolated by micropipette, fixed with 2% glutaraldehyde for 24 h at 4° C, rinsed with seawater, and postfixed in 1% OsO_4 for 1 h. After ethanol dehydration, cysts were CO_2 critical point-dried, Au-Pt sputter coated, and examined with a JEOL electron microscope.

Sample processing. Cyst/detritus material was subjected to acetolysis treatment as follows: 15 mL of sample was centrifuged, the supernatant removed and the pellet mixed with 6 mL of glacial acetic acid and centrifuged again. This procedure was repeated twice. Then 4.5 mL of acetic anhydride and 0.5 mL of concentrated H_2SO_4 were added to the pellet and the mixture placed in a boiling water bath for 20 min. After several washings with acetic acid and finally distilled water, the acetolyzed pellet was resuspended in distilled water and examined under the microscope for resistant cyst walls.

Plankton and bottom mud samples were also subjected to standard palynological processing techniques (Barss and Williams 1973). These rigorous procedures are used to extract fossil dinoflagellate cysts from sediments and solid rock. Each sample was washed to remove salt water, placed in 90 mL of 10% HCl (to remove calcareous material), washed to neutrality, placed in 90 mL of 52% hydrofluoric acid (to remove siliceous material) and again washed to neutrality. The samples were then placed in 10% hot HCl in order to remove any calcium fluoride that might have



FIGS. 7–12. *Gymnodinium catenatum* cyst morphology (SEM). Scale bars = 10 μm unless specified. FIG. 7. Cyst, ventral view. Flagellar pore region (boxed) enlarged in Figure 8. Note paracrobasis tail (a), paraculcal row of paravesicles (s) and paracingulum (c). FIG. 8. Flagellar pore region with tail of paracrobasis (a). Scale bar = 5 μm . FIG. 9. Ruptured cyst revealing smooth interior surface. Protrusion on right side is an artifact. FIG. 10. Dorsal region of paracingulum. Note narrow margin paravesicles (arrow). Scale bar = 5 μm . FIG. 11. Paracrobasis (a) situated on cyst apex. Scale bar = 5 μm . FIG. 12. Cyst wall rupture along paracingulum, showing wall thickness. Scale bar = 1 μm .

formed during the previous steps and washed to neutrality. The samples were oxidized in 70% nitric acid, washed to neutrality and screened through a 20 μm mesh sieve using an ultrasonic probe. Permanent slides were made of the >20 μm size fraction using Clearcol as a mounting medium.

RESULTS

Cyst description. Incubation of plankton samples from two *G. catenatum* blooms resulted in the formation of numerous spherical cysts approximately 50 μm in diameter (Fig. 1). Contents were difficult to discern through the cyst wall, but with slight pressure on the cover glass the cytoplasm could be forced from the outer covering, revealing the typical characteristics of other dinoflagellate cysts—a thin, inner wall, starch grains, lipid bodies, cytoplasm in Brownian motion, and a red pigmented accumulation body (Fig. 2). The actual cyst wall or autophragm (Evitt 1985) was pigmented brown, with nu-

merous microreticulations over its entire surface in a pattern similar to the surface of a canteloupe (Figs. 3, 10). Individual polygons varied in size (from 1–3 μm) and shape (3–8 sides), numbering nearly 1000 per cell (Fig. 7). Closer examination with SEM showed that the surface markings do not extend to the inside surface of the wall (Fig. 9). The wall thickness is approximately 0.6 μm (Fig. 12).

To clarify the following discussion, a complete ventral view of the *G. catenatum* cyst is sketched in Figure 13. A noteworthy characteristic of the surface markings on the cyst is that they reflect certain features of the motile cell. Following the terminology of Evitt et al. (1977) and Evitt (1985), they will hereafter be referred to with the prefix “para.” The small polygons provisionally can be termed paravesicles since they appear to be reflections of the cortical vesicles described for the motile stages of

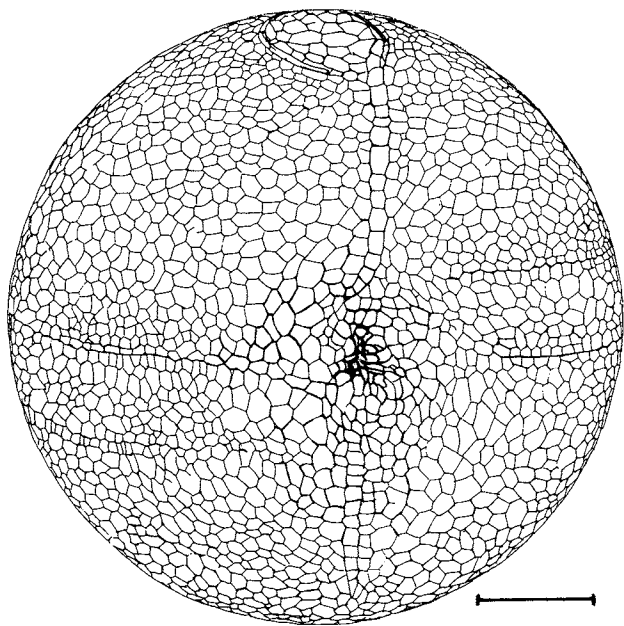


FIG. 13. Sketch of ventral surface of *G. catenatum* cyst. Scale bar = 10 μm .

numerous unarmored dinoflagellates (Biecheler 1952, Chatton 1952, Netzel and Durr 1984). Such features have not yet been described for *G. catenatum* motile cells, although smooth mounds of the same general size and number have been observed over the entire surface of certain SEM preparations of cells of this species (T. Rees, G. Hallegraeff, pers. comm.). The paracingulum can be readily recognized from the pattern of individual paravesicles (Figs. 7, 10). The outer edges are prominent because the paravesicles in those areas are smaller, more uniform in shape, and form continuous, straight lines rather than being randomly oriented as is the case elsewhere on the general cyst surface. The paracingulum has a displacement of 1–2 cingulum widths (Fig. 7).

Other reflected features include an 8–10 μm diameter flagellar pore region (Fig. 8), a parasulcus marked by a single ordered row of paravesicles extending into the antapex (Fig. 7), and a loop structure extending from the flagellar pore area to the apical region (Fig. 11). This latter feature is clearly a reflection of the acrobase found on many naked dinoflagellates including *G. catenatum* (Morey-Gaines 1982, Estrada et al. 1984) and hereafter is termed the paracrobase.

The cyst wall ruptures relatively easily with sonication or simple mechanical pressure but is resistant to standard acetolysis. A more rigorous palynological treatment with hydrochloric, hydrofluoric and nitric acids did not destroy the cyst wall, although it did change the brown coloration to an amber or light yellow tone.

No consistent archeopyle is present in germinated cysts. Ruptures often follow a paracingular margin

for some of their distance, but non-oriented tearing of the cyst wall is also common (Figs. 3, 9, 12). Matsuoka (1985) termed this a "chasmic" archeopyle.

Cyst germination. When a cyst germinates, a single cell emerges that has a morphology notably different from the typical *G. catenatum* vegetative cell. The golden-brown pigmented germlings are longer than they are wide, averaging $44 \times 56 \mu\text{m}$, and are easily recognized due to a flattened area at the top of the epicone and a deep invagination in the sulcal area of the hypocone (Fig. 4). Two longitudinal flagella are present, as is the red accumulation body seen in the cyst. As a result of the first division, the daughter cells become smaller and less elongate, averaging about 40 μm in diameter (Fig. 5). Subsequent divisions produce long chains of vegetative cells that are as wide as they are long, also averaging 40 μm in diameter (Fig. 6).

DISCUSSION

Morphological examination of motile cells in cultures established from our cysts indicate that the species is *G. catenatum* Graham as described by Estrada et al. (1984) and Fraga and Sanchez (1985). The cells have the same excavated cingulum and sulcus, cingular displacement, central nucleus, and acrobase in the epicone. These characteristics also coincide with the description of this species by Morey-Gaines (1982) but differ from the original description by Graham (1943) in that the cingulum is longitudinally displaced and chloroplasts are present. The latter were probably bleached in Graham's preserved material. Our cultures also differ from Balch's (1964) description with respect to the shape of the cells. This is not considered a diagnostic difference since major changes in cell shape were observed under varying nutrient regimes (Reguera and Yentsch, unpubl. data). No detailed description of the Tasmanian *G. catenatum* is available at this time, but it will likely be shown to be the same species since cysts collected from Tasmanian sediments appear to be identical to those described here (Hallegraeff, pers. comm.).

The cyst of *Gymnodinium catenatum* is unique in several ways. First, its surface reticulation is different from that seen on any living or fossil dinoflagellate cyst. Second, these markings reflect features from the cell wall of the motile stage of this naked dinoflagellate. Reflected markings are quite common on dinoflagellate cysts, especially in many fossil forms (Evitt 1985), but to our knowledge a reflected acrobase, minute vesicles, or other surface features common to naked dinoflagellates have not been reported. The fact that the *G. catenatum* cyst wall withstands acetolysis and standard palynological processing procedures is also noteworthy, especially in light of Wall and Dale's (1968) observation that the cysts of naked dinoflagellates are seldom very durable or resistant. We thus have an easily identifiable cyst that should be preserved in sediments for many

years and that in all probability could become fossilized as well. However, this cyst has not been previously reported by either neontologists or paleontologists. One possible explanation for this obscurity may be that the only published observations of *G. catenatum* motile cells are from four locations—Mexico (Graham 1943, Morey-Gaines 1982, Mee et al. 1986), Argentina (Balech 1964), Spain (Estrada et al. 1984, Fraga and Sanchez 1985) and Tasmania (Hallegraeff and Sumner 1986). We note also that a cyst similar to that described here was found in Omura Bay, Japan (K. Matsuoka, pers. comm.), but that the motile cells produced upon germination were tentatively identified as *Gymnodinium* sp. A₅ (Japan Fisheries Resource Conservation Association 1987). That species is now considered to be identical to *G. catenatum* (K. Matsuoka, pers. comm.). The known species distribution is clearly rather limited on a global basis. Since the motile stage forms long, easily-identified chains and produces a potent neurotoxin that accumulates in shellfish, its presence would be noted quickly in other parts of the world where phytoplankton are studied or where shellfish are consumed.

One interesting speculation (and an alternative explanation for the absence of this cyst in the fossil record) is that *G. catenatum* is evolutionarily derived from saxitoxin-producing species within the genus *Gonyaulax*. The arguments for this hypothesis were first presented by Morey-Gaines (1982) and include similarities in size, shape, nuclear orientation, toxin production, cingular displacement, wall layer type, and tendency to form chains. Morey-Gaines (1982) also described a possible smooth-walled cyst for *G. catenatum*, but the description was provisional since none of the cysts were germinated to produce an identifiable motile stage. We now believe that the cyst described by Morey-Gaines belongs to a different dinoflagellate species and is not *G. catenatum*.

The proposed evolutionary link between *G. catenatum* and the armored *Gonyaulax* species would certainly have been strengthened if the cysts were similar, but they clearly are not. *Gonyaulax catenella* and *G. tamarensis* cysts are smooth-walled, elongate, clear in color, and not resistant to acetolysis (Dale 1977, Anderson and Wall 1978, Turpin et al. 1978), whereas *G. catenatum* cysts have surface reticulations, are spherical, brown in color, and resist acetolysis. We acknowledge that these differences are not *a priori* proof that no direct evolutionary linkage exists between these species (i.e. that *G. catenatum* represents a strain of *Gonyaulax* that simply lost its ability to form cellulose thecal plates), but the hypothesis now seems less attractive.

In summary, we describe the resting cyst of *G. catenatum*, a naked dinoflagellate linked to several recent episodes of paralytic shellfish poisoning. The cyst is strikingly different from those of armored saxitoxin-producing dinoflagellates, arguing against a close evolutionary relationship. The cyst is also

noteworthy because it is resistant to harsh palynological processing techniques and because its surface markings reflect several features of the motile stage—two characteristics commonly attributed to the cysts of armored species. From an ecological standpoint, the existence of a *G. catenatum* cyst must now be recognized in research on the bloom dynamics and geographic distribution of this important species. Since the cyst wall is highly resistant and easy to recognize, even in fragments, it may be possible to examine deep sediments from cores in areas such as Spain or Tasmania to determine whether *G. catenatum* is a recently-introduced species, a common inference based on the sudden appearance of shellfish toxicity in those regions.

Systematics

Division Pyrrophyta Pascher 1914

Class Dinophyceae Fritsch 1929

Order Gymnodiniales Lemmermann 1910

Family Gymnodiniaceae (Bergh) Lankester 1885

Genus *Gymnodinium* Stein 1878

Gymnodinium catenatum Graham 1943 emend.

(Figs. 1–16)

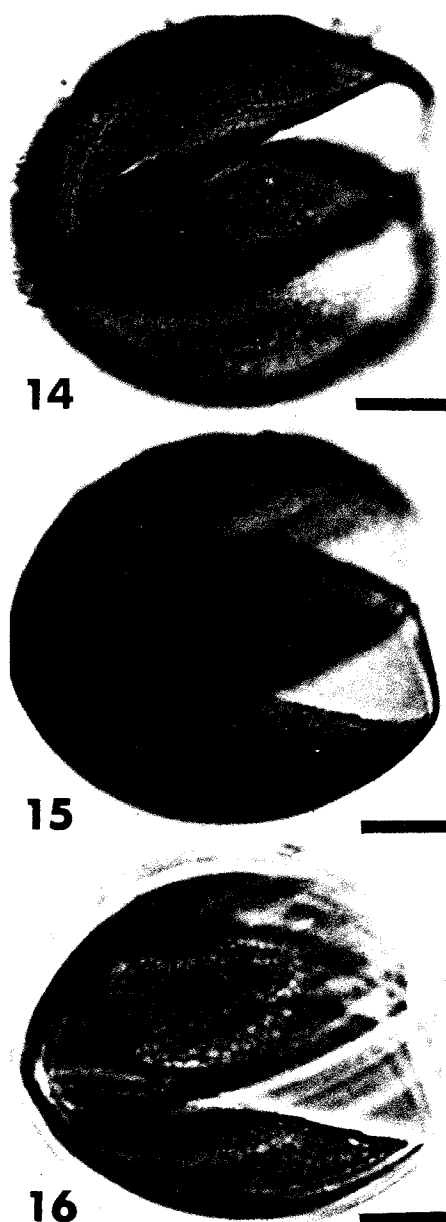
Gymnodinium catenatum Graham 1943:259–61.

Diagnosis. Small chain-forming dinoflagellate in which the cytoplasm is continuous throughout. Body circular to squarish in ventral view and subcircular in anterior view, with approximately a 40 μ m diameter. Color is golden-brown due to numerous small chloroplasts. Hypocone truncated posteriorly with a deep notch at sulcus; epicone broadly rounded in anterior cells or bearing a short apical protrusion in subsequent cells by which attachment to the cell ahead is made. An open circular to egg-shaped crest or acrobase up to 10 μ m in length is located on the ventral side of the epicone near the apex. Cell length to transdiameter ratio varies from 0.64–1.0. The wide girdle (0.16 transdiameters) is deeply impressed, median, and is displaced 0–1 girdle widths, and occur without striae or other surface markings. The large, centrally located nucleus is moniliform and slightly lunate.

The encysted stage is characterized by a thin walled, spherical cyst covered with an irregular microreticulum. The apex is marked by a subcircular to egg-shaped paracrobasis; a short line of oriented paravesicles extends posteriorly from the paracrobasis. The laevorotatory paracingulum is marked by two parallel rows of oriented paravesicles. The chasmic archeopyle is irregularly developed.

Cyst Description

Shape. Spherical, unless excystment has occurred. Excysted specimens may resemble taxodium-type pollen in that the cyst splits and gapes open to a varying degree subequatorially. The halves may or may not be attached along a narrow isthmus of cyst wall.



FIGS. 14–16. *Gymnodinium catenatum* Graham 1943 reference cyst. Scale bars = 10 μm . FIG. 14. Note small, oriented paravesicles trending subparallel to the upper margin of the archeopyle. Parasulcus lies along the left margin of the cyst. Low focus; bright field illumination. FIG. 15. Note the shape of the archeopyle margin and the line of small oriented paravesicles that are nearly coincident with the lower archeopyle margin. High focus; bright field illumination. FIG. 16. Note the shape of the paravesicles on the dinocyst surface. High focus; phase contrast illumination.

Phragma. Autophragm; internally smooth but covered externally by a dense microreticulum of low ($<0.2 \mu\text{m}$) rounded ridges. The ridges delineate minute three to eight sided paravesicles less than 3 μm across. Five and six sided paravesicles are the most common. The cyst surface within the paravesicles may bear submicron granulae (perforations were observed within some paravesicles but may be pres-

ervational artifacts). The paravesicles are randomly distributed over the cyst surface, except for oriented bands that delimit the paracingulum, parasulcus and paracrobasis.

Paratabulation. None evident.

Paracingulum. The paracingulum is bordered by two parallel bands of aligned paravesicles. Each band consists of two or more rows of oriented, primarily five or six sided paravesicles, although in some locations this pattern appears less distinct and the paravesicles more random (Fig. 10). These areas are approximately the same size, whereas those forming the general microreticulum vary in shape and range greatly in size. The adequatorial row of each band generally consists of five or six sided, often gabled paravesicles, the gables of which point toward the equator. The opposite side of each gabled paravesicle is flat or nearly so. The alignment of these flat sides emphasizes the straightness of the oriented row of paravesicles. The other row of each parallel band, the adpolar row, often consists of narrow paravesicles that are elongate parallel to the cyst equator (Fig. 10). Sometimes the small paravesicles of the adpolar row are elongate transverse to the cyst equator (Fig. 7). Most paravesicles are four to six sided, and one of their long sides is shared with the flat side of a gabled paravesicle area. There is considerable range in the shape of the paravesicles in both rows, but the adpolar row is composed of smaller paravesicles than is the adequatorial row.

Parasulcus. The parasulcus is marked by a longitudinal line of approximately 15 oriented paravesicles which begin at the lower margin of the flagellar pore region and extend approximately 15 μm toward the antapical pole. The reflected flagellar pore region is marked by a “whorled” pattern of paravesicles that seems to have two central foci (Fig. 8). The region is oblong and approximately $10 \times 8 \mu\text{m}$ in size.

Paracrobasis. The circular to egg-shaped paracrobasis is approximately 10 μm long and almost surrounded by two parallel rows of minute, elongated polygonal paravesicle areas. The small, elongate polygons merge with larger paravesicles towards the adcingular margin of the acrobasis. The paravesicles within the acrobasis margin are as large or larger than those outside the margin. These paravesicles are more or less circularly arranged around the innermost paravesicles of the acrobasis. A short (10–15 μm) row of small, four to six sided oriented paravesicles extends posteriorly from the acrobasis. This row of paravesicles, or tail, is reminiscent of those bordering the paracingulum, though it is simpler and shorter. The tail points toward the flagellar pore region and the break in the track of the paracingulum. The posterior terminus of the tail is bent at an angle of 20–40° to the right as it reaches the flagellar pore region. At this point the tail is opposite the anterior margin of the paracingulum.

Archeopyle. The cyst splits roughly in half. The

archeopyle margin often coincides with one edge of the paracingulum, but in many instances the tear occurs randomly. It has the characteristics of a chasmic archeopyle (Matsuoka 1985). A step-like feature is often evident along one part of the archeopyle margin. The significance of this is unknown and the feature does not seem to be consistently related to other surface characteristics of the cyst.

Dimensions. Diameter: 38–60 μm (mean 49 μm); wall thickness: 0.6–1 μm ; polygonal areas, longest dimension: 3 μm . Twenty specimens were measured.

Reference cyst. The cyst reference specimen (Figs. 14–16) was formed in an incubated plankton sample collected from a bloom off Ria de Vigo, Spain. Slide 5, specimen coordinates on Zeiss Universal Microscope No. 038295, with a rotating stage, are: 102.9X 22.7. (Alternatively, the specimen location is 7.1 mm right of and 0.1 mm below the upper left hand corner of the cover glass.) The slide containing the reference cyst resides, temporarily, in the slide collection of J. H. Wrenn.

Comments. This species diagnosis is emended to include, for the motile form, the presence of an acrobase on the ventral side of the epicone and golden-brown chloroplasts scattered throughout the cytoplasm. In addition, this form includes a resistant, microreticulate cyst which bears morphologic features comparable to those on the motile stage. The microreticulum is clearly seen through a compound microscope at a magnification of $\times 400$ or more (Fig. 3), though some of the details of the oriented rows of paravesicles are best seen with a scanning electron microscope. For example, the narrow rows of elongate paravesicles bordering the acrobase are evident on SEM photomicrographs but look like dark lines or shadows through the compound light microscope. The paravesicles bordering the paracingulum, which are also evident on SEM photomicrographs, look like sewing-machine stitching or postage stamp perforations under phase illumination.

Cyst color changed from brown or grey-brown to amber or light yellow during palynological processing in HCl and HF acids, but before oxidation with nitric acid. Folding and/or fracturing of the cysts commonly occurred during sample processing. Protoplasm was noted within some cysts, both before and after processing. Accumulation bodies were present in some open cysts after processing, commonly remaining associated with cysts even if they were broken in half, as if they were in some way attached to the cyst wall. These were similar to brown accumulation bodies noted in the literature treating fossil dinoflagellate cysts.

Comparisons. The cysts are reminiscent of the round-brown cysts of protoperidiniacean taxa such as *Brigantedinium*, *Diplopsalis*, *Diplopsalopsis*, and *Zygabikodinium*. However, *G. catenatum* differs from those taxa by having a microreticulate surface, an irregular archeopyle, and bands composed of two

or more rows of oriented paravesicles that reflect morphological features of the motile stage including a tail-bearing acrobase.

Known stratigraphic range. Holocene.

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