

Distribution of microreticulate dinoflagellate cysts from the Galician and Portuguese coast*

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SUMMARY: In May 1993, surface sediment samples from the west coast of the Iberian Peninsula were collected with the aim of studying the geographic distribution of *G. catenatum* resting cysts. The sampling area ranged from 40° 39' N (Portugal) to 42° 38' N (Galicia, Spain). Cysts with exactly the same morphology as *G. catenatum* were found in a maximum concentration of 504 cysts·cm⁻³ of sediment. Similar cysts but smaller (24-36 µm) were found in a maximum concentration of 4488 cysts·cm⁻³ of sediment. A bimodal size distribution of microreticulate cysts from the sediment indicates the probable existence of two cyst species. Differences in the microreticulation of the paracingulum were also observed. We compared the cysts from the sediment with *G. catenatum* cysts produced in cultures. The study on the distribution of cysts in the sediment also showed significant differences in the vertical profile concentrations of both cyst types. Differences in both the size and microreticulation morphology of the cysts found in the sediment of the continental shelf of Galicia and Portugal are also observed in the literature concerning other countries. The cysts of this species cited for the coasts of Northern Europe present characteristics similar to the cysts referred to in this communication as *G. catenatum*-like or small microreticulate cysts. The need to clarify the identity of cysts with microreticulation in the wall is discussed in order to identify the geographical distribution of the *G. catenatum* cyst.

Key words: cyst morphology, microreticulate cysts, Galicia, *Gymnodium catenatum* cyst.

INTRODUCTION

Gymnodium catenatum Graham is a naked, chain-forming dinoflagellate causing PSP intoxications in several parts of the world (Hallegraeff and Fraga, 1998). It was associated with this type of episode for the first time in Galicia (NW Spain) in 1976, and has frequently been observed since then in these coastal waters and others in a wider area covering the entire west coast of the Iberian Peninsula. The *Gymnodium catenatum* cyst was first described by Anderson *et al.*, 1988; they reported a brown, spherical 38-60 µm diameter cyst (mean 49µm) from incubated plankton samples. These

authors noted the morphological characteristics for the *Gymnodinium catenatum* cyst which were unique in various aspects. This was the only described dinoflagellate cyst whose external wall has a microreticulation comprising hundreds of polygons measuring 1-3 µm. Also, the microreticulation adopts an orientation which reflects the morphological characteristics of the vegetative cell, namely the paracingulum, parasulcus and paracrobases.

After the original description of the *G. catenatum* cyst, the presence of cysts from this dinoflagellate has been noted in various parts of the world. Identification was based on the microreticulation cited as the main morphological characteristic. The *G. catenatum* cysts described by Blackburn *et al.*, (1989)

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and Bolch and Hallegraeff (1990) from Tasmania and Matsuoka and Fukuyo (1994) from sediment of Japan have the same morphology and size as in the original description (49 μm mean). Conversely, the cyst described for the north coasts of Europe in recent sediment and in older sediments are somewhat smaller (30-40 μm) than that of the original description (Dale *et al.*, 1993; Ellegaard *et al.*, 1993; Nehring, 1995). Bolch and Hallegraeff (1990) noted a third, still smaller, microreticulate cyst (17-22 μm), naming it *Gymnodium* sp. Recent genetic, morphological and ultrastructural studies have demonstrated that there are species other than *G. catenatum* which produce microreticulate cysts (Bolch *et al.*, 1998; Ellegaard and Moestrup, in press).

This paper describes two types of cysts, with microreticulation in the wall, found in the continental shelf sediment of Galicia and Portugal. A comparison is also made between the morphology and size of these cysts and those of the *G. catenatum* cyst produced in cultures isolated from Galician waters and those described in the literature.

MATERIAL AND METHODS

Sediment samples were collected on 24th and 25th May, 1993, in the area shown in Figure 1, between 40° 39' N (Portugal) and 42° 38' N (Galicia) at water depths of between 60 and 200 m. A box-corer was used for sampling and, once on board, a cylinder of 20 cm length x 3 cm base diameter was inserted into the sediment box to extract a subsample. Depending on the percentage of clay/lime matter, the box-corer penetrated to greater or lesser depth in the sediment, obtaining the deepest samples in the limy sediment stations. Therefore, vertical profile resulted in a sediment depth range from 0 to 8-16 cm. Samples of 5 cm³ were processed each 2 cm to quantify cyst concentrations.

The sediment suspended in filtered sea water was disaggregated with an ultrasonic bath for 10 minutes, sieved to retain the 20-75 μm size fraction, and suspended in 50 ml of distilled water. 10 ml of the suspension was centrifuged at a density gradient using a Ludox TM Colloidal Silica Tech (density of 1.4 g·cm⁻³). The gradient was performed according to a modification of the technique described by Blanco (1986). In order to avoid osmotic changes in processing cysts, a solution of distilled water with 24% saccharose was used to perform the gradients and cleansing of the sediment in place of distilled

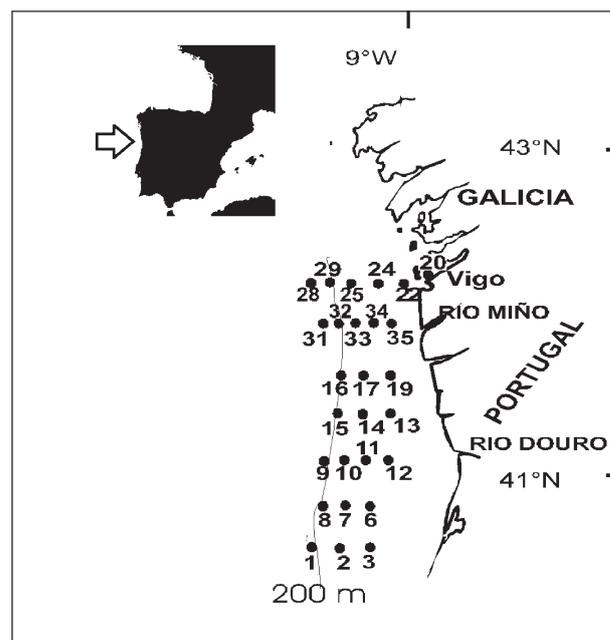


FIG. 1. – Position of the sampling area and stations where sediment samples were collected.

water, as described by that author. The density gradients were carried out in centrifuge tubes of 50 ml, where step gradient solutions of 100%, 80% and 60% Ludox TM were carefully delivered. 10 ml of sediment sample were added and centrifuged at 600 rpm for 15 min. To avoid possible bias in the results of concentrations of the different types of cyst due to their having different densities, all cysts of the whole fraction over 1.4 g·cm⁻³ density were counted. In addition, the loss of microreticulate cysts in the fraction remaining at the bottom was observed to be less than 1%.

The fraction separated with the cysts was resuspended in filtered seawater, microreticulate cysts (*G. catenatum* and *G. catenatum*-like or small microreticulate cysts) and total cysts (no microreticulate cysts included) were counted using the Utermöhl method (Utermöhl, 1958) without fixation.

G. catenatum cysts from culture were obtained by crossing two strains from the culture collection of the Centro Oceanografico de Vigo (Bravo *et al.*, 1998).

RESULTS

In the sediment samples, two types of cysts were observed with the microreticulation described for *G. catenatum*. Under inverted microscopy, the most

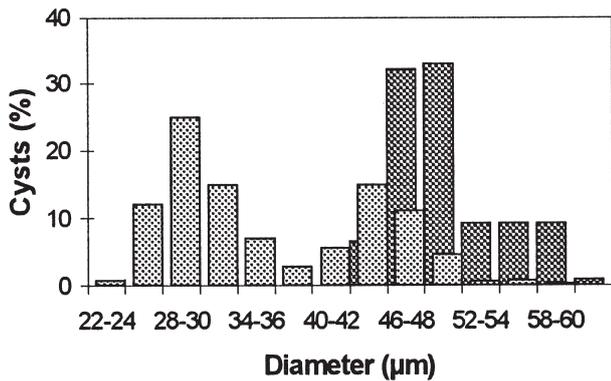


FIG. 2. – Size distribution of cysts,  microreticulate cysts from sediment.  *G. catenatum* cysts produced in cultures.

striking differentiating characteristic between the two cyst types was size, since brownish colour was very similar in both. Measurements of the diameters of 289 cysts were taken (Figure 2). All microreticulate cysts were classified into two groups depending

on size: *G. catenatum* cysts and small microreticulate cysts (*G. catenatum*-like cysts). Figure 2 shows a bimodal size distribution which corresponds to the two groups of cysts cited, one centred around 28-30 µm and another at 43-45 µm, while 36 µm was chosen as the diameter of separation between the two. This criterion of classification came out true on all occasions that the microreticulation was tested, though the possibility of a small overlap zone at around 36 µm in a very small number of cysts can not be discarded. Figure 2 also shows the size distribution

of *G. catenatum* cysts produced in the laboratory from cultures. The mean size of these cysts was 50 µm (SD= 3.95, n=108). Differences were also observed in the ornamentation of the outer wall in both types of cysts mentioned. The wall of cysts from the sediment corresponding to the larger size group shows characteristics which are exactly the same as those originally described for *G. catenatum* (Anderson *et al.*, 1988). Furthermore, both the size and the microreticulation (Figure 3a) are the same as the *G. catenatum* cysts produced in cultures. When these cysts are compared with the smallest ones (*G. catenatum*-like cysts, 24-36 µm, Figure 3b), differences are noted in microreticulation. The ornamentation of *G. catenatum*-like cyst walls is formed of a lower number of polygons, which together with the fact that these cysts are far smaller, makes them appear to have a less fine microreticulation than in *G. catenatum* cysts. The paracingulum of the smallest cysts has

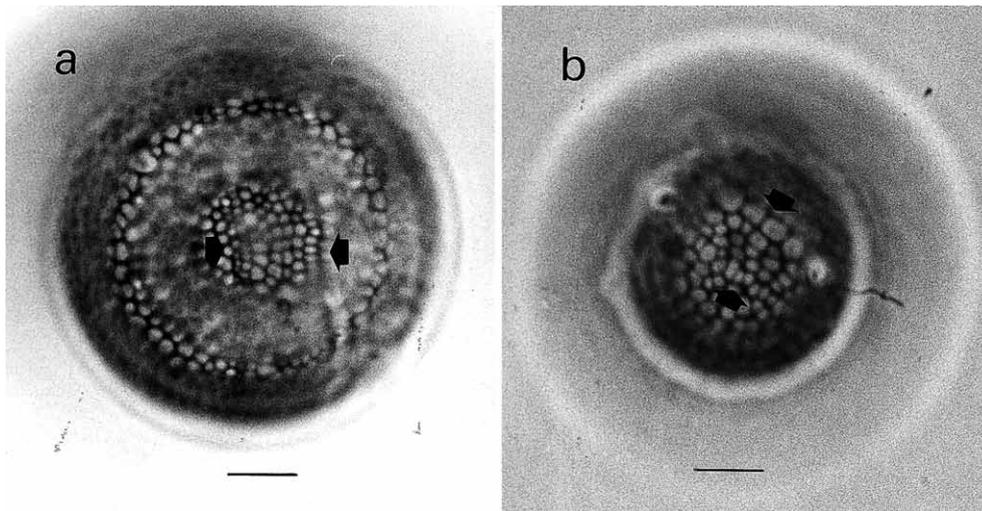


FIG 3. – Microreticulate cysts observed in the sediment from the Galician and Portuguese coast. **a)** *Gymnodinium catenatum* cyst (36-60 µm). **b)** small microreticulate cyst (24-36 µm). Arrowheads indicate the paracingulum in both cyst types. Bar = 10 µm.

fewer rows of polygons (five rows) than those described for *G. catenatum* (seven rows). Also, the polygons in the paracingulum are larger in the smallest cysts (up to 3.7 µm) than in *G. catenatum* cysts (up to 2 µm), as can be observed when comparing Figures 3a and 3b. Figure 4 shows the mean concentrations of both types of cysts in the upper 8 cm of the sediment. The values ranged from 0-504 cysts·cm⁻³ and revealed no notable differences between both types of cysts. If, however, the complete vertical profiles are com-

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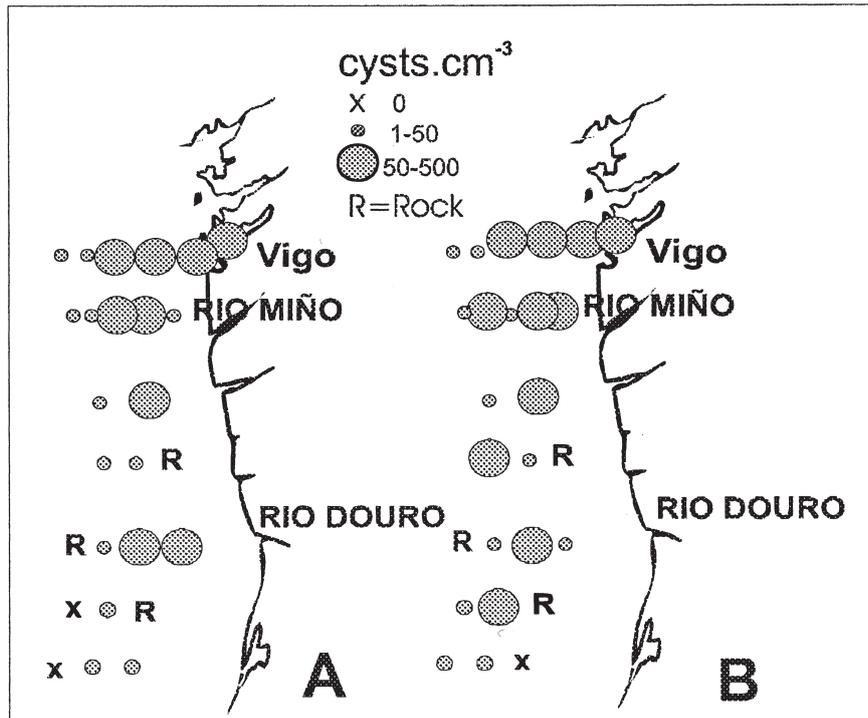


FIG 4. – Concentrations of microreticulate cysts in the upper 8 cm of the sediment (averaged values). A) *G. catenatum* cysts (36-60 μm); B) small microreticulate cysts (24-36 μm).

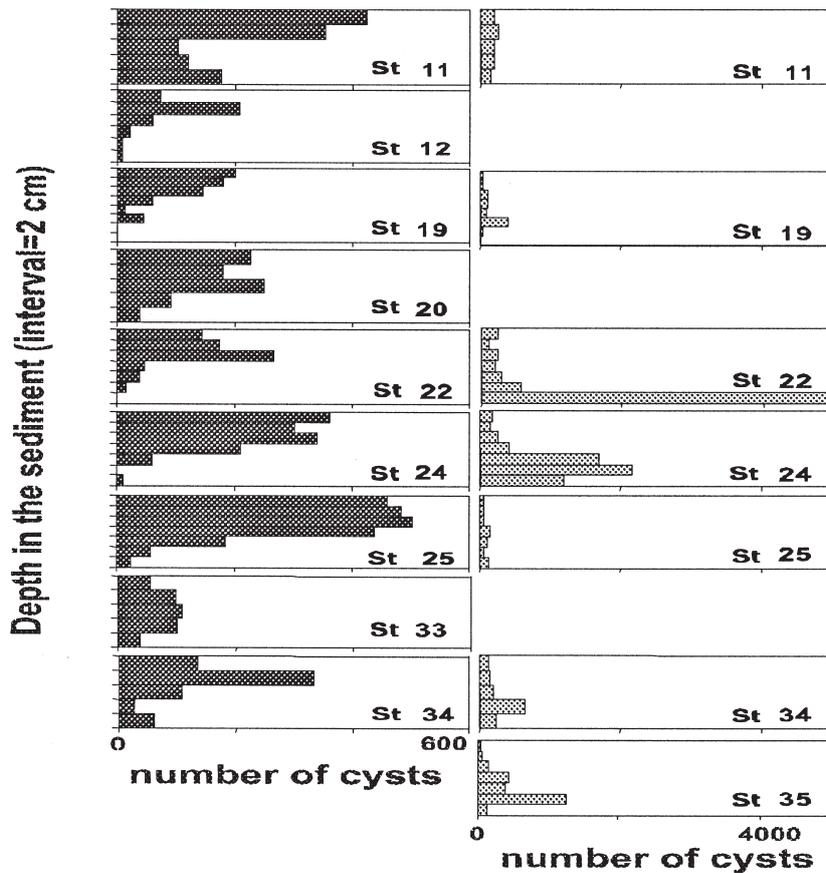


FIG 5. – Vertical distribution of microreticulate cysts in the sediment. ■ *G. catenatum* cysts (36-60 μm); ▨ small microreticulate cysts (24-36 μm).

pared (Figure 5), differences in depth distribution are observed. The concentration of *G. catenatum* cysts (36-60 μm) decreases with the depth in the sediment. Conversely, in the case of the small cysts (24-36 μm), the concentration is either uniform or shows a clearly defined increase in the sediment (Figure 5, see stations 22 and 24). The highest concentrations for the smaller 36 μm cysts were below 8 cm.

In the case of *G. catenatum* cysts, the maximum concentration achieved was 504 cysts $\cdot\text{cm}^{-3}$ of sediment at station number 25, off the Ría of Vigo at a sediment depth of 4-6 cm (Figure 5). *G. catenatum* cysts accounted for 38% of the total cysts. In the case of the smallest cysts, the maximum concentration was 4488 cysts $\cdot\text{cm}^{-3}$ at station 22, and at 12-14 cm depth, accounting for 88% of the total.

DISCUSSION

Widely varying interpretations may be deduced from the data presented here, depending on the identity of the microreticulate cysts. The authentic *G. catenatum* cysts identified were not over 504 cysts $\cdot\text{cm}^{-3}$ of sediment. The smallest microreticulate cysts presented concentrations of up to 4488 cysts $\cdot\text{cm}^{-3}$. Due to the fact that these high concentrations were found in depths of sediment of over 8 cm, the likelihood that they are involved in the initiation of a bloom appears low, whether they are *G. catenatum* or otherwise. It may be deduced, however, that the species (or *G. catenatum* morphotypes) to which the *G. catenatum*-like cysts belong was more abundant in previous decades. Also, the concentrations of cysts appear to reflect that the current form of *G. catenatum* is gradually becoming more abundant in these waters.

Blanco (1989) refers to two types of cyst in sediment from Galicia, which differ in size and are very similar to *G. catenatum*. The cyst he describes for this species appears to have more in common with what is termed *G. catenatum*-like in this article. The cysts mentioned by Bravo and Anderson (1994) in the cyst distribution data on this dinoflagellate in the Ria of Vigo (Galicia, Spain) we consider as belonging to the group identified as *G. catenatum*, i.e. in the 36-60 μm size range (Bravo, unpublished data). Although the role of *G. catenatum* cysts is unknown in the bloom dynamics of this species in the western coast of the Iberian Peninsula, it would be important to be able to quantify the cysts in the sediment in the

area. The description of *G. catenatum* cysts involved a step forward in the study of the bloom dynamics of this species. At present, however, it is necessary to clarify and differentiate the various types of cysts with microreticulation in the wall since there may be cases of erroneous identifications that could lead to mistaken interpretations.

In the literature where reference is made to *G. catenatum* cysts, both types of cysts mentioned in this text are also cited. There are descriptions which correspond exactly to the original description of the *G. catenatum* cyst, such as the description by Blackburn *et al.*, (1989) and Bolch and Hallegraeff (1990) for cysts observed in sediment from Tasmania. In that country, however, the last authors mentioned also refer to a cyst very similar, but smaller (17-22 μm) which they referred to as *Gymnodium* sp. 1. Recently Bolch *et al.*, 1998 has also reported a widespread distribution of three distinct size morphotypes of microreticulate cysts: the largest type (43-56 μm) corresponds to *G. catenatum* cysts, a "small-form" (17-28 μm) that produced small vegetative cells of a non-chain-forming Gymnodinioid genetically distinct from *G. catenatum* and an "intermediate-form" (36-43 μm) of cysts that germinated in typical chain-forming cells similar to those of *G. catenatum*.

The microreticulate cyst mentioned by several authors from Northern Europe and identified as *G. catenatum* due to its characteristic microreticulate wall is also undergoing revision. Ellegaard *et al.*, (1993) and Nehring, (1995) refer to *G. catenatum* cysts in sediment from the Danish and German coast, and Dale *et al.*, (1993) refers to a fossil cyst of this dinoflagellate in Scandinavian sediment. Recently Ellegaard and Moestrup (in press) describe the vegetative cells produced by germinating microreticulate cysts from Danish marine sediments as a new species named *Gymnodinium nolleri* Ellegaard et Moestrup *sp. ined.* These authors separate this species and *G. catenatum* on the basis of molecular sequencing, ultrastructure of the flagellar root system and morphological differences between the two cysts and vegetative cells. There would seem to be two species of dinoflagellates with microreticulate cysts already described: *G. catenatum* and *G. nolleri*. Besides, the Australian small-form cysts belong to other species which must be described (Bolch *et al.*, 1998).

The cysts size, colour and the characteristics of the paracingulum microreticulation of the smallest *G. catenatum*-like cysts we describe in the present

publication appear to be very similar to *G. nolleri* cysts described by Ellegaard and Moestrup (in press). To date, our attempts to germinate small *G. catenatum*-like cysts have been unsuccessful and since the species with microreticulate cysts are still not known definitively we are unable to determine which species we are dealing with. Nevertheless, further efforts should be made in order to be able to compare and distinguish between different dinoflagellate species producing a microreticulate cyst.

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