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MORPHOLOGICAL AND NUCLEAR ANALYSIS OF THE BLOOM-FORMING DINOFLAGELLATES *GYRODINIUM* CF. *AUREOLUM* AND *GYMNODINIUM NAGASAKIENSE*[1]

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ABSTRACT

Two red-tide forming naked dinoflagellates, the Japanese species Gymnodinium nagasakiense *Takayama et Adachi and the north European species* Gyrodinium *cf.* aureolum *Hulburt, were compared with regard to their external morphology, chromosome number and DNA content. Morphological studies were performed using both light and scanning electron microscopy; analytical flow cytometry was used to compare DNA contents. Our strains of* G. *cf.* aureolum *and* G. nagasakiense *were morphologically indistinguishable, and both closely fit the original description of the latter species. In contrast, several details, including the shape and position of the nucleus, differed between our strains and the original description of* Gyrodinium aureolum. *However, despite an equal number of chromosomes, the Japanese taxon possessed about 40% more DNA than the European one. Moreover, the latter taxon was able to form two subpopulations of vegetative cells, which we called "small" and "large," whereas the former one only formed "large" cells. These discrepancies cast doubt upon the conspecificity of* G. *cf.* aureolum *and* G. nagasakiense. *The appellation* Gymnodinium *cf.* nagasakiense *is therefore proposed for the species encountered in the northern European seas, waiting for a future reconsideration of both the genera and the species.*

Key index words: dinoflagellate; DNA content; flow cytometry; Gymnodinium nagasakiense; Gyrodinium aureolum; *species concept*

The morphological similarity of two marine naked dinoflagellates, *Gyrodinium aureolum* Hulburt and *Gymnodinium nagasakiense* Takayama et Adachi, has been often cited (Tangen 1977, Takayama and Adachi 1984, Taylor 1985, Partensky and Sournia 1986). Both species form red-tides frequently associated with mortalities of marine fauna (Helm et al. 1974, Iizuka 1979, Takayama 1981, Dahl et al. 1982).

Gyrodinium aureolum originally was found by Hulburt (1957) in a pond near Woods Hole (U.S.A.). In 1966, Braarud and Heimdal (1970) identified a dinoflagellate responsible for a huge bloom along the southern coast of Norway as *Gyrodinium aureolum.* Since then, the distribution of this dinoflagellate has been reported for all the northern European seas (45–65° N) and the specific epithet *aureolum* retained in later descriptions (Ballantine and Smith 1973, Tangen 1977, Boalch 1979, Ottway et al. 1979). However, the use of the more cautionary designation *Gyrodinium* cf. *aureolum* recently was suggested for European specimens until firm taxonomic features are found for the determination of this organism (Partensky and Sournia 1986).

Gymnodinium nagasakiense was initially called *Gymnodinium* type-'65 because it was first observed in the summer of 1965 during an extensive bloom in Omura Bay, Kyushu Island, Japan (Iizuka and Irie 1969). Like *G.* cf. *aureolum, G. nagasakiense* is known to be widely distributed. It has been found along many parts of the southwest coast of Japan (32–36° N) and recently in a bay of the south coast of Korea (Takayama and Adachi 1984). *G. nagasakiense* has also been implicated in fish kills along the coast of New Zealand in 1987 (G. M. Hallegraeff, pers. comm.).

Despite their different geographical distributions, a common origin or even a synonymy have been suggested for *G.* cf. *aureolum* and *G. nagasakiense* (Taylor 1985, Partensky and Sournia 1986). Very few data on the biochemistry, toxicology and life histories of these species, which could help solve the puzzle of conspecificity, are available. Biochemically, *G.* cf. *aureolum* is characterized by the presence of 19'-hexanoyloxyfucoxanthin as a major carotenoid, a pigment known only in a few species of dinoflagellates and in the coccolithophorid *Emiliania huxleyi* (Lohm) Hay et Mohler (Tangen and Björnland 1981). Preliminary HPLC analyses suggest that *G. nagasakiense* also contains this particular carotenoid (F. Partensky and S. Roy, unpubl. data). Crude ethanolic extracts from cultures of both taxa have a similar slight cytotoxicity on cultured human cells. Although partial purification has confirmed the presence of active biotoxins within *G.* cf. *aureo-*

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lum cells, these compounds are very scarce and have yet to be characterized (F. Partensky and J. F. Verbist, unpubl. data). Finally, the sexual life history of both these organisms is still unknown.

The present paper focuses on the comparison of some morphological and nuclear features of *G. nagasakiense* and *G.* cf. *aureolum* to tentatively decide if they are conspecific.

MATERIAL AND METHODS

Culture conditions. Three unialgal strains of *Gyrodinium* cf. *aureolum* were used for this study. Strain PML-497A, isolated from the Plymouth (United Kingdom) area, was kindly provided by Dr. J. C. Green (Plymouth Marine Laboratory). Strain Iroise was isolated in August 1986 during a cruise off Ushant (Brittany, France). Strain Tinduff was isolated in July 1987 from aquaculture tanks naturally contaminated by *G.* cf. *aureolum* (Tinduff shellfish farm, Brest Bay, Brittany). Wild samples collected the same month either in Tinduff or in the "Baie de Vilaine," an embayment located along the south coast of Brittany, were also analyzed.

Three clonal strains of *Gymnodinium nagasakiense,* Buzen-'82, Buzen-'85-2, and Katsuura, were obtained courtesy of Prof. M. Kodama (Kitasato University, Japan). Another clonal strain of the same species, NIES-249, was also obtained from the National Institute for Environmental Studies collection (Tsukuba, Japan).

Batch cultures of these dinoflagellates were grown in either f/2 (Guillard and Ryther 1962) or K medium (Keller and Guillard 1985), under either a 12:12 h L:D cycle or continuous light. Illumination was provided by cool white and Grolux fluorescent tubes giving a mean photon fluence rate of 100 $\mu E \cdot m^{-2} \cdot s^{-1}$. A temperature of 20 ± 1° C was suitable for the growth of both species. Cultures were grown in Erlenmeyer flasks of various sizes. Under these conditions, minimum generation times were 2.0 days for *G. nagasakiense* and 1.7 day for *G.* cf. *aureolum.*

Light microscopy. Live cells of *G.* cf. *aureolum* and *G. nagasakiense* were observed and photographed with an epifluorescence microscope Olympus BH2 (Tokyo, Japan). Body length, width, and cingular displacement were measured on individual cells. The number of chloroplasts as well as shape and position of the nucleus, stained with 0.1% of a 1 $g \cdot L^{-1}$ Hoechst 33342 stock solution in Phosphate Buffered Saline (both chemicals from Sigma, Saint Louis, Missouri), were examined under epifluorescence using either blue or UV excitation light.

The chromosome number was determined on one strain of each species (Katsuura and PML-497A) and on a natural sample of *G.* cf. *aureolum* harvested in Tinduff. Cells were concentrated by centrifugation (5000 × *g,* 5 min). Cell pellets were then resuspended in distilled water in order to provoke cell disruption. Cells were stained with Hoechst 33342 (1 $mg \cdot L^{-1}$) and squashed between slide and cover slip. Chromosomes were counted on micrographs taken under UV light. This fluorescence method was simpler than the classical acetocarmine staining techniques (Dodge 1963, Loper et al. 1980). Counts were preferentially made on non-dividing cells, the chromosomes of which are short and rod-shaped, whereas dividing cells possess long, thin chromosomes (Tangen and Björnland 1981).

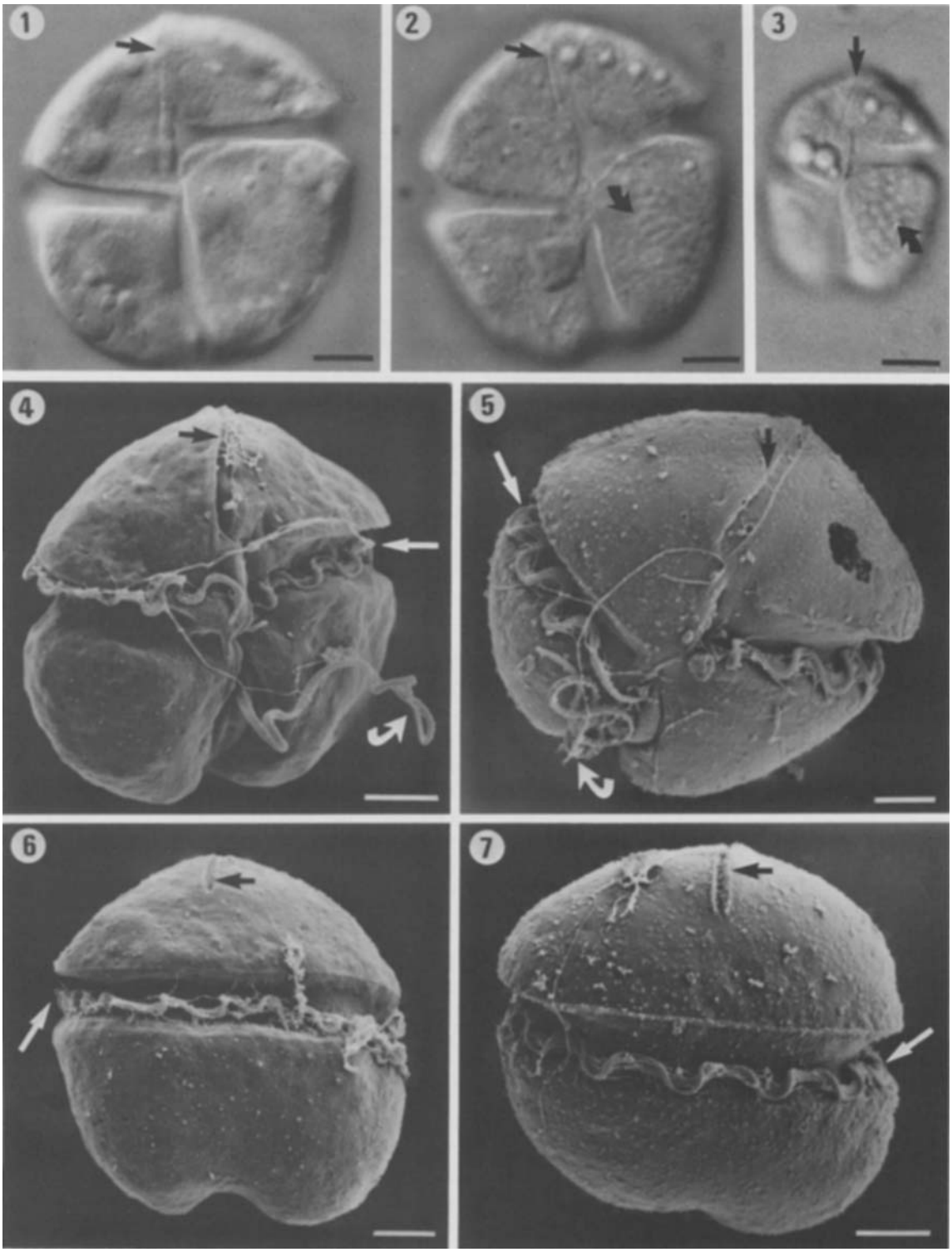
Scanning electron microscopy. After gentle centrifugation (1500 × *g,* 10 min), cells of *G.* cf. *aureolum* (strain PML-497A) and *G. nagasakiense* (strain Katsuura) were fixed for 1 h at 4° C in a 2% osmium textroxide solution in culture medium. Cells were then rinsed with distilled water, taken through a graded ethanol series, critical-point dried with CO_2, and metal coated with a gold : palladium mixture (60:40). Observations were made with a JEOL JSM 840A scanning electron microscope.

Flow cytometric analysis of DNA content. Aliquots of dense cultures of *G.* cf. *aureolum* and *G. nagasakiense* were centrifuged at about 2000 × *g* for 10 min. Cell pellets were fixed in cold methanol according to the method of Olson et al. (1983). Two days after fixation, cells were rinsed, resuspended in PBS and stained for DNA using Hoechst 33342 (3 $mg \cdot L^{-1}$). This externally binding fluorochrome was preferred to DNA intercalating dyes such as acridine orange or ethidium bromide because the fluorescence yield of the latter has been found to depend upon culture phase in the unicellular alga *Euglena gracilis* Klebs (Bonaly et al. 1987). Nuclei of calf thymocytes (Ortho Diagnostic Systems Inc., Westwood, Massachusetts) were added to the cell suspension prior to staining to serve as an internal standard.

The relative DNA content of stained cells was analyzed with an EPICS 541 flow cytometer (Coultronics Inc., Hialeah, Florida) using the 320 nm (UV) laser line as the excitation light source (250 mW). Each algal population produced a well-defined DNA peak formed by the vegetative cells within the interphasic stage of the cell cycle (G_1 phase). A second peak, only visible during exponential growth, was formed by the cells with a double stock of chromosomes, i.e. preparing for or undergoing mitosis (G_2 + M phases). A continuum between these two peaks was produced by cells duplicating their DNA (S phase). The terms G_1, G_2 and S were used by homology with the classical eukaryotic cell cycle, although the interphasic vegetative cells of both *G.* cf. *aureolum* and *G. nagasakiense* are probably haploid like most dinoflagellates (Pfiester 1984), whereas the term G_1 phase commonly refers to diploid cells. The G_1 peak of both species was normalized to the G_1 peak of the calf thymocyte nuclei.

RESULTS

Light microscopic observations of typical cells of *Gyrodinium* cf. *aureolum* and *Gymnodinium nagasakiense* confirmed the morphological similarity of the two species (Figs. 1, 2). Both were in closer agreement with the diagnosis of *Gymnodinium nagasakiense* by Takayama and Adachi (1984) than with the original description of *Gyrodinium aureolum* by Hulburt (1957), as discussed below. Intraspecific morphological variability was generally greater than interspecific variability. In particular, all our strains of *G.* cf. *aureolum* produced two subpopulations of different sizes, which we called "large" and "small" cells (Figs. 2, 3). The proportion of each cell type varied during growth, as confirmed by Coulter volume measurements (Partensky, unpubl. data). In culture, "small" cells were always longer than wide, whereas "large" cells were either spindle-shaped or subspherical. Both cell types of *G.* cf. *aureolum* were also observed in natural samples but were somewhat more elongated than cultured ones (Table 1). The cell size of cultured *G. nagasakiense* was also variable, with a body length varying between 21–34 μm and a body width between 16.1–29.2 μm (Table 1), but the presence of two distinct subpopulations within the same culture was rarely observed, whatever the strain. Typical *G. nagasakiense* cells were therefore similar to "large" cells of *G.* cf. *aureolum.* The ratio of the girdle displacement to the total body length, which should have been a distinctive feature between the genera *Gymnodinium* and *Gyrodinium,* varied similarly in both species, i.e. between 17 and 27% of the total body length (Table 1). Chloroplasts were generally rod-shaped or round, sometimes asymmetrical. Chloroplast number varied considerably in both



FIGS. 1–7. Morphology of *Gymnodinium nagasakiense* and *Gyrodinium* cf. *aureolum* with light and scanning electron microscopy. FIG. 1. Typical cell of *Gymnodinium nagasakiense*. FIGS. 2, 3. "Large" (Fig. 2) and "small" (Fig. 3) specimens of *Gyrodinium* cf. *aureolum*. FIG. 4. *Gymnodinium nagasakiense*, ventral view. FIG. 5. *Gyrodinium* cf. *aureolum*, ventral view. FIG. 6. *Gymnodinium nagasakiense*, dorsal view. FIG. 7. *Gyrodinium* cf. *aureolum*, dorsal view. All scale bars = 5 μm. Black straight arrow = apical groove; black curved arrow = nucleus; white straight arrow = transverse flagellum; white curved arrow = longitudinal flagellum.

TABLE 1. *Cell size, girdle displacement and chloroplast number of different strains or samples of* Gymnodinium nagasakiense *and* Gyrodinium *cf.* aureolum. *C = cultured strain; N = natural sample. Mean ± SD.*

Species	Origin	Strain or sample	Cell no.	Cell type	Body length (μm)	Body width (μm)	Body length to width ratio	Girdle displacement to body length ratio (%)	Chloroplast number
Gymnodinium nagasakiense	C	Katsuura	58	typical	28.3 ± 2.8	23.5 ± 2.7	1.2 ± 0.1	22 ± 3	18 ± 6
Gyrodinium cf. *aureolum*	C	PML-497A	57	large	28.3 ± 2.7	26.4 ± 3.2	1.1 ± 0.1	22 ± 3	24 ± 6
	N	Tinduff	51	large	30.7 ± 3.8	24.8 ± 3.5	1.3 ± 0.2	20 ± 3	20 ± 8
	C	Iroise	43	small	23.6 ± 3.3	19.2 ± 3.2	1.2 ± 0.1	22 ± 4	7 ± 5
	N	Baie de Vilaine	24	small	23.6 ± 1.8	17.4 ± 2.0	1.4 ± 0.1	19 ± 3	9 ± 3

species, from a minimum of 1 or 2 in the smallest specimens to a maximum of 40 in the largest ones (Table 1). In wild or cultured specimens of *G.* cf. *aureolum* and in cultured *G. nagasakiense,* the nucleus was always located in the left lobe of the cells (Figs. 2, 3), although in "small" cells it sometimes extended into the right lobe. The most common shape of the nucleus was antero-posteriorly elongate, but pear-shaped, kidney-shaped, or ovoid (in the smallest specimens) nuclei also were observed.

Scanning electron microscopy clarified some of the morphological features of *G.* cf. *aureolum* and *G. nagasakiense* (Figs. 4–13). Both possessed an apical groove set to the right of the sulcal axis in ventral view and extended in a straight line to the upper third of the dorsal epicone (Figs. 4–10), as previously described on *G. nagasakiense* by Takayama (1981) and Takayama and Adachi (1984). The dorso-ventral cell flattening, easily seen on anterior or lateral views (Figs. 8, 9, 11, 12), was similar in both species. Cell thickness to cell length ratio was estimated from the micrographs to be 2/3. Numerous pores of various sizes, most of them presumably trichocyst pores, and small white granules were observed on the cell surface (Fig. 13). We think that the granules are bacterial particles (mycoplasms) rather than ejectile organelles, as previously suggested by Takayama (1981). This hypothesis was supported by fluorescence microscopic observations of Hoechst 33342 stained cells, which were covered with numerous blue-fluorescing (i.e. DNA containing) particles. The transverse flagellum had the classical ribbon-like structure (Leadbeater and Dodge 1967) and was bordered with a unilateral array of fine mastigonemes (not shown). The longitudinal flagellum had a simple thread-like structure.

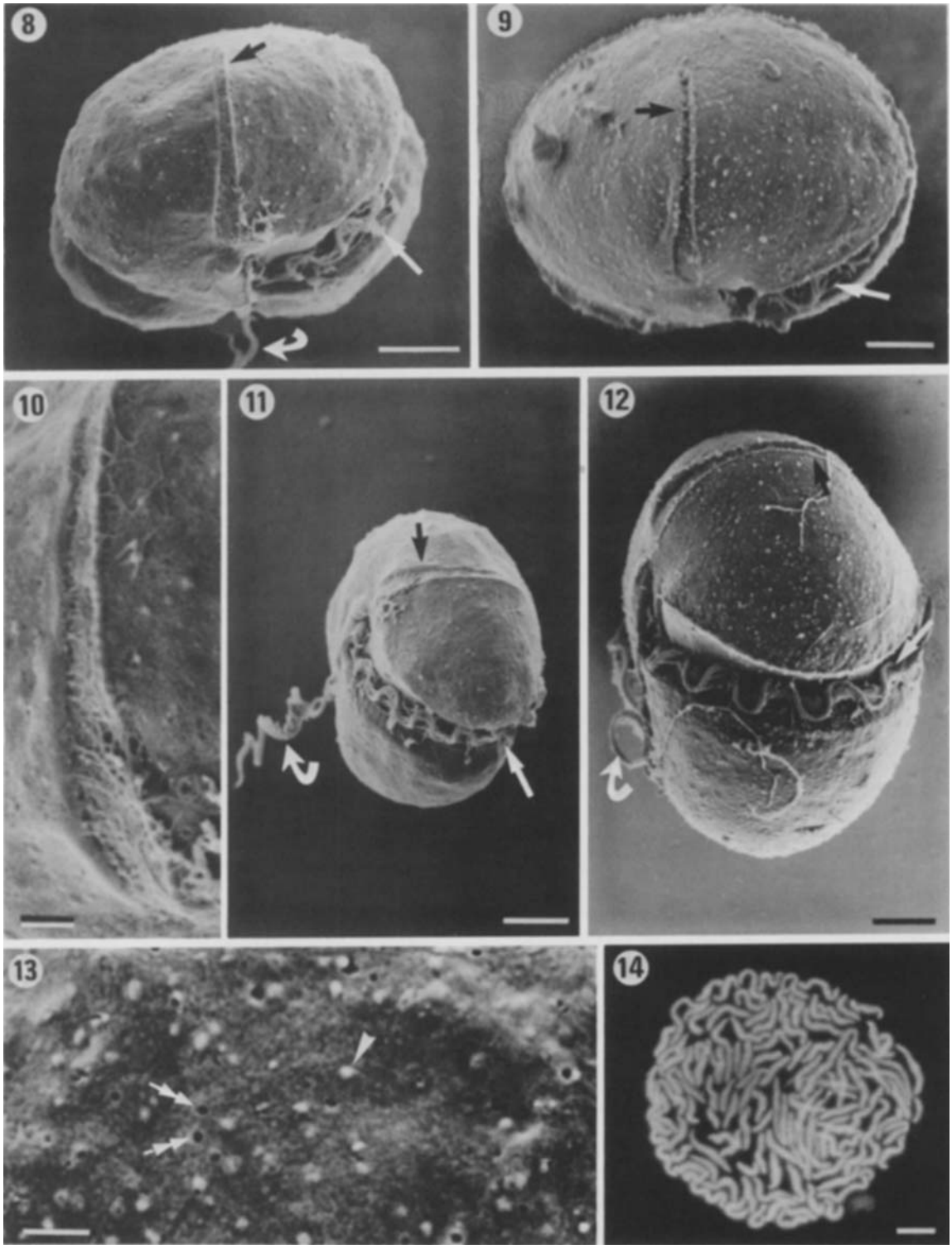
The mean chromosome number of cultured *G. nagasakiense* and cultured or wild *G.* cf. *aureolum* (Fig. 14) was identical (117 ± 3; mean ± SE; n = 5 for each strain).

Flow cytometric analyses revealed that either a single strain or a mixture of two strains of the same species produced a single peak of cells within the G_1 cell cycle stage (Fig. 15A, B), whereas a mixture of the two species produced two distinct peaks of G_1 cells (Fig. 15C). The DNA content of the different strains of *G.* cf. *aureolum* (normalized to the DNA content of calf thymocytes) was remarkably constant (Table 2) and identical in "large" and "small" cells. Moreover, both subpopulations included G_1, S and G_2 cells but no tetraploid cells (data not shown), indicating that cell size differentiation was probably not linked to a sexual process. The greater variation in the DNA content appearing among the different strains of *G. nagasakiense* is not significant (mean DNA contents of strains Katsuura and Buzen-'82 were compared using a Student *t*-test at 5%) and is thought to be chiefly due to experimental inaccuracies. On the contrary, the differences in DNA content among the Japanese and European species (Table 2) are quite large, since the latter taxon possesses 41% more DNA than the former. The absolute DNA content of *G.* cf. *aureolum* and *G. nagasakiense* was estimated to be 44 and 62 pg per cell respectively, assuming that calf thymocyte nuclei contained 7.15 pg DNA (Mirsky and Ris 1949). These values fall within the range measured in other naked dinoflagellates such as *Gymnodinium resplendens* Hulburt (66 pg per cell; Allen et al. 1975), but they are markedly less than the 113 pg per cell found by Rizzo et al. (1982) for *Gymnodinium breve* Davis (= *Ptychodiscus brevis* (Davis) Steidinger), a species close to the ones we studied both morphologically and by its chromosome number (121; Loper et al. 1980).

DISCUSSION

None of the identifying characters used classically in the taxonomy of Gymnodiniaceae (listed in Partensky and Sournia 1986), including observations of scanning electron micrographs and chromosome number, were sufficient to distinguish *Gymnodinium nagasakiense* from *Gyrodinium* cf. *aureolum.* In contrast, flow cytometric DNA analysis proved useful in differentiating the taxa since the Japanese strains possessed 41% more DNA than the European ones. DNA content therefore appears to be a distinguishing feature, although it has been generally neglected in previous taxonomic studies of sibling species (Fine and Loeblich 1976, Loeblich et al. 1981).

Although morphologically indistinguishable, *G.* cf. *aureolum* and *G. nagasakiense* have been placed more or less arbitrarily in two different genera. Conventionally, the main distinctive criterion between the genera *Gyrodinium* and *Gymnodinium* rests on the ratio of girdle displacement to total body length, which is higher or lower than 20%, respectively (Kofoid and Swezy 1921). In the present case, both



FIGS. 8–14. Morphology of *Gymnodinium nagasakiense* and *Gyrodinium* cf. *aureolum* with SEM. FIGS. 8–9. Anterior views, showing apical groove and cell flattening. Scale bars = 5 μm. FIG. 8. *Gymnodinium nagasakiense.* FIG. 9. *Gyrodinium* cf. *aureolum.* FIG. 10. Detail of the apical groove of *Gymnodinium nagasakiense.* Note the fibrous aspect of the groove. Scale bar = 1 μm. FIGS. 11–12. Lateral left views. Scale bars = 5 μm. FIG. 11. *Gymnodinium nagasakiense.* FIG. 12. *Gyrodinium* cf. *aureolum.* FIG. 13. Detail of the theca of *Gymnodinium nagasakiense,* showing trichocyst pores (double headed arrows) and mycoplasms (arrowhead). Scale bar = 1 μm. FIG. 14. Chromosomes of *Gyrodinium* cf. *aureoleum* stained by Hoechst 33342, as seen with epifluorescence microscopy (UV light). Scale bar = 5 μm. Black straight arrow = apical groove; white straight arrow = transverse flagellum; white curved arrow = longitudinal flagellum.

species lie at the borderline (17–27%), as previously noted by Tangen (1977) and Taylor (1985). Takayama and Adachi (1984) chose to put the Japanese taxon within the genus *Gymnodinium* because they found that most cells in their cultures had a girdle displacement less than or equal to one fifth of the body length, whereas we found it to be slightly more than one fifth. This contradiction may be partly due to the unavoidable inaccuracy of the optical measurements and also to a possible modification of this ratio with the physiological state of the cells (Taylor 1985). The existence of borderline species suggests a long-needed reconsideration of the genera *Gyrodinium* and *Gymnodinium,* which probably should be fused into the first described genus *Gymnodinium* (Stein 1878), as was done previously for *Exuviaella* and *Prorocentrum* or *Phalacroma* and *Dinophysis* (see Sournia 1987).

Several cellular features other than girdle displacement are similar in *G.* cf. *aureolum* and *G. nagasakiense.* In both species, including wild specimens of the European taxon, most non-dividing cells possess a large elongate nucleus in the left lobe of the cell. This observation is in good agreement with the original diagnosis of *Gymnodinium nagasakiense* (Fig. 1 in Takayama and Adachi 1984) but does not match the original description of *Gyrodinium aureolum,* in which the nucleus was spherical or wider than long and was located subcentrally (Plate 2, Figs. 8, 9 in Hulburt 1957). Several descriptions of wild *Gyrodinium* from European waters reported a nucleus similar in shape and position to the species described by Hulburt (Ballantine and Smith 1973, Tangen 1977, Ottway et al. 1979). These apparent discrepancies could indicate that several different species have been attributed to *Gyrodinium aureolum.* Nevertheless, it is noteworthy that Tangen and Björnland (1981), in contradiction with their own previous observations (Tangen 1977), observed that most cells in their cultures had a nucleus located in the left portion of the hypocone. The problem is therefore more complicated than it appears and may be due to observations made either on dividing cells or on badly preserved material.

Among the features common to *G.* cf. *aureolum* and *G. nagasakiense* is the presence of a similar apical groove, which is a distinctive character among unarmored dinoflagellates (Takayama 1985). In his original description of *Gyrodinium aureolum,* Hulburt (1957) probably misinterpreted this feature as an extension of the sulcus into the epicone. The same misinterpretation has been made by other workers in more recent descriptions of European isolates (Braarud and Heimdal 1970, Ballantine and Smith 1973, Tangen 1977).

Among our strains, *G.* cf. *aureolum* appears to be morphologically much closer to *G. nagasakiense* than to *Gyrodinium aureolum,* as diagnosed by Hulburt (1957). One could be tempted to consider the two former taxa as a single species and the latter taxon

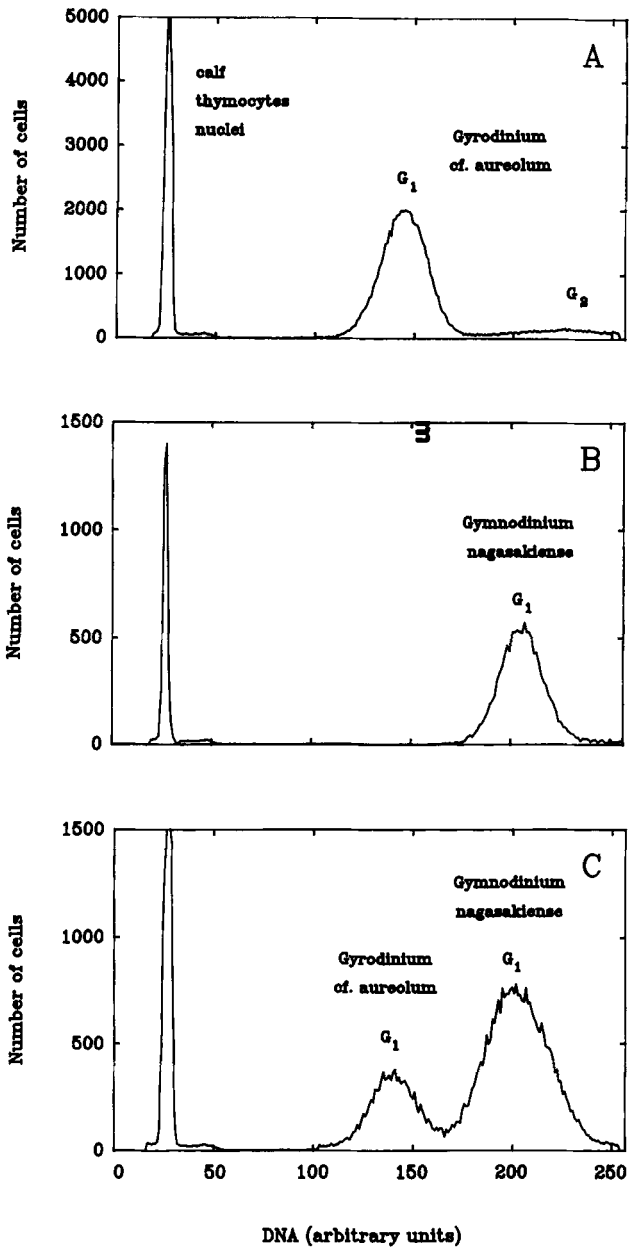


FIG. 15A–C. Flow cytometric DNA analysis of (A) a mixture of two strains of *Gyrodinium* cf. *aureolum* (PML-497A and Iroise), (B) a strain of *Gymnodinium nagasakiense* (Katsuura) and (C) a mixture of *Gymnodinium nagasakiense* (Katsuura) and *Gyrodinium* cf. *aureolum* (Iroise). The left-handed peak is produced by calf thymocyte nuclei (used as an internal standard) in the G_1 cell cycle stage, whereas the right-handed peaks are produced by the G_1 cells of either *Gymnodinium nagasakiense* or *Gyrodinium* cf. *aureolum.* The number of G_2 cells is low because samples were collected at the end of the exponential phase of growth.

as a distinct species. However, the significant discrepancies in the DNA contents of *G.* cf. *aureolum* and *G. nagasakiense* has led us to doubt that even they are conspecific. According to recent definitions of the species concept, that is a "a population (or set of populations) potentially sharing a common gene pool, gene exchange occurring sexually or parasexually" (Lewin 1981) or "an evolutionary

TABLE 2. *DNA content of different strains or samples of* Gymnodinium nagasakiense *and* Gyrodinium *cf.* aureolum. *Mean ± SD.*

Species	Strain or sample	Strain DNA content[a]	Species DNA content[a]
Gymnodinium nagasakiense	Katsuura	8.2 ± 0.4 (n = 5)	
	Buzen-'82	8.8 ± 0.4 (n = 5)	8.6 ± 0.6 (n = 12)
	Buzen-'85-2	9.1 (n = 1)	
	NIES-249	9.4 (n = 1)	
Gyrodinium cf. *aureolum*	PML-497A	6.1 ± 0.3 (n = 20)	
	Tinduff	6.0 ± 0.4 (n = 7)	6.1 ± 0.3 (n = 33)
	Iroise	6.3 ± 0.4 (n = 4)	
	Baie de Vilaine[b]	6.0 ± 0.1 (n = 2)	

[a] Normalized to calf thymocyte DNA content.
[b] Natural sample.

group (population) . . . capable to produce generations identical in all fundamental genetic features" (Ettl and Popovsky 1986), two taxa with significantly different DNA contents (i.e. with partial chromosomal inhomologies) may be theoretically considered as two different species, even if they are similar in all other characters. The recent finding by Hayhome et al. (1988) that two clones of *Peridinium volzii* Lemmerman were interfertile despite their distinctly different DNA contents (131.9 and 172.1 pg DNA per cell for OK-75 and NE-1, respectively), however, makes the value of this feature by itself for species determination uncertain. Since we never observed sexuality in our cultures, including mixtures of clones of *G. nagasakiense* or strains of *G.* cf. *aureolum,* the interfertility of these taxa is not easy to check. However, it is noteworthy that the European and Japanese taxa also differ in their growth physiology, since only the former can form both "small" and "large" cells. This, added to the difference in DNA content, is a serious argument for the non-conspecificity of the taxa. The diagnosis of *Gymnodinium nagasakiense* by Takayama and Adachi (1984) still fits the European taxon, however, since the DNA content was not mentioned. We thus suggest that the para-taxonomical appellation *Gymnodium* cf. *nagasakiense* replace *Gyrodinium* cf. *aureolum* (currently called *Gyrodinium aureolum* in the literature), assuming that a revision of both the genera and species is likely to occur in the next few years.

Absence of data on DNA content, some observational inaccuracies, and the large intraspecific morphological variability of the specimens, cast doubt upon the identity of the original *Gyrodinium aureolum* Hulburt. Several other taxa share some similarities with the species we studied (Taylor 1985, Partensky and Sournia 1986). Most of them, including *Gymnodinium gelbum* Kofoid, *G. mikimitoi* Miyake and Kominami, *G. schaefferi* Morris, *G. chuckwanii* Ballantine and *Spirodinium aureum* Conrad have been rarely observed, sometimes only by the describing authors, and living material is generally not available. In these cases, comparisons remain speculative. For some other species, such as *Gymnodinium flavum* Kofoid and Swezy, and also for isolates from different parts of the world referred to as *Gymnodinium nagasakiense* or *Gyrodinium aureolum,* only comparative studies on strains grown under the same culture conditions are likely to elucidate the problems of conspecificity.

The significant discrepancy in DNA contents of *Gymnodinium nagasakiense* and *Gymnodinium* cf. *nagasakiense* (ex *Gyrodinium* cf. *aureolum*) also leads to speculations about the relationship between these two taxa. If they actually belong to two different species, it is reasonable to assume that they possess a recent common ancestor (see evolution tree; frontispiece in Taylor 1987). Further biochemical investigations could thus provide a great deal of information concerning the first steps of a speciation mechanism different from polyploidy, which has been previously invoked for the sibling species *Heterocapsa pygmaea* Loeblich, Schmidt et Sherley and *H. niei* (Loeblich) Morrill et Loeblich (Loeblich et al. 1981). In the present case, a series of deletions or repetitions of gene sequences (redundancy), not affecting the chromosome number, could be largely responsible for the difference noted in DNA contents. This genetic recasting, which may be related to the initial geographical isolation of the populations, is probably recent according to an evolutionary scale but may be relatively ancient according to the human time scale. Thus, the hypothesis of an introduction of the Japanese taxon into European waters in the past thirty years is invalidated (Partensky and Sournia 1986). This is supported by the observation that DNA content is relatively constant among the different strains of the same taxon, strains which have been isolated from very different locations (i.e. belonging to populations probably separated for years).

Our work points out the limitations of classical Linnean taxonomy. It is clear that the descriptions of most naked dinoflagellates are not useful for separating species if based solely on morphological characters. The problem was previously noted by Kimball and Wood (1965), who observed pleomorphism in a clonal strain of *Gymnodinium mirabile* Penard. Modern taxonomic methods, which include the study of biochemical features such as DNA content, pigment or isozyme composition, or toxin production, still seem impractical for routine studies or

plankton surveys. However, these methods may be useful in elucidating relationships among sibling species.

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