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COMPARATIVE STUDY OF *GYMNODINIUM MIKIMOTOI* AND *GYMNODINIUM AUREOLUM*, COMB. NOV. (=*GYRODINIUM AUREOLUM*) BASED ON MORPHOLOGY, PIGMENT COMPOSITION, AND MOLECULAR DATA[1]

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Light and electron microscopy, nuclear-encoded LSU rDNA sequences, and pigment analyses were performed on five geographically separate isolates of *Gymnodinium mikimotoi*. The morphological variation between the isolates equals that found within the isolates. The nuclear-encoded LSU rDNA sequences were nearly identical in all isolates, and molecular analyses using maximum likelihood, parsimony, and neighbor joining showed the geographical isolates as an unresolved clade. Based on the available data it is concluded that the European isolates, formerly identified as *Gyrodinium aureolum*, *Gyrodinium* cf. *aureolum*, or *Gymnodinium* cf. *nagasakiense*, are conspecific with the Japanese *Gymnodinium mikimotoi*. An isolate from the Pettaquamscutt River, USA, is suggested to represent what Hulburt (1957) described as *Gyrodinium aureolum*. The LSU rDNA sequence data and ultrastructural characters in this isolate closely resemble those of *Gymnodinium fuscum*, the type species of *Gymnodinium*, and *Gyrodinium aureolum* Hulburt is therefore renamed *Gymnodinium aureolum* (Hulburt) G. Hansen, comb. nov.

Key index words: **accessory pigments; *Gymnodinium aureolum*; *Gymnodinium mikimotoi*; *Gyrodinium aureolum*; LSU rDNA, molecular phylogeny; taxonomy**

Abbreviations: **lf, longitudinal flagellum; LSU, large ribosomal subunit; ML, maximum likelihood; n, nucleus; NJ, neighbor joining; py, pyrenoid; se, sulcal extension; SSU, small ribosomal subunit; tf, transverse flagellum**

In 1966, a massive dinoflagellate bloom, accompanied by death of caged sea trouts, occurred in coastal waters of southern Norway (Braarud and Heimdal 1970). The causative organism was identified as *Gyrodinium aureolum* Hulburt, a species originally described from small embayments in the Woods Hole area in Massachusetts (Hulburt 1957). Subsequently it has become one of the most commonly reported bloom-forming dinoflagellates in northern temperate waters (e.g. Tangen 1977, 1979, Pybus 1980, Jones et al. 1982, Lindahl 1983, Potts and Edwards 1987). However, there is considerable taxonomic confusion with regard to identity of this species (e.g. Partensky and Sournia 1986, Partensky et al. 1988). It is now generally accepted that the European "*Gyrodinium aureolum*" is very closely related to or even synonymous with the earlier described *Gymnodinium mikimotoi* (Miyake et Kominami) ex Oda from Japanese waters (=*Gymnodinium nagasakiense* Takayama et Adachi) (Partensky and Sournia 1986, Partensky et al. 1988, Taylor et al. 1995). Nevertheless, the name *Gyrodinium aureolum* is still widely used for European material (e.g. Bjørnsen and Nielsen 1991, Hansen 1995, Dahlberg et al. 1998), despite discrepancies with Hulburt's original description. To further characterize Hulburt's *G. aureolum*, material should preferably be collected at the type locality, but extensive sampling for *Gyrodinium aureolum* at Hulburt's collection sites during late March 1996 was unsuccessful (G. Hansen, personal observation). A culture collected in the Pettaquamscutt River, about 65 km from Woods Hole, Massachusetts (Fig. 1), was subsequently obtained from Lucie Maranda, University of Rhode Island. It showed striking resemblance to Hulburt's *Gyrodinium aureolum* and is therefore considered to represent Hulburt's species. LSU rDNA sequences and ultrastructural data suggest, however, that this species belongs to the genus *Gymnodinium* rather than *Gyrodinium*. Here we report on light microscopical observations, partial

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LSU rDNA sequence data, and pigment composition of the isolate, which is compared with an isolate from Danish waters and with five other geographically different isolates of *Gymnodinium mikimotoi*.

MATERIALS AND METHODS

Cultures. The cultures studied are listed in Table 1. Strains were obtained from Provasoli-Guillard National Centre for Marine Phytoplankton (CCMP strains numbers), Scandinavian Culture Centre for Algae and Protozoa (K strain numbers), J. Larsen (JL strain numbers), M. Ellegaard (DK4 strain), L. Maranda (S1-30-6 strain), C.A. Scholin (A3 strain), K. Tangen (KT77B strain), and Ikeda and Mutsuno (Japanese isolate of *Gymnodinium mikimotoi*).

The different isolates of *Gymnodinium mikimotoi* are referred to in the text by their country of origin rather than their strain number.

All marine cultures were grown at 15, 20, or 25° C in T or TL medium (see http://www.sccap.bot.ku.dk/) at a salinity of ≈30 psu, and a 12 h light:12 h dark cycle. Illumination was ≈50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. *Gymnodinium fuscum* was grown in DYIV medium (see Keller and Andersen: http://ccmp.bigelow.org/CI/CI_01d.html).

Light microscopy. Live cells were examined using either an Olympus BHS or an Olympus Provis AX 70 microscope, fitted with a ×60 planapochromat, N 1.40, oil-immersion objective. A Wratten Blue filter was used to better visualize the chloroplasts.

Scanning electron microscopy. The culture was fixed in 1% OsO_4 for 30 min, filtered onto a Nucleopore filter (8 μm), thoroughly washed in distilled water, dehydrated in an ethanol series and critical-point dried via liquid CO_2 in a Balzer CPD 010 critical point drying apparatus. The filters were subsequently glued to the SEM stubs with double-adhesive carbon disks, sputter coated with gold, and examined in a Phillips 515 scanning electron microscope.

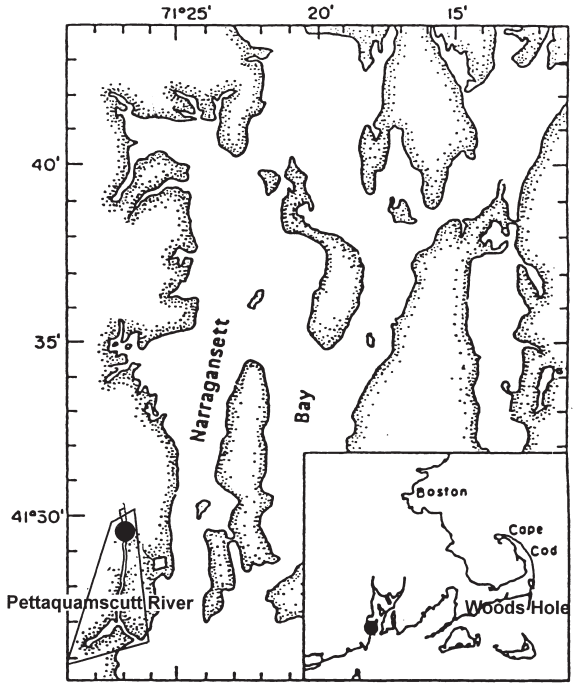


FIG. 1. Sampling locality (Pettaquamscutt River, Massachusetts) of the *Gymnodinium aureolum*, comb. nov. used in this study.

Transmission electron microscopy. Two fixation schedules were used. Schedule 1: The culture was fixed initially in a mixture of 2% glutaraldehyde and 0.1% OsO_4 (final concentrations) for 20 min, pelleted by centrifugation and washed in culture medium. Postfixation was for 1 hour in 1% OsO_4 made up in 0.1 M Na-cacodylate buffer. Schedule 2: The culture was gently concentrated by gravity filtration and fixed in a mixture of 2% glutaraldehyde and 0.4% OsO_4 in 0.16 M phosphate buffer (pH 7.5) and 0.33 M NaCl (final concentrations) for 15 min. After thorough washing in buffer, the material was covered by 1.5% warm agar. The agar was allowed to cool and solidify and then peeled off the filter, divided into smaller pieces, and postfixed for 1 h in 2% OsO_4 made up in 0.1 M phosphate buffer and 0.2 M NaCl. In both schedules, the material was dehydrated in an alcohol series and embedded in Spurr's resin via propylene oxide. The material was sectioned on a Reichert Ultracut E microtome using a diamond knife, and the sections were collected on a slot grid and placed on a Formvar film. After staining in uranyl acetate and lead citrate, sections were examined in a JEOL 100CX electron microscope (JEOL, Peabody, MA).

Measurements. The cultures used for morphometric analyses were transferred to fresh medium one month prior to the measurements. They were presumed to be in the late exponential growth phase, but growth measurements were not carried out. The reason for using relatively old cultures was to get an impression of intraspecific size variation. The Danish strain of *Gymnodinium mikimotoi* grew very slowly, and measurements were also made on a 2-month-old very thin culture and on a 4-month-old dense culture, referred to as "fresh" and "old," respectively. The strains were photographed by video microscopy, and the video sequences were subsequently digitized using an AVID-Express video editing system for the Macintosh computer. Video sequences were carefully examined frame by frame and individual frames were exported in the TIFF file format. Measurements on randomly selected cells were performed on a PC using the UTHSCSA Imagetool program (developed at the University of Texas Health Science Center at San Antonio, Texas).

Pigment analyses. Cells were gently filtered onto 25-mm Advantec GF 75 glass fiber filters and immediately stored in liquid nitrogen. For extraction of pigments, filters were placed in 3 mL acetone, sonicated on ice for 15 min and left for 24 h at 4° C. Extracts were subsequently filtered (0.2-μm filters) into HPLC vials containing 1 mL water and analyzed on a Shimazu LC 10A system with a Supercosil C18 column (250 by 4.6 mm, 5 μm). A slight modification of the method of Wright et al. (1991) was used, as described by Schlüter and Havskum (1997). Pigments were identified by retention times and absorption spectra identical to those of authentic standards. They were quantified against standards purchased from the International Agency for ^{14}C Determination, Hørsholm, Denmark. Analyses were made on the Japanese and Norwegian strains of *Gymnodinium mikimotoi* and *G. aureolum* from the Pettaquamscutt River.

DNA extraction and amplifications of the LSU rDNA gene (domain D1–D3). Approximately 15 mL of nonaxenic, exponentially growing cultures was harvested by centrifugation (1500–2000 rpm) for 10 to 15 min at room temperature and transferred to a 1.5-mL Eppendorf tube. Pelleted cultures were frozen at −20° C. Total genomic DNA was extracted as previously outlined (Daugbjerg et al. 1994). Subsequently total cellular DNA was used as template to amplify ≈1000 base pairs of the LSU rDNA gene using terminal primers D1R and D3B. PCR amplifications were performed in volumes of 50 μL (67 mM Tris-HCl, pH 8.8, 2 mM $MgCl_2$, 16.6 mM $(NH_4)_2SO_4$, 10 mM β-mercaptoethanol, 200 μM dNTP, 0.5 μM of each primer, and 0.2 units of Pharmacia Biotech Taq DNA polymerase). PCR conditions were one initial cycle of denaturation at 94° C for 3 min, followed by 30 to 32 cycles of denaturation at 94° C for 1 minute, annealing at 52° C for 1 minute, and extension at 72° C for 3 min. The temperature profile was completed by a final extension cycle at 72° C for 6 min. DNA fragments were checked in 2% agarose gels containing ethidium bromide and visualized by ultraviolet illumination. PCR products were purified using the QIAquick PCR purification

TABLE 1. Strains of dinoflagellates included in the molecular study.

Taxon	Origin	Clonal isolate	Culture Number	Isolated by	Accession number
Alexandrium catenella	Monterey Bay, USA	+	A3	C.A. Scholin	AF200667
Alexandrium catenella	Port Phillip Bay, Australia	+	K-0270	Ø. Moestrup	AF200666
Alexandrium tamarense	Tjaldavik, Faroe Islands	−	K-0055	Ø. Moestrup	AF200668
Gymnodinium aureolum	Nivå Bugt, Denmark	+	K-0303	G Hansen	AF200671
Gymnodinium aureolum	Pettaquamscutt R., USA	+	S1-30-6	L. Maranda	AF200670
Gymnodinium breve	Florida, USA	+	JL32	W.B. Wilson	AF200677
Gymnodinium catenatum	Vigo, Spain	?	?	?	AF200672
Gynmodinium chlorophorum	Sylt, Germany	+	K-0539 (=BAH ME 100)	M. Elbrächter	AF200669
Gymnodinium fuscum	La Trobe, Australia	?	CCMP 1677	D.R.A. Hill	AF200676
Gymnodinium galatheanum	Oslo Fjord, Norway	?	K-0522	K. Tangen	AF200675
Gymnodinium mikimotoi	Sutton Harbour, Plymouth, UK	?	CCMP 429 (=PLY 497a)	D. Harbor	AF200678
Gymnodinium mikimotoi	Oslo Fjord, Norway	?	KT77B	K. Tangen	AF200680
Gymnodinium mikimotoi	Øresund, Denmark	+	K-0579	G. Hansen	AF200682
Gymnodinium mikimotoi	Tokuyama Port, Seto Island Sea, Japan	+	?	T. Ikeda & S. Mutsuno	AF200681
Gymnodinium mikimotoi	Hobson Bay, Australia	−	K-0286	J. Larsen	AF200679
Gymnodinium nolleri	Øresund, Denmark	−	DK4	M. Ellegaard	AF200673
Gyrodinium impudicum	Naples, Italy	?	JL30	M. Montresor	AF200674

Kit (Qiagen) and nucleotide sequences were determined following the recommendations of the manufacturer for the Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). The cycle sequencing reactions were run on an ABI PRISM 377 DNA Sequencer (Perkin Elmer). Table 2 shows the nucleotide sequences of terminal and internal primers used to determine the first three domains of the LSU rDNA gene including the conserved core region upstream D3.

Alignment and phylogenetic analyses. Sequences were aligned by incorporating information from the secondary structure of LSU rRNA molecule (De Rijk et al. 1998). The alignment also included information on the secondary structure of *Prorocentrum micans*, *Tetrahymena pyriformis*, *T. thermophila*, *Toxoplasma gondii*, and *Plasmodium falciparum*. However, these taxa were omitted prior to the phylogenetic analyses. Out of 1022 aligned nucleotide positions (including gaps), 931 were considered unambiguous and examined using parsimony, maximum-likelihood (ML), and neighbor-joining (NJ) analyses. Parsimony analysis was performed with PAUP* (version 4.0.d65, D.L. Swofford, unpublished) using the heuristic search option with random addition of sequences (1000 replicates) and a branch-swapping algorithm (tree bisection–reconnection). All characters were weighted equally and gaps were treated as missing data. Maximum likelihood analysis using the F84 model was also performed using PAUP*. In order to find the tree topology with the best log likelihood score, searches were repeated by varying the transitions/transversions ratio. PAUP* was used to compute dissimilarity values, which were converted into evolutionary distances by correction for multiple substitutions according to the Kimura two-parameter model. Using the distance matrix, an evolutionary tree was constructed with the NJ method. Bootstrap analyses (1000 replications in parsimony and NJ and 100 replications in ML) were used to determine the robustness of topologies (Felsenstein 1985). The filter trees option in PAUP* was used to find the support index (decay index sensu Donoghue et al. 1992). PAUP* was used to calculate genetic distances (Kimura two-parameter model) between geographically different isolates of *Gymnodinium mikimotoi* and *G. aureolum* comb. nov. The total number of differences (excluding gaps) between these were calculated using Compare Sequences version 2.0 (H. Sigiesmund, unpublished). The LSU rDNA sequences determined in this study have been deposited in Genbank and accession numbers are given in Table 1.

Outgroup. The LSU rDNA sequences from two thecate species of *Alexandrium* (two isolates of *A. catenella* and *A. tamarense*) were used to polarize the ingroup.

TABLE 2. List of oligonucleotide primer sequences used to amplify and determine the LSU rDNA gene (domains 1–3) in dinoflagellates.

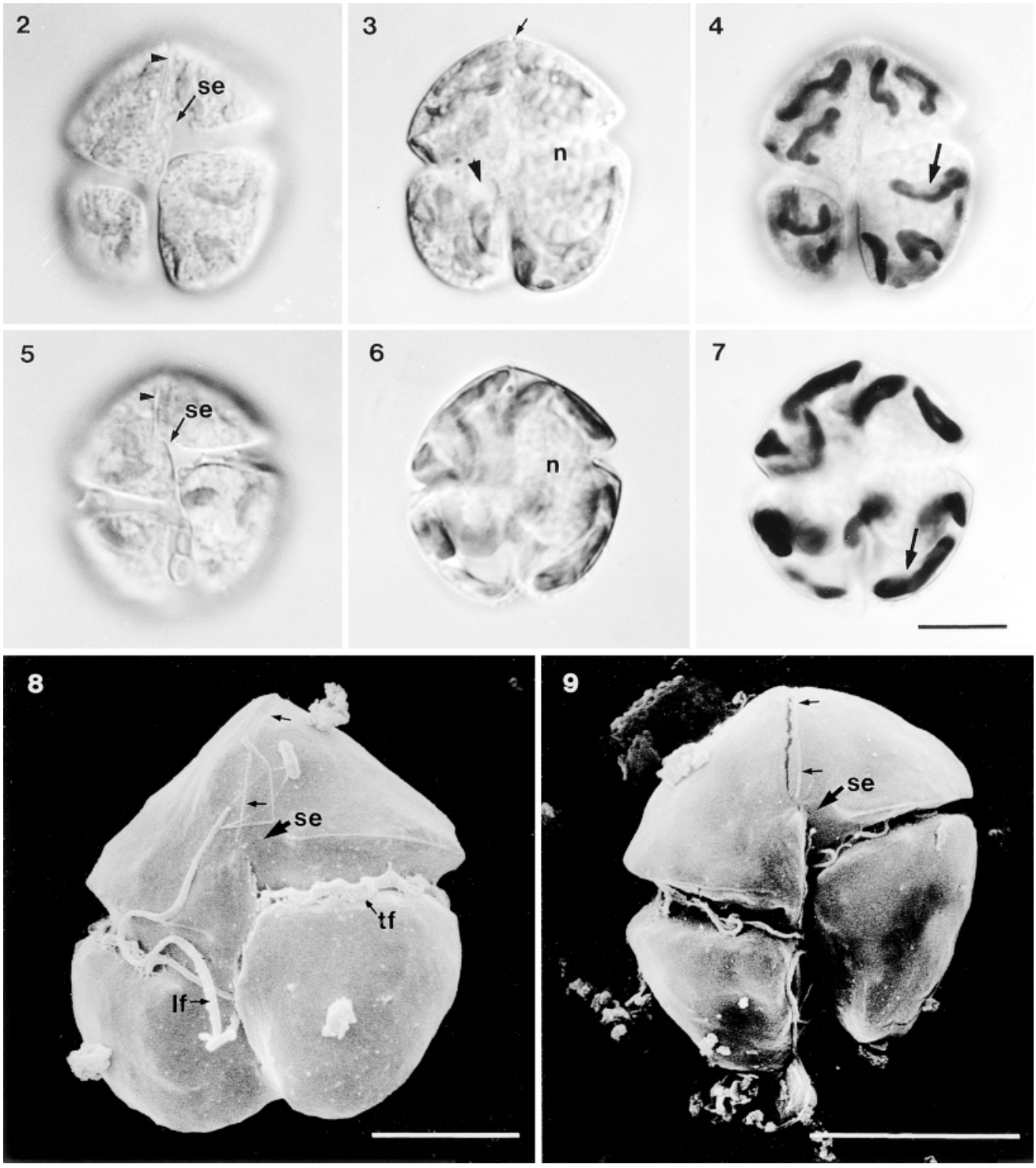
Direction/ Primer name	Annealing position[a]	Primer sequence (5′–3′)
Forward		
D1R[b]	24–31	ACCCGCTGAATTTAAGCATA
D2Ra[b]	365–384	TGAAAAGGACTTTGAAAAGA
D3A[c]	708–727	GACCCGTCTTGAAACACGGA
Reverse		
D2C[b]	733–714	CCTTGGTCCGTGTTTCAAGA
D3B[c]	1011–992	TCGGAGGGAACCAGCTACTA

[a]Annealing positions refer to the LSU rDNA of *Prorocentrum micans* (Lenaers et al. 1989).
[b]Primer sequences made by Scholin et al. (1994).
[c]Primer sequences made by Nunn et al. (1996).

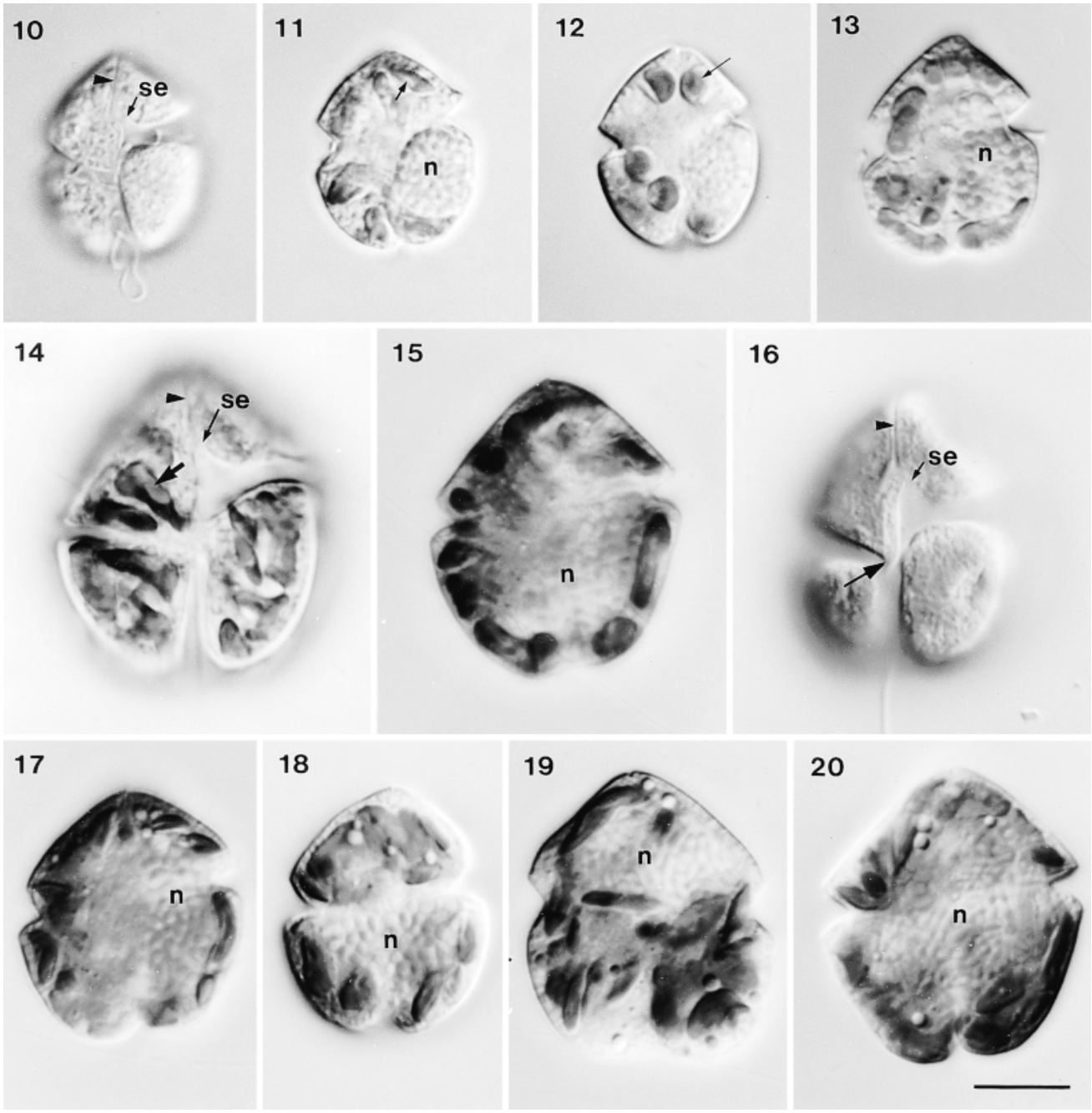
RESULTS

Gymnodinium mikimotoi

Morphological observations. All *Gymnodinium mikimotoi* strains examined had basically the same morphology (Figs. 2–20). The morphometric measurements are assembled in Table 3 and the variation in body length is shown graphically in Fig. 21. The average body length was 23.6–35.1 μm, with a range of 18.9–41.2 μm. The average body width was 19.4–30.6 μm, with a range of 15.1–44.3 μm. The cell size of the Australian strain was considerably smaller, but still within the range of the other strains. The average cell size of a fresh culture of the Danish strain was larger than the old cultures, due to a larger proportion of small cells in the old cultures (Fig. 21). However, it was not determined whether small cells of the different isolates constituted distinct subpopulations. There was no difference in the average body width/length ratio (0.8–0.9) between the strains. The cells were dorsoventrally flattened, with an average thickness/body length ratio of 0.6–0.7 (Table 3). When carefully examined by



FIGS. 2–9. *Gymnodinium mikimotoi*. Scale bars = 10 μm. FIGS. 2–4. Light microscopy of the Japanese isolate of *Gymnodinium mikimotoi* (same cell). FIG. 2. High focus showing the apical groove (arrowhead) and the sulcal extension (se). FIG 3. Low focus, the elongated nucleus (n) is visible, notice also the small apical indentation from the apical groove (arrow) and the pusule (arrowhead). FIG. 4. Chloroplasts visualized by a Wratten Blue filter. The pyrenoid is marked by an arrow. FIGS. 5–7. Norwegian isolate of *Gymnodinium mikimotoi* (same cell). FIGS. 8 and 9. Scanning electron microscopy of the Japanese and Norwegian isolates of *Gymnodinium mikimotoi*, respectively. The straight apical groove (arrows) and the sulcal extension (se) are evident. Transverse flagellum (tf) and longitudinal flagellum (lf) are indicated.



FIGS. 10–20. *Gymnodinium mikimotoi*. Scale bar = 10 μm. FIGS. 11–13. Australian isolate. FIGS. 10–11. High and low focus of the same cell. Notice the rounded nucleus (n) situated in the left lobe of the hypocone, and the pyrenoid (arrow). FIG. 12. Specimen with almost spherical chloroplasts (arrow). FIG. 13. Specimen with elongated nucleus (n) in the left side of the cell. FIGS. 14–15. Danish isolate. FIG. 14. The pyrenoid is marked by an arrow. FIG. 15. The nucleus (n) extends to the right lobe of the hypocone. FIGS. 16–20. English isolate. FIG. 16. Small terminal protrusion (arrow) of the ventral ridge or flange is visible. FIG. 17. Nucleus situated in the left side of the cell. FIG. 18. Nucleus in the hypocone. FIG. 19. Nucleus in the epicone. FIG. 20. Large nucleus occupying most of the dorsal part of the cell.

video sequences some cells appeared to be concave ventrally, but this is not apparent in the light or scanning electron micrographs. The epicone was conical or hemispherical (Figs. 8, 9); the hypocone was hemispherical, with a distinct antapical notch caused by the sulcus. The cingulum was slightly premedian, with an average displacement of 19.2%–21.2% of the body length and with a range of 11.3%–28.1% (Table 3). The sulcus extended from the antapex to slightly above the epicone–cingular border, the so-called sulcal extension. It was narrow, but widened slightly towards the antapex. The left sulcal border was gener-

TABLE 3. Cell size and cingular displacement of different strains of *Gymnodinium mikimotoi* and *G. aureolum*.[a]

Species	Origin	Body length (μm)	Body width (μm)	Body thickness (μm)	Body width to length ratio	Body thickness to length ratio	Cingular displacement to body length ratio (%)
Gymnodinium mikimotoi	Japan	32.8 ± 3.4	30.6 ± 3.8	19.0 ± 2.4	0.9 ± 0.1	0.6 ± 0.0	21.2 ± 2.8
	min.	23.9	21.6	15.6	0.7	0.5	14.9
	max.	37.7	36.4	23.9	1.1	0.7	28.1
	n	28	28	17	28	17	26
	Norway	27.0 ± 2.1	23.2 ± 2.3	15.9 ± 1.2	0.9 ± 0.1	0.6 ± 0.1	20.1 ± 3.1
	min	22.7	18.4	13.7	0.7	0.5	11.3
	max	31.9	28.3	18.1	1.0	0.7	25.5
	n	59	59	16	59	16	30
	England	29.4 ± 2.7	24.3 ± 2.4	17.5 ± 1.6	0.8 ± 0.1	0.6 ± 0.1	20.5 ± 2.9
	min	23.9	18.6	14.9	0.6	0.5	14.6
	max	34.1	29.2	20.3	1.0	0.7	27.4
	n	59	59	21	59	21	50
	Australia	23.6 ± 2.1	19.4 ± 2.3	14.6 ± 1.1	0.8 ± 0.1	0.7 ± 0.1	20.5 ± 3.7
	min	18.9	15.1	12.6	0.7	0.6	11.9
	max	29.1	26.3	16.5	1.0	0.8	28.1
	n	54	54	25	54	25	30
	Denmark, fresh culture	35.1 ± 2.8	30.6 ± 4.1	23.2 ± 2	0.9 ± 0.1	0.7 ± 0.1	19.2 ± 3.7
	min	29.3	21.7	19.5	0.7	0.6	12.0
	max	41.2	44.3	28.6	1.1	0.9	25.7
	n	32	32	18	32	18	13
	Denmark, old culture	31.9 ± 4.0	26.6 ± 4.0	20.9 ± 2.9	0.8 ± 0.1	ND	20 ± 2.9
	min	20.5	16.6	13.5	0.6		13.5
	max	40.6	36.5	24.3	1.0		24.3
	n	103	103	14	103		14
Gymnodinium aureolum	USA	28.4 ± 4.3	22.8 ± 4.1	19 ± 1.9	0.8 ± 0.1	0.7 ± 0.1	19.5 ± 0.9
	min	18.9	13.9	15.5	0.6	0.6	17.3
	max	38.7	32.7	21.6	1.0	0.9	20.7
	n	53	53	17	53	17	11

[a]For details of the origin see Table 1. Mean ± SD; ND = not determined; *n* = number.

ally slightly convex but sometimes had a small indentation (Figs. 5, 9). The right epicone–sulcal border, sometimes referred to as the ventral ridge or ventral flange (see Steidinger and Tangen 1996), was slightly curved (Figs. 2, 5, 14). The anterior part curved towards the apical groove (see below), while the posterior part terminated into a small pointed projection (Fig. 16), a feature which was often difficult to visualize. A very delicate and narrow apical groove was situated to the left of the sulcal axis (Figs. 2, 5, 8–10, 14, 16). It extended from slightly above the sulcal extension on the ventral side of the cell and continued about 1/3 of the way down the dorsal side of the epicone (not shown). A diminutive indentation caused by the apical groove was usually visible at the apex (Fig. 3). A reniform or pyriform nucleus was generally situated in the left part of the cell (Figs. 3, 6, 13, 17). However, in the Australian strain the nucleus was usually rounded and situated in the left lobe of the hypocone (Fig. 11), although a normal nucleus was also seen (Fig. 13), and in a few cells the nucleus was located in the epicone (not shown). In the Danish strain, the nucleus usually extended into the right lobe of the hypocone (Fig. 15), but a normal nucleus was also present (not shown). In a small number of cells of the English strain, the nucleus was situated either in the hypocone (Fig. 18) or the epicone (Fig. 19), or it occupied most of the dorsal part of the cell (Fig. 20). The latter situation might be a predivision stage. The number, shape, and color of the chloroplasts were quite variable, both within and between the strains. The number of chloroplasts was generally 10–15 or more. The exact number was not determined, but as cultures aged the number decreased to only a few, 3–4 at the most. The Australian strain had considerably fewer chloroplasts, usually only 4–5 and rarely more than 10. The shape of the chloroplasts was generally somewhat elongated, irregular without any particular shape, but might also be rounded (Fig. 12). Each chloroplast contained a single pyrenoid (Figs. 4, 7, 14), which in some viewing angles had a triangular appearance (see also Fig. 6 in Takayama and Adachi 1984). The similarity in pyrenoid structure was confirmed by transmission electron microscopy of the Japanese and Norwegian strains (Figs. 37–39). The color was somewhat variable between strains. It appeared yellow with a greenish tinge in the Japanese strain and yellow-brown or almost orange in the Norwegian strain. The color of the other strains were intermediate between the Japanese and Norwegian strains. No cysts were observed in any of the strains.

Gymnodinium aureolum (Hulburt) G. Hansen, comb. nov. (basionym: *Gyrodinium aureolum* Hulburt 1957; pp. 209–10; Plate 2, Figs. 8–9)

The cell shape is generally ovoid or ellipsoid (Figs. 22–23, 26), but small cells may be biconical (Fig. 24).

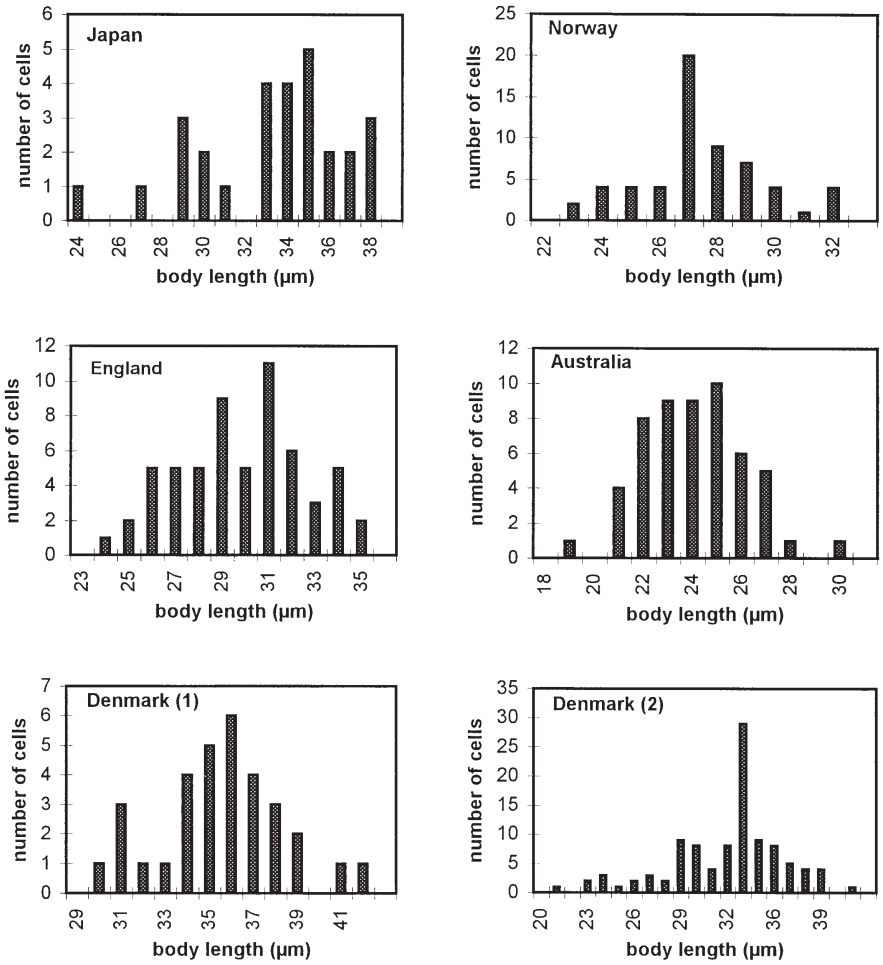
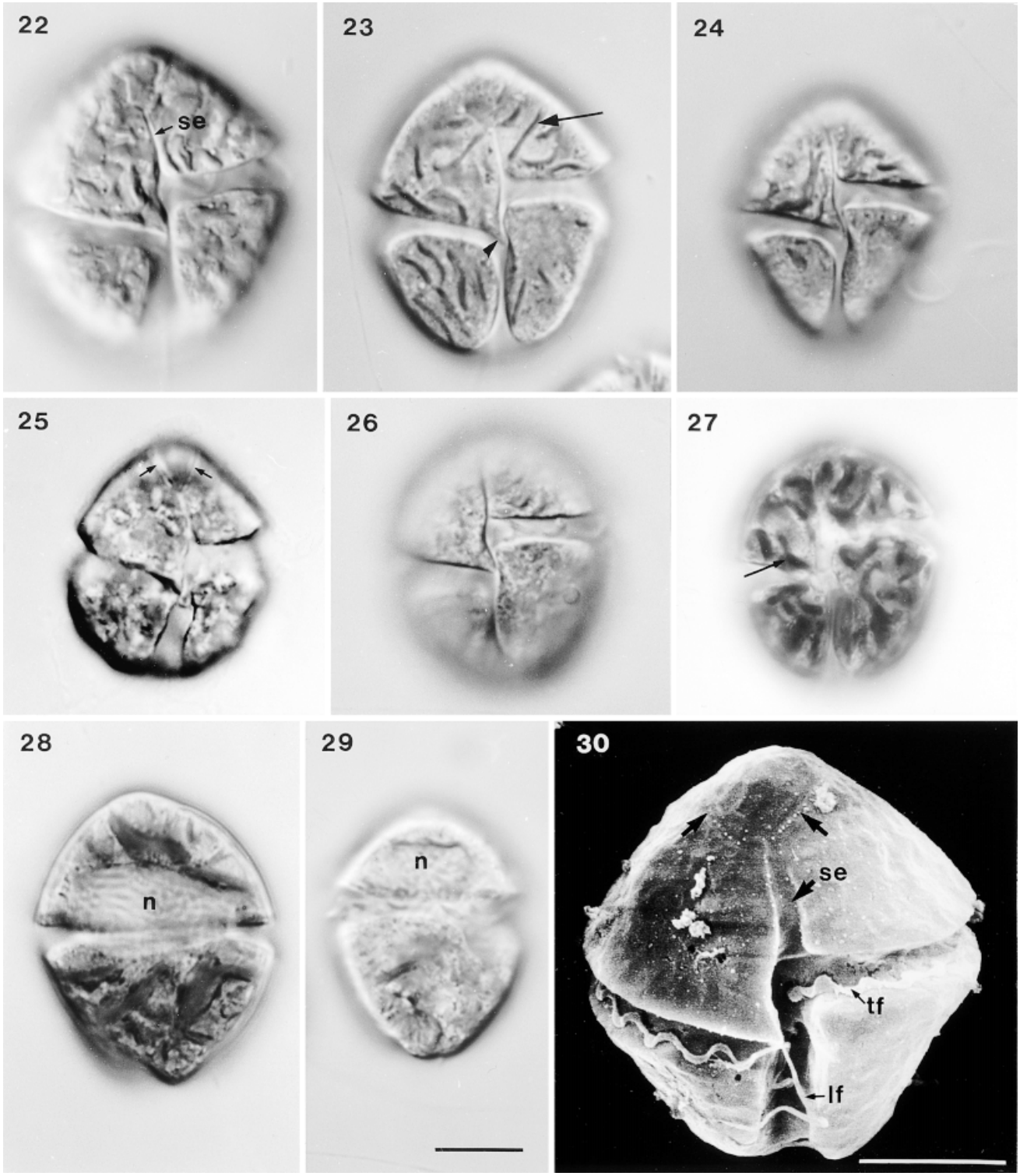


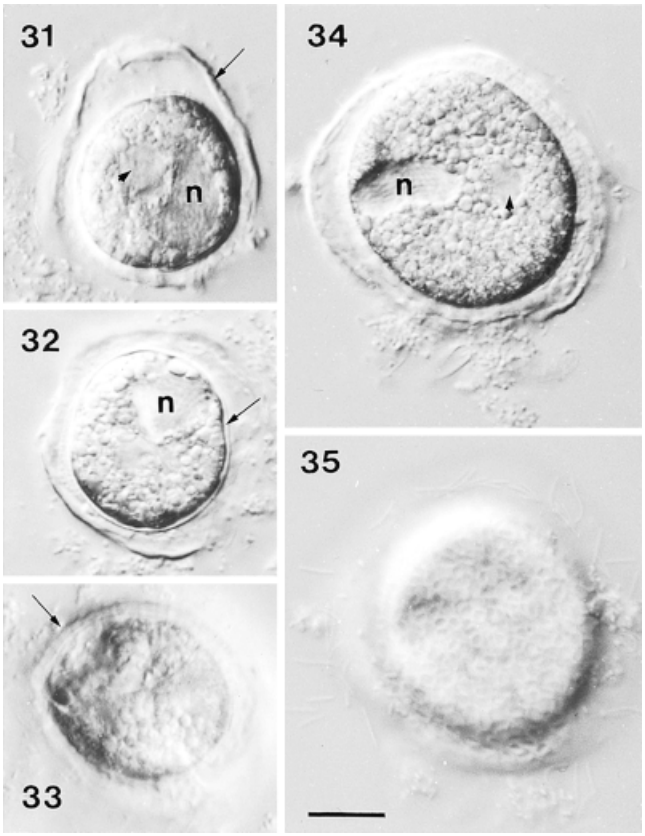
FIG. 21. Body length distribution in geographically different isolates of *Gymnodinium mikimotoi*. 1 = fresh culture; 2 = old culture.

The morphometric measurements are summarized in Table 3 and Fig. 36. The average body length was 28.4 µm, with a range of 18.9–38.7 µm. The average body width was 22.8 µm, with a range of 13.9–32.7 µm. In senescent cultures the proportion of small cells increased, but again it was not determined whether these represented distinct subpopulations. The cell was usually slightly longer than wide, the average body width/length ratio being 0.8. The average thickness/body length ratio was 0.7, with a range of 0.6–0.9. No ventral concavity was observed. The cingulum was usually median and relatively wide (Figs. 22–26), but might be premedian in small cells (Fig. 29). The cingular displacement was 17.3%–20.7% (average 19.5%) of the body length. An inconspicuous antapical indentation was sometimes present (Figs. 28, 30). The sulcus extended from the antapex and about 2/3 to 3/4 of the way onto the epicone (Figs. 22–26, 30). It was slightly wider towards the antapex (Figs. 22, 25, 30). The sulcal extension on the epicone was narrow but distinct. The right epicone–sulcal border or ventral ridge was more or less deflected to the left, and usually had a slightly sigmoid outline (Figs. 22–24, 26, 30). In some specimens it terminated into a small projection (Figs. 23–24, 26). A very indistinct loop-shaped apical groove ran counterclockwise from the sulcal extension, encircling the apex, and again reaching the ventral side of the cell a short distance from its origin. Usually, this structure was visible only in SEM (Fig. 30), rarely in light microscopy (Fig. 25). The nucleus was wider than long and generally located in the central part of the cell (Fig. 28), but in small cells it was sometimes situated in the epicone (Fig. 29). The cell contained numerous irregular or branching chloroplasts (Fig. 27). Generally they were not arranged in any particular pattern, but sometimes showed an indistinct radiating arrangement (Fig. 27). In some cells the chloroplasts were elongated and narrow (Fig. 23). Pyrenoids were not visible in the light microscope, but electron microscopy of sectioned material revealed a single somewhat branching pyrenoid complex situated in the area between the nucleus and the ventral side of the cell (Fig. 40). Serial sections indicated that what appeared to be many chloroplasts in the light microscope might represent only a single ramifying chloroplast (not shown).

In very old cultures, numerous cysts occurred at the bottom of the culture tubes (Figs. 31–35). They might



FIGS. 22–30. *Gymnodinium aureolum*. Scale bars 10 = μm. FIG. 22. Typical cell. Notice the sulcal extension (se). FIG. 23. Cell with elongated chloroplasts (arrow) and a finger-like terminal protrusion of the ventral flange. FIG. 24. Relatively small cell with somewhat biconical shape. FIG. 25. Apical groove (arrows) is visible. FIGS. 26–27. Same cell with hemispherical epicone and hypocone after prolonged observation under the microscope. The chloroplasts (arrow) are visualized by a Wratten Blue filter in FIG. 27. FIG. 28. Typical shape and position of the nucleus (n). FIG. 29. Small cell with the nucleus (n) situated in the epicone. FIG. 30. Scanning electron microscopy. The apical groove is marked with arrows. Notice also the very distinct sulcal extension (se).



FIGS. 31–35. Cysts of *Gymnodinium aureolum*. Scale bar = 10 μm. FIGS. 31–32. The outer and inner layers of the cyst wall are marked by arrows in FIGS. 31 and 32, respectively. FIG. 33. Cyst with more firmly attached outer layer and a columnar appearance. FIGS. 34–35. High and low focus of the same cyst. The outer layer has a microreticulate pattern clearly seen in FIG. 35. Notice also the nucleus (n) and the residual body (arrowhead).

represent sexual cysts or hypnozygotes, but the stages in the life history giving rise to these cysts were not followed, nor their germination. The cysts were colorless and spherical or ovoid in shape. They sometimes contained a yellow-greenish residual body (Figs. 31, 34), which most likely represented the remnants of the plastidial complex. No obvious accumulation bodies were observed. Numerous starch grains were present (Figs. 33–34). The nucleus was usually visible (Figs. 31, 34), but might also be indiscernible. The latter situation may represent a later cyst stage. Two layers surrounded the cyst, an inner smooth and relatively thick cyst wall (Fig. 32) and an outer more loosely attached layer (Fig. 31). The latter was usually covered with debris and bacteria, but a faint microreticulated ornamentation was sometimes observed (Fig. 35). In some specimens the outer layer was more firmly attached and then had a columnar appearance (Fig. 33).

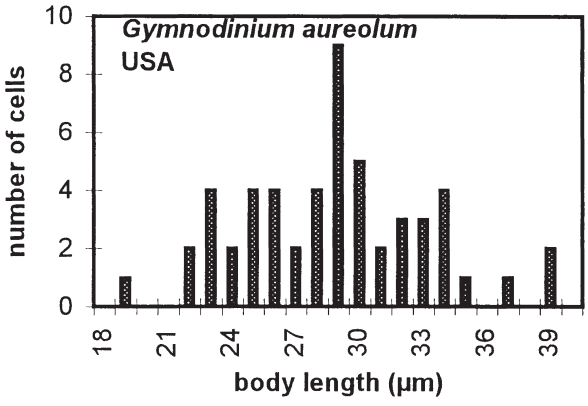
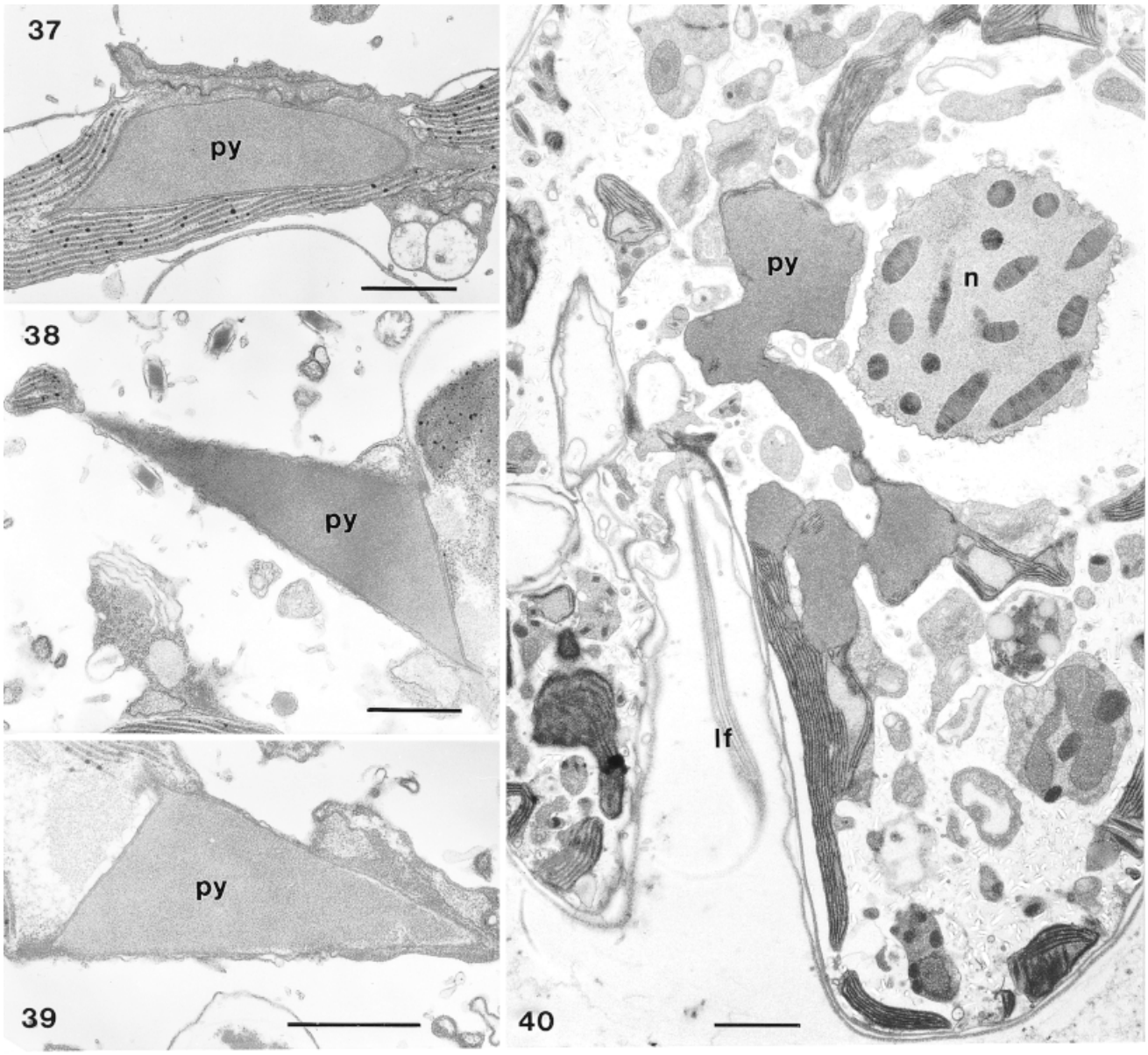


FIG. 36. Cell body length in *Gymnodinium aureolum*.

A morphologically identical species had previously been isolated from Danish waters (not shown). This isolate was confirmed to be the same species by determination of LSU rDNA sequences (see below).

Pigment composition. The pigment profiles of the Japanese and Norwegian isolates of *Gymnodinium mikimotoi* were identical but differed from *G. aureolum* (Fig. 41). In addition to chl *a*, both isolates of *G. mikimotoi* contained chl c_1/c_2 (not separated by the present HPLC method) and minor amounts of chl c_3. Chlorophyll c_3 was identified from absorption spectra only (Jeffrey et al. 1997), as no standard was available. In *G. aureolum*, the very small peak eluting prior to chl c_1/c_2 could not be verified as chl c_3 (Fig. 41). The carotenoid composition of the two isolates of *G. mikimotoi* was dominated by fucoxanthin; additional carotenoids were 19′-butanoyloxy- and 19′-hexanoyloxyfucoxanthin, diadinoxanthin, β,ϵ- and β,β-carotene, and an unidentified peak (retention time 22.5 min) with an absorption spectrum identical to gyroxanthin-diester as described in Johnsen and Sakshaug (1993). In addition, a peak eluting immediately prior to 19′-hexanoyloxyfucoxanthin and of similar size and with a similar absorption spectrum was found in both *G.*



FIGS. 37–40. Transmission electron microscopy of pyrenoids (py). FIGS. 37–38. Japanese isolate of *Gymnodinium mikimotoi*. Scale bars = 1 μm. FIG. 39. Norwegian isolate of *Gymnodinium mikimotoi*. Scale bar = 2 μm. FIG. 40. *Gymnodinium aureolum*. Scale bar = 2 μm.

mikimotoi isolates. In contrast, peridinin was the major carotenoid in *G. aureolum*. Minor carotenoids were diadinoxanthin, β,ϵ-carotene, and an unidentified carotenoid with an absorption spectrum similar to dinoxanthin (Jeffrey et al. 1997) eluting prior to diadinoxanthin.

Large subunit rDNA data. The first three domains (D1, D2, and D3 including the conserved core region upstream domain D3) constituted ≈28% of the complete nuclear-encoded LSU rDNA. Domains D1 and D2 are known to be hypervariable and constitute one of the fastest evolving segments in rRNA-encoded eukaryotic DNA (e.g. Lenaers et al. 1989, 1991). The length of the sequences determined varied from 942 base pairs in *Alexandrium tamarense* (Lebour) Balech to 1011 base pairs in *Gymnodinium galatheanum* Braarud. Determination of nucleotides in domains D1–D3 from five geographically different isolates of *Gymnodinium mikimotoi* revealed these to be nearly identical. A comparison between isolates from England and Norway showed only 2 out of 925 positions (excluding gaps) to differ (≈0.2%), whereas the Danish, Australian, and Japanese strains were identical. The strains of *Gymnodinium aureolum* isolated from Denmark and North America were identical. Table 4 summarizes all pairwise comparisons between isolates of *G. mikimotoi* and *G. aureolum* with respect to the total number of substitutions and the genetic distance based on Kimura's two-parameter model.

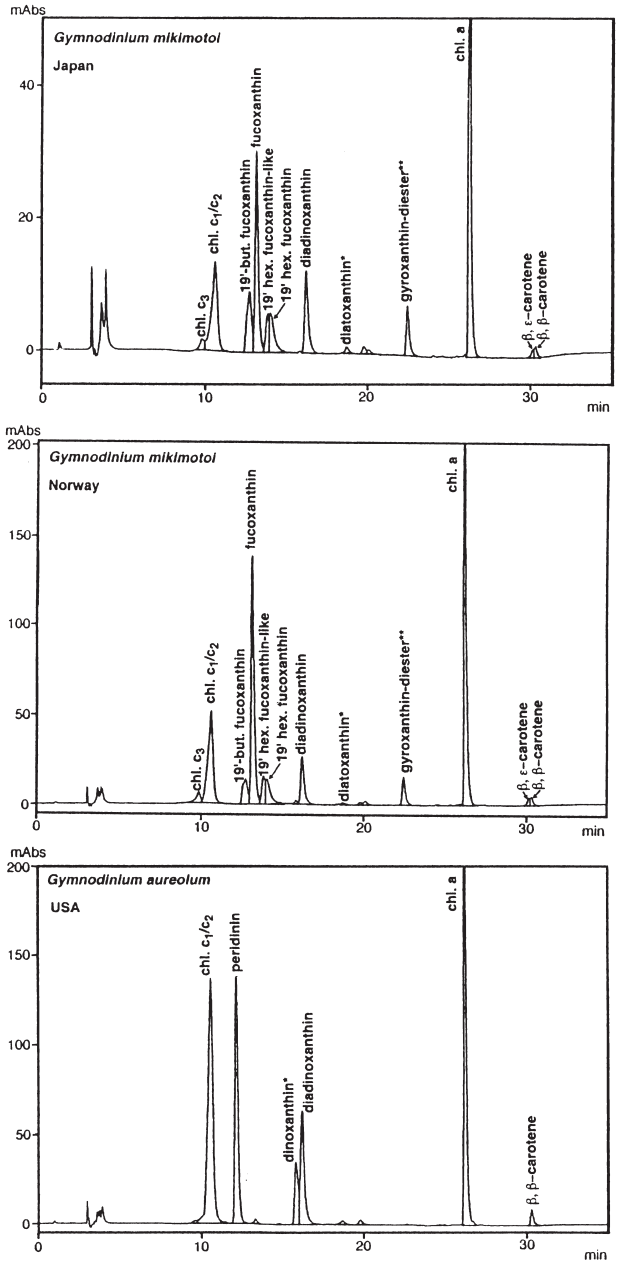


FIG. 41. Pigment profiles of Japanese and Norwegian isolates of *Gymnodinium mikimotoi*, and *Gymnodinium aureolum* from the USA. *Identified from absorption spectra only (Jeffrey et al. 1997). **Absorption spectrum identical to gyroxanthin-diester as described by Johnsen & Sakshaug (1993).

The alignment incorporating the secondary structure of the mature ribosomal gene was analyzed using parsimony, ML, and NJ. The branching patterns obtained in ML and NJ analyses were identical and are shown in Fig. 42. The topology from the cladistic analysis shown in Fig. 43 is congruent with the branching pattern obtained with ML and NJ, except for the position of *Gymnodinium fuscum* (Ehrenberg) Stein. In ML and NJ analyses, *G. fuscum* forms a sister group relationship with the clade containing *Gyrodinium impudicum* Fraga et Bravo, the two isolates of *Gymnodinium aureolum*, and *G. chlorophorum* Elbrächter et Schnepf, whereas parsimony analysis shows *G. fuscum* as a sister species to a clade comprising *G. catenatum* Graham and *G. nolleri* Ellegaard et Moestrup. The inconsistent position of *G. fuscum* is also revealed by the lower bootstrap and support index values. (The support index indicates the added number of steps before a clade is no longer present when compared with the tree with the minimum length.) Otherwise the bootstrap analyses strongly indicate a robust tree topology. The five geographically separate isolates of *G. mikimotoi* form a sister group to *Gymnodinium breve* Davis. The isolates of *G. mikimotoi* show an unresolved topology, as their sequences are almost identical. *Gymnodinium chlorophorum* is the closest relative to the strains of *G. aureolum*. *Gymnodinium nolleri* clusters strongly (100% bootstrap) with a Spanish isolate of *G. catenatum*. The sequence divergence between these dinoflagellate taxa was 2.1%.

DISCUSSION

North European Gymnodinium mikimotoi (= "Gyrodinium aureolum," G. *cf.* aureolum, Gymnodinium nagasakiense, G. *cf.* nagasakiense)

According to Hulburt (1957) the main characteristics of *Gyrodinium aureolum* are a cell length of 30–35 μm, a globular or ellipsoid cell shape, and slightly dorsoventrally flattened cells. The sulcus extended onto the epicone with a slight deflection to the left in the cingular region. Numerous elliptical chloroplasts were present in the cells, usually arranged in a somewhat radiating manner, and the central nucleus was usually wider than long (see also Fig. 44A). The cingular displacement was about 1/5 of the cell length and this figure represents the separating character between the genera *Gymnodinium* and *Gyrodinium*; more than 1/5 in *Gyrodinium*, less than 1/5 in *Gymnodinium* (Kofoid and Swezy 1921). Hulburt, however, placed his taxon in *Gyrodinium*.

In 1965, a massive bloom of a gymnodinioid dinoflagellate occurred in Omuru Bay, Japan. The causative species was tentatively named *Gymnodinium* sp., *Gymnodinium* sp. 1, or *Gymnodinium* type-'65 (Takayama and Adachi 1984, and references therein) and it subsequently became a very common bloom-forming species in coastal waters of Japan (e.g. Matsuoka et al. 1989). It was formally described as *Gymnodinium nagasakiense* (Takayama and Adachi 1984) (see also Fig. 44B). The main characteristics were a cell length of 18–37 μm (average 25–35 μm), dorsoventrally flattened cells, numerous yellow-brown chloroplasts of various shape each containing a single pyrenoid, and an ellipsoid or reniform nucleus situated in the left side of the cell. The sulcus extended from slightly above the cingulum to the antapex. A very distinct feature was the presence of a straight apical groove: i.e. a very delicate narrow furrow extending from the ven-

TABLE 4. Pairwise comparison between five isolates of *Gymnodinium mikimotoi* and two isolates of *Gymnodinium aureolum* based on 925 unambiguous aligned LSU rDNA nucleotides. The upper half of the matrix shows the total number of substitutions for all pairwise comparisons and the lower half shows distances calculated using the Kimura two-parameter model and PAUP*.

	G. mikimotoi (DK)	*G. mikimotoi* (AUS)	*G. mikimotoi* (UK)	*G. mikimotoi* (JPN)	*G. mikimotoi* (NOR)	*G. aureolum* (DK)	*G. aureolum* (USA)
G. mikimotoi (DK)	—	0	1	0	1	151	151
G. mikimotoi (AUS)	0	—	0	0	1	151	151
G. mikimotoi (UK)	0.0011	0	—	1	2	152	152
G. mikimotoi (JPN)	0	0	0.0011	—	1	151	151
G. mikimotoi (NOR)	0.0011	0.011	0.0022	0.0011	—	152	152
G. aureolum (DK)	0.1875	0.1878	0.1890	0.1875	0.1890	—	0
G. aureolum (USA)	0.1875	0.1878	0.1890	0.1875	0.1890	0	—

tral to the dorsal side of the cell, causing a small indentation at the apex. The cingular displacement was found to range from 1/9 to 1/4 of the cell length, but usually from 1/7 to 1/5. Takayama and Adachi discussed the striking similarities between *Gymnodinium nagasakiense* and the earlier described *Gymnodinium mikimotoi* (Miyake et Kominami) ex Oda, which had previously caused mortality of pearl oysters during a bloom in Gokasho Bay, Japan (Oda 1935, cited from Takayama and Adachi 1984; see also Fig. 44C). Takayama and Matsuoka (1991) presented a reassessment of the morphological and physiological charac-

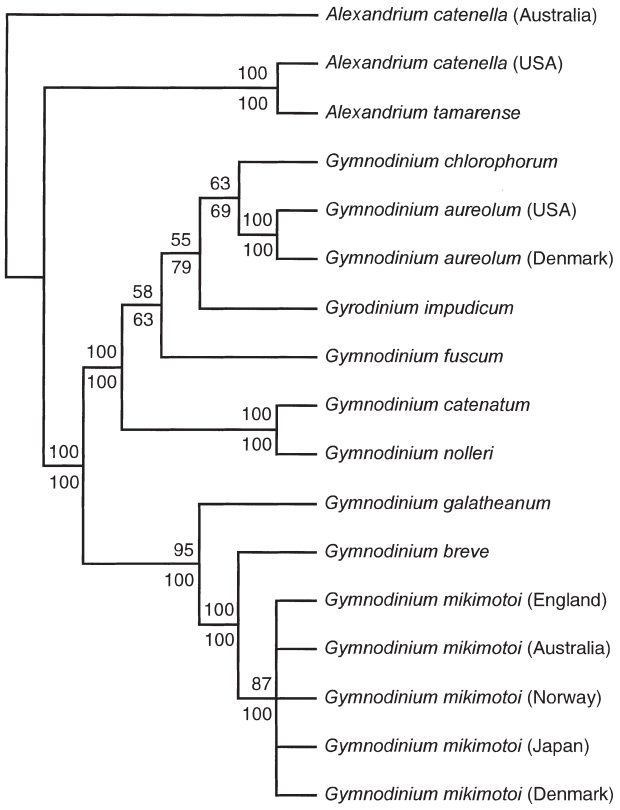


FIG. 42. Phylogeny of 17 species of dinoflagellates inferred from 931 base pairs of nuclear-encoded LSU rDNA (domains D1–D3 including the conserved core region upstream of domain D3). The reconstruction was based on maximum likelihood (ML) and neighbor-joining (NJ) methods. The best ln likelihood score was −4669.891, obtained with a transition/transversion ratio equal to 1.4. Bootstrap numbers above branches are calculated from ML analysis (100 replicates) and numbers below branches are calculated from NJ (1000 replicates using the Kimura two-parameter model for distance estimates). Three isolates of *Alexandrium* were used to root the trees.

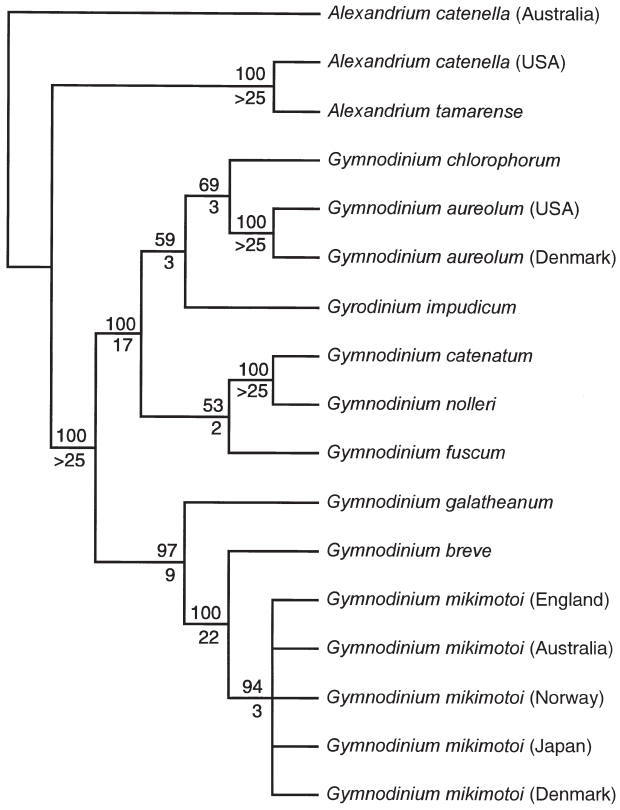


FIG. 43. Phylogeny of 17 species of dinoflagellates inferred from 931 base pairs of nuclear-encoded LSU rDNA (domains D1–D3). The reconstruction was based on parsimony analysis with equal weighting of all positions using PAUP*. The parsimony analysis produced six equally parsimonious trees (725 steps) using the heuristic search option with random addition of sequences (1000 replicates). The consistency index was 0.7453 and the retention index was 0.8651. Bootstrap numbers above branches are calculated from parsimony analyses (1000 replicates) and numbers below are the support index values. Three isolates of *Alexandrium* were used to root the tree.

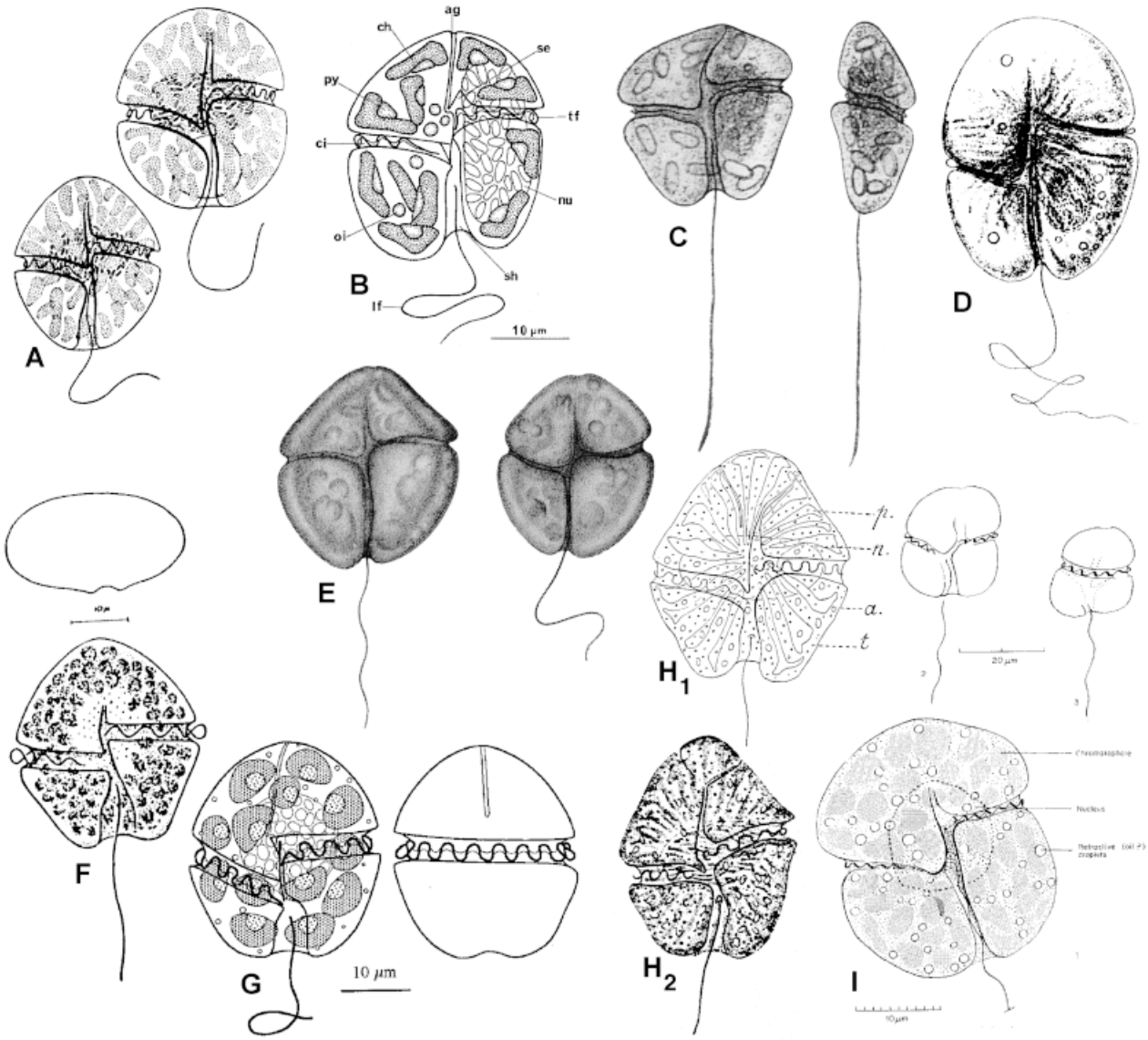


FIG. 44. (A) *Gyrodinium aureolum* (from Hulburt 1957). (B) *Gymnodinium nagasakiense* (from Takayama and Adachi 1984). (C) *Gymnodinium mikimotoi* (from Oda 1935). (D) *Gyrodinium aureum* (from Conrad 1926, as *Spirodinium aureum*). (E) *Gyrodinium aureolum* (from Braarud and Heimdal 1970). (F) *Gyrodinium aureolum* (from Campbell 1973). (G) *Gyrodinium aureolum* (from Takayama et al. 1998). (H_1 and H_2) *Gymnodinium maguelonnense* (from Biecheler 1939, 1952). (I) *Gyrodinium aureolum* (from Ballantine and Smith 1973).

teristics of the two species, based on material collected from the type localities, and concluded that they were conspecific, *G. mikimotoi* being the older and therefore valid name.

The uncertain taxonomy of the European "*Gyrodinium aureolum*" arose by the apparent misidentification of the North Atlantic material by Braarud and Heimdal (1970). This material deviated from Hulburt's *G. aureolum* by the position and shape of the nucleus, which was situated in the left side of cell (clearly seen in Fig. 1c of Braarud and Heimdal). In addition, what was termed the sulcus reached the apex, as shown in the drawings (see Fig. 44E), as there was a distinct apical indentation. Tangen (1977) subsequently described material from a bloom in 1976 and found this material to be in good agreement with Hulburt's (1957) original description, but an affinity to the Japanese *Gymnodinium* type-'65 (=*Gymnodinium nagasakiense* = *G. mikimotoi*) was suggested. The nucleus was situated subcentrally, though in some specimens it was located in the left side of the cell, considered to represent predivision stages. According to Tangen, the sulcus extended halfway between the cingulum and apex; however, the presence of an apical indentation indicated that it might have reached the apex. Subsequently, based on cultured material established from the Oslo Fjord in 1977, it was noted that an apical indentation was a common feature, and the sulcus

was found to extend to the dorsal side of the epicone. The nucleus was usually situated in the left side of the cell (Tangen and Björnland 1981). The present study clearly demonstrates that Tangen's *Gyrodinium aureolum* KT77B isolate is morphologically very similar to the Japanese isolate of *Gymnodinium mikimotoi*. The apical groove of the Norwegian material was obviously misinterpreted as a sulcal extension (Tangen 1977, Tangen and Björnland 1981); however, this mistake was also made in the original description of *Gymnodinium mikimotoi* (Oda 1935). The LSU rDNA sequences (see below), the chloroplast and pyrenoid ultrastructure, and the pigment profiles are also identical in the two isolates. Contrary to typical dinoflagellate pigments characterized mainly by peridinin, pigments of the two *G. mikimotoi* strains examined are dominated by fucoxanthin and 19′-acyloxyfucoxanthins and gyroxanthin-diester, typical of haptophytes and pelagophyceans (Johnsen and Sakshaug 1993). Previous studies of the pigment composition of European "*Gyrodinium aureolum*" and Japanese *Gymnodinium mikimotoi* used different methods, thus complicating a direct comparison (Tangen and Björnland 1981, Suzuki and Ishimaru 1992, Johnsen and Sakshaug 1993). Using thin-layer chromatography and mass spectrometry, Tangen and Björnland (1981) found that 19′-hexanoyloxyfucoxanthin constituted 71% of the total carotenoid content of "*G. aureolum*" isolated from the Oslo Fjord, Norway, but mass spectrometry also indicated fucoxanthin. This was confirmed by HPLC, which showed up to more than three times as much fucoxanthin as 19′-hexanoyloxyfucoxanthin in "*G. aureolum*" KT77B from the Oslo Fjord (Johnsen and Sakshaug 1993). Neither of these studies detected any 19′-butanoyloxyfucoxanthin. In *G. mikimotoi* from Tenma Bay, Japan, HPLC analysis identified fucoxanthin as the major carotenoid, together with smaller amounts of 19′-butanoyloxy- and 19′-hexanoyloxyfucoxanthin (Suzuki and Ishimaru 1992). The apparent discrepancies in pigment composition between the Norwegian and the Japanese strains were reinvestigated in the present study, using identical analytical methods on all isolates. In addition to the identified carotenoids, the combined use of retention time and absorption spectra of peaks in chromatograms allowed us to establish that the unidentified carotenoids were identical in the European and Japanese isolates. The carotenoid composition of both isolates was similar and showed only minor differences from strain 1 isolated from Tenma Bay (Suzuki and Ishimaru 1992).

The Danish and English strains were also morphologically identical to the Japanese strain of *Gymnodinium mikimotoi*, and the morphological difference between the different strains was not larger than the variation within a strain. The similarity was also reflected in the LSU rDNA sequence data, which clearly showed the strains to represent the same species. The significantly smaller size of the Australian strain was not reflected in the LSU rDNA sequences, as this strain was otherwise identical to the Japanese and Danish strains. We conclude that the Australian strain is also *Gymnodinium mikimotoi*. Likewise, we do not consider that the sequence divergence (≈0.2%) between the English and Norwegian strains justifies separation as different species. For comparison, *Gymnodinium catenatum* and *Gymnodinium nolleri*, which share many morphological and ultrastructural features (Ellegaard and Moestrup 1999), show a 2.1% sequence divergence. Based on LSU rDNA sequences, Scholin et al. (1994) were able to separate isolates of different *Alexandrium* species according to their geographic origin rather than their morphotype. However, we were unable to resolve the relationship of our isolates of *Gymnodinium mikimotoi*, which further strengthens the conclusion that they represent the same species. This confirms with previous observations of four European and two Japanese isolates of *Gymnodinium mikimotoi* (as *Gyrodinium aureolum* and *Gymnodinium nagasakiense*, respectively), based on LSU rDNA sequences (Engelen 1995). Using ITS sequences and information given by the presence and absence of introduced alignment gaps, Engelen (1995) was able to separate the Japanese and European isolates. However, this phylogenetic relationship was based on only six informative sites, and this was considered insufficient to separate the isolates into two different species (Engelen 1995). Monoclonal antibodies against surface antigens prepared from an European isolate of *G. mikimotoi* (as *Gyrodinium* cf. *aureolum*) cross-react only with Australian and Japanese *G. mikimotoi* (as *G. nagasakiens*e) and not with a number of other gymnodinioid species (Vrieling et al. 1994). Nevertheless, Partensky et al. (1988) found that the DNA content of Japanese *Gymnodinium mikimotoi* strains (as *Gymnodinium nagasakiense*) was 41% higher than in the European strains. The European isolates formed two distinct populations of large and small cells, respectively. The small cells apparently formed by a budding-like division process, a feature not observed in the Japanese strains (Partensky and Vaulot 1989). These discrepancies led Partensky et al. (1988) to suggest the designation *Gymnodinium* cf. *nagasakiense* for the European strains.

All isolates of *Gymnodinium mikimotoi* used in this study produced small cells in aging cultures, but it was not determined whether they represent distinct populations. We did not observe any budding-like divisions in any of the isolates. Such divisions occur very rarely in batch cultures, according to Partensky and Vaulot (1989), and we may have overlooked this phenomenon. The formation of small cells in senescent cultures has been described for a number of dinoflagellates (see Silva and Faust 1995 for a review) and is probably a general feature.

The differences in DNA content between the European and Japanese strains observed by Partensky et al. (1988) are puzzling, though the difference in DNA contents was not reflected in the number of chromosomes or in ploidy level. The higher DNA contents may represent additive structural DNA; see, for example, Sigee (1984), who suggested that the extraordi-

nary high DNA contents of dinoflagellates is mainly structural. If the higher DNA content of Japanese strains is a general feature, it may become an interesting geographical marker at the population level.

North American Gyrodinium aureolum. It may be argued that Hulburt's description of *Gyrodinium aureolum* was inaccurate and that his material should be regarded as a synonym of *Gymnodinium mikimotoi* (=*Gymnodinium nagasakiense*). However, Hulburt's (1957) illustrations and descriptions are generally very accurate and there are too many discrepancies between *Gymnodinium mikimotoi* and Hulburt's original description of *Gyrodinium aureolum*. We therefore agree with Partensky and Sournia (1986) and Taylor et al. (1995) that they represent different species. For example the cingulum–sulcus juncture is very different in the two species (compare Figs. 44A and 44B). The short oblique sulcal extension in *G. mikimotoi* gives the ventral ridge or flange a bulge-like appearance, a detail Hulburt would not have overlooked. It may be argued that the very distinct sulcal extension onto the epicone in *Gyrodinium aureolum* represents part of a straight apical groove misinterpreted as a sulcal extension by Hulburt. However, Hulburt depicted it as relatively broad, indicating that it represents a sulcal extension and not a delicate apical groove. *Gymnodinium mikimotoi* also possesses a distinct antapical notch formed by the sulcus, a feature which is not present in *Gyrodinium aureolum* (Hulburt: "antapex faintly indented at times"). Other differences between the two species are found in the shape and position of the nucleus, which according to Hulburt usually is wider than long in *Gyrodinium aureolum* and centrally located, as opposed to the kidney-shaped nucleus situated in the left part of the cell of *Gymnodinium mikimotoi*. This difference is not always reliable, however; our observations showed deviations from the typical position of the nucleus in *G. mikimotoi*, a variation also observed by Tangen (1977). We nevertheless consider the position of the nucleus to be a valuable taxonomic character, although it should be used cautiously. Hulburt did not observe any pyrenoids in *Gyrodinium aureolum* as opposed to the distinct pyrenoids of each chloroplast of *Gymnodinium mikimotoi*. A few additive studies have illustrated material that somewhat resemble Hulburt's *Gyrodinium aureolum*. Thus, the causative organism of a bloom along the coast of the northern Wales in October 1971 was identified as *Gyrodinium aureolum* by Ballantine and Smith (1973). Based on their illustrations (Fig. 44I), the location of the nucleus, the chloroplast, the shape of the cell, and to some extent the sulcal extension are all in agreement with Hulburt's description. However, the shape of the nucleus seems to be different and an apical indentation, believed to represent predivision stages, was present in some specimens. The apical indentation may be indicative of the presence of a straight apical groove. This material may indeed have been *Gyrodinium aureolum*. One of the very few illustrated reports of North American *Gyrodinium aureolum* is by Campbell (1973). His material from Gales Creek, North Carolina (Fig. 44F), is in good agreement with Hulburt's description, though the sulcal extension on the epicone is very short and much wider on the hypocone than depicted by Hulburt. Recently, Takayama et al. (1998) described *Gyrodinium aureolum* from Japanese waters. Their material is in agreement with Hulburt's description in the position of the nucleus and the shape of the cell (see Fig. 44G). It differs in the shape of the ventral flange or ridge, the lack of a sulcal extension, the presence of a straight apical groove, and the shape of the chloroplasts. We do not consider this material to be *Gyrodinium aureolum* but to represent an undescribed species related to *Gymnodinium mikimotoi*.

The *Gymnodinium* isolate from the Pettaquamscutt River is very similar to Hulburt's original material of *Gyrodinium aureolum*. Cell size and cingular displacements are identical. The cell shape is similar, and although measurements actually revealed an average body thickness/body length ratio similar to *Gymnodinium mikimotoi*, the range 0.9–0.6 may be acceptable as "only slightly" dorsoventrally flattened, in agreement with the description of Hulburt. The position and shape of the nucleus are also identical. The chloroplast shape of the Pettaquamscutt material is somewhat irregular, branching, and sometimes elongated, as opposed to the elliptical shape in Hulburt's description. However, in his figures 8 and 9 (see Fig. 44A), the shape and arrangement of the chloroplasts look very similar to the Pettaquamscutt material. Furthermore, the present study and others (e.g. Tangen 1977) have shown that chloroplast number and shape may vary considerably even within strains. Some of the most significant characters are the sulcal extension onto the epicone as well as the shape of the ventral flange or ridge. Both of these features are identical with Hulburt's description. It has been argued that the sulcal extension of Hulburt's *G. aureolum* is a misinterpreted apical groove, but our study has clearly demonstrated that it is an extension of the sulcus. The material from the Pettaquamscutt River has a delicate loop-shaped apical groove, a structure not mentioned by Hulburt, who may not have been able to see this delicate structure. Most naked dinoflagellates possess an apical groove (see Takayama 1985) and we find it very unlikely that this feature is absent in *Gyrodinium aureolum*. In conclusion, we believe that the Pettaquamscutt material is identical to Hulburt's *Gyrodinium aureolum*. LSU rDNA data (this study), as well as ultrastructural comparisons with the type species *Gymnodinium fuscum* and *Gyrodinium spirale* (Bergh) Kofoid et Swezy (Hansen, unpublished data), have shown that *Gyrodinium aureolum* belongs to genus *Gymnodinium* rather than *Gyrodinium*. Hulburt regarded *Gymnodinium aureolum* (=*Gyrodinium aureolum*) to be "rather similar" to *Gyrodinium aureum* (Conrad) Schiller, differing in "its less elongate chromatophores, wider grooves, greater width for the same length, more conical outline of the epicone and hypocone, and somewhat less displacement of girdle ends." To this can be added the presence of a distinct antapical notch in *Gyrodinium au-*

reum (see Fig. 44D) and the different shape and position of the nucleus. *Gymnodinium maguelonnense* Biecheler may be related to *Gymnodinium aureolum*. The sulcal extension, the nucleus, the apical groove and to a large extent the cell shape of this species are similar to that of *G. aureolum*. However, this species differs by having lamellate green chloroplasts arranged in a radiating pattern (Biecheler 1939, 1952), indicating that it contains chl *b*. The *Gyrodinium* sp. 1 of Bolch and Hallegraeff (1990) may be *Gymnodinium aureolum*. The morphology is similar to the Pettaquamscutt material and the cysts are also quite similar. However, an outer columnar covering was observed in the cysts of *Gyrodinium* sp. 1. This layer is not obvious in the Pettaquamscutt material, though in some specimens the outer layer resembled the columnar covering of *Gyrodinium* sp. 1. The outer layer of the Pettaquamscutt material has a microreticulate pattern not observed in *Gyrodinium* sp. 1. This pattern has some resemblance with the microreticulated outer cyst walls of *Gymnodinium catenatum* and *Gymnodinium nolleri*. However, in those species the layer is more firmly attached, appears to be more resistant, and shows what is termed paratabulation of the apical groove, the cingulum, and sulcus (Anderson et al. 1988, Ellegaard et al. 1993, Ellegaard and Oshima 1998). The morphology of the vegetative cell of these species, as well as *Gyrodinium impudicum* and the green *Gymnodinium chlorophorum* are very similar. This agrees with the molecular phylogenies based on LSU rDNA (this study) and SSU rDNA sequences (Saunders et al. 1997), which show a close relationship between these species. *Gymnodinium fuscum*, the type species of *Gymnodinium*, is also situated within the clade of these species, whereas the *mikimotoi* complex is more distantly related to *Gymnodinium fuscum*. The branching pattern clearly indicates the polyphyletic origin of the gymnodinioids and the need for a taxonomic revision of the genera *Gymnodinium* and *Gyrodinium*.

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