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Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSU rDNA sequence data, including the erection of three new genera of unarmoured dinoflagellates

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Evidence from partial large-subunit (LSU) rDNA sequencing has been combined with ultrastructure, including details of the flagellar apparatus, in a number of phototrophic dinoflagellates, with the aim of trying to solve some of the most pressing taxonomic problems and to contribute to an improved understanding of the phylogeny within the group. Special attention has been paid to the unarmoured (naked) genera, many of which were described during the 1800s or early 1900s and whose taxonomy is artificial and misleading. This is particularly unsatisfactory because many of the species cause extensive plankton blooms, fish kills and other harmful events. Our studies have indicated that the path of the so-called apical groove (acrobase) is of particular importance for the taxonomy of the unarmoured genera of dinoflagellates. Features presently used to characterize many of the genera, such as the relative size of the epicone and hypocone, are misleading. Our data have resulted in the splitting of the large genus *Gymnodinium* into four genera. The fish-killing species are confined to two genera, *Karenia* G. Hansen & Moestrup *gen. nov.* and *Karlodinium* J. Larsen *gen. nov.* The paralytic shellfish poisoning-producing species *Gymnodinium catenatum* is retained within *Gymnodinium*, together with a number of harmless species. The fourth genus, *Akashiwo* G. Hansen & Moestrup *gen. nov.*, presently comprises only the large nontoxic species previously known as *Gymnodinium sanguineum*. The genus *Gyrodinium* is redefined. The genus *Amphidinium* is artificial, but more data are needed before redescription of the genus can be made with any confidence. Within the armoured dinoflagellates, LSU and previously published small-subunit rDNA data show the Gonyaulacales to be a natural group. *Peridiniella catenata*, sometimes included in the Gonyaulacales based on gross morphology, falls outside this order both genetically and ultrastructurally. Based on the DNA data, the genus *Peridinium* appears to be artificial. Ultrastructure as well as gene sequences confirm that the genus *Heterocapsa* is unusual, since it includes both apparently unarmoured species (but with very thin plates) and armoured species.

INTRODUCTION

Most of the major genera of dinoflagellates were described during the late 1800s or early 1900s (e.g. *Gymnodinium* F. Stein, *Prorocentrum* Ehrenberg, *Amphidinium* Claparède & Lachmann and *Peridinium* Ehrenberg) and were therefore defined based on morphological criteria visible with the light microscope. In particular, following the work of Enrique Balech in Argentina, one of the main genera of armoured dinoflagellates, *Peridinium*, was redefined in the 1960s and was divided into several new genera, each was characterized by a particular type of plate pattern. The number of cingular plates was given particular importance (e.g. Balech 1974). Based on plate patterns, Balech also accepted the previously erected genus *Alexandrium* Halim as a genus separate from *Gonyaulax* (Balech 1989, 1995). However, in the case of the genera lacking well-defined thecal plates – the so-called naked or unarmoured species – very little progress has been made since these genera were erected, and the taxonomic system has remained almost unchanged since the 19th century. The fact that large genera such as *Gymnodinium*, *Gyrodinium* Kofoid & Swezy, *Amphidinium*, *Katodinium* Fott and others are assemblies of unrelated species has been known for many years, but alternative taxonomic definitions have not been forthcoming. These genera were defined on the basis of the relative sizes of the epicone and hypocone (e.g. Kofoid & Swezy 1921), but in fact there is a continuous series of species, from those with equally sized epicone and hypocone to species in which the cones are very different in size. In this continuum, generic boundaries can only be arbitrary. The separation between unarmoured and armoured species has also been questioned as a generic feature: when the common and apparently unarmoured marine species generally known as *Katodinium rotundatum* (Lohmann) Loeblich was examined in detail ultrastructurally, it was found to be related not to other unarmoured species but rather to the armoured genus *Heterocapsa* F. Stein (Hansen 1995). The absence of a satisfactory taxonomy for unarmoured species has become critical following the discovery that several of these species cause huge economic losses, killing fish in aquaculture farms, etc. (e.g. Hallegraeff 1993).

We have studied the ultrastructure of dinoflagellates for a number of years with the aim of constructing a more satisfactory taxonomic system based on fine structural features, notably features of the flagellar apparatus. Because of the com-

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paratively large size of many dinoflagellate cells – often combined with difficulties related to fixing the cells satisfactorily for reconstruction of the flagellar apparatus by serial sectioning – these studies have been time-consuming. In the case of the type species of *Gyrodinium*, more than 1000 serial sections were required to reconstruct the relevant part of the cell. Studies of the type species are essential when defining genera, and studies of the type species of *Gymnodinium* and *Gyrodinium* are being published separately (Hansen *et al.* 2000b). In the present paper we provide an overview of the systematics of unarmoured dinoflagellates by combining ultrastructural studies with gene sequence data, notably from the small-subunit (SSU) rDNA studies of Saunders *et al.* (1997) and our own studies on large-subunit (LSU) rDNA. Our LSU data now comprise nearly 1400 base pairs (bp) of this nuclear-encoded gene for 40 species (> 100,000 bases). We do not claim to have the final answer to all of the questions: determination of the generic affinity of many species of unarmoured dinoflagellates will require careful examination of ultrastructure and this will take a long time. However, some patterns are now emerging, and these are reported here, together with a discussion of some of the major problems that remain to be addressed. A preliminary report was given at *DINO 6* in Trondheim (Hansen 1998a, b; Moestrup *et al.* 1998). New combinations are listed in an Appendix.

MATERIAL AND METHODS

Cultures

Nonaxenic dinoflagellate cultures were obtained from various sources: Scandinavian Culture Centre for Algae and Protozoa (K strains); Provasoli–Guillard National Center for Marine Phytoplankton (CCMP strains); North East Pacific Culture Collection (NEPCC strains); and Drs A. Calado (AJC strains), M. Ellegaard (DK4 strain), P.J. Hansen (Danish isolates of species of *Ceratium*), T. Ikeda and S. Mutsuno (Japanese isolate of *Gymnodinium mikimotoi*), J. Larsen (JL strains), L. Maranda (S1-30-6 strain), C. Scholin (A3 strain), and K. Tangen (KT77B strain). Growth conditions for some of the cultures studied can be retrieved at http://www.sccap.bot.ku.dk or http://ccmp.bigelow.org or in Andersen *et al.* (1997). A list of species included in the phylogenetic reconstructions is given in Table 1.

DNA extraction, amplification and determination of partial LSU rDNA sequences

Volumes of 10–15 ml of exponentially growing cultures were collected by centrifugation at room temperature (1500 rpm for 10 min). Prior to extraction of total genomic DNA, the pellet was kept frozen (−20°C) for a minimum of 2 days. DNA was extracted using the CTAB method (Doyle & Doyle 1987) and precipitated using ethanol, as described in Daugbjerg *et al.* (1994). Extracted DNA was used as a template to amplify approximately 1400 bp of the nuclear-encoded LSU rDNA gene using terminal primers D1R (Scholin *et al.* 1994) and 28-1483R (5′-GCTACTACCACCAAGATCTGC-3′). Internal primers used to determine the LSU rDNA gene sequences and conditions for polymerase chain reaction (PCR) amplification and thermal cycling are outlined in Hansen *et al.* (2000a). The

Table 1. List of dinoflagellates included in the phylogenetic study. Available strain numbers and GenBank accession numbers are also provided.

Species	Strain numbers	GenBank accession numbers
Akashiwo sanguinea (Hirasaka) G. Hansen & Moestrup (= *Gymnodinium sanguineum* Hirasaka)	NEPCC354	AF260397
Akashiwo sanguinea	JL36	AF260396
Alexandrium catenella (Whedon & Kofoid) Balech (USA)	A3	AF200667
Alexandrium catenella (AUS)	K-0270	AF200666
Alexandrium tamarense (Lebour) Balech	K-0055	AF200668
Amphidinium carterae Hulburt	JL3	AF260380
Amphidinium operculatum Claparède & Lachmann	JL9	AF260381
Ceratium fusus (Ehrenberg) Dujardin	—	AF260390
Ceratium lineatum (Ehrenberg) Cleve	—	AF260391
Ceratium tripos (O.F. Müller) Nitzsch	—	AF260389
Dinophysis acuminata Claparède & Lachmann	—	X98250
Fragilidium subglobosum (von Stosch) Loeblich III	—	AF260387
Gonyaulax spinifera Diesing	K-0487	AF260388
Gymnodinium aureolum (Hulburt) G. Hansen (DK)	K-0303	AF200671
Gymnodinium aureolum (USA)	S1-30-6	AF200670
Gymnodinium catenatum L.W. Graham	—	AF200672
Gymnodinium chlorophorum Elbrächter & Schnepf	K-0539	AF200669
Gymnodinium fuscum F. Stein	CCMP1677	AF200676
Gymnodinium impudicum (Fraga & Bravo) G. Hansen & Moestrup (= *Gyrodinium impudicum* Fraga & Bravo)	JL30	AF200674
Gymnodinium nolleri Ellegaard & Moestrup	DK4	AF200673
Gymnodinium palustre Schilling	AJC14-732	AF260382
Gymnodinium cf. *placidum* Herdman	K-0308	AF260383
Heterocapsa rotundata (Lohmann) G. Hansen	K-0479	AF260400
Heterocapsa triquetra (Ehrenberg) F. Stein	K-0447	AF260401
Heterocapsa sp.	—	AF260399
Karenia brevis (Davis) G. Hansen & Moestrup (= *Gymnodinium breve* Davis)	JL32	AF200677
Karenia mikimotoi (Miyake & Kominami *ex* Oda) G. Hansen & Moestrup (= *Gymnodinium mikimotoi* Miyake & Kominami *ex* Oda) (DK)	K-0579	AF200682
Karenia mikimotoi (Japan)	—	AF200681
Karlodinium micrum (Leadbeater & Dodge) J. Larsen (= *Gymnodinium micrum* (Leadbeater & Dodge) Loeblich III	K-0522	AF200675
Peridiniella catenata (Levander) Balech	K-0543	AF260398
Peridinium bipes F. Stein	AJC8-847	AF260385
Peridinium cinctum Ehrenberg	AJC4cl-a	AF260394
Peridinium pseudolaeve Lefèvre	AJC2-798	AF260395
Peridinium willei Huitfeld-Kaas	AJC2-675	AF260384
Prorocentrum mexicanum Tafall	JL35	AF260378
Prorocentrum micans Ehrenberg	K-0335	AF260377
Prorocentrum minimum (Pavillard) Schiller	K-0010	AF260379
Protoceratium reticulatum Bütschli	K-0485	AF260386
Scrippsiella sp.	K-0399	AF260392
Scrippsiella trochoidea (F. Stein) Loeblich III var. *aciculifera*	K-0500	AF260393
Woloszynskia pseudopalustris (Woloszynska) Kisselew	AJC12cl-915	AF260402

Table 2. A comparison of LSU rDNA sequences from three *Prorocentrum* species. A total of 1397 nucleotides were included, starting at position 74, relative to the sequence of *P. micans* determined by Lenaers *et al.* 1989. Only those positions that differ from *P. micans* are shown. *Prorocentrum micans*[1] refers to the sequence retrieved from GenBank, and *P. micans*[2] refers to the Danish strain K-0335.

```
P. micans[1]      CC-CTAAAGG-CGGGGTACCTTTGGGTGCAGCCCGACTACCCTGTGTGTGTTCTCGATC
P. micans[2]      ..G.......C...-....-.......CGCCAG..............C..........
P. mexicanum      .AG....T..CA..-A...-.......CGCCAGT....GTTTC.C...CT.A.C.TTCT
P. minimum        T.GTCGG.AACAAA-.GGTTGACTATCCGCCAG.TTTCGTTACA.AGC..C.T.-CGCA

P. micans[1]      TGTG----C-GGGA-GG-ACCTC-AAACCT-CCTCTAAGTCCAGCT-AT-TGT--C-
P. micans[2]      ....TTGG.T...GG..G.....C..C...G.........T.....AG-GCT.GGGG
P. mexicanum      CCGAC..GTT...GG..G.T...CG.C..AG.GCGGGTAC-GG...AG-GCT.NNNN
P. minimum        .C....GAA.---CGCTTG.TCAGGGGGG.A..-GGGTACTGGATAAG-GCTCNNNN
```

QIAquick PCR Purification Kit (Qiagen) was used to purify PCR products, and nucleotide sequences were determined using the Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). The sequence reactions were run on an ABI PRISM™ 377 DNA sequencer (Perkin Elmer), following the recommendations of the manufacturer.

The LSU rDNA gene sequences that were determined (covering domain D1–D3 and including the conserved core region of D3) have all been submitted to GenBank. Accession numbers are provided in Table 1.

Sequence alignment and phylogenetic analyses

In order to optimize the alignment of the dinoflagellate gene sequences, we used information from the secondary structure of the LSU rRNA molecule, using the alveolate taxa available at the rRNA WWW server (http://www-rrna.uia.ac.be/lsu/index.html) (de Rijk *et al.* 2000). The data matrix comprised 1296 aligned positions (including introduced gaps) and of these 822 bp were considered unambiguous and examined using maximum parsimony (MP) and neighbour-joining (NJ) analyses. MP was performed with PAUP* version 4.0b2a (Swofford 1998), using the heuristic search option with random addition of sequences (100 replicates) and a branch-swapping algorithm (tree-bisection-reconnection). Characters were weighted equally and gaps were treated as missing data. PAUP* was also used to compute the phylogenetic distance between pairs of organisms by means of dissimilarity values based on a maximum likelihood model. The distance matrix was converted to an evolutionary tree using the NJ method. Bootstrap analyses (100 replications in MP and NJ) were applied to determine the robustness of the topologies (Felsenstein 1985).

Comparative studies using ultrastructural characters and phylogenetic reconstructions based on molecular data (e.g. van de Peer *et al.* 1996) have indicated that ciliates and apicomplexans form the sister groups to the dinoflagellates. Hence we used two ciliates [*Tetrahymena pyriformis* (GenBank X01533) and *Tetrahymena thermophila* (X54512)] and two apicomplexans [*Plasmodium falciparum* (U21939) and *Toxoplasma gondii* (X75429)] to polarise the ingroup.

RESULTS AND DISCUSSION

For the past two or three years, phylogenetic studies based on LSU rDNA sequence data have begun to appear more frequently in the literature, as attention has turned to relationships within the major eukaryotic lineages. This may be explained by the fact that molecular evolution of the LSU rDNA gene is faster than that of the more frequently used SSU rDNA gene. The LSU rDNA gene comprises highly variable regions intermixed with very conservative areas and may therefore be used to address phylogeny and evolutionary history at different systematic levels (Hillis & Dixon 1991). Because it is almost twice the size of the SSU rDNA gene, the LSU rDNA gene is a promising candidate for phylogenetic studies.

Prior to our study, the only complete dinoflagellate LSU rDNA sequence available in GenBank was that of *Prorocentrum micans* (GenBank accession X15973) and we used this, together with sequences from ciliates and apicomplexans, to align our data matrix of 40 dinoflagellates. Determination of the LSU rDNA sequence from two other species of *Prorocentrum* Ehrenberg (*P. mexicanum* and *P. minimum*) revealed that the sequence of *P. micans* from GenBank deviates at a few positions in conservative regions, indicating that the sequence is not entirely reliable. These differences prompted us to determine the first *c.* 1400 bp of a further strain of *P. micans* (from Denmark; K-0335). A sequence comparison of the two isolates of *P. micans, P. minimum* and *P. mexicanum,* is illustrated in Table 2. Based on a comparative study of the much larger LSU rDNA sequence database established, we included the Danish strain of *P. micans* rather than that presently available from GenBank.

The MP and NJ methods applied for phylogeny reconstructions resulted in tree topologies with almost the same branching pattern. Most of the clades for the terminal taxa are sup-

ported by high bootstrap values, whereas some of the deeper branches and the relationship of the clade containing the gymnodinioid, peridinioids and prorocentroid taxa (the GPP complex: Saunders *et al.* 1997) received less support and could not be resolved with confidence, based on the bootstrap method. Branches with less than 50% support were characterized by very short branch lengths (not shown). The short branches indicate either that the ancestors of these dinoflagellate lineages evolved within a relatively short period of time or that the LSU rDNA sequences determined are insufficient to resolve relationships. Similarly, analyses of dinoflagellate SSU rDNA sequence data resulted in short branch lengths for the deep branches (Saunders *et al.* 1997) and thus in an unresolved relationship for this particular part of the tree topology. Whether there really was a rapid radiation of some dinoflagellate ancestors will have to be tested by applying sequence information from genes other than those encoding nuclear ribosomal DNA, or by determination of the complete LSU rDNA sequence, approximately 3600 bp in dinoflagellates.

The phylogenetic reconstruction illustrated in Fig. 1 reveals six distinct clades: *Amphidinium, Dinophysis* Ehrenberg, the gonyaulacoids, *Woloszynskia pseudopalustris, Peridinium bipes/Peridinium willei,* and a large, more or less unresolved clade (i.e. low bootstrap values) consisting of the GPP complex. The latter contains several well-supported groups, but their interrelationships are uncertain and are not resolved. The tree in Fig. 1 forms the basis for the following discussion, combined with morphological and biochemical data. The genera will be discussed separately.

Unarmoured (naked) dinoflagellates

With the exception of *Amphidinium,* the unarmoured dinoflagellates are situated within the GPP complex in the following three separate but distinct clades.

Gymnodinium F. Stein emend. G. Hansen & Moestrup

Unarmoured unicellular or colony-forming dinoflagellates with horseshoe-shaped apical groove running in an anticlockwise direction. Nuclear envelope with vesicular chambers. Cingulum displacement one or more cingulum widths. Nuclear or dorsal fibrous connective present.

TYPE SPECIES: *G. fuscum* F. Stein.

OTHER SPECIES THAT WE CONSIDER TO BELONG TO GYMNODINIUM:

G. acidotum Nygaard

G. allophron J. Larsen

G. aureolum (Hulburt) G. Hansen *in* Hansen *et al.* (2000a)

G. catenatum H.W. Graham

G. chlorophorum Elbrächter & Schnepf

G. cryophilum (Wedemayer, Wilcox & Graham) G. Hansen & Moestrup *comb. nov.*

BASIONYM: *Amphidinium cryophilum* Wedemayer, Wilcox & Graham (p. 14; see further below)

Gymnodinium impudicum (Fraga & Bravo) G. Hansen & Moestrup *comb. nov.*

BASIONYM: *Gyrodinium impudicum* Fraga & Bravo (1995, p. 515).

G. maguelonnense Biecheler

G. nolleri Ellegaard & Moestrup

G. palustre Schilling

G. cf. *placidum* Herdman

Gymnodinium sp. *sensu* Roberts (1986)

? *G. microreticulatum* Bolch (judging from the shape of the anterior groove)

The green species *Lepidodinium viride* Watanabe, Suda, Inouye, Sawaguchi & Chihara almost certainly also belongs in the *Gymnodinium* clade, though not necessarily in the genus *Gymnodinium.* It has a similar apical groove but differs by the presence of an outer layer of body scales (Watanabe *et al.* 1987). Details of the flagellar apparatus are not known. Analysis of SSU rDNA sequences places this species within or close to *Gymnodinium sensu stricto* (Saunders *et al.* 1997), in agreement with the type of apical groove present.

In addition to the type species of *Gymnodinium, G. fuscum,* which is a species of oligotrophic freshwaters, the clade revealed by LSU rDNA data (Fig. 1) comprises six marine species [*G. aureolum, G. catenatum, G. chlorophorum* (with green chloroplasts), *G. impudicum, G. nolleri,* and *G.* cf. *placidum*] and one freshwater species (*G. palustre*). Both light microscopical and ultrastructural characters confirm that these species form a natural group. The apical grooves of *G. palustre* and *G.* cf. *placidum* are not known, but all of the other species have a delicate horseshoe-shaped apical groove running anticlockwise around the apex of the cell (Fig. 2A, B). In *G. fuscum* this groove is situated further away from the apex, and it is very faint and only visible in SEM (Hansen *et al.* 2000b). However, we believe it to be essentially the same as in other species of the clade.

Some striking ultrastructural features characterize all the members of this group that have been examined, which together comprise *G. fuscum* (Dodge & Crawford 1969; Hansen *et al.* 2000b), *G. aureolum* (Hansen *et al.* 2000a), *G. catenatum* (T. Reese & Ø. Moestrup, unpublished observations), and *G. nolleri* (Ellegaard & Moestrup 1999). All these species possess a so-called nuclear fibrous connector (NFC), also known as the dorsal connective, a very distinct fibre that interconnects the longitudinal microtubular root R1 (LMR) with the nucleus (for root terminology, see Moestrup 2000). In addition, the nuclear envelope of these species has peculiar chambers, in which the nuclear pores are situated. Such chambers were first observed in *G. fuscum* (Dodge & Crawford 1969), but subsequent studies have shown them to be present in *G. aureolum, G. catenatum* and *G. nolleri* also (Ellegaard & Moestrup 1999; G. Hansen, unpublished observations; T. Reese & Ø. Moestrup, unpublished observations). Similar chambers appear to be present in the vegetative stage of the aberrant dinoflagellate *Noctiluca scintillans* (Afzelius 1963; Soyer 1969), but they are apparently absent in the zoospores (Höhfeld & Melkonian 1995, fig. 1). A phylogeny based on SSU rDNA sequences revealed *Noctiluca* Suriray as the earliest lineage of the taxa analysed (Saunders *et al.* 1997), and indicated that it was apparently not closely related to *G. sensu stricto* or other 'gymnodinioids'. The phylogenetic significance of the nuclear chambers is therefore uncertain at present.

Gymnodinium fuscum differs from other members of this group in several respects. The pusule has an internal collection chamber, which is absent in the other species, and the pusule is connected to the flagellar canal by a complex tubular structure (Dodge 1972). The cortical microtubuli are grouped in characteristic triangular bundles (Dodge & Crawford 1969), not seen in other species. In addition, there are no striated collars surrounding the flagellar canals, nor is there a trans-

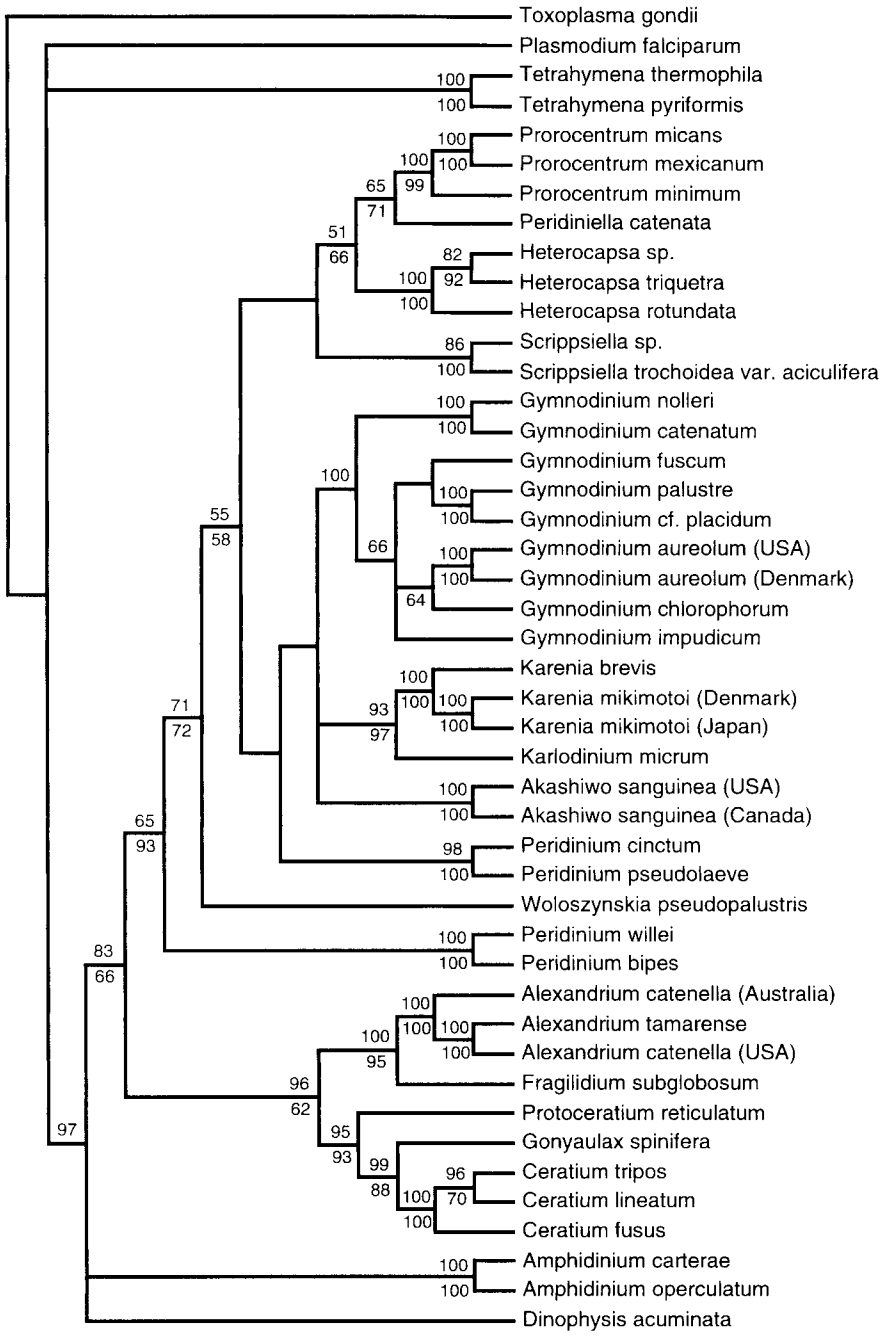


Fig. 1. Strict consensus of the 10 equally parsimonious trees obtained with the heuristic search option in PAUP* and based on LSU rDNA domain D1–D3, including the conserved core region of D3. Tree length = 2842, consistency index = 0.427 and retention index = 0.601. The bootstrap values shown above internal nodes are inferred from MP analysis using a weighted rescaled consistency index over an interval of 1–1000. The bootstrap values below the internal nodes are inferred from distance analyses of the same data set and are based on a maximum likelihood model to calculate dissimilarities (the Felsenstein 1984 model available in PAUP*) and are used as input for NJ analyses. The two ciliates and the two apicomplexans were used to root the tree.

verse striated flagellar root (TSR) associated with the transverse basal bodies (Hansen *et al.* 2000b). Lack of a striated flagellar root has not yet been observed in any other dinoflagellate. Trichocysts are also absent in *G. fuscum* (Dodge & Crawford 1969), an unusual but not a unique feature among the dinoflagellates. The molecular phylogeny shown in Fig. 1 indicates that *G. fuscum, G. palustre* and *G.* cf. *placidum* are more closely related to the clade comprising *G. aureolum, G. chlorophorum* and *G. impudicum* than to that comprising *G. catenatum* and *G. nolleri.* The ultrastructural evidence does not support this relationship, as *G. aureolum* is ultrastructurally very similar to *G. catenatum* and *G. nolleri* (Hansen, personal observations). Though only weakly supported, the relationship of *G. palustre* and *G.* cf. *placidum* with *G. fuscum* is interesting, since the gross morphology of these species appears to be somewhat similar. In culture, both *G.* cf. *pla-*

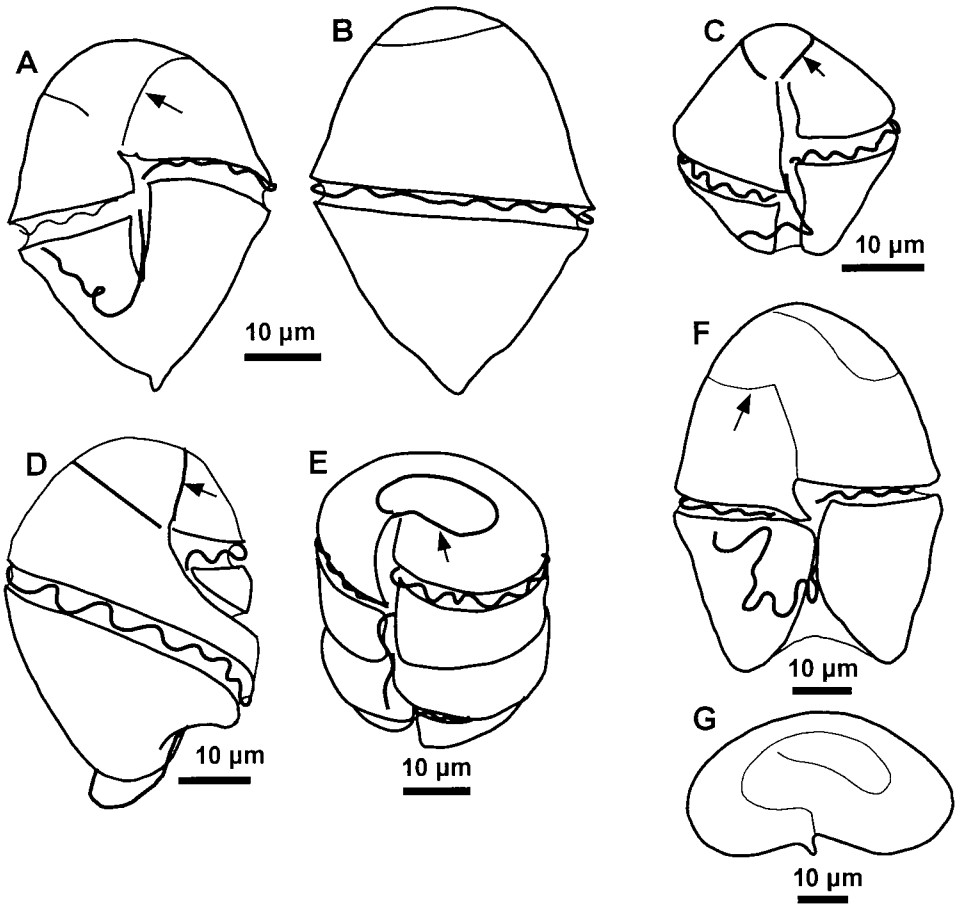


Fig. 2. Apical grooves (arrows) of unarmoured dinoflagellates (drawings based on Hansen *et al.* 2000b; Takayama 1985, 1998; Roberts *et al.* 1992; Hansen, unpublished observations). (A, B) *Gymnodinium fuscum,* ventral and dorsal view, respectively. (C) *Gymnodinium aureolum.* (D) *Nematodinium armatum.* (E) *Polykrikos kofoidii.* (F, G) *Akashiwo sanguinea,* ventral and apical views, respectively.

cidum and *G. fuscum* produce extensive amounts of mucilage, in which the cells remain motionless or move very slowly (G. Hansen, unpublished observations). The difference between *G. fuscum* and the other species suggests that *G. fuscum* and its relatives should be placed in a genus of their own, requiring the removal of all other species into one or more new genera. However, such a move would reduce the genus *Gymnodinium* from being one of largest genera of unarmoured dinoflagellates (*c.* 200 described species) to one of the smallest, with only a few species. We consider such a move premature, especially since ultrastructural data are not available for *G. palustre* and *G.* cf. *placidum.* In addition, the somewhat ambiguous position of *G. fuscum* within *G. sensu stricto,* based on LSU rDNA sequencing, needs further investigation.

A NFC is also present in the *Gymnodinium* species studied by Roberts (1986), and the 'large fibrous connective' of *G. acidotum* (Farmer & Roberts 1989) is most likely homologous with a NFC. It extends from flagellar root R1 to the dorsal side of the cell but without reaching the nucleus. The same probably applies to the dorsal fibre of *Amphidinium cryophilum* (Wilcox *et al.* 1982). The pusular type and nuclear envelope of both *G. acidotum* and *A. cryophilum* appear to be similar to those of *G. fuscum* (Wilcox *et al.* 1982, figs 1, 6; Wilcox & Wedemayer 1984, figs 4, 5). These species therefore almost certainly also belong to *G. sensu stricto,* requiring the formal transfer of *A. cryophilum.*

The presence of a NFC in the heterotrophic species *Actiniscus pentasterias* (Ehrenberg) Ehrenberg (Hansen 1993), *Nematodinium armatum* (Dogiel) Kofoid & Swezy (Roberts & Taylor 1995), and *Polykrikos kofoidii* Chatton (Bradbury *et al.* 1983) points towards a phylogenetic relationship between these species and *Gymnodinium sensu stricto. Polykrikos* Bütschli usually forms chains of six cell-like entities, but containing only three nuclei. Such colonies are at first sight very unlike *Gymnodinium.* Under suboptimal conditions, however, the chains break up into single *Gymnodinium*-like cells (Morey-Gaines & Ruse 1980).

The nuclear envelopes of *A. pentasterias* and *P. kofoidii,* which are identical, could have developed from *Gymnodinium,* or vice versa, as the nuclear pores in these species are located in vesicular differentiations (invaginations) of the nuclear envelope. However, an additional bilayered wall-like layer or nuclear capsule is situated underneath the nuclear envelope in these species (Bradbury *et al.* 1983; Hansen 1993). Details of the nuclear envelope in *N. armatum* are not known. The apical grooves of *N. armatum* and *Polykrikos schwartzii* (Fig 2D, E) appear to be of the same type as those observed in *Gymnodinium sensu stricto* (Takayama 1985, pl.

II, figs 18, 19), further supporting the idea of a phylogenetic relationship between these organisms. The SSU rDNA sequence of *P. schwartzii* was determined by Saunders *et al.* (1997), whose phylogenetic tree did not show a close relationship with *Gymnodinium sensu stricto.* Instead, *Polykrikos* grouped with *G. mikimotoi,* a grouping that is supported neither by external morphology (SEM) nor by internal ultrastructural characters (see below).

Akashiwo G. Hansen & Moestrup, *gen. nov.*

Dinoflagellata inarmata chloroplastis cum peridinin pro pigmentis principalibus accessoriisque. Involucrum nuclei in facie eukaryotum typicum, id est sine loculis in involucro. Connectivum dorsale inter apparatum flagellarem et nucleum absens. Canalis apicalis secundum horologii motum curvus.

Unarmoured dinoflagellates with chloroplasts containing peridinin as major carotenoid. Nuclear envelope of typical eukaryotic appearance (i.e. lacking envelope chambers). Dorsal connective between the flagellar apparatus and the nucleus absent. Apical groove curves around the apex in a clockwise direction.

ETYMOLOGY: *akashiwo* (Japanese) = red tide.

TYPE SPECIES: *Akashiwo sanguinea* (Hirasaka) G. Hansen & Moestrup *comb. nov.*

BASIONYM: *Gymnodinium sanguineum* Hirasaka (1922, p. 162).

SYNONYMS: *Gymnodinium splendens* Lebour, *G. nelsonii* Martin.

Akashiwo sanguinea is one of the largest and most conspicuous naked dinoflagellates and is thought to include as synonyms *G. splendens* and *G. nelsonii* (e.g. Steidinger & Tangen 1996). *Akashiwo sanguinea* is widely distributed and often forms blooms, but there are no confirmed reports of toxicity. In the SSU rDNA (Gunderson *et al.* 1999) and the LSU rDNA (Fig. 1) trees, *A. sanguinea* does not cluster with other gymnodinioids. At first sight this seems surprising, since *A. sanguinea* has a 'typical' *Gymnodinium*-like appearance and cells contain peridinin as the major carotenoid (Johansen *et al.* 1974, as *G. nelsonii* and *G. splendens*). However, *A. sanguinea* also differs from *Gymnodinium sensu stricto* in several significant morphological features. The apical groove of *A. sanguinea* appears to be a large clockwise spiral when seen from the front of the cell [Fig. 2F, G, based on SEM photographs of Takayama (1998, pl. 14, figs 4, 8)], rather than straight, as in the *mikimotoi* group, or like the anticlockwise horseshoe of *Gymnodinium sensu stricto.* The apical groove of *A. sanguinea* is not usually visible in the light microscope. However, after immunofluorescence labelling with anti-tubulin or anti-centrin, an 'apical transverse band' appears (Roberts & Roberts 1991; Roberts *et al.* 1992), undoubtedly the apical groove. This indicates that the groove is supported by microtubules and that it also contains the Ca^{2+}-modulated protein centrin or a homologue. In addition, the pusular vesicles of *A. sanguinea* do not open into a collecting chamber, as they do in *Gymnodinium sensu stricto* or in the *mikimotoi* group, but rather they connect directly to the flagellar canal. They possess a 'fuzzy coat' on their inner margin (Dodge 1972, as *G. nelsonii*).

The flagellar apparatus of *A. sanguinea* includes most of the typical dinoflagellate components. However, the striated collars are very reduced, an unusual multilayered structure is present near the anterior part of R1, and a NFC (dorsal connective) is absent (Roberts 1991; Roberts & Roberts 1991; Roberts & Bunnell 1998; Roberts, personal communication). One of the most significant features of *A. sanguinea* is the absence of vesicular differentiations of the nuclear envelope. A 40–120 nm wide electron-dense granular layer is situated immediately beneath the nuclear envelope (Stone & Vesk 1982).

Reduction or even loss of micromorphological structures has obviously taken place many times during the evolutionary history of dinoflagellates, and this probably applies to the striated collars of both *G. fuscum* and *A. sanguinea.* Whether a dorsal connective (homologous with a NFC) has been lost in *A. sanguinea,* or whether it was never present in this and related species, is a question that cannot be answered at the moment. The lack of nuclear envelope chambers and the path of the apical groove are presently the most characteristic features of *A. sanguinea.* Together with the other ultrastructural data and the data from the rDNA trees, they justify the transfer of this species to a genus of its own.

The apical groove in *Gyrodinium resplendens* Hulburt also extends in a clockwise direction (Takayama 1998), indicating that this species may belong to *Akashiwo.* In *Gymnodinium pulchellum* J. Larsen, the apical groove begins proximally as in *A. sanguinea* (Larsen 1994), but distally it bends away from the apex, in the direction of the dorsal side (Fig. 3E, F). The ultrastructure and LSU rDNA sequence have not been studied in either of these species, and it is not possible to determine their generic affiliation.

Karenia G. Hansen & Moestrup, *gen. nov.*

Dinoflagellata inarmata cum fucoxanthin et/aut 19′-hexanoyl-oxyfucoxanthin et/aut 19′-butanoyl-oxyfucoxanthin pro pigmentis principalibus accessoriisque. Nucleus sine loculis in involucro et sine capsula. Canalis anticus rectus.

Unarmoured dinoflagellates whose major carotenoid is fucoxanthin, 19′-hexanoyl-oxyfucoxanthin and/or 19′-butanoyl-oxyfucoxanthin. Cell nucleus without nuclear envelope chambers and nuclear capsule. Apical groove straight.

ETYMOLOGY: Named after Karen Steidinger in recognition of her many contributions to dinoflagellate research.

TYPE SPECIES: *Karenia brevis* (Davis) G. Hansen & Moestrup *comb. nov.*

BASIONYM: *Gymnodinium breve* Davis (1948, p. 358).

SYNONYM: *Ptychodiscus brevis* (Davis) Steidinger.

OTHER SPECIES WE BELIEVE BELONG TO KARENIA: *Karenia mikimotoi* (Miyake & Kominami *ex* Oda) G. Hansen & Moestrup *comb. nov.*

BASIONYM: *Gymnodinium mikimotoi* Miyake & Kominami *ex* Oda (1935, pp. 38, 39).

SYNONYM: *G. nagasakiense* Takayama & Adachi.

Karenia brevisulcata (Chang) G. Hansen & Moestrup *comb. nov.*

BASIONYM: *Gymnodinium brevisulcatum* Chang (1999, p. 379).

Karenia mikimotoi and *K. brevis* form a strongly supported clade (100% bootstrap support in Fig. 1), with *Gymnodinium micrum* (as *Karlodinium micrum*) as a strongly supported sister group (> 93% bootstrap). The relationship is also supported by morphological and ultrastructural evidence. All these species possess a very characteristic apical groove that is morphologically unlike that of *G. sensu stricto.* The apical groove is straight when seen from a position in front of the cell (Fig. 3A, B), extending from the ventral side of the epicone, passing over the apex, and continuing down on the dorsal side. Its ventral termination is near the dorsal extension of

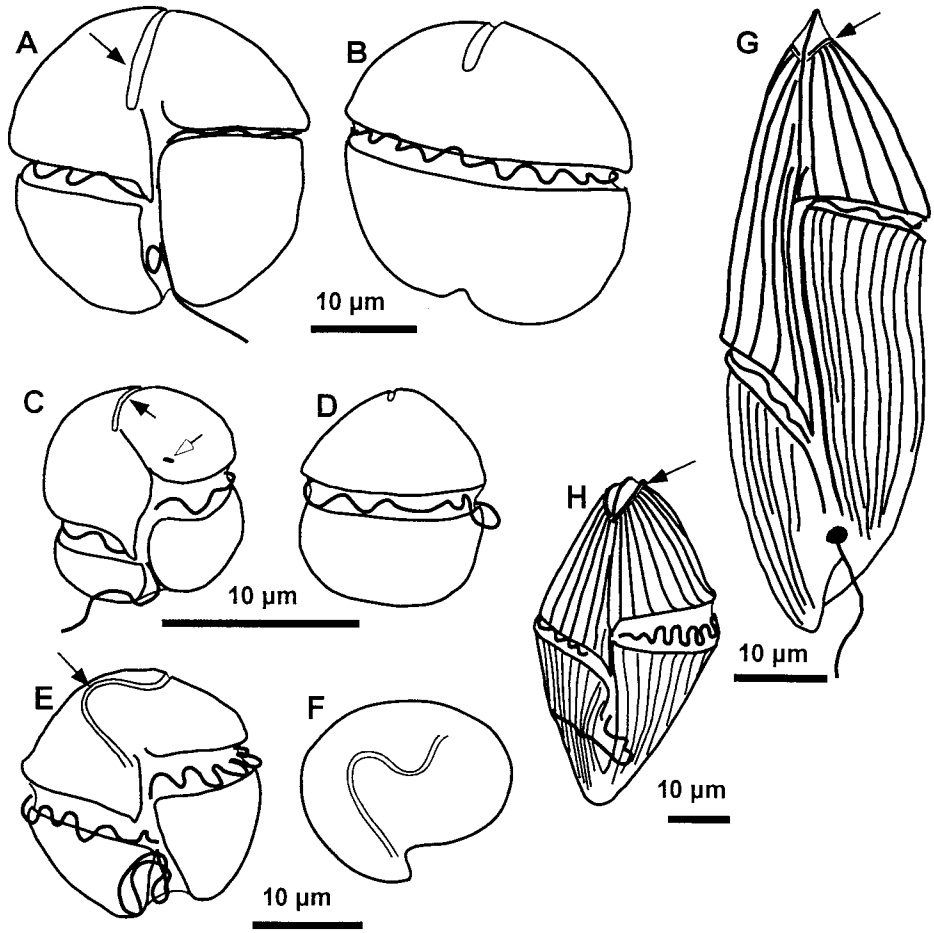


Fig. 3. Apical grooves (arrows) of unarmoured dinoflagellates [drawings based on Takayama (1985, 1998) and on unpublished observations of *Karlodinium micrum* by K.R. Roberts (as *Gymnodinium galatheanum*)]. (A, B) *Karenia mikimotoi*, ventral and dorsal views, respectively. (C, D) *Karlodinium micrum*, ventral and dorsal views, respectively. The ventral pore is marked with a white arrow. (E, F) *'Gymnodinium' pulchellum*, ventral and apical views, respectively. (G) *Gyrodinium fusiforme/G. spirale*. (H) *Gymnodinium aureum*.

the sulcus. The chloroplast pigments are fucoxanthin or fucoxanthin derivatives rather than the peridinin typical of most other dinoflagellates [*K. brevis*: Bjørnland *et al.* 1984; Liaaen-Jensen 1985, in both cases as *Gymnodinium breve*; *K. mikimotoi*: Larsen & Rowan *in* Rowan 1989, as *Gymnodinium nagasakiensis* (sic)]. Preliminary studies of the flagellar apparatus indicate that it is very similar in *Gymnodinium galatheanum* (Roberts *et al.* 1996) and *K. mikimotoi* (Hansen, unpublished observations). A NFC is absent, and both species have a 'normal' nuclear envelope without envelope chambers. The *mikimotoi* complex is neither closely related to *G. sensu stricto* nor to *Gyrodinium* but rather constitutes a genus of its own.

Karenia brevis, K. mikimotoi and *K. brevisulcata* may all be ichthyotoxic, and several toxins have been described: *K. brevis* produces brevetoxins, whereas the toxin(s) of *K. brevisulcata* resemble gymnodimine (Chang 1999). *Karenia mikimotoi* produces galactolipids (Yasumoto *et al.* 1990, as *Gyrodinium aureolum* Hulburt).

Karlodinium J. Larsen, *gen. nov.*

Dinoflagellata inarmata. Chloroplasti pyrenoidibus internis lenticularibusque et fucoxanthin aut oriundis ex fucoxanthin pro pigmentis principalibus accessoriisque. Amphiesma seriebus magnificis structurarum similium obturamentis in forma sexangulari. Canalis rectus et porus ventralis.

Unarmoured dinoflagellates with chloroplasts containing internal, lenticular pyrenoids and fucoxanthin or fucoxanthin derivatives as main accessory pigments. Amphiesma with arrays of pluglike structures in a hexagonal configuration. Apical groove straight; ventral pore present.

ETYMOLOGY: Named after Karl Tangen, who isolated the culture on which this work is based.

TYPE SPECIES: *Karlodinium micrum* (Leadbeater & Dodge) J. Larsen *comb. nov.*

BASIONYM: *Woloszynskia micra* Leadbeater & Dodge (1966, p. 1).

SYNONYMS: *Gymnodinium galatheanum* Braarud *sensu* Kite & Dodge (1988), *Gymnodinium micrum* (Leadbeater & Dodge) Loeblich III, *Gyrodinium galatheanum* (Braarud) Taylor *sensu* Taylor.

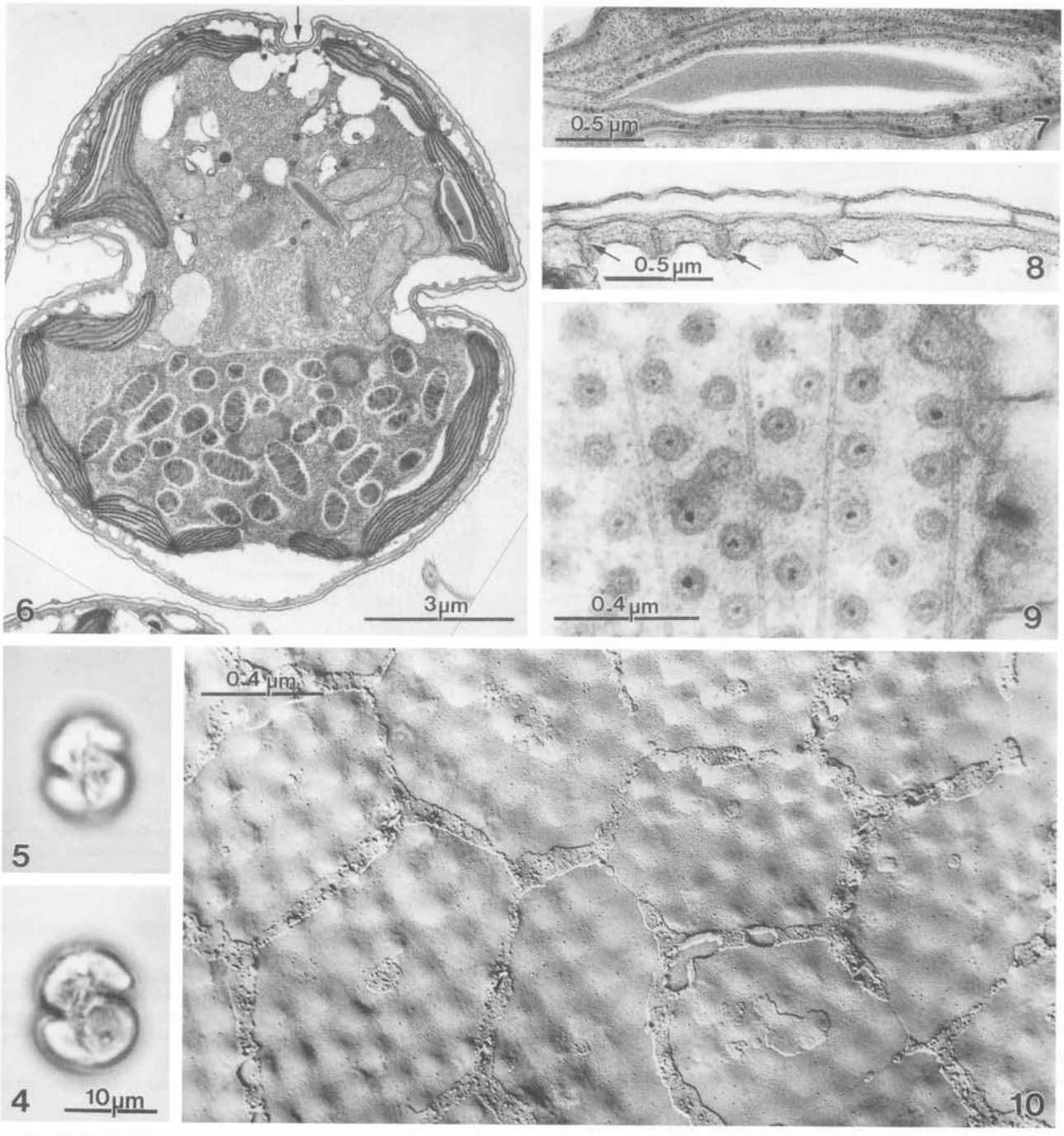
OTHER SPECIES: *Karlodinium veneficum* (Ballantine) J. Larsen *comb. nov.*

BASIONYM: *Gymnodinium veneficum* Ballantine (1956, p. 468).

Karlodinium vitiligo (Ballantine) J. Larsen *comb. nov.*

BASIONYM: *Gymnodinium vitiligo* Ballantine (1956, p. 467).

The species that we studied (Figs 4–10) was isolated into culture from the Oslofjord, Norway, and has been previously identified as *Gymnodinium galatheanum* Braarud (Kite &



Figs 4–10. *Karlodinium micrum.*
Figs 4–5. Light microscopy, cells in ventral view, same scale.
Figs 6–9. Electron microscopy of cells fixed as described by Larsen (1988).
Fig. 6. Longitudinal section, note the apical groove (arrow).
Fig. 7. Detail showing the internal, lenticular pyrenoid.
Fig. 8. Transverse section of the amphiesma showing the array of plug-like material beneath the amphiesma vesicles (arrows) and the thin plates inside the vesicles.
Fig. 9. Tangential section of the amphiesma showing the structure of the plugs in more detail.
Fig. 10. Freeze-etch preparation illustrating the hexagonal amphiesmal vesicles underlain by the plugs. Cells were prepared as described by Hill & Wetherbee (1986).

Dodge 1988). Arrays of pluglike structures are present in the amphiesma (Figs 8–10; Kite & Dodge 1988, fig. 10). Similar structures have been described in *Woloszynskia micra* by Leadbeater & Dodge (1966, figs 19–20, 22), *Gymnodinium veneficum* and *G. vitiligo* (Leadbeater & Dodge 1966; Leadbeater 1967, cited in Dodge & Crawford 1970), and in three unnamed species in the Plymouth Culture Collection: Plymouth D, Plymouth 370, and Plymouth 417 (Dodge & Crawford

1970). This type of amphiesma is otherwise unknown in the dinoflagellates. The plug-like structures somewhat resemble the collared pits found in most dinoflagellates, particularly in the flagellar canal. Internal, lenticular pyrenoids are another feature shared by the present species (Fig. 7; Kite & Dodge 1988, fig. 11), *W. micra* (Leadbeater & Dodge 1966; Dodge 1975, as *Gymnodinium micrum*) and *G. veneficum* (Dodge 1975).

Pigment analyses of the culture studied here have shown that the cells lack peridinin as the major accessory pigment but possess 19′-hexanoyl-oxyfucoxanthin (Bjørnland & Tangen 1979; Tangen & Bjørnland 1981 – both as *Gyrodinium* sp. A; Johnsen & Sakshaug 1993). Fucoxanthin or fucoxanthin derivatives have been previously demonstrated in *W. micra* (Whittle & Casselton 1968) and *G. veneficum* (Riley & Wilson 1967). Bjørnland & Tangen (1979), however, deemed the methods inadequate to establish whether the pigment in these species is 19′-hexanoyl-oxyfucoxanthin.

Karlodinium micrum and *K. vitiligo* share many features with *Karenia*: they possess the same pigments, the apical groove is identical (K.R. Roberts, personal communication), and the flagellar apparatus is very similar to that described above. However, they differ from *K. brevis* and *K. mikimotoi* in some significant features. Both possess a ventral pore of unknown function (K.R. Roberts, personal communication). A pore has also been observed in *Gyrodinium corsicum* Paulmier, Berland, Billard & Nezan (Paulmier *et al.* 1995), a species that most likely is closely related to *G. galatheanum sensu* Kite & Dodge. The pyrenoid structure of *Karlodinium micrum* is slightly different from that of *Karenia mikimotoi* (Kite & Dodge 1988, as *G. galatheanum* and *Gyrodinium aureolum*). This difference agrees with plastid 16S rDNA sequences, which indicate that the plastids of *K. mikimotoi* (as *Gyrodinium aureolum*) and *K. breve* are more closely related to each other than they are to *G. galatheanum sensu* Kite & Dodge (Dahlberg *et al.* 1998). The plastids are related to haptophyte plastids (Dahlberg *et al.* 1998; Takishita *et al.* 1999; Tengs *et al.* 2000).

The original description of *G. galatheanum* is very incomplete, being based on formaldehyde-preserved material from the South Atlantic (Braarud 1957); in addition, the original illustration is believed to be a mirror image, as the cingulum shows a so-called right-hand displacement (Braarud 1957, fig. 1), contrary to what is observed in the present species and in other similar species. The material illustrated in Figs 4–10 has been described by light microscopy by Bjørnland & Tangen (1979) and by Larsen & Moestrup (1989), whose observations agree with *W. micra* (Leadbeater & Dodge 1966). Thus, the present material is considered to represent *W. micra,* leaving the identity of Braarud's (1957) *G. galatheanum* unknown. There is no detailed morphological study by SEM, but according to Taylor (1992), who studied the same culture, the cell possesses a peduncle and a ventral pore on the epicone (illustrations were not provided).

The features observed in *Karlodinium micrum,* particularly the unique structure of the amphiesma, supported by the phylogenetic analyses (Fig. 1), warrant the erection of the new genus, one that is different from but related to *Karenia.*

Karlodinium micrum is morphologically very similar to *K. veneficum* and *K. vitiligo,* and the available information on the ultrastructure and pigment composition suggests that these species may perhaps all be synonymous. However, we are reluctant to draw this conclusion before additional morphological studies have been conducted. *Karlodinium veneficum* has been reported to be toxic to a range of marine invertebrates and fish (Abbott & Ballantine 1957).

Gyrodinium corsicum (Paulmier *et al.* 1995) and *Gyrodinium esturiale* Hulburt (Hulburt 1957) show morphological similarities to *K. micrum.* However, the amphiesma structure of *G. corsicum* is unknown, whereas *G. esturiale* has a different amphiesma structure (Gardiner *et al.* 1989) and therefore does not belong in *Karlodinium.*

Amphidinium Claparède & Lachmann

TYPE: *Amphidinium operculatum* Claparède & Lachmann.

SYNONYM: *A. klebsii* Kofoid & Swezy.

OTHER PRESENTLY KNOWN SPECIES OF AMPHIDINIUM:

A. carterae Hulburt

A. rhynchocephalum Anissimowa

The *Amphidinium* clade, here represented by *A. operculatumi* and *A. carterae,* is very well supported by the LSU rDNA sequence data (100% bootstrap support). The tree in Fig. 1 suggests an early divergence of *Amphidinium,* which is in agreement with the SSU rDNA data of Saunders *et al.* (1997), although *Noctiluca scintillans* (Macartney) Kofoid, which is not included in the present study, had an even earlier divergence. Traditionally, *Amphidinium* has been grouped within the gymnodinioids, more formally in the order Gymnodiniales (e.g. Fensome *et al.* 1993). However, the molecular data indicate that *Amphidinium* is not closely related to the other gymnodinioids. Interestingly, Bergh (1881) classified *Amphidinium* together with *Dinophysis* in the subfamily Dinophyida, whereas *Gymnodinium* and *Polykrikos* were placed in Gymnodinida. The present data provide some support for this idea, and Taylor (1980) also discussed whether the amphidinioid morphotype has given rise to the dinophysoid type. Unfortunately, the phylogenetic position of *Dinophysis acuminata,* based on the molecular data presently available in GenBank, is only indicative and is not well supported (see below).

Ultrastructural data on other *Amphidinium* species suggest that this genus, as presently defined, is polyphyletic. It was erected in 1859 by Claparède & Lachmann to include unarmoured species whose epicone is smaller than the hypocone. The genus presently includes heterotrophic as well as phototrophic and a few cyanelle-containing species, and there is considerable morphological diversity within the genus. In some species, the epicone and the hypocone are almost equal in size, whereas in others the epicone is a small finger-like protrusion on a much larger hypocone. The type species of *Amphidinium, A. operculatum,* has a finger-like epicone, and the ultrastructure of the closely related species *A. carterae* and *A. rhynchocephalum* Anissimowa was examined by Dodge & Crawford (1968) and Farmer & Roberts (1989), respectively. This species complex is therefore the true *Amphidinium.* It is an assemblage of benthic species with more or less flattened cells, which, at least in some cases, are known to be toxic to invertebrates and perhaps fish. The toxin was identified as a galactolipid by Yasumoto *et al.* (1990). Another species, *A. cryophilum* Wedemayer, Wilcox & Graham, was examined ul-

trastructurally by Wilcox *et al.* (1982) and is very different from the *operculatum*-complex. In *A. cryophilum,* the epicone and hypocone are almost equal in size, and the ultrastructure shows very clear similarities to *Gymnodinium sensu stricto.* Thus, the nucleus has nuclear pores located in invaginations of the nuclear envelope, one of the main characteristics of *Gymnodinium sensu stricto.* Other features shared between *A. cryophilum* and *Gymnodinium* are the striated dorsal connective and the structure of the apical groove, which appears to curve in an anticlockwise direction around the cell apex (Wedemayer *et al.* 1982, figs 3, 6). Large mucocysts are present in both *A. cryophilum* and *G. fuscum. Amphidinium cryophilum* clearly does not belong in *Amphidinium* as defined by the type species *A. operculatum.*

Another species of *Amphidinium, A. lacustre* F. Stein, a heterotrophic freshwater species, was recently examined ultrastructurally by Calado *et al.* (1998), who found several unusual morphological features. These include a very peculiar eyespot of brick-like subunits, each 'brick' located in a vesicle. The eyespot associates with flagellar root R1 (LMR), as in other eyespot-containing dinoflagellates. This type of eyespot is presently known only in four dinoflagellates, all unarmoured but included in three different genera: *Amphidinium lacustre,* mentioned above; *Gymnodinium natalense* Horiguchi & Pienaar, a benthic marine species from South Africa (Horiguchi & Pienaar 1994a, b); *Gymnodinium linuchiae* Trench & Thin, an endosymbiont of the jellyfish *Linuche unguiculata* Swartz (Trench & Thin 1995); and *Polarella antarctica* Montresor, Procaccini & Stoecker (1999), a photosynthetic phytoplankton species from Antarctic waters. These species all deserve to be examined in detail by transmission electron microscopy and gene sequencing to determine their generic status. It is clear that none of them belong to *Amphidinium,* but it is not clear whether they should be classified into one or more other genera. Of particular interest is the finding that *Polarella* may be related to the many extinct species of armoured dinoflagellates known to geologists as members of the Suessiales (Montresor *et al.* 1999).

Based on partial SSU analysis, McNally *et al.* (1994) found that *Amphidinium belauense,* an endosymbiont of acoel flatworms, is closely related to *A. carterae.* It is therefore probably a true member of *Amphidinium.*

Amphidinium cryophilum belongs in the genus *Gymnodinium*; in other words, it represents a *Gymnodinium* species in which the epicone is slightly smaller that the hypocone. The generic circumscription of *Amphidinium* given by Claparède & Lachmann (1859) therefore does not hold, and all species of *Amphidinium,* except those resembling *A. operculatum,* need to be examined ultrastructurally and genetically to determine their generic affiliation. Because of the small number of species studied, we refrain from emending the generic circumscription of *Amphidinium.*

Gyrodinium Kofoid & Swezy emend. G. Hansen & Moestrup

Naked, heterotrophic dinoflagellates. Cingulum displacement from one to more cingulum widths. Apical groove elliptical. Amphiesma with longitudinal striations.

TYPE SPECIES: *G. spirale* (Bergh) Kofoid & Swezy.

OTHER SPECIES: Species illustrated by SEM by Takayama (1998) and clearly belonging to *Gyrodinium* as the genus is emended above:

Gymnodinium aureum Kofoid & Swezy (but not *Gyrodinium aureum* (Conrad) Schiller. This probably belongs in *Karenia.*)

Gyrodinium fusiforme Kofoid & Swezy

G. grossestriatum Campbell

G. heterogrammum J. Larsen

G. pepo (Schütt) Kofoid & Swezy

Gyrodinium striatissimum (Hulburt) G. Hansen & Moestrup *comb. nov.*

BASIONYM: *Gymnodinium striatissimum* Hulburt (1957, p. 206).

In addition, the following heterotrophic species included in *Gyrodinium* by Dodge (1982) appear to fit the emendation of the genus:

Gyrodinium britannicum Kofoid & Swezy

G. cochlea Lebour

G. crassum (Pouchet) Kofoid & Swezy

G. cuneatum Kofoid & Swezy

G. fissum (Levander) Kofoid & Swezy

G. glaucum (Lebour) Kofoid & Swezy

G. lachryma (Meunier) Kofoid & Swezy

G. obtusum (Schütt) Kofoid & Swezy

G. opimum (Schütt) Lebour

G. pingue (Schütt) Kofoid & Swezy

The genus *Gyrodinium* is presently circumscribed as containing those gymnodinioid dinoflagellates in which the two ends of the cingulum are separated in the longitudinal direction of the cell by a distance exceeding one fifth of the cell length. That this generic circumscription is unsatisfactory has been known for a long time. Several gymnodinioid dinoflagellates possess a cingulum whose ends are separated by approximately this distance (e.g. *K. mikimotoi*). In some cells of a clonal culture, the two ends may be separated by slightly less than one fifth of the cell length; in others, the two ends may be separated by slightly more than one fifth of the cell length (see also Dodge 1982, p. 98). A study of the ultrastructure of the type species of *Gyrodinium, G. spirale,* is being published separately (Hansen, in preparation). Based on this work and on SEM micrographs by Takayama (1985, 1998), it is now clear that *Gyrodinium* is readily distinguished in the SEM. The characteristic feature of *Gyrodinium* is not so much the cingulum displacement as the morphology of the apical groove system. The apical groove is an elliptical structure situated around the apical end, perpendicular to the longitudinal axis of the cell (Fig. 3H, the 'apical ring' of Larsen 1996). The ellipse is bisected into two equal parts by a central line (Fig. 3H). The long axis of the apical groove is middorsal to midventral. If present, an anterior extension of the sulcus extends toward one end of the apical groove.

Gyrodinium spirale and *G. fusiforme* differ from the other species in having an apical projection or cap emerging from the apical groove (Fig. 3G), a feature suggesting that these species form a subgroup within *Gyrodinium* (perhaps a subgenus). All of the above-mentioned species have cells ornamented with longitudinal striations, and in the *G. spirale* group, one of the striae extends onto the apical cap, eventually reaching the tip of the cell (Fig. 3G).

Armoured dinoflagellates:

***Woloszynskia* Thompson**

The phylogenetic position of *Woloszynskia* is interesting, as this genus has been suggested to be intermediate between peridinioids and gymnodinioids (e.g. Taylor 1980). Netzel & Dürr (1984) even suggested that this genus might represent a distinct dinoflagellate morphotype, the woloszynskioid type. The woloszynskioids generally have fewer amphiesmal vesicles than the gymnodinioids, but more than the peridinioids (*c.* 47–360; Netzel & Dürr 1984). In addition, the vesicles are not arranged in five distinct latitudinal series as they are in the peridinioids and gonyaulacoids. The amphiesmal vesicles of woloszynskioids usually contain thin plates, which can be stained by the fluorochrome CalcoFluor White (e.g. *W. pseudopalustris*; A. Calado, personal communication), indicating their homology with the thecal plates of the armoured dinoflagellates. Cells of the type species, *W. reticulata* Thompson, however, are covered with thin plates on the epicone but thick plates on the hypocone (Thompson 1950). It has a distinct carina on the epicone, approximately at the position of the anterior groove in *Karenia* and *Karlodinium.* The ultrastructure of woloszynskioids has been studied in detail (Crawford *et al.* 1970; Crawford & Dodge 1971; Dodge 1984; Roberts & Timpano 1989; Roberts *et al.* 1995), the most significant feature being the presence of a very complicated pusular arrangement, so far unique to woloszynskioids. It consists of elaborate convoluted collecting tubules originating from each of the flagellar canals and continuing deep into the cell. The distal parts of these tubules are lined with peculiar projections or a 'tomentum' (Dodge 1972). The pusular vesicles merge with the collecting tubules.

The present LSU rDNA data indicate that *Woloszynskia pseudopalustris* emerged after the *Peridinium bipes/P. willei* clade but before the GPP complex (Fig. 1). This suggests that the woloszynskioid morphology preceded the major gymnodinioid lineages. However, their relationship to the peridinioids is still open because of the ambiguous position of the peridinioid taxa outside and within the GPP complex.

***Peridinium* Ehrenberg**

Somewhat surprisingly, the nucleotide sequences determined from the four *Peridinium* species included in this study fall into two relatively distant groups (Fig. 1). *Peridinium cinctum* and *P. pseudolaeve* form a well-supported clade within the GPP complex, whereas *P. bipes* and *P. willei* form a strongly supported clade outside it. The genus *Peridinium* has been subdivided into two subgenera, *Poroperidinium* (e.g. *P. bipes*) and *Cleistoperidinium* (e.g. *P. cinctum, P. pseudolaeve, P. willei*), based on the presence or absence of an apical pore complex (e.g. Popovský & Pfiester 1990). The molecular data do not support this separation, since *P. bipes* groups with *P. willei* rather than with the *P. cinctum/P. pseudolaeve* clade. According to Bujak & Davies (1983), *Peridinium sensu stricto* comprises two distinct but closely related lineages, the cinctoid and bipesoid types. The bipesoid tabulation is characterized by a linteloid 2a-plate (i.e. the boundary with plate 4″ is parallel to the cingulum), which is symmetrically arranged anterior to the 4″-plate. The 3′-, 2a- and 4″-plates are usually symmetrically arranged relative to the dorsal midline. The cinctoid tabulation differs in having a fastigiate 2a-plate (the boundary with the precingulars is zigzag) situated anterior to the boundary of plates 3″ and 4″. Bujak & Davies (1983) argued that the two lineages may have originated from a marine bipesoid ancestor that moved into the freshwater environment. Subsequently, the tabulation was modified into a cinctoid type. Interestingly, the LSU rDNA data appear to support this hypothesis, but the wide separation of the two lineages in Fig. 1 needs further investigations by sequence determination of other genes and/or increased taxon sampling.

Other peridinioid genera

Heterocapsa and *Scrippsiella* Balech *ex* Loeblich III, two other peridinioid genera, form strongly supported clades within the polytomous GPP complex (100% and > 86% bootstrap support, respectively), but their interrelationship, as well as their relationship to *Peridinium*, are not well resolved in this investigation. The transfer of *Heterocapsa rotundata* from the genus *Katodinium* to the genus *Heterocapsa,* based on ultrastructural evidence and plate tabulation (Hansen 1995), is strongly supported by the molecular data, as *H. rotundata* forms a sister group to *Heterocapsa triquetra* and *Heterocapsa* sp.

Gonyaulacales

The LSU rDNA data show that the gonyaulacoid dinoflagellates, here represented by the genera *Alexandrium, Ceratium* Schrank, *Fragilidium* Balech *ex* A.R. Loeblich III, *Gonyaulax* Diesing, and *Protoceratium* R. Bergh, form a monophyletic assemblage supported by bootstrap values of 96% in MP and 62% in NJ analyses, respectively. *Alexandrium* and *Fragilidium* form a sister group to *Ceratium, Gonyaulax* and *Protoceratium.* This finding agrees with the SSU rDNA data of Saunders *et al.* (1997), whose SSU data weakly supported *Alexandrium* as a sister group to a clade comprising *Ceratium, Gonyaulax* and *Ceratocorys.* Hansen & Moestrup (1998a) discussed this branching pattern, because the ultrastructural data, primarily details of the flagellar apparatus, do not support such a phylogenetic relationship. They suggested that *Ceratium* is more distantly related to *Alexandrium, Gonyaulax* and *Protoceratium.* Thus, in *Ceratium furcoides* (Levander) Langhans, both a dorsal and a ventral fibre are present, associated with the longitudinal microtubular root (R1 or LMR); such fibres are not present in *Alexandrium catenella* (Whedon & Kofoid) Balech, *Gonyaulax spinifera* (Claparède & Lachmann) Diesing, or *Protoceratium reticulatum* Bütschli. Furthermore, the so-called accessory striated collar connective, a second connective interconnecting the striated collars, has not been observed in *Ceratium* (Roberts 1989) but is present in *Alexandrium, Gonyaulax, Peridiniella* Kofoid & Michener (see below) and *Protoceratium,* and was suggested to be unique for gonyaulacoid dinoflagellates (Hansen *et al.* 1996, 1997; Hansen & Moestrup 1998a, b). However, immediately after this statement was made, the connective was found in *Peridinium cinctum* (Calado *et al.* 1999). Its absence in *Ceratium* is therefore puzzling. An ultrastructural character unique for the gonyaulacoids is presently unknown. Plate patterns, and to some extent cyst details, appear to be the features that define this order. Based on plate patterns, Fensome *et al.* (1993) subdivided the order Gonyaulacales into five subor-

ders: Rhaetogonyaulacineae (fossil only), Cladopyxiineae, Gonyaulacacineae (with *Gonyaulax, Protoceratium*), Ceratineae (with *Ceratium*), and Goniodomineae (with *Alexandrium, Fragilidium*). The LSU rDNA data clearly show the Goniodomineae and Ceratiineae to represent monophyletic groups. Fensome *et al.* (1993) separated *Protoceratium reticulatum* and *Gonyaulax spinifera* into the subfamilies Cribroperidinioideae and Gonyaulacoideae, respectively. Our molecular data also seem to support this separation, which should probably be reflected at an even higher taxonomic level.

Peridiniella catenata

This species has had a very changeable taxonomic position. Originally described as *Peridinium catenella* Levander (1894), it was transferred to *Amylax* Meunier (Meunier 1910), *Gonyaulax* (Kofoid 1911) and finally *Peridiniella* (Balech 1977). It has gonyaulacoid traits in the arrangement of the plates on the hypocone, but it shows peridinialean affinities in its epicone structure, e.g. the presence of a canal plate in conjunction with the apical pore plate. The latter feature is usually absent in typical gonyaulacoids, but it is common in the Peridiniales (Fensome *et al.* 1993). In addition, *P. catenata* shows some ultrastructural differences when compared to gonyaulacoids (Hansen & Moestrup 1998b), e.g. in the pusular arrangement and the type of pyrenoid. The presence in *P. catenata* of a peculiar scale-like outer layer and two size classes of trichocysts has also not been observed in gonyaulacoids. Based on these differences, Hansen & Moestrup (1998b) suggested that *P. catenata* was not closely related to the gonyaulacoids. The presence of two striated collar connectives in *P. catenata* was an indication of gonyaulacoid affinity, but two striated collar connectives have now also been found in *Peridinium cinctum* (Calado *et al.* 1999).

The molecular data confirm that *P. catenata* is not related to the gonyaulacoids but rather that it shows a relationship to the GPP complex. Fensome *et al.* (1993) placed *Peridiniella* in an 'uncertain family' within the Gonyaulacales, but it is now clear that *P. catenata* does not belong here. Whether this applies also to *P. sphaeroidea* Kofoid & Michener, the type species, is not known, as this species has not been analysed in detail.

Dinophysis and *Prorocentrum*

The LSU rDNA sequences indicate an early divergence of *Dinophysis*. However, this is based on the analysis of only 326 bp from one species (*Dinophysis acuminata*), and more taxa need to be included before the phylogenetic position of the dinophysoids can be ascertained with confidence. Partial SSU rDNA sequences did not suggest an early divergence of *D. acuminata* but placed this species within the GPP complex, in a clade together with *K. mikimotoi* (as *Gymnodinium mikimotoi*) and *Polykrikos swartzii* Bütschli (Saunders *et al.* 1997). However, this position was not well supported. The dinophysoids were considered by Taylor (1980) to represent one of the basic dinoflagellate morphotypes, and their phylogenetic position is therefore of particular interest. Bergh (1881) considered dinophysoids as the link between the prorocentroids and the peridinioids, and Pascher (1914) grouped Prorocentraceae and Dinophysidaceae in the order Desmomonadales. The prorocentroids, with their anterior flagellar insertion and an armour consisting mainly of two large plates (valves), have been suggested to represent the most primitive dinoflagellates (Loeblich 1976; Taylor 1980) or, alternatively, the more advanced dinoflagellates (Dodge 1983). Our data do not suggest a close relationship between dinophysoids and prorocentroids, but there is some indication of an early divergence of the Dinophysales. This does not apply to the Prorocentrales, which is situated within the GPP complex (Fig. 1).

Closing remarks

The molecular phylogeny based on partial LSU rDNA is basically similar to the phylogeny based on SSU rDNA sequences. The bootstrap support for the branching pattern is generally greater, however, indicating that LSU rDNA sequences are more suitable for studies of phylogenetic relationships at the generic and species level than are SSU rDNA sequence data. The molecular reconstructions have provided support for conclusions based on ultrastructural features, notably features associated with the flagellar apparatus, and biochemical features, notably photosynthetic pigments. The combination of these different approaches has enabled us to reach conclusions on taxonomy and phylogeny of the dinoflagellates that would have been difficult to reach if only one of the techniques had been employed. Future studies based on LSU rDNA should include heterotrophic dinoflagellates in order to allow us to better understand the systematics and evolutionary history of this highly diverse assemblage of protists.

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APPENDIX

New combinations or synonymy are proposed here for the following:

Amphidinium cryophilum Wedemayer, Wilcox & Graham
= *Gymnodinium cryophilum* (Wedemayer, Wilcox & Graham) G. Hansen & Moestrup

Gymnodinium breve Davis
= *Karenia brevis* (Davis) G. Hansen & Moestrup

Gymnodinium brevisulcatum Chang
= *Karenia brevisulcata* (Chang) G. Hansen & Moestrup

Gymnodinium galatheanum Braarud *sensu* Kite & Dodge
= *Karlodinium micrum* (Leadbeater & Dodge) J. Larsen

Gymnodinium micrum (Leadbeater & Dodge) Loeblich III
= *Karlodinium micrum* (Leadbeater & Dodge) J. Larsen

Gymnodinium mikimotoi Miyake & Kominami *ex* Oda
= *Karenia mikimotoi* (Miyake & Kominami *ex* Oda) G. Hansen & Moestrup

Gymnodinium nagasakiense Takayama & Adachi
= *Karenia mikimotoi* (Miyake & Kominami *ex* Oda) G. Hansen & Moestrup

Gymnodinium nelsonii Martin
= *Akashiwo sanguinea* (Hirasaka) G. Hansen & Moestrup

Gymnodinium sanguineum Hirasaka
= *Akashiwo sanguinea* (Hirasaka) G. Hansen & Moestrup

Gymnodinium splendens Lebour
= *Akashiwo sanguinea* (Hirasaka) G. Hansen & Moestrup

Gymnodinium striatissimum Hulburt
= *Gyrodinium striatissimum* (Hulburt) G. Hansen & Moestrup

Gymnodinium veneficum Ballantine
= *Karlodinium veneficum* (Ballantine) J. Larsen

Gymnodinium vitiligo Ballantine
= *Karlodinium vitiligo* (Ballantine) J. Larsen

Gyrodinium galatheanum (Braarud) Taylor *sensu* Taylor
= *Karlodinium micrum* (Leadbeater & Dodge) J. Larsen

Gyrodinium impudicum Fraga & Bravo
= *Gymnodinium impudicum* (Fraga & Bravo) G. Hansen & Moestrup

Ptychodiscus brevis (Davis) Steidinger
= *Karenia brevis* (Davis) G. Hansen & Moestrup

Woloszynskia micra Leadbeater & Dodge
= *Karlodinium micrum* (Leadbeater & Dodge) J. Larsen