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Illumination or confusion? Dinoflagellate molecular phylogenetic data viewed from a primarily morphological standpoint

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This paper is dedicated to the memory of Bill (W.A.S) Sarjeant 1935–2002, friend and colleague.

SUMMARY

Recent molecular sequencing results involving multiple genes require evaluation in the light of pre-existing morphological data, particularly as different methodologies and genes produce trees that are incongruent in some respects or have major areas with poorly supported branch resolution. The present paper summarizes the current situation, primarily from a morphologist's perspective. Most of the tabulation-based groups are coherent in small subunit (SSU) and large subunit (LSU) trees; but some, notably the prorocentroids and peridinioids, are not. In prorocentroids this is primarily because of intrinsic inadequacies of the molecules to resolve their phylogeny. In peridinioids it seems to be because of paraphyly of the group. Other artefacts are noted, such as the drastically different positions of *Oxyrrhis* in phylogenetic trees based on SSU and protein genes, and of *Noctiluca* in SSU trees that include analyses with different numbers of nucleotides. Polyphyly in non-tabulate or poorly known groups has been confirmed, as has been the presence of cryptic thecae in members of those groups (group misattribution). Whether or not some extant groups of athecate, wholly dinokaryotic forms originated prior to polytabulate groups, like the suessioids, peridinioids and gonyaulacoids, remains unclear. Gymnodinioids with a spiral acrobase seem to have given rise to the more complex athecate forms, whereas morphological features of the genus *Gymnodinium* are consistent with it being a sister group to polytabulate taxa such as *Woloszynskia* and the suessioids. Peridinioids and gonyaulacoids appear to have originated after that split. Dinophysoid and prorocentroid dinoflagellates appear to be derived from peridinioid forms. Trees based on protein genes, such as actin or α- and β-tubulin, may help resolve some of the positions of key groups, but they do not include enough taxa to be widely useful as yet.

Key words: dinoflagellate, evolution, molecular phylogeny, morphology, relationships.

INTRODUCTION

At the present time three main types of data can be used to unravel the patterns of dinoflagellate evolution: a rich, but incomplete, fossil record (mostly morphological but with a small paleogeochemical component), morphological/ultrastructural (with some biochemical) data on contemporary forms, and molecular sequence comparisons. Until the early 1990s, dinoflagellate classification of both living and fossil taxa relied almost exclusively on morphological characters. Where it was possible to determine tabulation (= thecal plate) patterns, these were considered of primary importance because a great deal was known about their conservativeness, in a similar way to the remarkably precise and fixed pore patterns on diatom frustules (see Taylor 1980, 1987a,b; Fensome *et al*. 1993 for all tabulations referred to here). Groups created for dinoflagellates without a clear tabulation (athecate or 'unarmoured' taxa like Gymnodiniales, Blastodiniales, Phytodiniales etc.) have long been assumed to be polyphyletic (Taylor 1980; Fensome *et al*. 1993), although some athecate groups such as the noctilucoids or syndinians could be also distinguished by their nuclear state, lacking a dinokaryon for part or all of their life cycle (Soyer 1972). Their basal position within the dinoflagellates in early small subunit (SSU) trees supported the hypothesis that histones were lost gradually in the dinoflagellate lineage (Saunders *et al*. 1997). Ornamentation on cyst surfaces (ridges, spines etc.), shape of the archeopyle and degree of cavation formed the basis of earlier classifications of dinoflagellate fossils (e.g. Sarjeant & Downie 1966), independent of the tabulation-dominant system for living taxa. The first attempt at combining data from living and fossil taxa was by Bujak and Williams (1981), but apparent tabulation on cysts (paratabulation) was not available for many genera.

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Evitt (1967) pioneered the methods of inference of tabulation patterns from archeopyle shapes and, later (Evitt 1985), the interpretation of the arrangement of processes on many cyst surfaces, thereby showing that many hystrichospheres were dinoflagellate cysts. The reflection of sulcal, and sometimes apical pore complex plates (paratabulation), is often lacking on cyst walls, a notable exception being *Nannoceratopsis* (Piel & Evitt 1980). Scanning electron microscopy greatly assisted in determining probable paratabulation (e.g. Below 1987), and the combined classification was taken to a more mature state by Fensome *et al.* (1993). All these classifications were based on morphological characters. The selectivity of the fossil record imposes considerable constraints regarding phyletic interpretation (Evitt 1981), although Fensome *et al.* (1996) argued that the record of most groups represented in the Mesozoic does provide a reasonable basis for evolutionary interpretation. Goodman (1987) and Head (1996) have provided a thorough and comprehensive review of the interpretation of dinoflagellate cysts in both older and modern sediments, which is of particular value to neontologists.

Molecular phylogeneticists attempt to construct hypothetical phylogenetic trees from the comparison of the sequences of homologous genes by methods based on various hypotheses as to how the genes have evolved, using correction factors to deal with inherent methodological biases such as long-branch attraction (Philippe & Adoutte 1998). Other factors, such as alignment choices, 'sampling' involving the number and selection of taxa (photosynthetic species of dinoflagellates far outnumber non-photosynthetics in present dinoflagellate molecular trees because cultures of them are readily available) and choice of outgroups, can greatly influence the resultant trees (see below). The genes chosen can have different histories, resulting in conflicting trees. There are also just plain methodological anomalies (see *Oxyrrhis* below). The most commonly used molecules are ribosomal RNA genes (SSU and large subunit (LSU)) but also proteins such as actin, α- and β-tubulin or rubisco (for plastids). 5S ribosomal DNA is considered too short a sequence (roughly 120 nucleotides) but it was adequate to indicate that dinoflagellates were not basal in the eukaryotic tree as required by the Mesokaryote hypothesis (Hinnebusch *et al.* 1981). Mitochondrial DNA has been phylogenetically useful in many protists but has proved to be very difficult to work with in dinoflagellates (Gray *et al.* 1998).

The author has addressed this topic on several previous occasions as the molecular information became available (e.g. Taylor 1999a,b). At the previous conference in this series, 'DINO 6', in Trondheim, Norway in 1999, we made an attempt to review the state of knowledge of dinoflagellate phylogeny from combined morphological and molecular data (Fensome *et al.* 1999). This has been added to in more detail in a series of papers by us (Saldarriaga *et al.* 2001, 2003a, 2004) and others (Tengs *et al.* 2000; Litaker *et al.* 1999; Montresor *et al.* 1999; and Takishita *et al.* 2003a,b). The present contribution builds on these and discusses aspects not considered previously. At the same conference Sarjeant and Taylor (1999) addressed more functional questions, such as the origin of paratabulation and the function of processes on cysts, which will not be considered here.

BROAD FEATURES OF DINOFLAGELLATE EVOLUTION

One of the most strongly supported macrolineages in protist evolution is the superphylum Alveolata (sometimes considered a subkingdom or even a kingdom). It contains three main phyla: the Dinoflagellata, the Apicomplexa and the Ciliata. Most recently a member of the parasitic, fungal-like ellobiopsids, *Thalassomyces,* has been shown to be an alveolate by its molecular sequence (Silberman *et al.* 2004). It has a dinoflagellate-like arrangement of its motile spore flagella.

Early ultrastructural evidence (peripheral alveoli/vesicles) strongly linked the dinoflagellates and the ciliates (Taylor 1976), and molecular data from ribosomal RNA genes reinforced this, adding the apicomplexans (sporozoans) to the clade (Gajadhar *et al.* 1991). Molecular data have also shown that the ciliates are the sister group to an apicomplexan/dinoflagellate clade (Van de Peer & De Wachter 1997; Fast *et al.* 2002). Using a molecular clock based on SSU with an estimated overall substitution rate of 0.85×10^{-8} site^{-1}year^{-1}, Escalante and Ayala (1996), estimated possible divergence times of dinoflagellates/apicomplexans and apicomplexans/ciliates at 900 Ma and 1317 Ma, respectively. While there must be many caveats when such data are used (rates of evolution vary in different lineages), corrections were made for many of these and the estimations obtained agree with current views on divergence times of major groups in eukaryotic evolution. Such results place these events in the Neoproterozoic, when the crown of the eukaryotic radiation apparently underwent rapid radiation (Sogin 1989; Van de Peer & De Wachter 1997; Baldauf *et al.* 2000). Older ideas about an early eukaryotic divergence of the group have been abandoned. The presence of the dinoflagellate lineage in the Neoproterozoic is also supported by the presence of dinosterane (Moldowan & Talyzina 1998), a derivative of dinosterol, which is unique to the group (synapomorphic). Putative dinoflagellate fossils from this period (Butterfield & Rainbird 1998) are not strongly convincing, although some might have been present. It is equally possible that members of the lineage did not produce fossilizable remains during the Neoproterozoic.

Unfortunately, this is also true for the Paleozoic microfossils that have been attributed to the group, such as the Silurian *Arpylorus* and the Devonian *Paleodinophysis* (see Discussion in Fensome *et al.* 1999). The most likely Paleozoic dinoflagellate fossils are among the acritarchs (organic-walled cyst-like fossils showing insufficient morphological features to confidently place them in a known group), particularly the galeates from the Late Cambrian-Ordovician period (Servais *et al.*, pers. comm., 2003). Biogeochemical analyses are being made on purified galeate and undoubted dinoflagellate cyst samples (Versteegh *et al.*, pers. comm., 2003), which may provide stronger evidence of the group's presence. Essentially, although there are good reasons, from multiple lines of evidence, to view the origin of the dinoflagellate lineage as pre-Paleozoic, we have no idea of their characteristics until the Triassic when the well known Mesozoic radiation of producers of recognizable cyst forms began (extensive discussion in Fensome *et al.* 1996).

The 'pre-dinoflagellates'

Perhaps present-day 'pre-dinoflagellates' (an informal name referring here to those taxa diverging from the lineage between the apicomplexan divergence and the fully recognizable dinoflagellates) can tell us something about those early ancestors of dinoflagellates. Botanists had long included dinoflagellates in the Chromophyta (e.g. Christensen 1962), but this assemblage was based primarily upon plastid characters (chromophytes were defined as containing chlorophylls a plus c, but many of them also had compound hairs on their anterior flagellum). Because of the recognition that plastids possibly may be unreliable indicators of the organisms that contain them and the presence of simple rather than compound flagellar hairs, Taylor (1976, 1980) made dinoflagellates a sister-group to the chromophytes, noting that they also shared tubulocristate mitochondria. More recently, Cavalier-Smith (1998, 1999) proposed the chromalveolate hypothesis, in which the common plastid type of chromophytes is hypothesized to be a result of a single secondary symbiotic event in a common photosynthetic ancestor of both chromists (heterokonts, haptophytes and cryptomonads) and alveolates. The implication of this hypothesis is that there have been multiple instances of plastid loss in many lineages within this group, notably in ciliates, pre-dinoflagellates, oomycetes and others. There is molecular evidence that has been interpreted as supporting a single plastid origin (photosynthetic ancestor) for the alveolates (Zhang *et al.* 2000), particularly early results with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Fast *et al.* 2001, 2002; Harper & Keeling 2003). The GAPDH evidence has been complicated by the discovery of two further types, raising the possibility of lateral gene transfer in one of these cases (see Takishita *et al.* 2003a,b for recent discussion). To this author the simpler alternative is that ciliates and predinoflagellates never had plastids, the earliest dinoflagellates also being non-photosynthetic phagotrophs. The apicomplexans acquired theirs (now vestigial) in a secondary symbiotic event separately from dinoflagellates. The presence of vestigial plastids or relict plastid genes in ciliates and predinoflagellates would argue in favor of a single origin but they have not been found yet.

The flagellates *Oxyrrhis*, *Perkinsus* and *Parvilucifera* (the latter two parasitic) have been shown by molecular sequencing (Norén *et al.* 1999; Saldarriaga *et al.* 2003a, 2004) to occupy positions between the Apicomplexa and the dinoflagellates *sensu stricto*. *Oxyrrhis* had long been considered to be a dinoflagellate, but was excluded by Fensome *et al.* (1993) because of its lack of most dinoflagellate features. For example it lacks a dinokaryon (histone-like proteins are present and the chromosomes decondense during interphase, dinomitosis (it has an intranuclear spindle), a girdle, a sulcus etc. The flagella are only slightly differentiated from each other. Disturbingly, the position of *Oxyrrhis* in SSU trees generated by the favored maximum likelihood method is highly derived (Fig. 2b). If this were true then it would require multiple reversions to a state resembling pre-dinoflagellates, including movement of the spindle back into the nucleus and loss of the fibrillar chromosomal state: both highly unlikely. Other methods using SSU and other gene phylogenies (actin, α- and β-tubulin, HSP90) place *Oxyrrhis* as a sister to the dinokaryotic dinoflagellates (Fig. 2a), and so the SSU maximum likelihood result is clearly an anomaly (Saldarriaga *et al.* 2003b). All these predinoflagellates are non-photosynthetic, naked flagellates. Unfortunately, none produce resistant cysts, and so are not known from the fossil record.

Noctilucoids have been considered as possibly early diverging among the true dinoflagellates because they have a dinokaryon during only part of their life cycle (Soyer 1972) and earlier SSU molecular trees (e.g. Saunders *et al.* 1997; Gunderson *et al.* 1999), supported this reasonably strongly. However, as more taxa were added, the support grew weaker statistically until it reached a point where the position of *Noctiluca* in SSU trees could be influenced by the number of nucleotides used in the alignment (Figs 1a,b). SSU is therefore too weak to place *Noctiluca* with confidence at present. The sequencing of other noctilucoids, such as *Kofoidinium*, and the use of other genes, should make the placement stronger.

The athecate, non-photosynthetic syndinians are parasites of protists (including dinoflagellates). They have usually been included in the dinoflagellates, even though they do not have a dinokaryon. Judging by *Amoebophrya,* the nucleus undergoes changes in chromatin appearance as the numerous mitoses take place,

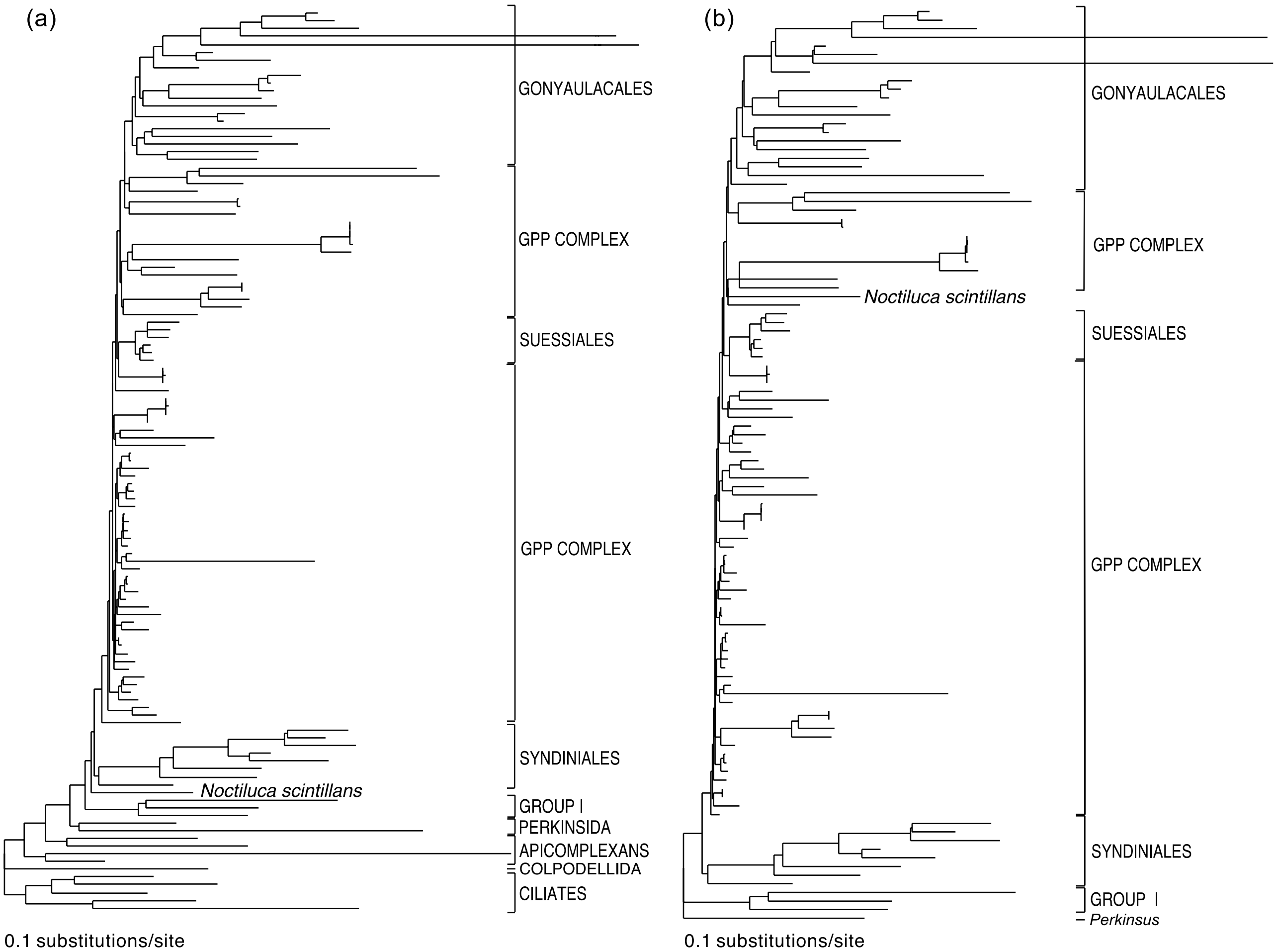

Fig. 1. Examples of volatility in the placement of key taxa in the molecular phylogenetic trees (see Figs 3 and 4 for complete trees). 1(a). The position of *Noctiluca scintillans* using 1479 nucleotides of small subunit (SSU) ribosomal DNA in a gamma-corrected distance matrix tree using BioNJ. This is a similar position shown by most other methods. 1(b). The position of *Noctiluca scintillans* in a similar SSU distance matrix tree using 1649 nucleotides.

the condensed chromosomes not being fibrillar. The spindle is extranuclear. In the dinospore stage they have a pair of laterally inserted flagella, one of which winds around the cell and is wavy, but it has not been examined in detail. Molecular trees place them as basal in the dinoflagellate cluster (Gunderson *et al.* 1999). Also occupying a position more basal to undoubted dinoflagellates in molecular trees are two clusters of entities known only from their SSU gene sequences, isolated from field samples of sea water (López-García *et al.* 2001; Moon-van der Staay *et al.* 2001). These so-called 'environmental taxa' have no corporeal identity, being phantoms at present. Judging by their positions on the SSU tree some could be syndinean-like parasites and/or pre-dinoflagellates (Saldarriaga *et al.* 2004).

All these probably basal groups in the dinoflagellate lineage are non-photosynthetic, suggesting a non-phosynthetic ancestry for the group as a whole, for it is unlikely that all these groups lost plastids independently. The presence of a feeding apparatus in many photosynthetic taxa and widespread mixotrophy in the group (Gaines & Elbrächter 1987; Stoecker 1999) also suggests that the presence of plastids occurred after the early radiation of the group. It should be noted that the presence of secondary or tertiary plastids requires engulfment of the donor eukaryote by the ultimate host; that is, it must have been a phagotrophy or mixotrophy (see McFadden & Gilson 1995; Delwiche 1999; for a full exposition of primary, secondary and even tertiary plastid acquisition in protists).

Several major events had to happen within the dinoflagellate lineage to create the group as it is today: the differentiation of the flagella into a ribbon-like, hair-bearing, transverse flagellum, and more conventional, posterior, longitudinal flagellum (selected as the key dinoflagellate synapomorphy by Fensome *et al.* 1993); development of the dinokaryon (presumably in response to a potentially catastrophic mutational loss of histones); dinomitosis, with its shift of the spindle to

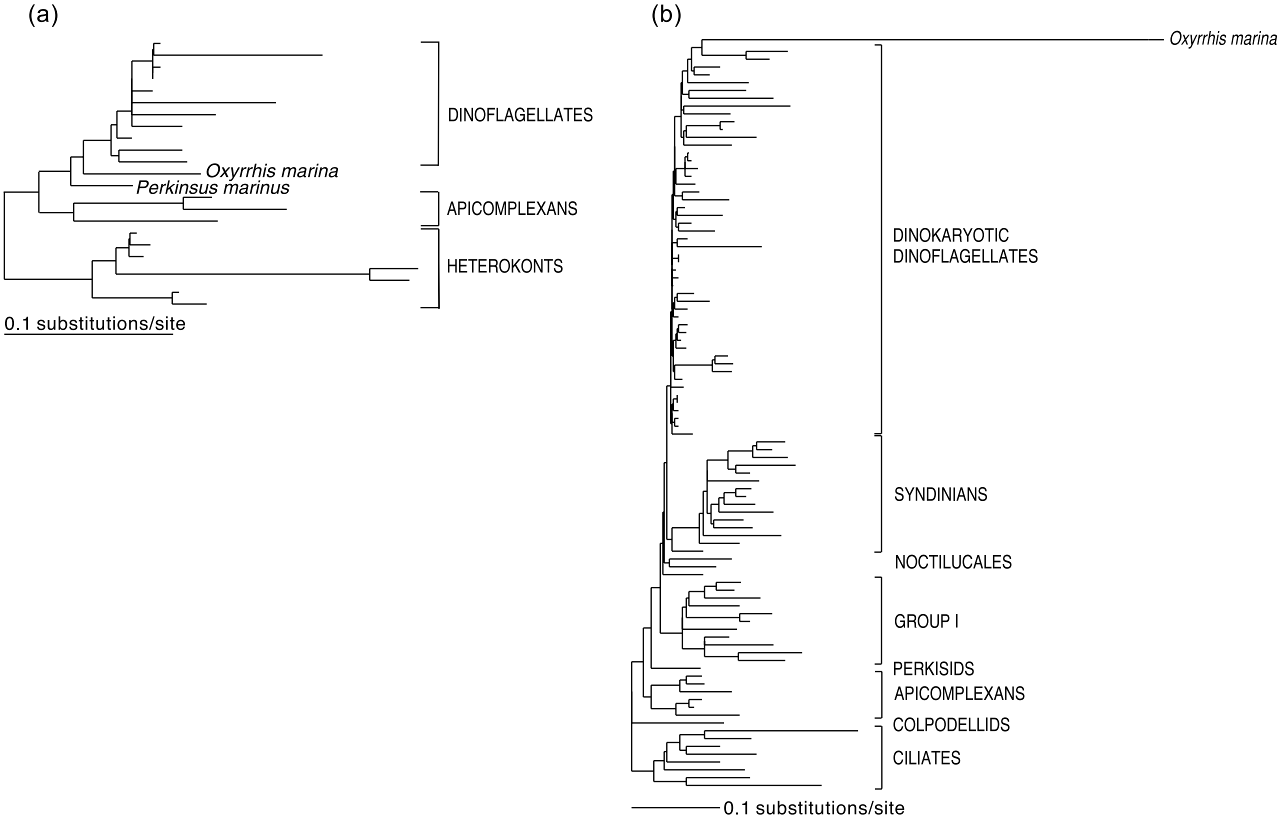

Fig. 2. (a) The position of *Oxyrrhis marina* in a weighted neighbor-joining, gamma-corrected distance matrix tree (1488 nucleotides). Most other methods and molecules (e.g. protein genes) support this position (Saldarriaga *et al.* 2003a). (b). The highly anomalous, apparently derived position of *Oxyrrhis marina* in a maximum likelihood SSU tree. This position would require at least three major character reversions.

the exterior of the still closed nucleus; and the eventual development of cellulose thecal plates in the peripheral vesicles. The life cycle of some developed an alternate, benthic, dormant stage surrounded by a tough organic wall, homologous to the pellicle layer present in some motile cells. If the ancestor was non-photosynthetic, then plastids had to be acquired by secondary symbiosis. When these events took place, and in what sequence, cannot be determined with certainty.

The Triassic fossils clearly show several of these apomorphic dinoflagellate characters, indicating that major developments had already occurred at an undetermined earlier time (Head 1996). Paratabulation in patterns resembling thecal tabulation suggests that they reflect the presence of thecal plates on the motile cell. Plates were well developed by the late Triassic, judging by the suessioid paratabulations. The presence of a girdle and sulcus on the cysts implies (but does not prove) the presence of the typical transverse flagellum winding around the cell, the other posteriorly directed. Sulcal plates are often not discernible in cysts, but exceptions exist. In *Nannoceratopsis,* for example, the sulcal plate pattern is very clearly revealed. In *Paleoperidinium* even the transient intercalary growth bands, usually formed on the theca to accommodate the enlarged zygote, are evident on the cyst wall.

Nothing of the nuclear state or presence and type of plastid can be determined. However, if dinosporin was a polymer of carotene, as thought to be the case for sporopollenin at one time (e.g. Brooks & Shaw 1977), it could be argued that plastids were present because these organelles are the usual source of carotene. This raises the interesting possibility that the rapid radiation in the Mesozoic could have been fueled by the acquisition of the peridinin (red type) plastid, opening a huge new niche for the formerly non-photosynthetic group. However, more recent evidence suggests involvement of the shikimate pathway in cyst formation rather than the mevalonate or methyl-erythritol pathways in the production of carotenes (Versteegh, pers. comm., 2003). Modern heterotrophic dinoflagellates, such as *Protoperidinium*, for example, accumulate large quantities of carotene from their food (mostly diatoms) prior to cyst formation and so this accumulation of carotene must have another function.

The more usual explanation for the dramatic radiation in the Mesozoic has to do with resting cyst function and availability of an environment appropriate for this function. Resting cysts (hypnocysts) are the means by which approximately 10–15% of living dinoflagellates survive unfavorable conditions (see multiple papers in the workshop publication edited by Garcés *et al.* 2002). Typically, starvation induces sexuality and cells of relatively normal appearance transform into gametes, fusing to form a swimming zygote (planozygote). If resting cysts are formed (encystment) they sink to the sediments and become dormant. After a period, typically months but sometimes years, they escape through the archeopyle (excystment) in response to a cue, usually assumed to be external (temperature, light). It is widely believed that this strategy is only feasible in the shallow water of the continental shelf. The deeper waters of the open ocean (average depth 4000 m) would impose prohibitive time constraints and a lack of any conceivable external excystment cue.

The formation and subsequent break-up of Pangea dominated the Mesozoic. As the continental pieces drifted apart the available coastline presumably increased significantly. The rise in sea level also flooded large continental areas. These would seem to be ideal for the usual benthic cyst strategy of dinoflagellates, which is based on dormancy in relatively shallow, shelf waters (pelagic cysts in which the cells are photosynthetically active occur in the genera *Pyrocystis* and *Thoracosphaera*). There is a clear match between sea level increase and dinoflagellate fossil taxa (Fensome *et al.* 1996; MacRae *et al.* 1996). Therefore, it can be argued that this increase in shallow shelf areas opened this survival niche to cyst-forming dinoflagellates. However, other photosynthetic protist groups, such as coccolithophorids and diatoms, also radiated in the Mesozoic, but later than dinoflagellates and for different, less clear reasons (see Falkowski *et al.* 2004 for hypotheses concerning the three groups) while the subdivision of Panthalassa altered ocean circulation (John *et al.* 2003). Extensive shallow areas could also have been present in the Paleozoic (H. Brinkhuis, pers. comm., 2003) but did not result in classic dinoflagellate cyst types. By the mid Jurassic nearly all the main tabulational types were present (Fensome *et al.* 1996, 1999). While other groups were significantly impacted by the Cretaceous/Tertiary bolide impact, dinoflagellates were much less so (a decline began in the Maastrichtian before the K/T boundary; see Discussion in MacRae *et al.* 1996) and all the major lineages survived through to the present day.

COHERENCE OF MODERN GROUPS, RELATIONSHIPS, SEQUENCE OF APPEARANCE AND MOLECULAR TREES

Five basic types of tabulation in extant taxa were recognized by Taylor (1980): gymnodinioid, peridinioid, gonyaulacoid, dinophysoid and prorocentroid, and these are still the basis for five orders of dinoflagellates. A sixth type, suessioid, includes mostly fossil forms, but also living genera such as *Symbiodinium* and the recently described *Polarella* (Montresor *et al.* 1999; Taylor 1999c), while a further basic type, nannoceratopsioid, is known only from the fossil record (Fensome *et al.* 1993). It is not the intention to discuss all the families of dinoflagellates here but it is possible to discuss key relationships in the light of both morphology/biochemistry and molecular data.

In essence gymnodinioids have a large number of polygonal vesicles with or without delicate platelets that do not form clear latitudinal series, suessioids have nine series (including the girdle or cingular plates), peridinioids and gonyaulacoids six, but differing in symmetry, dinophysoids are bilateral with a girdle and sulcus, and prorocentroids lack a girdle and sulcus, having a cluster of very small platelets around two pores. The extinct nannoceratopsids have a peridinioid epthecal pattern and a dinophysoid hypotheca, constituting a true missing link between peridinioids and dinophysoids. Prorocentroids are very similar to dinophysoids in being laterally subdivisible by the megacytic suture but lack any sign of the girdle or sulcus.

Athecate taxa

In view of the early, relatively crude, characterization of genera in classical monographs such as that of Kofoid and Swezy (1921), in which position and shape of the girdle was a pre-eminent criterion (and presence or absence of plastids was irrelevant), it has long been considered that genera such as *Gymnodinium, Amphidinium, Katodinium* and *Gyrodinium* intergrade, and also would prove to contain taxa that were not closely related (e.g. Taylor 1980, 1987a). In addition, there are athecate dinoflagellates that also have many amphiesmal vesicles (cortical alveoli) but are distinguished by other, more derived characters; examples include the noctilucoids, warnowiaceans (with ocelli), the multicellular *Haplozoon* and the pseudo-multicellular *Polykrikos*.

Gymnodinoid dinoflagellates were at first defined by a negative criterion: the absence of a theca. However, the clear visualization of the amphiesmal/cortical vesicular pattern, begun by Biecheler using the Chatton-Lwoff silver-impregnation method (Biecheler 1934) and, later, much more readily, by modern scanning electron microscopy, allowed positive morphological distinctions that were not possible before (Netzel & Dürr 1984). Biecheler saw and named the acrobase, a narrow anterior extension of the sulcus to or near the apex. Takayama (1985) showed that several distinct types occur, some winding in opposite directions around the apex and others not contacting the sulcus. These have been used as the basis for new genera (Daugbjerg *et al.* 2000) and should be valuable in linking to the more complex genera such as *Cochlodinium, Warnowia* and *Polykrikos*. *Gymnodinium fuscum*, the type species of the genus *Gymnodinium*, has a simple apical furrow and its amphiesmal vesicles have only a very delicate, membraneous layer within them (Dodge & Crawford 1969; Hansen *et al*. 2000). Additionally, cryptic thecae have been found in formerly gymnodinioid species, which place them in existing or new thecate genera, for example *Crypthecodinium, Lessardia* (a podolampid; Saldarriaga *et al*. 2003b) and *Pfiesteria* (a peridinioid; Steidinger *et al*. 1996; Fensome *et al*. 1999; Litaker *et al*. 1999). These are referred to here as pseudo-gymnodinioids, for convenience. Once they are removed from the Gymnodiniales, do the remaining gymnodinioids form a discrete clade, and is it basal to the thecate groups as might be expected (Fensome *et al*. 1993)?

Molecular data in the form of rRNA gene sequences have been obtained for many truly gymnodinoid dinoflagellates; that is, for dinoflagellates with large numbers of small alveolae as determined by ultrastructural studies. These taxa, many species in the genera such as *Gymnodinium, Amphidinium, Gyrodinium, Karenia, Karlodinium and Akashiwo*, never form a single clade in any extant molecular trees. They are always interspersed with peridinioid, prorocentroid and dinophysoid forms (Saldarriaga *et al*. 2004). Murray (2003) made a particular study of the genus *Amphidinium* and found that while most clustered in one clade, others were far removed from it, much the same as in the genus *Gymnodinium.* Artificial trees, where these species are forced together to the exclusion of other forms, are always rejected by statistical tests, irrespective of where this artificial clade is put with respect to the rest of the dinoflagellates. Therefore, molecular data suggest fairly strongly that gymnodinoid dinoflagellates (even excluding the pseudo-gymnodinoid forms) are polyphyletic. Whether or not some (or all) of these gymnodinoid lineages originated before the appearance of the theca is, however, still unclear, although it is possible that at least some lineages may have had thecate ancestors (Saldarriaga *et al*. 2004). Those with a spiral acrobase seem most likely to have diverged before the origin of the theca.

Many groups of gymnodinoid dinoflagellates have not yet been included in molecular studies. An example is the order Ptychodiscales (Fensome *et al*. 1996), a group that includes forms in which a continuous layer, the pellicle, forms a flexible wall beneath the vesicles (pelliculate genera; Taylor 1987b). A modern example would be *Balechina*, whereas fossils include *Dinogymnium* (Late Cretaceous). These possibly provide the best examples of preservable vegetative cells in dinoflagellates. Other athecate groups that have been neglected by molecular studies include the genera with internal skeletal elements (e.g. *Actiniscus* or *Dicroerisma*), multinucleate forms (*Polykrykos*), and the forms with ocelli (e.g. *Warnowia* and *Erythropsidinium*).

Peridinioids and Gonyaulacoids

These two groups dominate the thecate dinoflagellates, both Cenozoic and Mesozoic. They are basically similar in that they both have five latitudinal plate series (apicals, anterior intercalaries, precingulars, postcingulars and antapicals) plus the cingulars and sulcals (Kofoidean gonyaulacoid posterior intercalaries were relegated to antapicals when redefined by Balech 1980). Because of these similarities they were usually combined in a single order Peridiniales. However, Taylor (1980) proposed that the gonyaulacoids should be a separate order, the Gonyaulacales, based primarily on symmetry but also sulcal plate/flagella relationships; this has been followed by most authors subsequently, (e.g. Fensome *et al*. 1993). As a result of a comparative study of extant tabulation, Taylor (1980) found that both tabulations could be reduced to an almost radially symmetrical, hypothetical model that could, and has been (Evitt 1985; Fensome *et al*. 1993) used to determine plate homologies in comparisons of genera.

Peridinioids tend to be bilaterally symmetrical, lacking much torsion, with two or three well-developed anterior intercalaries. There is usually a small apical pore plate associated with a narrow, elongate preapical or canal platelet between it and the first apical plate. Peridinioids are most clearly recognizable by having predominantly bilateral symmetry, with two relatively large, subequal antapical plates and a single posterior sulcal plate between the single, relatively large flagellar pore, and the antapicals. They have both photosynthetic and non-photosynthetic members. Most of the latter seem to use a feeding veil/pallium to engulf their prey.

Gonyaulacoids, in contrast, show significant left-handed torsion of the epitheca (extreme in *Gonyaulax*), the anterior intercalaries do not form a strong epithecal series but tend to be small plates on the right side, cut off from the apex by other small plates. The apical pore complex often has a hook-like groove on the border of an inner and outer pore plate. The first apical plate is usually asymmetrical and a ventral pore is often situated on its upper right margin. The transverse flagellum arises from under the anterior sulcal plate, whereas the longitudinal flagellum arises more posteriorly within the sulcus. Their antapical plates show considerable asymmetry, the right antapical (Z in Taylor-Evitt notation) being much larger and mid-antapical, the left (Y, the old Kofoidean I^{p}) being smaller and shifted to the left side as a result of torsion. They are nearly all photosynthetic except for *Crypthecodinium* and one species of *Gonyaulax*, *Gonyaulax alaskensis* (F.J.R. Taylor and D. M. Jacobsen, unpubl. obs.) for both east and west coast populations of North America.

Molecular data support the separation of peridinioids and gonyaulacoids: members of the order Gonyaulacales form a clade in most molecular trees examined to date (*Crypthecodinium* and some species of *Thecadinium* may fall outside in some trees), and this clade always excludes all members of the Peridiniales. However, whereas the Gonyaulacales tend to form a cohesive, monophyletic group, the Peridiniales do not: they are always interspersed with gymnodinialean, prorocentralean and dinophysialean forms. This topology is consistent with the peridinioid dinoflagellates being a paraphyletic group that gave rise to both prorocentroids and dinophysoids, as well as possibly to several groups of gymnodinoids, and perhaps even the gonyaulacoids.

Photosynthetic Peridinioids

Most extant photosynthetic peridinioids, in particular the genera *Peridinium*, *Peridiniopsis* and *Glenodinium*, are found in freshwater habitats, but species of *Heterocapsa* and *Scrippsiella* are common marine, coastal taxa and *Durinskia* is brackish (containing a diatom endosymbiont: see Plastids below). They all share an anterior intercalary series of four (*Glenodinium*) or, most typically, three plates, an apparently derived state that is unlikely to have evolved more than once. The sulcus of *Heterocapsa* is simpler than that of other peridinioids, with two distinct flagellar pores surrounded by a rosette of four or five platelets, and was placed in its own suborder by Fensome *et al*. (1993), although the basis for this was the number of apical plates (which included some fossil genera in which the sulcal platelets were not reflected). The other photosynthetic peridinioids, including *Scrippsiella*, have sulcal and girdle plates that are all quite similar, with five or six girdle plates and a single flagellar pore. Some distinctive cyst types are found within *Scrippsiella*, notably the modern calcareous-walled cyst producers and the fossil calciodinellids. The vegetative cyst (see Taylor 1987b) of *Thoracosphaera* drifts in the upper ocean as a calcareous ball, essentially mimicking a coccolithophorid. It is possible that some of the other oceanically distributed calcareous ‘cysts’ may also be of vegetative stages, rather than the resting cysts of the coastal *Scrippsiella* relatives.

Interestingly, phylogenetic trees based on combined SSU and LSU data often put the genus *Heterocapsa* as a sister group to the rest of the dinokaryotic dinoflagellates, although with weak support; if this placement is correct, it would be congruent with the simple organization of the sulcal plates in the genus. In most trees, calcareous cyst formers (*Scrippsiella* and *Pentapharsodinium*) form a monophyletic group that also includes the genus *Thoracosphaera*; the same is true for diatom-containing dinoflagellates (*Kryptoperidinium* and *Durinskia*) and for two clades of non-photosynthetic forms (*Lessardia/Roscoffia* on the one hand, the genus *Protoperidinium* on the other). However, as explained before, peridinioid dinoflagellates do not form a clade to the exclusion of other forms. Furthermore, all phylogenetic trees examined to date lack resolution in the section that contains the peridinialean forms, and as a consequence it is as yet impossible to determine whether the photosynthetic forms gave rise to the non-photosynthetic ones or vice versa, or indeed whether either of those groupings is monophyletic to the exclusion of the other.

Non-photosynthetic peridinioids

Heterotrophic peridinioids consist of *Pfiesteria,* the large marine genus *Protoperidinium,* the diplopsaloids (e.g. *Diplopsalis* and *Zygabikodinium*) and the podolampids (e.g. *Podolampas, Blepharocysta* and *Lissodinium*). With the exception of *Pfiesteria,* these genera have a peridinioid tabulation but show a reduction of the girdle plate number to three. In some diplopsaloids the mid-antapical suture is lost (see Taylor 1980) and in podolampids the girdle indentation is lost, although the plates remain. Therefore, there are several derived features that unite these groups as a distinct morphological clade with three main branches. The fossil *Deflandrea* and similar genera show shapes and paratabulations that are very similar to those of *Protoperidinium*, but usually have no girdle plate reflection. It is, of course, not known whether these fossil genera were photosynthetic or not. Only four genera of non-photosynthetic peridinioids are included in molecular trees: *Lessardia*, *Roscoffia*, *Protoperidinium* and *Pfiesteria*; the diplopsaloids are not represented at all but, on a morphological basis, would be expected to group with *Protoperidinium*. Interestingly, although *Lessardia* and *Roscoffia* do make a clade in most trees, they never group together with either *Protoperidinium* or *Pfiesteria*. *Pfiesteria* has a much simpler peridinioid tabulation, with unusual sulcal plates, no girdle plate reduction, and only one small anterior intercalary. These tabulational characteristics of *Pfiesteria* suggest that there are no close relationships with the other heterotrophic peridinioids in this assemblage. In some molecular trees *Pfiesteria* has a relatively basal position (Litaker *et al*. 1999). It is unclear whether its feeding behavior is phylogenetically significant.

Gonyaulacoids

As noted, the gonyaulacoid clade is well supported by molecular data, supporting the separation at the ordinal level as proposed by Taylor (1980). Comparison of tabulations provided nice examples of gradients of tabulation (morphoclines) (e.g. Taylor 1979, 1980), but left polarity open; that is, in which direction did evolution proceed: from less asymmetrical to more, or vice versa? Molecular data, with strongly supported branches, place *Gonyaulax* and *Ceratium* near the base of the clade, with the goniodomineans, such as *Alexandrium* and *Goniodoma*, as the most derived. In an interesting exercise combining fossil with molecular data, John *et al.* (2003) used fossils to date the nodes of the goniodominean and, in particular, the *Alexandrium tamarense* clade. The latter was concluded to be not earlier than 45 Ma and could be as late as 23 Ma. Paleobiogeographic distribution was inferred, using plate tectonics and paleocurrent systems. Unfortunately, southern hemisphere records were ignored, but this may not have altered their hypothesis much.

Prorocentroids and dinophysoids

These two orders share a major synapomorphic feature: the division of the theca into lateral halves by a sagittal suture. No other groups have this feature and so, although this could be a result of convergent evolution, it is unlikely. Both have two pores, a large and a smaller one, in which both the flagella arise from the larger one. The prorocentroids are highly unusual in lacking a girdle and sulcus, but their tiny periflagellar platelets may be homologous to sulcals and some epithecal plates of dinophysoids (Taylor 1980, 1987b). Therefore, both form well defined, coherent groups and should share common ancestry as sister groups. In molecular trees, both groups are included in the same clade as peridinioids and many gymnodinoids, but seldom branch close to each other. Again, the support for the branching orders in this section of the tree are always very low, and so it is unclear whether the topologies obtained have real phylogenetic significance or not.

PLASTIDS

Dinoflagellates have become notorious for the variety of plastid types that they have acquired by secondary, or even tertiary symbiosis (McFadden & Gilson 1995). One could easily get the false impression that the five types recognized so far are semi-randomly scattered throughout the families. However, there appears to be a distinct pattern, with each of them having been apparently acquired only once; that is, present in monophyletic clades. The predominating, synapomorphic plastid is the peridinin type, found in gonyaulacoids, most photosynthetic members of the gymnodinioids and peridinioids, and the prorocentroids. It is usually surrounded by an envelope consisting of three membranes. Given the wide distribution and unlikelihood of independent acquisition of this unique type, the above groups must have had a common ancestor that was basal to most modern dinoflagellates (Takishita & Uchida 1999). They should plot coherently on the molecular trees. Each of the other types (e.g. forms containing fucoxanthin, 19′ oxyfucoxanthin, chlorophyll a and b, or phycobilin) forms a clade that is derived from the peridinin- containing clades (Saldarriaga *et al.* 2001). The fucoxanthin type has been shown to be of diatom origin (Chesnick *et al.* 1997). In the genera *Durinskia and Kryptoperidinium* the symbiont nucleus is still present.

The weakly pigmented, pale yellowish-green, 19′ hexanoyloxyfucoxanthin type, is found only in a closely similar cluster of gymnodinioids containing the fish-killing genera *Karenia* and *Karlodinium*. This plastid type is believed to have its origin in a haptophyte (Tengs *et al.* 2000) and, if the chlorophyll-c type plastids had a single origin, this should have been the type from which the peridinin plastids evolved. Yoon *et al.* 2002) found evidence in plastid genes of strong similarity between some peridinin plastids and haptophyte plastids, leading to the proposal that the former arose from the latter by tertiary symbiosis. However, Inagaki *et al.* (2004) found that codon bias could provide a misleading impression in this case.

The phycobilin-containing dinophysoids *Dinophysis, Amphisolenia* and *Triposolenia*, seem more closely related (all having a reduced epitheca and, consequently, a strongly apically displaced cingulum) than more morphologically derived genera, such as *Ornithocercus, Histioneis* and *Citharistes*, which lack plastids but have an extracellular association with coccoid cyanobacteria (Taylor 1980, 1982, 1990). The apparently less-derived taxa (epitheca less reduced), formerly in the genus *Phalacroma*, are also mostly without plastids. One of these has been sequenced and is basal to *Dinophysis* in the LSU trees and so it might be argued that it was within this group that the cryptomonad plastids were acquired. Schnepf and Elbrächter (1999) showed that all were of similar structure and proposed myzocytosis as the method of ingestion. Hackett *et al.* (2003) provided molecular evidence that they believe supports a single origin for these cryptomonad plastids. Some have asserted that the phycobilin plastids are not permanent but instead are acquired annually from surrounding cryptomonads; that is, they are kleptochloroplasts. The sister group to dinophysoids, the prorocentroids, contain the usual peridinin type.

The chlorophyll a and b plastid-containing taxa are the gymnodinioids *Gymnodinium chlorophorum* and the scaley *Lepidodinium.* Prasinomonads are much more common than chlorophycean green algae in the planktonic marine environment and so it might be expected that they are the source of these plastids (this is currently under study, D. Grzebyk. pers. comm.).

Given their position in rRNA molecular trees (e.g. Figs 3,4) Saldarriaga *et al.* (2001) argued that the peridinin plastids have been replaced in each of these other types, rather than being acquired independently by non-photosythetic taxa. In partial support of this hypothesis, electron microscopy showed that *Durinskia*

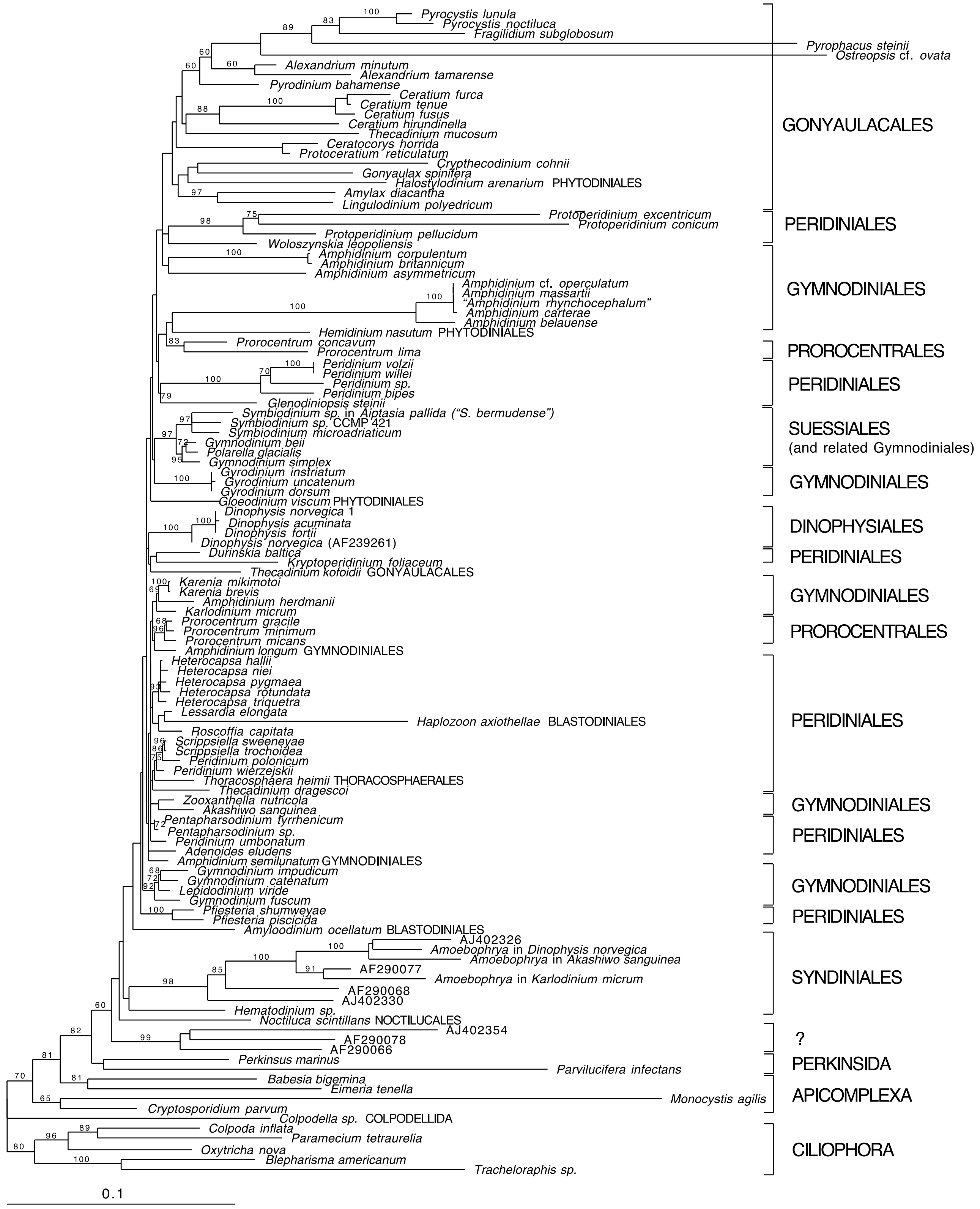

Fig. 3. A tree of small subunit (SSU) ribosomal DNA sequences for 98 dinoflagellates plus 7 undescribed species using BioNJ, gamma corrected distance matrix (from Saldarriaga *et al.* 2004).

and *Kryptoperidinium* have an eyespot within a three-membraned envelope that has been interpreted as a vestigial prior, presumably peridinin plastid. The mechanism implicit in this process is a mixotrophic ingestion of a photosynthetic foreign organism by a photosynthetic dinoflagellate.

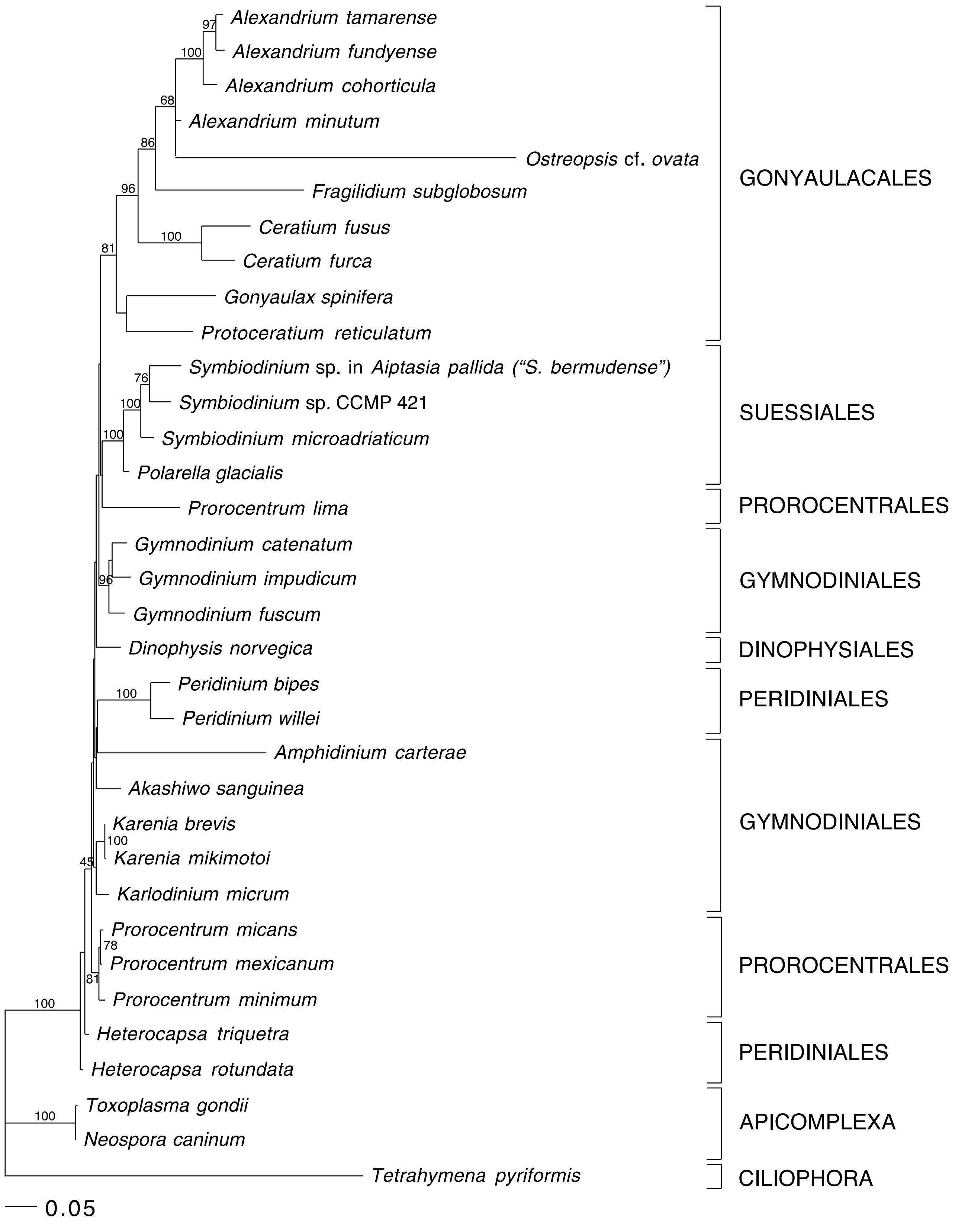

Fig. 4. A maximum-likelihood tree constructed from concatenated large subunit (LSU) (domains D1 and D2) and small subunit (SSU) ribosomal DNA sequences (2100 nucleotides) from 34 alveolates, 31 from dinoflagellates (from Saldarriaga *et al.* 2004).

DISCUSSION

Taking the above plastid and early branching features, we can first address the issue of whether or not the ancestral dinoflagellate was photosynthetic. Given the vestigial plastids in the sister group, the apicomplexans, and molecular evidence suggesting that even the common ancestor of all the alveolates might have been photosynthetic, it seems, nevertheless, that a hypothesized photosynthetic ancestry is not supported by the overall morphological or molecular picture. No plastid vestiges have been found in ciliates, the most basal alveolates. The extant basal groups on the dinoflagellate lineage, including the pre-dinoflagellates and groups with chromosomal decondensation at some stage in their life cycle, are not photosynthetic. A photosynthetic ancestor for all dinoflagellates, such as that proposed by Yoon *et al.* (2002) or the chromalveolate hypothesis (see above), would require invocation of highly unparsimonious, repeated losses of plastids.

In general, despite some molecular evidence to the contrary, dinoflagellates appear to be fundamentally heterotrophic and, like euglenoids, often retain a feeding capacity (or vestiges) even when photosynthetic; that is, are often mixotrophic. This does not apply only to small particles, but also to other eukaryotic cells. Indeed, this is a requirement for the postulated frequency of secondary symbioses and plastid replacements (Saldarriaga *et al.* 2001).

Given the synapomorphies found in dinoflagellates one can readily infer changes from a more generalized flagellate ancestor. Several extant genera exhibit postulated transition states in dinoflagellate evolution. *Oxyrrhis* exhibits a possible transient state in flagellar insertion and differentiation consistent with its position in some trees. Paraflagellar material is present in differing degrees in the two flagella but a true transverse, undulating ribbon is not present. Syndineans have an external spindle but still have unusual, non-dinokaryotic, nuclei. Dinokaryotic nuclei (condensed fibrillar chromosomes) appear in the life cycle of noctilucoids, alternating with more typical eukaryote-like nuclei. This is also true of the poorly studied, parasitic Blastodinians (probably polyphyletic), which otherwise show little resemblance to the highly specialized noctilucoids with their huge vacuoles. Both blastodineans and noctilucoids tree out basal to the rest of the dinoflagellates (but, the position of *Noctiluca* is weakly supported at present, as noted earlier). *Blastodinium* has been reported to be pigmented but there has been no recent examination of the cause of this. If it contains true plastids, then it could be the most basal photosynthetic dinoflagellate. All other dinoflagellates have the dinokaryon throughout their lifecycle.

Another derived feature is the formation of cellulose thecal plates within the amphiesmal vesicles. The vesicles of *Oxyrrhis* are empty, and it lacks a true girdle and sulcus. How many gymnodinioids also have empty vesicles is unknown. Some gymnodinioids that have a girdle and sulcus have extremely thin, membraneous-like leaflet structures within their amphiesmal vesicles, including the type of the genus *Gymnodinium. Woloszynskia* is a gymnodinioid with a thin theca consisting of many plates not forming obvious series. *Polarella* and *Symbiodinium* show the suessioid pattern of forming numerous distinct latitudinal plate series. Therefore, it might be expected that these genera should form the base of the thecate dinoflagellate clades (Fig. 5). In both LSU and SSU trees these do form a cluster with a branching pattern in the expected order, but they are not basal to other thecate forms. The main *Gymnodinium* cluster is sometimes a sister group to these. The more derived gymnodinioids (e.g. *Cochlodinium*, the warnowiaceans and *Polykrikos*) appear to have arisen from an ancestor possessing a spiral acrobase (e.g. resembling *Akashiwo*).

Patterson's (1981) assertion that inclusion of fossil data rarely modifies hypotheses inferred from the comparative study of living taxa, can be tested by the study of the cyst formers that contributed to the fossil record. Unfortunately, none of the putatively earliest diverging dinoflagellates produce preservable cysts, nor do the pre-dinoflagellates. The most basal extant dinoflagellate producing a fossilizable cyst is *Polarella*, a suessioid (Montresor *et al.* 1999; Taylor 1999c) which is consistent with the suessioids being one of the two earliest (Triassic) members of the Mesozoic radiation of undoubted dinoflagellate fossils: the others are the rhaetogonyaulacoids, with a more modern reduced number of plate series but most with four antapical plates. Bujak and Williams (1981) presented several alternative trends in thecal pattern evolution. One was a plate fragmentation model in which few plates are the more basal state, for example *Prorocentrum.* Although the latter is near-basal in some trees, the consensus suggests that it is a derived taxon linked to peridinioids. Another alternative, the plate fusion model, implies that few plates are the more derived state and this is supported by the position of *Prorocentrum,* with *Woloszynskia* and many-plated suessioids near the base and the simple, almost radially symmetrical *Goniodoma* in a highly derived position in the gonyaulacoid. Therefore, although these models are simplistic since both may have occurred, most evidence supports a trend toward sutural loss.

The extension of a single elongated plate, traditionally designated the first apical plate, from the sulcus to the apical pore complex, is another derived development (Fig. 5). Genera such as *Heterocapsa* and the fossil *Rhaetogonyaulax*, have an essentially complete precingular series that creates the impression of two plates between the sulcus and the apex. It appears as if, in most peridinioids and gonyaulacoids, the single

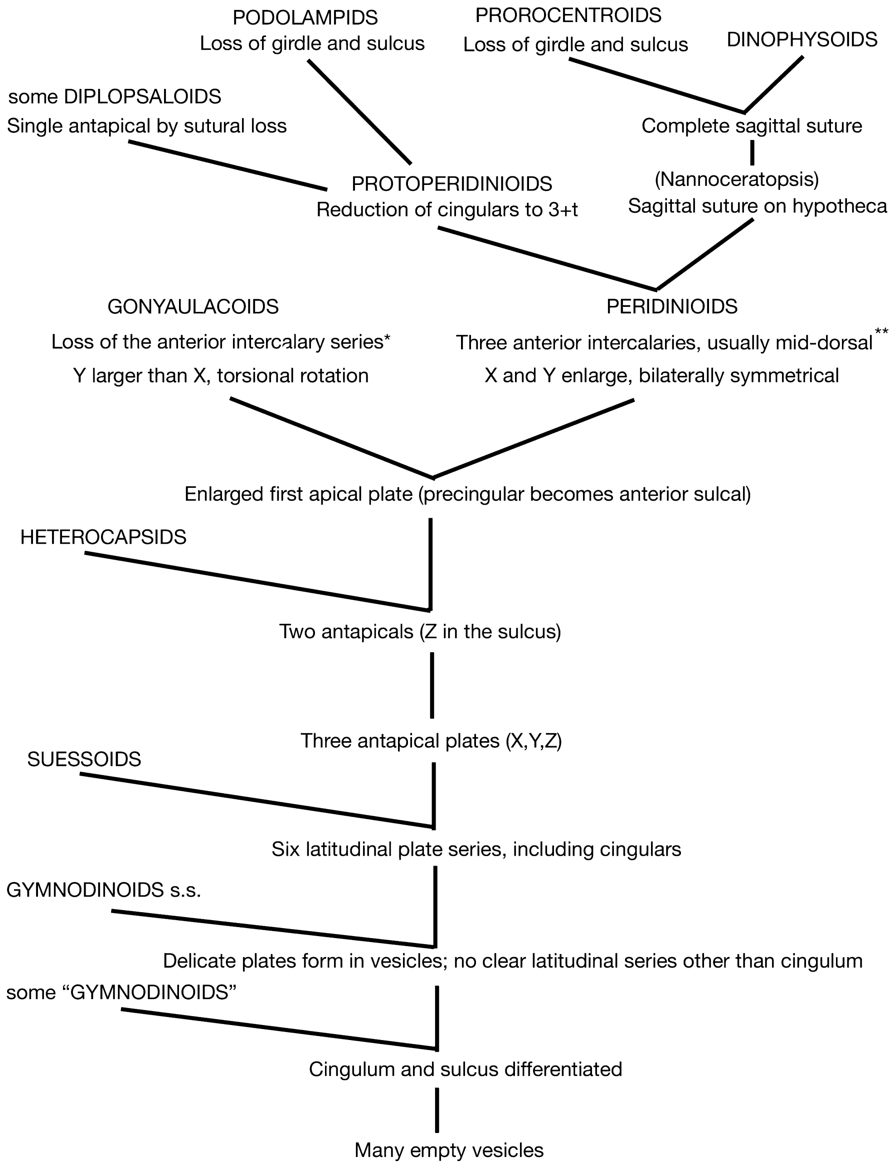

Fig. 5. Example of a hypothetical, parsimonious, morphology-based model of the evolution of the dinoflagellate theca, assuming a single origin (original). Conceptual models like this have proved difficult to test with existing molecular data. *Some small platelets on the right side, near the apex of some gonyaulacoids, are technically Kofoidean anterior intercalaries but not homologous to those in peridinioids. **Reduced to two in Archaeperidinium. The first apical plate of peridinioids is often displaced from the apical pore plate by a small, narrow platelet.

first apical plate is caused by the incorporation of one of the precingulars into the sulcus, becoming the anterior sulcal plate. *Scrippsiella* shows this condition. In *Pfiesteria* and related genera, this has not happened, raising the possibility that these taxa diverged before this came about.

The primary evolutionary feature of the gonyaulacoids has been torsion, with left-handed (descending) girdle displacement, linked to an antapex in which one plate (Y) is larger than the others. Also, the typical peridinealean anterior intercalaries are absent, small platelets close to the apex in *Gonyaulax* being technically designated as anterior intercalaries by the Kofoid system, but not apparent homologs. The fossil record suggests that gonyaulacoids may have preceded peridinioids, with a link between them in the partiform taxa. However, although poorly resolved, the molecular data suggest the opposite.

The key problem in using dinoflagellate molecular trees, especially of ribosomal DNA genes, has been the short branches and very poor resolution in the main 'backbone' (Figs 3,4), the so-called GPP complex (*Gymnodinium, Peridinium, Prorocentrum*) of Saunders *et al.* (1997) who were the first to run into this difficulty. Unfortunately, adding more taxa (now approximately a hundred in SSU trees: Saldarriaga *et al.* 2004), has not helped to improve resolution in the backbone, although questions concerning more closely related taxa can be resolved, especially in the gonyaulacoids (e.g. John *et al.* 2003). The reason for the slight amount of divergence of these genes over more than 100 million years is unclear. The relatively abrupt radiation of the main thecate lineages in the late Triassic (Fensome *et al.* 1996) seems to have been followed by relatively little change in the ribosomal genes for most of the subsequent period.

Molecular phylogenies in dinoflagellates have been useful for identifying some of the close relatives of the group itself (apicomplexans, ellobiopsids, *Perkinsus* and *Parvilucifera*) and for determining phylogenetic relationships of many taxa, in particular athecate or pseudo-gymnodinoid groups. For example, rRNA data has shown that the Suessiales, the order that contains *Polarella* and *Symbiodinium,* is larger than previously thought. It contains many pseudo-gymnodinoid forms previously thought to be related to *Gymnodinium*. Resolution of closely related taxa, such as species within a genus, has been practicable but anomalies, such as the splitting of *Prorocentrum* (Grzebyk *et al.* 1998) into two clusters interspersed by gymnodinioids, or dinophysoids never grouping with any prorocentroids in any molecular trees, are problematic. New SSU (18S) or LSU sequences will probably be useful to determine close relatives of particular taxa, but are not likely to be helpful in addressing the big picture. The inclusion of more heterotrophic taxa should help to answer some fundamental questions, such as the nutritional type of more basal dinoflagellates or the position of the noctilucoids. Phylogenetic trees based on other (protein) genes should be useful in providing a better consensus. It is also worth noting that the evolution of thecal patterns over a wide scale has never been fully explored, although attempts regarding major events were made long ago (e.g. Taylor 1980, 1999b), and in Fig. 5 tabulations have been summarized and used in the classification of Fensome *et al*. (1993). There is much unfinished work to do, even on the morphological side.

It is gratifying to see that this fascinating group of protists, with so many intriguing, idiosyncratic features (Taylor 1987a), still attracts the interest of so many excellent cell biologists, taxonomists, ecologists and palynologists. It is surprising that there is no society dedicated to their study (a better name than 'dinoflagellatologist' seems to be needed). Synthesis of our growing knowledge of dinoflagellates is presently served primarily by the 'DINO' conferences, which surely must carry on into the future.

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REFERENCES

Baldauf, S. L., Roger, A. J., Wenk-Siefert, I. and Doolittle, W. F. 2000. A kingdom-level phylogeny of eukaryotes based on combined protein data. *Science* **290**: 972–7.

Balech, E. 1980. On thecal morphology of dinoflagellates with special emphasis on circular (sic) and sulcal plates. *An. Centro Cienc. Mar. Limnol., University Nat. Auton. Mexico* **7**: 57–68.

Below, R. 1987. Evolution und Systematik von Dinoflagellaten-Zysten aus der Ordnung Peridiniales. I. Allgemeine Grundlagen und Subfamilie Rhaetogonyaulacoideae (Familie Peridiniaceae). *Palaeontographica Abt. B.* **205**: 1–164.

Biecheler, B. 1934. Sur le réseau argentophile et la morphologie de quelques Péridiniens. *C.R. Soc. Biol.* **115**: 1039.

Brooks, J. and Shaw, G. 1977. Recent advances in the chemistry and geochemistry of pollen and spore walls. *Trans. Bose Res. Inst* **40**: 19–38.

Bujak, J. P. and Williams, G. L. 1981. The evolution of dinoflagellates. *Can. J. Bot.* **59**: 2077–87.

Butterfield, N. J. and Rainbird, R. H. 1998. Diverse organic-walled fossils, including 'possible dinoflagellates', from the early Neoproterozoic of arctic Canada. *Geology* **26**: 963–6.

Cavalier-Smith, T. 1998. A revised six kingdom of life. *Biol. Rev. Camb. Philos. Soc.* **73**: 203–66.

Cavalier-Smith, T. 1999. Principles of protein and lipid targeting in secondary symbiogenesis: euglenoid, dinoflagellate, and sporozoan plastid origins and the eukaryote family tree. *J. Eukaryot. Microbiol.* **46**: 347–66.

Chesnick, J. M., Kooistra, W. H. C. F., Wellbrock, U. and Medlin, L. K. 1997. Ribosomal RNA analysis indicates a benthic pennate diatom ancestry for the endosymbionts of the dinoflagellates *Peridinium foliaceum* and *Peridinium balticum* (Pyrrophyta). *J. Eukaryot. Microbiol.* **44**: 314–20.

Christensen, T. 1962. Alger *In* Böcher, T.W., Lange, M. and Sørensen, T. (Eds.) *Botanik 2., Systematisk Botanik.* Munksgaard, Copenhagen, pp. 56–61.

Daugbjerg, N., Hansen, G., Larsen, J. and Moestrup, Ø. 2000. 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. *Phycologia* **39**: 302–17.

Delwiche, C. F. 1999. Tracing the thread of plastid diversity through the tapestry of life. *Amer Nat.* **154** (Suppl): 164–77.

Dodge, J. D. and Crawford, R. M. 1969. The fine structure of *Gymnodinium fuscum* (Dinophyceae). *New Phytol.* **68**: 613–8.

Escalante, A. A. and Ayala, F. J. 1996. Molecular paleogenetics: the evolutionary history of *Plasmodium and* related protests. *In* Jablonski, D., Erwin, D. H. and Lipps, J. H. (Eds) *Evolutionary Paleobiology*. University of Chicago Press, Chicago, 21–41.

Evitt, W. R. 1967. Dinoflagellate studies II: the archeopyle. *Stanford University Pubs. Geol. Sci.* **10**: 1–83.

Evitt, W. R. 1981. The difference it makes that dinoflagellates did it differently. *Int. Comm. Palynol. Newsletter* **4**: 6–7.

Evitt, W. R. 1985. *Sporopollenin Dinoflagellate Cysts: Their Morphology and Interpretation*. American Association of Stratigraphic Palynologists, Dallas, USA, 333 pp.

Falkowski, P. G., Katz, M. E., Knoll, A. H. *et al.* 2004. The evolution of modern eukaryotic phytoplankton. *Science* **305**: 354–360.

Fast, N. M., Kissinger, J. C., Roos, D. S. and Keeling, P. J. 2001. Nuclear-encoded, plastid-targeted genes suggest a single common origin for apicomplexan and dinoflagellate plastids. *Mol. Biol. Evol.* **18**: 418–26.

Fast, N. M., Xue, L., Bingham, S. and Keeling, P. J. 2002. Re-examining alveolate evolution using multiple protein molecular phylogenies. *J. Eukaryot. Microbiol.* **49**: 30–7.

Fensome, R. A., MacRae, R. A., Moldowan, J. M., Taylor, F. J. R. and Williams, G. L. 1996. The early Mesozoic radiation of dinoflagellates. *Paleobiology* **22**: 329–38.

Fensome, R. A., Saldarriaga, J. F. and Taylor, F. J. R. 1999. Dinoflagellate phylogeny revisited: reconciling morphological and molecular based phylogenies. *Grana* **38**: 66–80.

Fensome, R. A., Taylor, F. J. R., Norris, G., Sarjeant, W. A. S., Wharton, D. I. and Williams, G. L. 1993. *A Classification of Living and Fossil Dinoflagellates*. Micropaleontology Special Publication 7, Sheridan Press, Hanover, Pennsylvania, USA.

Gaines, G. and Elbrächter, M. 1987. Heterotrophic nutrition. *In* Taylor, F. J. R (Ed.) *The Biology of Dinoflagellates*. Blackwell, Oxford, pp. 224–68.

Gajadhar, A. A., Marquardt, W. C., Hall, R., Gunderson, J., Ariztia-Carmona, E. V. and Sogin, M. L. 1991. Ribosomal RNA sequences of *Sarcocystis muris*, *Theileria annulata* and *Crypthecodinium cohnii* reveal evolutionary relationships among apicomplexans, dinoflagellates, and ciliates. *Mol. Biochem. Parasitol.* **45**: 147–54.

Garcés, E., Zingone, A., Montresor, M., Reguera, B. and Dale, B. (Eds). 2002. Report of the workshop on LIFEHAB: life histories of microalgal species causing harmful blooms. Research in Enclosed Seas Series 12, European Commision Office of Official pubs., Luxembourg, 189 pp.

Goodman, D. E. 1987. Dinoflagellate cysts in ancient and modern sediments. *In* Taylor, F. J. R. (Ed.) *The Biology of Dinoflagellates.* Blackwell, Oxford, pp. 649–722.

Gray, M. W., Lang, B. F., Cedergren, R. *et al.* 1998. Genome structure and gene content in protist mitochondrial DNAs. *Nucleic Acids Research* **26**: 865–78.

Grzebyk, D., Sako, Y. and Berland, B. 1998. Phylogenetic analysis of nine species of *Prorocentrum* (Dinophyceae) inferred from 18S ribosomal DNA sequences, morphological comparisons and description of *Prorocentrum panamensis* sp. nov. *J. Phycol.* **34**: 1055–68.

Gunderson, J. H., Goss, S. H. and Coats, D. W. 1999. The phylogenetic position of *Amoebophrya sp.* infecting *Gymnodinium sanguineum*. *J. Eukaryot. Microbiol.* **46**: 194–7.

Hackett, J. D., Maranda, L., Yoon, H. S. and Bhattacharya, D. 2003. Phylogenetic evidence for the cryptophyte origin of the plastid of *Dinophysis* (Dinophysiales, Dinophyceae). *J. Phycol.* **39**: 440–8.

Hansen, G., Moestrup, Ø. and Roberts, K. R. 2000. Light and electron-microscopical observations on the type species of *Gymnodinium*, *G. fuscum* (Dinophyceae). *Phycologia* **39**: 365–76.

Harper, J. T. and Keeling, P. J. 2003. Nucleus-encoded, plastid-targeted glyceraldehyde-3-phosphate dehydrogenase (GAPDH) indicates a single origin for chromalveolate plastids. *Mol. Biol. Evol.* **20**: 1730–5.

Head, M. J. 1996. Modern dinoflagellate cysts and their biological affinities. *In* Jansonius, J. and McGregor, D. C. (Eds) *Palynology, Principles and Applications*, Vol. 3. American Association of Stratigraphic Palynologists Foundation, Dallas, USA, pp. 1197–248.

Hinnebusch, A. G., Klotz, L. C., Blanken, R. L. and Loeblich, A. R. III. 1981. An evaluation of the phylogenetic position of the dinoflagellate *Crypthecodinium cohnii* based on 5S rRNA characterization. *J. Mol. Evol.* **17**: 334–47.

Inagaki, Y., Simpson, A. G. B., Dacks, J. B. and Roger, A. J. 2004. Phylogenetic artifacts can be caused by Leucine, Serine and Arginine codon usage heterogeneity: dinoflagellate plastid origins as a case study. *Syst. Biol.* (in press).

John, U., Fensome. R. A. and Medlin, L. K. 2003. The application of a molecular clock based on molecular sequences and the fossil record to explain biogeographic distributions within the *Alexandrium tamarense* 'species complex' (Dinophyceae). *Mol. Biol. Evol.* **20**: 1015–27.

Kofoid, C. A. and Swezy, O. 1921. The free-living unarmored Dinoflagellata. *Mem. University Calif.* **5**: 1–562.

Litaker, R. W., Tester, P. A., Colorni, A., Levy, M. G. and Noga, E. J. 1999. The phylogenetic relationship of *Pfiesteria piscicida,* cryptoperidiniopsoid sp., *Amyloodinium ocellatum, and* a *Pfiesteria*-like dinoflagellate to other dinoflagellates and apicomplexans. *J. Phycol.* **35**: 1379–89.

López-García, P., Rodríguez-Valera, F., Pedrós-Alió, C. and Moreira, D. 2001. Unexpected diversity of small eukaryotes in deep-sea Antarctic phytoplankton. *Nature* **409**: 603–7.

MacRae, R. A., Fensome, R. A. and Williams, G. L. 1996. Fossil dinoflagellate diversity, originations, and extinctions and their significance. *Can. J. Bot.* **74**: 1687–94.

McFadden, G. and Gilson, P. 1995. Something borrowed, something green: lateral transfer of chloroplasts by secondary endosymbiosis. *Trends Ecol. Evol* **10**: 12–7.

Moldowan, J. M. and Talyzina, N. M. 1998. Biogeochemical evidence for dinoflagellate ancestors in the early Cambrian. *Science* **281**: 1168–70.

Montresor, M., Procaccini, G. and Stoecker, D. K. 1999. *Polarella glacialis* General nov., sp. nov. (Dinophyceae): Suessiaceae are still alive!. *J. Phycol.* **35**: 186–97.

Moon-van der Staay, S. Y., De Wachter, R. and Vaulot, D. 2001. Oceanic 18S rDNA sequences from picoplankton reveal unsuspected eukaryotic diversity. *Nature* **409**: 607–10.

Murray, S. 2003. Diversity and Phylogenetics of Sand-Dwelling Dinoflagellates from Southern Australia. PhD Thesis, University of Sydney, Australia, 202 pp.

Netzel, H. and Dürr, G. 1984. Dinoflagellate cell cortex, *In* Spector, D. L. (Ed.) *Dinoflagellates*. Academic Press, Orlando, pp. 43–105.

Norén, F., Moestrup, Ø. and Rehnstam-Holm, A. S. 1999. *Parvilucifera infectans* Norén and Moestrup General et sp. nov. (Perkinsozoa phylum nov.): a parasitic flagellate capable of killing toxic microalgae. *Eur. J. Protistol.* **35**: 233–54.

Patterson, C. 1981. Significance of fossils in determining evolutionary relationships. *Ann. Rev. Ecol. Syst.* **12**: 195–223.

Philippe, H. and Adoutte, A. 1998. The molecular phylogeny of Eukaryota: solid facts and uncertainties. *In* Coombs, G. H., Vickerman, K., Sleigh, M. A. and Warren, A. (Eds) *Evolutionary Relationships Among Protozoa*. Chapman & Hall, London, pp. 25–56.

Piel, K. M. and Evitt, W. M. 1980. Paratabulation in the Jurassic dinoflagellate genus *Nannoceratopsis* and a comparison with modern taxa. *Palynology* **4**: 79–104.

Saldarriaga, J. F., Leander, B. S., Taylor, F. J. R. and Keeling, P. J. 2003b. *Lessardia elongata* General et sp. nov. (Dinoflagellata, Peridiniales, Podolampaceae) and the taxonomic position of the genus *Roscoffia*. *J. Phycol.* **39**: 1–12.

Saldarriaga, J. F., McEwan, M. L., Fast, N. M., Taylor, F. J. R. and Keeling, P. J. 2003a. Multiple protein phylogenies show that *Oxyrrhis marina* and *Perkinsus marinus* are early branches of the dinoflagellate lineage. *Int. J. Syst. Evol. Microbiol.* **53**: 355–65.

Saldarriaga, J. F., Taylor, F. J. R., Cavalier-Smith, T., Menden-Deuer, S. and Keeling, P. J. 2004. Molecular data and the evolutionary history of dinoflagellates. *Eur. J. Protistol.* **40**: 85–111.

Saldarriaga, J. F., Taylor, F. J. R., Keeling, P. J. and Cavalier-Smith, T. 2001. Dinoflagellate nuclear SSU rRNA phylogeny suggests multiple plastid losses and replacements. *J. Mol. Evol.* **53**: 204–13.

Sarjeant, W. A. S. and Downie, C. 1966. The classification of dinoflagellate cysts above generic level. *Grana* **6**: 503–27.

Sarjeant, W. A. S. and Taylor, F. J. R. 1999. Dinoflagellates, fossil and modern: certain unresolved problems. *Grana* **38**: 186–92.

Saunders, G. W., Hill, D. R. A., Sexton, J. P. and Andersen, R. A. 1997. Small-subunit ribosomal RNA sequences from selected dinoflagellates: testing classical evolutionary hypotheses with molecular systematic methods. *Pl. Syst. Evol.* **11** (Suppl): 237–59.

Schnepf, E. and Elbrächter, M. 1999. Dinophyte plastids and phylogeny: a review. *Grana* **38**: 81–97.

Silberman, J. D., Collins, A. G., Gershwin, L.-A., Johnson, P. J. and Roger, A. J. 2004. Ellobiopsids of the genus *Thalassomyces* are alveolates. *J. Eukaryot. Microbiol.* **51**: 246–52.

Sogin, M. L. 1989. Evolution of eukaryotic microorganisms and their small subunit ribosomal RNA's. *Amer. Zool.* **29**: 487–99.

Soyer, M.-O. 1972. Les ultrastructures nucléaires de la noctiluque (dinoflagellé libre) au cours de la sporogenèse. *Chromosoma (Berlin)* **39**: 419–41.

Steidinger, K. A., Burkholder, J. M., Glasgow, H. B. *et al.* 1996. *Pfiesteria piscicida* General et sp. nov. (Pfiesteriaceae fam. nov.), a new toxic dinoflagellate with a complex life cycle and behaviour. *J. Phycol.* **32**: 157–64.

Stoecker, D. K. 1999. Mixotrophy among dinoflagellates. *J. Eukaryot. Microbiol.* **46**: 397–401.

Takayama, H. 1985. Apical grooves of dinoflagellates. *Bull. Plankton Soc. Japan* **32**: 129–40.

Takishita, K., Ishida, K. and Maruyama, T. 2003b. An enigmatic GAPDH gene in the symbiotic dinoflagellate genus *Symbiodinium and* its related species (the Order Suessiales): possible lateral gene transfer between two eukaryotic algae, dinoflagellate and euglenophyte. *Protist* **154**: 443–54.

Takishita, K., Ishikura, M., Koike, K. and Maruyama, T. 2003a. Comparison of phylogenies based on nuclear-encoded SSU rDNA and plastid-encoded *psbA* in the symbiotic dinoflagelate genus *Symbiodinium*. *Phycologia* **42**: 285–91.

Takishita, K. and Uchida, A. 1999. Molecular cloning and nucleotide sequence analysis of *psbA* from the dinoflagellates: origin of the dinoflagellate plastid. *Phycol. Res.* **47**: 207–16.

Taylor, F. J. R. 1976. Flagellate phylogeny: a study of conflicts. *J. Protozool.* **23**: 28–40.

Taylor, F. J. R. 1979. The toxigenic gonyaulacoid dinoflagellates. *In* Taylor, D. L. and Seliger, H. H. (Eds) *Toxic Dinoflagellate Blooms.* Elsevier North Holland, New York, pp. 47–56.

Taylor, F. J. R. 1980. On dinoflagellate evolution. *Biosystems* **13**: 65–108.

Taylor, F. J. R. 1982. Symbioses in marine microplankton. *Ann. Inst Oceanogr. Paris* **58**: 61–90.

Taylor, F. J. R. 1987a. General group characteristics, special features, short history of dinoflagellate study. *In* Taylor, F. J. R. (Ed.) *The Biology of Dinoflagellates. Botanical Monographs*, Vol. 21. Blackwell Scientific Publications, Oxford, pp. 1–23.

Taylor, F. J. R. 1987b. Dinoflagellate morphology. *In* Taylor, F. J. R. (Ed.) *The Biology of Dinoflagellates. Botanical Monographs*, Vol. 21. Blackwell Scientific Publications, Oxford, pp. 24–91.

Taylor, F. J. R. 1990. Symbiosis in marine protozoa. *In* Capriulo, G. M. (Ed.) *The Ecology of Marine Protozoa.* Oxford University Press, New York, pp. 323–40.

Taylor, F. J. R. 1999a. Ultrastructure as a control for protistan molecular phylogeny. *Am. Naturalist* **154** (Suppl): 127–136.

Taylor, F. J. R. 1999b. Charles Atwood Kofoid and his dinoflagellate tabulation system: an appraisal and evaluation of the phylogenetic value of tabulation. *Protist* **150**: 213–20.

Taylor, F. J. R. 1999c. Morphology (tabulation) and molecular evidence for dinoflagellate phylogeny reinforce each other. *J. Phycol.* **35**: 1–3.

Tengs, T., Dahlberg, O. J., Shalchian-Tabrizi, K. *et al.* 2000. Phylogenetic analyses indicate that the 19′ hexanoyloxyfucoxanthin-containing dinoflagellates have tertiary plastids of haptophyte origin. *Mol. Biol. Evol.* **17**: 718–29.

Van de Peer, Y. and De Wachter, R. 1997. Evolutionary relationships among the eukaryotic crown taxa taking into account site to site rate variation in 18S rRNA. *J. Mol. Evol.* **45**: 619–30.

Yoon, H. S., Hackett, J. D. and Bhattacharya, D. 2002. A single origin of the peridinin- and fucoxanthin-containing plastids in dinoflagellates through tertiary endosymbiosis. *PNAS* **99**: 11724–9.

Zhang, Z., Green, B. R. and Cavalier-Smith, T. 2000. Phylogeny of ultra-rapidly evolving dinoflagellate chloroplast genes: a possible common origin for sporozoan and dinoflagellate plastids. *J. Mol. Evol.* **51**: 26–40.