Dinoflagellate macroevolution: some considerations based on an integration of molecular, morphological and fossil evidence

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Abstract: Dinoflagellates have been regarded as bizarre products of evolution. They belong to one of the most strongly supported macrolineages among the protists, the superphylum/kingdom Alveolata, which contains three main phyla: the Dinoflagellata, the Apicomplexa and the Ciliata. These organisms all have cortical alveoli and micropores. Until the early 1990s, living and fossil dinoflagellates were classified separately and both relied almost exclusively on morphological characters. During the early 1990s, fossil and living taxa were brought together in a detailed morphological classification that emphasized tabulation. Since that time, molecular studies have supported many morphological groups, but have shown others to be paraphyletic. Our understanding of phylogenetic relationships within the dinoflagellates has changed as more taxa have been described and more genes have been analysed. Relationships among the lineages also vary widely with the algorithm used to analyse the data. A highly unusual and notable feature of dinoflagellates is the variety of plastid types that they have acquired by secondary and even tertiary symbiosis; indeed, they possess the most diverse array of plastids of any eukaryotic lineageand they are truly the kings of symbioses. Genome rearrangements have taken place as the plastids evolved. The genes that have moved to the nucleus in dinoflagellates with peridinin plastids are different from those moved in all other eukaryotes; moreover the few genes left behind in the peridinin plastid have become uniquely arranged into mini-circles. Where tertiary endosymbiosis has taken place, the plastid genome was rearranged again. Mitochondrial modifications in the dinoflagellates are also unique among the eukaryotes. While study of these factors remains critical in understanding dinoflagellate phylogeny, the fossil record continues to contribute by presenting morphologies that are unrepresented (or underrepresented) among extant taxa; such observations can suggest relationships to be tested by molecular analyses.

Dinoflagellates are protists that typically possess a unique type of nucleus in which the chromosomes are permanently condensed and in which the motile cell typically possesses two distinctive, dissimilar flagella. Other aspects of dinoflagellates are also very unusual; for example, their metabolic processes rarely follow the norm and the origin of their plastids is enigmatic. Unlike many related protistan groups, dinoflagellates have left a significant (albeit incomplete) fossil record, mainly in the form of resistant organic-walled or calcareous resting cysts. In this review, we will discuss dinoflagellate origin and evolution as evidenced by both molecular methods and morphology, the latter including extensive observations from fossils. We will also review the relationship between dinoflagellates and other protists, and the evolution of dinoflagellate plastids and mitochondria.

Origin and relationships with other protists

Fensome *et al.* (1993) developed a phylogenetic tree for dinoflagellates and their closest allies based on ultrastructure (Fig. 1). It shows that dinoflagellates (dinokaryotes and syndinians) share the possession of cortical vesicles (alveolae) with apicomplexans and ciliates, a grouping of protists classified together as the alveolates (see also Adl *et al.* 2005). According to this tree, alveolates in turn share the possession of tubular mitochondrial cristae, an intranuclear spindle (which in dinoflagellates purportedly became secondarily extranuclear) and rod trichocysts with chromophytes. At the base of the lineage to dinokaryotes (dinoflagellates 'proper') and their sister group, the syndinians,

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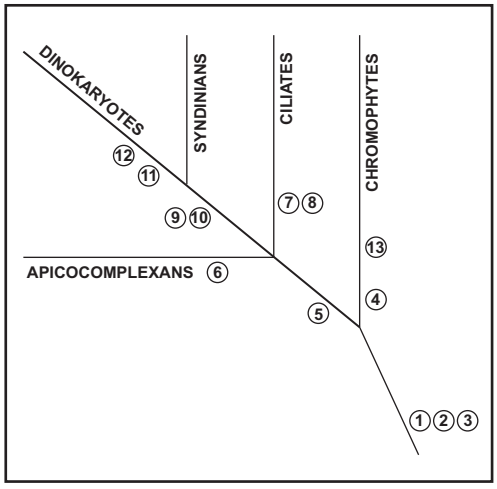


Fig. 1. A phylogenetic tree for dinoflagellates and their allies based on ultrastructure as proposed by Fensome *et al.* (1993). The length of the lines is not significant. Derived character states (indicated by numbers): 1, tubular mitochondrial cristae; 2, intranuclear spindle; 3, rod trichocysts; 4, compound flagellar hairs; 5, cortical (amphiesmal) vesicles (alveolae); 6, apical complex; 7, polykineties; 8, nuclear dimorphism; 9, donokont flagella; 10, extranuclear spindle; 11, dinokaryon (temporary); 12, dinokaryon (permanent); 13, dinokaryon (temporary); loss of trichocysts.

Fensome *et al.* (1993) noted the development of the characteristic dinoflagellate flagella and redevelopment of an extranuclear mitotic spindle. In the dinokaryote clade, Fensome *et al.* (1993) interpreted the development of the temporary dinokaryon (as in Noctilucales, which only have condensed chromosomes in the gamete stage, and Blastodiniales) as a basal trait, followed by the development of a permanent dinokaryon in all other dinoflagellates. Saunders *et al.* (1997, fig. 4) built upon this model by interpreting the peduncle mode of feeding (myzotosis) as a basal trait leading to free-living autotrophic dinoflagellates.

Subsequent molecular data confirm that the dinoflagellates are a monophyletic group, with the so-called pre-dinoflagellate *Oxyrrhis* lying outside core dinoflagellates. In the Fensome *et al.* (1993) tree, *Oxyrrhis* would diverge between ciliates and syndinians prior to development of dinokont flagella and an extranuclear spindle. Molecular studies have confirmed dinoflagellates as the sister group to the apicomplexans (perkinsid flagellates) with high bootstrap support, which are in turn sister to the ciliates, again with high bootstrap support (Leander & Keeling 2004). A less robust sister relationship (<50%) has been commonly recovered between alveolates (apicomplexans) and the stramenopiles (=heterokont organisms) (Leander & Keeling 2004), leading to a grouping of the alveolates with the cryptomonads and haptophytes to form the chromalveolates. The chromalveolates have the least bootstrap support and lack firm molecular evidence, but are nevertheless still thought to be monophyletic. Recent re-analysis of 108 genes from nuclear, plastid and mitochondrial genomes have failed to recover a well-supported host-cell lineage for the chromalveolates (Baurain *et al.* 2010).

A date for the divergence of stramenopiles (heterokonts) and alveolates at 950 Ma has been determined by molecular clocks using fossils other than dinoflagellates for calibration (Douzery *et al.* 2004). Using fossil dinoflagellates to calibrate the clock, Medlin (2008, 2011) dated the divergence of the dinoflagellates from the apicomplexans at 650 Ma. The presence of triaromatic dinosteroids, assumed to be derived from dinoflagellates, has been recorded in some acritarchs (organic-walled microfossils of uncertain origin on morphological grounds) of pre-Carboniferous age (Moldowan *et al.* 1996; Moldowan & Talyzina 1998). These biogeochemical markers could therefore reflect the existence of ancestral members of the dinoflagellate lineage. An alternative suggestion could be that at least some acritarchs represent one or more extinct lineages with no surviving descendants.

Although biogeochemical evidence of the existence of pre-Mesozoic dinoflagellates is compelling, it is not backed up by fossil morphological evidence either because pre-Mesozoic members of the dinoflagellates did not leave a preservable record, or because pre-Mesozoic dinoflagellate remains are not clearly recognizable as such. The few claims based on morphology for dinoflagellate affinity among pre-Mesozoic fossils have not been convincing (see Fensome *et al.* 2000). The most famous of these is the Silurian fossil *Arpylorus*, which Le Herisse *et al.* (2012) have convincingly shown not to be a dinoflagellate but part of a more complex biological structure possibly associated with eurypterids. One recent intriguing observation by Servais *et al.* (2009) was that some Ordovician calcareous microfossils display wall structures that are surprisingly similar to those of calcareous dinoflagellate cysts. All confirmed calcareous cysts are peridinioids, a group that on compelling evidence aside from crystal structure originated in the Jurassic. Speculation by Servais *et al.* (2009) that these fossils could represent the ancestors of dinoflagellates, if borne out, would therefore be a fundamental challenge to current ideas on the pattern and timing of dinoflagellate evolution.

Plastid evolution

A series of endosymbiotic events has given rise to all photosynthetic organisms. Initially a heterotrophic ancestor engulfed a cyanobacterium and transformed it into a plastid to form the first photosynthetic cell. This single primary endosymbiosis resulted in the red, green and glaucophyte algae (see reviews in Archibald & Keeling 2002 and Sanchez-Puerta & Delwiche 2008). Molecular analyses have shown that the host and the plastid lineages in this event are both monophyletic, so this event happened only once. Another heterotrophic organism then engulfed either a red or green alga from the first endosymbiosis to form the secondary event. If plastids in a particular alga are surrounded by two membranes, then the alga was derived from the primary endosymbiosis; if three to four membranes surround the plastid, then the alga is derived from a secondary endosymbiosis (Keeling 2004). The hypothesis that this secondary endosymbiosis also happened only once has been controversial (Yoon *et al.* 2002); the recent re-analysis of multiple genes from multiple genomes that failed to recover a monophyletic origin for all plastids from the secondary endosymbiosis (Baurain *et al.* 2010) has resurrected the hypothesis of multiple secondary endosymbiosis events for each plastid in the secondary endosymbiosis.

Recently, Moustafa *et al.* (2009) have uncovered unusual evidence from whole genome and expressed sequence tag (EST) analyses, which suggests that a sequence of unusual endosymbiotic events led to the chromalveolate lineage. Traces of green genes can be found in this lineage, which today is a red algal plastid lineage. This has been interpreted to mean that algae in the lineage first had green plastids, but this green plastid type was subsequently replaced by a red plastid. Falkowsky *et al.* (2004) hypothesized that red plastids had an adaptive advantage only after the end-Permian mass-extinction event at 251 Ma. At that time, oceans became anoxic and their trace-metal chemistry changed such that iron became abundant. The red plastid-bearing microalgae have iron-containing cytochrome c6 in their photosynthetic electron carrier complex instead of the copper-containing plastocyanin of the photosystem of green plastid-bearing algae.

If chromalveolates underwent multiple secondary endosymbioses (Baurain *et al.* 2010), then each lineage should have acquired a red algal plastid and dumped the green one at different times. Medlin (2011) constructed a molecular clock to determine if the radiations in the different chromalveolates lineages relate to the end-Permian event. She found that it corresponded to different taxonomic levels of radiation in the various lineages, and that this supported the idea that the green plastid was replaced by the red plastid multiple times.

Dinoflagellates have many different types of plastid. The predominant peridinin plastid is believed to have originated from a red algal secondary endosymbiosis. Supposedly, a tertiary endosymbiosis occurred in the dinoflagellates when the original plastid from the secondary endosymbiosis (this should be the peridinin plastid) was replaced by another plastid from the secondary endosymbiotic algae. Cryptophyte, prasinophyte and haptophyte plastids, which are each originally derived from a red algal secondary endosymbiosis, were therefore incorporated into the dinoflagellate lineages by various species (see Saldarriaga *et al.* 2001). However, the peridinin lineage does not appear independent in any of the plastid gene trees in dinoflagellates as it does in the heterokont, cryptophyte and haptophyte plastid lineages (Yoon *et al.* 2005; Verbruggen 2011; A. Moustafa, pers. comm., 2012). In contrast, the peridinin lineage is either (1) embedded in the heterokont lineage as sister to the diatoms (Yoon *et al.* 2005) or, with better taxon sampling, as sister to the chrysophytes/synurophyte lineage, which is sister to the diatoms (Verbruggen 2011); or (2) embedded in the green lineage. Evidence therefore suggests that all dinoflagellate plastids are the result of at least four tertiary endosymbioses (heterokont = diatom, cryptophyte, haptophyte and peridinin = heterokont chrysophytes). This means that in the dinoflagellates, there was no transformation of either a red or a green plastid into the peridinin plastids and all modern plastids in the dinoflagellates are the result of tertiary endosymbioses. Furthermore, it also suggests that it was the green plastid that was eliminated at the time of the four tertiary endosymbiosis. If the timing of the end-Permian event is placed over the dinoflagellate tree using a molecular clock, this extinction event corresponds to radiation at the generic level in the dinoflagellates, at the order and family level radiation in the haptophytes and at the phylum level radiation in the heterokonts (Medlin 2011). Generic level radiation of the extant dinoflagellates also is also supported by their early Mesozoic fossil record (Fensome *et al.* 1996). After the end-Permian mass extinction, the dinoflagellates likely evolved from a heterotrophic lineage to an autotrophic or mixotrophic lineage by only tertiary endosymbioses of several different algal groups replacing the original green plastid. However, it makes no evolutionary sense to have re-engulfed a green algal cell if the green algal plastid was a disadvantage at this time; it is more likely that the dinoflagellate/green lineage represents a relict green plastid that was originally present in the entire alveolate lineage. There is evidence that the last common ancestor of dinoflagellates, apicomplexans and ciliates likely

had a green plastid, because there are traces of green plastid genes in all of the host lineages (Takishita *et al.* 2003; Hackett *et al.* 2004; Patron *et al.* 2006; Moustafa *et al.* 2009). The common mode of feeding by a peduncle in the ancestral dinoflagellates would mean that the host cell membrane was left behind after the dinoflagellate finished sucking out its contents, which would result in typically only three membranes around the dinoflagellate plastid instead of the four membranes found around the plastid in the other algal groups involved in the secondary endosymbiosis.

The transformation of the heterokont plastid into the peridinin plastid after it was engulfed provides some of the most intriguing genomic rearrangement known in the eukaryotic world. Only about 12 genes coding for plastid function are left behind following the massive transfer of genes from the plastid into the nucleus. These 12 genes form mini-circles of genes of different sizes in different species of the same genus but the mini-circles from different species within a genus share nearly identical spacer regions between the genes (Zhang *et al.* 2002). This same massive gene transfer and gene rearrangement did not happen in the haptophyte and cryptophyte plastid-bearing dinoflagellates, which adds more evidence that the tertiary endosymbioses are independent events and that the red algal plastid in each of these lineages came from a different, independent secondary endosymbiosis. The genes encoded by the plastid possess another different feature. The normal poly A tail of the messenger ribosomal nucleic acids (mRNA) in the plastid is replaced by a poly T tail (Wang & Morse 2006), so when converting mRNA to complementary Deoxyribonucleic acid (cDNA) all plastid-encoding ESTs from dinoflagellates can be differentially separated from other plastids.

Evolution within the dinoflagellates: morphological background

Prior to the late 20th century, little effort was made to develop a view of dinoflagellate evolution that accommodated evidence from both fossils and modern forms. The first concerted attempt to relate the two realms was that of Evitt (1985), who made detailed and extensive comparisons of tabulation patterns reflected on fossil cysts with the tabulation patterns of living taxa. However, Evitt (1985) made little attempt to couch his comparisons in phylogenetic terms; that was left to Fensome *et al.* (1993) who, building on Evitt's work, produced the first modern phylogenetically based classification that incorporated both fossil and living dinoflagellates.

A key feature of dinoflagellate anatomy is tabulation. As alveolates all dinoflagellates have vesicles in the cortex, beneath the cell's outer cell membrane. In dinoflagellates, this cortical region is referred to as the amphiesma, and the vesicles are known as amphiesmal vesicles. Amphiesmal vesicles may remain empty or they may contain cellulosic plates, termed thecal plates. Collectively, thecal plates form a cell wall known as a theca. The arrangement of amphiesmal vesicles (with or without thecal plates) is called tabulation. (The term paratabulation is sometimes used to refer to 'reflected' tabulation on cysts.) Arrangement of the thecal plates varies to form tabulation patterns, many elements of which tend to be consistent and stable within related taxa or lineages. Tabulation and tabulation patterns are therefore important in unravelling phylogenetic relationships among many groups of dinoflagellates. Fensome *et al.* (1993) recognized six basic tabulation pattern types (Fig. 2):

(1) gymnodinioid, in which amphiesmal vesicles (usually without thecal plates) are numerous and arranged randomly or in more than ten latitudinal series
(2) suessioid, in which amphiesmal vesicles (usually with thecal plates) are arranged into seven to ten latitudinal series and a cingulum that tends not to be distinctly differentiated
(3) gonyaulacoid-peridinioid, in which amphiesmal vesicles (with thecal plates) are arranged in five or six latitudinal series, with a clearly differentiated cingulum
(4) dinophysioid, in which amphiesmal vesicles can be attributed to four latitudinal series with a distinctive cingulum, and in which a sagittal (longitudinal) suture, perpendicular to the plane of the cingulum, divides the theca into left and right halves
(5) nannoceratopsioid, in which the hyposome (that part of the cyst posterior to the cingulum) is 'dinophysioid' and the episome (anterior to the cingulum) is 'gonyaulacoid-peridinioid'
(6) prorocentroid, in which the vesicles are arranged into two very large plates separated by a sagittal suture and an area of small plates surround the flagellar apertures.

The earliest confirmed fossil dinoflagellates are from the (possibly) Middle–Late Triassic, 240–200 Ma. Distinctive among these were forms with a suessioid tabulation pattern, although a variety of tabulation patterns were represented by Late Triassic and Early Jurassic forms. From the Middle Jurassic, most fossil dinoflagellates have a clearly gonyaulacoid-peridinioid tabulation pattern. In an early attempt to make sense of the dinoflagellate fossil record, Bujak & Williams (1981) proposed three possible evolutionary models: the plate increase model, in which early tabulation involved

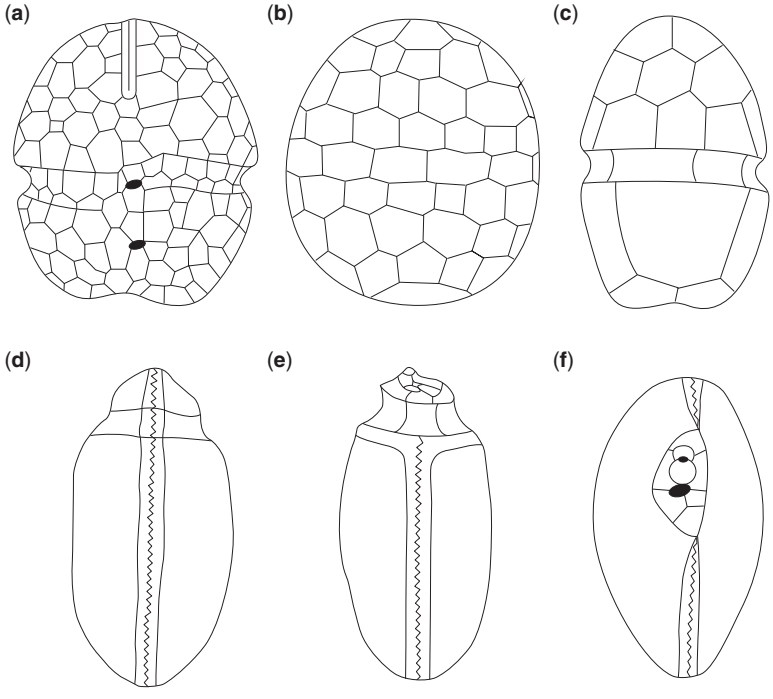


Fig. 2. Dinoflagellate tabulation types: (**a**) gymnodinioid, (**b**) suessioid, (**c**) gonyaulacoid-peridinioid, (**d**) dinophysioid, (**e**) nannoceratopsioid, (**f**) prorocentroid.

relatively few plates, the number of plates tending to increase with time; the plate reduction model with the reverse trend (a model which would make sense if forms such as *Suessia* were indeed early-derived); and the plate fragmentation model, which would involve early, perhaps prorocentroid-like, forms with few plates leading to later multi-plated forms. As has become apparent with hindsight and armed with molecular data, these models are too simplistic. They did provide a timely stimulus for tying fossils and living forms together from an evolutionary perspective, however.

A question among fossil dinoflagellate researchers in the latter decades of the 20th century was whether the appearance of dinoflagellates during the later Triassic–Early Jurassic reflected a real evolutionary event, or perhaps the 'switching on', of preservable cyst-forming capability in one or more lineages with a long pre-Mesozoic ancestry. Researchers at the time were concerned about the incompleteness of the fossil record and worried that this might hamper anything meaningful being determined from fossils about the evolution of the group (Evitt 1981). However, Fensome *et al.* (1996) reasoned that the early Mesozoic record of dinoflagellates bore clear hallmarks of a true evolutionary radiation, with highly variable 'experimental' morphologies (at least as reflected by tabulation) over a few tens of millions of years during the later Triassic and Early Jurassic, before stabilization into a few 'standard' tabulation types from about 175 Ma to the present day (again in terms of mostly gonyaulacoid and peridinioid tabulation patterns). Although multi-plated *Suessia*, as well as forms such as *Rhaetogonyaulax* with a plate-rich gonyaulacoid-peridinioid tabulation, were prominent during the Late Triassic, *Valvaeodinium*, a gonyaulacoid-peridinioid with below-average plate numbers for the group, was also a member of the Late Triassic assemblage. Also present among Jurassic assemblages was *Nannoceratopsis*, a strange combination of dinophysioid and gonyaulacoid-peridinoid tabulation features (Piel & Evitt 1980).

In their intra-dinoflagellate tree, Fensome *et al.* (1993, p. 206; slightly modified as Fig. 1 here) placed the Noctilucales and Blastodiniales as basal groups because of their 'part-time' dinokaryon, with the Gymnodiniales basal to the remainder of the dinoflagellates, followed in order of derivation by the Suessiales, Gonyaulacales, Peridiniales, Dinophysiales and Prorocentrales. No major advances based on dinoflagellate morphology have been proposed since this scheme was introduced. Below we will explore how this scheme holds up against subsequently established molecular trees.

Evolution of the dinoflagellates: molecular results

Unfortunately, most molecular trees for dinoflagellates have concentrated on closely related genera or groups of species rather than the entire range of the group. An exception was the tree produced by Saldarriaga *et al.* (2004). Using ciliates as the outgroup, parasitic and atypical taxa such as *Amoebophrya* and *Cryptosporidium* diverge early, as predicted by their morphological features, before the core dinoflagellates radiate. In this radiation, gymnodinioid clades (in which all taxa are athecate or naked) alternate in divergence with clades that contain peridinioid taxa. Although most thecate genera were well supported and monophyletic in the various peridinioid clades, *Gymnodinium* was paraphyletic and relationships between the gymnodinioid clades were not supported. The Gonyaulacales were in the final divergence and the Prorocentrales were paraphyletic.

A maximum likelihood analysis of 1246 dinoflagellate 18S rRNA gene sequences has not improved the resolution but has added a few new surprises (Fig. 3). The core dinoflagellates diverge simultaneously into four major clades. The first major clade contains a mixture of gymnodinioid and peridinioid taxa; *Amphidinium* commonly occurs as a basal divergence in a peridinioid clade; prorocentroids are split into benthic and planktonic clades not too distantly related; and dinophysioids are a basal not derived divergence. The second major but smaller clade is a gymnodinioid clade. The third major clade also contains a mixture of peridinioid and gymnodinioid taxa; surprisingly, the Noctilucales are embedded in this clade, as also seen by Hoppenrath & Leander (2010) in their heat shock protein tree, as are the Blastodiniales. Gonyaulacoids are a final divergence in this clade. The fourth major clade is primarily composed of naked forms, with the extant suessioids as a final divergence. Most clades consist of either thecate or non-thecate forms, the exception being in the first major clade, in which *Amphidinium* spp. are at the base of some peridinioid lineages. Basically, this tree shows that the taxa conventionally attributed to the Gymnodiniales and Peridiniales have evolved multiple times and only a few conventional orders, such as the Gonyaulacales, are monophyletic.

More recent trees with more genes show a similar pattern of divergences from the ciliates to the core dinoflagellates along with other significant relationships. Using three genes and a reduced taxon sampling, Zhang *et al.* (2007) found that *Amphidinium* was at the base of the entire dinoflagellate lineage. In the tree of Zhang *et al.* (2007), the core dinoflagellates diverge into two major well-supported clades: an endosymbiotic clade and a free-living clade in which the gonyaulacoids are monophyletic with one exception. Zhang *et al.* (2007) contended that the prorocentroids are a monophyletic group, as also recovered by Hoppenrath & Leander (2010) using the heat-shock protein gene.

Toward a reconciliation of morphological and molecular approaches

We can now test the tree proposed by Fensome *et al.* (1993) (slightly modified as in Fig. 4) against molecular results. Fensome *et al.* (1993) considered a temporary dinokaryon (a dinoflagellate-style nucleus that occurs only in part of the life cycle) to be a primitive feature and so placed the Noctilucales and Blastodiniales at the base of the tree, derived early from the core dinoflagellates. This prediction is supported by the Saldarriaga *et al.* (2004) tree but it is not supported in the tree in Figure 3 or in the heat-shock protein tree by Hoppenrath & Leander (2010). This suggests that the temporary dinokaryon is not (consistently) a symplesiomorphic feature but can be re-instated from time to time among different lineages.

The most recent molecular trees suggests that the athecate (naked, unarmoured) condition traditionally grouped as the Gymnodiniales is paraphyletic, indicating that loss and (re)gain of thecal plates has happened repeatedly throughout dinoflagellate evolution. This makes sense when considering that the athecate state is not the equivalent of gymnodinioid tabulation pattern, and presumably gymnodinioids in the sense of athecate forms may arise in lineages with non-gymnodinioid tabulation patterns.

Molecular trees (Saldarriaga *et al.* 2004; Zhang *et al.* 2007, fig. 3) show that modern dinoflagellates attributed to the Suessiales (primarily *Symbiodinium*) appear to be derived, suggesting that the modern forms are not closely related to the Triassic fossil taxon *Suessia*. This is perhaps no surprise as Fensome *et al.* (1993) based the grouping of *Symbiodinium* with fossils, such as *Suessia*, generally on a particular range in the number of plate series and not on tabulation details. However, the indication that *Symbiodinium* and *Suessia* are not closely related does make the suggestion that *Suessia* was in some way related to the rise of scleractinian corals through symbiosis less compelling, even though the first appearance of *Suessia* broadly coincides with the time of origin of the modern corals in the Triassic.

The separation of gonyaulacoids and peridinioids as a fundamental split has long been recognized among palaeontologists. The molecular

results (Saldarriaga *et al.* 2004; Zhang *et al.* 2007, Fig. 3) support a strong gonyaulacoid clade but indicate that modern peridinioids are paraphyletic. In the tree published by Saldarriaga *et al.* (2004, fig. 1), within the gonyaulacoids, goniodomoids and ceratioids fall out very clearly as separate clades. The goniodomoids are represented by the clade at the top of the diagram that connects species of *Pyrocystis*, *Fragilidinium*, *Alexandrium* and *Pyrodinium*. Below these in the same tree, species of *Ceratium*, the modern representative of an important and coherent fossil-rich family, fall neatly into a single lineage. Below these, with common derivation, are clades containing other forms that would generally be considered gonyaulacacean on the basis of morphology occur on the remaining major gonyaulacoid clade. The molecular evidence thus accords well with a gonyaulacoid lineage that, based on fossils, has been separate for the past 175–180 Ma. Interestingly, Saldarriaga *et al.* (2004) indicated that part of the grouping that Fensome *et al.* (1993) had assembled as the Phytodiniales is molecularly associated with *Gonyaulax spinifera*, the type of *Gonyaulax*. Other forms considered by Fensome *et al.* (1993) to be phytodinialeans fell out in very different lineages. Like the presence of an athecate amphiesmal and a part-time nucleus, the inclusion of a prominent coccoid life-cycle stage is therefore a feature developed or retained by several separate lineages, not a synapomorphy that can be used to define high-level taxa.

In all trees so far published (e.g. Saldarriaga *et al.* 2004; Zhang *et al.* 2007, Fig. 3) peridinioids resolve into several clades and thus the morphologically based Peridiniales is shown to be polyphyletic. This is surprising from a palaeontological perspective because the tabulation among fossil peridinioids is so consistent and apparently stable, especially from the Late Jurassic to the Miocene, far more so than the gonyaulacoid fossil record. To understand this discordance between the fossil and molecular evidence, it may help to delve a little deeper into the nature of the former for peridinioids. Fensome *et al.* (1993) designated a 'standard' peridinioid tabulation involving 4 apical plates, 3 anterior intercalaries symmetrically arranged on the dorsal surface, 7 precingulars, 5 postcingulars and 2 symmetrically disposed antapicals. Especially characteristic and commonly recognizable through archeopyle formation, even if the rest of the tabulation is not reflected, is the mid-dorsal second anterior intercalary plate (2a), which is most often 6-sided. These tabulation characteristics are present in the vast majority of peridinioid genera including calcareous forms from the Middle Jurassic to the Palaeogene, and the stability of this tabulation pattern despite other types of morphological variation (overall shape, cyst-wall composition, archeopyle details) strongly suggests a single clade, even though this can never be confirmed by molecular evidence.

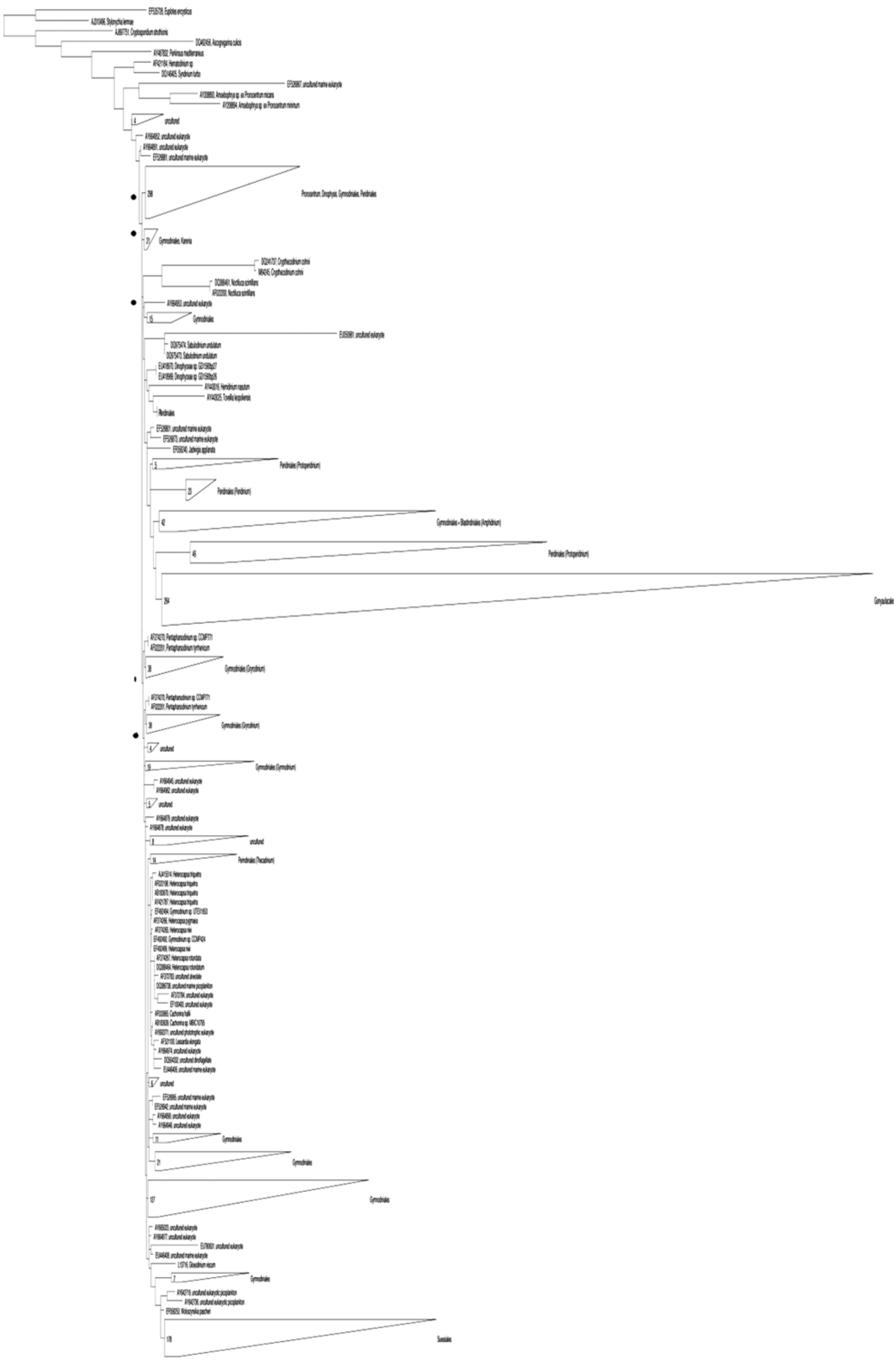
From primarily fossil evidence, Fensome *et al.* (1993) (and palaeontologists in general over recent decades) recognized a protoperidinioid lineage separate from the peridinioid mainstream from the Late Cretaceous. Partly recognized by cyst-wall characteristics and by a distinctive cingular tabulation evidenced by modern forms (cingular details are almost always not discernible on fossils), fossil and modern protoperidinioids are characterized by a variation in episomal tabulation, especially dorsally; this is in contrast to the stability of this feature among fossil peridinioids. As illustrated by the molecular tree published by Saldarriaga *et al.* (2004; as also represented by unlabelled clade 5 in Fig. 3), it is not surprising that protoperidinioids resolve into a clade separate from other peridinioids.

What is unexpected from a palaeontological perspective is that peridiniaceans (*sensu stricto*, thus excluding protoperidiniaceans) resolve onto a number of clades, some quite distant from others. The youngest-known fossil organic-walled peridiniacean, *Palaeocystodinium*, made its last appearance in the Miocene. Perhaps, non-calcareous fossils with a standard peridinioid tabulation can be considered part of a clade, the last representative of which became extinct in the Miocene. The stable tabulation of fossil peridiniaceans is most like modern *Peridinium bipes*; indeed the symmetrical dorsal episomal tabulation of fossil peridinioids is sometimes referred to as 'bipesioid'. Perhaps, the fossil peridiniacean clade is related to Peridinium bipes. However, fossil 'bipesioids' (peridiniaceans) are all marine, like modern protoperidiniaceans, whereas the modern peridiniaceans in the clade containing *Peridinium bipes* are all non-marine.

Because the Early Jurassic peridinioids and a few later ones such as Cretaceous *Angustidinium* had five apical plates (in contrast to four in the vast majority of later fossil peridinioids; see Below 1987), Fensome *et al.* (1993) placed these forms with modern *Heterocapsa* in the suborder Heterocapsineae. From the molecular trees of Saldarriaga *et al.* (2004) and Figure 3, *Heterocapsa* appears to be a relatively derived peridinioid. The presence of five apical plates in early peridinioids and *Heterocapsa* is a very broad similarity, but in detail the apical thecal plates of the two entities are not closely similar in arrangement, and the similarity in apical plate number most probably represents convergence. Early fossil forms with five apical plates are therefore probably not related to *Heterocapsa*, but may well represent a group ancestral to other Jurassic–Miocene peridinioids.

Among dinoflagellates with calcareous life-cycle stages (primarily if not always cysts),

(a)


Prorocentrum, Dinophysis, Gymnodiniales, Peridiniales
Gymnodiniales, Karenia
Gymnodiniales
Peridiniales
Peridiniales (Protoperidinium)
Peridiniales (Peridinium)
Gymnodiniales + Blastodiniales (Amphidinium)
Peridiniales (Protoperidinium)
Gonyaulacales
Gymnodiniales (Gyrodinium)
Gymnodiniales (Gyrodinium)
uncultured
Gymnodiniales (Gymnodinium)
uncultured
uncultured
Peridiniales (Thecadinium)
uncultured
Gymnodiniales
Gymnodiniales
Gymnodiniales
Gymnodiniales
Suessiales

(**b**)

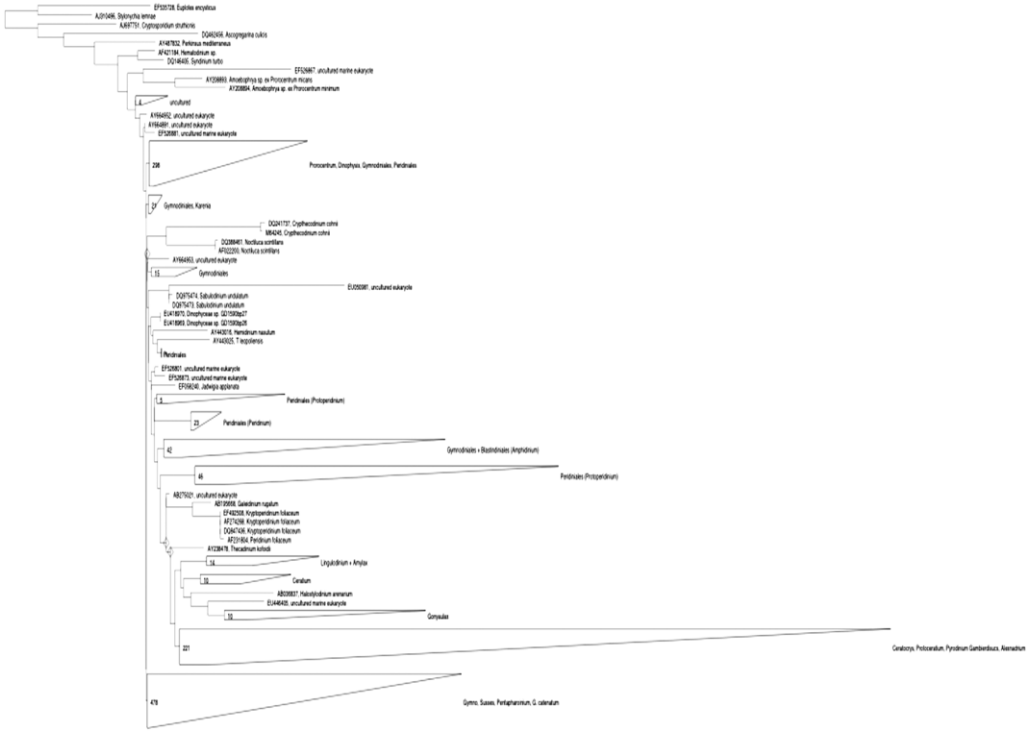


Fig. 3. (**a**) Maximum likelihood analysis of 1296 SSU rRNA sequences currently held in the ARB database and alignment by secondary structure. Black dots indicate the simultaneous divergence of four clades. (**b**) The opening of the gonyaulacoid clade from A.

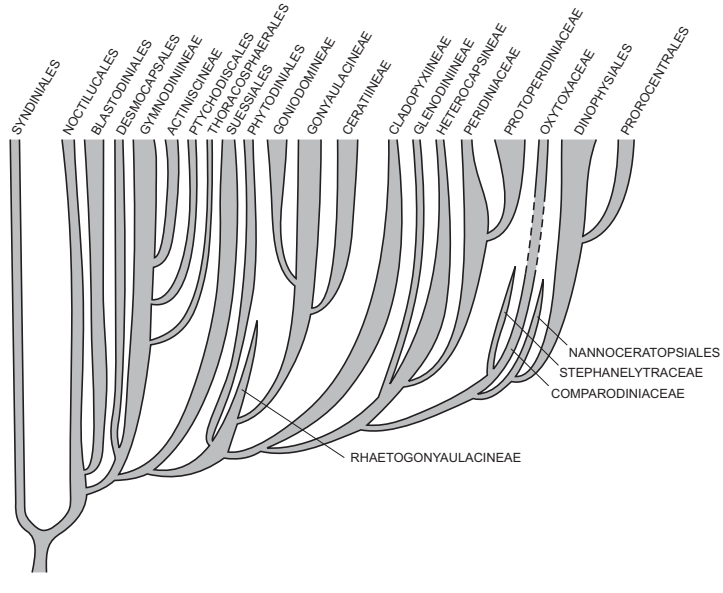


Fig. 4. Slightly modified phylogenetric tree with principle dinoflagellate taxa, originally developed by Fensome *et al.* (1993).

Fensome *et al.* classified the Calciodinelloideae as a subfamily of the Peridiniaceae and treated *Thoracosphaera* (which lacks indications of tabulation and had been interpreted not to be a cyst) in its own order, the Thoracosphaerales. The tree published by Saldarriaga *et al.* (2004) clearly associated *Thoracosphaera* with *Scrippsiella*, which is a modern representative of the calciodinelloids; the separation of *Thoracosphaera* from other dinoflagellates with calcareous life-cycle stage is therefore not upheld. However, neither is a close relationship between calciodinelloids and *Peridinium bipes*. The tabulation of the calciodinelloids, where it can clearly be seen on fossil cysts, is consistently the 'standard peridiniacean pattern' and thus conforms with that of most Jurassic–Miocene organic-walled cysts. Other than cyst-wall composition, the most significant difference between fossil peridiniaceans with organic-walled cysts and those with calcareous cysts (calciodinellods) is the archeopyle type. In organic-walled forms, the archeopyle in almost all genera is focused on the second anterior intercalary plate; in calcareous forms it is generally apical however, a fundamental difference going back into the Mesozoic.

One aspect of the fossil record is that it provides unique evidence for whole groups of taxa not represented today and of which we have no other record. *Nannoceratopsis*, cited above, is an excellent example; the basal position of the dinophysioids in Figure 3 seems in accordance with the early presence of *Nannoceratopsis* in the fossil record. Another exclusive fossil lineage is represented by the wetzelielloids, a group of peridinioids with an episomal tabulation that is basically bipesioid, but with a four-sided rather than a five-sided 2a plate. This may seem a subtle difference, but wetzelielloids were a highly distinctive and prominent group of Palaeogene dinoflagellates. They appear to have left no descendants, despite early and now-disproven claims that they were protoperidinioids. Wetzelielloids appear to have had a peridiniacean cingular tabulation and a tabulation overall that was symmetrical and stable, like that of other fossil peridiniaceans but in contrast to the variable and commonly asymmetrical protoperidiniacean tabulation.

The fossil record also reveals what can be thought of as 'iceberg' taxa, ones with far greater representation in the past that at the present time. An example is the cladopyxioid lineage, represented by just two or three modern genera (including *Cladopyxis*) but which has a significant, mainly Jurassic–Cretaceous, fossil record. Indeed, from tabulation details, the cladopyxoids appear to be transitional between gonyaulacoids and peridinioids, so a molecular analysis of a modern member of the lineage would be very interesting.

Mitochondrial evolution

The mitochondria of dinoflagellates are another example in which the group has taken a bizarre evolutionary pathway. Lukes *et al.* (2009) documented that within the alveolate lineage, ciliates have a normal-sized circular mitochondrial genome; apicomplexans and dinoflagellates however have a reduced genome with only three genes and, after transcription, the mRNAs can be modified to change the codons or the amino acid sequence into other proteins. It therefore does not matter that they have reduced their mitochondrial genome to only three genes because they have post-transcriptional modification of the mRNAs.

An unusual case of convergent evolution?

Although the dinoflagellates have evolved many unusual features, similar features also occur in the Euglenozoa (Lukes *et al.* 2009). Are such similarities the result of convergent evolution? Shared similarities include flagella with a paraflagellar rod; a large nucleolus and permanently condensed chromosomes, mucocysts or trichocysts ejected through pores; cell walls composed of 'proteinaceous/cellulosic' strips or plates; thylakoids with three lamellae; and chloroplast endoplasmic reticulum (CER) composed of three membranes. Another comparison is that the dinoflagellate mitochondrion has only three genes and euglenoid mitochondria have mini-circles with three genes. It is tempting to suggest that the reduction of the mitochondrial genome to only three genes is the next evolutionary step before the formation of the mini-circles. Both dinoflagellates and euglenoids have post-transcriptional editing of their mitochondrial mRNA. The unique splice leaders to the mRNA are conserved at the class level in dinoflagellates and at the species level in euglenids. Both groups have mRNAs transcribed with multiple genes. In the dinoflagellates they are tandem repeats of the same gene, but these are different genes in the euglenophytes. Each of these strange features likely conveys some evolutionary advantage in these two very different lineages of eukaryotic microalgae.

It would therefore seem that, although the dinoflagellates exhibit some rare and unusual cellular features, they are not alone in having these features and their bizarre evolutionary pathways are not as unique as once believed.

Conclusions

As with the two 'solitudes' of fossil and extant dinoflagellate studies until the 1980s and 1990s, the morphological (primarily fossil) and molecular

approaches to dinoflagellate evolution have, until now, largely developed separately in recent years. Our attempt here to bring the two approaches together will, we hope, pave the way for greater collaboration in the quest to understand dinoflagellate macroevolution.

Current combined evidence suggests that dinoflagellates separated from their apicomplexans cousins some 650 Ma. Although they did not leave a convincing morphological record until the Mesozoic, biogeochemical evidence provides some evidence for the group during the Palaeozoic. Evidence from molecular analyses and plastids supports the fossil evidence for an early Mesozoic radiation of dinoflagellates, during which it seems that the morphology of the group as we know it today, with cingulum, sulcus and characteristic flagella and tabulation patterns, first appeared. Some lineages reflected by the fossil record, such as ceratioid gonyaualacaleans and protoperidiniod peridinialeans, are strongly supported by molecular evidence. Other relationships, such as that of the Triassic fossil *Suessia* with modern *Symbiodinium*, appear to be unfounded by molecular data. Molecular evidence is clearly our strongest indication for phylogenetic relationships, but any overall evolutionary tree must accommodate morphologies represented only by fossils.

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