Evolution of the Dinoflagellates: from the origin of the group to their genes

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Abstract

Dinoflagellates have often been regarded as bizarre examples of evolution. They belong to one of the most strongly supported macrolineages among the protists: the superphylum/kingdom Alveolata containing three main phyla: the Dinoflagellata, Apicomplexa and Ciliata. These organisms share the features of cortical alveoli and micropores. Until the early 1990s, dinoflagellate classification of both living and fossil taxa relied almost exclusively on morphological characters, such as plate tabulation. Molecular data have supported many morphological groups, whereas others have been shown to be paraphyletic. Phylogenetic relationships within the dinoflagellates have changed as more taxa and more genes have been added. There is also much variation with the algorithms used to analyse the data. Dinoflagellates have become notorious for the variety of plastid types that they have acquired by secondary and tertiary symbiosis. They possess the most diverse array of plastids of any eukaryotic lineage and are truly the kings of symbioses. Genome rearrangements have taken place as the plastid has evolved. The genes that have been moved to the nucleus in the peridinin plastid dinoflagellates is different from all other eukaryotes, as well as the genes left in the plastid, being arranged in mini circles. As tertiary endosymbiosis has taken place, the plastid genome has become rearranged. At the species level, some, but not many, cryptic species have been uncovered. However, below the species level, microsatellites have shown immense spatial fragmentation in dinoflagellate populations. EST libraries have been constructed for several species. Initial annotation results indicated that a low percentage of the genes could be annotated. Recent advances have pushed this level to nearly 29% of the ESTs. Gene expression has been studied in relationship to stress conditions for several species. Some common responses to stress conditions have been noted. Only in EST libraries that have been screened by 454 sequencing (Illumina) have toxin genes been identified. The complete genome of *Heterocapsa circularisquama* will be done within the year.

Introduction

Most scientists who work on dinoflagellates have said themselves or heard it said that dinoflagellates break all the rules. Certainly, there are many examples, where the dinoflagellate's way of doing certain metabolic processes, from organisation of their chromatin down to acquisition of plastids has not followed the norm. This has lead to some very remarkable features being discovered in the dinoflagellates, of which I will try to review some of the more interesting points here.

Origin of the group. The dinoflagellates as a group are monophyletic with *Oxyrrhis* (pre-dinoflagellate) lying outside the core dinoflagellates and are sister to the perkinsid flagellates (Apicomplexa) with high bootstrap support to form a clade that is sister to the ciliates, again with high bootstrap support (Leander and Keeling 2004). This forms a group, termed the Alveolata, which is recognised at the superphylum/kindgom level (Adl et al. 2005) and defined morphologically by the series of cortical membranes beneath the plasmalemma and tubular cristae. A less robust sister relationship (<50%) is recovered in most trees with the stramenopiles or heterokont organisms (Leander and Keeling, 2004), which together with the cryptomonads and haptophytes form the chromalveolates. Recent re-analysis of 108 genes from the nuclear, plastid and mitochondrial genomes have failed to recover a well supported host cell lineage for the chromalveolates, which has resurrected the hypothesis of multiple secondary endosymbiosis for the chromalveolates (Baurain et al. 2010). Molecular clocks have used other eukaryotic fossil dates to date the divergence between the stramenopiles and the alveolates at 950 MA (Douzer al. 2004) and Medlin (2008) used the dinoflagellate fossil record to date the divergence of the dinoflagellates from the apicomplexans at 650MA. The presence of triaromatic dinosteroids in fossils of pre-Carboniferous age (Moldowan et al. (1996), which have been assumed to be dinoflagellates, were likely in the last common ancestor (LCA) of the apicomplexans and the

dinoflagellates and likely in the LCA of this clade and the ciliates.

Evolution within the dinoflagellates. Saunders et al. (1997) have reviewed the morphological steps that have evolved leading to a final radiation of the free-living autotrophic dinoflagellates (Fig 1). Peduncle feeding (myzotosis) evolved early in the dinoflagellates. Noctilucales are basal to the core dinoflagellates. Once the core dinoflagellates radiate, there are three different theories of evolution to explain the separation of thecate and non-thecate genera (Bujak and Williams 1971). These are the plate increase, the plate reduction and the plate fragmentation models, with the first and third models placing the Gymnodiniales in a derived position. The peridinioid taxa are in a different evolutionary position in each of the three models. It is interesting to assess new molecular trees in light of these evolutionary schemes based on morphology. However, there are few molecular trees that sample the entire range of the dinoflagellates, with most molecular trees concentrating on a group of species or closely related genera. Saldarriaga et al. (2004) have produced one of the earliest trees that sampled across most major core dinoflagellates. Using ciliates as an outgroup, the parasitic and atypical taxa diverge in exactly the sequence predicted by their morphological features until the divergence of the core dinoflagellates. After that clades of gymnodinioid dinoflagellates alter in divergence with clades of peridinioid dinoflagellates. Each clade consists of monophyletic well-supported genera, with the exception of *Gymnodinium*, which is paraphyletic. However relationships between the clades are not supported. There is a final divergence of the Gonyaulacales, but the Prorocentrales are paraphyletic; thus no real support for any of the morphological models. Adding more taxa to the tree has not really improved the situation but has added a few new surprises. Fig.2 shows a tree of 1246 dinoflagellates from a maximum likelihood analysis of the 18S rRNA gene. Divergences from the ciliates to the core dinoflagellates follow a similar pattern as seen in the other trees. The core dinoflagellates diverge simultaneously into four major clades. The first major clade contains a mixture of gymnodinioid and peridinioid taxa with *Amphidinium* often occuring as a basal divergence in a peridinioid clade. Dinophysiales are a basal divergence and the Prorocentrales are split into benthic and planktonic clades not too distantly related. The second major but smaller clade is a gymnodinioid clade. The third major clade contains a mixture of gymnodinioid and peridinioid taxa. However, Noctilucales are embedded in this clade, a position also recovered in the heat shock protein tree by Hoppenrath and Leander (2010). Gonyaulacales are a final divergence. The fourth major clade is primarily a naked clade with the Suessiales as a final divergence. However, there is one smaller clade in which there is a mixture of thecate and non-thecate taxa in the same clade. This is an unusual feature in this tree as most clades are either one or the other, the only exception being the basal position of *Amphidinium* spp. at the base of some of the peridinioid clades in the first major clade. Zhang et al. (2007a) used three genes with a reduced taxon sampling and recovered other significant relationships. With a three gene concatenated data set, *Amphidinium* was at the base of the lineage. The major well-supported divergence in this tree was between endosymbiotic taxa and free-living taxa with the Gonyaulacales being monophyletic with one exception. Prorocentrales were now a monophyletic group, which was also recovered in the heat shock protein tree of Hoppenrath and Leander (2010).

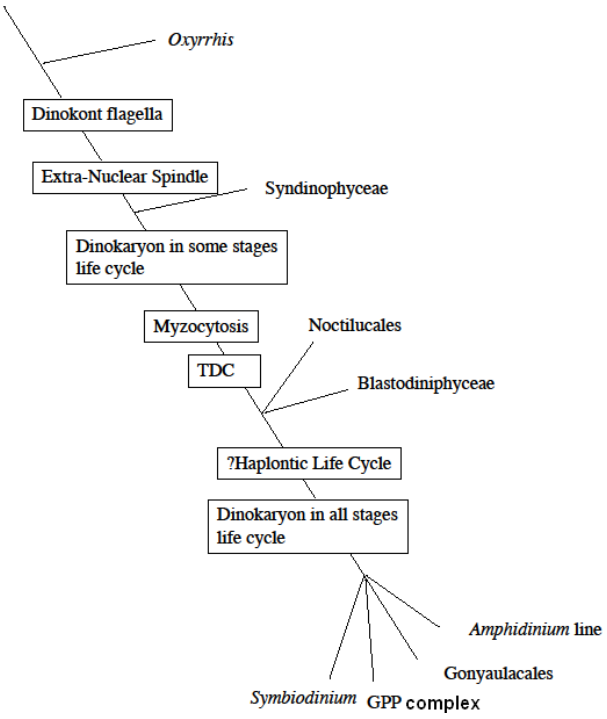

Figure 1. Scheme of the morphological steps in the evolution of the dinoflagellates

Evolution at the species level (cryptic diversity). Molecular analyses have wreaked havoc at the

species level in nearly all groups and the dinoflagellates are no exception. As expected in the non-thecate taxa, relationships are difficult to ascertain morphologically and many cryptic species have been discovered molecularly. The best example can be found in the Suessiales where 8 clades of symbiotic dinoflagellates have been recovered (Pouchon et al. 2006) where originally only one species had been described from corals. The *Alexandrium tamarense/fundyense/catenella* species complex has shown that these taxa are related by their geographic origin and not by their morphological features (Scholin et al. 1994, John et al. 2003). Using a molecular clock John et al. (2003) proposed a scenario for the historical biogeographic history of this complex from a single global ancestor. The later divergences of the non-toxic Western European clade from the toxic Temperate Asian could be dated to the closing of the Tethys Sea, which likely explains presence of relict cells of this clade in the Mediterranean. The separation of the non-toxic Mediterranean Clade from the toxic North American clade was dated to the rising of the Isthmus of Panama. With time, the Mediterranean clade went extinct in the western tropical Atlantic waters and it thrives today in the Mediterranean where it is now endemic.

Cryptic diversity at the species level is usually recovered first with sequence data from ribosomal genes and then reconfirmed with data from other genes. Piganeau et al. (2011) have analyzed the rate of evolution in protein coding genes v. 18S ribosomal genes using whole genomes. They found that the rate of evolution between them is different among unicellular organisms and multicellular organisms. In unicells, the rate of evolution in the 18S rRNA gene is ca. 5x faster than the rate of evolution in the protein genes as compared to multicellular organisms. They attribute this to differences in their effective population sizes with the smaller population sizes having a faster rate. If this is the case, then in the dinoflagellates, among the Suessiales are two different endosymbiotic lineages with two different rates of evolution (Shaked and de Vargas 2006) occur in two lineages with different effective population sizes. The endosymbionts within planktonic forams have an effective larger population size and therefore evolve much slower than the endosymbionts within the corals with a very much smaller effective population size with some lineages being endemic to a single coral head. Piganeau et al. (2011) feel that a single base difference in the 18S rRNA gene denotes a different species.

Plastid Evolution. Photosynthetic organisms have arisen through a series of endosymbiotic events. The primary endosymbiosis arose from a heterotrophic ancestor which engulfed and transformed a cyanobacterium into a plastid. This resulted in the red, green and glaucophyte algae (see reviews in Archibald and Keeling 2002, Sanchez-Puerta and Delwich 2008). These host and plastid lineages are both monophyletic supporting that this event happened only once. A secondary event took place between another heterotrophic organism and either a red or green alga from the first endosymbiosis. Counting the number of membranes around a plastid can determine whether the alga was derived from a primary endosymbiosis (2 membranes) or a secondary endosymbiosis (3-4 membranes). Recent evidence from whole genome and EST analyses has uncovered an unusual sequence of events leading to the chromalveolates lineages (Moustafa et al. 2008). Traces of green genes can be found in this lineage, which, today, is a red algal plastid lineage. Current opinion is that there was originally a green gene in the lineage, which was replaced by a red one. Falkowski et al. (2004 have hypothesized that the red algal plastid only had an adaptive advantage after the Permium Triassic extinction when the ocean became anoxic and trace metal chemistry changed and Fe is abundant and needed for cytochrome 6 in the plastid but Zn and Cu are depleted, which is needed for plastocyanin in the green plastid. Medlin (2011) constructed a molecular clock to determine if the radiations in the different chromalveolates lineages corresponded to 250 MA, the timing of the P/T extinction to provide supporting evidence that the green plastid was replaced by the red one and found that the P/T boundary corresponded to different taxonomic levels of radiation in the various lineages. If it is correct that there were multiple secondary endosymbioses (Baurain et al. 2010), then each lineage could have acquired a red algal plastid at a different time and dumped the green one at different time points in their radiation. In the dinoflagellates, there are several different types of plastids: the predominate peridinin plastid, which is believed to have been derived from the red algal secondary endosymbiosis and cryptophyte, prasinophyte and haptophyte plastids derived from a so-called tertiary endosymbiosis when the peridinin plastid was replaced by another from

either of these three lineages (see review in Saldarriaga et al. 2001). The perplexing observation about the peridinin plastid is that none of the plastid gene trees show the peridinin lineage as an independent lineage as are the stramenopiles, cryptophytes and haptophytes (Yoon et al. 2005, Verbruggen 2011, Moustafa, pers.comm.); instead the peridinin lineage is embedded in the stramenopiles lineage either sister to the diatoms (Yoon et al. 2005) or with better taxon sampling sister to the chrysophytes /synurophyte lineage, which is sister to the diatoms (Vergegroen 2011) or its closest sister in the green lineage. This implies that the dinoflagellates did not have a red plastid that was transformed into a peridinin plastid and that all of the plastids in the dinoflagellates are tertiary plastids and it was the green plastid that was eliminated at time of the four tertiary endosymbiosis. This hypothesis fits using a molecular clock to place the timing of the P/T extension over the dinoflagellate tree, which corresponds to generic level radiation in the dinoflagellates and to phylum level radiation in the stramenopiles (Medlin 2011). Generic level radiation of the extant dinoflagellates also occurred after this time according to their fossil record (Fensome et al. 1997). After the P/T extinction, the dinoflagellates likely underwent several tertiary endosymbioses of several different algal groups to evolve from a heterotrophic lineage to an autotrophic or mixotrophic lineage. One puzzling point is why they would have re-engulfed a prasinophyte algae if the green algal plastid was a disadvantage at this time. It is more parsimonious to predict that the dinoflagellate/ prasinophyte lineage is a relict of the green plastid that was originally present in all the alveolate lineage because there is evidence that the LCA of the dinoflagellates, apicomplexans and ciliates likely had a green plastid, because there are traces of green genes in all of these host lineages (Takishita et al. 2003, Patron et al. 2006, Hackett et al. 2004, Moustafa et al. 2008). The presence of only 3 membranes around the dinoflagellate plastid is likely the results of peduncle feeding where the host cell membrane was left behind after the dinoflagellate finished sucking out its contents.

Still even more interesting is the transformation of the heterokont plastid into the peridinin plastid after it was engulfed. There is a massive transfer of genes from the plastid into the nucleus, leaving behind about 12 genes coding only for plastid function, which form minicircles of genes of different sizes in different species of the same genus but sharing nearly identical spacer regions between the genes within a genus (Zhang et al. 2002). This did not happen in the haptophyte and cryptophyte plastid bearing dinoflagellates, adding further evidence that the tertiary endosymbioses are independent events and that the red algal plastid in each of these lineages resulted from different, independent secondary endosymbioses. The genes encoded by the plastid possess another different feature. When their rRNAs are converted to cDNA, the normal poly A tail is replaced by a poly T tail (Wang and Morris 2006), so plastid-encoding ESTs from dinoflagellates can be differentially separated from other plastids.

Mitochondrial Evolution. Within the alveolate lineage, the ciliates have a normal sized circular genome, both the apicomplexans and the dinoflagellates have a reduced genome with only three genes and after transcription the mRNAs can be modified to change the coding region into another protein (Lukes et al. 2009).

Table 1. Summary of microsatellite studies on dinoflagellates

Species	Source
Alexandrium tamarense/ fundyense/catenella NA	Nagai et al. 2004, Alpermann 2009
Alexandrium tamarense/ fundyense/catenella TA	Nagai et al. 2004, Nishitani et al. 2007a
Alexandrium minutum	Nagai et al. 2006
Cochlodinium polykrikoides	Nishitani et al. 2007b
Heterocapsa circularisquama	Nagai et al. 2007
Symbiodinium sp. Clades B & C	Bay et al. 2009, Santos & Coffroth 2003
Karenia brevis	Renshaw et al. 2006
Lingulodinium polyhedrum	Frommlet & Iglesias-Rodriguez 2

Evolution below the Species Level (Genetic Diversity). Diversity below the species level is most robustly measured by fingerprinting methods of which microsatellites are the most computationally intensive. MS are short repeated sequences of 1-6 nucleotides in length, e.g., (GT)n. MS are codominantly inherited markers that provide very high levels of heterozygosity and the ability to measure gene flow from one population to another. Microsatellites have

been established in a number of dinoflagellates and each study has shown distinct population structure and reduced gene flow between close areas (Table 1). Population structure in two clades of *Symbiodinium* is different depending on the inheritance type of the endosymbiont (Thornhill et al.

2009). In clade B, which has horizontal inheritance (coral larvae take endosymbionts from the plankton), has host, within host and even reef endemism and strong population structure and are temporally stable. In contrast, Clade C with vertical inheritance (endosymbionts in the coral larvae) has no population structure. The endemism in Clade B could also be the result of the host coral expelling other genotypes to maintain a specific population and its density. Microsatellites have clarified the origin of the Temperate Asian of the *Alexandrium tamarense/fundyense/caten-ella* species complex. Lilly et al. (2002), comparing Mediterranean and Asian strains with LSU rRNA sequence data, concluded that the presence of TA strains in the Mediterranean was a clear case of ballast water introduction. Penna et al. (2005), using ITS sequence data concluded that it was likely a case of ballast water introduction. Masseret et al. (2009), using micro-satellites, showed the Mediterranean populations to be clearly distinct and distant from the Asian strains but could not offer any reasonable explanation for their occurrence in the Mediterranean. However, referring back to the historical biogeography of the species complex by John et al. (2003), the closing of the Tethys Sea, which is the vicariant event separating the West European Strains from the Temperate Asian strains, the presence of the TA genotypes in the Mediterranean is likely a relict population from when the two groups were once joined and their presence in the French Lagoons is likely caused by a change in environmental conditions that have caused them to bloom and be noticed. The first hierarchical study of genetic diversity has been conducted on populations of *Alexandrium tamarense* (NA clade) in the Orkney Islands, UK (Alpermann 2009). On a global scale, the Orkney Island populations are more closely related to Pacific Isolates from Japan than to populations on the east coast of North America, which suggests that these populations were introduced to the region by cells directly coming across the Arctic Ocean in contrast to the hypothesis put forward by Medlin et al. (1998) that these populations entered from the Pacific and moved along coastal pathways of the eastern side of North America until they reached the Gulf Stream, which carried them across to the Orkney Islands. Moving to the diversity of local populations, at one site in the Orkney Islands, four populations were discovered that could interbreed and these populations were determined to be different year classes that had hatched from local cyst beds.

Evolution of their genes and their transcription. The dinoflagellates have long been known to possess an unusual genome structure (Hackett & Bhattacharya 2006). The chromosomes are permanently condensed. The DNA content of the nucleus contains 2-200 pg/cell as compared to 0.5 pg/cell in other algae with many copies of each gene with long stretches of non-coding regions, which may not be so different in length to other organisms (Triplett et al. 1993).

Table 2. Summary of EST libraries made to date and the most interesting genes recovered.

Author	Species	EST % identified	Most interesting gene identified
John et al. 2005	*Alex. tamarense (NA)*	9% of 2500 ESTs	Polyketides genes
Lidie et al. 2005	*Karenia brevis*	29% of 7001 ESTs	Microarray generated SNPs in multiple gene copies
Hackett et al. 2005	*Alexandrium tamarense (NA)*	20% of 6723 ESTs	Histone genes
Yang et al. 2010	*Alexandrium minutum*	28% of 3000 ESTs	4 stress libraries, toxic and non-toxic strains express different genes
Toulza et al. 2010	*Alexandrium catenella (TA)*	24% of 21236 ESTs	Alveolin protein genes
Lin et al. 2010	3 SL environ mental libraries	27% of clones	Nucleosome histone core, modification and assembly genes, Rhodopsin

All of the copies of each gene are transcribed on a single mRNA. What is not known is if the long stretches of non-coding regions are in between each multiple copy of a gene or between clusters of multiple copies of a gene. If the former, then crossing over during meiosis will likely be more difficult as will the transcription of multiple genes into a single

mRNA. In contrast, the plastid and the mitochondrial genomes are dramatically reduced. These questions, among others, will likely be answered when the first genome of a dinoflagellate is completed. Its immense size has daunted many labs to avoid the dinoflagellates and even isolating a single chromosome for sequencing has resulted in so much non-coding data that contig overlaps were impossible (John pers. comm.). *Heterocapsa circularisquama* has been targeted for genome sequencing by the Bhattacharya lab at Rutgers University USA primarily because its virus has had its genome completely sequenced (Nagasaki et al. 2005).

A comparison of expressed sequence tags (ESTs) from dinoflagellate has shown that they possess a splice leader sequence that is unique to all mRNAs of the dinoflagellates (Zhang et al. 2007, Lidie and Van Dolah 2007). Because of this, it is possible to retrieve all mRNAs from dinoflagellates separated from all other mRNAs in an environmental sample. Environmental clone libraries have now been made using exactly this technique and have discovered the expression of genes not known to exist in dinoflagellates, e.g., nucleosome core histones and rhodopsin, the latter gene implicated in non-photosynthetic solar energy capture (Lin et al. 2010). As sequencing capacities have increased and more whole genomes have been edited, the annotation of ESTs has improved (Table 2). Recently 454 sequencing of EST libraries has recovered the entire operon for the saxitoxin genes (Hackett et al. 2010). Of the eight genes involved, three are of cyanobacterial origin, one is of eukaryotic origin and four are of unknown origin, so there has not been a lateral gene transfer from the Cyanobacteria into the dinoflagellates. Interestingly, these same genes have not been recovered in lower sequencing capacity EST clone libraries, suggesting some unique control over the production of toxins. In *Karenia*, type 1 polyketide genes for brevetoxins have been recovered from EST libraries but with a type 2 polyketide protein complex (Snyder et al. 2003, Monroe and van Dolah 2008).

In summary the dinoflagellates have evolved many unique features but a comparison of other eukaryotic cells also reveals similar features in the Euglenozoa (Lukes et al. 2009). Both groups share flagella with paraflagellar rod, large nucleolus and permanently condensed chromosomes, cell walls composed of "proteinaceous/cellulosic" strips or plates, mucocysts or trichocysts ejected through pores, thylakoids with 3 lamellae, CER composed of 3 membranes. Is this a strange case of convergent evolution? Dinoflagellates only have 3 genes in the mitochondrion; euglenids have mini circles with three genes (?just the next evolutionary step). Both can edit the mitochondrial mRNA after transcription to change the protein desired. Both have unique splice leaders to the mRNA, conserved at the group level in dinoflagellates and at the species level in euglenids. Both have mRNAs transcribed with multiple genes: in the euglenophytes these are different genes but in the dinoflagellates they are tandem repeats of the same gene. More likely each of these strange features conveys some evolutionary advantage and are not just quirks of nature because we all know that God does not shoot craps to paraphrase an observation made by Albert Einstein on the organisation of the universe.

References

Alpermann, T. (2009) PhD Thesis University of Bremen.

Adl, S.M., et al. (2005) J. Euk Microbiol. 52: 399-451.

Archibald, J.M. & Keeling, P.J. (2002) Trends in Gen. 18: 577-584.

Baurain, D., et al. (2010) Mol. Biol. Evol. 27: 1698-1709.

Bay, L. K., Howells, E.J. & van Oppen, M.J.H. (2009) Cons. Genet. Resour. 1: 199-203.

Douzery E.J.P., et al. (2004) PNAS, USA 101:15386-15391.

Falkowsky, P.G., et al. (2004) Science 305: 354-359.

Fensome, R.A., et al. (1996) Paleobiol. 22: 329-338.

Frommlet, J.C. & Iglesias-Rodriquez, M.D. (2008) J. Phycol. 44: 1116-1125

Hackett, J.D., et al. (2004) Am. J. Bot. 91: 1523-1524.

Hackett, J.D. & Bhattacharya, D., (2006) in Katz, L.A. & Bhattacharya, D. Genomic and evolution of microbial eukaryotes. Oxford Press, pp. 48-63.

Hackett, J.D et al. (2005) BMC Gen. 6:80-93.

Hoppenrath, M. & Leander, B.S. (2010) PLOS One 5: e13220.

John, U, Fensome, R.A. & Medlin, L.K. (2003) Mol. Biol. Evol., 20:1015-1027.

John, U., et al. (2005) Proc Xth International Conf Harmful Algae. Steidinger, K.A., Landsberg, J.H., Tomas, C.R., & Vargo, G.A. (Eds.) Florida Fish and Wildlife Conservation Commission and Intergovernmental Oceanographic Commission of UNESCO.

Keeling, P. (2004) Protist 155: 3-7.

Leander, B.S. & Keeling, P. (2004) J. Phycol. 40: 341-350.

Lidie, K., Ryan, J.C., Barbier, M. & van Dolah (2005)

Mar. Biotech. 7: 481–493.
Lidie, K. & van Dolah, F. (2007) J. Euk Microbiol. 54: 427–435.
Lilly, E.L., Kulis, D.M., Gentian, P. & Anderson, D.M. (2002) J. Plank. Res. 24: 443-452.
Lin, S., Zhang, H., Zhuang, Y. & Gill, J. (2010) PNAS 46: (2003)3-(2003)8.
Lukes, J., Leander, B.S. & Keeling, P.J. (2009) PNAS 106: 9963-9970.
Masseret, E., et al. (2009) Appl. Env. Micro. 75: 2037-2045.
Medlin, Linda K. (2008) Origin and Evolution of Natural Diversity, Ed. By H. Okada, S.F. Mawatari, N. Suzuki, P. Gautam, pp. 31-42.
Medlin, Linda (2011) Phycologia, in press.
Medlin, L.K, et al. 1998. Eur. J. Protist., 34: 329-335
Moldowan J.M., Dahl, J. Jacobson, S.R., Huizinga, B.J., Fago, F.J., Shetty, R. Watt, D.S. & Peters, K.E. (1996) Geology 24: 159-162.
Monroe E.A. and Van Dolah F.M. (2008) Protist /159: 471-482.
Moustafa A., Beszteri B., Maier U.G., Bowler C., Valentin K. & Bhattacharya, D. (2009) *Science*, 324:1724-1726..
Nagai, S. Lian, C., Hamaguchi, M., Matsuyama, Y., Itakaru, S. & Hogetsu, T. (2004) Mol. Ecol. Notes 4:83-85.
Nagai, S., et al. (2006) Mol. Ecol. Notes 6: 756-758.
Nagai, S., Nishitani, G., Yamaguchi, M., Yasuda, N. Lian, C.L., Itakura & Yamaguchi, M. (2007) Mol. Ecol. Notes 7; 993-995.
Nagai, S., Sekino, M., Matsuyama, Y. & Itakura, S. (2006) Mol. Ecol. Notes 6: 120-122.
Nagasaki, K., Shirai, Y., Takao, Y., Mizumoto, H., Nishida, K. & Tomaru, Y. (2005) Appl. Environ. Microbiol. 71: 8888-8894.
Nash, E.A., Nisbet, E.R., Barbrook, A.C. & Howe, C.J. (2008) Dinoflagellates: a mitochondrial genome all at sea. Trends Gen. 24: 328-335.
Nishitani, G et al. (2007)a. Plank. Benthos Res. 2:128-133.
Nishitani, G., Nagai, S., Sakamoto, S., Sugaya, T., Lee, C.K., Kim, C.H., Itakura, S. & Yamaguchi, M. (2007)b. Mol. Ecol. Notes 7: 827-829.
Patron, N. J., Waller, R.F. & Keeling, P.J. (2006) J. Mol. Biol. 357: 1373-1382.
Penna, A., Garces, E., Vila, M., Giacobbe, M.G., Fraga, S., Luglie, A., Bravo, I., Bertozzini & Vernesi, C. (2005) Mar. Biol. 148: 13-23.
Piganeau, G., Eyre-Walker, A., Grimsley, N. & Moreau, H. (2011) PLOS one, in press.
Pouchon, X., Montoya-Burgos, J.I., Stadelmann, B. & Pawlowski, J. (2006) Mol. Phylo. Evol. 38: 20-30.
Renshaw, M.A., et al. (2006) Mol. Ecol. Notes 6: 1157-1159.
Saldarriaga, J.F., Taylor, F.J.R., Keeling, P.J. & Cavalier-Smith, T. (2001) J. Mol. Evol. 53:204-213.
Saldarriaga, J.F., et al. (2004) Eur. J. Prot. 40: 85-111.
Sanchez-Puerto, M.V. & Delwiche, C.F. (2008) J. Phycol. 44: 1097-1107.
Santos, S.R. & Coffrother, M.A. (2003) Biol. Bull. 204:10-20.
Saunders, G.W., et al. 1997 Pl. Syst. Evol. [Suppl.] 11: 237-259.
Scholin, C.A., Herzog, M., Sogin, M. & Anderson, D.M. 1994. J. Phycol. 30: 999-1011.
Shaked, Y. & de Vargas, C. (2006) Mar. Eco. Prog. Ser. 325: 59-71.
Snyder RV, Gibbs PDL, Palacios A, Abiy L, Dickey R, Lopez JV, Rein KS (2003) Mar Biotechnol 5(1), 1–12.
Taylor, F.J.R. (2004) Phyco. Res. 52: 308-324.
Takishita, K., Ishida, K. & Maruyama, T. (2003) An Protist 154: 443-454.
Thornhill, D.J. Xiang, Y. Fitt., W.K. & Santos, S.R. (2009). Plos One 4: 1-11.
Toulza, E., Shin, M.S., Blanc, G., Audic, S., Laabir, M., Collos, Y., Claverie, J.M., & Grzebyk, D. 2010 Appl. Env. Micro. 76: 4521-4529
Triplett, E.L., Govind, N.S, Roman, S.J., Jovine, R.V.M. and Prezelin, B. 1993. Mol. Mar. Bio. Biotech. 2: 239-245.
Verbruggen, H. (2011) Phycologist 80: xx.
Wang, Y. & Morse, D. (2006)Nuc. Ac. Res. 34: 613-619.
Yang, I., John, U., Beszteri, S., Gloeckner, G., Krock, B., Goesmann, A. & Cembella, A. D.(2010). BMC Genomics 11:248-266.
Yoon, H.S., Hackett, J.D., Pinto, G. & Bhattacharya, D. (2002) PNAS 99:15507-15512.
Yoon, H.S., Hackett, J.D., van Dolah, F.M. Nosenk, T., Lidie, K.L. & Bhattacharya, D. (2005) Mol. Biol. Evol. 22:1299-1308.
Zhang, H. Bhattacharya, D. & Lin, S. (2007)a. J. Mol. Evol. 65: 463-474.
Zhang, H. et al. (2007)bPNAS 104: 4618-4623.
Zhang, Z., Cavalier-Smith, T. & Green, B.R. (2002) Mol. Biol. Evol. 19: 489-500., G., Töbe, K. & Medlin, L.K. (2007). J. Plankton Res 29: 629-640.

Figure 2. Maximum likelihood analysis of 1296 SSU rRNA sequences currently held in the ARB database and alignment by secondary structure. A = clade 1 and 2, B = clade 3, C = clade 4.