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The evolution of the diatoms (Bacillariophyta). I. Origin of the group and assessment of the monophyly of its major divisions

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The diatoms are one of the best characterised algal groups. Despite this, little is known of the evolution of the group from the earliest cell to the myriad of taxa known today. Relationships among taxa at the family or generic level have been recognised in some diatoms. However, relationships at higher taxonomic levels are poorly understood and have often been strongly influenced by the first appearances of key taxa in the fossil record. An independent assessment of relationships among the diatoms at these higher taxonomic levels has been made using rRNA sequence data to infer phylogenetic relationships. In this paper we present an analysis of 18S rRNA data from several chosen centric, araphid and raphid pennate taxa. The phylogenetic inferences from these 18S rRNA sequences are supported by evidence from the fossil record and evidence from ontogenetic data. Ribosomal RNA data indicate that both the centric and araphid pennate lineages may not be monophyletic.

Key words: Bacillariophyta, Diatoms, molecular evolution, phylogeny, small subunit rRNA.

Introduction

The diatoms are one of the best circumscribed algal groups. They possess a siliceous cell wall (frustule) composed of two overlapping parts (valves). In addition, each valve is subtended by a series of siliceous hoops (girdle bands) that effectively cover and protect the middle of the cell. The siliceous cell wall imposes a growth restriction on the cell. This results in a cell size reduction with each mitotic division. Eventually the diatoms reach a size when sexual reproduction can be initiated. The gametes, formed either through oogamy, anisogamy or isogamy, are released from the parental cell walls and fuse to form a zygote (auxospore), which enlarges to restore the cell line to its maximum size. These features of size reduction and restitution coupled with those of the highly ornamented siliceous cell wall are unique to the diatoms and have been known since the last century (for review see Mann & Marchant, 1989).

Round (1981) and Round & Crawford (1981, 1984) and later Mann & Marchant (1989) suggested that the diatoms were derived from a scaly ancestor. In their schemes, two scales evolved into the valves, while others formed the girdle bands. It is thus suggested that the first diatom had two domed-shaped valves and many scale-like bands. The radiation of this ancestral hypothetical cell is virtually unknown because the earliest fossil record of the diatoms indicates that the group was already very diverse by the lower Cretaceous (Gersonde & Harwood, 1990). Many genera/species present in late Cretaceous material are morphologically unchanged from their counterparts in modern floras and some retain cell wall features characteristic of this hypothetical Ur-diatom. Nevertheless, the first recognisable diatoms from the early Cretaceous are centric diatoms (those with radial symmetry) (Gersonde & Harwood, 1990), while pennate diatoms (cells with bilateral symmetry) are first recorded in the late Cretaceous (65–75 million years ago) (Strel'nikova, 1974; Hajós & Stradner, 1975; Harwood, 1988). These pennate diatoms are araphid (non-motile). The raphid (motile) pennate diatoms first appear in reasonable numbers in the middle Eocene.

These appearances in the fossil record suggest that centric diatoms arose first, followed by the araphid diatoms and finally the raphid diatoms. Such evolutionary schemes can be traced in the phylogenetic reconstructions of the diatoms given in Simonsen (1979) and in Nikolaev (1984). They are also reflected in the classification system of the diatoms.

As long ago as 1896, Schütt separated the diatoms into genera with a centric or pennate morphology. The centric and pennate diatoms have different pattern centres from which valve morphogenesis commences (Mann, 1984), different modes of sexual reproduction (Drebes, 1977), and different types of plastids (Mereschkowsky, 1902–1903).

Many other classification systems (e.g. Hustedt, 1961–1966) further subdivide the pennate diatoms into raphid diatoms, which possess a slit or raphe in the cell wall associated with cell movement (Edgar & Pickett-Heaps, 1984), and araphid diatoms, which lack this structure.

However, in the latest taxonomic treatment of the diatoms (Round *et al.*, 1990) the araphid diatoms are given equal taxonomic rank alongside the centric and raphid pennate diatoms.

Significantly, there are diatoms traditionally placed in the araphid section that have features diagnostic of centric diatoms, such as oogamous reproduction in *Rhabdonema* Lyngb. (Stosch, 1958) and a 'pseudo'-oogamous reproduction in *Grammatophora* Ehrenb. (Magne-Simon, 1962). Many araphid diatom genera have numerous small discoid plastids like the centric diatoms (e.g. *Thalassionema* Grun. ex Hust. [Hallegraeff, 1986] and *Catacombas* Williams and Round [Williams & Round, 1986]). The occurrence of these diagnostic characters in the centric and araphid diatoms suggests that the araphid diatoms may be paraphyletic; that is, a group with no characters of its own (Kociolek *et al.*, 1989). Furthermore, this suggests either that these characters may have arisen more than once (that is separately in both centric and araphid lineages) or else that both the araphid and centric diatoms are not monophyletic as currently understood.

The independent assessment of relationships based upon rRNA sequence data can be used to infer phylogenetic relationships among taxa at all hierarchical levels (Elwood *et al.*, 1985; Sogin & Elwood, 1986; Bhattacharya & Druehl, 1988; Bhattacharya *et al.*, 1989; Rausch *et al.*, 1989; Huss & Sogin, 1990; Medlin *et al.*, 1991; Buchheim & Chapman, 1992). Recently, Bhattacharya *et al.* (1992) have shown, using small subunit (ssu) rRNA sequence data, that the diatoms are correctly placed in a chromophyte/oomycete lineage that excludes dinoflagellates and prymnesiophytes. We have employed this same technique to assess the relationships among diatoms and the relationship of the diatoms to other groups within the chromophyte/oomycete lineage. In this paper we present an analysis of 18S rRNA data from 11 diatoms: four centric, four araphid and three raphid taxa. The phylogenetic inferences from these 18S rRNA sequences are compared with those previously generated from a study of frustule morphogenesis (Round & Crawford, 1981, 1984) and with evidence from the fossil record to assess the monophyly of the major subdivisions of the diatoms.

Materials and methods

Cultures

The diatom species grown for this study are shown in Table 1. All isolates were grown in an artificial seawater medium (Medlin *et al.*, 1986) under a 16:8 light/dark cycle at 16 or 20 °C and stirred manually on a daily basis. We have confined our primary analysis to marine taxa assuming that the diatoms originated first in marine waters and later spread into freshwater systems (Round & Sims, 1981). The marine *Fragilaria striatula* Lyngb. examined in this study should not be regarded as being typical of the freshwater genus *Fragilaria* Lyngb. (Williams & Round, 1987).

Microscopy

Cultures were rinsed free of media and mounted for electron microscopy either onto glass coverslips attached to stubs for scanning electron microscopy (SEM) or onto formvar grids for transmission electron microscopy (TEM). Additional material was cleaned of organic matter following Simonsen (1974) and similarly mounted. Material mounted for electron microscopy was examined with a Philips 501 or a Hitachi S 800 field emission scanning electron microscope or a JEOL 1200 EX transmission electron microscope.

Isolation of DNA

Cultures were harvested during log phase by filtration through a 3 μm Nuclepore filter, rinsed, and resuspended in extraction buffer (100 mM Tris, pH 8·5; 100 mM NaCl, 50 mM EDTA). Total nucleic acids were extracted by vortexing the cells in the presence of 2% SDS, glass beads (40 mesh size), and buffered phenol/chloroform/isoamyl alcohol (50:48:2, v/v/v).

The supernatant was extracted twice with phenol/chloroform/isoamyl alcohol and once with chloroform/isoamyl alcohol (48:2, v/v) before the nucleic acids were concentrated by ethanol precipitation. Total nucleic acid preparations or DNA purified with an ion resin exchanger (Qiagen) were used as template for the amplification of the nuclear gene coding for the 18S rRNA molecule using polymerase chain reactions (PCR) (as modified in Medlin *et al.*, 1988). A minimum of five PCR reactions were performed and pooled for each species.

Cloning and sequencing

Amplification products were purified using BRL glass max spin columns following a 5 min precipitation at room temperature with 1/2 volume ammonium acetate and 2 volumes 100% ethyl alcohol. Purified gene products were then ligated into the replicative form of M13 mp18 and M13 mp19 (Medlin *et al.*, 1988; Messing, 1973). Single-stranded templates were prepared from as many as 19 pooled recombinant M13 phages in each orientation for each species. Oligonucleotide primers that are well conserved in eukaryotic 18S rRNA genes (Elwood *et al.*, 1985) were used to initiate DNA synthesis in dideoxynucleotide chain-termination sequencing reactions (Sanger *et al.*, 1977).

Molecular character analysis

The diatom sequences were compared with previously published small subunit rRNA sequences from other chromophyte/oomycete species (Table 2, Neefs *et al.*, 1991). In addition to diatom sequences presented in Bhattacharya *et al.* (1992), we have included sequence data for five more diatom species (Table 2), which have been deposited in Genbank.

Table 3. Distance values calculated using the Jukes/Cantor model in the upper right triangle for those species listed in Table 2. In the lower left triangle are the uncorrected pairwise differences for the same species

Organism	Och	Mal	Nit	Cyl	Bac	Skel	Cosc	Step	Frag	Rhap	Rhiz	Thal	Ast	Lag	Ach	Phy	Trib	Cost
Och		0·0593	0·0906	0·0864	0·0884	0·1170	0·1248	0·1075	0·0915	0·1056	0·1114	0·0974	0·0871	0·1125	0·1015	0·1101	0·0920	0·1003
Mal	67		0·0834	0·0792	0·0806	0·1031	0·1107	0·0945	0·0836	0·0969	0·1011	0·0902	0·0807	0·1073	0·0978	0·1099	0·0731	0·0838
Nit	119	114		0·0106	0·0081	0·0721	0·0996	0·0653	0·0240	0·0467	0·0763	0·0285	0·0284	0·1070	0·1006	0·1091	0·0765	0·0929
Cyl	114	109	12		0·0100	0·0680	0·1017	0·0646	0·0233	0·0441	0·0776	0·0285	0·0271	0·1006	0·0949	0·1027	0·0758	0·0929
Bac	117	111	8	12		0·0686	0·0967	0·0619	0·0208	0·0428	0·0728	0·0253	0·0239	0·1019	0·0942	0·1040	0·0723	0·0886
Ske	138	123	85	81	81		0·1195	0·0898	0·0709	0·0798	0·0900	0·0787	0·0694	0·1263	0·1183	0·1241	0·1044	0·1149
Cosc	131	119	109	113	106	117		0·1065	0·0984	0·1149	0·1010	0·1037	0·0989	0·1394	0·1386	0·1408	0·1251	0·1338
Step	122	109	73	73	69	88	100		0·0634	0·0832	0·0810	0·0718	0·0687	0·1197	0·1125	0·1182	0·0972	0·1040
Frag	119	113	31	31	27	82	106	69		0·0442	0·0799	0·0170	0·0259	0·1058	0·1008	0·1065	0·0794	0·0874
Rhap	126	119	53	50	48	81	115	84	48		0·0904	0·0503	0·0363	0·1195	0·1151	0·1202	0·0962	0·1067
Rhiz	127	118	90	93	86	91	92	75	94	97		0·0843	0·0763	0·1185	0·1185	0·1156	0·1045	0·1078
Thal	121	116	32	33	28	87	107	75	13	51	94		0·0318	0·1111	0·1068	0·1133	0·0838	0·0962
Ast	116	112	41	40	35	83	111	80	36	39	92	39		0·0999	0·0928	0·1006	0·0792	0·0879
Lag	151	149	156	148	150	163	166	152	153	159	151	154	148		0·0327	0·0212	0·1077	0·1160
Ach	136	137	148	142	140	154	165	143	147	154	152	149	139	48		0·0378	0·1047	0·1137
Phy	147	153	159	151	153	160	167	150	154	160	147	157	149	29	57		0·1111	0·1210
Trib	112	90	103	103	98	125	136	111	106	117	122	107	109	149	154	146		0·0710
Cost	112	94	113	114	108	124	136	108	105	118	113	110	107	147	153	73	144	

Species are referred to by the first three or four letters of their generic names. For full names see Table 2.

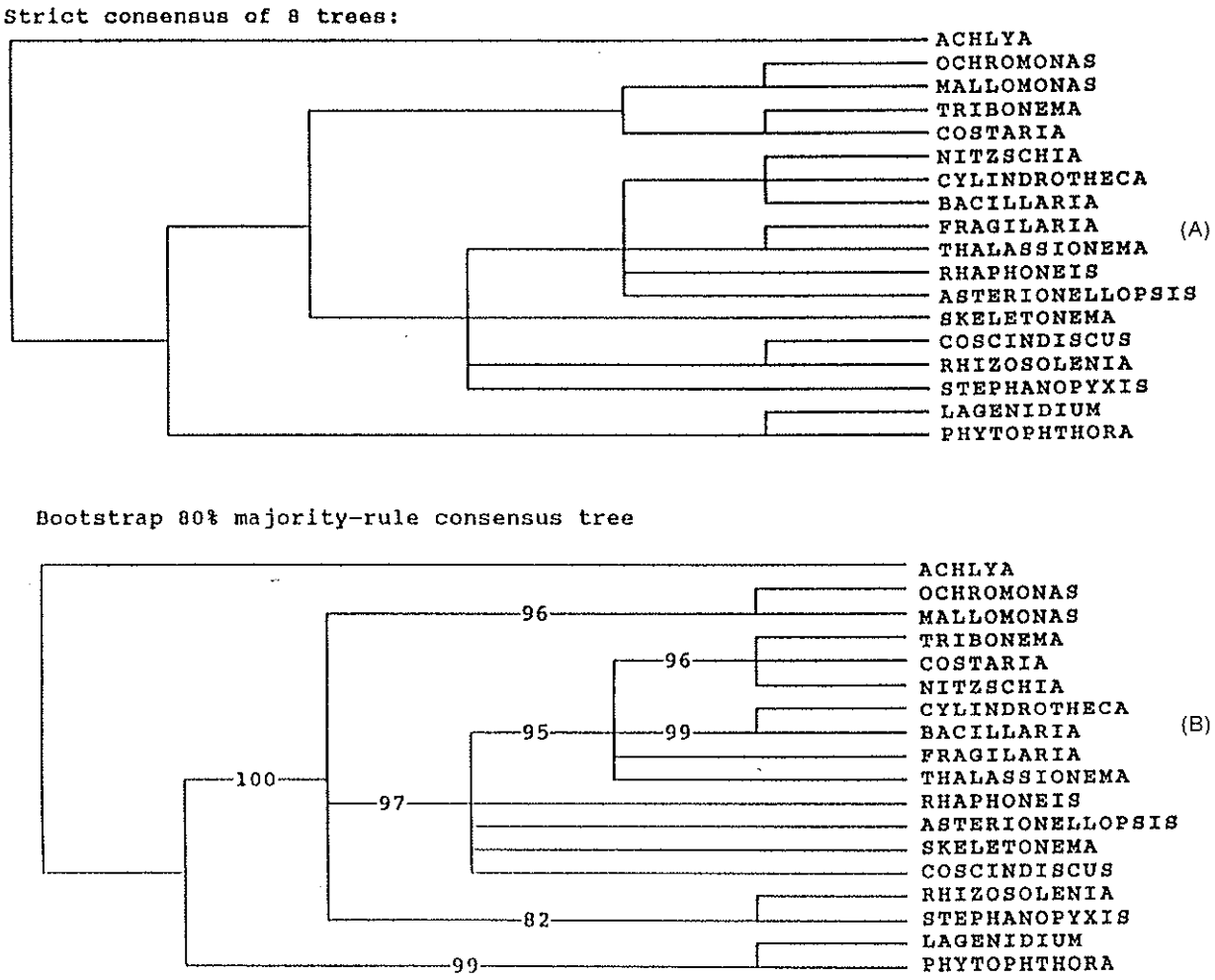


Fig. 2. Diatom phylogeny inferred from maximum parsimony analysis of the CLUSTAL V alignment of nucleotide positions in the 18S rRNA coding regions using the heuristic search within PAUP. (*A*) Strict consensus tree. (B) Consensus bootstrap tree using an 80% majority rule.

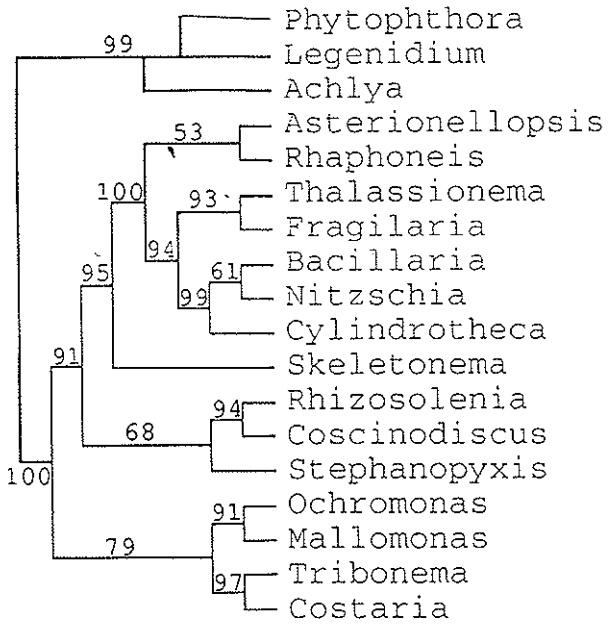


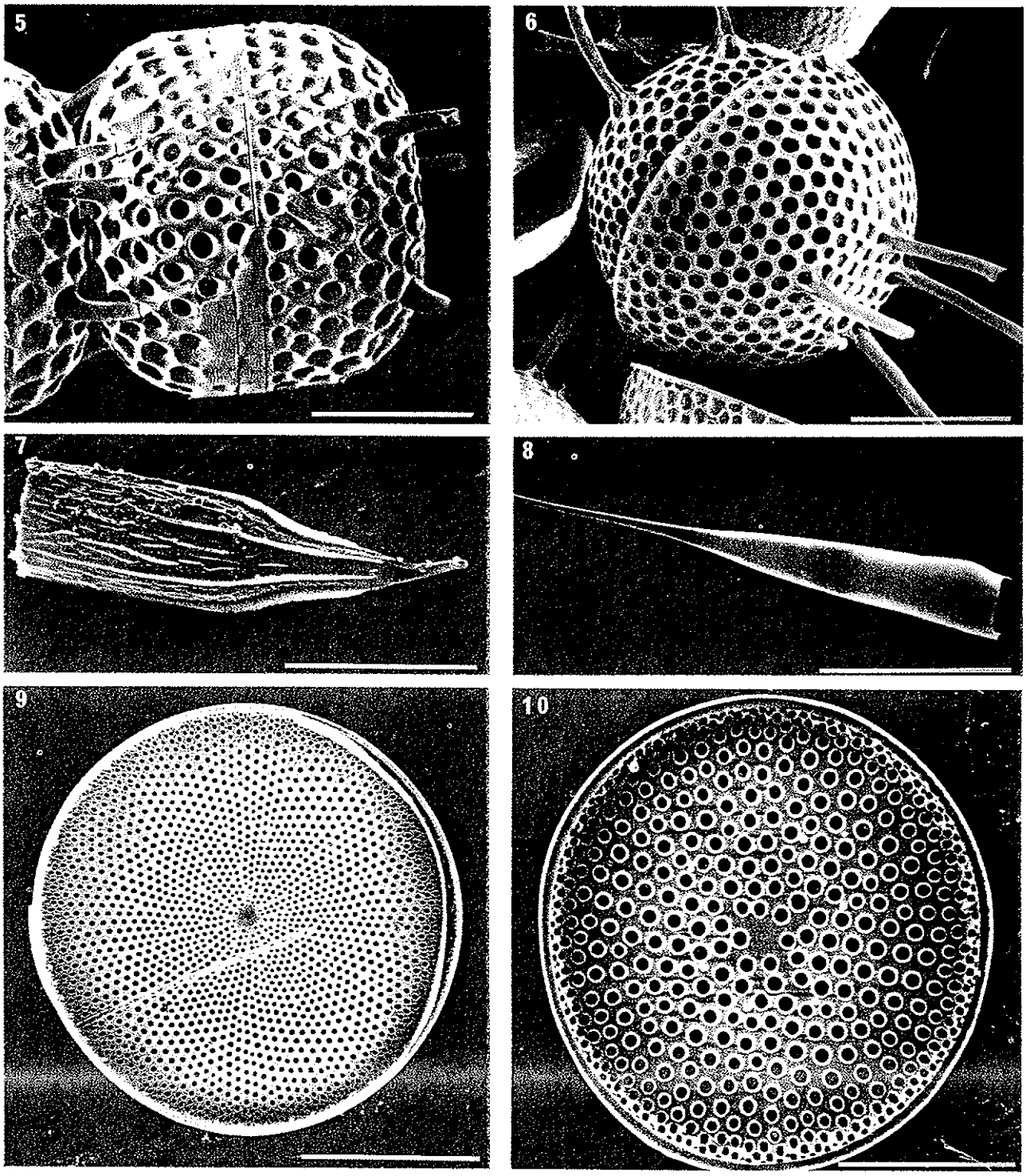
Fig. 3. Diatom phylogeny inferred from maximum parsimony analysis of the secondary structure alignment of nucleotide positions in the 18S rRNA coding regions using the heuristic search within PAUP. Bootstrap values at the branch nodes are based on a 50% majority rule.

Results

Complete 18S rRNA sequences were determined for *Coscinodiscus, Asterionellopsis, Rhaphoneis, Thalassionema* and *'Fragilaria'* (Table 2). Microheterogeneity/ambiguities were noted in most species. Multiple PCR reactions and multiple clones in each orientation were pooled for our single-stranded sequencing reactions. These precautions should ensure that base changes were not PCR artefacts. It should also be noted that microheterogeneities/ambiguities were almost never found in highly conserved regions but were instead restricted to variable regions which would be evolving at a faster rate. Assuming PCR-induced base changes to be random, one would expect to find such ambiguities in conserved regions as well. Therefore, it seems reasonable to assume that where microheterogeneity/ambiguity exists, it is not a PCR-induced artefact in this study.

Distance analysis

Pairwise distance values among the taxa are presented in Table 3. These values range from 0·1195 to 0·0081 among the diatoms, which are comparable to those obtained



Figs 5–10. Scanning electron micrographs of valves of fossil and recent representatives of centric diatoms. Fig. 5. *Stephanopyxis turris* from the Cretaceous, Moreno Shale, California. Note domed pseudoloculate valve. Fig. 6. *Stephanopyxis* cf. *broschii*, recent plankton. Note domed pseudoloculate valve. Fig. 7. *Rhizosolenia* sp. from the Cretaceous, USGS site Fi 437-5C, Arctic Ocean, Alpha Ridge. Specimen is probably an asexual resting spore. Fig. 8. *Rhizosolenia setigera*, recent plankton. Note domed poroid valve. Fig. 9. *Coscinodiscus* cf. *radiatus* from the Cretaceous, Core 82, Ust'Man'ya, Western Siberia. Note coin-shaped loculate valve. Fig. 10. *Coscinodiscus radiatus*, recent plankton. Note coin-shaped loculate valve. Cell at the small end of its size range. Scale bars represent: Figs 5, 6 and 10, 15 μm; Figs 7 and 8, 20 μm; Fig. 9, 60 μm.

relatively derived (morphologically) raphid diatom family, the branch lengths separating these groups are short, indicating that once these taxa diverged they rapidly radiated and diversified. It appears that morphological evolution was more rapid than the small subunit rRNA nuclear gene molecular evolution during this time interval.

The first known appearance of nearly all of the diatom taxa in our study can be documented from the fossil record (Fig. 4). These records corroborate the phylogeny of the major lineages inferred from the rRNA sequence data. The centric taxa *Coscinodiscus*, *Rhizosolenia* and *Stephanopyxis* are present in the records from the late Cretaceous;

however, so is the araphid genus *Rhaphoneis*. *Skeletonema* appears later in the Pliocene, but centric diatoms with strutted processes have been recorded earlier. The remaining three araphids appear from middle Eocene to recent. The recent appearance of *Asterionellopsis* may be artefactual because this delicate taxon would not preserve well. Of the three raphid taxa, *Nitzschia* appears in the fossil record at about the same time as the two araphid taxa, *'Fragilaria'* and *Thalassionema*, its sister taxa.

Parsimony analysis

Using the alignment without the secondary structure imposed on the nucleotide positioning, eight equally parsimonious trees were obtained using both Hennig86 and PAUP (tree length 987 steps; CI, excluding uninformative characters=0·531; RI=0·605, data not shown). Four of the eight trees have the centric diatoms as monophyletic, while two have the araphid diatoms as monophyletic. All eight trees support the raphid taxa as monophyletic. A strict consensus tree and a bootstrap consensus tree (80% majority rule) for these eight trees are presented in Fig. 2*a* and *b*, respectively. These trees agree in principle with the distance tree and show the diatoms as a monophyletic group. However, the branching order between the centric and the three araphid diatoms, (*Asterionellopsis*, *Rhaphoneis* and *Thalassionema*), is not resolved. *Skeletonema* emerges as a separate lineage not linked to the araphid group in the consensus tree. In the consensus tree and the bootstrap consensus tree, the araphid taxa are placed as sister taxon to the raphid diatoms (95%). Of the araphid diatoms only *'Fragilaria'* and *Thalassionema* consistently pair with a high bootstrap value (99%).

Using the secondary structure alignment, a single most parsimonious tree was obtained using both the heuristic (Fig. 3) and branch and bound (tree not shown) searches within PAUP (tree length 1199 steps; CI excluding uninformative characters=0·532; RI=0·599; bootstrap values [using 50% majority rule] reported at branch nodes). The tree agrees with the topology obtained in the distance analysis and shows that neither the centric nor the araphid diatoms are monophyletic.

With the addition of four more steps, eight more trees were obtained (data not shown). These trees differed in the position of *Cylindrotheca* and *Bacillaria* within the Bacillariaceae and in the position of *Rhaphoneis*, which emerged as a separate lineage. These topology changes are reflected in the low bootstrap values at these branch nodes. The position of *Skeletonema* as sister taxon to the araphid taxa is supported by a 95% bootstrap value; however, this species may be evolving rapidly (see branch lengths on the distance tree), which may confuse its correct placement in all our trees recovered and is a relationship we view with caution. The use of the secondary structure of the rRNA molecule in sequence alignment appears to achieve slightly better resolution among the branching order of the centric and araphid taxa sampled in this study.

Discussion

Our phylogenetic analysis using rRNA sequence analysis continues to provide unequivocal evidence that the diatoms are a major monophyletic group within the chromophyte/oomycete lineage (see also Bhattacharya *et al.*, 1992 and from 28S rRNA sequence data, A. Adoutte, personal communication). Earlier work has also shown that the Dinoflagellata and the Prymnesiophyta are excluded from this lineage (Perasso *et al.*, 1989; Bhattacharya *et al.*, 1992). However, one issue that continues to be completely unresolved is the branching order within the chromophyte/oomycete lineages (Williams, 1991*a*; Bhattacharya *et al.*, 1992). With the addition of four diatoms to the analysis, the oomyctes and the diatoms group together as do the remaining two lineages: the Xanthophyta/Phaeophyta and the Synurophyta/Chrysophyta. However, the inclusion of the thraustochytrids (Labyrinthulomycota) in the dataset changes the divergence of the taxa such that the diatoms emerge after the Oomycophyta but still there is little resolution in the branching order of the pigmented forms (Cavalier-Smith, 1994). The two heterotrophic groups, the thraustochytrids followed by the oomycetes, are now the earliest known divergences in this lineage.

In the scheme for the early evolution of the diatoms proposed by Round (1981) and Round & Crawford (1981, 1984) the diatoms arose from a scaly ancestor. Two scales become polarized and evolve into the two valves of the diatom cell wall; other scales form the girdle bands. This Ur-diatom, initially characterised by two domed-shaped valves and many scale-like girdle bands, then underwent a massive radiation to evolve the major groups of diatoms known today. Valve perforations changed from simple poroids to three-dimensional structures known as pseudoloculae and true loculae by increased silicification in a vertical direction above a basal layer (Round & Crawford, 1981, 1984). Notably, Gersonde & Harwood (1990) found that the majority of early Cretaceous species have a pseudoloculate valve structure.

To support their phylogeny, Round & Crawford (1981, 1984) proposed a character transformation from multiple, minute siliceous scales and hoops found on the organic auxospore (zygote) walls of the diatoms (Figs 15, 16) to the developing siliceous valves and girdle bands of the vegetative cell (Fig. 17). This proposal suggests a morphogenetic link between the valves, girdle bands and scales of the diatoms.

This evolutionary scheme undoubtedly prompted later workers to interpret Round & Crawford's hypothesis as evidence that Synurophyta was sister taxon to the diatoms, and the relationship between diatoms and other chromophyte algae has often resolved the diatoms as sister taxon to the Synurophyta (Cavalier-Smith, 1986; Andersen, 1987) or assumed that such a relationship exists (Kociolek 1986; Kociolek & Williams, 1987; Crawford & Round, 1989). However, not all morphological analyses have supported a close relationship between the Syunurophyta and the diatoms (Andersen, 1989, 1991; Williams,

1991*b*), and neither does this present rRNA analysis (see also Bhattacharya *et al.*, 1992). The phylogenetic position of the diatoms presented here shows that they are not a sister taxon to either the Chrysophyta or the scaly Synurophyta.

However, if the ancestor of the diatoms was scaly, then this scaly precursor cell will have also given rise to the other chromophyte/oomycete groups and the presence of scales or their later homologues can be interpreted as a primitive character for the entire lineage. In this context, it should be noted that the vegetative thallus of the thraustochytrids and the closely related Labyrinthuliidae possesses a wall of thin scales, while only the heterokont zoospores of the thraustochytrids are covered with scales (Porter, 1989).

Another diatom feature that suggests that the diatoms may have emerged before the other pigmented forms in this lineage is their ploidy state. The diatoms are virtually unique in the chromophyte algae in having only a diploid stage. However, all oomycetes are diploid (Beakes, 1989) and meiotic sexuality has been reported in at least one species of Labyrinthulomycota (Porter, 1989). Thus, the two earliest known divergences in this lineage are diploid, which may suggest that diploidy is an ancient feature. Other chromophytes which have only a diploid phase are the Fucales in the Phaeophyceae (Bold & Wynne, 1985) and the xanthophyte *Vaucheria* (Al-Kubaisy *et al.*, 1981). Synurophytes and chrysophytes are haploid, and many other Phaeophyceae have an alternation of generations (Bold & Wynne, 1985). These groups are one of the last to emerge in the chromophyte phylogeny using the 18S gene (Bhattacharya *et al.*, 1992 and this study). A different resolution could possibly be obtained with the variable regions of the 28S.

The diploid nature of the diatoms prompted Mann & Marchant (1989) to propose an alternative scheme for the evolution of the diatoms to that proposed by Round & Crawford (1981). They suggested that a diploid cyst with multiple scales alternated with a scaly flagellate haploid vegetative cell. The life cycle of modern Thraustochytriidae and Labyrinthuliidae is not unlike that proposed by Mann & Marchant (1989) for the early diatoms except that the scaly flagellate cells in the Thraustochytriidae and Labyrinthuliidae are zoospores rather than gametes. To date there is no report of a scaly flagellate cell in any known diatom.

In the evolution of the Ur-diatom in Mann & Marchant's scheme (1989), the cyst evolves into the modern vegetative cell of the diatoms; the original flagellate haploid cells are reduced to gametes. However, the scaly cyst (auxospore) exists in all investigated modern species. With subsequent divisions, this cyst forms the modern vegetative cell. The early divergence of the diploid diatoms and oomycetes in the chromophyte/oomycete lineage as shown by our 18S rRNA data and of the thraustochytrids and oomycetes in Cavalier-Smith's 18S rRNA tree (Cavalier-Smith, 1994) suggests that the ancestral cell to this entire lineage may have contained a diploid stage. This stage is the dominant stage in the life cycle in the two earliest known divergences in this lineage and in the diatoms.

Although in the ancestral chromophyte/oomycete cell sexual reproduction probably involved isogamy and anisogamy, it appears that oogamy was selected for in certain genera of the diatoms once size reduction and the need for rapid size restitution via a maximum zygote size became permanent diatom features (Mann & Marchant, 1989). Beakes (1989) suggested that the Oomycetes also evolved from anisogamous or isogamous ancestors. However, in the diatoms, oogamy is restricted to the centric taxa and one, possibly two, araphid diatoms, while isogamy/anisogamy is the rule among the pennate diatoms (Round *et al.*, 1990). Our rRNA analysis substantiates that the centric diatoms emerged prior to the pennate forms, thus suggesting that isogamy/anisogamy rather than oogamy may be the derived character state in the diatoms (see also Kociolek, 1986).

The questions then arise as to why this apparent reversal of evolution has occurred and what adaptive advantage isogamy/anisogamy has conferred on the pennate taxa to become the derived character state. Mann & Marchant (1989) suggested that this reversal occurred because in the pennate diatom life cycle the gametangia are brought together within a mucilaginous envelope prior to gamete release, thus abating the need for oogamous reproduction. Lewis (1984) explained that there is a high cost in having differentiated sexual cells in unicellular organisms with intermittent sexuality. The evolution of the juxtaposed gametangia in the pennates coupled with isogamy/anisogamy probably further 'ensures that sexuality is less likely to be lost because of its short-term disadvantages' (Lewis, 1984) in a group of algae controlling their sexuality at intervals greater than 1 year (Mann, 1988; Jewson, 1992).

Although our analyses show that the diatoms are monophyletic, the major subdivisions within the group are not. If Round *et al.* (1990) are correct in the recognition of three major groups within the diatoms (the centric, the araphid pennate, and the raphid pennate lineages), then the trees inferred from both the distance and parsimony analyses indicate that at least two of these groups are not monophyletic. Within the centric lineage, those taxa emerging first (*Stephanopyxis* and *Rhizosolenia*) are those whose valve and girdle band morphology most closely resembles the hypothetical Ur-diatom. *Stephanopyxis* has pseudoloculate valves. Our rRNA analysis is consistent with the first appearance of these diatoms in the fossil record (Fig. 4) and with the predictions of the morphotype of the first diatom from the ontogenetic evidence presented in Round & Crawford (1981, 1984). The separation of *Skeletonema* (and probably related genera with strutted processes) from the centric diatoms is also consistent with the later appearance of these centric diatoms with strutted processes. *Skeletonema* appears as a sister taxon to the

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