Evolution of the Diatoms (Bacillariophyta). II. Nuclear-Encoded Small-Subunit rRNA Sequence Comparisons Confirm a Paraphyletic Origin for the Centric Diatoms

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A phylogeny of the diatoms was inferred from comparisons of nuclear-encoded small subunit ribosomal RNA coding regions using maximum likelihood, weighted maximum parsimony, and neighbor-joining distance methods with Jukes and Cantor, Kimura, Gamma, van de Peer, and LogDet evolutionary models. Analyses of 30 taxa in 11 orders recovered two clades (Clades I and II). Neither of these clades correspond to the three classes of diatoms presently recognized or to the traditionally recognized radially symmetrical centric diatoms or bilaterally symmetrical pennate diatoms. All analyses show that the centric diatoms are a paraphyletic lineage. Tests of alternative phylogenies that address existing hypotheses regarding diatom systematics with the maximum likelihood and maximum parsimony methods support the two clades. Clade I is defined by centric diatom orders with specialized tubes, termed labiate processes, located peripherally in the cell wall. Clade II contains (1) bi(multi)polar centric diatoms with centrally located labiate processes, (2) centric diatoms with other central tubes termed strutted processes, and (3) pennate diatoms. Morphological evidence from fossil assemblages and cytological architecture support the results of the molecular analyses, whereas morphological features of extant diatoms are too derived to resolve the deeper branches in the tree.

Introduction

The diatoms (Bacillariophyta) are one of the most successful groups of microalgae, with over 10,000 known species occurring in both aquatic and terrestrial habitats. They are renown for the intricate and precise geometric designs of their silica cell walls (valves and girdle bands) and the unusual pattern of reduction in cell size of one of the daughter cells following mitosis (Mann and Marchant 1989). Small-subunit ribosomal RNA (ssu RNA) sequence comparisons (Bhattacharya et al. 1992; Medlin, Williams, and Sims 1993) support a monophyletic origin of the diatoms within the pigmented protistan heterokont lineage (those organisms bearing tubular mastigonemes or hairs on their leading flagellum). Recent rRNA analyses have shown the diatoms to be related specifically to the Pelagophyceae, i.e., heterokont algae with a reduced flagellar apparatus that lacks the central two microtubules (Saunders et al. 1995).

Central to diatom taxonomy and phylogeny has been an assumption that the group contains two forms based on mode of sexual reproduction, pattern centers or symmetry, and plastid number and structure (Round, Crawford, and Mann 1990, pp. 58–100). Familiar to most aquatic and cell biologists are the oogamous, radially symmetrical centric diatoms with numerous cold plastids, and the isogamous, bilaterally symmetrical pennate diatoms with fewer plate-like plastids. These characters have led most systematists to treat the centric and pennate diatoms as two separate classes. It is generally agreed that the pennate diatoms evolved from the centric forms due to their later appearance in the fossil record. In a recent systematic revision (Round, Crawford, and Mann 1990, p. 125), the raphid pennate diatoms (those with a slit [raphe] in the cell wall for movement) and the araphid pennate diatoms (those without this slit) were given equal taxonomic ranking. Thus, the present classification system of the diatoms recognizes three classes: Coscinodiscophyceae (centric diatoms), Fragilariphycyceae (raphid pennate diatoms), and Bacillariophyceae (raphid pennate diatoms).

Classification of the diatoms at the order level and below is almost exclusively based on morphological features of the siliceous cell wall (Round, Crawford and Mann 1990, pp. 37–49). Key characters limiting centric diatom orders are valve structure and symmetry, and the type, number, and arrangement of tubes through the valve. These features, plus the presence or absence of the raphe and its structure, figure prominently in the subdivision of raphid and araphid taxa. The raphe and the tubes (labiate or strutted processes) through the cell wall are probably the most important features used to infer phylogenetic relationships among the diatoms. The labiate process normally has elaborate lip-like internal extensions, but some labiate processes are reduced, with only a simple slit-like internal opening. The function of the labiate process is not well established, although in a restricted number of centric and pennate taxa mucilage
is secreted through the labiate process for movement (Medlin, Crawford, and Andersen 1986; Pickett-Heaps, Hill, and Weatherbee 1986; Pickett-Heaps, Hill, and Blažé 1991), and the internal part of the tube is used as a cytological anchor for the nucleus during interphase and new valve formation (Schmid 1994). It has been hypothesized that the labiate process evolved into the raphe of the pennate diatoms (Hasle 1974). The strutted process, through which chitin threads are secreted for chain formation and flotation, is restricted to the centric order Thalassiosirales and is a simple tube with adjacent pores (Round, Crawford, and Mann 1990, pp. 35-36).

Relationships at higher taxonomic levels in the diatoms are virtually unexplored with modern phylogenetic and morphometric tools. This likely reflects the unique, highly derived morphologies of the diatom orders. In this regard, RNA sequence comparison has proven to be a powerful tool for resolving phylogenetic relationships at all taxonomic levels because it is a functionally stable evolutionary marker that is independent of morphometric characters (Woese, 1987; Bhattacharya et al. 1992). It is functionally conserved in all cells with no documented evidence of lateral gene transfer (Woese 1987).

Using rRNA sequence comparisons, Medlin, Williams, and Sims (1993) presented the first evidence that suggested centric and araphid pennate diatoms had a paraphyletic origin. One centric diatom, Skeletonema costatum, was most closely related to the pennate diatoms, with high bootstrap support in phylogenetic analyses; the araphid taxa were a paraphyletic lineage within the pennates. An analysis of partial sequences from the 28S large-subunit (lsu) rRNA coding region from eight diatoms also showed that centric and araphid taxa were paraphyletic (Sörhannus et al. 1995). These preliminary data suggest that higher level diatom systematics do not reflect their evolutionary history.

To investigate the phylogenetic relationships among the diatoms at higher taxonomic levels we compared the nucleotide sequence of the nuclear-encoded ssu rRNA coding regions from 30 taxa representing 11 orders and compared these sequences to homologous coding regions from other heterokonts. Mapping of morphological characters onto the rRNA phylogenies allowed deeper insights into the origins of taxonomically important features of the diatoms.

Materials and Methods

Cultures

Representatives of each diatom class and at least one species from most major orders of centric diatoms were selected (Appendix). New isolates were grown as described (Medlin, Crawford, and Andersen 1986) and deposited in the Culture Centre for Algae and Protozoa (Oban, Scotland) or in the Antarctic algal collection at the Alfred Wegener Institute. Permanent slides of each taxon are available from L.K.M.

DNA Methods

Nucleic acids were extracted as described (Medlin et al. 1988) or with a 3% CTAB (hexadecyltrimethylammonium bromide) procedure (Doyle and Doyle 1990). Small-subunit rRNA coding regions were amplified using the polymerase chain reaction (PCR; Saiki et al. 1988) with eukaryote-specific primers, and single-stranded templates were produced for sequencing (Dynabeads M-280 Streptavidin, DY-NAL A.S. Oslo, Norway). Both coding and noncoding strands were completely sequenced (Sanger, Nicklen, and Coulsen 1977; Elwood, Olsen, and Sogin 1985). No fewer than six PCR reactions in each orientation were pooled for the direct sequencing reactions.

Sequence Analysis

The ssu rRNA sequences of 16 centric diatoms were aligned with previously published diatom (Medlin et al. 1991; Bhattacharya et al. 1992; Medlin, Williams and Sims 1993) and other chromophyte/oomycete, dinoflagellate, and prymnesiophyte rRNA sequences (Neefs et al. 1991; Andersen et al. 1993, Medlin, Lange, and Baumann 1994), using maximum primary and secondary structural similarity. Positional homology was assumed for 1,739 positions, of which 528 were informative for parsimony analyses. The final data set contained 34 taxa (Appendix). Three oomycetes and one pelagophyte were used to root the trees. The use of multiple outgroups stabilizes the phylogenetic analyses (Swofford and Olsen 1990). The oomycetes, sister group to the pigmented heterokont lineage (Saunders et al. 1995), are not included in the figures for clarity. Complete phylogenies are available from L.K.M.

Maximum parsimony analyses were implemented with the PAUP computer program (Swofford 1993). Introduced gaps were treated as missing data; informative characters were treated as multistate unordered. Unweighted maximum parsimony trees were obtained using the tree-bisection-reconnection (TBR) branch swapping option in a heuristic search with random taxon addition. The most parsimonious trees (MPT) and the data matrix were loaded into the MacClade computer program (Maddison and Maddison 1992) to infer a weighted data set in which the frequency of nucleotide substitutions at each position was inversely related to the number of changes at that position (scale 1-100). The
type of substitution, i.e., purine → purine, purine → pyrimidine, (i, j), at each position was also weighted
(Kij) as the cost of going from one state to another: Kij
= −ln(Xij/Xi) where Xij is the number of i → j changes,
Xi is the number of changes from i to any state, and X
is the number of changes on the tree (scale 1–100).
These weighting schemes greatly enhance the ability of
the maximum parsimony analyses to recover the correct
tree when multiple substitutions have occurred over sites
(Hillis, Huelsenbeck, and Cunningham 1994). Stability
of monophyletic groups in weighted maximum parsim-
ony trees was estimated with a bootstrap analysis (100
replicates; Felsenstein 1985). The information content of
this data set was measured with the G1 statistic, which
tests for the distribution skewness of 10,000 random
trees (Hillis and Huelsenbeck 1992).
Distance analyses were performed using the PHY-
LIP computer program (Felsenstein 1993). Dissimilarity
values (Fitch and Margoliash 1967), based on pairwise
comparisons of sequences, were transformed into dis-
tances using both the Jukes and Cantor (1969) and the
Kimura two-parameter models (Kimura 1980). Distance
matrices were converted into trees using the neighbor-
joining method (Felsenstein 1993); a bootstrap analysis
(100 replicates; Felsenstein 1985) was also performed.
Weighted distance analyses were performed (a) by using
the weighting scheme for the frequency of base substi-
tution from MacClade to generate a data set with each
position weighted, (b) by using a weighting scheme in
which the positions were grouped into variability classes
and only four possible weights assigned to any position
(van de Peer et al. 1993), and (c) by using the gamma
distribution option (Jin and Nei 1990) in the MEGA
computer program (Kumar, Tamura, and Nei 1993).
Neighbor-joining methods were used to construct trees
from each of the weighted distance matrices.
Maximum likelihood analyses were done using the
fastDNAm1 program (version 1.0, Larson et al. 1993).
The maximum likelihood tree was used to construct
user-defined trees (RETREE, PHYLIP) to constrain
maximum parsimony (PAUP) and maximum likelihood
(DNAML, PHYLIP) analyses. Three alternative hypoth-
eses of relationships (trees) were compared with the
likelihood ratio test (Kishino and Hasegawa 1989). This
test uses the mean and variance of log-likelihood dif-
fences between trees, taken across sites. If the mean
is >1.96 standard deviations different between any two
trees, then these trees are declared significantly worse
(Felsenstein 1993).
A concern regarding phylogenetic analyses is that
relationships may reflect shared base compositional bias
rather than a monophyletic origin. Therefore, a LogDet
transformation analysis, which attempts to correct for
base compositional bias (Lockhart et al. 1994), was also
done to determine if this had any affect on the phylo-
genetic analyses. The percentage composition of the
four nucleotides was calculated for each taxon.

Results

Of the 16 complete ssu rRNA sequences, 14 varied
little from approximately 1,800 nucleotides (nt) in
length, whereas two centric taxa, Aulacoseira ambiguа
and Cymatosira belgica, had insertions that increased
the length of their ssu rRNA coding regions to 1,847
and 2,317 nt, respectively, including amplification prim-
ers (Medlin et al. 1988). Cymatosira belgica has three
separate insertions of size 152, 199, and 122 nt. Compar-
ison of these insertions with ssu rRNA Group I intron
sequences from green algae showed no significant simi-
larity. These insertions were excluded from the data
analysis.

Results of maximum likelihood, neighbor-joining,
and weighted maximum parsimony analyses of nuclear-
coded small subunit rRNA coding regions are sum-
marized in figure 1. Bootstrap values (>50%) from
weighted maximum parsimony and neighbor-joining
analyses are placed at the internal nodes that were
shared by these and the maximum likelihood analysis.
Two MPTs, differing only in the position of the two S.
costatum strains, were generated in the unweighted
and weighted maximum parsimony analyses. The consisten-
cy index improved from 0.492 to 0.718 in the weighted
analysis.

The analyses show that the diatoms diverge ini-
tially into two clades (fig. 1, Clades I and II). These
clades do not correspond to either centric or pennate
diatoms or to the three classes defined in Round, Craw-
ford, and Mann (1990, p. 125). These clades are strongly
supported in both bootstrap analyses. Other lineages
within these two major clades with high bootstrap values
match, in general, to order level systematics in the
diatoms. The phylogenetic relationships inferred from
the maximum likelihood methods were identical to those
found in the weighted maximum parsimony bootstrap
analyses; whereas the neighbor-joining bootstrap anal-
sis showed minor rearrangements. In Clade I, the
branching order of Melosira and Stephanopyxis was
reversed, and the Coscinodiscus was sister taxon to the
Melosirales. In Clade II, Eucampia was sister taxon to
the Cymatosirales. The three weighting schemes used in
the distance analysis also recovered Clades I and II with
minor rearrangements of the branching orders within
each clade and minor differences in branch lengths (data
not shown). The Gi test provided further support for the
resolving power of this data set (Hillis and Huelsenbeck
1992). A Gi value of −0.938660 is highly significant (P
< 0.01) and indicates a strong phylogenetic signal.
Fig. 1.—(A) Phylogeny of the diatoms from ssu rRNA sequence comparisons of 30 diatom taxa with *Pelagomonas calceolata* and three Oomycetes as outgroup with the ML methods (fastDNAm1). Oomycetes were pruned from the far left of the tree. Clades I and II are labeled. Percentages of adenine are shown in (). Encircled figures above the internal nodes are bootstrap values based on neighbor-joining analysis. Italic figures below the internal nodes are bootstrap values based on a weighted maximum parsimony analysis. Centric taxa with peripheral labiate processes are in hatched lines; those with a central labiate process are in stippled lines; those with central strutted processes are in striped lines. The period of time before the diversification into the two major clades is represented by a "*." (B) Results of user-defined tree analyses using the DNAML program (PHYLIP) and maximum parsimony analyses (PAUP) in which the tree in figure 1A is compared to alternative hypotheses concerning the monophyly of the centrics and lineages therein. The log-likelihoods of the "best" and the user-defined trees were statistically compared (Kishino and Hasagawa 1989).
Clade I contains radial centric diatoms of the orders Coscinodiscales, Rhizosoleniales, Corethrales, and Melosirales (fig. 1). These taxa have tubes (i.e., labiate processes) located in most cases, in a peripheral position in the cell wall. These tubes can have large and elaborate internal openings, such as those in the Coscinodiscales and Rhizosoleniales, or openings that are small and slit-like, as in the Melosirales.

Clade II is composed of three groups (fig. 1). The bi(multi)polar centric diatoms are polyphyletic and can be found in all three groups. The Chaetocerotales and true Hemiaulales diverge together in the first lineage. The second lineage contains the Cymatosirales. The third lineage gives rise to the pennates, which appear to diverge at the same time as another clade containing the Lithodesmiales, Streptotheca (presently misclassified as a hemiauloid diatom), and the Thalassiosirales. Taxa in the bi(multi)polar orders have simple, usually centrally located tubes, i.e., labiate processes. The radial centric diatoms of the order Thalassiosirales also have centrally located tube(s), but these are strutted processes. They do have a labiate process, but it is elaborate and lies in a peripheral position. Significantly in all analyses, Streptotheca and Ditylum, two taxa with a central tube-like labiate process and similar cytological detail (von Stosch 1977), diverge with the Thalassiosirales. Among the pennate diatoms, only the araphid taxa possess tubes through the cell wall (labiate processes) usually at the cell apices. Weak bootstrap support for the branching order within Clade II in both the neighbor-joining and weighted maximum parsimony analyses implies that a preferred branching order cannot be determined. This can reflect either a rapid radiation in these diatoms or a slow-down in sequence divergence at the time of the radiation (see Leipe et al. 1994).

An analysis of the base composition of the rRNA coding regions showed that some taxa had biased nucleotide compositions (fig. 1). The difference between the taxa with the two most varying adenine compositions is 3%. When taxa with adenine values greater than 28% were deleted from distance and maximum parsimony analyses, tree topologies did not change (data not shown). In the LogDet transformation analysis, the same two primary clades were recovered (data not shown). The topology of the first clade is identical to that in the maximum likelihood tree. However, in the second clade, there are minor rearrangements of the branching order of the major lineages as found in the distance and the weighted maximum parsimony analyses. The differences in the branching order in each of the analyses is reflected by the short branch lengths separating the major lineages and the lack of bootstrap support for their order in the distance and parsimony analyses. Thus, elevated adenine compositions in some taxa may not be significant because the inclusion of these taxa in the analysis does not appear to alter significantly the phylogenetic relationships recovered in the other analyses. In all analyses, the same two major clades are recovered.

Given that the recovered clades do not correspond to morphological-based systematics of the diatoms, three alternative topologies were tested. To address the monophyly of the centrics, we exchanged Clade I with the pennate diatoms. To address the monophyly of the centrics with three independent lineages, we maintained the first rearrangement and then grouped the centric diatoms into three lineages: the radial centrics, the bipolar centrics and the centrics with strutted processes. The third user-defined tree maintained the original maximum likelihood tree topology but removed Ditylum and Streptotheca to a separate branch so that the pennates and the Thalassiosirales were sister clades. These trees were used to constrain the maximum likelihood analysis and the maximum parsimony analysis. Statistics associated with each analysis were compared to those obtained in the original analyses, which were assumed to represent the best tree. The user-defined tree analyses (fig. 1B and which required more steps in the maximum-parsimony analyses.

**Discussion**

The diatoms represent an important monophyletic radiation within the heterokont algae (Mann and Marchant 1989; Round, Crawford, and Mann 1990; Bhattacharya et al. 1992; Leipe et al. 1994) and, taken together with recent evidence for their monophyletic grouping with other heterokonts with a reduced flagellar apparatus (Saunders et al. 1995) may constitute a new algal taxon. The presumed dichotomy of the diatoms into centric and pennate taxa based on pattern centers, reproduction, and plastid morphology is not supported by either 18S or 28S rRNA sequence comparisons (Söhrmann et al. 1995). User-defined trees also do not support a dichotomy into centric and pennate taxa.

The divergence in the diatoms revealed by the rRNA tree is difficult to reconcile using morphological features that currently define diatom classes and orders. Valve polarity has been useful in defining several of the major lineages within each clade. However, our analyses also suggest that certain morphological features are not useful markers for delineating the deeper branches in the rRNA tree. A mapping of the presence of labiate processes onto the tree (fig. 1) shows that they are found in both clades, although its position in the cell wall can
separate the two clades. The strutted process has a unique derived origin within Clade II taxa. However, details of cellular architecture, where available, can distinguish the two clades.

In Clade I, the labiate processes are located peripherally in the cell wall. In many taxa assigned to this clade, cytoplasmic strands extend from the nucleus to the peripheral labiate processes to anchor the nucleus during interphase and new valve formation (Schmid 1994). In Coscinodiscus, Stephanopyxis, and Ellerbeckia (Melosirales), Golgi bodies are associated with a mitochonridion in an endoplasmic reticulum cisternae (Schmid 1988 and personal communication), a character also found in the Oomycetes and, therefore, likely a primitive feature of all diatoms.

The bi(multi)polar centrics (except for Ditylum/ Streptotheca) are the first two divergences in Clade II, although their branching order is not resolved. Their labiate processes are in a central position in the cell wall and are a simple tube structure with a slit-like internal opening. The Thalassiosirales, which have centrally located strutted processes, diverge at the same time as Ditylum and Streptotheca, which have a simple tube-like central labiate process. Among the pennate diatoms, only the araphid taxa have labiate processes. These are generally located at the cell apices and are separated by a siliceous rib extending the length of the cell wall. Cytoplasmic threads anchor the nucleus to the central labiate processes of the bipolar taxa (Schmid 1994) or to the central strutted processes in the Thalassiosirales (Schmid 1984). The marginally located labiate process of the Thalassiosirales is not involved in these anchoring mechanisms and is likely of an independent origin. There is no available information for the possible cytoplasmic anchoring function of the labiate process in araphid pennate diatoms; however, in the raphid pennate diatoms, cytoplasm remains attached to the central siliceous raphe ribs (Höfler 1940) during plasmolysis, indicating that this structure functions as an anchoring site for the cytoplasm. In the Thalassiosirales and the pen nates, the Golgi bodies are arranged in a perinuclear shell or 'Plattenband' (Schmid 1989), but we are unaware of any documentation of the position of the Golgi bodies in the bipolar centrics.

These data suggest that the strutted process is derived from a labiate process or a tube-like structure that has been termed a labiate process. When Ditylum and Streptotheca, taxa with central labiate processes, were removed as sister taxa to the Thalassiosirales, the tree was significantly worse. There is a structural similarity between the perforations in the cell walls of Ditylum and the adjacent pores associated with the strutted processes of the Thalassiosirales (see illustrations in Li and Vol-
structure begins as an invagination of the cell wall and gradually transforms into a distinct tube (Gersonne and Harwood 1990; Nikolaev and Harwood 1995). This tube may be a predecessor for either the modern-day strutted or labiate processes; however, the poor preservation of the diatom assemblages between the Lower (115–110 Mya) and Upper Cretaceous (87–65 Mya) preclude the establishment of a direct link between the tube structures found in the Lower Cretaceous taxa and the tube structures found in modern day taxa. Nevertheless, overall valve structure would suggest that the fossil taxa with a central tube from the Upper Cretaceous are related to Clade II taxa with a central labiate or strutted process in the cell wall. Fossil taxa lacking any central tube from the Upper Cretaceous have an overall valve structure like Clade I taxa and more closely resemble the taxa from the Jurassic. Thus, this early, best preserved diatom deposit provides speculative ancestors for both clades.

The taxonomic implications of the phylogeny inferred from the ssu and the limited data set from the lso rRNA coding regions (Sörmanns et al. 1995) support a major reclassification of the diatoms at higher taxonomic levels. Clearly, the features considered essential in present-day systematics of the diatoms represent derived characters that cannot be used as they are presently defined to characterize the separation of the two major clades of diatoms. Cladistic analyses of extant morphological features would not, for example, recover the molecular tree because structures, such as the labiate processes, believed to be homologous in current systematics are shown to have a paraphyletic origin. However, if labiate process are redefined, then the molecular and molecular data correlate well. Our analyses have highlighted the incongruencies in the two data sets that should be further studied. It is clear that a modern ultrastructural study of labiate process development/morphogenesis for the two clades is needed. The position of the labiate process appears to be highly significant and may be the result of a basic difference in valve morphogenesis between the two clades. The strutted process of the Thalassiosirales, the central labiate process of the bipolar centrics, and the raphe are likely derived from the same ancestral structure. Furthermore, the molecular analysis provide insights into the major period of diatom radiation that cannot be reconstructed on the basis of the existing fossil record.

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APPENDIX

**OOMYCETES:** Lagenidium giganteum Couch, X54266; Phytophthora megasperma Drech., X54265; Achlya bisexualis Coker M32705. **PELAGOPHYCEAE:** Pelagomonas calceolata And. and Saund., U14389. **BACILLARIOPHYTA:** Coscinodiscophyceae: Thalassiosira eccentrica (Ehrenb.) Cl., X85396; T. rotula Meinier, X85397; Porosira glacialis (Grun.) Jörg, X85398; Skeletonema costatum (Grev.) Cl., X52006; S. costatum, X85395; S. pseudocostatum Medl., X85393; S. pseudocostatum Medl., X85394; Lauderia borealis Gran, X85399; Melosira varians C. Ag., X85402; Stephanopyxis cf. hroschi Gran, M87330; Aulacoseira distans (Ehrenb.) Sim., X85403; A. ambiguia (Grun.) Sim., X85404; Coscinodiscus radiatus Ehrenb., X77705; Actinocyclus curvatus Jan., X85401; Eugamnium antarctica (Castr.) Mang., X85389; Strepheotheca thamesis Shrubs., X85385; Ditylum brightwelli (West) Grun., X85386; Corethron criophilum Castr., X85400; Cymatiosira belgica Grun. in V. H., X85387; Papillocellulus elegans Hasle, von Stoch and Syv., X85388; Rhizosolenia setigera Brightw., M87329; Chaetoceros didymus Ehrenb., X85392; C. rostratus Laud., X85391; Chaetoceros sp., X85390. **FRAGILARIOPHYCEAE:** Fragilariella sratula Lyngb., X77704; Asterionellopsis glacialis (Castr.) Round, X77701; Rhaphoneis cf. belgica (Grun.) Grun., X77703; Thalassionema nitzschioide (Grun.) V.H., X77702. **BACILLARO-IOPHYCEAE:** Nitzschia apiculata (Greg.) Grun., M97334; Bacillaria paxillifer (Müll.) Hend., M87325; Cylindrothea closterium (Ehrenb.) Reim. and Lewin, M87326.

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