Three extant genera of freshwater thalassiosiroid diatoms from Middle Eocene sediments in northern Canada[1]

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The evolutionary history of diatoms is only constrained partially by the fossil record. The timing of several key events, such as initial colonization of freshwater habitats by marine taxa, remains poorly resolved. Numerous specimens of the genera *Cyclotella*, *Discostella*, and *Puncticulata* (Ochrophyta: Thalassiosirales) have been recovered in Middle Eocene lacustrine sediments from the Giraffe Pipe locality in the Northwest Territories, Canada. These diatoms extend the fossil record of the family Stephanodiscaceae to at least 40 million years before present (Ma) and thus provide a new evolutionary milepost for the thalassiosiroid diatoms, an important clade of centric diatoms with global representation in both marine and freshwater environments. The quality of the fossil material enables detailed investigations of areolae, fultoportulae, and rimoportulae, revealing direct morphological affinities with a number of extant taxa. These observations extend the antiquity of several characters of phylogenetic importance within the thalassiosiroid diatoms, including the fultoportula, and imply that the entire lineage is considerably older than prior constraints from the fossil record, as suggested independently by several recent molecular phylogenies.

Key words: *Cyclotella*; *Discostella*; Eocene; evolution; fossil diatoms; fultoportula; morphology; *Puncticulata*; Thalassiosirales.

Diatoms are a highly successful clade of microscopic photoautotrophs, accounting for approximately 20% of global photosynthesis (Falkowski and Raven, 1997). However, the evolutionary history of the diatoms is poorly understood relative to other major phytoplankton groups, ultimately limiting the extent to which hypotheses concerning their rise to ecological prominence can be evaluated (Falkowski et al., 2004). Poor concordance between molecular and paleontological estimates of major events in diatom evolution places a premium on fossil localities with exceptional preservation and adequate geochronology.

The order Thalassiosirales Glezer and Makarova contains about 40 validly published genera of living and fossil diatoms that are distributed globally in freshwater and marine environments (Table 1). These diatoms present an impressive array of morphological characters, including fultoportulae (strutted processes), rimoportulae (labiate processes), and in some taxa, loculate areolae with internal cribrae. Valve-face areolae frequently form striae with either linear or radial arrangements. In several genera, areolae are organized as distinct fascicles with or without internal radial costae on the interstriae. Despite considerable morphological and ecological diversity, the Thalassi-

osirales form a robust natural group in all recent molecular phylogenies, with the nonfultoportulate Lithodesmiales Round and Crawford as the sister clade (Fox and Sörhannus, 2003; Sörhannus, 2004; Kaczmarska et al., 2006; Sims et al., 2006). Within the thalassiosiroid lineage, the families Thalassiosiraceae Glezer and Makarova and Stephanodiscaceae Lebour are the most common and diversified (Table 1). *Thalassiosira* Cleve is the most speciose genus in the order, with about 180 and 12 marine and fresh-water species, respectively (Round et al., 1990). This genus includes the first diatom for which the entire genome was sequenced, *T. pseudonana* Cleve, yielding a relatively compact genome (34.5 mega base pairs) that nonetheless elucidates the complexity of diatom origins (Armbrust et al., 2004). Furthermore, the culturing of *Thalassiosira* has been central to major developments in algal toxicology, physiology, and biochemistry (e.g., Rueter and Morel, 1981; Harrison and Morel, 1986; Roberts et al., 2007). Marine representatives of *Thalassiosira* appear closely related to several freshwater genera of the family Stephanodiscaceae, the so-called cyclostephanoid group that includes *Cyclostephanos* Round, *Cyclotella* Kützing ex de Brébisson, *Discostella* Houk and Klee, *Puncticulata* Håkansson, and *Stephanodiscus* Ehrenberg. It has been proposed that these genera originated from multiple events of freshwater colonization by marine thalassiosiroid taxa (Kaczmarska et al., 2006; Sims et al., 2006). Additional molecular approaches involving both nuclear and chloroplast gene sequences confirm elegantly this polyphyletic model of diversification into freshwater habitats (Alverson, 2007; Alverson et al., 2007). However, the timing of these hypothesized evolutionary radiations remains poorly constrained. Continued interrogation of the fossil record is therefore necessary to furnish mileposts for various evolutionary events among the thalassiosiroid diatoms.

In this communication, we report unambiguous specimens of the genera *Cyclotella*, *Discostella*, and *Puncticulata* in lake sediments ≥40 Ma from northern Canada. These fossil diatoms are morphologically similar to the modern species *C. michiga-*

TABLE 1.   A classification scheme for the thalassiosiroid diatom genera, integrating currently available molecular, morphological, and paleontological data and including both extant and fossil representatives. Genera only known from the fossil record and presumed extinct are in boldface. Five additional validly published genera are not included due to either uncertain taxonomic positions at the family level, or possible elements of synonymy: *Cymatodiscus* Hendey 1958, *Cymatotheca* Hendey 1958, **Ectodictyon** Khursevich and Cherniaeva 1989, *Pleurocyclos* Casper and Scheffler 1986, and *Stephanocyclus* Skabitschevsky 1975.

Taxon, authority, and year of publication

Domain Eukarya Whittaker and Margulis 1978
  Kingdom Chromista Cavalier-Smith 1981
    Subkingdom Chromobiota Cavalier-Smith 1991
      Infrakingdom Heterokonta (Cavalier-Smith) emend. Cavalier-Smith, 1995
        Phylum Ochrophyta Cavalier-Smith 1986
          Subphylum Diatomeae (Dumortier) Cavalier-Smith 1991
            Class Coscinodiscophyceae Round, Crawford and Mann 1990
              Subclass Thalassiosirophycidae Round, Crawford and Mann 1990
                Order Thalassiosirales Glezer and Makarova 1986
                  Family Lauderiaceae (Schütt) emend. Medlin and Kaczmarska 2006
                    Genera
                      Lauderia Cleve 1873
                      Porosira Jørgensen 1905
                  Family Skeletonemataceae Lebour 1930
                    Genera
                      Cyclotubicoalitus Stoermer, Kociolek and Cody 1990
                      Detonula Schütt ex DeToni 1894
                      Schroederella Pavillard 1913
                      Skeletonema Greville 1865
                  Family Stephanodiscaceae Glezer and Makarova 1986
                    Genera
                      Concentrodiscus Khursevich, Moisseeva and Sukhova 1989
                      Crateriportula Flower and Håkansson 1994
                      Cyclostephanos Round 1987
                      Cyclostephanopsis Loginova 1993
                      Cyclotella Kützing ex de Brébisson 1838
                      Discostella Houk and Klee 2004
                      Mesodictyon Theriot and Bradbury 1987
                      Mesodictyopsis Khursevich, Iwashita, Kociolek and Fedenya 2004
                      Pelagodictyon Clarke 1994
                      Pliocaenicus (Round and Håkansson) emend. Flower, Ozornina, Kuzmina and Round 1998
                      Puncticulata Håkansson 2002
                      Stephanocostis Genkal and Kuzmin 1985
                      Stephanodiscus Ehrenberg 1845
                      Stephanopsis Khursevich and Fedenya 2000
                      Tertiariopsis Khursevich and Kociolek in Khursevich, Kociolek and Fedenya 2002
                      Tertiarius Håkansson and Khursevich 1997
                  Family Thalassiosiraceae Lebour 1930
                    Genera
                      Bacteriosira Gran 1900
                      Coenobiodiscus Loeblich, Wight and Darley 1968
                      Coscinosira Gran 1900
                      Lomonycus Komura 1996
                      Microsiphona Weber 1970
                      Minidiscus Hasle 1973
                      Nephrodiscus Komura 1996
                      Parthaea (Schmidt) Gowthaman 1996
                      Planktoniella (Wallich) Schütt 1892
                      Poloniasira Kaczmarska and Ehrman 2008
                      Roundia (Round) Makarova 1994
                      Shionodiscus Alverson, Kang and Theriot 2006
                      Takanoa Makarova 1994
                      Thalassiocyclus Håkansson and Mahood 1993
                      Thalassiosira Cleve 1873
                      Thalassiosiropsis Hasle and Syvertsen 1985
                      Tryblioptychus Hendey 1958

niana Skvortzow, *D. stelligera* (Cleve and Grunow) Houk and Klee, and *P. radiosa* (Lemmermann) Håkansson. These observations extend the fossil record of the fultoportula by approximately 10 million years. In this light, diatom phylogenetic schemes that integrate paleontological and molecular data are in need of substantial revision.

MATERIALS AND METHODS

Study site—The Giraffe Pipe locality (64°44′N, 109°45′W) is a kimberlite diatreme that has been infilled by a sequence of Middle Eocene lacustrine and paludal sediments and subsequently back-filled by Neogene glacigenic sediments (Fig. 1). The Giraffe Pipe is one of many kimberlites in the Lac de Gras

field, most of which have Cretaceous or Paleogene emplacement ages (Heaman et al., 2004). A 165-m drill core collared at 47° was raised by BHP Billiton Diamonds in 1999 (core BHP 99–01) to assess the diamond potential of kimberlite underlying the crater fill. Core BHP 99–01 contains 113.1 m of stratified organic sediment of Middle Eocene age, including 44.8 m of peaty material underlain by 68.3 m of lacustrine mudstone. Conversion of these core depths to their vertical equivalents implies stratigraphic thicknesses of 32.7 m for the peat and 51.1 m for lake sediments (Fig. 1). Two air-fall tephra beds occur at the transition between lacustrine and paludal sedimentation, which marks the final infilling of the maar and incipient colonization by a forest composed dominantly of *Metasequoia* Miki ex Hu and Cheng (Cupressaceae).

Lake sediments—The recovered lake sediments include both massive and fissile core sections that part along bedding planes, commonly preserving fine laminations and mottling with nodular opal-A (Fig. 2). The opal-A is believed to be an early diagenetic product that formed as silica-rich groundwater entered the lake via bedrock fractures formed during kimberlite intrusion. Organic matter represents 10–40% of the bulk mudstone by mass, with minimal evidence of thermal maturation. In several intervals of the core, diatom cellular contents are preserved, implying some combination of anoxic bottom-waters, rapid sedimentation, and low energy in the depositional environment, coupled to little post-eruptive thermal and tectonic activity (Wolfe et al., 2006). The current study is based on samples from every ~50 cm in the lower core (85–110 m equivalent vertical depth, Fig. 1C). Below this depth, diatoms and other siliceous microfossils are scarce, but colonies of *Botryococcus* Kützing (Chlorophyceae) are profuse. Above the studied interval, diatoms belonging to the families Aulacoseiraceae Crawford and Eunotiaceae Kützing become abundant, as do scaled chrysophytes of the class Synurophyceae Andersen, all of which are exquisitely preserved (Siver and Wolfe, 2005, 2007; Wolfe et al., 2006). The interval considered here thus reflects the lake's initial diatom communities.

Geochronology—The age of the lacustrine sediments is assessed in several ways. First, a ^{87}Rb/^{87}Sr model age of 47.8 ± 1.4 Ma from kimberlitic phlogopite provides a maximum age for the overlying sediments (Creaser et al., 2004).

Second, diameter-corrected ($N = 2$) and isothermal-plateau ($N = 1$) fission track dates from the tephra beds are highly coherent and statistically indistinguishable from 40 Ma (J. Westgate, Unversity of Toronto, personal communication, 2007). This provides a minimum age for the underlying lacustrine sequence. Third, pollen assemblages throughout the core contain a number of diagnostic Middle Eocene taxa (Rouse, 1977; Wolfe et al., 2006). We therefore envisage that, following the phreatomagmatic kimberite emplacement event, a lake formed within the crater and persisted for ~7–8 million years before being infilled by sediment and colonized by terrestrial vegetation.

Sample preparation—Specimens were prepared for scanning electron microscopy (SEM) as follows. Small chips (~200 mg) of mudstone were first oxidized with 30% H_2O_2 overnight to attack organic matter, and then centrifuged and rinsed several times with deionized water (reverse-osmosis filtration). Samples of cleaned slurry were air-dried onto heavy-duty aluminum foil, trimmed, and mounted to aluminum stubs with Apiezon wax. Additional examinations were made of unprepared material. In this case, fresh fractures perpendicular to bedding planes were mounted onto stubs with double-sided carbon tape, and ringed with silver paint. These stubs were sputter-coated with Au or Au-Pd mixtures, prior to examination with either a Leo 982 (University of Connecticut) or a JEOL-6301F (University of Alberta) field-emission SEM. For light microscopy (LM), both H_2O_2-digested slurries and untreated disaggregated mudstone (<125 μm fraction) were mounted in Naphrax and examined under oil immersion objectives.

RESULTS

Despite excellent and continuous preservation of biogenic silica in the lower lacustrine facies of the Giraffe pipe core, diatoms are relatively uncommon. Chrysophyte stomatocysts, scales, and bristles are by far the most abundant siliceous microfossils. Cysts often constitute discrete laminae, but are also abundant in the organic-rich sediment matrix (Fig. 2D, E).
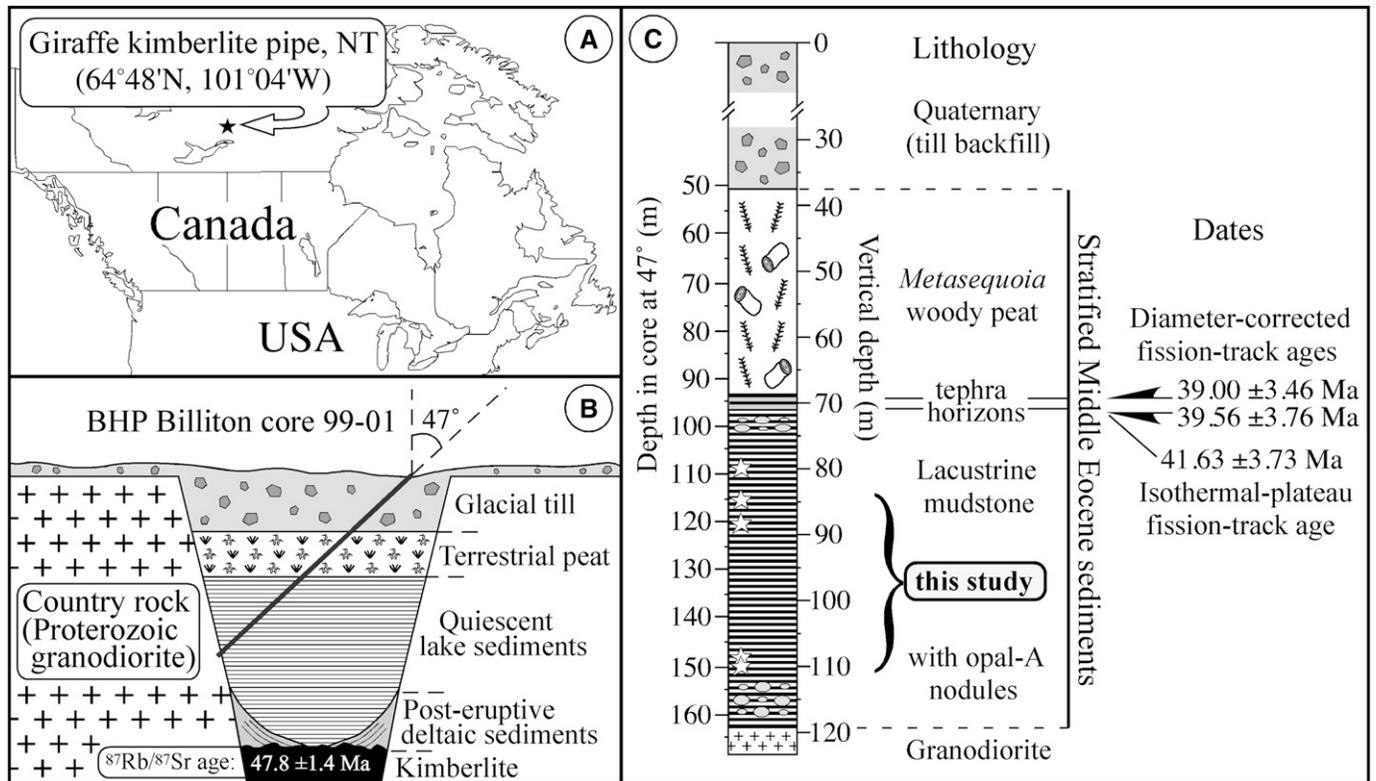

Fig. 1.   (A) Location of the Giraffe pipe fossil locality, (B) schematic stratigraphy of posteruptive sediments in the kimberlite diatreme, and (C) detailed lithostratigraphy and chronology of core 99–01. The interval of core considered in the current study is in boldface in (C), whereas stars indicate the depths illustrated in Fig. 2.
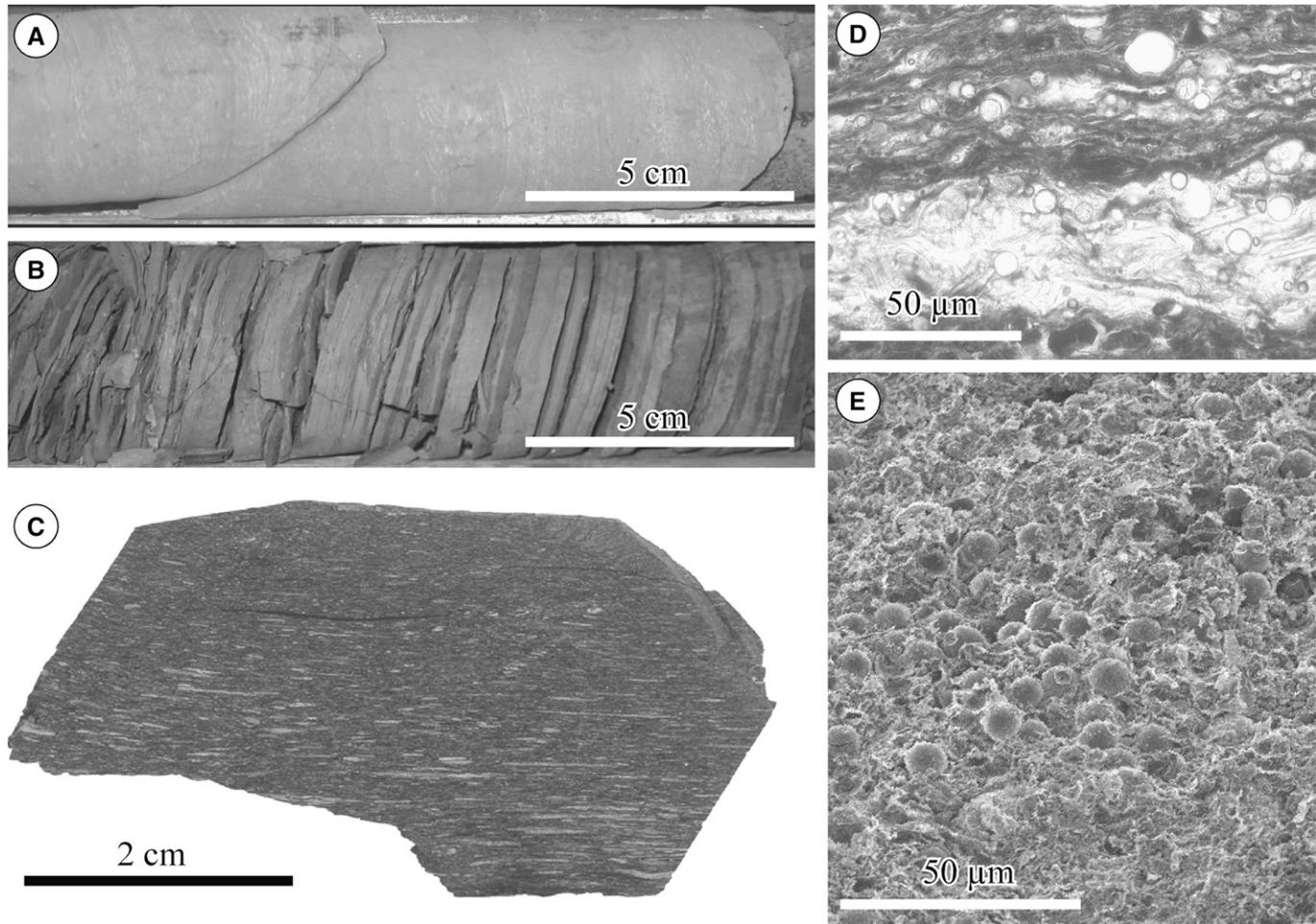
Fig. 2.   Examples of Giraffe Pipe lacustrine sediments. The core contains mudstone that ranges from (A) massive to (B) fissile. In many sections, (C) macroscopic opal-A nodules are evident as subparallel partings. (D) Petrographic thin sections indicate that organic-rich mudstones are frequently finely laminated. Dark laminae comprise amorphous organic matter and chrysophyte cysts, whereas light laminae are composed of chrysophyte cysts and occasional diatom valves. (E) Surfaces of individual bedding planes often reflect massive chrysophyte cyst deposition events. Intact diatoms, including the taxa reported here, can occur in association with these cyst beds in trace abundances. These images are centered on the following core depths: 116.1 m (A), 109.9 m (B), 149.7 m (C), 150.1 m (D), and 121.3 m (D), as indicated in Fig. 1C.

Siliceous sponge spicules and gemmoscleres (birotules), as well as euglyphid protozoan scales (testate amoebae), are also more abundant than diatoms. From the census of SEM observations, we estimate that diatoms represent 0.5–5.0% of total siliceous microfossils. The most common diatoms in this portion of the core are araphid pennate taxa, including *Fragilaria* Lyngbye, *Fragilariforma* (Ralfs) Williams and Round, *Oxyneis* Round, *Staurosira* (Ehrenberg) Williams and Round, and *Staurosirella* Williams and Round, which will be reported upon separately. However, in the course of intensive SEM efforts (i.e., >100 h), regular observations of several morphotypes of thalassiosiroid diatoms were made. These account for ≤10% of the total diatoms encountered, implying that between a few hundred and several thousand siliceous microfossils were examined for each thalassiosiroid diatom. Nonetheless, given the sheer richness in most samples, a typical SEM session would yield several observations of the thalassiosiroid forms.

Given the resemblance of these diatoms to modern taxa, serious concerns were raised concerning potential contamination, both in the laboratory and from stratigraphic remobilization.

Multiple additional and independent preparations were conducted in our respective laboratories with previously unused glassware, revealing rare but consistent occurrences of these diatoms across a range of samples from the lower core. Subsequently, fragments of these diatoms, including broken valves and copulae, were observed embedded within the matrix of undigested freshly fractured surfaces, confirming that they are part of the primary sedimentary material. We found no evidence in any of our preparations for contamination by species that commonly co-occur with these diatoms in modern lakes, which we believe would have been conspicuous. The possibility that these microfossils were leaked from younger strata can also be discounted because the ~30 m peat facies that overlies the lake sediments is devoid of diatoms and chrysophyte microfossils. Furthermore, material originating from the outer core surface was consistently excluded by sampling only freshly fractured surfaces well beneath the surface, hence obviating the possibility of contamination during drilling. Finally, high-magnification SEM shows that the surfaces of these diatoms are lightly but visibly etched, to an extent not predicted for modern contami-

nants. Fortunately, this degree of etching does not impede observations of fine structures. Through these efforts, we have convinced ourselves that the diatoms we report here are indeed of Middle Eocene age. If additional authentication is warranted, voucher material is available upon request from the authors.

***Cyclotella* Kützing ex de Brébisson 1838 (Figs. 3–10)**—Specimens observed from Giraffe pipe sediments conform in every way to current concepts of the genus *Cyclotella* (Round et al., 1990; Håkansson, 2002). Accordingly, Giraffe pipe *Cyclotella* have an undulating central area and robust radial costae (Figs. 3–5). Valve margin fultoportulae occur primarily adjacent to every fourth stria (Fig. 6), or occasionally every fifth or sixth (Fig. 8). Valve-face fultoportulae are clustered asymmetrically, opposite to both the prominent convex undulation (Figs. 3–7) and the single sessile rimoportula (Figs. 9–10). Valve-face fultoportulae generally have two satellite pores, although most specimens also possess a single fultoportula bearing three satellite pores (Fig. 7).

Although we refrain from attaching a specific epithet to *Cyclotella* specimens from the Giraffe locality, we note that the morphology and dimensions of Middle Eocene forms recall the modern lacustrine diatom *Cylotella michiganiana* Skvortzow. For example, Skvortzow (1937) treated the undulating central area with "large beads" disposed on one side only as defining characters of this species. Undoubtedly, the large beads alluded to represent valve-face fultoportulae (Figs. 3–5), as observed by Skvortzow in LM.

***Discostella* Houk and Klee 2004 (Figs. 11–22, 29)**—A second group of thalassiosiroid diatoms observed in Giraffe pipe sediments is characterized by the stellate organization of the central area. Valves have a convex or concave central area, frequently with a colliculate external morphology (Fig. 11). Stellate alveoli are occluded by cribrae (Figs. 12–14), but in some cases do not fully perforate the valve face, resulting in depressions that express variably the stellate pattern (Figs. 17, 20, 22). Marginal fultoportulae have two satellite pores and are situated between costae, adjacent to every second to fourth stria (Figs. 15–21). A simple single rimoportula also occurs on the valve mantle (Figs. 18–21), but neither rimoportulae nor fultoportulae are present on the valve face. All these features are entirely consistent with the diagnosis of *Discostella* by Houk and Klee (2004).

Discostella is a common freshwater genus in the plankton of oligotrophic lakes and rivers. There exist myriad accounts of the dominant species, *D. stelligera* (Cleve and Grunow) Houk and Klee, under its prior name *Cyclotella stelligera* (Cleve and Grunow) Van Heurck (e.g., Kling and Håkansson, 1988; Krammer and Lange-Bertalot, 1991; Siver et al., 2005). Although *D. stelligera* has a rich fossil record, it has not been reported previously from sediments older than Miocene (Fourtanier et al., 1993). Moreover, most Neogene fossil specimens differ little, if at all, from either modern or Giraffe pipe specimens. Many of the *Discostella* specimens recovered from Giraffe have compelling similarities to *D. stelligera* (Figs. 11–16). These elements of similarity are especially evident in LM (Fig. 29), in comparison to modern specimens (Fig. 32). The smaller Eocene forms lacking internal valve-face alveoli are more enigmatic (Figs. 17–22), although the placement fultoportulae and the rimoportula enables their conclusive assignment to *Discostella*.

***Puncticulata* Håkansson 2002 (Figs. 23–28, 30–31)**—A third group of specimens from the Giraffe pipe belong to a species cluster of morphologically similar diatoms that formerly included *Cyclotella bodanica* (Grunow) Lemmermann, *C. comta* (Ehrenberg) Kützing, and *C. radiosa* Grunow, all of which were transferred to *Puncticulata* by Håkansson (2002). The presence of both areolae and fultoportulae on the valve face enables differentiation from *Cyclotella* sensu stricto (Figs. 23–26). The valve face is either convex or concave. Valve-face areolae have internally domed cribrae, whereas valve-face fultoportulae are surrounded by either two or more commonly three satellite pores (Fig. 24). Marginal fultoportulae have two satellite pores only, which rise internally from every fourth (rarely fifth or sixth) interstria. One or two rimoportulae open internally to a labium on the valve face, marginally to the central area (Fig. 26). Collectively, these characters enable the Giraffe pipe specimens to be assigned confidently to *Puncticulata*.

Puncticulata is defined from fossil material and has been presumed to be the oldest genus within Stephanodiscaceae, extending to the Oligocene (Håkansson, 2002). On the other hand, extant representatives are distributed globally in the phytoplankton of oligotrophic lakes, typically forming species complexes that are notoriously difficult to differentiate taxonomically (Kling and Håkansson, 1988; Krammer and Lange-Bertalot, 1991; Siver et al., 2005). We demonstrate the similarity between Giraffe pipe and modern *Puncticulata* through SEM comparisons with recent specimens from west Greenland (Figs. 27, 28) and LM comparisons with specimens from Cape Cod, Massachusetts, USA (Figs. 33–34). In LM, the internal or external doming of the central area is especially evident, requiring multiple focal planes to appreciate fully. LM illustrates advantageously how both modern and Eocene *Puncticulata* have striae that occasionally bifurcate distally to the central area (Figs. 30, 33), as well as the distinct thickenings of every fourth marginal interstria (Figs. 31, 33), which mark the position of marginal fultoportulae (Figs. 23, 28). These characters confer some degree of affinity between both the modern and ancient specimens and *P. radiosa* (Lemmerman) Håkansson, as construed by Håkansson (2002).

DISCUSSION

The documentation of Middle Eocene freshwater thalassiosiroid diatoms with morphological affinities to modern taxa has numerous implications for the evolution of the lineage. The emergence of these diatoms in lakes of western North American had been hitherto assigned to the Middle and Late Miocene (*Cylotella* sensu lato followed by *Mesodictyon* Theriot and Bradbury), with further speciation during the Pliocene as *Stephanodiscus* and *Cyclostephanos* evolved (Krebs et al., 1987). These authors stated (p. 506) that they "are confident, however, that the general outline of lacustrine diatom biochronology presented herein will remain intact." While their biostratigraphic scheme has endured with respect to *Mesodictyon*, which appears restricted to the Late Miocene globally (Fourtanier et al., 1993; Khursevich, 2006), it is now concluded that the cyclostephanoid genera *Cyclotella*, *Discostella*, and *Puncticulata* had evolved by the Middle Eocene, in the order of 20 million years prior to previous estimates.

Central to arguments concerning thalassiosiroid evolution is the emergence of the fultoportula, which, while representing the defining character of the order Thalassiosirales, mandates both excellent preservation and the use of SEM to document
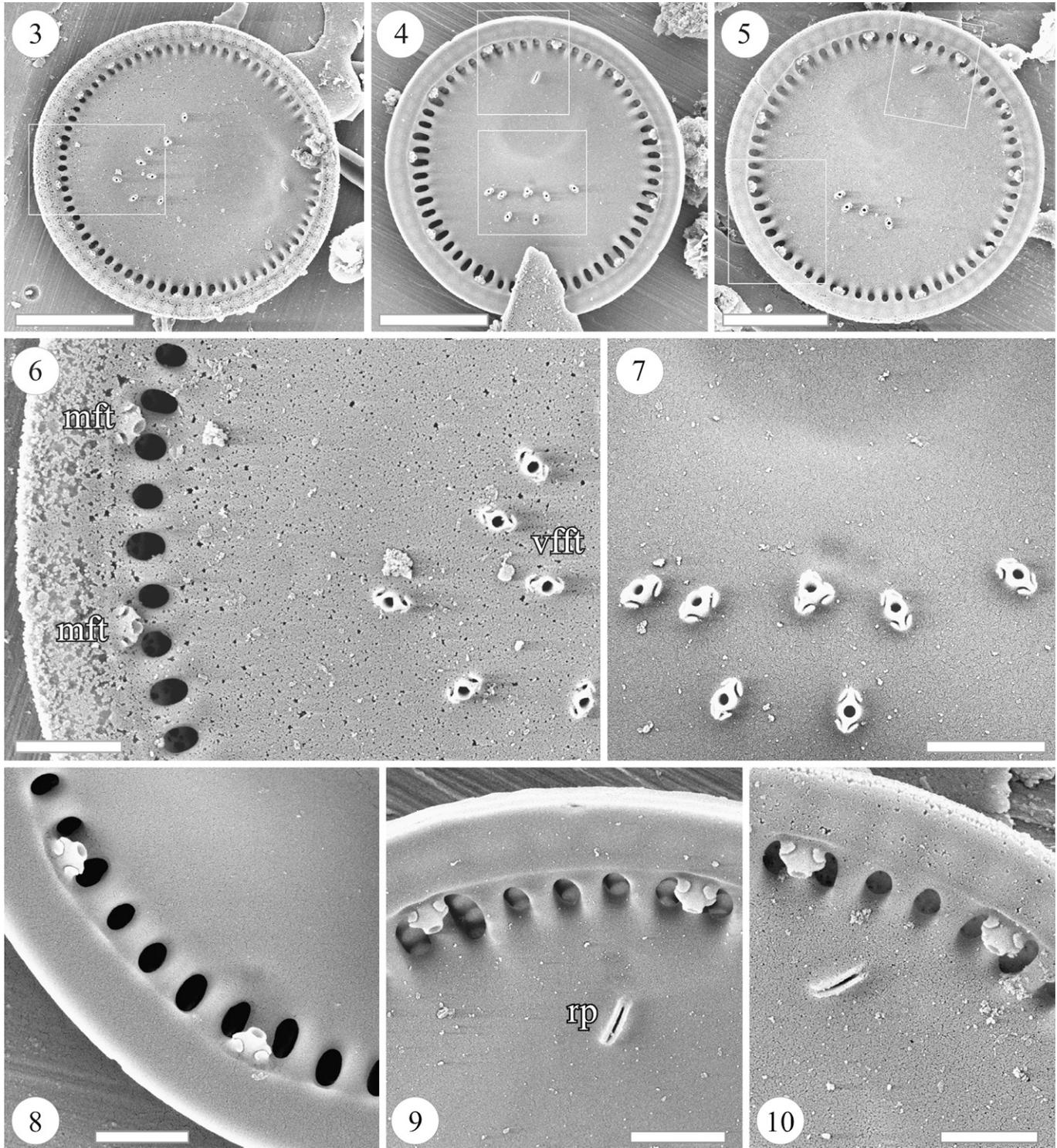
Figs. 3–10.   Diatoms from the Giraffe pipe locality identified as *Cyclotella*. **3–5.** Internal views of whole valves reveal the asymmetric disposition of valve-face fultoportulae opposite to the convex undulation of the central area and the single rimoportula, much as in the extant species *C. michiganiana*. **6–10.** Magnifications of the areas outlined by white boxes reveals (Figs. 6, 8) marginal fultoportulae with two satellite pores, (Fig. 7) a single valve-face fultoportula with three satellite pores, and (Figs. 9–10) the single sessile rimoportula. Abbreviations: rp = rimoportula, mft = marginal fultoportula, and vfft = valve-face fultoportula. Scale bars are 10 µm for Figs. 3–5 and 1 µm for Figs. 5–10.
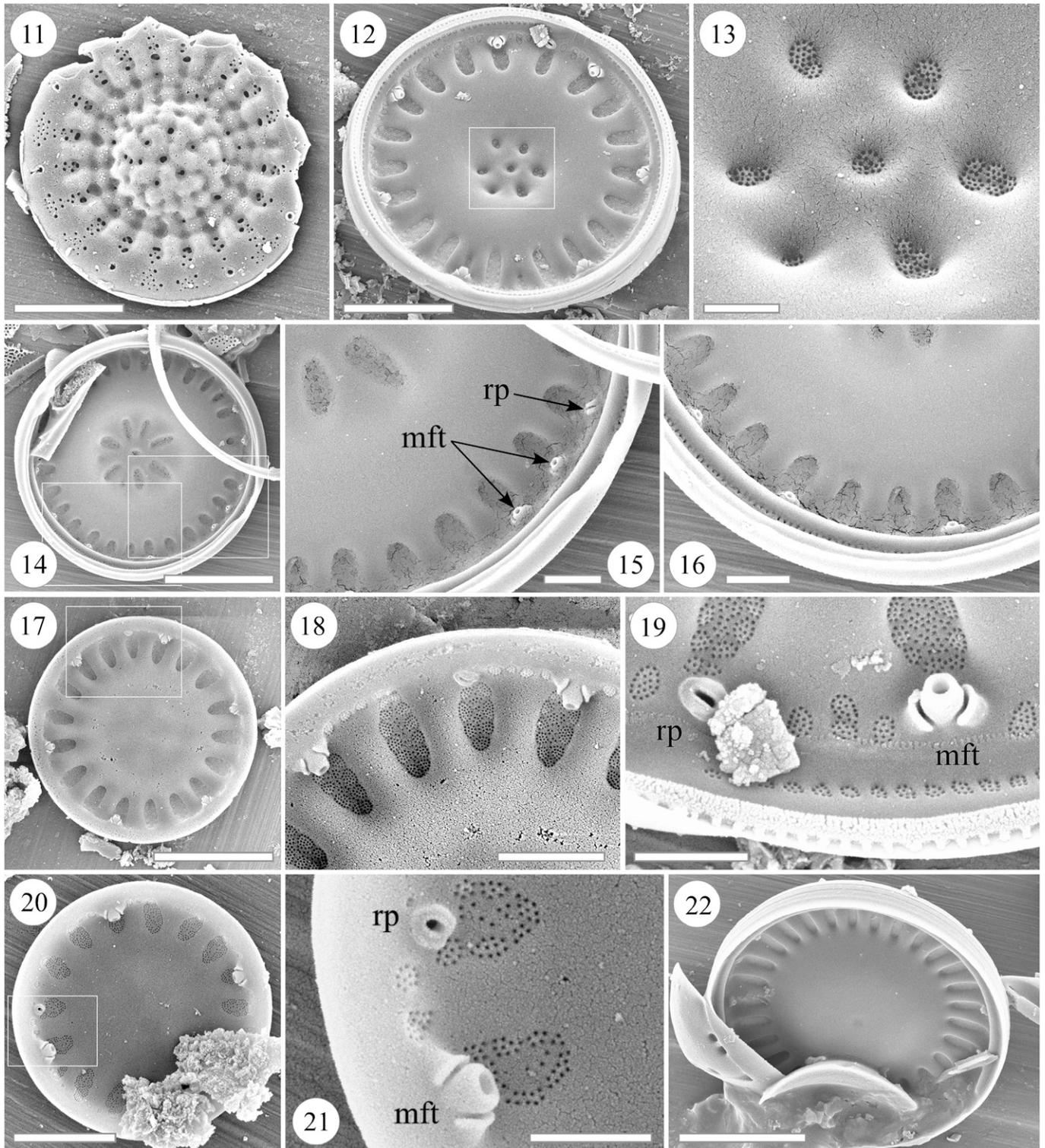
Figs. 11–22.   *Discostella* from the Giraffe pipe material. Valves have either a convex central area with colliculate external morphology (Fig. 11) or are concave (Fig. 22). Internally, a stellate organization of alveolar openings with cribrate occlusions is present in some (Figs. 12–16), but not all (Figs. 17–22) specimens. White boxes indicate areas magnified in successive images. Abbreviations are as in the previous images. Scale bars are 5 μm for Figs. 11, 12, 14, 17, 20, and 22; 1 μm for Figs. 13, 15–16, and 18; and 0.5 μm for Figs. 19 and 21.
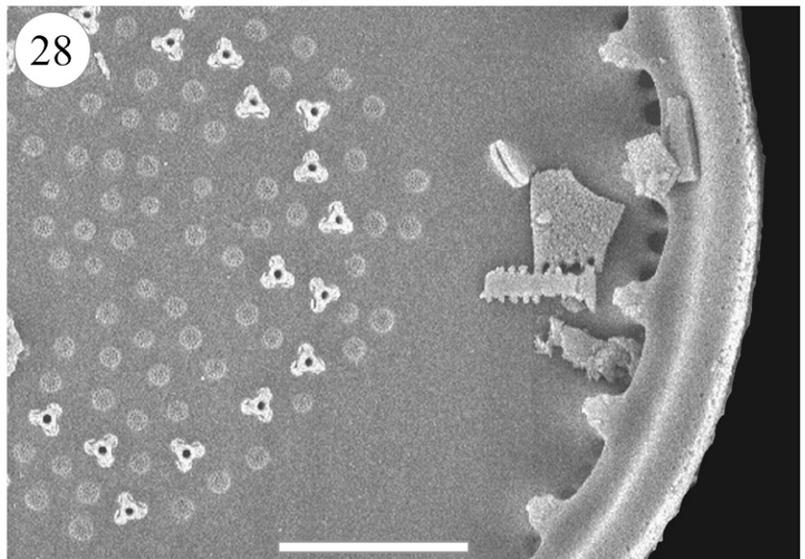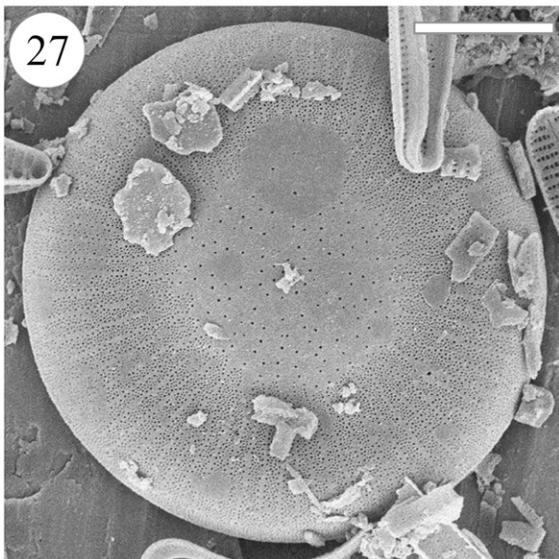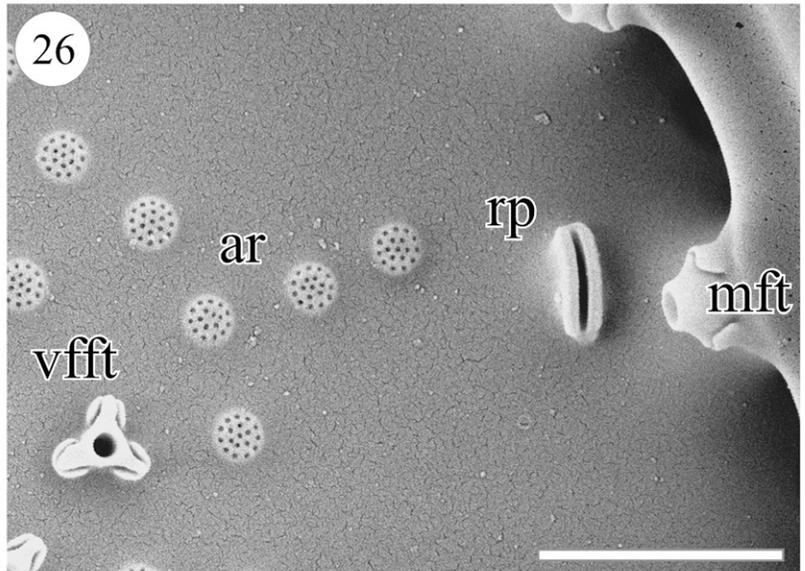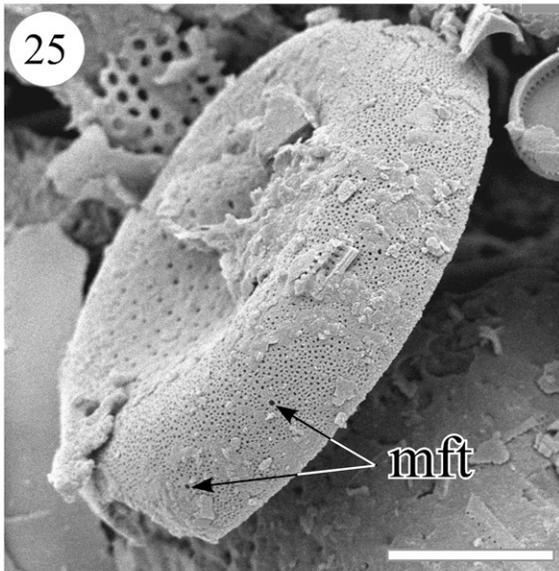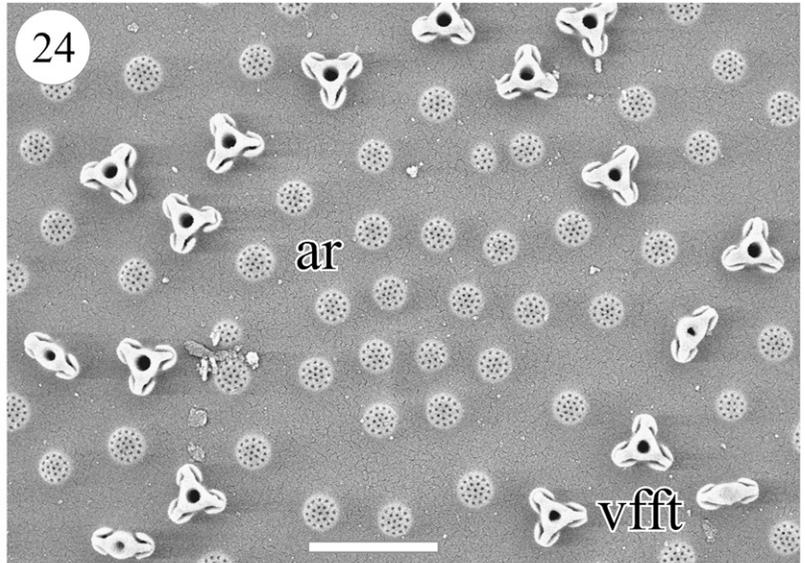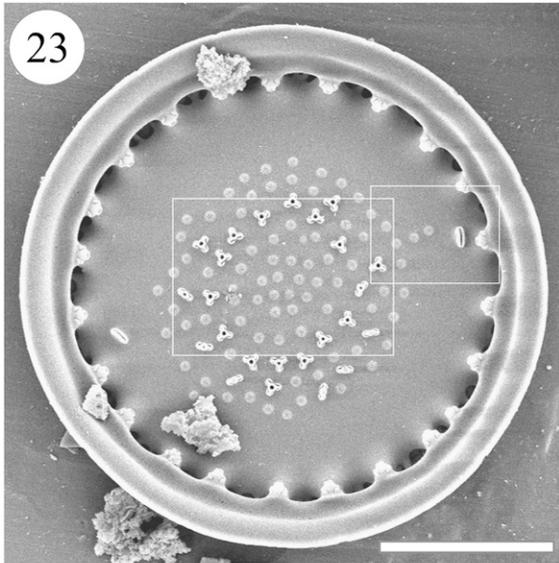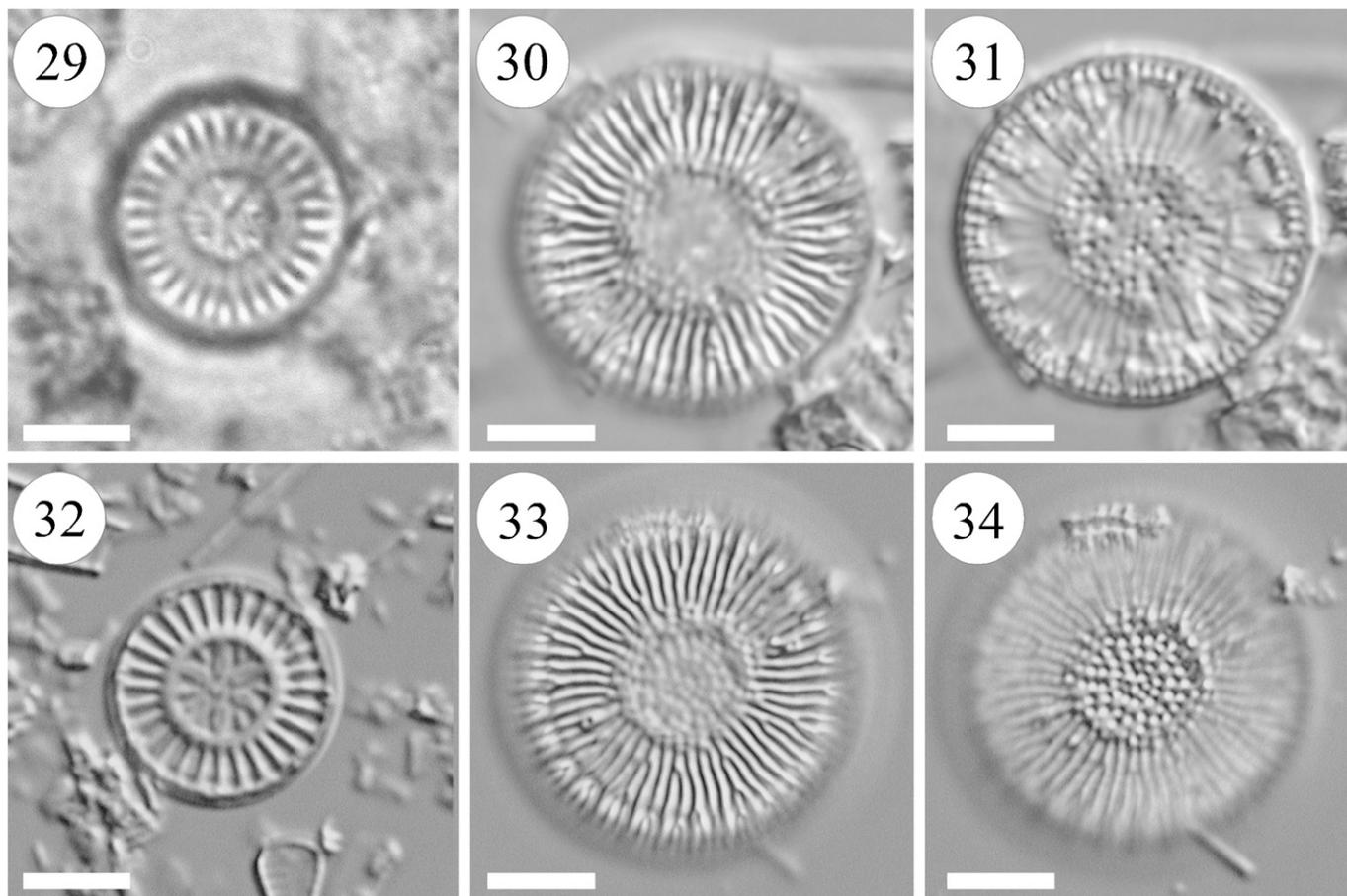
Figs. 29–34.   Paired comparisons of Middle Eocene and modern *Discostella* and *Puncticulata* in LM. The upper row (Figs. 29–31) includes specimens from the Giraffe locality, whereas the lower row (Figs. 32–34) is from Ashumet Lake, Mashpee, Massachusetts, USA. (41.63°N, 70.54°W), and identified as *D. stelligera* (Fig. 32) and *P. radiosa* (Fig. 33–34). The *Puncticulata* pairs in each row are the same specimens viewed in different focal planes. Scale bars = 5 μm.

satisfactorily. As the locus of β-chitin thread synthesis (Herth, 1978), the fultoportula is a synapomorphic trait for the thalassiosiroid lineage, to which this secondary metabolism appears unique. Threads produced by valve-face fultoportulae link adjacent cells into filamentous colonies, whereas those emerging from valve margin fultoportulae appear involved in cell buoyancy regulation (Walsby and Xypolyta, 1977). The oldest diatoms with fultoportulae unambiguously homologous to those of modern thalassiosiroids had recently been assigned to *Poloniasira* Kaczmarska and Ehrman, from the Late Oligocene (~31 Ma) of Poland (Kaczmarska and Ehrman, 2008). The current study suggests that both marginal and valve-face fultoportulae were already present in nonmarine diatoms by the Middle Eocene. Concerning the evolution of the fultoportula, Kaczmarska et al. (2006, p. 132) conceded cautiously that "the sequence of events leading to its formation is not known either from the fos-

sil record or ontogenetically." However, only the scenarios of fultoportula evolution from either the areola or the multistrutted process pass the suite of homology tests required to be retained as testable hypotheses (Theriot, 2008). The relationship between the fultoportula and the multistrutted process of Late Cretaceous and Paleogene *Thalassiosiropsis* has been suspected since the genus was erected (Hasle and Syvertsen, 1985), and the inclusion of *Thalassiosiropsis* in Table 1 reflects our conviction that homology can be postulated between the multistrutted process and the fultoportula. The architecture of fultoportulae in the Giraffe pipe specimens implies that their morphology is highly conserved over the last 40 million years in the documented genera. This narrows the stratigraphic window in which to search for key transitional morphotypes: sediments of Late Cretaceous to Middle Eocene age, but capturing both marine and freshwater environments (Harwood et al., 2007). In a more

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Figs. 23–28.   Middle Eocene and modern specimens of *Puncticulata*. Internal view of a complete valve from the Giraffe pipe material (Fig. 23), showing the distribution of areolae, fultoportulae, and rimorportulae. The white boxes outline areas shown in high magnification (Figs. 24, 26). Note the presence of valve-face fultoportulae with both two and three satellite pores (Fig. 24). Oblique external view of another specimen from the Giraffe pipe, illustrating the concave central area and external openings of marginal fultoportulae (Fig. 23). Holocene specimens of *P. radiosa* from the Kangerlussuaq region of west Greenland (Lake SS32, 66.97°N, 49.8°W) are shown for comparison (Figs. 27, 28). Areolae with internally domed cribrae are abbreviated ar; other abbreviations are as in the previous images. Scale bars are 5 μm for Figs. 23, 25, and 27; 1 μm for Figs. 24 and 26; and 2 μm for Fig. 28.

general sense, the Giraffe pipe specimens indicate that the full complement of morphological characters that define modern thalassiosiroid diatoms had evolved much earlier than previously suspected. Two hypotheses can be invoked with respect to these observations.

First, the evolution of Thalassiosirales from extinct marine centric lineages of Cretaceous age may have occurred at an accelerated tempo. This possibility may be explored through analyses of three of the Early Cretaceous genera erected by Gersonde and Harwood (1990), namely *Gladiopsis*, *Praethalassiosiropsis*, and *Rhynchopyxis*. If any of the features that characterize these genera, including the central annular, perforate, or rhyncho-shaped processes, can be proven homologous to the fultoportulae of thalassiosiroid diatoms, one or more of these taxa may yet be confirmed as the ancestral stock. Support exists for the potential of rapid speciation within the Thalassiosirales, as implied by a wealth of molecular (Kooistra and Medlin, 1996; Armbrust and Galindo, 2001) and stratigraphic (Edlund et al., 2000; Khursevich, 2006; Theriot et al., 2006) evidence. The scenario of accelerated evolution during the Cretaceous is not only consistent with these and other elements of the diatom fossil record (Sinninghe Damsté et al., 2004), but also mirrored by the spermatophyte and pteridophyte lineages on land (Schneider et al., 2004). Possibly, accelerated diversification occurred broadly across autotrophic clades at this time, in response to unique but unspecified environmental cues. One caveat to this scenario is that the backbone of the thalassiosiroid tree is relatively well resolved (Kaczmarska et al., 2006; Alverson et al., 2007), which likely places an upper limit on potential rates of speciation within the group.

A second possibility is that the entire thalassiosiroid lineage is considerably older than currently established, and inferences concerning its history have been complicated by the incomplete nature of the fossil record (Theriot, 2008). This view is consistent with molecular clocks using small subunit rRNA and various constraints from the fossil record, which suggest that the order Thalassiosirales is considerably older than implied by the available fossil record (Kooistra and Medlin, 1996; Sörhannus, 2007). Of course, the two hypotheses presented are not mutually exclusive. Moreover, the observations from the current study allow only the second to be addressed, until a fuller range of Cretaceous diatoms, including putative freshwater forms (Chacón-Baca et al., 2002), can be appraised in full morphological detail using SEM.

We argue here that it is primarily the limiting quality of the diatom fossil record that has led to discrepant age estimates for the origin of phylogenetically important characters such as the fultoportula. In this regard, exceptional fossil localities with well-constrained ages, such as the Giraffe pipe locality for Middle Eocene lakes, gain considerable importance. For example, this site has already extended by millions of years the fossil records of eunotioid diatoms (Siver and Wolfe, 2007) and of scaled chrysophytes (Siver and Wolfe, 2005). From the simple perspective of the outstanding preservation witnessed in this material, the discovery of thalassiosiroid diatoms is perhaps less surprising, all the more since the vast majority of siliceous microfossils observed to date can be attributed to extant genera. It is not only the morphology of these organisms, but also their autecologies, that appear largely unchanged since the Middle Eocene. For example, the three cyclostephanoid diatom genera reported here commonly co-occur in the phytoplankton of modern oligotrophic to mesotrophic lakes (Kling and Håkansson, 1988; Siver et al., 2005), much as they apparently did in the Middle Eocene Giraffe lake ecosystem. From the integration of these observations, we share the view that the diatom fossil record is likely considerably older than presently known, in keeping with the fact that molecular clocks place the origin of diatoms at least 100 Ma before the earliest unambiguous fossils, but unlikely earlier than 266 Ma (Kooistra and Medlin, 1996; Sims et al., 2006). As for the thalassiosiroid lineage, an age of at least 100 Ma seems most probable, in keeping with current molecular estimates that predict an origin for the clade between 125 and 149 Ma (Sörhannus, 2007).

Finally, we state with assurance that initial colonization of freshwater habitats by cyclostephanoid diatoms evolved from marine Thalassiosiraceae had occurred by 40 Ma, considerably earlier than longstanding biostratigraphic estimates placing these events in the Miocene (e.g., Krebs et al., 1987). Given the relative abundance of Cretaceous kimberlites in western and central North America (Heaman et al., 2004), as well as in southern Africa (Smith et al., 1985), concerted efforts should be directed at exploring their post-eruptive sedimentary fills for well-preserved early diatom floras. Given the results obtained thus far from Giraffe pipe sediments, the kimberlite maar depositional environment is likely to continue informing the fossil record of nonmarine diatoms.

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