

Taxonomic descriptions and evolutionary implications of Middle Eocene pennate diatoms representing the extant genera *Oxyneis*, *Actinella* and *Nupela* (Bacillariophyceae)

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Background and aims – The majority of freshwater diatom lineages appear to have evolved before or during the Eocene, with rapid radiation occurring in the Miocene. However, only a few accurately dated fossil localities are available to test this assertion, resulting in an overall poor understanding of the biostratigraphy and early evolution of these organisms. Exquisitely preserved diatoms have been uncovered from Eocene lake sediments deposited post-eruptively in the Giraffe kimberlite pipe (Northwest Territories, Canada). We describe three new pennate diatoms from this deposit and discuss their evolutionary implications.

Methods – Both oxidized preparations and whole-rock mudstone samples from the Giraffe Pipe core were examined with light and scanning electron microscopy for diatom remains.

Key results – Species belonging to the genera *Actinella* F.W.Lewis, *Oxyneis* Round and *Nupela* Vyverman & Compère are described from the Giraffe Pipe sediments. *Oxyneis apporrecta* is a unique species with valves that are centrally constricted, inflations midway between the center and apices, and protracted and rostrate apices. *Actinella giraffensis* is a small taxon that lacks a highly complex head pole, and is most closely related to a species known today only from the highlands of New Zealand. *Nupela mutabilis* has variably shaped valves possessing a raphe and unique areolae characteristic of this genus. These findings represent the first known fossils for both *Oxyneis* and *Nupela*, and the oldest record for *Actinella*.

Conclusions – None of the species are known from extant floras and are therefore considered extinct. However, the morphological features they present, including the structure of the areolae, raphe, rimoportula and girdle bands, share pronounced affinities with modern congeneric taxa. The well-developed raphe found on *Nupela mutabilis* confirms that the evolution of this structure dates to at least the Middle Eocene, and supports the hypothesis that it may be older than the Paleocene.

Key words – *Actinella*, Eocene, freshwater, fossil diatoms, *Nupela*, *Oxyneis*, raphe.

INTRODUCTION

Actinella F.W.Lewis, *Oxyneis* Round and *Nupela* Vyverman & Compère are three genera of pennate diatoms known exclusively from freshwater environments that are predominantly dilute and acidic (Round et al. 1990, Sabbe et al. 2001, Siver et al. 2007). Of the three, *Oxyneis* is the smallest genus and comprises only three species (Round et al. 1990, Metzeltin & Lange-Bertalot 2007), although only two have formally been described within the genus. *Oxyneis* is an araphid taxon, possessing one rimoportula per valve and a large number of girdle bands, each equipped with a septum (Round et al. 1990, Kingston 2003). In this regard, *Oxyneis* is closely related to *Tabellaria* Ehrenb. ex Kütz., a common araphid freshwater

diatom. *Actinella* and *Nupela* both possess a raphe, although the morphology of this feature is very different, yielding placement of the two genera into separate subclasses (Round et al. 1990). *Actinella* is characterized by reduced raphe slits, one rimoportula per valve, and frustules that are asymmetric along all three axes. *Nupela* has a fully formed raphe on one (heterovalvar forms) or both (isovalvar forms) valves and symmetric frustules. Although *Nupela* was established by Vyverman & Compère in 1991, the majority of species now contained within the genus were originally described as *Navicula*, a genus that has been split into numerous taxa in recent years (see Metzeltin & Lange-Bertalot 1998, 2007 and references therein). Phylogenies based on gene sequences

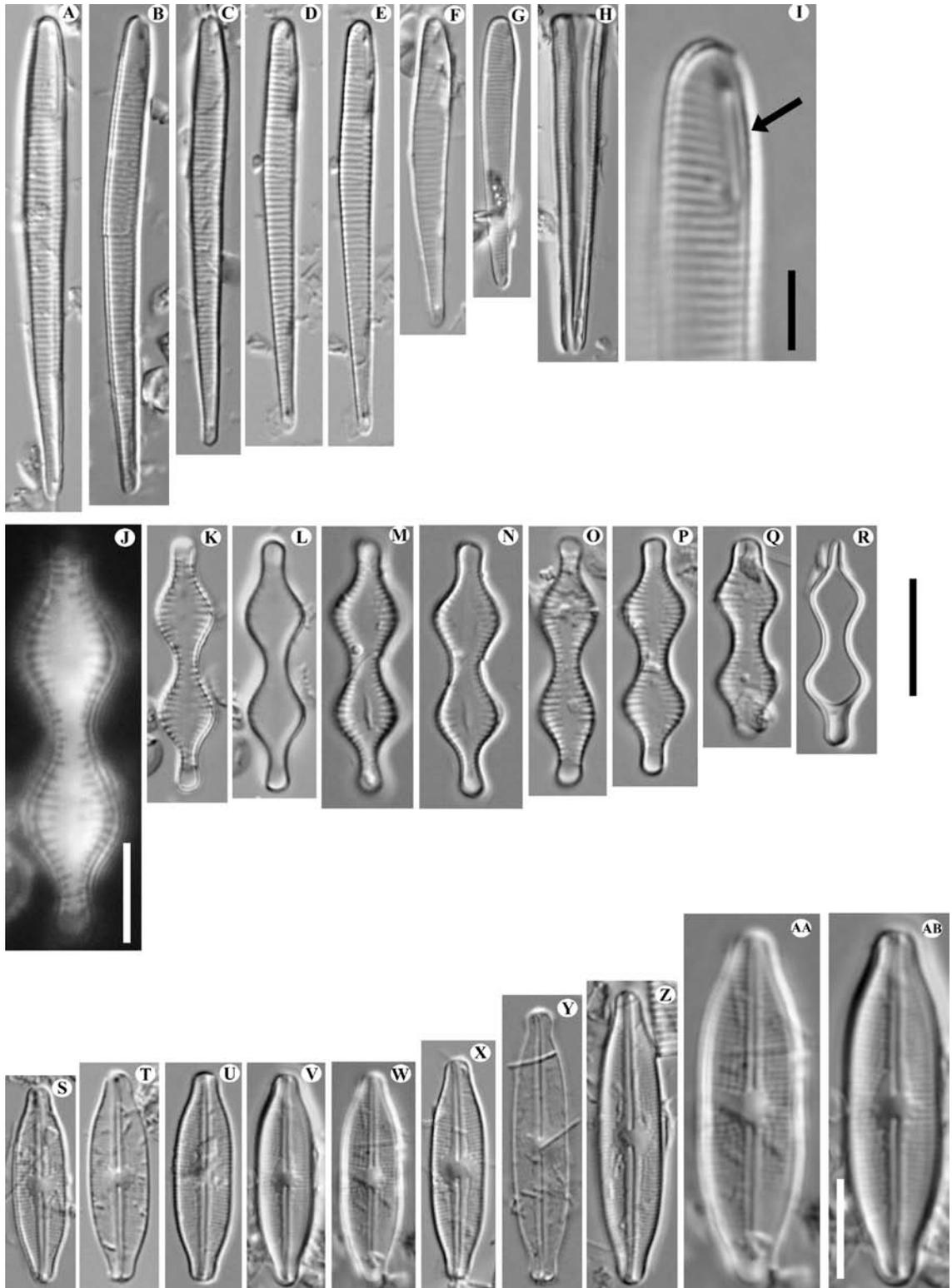


Figure 1 – Light micrographs of *Actinella giraffensis* (A–I), *Oxyneis apporrecta* (J–R) and *Nupela mutabilis* (S–AB). D & E represent slightly different focal planes of the type specimen for *A. giraffensis* deposited at ANSP, and H is a girdle view of this species. The arrow on fig. I denotes the raphe slit on the head pole. K & L are different focal planes of the same specimen, and J represents a close-up of this same specimen taken with reflected interference contrast optics. The extensive apical pore fields appear dark on this image. M & N represent the same specimen, and P is of the type specimen for *O. apporrecta* deposited at the ANSP. V–W and AA–AB denote both valves of the type specimen for *N. mutabilis* deposited at ANSP taken at different magnifications. Note the slight difference in distances between the proximal raphe fissures on both valves. Scale bars: I = 2 μ m; J = 5 μ m; AB (also for AA) = 5 μ m; separate bar = 10 μ m for all other images. Except for J, all others taken with differential interference contrast optics.

consistently place all three genera into distinct evolutionary lineages (Alverson & Theriot 2005, Sorhannus 2007).

The oldest known freshwater diatoms date to the late Cretaceous and include both radial centric (Ambwani et al. 2003) and pennate forms (Chacón-Baca et al. 2002). Although the identity of some of the fossil forms reported by Chacón-Baca et al. (2002) from 70 My old sediments in Mexico, especially the ones possessing a raphe, has been questioned (Sims et al. 2006), most fossil evidence suggests that the majority of freshwater lineages evolved in the Eocene or later with rapid radiation occurring in the Miocene (Wornardt 1972, Bradbury et al. 1985, Krebs 1994, Sims et al. 2006, Siver & Wolfe 2007). In general, the lack of well preserved and accurately dated fossil remains has resulted in an overall poor understanding of the biostratigraphy and evolution of freshwater diatoms (Barron 1993, Sims et al. 2006), and a lack of geologic mileposts for use in phylogenetic investigations (Sims et al. 2006, Wolfe & Siver 2009). On the contrary, freshwater sediments contained in the Giraffe Pipe kimberlite crater

Table 1 – Samples from the Giraffe Pipe core containing specimens of *Nupela mirabilis* (*N.m.*), *Actinella giraffensis* (*A.g.*) and *Oxyneis apporrecta* (*O.a.*).

The corrected vertical depth in the core and estimated depth within the lake are also shown.

Sample	Presence of			Corrected Depth (m)	Depth in Lake (m)
	<i>N. m.</i>	<i>A. g.</i>	<i>O. a.</i>		
11-3-90	****			70.09	0.51
11-3-147	****			70.66	1.08
13-3-126	****			77.08	7.5
13-2-120	****			78.14	8.56
13-1-33	****			78.6	9.02
14-2-22			****	80.71	11.13
14-2-56			****	80.96	11.38
14-2-148		****		81.63	12.05
15-3-24		****			
15-3-75		****		83.29	13.71
15-2-40	****	****		84.21	14.63
15-1-15		****		85.05	15.47
15-1-65		****		85.41	15.83
15-1-131		****	****	85.9	16.32
16-3-23		****		86.2	16.62
16-3-81		****	****	86.63	17.05
16-3-111		****			
16-2-22			****	87.29	17.71
17-3-26	****			89.52	19.94
17-2-25	****			90.61	21.03
17-2-94	****			91.11	21.53
19-3-67	****			96.4	26.82
20-2-25	****			100.48	30.9
20-1-95	****			102.09	32.51
23-2-68	****			110.67	41.09

situated near the Arctic Circle in northern Canada harbour numerous and exquisitely preserved diatom and chrysophyte microfossils (Wolfe et al. 2006, Siver & Wolfe 2005, 2007). The high quality of fossil preservation, coupled with a well-constrained Middle Eocene age, make this an exceptional deposit for understanding the early history of freshwater ochrophyte algae.

In this communication, we describe three freshwater diatoms representing the genera *Actinella*, *Oxyneis* and *Nupela* from Giraffe pipe. Other than initial observations of the former genus (Siver & Wolfe 2009), none of these diatoms have previously been recorded from the Paleogene fossil record. Although none of these fossil species have been observed in extant floras, the morphological structures they possess share pronounced affinities with modern congeneric taxa.

MATERIALS AND METHODS

As described in earlier communications, the Giraffe Pipe locality (64°44'N 109°45'W) is a kimberlite diatreme that was infilled by a sequence of lacustrine and paludal sediments, and subsequently covered by Neogene glacial deposits (Siver & Wolfe 2005, Wolfe et al. 2006, Wolfe & Siver 2009). BHP Billiton retrieved a 165 m long drilled core (BHP 99-01) from the Giraffe Pipe diatreme in 1999 to assess the kimberlite deposit for potential diamond exploration. The core was taken at 47° and included 68.3 m of lacustrine mudstone sediments overlaid with 44.8 m of peat and terrestrial remains and capped with glacial till. Converting the core to vertical equivalents results in 51.1 m and 32.7 m of lacustrine and peat sediments, respectively. The estimated depth in the Giraffe Pipe waterbody that each sample represents was measured from the point where the lacustrine sediments transition to true peat layers. Based on a ⁸⁷Rb/⁸⁷Sr model from kimberlite phlogopite, emplacement of the kimberlite occurred at 47.8 ± 1.4 My, representing a maximum age for the lacustrine sediments (Creaser et al. 2004). Fission track estimates using tephra beds lying near the termination of the lacustrine phase establish a minimum age for the underlying lacustrine sediments of 40 My (Wolfe & Siver 2009). The portions of the core containing lacustrine sediments are stored in boxes labeled 11–27. Each box has three channels (1–3) and each channel has 1.5 m of core (4.5 m total per box). Samples are recorded as the box # - channel # - cm from the top of the box. Thus, the sample noted as 11-3-90 represents a sample taken from 90 cm along the core length in channel three from box 11.

A total of 300 specimens, 150 of *Nupela*, 100 of *Actinella* and fifty of *Oxyneis*, in 25 samples from the Giraffe Pipe core were examined as part of this investigation (table 1). The samples correspond to corrected vertical depths within the core ranging from 70.09 to 110.67 m, and lakewater depths ranging from 0.51 to 41.09 m. Samples consisted of either untreated chips of mudstone or aliquots from digested slurries.

Approximately 0.5–1.0 g of organic mudstone from each lacustrine layer was oxidized with 30% H₂O₂ under low heat for several hours, centrifuged, rinsed several times with distilled water and stored as a final volume of 10 ml. Aliquots

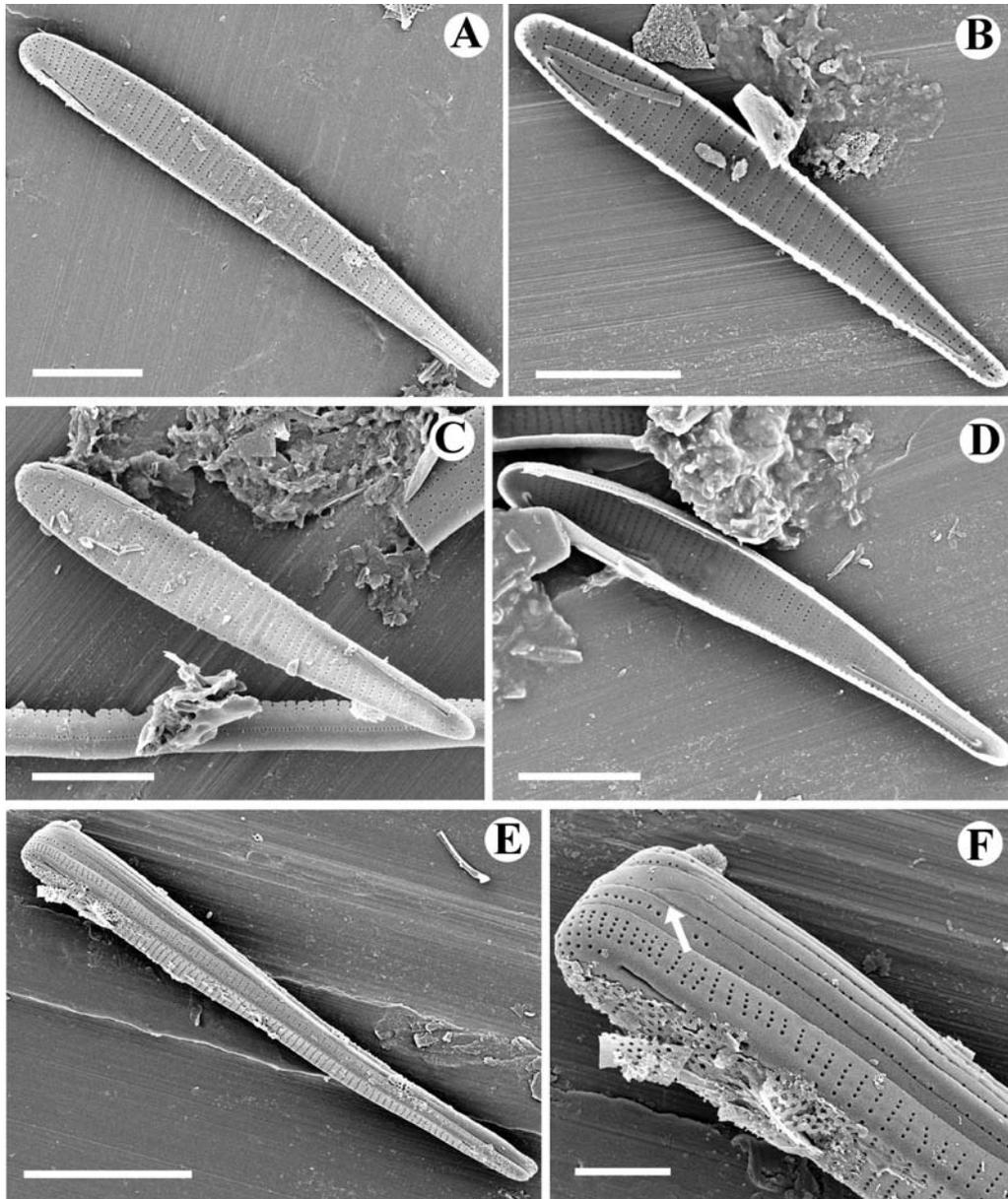


Figure 2 – Scanning electron micrographs of *Actinella giraffensis* depicting external (A, C), internal (B, D) and girdle (E–F) views. Note the short raphe slits of variable lengths situated on the valve margin and terminating as small helictoglossa (B, D). Frustules are clavate in girdle view (E) and possess open girdle bands (arrow, F). Scale bars are 2 μm (F), 5 μm (A–D) and 10 μm (E).

of these slurries were air dried onto heavy duty aluminum foil, attached onto aluminum SEM stubs with Apiezon[®] wax and coated with a gold and palladium mixture for two minutes with a Polaron model E5100 sputter coater (Siver 1987). Small chips of mudstone from a few of the lacustrine facies were also mounted directly onto aluminum SEM stubs using carbon tape, coated with the same gold and palladium mixture and grounded with silver paint. All samples were observed with a Leo (Zeiss) 982 SEM.

RESULTS

Actinella giraffensis Siver, Wolfe & Edlund, sp. nov.

Description: Specimens of *Actinella giraffensis* have a slight dorsi-ventral design, acutely rounded and unmodified

foot and head poles, and are clavate in girdle view (figs 1A–I & 2A–F). Valves are small, narrow, club-shaped and range in size from 18–40 μm \times 1.8–3 μm . The striae are parallel to the transapical axis except at the apices where they become radially aligned around the poles (fig. 3A–F). The striae become more closely spaced at the apices, continue onto the mantle and range in density from 22–27 in 10 μm . The areolae are small, circular, evenly spaced pores that lack coverings on both the exterior (fig. 3A–B) and interior (fig. 3C–F) surfaces. The mantle is shallow, ranging in depth from 0.8–1.3 μm at the head pole to 0.5–0.8 μm at the foot pole, yielding the slender clavate shape (figs 1H & 2E–F). The raphe slits are straight, restricted to the ends of the valve, of unequal lengths and range in length from 1.5–5 μm (figs 1I & 3A–F). Each raphe slit lies on the valve face along the ventral margin. On

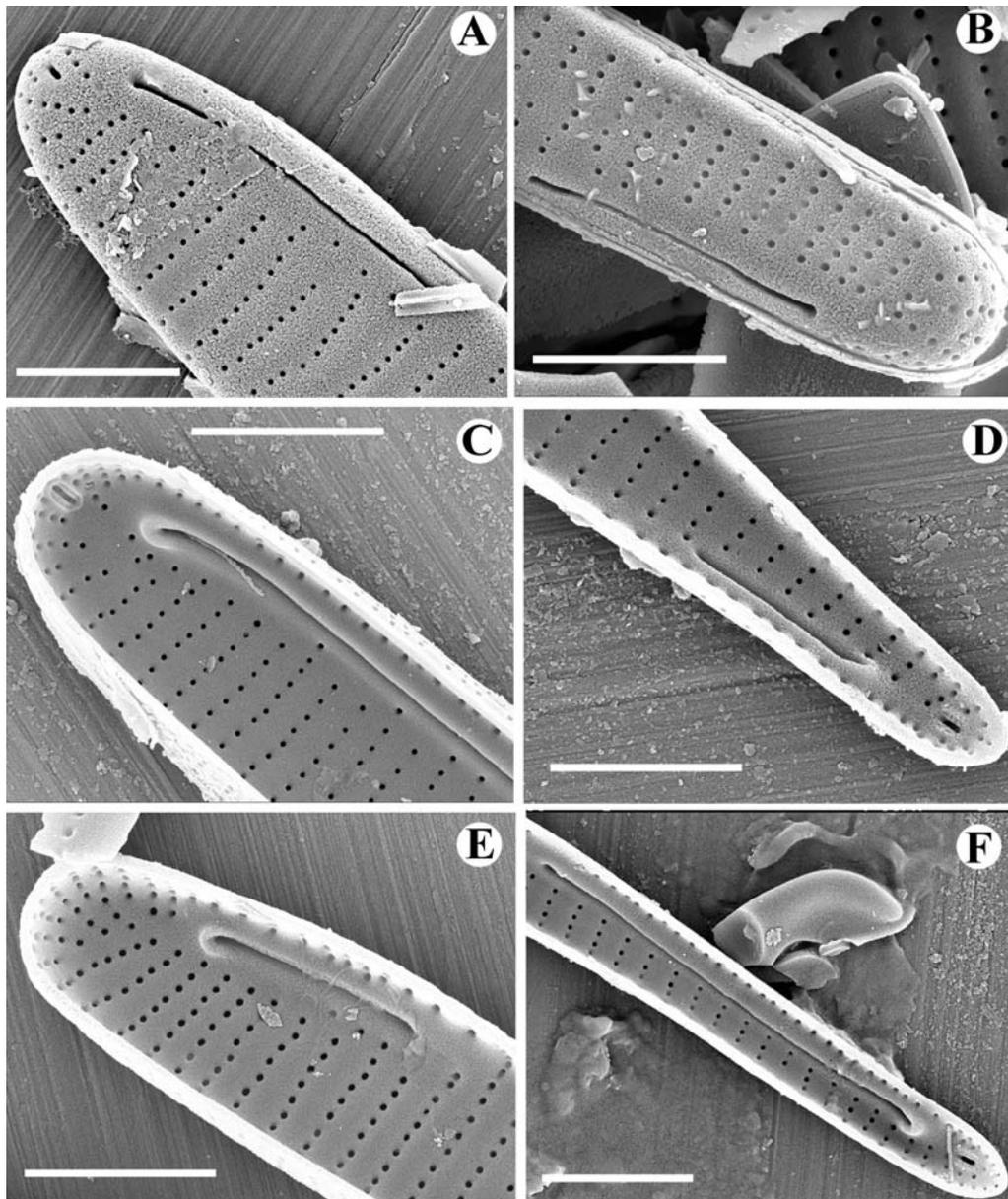


Figure 3 – Scanning electron micrographs of *Actinella giraffensis* revealing the external (A, B) and internal (C–F) morphology of head (A, C, E) and foot (B, D, F) poles. Note the short raphe slits of variable lengths positioned on the valve margin (A–F) and terminating as small helictoglossa (C–F). The rimporulae are parallel with the apical valve axis and can be found on either the head (A, C) or foot (D, F) pole. Scale bars are 2 μm .

the exterior surface, the distal and proximal ends of the raphe are slightly rounded, but otherwise unmodified (fig. 3A–B). On the interior surface, each raphe slit terminates as a well-formed helictoglossa (fig. 3C–F). There is a single rimporula per valve, usually orientated parallel to the apical axis and positioned at the apex (fig. 3A, C–D & F). There are up to four, and most often three, girdle bands per frustule, each opened at the apex and with one row of pores (fig. 2F). The specific epithet refers to the discovery site.

Location in core: *Actinella giraffensis* has been found in eight sections of the core, all from core boxes 14–16 (table 1). The samples containing this species range in corrected vertical depth from only 81.63–86.63 m, representing

estimated lake depths of between 12.05–17.05 m. – Type: holo-: here designated as the circled specimen on microscope slide marked “Giraffe Pipe, 15-2-40, LM2“, deposited at the Academy of Natural Sciences of Philadelphia, PA, USA, as ANSP GC 64856, and illustrated in figure 1D–E. – Type locality: sample 15-2-40; corrected vertical depth = 84.21 m; estimated depth in lake = 14.63 m. – Type material: cleaned sediment deposited at the Academy of Natural Sciences of Philadelphia, PA, USA, as ANSP GC M5515.

***Oxyneis apporrecta* Siver, Wolfe & Edlund, sp. nov.**

Description: Valves are biundulate, constricted in the center, with extended rostrate apices and rounded margins (figs 1J–R, 4A & C). Valves range in size from 16–23 μm

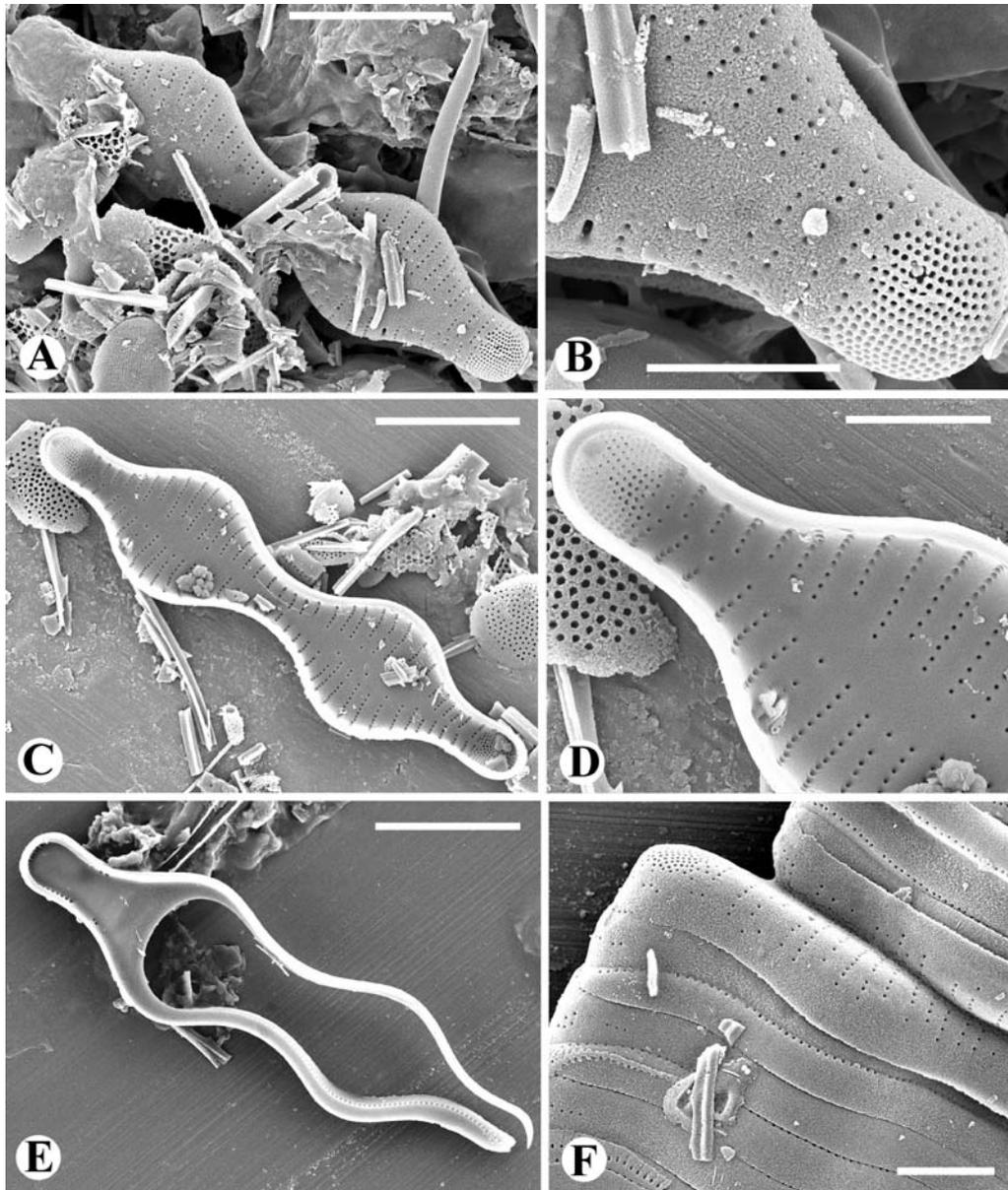


Figure 4 – Scanning electron micrographs of *Oxyneis apporrecta* showing external (A, B, F) and internal (C–E) surfaces of valves (A–D), girdle (F) and girdle bands (E). Note the biundulate shape of the valve (A, C), rostrate apices (A–E), marginal and more apical position of the rimoportulae (B–D), and the extensive apical pore fields (A–D, F). Girdle bands are open and possess a septum that extends to the valve inflation (E). Scale bars are 2 μm (B, D, F) and 5 μm (A, C, E).

$\times 1.8\text{--}2.1 \mu\text{m}$ (center) $\times 4.5\text{--}5.5 \mu\text{m}$ (inflation). The narrow portion of the middle of the valve is approximately the same width as the rostrate apices, and about one-third that of each inflated portion (fig. 4C). The mantle is of the same width, except around the apices and sometimes at the inflations where it often widens slightly (fig. 4F). Striae are uniseriate, parallel to slightly radiate, linear to slightly curved, often unevenly and alternately spaced and consist of very small and round areolae (fig. 4A–D). Striae continue over the rounded valve margins and terminate half way down the mantle (fig. 4F). On our specimens the areolae lacked coverings. The axial area is thin and narrow within the constricted center of the valve and along the rostrate apices, but is broadly lanceolate within the expanded portions of the valve (fig. 4C–D). Extensive and

well-developed apical pore fields are found on both apices, each consisting of numerous rows of small pores (figs 1J, 4B & D). A single rimoportula is found within a stria near the margin close to the point where the valve is extended to form the rostrate apex (fig. 4B–D). The rimoportula is oriented perpendicular to the apical axis. Copulae are open, have a well-developed septum that extends approximately the length of the rostrate apex, and a single row of pores along the margin (fig. 4E–F). The closed end of each copula is expanded and contains three to four short rows of pores. Frustules with up to 10 copulae were observed. Valve ridges were not observed. The specific epithet refers to the extended rostrate apices.

Location in core: *Oxyneis apporrecta* has been found in four sections of the core, all from core boxes 14–16

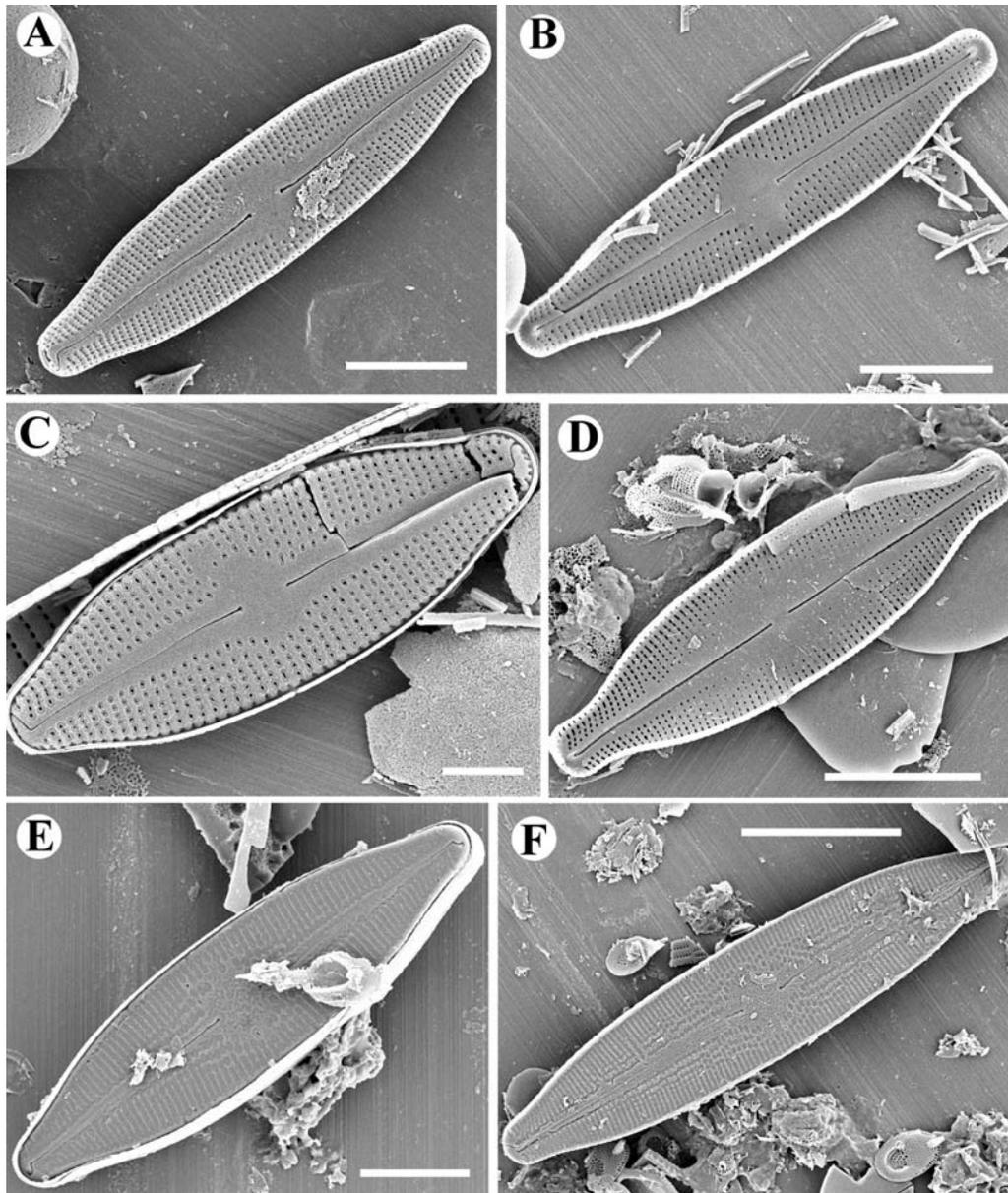


Figure 5 – Scanning electron micrographs of external (A, C, E–F) and internal (B, D) views of *Nupela mutabilis*. Note the linear to linear-lanceolate outline of valves, rostrate apices, wide range in the L:W ratio, deflected distal raphe fissures (A, C, E), lanceolate axial area and circular to elliptical central area (A–C, E, F). Scale bars are 2 μm (C), 5 μm (A, B, D, E) and 10 μm (F).

(table 1). The samples containing this species range in corrected vertical depth from only 80.71–87.29 m, representing estimated lake depths of between 11.13–17.71 m. – Type: holo-: here designated as the circled specimen on microscope slide marked “Giraffe Pipe, 15-1-131, LM2”, deposited at the Academy of Natural Sciences of Philadelphia, PA, USA, as ANSP GC 64857, and illustrated in figure 1P. – Type locality: sample 15-1-131; corrected vertical depth = 85.9 m; estimated depth in lake = 16.32 m. – Type material: cleaned sediment deposited at the Academy of Natural Sciences of Philadelphia, PA, USA, as ANSP GC M5516.

***Nupela mutabilis* Siver, Wolfe & Edlund, sp. nov.**

Description: Valves are linear to linear-lanceolate with rostrate to subcapitate apices, and range in size from 15–36

$\mu\text{m} \times 5\text{--}7.9 \mu\text{m}$ (figs 1S–AB & 5A–F). Valves have a length to width (L:W) ratio ranging from 3 (e.g. fig. 5C) to 4.5 (e.g. fig. 5F), and become more broadly lanceolate with size reduction. Striae are parallel to slightly radiate becoming convergent at the apices, evenly spaced, and range in density from 32–35 (40) per 10 μm . The axial area is lanceolate, expanding into an elliptical to circular central area (figs 5A–F & 6C–D). On some valves, the axial and central areas can be very broad, and the distinction between the two less apparent (fig. 5D). A fully developed raphe is found on each valve of the frustule, however, the distances between the proximal fissures are usually not equal making the frustule slightly heteromorphic (fig. 1AA–AB). The raphe is linear to slightly curved with distal raphe fissures that deflect strongly in the same direction down onto the mantle, where they become recurvate (figs 5A & E,

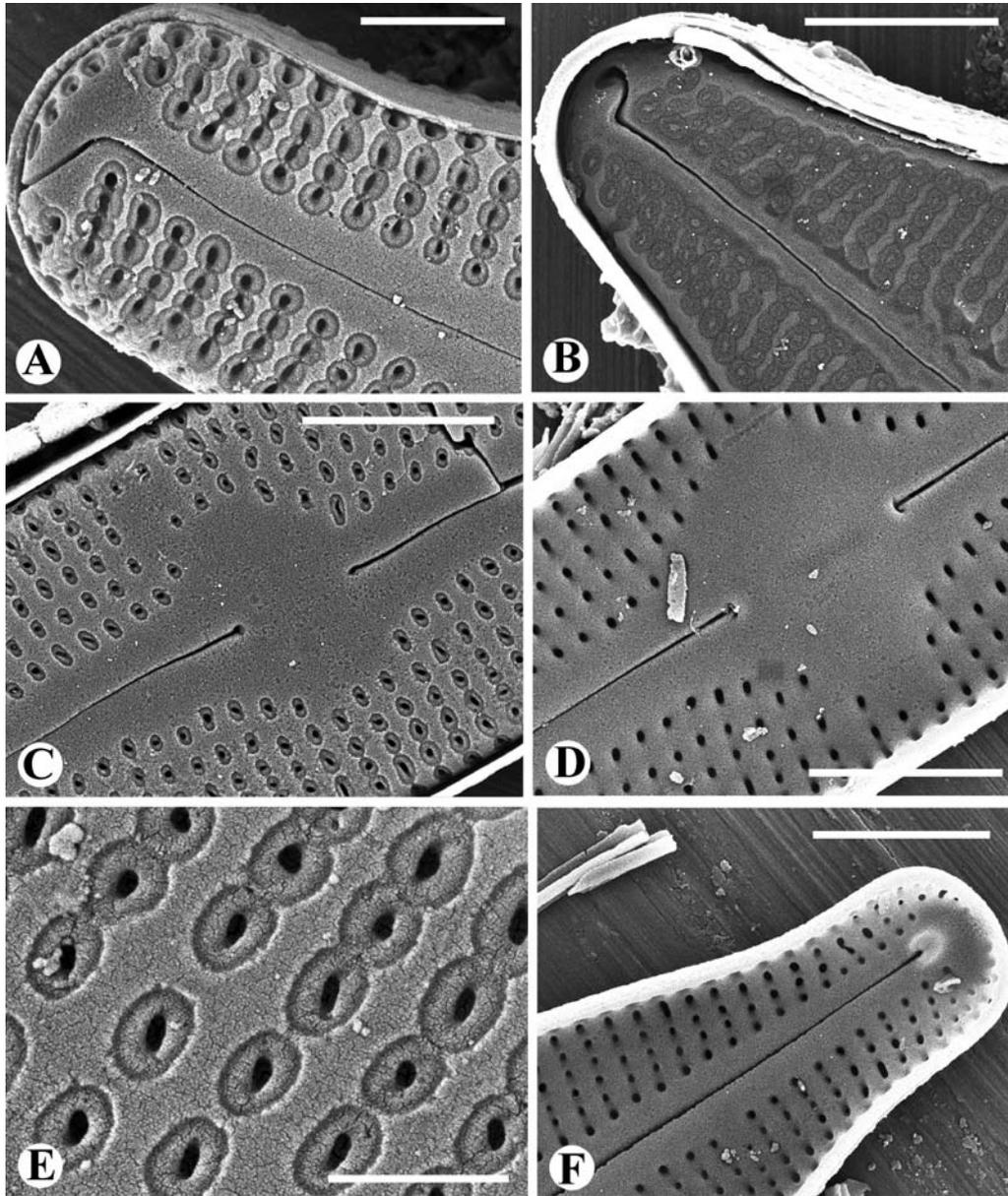


Figure 6 – Additional scanning electron micrographs of external (A–C, E) and internal (D, F) views of *Nupela mutabilis*. Note the highly deflected distal raphe fissures (A–B), the small and straight proximal raphe fissures (C), T-shaped internal fissures (D) and helictoglossa (F). Hymen coverings are eroded from some valves (A, C, E) and present on others (B). The often coalesced nature of the hymenes is shown in A and E. Scale bars are 500 nm (E), 1 μ m (A) and 2 μ m (B–D, F).

6A–B). The proximal raphe fissures are straight and terminate as small pores on the external surface (figs 5A, C & E–F, 6C). Internally, the distal raphe ends terminate as small, well formed, helictoglossa (fig. 6F), and the proximal raphe ends form small recurved “T-shaped” structures that are deflected moreso to the secondary side of the valve (fig. 6D). Externally, the areolae are circular to elliptical and covered with hymenes that are flush with the external valve surface (fig. 6A–C & E). Hymenes between adjoining areolae are often fused together (fig. 6A–B & E). The diameter of the areola decreases significantly between the external and internal valve surfaces, and areolae open internally as small circular to elliptical pores that lack coverings (fig. 6D & F). The striae continue onto the mantle, usually as a single row of elongated

areolae. The specific epithet refers to the variable nature of the valve outline.

Location in core: *Nupela mutabilis* has been found in thirteen sections of the core, spanning a wide range of depths (table 1). Samples containing this species range in corrected vertical depth from only 70.09–110.67 m, representing a wide range of estimated lake depths from 0.51–41.09 m. – Type: holo-: here designated as the circled specimen on microscope slide marked “Giraffe Pipe, 11-3-90, LM2”, deposited at the Academy of Natural Sciences of Philadelphia, PA, USA, as ANSP GC 64858, and illustrated in fig. 1V–W & AA–AB. – Type locality: sample 11-3-90; corrected vertical depth = 70.09 m; estimated depth in lake = 0.51 m. – Type material:

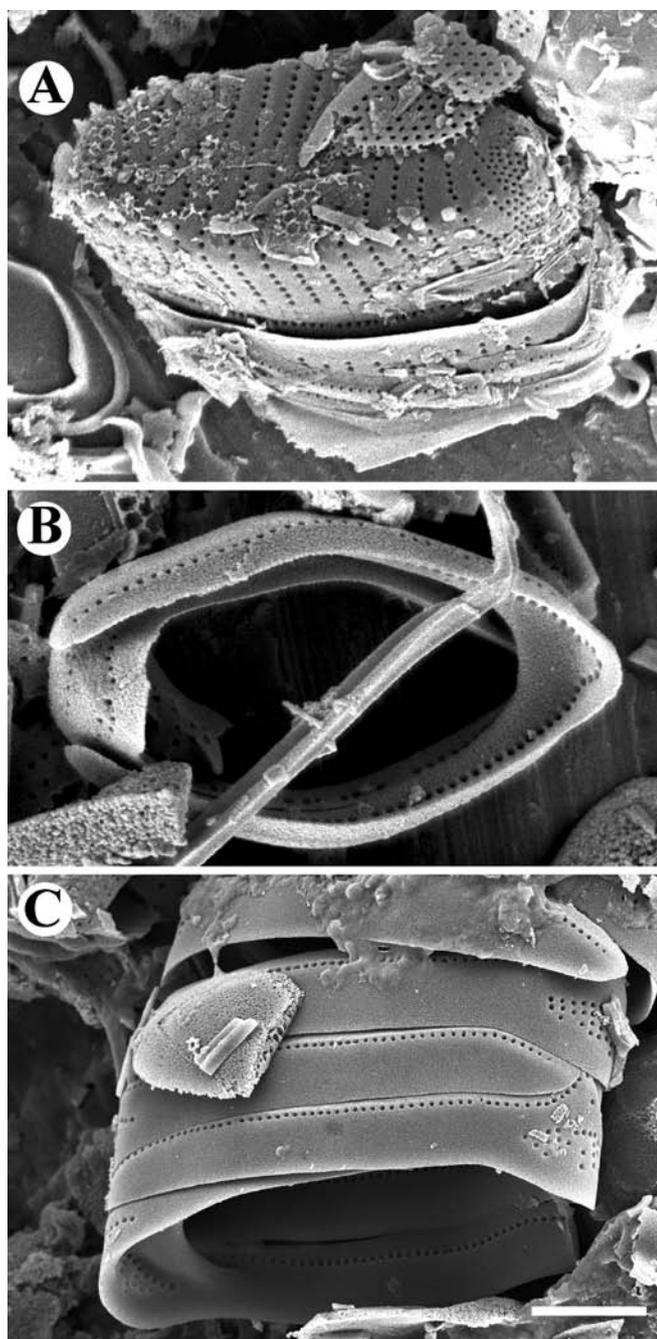


Figure 7 – Scanning electron micrographs of a frustule (A) and girdle bands (B, C) of a diatom from Giraffe Pipe sediments that is largely identical to the modern taxon *Oxyneis binalis* var. *elliptica* (Flower) Kingston (Kingston 2000). Scale bar is 2 μ m.

cleaned sediment deposited at the Academy of Natural Sciences of Philadelphia, PA, USA, as ANSP GC M5517.

DISCUSSION

Oxyneis is a small genus containing only two named taxa, *O. binalis* (Ehrenb.) Round in Round et al. 1990, and *Oxyneis binalis* var. *elliptica* (Flower) Kingston (Kingston 2000). A third taxon recently described from Florida peat has been placed within *Tabellaria* as *T. binalis* var. *brevistriata* Metz-

tin & Lange-Bert. This diatom is nearly identical to *O. binalis*, with striae restricted to the margins (Metzeltin & Lange-Bertalot 2007). *Oxyneis apporrecta* represents a fourth taxon that has not been observed in modern collections and must be considered extinct. In this regard, it is noteworthy that a small elliptical taxon virtually identical to *O. binalis* var. *elliptica* is also present in Giraffe Pipe sediments (fig. 7), however, we have not observed specimens belonging to *O. binalis*. Reduction of the rostrate apices on *O. apporrecta* specimens would essentially yield a taxon similar to *O. binalis*.

As originally described by Round (in Round et al. 1990), *Oxyneis* differs from the closely related genus *Tabellaria* in spine complexity, position of the rimoportula, details of the girdle bands and valve shape. Some, but not all, species of *Tabellaria* form a row of short spines along the valve margin. In many diatom genera spines are used to join frustules into filaments. This is not the case in *Tabellaria* where commonly observed zig-zag, linear and stellate arrangements of cells are accomplished by mucilage secretions through the apical pore fields. Round (in Round et al. 1990) noted that the marginal spines on valves of *Tabellaria binalis* (Ehrenb.) Grunow (in Van Heurck 1881) were significantly more complex than those on other *Tabellaria* species, often fused together, and used to connect frustules valve to valve to form straight chains. The more complex spine development was a primary character used by Round to establish *Oxyneis*.

Interestingly, Round (in Round et al. 1990) stated that the elliptical valve morphotype of *O. binalis* (= *Oxyneis binalis* var. *elliptica*) formed zig-zag colonies, presumably through mucilage secretions of the pore fields and not using marginal ridges. In this regard, *O. binalis* var. *elliptica* was perhaps more similar to *Tabellaria*. Siver & Hamilton (in press) present specimens of both *O. binalis* and *Oxyneis binalis* var. *elliptica* with massive marginal ridges, structures readily distinguished from individual spines and not reported for *Tabellaria*. However, Siver & Hamilton (in press) also note that in both of these extant *Oxyneis* taxa ridges were primarily restricted to terminal valves within a filament. In fact, most isolated valves lacked ridges and were presumably held together, valve-to-valve, by mucilage secretions from the extensive pore fields. Perhaps ridge formation in *Oxyneis* serves as a means of separating valves from one another, much like separating spines in *Aulacoseira*, and are not used for linking cells together. Thus, spine and ridge formation is not a universal feature found on all frustules of modern *Oxyneis* taxa. In fact, Metzeltin & Lange-Bertalot (2007) do not recognize *Oxyneis*, in part, because they believe differences in spine and ridge structure should be used to differentiate at the species, not genus, level. Further, Kingston (2003) does not even mention spine or ridge structure in his description of *Oxyneis*. We have yet to observe marginal spines or ridges on any *Oxyneis apporrecta* valves, and we must conclude at this time that these structures are not formed by this organism. We further presume that valves of *Oxyneis apporrecta* were held together through mucilage secretions from the extensive apical pore fields that extend over the mantle and well onto the valve face.

The position of the rimoportula, being close to the ends of the cell as opposed to near the central region, was another character used by Round (Round et al. 1990) to differentiate

Oxyneis from *Tabellaria*. Our review of published records for *Oxyneis* clearly indicates a more apical position for the rimoportula, and *Oxyneis apporrecta* is no exception. On valves of *Tabellaria*, rimoportulae are almost always positioned near the center of the valve or close to the central inflation (Round et al. 1990, Krammer & Lange-Bertalot 1991). A notable exception is *Tabellaria ventricosa* Kütz., a species possessing short valves with a wide central inflation and a single rimoportula near one apex (Krammer & Lange-Bertalot 1991, Metzeltin & Lange-Bertalot 2007). This fact also caused Metzeltin & Lange-Bertalot (2007) to question the validity of the genus *Oxyneis*.

Features of the girdle bands and overall valve shape also differ between species of *Oxyneis* and *Tabellaria*. Frustules of *Oxyneis* species have open girdle bands that possess a septum on the end opposite of the opening, and a row of pores that usually lines the entire structure. In contrast, *Tabellaria* species have two distinct types of girdle bands, including complete bands with one or two septa and open bands that often lack septa altogether. The septa on *Oxyneis* girdle bands are short and less developed compared to those of *Tabellaria* where the septum often extends to the central inflation (Patrick & Reimer 1966, Round et al. 1990, Kingston 2003). Lastly, *Tabellaria* girdle bands lack the characteristic row of areolae found on *Oxyneis*.

All species of *Tabellaria* have valves with terminal and central inflations, whereas species of *Oxyneis* lack central inflations and, in fact, can be highly constricted in the central region. Inflated regions of the valve on *Oxyneis* specimens are either in an apical position (e.g. *O. binalis*) or along the valve (e.g. *Oxyneis apporrecta*), but not in the center of the valve. The degree of development of the septa on *Oxyneis* and *Tabellaria* may simply be related to the position of inflations along the valve. In conclusion, despite the controversy over whether *Oxyneis* should remain a separate genus (Round et al. 1990) or be merged with *Tabellaria* (Metzeltin & Lange-Bertalot 2007), it is clear that both taxa are very closely related. Since most recent authors have maintained separation of these two genera, we are electing to describe the taxon from Giraffe Pipe sediments as *Oxyneis* because of valve shape, position of the rimoportula and similarities of the girdle bands with modern taxa.

When the genus *Nupela* was originally described, Vyverman & Compère (1991) noted it to be isovalvar with both valves possessing a fully formed raphe and, more importantly, to have uniquely structured areolae. Since the original description, over 38 species have been described or transferred into *Nupela* (Monnier et al. 2003, Potapova et al. 2003, Siver et al. 2007), many of which are heterovalvar with respect to the degree of development of the raphe (Lange-Bertalot 1993, Metzeltin & Lange-Bertalot 1998, Potapova et al. 2003). In this regard, the slight heterovalvar condition found on *N. mutabilis* frustules due to differences in the lengths of the raphes fits the situation found in many species within the genus. The unique structure of the areolae on all *Nupela* species, including those on *N. mutabilis*, is a very stable and reliable character of the genus (Potapova et al. 2003, Siver et al. 2007). In addition to the structure of the areolae, *N. mutabilis* possesses all of the characters identified by Siver et al. (2007) as shared among *Nupela* species, including external hymenes

covering one or more areolae, distal raphe fissures deflected to the same side of the valve, straight external proximal raphe fissures ending as small pores, a single row of elongated areolae on the mantle and small helictoglossae. All samples containing *N. mutabilis* consistently had specimens with valve shapes ranging from linear to linear-lanceolate and possessing a relatively wide range of L:W values. Such variability in valve shape is not uncommon for *Nupela* taxa (Siver et al. 2007).

Specimens of *Actinella giraffensis* can be reliably assigned to this genus because of the heteropolar nature of valves, the clavate shape of frustules in girdle view and the position and nature of the raphe slits. The location of the raphe slits on the valve face margin, as opposed to on the mantle, also separates it from the closely related genus *Eunotia*. Within the genus, *A. giraffensis* does not match any extant species, but certainly belongs with species possessing simple, as opposed to complex, head poles. As noted in a previous communication (Siver & Wolfe 2009), *A. giraffensis* is most closely aligned with *Actinella parva* Vanhoutte & Sabbe, a species only known from the highlands of Tasmania (Sabbe et al. 2001). It is not yet known if *Actinella* species possessing simpler head poles and lacking marginal spines are basal or derived taxa within the genus, but it is noteworthy that the few species from the fossil record have less complex valve shapes compared to many tropical species known today from South America (Kociolek et al. 2001, Metzeltin & Lange-Bertalot 2007) and Africa (Kociolek & Rhode 1998, Sabbe et al. 2001), as well as most Australian endemics (Sabbe et al. 2001).

As already discussed for *Eunotia* (Siver & Wolfe 2007) and the thalassiosiroid genera *Cyclotella*, *Discotella* and *Puncticulata* (Wolfe & Siver 2009), our findings significantly extend the fossil record for *Actinella*. As noted by Siver & Wolfe (2009), in addition to the presence of *Actinella* in Giraffe Pipe sediments, three other fossil records exist for this genus. *Actinella penzhica* Lupikina & Dolmatova was described from Oligocene to late Eocene deposits on the Kamchatka Peninsula (Lupikina & Dolmatova 1982), *A. mioce-nica* J.Y.Li from Miocene sediments in northeastern China (Li 1988), and *A. pliocenica* from Pliocene remains in France (Héribaud 1902). Interestingly, none of the fossil remains of *Actinella* were found in presently tropical areas and none from the Southern Hemisphere, regions of the world where most *Actinella* species are found today. The presence of *Actinella* in Giraffe Pipe with a minimum age of 40 My significantly extends the stratigraphic range of this freshwater genus.

To our knowledge, Giraffe Pipe sediments also represent the first fossil records for the araphid genus *Oxyneis* and the raphe-bearing genus *Nupela*. Although fossils of *Oxyneis* are previously lacking from the fossil record, specimens of the closely related genus *Tabellaria* are known from late Eocene–early Oligocene (Lupikina & Dolmatova 1975) and Miocene (Wornardt 1972, Bradbury et al. 1985) sediments. In addition, *Tetracyclus* Ralfs (Ralfs 1843), another genus within the family Tabellariaceae Kütz. (Kützing 1844), also dates to at least the late Eocene–early Oligocene (Lupikina & Dolmatova 1975). Thus, our discovery of *Oxyneis* in the Middle Eocene is consistent with these previous finds, and

collectively these records support an Eocene or earlier evolution for the Tabellariaceae.

As reviewed by Sims et al. (2006), the diatom raphe had evolved by the Paleocene and it is likely that this structure is even older, perhaps originating in the Cretaceous. Lohman & Andrews (1968) reported 34 freshwater diatom taxa, including twenty raphid forms, from Late Eocene sediments in Wyoming, U.S.A., supporting the view that most modern raphid lineages had evolved by this time (Sims et al. 2006). Thus, the presence of *Nupela mutabilis* in the Giraffe Pipe core is consistent with the known fossil record in regards to evolution of the raphe. On the basis of SSU rRNA sequences and preliminary molecular clock calculations, Sorhannus (2007) predicted that early representatives of modern araphid diatoms were present by the Early Cretaceous (~ 100 My), whereas raphid taxa had evolved by the Late Cretaceous (~ 75 My). In this regard, it is of interest that details of the raphe found on *Nupela mutabilis*, as well as other Eocene pennate diatoms (Lohman & Andrews 1968), are strikingly similar to those found on modern congeneric forms, suggesting that the fossil record of raphe origins converges with molecular estimates (Sims et al. 2006, Sorhannus 2007). In fact, Bradbury et al. (1985) noted that lacustrine diatoms from the Miocene, Oligocene and late Eocene had structures such as areolae, rimoportulae and raphes that made them “morphologically modern”, a theme also echoed by Lohman & Andrews (1968). Our findings from Giraffe Pipe sediments support these previous works and extend them to include additional structures such as the fuloportulae (Wolfe & Siver 2009).

The genera *Oxyneis* and *Actinella* are largely restricted to very acidic habitats (Round et al. 1990), and although *Nupela* is more widespread, this genus is also most often associated with acidic waterbodies (Siver et al. 2007 and references therein). Flower (1989) reported both *Oxyneis binalis* and *O. binalis* var. *elliptica* (under *Tabellaria*) as being confined to lakes usually with pH < 5, and Camburn & Charles (2000) reported an average weighted mean pH for *O. binalis* (including specimens of *O. binalis* var. *elliptica*) of 5.1 for lakes in the Adirondack Mountains of New York. In a forthcoming publication, Siver & Hamilton (in press) observed both *Oxyneis binalis* and *O. binalis* var. *elliptica* as common elements of the diatom floras of very acidic and often humic-stained waterbodies along the Atlantic Coastal Plain, U.S.A. Kingston (2003) also noted *Oxyneis* as typical of very acidic lakes, including peat bog ponds, and Patrick & Reimer (1966) and Siver & Hamilton (in press) found this genus typical of waterbodies low in nutrients and dissolved salts.

Like *Oxyneis*, *Actinella* taxa are primarily recorded from very acidic environments, often high in dissolved humic content (Round et al. 1990, Kociolek & Rhode 1998, Sabbe et al. 2001, Kociolek et al. 2001, Metzeltin & Lange-Bertalot 2007). The acidic nature of *Actinella* holds true for tropical taxa from South America (Kociolek et al. 2001, Metzeltin & Lange-Bertalot 2007) and Africa (Kociolek & Rhode 1998), Australasian endemics (Sabbe et al. 2001) and the widely distributed and common species *A. punctata* F.W.Lewis. To date, both *A. giraffensis* and *O. apporrecta* have only been uncovered in samples from core boxes 14, 15 and 16. Coupled with the fact that many of these samples also contain sig-

nificant numbers of *Eunotia* specimens (Siver & Wolfe 2007) and several chrysophyte species aligned with modern acidophilic taxa (P.A. Siver, Connecticut College, U.S.A., unpubl. res.), we conclude that the waterbody that existed between approximately 11 and 18 m was very acidic.

Interestingly, although *Nupela mutabilis* is found widely throughout the core, ranging from boxes 23 to 11, it is largely absent from the section of the core containing *A. giraffensis* and *O. apporrecta*, suggesting that the former taxon is less abundant in very acidic waters. Although much more work needs to be done before a complete reconstruction of the paleoenvironment represented by the Giraffe Pipe waterbody can be appreciated, our preliminary observations support the concept that the initial lake was more alkaline and acidified over time.

CONCLUSION

In conclusion, the diatoms reported here provide valuable milestones that refine the existing fossil record and provide new temporal constraints for molecular phylogenetic investigations. By the Middle Eocene (≥ 40 My), the orders Tabellariales, Eunotiales and Naviculales were already established in North American freshwater habitats, unambiguously displaying the range of morphological characters that define each of these orders. The search for primitive forms that inform more directly the evolution of the raphe system in pennate diatoms and the initial colonization of nonmarine habitats must therefore address older sedimentary sequences.

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REFERENCES

- Alverson A.J., Theriot E.C. (2005) Comments on recent progress toward reconstructing the diatom phylogeny. *Journal of Nanoscience and Nanotechnology* 5: 57–62.
- Ambwani K., Sahni A., Kar R.K., Dutta D. (2003) Oldest known non-marine diatoms (*Aulacoseira*) from the uppermost Cretaceous Deccan Intertrappean beds and Lameta Formation of India. *Revue de Micropaléontologie*, v. 46: 67–71.
- Barron J.A. (1993) Diatoms. In: Lipps J.H. (ed.) *Fossil prokaryotes and protists*: 155–167. Boston, Blackwell Scientific Publications.
- Bradbury J.P., Dieterich K.V., Williams J.L. (1985) Diatom flora of the Miocene lake beds near Clarkia in northern Idaho. In: Smiley C.J. (ed.) *Late Cenozoic history of the Pacific northwest*: 33–59. San Francisco, California Academy of Sciences.
- Camburn K.E., Charles D.F. (2000) Diatoms of low-alkalinity lakes in the northeastern United States. Special Publication 18. Philadelphia, The Academy of Natural Sciences of Philadelphia.
- Chacón-Baca E., Beraldi-Campesi H., Cevallos-Ferriz S.R., Knoll A.H., Golubic S. (2002) 70 Ma nonmarine diatoms from northern Mexico. *Geology* 30: 279–281.

- Creaser R., Grütter H., Carlson J., Crawford B. (2004) Macrocrystal phlogopite Rb-Sr dates for the Ekati Property kimberlites, Slave Province, Canada: Evidence for multiple intrusive episodes in the Paleocene and Eocene. *Lithos* 76: 399–414.
- Flower R.J. (1989) A new variety of *Tabellaria binalis* (Ehrenb.) Grunow from several acid lakes in the U.K. *Diatom Research* 4: 21–23.
- Héribaud F.J. (1902) *Les Diatomées d’Auvergne*. Premier Mémoire. Paris, Librairie des Sciences Naturelles.
- Kingston J.C. (2000) New combinations in the freshwater Fragilariaceae and Achnantheaceae. *Diatom Research* 15: 409–411.
- Kingston J.C. (2003) Araphid and Monoraphid Diatoms. In: Wehr J.D., Sheath R.G. (eds) *Freshwater Algae of North America*: 595–636. New York, Academic Press.
- Kociolek J.P., Rhode K. (1998) Raphe vestiges in “*Asterionella*” species from Madagascar: evidence for a polyphyletic origin of the araphid diatoms? *Cryptogamie Algologie* 19: 57–74.
- Kociolek J.P., Lyon D., Spaulding S. (2001) Revision of the South American species of *Actinella*. In: Jahn R., Kociolek J.P., Witkowski A., Compère P. (eds) *Studies on diatoms*. H. Lange-Bertalot-Festschrift: 131–165. Ruggell, A.R.G. Gantner Verlag K.G.
- Krammer K., Lange-Bertalot H. (1991) *Bacillariophyceae*, Teil 3, Centrales, Fragilariaceae, Eunotiaceae. Band 2/3: Süßwasserflora von Mitteleuropa (begr. von A. Pascher). Stuttgart, Gustav Fischer Verlag.
- Krebs W.N. (1994) The biochronology of freshwater planktonic diatom communities in western North America. In: Kociolek J.P. (eds) *Proceedings of the 11th International Diatom Symposium*: 485–499. San Francisco, California Academy of Sciences.
- Kützing F.T. (1844) *Die kieselschaligen Bacillarien oder Diatomeen*. Nordhausen, W. Köhne.
- Lange-Bertalot H. (1993) 85 new taxa and much more than 100 taxonomic clarifications supplementary to Süßwasserflora von Mitteleuropa Vol. 2/1–4. *Bibliotheca Diatomologica* 27: 1–454.
- Li J.Y. (1988) A new fossil species (diatom) recorded and its significance. *Bulletin of Botanical Research, Harbin* 8: 129–132.
- Lohman, K.E., Andrews, G.W. (1968) Late Eocene nonmarine diatoms from the Beaver Divide area, Fremont County, Wyoming. *Contributions to Paleontology*. U.S. Geological Survey professional paper 593-E: 1–26.
- Lupikina E.G., Dolmatova L.M. (1982) On new Paleogene species of the family Eunotiaceae (Bacillariophyta) from Kamchatka. *Botanicheskii Zhurnal (Moscow & Leningrad)* 69: 1406–1408.
- Lupikina Y.G., Dolmatova L.M. (1975) The Paleocene lagoonal diatom flora of Kamchatka. *Paleontologicheskii Zhurnal* 1: 120–128.
- Metzeltin D., Lange-Bertalot H. (1998) Tropical diatoms of South America. I. About 700 predominantly rarely known or new taxa representative of the neotropical flora. *Iconographia Diatomologica* 5: 1–695.
- Metzeltin D., Lange-Bertalot H. (2007) Tropical diatoms of South America. II. Special remarks on biogeographic disjunction. *Iconographia Diatomologica* 18: 1–877.
- Monnier O., Lange-Bertalot H., Bertrand J. (2003) *Nupela exotica* species nova: une diatomée d’un aquarium tropical d’eau douce. Avec des remarques sur la biogéographie du genre. *Diatom Research* 18: 273–291.
- Patrick R., Reimer C.W. (1966) *The diatoms of the United States*. Volume I: Fragilariaceae, Eunotiaceae, Achnantheaceae, Naviculaceae. Philadelphia, Academy of Natural Sciences of Philadelphia.
- Potapova M.G., Ponader K.C., Lowe R.L., Clason T.A., Bahls L.L. (2003) Small celled *Nupela* species from North America. *Diatom Research* 18: 293–306.
- Ralfs J. (1843) On the Diatomaceae. *Annals and Magazine of Natural History* 12: 104–111.
- Round F.E., Crawford R.M., Mann D.G. (1990) *The diatoms: biology and morphology of the genera*. Cambridge, Cambridge University Press.
- Sabbe K., Vanhoutte V., Lowe R.L., Bergey E.A., Biggs B.J.F., Francoeur S., Hodgson D., Vyverman W. (2001) Six new *Actinella* (Bacillariophyta) species from Papua New Guinea, Australia and New Zealand: further evidence for widespread diatom endemism in the Australasian region. *European Journal of Phycology* 36: 321–340.
- Sims P.A., Mann D.G., Medlin L.K. (2006) Evolution of the diatoms: Insights from fossil, biological and molecular data. *Phycologia* 45: 361–402.
- Siver P.A. (1987) The distribution and variation of *Synura* species (Chrysophyceae) in Connecticut, USA. *Nordic Journal of Botany* 7: 107–116.
- Siver P.A., Hamilton P.B. (in press) Diatoms of North America, the freshwater flora of acid ponds along the Atlantic Coastal Plain, U.S.A. *Iconographia Diatomologica*.
- Siver P.A., Hamilton P.B., Morales E.A. (2007) Notes on the genus *Nupela* (Bacillariophyceae) including the description of a new species, *Nupela scissura* sp. nov. and an expanded description of *Nupela paludigena*. *Phycological Research* 55: 125–134.
- Siver P.A., Wolfe A.P. (2005) Eocene scaled chrysophytes with pronounced modern affinities: *International Journal of Plant Science* 66: 533–536.
- Siver P.A., Wolfe A.P. (2007) *Eunotia* spp. (Bacillariophyceae) from Middle Eocene lake sediments and comments on the origin of the diatom raphe. *Canadian Journal of Botany* 85: 83–90.
- Siver P.A., Wolfe A.P. (2009) Tropical ochrophyte algae from the Eocene of northern Canada: a biogeographic response to past global warming. *Palaios* 24: 192–198.
- Sorhannus U. (2007) A nuclear-encoded small-subunit ribosomal RNA timescale for diatom evolution. *Marine Micropaleontology* 65: 1–12.
- Van Heurck H. (1881) *Synopsis des Diatomées de Belgique*. Atlas. Anvers, Ducaju.
- Vyverman W., Compère P. (1991) *Nupela giluwensis* gen & spec. nov. a new genus of naviculoid diatoms. *Diatom Research* 6: 175–179.
- Wolfe A.P., Edlund M.B., Sweet A.R., Creighton S. (2006) A first account of organelle preservation in Eocene non-marine diatoms: observations and paleobiological implications. *Palaios* 21: 298–304.
- Wolfe A.P., Siver P.A. (2009) Three extant genera of freshwater Thalassiosiroid diatoms from Middle Eocene sediments in northern Canada. *American Journal of Botany* 96: 487–497.
- Wornardt W.W. (1972) Stratigraphic distribution of diatom genera in marine sediments in western North America. *Palaeogeography, Palaeoclimatology, Palaeoecology* 12: 49–74.

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