Phytotaxa 567 (1): 021–035
https://www.mapress.com/pt/
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Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.567.1.2

Descriptions of three new diatom species in the genus *Eunotia* (Eunotiaceae, Bacillariophyta) from the Eocene Arctic

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Abstract

Eunotia is the largest and most diverse genus within the family Eunotiaceae, a primarily freshwater group of diatoms often found in dilute, acidic and humic-stained environments. Species in this genus are characterized by being asymmetric along their apical axis, symmetric about the transapical axis, and with a simple and reduced raphe system situated largely on the mantle and restricted to the apical ends of the valve. In addition, *Eunotia* taxa have one or more rimoportula per valve, usually close to the apex. Because of their reduced raphe system, coupled with the presence of rimoportulae, *Eunotia* and its relatives are often viewed as the oldest lineage of raphe-bearing diatoms. To date, the oldest remains of *Eunotia* species have been reported from the early to middle Eocene, including from the Giraffe Pipe locality, an ancient Eocene fossil site located in northern Canada near the Arctic Circle. Rocks from this site contain a large and diverse assemblage of *Eunotia* taxa. The purpose of this study is to begin to characterize this assemblage with descriptions of three new species, *Eunotia giraffensis sp. nov*., *E. petasum sp. nov*. and *E. pseudonaegelii sp. nov*. The new species, representing the longest specimens found at the Giraffe Pipe locality, each possess characteristics common to *Eunotia* making them easily assigned to this genus. Because the *Eunotia* lineage was well established by the early part of the Eocene, it is likely to be significantly older.

Keywords: acidic, ecology, freshwater, Giraffe Pipe, new species, raphe

Introduction

Eunotia Ehrenberg (1837: 45) is a large genus of mostly freshwater diatoms that overwhelmingly inhabit acidic waterbodies (Camburn & Charles 2000, Siver & Hamilton 2011, Costa *et al*. 2017). *Eunotia* is in the family Eunotiaceae Kützing 1844 within the subclass Eunotiophycidae Mann (in Round *et al*. 1990), the latter characterized by a reduced raphe and the presence of rimoportulae. The family Eunotiaceae consists of 13 genera, and of the 773 recognized species, 663 belong to *Eunotia* (Guiry & Guiry 2022). The closely related genus, *Actinella* Lewis (1864: 343), has 52 species and many of the remaining species in the family were originally described under *Eunotia*.

Frustules of *Eunotia* are asymmetric about the apical axis, symmetric about the transapical axis, and most species possess a dorsiventral lunate shape (Round *et al*. 1990). The short raphe branches are situated on the ventral mantle, with the distal ends curving up onto the valve face at the apices. Internally, the distal end of the raphe terminates within a well-formed helictoglossa. Striae cross the valve face and are most often continuous on both the ventral and dorsal mantle, including between the raphe branches. As a result, a central nodule is lacking. Unlike other raphe-bearing diatoms, species in the subclass Eunotiophycidae possess rimoportulae, and most *Eunotia* species have a single rimoportula per valve located on one of the valve apices, typically on the mantle (Mayama & Kobayasi 1991, Williams & Reid 2006, Siver & Hamilton 2011).

Based on most fossil evidence, the earliest freshwater diatoms are found in the Late Cretaceous (Chacón-Baca *et al*. 2002, Ambwani *et al*. 2003, Singh *et al*. 2006, Siver *et al*. 2018), and by the middle to end of the Eocene numerous sites and lineages are documented (Lohman & Andrews 1968, Sims *et al*. 2006, Siver & Wolfe 2007, Benson *et al*. 2012). However, the potential that freshwater diatoms being considerably older than Cretaceous can't be ruled out.

Accepted by Eduardo A. Morales: 16 Sept. 2022; published: 30 Sept. 2022

Siemińska (2000) provided an excellent review of earlier reports of fossil diatoms from Mesozoic, Paleozoic and even in Proterozoic rocks, many of which illustrate freshwater taxa clearly linked to modern lineages, including those of *Eunotia* from the Upper Triassic (Zanon 1929). Siemińska (2000) noted that many of these reports were considered by other researchers to be the result of contamination. Still, the finding of diatom remains from Proterozoic marbles (Siemińska & Kwiecińska 2000), and multiple freshwater diatoms from the Early Permian (295 Ma) Manjir Formation in India (Farooqui *et al*. 2015), both including nitzschioid forms with complex raphe systems, is certainly intriguing.

Besides the report by Zanon (1929), the oldest known fossil records for *Eunotia* are from early to middle Eocene localities, including the 51 Ma Horsefly deposit in British Columbia, Canada (Benson *et al*. 2012), the 48 Ma Giraffe Pipe site situated near the Arctic Circle in northern Canada (Siver & Wolfe 2007), and the 45 Ma Dewey beds in Idaho, USA (Benson *et al*. 2012). Only a few specimens (mostly fragmented pieces) were uncovered from the Horsefly and Dewey beds localities, whereas numerous, well-preserved specimens were reported from the Giraffe Pipe site. In addition to *Eunotia*, species of the closely related genus *Actinella* have also been uncovered from Giraffe Pipe (Siver *et al*. 2010).

Since the Siver & Wolfe (2007) report of *Eunotia* from the Giraffe Pipe locality, further investigation of rocks from this locality have been undertaken and numerous species of chrysophytes (Siver 2018a, 2018b), euglyphid testate amoebae (Barber *et al*. 2013), sponges (Pisera *et al*. 2013, 2016), rotosphaerids (Siver & Skogstad 2022), and additional diatoms (Wolfe & Siver 2009, Siver *et al*. 2010, Siver 2019, 2021) have been reported. In addition, an 18 m section of an extensive core from Giraffe Pipe has been found to be dominated by an extensive number of eunotioid diatoms. The objective of this paper is to begin to describe members of *Eunotia* from this Eocene locality, with descriptions of three of the more common species.

Material & methods

Site and Core Description

Detailed descriptions of the Giraffe Pipe locality are given in Siver *et al*. (2015), Wolfe *et al*. (2017), and references therein. Briefly, the Giraffe Pipe locality (64°44' N, 109°45' W) is located within a crater formed as a result of emplacement of a kimberlite diatreme into the Slave Craton, situated in the Northwest Territories, Canada. The emplacement occurred approximately 47.8 Ma between the Ypresian (56–47.8 Ma) and Lutetian (47.8–41.2 Ma) of the Eocene (Siver & Wolfe 2005a, Wolfe *et al*. 2006). Post emplacement, the diatreme crater harbored an aquatic environment that remained for what has been estimated to be thousands of years, eventually transitioning to a terrestrial environment, and later capped by Neogene glacial deposits (Siver & Wolfe 2005a, Wolfe *et al*. 2006).

A 163 m long core, drilled at a 47° angle, was recovered from the Giraffe maar in 1999 by BHP Billiton Inc. (Wolfe *et al*. 2006, Siver & Wolfe 2009). The lower 113 m of the core contains 68 m of lacustrine mudstones, overlain with 45 m of peaty and terrestrial remains. The lake and terrestrial sediments underwent little to no post-deposition alteration, yielding exceptionally preserved and abundant concentrations of fossils. The core is archived at the Geological Survey of Canada's core and cuttings repository in Calgary, Alberta, Canada. All samples taken from the core and used in this study are archived at the Connecticut College core facility, New London, Connecticut, U.S.A.

The core was cut on site and stored in core boxes. Each core box contains 4.5 meters of core material, divided into three 1.5 meter-long sections stored in separate channels. Samples from the core are identified with a three part number (Siver 2015). The first number indicates the core box, numbered sequentially from the surface down to the bottom of the core. Eighteen boxes, numbers 11 through 27, contain the remains of the lacustrine mudstones, with box 11 representing the termination (most recent) of the aquatic environment. The second number represents the channel within the box. The third number is a measurement in cm along a length of core within a given channel.

Laboratory methods

We examined 175 samples distributed over the 68 m lacustrine section of the core for remains of *Eunotia* specimens. Mudstone fragments (0.5–1.0 g) from each sample were oxidized using 30 % H_2O_2 under low heat for a minimum of 1–3 hr, rinsed multiple times with distilled water, and the resulting slurries stored in glass vials at ~4 ºC. This mild oxidation procedure resulted in separation of numerous microfossil specimens from the majority of mudstone samples. A few samples were treated more aggressively with a sulfuric acid-potassium dichromate solution after the procedure of Marsicano & Siver (1993), in addition to treatment with H_2O_2.

Aliquots of each slurry were used to prepare samples for observation with scanning electron microscopy (SEM) and light microscopy (LM). For SEM, an aliquot was diluted and air dried onto a piece of heavy duty aluminum foil, trimmed, and attached to an aluminum SEM stub with Apiezon® wax. Samples were coated with a mixture of gold and palladium for 2 min with a Polaron Model E sputter coater, and examined with a Leo (Zeiss) 982 FESEM or a FEI Nova NanoSEM 450 FESEM. For LM, aliquots were air dried onto coverslips, mounted onto glass slides using Naphrax, and examined at 1000× magnification using Differential Interference Contrast optics with either an Olympus BH51 microscope (N.A. 1.35) coupled with a SONY DKC-ST5 digital camera, or a Leica DMR microscope (N.A. 1.4) coupled with a Zeiss Axiocam 503 digital camera.

Terminology used to describe the new species is based on Ross *et al*. (1979) and Siver & Hamilton (2011). Measurements of valve sizes and striae densities were made directly from the SEM or LM images, with the latter measured at the center of the valve. Except for material submitted and archived at the Canadian Museum of Nature, all slurry and slide preparations, and SEM stubs, used in the study are stored in Siver's research laboratory at Connecticut College.

Results

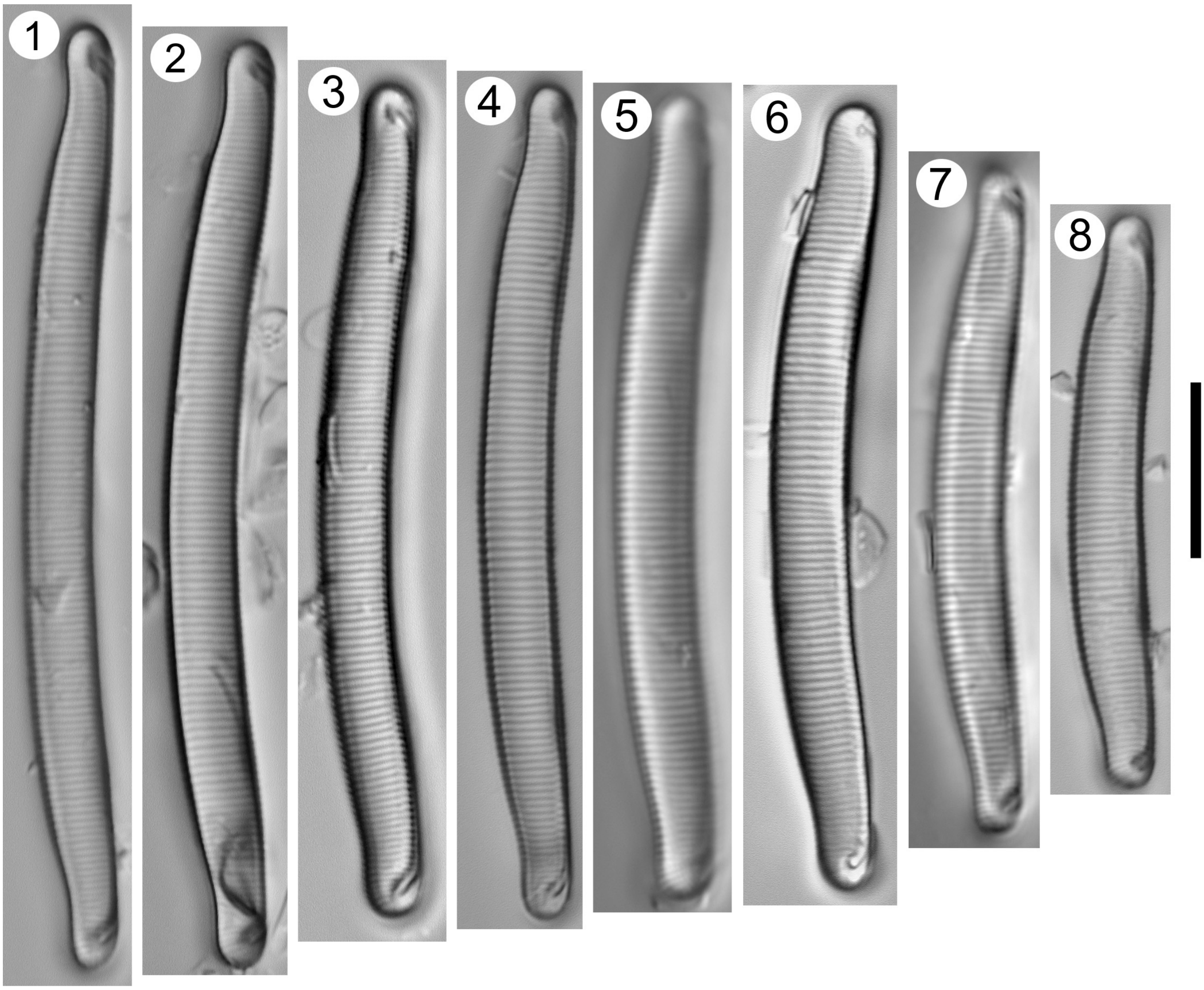
Seventy-one of the 175 core sections examined contained specimens of either *Eunotia*, *Actinella* or both. The genus *Eunotia* was especially abundant over a 13 m section ranging in depth from 122–109 m along the core, and contained in boxes 14–16. This section is estimated to contain over 20 species of *Eunotia*, and we now have examined a sufficient number of specimens to make formal species descriptions for three taxa.

Taxonomic results

Division Bacillariophyta
Class Bacillariophyceae
Subclass Eunotiophycidae
Order Eunotiales
Class Eunotiacea
Genus *Eunotia*

***Eunotia giraffensis* Siver, Oddsund & Lott *sp. nov*.** (LM Figs 1–8, SEM Figs 9–14)

Description:—Valves are slightly dorsi-ventral, elongate, with broadly rounded and obliquely rostrate apices that are deflected slightly towards the dorsal margin (Figs 1–8). Valves range in length from 26–68 µm with a mean of 39 µm, and in diameter from 3.2–6.5 µm with a mean of 3.7 µm (n = 40). The ventral margin is slightly concave, becoming almost linear on smaller valves, and is more or less parallel with the dorsal margin, and of equal diameter throughout except at the apices. Small spines may be present on the virgae between the striae on the valve margin. Striae range from 16–25 per 10 µm, with a mean of 22. Striae are evenly spaced, parallel over most of the valve, becoming more closely spaced towards the apices (Figs 1–8). Striae are continuous from the valve face onto the mantle on both the ventral and dorsal sides (Figs 10, 12–14). Areolae are small, circular, closely spaced, and open on both the external and internal valve surfaces. The mantle is deep, approximately 3.5–4 µm, and forms a right angle with the valve margin on both the ventral and dorsal sides (Figs 11–14). The margin of the mantle is thickened around each apex (Figs 10, 12). The proximal end of the raphe commences midway down the mantle (Figs 13–14). From this point, the raphe rises slowly until it reaches the valve margin, then turns approximately 45º up onto the valve face, terminating midway across the valve face close to the apex. The portion of raphe on the valve face is straight and the distal end is not curved or recurved (Figs 9, 11). A distinct hyaline zone extends approximately 3 µm from the proximal end of the raphe towards the center of the valve (Figs 13–14), is continuous along the lower side of the raphe, and extends up onto the valve face terminating near the apex. The striae are more closely spaced on the mantle below the raphe (Figs 13–14), and a discontinuity zone is often observed midway along the raphe. Internally, the distal raphe fissure terminates within a thick and well-formed helictoglossa (Figs 10, 12), which in turn is surrounded by a hyaline zone. A single rimoportula is found at one of the valve apices (Fig. 12).



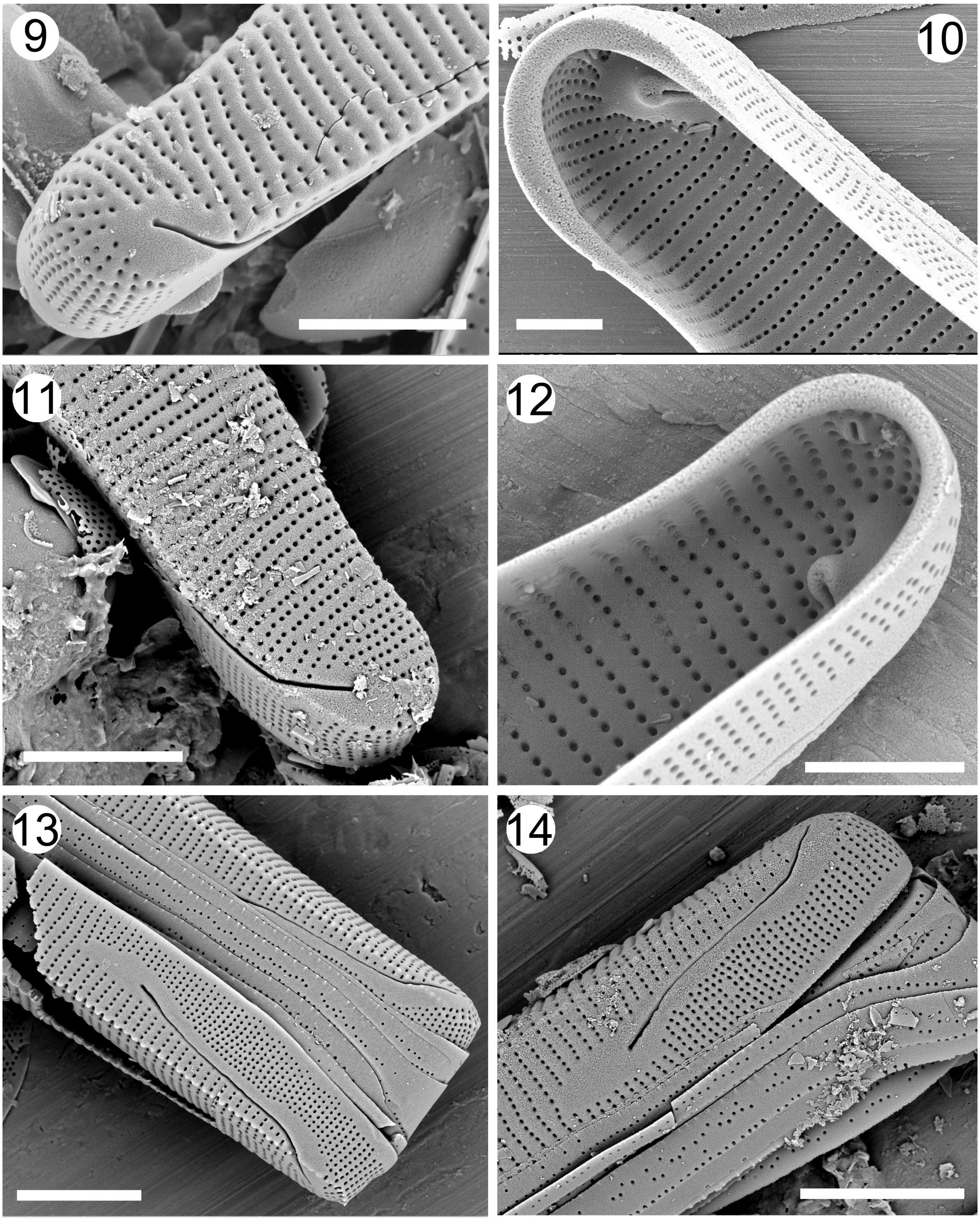
FIGURES 1–8. LM micrographs of *Eunotia giraffensis sp. nov.* from the Giraffe Pipe fossil locality. Figure 5 is the isotype specimen circled on slide "GP 16-3-42 C" in P. Siver's collection. Figure 7 corresponds to the type specimen circled on slide "GP 16-3-42 B" (CANA 129307). Scale bar = 10 µm.

Type:—CANADA. Lac de Gras kimberlite field region, Northwest Territories: Rock specimen sub sampled from section 16-3-42 of the Giraffe Pipe core (holotype circled specimen on slide "GP 16-3-42 B" Canadian Museum of Nature CANA 129307 = Fig. 7. Isotype circled specimen on slide "GP 16-3-42 C" P. Siver's personal collection = Fig. 5).

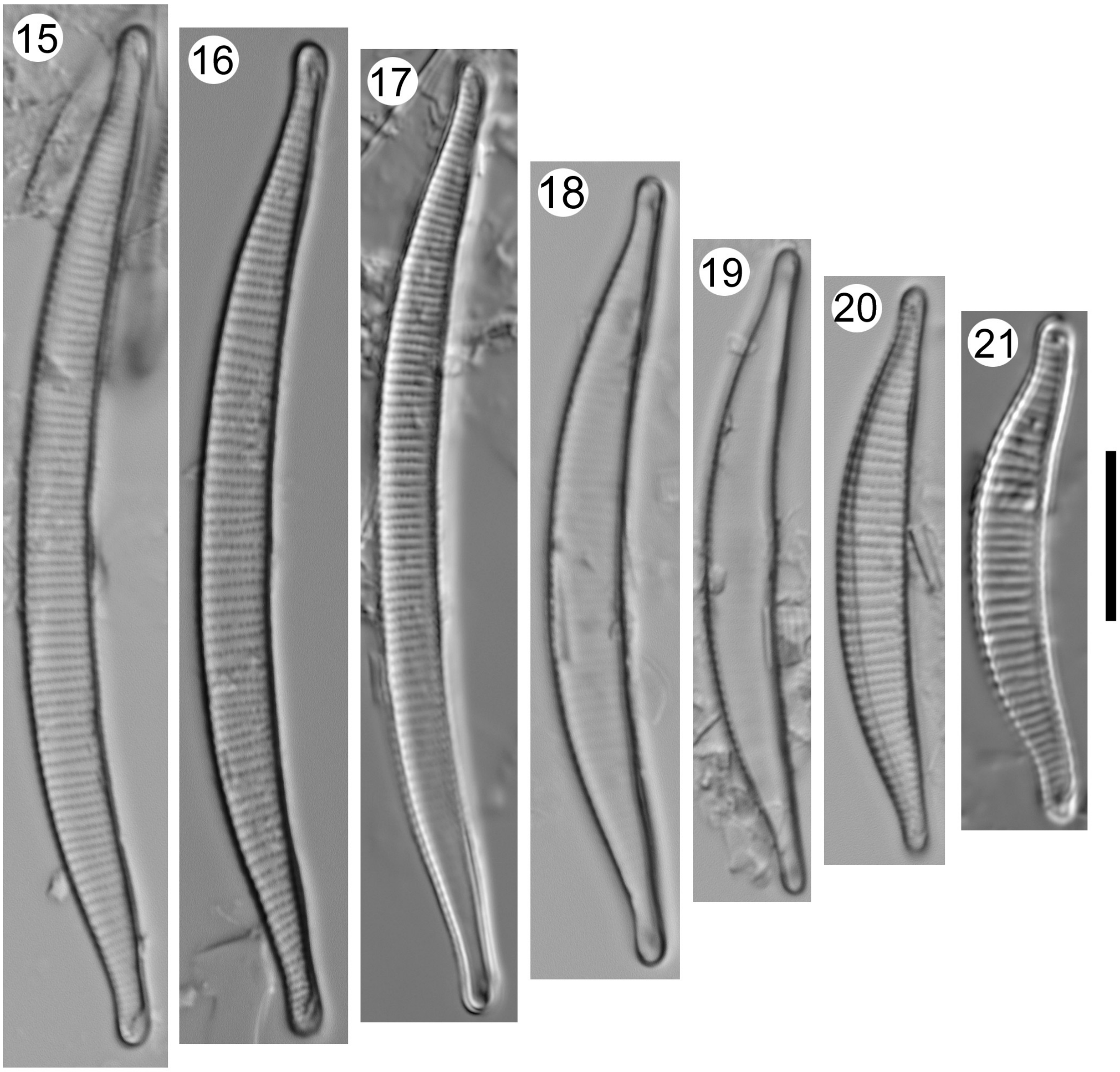
Etymology:—The species epithet refers to the type locality.

***Eunotia petasum* Siver, Oddsund & Lott *sp. nov*.** (LM Figs 15–21, SEM Figs 22–27)

Description:—Valves are dorsi-ventral, elongate, widest in the middle of the valve, and becoming tapered and drawn out at the apices forming protracted ends (Figs 15–21). The ventral margin is concave, becoming slightly linear at the apices. The dorsal margin is more convex relative to the concave ventral margin such that the valve becomes progressively narrower from the center to the apices (Figs 24–27). Valves range in length from 19.6–57.6 µm with a mean of 38 µm, and in diameter at the valve center from 2.9–5.1 µm with a mean of 3.8 µm (n=25). At the apices, valve width ranges from 1.5–2.2 µm, with a mean of 1.8 µm. The margins of the valve face, especially along the ventral side, are slightly thickened and form right angles with the mantle (Figs 23–24). Striae are parallel, widely spaced with 13.5–19 per 10 µm, a mean of 16 µm, and consist of small, circular areolae that are opened on both the external and internal valve surfaces. Striae are continuous from the valve face onto the dorsal mantle, but may be interrupted and discontinuous with those on the ventral mantle (Figs 23–24). On smaller valves, the areolae on the ventral mantle may become more randomly spaced, especially below the raphe branches (Fig. 23). The mantle is shallow, approximately 1.5–2 µm, and the margin slightly thickened around each apex. The proximal end of the raphe commences near the middle of the mantle approximately 1/4 to 1/3 of the valve length from the apex, rises slowly on a linear path to the valve margin, and then bends up and onto the valve face terminating close to the end of the valve (Figs 23, 25). The proximal and distal raphe fissures both terminate as small round pores (Figs 23–25). On most specimens, the portion of the mantle below the raphe consists of short striae composed of a few pores, but on some valves the pores are randomly spaced and not organized into distinct striae. Internally, the distal raphe fissure terminates within a small helictoglossa positioned about 1/3 the distance across the valve face (Figs 26–27). A single rimoportula is found on one of the valve apices.



FIGURES 9–14. SEM micrographs of *Eunotia giraffensis sp. nov*. from the Giraffe Pipe fossil locality. 9, 11. Close-ups of the end of a valve face showing the straight distal raphe end and the closer-spaced striae at the valve apex. 10, 12. Internal views of the helictoglossa and distal raphe end. Note the extended hyaline region surrounding the helictoglossa, thickened margin of the valve around the apex, densely-spaced striae on the mantle below the raphe, and the presence of a rimoportula. 13, 14. Girdle views of the ventral mantle depicting the position of the raphe, hyaline region surrounding the raphe, and the densely-spaced striae on the mantle below the raphe. Scale bars = 2 µm (Figs 9–12, 14), 3 µm (Fig. 13).



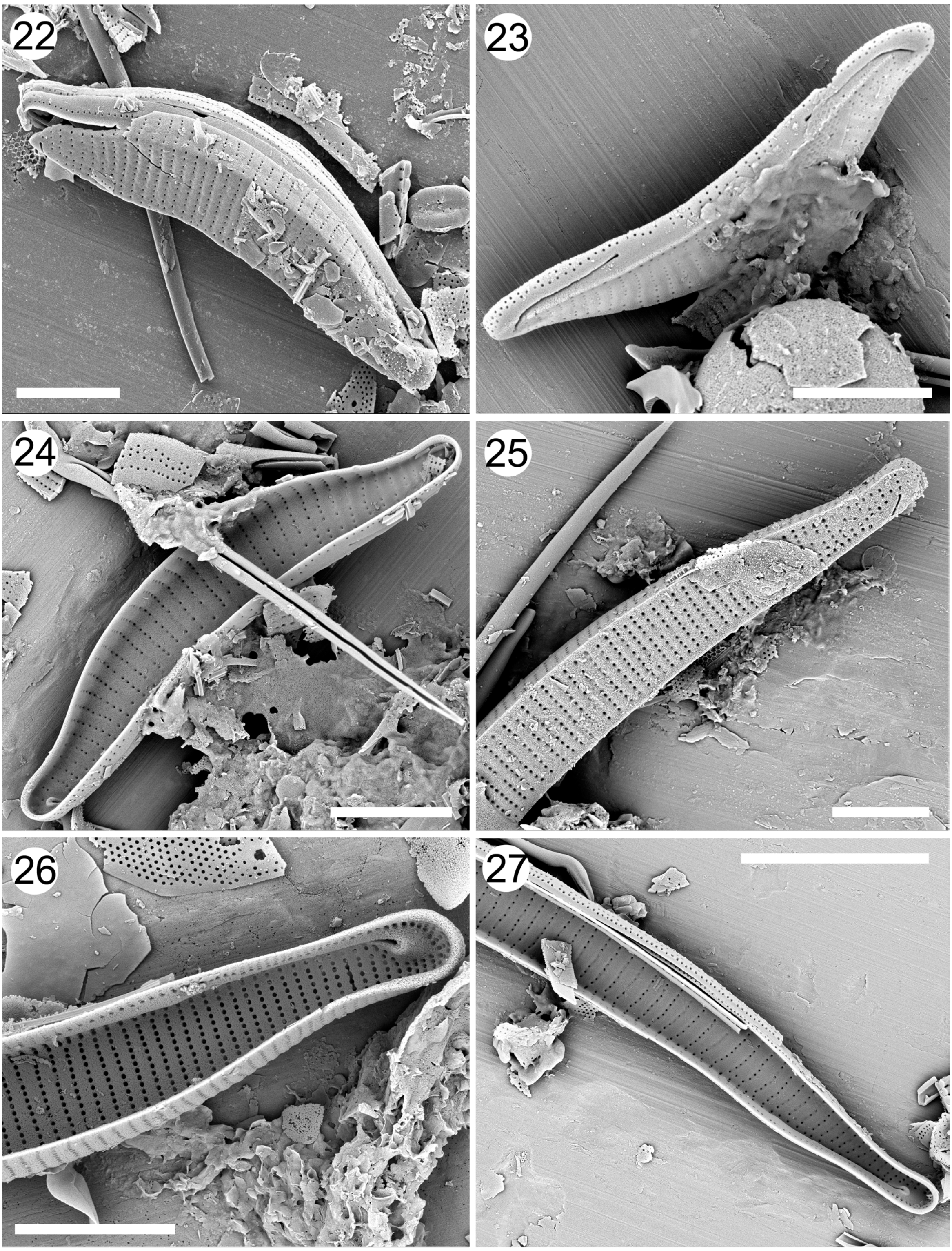
FIGURES 15–21. LM Micrographs of *Eunotia petasum sp. nov.* from the Giraffe Pipe fossil locality. Figure 17 is the type specimen circled on slide "GP 15-3-75D, LM5" (CANA 129308). Figure 21 corresponds to the isotype specimen circled on slide "GP 15-3-75 LM4" in P. Siver's collection. Scale bar = 10 µm.

Type:—CANADA. Lac de Gras kimberlite field region, Northwest Territories: Rock specimen sub sampled from section 15-3-75 of the Giraffe Pipe core (holotype circled specimen on slide "GP 15-3-75 D, LM 5" Canadian Museum of Nature CANA 129308 = Fig. 17. Isotype circled specimen on slide labeled "GP 15-3-75 LM4" P. Siver's personal collection = Fig. 21).

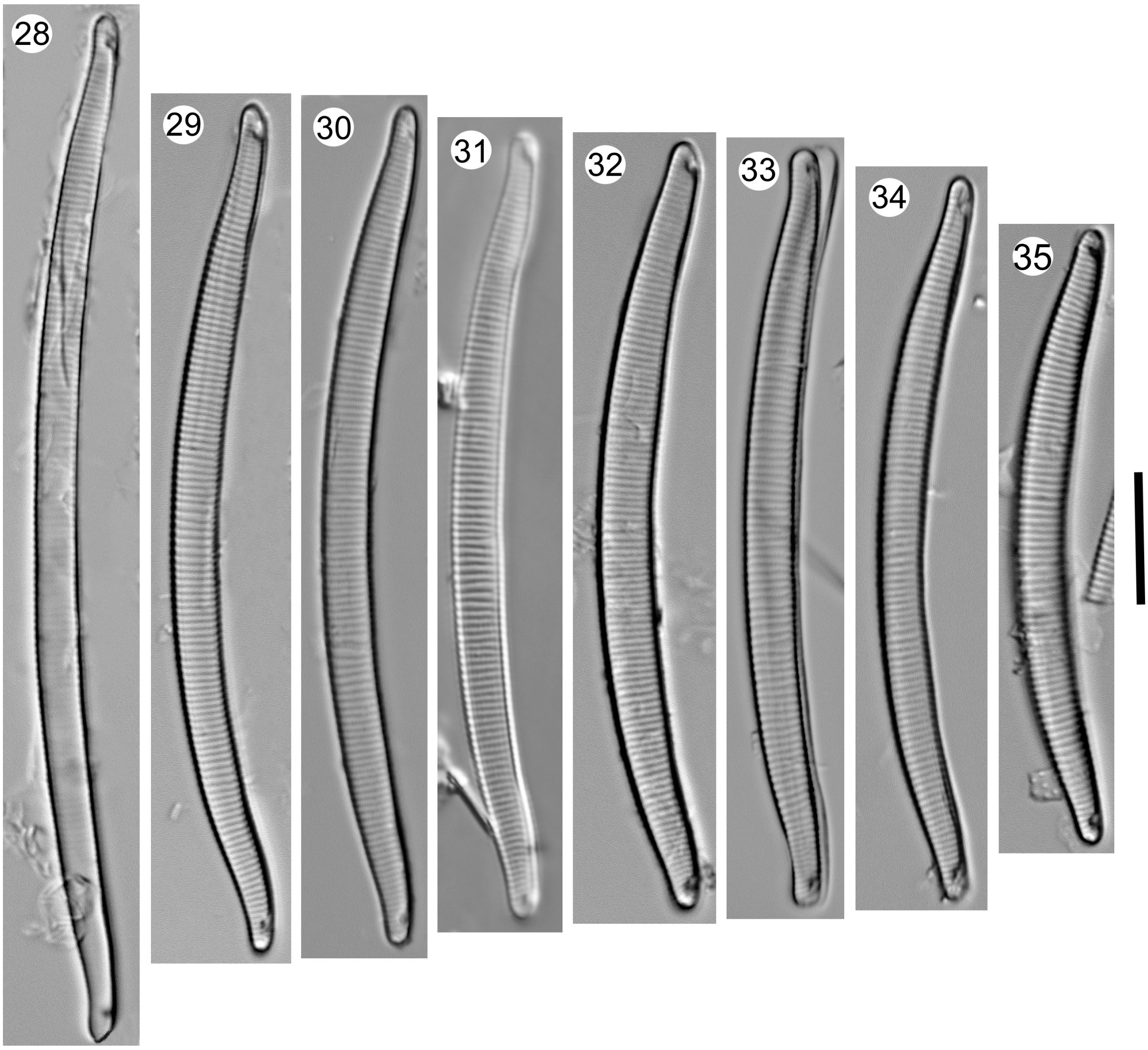
Etymology:—The name refers to a hat, whereby smaller specimens give the impression of a hat worn, for example, by Napoleon.

Eunotia pseudonaegelii **Siver, Oddsund & Lott *sp. nov*.** (LM Figs 28–35, SEM Figs 36–41)

Description:—Valves are long and narrow, linear to slightly lunate, and with slightly protracted apices (Figs 28–35). Valves range in length from 44–91 µm with a mean of 64, and in diameter from 2.4–4.3 µm with a mean of 3.4 (n=30). The ventral and dorsal margins are more or less parallel resulting in a similar diameter over most of the valve. Small spines are present on the virgae along both valve margins, and often around the apices (Figs 36–38, 40–41). Striae are evenly spaced, parallel, continue from the valve face onto the mantle, range in density from 17–20 per 10 µm and a mean of 19. Areolae are small, circular, closely spaced, and open on both the external and internal valve surfaces (Fig. 36–41). The mantle is shallow, often not more than 1 µm in depth, and forms a right angle with the valve margin on both the ventral and dorsal sides (Figs 38–39, 41). The raphe is approximately 6.5–8 µm long. The proximal end of the raphe is positioned near the bottom of the ventral mantle, rises on a more or less 45º linear path, curves up onto the valve face, and with the distal end terminating in a small pore about 1/3 the distance across the valve face (Figs 36, 38, 40). Internally, the raphe is slightly elevated from the mantle, especially near the proximal end, and the distal end terminates within a small, well-formed, helicoglossa (Figs 37, 39). The striae below the raphe are typically reduced to a single row of pores (Figs 37, 39). Internally, the distal raphe fissure terminates within a thick and well-formed helictoglossa, which in turn is surrounded by a hyaline zone (Figs 37, 39). A single rimoportula is found per valve (Figs 37, 39).



FIGURES 22–27. SEM micrographs of *Eunotia petasum sp. nov.* from the Giraffe Pipe fossil locality. 22. Dorsal view of a frustule showing the continuation of the striae from the valve face onto the mantle. 23. Ventral view of a valve depicting the raphe and reduced striae, often consisting of only 1–2 pores. 24. Interior view of a valve showing the continuation of the striae from the dorsal mantle onto the valve face. Striae on the ventral mantle are often reduced to random pores. 25. Exterior view of a valve depicting the narrow and protracted end and the distal raphe fissure. 26, 27. Internal views showing the striae, the open nature of the pores, the protracted apex, and the small rounded helictoglossa. Scale bars = 5 µm (Figs 22–26), 10 µm (Fig. 27).



FIGURES 28–35. LM micrographs of *Eunotia pseudonaegelii sp. nov*. from the Giraffe Pipe fossil locality. Figure 31 corresponds to the type specimen circled on slide "GP 16-3-42E" (CANA 129309). Scale bar = 10 µm.

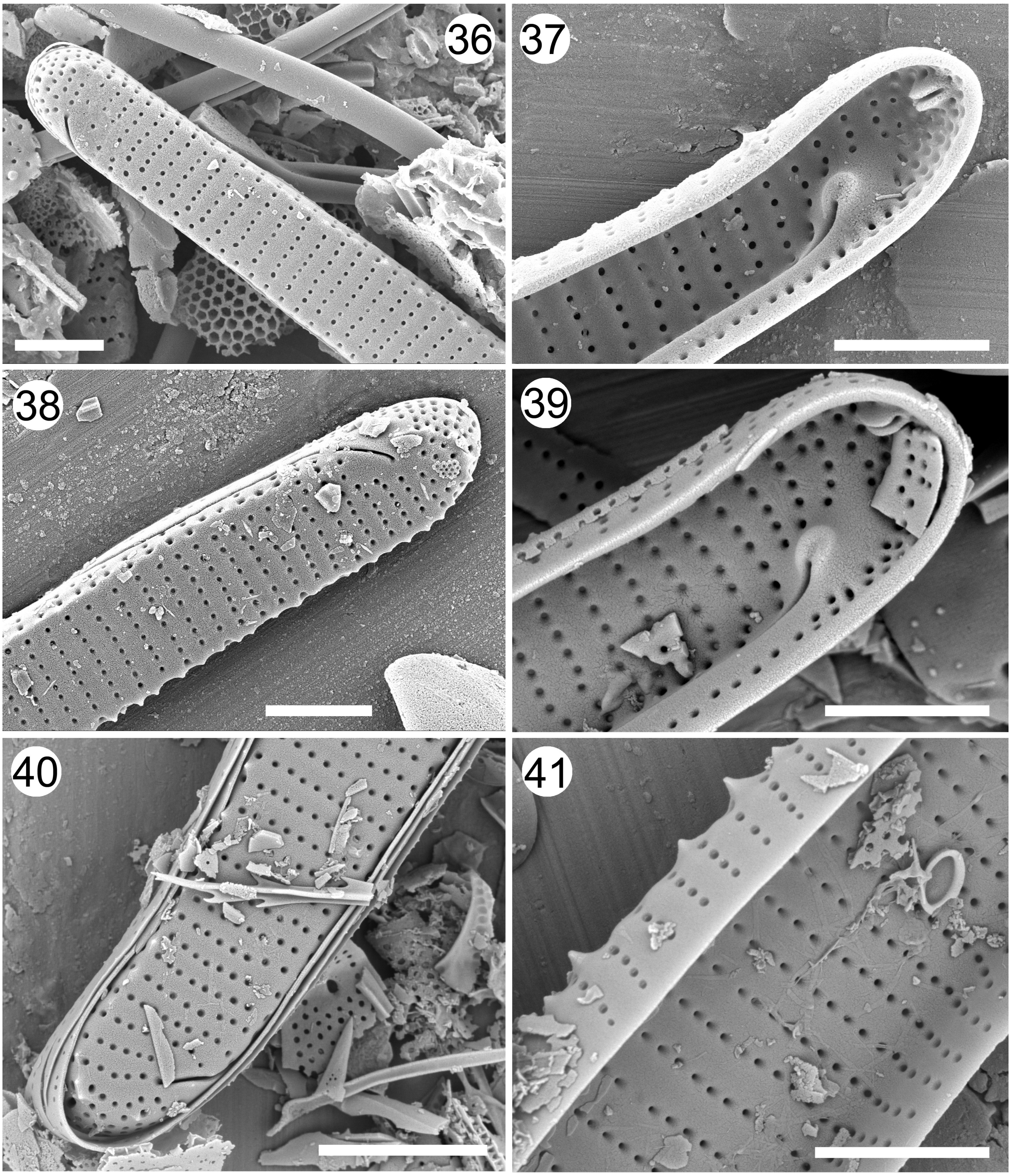
Type:—CANADA. Lac de Gras kimberlite field region, Northwest Territories: Rock specimen sub sampled from section 16-3-42 of the Giraffe Pipe core (holotype circled specimen on slide "GP 16-3-42 E" Canadian Museum of Nature CANA 129309 = Fig. 31).

Etymology:—The name refers to the similarity in shape with *Eunotia naegelii*, but reflects the fact they are not the same organism.

Co-occurring organisms:—The extensive portion of the core harboring all three of the new *Eunotia* species contained other species of *Eunotia*, species of the closely related genus, *Actinella*, and extensive concentrations of chrysophyte microfossils. In addition to numerous and many types of chrysophyte cysts, remains of three synurophytes, *Mallomonas lychenensis* Conrad (1938: 1), *M. porifera* Siver & Wolfe (2005b: 300) and *Synura cronbergiae* Siver (2013: 181) were also dominant throughout this section of the core. Remains of euglyphid testate amoebae and sponge spicules were consistently present as well, but in smaller numbers relative to lower sections representing earlier time periods in the history of the lake.

Discussion

The Giraffe Pipe fossil locality (Siver & Wolfe 2007), along with the Horsefly deposit in British Columbia (Benson *et al*. 2012), represent the oldest sites harboring remains of *Eunotia*, confirming that this genus was well established by the Ypresian. Other fossil sites containing remains of *Eunotia* species appear soon after the Ypresian, including the Dewey Beds in Idaho, USA, at 45 Ma (Benson *et al*. 2012), the Florissant Formation, Colorado, USA, at 34 Ma (Benson *et al*. 2012), and diatomite from Oamaru, New Zealand, at 32–35 Ma (Novitski & Kociolek 2005, Williams & Reed 2006). All of these localities represent freshwater sites with the exception of the nearshore marine sediments from Oamaru. There remains some question whether the *Eunotia* specimens uncovered from Oamaru represent marine taxa, or if they originated in freshwater habitats and were transported to the marine sediments. Novitski & Kociolek (2005) concluded that because other diagnostic freshwater diatoms have not been uncovered from Oamaru, the two *Eunotia* species described from this site were true marine taxa. Nonetheless, given the abundance and diversity of *Eunotia* specimens from Giraffe Pipe, it is clear this genus was well established in freshwater by the end of the Ypresian.



FIGURES 36–41. SEM micrographs of *Eunotia pseudonaegelii sp. nov*. from the Giraffe Pipe fossil locality. 36, 38, 40. Exterior views of the end of a valve showing the position of the distal raphe end that extends only a short distance onto the valve face. Note the small spines along the dorsal margin and apex. 37, 39. Internal views of the distal raphe end, helictoglossa and position of the well-developed rimoportula. Note the shallow depth of the mantle. 41. Internal view depicting continuation of the striae on the valve face onto both the ventral mantle and the dorsal mantle. Note the spines along the dorsal valve margin. Scale bars = 2 µm (Figs 36–39, 41), 3 µm (Fig. 40).

All three of the newly described species display clear characteristics found on modern taxa, further emphasizing that the genus was not only established by the early Eocene, but supporting the idea that it is likely considerably older. The size and long slender shape of *E. pseudonaegelii sp. nov.* valves are similar to a number of modern species, including *E. naegelii* Migula (1905: 203), *E. flexuosa* (Brébisson ex Kützing) Kützing (1849: 6), *E. desmogonioides* Metzeltin & Lange-Bertalot (2002: 27), *E. juettnerae* Lange-Bertalot (in Lange-Bertalot *et al.* 2011: 127) and *E. genuflexa* Nörpel-Schempp (in Lange-Bertalot & Metzeltin 1996: 50). Distinguishing features between the modern species can be slight, and often reflect differences in striae density or valve length and width (Lange-Bertalot *et al.* 2011, Siver & Hamilton 2011, Costa *et al.* 2017). Indeed, isolated valves of this suite of modern species can be difficult to identify based on overall shape and size. However, all of these modern species with long slender valves have distal raphe fissures that bend backwards 180º on the valve face, forming fish-hook designs. This distinctive, and relatively common, feature is also found on species in the *E. bilunaris* complex (Alles *et al.* 1991, Lange-Bertalot *et al.* 2011), possibly indicating a close evolutionary relationship between taxa that share this characteristic. However, this feature is clearly absent on *E. pseudonaegelii sp. nov.* valves, and, in fact, we have not observed recurved distal raphe ends on any of the numerous *Eunotia* specimens found in the Giraffe Pipe core. Nor has it been observed on any fossil *Eunotia* species from the Eocene. The advantage of a recurved distal fissure, if any, is not known, but perhaps it aids in the motility mechanism. The evidence to date supports the idea that this feature evolved after the Eocene.

The overall valve appearance of *Eunotia giraffensis sp. nov.* is very similar to the modern taxon *Eunotia lewisii* Siver & Hamilton (in Siver *et al.* 2009: 410), a species described from shallow, dilute, highly acidic and humic-stained ponds in the Pinelands of southern New Jersey (Siver & Hamilton 2011). The valve shape, configuration of the raphe and striae, hyaline region surrounding the raphe, and the densely packed striae below the raphe are strikingly similar on both species. In addition, the stria density on the valve face is also similar on both species, becoming denser at the apices. The species differ in the internal structure of the distal raphe end. On *E. lewisii* valves, the distal end of the raphe ends in a helictoglossa associated with a large, solid, hyaline structure that extends across the valve face. This large solid structure is unique among *Eunotia* species and significantly different from the configuration found on valves of *E. giraffensis sp. nov.* In addition, although there is overlap, valves of *E. giraffensis sp. nov.* tend to be smaller than those of *E. lewisii*.

Other modern *Eunotia* species that share some similarities to *E. giraffensis sp. nov.* include *E. gustavoi* Costa (in Costa *et al.* 2017: 25), *E. intricans* Lange-Bertalot & Metzeltin (2009: 141) and *E. xystriformis* Manguin (in Bourrelly & Manguin 1952: 49), all known from South America, and *E. lapponica* Grunow ex A. Cleve (in Cleve [Cleve-Euler] 1895: 29) first described in Europe. Valves of *E. gustavoi* have a similar shape, size and pattern of the striae, but have very different distal raphe ends and more protracted apices. *Eunotia intricans* valves are also of similar shape and the striae become more closely spaced at the poles, but *E. giraffensis sp. nov.* valves have a greater striae density and are larger. In addition, the striae pattern on the mantle below the raphe differs on both taxa, and the distal raphe ends on *E. intricans* are slightly recurved. Similarities between *E. giraffensis sp. nov.* and *E. xystriformis* include valve shape, a similar hyaline region surrounding the raphe, and the closer-spaced striae on the mantle below the raphe, but valves of *E. xystriformis* are considerably larger, possess a lower striae density and apical spine, and have a very different distal raphe end. Some specimens of *E. lapponica* illustrated in Lange-Bertalot *et al.* (2011) also resemble those of *E. giraffensis sp. nov.* and both taxa have a similar hyaline region surrounding the raphe, coupled with a greater striae density on the mantle below the raphe. However, *E. lapponica* is significantly larger and most specimens have protracted apices that are deflected towards the dorsal side.

Only a few modern species, including *E. pexii* Lange-Bertalot (in Werum & Lange-Bertalot 2004: 155) and *E. ferefalcata* Kulikovskiy & Lange-Bertalot (in Lange-Bertalot *et al.* 2011: 101), have an overall shape with similarities to *E. petasum sp. nov*. *Eunotia pexii* differs in being a smaller taxon with a more strongly dorsi-ventral shape, and obliquely shaped rostrate apices that are slightly deflected towards the dorsal margin. Valves of *E. ferefalcata* have a similar size range, but have less protracted ends and a slightly higher striae density. Although valves of *E. petasum sp. nov.* do not have undulated dorsal margins and could not be confused with *E. longicamelus* Costa, Bicudo and Wetzel (in Costa *et al.* 2017: 32), the degree of curvature of the ventral surface and shape of the protracted apices are similar on both taxa.

Development of the raphe used for movement represents one of the most significant events in the evolution of the diatoms (Sims *et al.* 2006, Medlin 2016). Morphological and molecular data support development of the raphid diatoms from araphid pennate ancestors (Sims *et al.* 2006, Sorhannus 2007). An unsettled question is whether the initial raphe was similar to a eunotioid design consisting of a pair of short apical slits on the valve mantle, which later migrated to and became incorporated with the central sternum, a hypothesis advanced by Mann (1984) and others. This hypothesis is supported by molecular data presented by Sorhannus (2004) and Brown & Sorhannus (2010) who reported the eunotioid clade to be basal within the raphe-bearing Bacillariophyceae. An alternative, and essentially reverse hypothesis, was that the eunotioid raphe system was derived within the raphid clade, moving from a central position on the valve face to a marginal position on the mantle. Molecular works of Medlin & Kaczmarska (2004) and Sorhannus (2007) placed the eunotioid lineage in a derived position within the raphid clade, lending support to the alternative hypothesis. Unfortunately, the *Eunotia* species we describe herein do not present any new data in support of either hypothesis. On the other hand, our findings clearly show that the characteristics, including those of the raphe, that define *Eunotia* were well established by 48 Ma and that we need to dig deeper in the geologic record for fossil evidence that can help determine the full evolutionary history of the formation of the raphe.

The section of the Giraffe core containing abundant numbers of *Eunotia* remains, including the three new species, most likely represents the remains of a shallow, acidic and humic-stained pond. Species of *Eunotia* are common elements of such waterbodies, and this genus is known as one of the most acidophilic diatom genera reported from freshwater environments (Camburn & Charles 2000, Gaiser & Johansen 2000, Siver & Hamilton 2011, Wetzel *et al*. 2011, Costa *et al*. 2017). Indeed, a large number of acidic ponds and lakes, mostly with elevated dissolved colored organic matter, stretching from the Atlantic Coastal Plain to the Adirondack Mountains along the eastern portion of the U.S.A. were found to contain high numbers and diversities of *Eunotia*, with weighted mean pH estimates for almost all of the species below 6 (Camburn & Charles 2000, Gaiser & Johansen 2000, Siver *et al*. 2005, Siver & Hamilton 2011). Similar findings have been reported for the Neotropical region of South America, also known to harbor numerous acidic and darkly-stained ponds and lakes containing a high diversity of *Eunotia* species (Metzeltin & Lange-Bertalot 1998, 2007, Burliga *et al*. 2007, Wetzel *et al*. 2011).

Other organisms found with the *Eunotia* remains further support the idea that the waterbody was shallow, acidic and humic-stained. For example, the section also contains multiple species of *Actinella* and *Oxyneis* Round (in Round *et al*. 1990: 402, 673), two additional diatom genera almost exclusively reported from these types of waterbodies (Flower 1989, Kociolek & Rhode 1998, Sabbe *et al.* 2001, Kingston 2003, Melo *et al*. 2010, Siver *et al*. 2010, 2015). In addition, the presence of high concentrations of chrysophyte cysts, synurophyte species associated with acidic ponds, and testate euglyphids (Siver *et al*. 2020) further supports a shallow, acidic, and humic-stained hypothesis.

In summary, the Giraffe Pipe fossil locality harbors an extensive diversity of *Eunotia* diatoms, indicating that the genus was well established by the early to middle Eocene and clearly well distributed in freshwater habitats at northern latitudes.

Acknowledgements

This project was funded, in part, with grants to PAS from the National Science Foundation (EAR-1725265 and EAR-1940070), and the American Philosophical Society. Special thanks to Xuanhao Sun from the Bioscience Electron Microscopy Laboratory (BEML) at the University of Connecticut for help with SEM facilities. We also thank Nadja Ognjanova-Rumenova and two anonymous reviewers for constructive comments.

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