

# The novel species *Navicula eileeniae* (Bacillariophyta, Naviculaceae) and its recent expansion in the Central Appalachian region of North America

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**Background and aims** – A novel species of *Navicula* has been recently discovered in rivers of the central Appalachian Mountains, USA. The purpose of this paper is to formally establish this new species and to determine whether it had been overlooked in the past or is a recent arrival to the area.

**Methods** – We studied historical collections made in Pennsylvania, Virginia and adjacent areas since the 1940s and housed at the ANSP Diatom Herbarium. Light and scanning electron microscopy was employed to study the morphology of the new species and similar *Navicula* taxa, including those commonly reported at the same locations, or originally described from the rivers of the eastern United States, such as *N. radiosafallax*, initially established as *N. radiosa* var. *parva* by J.H. Wallace.

**Key results** – The new species has an unusual shape of the external raphe slit, which is undulate toward the proximal ends. This character was so far found in a single other *Navicula* species known only from Europe. Examination of historical materials confirmed that *N. eileeniae* was absent from collections made in the eastern United States before 2007, but has become progressively more common and abundant in the last decade. The prevalence of this species in streams of low to moderate mineral and nutrient content suggests that its recent expansion cannot be explained by pollution. Climate change is also unlikely to have caused the northward expansion of *N. eileeniae*, as it has never been recorded south from central Appalachia.

**Conclusions** – The results of this study confirm that a population of previously unknown *Navicula* species has been expanding in Central Appalachia and suggest that rapid shifts of distributional ranges not readily explainable by environmental changes may occur in protists.

**Key words** – Bacillariophyta, diatoms, morphology, new species, *Navicula*, rivers, taxonomy, United States.

## INTRODUCTION

The genus *Navicula* Bory is ecologically diverse and exceptionally species-rich, although the number of species currently assigned to this genus is difficult to estimate because of its continuous and rapid taxonomic re-assessment. The current concept of *Navicula* is largely based on seminal studies of Dr. Eileen Cox, who redefined the genus (Cox 1979, 1999), building on ideas of the significance of protoplast features and frustule morphogenesis for systematics of naviculoid diatoms (Cox 1981, 1982, 1999b, 2010, 2012, Cox & Reid 2004, Cox & Williams 2000, 2006). A valve structure rich

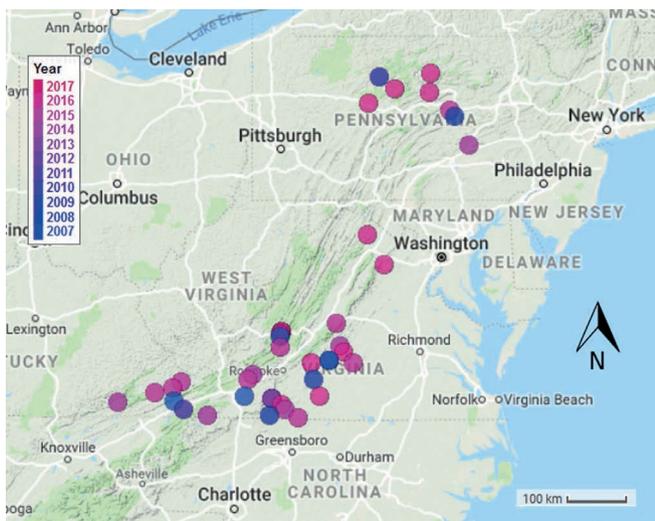
in taxonomically useful characters in *Navicula* is the raphe and its associated system of ribs, grooves and “nodules” or valve thickenings. Cox (1995, 1999a) revealed considerable variability of raphe morphology in *Navicula* and showed that configurations of the raphe slits on external and internal valve surface can be important taxonomic characters. The most comprehensive taxonomic treatise of the genus to date (Lange-Bertalot 2001) pays special attention to features of the raphe, such as the shape of the proximal raphe ends, especially at the external valve surface.

While examining recent diatom collections from streams and rivers of Pennsylvania and Virginia, USA, we found a

novel species of *Navicula* with an unusual shape of the proximal part of the external raphe slit. To determine whether this species had been overlooked in the past or was a recent arrival to the area, we studied historical collections made from streams and rivers of these states and surrounding areas since the 1940s. The purpose of this paper is to formally establish this new species and to report our findings on its past and present distribution. We also examined the type material of *N. radiosafallax* Lange-Bert., a morphologically similar species common in the eastern United States. The frustule ultrastructure of this species had not been previously studied under scanning electron microscopy (Lange-Bertalot 2001).

## MATERIAL AND METHODS

We examined a total of 762 diatom slides from the ANSP Diatom Herbarium (Academy of Natural Sciences, Philadelphia). All were benthic stream samples collected from Pennsylvania, Virginia, Tennessee and Maryland between 1941 and 2017 (electronic appendix 1). The oldest collections were made by Dr. Ruth Patrick during a road trip across Virginia and Tennessee in 1941. Some slides originated from river and stream surveys conducted by the Academy of Natural Sciences beginning in the early 1950s, including the James River (1951), North Anna River (1954), Holston River (1954–1980), and Jackson River (1997–2000) surveys, and surveys of North-Central Pennsylvania (2009) and South-Western Pennsylvania (2013). Other collections were made by federal and state agencies, such as the US Geological Survey, the US Environmental Protection Agency, Virginia Department of Environmental Quality and Pennsylvania Department of Environmental Protection since the 1990s.



**Figure 1** – Map of the Eastern United States showing locations of river sites where *Navicula eileeniae* was recorded. The colour scale shows transition from older (blue) to more recent (purple) samples. Map produced using GPS Visualizer (<http://www.gpsvisualizer.com/>) and underlying Google Maps API (©Google). This image is not covered by the terms of the Creative Commons licence of this publication. For permission to reuse, please contact the rights holders (Adam Schneider, <http://www.gpsvisualizer.com/>; and Google).

Samples containing the new *Navicula* species are listed in table 1 and their locations are shown in fig. 1.

To compare the new species with the most similar *Navicula* species commonly reported from the eastern United States, we examined specimens from several additional samples under light (LM) and scanning electron (SEM) microscopy. Their provenance is also reported in electronic appendix 1. The type material of one of these species, *Navicula radiosafallax* (replaced synonym: *Navicula radiosa* var. *parva* J.H.Wallace), is studied here under SEM for the first time. This acid-treated sample [ANSP GCM 3555(1)\_2(8)] contained fine clay particles that were extremely difficult to separate from diatoms. This sample was repeatedly treated with hydrogen peroxide and Kodak Photo-Flo solution, which helped to disperse sediment particles to some degree, but the quality of the preparation remained quite poor, with most diatom frustules still obscured by clay. We also studied the holotype slide (ANSP GC4373b), made from the same original material.

Most of the remaining samples we examined were prepared using standard nitric acid digestion protocols (Charles et al. 2002). Slides prepared since 1990 have been permanently mounted using Naphrax medium (Brunel Microscopes, UK), while older slides were mostly mounted using Hyrax. We examined the slides with a Zeiss AxioImager A1 light microscope equipped with DIC optics and AxioScopeMRm digital camera (Zeiss, Jena). For SEM examination, diatom slurries were dried on aluminum stubs, sputter-coated with Pt-Pd and observed with a Zeiss Supra 50 scanning electron microscope under 10 kV accelerating voltage without tilting. Environmental data were obtained from the Water Quality Portal (<https://www.waterqualitydata.us/>). Terminology follows Round et al. (1990) and Cox (1999a). Data sources for the morphology of species similar to the new species are given in table 2.

## RESULTS

### *Navicula eileeniae* Potapova & Ciugulea, sp. nov.

Figs 2A–I, 3A–F & H

**Type material** – United States, Virginia, Jordan River, sample ANSP VA000696, 38.7829N, 78.0807W, 4 Oct. 2016, staff of the Virginia Department of Environmental Quality (holo-: ANSP, circled specimen on slide ANSP GC 59332 (fig. 6); iso-: ANSP, circled specimens on slide ANSP GC 59333, & CANA, circled specimen on slide CANA 128320).

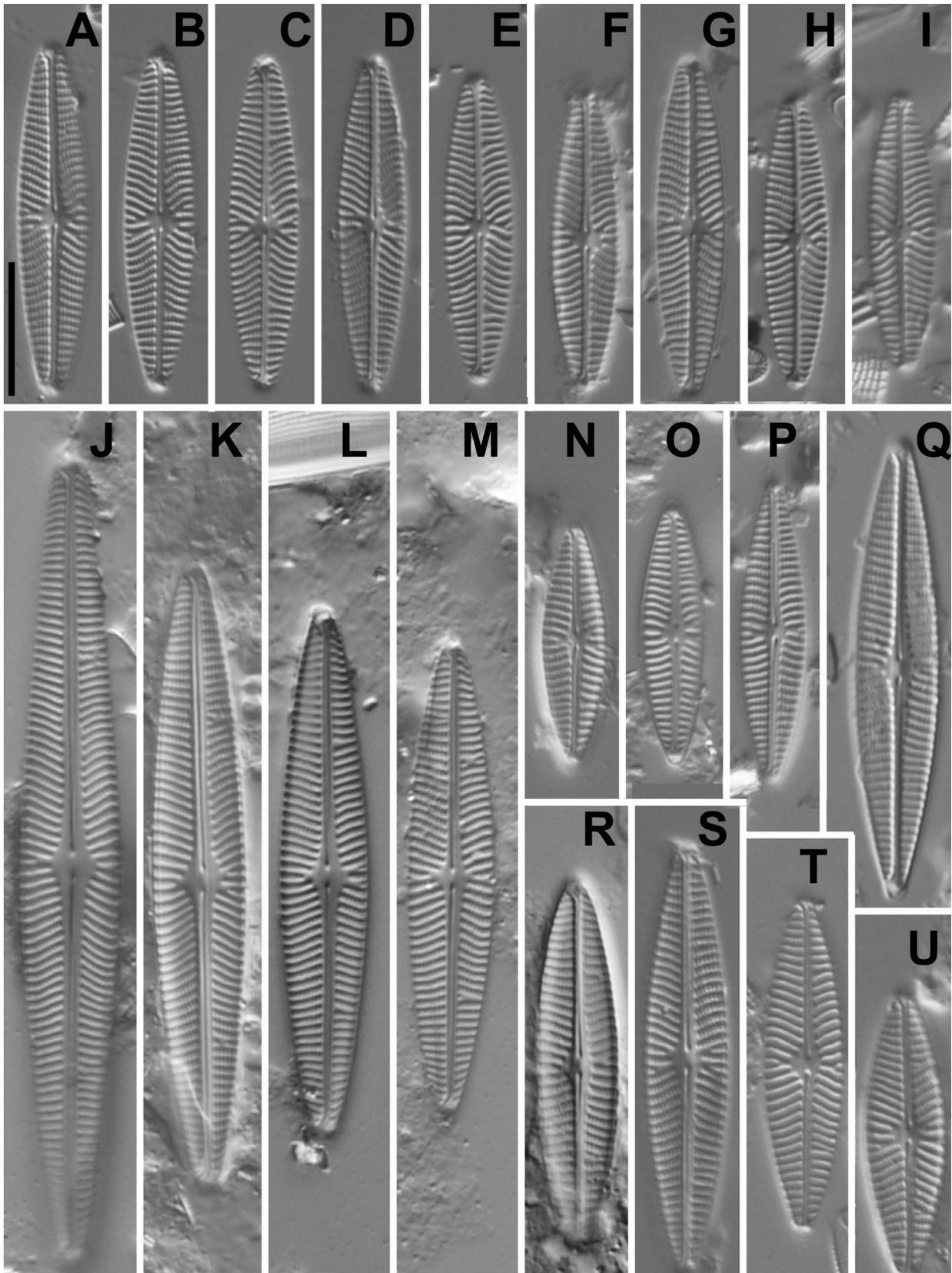
**Description** – Valves lanceolate, 4.2–5.4  $\mu\text{m}$  wide, 17.8–26.5  $\mu\text{m}$  long ( $n = 40$ ) with wedge-shaped obtusely rounded apices. Axial area narrow, linear. Central area small, symmetrical, rhombic to rounded (fig. 2A–I). Raphe lateral, with hook-shaped terminal raphe fissures (fig. 3A, F & H). Proximal part of the external raphe slits undulating, central pores expanded and bent towards secondary valve side (fig. 3A, B, D, F & H). Internal proximal raphe ends connected by a shallow groove across the central nodule and each with a short extension sharply bent towards secondary valve side (fig. 3C & E). Striae strongly radiate and bent in the valve centre, parallel at the ends, 14–15 in 10  $\mu\text{m}$ . Long and short striae alternate in the central part of the valve. Areolae ap-

**Table 1 – Records of *Navicula eileeniae* and corresponding water quality data.**PA: Pennsylvania; VA: Virginia; R.: River; Cr.: Creek; Cond.: conductivity; TN: total nitrogen; TP: total phosphorus; RA: relative abundance of *N. eileeniae*.

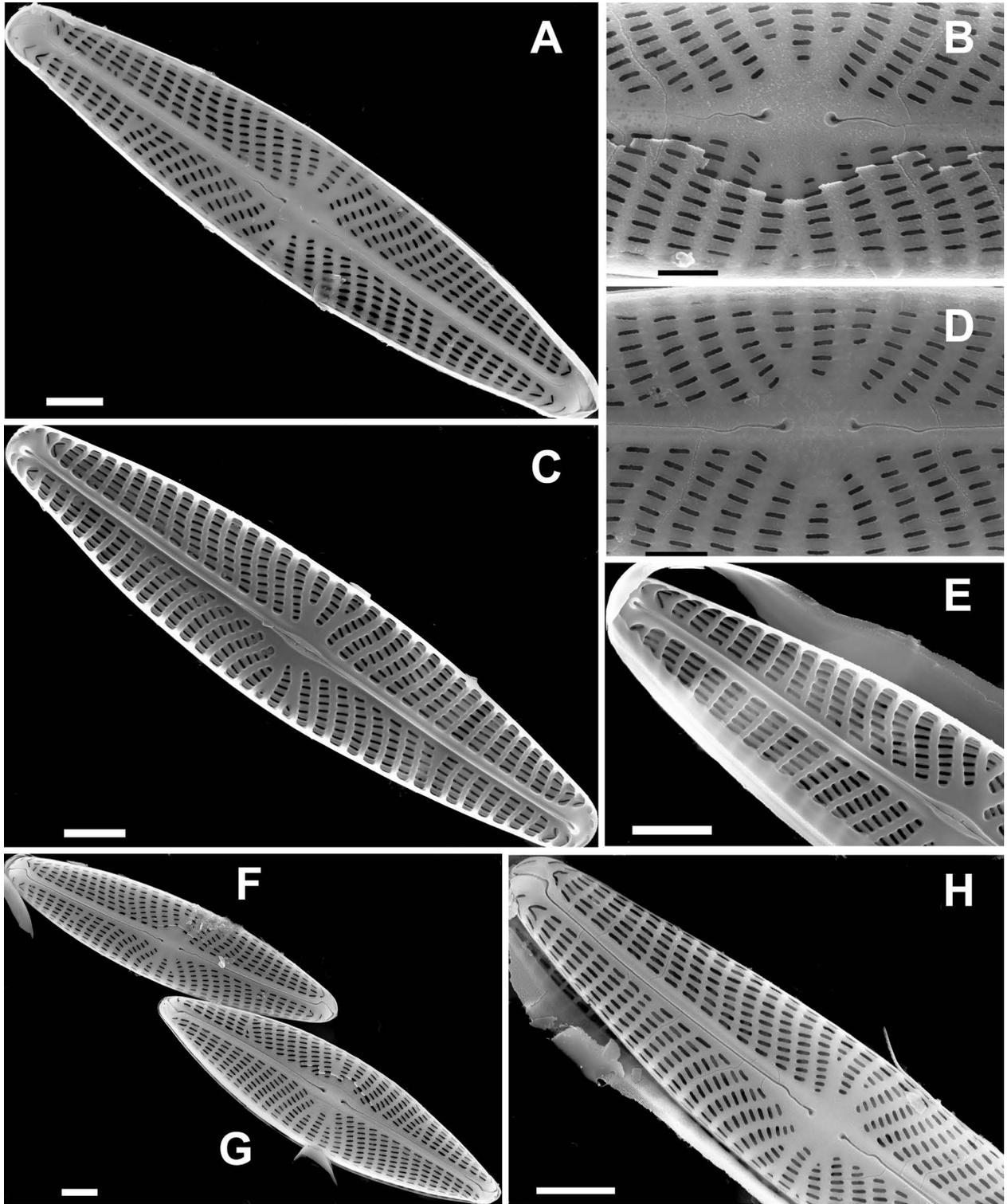
Waterbody, State	Latitude	Longitude	Collection date	ANS sample #	Cond., $\mu\text{S cm}^{-1}$	pH	TN, $\text{mg L}^{-1}$	TP, mg $\text{L}^{-1}$	RA, %
Blockhouse Cr., PA	41.4739	-77.2302	24 Aug. 2017	PADEP225	149	8.3	0.28	-	0.8
Driftwood Branch, PA	41.4326	-78.1793	20 Sep. 2009	NCPA0301	118	6.8	-	-	2.0
Sinnemahoning Cr., PA	41.2609	-77.9039	16 Aug. 2017	PADEP215	112	7.2	-	0.011	0.3
Swatara Cr., PA	40.4811	-76.5325	20 Oct. 2015	PADEP116	-	-	-	-	3.5
West Branch Susquehanna R., PA	40.8858	-76.8014	3 Sep. 2008	NRSA0191	-	-	-	-	11.2
West Branch Susquehanna R., PA	40.9649	-76.8782	19 Jul. 2016	PADEP145	-	-	-	-	0.7
West Branch Susquehanna R., PA	41.2071	-77.2501	17 Aug. 2017	PADEP219	316	7.8	0.665	0.007	1.8
West Branch Susquehanna R., PA	41.0592	-78.3774	15 Aug. 2017	PADEP212	-	-	-	-	0.5
Youghiogheny R., PA	39.8751	-79.4958	4 May 2013	SWPA0001	220	6.9	-	-	0.2
Back Cr., VA	37.9370	-78.9714	22 Sep. 2016	VA000676	81	7.1	0.12	0.01	13.3
Back Cr., VA	39.2084	-78.4010	14 Sep. 2017	VA000719	238	7.4	0.2	0.01	3.8
Big Otter R., VA	37.3782	-79.4384	30 Aug. 2017	VA000730	74	7.9	0.37	0.05	3.2
Buffalo R., VA	37.6197	-78.8980	16 Sep. 2015	VA000606	75	6.0	0.23	0.04	1.0
Cascade Cr., VA	36.5595	-79.6798	22 Sep. 2016	VA000662	88	7.8	0.2	0.02	4.0
Craig Cr., VA	37.5947	-79.9989	1 Sep. 2016	VA000686	147	8.0	0.21	0.003	0.7
Fishpond Cr., VA	37.3668	-78.6540	3 Nov. 2016	VA000657	53	7.6	0.2	0.02	5.8
Greasy Cr., VA	36.8865	-80.6592	13 Nov. 2007	VA000215	50	7.5	0.32	0.02	0.1
Honey Branch, VA	36.9356	-82.3121	7 Nov. 2016	VA000666	483	7.4	0.13	0.01	23.1
Jackson R., VA	37.8227	-79.9894	22 Aug. 2017	VA000727	169	8.4	0.38	0.003	4.0
Jackson R., VA	37.7886	-80.0008	23 Aug. 2017	VA000726	807	7.7	0.54	0.06	0.5
Jackson R., VA	37.7615	-80.0090	7 Nov. 2007	VA000171	1110	7.8	0.72	0.12	0.1
Jackson R., VA	37.8227	-79.9894	24 Aug. 2015	VA000634	168	6.3	0.27	0.01	7.3
James R., VA	37.5357	-78.8338	3 Oct. 2017	VA000705	472	8.7	0.27	0.02	0.5
Jordan R., VA	38.7829	-78.0807	4 Oct. 2017	VA000696	99	6.6	0.22	0.2	6.8
Lewis Cr., VA	37.0047	-81.9736	3 Nov. 2016	VA000667	552	7.8	0.69	0.12	0.7
Mill Cr., VA	36.8755	-79.2766	2 Nov. 2017	VA000740	63	8.4	0.2	0.03	1.8
Mudlick Cr., VA	37.0970	-81.8351	20 Sep. 2016	VA000668	200	7.9	0.17	0.01	23.4
New R., VA	37.1230	-80.5959	31 Aug. 2016	VA000694	144	6.3	0.51	0.01	0.7
New R., VA	37.1945	-80.5170	13 Oct. 2015	VA000635	123	6.1	1.12	0.03	0.3
North Fork Holston R., VA	36.8100	-81.9596	4 Oct. 2007	VA000211	1205	8.0	0.28	0.01	5.0
North Fork Powell R., VA	36.7965	-83.0017	29 Sep. 2015	VA000614	632	8.0	0.1	0.01	0.9
Smith R., VA	36.7543	-79.9856	28 Aug. 2017	VA000734	60	7.9	0.29	0.01	0.3
Smith R., VA	36.8436	-80.1617	22 Oct. 2012	VA000452	70	6.1	0.11	0.01	7.5
Smith R., VA	36.8436	-80.1617	21 Oct. 2014	VA000538	65	7.4	0.13	0.02	9.4
Smith R., VA	36.8436	-80.1617	25 Aug. 2016	VA000692	53	8.0	0.24	0.04	19.4
South Fork Holston R., VA	36.6781	-81.7900	3 Nov. 2010	VA000351	258	7.7	0.59	0.01	6.2
South Mayo R., VA	36.5963	-80.1967	27 Oct. 2008	VA000255	62	6.5	0.45	0.02	1.7
South Mayo R., VA	36.5963	-80.1967	29 Oct. 2008	VA000256	62	6.5	0.45	0.02	1.7
Staunton R., VA	37.1195	-79.3825	3 Nov. 2008	VA000233	177	7.9	0.36	0.01	2.0
Williams Run, VA	37.4090	-79.0980	6 Oct. 2008	VA000272	107	7.7	0.96	0.03	2.0
Williams Run, VA	37.4090	-79.0980	3 Oct. 2007	VA000178	100	7.5	0.94	0.08	5.0
Wilson Cr., VA	36.5905	-81.3507	15 Oct. 2015	VA000611	44	8.3	0.37	0.01	0.6
Yellowstone Branch, VA	36.6915	-79.9067	17 Sep. 2015	VA000642	159	7.4	0.69	0.02	15.0

Table 2 – Comparison of *Navicula eileeniae* with similar species.

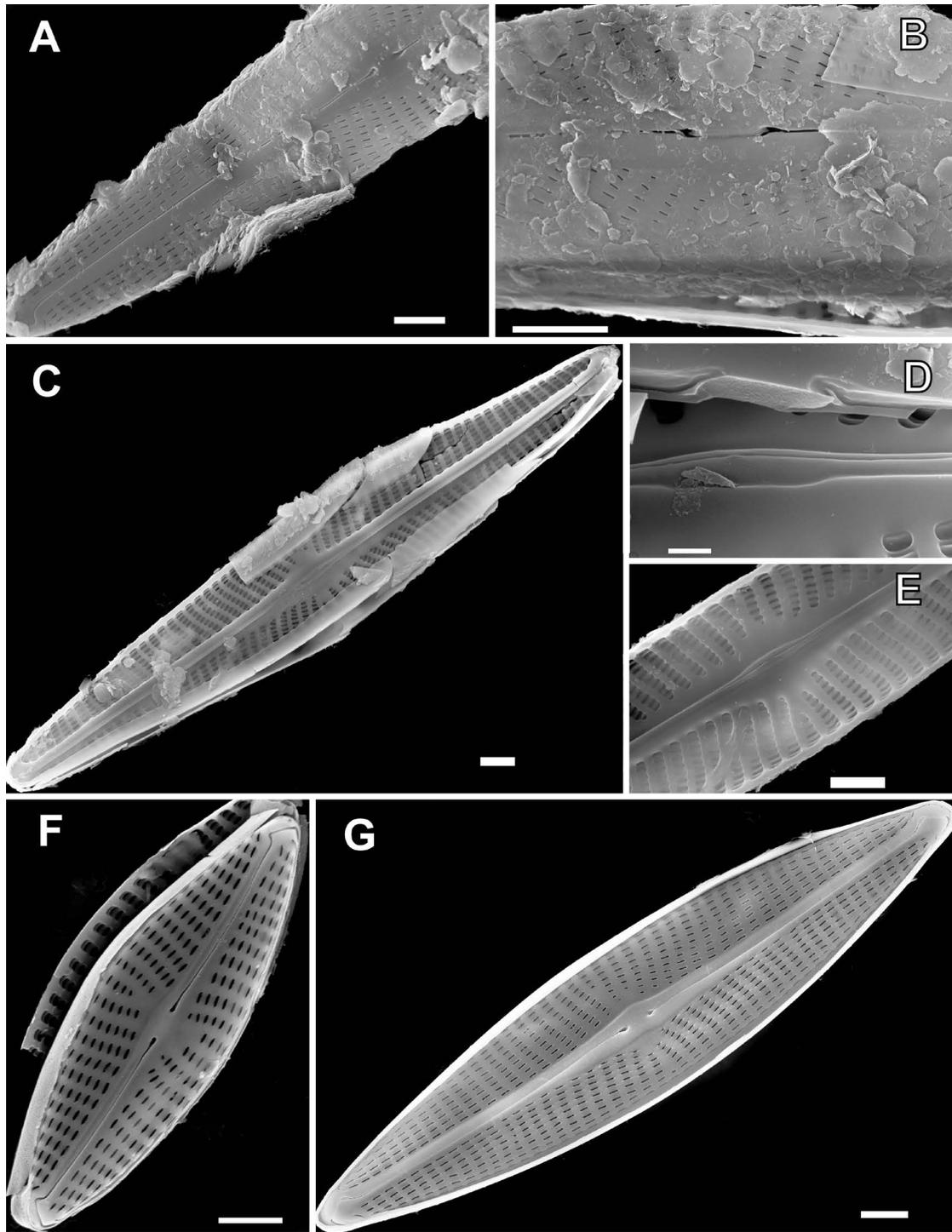
Species	Data sources	Valve width, $\mu\text{m}$	Valve length, $\mu\text{m}$	Valve shape	Valve ends	Striae / 10 $\mu\text{m}$	Arcolae / 10 $\mu\text{m}$	Central area	External proximal raphe ends	Central external raphe pores
<i>N. eileeniae</i>	this study	4.2–5.4	18–27	lanceolate	obtusely rounded	14–15	32–35	small, rounded	undulate	turned to secondary side
<i>N. cryptotenella</i>	Lange-Bertalot 2001	5–7	12–40	lanceolate	acutely rounded	14–16	~38	small, irregular	slightly bent towards primary side	tear-shaped, turned towards secondary side
<i>N. cryptotenelloides</i>	Lange-Bertalot 2001, this study	3.7–4.2	9–18	lanceolate	acutely rounded	16–18	42–44	very small	slightly bent towards primary side	very slightly expanded, straight
<i>N. radiosafallax</i>	this study	6.1–7.6	27–60	lanceolate	acutely rounded	13–14	–	rhombic-lanceolate	almost straight	tear-shaped, turned towards secondary side
<i>N. lundii</i>	Bahls 2012a, Lange-Bertalot 2001	4–6.3	13–35	lanceolate	slightly protracted, narrowly rounded	14–15	–	rounded	–	–
<i>N. wildii</i>	Bahls 2012b, Lange-Bertalot 2001	5.5–7.8	23–50	lanceolate	slightly protracted, narrowly rounded	11–14	30–35	lanceolate to rhombic	slightly bent towards primary side	hook-shaped, turned to secondary side
<i>N. recens</i>	Lange-Bertalot 2001, Potapova 2009	5.5–9	16–51	elliptic-lanceolate to linear-lanceolate	acutely to obtusely rounded	10.5–14	28–32	rounded	slightly bent towards primary side	expanded, straight
<i>N. reichardtiana</i>	Lange-Bertalot 2001, Potapova 2011	4.5–6	12–22	lanceolate	substrate	14–16	32–36	small, rounded	slightly bent towards primary side	expanded, slightly turned towards primary side
<i>N. scaniae</i>	Van de Vijver et al. 2010	5.6–6.5	25–50	linear-lanceolate	obtusely rounded	11–13	35–38	small, variable	undulate	turned to secondary side
<i>N. microcari</i>	Lange-Bertalot 1993, Dobosz et al. 2017	4.8–5.5	19–24	elliptic-lanceolate to lanceolate	weakly protracted obtusely rounded	13–14	40–45	transverse, rectangular	almost straight	almost straight



**Figure 2** – *Navicula* species from eastern USA, LM: A–I, *N. eileeniae* type population, sample ANSP VA000696 from Jordan River, Virginia; E, the holotype specimen, slide ANSP GC 59332; J–M, R, *N. radiosafallax*, holotype slide ANSP GC4373b, Escambia River, Florida; N–P, *N. cryptotenelloides*, sample ANSP VA000055, Roanoke River, Virginia; Q, S–U, *N. cryptotenella*; Q, sample ANSP NCPA0206, Stony Lick Run, Pennsylvania; S–U, sample VA000676, Back Creek, Virginia. Scale bar = 10  $\mu$ m.



**Figure 3** – *Navicula* species from eastern USA, SEM, sample ANSP VA000696 from Jordan River, Virginia: A–F & H, *N. eileeniae*, type population; A, external view of a whole valve with attached girdle band; B & D, external views of central valve area, showing undulate proximal raphe ends, expanded central pores turned towards secondary valve side, external areolae openings and hair-like, irregularly shaped grooves branching off the raphe slit and extending along interstriae; C, internal view of a whole valve; E, internal view of half of a valve, showing the raphe-sternum moderately expanded in its central part, internal raphe slit turned towards secondary valve side, internal proximal raphe ends with small transapically oriented extensions turned to secondary valve side and plain valvocopula with undulate margin abutting the valve; F & H, external valve views; G, *N. cryptotenella*, external valve view showing raised sternum slightly expanded towards primary valve side at the valve center, proximal raphe portions slightly bent towards primary valve side and expanded central pores. Scale bars: A, C & E–H = 2  $\mu\text{m}$ ; B & D = 1  $\mu\text{m}$ .



**Figure 4** – *Navicula* species from eastern USA, SEM: A–E, *N. radiosafallax*, type population, sample ANSP GCM 3555(1)\_2(8), Escambia River, Florida; A, external view of a partial raphe valve showing axial area gradually widening into rhombic-lanceolate central area, sternum slightly raised above valve surface, straight raphe slit with expanded proximal ends curved towards secondary valve side.; B, external view of the central part of a valve showing slightly curved proximal raphe ends with a fracture across central nodule; C, internal view of a valve with fragments of an attached plain girdle band; D, a frustule fragment showing internal view of a central nodule with straight proximal raphe ends and a cross-section of the central nodule of another valve of the same frustule with visible external proximal raphe ends; E, internal view of the central part of a valve showing rhombic central area and the central nodule; F, *N. cryptotenelloides*, sample ANSP VA000055, Roanoke River, Virginia, external view of a valve showing proximal parts of the raphe slits slightly deflected towards the primary valve side and almost straight central pores; G, *N. cryptotenella*, sample ANSP NCPA0206, Stony Lick Run, Pennsylvania, external view of the whole valve showing sternum raised above valve surface, proximal raphe portions slightly bent towards primary valve side and expanded central pores slightly turned towards secondary valve side. Scale bars: A–C & E–G = 2  $\mu\text{m}$ ; D = 0.5  $\mu\text{m}$ .

proximately 32–35 in 10  $\mu\text{m}$ , often merging into V-shapes near valve apices.

**Etymology** – The species is named after Dr. Eileen Cox, in recognition of her outstanding contribution to the knowledge of naviculoid diatoms.

**Details of frustule morphology** – There is little recognizable variation in the size and shape of the valves of *Navicula eileeniae* under LM. Valves have non-protracted bluntly rounded ends, strongly radiate curved striae near the central area, and visible areolae (fig. 2A–I). The external openings of the areolae are relatively long, so that striae appear to be wider than virgae (interstriae). Shortened striae inserted between the longer striae around the central area give the interstriae a ‘branching’ appearance. Thin irregular grooves on the external valve surface often run in the middle of interstriae or may cross a few striae (fig. 3B, D & H). These grooves may be short or long, reaching the valve margin. The most prominent ultrastructural feature of this species is the undulating proximal external raphe slits, about 1.2–1.5  $\mu\text{m}$  long, starting distally by curving towards the primary valve side and ending by a round expanded central pore bent towards secondary valve side (fig. 3A, B, D, F & H). The numbers of undulations vary and may be different between the two raphe branches on the same valve (fig. 3B). On the internal valve surface the raphe-sternum rib is moderately and symmetrically expanded in its central part, but no additional ribs or deep grooves are present alongside it (fig. 3C & E). The internal raphe slit faces the secondary valve side, except near the poles and central area, as in most other *Navicula* species. The central internal raphe endings have a somewhat unusual appearance because of the small transapically oriented extensions, which are superficial grooves (fig. 3C & E). Wide open girdle bands without any ornamentation have occasionally been observed under SEM (fig. 3E).

**Distribution** – *Navicula eileeniae* has been found in 43 benthic samples collected between 2007 and 2017 from 30 rivers and streams across the states of Virginia and Pennsylvania, USA (fig. 1, table 1). We did not find this species in any of the 210 examined samples collected in Central Appalachian rivers from 1941 to 2006. This suggests that *N. eileeniae* was either absent or very rare in the area before 2007. However, only rarely were sites repeatedly sampled prior to 2017. One locality that was sampled repeatedly from 1997 to 2017 was the Jackson River in Covington, Virginia. *Navicula eileeniae* first appeared in this location in 2007 and was more abundant in samples collected upstream of the industrial effluents that have increased water mineralization and nutrient content. The upper portion of the South Fork of the Holston River, Virginia was sampled by the VADEQ in 2004 and 2010, but *N. eileeniae* only appeared in the latter sample. We did not find this species in any samples from a downstream locality on the same river near Kingsport, Tennessee, which had been repeatedly sampled since 1951 (electronic appendix 1).

The rivers where *N. eileeniae* was found had circumneutral water (pH 6–8.7, median 7.7) of variable mineral content (conductivity 44–1205  $\mu\text{S cm}^{-1}$ ), but most sites had rather soft water (median conductivity 123  $\mu\text{S cm}^{-1}$ ). These rivers were either nutrient-poor or had slightly elevated nutrient concentrations. Total nitrogen varied from 0.1 to 1.12  $\text{mg L}^{-1}$ ,

with a median value of 0.29  $\text{mg L}^{-1}$ , while total phosphorus measured 0.003–0.3  $\text{mg L}^{-1}$ , with a median value of 0.02  $\text{mg L}^{-1}$ . *Navicula eileeniae* reached its highest relative abundance (23.4%) in a sediment sample from Mudlick Creek, a small Virginia stream with water pH of 7.9 and conductivity of 200  $\mu\text{S cm}^{-1}$ .

*Navicula radiosafallax* Lange-Bert.

*Navicula radiosa* var. *parva* J.H. Wallace

Figs 2J–M & R, 4A–E

**Type material** – United States, Florida, Escambia River, April 1953, J.H. Wallace (holo-: ANSP, slide ANSP GC 4373b).

**Frustule morphology** – Valves are lanceolate, 6.1–7.6 (mean 6.7)  $\mu\text{m}$  wide, 27–60 (mean 42)  $\mu\text{m}$  long ( $n = 32$ ), with acutely rounded apices (fig. 2J–M & R). The axial area is narrow and linear, but gradually expanding into a relatively small rhombic central area and slightly raised above the external valve surface (fig. 4A). The raphe is lateral, with hook-shaped terminal raphe fissures (fig. 4A). The external raphe slit is almost straight and the central pores are expanded and slightly bent towards secondary valve side (fig. 4A & B). Internal proximal raphe ends simple, straight (fig. 4C). Striae radiate and almost straight, convergent at the ends, 13–14 in 10  $\mu\text{m}$ . Short striae are often inserted between long striae in the central part of the valve. Areolae number approximately 34–36 in 10  $\mu\text{m}$ .

**Distribution** – The type locality of *N. radiosafallax* is Escambia River, Florida, United States. Although there are numerous records of this species in the United States (Wallace 1960, Patrick & Reimer 1966), we did not observe it in the examined slides from the Central Appalachian region any populations that would morphologically be identical to the type population. Bishop et al. (2017) illustrate it, however, from rivers of the southeastern United States.

## DISCUSSION

Our examination of diatom materials from the eastern USA showed that *N. eileeniae* has been previously recorded under the names of *N. cryptotenella* Lange-Bert., *N. recens* Lange-Bert., *N. lundii* E.Reichardt, or a few provisional names. *Navicula eileeniae* and *N. cryptotenella* often co-occur (fig. 3F & G) and are indeed similar, but *N. cryptotenella* can be distinguished under LM by the wider valves and acute valve apices (fig. 2Q & S–U, table 2). *Navicula cryptotenelloides* Lange-Bert. also has acute valve ends, but is on average smaller than *N. eileeniae* (figs 2N–P & 4F, table 2). The valves of *N. radiosafallax* are even wider and longer than the valves of *N. cryptotenella*, and therefore are unlikely to be confused with *N. eileeniae*. The boundaries of *N. radiosafallax* and *N. cryptotenella* are, however, unclear as smaller valves in the type population of *N. radiosafallax* cannot be distinguished from the larger valves of *N. cryptotenella* (fig. 2R). Unlike *N. eileeniae*, *N. cryptotenella* and *N. radiosafallax* have a sternum raised above the external valve surface (fig. 4A & G) and almost straight striae in the central part of the valve. Both *N. cryptotenella* and *N. radiosafallax* have expanded central raphe pores slightly turned to the secondary

valve side (fig. 4A, B & G). Besides size differences, morphological characters that may separate these two species are stria orientation at the valve ends and the size and shape of the central area. *Navicula radiosafallax* has convergent striae at the valve ends (fig. 2J–M & R), while *N. cryptotenella* has parallel striae (fig. 2Q & S–U). The central area is rather small in *N. cryptotenella*, but in *N. radiosafallax* it is larger and merges with the axial area (fig. 4E). Our SEM observations also confirm that *N. radiosafallax* differs from similar species, such as *N. madeirensis* Lange-Bert. by the shape of the central raphe pores, which are strongly hooked in the latter species (Lange-Bertalot 1993).

*Navicula lundii* is similar to *N. eileeniae* in size and stria density, but has more acute valve ends and larger rounded central area, while *N. wildii* Lange-Bert. can be distinguished by larger size, protracted ends, straight striae, and an axial area gradually widening towards the valve centre (table 2). *Navicula wildii* also has rather distinct external proximal raphe ends, which are strongly curved to the secondary valve side.

Both *N. recens* and *N. reichardtiana* Lange-Bert. resemble *N. eileeniae* in the striation pattern in the central part of the valve, where strongly radiate and curved long striae alternate with short striae. The shape of the striae is especially similar between *N. recens* and *N. eileeniae*, but the former is larger with coarser striae and areolae (table 2). *Navicula reichardtiana* is similar to *N. eileeniae* in size, but has valves with protracted subrostrate ends (table 2).

The species that appear to be most similar to *N. eileeniae* are *N. scaniae* Van de Vijver, Jarlman & Lange-Bert. and *N. microcari* Lange-Bert. *Navicula scaniae* is very similar to *N. eileeniae* in valve shape, striae pattern and undulating proximal parts of the raphe slits (Van de Vijver et al. 2010). *Navicula scaniae* and *N. eileeniae* do not overlap, however, in the valve width and stria density (table 2). On the other hand, the size, shape, and stria pattern and density of *N. eileeniae* and *N. microcari* are practically indistinguishable (table 2). The differences that can be observed under LM are very slightly protracted valve ends, at least in some specimens, a wider transversally oriented rectangular central area, and unresolvable areolae in *N. microcari*. The proximal parts of the external raphe slits of *N. microcari* are not undulate and are only slightly deflected towards the secondary valve side (Lange-Bertalot 1993: pl. 58, fig. 1).

The undulate proximal (1.2–1.5 µm long) part of the external raphe slit is a character so far found only in *N. eileeniae* and *N. scaniae*, but this part of the slit appears to be slightly deflected towards primary valve side in many *Navicula* species (Cox 1999a: table 2). Sometimes the raphe slit gradually slopes towards the primary side in the central part of the valve (e.g. Cox 1999a: fig. 2), but more commonly there is an abrupt shift, 1–2 µm from the central pore (Cox 1999a: figs 7, 8, 10, 20). This shift is likely homologous to the first “wave” in the undulating part of the raphe in *N. eileeniae*. The internal central raphe ends turned towards the secondary side are also an unusual feature of this new species. This structure superficially resembles the internal raphe ends in gomphocymbelloid diatoms that have their internal raphe ends sharply turned to primary valve side. The signifi-

cance of these morphological characters is difficult to interpret, but since the main function of raphe is locomotion, they may be adaptations to physical characteristics of habitat, such as particle sizes, which may affect the motility of raphid diatoms (Harper 1977).

It is notable that *N. eileeniae* has not been found in samples collected before 2007, but it is also true that the number of sampled river sites collected in Central Appalachia has increased considerably in recent years. It is therefore impossible to prove that this species is a recent arrival and was missing completely in this region in the past. The most probable explanation of the sudden “appearance” of this diatom is its relative rarity before the recent increase in frequency due to unknown factors. The river sites where the new species was found mostly have good water quality and are considered oligotrophic based on the values of total N and P (Dodds 2013). Therefore, water pollution cannot be evoked as a plausible explanation of the current spread of this diatom.

Another example of an apparent “expansion” of a diatom species in rivers of North America is *Didymosphenia geminata* (Lyngb.) M.Schmidt (Spaulding et al. 2010). Similarly to *N. eileeniae*, *D. geminata* also “appeared” in the 1990s and 2000s in many nutrient-poor rivers of North America. Although the reasons for the spread of *D. geminata* are still debated, no specific environmental factor or combination of factors has been confirmed as a trigger of its expansion. It is also not clear whether *D. geminata* is indeed a new arrival to regions of North America where it has not previously been found, or if it was simply undetected because of its rarity and a lesser sampling effort. In addition to *N. eileeniae*, we also noticed a recent appearance in Virginia and Pennsylvania of another diatom species, *Achnanthisidium delmontii* F.Pérès, Le Cohu & Barthès previously unreported in North America and originally described from France, where it also appeared for the first time in 2007 and then began rapidly spreading in rivers of the Languedoc-Roussillon and Rhine–Meuse regions (Pérès et al. 2012). Curiously, both species are often abundant in the same samples from central Appalachia, although *A. delmontii* is more frequent and abundant than *N. eileeniae*. Simultaneous expansion of a novel diatom species on two continents is enigmatic and raises a host of questions on the modes and rates of speciation and dispersal of diatoms. It may be possible that rapid range expansions are common in protists and only now are being noticed because of increased sampling efforts. Approaches enabling detection of rare species, such as environmental DNA metabarcoding (Kerमारrec et al. 2013) and automated diatom identification coupled with large-scale digitization of diatom slides (Kloster et al. 2017), will be instrumental for tracking changes in diatom assemblages over a range of temporal and spatial scales.

#### SUPPLEMENTARY DATA

Supplementary Data are available at *Plant Ecology and Evolution*, Supplementary Data Site (<https://www.ingentaconnect.com/content/botbel/plecevo/supp-data>) and consists of a list of studied materials (Excel spreadsheet).

## ACKNOWLEDGEMENTS

This paper is dedicated to Dr. Eileen Cox in recognition of her outstanding contributions to the field of diatom research. We thank all diatom analysts at the Academy of Natural Sciences of Philadelphia who counted innumerable diatom slides and contributed data to the ANS databases over the years. The use of the Centralized Research Facilities, Drexel University for the SEM is gratefully acknowledged.

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- Managing Editor: David G. Mann  
Submission date: 15 Oct. 2018  
Acceptance date: 22 Nov. 2018