ELSEVIER

Diatoms from the genus *Achnanthidium* in flowing waters of the Appalachian Mountains (North America): Ecology, distribution and taxonomic notes

Karin C. Ponader[a],*, Marina G. Potapova[b]

[a]*Harvard University Herbaria, 22 Divinity Avenue, Cambridge, MA 02138, USA*
[b]*Patrick Center for Environmental Research, The Academy of Natural Sciences, Philadelphia, PA 19103, USA*

Abstract

Diatoms from the genus *Achnanthidium* are abundant in rivers, streams, and springs of the Appalachian Mountains. They inhabit clean and polluted waters, including those affected by acid mine drainage. The identification of *Achnanthidium* taxa is difficult due to their small cell size and insufficient information in the diatom floras. We studied the taxonomy and ecology of *Achnanthidium* in Appalachian rivers by analyzing a data set of benthic diatom samples and corresponding water chemistry data collected during several water-quality surveys from 181 sampling sites. Ten species were identified using scanning electron and light microscopy: *A. alpestre* (Lowe & Kociolek) Lowe & Kociolek, *A. atomus* (Hustedt) Monnier, Lange-Bertalot, & Ector, *A. deflexum* (Reimer) Kingston, *A. duthii* (Sreenivasa) Edlund, *A. eutrophilum* (Lange-Bertalot) Lange-Bertalot, *A.* cf. *gracillimum* (Meister) Lange-Bertalot, *A.* cf. *latecephalum* Kobayasi, *A. minutissimum* (Kützing) Czarnecki (sensu lato), *A. reimeri* (Camburn) comb. nov., and *A. rivulare* Potapova & Ponader. The distribution of common taxa in relation to water chemistry was studied by fitting non-parametric regression models (generalized additive models, GAM, and non-parametric multiplicative regression models, NPMR) to species relative abundances. Studied *Achnanthidium* species differed considerably in their responses to water chemistry. These results suggest that species-level identifications will lead to more accurate bioassessments.
© 2007 Elsevier GmbH. All rights reserved.

Keywords: Diatoms; *Achnanthidium*; Appalachian Mountains; Rivers; Taxonomy; Ecology; Distribution; Generalized additive models; Non-parametric multiplicative regression models; Water-quality assessment

Introduction

Diatoms from the genus *Achnanthidium* are common in North American rivers; however their identification remains difficult and their ecology is insufficiently studied (Potapova & Ponader, 2004). There are several reasons why the identification of *Achnanthidium* taxa is complicated. First, because of the small cell size of these diatoms, some morphological features can be difficult to observe. Second, there is a considerable overlap among species in quantitative characters traditionally used for species identification. Difficulties in separating this inter-specific variability from intra-specific variability led to grouping of some *Achnanthidium* taxa into "species complexes" (Krammer & Lange-Bertalot, 1991). Third, several species that are common in North America have

*Corresponding author. Tel.: +1 (671) 384 5871; fax: +1 (617) 495 9484.

E-mail address: kponader@oeb.harvard.edu (K.C. Ponader).

been described in local floras (Camburn, Lowe, & Stoneburner, 1978; Johansen, Lowe, Gomez, Kociolek, & Makosky, 2004; Kobayasi, 1997; Lowe & Kociolek, 1984), but not included in floras commonly used for diatom identifications, such as Krammer & Lange-Bertalot (1991) and Patrick & Reimer (1966). Finally, the nomenclature of *Achnanthidium* is complicated because the genus concept has changed several times since its establishment by Kützing (1833). According to the current concept of *Achnanthidium* (Round & Bukhtiyarova, 1996), this genus encompasses monoraphid freshwater species with small linear-lanceolate to lanceolate elliptic cells less than 30 μm long and less than 5 μm wide, a concave raphe valve (RV) and a convex rapheless valve (RLV), uniseriate striae that are spaced wider in the center and denser towards the apices on the RV and that are more evenly and wider spaced on the RLV, and a fine raphe with terminal raphe fissures that can be either straight or turned to one side.

Examining diatom samples collected in the course of several water-quality surveys and stored at the Diatom Herbarium at the Academy of Natural Sciences (ANS) of Philadelphia (ANSP), we found that representatives of *Achnanthidium* are among the most abundant benthic diatoms in rivers of the Appalachian Mountains of Eastern North America. *Achnanthidium* species in Appalachia inhabit clean and polluted waters, including those affected by acid mine drainage. This led us to further investigate *Achnanthidium* species, especially with regards to their potential use as indicators of water quality. The goal of this paper was to describe the diversity of *Achnanthidium* species in the Appalachian Mountains and to explore their ecology and related distribution patterns.

Study area and methods

The Appalachian Mountain range parallels the North American East Coast, and stretches from Northern Alabama, Southern Tennessee and North Carolina into the Gaspé Peninsula (Québec, Canada) in its northernmost extent. Based on the geology, the Appalachian Mountains can be divided into the mostly crystalline northern Appalachian chain (Newfoundland, Québec and New England) and the southern Appalachian chain (from the Hudson Valley to Alabama) of diverse crystalline and sedimentary deposits (USGS, 2003). In this paper, when using the term "Appalachian Mountains", or "Appalachia" we refer to this southern portion of the Appalachian chain.

To study the distribution of *Achnanthidium* species in the Appalachian Mountains we used samples of benthic diatoms and environmental data collected during the course of three river surveys: the United States Geological Survey (USGS) National Water Quality

Assessment (NAWQA) Program (93 sites), the Virginia Department of Environmental Quality (DEQ) stream survey (24 sites), and the USGS Northern Appalachian Research Laboratory Analysis of Periphyton for an Acid Mine Drainage Study of the West Branch Susquehanna River watershed (64 sites). The samples were collected from rocks and occasionally from other substrates (soft sediment or submerged wood) following standard protocols (Moulton, Kennen, Goldstein, & Hambrook, 2002). Samples were treated with nitric and sulfuric acid and diatoms were mounted on microscope slides using Naphrax mounting medium. Six hundred diatom valves were counted per slide (Charles, Knowles, & Davis, 2002). Light microscope (LM) digital images were taken using a Zeiss Axioscope 2 equipped with Spot Insight QE Camera. For scanning electron microscopy (SEM), diatom samples were placed on aluminum stubs, coated with gold or gold and palladium, and studied with a JEOL JSM-6300FV, an FEI XL30 ESEM, or a Zeiss Supra 50 SEM. Chemistry data were collected by USGS and Virginia DEQ staff.

The ecology of the *Achnanthidium* species was studied by fitting regression models of their distribution in relation to several water chemistry characteristics available for all sites: conductivity, pH, concentrations of major ions (Cl^-, SO_4^{2-}, Ca^{2+}, Mg^{2+}, Na^+, K^+), and nutrients (PO_4^{3-}, $NO_3^- + NO_2^-$). Distribution of selected water chemistry characteristics in the studied sites is shown in Table 1. Non-parametric regression methods were used to avoid making assumptions about the possible shape of the species "response" curves. Generalized additive models (GAMs) were used to explore species distribution in relation to each individual chemistry characteristic. GAMs assume that the mean of the dependent variable depends on an additive predictor through a nonlinear link function (Hastie & Tibshirani, 1990), which is a log-link for Poisson-distributed data, such as diatom relative abundance. The process of fitting a GAM is essentially an estimation of non-parametric smoothing functions. In this study, cubic spline smoothers were used as smoothing functions. The Cano Draw for Windows 4.0 Program

Table 1. Summary of chemical characteristics of rivers sampled in the study area

Parameter	Minimum	Maximum	Mean	Median
Conductivity ($\mu S\,cm^{-1}$)	16.0	886.0	213.4	152.0
pH	3.4	8.8	7.4	7.4
Alkalinity ($mg\,L^{-1}$)	0.0	290.0	46.2	22.2
Cl^- ($mg\,L^{-1}$)	0.4	378.2	20.6	5.7
SO_4^{2-} ($mg\,L^{-1}$)	0.5	2282.3	75.2	22.4
Ca^{2+} ($mg\,L^{-1}$)	0.9	87.2	22.1	15.0
Mg^{2+} ($mg\,L^{-1}$)	0.32	31.2	6.9	3.65
$NO_3^- + NO_2^-$ ($mg\,L^{-1}$)	0.05	3.70	0.53	0.32
PO_4^{3-} ($mg\,L^{-1}$)	0.001	0.465	0.029	0.013

(ter Braak & Šmilauer, 2002) was used to fit GAMs. Non-parametric multiplicative regression (NPMR) was used to determine which factors were most important for distribution of each species (McCune & Mefford, 2004). In NPMR predictors are combined multiplicatively rather than additively, which means that the effect of one variable may depend on the value of other variables. NPMR implemented by the software Hyper-Niche 1 (McCune, 2004) uses kernel functions as a smoothing technique and cross-validation to select best-fitting and at the same time most parsimonious models. A local linear estimator and Gaussian kernel functions were used to construct models for this study. The models reported in this paper are those with the best fit for a particular number of predictors. Predictors were added until the cross-validated coefficient of multiple determination (xR^2) was increasing, so that the model with the highest number of predictors had the highest xR^2.

Results

Achnanthidium minutissimum (Kützing) Czarnecki (Plate 1, Figs. 1–10; Plate 2, Figs. 1–3)
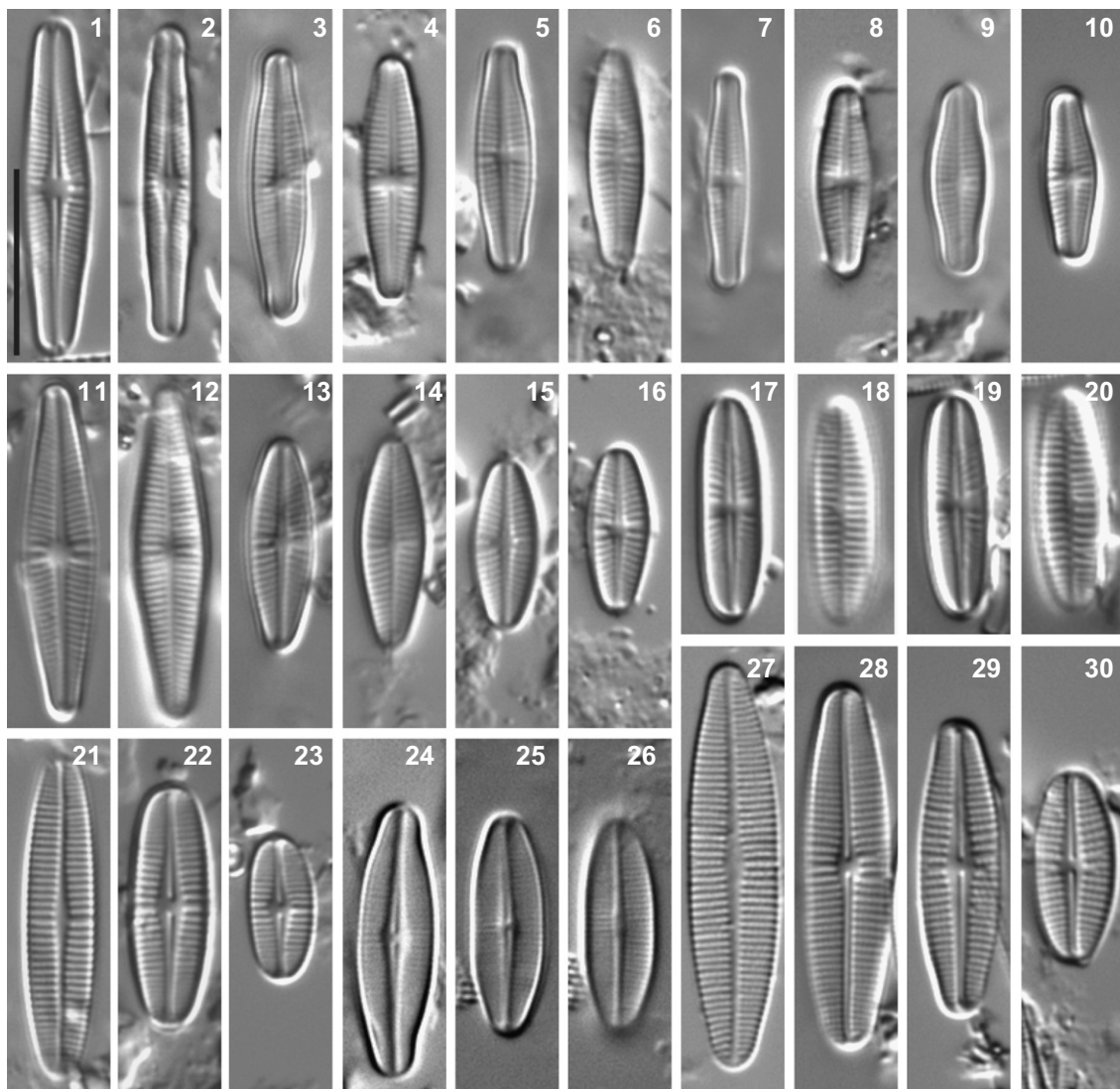
Synonym. *Achnanthes minutissima* Kützing.

Plate 1. LM, Scale bar 10 μm. **1–10.** *Achnanthidium minutissimum*. **1–2, 7.** Williams River, West Virginia. **3.** Clear Fork, West Virginia. **4.** Sinking Creek, Tennessee. **5–6.** Watuaga River, Tennessee. **8.** Williams River, West Virginia. **9.** Big Creek, Tennessee. **10.** Daddy Run, Virginia. **11–16.** *Achnanthidium eutrophilum*. **11–12.** Big Creek, Tennessee. **13–14.** Indian Creek, Tennessee. **15–16.** Sinking Creek, Tennessee. **17–20.** *Achnanthidium atomus*. Peak Creek, Virginia. **21–23.** *Achnanthidium rivulare*. Pigeon River, Tennessee. **24–26.** *Achnanthidium alpestre*. Mills River, North Carolina. **27–30.** *Achnanthidium deflexum*. **27, 30**. Copper Creek, Virginia. **28–29**. Mason Spring, Tennessee.
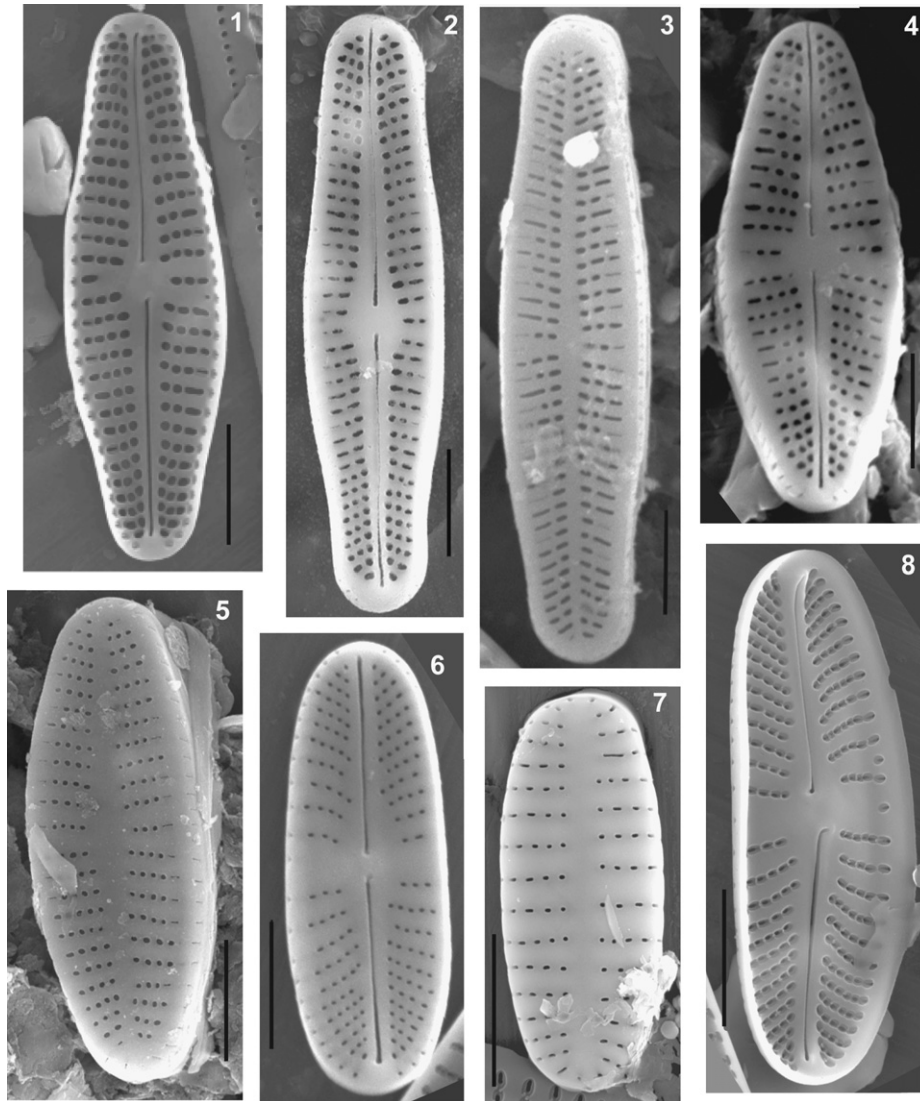
Plate 2. 1–8. SEM, Scale bar 2 µm. **1–3.** *Achnanthidium minutissimum.* **1.** Internal view of the raphe valve, Antes Creek, Pennsylvania. **2–3.** Sinnemahoning Creek, Pennsylvania. **2.** External view of the raphe valve, **3.** External view of the rapheless valve. **4–5.** *Achnanthidium eutrophilum.* **4**. External view of the raphe valve, Antes Creek, Pennsylvania. **5.** External view of the rapheless valve, Antes Creek, Pennsylvania. **6–8.** *Achnanthidium atomus.* Holston River, Virginia. **6.** External view of the raphe valve. **7.** External view of the rapheless valve. **8.** Internal view of the raphe valve.

Illustrations of type material. Krammer & Lange-Bertalot (1991), Plate 32, Figs. 1–9.

Taxonomy and morphology: In modern diatom floras *A. minutissimum* was treated as a "species complex" (Krammer & Lange-Bertalot, 1991; Lange-Bertalot, 2004) because it was often impossible to find morphological criteria unambiguously distinguishing among type populations of many species and infraspecific taxa similar to *A. minutissimum*. Following this concept no attempt was made here to separate taxa that are listed as varieties of *A. minutissimum* in the contemporary floras (Lange-Bertalot, 2004), although considerable variation in the morphology of *A. minutissimum* was found in this study (Plate 1, Figs. 1–10). For instance, specimens with stauros-like central area on the RV (Plate 1, Figs. 7–10)

could be identified as *Achnanthidium jackii* Rabenhorst because this feature appears to distinguish *A. minutissimum* and *A. jackii* if the illustrations in Krammer & Lange-Bertalot (1991) are compared and used as an identification guide. According to the descriptions in the same flora (Krammer & Lange-Bertalot, 1991), the character discriminating between these two taxa is, however, striation density. In reality, both characters, presence of stauros and striation density, are overlapping in type populations of *A. minutissimum* and *A. jackii* (Potapova & Hamilton, in press).

Ecology and distribution

In Appalachia *A. minutissimum* was widespread (Fig. 1A, Table 2) and was absent or had low abundance

only in rivers polluted by nutrients (Fig. 2E). Compared to other species of *Achnanthidium* it had a much wider tolerance range to various environmental factors (Fig. 2A, C, E). In streams polluted by acid mine drainage it was usually the only reported *Achnanthidium* species (Fig. 2C). NPMR modeling showed that higher abundances of *A. minutissimum* were mostly associated with low nutrient and ionic content (Table 3).

Achnanthidium eutrophilum (Lange-Bertalot) Lange-Bertalot (Plate 1, Figs. 11–16; Plate 2, Figs. 4–5)

Synonym. *Achnanthes eutrophila* Lange-Bertalot.
Illustrations of type material. Lange-Bertalot & Metzeltin (1996), Plate 78, Figs. 29–38.
Taxonomy and morphology: *A. eutrophilum* differs from *A. minutissimum* mostly by the rhombic valve shape (Plate 1, Figs. 11–16). We found that *A. eutrophilum* has been often identified in North America as *A. minutissimum* or *A. exile* (Kützing) Round & Bukhtiyarova.

Ecology and distribution

In the Appalachian data set, *A. eutrophilum* was found only at 4 sites (Table 2) characterized by higher than average pH (8.1–8.3) and conductivity (291–583), and by a wide range of nutrient concentrations ($NO_3^- + NO_2^-$: 0.7–2.2 mg L^{-1}; PO_4^{3-}: 0.010–0.026 mg L^{-1}).

Achnanthidium atomus (Hustedt) Monnier, Lange-Bertalot & Ector (Plate 1, Figs. 17–20; Plate 2, Figs. 6–8)

Synonym. *Achnanthes atomus* Hustedt.
Illustrations of type material. Simonsen (1987), Plate 325, Figs. 32–38, Monnier, Lange-Bertalot, Rimet, Hoffmann & Ector (2004), Figs. 73–116, 124–130.
Taxonomy and morphology: *A. atomus* has strongly radiate striae and a straight raphe on the RV (Plate 1, Figs. 17 and 19) and parallel striae on the RLV (Plate 1, Figs. 18 and 20). *A. atomus* from Appalachian rivers was slightly bigger than specimens from the type population. Hustedt (1938) recorded a length of 7–16 μm and a width of 2.5–3 μm on the RV. In this study we measured

Table 2. Occurrence of *Achnanthidium* species in the study area

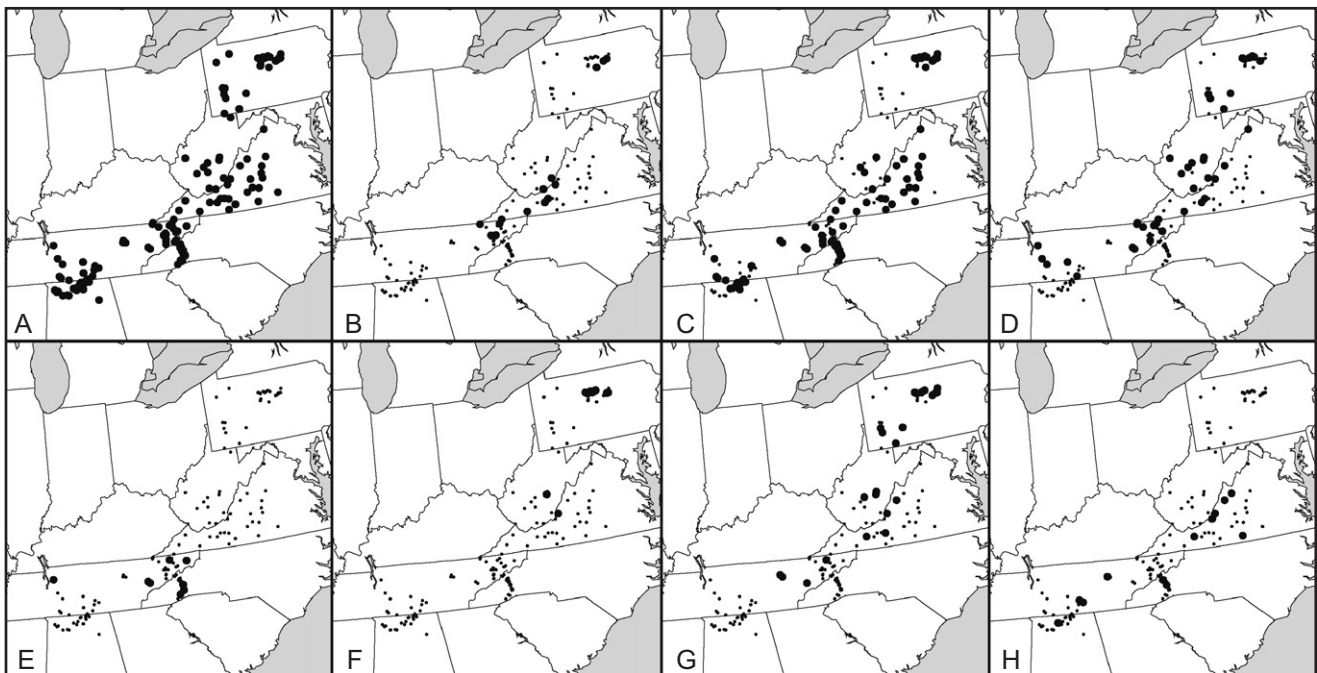| Species	% occurrence
A. minutissimum	98
A. rivulare	68
A. deflexum	28
A. cf. *latecephalum*	19
A. cf. *gracillimum*	18
A. reimeri	7
A. atomus	6
A. alpestre	4
A. eutrophilum	2
A. duthii	<1

Fig. 1. Geographic location of sites and species occurrence. Circles indicate all site locations included in the analysis; Small circles: sites where species was not found; Large circles: sites where species was found: (A) *Achnanthidium minutissimum*, (B) *A. atomus*, (C) *A. rivulare*, (D) *A. deflexum*, (E) *A. alpestre*, (F) *A.* cf. *gracillimum*, (G) *A.* cf. *latecephalum*, (H) *A. reimeri*.
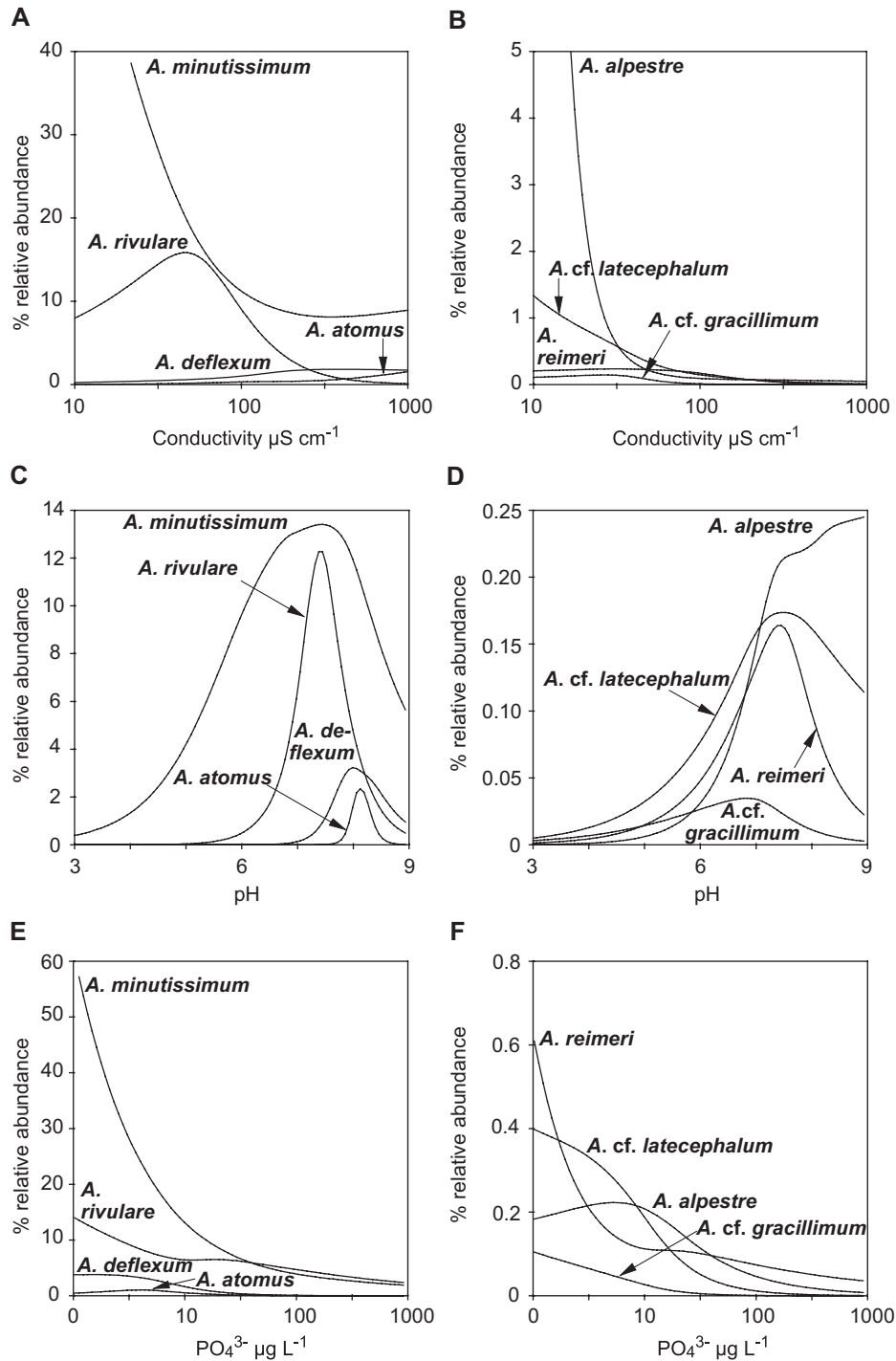
Fig. 2. Response curves of common (A, C, E) and rare (B, D, F) *Achnanthidium* species to conductivity, pH, and PO_4^{3-} fitted by generalized additive regression models.

specimens with an apical axis of 9.9–21 μm, and a transapical axis of 3–3.5 μm. However, the striae density on both valves fell within the range given by Hustedt (RV: 28–30 in 10 μm, RLV: 22–25 in 10 μm).

Ecology and distribution

A. atomus in our material was generally confined to two regions within Central and Northern Appalachia

(Fig. 1B). It was mostly found in nutrient-poor rivers (Figs. 2E, 3A) within a narrow pH range from 8 to 8.3 (Figs. 2C, 3A), but over a wide range of conductivity levels with a preference towards higher values (Fig. 2A). NPMR modeling showed that pH and nutrients were major factors driving the distribution of this species in Appalachia, although measured chemical characteristics were weak predictors of this species relative abundance (Table 3).

Table 3. Results of the NPMR modeling of relative abundance of 7 *Achnanthidium* species in relation to water chemistry characteristics

Response variable	Number of predictors	xR^2	Predictors
A. minutissimum	1	0.14	Conductivity
	2	0.19	Conductivity, $NO_3 + NO_2$
	3	0.23	Conductivity, $NO_3 + NO_2$, PO_4
	4	0.28	Conductivity, alkalinity, $NO_3 + NO_2$, PO_4
	5	0.29	Conductivity, alkalinity, $NO_3 + NO_2$, PO_4, SO_4
	6	0.29	Conductivity, alkalinity, $NO_3 + NO_2$, PO_4, Ca, SO_4
A. atomus	1	0.02	pH
	2	0.03	pH, PO_4
A. rivulare	1	0.33	Ca
	2	0.62	Ca, Cl
	3	0.64	Ca, SO_4, Cl
	4	0.68	PO_4, Ca, SO_4, Cl
	5	0.68	$NO_3 + NO_2$, PO_4, Ca, SO_4, Cl
	6	0.69	Conductivity, $NO_3 + NO_2$, PO_4, Ca, SO_4, Cl
A. deflexum	1	0.04	pH
	2	0.12	pH, Cl
	3	0.16	pH, alkalinity, Cl
	4	0.17	pH, alkalinity, $NO_3 + NO_2$, Cl
	5	0.17	pH, alkalinity, $NO_3 + NO_2$, PO_4, Cl
	6	0.17	pH, alkalinity, $NO_3 + NO_2$, PO_4, Ca, Cl
A. alpestre	1	0.02	Cl
	2	0.08	Cl, SO_4
	3	0.12	PO_4, SO_4, Cl
	4	0.12	pH, PO_4, SO_4, Cl
A. cf. *gracillimum*	1	0.00	$NO_3 + NO_2$
	2	0.00	$NO_3 + NO_2$, Cl
	3	0.01	$NO_3 + NO_2$, Ca, Mg
	4	0.04	Conductivity, $NO_3 + NO_2$, Ca, Cl
	5	0.04	Conductivity, $NO_3 + NO_2$, Ca, Mg, Cl
A. cf. *latecephalum*	1	0.17	Cl
	2	0.36	SO_4, Cl
	3	0.47	Mg, SO_4, Cl
	4	0.50	$NO_3 + NO_2$, Mg, SO_4, Cl
	5	0.50	$NO_3 + NO_2$, PO_4, Mg, SO_4, Cl

Coefficients of determination (R^2) and predictors are listed for the best models obtained for each number of predictors.

Achnanthidium rivulare Potapova & Ponader (Plate 1, Figs. 21–23)

Illustrations of type material. Potapova & Ponader (2004), Figs. 1–18, 28–43.

Taxonomy and morphology: *A. rivulare* has a raphe with curved terminal fissures and small round areolae arranged in parallel striae, which can be slightly convergent at the ends of the RV and slightly radiate at the ends of the RLV. This species has often been confused with *A. deflexum* (or *Achnanthes deflexa*) by diatomists using Patrick & Reimer (1966) as an identification guide or with *Achnanthes biasolettiana* Grunow in Cleve & Grunow by those using Krammer & Lange-Bertalot (1991).

Ecology and distribution

In Appalachia *A. rivulare* was widespread (Fig. 1C, Table 2) and had a pH optimum of around 7.3 (Fig. 2C). Compared to other species of *Achnanthidium* in Appalachia it has a greater tolerance towards elevated nutrients (Fig 2E). NPMR modeling showed that the distribution of this species was driven mostly by the ionic composition of the water (Table 3). *A. rivulare* had affinity towards relatively low calcium and high chloride concentrations.

Achnanthidium deflexum (Reimer) Kingston (Plate 1, Figs. 27–30)

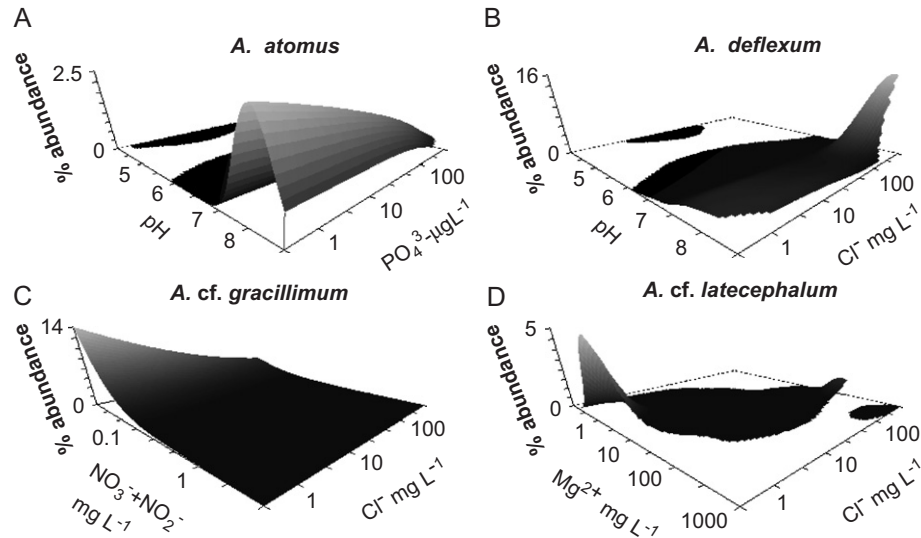Synonym. *Achnanthes deflexa* Reimer.

Fig. 3. (A–D) Graphs showing species response to two variables with highest explanatory power in NPMR models.

Illustrations of type material. Kingston (2003), Figs. 15F, G; Potapova and Ponader (2004), Figs. 51–60, 81–96, 102, 103.

Taxonomy and morphology: *A. deflexum* has a raphe with terminal fissures curved to the same side and transapically elongated areolae (Figs. 91, 94, 100 and 102 in Potapova & Ponader, 2004). Appalachian populations of *A. deflexum* (Plate 1, Figs. 27–30) were very similar to the type population from Indiana. *A. deflexum* and *A. rivulare* can be difficult to distinguish under LM because of their overlapping shape and size. These two species differ mostly by the shape of the areolae, which are small and rounded in *A. rivulare* but transapically elongated in *A. deflexum*. In *A. rivulare* areolae are indistinguishable under LM, while in *A. deflexum* they are usually visible. Moreover, *A. deflexum* often has very short striae in the central portion of the valve that can be visible near the valve margin between striae of the normal length (Plate 1, Figs. 28–30). *A. rivulare* does not have these shortened striae.

Ecology and distribution

A. deflexum is common in North American waters that are relatively rich in electrolytes, poor in nutrients, and have a pH above 7.5 (Potapova & Ponader, 2004). In Appalachia *A. deflexum* was recorded in approximately a third of all sites (Fig. 1D, Table 2) and had a tendency to occur in waters of higher mineral content (Fig. 2A), including those affected by mining, but not acidic (Fig. 2C). NPMR showed that the two chemistry characteristics explaining most variation in relative abundance of *A. deflexum* were relatively high pH, alkalinity, and chloride concentration (Table 3, Fig. 3B).

***Achnanthidium alpestre* (Lowe & Kociolek) Lowe & Kociolek (Plate 1, Figs. 24–26; Plate 3, Figs. 1–5)**

Synonym. *Achnanthes deflexa* var. *alpestris* Lowe & Kociolek.

Illustrations of type material. Lowe & Kociolek (1984), Figs. 2, 3; Potapova & Ponader (2004), Figs. 111–119.

Taxonomy and morphology: *A. alpestre* is similar to *A. deflexum* in shape, size, and raphe structure, but has higher striae density (Plate 1, Figs. 24–26; Plate 3, Figs. 1–5). After inspecting the holotype slide (ANSP G.C. 53918) of *A. alpestre* deposited in the Diatom Herbarium of ANSP we came to the conclusion that only Figs. 2 and 3 in the original description of *A. alpestre* illustrate specimens of the same taxon as circled on the holotype slide. Figs. 1, 4, and 5 illustrate another taxon, possibly *A. rivulare*.

Ecology and distribution

A. alpestre was found only in Tennessee and North Carolina (Fig. 1E), mostly in or around the Great Smoky Mountains National Park, which is its type locality (Lowe & Kociolek, 1984). Water chemistry characteristics that appeared to determine its distribution were low ionic strength (Fig. 2B), pH (7.3–8.2) (Fig. 2D), low nutrients (Fig. 2F), and lower than average proportions of Cl^- and SO_4^{2-} (Table 3).

***Achnanthidium* cf. *gracillimum* (Meister) Lange-Bertalot (Plate 3, Figs. 6–11; Plate 4, Figs. 1–10)**

Illustrations of type material of *A. gracillimum*. Meister (1912), Plate 12, Figs. 21–22.

Taxonomy and morphology: *A.* cf. *gracillimum* from Appalachia is similar to the diatoms illustrated in Plate 33, Figs. 1–12 of Krammer & Lange-Bertalot (1991) under the name of *Achnanthes minutissima* var. *gracillima* (Meister) Lange-Bertalot. It is distinguishable by its linear-lanceolate, sometimes asymmetric valves, with rostrate to sub-capitate ends (Plate 3, Figs. 6–11; Plate 4, Figs. 1–10). Investigation of material from Appalachia under the SEM showed that its terminal raphe fissures are abruptly bent (Plate 3, Figs. 9, 10), as it is also depicted in SEM illustrations of "*Achnanthes altera-gracillima* Lange-Bertalot" (Plate 35, Figs. 2–3 in Lange-Bertalot, 1993). In addition the terminal raphe endings extend all the way to the valve margin. This characteristical feature sets this taxon apart from other *Achnanthidium* taxa with gradually curved terminal raphe endings. The length of the Appalachian specimens was generally below 20 μm and they were therefore smaller than what was reported (25–40 μm) in the original description (Meister, 1912). Also, the specimens illustrated in Meister (1912, Plate 12, Fig. 22) have less capitate and slightly more drawn-out apices. All other features correspond to Meister's description.

The nomenclature of *A. gracillimum* is complicated. Its basionym is *Microneis gracillima* (Meister, 1912). Although *M. gracillima* has been transferred to *Achnanthes gracillima* by Mills (1933), Lange-Bertalot (1993) established the nomen novum *Achnanthes alter-agracillima* because the name "*Achnanthes gracillima*" was already used in 1927 by Hustedt for another diatom (Lange-Bertalot, 2004). Soon after, *Achnanthes alter-agracillima* was transferred to *Achnanthidium altergra-cillima* by Round and Bukhtiyarova (1996). Finally, Lange-Bertalot (2004) created the new combination *Achnanthidium gracillimum* because with the transfer of this species to *Achanthidium* the specific epithet '*gracilli-mum*' was available again.

However, it is not known whether *A. gracillimum* sensu Lange-Bertalot is the same diatom as "*Microneis gracillima*" sensu Meister because the type material of *M. gracillima* has never been photographed under LM or SEM. The shape of *M. gracillima* in Meister's publication is slightly different from the shape of "*A. minutissima* var. *gracillima*" in Lange-Bertalot & Krammer (1989). Unfortunately, the identity of *M. gracillima* is difficult to verify because there is no mention of type material or a specific type location of this species in Meister (1912). The shape of *A.* cf. *gracillimum* found in Appalachia is very similar to the shape of "*A. minutissima* var. *gracillima*" illustrated in Krammer & Lange-Bertalot (1989), but the name of this diatom can not be currently confirmed.

In addition to *A. gracillimum*, *A.* cf. *gracillimum* from Appalachia is somewhat similar to *Achnanthidium thienemannii* described from Sweden (Hustedt, 1942),

but differs from it in the overall valve shape. The type of *A. thienemannii* (Simonsen, 1987; Potapova, 2006) has a more linear shape than the taxon found in this study, which has more rounded valve sides. Also, the terminal raphe endings of *A. thienemannii* are gradually curved (Potapova, 2006) and not sharply bent as in *A.* cf. *gracillimum*.

Ecology and distribution

In Appalachia *A.* cf. *gracillimum* occurred occasionally (Table 2, Fig 1F), in higher abundances in low-conductivity, nutrient-poor rivers (Figs. 2B, F and 3C, Table 3) and sometimes in streams slightly affected by mining.

Achnanthidium cf. *latecephalum* Kobayasi (Plate 4, Figs. 11–20; Plate 5, Figs. 1–5)

Illustrations of type material of *A. latecephalum*. Kobayasi (1997), Figs. 19–40.

Taxonomy and morphology: *A.* cf. *latecephalum* from North America has a slightly more linear shape (Plate 4, Figs. 11–20) than *A. latecephalum* from Japan (Kobaya-si, 1997) and France (Rimet, Tudesque, Peeters, Vidal, & Ector, 2003) and sometimes lacks the apically oriented areola at the apex of the RLV, characteristic for *A. latecephalum* (Plate 5, Fig. 3). In addition, the transapical axis of the specimens found in this study measured 3.5–3.9 μm, whereas the original description reported the valves to be 4.0–4.5 μm wide. *A.* cf. *latecephalum* is similar to *Achnanthes nollii* O. Bock & W. Bock (1953) and was sometimes reported under this name by American diatomists. However, since the type of *A. nollii* is "currently not accessible" (Krammer & Lange-Bertalot, 1991), a potential synonomy could not be verified.

Ecology and distribution

In Appalachia *A.* cf. *latecephalum* was found mostly in nutrient-poor rivers of regions furthest from the seacoast (Fig. 1G), over a wide range of conductivity and pH (Fig. 2B, D), but relatively poor in SO_4^{2-}, Cl^-, and Mg^{2+} (Fig. 3D, Table 3).

Achnanthidium reimeri (Camburn) Ponader & Potapova comb. nov. (Plate 4, Figs. 21–30, Plate 5, Figs. 6–8

Basionym. *Achnanthes reimeri* Camburn in Camburn et al. (1978), Nova Hedwigia, 30, p. 173, Plate 4, Figs. 53–55.

Taxonomy and morphology: *A. reimeri* is easily distinguished from other *Achnanthidium* species by the rhomboid central area on the RLV (Plate 4, Figs. 21, 23,
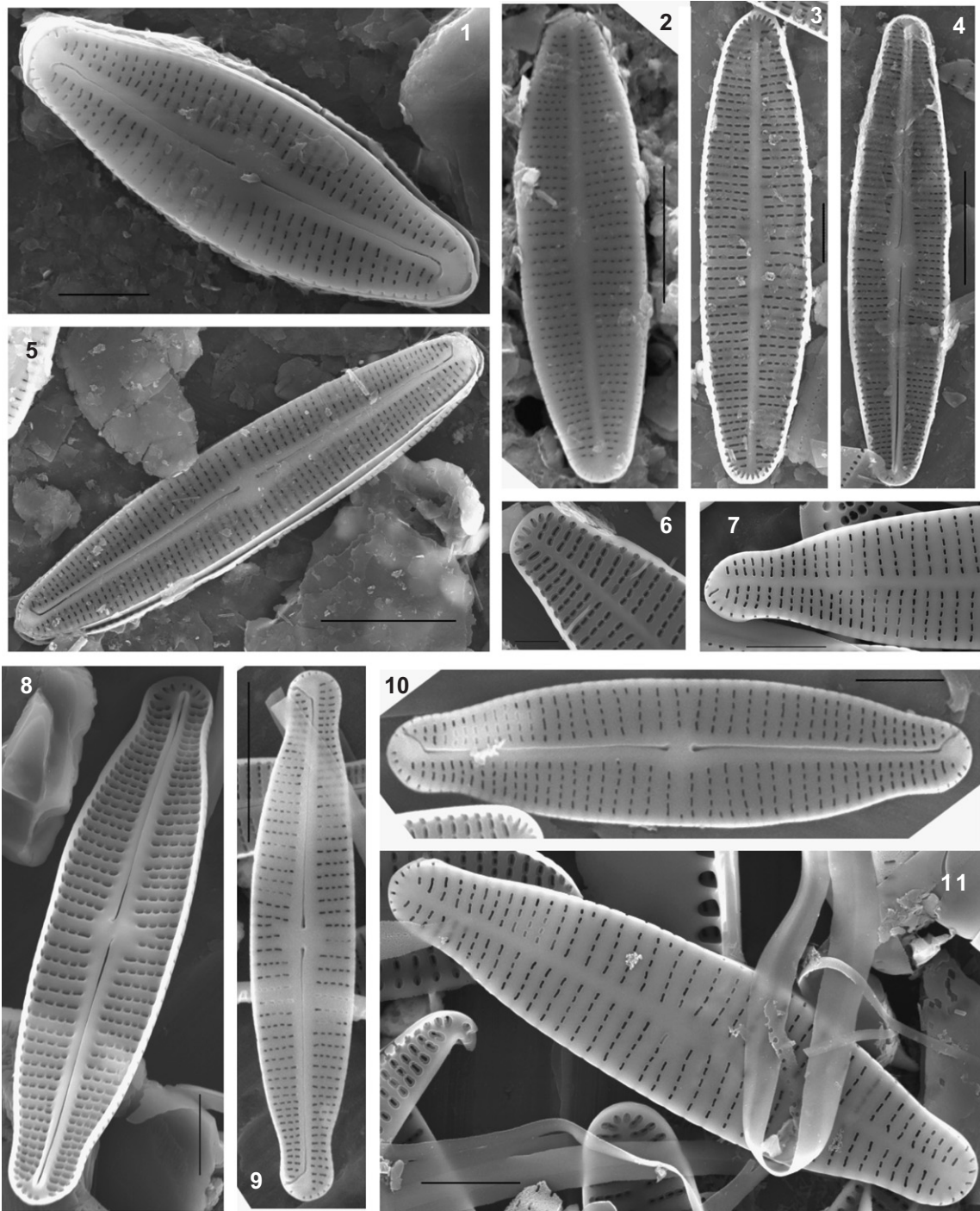
Plate 3. 1, 7, 8, 10. SEM, Scale bar 2 µm. **2–5, 9, 11.** SEM, Scale bar 5 µm. **6.** SEM, Scale bar 1 µm. **1–5.** *Achnanthidium alpestre.* **1.** External view of the raphe valve, Mills River, North Carolina. **2.** External view of the rapheless valve, Big Creek, Tennessee. **3–5.** Jacob Fork, North Carolina. **3.** Internal view of the rapheless valve. **4.** Internal view of the raphe valve. **5.** External view of the raphe valve. **6–11.** *Achnanthidium* cf. *gracillimum.* **6–7.** Antes Creek, Pennsylvania. **8–11.** Holston River, Virginia. **6.** Internal view of the rapheless valve. **7.** External view of the rapheless valve. **8.** Internal view of the raphe valve. **9–10.** External view of the raphe valve. **11.** External view of the rapheless valve.

125, 27, 30; Plate 5, Fig. 7). This species has terminal raphe ends curved to the same sides and small, round or transapically elongated areolae (Plate 5, Figs. 6 and 7).

Ecology and distribution

A. reimeri was previously reported only from South Carolina (Camburn et al., 1978) and from the Great

Smoky Mountains National Park in North Carolina and Tennessee (Johansen et al., 2004). No significant NPMR model could be constructed with habitat parameters measured in this study. It appears, however, that *A. reimeri* was mostly found in silty rivers of relatively low nutrient and ionic content (Fig. 2B, 2F).

Achnanthidium duthii (Sreenivasa) Edlund (Plate 5, Figs. 9–10; Plate 6, Figs. 1–8)

Synonyms. *Achnanthes duthii* Sreenivasa, *Rossithidium duthii* (Sreenivasa) Kingston.

Illustrations of type material. Sreenivasa (1971), Fig. 1A–D.
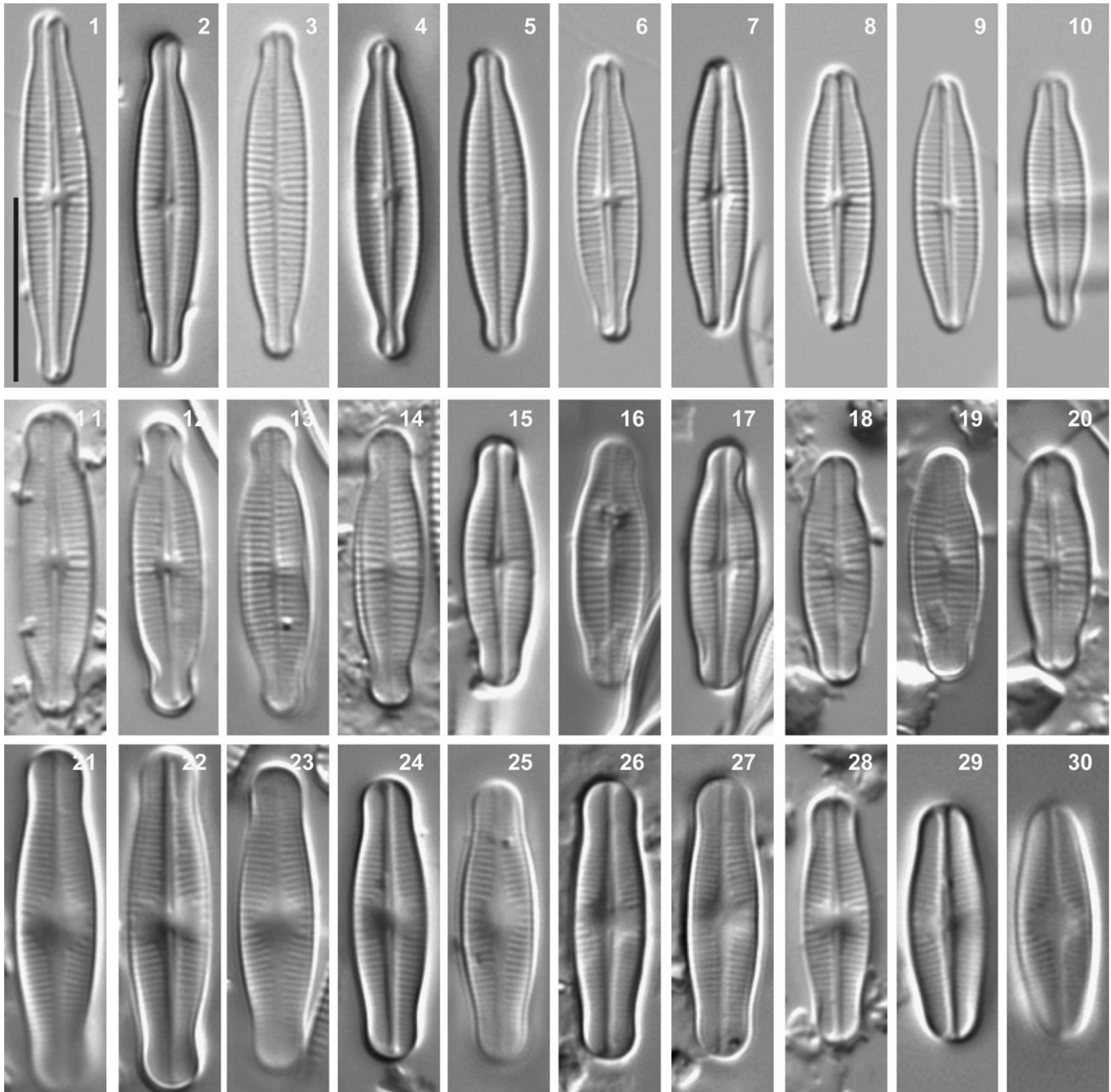
Plate 4. LM, Scale bar 10 μm. **1–10.** *Achnanthidium* cf. *gracillimum*. **1, 5–10.** West Branch Susquehanna River, Pennsylvania. **2–4.** Holston River, Virginia. **11–20.** *Achnanthidium* cf. *latecephalum*. **11–13, 15–16.** West Branch Susquehanna River, Pennsylvania. **14, 19–20.** Big Creek, Tennessee. **17.** Clear Fork, West Virginia. **21–30.** *Achnanthidium reimeri*. **21–25.** Daddy Run, Virginia. **26–27.** Two valves from the holotype slide (ANSP GC#53752), Long Branch Creek, South Carolina. **28–30.** Shawver's Run, Virginia.

Taxonomy and morphology: *A. duthii* has curved terminal raphe fissures and transapically elongated areolae (Plate 5, Figs. 9–10). It differs from other *Achnanthidium* species with the same structure of raphe and areolae by the characteristic capitate shape of the valve (Plate 6, Figs. 1–8). This diatom belongs to the genus *Achnanthidium*, but not to *Rossithidium* as suggested by Kingston (2000), because the latter genus is characterized by the straight raphe ends and a different valve fine structure than *Achnanthidium*.

Ecology and distribution

A. duthii was found in only one site (Big Creek, Rogersville, Tenessee) (Table 2) with high pH (8.3) and
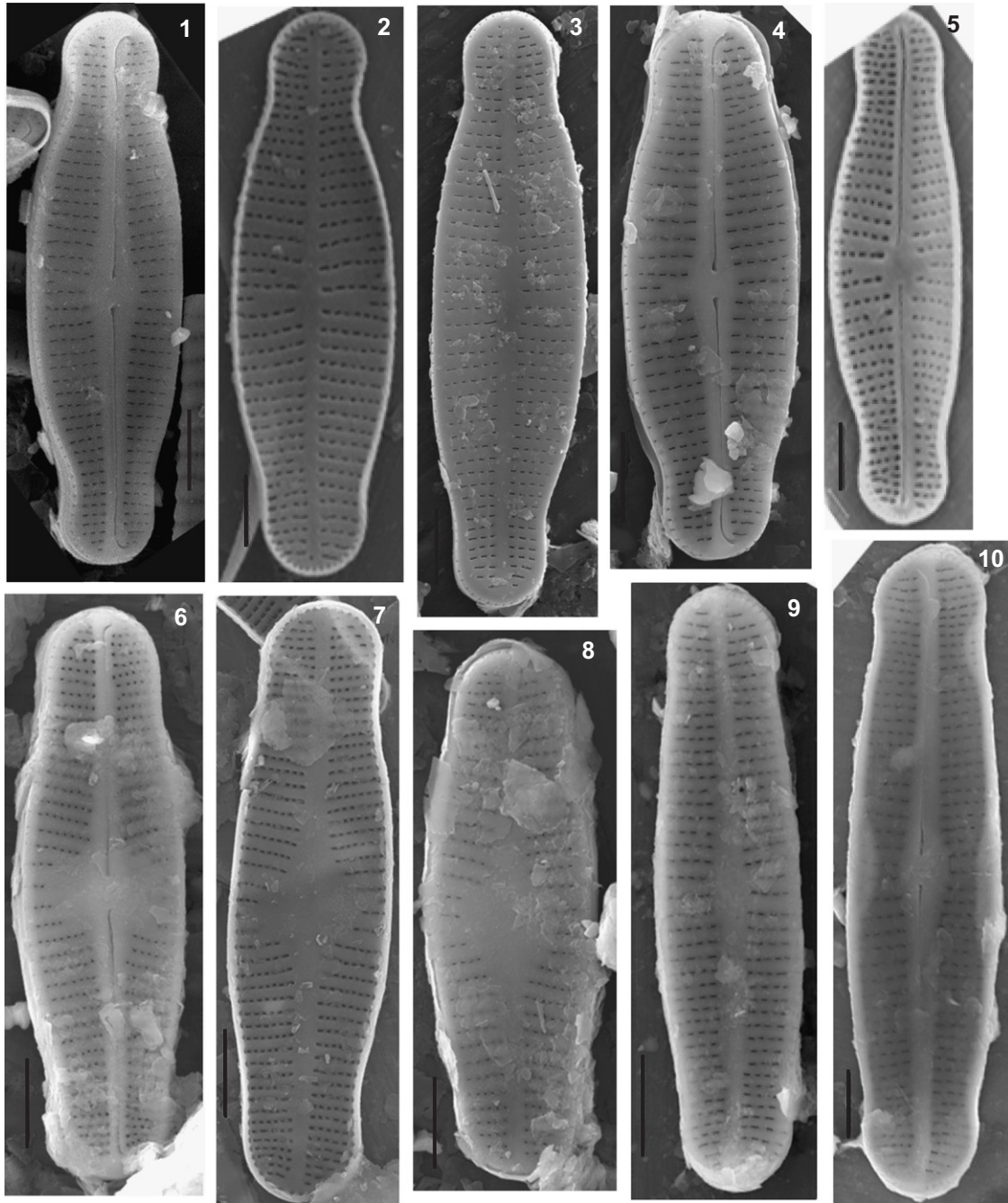

Plate 5. 1–8, 10. SEM, Scale bar 2 μm. **9.** SEM, Scale bar 3 μm. **1–5.** *Achnanthidium* cf. *latecephalum*. **1.** External view of the raphe valve, Sinnemahoning Creek, Pennsylvania. **2.** Internal view of the rapheless valve. Peters Creek, West Virginia. **3.** External view of the rapheless valve. Hunts Creek, Virginia. **4.** External view of the raphe valve, Antes Creek, Pennsylvania. **5.** Internal view of the raphe valve. Peters Creek, West Virginia. **6–8.** *Achnanthidium reimeri*. **6.** External view of the raphe valve, Daddy Run, Virginia. **7.** Internal view of the rapheless valve, Jacob Fork, North Carolina. **8.** External view of the rapheless valve, Daddy Run, Virginia. **9–10.** *Achnanthidium duthii*, Big Creek, Tennessee. **9.** External view of the rapheless valve. **10.** External view of the raphe valve.
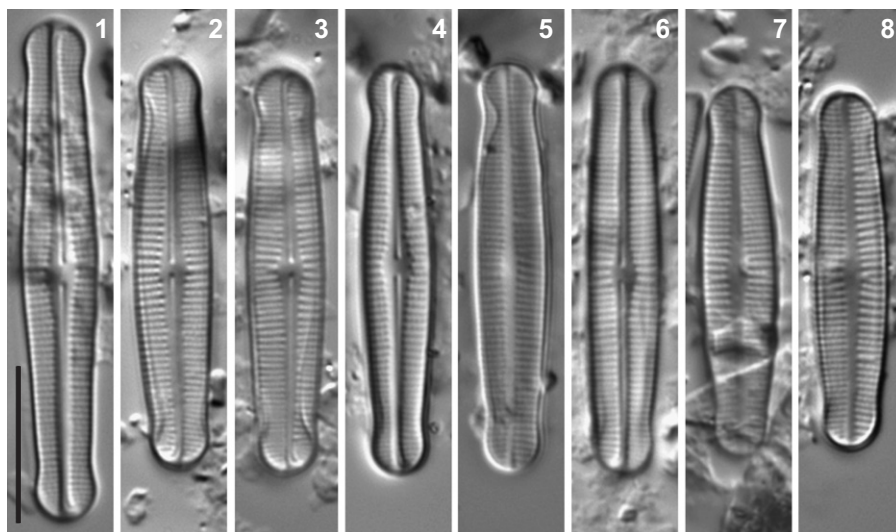
Plate 6. LM, Scale bar 10 μm. **1–8.** *Achnanthidium duthii*. Big Creek, Tennessee.

high alkalinity (159 mg L^{-1}), high inonic content (calcium: 51.7 mg L^{-1}; magnesium: 19.4 mg L^{-1}), higher conductivity than average (335 μS cm^{-1}), and low nutrients (NO$_3^-$ + NO$_2^-$: 0.794 mg L^{-1}, PO$_4^{3-}$: 0.019 mg L^{-1}).

Discussion

It has been suggested that diatom-based water-quality assessment methods can be based on genus-level identifications because there is considerable similarity in ecological characteristics of species within a genus (Chessman, Growns, Currey, & Plunkett-Cole, 1999; Hill et al., 2000; Wu, 1999). This study shows that various *Achnanthidium* species inhabiting Appalachian waters are different in some aspects of their ecology. Although most representatives have a tendency to occur in low-nutrient environment, the ranges of their distribution along the nutrient gradient vary considerably.

We also found large differences in species distributions in relation to pH and ionic content. These findings suggest that species-level identifications would help to improve the accuracy of bioassessments.

Species-level identifications are, however, often precluded by the absence of comprehensive diatom floras for many areas of the world. Among the ten diatom taxa covered by this paper only two, *A. minutissimum* and *A. eutrophilum,* could be found in the diatom flora of Middle Europe (Krammer & Lange-Bertalot, 1991), the most commonly used reference for routine identification of diatoms around the world. In fact, *A. eutrophilum* was listed as a "Sippe" (meaning a "clan" or "tribe" in German) of *A. minutissimum* in the first edition of this flora, as it was not yet formally described as a species in

1991. Most of the other eight *Achnanthidium* species (*A. rivulare, A. deflexum, A. alpestre, A. reimeri, A. duthii),* have been so far found only in the Americas, and are therefore absent in European floras. *A.* cf. *latecephalum* and *A.* cf. *gracillimum* are quite similar morphologically to Eurasian taxa, but small differences in shape, size and frustule structure suggest that they may be separate species. The need to base water-quality assessment on the detailed studies of regional diatom floras is obvious.

Our study also shows that it is beneficial for bioassessments to use ecological characteristics of diatoms obtained from regional datasets. For example, the distribution of *A. rivulare* in relation to nutrient and pH in Appalachia was slightly different from its distribution at the large US-scale (Potapova & Ponader, 2004). It is possible that several morphologically indistinguishable populations can represent different ecotypes or even cryptic species that vary in their responses to the environment. This is probably the case of *A. minutissimum*, which is usually characterized as a cosmopolitan and ubiquitous diatom, tolerant to various types of stressors (hydrologic disturbance, low pH, heavy metals) and an early colonizer (Nakanishi, Sumita, Yumita, Yamada, & Honjo, 2004; Stevenson & Bahls, 1999). It may consist of many entities (ecotypes or species) that are very similar morphologically, but differ in their ecology.

Although this study dealt only with *Achnanthidium* species, similar regional studies on other diatom genera are also likely to reveal a high diversity even in areas so seemingly well studied as North America, not to mention more remote areas of the world. We believe that further detailed studies of diatoms from the floristic and ecological perspectives are critical for their successful use as accurate environmental indicators.

Acknowledgments

References

Bock, O., & Bock, W. (1953). Das Phytoplankton des Freigerichtsees und des Langen Sees bei Kahl am Main. *Nachrichten des Naturwissenschaftlichen Museums der Stadt Aschaffenburg*, *38*, 1–105.

Camburn, K. E., Lowe, R. L., & Stoneburner, D. L. (1978). The haptobenthic diatom flora of Long Branch Creek, South Carolina. *Nova Hedwigia*, *30*, 149–279.

Charles, D. F., Knowles, C., & Davis, R. (Eds.). (2002). *Protocols for the analysis of algal samples collected as part of the US Geological Survey, National Water-Quality Assessment Program. Report No. 02-06. The Academy of Natural Sciences*. Philadelphia, PA: Patrick Center for Environmental Research.

Chessman, B., Growns, I., Currey, J., & Plunkett-Cole, N. (1999). Predicting diatom communities at the genus level for the rapid biological assessment of rivers. *Freshwater Biology*, *41*, 317–331.

Hastie, T., & Tibshirani, R. (1990). *Generalized additive models*. London: Chapman & Hall.

Hill, B. H., Herlihy, A. T., Kaufmann, P. R., Stevenson, R. J., McCorkick, F. H., & Burch Johnson, C. (2000). Use of periphyton assemblage data as an index of biotic integrity. *Journal of the North American Benthological Society*, *19*, 50–67.

Hustedt, F. (1927). Bacillariales aus dem Aokikosee in Japan. *Archiv für Hydrobiologie*, *18*, 155–172.

Hustedt, F. (1938). Systematische und ökologische Untersuchungen über die Diatomeen-Flora von Java, Bali und Sumatra nach dem Material der deutschen limnologischen Sunda-Expedition. Teil I. Systematischer Teil. *Archiv für Hydrobiologie* (Suppl. 15), 131–506.

Hustedt, F. (1942). Diatomeen aus der Umgebung von Abisko in Schwedisch-Lappland. *Archiv für Hydrobiologie*, *39*, 82–174.

Johansen, J. R., Lowe, R. L., Gomez, S. R., Kociolek, J. P., & Makosky, S. A. (2004). New algal species records for the Great Smoky Mountains National Park, USA, with an annotated checklist of all reported algal species for the Park. *Algological Studies*, *111*, 17–44.

Kingston, J. C. (2000). New combinations in the freshwater Fragliariaceae and Achnanthidiaceae. *Diatom Research*, *15*, 409–411.

Kingston, J. C. (2003). Araphid and monoraphid diatoms. In J. D. Wehr, & R. G. Sheath (Eds.), *Freshwater Algae of North America: Ecology and classification* (pp. 595–636). New York: Academic Press.

Kobayasi, H. (1997). Comparative studies among four linear-lanceolate *Achnanthidium* species (Bacillariophyceae) with curved terminal raphe endings. *Nova Hedwigia*, *65*, 147–163.

Krammer, K., & Lange-Bertalot, H. (1991). *Bacillariophyceae*. 4. Teil: *Achnanthaceae*, Kritische Ergänzungen zu *Navicula* (Lineolatae) und *Gomphonema*. In H. Ettl, G. Gärtner, J. Gerloff, H. Heynig, & D. Mollenhauer (Eds.), *Süßwasserflora von Mitteleuropa*, Vol. 2(4) (pp. 1–43). Stuttgart: Gustav Fischer.

Kützing, F. T. (1833). Synopsis Diatomacearum oder Versuch einer systematischen Zusammenstellung der Diatomeen. *Linnaea*, *8*, 529–620.

Lange-Bertalot, H. (1993). 85 Neue Taxa und über 100 weitere neu definierte Taxa ergänzend zur Süßwasserflora von Mitteleuropa 2 (1–4). *Bibliotheca Diatomologica*, *27*, 1–454.

Lange-Bertalot, H. (2004). Ergänzungen und Revisionen. In H. Ettl, G. Gärtner, J. Gerloff, H. Heynig, & D. Mollenhauer (Eds.), *Süßwasserflora von Mitteleuropa*, Vol. 2(4) (2nd ed.) (pp. 427–459). Stuttgart: Gustav Fischer.

Lange-Bertalot, H., & Krammer, K. (1989). *Achnanthes*, eine Monographie der Gattung mit Definition der Gattung *Cocconeis*. *Bibliotheca Diatomologica*, *18*, 1–393.

Lange-Bertalot, H., & Metzeltin, D. (1996). Indicators of oligotrophy. 800 taxa representative of three ecologically distinct lake types. Carbonate buffered – Oligodystrophic – weakly buffered soft water. In H. Lange-Bertalot (Ed.), *Iconographia Diatomologica*, Vol. 2 (pp. 1–390). Koenigstein: Koeltz Scientific Books.

Lowe, R. L., & Kociolek, J. P. (1984). New and rare diatoms from Great Smoky Mountains National Park. *Nova Hedwigia*, *39*, 465–476.

McCune, B. (2004). Nonparametric Multiplicative Regression for Habitat Modeling. Available at ⟨http://www.pcord.com/NPMRintro.pdf⟩.

McCune, B., & Mefford, M. J. (2004). *Nonparametric multiplicative regression for habitat modeling version 1. 00.60 beta*. Gleneden Beach, OR: MjM Software.

Meister, F. (1912). Die Kieselalgen der Schweiz. *Beiträge zur Kryptogamenflora der Schweiz*, *4/1*, 1–254.

Mills, F. W. (1933). *An index to the genera and species of the Diatomaceae and their synonyms: 1816–1932. Parts 1–7*. London: Wheldon & Wesley.

Monnier, O., Lange-Bertalot, H., Rimet, F., Hoffmann, L., & Ector, L. (2004). *Achnanthidium atomoides* sp. nov., a new diatom from the Grand-Duchy of Luxembourg. *Vie et Mileu*, *54*, 127–136.

Moulton, S. R., Kennen, J. G., Goldstein, R. M., & Hambrook, J. A. (2002). *Revised protocols for sampling algal, invertebrate and fish communities as part of the*

National Water-Quality Assessment Program. Open-File Report 02-150. Reston, VA: US Geological Survey.

Nakanishi, Y., Sumita, M., Yumita, K., Yamada, T., & Honjo, T. (2004). Heavy-metal pollution and its state in algae in Kakehashi river and Godani river at the foot of Ogoya mine, Ishikawa Prefecture. *Analytical Sciences*, *20*, 73–78.

Patrick, R., & Reimer, C. (1966). The diatoms of the United States. *Vol. 1. Monographs of the Academy of Natural Sciences of Philadelphia*, Vol. 13 (pp. 1–688).

Potapova, M. (2006). *Achnanthidium zhakovschikovii* sp. nov. (Bacillariophyta) and related species from rivers of North-western Russia. *Nova Hedwigia*, *82*, 399–408.

Potapova, M., & Hamilton, P. Morphological and ecological variation within the *Achnanthidium minutissimum* (Bacillariophyta) species complex. Journal of Phycology, in press.

Potapova, M. G., & Ponader, K. C. (2004). Two common North American diatoms, *Achnanthidium rivulare* sp. nov. and *A. deflexum* (Reimer) Kingston: morphology, ecology and comparison with related species. *Diatom Research*, *19*, 33–57.

Rimet, F., Tudesque, L., Peeters, V., Vidal, H., & Ector, L. (2003). Assemblages-types de diatomées benthiques des rivières non-polluées du bassin Rhône-Méditerranée-Corse (France). *Bulletin de la Société des Sciences Naturelles de l'Ouest de la France, 2ème supplément hors série*, 272–287.

Round, F. E., & Bukhtiyarova, L. (1996). Four new genera based on *Achnanthes* (*Achnanthidium*) together with a re-definition of *Achnanthidium. Diatom Research*, *11*, 345–361.

Simonsen, R. (1987). *Atlas and catalogue of the diatom types of Friedrich Hustedt, Plates 1–395*, Vol. 2. Berlin: J. Cramer.

Sreenivasa, M. R. (1971). New fossil diatoms from Ontario. II. *Achnanthes duthii* sp. nov., *Achnanthes undulatus* sp. nov., and *Neidium distincte-punctatum* Hustedt var. *major* var. nov. *Phycologia*, *10*, 79–80.

Stevenson, R. J., & Bahls, L. L. (1999). Periphyton protocols. In M. T. Barbour, J. Gerritsen, & B. D. Snyder (Eds.), *Rapid bioassessment protocols for use in wadeable streams and rivers: Periphyton, benthic macroinvertebrates, and fish. EPA 841-B-99-002* (2nd ed., pp. 6.1–6.22). Washington: United States Environmental Protection Agency.

ter Braak, C. J. F., & Šmilauer, P. (2002). *CANOCO reference manual and CanoDraw for Windows user's guide: Software for Canonical Community Ordination. Version 4.5*. Ithaca, NY: Microcomputer Power.

USGS (United States Geological Survey) (2003). Map o the Appalachian Mountains region. Fig. 51 at ⟨http://3dparks.wr.usgs.gov/nyc/images/fig51.jpg⟩. Figure captions at ⟨http://3dparks.wr.usgs.gov/nyc/common/captions.htm⟩.

Wu, J.-T. (1999). A generic index of diatom assemblages as bioindicator of pollution in the Keelung River of Taiwan. *Hydrobiologia*, *397*, 79–87.