Supplementary Materials

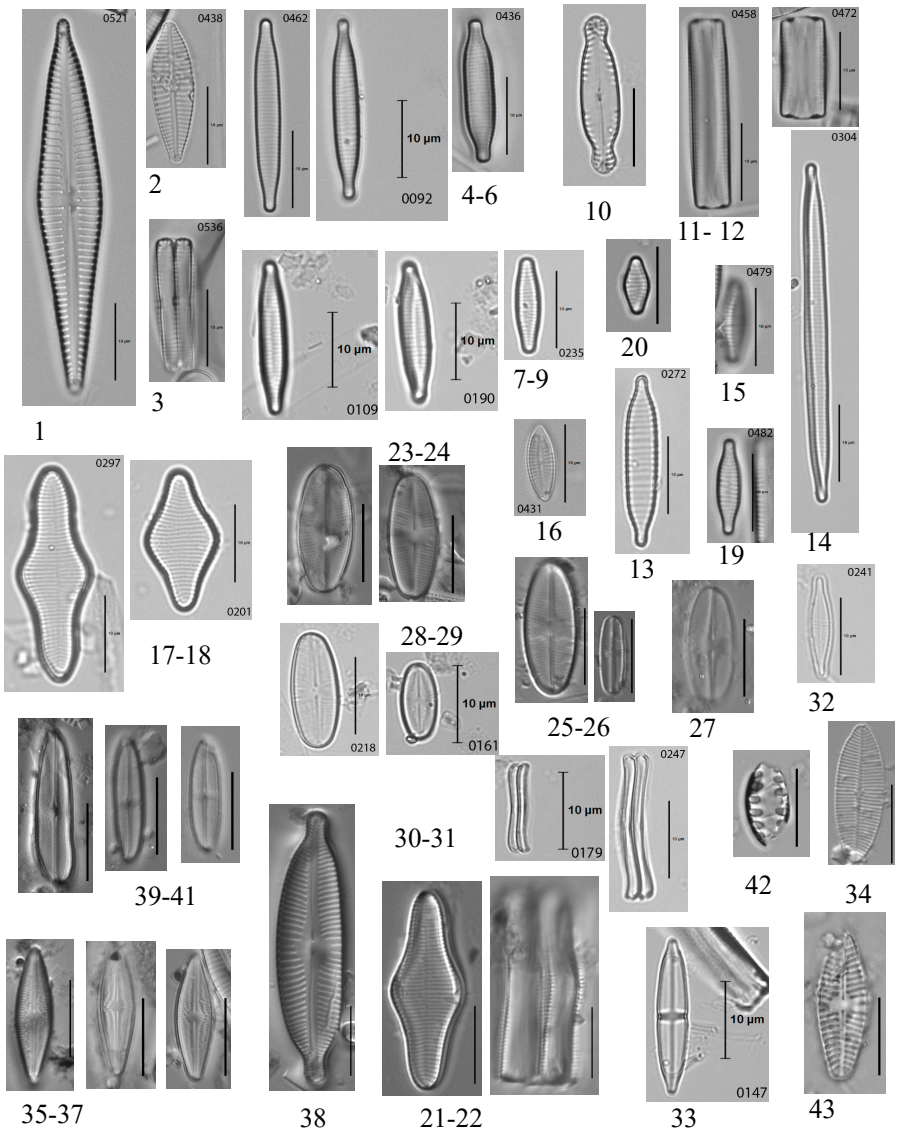

Plate S1. Diatoms at Upper Three Runs Creek site in 2018: Fig 1. *Gomphonema* sp. M1; Fig 2 *Gomphonema parvulum* Morphotype 3; Fig 3. *Gomphonema* spp. girdle*; Figs 4–6. *Fragilariforma virescens* var. 1*; Figs 7-–9. *Fragilariforma virescens* var. 2; Fig 10. *Pinnularia* sp.1; Figs 11–12. *Fragilariforma* spp. girdle*; Fig 13. *Fragilariforma* "coarse"; Fig 14. *Fragilaria* sp.1; Fig 15. *Fragilaria exigua*; Fig 16. *Achnanthidium subhudsonis* var. *kraeuselii*; Figs 17–18 *Fragilariforma* cf. *floridana*; Fig 19. *Fragilariforma bicapitata*; Fig 20. *Fragilariforma virescens*; Figs 21–22. *Fragilariforma polygonota*; Figs 23–24. *Psammothidium altaicum*; Figs 25–26. *Psammothidium helveticum*; Fig 27. *Psammothidium bioretii*; Figs 28–29. *Psammothidium subatomoides*; Figs 30–31. *Achnanthidium catenatum* girdle; Fig 32. *Achnanthidium minutissimum*; Fig 33. *Stauroneis smithii* var. *incisa;* Fig 34. *Lemnicola hungarica;* Figs 35–37. *Brachysira brebissonii*; Fig 38. *Cymbopleura naviculiformis;* Figs 39–41. *Neidium alpinum*; Fig 42. *Staurosirella martyi*; Fig 43. *Planothidium lanceolatum.* Scale bars = 10 µm. *Denotes taxon or OTU found at > 1% relative abundance (RA) identified from 2018 samples and 1956 archived slides collected from Upper Three Runs Creek, South Carolina.

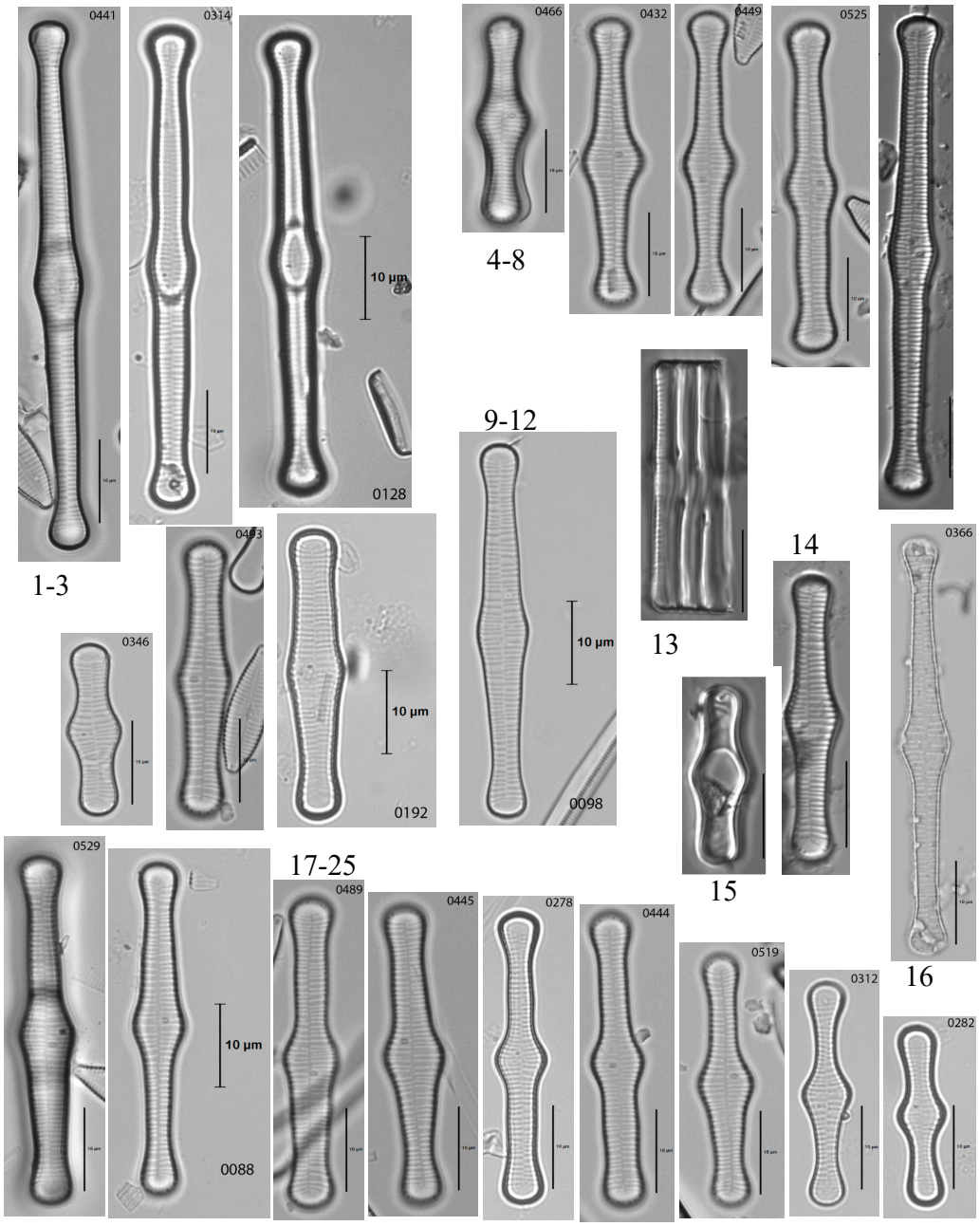

Plate S2. Diatoms at Upper Three Runs Creek site in 2018: Scale bars = 10 µm. Figs 1–3. *Tabellaria quadriseptata*; Figs 4–8. *Tabellaria fenestrata*; Figs 9–12. *Tabellaria flocculosa* strain III; Fig 13. *Tabellaria* spp. girdle*; Fig 14. *Tabellaria flocculosa*; Fig 15. *Tabellaria flocculosa* girdle band; Fig 16. *Tabellaria* sp. Transitional; Figs 17–25. *Tabellaria flocculosa* intermediate*. *Denotes taxon or OTU found at > 1% relative abundance (RA) identified from 2018 samples and 1956 archived slides collected from Upper Three Runs Creek, South Carolina.

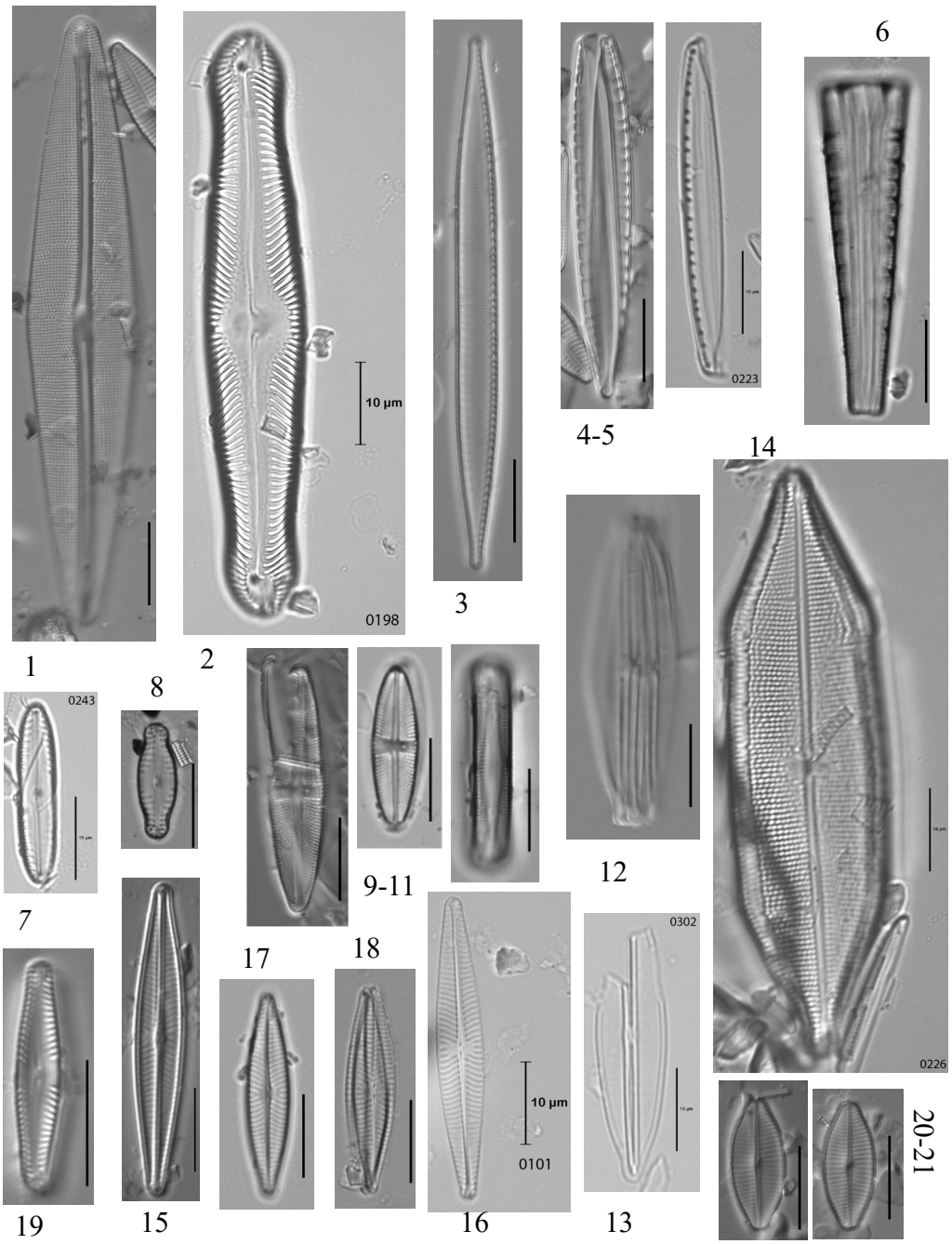

Plate S3. Diatoms at Upper Three Runs Creek site in 2018: Scale bars = 10 µm. Fig 1. *Frustulia rhomboides*; Fig 2. *Pinnularia* cf. *mesogongyla*; Fig 3. *Nitzschia intermedia*; Figs 4–5. *Nitzschia recta**; Fig 6. *Meridion alansmithii*; Fig 7. *Pinnularia obscura*; Fig 8. *Pinnularia metzeltinii* Krammer; Figs 9–11. *Luticola goeppertiana* and girdle*; Fig 12. *Frustulia* spp. girdle; Fig 13. *Frustulia crassinervia* *; Fig 14. *Neidium sacoense*; Fig 15. *Navicula notha**; Fig 16. *Navicula leptostriata**; Fig 17. *Navicula exilis*; Fig 18. *Navicula* cf. *cryptotenella*; Fig 19. *Pinnularia appendiculata*; Figs 20–21. *Geissleria kriegeri.* *Denotes taxon or OTU found at > 1% relative abundance (RA) identified from 2018 samples and 1956 archived slides collected from Upper Three Runs Creek, South Carolina.

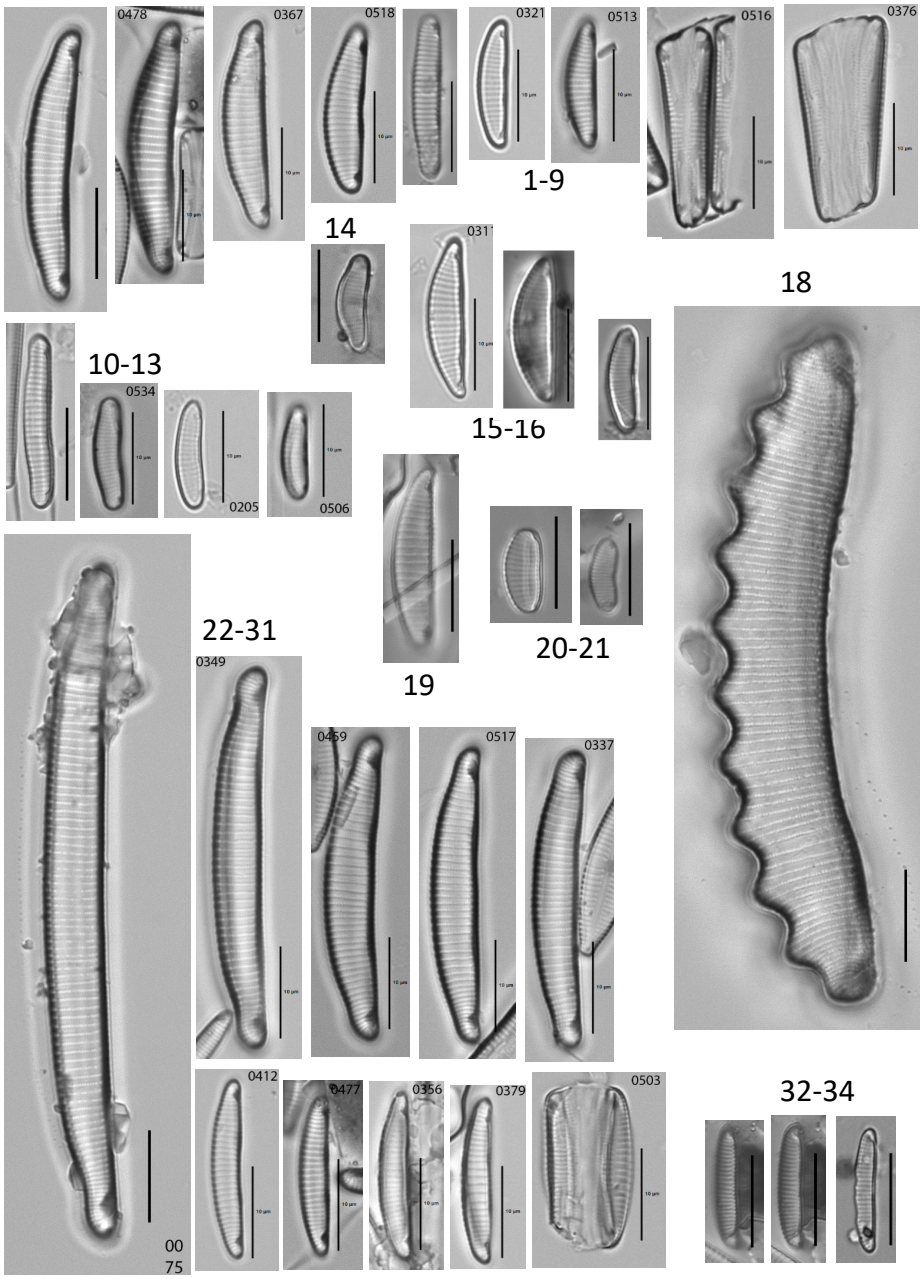

Plate S4. Diatoms at Upper Three Runs Creek site in 2018: Figs 1–9. *Eunotia rhomboidea* with girdle (Figs. 8–9)*; Figs 10-13. *Eunotia mucophila*; Fig 14. *Eunotia schwabei*; Figs 15–16. *Eunotia pirla*; Fig 17. *Eunotia mucophila* var. 1; Fig 18. *Eunotia serra*; Fig 19. *Eunotia veneris*; Figs 20–21. *Eunotia subarcuatoides*; Figs 22–31. *Eunotia incisa* with girdle (Fig. 31)*; Figs 32–34. *Eunotia* cf. *botulitropica.* *Denotes taxon or OTU found at > 1% relative abundance (RA) identified from 2018 samples and 1956 archived slides collected from Upper Three Runs Creek, South Carolina. Scale bars = 10 μm.

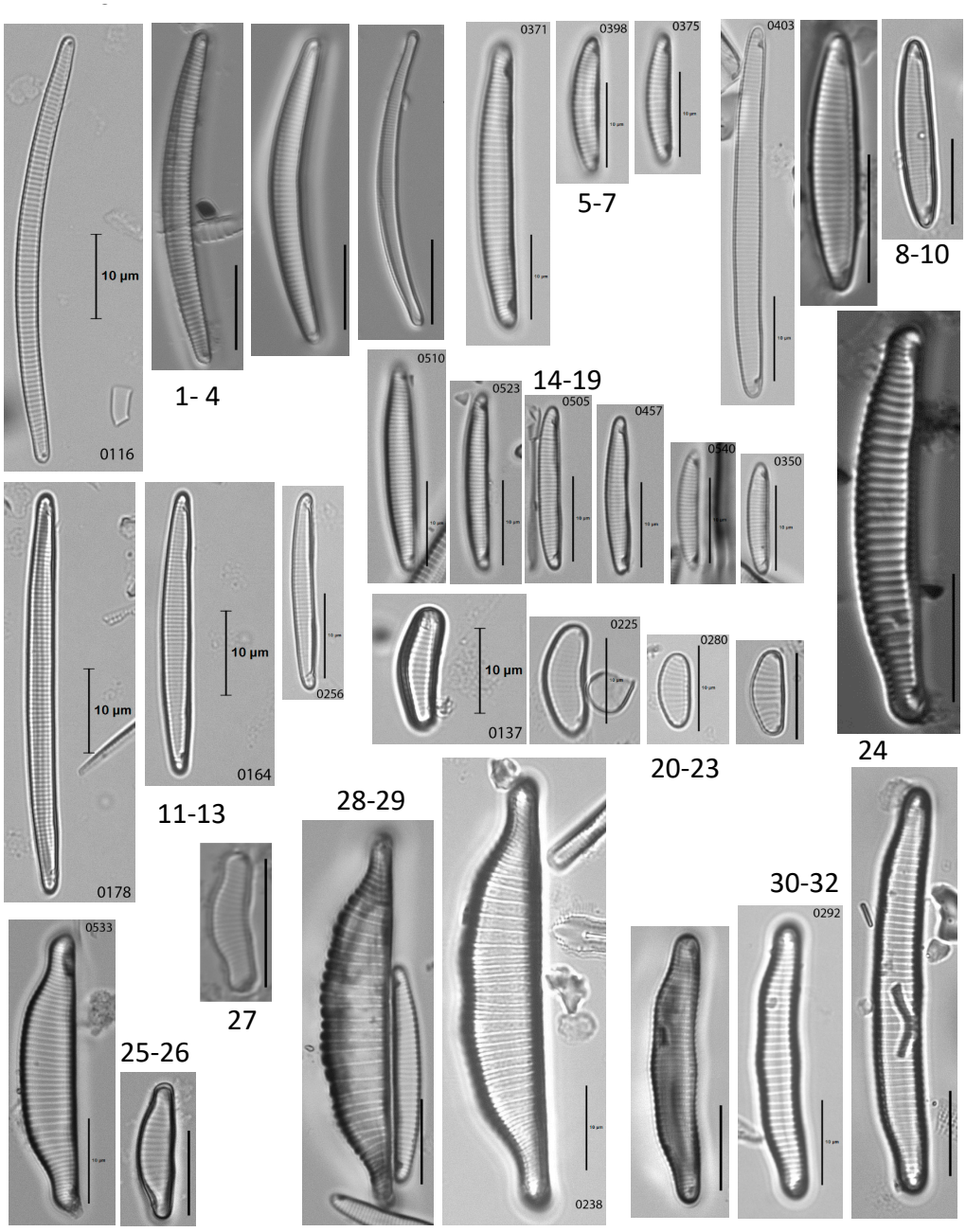

Plate S5. Diatoms at Upper Three Runs Creek site in 2018: Scale bars = 10 µm. Figs 1–4. *Eunotia bilunaris*; Figs 5–7. *Eunotia* site 4; Figs 8–10. *Eunotia* site 2; Figs 11–13. *Eunotia* site 3; Figs 14–19. *Eunotia* site; Figs 20–23. *Eunotia boreotenuis*; Fig 24. *Eunotia* cf. *macroglossa*; Figs 25–26. *Eunotia carolina*; Fig 27. *Eunotia exigua*; Figs 28–29. *Eunotia* 238 new; Figs 30–32. *Eunotia pectinalis* var. *undulata.*

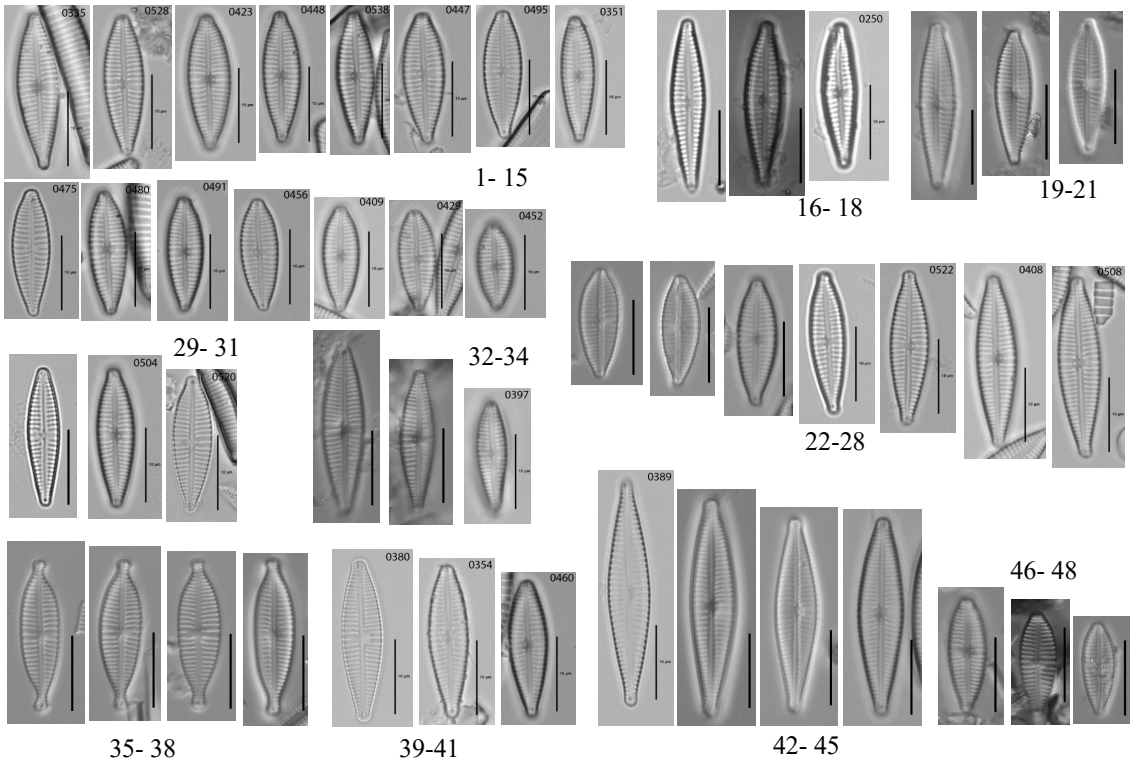

Plate S6. Light micrographs of *Gomphonema parvulum sensu lato* species complex separations used in this study: Figs 1–15. *Gomphonema parvulum sensu lato*; Figs 16–18. *Gomphonema exilissimum*; Figs 19–21. *Gomphonema parvulum* Morphotype 2; Figs 22–28. *Gomphonema parvulum "*protracted and off-center"*; Figs 29–31. *Gomphonema parvulum* "intermedio"; Figs 32–34. *Gomphonema parvulum* "radial—rhombic"; Figs 35–38. *Gomphonema lagenula*; Figs 39–41. *Gomphonema parvulum* "rhombic"; Figs 42–45. *Gomphonema parvulum "*Cox"; Figs 46–48. *Gomphonema confusum.* *Denotes taxon or OTU found at > 1% relative abundance (RA) identified from 2018 samples and 1956 archived slides collected from Upper Three Runs Creek, South Carolina. Scale bars = 10 µm.

Table S1. Morphological features distinguishing taxa originally belonging to *Gomphonema parvulum sensu lato* species complex for 2018 Upper Three Runs Creek study.

Taxon/OTU	Length (µm)	Width (µm)	Striae density (in 10 µm)	Raphe	Central area	Striae orientation	Valve and Pole shape	Previous literature
Gomphonema parvulum sensu lato **Plate A6, Figs. 1–15**	12.6-31	4.5-5.7	14-20	Narrow	Small-moderate depending on area opposite isolated punctum	Not radial across transapical axis, parallel or curved toward isolate puncta	Ends rostrate, valves elliptic-lanceolate	[13] [16]
G. exilissimum **Plate A6, Figs. 16–18**	19.9-23.5	4.5-5.2	15-17	Narrow, weakly undulate	Small, rectangular depending on area opposite isolated punctum	Mostly radiate throughout	Ends rostrate and rounded, valves linear-lanceolate	[16]
G. parvulum morphotype 2 **Plate A6, Figs. 19–21**	12.4-18.9	4.5-5.2	17-20	Similar to *G. parvulum sensu lato*	Similar to *G. exilissimum*	Similar to *G. exilissimum* and radial across transapical axis	Sub-capitate headpole, valve more lanceolate	[16]
*G. parvulum "*off- center and protracted" **Plate A6, Figs. 22–28**	18.8-24.6	4.5-5.6	16-21	Asymmetrical along the apical axis, also bisecting foot poles asymmetrically	Small	Variable-weakly radiate, curved near isolate punctum in central area	Ends protracted and headpoles capitate, valves lanceolate	n/a

*G. parvulum "*intermedio" **Plate A6, Figs. 29–31**	18.3-19.1	5.3-5.4	16-18	Similar to *G. parvulum sensu lato*	Small	Not radial across transapical axis, radiate or curved on one side of the apical axis	Ends weakly protracted and headpoles weakly capitate, valves lanceolate-rhombic	n/a
*G. parvulum "*radial–rhombic" **Plate A6, Figs. 32–34**	15.1-20.5	3.5	15-20	Similar to *G. parvulum sensu lato*	Small	Radial across transapical axis	Valves rhombic	n/a
G. lagenula **Plate A6, Figs. 35–38**	16-20.1	4.6-5.7	17-18	Straight and filiform	Asymmetric, depending on area opposite isolated punctum	Parallel–slightly radiate	Ends distinctly capitate, valves slightly narrow lanceolate to elliptic	[13] [36]
G. parvulum "rhombic" **Plate A6, Figs. 39–41**	19.3-23.2	4.9-5.7	16-17	Similar to *G. parvulum sensu lato*	Small	Not radial across transapical axis	Valves rhombic	n/a
*G. parvulum "*Cox" **Plate A6, Figs. 42–45**	23.2-34.6	3.3-10.6	(10)-14-18 (21)	Narrow, weakly undulate	Small to moderate, rectangular depending on cell size and area opposite isolated punctum	Slightly radiate throughout	Foot poles acutely rounded and head poles broader and more obtuse, valves lanceolate	[37]
G. confusum **Plate A6, Figs. 46–48**	14.8-22.3	4.5-5.1	15-18	Similar to *G. parvulum sensu lato*	Small, rectangular depending on area opposite isolated punctum	Radiate to parallel across transapical axis	Ends acutely rounded and protracted, valves lanceolate to rhombic-lanceolate	[16]

Table S2. List of diatom taxa and operational taxonomic units (OTUs) found at > 1% relative (RA) identified from 2018 samples and 1956 archived slides collected from Upper Three Runs Creek, South Carolina. In sample type, R= right diatometer, L= left diatometer, NC= 2018 composite, OC= 1956 composite, and A= present in all sample types. * Denotes relative abundance with taxa not split from species complexes for *Gomphonema parvulum sensu lato*, *Eunotia incisa sensu lato*, and *Tabellaria flocculosa sensu lato*. In RA, (-) represents taxa not found in sample year or scored < 1% relative abundance.

Scientific name/ OTU	2018	1956	Sample type	%RA 2018	%RA 1956	Ecological indication	Literature
Eunotia spp. girdle	X	X	A	31.5	10.0	Genus ecology: found in soft-water lakes, oligotrophic, areas with low light, low turbulence, narrow temperature gradient; acidophilic	[38-39]
Eunotia incisa **Plate A4, Figs. 22–31**	X	X	A	3.7, 4.8*	- , 2.3*	Acidobiontic; optimums: pH 5.87, TP 9.85, DOC 9.21, acidophilic; epiphytic on bryophytes in streams and observed on wet walls; common and abundant in peat bogs, acidic waters, and low electric conductance, most frequent taxon in Holarctic zone; acidophilic, high (100% saturation) oxygen requirements, tolerating small concentrations of organic nitrogen, oligosaprobous, oligotraphentic, mainly occurring in water, but sometimes in wet places out of water bodies	[38- 42]
Eunotia rhomboidea **Plate A4, Figs. 1–9**	X	X	A	8.3	2.0	Optimums: pH 4.89, TP 7.48, DOC 6.95, acidophilic; epiphytic on bryophytes in streams; cosmopolitan, found in oligotrophic to dystrophic waters, and in mineral poor and acidic environments; also found in waters with high nutrient concentrations; acidophilic, high (100% saturation)	[39-43]

						oxygen requirements, can tolerate small concentrations of organic nitrogen, oligosaprobic, oligotraphentic, and mainly found in water, but regularly found in wet places	
Fragilaria spp. girdle	-	X	OC	-	1.1	Genus ecology: dominate headwater reaches, nutrient rich environments, and settle rapidly	[38]
Fragilariforma spp. girdle **Plate 1A, Figs. 11–12**	X	X	A	9.8	-	Acidophilic, and occurring in environments high in tannic acid	This 2018 study
Fragilariforma virescens var. 1 **Plate 1A, Figs. 4–6**	X	-	NC, R, L	1.1	-	pH indifferent, oligosaprobic, current (flow) indifferent, found in streams and ditches, and seasons spring and fall	[44]
Frustulia crassinervia **Plate 3A, Figs. 13**	X	X	R, L, OC	-	1.8	Acidophilic; genus found in soft-water lakes; acidophilic; acidobiontic, high (100% saturation) oxygen requirements, tolerating small concentration of organic nitrogen, oligosaprobic, oligotraphentic, mainly in water regularly in wet places	[38, 40, 42]
Frustulia saxonica	-	X	OC	-	1.1	Genus ecology: found in soft-water lakes; Species ecology: pH optimum 5.49; acidophilic; acidobiontic, high (100% saturation) oxygen requirements, tolerating small concentrations of organic nitrogen, oligosaprobic, oligotraphentic, mainly found in water, but regularly observed in wet places	[38, 40, 42]
Gomphonema spp. girdle **Plate A1, Fig. 3**	X	X	A	5.3	8.1	Genus ecology: found in areas of high light, high turbulence, broad temperature ranges, found in nutrient rich environments, and oligotrophic hard water	[38]
Gomphonema parvulum Morphotype 2 **Plate A6, Figs. 26–28**	X	X	A	2.5	-	Found in eutrophic and moderately polluted waters	[16]
Gomphonema parvulum "protracted off-center" **Plate A6, Figs. 22–28**	X	-	NC, R, L	3.1	-	Acidophilic, and occurring in environments high in tannic acid	This 2018 study
Gomphonema parvulum sensu lato **Plate A6, Figs. 1–15**	X	X	A	7.2, 16.2*	1.5, 3.4*	Optimums: pH 6.66, DOC 5.86; pH indifferent, mesosaprobic, thriving in flowing water, periphytic, mesothermal and stenothermal; found in eutrophic and moderately to highly polluted waters, observed in oligotrophic environments; circumneutral, found in	[16, 40, 42, 44]

						fresh-brackish, needs periods of high organic nitrogen concentrations, low (above 30% saturation) oxygen requirements, mainly occurring in water, but regularly observed in moist and wet places	
Gomphonema preliciae	-	X	OC	-	1.2	Epiphytic on macrophytes, found in shallow areas, and in eutrophic to hyper-eutrophic environments	[16]
Luticola spp. girdle	X	X	NC, OC	-	1.5	Acidophilic, and occurring in environments high in tannic acid	This 2018 study
Luticola goeppertiana **Plate A3, Figs. 9–11**	X	X	NC, OC, L	-	5.4	Found in eutrophic and eusaprobic environments; circumneutral, found in fresh-brackish water, needs periods of high organic nitrogen concentrations, low (above 30% saturation) oxygen requirements, mainly occurring in water, but regularly observed in moist and wet places; found in " electrolyte-rich running waters" and rivers with high industrial pollution	[16, 42, 45]
L. goeppertiana girdle **Plate A3, Fig. 11**	X	X	NC, OC	-	2.0		
Navicula leptostriata **Plate A3, Fig. 16**	X	X	R, L, OC	-	1.3	Genus ecology: found in eulittoral environments and areas of low light, can survive in groundwater, and resettle rapidly from suspension; Species ecology: optimums: pH 6.26, TP 13.07, DOC 15.48, acidophilic; acidophilic, high (100% saturation) oxygen requirements, tolerates small concentrations of organic nitrogen, oligosaprobous, oligo-mesotraphentic, mainly in water, and regularly observed in wet places	[38, 40, 42]
Navicula notha **Plate A3, Fig. 15**	X	X	NC, OC	-	2.1	Genus ecology: found in eulittoral environments and areas of low light, can survive in groundwater, and resettle rapidly from suspension; Species ecology: Alkaliphilous, found in eutrophic environments, halobionts indifferent, current (flow) indifferent, observed in lakes and ponds, periphytic	[38, 44]
Past "*Navicula minima* group" (*Navicula difficillima*/ *Sellaphora difficillima*/ *Adlafia* spp.)	X	X	L, OC	-	2.1	Alkaphilous, found in fresh- brackish waters, needs periods of high concentrations of organic nitrogen. Low (above 30% saturation) oxygen requirements, eutraphentic, mainly found in water, but regularly observed in wet places	[42]
Nitzschia spp. girdle	-	X	OC	-	2.6	Diurnal migration, found in low light and nutrient rich environments, remain in suspension longer instead of settling immediately	[38, 46]
Nitzschia recta **Plate A3, Fig. 4–5**	X	X	NC, L, OC	-	2.6	Alkaphilous, found in fresh-brackish water, can tolerate high concentrations of organic nitrogen, fairly high (above 75% saturation) oxygen requirements	[42]

						mesosaprobous, oligo-eutraphentic, never to rarely observed outside of water bodies	
Pinnularia spp. girdle	-	X	OC	-	2.0	-	-
Tabellaria flocculosa *"*intermediate*"* **Plate A2, Figs. 17–25**	X	X	A	1.3, 2.6*	-	*Sensu lato* ecology: optimums: pH 5.22, TP 7.45, DOC 8.83 acidophilic; acidophilous, mesotrophic-oligotrophic-dystrophic, halophobous, found in ponds, periphytic, and tychoplanktonic, seasons spring and fall; acidophilous, fresh water species, tolerating very small concentrations of organic nitrogen, high (about 100% sat) oxygen requirements mesosaproubous, mesotraphentic, mainly occurring in water bodies, and sometimes observed in wet places; oligotrophic, found in areas with high light, high turbulence, and broad temperature changes	[38, 40, 42, 44]
Tabellaria spp. girdle **Plate A2, Fig. 13**	X	X	A	-	1.4	Genus ecology: found in soft-water lakes	[38]