

# Notes on the genus *Nupela* (Bacillariophyceae) including the description of a new species, *Nupela scissura* sp. nov. and an expanded description of *Nupela paludigena*

Peter A. Siver,<sup>1\*</sup> Paul B. Hamilton<sup>2</sup> and Eduardo A. Morales<sup>3</sup>

<sup>1</sup>Department of Botany, Connecticut College, New London, Connecticut 06320, USA, <sup>2</sup>Research Division, Canadian Museum of Nature, Ottawa, Canada K1P 6P4; and <sup>3</sup>Patrick Center for Environmental Research, The Academy of Natural Sciences of Philadelphia, Philadelphia, Pennsylvania 19103-1195, USA

## SUMMARY

Two species of the diatom genus *Nupela* are reported from freshwater lakes situated along the Atlantic Coastal Plain of North Carolina, USA. One of the species, *N. scissura* sp. nov., is described as new and the description of the other, *Nupela paludigena* (Scherer) Lange-Bertalot, is expanded to include a full suite of ultrastructural characters. *Nupela scissura* is a heterovalvar taxon with one valve having a fully developed raphe identical in structure to those found on other *Nupela* species. The other valve lacks a true raphe, but presents one to several elongated slits in the apical portion of the sternum. The true identity of the slits, whether they represent a highly reduced raphe, a rudimentary raphe, or neither is unknown. In addition, both valves of a single *N. scissura* frustule differ in the structure of the axial and central areas. The structure of the areolae and details of the raphe clearly place this species in *Nupela*. *Nupela paludigena* (Scherer) Lange-Bertalot was originally described in the genus *Anomoeoneis* from acidic and humic localities from the south-eastern part of the Atlantic Coastal Plain and later transferred to *Nupela*. Although this species has rarely been reported since its description, our findings indicate that it is a common component of the floras in the Carolina bays of North Carolina. The characteristics currently used to circumscribe taxa within the genus *Nupela* are discussed in light of published work and our own observations.

Key words: Bladen Lakes State Forest, Carolina bays, Croatan National Forest, diatoms, new species, North Carolina, *Nupela*, taxonomy.

Since the original description of the genus, 37 species have been either validly described or transferred into *Nupela* (Monnier *et al.* 2003; Potapova *et al.* 2003; Siver & Hamilton 2005). Because of the minute nature of the *Nupela* species, most being less than 20 µm in length, and the fact that light microscopy is generally insufficient to document an identification (Potapova *et al.* 2003), additional species will most likely be described in future surveys incorporating the use of scanning electron microscopy (SEM). Although many *Nupela* species have been described from tropical areas, records also now exist for temperate, subpolar and mountainous localities, as well as for most major continents, including North and South America, Europe, Asia and Africa (Monnier *et al.* 2003).

In the original description of the genus, Vyverman and Compère (1991) described *Nupela* as isovalvar and made note of the unique nature of the areolae. However, since the original description, many species have been described as being heterovalvar with respect to the degree of development of the raphe (e.g. Lange-Bertalot 1993; Metzeltin & Lange-Bertalot 1998; Rumrich *et al.* 2000; Potapova *et al.* 2003). On these heterovalvar taxa, one valve always has a fully formed raphe, while the opposing valve has a significantly reduced raphe. The structure of the areolae on all species examined with SEM appears to be an excellent and stable character of *Nupela* (Potapova *et al.* 2003; Siver & Hamilton 2005). The external opening of an areola is larger than the internal opening and covered with a hymen. The internal opening is smaller, due to a siliceous constriction, and not covered with a hymen. The taxonomic position of *Nupela* relative to the morphology-based classification of Round *et al.* (1990) remains questionable. Vyverman and Compère (1991)

## INTRODUCTION

The genus *Nupela* was established by Vyverman and Compère (1991), with *N. giluwensis* as the type species originally collected from Papua New Guinea.

\*To whom correspondence should be addressed.

Email: pasiv@conncoll.edu

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and Vyverman (1991) noted similarities between *Nupela* and both *Brachysira* Kützing and *Diadesmis* Kützing and suggested that *Nupela* be placed in the suborder Neidineae. Despite similarities to these other genera, Potapova *et al.* (2003) noted differences in the position of the hymen coverings in *Nupela* taxa relative to other genera in the Neidineae and questioned its placement within that suborder. The fact that numerous *Nupela* species are heterovalvar, especially in regards to raphe structure, constitutes another difference with respect to other genera in the Neidineae.

During our investigations of chrysophytes and diatoms in freshwater lakes and ponds along the eastern coast of North America, we observed two distinctive species belonging to *Nupela* in waterbodies situated along the Atlantic Coastal Plain of North Carolina, USA. One of these species, *Nupela paludigena* (Scherer) Lange-Bertalot, is a rarely reported taxon originally described by Scherer (1988) from acidic and dystrophic localities in the south-eastern United States. We found *N. paludigena* in relatively high abundances in six waterbodies enabling us to thoroughly examine its ultrastructure with SEM. The second species, observed in only one locality, presented a unique suite of characters within the genus, including what may be either a rudimentary raphe or the vestigial remains of such a structure. The objectives of this contribution are to expand the species description of *N. paludigena*, formally describe the second taxon as a new species, and to discuss the relative importance of the heterovalvar condition and areolae structure in regard to the definition of the genus.

## MATERIALS AND METHODS

Periphytic and surface sediment samples were collected from 27 freshwater waterbodies along the Atlantic Coastal Plain of North Carolina in May and June, 2001. Representative periphyton samples were collected from microhabitats around the perimeter of each lake and stored in whirl-pack bags. Surface sediments were taken from the center of each waterbody, usually from the deepest point, using a Glew gravity corer (Glew 1989) and sectioned on site with a mechanical extruder (Glew 1988). Water samples for chemical analyses were taken at a depth of 1 m from the center of each waterbody. The Secchi disk depth and geographic coordinates were taken with a 20 cm black and white disk and a Trimble Geoexplorer GPS unit, respectively. Specific conductance and temperature were measured with a Hydrolab DataSonde 4 A and pH with a Fisher Acromet meter. Chemical analyses followed the procedures of Ahrens and Siver (2000) or as modified by Lott and Siver (2005). Physical and chemical data for the waterbodies containing both *Nupela* taxa presented in this work are given below; however, data for all study

locations can be found online (<http://silicasecchidisk.conncoll.edu>).

For the purposes of this study, aliquots of all periphyton collections from a given waterbody were mixed into a combined sample. The surface sediment (0–1 cm) samples and combined periphyton samples were oxidized with a sulfuric acid-potassium dichromate solution according to Marsicano and Siver (1993), and an aliquot from each resulting slurry was air dried onto both aluminum foil and glass coverslips. The aluminum foil samples were used for observation with SEM according to the procedures presented by Siver (1987). Essentially, samples were attached onto an aluminum stub with Apiezon wax, coated with a gold and palladium mixture for one minute with a Polaron model E5100 sputter coater (Quorum Technologies, East Sussex, UK) and observed with a Leo-Zeiss 982-DSM SEM (Zeiss, Oberkochen, Germany). Glass coverslips were mounted onto glass slides with Naphrax mounting medium, observed with a Leica DMR light microscope equipped with differential interference contrast and reflected interference contrast optics (Siver & Hinsch 2000). Imaging was done using a Sony DKC-ST5 digital camera and photographic plates assembled with Adobe Photoshop v. 4.1 (Adobe Systems, San Jose, CA, USA). Specimens selected as holotypes and isotypes were circled with the aid of a diamond scribe. Slides and aliquots of slurries have been deposited into herbaria as described below. Morphometric ranges were established by measuring a minimum of 25 specimens. The terminology used in this paper follows Ross *et al.* (1979) and Krammer and Lange-Bertalot (1991).

## RESULTS

Carolina bays are shallow, elliptical basins with a northwest–southeast orientation scattered over the Atlantic Coastal Plain from Florida to New Jersey. The highest concentration of Carolina bays is found in North Carolina. The majority of Carolina bays examined as part of this project were largely acidic, humic stained, poorly buffered, dilute waterbodies often with low light penetration. Although numerous diatom genera are represented in this suite of waterbodies, the flora is largely dominated by species within the genera *Eunotia* and *Frustulia*, with lesser contributions from *Pinnularia*, *Aulacoseira*, *Neidium*, *Nupela*, *Brachysira* and *Stenopterobia*. Other genera, such as *Fragilaria*, *Synedra*, *Achnanthes*, *Navicula* and closely related taxa, are poorly represented in these Carolina bays. A complete description of the flora will be reported in a subsequent monograph. Details of the ultrastructure of the new species of *Nupela* and *N. paludigena* are given below.

*Nupela scissura* Siver, Hamilton & Morales  
sp. nov.

*Description*

Valvae late ellipticae-lanceolatae cum apiculatus apicibus. Longitudo 9–15  $\mu\text{m}$ , latitudo 4.5–5  $\mu\text{m}$ . Valvae heteromorphae; una valva cum completa raphe, opposita valva raphe destituta, cum 1–4 elongatis scissuris in distali axiali area. Striae 48–58/10  $\mu\text{m}$ , leviter radiatae ad parallelas in rapho-valva, plus radiatae in valva sine raphe. Axialis area rapho-valvae linearis ad lanceolatam, expandens in secundam centralem aream; centralis fascia rectangularis ad leviter stellariformem. Valva cum pseudoraphis scissuris, axialis area angusta prope apices, expandens in late lanceolatam aream centralem; centralis area cum secunda stellariformi fascia. Fascia in valva cum pseudoraphis scissuris latis, bis latitudo fasciae in rapho-valva. Raphe linearis, recta, distalis raphis fissurae deflectunt in eadem directione deorsum in limbo. Proximalis raphis fissurae rectae, desinentes in parvos lacrimiformes poros. Interne, distalis raphis fissura parva helictoglossa; proximalis raphis fissura recta, non-deflexa. Scissurae in valva sine raphe 1–4, dispositae in axiali area ad apicem. Extra, aerolae ellipticae ad rectangularem, dispositae cum transapicali valvae axe, tectae cum velo. Interne, aerolae parvae diametro, circulares ad ellipticas et destituta tegentes. Aerolae plus minusve contractae ex latis externis orificiis ad parvis internis orificiis. Limbus cum singularibus seriebus elongatis areolis circumscribens valvam includens apices. Silicea porca secus valvae marginem adest, saepe plus notata in valva sine raphe.

*Holotypus*

Circled specimens on microscope slide marked 'C', deposited at the California Academy of Sciences, San Francisco, California, USA (CAS # 222032). Four specimens within the scribed circle are illustrated in Figure 1.

*Isotypi*

Circled specimens on microscope slide marked 'A' deposited at Canadian Museum of Nature, Ottawa, Canada (CANA 79748), slide marked 'B' deposited at the Academy of Natural Sciences of Philadelphia, Pennsylvania, USA (ANSP G.C. 58610) and slide marked 'E' in P.A. Siver's personal collection.

*Type material*

Cleaned surface sediment material deposited at CAS (625096) and the Canadian Museum of Nature, Ottawa, Canada (CANA # 79748). Collector: P.A. Siver, June, 2001.

Type locality: Bay Tree Lake, Bladen County, North Carolina, USA, N 34°40' 59.36"-W 78°24' 58.77".

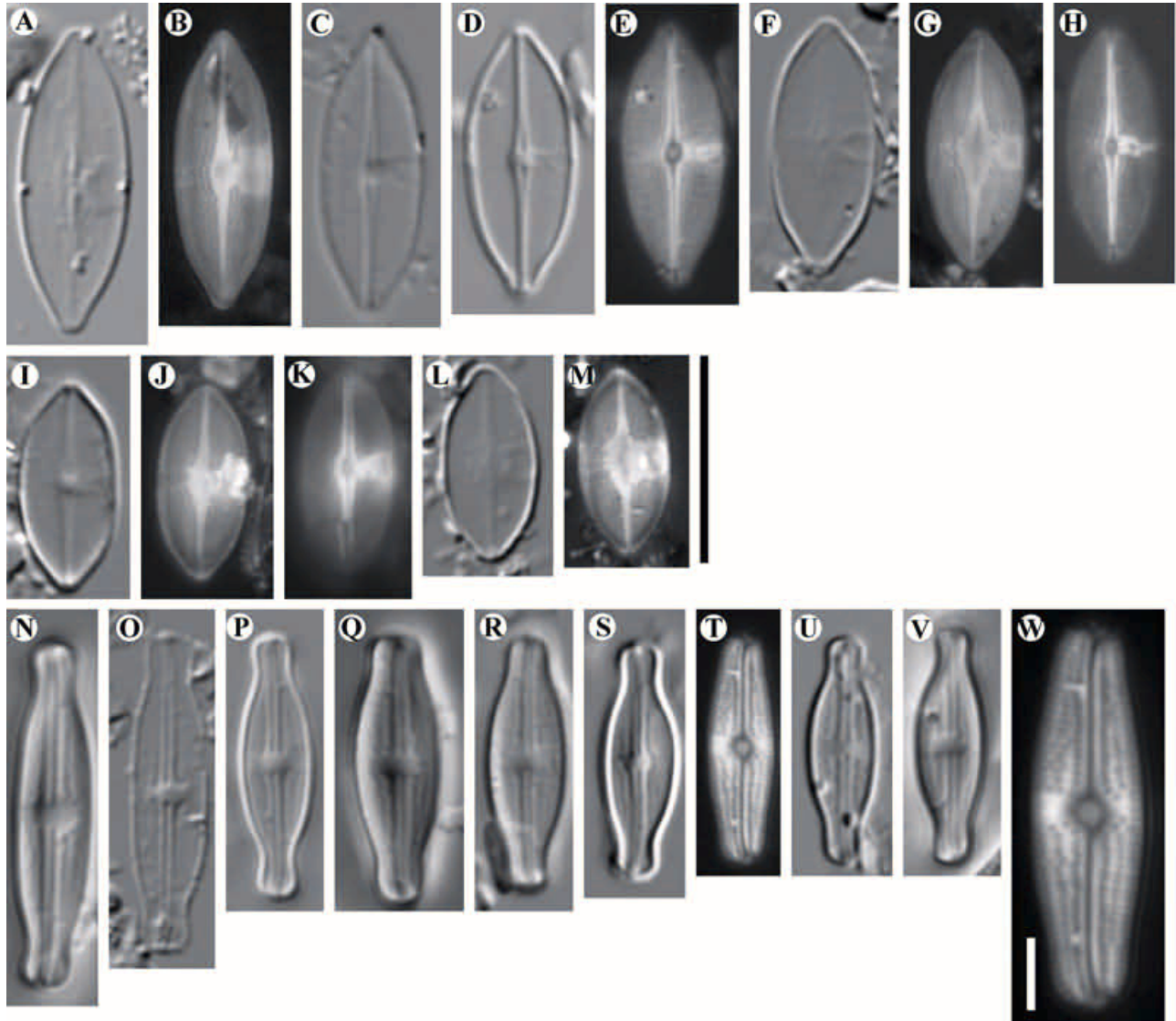
*Etymology*

The specific epithet refers to the slits found in the axial area.

*Description*

Valves are broadly elliptical-lanceolate with apiculate apices and range in size from 9 to 15  $\times$  4.5–5.0  $\mu\text{m}$  (Figs 1A–M and 2A–F). Valves of a frustule are heteromorphic with respect to raphe development and the nature of the central and axial areas. One valve possesses a fully formed raphe (Figs 1A,C–E,H,K and 2A–C) and the other lacks a raphe (Figs 1B,F,G,I,K–M and 2D–F), but has one to four elongated slits positioned in the distal part of the axial area (Fig. 3E–H). Striae are slightly radiate to parallel on the raphe-bearing valve, more radiate on the opposite valve and range from 48 to 58 in 10  $\mu\text{m}$ . On the raphe-bearing valve, the axial area is linear to lanceolate and expands into a one-sided fascia that is rectangular to slightly bow-tie shaped (Figs 2A–C and 3A,B). On the valve bearing the raphe-like slits, the axial area is narrow near the apices, but expands into a broadly lanceolate central area with an equally broad one-sided bow-tie shaped fascia (Figs 2E,F and 3C). The fascia on this same valve is typically twice as wide as the one on the raphe-bearing valve. The raphe is linear, straight and has distal raphe fissures that deflect in the same direction down onto the mantle (Fig. 2B). The proximal raphe fissures are straight and end as small tear-drop shaped pores (Fig. 3A). Internally, the distal raphe ends terminate as small helictoglossa and the proximal raphe ends are straight and do not deflect in any direction (Fig. 2A). The valve opposite to the raphe-bearing one has one to four elongated slits aligned in a row within the distal end of the apical area (Fig. 3E–H). Externally, the areolae are elliptical to rectangular, aligned with the transapical valve axis, and covered with a hymen (Fig. 2D). Internally, the areolae are smaller in diameter, circular to elliptical and lack coverings. As a result of the different size openings, the sides of the areolae more or less taper from the wider external opening to the smaller internal openings situated in the bottom of the structure. The mantle consists of a single row of elongated areolae that also encircles the apices (Fig. 3E–H). A siliceous ridge is present along the valve margin that is often more pronounced on the rapheless valve (Fig. 2D). It should be noted that the slits on *N. scissura* specimens can be difficult to observe at low magnification on the external surface of valves with intact hymen coverings (e.g. Fig. 2D).

The type locality, Bay Tree Lake, is an acidic, oligotrophic to mesotrophic Carolina bay low in dissolved salts and with a slight humic color situated in the Bladen Lakes State Forest region of North Carolina (Table 1).



**Fig. 1.** Light micrographs of *Nupela scissura* sp. nov. (A–M) and *N. paludigena* (N–W). Raphe-bearing valves of *N. scissura* are depicted in A, C–E, H and K. Valves of *N. scissura* lacking raphes are illustrated in B, F, G, I and K–M. Four holotype specimens found within the scribed circle on the holotype slide (CAS # 222032) are included here: (D,E) raphe-bearing valve; (C) raphe-bearing valve; (J,K) both valves of a frustule and; (F,G) valve lacking a raphe. Valves in (A) and (H) represent isotype specimens circled on CANA # 79748 and P.A. Siver's personal collection (Slide E), respectively. The specimen of *N. paludigena* depicted in (T) and magnified in (W) is within the scribed circle on Siver's isotype slide of *N. scissura*. Images B, E, G, H, J, K, M, T & W were taken with reflected interference contrast; all others were taken with differential interference contrast (DIC) optics. Scale bar, 10  $\mu\text{m}$ . Scale bar on (W), 2  $\mu\text{m}$ .

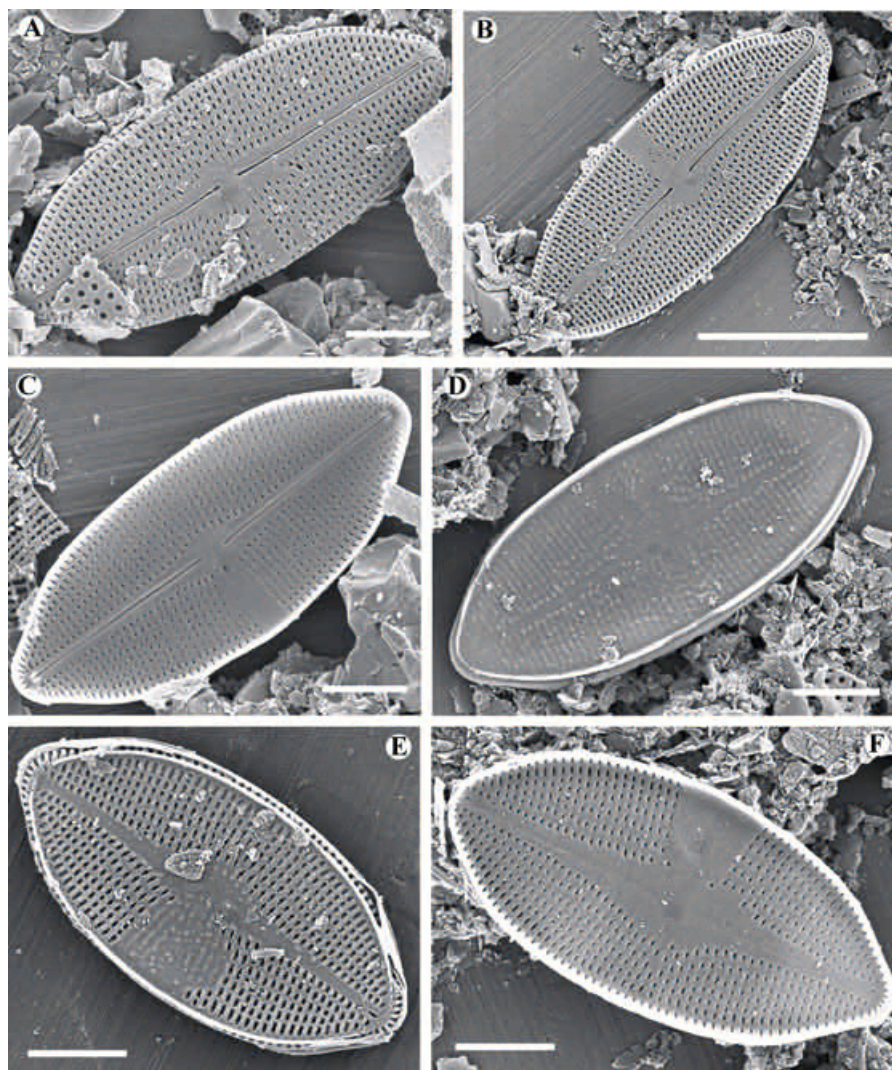
### *Nupela paludigena* (Scherer) Lange-Bertalot

This species was originally described by Scherer (1988) as *Anomoeoneis paludigena* from acidic and humic-stained localities in the southeastern portion of the Atlantic Coastal Plain and later transferred to *Nupela* by Lange-Bertalot (Lange-Bertalot 1993). Even though the original paper included several SEM images, our observations based on the examination of six populations with SEM yielded additional ultrastructural details that

were not included in the protologue of this species. We offer the following expanded description:

#### *Description*

Valves are elliptical-lanceolate to linear-lanceolate, rarely slightly dorsiventral, with rostrate to capitate apices, 10–20  $\times$  3–5  $\mu\text{m}$  in size (Figs 1N–W and 4A–D). Striae are slightly radiate to parallel in the center, becoming parallel towards the apices and range from 38 to 45 in 10  $\mu\text{m}$ . The axial area is narrow and



**Fig. 2.** Scanning electron microscopy (SEM) micrographs of *Nupela scissura* sp. nov. (A,B) External views of raphe-bearing valves depicting the arrangement of striae, the narrow one-sided fascia, details of the raphe and the single row of elongated mantle areolae. Scale bars, 2 & 5  $\mu\text{m}$ , respectively. (C) Internal view of a whole raphe-bearing valve. Note the linear raphe and small helictoglossa. Scale bar, 2  $\mu\text{m}$ . (D,E) External views of whole valves lacking a fully developed raphe. Note the broadly expanded one-sided fascia and prominent marginal ridge. The areolae on the valve in (D) are with hymen coverings. Scale bars, 2  $\mu\text{m}$  (F) Internal view of a valve with slits depicting the axial area widening into a broad central area with an equally broad one-sided fascia. Note the single slit at each apex. Scale bar, 2  $\mu\text{m}$ .

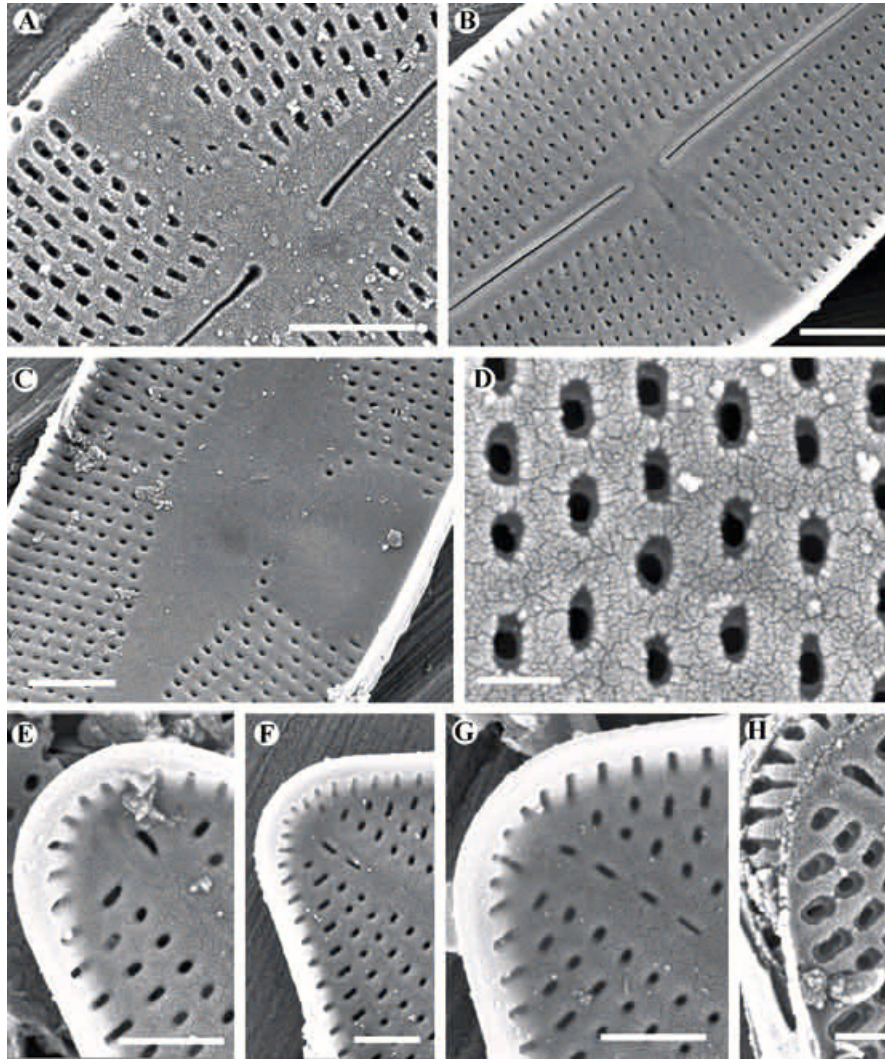
**Table 1.** Physical and chemical characteristics of ponds and lakes along the Atlantic Coastal Plain of North Carolina, USA with populations of *Nupela scissura* (Baytree) and *N. paludigena* (all waterbodies listed)

Lake	Latitude	Longitude	SD depth (m)	TP ( $\mu\text{g/L}$ )	Spec Cond ( $\mu\text{S/cm}$ )	pH	Color (Pt-Co)
Bay Tree	N 34°40' 59.36'	W 78°24' 58.77"	0.99	13	68	4.6	23
Catfish	N 34°55' 58.95'	W 77°05' 16.98"	0.35	22	75	4	250
Jessup	N 34°51' 46.30'	W 78°43' 46.74"	0.56	10	93	3.7	875
Jones	N 34°41' 02.21'	W 78°35' 50.51"	1.06	7	71	4	117
Long	N 34°53' 53.62'	W 76°59' 47.82"	0.31	32	85	3.9	192
Salters	N 34°41' 55.60'	W 78°37' 35.98"	0.77	16	62	4.1	125

SD, secchi disk; TP, total phosphorus.

slightly wider on the secondary side of the raphe, although this feature can best be discerned with SEM (Fig. 4A–D). The central area is variable, ranging from elliptical to rectangular in shape and most often unevenly spaced on each side of the valve (Fig. 4A–E). The raphe is slightly wavy with distal raphe fissures that run down onto the apical mantle and are deflected

towards the secondary side of the valve (Figs 4A–D, F and 5C). The proximal raphe fissures are straight and end as small teardrop-shaped pores (Fig. 5B). Internally, the distal raphe ends terminate in small helictoglossa and the proximal raphe ends are t-shaped and slightly deflected towards the secondary side of the valve (Fig. 5D, E). The areolae are elliptical to rectan-



**Fig. 3.** Scanning electron microscopy (SEM) micrographs of *Nupela scissura* sp. nov. (A,B) External (A) and internal (B) views of the center of the valve. Note the large external openings *versus* the smaller internal openings, details of the fascia and proximal raphe ends. Scale bars, 1 µm (C) Internal view of the center of a valve with axial slits showing the central area and one-sided fascia. Scale bar, 1 µm (D) Close-up of the external surface depicting the nature of the areolae. Scale bar, 200 nm. (E–G) Close-up views of the internal apical region of a valve with axial slits depicting one, two and four elongated slits, respectively. Scale bars, 500 nm (H) Close-up of the external apical region of a valve with one axial slit. Scale bar, 250 nm.

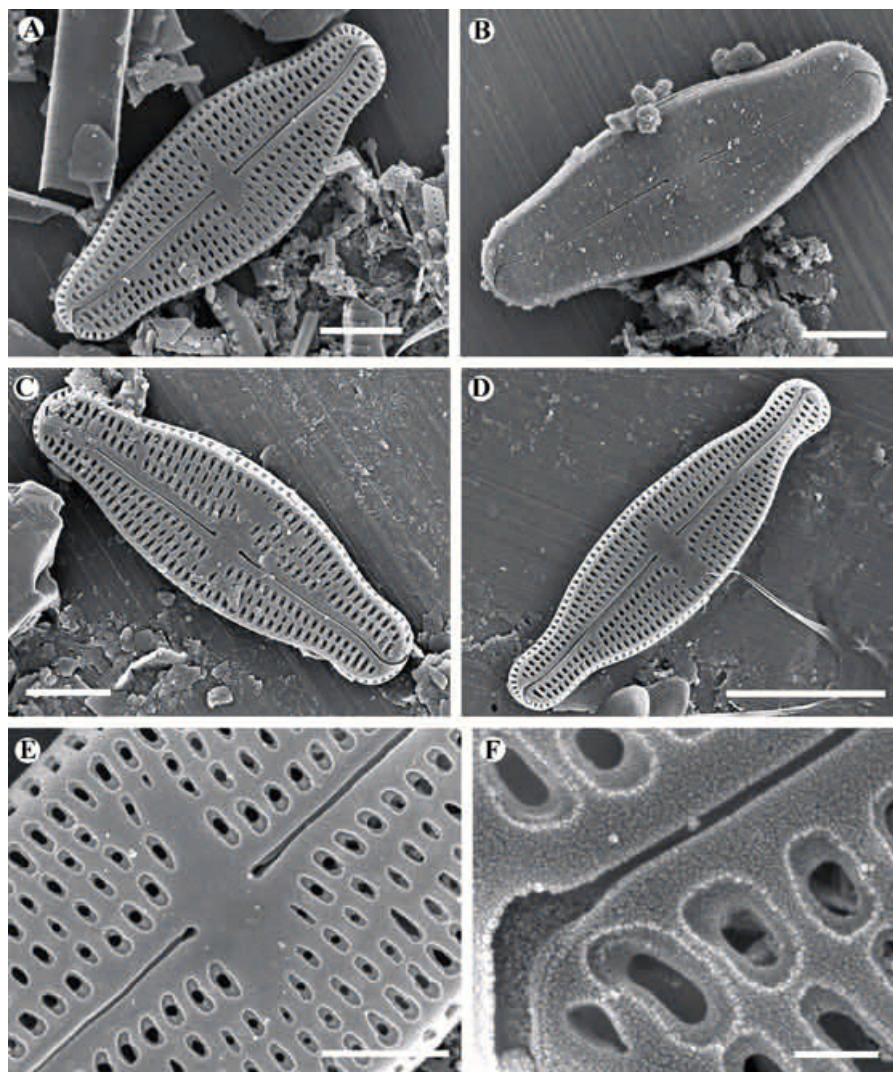
gular, expanded along the transapical valve axis (Fig. 4A–E), and have a wider opening on the external surface than the internal surface (Fig. 4E,F). Undulating or wavy longitudinal hyaline regions can often be discerned between the areolae, especially with reflected interference contrast optics (e.g. Fig. 1W). As a result of the different size openings, the sides of the areolae more or less taper from the wider external opening to the smaller internal openings situated in the bottom of the structure (Fig. 4F). The areolae are covered externally with a hymen that is flush with the valve surface (Fig. 5A–C), but are not covered on the internal surface of the valve (Fig. 5D–F). The mantle consists of a single row of elongated areolae (Fig. 4). Voigt faults are evident as slight alterations in the striae pattern (Figs 4A,C,D and 5D).

We found populations of *N. paludigena* in six Carolina bays situated in either the Bladen Lakes region or the Croatan National Forest along the Atlantic Coastal Plain in North Carolina (Table 1). The lakes harboring *N. paludigena* are very acidic, poorly buffered waterbodies low

in dissolved salts and most are significantly stained with humic acids (Table 1). The range in pH of the waterbodies with this taxon was only 3.7–4.6. As a result of the darkly stained water, the Secchi disk depths for the majority of waterbodies harboring this taxon are very low ranging from only 0.31–1.06 m. Sodium and chloride are the most dominant cation and anion, respectively, in all of the Carolina bays harboring this taxon.

## DISCUSSION

The suite of characters used to circumscribe *N. scissura* is unique and distinctively different from all other known species. The broad elliptical shape of the valve differs from the majority of *Nupela* species with the possible exceptions of *N. chilensis* (Krasske) Lange-Bertalot and *N. vyvermanii* Lange-Bertalot, but these latter two taxa are quite different from *N. scissura* in regards to raphe development and the shapes of the axial and central areas (Lange-Bertalot & Moser 1994; Moser *et al.* 1998). As noted below, there are heterov-



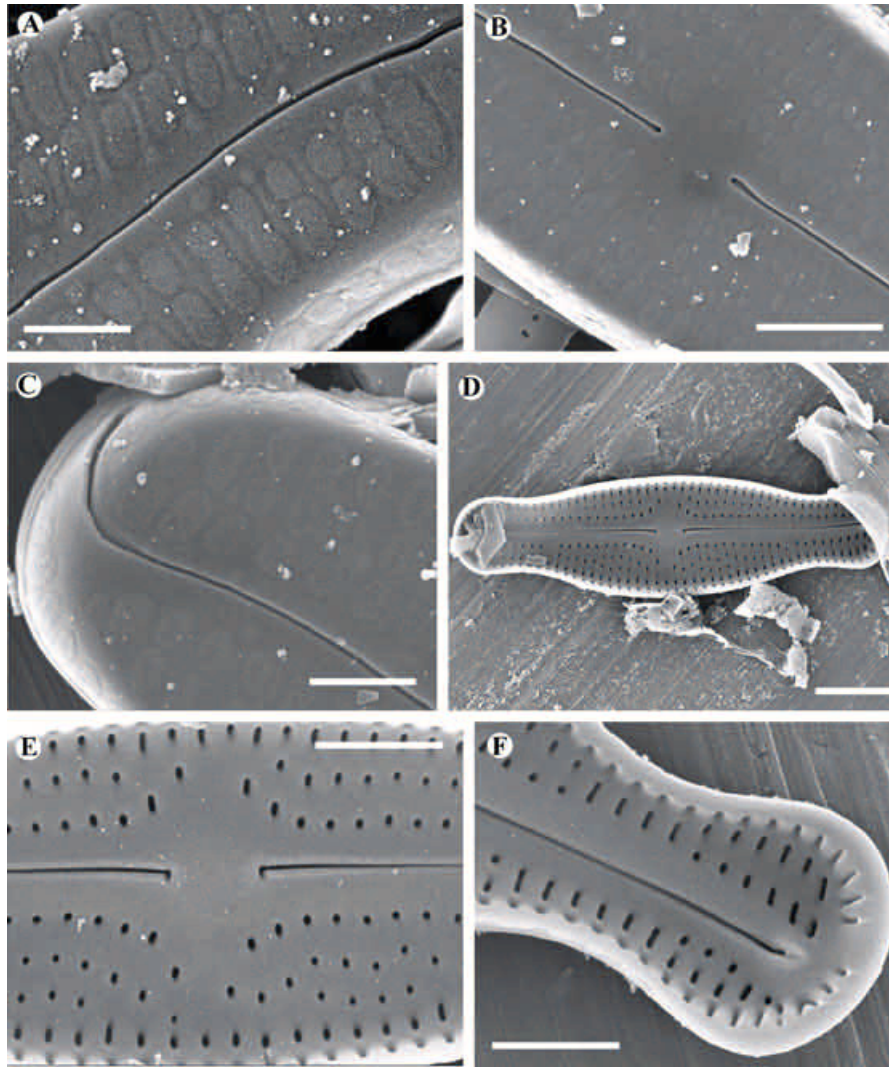
**Fig. 4.** Scanning electron microscopy (SEM) micrographs of *Nupela paludigena*. (A–D) External views of whole valves depicting specimens with rostrate to subcapitate apices. Note details of the striae, raphe and the single row of elongate mantle areolae. The external hymen coverings are still intact on the specimen in B. Scale bars, 2  $\mu\text{m}$  for A–C and 5  $\mu\text{m}$  for D. (E) External view of the center of the valve showing proximal raphe fissures, the central area and details of the areolae. Scale bar, 1  $\mu\text{m}$  (F) Close-up of the distal raphe fissure and areolae. Scale bar, 200 nm.

alvar species within the genus where one valve has a reduced raphe or where the raphe is essentially lacking altogether. The presence of one to several slits on the apical part of the axial area, as observed for *N. scissura*, has not been previously reported for *Nupela*. Finally, the heterovalvar nature of the one-sided fascia and the greater development of the marginal ridge on the raphelless valve are also unique characters for *N. scissura*.

The valve morphology of our *N. paludigena* specimens clearly match those in the original description of *Anomoeoneis paludigena* in all respects except that ours have a slightly higher striae density than that reported by Scherer (1988). However, the striae density measured on the SEM image presented by Scherer (Fig. 72, 1988) and one by Camburn *et al.* (1984–86), indicated by Scherer (1988) as matching his taxon, are both within the range of our specimens. *Nupela paludigena* is very similar to *N. giluwensis* Vyverman & Compère, the type species for the genus, and according to Vyverman (1991) both taxa can be distinguished

based on slight differences in striae density, valve outline and the shape of the central area. Based on our findings, there is no difference in striae density and there is certainly overlap in the shape of the central area, leaving the distinction between these two taxa less obvious and raising the possibility that they are conspecific. *Nupela subinuicta* (Krasske) Lange-Bertalot, originally described by Krasske from Chile, is similar to *N. paludigena* but has much more capitate apices and a broader, more symmetrical central area. It is of interest that Potapova *et al.* (2003) did not report *N. paludigena* in a study of the genus from rivers and streams situated in the same geographic region, which perhaps indicates that *N. paludigena* has an ecologic preference for lentic water systems.

In the original description of *Nupela* (Vyverman & Compère 1991), with *N. giluwensis* as the type species, the genus was noted to be isovalvar with both valves bearing an equal and fully formed raphe. Vyverman (1991) described a second isovalvar species, *Nupela*



**Fig. 5.** Scanning electron microscopy (SEM) micrographs of *Nupela paludigena*. (A–C) Close-up views of the external surface depicting the nature of the external hymen coverings, proximal (B) and distal (C) raphe fissures. Scale bars = 500 nm (A, C) or 1  $\mu$ m (B). (D) Internal view of a whole valve denoting the slightly deflected proximal raphe fissures and the longitudinal arrangement of the areolae. Scale bar, 2  $\mu$ m. (E,F) Close-up views of the internal surface showing the central (E) and apical (F) regions. Note the decreased diameters of the areolae, the slightly deflected proximal raphe fissures and the helictoglossa. Scale bars, 1  $\mu$ m.

*hagensis*, and additional isovalvar taxa, including *N. neotropica* Lange-Bertalot (Lange-Bertalot & Moser 1994; Siver *et al.* 2005) and *N. vitiosa* (Siver & Hamilton 2005), have been added since the original description of the genus. However, the majority of newly described *Nupela* species have been depicted as heterovalvar, not isovalvar (e.g. Lange-Bertalot 1993; Metzeltin & Lange-Bertalot 1998; Rumrich *et al.* 2000; Potapova *et al.* 2003). In all of the heterovalvar taxa, one valve always possesses a fully formed raphe. The second valve may have a shorter, partially formed raphe restricted towards the distal end of the valve (e.g. *N. tenuistriata* (Hustedt) Metzeltin & Lange-Bertalot, *N. lesothensis* (Schoeman) Lange-Bertalot and *N. neglecta* Ponader, Lowe & Potapova), or apparently lack a raphe altogether (*N. praecipua* (Reichardt) Reichardt and at least some specimens of *N. rumrichorum* Lange-Bertalot (see Plates 50, Figs 5,6 of Lange-Bertalot & Moser 1994)). Internally, the distal end of the raphe terminates as a small helictoglossa in *Nupela* taxa, even

on species with a reduced raphe. Similarly, the external distal raphe fissures on the reduced raphe, such as that of *N. jahniae-reginae* Lange-Bertalot & Metzeltin, bend strongly to the same side of the valve as they do on valves having a full raphe system on both valves (Rumrich *et al.* 2000).

All aspects of the raphe on the first valve of *Nupela scissura* clearly match those of the genus, including proximal and distal raphe fissures, helictoglossa structure and position within the axial area. Whether *N. scissura* has a rudimentary raphe on the second valve, a vestigial raphe structure or some other feature altogether is unclear. What is apparent is that each apex of the second valve has at least one, sometimes two to four, elongated slits situated within the axial area and oriented with the apical axis. Although it can be hypothesized that the slits are modified rimoportula, we found no thickened rims to suggest such a connection to this structure. The vast majority of valves had one slit per apex. A number of valves had what may be viewed as a



short depression situated where the raphe would be located and a few valves had multiple slits aligned in a row. We found no valves lacking slits altogether. We believe *N. scissura* may be closer than *N. rumrichorum* to a species bearing a truly rapheless valve since it does not have a distinct helictoglossa on the internal surface as does *N. rumrichorum*.

Interestingly, in addition to heterovalvar raphe structures within the genus *Nupela*, there can also be heterovalvy with respect to the central area in some species. Differences in the central area between valves are especially notable, and perhaps restricted to species that are heterovalvar with respect to raphe structure. For example, the central area on the raphe valve of *N. praecipua* is small and elliptical, but it is essentially lacking on the second valve. It is also not uncommon for species of *Nupela* to have asymmetrical central areas and to form one-sided fascia extending to the valve margin. Examples of species with such an asymmetrical central area include *N. vitiosa* (Siver & Hamilton 2005), *N. neotropica* (Lange-Bertalot & Moser 1994) and *Nupela tenuicephala* (Hustedt) Lange-Bertalot. All three of these taxa with one-sided fascia are not heterovalvar. In the case of *N. scissura*, these two characters, heterovalvy with respect to central area and one-sided fascia are combined. Both valves of *N. scissura* have one-sided fascia, but the fascia on the raphe valve is much smaller relative to the one on the opposite valve.

The degree of variability in raphe structure between valves on a frustule in the genus *Nupela* could possibly be used to split the genus. That is, the isovalvar versus heterovalvar character is commonly recognized by many authors as unique at the genus level in diatom taxonomy (Round *et al.* 1990). However, the significance of differential valve formation is not fully understood in terms of taxonomic affinities or even if they represent distinct but parallel morphological developmental processes across orders, families and even genera. In the case of *Nupela*, the differences in raphe structure observed on the second valve could conceivably be used to split the current genus into two, or even more, genera. In an earlier communication (Siver & Hamilton 2005) we noted the fact that *Nupela* included isovalvar and heterovalvar species and stated that this would typically be a justified reason to split the genus. However, after examination of many more *Nupela* specimens we currently believe that such a split based solely on the degree of development of the second raphe may be premature, especially since it seems to form a continuum from lacking altogether to being fully formed. Instead, we consider that the distinct and unique character of the areolae, and the fact that the nature of the fully developed raphe is similar in all species, are sufficient to maintain a single genus at this time. We propose that the follow-

ing characters be used to define *Nupela*. First, the areolae have a wider opening on the external surface of the valve that tapers to a much smaller opening on the internal surface. Second, the external opening tends to be elongated, rectangular in outline, aligned with the transverse axis and covered with a hymen that is flush with the valve surface. Third, the internal opening is generally much smaller, more rounded and lacks a covering. Externally, the distal raphe fissures are deflected in the same direction and towards the secondary side of the valve, while the proximal raphe fissures are normally straight and end as small pores. Internally, the proximal raphe fissures are often slightly bent in the same direction towards the secondary side of the valve, and the distal raphe fissures terminate as small helictoglossa. The mantle consists of a single row of elongated areolae.

In addition to the well-defined and consistent characters identified above, *Nupela* species tend to be small and many have unevenly aligned areolae that yield longitudinal hyaline zones on the valve face reminiscent of *Brachysira* taxa (Lange-Bertalot & Moser 1994). It is our experience that Voigt faults, indicating the secondary side of the valve, can be discerned on most, if not all, *Nupela* taxa and that the raphe is often positioned off centered within the axial area such that the secondary side is slightly larger. It is important to remember that if the outer hymen coverings are intact and the internal structure of the areolae can not be discerned with SEM, taxa within *Nupela* could be confused with genera like *Adlafia* and *Eolimna*. Nonetheless, the importance of the flush external areolae hymen coverings as a distinguishing feature of *Nupela* was also pointed out by Potapova *et al.* (2003). Within this concept of *Nupela*, the degree of development of the raphe on the second valve and the shape of the central area are variable characters.

It remains unclear if the genus *Nupela* is more closely related to taxa within the Achnanthes or Naviculales as defined by Round *et al.* (1990). Vyverman and Compère (1991) and Vyverman (1991) suggested an affinity with genera within the suborder Neidiineae of the Naviculales, but this placement was questioned by Potapova *et al.* (2003). Some *Nupela* species, such as *N. praecipua* and *N. scissura*, are essentially monoraphid species similar to taxa within the Achnanthes, but the majority of *Nupela* species clearly do not belong in this order. Kociolek and Stoermer (1986) suggested that the monoraphid condition evolved a number of times within different lineages of raphid diatoms, a concept that has been supported by recent studies (Medlin & Kaczmarska 2004; Sorhannus 2004). It seems likely that *Nupela* is following a similar evolutionary trend towards forming a monoraphid condition. Given the fact that ordinal-level classification of diatoms as proposed by Round *et al.* (1990) has been

largely unsupported by molecular work (Sorhannus 2004) it seems premature to speculate any further on the position of *Nupela* among raphid diatoms at this time. Future molecular studies that include *Nupela* species should help resolve the questions regarding the validity of the genus and its taxonomic affinity within the raphid diatoms.

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## REFERENCES

- Ahrens, T. D. and Siver, P. A. 2000. Trophic conditions and water chemistry of lakes on Cape Cod, Massachusetts, USA. *Lake Reservoir Manage.* **16**: 268–80.
- Camburn, K. E., Kingston, J. C. and Charles, D. F. 1984–86. *Paleoecological investigation of recent lake acidification. PIRLA diatom iconograph*. PIRLA unpublished report series, Report 3. 53. Plates. Contributors: Anderson, D. S., Camburn, K. E., Charles, D. F., Ford, J., Kingston, J. C., Sweets, P. R., Turner, F. R. and Whiting, M. C.
- Glew, J. R. 1988. A portable extruding device for close interval sectioning of unconsolidated core samples. *J. Paleolimnol.* **1**: 235–9.
- Glew, J. R. 1989. A new trigger mechanism for sediment samplers. *J. Paleolimnol.* **2**: 241–3.
- Kociolek, J. P. and Stoermer, E. F. 1986. Phylogenetic relationships and classification of monoraphid diatoms based on phonetic and cladistic methodologies. *Phycologia* **25**: 297–303.
- Krammer, K. and Lange-Bertalot, H. 1991. Bacillariophyceae, Teil 3, Centrales, Fragilariaceae, Eunotiaceae. In Ettl, H., Gerloff, J., Heynig, H., and D. Mollenhauer (Eds) *Süßwasserflora Von Mitteleuropa*, Bd. 2/3, 576 pp. Gustav Fischer Verlag, Stuttgart, pp. ●●–●●.
- Lange-Bertalot, H. 1993. 85 new taxa and much more than 100 taxonomic clarifications supplementary to Süßwasserflora von Mitteleuropa Vol. 2/1–4. *Biblioth. Diatomol.* **27**: 1–454.
- Lange-Bertalot, H. and Moser, G. 1994. *Brachysira* monographie der gattung und *Naviculadicta* nov. gen. *Biblioth. Diatomol.* **29**: 1–212.
- Lott, A. M. and Siver, P. A. 2005. An inventory of scaled chrysophytes from the Atlantic Coastal Plain of North Carolina, USA. In Kristiansen, J. and G. Cronberg (Eds) *Chrysophytes: Past and Present. Proceedings of the Sixth International Chrysophyte Symposium*, Lami, Finland. *Nova Hedwigia Beih.* **128** : 211–29.
- Marsicano, L. J. and Siver, P. A. 1993. A paleolimnological assessment of lake acidification in five Connecticut lakes. *J. Paleolimnol.* **9**: 209–21.
- Medlin, L. K. and Kaczmarska, I. 2004. Evolution of the diatoms. V. Morphological and cytological support for the major clades and a taxonomic revision. *Phycologia* **43**: 245–70.
- Metzeltin, D. and Lange-Bertalot, H. 1998. Tropical diatoms of South America. I. About 700 predominantly rarely known or new taxa representative of the neotropical flora. *Iconogr. Diatomol.* **5**: 1–695.
- Monnier, O., Lange-Bertalot, H. and Bertrand, J. 2003. *Nupela exotica* species nova: une diatomée d'un aquarium tropical d'eau douce. Avec des remarques sur la biogéographie du genre. *Diatom Res.* **18**: 273–91.
- Moser, G., Lange-Bertalot, H. Metzeltin, D. and A. Witkowski. 1998. Insel der Endemiten. Geobotanisches Phänomen Neukaledonien. *Bibliotheca Diatomologica* **38**: 1–464.
- Potapova, M. G., Ponader, K. C., Lowe, R. L., Clason, T. A. and Bahls, L. L. 2003. Small celled *Nupela* species from North America. *Diatom Res.* **18**: 293–306.
- Ross, R., Cox, E. J., Karayeva, N. I. *et al.* 1979. An amended terminology for the siliceous components of the diatom cell. *Nova Hedwigia Beih.* **64**: 513–33.
- Round, F. E., Crawford, R. M. and Mann, D. G. 1990. *The Diatoms: Biology and Morphology of the Genera*. Cambridge University Press, Cambridge.
- Rumrich, U., Lange-Bertalot, H. and Rumrich, M. 2000. Diatomeen der Anden, von Venezuela bis Patagonien/Feuerland. *Iconogr. Diatomol.* **9**: 1–649.
- Scherer, R. P. 1988. Freshwater diatom assemblages and ecology/paleoecology of the Okefenokee swamp/marsh complex, Southern Georgia, U.S.A. *Diatom Res.* **3**: 129–57.
- Siver, P. A. 1987. The distribution and variation of *Synura* species (Chrysophyceae) in Connecticut, USA. *Nord. J. Bot.* **7**: 107–16.
- Siver, P. A. and Hamilton, P. B. 2005. Observations on new and rare species of freshwater diatoms from Cape Cod, Massachusetts, USA. *Can. J. Bot.* **83**: 362–78.
- Siver, P. A., Hamilton, P. B., Stachura-Suchoples, K. and Kociolek, J. P. 2005. Diatoms of North America: the Freshwater Flora of Cape Cod. *Iconogr. Diatomol.* **14**: 1–463.
- Siver, P. A. and Hinsch, J. 2000. The use of interference reflection contrast in the examination of diatom valves. *J. Phycol.* **36**: 616–20.
- Sorhannus, U. 2004. Diatom phylogenetics inferred based on direct optimization of nuclear-encoded SSU rRNA sequences. *Cladistics* **20**: 487–97.
- Vyverman, W. 1991. Diatoms from Papua New Guinea. *Biblioth. Diatomol.* **22**: 1–223.
- Vyverman, W. and Compère, P. 1991. *Nupela giluwensis* General & spec. nov. A new genus of naviculoid diatoms. *Diatom Res.* **6**: 175–9.

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