

**A reevaluation of *Aulacoseira nygaardii* (Camburn)
Camburn & D.F. Charles (Bacillariophyceae):
based on scanning electron microscopy of type material**

by

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With 16 figures

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Abstract: *Aulacoseira nygaardii* was originally described by Camburn (as *Melosira nygaardii* in Camburn & Kingston 1986) based on light microscopy. Because the fine structure of this taxon can be difficult to resolve with light microscopy, positive identifications can also be difficult. We present an emended description of this taxon based on an evaluation of type material with scanning electron microscopy (SEM). Although SEM micrographs reported as *A. nygaardii* were presented by Scherer (1988), our observations indicate that the specimens examined by him possess several characters that differ from those in the type material and may represent a different organism.

Key words: *Aulacoseira*, *Aulacoseira nygaardii*, diatom morphology, *Melosira nygaardii*, *Melosira fennoscandica*, scanning electron microscopy, type material.

Introduction

Aulacoseira nygaardii (Camburn) Camburn & D.F. Charles 2000 was originally described by Camburn as *Melosira nygaardii* in surface sediments from Sand Lake, Alger County, Michigan, U.S.A. (Camburn & Kingston 1986). This species was

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distinguished from other closely related taxa based, in part, on its small size, finely punctuated and closely spaced striae, a peripheral ring of short striae on the valve face and marginal spines located every 2-3 striae (Camburn & Kingston 1986). According to Camburn, *Aulacoseira nygaardii* most closely resembles *Melosira laevis* (Ehrenberg) Ralfs, of which he presented a detailed history. Camburn & Kingston (1986) further noted that *A. nygaardii* was widespread in the United States with records from Michigan, Wisconsin, Minnesota, New York (Adirondack Mountains), New Hampshire, Florida and Georgia. The original description of *A. nygaardii* was based solely on light microscopy.

A number of populations of an organism were found in glacial kettle ponds on Cape Cod, Massachusetts, U.S.A. (Siver et al. 2004) that we suspected were *A. nygaardii*, but had difficulty making a comparison with the original light micrographs presented by Camburn & Kingston (1986). In order to verify this identification, we examined with scanning electron microscopy (SEM) material from the type locality, Sand Lake, originally deposited by John Kingston at the Canadian Museum of Nature (CANA 32605), Ottawa, Canada. The holotype slide for *M. nygaardii* is deposited at the Academy of Natural Sciences of Philadelphia (ANSP A.-G.C. 54240). Subsequent to the examination of the Sand Lake sediment material, we determined that the Cape Cod organism was indeed *A. nygaardii*. The purpose of this paper is to present an emended description of *A. nygaardii* based on SEM analysis of type material from Sand Lake.

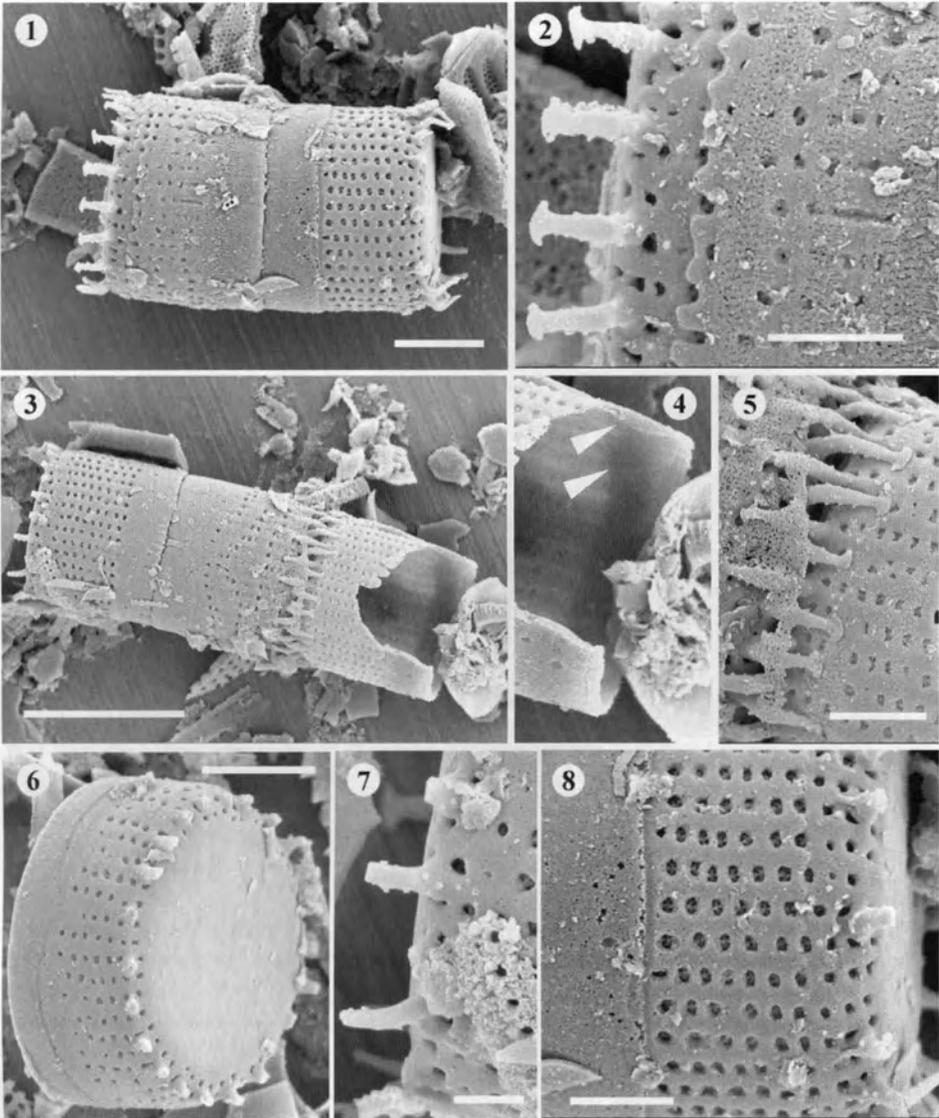
Methods

Type material from Sand Lake deposited by John Kingston at the Canadian Museum of Nature (CANA 32605) was used in this study. The material is labeled as being from the 0-1 cm layer of the surface sediments. Approximately 0.2 g of the surface sediment sample was oxidized with a mixture of sulfuric acid and potassium dichromate and cleaned according to Marsicano & Siver (1993). Aliquots of the resulting clean slurry were air dried onto pieces of heavy duty aluminum foil and glass coverslips. The aluminum foil samples were trimmed, attached to aluminum stubs with Apiezon® wax, coated with a mixture of gold and palladium for 1 min with a Polaron Model E sputter coater and observed with a Leo 982 field emission SEM. The coverslips were mounted onto glass slides with Naphrax® and specimens observed with a Leica DMR.

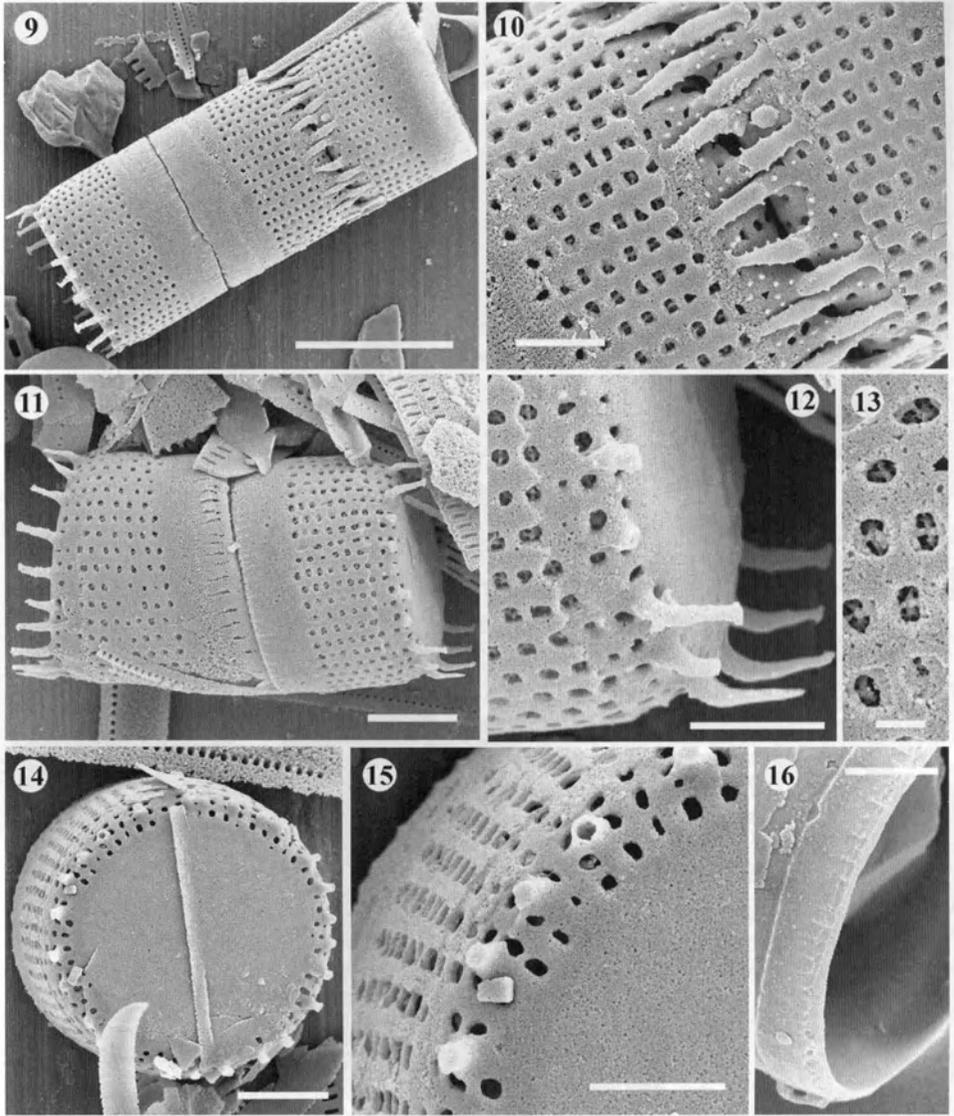
Results

Aulacoseira nygaardii (Camburn) Camburn & D.F. Charles 2000

The following emended description is based on the original description given by Camburn (in Camburn & Kingston 1986) and our LM and SEM observations of type material. Frustules are square to rectangular in girdle view with a rounded valve face/mantle junction and a mantle height to valve diameter ratio slightly less than, or close to, 1 (Figs 1-16). A step-like demarcation is present approximately ¼ of the distance down on the mantle from the valve face (Figs 1-2, 8-10, 12). Spines from the adjacent cell abut along the demarcation in such a way that the spines are more or less flush with the lower portion of the mantle (Fig. 10). The valve face is



Figs 1-8. SEM micrographs of *Aulacoseira nygaardii* from type material CANA 32605, Sand Lake, Michigan, U.S.A. Fig. 1. Whole frustule showing the structure of the striae, collum and spines. Scale bar = 2 μm. Fig. 2. Close up of the specimen in Fig. 1. Note the anchor-shaped spines and the secondary layer of silica on the mantle. Scale bar = 1 μm. Fig. 3. Whole frustule depicting spine arrangement and the structures of the striae and collum. Scale bar = 5 μm. Fig. 4. Close up of the specimen in Fig. 3 showing the shallow Ringleist (arrowheads). Fig. 5. Close up depicting the interlocking and spacing of spines. Note the anchor-shaped spine apices. Scale bar = 1 μm. Fig. 6. View of the valve face. Note the short marginal striae consisting of only 1-2 areolae. Scale bar = 2 μm. Fig. 7. External view showing the spacing of the spines. Scale bar = 0.5 μm. Fig. 8. Close up view of the mantle. Note the recessed rotulae of the areolae. Scale bar = 1 μm.



Figs 9-16. SEM micrographs of *Aulacoseira nygaardii* from type material CANA 32605, Sand Lake, Michigan, U.S.A. Fig. 9. Three valves depicting the parallel striae, collum and arrangement of spines. Scale bar = 5 μm . Fig. 10. Close up of the specimen in Fig. 9 showing the interconnecting spines and step-like demarcation on each valve. Note that the anchor-shaped tips of the spines are flush with the step. Sometimes a random distribution of papillae can be observed on the spines or along the step. Scale bar = 1 μm . Fig. 11. Whole frustule showing details of the mantle and spines. Scale bar = 2 μm . Fig. 12. Close up of the specimen in Fig. 11. Note the distinct step-like demarcation and smooth valve face. Scale bar = 1 μm . Fig. 13. Close up of the specimen in Fig. 11 depicting the cross-shaped recessed rotulae of the areolae. Scale bar = 200 nm. Fig. 14. Valve view of a specimen depicting the short marginal striae and smooth valve face. Scale bar = 2 μm . Fig. 15. Close up of the specimen in Fig. 14. Note the continuation of the mantle striae onto the otherwise smooth valve face. Scale bar = 1 μm . Fig. 16. Internal view of the shallow Ringleist. Scale bar = 1 μm .

smooth except for short, radially aligned striae along the periphery that consist usually of only one, or two, areolae (Figs 6, 14-15). These marginal areolae/striae are continuous with the striae on the mantle (Fig. 15). The striae on the mantle are parallel to the perivalvar axis, closely spaced, and consist of small circular to rectangular areolae (Figs 1, 3, 8-9). The collum is well defined, unornamented and approximately 1/3 of the mantle height (Figs 1, 9). The Ringleist is very shallow, consisting of only a slight swelling of the valve (Figs 4 [arrowheads], 16). A row of spines is present at the junction of the valve face and mantle. Spines are short, 0.7-1.5 μm long, of equal length, somewhat flattened, tapered, and with anchor-shaped tips (Figs 2, 5, 10, 12). Spines may also be ornamented with small papillae or serrations (Fig. 10). Spines are positioned between every 2-3 (rarely 4) mantle striae (Figs 1-5, 10-12). Areolae have vela consisting of recessed rotae (Fig. 8). The rotae, attached down into and near the base of the areola, usually consist of two siliceous bars that most often form a cross (Figs 8, 10, 13). A thin siliceous layer covers the areola on the inner mantle wall. Pervalvar length: (4) 7-17 (19) μm ; Valve diameter: (5.0) 6.9-11.0 μm ; Striae density (on both the mantle and valve face): 20-30/10 μm ; Areolae density: 25-48.

Discussion

Although other species of *Aulacoseira* were present in the Sand Lake sample, including *A. ambigua* (Grunow) Simonsen, *A. lacustris* (Grunow) Krammer, *A. lirata* (Ehrenberg) Ross in Hartley and *A. perglabra* (Østrup) Haworth, they could not be confused with *A. nygaardii*. Even though the specimens of *A. nygaardii* that we observed in Sand Lake matched the original description by Camburn (Camburn & Kingston 1986), we did note several differences. Firstly, our Sand Lake specimens had higher striae densities than the 20-22 in 10 μm reported by Camburn & Kingston (1986). Although we retained the lower striae density in the emended description presented above because Camburn & Kingston (1986) observed populations from many localities, the majority of specimens in Sand Lake had ca. 30 striae in 10 μm . It is also worth noting that striae densities of the specimens presented in the original description (e.g. figs 48 and 52 in Camburn & Kingston 1986) appear to be greater than the range of 20-22 in 10 μm . Secondly, the density of areolae within striae was also higher in Sand Lake specimens (38-48 in 10 μm) than the range of 25-30 in 10 μm presented in the original description. We were not able to resolve areolae with LM on most Sand Lake specimens, which is consistent with the higher density range we established with SEM. In addition, we are not able to resolve areolae on the specimens illustrated by Camburn in the original description. Since Camburn & Kingston (1986) did not examine material with SEM, they would not have been able to determine this upper range in areolae density since it is beyond the limit of resolution with LM. On all specimens showing the valve face, it was clear that the short marginal valve face striae were continuous with those on the mantle which would yield a similar density at the valve periphery as found on the mantle. On some valves striae were not easily resolved with LM, perhaps due to the remnants of girdle bands. Our specimens of *A. nygaardii* from Cape Cod also had higher striae

and areolae densities than those originally reported in Camburn & Kingston (1986) and closer to those that we observed for the population in Sand Lake. In all other respects, the Cape Cod specimens matched those observed from Sand Lake.

Several authors, most notably Likhoshway & Crawford (2001), have indicated the usefulness of rimoportulae as a character for distinguishing between species in the genus *Aulacoseira*. Siver & Kling (1997) commented that the position of rimoportulae appeared to be a constant trait in many species, and the work of Likhoshway & Crawford (2001) significantly advances this concept and further discusses the potential evolutionary importance of this structure. For a given species, the characteristics of the rimoportulae are stable and do not vary with changes in environmental conditions (Likhoshway & Crawford 2001). The rimoportulae are always situated on the mantle in *Aulacoseira*, most often on or slightly above the Ringleist. Despite examination of numerous specimens of *A. nygaardii*, we were unable to find rimoportulae on the interior surfaces of broken valves, and the openings of these structures onto the exterior surface were not evident. Some species of *Aulacoseira*, including *A. crassipunctata* Krammer, are believed to lack rimoportulae (Likhoshway & Crawford 2001), and on other taxa the opening onto the external surface is notably inconspicuous (Denys et al. 2003). Although we were not able to verify the presence of rimoportulae on *A. nygaardii* specimens from Sand Lake, we are not yet convinced that they are lacking.

Crawford & Likhoshway (2002) provide an excellent summary of variation in vela structures among species of *Aulacoseira* and concluded that with further examination it may be possible to trace an evolutionary sequence of this structure within the genus. Although it is beyond the scope of this paper to review all types of known vela structures, it is worth noting some of the variation found among species. In some taxa, such as *A. ambigua*, the velum consists of only a few thick ribs (a rotae-like velum) that are attached to the side of the areola close to the inner surface of the valve and extend into and meet in the center of the opening (Crawford & Likhoshway 2002). The velum of *A. granulata* (Ehrenberg) Simonsen consists of a larger number of ribs that attach near the external, not internal, surface of the valve. On other species, such as *A. perglabra*, the velum consists of a much more complex series of thinner ribs. In addition to ribs, most species appear to also have a thin layer of material covering the vela on the internal surface. Crawford & Likhoshway (2002) suggested that this layer, which varies in structure between taxa, was composed of both silica and organic material. The velum of *A. nygaardii* consists of only a few, usually four, ribs attached relatively deep within the areola, similar to what has been described for *A. ambigua*. A thin layer of siliceous material also covers the areola on the inside surface of the valve as has been shown for other species.

Several previously published micrographs share some similarities with specimens of *A. nygaardii* from Sand Lake, including ones in Florin (1981), Scherer (1988) and Siver & Kling (1997). Florin (1981) presented six micrographs of a taxon she referred to as *Melosira fennoscandica* Cleve-Euler (figs 46-51 in Florin 1981). Three of these images (figs 47-49) are of a single specimen that is very similar to, if not identical with, the specimens of *A. nygaardii* we observed from both Sand Lake and Cape Cod. However, the other three images (figs 46, 50-51) appear to represent a different taxon altogether from the one illustrated in figs 47-49, and one that is definitely not the same as *A. nygaardii*. According to Camburn & Kingston (1986),

Florin came to believe that Cleve-Euler's *M. fennoscandica* was identical to *A. perglabra*, and she later recognized that the specimen in her figs 47-49 was of another species altogether. Krammer & Lange-Bertalot (1991) listed *M. fennoscandica* as a possible synonym of *A. perglabra*. Clearly, the specimen represented in figs 47-49 (Florin 1981) is not *A. perglabra*, but may indeed represent *A. nygaardii*. Curiously, in the original description of *A. nygaardii*, Camburn & Kingston (1986) does not mention the possibility that the specimen in figs 47-49 of Florin (1981) could be of *A. nygaardii*, and further, they discuss Florin's micrographs under *Melosira perglabra* var. *florinae* Camburn. We attribute this to the fact that since Camburn & Kingston (1986) did not examine their material with SEM, they did not fully realize the morphological features of *A. nygaardii*.

According to Camburn & Kingston (1986), the majority of Cleve-Euler's diatom collection was destroyed after her death in 1968. Marianne Hamnede confirms that there is no labeled holotype slide for *Melosira fennoscandica* in the Cleve-Euler materials that were deposited at the Swedish Museum of Natural History (Stockholm). However, in the original description, Cleve-Euler (1951) lists nine current localities and five fossil localities for *M. fennoscandica*. The Swedish Museum of Natural History has 27 slides from 9 of the 14 localities with the name of the relevant collector and/or Astrid Cleve-Euler's initials (A. Cl.). These samples include Farsjön Småland (Prof. Dr. S. Thunmark collector/donator, 1942 one slide, with A. Cl. initial), Flögen (Å. Berg collector/donator Aug. 1941 one slide, with A. Cl. initial), Emån (August 1841, 4 slides, with A. Cl. initials), O. Skirösjön (July 1941, one slide, with A. Cl. initial [no coverslip]), V.G. Tolken (July 1941, 4 slides, with A. Cl. initials), Växnan (1940, 4 slides, A. Cl. initials), Härno gytta interglacial (Prof. H. Munthe collector/donator, 1915, 2 slides), Krön Småland (U. Sundelin collector/donator, 1914, two slides [186.2, 186.4] and SW Finland, Hindersmossen (Dr. H. Lindberg, 1911, 12 slides and an additional slide with A. Cl. initial). Although Cleve-Euler did not present sample dates in her treatise of the Diatoms of Sweden and Finland (1951), her initials on the slides mentioned above clearly indicates that she examined them. Although we cannot be certain, the probability that these are the slides she used in her treatise would appear to be quite high. Even though many of the slides are not in good condition, we examined all 27 of them and found *A. perglabra* and possibly *A. perglabra* var. *florinae* on 21 slides and no apparent examples of *A. nygaardii* (photomicrographs deposited in the CANA photo collection). Cleve-Euler (1951) also illustrates a puzzling line drawing for *M. fennoscandica* (12d), which we believe is represented on the slides as *Staurosira construens* var. *venter* (Ehrenberg) Vyverman. Although we cannot definitively say that the slides mentioned above represent type material or syntypes, the abundance of *Aulacoseira* specimens on the slides, the appropriate localities and collectors named on the slides, and Cleve-Euler initials all suggest that these are slides she used to describe *M. fennoscandica*. Based on our examination of the slides, the drawings presented by Cleve-Euler for *M. fennoscandica* are clearly of the *A. perglabra* complex.

Scherer published four SEM micrographs (1988, figs 62-64, 66) of a taxon he reported as *Melosira nygaardii* from the Okefenokee swamp, Georgia, U.S.A. Although the Scherer (1988) specimens are very similar to *A. nygaardii* they do not match ones

we observed from the type material in several important ways. Details of the striae, the rounded valve face/mantle junction, the presence of a secondary layer of silica covering part of the mantle and overall dimensions of the specimens illustrated by Scherer (1988) are very similar to the population in Sand Lake. The Sand Lake population differed in three significant ways. Firstly, the spines on the Okefenokee specimens do not have anchor-shaped tips, but rather numerous small finger-like projections. Secondly, the valve face illustrated in Fig. 62 of Scherer (1988) is completely covered with areolae, which is not consistent with the original description for *A. nygaardii*, nor with our observations of type material with SEM. Thirdly, the costae on Okefenokee specimens bearing the marginal spines appear to be thickened relative to those not bearing spines. Similar thickened ribs were not found on Sand Lake specimens. Despite the differences, the Okefenokee taxon is closely related to *A. nygaardii* and further study is needed to discern its true identity.

Siver & Kling (1997, figs 78-82) illustrated specimens of what may represent *Aulacoseira distans* var. *nivaloides* (Camburn) Siver & H.J. Kling that share a few similarities with specimens of *A. nygaardii*. The most notable similarities concern the structure and spacing of the spines. Both taxa have flat, tapering spines of similar length and anchor-shaped tips that are spaced on every 2-3 mantle striae. Both taxa also have a similar mantle height to valve diameter ratio and parallel striae. However, the distinctly convex valve face covered with large areolae on the Siver & Kling (1997) specimens clearly serve to separate this taxon from *A. nygaardii*. In addition, the vela on the Siver & Kling (1997) specimens are much more branched, and the mantle areolae more elongate, than those on *A. nygaardii*.

In the original description Camburn & Kingston (1986) reported *A. nygaardii* to be widespread, including in lakes from the midwest (e.g. Michigan, Minnesota and Wisconsin), the northeast (e.g. Adirondacks of New York and New Hampshire) and the southeast (e.g. Florida and Georgia). In addition, Fallu et al. (2000) reported it from the northern Quebec and Labrador region and, as noted above, we have documented this species from Cape Cod ponds. If the images presented in Florin (1981) are indeed of *A. nygaardii*, then this species is also present in northern Europe. Interestingly, although Camburn & Charles (2000) reported *A. nygaardii* from 35 waterbodies in the northeastern U.S., Siver & Kling (1997) did not observe it in a detailed survey of the genus with SEM from Connecticut. Given the fact that Scherer (1988) has observed a morphologically similar taxon in southern Georgia, we suggest that records of *A. nygaardii* from the southeastern U.S. be confirmed with SEM. Camburn & Charles (2000) reported this taxon to have abundance weighted mean pH and total phosphorus values of 6.1 and $6 \mu\text{g L}^{-1}$, respectively. On Cape Cod, we also observed this taxon in acidic to slightly acidic ponds with a range of pH of 4.9-6.9.

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