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Source: *Proceedings of the Academy of Natural Sciences of Philadelphia*, Vol. 151 (Dec. 31, 2001), pp. 95-103

Published by: Academy of Natural Sciences

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Identification of diatoms (Bacillariophyceae) during ecological assessments: Comparison between Light Microscopy and Scanning Electron Microscopy techniques

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ABSTRACT—Identification of diatom frustules, a vital step during ecological analyses, is greatly influenced by the magnification at which samples are examined. The striking differences between Light Microscopy (LM) and Scanning Electron Microscopy (SEM) reveal inconsistencies in the identification of diatoms at low magnifications. These inconsistencies hinder comparative analyses between data sets collected by several research centers and may be hiding information of potential value in the assessment of ecosystem health. Additionally, SEM research has had a marked impact on diatom taxonomy rendering traditional identification methods insufficient for recognition of newly created taxa. Many diagnostic features of small representatives of these newly erected taxa can not be recognized even at higher LM magnifications. The consequent lack of taxonomic resolution at the LM level may be leading to overestimation of geographical distributions, ranges of tolerance to environmental parameters, and optimal conditions for growth. Qualitative aspects of the differences between LM and SEM approaches are discussed in the present paper.

INTRODUCTION

In the last two decades numerous ecological studies have concentrated on the use of diatoms as indicators of past and present changes in water ecosystems (*e.g.*, Agbeti and Dickman, 1989; Bennion, 1994; Christie and Smol, 1993; Renberg and Hultberg, 1992; Stoermer and Smol, 1999; Stoermer et al., 1996; Underwood, 1994; Underwood et al., 1998). There are several advantages to using diatoms as ecological indicators. First, diatoms are abundant and widely distributed in almost all aquatic ecosystems, marine, brackish, or freshwater. Second, diatom communities are very diverse and are represented in various habitats within an aquatic ecosystem and its surrounding drainage basin. Third, many diatom species are highly sensitive to alterations in water physico-chemistry; ecological optima and tolerances for many species are well documented (*e.g.*, Beaver, 1981; Douglas and Smol, 1993; Lowe, 1974; Patrick, 1977; Patrick and Reimer, 1966; van Dam et al., 1994). Fourth, taxa are delimited by features of the cell wall components, which often are well preserved in the sediments. Finally, since microscope preparations can be made permanent, specimens can be viewed at a later date for comparative purposes.

To this date, many ecological studies are mainly based on LM examination of diatom material (*e.g.*, see papers presented in Stoermer and Smol, 1999). For this, permanent slides are prepared following standard procedures (Battarbee, 1986), and specimens are analyzed using immersion objectives at magnifications of

ca. 1000 \times . However, when dealing with smaller specimens (*i.e.*, those in the 2–10 μm range) the limitations of LM studies become evident. Furthermore, there are groups of species that share typical diagnostic features such as the presence and location of spines, presence of apical pore fields, and areolae structure (*e.g.*, species in *Aulacoseira*; Siver and Kling, 1997). Differentiation of such characteristics with the light microscope requires considerable expertise, yet distinguishing between some of these taxa can be a very difficult, if not impossible, task.

Although the insufficiency of LM for the segregation of diatom taxa has already been discussed elsewhere (*e.g.*, Archibald, 1984; Battarbee et al., 1999; Cox, 1975; 1998; Flower, 1993; Haworth, 1975; Miller, 1969; Rosen and Lowe, 1981; Siver and Kling, 1997), the fact that small taxonomically difficult organisms may bear additional important ecological information has not received sufficient attention. The lack of taxonomic resolution among small taxa with similar appearance (leading to their "lumping" into a single category) results in the overestimation of environmental parameters, since each taxon contributes with its own range of tolerance and optimum (Siver, 1995). Thus, lumping may mask the ecological relevance that each taxon may potentially hold if considered separately (Sabbe and Vyverman, 1995; Siver, 1995).

Cox (1998), also stressed the importance of accurate identification in the application of the concept of indicator species. When particular taxa are used for the diagnosis of certain ecological conditions, investigators

must be sure of their identity, for reliable identifications become crucial during comparative analyses and constitute solid bases for communication, providing at the same time a consistent foundation for further advancement of ecological studies. Unfortunately, despite current efforts toward the standardization of terminology and identification protocols (e.g., Camburn et al., 1986; Juggins et al., 1994; Munro et al., 1990), attribution of names is still largely dependent on the laboratory where the study is being conducted and on the literature used for identification.

In recent years, identification protocols have incorporated relatively new tools. For example, SEM and molecular work on the marine genus *Pseudo-nitzschia* have enabled taxonomical delimitation and reliable identification of strains that might be harmful to humans (Fritz et al., 1992; Hasle, 1994; 1995; Manhart et al., 1995; Miller and Scholin, 1996; Villac and Fryxell, 1998). Many of the morphological features used for identification of representatives of *Pseudo-nitzschia* at the SEM level are extremely difficult to recognize under the light microscope. A similar problem arises during identification of araphid genera; i.e., those in *Fragilaria*, *Synedra*, and *Opephora* (Rosen and Lowe, 1981; Williams, 1986; Williams and Round, 1986; 1987; Witkowski, 1993). The use of SEM has revealed a higher morphological diversity among fragilarioid diatoms than previously recognized with LM. Many new genera and species have been erected based on features that are practically invisible under LM yielding the establishment of two parallel approaches for the recognition of these taxa. Unfortunately, the vast majority of ecological studies incorporating diatoms are still based primarily on LM due to reduced availability and high costs of electron microscopes. The results presented in this paper render LM insufficient for identification of smaller diatom taxa and strongly advocate the use of higher magnifications. Qualitative differences between LM and SEM data, with emphasis on fragilarioid diatoms, constitute the focus of this study.

MATERIAL AND METHODS

Samples were processed following standard procedures for the preparation of permanent slides in paleolimnological studies (Battarbee, 1986). Sediment cores were obtained from the deeper parts of 60 Connecticut lakes (USA) using a K-B type gravity corer, and immediately sectioned with an extruding device (Glew, 1989). The sections were stored in Whirl-pac plastic bags and maintained in a dark moist environment at 4° C. Even though identification problems in fragilarioid and other diatom groups were common to our set of 60 lakes, we present information from only 4 lakes, namely, Avery Pond, Black Pond (Meriden), Lake of Isles, and Mount Tom Pond. Examples found in these ecosystems best illustrate our main points of discussion.

For details on the morphometry and physico-chemistry of these bodies of water refer to Canavan and Siver (1995). An additional sample used in this study came from a long core collected from Lake o' Pines, Wisconsin (USA) in 1995. Specimens analyzed corresponded to material taken from a depth of 7.5 m in the core.

Subsamples (0.5 to 1.0 g) from the top sections of each CT sediment core and Lake o' Pines material were digested with an acid-dichromate solution to eliminate organic matter (Marsicano and Siver, 1993; Battarbee, 1986). Siliceous remains were washed several times with distilled water in order to dilute the acid. Aliquots of each slurry were dried onto aluminum foil, trimmed, mounted on an aluminum stub with double-sided tape, coated for 30 sec at 1.8 kV with gold/palladium using a Polaron sputter coater, and observed with a Leo/Zeiss DSM 982 Digital Scanning Electron Microscope. Preparation of permanent slides for LM analyses was performed by using a Battarbee chamber; coverslips were then mounted on glass slides with Hyrax mounting medium. A Zeiss light microscope equipped with DIC optics and built-in 35 mm photographic camera was used to analyze permanent preparations at 1600 \times . LM photographs were taken using Kodacolor 100 print film and subsequently scanned into digital form. SEM images were either stored directly in zip disks or printed in black and white Kodak Type 55 film. Plates presented in this paper were mounted using Adobe Photoshop ver. 4.0 for Macintosh. Morphological terminology follows definitions in Anonymous (1975) and Ross et al. (1979). Use of taxonomic names follows Round et al. (1990) and Williams and Round (1987; 1988).

RESULTS AND DISCUSSION

During routine qualitative and quantitative ecological protocols, one frequently encounters organisms that are extremely difficult to discriminate from others with similar valve outline and frustule ornamentation. Many small fragilarioid species possessing ovoid, clavate, or slightly rounded valve contours fall into this problematic group. LM observations of these organisms are complicated by the fact that the already smaller frustule sizes are further reduced during asexual reproduction. As a result, many of the features of the cell wall approach or fall below the limit of resolution of the light microscope leading to confusion during their identification (Fig. 1). Ambiguity in taxonomical descriptions and overlap of size ranges presented in the literature deepen identification uncertainties. Similarity in valve dimensions and number of striae per 10 μ m in organisms presented in Fig. 1 could lead to their inclusion into a single taxon. Additionally, characteristics such as striae structure, spine presence, and apical pore fields used in more specialized descriptions (Round et al., 1990; Williams and Round, 1987) may not be self-

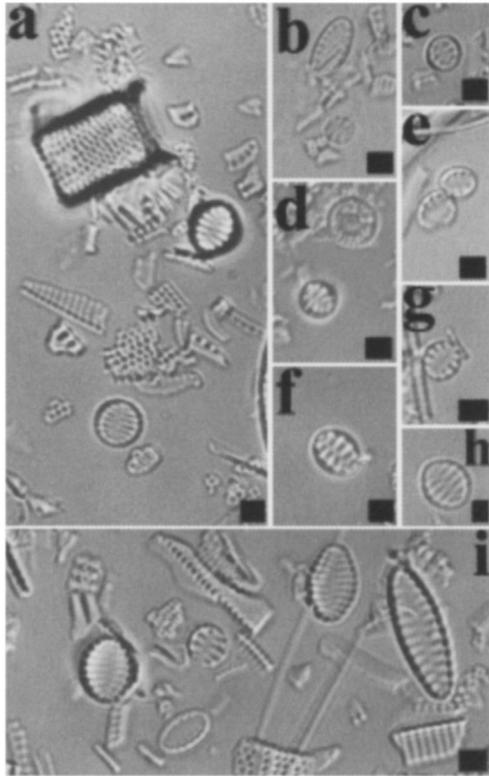


Fig. 1. LM photographs of some small representatives of fragilarioid diatoms. Each organism pictured here falls within size ranges presented in the literature for two or more taxa. Valves depicted here all from Avery Pond material. Scale bars: a–g, i, 2 μm ; h, 1 μm .

evident; neither frustule shape constitutes a reliable feature. For example, all valves presented in Fig. 1 produce a round configuration, yet they may not belong to the same taxon (see evidence later).

Some researchers may argue that in cases such as the one presented in Fig. 1 examination of large numbers of individuals could be a plausible solution to taxonomic uncertainty under LM. Analysis of more individuals could reveal trends in size reduction and variation of key features in populations. When similar morphologies are being produced by sympatric populations, however, such trends can only be tracked down to a certain size, after which similarities are markedly accentuated. Some organisms depicted in Fig. 1, for example, are practically indistinguishable from each other, yet they were all found in Avery Pond material. SEM analysis of this sample reveals the existence of at least six different morphs at this size range (Fig. 2). Three of the morphs (Figs. 2a–c) may be assigned to *Staurosira construens* var. *venter* based on striae structure and number, apical pore fields, and location of spines—although some valves such as those depicted in Fig. 2c possess unusually short striae, wide areolae, and lack spines (see discussion later). A fourth morph may be identified as

Staurosira elliptica, characterized by small round areolae, spines always located between striae, and apical pore fields absent or consisting of a few poroids (Fig. 2d). A fifth morph corresponds to an unknown fragilarioid with strong affinities to the genus *Pseudostaurosira*. Closing plates in this morph are very similar to those of *P. brevistriata*, but it can be distinguished from this latter taxon by the higher number of areolae on the valve face and valve mantle (Fig. 2e). Finally, a sixth unknown fragilarioid morph with uncertain affinities was encountered. This latter morph is characterized by unusual flattened circular closing plates that almost entirely occlude each of the areolar openings—a feature that is completely invisible under LM (Fig. 2f). Spines in this morph are positioned on the striae and lie at the edge of the valve face. Apical pore fields tend to be very reduced, usually composed of a few poroids.

Another example of taxonomic confusion is represented by small cruciate and elliptical morphs of *Staurosira construens* var. *venter* (Fig. 3a). During LM analysis, such forms may be identified as representatives of *Pseudostaurosira brevistriata* (Figs. 3c, e). Both, frustule dimensions and number of striae per 10 μm greatly overlap (see Krammer and Lange-Bertalot, 1991) making their distinction virtually impossible. Analysis under SEM, however, reveals the actual structure of the striae in these taxa (Figs. 3b, d, f). Even though the striae in this particular population of *S. construens* var. *venter* are very reduced delimiting a very wide central sternum, they are composed of laterally expanded instead of the wide rounded areolae characteristic of *Pseudostaurosira* spp. Furthermore, the spines in *Staurosira* are localized on the costae while in *Pseudostaurosira* they interrupt the striae (contrast Fig. 3b with either 3d or 3f). A large population of elliptical *Staurosira* with short striae was found in Avery Pond. At the SEM level, some of the individuals were characterized by the usual laterally expanded areolae, but lacked spines (Fig. 4a). Analysis of a larger number of valves in the sample reveals the presence of rudimentary spines between the short striae. This feature linked to the arrangement of the striae on the valve mantle and the typical rimmed apical pores (the latter not seen under LM) associate this population with *Staurosira construens* (Fig. 4b).

The case of *Synedra parasitica* is yet another example in which shape of the frustule and even valve ornamentation may be misleading during LM analyses. There are at least two other genera that can be confused with representatives of *S. parasitica*; i.e., *Pseudostaurosira* and some forms of *Staurosira* (see for example Krammer and Lange-Bertalot, 1991, p. 491, figs. 1–5, 9–16, and p. 495, figs. 32, 35). Also compare Fig. 5a with 3a, c, and e in this paper). Besides similarities in overall frustule shape among these taxa, there is also an overlap in cell wall dimensions and the number of striae per 10 μm (see dimensions presented by the same authors). Even though in many instances it is possible to ade-

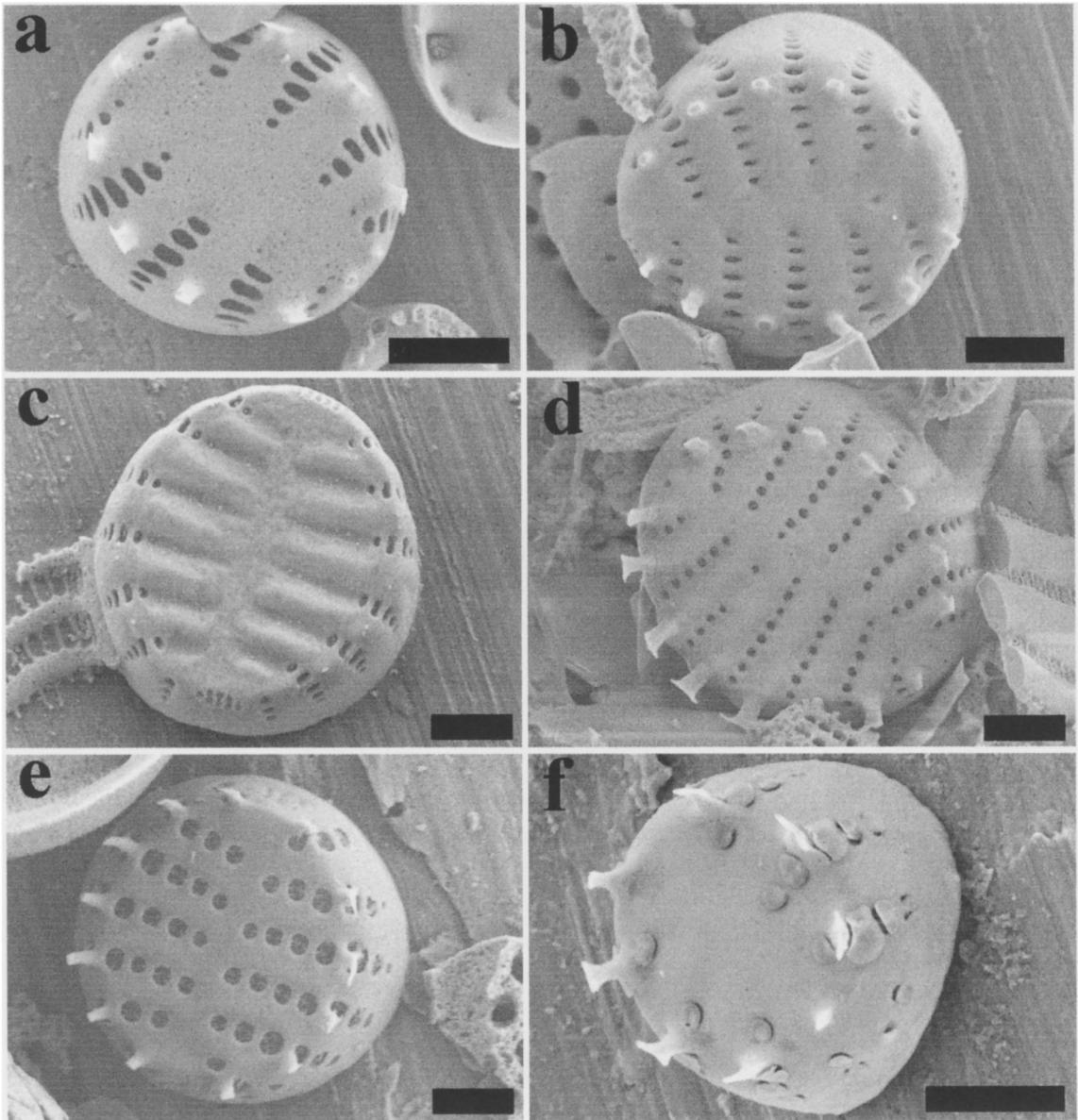


Fig. 2. SEM photomicrographs of smaller fragilarioid diatoms present in Avery Pond material. **a**, *Staurosira construens* var. *venter* with unusually wide areolae. **b**, Long-striated form of the same taxon possessing more characteristic areolae. **c**, short-striated form on the same taxon. **d**, *Staurosira elliptica*. **e**, Unidentified fragilarioid with affinities to *Pseudostaurosira* spp. **f**, Unknown fragilarioid exhibiting numerous representatives in the sample. Scale bars = 1 μm .

quately identify either of these species, as additional material from different localities is examined their boundaries become difficult to recognize. At the SEM level, one of the most characteristic structures in *Synedra parasitica* is the ocellulimbus (Williams, 1986) or “sunken” apical pore field (Fig. 5c). This structure coupled with the absence of spines (Fig. 5d) separate *S. parasitica* from the other two taxa. However, the ocellulimbus is not clearly seen under LM, so it becomes difficult to decide whether one is seeing a valve with apical regions devoid of such ornamentation, or highly

refractive zones corresponding to the ocellulimbus. In fact, most araphid valves with rostrate apices show refractive poles due to the refraction of light through the curving relief at these regions commonly lacking any other ornamentation (see Figs. 3a, c, and e). If we realize that the presence of spines can not be determined with certainty and that the ocellulimbus is not a reliable LM feature, then fair identification of some morphs of *S. parasitica* under the LM is practically impossible.

Analysis of material presented in this paper yields at least two situations when dealing with problematic taxa.

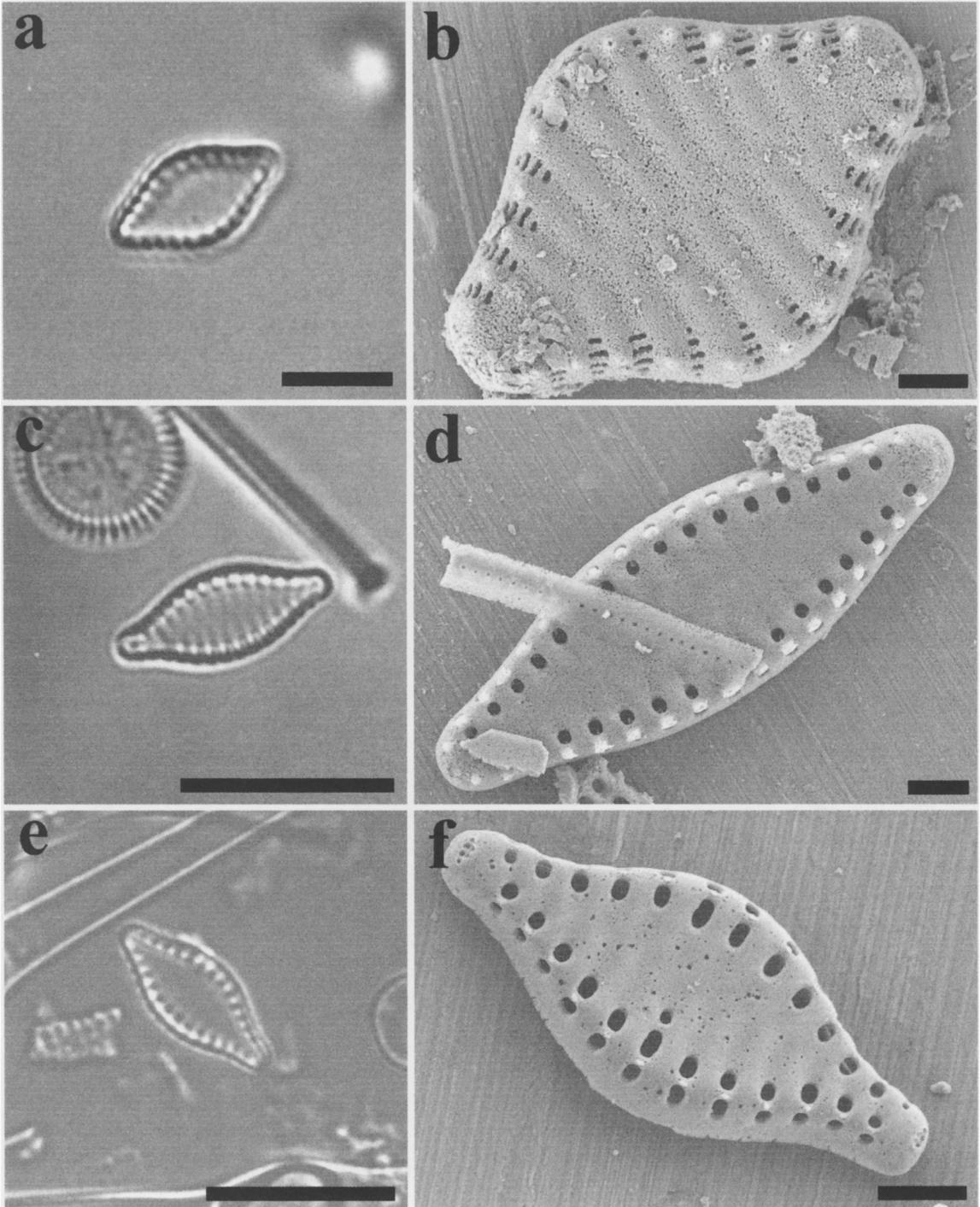


Fig. 3. Three fragilarioid diatoms that may be confused at LM magnifications because they produce some morphs that resemble each other and because there are overlaps in descriptions given in the literature. **a**, *Staurosira construens* var. *venter* (Lake o' Pines). **b**, Same taxon as seen under SEM (Black Pond, Meriden). **c**, *Pseudostaurosira brevistriata* (Lake o' Pines). **d**, SEM photomicrograph of the same taxon (Lake of Isles). **e**, *Pseudostaurosira brevistriata* (Mount Tom Pond). **f**, SEM image of the same species (Avery Pond). Scale bars: **a**, **c**, **e**, 10 μm ; **b**, **d**, **f**, 1 μm .

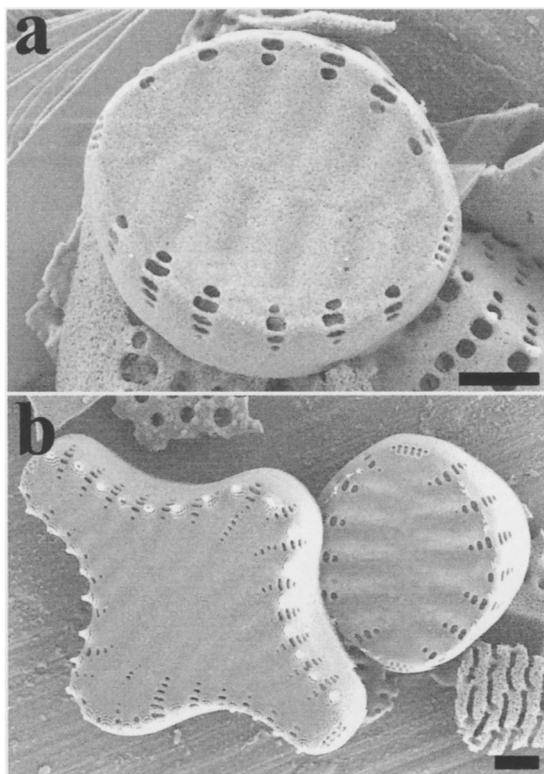


Fig. 4. a, SEM photomicrograph of the short-striated representative of *Staurosira construens* var. *venter* (Avery Pond), which might be confused with morphs of *Pseudostaurosira brevistriata* at LM magnifications. b, *Staurosira construens* var. *construens* (left) and the *venter* form in the same sample. Scale bars = 1 μm .

First, during LM observations one might encounter organisms whose features are not clearly visible and for which examination with SEM will surely enhance the assignment of a given name (e.g., the case of *S. parasitica*). In this case, reliable attribution of names is still possible, although some problems may arise when LM and SEM data are compared (Haworth, 1975). Second, the features of some small organisms may not be distinguished by LM, and the application of a name completely depends upon SEM analysis (as in the case presented in Fig. 1). For this latter scenario, one of the greatest difficulties in trying to identify diatoms under SEM is the lack of supporting literature. The majority of taxonomic keys are based on LM features, and even though there are SEM treatments of certain diatom groups, this literature still does not cover the vast diatom diversity. An additional shortcoming to the use of ultrastructural information is that electron microscopes and associated tools are extremely expensive, and often require experienced personnel for their care and maintenance.

As illustrated by the examples presented above, naming smaller organisms using the light microscope can

be ambiguous in many cases. Bias introduced by the exclusive use of LM can be significant if we consider that smaller individuals often represent a large fraction of the organisms in a sample. Yet, during ecological analyses the researcher is still compelled to make decisions regarding application of names even when only LM is available. However, such decisions must be founded on universal and well-established ground.

Several authors have expressed the unequivocal importance of taxonomic precision in limnology (Battarbee et al., 1999; Birks, 1994; Imbrie and Kipp, 1971; Munro et al., 1990). The need for accurate identification resides on the importance of recognizing morphological variants among and within particular taxa, which in turn, increases the predictive value of organisms by linking them to particular ecological conditions (Anderson et al., 1993; Cox 1995). Additionally, ecological inferences based on taxonomically precise determinations can be used later for comparative purposes over wide geographical areas or at a single site (e.g., during determination of geographical ranges and paleoenvironmental reconstruction, respectively). We have demonstrated that the use of SEM improves significantly the accuracy of identifications since diagnostic features can be clearly observed. Consequently, characterization of discrete morphological variants of potential ecological value becomes a more reliable process. Additionally, SEM information (image files or photographs) can be reinterpreted years later or used directly as a basis for further studies. This latter quality of SEM data becomes relevant when we realize that most of current publications lack any explanation about how identifications were made and/or do not provide supporting illustrations, hence, hindering any further use of information thus generated.

The authors are aware that the general conclusions presented here are not new to diatom science. These results, however, may further contribute to the realization concerning the usefulness of SEM data in diatom analyses, especially when important decisions about the fate of aquatic ecosystems are drawn from such analyses.

ACKNOWLEDGMENTS

We are indebted to A. M. Lott for preparing the Connecticut samples for both LM and SEM. We also thank Dr. M. E. Cantino, J. S. Romanow, and S. B. Daniels for their guidance during the SEM sessions and during the preparation of figures at the Electron Microscopy Laboratory (University of Connecticut). We deeply appreciate the valuable suggestions, and revision of the manuscript in its early stages of preparation made by Dr. E. F. Stoermer, and R. J. Bixby (University of Michigan). Our special thanks to H. Ewing (University of Minnesota) for kindly providing the sample from Lake o' Pines, Wisconsin. This research has been fund-

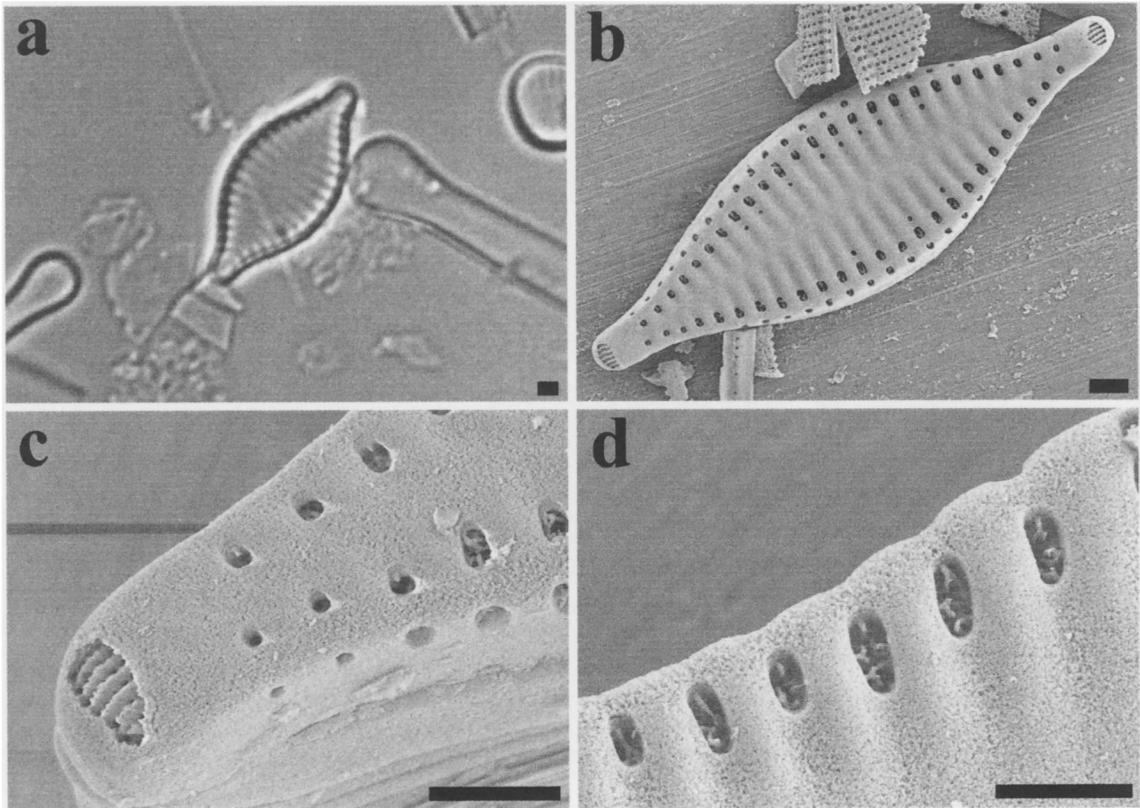


Fig. 5. *Synedra parasitica*. **a**, LM photomicrograph (Mount Tom Pond). **b**, SEM image (Avery Pond). **c**, Detail of the ocellulimbus (Lake o' Pines). **d**, Detail of areolae and closing plates (Lake o' Pines). Scale bars = 1 μ m.

ed in part by the Francis R. Trainor Endowment to the Department of Ecology and Evolutionary Biology at the University of Connecticut (E. Morales); NSF Grants #DEB-9306587 and #DEB-9615062, and the Nancy Rash Scholarship Fund (P. Siver).

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