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Observations on the Tube-Dwelling Diatom *Navicula Tripunctata* var. *Schizonemoides* (V.H.) Patr. Using Light and Electron Microscopy¹

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The valve morphology of specimens from four different collections, each identified as *Navicula tripunctata* var. *schizonemoides* (V.H.) Patr., was examined by light and electron microscopy. Light microscopy revealed little variation between populations. Variability in the valve shape and striae arrangement within a population, however, was observed. Observations by electron microscopy revealed structures common to other lineolate *Navicula* and pennate diatoms. The continued use of electron microscopy as a supplement to light microscopy for proper interpretation of a diatom's valve morphology is stressed. Notes on the ecology of this taxon are made where appropriate.

INDEX DESCRIPTORS: Diatom, tube-dwelling *Navicula*, light microscopy, electron microscopy.

Many diatoms produce mucilaginous tubes within which individual cells move and divide (Cox, 1977; Drum, 1969). The genus *Schizonema* was established by C.A. Agardh in 1824 to include all diatoms which inhabit tubes (Aleem, 1949). Initially, species were distinguished based on differences of tube color, texture, and mode of branching. Since frustules of two or more species were found sometimes to occur in the same tube and the tubes were seen to change with different environmental conditions, Grunow in 1890 differentiated the naviculoid tube-dwellers into the genera *Schizonema* and *Dickeii* based on frustule characteristics (Aleem, 1949.) Later, Cleve (1894) incorporated these genera into the genus *Navicula*. The subsequent fragmentation of *Navicula* into several subgenera is well known and does not require further discussion.

In a recent study (Millie, 1979), a tube-dwelling diatom was observed as a dominant fall taxon in a Lake Erie marsh. This taxon, originally designated as *Navicula* sp. 38, has been subsequently identified as *N. tripunctata* var. *schizonemoides* (V.H.) Patr. based on herbarium specimens at the Academy of Natural Sciences of Philadelphia (general collection slides 6803a, 44619a, and 62002). Patrick and Reimer (1966) based their description of this taxon on specimens which Van Heurck (1885) initially described as *N. gracilis* var. *schizonemoides*. The variability of the observed diatom's morphology, however, was seen to be greater than that described by Patrick and Reimer and Van Heurck. In addition, a discrepancy in the manner by which this taxon has been identified and reported was found to exist. Specimens which were observed to be identical to our sp. 38 have been previously identified by Stevenson (1977) and Hoagland, Roemer, and Rosowski (personal communication, 1979) as *N. tripunctata* var. *schizonemoides* and *N. graciloides* A. Mayer respectively. Collins and Kalinsky (1977) and Carter (1979) illustrated specimens similar to our taxon as *N. cincta* (Ehr.) Ralfs. Examination of herbarium specimens at the Academy of Natural Sciences of Philadelphia of *N. graciloides* (general collection slides 1715 and 1950) and *N. cincta* (general collection slides 2359a and 11874) indicates that these taxa are distinct entities from the diatom analyzed in this study.

The intent of this paper is to present information of the morphological variability observed in *N. tripunctata* var. *schizonemoides* from four populations using light microscopy. Structural features of the frustule observed by electron microscopy (EM) and notes on the taxon's ecology are also reported.

MATERIALS AND METHODS

An epiphytic population of *N. tripunctata* var. *schizonemoides* was collected from a Lake Erie marsh during the fall of 1977. Additional collections in which this diatom was dominant or sub-dominant were obtained from R. Jan Stevenson (Great Lakes Research Division, University of Michigan), Kyle D. Hoagland and Steve C. Roemer (School of Life Sciences, University of Nebraska) and Resource Consultants, Inc. (Bentford, Tennessee). Information concerning the date and type of habitat from which the diatom assemblages were sampled and the taxon's relative abundance within the assemblage are presented in Table 1. Specimens were "cleaned" by a modified nitric acid technique (Rohr, 1977). Permanent slides were prepared using Hyrax Mounting Medium (I.R. = 1.71).

Using light microscopy, 100 diatom valves in each population were measured for the valve characters presented in Table 2. A point on the edge of the coverslip was established for a suitable starting point for a transverse transect. The transect continued across the coverslip until the required number of valves had been examined. All examination was done using a 90× apochromatic oil immersion objective (1.32 N.A.).

For scanning electron microscopy (SEM), "cleaned" specimens were air dried on a SEM stub and coated with approximately 300Å of gold in a Polaron ES5100 sputter coater. Specimens were observed in a JEOL JSM-35 scanning microscope at 20KV. Carbon replicas for transmission electron microscopy (TEM) were prepared by techniques outlined by Wiemers (1974). Replicas were examined in a Hitachi HU-11C transmission microscope.

Table 1. Populations of *Navicula tripunctata* var. *schizonemoides* (V.H.) Patr. analyzed

Sampling Location	Collector	Date Sampled	Habitat	Maximum Relative Abundance Observed (%)
Lake Erie, Ohio	D.F. Millie	7 October 1977	epiphytic	62.6
Sandusky River, Ohio	R.J. Stevenson	19 September 1975	epilithic	16.0
Tennessee River, Tennessee	Resource Consultants, Inc.	Summer 1978	epiphytic	38.2
Yankee Hill Reservoir, Nebraska	K. Hoagland/ S. Reimer	5 August 1977	periphytic on plexiglass tabs	43.0

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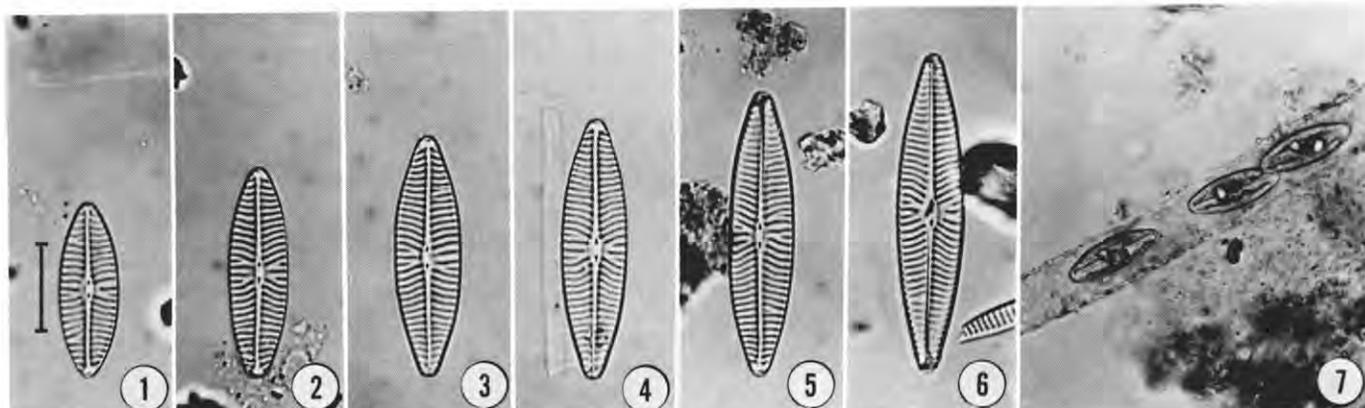


Fig. 1-7. Light micrographs of cleaned and preserved specimens. Fig. 1-6. Morphological series of the size range from the Tennessee River population. Bar represents 10 μm . X1000. Fig. 7. Preserved specimens from the Lake Erie population. X250.

RESULTS

Specimens of *N. tripunctata* var. *schizonemoides* were seen living in mucilaginous tubes in uncleaned samples. Frustules within the tubes were most often observed lying in a linear fashion (Fig. 7). In many instances, however, rapid cell division apparently caused the frustules to become stacked upon one another, producing a "multi-seriate" effect. The tubes also provided a suitable substrate for attachment of diatom taxa which produce mucilaginous stalks, especially *Gomphonema parvulum* Kütz.

Character variability of the valve morphology between the four populations are presented in Table 2. No distinct differences between populations concerning valve length, breadth, or length-breadth ratio were observed. Valve length varied from 19 to 34 μm in almost all populations. One specimen measuring 38 μm in length was seen during random observation of the Tennessee River population (Fig. 6). No specimens smaller than 19 μm were observed in any population. Approximate values for valve breadth, 7.3 μm , and length-breadth ratio, 3.7:1, were consistent between populations.

Distinct differences in valve shape were noted throughout the entire size range within a population. Figures 1 to 6 represent the observed size range of the Tennessee River population. Valve shape was seen to change from elliptical lanceolate (Figs. 1, 2) in the lower portion of the size range to linear elliptical (Figs. 3, 4) to almost linear lanceolate in the largest specimens (Figs. 5, 6). Corresponding with these size differences was an expected change in apex morphology. Smaller specimens possessed slightly rounded apices (Figs. 1, 2) while apices of specimens in the mid to upper portion of the size range were more sub-rostrate in shape (Figs. 3-6, 8).

Striae density was consistent throughout the taxon's entire size range numbering 12 to 14 in 10 μm at the apices. Density of the central striae was usually less numbering 10 to 14 μm (Figs. 1-6, 8, 11, 14). Striae were slightly radiate near the valve center to parallel or slightly convergent at the apices. A distinct central area is produced due to differential extension of the striae into the center of the valve. Striae on one side extend farther into the center of the valve than the other side (Figs. 1, 2, 6, 8, 11, 14). This produced a central area which is slightly rounded on one side and more rectangular on the other.

The presence of a marked interruption in the striae (Figs. 1, 5, 6, 12), called by Voigt (1956) "défaut régulier," but termed "Voigt's fault" by Ross *et al* (1979), was observed in all populations (Table 2). Generally, 60 percent of all valves observed possessed this interrup-

tion. However, only 31 percent of the valves observed from the Tennessee River possessed this feature.

Striae extended from the valve face onto the valve mantle (Fig. 9). Puncta were observed at the outermost portion of the apex (Figs. 10, 15). No specialized pores for mucilage secretion were seen. Individual puncta were shown by EM to be lineate in shape (Figs. 8-12, 16). In the interior of the valve, silicious thickenings were observed to border each individual punctum (Figs. 13, 16). Examination of the puncta with TEM revealed no sieve membrane (Fig. 16).

Externally, the proximal raphe endings appear slightly deflected (Fig. 11). The deflection is so slight, however, that it is not usually observed with the light microscope. The distal raphe ends appear "comma shaped" (Figs. 8, 10) and extend onto the valve mantle (Fig. 15). With light microscopy, this shape is often obscure due to the extension onto the valve mantle (Figs. 1-6). Internally, the raphe is seen to lie in a pronounced rib-like structure (Figs. 13, 14). The inner proximal raphe ends are deflected more to one side than the external

Table 2. Character variability of *Navicula tripunctata* var. *schizonemoides* (V.H.) Patr.

Character	Sampling Location			
	Lake Erie	Sandusky River	Tennessee River	Yankee Hill Reservoir
Length (μm)	27.7 \pm 2.5 ^a (21.7-34.0) ^b	27.1 \pm 2.9 (19.4-34.0)	26.8 \pm 2.8 (20.2-34.0)	25.5 \pm 2.7 (19.2-34.0)
Breadth (μm)	7.3 \pm .2 (6.9-7.9)	7.3 \pm .3 (6.4-8.0)	7.4 \pm .3 (6.9-8.3)	6.8 \pm .4 (6.0-7.5)
Length/Breadth Ratio	3.8 \pm .4 (3.1-4.6)	3.7 \pm .4 (2.76-4.74)	3.6 \pm .5 (2.84-4.59)	3.7 \pm .4 (2.9-5.4)
Apical Striae (per 10 μm)	12.9 \pm .7 (12-14)	13.1 \pm .7 (12-14)	12.7 \pm .7 (12-14)	13.6 \pm .6 (12-14)
Central Striae (per 10 μm)	12.8 \pm 1.0 (10.7-14.3)	11.6 \pm 1.1 (9.1-14.0)	12.3 \pm .9 (10.7-14.3)	12.4 \pm 1.1 (10.7-14.3)
% valves with Voigt's fault	58%	80%	31%	68%

^aMeans and standard deviations based on 100 valves per population.

^bNumbers in parenthesis represent ranges for values observed.

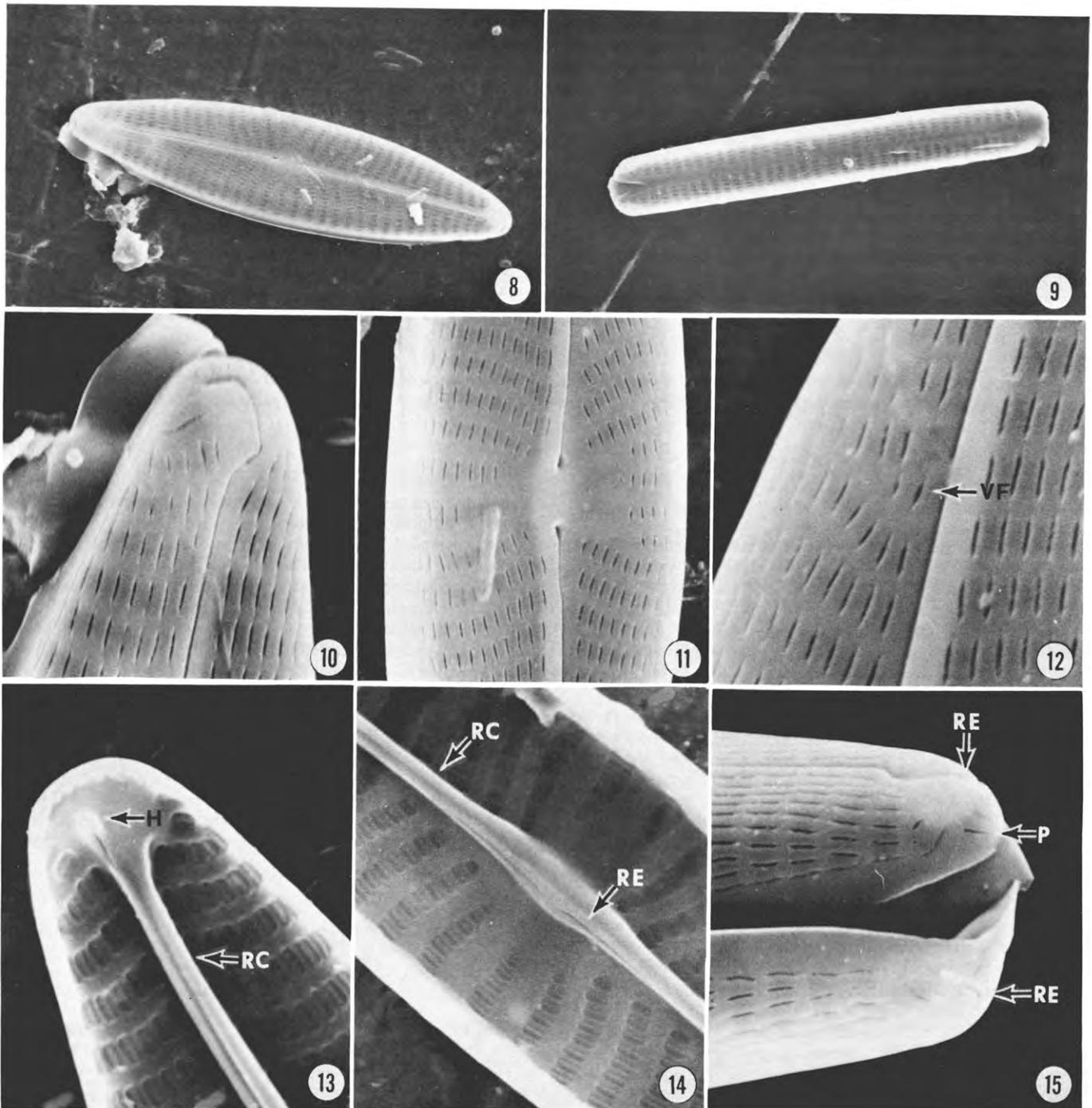


Fig. 8-15. Scanning electron micrographs of cleaned specimens. Fig. 8. Valve view. X2,400. Fig. 9. Girdle view. X2,400. Fig. 10. External view of the valve apex. X9,400. Fig. 11. External view of the central area. X6,600. Fig. 12. External view of Voigt's Fault (VF). X13,000. Fig. 13. Internal view of the valve apex. Note the helictoglossa (H) and the thickened raphe canal (RC). X11,000. Fig. 14. Internal view of the central area. Note the slightly deflected proximal raphe endings (RE) and the thickened raphe canal (RC). X8,600. Fig. 15. Girdle view of the apex of a slightly separated frustule. Note curvature of the distal raphe ending (RE) onto the valve mantle and the presence of puncta (P) along the valve mantle at the apex. X9,400.

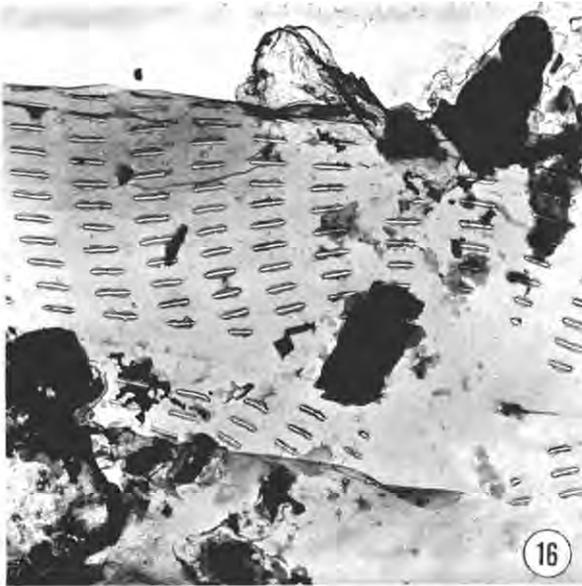


Fig. 16. Transmission electron micrograph of a carbon replica. Note silicious thickenings bordering each punctum. X8,500.

proximal raphe ends. The distal raphe endings end in a silicious thickening. This helictoglossa (Ross *et al.*, 1979) is slightly eccentric and deflected to one side.

DISCUSSION

Specimens of *N. tripunctata* var. *schizonemoides* in this study deviated from Patrick and Reimer's (1966) description of this taxon. They described this taxon to be "almost linear with obtuse rounded ends." Valve shape of our specimens varied from elliptical lanceolate to almost linear lanceolate. Apex morphology varied from slightly rounded in the smaller specimens to sub-rostrate in the larger ones. This agrees with Geitler's (1932) observation that diatoms generally become rounder as they approach the lower portion of their size range.

Navicula tripunctata (O.F. Mull) var. *tripunctata* is described by Patrick and Reimer (1966) to possess a rectangular central area. *N. tripunctata* var. *schizonemoides* is differentiated from the nominate variety by possessing a central area that does not have a rectangular shape. The central area in our specimens, however, approached, at times, a rectangular shape. Observations in this study also support Carter's (1979) delimitation of "two sides" within the central area. He proposed that the terms "staff" (referring to the side possessing a semi-lanceolate central area) and "distaff" (referring to the side of the central area possessing a rectangular shape) be adopted for diatoms similar to *N. cincta*. While he does not propose that this terminology is applicable to all pennate diatoms, the terms were quite appropriate for the specimens in this study.

Carter (1979) noted an interruption of the striae (Voigt's fault) halfway between the central area and the valve apex to always be present on the "staff" side of the valve. Specimens from the four populations analyzed here possessed this character in a variable manner. While the majority of the Lake Erie, Sandusky River and Yankee Hill Reservoir specimens possessed this fault, it was observed in only

31 percent of the Tennessee River specimens. Patrick and Reimer (1966) made no mention of this character in their description of the taxon.

Internally, the structure of *N. tripunctata* var. *schizonemoides* is similar to other taxa in the lineolate *Navicula* and several other pennate diatoms. Cox (1975; 1976) and Jackson and Lowe (1978) have noted the presence of a thickened rib-like structure accommodating the raphe slit in several pennate taxa. The presence of the helictoglossa at the distal raphe ending appears to be common in naviculoid diatoms (Cox, 1975). However, the extent of variability to which this structure is raised or twisted appears to be characteristic to a specific taxon (Cox, 1975; 1976; 1979).

Studies have shown certain pennate diatoms to possess specialized pores for mucilage secretion at one or both ends of the frustule (Dawson, 1972, 1973a, 1973b; Hufford and Collins, 1972; Cox, 1976). The absence of these pores in *N. tripunctata* var. *schizonemoides* is explainable. Several researchers (Stoermer, *et al.*, 1965; Drum, 1969; Cox, 1975) have accounted for their absence by surmising that special intercalary band structures exist which are presumably associated with polysaccharide secretion. Cox (1976) theorized that the sieve plate on the internal surface of a stria pore, a characteristic feature of pennate diatoms, would prevent mucilage extrusion. In this study, examination by transmission electron microscopy revealed no pore membranes to exist. Their absence, however, could be accounted for by the harsh cleaning technique utilized. Individual puncta were also observed to be bordered internally by silicious thickenings. Cox (1976) noted this feature in several of the tube-dwelling *Cymbella*.

Very little work has been published concerning the ecology of tube-dwelling diatoms. The presence of a mucilaginous tube would allow a diatom to live in an environment more stable than an extratubular environment (Drum, 1969). Drum also proposed that not only does the tube material serve as a protective boundary, but functions as a nutrient trap. Previous researchers, (Drum, 1969; Cox, 1975) have observed the tube used as substrate by epiphytic bacteria, other algae, and fungi. This was seen in the present study as several stalked *Gomphonema parvulum* specimens were observed attached to the mucilage.

All the populations analyzed were collected from firm substrates during the summer or fall. This taxon has been observed to be at its highest relative abundance throughout the mid-west in the late summer or early fall (Stevenson, personal communication 1978; Millie, 1979). The growth of a tube-dwelling taxon would be best on firm substrates. Tubes adhering to transient substrates would detach and settle to the bottom where the colony would become buried within the sediment and subsequently die (Cox, 1977). Since an epilithic sample in a lotic habitat would be constantly exposed to shifting sediment, the low relative abundance of *N. tripunctata* var. *schizonemoides* in the Sandusky River sample can be explained.

The primary intent of this study was to present the morphological variability of specimens, each identified as *N. tripunctata* var. *schizonemoides* by light microscopy, from four different populations. This taxon appears to have been previously identified and reported as four different taxa by researchers. This taxonomic confusion has resulted from the taxon's variability in valve shape and striae arrangement. The use of electron microscopy correlated to light microscopy can frequently reduce this confusion. Morphologic features are more precisely interpreted when EM observations supplement those made with light microscopy. This information is a useful aid for the observer who does not have routine access to EM or who must process large numbers of samples e.g. for ecological work. With the present day use of determining the environmental status of a habitat through the ecological requirements of the dominant taxa, the need for taxonomic consistency within the literature is apparent.

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