Morphological variability and cell wall composition of *Phaeodactylum tricornutum* (Bacillariophyceae)

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MORPHOLOGICAL VARIABILITY AND CELL WALL COMPOSITION OF *PHAEODACTYLUM TRICORNUTUM* (BACILLARIOPHYCEAE)

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ABSTRACT.—Five isolates of *Phaeodactylum tricornutum* Bohlin were examined in light and transmission electron microscopes. Although isolated from widely separated sites including both coastal marine and inland saline habitats, the morphology of the siliceous frustules of all isolates was very constant. The fusiform and triradiate forms, which have been reported as being non-siliceous in the past, were found to have an amorphous particulate nature in TEM. These forms were examined using EDAX SEM elemental analysis and were found to contain silica in their cell walls.

Key words: diatom, brackish-water; diatom, morphology; Great Salt Lake, Utah; Phaeodactylum; *Soap Lake, Washington.*

The diatom *Phaeodactylum tricornutum* Bohlin (1897) was first described from coastal marine waters in Sweden in 1897. Since then it has been observed in other marine coastal waters, particularly in areas of high enrichment. Rushforth et al. (1988) gave the first report of this species occurring in inland waters after discovering the taxon in phytoplankton samples from Farmington Bay, Great Salt Lake, Utah. *Phaeodactylum,* a monotypic genus, differs from other diatom genera in that it produces several types of cells, one bearing a weakly silicified naviculoid diatom valve, the others reportedly lacking siliceous valves and having either a fusiform, triradiate, or rarely cruciform shape (Wilson and Lucas 1942, Wilson 1946, Bourelly and Dragesco 1955, Borowitzka and Volcani 1978). The typically single-celled diatom can occasionally form chains of variable length (Borowitzka et al. 1977). The long and confused taxonomic history of this peculiar species has been covered elsewhere (Hendey 1954, Rushforth et al. 1988).

Subsequent to observation of *Phaeodactylum* populations in Great Salt Lake, I isolated the taxon from Soap Lake, Grant County, Washington, an unusual alkaline lake in the arid western part of the state. Originally, I thought my isolate was a small *Navicula* in the *Navicula saprophila/permitis/pelliculosa* complex. However, after careful electron microscope analysis I realized that it was indeed a *Phaeodactylum* species, although the alternate fusiform and triradiate cells have never been observed. I decided to undertake a comparative study of *Phaeodactylum* isolates to determine if morphological differences exist between marine and inland populations. This paper reports the results of that study.

METHODS

Five isolates of *P. tricornutum* were examined, all of which are maintained as part of the SERI Microalgae Culture Collection in Golden, Colorado. PHAEO1 (also designated TFX-1) was isolated from culture ponds at Woods Hole, Massachusetts, in September 1956 by Joyce and Ralph Lewin (Lewin et al. 1958). PHAEO2 (also designated BB) was isolated from coastal waters near Scripps Institution of Oceanography, La Jolla, California, by William H. Thomas (Barclay et al. 1986). PHAEO3 and PHAEO4 were isolated by myself from Farmington Bay, Great Salt Lake, Utah, 23 October 1986 and 26 March 1987, respectively. DIATO1 was isolated by myself from Soap Lake, Washington, 6 March 1987.

Marine isolates are routinely grown in GPM artificial seawater media, while inland strains are normally grown in SERI Type II/25 media (Barclay et al. 1986). However, all strains grow in SERI Type II/25. To eliminate morphological differences due to media differences, all cultures were grown in Type

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II/25 for one month prior to electron microscopical analysis.

P. tricornutum frustules are very fragile and are destroyed by boiling in nitric acid, a traditional diatom clearing procedure. To remove organic matter from the cultures, several techniques were employed, the best of which was to treat samples with concentrated H_2O_2 for 12–24 hours, followed by heating in a boiling water bath to degrade unreduced H_2O_2. Samples were then cleared of soluble salts by repeated washing and centrifugation. Cleared samples were mounted in Naphrax resin for light microscope analysis. It should be noted that this method was gentle enough that even the valves traditionally considered unsilicified were not dissolved. Diluted cleared samples were also mounted on formvar coated hexagonal copper grids for transmission electron microscopy (TEM). Grids were examined on a JEOL 100CX transmission electron microscope at an operating voltage of 80 kV. Additional cleared material was mounted on carbon stubs, carbon coated by vacuum evaporation, and examined with an EDAX 9100 attached to a Hitachi HHS-2R scanning electron microscope (SEM) at an operating voltage of 20 kV.

RESULTS

Simple elongated chloroplasts and circular lipid droplets are present in all cell types. In culture the naviculoid frustules produce more extracellular polysaccharides and form cohesive aggregates of cells that cannot be segregated by agitation. Fusiform and triradiate cells do not form cohesive aggregates. Naviculoid frustules were found in all five strains (Figs. 1, 5). Fusiform cells have been observed in PHAEO1, PHAEO2, PHAEO3, PHAEO4, and dominate cultures of PHAEO1 and PHAEO2 (Figs. 3, 4). During the past four years of culture history, triradiate cells have been observed only in PHAEO3 and PHAEO4, and they dominate cultures of those strains (Fig. 2). Chains like those recorded by Borowitzka et al. (1977) were seen rarely in cultures of PHAEO1 and PHAEO2.

Since naviculoid frustules were observed in all strains, morphological comparisons based on these cells can be made. Valves of all strains have overlapping dimensions and are morphologically indistinguishable. For this reason a summary of the traits of oval cells will be given without reference to strain. Cells are 7.5–16 μm long by 2.5–6.5 μm wide, with most cells 8–10 μm by 3–4.5 μm. The few larger cells were found in cultures of PHAEO1 dominated by the fusiform cells and may represent transitional forms between the two forms. In living material, two types of naviculoid cells were observed, long thin cells and cells of similar length but greater width. Wilson (1946) also observed these cell types and noted that the thinner cells are characteristic of rapidly growing cultures, whereas the larger cells are more typical of nutrient-deficient conditions.

When naviculoid cells are cleared of organic matter, only the axial areas are distinguishable in light microscope mounts (Fig. 1). In TEM mounts, ultrastructure of the siliceous valve is revealed. Striae are usually parallel (Fig. 5), but they may be radiate (Fig. 7), especially around the central area (Figs. 6–8). Striae are evident as less thickly silicified areas of the valve that are perforated irregularly with simple pores (Figs. 5–8). Striae density ranges from 90 to 105 in 10 μm. The axial area is thickly silicified, wider at the center, and flexed to one side, giving the valves a slightly assymetrical aspect. The central area is further set off from the rest of the valve by having "ghost striae" not perforated by pores in an orbicular region around the center of the axial area (Figs. 5–6). The raphe is distinctly lateral, becoming filiform at both distal and proximal ends. Proximal raphe ends are hooked consistently toward the dorsal margin (Fig. 6).

Triradiate cells were common in the isolates from the Great Salt Lake (PHAEO3 and PHAEO4). The forms figured in this paper (Fig. 2) have shorter arms than those from the cultures first isolated (see Rushforth et al. 1988, Figs. 2, 3). The length of the arms as measured from valve center to tip ranges from 6 to 15 μm. In material cleared with hydrogen peroxide, the valve appears to be made of an amorphous particulate matrix and is clearly divided into valve face and mantle areas (Figs. 9, 10).

Fusiform cells are dominant in the marine isolates (PHAEO1 and PHAEO2). Cell dimensions are 15–27 μm long by 3–5 μm wide, dimensions similar to those reported by
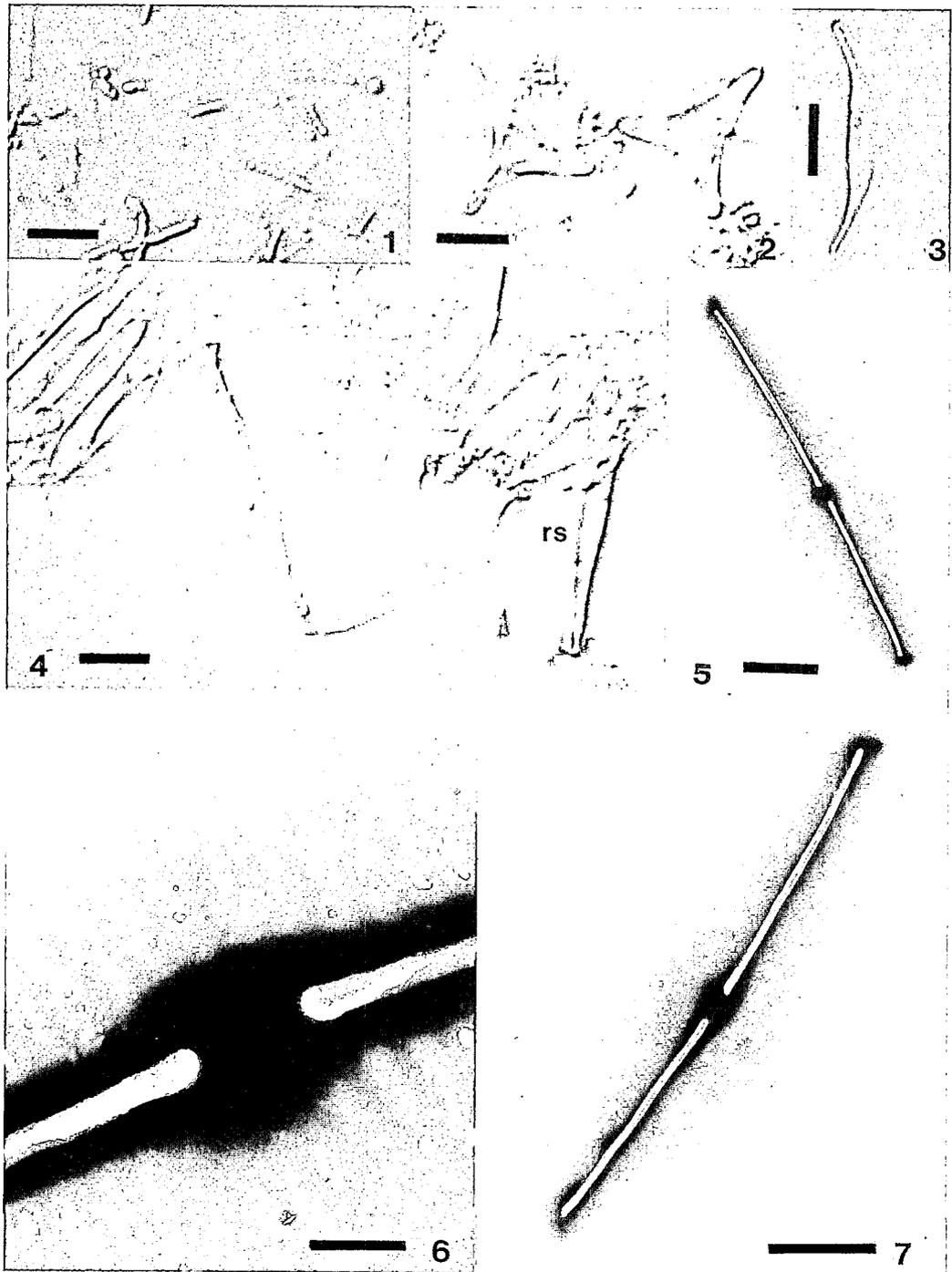
Figs. 1–7. *Phaeodactylum tricornutum,* cleared specimens: 1, naviculoid valves of DIATO1 showing axial areas; 2, triradiate cells of PHAEO4; 3, dorsiventrally arched fusiform cell from PHAEO4 cultures; 4, fusiform cells of PHAEO2 (note the highly refractive specimen [rs] in the lower right, which is apparently more heavily silicified than other cells in the micrograph [scale = 5 μm for Figs. 1–4]); 5–7, DIATO1 naviculoid cells showing morphology of the striae, raphe, and central area (scales = 1 μm, 0.2 μm, and 1 μm, respectively).
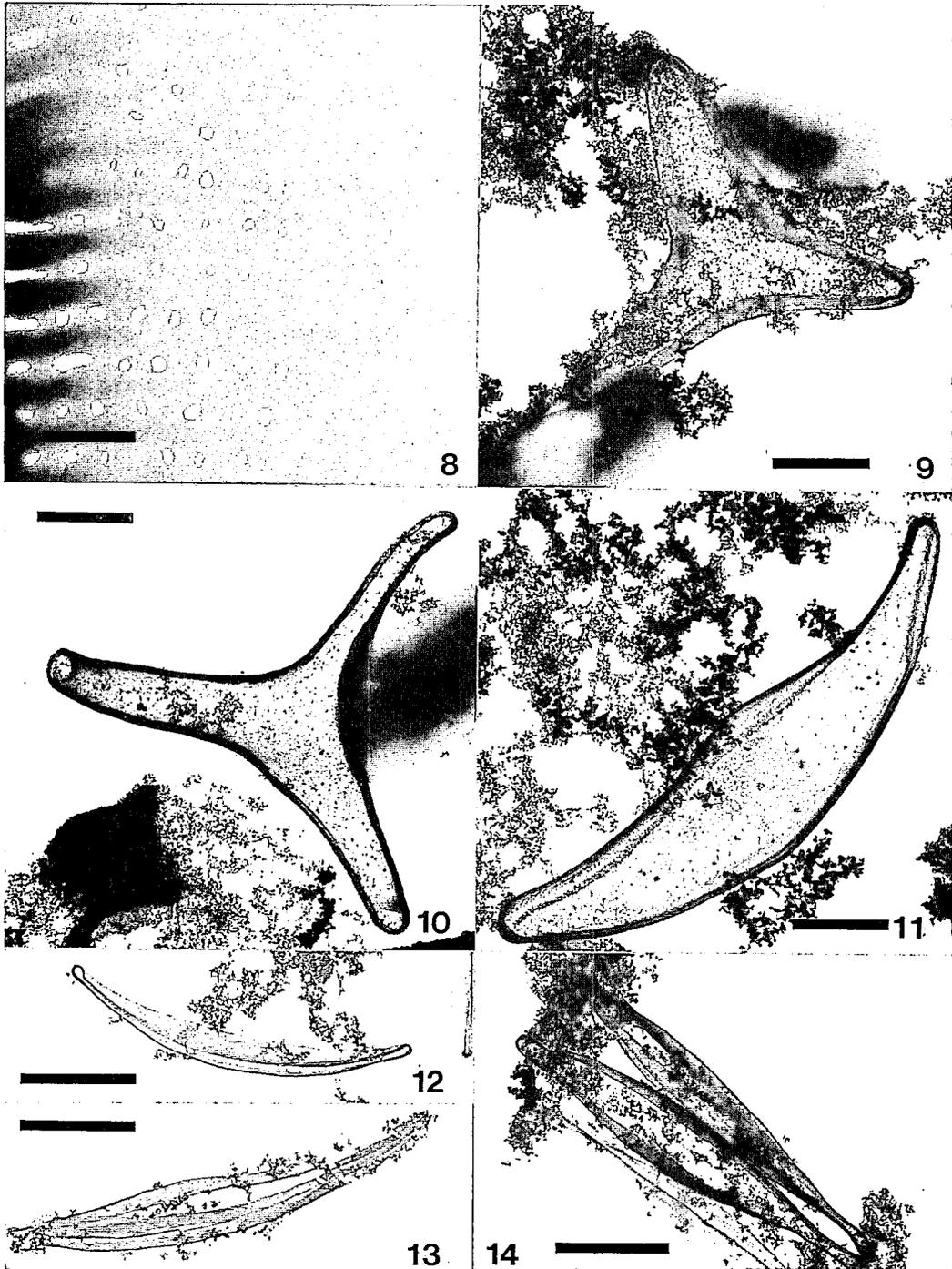
Figs. 8–14. *Phaeodactylum tricornutum,* cleared specimens: 8, DIATO1 naviculoid valve showing details of striae ultrastructure (note irregular margin of the valve [scale = 0.2 μm]); 9, 10, PHAEO4 triradiate specimens showing amorphous particulate matrix of the valve (scale = 2 μm); 11, tumid dorsiventrally arched fusiform cell of PHAEO4 (scale = 2 μm); 12, PHAEO4; 13, PHAEO2 striated girdle bands; 14, PHAEO2 chain of cells showing striated girdle bands (scale = 5 μm for Figs. 12–14).

Wilson (1946), but shorter than those reported by Lewin (1958). Many fusiform valves have a distinctly dorsiventral appearance (Fig. 3), cells which are thought to be transitional between fusiform and triradiate cells (Borowitzka and Volcani 1978). In TEM the valves appear to be composed of the same particulate matrix as the triradiate cells (Fig. 11). The ultrastructure of these walls has been covered in detail elsewhere. They consist of an external corrugated layer subtended by a thicker fibrillar layer embedded in an amorphous matrix (Reimann and Volcani 1968, Borowitzka and Volcani 1978). All of these wall components have been assumed to be completely organic. Valve face and mantle regions are evident (Figs. 11, 12). The cingulum consists of a single siliceous striated band (Figs. 13, 14), which has been examined in detail elsewhere (Neuville et al. 1971, Borowitzka and Volcani 1978).

In cleared material some fusiform frustules appeared more refractive than others (Fig. 4). These cells had the appearance of having silica in their valves, even though previous workers reported that fusiform cell walls were unsilicified (Lewin 1958, Lewin et al. 1958, Borowitzka and Volcani 1978). In light of this finding, elemental analysis of cleared cell walls of cells grown in silica-deficient media was undertaken. The EDAX readout indicated the presence of silica, as well as smaller amounts of Na, Mg, Al, P, S, and Ca (Fig. 15).

DISCUSSION ·

The morphological observations on inland and marine strains of *Phaeodactylum tricornutum* presented in this paper agree with the observations made by others (Wilson 1946, Lewin et al. 1958, Reimann and Volcani 1968, Borowitzka et al. 1977, Borowitzka and Volcani 1978). The inland strains did not differ morphologically from the marine strains. It is interesting to note that the Great Salt Lake strains were typically triradiate, the Soap Lake form produced only oval cells, and the marine forms were typically fusiform. However, given the past history of this species in culture (Wilson 1946), little significance can be attached to such differences.

External mucilaginous polysaccharides were associated only with oval cells, in agreement with the observations of Lewin et al. (1958).
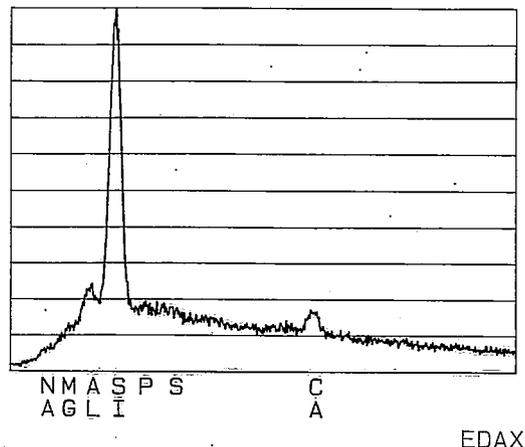

Fig. 15. EDAX elemental analysis of fusiform cell of *Phaeodactylum tricornutum.* Note the strong silica peak.

The ability of oval cells to grow on agar, the absence of mucilage from triradiate and fusiform cells, and the higher buoyancy of triradiate and fusiform cells suggest that the oval form is benthic, while the other forms are planktonic (Round et al. 1990). One wonders if in a small inland lake such as Soap Lake there is less opportunity for planktonic forms, and hence the dominance of the oval form.

By far the most surprising observation in this paper is the presence of silica in the cell walls of the fusiform and triradiate cells. Because this finding contradicted the assumptions of other workers, a number of valves were studied with EDAX elemental analysis. Care was taken to focus the beam on a very small region in the center of the valve to avoid picking up a silica signal from the siliceous bands. Lewin et al. (1958) reported the presence of flocculent, structureless silica following nitric acid digestion of fusiform cells. They assumed that the silica was from internal reserves, not from walls. Reimann and Volcani (1968) and Borowitzka and Volcani (1978) presented high-resolution TEM micrographs of the cell walls of *Phaeodactylum* fusiform cells. From examination of these micrographs, it seems possible that the fibrillar electron-dense layer of the wall may contain silica embedded in an organic matrix. Alternatively, silica may be present in some amorphous form. Since the silica is not organized into a solid siliceous structure as in other diatoms, it has been disregarded as a component of the

walls, which have been falsely assumed to be totally organic.

It appears that silica is present to varying degrees within fusiform and triradiate cells (Fig. 4). The cultures in this study, routinely grown with fairly high available silica, were transferred to silica-free medium only a few days before analysis. It may be that others (Reimann and Volcani 1968, Borowitzka and Volcani 1978) have used low-silica media and thus not favored facultative silica deposition in fusiform and triradiate cells.

As this enigmatic and peculiar diatom continues to be found in new localities, it will probably continue to draw the interest of phycologists. It seems very possible that the taxon is widely distributed in inland saline water and has avoided detection because the cell walls tend to be destroyed in standard diatom preparation procedures.

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