Scanning Electron Microscope: Potentials in the Morphology of Microorganisms

Abstract. *Morphologic characteristics related to ecology and evolutionary sequences, and to specific, generic, and familial relations, can now be determined with the scanning electron microscope. These detailed characteristics will help to establish a more natural faunal classification and will enable more accurate ecologic and biostratigraphic correlations.*

Detailed study of microorganisms has always been hampered by our inability to observe minute structures of test morphology. The imaging capability of the scanning electron microscope now enables the researcher to observe effectively many of the fine details upon which faunal classifications are based; the microscope also provides information on and permits illustration of features that have not been observed previously (see cover).

This microscope became commercially available in 1966 and differs from the transmission instrument in many ways. The depth of field (500 times that of a light microscope) and high resolution (ten times that of a light microscope) are complemented by a very wide range of magnifications (× 50 to greater than × 100,000); development and operation of this microscope have been discussed (*1*, *2*). Specimens can be observed directly within a few minutes of being mounted; troublesome delays, due to faulty replicas, normally experienced by the transmission electron microscopist are completely eliminated, and suitably prepared whole specimens are used rather than thin replicas.

The following technique is used for the study of various microorganisms such as foraminifers, diatoms, radiolarians, and ostracodes. Specimens are mounted with a diluted solution of tragacanth containing a small amount of glycerin. The mounts are allowed to dry at room temperature before being placed on a rotary turntable in a high-vacuum coating unit. Two coating runs (coating angles, 45° and 10°) are made, and the specimens are coated with gold to a thickness of 300 Å; conductive coatings of 100 to 200 Å also have been used. Photographs are taken at an accelerating voltage of 25 kv. Scanning time required to record the pictures, with a single-line scan, is 20 seconds. Panatomic-X 35-mm film is used. Morphologic characteristics of specimens are then studied in detail.

The thinnest conductive coatings applicable to microorganisms have not yet been determined. Pease *et al.* (*2*) have photographed living insects without conductive coatings, but I was unsuccessful with foraminifers, radiolarians, diatoms, ostracodes, and dinoflagellates without use of conductive coatings. Much higher contrast in image of the specimen is possible if nonmetallic specimens are coated.

Expensive thin-film monitoring equipment is necessary to determine accurately the minimum thickness of coating that can be applied. New techniques incorporating high-vacuum evaporation are being devised for the deep penetration of metallic molecules within individual pore structures. Beam angle, beam collimation, and metal-particle speed, together with a variable substrate temperature, are a few of the many important aspects of high-vacuum evaporation now being investigated.

Differences in spinal development and shell strengthening with depth in the marine environment are exemplified by *Globigerina bulloides* d'Orbigny (Figs. 1 and 2). Strong spinal development in the apertural region is present in both specimens shown, but the specimen collected at 200 m (Fig. 1) displays distinct thickening and orientation of "flying buttress" support spines, which are evident in both the apertural and sutural regions. The specimen of *G. bulloides* collected at 50 m (Fig. 2) is characterized by extremely long, narrow, pointed spines lacking the thickening mentioned.

Most specimens of planktonic Foraminifera collected at depths greater than 100 m exemplify some form of primary bilamellar shell growth and secondary thickening. Bilamellar growth is shown in spinal thickening originating at the base of each spine and progressing to the apical end. Secondary growth (usually layering) consists of thick calcite crusts deposited over the primary shell.

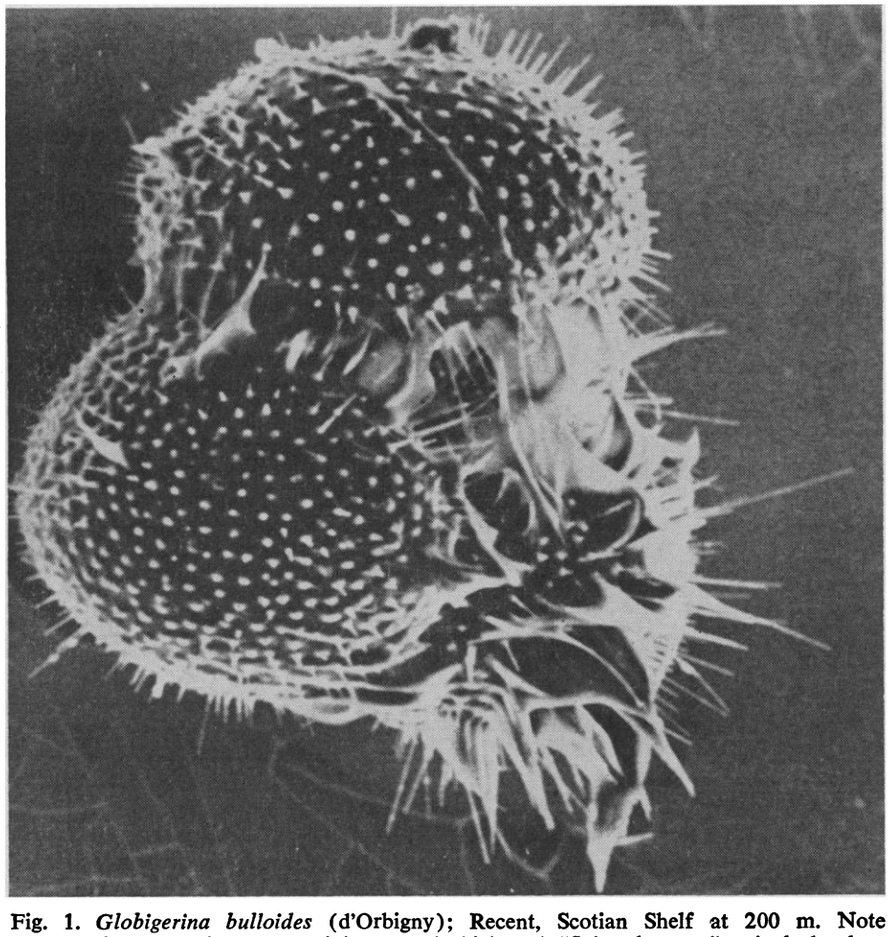

Fig. 1. *Globigerina bulloides* (d'Orbigny); Recent, Scotian Shelf at 200 m. Note narrow elongate spines on periphery, and thickened "flying buttress" spinal development in the apertural region (× 400).

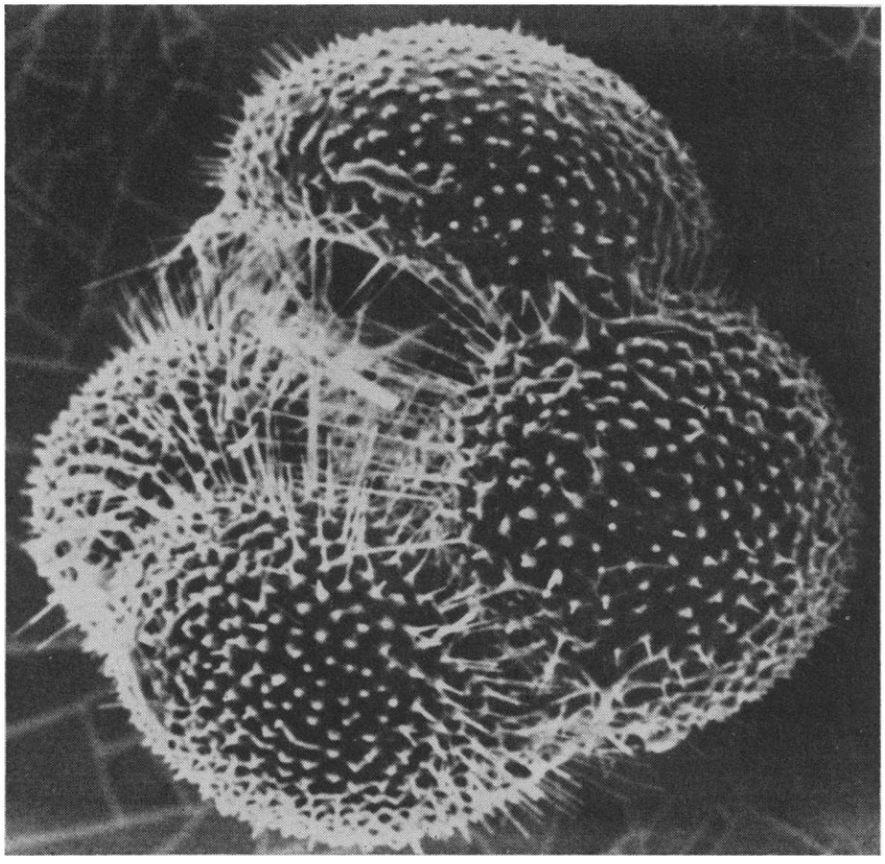

Fig. 2. *Globigerina bulloides* (d'Orbigny); Recent, Scotian Shelf at 50 m. Note narrow elongate spines over entire surface, concentration of spines in apertural region, and absence of any form of bilamellar or secondary thickening (× 400).

The secondary layers can be counted by focusing on individual pores. Both bilamellar and secondary thickening support the hypothesis (*3*) that shell thickening occurs at depth in adult stages of planktonic Foraminifera. Spinal development in *G. bulloides* at depth is contrary to findings of Bé and Hamlin (*4*), who found spines only in juveniles living at or near the surface of the ocean.

I have also investigated differences in microstructure of other foraminiferal species (*5*). *Globoquadrina dehiscens dehiscens* (Chapman, Parr, and Collins) differs radically from *Globigerina* in wall structure as well as in having prominent apertural flaps covering each aperture. *Globigerinoides trilobus trilobus* (Ruess) displays a heavy cancellate pore pattern, characteristic of the *Globigerinoides* group. However, *Globigerinoides trilobus immaturus* LeRoy, considered by many to be a member within the *G. trilobus* (s.l.) evolutionary sequence, has a surface covered with irregularly spaced knobs and small circular pores; this wall structure resembles that of *Globigerina*.

I have mentioned only a few morphologic characteristics. Many detailed features of various microorganisms, hitherto unavailable, are being investigated and will now add greatly to the determination of evolutionary sequences and specific, generic, and familial relations; they will enable more natural classification of these microorganisms. This new insight can be attributed to the scanning electron microscope.

GRANT A. BARTLETT

Micropaleontology, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, Nova Scotia

References and Notes

1. C. W. Oatley, *Sci. Progr. Oxford* **54**, 483 (1966); ———, W. C. Nixon, R. F. W. Pease, *Advan. Electron. Electron Phys.* **21**, 181 (1965); T. L. Hayes, R. F. W. Pease, L. W. McDonald, *Lab. Invest.* **15**, 1320 (1966).
2. R. F. W. Pease, T. L. Hayes, A. S. Camp, N. M. Amer, *Science* **154**, 1185 (1966).
3. A. W. H. Bé, *Micropaleontology* **11**, 1 (1965); ——— and L. Lott, *Science* **145**, 823 (1964); A. W. H. Bé, A. McIntyre, D. L. Breger, *Ecol. Geol. Helv.* **59**, 2 (1966).
4. A. W. H. Bé and W. H. Hamlin, *Micropaleontology*, **13**, 1, 87 (1967).
5. G. A. Bartlett, *Can. J. Earth Sci.*, in press.
6. I thank Charles Godden, Marine Geology Division, Bedford Institute of Oceanography, for his active interest and the photographs [taken with the aid of the Jeolco scanning electron microscope; Jeolco (U.S.A.) Inc., 477 Riverside Drive, Medford, Mass.].

21 August 1967

Electrophoretic Variants of α-Glycerophosphate Dehydrogenase in Drosophila melanogaster

Abstract. *Two alleles of* Gdh, *the locus specifying the electrophoretic mobility of α-glycerophosphate dehydrogenase, are found in* Drosophila melanogaster. *The gene is located on the second chromosome at a map position of 17.8. Hybrid enzyme molecules are found in heterozygotes.*

The enzyme α-glycerophosphate dehydrogenase (GDH) catalyzes the oxidation of α-glycerophosphate to dihydroxyacetone phosphate and the reverse reaction. Extraordinarily high activity of this enzyme is found in thoracic muscle of insects (*1*, *2*), where it plays an important role in the rapid production of energy from carbohydrate [see reviews by Sacktor (*3*) and Chefurka (*4*)].

Electrophoretic variants of at least 12 enzymes are known in *Drosophila melanogaster* [see review by Shaw (*5*)]. More than one electrophoretic type of an enzyme is more the rule than the exception in this species. The genetic loci responsible for the variations have in most cases been located on the linkage map of *Drosophila*.

A method for acrylamide gel electrophoresis of α-glycerophosphate dehydrogenase of *Drosophila* has been described by Sims (*6*). Hubby and Throckmorton (*7*) examined GDH electrophoretic patterns of several species of the virilis group of *Drosophila* and found differences between but not

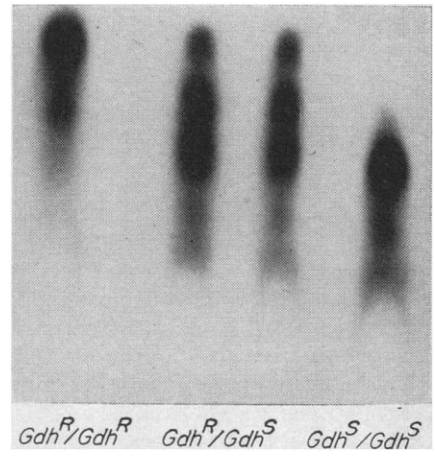

Fig. 1. Acrylamide-gel electrophoresis of α-glycerophosphate dehydrogenases of *Drosophila melanogaster*. On the left is the homozygote of the rapid type of GDH. On the right is the homozygote of the slow type of GDH. In the center are two heterozygotes of the two alleles.