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APPLICATION OF THE SCANNING ELECTRON MICROSCOPE TO THE STUDY OF TROPICAL MICROPLANKTON*

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ABSTRACT

The author describes the assets and limitations of the scanning electron microscope used under routine conditions for the floristic analysis of marine microplankton. Drawing on experience gained in the examination of Indian Ocean material collected by the R.V. *ANTON BRUUN* the examination of diatoms, tintinnids, radiolaria, and in particular, dinoflagellates is discussed. The considerable simplicity of the techniques involved, combined with the type of image obtained, make the instrument highly suitable for the examination of field material for taxonomical, morphological and ecological purposes. This is counterbalanced to a certain extent by the great cost of the instrument.

INTRODUCTION

IN the study of the community structure of marine plankton the instrument still most extensively used is the light microscope. Typically, high quality standard microscopes are used for qualitative purposes and the inverted 'plankton micrscope' is used for quantitative, species-level analyses. Only in the study of coccolithophorids has the electron microscope become of routine necessity (Braarud, 1955), a feature which has no doubt discouraged many from their study. In reality the direct transmission examination of wall structures in coccolithophorids and diatoms has not proved very difficult, although the manipulation preliminaries require patience and the transmission electron microscope (T.E.M.) requires further care and skill in operation. Certainly the examination of the cytoplasm, involving careful fixation, embedding, cutting and staining is sufficiently demanding and time consuming to rule out its usefulness in routine plankton analysis. Allen (1968) has reported that glutaraldehyde alone may be used as a preservative in field studies such that the cells can be subsequently cut for T.E.M. examination, thus eliminating the necessity for the highly dangerous use of osmium tetroxide. However, glutaraldehyde is also unpleasant to work with and the laboratory manipulations are still tedious. Furthermore, one of the chief drawbacks to the field ecologist/taxonomist in the use of the T.E.M. is that it is difficult to relate such high power sections with conventional taxonomic descriptions.

In the last four years many papers have appeared extolling the virtues of the scanning electron microscope (S.E.M.) in the examination of many types of organisms, particularly in palynology (Echlin, 1968) and in micropaleontology (Hay and Sandburg, 1967). In essence it differs from the transmission image in that it pro-

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vides a surface image rather similar to that provided by carbon replicas used with the T.E.M. However, the specimen itself is examined while thinly coated with metal. By this means not only hard exoskeletal structure can be examined, but also, with suitable care in drying and fixing, outer cytoplasmic features such as the pellicle of ciliates (Small and Marszalek, 1969 ; Horridge and Tamm, 1969), the pseudopodia of foraminifera (Marszalek, 1969) and the folded outer layers of gregarines (Vavra and Small, 1969).

The S.E.M. commends itself to microplankton work in three particular respects. The external image, seen at a range of magnifications which overlaps the light microscope at the lower end of the scale, is readily comparable (although much less equivocal) to descriptions based on light microscopy. The specimens can be tilted through 90° and rotated through 360°, allowing the type of examination of one specimen formerly limited to cases where many specimens of the same species were available. Finally, and most importantly from the ecologist's point of view, the techniques necessary for the detailed examination of most of the common microplankton species are very simple and the instrument is relatively simple to operate. At present the price of the S.E.M. severely restricts the number of people having access to one, but specimens can be prepared and stored under vacuum in a dessicator for long periods of time until such an instrument is obtained, or until another worker consents to examine the material.

For two years the author has been using a Cambridge Mark II A Stereoscan microscope on formalin-preserved microplankton collected by the R. V. *ANTON BRUUN* during the International Indian Ocean Expedition. Essentially this was a re-examination of 213 samples previously analysed with the light microscope. Apart from exploring the instrument's potential by applying it to the examination of dinoflagellates, diatoms, tintinnids, radiolarians and a few foraminifera, it has been used specifically to elucidate some problems of structural interpretation that had arisen during the previous light microscope examination. As a result more than two thousand micrographs have been obtained. This short paper will report on the assets and drawbacks that have been noted so far, particularly with regard to the dinoflagellates studied.

Methods Used

The simplest technique used by the author was as follows: The specimens were already preserved in formalin sea-water and so the first step involved washing the organisms to remove the salt. This was carried out by transferring the cells individually with a fine pipette through three washes of distilled water under a good dissecting microscope. They were transferred to an aluminium disc, a 'stub', with a highly polished, clean surface, and allowed to dry in small drops of water with no special precautions taken other than to keep out dust. Frequently twenty or more specimens were placed on stubs 1.3 cm in diameter. The cells could be aligned somewhat in the drops before drying by using a hair from a paint brush.

When dry the stubs were placed, usually four at a time, in a 'shadowing' apparatus and coated in a vacuum with metal evaporated at an angle of 45° to the rotating specimens. Usually fine gauge wire consisting of a 40% Palladium, 60% Gold mixture was evaporated (No. 1222, Ernest Fullam, Schenectady, N. Y.). Approximately 14 cm of wire gave a coating which did not obscure detail but which could withstand 'burning' by the electron beam. As an added precaution a fur-

ther four to six cm was evaporated at a lower angle when examining very rugose, spherical specimens.

The stubs could then be immediately examined and also stored in a dessicator for future study. In the microscope contrast settings of gamma 1 or 2, and an accelerating voltage of 20 Kv, were most frequently used.

Variations on the above procedure included the batch preparations of plankton ' spreads ', washing the unselected sample by settling through four or five transfers of distilled water. This can produce a great quantity of material for examination but the result is not as clean, it being difficult to remove the salt adequately. Also, the right concentration of material to minimise the obscuring of specimens by others overlying them requires skilled judgement.

Aluminium metal can be used as a cheaper material for shadowing but the coating deteriorates rapidly and the specimens should not be stored before examination. Gold can also be used, but the author has found the Palladium/Gold wire easier to handle and the results equally good.

In the comments that follow mention will be made of the more critical techniques used for special purposes (rapid fixation, freeze-drying, critical-point-drying). However, the theme of this paper is practicability in routine situations and so these other techniques will not be expanded upon even though even they are not particularly difficult to perform.

Observations

The great bulk of organisms studied by the author with the S.E.M. were dinoflagellates as this was the group subjected to particular study in the previous analysis of the material. However, before providing a summary of the observations on dinoflagellates, brief mention can be made of the results of examining members of other groups.

In general the oceanic diatoms (still containing their cell contents) were fragile and liable to collapse if they were air-dried on the discs, although much useful information could be obtained from the more robust specimens and fragments. Whereas strongly silicified diatoms from benthic deposits can be acid-cleaned for the S.E.M. in a similar manner to that classically used for permanent mounts for light microscopy, this technique is often too severe for most planktonic species. Hasle (1968 *a*, *b*) has apparently had some success with regular cleaning*. The best technique for delicate species in which the cytoplasm is still present seems to be quick-freezing on a thin piece of copper sheet plunged into liquid freon (preceded as usual by washing in distilled water) followed by slow drying in a freeze-drying apparatus, as used for ciliates by Small and Marszalek (1969). The small copper disc containing the specimens can then be cemented to the aluminium stub by using metallic paint such as ' silver dag '.

Tintinnids are usually represented in preserved samples by their empty loricae only, unless a very rapid killing agent (such as osmium tetroxide) is used. Con-

* Hasle and Fryxell (*Trans. Amer. Micr. Soc.*, 89 : 469-474, 1970) have developed a more gentle cleaning technique which works well for scanning electron microscopy.

sequently most loricas can be studied with ease by the simplest technique described earlier. Those with delicate prismatic walls tend to collapse into a shapeless mass. Although it has not been tried yet, the freeze-drying may prove the solution in these cases as well. One subject of considerable interest to the author has been the examination of accreted wall structures derived from other organisms. It is well known that many genera such as *Dictyocysta* and *Codonella* contain coccoliths incorporated within their walls. Diatom frustules are also commonly found in the loricae of many species, particularly in the genera *Codonellopsis*. Most surprising are the instances when the loricae appear to contain a great many structures from only one species (see figures in Kofoid and Campbell, 1939). A case is illustrated here of the bowl of *C. orthoceras* (Haeckel) Joergensen which was entirely covered with the frustules of an unidentified naviculoid diatom species. Other species, such as *C. brevicaudata* (Brandt) Kofoid et Campbell, commonly have valves of *Coscinodiscus* or *Thalassiosira* species imbedded within them. If these are derived from food by a similar mechanism to that in which the testacean amoeba *Difflugia* derives foreign particles for its test, these observations could provide interesting trophic information.

Coccolithophorids have not yet been examined by the author, but Gaarder (1970), as well as others, has used the S.E.M. to good effect.

Perhaps the simplest of all microplankton for examination of the skeletal structures are the radiolaria. The cytoplasm, if present, is often retracted so that only the central parts are obscured. The skeletons are generally very strong so that drying can be crudely effected without distortion, and most species can be acid-cleaned if central skeletal details are to be examined. However, this common practice may destroy hitherto unsuspected features such as the membraneous structure (possibly chitinous) seen closing the pores in the outer cortical shell of some species of *Hexalonche*. The open lattice construction of most species allows the metal coating to be quite thick without obscuring detail.

The foraminifera are somewhat similar to the radiolaria from the point of view of the S.E.M. although naturally the calcium carbonate test precludes the same acid treatment. The remarkable study of Marszalek (1969) on carefully fixed and freeze-dried *Iridia diaphana* pseudopodia suggests that this approach could produce much original information on the outer cytoplasmic features of both foraminifera and radiolaria.

The thecate dinoflagellates, and particularly those from tropical waters, possess some of the most elaborately differentiated wall structures of all protists. While the diatoms or raidolaria may appear at first sight to be as complex, in fact their structures usually consist of many repeating units. On the other hand the dinoflagellate theca, while often containing pore patterns of equal complexity, also is differentiated into decidedly different left and right sides, up and down, ventral and dorsal sides. The thecal units, the plates, closely fitted together, are used extensively in the taxonomy of the group. Consequently during field analyses one is frequently confronted with the necessity to view specimens from as many angles as possible and to attempt to discern the sutures demarcating the plates. Further, as most of the species are highly rounded, focussing problems abound. Like other planktonic groups long spines and horns often extend out in various planes which again may be difficult to determine.

It was hoped, naturally, at the beginning of the study, that the S.E.M. would solve all these problems, particularly with regard to details within the ventral area

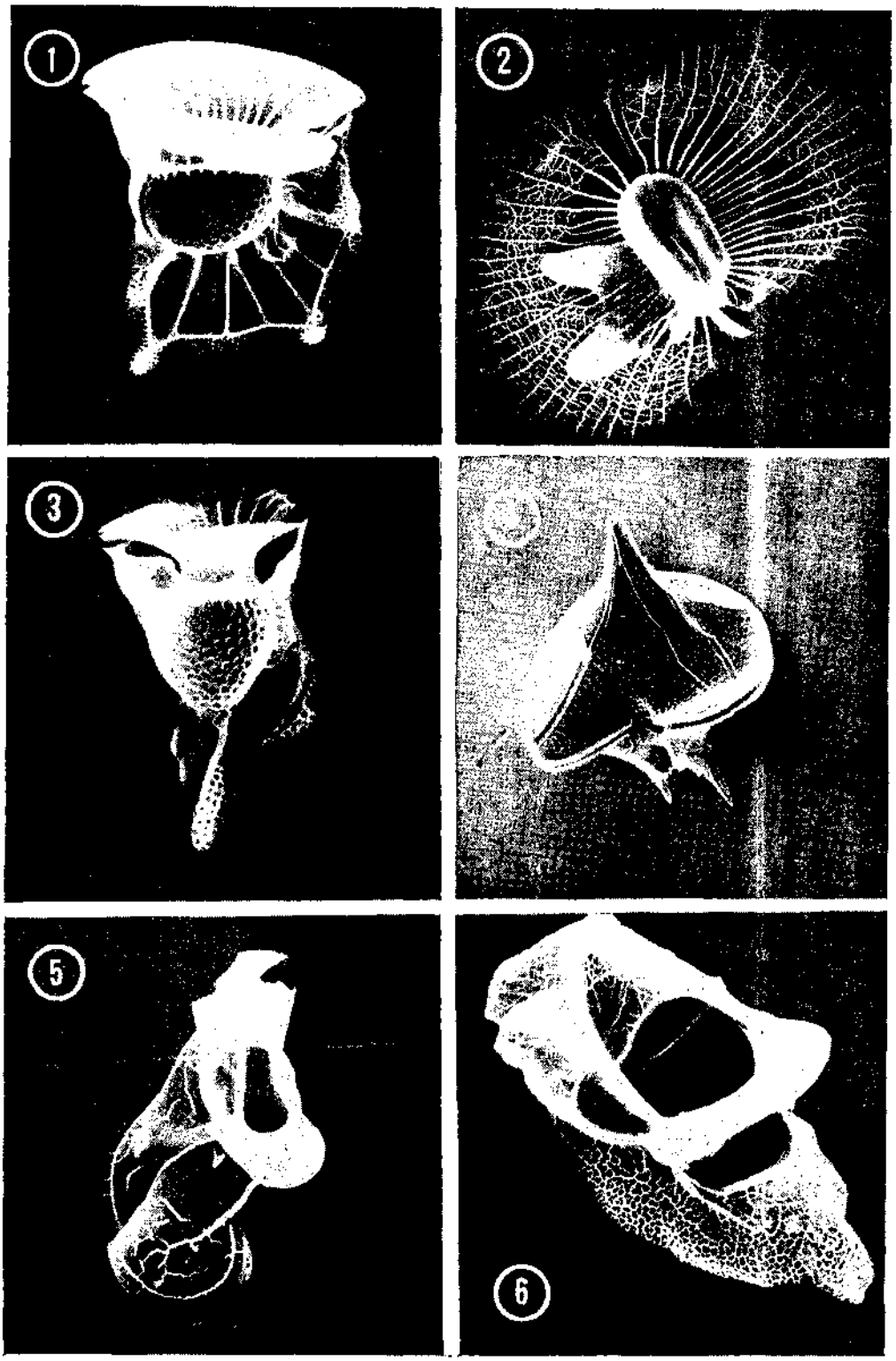

PLATE I. Scanning electron micrographs of Indian Ocean dinoflagellates. 1. *Ornithocercus quadratus* Schuett, right side, X 344 ; 2. *O. splendidus* Schuett, antapical view with left sulcal list folded over left side, X 314 ; 3. *Parahistioneis conica* Boehm, right side, X 656 ; 4. *Peridinium divergens* Ehrenberg ventroapical view, X 318 ; 5. *Histioneis dolon* Murray et Whitting, left side of young individual, X 433; and 6. *H. mitchellana* Murray et Whitting, left side, lists partly ruptured in drying, X 656.

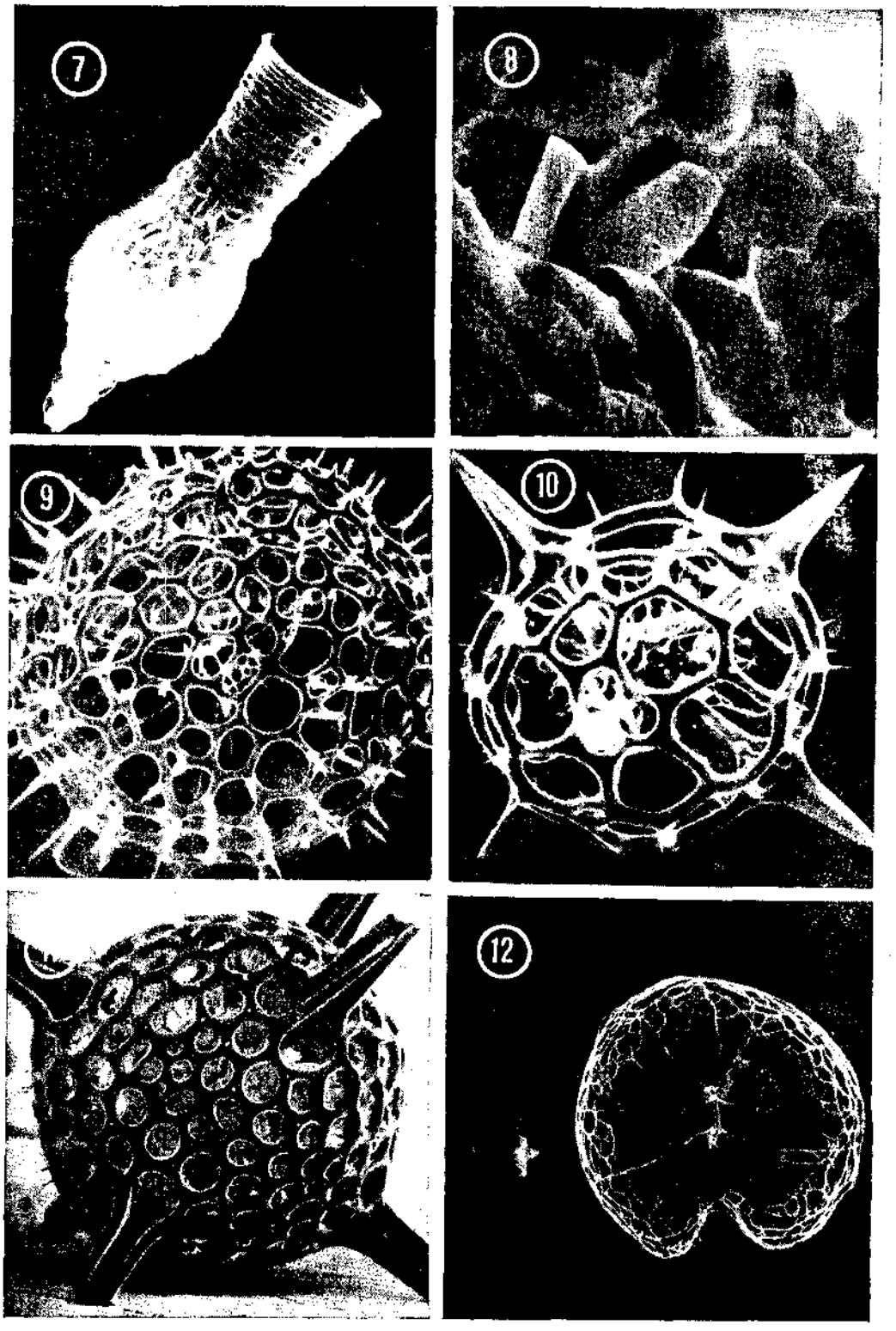

PLATE II. Indian Ocean microplankton. 7. A tintinnid, *Codonellopsis orthoceras* (Haeckel), the bowl of which is covered with diatom frustules (see text), X 360; 8. Detail of the diatoms in Fig. 7. The species of pennate, posessing pseudoraphes on both valves, is unknown, X 3116; 9. A radiolarian skeleton: *Haliomma erinaceus* Haeckel, X 354; 10. The skeleton of *Hexacontium enthacanthum* Joergensen, X 373; 11. A radiolarian, *Hexalonche* sp., in which the apertures in the cortical shell were apparently closed by a membrane, X 557; and 12. A large radiolarian, *Nephrospyris* spl., with a dinoflagellate, *Certocorys bipes* (Cleve) Kofoid to the left beside it, X 137.

(from where the flagella arise). It was soon discovered that, as is usually the case, the instrument was of greater value than hoped for most purposes, but was not able to aid in some of the difficulties. The 'manipulability' of the species was a considerable asset in resolving the spatial relations of projections from the theca. For example, in the rare genus *Cladopyxis* it was discovered that the projections arise in a single plane which, however, transects the girdle plane, a feature causing interpretational difficulties in the past. Very flat species can be examined on their sides, a virtual impossibility in water mounts.

Little help was given in sutural analysis as in the cases where sutures are difficult to see, as in the genus *Diplopsalis* or some peridinia, there is little relief associated with the suture. Consequently, apart from the great depth of focus, sutures show more clearly by their shadows as seen with light microscopy. Where the sutures were accompanied by ridges they could be very clearly resolved as, for example, in *Peridinium elegans* Cleve. The ventral areas were frequently obscured as a result of folding of the flange-like lists that often surround them. However, a great deal of information has been obtained concerning the list structures, especially within the dinophysoid genera *Ornithocercus*, *Parahistioneis*, *Histioneis* and *Citharistes*. New taxonomic insights into *Ornithocercus* have been gained as a result of examining the ribs on the lists (Taylor, 1971). The other three dinophysoid genera mentioned above are extremely rare and it was fortunate that they could be subjected to S.E.M. examination.

One of the assets of the use of the S.E.M. on organisms such as these is that, although the primary purpose may be accurate floristic analysis, if one is aware of the key problems in the biology of the groups, there is always a 'spin-off' in valuable information through chance as a result of the examination of large amounts of material. For this reason broken specimens, accidentally or deliberately produced, can yield much valuable information. With the aid of suitable adhesives (electro-conductive, quick drying), thecae can be attached to discs and then delicately probed until they separate into their components.

Finally, as an instrument of illustration for whole microplankton organisms, the S.E.M. appears to be unsurpassed and one can forsee the compilation of large atlases as identification aids in the near future, providing publication funds are available.

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