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ON PROCESSING FIELD AND CULTURE SAMPLES OF DESMIDS (DESMIDIALES, CHLOROPHYTA) FOR SCANNING ELECTRON MICROSCOPY

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A procedure is outlined for preparing both field collected and laboratory grown desmid populations for scanning electron microscopy which avoids the use of the potentially hazardous chemicals glutaraldehyde and osmium tetroxide and reduces preparation time substantially. FAA is utilized both in fixation and mucilage removal, and living material can be prepared for critical point drying in as little as 90 min. Limitations of this procedure are outlined briefly and the use of SEM material in systematic studies is commented upon.

Recognition of the potential of scanning electron microscopy (SEM) as a tool in studies of morphogenesis, cell wall ornamentation, and systematics of desmids (Desmidiales, Chlorophyta) has led to the development of several techniques (Lyon, 1969; Pickett–Heaps, 1973, 1974) for the preparation of desmids for SEM examination. These procedures utilize specimens grown in culture and involve apparent air drying from water or alcohol (Lyon, 1969, see Pickett–Heaps, 1973, p. 114) or critical point drying (Pickett–Heaps, 1973, 1974) after pretreatment in "Glusulase" (a polysaccharidase) for mucilage removal, fixation in glutaraldehyde, post-fixation in osmium tetroxide, and dehydration via an acetone series.

Although these techniques have yielded some excellent scanning electron micrographs (see Pickett–Heaps, 1975), certain limitations apparently exist. Thus, for example, the procedure seems applicable only to specimens growing in unialgal culture. In addition, handling prior to critical point drying involves the use of the potentially hazardous chemicals glutaraldehyde and osmium tetroxide and requires up to 6·5 h. Finally, removal of the copious quantities of mucilage from desmid cell walls commonly appears difficult (Pickett–Heaps, 1973, p. 116; 1975, p. 408–409, 414) and leads to inconsistent results and many unusable preparations.

In conjunction with studies of polymorphic behaviour in field and experimental populations of various desmids, we have developed an SEM preparation procedure which can be utilized directly on field populations as well as on experimental cultures, which avoids the use of glutaraldehyde and osmium tetroxide and which reduces preparation time substantially. In addition, the problem of mucilage removal appears to be minimal, but it has not been solved entirely. This paper outlines the procedure and evaluates its potential in quantitative and qualitative morphological and systematic investigations of desmids.

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MATERIALS AND METHODS

COLLECTION AND MAINTENANCE

Field populations of desmid plankton were gathered by passing 10–20 l of surface water through a No. 25 silk mesh plankton net and transferring the concentrate to a 50 ml storage vial prior to fixation (see below). Desmids in the epiphyton were obtained by hand squeezing quantities of the macrophyte host and collecting the run off in a jar or by retaining the macrophyte sample and subsequently removing the desmids by agitation (Gough & Woelkerling, 1976). Clonal cultures of taxa isolated from acid waters were grown in liquid Waris medium (Waris, 1953) at a pH of 6·0 while taxa isolated from alkaline waters were grown in liquid Bristol's medium (Starr, 1964) at a pH of 8·0; 1 $\mu g\ l^{-1}$ of vitamin B_{12} was added to all containers. Material was maintained at 20°C on a 16/8 h light/dark cycle. References used for desmid identification include Iréneé-Marie (1939), Krieger (1935, 1937, 1939), and Smith (1924).

FIXATION AND MUCILAGE REMOVAL

All fixation was effected with the addition of sufficient FAA (7 : 2 : 1 : 1 :: water : formalin : glacial acetic acid : 95% ethanol) to double the volume of liquid in which the desmids were suspended. Field material was fixed at the time of collection and stored in FAA until studied. Material preserved in FAA for over 15 months yielded good results, and it appears that samples destined for SEM work can be kept in FAA indefinitely. Cells grown in culture were fixed in FAA for a minimum of 30 min but in most cases for 2–48 h.

While FAA itself apparently facilitates the removal of most excess mucilage from the cell walls by acid hydrolysis (see Gough & Woelkerling, 1976), cleaner cells sometimes could be obtained by sonication for 5–45 s in 1·0 N HCl. Caution must be exercised, however, since the use of formaldehyde and HCl may lead to formation of vapours of Bis-chloromethylether, a potentially powerful carcinogen.

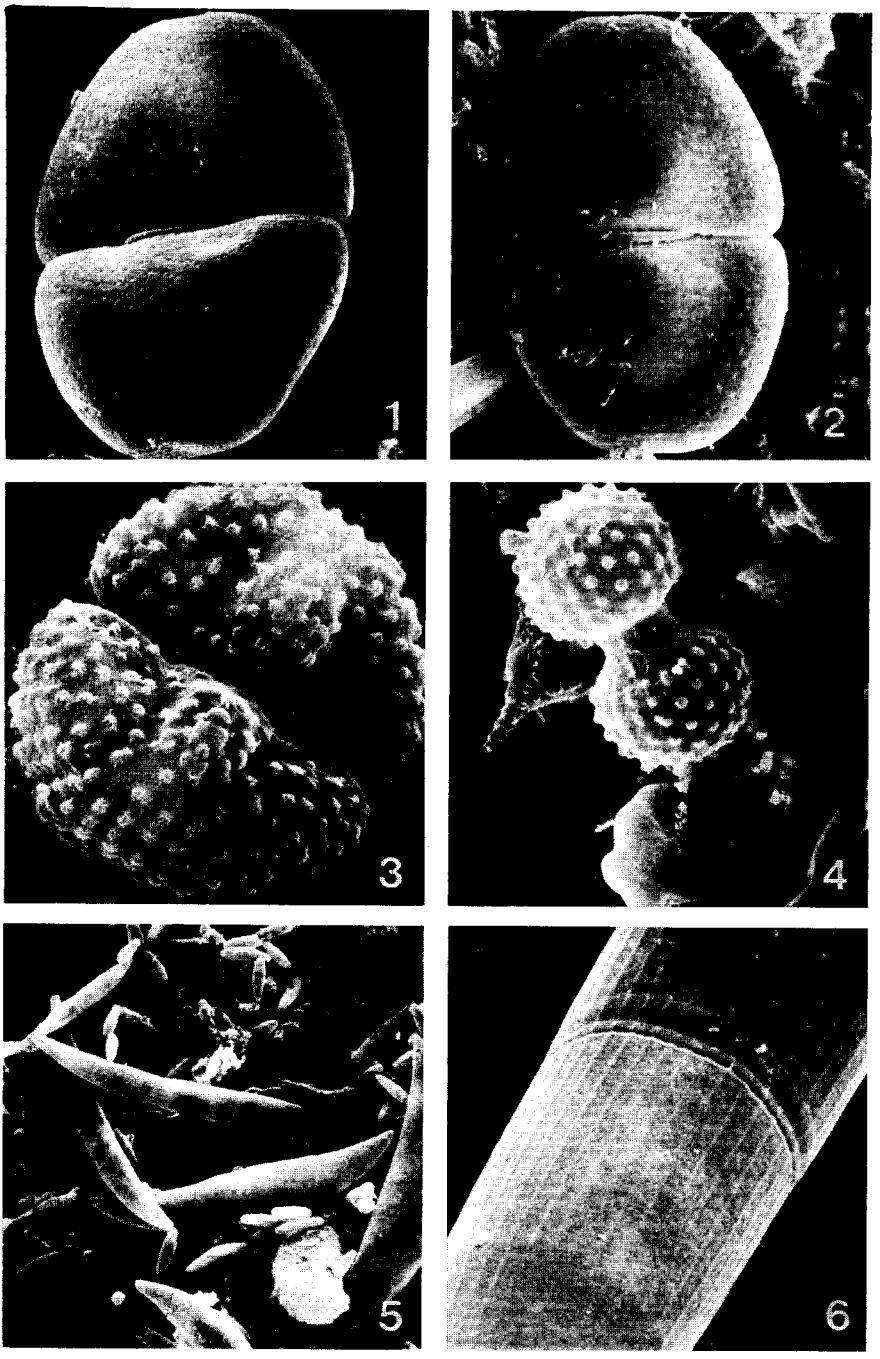
FURTHER HANDLING

After fixation, the cells were collected by suction-filtration from FAA onto a membrane filter fitted to a membrane filter flask assembly. Filter selection was based on criteria outlined by Pickett-Heaps (1973). Pore sizes of 10 μm and 0·45 μm both worked satisfactorily, and the use of gridded filters facilitated recognition of top and bottom sides during later handling. Once on the filters, specimens were partially dehydrated with three successive aliquots of 95% ethanol. Then filters were transferred quickly to petri dishes of 100% ethanol, cut into 6 mm squares with a scalpel, and transferred to vials of 100% ethanol for temporary storage. It is vitally important that filters are not allowed to dry out during the above sequence of steps, or severe collapse and distortion of cells will result.

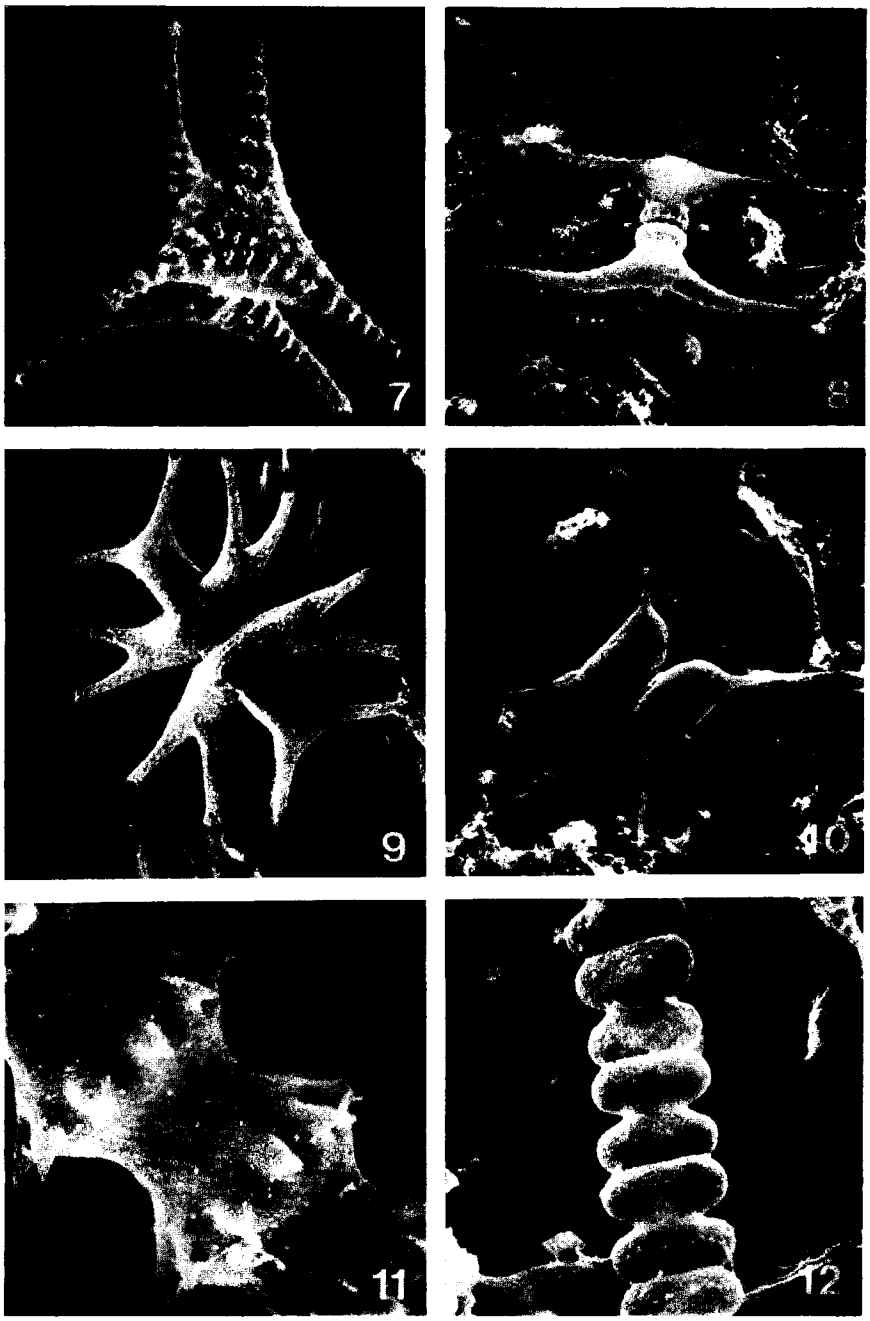
Final processing involved placing the 6 mm squares in plastic baskets, critical point drying (see Pickett-Heaps, 1973 for details) from 100% ethanol in a Denton CPD apparatus, affixing the dried squares to aluminium specimen stubs with double-stick tape, grounding the squares with an edging of silver paint, and coating the specimens with gold-palladium in a Denton Vacuum Evaporator. Specimens were examined at 20 μV using a JOEL JSM-U3 scanning electron microscope, and Polaroid no. 55 P/N film was used for photography.

RESULTS AND DISCUSSION

The above procedure has been applied successfully to a variety of desmid genera obtained directly from field samples as well as from clonal cultures, and morphological features of systematic importance are readily visible. In *Cosmarium* (Figs 1–4), for example, punctate and verrucate wall ornamentation patterns are both readily discernable, and comparable results can be obtained for the same species whether cells come from experimental (Fig. 1) or field (Fig. 2) populations. Similarly, cells from cultures of *Closterium* (Figs 5, 6) can be collected on filters in sufficient numbers to enable measurement of cell size and curvature, and data on bands, ribs and other morphological characters of systematic significance can be gathered as well. Cells of radiate taxa (Figs 7–9),



FIGS 1–6. Fig. 1. *Cosmarium granatum* Brebisson in Ralfs 1848: 96. ×1600. Fig. 2. *Cosmarium granatum* Brebisson in Ralfs 1848: 96. ×1600. Fig. 3. *Cosmarium punctulatum* Brebisson 1856: 129. ×2200. Fig. 4. *Cosmarium portianum* Archer 1860: 235. ×925. Fig. 5. *Closterium* sp. A mixed population of several species. ×145. Fig. 6. *Closterium regulare* Brebisson 1856: 148. ×1600.



FIGS 7–12. Fig. 7. *Staurastrum manfeldtii* Delponte 1877: 160. ×660. Fig. 8. *Staurastrum leptocladum* Nordstedt 1869 : 228. ×780. Fig. 9. *Micrasterias radiata* Hassall in West and West 1905: 113. ×475. Fig. 10. *Arthrodesmus bulnheimii* Raciborski 1889: 95. ×200. Fig. 11. *Triploceras gracile* Bailey 1851: 38. Portion of a semicell showing whorls of spines. ×1750. Fig. 12. *Spondylosium planum* (Wolle) W. and G. S. West 1913: 430. ×1400.

spined taxa (Figs 10, 11), and filamentous taxa (Fig. 12) commonly emerge from the processing without collapse, distortion, or excessive mucilage.

In addition to its applicability to both laboratory grown and field populations, this procedure eliminates (assuming HCl is not used) the need for potentially hazardous chemicals such as glutaraldehyde and osmium tetroxide. Living material can be prepared for critical point drying in as little as 90 min, or it can be preserved and stored indefinitely for later use.

The problem of mucilage removal has not been solved entirely. The need for a 3 h polysaccharidase treatment and washing prior to fixation (Pickett–Heaps, 1973) has been eliminated because of the apparent mucilage removal capabilities of FAA (see Gough & Woelkerling, 1976). Total mucilage removal, however, has not been possible with consistency, and in some preparations, no more than approximately 25% of the cells emerged in an acceptably clean condition (i.e., with all features of wall ornamentation clearly evident). A number of other methods of mucilage removal have been attempted (e.g. use of ammonium oxalate, EDTA, dichromate cleaners, sodium hydroxide, detergent) in hopes of obtaining more consistent results, but in all cases the cells were either completely destroyed or the outcome was very erratic as compared with the FAA treatment.

A second difficulty was sometimes encountered in the preparation of large-celled species of *Closterium* where many of the cells would collapse during preparation. Such collapse was minimized, however, if sonication times of only 5 s were used and critical point drying was very carefully regulated.

Excessive detritis or plant fragments (e.g., *Sphagnum* leaves) in field samples occasionally caused membrane filter pores to clog quickly, thereby reducing the ability of desmid cells to adhere to the filter during dehydration and critical point drying. Such debris also commonly obscured cell wall ornamentation (e.g. note debris on cells of *Euastrum* in background of Fig. 4). These problems, however, were usually overcome by filtering potentially troublesome field samples through cheesecloth prior to membrane filtration.

The use of SEM preparations for studies of cell wall morphogenesis and for desmid identification are readily apparent, especially in attempting species determinations in genera such as *Cosmarium* and *Staurastrum* where large numbers of taxa have been separated on differences in wall ornamentation. Their application in gathering quantitative data on intraspecific polymorphism in cell wall morphology requires further study. Present attempts of the authors (unpublished data) to gather quantitative data have been hampered by the expense involved in SEM photography and the time required to obtain the series of photos on populations needed for proper statistical assessment. Especially troublesome is the absolute necessity (for mathematical analyses) of obtaining both end views and face views of the same cell of species of *Cosmarium* and *Staurastrum*, and this difficulty has not been resolved as yet. Similarly we do not know whether cells adhere to membrane filters in random fashion or whether slight differences in morphology result in sufficiently great selectivity to cause subsequent bias in cells used for data collection.

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