A Simple and Rapid Scanning Electron Microscope Preparative Technique for Delicate "Gymnodinioid" Dinoflagellates

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ABSTRACT Light microscopy (LM) is routinely used to investigate delicate (unarmoured and lightly armoured) "gymnodinioid" dinoflagellate species but at this level of resolution, morphological features such as apical grooves, apical pores, thin thecal plates, and scales are often difficult to observe, thereby necessitating the use of scanning electron microscopy (SEM). Good results were obtained when harvested cells were fixed with osmium tetroxide (OsO_4) as the primary fixative, adhered with poly-L-lysine to round glass coverslips, dehydrated in an ethanol series, and dried with hexamethyldisilazane (HMDS). Poly-L-lysine has in the past effectively been used to adhere biological material such as human red blood cells, mouse leukemic cells, and marine dinoflagellates to glass coverslips. HMDS has been used to substitute critical point drying (CPD) to dry soft insect tissues, rat hepatic endothelial cells, and the cilia of rat trachea. By combining and fine-tuning these two protocols in SEM studies of delicate "gymnodinioid" dinoflagellates, it is possible to overcome cell distortion such as shrinking and collapsing that result from centrifuging, filtering, and CPD. The combination of poly-L-lysine and HMDS not only produces good results but also requires limited expertise and equipment, is inexpensive, and is less time-consuming. *Microsc. Res. Tech. 59:128–130, 2002.*

INTRODUCTION

Considerable confusion exists with regard to the identity of many delicate (unarmoured and lightly armoured) "gymnodinioid" dinoflagellate species. Genera such as *Gymnodinium, Gyrodinium, Amphidinium*, and *Katodinium* were erected during the late 1800s and early 1900s and were based on light microscope observations (Daugbjerg et al., 2000). However, at this level of resolution morphological features such as apical grooves, apical pores, thin thecal plates, and scales are often difficult to observe and can result in the misidentification of dinoflagellates, thereby necessitating the use of scanning electron microscopy (SEM) (Hansen, 1995; Steidinger et al., 1996). Attention has recently been focused on constructing a more satisfactory taxonomic system for unarmoured species by combining results obtained from light microscopy, electron microscopy as well as pigment and phylogenetic analysis (Daugbjerg et al., 2000; Hansen et al., 2000). Unfortunately, unlike their armoured counterparts, delicate dinoflagellate species lack thick cellulosic plates within their thecal vesicles, which render them highly susceptible to distortion during standard SEM techniques that involve centrifugation, filtration, fixation, dehydration, and critical point drying (CPD) (Truby, 1997). By using poly-L-lysine in combination with hexamethyldisilazane (HMDS), we have overcome cell distortion thereby obtaining results in which morphological features such as the apical grooves and cingulum displacement are clearly visible. Poly-L-lysine has in the past effectively been used to adhere biological material such as human red blood cells, mouse leukemic cells, as well as dinoflagellates to glass coverslips (Takayama, 1985; Tsutsui and Kumon, 1976). The adhesion of biological material to the surface of poly-L-lysine coated coverslips is a consequence of the attraction between the polyanionic surface of the biological material and the polycationic surface of the coverslip. HMDS, which has a reduced surface tension when evaporating, has effectively been used to dry soft insect tissues, rat hepatic endothelial cells, and the cilia of rat trachea (Braet et al., 1997; Bray et al., 1993; Nation, 1983).

The objective of this study, therefore, was to develop a technique for obtaining scanning electron micrographs of delicate "gymnodinioid" dinoflagellate species that will minimise cell distortion, require limited expertise and equipment, and is inexpensive and less time-consuming.

MATERIALS AND METHODS

Cultures of various delicate "gymnodinioid" dinoflagellate species present on the south and west coasts of South Africa were grown at 18°C in F_2 medium (Guillard and Ryther, 1962) under a 12h light: 12h dark cycle. To avoid centrifugation, cells of the

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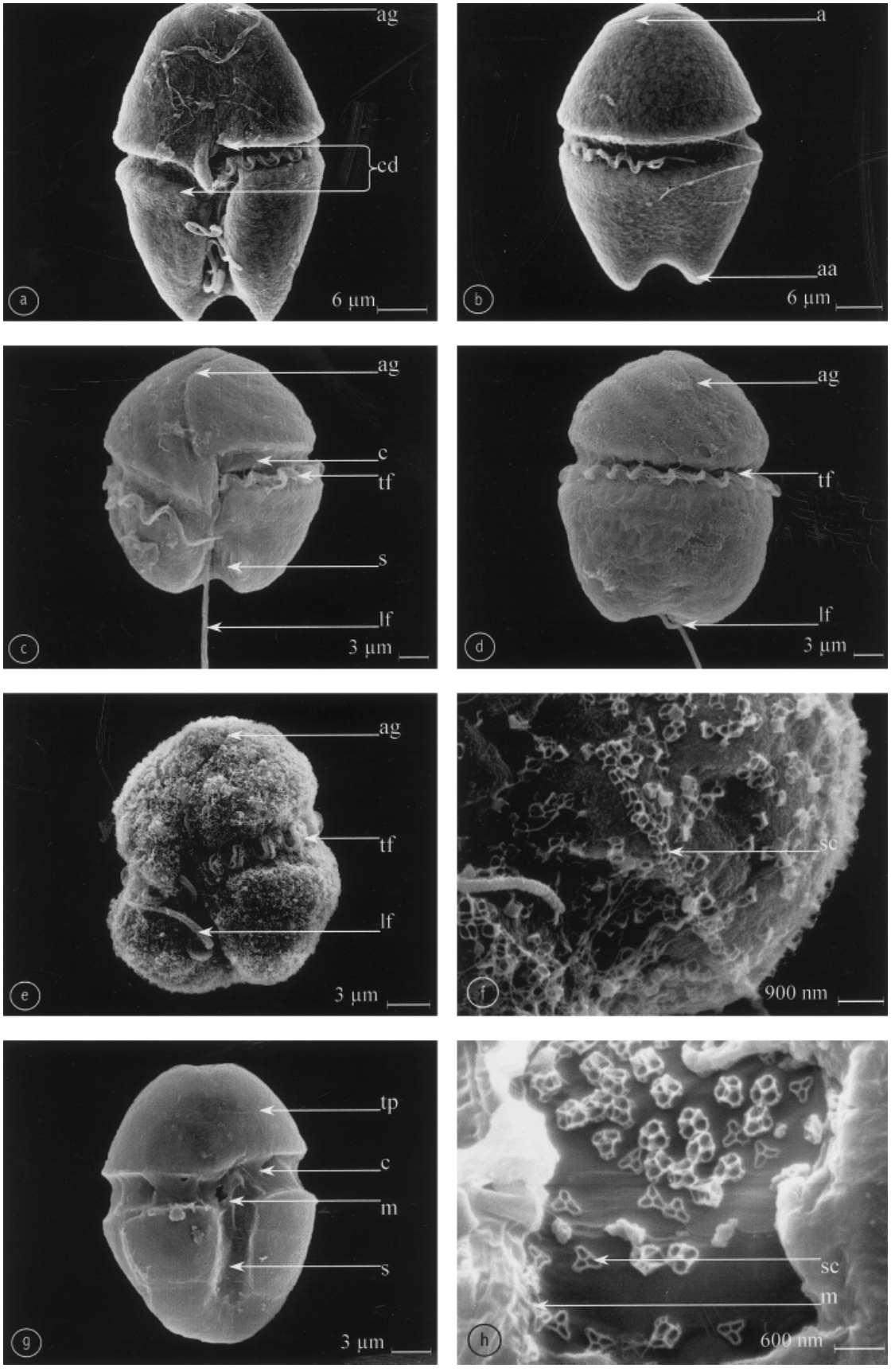

Fig. 1. Scanning electron micrographs of delicate "gymnodinioid" dinoflagellate species. **a,b:** *Akashiwo sanguinea* (Hirasaka) G. Hansen & Moestrup comb. nov. (2000) with apical groove encircling the apex. **c,d:** *Gymnodinium pulchellum* J. Larsen. (1994) with a sigmoid apical groove. **e,f:** *Lepidodinium viride* Watanabe *et al.* (1990) with a horseshoe-shaped apical groove and square-shaped scales covering the cell surface. **g,h:** *Heterocapsa* sp. with thin thecal plates and triangular-shaped scales underneath a membrane-like structure. a: apex, aa: antapex, ag: apical groove, c: cingulum, cd: cingulum displacement, lf: longitudinal flagellum, tf: transverse flagellum, s: sulcus, sc: scales, tp: thecal plate, m: membrane-like structure.

various cultures were harvested just before the light phase when swarming at the bottom of the culture flask.

Harvested cells in culture medium were fixed 1:1 (for 1 hour at room temperature) with 2% osmium tetroxide (OsO_4), which was made up with filtered seawater. While cells were being fixed, glass coverslips (10 mm in diameter) were washed with acetone, placed on a heating block, and coated with poly-L-lysine (molecular weight 70,000–150,000). To ensure that the coverslips were well coated, poly-L-lysine was applied repeatedly. Once the coverslips had cooled, they were mounted onto SEM stubs with silver paint. The 1% solution of fixed cells and OsO_4 was applied to the coverslips and left for ~30 minutes to allow the now negatively charged cells to adhere to the now positively charged coverslips. Cells were washed by submerging the stubs for 10 minutes in a 1:1 solution of distilled water and filtered seawater, followed by a second wash in distilled water for 10 minutes. The stubs were then taken through a graded ethanol series (30, 50, 70, 80, 90, 95% and 3X in 100%, 10 minutes at each step). Following removal from the 100% ethanol, a few drops of HMDS were immediately dispensed onto the coverslips. If necessary, once the HMDS had evaporated (5 minutes), more HMDS could be added to ensure a well-dried sample. The stubs were sputter coated with gold-palladium (60:40) and viewed with a Leo S440 SEM. This procedure was completed in ~3 hours and 30 minutes.

RESULTS

Four delicate "gymnodinioid" dinoflagellate species were investigated using the technique described above (Fig. 1). Cells were well preserved with very little morphological distortion and features such as the apical groove, cingulum-sulcus juncture, and the transverse and longitudinal flagella were clearly visible. An apical groove (Figs. 1a and b) encircling the apex of *Akashiwo sanguinea* (Hirasaka) G. Hansen & Moestrup *comb. nov.* (2000), was clearly visible. The species, *Gymnodinium pulchellum* J. Larsen (1994) displayed a sigmoid apical groove on the ventral side (Fig. 1c), extending on to the dorsal side (Fig. 1d) of the cell. A horseshoe-shaped apical groove (Fig. 1e) and square-shaped scales (Fig. 1f) were clearly visible on the cell surface of *Lepidodinium viride* Watanabe *et al.* (1990). Thin thecal plates (Fig. 1g) and triangular-shaped scales (Fig. 1h), obscured by a membrane-like structure and not visible with LM, were present on the "gymnodinioid" species, *Heterocapsa* sp.

DISCUSSION

Standard SEM techniques involve gentle centrifuging, filtering, fixing, dehydrating, and CPD. Apart from the negative effect of centrifugation and filtration on delicate dinoflagellate species and apart from the little information available in the literature on the CPD parameters, and the negative effect of the vigorous solvent exchanges on the cells, as well as temperature and pressure changes during CPD, we found the standard SEM techniques to be very time-consuming. With minimisation of cell distortion as the main objective, we obtained good scanning electron micrographs of various delicate dinoflagellate species present on the south and west coasts of South Africa in a quick and simple manner by using poly-L-lysine in combination with HMDS. Features such as apical grooves, cingulum-sulcus juncture, thin thecal plates, and scales were clearly visible. Limited expertise and equipment are needed and the technique is inexpensive and less time-consuming.

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