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An embedding technique for observation of internal microfossil structure by scanning electron microscopy

ABSTRACT

A hardened Canada balsam is a useful embedding medium, since it can be dissolved by xylene, for observation of the inner structure of microfossils with the scanning electron microscope. Preparation of the Canada balsam-xylene method and its advantages over previously presented techniques are described.

INTRODUCTION

Many methods have been employed for studies of the inner structure of microfossils. During the last decade, the scanning electron microscope has gained a prime position among equipment used for these studies.

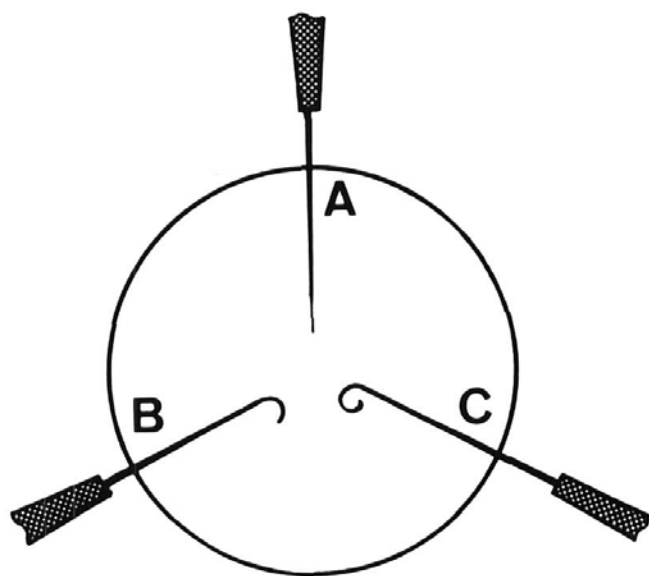
Preparation of specimens for scanning electron microscopic observation has been made by any of the following methods: cracking with a sharp-pointed needle (Glacon and Sigal, 1974; Ujiié, 1975; Hemleben et al., 1977; Banner and Culver, 1978); etching with a dilute acid solution (Crouch and Poag, 1979); and selection of naturally broken or etched specimens. However, these methods seem to be fraught with a number of drawbacks and risks, and often present difficulties in observing desirable parts for analyses. The reasons for these defects are:

- 1) In the case of very small specimens (e.g., less than 0.2 mm in length), it is almost impossible to remove desired portions by the use of a sharp-pointed needle.
- 2) It is hazardous to dissolve chamber walls, because dissolution of the fine structure of internal parts may also occur.
- 3) A considerable length of time is often needed to prepare specimens exhibiting a desired structure.

Besides these methods, Hansen and Lykke-Andersen (1976) have used Lakeside-70 cement for sectioning, and have made the sectioned specimen free from the embedding medium by placing it in ethanol, using a fine brush. By this method, however, it is not easy to remove the medium from the specimen. Undesirable damage can be caused by using a brush. The Lakeside cement is more soluble in warmed ethanol (40–50°C) and the specimen must be washed repeatedly by the ethanol, sometimes using an ultrasonic bath. Kennedy (1978) introduced a polyvinyl acetate-acetone method, in which the embedding medium of acetate is dissolved with acetone. However, as in the Lakeside cement-ethanol method, it is often difficult to obtain complete removal of acetate from specimens with complicated internal structure. It is also necessary to warm acetone. Coleman (1979, 1980), who adopted Hofker's method (1933), used a paraffin wax-xylene method to reveal internal test morphology. But some difficulties encountered in this method are in the orientation of pointed specimens in a given direction and the sectioning of hyaline calcareous specimens.

PROCEDURE

Recently, I used a hardened Canada balsam to avoid some of the



TEXT-FIGURE 1

Three types of fine-pointed needles used in preparation of Canada balsam-xylene method. A- or B-type is usually used for placing specimen in desired position. B- and C-type are used for picking.

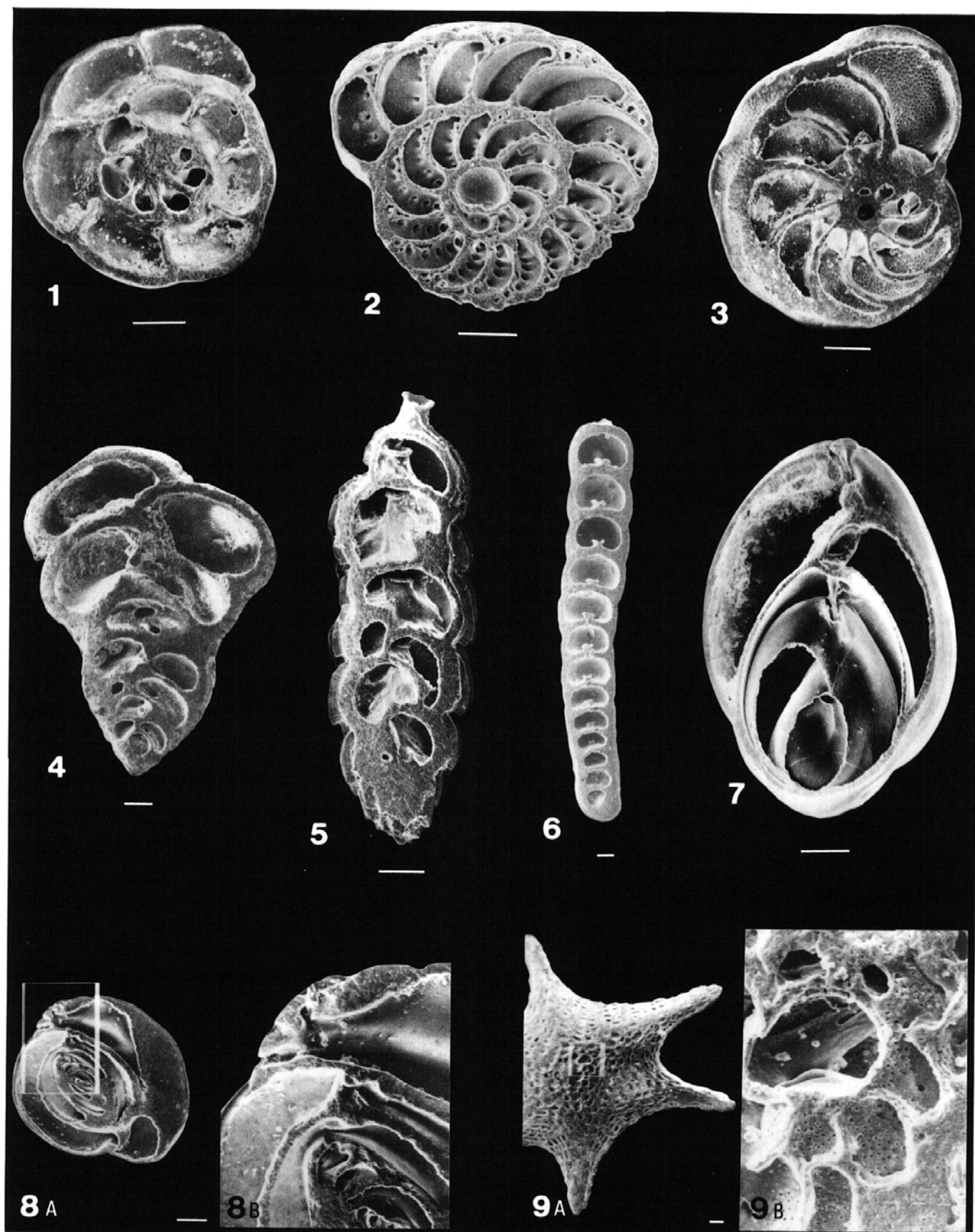
aforementioned problems. Employment of this technique enabled me to prepare specimens successfully and more quickly (pl. 1). The technique is summarized as follows:

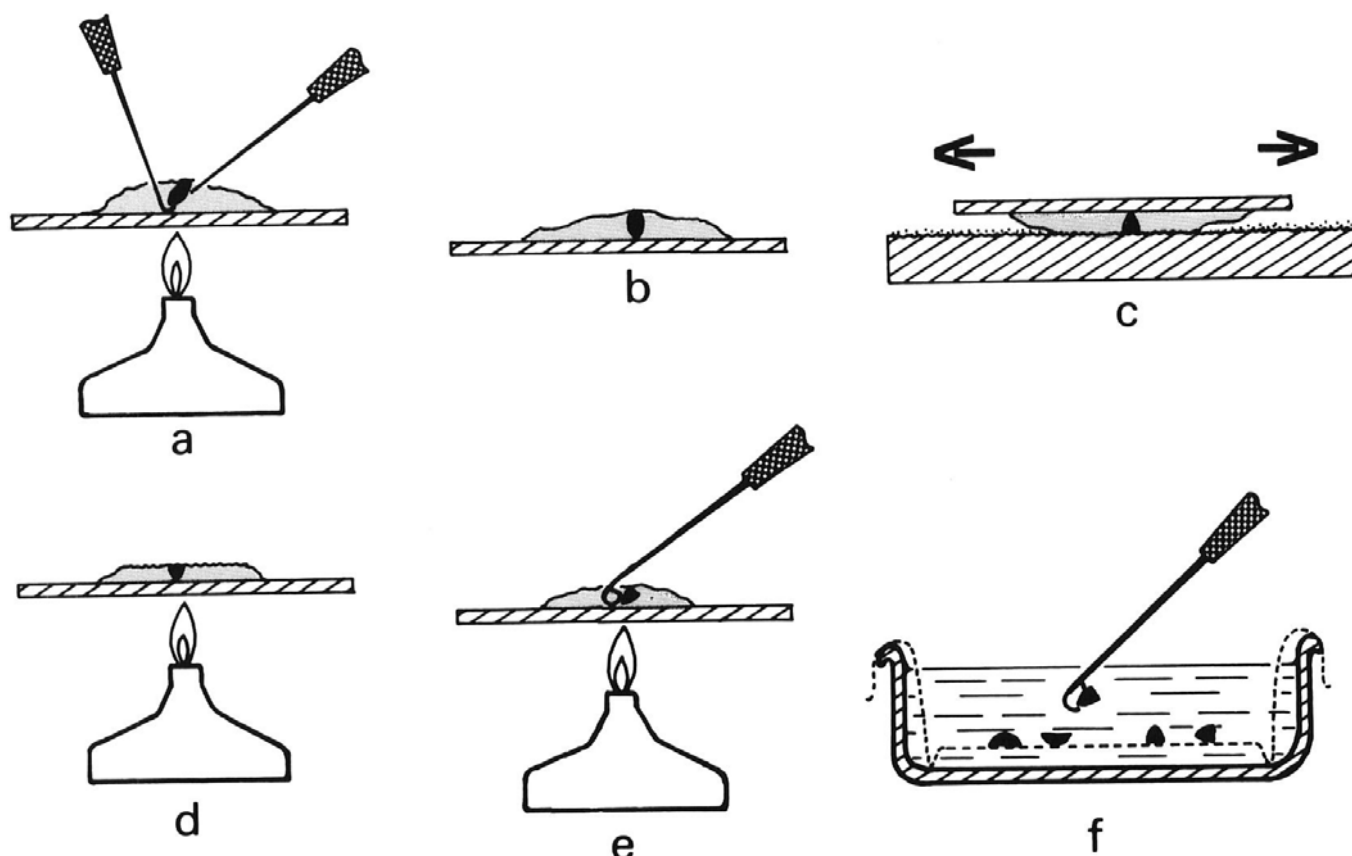
- 1) Canada balsam is moderately warmed for 20 to 30 minutes. When heating the balsam, it should be kept in mind that an immoderate amount of heating will cause difficulties in grinding.
- 2) The slide glass is cleaned by placing it in an ultrasonic bath.
- 3) Slightly hardened Canada balsam is heated on a slide glass by means of an alcohol lamp, and the specimen is completely embedded (text-fig. 2a).
- 4) Positioning of the embedded specimen is done with a hot needle of A- or B-type (text-fig. 1) while the Canada balsam is still soft enough to be moved (text-fig. 2a).
- 5) After the Canada balsam cools, it is ground manually on a plate of finely frosted glass with carborundum powder no. 2000 or 3000 until a desired cross section is exposed (text-figs. 2b-c).
- 6) The slide glass is reheated, and the specimen is picked out with the aid of a hot needle of B- or C-type while the Canada balsam is still soft (text-figs. 1, 2d-e).
- 7) The specimen is then immediately put into a dish filled with xylene, where the Canada balsam will dissolve in a few minutes. This treatment should be carried out on a 200-mesh screen with a raised bottom on a dark background dish (text-fig. 2f).
- 8) After the specimen is completely dry, it is placed on a specimen stub using a piece of double-sided adhesive tape.

PLATE 1

All specimens of benthonic foraminifera are equatorial sections with the embedding medium removed. Scale bar equals 100 μm .

- 1 *Oridorsalis umbonatus* (Reuss), from the Nobori Formation, Pliocene, Kochi Prefecture, $\times 90$.
- 2 *Elphidium crispum* (Linné), from the Miyata Formation, Pleistocene, Kanagawa Prefecture, $\times 110$.
- 3 *Hanzawaia nipponica* Asano, from the Ofuna Formation, Pleistocene, Kanagawa Prefecture, $\times 85$.
- 4 *Eggerella bradyi* (Cushman), from the Nobori Formation, Pliocene, Kochi Prefecture, $\times 50$.
- 5 *Uvigerina akitaensis* Asano, from the Kitaura Formation, Pleistocene, Akita Prefecture, $\times 80$.
- 6 *Martinottiella communis* (d'Orbigny), from the Funakawa Formation, Pliocene, Akita Prefecture, $\times 85$.
- 7 *Globobulimina auriculata* (Bailey), from the Wakimoto Formation, Pleistocene, Akita Prefecture, $\times 85$.
- 8A-8B *Hauerina fragilissima* (Brady), from the Shibikawa Formation, Pleistocene, Akita Prefecture. 8B, detail of specimen in figure 8A. 8A, $\times 50$; 8B, $\times 165$.
- 9A-9B *Baculogypsina sphaerulata* (Parker and Jones), from the Recent beach sand of Miyakojima, Okinawa Prefecture. 9B, detail of specimen in figure 9A. 9A, $\times 23$; 9B, $\times 200$.





TEXT-FIGURE 2

Procedures of Canada balsam-xylene method. a, adjusting specimen on slide glass while Canada balsam is soft; b, cooling; c, grinding on plate of frosted glass with carborundum; d, reheating specimen to melt Canada balsam; e, picking; f, dissolving Canada balsam with xylene. A broken line indicates a 200-mesh screen. Arrows in figure c indicate movement of slide glass.

For grinding the specimen, a good result can be obtained also by the use of a plate of rough- and medium-frosted glass without carborundum powder. In this case, the preparation should be made with oil instead of water.

By this simple method, the inner structure of microfossils may be well exposed and can be clearly observed with the scanning electron microscope.

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