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## **“Grouping” preparation of fossil dinoflagellates**

### **ABSTRACT**

A method of multispecimen mounting that has been developed for the preparation of fossil dinoflagellates is described. This method requires little technical investment and presupposes no great manual skill. When required, single specimens can be mounted with exact orientation. The procedure described in the present paper is most suitable for samples containing abundant individuals. Any specimen among tens of thousands can be found quickly, without the use of a mechanical stage.

### **INTRODUCTION**

When a large quantity of microfossils, such as pollen and spores, is handled in transmitted light, the method by which the specimens are distributed on the slide can contribute substantially to the progress of the investigation. It is often necessary to refer back to some specimens of a preparation in order to compare them with others. Such referral requires a kind of laboratory procedure that enables one to find a particular specimen among tens of thousands in a matter of seconds. Besides pollen and spores, the range of application of such preparations includes primarily acritarchs and fossil dinoflagellates. These are similar in order of magnitude and chemical resistance to the sporomorphs, and for this reason often occur together with them in palynological investigations. The practical method for the preparation of fossil dinoflagellates that is explained here is therefore applicable without restriction to the other groups mentioned above.

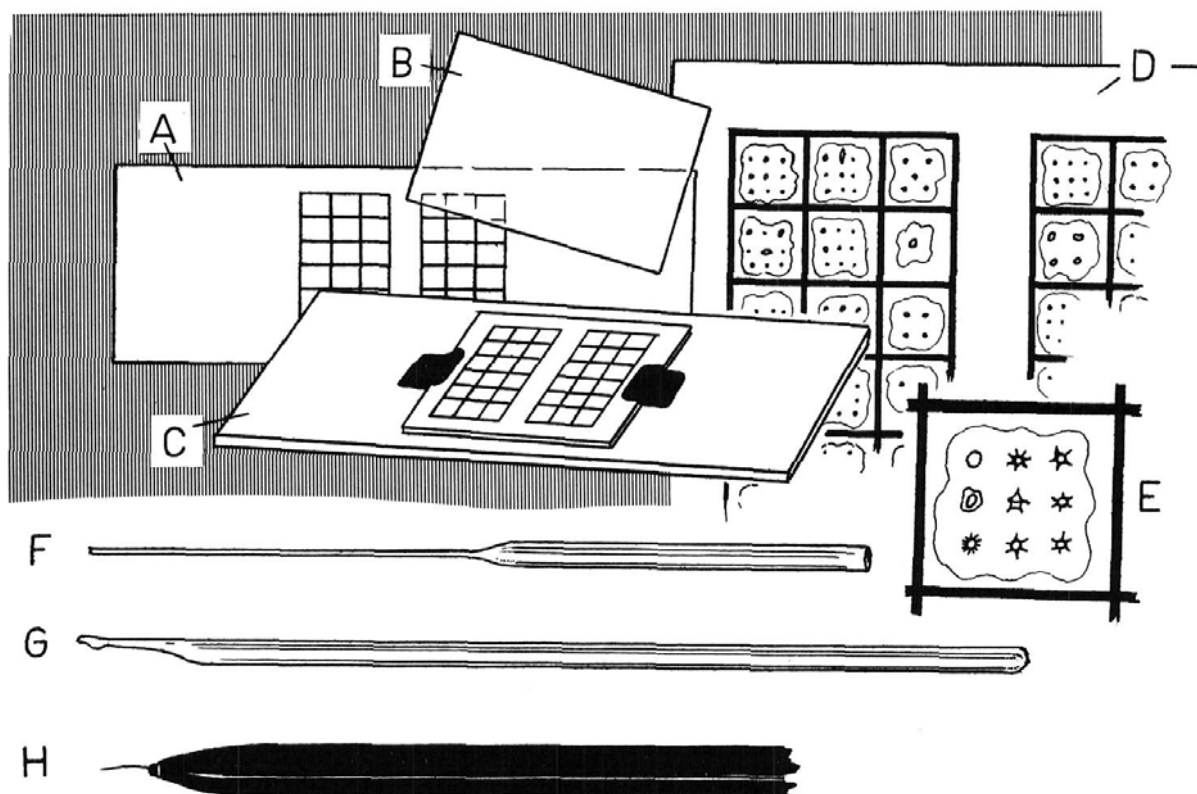
### **PREPARATION**

There are two principal ways of preparing palynological material. Both methods have long been used, and the choice between them depends largely on one's aims.

- 1) The total residue of a sample is mixed with the mounting medium, which is then spread out between slide and cover slip (strewn mounts).
- 2) Single specimens are removed from the residue and then embedded (single-specimen mounts). A development of this method is the embedding of several or many specimens either irregularly or in a certain mode of grouping (multispecimen mounts).

For details concerning practical aspects of these methods, the reader is referred to the following literature, which contains further bibliographical data: Andersen (1965), Erdtman (1943), Klaus (1953), Mädler (1956).

The great advantages of strewn mounts are the short time involved in preparation and the speed with which individual specimens can be located with the help of a mechanical stage. In addition, either the entire residue of a sample or at least a representative part of it is processed. This precludes a subjective selection of certain specimens during preparation and also results in the inclusion of small forms. Finally, the remains of other organisms are prepared along with those of the organisms under investigation and may throw some light on environmental conditions. This very advantage may easily turn into a drawback, however, as the remains of organic substances, fragments of wood, cuticles, and other objects are scattered throughout the preparation and may obscure the specimens to be studied or render them difficult to find.



TEXT-FIGURE 1

Making preparations. A cover slip (B) is attached by adhesive tape (C) to a slide on which squares have been drawn (A). In each square, a group of specimens or a single specimen is mounted with the use of glycerin jelly (D-E: state prior to being covered). Further utensils: Capillary pipette (F), glass rod with one end drawn out (G) to obtain fine drops, bristle attached to a rod (H) for aligning and orienting the specimens.

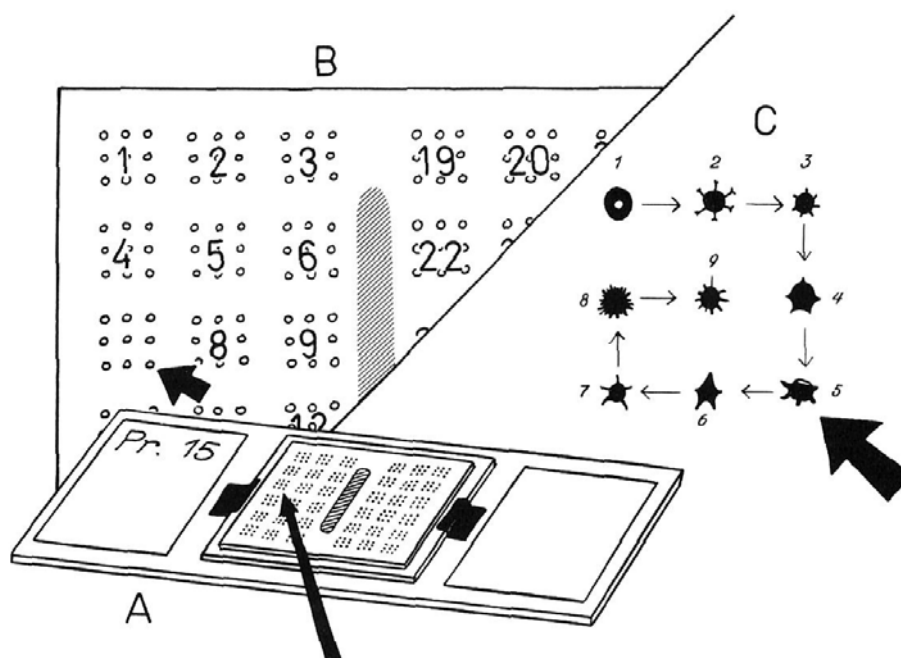
Added to these difficulties is the fact that all specimens, and especially those preserved in all three dimensions, assume a random position and can not be oriented as required. Only simple, flat shapes, such as compressed sporomorphs, orient themselves, prior to hardening of the medium, parallel to the surface of the slide. Searching for a specimen with the use of a different mechanical stage can also result in difficulties.

On the other hand, one may argue that these disadvantages can be largely compensated for by careful procedure (e.g., use of a moderately dense suspension medium). Furthermore, it might be expected that, in rich material, sufficient specimens would in any case come to lie as required and would not be obscured by foreign bodies. A short glance at the literature, however, proves otherwise. Few students of fossil dinoflagellates have been able to produce optimal strewn mounts. Others have had difficulty in finding enough useful (or reproducible) specimens to serve as holotypes. In such cases, one can recommend the use of single-specimen mounts or, if the material is abundant, the method described below.

#### "GROUPING" PREPARATION

##### Generalities

The idea of arranging individual specimens on a slide according to certain principles is by no means new, although the method described here was developed independently by the author. In many details the method is similar to that of Mädlar (1956). Nevertheless, multispecimen mounts are seldom employed at present, at least for dinoflagellates. Numerous conversations with colleagues have shown that the degree of dexterity and the amount of time needed are often greatly overestimated. Perhaps comparison with classical diatom "mosaic slides" plays a subconscious role, but these are more difficult to produce, largely because of the smallness of the siliceous algae. With suitable equipment and a little practice, the manual part of preparation should present no difficulty and can easily be learned by a skilled assistant. The increased expenditure of time is quickly compensated for by a lucid and uncluttered arrangement and also by the desired orientation of the specimens. The arrangement of the specimens in groups is not an esthetic pastime, but a system that assists in the quick location of specimens.



TEXT-FIGURE 2

Location of specimens. Each specimen is given a number with three sets of figures. If one wants to examine, for example, dinoflagellate 15/7/5, one refers to preparation 15, group 7, and then finds specimen 5 (C) in a clockwise direction, starting from the upper left corner. The preparation figured (A) consists of 36 groups, each of nine specimens, a total of 324 specimens. The thick line down the middle of the preparation serves as a useful landmark. The use of group and specimen numbers is quickly learned; they are employed here solely for describing the figures.

The entire procedure of preparation is strictly divided into separate operations and requires calm and conscientious workmanship without interruptions. When one is acquainted with the process, it is easily possible to prepare 2000 or more specimens per day. The method is worthwhile only when abundant material is available. It proved itself during the investigation of an abundant flora of Tertiary dinoflagellate cysts (Gocht, 1969).

#### Mounting medium

Glycerin jelly, which has found wide use in palynology, is also suitable for grouping preparations. It hardens quickly and does not permit the specimen to move in position, even after months or years. Furthermore, this medium has satisfactory optical properties and does not require drying of the material, the latter being in water after acid etching. The greater durability of some resins is a fact which must be taken into consideration.

#### Isolation of specimens

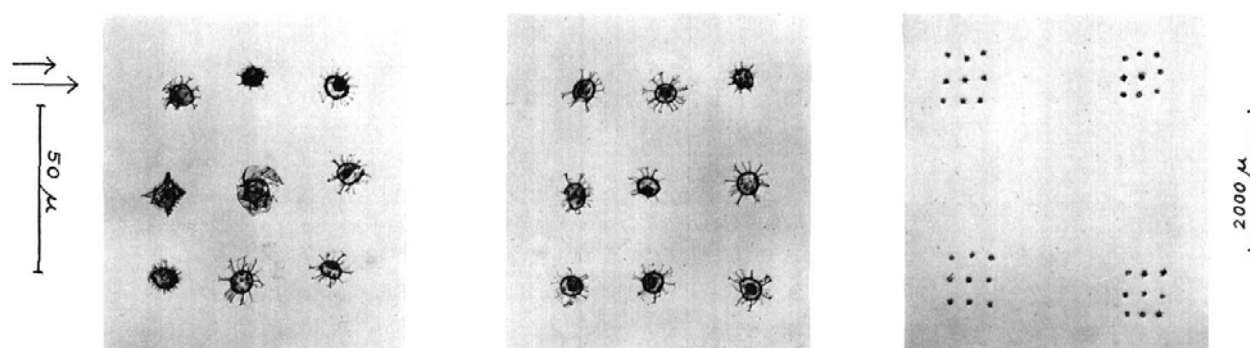
A binocular magnifier fitted with a glass plate for transmitted light is suitable for the isolation and mounting of specimens. The residue to be examined should be placed in a Petri dish and examined at a magnification of from  $\times 50$  to  $\times 100$ . When an interesting specimen is

found, it should be isolated by means of a glass capillary pipette (text-figure 1F) with an internal diameter of from 200 to 300  $\mu$ . The end of the pipette should be quickly positioned just above the specimen, which is then sucked into the tube by capillary force. If the pipette is then quickly withdrawn from the water, the procedure can be repeated several times until sufficient specimens for one group have been sucked into the tube.

#### Mounting

A slide on which two rectangles have been drawn with India ink serves as a base plate. These rectangles can be protected from scratches by a thin coat of varnish. Each rectangle is divided into 18 squares (three horizontal, six vertical; see text-figure 1A, C), which serve solely as a template. Specimens are mounted on a large cover slip (text-figure 1B), which is held to the slide by adhesive tape (text-figure 1C). In each square a group of as many as nine specimens may be mounted simultaneously.

On a hot plate a small quantity of glycerin jelly is constantly kept warm. Under the binocular magnifier a small drop of the jelly is transferred to the first square and there spread out. With a glass rod of the type



TEXT-FIGURE 3  
Photomicrographs showing fossil dinoflagellate cysts in "grouping" preparation.

pictured in text-figure 1G it is possible to transfer very small drops of the medium. The water, together with the specimens, is next blown out by mouth from the pipette, directly beside the drop of glycerin jelly. The specimens are quickly transferred one after another by means of a single bristle pasted onto a rod (text-figure 1H), which is then used to arrange the specimens and spread out the jelly farther, if necessary. The jelly hardens shortly after this operation is completed. The drop of water, which often contains impurities, is then removed. The next group is then prepared, and the process is repeated until all 36 squares are occupied.

It is often necessary to orientate dinoflagellates in a particular position, so that the dorsal or apical side points upwards. Similarly, flattened objects must sometimes be placed on edge. In such cases, it is recommended that only one specimen be included in a drop and that its position be checked (and, if necessary, corrected) until the jelly has virtually hardened. Checking can be carried out under increased magnification with the use of a microscope. With increased experience it becomes possible to include in one group several specimens of the same species, each specimen being oriented as required. In all such instances, the bristle proves to be an ideal instrument.

Text-figure 1D-E shows a completed cover slip with various numbers of specimens in each square.

#### Covering

Covering should take place only after all drops have hardened. In the middle of a second and somewhat smaller cover slip, a proper amount of glycerin jelly is deposited and then spread out toward the four corners. The jelly should be drawn out evenly and as far as possible to the edges of the slip. This operation should

be carried out without reheating the preparation, which might result in a shifting of the specimens.

After cooling, the preparation can be attached to the slide by means of two pieces of adhesive tape. Should it be necessary to examine the specimens from the bottom, the tape strips can be easily detached and the preparation turned over.

It is recommended that after a certain time the gap between the two cover slips be sealed with an acetone-based varnish. Under no circumstances should resins that are soluble in xylene be used for this purpose. In earlier preparations made by the author in a slightly different manner and then sealed with Canada balsam, it was noticed that xylene vapours had penetrated into the jelly and caused undesirable bubbles.

To avoid mistakes, it is good policy to draw a thick line on the larger cover slip between the two rectangles. This line serves as a useful landmark. It can be drawn before or after the embedding operation. A completed preparation is shown in text-figure 2A.

#### Finding the specimens

Each specimen is given a reference number of three sets of figures, each set separated by slant lines. As an example, for specimen 15/7/5 (text-figure 2), 15 is the number of the preparation, 7 is the number of the group, and 5 refers to the position of the specimen within this group. Preparation 15 is first placed under the microscope, then group 7 is found under low magnification (see text-figure 2B). Within group 7 the specimens are counted clockwise, the number of specimens belonging to a particular group (text-figure 2C) being of no importance. Specimen 5 is then brought into focus under higher magnification. This

procedure of finding a particular specimen requires scarcely any more time than the use of a mechanical stage.

The above system of counting can be employed in the sense of a "mirror image" when specimens are examined from the bottom. Because a microscope inverts an image through 180 degrees, one should insert the preparation with the writing upside down. Alternatively, one can employ a symbol to denote the upper (or lower) side of the preparation.

#### Other methods

Mädler (1956) frequently stressed the possibility of varying the individual operations according to the preference of the individual worker. If only a few specimens are to be investigated, or if interruptions are inevitable, it is practical to make more preparations, with each one having fewer specimens. In such cases, the investigator can omit the squares, recording the distribution of individual specimens or groups in short sketches—much in the fashion of the individual stars of a constellation. One must pay attention only to the fact that later workers using the preparations must be able to find their way about. Whether one makes strewn mounts, single-specimen mounts, or multispecimen mounts, the important point, for all, is the success of the result, not the method employed.

#### ACKNOWLEDGMENT

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