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PREPARATION OF NEMATODES FOR SCANNING ELECTRON MICROSCOPY

by
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This simplified procedure, using a chamber to contain the specimens during processing (McClure and Stowell, 1978) has been used successfully on a large number of nematode species. Because handling is minimised, a high proportion of nematodes has been retrieved, with little damage or contamination.

Materials and Methods

Nematodes are killed, either by heating in a drop of water or with hot fixative (F:P 4:1 or 4% formalin at 70-80 °C), then fixed for at least 48 hours.

Cleaning of fixed specimens, to remove adhering debris, is accomplished by hand picking into approximately 0.2 ml distilled water in a glass tube, adding two drops of 0.03% Agral ([1]) solution, then sonicating for 45 seconds in an ultrasonic cleaner. To ensure that the nematodes are rinsed free of detergent, this step is repeated four or five times, without the addition of Agral.

The processing chamber (Fig. 1A) is constructed from glass tubing, teflon tubing and nylon mesh - pore size 5 or 10 μm (McClure and Stowell, 1978). Clean specimens are hand picked into a partially constructed chamber, filled with distilled water. Then nylon mesh and teflon tubing are inserted into the lower end of the glass tubing

([1]) Agral 60 - Wetting agent, ICI Australia.

only, to form a « cup ». The chamber is partly immersed in distilled water, in a clean glass tube (12 mm diameter), to prevent drainage. Once it is loaded, nylon mesh and teflon tubing are inserted in the upper, open end, to form an enclosed chamber.

A syringe, connected to teflon tubing with a short piece of glass tubing inserted in one end, is used to facilitate liquid exchange. This short piece of glass tubing is inserted into the teflon tubing, at the upper end of the processing chamber. A small quantity of distilled water is drawn through the chamber.

Each exchange is performed whilst the chamber is immersed in 4-5 ml of the liquid in a glass tube. This quantity of liquid is drawn through the chamber using a process of continuous flow, in which one liquid gradually replaces another. The chamber remains full of liquid at all times, until the critical point is reached in the drying procedure.

The sample is postfixed, for one hour, in unbuffered 1% osmium tetroxide. Specimens are then washed in several exchanges of distilled water and left in distilled water overnight.

Dehydration of nematodes is achieved using a 'gradual' sequence of distilled ethanol/distilled water concentrations: 2.5%, 5%, 10%, 20%, 30% and 40% each for 10 minutes, then 60%, 80% and 100% each for 5 minutes.

The transfer to amyl acetate takes place immediately after dehydration is complete, with a graded series of amyl acetate/distilled ethanol concentrations: 10%, 20%, 30%, 40%, 60%, 80%, 100% and 100% each for 5 minutes. The processing chamber (filled with amyl acetate) is placed in the pressure container of the critical point dryer unit.

The standard critical point drying procedure is used, with CO_2 as the drying liquid (miscible with amyl acetate). To ensure that all the amyl acetate has been replaced, before the temperature and pressure are increased to the critical point, the pressure container must be flushed with CO_2 at least 5 or 6 times.

The dried nematode specimens are removed, individually, from the chamber and mounted on standard aluminium specimen stubs which have been prepared in the following way. Specimen mounts are manufactured from small pieces of brass sheet (0.1 mm gauge, cut approximately 6 x 10 mm) bent lengthways to an angle of about 45°. The mounts are attached to the stub, along one of the angled

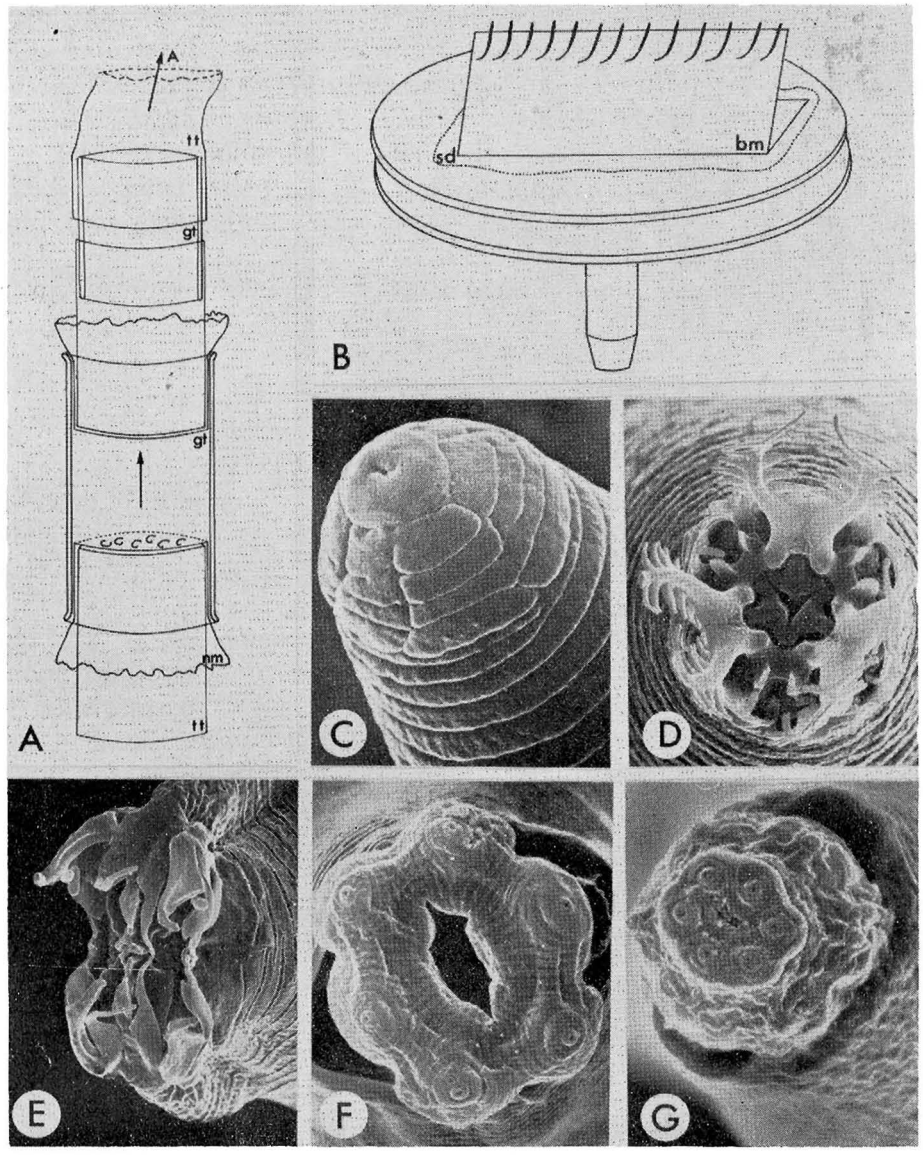

Fig. 1 - A: Processing chamber (McClure and Stowell, 1978) with syringe connected from A. Arrows indicate the direction fluids are drawn through the chamber towards the barrel of the syringe; gt = glass tubing; tt = teflon tubing; nm = nylon mesh. B: Stub prepared for nematode examination; bm = brass mount; sd = silver dag. C - G, face views. C: *Scutellonema brachyurum*. D: *Acroboles complexus*. E: *Tylocephalus auriculatus*. F: *Aporcelaimellus* sp. G: *Tylencholaimellus* sp.

surfaces, with silver conducting paint (2) (Fig. 1B). A very thin layer of adhesive solution [four or five 2 cm lengths of adhesive tape in 5-10 ml of chloroform (Stone and Green, 1971)] is coated on the exposed, angled surface. Each nematode is positioned on this surface with its head protruding above the level of the mount (Fig. 1B).

Finally, the mounted nematodes are gold coated using a diode sputtering system to make them electrically conductive (gold film about 50 nm thick).

The specimens, illustrated here (Fig. 1), were examined in a Philips PSEM 500 at an accelerating voltage of 25 kV.

Results and Discussion

This method has a number of advantages over previously described techniques. Post fixation has been shown to reduce specimen shrinkage and collapse (Högger and Estey, 1975). Osmium may also act as an electron conductor (De Grisse, 1973).

The dehydration sequence, using small increases in distilled ethanol/distilled water concentrations and the gradual replacement of one concentration with the next, minimises osmotic pressure differences. This reduces specimen shrinkage and distortion, avoiding the need to make an incision in each nematode to facilitate the penetration of dehydration fluids (Högger and Bird, 1974; Nickle and Högger, 1974).

Once dried, the specimens can easily be retrieved from the processing chamber. Up to 50 nematodes can be arranged on a single stub if brass mounts are used. The examination of head morphology is simplified by this mounting procedure, as enface and other views can be easily obtained.

Samples from nematode species within the orders Tylenchida (Fig. 1C), Rhabditida (Fig. 1D), Araeolaimida (Fig. 1E) and Dorylaimida (Fig. 1 F) have been successfully prepared using this schedule. However, this preparation schedule did not produce successful results for a number of Dorylaim species. Other fixation and postfixation techniques may be required to reduce specimen shrinkage and distortion of the cuticle (Fig. 1G). Also a different method of cleaning these

(2) Balzers Union.

nematodes is needed, as damage can result from sonication, because of their greater volume and fragile cuticle.

SUMMARY

A simplified procedure is described for the preparation of nematodes for scanning electron microscopy. The method uses a chamber to contain the specimens, during processing (McClure and Stowell, 1978). The nematodes are killed by heating, fixed in either 4% formalin or F:P 4:1, cleaned by sonication, post fixed in unbuffered 1% osmium tetroxide, slowly dehydrated to 100% ethanol, transferred to amyl acetate and then critical point dried (using CO_2). Finally the nematodes are mounted and gold coated. Samples from nematode species within the orders Tylenchida, Rhabditida, Araeolaimida, and Dorylaimida have been successfully prepared using this schedule.

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