Bulletin of the British Myriapod Group 16 (2000)

THE PREPARATION OF CENTIPEDES FOR MICROSCOPICAL EXAMINATION WITH PARTICULAR REFERENCE TO THE GEOPHILOMORPHA

Luis Alberto Pereira

Museo de La Plata, Paseo del Bosque s/n, (1900) La Plata, Argentina
myriapo@infovia.com.ar

INTRODUCTION

Traditional methods of clearing and mounting geophilomorph centipedes have proved to be unsatisfactory. Both 60 per cent lactic acid and lactophenol, which are often used for temporary mounts, do not clear specimens adequately and cause slight swelling of the appendages, altering their shape. In addition, lactophenol contains glycerol which is hygroscopic and therefore takes up some water from the air, which is an additional disadvantage. Many workers, for example Attems and Verhoeff, made permanent mounts, macerating specimens by heating them in 10 per cent potassium hydroxide, washing, then dehydrating in a series of alcohols. They were cleared in xylene and mounted in Canada balsam. Potassium hydroxide, however, damages poorly sclerotized structures as well as the internal organs which are important for classification (for example the structure of the coxal organs) and also for assessing the maturity of the specimen (presence of spermatozoa in the seminiferous tubules of males and spermathecae of females). In addition specimens treated with potassium hydroxide become deformed under the pressure of the cover glass when mounted.

Other mountants which also act as clearing agents have been used such as polyvinyl lactophenol, which shrinks after some time, and Hoyer's mountant which may cause the specimen to swell and split, may crystallise and does not always ring satisfactorily.

Until recently (Pereira and Hoffman, 1993) I have used vegetable creosote (refined from beech tar) for temporary mounts. This is very satisfactory as it clears and dehydrates the specimens very well and does not alter the shape of the body and appendages (they are neither collapsed, nor inflated) and they remain pliable. Nevertheless, the smell presents a problem and if the specimens are kept in the creosote for a long time they are irreversibly darkened. Currently, however, I use ethylene glycol (ethylene glycol monophenyl ether = phenoxetol). Propylenephenoxetol (=3-phenoxypropanol) is similar and also functions well. Ethylene glycol has previously been used as a preservative for arthropods in pitfall traps by Szekelyhidy & Loksa, (1979) and Geoffroy & Celerier (1996). The great advantage of the ethylene glycol as opposed to creosote is that it has not have a strong unpleasant smell and in addition the specimens can remain in it without undergoing any alteration.

RECOMMENDED PROCEDURES

Dissection

As the anatomy of the mouth-parts, including the mandibles, is important in the taxonomy of the Geophilomorpha, it is often necessary to dissect these off. Specimens are dissected in ethanol not in creosote or ethylene glycol. The procedure is as follows:

1. Detach the head from the body. Dissection is carried out with a very fine entomological pin (a second pin bent at an angle of about 90 degrees is used to maintain body in an appropriate position). The pin can be mounted in an old biro by melting the end of the latter with a Bunsen and then introducing the head of the pin and holding it in place until the plastic solidifies.

2. Detach the maxillae on one side of the head capsule at the level of the membrane between the pleura of the head capsule and the coxosternum of the second maxillae.

3. Turn back the maxillae (i.e. turn through 180 degrees). This will expose their dorsal surfaces. They will still be attached to the head capsule by one side. Do not detach the mandibles, simply adjust them to provide the best position for their examination. In a temporary mount the preparation can be turned over to expose the dorsal side of the head capsule and the ventral surface of the maxillae.

If the dissection is done with care, the specimen is not damaged (this may be a time consuming process).

Temporary mounts

Specimens are normally stored and dissected in 70-75 per cent ethanol. For temporary mounts they are removed to undiluted ethylene glycol which clears and dehydrates them in similar way to creosote. The dissected head clears in about half an hour or even a few minutes, the trunk takes longer, especially if robust.

A large cover glass is often required to examine the trunk and it may be necessary to cut the trunk into two or even three pieces if it is very long. Perforation of the pleura with a fine pin facilitates the entry of ethylene glycol and thus speeds the process of clearing. If large cover slips are used, there is a danger of spillage of the ethylene glycol onto the microscope stage. To avoid this, larger slides may be used. The simplest way to obtain these is to have them cut to order by a local glass merchant.

After examination the specimens are returned to ethanol, the head and mouth-parts are usually placed in a micro-vial.

Permanent mounts

Permanent mounts are not normally made even for the very small specimens. It is much more convenient to store specimens in ethanol and make temporary mounts when needed because they can be observed in all positions (not only from ventral or dorsal sides). If however a permanent mount is required (for instance to avoid the loss of very small mouthparts accidentally detached from head during dissection) the specimen is removed from 70-75 per cent ethanol and placed in creosote (or in

ethylene glycol) in a Petri dish for a few hours and mounted on a slide in natural Canada balsam (obtainable from BDH, Laboratory Chemicals Division, Poole, Dorset, UK) diluted with a little creosote or ethylene glycol (NOT with xylene). The specimen is mounted on a large cover glass rather than a glass microscope slide and then covered with a small cover glass. This allows examination of the specimen from either side and at all magnifications. Such preparations are, however, very delicate and require protection both during storage and examination. This is provided by attaching them to a glass microscope slide with a band of paper glued to it by only one side. To examine from the other side remove the preparation, turn it over and slip back under the band of paper. A diamond pencil used for marking or engraving glass, metals and other hard materials is used to cut the small cover slip. For permanent mounts I prefer to clear the specimens with creosote because when mounted in Canada balsam the form of less sclerotized structures (such as the palps of the first maxillae) is better preserved than when cleared with ethylene glycol.

If the specimen has been in glycerine it should be washed in a Petri dish in 70-75 per cent ethanol for a few hours.

Temporary mounts may be made permanent by direct transfer from the creosote or ethylene glycol into Canada balsam.

General remarks
Specimens too large to mount on slides, for example some lithobiomorphs and scolopendromorphs, may be examined in Petri dishes containing ethylene glycol (or creosote) and held in place beneath a microscope slide. Perforation of the pleura facilitates clearing but this may take several days for large specimens.

The importance of accurate drawings cannot be over-emphasised. These are most easily and precisely obtained using a microscope with drawing tube. If this is not available then a squared eyepiece graticule may be used and the specimen drawn on squared paper.

Techniques to remount specimens on slides
Specimens on old slides and apparently in poor condition can be renovated by remounting.

1. Firstly the slide is soaked in water in a Petri dish to remove the original labels. Then if necessary, these are washed to clean them further, then dried and attached to a new slide. For specimens mounted in gelatine, place the slide vertically in a tube containing sufficient water to cover only the slide label.

2. Further treatment depends on the mountant that has been used.

a) For Hoyer's mountant. The slide is soaked in water, possibly for several days, until the cover glass can be removed with ease. The parts of the specimen are transferred to a small container of 70-75 per cent ethanol for a day or so, then to creosote and then mounted on the new slide in natural Canada balsam diluted with a little creosote.

b) For Canada balsam. Remove the slide from water, dry and transfer to creosote

until the cover glass and specimen parts can be easily removed. Then follow the procedure as in a).

c) For gelatine. Remove the slide from the water and dry it. Place in a Petri dish with glacial acetic acid until the cover glass and specimen parts can easily removed. Then follow the procedure as in a).

ACKNOWLEDGEMENTS

I am very grateful to Dr. J.G.E. Lewis for suggesting that I publish this note in the Bulletin of the British Myriapod Group and for advice and suggestions on my drafts.

REFERENCES

Geoffroy, J.-J. & Célérier, M.-L. (1996) Cycles d'activité comparés de populations de Diplopodes édaphiques dans un écosystèm forestier tempéré. In: Geoffroy, J.-J., Mauriès, J.-P. & Nguyen Duy-Jacquemin, M. (eds), Acta Myriapodologica. *Mém. Mus. natn. Hist. nat.*, **169:** 533-544.

Pereira, L. A. & Hoffman, R. L. (1993) The American species of *Escaryus*, a genus of Holarctic centipedes (Geophilomorpha: Schendylidae). *Jeffersoniana*, Number 3: 1-72.

Szekelyhidy, E. H. & Loksa, K. D. (1979). Oniscoiden- Diplopoden- und Chilopoden- Gemeinschaften im Untersuchungensgebiet "Sikfökut-Projekt" (Ungarn). *Opusc. Zool. Budapest*, **16:** 151-174.