III. INTERNATIONAL WORKSHOP ON TAXONOMY AND ECOLOGY OF TESTATE AMOEBA

Aachen October 10-13, 1983

Kind of communication: Contributed paper ■
Poster ☐
Other ☐

Abstract of communication

Author(s): Wilhelm FOISSNER
Adress: Zoologisches Institut der Universität Salzburg, Akademiestraße 26, A-5020 Salzburg (Austria/Europe)

Title: Protargol silver staining: a useful method in testacean taxonomy at the light microscopical level

Abstract:

General remarks: This method is the same as I use for years to impregnate the infraciliature of ciliated protozoa. It is a modification of the original method described by TUFFRAU in 1964. Testacea can be impregnated very well because the organic cement stains heavily, especially in non agglutinated shells. Very frequently, the nucleus and other cytoplasmatic details are impregnated too.

Method (steps 1-4 can be done in a centrifuge tube, steps 7-11 should be performed in staining jars; for preparation of BOUIN's-Fluid, MAYER's albumen, and the Protargol solution see general textbooks, e.g. ROMEIS):

1) If possible, concentrate material by slight centrifugation.

2) Fix in BOUIN's Fluid for about 10 min.

3) Wash 2-4x in A. dest. until the solution is colorless.

4) Now you may dehydrate in a graded isopropyl alcohol series (30-50-70%) for 5 min. each change and store the material in 70% isopropyl alcohol for months, or you can go on immediately with step 5 (stored material must be rehydrated before use!).

5) Place a small drop of MAYER's albumen on a very clean slide, add a small drop of the concentrated material, mix and spread out with a needle until the layer is about as thick as the animals. Drain off excess fluid.

6) Let dry over night at room temperature or at 60°C in an oven for 30-60 min.

7) Place slides for about 30 min. into 98% isopropyl alcohol, then for about 5 min. into 70% alcohol, for about 5 min. into A. dest. (change twice), for about 60-150 sec. into 0,2% $KMnO_4$, for about 60 sec. into A. dest., for 160-230 sec. into 2,5% oxalic acid, and again for about 10 min. (change twice) into A. dest. The time of bleaching ($KMnO_4$/oxalic acid) influences the results very much - try several times within the given limits! Voluminous animals generally require longer times.

8) Impregnate in 0,4% Protargol solution for 10-20 min. at 60°C.

9) Remove slides from Protargol and place a drop of hydroquinone solution (1% w/v hydroquinone dissolved in 2,5% w/v sodium sulfite solution; should be made 1-2 weeks before use; developers with a slight brownish color work best) on the material for about 10-120 sec. Watch and control the development of the stain microscopically. Start with the slides which are bleached the shortest time. Stop development by dipping the slide into A. dest. (2 changes) for about 30 sec. each.

10) Then place slides immediately into 2,5% (w/v) sodium thiosulfate for 3 min.

11) Wash slides twice in A. dest. for about 60 sec. each and dehydrate immediately in 70-98-100-100% isopropyl alcohol for about 5 min. each. Clear by transfer through two xylene steps for about 10 min. each. Mount in synthetic neutral mounting medium.