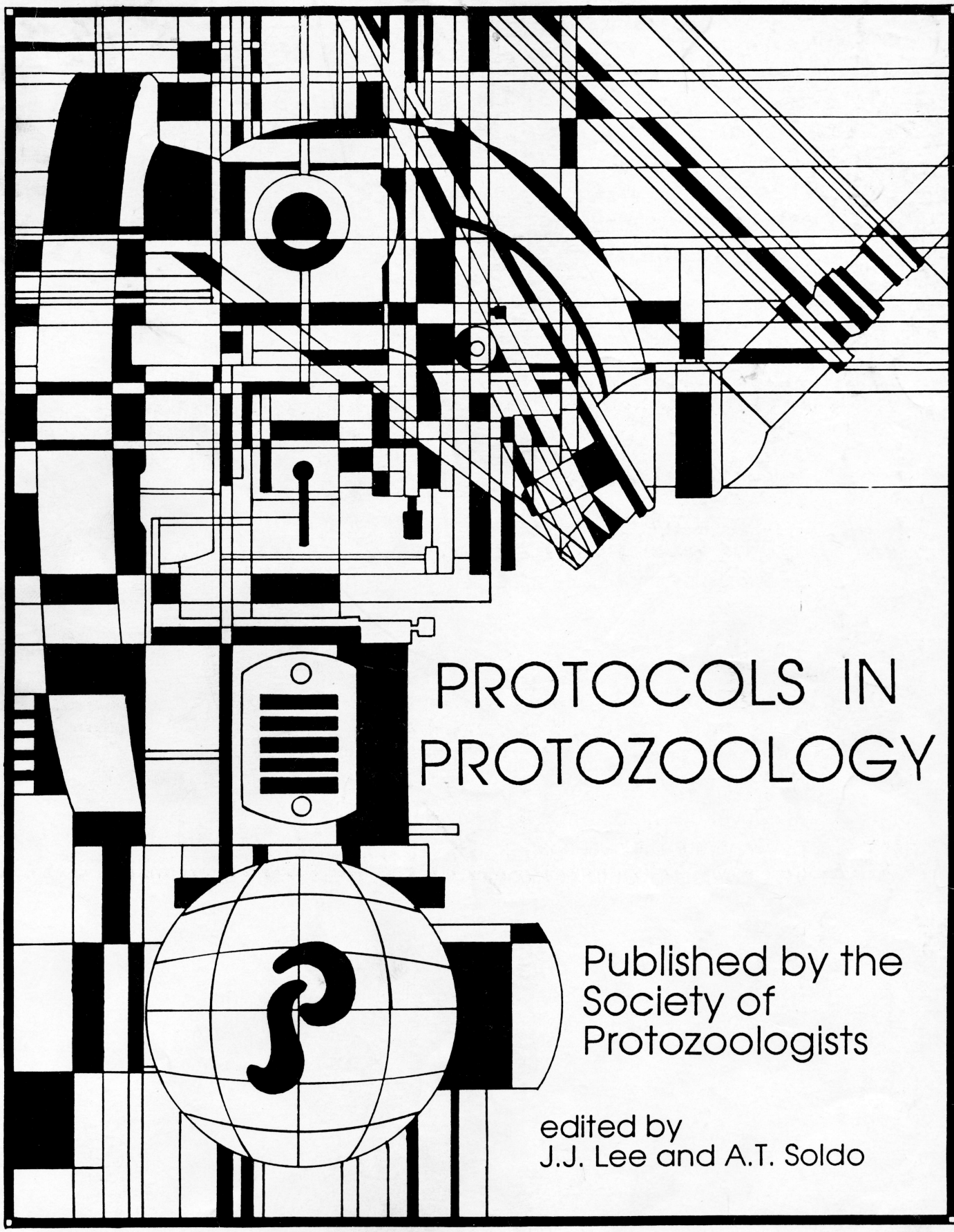

PROTOCOLS IN PROTOZOOLOGY

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ENUMERATING SOIL TESTATE AMOEBAE BY DIRECT COUNTING

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INTRODUCTION

Testate amoebae are usually enumerated by direct microscopy of aqueous soil suspensions (4-9). Various modifications are used worldwide (e. g., 1, 2). We recommend the method of Lüftenegger et al. (6) because recovery experiments revealed a mean efficiency of 86% and the inspected sample mass is sufficient to record even the more rare, euedaphic species (Fig. 2, 3).

PROTOCOL

1. Put a certain amount of fresh (wet) soil, i. e. 1-2 g arable land or grassland or 0.5 g forest litter, in a centrifuge tube by taking 10-20 portions with tweezers from different sites of the sample.
   Remarks: No systematic studies about distribution of testate amoebae in soil are known. Usually, 10-20 soil cores are collected from the area studied and thoroughly mixed to a bulk sample.
2. Fix and stain sample with about 7 ml phenolic aniline blue at least overnight. Mix thoroughly by shaking at least ten times.
   Remarks: Samples can be stored in this condition for years. Centrifuge tubes with screw-tops are ideal for mixing and storing such samples. If suspension becomes colourless after a few hours (sometimes with calcareous soils), centrifugate sample and replace colourless solution by fresh phenolic aniline blue.
3. Wash content of storage vessel into a calibrated cylinder and fill up to 100 ml with distilled water. Close cylinder with parafilm and mix thoroughly by shaking at least ten times.
4. Take a 1 ml subsample from suspension using a 5 ml calibrated pipette cut off at the 1 ml marking to prevent selective sampling of small soil particles.
   Remarks: This step must be done quickly to minimize sedimentation. Collected sample mass corresponds to 0.01-0.02 g and 0.005 g fresh (wet) soil and litter, respectively (see step 1). Dilution depends mainly on soil type. Soils with a high clay content or with high numbers of testate amoebae need a higher dilution than humic or weakly populated soils. The 1 ml sample should be diluted with some water if suspension is too dense.
5. Examine whole subsample by placing suspension dropwise (about 0.1 ml) on grease-free slide. Use a compound microscope and a magnification of at least X100 (objective 10:1, ocular X10). Full (dark blue stained cytoplasm) and empty tests (unstained or light blue) are easily distinguished from unstained, inorganic soil particles.
   Remarks: Add 0.1 ml albumen-glycerol to 1 ml soil suspension if soil particles tend to aggregate on the slide. Preparations should be investigated without coverslip because species identification often requires that tests are turned with a mounted eye lash or isolated with a micropipette. Isolated species can be stored in a moist chamber (e. g., a covered petri dish with damp filter paper covering its bottom) for later identification. However, it is recommended to get acquainted with the respective species inventory

beforehand to restrict time-consuming identification during enumeration.

Stable voucher specimens can be obtained with the following method: collect tests with a micropipette and place them onto a slide covered with a thin, dry layer of albumen-glycerol; dry preparation at room temperature; transfer slide to xylene overnight and mount in synthetic medium (e. g., Eukitt, Euparal). To avoid destruction of voluminous tests support coverslip corners by small pieces of coverslip glass.

6. Repeat steps 4 and 5 to record at least 15-30 (arable land) or 50-70 (grassland, forest) full tests.
   Remarks: An experienced worker needs about 8 hours for the microscopical examination (counting) of 0.1 g soil from arable land or grassland and about 4 hours for 0.005 g forest litter. In bulked samples, the individual minimal area is usually approached with the masses mentioned above. A complete species inventory needs more detailed investigations, e. g., the flotation of empty tests by gas bubbles (3, 8). Repeated investigations of some soils with the method described showed that 2-5 samples distributed over one year yield approximately 50-80% of the species found in 10 samples investigated over two years (Fig. 1).

REAGENTS

a) Phenolic aniline blue (mix components and filter; stable for years)
   15 parts phenol solution (C_6H_5OH; preparation: dissolve 5 g phenol in 100 ml distilled water)
   1 part aniline blue solution ($C_{32}H_{25}N_3Na_2O_9S_3$; preparation: dissolve 1 g aniline blue in 100 ml distilled water)
   4 parts glacial acetic acid (= concentrated acetic acid; $C_2H_4O_2$)
b) Albumen-glycerol
   Use self-made (see Foissner's protargol protocol) or commercial product (e. g., Merck)

CALCULATION

Numbers are calculated per g dry mass of soil and/or as individuals per square meter. Accordingly, the water content and/or the bulk density of the respective soil layer must be determined by standard methods (see textbooks on soil investigation).

$$I\ g^{-1}\ dm = \frac{lwm}{wm \cdot dm} \qquad I\ m^{-2} = \frac{lwm}{wm \cdot dm} \cdot b \cdot d \cdot 10^4$$

b bulk density in g cm^{-3}
d depth (cm) of soil layer sampled (e. g., 5 cm)
dm dry mass of soil expressed from 0.0 to 1.0 (e. g., 0.4 if soil contained 60 % water)
I individual number (abundance)
lwm total individual number counted in wet mass (wm) of soil
wm wet (fresh) mass (in gram) of soil examined (e. g., 0.005 g forest litter)
10^4 factor to relate bulk density to 1 m^2 (= 10000 cm^2)

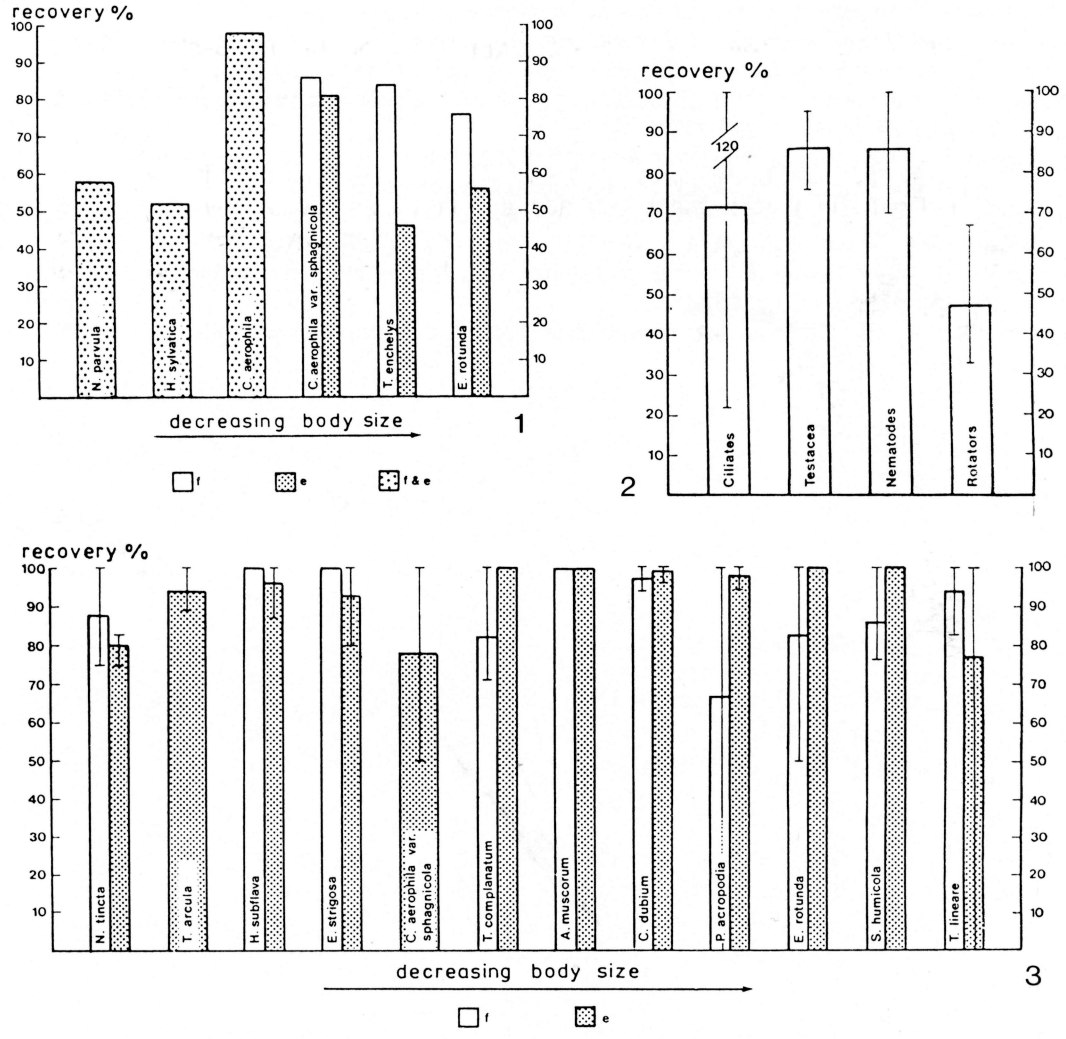

Fig. 1. Cumulative totals of testacean species from 10 sampling occasions over 27 months at three sites in Austria (from [4]). ---------- beech forest, - - - - - xerothermic uncultivated grassland, ... wheat field (see step 6 of protocol for explanation).

Fig. 2, 3. Recovery rates of testacean species (from [6]). **2.** Single species experiments (n = 1) with mineral soil from a levelled ski slope (0-3 cm) and from a mixed deciduous forest (5-10 cm), respectively. **3.** Multiple species experiments (n = 3) with spruce forest litter. e, empty tests; f, full tests.

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