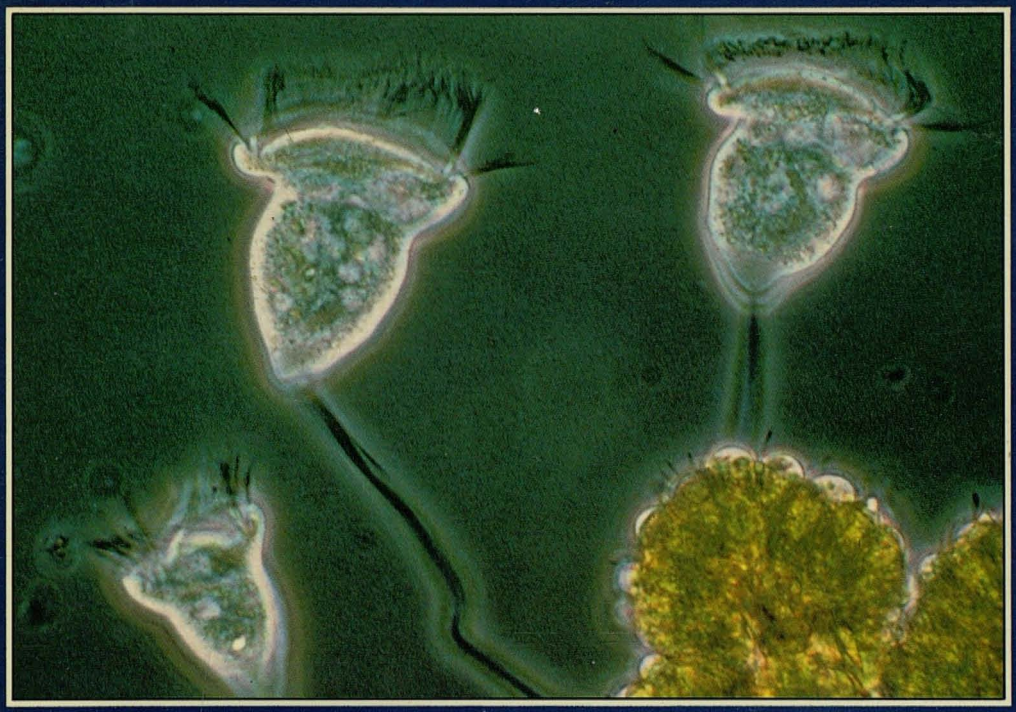
A BEGINNERS GUIDE TO THE COLLECTION, ISOLATION, CULTIVATION AND IDENTIFICATION OF

FRESHWATER PROTOZOA



Culture Collection of Algae and Protozoa
NATURAL ENVIRONMENT RESEARCH COUNCIL

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A beginner's guide to the

Collection, Isolation, Cultivation and Identification of Freshwater Protozoa

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Culture Collection of Algae and Protozoa
(CCAP)

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INTRODUCTION

The protozoa, or 'first animals', are a diverse group of unicellular, animal-like organisms. Many protozoa are parasites of man, causing such diseases as malaria and sleeping sickness, which kill millions of people each year. There are also many types of protozoa that are free-living in the natural aquatic environment. It is the purpose of this booklet to introduce the reader to the diversity of these free-living forms and to some basic practical aspects of their biology and ecology.

The free-living protozoa are characteristically PHAGOTROPHIC: they ingest bacteria, algae and other small organic particles, including other protozoa. Each organism consists of only a single cell, but the size, shape and composition of that cell are extremely variable and very adaptable; protozoa have colonised almost all aquatic environments and they have evolved into a vast number of distinct types. The number of extant species of protozoa is not known with any certainty but about 20,000 species have been described so far. There are certainly many more species waiting to be discovered.

The traditional practice has been to allocate different species names to forms that look different from each other when viewed with the aid of a light microscope. This practice is still valid but as taxonomists have searched for more rigid characters to be used in identification, so they have tended to place new emphasis on intracellular characters and to make use of the much higher magnifications provided by the ELECTRON MICROSCOPE.

The great diversity of protozoa is usually classified by dividing them into broad categories containing similar types. The free-living species can be divided into three large groups; the SARCODINES (amoeboid protozoa), the FLAGELLATES (using flagella for swimming and feeding), and the CILIATES (using cilia for swimming and feeding). Many flagellates (e.g. *Euglena*) are AUTOTROPHIC - they have the green photosynthetic pigment chlorophyll which enables them to fix CO_2 into organic carbon compounds. It is now known that some of these protozoa can also phagocytise (ingest) bacteria and other small particles and use these as a source of carbon (i.e. they can be HETEROTROPHIC). Organisms such as these that can be autotrophic or heterotrophic are often referred to as being MIXOTROPHIC. Under certain circumstances, especially in laboratory cultures and in habitats affected by much organic pollution, many protozoa can also be OSMOTROPHIC whereby at least some of the carbon they require diffuses into the cell from the surrounding water. However, most protozoa

have mouths or specialised feeding organelles, and a capacity for phagotrophy is the principal characteristic which separates protozoa from other, similarly-sized unicellular organisms.

But this definition is not completely satisfactory, and the boundaries between 'animal-like' PROTOZOA and 'plant-like' ALGAE are ill-defined. Some protozoa are phagotrophic but they also have features more commonly seen in algae (chloroplasts, eyespots, pigments) although these are often rudimentary or non-functional. Consequently, some of the organisms featured in this booklet (especially some of the flagellates) are referred to as protozoa by protozoologists and as algae by algologists. The practice of referring to the complete vast collection of protozoa, algae and lower fungi as PROTISTA has the twin merits of disposing with some chauvinism while recognising the close evolutionary relationships that exist between these groups.

The unicellular nature of protozoa means that they are limited in size. Heterotrophic flagellates are usually less than 20 μm (0.02 mm) in length. Most sarcodines are also about 20 μm although some (e.g. *Amoeba*, *Pelomyxa*) may grow to several mm and be visible to the naked eye. Most ciliates are between 20 and 100 μm (0.02-0.1 mm) in length. A few (e.g. *Stentor*, *Spirostomum*) grow up to 1 mm or more.

Protozoa can reproduce asexually, sexually, or by both means. The sexual process of CONJUGATION (when cells fuse and exchange genetic material) is unmistakable in the ciliates, but the asexual process of BINARY FISSION, in which a parent cell grows and divides into two equal sized daughter cells is the most frequently observed reproductive process in protozoa.

Protozoa, like micro-organisms in general, tend to be more abundant in habitats where biological productivity is high. In the open water of a deep lake, one millilitre (ml) of water will usually contain about 1,000 heterotrophic flagellates which feed on planktonic bacteria. The soft sediments of biologically productive ponds and lakes also support large numbers of protozoa, especially ciliates and flagellates, but also amoebae, including the giant naked amoeba *Pelomyxa* and the testate amoeba *Difflugia*. As a rough guide, one ml of lake sediment might contain several thousand ciliates and tens of thousands of flagellates. Many of these BENTHIC (sediment-dwelling) protozoa can live in the absence of oxygen (they are ANAEROBIC) and recent discoveries have shown that many of them contain SYMBIOTIC BACTERIA (i.e. bacteria that live and grow in the cytoplasm of the protozoon) which produce methane (CH_4) gas.

The capacity of protozoa to thrive in organically-polluted environments has also benefitted mankind. By the late 1960's it was known that the protozoa living in sewage-treatment plants, and especially in ACTIVATED SLUDGE plants, were largely responsible for the clarity of the effluent. Much of the dissolved organic matter in sewage is consumed by bacteria. The protozoa then consume the suspended bacteria, and, through this grazing activity, control bacterial abundance in effluents. This control probably maintains the physiological capacity or 'vigour' of the bacterial population. The protozoa also secrete substances which FLOCCULATE many of the other bacteria, removing them from suspension. The end result is that much of the dissolved organic material ends up as particulate material (protozoan cells and flocs) which is removed by sedimentation, allowing a relatively clean effluent to be discharged. One ml of activated sludge will usually contain more than 100,000 protozoa.

In the natural environment, the ecological significance of protozoa also hinges on their grazing activities. To take one example, heterotrophic flagellates graze the bacteria in the sea and in lakes and probably control bacterial abundance at about one million per ml. They digest the bacteria and excrete waste materials which are then taken up as nutrients (especially NITROGEN and PHOSPHORUS) by algae and other photosynthetic micro-organisms. Algae photosynthesise and subsequently excrete much of the organic matter they produce. This organic matter then serves as a source of carbon for the bacteria, which feed the flagellates and the cycle continues. Heterotrophic flagellates probably have an important role to play in this so-called MICROBIAL LOOP.

Most protozoa are COSMOPOLITAN - they are usually found in a specific type of habitat wherever that habitat occurs worldwide. The protozoa living in peat soils and penguin guano in the Antarctic are often apparently identical to those living in moorland peat soils and cow pats in Britain. A useful consequence of this is that identification keys to the protozoa, unlike those to most groups of higher animals, have general applicability worldwide. Many keys and guides to the protozoa have been published although surprisingly few have been prepared with the beginner in mind. This booklet is aimed at the beginner who has access to a microscope, who may be interested in identifying (albeit tentatively) protozoa collected from the natural freshwater environment, and who would be interested in isolating and cultivating some of these in the laboratory. It complements and overlaps slightly the short guide produced by F. Page in 1981 (*The Culture and Use of Free-living Protozoa in Teaching*, published by Institute of Terrestrial Ecology) which is now out of print.

COLLECTION

Protozoa are much more abundant in small, shallow ponds surrounded by agricultural land than in deep lakes at high altitude. Anyone interested in merely obtaining large numbers of protozoa would do well to concentrate their efforts on the more productive habitats. It is certainly easier to obtain water samples from a drainage ditch polluted with farm effluent than from the benthos of a deep lake.

Ponds and Lakes

The traditional way to collect algae and the small animals that live in the open water of lakes (phytoplankton and zooplankton) is to use nets with the appropriate mesh size and to pull these relatively slowly through the water. Nets are rarely of any use in collecting the smallest zooplankters (i.e. the protozoa): the mesh has to be so small that it quickly clogs with the larger phytoplankton and the more fragile species are often broken or distorted. The simplest way to collect protozoa from lakes and ponds is to wade in from the shore, submerge a bottle of at least 1 litre capacity and return it, about two-thirds full, to the laboratory. Most protozoa will remain alive for several hours if the bottle is kept cool and out of direct sunlight. If the bottle is left undisturbed, many of the protozoa will settle on the bottom after about 1 hour. If left overnight, even more protozoa will accumulate on the bottom but by that time it is likely that some of the more fragile and sensitive species will have died, and that some other species will have begun to grow up. The easiest way to remove these sedimented protozoa is by sucking them up in a 10 ml pipette fitted with a rubber bulb.

If a boat is available or if it is otherwise possible to have access to deeper water, a simple weighted bottle can be constructed and operated to fill at the required depth by tugging sharply on the supporting string (Fig. 1) which releases the stopper. We have successfully used such bottles at depths of more than 20 m. At such depths, the water in productive lakes is often devoid of oxygen (i.e. ANAEROBIC), especially in the summer months. If the intention is to collect anaerobic water it is obviously necessary to stopper the bottle as soon as it is hauled to the surface. Several more sophisticated types of bottle samplers are commercially available (e.g. Niskin, Ruttner, van Dorn, Friedinger) but they are expensive. It will not usually be necessary to collect such large volumes of water when collecting only heterotrophic flagellates, which are usually abundant in the water column. In such cases, sterile 10 ml syringes can be used to sample the water directly.

Having collected an unconcentrated water sample, it is usually necessary to concentrate the protozoa it contains. They can be concentrated and kept alive by either of the following methods:

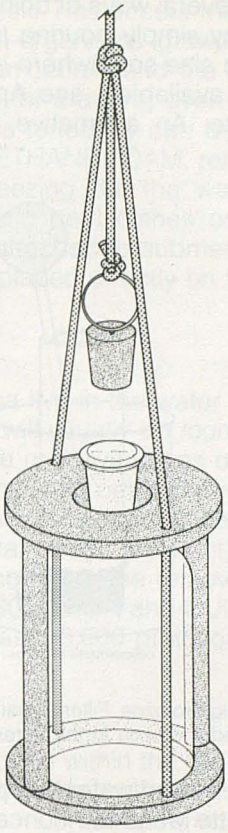


Fig. 1. A simple, weighted bottle sampler. The bung is removed by tugging sharply on the string.

1. Centrifugation. It is necessary to experiment: the speed and duration required depend on the size and density of the protozoa. A very low speed (e.g. 100-200 rpm) for about half a minute is usually all that is required. The large protozoa can be sedimented quite easily. Some fragile species may not survive and the smallest flagellates will probably stay in suspension. In our experience, Pyrex centrifuge tubes with a conical base and a capacity of about 15 ml are the most useful. A hand lens with a magnification of 8 or 10 times should be used to check the efficiency of the centrifugation. Hold the tube up to the light and scan it with the lens. Most swimming ciliates can be seen with this procedure. A hand-operated centrifuge is another useful tool, as indeed is any centrifuge that can be made to come to rest before the protozoa have swum back into the overlying water.

2. Sieving. There are several ways of doing this. Most ciliate protozoa can be concentrated by simply pouring the water sample through a nylon sieve with a pore size somewhere in the range 5-30 μm (nylon mesh is commercially available - see Appendix) and removing the retentate with a pipette. An alternative, more gentle procedure is

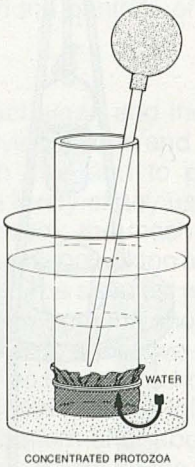


Fig. 2. Device for concentrating protozoa. Filtered water is removed from the inner plastic cylinder, leaving concentrated protozoa in the outer cylinder.

illustrated in Fig. 2. The filtered water that passes up through the sieve is removed with a pipette while the concentrated protozoa remain in the larger container.

Smaller protozoa, especially flagellates, can be trapped on membrane filters. Again, the pore size of the filter will dictate the types of protozoa that can be retained and the speed of filtration. Membrane filters with porosities down to 0.8 μm can be used to concentrate most of the flagellates and many of the less fragile amoebae and ciliates from 200 - 500 ml of relatively clear water. Do not use a high vacuum, and stop filtration when there is still several mm of water on the filter. The concentrate can then be removed with a pipette. A cruder method is to use a paper coffee filter. This filter material tends to clog quite easily but it is a quick and simple method for concentrating at least some of the protozoa.

3. Other Methods. Protozoa can also be encouraged to concentrate themselves. Many protozoa tend to become associated with surfaces (e.g. sediment, ropes, jetties, the surfaces of leaves) that remain

submerged for at least a week. This occurs mainly because they are attracted to the other micro-organisms growing on these surfaces. Many protozoa (e.g. most amoebae, and the ciliates *Aspidisca* and *Vorticella*) are also adapted for crawling over or becoming attached to submerged surfaces. This tendency can be encouraged by submerging pieces of POLYURETHANE FOAM, retrieving them after several weeks, and then squeezing out the water and concentrated protozoa. Alternatively, plastic petri dishes or glass slides can be suspended in the water or attached to submerged twigs. When they are recovered they can be placed directly on the microscope stage.

Rivers

Few truly planktonic protozoa live in the water column of any but the slowest flowing rivers. Most will usually be found in the sediment and on submerged surfaces. The under surfaces of duckweed and lilies, and the stems of *Iris*, bulrushes and other macrophytes growing close to the banks, are often rich sources of protozoa. The nodes of *Ranunculus penicillatus* (chalk-stream water crowfoot) are a particularly rich source. The water within a node can be removed with a finely-drawn glass Pasteur pipette (see ISOLATION and CULTIVATION). One ml of this water may contain at least 10,000 protozoa.

Sediment

Most benthic protozoa are found within the top 1 cm of sediment and if the intention is to collect as many protozoa as possible there is little point in taking sediment from much deeper (incidentally, this does not apply to marine and intertidal sediments where the larger grain sizes and periodic tidal flushing ensure that the protozoa and other interstitial fauna penetrate much deeper). Surface sediments containing a lot of partially degraded organic matter are particularly rich in protozoa. If the water is not too deep, this surface sediment can usually be removed with a teaspoon or similar implement, or with a 10 ml pipette (fit a large rubber bulb on the end and snap off the point of the pipette to allow the organic aggregates and larger particles to travel up the tube). Greater volumes can also be sucked up with a 'pooter', which is usually used for collecting insects. Most quantitative studies of benthic protozoa involve taking relatively undisturbed core samples with plastic tubes. They usually have internal diameters of 4-6 cm and there are many designs.

In larger lakes the wind often causes much DETRITUS (decomposing plant and animal material) to be swept on to the shore. When this is collected wet it often contains many protozoa. The same can be said

for the thick layers of BLOOM-FORMING ALGAE (usually CYANOBACTERIA) that are sometimes swept to one end of a lake.

Bogs

Protozoa are abundant in *Sphagnum* bogs. TESTATE AMOEBAE, NAKED AMOEBAE and some of the larger flagellates are particularly abundant. Collecting them is relatively simple and requires nothing more than a pair of rubber boots and a jam jar. Stand in the bog and allow the water to flood around the boots. Alternatively, collect some of the wet *Sphagnum* and squeeze out the water into a collecting jar.

OBSERVING FRESH WATER SAMPLES

It is possible to see some of the larger protozoa with the naked eye but it is impossible to discern any of their internal structures without the aid of a microscope. There are few standard procedures for the examination of protozoa living in samples of water and sediment. If we assume total ignorance of what the sample contains, the following guidelines may be of some use.

It is always best to keep interference with the protozoa to a minimum and to observe undisturbed living material for as long as possible. The free-swimming behaviour of protozoa is most easily observed when they are contained in glass chambers, e.g. under cover slips supported at all corners by vaseline. Larger chambers can be constructed quite easily from glass slides and strips of glass or pieces of cut glass tubing. The Sedgewick-Rafter Cell is a commercially available glass chamber (Fig. 3): it holds 1 ml spread 1 mm deep over a

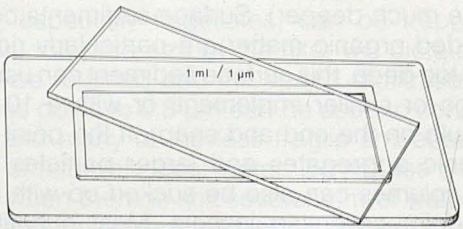


Fig. 3. A commercially available glass cell holding 1 ml.

large area. The chamber is supplied with a thick cover glass which can be replaced by a large, thin cover glass, allowing objectives with magnifications up to about 16× to be used. Begin observation with a low power (e.g. 4×) objective and observe as many different types of protozoa and their different swimming behaviours as possible. The

fastest swimming protozoa will be the ciliates. The flagellates move significantly more slowly, and often with a distinctive behaviour, e.g. gliding, rotating, vibrating or a gentle side-to-side rocking motion. The slowest moving protozoa are the amoebae, which move by extension or eruption of the cell mass. Naked amoebae are not usually observed in open water samples (although they can usually be isolated - see ISOLATION and CULTIVATION); they are more common in sediments, in detritus and on leaf surfaces. There may be a few suspended organisms which are almost spherical, with many spines - these will probably be HELIOZOANS. It is sometimes possible to see other organisms which are similar but much smaller - these could be spine-bearing CHRYSOMONAD FLAGELLATES which can be very abundant. They are unlikely to be seen with an objective lens of less than 10x magnification.

Most protozoa can be kept alive in chambers like the Sedgewick-Rafter Cell for about 30 min. They can be kept alive longer if the chamber contains some air and if the water can be kept below about 20°C.

It is unlikely that protozoa will be the only micro-organisms swimming in the water sample. There will probably be some free-swimming dinoflagellates and green algae, both of which are pigmented. There may also be some small planktonic invertebrates (metazoans), some of which superficially resemble ciliates. These will almost always be rotifers and gastrotrichs in the water column, and nematodes, turbellarians and tardigrades in sediments and detritus.

Samples of sediment should be diluted with some of the overlying water and placed directly on a microscope slide without a cover slip. A volume of about 50 µl (0.05 ml) is sufficient. Use a needle to move the particles around and to break up the larger organic aggregates. It is best to do this while observing the material under the microscope. Use BRIGHT FIELD illumination and an objective lens with a magnification of 4× or 10×. You will certainly see some small ciliates darting about (these will probably be SCUTICOCILIATES) and possibly crawling over sediment aggregates (e.g. *Aspidisca*). You may also see some larger ciliates (e.g. *Loxodes*, *Spirostomum*, *Stentor*) and large naked amoebae (e.g. *Pelomyxa*). If a low power binocular microscope with DARKFIELD illumination is available (the latter effect may be obtained with a rotating mirror beneath the stage) it is often worth using this to get some impression of the great abundance of protozoa in sediments: swimming flagellates (and some bacteria) will also be readily observed. Some TESTATE AMOEBAE (e.g. *Difflugia*, *Centropyxis*) have lumpy, opaque shells which can look remarkably like sand

grains or the sediment particles themselves. Others (e.g. *Euglypha*, *Nebela*) have transparent shells within which the amoeba can be seen. Look for a regular shell shape, possibly with spines, and an opening (aperture) through which pseudopodia may protrude, sometimes making the whole shell move.

The next step is to increase the magnification on the microscope. The working distance and the depth of field both get smaller as the objective magnification increases and it also becomes necessary to add a cover slip. Apply a little vaseline on each corner of a square cover slip and place this 'raised' cover slip on a small water sample on a slide. This will allow a 20-25× objective to be used, and if the cover slip is pressed down gently, a 40× objective can be swung into place. Two things will be discovered while doing this; the large amoebae and large ciliates will be restrained and possibly squashed, while the smallest protozoa will be unhindered. Most ciliates, especially the small scuticociliates will still be swimming too fast to be observed properly but many flagellates and perhaps small amoebae will become apparent.

Coping with fast-swimming ciliates can be more of a problem and there is no simple solution that works for all species. Most ciliates do periodically come to rest, usually for a few seconds at a time, and patient observation will invariably bring its rewards. Moreover, as the water beneath the cover slip evaporates, so the ciliates are gradually restrained, although their shape becomes distorted and they eventually die. The main advantage associated with these flattened cells is that it is possible to use the highest power objectives, including oil immersion lenses, to resolve much of the internal detail. Contractile vacuole (see glossary diagrams) activity slows down and the vacuole swells up. Food vacuoles grow steadily larger as they are squeezed - often allowing their contents to be identified.

The use of the viscous substance methyl cellulose (sometimes marketed as 'Methocel'; prepared as a 10% aqueous solution) is often advocated for slowing down very active protozoa. The technique is sometimes messy to use, but protozoa are extremely sensitive to the viscosity of the liquid that surrounds them and the method will significantly slow down cells or bring them to a halt. Most textbooks prescribe a variety of other compounds and concoctions which can be used as slowing agents, including nickel sulphate (2%) and formalin (4%), which can be applied to the water drop on the end of a needle. Strands of cotton wool, growths of filamentous bacteria and clumps of detritus all help to restrain and hinder the movements of the larger protozoa.

IDENTIFICATION

It is often difficult or time-consuming to identify protozoa to the level of species but it is usually quite easy to place them within one of the higher levels of classification. It is not intended that this guide should facilitate the unambiguous identification of protozoa to the level of species but it should enable the identification of many of the more common freshwater protozoa, at least to the level of the three broad categories of protozoa (see page 66) and at best, to the level of genus.

The various methods used in identifying protozoa are largely dictated by the nature of the protozoa themselves. Ciliates show an enormous diversity of size and shape and identifying some of them to genus may require nothing more than a little casual observation of living cells with a light microscope. However, most flagellates and amoebae will require considerably more patience and care. For example, although amoebae may survive under a cover slip, particularly if it is supported by vaseline, the presence of the glass usually interferes with their locomotion. The mode of locomotion is an important diagnostic character for identifying amoebae. The solution is simple but time-consuming since it relies on the CULTIVATION (see page 66) of sufficient cells for the preparation of 'hanging drops'. A drop containing many cells (hence the need for prior cultivation) is placed on a cover slip, which is then left in a MOIST CHAMBER (e.g. Petri dish lined with wet cotton

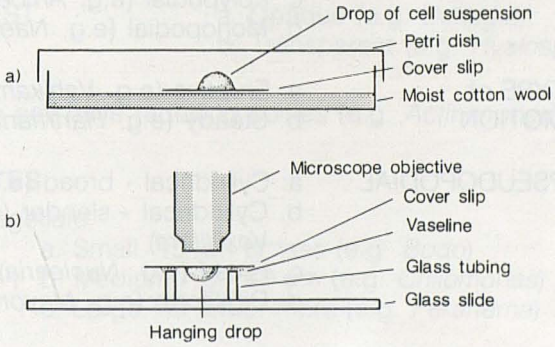


Fig. 4. A hanging drop preparation.

wool; see Fig. 4) until the cells become attached to the glass surface. The cover slip is then turned over and placed on a section of glass tubing glued to a microscope slide. The cells on the under side of the cover slip can be viewed with a high power microscope. If the cover slip is sealed against the tubing with vaseline, the amoebae should remain active for several days.

Checklist of Characters Useful in Identifying Protozoa

We have assembled a list of characters which are useful in identifying protozoa in each of the three principal categories (sarcodines, flagellates and ciliates). These are all key features, which in most cases can be easily observed without sophisticated staining procedures (see also IMAGE ENHANCEMENT and FIXATION). The reader might find it useful to use the list in conjunction with the glossary diagrams and the illustrations of some common protozoa that are provided. It often helps to know the size of protozoa when trying to identify them. Although there is always some variation in size between representatives of a species, the species that are characteristically small (e.g. *Cyclidium*, *Acanthamoeba*) are usually significantly smaller than those that are characteristically large (e.g. *Stentor*, *Chaos*). *Acanthamoeba* never grows to 500 μm and no *Stentor* is as small as 20 μm.

Size is measured with an eyepiece graticule - a glass disk bearing a printed scale which is inserted into the eyepiece. This scale is calibrated for each objective using a known measurement (usually 1 mm divided into 100 parts) engraved or printed on a slide.

SARCODINES

1. Is the LOCOMOTIVE FORM
   a. Flattened (e.g. *Vexillifera*)
   b. Cylindrical (e.g. *Pelomyxa*)
   c. Polypodial (e.g. *Amoeba*)
   d. Monopodial (e.g. *Naegleria*)

2. Is the TYPE of LOCOMOTION
   a. Eruptive (e.g. *Vahlkampfia*)
   b. Steady (e.g. *Hartmannella*)

3. Is the PSEUDOPODIAL TYPE
   a. Cylindrical - broad (e.g. *Chaos*)
   b. Cylindrical - slender (e.g. *Vexillifera*)
   c. Filose (e.g. *Nuclearia*)
   d. Digitiform (e.g. *Mayorella*)

4. Does the SUSPENDED or FLOATING form have radiating pseudopodia (e.g. *Vannella*)?

5. What is the form of the POSTERIOR END (e.g. *Saccamoeba*, with obvious uroid)?

6. Are UROIDAL FILAMENTS present (e.g. *Vahlkampfia*)?

7. What is the LENGTH of the locomoting amoeba?

8. What is the number and appearance of the NUCLEI (e.g. presence of central nucleolus (*Saccamoeba*), granular appearance (*Trichamoeba*), or multinucleate (*Chaos*)?

9. Are CYTOPLASMIC CRYSTALS present (e.g. *Cochliopodium*)?

10. Are CYSTS present (e.g. *Acanthamoeba*)?

11. What is the extent of the HYALINE ZONE (e.g. broad (*Hartmannella*), or narrow (e.g. *Cashia*))?

12. Does the organism transform from an amoeba into a FLAGELLATE (e.g. *Naegleria*)?

13. Is a TEST present (e.g. *Nebela*)?

14. What is the shape of the TEST (e.g. flask-shaped *Hyalosphenia*)?

15. What is the position and shape of the TEST APERTURE (e.g. central (*Arcella*), or terminal (*Cyphoderia*)?

16. Is the TEST
    a. Opaque (e.g. *Difflugia*)
    b. Transparent (e.g. *Hyalosphenia*)

17. Does the cell have radiating spines (e.g. *Actinospherium*)?

FLAGELLATES

1. Is the flagellate
    a. Small, 15 μm or less (e.g. *Bodo*)
    b. Medium, 16 - 40 μm (e.g. *Chilomonas*)
    c. Large, 40 μm or more (e.g. *Peranema*)

2. Is the usual shape or form of the flagellate
    a. Ovoid (e.g. *Cryptomonas*)
    b. Spherical (e.g. *Paraphysomonas*)
    c. Elongate (e.g. *Heteronema*)
    d. Twisted (e.g. *Rhabdospira*)
    e. Cylindrical (e.g. *Rhabdomonas*)
    f. Flattened (e.g. *Petalomonas*)
    g. Roughly spherical (e.g. *Peridinium*)

3. How many FLAGELLA are present or visible?
    a. One long anterior only (e.g. *Menoidium*)
    b. Two: one short anterior, one long posterior (e.g. *Anisonema*)
    c. Two anterior, equal length (e.g. *Chilomonas*)
    d. Two: one anterior, one close to body (e.g. *Peranema*)
    e. Two: one very short, one long anterior (e.g. *Spumella*)

4. What is the motility of the FLAGELLA?
    a. Two anterior, both motile (e.g. *Polytoma*)
    b. One anterior, pointing straight ahead, motile (e.g. *Petalomonas*)
    c. One anterior motile, one posterior trailing (e.g. *Entosiphon*) or frequently attached to surfaces (e.g. *Bodo*)

5. How does the flagellate body move?
    a. Rapidly vibrating (e.g. *Heteromita*)
    b. Slow or steady gyration (e.g. *Entosiphon*)
    c. Free-swimming individuals (e.g. *Gymnodinium*) or colonies (e.g. *Sphaeroeca*)
    d. 'Creeping' (e.g. *Anisonema*)
    e. 'Gliding' (e.g. *Petalomonas*)
    f. Amoeboid (e.g. *Cercomonas*)
    g. Euglenoid (e.g. *Khawkinea*)

6. Does the flagellate have any obvious appendages?
    a. Anterior stiff arms or TENTACLES (e.g. *Pteridomonas*)
    b. Collar encircling an anterior flagellum (e.g. *Monosiga*)
    c. Faintly visible spines (e.g. *Paraphysomonas*)
    d. A secreted LORICA in which it lies (e.g. *Bicoeca*)
    e. A STALK, to which it is attached as a colony (e.g. *Anthophysa*)

7. Is there an anterior opening leading to a tube-like CANAL and wider RESERVOIR (euglenid flagellates, e.g. *Astasia*)? The opening of the canal where the flagella are inserted may be either at the tip (APICAL, e.g. *Menoidium*) or just below (SUB-APICAL, e.g. *Khawkinea*) or sometimes either (e.g. *Distigma*).

8. Are there faint lines (striations) or ridges on the PELLICLE (e.g. *Rhabdomonas*), or is there an EYESPOT - a small pigmented (usually red) light-sensitive area near the anterior (e.g. *Hyalophacus*)? Both are typically euglenid features, although other forms may also have eyespots (e.g. *Polytomella*, *Spumella*).

9. Are there storage (PARAMYLON) granules - usually oval, rod or ring-shaped - in the cytoplasm? These are found in this form only in euglenid flagellates (e.g. *Menoidium*, *Hyalophacus*).

10. Does the cell have an INGESTION APPARATUS (euglenid flagellates only) consisting of two rods with an opening near, but separate from, the canal (e.g. *Heteronema*, *Peranema*) or a tube-like SIPHON, with a similar opening (*Entosiphon* only)?

CILIATES

1. Is the cell WITHOUT CILIA, but with TENTACLES and usually with a STALK (e.g. *Podophrya*)?

2. Is the cell usually SESSILE (i.e. attached to a surface)?
   a. Size usually <100 μm, with a thin stalk (e.g. *Vorticella*)
   b. Size usually >100 μm, without a stalk (e.g. *Stentor*, especially if contractile and trumpet-shaped)

3. Does the cell have a LORICA (e.g. *Tintinnopsis*, especially in open water)?

4. If the cell has no obvious AZM and (usually) no CIRRI, does it have a SIMPLE MOUTH (usually just a hole or slit) at, or close to, the anterior end (e.g. *Prorodon* if cell approximately SPHERICAL or OVOID; *Dileptus*, *Amphileptus* or *Lacrymaria* if ELONGATE and/or FLASK-SHAPED)?

5. Is the mouth in a DEEP NOTCH in the ventral surface (e.g. *Plagiopyla*, especially if KIDNEY-SHAPED and ANAEROBIC)?

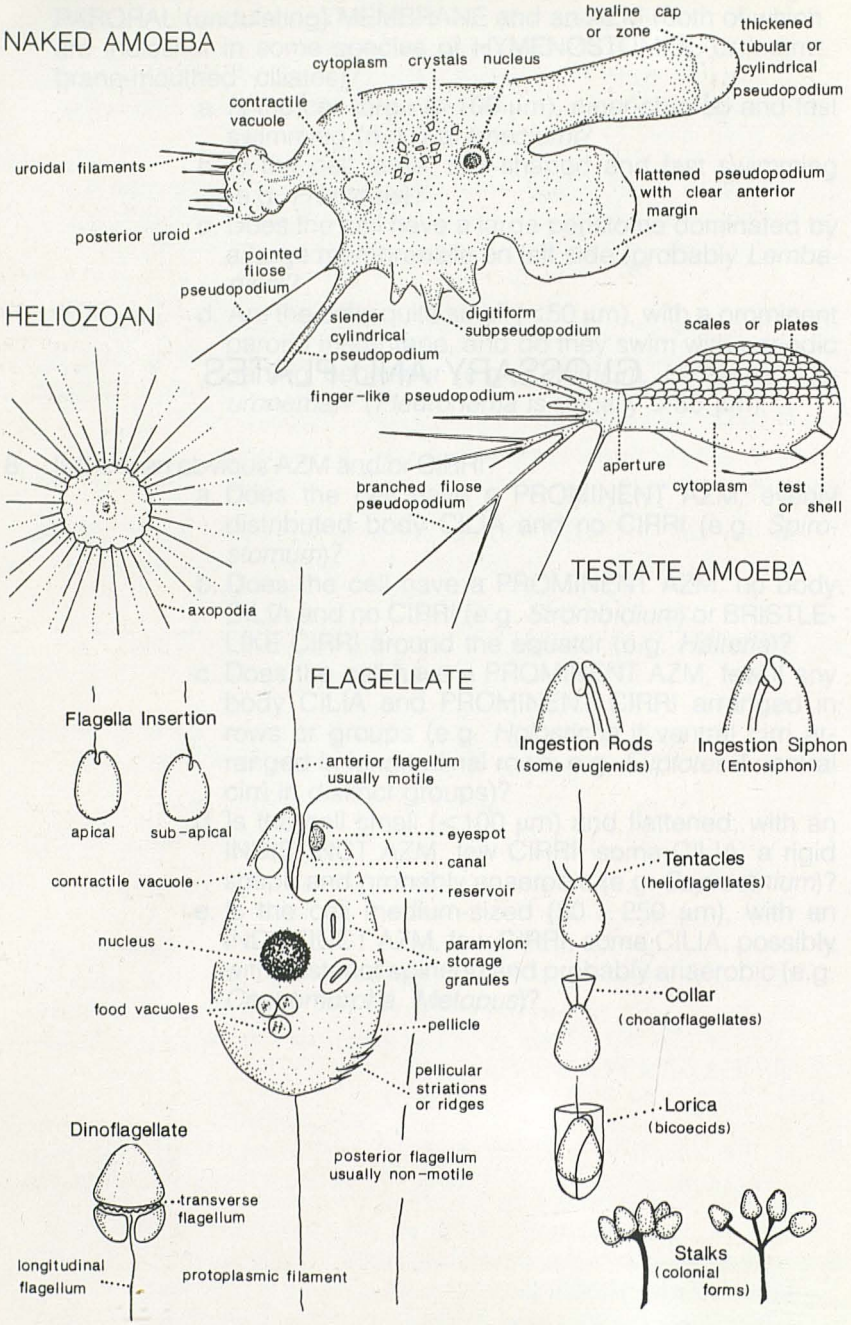
6. Is the mouth on the VENTRAL SURFACE, with a RIGID TUBE (the cytopharyngeal basket) leading from it, into the cell (e.g. *Chilodonella*, especially if dorso-ventrally flattened)?

7. Does the cell have SPECIALISED ORAL CILIATURE, including a PARORAL (undulating) MEMBRANE and an AZM (both of which are indistinct in some species of HYMENOSTOMES, or 'membrane-mouthed' ciliates)?
    a. Is the cell large (>100 μm), cigar-shaped and fast swimming (e.g. *Paramecium*)?
    b. Is the cell large, foot-shaped and fast swimming (e.g. *Frontonia*)?
    c. Does the cell have a large peristome dominated by a large membranelle on left side (probably *Lembadion*)?
    d. Are the cells quite small (<50 μm), with a prominent paroral membrane, and do they swim with periodic darting behaviour (e.g. *Cyclidium*, *Uronema*, *Pleuronema*)? (*Pleuronema* is usually >50 μm).

8. Is there an obvious AZM and/or CIRRI?
    a. Does the cell have a PROMINENT AZM, evenly distributed body CILIA and no CIRRI (e.g. *Spirostomum*)?
    b. Does the cell have a PROMINENT AZM, no body CILIA and no CIRRI (e.g. *Strombidium*) or BRISTLE-LIKE CIRRI around the equator (e.g. *Halteria*)?
    c. Does the cell have a PROMINENT AZM, few if any body CILIA and PROMINENT CIRRI arranged in rows or groups (e.g. *Holosticha* if ventral cirri arranged in longitudinal rows; e.g. *Euplotes* if ventral cirri in distinct groups)?
    d. Is the cell small (<100 μm) and flattened, with an INDISTINCT AZM, few CIRRI, some CILIA, a rigid shape and probably anaerobic (e.g. *Saprodinium*)?
    e. Is the cell medium-sized (50 - 250 μm), with an INDISTINCT AZM, few CIRRI, some CILIA, possibly with posterior spine(s) and probably anaerobic (e.g. *Caenomorpha*, *Metopus*)?

GLOSSARY AND PLATES

GLOSSARY DIAGRAM 1



GLOSSARY DIAGRAM 2 CILIATES

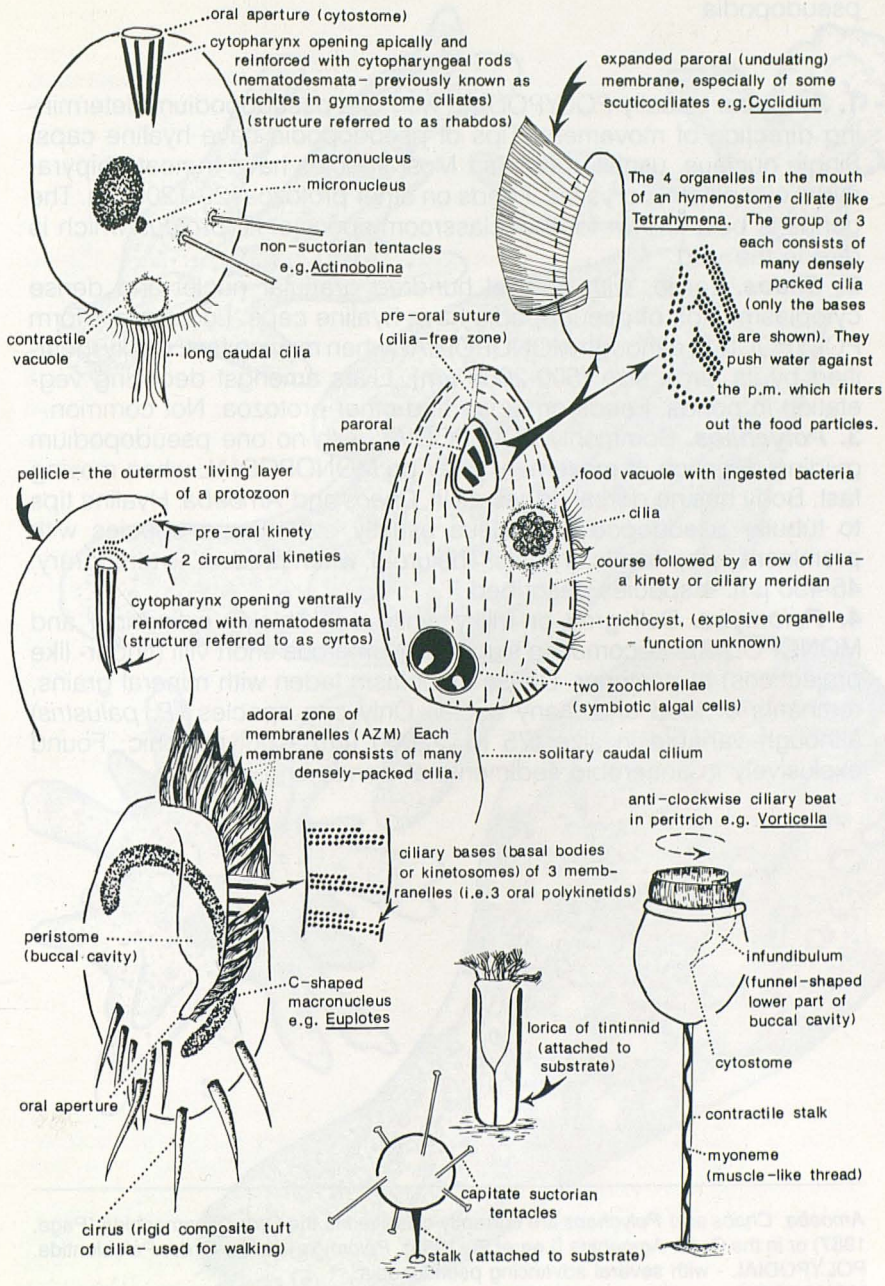


Plate 1. NAKED AMOEBAE; with thickened tubular or cylindrical pseudopodia

1. *Amoeba.* Usually POLYPODIAL with one pseudopodium determining direction of movement. Tips of pseudopodia have hyaline caps. Single nucleus, usually granular. Most species have truncate bipyramidal cytoplasmic crystals. Feeds on other protozoa. 20-1200 μm. The genus is best known for the 'classroom species' *A. proteus* which is rare in the wild.

2. *Chaos.* Large, with several hundred granular nuclei and dense cytoplasm. Tips of pseudopodia have hyaline caps. Locomotory form POLYPODIAL, although MONOPODIAL when moving fast. Easily identified by its large size (500-2000 μm). Lives amongst decaying vegetation in ponds. Feeds on algae and other protozoa. Not common.

3. *Polychaos.* Commonly POLYPODIAL with no one pseudopodium guiding direction of movement. May be MONOPODIAL when moving fast. Body has no dorsal ridges as in *Chaos* and *Amoeba.* Hyaline tips to tubular pseudopodia. Nucleus usually oval. Some species with prominent cytoplasmic crystals. The uroid, when present, is temporary. 45-450 μm. 4 species described.

4. *Pelomyxa.* Dull grey or milky white cell, thickly cylindrical and MONOPODIAL. Locomotion sluggish. Numerous short villi (finger- like projections) at posterior. Dense cytoplasm laden with mineral grains, remnants of food and many nuclei. Only one species (*P. palustris*) although variable in size (75 to >2000 μm) - polymorphic. Found exclusively in anaerobic sediments of ponds and lakes.

Amoeba, Chaos and *Polychaos* are currently classified in the Order Euamoebida (Page, 1987) or in the Order Amoebida (Lee *et al.*, 1985). *Pelomyxa* is in the Order Pelobiontida.
POLYPODIAL - with several advancing pseudopodia.
MONOPODIAL - with one pseudopodium; cell moves as a single mass

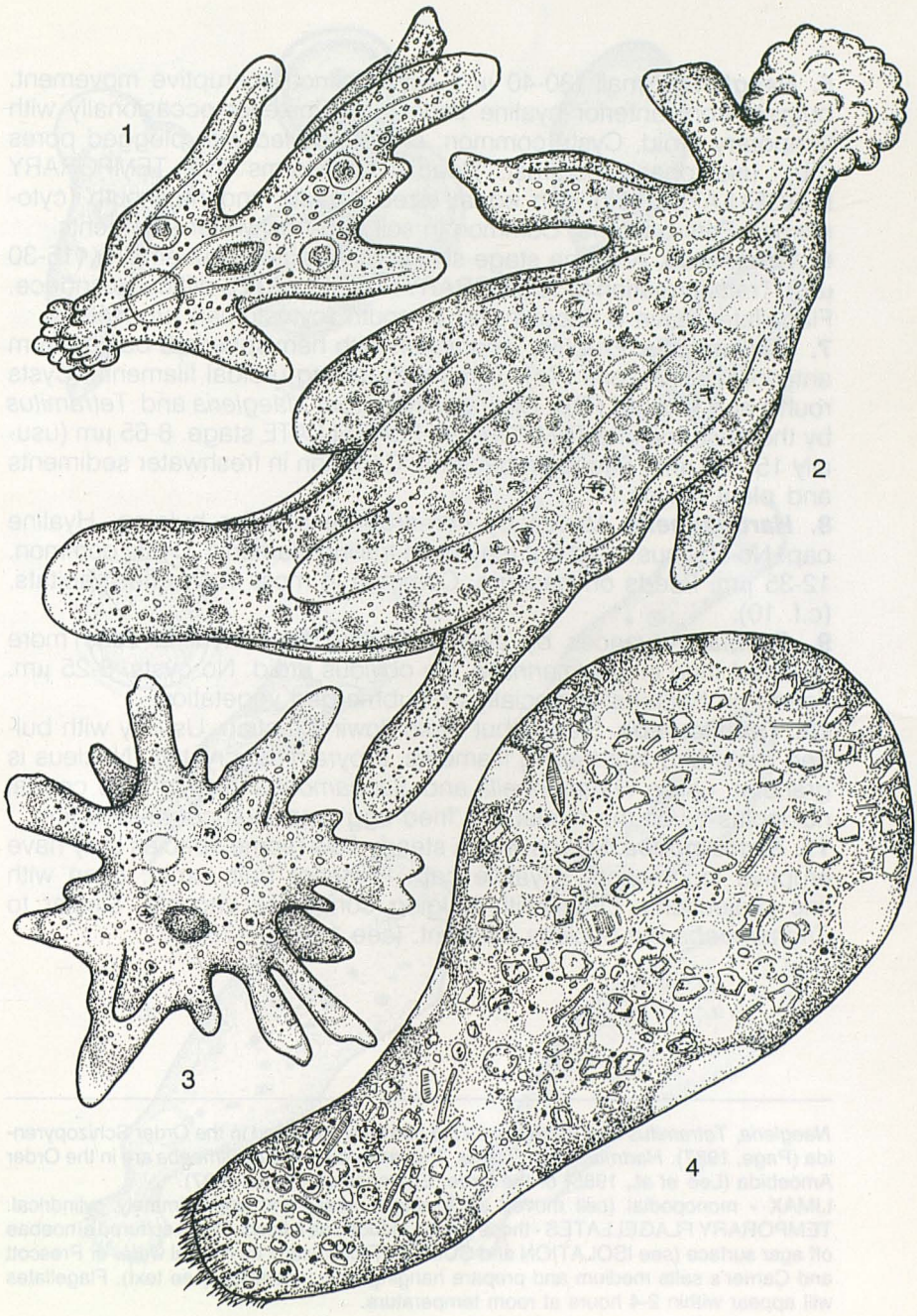

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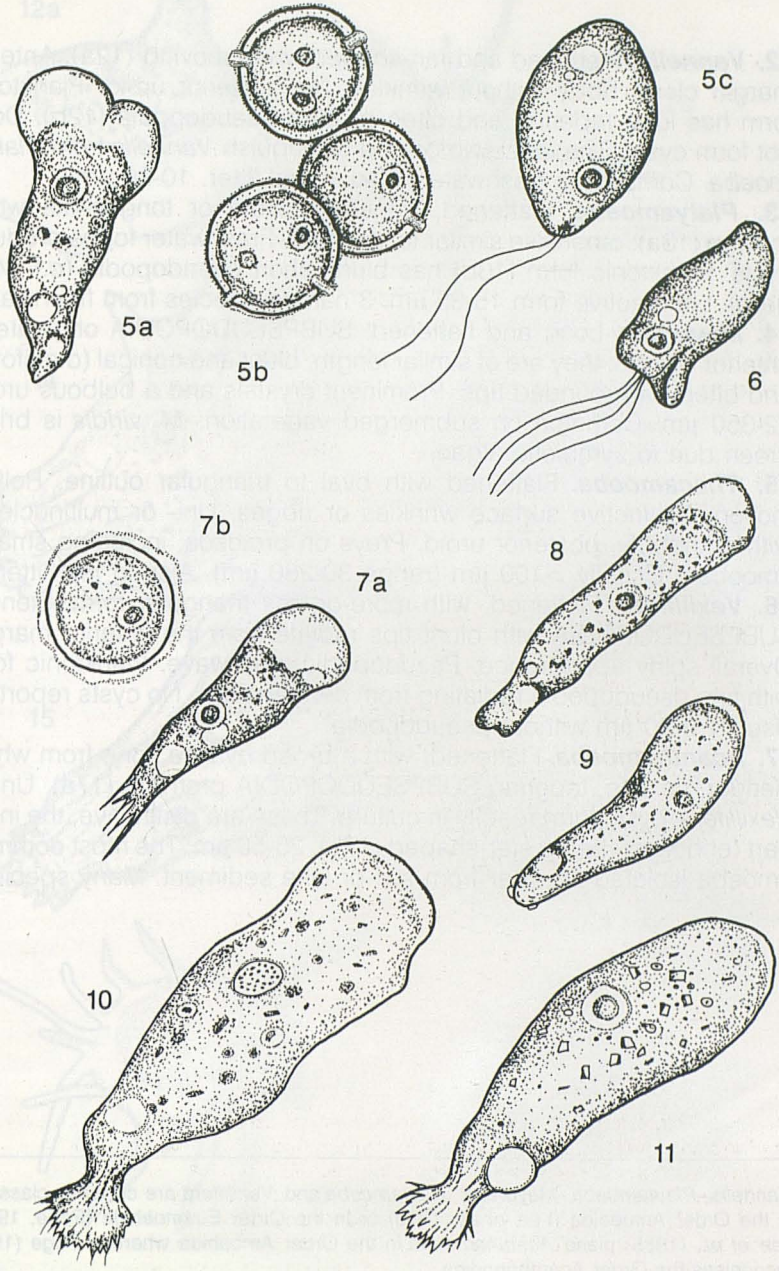
Plate 2. NAKED AMOEBAE; small LIMAX

5. *Naegleria.* Small (30-40 μm) with distinctive eruptive movement, bulging from anterior hyaline zone (5a). Posterior occasionally with temporary uroid. Cysts common, smooth-walled with plugged pores (5b); use phase contrast. Readily transforms into TEMPORARY FLAGELLATES (5c) with equal sized flagella and no mouth (cytostome). Eats bacteria. Common in soil and freshwater sediments.
6. *Tetramitus.* Amoeba stage similar to *Naegleria* but smaller (15-30 μm). Transformation to TEMPORARY FLAGELLATES difficult to induce. Flagellates have 4 flagella and a mouth (cytostomal) groove.
7. *Vahlkampfia.* Eruptive locomotion with hemispherical bulges from anterior hyaline zone (7a). Some with trailing uroidal filaments. Cysts round and smooth (7b). Distinguished from *Naegleria* and *Tetramitus* by the absence of a TEMPORARY FLAGELLATE stage. 8-65 μm (usually 15 - 35 μm). Feeds on bacteria. Common in freshwater sediments and plant debris.
8. *Hartmannella.* Moves by non-eruptive anterior bulging. Hyaline cap. No obvious uroid but may have trailing filaments. Cysts common. 12-35 μm. Feeds on bacteria. Common in most freshwater habitats. (c.f. 10).
9. *Cashia.* Advances by steady forward flow. Hyaline zone more reduced than in *Hartmannella.* No obvious uroid. No cysts. 8-25 μm. Feeds on bacteria, especially on submerged vegetation.
10. *Trichamoeba.* Steady but rapid flowing motion. Usually with bulbed uroid and fine trailing filaments. Bipyramidal crystals. Nucleus is granular, unlike *Hartmannella* and *Saccamoeba* in which the central nucleolus gives the nucleus a 'fried-egg' appearance. 45-300 μm.
11. *Saccamoeba.* Moves with a steady flow (some species may have eruptive locomotion). Hyaline cap. Posterior bulb uroid, often with fine projections. Often with bulging contractile vacuole. Similar to *Trichamoeba* but nucleus different. (see 10). 30-175 μm.

Naegleria, *Tetramitus* and *Vahlkampfia* are currently classified in the Order Schizopyrenida (Page, 1987). *Hartmannella*, *Cashia*, *Trichamoeba* and *Saccamoeba* are in the Order Amoebida (Lee *et al.*, 1985) or the Order Euamoebida (Page, 1987).
LIMAX - monopodial (cell moves as a single mass) and approximately cylindrical. TEMPORARY FLAGELLATES - these can be induced as follows. Wash cultured amoebae off agar surface (see ISOLATION and CULTIVATION) with 1 ml natural water or Prescott and Carrier's salts medium and prepare hanging drop prepartion (see text). Flagellates will appear within 2-4 hours at room temperature.


5a
5b
5c
6
7b
7a
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11

Plate 3. NAKED AMOEBAE; flattened

12. *Vannella.* Flattened and fan-shaped when moving (12a). Anterior margin clear. Body without wrinkles. No posterior uroid. Planktonic form has long tapering and often pointed pseudopodia (12b). Does not form cysts. The latter two criteria distinguish *Vannella* from *Platyamoeba*. Common in freshwater. Also in leaf litter. 10-80 μm.

13. *Platyamoeba.* Flattened, generally ovoid or tongue-like when moving (13a); otherwise similar to *Vannella*. Freshwater forms produce cysts. Planktonic form (13b) has blunt, short pseudopodia (c.f. *Vannella*). Locomotive form 15-35 μm. 3 named species from freshwater.

14. *Mayorella.* Long and flattened. SUBPSEUDOPODIA originate at anterior margin: they are of similar length, blunt and conical (digitiform) and often with rounded tips. Prominent crystals and a bulbous uroid. 12-350 μm. Common on submerged vegetation. *M. viridis* is bright green due to symbiotic algae.

15. *Thecamoeba.* Flattened with oval to triangular outline. Rolling motion. Distinctive surface wrinkles or ridges. Uni- or multinucleate with a knobbly, posterior uroid. Preys on protozoa, including smaller amoebae. Usually >100 μm (range 30-280 μm). Also in leaf litter.

16. *Vexillifera.* Flattened, with more-or-less triangular form. Slender SUBPSEUDOPODIA with blunt tips radiate from the anterior margin. Overall spiny appearance. Pseudopodia may wave. Planktonic form with fine pseudopodia radiating from central mass. No cysts reported. Usually 8-20 μm without pseudopodia.

17. *Acanthamoeba.* Flattened, with a broad hyaline zone from which slender, flexible, tapering SUBPSEUDOPODIA protrude (17a). Unlike *Vexillifera*, cysts form readily in culture. These are distinctive, the inner part (endocyst) being star-shaped (17b). 25-50 μm. The most common amoeba isolated on agar from soil or lake sediment. Many species.

Vannella, *Playtamoeba*, *Mayorella*, *Thecamoeba* and *Vexillifera* are currently classified in the Order Amoebida (Lee *et al.*, 1985) or in the Order Euamoebida (Page, 1987). Lee *et al.*, (1985) place *Acanthamoeba* in the Order Amoebida whereas Page (1987) recognises the Order Acanthopodida.

SUBPSEUDOPODIA - projections from a broad hyaline zone.

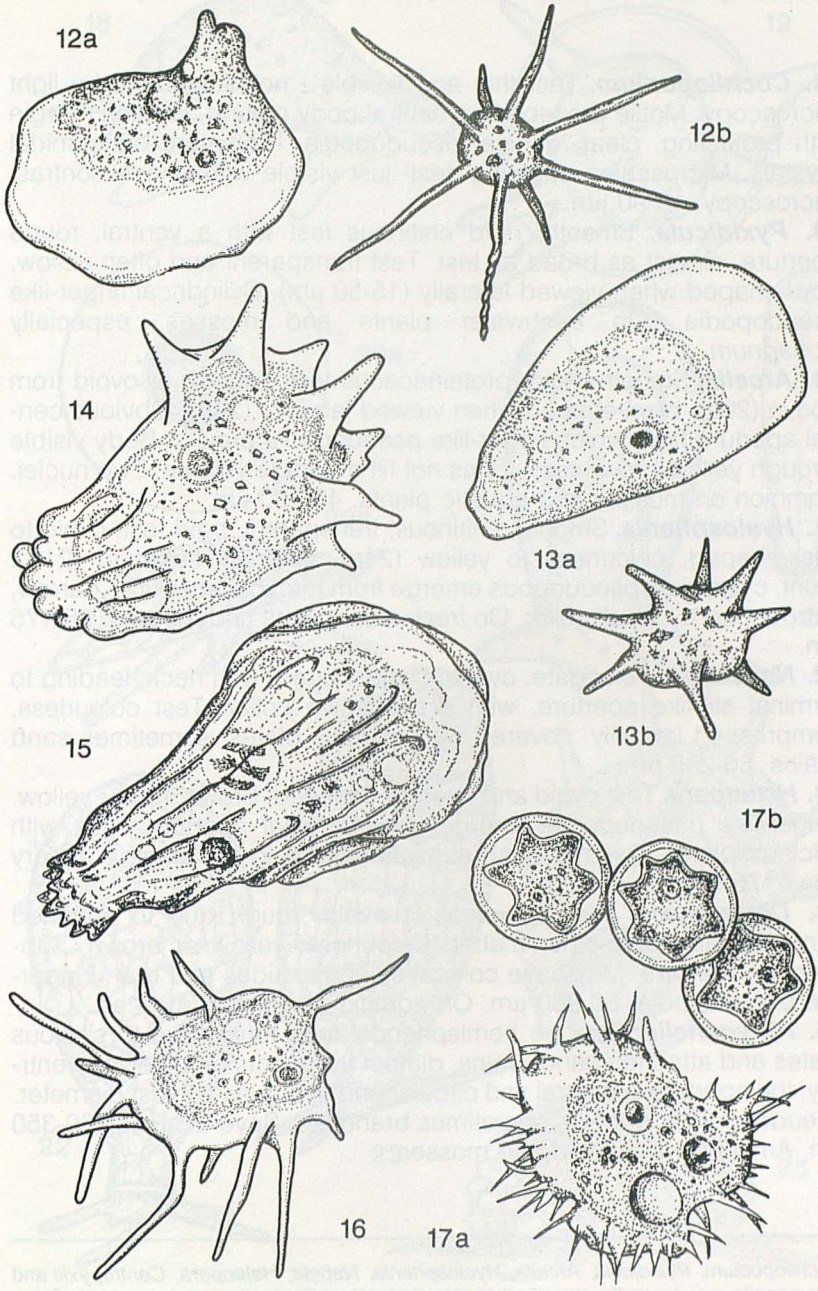

12a
12b
14
13a
13b
15
17b
16
17a

Plate 4. TESTATE AMOEBAE; with finger-like pseudopodia

18. *Cochliopodium.* Test thin and flexible - not detectable by light microscopy. Motile amoeba has central body mound. Hyaline margin with projecting, clear, conical pseudopodia. Prominent bipyramidal crystals. Microscales covering 'test' just visible with phase contrast microscopy. 15-90 μm.

19. *Pyxidicula.* Smooth, rigid chitinous test with a ventral, round aperture, almost as broad as test. Test transparent and often yellow, bowl-shaped when viewed laterally (15-50 μm). Cylindrical finger-like pseudopodia. On freshwater plants and mosses, especially *Sphagnum.*

20. *Arcella.* Smooth, rigid, proteinaceous test, circular or ovoid from above (20a), bowl-shaped when viewed laterally (20b). Obvious central aperture from which finger-like pseudopodia project. Body visible through yellow-brown test - does not fill available volume. Two nuclei. Common on mosses and aquatic plants. 45-200 μm.

21. *Hyalosphenia.* Smooth, chitinous, transparent, rigid test, ovoid to flask-shaped, colourless to yellow (21a). Laterally flattened (21b). Blunt, cylindrical pseudopods emerge from terminal aperture - narrow, surrounded by small collar. On freshwater plants and mosses. 55-175 μm.

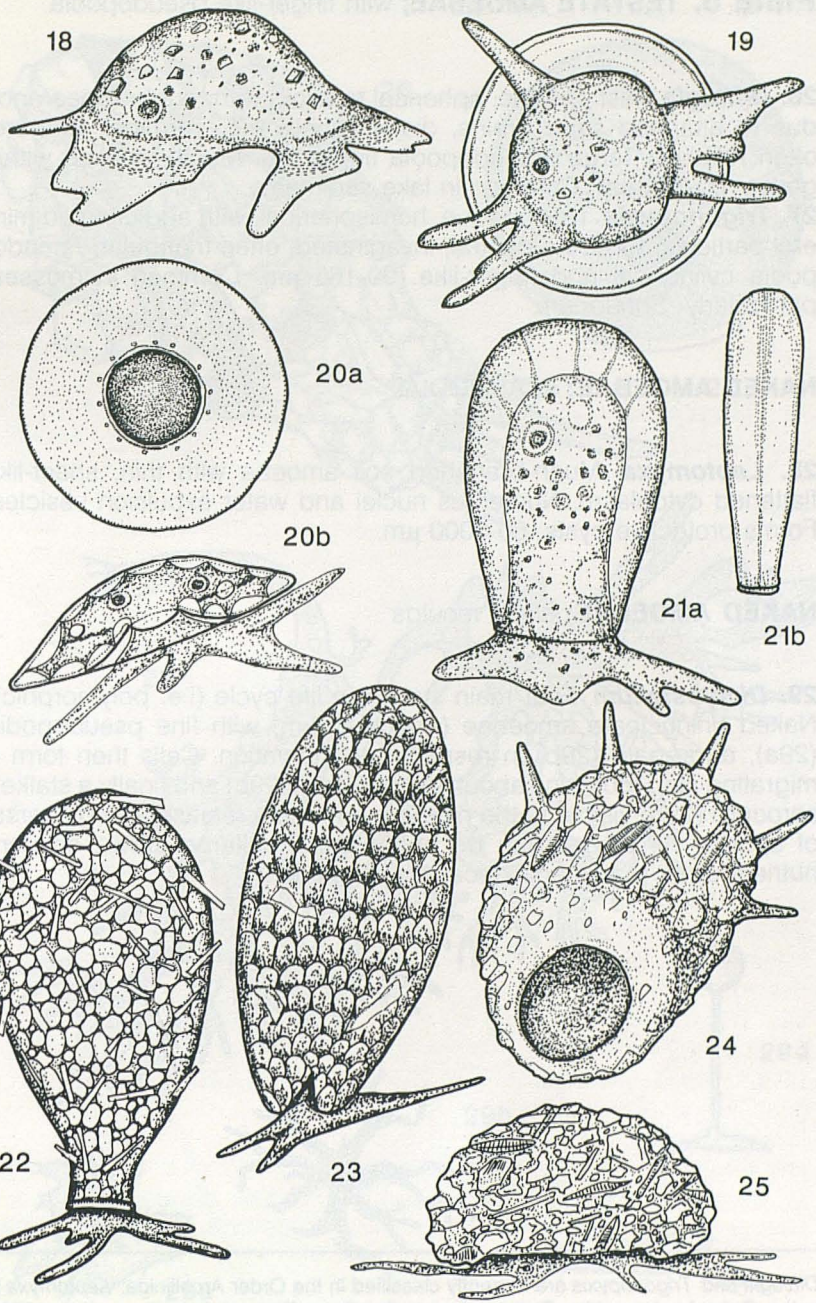
22. *Nebela.* Test elongate, oval to flask-shaped, with neck, leading to terminal slit-like aperture, with a distinctive collar. Test colourless, compressed laterally, covered with silicious plates, sometimes sand grains. 50-280 μm.

23. *Heleopera.* Test ovoid and laterally flattened, brown, red or yellow. Finger-like pseudopodia emerge from terminal aperture. Test with silicious plates - sometimes seen after allowing the sample to dry down. 75-145 μm.

24. *Centropyxis.* Test spherical or ovoid, rough due to attached mineral grains and other matter. Colourless, yellow or brown. Subterminal aperture. May have conical spines at sides and rear. Finger-like pseudopodia. 50-260 μm. On aquatic plants and mosses.

25. *Phryganella.* Opaque hemispherical test, rough due to silicious plates and attached sand grains, diatom fragments etc. Viewed ventrally, the aperture is central and circular and occupies 2/3 test diameter. Pseudopodia finger-like, sometimes branched. Several nuclei. 30-350 μm. Amongst vegetation and mosses.

Cochliopodium, *Pyxidicula*, *Arcella*, *Hyalosphenia*, *Nebela*, *Heleopera*, *Centropyxis* and *Phryganella* are currently classified in the Order Arcellinida (Lee *et al.*, 1985). Page (1987) views *Cochliopodium* as a 'naked' amoeba in the new Order, Himatismenida.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20a
20b
21a
21b
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Plate 5. TESTATE AMOEBAE; with finger-like pseudopodia.

26. *Difflugia.* Test opaque, spherical to ovoid with rough appearance due to attached sand grains, diatom shells etc. Terminal aperture, often relatively large. Pseudopodia finger-like. Much variation within genus. 60-395 μm. Common in lake sediments.
27. *Trigonopyxis*. Test opaque, hemispherical, with agglutinated mineral particles. Aperture central, invaginated, often triangular. Pseudopodia cylindrical and finger-like. 90-165 μm. Common in mosses, particularly *Sphagnum*.

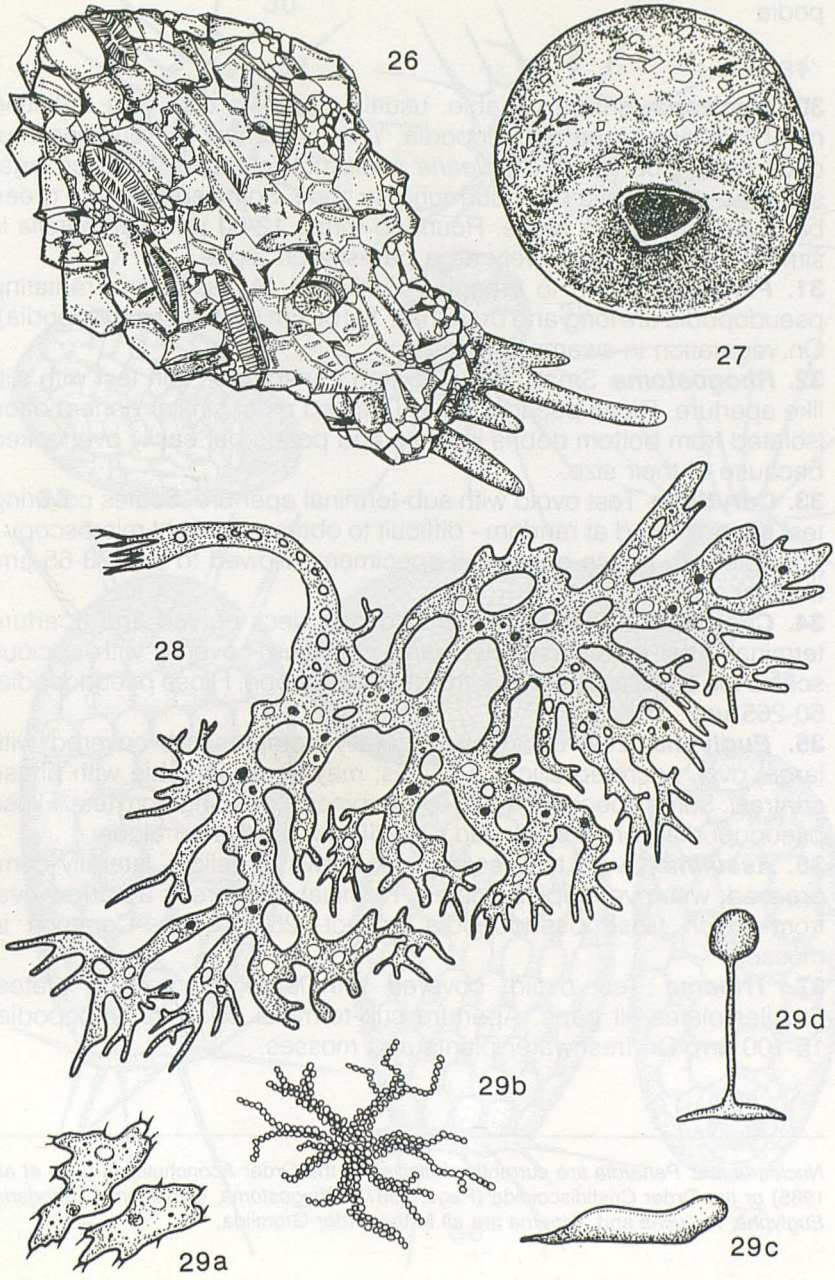
NAKED AMOEBAE; PLASMODIAL

28. *Leptomyxa*. Much-branched soil amoeba with thin, sheet-like flattened cytoplasm. Numerous nuclei and water expulsion vesicles. Forms protective cysts. 60-3000 μm.

NAKED AMOEBAE; slime moulds

29. *Dictyostelium*. Four main stages in life cycle (i.e. polymorphic). Naked uninucleate amoebae (about 10 μm) with fine pseudopodia (29a), aggregate (29b) in response to starvation. Cells then form a migrating slug (contains about 100,000 cells; 29c) and finally a stalked sorocarp (29d; visible to the naked eye) for the release and dispersal of spores. All stages can be observed in cultures grown on non-nutrient agar. In soil and decaying vegetation.

Difflugia and *Trigonopyxis* are currently classified in the Order Arcellinida. *Leptomyxa* is in the Order Leptomyxida; *Dictyostelium* in the Order Dictyosteliida (Lee *et al.*, 1985).
PLASMODIUM - multinucleate mass of protoplasm.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29d
29b
29a
29c

Plate 6. NAKED AND TESTATE AMOEBAE; with filose pseudopodia

30. *Nuclearia.* Shape variable, usually round to oval with radiating non-branching filose pseudopodia. With or without surrounding mucous coat. Coat gives *Nuclearia* a distinctive appearance on agar surfaces - cells have clear surrounding zone. Some species are green because they graze algae. Rounded forms 12-60 μm. *Vampyrella* is similar but lives exclusively as a parasite on algae.

31. *Penardia*. Ovoid to irregular. Similar to *Nuclearia*, but radiating pseudopodia are long and branched. 8-100 μm (without pseudopodia). On vegetation in swampy ponds.

32. *Rhogostoma*. Small (about 15 μm) transparent, thin test with slit-like aperture. Filose pseudopodia. This and other similar genera often isolated from bottom debris in lakes and ponds but easily overlooked because of their size.

33. *Corythion*. Test ovoid with sub-terminal aperture. Scales covering test are scattered at random - difficult to observe by light microscopy - possible with phase contrast if specimens allowed to dry. 23-65 μm. In mosses.

34. *Cyphoderia*. Test spherical or ovoid. Neck curved and aperture terminal. Test colourless, clear and chitinous, covered with silicious scales too small to observe with light microscope. Filose pseudopodia. 60-265 μm.

35. *Euglypha*. Test ovoid and laterally compressed, covered with large, oval, secreted silicious scales; may be observable with phase contrast. Some specimens with a few spines extending from test. Filose pseudopodia, rarely branched. 20-140 μm. Feeds on algae.

36. *Assulina*. Ovoid test, colourless, brown or yellow, laterally compressed, with overlapping scales. Terminal aperture is a narrow oval from which filose pseudopodia project. 25-115 μm. Common in mosses.

37. *Trinema*. Test ovoid, covered with large overlapping plates. Smaller plates fill gaps. Aperture sub-terminal. Filose pseudopodia. 15-100 μm. On freshwater plants and mosses.

Nuclearia and *Penardia* are currently classified in the Order Aconchulinida (Lee *et al.*, 1985) or the Order Cristidiscoidida (Page, 1987). *Rhogostoma*, *Corythion*, *Cyphoderia*, *Euglypha*, *Assulina* and *Trinema* are all in the Order Gromiida.

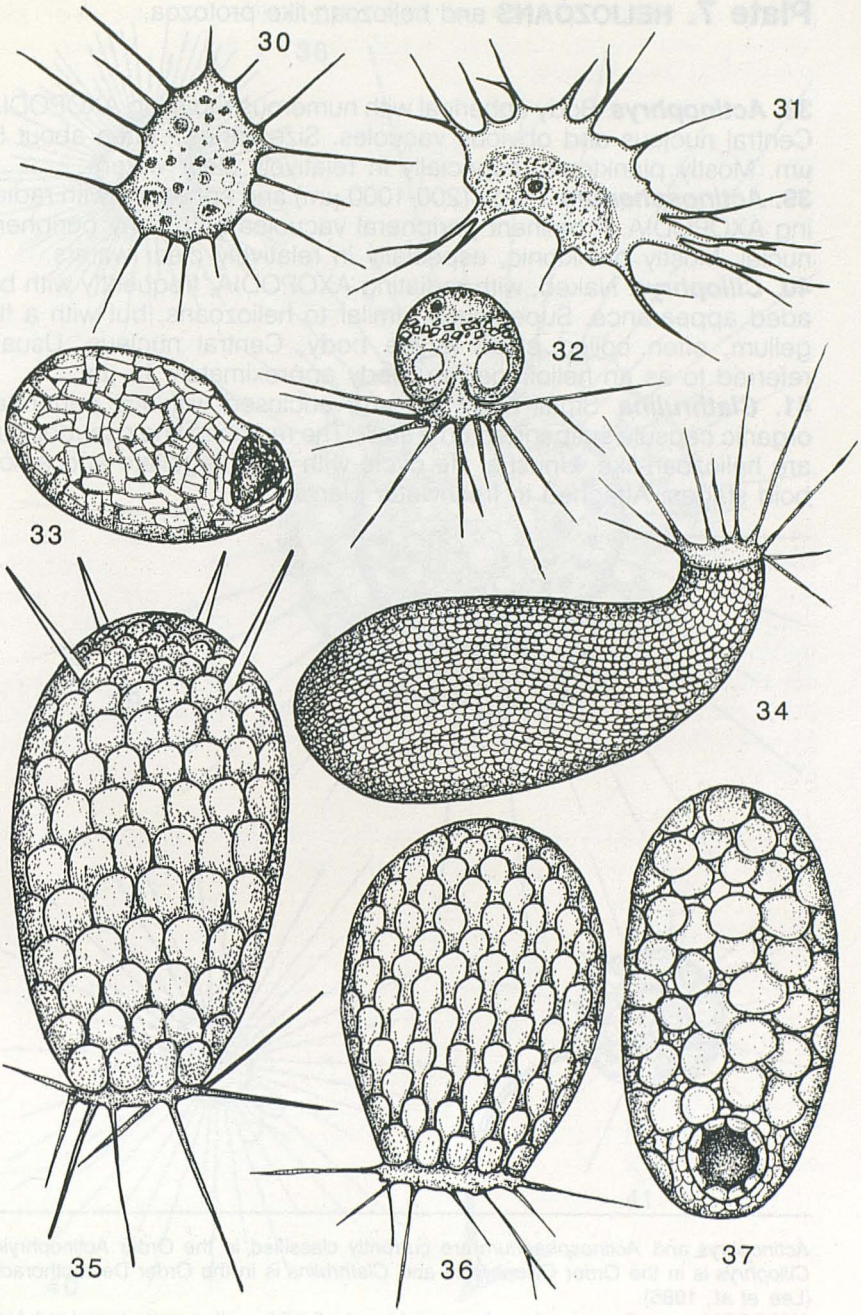
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Plate 7. **HELIOZOANS** and heliozoan-like protozoa.

38. *Actinophrys*. Body spherical with numerous radiating AXOPODIA. Central nucleus and obvious vacuoles. Size varies - often about 50 μm. Mostly planktonic, especially in relatively clear waters.
39. *Actinospherium*. Large (200-1000 μm) and spherical, with radiating AXOPODIA. Prominent peripheral vacuoles and many peripheral nuclei. Mostly planktonic, especially in relatively clear waters.
40. *Ciliophrys*. Naked, with radiating AXOPODIA, frequently with beaded appearance. Superficially similar to heliozoans, but with a flagellum, often coiled close to the body. Central nucleus. Usually referred to as an helioflagellate. Body approximately 15 μm.
41. *Clathrulina*. Small body (20 μm) enclosed within a perforated organic capsule suspended on a stalk. The radiating fine pseudopodia are heliozoan-like. Unusual life cycle with both flagellate and amoeboid stages. Attached to freshwater plants.

Actinophrys and *Actinosphaerium* are currently classified in the Order Actinophryida. *Ciliophrys* is in the Order Ciliophryida and *Clathrulina* is in the Order Desmothoracida (Lee *et al.*, 1985).
AXOPODIA - very fine pseudopodia, straight and inflexible, with a central axial rod. Used for feeding.

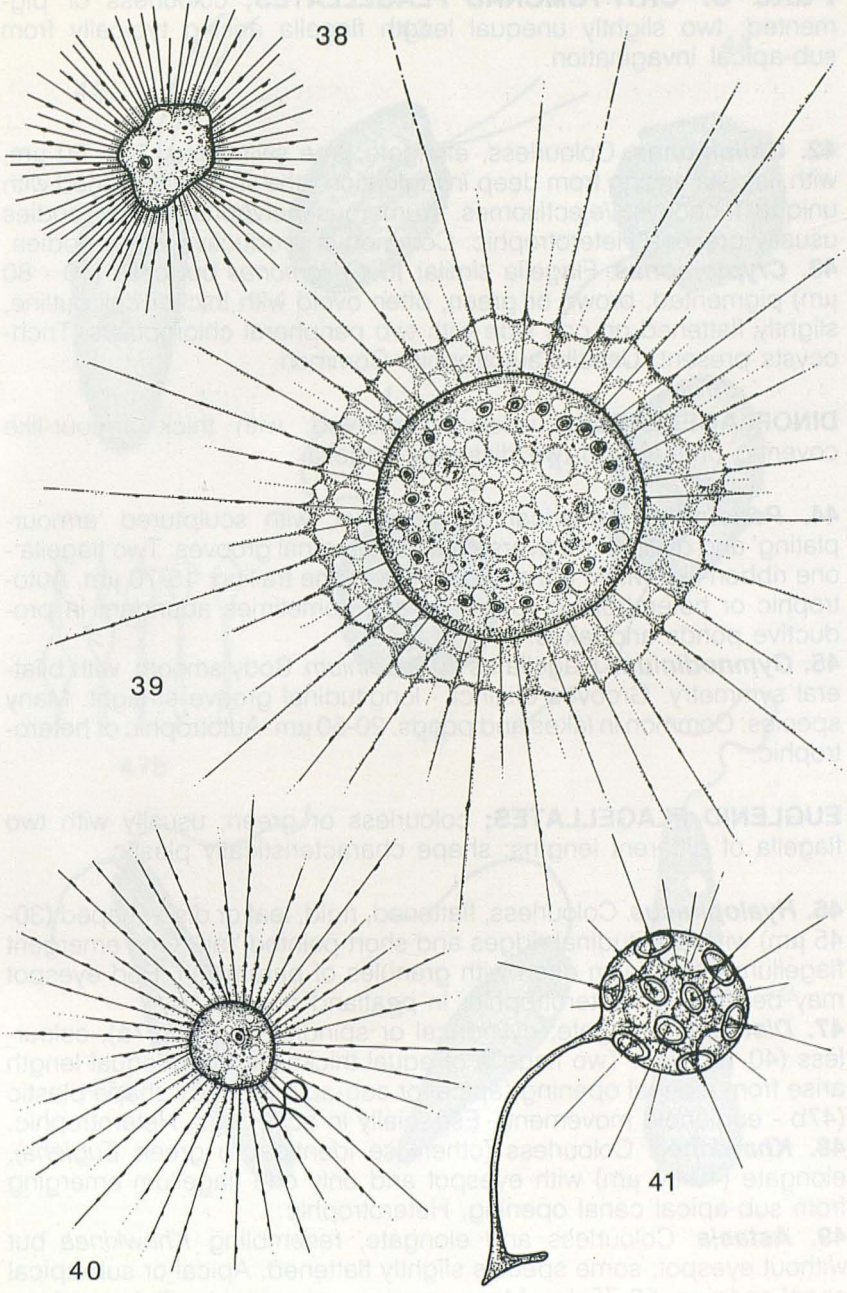
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Plate 8. CRYPTOMONAD FLAGELLATES; colourless or pigmented; two slightly unequal length flagella arising typically from sub-apical invagination.

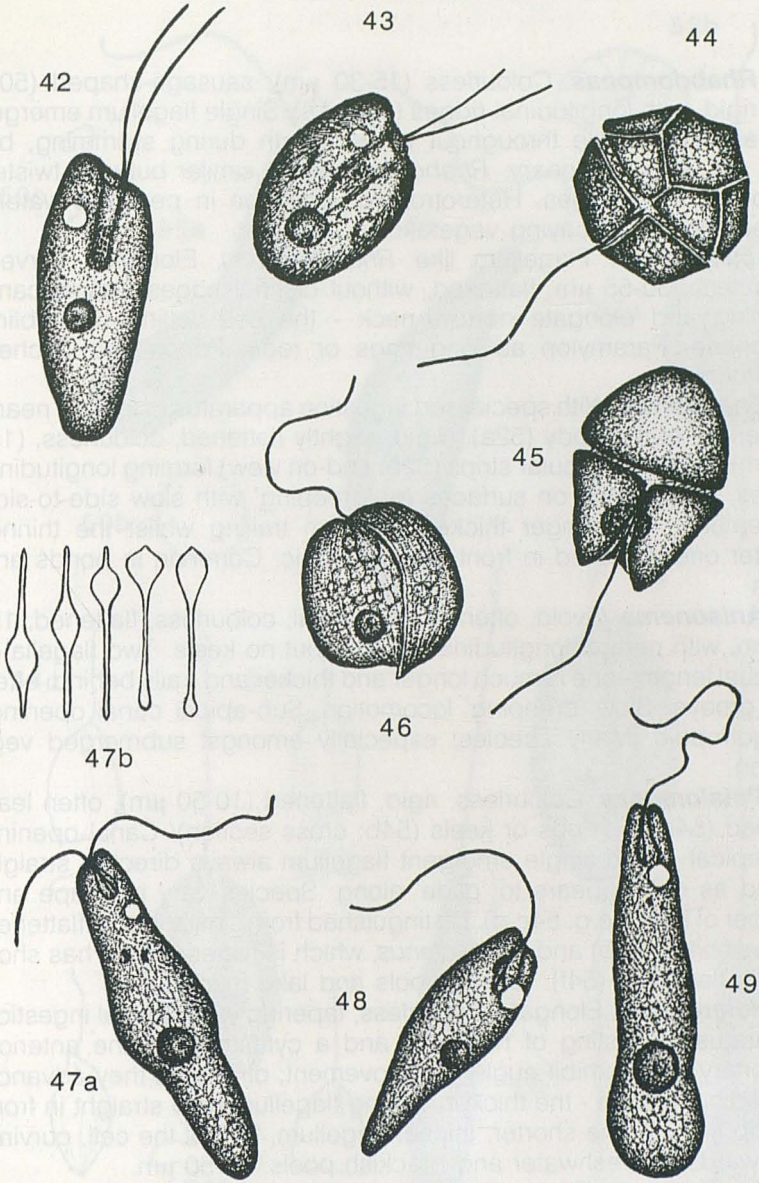
42. *Chilomonas.* Colourless, elongate, free swimming, 20 - 50 μm, with flagella arising from deep invagination - the vestibulum, lined with unique trichocysts/ejectisomes. Numerous polygonal starch bodies usually present. Heterotrophic. Common in productive water bodies.
43. *Cryptomonas*. Flagella similar to *Chilomonas* but cells (10 - 80 μm) pigmented, brown or green, often ovoid with thicker cell outline, slightly flattened on one side with two peripheral chloroplasts. Trichocysts present. Usually autotrophic. Common.

DINOFLAGELLATES; usually pigmented; with thick armour-like covering (theca) and two dissimilar flagella.

44. *Peridinium*. Spherical or polygonal, with sculptured 'armour-plating' and distinct transverse and longitudinal grooves. Two flagella - one ribbon-like within transverse groove, one trailing. 15-70 μm. Autotrophic or heterotrophic. Common and sometimes abundant in productive ponds and lakes.
45. *Gymnodinium*. Flagella as in *Peridinium*. Body smooth, with bilateral symmetry. Grooves distinct - longitudinal groove straight. Many species. Common in lakes and ponds. 20-50 μm. Autotrophic or heterotrophic.

EUGLENID FLAGELLATES; colourless or green; usually with two flagella of different lengths; shape characteristically plastic.

46. *Hyalophacus*. Colourless, flattened, rigid, leaf or disc-shaped (30-45 μm) with longitudinal ridges and short pointed 'tail'. One emergent flagellum. Cytoplasm often with granules of paramylon. Red eyespot may be present. Heterotrophic. In peatland pools.
47. *Distigma*. Elongate, cylindrical or spindle-shaped (47a), colourless (40-120 μm). Two flagella of equal thickness but unequal length arise from a canal opening (apical or sub-apical). Body shape plastic (47b - euglenoid movement). Especially in acid bogs. Heterotrophic.
48. *Khawkinea*. Colourless (otherwise identical to green *Euglena*), elongate (40-60 μm) with eyespot and only one flagellum emerging from sub-apical canal opening. Heterotrophic.
49. *Astasia*. Colourless and elongate, resembling *Khawkinea* but without eyespot; some species slightly flattened. Apical or sub-apical canal opening. 50-75 μm. Many species, common in polluted waters. Heterotrophic.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47b
47a
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49

Plate 9. EUGLENID FLAGELLATES (continued)

50. *Rhabdomonas*. Colourless (15-30 μm), sausage-shaped (50a) and rigid, with longitudinal ridges (50b; TS). Single flagellum emerges sub-apically; motile throughout whole length during swimming, but held still when stationary. *Rhabdospira* (50c) similar but with twisted shape and no ridges. Heterotrophic. Common in peatland waters, especially with decaying vegetation.

51. *Menoidium*. Flagellum like *Rhabdomonas*. Elongate, curved, colourless, 30-55 μm, flattened, without distinct ridges. Apical canal opening and elongate narrow neck - the cell outline resembling a banana. Paramylon as long rings or rods. Ponds and ditches. Heterotrophic.

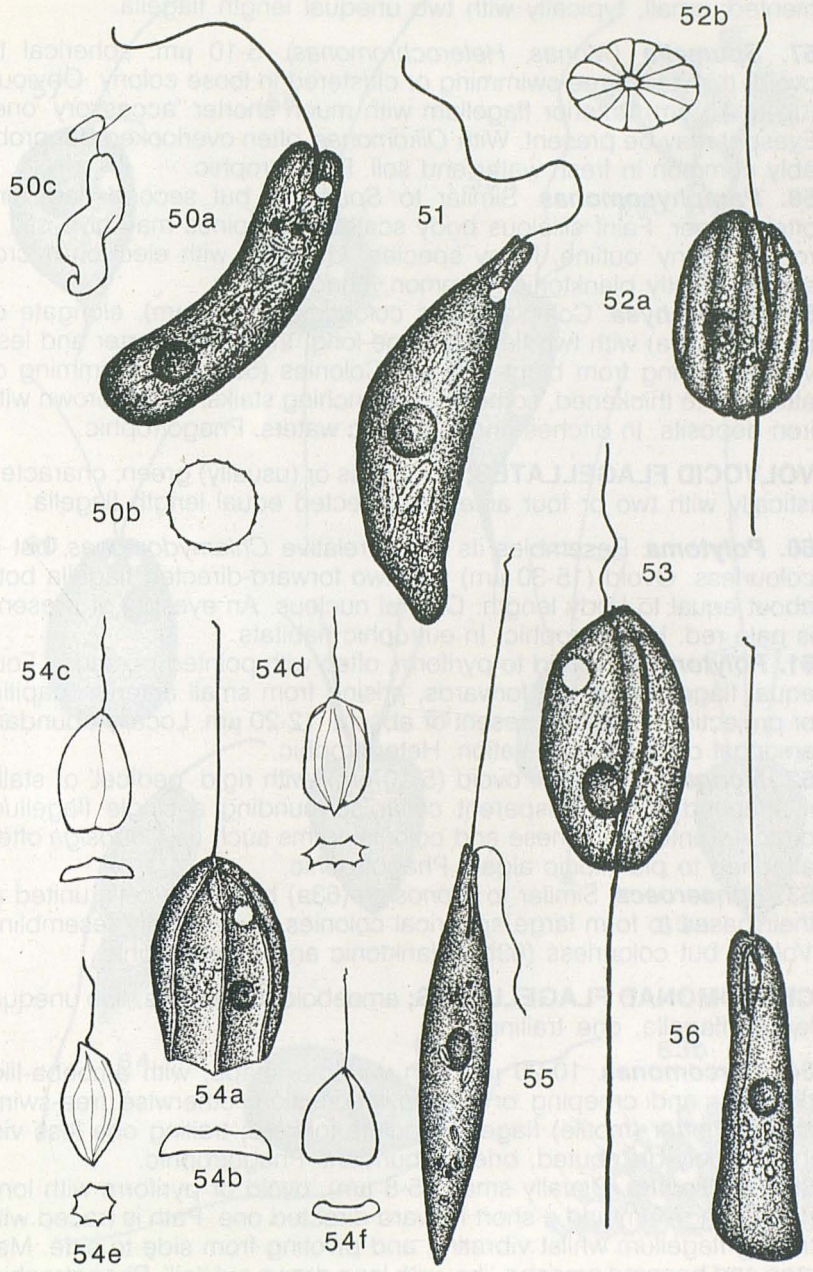
52. *Entosiphon*. With specialised ingestion apparatus ('siphon') nearly the length of the body (52a). Rigid, slightly flattened, colourless, (15-25 μm), curved pellicular strips (52b; end-on view) forming longitudinal ridges. Locomotion on surfaces by 'creeping' with slow side-to-side movement - the longer thicker flagellum trailing whilst the thinner shorter one is flicked in front. Phagotrophic. Common in ponds and lakes.

53. *Anisonema*. Ovoid, often asymmetrical, colourless, flattened, 15-60 μm, with central longitudinal groove, but no keels. Two flagella of unequal length - one is much longer and thicker and trails behind, often in a groove. Slow 'creeping' locomotion. Sub-apical canal opening. Phagotrophic. Many species; especially amongst submerged vegetation.

54. *Petalomonas*. Colourless, rigid, flattened (10-50 μm), often leaf-shaped (54a) with ribs or keels (54b: cross section). Canal opening sub-apical with a single emergent flagellum always directed straight ahead as cell appears to 'glide' along. Species vary in shape and number of keels (e.g. 54c,d). Distinguished from similar but unflattened *Calycimonas* (54e) and *Notosolenus*, which is flattened, but has short trailing flagellum (54f). In bog pools and lake muds.

55. *Heteronema*. Elongate, colourless, tapering with special ingestion apparatus consisting of two rods and a cytostome at the anterior. Stationary cells exhibit euglenoid movement; otherwise they advance by 'gliding' motion - the thicker leading flagellum held straight in front with tip flicking; the shorter, thinner flagellum, free of the cell, curving backwards. In freshwater and brackish pools. 40-50 μm.

56. *Peranema*. 20-70 μm. Similar to *Heteronema*, but with the thinner, trailing, curved flagellum pressed close to the body, difficult to see. Shape can change considerably when stationary or during feeding. Common in ditches and ponds. Phagotrophic, able to ingest other protozoa.


50c
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52b
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54c
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54f
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56

Plate 10. CHRYSOMONAD FLAGELLATES; colourless or pigmented; small, typically with two unequal length flagella.

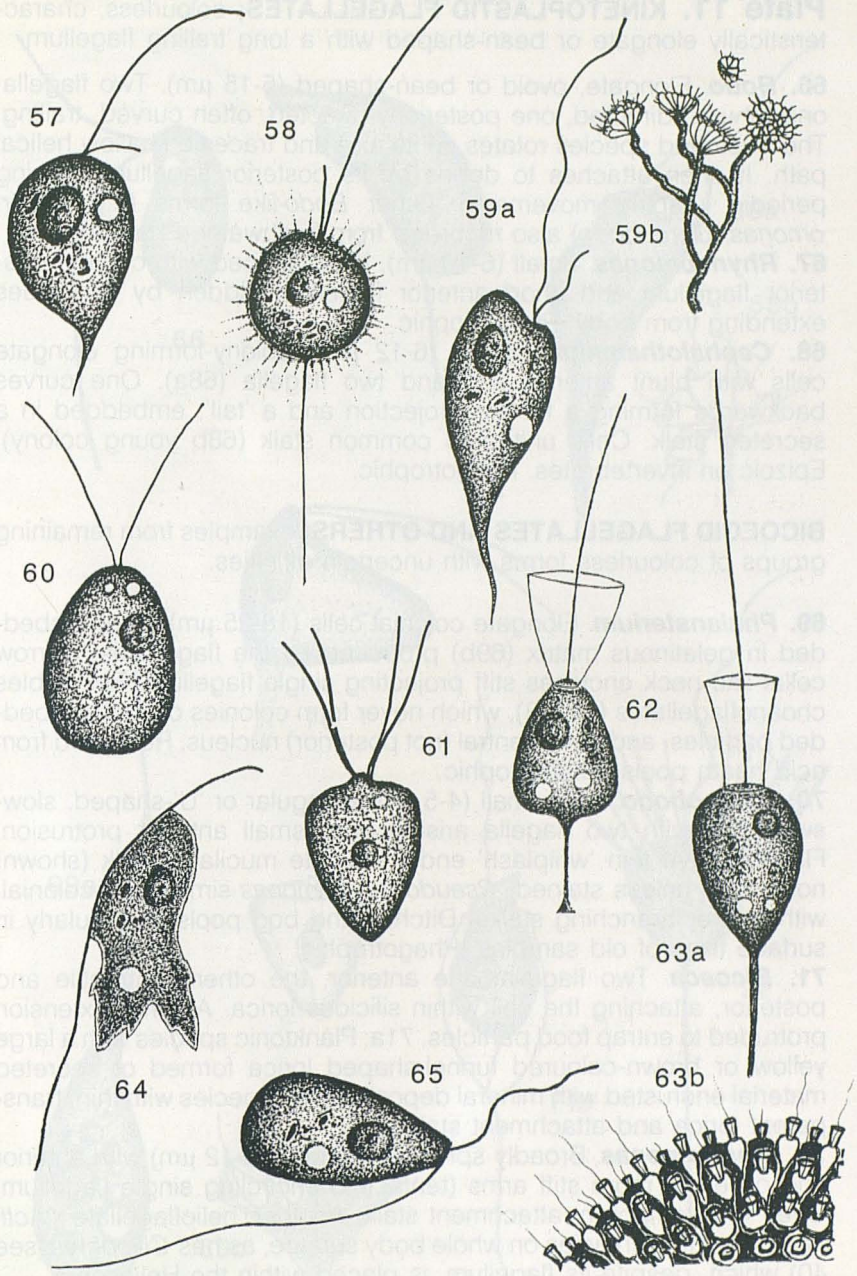
57. *Spumella*. (*Monas*, *Heterochromonas*). 5-10 μm, spherical to ovoid; thin 'tail'. Free-swimming or clustered in loose colony. Obvious (up to 15 μm) anterior flagellum with much shorter 'accessory' one. Eyespot may be present. With *Oikomonas*, often overlooked but probably common in fresh water and soil. Phagotrophic.
58. *Paraphysomonas*. Similar to *Spumella*, but second flagellum often longer. Faint silicious body scales and spines may give cell a rough, 'spiny' outline. Many species, identified with electron microscope. Mostly planktonic; common. Phagotrophic.
59. *Anthophysa*. Colonial. Cells colourless, (5-15 μm), elongate or pyriform (59a) with two flagella - one long, the other shorter and less visible, arising from blunt anterior. Colonies (59b) free-swimming or attached to thickened, sometimes branching stalks. Stalks brown with iron deposits. In ditches and eutrophic waters. Phagotrophic.

VOLVOCID FLAGELLATES; colourless or (usually) green; characteristically with two or four anteriorly-directed equal length flagella.

60. *Polytoma*. Resembles its green relative *Chlamydomonas*, but is colourless. Ovoid (15-30 μm) with two forward-directed flagella both about equal to body length. Central nucleus. An eyespot, if present, is pale red. Heterotrophic. In eutrophic habitats.
61. *Polytomella*. Ovoid to pyriform, often with pointed posterior. Four equal flagella directed forwards, arising from small anterior 'papilla' or projection. Eyespot present or absent. 12-20 μm. Locally abundant amongst decaying vegetation. Heterotrophic.
62. *Monosiga*. Solitary, ovoid (5-10 μm); with rigid 'pedicel' or stalk, if attached. With transparent collar surrounding a single flagellum directed anteriorly. These and colonial forms such as *Codosiga* often attached to planktonic algae. Phagotrophic.
63. *Sphaeroeca*. Similar to *Monosiga* (63a) but many cells united at their bases to form large spherical colonies, superficially resembling *Volvox*, but colourless (63b). Planktonic and phagotrophic.

CERCOMONAD FLAGELLATES; amoeboid, colourless; two unequal length flagella, one trailing.

64. *Cercomonas*. 10-20 μm with variable shape; with amoeba-like posterior and creeping or gliding locomotion; otherwise free-swimming. Shorter (motile) flagellum points forward; trailing one less visible. Widely distributed, briefly abundant. Phagotrophic.
65. *Heteromita*. Usually small, (5-8 μm), ovoid or pyriform with long trailing flagellum and a short forward directed one. Path is traced with trailing flagellum whilst vibrating and pivoting from side to side. May stop and become amoeba-like, with long drawn out 'tail'. Phagotrophic. Freshwater and soil.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59a
59b
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63a
63b
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Plate 11. KINETOPLASTID FLAGELLATES; colourless, characteristically elongate or bean-shaped with a long trailing flagellum.

66. *Bodo*. Elongate, ovoid or bean-shaped (5-15 μm). Two flagella; one forward-directed, one posteriorly-directed, often curved, trailing. The illustrated species rotates on its axis and traces a shallow helical path. It often attaches to debris by its posterior flagellum, making periodic jumping movements. Other *Bodo*-like forms (e.g. *Pleuromonas*, *Cercobodo*) also recorded from freshwater. Phagotrophic.

67. *Rhyncomonas*. Small (6-10 μm), bean-shaped with curving posterior flagellum and short anterior flagellum hidden by two lobes extending from body. Phagotrophic.

68. *Cephalothamnium*. Small (6-12 μm), colony-forming elongate cells with blunt anterior end and two flagella (68a). One curves backwards forming a fin-like projection and a 'tail', embedded in a secreted stalk. Cells united to common stalk (68b young colony). Epizoic on invertebrates. Phagotrophic.

BICOECID FLAGELLATES AND OTHERS; examples from remaining groups of colourless forms with uncertain affinities.

69. *Phalansterium*. Elongate colonial cells (18-25 μm) (69a) embedded in gelatinous matrix (69b) produced by the flagellates. Narrow collar-like neck encloses stiff projecting single flagellum. Resembles choanoflagellates (62, 63), which never form colonies of such embedded particles, and have central (not posterior) nucleus. Recorded from acid heath pools. Phagotrophic.

70. *Cyathobodo*. Very small (4-5 μm) triangular or 'U'-shaped, slow-swimming with two flagella arising from small anterior protrusion. Flagella have thin 'whiplash' ends. Delicate mucilage stalk (shown) not visible unless stained. *Pseudodendromonas* similar but colonial, with thicker branching stalks. Ditches and bog pools; particularly in surface films of old samples. Phagotrophic.

71. *Bicoeca*. Two flagella; one anterior, the other contractile and posterior, attaching the cell within silicious lorica. Anterior extension protruded to entrap food particles. 71a; Planktonic species with a large yellow or brown-coloured funnel-shaped lorica formed of secreted material encrusted with mineral deposits. 71b; Species with thin, transparent lorica and attachment stalk.

72. *Pteridomonas*. Broadly spherical (approx. 8-12 μm) with anterior ring of ten or more stiff arms (tentacles) encircling single flagellum. Often with temporary attachment stalk. Another 'helioflagellate', *Actinomonas*, has tentacles on whole body surface, as has *Ciliophrys* (see 40) which, despite its flagellum, is placed within the Heliozoa.

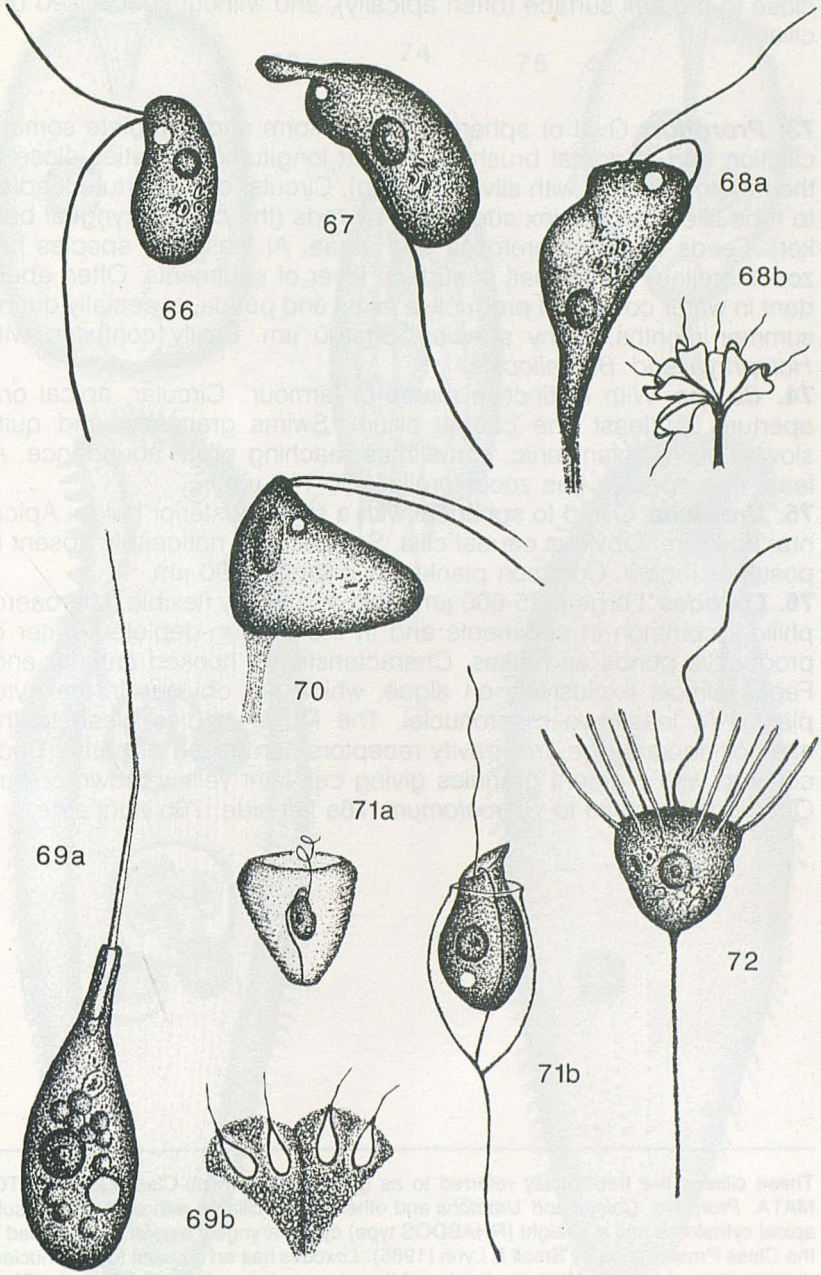

66
67
68a
68b
70
71a
71b
72
69a
69b

Plate 12. Ciliates with mouths which are simple, situated at or close to the cell surface (often apically), and without specialised oral ciliature.

73. *Prorodon*. Oval or spherical with uniform and complete somatic ciliation and a 'dorsal brush' of 3 short longitudinal kineties close to the mouth (obvious with silver staining). Circular oral aperture leading to tube-like cytopharynx supported by rods (the cytopharyngeal basket). Feeds on other protozoa and algae. At least one species has zoochlorellae. Sometimes in surface layer of sediments. Often abundant in water column of productive lakes and ponds, especially during summer months. Many species; 50-400 μm. Easily confused with *Holophrya* and *Bursellopsis*.

74. *Coleps*. With distinctive plates of 'armour'. Circular, apical oral aperture. At least one caudal cilium. Swims gracefully and quite slowly. Mainly planktonic, sometimes reaching great abundance. At least one species has zoochlorellae. 50-110 μm.

75. *Urotricha*. Ovoid to spherical with a slight posterior bulge. Apical oral aperture. Obvious caudal cilia. Somatic cilia noticeably absent in posterior region. Common planktonic ciliate. 18-90 μm.

76. *Loxodes*. Large (125-600 μm), thin and highly flexible. Microaerophilic - common in sediments and in the oxygen-depleted water of productive ponds and lakes. Characteristically hooked anterior end. Feeds almost exclusively on algae, which are obvious in the cytoplasm. At least two macronuclei. The Müller bodies close to the anterior dorsal ridge are gravity receptors composed of barite. Body covered with pigment granules giving cell faint yellow-brown colour. Often occurs close to *Spirostomum*. 76a left side; 76b right side.

These ciliates are traditionally referred to as gymnostomes (Sub-Class GYMNOSTOMATA. *Prorodon*, *Coleps* and *Urotricha* and other similar ciliates with an apical or subapical cytostome and a straight (RHABDOS type) cytopharyngeal basket are included in the Class Prostomatea by Small & Lynn (1985). *Loxodes* has an unusual form of nuclear division (the macronuclei form by division of the micronuclei) and it is placed in the Class Karyorelictea by Small & Lynn (1985).

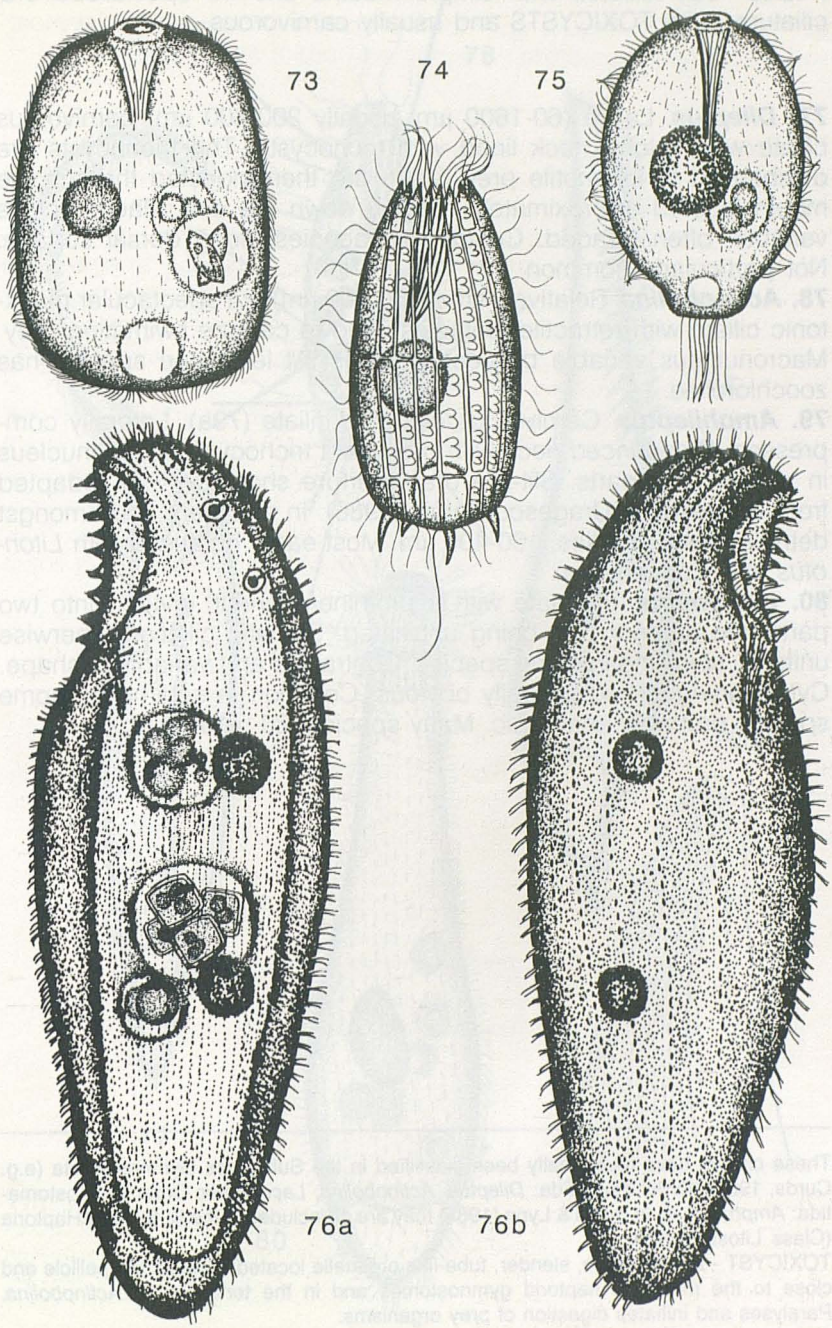

73
74
75
76a
76b

Plate 13. Ciliates with simple mouths and no specialised oral ciliature. With TOXICYSTS and usually carnivorous.

77. *Dileptus*. Large (60-1600 μm; usually 200-600 μm) carnivorous ciliate with a long neck lined with trichocysts. The trichocysts are discharged to kill motile prey which are then ingested through the mouth located approximately halfway down the cell. Macronucleus variable, often beaded. Contractile vacuoles along dorsal surface. Not particularly common.

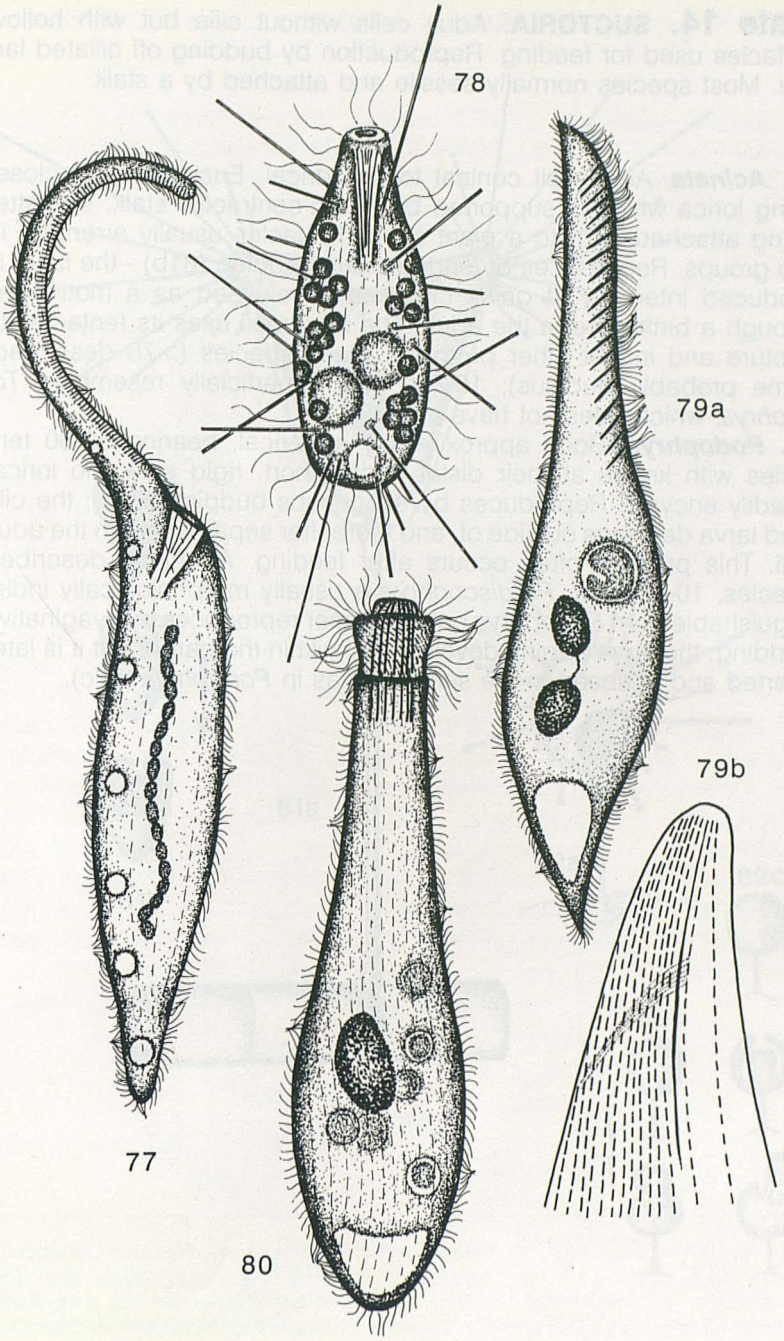
78. *Actinobolina*. Relatively small (50-200 μm) but spectacular planktonic ciliate with retractile tentacles used to capture swimming prey. Macronucleus variable between species. At least one species has zoochlorellae.

79. *Amphileptus*. Carnivorous elongate ciliate (79a). Laterally compressed. Pronounced neck with prominent trichocysts. Macronucleus in at least two parts. Slit-like oral aperture shown in 79b (adapted from Dragesco & Dragesco-Kernis, 1986). In sediment and amongst detritus. Many species, 100-400 μm. Most easily confused with *Litonotus* and *Loxophyllum*.

80. *Lacrymaria*. Elongate with a prominent 'snout' divided into two parts, the anterior part being unciliated. Somatic ciliation otherwise uniform, spiralling in some species. Contractile and variable in shape. Cytopharyngeal rods usually obvious. Common in sediments; some species probably anaerobic. Many species, 30->1000 μm.

These ciliates have traditionally been classified in the Sub-Class Gymnostomata (e.g. Curds, 1982) (Order Haptorida: *Dileptus*, *Actinobolina*, *Lacrymaria*; Order Pleurostomatida: *Amphileptus*). In Small & Lynn (1985) they are all included in the Sub-Class Haptoria (Class Litostomatea).

TOXICYST - an extrusible, slender, tube-like organelle located beneath the pellicle and close to the mouth in haptorid gymnostomes and in the tentacles of *Actinobolina*. Paralyses and initiates digestion of prey organisms.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79a
79b
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Plate 14. SUCTORIA. Adult cells without cilia but with hollow tentacles used for feeding. Reproduction by budding off ciliated larvae. Most species normally sessile and attached by a stalk.

81. *Acineta*. Adult cell conical to cylindrical. Enclosed in a close-fitting lorica which is supported by a non-contractile stalk, the latter being attached, e.g. to a plant stem. Tentacles usually arranged in two groups. Reproduces by endogenous budding (81b) - the larva is produced internally, it gains cilia and is released as a motile cell through a birth pore in the adult. The adult cell uses its tentacles to capture and ingest other protozoa. Many species (>75 described, some probably dubious); 15-330 μm. Superficially resembles *Tokophrya*, which does not have a lorica.

82. *Podophrya*. Body approximately spherical, bearing 30-60 tentacles with knobs at their distal ends. Short, rigid stalk; no lorica. Readily encysts. Reproduces by exogenous budding (82b); the ciliated larva develops outside of, and thereafter separates from the adult cell. This process often occurs after feeding. About 50 described species, 10-105 μm. *Prodiscophrya* is usually morphologically indistinguishable from *Podophrya* but the former reproduces by evaginative budding: the larva begins development within the parent but it is later everted and released in the same way as in *Podophrya* (82c).

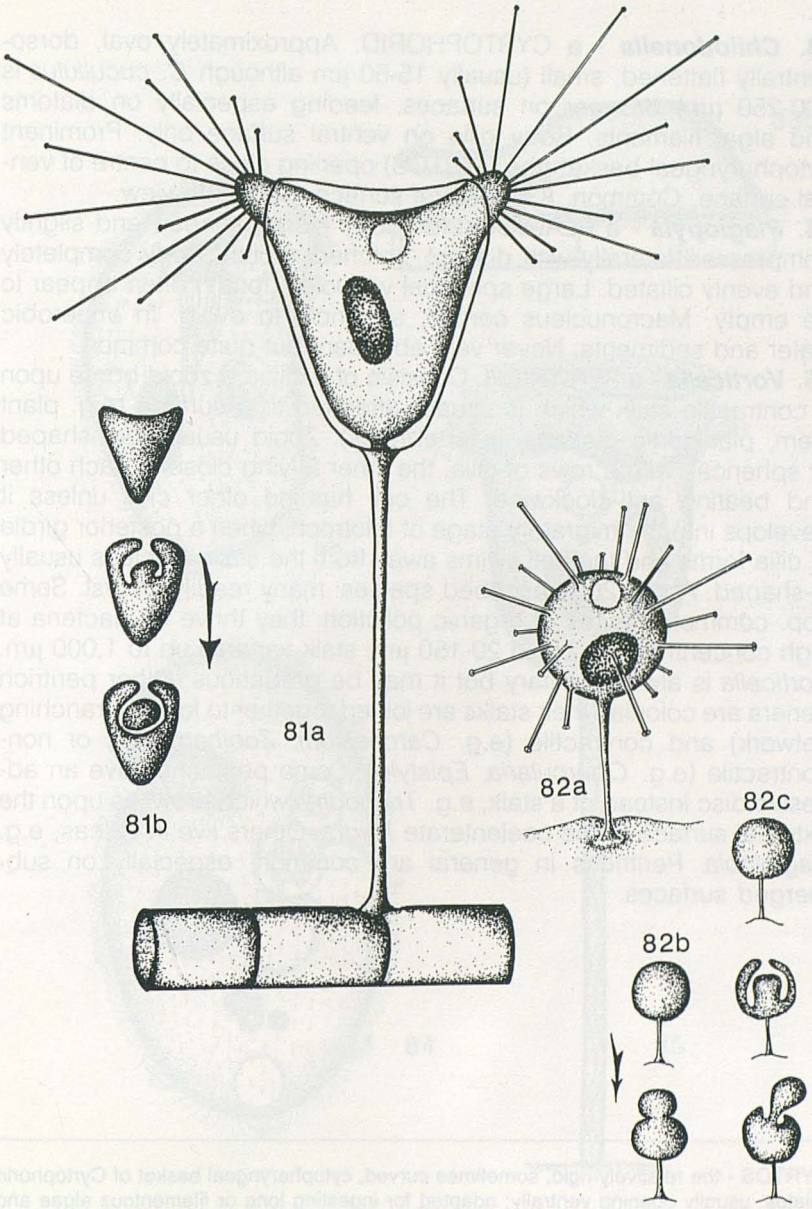

81a
81b
82a
82c
82b

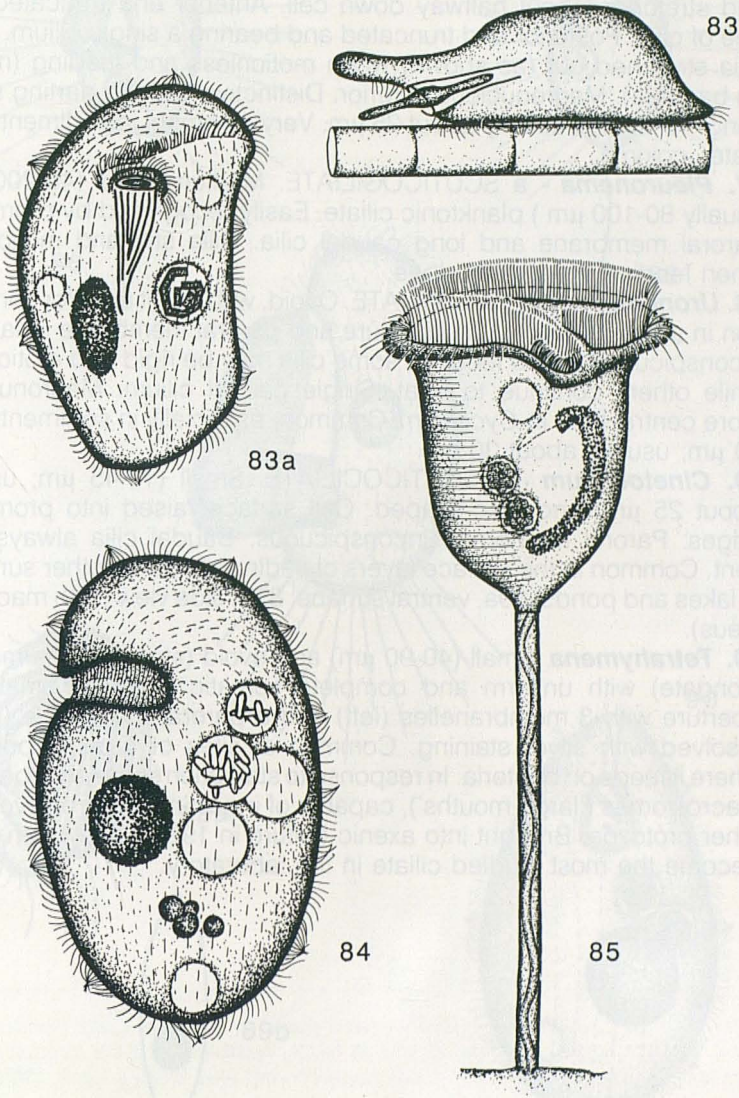
Plate 15. CYRTOPHORIDS, PLAGIOPYLIDS and PERITRICHS

83. *Chilodonella* - a CYRTOPHORID. Approximately oval, dorso-ventrally flattened, small (usually 15-50 μm although *C. cucullulus* is 100-250 μm) browser on surfaces, feeding especially on diatoms and algal filaments. Body cilia on ventral surface only. Prominent cytopharyngeal basket (the CYRTOS) opening close to centre of ventral surface. Common. 83a, ventral surface; 83b, side view.

84. *Plagiopyla* - a PLAGIOPYLID. Body kidney-shaped and slightly compressed laterally with distinct 'notched' mouth. Body completely and evenly ciliated. Large spherical vacuoles (food?) often appear to be empty. Macronucleus central, spherical to ovoid. In anaerobic water and sediments. Never very abundant but quite common.

85. *Vorticella* - a PERITRICH. Consists of a ciliated zooid borne upon a contractile stalk which is usually attached to a surface (e.g. plant stem, planktonic diatoms, invertebrates). Zooid usually bell-shaped or spherical, with 3 rows of cilia, the inner 2 lying close to each other and beating anti-clockwise. The cell has no other cilia unless it develops into the migratory stage of telotroch, when a posterior girdle of cilia forms and the cell swims away from the stalk. Nucleus usually C-shaped. About 200 described species: many readily encyst. Some spp. common at sites of organic pollution: they thrive on bacteria at high concentrations. Zooid 20-150 μm; stalk variable up to 1,000 μm. *Vorticella* is always solitary but it may be gregarious. Other peritrich genera are colonial (their stalks are joined together to form a branching network) and contractile (e.g. *Carchesium*, *Zoothamnium*) or non-contractile (e.g. *Opercularia*, *Epistylis*). Some peritrichs have an adhesive disc instead of a stalk, e.g. *Trichodina* which browses upon the external surface of the coelenterate *Hydra*. Others live in loricas, e.g. *Vaginicola*. Peritrichs in general are common, especially on submerged surfaces.

CYRTOS - the relatively rigid, sometimes curved, cytopharyngeal basket of Cyrtophorid ciliates; usually opening ventrally; adapted for ingesting long or filamentous algae and cyanobacteria. c.f. RHABDOS, the straight but more easily distended and supposedly less complex cytopharyngeal basket of the gymnostome ciliates which enables ingestion of bulkier prey.



83b

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Plate 16. SCUTICOCILIATES and other **HYMENOSTOME** ('membrane-mouthed') ciliates.

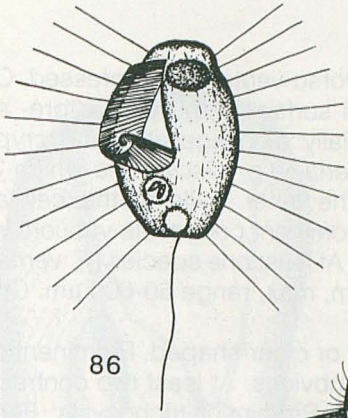
86. *Cyclidium* - a SCUTICOCILIATE. Paroral membrane conspicuous and stretches about halfway down cell. Anterior end truncated and free of cilia. Posterior end truncated and bearing a single cilium. Body cilia stretched out (as shown) when motionless and feeding (mainly on bacteria). Macronucleus anterior. Distinctive sudden darting swimming. 15-70 μm; usually about 25 μm. Very common, in sediments and water column.

87. *Pleuronema* - a SCUTICOCILIATE. Medium-sized (45-200 μm; usually 80-100 μm) planktonic ciliate. Easily recognised by prominent paroral membrane and long caudal cilia. Cilia stiff and motionless when feeding. Feeds on algae.

88. *Uronema* - a SCUTICOCILIATE. Ovoid, with slight anterior constriction in some species. Oral aperture and paroral membrane small and inconspicuous. While feeding, some cilia may be rigid and motionless while others continue to beat. Single caudal cilium. Macronucleus more central than in *Cyclidium*. Common, especially in sediments. 25-90 μm; usually about 30 μm.

89. *Cinetochilum* - a SCUTICOCILIATE. Small (15-45 μm; usually about 25 μm) and disc-shaped. Cell surface raised into prominent ridges. Paroral membrane inconspicuous. Caudal cilia always present. Common in the surface layers of sediment and on other surfaces in lakes and ponds. 89a, ventral surface; 89b, side view (with macronucleus).

90. *Tetrahymena*. Small (40-90 μm) and ovoid (starved cells may be elongate) with uniform and complete somatic ciliature. Small oral aperture with 3 membranelles (left) and a paroral membrane (right), resolved with silver staining. Common at sites of organic pollution where it feeds on bacteria. In response to starvation some cells become macrostomes ('large mouths'), capable of ingesting their relatives and other protozoa. Brought into axenic culture in 1923 since when it has become the most studied ciliate in the laboratory.



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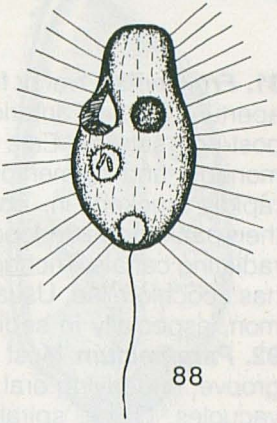

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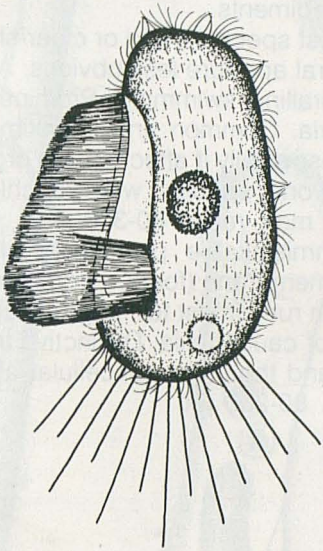

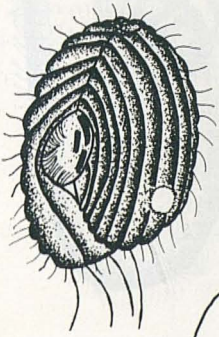

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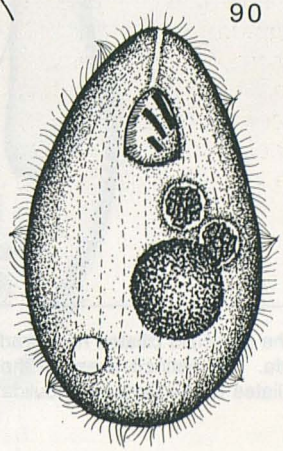


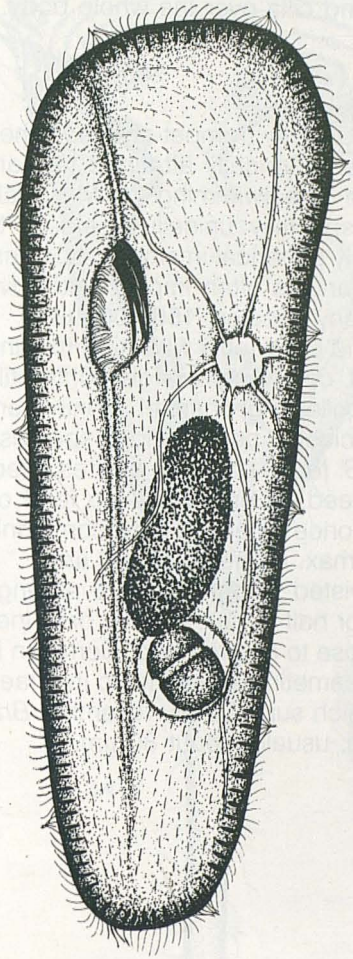
Plate 17. PENICULID Ciliates; typically with 3 oral polykinetids (the peniculi) lying within, and parallel to long axis of oral cavity.

91. *Frontonia*. Oval or foot-shaped. Dorso-ventrally compressed. Oral aperture located anteriorly on ventral surface with obvious pre- and post-oral sutures. Eats algae, especially dinoflagellates and cryptomonads, and filamentous cyanobacteria, e.g. *Oscillatoria* which are rapidly 'sucked' in, coiled around the inner walls of the cell and then packaged into food vacuoles. Prominent contractile vacuole with radiating canals. Trichocysts obvious. At least one species (*F. vernalis*) has zoochlorellae. Usually 100-300 μm; max. range 60-600 μm. Common, especially in sediments.

92. *Paramecium*. Most species ovoid or cigar-shaped. Prominent oral groove; underlying oral aperture less obvious. At least two contractile vacuoles. Quick, spiralling swimming. Prominent trichocysts. Feeds principally on bacteria. Common and sometimes abundant in sediments and detritus, especially if affected with organic pollution. Rarely planktonic. At least one species with zoochlorellae (*P. bursaria*). Usually 100-200 μm; max. range 60-300 μm.

93. *Lembadion*. Unmistakable planktonic ciliate. Ventral surface dominated by large membrane (formed from peniculi) on left of massive oral aperture: both run almost full length of cell. Paroral membrane inconspicuous. Tuft of caudal cilia. Distinctive translucent cytoplasm. Feeds on protozoa and the larger unicellular algae (e.g. cryptomonads, dinoflagellates). 80-200 μm.

The peniculid ciliates have traditionally been classified within the Order Hymenostomatida. The distinctiveness of the peniculi is largely responsible for the removal of these ciliates to the Order Peniculida (Class Nassophorea) (see Small & Lynn, 1985).



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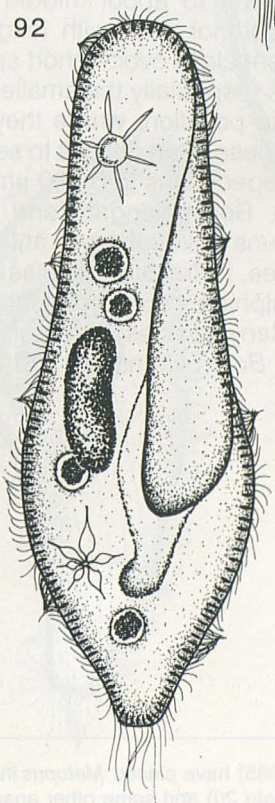

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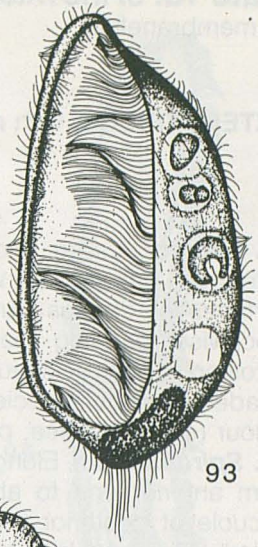

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Plate 18. SPIROTRICH CILIATES; with a conspicuous adoral zone of membranelles.

HETEROTRICHS; with a large AZM and cilia over the whole body.

94. *Stentor*. Large and contractile. Usually trumpet-shaped when extended, bell-shaped when contracted. Typically attached to plant surfaces or detritus but may also be free-swimming. AZM spirals clockwise, carrying food particles (especially unicellular algae) to cytostome. Macronucleus approximately spherical in smaller species; beaded in larger species. Pigment granules often impart distinctive colour (e.g. turquoise, pink, black). Many species, 150-3000 μm.

95. *Spirostomum*. Elongate, narrow and contractile with AZM running from anterior end to about middle of cell. Single large contractile vacuole at posterior end, with long collecting channel running anteriorly. Macronucleus ovoid (short species) or beaded (long species). Some species (especially the smaller *S. teres*) common at sites affected by organic pollution, where they feed on bacteria. Also feed on small algae - these often difficult to see once ingested. Most commonly encountered specimens 200-300 μm; max. range 150-3000 μm.

96. *Metopus*. Body elongate and twisted. Frontal lobe overhangs AZM, which runs obliquely over anterior half of cell. No cirri or spines in most species. Refractile granules close to anterior end. Common in anaerobic, sulphide - rich sediments; sometimes planktonic in anaerobic water. Many species, some of which superficially resemble *Brachonella* and *Bothrostoma*. 30-300 μm; usually about 80 μm.

Small & Lynn (1985) have placed *Metopus* in the Order Armophorida along with Caenomorpha (see Plate 20) and some other anaerobic ciliates.

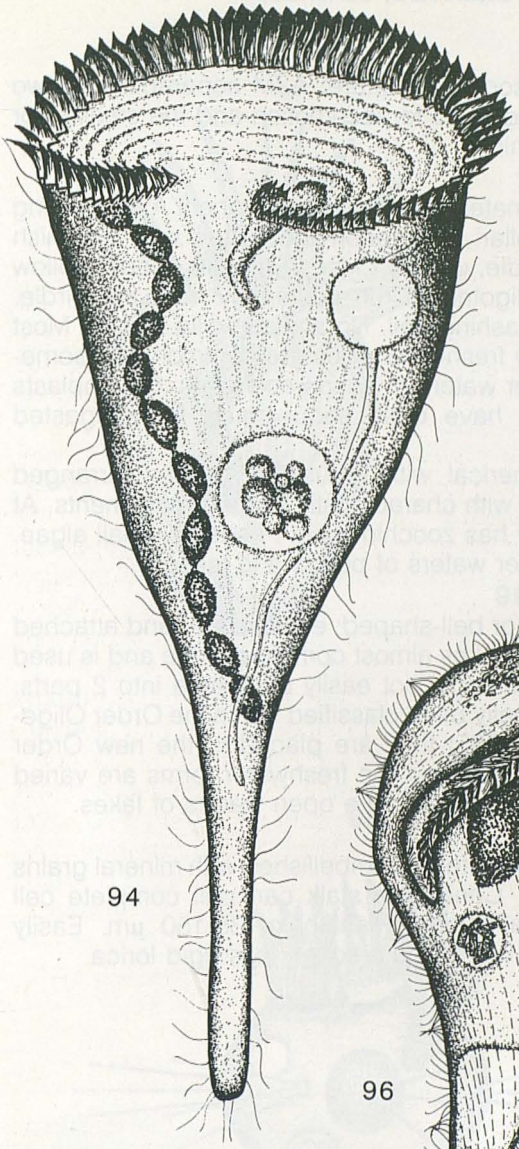

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Plate 19. SPIROTRICH CILIATES; continued

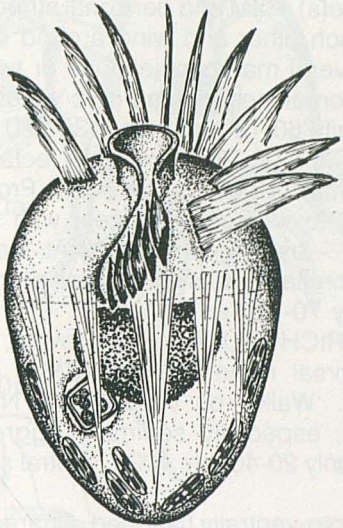
OLIGOTRICHS; reduced somatic ciliature; AZM separable into two parts - the larger part being used for locomotion and the smaller for feeding; no lorica; planktonic.

97. *Strombidium*. Approximately ovoid with a prominent AZM winding round slightly elevated 'collar' at anterior end. Often with brownish appearance. Prominent girdle, usually close to middle of cell. Hollow skeletal rods (unique to oligotrichs) run posteriorly from the girdle. Rods easily seen after squashing cell. No somatic cilia or cirri. Most species marine but the few freshwater ones are common and sometimes abundant in the upper waters of ponds and lakes. Chloroplasts sometimes obvious: these have been sequestered from ingested algae. 20-170 μm.

98. *Halteria*. Roughly spherical with cirrus-like 'bristles' arranged around the equator. Swims with characteristic darting movements. At least one species probably has zoochlorellae. Feeds on small algae. 20-50 μm. Common in upper waters of ponds and lakes.

TINTINNIDS; body conical or bell-shaped; enclosed in, and attached to inner wall of lorica. AZM makes almost complete circle and is used for both locomotion and feeding - not easily separable into 2 parts. The Tintinnids have traditionally been classified within the Order Oligotrichida. In Small & Lynn (1985) they are placed in the new Order Choreotrichida. Most are marine but the freshwater forms are varied and sometimes relatively abundant in the open waters of lakes.

99. *Tintinnopsis*. Lorica elongate and embellished with mineral grains and fragments of diatoms. Contractile stalk can pull complete cell into lorica. Feeds on small algae. Planktonic. 35-150 μm. Easily confused with *Tintinnidium* which has a softer, less rigid lorica.



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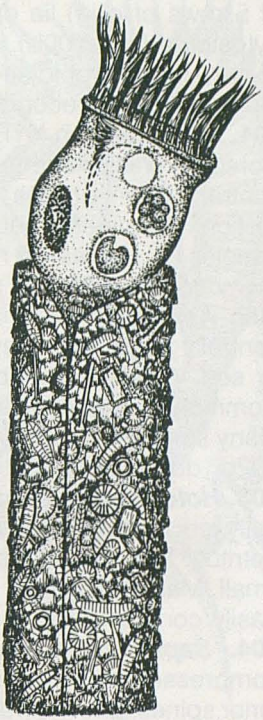

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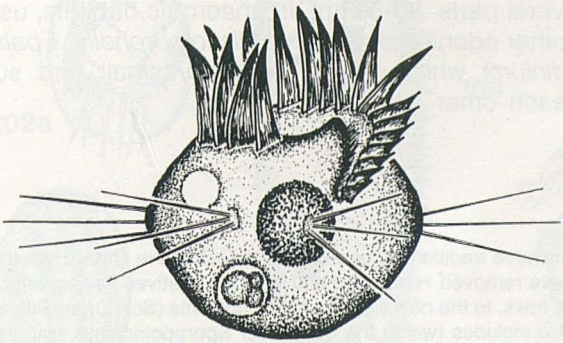


Plate 20. SPIROTRICH CILIATES; continued

100. *Caenomorpha* - an ARMOPHORID HETEROTRICH. Distinctively medusoid, with rigid posterior spine(s). AZM and perizonal stripe (band of 5 rows of cilia) lie opposite each other and wind around body to cytostome (see 100b). One to several macronuclei. One or two rows of cirri. In anaerobic sediments; sometimes in anaerobic water. Common and easily recognised. Usually 60-150 μm; max. 30-220 μm.

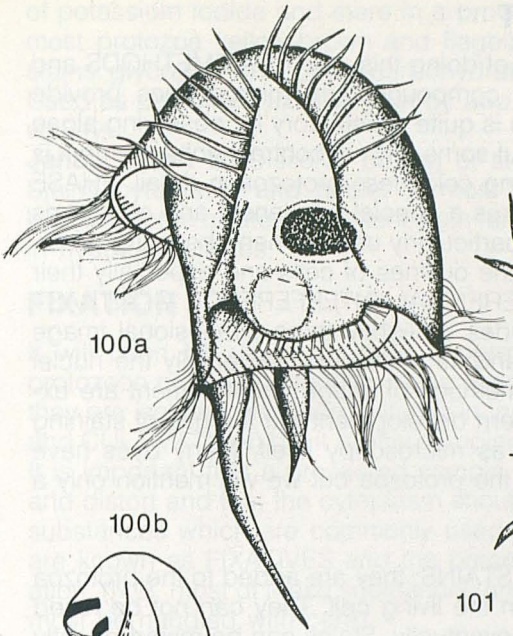
101. *Euplotes* - an HYPOTRICH CILIATE. Rigid, ovoid to rectangular. Dorso-ventrally flattened with dorsal ridges. Large AZM. Prominent ventral cirri in groups. Uses cirri to walk on surfaces, with a jerky motion. Prominent macronucleus - usually C-shaped. At least one species (*E. daidaleos*) has zoochlorellae and is sometimes planktonic. Many species, 35-250 μm; usually 70-140 μm.

102. *Aspidisca* - a small HYPOTRICH CILIATE. Rigid, ovoid, dorso-ventrally flattened. May have dorsal ridges. Small AZM - difficult to see. C-shaped macronucleus. 'Walks' on ventral cirri. No cilia. Commonly crawls over surfaces, especially sediment aggregates. Many species, 20-150 μm; commonly 20-40 μm. 102a, ventral surface; 102b, dorsal surface.

103. *Holosticha*. Elongate and dorso-ventrally flattened. Characteristically jerky movement. Usually associated with particles of sediment or detritus. Two mid-ventral rows or cirri running obliquely. AZM relatively small. Macronucleus in two to many parts. Many species, 50-300 μm. Easily confused with many other Stichotrich genera.

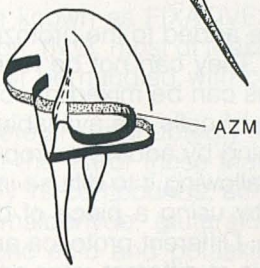
104. *Saprodinium* - an ODONTOSTOME CILIATE. Rigid, laterally compressed and ridged; roughly circular outline and prominent posterior spines. Short rows of somatic cilia. AZM inconspicuous. Macronucleus in one to several parts. 35-80 μm. In anaerobic habitats, usually accompanied by other odontostomes (e.g. *Discomorphella*, *Epalxella*, *Mylestoma*, *Pelodinium*) which are all relatively small and superficially similar to each other.

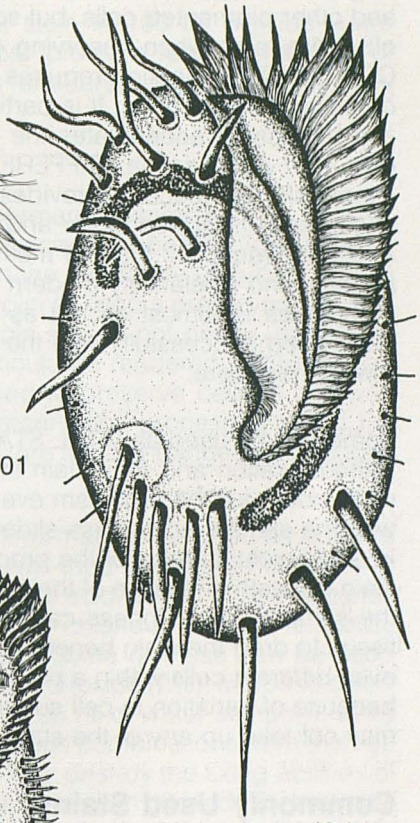
Euplotes and *Holosticha* have traditionally been classified within the Order Hypotrichida. Small & Lynn (1985) have removed *Holosticha* and its close relatives having ventral cirri arranged in longitudinal rows, to the new Sub-Class Stichotrichia (Sub-Order Stichotrichina). The Sub-Class also includes (within the Sub-Order Sporadotrichina), ciliates such as *Oxytricha* and *Stylonychia* which sometimes superficially resemble *Euplotes*. *Euplotes* and *Aspidisca* remain within the Sub-Class Hypotrichia, but with the removal of the latter taxon to the Class Nassophorea, these two genera are no longer, technically, 'spirotrichs'.



100a

100b





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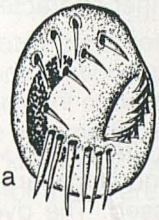
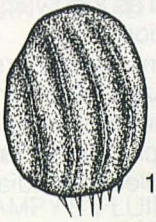


102a



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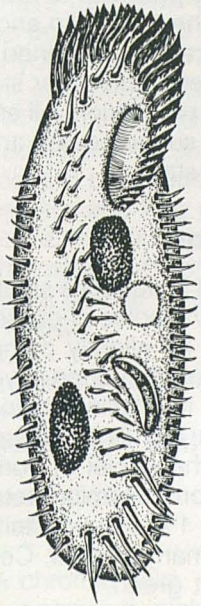

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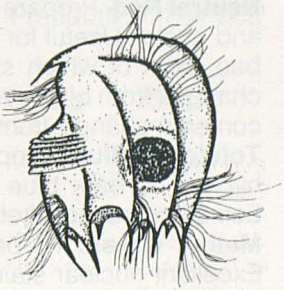

104

IMAGE ENHANCEMENT

There are two principal ways of doing this - OPTICAL METHODS and the use of DYESTUFFS. All compound light microscopes provide bright field illumination, which is quite satisfactory for observing algae and other pigmented cells, but some form of contrast enhancement is often desirable when observing colourless protozoa in detail. PHASE CONTRAST illumination requires a special condenser and objectives containing phase rings. It is particularly useful when observing protozoa because it accentuates the outlines of cells and especially their cilia, cirri and flagella. DIFFERENTIAL INTERFERENCE CONTRAST (Nomarski) microscopy provides a slightly three dimensional image of the cell, its appendages and its organelles, especially the nuclei and food vacuoles. Optical methods of image enhancement are expensive and a relatively modern development but the use of staining techniques is almost as old as microscopy itself. Many dyes have been used successfully with the protozoa but we will mention only a few of them here.

Some can be used as VITAL STAINS; they are added to the protozoa without fixation and they stain the living cell. They can not be relied upon not to kill the organism eventually. Stains can be mixed directly with the sample on a glass slide but if plenty of cells are available it is often useful to monitor the progress of staining by adding a drop of stain beneath one edge of the cover slip and allowing it to diffuse into the sample. This process can be hastened by using a piece of dry tissue to drag the stain beneath the cover slip. Different protozoa and even different cells within a population will stain at different rates and, because of variation in cell surface coats and other characters some may not take up any of the stain.

Commonly Used Stains

Neutral Red. Prepare a 0.01% aqueous solution. Stains food vacuoles and nuclei. Useful for food vacuoles of ciliates and some large amoebae, both of which should be slightly compressed. The dye colour changes from bluish-red through orange to yellow-red as food vacuole contents change from acid to alkaline during digestion.
Toluidine Blue. Prepare a 0.01% aqueous solution. Stains cirri of hypotrichs dark blue and the nuclei of some protozoa blue-purple. Sometimes used after fixation in osmium tetroxide fumes.
Methyl Green. Prepare a 1% solution in 1% glacial acetic acid. Excellent nuclear stain for many ciliates. Cells are killed immediately and the nuclei stain brilliant green.
Lugol's Iodine. Iodine is almost insoluble in water but it is soluble in solutions of potassium iodide. Dissolve 4g iodine solid in a 6% solution

of potassium iodide and store in a brown bottle. Stains cytoplasm of most protozoa yellow-brown and flagella, dark brown. Preferentially stains glycogen and other carbohydrates. May also stain nucleus. Used as combined fixative-stain by adding acetic acid to 10% of the volume.

Janus Green B. Dissolve 50 mg of stain in 100 ml distilled water Stains cytoplasm and nuclei of most protozoa and trichocysts of hymenostome ciliates. Excellent stain for adoral zone of membranelles in hypotrich ciliates.

FIXATION

It will often be useful to keep a permanent sample of a specific protozoon or of a community. Many species can be kept indefinitely if they are isolated and then cultured in the laboratory (see ISOLATION and CULTIVATION) but it is often simpler to keep a preserved sample. It is important that a preserved sample should not decay, nor shrink and distort and that the cytoplasm should be rendered insoluble. The substances which are commonly used to preserve cells in this way are known as FIXATIVES and the preservation process is called fixation. (N.B. most of these substances are toxic or dangerous and they must be handled with care).

There are undoubtedly many substances that can be used as fixatives but the list of those that have been well-tried and tested consists of only 10 compounds; acetic acid, chromic acid, cobalt nitrate, ethanol, formaldehyde, glutaraldehyde, mercuric chloride, osmium tetroxide, picric acid and potassium dichromate. Some of these can be used singly (e.g. formaldehyde (4%), osmium tetroxide (2%), mercuric chloride (add 5 ml of a saturated solution to 95 ml of water sample)), while some fixative treatments consist of various combinations from this list. Some combinations are useless and they destroy the fixing abilities of the constituent substances: ethanol and osmium tetroxide for example are quite incompatible. The more commonly used combinations have become known by the names of their inventors. Preparation methods for three of these are as follows:

BOUIN'S FLUID	saturated solution of picric acid	75 ml
	formalin	25 ml
	glacial acetic acid	5 ml

Add cells to freshly prepared fixative in the ratio 19 parts protozoan suspension to 1 part fixative.

CHAMPY'S FLUID	1% chromic acid	7 parts
	3% potassium dichromate	7 parts
	2% osmium tetroxide	4 parts

Add cells to freshly prepared fixative (the first two constituents can be mixed and kept indefinitely). When fixing freshly collected samples, add 5.5 ml to 4.5 ml of fixative in a glass, 15 ml centrifuge tube. Leave for 5 minutes, then centrifuge, decant the supernatant fixative and add da Fano's fluid. Repeat until the colour of Champy's fluid is no longer obvious.

DA FANO'S FLUID	cobalt nitrate	1 g
	sodium chloride	1 g
	formalin	10 ml
	distilled water	90 ml

This fixative can be kept mixed for long periods.

Combinations of fixatives like these are usually used before the cells are subjected to specific staining procedures. Bouin's fluid is used before staining ciliates with protargol (protein silver; see Curds, 1982) and Champy's fluid followed by da Fano's fluid is used before staining with silver nitrate. The silver line system is the complex pattern of structures (especially the ciliary basal bodies, also known as kinetosomes) lying just beneath the pellicle, which can be visualised because it takes up silver. Each ciliate species has a characteristic pattern so 'silver staining' is often used to identify species. There are several methods and many variations of each one. We have had some success in using a version of the Chatton-Lwoff method.

Silver-staining (Chatton-Lwoff)

1. Fix cells in Champy's fluid for a minimum of 5 min and a maximum of two hours.
2. Centrifuge and transfer cells to da Fano's fluid. Cells can be left in da Fano's for long periods.
3. Centrifuge and wash once in distilled water.
4. Prepare some fresh gelatine in solution by adding 1 g to 9 ml distilled water in a small beaker. Keep on a hotplate at 45-50°C.
5. Half fill a 100 ml beaker with warm water and place it on the hotplate. Place some cover slips on the hotplate.
6. Transfer 1 ml of the gelatine to a clean, warm, glass centrifuge tube and place it in the beaker of warm water.
7. Transfer 0.5 ml of the cell suspension in da Fano's fluid to the warmed gelatine and mix with a warm needle. Leave for a few minutes to allow the cells to settle. It may be necessary to centrifuge them briefly and then return them to the hotplate.
8. Use a warm, glass, Pasteur pipette to remove the protozoan pellet. Dispense a small drop to each of the warmed cover slips.

Use a warmed needle to spread the suspension evenly and thinly over each cover slip. Try to do this as quickly and as carefully as possible to avoid the gelatine solidifying.

9. Place cover slips in a moist chamber (petri dish + absorbent paper + water) in the refrigerator for 5 min. This will solidify the gelatine.
10. Submerge cover slips in cold (<10°C) 3% silver nitrate in a glass petri dish and leave in the dark, in the refrigerator, for 5-10 min.
11. Transfer cover slips to refrigerated distilled water in a glass petri dish. Expose to bright sunlight or, preferably, the light from an ultraviolet lamp (take care!). The exposure required depends on the type and intensity of the source of short wavelength radiation. After 5-15 min the gelatine should begin to turn yellow and the cells should be checked with the microscope every few minutes. Do not allow the cells to blacken completely.
12. Wash cover slips in cold distilled water.
13. Dehydrate with alcohol (50%, 70%, 95%, 100%, 100%). We use Columbia staining jars and isopropanol and the cover slips are left in each dilution for only a few minutes.
14. Transfer to xylene for clearing.
15. Mount with Canada balsam or similar mountant (we use DPX mounting medium from BDH Limited, Poole, England).
16. Observe with bright field illumination. If the preparation is only lightly stained it may be necessary to use an objective lens with at least 40x magnification.

Fixation and Staining of Amoebae

To enhance contrast for photomicroscopy, to stain nuclei and other cytoplasmic inclusions, or to make permanent preparations, fixation with NISSENBAUM and ACIDIFIED MERCURIC CHLORIDE followed by staining with HEIDENHAIN'S IRON HAEMATOXYLIN can be recommended.

1. Place a drop of medium containing amoebae on a slide or cover slip and leave in a moist chamber until the cells have adhered firmly to the glass.
2. Add several drops of fresh NISSENBAUM'S fixative and leave for 2 min.
3. Transfer the slide or cover slip into fresh ACIDIFIED MERCURIC CHLORIDE and leave for 10 min.
4. Transfer through 4 changes of 50% ethanol, one of 35% ethanol and one of distilled water; 3 min each.
5. Mordant in 2% ferric ammonium sulphate for 2 hours, then rinse in tap water (2 min).
6. Stain with 0.5% HAEMATOXYLIN for 2 hours, rinse briefly with distilled water, then tap water for 2 min.

7. De-stain with 2% ferric ammonium sulphate. After 1 min check progress of decolourisation and continue if necessary: cytoplasm should be lightly stained with a distinct nucleus. Stop the de-staining by washing in tap water for 30 min.
8. Dehydrate with 3 min each in 35%, 50%, 70%, 80%, 90%, 95% and 100% ethanol. Transfer to fresh 100% ethanol for 10 min. Wash briefly in a 1:1 mixture of 100% ethanol and xylene and then 100% xylene for 5 min. Mount in DPX or similar mountant.

Note: Some amoebae adhere poorly to glass. Try coating a slide with a thin layer of Mayer's albumen (1 part glycerol: 1 part egg white). Large cells can be pipetted through the various stages, or transferred by low speed centrifugation.

NISSENBAUM'S FIXATIVE

saturated aqueous mercuric chloride	5 ml
glacial acetic acid	1 ml
formaldehyde (38-40%)	1 ml
tertiary butyl alcohol	2.5 ml

ACIDIFIED MERCURIC CHLORIDE

saturated aqueous mercuric chloride	50 ml
glacial acetic acid	2.5 ml

HAEMATOXYLIN

haematoxylin	1 g
100% ethanol	10 ml

This is the stock solution of haematoxylin. Store in the dark. Prepare working solution by adding 1 part stock to 19 parts distilled water.

ISOLATION and CULTIVATION

It is possible to cultivate a great variety of free-living protozoa in the laboratory. Some are easier to grow than others: the protozoa that thrive in high bacterial concentrations can usually be cultured without much effort whereas some planktonic species, especially those with specific food requirements may demand much patience and ingenuity if they are to be obtained in dependable cultures. All protozoa have some basic requirements which need to be considered before we begin cultivation. We would also draw the reader's attention to the safety considerations mentioned under TEMPERATURE.

Water. Protozoa must be in contact with, or surrounded by water if they are to move and feed. This also applies to agar cultures in which many

small amoebae thrive: they do not penetrate the agar but they migrate in the thin film of water on the surface. They soon stop moving when the agar dries out.

Temperature. Most protozoa will not grow at temperatures higher than 45°C. Many free-living species can be cultured at temperatures between 25° and 35° and they usually grow very quickly at these temperatures. This may or may not be an advantage; at such high temperatures the chemical conditions in the culture will change very quickly and the protozoon may quickly exhaust its food source. It is often as well to opt for the slower growth at lower temperatures. Virtually all protozoa in temperate latitudes that can be isolated and grown will do so at some temperature within the range 15°-25°. Our experience is that temperatures close to the bottom end of this range more frequently bring success.

The question of SAFETY should also be considered when selecting a temperature for cultivation. The LETHAL PATHOGEN *Naegleria fowleri* is a naked amoeba which grows at temperatures up to 45°C, and at temperatures higher than about 37°C its growth may be favoured over the many other non-pathogenic amoebae. There is little likelihood of isolating *N. fowleri* at temperatures less than 25°C in samples collected from temperate habitats. However, we would suggest that anyone attempting to isolate and grow protozoa should adopt at least the same standards of CLEANLINESS and SAFE PRACTICE that they would apply to other micro-organisms. The absolute minimum requirements are: a. the prohibition of MOUTH PIPETTING, b. the prohibition of EATING and DRINKING in the laboratory, and c. facilities for the safe cleansing (e.g. by use of disinfectants or autoclaving) or disposal, of glassware, consumables (especially glass slides and pipettes) and discarded cultures.

Oxygen. Some protozoa are anaerobic and they cannot be cultured in the presence of oxygen. Many other protozoa can live for short periods without access to oxygen but they require oxygen for their continued survival, growth and reproduction. Many protozoa are MICROAEROPHILIC, i.e. they grow best when they have access to only very low levels of oxygen and, if it is at all possible for them, they seek out water with a low oxygen concentration. This applies especially to many ciliate species that normally live in sediments: they may be aerobic but high oxygen concentrations may be toxic. These species usually grow best in test tubes.

Food. Protozoa are phagotrophic and their natural method of feeding is to ingest bacteria, algae and other micro-organisms. This means

that food organisms must normally also be present in protozoan cultures. There are two ways of doing this; if the protozoa are living in a NON-NUTRIENT MEDIUM, micro-organisms added to this medium will not be able to grow and divide. The protozoa will consume and exhaust the supply of micro-organisms which must be replenished at intervals. The alternative is to suspend the protozoa in a medium which contains some nutrients (e.g. soil extract medium, see below) which sustains the micro-organisms. The latter continue growing until they have exhausted the nutrients or until the build-up of waste products in the medium prevents their further growth. Their continued growth provides a continuous food supply for the protozoa. The main disadvantage with this type of method is that it is often difficult to control the growth rate of the micro-organisms. Bacteria in particular can often swamp the culture if they are supplied with an excess of nutrients.

Obtaining the First Culture

Keeping these basic requirements of protozoa in mind, the first step is usually to stimulate the growth of a particular protozoon. To do this it is necessary to know something about the habitat from which the sample was obtained and about the likely natural food supply of the protozoon. For example, many of the bacteria-feeding protozoa in sediments can be encouraged to grow by simply adding a FEW cereal grains (e.g. rice, wheat) or some soil extract medium. The increase in the quantity of dissolved organic compounds will stimulate bacterial growth and, as a consequence, the growth of the protozoa. A few of the faster growing species, like the ciliates *Paramecium* and *Colpidium*, will in all likelihood soon become the dominant protozoa in such a culture. By taking a sample of this culture and adding it to more soil extract medium, the bacteria that are transferred will grow with renewed vigour and growth of the protozoa will follow. For example, suppose the object of the exercise is to obtain a culture of the ciliate *Paramecium*. We now have a so-called POLYXENIC culture - the organism we are interested in is surrounded in culture by many other types of micro-organisms. We do not know the identity of many of these organisms so the polyxenic culture of *Paramecium* is also said to be AGNOTOXENIC or AGNOTOBIOTIC. Most protozoan cultures start off in this way. The next step is to try and isolate the *Paramecium* from some of the other organisms in the culture. There are two simple ways of doing this:

1. Isolation by Dilution. The idea is to continue to dilute the culture with fresh medium, using a series of containers, until only one or a few cells of the wanted species plus the food organisms remain. The chances of doing this successfully are obviously increased if the species in question is one of the most abundant in the mixed culture.

The unwanted organisms will then be diluted out more quickly. The technique is most useful when attempting to isolate very small protozoa, e.g. flagellates or small ciliates (small amoebae are best isolated on agar surfaces, see 3 below). The scale and number of dilutions depend on the abundance of the wanted organism. It is also advisable to make several duplicates at each dilution level. Many companies now supply disposable plastic, multichambered 'repli-dishes' which are ideal for this purpose.

2. Isolation with Pipettes. This is the simplest and quickest method of isolating relatively big protozoa like *Paramecium*. It is necessary to prepare some finely-drawn glass Pasteur pipettes. Hold each end of a pipette and place the neck in a fairly hot bunsen flame. After a few seconds the glass will turn soft. Remove the pipette from the flame and then gently but decisively pull the two ends in opposite directions. The region of hot glass can be drawn out to about 20 cm of capillary tubing. Break this to produce a fine bore pipette with the required tube diameter (usually about 0.5-1 mm). The rough end can be polished by passing the tip rapidly back and forth through a cold (yellow) flame. The pipette is then used in the normal way, with a rubber bulb attached, to pick up individual protozoa and transfer them through a series of washings in sterile culture medium. It is usually necessary to do this with the aid of a microscope. A compound microscope with the lowest power objective in position will be satisfactory but the extra working distance afforded by low power (e.g. 10-40x) stereo microscopes makes the task much easier.

The objective in both of these procedures is to produce a so-called MONOXENIC culture - in which only one other species of organism (usually the food organism) is present in addition to the species that has been purposefully isolated. There is another way of doing this if the organism will grow and migrate over solid agar.

3. Isolation on Agar Surfaces. This method is best for isolating small amoebae which readily grow and migrate on agar surfaces. Ciliates and flagellates are rarely capable of doing this. One or two drops of sample (e.g. untreated water, sediment, washings of vegetation surfaces) are placed on a non-nutrient agar plate that has been streaked with a suitable food organism (the bacterium *Escherichia coli* is a sensible first choice). The initial sample that is added to the plate may contain ciliates, flagellates, fungi and other micro-organisms but only the small amoebae will be able to both consume the food bacteria supplied and migrate over the agar surface following local exhaustion of the food supply. As a consequence, the amoebae isolate themselves

from the rest of the natural microbial community, which is often dominated by fungal hyphae. Isolated amoebae should be picked off the agar surface with a sterile scalpel blade and sub-cultured onto a fresh agar plate with bacteria.

The ultimate goal in the business of isolation and cultivation is to obtain an AXENIC culture, in which no other organisms are present, or better still, a culture in a DEFINED MEDIUM, with a chemical composition that is known exactly. Many protozoa have been obtained in axenic culture (e.g. *Tetrahymena*, *Paramecium*, *Astasia*, *Acanthamoeba*). The process usually involves the cleaning up of a monoxenic culture with selective antibiotics, but the details of the process are outwith the scope of this booklet.

Types of Media and Methods of Cultivation

The great variety of media and methods used in the cultivation of protozoa can be divided into six broad categories:

Water Tap water and rain water have traditionally been used to culture protozoa. The main problem with these sources is that they are extremely variable in composition: some waters are harder than others and some may contain variable quantities of both organic and inorganic contaminants. It is possible to alleviate some of these problems by filtration and by using chelating agents but a far simpler solution is to use one of the commercially available BOTTLED MINERAL WATERS (non-carbonated), many of which also provide a fairly comprehensive account of their chemical composition. We have had some success using the brand name 'Volvic' but it is likely that most other brands are equally suitable. With few exceptions these waters contain only negligible quantities of dissolved organic matter, so although the ionic composition is suitable for the growth of many protozoa, they will not grow unless a food supply is introduced. Heliozoans grow well in these mineral waters if they are periodically supplied with food organisms. In the case of *Actinophrys* the food is usually *Tetrahymena* or some other similar ciliate that is concentrated by centrifugation before being introduced.

Inorganic Salt Solutions Like mineral water, these solutions are designed to provide a balanced medium which in itself has no nutritional value. The advantage of these over mineral water is that the chemical composition is known more precisely. The following two recipes are used mainly for the cultivation of large naked amoebae (e.g. *Amoeba proteus* in PC solution). Again, because these media contain no added organic matter, they support growth of protozoa only

when food organisms (e.g. the ciliates *Tetrahymena* or *Colpidium*) are added.

PRESCOTT AND CARRIER'S SOLUTION Make up two stock solutions (A and B), each with 1 litre glass-distilled water

Stock solution A	$MgSO_4.7H_2O$	0.2 g
	KCl	0.5 g
	$CaCl_2$	1.0 g
	NaCl	1.0 g
Stock solution B	$CaHPO_4$	0.36 g

Prepare the culture medium by adding 10 ml of each stock solution to 980 ml glass-distilled water.

MODIFIED NEFF'S AMOEBA SALINE (this is also used in the preparation of non-nutrient agar) Prepare five stock solutions by dissolving each of the following in 100 ml glass-distilled water:

NaCl	1.20 g
$MgSO_4.7H_2O$	0.04 g
$CaCl_2.2H_2O$	0.04 g
Na_2HPO_4	1.42 g
KH_2PO_4	1.36 g

The culture medium is prepared by mixing 10 ml of each solution and making up the volume to 1 litre with glass-distilled water.

Non-nutrient Agar Media These are used for the cultivation of small to medium-sized amoebae (e.g. *Vahlkampfia* and *Saccamoeba*). Dissolve 15 g of non-nutrient agar in 1 litre of modified Neff's amoeba saline. Bring to the boil. Dispense into 500 ml flasks or bottles (250 ml in each) and sterilise in an autoclave or pressure cooker (about 15 psi, 121°C, 15 min). Pour into 9 cm plastic Petri dishes. Use mineral water or tap water if amoeba saline is not available. Grow the food bacteria (e.g. *Escherichia coli*) by inoculating plates of nutrient agar. Use commercially available nutrient agar and follow the manufacturer's instructions. Use a bacteriological loop to streak the non-nutrient agar with food bacteria. The amoebae are then added to the plate as a suspension of cells in modified Neff's amoeba saline or on a block of agar cut from an older culture. A dense population of amoebae should have grown within 1-2 weeks. They can be observed by turning the plate upside down and using a microscope with the 10× objective in place. Alternatively, some cells can be washed off the plate with a little saline dispensed from a pipette.

Plant Infusions These have long been used for the cultivation of a diversity of protozoa, especially flagellates and bactivorous ciliates like *Colpidium* and *Paramecium*. The underlying principle is that various organic compounds leach out, or are extracted from various plant parts. Polished rice grains are the simplest to use - just add them to the culture medium (either water or an organic medium). The amount of bacterial growth will be roughly proportional to the number of grains added. As a rough guide, allow one grain per 10 ml of medium. Barley and wheat grains can be used in the same way after boiling them for a few minutes.

Hay infusions are also excellent for the cultivation of many bactivorous protozoa. Boil 10 g of hay in a litre of water (tap water will do) for about 5 min. Allow the hay to settle and use the supernatant as a culture medium. It may be necessary to dilute it with water (e.g. 1 part infusion to 4 parts water).

Dehydrated cereal leaves are also commercially available (e.g. from Sigma Chemical Co., Fancy Rd, Poole, Dorset, U.K.; sometimes sold in the U.S.A. under the brand name 'Cerophyl'). Prepare a 0.15% (w/v) suspension of 'Cerophyl' in distilled water. Bring to the boil and simmer for 10 min. Clarify the infusion by passing it through a glass fibre filter or paper filter, e.g. coffee filter. Adjust the pH to 7.0-7.2 using 0.1M NaOH. Sterilise by autoclaving (a pressure cooker is quite satisfactory). The final pH when cool is about 6.8.

Soil Extract Media The underlying principle is the same as for plant infusions - the object is to extract some of the organic compounds from soil that will sustain bacterial growth. There are many ways of doing this and there are of course many soil types so it is difficult to give a precise method that anyone can use anywhere. There are however a few points worth remembering.

1. Try to use a garden soil or the surface soil of arable land. Avoid forest soils and those with an excess of sand or clay.
2. Check the pH of the prepared medium and adjust it to about pH 7 if necessary. Some soil extract media can be very acid.
3. Remove stones and debris before using the soil.
4. Some media recipes specify 'steaming' on successive days; others specify autoclaving. The latter is a harsher treatment, it obviously brings more substances into solution and it is more likely to kill all soil organisms. It will also undoubtedly denature some organic compounds and effect other chemical changes, the relevance of which is usually unknown.

Soil extract medium can be prepared in any glass vessels that can be autoclaved. The following instructions cover three sizes of container.

Collect garden soil and dry it in the sunshine or in a warm room. When dry, pass it through a series of sieves, e.g. 16, 8 and 3.4 mm to remove stones and coarser materials. Mix with water in the following proportions:

	Sovirel bottle (500 ml)	Sovirel bottle (250 ml)	Test tube
Garden soil	52 g	35 g	2 g
Tap water	330 ml	220 ml	15 ml

Autoclave at 15 psi, 121°C for 15 min and then allow contents to settle for at least one week before use. When completely clear, the medium can vary in colour from a deep, rich, gold colour to pale yellow. If the medium does not clear it may be contaminated with bacteria and it is best to discard it. The medium can be decanted and filtered or it can be used with the sediment remaining. In the latter case it is known as a BIPHASIC culure. The pH is normally about 6.5 which is suitable for most benthic protozoa. It is not usually necessary to dilute the medium.

There are many documented variations of this procedure including the deposition of a little calcium carbonate beneath the soil in test tube preparations and the inclusion of a few barley grains to promote faster bacterial growth.

Rich Organic Media At high concentrations of dissolved organic carbon many protozoa can live without ingesting particulate food - they are said to feed osmotrophically, with the organic compounds passing directly across the cell membrane. The main advantage of these media is that they make it possible to dispense with food organisms, and the protozoon can be grown axenically. However, it should be pointed out that these rich organic media should only be used undiluted for axenic cultures. If bacteria are present these will quickly grow and invariably swamp the protozoa. Aside from their designated purpose these media may also be used, greatly diluted, to stimulate rapid heterotrophic microbial growth in water or sediment samples collected from the wild. A few recipes follow.

PROTEOSE PEPTONE/YEAST EXTRACT MEDIUM (especially for *Tetrahymena*)

Proteose peptone	10 g
Yeast extract	2.5 g
Distilled water	1000 ml

Add the solids to a little water, heat and dissolve. Be careful that the solids do not stick to the bottom of the vessel. Boil until completely dissolved and the medium appears clear and yellow. Dispense into glass vessels and autoclave.

MEDIUM FOR ACETATE FLAGELLATES (e.g. many euglenids and cryptomonads)

Sodium acetate (tri-hydrate)	1 g
Beef extract	1 g
Yeast extract	2 g
Bacto tryptone	2 g
Calcium chloride	0.01 g
Distilled water	1000 ml
(Agar	10 g)

Dissolve the beef extract first. The agar is optional: some flagellates, e.g. many euglenids, can be grown heterotrophically on this solid medium in the dark. Autoclave and dispense into glass tubes or Petri dishes. Glass tubes should be left inclined at an angle to allow the agar to solidify as a slant.

MEDIUM FOR THE SMALL NAKED AMOEBA *Acanthamoeba*

Proteose peptone	10 g
Glucose	18 g
Amoeba saline	1000 ml

Autoclave and dispense into test tubes.

APPENDIX 1. Some useful suppliers

Sedgewick Rafter Cells are made in glass and in plastic. Both types can be purchased from Graticules Ltd, Morley Rd, Tonbridge, Kent, TN9 1RN (tel. 0732 359061) and they cost about £40 each + VAT (glass) or £8 + VAT (plastic) (1988 prices). Plastic cells can also be obtained from general laboratory suppliers e.g. A. Gallenkamp & Co. Ltd, Belton Rd West, Loughborough, Leics. LE11 0TR (tel. 0509 237 371).

Nylon Mesh with defined pore sizes is sold under the trade names NYTAL and NYBOLT. NYTAL is imported into the U.K. from Switzerland by Henry Simon, PO Box 31, Stockport, Cheshire (tel. 061 428 3600). NYBOLT can be obtained from John Staniar & Co., Manchester Wire Works, Sherborne St., Manchester M3 1FD (tel. 061 834 0330). Both companies have minimum order charges.

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