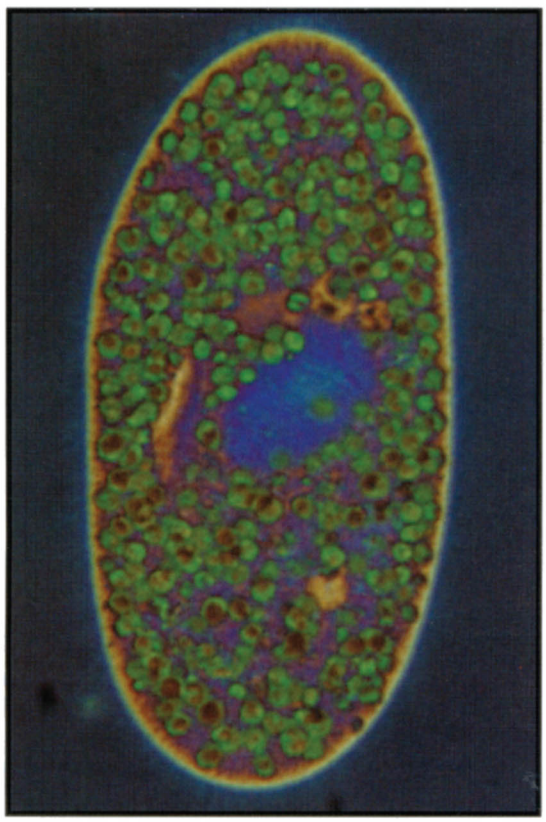
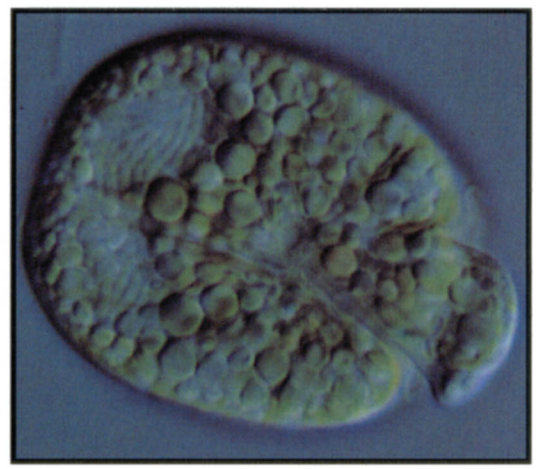
Free-Living Freshwater Protozoa

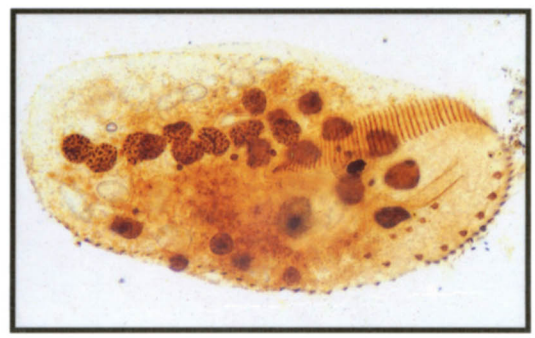
A Colour Guide

D.J. Patterson

Drawings by Stuart Hedley







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Daytona

Preface

'Thou knowest that the ancient trees seen by thine eyes have fruit,
But knowest thou that trees and fruit flourish upon the earth
To gratify senses unknown? trees, beasts and birds unknown;
Unknown, not unperceiv'd, spread in the infinite microscope,
In places yet unvisited by the voyager, and in worlds
Over another kind of seas, and in atmospheres unknown.'

Visions of the Daughters of Albion, William Blake
1757–1827

Protozoa are important consumers in many aquatic ecosystems including some of economic importance, such as waste water-treatment plants. The biology of such habitats cannot be properly described unless the protozoa are taken into account. Protozoan communities respond rapidly to changing physical and chemical characteristics, and can be useful indicators of changes in natural communities. In spite of these facts, the inclusion of a protozoan perspective in studies of the biology of aquatic habitats, especially by non-protozoologists, is relatively rare. The purpose of this Guide is to make free-living protozoa a little more accessible to students and professionals who need to study protozoa in the course of their work.

The emphasis of the Guide is on those organisms that are most likely to be encountered in freshwater habitats. It is assumed that most readers wanting to develop a basic knowledge of free-living protozoa will not use specialist staining or preservation techniques, rather they will observe living organisms. The identification of most genera is relatively straightforward, but that of most species is more difficult, requiring specialist techniques. Those who wish to work at the species level will find reference to suitable literature in the Bibliography.

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A variety of people have provided advice or other forms of support. I would like to thank Hilda Canter, Marina Christopher, Tore Lindholm, Craig Sandgren and Helge Thomsen for photographic material; Klaus Hausmann for teaching me the techniques of photomicrography; Willi Foissner, Jacob Larsen, Øjvind Moestrup, Colin Ogden, Ferry Siemensma, Michael Sleigh, Naja Vørs and Michael Zölffel for confirming and/or correcting identifications, or for commenting on the key and the text, and E.G. Bellinger, Hilary Kelle and Richard Foot (Wessex Water) for advice on water treatment. The assistance of Claire Wyatt and Kevin Williams is also deeply appreciated. The University of Bristol Publication Fund provided financial assistance. Finally, I would like to thank Stuart Hedley, without whose interest and skills this Guide would not have appeared.

D.J.P.

Introduction

Protozoa may be found in almost every aquatic habitat, from cesspit to mountain stream, from garden birdbath to the Amazon. Natural communities typically contain dozens of species, and this diversity is retained when collections are made in large jars and returned to the laboratory. The richness is expressed as a spectacular array of body forms, reflecting the wide range of niches occupied. The number of species, the number of individuals within each species, and the types of species can all provide valuable insights into the nature of the habitat from which a sample was taken. For these reasons, protozoa can be a convenient source of material to illustrate biological principles.

In recent years it has become clear that, despite their small size, the contribution of protozoa to the metabolism of aquatic and terrestrial ecosystems can be very substantial. Protozoa occupy a significant, sometimes dominant, position among the consumers within a community. Their importance is closely linked with their use of bacteria as a source of food. Progress in understanding the role of particular protozoa and the nature of the transactions that occur among members of the microbial communities has been held back because there is little familiarity with the organisms.

Protozoan communities are very dynamic structures, with numbers of cells changing rapidly by cell division, encystment or excystment. The structure of the protozoan community quickly responds to changing physical and chemical characteristics of the environment, suggesting a potential use of the diversity of protozoa and the occurrence of particular species as indicators of changes in ecosystems. However, such suggestions should be followed with caution as there are major difficulties involved in finding the right way to sample the habitats occupied by protozoa, and in accurately identifying species.

In order to understand or to use the protozoan community (particularly as an indicator of change) in teaching or research, it is necessary to be able to identify individual protozoa. Developing familiarity with the diversity of any group of organisms can be a daunting task. In the case of protozoa, there are few English-language books offering an authoritative understanding of the group. Many books claiming to be guides to the protozoa (see Bibliography, for a list of such books) often require knowledge of special preservation and/or staining techniques, or they rely on codified drawings of organisms. The latter may be suitable for specialists, who understand the way in which the information has been coded, but such drawings add to the hurdles faced by beginners. This Guide relies heavily on photographs because they show protozoa as they would appear to an observer looking down a microscope, and make the learning and recognition processes a little more exact.

This Guide deals only with protozoa from freshwater sites, and the organisms illustrated have been obtained from ditches, small ponds, puddles, lakes, aquaria and water-treatment plants. The common and accessible organisms are emphasized in preference to rarer organisms or organisms less likely to be collected (e.g. from open lakes or from anoxic habitats). As the book is not comprehensive, readers will find that some organisms are not included here. For these, and for identification beyond genus, the user should make use of the specialist literature cited in the Key and the Bibliography.

The terms 'free-living', 'protozoa' and 'freshwater' have been rather freely interpreted: included are a few organisms that may be found in soils, mosses and low-salinity brackish water habitats. There is considerable species overlap between communities from these habitats and from more usual freshwater sites. Indeed, some species can be found in both marine and freshwater sites. Soil protozoa are of great importance in cycling nutrients, and should not be ignored. Also included are a couple of protozoa that are found living attached to other organisms. Ectosymbionts rarely harm their hosts, and are best regarded as free-living species exploiting their hosts for attachment. Their location usually secures a better supply of food.

What are protozoa?

Protozoa cannot be easily defined because they are diverse and are often only distantly related to each other. They are unicellular eukaryotes. Together with the unicellular algae and the slime moulds, they make up the protists. Protozoa have usually been distinguished from algae because they obtain energy and nutrients by heterotrophy, that is, by taking in complex organic molecules, either in soluble form (osmotrophy) or as particles such as bacteria, detritus or other protists (phagotrophy). Protozoa ('first ani-

mals') get their name because they employ the same type of feeding strategy as animals. Heterotrophy contrasts with photosynthesis, the use of radiant energy (sunlight), as a source of energy for metabolism (as in algae and plants). In unicellular organisms, these two nutritional strategies are not mutually exclusive (as they are in multicellular plants and animals). Indeed, quite a large number of flagellates are mixotrophic and can use both types of nutrition; many heterotrophic protozoa harbour photosynthetically active endosymbionts.

Protozoa include amoeboid, flagellated and ciliated organisms that are capable of heterotrophic nutrition, whether or not they also contain chloroplasts (see p. 19, for the composition of protozoa). Some heterotrophic protists evolved before the ability to photosynthesize was acquired, but others evolved from algal protists by loss of their chloroplasts. Not only has the protozoan state been achieved independently in different lines of evolution, but these organisms cover an immense area of evolutionary territory; measured in molecular terms, two protozoa may have less in common than do a plant and an animal. Furthermore, not all protozoa are equally equipped to deal with the demands of living. Having appeared over the period during which the eukaryotic cell was being assembled, some have relatively few organelles at their disposal, whereas others have been very inventive in the development and application of organelles. One should therefore be very wary of making generalizations about this diverse group.

Distinguishing protozoa from other microbes

The microscopic community includes bacteria and blue-green algae (both are prokaryotic, having no nuclei or other discrete cellular organelles), algae (both motile and non-motile, unicellular and multicellular), slime moulds, and some small (multicellular) metazoa (especially rotifers, gastrotrichs, copepods, flatworms and nematodes). Typical representatives have been illustrated (Figs 4–19) to aid in distinguishing protozoa from non-protozoa.

In principle, distinguishing protozoa from metazoa is simple: protozoa are single-celled; metazoa are comprised of many cells. In practice, it is extremely difficult to see the boundaries of cells within microscopic metazoa, and other features may have to be relied upon for positive identification: the exoskeleton and jointed appendages of small arthropods (Fig. 231) allow them to be easily recognized; rotifers have a distinctive mastax (Fig. 15) behind the mouth, anterior cilia and a forked 'tail'; and the round, smooth surface of most nematodes (Fig. 18), together with their stiffness and their serpentine motion, makes them easy to recognize. Only a few gastrotrichs (Fig. 16) and tardigrades (Fig. 19) are likely to be found, and they are quickly learned.

Equally simple on paper is the distinction between protozoa and prokaryotes. The latter have no internal organelles, and are usually very much smaller than protozoa, with one dimension restricted to about 1 μm. However, some protozoa are very tiny, and some bacteria are rather large and may have various inclusions, so identification is not always straightforward. Most bacteria have a simple shape (spherical, sausage-shaped, helical), and most, but not all, are rigid. Many are capable of swimming or gliding, but none have the lashing cilia or flagella of eukaryotic single cells, nor are they able to form pseudopodia.

Protozoa may be amoeboid, flagellated or ciliated. There is no clear boundary between flagellated protozoa and flagellated algae. If definitions of algae and protozoa based on their respective nutritional strategies are accepted, then some organisms are both algae and protozoa. Some protists that rely exclusively on heterotrophy (i.e. are protozoa) may be closely related to some species that rely exclusively on autotrophy (i.e. are algae). Exceptionally, autotrophic and heterotrophic species may be so closely related that they are placed in the same genus. As no clear boundary can be drawn here, this Guide includes some algal cells that may be a source of confusion, or that are closely related to protozoa.

Just as there is an unclear boundary between algae and colourless flagellates, so there are problems in distinguishing between slime moulds and amoebae. Slime moulds are amoeboid organisms with two stages in their life cycle that are not encountered in conventional amoebae. They can form large amoeboid masses, and they may produce a mass of spores (cysts) that is lifted away from the substrate on a stalk, allowing the spores to be released into air or water currents to aid in the dispersal of the organism. The large amoeboid stages (plasmodia) are rarely observed, except when special growth media are employed, and have not been included here. Slime moulds may have unicellular (amoeboid or flagellated (Fig. 20)) stages in their life cycle, which may be misidentified as protozoa. Some hyphal fungi also produce flagellated stages (zoospores) which may be mistaken for protozoa.

Equipment

For effective protozoological work, the following equipment is essential: a compound microscope, 2.6 × 7.6 cm glass slides with coverslips, glass dropping (Pasteur) pipettes and teats, small glass Petri dishes, collecting jars, soft tissues and filter paper (blotting paper). Ideally, the following equipment should also be on hand: a dissecting (low-power or binocular) microscope, an alcohol or bunsen burner, lens tissue, a measuring eyepiece and micrometer slide, a filter apparatus (filter funnel/coffee filter), plankton net (less than 20μm mesh), a can of compressed air with a nozzle, a collection of bottles and jars, solid watch glasses, barley, wheat and/or rice grains, agar powder, autoclave (pressure cooker), photographic facilities and a centrifuge.

Microscopes

The choice of microscope is important in the study of protozoa, in that a good choice will allow you to see more, and to see it with ease. Microscopes with built-in illuminators and binocular eyepieces are more convenient than those with separate light sources, and protozoologists also benefit greatly from phase contrast optics. The condenser should be equipped with an iris. An option for photography, such as a trinocular head with a vertical tube to which a camera can be attached, is desirable.

The components requiring the most critical consideration are objectives: the best affordable should be used. Normally several objectives will be needed, and the magnifications should range between x10 and x100.

Magnifying power is less important than resolving power, that is, it is far better to see details clearly than to have them appear large but blurred. If finances are restricted, it is preferable to buy a smaller range (minimum of two, about x10 and x40) of good-quality objectives than several of poor-quality. Higher-power objectives are usually of the oil-immersion type. Ideally, if the microscope is capable of phase contrast microscopy, phase objectives should be bought in preference to those of the bright-field (normal) variety. The eyepieces should have a magnification of between x8 and x12.

A dissecting microscope is a lower-power microscope with greater depth of field than a compound microscope, a longer working distance (between the lenses and the specimen), and usually with stereoscopic vision. It is ideally suited to hunting around a sample before material or organisms are selected for observation or culture. Lighting on such microscopes may either be from above (top lighting) or transmitted through the specimen. The latter is desirable when examining protozoa, with the light source removed as far as possible from the organisms to increase contrast and to reduce the risk of heating the specimen. Good dissecting microscopes have built-in illuminators.

Compact field microscopes are also available.

Basic care of microscopes

Microscopes are expensive and delicate, and the glass surfaces are most vulnerable to dirt and damage. Both cause reduction in image quality. For example, mascara-laden eyelashes can damage the surfaces of eyepieces! However, a properly maintained microscope can last for decades.

As far as possible, keep dust off microscopes by protecting them with a plastic bag or cover. Do not leave any open tubes uncovered (e.g. the eyepiece tubes), as dust will get inside the microscope. Avoid sudden changes in temperature, since this can lead to condensation inside the microscope. Do not place a microscope where it can be splashed with water or other chemicals. Salt water should be removed quickly if it gets onto the microscope. As a general rule, microscopes should be kept in a dry atmosphere and at an even temperature.

If possible, avoid touching glass surfaces with any material. Most dirt will be in the form of dust, and it is best cleaned off using compressed air from canisters (such as can be bought at photographic agencies). If surfaces have to be cleaned by wiping them (e.g. to remove immersion oil), avoid using any materials that may contain grit, such as cheap paper tissues. Special lens tissues are available (usually from photographers and opticians) for cleaning optical glass surfaces, but clean, soft cotton is also very good.

Lubrication of moving parts (stage movement, objective turret, focusing mechanism, iris diaphragms) is best left to experts.

Basic microscopy

Familiarize yourself with the principle components of the microscope. These include the light source, condenser, stage, objectives, and eyepieces.

- **The light source:** almost all modern microscopes have built-in illuminators, typically equipped with a diffuser to give even illumination, and a regulator to control the level of illumination. There is no 'perfect' level of illumination: light intensity should be adjusted for personal convenience. More intense lights tend to heat the specimen being observed, and will lead to physiological distress and morphological distortion of cells. Minimal illumination, best achieved by working in a dimly lit or darkened room, is desirable.

Some microscopes have meters that indicate the relative intensity of illumination. In some, the upper extreme is marked (usually in red), and if the intensity remains at this level for a long time, it will shorten the life span of the bulb. Some meters also have a marked zone in the middle of the range, within which the best colour balance in photographs will be achieved.

- **The condenser** is a lens system that focuses light onto the specimen. The condenser can be moved up and down relative to the stage, and on some microscopes it may also be possible to move it to the left and the right, and backwards and forwards. It may contain an iris, and may have removable or optional components for different contrast enhancement techniques.
- **The stage** usually has a clip that is pressed against the end of a slide to hold it in place. Additional clips that press onto the top of a slide are entirely unnecessary.
- **The objectives** are usually located on a rotating turret, and will click into place. If all the objectives have been bought from the same manufacturer, they should all focus at the same level, eliminating the need to change the focus as you switch from one objective to another, and helping to prevent accidental damage to specimens, or to the objectives themselves.

The highest-power objectives are usually of the oil-immersion type. A drop of special immersion oil, which should be obtained from the microscope manufacturer, is placed on the coverslip above the specimen, and the objective is then rotated into place so that it touches the oil.

To set a microscope up for basic bright-field (no contrast enhancement) microscopy, the steps are as follows:

- **First select a low-power objective (x10 or lower). Place the slide (with coverslip) on the stage, switch on the lamp, check that all irises (lamp housing and condenser) are wide open, and focus on the specimen or on the edge of the slide or the coverslip.**

- **If there is an iris in the lamp housing, close it down; if not, place an object with a distinct edge (e.g. a piece of paper) on the glass surface of the lamp housing that is nearest the specimen. Looking down the microscope, adjust the condenser until the edge of the iris or of the piece of paper is in focus. The condenser is now focused to project light onto the specimen.**

- **If there is a lamp iris, make sure that its image is in the middle of the field of view. If this is not the case, then the condenser is projecting the light to one side of the objective rather than along its optical axis. Check that the objective is screwed in tightly and that it is clicked into its proper position. If the light is still directed to one side, the condenser may be incorrectly fitted, or you may have to adjust its side-to-side or to-and-fro position in order to align it along the optical axis of the objective. There are usually two knobs or screws for this purpose. Having centred the condenser, open the lamp iris.**

The above steps need only be repeated at the outset of each session. The following steps should be carried out every time the objective is changed:

- **Remove one eyepiece, and, looking straight down the tube, close the condenser iris if there is one. Open the iris until any change in its position neither enlarges the area being illuminated nor increases the amount of illumination. Having closed the iris slightly, replace the eyepiece. The microscope is now ready for use.**

The illumination achieved by setting up the microscope in this way is called bright-field microscopy. Specimens with colour and great inherent contrast can be seen clearly, but most protozoa will be almost impossible to see. Consequently, some form of contrast enhancement will be required.

Contrast enhancement

Contrary to the recommendations of many books on microscopy, resolving power is less important to protozoologists than is visibility. The lack of optical contrast in many protozoa means that very little can be seen using normal bright-field microscopy. The photographs of *Paramecium bursaria* (Figs 349–358) illustrate some of the techniques that may be employed to enhance contrast. Special accessories are required for most of these.

The simplest way of enhancing contrast is to close the iris in the condenser, or to lower the condenser so that it is below its optimal position (compare Figs 349 & 350). Dark-ground effects (Fig. 353) can also be achieved by adjusting the lighting so that light is directed through the object, but passes to one side of the objective. Both techniques make specimens visible, but they can only reveal a limited amount of detail in an object.

Phase contrast facilities are widely available. Special objectives and a condenser are required. If starting from scratch, it is probably more economical to buy phase facilities at the outset. Phase contrast is a relatively cost-effective way of getting good, high-contrast images. Nomarski (differential interference) contrast also requires special accessories; these are relatively expensive, but the resulting images (Fig. 352) have great clarity as well as good contrast.

Microscopical examination

Normally, preparations are made on glass microscope slides; a coverslip should always be used, as it protects the objectives from contamination and improves image quality.

2.6 x 7.6 cm reusable glass slides are widely employed. They should be cleaned and polished with tissue before use. Coverslips are available in various sizes and thicknesses, ranging from No.0 to No.2, which are thin and thick respectively. The author recommends 22 x 22 mm (square) or 32 x 22 mm No.1 coverslips. Like slides, coverslips should be cleaned before use, as small glass fragments, or greasy films reduce image quality. Coverslips are cleaned by carefully drawing them between folds of tissue held between the thumb and forefinger.

Heat and oxygen-depletion can cause cells to become moribund. The lamp of a microscope tends to warm specimens, and cells may only remain healthy for a few minutes. Bringing samples from bodies of water into a laboratory can involve a 10–20°C temperature change, which is enough to cause extensive physiological distress. Samples taken from organically enriched sites (e.g. sewage treatment plants) and placed under a coverslip will rapidly use up the available oxygen, and the community structure will begin to change within a few minutes. Thus, rapid processing is usually desirable if you wish to observe healthy cells behaving normally.

It is usually more convenient to add only a small drop of the sample to the slide. If it is possible to move the coverslip around freely, there is too much fluid, and protozoa will move not only in the lateral plane but also in a vertical plane, making careful observation almost impossible.

The movements of protozoa often cause problems. Usually, active motility is a sign of distress. Typical causes might be pressure from a coverslip, overheating, or depletion of oxygen. The cells move until they find a more favourable site. The use of minimal illumination or gently blowing on a preparation as you observe it often 'calms' protozoa. Filters that remove heat can be obtained from microscope suppliers. If these devices do not work, various narcotizing agents or viscous slowing agents can be used. Narcotizing agents include the solutions of heavy metals, such as nickel or copper chloride (used at a concentration of 5–10 mMol/l), while methyl cellulose can be used to increase viscosity. Iodine or formalin will kill protozoa. All of these treatments may cause distortion of one sort or another, and, as one of the great pleasures of watching protozoa is to see them behaving naturally, all can be regarded as unsatisfactory

An alternative means of immobilizing active organisms is to use a small piece of tissue paper to draw excess fluid from under the coverslip. The coverslip is pulled down towards the slide, and protozoa can then be trapped. Such samples can be observed for about five minutes before cells become distorted. A small pipette and some fluid should be kept handy, as it may be necessary to add a small drop of fluid to the edge of the coverslip to release the cell from terminal compression.

A few protozoa may go unnoticed because of their inactivity. Amoebae, in particular, require a few minutes to recover from the trauma of being placed on a slide. Other organisms may be located in detritus and will not become visible until they have been given sufficient time to disperse from it.

Complete beginners are advised to work with material that is known to contain many protozoa, for

example, natural samples that have been checked using a binocular microscope, cultures from biological suppliers, sludge samples from treated sewage, coverslips left for three days on mud collected when there was an orange or green patch on the surface, or water with a soup-like consistency. With samples maintained in bottles in the laboratory, the fluid in the middle section of the bottle will have relatively few organisms; most protozoa will be found near the sediment or associated with the surface film. This can be sampled by placing the flat side of a coverslip against it.

Using one of the methods for enhanced contrast, and making sure that the microscope is focused on the sample (check the edge of the coverslip) at a low magnification (about x 10), scan the slide methodically to find protozoa.

In order to examine rare or specific types of protozoa, it may be necessary to soften the glass of a Pasteur pipette in a burner, remove it from the flame, and, with a smooth movement, draw it out to the thickness of a hair. This pipette can then be broken to give an aperture with a diameter 2–5 times greater than that of the cell, and can be used to pick up individual cells with the minimum amount of fluid while using a dissecting microscope. Protozoa collected individually or in small numbers have the peculiar ability to disappear after being added to a slide: they may be killed as they are pulled into the pipette, adhere to the inner surface of the pipette, be smashed as the coverslip is added, or move to the thin film of fluid around the outside of the coverslip. Care and regular examination of the cells throughout the procedure are advisable if it is important that a particular species is observed.

Large protozoa may be crushed by coverslips, and should be protected by creating a chamber on the slide. This is achieved by placing two shards of broken coverslips on either side of a drop of fluid and then laying another coverslip across them.

Recording protozoa

It is strongly recommended that protozoa should not merely be observed, but also recorded. The most simple and often the best way of recording protozoa in a sample is to make line drawings. This directs the eye to important features. The copying of drawings from books should be avoided, as many are inaccurate and often contain information in a coded form.

A picture of the organism should be built up, beginning with outline sketches and a measure of size (see below), and including a number of typical profiles. The location of the nucleus, mouth and contractile vacuoles, together with the density, length, width and location of flagella or cilia, should then be added. Separate drawings of details of, for example, the behaviour of the contractile vacuole, the contents of food vacuoles, the patterns of locomotion, the structure of the mouth, and the presence of extrusomes, should also be prepared. Written notes are often very useful.

Drawings need to be accompanied by an estimate of size, which may be made in two ways. The first is to measure the diameter of the field of view (the area that can be seen when looking down the eyepiece) before making observations, by looking at a micrometer slide with a scale (usually 1 mm) etched onto its surface. Measurements of the field of view have to be made for each objective. The size of an organism may then be estimated as a proportion of the field of view. Alternatively, a measuring eyepiece may be used. This contains a scale which is in focus when observing the specimen. Micrometer graticules are inserts which convert normal eyepieces into measuring eyepieces, and they can be bought for most types of eyepieces. Measuring eyepieces have to be calibrated against a micrometer slide. This has to be done for each objective. Organisms are measured as a number of eyepiece units, and this is converted into microns. Multiplying the magnification of the objective and of the eyepiece does not give the magnification of the object being observed.

Although drawings are best made in a firm plain paper notebook, one option is to use large punchcards. The holes may be cut out according to a predetermined code (e.g. to indicate the presence of cilia, flagella or pseudopodia, or to indicate colour, habitats, etc.), and, by using a knitting needle, all previously made drawings with a particular feature can be selected and compared.

Uninterpreted records

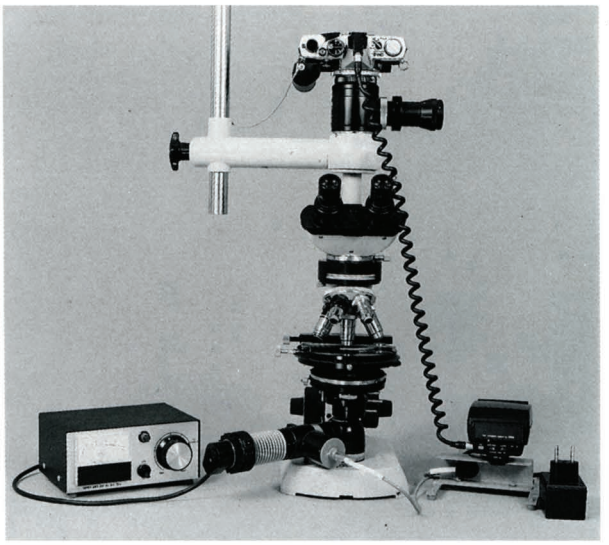
The extent to which errors of interpretation of the protozoan form may occur is quite remarkable. Thus, uninterpreted records are highly desirable and ought to be included in professional surveys. Such records can be made by photography, ciné or video. However, since ciné has been rendered obsolete by advancing video technology, it will not be discussed any further. If photography or video are to be used, it is best to have a microscope equipped with separate ports to which cameras may be attached: the usual arrangement is to have a microscope with a trinocular head. Usually, the camera is attached to the vertical tube (Fig. A).

Photomicrography of protozoa requires a camera from which the lens can be removed, an adaptor that will allow attachment to a microscope, and a projection eyepiece. Only if the camera is attached to a separate port will it be possible to make unhindered observations of protozoa while photographs are being taken.

Cameras with a heavy shutter movement (focal plane shutters) will cause vibration in the microscope and movement of the fluid on the slide. Such movement is greatly exaggerated on the film plane because of the magnification factor, and the object will appear blurred or out of focus. It may be necessary to support such cameras on a stand, rather than attach them directly to the microscope. Cameras with diaphragm shutters are to be preferred.

Whereas photography of fixed and stained preparations is straightforward, and any camera with automatic exposure control can be used, fast moving living organisms can only be photographed satisfactorily with an electronic flashgun. There are many cameras on the market that can adjust exposure of subjects illuminated by electronic flashes, but as yet none is available specifically for microscopes. For use in photomicrography, domestic cameras need to be modified: the flash tube must be placed in the light path either by dismantling it from the electronic flash gun and fixing it in an appropriate location in the light path, or by redirecting the flash into the light path of the microscope via a mirror system (Fig. A, Patterson, 1982). Variations and improvements of such systems may be found in the pages of *Microscopy* (the journal of the Quekett Microscopical Club) or in *Mikrokosmos* (in German), both of which cater for the amateur microscopist.

Without an automatic flash exposure system, the exposure will differ with each magnification and with each type of contrast enhancement system.



A

Obtaining the correct exposure involves a series of trials with neutral density (grey) filters, such that the light is attenuated to the appropriate intensity.

Photomicrographers should have basic photographic skills and access to a darkroom. Microscopically viewed objects have much poorer contrast than conventional subjects, and specialist films and developing or printing techniques may be needed. Photography of living protozoa has a high film-wastage rate, as the organisms move at inappropriate moments or with unkind rapidity. The best black and white films, for example, *Kodak Technical Pan*, are those that have very high contrast, adjustable sensitivity, and an insignificant grain. For colour work, the author has used conventional colour-reversal (slide) film. Special photomicrographic films are available, but they are difficult to obtain, expensive, and offer marginal improvement over normal films. If an electronic flash is to be used, daylight colour films are appropriate; without a flash, a blue filter or an artificial light film will be needed.

Photographs are often marred by dust within the optical system. The most common sites from which images of dust are projected onto the picture are the glass surfaces between the objective and camera, and the front face of the lamp housing of the microscope. Dust should be removed with compressed air (see p.11). Slight unfocusing of the condenser usually eliminates any images of dust inside the lamp housing.

Video microscopy

Video microscopy is an ideal medium for teaching microscopy and protozoology, as many smaller organisms can be identified from their movements and the images recorded on video tape. VHS tapes give poor-quality results if edited, but S-VHS, BETACAM and U-MATIC formats are suitable if editing is required. The sound-track can be useful for recording verbal comments.

It is now as cheap to buy a colour camera as a black and white one, and colour is recommended. Camcorders can be set to focus on infinity and simply directed down the eyepiece, but the result is far from satisfactory. It is preferable to use surveillance-style cameras without a lens, which are attached to the microscope using a special adaptor. Adaptors may be bought from microscope suppliers.

Where to find and how to collect protozoa

Protozoa occur in trophic or encysted states in virtually any kind of natural habitat which is temporarily or permanently wet. The numbers of active, trophic individuals will be determined by the amount of food available and by the prevalence of predators. Many protozoa directly or indirectly rely on decaying organic (vegetable) debris or on unicellular algae for food. The richest sources of protozoa in natural habitats are sites of high productivity, such as shallow ponds, or the borders of larger standing bodies of water where leaves and other plant matter accumulate and where the sun penetrates in sufficient strength to support algal growth. Any small body of water that has developed a strong colour or a green mat of material (either across the bottom or within the water) will probably prove to have an algal bloom. Protozoa are often present as consumers of these algae.

Very small ponds and puddles are rarely particularly good sources because there may be a paucity of nutrients, or because continual drying and rewetting creates very demanding conditions in which only a few species can survive. Similarly, flowing waters do not usually contain many protozoa. However, protozoa may be found in and around associated plant material.

Protozoan communities are very changeable and when collecting, transporting and maintaining samples, care must be taken to prevent severe changes in the communities. If samples are kept in a cool place in the laboratory, a succession of different organisms will appear over a period of several weeks. Collections should be made in relatively large glass jars and samples should contain some detritus, but soil and mud are best avoided. Sufficient organic matter to form a loose layer at the bottom of the jar should be included.

Most pond/ditch/lake samples will be collected from clean, organically enriched, or anoxic sites; each type of sample has to be handled differently. If the water from the collection site is clean and aerated, a large amount of air should be enclosed with the sample to prevent it becoming anoxic. Samples from anoxic sites (they usually look black or smell strongly) should not be mixed with clean-water samples, as the sulphides that are present will kill the protozoa from aerated sites, and the oxygen will kill protozoa living in anoxic sites. For this reason, one should also avoid mixing air with the sample. Protozoa from organically enriched sites (e.g. polluted sites and sewage works) usually need some oxygen, but as this can be very rapidly depleted by bacterial degradation of organic matter, samples should be stored as a thin layer of fluid with plenty of air.

Open bodies of water are worth sampling, especially if they have a distinctive greenish, olive or brown colour, as this may suggest a bloom of algal growth. However, samples from open waters have to be concentrated; this is usually done in the laboratory (see below), but it can be done on site. The most simple way is to pour the sample through a coffee filter, shake it (before all the fluid has gone) to resuspend collected material, and tip the concentrated sample into a container. Many species die soon after being concentrated. Samples may also be taken with a plankton net, ideally with a mesh size of less than 20 µm if the net is to retain protozoa. Such nets are expensive. A slower but convenient method is to reverse filter a sample. A plastic or glass cylinder with a membrane (e.g. 0.45 µm Nuclepore) filter or a 20 µm (or finer) mesh filter (obtainable from Staniar, see p.220) over one end is placed into a sample. Water passes into the cylinder, but the protozoa are held back by the mesh. The water can be removed from inside the cylinder with a pipette.

Alternatively, sponges or slides inserted into expanded polystyrene foam may be suspended in a water column for a week or so, and communities will develop on these.

If a sample is left to stand undisturbed for several

hours, various species may move towards the light, or accumulate at the top, bottom or sides of the container. Such aggregations are sometimes visible with the naked eye, but flat glass containers (such as Petri dishes) and a dissecting microscope do make it much easier to find them. Generally, samples taken from the bottom of a jar will contain most protozoa.

Muds, sands, peat slurries and other sediments are often rich in protozoa. Samples of the top few millimetres (i.e. without the deeper anoxic material) may be taken with a spoon (wok spoons are ideal). In the laboratory the sediment may be used to inoculate a fluid culture (see below). Most protozoa in sediments are motile and will move upwards; they may be collected by placing the sediment in a dish, removing the excess fluid after a few hours, placing a layer of lens tissue over the mud, and adding some coverslips. Two or three days later, a community of organisms will have developed on the undersurface of the coverslips. Older preparations usually become anoxic.

Soils contain many protozoa, and may be used to inoculate fluid cultures (see below). Virtually every species found in soils has the capacity to encyst, and good results are obtained if the soil is first allowed to dry out completely. Dry crumbs of soil are then used to inoculate fluid or agar cultures. Alternatively, the soil may be dampened so that fluid and associated protozoa may be squeezed out by finger pressure.

Mosses, such as *Sphagnum*, also contain many protozoa, especially testate amoebae and various species with symbiotic algae. Handfuls of the filaments that extend to the ground should be collected, placed in a plastic bag and returned to the laboratory. Protozoa will be found in the water that is obtained by squeezing the sample several times.

Other sites that are rich in protozoa include slimes on solid surfaces over which water is running, areas under ice, and the gutters of houses. Protozoa also occur in abundance in sewage works, where they play an important role in clarifying the water (they remove suspended bacteria). The protozoa are found in the sludge of activated sludge plants, in the organic layer in trickle filters, or on biodiscs.

The relative abundance of protozoa varies according to the time of year. The greatest diversity occurs in late winter and very early spring, when there are relatively few metazoa. Freshwater protozoa with symbiotic algae appear to be relatively abundant early in the year in temperate climates.

Samples that contain large numbers of animals (small crustacea, worms, midge larvae, etc.) will not provide as rich a variety of protozoa as those without metazoa. If metazoa are present, the protozoa will soon disappear after the material has been returned to the laboratory, and it is advisable to pass the sample through a sieve or other crude filter to remove larger organisms if a long-lived sample is required.

Keeping samples

Once collected, protozoan samples should be protected from temperature changes and kept out of direct sunlight. If it is important to report accurately the most common species that are present, a list must be made within 12 hours of collection.

If stored out of direct sunlight (e.g. near a north-facing window in the northern hemisphere), and if protected from cold and heat, samples will usually provide a changing community of protozoa for several weeks. If placed in large, flat containers, the samples can be monitored with a dissecting microscope to establish the diversity and abundance of organisms.

It should be noted that an excess of organic matter can cause cultures to 'go off': the organic matter creates a biological demand for oxygen that cannot be met by diffusion from the surface. The cultures first become milky with bacterial growth, and anoxic (even reduced) conditions may follow. This can be avoided by keeping the amount of organic matter to a minimum and by ensuring that the sample has a large surface area.

Cultures

In order to maintain long-term cultures, it is necessary to provide a medium that suits each species, and a supply of appropriate food. Glassware that has been cleaned in hot water and rinsed of all detergent provides the best culture vessels.

Cultures can be selective or non-selective. The latter are usually samples of water enriched with some food material. Since most protozoa eat bacteria or bacterial detritus, the simplest way to enrich a culture is to add several boiled barley, wheat or rice grains. However, as cultures enriched in this way tend to distort the community structure, this approach cannot be used to provide a list of all the protozoan species initially present in a sample.

Non-selective cultures often produce similar species (equivalent to garden weeds). Genera that commonly emerge in organically enriched cultures include the flagellates *Chilomonas*, *Bodo* and *Para-*

physomonas; and the ciliates *Paramecium*, *Cinetochilum*, *Cyclidium*, *Halteria*, and *Colpidium* (pp.186–187).

More selective cultures are obtained either by offering food that will suit particular protozoa only, or by collecting one or more individuals of one species and inoculating them into a suitable medium with food. The best medium is filtered fluid from the sampling site, but most freshwater and soil species will also grow in commercially available, non-carbonated spring waters with a low mineral content.

For the most selective cultures, it is necessary to catch individual protozoa with a fine pipette. This can be frustrating at first. It helps to hold the pipette so that it does not shake: it can be braced across several fingers, or held with two hands. The pipette is kept relatively still under the microscope, and the sample is gently moved around to bring the organisms to the pipette, rather than the other way around. Alternatively, a small drop of fluid can be drawn up via a teat or a tube to the mouth. For very small organisms, it may be necessary to carry out this process under a compound microscope, using a mechanically driven (hypodermic) syringe to draw up small quantities of fluid.

If absolute purity is required of a sample, around 20 selected cells are placed in several millilitres of medium. The cells are collected a second time and the process repeated. This 'washes' the protozoa, and also removes any contaminating species. The target species can then be inoculated into fresh medium with a source of food. The most rewarding growth is usually achieved if the initial cultures are of small volume (no more than several millilitres), and it is advisable to build up the size of the cultures gradually.

The technique of inoculating cultures with small numbers of selected cells often fails because the right kind of food is not available, or because the composition of the medium is not ideally suited to the target organism. Some species grow best in mixed cultures, and this is especially true of larger genera such as *Amoeba* or *Stentor*.

Some protozoa do not grow well, or conveniently, in a fluid medium, being more suited to thin films of water. They can be grown on solidified 1.5–2 % agar: 1.5 g of agar are added to 100 mls of medium, placed in a boiling water bath until molten, poured into shallow (usually Petri) dishes, and left to gel. Agar is suitable for many amoebae and soil protozoa, and some flagellates. The dishes should be covered to prevent evaporation while the protozoa grow. If used with soils, this approach is particularly successful in cultivating small amoebae. Fluid cultures of soils tend to produce the ciliate *Colpoda*.

Most protozoa are selective feeders and cultures must seek to provide appropriate food. The principal categories of food comprise bacteria in suspension, bacteria adhering to surfaces, other protozoa, algae, and detritus. The simplest organisms to culture are often those that eat bacteria, a supply of which can be guaranteed by adding boiled barley, wheat, or rice grains to support bacterial growth.

Other media and methods of culture are to be found in, for example, Finlay *et al.* (1988), Kirby (1941) and Lee *et al.* (1985).

Classification of protozoa

Classification schemes for organisms fulfil two functions:

- A filing system from which data may be conveniently retrieved.
- A means for expressing ideas about evolution.

However, because ideas about patterns of evolution are always changing, classification schemes are inevitably unstable. This is especially true at the moment for protozoa. Consequently, given below is a short list of the major types of protozoa (along with a few distinguishing features), which is intended to be a simple filing system; evolutionary relationships are not implied. It should also be noted that some groups appear more than once, and that included in each group are those species that are illustrated or mentioned in this book.

1 CILIATES: protists with cilia in lines (kineties) at some stage in the life cycle. Two kinds of nuclei.

Chonotrichs: ectosymbiotic ciliates with a spiral fold of cytoplasm around the unattached end. *Spirochona.*

Colpodids: mostly filter-feeding ciliates, using tightly packed feeding cilia clustered around the mouth. No undulating membrane. Somatic cilia arranged in pairs. *Bursaria, Colpoda, Cyrtolophosis.*

Cyrtophores: motile ciliates with a cluster of strongly developed microtubular rods or nematodesmata, normally used for manipulating algae or large lumps of debris into the mouth. Either flattened (hypostomes) or rounded. *Chilodonella, Chlamydodon, Drepanomonas, Nassula, Phascolodon, Pseudomicrothorax, Trithigmostoma, Trochilia.*

Haptorids: predatory ciliates with an armoury of killing and/or holding extrusomes around the mouth region. The mouth may be apical or arranged along one flattened margin of the cell. *Amphileptus, Chaenea, Didinium, Dileptus, Homalozoon, Lacrymaria, Litonotus, Loxophyllum, Monodinium, Phialina, Spathidium, Trachelius, Trachelophyllum.*

Karyorelicts: ciliates with non-dividing macronuclei. *Loxodes.*

Oligohymenophora: ciliates with a specialized buccal ciliature comprising only three membranelles (blocks of cilia) and an undulating membrane. These organelles can nevertheless be difficult to see. Mostly filter feeders, eating bacteria. Common.

(i) Hymenostomes: oligohymenophora with short membranelles and an undulating membrane. Mouth usually small and difficult to see. Common, especially in organically enriched sites. *Cinetochilum, Colpidium, Glaucoma, Tetrahymena.*

(ii) Scuticociliates: oligohymenophora in which the undulating membrane is typically a long and well-developed, veil-like structure to the right of the mouth. *Calyptotricha, Cohnilembus, Cyclidium, Lembadion, Pleuronema.*

(iii) Peritrichs: oligohymenophora with buccal ciliature forming one or more wreaths around the broad anterior part of the cell. Usually bell-shaped. Mostly sessile. *Astylozoon, Carchesium, Cothurnia, Epistylis, Hastatella, Orbopercularia, Opercularia, Ophrydium, Platycola, Rhabdostyla, Vaginicola, Vorticella.*

(iv) Peniculines: oligohymenophora in which the membranelles are drawn out as relatively elongate structures. Undulating membrane weakly developed. Usually with trichocysts and star-shaped contractile vacuole complexes. *Frontonia, Paramecium, Neobursaridium, Urocentrum.*

Polyhymenophora (spirotrichs): ciliates that feed using a band of membranelles stretching from the anterior pole of the cell to the cytostome. The band is called the adoral zone of membranelles (AZM).

(i) Hypotrichs: polyhymenophora that walk on the substrate using cirri (blocks of cilia). Usually dorsoventrally flattened. *Amphisiella, Aspidisca, Chaetospira, Euplotes, Holosticha, Oxytricha, Paruroleptus, Pattersoniella, Stichotricha, Stylonychia, Tachysoma, Uroleptus, Urostyla.*

(ii) Heterotrichs: polyhymenophora that move with somatic cilia arranged in kineties. *Blepharisma, Brachonella, Caenomorpha, Climacostomum, Condylostoma, Metopus, Spirostomum, Stentor.*

(iii) Oligotrichs: polyhymenophora in which the somatic cilia are absent or reduced to a circumferential band of spines. AZM is apical and well developed. Mostly open-water organisms. *Halteria, Strombidium, Strobilidium, Tintinnidium.*

(iv) Epalcids: polyhymenophora with a flattened sculpted body. Somatic cilia reduced or absent. AZM usually near the middle of the body and reduced. Mostly from anoxic sites. *Discomorphella, Epalxella.*

Prostomes: ciliates with an apical mouth (normally quite distensible) used mostly for ingestion of de-

bris, detritus, damaged cells or tissue. Mostly associated with detritus. *Coleps, Mesodinium, Urotricha, Prorodon.*

Suctoria: ciliates without cilia during the trophic stage. This stage is usually sessile and immotile, and feeding is by means of one or many radiating arms (= mouths), each of which is equipped with holding extrusomes at its tip. *Acineta, Dendrocometes, Podophrya, Tokophrya, Trichophrya.*

2 FLAGELLATES: protists with 1–8 flagella, usually located apically or subapically. With chloroplasts or without.

Bicosoecids: sessile cells of a single genus. Two flagella insert anteriorly, but one is directed backwards (recurrent) and attaches the cell to the bottom of a vase-shaped, organic lorica. Eat suspended bacteria. Without chloroplasts. *Bicosoeca.*

Bodonids: small, biflagellated organisms. Flagella insert subapically or laterally, with one directed laterally or anteriorly, and one recurrent. One genus is attached; the others usually move by gliding or skipping. Usually eat individual adhering bacteria, taken in via a discrete mouth. Without chloroplasts. *Bodo, Cephalothamnium, Rhynchomonas.*

Cercomonads: gliding flagellates with two flagella, one of which trails on the ground, often with cytoplasm being pulled out behind the cell, and one active anterior one. Colourless. Feed on bacteria by pseudopodial engulfment. *Cercomonas, Heteromita.*

Chrysophytes = Chrysomonads: cells with two flagella: typically, one is short and flaccid, and the other is longer. Either with chloroplasts (golden) or without, capable of phagocytosis or not, with or without a layer of surrounding siliceous plates, sessile or motile, and solitary or colonial. *Anthophysa, Chrysamoeba, Chrysosphaerella, Dendromonas, Dinobryon, Mallomonas, Ochromonas, Paraphysomonas, Poterioochromonas, Spumella, Syncrypta, Synura, Uroglena.*

Collar flagellates: with single apical flagellum, around which lies a collar comprised of fine cytoplasmic 'fingers'. Mostly sessile. May be solitary or colonial, naked or loricated. Without chloroplasts. Eat by filtering suspended bacteria or other small particles. *Codonosiga, Diploeca, Diplosigopsis, Monosiga, Pachysoeca, Sphaeroeca.*

Cryptophytes = Cryptomonads: rigid cells with two flagella arising within an anterior depression. The depression is lined with ejectisomes. Either with chloroplasts (off-green, orange, blue-green, red) or colourless (in which case osmotrophic). *Chilomonas, Cryptomonas, Cyanomonas, Goniomonas.*

Dinoflagellates: rigid cells with two flagella: one passes horizontally around the body, usually in a groove; the other passes longitudinally, often trailing behind the cell. Rounded. May occasionally be drawn out into spines. With (orange or off-green) chloroplasts or without. Colourless species are osmotrophic, consume detritus, or prey on other protists. *Gymnodinium, Gyrodinium, Ceratium, Amphidinium, Peridinium.*

Dilpomonads: cells with two nuclei and two clusters of four flagella, each arising at the head of a longitudinal groove, with some flagella projecting laterally and others trailing behind. Usually from anoxic or organically enriched sites. Without chloroplasts. Osmotrophic or eating bacteria. *Trepomonas, Hexamita.*

Euglenids: small to medium-sized cells with (usually) two flagella arising in an anterior flagellar pocket, both, one or none of which may emerge. Move by swimming or gliding, or squirming. Body may be pliable. Some with ingestion apparatus. Eat bacteria, detritus or other protists. Some have one or more bright green (chlorophyll b present) chloroplasts. *Anisonema, Astasia, Entosiphon, Euglena, Heteronema, Menoidium, Notosolenus, Peranema, Petalomonas, Phacus, Trachelomonas, Urceolus.*

Heterolobosea: protists with an amoeboid and a flagellated stage in the life cycle. The flagellated stage has two or four flagella, a flexible body, and usually does not eat. Includes facultative pathogens. *Naegleria.*

Others: there are about 70 genera of heterotrophic flagellates that cannot be confidently assigned to any of the groups of flagellates listed in this Guide (Patterson and Larsen, 1991). They include *Artodiscus, Clautriavia, Helkesimastix, Kathablepharis,* and *Multicilia.* In addition, oomycete fungi, protostelid and eumycetozoan (e.g. *Ceratiomyxa*) slime moulds, and desmothoracid heliozoa (among others) produce flagellated organisms as part of their life cycle.

Pedinellids: cells with a single apical flagellum. Usually with a stalk, although they may swim trailing the stalk behind. When sessile, the flagellum is surrounded by a small number of discrete arms which may be used to intercept particles of food. *Actinomonas, Pteridomonas, Ciliophrys.*

Pelobionts: amoeboid cells, usually with a single, relatively long, stiff flagellum. From anoxic sites. *Mastigamoeba, Mastigella, Pelomyxa.*

Phalansteriids: flagellates with a single flagellum

that has a tight basal collar. Live in colonies supported by globular mucus. Eat bacteria. *Phalansterium.*

Pseudodendromonads: Biflagellated cells. The two similar flagella are located at one edge of flattened triangular cells. One genus colonial, supported on a stiff, dichotomously branching stalk system. Eat bacteria and other small suspended particles. *Pseudodendromonas.*

Spongomonads: biflagellated cells living in colonies supported by globules of mucus. *Rhipidodendron, Spongomonas.*

Volvocales: flagellates with two or four apical flagella. Most species have a bright green chloroplast containing chlorophyll b. With rigid cellulosic wall. Often forming rounded, swimming colonies. *Brachiomonas, Carteria, Chlamydomonas, Chlorogonium, Eudorina, Gonium, Haematococcus, Pandorina, Polytoma, Polytomella, Volvox.*

3 AMOEBAE: traditionally, organisms that move and/or feed using temporary extensions of the cell body (pseudopodia). Usually include star-like protists with stiffened pseudopodia (the heliozoa), and rhizopod amoebae, which produce short-lived pseudopodia.

Actinophryid: heliozoan body form. Arms taper from base to tip. Two genera only: one has a single central nucleus; the other has a layer of nuclei underlying a layer of vacuoles. *Actinophrys, Actinosphaerium.*

Centrohelid: heliozoan body form. Arms are relatively thin and do not taper. Extrusomes prominent. Microtubular supports terminate on a central granule. With or without a layer of scales or spines on the body surface. *Acanthocystis, Chlamydaster, Heterophrys, Oxnerella, Raphidocystis, Raphidiophrys.*

Desmothoracids: sessile, heliozoan-like cells living within a perforated lorica, out of which the arms project. With a conventional amoeboid and flagellated stage in the life cycle. *Clathrulina, Hedriocystis.*

Diplophryids: cell body enclosed in a delicate organic shell. With two tufts of fine pseudopodia emerging from opposing ends of the cell, and with one or more large orange lipid droplets. From organically enriched sites. *Diplophrys.*

Euamoebae: rhizopod amoebae with one or more broad pseudopodia, and without a firm shell. May have short, stubby, filose subpseudopodia emerging from a larger pseudopodial region. Pseudopodia usually develop either gradually (progressive) or suddenly (eruptive). Some species have a flagellated stage; others are facultative pathogens. *Acanthamoeba, Amoeba, Astramoeba, Cashia, Chaos, Cochliopodium, Hartmanella, Mayorella, Saccamoeba, Thecamoeba, Vannella.*

Heterolobosea: protists with amoeboid and flagellated forms. Amoebae usually small (less than 50 μm), with eruptive bulging of the pseudopodia. Includes facultative pathogens. *Naegleria.*

Leptomyxids: naked amoeboid organism, with cytoplasm forming anastomosing channels. *Leptomyxa.*

Nucleariid filose amoebae: with thin pseudopodia, usually arising at any part of the body. Flattened or spherical. Naked or with mucus sheath, or with adhering siliceous particles. No extrusomes (or extrusome-like granules) on the pseudopodia. *Nucleariа, Pompholyxophrys, Pinaciophora.*

Others: there are numerous amoeboid organisms that have yet to be properly described. Those least studied have very thin pseudopodia, either bearing extrusomes (*Gymnophrys, Biomyxa, Microcometes, Reticulomyxa*) or smooth (*Belonocystis, Elaeorhanis*). Some large, shelled species (*Allelogromia, Gromia, Lieberkuhnia*) may be related to marine foraminifera and are in need of further study.

Pelobionts: typically amoeboid organisms with a single long flagellum. However, in one genus (*Pelomyxa*) the flagella are very difficult to see and so this genus has usually been described as an amoeba. *Mastigamoeba, Mastigella, Pelomyxa.*

Testate amoebae: amoeboid organisms with a shell of organic matter, or adhering particles around the body. Pseudopodia emerge from one or two apertures. Either with filose pseudopodia (*Amphitrema, Assulina, Cyphoderia, Euglypha, Trinema*) or with lobose pseudopodia (*Arcella, Cyphoderia, Centropyxis, Cryptodifflugia, Difflugia, Lecquereusia, Nebela, Quadrulella*).

Vampyrellids: flattened amoeboid organisms with cell margins giving rise to numerous very delicate pseudopodia. Often eat algae or fungi, and often orange in colour, with a granular texture to the cytoplasm. *Arachnula, Vampyrella*

4 NON-PROTOZOAN TAXA INCLUDED:

Algae: protists with chloroplasts. Only those without flagella are included here; the remainder are listed under 'Flagellates' (above). Mostly unicellular organisms are included here, but many algae are multicellular.

(i) Diatoms: with a siliceous wall and orange or golden chloroplasts. Filamentous or solitary, motile or immotile, centric (pill-box shape) or pennate (cell with discrete poles). *Melosira, Navicula, Nitzschia, Pinnularia, Stephanodiscus, Tabellaria.*

(ii) Green algae (Chlorophyta): cells with cellulosic cells walls and bright green (chlorophyll b) chloroplasts. With a rich variety of shapes (e.g. coccoid and filamentous). *Closterium, Eurastrum, Hyalotheca, Micractinium, Micrasterias, Mougeotia, Spirogyra, Spondylosium.*

Prokaryotes: (bacteria). Without internal organelles, but may have inclusions. Typically with one dimension restricted to about 1 μm. Usually distinguished by colour (e.g. blue-green algae), form or metabolic pathways (e.g. spiral, coccoid, filamentous, red sulphur, etc.).

Blue-green algae: (cyanobacteria). Prokaryotic organisms carrying out photosynthesis. Either solitary cells or filamentous – the latter are more obvious. A type of bacterium, but forms an ecologically distinct group. Some are endosymbionts in protists. *Oscillatoria.*

Metazoa: organisms with many cells which are arranged in epithelia (layers attached to collaginous sheets). Different cells may have different functions.

(i) Gastrotrichs: pliable bodies, usually with locomotor cilia, a mouth that opens anteriorly, and two posterior adhesive structures. *Chaetonotus.*

(ii) Rotifers: cilia usually reduced to two anterior clusters used for feeding. Body surface rigid and normally made of articulating elements. Usually has a mastax located near the anterior end of the digestive system, and two posterior adhesive structures. *Polyarthra, Squatinella.*

(iii) Nematodes: elongate stiff bodies that are rounded in cross section. Move by writhing or serpentine gliding through the substrate. Typically with a muscular pharynx near the front end.

(iv) Flatworms: very pliable bodies. Move using a combination of muscular activity and the cilia which cover the entire body. Typically with a muscular pharynx opening on the ventral surface, away from the anterior end of the cell.

(v) Tardigrades: rigid bodies with an exoskeleton made of a number of articulating elements. With eight stubby legs ending in claws. *Macrobiotus.*

How to use the key

What follows is a simple, dichotomous key. At any given step (e.g. Step 1) you are presented with a pair of options (A and B). Decide which statement best fits the organism you are looking at. At the end of the option, you are advised to go to another step. There you repeat the process, continuing until you are provided with a generic name instead of being directed to another step. An illustration is provided so that you can check whether you have the correct organism.

Unfamiliar objects can only be understood with the acquisition of new concepts and new terms. Thus, successful use of the key will involve a learning process and necessitate effort from the user. The key is followed by a glossary to ease the process.

The key makes little provision for cells which are damaged, for example, through being squashed. You should therefore try to find several individuals of each species, and it should then be possible to distinguish normal features (present in all cells) from those which are abnormal (peculiarities of individual cells). A good example of the need for careful observation occurs where the key asks if the cells swim or glide. If one cell only was observed, and this has been compressed, such a question cannot be answered reliably.

This key does not claim to be comprehensive. You may encounter organisms that are not included. The drawings, which have been simplified for clarity, may generalize the characteristics of a genus. However, the true appearance of organisms will be evident from the photographs.