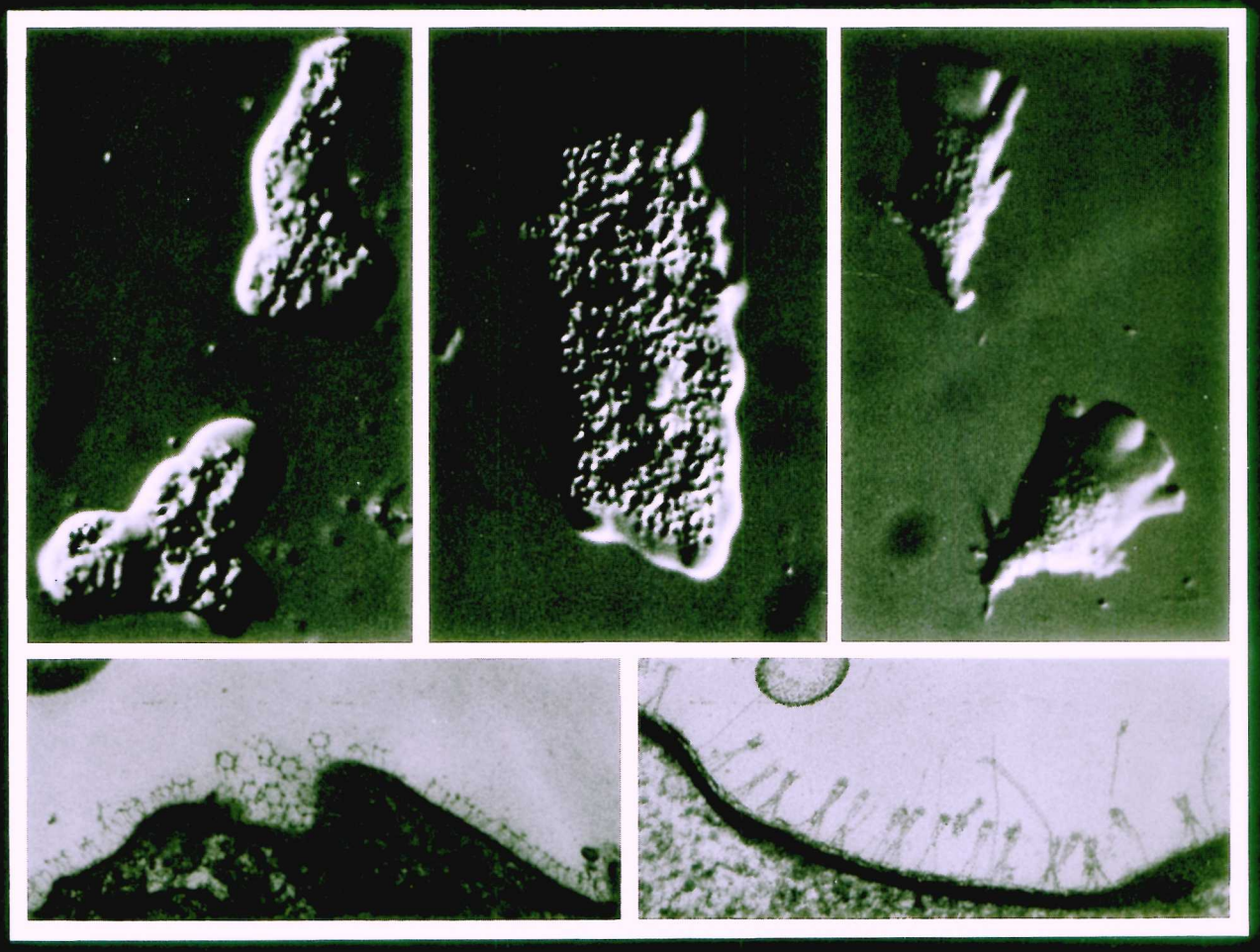
MARINE GYMNAMOEBAE



Institute of Terrestrial Ecology

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MARINE GYMNAMOEBAE

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Cover photograph shows (light micrographs) *Vahlkampfia dumnonica*, *Mayorella gemmifera*, and *Flabellula citata*; and (electron micrographs) surface structures of *Vexillifera minutissima* and *Vannella caledonica*.

The *Institute of Terrestrial Ecology (ITE)* was established in 1973, from the former Nature Conservancy's research stations and staff, joined later by the Institute of Tree Biology and the Culture Centre of Algae and Protozoa. ITE contributes to, and draws upon, the collective knowledge of the fourteen sister institutes which make up the *Natural Environment Research Council*, spanning all the environmental sciences.

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Foreword

This publication is intended as a source-book for the investigation of naked lobose amoebae in the marine environment. It aims to encourage the inclusion of these organisms in ecological studies and, through information on culture, to make them available for experimental work. It reflects the present state of knowledge and indicates areas where that knowledge is still particularly deficient. Although it draws on the literature, it is based chiefly on work carried out at the Culture Centre of Algae and Protozoa.

Because this publication is intended especially to give the marine biologist confidence in identification, electron microscopy is an essential tool for full utilization of the taxonomic section. The key for identification is the first to employ fine structure as a source of diagnostic characters for the Gymnamoebia of any environment. Nevertheless, every effort has been made to correlate light microscopical characters with fine structure so that lack of electron microscopy is not an insuperable handicap in most cases.

The title *Marine Gymnamoebae* is meant to suggest that, while most of these organisms would be included in the subclass Gymnamoebia, other naked lobose amoebae (the marine Acarpomyxea) are also dealt with.

I should like to thank the following colleagues: Mr Nigel C Pennick for use of 2 electron micrographs of scales (Figures 138 and 139); Dr P G Carey for consultation on *Heteramoeba clara*; Dr Lynn Margulis and Dr Laurie K Read for permission to mention their finding of *Paratetramitus* at Laguna Figueroa and the resulting discovery of euryhalinity in that organism; Dr N B S Willumsen for his collaboration on *Paraflabellula reniformis* (to be published in more detail elsewhere) and for sharing other findings of his Baltic explorations; and Dr J C Green for directing my attention to the rock pools at Hannafore Point, Cornwall.

Contents

Introduction

Naked, lobose amoebae, the majority belonging to the subclass Gymnamoebia (Page 1976b; Levine *et al.* 1980), have been found in many different marine habitats (Anderson 1977; Bunt 1970; Caron *et al.* 1982; Davis 1978; Davis *et al.* 1978; Sawyer 1971b, 1980). The author has never failed to isolate amoebae from a sample of seawater. Furthermore, as discussed in a later section, amoebae feed on such diverse micro-organisms as bacteria, blue-greens, diatoms and other algae, yeasts, ciliates, flagellates, and other amoebae. Many are highly euryhaline (Page 1971a, 1971b, 1974b). Nevertheless, few studies of the possible rôle of amoebae in marine habitats have been reported (Mitchell & Yankofsky 1969; Bunt 1970; possibly McCambridge & McMeekin 1980). As pointed out previously (Page 1980b), this neglect of these ubiquitous organisms in marine ecology contrasts with the investigations of their rôle in the soil.

Compared with the diversity of Sarcodina in marine environments (including actinopods, foraminiferans, testaceans, xenophyophores and others), the taxonomic scope of this publication is rather limited. It covers principally those free-living members of the Gymnamoebia found in coastal waters and the lower reaches of estuaries. Also included are the Leptomyxidae and Stereomyxidae, though these have been classified (Levine *et al.* 1980) in a class Acarpomyxea rather than in the Lobosea; members of the freshwater/soil family Acanthamoebidae, repeatedly isolated from saltwater; and the amoeboflagellate *Paratetramitus*, recently discovered to include euryhaline strains though not yet isolated from a marine source. Attention is also drawn to the common but morphologically unusual testacean *Trichosphaerium*, which in one of its stages resembles a naked amoeba and has even been given another name for that reason. A brief section lists some amoebae parasitic in marine organisms.

Ecologically and geographically, seas of normal oceanic salinity, the lower reaches of estuaries and that great body of brackish water, the Baltic (Jansson 1978), fall within the scope of this publication. The reports of Schmoller (1961, 1964) and Schmoller *et al.* (1982), and unpublished observations of N B S Willumsen (personal communication) suggest the occurrence of both freshwater and marine forms in various areas of the Baltic. Certain isolated salt lakes (Butschinsky 1897; Jones 1945) are outside its scope.

Some marine amoebae are already known to be widely distributed over the globe. In the course of work leading to this publication, two unusual organisms previously known only from the Mediterranean were found in Cornish rock pools. Therefore, though emphasizing British waters, the key is usable anywhere to generic and often to specific level, and some strains on which the descriptions are based were in fact isolated far from Britain. In reports of distribution, the name of a large body of water such as an ocean has usually been extended to its bays and estuaries.

While this publication is in some ways a companion, generally at a more advanced level, to the author's *Illustrated key to freshwater and soil amoebae* (Page 1976a), it may seem on first inspection to be less comprehensive in its coverage of genera and species. This is due to an emphasis on those organisms which have been studied at first hand, most of those known to the author only from the literature being listed (with descriptive details) as 'other genera' or 'other species' and not included in the dichotomous keys. The reason for this emphasis is the importance attached to electron microscopical observations, as well as to studies based on clonal cultures. With 3 exceptions (Anderson 1977; Hollande *et al.* 1981; Schmoller *et al.* 1982), reports on fine structure of free-living marine amoebae (other than abstracts) have been published only by Professor Grell and his colleagues, and from our Institute. However, the coverage of described taxa is inclusive. The dichotomous 'Key to genera' includes 22 genera, while 21 non-parasitic genera are listed elsewhere, the majority under families. The dichotomous keys of genera to species (arranged by families) include 42 species, while 45 'other species' (not including Acanthamoebidae) are listed under genera, and a further 30 in the list of species 'not listed elsewhere'. References are included for all these 43 named genera and more than 120 species (including Acanthamoebidae), as well as a few strictly parasitic organisms, so that the entire original literature is accessible.

The principal recent taxonomic reference is the key of Bovee and Sawyer (1979), emphasizing the fauna of the north-eastern United States.

This publication, therefore, presents both a summary of present knowledge of marine Gymnamoebia and similar amoebae, and an idea of the many gaps in that knowledge. Even the taxonomic picture is not yet complete. Further advances will continue to benefit from both light and electron microscopy, but resolution of species distinctions is likely to require non-morphological investigation. The application of isoenzyme electrophoresis to *Platyamoeba*, the most commonly isolated marine genus, is one obvious project. These non-morphological approaches require the refinement of culture methods. Quite apart from the need for such refinement, an increased understanding of the eukaryotic food of marine amoebae in natural habitats should be an early result of further work. Euryhalinity is another obvious area for further investigation.

Strains of many species of marine Gymnamoebia, including most of those in the dichotomous keys, are available from the Culture Centre of Algae and Protozoa.

Culturing marine amoebae

Little of value can be learned about the amoebae of a marine habitat or collected sample without bringing them into culture. Most will not be seen on direct examination of field samples, and concentration of a collected sample also concentrates all the other, more numerous organisms. Enrichment is necessary, even if only in crude, mixed cultures. The advice along these lines given by Uhlig (1968) in his instructions for collecting and processing marine protozoa is especially true of naked amoebae. Any rigorous work further requires clonal isolation.

Grell (1968b) and Bovee and Sawyer (1979) have offered some suggestions for culturing, and the procedures and (using seawater) even the media presented by Page (1976a) for freshwater/soil amoebae can be adapted. In this section, methods which have proved their value are summarized briefly. The compositions of the media are given on pp 7-8. In the dichotomous keys to species, suggestions are given of appropriate media for each species.

Collection and initial handling

Collection vessels should be sterile, though the lack of cysts in most if not all truly marine (rather than intrusive or brackish-water) amoebae reduces the risk of contamination. (One cannot count on the absence of the Acanthamoebidae from any part of the biosphere; see p. 41). The author's purpose has always been to collect any amoebae present in the hope that some were of interest, a permissible aim in a relatively unexplored field, allowing observance of minimal precautions against environmental contamination. A sterile jar is opened and scraped along the bottom to collect some bottom material and enough water to fill the jar except for an air space. Sometimes a bit of algal material is added to the jar with the fingers. Since all collections at any one time are from adjacent sites, eg on a single beach, no essential information about occurrence is lost. If one is comparing the fauna of neighbouring rock pools or intends to plate out material for *Acanthamoeba*, one cannot be so casual, and quantitative studies require rigorous procedures. For places that are difficult to reach (eg the centre of a large rock pool), a simple collecting apparatus with a dip tube, as described earlier (Page 1976a), is very useful for qualitative work.

The material should be examined and processed within a day or 2 if possible, without being permitted to warm up. Bits of algae should be washed in the collected water to leave behind any amoebae, then discarded.

Initial observations, which seldom reveal any amoebae, should be made before or when cultures are inoculated, examining preparations of drops from the bottom and sides of a jar which has been standing for an hour or so, then of water immediately after the jar has been inverted a few times. The next step is enrichment culture, but a few basic principles must be considered first.

Conditions for culturing amoebae

Food. At present, the culture of marine gymnamoebae is reasonably successful but, with few exceptions, crude. None are in *axenic* culture, living wholly on dissolved nutrients such as proteose peptone in the absence of any other species. Only a few are *monoxenic*, feeding on a single known organism. Most are in *polyxenic* culture, ie with 2 or more other species present, not all of which may be serving as food. Organisms on which marine amoebae in culture are known to feed include bacteria, diatoms and other algae, green and colourless flagellates, smaller amoebae and ciliates such as *Cyclidium*.

In the early stages of culture, the first aim is to ensure survival of the strain, even if the culture is not as clean biologically as one might wish, and healthy, abundant cultures have been the main requirement for the descriptive work carried on till now. When such crude but thriving cultures have proved long-lasting, they can be maintained in that state while they are drawn on for attempts to establish monoxenic and eventually axenic cultures. Such attempts at refinement are based at first on the use of food organisms observed to serve that rôle in the polyxenic cultures or on food organisms like those used with freshwater amoebae. Thus, *Paramoeba eilhardi* can be cultured on a known diatom (though bacteria are also present); *Heteramoeba clara* grows on *Escherichia coli*; and *Oceanospirillum* has been used by some workers as food for marine amoebae.

Salinity. Since amoebae in coastal and estuarine habitats experience fluctuations in ambient salinity, it is no surprise that they can be cultured on media with salinity below the normal range of 30-35 o/oo (Page 1971a, 1971b, 1974b). Following the observation that many strains of *Platyamoeba* seemed to do better at 75% of oceanic salinity, ie at approximately 26 o/oo, other genera were tried at the same concentration with similar results, and that concentration was adopted for all agar media. Twenty samples from rock pools were tried in both full-strength and 75% seawater *liquid* media (including Føyn's Erdschreiber, number 1 in the list of media, which has a salinity near that of full-strength seawater). Most did best at a concentration of 75% oceanic salinity, ie in media 3 and 5 rather than 2 and 4 (p. 8).

Even from marine habitats, *Acanthamoeba* is isolated on freshwater rather than marine media, most commonly on non-nutrient agar streaked with *Escherichia coli*. Appropriate methods are described by Page (1976a) and in the references on *Acanthamoeba* (p. 41). Before undertaking the isolation of this genus, the comments on sporadic pathogenicity (p. 43) should be noted.

Phase. Most smaller and a good many medium-sized amoebae do well on agar. Other medium-sized

amoebae such as *Mayorella* and *Gruberella* have succeeded only in a liquid medium. In such cultures, the liquid should be about 1 cm deep in a culture dish with a diameter of 50 mm. Some grown in liquid become abundant on agar (usually medium 7) with an overlay of liquid (usually medium 1), though these cultures contain more bacteria than does liquid alone and may become somewhat gelatinous; such strains are *Paramoeba eilhardi* and *Gruberella flavescens*.

Temperature. All our marine strains are grown at room temperature, about 18-22°, though they tolerate slightly higher temperatures during the day-time in the summer. *Paramoeba eilhardi* feeding on *Amphiprora* in liquid can be kept at 15°. If cultures can be kept at a constant temperature, settings between 18° and 25° should be tried.

Light. There is no evidence that Gymnamoebia need a light/dark cycle, and only cultures containing photosynthetic food organisms *need* to be kept in the light, never in direct sunlight.

Isolation and maintenance

The first step, enrichment, should be started as soon as possible after collection. Unless enriching only for a particular organism with known requirements, collected material should be inoculated into several media to bring out different amoebae and their food organisms, eg a liquid medium such as (5) (cf media, pp 7-8) with a rice grain in a 50 mm culture dish; an agar such as (7) or (9), inoculating no more collected liquid than will be absorbed in a few hours; and an agar with enough collected liquid to provide an overlay about 3 or 4 mm deep. Some collected water can be put into a 50 mm culture dish and a rice grain added. If possible, media of both full-strength oceanic salinity (c. 35 o/oo) and 75% of that concentration (c. 26 o/oo) should be used for this step.

When the amoebae have grown out, any of interest should be isolated into clonal culture, using an appropriate medium. Medium-sized and large amoebae (more than 50 or 60 μm long) can be cloned by transferring them with sterile, fine-tipped pipettes through several sterile drops until only one amoeba is left. An easier method, which should always be used for smaller amoebae to be grown on agar and is often useful even for larger ones which will survive only a short time on agar, is to spread them out on a fresh agar surface for selection of clone founders. They can be spread out on the plate by placing 3 drops of cell suspension from a mixed culture about 1 cm apart at one side of the agar, immediately tipping the plate so that the drops run in parallel paths down the agar, and leaving the plate overnight for the amoebae to separate farther by migration. When picking out single cells to start clones, one should examine the area carefully for other cells (either on the surface or burrowed into the agar). The location of the single amoeba on the isolation surface can conveniently be marked with the light spot from the condenser (Page 1976a) so that the amoeba need not be kept in view during manipulation. Then, with a fine scalpel blade, pick out a small block of agar containing only one amoeba, and turn it over on to a fresh agar surface or put it into sterile liquid medium. Unless one is supplying a known food organism, one should include on the block any accompanying organisms which may serve as food: bacteria at least, and possibly flagellates, small ciliates, and even amoebae which are much smaller than the one being isolated. Most amoebae which grow on agar without a liquid overlay will feed on bacteria only, except *Mayorella* and species of *Thecamoeba* larger than *T. orbis*. In the case of such bacteria-feeders, it is preferable to eliminate all eukaryotes except the clone-founding amoeba.

Clones on agar should show growth in a week (*Thecamoeba* and *Mayorella* often more slowly), those in liquid within about 3 weeks, but in both media they should be given at least one week longer. Once a clone is established, various media (including different salinities) and possibly various food organisms should be tried. All our marine strains on agar are routinely maintained on medium 7, but medium 9 may be better for the initial clonal isolation because bacteria grow less abundantly. For liquid cultures with mainly non-photosynthetic food organisms, medium 5 with a rice grain has proved generally useful, but medium 1 with or without rice should be tried. Media 2 and 3 often give good growth, but sometimes the bacterial population becomes excessive.

Many strains, if thriving, can be left 7 or 8 weeks without subculturing, but others need to be transferred every 3 or 4 weeks. Attempts to store marine strains on agar slopes at 6-9° for periods up to 6 months have not usually been successful. Some marine amoebae have been cryopreserved.

Media

All these media are for amoebae feeding on bacteria or eukaryotic protists.

Seawater. This is the basic component. Most commonly, natural seawater is used. It should be filtered as soon as possible after collection and stored in a dark place or opaque vessel, preferably at 4-6°.

For many purposes, the synthetic 'seawater' preparations for aquarists are suitable. We have compared, in parallel cultures, results with agar media prepared with natural seawater and with a synthetic preparation, 'Natura' (Waterlife Research Industries Ltd, 476 Bath Road, Longford, West Drayton, Middlesex UB7 0ED). Both these liquids were used in MY75S agar (medium 7), with a salinity near 26 o/oo. The pH of the natural seawater was 7.9, that of the dilute 'Natura' 7.6. Most strains grew well on either medium. Very often growth appeared more abundant on the medium made with the synthetic mixture, though growth of a few strains on the synthetic seawater was poor.

Comparisons of parallel cultures of 5 strains grown on the 2 media revealed no important difference in mean cell size within a strain, and comparisons of 2 strains showed little or no difference in mean length:breadth ratio.

The indications that some strains multiply more rapidly on media made with the synthetic liquid suggest that it may help to increase the yield of cells per plate for such purposes as electron microscopy. One of its features suggests a use at the crucial step to axenic or monoxenic culture: the 'growth substances' are contained in a package with trace elements, separate from the bulk salts. It would therefore be possible to make up an agar lacking all organic nutrients by using only the bulk salts and distilled water. This would permit migration of the amoebae without attendant multiplication of bacteria, a technique which is used to free amoebae from their original bacterial companions. All natural seawater, of course, contains organic nutrients in various quantities.

One *possible* disadvantage (not actually demonstrated) of medium made with such a synthetic seawater is that the population might reach its maximum sooner, and therefore require more frequent subculturing. Furthermore, one must also guard against bacterial overgrowth whenever the nutrient content is increased, but this drawback was not observed in our tests.

The mention of a particular brand of synthetic 'seawater' is not intended to imply that it is necessarily superior to other products. At one time, an American product, 'Instant Ocean', was used as a temporary substitute for natural seawater in making agar.

Other components. Cerophyl, used by many protozoologists in liquid media for ciliates and included as an ingredient of some media below, is a commercial product made from dehydrated cereal grass leaves (Cerophyl Laboratories Inc., Kansas City, Missouri, USA; supplied by International Marketing Corporation, 36 Lenexa Business Centre, 9900 Pflumm Road, Lenexa, Kansas 66215, USA). Readers wishing to try their own substitute should dry the leaves overnight at 80°, then grind them with a mortar and pestle.

When amoebae are grown in liquid media, feeding on bacteria and eukaryotic protists, a cereal grain is usually added to each dish, unless the liquid contains malt and yeast extracts.

Preparation. All media should be autoclaved, after which agar can be poured into petri dishes and liquid media stored in the flasks in which they were autoclaved.

Media. The agar media derived from liquid media 2-5 are listed separately for ease of reference.

1 Føyn's Erdschreiber
The proportions of components vary widely from one author to another. Soil extract: Mix 1 kg unmanured garden soil and one litre distilled water. Bring to pH 8 with NaOH. Autoclave for one hour at 15 lbs pressure. Decant or filter supernatant liquid, which is the soil extract.
Stock solutions:
$NaNO_3$ . . . . . 20 g in 100 ml distilled water
Na_2HPO_4 . . . . . 1.18 g in 100 ml distilled water
Final composition:
Filtered seawater . 950 ml
Soil extract . . . . . 50 ml
Each stock solution 1 ml

2 MY100S liquid
Filtered seawater . 1 l
Malt extract . . . . . 0.1 g
Yeast extract . . . . . 0.1 g
Dissolve the powders completely before autoclaving.

3 MY75S liquid
Make as (2), using 750 ml seawater and 250 ml distilled water.

4 C100S liquid
Filtered seawater . 1 l
Cerophyl . . . . . 1 g
Bring to boil, boil 5 minutes, and filter. Restore to volume with distilled water.

5 C75S liquid
Make as (4), using 750 ml seawater and 250 ml distilled water.

6 MY100S agar
Add 15 g non-nutrient agar (eg Oxoid no. 1) to the ingredients of 2, and bring to boil to dissolve agar.

7 MY75S agar
Add 15 g non-nutrient agar to the ingredients of 3.

8 C100S agar
Add 15 g non-nutrient agar to the ingredients of 4.

9 C75S agar
Add 15 g non-nutrient agar to the ingredients of 5.

Methods of observation and study

Light microscopy

Living amoebae. Observations of living amoebae are essential, whatever other techniques are employed.

The basic observations are of amoebae in locomotion on a glass substratum, with adequate oxygen and without either mechanical pressure or a significant increase in salinity by evaporation. These conditions are best fulfilled in hanging drops, with the amoebae attached to and moving on the under-side of cover glasses.

Prepare a cell suspension by washing amoebae from an agar surface or concentrating them from a liquid

culture. Place a drop of suspension on a cover glass, and place over this either a slide with a concavity or one with a ring about 2 mm deep cemented to it. Petroleum jelly is used to seal the connection between slide and cover glass. Let the preparation stand overnight, turn it over, and let it stand about an hour so that some of the accompanying bacteria sink to the bottom of the drop. To ensure adequate numbers of amoebae, prepare several drops from each culture.

Not all amoebae adhere well. Sometimes adhesion is improved by treating the cover glasses as follows: heat a 1% solution of NaOH to 80-90°, immerse the cover glasses for a few minutes, then rinse them thoroughly through several changes of tap and distilled water. If amoebae do not adhere to the cover glasses and must be observed on flat slides, support the cover glass with a thin ridge of petroleum jelly on each of 2 sides. Unless from a liquid culture with a low bacterial population, the amoebae may not continue moving normally for very long in such preparations, so that new preparations must be made frequently.

Other ways of getting the necessary conditions are observation of amoebae moving in a very thin layer of liquid in a 9 cm petri dish, or use of an inverted microscope.

Both lengths and breadths of 50-100 amoebae moving under these conditions should be measured. To measure 2 dimensions before the amoeba changes shape requires fast work, but gives reproducible results and is more accurate than measuring shrunken cells in fixed preparations. The locomotive rates (μm per minute) of 10 amoebae and lengths of the same 10 should be measured. The locomotive rate of amoebae attached to the under-side of such a substratum does not differ from that of amoebae on top of a substratum (Davies *et al.* 1981). Amoebae should be measured at the highest possible magnification, which for amoebae up to about 90 μm long means under an oil immersion objective.

Although the nucleus can be measured in a hanging drop, it is better to transfer the cover glass to a flat slide and measure under phase contrast optics. Amoebae still attached to the cover glass and well extended will give better results than those that have come loose. For each strain, 25 nuclei and their nucleoli, if the latter are single and central, should be measured.

The use of a polarizer is strongly recommended for detecting small crystalline inclusions, especially in *Mayorella*-like amoebae, where their presence may help to distinguish *Mayorella sensu stricto* from those forms here designated *Dactylamoeba*.

Stained preparations. The methods applicable to nuclear studies include Heidenhain's iron haematoxylin and the Feulgen procedure; the latter is also valuable in showing up the parasomes of *Paramoeba* for counting and other purposes. A fast method uses the dye Kernechtrot, manufactured by the Chroma-Gesellschaft. Methods are available in standard references and from manufacturers.

The principal problem, other than adjustment of times to the amoebae under investigation, is adhesion. I prefer to fix the amoebae on cover glasses and stain them in cover glass staining jars (Columbia jars). The cover glasses, often pre-treated with NaOH as described above, can be put into moist chambers made with a piece of filter paper or paper towel (moistened with seawater) in a petri dish, and a drop of cell suspension placed on each cover glass. They can be left overnight, though sometimes the amoebae are in better condition if the drops are set up first thing in the morning and fixed about 2 hours later.

Small and many medium-sized amoebae can be fixed first with Nissenbaum's fixative (Nissenbaum 1953): 10 ml saturated $HgCl_2$, 2 ml glacial acetic acid, 2 ml formalin (ie 2 ml of 40% HCHO), 5 ml *tert*-butanol, mixed fresh. Place one drop on to the cell suspension on the cover glass, then flood the cover glass. After one minute, transfer to a staining jar containing acidified $HgCl_2$ (saturated $HgCl_2$ and glacial acetic acid, 19:1) and leave for 10 minutes. Put through several changes of 50% ethanol, then bring down through 35% ethanol to distilled water before staining. If the cells adhere well, Bouin's fixative alone (not combined with a fixative containing $HgCl_2$) can be used for mitotic preparations. If the amoebae adhere poorly and come loose when agitated by the waves caused by Nissenbaum's fixative, acidified $HgCl_2$ can be used alone. Some of the best preparations of *Gruberella flavescens* were fixed by putting drops of $HgCl_2$ all around the drop of cell suspension on the cover glass (after it had stood in a moist chamber about 2 hours), then causing the fixative to flow gently into the culture drop from the sides.

Age of cultures for observation. In any observations, cultures of a standard age should be used. Most of the original measurements in this publication (those not taken from publications of other authors) were made on 7-day cultures. Especially with amoebae growing in liquid (usually less abundant than on agar), this may necessitate bringing the population to a high level by several successive transfers from near-peak cultures before achieving one suitable for observation. Mitotic figures are usually easier to find when preparations are made from 1- to 2-day cultures which have been preceded by such successive transfers. However, this recommended age may need to be modified with slowly growing strains. Mitotic figures are much more difficult to find in some species than others.

Euryhalinity. The method of testing salinity tolerance is described in the discussion of euryhalinity under 'Characters for identification'.

Electron microscopy

Here, too, populations can be built up by successive subculturing, and the cells must be concentrated. For amoebae growing on agar, 5 or 6 7-day cultures usually provide an adequate pellet.

In general, the results of a fixation procedure vary from one strain to another, even in the same genus, although some procedures are consistently more successful than others. For smaller marine amoebae, a short procedure often seems better than a longer one (a suggestion by Dr Joe L Griffin). In some successful procedures, the fixative is in seawater, from half to full-strength oceanic salinity. Sucrose appears of no value with these organisms. Fixatives osmotically identical with those used for freshwater amoebae were sometimes very successful. Greater difficulty was experienced with glutaraldehyde followed by OsO_4 than with OsO_4 alone, and both cytoplasmic and (once) intranuclear microtubules were preserved even by OsO_4 fixation carried out in an ice bath.

A procedure which gave good results for *Gruberella*, *Heteramoeba* and, to a lesser degree, some strains of *Mayorella* was the following. (1) Centrifuge to concentrate, then re-suspend in 15 drops of Erdschreiber or 100% seawater. (2) Let stand 10 minutes to take on locomotive form. (3) Add 15 drops of 2% OsO_4 in 0.1 M sodium cacodylate buffer, pH 7.0. (4) After 15 minutes (including any necessary centrifugation), replace fixative with 2% OsO_4 in buffer without any seawater, and leave 45 minutes longer. (5) Wash 4 times in distilled water, and treat for 20 minutes with 1% uranyl acetate in distilled water. (6) Wash twice with distilled water, dehydrate, and embed. Steps 2-5 are carried out in an ice bath.

Other procedures applied to various genera have been reported previously: *Vexillifera* (Page 1979a); *Pseudoparamoeba* (Page 1979b); *Vannella* (Page 1979b, 1980b); *Platyamoeba* (Page 1980a); *Hartmannella, Vahlkampfia*, *Flabellula*, *Rhizamoeba* (Page 1980c); small *Paramoeba* (Cann & Page 1982). Leaving *Vannella* in OsO_4 too long appears to destroy the glycostyles. Other methods are given by Anderson (1977), Benwitz and Grell (1971a, 1971b), Grell and Benwitz (1970, 1978), and Hollande *et al.* (1981).

Taxonomic introduction

General comments

As this is a practical publication, identification has taken precedence over presentation of a formal taxonomic scheme and nomenclatural innovation has been kept to a minimum. No change above the rank of genus has been made, and the system of Levine *et al.* (1980) is followed despite recent indications that some changes at higher levels are desirable.

This first utilization of ultrastructural characters in a key to naked lobose amoebae gives further support to the idea that generic diagnoses carry a higher degree of certainty than do species diagnoses in these organisms. The electron microscope has not, in most genera, given a great deal of additional information for distinguishing amongst species of the same genus, though it has firmly established a number of genera. However, it will undoubtedly provide clear distinctions amongst the scale-bearing *Mayorella*-like amoebae here tentatively designated *Dactylamoeba*, and some intrageneric differences in surface structure are perceptible in *Thecamoeba* (Page and Blakey 1979), even leaving the new genus *Dermamoeba* out of consideration, and in *Platyamoeba*. Possibly this approach can be further refined to provide species distinctions. However, at least in *Platyamoeba*, apparently the most common marine member of the Amoebida, non-morphological approaches such as those now being applied to *Acanthamoeba* and *Naegleria* will probably be necessary in the long run.

New taxa included in this publication are:
Gruberella n. g. (p. 17).
Nolandella n. g. (p. 18)
Paraflabellula Page & Willumsen n.g. (p. 33)
Vahlkampfia dumnonica n. sp. (p. 19)
Platyamoeba australis n. sp. (p. 28)

New combinations are:
Gruberella flavescens (Gruber 1889)
Nolandella hibernica (Page 1980)
Platyamoeba leei (Sawyer 1975)
Paraflabellula reniformis (Schmoller 1964)
Paraflabellula hoguae (Sawyer 1975)
Mayorella kuwaitensis (Page 1982)

Type slides which have been deposited in the British Museum (Natural History) have received the following accession numbers:
Platyamoeba australis n. sp. Holotype 1983:6:8:1; paratype 1983:6:8:2.
Gruberella flavescens (Gruber, 1889). Neotype 1983:6:8:3; paraneotype 1983:6:8:4.

For easy reference, a summary of the present taxonomic system for organisms included in this publication is presented. It accords on the whole with that of Page (1976a, 1976b) and Levine *et al.* (1980). Taxa in parentheses are not known to be represented in marine habitats.

Superclass Rhizopoda von Siebold 1845
Class Lobosea Carpenter 1861
Subclass Gymnamoebia Haeckel 1862
Order Schizopyrenida Singh 1952

Family Vahlkampfiidae Jollos 1917; Zulueta 1917
Order Amoebida Ehrenberg 1830
Family Amoebidae Diesing 1848
Family Thecamoebidae Schaeffer 1926
Family Hartmannellidae Volkonsky 1931
(Family Entamoebidae Chatton 1925)
Family Flabellulidae Bovee 1970
Family Hyalodiscidae Poche 1913
Family Paramoebidae Poche 1913
Family Acanthamoebidae Sawyer & Griffin 1975
(Family Echinamoebidae Page 1975)
(Order Pelobiontida Page 1976)
(Family Pelomyxidae Schulze 1877)
Subclass Testacealobosia De Saedeleer 1934
Order Arcellinida Kent 1880
Order Trichosida Möbius 1889
Class Acarpomyxea Page 1976
Order Leptomyxida Pussard 1976
Order Stereomyxida Grell 1966

Characters for identification

The emphasis in this section is on facilitating identification, though these characters overlap with those by which a taxonomic system is established. Methods of observing the characters are explained in an earlier section (pp 8-10).

Locomotive form. The appearance of amoebae moving on a substratum is the first clue to their identity. Methods of observation are important to avoid distortions, and the suggestions made earlier should be taken into consideration. Such observations require several or many amoebae and patience to wait for the appearance of diagnostic characters, particularly transitory ones such as the uroidal villi of *Saccamoeba* or the dactylopodia of those species of *Mayorella* which lack them at times. No species has a single shape, and attempts to match an amoeba as it appears in a fleeting moment with a single illustration are usually futile. Few species can be identified from one cell at one moment. The multiple illustrations of any one species in this publication are intended to convey something of the range of variation, and further illustrations are found in the references cited.

When an amoeba is advancing, its cytoplasm can usually be divided according to appearance into the *granuloplasm*, containing the inclusions visible in the light microscope, and the *hyaloplasm*, more or less clear. The hyaloplasm may occupy the entire anterior half of the amoeba, or form only a crescent-shaped hyaline cap, or even be absent temporarily. In general, the anterior half of an amoeba tends to be broader and the posterior half to taper toward a more or less narrowed posterior end, the *uroid*, though this difference is more marked in some amoebae than in others and the broadest point is often about one-half to one-third back from the anterior end.

A convenient dichotomy in describing the general forms of amoebae is cylindrical versus compressed or flattened. The cylindrical locomotive form can be branched or polypodial, as that of *Amoeba proteus* often is, or unbranched or monopodial. Only monopodial, cylindrical amoebae should be described as 'limax' (Page 1974a). The cross-section of a limax amoeba would seldom be circular, usually oval or thickly elliptical. Limax amoebae can be further divided, morphologically, into those whose locomotive activity is eruptive and those whose locomotion is more steadily flowing. Amoebae with markedly eruptive activity tend to advance with the production of hemispherical, usually hyaline, bulges at one side or the other on the anterior end, and the hyaloplasm may spill back along one side. The steadier pattern of locomotion often does involve a gentle antero-lateral bulging to one side or the other, but hyaloplasm is not reflected back along the side. In general, limax amoebae with eruptive activity tend to be thicker (with a lower length:breadth ratio) and to advance more rapidly than the more steadily flowing ones, but these distinctions, especially the second one, have exceptions.

Compressed amoebae include those which are much flattened against the substratum and others which are somewhat compressed, and those with and without *subpseudopodia*.

Truly flattened amoebae are generally broad, sometimes even with the breadth the greater dimension. In such amoebae, the hyaloplasm tends to be especially conspicuous, sometimes taking up the entire anterior half of the cell in locomotion, and flatter than the granuloplasm, which may have the form of a posterior hump. Such amoebae are usually distinguished by their outline, eg oblong, oval, flabellate or spatulate.

While the entire advancing, usually hyaline, anterior end of limax and many truly flattened amoebae is functionally a single lobose pseudopodium, many somewhat compressed and even some markedly flattened amoebae produce, usually from the hyaloplasm, conical or fine projections, *subpseudopodia*. Most have no demonstrated locomotive or feeding functions, and such subpseudopodia, arising from a lobopodium which is itself actively advancing, are not correctly called filopodia, no matter how fine their shape. They may be blunt and digitiform or mamilliform, like the *dactylopodia* of *Mayorella* (Figure 122); or slender and non-furcate (unbranched), either long as in *Vexillifera* (Figure 143) or short as in *Paraflabellula* (Figure 96); or markedly tapering, seldom straight, and sometimes furcate, as the *acanthopodia* of *Acanthamoeba* (Figure 155). Amoebae with subpseudopodia sometimes have very irregular forms, though in more or less steady locomotion amoebae of some genera with subpseudopodia may have a triangular outline with the base foremost.

The posterior end is properly called the *uroid* whether or not it shows any morphological peculiarities. It may be a

simple, smooth, round posterior end (Figure 4) or knobby or *morulate* (Figure 124). A uroid dragging shrivelled remnants of pseudopodia or subpseudopodia is *fascicular*; some of the projections trailed by *Vexillifera* or *Acanthamoeba* are of that sort. *Uroidal filaments* result from a common phenomenon: the surface of many or all amoebae is somewhat adherent to the substratum, and the posterior end is often drawn out when it adheres as the amoeba moves forward. Sooner or later it breaks loose from the substratum, and in certain amoebae trailing uroidal filaments are formed then (Figures 28, 85). This is characteristic of amoebae with no more than a thin glycocalyx; such filaments are not formed by, for example, *Thecamoeba* or *Platyamoeba*, but are common in the vahlkampfiids. A cluster of many such filaments, flexible and often with some furcate, is a *collopodium* (Figures 5, 36, 97). This is not a pseudopodium or group of pseudopodia, as the filaments have been drawn out rather than actively put out by the cell. Some amoebae, notably *Saccamoeba*, often have a posterior bulb carrying many more or less rigid, non-furcate, fine villi, the *villous bulb*, which appears to be produced by a different process from that producing the collopodium.

Measurements. Lengths and breadths of 100 amoebae are measured as they move on the under-side of a cover glass, in a hanging drop made (with rare exceptions) from a culture inoculated 7 days earlier.

Variations in measurements obtained at different times for a single strain or species could have several causes, such as position on the growth curve, which may be affected by temperature or food. An example of apparent size increase over the years has been illustrated by *Paramoeba pemaquidensis* (Cann & Page 1982). Other apparent increases over periods of 7 to 13 years in culture have been found in strains of *Flabellula citata*, *Platyamoeba plurinucleolus*, and *Platyamoeba calycinucleolus*, but strains of *Thecamoeba orbis*, *Flabellula calkinsi* and *Vahlkampfia damariscottae* changed little over 13 years, despite differences in media and in the ages of the cultures from which samples were taken; a strain of *Platyamoeba bursella* showed no change over 10 years. Some observations, not systematic, suggest that amoebae in culture generally tend to be smaller than the same species in nature, possibly due to differences in generation time. Caution should be exercised in both measurement and in application of published data to the material being studied.

Cytoplasmic inclusions. Some genera contain crystals, including 'paired bodies' such as those of *Mayorella*; in others, even the polarizer does not reveal any. The parasome, a complex DNA-containing body (Figures 115, 116), probably a symbiont (Grell 1961; Perkins and Castagna 1971; Hollande 1980; Cann & Page 1982), is found in *Paramoeba* and the parasitic amoeba *Janickina*. In one species of *Vexillifera*, easily distinguishable trichocyst-like bodies (Figure 146) have persisted during 5 years in culture. Symbiotic bacteria are one characteristic of *Pelomyxa*, though both symbiotic and parasitic bacteria are found in other genera and species. Zoochlorellae are known in 2 freshwater amoebae, but have not been reported in any marine species.

Nuclear structure and division pattern. For identification, observation of the interphase nucleus is essential, but elucidation of the mitotic pattern is necessary in only a few cases.

Using the terminology of Raikov (1982), the great majority of free-living amoebae, including most marine species, have *vesicular* nuclei. Such a nucleus usually has a central, more or less spherical nucleolus; in one strain of *Platyamoeba*, the nucleolus is consistently eccentric. The nucleolar material may occur not as a single central body, but as 2 or more lobes in a parietal (peripheral) position (Figures 24, 25, 73), though careful examination often shows these lobes to be part of a single body extending around much of the periphery of the nucleus. Nuclei with parietal nucleoli are found in a number of genera, all of which except *Heteramoeba* also contain species with different kinds of nuclei. The other principal kind of nucleus in free-living amoebae is the *ovular* nucleus, a larger nucleus found in some of the larger amoebae, with many peripheral nucleoli, as in *Amoeba proteus*. No marine amoeba which I have found has such a nucleus, though one is described for *Amoeba crystalligera*. Intermediate cases between these major categories are described by Raikov (1982) and Page (1976a).

For each strain, I measure the greatest dimension of 25 nuclei of 'normal' shape, usually spherical or ovoid, and, if the nucleolus is central, their nucleoli, under conditions described under 'Methods of observation and study' (pp 8-10).

Despite their value in establishing some groups of amoebae, mitotic patterns usually need not be observed for identification, except with isolates of limax amoebae. To ensure accurate identification of any limax amoeba, except those otherwise identifiable as *Saccamoeba* or members of the Amoebidae (the latter larger than those usually called 'limax'), permanent stained preparations should be made. (Pussard (1973) has obtained convincing results with *in vivo* observations of some amoebae, none marine.) Such preparations are necessary, for example, to distinguish *Nolandella* or perhaps even a small *Rhizamoeba* from *Vahlkampfia* and to settle cases where the locomotive habit may be atypical.

All division patterns likely to be found are orthomitotic (Raikov 1982). The closed orthomitosis of vahlkampfiids has long been called *promitosis* (Figures 1a, 17), though that term no longer implies that it is primitive. The nucleolar material, rather than disintegrating, forms 2 polar masses with the spindle between them, and the

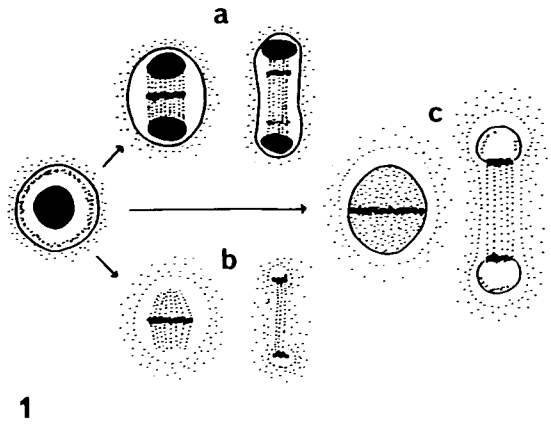


Figure 1. Some mitotic patterns. a, promitosis, a variety of closed orthomitosis. b, mesomitosis with early disintegration of nuclear membrane, an acentric open orthomitosis. c, a variety of mitosis found in some Platyamoeba, *with nuclear membrane present (and spindle not always discernible) in metaphase and remnants of nuclear membrane forming polar caps in anaphase.*

nuclear membrane remains intact until separation of the daughter nuclei. No centriole or other microtubular organizing centre is seen. Most amoebae have various patterns of acentric orthomitosis, either open or closed, which I shall group for convenience under the term *mesomitosis* (Figures 1b, 74). No centriole or other microtubular organizing centre has been found, the nucleolus disappears and the nuclear membrane has usually disintegrated by metaphase, though it may persist longer. Since the mitotic process has been studied electron microscopically in few amoebae, the fate of the nuclear membrane at any particular stage is not certain in most. In some species of marine *Platyamoeba*, a remnant of the nuclear membrane forms a polar cap at each end of the mitotic figure (Figure 1c).

Mitotic patterns involving a centriole or microtubular organizing centre (MTOC) can be classified as centric orthomitosis or *metamitosis*. Amongst organisms included in this publication, MTOC's are known only in the Acanthamoebidae and the Stereomyxidae, though the mitotic process itself has not been described electron microscopically in those groups. Observation of mitosis is not necessary to identify them.

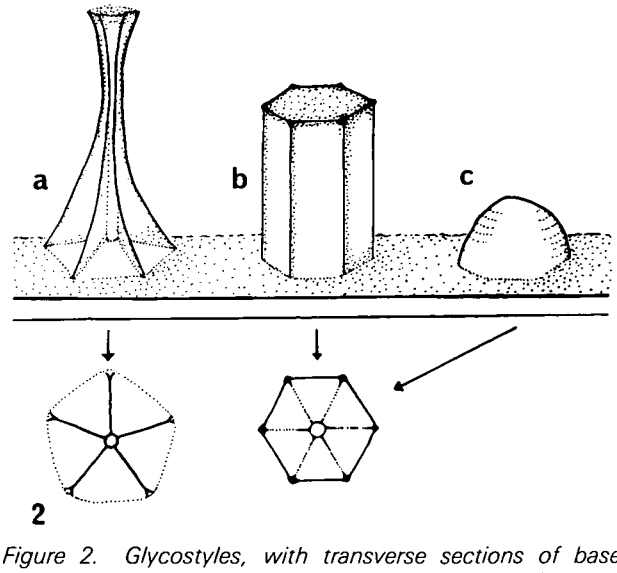


Figure 2. Glycostyles, with transverse sections of bases below. a, Vannella. *b,* Vexillifera. *c,* Pseudoparamoeba.

Floating form. This form, taken when an amoeba is suspended in liquid, is of no help with identification of, for example, limax amoebae, where it is irregularly rounded up. When it has pseudopodia radiating from a central mass, the nature of these may be of some assistance, eg in distinguishing between *Vannella* and *Platyamoeba*, but even then the lack of slender radiate pseudopodia in floating forms of some *Vannella* limits its usefulness.

Flagellate phase. Its occurrence and structure are essential for identifying genera of Vahlkampfiidae. Means of obtaining such stages are suggested in the section on that family, though methods for inducing transformation are less well understood for marine than for freshwater species. The flagellates should be examined while living and then in temporary fixed preparations made by adding one drop of saturated $HgCl_2$ solution to a drop of cell suspension.

Cysts. These are useful for the identification of many freshwater/soil amoebae, but amongst amoebae found in marine environments are known only in intrusive (Acanthamoebidae, *Paratetramitus*) or brackish *(Heteramoeba)* groups.

Physiological characters. Several have been examined sporadically. The *maximum relative locomotive rate* is the maximum ratio of μm/min to length of amoeba at temperatures near 22°; usually the rates of 10 amoebae are measured. It is of value with limax amoebae. For hartmannellids, it is usually less than 3.0, and for vahlkampfiids often more than 5.0, though it may be less (Page 1978a). A new species described in this publication, *Vahlkampfia dumnonica*, is an example of a slow vahlkampfiid.

Euryhalinity, investigated mainly to determine the possible ecological range of a species, has sometimes appeared to correlate well with morphological characters (Page 1974b). In these experiments, amoebae were subcultured from their normal medium to media of progressively lower salinities, usually starting with MY100S (medium 6) and moving down to agars made with 75%, 50%, 25% and 10% seawater, and then to agar made with amoeba saline (Page 1976a). At each step, a control medium of the same salinity as the parent culture was also inoculated. Population growth was taken as a positive result, and the test was repeated 2 or 3 times if negative. The method needs further development, including quantification.

Generation time might be of some value, as it seems to be with large freshwater amoebae (Page and Kalinina, in press), but observations suggest a strong possibility of intraspecific variation in smaller amoebae.

Some amoebae show growth habits that seem characteristic, such as burrowing into the agar or forming low mounds of cells on the agar, but the latter habit needs to be separated experimentally from effects of the bacterial population.

No electrophoretic or other biochemical or immunological information is yet available on truly marine amoebae, ie excluding *Acanthamoeba* and *Paratetramitus*.

Fine structure. Although the key has been written as far as possible for identification by light microscopy alone, some isolates will require electron microscopy, eg to distinguish certain *Vannella* species from *Platyamoeba* and to confirm a distinction between *Mayorella* and *'Dactylamoeba'*. For the non-specialist or the worker who needs only identification to genus in uncloned material, electron microscopy may actually save time.

The most useful character is surface structure. The glycocalyx may be amorphous and thin *(Gruberella, Heteramoeba, Flabellula)* or thick *(Thecamoeba)*. It may consist of various configurations which are not resolvable into separate structures: fuzzy, with a suggestion of an organized pattern *(Vahlkampfia, Hartmannella)*, hexagonal *(Platyamoeba, Nolandella)*, or tubular (*Paramoeba* spp. but not *P. eilhardi*). More discrete structures are *glycostyles*, complex, flexible differentiations, each set apart from its neighbours in a definite pattern and not removable intact from the surface (Figure 2). These are pentagonally symmetrical in *Vannella* (Figures 81-83), and hexagonal in *Vexillifera* (Figures 152, 153) and the related *Pseudoparamoeba* (Figure 154). Discrete, boat-shaped, more or less rigid scales, which may be separated from the surface in some whole-mount preparations, occur on *Paramoeba eilhardi* (Figure 117) and the *Mayorella*-like amoebae here tentatively designated *Dactylamoeba* (Figures 138, 139). A thick cuticle, made up of funnel-like elements embedded in a fibrous matrix, is found on amoebae which are here recognized as true representatives of the genus *Mayorella*, though they were earlier separated as *Hollandella* (Page 1981c, 1983) (Figures 134-137).

Ordinarily, the Vahlkampfiidae should be distinguishable from other limax amoebae (eg Hartmannellidae, *Nolandella*) by light microscopy, but another distinctive character is the absence of dictyosomes with the classical structure of a stack of flattened saccules. These have not been found in any vahlkampfiids, including my sectioned material of *Heteramoeba*. On the other hand, *Nolandella hibernica*, which has a vahlkampfiid-like eruptive locomotive habit, possesses a conspicuous dictyosome, and dictyosomes are also present in the somewhat eruptive *Rhizamoeba* and in *Hartmannella*.

Mitochondrial structure is unlikely to be of great help, though the envelopment of each mitochondrion by rough endoplasmic reticulum appears consistent in certain species.

The presence of oriented fibrillar material in subpseudopodia and radiate pseudopodia of certain genera is of considerable importance for establishing the taxonomy, but cannot be considered a practical character for identification.

Finally, the structure of the nuclear envelope (presence or absence of distinct internal lamina and, if present, its structure; Page 1978b) is of value in freshwater members of the Amoebidae, but that family appears to be rare in saltwater.

Use of the keys

The initial key is to genera rather than families, because the familial classifications of 2 new genera have not yet been determined but also, more importantly, because most families include genera for which electronmicroscopical information has not yet been obtained. Since the generic key includes only genera for which some ultrastructural information is available, absence of a named genus does not imply that it is invalid. Some genera not included in the key are listed and characterized under their families, others under 'Genera and species not listed elsewhere'. All genera are included in the taxonomic index.

From the generic key, the user is led into the key to species of each genus by the page number after each generic name. This second set of keys is grouped according to families, and it is in the section on families that an overall picture is given, whether or not ultrastructural and other information is available. The families are arranged in the same order in which their constituent genera appear in the key to genera, not in any order of taxonomic relationship. Under each family are keys to species of genera included in the generic key, lists of other species which have not been studied first-hand and for which certain information is not available, and lists of other genera and their constituent species. Both 'other genera' and 'other species' are characterized briefly, so that some idea of them can be obtained. Finally, attention is called to references where further information about each family and genus can be found.

Terms used in the keys have been defined in the section 'Characters for identification'. L = length of locomotive form; B = breadth of locomotive form; L:B = length/breadth ratio of locomotive forms. Where not otherwise stated, the nucleus is vesicular with a central nucleolus. When a range of means is given (eg $\bar{x}$ 45-55 µm), this indicates a considerable difference in the means obtained from either different strains of the same species or from different sets of measurements of the same strain. Since this publication is intended for identification, such an indication of diversity is considered the most useful one for practical purposes. See discussion under 'Measurements', p. 12.

Information not strictly necessary for the dichotomies but helpful in making the correct choice is included. In the keys to species and the short characterizations of

species not included in the dichotomous keys, as much diagnostic information as possible is included.

Parasitic species, whether or not they belong to genera which also include free-living species, are listed in the later section, 'Some parasitic marine amoebae'. Attention is also called to the separate section on *'Pontifex maximus'*, described by Schaeffer (1926) as a naked amoeba but actually a stage of the testacean *Trichosphaerium*.

Key to genera

1 Locomotive form always limax; no flattened flabellate or spatulate form 2

– Locomotive form not limax, or, if limax, also with less active flabellate or spatulate form 8

2 Locomotion with markedly eruptive bulging, occasionally with hyaloplasm spilling back along sides 3

– Locomotion with steady flow, often slight anterolateral bulging, but not markedly eruptive and without spilling of hyaloplasm along sides 7

3 Normally multinucleate thick cylinder; in sections, often with dense bodies throughout cytoplasm and around cell periphery; very thin, smooth glycocalyx ..... ***Gruberella,*** p 17

– Normally uninucleate 4

4 Nuclear division mesomitotic; surface coat of filaments, in tightly packed hexagonal elements, rising to distinct line 30 nm above plasma membrane; prominent dictyosome often adjacent to nucleus ..... ***Nolandella,*** p 18

– Nuclear division promitotic; surface coat much thinner than that of *Nolandella*; Golgi system not in form of dictyosomes 5

5 No flagellate stage; no marine species (except one from low salinity in Baltic) known to form cysts ..... ***Vahlkampfia,*** p 19

– Cyst-forming, with flagellate stage which is often difficult to obtain 6

6 Flagellate globose, with 2 long flagella, prominent anterior collar, and anterior nucleus; cyst wall smooth, often with delicate, somewhat wrinkled outer membrane ..... ***Heteramoeba,*** p 20

– Flagellate elongate, normally biflagellate but with much variation of number, with anterior rostrum and with nucleus in middle or posterior region; cyst often with outer wall thrown into waves but not wrinkled; a euryhaline soil/freshwater organism ..... ***Paratetramitus,*** p 22

7 Hyaline cap almost always present in continuing locomotion; no villous-bulb uroid; surface coat fuzzy ..... ***Hartmannella,*** p 22

– Hyaline cap often absent in continuing locomotion; villous-bulb uroid often present; glycocalyx very thin ..... ***Saccamoeba,*** p 23

8 Locomotive forms either limax (but also with less active flabellate or spatulate form) or markedly flattened; no subpseudopodia, except very short, non-furcate ones on flabellate forms of one genus; never slender, branched cells 9

– Locomotive forms never limax; either compressed and with subpseudopodia, or long, branched floating and attached forms, or branching plasmodia or pseudoplasmodia 14

9 Locomotive form often limax, sometimes flabellate or spatulate, often markedly eruptive, often with prominent collopodium; stationary forms often flabellate, spatulate or irregular, often with fine holdfasts around periphery; dense collosomes (Figure 42) around cell periphery in species whose fine structure is known; one or more nuclei ..... ***Rhizamoeba,*** p 23

– Locomotive forms flattened only, never limax, though flattened forms of some species occasionally elongate 10

10 Pellicle-like surface with a few parallel, longitudinal dorsal folds or more numerous, less regularly orientated wrinkles; all species *except one* oblong, with L:B often more than 1.5, and (except in one species) hyaloplasm a crescent extending part of the way back along sides; glycocalyx thick (at least 16 nm, usually more), dense, not composed of discrete elements ..... ***Thecamoeba,*** p 25

– Granuloplasmic mass preceded by extensive flattened hyaloplasm, which may extend around sides; amoebae often broad; no parallel, longitudinal dorsal folds 11

11 No marked eruptive activity, no uroidal filaments; outline usually smooth, may be flabellate, oval, semicircular, elliptical, oblong, sometimes spatulate; L:B $\bar{x}$ commonly near 1.0 12

– Commonly flabellate, sometimes spatulate or elongate, with anterior edge often irregular;

frequently with trailing uroidal filaments; rapidly and frequently changing shape, with some eruptive activity; no collosomes; glycocalyx thin 13

12 Outline seldom if ever spatulate; floating form with a few hyaline pseudopodia not tapering greatly from base, or no pseudopodia; glycocalyx, usually 20-30 nm thick above plasma membrane, composed of tightly packed, filamentous hexagonal elements, each with diameter about 40 nm ..... ***Platyamoeba,*** p 26

— Outline often flabellate, occasionally spatulate with long tail; floating forms of *some* species with long, slender, radiate pseudopodia; surface covered with pentagonally symmetrical glycostyles, 95-120 nm tall above plasma membrane, composed of 5 radially arranged wings around central tubule..... ***Vannella,*** p 28

13 No subpseudopodia from hyaline zone, though that zone may become cleft ... ***Flabellula,*** p 31

— Short, unbranched subpseudopodia produced from hyaline zone..... ***Paraflabellula,*** p 33

14 Locomotive form with conical, hyaline subpseudopodia, usually produced from hyaloplasm; L:B $\bar{x}$ commonly 1.5 or more, often 2.0 or more 15

— Long, slender, branched, relatively rigid floating or attached forms, or branching and anastomosing plasmodia or pseudoplasmodia, or both..... 21

15 Subpseudopodia not furcate; no centriole-like body known; no cysts known; surface coat highly differentiated 16

— Subpseudopodia often furcate at base, tapering to narrow tip; centriole-like microtubular organizing centre; double-walled cysts; surface coat a thin glycocalyx; soil/freshwater organisms often isolated from marine sources 20

16 One or more DNA-containing parasomes adjacent to nucleus; one species with many short, dactyloid subpseudopodia and boat-shaped scales (indiscernible with light microscope); others often with one or more dactyloid subpseudopodia from dorsal ridge or extensive hyaloplasm, and glycocalyx about 10 nm thick, possibly consisting of tightly packed tubular elements .... ***Paramoeba,*** p 34

— No parasome 17

17 Medium-sized amoebae (mean L usually more than 50 μm), with short, round-tipped dactylopodia which may be temporarily lacking; cell covered with thick, fibrous cuticle or complex scales, both discernible only with electron microscope 18

— Somewhat smaller amoebae, often with slender, relatively long subpseudopodia; surface covered with hexagonal cylindroid or blister-like glycostyles 19

18 Surface covered with cuticle about 200 nm thick composed of thin-walled, funnel-shaped elements perpendicular to cell surface in fibrillar matrix; dactylopodia often temporarily absent in some species; floating form usually without distinctly spherical central mass ..... ***Mayorella,*** p 34

— Surface covered with complex, boat-shaped latticework scales, 0.5 μm or slightly longer; locomotive form like that of *Mayorella* but with less tendency to lack dactylopodia; floating form more likely to have spherical central mass and slender radiate pseudopodia..... ***Dactylamoeba,*** p 36

19 Locomotive form with thin, sometimes long and linear subpseudopodia; surface covered with discrete glycostyles in form of hexagonal cylinders rising approximately 60-70 nm above plasma membrane ..... ***Vexillifera,*** p 39

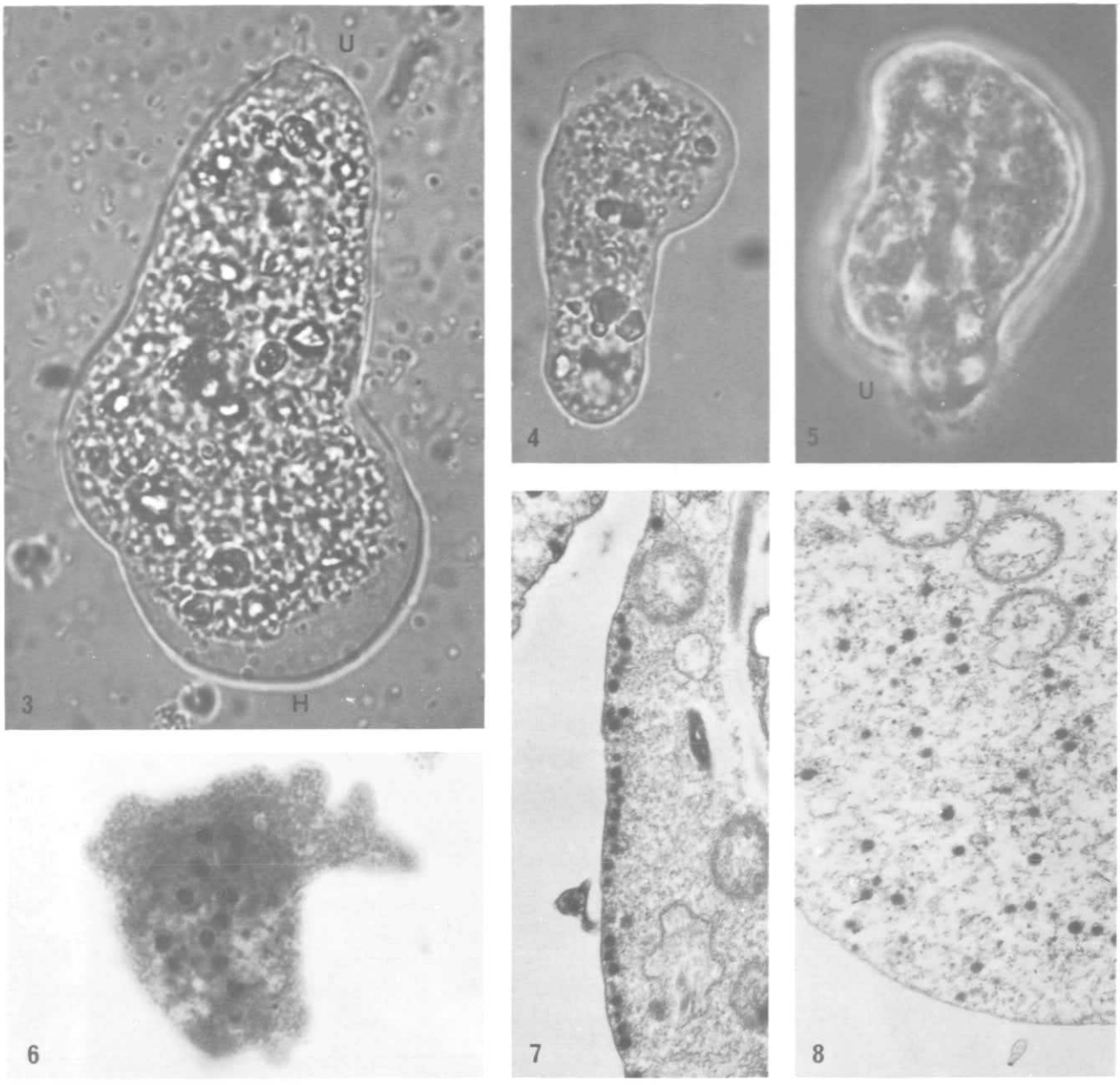
— Locomotive form like small *Paramoeba*, often with one to 3 long, narrow pseudopodia, often as anterior continuations of ridges in extensive hyaloplasm; floating form characteristically with spherical central mass and fine, long, radiate pseudopodia; surface covered with blister-like glycostyles rising to about 35 nm above plasma membrane, with hexagonal basal structure identical to that of *Vexillifera* glycostyles..... ***Pseudoparamoeba,*** p 39

20 Excystment by removal of opercula at points of contact between inner and outer cyst walls; inner cyst wall polygonal or stellate in some species, more rounded in others . ***Acanthamoeba,*** p 41

— No preformed pores or opercula in cyst wall; excystment through opening broken in wall; cysts usually circular or oval in outline, with 2 layers of wall not always distinguishable ..... ***Protacanthamoeba,*** p 43

21 Multinucleate, reticulate plasmodia on substratum; long, branched, relatively rigid multinucleate or uninucleate floating forms . ***Corallomyxa,*** p 43

— Reticulum of uninucleate amoebae on substratum; floating or attached uninucleate hunger forms .... ***Stereomyxa,*** p 43



Figures 3-8. Gruberella flavescens. *3-5, living amoebae; × 1000. 6, haematoxylin, showing nuclei; × 1000. 7,8, sections, showing dark bodies (7, concentrated at surface; 8, dispersed in cytoplasm) and surface; × 10000. H, anterior hyaloplasm; U, uroid.*

Families

INCERTAE SEDIS

These 2 new genera do not clearly belong to any existing family of Gymnamoebia or Leptomyxida. Both have a limax locomotive form and a somewhat eruptive habit. They are excluded from the Vahlkampfiidae because their nuclear division is not promitotic and the Golgi system (in at least one of these genera) is organized into recognizable dictyosomes. *Gruberella* bears some resemblance to some Leptomyxidae, but differs in lacking a flabellate or other regular flattened form. It is more consistently multinucleate than leptomyxids in its size range. *Nolandella* likewise bears some resemblance to the leptomyxids, but is distinguished by its surface structure and prominent dictyosome, usually with a definite position, and also by its lack of the flabellate form. It is regularly uninucleate, with no more tendency to supernumerary nuclei than other ordinarily uninucleate genera.

Genus *Gruberella* n. gen.

Reference: Gruber (1889)

Diagnosis: Normally multinucleate; thickly limax, moving by rapid flow accompanied by production of hemispherical hyaline bulges and some eruptive activity; few to many collopodial filaments on posterior end; nucleolus disintegrating during mitosis; mitochondria present; surface coat a thin glycocalyx.

Type species: *Gruberella flavescens* (Gruber 1889) n. comb.

Amongst the characters distinguishing this genus from *Pelomyxa*, in which even some recent authors have classified all multinucleate lobose amoebae, are: locomotive activity, presence of mitochondria and possibly dictyosomes, absence of bacterial endosymbionts, and inclusion of non-pigmented protists in its diet. The occurrence of mitosis also separates it from *Pelomyxa*, if that process is indeed lacking from the latter genus. Multinuclearity and the occurrence of posterior collopodial filaments are not in themselves, together or separately, distinctive of any one genus or even family.

The type species is the only one definitely known to belong to the genus:

Length in locomotion 26-163 μm (x̄ 60 μm); L:B 1.5-3.7 (x̄ 2.4); hyaline cap sometimes briefly absent; nucleus with single nucleolus, central or sometimes eccentric; observed nuclear numbers 1-37, with few amoebae uninucleate and mean nuclear number about 6; nuclear diameter in fixed preparations 3.2-5.1 μm (x̄ 3.9 μm); no cytoplasmic crystals (Figures 3-8) ..... ***G. flavescens*** (Gruber 1889).

This species will be described more fully elsewhere. Since multinucleate amoebae in general show considerable variation in size and nuclear number, findings greater than those reported above may be expected.

There is little reason to doubt that the strains in culture, isolated from a rock pool at Hannafore Point, Cornwall, belong to the species which Gruber described from the harbour of Genoa, 'the first true multinucleate amoeba which I have found up to now in the sea'. Very similar organisms have been found on the coasts of Essex and the north-eastern USA.

Media: 1 with rice grain; 7 with an overlay of 1. Observed to feed on unicellular algae, *Cyclidium* sp., colourless flagellates, *Vahlkampfia dumnonica*, and possibly a heliozoan-like organism.

Goodkov *et al.* (1982) have just described under the name *Hyperamoeba fallax* Seravin & Goodkov 1982, another multinucleate limax marine amoeba, isolated from an aquarium containing algae and invertebrate animals from Peter the Great Bay on the Sea of Japan. It was 92-230 μm long (x̄ 141 μm), with a villous-bulb uroid, usually multinucleate (nuclear number to several 10's in large specimens), with no known flagellate stage or cyst. Since cytoplasmic inclusions made the nuclei indistinguishable in living amoebae, the diameters of nuclei (5-6 μm) presumably were measured in fixed preparations. Goodkov *et al.* described a process of fusion and later separation of amoebae which Seravin *et al.* (1982) interpreted as 'paracopulation'.

This Far Eastern amoeba does not appear to belong to the same species as *G. flavescens*. Although both have vesiculate nuclei, the nucleolus of *H. fallax* apparently differs in details from that of *G. flavescens*, locomotive activity seems to differ, and the collopodial filaments of *G. flavescens* do not constitute a villous-bulb uroid (Page 1972a). Further comparisons will be included in the later publication on *G. flavescens*.

The name *Hyperamoeba* Seravin and Goodkov 1982 (in Goodkov et al. 1982) is a junior homonym of *Hyperamoeba* Alexeieff 1924, which Alexeieff (1924) applied to a copricolous amoeboflagellate. The family name Hyperamoebidae Seravin and Goodkov 1982 can therefore not be used. Seravin and Goodkov (personal communication) intend to propose *Euhyperamoeba* as a substitute.

There are no certain indications that *G. flavescens* and *H. fallax* belong to the same genus or family, nuclear number and size being inadequate to determine their relationships. Possibly the freshwater amoeba which Penard designated *'Amoeba peritissima'* on one of his prepared slides (Page 1981b) belongs to the same genus as one or both of these marine organisms.

Genus *Nolandella* n. gen.

Reference: Page (1980c)

Diagnosis: Uninucleate; in locomotion, limax-like, with some eruptive activity producing hyaline bulges and frequent changes of shape; nucleolus disintegrating during mitosis; surface coat of tightly packed hexagonal elements with diameter approximately 35 nm; dictyosome present.

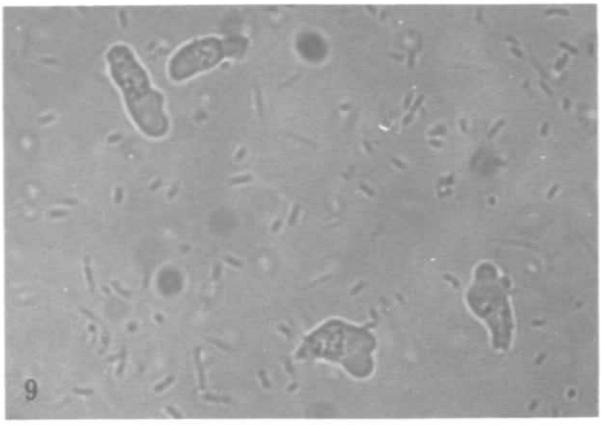
Type species: *Nolandella hibernica* (Page 1980) n. comb.

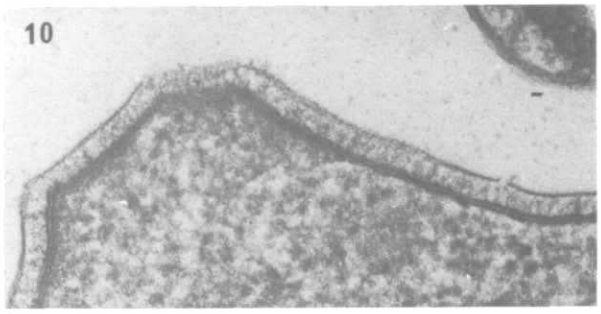
This genus is named in honour of the late Professor L E Noland of the University of Wisconsin.

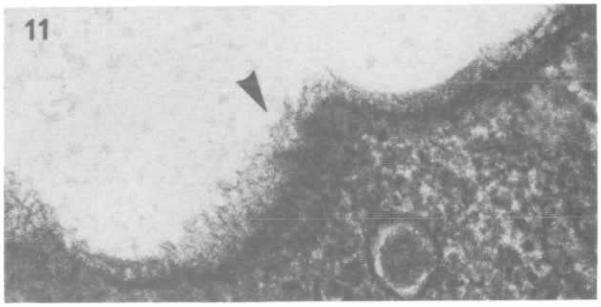
A single known species:

Length in locomotion approximately 8.5-19 μm (x̄ 11.8 μm); L:B 1.7-5.0 (x̄ 2.9); nucleus 1.9-3.7 μm (x̄ 2.6 μm). Floating form often with distinct central mass and approximately 5-12 hyaline, blunt, radiating pseudopodia with length usually slightly less than diameter of central mass. Glycocalyx approximately 30 nm thick above plasma membrane, terminating at dense line parallel to plasma membrane; a single prominent dictyosome often but not always adjacent to nucleus; mitochondria wrapped loosely in rough endoplasmic reticulum. (Figures 9-11) .... ***N. hibernica*** (Page 1980) (Atlantic coast of Ireland; medium 7)

Although this species was provisionally classified in *Hartmannella* when it was described, some differences from that genus were noted (Page 1980c). These included eruptive activity (though sluggish) and shape,







Figures 9-11. Nolandella hibernica. *9, living amoebae;* × *1000. 10, 11, sections of surface; 10,* × *100000; 11,* × *75000, showing tangential section of surface with cross-sections of apparently hexagonal elements (arrow).*

more like the Vahlkampfiidae, from which it differs in both mitotic pattern and presence of the dictyosome. The surface structure differs from that of both Hartmannellidae and Vahlkampfiidae and resembles that of *Platyamoeba* (q. v.).

Our strains have a strong tendency to burrow in the agar.

Family VAHLKAMPFIIDAE Jollos 1917

References: Darbyshire et al (1976); Page (1967a, 1974a, 1978a, 1980c)

Limax amoebae with locomotion by hemispherical, hyaloplasmic eruptions, alternating to either side at anterior end and sometimes running back along side toward posterior end; mean L:B commonly less than 4.0, often less than 3.0; nuclear division promitotic; flagellate phase in all genera but one; Golgi system not in form of dictyosomes.

Essentially, these are limax amoebae with promitosis. With a few exceptions, they are also more or less distinguished from limax amoebae of the family Hartmannellidae by several other characters: (i) markedly eruptive locomotive activity, which is, however, also found in some other limax and flabellate amoebae; (ii) mean L:B *usually* less than 3.0 (Page 1978a); (iii) maximum relative locomotive rate (see p. 13) *usually* more than 4.0 (Page 1978a); (iv) tendency in many species to supernumerary nuclei, ie for proportionately more cells to have 2 or more nuclei than in most uninucleate species (Page 1974a, 1978a); (v) absence from all vahlkampfiids so far examined electron-microscopically of the dictyosome in the form of a stack of flattened cisternae, a structure thus far found in all Amoebida. In some vahlkampfiids, the nucleolus is larger in proportion to the nucleus than in most other amoebae (Figure 16). However, the light microscopist should recognize the necessity in this case of seeing at least one metaphase or anaphase.

Genus *Vahlkampfia* Chatton and Lalung-Bonnaire 1912

References: Page (1967a, 1974a, 1980c); Schmoller (1961, 1964); Schmoller *et al.* (1982)

Vahlkampfiids without a flagellate stage

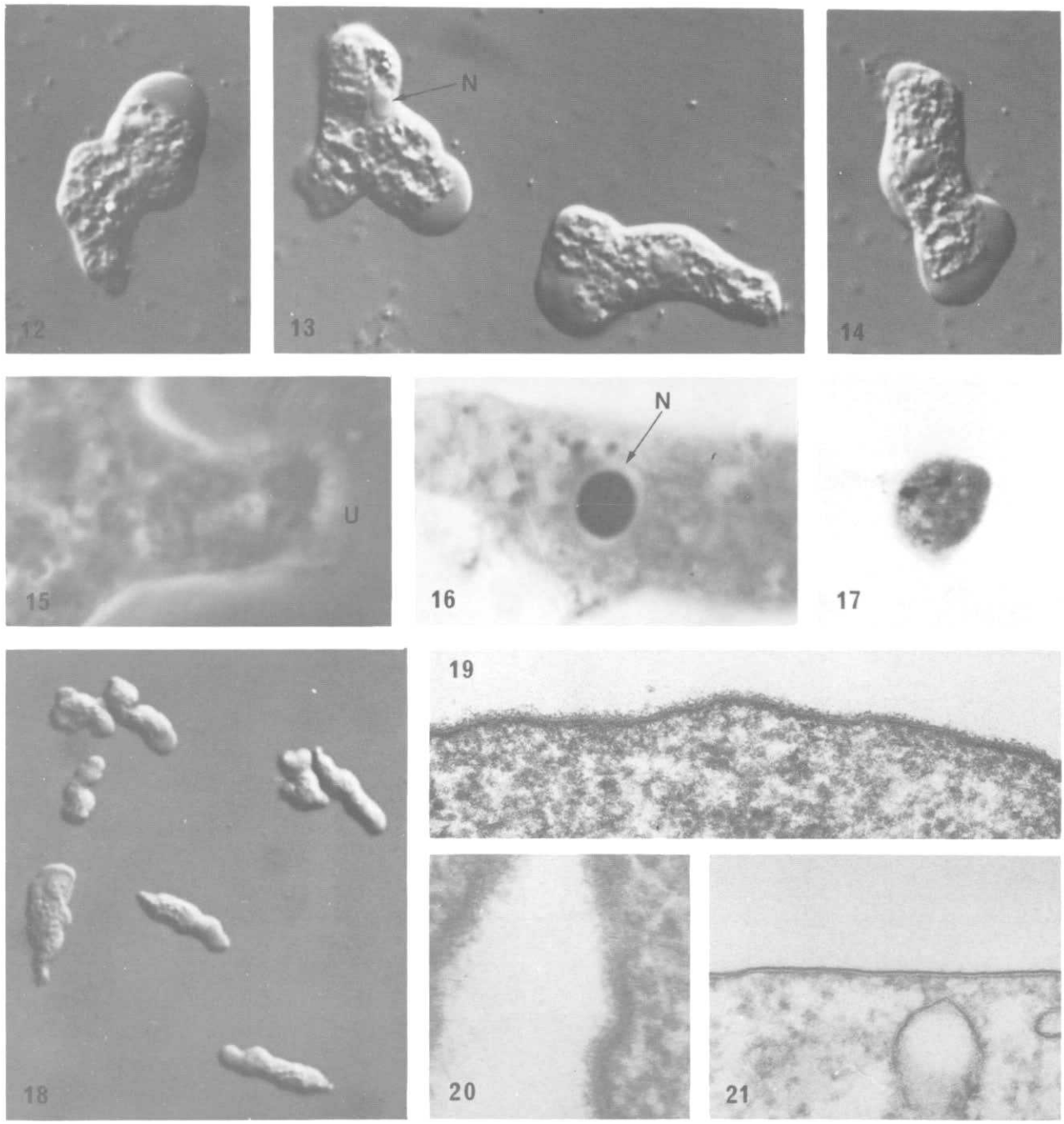
Because amoeba-flagellate transformation has been little investigated in marine organisms, the uncertainty about existence of a flagellate stage may be greater than with freshwater strains. Furthermore, no truly marine *Vahlkampfia* is known to produce a cyst (despite Schmoller 1961 and Schmoller *et al.* 1982; see below), so that species identification by cyst characters is not possible. The similarity amongst trophic amoebae of the genus makes it unlikely that all species are morphologically distinguishable.

1 L 24-54 μm ($\bar{x}$ 38 μm), L:B 1.8-3.9 ($\bar{x}$ 2.6); sometimes short uroidal filaments; nucleus 5.6-7.5 μm ($\bar{x}$ 6.3 μm), $\bar{x}$ nucleus/nucleolus 1.4; fine structure unknown; grows in liquid best c. 35 o/oo salinity. (Figures 12-17) . . . . . ***V. dumnonica*** n. sp. (Cornwall; medium 4 with rice)

– L 8-25 μm ($\bar{x}$ 14 μm), L:B 1.7-4.4 ($\bar{x}$ 2.7); a few uroidal filaments common; nucleus 2.4-4.7 μm ($\bar{x}$ 3.5 μm), $\bar{x}$ nucleus/nucleolus 1.7; glycocalyx c. 6 nm thick; occurs in salinity c. 26 o/oo, grows on agar with salinity 26-35 o/oo. (Figures 18-20) . . . . . ***V. damariscottae*** Page 1974. (Western North Atlantic; media 6-9)

Vahlkampfia dumnonica n. sp.

Diagnosis: Length 24-54 μm ($\bar{x}$ 38.3 μm), length:breadth ratio 1.8-3.9 ($\bar{x}$ 2.6); nucleus 5.6-7.5 μm ($\bar{x}$ 6.3 μm), nucleolus 3.7-5.6 μm ($\bar{x}$ 4.4 μm); typical vahlkampfiid form and locomotion, with eruptive formation of hemispherical bulges and reflection of hyaloplasm along



Figures 12-21. Vahlkampfiidae. 12-14, Vahlkampfia dumnonica, *living amoebae; × 1000. 15,* V. dumnonica, *uroid of living amoeba; × 2500. 16, 17,* V. dumnonica, *haematoxylin, showing (16, × 2500) interphase nucleus and (17, × 1000) promitotic division. 18,* Vahlkampfia damariscottae, *living amoebae; × 1000. 19, 20,* V. damariscottae, *sections of surface (19, × 100000; 20, × 150000). 21,* Heteramoeba clara, *section of surface; × 100000. N, nucleus; U, uroid.*

sides; sometimes short collopodial filaments on rounded posterior end. Maximum relative locomotive rate, recorded at 23°, twice length of amoebae per minute. No cyst known.

Observed habitat: south coast of Cornwall.

Other species:

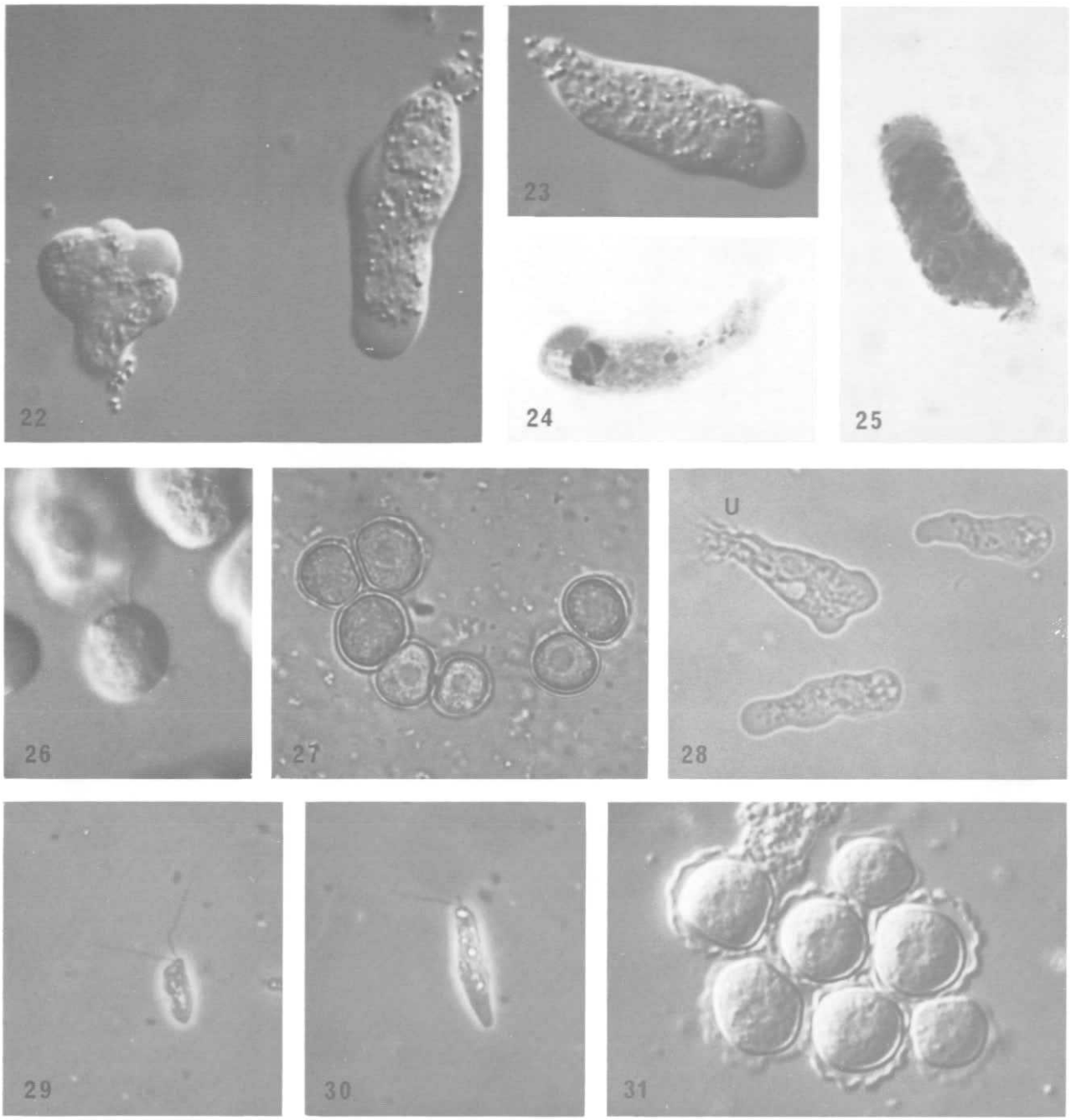
V. baltica Schmoller 1961. L 20 μm. The cysts pictured by Schmoller (1961) are those of an *Acanthamoeba*, and the cultures contained amoebae of *Acanthamoeba*. No mitotic figures reported. Baltic Sea, near Warnemünde; salinity presumably less than 10 o/oo.

V. longicauda Schmoller 1964. L 7.5-20 μm; uroidal filaments; promitosis. Baltic Sea, near Warnemünde.

V. trilaminata Schmoller, Jonas and Ludvik 1983 (publication dated 1982). L 10-20 μm; cyst 4-7.5 μm. Electron micrographs show no dictyosomes. Baltic Sea, near Warnemünde; perhaps an intrusive freshwater/soil organism or a brackish cyst-former like *Heteramoeba clara*.

Genus *Heteramoeba* Droop 1962

References: Carey (1978, 1979); Droop (1962, 1966); King and Preston (1979)



Figures 22-31. Vahlkampfiidae (continued). 22-27, Heteramoeba clara. *22, 23, living amoebae; × 1000. 24, 25, haematoxylin, showing uninucleate (24) and binucleate (25) amoebae; × 1000. 26, flagellate, × 1000. 27, cysts; × 1000. 28-31,* Paratetramitus jugosus. *28, living amoebae; × 1000. 29, 30, flagellates; × 1000. 31, cysts; × 1500. U, uroid.*

Vahlkampfiids with flagellate stage a globose cell, with two equal flagella, a deep cytostome between the main cell body and an encircling collar, and the nucleus near the flagellar insertion; feeding and dividing in both amoeboid and flagellate phases; cysts produced by cultures composed wholly or in part of amoebae.

A single known species

L of amoebae 12-47 μm ($\bar{x}$ 28 μm); nucleus 4.2-6.5 μm ($\bar{x}$ 5.3 μm), with nucleolar material in parietally arranged pieces; very thin, smooth glycocalyx. Mature flagellates to 30 μm, with flagella 60 μm long. Cysts 9.2-23.0 μm ($\bar{x}$ 15.5 μm), rounded to elliptical in outline, sometimes with thin outer layer somewhat wrinkled; excystment by breaking through preformed weak area in wall. (Figures 21-27) . . . . . ***H. clara*** Droop 1962 (Finland, Scotland; media below)

Droop (1962) reported amoebae to 70 μm long. Occasional binucleate amoebae and flagellates, less often cysts, were seen. Sizes of amoebae grown in liquid and on agar were very similar.

Indications that a sexual process was involved in the alternation of amoeboid and flagellate phases were reported by Droop (1962). Through 1975, flagellates were readily obtained from the strain maintained at

CCAP by suspending amoebae in full-strength or 75% seawater, but not all cultures yielded flagellates, and in the past few years none has been seen.

Droop considered this a brackish-water organism and grew it on several media of low salinity. It feeds on *Brachiomonas submarina*, *Dunaliella salina* or bacteria. When inoculated at the end of a streak of *Escherichia coli* on medium 7, it produces many amoebae and cysts. It also grows in a 1:1 mixture of medium 4 and Cerophyl-Prescott liquid (Page 1976a) (final salinity c. 17.5 o/oo), feeding on unidentified bacteria. Details of bacteria-free culture were given by Droop (1962).

Genus *Paratetramitus* Darbyshire, Page and Goodfellow 1976

References: Darbyshire *et al.* (1976); Margulis *et al.* (in press); Page (1967a); Read *et al.* (in press).

Vahlkampfiids with flagellate stage having 2 anteriorly inserted non-mastigonemate flagella, which are normally of equal length and originate in a depression; nucleus at or slightly posterior to mid-point of length; dividing in both amoeboid and flagellate phases; cysts formed in only known species.

A single described species:

L of amoebae 13-38 μm (x̄ 20-26 μm); nucleus c. 2.8-4.0 μm. Flagellates usually 14-19 μm long, with L:B ratio about 4.0. Cysts with single wall or with partly separated outer layer thrown into irregular waves, not wrinkled and without ostioles or opercula; diameter 5-16 μm, with mean of 10 μm or less in most strains. (Figures 28-31) ..... ***P. jugosus*** (Page 1967)

This species has never been reported from a marine habitat. However, its isolation from a hypersaline lagoon in Mexico (Margulis *et al.* in press) and the finding that those organisms were euryhaline (Read *et al* in press) have led to experiments with 2 of our strains, one from freshwater in the USA and the other from soil in Scotland, which demonstrated their euryhalinity. Strains of this species which have been tested grew on both non-nutrient agar, a freshwater medium (Page 1976a), and marine medium 7, both streaked with *Escherichia coli.* In view of the frequent findings of the common freshwater/soil genus *Acanthamoeba* in marine samples, the isolation of *P. jugosus* from such samples seems possible. This species is known from freshwater and soil in Europe and North America.

Flagellates have been obtained from 1 to 3-day cultures on non-nutrient agar (Page 1976a) by adding about 10 drops of sterile amoeba saline, distilled water, or tap water and preparing hanging drops. The drops should be observed at intervals for a day. A few strains otherwise identifiable as *P. jugosus* did not produce flagellates, and others gradually lost the ability to do so. Supernumerary nuclei and sets of flagella are fairly common in the flagellates.

The cyst is also useful in identification. Occurrence of cysts with and without a partly separated outer layer does not indicate a mixed culture (Darbyshire *et al.* 1976), and the separation of a pseudoexocyst and its wavy appearance are probably the result of splitting of a single wall while the cell is still dehydrating, as observed elsewhere (Page 1981a).

Other genera of Vahlkampfiidae

Tetramitus Perty 1852

Reference: Ruinen (1938)

These organisms are known primarily in their flagellate stage, with 4 flagella, and indeed amoeboid forms appear to be known for only one freshwater species, *T. rostratus* (Perty 1852), and one marine species, *T. salinus* Entz 1883. The flagellates are encountered occasionally in marine material. In view of the lapse of 70 years between the first description of *T. rostratus* and the discovery of its amoeboid stage, the possibility of further amoeboid phases in marine species cannot be ruled out. According to the late Professor William Balamuth (personal communication), '*"Tetramitus salinus"*, growing in 20% (=200 o/oo) salinity, produces a typical amoeboid stage upon demand. The flagellate stage is completely unlike *T. rostratus.*' I know of no published description of the *T. salinus* amoeba. Ruinen (1938) also described the species *T. cosmopolitus* and *T. ovoides*, in flagellate form only.

Family HARTMANNELLIDAE Volkonsky 1931; emend. Page 1974

Reference: Page (1974a)

Limax amoebae moving by steady, non-eruptive flow, sometimes with gentle antero-lateral bulging but never with hyaloplasm reflected back along side; mean L:B ratio commonly more than 4.0; nuclear division meso-mitotic; glycocalyx, when discernible, thin, and dictyosome(s) present in species whose fine structure is known.

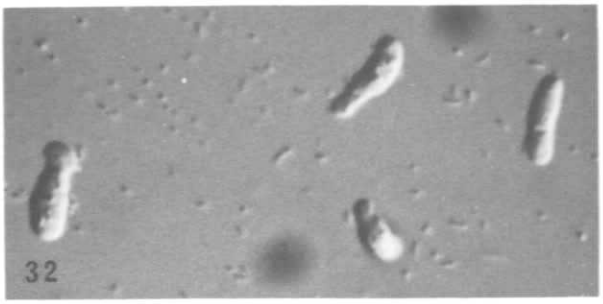
Genus *Hartmannella* Alexeieff 1912; emend. Page 1974

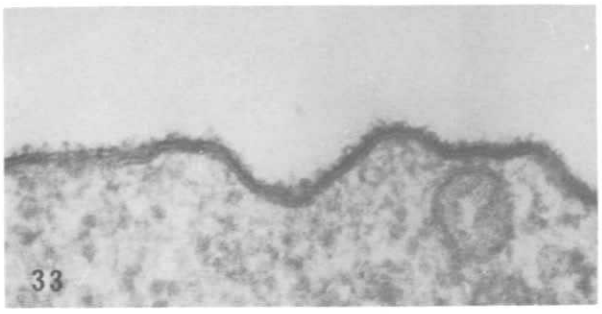
References: Page (1974a, 1980c)

Hyaline cap, usually at least as deep antero-posteriorly as broad, nearly always present in locomotion; no villous-knob uroid or collopodium.

A single described marine species:

L 7.5-19.0 μm (x̄ 11.5 μm), L:B 2.3-5.7 (x̄ 4.0); fixed nuclei 1.4-3.2 μm; glycocalyx c. 10 nm thick above plasma membrane, consisting of units like truncate pyramids, each with basal diameter c. 15 nm. (Figures 32, 33) ..... ***H. abertawensis*** Page 1980 (South Wales; medium 7)





Figures 32, 33. Hartmannella abertawensis. *32, living amoebae; × 1000. 33, section of surface; × 100000.*

Genus *Saccamoeba* Frenzel 1892; emend. Bovee 1972 and Page 1974

References: Bhowmick (1967); Bovee (1972); Gruber (1885); Page (1969a, 1974a)

Hyaline cap reduced to shallow crescent or absent in continuing locomotion, though always seen on initiation of pseudopodium; advance by steady flow, often with non-eruptive slight antero-lateral bulging; villous-bulb uroid with more or less rigid fine villi; where fine structure known, surface coat not distinguishable, dictyosome(s) present.

The presence of this genus in saltwater is not certain; an amoeba from Norfolk shown in Figure 40 of Page (1974a) may be a *Saccamoeba*. Freshwater species often contain conspicuous crystals (Bovee 1972; Page 1969a, under name *Hartmannella*), though cyst-forming strains from freshwater did not contain discernible crystals (Page 1974a). *Amoeba crystalligera* Gruber, 1885, from an aquarium filled from the North Sea, bore some similarity to *Saccamoeba*, though some details do not seem to support such a classification. Schaudinn (1894) also studied an amoeba under that name. A larger amoeba found by Levander (1894) in the Gulf of Finland, which he identified with hesitation as *A. crystalligera*, contained a nucleus unlike that of any known freshwater *Saccamoeba*, but another which Levander called '*Amoeba villosa* Wallich?' could be a *Saccamoeba* without crystals. The amoeba from the Kieler Bucht reported by Möbius (1889) as *A. crystalligera* does not seem to be a *Saccamoeba*.

The electron microscopical characters attributed to this genus were found in freshwater strains (Bhowmick 1967, under name *'Trichamoeba villosa'*; Page, unpublished).

Family LEPTOMYXIDAE Pussard and Pons 1976

References: Pussard and Pons (1976a, 1976b, 1976c)

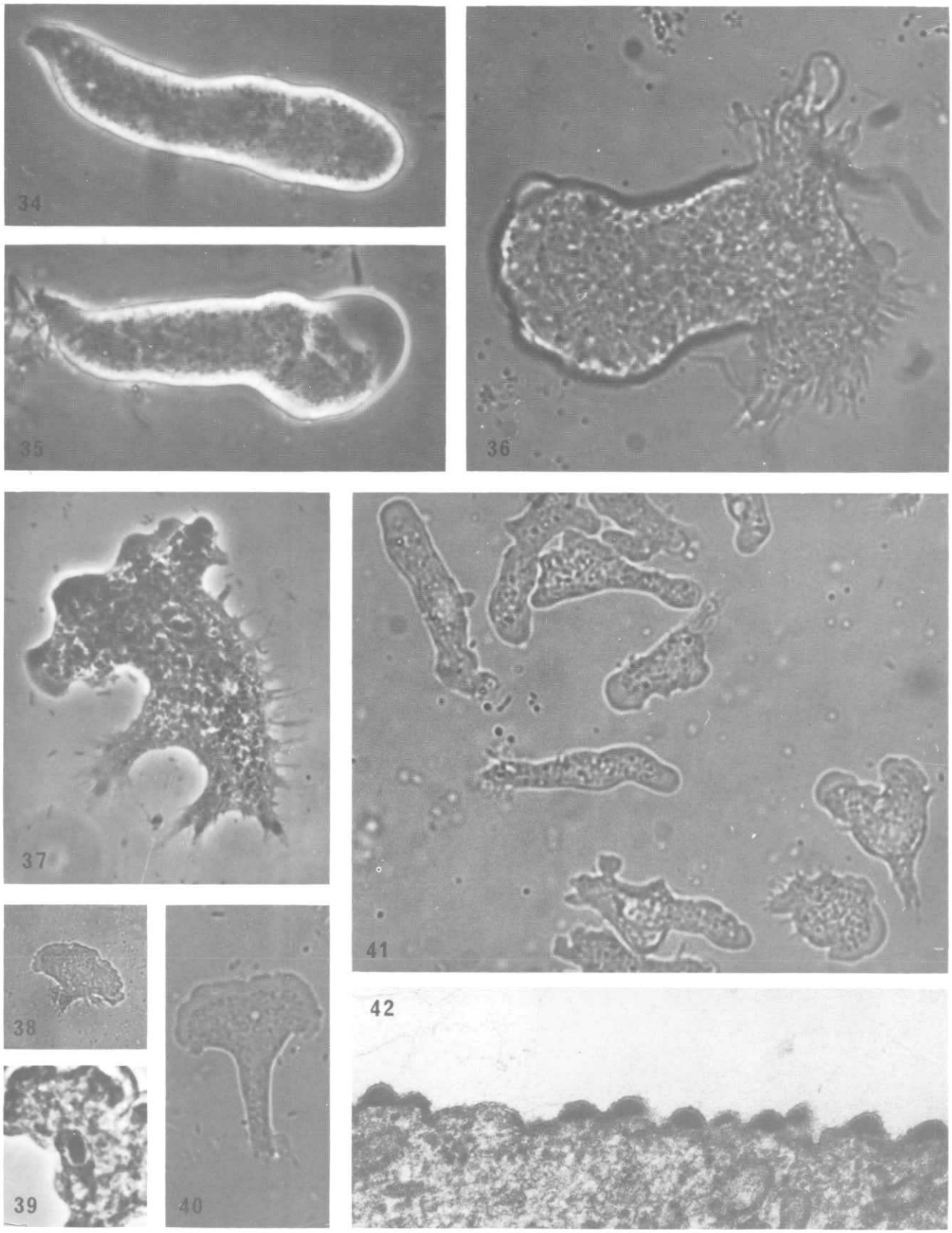
Multinucleate, uninucleate, or both in a given species, with uninucleate predominant in some and microplasmodia in others. Trophic form limax-like (in most active locomotion) and eruptive in some, flabellate, or extended as a thin sheet, with sheet-like forms often highly ramified, even reticulate, and even uninucleate flabellate cells sometimes multilobed; form influenced by substratum and presence or absence of layer of free water; locomotion uni- or polyaxial. Collopodial filaments conspicuous in some species. In those investigated with electron microscope, glycocalyx very thin or undetected, Golgi system in form of dictyosomes. Plasmotomy and fusion observed in some species. Cysts formed by freshwater/soil species, not known in marine species. No spore-producing fructifications.

The family as here defined is equivalent to the order Leptomyxida Pussard and Pons 1976, rather than to their family Leptomyxidae. The similarity between *Rhizamoeba* (to which the marine members belong) and one species of *Leptomyxa* (Page 1976a) gave rise to the proposal (Pussard and Pons 1976b) that *Rhizamoeba* be classified 'à côté des genres *Leptomyxa* et *Gephyramoeba*'. Acceptance of this suggestion (Page 1980c; Levine *et al.* 1980) reveals a problem, since neither Leptomyxidae nor Gephyramoebidae were defined (Pussard and Pons 1976a) in a way permitting inclusion of *Rhizamoeba*. A certain broad interpretation of the Gephyramoebidae could formally accommodate *Rhizamoeba*, but on the basis of trophic form it is unlikely that *Rhizamoeba* is closer to *Gephyramoeba* than to *Leptomyxa*. Because of these unclarities, the present work includes all leptomyxids in a single family whose characterization above is only provisional. The interrelationships of leptomyxids and their relations to other naked lobose amoebae have not yet been satisfactorily settled.

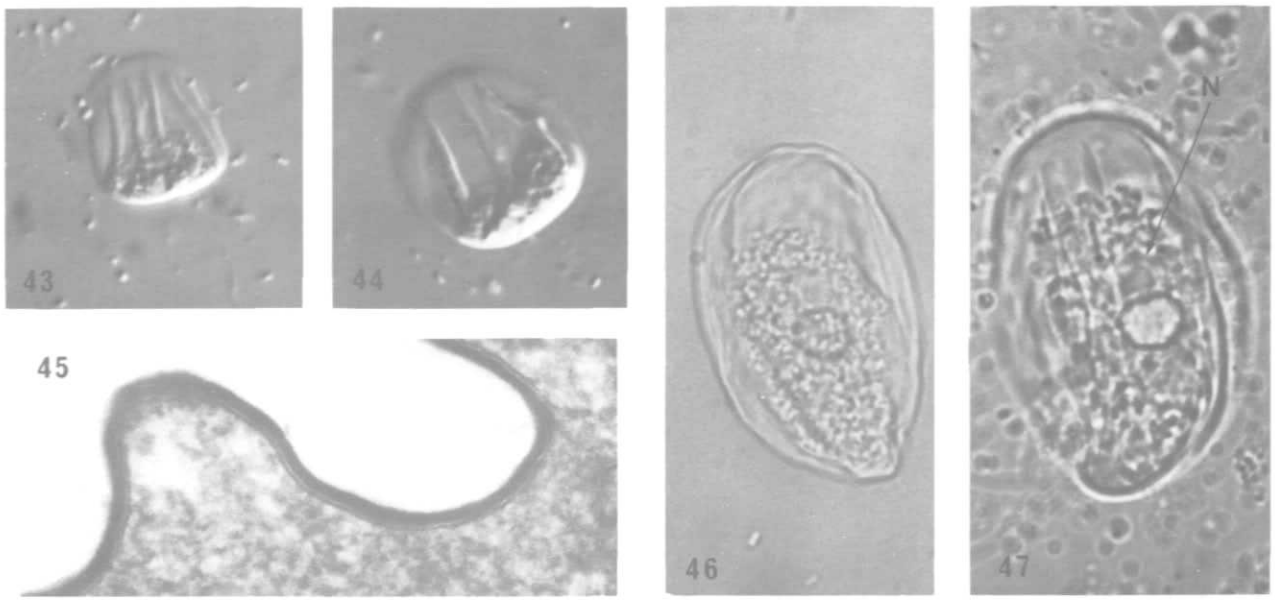
Genus *Rhizamoeba* Page 1972

References: Page (1972a, 1974a, 1980c); Gruber (1889); Hamburger (1905); Schaeffer (1926)

In rapid locomotion limax, often with hyaline cap and posterior collopodium of few to many pseudo-villi; in less active or stationary condition, flattened, often flabellate or spatulate with one or more expanded anterior lobes, each with narrow hyaline anterior border; often with collopodial holdfasts around periphery of stationary forms; occasional eruptive activity during locomotion or while stationary; majority uninucleate, with strong tendency to supernumerary nuclei in some species; nucleus, with central nucleolus, difficult to see in living amoebae; no cytoplasmic crystals; in one species studied electron microscopically, glycocalyx thin or undetectable, lenticular



Figures 34-42. Rhizamoeba. *34-36,* R. polyura, *living; × 1000. 37,* R. polyura, *Kernechtrot, showing many holdfast filaments; × 1000. 38,* R. polyura, *flabellate form; × 250. 39,* R. polyura, *Kernechtrot, showing comet-shaped nucleus; × 1000. 40,* R. saxonica, *flabellate form; × 1000. 41,* R. saxonica, *living amoebae in various forms; × 1000. 42,* R. saxonica, *section of surface, showing collosomes; × 100000.*



Figures 43-47. Thecamoeba. *43-45,* Thecamoeba orbis. *43, American strain in culture; × 1000. 44, amoeba in mixed material from Norfolk; × 1000. 45, section of surface; × 100000. 46, 47,* Thecamoeba hilla. *46, American strain in culture; × 1000. 47, amoeba in mixed material from Essex; × 1000. N, nucleus.*

collosomes just beneath plasma membrane associated with points of adhesion to substratum; nucleus and nuclear membrane disintegrate during mitosis.

1 L of limax form approximately 25-135 ($\bar{x}$ 72 μm), L:B $\bar{x}$ c. 4.3; nucleus rounded, spindle- or comet-shaped, greatest dimension on fixed preparation 4.9-13.7 μm ($\bar{x}$ 7.5 μm); majority uninucleate, many with two nuclei, rarely 16 or more. (Figures 34-39) . . . . . ***R. polyura*** Page 1972 (Western North Atlantic; media 6 and 8)

– L of limax form c. 12.5-45 μm ($\bar{x}$ 25-28 μm), L:B $\bar{x}$ c. 4.3; nucleus round or ovoid, diameter in fixed preparations approximately 2.4-4.6 μm ($\bar{x}$ 3.1 μm); usually uninucleate, sometimes bi- or tri-nucleate; surface fine structure as in generic description. (Figures 40-42). . . . . ***R. saxonica*** Page 1974 (North Sea; media 6 and 7)

Other species which may belong to this genus, as suggested by limax form, pseudo-villi, locomotive habit, inconspicuous nucleus, and occasionally by stationary form, include:

Amoeba globifera Gruber 1889. Diameter 100 μm (in what form?); uninucleate; nucleus described as homogeneous. Mediterranean.

Trichamoeba gumia Schaeffer 1926. 60-140 μm in ovoidal to limax locomotive form; uninucleate; nucleus 12 μm. The occasional thickness of the locomotive form casts some doubt on the position of this species, but it is very large for a vahlkampfiid, which is the other likely classification. Western North Atlantic.

Trichamoeba pallida Schaeffer 1926. 60 μm in locomotion; occasionally spreads out on substratum when stationary; uninucleate; nucleus 10 μm. Gulf of Mexico.

Amoeba salina Hamburger 1905. Small, in one case main cell mass said to be 10 μm long and 'pseudopodia' (term used for both anterior hyaloplasm and posterior collopodium) 14 μm; uninucleate; presumed cyst illustrated by author is fungal spore. Mediterranean.

Trichamoeba sphaerarum Schaeffer 1926. 20-30 μm in locomotion; uninucleate; nucleus 3.5 μm, with 'chromatin' apparently evenly distributed; Gulf of Mexico.

Family THECAMOEBIDAE Schaeffer 1926

Flattened amoebae, with fairly regular and symmetrical, usually oval, oblong, flabellate or semi-circular outline; no sub-pseudopodia or uroidal filaments in locomotive forms; hyaloplasm usually well-developed; sometimes with appearance of a pellicle with longitudinal or irregular folds; a variety of nuclear structures; well-developed glycocalyx.

Genus *Thecamoeba* Fromentel 1874

References: Biernacka (1963); Page (1971a, 1977); Page and Blakey (1979); Schaeffer (1926)

Pellicle-like surface with folds or wrinkles; hyaloplasm an anterior and lateral crescent or an anterior region, but with granuloplasm not set off as thicker, separate mass; L:B often 1.5 or more; glycocalyx dense, not composed of discrete individual elements, usually 20 nm or more thick; nuclear envelope usually with an internal fibrous lamina (Page 1978b). More commonly isolated from freshwater and soil than from saltwater.

1 Greatest dimension 11-25 μm (x̄ 16 μm), some to 30 μm; L:B x̄ 1.0-1.1; anterior outline more or less semi-circular, posterior edge somewhat convex; hyaloplasm occupying anterior half; usually several parallel longitudinal dorsal folds; nucleus 3-6 μm (x̄ 3.7-4.2 μm) with single central nucleolus, nucleus and nucleolus sometimes pushed into elongate form; glycocalyx 16-22 nm thick; nuclear envelope without internal fibrous lamina. (Figures 43-45). . . . . ***T. orbis*** Schaeffer 1926 (North Sea, western North Atlantic, Indian Ocean, Gulf of Mexico; possibly most common marine *Thecamoeba*; media 6-9)

– L 32-68 μm (x̄ 50 μm), some to 80 μm; L:B x̄ 1.5; outline oval with greatest breadth usually in anterior half; hyaloplasm commonly a deep crescent; commonly several parallel longitudinal dorsal folds; nucleus 7.5-12.5 μm, with central nucleolus, both nucleus and nucleolus often elongate with L 2X B; contractile vacuole reported in some; fine structure not known (Figures 46, 47) . . . . . ***T. hilla*** Schaeffer 1926 (North Sea, western North Atlantic, Gulf of Mexico; media 7, 8)

Other species:

T. munda Schaeffer 1926. L 45 μm, B 35 μm; flattened ovoid; anterior quarter hyaloplasmic; 3 or 4 prominent longitudinal folds; nucleus (10 μm) with parietal nucleolar material. Gulf of Mexico.

T. pulchra (Biernacka 1963). L c. 75 μm; outline oval; numerous longitudinal folds; spherical vesicular nucleus; contractile vacuole. Gulf of Gdánsk, salinity 6.5-7.5 o/oo.

T. rugosa Schaeffer 1926. L 60-80 μm, B 35-50 μm; outline oval, more or less irregular; hyaloplasm an antero-lateral crescent; large and indefinite number of irregular folds; nucleus (10 μm) with homogeneous central nucleolus. Gulf of Mexico.

Levander (1894) found in the Gulf of Finland a *Thecamoeba* which he called *Amoeba verrucosa* but which undoubtedly did not belong to that species (Page 1976a, 1977). His Figure 1, Plate I, shows a smooth *Thecamoeba* about 145 μm long.

Genus *Platyamoeba* Page 1969

References: Page (1968 (as *Rugipes*), 1969b, 1971a, 1974b, 1980a); Page and Blakey (1979); Sawyer (1975b, 1975c)

Flattened hyaloplasm occupying approximately anterior one-third to one-half, often extending around sides of thicker granuloplasmic mass; outline oval, semi-circular, flabellate, or linguiform; in some species, occasional transient folds or wrinkles near edges but no dorsal ridges. Floating forms with or without blunt, hyaline, radiate pseudopodia, which never taper to a fine tip. Glycocalyx composed of tightly packed, filamentous, hexagonal elements (not discrete glycostyles), each with diameter about 40 nm and usually extending 10-30 nm above plasma membrane.

This is the marine genus which I have most often isolated. As here defined, it includes *Lingulamoeba* Sawyer 1975, subject to the reservation that the surface structure of Sawyer's marine *Lingulamoeba* is not known. (A similar freshwater amoeba has the surface structure characteristic of *Platyamoeba*; see Page and Blakey 1979). It is not known whether any species classified by Sawyer (1975b, 1975c) in *Clydonella* and *Hyalodiscus* or those classified by Sawyer (1975b) and Schaeffer (1926) in *Unda* would be placed into this genus as distinguished by surface structure. Likewise, *Rugipes vivax* Schaeffer 1926 cannot be assigned with certainty to either *Platyamoeba* or *Vannella*, the 2 similar genera recognized here. The possibility should be noted that some workers have used Schaeffer's name *Flabellula mira* for strains of *Platyamoeba*, though it is almost certain that the original description was not based on a *Platyamoeba*.

Remarks on the distinctions between *Platyamoeba* and *Vannella* are included under the latter genus.

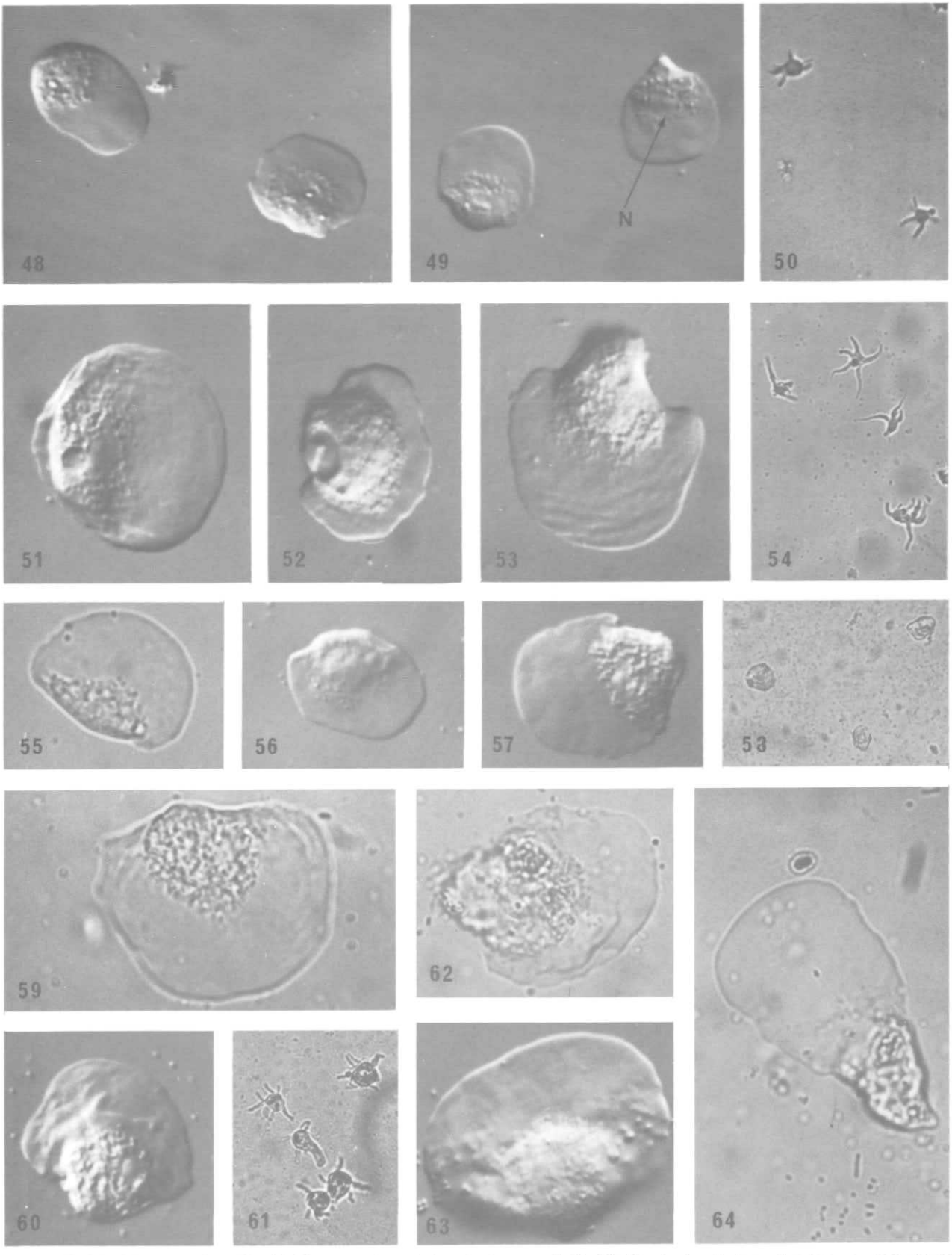
Although both freshwater species for which this genus was originally erected form cysts, no cysts have been found in any marine strain.

With one exception, the characters in this key to the genus are drawn exclusively from the original strains of these species, but the measurements differ somewhat from those reported earlier (Page 1971a, 1974b) as the result of new measurements made under the conditions set out under 'Characters for identification'. The exception is *P. plurinucleolus*, the measurements of which include those of 10 new strains of these relatively small amoebae.

Because of its commonness, this genus raises acutely the question of intra-specific variation on both light and electron microscopical levels as well as non-morphologically. It is not yet certain that glycocalyx thickness is a species-specific character in *Platyamoeba*. For these reasons, and because of the probability of undescribed species, it may not always be possible to assign an isolate to a named species with certainty.

1 Nucleolar material parietal; greatest dimension 8-34 μm (x̄ 11-26 μm in different strains); L:B x̄ 0.9-1.2, commonly 1.0 or 1.1; nuclear diameter 2.4-6.5 μm (x̄ 2.5-5.5 μm in different strains); glycocalyx commonly 25-27 nm. (Figures 48-50, 78) . . . . . ***P. plurinucleolus*** Page 1974 (East and west coast of Britain, western Atlantic; media 6-9)

– Nucleolus central. 2



Figures 48-64. Platyamoeba. *48, 49,* Platyamoeba plurinucleolus; × *1000. 50,* P. plurinucleolus, *floating;* × *250. 51-53,* Platyamoeba calycinucleolus; × *1000. 54,* P. calycinucleolus, *floating;* × *250. 55, 56,* Platyamoeba bursella; × *1000. 57,* Platyamoeba mainensis; × *1000. 58,* P. mainensis, *floating;* × *250. 59, 60,* Platyamoeba australis; × *1000. 61,* P. australis, *floating;* × *250. 62, 63, 64,* Platyamoeba flabellata; × *1000. N, nucleus.*

2 L:B $\bar{x}$ usually or always less than 1.0; outline often oval or semi-circular, with thickened granular region as a spindle-shaped or ovoid posterior mass; posterior edge often straight or slightly convex. 3

– L:B $\bar{x}$ 1.0 or more; posterior edge often rounded, convex, triangular 4

3 L:B $\bar{x}$ 0.8-0.9; greatest dimension 22-56 μm ($\bar{x}$ 32-39 μm); nucleus 4.7-7.5 μm ($\bar{x}$ 6.3 μm); nucleolus sometimes shallowly calyciform; nuclear membrane disappearing before end of metaphase; glycocalyx c. 40 nm. (Figures 51-54, 75) ..... ***P. calycinucleolus*** Page 1974 (North Sea; media 6-9)

– L:B $\bar{x}$ 0.9-1.0; greatest dimension 14-37 μm ($\bar{x}$ 19-25 μm); nucleus 4.6-6.5 μm ($\bar{x}$ 5.3 μm); nucleolus spherical; nuclear membrane present through metaphase; glycocalyx c. 26 nm, possibly less in some. (Figures 55, 56, 76, 79) ..... ***P. bursella*** Page 1974 (East coast of England, media 6-9)

4 L of radiate pseudopodia sometimes more than twice diameter of central mass of floating form; greatest dimension of locomotive form 22-44 μm ($\bar{x}$ 32 μm); L:B $\bar{x}$ 1.0; nucleus 5.6-8.4 ($\bar{x}$ 6.7 μm); glycocalyx c. 30 nm. (Figures 59-61; electron micrographs as strain 236 in Page 1980a) ..... ***P. australis*** n. sp. (East coast of Australia; media 6-9)

– L of radiate pseudopodia of floating forms seldom greater than diameter of central mass; L:B $\bar{x}$ 1.0-1.1; glycocalyx less than 30 nm 5

5 L of radiate pseudopodia often approximately equal to diameter of central mass of floating form; greatest dimension of locomotive form 19-37 μm ($\bar{x}$ 26 μm); tendency to oblong outline; nucleus 3.7-6.5 μm ($\bar{x}$ 5.6 μm); spindle caps derived from nuclear membrane present in anaphase (Figure 1c); glycocalyx 20-22 nm. (Figures 57, 58, 77) ..... ***P. mainensis*** Page 1971 (Western North Atlantic; media 6-9)

– Radiate pseudopodia often absent from floating forms, L when present usually less than diameter of central mass; greatest dimension of locomotive form 20-43 μm ($\bar{x}$ 28-33 μm); tendency to flabellate outline; nucleus 5-9 μm ($\bar{x}$ 6.5 μm); no spindle caps in anaphase; glycocalyx 26-28 nm. (Figures 63, 64) ..... ***P. flabellata*** Page 1974 (North Sea, western North Atlantic; media 6-9)

Platyamoeba australis n. sp.

Diagnosis: Greatest dimension 22-44 μm ($\bar{x}$ 32.3 ± 0.5 μm); strong tendency to broad forms, with L:B 0.6-2.5 ($\bar{x}$ 1.0), hyaline zone often occupying entire anterior half, posterior end often triangular, giving broad, flabellate form with extensive hyaloplasm; nucleus 5.6-8.4 μm ($\bar{x}$ 6.7 μm), nucleolus 2.8-4.7 μm ($\bar{x}$ 3.8 μm); floating forms with radiate pseudopodia whose length is sometimes more than twice diameter of central mass; glycocalyx approximately 30 nm thick.

Observed habitat: East coast of Australia.

The species is described from strain 236, isolated from material collected at Maroochydore, north of Brisbane, by E A George. The surface structure is shown in Figures 10-12 of Page (1980a) and the nucleus in Figure 17 of the same paper. Because of its size and good growth on medium 7, this species is especially suited for research and teaching.

Other species (Sawyer 1975b):

P. douvresi Sawyer 1975. L 12-15 μm ($\bar{x}$ 13 μm); discoid to broadly oval with straight to slightly convex posterior edge; L:B c. 1.0 in rapid locomotion, 0.5 in slow locomotion, nucleus c. 3 μm, with central nucleolus; L of pseudopodia of floating form approximately equal to diameter of central mass. Western North Atlantic.

P. langae Sawyer 1975. L 7-12 μm ($\bar{x}$ 9 μm); broadly triangular to oval outline, with slightly convex posterior edge; L:B c. 1.0; nucleus c. 2.5 μm, with central nucleolus; floating forms 'peg-shaped', ie elongate. Western North Atlantic, Gulf of Mexico.

P. leei (Sawyer 1975) n. comb. L 16-23 μm ($\bar{x}$ 20 μm); linguiform, with L:B 2.0; nucleus c. 3.5 μm, with central nucleolus; floating form without radiate pseudopodia. Western North Atlantic.

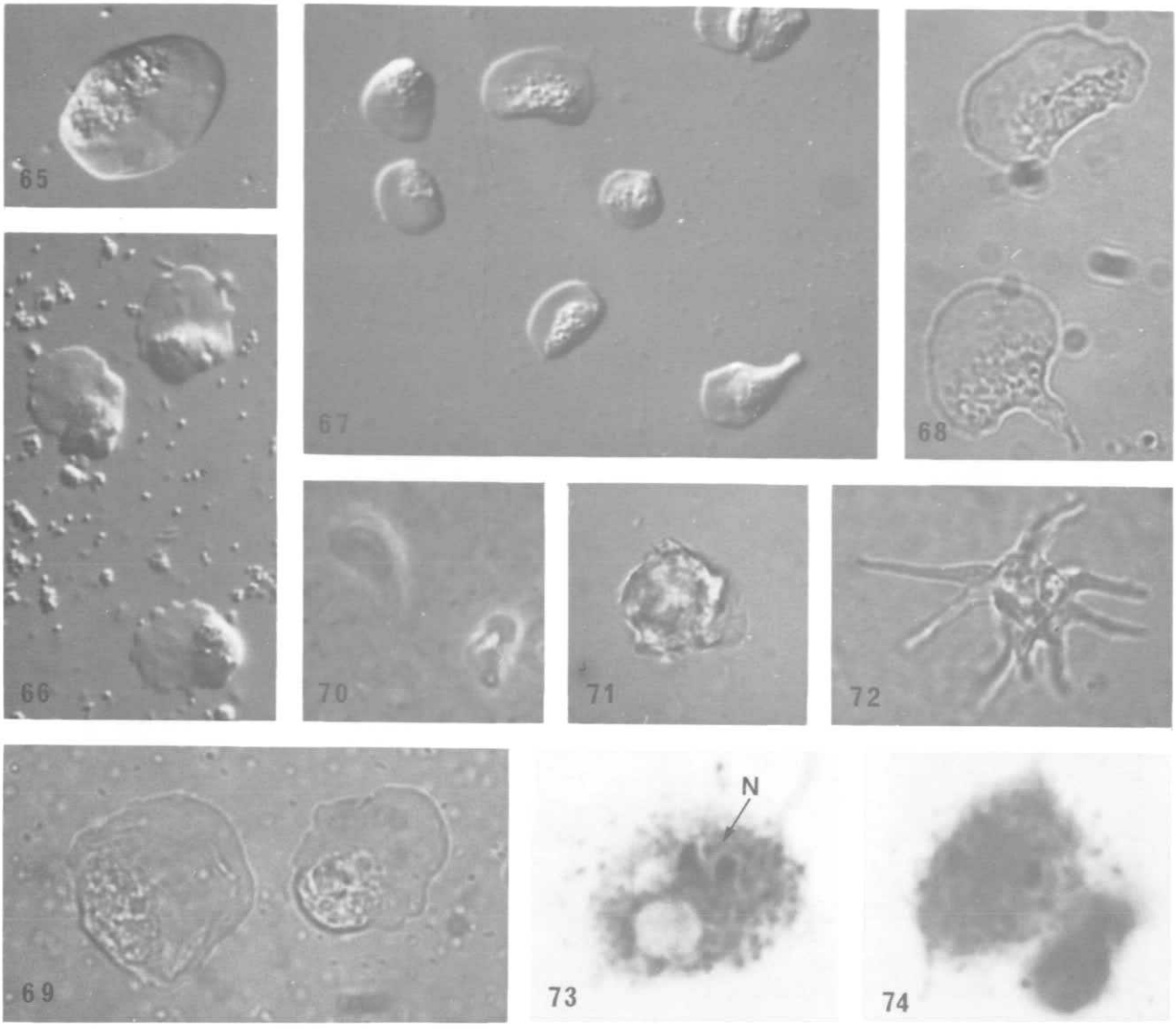
P. murchelanoi Sawyer 1975. L 8-13 μm ($\bar{x}$ 11 μm); outline oval with straight posterior edge; L:B c. 1.0; nucleus 2-2.5 μm, with central nucleolus; floating form without radiate pseudopodia. Western North Atlantic, Gulf of Mexico.

P. weinsteini Sawyer 1975. L 11-14 μm ($\bar{x}$ 12 μm); outline oval to discoid with straight or slightly convex posterior edge; L:B c. 1.0; nucleus c. 3-4 μm, with central nucleolus; floating form with pseudopodia about as long as diameter of central mass. Western North Atlantic, Gulf of Mexico.

Genus *Vannella* Bovee 1965

References: Bovee (1965; Bovee and Sawyer (1979); Page (1968 (as *Flabellula*), 1979b, 1980b); Page and Blakey (1979); Schaeffer (1926)

Flattened hyaloplasm occupying approximately anterior quarter to half, usually extending around sides of thicker granuloplasmic mass; outline flabellate, spatulate, occasionally oval. Floating forms with or without slender, hyaline, radiate pseudopodia, which in some species taper to a narrow tip. Surface covered



Figures 65-74. Vannella. *65,* Vannella devonica; × *1000. 66,* Vannella caledonica; × *1000. 67,* Vannella aberdonica; × *1000. 68,* Vannella septentrionalis; × *1000. 69,* Vannella anglica; × *1000. 70-72, floating forms of (70)* V. aberdonica, *(71)* V. arabica, *and (72)* V. septentrionalis; × *1000. 73, 74,* V. devonica, *haematoxylin, showing (73) interphase nucleus with parietal nucleolar material and (74) nuclear division;* × *2500. N, nucleus.*

with pentagonally symmetrical glycostyles, 95-120 nm tall above plasma membrane, composed of 5 wings arranged radially around a central tubule.

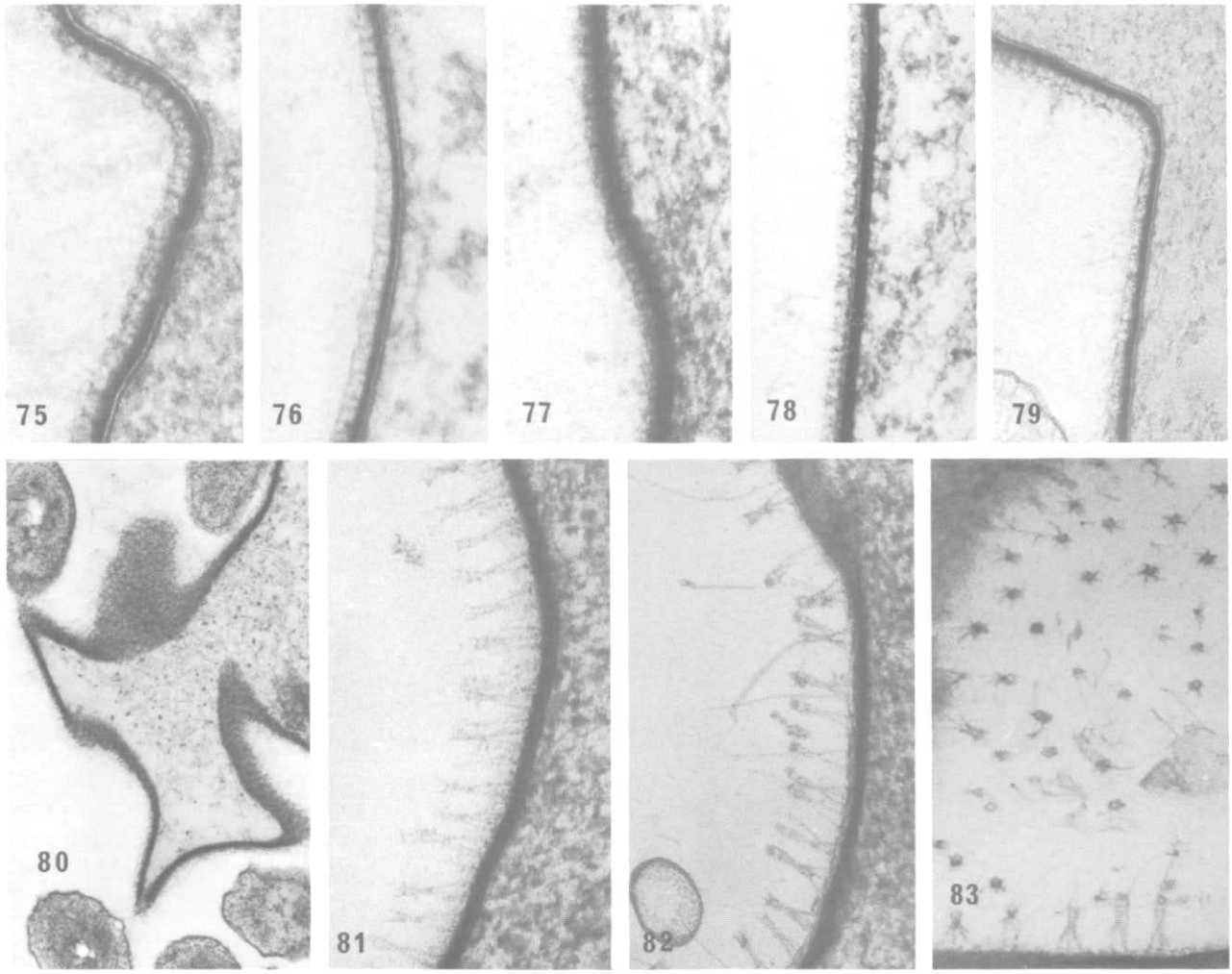
This genus, fairly common in both freshwater and marine habitats, has as type species *V. mira* (Schaeffer 1926), described by Schaeffer from saltwater, to which freshwater isolates have also been attributed (Page 1968). Unfortunately, as with some other genera of Gymnamoebia, the type species seems identifiable with less certainty than some more recently isolated species.

The best diagnostic character is the glycostyles (Figures 2a, 81-83), identical in structure and almost identical in dimensions on freshwater strains from Germany and America and marine strains from western Scotland to the Persian Gulf. Distinguishing between *Vannella* and *Platyamoeba* with the light microscope may be relatively easy or impossible, depending on the isolate. An amoeba with a *Vannella/Platyamoeba*-like locomotive form and *slender, tapering* pseudopodia on the floating form can be safely classified as *Vannella*. The locomotive forms of *Vannella* are somewhat more variable than those of *Platyamoeba*, so that a long, spatulate posterior end and the temporary possession of 2 hyaloplasmic zones moving in opposite directions are good indications that an isolate is *Vannella*. Unfortunately, some marine isolates of *Vannella* seem indistinguishable from *Platyamoeba* with the light microscope. No species of *Vannella*, marine or freshwater, is known to encyst.

1 Nucleolus parietal; locomotive form 14-33 μm (x̄ 22 μm), L:B x̄ 0.9; nucleus 3.7-5.6 μm (x̄ 4.8 μm); no simple filaments amongst glycostyles. (Figures 65, 73, 74, 81) ..... ***V. devonica*** Page 1979 (Channel Coast; media 6-9)

– Nucleolus central; at least a few simple filaments amongst glycostyles 2

2 Floating form thickly flattened and twisted, never



Figures 75-80. Platyamoeba, *cell surfaces in section; all × 100000 except 80, which is × 30000. 75,* Platyamoeba calycinucleolus. *76,* Platyamoeba bursella. *77,* Platyamoeba mainensis. *78, a strain with parietal nucleolar material which by the present diagnosis is* Platyamoeba plurinucleolus. *79, another possible strain of* P. bursella *(cf. Fig. 76). 80, tangential section of surface showing arrangement and hexagonal cross-sections of individual elements of glycocalyx.*
Figures 81-83. Glycostyles of Vannella; *× 100000. 81,* Vannella devonica. *82,* Vannella caledonica *(note also simple hairs). 83,* V. caledonica, *showing cross-sections of glycostyles at different levels; cf Figure 2.*

rounded or radiate; locomotive form 6.5-13 μm (x̄ 9 μm), L:B x̄ 1.0; nucleus 2-3.2 μm (x̄ 2.6 μm). (Figures 67, 70) ..... ***V. aberdonica*** Page 1980 (North Sea, media 6-9)

– Larger, floating form rounded up, often radiate 3

3 Floating form rounded up but always without radiate pseudopodia; locomotive form 10-25 μm (x̄ 16 μm); L:B x̄ 1.2; posterior end sometimes spatulate; nucleus 2.8-4.7 μm (x̄ 3.2-3.5 μm). (Figures 66, 82, 83) ... ***V. caledonica*** Page 1979 (Western Scotland; media 6-9)

– Floating form sometimes or often with radiate pseudopodia 4

4 Floating form rarely with radiate pseudopodia, which when present are usually short; locomotive form 17-37 μm (x̄ 27 μm); L:B x̄ 0.8; nucleus 4.2-6.5 μm (x̄ 5.4 μm). (Figure 71) ..... ***V. arabica*** Page 1980 (Persian Gulf region; media 6-9)

– Floating form often with radiate pseudopodia; amoebae and nuclei often somewhat smaller than preceding species 5

5 Pseudopodia of floating form often long; locomotive form 15-33 μm (x̄ 21-23 μm); L:B x̄ 0.8-0.9; posterior end sometimes spatulate; nucleus 3.7-5.6 μm (x̄ 4.2-4.8 μm). (Figures 68, 72) ..... ***V. septentrionalis*** Page 1980 (North Sea; media 6-9)

– Pseudopodia of floating form, when present, short to medium; locomotive form 15-37 μm (x̄ 21-24 μm); L:B x̄ 1.0-1.2; posterior end seldom or never spatulate; nucleus 3.8-6.2 μm (x̄ 4.9-5.3 μm). (Figure 69)..... ***V. anglica*** Page 1980 (North Sea; media 6-9)

Other species:

V. crassa (Schaeffer 1926). Locomotive form 50-75 μm in L and B; posterior end sometimes spatulate; nucleus 12-15 μm; pseudopodia of floating form blunt, short to medium. Gulf of Mexico.

V. mira (Schaeffer 1926). L 20-30 μm, B 15-25 μm; seldom spatulate; nucleus 4.6 μm; pseudopodia of floating form long. Western North Atlantic, Gulf of Mexico, Pacific Coast of USA, Indian Ocean. This name may have been applied to diverse species of *Vannella* and *Platyamoeba*.

V. sensilis Bovee and Sawyer 1979. L 13-20 μm, B 15-24 μm; posterior end sometimes spatulate; nucleus 3 μm; pseudopodia of floating form short. Atlantic and Pacific coasts of USA.

Other genera of Thecamoebidae

Clydonella Sawyer 1975

References: Sawyer (1975b, 1975c)

This genus was distinguished from *Vannella* and *Platyamoeba* before the surface fine structure of those 2 genera had been compared, and no description of surface structure of *Clydonella* has been published. Light microscopical characters appear intermediate between those of *Vannella* and *Platyamoeba*. Sawyer made *Rugipes vivax* Schaeffer 1926 (see below) the type species of the new genus. Three new species were described by Sawyer (1975b), whose descriptions should be consulted for fuller information.

C. rosenfieldi Sawyer 1975. L 14-19 μm (x̄ 17 μm); L:B c. 1.0 in slow locomotion; nucleus c. 4.5 μm, with central nucleolus. Western North Atlantic, Gulf of Mexico.

C. sindermanni Sawyer 1975. L 21-40 μm (x̄ 28 μm), L:B slightly less than 1.0; nucleus c. 4.5 μm, with central nucleolus. Western North Atlantic, Gulf of Mexico.

C. wardi Sawyer 1975. L 14-20 μm (x̄ 18 μm), L:B slightly less or more than 1.0; nucleus c. 3-3.5 μm, central nucleolus. Western North Atlantic, Gulf of Mexico.

Lingulamoeba Sawyer 1975

Reference: Sawyer (1975b)

Included in genus *Platyamoeba*, q. v.

Rugipes Schaeffer 1926

References: Page (1969b); Sawyer (1975b); Schaeffer (1926)

This genus is no longer accepted, for reasons given by Page (1969b) and Sawyer (1975b). It includes a marine species, *R. vivax* Schaeffer 1926, whose identity and position according to the present system are uncertain, though Sawyer (1975b) made it the type species of *Clydonella*. It was a small (L 12 μm) amoeba resembling *Platyamoeba* or *Vannella*.

Striamoeba Bovee and Jahn 1966

References: Bovee and Jahn (1966); Jahn *et al.* (1974)

This is an invalid name (for reasons given by Page 1977) applied to some species of *Thecamoeba*.

Family FLABELLULIDAE Bovee 1970

Reference: Bovee (1970)

Flattened, with prominent anterior hyaloplasm; flabellate, spatulate, often with L:B less than 1 but sometimes elongate; form commonly irregular and rapidly changing, often with eruptive cytoplasmic activity, often with trailing uroidal filaments; with or without short, non-furcate subpseudopodia produced from anterior hyaloplasm; no collosomes; glycocalyx thin; nuclear division mesomitotic. Note (superficial?) similarity of some locomotive forms (especially *F. calkinsi*) to *Rhizamoeba* (Leptomyxidae) in general shape and adherent filaments, though *Flabellula* has a deep hyaline zone rather than a laterally expanded hyaline cap.

Genus *Flabellula* Schaeffer 1926

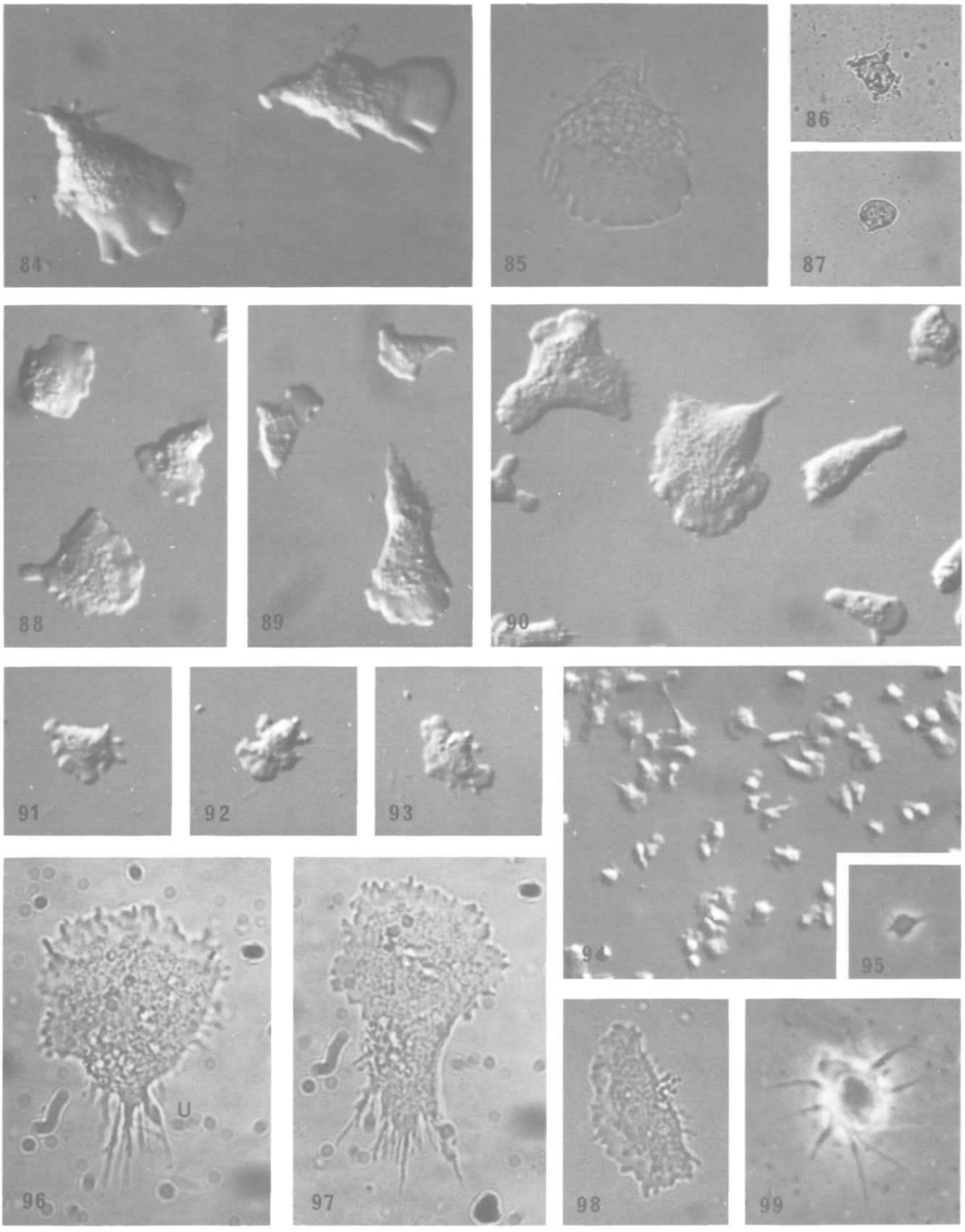
References: Bovee (1965); Hogue (1914, 1921); Page 1971b, 1980c)

No subpseudopodia, though clefts occurring during advance may separate off narrow part of hyaloplasm with appearance of conical pseudopodium to one side; floating form usually irregularly rounded, usually without radiate pseudopodia.

1 Greatest dimension 15-75 μm (x̄ 24-40 μm), L:B x̄ 1.2 but broad forms fairly common; usually more or less fan-shaped; anterior edge of hyaloplasm often smooth but antero-lateral clefts common; uroidal filaments frequent; nucleus 4.0-12.4 μm (x̄ 5.7 μm); often some binucleate cells; surface coat outside plasma membrane usually not discernible; electron microscope shows local concentrations of small vesicles along some parts of cell periphery. (Figures 84-87, 100) ..... ***F. citata*** Schaeffer 1926 (Western North Atlantic, possibly east coast of Britain; euryhaline; media 6-9)

– Smaller; less regularly fan-shaped; glycocalyx thin but discernible 2

2 Greatest dimension 8-30 μm (x̄ 13-15 μm); L:B x̄ 1.5; less regularly fan-shaped; often producing elongate but not truly limax (cylindrical) form, no lateral pseudopodium-like projections produced by rifts in hyaloplasm, occasionally uroidal filaments; cannibalism especially noticeable; nucleus 2.0-3.7 μm (x̄ 2.6 μm); binucledate cells may exceed 10% in young cultures, with occasional 3-5-nucleate cells and multiple fission; glycocalyx amorphous, c. 8 nm thick above plasma membrane. (Figures 88-90, 101) ..... ***F. calkinsi*** (Hogue 1914)

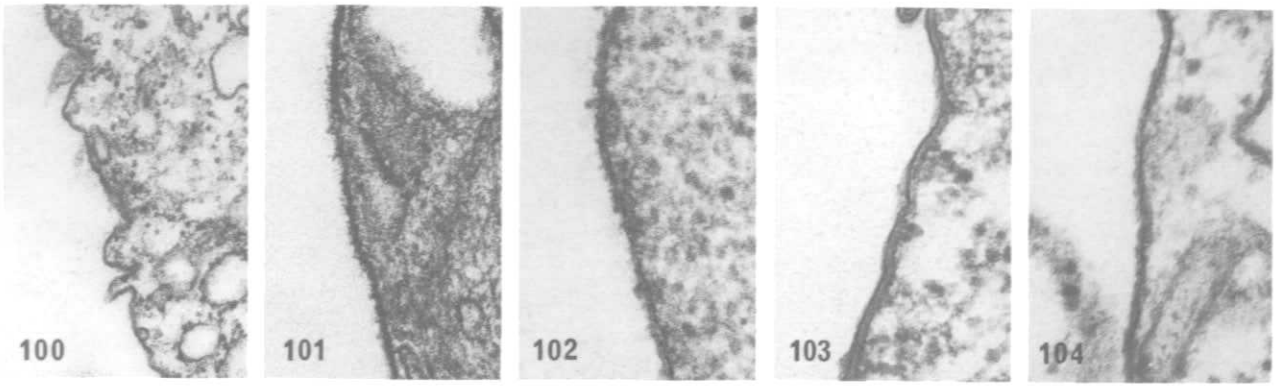


Figures 84-99. Flabellulidae, living amoebae. 84, 85, Flabellula citata; × *1000. 86, 87,* F. citata, *floating;* × *250. 88-90,* Flabellula calkinsi; × *1000. 91-93,* Flabellula trinovantica, *views of a single amoeba photographed in quick succession to show change of form;* × *1000. 94,* Flabellula demetica; × *1000. 95,* F. demetica, *floating;* × *1000. 96-98,* Paraflabellula reniformis; × *1000. 99,* P. reniformis, *floating;* × *1000. U, uroid.*

(Western North Atlantic, probably east coast of Britain; euryhaline, described by Hogue from digestive tract of oysters, with no evidence of pathogenicity and clear indications that cultures contained more than one species; recent isolates from free-living habitats; media 6-9)

— Smaller, uroidal filaments rare; shapes of locomotive forms very irregular. 3

3 Greatest dimension 7.4-16 μm (x̄ 10 μm); L:B x̄ 1.1 but of little significance because of irregularity of shape; floating forms spherical, smooth; fixed



Figures 100-104. Flabellulidae, surfaces in section; all × 100000 except 100, which is × 50000. 100, Flabellula citata. *101,* F. calkinsi. *102,* F. trinovantica. *103,* F. demetica. *104,* Paraflabellula reniformis.

nuclei c. 2-2.8 μm (x̄ 2.3 μm); glycocalyx amorphous, to about 10 nm above plasma membrane. (Figures 91-93, 102) ..... ***F. trinovantica*** Page 1980 (Eastern England; media 6-9)

– Greatest dimension 2.8-7.4 μm (x̄ 4.5 μm); some amoebae with 2 hyaloplasmic zones advancing in opposite directions; rounded floating forms sometimes with 2 or 3 very fine pseudopodia, nuclei in electron microscopical preparations c. 0.8-1.6 μm; glycocalyx dense, about 2.5 nm thick, separated from plasma membrane by space of about 4 nm. (Figure 94, 95, 103) ..... ***F. demetica*** Page 1980 (The smallest named free-living amoeba; south Wales; media 6-9)

Other species:

Vahlkampfia patuxent Hogue 1921 is a name based on a culture which clearly contained more than one species, of which one was almost certainly a *Flabellula*, to which this species has been attributed since the work of Schaeffer (1926). Because the characters differentiating it from *F. calkinsi* are derived from the contaminants in the cultures of both strains, this species is unacceptable.

Genus *Paraflabellula* n. g. Page and Willumsen

References: Sawyer (1975a); Schaeffer (1926); Schmoller (1964)

Diagnosis: With short, narrow but not sharply tipped, non-furcate subpseudopodia produced from anterior hyaline zone; uroidal filaments common, sometimes furcate and sometimes numerous; floating form with radiate pseudopodia, sometimes furcate near base, tapering to fine tip. Other characters those of family.

Type species: *Paraflabellula reniformis* (Schmoller 1964) n. comb.

Distinguish from: (1) *Flamella* Schaeffer 1926, which lacks the numerous uroidal filaments and has many long, marginal subpseudopodia, which Schaeffer's description suggests are not the same kind as the short ones of *Paraflabellula*. Schaeffer's inability to find a nucleus suggests multinuclearity, some of his observations suggest a thick coat, and his amoeba contained crystals. (2) *Gibbodiscus* Schaeffer 1926, which has the granuloplasm entirely surrounded by a hyaline margin, appears to differ in the nature of its subpseudopodia, and contains crystals.

We have studied one species:

Greatest dimension 8.4-45 μm (x̄ 17.5-23.5 μm); L:B x̄ 0.9, with some broad forms having L:B 0.5 and triangular forms with L:B greater than 1.0 tapering toward posterior end; subpseudopodia to about 4 μm long; floating form with few to many radiate pseudopodia which may reach a length 3-5 times diameter of central mass; nucleus c.3-6 μm (x̄ 3.5-5 μm); glycocalyx with fuzzy appearance, about 5-6 nm above plasma membrane; electron microscope sometimes shows many small vesicles at cell periphery (cf. *F. citata*). (Figures 96-99, 104) ..... ***P. reniformis*** (Schmoller 1964) (Appears to be very common in Baltic; apparently very euryhaline; grows slowly on medium 7; lower salinity may be preferable for some strains)

Other species:

Paraflabellula hoguae (Sawyer 1975) n. comb. L 18-27 μm (x̄ 26 μm); B 18-45 μm (x̄ 34 μm); nucleus c. 4.5 μm. Appears similar to *P. reniformis*. Western North Atlantic.

Flabellula pellucida Schaeffer 1926. This species, reported to have 15-20 nuclei (Schaeffer 1926), needs further study before being placed into the genus *Paraflabellula*, which it resembles. L 20-40 μm, B 60-100 μm. Gulf of Mexico.

Other genera of Flabellulidae

Flamella Schaeffer 1926

Reference: Schaeffer (1926)

This genus is placed into the Flabellulidae, following Bovee and Sawyer (1979), for lack of information to indicate a better classification. It changes shape rapidly; the common form is oval and broad. The possibility of

its having a more highly developed surface coat than *Flabellula* and *Paraflabellula* is suggested by the lack of uroidal filaments, the web-like appearance of the hyaline zone, and the vein-like thickenings.

The only marine species is the type species, *F. magnifica* Schaeffer 1926, with both L and B reported as 30-60 μm, which 'throws out at the same moment hundreds of pseudopods' of different shapes. Schaeffer could not identify a nucleus.

Family PARAMOEBIDAE Poche 1913

References: Bovee (1970); Page (1972b); Poche (1913); Schaeffer (1926)

Digitiform or mamilliform subpseudopodia (dactylopodia) or finely conical or linear subpseudopodia, all hyaline and non-furcate and seldom numerous, usually produced from anterior hyaloplasmic lobe of locomotive form; L:B usually more than 1.0; surface structure usually highly differentiated.

This is the same family as the Mayorellidae Schaeffer 1926, a name which would be preferable but is unfortunately a junior synonym (Page 1972b). For reasons beyond the scope of this publication, recent findings suggest an eventual dichotomy with *Mayorella*, *Dactylamoeba* and *Paramoeba eilhardi* on the one hand, and other amoebae now classified here on the other.

Genus *Paramoeba* Schaudinn, 1896

References: Cann and Page (1982); De Faria *et al.* (1922); Grell (1961); Grell and Benwitz (1966, 1970); Hollande (1980); Page (1970, 1973); Perkins and Castagna (1971); Schaudinn (1896)

One or more DNA-containing parasomes adjacent to nucleus; dactylopodia or finely conical subpseudopodia; floating form spherical with fine radiating pseudopodia; surface covered with scales or glycocalyx composed of tightly packed, apparently tubular elements.

The parasome or *Nebenkörper* is characteristic of this genus but not unique to it, occurring also in certain parasitic amoebae classified in the genus *Janickina* Chatton 1953. It consists of a middle-piece, which gives a densely positive Feulgen reaction (used in this laboratory as a positive control for Schiff's reagent), and 2 end-pieces. Its fine structure has been studied in various species of *Paramoeba* by Grell and Benwitz (1970), Perkins and Castagna (1971), and Cann and Page (1982). After an ultrastructural study of the structurally identical parasome of *Janickina*, Hollande (1980) concluded that it was a kinetoplastid symbiont, which he named *Perkinsiella amoebae*. Although a single parasome per nucleus is the norm, supernumerary parasomes are very common.

The parasite *P. perniciosa* is included in the section 'Some parasitic marine amoebae', as are the species of *Janickina*, and the following brief key includes only free-living species.

1 Greatest dimension usually 45-100 μm; short, blunt dactylopodia from narrow hyaloplasmic margin; nucleus often 10 μm or more; parasome up to 11 μm long; supernumerary parasomes common in some strains; cell surface covered with boat-shaped scales, usually 0.35-0.60 μm long, sometimes longer, consisting of base, rim, and 8 or more upright bars. (Figures 105, 106, 117) ..... ***P. eilhardi*** Schaudinn 1896 (Mediterranean, Indian Ocean; medium 1, with the diatom *Amphiprora hyalina* as food, or 6 or 7 with an overlay of 1 and accompanying bacteria as food)

— Smaller, with flattened hyaloplasm often occupying anterior one-fifth to one-third; without scales; glycocalyx c. 10 nm thick 2

2 L:B $\bar{x}$ c. 2.0; often a few short, blunt projections from anterior edge of hyaloplasm, rarely with prominent longitudinal ridges; mean greatest dimension usually less than 22 μm; nuclear diameter usually less than 5 μm; L of parasome usually 4 μm or less; supernumerary parasomes rare. (Figures 107-109, 118) ..... ***P. aestuarina*** Page 1970 (North Atlantic, Gulf of Mexico; media 6-9; grows less well in culture than does next species)

— L:B seldom 2.0, $\bar{x}$ usually near 1.5; often one or more conspicuous longitudinal ridges, which may continue anteriorly as conical subpseudopodia; some strains commonly with more or less asymmetrically expanded form (Figure 113); mean greatest dimension usually more than 25 μm; nuclear diameter usually more than 5.0 μm; L of parasome usually more than 4.5 μm; some strains with tendency to supernumerary parasomes. (Figures 110-116, 119, 120) ..... ***P. pemaquidensis*** Page 1970 (One of the most common marine amoebae; North Atlantic, Gulf of Mexico, common on British coasts, western end of Baltic; media 6-9)

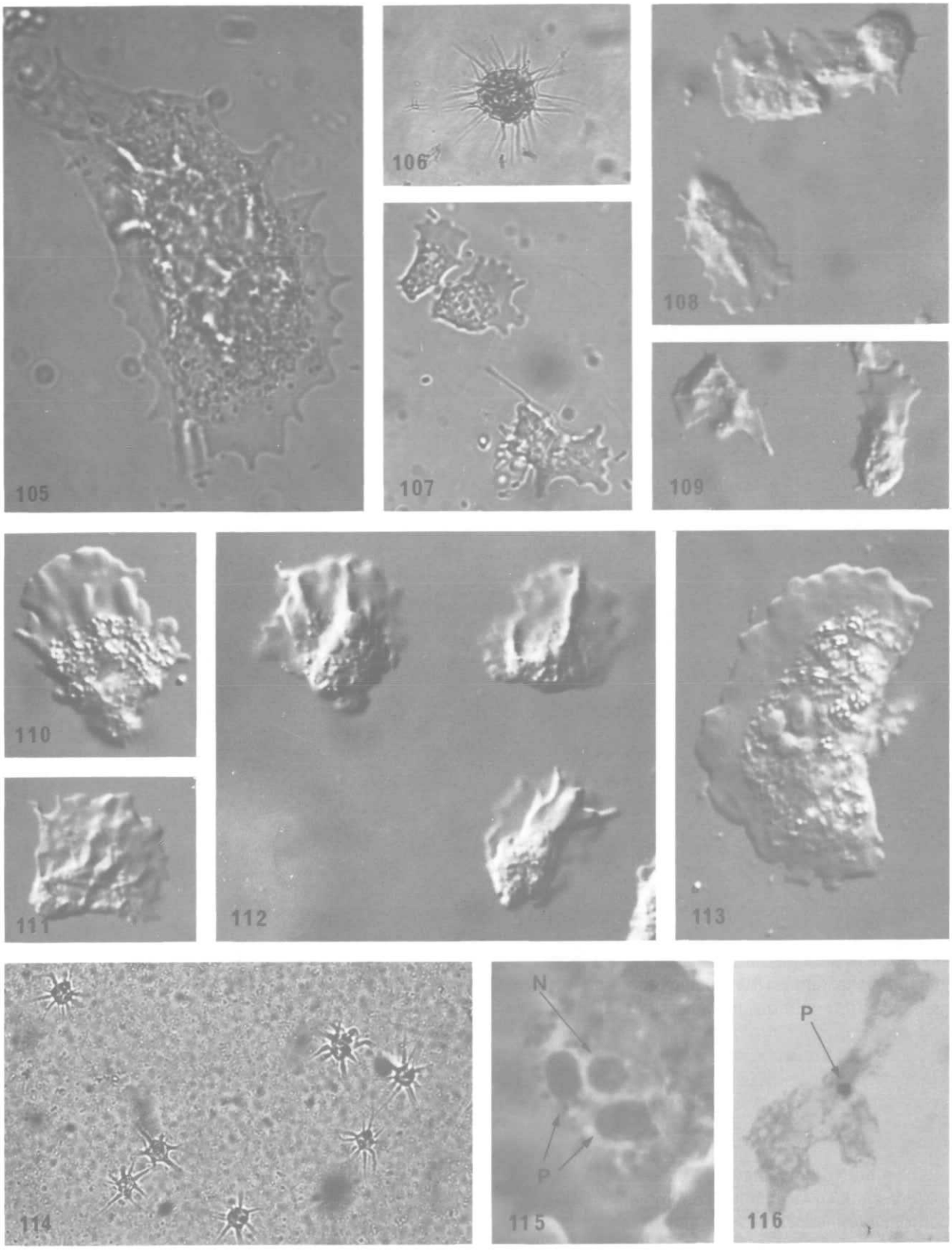
Other species:

P. schaudinni de Faria, da Cunha and Pinto 1922. Description inadequate for inclusion in key or determination of synonymy with later names. Described from aquarium at Rio de Janeiro, reported from Gulf of Mexico.

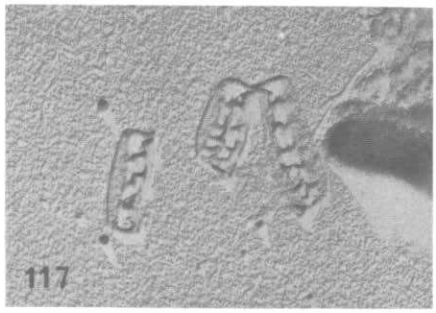
Genus *Mayorella* Schaeffer 1926

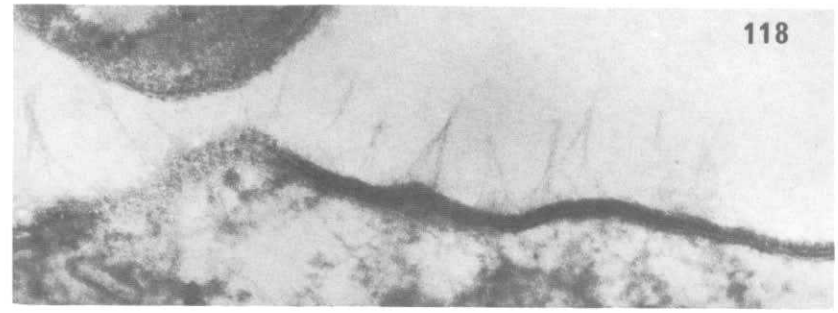
References: Delphy (1938); Hollande *et al.* (1981); Mereschkowsky (1879); Page (1981c, 1982, 1983); Sawyer (1975a); Schaeffer (1926)

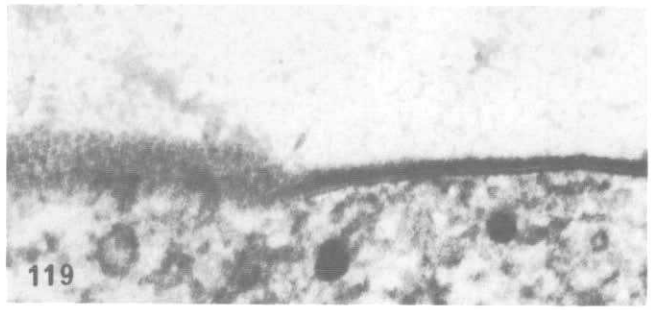
A few dactyloid, blunt, hyaline, non-furcate subpseudopodia of similar lengths usually produced from a hyaline lobe, often temporarily lacking in some species; floating forms with tapering pseudopodia on a cell mass that is not a regular sphere; cuticle about 200 nm thick composed of thin-walled, laterally compressed, funnel-shaped cylinders in fibrillar matrix; numerous Golgi bodies distributed throughout endoplasm.

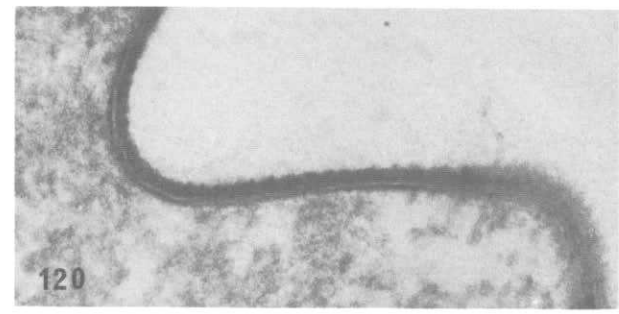


Figures 105-116. Paramoeba. *105,* P. eilhardi; × *1000. 106,* P. eilhardi, *floating;* × *250. 107-109,* P. aestuarina; × *1000. 110-113,* P. pemaquidensis; × *1000. 114,* P. pemaquidensis, *floating;* × *250. 115,* P. pemaquidensis, *phase contrast, to show 2 parasomes and nucleus (nuclear membrane not in focus);* × *2500. 116,* P. pemaquidensis, *Feulgen-fast green, showing densely positive parasome;* × *1000. N, nucleus; P, parasome.*









Figures 117-120. Paramoeba, *surface structures. 117,* Paramoeba eilhardi, *detached scales;* × *25000. 118,* Paramoeba aestuarina, *section of surface, tangential toward left and with hair-like filaments;* × *100000. 119, 120,* Paramoeba pemaquidensis, *2 strains, with tangential section at left of 119 and at right of 120;* × *100000.*

This is the genus recently diagnosed on the basis of its fine structure under the name *Hollandella* (Page 1981b). Because new information strongly suggests that the type species of *Mayorella*, the freshwater *M. bigemma* (Schaeffer 1918), has such a cuticle (Page 1983), the name *Hollandella* unfortunately can no longer be used. The name *Mayorella* is retained, despite the slight possibility that it is a junior synonym of *Dactylamoeba* Korotneff 1880 (Page 1982), as discussed below under the latter genus. Amoebae which, by the original diagnosis (Schaeffer 1926), would be members of a single genus, are now distributed into 2 genera (Page 1981c, 1983), but it is, of course, not possible to be certain of those whose fine structure is unknown.

Mayorella is widely distributed in the seas as well as in freshwater. These small to medium-sized amoebae are cultured in liquid media and feed on accompanying eukaryote protists, including flagellates, small amoebae, unicellular algae, and even ciliates such as *Cyclidium*, as well as bacteria.

1 L 17-20 μm; nucleus 3-4 μm; cuticle 250 nm thick . . . . ***M. pussardi*** Hollande, Nicolas and Escaig 1981 (Mediterranean; for illustrations, medium, and food see Hollande *et al.* 1981)

— Larger, with crystals to 1.5 μm long, demonstrable with polarizers and sometimes paired, cuticle c. 180 nm 2

2 Dactylopodia usually present; knobby, sometimes elongate uroid common; L 29-86 μm (x̄ 52-59 μm); L:B x̄ 2.2-2.3; nucleus 4.6-8.4 μm (x̄ 5.4-7.6 μm). (Figures 121-123, 136, 137). . . . . ***M. kuwaitensis*** (Page 1982) n. comb. (Persian Gulf region; medium 1 without rice or 5 with rice grain. Description actually published in 1982 despite 1981 date on journal)

— Dactylopodia often absent; L 30-88 μm (x̄ 46-63 μm); L:B x̄ usually 1.8-2.0; nucleus 5.6-9.3 μm (x̄ 7.2-8.7 μm). (Figures 124-129, 134, 135). . . . . ***M. gemmifera*** Schaeffer 1926 (Cornwall, Norfolk, western North Atlantic, Gulf of Mexico; media 1, 4, 5, always with rice grain; food includes flagellates, unicellular algae, *Cyclidium*, as well as bacteria)

Other species whose form suggests that they may have a cuticle are:

Amoeba alveolata Mereschkowsky 1879. L approximately 45 μm. Description does not permit determination of synonymy with any more recently described species. White Sea.

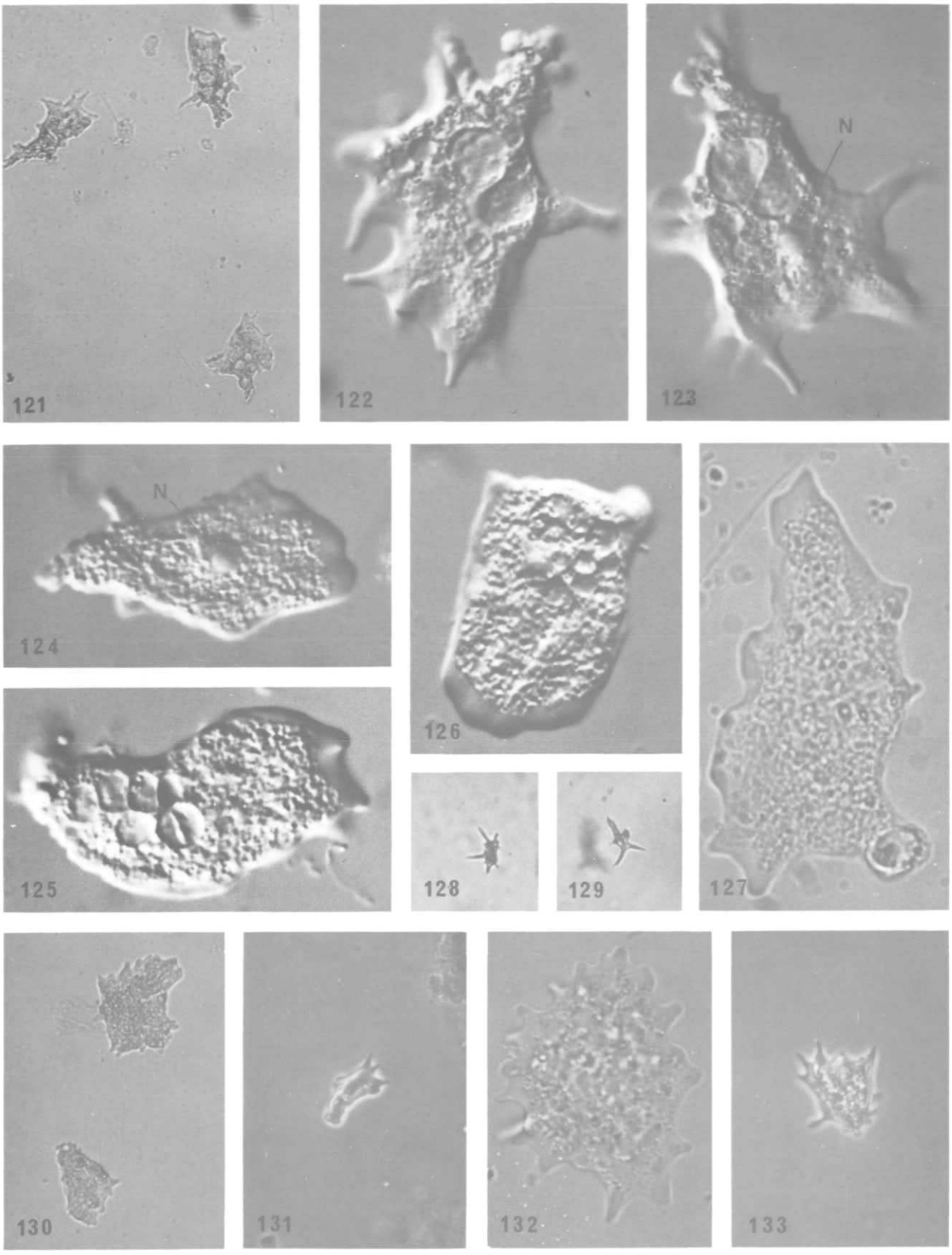
Mayorella conipes Schaeffer 1926. Schaeffer (1926) recognized that his 5 'varieties' may have included more than one species. Western North Atlantic, Gulf of Mexico.

Amoeba mustela Delphy 1938. Mean L 30-35 μm. 'Corps très anguleux'. Bay of Biscay.

Genus *Dactylamoeba* Korotneff 1880

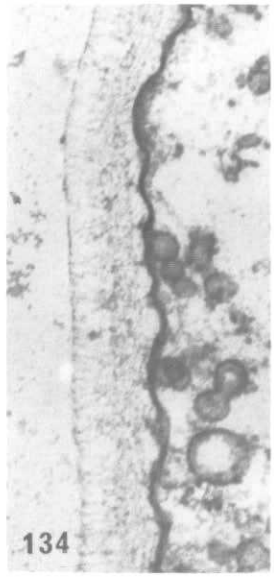
References: Anderson (1977); Grell and Benwitz (1970); Korotneff (1880); Page (1981c, 1982, 1983); Pennick and Goodfellow (1975)

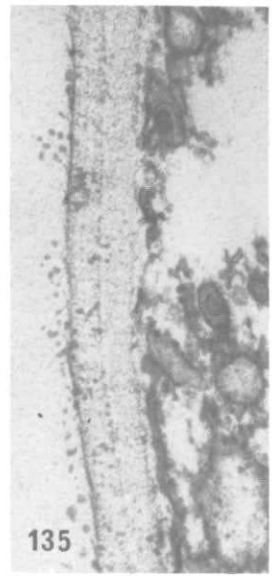
A few dactyloid, blunt, hyaline, non-furcate subpseudopodia of similar lengths usually produced from a hyaline

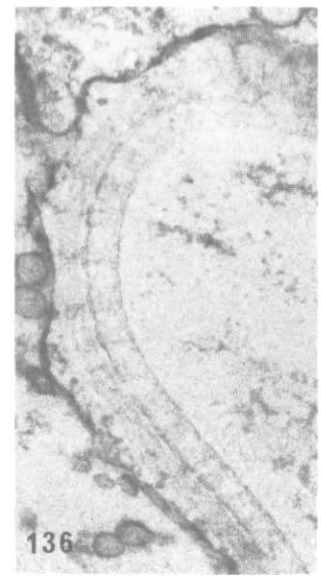


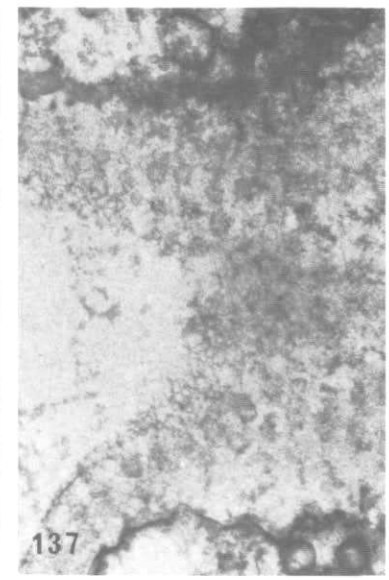
Figures 121-129. Mayorella, *strains known to have cuticle. 121,* Mayorella kuwaitensis; × *250. 122, 123,* M. kuwaitensis; × *1000. 124-127,* Mayorella gemmifera; × *1000. 128, 129,* M. gemmifera, *floating;* × *100.*

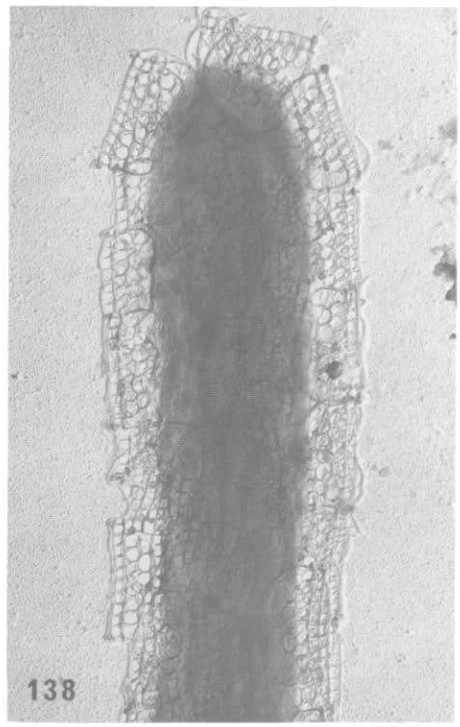
Figures 130-133. Strains of Mayorella *or* Dactylamoeba *in mixed cultures from 4 places; all* × *250 except 132, which is* × *1000. N, nucleus.*

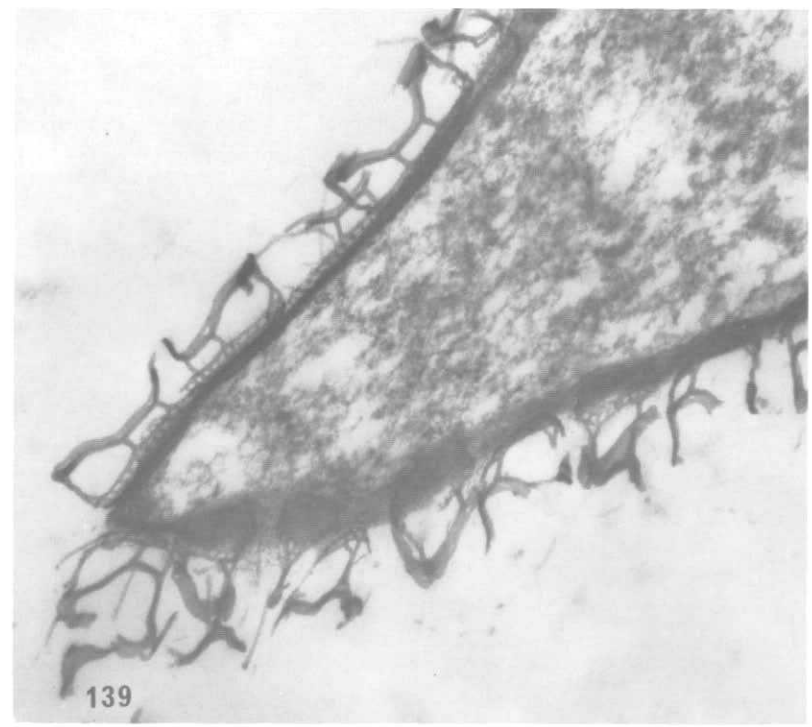












Figures 134-137. Cuticle of 3 strains of Mayorella *(in section); × 50000. 134,* Mayorella gemmifera. *135, another strain of* M. gemmifera. *136, 137,* M. kuwaitensis, *with tangential section of surface showing cross-sections of elements of cuticle in 137.*

Figures 138, 139. Scales of 2 strains of Mayorella*-like amoebae, which belong to the group tentatively designated as* Dactylamoeba. *138, shadow-cast whole mount; × 25000. 139, section; × 50000. Figures 138 and 139 by Mr N C Pennick.*

lobe; floating form often with distinctly spherical central mass and slender radiating pseudopodia; surface covered with complex, boat-shaped scales, often of 2 sizes and patterns; large Golgi region, site of scale synthesis, adjacent to nucleus. This is the group to which the name *Mayorella* was first applied in dividing Schaeffer's genus (Page 1981c), but it has since become apparent that *M. bigemma*, type species of *Mayorella*, was probably a cuticle-bearing amoeba (Page 1983). As the type species of *Dactylamoeba*, *D. elongata* Korotneff 1880, had a form resembling at least 2 scale-bearing amoebae, *M. riparia* Page 1972 (Pennick and Goodfellow 1975) and *M. stella* (Schaeffer 1926) (Page 1981c), it is suggested that the name *Dactylamoeba* be provisionally applied to this group.

At the same time, no named marine species can yet be classified as *Dactylamoeba*. None of the marine *Mayorella*-like amoebae known to bear scales (Anderson 1977; Grell and Benwitz 1970; Pennick and Goodfellow 1975) has been identified to species. Other named species of *Mayorella*-like amoebae await electron microscopical examination. Sawyer's (1975a) figure of the floating form of *M. corlissi* resembles that of scale-bearing amoebae, though his *M. smalli* seems unlike larger amoebae of either cuticle- or scale-bearing group. The pseudopodia of *M. crystallus* Schaeffer 1926 resemble those of scale-bearing strains, but no crystals have been found in identified freshwater scale-bearing strains, even with the polarizer.

This is therefore a group which is known to exist in saltwater without any named marine species yet being classified in the genus.

Genus *Vexillifera* Schaeffer 1926

References: Bovee (1956); Bovee and Sawyer (1979); Bunt (1970); Mitchell and Yankovsky (1969); Page (1972b, 1979a); Sawyer (1975a); Schaeffer (1926)

Subpseudopodia produced from anterior hyaloplasm as slender conical projections, which may elongate into linear form sometimes as long as main cell mass; subpseudopodia carried back along sides to posterior end, giving spiny appearance to amoeba; L:B usually greater than 1.0, with amoeba often narrow posteriorly, but outline often very irregular; floating form irregularly rounded with no or a few fine, bent, asymmetrically distributed pseudopodia; where fine structure is known, surface of marine species covered with discrete glycostyles in form of hexagonal cylinders approximately 50 nm in diameter rising approximately 50-70 nm above plasma membrane (Figures 2b, 152, 153).

These small (except one species) amoebae are the most spiny-looking, non-encysting marine Gymnamoebia, except perhaps *Paraflabellula*. Although their spiny appearance recalls that of *Acanthamoeba*, they should not be confused, because the subpseudopodia are never furcate and cysts are not formed. Two studies on the role of amoebae as consumers in the marine environment (Bunt 1970; Mitchell and Yankovsky 1969) employed strains attributed to this genus.

1 Nucleolar material in parietal lobes; greatest dimension of amoebae 5-16 μm (x̄ 10 μm); nucleus about 1-3 μm, observed under light microscope with difficulty. (Figures 140-142, 152) ..... ***V. minutissima*** Bovee and Sawyer 1979 (Western North Atlantic, Gulf of Mexico, South Wales; media 6-9)

– Nucleolus central 2

2 Amoebae containing several splinter-shaped trichocyst-like bodies, c. 5 μm long, with no fixed location in cytoplasm; greatest dimension of amoebae 10-23 μm (x̄ 15.5 μm), L:B x̄ 1.6; nucleus 2.8-4.2 μm (x̄ 3.6 μm). (Figures 143-147, 153) ..... ***V. armata*** Page 1979 (South Devon; media 6-9)

– No trichocyst-like bodies; greatest dimension 7-17 μm (x̄ 11 μm), L:B x̄ 1.6; nucleus 1.9-2.8 μm (x̄ 2.0 μm) in fixed preparations, reported as 3.0 μm by Bovee (1956); fine structure not known. (Figure 148) ..... ***V. telmathalassa*** Bovee 1956 (Western North Atlantic, Gulf of Mexico, North Sea; media 6-9)

Other species:

V. aurea Schaeffer 1926. L 60-80 μm; nucleus 15 μm. Gulf of Mexico.

V. browni Sawyer 1975. L 14-23 μm (x̄ 16.5 μm); nucleus 2.5-3 μm. Western North Atlantic.

V. ottoi Sawyer 1975. L 18-27 μm (x̄ 21 μm); nucleus 5-6 μm. Western North Atlantic, Gulf of Mexico.

Genus *Pseudoparamoeba* Page 1979

References: Page (1979b); Sawyer (1975a)

Expanded, flattened hyaloplasm occupying approximately anterior one third, with a few, short conical subpseudopodia produced from hyaloplasm and 1-3 long, narrowly conical subpseudopodia by elongation of short ones or, commonly, as anterior prolongation of ridge extending from granuloplasm through hyaloplasm; floating form with spherical central mass and characteristically with slender radiate pseudopodia tapering to very fine tip, length of pseudopodia to 4 times diameter of central mass; surface covered with blister-like structures rising about 35 nm above plasma membrane, with basal diameter about 55 nm, and hexagonal basal structural identical to that of *Vexillifera* glycostyles.

One known species:

Greatest dimension 7-23 μm (x̄ 13-14 μm), L:B x̄ 1.5-2.0; nucleus 2.4-4.5 μm (x̄ 2.7-4.0 μm); central mass of floating form with mean diameter c. 7 μm, 3-11 radiate pseudopodia, which extend to full length several minutes after amoebae suspended in liquid. (Figures 149-151, 154) .... ***P. pagei*** (Sawyer 1975)

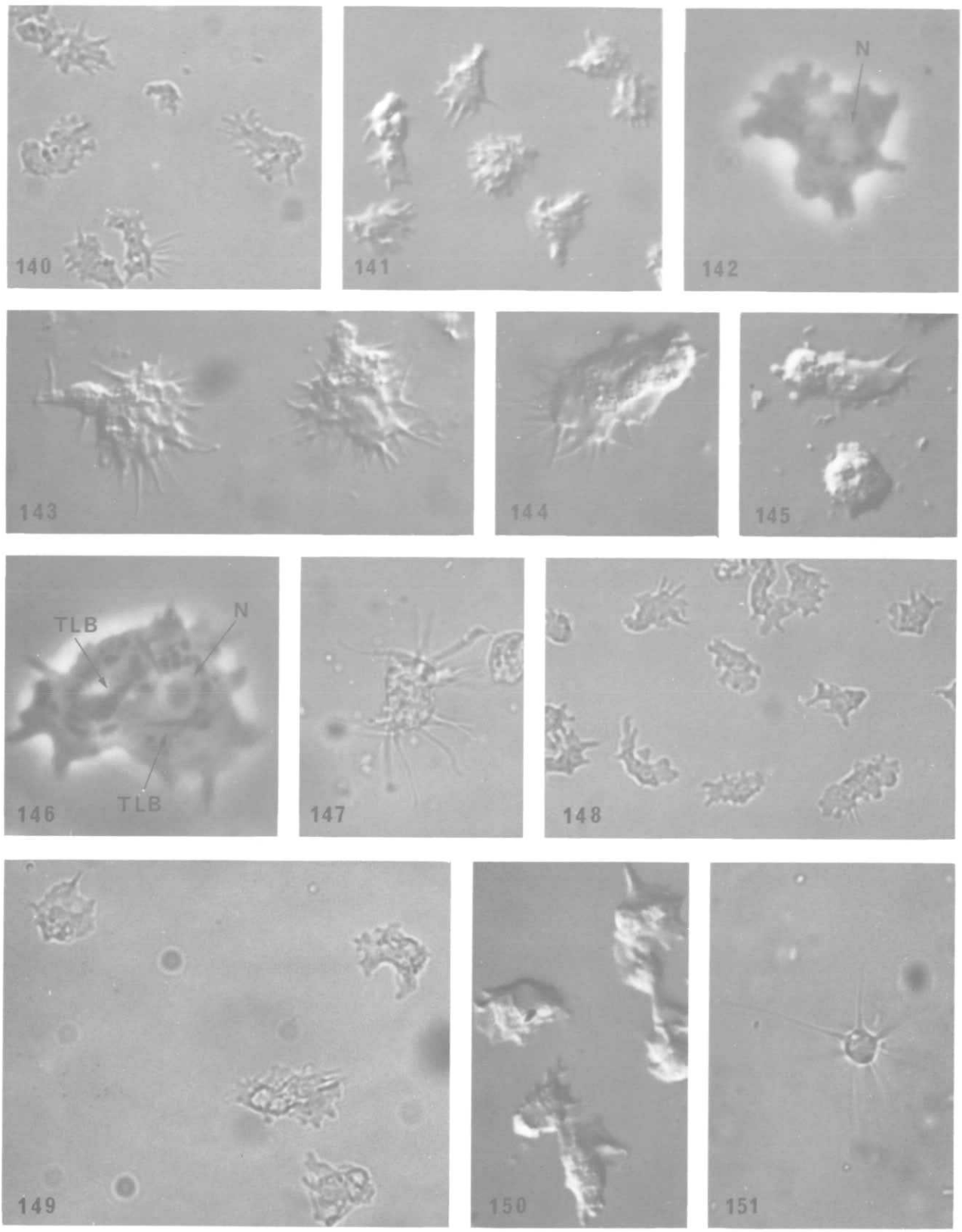
This amoeba looks almost exactly like a small variety of *Paramoeba pemaquidensis*, even to the somewhat parchment-like appearance of the expanded hyaloplasm; absence of the parasome must be confirmed, if necessary by the Feulgen procedure, to distinguish it from that more common species. The surface structure, identical on strains from south Devon, western Scotland, and Maine (USA), confirms its distinctness from, but relationship to, *Vexillifera*. Because subpseudopodia are not often carried back along the side, these amoebae do not have the spiny look of *Vexillifera*. (Western North Atlantic, British coastal waters, Gulf of Mexico; media 6-9)

Other genera of Paramoebidae

Boveella Sawyer 1975

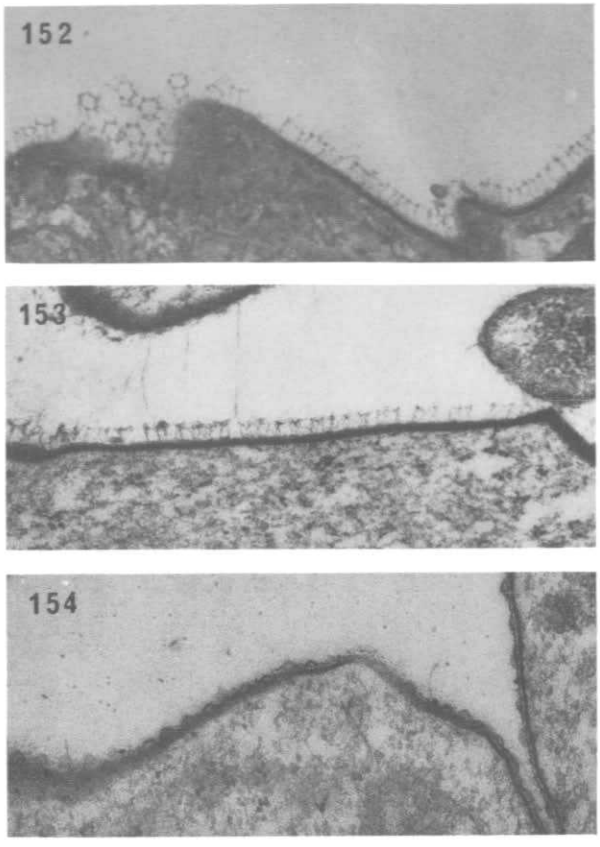
Reference: Sawyer (1975a)

Locomotive form unknown. Resting form a spherical mass, covered with organic detritus, from which numerous long, slender pseudopodia extend to a length approximately twice the diameter of the central mass. Pseudopodia may also be withdrawn.



Figures 140-148. Vexillifera. *140, 141,* Vexillifera minutissima; × *1000. 142,* V. minutissima, *phase contrast photograph to show nucleus with parietal nucleolar material;* × *2500. 143-145.* Vexillifera armata; × *1000. 146,* V. armata, *to show nucleus and trichocyst-like bodies;* × *2500. 147,* V. armata, *floating;* × *1000. 148,* Vexillifera telmathalassa; × *1000. N, nucleus; TLB, trichocyst-like body.*

Figures 149-151. Pseudoparamoeba pagei; × *1000. 151, floating.*



Figures 152-154. Sections of cell surfaces of (152) Vexillifera minutissima, *(153)* Vexillifera armata, *and (154)* Pseudoparamoeba pagei; × *50000. Cf Figure 2.*

B. obscura Sawyer 1975. Maximum diameter c. 20 μm, with c. 12 pseudopodia projecting at all angles, to a length of about 30 μm. Nucleus not observed. Chincoteague Bay, Virginia, USA.

Dactylosphaerium Hertwig and Lesser 1874

References: Hertwig and Lesser (1874); Schaeffer (1926)

Spherical or sub-spherical, with many digitiform or conical subpseudopodia produced from a hyaline edge and radiating in all directions, though most at anterior end in locomotion. Locomotion slow, with little change of shape. Radiate stage considered typical.

D. acuum Schaeffer 1926. L and B in locomotion 40-60 μm. Usually ovoidal with many tapering pseudopodia. Nucleus 12 μm, often obscured by remnants of algal food. Gulf of Mexico.

Triaenamoeba Bovee 1970

References: Bovee (1970); Sawyer (1975a)

Resembles *Vexillifera* in general appearance. Long, slender subpseudopodia produced in groups of 2-4, usually 3, from anterior hyaloplasm; short subpseudopodia also produced.
T. jachowskii Sawyer 1975. With 2 or more slender subpseudopodia. L of amoebae 13.5-22.5 μm ($\bar{x}$ 17 μm). Nucleus c. 3 μm. Western North Atlantic.

Family ACANTHAMOEBIDAE Sawyer and Griffin 1975

Reference: Sawyer and Griffin (1975)

Somewhat flattened amoebae with slender, flexible, tapering, occasionally furcate subpseudopodia containing oriented, finely fibrillar material (acanthopodia) produced from a broad, hyaline lobopodium; outline irregular, sometimes triangular with hyaline lobopodium at base of triangle; microtubular organizing centre in form of plaque- or bar-shaped centriole-like body; glycocalyx very thin or not detected by usual electron microscopical methods; cyst-forming.

Freshwater/soil amoebae which occur widely in saltwater, from which they are cultured by use of non-nutrient agar streaked with *Escherichia coli* (Page 1976a).

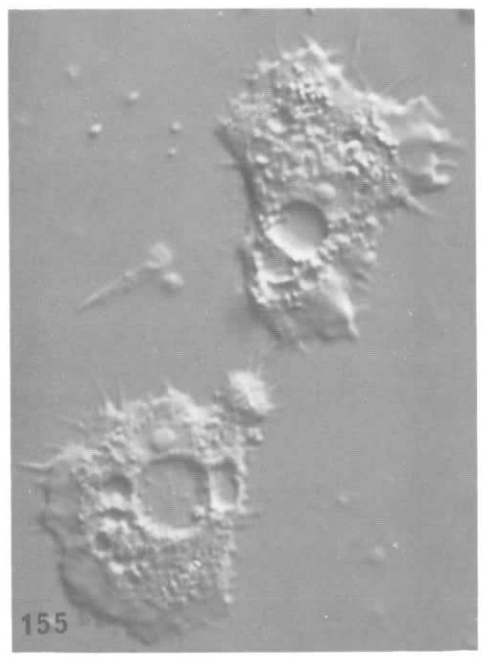
Genus *Acanthamoeba* Volkonsky 1931; emend. Page 1967 (Figures 155-159)

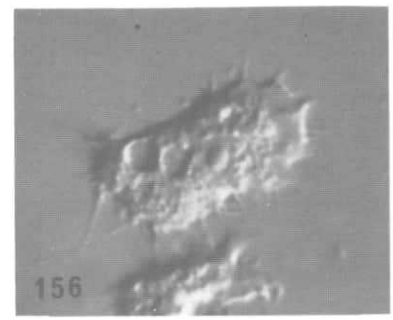
References: (1) Taxonomy: Costas and Griffiths (1980); Daggett *et al.* (1980); De Jonckheere (1982); Page (1967b, 1976a); Pussard and Pons (1977); Sawyer (1971a); Sawyer *et al.* (1977); Willaert (1976). (2) Occurrence in salt water and euryhalinity: Davis *et al.* (1978); Nerad *et al.* (1982); Page (1976c); Sawyer (1970, 1971a, 1980); Sawyer and Bodammer (1982); Sawyer and Buchanan (1971); Sawyer *et al.* (1977, 1982). (3) Pathogenicity: Griffin (1978); Pernin and Riany (1980)

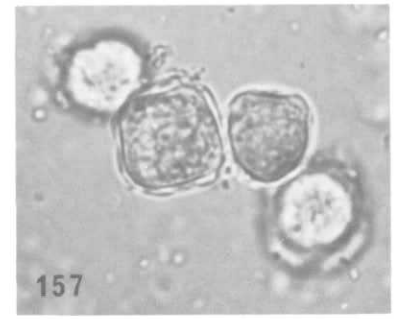
Excystment by removal of opercula at points of contact between inner and outer cyst walls. Inner wall (endocyst) often more or less polygonal or stellate (nearly rounded in some species); outer wall (ectocyst) more or less rippled.

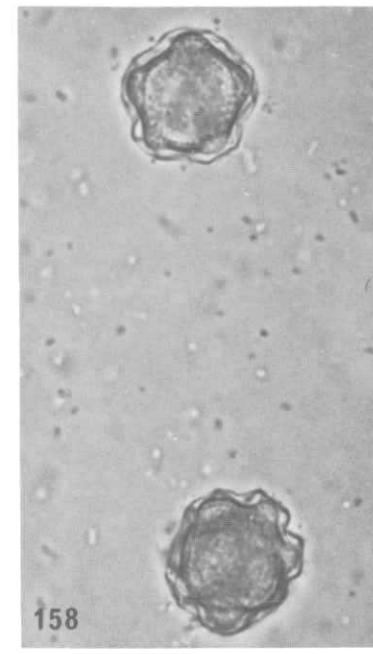
Three new species have been described on the basis of material collected from salt water: *A. gigantea* Schmoller 1964, from the Baltic, which must be considered brackish, with a surface salinity in most areas of 6-7 o/oo (Jansson 1978); *A. griffini* Sawyer 1971; and *A. hatchetti* Sawyer, Visvesvara and Harke 1977, which was found pathogenic to mice by intranasal introduction. Other species found in marine material include *A. castellanii* (Douglas 1930) (Sawyer 1980; Sawyer and Bodammer 1982); *A. culbertsoni* (Singh and Das 1970), the species most consistently found to be pathogenic (Sawyer *et al.* 1977; Sawyer 1980; Sawyer and Bodammer 1982); *A. polyphaga* (Puschkarew 1913) (Davis *et al.* 1978; Page 1976c; Sawyer 1980; Sawyer and Bodammer 1982); *A. rhysodes* (Sawyer 1980; Sawyer and Bodammer 1982); and *A. triangularis* Pussard and Pons 1977 (Sawyer and Bodammer 1982).

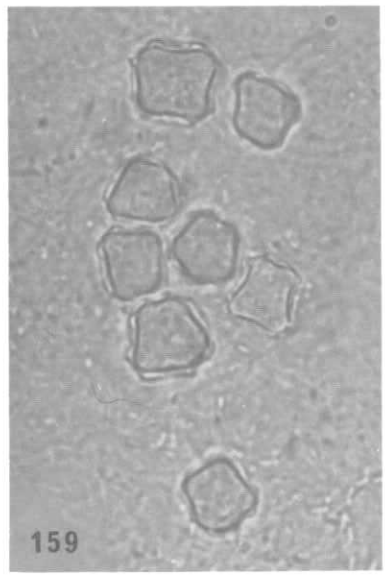
Acanthamoeba is essentially a ubiquitous, very adaptable freshwater/soil organism which tolerates relatively severe environmental stresses and can survive, at least as cysts, in waters of varying salinities. Its presence in marine habitats may be connected with the introduction of freshwater/soil materials by either natural processes or human activities (Sawyer 1980). Furthermore, although some *Acanthamoeba* isolates grow on agar of oceanic or near-oceanic salinities,

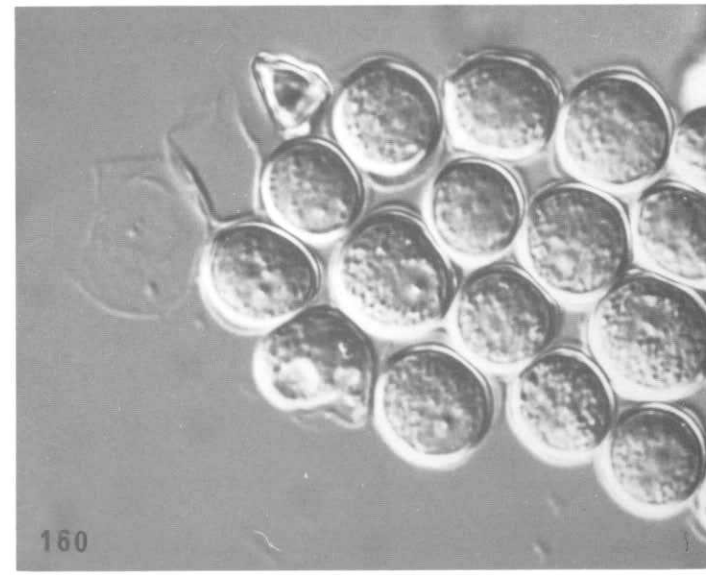












Figures 155-160. Acanthamoebidae; × 1000. 155, 156, Acanthamoeba castellanii. *157,* Acanthamoeba griffini, *cysts produced on marine agar (medium 7). 158,* A. griffini, *cysts produced on freshwater agar (non-nutrient agar; Page 1976a). 159,* Acanthamoeba polyphaga, *cysts produced on freshwater agar by material collected from estuary of River Stour, Essex, salinity 23 o/oo. 160,* Protacanthamoeba caledonica, *cysts produced on freshwater agar by clone isolated from Morar estuary. Note walls of empty cysts at left.*

material from a single sample often yields *Acanthamoeba* on freshwater but not saltwater agar (Page 1976c). The author has never isolated *Acanthamoeba* from inoculations on to agar with salinities of 26-35 o/oo, and other workers have also found agar with a salinity of 5 o/oo or less appropriate for isolation of *Acanthamoeba* from marine samples (Davis *et al.* 1978; Sawyer *et al.* 1977; Sawyer 1980). We have found non-nutrient agar (Page 1976a) most suitable for *A. griffini* and culture it axenically in the medium used for freshwater and soil strains.

Since *Acanthamoeba* seldom if ever grows out on the media recommended in this publication for marine amoebae, unintentional isolation of a genus containing facultative pathogens presents no problem. Workers intending to isolate *Acanthamoeba* from marine habitats can use the methods and media suggested for freshwater and soil amoebae (Page 1976a) or those employed in the cited works on marine findings. The recommended food organism is *Escherichia coli*. Many strains can also be grown axenically.

Identification of *Acanthamoeba* strains to species is beyond the scope of a publication on marine amoebae. Non-morphological characters (Costas and Griffiths 1980; Daggett *et al.* 1980; De Jonckheere 1982) may well provide the best approach to this difficult genus. The principal references for identification by morphological characters are Page (1976a) and Pussard and Pons (1977), but several species have been described since those dates.

Because of the sporadic pathogenicity attributed to this most common genus of free-living amoebae, investigators intending to carry out projects involving *Acanthamoeba* should consult the appropriate body (in Britain, the Health and Safety Executive) for procedures to be observed. The literature on pathogenicity has continued to grow since the review by Griffin (1978) and is beyond the scope of this publication.

Genus *Protacanthamoeba* Page 1981

References: Page (1981a); Singh and Hanumaiah (1979)

Cyst without preformed pores or opercula, usually circular or oval in outline, with 2 layers not always distinguishable; excystment by break in cyst wall.

One species has been reported from saltwater, *P. caledonica* Page 1981, isolated from the Morar estuary (Figure 160). This is probably the same species which, when isolated from soil, has been misidentified by some workers as *Amoeba glebae* Dobell 1914. L usually 20-50 μm, L:B 1.0 or more; mean nuclear diameter c. 8 μm. Cysts usually smooth-walled, though often with slight separation of wall into layers at some points, sometimes with slight wrinkling of separated outer part; greatest cyst diameter 11-21 μm ($\bar{x}$ 16.5 μm), with thickness (least diameter) of cysts on average 0.6 × greatest diameter. This strain did not grow at 37°. Cysts produced on non-nutrient (= freshwater) agar were viable after one week in full-strength seawater.

Singh and Hanumaiah (1979) described, under the name *Acanthamoeba invadens*, strains of *Protacanthamoeba* isolated from sewage sludge and lake mud in India, which grew at 42° and were pathogenic to mice when introduced intracerebrally or intranasally.

Family STEREOMYXIDAE Grell 1966

References: Grell (1966); Grell and Benwitz (1978)

Long and branched, relatively rigid floating or attached forms, or branching and anastomosing plasmodia or pseudoplasmodia, or both. In several species, a microtubular organizing centre (centriole-like body) with same structure as that of the Acanthamoebidae.

Genus *Stereomyxa* Grell 1966

References: Grell (1966); Benwitz and Grell (1971a, 1971b)

Floating or attached uninucleate 'hunger forms'; reticulum of uninucleate amoebae on substratum; feeding form round and attached. Neither species has been found in British waters, and the references must be consulted for both illustrations and culture methods.

1 Hunger form suspended in water; centriole-like body anywhere in cytoplasm; Golgi bodies distributed in cell; cell surrounded by broad slime layer ..... ***S. angulosa*** Grell 1966 (Coral reef, Madagascar, Indian Ocean)

– Hunger forms attached, forming network on substratum; Golgi region adjacent to nucleus; centriole-like body with radiating microtubules in Golgi region; cell membrane with no special differentiation..... ***S. ramosa*** Grell 1966 (Coral reef, Madagascar, Indian Ocean)

Genus *Corallomyxa* Grell 1966

References: Grell (1966); Grell and Benwitz (1978)

Multinucleate, reticulate plasmodia on substratum; long, unbranched, relatively rigid multinucleate or uninucleate floating forms, formed by detachment of dendriform buds from plasmodia in conditions of hunger.

1 Hunger form a uninucleate bud; centriole-like body and Golgi bodies adjacent to nucleus; no special differentiation of cell surface but at places osmiophilic bodies in great numbers under plasmalemma. (Figures 161-165)..... ***C. chattoni*** Grell and Benwitz 1978 (Mediterranean, Cornwall; culture methods in Grell and Benwitz 1978)

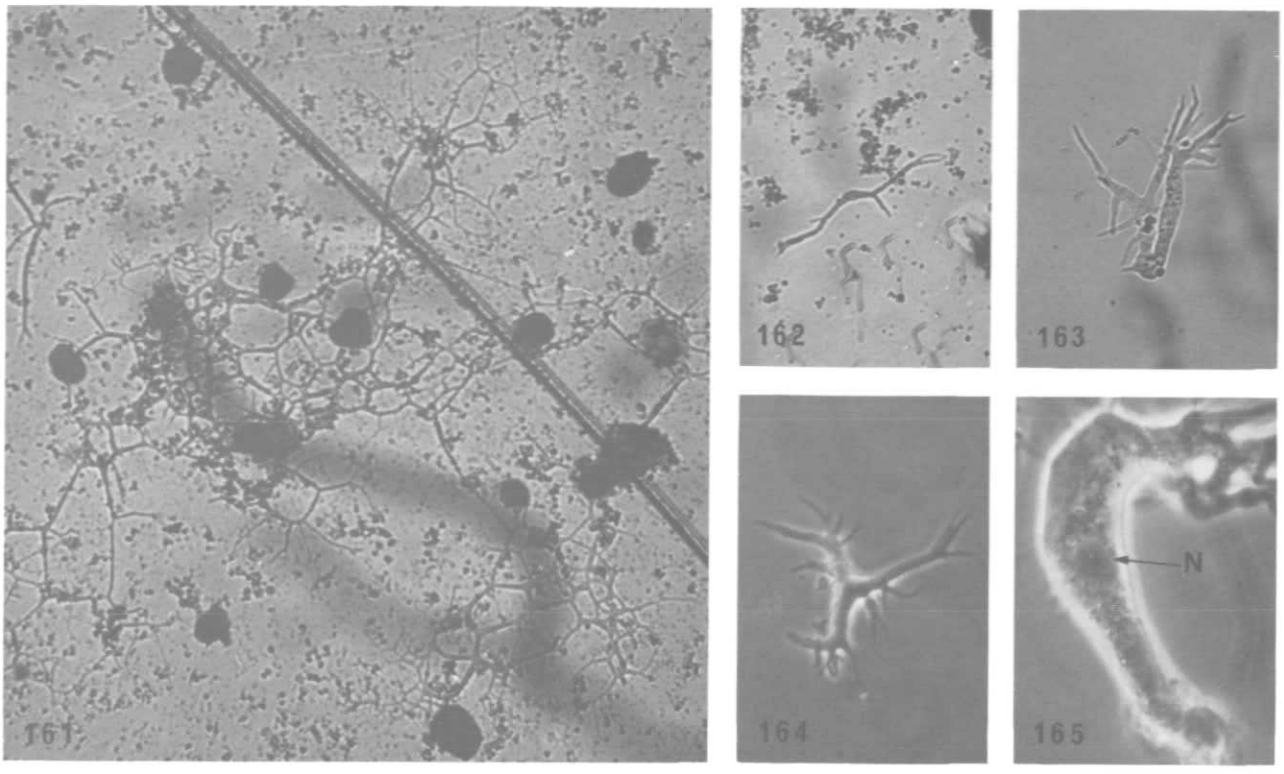
– Hunger form a multinucleate bud; no centriole-like body found; Golgi bodies mostly in pairs in vicinity of nucleus; thin-walled tubules on cell surface .... ***C. mutabilis*** Grell 1966 (Coral reef, Madagascar, Indian Ocean; illustrations and culture method in Grell 1966)

Other genera of Stereomyxidae

Stygamoeba Sawyer 1975

Reference: Sawyer (1975b)

Thin, elongate, monopodial locomotive form; branched or unbranched, sometimes with thin lateral expansions giving leaf-like appearance, in feeding form. Locomotion by slow gliding without evidence of protoplasmic streaming. Uninucleate.



Figures 161-165. Corallomyxa chattoni, *from Cornish rock pool. 161, reticulate plasmodium in culture dish (dark bodies not part of plasmodium); × 100. 162-165, uninucleate floating 'buds' (162, × 100; 163 and 164, × 250; 165, × 800). N, nucleus.*

S. polymorpha Sawyer 1975. Thin locomotive forms 13-17 μm long (x̄ 13.5 μm); leaf-like forms 7-13.5 μm (x̄ 10 μm). Nucleus 1.5-2 μm, difficult to see. Floating forms radiate, with fine pseudopodia. Western North Atlantic.

Family AMOEBIDAE Diesing 1848

References: Page (1976a); Radir (1927)

Usually polypodial; 2 genera (*Trichamoeba* and *Hydramoeba*) normally monopodial, with pseudopodial tips hemispherical and usually hyaline; pseudopodia of radiate floating form granular for most of length, not tapering strongly, with broadly rounded tips; nucleus in known species ovular (see p 12) or, rarely, with parietal nucleolus of one or a few large lobes.

The only well-described marine amoeba which appears likely to belong to this family is *Trichamoeba schaefferi* Radir 1927. The monopodial form agrees with Schaeffer's (1926) diagnosis of *Trichamoeba* Fromentel 1874, but *T. schaefferi* lacks the 'numerous, thin, hair-like projections extending from the posterior end' considered an important character by Schaeffer. Its re-classification must await its rediscovery and study by modern methods, including electron microscopy. It was found on the coast of California.

Radir described *T. schaefferi* as 226-306 μm long, with crystals of a shape different from that known in other Amoebidae, and with a spherical nucleus about 30 μm in diameter with a cup-shaped nucleolus shaped somewhat like the parietal nucleolus of amoebae such as *Polychaos fasciculatum* (Penard 1902) (Page and Baldock 1980).

Some parasitic marine amoebae

This list is intended as an introduction to the literature about Gymnamoebia parasitic in marine organisms. It does not include parasites of salmonids or strains of *Acanthamoeba* isolated from marine sources and found pathogenic to laboratory animals. Some of the organisms in this list were undoubtedly parasitic and the cause of pathological conditions; others appear more likely to have been commensals. Free-living amoebae such as *Flabellula calkinsi* have been found in association with marine invertebrates.

Parasite	*Host(s)*	*References*
Amoeba biddulphiae Zuelzer 1927	*Biddulphia sinensis*	Zuelzer 1927
Hartmannella tahitiensis Cheng 1970	*Crassostrea commercialis*	Cheng 1970
Janickina chaetognathi (Grassi 1881)	*Spadella* spp.	Janicki 1912; Chatton 1953
Janickina pigmentifera (Grassi 1881)	*Spadella* spp. *Sagitta* spp.	Janicki 1912, 1928, 1932; Chatton 1953; Hollande 1980
Paramoeba perniciosa Sprague, Beckett and Sawyer 1969	*Callinectes sapidus,* *Cancer irroratus* *Homarus americanus*	Sprague and Beckett 1966, 1968; Sawyer 1968, 1976; Sprague *et al.* 1969; Perkins and Castagna 1971; Newman and Ward 1973; Johnson 1977
Pseudovahlkampfia emersoni Sawyer 1980	*Callinectes sapidus* *Ovalipes ocellatus*	Sawyer 1980
Vahlkampfia discorbini Le Calvez 1939	*Discorbis mediterranensis*	Le Calvez 1939
Vahlkampfia mucicola (Chatton 1909)	*Symphodus* spp.	Chatton 1909, 1910
Vahlkampfia paedophthora	*Peltogaster curvatus*	Caullery 1906

'Pontifex maximus'

References: Angell (1976); Griffin and Spoon (1977); Gruber (1881); Schaeffer (1926); Schaudinn (1899)

The organisms shown in Figures 166-172 appear, especially at low magnification, to be Gymnamoebia, perhaps an unusual *Thecamoeba*. However, observation of the dactylopodia, indicated by arrows, at various points immediately identifies them as the organisms Schaeffer (1926) named *Pontifex maximus* and classifed in his family Mayorellidae (= Paramoebidae). Even if this were, as Schaeffer believed, a naked amoeba, *P. maximus* would not be the valid name as it had already been described by Gruber (1881) as *Amoeba tentaculata*.

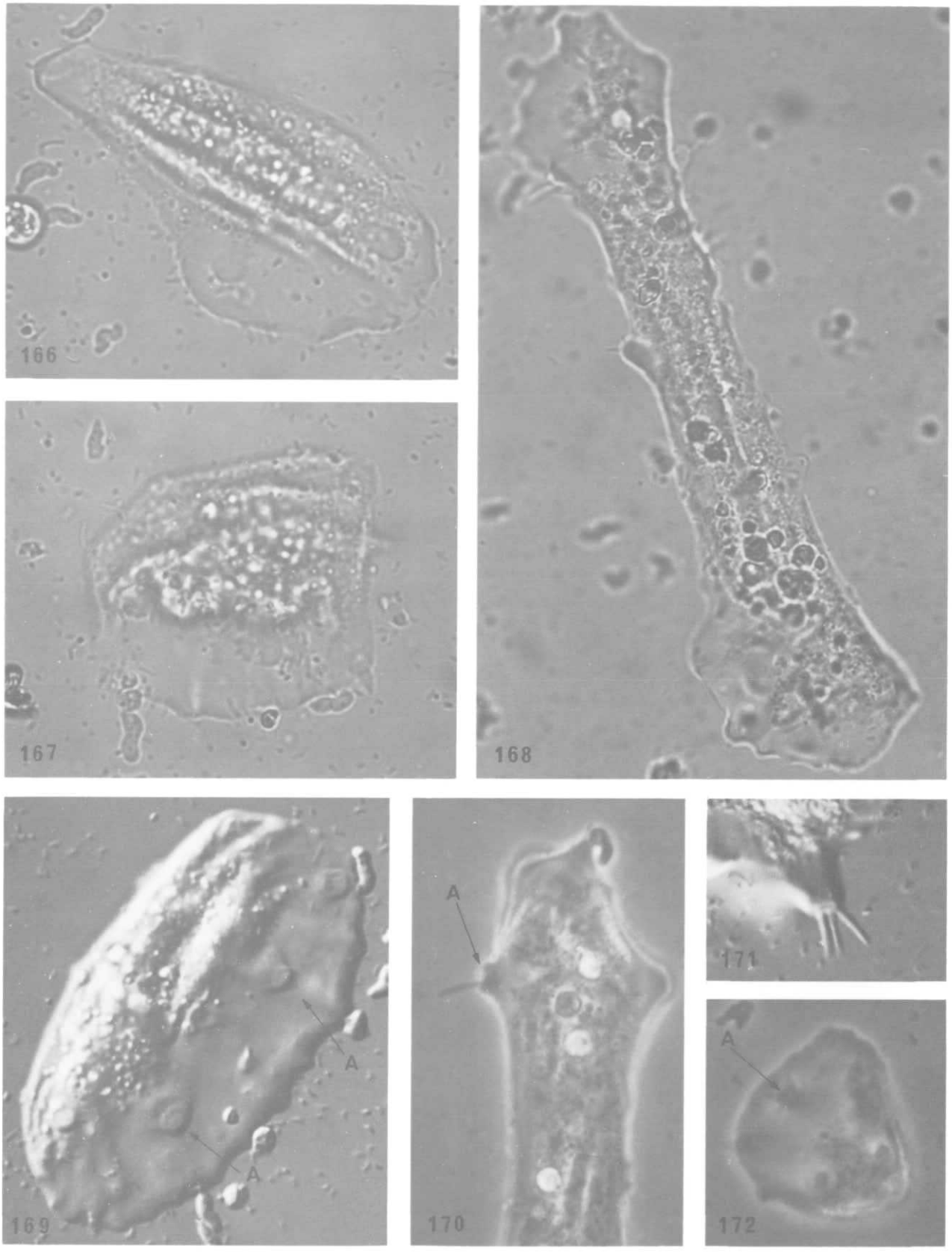
P. maximus/A. tentaculata is, however, not a naked amoeba, but one of the 2 alternate forms of the common marine testacean *Trichosphaerium* Schneider 1878. Although the spicule-less 'gamont' (Schaudinn 1899) or 'smooth' form (Griffin and Spoon 1977) of *Trichosphaerium* has thus been known for more than a century and light micrographs have been published (Grell 1968a; Angell 1976), the identity of *Pontifex maximus* with that form of *Trichosphaerium* appears to have been recognized first by Griffin and Spoon (1977). It is included here because it is common and easily mistaken for a naked amoeba, as it was by Gruber and Schaeffer, though Gruber recognized the indications of a 'skin-like cortical layer', which he mistakenly thought had to be broken through before a dactylopodium could appear. (*Amoeba tentaculata* Biernacka 1963 is a homonym, not the same organism.)

Trichosphaerium is common in marine habitats throughout the world. Spicule-bearing *Trichosphaerium* (the more commonly found form) have been reported from the North Sea (Schneider 1878); the Baltic (Möbius 1889); Wales (Sheehan and Banner 1973); Florida, California, Australia, Fiji (Angell 1975, 1976); New York (Schuster 1976); and Virginia (Griffin and Spoon 1977). A spicule-bearing strain from Essex has been maintained for years at CCAP, and spicule-bearing forms were common in rock pools at Hannafore Point, Cornwall. Smooth, or spicule-less, forms occurred in material from an aquarium in Germany, possibly originating from the Mediterranean (Gruber 1881); the eastern USA (Schaeffer 1926; Griffin and Spoon 1977); Australia and Fiji (Angell 1976); they have been isolated in this laboratory from rock pools at Hannafore Point, Cornwall, and the sandy beach at Sheringham, Norfolk.

The relationship between the spicule-less forms and the more common ones with calcite spicules is not certain. Though most recent authors continue to use the terms 'gamont' (the 'sporont' of Schaudinn 1899) and 'schizont', the descriptive terms 'smooth' and 'fuzzy' (Griffin and Spoon 1977) are preferable until convincing evidence of the life cycle is presented. There is little doubt that both forms occur in at least one or 2 species of *Trichosphaerium*.

Trichosphaerium, with 3 named species, is the only genus in the order Trichosida, subclass Testacealobosia (Levine *et al.* 1980).

When extended on a substratum, the smooth form may resemble a somewhat angular *Thecamoeba* or have a more fan-like outline, or it may be highly irregular, especially large forms (sometimes more than 1 mm in length) in slowly multiplying cultures. The



Figures 166-172. 'Pontifex maximus', *the spicule-less stage of the trichosid testacean* Trichosphaerium; *all* × *1000 except 168, which is* × *800. Some apertures in flexible test, from some of which dactylopodia protrude, are indicated by A. These photographs show some of the many forms taken by this organism. The much smaller limax amoebae, probably* Vahlkampfia *sp., may serve as part of the diet of this stage of* Trichosphaerium.

greatest dimensions of 100 amoebae in a rapidly multiplying 8-day culture of the Sheringham strain were 26-144 μm, mean 41 μm. These amoebae are multinucleate; Angell (1976) reported 12-187 nuclei. The small cell in Figure 172 appears to have only a few nuclei, each with a diameter about 6 μm. The fibrous test (also present beneath the spicules of the 'fuzzy' form) contains apertures through which hyaline dactylopodia may be extended. These apertures can be seen to move forward on the dorsal or free side, over the front edge, and backward on the ventral side, that is the side applied to the substratum, particularly in forms such as those in Figures 169 and 172. The aperture is often on a raised papilla, like a volcanic crater. Sometimes a single dactylopodium is extended, thickens distally, and divides into 2 or 3 dactylopodia. The dactylopodia (found also in the 'fuzzy' forms) have not been found to be involved in either locomotion or feeding.

We maintain the spicule-bearing form in medium 1, feeding on accompanying organisms. Our strain of the smooth form thrives in medium 5 with a rice grain, feeding on accompanying organisms. However, the smooth form also grows abundantly on agar (medium 7) without a liquid overlay, kept in the dark and feeding on accompanying *Vahlkampfia*-like amoebae. It can be subcultured by turning a piece of agar from the parent culture over on to a fresh surface. Grell (1968b) listed diatoms as the food of *Pontifex maximus*. Angell (1976) reported that the smooth form feeds on diatoms and the spicule-bearing one on blue-greens.

Genera and species not listed elsewhere

The purpose of this section is to provide entry to the literature about genera and species of marine gymnamoebae which, usually for reasons given below, have not been included in either the keys or the lists of other genera and species under each family.

Genera

These are not included elsewhere either because their familial position is uncertain or because the diagnosis of the genus or its use for marine species is in some way unsatisfactory. Descriptions of these genera and of species classified in them will be found in the original works or, for some, in Bovee and Sawyer (1979).

Amoeba Bory de St. Vincent 1822. The great majority of free-living lobose amoebae described before Schaeffer (1926) were attributed to this genus, diagnosed vaguely and broadly. No well-described marine amoeba is now classified in this genus as now diagnosed (Page 1976a).

Gibbodiscus Schaeffer 1926. This has been mentioned (p 33) by way of comparison with *Paraflabellula*, but not classified to family. It is one of several nominal genera having the shape of a flattened disc consisting of a granuloplasmic hump surrounded by a hyaloplasmic zone, from the edge of which numerous long, slender subpseudopodia are extended and retracted. Other characters were described by Schaeffer (1926) and Sawyer (1975b), both of whom included it in the family Hyalodiscidae Poche 1913. Two species: *G. gemma* Schaeffer 1926 and *G. newmani* Sawyer 1975.

Hyalodiscus Hertwig and Lesser 1874. This generic name has presented problems ever since it was proposed, and various authors have attributed quite diverse fresh- and saltwater amoebae to the genus. It was the first genus described as disc-like and consisting of a granuloplasmic hump surrounded by hyaloplasm (Hertwig and Lesser 1874). The type species, a freshwater amoeba, *Hyalodiscus rubicundus* Hertwig and Lesser 1874, has been reported from the Baltic (Levander 1894). Marine amoebae classified in the genus are *H. korotnewi* Mereschkowsky 1879; *H. elegans* Schaeffer 1926; *H. caeruleus* Schaeffer 1926; *H. minimus* Lepşi 1960; and *H. angelovica* Sawyer 1975 (= *H. angelovici* in Bovee and Sawyer 1979). Because of the inadequacy of any generic diagnosis in the present state of knowledge, the family Hyalodiscidae cannot be used.

Metachaos Schaeffer 1926. There is some doubt about this genus, which if valid belongs to the Amoebidae. The doubt is due partly to uncertainty about the validity of the type species, *M. discoides*. The only marine species is *M. fulvum* Schaeffer 1926.

Pelomyxa Greeff 1874. This name has been ascribed indiscriminately to very diverse multinucleate or sluggish amoebae, especially large ones. It is correctly applied only to amoebae sharing some characters of *P. palustris* Greeff 1874 (Page 1976a), amongst which are the lack of mitochondria. One marine amoeba, *P. marina* Delphy 1938, has been classified in this genus, on the basis of an erroneous generic diagnosis.

Rhabdamoeba Dunkerly 1921. This genus was erected for a very small amoeba, *R. marina*, greatest dimension 10 μm, with pseudopodia which are 'prominent knoblike structures which contain generally 4 or 5 pointed rod-like bodies, and these project slightly from the surface of the pseudopodia like tiny spines' (Dunkerly 1921). The description and the fact that the cultures had been started with 'some *Trichosphaerium* material' make it very likely that this was a very small smooth form of *Trichosphaerium*, described in the section *'Pontifex maximus'*, pp 45-47.

Striolatus Schaeffer 1926. The type species *Striolatus tardus* Schaeffer 1926 was limax-like in locomotion and had long, slender subpseudopodia in its radiate form and a flattened stationary form. Schaeffer classified it in his family Mayorellidae (= Paramoebidae).

Unda Schaeffer 1926. This genus was diagnosed as a flattened ovoid with its length less than its breadth, the anterior part consisting of hyaloplasm that moves forward in waves. Both Schaeffer (1926) and Sawyer (1975b) classified it in the Hyalodiscidae, though its similarity to *Platyamoeba* suggests a position in the Thecamoebidae, if this is indeed a distinct genus. The species are *U. maris* Schaeffer 1926, and *U. schaefferi* Sawyer 1975.

Species

Some of these names have been given to organisms so scantily described that they are unrecognizable, but others undoubtedly represent valid species. The latter have not been included under the various families either because the familial position of their genus is not certain, according to the classification used here, or because certain characters (including fine structure) essential to the scheme employed are not known. In each case, the generic name is that to which the species was attributed when first described. Some of these species are included, under the original genus or another, in Bovee and Sawyer (1979).

Species	*Reference*
Hyalodiscus angelovica	Sawyer 1975b
Hyalodiscus caeruleus	Schaeffer 1926
Amiba crassa	Dujardin 1841
Hyalodiscus elegans	Schaeffer 1926
Amoeba filifera	Mereschkowsky 1879
Amoeba flava	Gruber 1885
Amoeba fluida	Gruber 1885
Metachaos fulvum	Schaeffer 1926
Gibbodiscus gemma	Schaeffer 1926
Amoeba hostilis	Kufferath 1952
Hyalodiscus korotnewi	Mereschkowsky 1879
Amiba marina	Dujardin 1841
Pelomyxa marina	Delphy 1938
Rhabdamoeba marina	Dunkerly 1921
Vahlkampfia marina	Delphy 1938
Unda maris	Schaeffer 1926
Hyalodiscus minimus	Lepşi 1960
Amoeba minuta	Mereschkowsky 1879
Amoeba monociliata	Gourret and Roeser 1888
Gibbodiscus newmani	Sawyer 1975b
Amoeba ostendensis	Kufferath 1952
Amoeba placida	Kufferath 1952
Amoeba prehensilis	Möbius 1889
Amiba ramosa	Dujardin 1841
Amoeba salinae	Frenzel 1893
Unda schaefferi	Sawyer 1975b
Striolatus tardus	Schaeffer 1926
Amoeba tentaculata	Biernacka 1963
Vexillifera vacillans	Wailes 1932
Vahlkampfia vacua	Delphy 1938

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Taxonomic index

This index includes only taxa of the classes Lobosea and Acarpomyxea, not food organisms or other organisms mentioned incidentally. Page references to *Amoeba* include only those in which the name is used in its modern restricted sense. Species are listed alphabetically under the specific epithets, to facilitate finding them no matter what generic name is known.

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