Eugène Penard's slides of Gymnamoebia: re-examination and taxonomic evaluation

Frederick C. Page

Culture Centre of Algae and Protozoa, Institute of Terrestrial Ecology (N.E.R.C.), 36 Storey's Way, Cambridge CB3 0DT

Contents

Synopsis

The slides of freshwater naked amoebae prepared early in this century by Eugène Penard have been examined with modern optical systems, and much of the material is presented in photomicrographs. The new genus *Thecochaos* is proposed for amoebae which resemble *Thecamoeba* except in being multinucleate, with *T. fibrillosum* (Greeff, 1891) as the type-species. Other new combinations include *Chaos nobile* (Penard, 1902), *Thecamoeba papyracea* (Penard, 1905), and *Thecochaos album* (Greeff, 1891). The validity of several named species of *Pelomyxa* is considered without a definite conclusion. The taxonomic positions of several species cannot be determined on the basis of the present material.

Bull. Br. Mus. nat. Hist. (Zool.) **40**(1): 1–32

Issued 26 February 1981

Introduction

Eugène Penard (1855–1954) was one of the classical students of protozoan natural history. From 1887 until his eyes failed him in 1922, he investigated diverse Sarcodina, ciliates, and flagellates, all collected from natural sources and studied alive and in fixed preparations. Most of his life was spent in his native Geneva, but his publications include material from such far places as Loch Ness, Sierra Leone, the Rocky Mountains, and the Himalayas. To the biography by Deflandre (1958) are appended a list of publications and a list of 24 genera and 426 species of Protozoa (including 31 species of Gymnamoebia) erected by Penard.

Altogether Penard produced at least 82 protistological publications, the great majority on Sarcodina. His most important work on rhizopods, including naked amoebae, is *Faune Rhizopodique du Bassin du Léman* (1902), foreshadowed by his 'Étude sur les Rhizopodes d'eau douce' in 1890. The *Faune Rhizopodique* is among the classical publications on naked amoebae, which also include those of Leidy (1879), Cash (1905), Cash & Wailes (1919), and Schaeffer (1926). All except the last also deal with testaceans, which indeed take up the larger part of the space.

Penard was interested especially in observations of living organisms, considering such 'physiology' more interesting and more important than systematics, which he did, however, regard as essential. Amongst the characters which he could observe in naked amoebae, he considered the nucleus especially important. To preserve his organisms and facilitate observations of such characters as the nucleus, he made many permanent preparations. His standard method (Penard, 1902) was fixation with absolute alcohol, staining with borax carmine, and mounting in Canada balsam. His observations were made without an immersion objective (Deflandre, 1958), and of course he depended on drawings rather than photographs to convey visual impressions. Deflandre praised these drawings highly, though it must be said that in the case of Gymnamoebia one might often wish for more details, more depth, and especially more individual amoebae. The subsequent reproduction by later authors of a single Penard drawing per species, which even if composite in origin could only represent a single cell at a single moment, has over the years proved inadequate for identification.

According to Deflandre (1958) Penard's slides are preserved in three major collections. When Deflandre was writing (he died in 1973), the smallest of these three main collections was in his own possession. It comprised 280 slides, of which 233 were of rhizopods, including 18 slides of *Amoeba* (in Penard's broad sense of the genus) and five of *Pelomyxa*. The second collection was at the Muséum d'Histoire Naturelle of Geneva, containing 663 preparations, according to Deflandre. In 1952 Deflandre found these preparations in good condition but could not summarize their contents for lack of an adequate catalogue. However, Grospietsch (1975), in a publication with limited distribution, listed 791 slides in the Geneva collection. These include 48 slides of 13 species of *Amoeba* (in Penard's broad sense) and 11 slides of five species of *Pelomyxa*. All these species of naked amoebae except two of *Amoeba* are also represented in the third and largest major collection, that at the British Museum (Natural History), consisting of 950 slides. The great majority of these are testaceans. The 55 slides in this third collection bearing naked lobose amoebae (Gymnamoebia) are the object of the present study. According to Penard's nomenclature, they contain specimens of 20 species, as well as one slide with no specific identification.

Besides the three major collections, Heal (1965) lists three smaller collections of Penard's slides in Britain, but these contain only three of Penard's species of Gymnamoebia, all represented in the collection at the British Museum (Natural History).

There are several reasons for re-examining Penard's slides now. Free-living amoebae are much better known than they were a few decades ago. They have been investigated by more workers with a greater diversity of aims than ever before, applying modern tools. Knowledge of taxonomic value has been developed in some cases by workers who initially had no particular interest in taxonomy. It is principally large amoebae which have been much used in cell biological investigations, with the exception of *Acanthamoeba*, and Penard's publications dealt with and his slides preserve mainly larger amoebae. Among the Penard material are represented several groups whose taxonomic status and boundaries are still matters of some uncertainty. For example, how many species of *Pelomyxa* did Penard actually have in front of him? There are also organisms which apparently have been seen by few other workers, so that on the basis of Penard's text and few drawings their existence and identification have been questioned: Are there, already described and identifiable, more species of *Chaos* than the two familiar to present-day cell biologists? Are there multinucleate amoebae which resemble *Thecamoeba* more than they resemble *Amoeba* and thus perhaps should not be classified as *Chaos*? With modern optical and photographic equipment, the Penard material can provide answers to some questions and give information for further consideration of those questions which remain unsettled.

Bright field, phase contrast, and differential interference contrast optics have been used to examine and photograph the preparations. In most cases, it was possible to examine and photograph the slides with an objective up to × 63 (giving × 800 final magnification for direct observation, × 315 on the photographic negative). In a few cases the thickness of the mount made use of an objective greater than × 10 impossible, but enlargement of the negatives provided further information, such as nuclear size and structure. Most of the material on the slides was photographed, the omissions being a very small quantity of useless material and some repetitive material. The same cells were usually photographed at two magnifications and with at least two optical systems. From these hundreds of photomicrographs, the most informative are included as illustrations. The optical systems are not consistent amongst the illustrations, since the most informative photomicrographs of one species or feature were not always obtained with the same system which was most useful for another. That this inconsistency does not prevent useful comparison can be seen by examining, for example, the figures of *Amoeba fibrillosa* and those of *Amoeba alba*.

All reasonably intact cells were measured. In uni- or binucleate species, all discernible nuclei were measured. In multinucleate cells, 25 nuclei were measured (if that many were present) in one or more cells.

Finally, it should be noted that Penard's over-all taxonomic system for rhizopods was simple and imprecise (Penard, 1902). He recognized two 'groups', the Lobosa and the Reticulosa, with the Filosa as a subgroup of the former. Within the naked lobose amoebae he classified most in the genus *Amoeba*, although he also used the two supposedly anucleate genera *Protamoeba* Haeckel, 1866, and *Gloïdium* Sorskine, as well as *Dinamoeba* Leidy, 1874, which is still recognized. He considered a subdivision of the genus *Amoeba sensu lato* premature at that time. *Pelomyxa* he defined as 'Amibes à mouvements lents, toujours pourvues de bactéries symbiotiques'. A modern classification of as many of Penard's organisms (on these slides) as possible will be suggested.

Observations

These are in no sense species descriptions but only summaries of the information derived from the slides. Because of the limited taxonomic usefulness of fixed light-microscopical preparations of Gymnamoebia (greater for these larger amoebae than for smaller ones), a fuller picture of the organisms requires consultation of the descriptions of living amoebae in the *Faune Rhizopodique* (1902) and Penard's other publications. It will be found that the measurements given in that publication differ somewhat from those derived from the fixed preparations. For some species listed here, Page (1976, 1977) gives more complete information, under modern generic names. The headings below use Penard's generic classification; some re-classifications with gender modifications of specific epithets are proposed later in this publication. The authorships given in the headings are those required by today's nomenclatorial regulations whether or not they correspond with those used by Penard. Dates are absent from many slides. All slides bearing dates of preparation were made between 1901 and 1903, but it is almost certain that some were made later. The first two digits of the slide numbers indicate the date of deposit in the British Museum (Natural

History): ·04 = 1904, etc. All these slides bear material collected in Geneva and vicinity, including Haute Savoie.

Fixation is as one would expect given the use of absolute alcohol alone, with some shrinkage of conical subpseudopodia (*Amoeba vespertilio* and *Dinamoeba mirabilis*) and blurring of the distinction between hyaloplasm and granuloplasm. The nuclear stain remains good. Most of the amoebae are located near the centres of the preparations. Some cells are broken. Only a minority of the amoebae are in the form taken during steady locomotion, with more irregular forms, e.g., producing branches in several directions or spreading on the substratum, common.

The references under each species heading list the publication(s) in which Penard described each species, since it is only his material which is being considered here. Publications of other authors are cited in the remarks where relevant.

Amoeba proteus Leidy, 1878
(Figs 1–6)

Penard, 1902, pages 57–60

Slide numbers. 04.5.9.19; 04.5.9.30; 20.12.8.10 (labelled *Amoeba laureata* with notation 'avec 1 *Amoeba proteus*'); 20.12.8.15; 20.12.8.16; 20.12.8.17; 20.12.8.18.

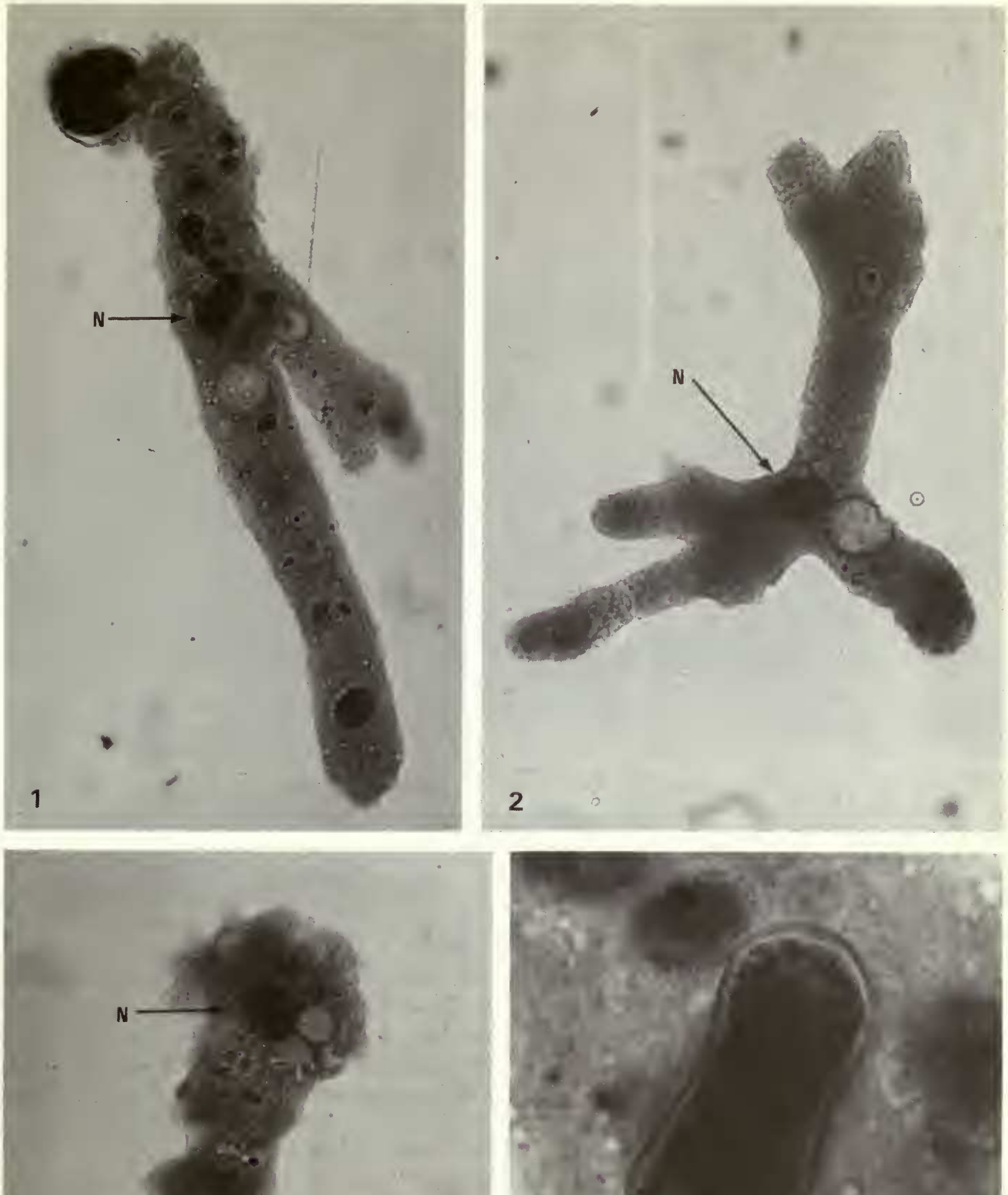
Total number of amoebae. 13.

Description and remarks. Several of these cells had the elongated form often seen in more rapid locomotion of *A. proteus*, the longest (Fig. 1), 524 μm long, with a second pseudopodium which may have been undergoing retraction at the moment of fixation. These preparations were examined in the light of Schaeffer's (1916) statement: 'I therefore suggest the specific name *dubia* for the organism named *proteus* by Penard.' None of the amoebae in these preparations had a cell form not reconcilable with that of *A. proteus* as understood since Schaeffer's (1916) more precise definition of that species, though one could equally say that these shapes could also be encountered in *Polychaos dubium* (Schaeffer, 1916). Certainly the three locomotive forms on slide 20.12.8.15 are fully compatible with *A. proteus*. Schaeffer pointed out the inconsistency between the discoid nuclear shape described for *A. proteus* by Leidy (1879) and that which Penard described as 'toujours ovoïde' except in a variety where it was 'toujours parfaitement globuleux'. The nuclei in most of Penard's specimens could indeed be ovoid, but at least one of those on slide 20.12.8.15 is discoidal (Figs 1, 4), and all the amoebae on that slide appear to be of the same type. Nuclear diameters in Penard's specimens are 27·5 to 54 μm, with only one below 37 μm.

Although it is therefore possible that some of Penard's slides labelled '*Amoeba proteus*' do bear members of other species, according to present specific distinctions, the amoebae on slide 20.12.8.15 certainly correspond to *A. proteus*. One or two amoebae on that slide appear to have the surface ridges which Schaeffer made diagnostic of *A. proteus*, though care is necessary in evaluating ridges or folds on these preparations. The slide 20.12.8.17, with the notation 'Variété', bears an amoeba which could be a *P. dubium* if any of these are (Fig. 3).

Most of these amoebae contain ingested diatoms, and a few other algae and possible protozoa were seen in some.

Amongst these slides is one, 20.12.8.18 ('avec prolongements cryptogamiques') with a single amoeba trailing a tuft of filaments (Fig. 5). These presumed hyphae (Fig. 6) are apparently nonseptate and about 2·5 μm in diameter, and the longest extends about 74 μm from the amoeba. Slide 04.5.9.19 is a preparation of an amoeba 'écrasée pour montrer les parasites'. These filaments are apparently nonseptate, with a diameter up to 3 μm or slightly more. In both cases, Penard's notation (not taxonomic label) refers to the '*Ouramoeba*' of Leidy (1879), now generally acknowledged to have consisted simply of such infected members of the genus *Amoeba*.



Figs 1–4 *Amoeba proteus.* (1) to (3) Whole cells × 200. (4) Nucleus × 1000. N = nucleus.


Figs 5, 6 *Amoeba proteus* with apparent fungal parasite. (5) × 250. (6) × 1000. D = diatom. F = fungal hyphae.

Amoeba nitida Penard, 1902
(Figs 7–11)

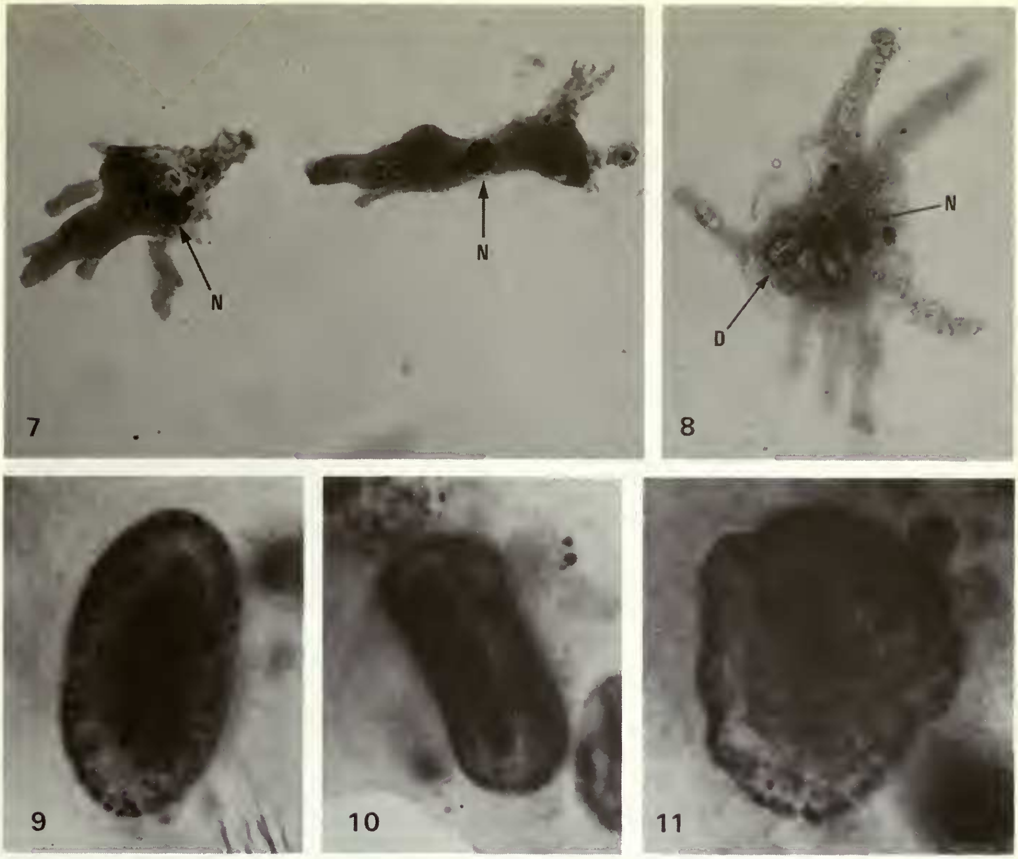
SLIDE NUMBERS. 04.5.9.23; 04.5.9.24; 20.12.8.12; 20.12.8.13.

TOTAL NUMBER OF AMOEBAE. 8.

DESCRIPTION AND REMARKS. At least four of these amoebae are in an elongate locomotive form (maximum length 408 μm) with one main pseudopodium, though some pseudopodia are found as separate fragments. In four amoebae (one binucleate) the nucleus is distinctly discoid (Figs 9, 10). Although the angle of viewing makes the other nuclei appear roughly circular or, in one case, oval in outline, a closer examination suggests that these nuclei, too, are discoid. The thickness is about half the greatest diameter or even less. The irregular outline of the nuclei in flat view (Fig. 11) recalls Penard's (1902) emphasis on the foldings and invaginations to which the nuclear envelope is susceptible. The nucleolar spherules are arranged in a layer just beneath the nuclear envelope, but in several nuclei there is also a central mass, perhaps a fixation artifact. The maximum diameters of nuclei in the uninucleate amoebae were 34 to 54 μm; the maximum diameters of the nuclei in the binucleate amoeba were 35 and 31 μm.

Ingested material included diatoms and possibly a few protozoa. At least one amoeba contained crystals, which appeared to be truncate bipyramids, though they were somewhat deteriorated.

Schaeffer (1916) asserted that Penard's *A. nitida* was equivalent to the *A. proteus* of Leidy (1879). My examination of Penard's slides labelled '*Amoeba proteus*' (see previous section) showed that the amoebae on at least one of them could not be *Polychaos dubium*, with which



Figs 7–11 *Amoeba nitida* (= *A. proteus*). (7), (8) Whole cells × 200. (9) to (11) Nuclei × 1000. D = diatom. N = nucleus.

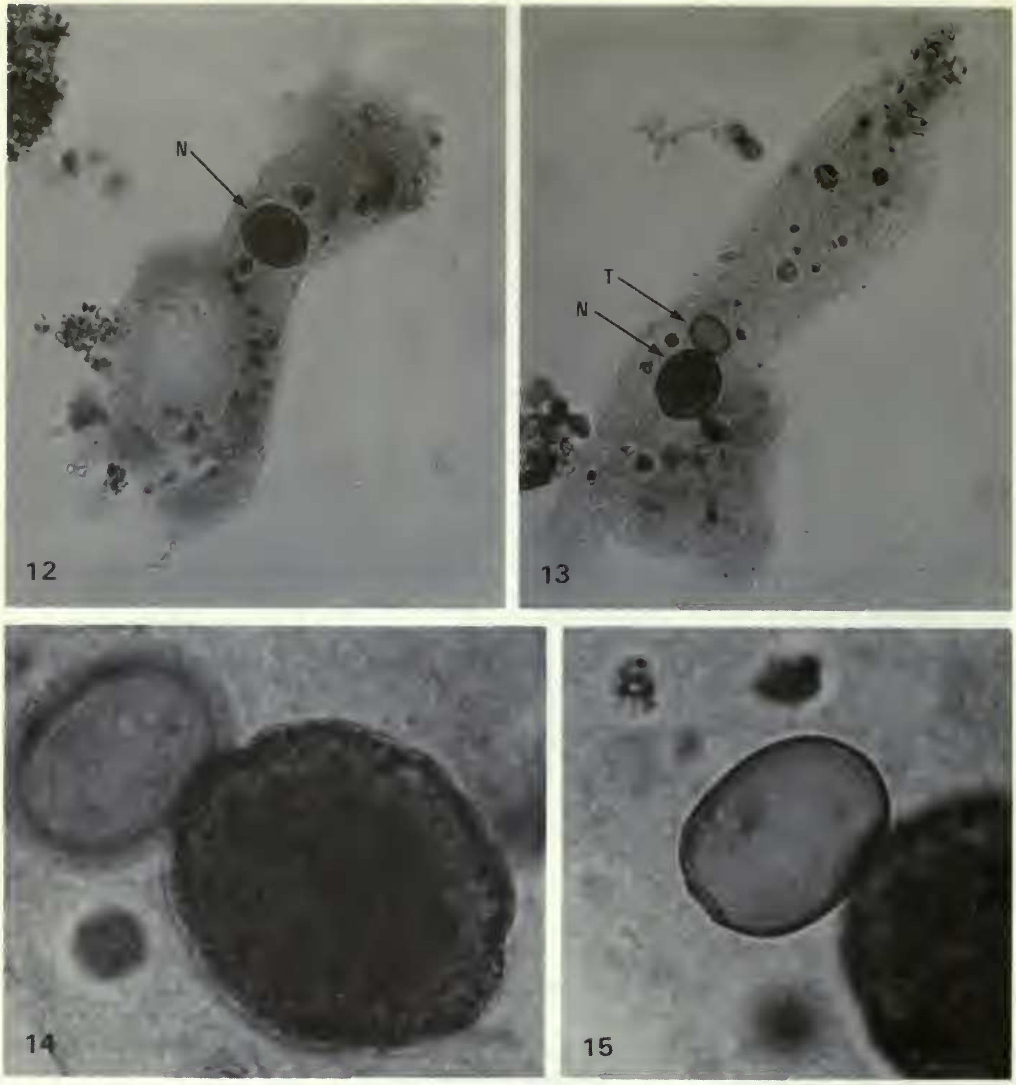
Schaeffer (using the name *Amoeba dubia*) equated the amoebae described by Penard (1902) as *A. proteus*, even if some of Penard's '*Amoeba proteus*' might not belong to the latter species as now defined. However, on the basis of these preparations as well as Penard's text, I accept Schaeffer's view that *A. nitida* is a junior synonym of *A. proteus*. The deformability of the nuclear envelope in '*Amoeba nitida*' is not a strong enough character for a specific separation. To separate the species on that basis would require isolation of a strain identifiable as *A. nitida* and demonstration that this deformability, leading to marked infolding and invagination, rests on an ultrastructural difference from the nuclear envelope of *A. proteus* (Flickinger, 1974).

Amoeba sp.
(Figs 12–15)

Slide number. 20.12.8.30.

Total number of amoebae. 2.

Description and remarks. This slide bears Penard's comment: 'Grande amibe à noyau curieux. L'un des individus renferme un petit rhizopode encore inconnu.' These amoebae (Figs 12 and 13) are 320 and 398 μm long. The nucleus (Fig. 14) has in each case an irregular outline, with maximum diameters of 46 and 49 μm. Careful focussing showed the thickness to be about half the greatest diameter or less, but the shape appeared to be lenticular rather



Figs 12–15 *Amoeba* sp. (12), (13) Whole cells × 200. (14) Nucleus, with rhizopod test out of focus × 1000. (15) Rhizopod test, with nucleus out of focus × 1000. N = nucleus. T = test.

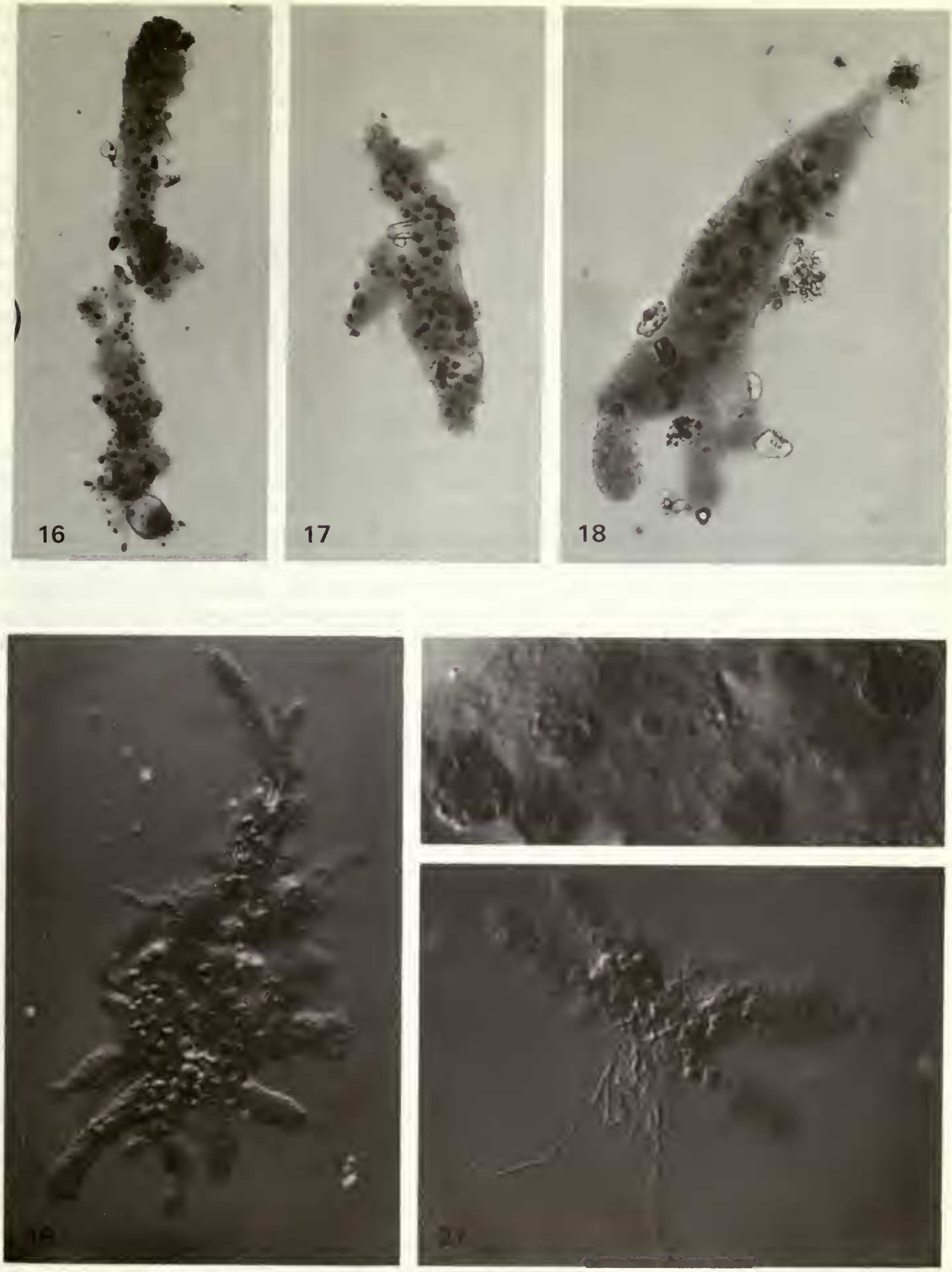
than discoid. There is a peripheral layer of granules, each slightly more than 1·5 μm in diameter, just beneath the nuclear membrane, and a less distinct, large central mass, which appears granular. Both amoebae contained ingested diatoms; one contained the test (Fig. 15) to which Penard's note refers (29 × 22 μm); and both contained what appeared to be small protozoa, which in one amoeba include apparent ciliates.

Given the difficulty of judging from fixed preparations alone, one cannot identify these amoebae with certainty, though they seem to be either *Amoeba* or *Polychaos*.

Amoeba nobilis **Penard, 1902**
(Figs 16–21)

Slide numbers. 04.5.9.21; 04.5.9.22; 20.12.8.14.

Total number of amoebae. 7.



Figs 16–21 *Amoeba nobilis* (=*Chaos nobile* comb. nov.) (16–18) × 200. (19) × 150. (20) Nuclei × 1000. (21) Amoeba apparently infected by fungus × 200.

DESCRIPTION AND REMARKS. These are multinucleate Amoebidae. The five amoebae on slide 20.12.8.14 are in the locomotive form; their lengths are from 262 to 446 μm. The largest amoeba in these preparations, on slide 04.5.9.22 (Fig. 19) is not a locomotive form and has pseudopodia projecting in several directions, with its greatest dimension 524 μm across, so that it would be much longer in locomotion; this resembles the cell described by Penard (1902) at the top of page 66. However, none of these fixed amoebae clearly shows the distal expansion of the pseudopodia pictured by Penard (1902) in Fig. 1 on page 66, which gives it the *Polychaos*-like appearance mentioned by Page (1976). It must be kept in mind that these fixed preparations undoubtedly do not show the pseudopodial form as accurately as do observations of live amoebae.

The number of nuclei counted in these seven amoebae ranged from 25 to 100 per amoeba, approximately, in the majority between 42 and 59, but these counts and those of other multinucleate species made from these slides are on the conservative side, since one cannot flatten the cells to observe all nuclei well. One hundred nuclei, 25 in each of four amoebae, had diameters ranging from 8·5 to 13·0 μm; the mean diameter varied from 9·8 μm in one amoeba to 12·2 μm in another. The nuclei (Fig. 20) are spherical to ovoid. The apparent nucleolar material is arranged in several small, irregular bodies on the inner side of the nuclear envelope, but there are also smaller granular bodies and filamentous material, apparently in the inner part of the nucleus.

Amongst the ingested material are diatoms and probably small protozoa.

One preparation, with the notation 'Avec cryptogames parasites', contains an amoeba, not in the locomotive form, with hypha-like filaments extending from one side to a maximum length of 184 μm from the amoeba (Fig. 21). Penard (1902) discussed this at some length.

Vonwiller (1913) isolated from an aquarium at Würzburg an amoeba which, after comparing his material with Penard's, he concluded was *A. nobilis*. Siemensma (1980) has found a similar amoeba in the Netherlands. Cysts have not been observed by these workers, although cysts are known to occur in the two recognized species of multinucleate Amoebidae, *Chaos carolinense* (Wilson, 1900) and *C. illinoisense* (Kudo, 1950) (Chapman-Andresen, 1979).

Amoeba nobilis, as seen in these preparations and in Penard's descriptions, is undoubtedly a member of the family Amoebidae as now defined (Page, 1976). It should therefore be known as *Chaos nobile* (Penard, 1902) comb. nov.

Amoeba laureata Penard, 1902
(Figs 22–24)

SLIDE NUMBERS. 20.12.8.9; 20.12.8.10 (also contains *A. proteus*).

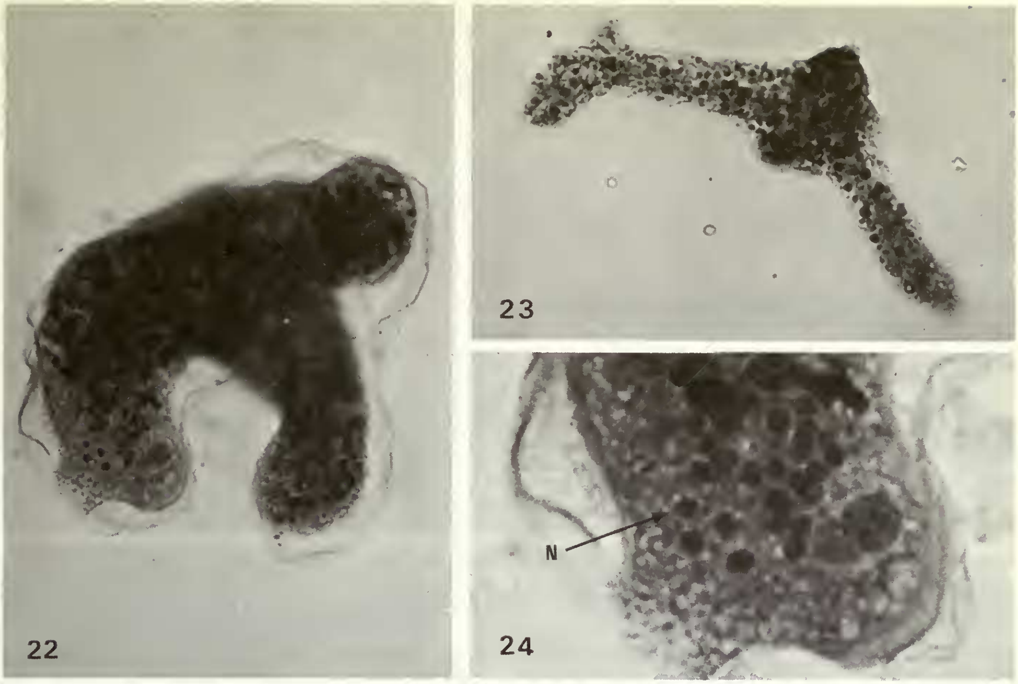
TOTAL NUMBER OF AMOEBAE. 2.

DESCRIPTION AND REMARKS. Observations on this species were limited by the facts that only two amoebae were present and the thickness of the preparations did not permit use of an objective lens above × 10. Furthermore, neither of the amoebae is a normal locomotive form.

The amoeba on slide 20.12.8.9 (Fig. 22) is made up of two thick branches and a knobby posterior end. The cell surface is separated from the cytoplasm around much of the periphery of the amoeba and is somewhat wrinkled. However, a comparison with the second amoeba (Fig. 23) suggests that this surface may not be a *Thecamoeba*-like pellicle. Possibly the fixation method is responsible for the separation. The second amoeba likewise has two arms or branches, but much longer and slender, proceeding from a main mass which includes the more or less knobby uroidal region. These amoebae do not look like the one shown by Penard (1902) in Fig. 1, page 132, which is an *Amoeba proteus*-like locomotive form, although Penard states that such a form is very rare in this species. Nor is the villous character of the uroid, described by Penard for this species, discernible in these preparations, perhaps having been distorted in fixation.

The length of the thicker amoeba, from the posterior end to the tip of the main branch, is 310 μm. The greatest extent of the more slender amoeba, from the tip of one pseudopodium to the tip of the other, is 314 μm.

Although the conditions of observation did not permit a count of the nuclei, it is obvious that there are hundreds per amoeba. (Penard said that the number sometimes exceeds 1000.)



Figs 22–24 *Amoeba laureata.* (22), (23) Whole cells × 200. (24) Enlargement of amoeba in (22) to show nuclei × 500. N = nucleus.

The micrographs of the nuclei (Fig. 24) do not show their structure very distinctly, but denser patches around the periphery suggest that many of them have the structure shown by Penard (1902) in his Fig. 4, page 132, with presumed nucleolar material in a few small parietal bodies. The greatest diameter of a nucleus measured on these photomicrographs was about 6·5 μm, somewhat below Penard's figure of 8–10 μm, which appears to be derived from live material.

A few diatoms and possibly a few other unicellular algae were seen, but many inclusions were not identifiable because of the conditions of observation.

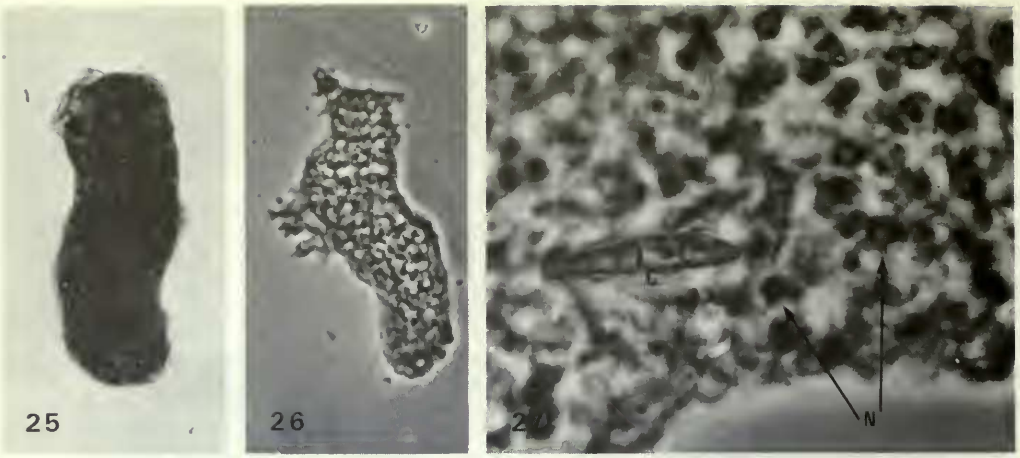
The taxonomic position of this species is probably not determinable from these preparations alone. The thickness of the amoeba in one preparation recalls *Pelomyxa*. However, the branching of these two amoebae is uncharacteristic of *Pelomyxa*. Furthermore, the possession of symbiotic bacteria was considered by Penard a characteristic of *Pelomyxa* and is so considered today (though bacteria occur in the cytoplasm of some Amoebidae). Penard explicitly mentions their absence in this species, which he would have classified as a *Pelomyxa* if he had found such endosymbionts. The presence of crystals, reported by Penard, also suggests that this is not a *Pelomyxa* (Griffin, 1961). Therefore, *A. laureata* may well be a *Chaos*, but the limitations of the available material make it advisable to reserve judgement.

'*Amoeba peritissima* Penard'
(Figs 25–27)

SLIDE NUMBER. 06.4.27.3.

TOTAL NUMBER OF AMOEBAE. 2.

DESCRIPTION AND REMARKS. These are thickly limax-shaped, multinucleate amoebae, if the two individuals available are representative. One cell measured 208 μm long by 68 μm broad; the other, 204 × 73 μm.



Figs 25–27 '*Amoeba peritissima*' (*nomen nudum*). (25), (26) Whole cells × 200. (27) Enlargement of part of amoeba in (26), to show nuclei. × 1000. C = conidium. N = nucleus.

Since the cytoplasm of one cell was less densely stained than that of the other, observations of nuclei were made on the former. This amoeba (Fig. 26) contained about 200 nuclei, on a conservative count. Observations of nuclear structure were not completely satisfactory. The nuclei (Fig. 27) appeared to contain a compact nucleolus, which at times appeared central and at other times eccentric in the nucleus. The central region of the nucleolus sometimes stained less densely than the outer part, leaving a lacuna. The diameters of 25 amoebae were 6·0 to 8·4 μm, mostly toward the lower end of that range.

One amoeba (Fig. 27) contained a multicellular conidium. The other (Fig. 25), which appeared to be ingesting an object at the time of fixation, contained several truncately bipyramidal crystals of sizes up to 12 × 9 μm.

Although this slide is labelled '*Amoeba peritissima* Penard', there is in fact no such specific name in the literature, and use of the name here is not intended as a publication to make it taxonomically available. It is a *nomen nudum*. A full taxonomic treatment would be possible only if this organism were found again and examined in sufficient numbers. Its generic position is uncertain. The thick limax form resembles *Pelomyxa*. However, the presence of crystals again suggests that it is not a *Pelomyxa* (Griffin, 1961). I can neither confirm nor rule out the presence of symbiotic bacteria on examination of these two preserved amoebae, but Penard's use of the generic name *Amoeba* indicates that he found no symbiotic bacteria. Again, this carefulness of Penard, who was familiar with diverse multinucleate amoebae, contrasts with the loose usage of some recent authors, who would throw all large multinucleate lobose amoebae into the genus *Pelomyxa* no mather how they differ in light- and electron-microscopical structure and in such basic physiological characters as locomotion and respiration.

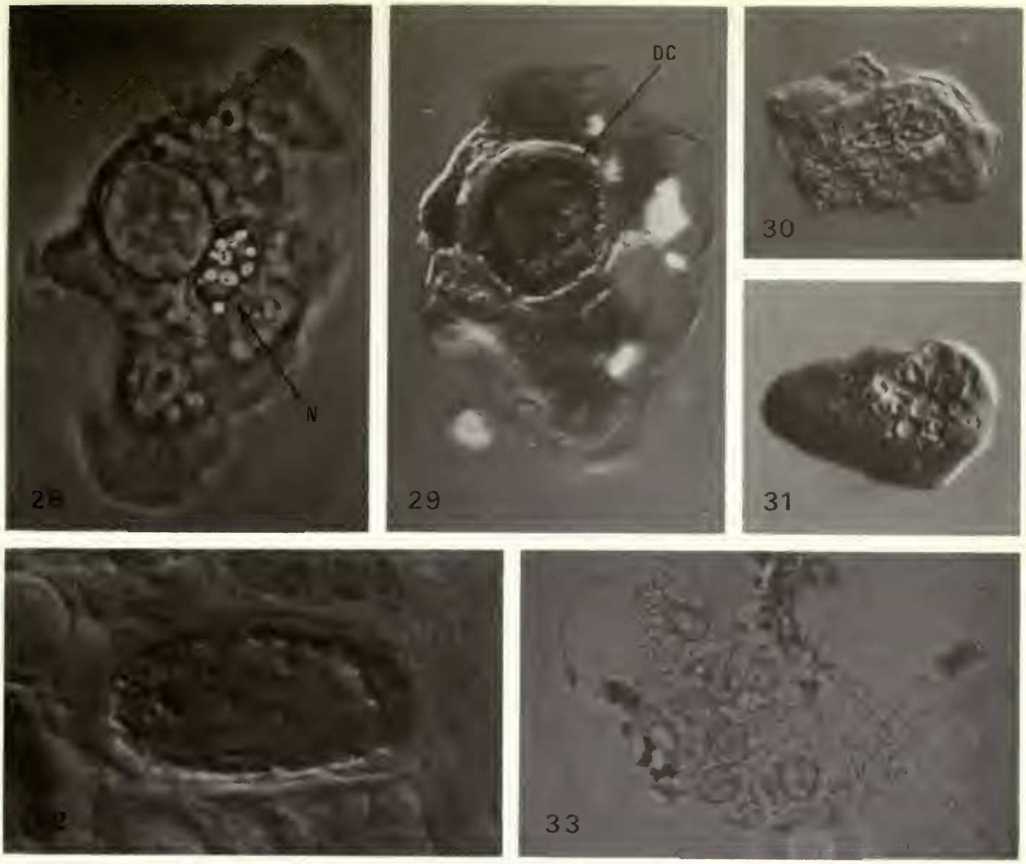
Amoeba terricola Greeff, 1866
(Figs 28–33)

Penard, 1902, pages 104–121; 1905; 1913.

Slide numbers. 20.12.8.20; 20.12.8.21; 20.12.8.22; 20.12.8.23; 20.12.8.24; 20.12.8.25; 20.12.8.26; 20.12.8.27; 20.12.8.28.

Total number of amoebae. 16 (not including six of 'forme *papyracea*').

Description and remarks. The slides designated as this species include one (20.12.8.25) with the notation 'forme *papyracea*' and another (20.12.8.28) labelled 'Variété'. As will be

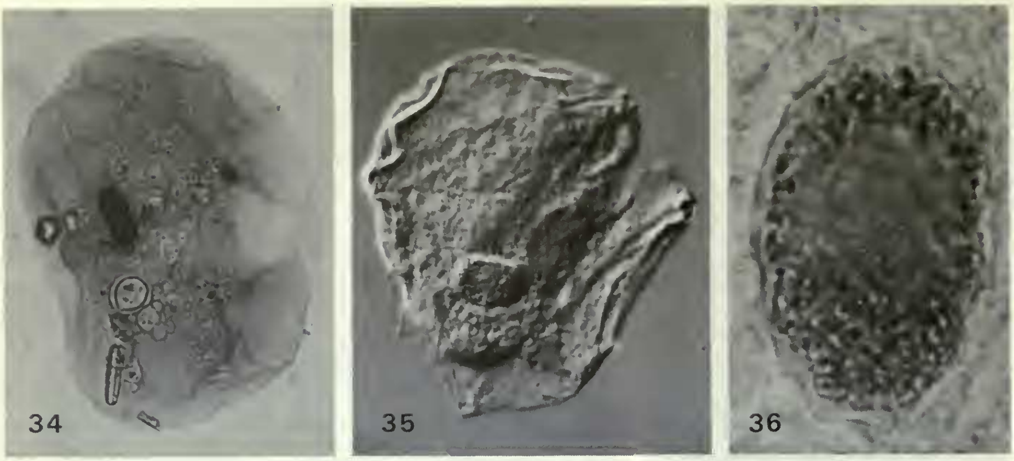


Figs 28–33 *Amoeba terricola* (= *Thecamoeba terricola*). (28) Whole cell × 500. (29) Amoeba with *Diplochlamys* in food vacuole × 200. (30) Whole cell × 200. (31) Whole cell labelled 'Variété' × 200. (32) Nucleus × 1000. (33) Bodies like collapsed sporangia or cyst walls in remains of amoeba, on slide labelled 'Parasitée' × 250. DC = *Diplochlamys*. N = nucleus.

seen below, this collection also includes one slide labelled '*Amoeba papyracea*'. Penard described the latter as a separate species in 1905, but in 1913 he decided to 'renounce' it and re-unite it with *A. terricola*. For the sake of clarity, the amoebae on slide 20.12.8.25 will be described under *Amoeba papyracea*, and the status of that species will be considered there. The present description is therefore derived from the amoebae on the other '*Amoeba terricola*' slides.

The maximum dimensions of these amoebae ranged from 94 to 262 μm, but most were 120 μm or more, and two of the smallest had been fixed 'après 32 jours d'isolement', in which time their size may well have decreased. The forms (Figs 28–31) were typical of *Thecamoeba*, though the majority did not appear to have been in locomotion when fixed, even if they were extended and flattened. The nuclei (Figs 28, 32) were the elongate ellipsoids or ovoids characteristic of the species, with a maximum length : breadth ratio of 2·3 and a mean of 1·7. In uninucleate cells (one was binucleate) the lengths of the nuclei were 24 to 55 μm (mean 34·7 μm), the majority between 24 and 38 μm. Elongate parietal nucleolar pieces, mostly at the ends of the nuclei as seen in living amoebae of the species (Page, 1977), did not stain well, in contrast to the presumed chromatin in the interior of the nucleus.

Identifiable ingested material included a few protozoa, including one identified by Penard as a *Diplochlamys* (Fig. 29) and a few small naked amoebae.



Figs 34–36 *Amoeba papyracea* (= *Thecamoeba papyracea* comb. nov.) (34), (35) Whole cells × 200. (36) Nucleus × 1000. N = nucleus.

One slide (20.12.8.21) bore the notation, 'Formation de petits kystes. Enveloppe déchirée.' The interpretation of this preparation is doubtful, which must be said also about 20.12.8.23, bearing the note, 'Parasitée' and containing bodies (Fig. 33) which may be empty fungal sporangia but could also be collapsed cyst walls of smaller amoebae.

The nuclei of these amoebae are somewhat larger than those found by Page (1977) in English strains of *Thecamoeba terricola*, but there seems little doubt that these amoebae and those investigated by other authors (comparison in Page, 1977) belong to the same species.

Amoeba papyracea Penard, 1905
(Figs 34–36)

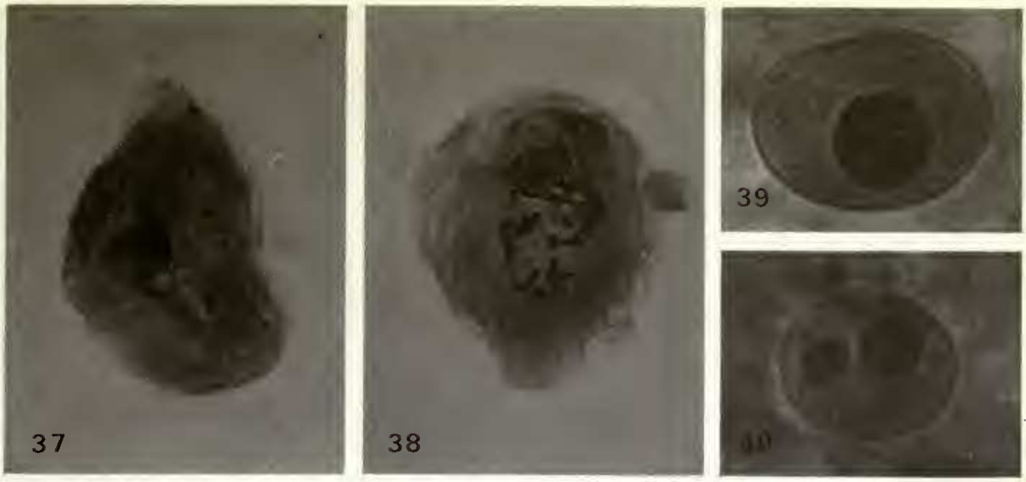
Penard, 1905, 1913.

SLIDE NUMBER. 06.4.27.1 (see below).

TOTAL NUMBER OF AMOEBAE. one on above slide; six of '*Amoeba terricola* forme *papyracea*'.

DESCRIPTION AND REMARKS. Penard's change of mind about the specific status of these organisms has been mentioned under the preceding species. The description given here is derived from both the single amoeba on slide 06.4.27.1 and the six on slide 20.12.8.25, 'forme *papyracea*' of '*Amoeba terricola*'.

As Penard says in both publications on this species, the amoebae appear more hyaline and more transparent than the usual *A. terricola*. Both his descriptions and the appearance of the fixed amoebae suggest that they are somewhat less rigid. The lengths of these seven amoebae ranged from 192 to 233 μm; all but one were more than 200 μm long. The nuclear structure is as shown by Penard in both illustrations. The nucleus (Fig. 36) is an elongate ovoid or ellipsoid. Although Penard describes it as broader than the nucleus of *A. terricola*, two of the amoebae had nuclei with a length : breadth ratio of 4·2. In the other five, the L : B was between 1·5 and 1·9. Possibly the nucleus is somewhat compressed in one direction. The lengths of the nuclei were 38 to 72 μm, mean 51·4 μm. There are no elongate nucleolar bodies as in the typical *A. terricola*. Rather there are many small spherules, diameter about 1·5 μm, arranged in the outer region of the nucleus, with the greatest concentration toward the poles, so that the central part of the nucleus appears free of them. These spherules at the poles reach to the nuclear membrane, whereas in the typical *A. terricola* those poles are occupied by the elongate nucleolar bodies.



Figs 37–40 *Amoeba sphaeronucleolus* (= *Thecamoeba sphaeronucleolus*). (37), (38) Whole cells × 250. (39), (40) Nuclei × 1000. N = nucleus.

In some of these amoebae little or no ingested material was evident. In others the food vacuoles contained bacteria, one or two diatoms, fungal conidia, and possibly one or two protozoa and algal filaments.

The more hyaline appearance and apparently greater plasticity of these organisms compared with the typical *A. terricola* could be due partly to their not having ingested many food organisms for some time before observation and fixation. The somewhat greater size than Penard found for *A. terricola* might likewise be due to their form being less thick and compact because of the paucity of ingested material. The nuclear structure differs from that found in *A. terricola* by Penard and other workers. I am inclined to consider this a separate species, but examination of living material and possibly investigation of surface fine structure (Page & Blakey, 1979) is advisable.

Amoeba sphaeronucleolus Greeff, 1891
(Figs 37–40)

Penard, 1902, pages 121–125; 1905; 1913.

Slide number. 20.12.9.19.

Total number of amoebae. 5.

Description and remarks. The concept of this species which we follow today is that of Penard, and there is some uncertainty whether his *A. sphaeronucleolus* is that of the original author (Greeff, 1891; Page, 1977).

The form of Penard's specimens on this single slide agrees with the usual description of the species. The lengths of these five amoebae are 92 μm, 108 μm, 143 μm, 156 μm, and 161 μm, thus rather large by Penard's statements that he found large individuals to about 150 μm but they are often much smaller (Penard, 1902) and that in their maximum elongation they measure 100 to 130 μm (Penard, 1913). The nuclei are approximately spherical or ovoid. Four have a single, more or less spherical nucleolus, while the nucleolus of the fifth (Fig. 40) is in two large fragments accompanied by four smaller pieces which may also be nucleolar fragments. The largest dimensions of the five nuclei range from 22 to 30 μm. The nucleolus is quite smooth and more or less homogeneous except sometimes for a few small achromatic lacunae.

Ingested bodies include three diatoms and a conidium in one amoeba and apparent bacteria and algae in others.

These details correspond fairly well with those reported by workers since Penard, though the size of the nucleus and the texture of the nucleolus differ somewhat from those reported for a North American strain by Page (1977). Even given the more homogeneous nature of the nucleolus in Penard's preparations, this species is easily distinguished from *Thecamoeba quadrilineata* (Carter, 1856), which is a 'smooth' *Thecamoeba* rather than a 'rough' one (Page, 1977). There may be some variation amongst strains of *Thecamoeba* from different parts of the world, since the literature suggests variation even within Europe. However, investigators should be alert to the possible existence of more than one species of 'rough' *Thecamoeba* with a single compact central nucleolus or endosome.

Amoeba fibrillosa Greef, 1891
(Figs 41–45)

Penard, 1913; mentioned in Penard, 1902, pages 123, 124.

SLIDE NUMBERS. 20.12.8.7 (labelled *Amoeba alba* 'avec 1 *Amoeba fibrillosa*'); 20.12.8.8.

TOTAL NUMBER OF AMOEBAE. 5.

DESCRIPTION AND REMARKS. These multinucleate amoebae have the wrinkled pellicle and general form of a *Thecamoeba*, even though the form shows a greater variety and, even in the fixed preparations, evidence of a greater fluidity than that of the more typically *Thecamoeba*-like *Amoeba alba* (next section). In this respect it may be compared with *Thecamoeba proteoides* Page, 1976 (Page, 1976, 1977). Long, slender forms occur, sometimes with temporary branching (which can, however, occur occasionally even in the more rigid *A. alba*; see Fig. 46). Undoubtedly this temporary branching is associated only with a change of direction. Further comments on this character will be found in both Greeff (1891) and Penard (1913).

The largest of these amoebae is that on slide 20.12.8.7, shown in Fig. 41, which is 320 μm long though certainly not in the most extended form possible. The lengths of the other four are 228 μm, 228 μm, 226 μm, and 158 μm, this last one an irregular form. The length : breadth ratio of the larger amoeba in Fig. 43 is 4·1, ignoring the lateral pseudopodium near the posterior end, which was probably being withdrawn at the time of fixation. In Fig. 42, the pseudopodium with the hyaline cap (arrow) was undoubtedly the active one, with the other branch being withdrawn in a change of direction at the time of fixation.

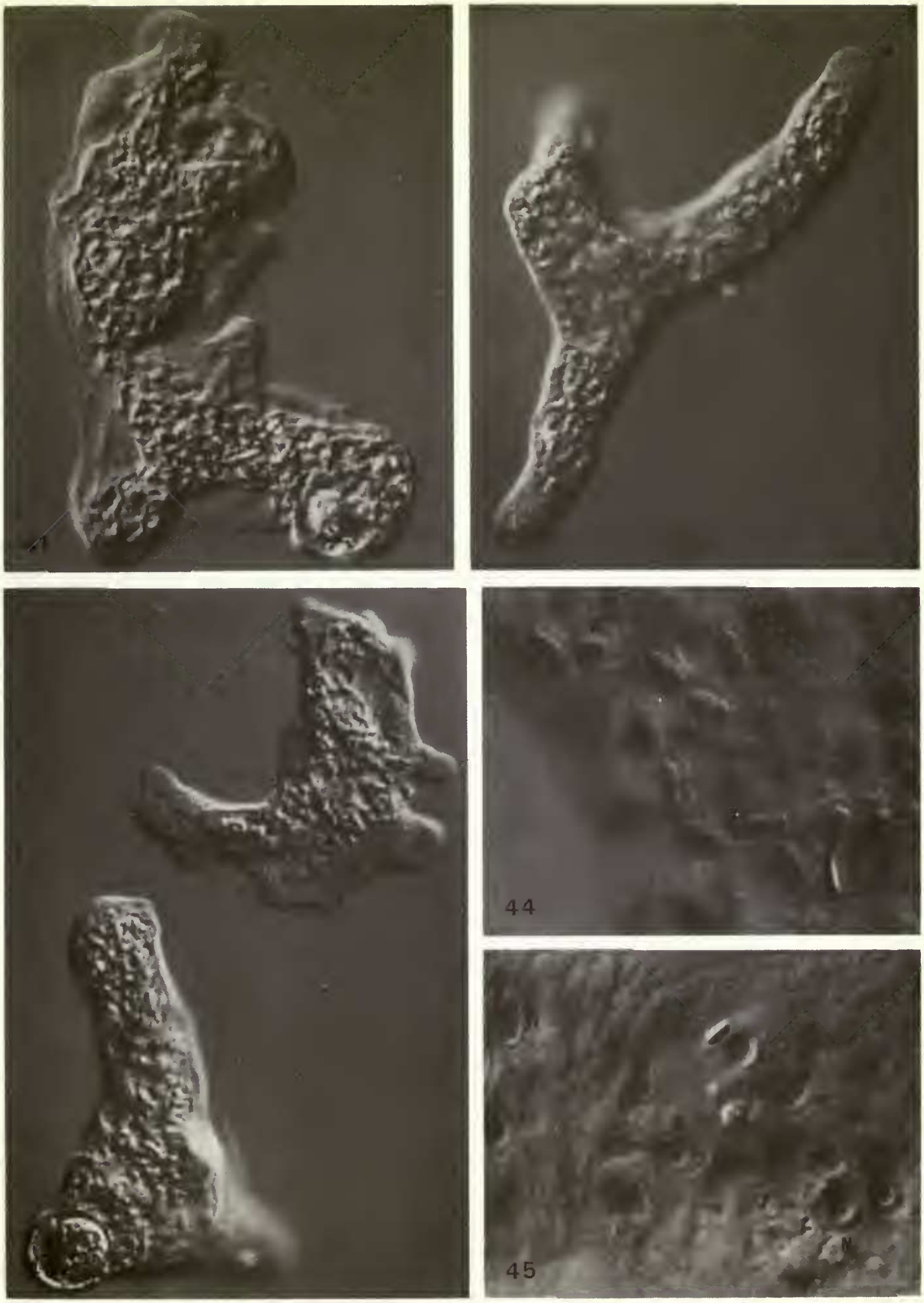
In the large amoeba in Fig. 41, 97 nuclei were counted, and in another amoeba 85 could be found. Both these numbers undoubtedly err on the low side.

Although Penard (1913) said that the nuclei are 'normalement globuleux' though fairly often elongate, the elongated condition appears normal in these preparations (Fig. 44). Furthermore, observations while focussing suggest that many if not all the more spherical and ovoid forms (Fig. 45) are actually due to polar and oblique views of elongate nuclei. The single central nucleolus has in general the shape of the nucleus, though it often appears even more elongate (with long sides straighter) than does the nucleus. It is sometimes constricted in the middle to a dumbbell-like shape, which appears to be merely another variation and not a prelude to division as Penard (1913) thought.

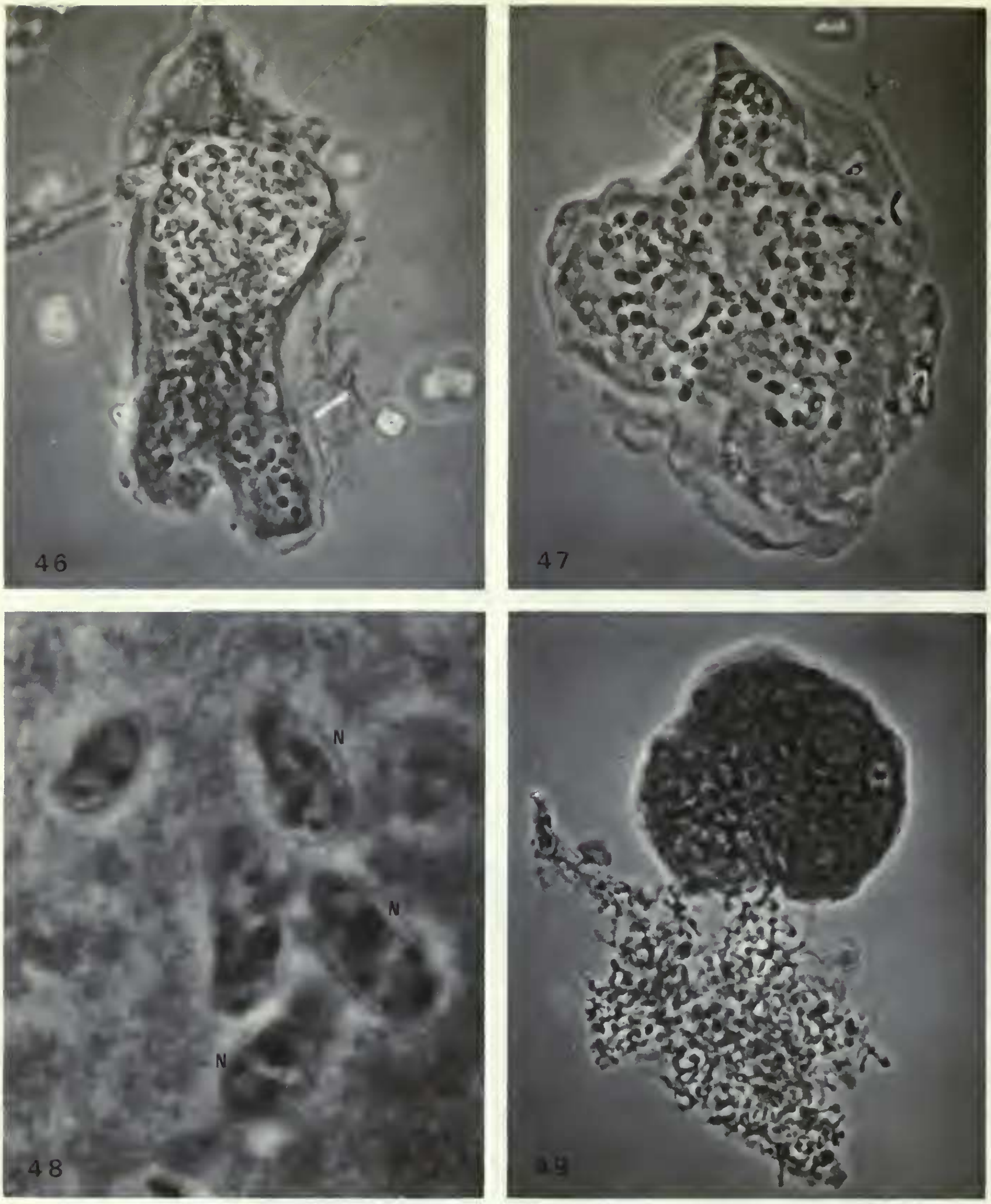
The measurements of 25 nuclei in the largest amoeba ranged from $7{\cdot}0 \times 6{\cdot}2\ \mu$m to $10{\cdot}8 \times 7{\cdot}0\ \mu$m, with a mean greatest dimension of $8{\cdot}9\ \mu$m.

A food vacuole in one amoeba contains an ingested organism which appears to be an amoeba, itself containing truncately bipyramidal crystals. Another amoeba also contains an ingested organism which appears to be a protozöon.

Greeff (1891) did not publish any illustrations of this species. (See remarks on *A. alba*.) Although I accept that this may well be the same species which Greeff saw, it must be pointed out that Greeff did not consider the nuclei to be elongate but described them as 'in der Regel rund, zuweilen leicht oval'. However, his description of the amoeba as a whole corresponds with this material. Since Greeff's description of *Amoeba fibrillosa* precedes in



Figs 41–45 *Amoeba fibrillosa* (= *Thecochaos fibrillosum* comb. nov.). (41) to (43) Whole cells; arrow indicates hyaline cap on main pseudopodium in (42) × 250. (44), (45) Nuclei × 1000. N = nucleus.



Figs 46–49 *Amoeba alba* (= *Thecochaos album* comb. nov.). (46), (47) Whole cells × 250. (48) Nuclei × 2000. (49) Amoeba apparently infected by fungus × 200.

the same publication his description of *A. alba* the former will be the type-species of the new genus being erected for the two. Penard, it will be noted, had more fixed material of *A. alba*, if this collection is representative.

Amoeba alba Greeff, 1891
(Figs 46–49)

Penard, 1902, pages 123–125; 1913.

SLIDE NUMBERS. 04.5.9.17; 20.12.8.1; 20.12.8.2; 20.12.8.3; 20.12.8.4; 20.12.8.5; 20.12.8.6; 20.12.8.7 ('avec 1 *Amoeba fibrillosa*').

TOTAL NUMBER OF AMOEBAE. 20.

DESCRIPTION AND REMARKS. This is another multinucleate species of Thecamoebidae, distinct in both locomotive form and nuclear structure from the preceding.

A number of these amoebae appear to have been in locomotion when fixed, thus representing the normal locomotive form well. Only the 15 amoebae which were apparently fixed before they died of bursting or other causes than fixation were measured. Their lengths were 166 to 276 μm, with a mean of 210 μm; their length : breadth ratios were 1·1 to 2·2, with a mean of 1·4, quite normal proportions for a *Thecamoeba*.

Use of an oil immersion objective and phase contrast optics permitted a closer look at the nuclei than Penard could have and resulted in a more accurate picture of their structure. However, the same problems which Penard encountered remained in counting the nuclei because, as he said, 'la plupart ne deviennent visibles qu'après compression de l'Amibe' (Penard, 1902), and compression was, of course, impossible. Attempts to count the nuclei in five favourable specimens yielded results of 94, 100, 102, 145, and 185, in each case certainly below the actual number. In 1902 Penard thought that the number might reach several hundreds, but in 1913 he said only that it often exceeded 100.

The nuclei (Fig. 48) appeared more ovoid/ellipsoid, i.e., more elongate, in some amoebae, and more spherical to ovoid, i.e., less elongate, in others. The more elongate nuclei, 25 from each of the two amoebae, measured from 7·0 × 5·6 μm to 12·0 × 5·6 μm, with a mean of 9·0 μm for the greatest diameter. The more spherical nuclei, 25 from one amoeba, had a greatest diameter of 6·5 to 7·5 μm, with a mean of 7·0 μm. The presumed nucleolar material was not scattered as spherules through the nucleus, as described by Penard (1913) ('disséminés . . . dans un suc nucléaire') and shown in Fig. 2, page 123, of Penard (1902) and Fig. 7 of Penard (1913). Rather, it was arranged parietally as variously shaped bodies, some band-shaped, which may all have been lobes of one or two parietal bands in each nucleus. These photomicrographs were made with an oil-immersion lens and phase-contrast optics, not available to Penard.

Ingested material included apparent algal filaments and a few diatoms. One slide (20.12.8.6) bears the notation 'Parasitée par cryptogame'. This preparation (Fig. 49) contains a more or less rounded amoeba with a mass of branching, non-septate filaments coming out of an invagination. The diameter of these filaments is about 2 μm or slightly more. Penard presumably examined this amoeba alive before fixing it; otherwise one might question whether the filaments were parasitizing the amoeba or the amoeba ingesting the filaments.

With his original description of *A. alba*, Greeff (1891) published no illustrations, an omission which led Page (1977) to doubt whether Greeff's organism was indeed a *Thecamoeba* and speculate whether it might not be a *Leptomyxa*, a fairly common genus of multinucleate amoebae in soil. However, Penard (1902) agreed with Greeff that *A. alba* is very rare. I have myself never seen a multinucleate *Thecamoeba*-like organism in many collections from nature and do not know of any reports of them by workers other than Greeff, Penard, and Cash & Wailes (1919). The figure published by Cash & Wailes is not very informative, but their text suggests that they may have had the same species as Penard. They also described *A. alba* as rare. A consideration of Greeff's description in the light of the Penard slides makes it quite likely that Penard's organism is the same as Greeff's.

Amoeba granulosa Gruber, 1885
(Fig. 50)

Penard, 1902, pages 46–48.

SLIDE NUMBER. 06.4.27.2.

TOTAL NUMBER OF AMOEBAE. 1.

Fig. 50 *Amoeba granulosa* × 250. N? = location of possible nucleus.
Fig. 51 *Amoeba lucens* (= *Saccamoeba lucens*), not normal locomotive form. × 800. N = nucleus.

DESCRIPTION AND REMARKS. The single amoeba of this species which Penard said that he found in great abundance furnishes little information. Because of the thickness of the mount, it could be examined only with the × 10 objective. At any rate, the amoeba does not appear well preserved. It is flattened but not circular in outline so presumably not dead or moribund when fixed. Inside the narrow hyaloplasmic border which occupies most of the periphery, the cytoplasm is filled with formed elements, somewhat less densely packed in the central region of the cell. These elements appear to be bipyramidal crystals, as Penard thought. A slightly stained area which may be the nucleus is indicated in Fig. 50 by an arrow. The dimensions of the cell are 142 × 88 μm; the diameter of the possible nucleus is about 29 μm.

Although this looks like the flattened cells figured by Gruber (1885), the identification is questionable. Gruber gave the diameter as 'ungefähr 0·03 mm', which Penard (1902) mistranslated as 'de 300 μ'. Their descriptions of the nucleus do not appear to agree, although the apparent difference may be due to either optics or terminology.

At any rate, one would not like to hazard a guess on the identity of this amoeba, though an amoeba with such an abundance of crystals (undoubtedly not silica, agreeing with Penard rather than Gruber) might be recognizable if found again. It might be mentioned that in this paper Gruber (1885) deplored the impossibility of identifying an amoeba with any degree of certainty.

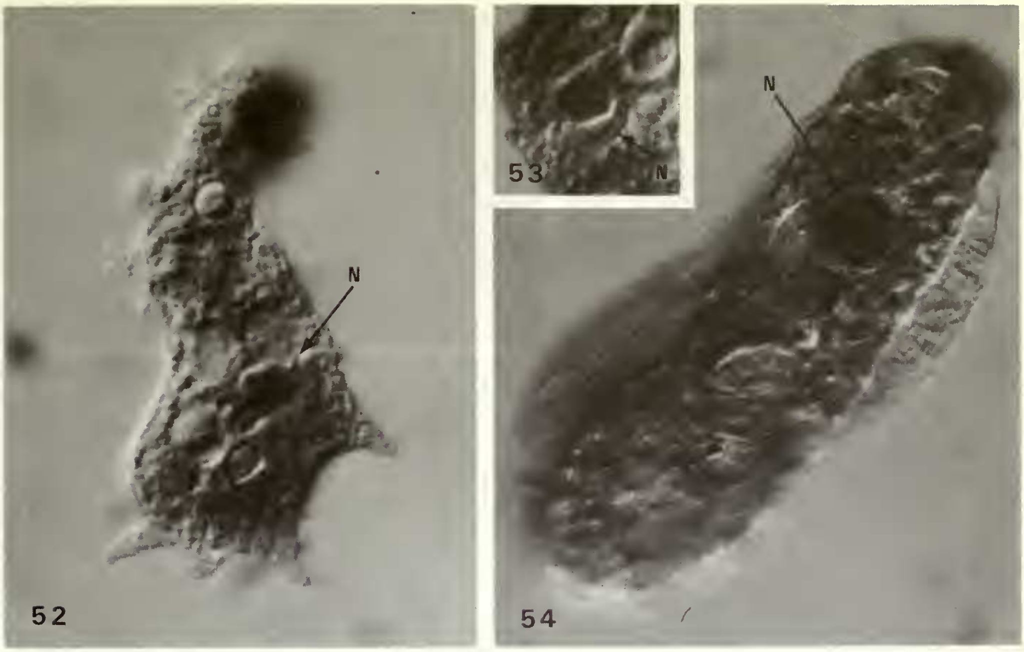
Amoeba lucens (Frenzel, 1892)
(Fig. 51)

Penard, 1902, pages 55–57.

SLIDE NUMBER. 04.5.9.18.

TOTAL NUMBER OF AMOEBAE. 1.

DESCRIPTION AND REMARKS. Although Penard (1902) illustrated his description of this species with a drawing (Fig. 1, page 56) of what is obviously a *Saccamoeba*, the single cell on this slide is not so unambiguous. In fact, it might be taken for a *Cochliopodium* with scales



Figs 52, 53 *Amoeba vespertilio* (= *Mayorella vespertilio*); both figures of same cell, focussed on pseudopodia in (52) and on nucleus in (53) × 1000. N = nucleus.

Fig. 54 Unidentified amoeba on same slide with *A. vespertilio* × 800. N = nucleus.

either lost or invisible to the light microscope, if it were not for Penard's label. This cell appears to be made up of a more or less discoid granular mass surrounded by a flattened hyaline border of varying width. The diameter of the granular region is 72×65 μm; including the hyaline border the cell is 90×73 μm. The most striking feature is, of course, the truncately bipyramidal crystals, of which there are about a dozen, the largest approximately 13×10 μm. Some crystals are slightly deteriorated. The nucleus seems poorly fixed, but using Penard's description as a guide, this appears to be a nucleus in which the diameter of the central nucleolus is only a little less than that of the nucleus. The dark area which appears to be the nucleolus has a maximum diameter about 15 μm. Although the nuclear membrane (usually quite distinct in *Saccamoeba*) is not preserved, the narrow clear halo around the nucleolus suggests a maximum nuclear diameter of 19 to 20 μm. The amoeba appears to contain at least one fungal conidium.

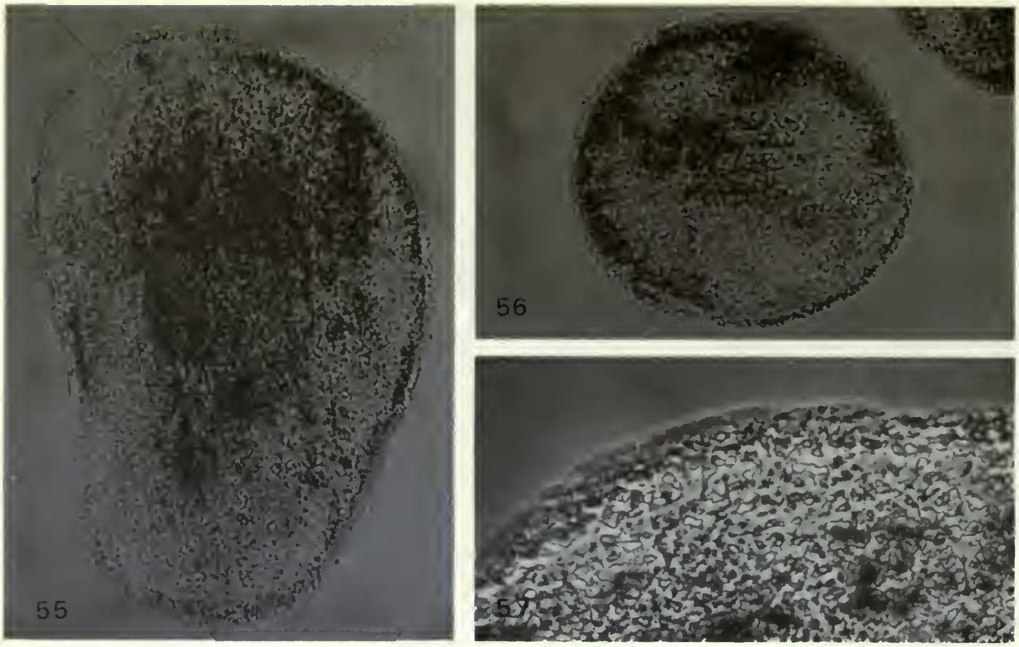
Despite the puzzling form of this preserved amoeba, Penard's account leaves no doubt of its identity, though this slide is of value chiefly for the structure of the crystals and, to a lesser degree, that of the nucleus. *Saccamoeba lucens* has been re-described by Bovee (1972) and is recognized as the type-species of the genus *Saccamoeba*. There is some inconsistency among the descriptions of Frenzel (1892), Penard, and Bovee (1972).

Amoeba vespertilio Penard, 1902
(Figs 52–54)

Slide number. 20.12.8.29.

Total number of amoebae. 1 of this species.

Description and remarks. Only one of the two organisms on this slide can belong to this species or even to the genus *Mayorella* Schaeffer, i926, in which *Amoeba vespertilio* is now classified. The other (Fig. 54) is elongate, apparently with flattened hyaline borders along the sides and with a different nuclear structure.



Figs 55–57 *Amoeba muralis.* (55), (56) Whole cells × 80. (57) Edge of cell, enlarged to show mineral grains × 250.

The amoeba which can be identified as *A. vespertilio* is 65 μm long × 31 μm wide, not including the conical pseudopodia, of which there are six or seven, counting those which have been relegated to the sides. These pseudopodia (Fig. 52), in their fixed and probably somewhat shrunken condition, are up to 9 μm long, with a basal diameter of about 4 μm. The appearance of the uroid (posterior end) suggests that as the amoeba advances the conical pseudopodia, after passing to the posterior end, form a small clump of blunt projections at the uroid before being resorbed. The nucleus (Fig. 53) has a diameter of 12·5 μm and the central nucleolus a diameter of 7·7 μm. The amoeba contains at least one ingested algal cell.

Despite the shrinkage accompanying fixation, this amoeba represents the typical *Mayorella* form much better than do the illustrations on page 94 in Penard (1902). It most resembles, among more recently described species, *Mayorella oclawaha* Bovee, 1970, and *M. riparia* Page, 1972, though both the amoeba and the nucleus are larger than the sizes reported for those species (Page, 1976). It would not be safe to derive a specific diagnosis from this single cell, since it is not possible even to know whether its length is large, small, or average for the species.

Amoeba muralis Penard, 1909
(Figs 55–57)

Slide number. 20.12.8.11.

Total number of amoebae. 11.

Description and remarks. Because Penard (1909*a*) considered this a naked amoeba rather than one with a flexible test and accordingly placed it into the genus *Amoeba*, it is included in this study.

This preparation contains eleven very flattened, mostly circular cells arranged in a ring. Four appear to have disintegrated, with their positions now marked chiefly by the foreign material which had covered their surfaces, though the outlines are still distinct enough for measurements of diameter. The thickness of the preparation did not permit use of an objective above × 10, but it is doubtful that a higher magnification would have yielded more information. All the amoebae were covered with foreign matter, apparently mineral grains with a maximum dimension of 5 to 10 μm. There are in some cells patches of denser material, probably internal and possibly the remains of ingested algal or other plant matter. The edge of the cell (or its endogenous covering) appears as a clear border extending 5 or 10 μm beyond the mineral grains around much of the periphery of some cells, with some extraneous particles helping to mark its outer edge. I could not find any nuclei, of which Penard said there might be 40, 50, or 60.

These cells are marked by multiple parallel streaks, as if scraped during preparation.

Penard (1909*a*) described this as a multinucleate amoeba which could secrete a mucilaginous envelope. According to him, this envelope kept particulate matter at a distance from the cell surface. However, when the amoeba began locomotion, the mucilage disappeared, first in the anterior region, then finally from the entire surface.

One genus of amoebae with a flexible test which can be detected only with difficulty is *Gocevia* Valkanov, 1932. The description given by Penard suggests that *A. muralis* may be covered by a cuticle which may accumulate foreign matter and which may stretch and become thinner during locomotion. His description of fine, digitiform pseudopodia also recalls some recently investigated organisms classified in that genus. The characters of *Gocevia* with descriptions of organisms which appear to belong to that genus are discussed most recently by Page & Willumsen (1980). *Gocevia* belongs to the family Cochliopodiidae, in the Testacealobosia. However, all known members of the genus are normally uninucleate except one which may be normally binucleate.

Dinamoeba mirabilis Leidy, 1874
(Fig 58, 59)

Penard, 1902, pages 134–137; 1909*b*; 1936.

Slide number. 04.5.9.154.

Total number of amoebae. 1.

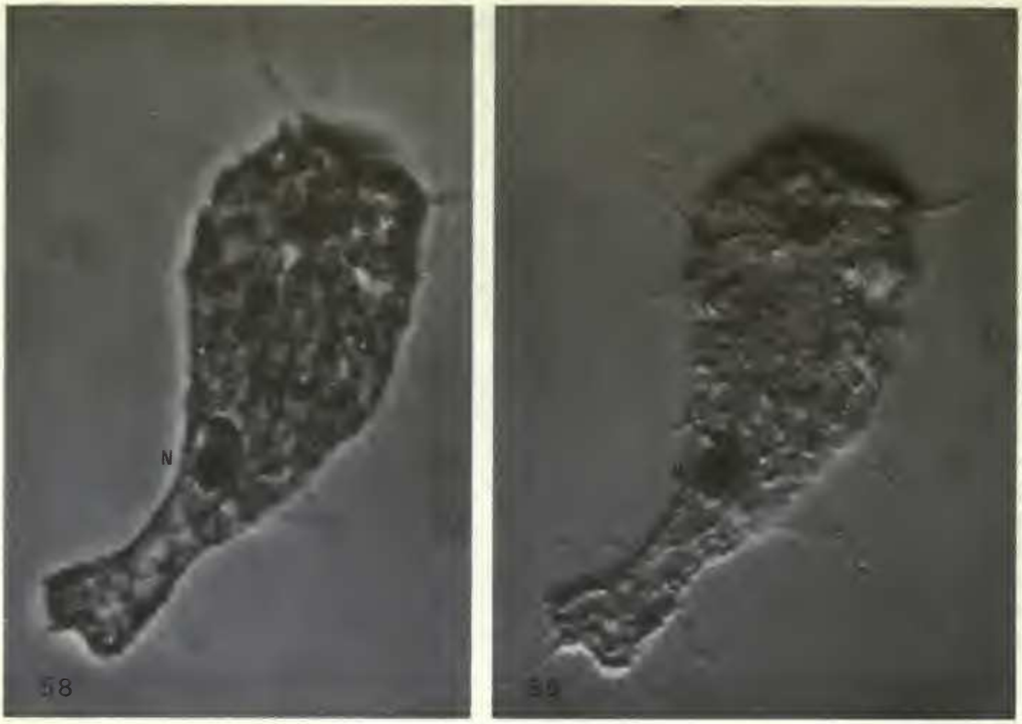
Description and remarks. This amoeba shows some signs of shrinkage in fixation in that some of the conical pseudopodia appear shrunken in diameter, though the hyaline pseudopodia of this genus are at any rate quite fine even in life (Fig. 59A, Page, 1976). The cell is 94 μm long, with a maximum breadth of 37 μm, neither measurement including the pseudopodia. On either side of the anterior end, which appears to have a shallow hyaline cap, is one pseudopodium, with lengths of 15·5 and 19 μm. There are several single pseudopodia along the sides, as well as one broad, flat, hyaline projection bearing three short pseudopodium-like extensions. At the posterior end are several uroidal filaments which appear to have originated by adhesion to the substratum but could be pseudopodial remnants.

Although Penard (1902, 1909b) emphasized that the organisms which he saw were as a rule binucleate, this cell contains only one nucleus, situated toward the posterior narrowed 'neck' and elongated by cytoplasmic movement. The diameters of the nucleus are 20 × 9 μm; of the compact central nucleolus, about 8·5 × 6·2 μm.

The cytoplasmic pigmentation, including granules, suggests an algal diet, and two or three ingested cells are distinguishable.

I could not make out any of the bacterium-like objects, adherent to the surface, which are characteristic of many reported *Dinamoeba*, though they may also be absent from living amoebae (see Fig. 59A, Page, 1976).

The possible identity of *D. mirabilis* with *Mastigamoeba aspera* Schulze, 1875, has been discussed by Penard (1909*b*, 1936), De Groot (1936), and Page (1970). This slide sheds no further light on that question.



Figs 58, 59 *Dinamoeba mirabilis*, both of same cell × 800. (58) Phase contrast, to show nucleus distinctly. (59) Differential interference contrast, to show pseudopodia distinctly. N = nucleus.

Pelomyxa palustris Greeff, 1874
(Figs 60, 61)

Penard, 1893; 1902, pages 138–143.

SLIDE NUMBERS. 04.5.9.206; 04.5.9.210; 06.4.27.7; 20.12.8.530; 20.12.8.531; 20.12.8.532; 20.12.8.533.

TOTAL NUMBER OF AMOEBAE. 16.

DESCRIPTION AND REMARKS. These are recognizable as the species universally designated by this name. Some of the cells are more or less rounded, others an elongated ovoid, i.e., the usual locomotive form. The longest reached 1478 μm; two 'jeunes individus' are 175 and 233 μm long. In some the mineral grains are so abundant as to hinder observations of other inclusions; some mineral grains measure more than 50 μm, but most are much smaller. Nuclei are numerous. A total of 125 nuclei, 25 in each of five amoebae, had diameters from 7·0 to 14·5 μm, with a mean of 9·2 μm; only in one of these five amoebae did the diameters exceed 10·8 μm, however. Some of the nuclei (Fig. 61) had a rather shrivelled appearance. In the nuclei there was usually a parietal layer of small granules, sometimes a few larger, darkly staining pieces of various shapes and sizes just beneath the nuclear membrane, sometimes a small body that appeared to be near the centre of the nucleus, and often some rather indistinct filamentous material. The amoebae often contained many diatoms, occasionally filamentous ones. Rods that appeared to be the characteristic symbiotic bacteria were up to 5 μm long. The cytoplasm was often highly alveolar.

Since there is no question about the identity of these amoebae and since the characters of the species are well known today, only two of the photomicrographs are reproduced here.

Figs 60, 61 *Pelomyxa palustris*. (60) Whole cell, with anterior end at top × 100. (61) Nuclei × 1000. N = nucleus.

Pelomyxa fragilis Penard, 1904
(Figs 62–65)

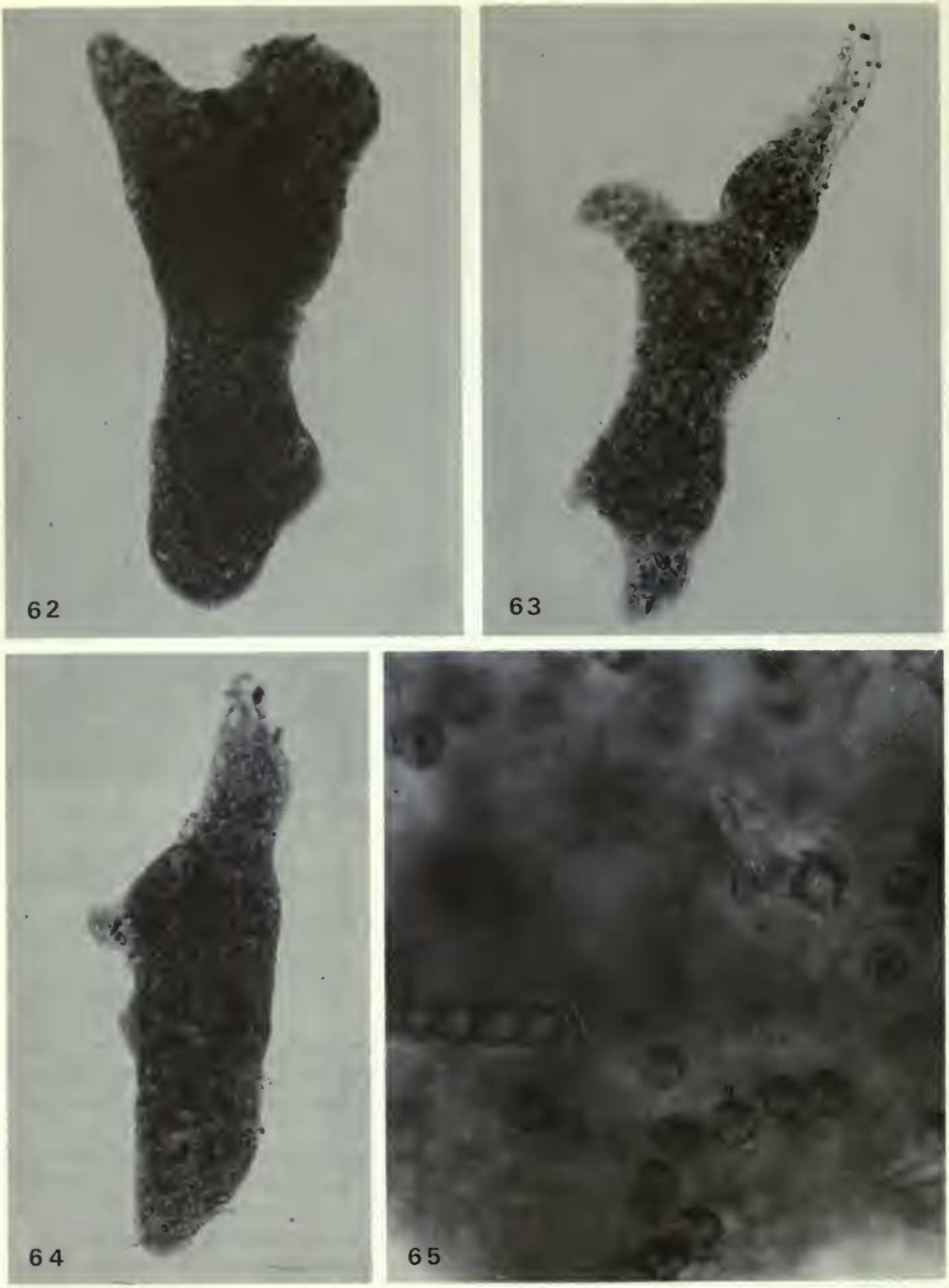
SLIDE NUMBERS. 04.5.9.209, 20.12.8.529.

TOTAL NUMBER OF AMOEBAE. 4.

DESCRIPTION AND REMARKS. Since the amoeba on slide 04.5.9.209 was so obscured by detritus that few useful observations could be made, this description is based entirely on the three amoebae on slide 20.12.8.529.

Although these amoebae certainly appear more changeable in form than the typical *Pelomyxa palustris*, none has pseudopodia which can be described, in Penard's term, as '*déchiquetés*', presumably referring to the form in Fig. 2 of Penard (1904). They do have secondary lateral pseudopodia, probably being retracted in a change of direction at the time of fixation. Shallow, crescent-shaped hyaline caps are distinguishable on two amoebae (Figs 62, 64). The uroidal regions of two (Figs 63, 64) appear somewhat drawn out as if by adhesion. The lengths and length : breadth ratios (not including lateral pseudopodia) are: 398 μm, L : B 3·0; 403 μm, L : B 3·6; and 456 μm, L : B 4·3.

These amoebae appear to contain hundreds of nuclei each; in one, there were at least 175 to 200. The diameters of the nuclei, 25 measured in each of the three cells, ranged from 5·4 to 7·7 μm, with a mean of 6·5 μm. The nuclei (Fig. 65) had a ring of darkly staining material just beneath the nuclear membrane and a roughly spherical or ovoid inner body which might



Figs 62–65 *Pelomyxa fragilis.* (62) to (64) Whole cells × 200. (65) Nuclei × 1000. N = nucleus.

be central or eccentric; sometimes there appeared to be two of these presumed nucleoli (see Penard, 1904).

Ingested material included many diatoms, other unicellular algae, a few short algal filaments, and, in one amoeba, possibly a *Colpoda*. I could not identify any mineral grains

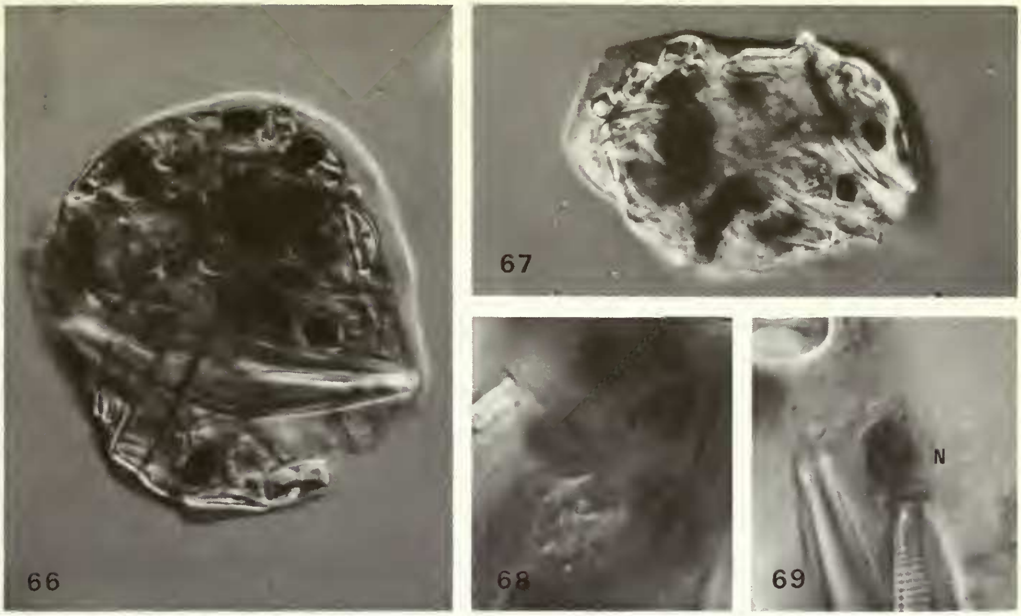


Fig 66–69 *Pelomyxa vivipara.* (66), (67) Whole cells × 250. (68), (69) Nuclei × 1000. N = nucleus.

with certainty within the amoebae, although a few possible mineral particles (or glass fragments?) appeared to be adherent to the outer surfaces. Nor could I identify with certainty the symbiotic bacteria, which Penard reported to be abundant. The cytoplasm appeared highly alveolar.

Accepting the presence of symbiotic bacteria, these amoebae differ from the typical *Pelomyxa palustris*, as far as can be determined from these fixed individuals, chiefly in their greater deformability and almost certainly greater pseudopodial activity, and in the absence of ingested mineral particles. It may be that their greater motility is, in fact, due to their not being packed with those particles. The difference in nuclear structure may not be of major importance, considering the variations reported for *P. palustris* (Daniels & Breyer, 1967; Andresen, Chapman-Andresen & Nilsson, 1968).

P. fragilis may therefore well be a synonym of *P. palustris*.

Pelomyxa vivipara Penard, 1902
(Figs 66–69)

SLIDE NUMBER. 20.12.8.534.

TOTAL NUMBER OF AMOEBAE. 2.

DESCRIPTION AND REMARKS. These two cells look like sacs packed with diatoms. Their measurements are 211 × 182 μm and 187 × 127 μm, and each is approximately 70–80 μm thick. Along part of the periphery of each cell is a narrow hyaline zone (extending inward up to 12 μm from the edge). Both are full of diatoms, and in one at least one desmid was seen. No mineral grains are present in either. Useful observations of the nuclei were possible in only one of the two amoebae, which contained well over 60 nuclei. The nuclei (Figs 68, 69) are circular to oval in outline, and much of the stained granular material is parietal in each nucleus. The diameters of 25 nuclei ranged from 7·7 to 9·2 μm, with a mean of 8·6 μm. Bacteria-like rods were discernible in the cytoplasm, particularly near the nuclei, as reported by Penard.

Penard described and figured 'embryos' in these amoebae, i.e., small amoebae, which might have been parasites or might have been ingested cells which were extruded before digestion. No information on that phenomenon could be gained from this preparation.

Modern workers would tend to regard these amoebae as another phase of *P. palustris* lacking mineral grains, a matter to which reference will be made in connection with the next two species.

Pelomyxa belevskii Penard, 1893
(Figs 70–74)

Penard, 1893; Penard, 1902, pages 144–146.

SLIDE NUMBER. 04.5.9.207.

TOTAL NUMBER OF AMOEBAE. 1.

DESCRIPTION AND REMARKS. This single cell measures $470 \times 398\ \mu m$ and appears considerably compressed. The first thing which strikes the eye is the ingested plant matter, all apparently derived from vascular plants, which Penard (1902) identified as decaying leaf fragments. The cell membrane is somewhat wrinkled around much of the periphery. Focussing carefully, one can find at some places on the cell fine projections, sometimes sharply pointed and single, at other times broader, irregular, and somewhat divided, if indeed these two kinds of projections are the same thing, as Penard thought (Figs. 72, 73). At one point there is also a broad, flat lobe bearing many fine projections (Fig. 71). On this preserved cell, the projections showed up best with differential interference contrast, and one must wonder at Penard's visual acuity that he could make them out with his optical system.

This amoeba contained no mineral grains, and the presence of symbiotic bacteria was not confirmed, though one must accept Penard's report of their presence. Twelve nuclei (Fig. 74) were seen, with finely granular material forming a layer against the inner surface of the nuclear membrane, though an area to one side of a nucleus might appear free of it, perhaps as the result of shrinkage during fixation. The nuclear diameters were strikingly greater than in the preceding three species of *Pelomyxa,* ranging from 24 to 29 μm, with a mean of 26 μm.

The fact that some of the tiny projections from the surface (*aiguillons* or *aspérités*) are very fine suggests the possibility that these are actually non-motile flagella of the type reported by Griffin (1972, 1979) for *P. palustris*, but the broad lobe in Fig. 71 has the appearance of an adhesion uroid with pseudovilli, and one cannot be certain that at least some of the other projections are not such pseudovilli, though the single ones look somewhat more like flagella.

P. belevskii is one of the named species which Chapman-Andresen (1978) has suggested may be a phase of *P. palustris.*

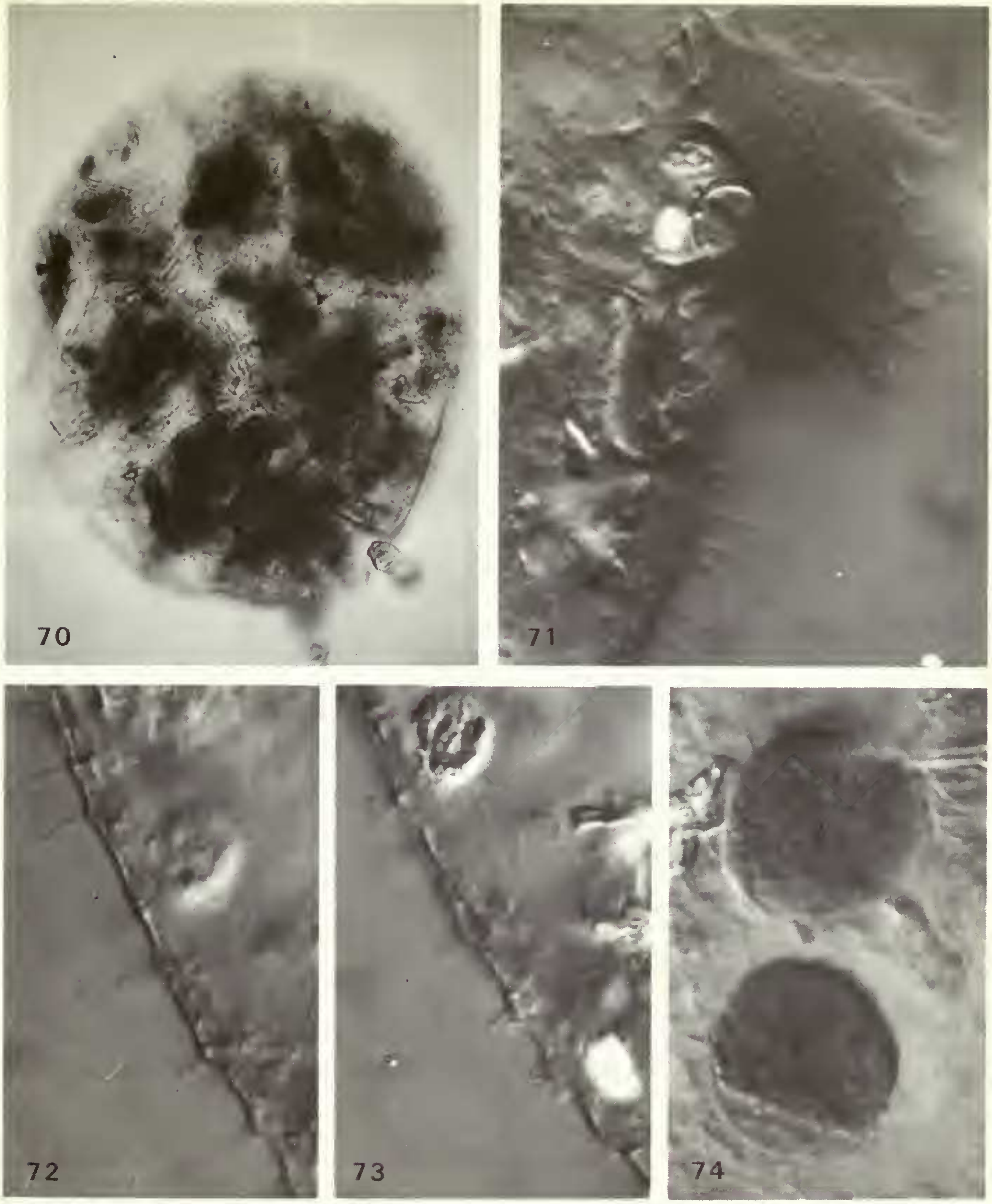
Pelomyxa binucleata (Gruber, 1885)
(Figs 75–81)

Penard, 1902, pages 147, 148.

SLIDE NUMBERS. 04.5.9.208; 20.12.8.528.

TOTAL NUMBER OF AMOEBAE. 13.

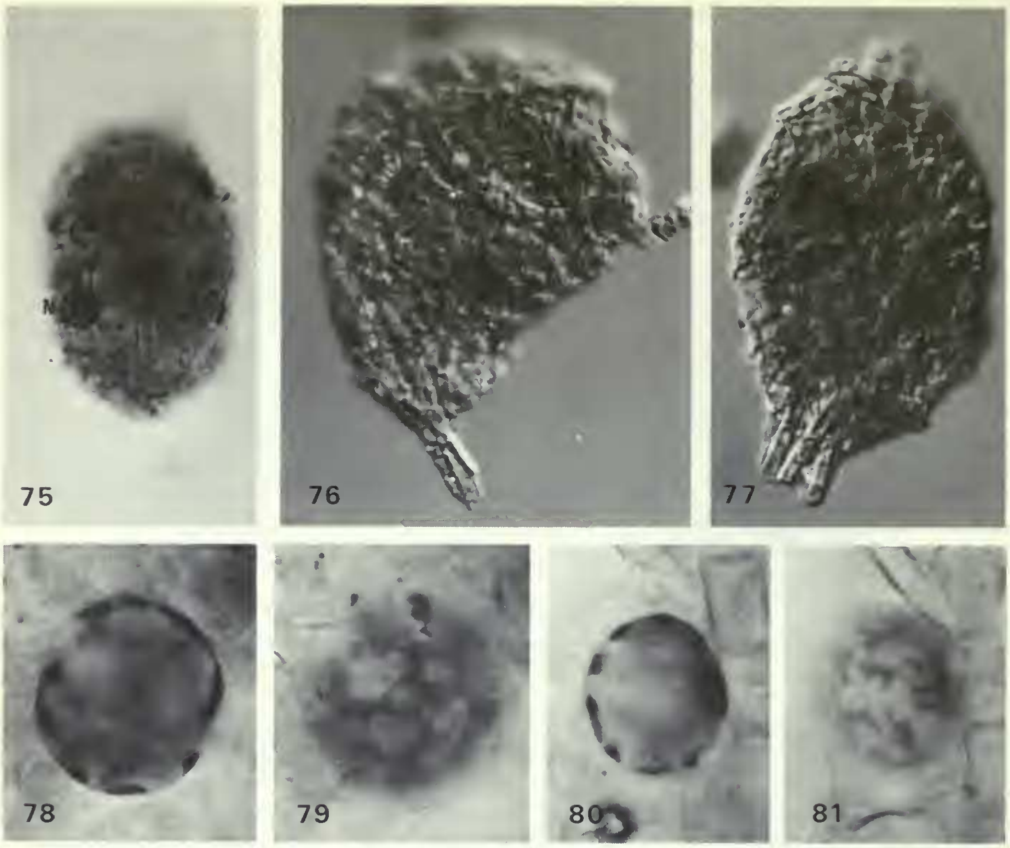
DESCRIPTION AND REMARKS. These amoebae are generally ovoid to ellipsoid, usually so packed with algae as to resemble sacs pushed out here and there by the ends of filaments. None of the uroidal villi pictured by Penard could be seen in the fixed preparations, although the posterior end is sometimes a little morulate or shrivelled. The lengths were from 96 to 240 μm, with a mean of 159 μm; length : breadth ratios 1·0–1·9, mean 1·4. Some of the many contained algal filaments are bent, but none appear to be reflected back upon themselves. Some amoebae also contain diatoms and other unicellular algae. Apparent symbiotic



Figs 70–74 *Pelomyxa belevskii.* (70) Whole cell, showing ingested remains of vascular plants and several nuclei, two of which are in focus at right of picture × 150. (71) Fine projections on both broad lobe (upper right of picture) and main cell body × 1000. (72), (73) Single and more complex projections from cell surface × 1000. (74) Two nuclei × 1000. N = nucleus.

bacteria could be distinguished with differential interference contrast. No mineral grains were identified with certainty.

All the amoebae are binucleate, with the two nuclei fairly close together in some cells but widely separated in others. Generally the diameters of the two nuclei in a given cell are similar, and the differences are slight enough to be accounted for by angle of viewing. The 26 nuclei had maximum diameters ranging from 19 to 34 μm, with a mean of 24·3 μm. Some nuclei were isodiametric, but in others the two diameters measured differed slightly. The



Figs 75–81 *Pelomyxa binucleata.* (75) Whole cell showing both nuclei × 250. (76), (77) Cells distorted by ingested algal filaments × 250. (78), (79) Same nucleus at different levels to show both parietal arrangement and reticulate form of nucleolar material × 1000. (80), (81) Another nucleus at two levels to show nucleolar structure × 1000. N = nucleus.

presumed nucleolar material was generally parietal, sometimes appearing to exist as fragmented bodies, sometimes as granules or larger clumps. However, in the best-preserved nuclei, what appeared to be small nucleolar pieces in one optical plane could be seen on focussing into another plane to be part of a reticulum just inside the nucleus, as shown in Figs. 78–81.

Again, one cannot say definitely whether *P. binucleata* is a distinct species or fits into the cycle of phenotypic change in *P. palustris* (Chapman-Andresen, 1978). The nuclei appear distinctive, but one could explain the absence of mineral grains associated with a shape differing from that of *P. palustris* as a stage on the way to maturation.

Generic diagnosis

Phylum **SARCOMASTIGOPHORA**
Subphylum **SARCODINA**
Superclass **RHIZOPODA**
Class **LOBOSEA**
Subclass **GYMNAMOEBIA**
Order **AMOEBIDA**
Family **THECAMOEBIDAE**

Genus ***THECOCHAOS*** nov.

Derivation of name. From *Theco-* + *Chaos* because *Thecochaos* (multinucleate) bears a relationship to *Thecamoeba* (uninucleate) in the family Thecamoebidae similar to that of *Chaos* (multinucleate) to *Amoeba* (uninucleate) in the family Amoebidae.

Diagnosis. Broad, flattened, often irregularly oval to oblong in outline but sometimes more elongate, always with length greater than breadth in locomotion, with surface folds and wrinkles and light-microscopical appearance of a thickened pellicle; hyaloplasm a more or less crescentic cap at anterior end, sometimes with slender lateral extensions; branching usually only when changing direction; multinucleate. Essentially a multinucleate *Thecamoeba.*

Type-species. *Thecochaos fibrillosum* (Greeff, 1891).

Classification

Subclass GYMNAMOEBIA
  Order AMOEBIDA
    Family AMOEBIDAE
      Amoeba proteus Leidy, 1878 (including *A. nitida*, junior synonym)
      Amoeba sp.
      Chaos nobile (Penard, 1902) comb. nov.

    Family THECAMOEBIDAE
      Thecamoeba terricola (Greeff, 1866)
      Thecamoeba papyracea (Penard, 1905) comb. nov.
      Thecamoeba sphaeronucleolus (Greeff, 1891)
      Thecochaos fibrillosum (Greeff, 1891) comb. nov.
      Thecochaos album (Greeff, 1891) comb. nov.

    Family HARTMANNELLIDAE
      Saccamoeba lucens Frenzel, 1892

    Family PARAMOEBIDAE
      Mayorella vespertilio (Penard, 1902)
      (*Dinamoeba mirabilis* Leidy, 1874?)

  Order PELOBIONTIDA
    Family PELOMYXIDAE
      Pelomyxa palustris Greeff, 1874
      (Validity of other species of *Pelomyxa* questionable.)

Incertae sedis: *Amoeba granulosa, Amoeba laureata, Amoeba muralis, 'Amoeba peritissima' (nomen nudum).*

References

Andresen, N., Chapman-Andresen, C. & Nilsson, J. R. 1968. The fine structure of *Pelomyxa palustris. C. R. Trav. Lab. Carlsberg* **36** : 286–317.

Bovee, E. C. 1972. The lobose amebas. IV. A key to the order Granulopodida Bovee & Jahn, 1966, and descriptions of some new and little-known species in this order. *Arch. Protistenk.* **114** : 371–403.

Cash, J. 1905. *The British freshwater Rhizopoda and Heliozoa* **1.** London.

—— **& Wailes, G. H.** 1919. *The British freshwater Rhizopoda and Heliozoa* **4.** London.

Chapman-Andresen, C. 1978. The life cycle of *Pelomyxa palustris. J. Protozool.* **25** : 42A (Abstract only.)

—— 1979. Comparative cytology of the genera *Amoeba, Chaos* and *Pelomyxa. In* S. H. Hutner, *Protozoological actualities—1977.* Lawrence, Kansas.

Daniels, E. W. & Breyer, E. P. 1967. Ultrastructure of the giant amoeba *Pelomyxa palustris. J. Protozool.* **14** : 167–179.

Deflandre, G. 1958. Eugène Penard (1855–1954). Correspondance et souvenirs. Bibliographie et bilan systématique de son oeuvre. *Hydrobiologia* **10** : 2–37.

De Groot, A. A. 1936. Einige Beobachtungen an *Dinamoeba mirabilis* Leidy. *Arch. Protistenk.* **87** : 427–436.

Flickinger, C. J. 1974. The fine structure of four 'species' of *Amoeba*. *J. Protozool.* **21** : 59–68.

Frenzel, J. 1892. Untersuchungen über die mikroskopische Fauna Argentiniens. *Bibliotheca Zoologica* **4**(12) : 1–82.

Greeff, R. 1981. Über den Organismus der Amöben. *Biol. Centralbl.* **11** : 633–640.

Griffin, J. L. 1961. Identification of ameba crystals. II. Triuret in two crystal forms. *Biochim. Biophys. Acta* **46** : 433–439.

—— 1972. Microfilaments, microtubules, pseudopods, villi, cilia, and cytoplasm of various amoebae. *J. Cell Biol.* **55**(2, part 2) : 96a (Abstract only.)

—— 1979. Flagellar and other ultrastructure of *Pelomyxa palustris*, the giant herbivorous amoeboflagellate: more evidence for evolutionary distance from carnivores. *Trans. Amer. Micros. Soc.* **98** : 157, 158. (Abstract only.)

Grospietsch, T. 1975. List of the slide collection made by E. Penard, which is deposited in the Muséum d'histoire naturelle at Geneva. *Informative Bulletin of Testacean Workers,* No. 3 : appendix (unpaginated).

Gruber, A. 1885. Studien über Amöben. *Zeitschr. wiss. Zool.* **41** : 186–225.

Heal, O. W. 1965. Some slide collections of Protozoa, especially Testacea. *J. Quekett Micros. Club* **30** : 1–6.

Leidy, J. 1879. *Fresh-water rhizopods of North America.* Washington.

Page, F. C. 1970. *Mastigamoeba aspera* from estuarine tidal pools in Maine. *Trans. Amer. Micros. Soc.* **89** : 197–200.

—— 1976. *An illustrated key to freshwater and soil amoebae.* Ambleside.

—— 1977. The genus *Thecamoeba* (Protozoa, Gymnamoebia). Species distinctions, locomotive morphology, and protozoan prey. *J. nat. Hist.* **11 : 25–63.**

—— **& Blakey, S. M.** 1979. Cell surface structure as a taxonomic character in the Thecamoebidae (Protozoa: Gymnamoebia). *Zool. J. Linn. Soc.* **66** : 113–135.

—— **& Willumsen, N. B. S.** 1980. Some observations on *Gocevia placopus* (Hülsmann, 1974), an amoeba with a flexible test, and on *Gocevia*-like organisms from Denmark, with comments on the genera *Gocevia* and *Hyalodiscus*. *J. nat. Hist.* **14** : 413–431.

Penard, E. 1890. Études sur les Rhizopodes d'eau douce. *Mém. Soc. Phys. Hist. nat. Genève* **31** : 1–230.

—— 1893. *Pelomyxa palustris* et quelques organismes inférieurs. *Arch. Sci. phys. nat. Genève* (3rd series) **29** : 165–184.

—— 1902. *Faune rhizopodique du bassin du Léman*. Genève.

—— 1904. Quelques nouveaux Rhizopodes d'eau douce. *Arch. Protistenk.* **3** : 391–422.

—— 1905. Observations sur les Amibes à pellicule. *Arch. Protistenk.* **6** : 175–206.

—— 1909*a*. Sur quelques Rhizopodes des mousses. *Arch. Protistenk.* **17** : 258–296.

—— 1909*b*. Sur quelques Mastigamibes des environs de Genève. *Rev. suisse zool.* **17** : 405–438.

—— 1913. Nouvelles recherches sur les Amibes du groupe Terricola. *Arch. Protistenk.* **28** : 78–140.

—— 1936. Rhizopode ou flagellate? Quelques refléxions à propos de la *Dinamoeba mirabilis. Bull. Soc. franc. Micros.* **5** : 136–140.

Schaeffer, A. A. 1916. Notes on the specific and other characters of *Amoeba proteus* Pallas (Leidy), *A. discoides* spec. nov., and *A. dubia* spec. nov. *Arch. Protistenk.* **37** : 204–228.

—— 1926. *Taxonomy of the amebas.* Washington.

Siemensma, F. J. 1980. Amoeben. *Natura* **77** : 62–72.

Vonwiller, P. 1913. Über den Bau der Amöben. *Arch. Protistenk.* **28** : 389–410.

Manuscript accepted for publication 2 September 1980.