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Diversity of free-living 'naked' amoeboid organisms

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Amoeboid organisms are phylogenetically diverse, some being more closely related to plants or metazoans than to each other. Amoeboid organisms are ecologically successful, having been isolated on all continents, including Antarctica, as well as being the main predators controlling bacterial populations in soil. The classification of these organisms has historically relied upon morphological characteristics. The application of electron microscopy, comparison of enzymic profiles after electrophoretic separation, and analysis of nucleic acid fractions have provided reliable bases for classifying amoeboid organisms. The extent of diversity of these organisms has been recognized, as methods to detect, culture, characterize and identify them has increased. It is reasonable to anticipate that the current 40 000 species of protists will increase substantially as amoeboid organisms are cultivated from poorly accessible niches and from extreme environs.

Keywords: free-living amoebae; biological diversity; molecular systematics; ecology of amoebae; amoebae as predators and prey; ecology of amoebae; phylogeny of amoebae

Biodiversity has been defined as the variety and variability of life-forms, both contemporary and extinct. An understanding of the origins and extent of biodiversity is basic to all other fields of comparative and applied biology. The diverse living organisms on earth interact in a vast array of complex ecosystems to maintain soils and their nutrients, to purify water, to control pest organisms, to maintain the gaseous composition of the atmosphere, and to stabilize the climate. A knowledge of biodiversity is essential for the formulation of sound public policy and for economic development based upon biotechnology and applied biology. Comprehensive systematic studies are essential for an analysis of genetic and ecosystem biodiversity at the level of the gene and species [78]. Classification systems serve as the knowledge base from which predictions about organisms can be made [58]. It has been estimated that the currently-described 1.5–2 million species represent only 10–20% of taxonomic biodiversity [20].

Free-living amoeboid organisms have been described for more than two centuries; however, van Leeuwenhoek did not include a description of these organisms in his pioneering work [51]. In 1755, Rösel von Rosenhof described an amoeboid organism that he referred to as 'little Proteus' [76]. The naming of this amoeba *chaos* by Linnaeus was perhaps prophetic. In 1758, Linnaeus named Rösel von Rosenhof's organism *Volvox chaos* [50], but in 1767 he renamed it *Chaos protheus* [51]. The epithet *Chaos chaos* was subsequently resurrected and applied to the giant amoeba *Pelomyxa carolinensis* [52]. Kudo [46] concluded that Linnaeus' *Chaos chaos* was an unidentifiable organism of historic interest only. The name *amoeba* was first applied to this morphological group of organisms by Ehrenberg in 1838. The obvious plasticity of the cell's shape and the superficially similar means of locomotion gave credence to the perception of *amoeba* as a natural group both in the lay language and in scientific nomenclature [45]. Bütschli [8] grouped the amoeboid organisms into the Sarkodina as a major taxon of the Protozoa or Infusoria. The concept of Sarcodina as a plausible taxon to encompass amoeboid organisms persisted for a century [43,48], although the diversity of these organisms was recognized, but ignored, throughout this period. Amoeboid locomotion, the only universally shared characteristic of the taxon Rhizopoda, is the consequence of an array of contractile mechanisms. It is now accepted, albeit reluctantly, that amoeboid organisms are remarkably diverse, with important biological properties that link some amoeboid organisms closer to plants or metazoans than to each other. The classification of amoebae has historically relied upon morphological characteristics [65]. As biochemical and molecular methods have been applied to the identification and classification of these organisms, it is obvious that morphological characteristics alone are not sufficient to delineate all important taxa, and that morphological differences may be misleading in classifying amoeboid organisms [70]. In the span of 10 years, the number of recognized species of *Naegleria* has grown from five to twelve, most of these distinguished from each other solely on molecular criteria. Taxonomy of amoeboid organisms is still in a state of flux, with advocates for morphological [12], biochemical [70] or molecular characters [39] as the bases for classification and identification.

Biology of amoebae

Amoeboid organisms have numerous features that make them particularly interesting organisms for study. They provide excellent tools for the study of cell differentiation [32]. Some amoeboid organisms transform into resting cysts or into flagellated cells, or aggregate into multicellular plasmodia. These processes, in selected systems, occur rapidly, synchronously and reproducibly. Several of these processes occur in the absence of added nutrients, and require that pre-formed proteins be degraded and new proteins synthe-

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sized, thereby providing an excellent tool for studying selective intracellular protein degradation [86]. The nuclear genome is relatively small, as compared with other eukaryotic cells. Relatively few intervening non-coding sequences (introns) have been described for amoeboid organisms; however, an intron is found in the extrachromosomal ribosomal DNA of *Naegleria*. Although introns of the type found in *Naegleria* rDNA are rare in nuclear DNA across the eukaryotic world, this group of introns is quite prevalent in mitochondrial and chloroplast genes [21]. Not all amoeboid organisms (eg *Entamoeba*) have mitochondria. It is not definitively resolved whether *Entamoeba* is secondarily amitochondriate or whether it represents an early branch prior to the acquisition of mitochondria as endosymbionts [40]. Many amoeboid organisms have life stages; for example, a feeding trophozoite stage and a thick-walled cyst stage that enable the organisms to survive during unfavorable periods of nutritional deprivation or during desiccation. Some amoeboflagellates (eg *Tetramitus*) are able to undergo cell division either as flagellates or as amoebae [45], while others are not able to divide in the flagellated stage (eg *Naegleria*). The distinction is blurred by the recent observation that quadriflagellate cells of one species of *Naegleria, N. minor*, are capable of division, whereas the biflagellate stage is not [25]. One of the most distinctive groups of amoeboid organisms (eg the cellular slime mold *Dictyostelium*) responds chemotactically to cyclic AMP, aggregates into a multicellular mass, and then undergoes differentiation to form spores on a stalk [35].

Ultrastructure

The application of electron microscopy for detailed study of amoeboid organisms revealed their diversity and provided a sound basis for developing phylogenetic reconstructions. The greater resolving power of electron microscopy allows for a more detailed description of organisms than can be achieved by light microscopy [68]. The genus *Neoparamoeba*, for example, was created to distinguish it from *Paramoeba* because the former organisms have microscales while the latter do not [66].

Prior to the 1970s, it was assumed that amoeboid organisms, as well as other protozoa, had evolved gradually from immediately ancestral taxa. Detailed studies on the mitotic processes in amoeboid organisms led a number of workers to propose that *Acanthamoeba* and *Naegleria* had separate origins, probably from non-amoeboid ancestors. *Naegleria* shares with *Euglena* a promitotic form of nuclear division in which centrioles are absent and the nucleolus and nuclear membrane persist throughout mitosis. In contrast, the metamitotic nuclear division of *Acanthamoeba* more closely resembles mitosis in higher plants and animals in that centrioles are present and the nuclear membrane and nucleolus disintegrate [16]. Moreover, it has become generally accepted that certain organelles of eukaryotes did not appear by gradual evolution but were acquired through endosymbiosis [72]. Patterson [68] proposed a progression in assemblage of the eukaryotic cell: nuclei with chromosomes, followed by flagella, then non-microtubule cytoskeleton, dictyosomes (Golgi), and finally mitochondria. *Acanthamoeba* has a well formed Golgi apparatus but this organelle is rarely observed in *Naegleria* [53]. Carosi *et al* [11] confirmed that *Acanthamoeba* has typical Golgi complexes with microvesicles and vacuoles whereas typical Golgi complexes were not observed in *Naegleria*. Rivera *et al* [73], however, reported that *N. lovaniensis tarasca* possesses nuclei, flagella, microtubules, dictyosomes (Golgi) and mitochondria. Amoeboid locomotion in *Naegleria* is based upon an extremely dynamic actin-based cytoskeleton that involves cytoplasmic streaming, pseudopod production, cell polarity and focal contact production [71]. *Acanthamoeba* and *Dictyostelium* have a multilamellar microtubule center, indicating that these two genera are related [6]. Alternatively, the similarities in microtubular structure and organization may reflect convergent evolution. Adhesion to solid substrates is essential for the normal functioning of bottom-dwelling amoeboid organisms. Ordered molecular contacts are required between the adhesion receptors on the membrane exterior and the cytoskeleton. In the case of amoeboid cells, the points of focal contact must provide increasing adhesiveness at the leading edge and decreasing adhesiveness at the trailing end, together with coordinated modifications of the cytoskeleton. The adhesion receptor contains a strongly conserved region that recognizes the tripeptide motif arginyl-glycyl-aspartic acid. Peptides containing this tripeptide not only impair the ability of the common marine amoeba *Neoparamoeba aestuarina* to attach to the substrate, but also affect cell shape [19]. Some of the alterations in cell shape observed in *Naegleria fowleri* grown in different media [55] may reflect effects on adhesion receptors as well as direct nutritional effects.

The vegetative or feeding trophozoite stage of *Naegleria fowleri* exhibits food cups or amoebastomes which are cytoplasmic extensions of the cell surface (Figure 1a). Mouse-passaged *N. fowleri* trophozoites have fewer amoebastomes than trophozoites grown in axenic medium, indicating that these food cups are used to ingest bacteria and organic particulate matter [54]. The remarkable surface feature of *Acanthamoeba* trophozoites is the sharp spiny acanthapoida (Figure 1b). Transmission electron micrographs of both *Naegleria* and *Acanthamoeba* display typical protozoan nuclei with a prominent nucleolus, well developed mitochondria and endoplasmic reticulum (Figure 2).

Ecology

Amoeboid organisms are ecologically successful, having been isolated from samples collected on all continents, including Antarctica. They are the predominant protozoans in many water and soil niches. Free-living amoeboid organisms are the most successful thermotolerant protists; several amoeboid species are able to survive and grow at temperatures above 45°C. In general, amoeboid organisms have been successful free-living organisms, voraciously eating living and dead bacteria and fungi, and are not serious pathogens of humans. The leptomyxid amoeba *Balamuthia mandrillaris*, however, was isolated from the brain of a mandrill baboon that died of meningoencephalitis and is now recognized as a human pathogen. *Balamuthia* can be cultivated as a predator of other amoebae such as *Acanthamoeba*, indicating that it might have the capability to

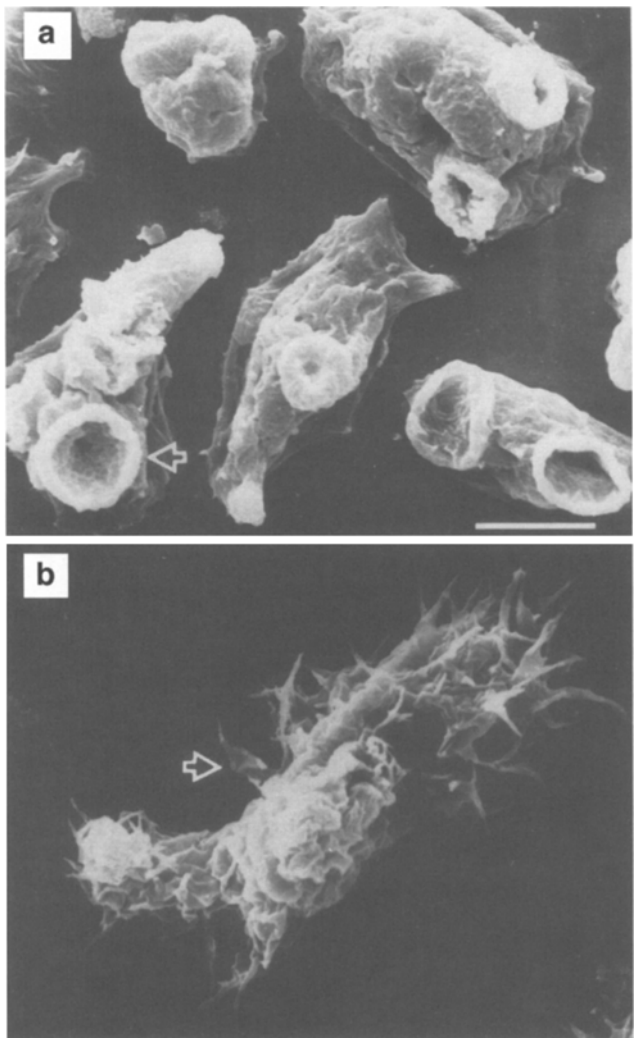

Figure 1 Scanning electron micrograph of (a) *Naegleria fowleri* LEE showing well developed amoebastomes or food cups (arrow) and (b) *Acanthamoeba castellanii* showing numerous acanthapodia (arrow). Marker bar denotes 10 μm.

live freely in nature. Various characteristics of *Balamuthia*, for example, breakdown of the nuclear membrane and dissolution of the nucleolus during nuclear division, link *Balamuthia* to the genus *Gephyramoeba* [83]. Most species of the genus *Entamoeba* have adapted to an intestinal environment, with the exception of *E. moshkovskii*, which appears to be free-living. This adaptation has been remarkably successful for *Entamoeba histolytica*, which infects 500 million humans worldwide. *Entamoeba* disease (amoebic dysentery) is not considered eradicable by the World Health Organization [44]. As humans alter their ability to resist infections, and as microorganisms gain entry into the body by alternative routes or in greater numbers, opportunistic amoebic infections are increasingly recognized; for example, *Acanthamoeba* causes amoebic keratitis in persons who wear contact lenses and serious granulomatous amoebic encephalitis in immunosuppressed humans. The severity of infections leading to primary amoebic meningoencephalitis (*Naegleria fowleri*) and granulomatous amoebic encephalitis (several species of *Acanthamoeba*) and the lack of efficacious therapy have discouraged most workers from extensive study of these amoeboid organisms. *Acanthamoeba* spp are reliable indicators of release of sewage wastes into rivers, bays, coastal waters and the open ocean. As brackish and sea water environments are examined more extensively, new species of *Acanthamoeba* are being described [60]. Several described species of *Acanthamoeba* are not readily identifiable on the basis of morphological features and require biochemical and molecular tools for definitive characterization. It is likely that additional species will be discovered in this environmental niche. Amoeboid organisms are readily cultured from marine samples. One of the most abundant marine species is *Vexillifera telmathalassa* [1]. *Vexillifera* has a rather typical protozoan nucleus with a prominent nucleolus, a well developed Golgi apparatus and mitochondria containing tubular cristae. The diversity of the described species of *Vexillifera* indicate that this is a heterogeneous group and that molecular studies are likely to reveal substantial evolutionary divergence.

Predators and prey

The ecology of amoeboid organisms is not known in great detail; however, we are beginning to understand interactions between bacteria and amoeboid cells that have practical implications as well as providing important biological insights [80]. Small, free-living amoebae are the main predators controlling bacterial populations in soil. The rhizosphere supports a diverse array of microorganisms: millions of bacteria per gram, hundreds of thousands of fungi and tens of thousands of protozoa and algae. Between 50% and 90% of the protozoa in litter are free-living amoebae. A number of amoeboid organisms feed on fungi as well as on bacteria [74]. Some of the common amoeboid genera in soil include *Naegleria, Acanthamoeba* and *Hartmannella* [3]. As many as 100 species of amoeboid organisms have been reported in marine sediments. Some of the more common marine genera are *Hartmannella, Neoparamoeba, Vannella, Platyamoeba* and *Stygamoeba* [7]. Bacteria metabolize detritus in marine sediments and amoeboid organisms, flagellates and ciliates consume these bacteria, thereby accelerating the rate of nutrient recycling [42]. The interactions between bacteria and amoebae may include amoebae as predators, amoebae as susceptible hosts, or bacteria as endosymbionts. *Bacillus licheniformis*, which commonly shares the same habitat as free-living amoebae, produces amoebolytic substances [17]. *Legionella pneumophila* is able to infect *Acanthamoeba* cells, proliferate inside the cell, and kill the cell liberating hundreds of bacteria [81]. *Legionella* also can be propagated in coculture with the pathogeneic amoeba, *Naegleria fowleri* [62]. Rowbotham [77] suggested that *Legionella*-laden debris of amoebae might be the infective particle rather than free bacterial cells. Amoebae that acquire bacterial endosymbionts may acquire the capability to kill amoebae lacking the endosymbiont [28]. About 25% of *Acanthamoeba* isolates appear to have bacterial endosymbionts. The endosymbionts are taxonomically diverse, and some have not been cultivated apart from the *Acanthamoeba* host [30]. The ability to establish an endosymbiotic association is strain-specific. Preparations of free endosymbionts may success-

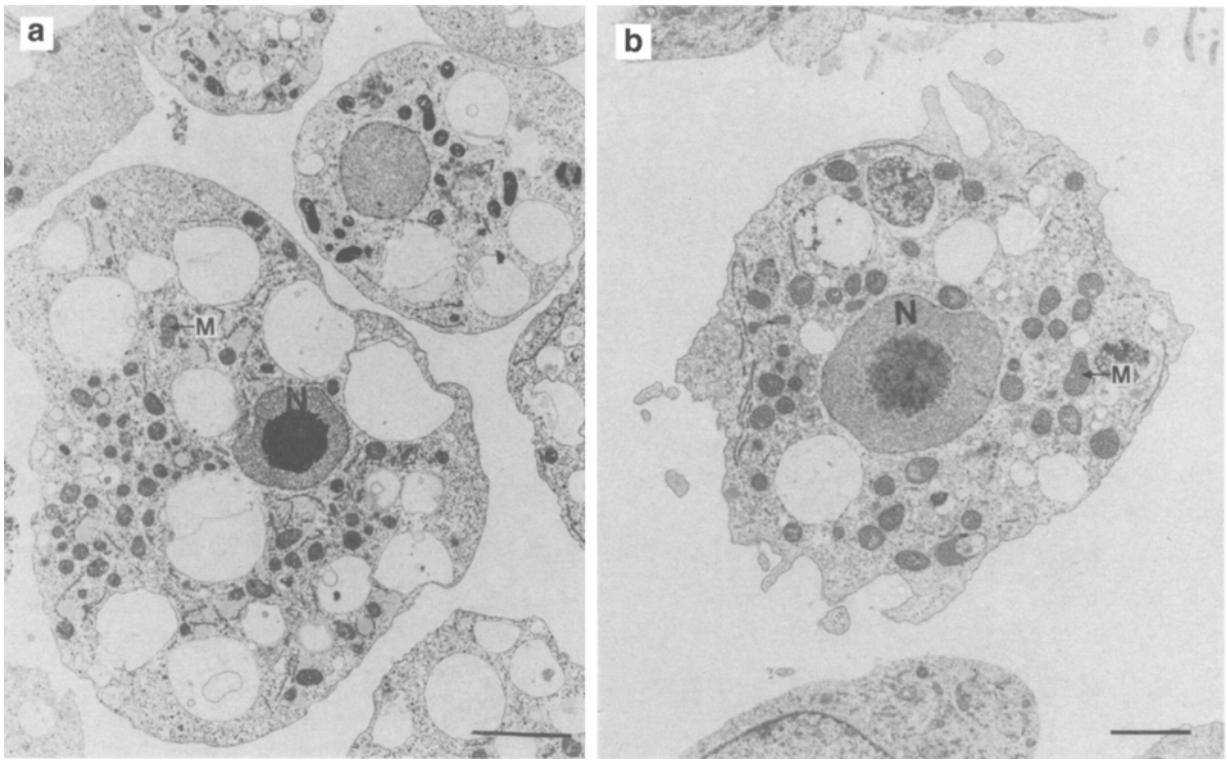

Figure 2 Transmission electron micrograph of (a) *Naegleria fowleri* LEE and (b) *Acanthamoeba castellanii* showing a typical protozoan nucleus (N) with a prominent nucleolus and mitochondria (M). Marker bars denote 5 μm.

fully establish an endosymbiotic association, or kill the challenged amoeba, or cause it to encyst [34].

Serologic systematics

Serologic techniques have been used extensively for the identification and classification of microorganisms, including free-living amoeboid organisms. Visvesvara and Healy [82], used gel diffusion and immunoelectrophoresis to demonstrate that *Naegleria fowleri* and *N. gruberi* share some antigens, and each possesses unique antigens. In that study, no antigenic cross reactivity was detected between *N. fowleri* and *Acanthamoeba castellanii, A. culbertsoni* or *Entamoeba histolytica*. DeJonckheere *et al* [26] analyzed the number of precipitin bands obtained after electrophoretic separation of proteins in extracts of 16 strains of *Naegleria*, and development with antisera against four species of *Naegleria*. They observed that strains designated *N. australiensis* were heterogeneous. Subsequent work led to the establishment of two subspecies of *N. australiensis*, and later elevation of *N. italica* to species rank. The resolution of serologic studies has been refined by the Western blot technique in which extracts are subjected to electrophoretic separation and then development with antibody. Using this technique, bands shared by three species of *Naegleria* are readily apparent and unique proteins are revealed (Figure 3).

Biochemical systematics

Biochemical analyses have documented the taxonomic diversity of amoeboid organisms. Nerad and Daggett [59] used starch gel electrophoresis to generate enzyme profiles of pathogenic and nonpathogenic strains of *Naegleria*. In this early study, leucine aminopeptidase and propionyl esterase isozyme patterns were able to differentiate the then known species and to provide evidence that the organisms belonging to *N. gruberi* were heterogeneous. The banding pattern of enzyme activities after isoelectric focusing has been used extensively to study the genus *Naegleria*. The enzymes lactate dehydrogenase, L-threonine dehydrogenase, superoxide dismutase, acid phosphatase, malic enzyme and leucine aminopeptidase was particularly useful for differentiating among *Naegleria* groups [70]. The isozyme patterns for pathogenic and non-pathogenic strains of *Naegleria* generate groups that are congruent with groups established by serologic analyses. Costas and Griffiths [18] found poor agreement between arrangement of strains of *Acanthamoeba* based on enzyme profiles versus morphological characteristics. During the past 20 years, the number of *Acanthamoeba* species has risen from 10 to more than 20, and these 20 named species do not appear to encompass the diversity of the genus. In a recent limited study (Flint J, P Dobson and B Robinson, 1996. Abstracts of the 7th International Conference on Small Freeliving Amoebae, Adelaide, Australia), one un-named species was found among 31 new isolates. Individual enzymes have been analyzed in detail to reveal phylogenetic relationships. For example, the lysosomal β-hexosaminidase A subunit of *Entamoeba histolytica* is 28% identical to that of the cellular slime mold *Dictyostelium*, and 32% identical to that of humans or mice [5]. β-Hexosaminidase in *Dictyostelium* is a homodimer whereas the lysosomal enzyme in *Acanthamoeba* is a 58-kD monomer [2], indicating that these two

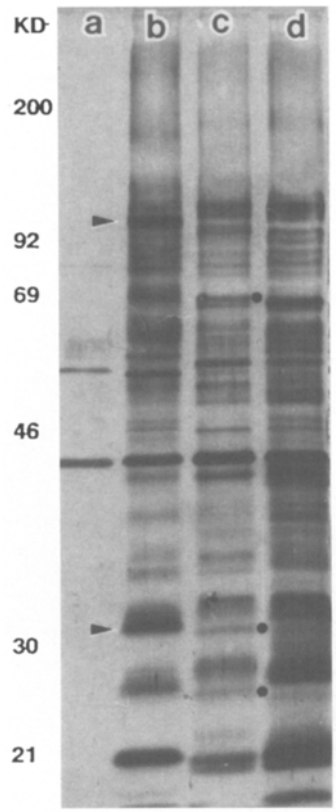

Figure 3 Western immunoblot analysis of membrane proteins from *Naegleria gruberi* (lane a), *N. lovaniensis* (lane b), *N. fowleri* LEEmp (lane c) and *N. fowleri* LEE (lane d), developed with rabbit antiserum to an extract of *N. fowleri* LEEmp. Arrows denote proteins unique to *N. lovaniensis*. Dots denote proteins more prominent in *N. fowleri* LEEmp than in *N. fowleri* LEE.

genera are only distantly related. The glycolytic enzyme phosphofructokinase in *Naegleria* uses pyrophosphate rather than ATP, a property shared with *Entamoeba, Trichomonas, Giardia, Toxoplasma, Euglena* and plants [56].

Molecular systematics

Molecular biological approaches have revolutionized microbial systematics. The application of these techniques to ecological studies of free-living microorganisms in natural ecosystems has begun only recently. The results of these studies are providing new insights into long-standing issues in protistan ecology [10]. *Naegleria* and *Entamoeba* are unusual genera in that the genes for ribosomal RNA (rDNA) are on a circular plasmid [15]. The plasmid is present in about 4000 copies in *Naegleria*, accounting for nearly 17% of the total cellular DNA. The plasmid is smaller in *Naegleria* than in other genera of Vahlkampfiidae: *Naegleria* 12–17.1 kb according to strain; *Vahlkampfia lobospinosa*, 21.4 kb; *Tetramitus rostratus*, 21.4 kb; and *Didasculus thorntoni*, 24 kb [24]. There is one rDNA transcription unit per plasmid. The nucleotide sequence of the small-subunit ribosomal RNA has proven to be valuable for assessing phylogenetic relationships among distantly related taxa because many regions are conserved over large periods. Woese [85] proposed that ribosomal RNA (rRNA) sequences may be the ultimate molecular clock in that the rate of molecular divergence appears to be relatively constant with time. Based upon an analysis of rRNA, Clark [14] concluded that *Naegleria* represents a relatively early branch in eukaryotic evolution whereas *Acanthamoeba* represents a much later branch. Phylogenetic trees based upon actin [6] however are not congruent with those based upon small-subunit ribosomal RNA analyses. In *Acanthamoeba*, the genes for rRNA are located in both the nucleus and the mitochondria. Both nuclear and mitochondrial small subunit rDNA sequences in *Acanthamoeba* include highly conserved and highly variable regions. Byers *et al* [9] have extensively sequenced the 18S rDNA from three strains of *A. castellanii* and found that sequence divergence was less than 3%. Gast and Byers [33] have developed genus and subgenus specific oligonucleotide probes for the identification of *Acanthamoeba*. Baverstock *et al* [4] examined four species of *Naegleria* and found divergence of 0.7–6.1% among the 18S rDNA of the four species. Moreover, there is relatively little homology between the rRNA of *Acanthamoeba* and *Naegleria* [4,16], confirming a distant evolutionary relationship between these two genera. An intron was found in the nuclear small subunit rDNA of only two species of *Acanthamoeba, A. griffini* and *A. lenticulata*, but not in mitochondial small subunit rDNA (Byers TJ, DR Ledee, RJ Gast, JM Diedrich, DR Stothard, MH Awwad and PA Fuerst, 1996. Abstracts of the 7th International Conference on Small Freeliving Amoebae, Adelaide, Australia). An intron has been detected in the small subunit rDNA of five of ten species of *Naegleria* examined. All isolates of the five species examined to date contain this intron. DeJonckheere [22] proposed that this intron may have been acquired at an early stage in the evolution of the genus and lost in the many *Naegleria* species.

Recombination

Few recombinational systems are available among free-living amoeboid organisms. A number of amoeboid species undergo cell fusion, potentially forming heterokaryons, as a normal part of their life cycle [45]. Cell fusion may also bring together new configurations of nuclear genes and mitochondrial genes. In addition, investigators have injected nuclei into cells of the same and different amoeboid species. Few studies have rigorously examined the genetic consequences of new complements of the nucleus, mitochondria and plasmids in amoeboid organisms. It is not unexpected that asexual haploid cells of *Dictyostelium discoideum* fuse on rare occasions to generate a relatively stable diploid strain that can be induced to segregate haploid progeny [61]. *D. discoideum* has seven chromosomes or linkage groups as established by parasexual analysis and by REMI-RFLP mapping [47]. A number of loci have been assigned to one of the chromosomes, allowing comparative studies by *in situ* hybridization. *Dictyostelium* possesses a novel sexual cannabalism. In this process, a giant zygote cell, having arisen from the fusion of gametic amoebae, attracts unfused nonzygotic amoebae and engulfs them as a food source [49]. Indirect evidence of genetic exchange in *Naegleria lovaniensis* has been presented [69] but rigorous experimental proof is lacking.

Diversity

Our understanding of the diversity of amoeboid organisms is rapidly expanding as methods to detect, culture, characterize and identify them increases. During the past 15 years, more than 20 new genera of free-living 'naked' amoeboid organisms have been described (Table 1). Investigators must be able to identify correctly the organisms with which they work [79]. Morphological characteristics are inadequate for classifying amoeboid organisms, leading to an underestimate of their diversity, and has limited the ability of workers to collect and analyze data on their ecology, biochemistry and genetics. Patterson [68] asserted that the protists, including the amoebae, are cytologically, organizationally, and molecularly more diverse than animals, plants or fungi. He concludes that 'phylogenetically, the protists are not equal to the animals, plants and fungi, but embrace them'. Patterson [68] estimated that prior studies described between 10% and 90% of flagellate diversity, and he selected the mid-points for his projections. He concluded that amoeboid organisms are derived polyphyletically from flagellates, and estimated the number of amoeboid lineages at 25 compared to 42 for flagellate lineages. Patterson projected about 200 ultrastructural lineages, each of which may encompass a thousand species, although some may encompass only a few species. It is reasonable to anticipate that the current 40 000 described species of protists will double during the coming few decades and is more likely to quadruple as protists are cultivated from poorly accessible niches and from extreme environs [78]. As just one modest example, DeJonckheere [23] proposed that the well-studied *Naegleria gruberi* is a complex consisting of at least four clusters or species. Henderson and Partridge (1996, Abstracts of the 7th International Conference on Small Freeliving Amoebae, Adelaide, Australia) identified 13 462 amoeboid isolates over a period between August 1985 and July 1995 from water samples collected in Western Australia. Another 45 isolates were not identified. Even in this rather homogeneous, well studied environment, not all biological diversity of free living 'naked' amoebae has been characterized.

Evolutionary relationships among amoeboid organisms have been inferred from ultrastructural studies and nucleic acid analyses such as sequencing the small subunit rRNA. There is increasing congruence among the various phylogenetic reconstructions but a number of issues remain unresolved. *Entamoeba*, for example, lacks cilia, mitochondria, peroxisomes and hydrogenosomes. *Entamoeba* may be a primitive Archezoa or may have lost organelles secondarily [12]. Analyses based upon alignment of rRNA sequences indicate that *Entamoeba* is secondarily amitochondriate and may be derived from mycetozoan amoebae [14]. The relatedness of the true slime mold *Physarum* to the cellular slime mold *Dictyostelium* is unresolved. Comparisons of 16S-like rRNA gene sequences indicate that these two lineages separated early and that *Dictyostelium* is as closely related to, or more closely related to *Entamoeba* than it is to *Physarum* [39]. Phylogenetic reconstructions based upon ultrastructure indicate that *Dictyostelium* and *Physarum* separated at a later date than *Entamoeba* [12]. A limited phylogenetic reconstruction, drawn from the work of several investigators using diverse methodologies, illustrates the diversity of amoeboid organisms (Figure 4). In this diagram, the Phylum Rhizopoda includes *Hartmannella* and *Acanthamoeba* whereas *Naegleria, Vahlkampfia, Tetramitus* and *Paratetramitus* are assigned to the Phylum Percolozoa [12]. Although *Tetramitus* and *Naegleria* have distinct life styles, the former predominately flagellated and the lat-

Table 1 Genera of free-living 'naked' amoeboid organisms described during the past 15 years

Genus	Habitat
Balamuthia Visvesvara, Schuster & Martinez, 1993 [83]	soil (?)
Deuteramoeba Page, 1987 [66]	fresh water
Gruberella Page, 1983 [64]	sea water
Leucodictyon Grell, 1991 [37]	sea water
Metamoeba Friz, 1992 [31]	fresh water
Nolandella Page, 1983 [64]	sea water
Neoparamoeba Page, 1987 [66]	sea water
Parachaos Willumsen, 1987 [84]	fresh water
Paraflabellula Page & Willumsen, 1983 [64]	fresh water
Paragocevia Page, 1987 [66]	soil and fresh water
Parvamoeba Rogerson, 1993 [75]	sea water
Pernina El Kadiri, Joyon & Pussard, 1992 [29]	sea water
Phreatamoeba Chávez, Balamuth & Gong, 1986 [13]	fresh water
Protacanthamoeba Page, 1981 [63]	soil and fresh water
Protonaegleria Michel & Raether, 1985 [57]	mud and fresh water
Pseudothecamoeba Page, 1988 [67]	fresh water
Reticulamoeba Grell, 1994 [38]	sea water
Thalassomyxa Grell, 1985 [36]	sea water
Thecochaos Page, 1981 [63]	fresh water
Willaertia DeJonckheere, Pussard, Dive & Vickerman, 1984 [27]	soil and fresh water

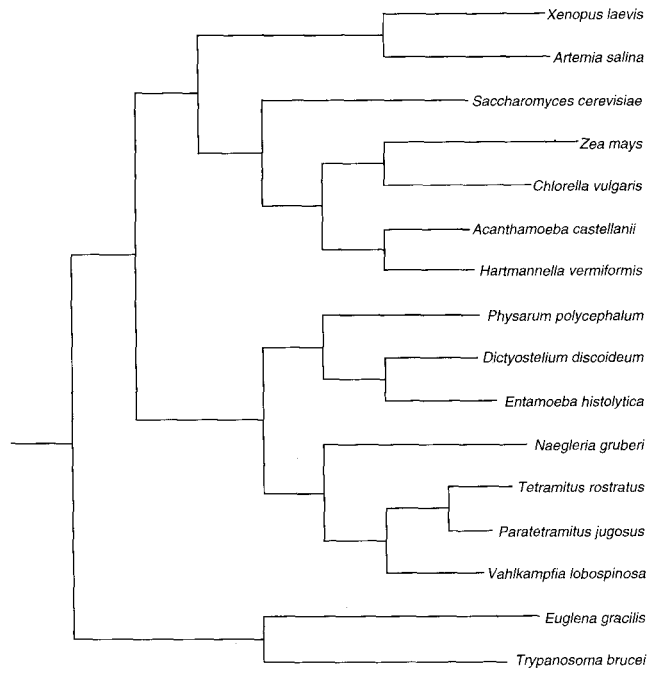

Figure 4 Evolutionary relationships inferred from data derived by diverse methodologies [12,14,39,41].

ter generally amoeboid, these organisms share many distinctive characteristics. They form cysts, have a limax morphology and undergo intranuclear mitosis [41]. It has been proposed that organisms that have 'germ-line' extrachromosal rDNA; for example *Euglena, Physarum, Dictyostelium, Entamoeba* and *Naegleria*, group together and that there is a progression from extrachromosomal rDNA, through chromosomally integrated but dispersed rDNA, to 'typical' tandemly arrayed rDNA [14]. The evolutionary scheme presented here does not subscribe to that proposal. This scheme does corroborate the opinion that the diversity of the protists is as great as that of animals, plants and fungi [68].

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