Amoebozoan Lobose Amoebae (Tubulinea, Flabellinea, and Others)

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Abstract

The Amoebozoans included here are amoeboid protists that locomote by forward flowing of the internal cytoplasm and protrusion of peripheral, fingerlike or fan-shaped pseudopodia, excluding the myxomycetes and other slime molds, and Archamoebae, which lack classical mitochondria. These lobose Amoebozoa

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J.M. Archibald et al. (eds.), *Handbook of the Protists*,
DOI 10.1007/978-3-319-32669-6_2-1

are an eclectic collection of amoeboid organisms. Some are naked without any surface covering, while other species may have a thin organic surface coat (glycocalyx) or delicate scales deposited on the outer cell membrane, with shapes that are species specific. Lobose testate amoebae are enclosed within an organic or mineralized shell (test) with an oral aperture where the tubular pseudopodia emerge. The lobose Amoebozoans consume prey (e.g., bacteria, algae, smaller protists, yeast, etc.) by phagocytosis. They are widely distributed globally in aquatic and terrestrial environments. They become dormant cysts under unfavorable conditions, such as lack of adequate food or drying of the environment, but excyst and become active when environmental conditions improve or form freeze-resistant, winter resting stages that are not encysted in some soil-dwelling amoebae in temperate regions. The Amoebozoan lobose amoebae are significant members of aquatic and terrestrial microbial communities and serve as important linkages in food webs between microbes and higher organisms, such as invertebrates. Like other Amoebozoa, the lobose amoebae typically have tubular mitochondrial cristae, which partially distinguish them from the Heterolobosean amoebae, with discoidal/flattened cristae. Molecular phylogenetic evidence indicates that Amoebozoans are monophyletic, with most, but not all, lobose amoebae falling into one of two subclades: Tubulinea (which includes the lobose testate amoebae or Arcellinida) and Discosea.

Keywords

Biogeography • Ecology • Evolution • Fine structure • Molecular phylogenetics • Naked amoebae • Protozoa • Taxonomy • Testate amoebae

Summary Classification

· **Amoebozoa**
·· **Tubulinea**
··· **Euamoebida** (e.g., *amoeba*, *cashia*, *Hartmannella*, *Saccamoeba*)
··· **Leptomyxida** (e.g., *Rhizamoeba*, *Flabellula*, *Leptomyxa*, *Paraflabellula*)
··· **Arcellinida** (e.g., *Arcella*, *Difflugia*, *Cryptodifflugia*, *Nebela*)
·· **Discosea**
··· **Flabellinia** (e.g., *Neoparamoeba*, *Paramoeba*, *Vannella*, *Vexillifera*)
··· **Himatismenida** (e.g., *Cochliopodium*, *Ovalopodium*)
··· **Stygamoebida** (e.g., *Stygamoeba*)
··· **Longamoebia** (e.g., *Acanthamoeba*, *Sappinia*, *Stenamoeba*, *Thecamoeba*)
·· **Variosea**
··· **Gracilipodida** (e.g., *Arachnula*, *Filamoeba*, *Flamella*)

[Note: Only taxa of Amoebozoa covered extensively in this chapter are listed here.]

Introduction

General Characteristics

The Amoebozoa broadly include amoeboid organisms, with or without an enclosing shell or test, that locomote largely by extension of pseudopodia and internal cytoplasmic streaming. Only the lobose amoebae with pseudopodia that are tubular and finger shaped (Figs. 1 and 2) or anteriorly broad and fan shaped, sometimes bearing extensions (e.g., subpseudopodia, Fig. 1c), are treated here. Other members of the Amoebozoa that are not (exclusively) lobose amoebae are treated in other chapters (see ▶ Archamoebae, ▶ Dictyostelia, ▶ Myxogastria, and ▶ Protosteloid Amoebozoa). In some species of lobose amoebae, locomotion is by protoplasmic streaming of the cytoplasm within the body of the amoeba that continuously propels the amoeba forward; while in others the elongated pseudopodia attach to the substrate and provide traction, drawing the body of the amoeba forward.

The lobose amoeboid protists were, until recently, included in the taxon Rhizopoda, defined originally by Von Siebold (1845) and described in the twentieth century by Levine et al. (1980) as protozoa that locomote "by lobopodia, filopodia or by protoplasmic flow without production of pseudopodia." The assemblage included the naked lobose amoebae, shell-bearing testate amoebae, Heterolobosea (amoeboid organisms with flagellated life stages), Foraminifera (with branching and anastomosing granular Rhizopoda), and other Rhizopodal amoeboid organisms (e.g., Margulis and Schwartz 1988). Prior classification schemes were based substantially on the morphology of the pseudopodia, including the Levine et al. (1980) system. However, fine structural and molecular genetic evidence confirms that these characteristics are not indicative of natural groups and in some cases are clearly a result of convergent evolution, thus leading to a substantial revision of the taxonomy based on more conservative features. Modern research has considerably refined our knowledge of the natural affinities among amoeboid organisms, and newer classifications no longer recognize Rhizopoda as a higher-level taxonomic group. The lobose amoeboid protists are currently included in the Amoebozoa (e.g., Adl et al. 2005, 2012). Additional more detailed taxonomic treatments of some of the other pseudopod-bearing organisms, based on modern revisions, are presented in other chapters of this book.

In this chapter, much of the focus will be on naked amoebae, with some attention to the lobose testate amoebae and their relatives. The naked amoebae, previously categorized as "Gymnamoebae," lack a substantial cell covering but may be enclosed by a thin or thickened organic surface coat (e.g., Page 1976, 1981, 1983, 1988), a variety of vertical, towerlike glycostyles (Page 1976, 1983), or in some cases mineral or organic scales adhering to a flexible organic matrix (Kudryavtsev 2006; Page 1983, 1988). The testate amoebae are enclosed by an aperture-bearing test or shell. The morphology of the test is species specific and its composition is of diagnostic value. In some species it is composed of organic subunits cemented together. In others it is simply an enclosing leathery coat, or a more complex matrix with mineral components embedded within it (Bovee 1985a, b; Clarke 2003; Ogden

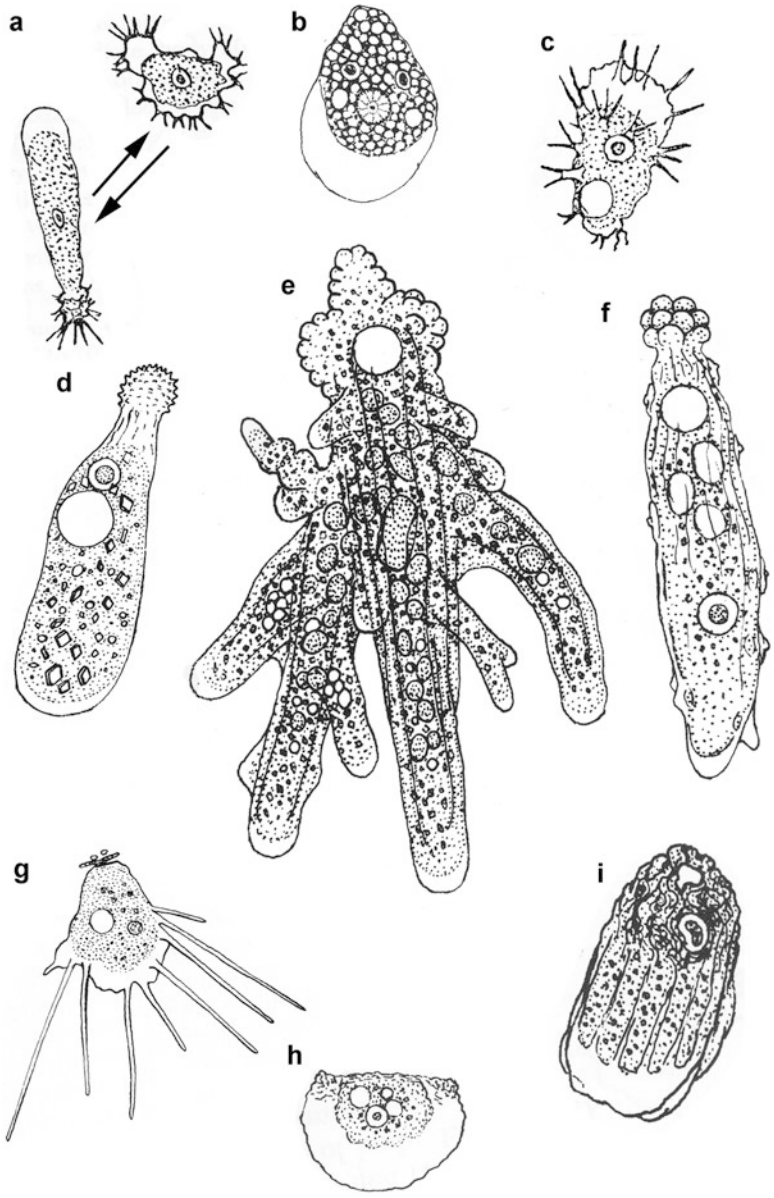

Fig. 1. Diagrammatic illustrations of some naked lobose amoebae. (**a**) *Rhizamoeba polyura*, showing an elongated motile stage and contracted stationary stage with fine lateral-radiating pseudopodia. (**b**) *Entamoeba histolytica* (actually a member of ▶ Archamoebae). (**c**) *Acanthamoeba castellanii*, bearing diagnostic bifurcated peripheral pseudopodia. (**d**) *Saccamoeba lucens*, a monopodial amoeba with prominent uroid. (**e**) *Amoeba proteus*, a polypoidal species with several lateral lobose pseudopodia. (**f**) *Mayorella limacis* in locomotion. (**g**) *Vexillifera lemani*, with characteristic of a triangular body and long tapered anterior pseudopodia. (**h**) *Vannella miroides*, a

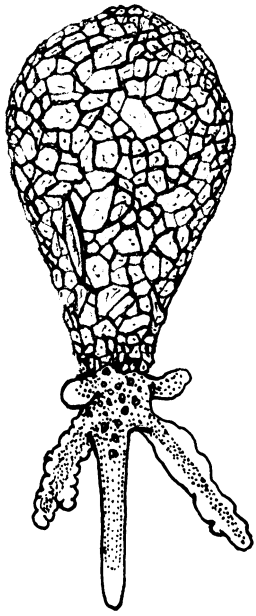

Fig. 2. Diagrams of the lobose testate amoeba *Difflugia pyriformis*, with extended pseudopodia (Adapted from Bovee (1985a) with permission (International Society of Protistologists))

and Hedley 1980). In some cases, the mineral components such as sand grains, diatom shell fragments, or other mineral particles are collected from the environment and attached to the organic matrix of the test. Typically, a single opening in the test (oral aperture) provides continuity between the internal cytoplasm and the protruding pseudopodia that extend into the surrounding environment (Fig. 2). There are two major groups: the lobose testate amoebae with lobopodia (e.g., Bovee 1985a; Smirnov 2008) and the filose testate amoebae with filopodia (e.g., Bovee 1985b). The latter are now included in the Cercozoa, within the subgroup Rhizaria, and are not considered here (see above).

Occurrence: Habitat, Distribution, and Abundance

Amoeboid protists are found in most habitats where other protists have been observed, including all major terrestrial habitats at low and high latitudes, freshwater ponds and bogs, brackish marshes and estuaries, and open ocean at near surface or great depth. Typically, distinctions are made during research between soil-dwelling, freshwater, and marine species. This distinction may be more a matter of convenience, as a way to focus and delimit a research agenda, rather than being a necessary

Fig. 1. (continued) flattened fan-shaped amoeba. (**i**) *Thecamoeba sphaeronucleolus*, exhibiting characteristic of longitudinal surface ridges. Illustrations are not to scale; some are enlarged relative to others to display significant morphological features (Adapted from Bovee (1985a) with permission (International Society of Protistologists))

restriction of habitat diversity. In general, however, there is good evidence that many marine species are stenohaline and dwell only in marine environments. Estuaries are of particular interest because the periodic tidal fluxes create markedly varied salinity gradients, thus subjecting microbiota to strong selection pressures and leading to wide salinity tolerances. Some species collected from extreme environments, including extreme cold as in arctic and Antarctic locales, often are obligate cryophiles. They exhibit rapid evidence of distress and soon die when introduced to more moderate temperatures. Some amoeboid protists are extremophiles that are found in highly polluted environments with low pH and/or high levels of potentially toxic minerals or industrial waste products (e.g., Amaral Zettler et al. 2003). Their mechanisms of survival are of increasing interest as evidence of the remarkable adaptive capacity of some protists and perhaps as guides to the properties of life-forms that may be found on other planets with more extreme environments ("exobiology").

Earlier research on amoeboid protists has provided substantial information on their habitats and distribution (e.g., Bovee 1979, 1985a; Kudo 1966; Leidy 1879; Loeblich and Tappan 1964; Page 1988). The distribution and adaptation of terrestrial naked and testate amoebae have been reviewed by Cowling (1994) and more recently for testate amoebae by Smith et al. (2008). Naked amoebae abundances, expressed as number per g soil dry weight, have been reported in the range of 10^5–2×10^6 g^{-1} for pine forest soil (Clarholm 1981), 10^2–5×10^3 g^{-1} in upland grassy plots in the USA (Anderson 2000), and with similar richness in grassland soils and the UK (Brown and Smirnov 2004). However, lower numbers (79–585 g^{-1}) were observed in sandy beach soil (Cowling 1994). Anderson (2009) reported data on the abundance of naked amoebae associated with major groups of plants, including moss (3.5×10^3–3.6×10^4 g^{-1}), ferns (2×10^3–4×10^6 g^{-1}), and seed plants (2×10^3–2×10^6 g^{-1}). He also reported similar data for testate amoebae, i.e., moss (3×10^2–6×10^3 g^{-1}), ferns (90–300 g^{-1}), and seed plants (10^4–4×10^5 g^{-1}). More recently, robust amoeba communities have been reported to be associated with terrestrial lichens (e.g., Anderson 2014), including other reports of the possible importance of testate amoebae (particularly the filose testate amoebae) in the silica biogeochemical cycle within lichen communities (Wilkinson et al. 2015). The distribution and abundance of terrestrial testate amoebae have been substantially investigated, in part because their tests persist for some time in the soil, especially in water-saturated sediments of peat bogs and marshes. Their diversity and abundance in soil strata provide evidence of their ecological and soil environmental histories (e.g., Smith and Coupe 2002). The abundance of testate amoebae of all kinds varies substantially within and across terrestrial sites but is generally in the range of 10^6–10^7 m^{-2} for forest and sphagnum-rich soils (e.g., Cowling 1994; Foissner 1987; Lousier 1982; Miesterfeld 1977).

Recently, abundances of naked amoebae in freshwater and marine habitats have been more extensively recorded, e.g., Rogerson and Laybourn Parry (1992) reported an annual mean abundance in the Clyde Estuary (Scotland) of 8300 amoebae L^{-1}. Similarly, Anderson and Rogerson (1995) examined the annual abundances of naked

amoebae in the Clyde, a more productive estuary, and the Hudson, a more turbid and less productive estuary. They found that maximum summer abundances in the Clyde were approx. 16,000 L^{-1}, while only 7000 L^{-1} were found in the Hudson. Moreover, increasing evidence indicates that naked amoebae in the Hudson may be major predators on bacteria, sometimes competing significantly with other bacteria-consuming protists in the food webs (e.g., Lesen et al. 2010). Naked amoebae can be particularly abundant in freshwater biofilms (Anderson 2013). In a freshwater pond in northern New York, biofilm amoeba densities ranged from 109 to 136 cm^{-2} biofilm surface area and 285 to 550 mg^{-1} biofilm dry weight. Sizes ranged from 13 to 200 μm. C-biomass ranged from 64 to 543 ng C cm^{-2} and 125 to 1700 μg C g^{-1} dry weight. Thirty morphospecies were identified, including very large amoebae in the range of 100–200 μm. Large amoebae (>50 μm) accounted for the largest proportion of the C-biomass.

With increasing interest in high-latitude biota, Mayes et al. (1998) examined naked amoeba abundances in the water column of two coastal sites off Eastern Antarctica. In general, numbers in the water column were highly variable (below detection to 2000 amoebae L^{-1}). There were no clear seasonal trends. Highest abundances, up to 2626 amoebae L^{-1}, were recorded at the ice-water interface. Abundance and diversity of amoebae in Alaskan tundra soils and their relationships to other terrestrial microbes in the carbon cycle and respiration of organic-rich, high-latitude soils have been reported by Anderson (e.g., Anderson 2012). Highly productive freshwater ponds support substantial numbers of naked amoebae, reaching densities close to 2000 mL^{-1} during the most productive periods in spring and early autumn when water temperatures are more favorable for growth (e.g., Anderson 1997). Organic-rich sediments are also highly favorable habitats for naked amoeba growth. The abundance and diversity have been reported in a variety of locales including brackish sediments of Niva Bay on the Baltic Sea (Smirnov 2002; Smirnov and Thar 2003, 2004) and calcareous sand sediments of coastal bays at Bermuda (Anderson 1998). Algal mats, fronds of thallose algae, and suspended floc are also favorable surfaces supporting diverse and/or abundant communities of naked amoebae (e.g., Armstrong et al. 2000; Rogerson 1991; Rogerson et al. 2003). The colony-forming cyanobacterium *Trichodesmium* supports a rich community of microbes, including naked amoebae (Anderson 1977), and as much as 50% of sampled colonies in the Sargasso Sea contained naked amoebae, among other eukaryotic microbes (Sheridan et al. 2002). Suspended floc in Antarctic lakes (e.g., Crooked Lake, Antarctica) may also support rich microbial communities that include attached amoebae and other eukaryotic microbes (Laybourn-Parry et al. 1992).

Practical Importance

Although most naked amoebae are free-living, some have also become pathogenic in humans and other primates. The amoeba *Entamoeba histolytica* (which is, phylogenetically speaking, a member of Archamoebae) invades the gut and causes amebic

dysentery (a serious diarrhea), and in more pronounced morbid pathologies, it invades other organs and can be fatal. It is estimated to infect about 50 million people worldwide. More information on the biology of *Entamoeba* is included in the treatment of ▶ Archamoebae. *Balamuthia mandrillaris*, *Acanthamoeba* sp., and *Sappinia* sp. can invade the central nervous system, causing serious amoebic encephalitis, particularly in individuals with compromised immune systems (Visvesvara et al. 2007). *Acanthamoeba* also causes a local serious infection of the eye (*Acanthamoeba* keratitis; e.g., Auran et al. 1987).

Morphology and Taxonomy

Light Microscopic and Fine Structural Morphology

The classification of naked amoebae, based on light-microscopic morphology, relies largely on their shape, size, mode of locomotion, and rate of movement (e.g., Jepps 1956; Page 1976, 1983; Patterson et al. 2002; Rogerson and Patterson 2002; Sawyer 1980; Smirnov et al. 2011). One of the defining morphological features of the Amoebozoa is their shape and pattern of locomotion. Some amoebae that are fan-shaped with a broad anterior lobe often lack subpseudopodia extending from the anterior margin and move largely by differences in cytoplasmic pressure from posterior to anterior that propels the amoeba forward by constantly expanding the anterior margin and retracting the posterior portion. Likewise, anterior extension of the lobopodia occurs through forward flow of the cytoplasm that expands the tip of the pseudopodium in the direction of motion, as the main body of the amoeba is drawn forward. In some species, the subpseudopodia extending from the anterior surface can become quite long and tapered relative to the body of the amoeba (e.g., Fig. 1g). In addition to the overall shape of the amoeba and its pattern of locomotion, other defining features include the presence or absence of a uroid. A uroid is a posterior projection of the amoeba cell and may be rather rounded, sometimes smooth, or with fine posterior cytoplasmic extensions (Fig. 1a); it may exhibit small surface pustules (Fig. 1d) or be decorated with larger surface protrusions (Fig. 1f) which can become quite elaborate in certain species (Fig. 1e). In some species the uroids are adhesive, that is, they become momentarily attached to the substratum as the amoeba moves forward.

Identification of naked amoebae based on their morphology and mode of locomotion requires expert knowledge. In some cases, the subtle distinctions among some species make clear identification difficult. Moreover, broad and overlapping variations in morphology sometimes make discrimination difficult among closely related species, especially for differences in testate amoebae based on shell morphology (Lahr and Lopes 2006). Consequently, in some cases, morphotypic categories are used, when appropriate for the research objectives. For example, Anderson and Rogerson (1995) used a typology with four types: Type 1, amoebae with lobose or filose protruding pseudopodia and/or locomotion by cytoplasmic streaming; Type 2, limax (worm-shaped) amoebae with steady, noneruptive locomotion; Type

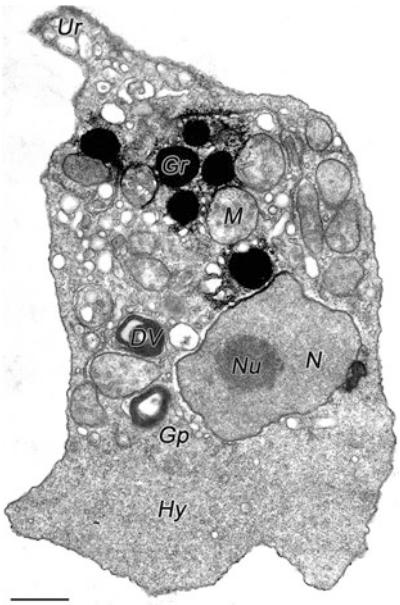

Fig. 3. Transmission electron microscopic image of a section through the nuclear region of a small naked amoeba showing the nucleus (*N*) with a denser nucleolus (*Nu*). The surrounding granular cytoplasm exhibits prominent vacuoles, including a digestive vacuole (*DV*) containing the non-digestible wall remains of a prey organism. Scale bar =1 μm

3, limax amoebae with anterolateral bulging pseudopodia and eruptive locomotion; and Type 4, discoid or fan-shaped flattened amoebae. Additionally, identified genera and species were included as subcategories of each morphotype. Subsequently, a refined typology with 16 morphotypes based on more detailed features was published by Smirnov and Brown (2004).

Fine structure features using electron microscopy (e.g., Fig. 3) are typical of eukaryotic cells, including a prominent nucleus (N, Fig. 3) surrounded by a nuclear envelope, sometimes including a denser nucleolus (Nu, Fig. 3). The cytoplasm contains membrane-enclosed organelles, including mitochondria (M, Fig. 3), digestive vacuoles (DV, Fig. 3), and a variety of smaller vacuoles. Mitochondria in most species have branched tubular internal cristae, but some variations occur, including more flattened cristae; however, the mitochondria are not enclosed by rough endoplasmic reticulum as found in most Heterolobosea. In some cases food reserves are present as electron-dense granular deposits (Gr, Fig. 3). In species with a broad anterior region, the cytoplasm is largely composed of very fine contractile filaments and is designated as hyaloplasm (Hy, Fig. 3) compared to the more granular cytoplasm (Gp, Fig. 3) that contains most of the membrane-bounded organelles. The hyaloplasm is the region of the cell that is continuously expanding as the amoebae move forward. Amoebozoa dwelling in freshwater also have contractile vacuoles that accumulate excess water from the cytoplasm and undergo rhythmic contractions to expel the water through a surface pore and into the surrounding environment.

Fine structure evidence has substantially improved our understanding of the cellular basis for making distinctions among taxa. For example, the amoeba's surface

coat, if present, has been used to distinguish among genera. A full account is beyond the scope of this chapter, but some examples are given. The surface coat of *Mayorella* spp. is a multilayered organic lamina and differentiates them from the morphologically similar *Korotnevella* (syn.: *Dactylamoeba*) spp. that possesses organic oval to "boat-shaped" complex surface scales. *Vexillifera* has distinctive hexagonal peg-like surface glycostyles, while the members of the "vannellid group" bear either pentagonal towerlike glycostyles *Vannella*) or less-prominent hexagonal prismatic projections (previously, *Platyamoeba*). Currently, however, molecular genetic evidence indicates that the fine structure of the scales is not a valid basis for distinguishing between species of *Vannella* and *Platyamoeba*. Therefore, it has been recommended that *Platyamoeba* species should be merged into the genus *Vannella* (Smirnov et al. 2007). In some cases, the surface coat is uniformly electron dense (e.g., *Thecamoeba* spp.) or thicker with chevron-like internal electron-dense structures (e.g., *Dermamoeba* spp.). The organization of the nucleus and the structure of mitochondrial cristae (tubular or flattened) are also important distinguishing characteristics (e.g., Page 1976, 1983, 1988). Internal inclusions such as crystals are also of significance, as are the arrangements of fibrillar substances and of microtubules.

The fine structure of the organic matrix and composition of surface components in the tests of testate amoebae have substantially enhanced our understanding of their morphology and systematics (e.g., Ogden and Hedley 1980), particularly in clarifying differences between surface components produced from within the cytoplasm (idiosomes) versus surface components gathered from the natural environment (xenosomes) (e.g., Anderson 1987, 1988a; Lahr and Lopes 2007; Miesterfeld 2002a, b).

Taxonomy

Modern taxonomy, based increasingly on fine structural and molecular phylogenetic evidence, is firmly rooted in the earlier systematics based largely on light microscopy. The literature base is substantial. Only some representative examples can be referenced here (e.g., Bovee 1985a, b; Cash et al. 1905/1909/1915; Chatton 1953; Deflandre 1953; De Saedeleer 1932; Page 1976, 1983; Penard 1902; Schaeffer 1926; Schaudinn 1899). Schaeffer's seminal publication (Schaeffer 1926) contains exquisite black ink-rendered illustrations produced by his own hand and, as he declared, with such attention to detail and lifelike features that they should look as though they could crawl off of the page. Our understanding of amoeboid protist systematics is still rapidly expanding, particularly with increasing insights from molecular genetics. Rogerson and Patterson (2002) identified 55 genera within 14 families in their survey of naked amoebae (Gymnamoebae). In the same publication, 71 genera of lobose testate amoebae were reported (Miesterfeld 2002a, b). In addition to those genera, further discoveries have been made including new naked amoebae:

Vermistella (Moran et al. 2007) isolated from Antarctica, morphologically similar to *Stygamoeba*, but presently not grouping with it in molecular phylogenetic analyses; *Pellita*, an amoeba with an unusual thickened surface coat (Smirnov and Kudryavtsev 2005; Kudryavtsev et al. 2014); *Squamamoeba*, a small scale-bearing species (Kudryavtsev and Pawlowski 2013); *Cunea*, with two species of small triangular marine amoebae (Kudryavtsev and Pawlowski 2015); and new species of *Cochliopodium* (e.g., Tekle et al. 2013). A novel, filose pseudopod-bearing, multinucleated amoeba (*Telaepolella tubasferens*) assigned to the Gracilipodida (Amoebozoa), a taxonomic group that also includes the genus *Flamella* (see Adl et al. 2012), has been described by Lahr et al. (2012), and more recently its molecular phylogenetic position has been further clarified (Berney et al. 2015; Kudryavtsev et al. 2009).

A number of recent publications have addressed improved classification schemes using modern evidence (e.g., Adl et al. 2005, 2012; Lee et al. 2002; Smirnov et al. 2005, 2007, 2011). The classification of Adl et al. (2005), published by the International Society of Protistologists, is used here with modifications. However, for a more detailed updated hierarchical classification, especially for higher-level groups of amoebae, see Smirnov et al. (2011) and Adl et al. (2012). Within the following text, relevant categories included in the Adl et al. (2012) classification are also cited. Only the naked amoebae (without stages producing flagella or fruiting bodies) and the lobose testate amoebae are considered here. In this scheme, the lobose naked and testate amoebae are included in the supergroup Amoebozoa (and placed in turn in the higher-order group Amorphea by Adl et al. (2012)). However, further research is needed to validate the phylogenetic validity of the supergroups (e.g., Pawlowski 2009; Yoon et al. 2008).

Taxonomic Outline

Some examples of taxa included in major subcategories of Amoebozoa within the classification scheme listed prior to the introduction are briefly described, including illustrative genera.

Tubulinea. Amoebae with tubular or finger-shaped pseudopodia. The major morphological features and some illustrative genera are presented.

Euamoebida. Naked amoebae with subcylindrical pseudopodia in locomotion (or the entire cell is monopodial and subcylindrical); without alteration of the locomotive form to a flattened expanded and branched one; no adhesive uroid. *Amoeba*, *cashia*, *Chaos*, *Deuteramoeba*, *Hartmannella*, Hydramoeba, *Saccamoeba*, and *Trichamoeba*

Leptomyxida. Naked, locomotive that forms a flattened expanded or reticulate one, becoming subcylindrically monopodial when in rapid movement or under specific conditions; adhesive uroid; uninucleate, tending to have more, or always

multinucleate in *Leptomyxa*. *Flabellula*, *Gephyramoeba*, *Leptomyxa*, *Paraflabellula*, and *Rhizamoeba*

Arcellinida. Testate amoebae with an organic or mineral extracellular test composed of either internally secreted components or mineral particles gathered from the natural environment and bounded together. Test with a single main opening. *Arcella*, *Cryptodifflugia*, *Difflugia*, *Nebela*, and *Pharynugula*

Disocosea. Flattened naked amoebae, never with tubular or subcylindrical pseudopodia and never altering the locomotive form, and cytoplasmic flow polyaxial (protruding outward around the periphery) or without a pronounced axis; subpseudopodia short or absent.

Flabellinia. Flattened, generally fan shaped, and discoid or irregularly triangular, never with pointed subpseudopodia; no centrosomes. *Korotnevella*, *Gocevia*, *Pellita Trichosphaerium*, *Paramoeba*, *Vannella*, and *Vexillifera*

Himatismenida. Dorsal surface containing a rigid coat without a defined aperture, ventral surface naked. *Cochliopodium*

Stygamoebida. Flattened, elongate amoebae resembling slender toothpicks or splinters, temporarily with a forked or branched form; extended area of anterior hyaloplasm. *Stygamoeba*

Longamoebia. Flattened and elongated amoeba with pointed subpseudopodia and cytoplasmic centrosomes in one lineage. *Acanthamoeba*, *Balamuthia*, *Dermamoeba*, *Mayorella*, *Sappinia*, *Stenamoeba*, and *Thecamoeba*

Gracilipodida. Amoebae without cilium or centrosomes; flattened, fan shaped, or irregularly branched, with short conical subpseudopodia or fine hyaline, hair-like subpseudopodia; cysts with smooth single-layered enclosing wall. *Arachnula*, *Filamoeba*, *Flamella*

Comment

There are a number of taxa previously included in earlier published treatises on the naked and testate amoebae that are not accommodated in the current classification scheme, largely due to uncertainties about their molecular phylogenetic affinities, lack of clear evidence whether they produce stages with flagella or not, and other issues pertaining to the fine structural characteristics such as the presence or absence of identifiable mitochondria versus their possibly derived organelles such as hydrogenosomes (Yarlett and Hackstein 2005). Moreover, until recently the Amoebozoa have been relatively undersampled in molecular phylogenetic studies, and with increasing attention to their phylogeny, classification systems will undoubtedly undergo significant revisions to better accommodate the emerging evidence of their natural affinities. Hence, the classification scheme outlined here will undoubtedly be modified as additional evidence is available.

Life Histories and Ecology

Most lobose Amoebozoa are free-living amoebae inhabiting aquatic and terrestrial environments. Reproduction is by mitosis. Nuclear division (karyokinesis) precedes cytoplasmic division (cytokinesis). Sexual reproduction has not been documented in naked lobose amoebae but is reported in the testate amoebae. More recently, a form of parasexual activity (cell fusion followed by nuclear fusion and subsequent cell division without a meiosis stage) has been reported in *Cochliopodium* spp. (Tekle et al. 2014). In general, the organization of the nucleus and its transformation during mitosis can be a taxonomic diagnostic feature. Vesicular nuclei have a single central nucleolus (e.g., Fig. 3) or two or more portions (often, but not always, joined) in a parietal (lateral) position. The other principal type is the ovular or granular nucleus with many nucleoli, typically but not always in a parietal layer. Intermediate conditions exist including a moderate number of rather small nucleolar bodies. Mitotic patterns include open mitosis where the nuclear membrane disintegrates during metaphase or closed mitosis where the nuclear membrane persists and may assist in the separation of the chromosomes during nuclear division.

Most of the amoeboid protists included here are exclusively heterotrophic, consuming bacteria, algae, or other small eukaryotes as prey. During ingestion, the prey is surrounded by the anterior pseudopodia and engulfed in intracytoplasmic digestive vacuoles (e.g., Fig. 3, DV). Some amoeba species contain intracellular algal symbionts (e.g., *Mayorella viridis*), but their role in host nutrition has not been established (Cann 1981). Bacterial endobionts are also present in some species, but their role also has not been described. However, an interesting example of co-adaptation has been reported in *Amoeba* by Jeon and Jeon (1976) that progressed from pathogenic bacterial infection (Jeon and Lorch 1967) to a mutually dependent status within several years, where the bacteria were required for the survival of the amoeba host. This relatively rapid evolution from a destructive to a mutually dependent relationship can be used as a model for the endosymbiotic origin of cellular organelles such as mitochondria (Margulis 1981).

Many terrestrial free-living Amoebozoan species are not obligate soil-dwelling biota and also are found in freshwater habitats. Some aquatic species are euryhaline, with a broad salinity tolerance. Others are strictly marine, or dwell in strong brackish water. Some species are cryophilic and grow only in cold temperatures, sometimes near the freezing point. Others require more moderate temperatures, and some thermophiles tolerate remarkably elevated temperatures, including those found in warm springs or shallow ponds subjected to elevated summer temperatures (e.g., Kyle and Noblet 1986, 1987). The capacity to form walled cysts, which resist desiccation, during unfavorable growth conditions (e.g., drought or insufficient food), especially for terrestrial and freshwater species, has enhanced the survival value of many amoeboid species and permitted widespread dispersal by wind or other transport mechanisms. Under favorable growth conditions, the encysted individuals excyst and emerge as actively feeding trophonts.

Earlier investigations on the habitats, feeding behavior, population growth dynamics, and life histories of pseudopodial-bearing protists (e.g., Bamforth 1985;

Bovee 1985a, b; Chatton 1953; Heal 1964; Sandon 1927) established a firm foundation for modern research on their life histories and ecology (e.g., Anderson 1988b; Fenchel 1985; Rodriguez-Zaragoza 1994; Smirnov 2008). Major advances have been made in our understanding of the significant ecological role of amoebae. Some recent representative studies on the life histories and ecology of amoeboid protists from aquatic and terrestrial environments are reviewed here within three broad ecological themes: (i) environmental variables, (ii) successions and seasonal abundances, and (iii) interactions with algae or plants, including biofilms.

Aquatic Ecology

Environmental variables. Temperature is a major variable determining the species composition and biogeographic distribution of Amoebozoa (e.g., Bonilla-Lemus et al. 2014). More generally, among other significant physicochemical variables, salinity is a major forcing function, segregating strictly freshwater amoebae (Page 1988) from marine species (Page 1983). In marine coastal marshes and estuaries, however, there are substantial populations of euryhaline amoebae (e.g., *Acanthamoeba Cochliopodium*, *Hartmannella*, *Mayorella*, *Vannella*, and *Vexillifera*) that have adapted to the diel cycles of tidal flushing where salinities may vary seasonally from 0 to 12 parts per thousand (e.g., Anderson and Rogerson 1995). The salinity tolerances of naked amoebae collected from widely different geographic sites, ranging from approximately 0 parts per thousand to 160 parts per thousand, were compared in laboratory experiments by Hauer and Rogerson (2005). Seven species were identified with remarkably wide tolerances in a range of 0 to 127 parts per thousand and six marine isolates that grew in the range of 2 to 127 parts per thousand. Further evidence of wide salinity adaptive tolerances of marine naked amoebae was reported by Cowie and Hannah (2006) who found substantial resilience to rapid salinity changes, including survival down to seven parts per thousand for the most resilient species.

Among other factors supporting naked amoeba population growth, the size, composition, and amount of suspended organic particles and floc in the water column are important variables. Naked amoebae must attach to surfaces while feeding on bacteria and other prey. Hence, suspended floc may be essential to support substantial planktonic populations of amoebae (e.g., Rogerson et al. 2003). Flocs may be "hot spots" for surface-dwelling eukaryotic microbes, especially amoebae (e.g., Anderson 2011), and represent significant centers for remineralization of nutrients through predation on bacteria (Arndt 1993; Zimmermann-Timm et al. 1998; Juhl and Anderson 2014).

Aquatic successions and seasonal abundance. The abundance of naked amoebae during seasonal successions is positively correlated with water temperature as exemplified by annual variation in abundances in some estuaries and ponds (Anderson and Rogerson 1995; Anderson 1997) with correlation values in the range of $r = 0.8$. Rivers and tidal estuaries offer unique environments to examine the effects of seasonal and tidal forcing functions on protists. Kiss et al. (2009) reported

maximum abundances of 3300 individuals L^{-1} in the Danube, particularly in April to July, with a secondary peak in October and November. Similar evidence of spring/summer and autumn blooms of naked amoebae was reported by Anderson and Rogerson (1995) for the Hudson Estuary and also in a shallow freshwater pond on the palisades above the Hudson Estuary (Anderson 1997, 2007). At maximum values, the amoeba carbon accounted for approximately 75% of the combined total carbon in the amoebae and ciliate fractions. Significant differences, however, may exist in the amoeba densities in sediments compared to the water column of some river systems, with amoebae dominating abundances in the sediment and ciliates in the water column (e.g., Gu et al. 1988). Weisse and Müller (1998), summarizing a 10-year analysis of seasonal standing stock of plankton in Lake Constance, reported that ciliates were found to be the single most important group, but naked amoebae were found in relatively high numbers and biomasses during phytoplankton peaks. A successional study of biofilms in a less-hospitable environment (the highly polluted Rio Tinto river, pH approx. 2) during 1 year by Aguilera et al. (2007) showed that amoebae and small flagellates were among the major eukaryotes after 1 month of biofilm development. Overall, the results suggest that some amoeboid eukaryotes are remarkably resilient, with potentials to adapt to highly mineral-polluted and low-pH environments.

Interactions within biofilms and with submerged phytobiota. Relatively little is known about the interactions of naked amoebae with prey bacteria in aquatic biofilms, but recent evidence suggests that naked amoebae may exert major top-down controls on biofilm bacteria (e.g., Anderson 2013; Zhang et al. 2014). Although ciliates are the most efficient predators in reducing bacterial biomass in the open water, amoebae can have a significant long-term negative effect on bacterial biomass both in the open-water phase and biofilms. Alga lamina and submerged stems and roots of plants, as well as floating colonies of algae (Anderson 1977), provide organically rich surfaces to support communities of naked amoebae, with surfaces of seaweeds supporting especially robust growth of potential bacterial prey (Armstrong et al. 2000). Additional studies of microbial populations on the surfaces of mangrove plant prop roots covered in epibiont film were reported by Maybruck and Rogerson (2004). No clearly discernible temporal pattern was detected throughout a 1-year sampling program, although naked amoebae were the second-most abundant group after flagellates. Some experimental trials comparing the growth of protozoa on tightly and loosely associated bacteria indicate that amoebae are more capable of removing tightly associated bacteria than are other micrograzers. Attached bacteria are likely to be significantly involved in the degradation of mangrove carbon; hence, predatory amoebae may serve an important ecological role in the film community.

Sediment and Soil Ecology

Environmental variables. The wide variation in the size and composition of organic and mineral particles in sediments and soils, as well as the intricate pore spaces,

produces a highly complex environment of microbial microniches, especially for amoeboid protists that typically attach to, or locomote upon, the elaborate surfaces of the solid substratum. Microniches have been characterized in sediments from Niva Bay (Baltic Sea) by Smirnov (2002) and subsequently extended to include oxygen analyses of the microenvironments within the microniches by Smirnov and Thar (2003). Naked amoebae were most abundant and diverse in the upper 1 cm of sediment. Their number and diversity decreased with increasing depth in the sediment. Species composition and abundance were highly heterogeneous, even at spatial scales of several centimeters, indicating the presence of microhabitats selectively occupied by particular suites of species. Amoebae were recovered from oxygenated upper layers as well as deeper anoxic layers. Some of the small sediment samples contained "hot spots" of amoebae biodiversity, with up to four species co-occurring in the same area. These may be loci of particularly favorable environmental growth conditions.

The distributions and biomass of amoebae and other protists in marine, brackish, and freshwater sediments were also examined by Lei et al. (2014) at 15 littoral stations across a relatively wide range of latitudes (arctic to European and North American sites). Amoeba abundance ranged from 0 to 937 cells mL^{-1} and biomass from 0 to 4.71 μg C mL^{-1}. Some of the highest naked amoebae densities were observed at marine tidal flats and contained only naked forms, whereas in a freshwater lake, only testate amoebae were observed. On an arctic ice floe, only naked amoeba forms were observed, and they contributed an average of more than 96% of the total protozoan abundance or biomass. At the other stations, both naked and testate amoebae were found.

Similar evidence of microhabitats (microbiocoenoses) in temperate forest soil has been reported by Anderson (2002). Samples of soil from four sites of varying soil porosity were analyzed in the laboratory, either unamended (NN) or amended (NE) with glucose solution to increase the organic content. Generally, the abundance of naked amoebae tended to increase with increasing soil particle size for both NN and NE treatments, possibly indicating that abundances increase with increasing porosity of the soil and the concurrent differences in physical and chemical properties that characterized the soil types. The NE cultures, moreover, showed consistently higher abundances and diversity of naked amoebae compared to the NN cultures. There also was evidence of growth "hot spots" where localized environmental conditions, such as sporadic nutrient loading or other favorable conditions, may have fostered proliferation of the amoebae.

Further evidence of the complexity of small-scale patchiness was obtained in a study carefully documenting the variation in numbers of amoeba morphotypes in small soil samples (Anderson 2003) to yield a mathematical model of biocomplexity using Euclidean spatial analysis. Three indices of amoeba abundance and distribution in the small volume samples were plotted as a three-dimensional graph: morphospecies richness (mean number of morphospecies counted in each small soil subsample), morphospecies diversity (number of morphospecies occurring in only one of the small soil subsamples but in no others), and morphospecies patchiness (the degree of aggregation or nonuniform distribution of morphospecies among

the soil subsamples). Soil samples were obtained from a freshwater bog, freshwater marsh, salt marsh, stream margin, and deciduous forest floor. Samples from the marsh rhizosphere were the most biocomplex, followed in decreasing order by the stream edge, salt marsh, and bog. Finally, forest soil samples and those from a nearby ravine were least complex.

In general, there is evidence that amoeba abundance decreases with depth in sediments (e.g., Decamp et al. 1999; Smirnov 2002), probably due to increasing anoxia and reducing conditions. However, there may be less stratification in soils, at least in the uppermost organically rich layers, especially if they are sufficiently hydrated (e.g., Bischoff 2002). Nonetheless, abundance usually declines within deeper layers of most stratified soils (Cowling 1994). Moisture content is seldom a major environmental variable in sediments, which are water-saturated in many cases. However, moisture is a major factor in determining naked amoeba abundance in terrestrial environments, as well as the proportion that is active versus encysted (e.g., Anderson 2000; Bischoff 2002).

Further evidence of the effects of soil water availability on terrestrial protists, including amoebae, was obtained by Geisen et al. (2014) using soil cores maintained under controlled environmental conditions in the laboratory. Total protist abundance differed eightfold between the two most extreme moisture treatments, and the higher number of total individuals was mainly attributed to an increased abundance of amoebae, which was 7.2-fold higher in the most moist treatment compared to the most dry. Some taxa reached highest numbers only in fully water-saturated soils and readily decreased when the habitable pore spaces became smaller, whereas other taxa were more resistant to decreasing water and only decreased at a later stage of water stress when the maximum size of water-filled pores (Pmax) was <60 μm. Overall, as the authors hypothesized, the largest protist species decreased with increasing soil dryness, but in particular nanoamoebae dominated in the dry soil, not flagellates as was initially predicted.

Successions and seasonal abundance. Abundances of terrestrial naked amoebae are typically lowest in winter and increase during early spring and summer, but precipitation and available moisture are much more significant factors than season (e.g., Anderson 2000; Bischoff 2002). Precipitation accounts for more variance in abundance than local organic content of the soil or its temperature at time of sampling, at least in a study of a temperate upland grassy site (Anderson 2000). Abundances of amoebae during mild winters with substantial precipitation may be comparable to those during warmer seasons of the year (Anderson 2000). Available water and water table depth in swamp and bogs may also be more important factors than is season in accounting for abundances of sphagnum-dwelling testate amoebae (Warner et al. 2007). Likewise, biological and microscale environmental factors may be important in explaining seasonal changes in testate amoebae, as documented above for soil-dwelling naked amoebae.

Testate amoebae serve an important role at the inception of succession on land. Wanner et al. (2008) examined the succession of testate amoebae in litter bags in four different soils that varied in nitrogen and phosphorous nutrients to document the early colonization (within less than 55 days) and establishment of testate amoebae

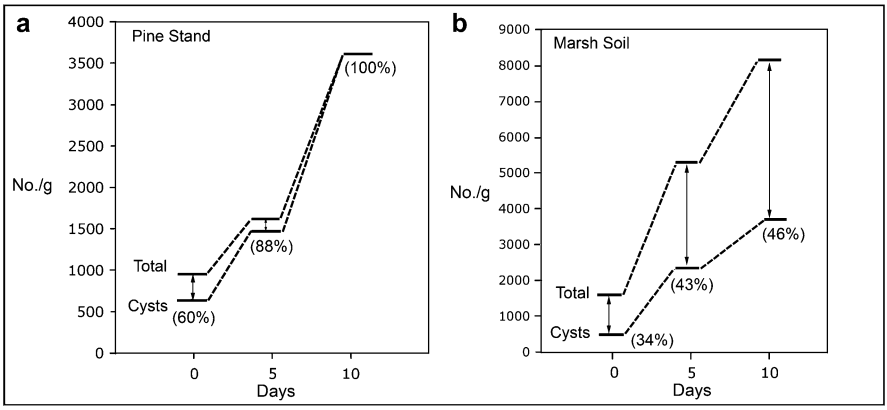

Fig. 4. Comparative plot of the densities of total amoebae (*upper graph*) and cysts (*lower graph*) including (percent encysted) at 0, 5, and 10 days in a laboratory microcosm study of a succession when winter soil was warmed to simulate spring temperatures. Plot of relative densities of total amoebae and encysted stages for a pine soil sample (**a**) and marsh soil sample (**b**) showing the gradual progression toward total encystment in the pine soil preparation and a more steady-state lesser ratio of encysted to total amoebae in the marsh soil preparation (Reproduced from Anderson (2010) with permission of the publisher (*Acta Protozoologica*))

communities. Substrates at the nutrient-poor sites were colonized more rapidly than reference sites where colonization was later and in lower densities. Both small-sized (r-strategist) and larger (K-strategist) species occurred in remarkably high densities on all sites. During later stages of colonization, the influencing environmental factors became more complex, and the composition of the testate communities changed from variability to stability.

The ability of amoebae to encyst and excyst relatively rapidly has contributed to their survival capacity, especially in temperate terrestrial environments where soils are subjected to intervals of drying and protracted periods of freezing during winter. However, the dynamics of encystment and excystment have not been extensively investigated during the development of successions following recovery from winter conditions. Anderson (2010) obtained winter soil samples from an organically rich swamp site and a less-moist mineral soil beneath a stand of pine and observed the dynamics of excystment in laboratory microcosms during warming simulating spring temperatures. The proportion of active and encysted naked amoebae was documented for 10 days during the ensuing succession (Fig. 4). The pine stand sample (ambient 18% moisture and organic content 6%) overall had lower initial total densities of naked amoebae and proliferated to lower total levels after 10 days compared to the swamp sample (ambient 47% moisture and organic content 15%). However, the proportion of encysted to total amoebae was more informative. In the drier pine stand sample, the proportion of encysted relative to total amoebae increased markedly during the 10-day rising from an initial 60% to a final 100% (Fig. 4a), whereas, the proportion of encysted to total amoebae in the swamp sample, though increasing moderately from 34% to 46% over 10 days, was much more stable

(Fig. 4b). The increase between day 5 and day 10 for the swamp sample was 43–46%, which is probably near a constant carrying-state value.

Overall, in both samples there is clearly a dynamic relationship between active and encysted stages, with evidence of substantial interconversion of active and encysted stages during the succession. More recently Anderson (2016) showed that, in addition to encysted stages, amoebae in temperate soil environments are capable of forming freeze-resistant resting stages without forming cysts. These "resting cells" are able to rapidly resume active feeding and metabolism when the soil unfreezes, either intermittently during winter or with the onset of spring, thus providing a more rapid exploitation of the environment than can be achieved if the amoebae had encysted. However, soil moisture must be sufficient at the time of freezing to support active amoebae; otherwise, they will encyst rather than forming resting stages.

In general, the typical circular cycle of alternation between encysted and active stages portrayed in textbooks, and some scientific treatises, should be expanded to include recognition of the dynamic balance in the alternation of the two stages during early succession. Moreover, further refinement is needed to include winter freeze-resistant resting stages that may provide more rapid resumed growth compared to cysts, requiring a more prolonged time for excystment in response to favorable growth conditions.

Given an increasing interest in high-latitude environments and climate change, Anderson (2008) examined the seasonal abundance of naked and testate amoebae during a succession from spring (June) to summer (August) at a tundra site (Toolik Lake, Alaska). Naked amoebae abundance (number per g of soil dry weight) increased from 2×10^4 to 3×10^4, and testate amoebae abundance increased more markedly from 1000 to 6000 during the seasonal succession. Interestingly, in terms of carbon content, the testate amoebae accounted for a larger part of the biotic carbon fraction than naked amoebae. Testate amoebae comprise a significant part of the microbial communities in moss-rich, high-latitude environments.

With evidence of global warming, what is the likely effect on these significant microbial communities? Beyens et al. (2009) examined the potential effects of global warming on the structure of testate communities by experimentally simulating a heat wave in Greenland arctic soils. Although the experimental heating of the soil was sufficiently severe to induce significant leaf mortality in the aboveground vegetation, overall there was no detectable effect on testate amoebae abundance. However, transient shifts in species populations occurred in the heated plots during the acute exposure, followed by increases in species richness weeks after the experimental heat wave had ended. Lobose pseudopod-bearing testate amoebae were more resistant to the heating and its associated desiccation than filose amoebae.

Interactions with plants. In general, substantially more information has been gathered on the interaction of testate amoebae with a wide variety of plant types, especially mosses, largely because their tests are more easily preserved and counted in samples (e.g., Cowling 1994). Naked amoebae are known to be abundant in the rhizosphere (root zone) of plants (e.g., Clarholm 1981), and there is increasing evidence that naked amoeba abundance is higher in the rhizosphere and soil beneath

plants compared to surrounding bare soil in a variety of ecosystems, including agricultural soils (Cowling 1994; Zwart et al. 1994, p. 102), arid lands (Robinson et al. 2002), and deserts (Rodriguez-Zaragoza and Mayzlish 2005; Rodriguez-Zaragoza et al. 2005). There is some variation, depending on the precipitation patterns and time of year. Within the limitations of methodological error, the ratio of protozoan biomass in the rhizosphere and to that in bulk soil is in the range of 4–6 (Zwart et al. 1994). This is attributed in part to the organic exudates released from plant roots and possibly also the higher moisture content of soil immediately surrounding the roots. The complex interactions of protists, including amoebae, with the plant rhizosphere have attracted considerable experimental research attention (e.g., Zwart et al. 1994) including a spatial analysis of the number of active and encysted amoebae in relation to the distance along the root axis (e.g., Coûteaux et al. 1988).

With evidence of increasing levels of atmospheric carbon dioxide concentrations, there has been an interest in documenting how atmospheric CO_2 affects plants, and, in turn, what effects (if any) there may be on rhizosphere microbial communities. Anderson and Griffin (2001) grew wheat plants in containers in controlled climate chambers with ambient and elevated carbon dioxide concentrations. Plant dry biomass was higher in the elevated CO_2 treatment (4.4 g/plant) compared to the ambient treatment (2.8 g/plant). The rhizosphere mean abundance of flagellates, ciliates, and amoebae, expressed as number/g dry weight, was greater in the elevated CO_2 treatment compared to the ambient treatment, with an approximate twofold difference in amoeba abundances. Comparable results using pot-grown wheat plants were reported by Rønn et al. (2003), who found that soil from pots with plants grown in elevated CO_2 had higher abundances of protozoa (especially bacterivorous amoebae) but similar abundances of bacteria. The bacteria may have been under grazing pressure by the predators, thus controlling their numbers.

The interactions of the protozoan and bacterial communities with mycorrhizal fungi in the soil may be complex (Rønn et al. 2002). In the absence of fungi, protozoan abundance was enhanced under elevated CO_2 treatments, but when fungi were present, the abundance of protozoa was reduced, possibly by adverse competitive effects of the fungi on the growth of food bacteria. Similar results were found in the natural environment for soil microbiota in grasslands exposed to elevated CO_2 (Hungate et al. 2000). Although the biomass of active fungi and flagellates increased, there was no difference in the abundance of ciliates and naked amoebae between the ambient and elevated treatments. In sum, there appear to be at least short-term effects of elevated atmospheric CO_2 concentrations on plant growth, root proliferation, and consequently increased sources of organic nutrients to support microbial communities, including an increased abundance of amoebae. However, complex interactions in the microbial communities, especially with fungi, may moderate these effects. Moreover, in some cases the relative peak in protozoan abundance during the first several weeks in the elevated CO_2 treatment was not sustained for longer time intervals. The reasons are not clear, but changes in trophodynamics, including increased top-down predation on the protozoa, may account for their decline in abundance with time.

Increasing evidence that rhizosphere eukaryotic microbes enhance plant growth has led to some interesting experimental studies to better understand the synergistic interactions. Bonkowski et al. (2001) examined the effects of amoebae on growth of Norway spruce seedlings in experimental cultivation. Spruce seedlings, cultivated with or without an ectomycorrhizal fungus, were grown for 10 months in microcosm chambers with defaunated forest soil, either supplemented with naked amoebae or without amoebae. The presence of amoebae resulted in the development of a more complex root system by increasing root length (51%), length of fine roots (64%), and number of root tips (43%). The effects of the amoebae were more pronounced in the absence of mycorrhizae. The explanation for enhanced growth of plants in the presence of protozoa is not fully determined, although the most direct effect is likely the remineralization of nutrients by predation on bacteria and perhaps by activation of bacteria that break down complex molecules into smaller, more available sources of plant nutrition.

In a more novel perspective, Bonkowski and Brandt (2002) evaluated the hypothesis that rhizosphere protozoa enhance plant growth by a grazing-induced stimulation of plant growth-promoting rhizobacteria that release plant growth substances (phytohormones). They investigated changes in root morphology of watercress seedlings and effects on the composition of the rhizosphere bacterial community, by adding *Acanthamoeba* sp. to the experimental treatments. They found that the presence of *Acanthamoeba* sp. induced changes in root morphology of watercress seedlings as soon as the root protruded from the seed, i.e., it was greater and more branched. These changes resembled hormonal effects and were accompanied by an increase in the proportion of auxin-producing rhizosphere bacteria. Evidence showed that the auxin (indole-3-acetic acid, IAA) did not originate from amoebal metabolism but resulted from changes in the composition and activity of the prokaryotic microbial community. They proposed a new mechanism based on hormonal effects of protozoa on root growth: protozoa function as "bacteria-mediated mutualists" promoting plant growth by hormonal feedback mechanisms and, as previously proposed, also due to nutrient effects based on nutrient release from grazed bacterial biomass, i.e., the microbial loop. There are undoubtedly multiple synergistic effects in the plant-protozoan association, but the preponderance of evidence, both experimental and from field studies, indicates that there is a mutual enhancing effect through the association of these two very diverse biotas.

Maintenance and Cultivation

Detailed instructions for collecting and laboratory cultivation of amoebae have been published by Page (1988). Some general information is presented here. A good source for collecting amoebae is the organic debris and decaying plant material usually present in the sediment of shallow ponds. Collect some of the debris from the pond using any convenient container such as a small plastic pitcher with a handle, or a large cup. Gently suspend the debris in the water and pour small portions into shallow culture dishes (e.g., 9 cm plastic or Pyrex Petri dishes). Keep the dishes

covered to prevent excessive evaporation and possible increase in solute and nutrient concentrations. Maintain a temperature in the range of 25°C. Avoid direct sunlight to prevent overheating. After several days, when the preparation has become more stable, add a small segment of a heat-killed wheat seed or a small rice grain to serve as a source of nutrients for food bacteria for the amoebae (wheat seeds and contaminant-free rice grains are available from organic food stores or from biological supply houses). After about 1–2 weeks, withdraw some water from the bottom of each dish and examine it with a microscope. Phase-contrast microscopes are preferred for visualization of smaller flattened amoebae. A 40x objective is usually necessary. If you find that there are sufficient amoebae to be visualized within a few milliliters of water, you can transfer aliquots into new culture dishes. Try to include some of the organic debris when making the transfer so there will be an initial source of food for the amoebae, and add a freshly prepared portion of a wheat seed, or rice grain, to the new dish. Usually, within a week to 2 weeks, you should obtain a fairly robust culture that can be maintained by periodic transfer of aliquots to new culture dishes prepared as above. Sometimes, a better yield of amoeba growth is obtained if you use a cube of nutrient-enriched agar to promote bacterial growth, instead of, or in combination with, wheat seed. Prepare the agar as follows: fully dissolve 0.1 g of malt extract and 0.1 g of yeast extract in 1 L of water from the culture site, or a good grade of noncarbonated bottled springwater may also suffice. For convenience, take a 100 mL portion and add 1.5 g of non-nutrient agar. Gently heat until the agar becomes a sol (a microwave oven is often preferable to prevent overheating the agar). Care must be taken not to allow the agar to froth and boil over. The agar sol is poured into a clean or preferably sterile Petri dish to about ¼ depth and solidified. The Petri dish can be wrapped in plastic film and kept in the refrigerator until needed. Portions about 1 cm square are cut from the solidified malt/yeast agar preparation and added to your culture dishes as a source of nutrients for food bacteria. You may increase the concentration of the malt and yeast extract twofold if you want a slightly more robust source of nutrients.

An improved yield of amoeba growth may be obtained by using one of several mineral media (Page 1988) such as modified Neff's amoeba saline. Prepare each of the following stock solutions by dissolving in 100 mL of glass-distilled water.

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

Prepare the final dilution by adding 10 mL of each stock solution to enough glass-distilled water to make 1 L. The very slightly saline solution reduces osmotic stress for some amoebae, but the culture medium must be prepared exactly as prescribed. You may be able to obtain a high quality of bottled distilled water at a local pharmacy or food store, but caution must be exercised to ensure that the water is as pure as possible.

Living amoeba cultures are available from biological supply houses and some culture collections. If you prefer to pursue your own collections, a key to success is to try collecting from a variety of sites to ensure as much diversity as possible. Avoid contaminating the cultures with toxic substances introduced in unclean containers or from impure water used to make the culture media. Persistence often leads to success if a good natural source of sample material is located. If you choose to sample brackish or marine sites, prepare your culture media using water from the source, again trying to find samples from rich organic sediments or where you see organic floc or plant debris. In general, whatever your source for samples, do not put too much debris in your culture dishes when you transfer your suspension; otherwise, an overgrowth of bacteria may make the culture medium too acidic and/or too anoxic for good amoeba growth.

Evolutionary History: In Light of Molecular Phylogenetics

The naked amoebae, without a substantial enclosing test or shell, leave no trace in the microfossil record, and therefore their evolutionary history must be inferred from other evidence, including interpretations based on comparative morphology, fine structure, life histories (e.g., Schönborn 1989; Schuster 1979, 1990), and, more recently, the significant insights obtained from molecular phylogenetics (e.g., Minge et al. 2009; Tekle et al. 2008). Testate amoeba tests are well preserved in some environments and provide a fairly robust microfossil record (Deflandre 1953). Classical evidence (e.g., Bradley 1931; Loeblich and Tappan 1964) has also been augmented by modern molecular phylogenetic analyses. Some of the most ancient microfossil specimens are from the middle Eocene epoch and are similar to extant species, including approximately 24 species (e.g., Schuster 1990). Moreover, fossils resembling the tests of lobose testate amoebae have been reported from the 740 MYA Chuar formation (Porter and Knoll 2000; Porter et al. 2003).

Although the evolutionary roots of the naked amoebae remain obscure, there is emerging strong molecular genetic evidence that they arose from flagellated ancestors (Cavalier-Smith et al. 2014, 2015; Minge et al. 2009; Paps et al. 2013), as previously inferred in earlier treatises (e.g., Bovee and Jahn 1973; Schuster 1990). Whether the naked amoebae (Amoebozoa) are monophyletic or polyphyletic has been a topic of considerable debate (e.g., Bovee and Jahn 1973; Chatton 1953; Page 1976). Currently, there is increasing evidence that the Amoebozoa are monophyletic (e.g., Cavalier-Smith et al. 2015; Lahr et al. 2011; Tekle et al. 2008). The order of evolutionary emergence of the major Amoebozoan groups is not fully resolved, but Tekle et al. (2008) position the Tubulinea at a deeper level in the phylogenetic tree than Flabellinea, and more recently, Cavalier-Smith et al. (2015) place the Discosea near the base of the Amoebozoa.

The Arcellinida (lobose testate amoebae) are grouped within the Tubulinea, with fairly good evidence of monophyly based on ribosomal RNA analyses, but not on actin analyses. The tree of Tekle et al. (2008) places *Echinamoeba* in the Tubulinea, basal to Leptomyxida, followed by Arcellinida, Hartmannellidae, Amoebidae, and

Thecamoebidae. Acanthamoebidae is basal relative to Dactylopodida and Vannellidae. However, this is a rapidly developing field, and further refinements and adjustments are to be expected. Although our knowledge of phylogeny of testate amoebae is advancing, the origin of the test during evolution remains unclear.

Acknowledgments Some of the published research by O.R.A. reviewed here was supported partially by funds from the National Science Foundation, International Polar Year (award no. 0732664). This is Lamont-Doherty Earth Observatory Contribution Number 7353.

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