Rhizopoda

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Advanced article

Article Contents

Rhizopoda are a broad group of protozoan amoeboid organisms placed in the kingdom Protista. They include the naked and testate amoebae, some members of the slime moulds and foraminifera. The latter are almost exclusively marine organisms, either benthic or planktonic. Locomotion is by extension of pseudopodia of varied morphology that are specific to the taxonomic group. Pseudopodial morphology includes broad and fan-shaped anterior extensions, finger-like tubular extensions, or a web-like network, the latter are called rhizopods. The shape of the pseudopodia, and the morphology of the enclosing shell or test, when present, are major defining taxonomic characteristics. The Rhizopoda are important aquatic and terrestrial protozoa at the base of food webs and thus provide a major link in the transfer of energy to higher order consumers. Some are capable of preying on fungi (e.g. large mycophagous amoebae) or other protists and in some cases small invertebrates (e.g. foraminifera).

Introduction

The Rhizopoda are an eclectic group of amoeboid organisms that move by cytoplasmic extensions from the cell surface known as pseudopodia. Pseudopodia vary in size and shape (Anderson, 1988a; Sleigh, 1989). Slender elongate forms are known as filopodia, whereas those that are more blunt and lobose (finger-shaped) are lobopodia as observed in the familiar *Amoeba proteus*. In some species, the pseudopodia form an interconnected, web-like network known as rhizopodia (e.g. planktonic foraminifera, **Figure 6**). However, none of these have stiffened internal arrays of microtubules as are found in the ray-like axopodia characteristic of the heliozoa and radiolaria. The form of the pseudopodia has been used in defining various taxonomic groups, although it is increasingly recognised that pseudopodial morphology is probably not a conservative feature and may have arisen in different evolutionary lineages by convergent adaptation. For example, molecular genetic evidence indicates that the radiolaria may be more closely related to some Rhizopoda than previously thought, and they are placed together with some of the members of Rhizopoda in the newer higher order group 'Rhizaria' (e.g. Adl *et al*., 2005). Among some groups, such as the testate amoebae and foraminifera (also included in the Rhizaria), the cell is enclosed by a species-specific shell or test that is secreted by the organism. The shape of the test, and its chemical composition, are important taxonomic characteristics used in many established classification systems. **See also**: Amoeba; Foraminifera; Heliozoa; Protist Systematics; Protozoan Organelles of Locomotion; Radiolaria

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Members of the Rhizopoda are widely distributed globally in terrestrial and aquatic habitats. There is increasing evidence that many species of this group serve an important role in ecosystem dynamics, especially as important food sources at the base of food webs. During feeding on smaller protists and bacteria, they also release particulate bound nutrients making them available to other organisms. This occurs partially during engulfment and degradation of the food, and subsequently in expelled waste matter. For example, terrestrial amoebae are increasingly recognised as numerically important microbiota inhabiting the rhizosphere (zone immediately adjacent to the roots) where they can increase soil fertility by remineralisation of major inorganic nutrients required by plants. The foraminifera, characterised by calcareous, porous shells, are significant members of marine ecosystems. The benthic species found in the sediments or attached to surfaces of plants in salt marshes are abundant members of coastal communities. Their shells accumulate in the sediments and over time form a fossil record that can be used to interpret the climatic and ecological conditions in that region. Planktonic foraminifera with spiral calcitic shells occur widely in the open ocean. Their shells also accumulate in the marine sediments and are used extensively by micropalaeontologists to reconstruct palaeoclimates and the palaeoecology of oceans. The succession of different shells of species with increasing depth in the sediments forms an excellent microfossil record that can be used in conjunction with other evidence to deduce the evolutionary pathways of the foraminifera. **See also**: Marine Communities; Palaeoclimatology; Palaeoecology; Protozoan Diversity and Biogeography; Rhizosphere; Soils and Decompositiont

Overall Taxonomic Scheme

A variety of classification schemes exist for the Rhizopoda, and increasing evidence from molecular genetic analyses has led to some major revisions in our thinking about how to classify the Rhizopoda. In the system of Levine *et al*. (1980), they included the Rhizopoda in a Superclass (Rhizopodea) within a subphylum Sarcodina that included all pseudopod-bearing protozoa. This system in general is also used by the *Zoological Record* (*ZR*), a standard taxonomic reference source. The ZR hierarchical taxonomic scheme is as follows: **See also**: Protozoan Taxonomy and Systematics

- Sarcomastigophora (phylum): amoeboid and flagellated protozoa.
  - Sarcodina (subphylum): amoeboid protozoa.
    - Rhizopoda (superclass): pseudopod-bearing protozoa.
      - Lobosea (class): lobate-pseudopod-bearing protozoa.
        - Gymnamoebia (subclass): naked amoebae, lacking a test.
          - Amoebida (order): typically uninucleate and possessing mitochondria.
          - Pelobiontida (order): one or two nuclei, living in anaerobic environments.
          - Schizopyrenida (order): heterolobsean amoebae with eruptive locomotion.
        - Testacealobosia (subclass): amoebae enclosed in a test.
          - Arcellinida (order): with lobose pseudopodia.
          - Himatismenida (order): with external scale-covered flexible test.
          - Trichosida (order): with external, flexible, spicule-containing test.
      - Acarpomyxea (class): much-branched forms, plasmodial or uninucleate.
      - Acrasea (class): slime moulds with limax-shaped amoeboid stages.
      - Eumycetozoea (class): true slime moulds with plasmodial stages.
        - Protosteliia (subclass): with long-stalked, single spore-containing sporecase.
        - Dictyosteliia (subclass): amoebae fuse into a motile slug that makes sporocarps.
        - Myxogastria (subclass): plasmodial, producing stalked fruiting bodies with spores.
      - Plasmodiophorea (class): minute plasmodial intracellular parasites.
      - Filosea (class): amoeboid protists with slender, long-tapered filopodia.
          - Aconchulinida (order): siliceous, scale-bearing testate amoebae.
          - Gromiida (order): with chitinous test varying from ovoidal to spherical.
      - Granuloreticulosea (class): amoeboid protists with granular, reticulate rhizopods.
          - Athalamida (order): lacking a test.
          - Foraminiferida (order): with calcareous, typically multichambered, porous test.
          - Monothalamida (order): single enclosing chamber or test and oral aperture.
      - Xenophyophorea (class): very large plasmodial-like, multinucleated marine protists.

The Sarcomastigophora includes the subphylum Mastigophora, encompassing the photosynthetic and nonphotosynthetic flagellates, and the subphylum Opalinata, a group of flagellated protists commonly found in the intestines of aquatic vertebrates such as fishes and amphibians. The third major subphylum, Sarcodina, are the 'amoeboid organisms' including the major group Rhizopoda.

Based on modern evidence, including electron microscopic fine structural features and molecular genetic data, recent changes have been recommended for the higher order classification categories (e.g. Adl *et al*., 2005). Other sources offer perspectives on interim categorisation schemes that help us put the emerging evidence into at least a reasonable approximation to a more natural system (e.g. Cavalier-Smith, 1998; Lee *et al*., 2000; Smirnov *et al*., 2005). Taxonomy, as with other fields of scientific inquiry, is a dynamic and evolving science. We are currently in an exciting time of gathering new and substantial evidence on the natural affinities of protists based on molecular evidence combined with established techniques of light and electron microscopy. Therefore, current taxonomic schemes can only be considered as works in progress, but they promise to bring much clearer resolution to solving the natural phylogenetic relationships among living organisms. A perspective, published by the International Society of Protistologists (Adl *et al*., 2005), illustrates current thought on the higher level relationships of some Rhizopoda and their relatives. Two major groups are included here: Amoebozoa (Shadwick *et al*., 2009) and Rhizaria (e.g. Nikolaev *et al*., 2004; Pawlowski and Burki, 2009).

AMOEBOZOA

Tubulinea Smirnov *et al*., 2005

Naked or testate amoeboid organisms, tubular, subcylindrical pseudopodia, or transforming from flattened and expanded to subcylindrical; monoaxial flow of cytoplasm in entire cell or within each pseudopodium; without centrosomes.

Flabellinea Smirnov *et al*., 2005

Flattened locomotive amoebae, without tubular subcylindrical pseudopodia; motile form is constant, not altered; polyaxial cytoplasmic flow, or without pronounced axis; without centrosomes.

Acanthamoebidae Sawyer and Griffin, 1975

Subpseudopodia prominent, flexible, and tapering to a fine or blunt tip; uninucleate; nonadhesive posterior uroid; glycocalyx extremely thin; cysts of most species double-walled, with operculate pores; centriole-like body present.

Entamoebida Cavalier-Smith, 1993

Pseudopodia clear, eruptive arising from hyaline ectoplasm and a granular or clear endoplasm; mitosis closed with endonuclear centrosome and spindle; mitochondria, peroxisomes and hydrogenosomes absent; reduced Golgi dictyosome.

RHIZARIA

Silicofilosea Adl *et al*., 2005 [Imbricatea Cavalier-Smith and Chao, 2003]

Testate amoebae with filose pseudopodia; internally secreted siliceous surface scales; mitochondria with tubular cristae.

Foraminifera d'Orbigny, 1826

Calcareous, porous-shelled protists with granular, reticulated pseudopodia.

Radiolaria Müller, 1858, emend. Adl *et al*., 2005

Siliceous skeletal protists with stiffened axopodia.

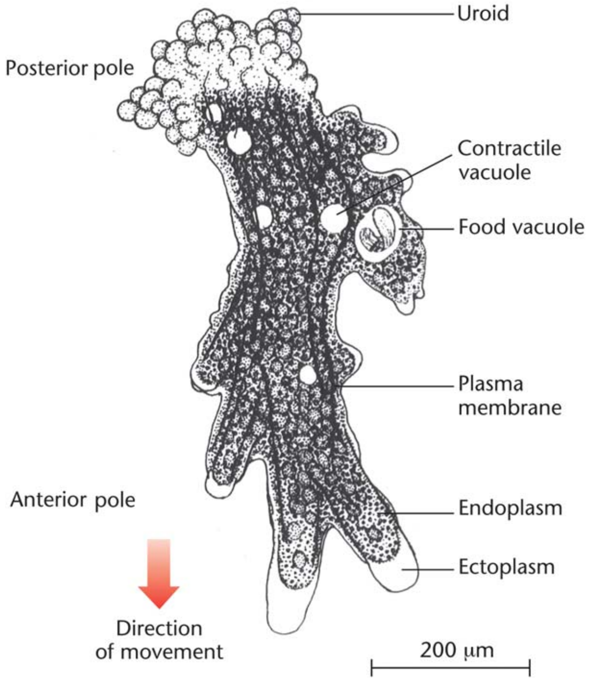

Figure 1 A lobose gymnamoeba (*Chaos carolinense*). With permission from Lee *et al.* (1985).

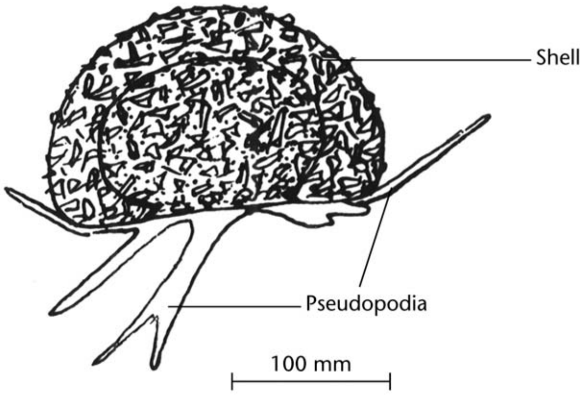

Figure 2 A lobose testate amoeba (*Phryganella nidulus*) showing the granular test enclosing the cell and emerging finger-shaped pseudopodia. With permission from Lee *et al.* (1985).

Major Taxonomic Groups

Lobose amoebae

This includes the so-called naked amoebae (**Figure 1**) (gymnamoebae) without a shell, but often with a thin organic surface coat or layer of scales, and the testate amoebae with lobose pseudopodia (**Figure 2**), enclosed within an organic or mineralised shell (Page, 1988). The naked amoebae are characterised by the familiar *A. proteus* often used in the classroom to exemplify the amoebae broadly. However, *A. proteus* is not so commonly encountered in samples from the natural environment. In general, the naked amoebae inhabit the surfaces of particles in soils and sediments or of suspended flocculent matter in the water column. They all move by pseudopodial formation. Some have rather blunt pseudopodia (e.g. *Amoeba* and *Mayorella*), whereas others have long, tapered pseudopodia as in *Vexillifera*. Other major groups are either discoid or fan-shaped (e.g. *Vannella*) and some are limax amoebae with a tubular or worm-like shape. The form of locomotion and rate of movement are often of taxonomic importance. Some of the limax amoebae move by a rather steady forward creeping motion including the genus *Hartmannella* and *Saccamoeba*. The amoebae with eruptive locomotion (e.g. *Naegleria* and *Vahlkampfia*) are characterised by genera that produce flagellated stages in their life cycle. They are classified in the Heterolobosea and current evidence indicates that they are not amoebae, but flagellates with an amoeboid life stage. Therefore, they have been moved into their own taxonomic group separate from the Rhizopoda. However, mention will be made of them when it contributes to clarifying some taxonomic or evolutionary relationships.

Naked amoebae are typically abundant protists in aquatic and terrestrial environments where they are significant members of the microbial community, preying on bacteria, small algae, or other microbes. Hence, they are important links in the food web transferring energy and matter to higher level consumers. They also contribute to the fertility of soils by releasing nutrients during predation on prey organisms. They are one of the important members of the so-called microbial loop, whereby prey-contained nutrients, including mineral and organic nutrients, are released during predation and assimilated by primary producers. The nutrients support photosynthesis and growth of primary producers, thus increasing the available sources of organic nutrients to sustain microbial food webs, and completing the loop. At least one amoeba (*Mayorella viridis*) is a host for symbiotic green algae. With proper illumination, the amoeba can be maintained in culture with only a mineral medium (Page, 1988, p. 79). The photosynthetic symbionts provide sufficient nutrition to sustain growth and reproduction of the amoeba. **See also**: Amoeba

The testate amoebae with lobose pseudopodia are included in the Testacealobosia (Ogden and Hedley, 1980). All possess some form of external test or shell whose shape and chemical composition are important taxonomic characteristics. In some species such as *Arcella*, the test is composed of numerous organic subunits secreted by the amoeba and organised into a somewhat flattened discoid shell. The ventral aperture is rounded. In other genera, the organic test is augmented by mineral matter, either secreted from the cytoplasm of the organism or gathered as mineral particles from the environment and organised in species-specific patterns on the surface of the test. For example, some species of *Difflugia*, with globose to elongated shells, selectively gather mineral particles from the environment by collecting them with their pseudopodia. The gathered particles are brought to the test by pseudopodial streaming and cemented into place on the surface. *Netzelia*, however, secretes siliceous particles within vacuoles in the cytoplasm, expels them on the cell surface, and cements them together to form a protective test (e.g. Anderson, 1988b). A terminal, rounded to stellate aperture permits extension of pseudopodia for locomotion and feeding. Other genera, such as *Quadrulella*, secrete small quadrangular, siliceous plates that form the vase-shaped test. *Lesquereusia* secretes vermiform or sigmoidal rodlets of silica that are arranged decoratively on the surface of the organic test.

Filose amoebae

Testate amoebae with thin, filose pseudopodia (**Figure 3**) are placed in the class Filosea (Ogden and Hedley, 1980). *Euglypha*, for example, has a vase-shaped test covered with imbricated (shingle-like) thin, oval, siliceous often spiny plates secreted by the organism. The hyaline, finely tapered pseudopodia emerge from a terminal ovate aperture surrounded by specialised, toothed scales. Other genera dwelling in beach sands, such as *Microamphora*, *Amphorellopsis*, *Chardezia* and *Psammonobioticus*, have elaborate tests whose terminal apertures bear outwardly flared rims. This may help secure them in the sand.

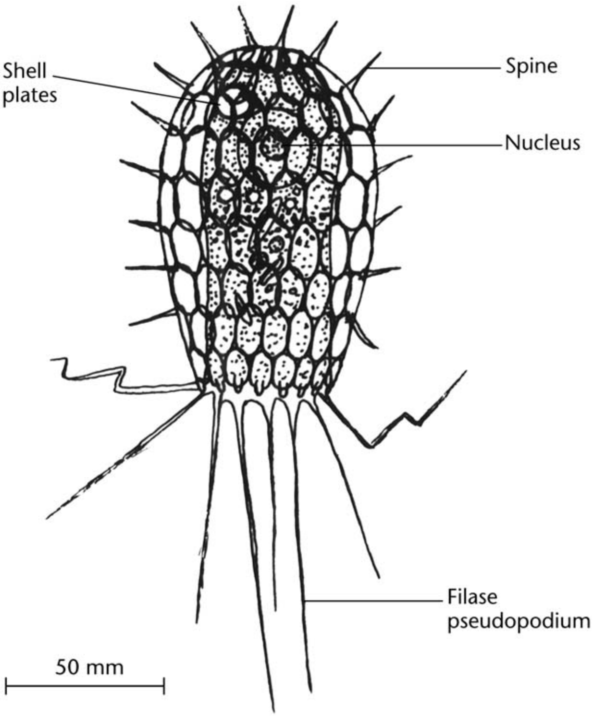

Figure 3 Euglyphid testate amoeba (*Euglypha ciliata*) with filose pseudopodia emerging from a spinose test composed of imbricated siliceous scales. With permission from Lee *et al.* (1985).

Acarpomyxea, Acrasea, Eumycetozoea and Plasmodiophorea ('slime moulds' and related genera)

The Acarpomyxea are a heterogeneous group of much-branched plasmodial forms that are multinucleate, or uninucleate, and dwell in freshwater, marine or terrestrial habitats (Margulis *et al.*, 1990). A prominent example is *Stereomyxa ramosa* (**Figure 4**), a uninucleate, cruciform cell with much-branched peripheral pseudopodia arising from the cell body that is covered with debris collected from the environment. Their position is uncertain, but they are sometimes grouped near the Acrasea and Eumycetozoea, two classes that encompass the so-called slime moulds included in the Amoebozoa of some modern classifications (e.g. Shadwick *et al.*, 2009). The Acrasea are cellular slime moulds (feeding stage is a limax-shaped amoeba known as myxamoebae). They are probably a polyphyletic group characterised by amoeboid stages that aggregate to form spore-containing sporocarps. Sporocarps are multicellular aerial stalked structures also known as fruiting bodies. Taxonomic characteristics of the Acrasea include pattern

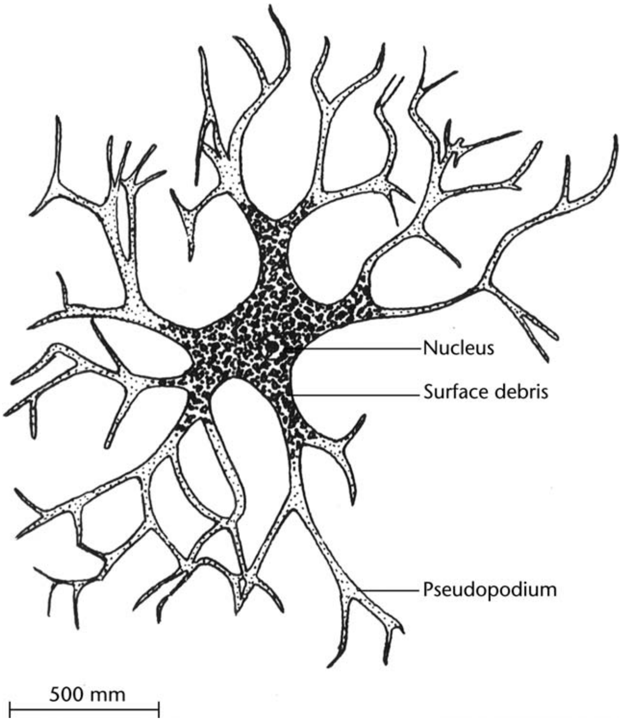

Figure 4 *Stereomyxa ramosa*, a species in the class Acarpomyxea showing the branching plasmodium and bifurcated terminal pseudopodia. With permission from Lee *et al.* (1985).

of sporogenesis, type of sporocarp, presence or absence of mastigote (flagellated) cells, and the type of mitochondrial cristae. The cristae are the internal membranes (whether tubular or plate-like) of the mitochondrion. *Acrasis rosea* exemplifies the Acrasids. It is characterised by a pink to orange tint of the cytoplasm of amoeboid stages and the delicate pink, branched, arborescent sporocarps produced by the aggregated amoebae at maturation. The Eumycetozoea, also known as the 'true slime moulds', encompass spore-forming slime moulds that have uninucleate to multinucleate amoeboid stages that sporulate either by: (1) differentiation of single amoeboid cells into simple stalked sporocarps (Protosteliia), (2) aggregation of individual amoebae to form multicellular pseudoplasmodia that produce stalked multispored sporocarps (Dictyosteliia), or (3) development of many spored sporangia from a multinucleate, often sexually derived, diploid plasmodium usually of relatively large, sometimes several centimetres, size (Myxogastria). The slime moulds are commonly found in moist terrestrial environments where the grazing amoebae consume bacteria, yeasts, spores of moulds and other small microbiota. The beautiful, golden-yellow, large plasmodium of *Physarum polycephalum* is often used in the classroom to illustrate the morphology and life habits of the Myxogastria. The Plasmodiophorea are all obligate, spore-forming, parasites living within the cells of plants, typically seed plants. Small plasmodia form within the host cells and give rise to resting spores. Each spore upon germination produces a biflagellated zoospore that disperses and infects the host tissue, completing the life cycle. **See also**: Slime Moulds

Granuloreticulate Rhizopods (foraminifera and related organisms)

These are organisms with anastomosing or net-like pseudopodia (**Figure 5**) with small intracellular granules moving along the pseudopodia, hence the class name 'Granuloreticulosea' (Lee *et al*., 1985; Lee and Anderson, 1991). There are three main groups: (1) Athalamida, without a test, (2) Foraminiferida, enclosed within single to multichambered tests, often composed of calcium carbonate and (3) Monothalamida, with single-chambered, organic or calcareous tests, commonly with attached debris, and lacking alternation of generations. The Athalamida include *Arachnula*, with branched, not anastomosed, pseudopodia, and *Biomyxa* and *Gymnophrys*, with branched, anastomosing pseudopodia. The foraminifera are a large and elegant group of marine Rhizopoda. Some form organic tests either unadorned or coated with mineral grains gathered from the environment. Others have elaborate multichambered, uniserial or biserial conical shells formed of calcite. Others have flattened or conical spiral shells of varied design. Members of the suborder Miliolina have tests resembling fired porcelain, including the genera *Spiroloculina* and *Quinqueloculina*. Others in the suborder Rotalina have glassy shells including the genera *Ammonia*, *Elphidium* and *Heterostegina*. The benthic species are typically nonspinose, whereas some planktonic (floating) species bear long spines anchored in the surface of the shells (Hemleben *et al*., 1989). The spines of planktonic species (e.g. **Figure 6**) support external cytoplasm and provide anchorage when capturing large prey such as copepods, crustacean larvae, and other invertebrate prey. Spinose species include *Globigerina*, *Orbulina*, with a porous spherical final chamber at maturity, and *Hastigerina*, one

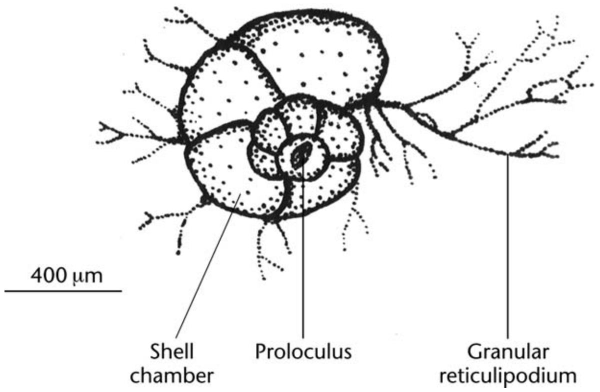

Figure 5 A general diagram of a benthic foraminifer showing the multichambered spiral shell with a small initial chamber (proloculus) at the centre and peripheral halo of granular rhizopodia. With permission from Lee *et al*. (1985).

Figure 6 A living planktonic foraminifer showing the translucent calcitic shell and radiating spines covered by, and supporting, a network of rhizopodia bearing yellow-green algal symbionts.

of the largest planktonic foraminifera with diameters in the millimetre range. Nonspinose planktonic foraminifera are widely distributed with some species preferring warmer and others colder water masses. They include the genera *Globoquadrina*, *Globorotalia* and *Pulleniatina*. Some benthic and planktonic foraminifera contain algal symbionts, which supply photosynthetically derived organic nutrients to the host. Among the benthic species, alternating generations consist of a gamont phase that produces gametes and an agamont phase that reproduces asexually. There are many variations on the following plan, which is offered as a general example. The multiple gametes released from a gamont fuse in pairs to form a zygote that gives rise to an agamont phase. After repeated asexual division, the agamonts eventually undergo multiple fission of the cytoplasm to produce numerous individuals of the gamont phase, thus completing the life cycle. Foraminifera are abundant and widely distributed. For example, the pink sand along the coasts of Bermuda is composed of the pigmented shells of dead foraminifera. The massive carbonate rocks used by the Egyptians to build the pyramids contain fossil remains of exceptionally large calcareous foraminifera. Sediments from productive salt marshes worldwide are likely to contain the elegant shells of benthic foraminifera. Open ocean planktonic foraminifera are a major sink (second to coccolithophore flagellates) for atmospheric carbon dioxide (CO_2) that is absorbed in the seawater and accumulated by the foraminiferan as calcium carbonate to build its calcareous shell. The shells sink to the ocean floor and become buried, some converted to calcareous rocks by geological processes, and thus help to sequester the atmospheric CO_2 and reduce the concentrations of this greenhouse gas. However, the large amounts of anthropogenic CO_2 being released into the atmosphere form carbonic acid when absorbed by the seawater and threaten to lower the pH of the ocean seawater to such an extent that the planktonic foraminiferans are unable to secrete their calcareous shells. **See also**: Foraminifera

Class Xenophyophorea

The Xenophyophorea are large (multimillimetre to centimetre size) sediment-dwelling Rhizopoda found in the deep sea (Lee *et al*., 1985). They are cosmopolitan in distribution. Xenophyophores are plasmodial and enclosed by a branching tube system made of a transparent, cement-like organic material. The tests of xenophyophores consist of aggregates of foreign matter including foram shells, radiolarian skeletons, sponge spicules and mineral grains cemented together within an organic matrix. The test of most species is lumpy in appearance and ranges in size from a few millimetres to several centimetres. They are often overlooked in sediment samples since their tests may resemble masses of detrital matter. Care is needed in collecting the sediment to preserve these delicate organisms. They have been overlooked in the past as nonliving debris or have been so badly destroyed during collection of the sediment that they were undetectable.

Reproduction

Asexual and sexual reproduction occur among species in the Rhizopoda. The naked amoebae reproduce asexually by binary fission. The cell rounds up or becomes discoid and divides mitotically to yield typically two identical daughter cells. The two cells are connected at first by a cytoplasmic bridge, but soon separate, assume the characteristic species shape and migrate away from each other. Sexual reproduction has not been convincingly observed. Testate amoebae reproduce asexually by binary fission. However, the process is somewhat more complex than in the naked amoebae (gymnamoebae) since a new shell must be produced to enclose the daughter cell. The parent cell produces a bulge of cytoplasm that is extruded from its aperture. This bulge forms a mould-like surface whereupon a new shell is deposited, the two shells being connected at their apertures. The cytoplasmic bulge is withdrawn into the parent shell in some species before mitosis but not in others. Subsequently, the nucleus divides mitotically. Eventually, the cell undergoes binary fission by migration of one nucleus and a portion of the cytoplasm into the new shell. This is followed by separation of the two daughter cells. Thus, two identical daughter cells with the characteristic species-specific shell shape are produced during each division cycle. The slime moulds reproduce asexually and sexually at different stages in their life cycle and there is a great deal of diversity among various taxonomic groups. For example, *P. polycephalum* produces haploid spores in sporangia that develop from the multinucleated amoeboid plasmodium during one phase of the life cycle. These are released and scattered by the wind. They settle on moist substrata and give rise to flagellated haploid swarmers that act as gametes. Fusion of two swarmers produces a diploid multinucleated amoeboid plasmodium that can reproduce asexually by fragmentation. Alternatively, during unfavourable growth conditions, the plasmodium produces a sclerotium (dehydrated, crust-like resting stage) that can fragment. Each fragment, containing cysts, produces a new plasmodium when environmental conditions are favourable for resumption of growth (e.g. Anderson, 1992). The plasmodium eventually produces sporangia with haploid spores, thus completing the life cycle. The foraminifera have some of the most elaborate reproductive cycles among the rhizopoda. There is considerable variability in life cycles among the species but a general description follows. During one phase of growth, the parent cell reproduces asexually by multiple fission. That is, the nucleus undergoes multiple divisions followed by fragmentation of the cytoplasm into many individual nucleated cell masses, each of which produces a new shell characteristic of the species (for example, **Figure 5**). In some species, this is followed by a sexual reproductive phase. Each parent foraminiferan produces haploid gametes, which in some species are flagellated, or in others amoeboid. The gametes fuse to form a diploid zygote that secretes a new shell and enlarges to form a parent organism thus completing the life cycle. In multichambered spiral-shelled species, the first formed chamber or proloculus is small and rounded (**Figure 5**) followed by secretion of successively larger chambers during maturation. **See also**: Binary Fission in Bacteria; Protozoan Asexuality; Protozoan Sexuality

Phylogenetic Evolutionary Considerations

Given the breadth of different groups of protists included in the Rhizopoda, it is difficult at present to make broad generalisations about their phylogenetic relationships and evolutionary history. It is generally recognised that the Rhizopoda are not monophyletic and include highly diverse groups that have evolved along many different lines (Cavalier-Smith and Chao, 1996; Hinkle and Sogin, 1993; Brown and De Jonckheere, 1994). The presence of an amoeboid habit undoubtedly represents convergent evolution and is no longer considered to be a conservative feature. However, current evidence indicates that the amoeboid protists evolved from ancestral flagellates. These flagellates initially comprised two groups the unikonts (with a single flagellum) and the bikonts (with two flagella). Amoeboid protists appear to have arisen from the unikonts – their flagellated stages, when present, bear one flagellum (e.g. Minge *et al*., 2009), whereas members of the Rhizaria may have branched from the bikonts (Burki and Pawlowski, 2006). However, there are alternative hypotheses for the origin of the eukaryotes indicating that a first evolutionary split may lie between photosynthetic and nonphotosynthetic forms (e.g. Rogozin *et al*., 2009). The Evidence for phylogenetic relationships among protozoa in general is derived from three sources: (1) microfossils for species that produce mineralised tests or skeletons, (2) electron microscopic, fine structural evidence of cytoplasmic organisation in living species and (3) molecular genetic analyses to determine likely phylogenetic affinities based on protein, deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) analyses (Patterson, 1994). Foraminifera and testate amoebae have excellent microfossil records. The naked amoebae and most ‘slime moulds’, however, leave no trace in the fossil record and therefore evidence for their phylogenetic relationships comes from fine structural and molecular genetic analyses (e.g. Fiore-Donno *et al*., 2010). Although it is not possible to set forth a grand scheme for the evolution of the Rhizopoda, there is some evidence for evolutionary and phylogenetic relationships within subgroups. Evidence is presented for the amoebae and foraminifera, which have been the subject of more intensive investigation. **See also**: Protozoan Evolution and Phylogeny

Naked and testate amoebae

There is increasing evidence that many of the classical taxonomic subgroups of the amoebae are artificial and that polyphyletic pathways are more common than previously assumed in some classification schemes, although experts in amoeba taxonomy have cautioned for some time that naked amoebae are probably polyphyletic, based on fine structural evidence and more refined light microscopic observations (e.g. Page, 1988). For example, molecular genetic analyses have confirmed that the subclass Gymnoamoebia is probably polyphyletic since the discoidal species *Vannella anglica*, for example, does not occur on the same branch of a taxonomic tree as two other widely occurring species in this subclass (*Acanthamoeba castellanii* and *Hartmannella vermiformis*) (Sims *et al*., 1999). Furthermore, small-subunit ribosomal RNA analyses (SS-rRNA) indicate that *Acanthamoeba*, *Dictyostelium* and *Naegleria* are sufficiently divergent that there is no evidence that they are monophyletic (Baverstock *et al*., 1989). Even among the heterolobosean limax amoebae with eruptive locomotion placed in the family Vahlkampfiidae in current taxonomic schemes, there is increasing evidence that they are polyphyletic. This family is characterised by members with flagellated stages (with the exception of those in the genus *Vahlkampfia*) and presumably may have been among the earliest amoeboid protists derived from a flagellated ancestor. *Paratetrimitus*, *Tetramitus* and *Vahlkampfia* appear to be closer relatives of each other than any of the three are to *Naegleria*, a widely studied genus occurring in soils and including a human pathogen (*Naegleria fowleri*). The depth of the evolutionary split in the branching pathway between *Naegleria* and the other three vahlkampfiids is sufficiently large to suggest a relatively ancient divergence. This line may have diverged as early as the divergence of plants and animals at about one billion years ago (Hinkle and Sogin, 1993).

The genus *Entamoeba*, which includes the human intestinal pathogen *Entamoeba histolytica*, has no mitochondria and forms multinuclear cysts in contrast to the others in this subclass that have uninuclear cysts. However, molecular genetic analyses of species within the genus *Entamoeba* support a monophyletic relationship of this group, and it is included in the Amoebozoa in some modern classifications (Adl *et al*., 2005). *Entamoeba* species producing cysts that have the same number of nuclei appear to form monophyletic groups. The most basal or earliest evolved *Entamoeba* species are those that produce cysts with eight nuclei. The most derived (evolutionary advanced) are those with four-nucleated cysts (Silberman *et al*., 1999). **See also**: *Entamoeba* and *Entamoeba histolytica*; Protozoan Cysts and Spores

Molecular genetic analyses of testate amoebae further indicate the polyphyletic quality of the amoeboid organisms. For example, gene sequence analyses show that the testate amoebae *Euglypha rotunda* and *Paulinella chromatophora* with filose pseudopodia share a monophyletic origin and are not closely related to any of the lobose amoebae analysed (Cavalier-Smith and Chao, 1996). Although much additional research remains to be done, there is strong evidence that the euglyphid testate amoebae, at least, evolved from a sarcomonad flagellate ancestor by the loss of flagella and that they are possibly closely related to the amoeboflagellate chlorarachnean algae. Moreover, certain members of the sarcomonads such as the thaumatomonad flagellates are coated with siliceous scales secreted from within the cell. They feed by branched pseudopodia emanating from a ventral groove and exhibit extensive amoeboid stages. The euglyphid testate amoebae are also covered by overlapping siliceous scales and feed by extruding filose pseudopodia through the terminal aperture of the shell. This further augments the conclusion that the euglyphid testate amoebae may have arisen from a thaumatomonad flagellate ancestor.

Foraminifera

Molecular genetic analyses of some benthic foraminifera indicate that they may be among the earliest known eukaryotes possessing mitochondria with tubular cristae and may have emerged much earlier than other amoeboid protists including the vahlkampfiids and some mycetozoan slime moulds such as *Physarum* (Pawlowski *et al*., 1996). Based on microfossil sedimentary analyses, it is generally assumed that the calcareous shelled species evolved from nonshelled species that build tests of agglutinated particles gathered from the environment. The oldest fossil evidence of foraminifera with agglutinated walls date from the early Cambrian, about 560 Ma, and may have evolved from earlier ancestors with simple organic tests as occur in living genera such as *Allogromia* (Tappan and Loeblich, 1988). Fossil evidence for evolutionary relationships is based on changes in morphology from more simple to more complex shells, growth patterns as observed in fossil shells and living representatives of major groups, apertural (major pore) characters of the shell, and pattern of occurrence over geological time as evidenced in sedimentary layers. It appears that two lines of evolution diverged from the Allogromiina with simple organic shells. One line diverged toward the agglutinated (granular) walled species, which also contain a certain amount of calcite deposition. This line eventually could have given rise to the more advanced forms with aragonite and calcite walls. The other major line that diverged from Allogromiina may have been by way of the Fusulinina with microgranular calcitic walls. This group may have given rise to several branches of evolutionary development including the Miliolina, which form shells with small calcite crystals that are arranged to give a porcellaneous appearance in reflected light. Although the fossil evidence suggests that the calcitic species emerged from groups with organic walls, molecular evidence points to a possible early emergence of some calcareous wall producing genera and this line of inquiry may be very productive in resolving the various lines of evolutionary descent among the highly diverse species of foraminifera.

Indeed, agglutinated and calcareous forms may have evolved independently along separate lines from a common ancestor. Although much progress has been made in elucidating the overall lines of evolutionary descent among the foraminifera, details of the origins of genera and species remains to be worked out. Palaeontological evidence combined with molecular genetic data and electron microscopic observations are likely to yield substantial evidence from a multidisciplinary perspective to help resolve the complexity of evolutionary pathways among these highly diverse and morphologically elaborate protists. **See also**: Cambrian Radiation; Fossils and Fossilization; Fossils in Phylogenetic Reconstruction; Mitochondria: Origin

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