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Major Features of Protistan Evolution: Controversies, Problems and a Few Answers

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Abstract

The major features of protist evolution are fraught with controversies, problems and few answers, especially in early Earth history. In general they are based on molecular data and fossil evidence that respectively provide a scaffold and details of eukaryotic phylogenetic and ecologic histories. 1. Their origin, inferred from molecular sequences, occurred very early (>3Ga). They are a chimera of different symbiont-derived organelles, including possibly the nucleus. 2. The initial diversification of eukaryotes may have occurred early in geologic time. Six supergroups exist today, each with fossils known from the Proterozoic and Phanerozoic. 3. Sex, considered an important development, may have been inherited from bacteria. 4. Precambrian protists were largely pelagic cyst-bearing taxa, but benthic forms were probably quite diverse and abundant. 5. Protists gave rise to animals long before 600 Ma through the choanoflagellates, for which no fossil record exists. 6. Acritarchs and skeletonized protists radiated in the Cambrian (544-530 my). From then on, they radiated and became extinct at all the major events recorded in the metazoan fossil record. 7. Protists dominated major environments (shelves and reefs) starting with a significant radiation in the Ordovician, followed by extinctions and other radiations until most died out at the end of the Permian. 8. In the Mesozoic, new planktic protozoa and algae appeared and radiated in pelagic environments. 9. Modern protists are important at all trophic levels in the oceans and a huge number terrestrial, parasitic and symbiotic protists must have existed for much of geologic time as well. 10. The future of protists is likely in jeopardy, just like most reefal, benthic, and planktic metazoans. An urgent need to understand the role of protists in modern threatened oceans should be addressed soon.

Keywords: Eukaryotes; protists; evolution; geologic record

1 Introduction

Protists are single-celled eukaryotes of wide genetic, phylogenetic, morphologic, ecologic, and functional variety (Cavalier-Smith, 2004, 2005; Patterson, 1999). Indeed hundreds of thousands of described species and probably millions of unknown or poorly-known species of protists exist today as free-living, parasitic and symbiotic forms in all environments from the deepest sea to the highest mountains, from deserts to reefs, and in and on almost every kind of organism (Patterson, 1999). Here the term is a convenient way to refer to the single-celled groups, including those with primary endosymbiotic chloroplasts, and not to a phylogenetically coherent group, yet they are solidly embedded within the eukaryotes. The terminology of eukaryotes can be confusing; as used in this paper; terms are defined in Table 1.

Term	Simplified Definition
Microorganisms	Single-celled prokaryotes and/or eukaryotes
Prokaryotes	Single-celled organism without nuclei
Eukaryote	Complex cell(s) with a nucleus
Protists	Single-celled eukaryotes
Protozoa	Non-photosynthetic protists
Algae	Photosynthetic protists
Fungi	Multicellular eukaryote*
Animal	Multicellular eukaryote*
Plant	Multicellular eukaryote*

Table 1 Definitions of terms eukaryote and related terms applied in this review. Asterisk indicates one of 7 groups of multicellular eukaryotes; the four others are included in several groups of protists.

Commonly considered “simple organisms” for a long time (Darwin, 1866), most protists are actually incredibly complex (Figure 1), accomplishing with a single cell most of the functions that animals or higher plants do with many cells and tissues. This diversity and complexity attest to a long and varied evolutionary history that has been ignored until recently by evolutionary biologists. Indeed, some workers have purposefully excluded them by repeating another fallacy: they are asexual and don’t obey the usual rules of evolution based on sexually reproducing organisms especially animals and higher plants. However, that is an error, and most protists include sexual reproduction in aspects of their life cycles that may be closely coordinated with environmental variables (see Erskian & Lipps, 1987, for a foraminiferal example). While their evolutionary rules may vary somewhat, they nevertheless show remarkable historical patterns preserved in the fossil record that commonly mirror those in metazoans (to compare foraminiferal

diversity to metazoan diversity, see Sepkoski, 1993), indicating strong environmental responses. Fossil protists therefore offer substantial tests of evolutionary hypotheses and environmental conditions through time due to their abundance and continuity through the geologic record, especially in the late Neoproterozoic and Phanerozoic. In fact, their record is superlative compared with the prokaryotic or multicellular organisms.



Figure 1 A complex living protist, the larger foraminiferan *Alveolinella quoyii*, embodying a CaCO_3 shell, endosymbiotic algal protists (diatoms in color), granuloreticulopods arrayed in the direction of locomotion, as well as many internal organelles each with complex functions (Lipps & Severin, 1986). Specimen is about 1.2 cm long.

Their evolution however makes sense only within the history of eukaryotes as a whole. The first eukaryote would likely be recognized as a protist with most of the organelles in one form or another that modern protists carry, and the obvious multicellular organisms (particularly animals, plants and fungi) had ancestral protists at the base of their clades.

For many protists, no fossil record exists at all, yet like metazoans and higher plants, a sufficient number of them left fossils so that we may broadly know their history (Figure 2). Although they are mostly microscopic, many manufacture characteristic biomolecules, shells or other cell coverings (Table 2) that preserve well and abundantly in the geologic record. This record begins over 2.7 Ga when the first possible eukaryote biomarkers are recorded (Brocks *et al.*, 2003) and continues with increasing fidelity right up to the present, at

least for the marine forms. A huge number of terrestrial, aquatic, parasitic and symbiotic protists exist today and must themselves have long evolutionary records as well, but those are not revealed by fossils.

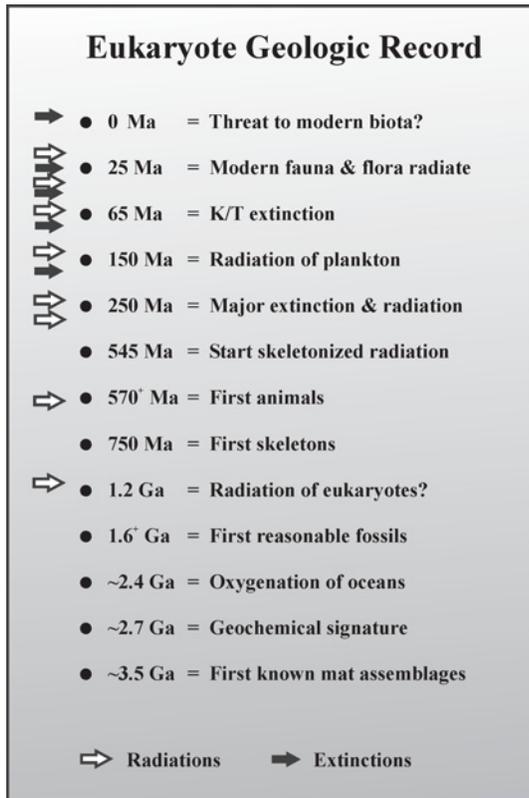


Figure 2 Major events in eukaryote history.

Protists have always important in marine communities, and indeed for a large part of the first 3.5 billion years of the geologic record, they were common in at least some benthic and pelagic ecosystems (Knoll, 2003; Lipps, 1993). Later when larger animals and plants appeared, they still remained important in ecologic interactions.

A good part of the evidence for all eukaryotes comes from the history that molecular phylogenetics reveals and, together with the fossil record, provides a framework for their evolution. Much of this remains controversial and problematic, so it is in need of enhancement, especially for the early parts of their history. Molecular data provide a useful scaffold on which to place the major features of eukaryote evolution while fossils provide confirmation and

<i>Prokaryotes & Eukaryotes with a Fossil Record</i>	<i>Habitat</i>	<i>Function</i>	<i>Age Range</i>	<i>Earliest Fossil Evidence</i>
Heterotrophic bacteria	All	Consumers	Archean-Recent	Stromatolites ~3.5 Ga*
Autotrophic bacteria, especially cyanobacteria	All	Primary producers	Archean to Recent	Stromatolites, mats ~3.5? Ga*
Acritarchs	Chiefly onshore/offshore pelagic ecosystems.	Primary Producers	MesoProterozoic-Recent	Cysts. ~1.6Ga
Dinoflagellates	Oceanic, nearshore, lacustrine	Primary Producers	PaleoProterozoic?-Recent	Biomarker: Steranes ~2.7Ga*
Coccolithophorids and related calcareous algae	Oceanic, eutrophic to oligotrophic	Primary Producers	Triassic-Recent	CaCO ₃ platelets. 210 Ma*
Silicoflagellates	Oceanic.	Primary Producers; secondary consumers?	Cretaceous-Recent	SiO ₂ H ₂ O rods. 115 Ma*
Diatoms	Oceanic, chiefly eutrophic; lacustrine	Primary Producers	Cretaceous-Recent	SiO ₂ H ₂ O valves. 115 Ma*
Foraminifera	Benthic. Pelagic.	Herbivorous, omnivorous, carnivorous	Cambrian-Recent. Jurassic-Recent for pelagic forms.	Agglutinated tests 545 Ma*
Radiolarians	Oceanic	Herbivorous, carnivorous	Cambrian-Recent	Siliceous skeletons 540 Ma*
Thecamoebians	Terrestrial, aquatic	Herbivorous, carnivorous	Neoproterozoic-Recent	Tests ~750 Ma
Tintinnids	Neritic, oceanic	Herbivorous, carnivorous	Ordovician-Recent	Theca in thin sections ~ 480 Ma
Animals	All environments	Herbivorous, carnivorous, parasitic	Ediacaran-Recent	Traces and body fossils. >580 Ma*
Charophytes	Chiefly freshwater, brackish	Primary Producers	Silurian-Recent	Chiefly the CaCO ₃ gyrogonites. ~444 Ma
Higher Plants	Terrestrial, shallow marine	Primary Producers	Silurian-Recent	Traces, body and parts fossils. 415 Ma

Table 2. Fossilized prokaryotes and eukaryotes, their nature, ecology, age range, and first appearances in the fossil record.

*see discussion in text. Modified from Lipps (1993).

the details of their phylogenetic and ecologic histories. Molecular data cannot answer questions about extinct lineages, which abound in the fossil record. Paleontology and molecular biology go together to clarify the entire history of a group. Much more data and information is required for a better understanding of eukaryote evolution in general.

The purpose of this paper is to provide an overview of the major events characterizing early protist evolution that can be built upon by future workers. It is an overview because so much of it is controversial and problematic for a variety of molecular and taphonomic reasons, and most answers remain tentative.

2 Origin of Eukaryotes

Origins are always fascinating but commonly difficult to understand. None is more so than for eukaryotes that emerged long ago, before skeletons were acquired, in environmental situations that were imperfectly or not preserved, and in a milieu of evolving prokaryotes. Two lines of evidence bear on the question of eukaryotic origins: molecular data from modern taxa and inferences from the geologic and fossil record. Neither line of evidence provides compelling support for how or when eukaryotes first appeared. As a result, multiple competing hypotheses have been suggested but none is completely accepted.

Eukaryotes may have arisen from a melding of prokaryotic cells at some time after those two domains were established (Baldauf *et al.*, 2000; Knoll, 1992). Such models derive from the simpler structure of prokaryotes and the rooting position of the eukaryotes in certain molecular phylogenetic models. At least a dozen hypotheses have been suggested to account for the origin of eukaryotes in this way (Cavalier-Smith, 2002; Doolittle, 1999; Markov & Kulikov, 2005), most based on how various organelles were developed or acquired within a basic eukaryote cell. All of these remain debatable and controversial. More recent data from both genomics and proteomics indicate that eukaryotes have been independent of the other two domains of life, the Bacteria and Archaea, since their emergence as coherent groups (Kurland; Collins & Penny, 2006). In this model, each domain originated independently from within an ancestral community of earlier cells of various types that interacted ecologically and exchanged genetic materials (Figures 3, 4). When this occurred is, of course, unknown but some molecular sequence data suggest (Gu, 1997) that it occurred very early (>3Ga). Possibly the three domains emerged from this milieu of cells simultaneously or perhaps at different times. We have no way of knowing at present.

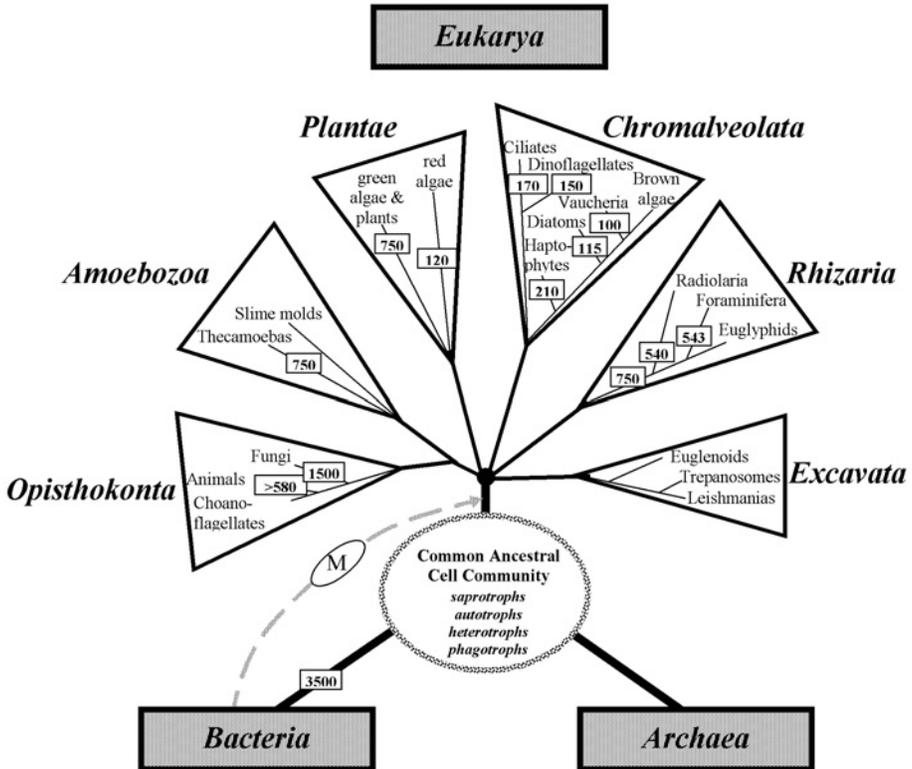


Figure 3 A simplified phylogeny of eukaryotes, showing their relationship with the Bacteria and Archaea through an ancestral mix of cells of different sorts (Kurland *et al.*, 2004) to the incorporation of the mitochondria to the six main monophyletic supergroups of eukaryotes (Simpson & Roger, 2004). Although the constituents of each supergroup will change, these groups currently appear to be monophyletic. Only the fossilizable groups within each supergroup are indicated (with thin lines) and dates of the first reliable fossil record compiled from the literature are indicated in the light grey boxes M and the arrowed dashed line indicate the early endosymbiotic acquisition of mitochondria from the Bacteria.

Modern eukaryotes are a chimera of different symbiont-derived organelles, including the mitochondria, plastids and possibly the nucleus, that were acquired at different times. The nucleus, if it was an early symbiont acquisition, and the mitochondria were incorporated into the cell by an early group not represented among the major modern groups of eukaryotes. Plastids were incorporated several times including possibly as late as the Mesozoic.

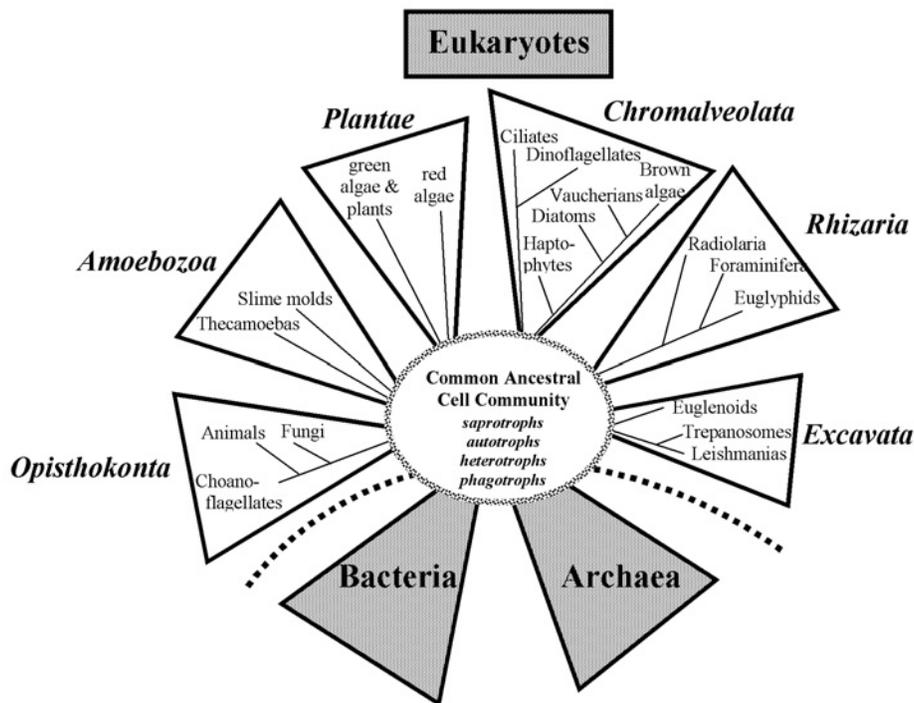


Figure 4 The “Big Bang” of life. In this view of life’s origins, all domains and the supergroups of Eukarya (above the dashed line) arose from a mix of cells of various trophic styles which were readily exchanging genes with one another. This view comes from the idea that the long stems on each domain and supergroup in the usual molecular phylogenetic diagrams are artifacts of long-branch attractions and other problems (Roger, 2006). Those long branches have been collapsed back to the common cell community in this diagram.

Does the geologic history of Earth provide constraints on the time of origin? The earliest time on Earth (prior to 3.8 Ga) can be inferred only from zircon grains incorporated into later-formed rocks and the records of extraterrestrial bombardment of nearby planetary bodies. The zircons yielded ages ranging from 4.3-4.4 Ga and they indicate the presence someplace on earth of granitic rock and surface water (Kramers, 2001) indicating oceans may have been present. Based on crater counts on nearby bodies in the Solar System, Earth was subjected to intense bombardment by bolides in its early history. The impacts were sufficient to impede the accumulation of water or oceans and the sustained development of life (Nisbet & Sleep, 2001). After a few hundred million years or so, the bombardment slowed and decreased in intensity as the bolides became smaller and fewer. These comets, asteroids,

and meteorites also brought to Earth organic material sufficient for life to start (Anders, 1989; Chyba *et al.*, 1990). The organic molecules provided the raw materials of life itself, and could have been utilized as food and nutrients for the new cellular organisms. Life had what was needed to form and survive very early in Earth's history; unfortunately, rocks old enough ($> \sim 4.0$ Ga) to preserve evidence of any life, if it were present then, are unknown.

Early Archean oceans and life are essentially unknown (Knoll, 2003), as those rocks constitute a small percentage of exposures on Earth today. While supposed bacterial fossils were described from 3.5 Ga rocks (Schopf, 1993; Schopf & Packer, 1987), they have been reinterpreted as non-biogenic kerogen artifacts (Brasier *et al.*, 2006; Brasier *et al.*, 2002). Nevertheless, the presence of life is indicated by isotopic evidence (Rosing, 1999) and by other fossil occurrences at about the same time or slightly later (see Schopf, 2006, for a summary).

Archean oceans contained little oxygen and abundant iron (Anbar & Knoll, 2002; Holland, 2006). Organisms present in those oceans were bacteria, probably Archaea and even early eukaryotes (Brocks *et al.*, 1999; Summons *et al.*, 1999), that likely inhabited very shallow benthic and open water habitats (Brocks *et al.*, 2003). Some molecular phylogenies suggest an early divergence of eukaryotes (Gu, 1997; Hedges *et al.*, 2001), leading by definition to ancestral protists, but this is an indirect conclusion subject to alternative interpretations (Katz, 1999). A benthic biota may have been confined to films and mats primarily composed of aggregations of bacteria, some of which formed stromatolites and laminated sedimentary rocks (Brocks *et al.*, 2003; Nisbet *et al.*, 2001; Simonson & Carney, 1999). In these mats and films, early eukaryotes may well have lived, but no evidence supports this idea. Eukaryotes that we know today require molecular oxygen, and the geologic record indicates that free oxygen was not yet present in the atmosphere and oceans (Holland, 2006). However, the steranes and biomarkers for oxygenic photosynthesis in late Archean rocks indicates that oxygen was present at least locally and perhaps generally in mat communities sufficient to support eukaryotes (Brocks *et al.*, 2003). Thus, arguments that eukaryotes could not have appeared before the oceans became oxygenated are problematic, indicating also that eukaryote origins may extend back even before the earliest known fossil mats and stromatolites (see Schopf, 2006). While this suggestion fits well with the hypothesis that all three domains arose independently (see Section 3) from a preexisting cell community (Figure 4), no evidence supports it either. The origin of eukaryotes and its timing thus remains unknown, and all possibilities must remain open pending further study.

3 The Diversification of Eukaryotes

The diversification of eukaryotes has been assumed to be significantly later in time than their origin based on molecular phylogenetics which shows a “crown” group of diverse forms and a long stem group leading to it (see Figure 3). Six supergroups, each with fossils known chiefly from the Phanerozoic, are recognized molecularly as part of this “crown”, while stem groups remain hypothetical. But the concept of “crown” and “stem” groups has also been seriously questioned because long-branch attraction in the molecular data could produce this kind of artifact (Roger & Hug, 2006). The alternative is that the diversification of eukaryotes into the supergroups was closely synchronous with eukaryote origins and that it occurred quickly in geologic time as a sort of “big bang” of evolution (see Figure 4; Cavalier-Smith, 2002; Dacks *et al.*, 2002; Roger *et al.*, 2006). Under this model, eukaryotes and prokaryotes are related through common ancestral unicells, but did not arise from each other. Each domain has been independent since their emergence. The supergroups of eukaryotes may well have arisen in this milieu of cells too, although later diversification within the supergroups clearly took place, and perhaps even some supergroups diverged from others (for example opisthokonts and amoebozoans) later in time. Whatever may have occurred, no fossil evidence suggests that the supergroups are a late development.

The six supergroups of eukaryotes have been named variously. Here I used a simplified nomenclature (Roger *et al.*, 2006; Simpson & Roger, 2004). A new higher level classification of eukaryotes and protists assembled by a large group of systematists using structural and molecular data recognizes these same six groups (Adl *et al.*, 2005). These groups seem to be more or less monophyletic, as far as recent research suggests, but the huge number of undescribed and unstudied smaller eukaryotes may change this view later. The molecular data has clarified the position of multicellular organisms among the eukaryotes. Each of these, whether they be animals, higher plants, fungi or one of the other smaller groups, are thoroughly embedded within different branches of single-celled eukaryotes. The traditional designation of Kingdoms for these and for all single-celled protozoa and algae is clearly incorrect, and should be abandoned completely, as the new classification has done (Adl *et al.*, 2005).

The supergroups include the Excavata, Rhizaria, Chromalveolata, Plantae, Amoebozoa, and Opisthokonta. These supergroups are well supported by multigene molecular sequencing. The time of diversification cannot be closely estimated by either molecular or paleontologic studies (see section 5) although much speculation has occurred. The best-known species assigned to the

Excavata are heterotrophic flagellates that may cause significant disease in humans (*Giardia* for example), although many forms are free-living as well (the euglenids, for example). Most have modified or no mitochondria, leading to the former belief that they were the most primitive and earliest protists. The **Rhizaria** include the fossilizable radiolarians with siliceous skeletons and the foraminifera usually with tests of various compositions. A large subgroup, the Cercozoa, contains flagellates and amoebae that have no skeletal materials and no fossil record, except for the euglyphids known from rocks ~750 Ma (Porter & Knoll, 2000; Porter; Meisterfeld & Knoll, 2003). **Chromalveolata** is a large group including those photosynthetic kinds that have undergone a secondary endosymbiosis with a red algal symbiont and many non-photosynthetic forms, of which ciliates are the most important. One group of ciliates, the tintinnids, has thecae that left a fossil record since the Ordovician. The algae with a secondary endosymbiosis include the fossilizable dinoflagellates, haptophytes (coccolithophorids), silicoflagellates, diatoms, and the generally-fossilizable large brown kelps. These algae are responsible for much of the primary production in the modern oceans. **Plantae** are not just higher plants but include all those photosynthetic forms that rely on a plastid derived from a primary endosymbiosis with a cyanobacterium. The supergroup includes higher plants, which left a fine fossil record, and green algae. The **Amoebozoa** contains those protists that have lobopodia, or wide pseudopodia, that capture and engulf their food. They are fairly common but poorly understood in marine ecosystems where they, along with foraminifera and ciliates, prey on a wide variety of other organisms including some relatively larger metazoans. A few amoebozoans are parasitic forms and spore-forming slime moulds. Some of the amoebae are thecate and have a fossil record extending back ~750 Ma (Porter *et al.*, 2000; Porter *et al.*, 2003), but the rest of the amoebozoans left no record. Of particular interest are the **Opisthokonta**, a supergroup that includes the animals and fungi. Animals share a common ancestor with the protistan choanoflagellates (King, 2005), also included in the supergroup.

4 The Origin of Sex

Sex, considered an important event, may not have originated with eukaryotes at all, but been inherited from bacteria. The definition of sex is often unclear. Here I mean that sex involves the reassortment of parental alleles at various loci (Roughgarden, 1991). All eukaryote lineages display sex in many of their members and some bacteria reproduce sexually as well, indicating that sex may be an early development before the emergence of the supergroups. All of these, of course, could be due to convergence on life history strategies selected for genetic exchanges, although the simplest answer is common inheritance. In

any case, it is an early development as far as can be inferred. Sex is often thought to confer positive selection on species by preserving genetic variability in the face of changing environments (Roughgarden, 1991; Williams, 1975). In single-celled eukaryotes and prokaryotes, sex enters into a variety of life history strategies. Forms may be diplont, haplont or haplodiplont, with sex dominating in the first, occurring occasionally in the second, and alternating although not necessarily regularly in the third. Hence sexual reproduction may alternate with many asexual generations, but this too is a way of conserving variability especially among populations in unfavorable environments where they preserve genetic variability, while asexual ones build their populations in benign or favorable environments. Sex and asexual modes are both under selection but for different reasons, resulting in a complex life cycle closely coordinated with changes in their environments (see Erskian and Lipps, 1987, for an example among the foraminifera), both expected and unexpected. Such systems should be selected commonly in Earth's seasonal and occasionally unexpected environmental changes.

5 Precambrian Fossilized Protists

Eukaryote history is clouded by a lack of a good preservation potential, inadequate preservation of enough environments, too few samples, and uncertain systematic assignments. In deeper time, conclusions are extremely difficult to draw, but the record improves somewhat into the Proterozoic starting about 1.8 Ga (Knoll *et al.*, 2006). Fossils occur sparsely but their affinities are commonly clouded as well. Most are probably eukaryotes but which supergroup, if any, they belong to is problematic (Knoll *et al.*, 2006). To date, samples from the Paleoproterozoic and Mesoproterozoic yield a maximum of about 10 taxa/sample or assemblage, increasing somewhat in the Mid Neoproterozoic to Early Ediacaran to 20 to 60 taxa/sample or assemblage. After the high of 60 in the Early Ediacaran, the preserved record falls to fewer than 10 again in the latest Early Ediacaran through to the end of the Period. The taxa undergo changes in the mid-Mesoproterozoic and Late Neoproterozoic, suggesting evolutionary changes (see Knoll *et al.*, 2006, for a complete discussion of these data and interpretations). Most of these taxa are assigned to the acritarchs, a diverse group of probable protists that inhabited both pelagic and benthic environments. Some might even be large prokaryotes. Rare filamentous (cellular ones included) and testate microfossils, assignable to various eukaryotes, occur from 1.8 to about .55 Ga, but megascopic eukaryotes (larger algae) appear a little earlier than .60 Ga. Whether or not these records come close to the actual biotic diversity of the times and thus presents a realistic view remains questionable. If the record is representative, then very low diversity protistan assemblages, compared to Phanerozoic times, occurred throughout the entire

Proterozoic, but they record evolutionary change in the broadest sense. Protists became more diversified, occupied more habitats, and developed morphologic variety including tests. Surely, just as today, a huge host of other protistan taxa lived during the Proterozoic but did not fossilize or were not in preserved environments. This suggests that in spite of a very general impression of evolutionary developments though this long interval, sampling and taphonomy remain critical problems for understanding the early evolution of eukaryotes. Windows into this potential diversity could be discovered at any time, and might change this general impression.

The acritarchs include cysts of pelagic phytoplankton, probably even at their first appearance at about 1.8 Ga. Benthic assemblages are also known (Knoll *et al.*, 2006), and together these suggest that all parts of the shallow marine environment were likely inhabited by complex assemblages that would have included unknown taxa that are not preserved. These ecosystems appear to be dominated by primary producers that may have fueled more trophic interactions by heterotrophic protists that could not be preserved (for example, naked amoeboid or ciliated types). Indeed, biomarkers for ciliates and other protists are reported from Proterozoic rocks (Summons & Walter, 1990) and, for eukaryotes in general, even earlier in the late Archean (Brocks *et al.*, 2003) nearly half a billion years before they appear as fossils. Thus, there are reasons to be wary of the fossil record of Proterozoic protists, and caution is necessary in evaluating its significance. Much diversification took place in the Proterozoic, although the timing and nature of it remain unclear.

6 Protists Gave Rise to Animals

The choanoflagellates, a flagellated protist group for which no fossil record exists (King, 2005), has long been thought to be related to sponges and the rest of the metazoans. While this relationship in general had been surmised by invertebrate morphologists a century ago, modern molecular phylogenetics has confirmed and clarified the relationship (Figure 5; King, 2004b; Medina *et al.*, 2003). Choanoflagellates or their ancestors acquired genes that allow the cohesion of cells, essential for multicellularity, first as colonies then as more complex sponges (King, 2004a, b). From these protistan ancestors, the multicellular animals, fungi and colonial choanoflagellates arose. The choanoflagellates, fungi, animals and a few small groups are placed by molecular phylogenetics in the supergroup Opisthokonta (Medina *et al.*, 2003). In this hypothesis, the choanoflagellates and mycetozoans share a common protistan ancestor with the sponges and all the other animals. Fungi and some other groups are sister groups to them (Figure 5).

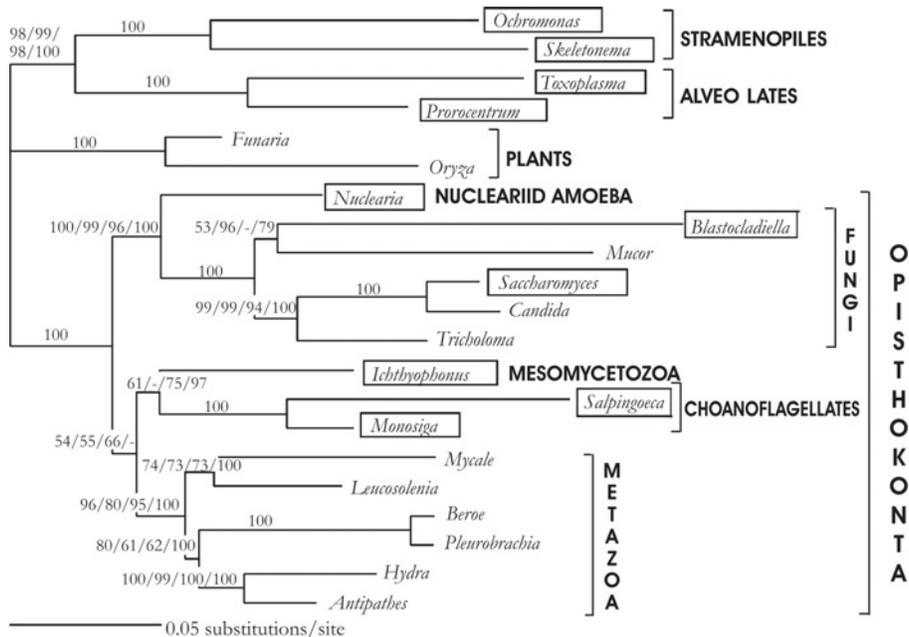


Figure 5 A phylogenetic hypothesis for the relationship of protists to fungi and animals (opisthokonts) based on analysis of SSU plus LSU rDNA. Best tree obtained by ML. The first three values above branches correspond to bootstrap values for ML, MP and ME, respectively. The fourth value corresponds to posterior probabilities from the Bayesian analysis (a single value of 100 indicates same support in all analyses). Protists are boxed and multicellular fungi and animals are not. The evolutionary distance is represented by the scale bar. From Medina *et al.* (2003).

Sponges first appear in the fossil record in the Ediacaran, over 550 Ma. They occur in the Doushantou Formation (China), formerly dated near 600 Ma and thought to contain the oldest confirmed animal fossils. The formation's age was reevaluated recently and is now considered younger than 580, but no younger than about 555 Ma (Condon *et al.*, 2005). This puts the formation, with preserved bilaterian and sponge embryos, within the range of known Ediacaran faunas with larger metazoans elsewhere in the world. Thus neither geologic nor paleontologic evidence constrains the time of origin of metazoans from their protist ancestors. Molecular estimates have yielded a range from 1200 to 670 Ma (Wray; Levinton & Shapiro, 1996) with a time near the younger date more likely (Ayala; Rzhetsky & Ayala, 1998).

7 Protists Participated Wholly in the Cambrian Radiation

The basal Cambrian is characterized by a sequence of metazoan fossils that represent many of the modern phyla of animals (Valentine, 2004) in a geologically rapid period of radiation. Metazoan evidence for increasing complexity appears over a period of 10-15 million years. In contrast to the Ediacaran, Cambrian animals show greater diversity of life styles, skeletons, and taxa. First to appear after the demise of the Ediacaran biota are infaunal bioturbators (Droser, Jensen, & Gehling, 2002; Seilacher, Buatois, & Mangano, 2005) that were not skeletonized, followed by a variety of small sclerites (Bengtson, 1994; Bengtson & Conway Morris, 1992), and finally, the familiar metazoans (brachiopods, trilobites, etc). Although relatively fast, this radiation was not particularly “explosive”. The Cambrian radiation is the most significant in the fossil record because it includes not only the first animals clearly assignable to modern clades but also protists and algae as well. Acritarchs and skeletonized protists (foraminifera, radiolaria, and perhaps tintinnids; see Figure 6) also radiated in the Cambrian between 544-530 Ma (Culver, 1991; Dong, Knoll, & Lipps, 1997, Lipps & Rozanov, 1996 among others). Whatever drove this radiation of protists, larger algae and metazoans is controversial.



Figure 6 First foraminiferan, *Platysolenites antiquissimus* Eichwald, found between 545-540 Ma, on the Baltic Platform, Avalonia, and western North America. Specimen is about 2 cm long and is from Newfoundland, above the Cambrian-Precambrian boundary. From Lipps & Rozanov (1996).

An important question is whether or not the record of the Cambrian radiation represents the actual timing of the evolution of these forms. Again, two alternatives are discussed in the literature: 1. the record as observed records evolutionary events more or less as they happened in time and space (Conway Morris, 2006); or 2. a longer Precambrian evolution of both animals (Valentine, 2002) and protists is required to account for certain observations. Evidence indicating a longer Precambrian period of evolution of both the invertebrates and protists are the advanced morphology of many of the taxa (trilobites, for example), modeling of speciation rates for Cambrian taxa (Lieberman, 2001), the presence of a few potential ancestors in the Ediacaran biota extending back in time for 40 million years, such as bilaterian embryos, mollusk-like *Kimberella*, and arthropod-like forms (Fedonkin, 1985; Fedonkin & Waggoner, 1997; Knoll, 2003), and vicariant biogeographic analysis of early Cambrian trilobites (Lieberman, 2003). Of this evidence, the last is critical because it is based on the phylogenetic analysis of well established lower Cambrian trilobites that shows vicariant events in their ancestry some 20-70 million years prior to the Cambrian. In addition molecular data of unknown reliability for metazoans (720-660 Ma; Valentine, 2002, 2004) and foraminifera (postulated to be abundant and diverse in the Neoproterozoic; see Pawlowski *et al.*, 2003) support a longer history for most of the elements of the Cambrian radiation.

If the Cambrian biota has a much longer history, then where is it? In a sense, the Cambrian radiation is like the radiations following the major extinction events of the Phanerozoic. For example, the Permo-Triassic event caused the demise of many metazoans and protists, and few if any are found in rocks immediately following the extinction event. It commonly took 4-10 million years for the biota to rediversify and leave a fossil record after extinctions. The same question arises after other extinction events, for example the Cretaceous-Tertiary event where several million years passed before the rediversification became apparent. Where were the forerunners of the diversifying taxa during this interval? They were surely present somewhere on Earth, for their ancestors and descendents were involved in both the extinctions and subsequent radiations. This is not a well-studied issue, but it must involve small populations living in less well preserved environments, perhaps in the deep sea or very marginal coastal habitats for which records are not commonly available. The same suggestion can be made for the ancestors of the Cambrian taxa, as has been done for foraminifera (Pawlowski *et al.*, 2003), but clearly more study is required.

Another major question is what caused this sequence of events at the base of the Cambrian involving so many disparate taxa? Much speculation surrounds this question as well, little of it supported by evidence. At least 20 hypotheses and ideas have been presented (see summary and list in Signor & Lipps, 1992), including the rise of oxygen in the atmosphere and the breakup of continents, among others. Most of these may account for diversification of metazoans (metazoan-bound hypotheses) and protists (usually omitted in these discussions) in one way or another, but few consider the morphology observed in both groups. Two possibilities are attractive that consider the diversity and morphology. One is that the newly radiating metazoans, either through adult or larval stages or both, developed the capability to prey actively on phytoplankton, on protists and on each other, resulting in bioturbation, skeletons in both benthic and pelagic protists and animals, calcification of benthic algae, and the production of spines and other ornamentation on phytoplanktonic acritarchs (Butterfield, 1997). Many questions are raised by this hypothesis and it is difficult to test it, even in modern biotas. Equally difficult to test, is the idea that paleoceanographic changes resulted in increased nutrient supply and primary production that were utilized by benthic and pelagic organisms, which then may well have developed the morphologic and behavioral characteristics seen in the metazoans, algae and protists of the Cambrian. The former is a top-down model, where top-level predators change the biota they prey on, while the second is a bottom-up hypothesis where primary production controls changes in higher trophic levels. Both of these models are under close scrutiny in modern ecosystems, and even there uncertainty prevails. This remains one of the important open questions in paleontology.

8 Protists Occupied Major Marine Environments

Phanerozoic protistan diversity changes in parallel with the general changes of the marine biota in general (see Sepkoski, 1993, for comparison of foraminiferal diversity with metazoans). The parallel evolution of single-celled eukaryotes and metazoans indicates similarity of evolutionary response to the environmental changes through the Era. Like the metazoans, protists began to dominate major environments (shelves and reefs) starting in the Cambrian with very low diversity assemblages (Culver, 1991, 1994) with a significant radiation in the Ordovician, followed by extinctions and other radiations until most died out at the end of the Permian (for details, see various chapters in Lipps, 1993). After the Paleozoic, benthic protists underwent extinctions and radiations at the same times as metazoans.

9 Mesozoic Radiations and Extinctions of Pelagic Protists.

Although protist parallel metazoan diversity changes throughout the Phanerozoic, the Mesozoic was a special time for protists. Starting in the Triassic and continuing into the Cretaceous, dinoflagellates, diatoms, coccolithophorids, silicoflagellates, radiolaria, and planktic foraminifera appeared and radiated in pelagic environments. The record for open ocean metazoans is very poor, except for ammonites, because most zooplankton do not today and apparently did not in the past possess preservable skeletons. The protist record, however, is excellent and records sequential radiations of various skeletonized phyto- and protozoan plankton (Falkowski *et al.*, 2004; Katz *et al.*, 2004; Lipps, 1970). The phytoplankton represent all the major contributors to primary production in modern seas, although Mesozoic taxa are significantly different taxa that are extinct today.

The radiation of pelagic protists was complex because they represent both autotrophic, secondarily autotrophic and heterotrophic forms. A variety of cell coverings also developed, including organic (dinoflagellates), silica (diatoms, radiolaria) and calcareous (coccolithophorids, foraminifera). These observations complicate evolutionary interpretations based on changing sea water chemistry, sea level fluctuations, or extensive shelf environments. The appearance of a variety of skeletal types indicates that a direct control by sea water chemistry (Ries; Stanley & Hardie, 2006) is unlikely as a general phenomenon for plankton. Since these five major groups (plus minor ones like silicoflagellates) are truly oceanic organisms independent from land or shelves, sea level changes and shelf availability are unlikely to have major effects on their evolution unless they changed oceanographic conditions. The evolution of dinoflagellates, diatoms, and coccolithophorids was enhanced by their secondary acquisition of plastids from red algal sources which appears to have allowed them to become dominant in Mesozoic and Cenozoic oceanic ecosystems (Falkowski *et al.*, 2004). The radiation then of radiolaria and planktonic foraminifera, and likely other zooplankton, in the Jurassic and Cretaceous would presumably be based on the new trophic resources made available by those phytoplankton.

The entire pelagic ecosystem was also undergoing physical changes, as Pangea broke up starting after 250 Ma. As the Atlantic Ocean opened, new oceanographic configurations also took shape. These likely provided opportunities for evolution of pelagic organisms in new vertical and horizontal partitions (Lipps, 1970) defined by sea water density, current patterns, and nutrient supply through upwelling, all of which would change in response to

new ocean basin configurations, associated climatic changes driving sea surface currents and current intensities, and haline-driven vertical circulation. These processes continued to change evolutionary patterns in the Cenozoic as well, long after the end-Cretaceous extinction event that eliminated all but a few species in each pelagic group. The mechanism of this extinction are debated as well, but the impact of an asteroid is the dominant hypothesis for the extinction of plankton, dinosaurs, shallow invertebrates, and reefs.

Also terrestrial ecosystems were occupied by protists. Diatoms first appear in these associations at about 70 Ma (Bradbury & Krebs, 1995; Chacón-Baca *et al.*, 2002), nearly 50 million years later than they first appeared in marine sediments (Gersonde & Harwood, 1990; Harwood & Gersonde, 1990). Other protists likely lived in terrestrial and freshwater habitats too, but their records in the Mesozoic are undocumented.

10 The Radiation of Modern Protists

Following the Cretaceous extinction, protists declined in reef, shallow marine, and pelagic ecosystems. For over three million years, diversity remained very low but eventually the protists and metazoans radiated. They attained similar diversities in much the same habitats in the later Paleocene and Eocene, but experienced another decline in the Oligocene and a radiation of mostly new species and genera in the lower Miocene (see summaries in Lipps, 1993). These taxa went on to become the modern protistan biota. The protist biota plays an important role at all trophic levels in modern marine environments, as primary producers (Falkowski *et al.*, 2004). A huge number of largely unknown terrestrial, parasitic and symbiotic protists must have existed for much of geologic time as well. These were surely important, as indicated by the impact of disease-causing forms in humans and other organisms. The modern protistan biota lives in just about every environment known on Earth, except for deep in rocks either on continents or in ocean basins. Otherwise, they do well in the deep sea to supratidal and pelagic marine habitats, running and standing freshwaters, damp moss and forest litter, in sea ice and snow, and as parasites in most other organisms. Some even parasitize other protists. The parasitic protists cause enormous ill among humans—amoebic dysentery, *Giardia* and malaria, for example, infect hundreds of millions of people and take millions of lives a year. Protists remain a significant component of oceanic primary production, on which the well-being of fisheries and marine ecosystems in general depend. In more restricted situations, protists may play a fundamental role—for example, foraminifera contribute up to 25% of the CaCO₃ deposited on reefs and sequester significant amounts of CO₂ (Langer; Silk & Lipps,

1997). Protists, invisible as they are to most humans, have major impact on human's and Earth's well being.

11 The Future of Protists may be in Jeopardy

The future of protists is likely in jeopardy, just like most reefal, benthic, and planktic metazoans. For many groups, we know well from the fossil record what happens to them under certain changing environmental conditions (green- or ice-houses, oceanographic pattern changes, sea levels, etc), but we do not know how human pollution and disruption of trophic structures may affect the protistan biotas that contribute significantly to the health and welfare of biotas everywhere. We can infer that their populations and assemblages have been changed by human activities. Chemical (and in limited places, radiation) pollution may take a toll on protists (for examples, see the deformations of foraminifera in Alve, 1995; Alve & Olsgard, 1999; Venec-Peyre, 1981). The disruption of marine trophic structures by over fishing is probably the greater threat and it has already changed nearly every marine habitat on Earth except perhaps the deepest seas (Jackson *et al.*, 2001). The removal of higher level predators allows the overgrowth of other, usually much smaller prokaryotes and protists that then kill the many of the remaining organisms (Pandolfi *et al.*, 2005). This urgent need to understand the role of protists in modern threatened oceans should be addressed soon.

12 Conclusions

Protists have played a significant role in the evolution of Earth's biota, probably for nearly the past 4 Ga. While many questions remain as topics for intensified research in paleontology and molecular biology, the general outline suggests low diversity assemblages of pelagic and benthic protists until the base of the Cambrian. In the Phanerozoic, protists became a large component of all ecosystems, probably including terrestrial ones as well as marine. They were so abundant and evolved so readily that they have provided detailed biostratigraphic schemes that have served well in geology and the petroleum industry. The major events in protistan and eukaryote evolution, however, remain clouded by taphonomy, uncertain systematic assignments, too few samples, molecular biologic artifacts, and rapidly changing views. All these efforts bode well for the future understanding of the early history of eukaryotes and protists. While protists did well throughout the last 65 million years, they could be imperiled now by human predation on top carnivores and pollution by chemical wastes. The effects on protists of these activities is unknown, but because protists provide many ecosystem functions, we should strive to understand them.

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