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Kingdom Protozoa and Its 18 Phyla

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INTRODUCTION

The classification of eukaryotic microorganisms, usually referred to as protists, has been in flux for over two centuries. During the past 20 years, there has been an increasing tendency to divide them into several kingdoms rather than to place them all in a single kingdom, as was proposed by the 19th century authors Owen (kingdom Protozoa, 1858), Hogg (kingdom Primigenum, 1860), and Haeckel (kingdom Protista, 1866). (These earlier kingdoms included bacteria, which were first formally removed as a separate kingdom by Copeland [48] in 1938.) Earlier attempts to subdivide protists simply into plants and animals, on the basis of the presence or absence of chloroplasts or phagotrophy (feeding by phagocytosis), were abandoned because three well-defined taxa (dinoflagellates, euglenoids, and heterokonts) have some members of each type, and in the case of dinoflagellates and heterokonts (and haptophytes) many species are both photosynthetic and phagotrophic. Since the early 1970s, new insights into protist ultrastructure arising from electron microscopic studies have been increasingly used to propose explicit phylogenies for protists (16–19, 21, 25–27, 29, 32, 34–43, 132, 133) and to apply more rigorous phylogenetic principles to the large-scale classification of protists. During the same period, the increasing availability of molecular sequences has been an increasingly valuable source of independent phylogenetic information. The establishment of the predominantly photosynthetic kingdom Chromista (brown algae and diatoms and their various relatives) in 1981 (17) and the primitively amitochondrial kingdom Archezoa in 1987 (26), and an ultrastructurally based redefinition of the kingdom Plantae (17, 29), excluded a large residue of mainly phagotrophic and aerobic protists whose classification is the subject of the present review. Although there might be some merit in subdividing these protists into several kingdoms along phylogenetic lines, I here adopt the more conservative approach of including them all in a single kingdom, Protozoa, and subdividing this into subkingdoms, infrakingdoms, parvkingdoms, and superphyla. The kingdom Protozoa in my present usage therefore includes all eukaryotes other than the primitively amitochondrial Archezoa and the four eukaryotic kingdoms (Animalia, Fungi [defined in reference 25], Plantae, and Chromista) that were independently derived from Protozoa.

Changing Views of Protozoa as a Taxon

Over 130 years ago, Owen raised Protozoa (originally a class, Goldfuss, 1818) to the rank of kingdom (107, 108), thus for the first time separating protists (as we now call them) from animals and plants at the highest classificatory level. But for many years neither this proposal nor Haeckel's proposal of a similar, but narrower, kingdom Protista (52, 67) became accepted, primarily because of the difficulty of demarcating Protozoa from the kingdoms Animalia and Plantae. Eventually, electron microscopy provided many new criteria for this demarcation and helped to reinforce a growing preference for multikingdom systems of classification over the old animal-or-vegetable dichotomy (16, 17, 19, 21, 31, 52, 76, 77, 90, 95, 96, 99, 101, 124, 147). Though it is widely agreed that Protozoa are too diverse to constitute a single phylum and must be distributed among a fairly large number of phyla (17, 31, 52, 77, 83, 89, 90, 98, 124), there has been no general consensus as to how this should be done or, indeed, whether or not Protozoa should even remain a formal taxon. At present, three fundamentally different viewpoints are enjoying an uneasy coexistence. The most conservative approach is to treat Protozoa as a subkingdom, but not to specify whether it belongs to Animalia or Protista, and to sidestep the problem of demarcation by failing to provide a diagnosis (89) or by providing a diagnosis that is too vague to be effective (83). The most radical approach is to abandon Protozoa altogether as a taxon (51, 90) and either to subsume its phyla into a broader kingdom, whether Protista (48, 52, 95, 96, 147), Protoctista Copeland 1947 (49, 97–99), or even Phytobiota (= Plantae) (77), or alternatively to subdivide it into several narrower kingdoms (86, 90, 101). A more eclectic middle way is to refine the concept of protozoa more precisely so as to produce a phylogenetically sound taxon that can be given a precise diagnosis (17, 21, 26, 35, 37).

The purpose of this review is to argue the merits of the third approach and to present a revised classification of this more rigorously defined kingdom Protozoa down to the level of subclass.

Table 1 shows the position of the kingdom Protozoa in the eight-kingdom system (31). [Note that the empire Eukaryota is equivalent in content to the domain Eukarya of Woese et al. (149a) Since the category empire was proposed (26) before that of domain (149a), it has historical priority. The renaming of the long established taxa Eukaryota, Archaebacteria, and Eubacteria as Eucarya, Archaea, and Bacteria is highly objectionable and should not be followed (40b), because it is entirely contrary to principles of stability and priority in nomenclature. The use of the term Bacteria as a junior synonym for Eubacteria is particularly confusing since it has often been used previously as a synonym for all prokaryotes. As I have long argued (17a, 24a, 27, 31, 35, 37, 41a), giving Eubacteria and Archaebacteria each the same rank as eukaryotes as a whole grossly inflates the importance of the differences between the two kingdoms Eubacteria and Archaebacteria. Contrary to what has so often been asserted in recent years, the differences in cellular and genetic organization between the empires Bacteria and Eukaryota are far more radical and fundamental than the differences between archaebacteria and eubacteria (35, 37, 41a). Both kingdoms of the empire Bacteria share many positive characters, e.g., polycistronic messengers (35, 37, 41a), that are absent from eukaryotes. Therefore, the frequent statement (e.g., see reference 111) that prokaryotes share only negative characters is false. Both Bacteria and Eubacteria are probably paraphyletic taxa, like the Protozoa, but this does not

TABLE 1. The 8 kingdoms of life and their 10 subkingdoms

EMPIRE BACTERIA[a]		
Kingdom 1. EUBACTERIA[a]		
Subkingdoms:	1. Negibacteria[a]	2. Posibacteria[a]
Kingdom 2. ARCHAEBACTERIA[a]		
EMPIRE EUKARYOTA		
Superkingdom 1. ARCHEZOA		
Kingdom ARCHEZOA		
Superkingdom 2. METAKARYOTA		
Kingdom 1. PROTOZOA		
Subkingdoms:	1. Adictyozoa	2. Dictyozoa
Kingdom 2. PLANTAE		
Subkingdoms:	1. Viridiplantae (green plants)	2. Biliphyta (red algae and glaucophytes)
Kingdom 3. ANIMALIA		
Subkingdoms:	1. Radiata	2. Bilateria
Kingdom 4. FUNGI		
Kingdom 5. CHROMISTA		
Subkingdoms:	1. Chlorarachnia	2. Euchromista (cryptomonads, *Goniomonas*, heterokonts, and haptophytes)

[a] My classification of these bacterial taxa into phyla and classes, taking into account both rRNA sequences and the distribution of many ultrastructural and biochemical characters, is summarized in reference 40a.

detract from their great utility. The original idea of three primary kingdoms was premature when it was proposed (149) and has since been refuted (37, 75). It is now generally accepted that Eubacteria is the only primary kingdom and that archaebacteria and eukaryotes are both secondarily derived holophyletic (3) taxa and sister groups to each other (37, 75, 149a), as argued in detail earlier (27). Both the "three primary kingdoms" concept and the identical but renamed "three domains" concept gave far too much classificatory weight to functionally relatively insignificant quantitative changes in a single molecule, 16S or 18S rRNA: this molecule is undoubtedly phylogenetically highly informative, but it should be regarded as complementary to other molecular, ultrastructural, and palaeontological data, which are too often ignored by rRNA enthusiasts.]

EXCESSIVE BREADTH OF PROTISTA OR PROTOCTISTA

For comparative studies, it is often very convenient to treat all protists together (16, 38, 52, 98), and no adequate understanding of protozoan phylogeny or systematics can be gained without considering algae and fungi (and indeed bacteria) together with protozoa in an integrated protistological perspective. However, it by no means follows from this that it is desirable to submerge protozoa into a broader protist or protoctist kingdom.

From the start, Haeckel's kingdom Protista was an arbitrary jumble of some (but not all) unicellular eukaryotes and some (but not all) prokaryotes: it included diatoms (and sometimes sponges) but excluded not only other algae and sometimes fungi (placed in the plant kingdom, contrary to more recent practice [8, 14, 25]) but also ciliates and sometimes gregarines (placed in the animal kingdom) and could not be given a proper diagnosis. In contrast to Owen's earlier proposal of a kingdom Protozoa, Haeckel's kingdom Protista was based on a fundamental phylogenetic error: the idea of a polyphyletic origin for the eukaryote cell. Haeckel thought that protist, animal, and plant cells originated independently from different precellular ancestors (an idea curiously similar to the equally erroneous [see references 35, 37, and 75] independent origin of eukaryotes, eubacteria, and archaebacteria from a primitive "progenote" proposed by Woese and Fox [149]): he thought that even Protista might be polyphyletic (68, p. 50).

Most 20th century proponents of a kingdom Protista (48, 52, 95, 146) have refined it by very properly excluding both bacteria (a few include these [102, 146]) and sponges but have broadened it by adding to it all protozoa and some or all fungi and some or all algae. Moreover, it is now thoroughly well established that eukaryotes are monophyletic (27, 35, 37, 127) and that animals, higher plants, and fungi all evolved from protists. Thus, Protista is a paraphyletic group. Contrary to Hennigian opinions (69, 90, 111), however, this is no reason in itself to reject the group. It is impossible to cut up a phylogenetic tree into purely holophyletic groups: every cut generating a holophyletic branch necessarily also generates a paraphyletic stem. Both holophyletic (3) and paraphyletic taxa are essential for systematics. It is merely more complicated to define a paraphyletic taxon than a holophyletic one. Holophyletic taxa can be simply defined by using positive shared derived characters that are unique to them (synapomorphies); a paraphyletic taxon, by contrast, has to be defined by using a combination of positive and negative characters, i.e., the presence of one or more synapomorphies that originated in the ancestral member of the taxon coupled with the absence of those synapomorphies that characterize the taxa that evolved from the paraphyletic taxon in question. (It is a myth that paraphyletic groups are purely negatively defined [111] or less real than holophyletic ones: all taxa are made by cutting the phylogenetic tree; the position of each cut, which should immediately precede the origin of an important new synapomorphy, simultaneously is used to define the derived holophyletic taxon and to be part of the definition of its paraphyletic ancestral taxon, in conjunction with the positive synapomorphy that marked its origin, and also the absence of all those synapomorphies that define any other taxa derived from it.)

What should be avoided, as all systematists agree, is the polyphyletic grouping together of several separately lopped branches: each taxon should correspond to a part of the tree having a single cut at its base: but it may either have no additional cuts (i.e., be holophyletic) or be bounded by one or more additional cuts higher up the tree.

We know now that Haeckel's three kingdoms were all polyphyletic, because the phylogenetic tree that he attempted to subdivide was incorrect. The kingdom Protoctista in Copeland's four-kingdom system (49, 50) and kingdom Protista in Whittaker's five-kingdom system (147) were great improvements; by clearly excluding both bacteria and sponges, and by grouping all green algae in a single kingdom (i.e. Plantae; though others [5, 95–97, 99, 118] confusingly transferred them to Protista), Protista became paraphyletic rather than polyphyletic. Most authors have accepted Whittaker's treatment of Fungi as a kingdom separate from Plantae (first suggested in 1832 by Fries) and also the separation of bacteria into two kingdoms (Archaebacteria and Eubacteria); thus, a six-kingdom system is now in effect in common use: Eubacteria, Archaebacteria, Protista, Animalia, Fungi, and Plantae.

The problem with this six-kingdom system is that there is no agreement about the boundaries between Protista, Fungi, Animalia, and Plantae. Whittaker's boundaries between these kingdoms were initially proposed in 1959 (146), before the advent of high-quality fixation (119) and epoxy embedding for ultrathin sectioning (66) and the revolution that these advances in electron microscopy caused in systematics (55), and are therefore now thoroughly obsolete.

Phylogenetic evidence from ultrastructure and molecular sequences has clearly shown that Whittaker's Plantae and Fungi were polyphyletic: brown algae are not specifically related to green plants (Viridiplantae [17]), and neither Mycetozoa nor the heterokont oomycetes and hyphochytrids are specifically related to Fungi sensu stricto (see Fig. 1). These taxa therefore cannot properly be included in Plantae or Fungi: they are now commonly placed in the Protista (52). Unfortunately, Rothmaler (118) and Barkley (5), followed by Margulis (95, 96), transferred green algae from Plantae to Protista, and Margulis (95, 96) transferred red algae from Plantae to Protista and Chytridiomycetes from Fungi to Protista, making the latter group even more heterogeneous. More recently, Margulis calls Protista sensu Margulis 1971 emend. 1974 Protoctista, a name first substituted for Protista by Copeland (49) in the erroneous belief that it had been used as a kingdom name by Hogg (72) before Haeckel's Protista. In fact, Hogg used Protoctista as a vernacular name: his formal name was kingdom Primigenum, which he proposed as a synonym for Owen's earlier kingdom Protozoa, solely because he did not like the suffix "-zoa" for the more plant-like protists, even though (as he himself pointed out) in Greek -zoa can refer to life in general and not merely to animal life. Copeland rejected Owen's Protozoa as a kingdom name solely because it had been used previously as a class and phylum name: he followed his own unique idiosyncratic rules of nomenclature according to which one should never change the rank of a name; for that reason, he also decided to call bacteria Mychota, fungi Inophyta, and sporozoa Fungilli! If we were to follow that curious dogma generally, we should have to change the familiar names of a very large number of major taxa that were initially named at a lower rank.

Copeland's Protoctista was therefore an entirely unnecessary junior synonym for both Protozoa and Protista and was based on multiple confusions and a personal nomenclatural dogma shared by no other taxonomists. To add to the confusion, Margulis has adopted the name Protoctista for a very different taxon: one that, unlike Copeland's, excludes nonflagellated Fungi and includes green algae (96–99).

The central problem with the kingdom Protista sensu Margulis 1974 (or the identical kingdom Protoctista sensu Margulis 1974) is not its name but its excessive diversity. Biologically, it is far more diverse than the other three eukaryote kingdoms. Consider at its two extremes the microsporidia and the brown algae. Microsporidia are minute unicellular amoeboid intracellular parasites with chitinous spores no bigger than most bacteria and, like them, having 70S ribosomes and lacking mitochondria, peroxisomes, chloroplasts, 9+2 cilia or flagella, and dictyosomes. By contrast, brown algae are free-living, multicellular, often gigantic seaweeds with varying degrees of cell differentiation (often quite elaborate), 80S ribosomes, cellulose walls, mitochondria, peroxisomes, 9+2 cilia (I use cilia to include eukaryotic flagella [19, 24, 69a]) with tubular mastigonemes, and dictyosomes and have chloroplasts and periplastid membranes located inside their rough endoplasmic reticulum (RER). There are thus very many more, really fundamental differences between microsporidia and brown algae than there are between mushrooms and sponges or between green algae and corals, which everyone places in separate kingdoms, and immensely more major differences than between bryophytes and Charophyceae, which Margulis (following Rothmaler [118] and Barkley [5] but in opposition to the vast majority of botanists) places in different kingdoms. For similar reasons, many authors have argued that a kingdom Protista is immensely too heterogeneous and needs to be split into several kingdoms (16, 17, 21, 23, 26, 31, 32, 76, 86, 90, 101). To say that a eukaryote is a member of the Protista sensu Margulis 1974 tells one nothing about it other than that it is a eukaryote. Not only is the kingdom too diverse, but its boundaries with the kingdoms Plantae, Fungi, and Animalia are not well chosen: they are not at the points of maximum biological discontinuity. Both the excessive breadth of the Protista and the arbitrariness of its boundaries can be solved by two major reforms: (i) splitting it into three major kingdoms (Archezoa, Protozoa, and Chromista), and (ii) realigning the boundaries between these and the classical kingdoms Plantae, Animalia, and Fungi (17, 21, 23, 25, 26, 29). In order to define the kingdom Protozoa, we must therefore consider in turn its delimitation from each of the other five kingdoms of eukaryotes recognized in this eight-kingdom system of life. Though I have argued against using Protista as a taxon, it is valuable to continue to use protist with a lowercase p to refer to eukaryotic unicells or simple multicellular aggregates having little or no cell differentiation.

DISTINCTION BETWEEN PROTOZOA AND PLANTAE

Classically, the distinction between the Linnean kingdoms Animalia and Vegetabilia (not Plantae, as so often incorrectly stated: it appears to be Haeckel [67] who replaced Linnaeus's kingdom Vegetabilia by a kingdom Plantae; the plant kingdom was thus actually originally introduced as part of a three-kingdom system of organisms!) was that animals moved and vegetables did not. For this reason, *Volvox* (like bacteria) was classically treated as an animalcule or infusorian rather than a plant (103a, 117), and to this day protozoologists have retained Volvocales and prasinomonads in the protozoa (83, 89), even though botanists who have studied them thoroughly and are more familiar with their other green algal relatives have correctly placed them in the green algae (currently division Chlorophyta Pascher, 1931) for over a century. It is totally inappropriate for these two taxa to be placed in the Protozoa merely because most of their life they move by cilia (or flagella; but the volvocalean cilium beats like a cilium and was called a cilium in classical works [117,

144]). Within the green algae, loss of cilia or flagella or of their motility has occurred several times, even within families, and changes in the proportions of the life cycle that are motile or immotile are very frequent. These sorts of differences are far too trivial to be used for a kingdom boundary. It is only a conservative historical carryover rather than sound positive taxonomic judgment that has caused these green algal taxa to remain within the Protozoa.

In my view, the kingdom Plantae comprises but two subkingdoms, Viridiplantae (green plants, including the green algae, divisions Charophyta and Chlorophyta [41], as well as the Embryophyta [so-called land plants]) and Biliphyta (i.e. red algae [Rhodophyta] and the Glaucophyta) (17, 23, 29, 31, 41). Whether these two taxa are correctly classified in a single kingdom or as two distinct kingdoms is not yet entirely clear but is irrelevant to the present paper because both can be sharply distinguished from protozoa. Green algae are sharply divided from protozoa by always having starch-containing plastids that are bounded by an envelope of two membranes, the synapomorphy defining the Viridiplantae (17). Their photosynthetic majority has stacked thylakoids containing chlorophylls *a* and *b* in their chloroplasts. These characters clearly define the subkingdom Viridiplantae Cavalier-Smith, 1981 (17), which following Copeland's (48–50) lead, botanists now agree is a monophyletic taxon (9, 125, 135). Protozoa (if Volvocales and prasinomonads are excluded, as they should be) never have such plastids. Moreover, most (but not all) protozoa are phagotrophic. Virtually no green plants feed by phagocytosis: the only published evidence for phagocytosis in any Viridiplantae is in a prasinomonad chlorophyte (class Prasinophyceae) (105). Since it is clear that green plants must have evolved from a phagotrophic protozoan by the symbiotic origin of chloroplasts (18, 29, 39, 97), and also is generally accepted that the Prasinophyceae are the ancestral green plants (100), it is not surprising that the ancestral phagotrophic character has been retained by at least one prasinomonad.

The Biliphyta (Glaucophyta [29], also known as Glaucocystophyta [80], and Rhodophyta) have never been included in the Protozoa and are also distinguished from Protozoa by the universal presence of plastids bounded by an envelope of two membranes and by the total absence of phagotrophy (17). Photosynthetic biliphytes (the vast majority; only a few parasitic red algae are nonphotosynthetic) have chloroplasts with single, unstacked thylakoids covered in phycobilisomes. Unlike Viridiplantae, biliphytes have starch in their cytosol not in their plastids. The combination of cytosolic starch and plastids bounded by only two membranes uniquely defines the Biliphyta. Glaucophyta, in addition, have cortical alveoli, whereas Rhodophyta do not (17).

Thus, Plantae sensu Cavalier-Smith, 1981 are characterized by plastids with double envelopes, the presence of starch either in their plastids or in the cytosol, and the almost universal absence of phagotrophy (17). Protozoa, by contrast, are mostly phagotrophic and rarely have chloroplasts; when they do have chloroplasts, they are never like those of plants but are of other types. Because of a widespread belief in the polyphyletic origin of chloroplasts (97), my concept of the Plantae is not yet widely accepted. However, as discussed in detail elsewhere (39, 41, 54, 102a), the evidence for a monophyletic origin of chloroplasts is substantial (and is now accepted by most students of chloroplast evolution [54, 66a, 102a, 110a]), and rRNA phylogeny, contrary to what is sometimes asserted (7), does not clearly contradict the monophyly of the group.

Two protozoan groups have perpetually plagued attempts to make a distinction between Protozoa and Plantae simply by the absence or presence of chloroplasts. These are the euglenoids and the dinoflagellates; both have a minority of phagotrophic species and a majority of species with chloroplasts. The phagotrophic and saprotrophic euglenoids are nothing like green plants and do not even have chloroplasts; the photosynthetic ones resemble green plants only in having chloroplasts of a similar grass green color with chlorophylls *a* and *b*, but these pigment similarities are relatively trivial and might even be convergent. Scholarly books on algae have very seldom treated euglenoids as green algae. They never contain starch, and their cell structure is radically different and much closer to that of the Kinetoplastea than to that of green algae (81, 137). Their chloroplasts are bounded by an envelope of three membranes. The chloroplasts of dinoflagellates also never contain starch, are usually bounded by three membranes, and always have stacked thylakoids containing chlorophylls *a* and *c*. If all dinoflagellate chloroplasts were bounded by an envelope of three membranes, one could make a very simple demarcation between plants and protozoa: plants invariably have plastids with envelopes of two membranes and are never phagotrophic; protozoa are usually phagotrophic and usually have no plastids; and if (rarely) present, protozoan plastids have envelopes of three membranes. Because a small minority of dinoflagellates have plastid envelopes (apparently) of only two membranes, it is necessary to add the rider: or very rarely envelopes of two membranes, in which case they contain chlorophyll *c*, and always lack chlorophyll *b*, starch, or phycobilisomes. Though this is a more complex distinction than the mere presence or absence of plastids, it does distinguish clearly between the totally nonphagotrophic and largely (but not entirely) photosynthetic Plantae and the largely phagotrophic Protozoa.

It is very clear from rDNA phylogeny (7, 121, 127) (Fig. 1) that Viridiplantae form a monophyletic and holophyletic group that includes the Volvocales and that dinoflagellates are entirely distinct from them and closer to the ciliates, whilst the euglenoids are very far removed indeed (but distantly allied to the Kinetoplasta). Thus, rDNA and ultrastructure are in total agreement on the great evolutionary distance that separates euglenoids from green plants. The apparent similarity of their chloroplasts alone may be due to the secondary acquisition by endosymbiosis of the euglenoid chloroplast from a primitive plant (34, 37, 64): in my present classification (see below), the subphylum Euglenoida is divided into three classes, two of which are entirely phagotrophic; whether these are primitively nonphotosynthetic or secondarily so is still unclear (39).

DISTINCTION BETWEEN PROTOZOA AND FUNGI

The distinction between Protozoa and Fungi has never presented such problems. However, Mycetozoa have usually been studied by mycologists rather than by protozoologists, even though the mycologist de Bary (53) long ago recognized their protozoan character. Mycetozoa are phagotrophic and have tubular mitochondrial cristae like most protozoa and have no walls in their trophic phase. In all three respects, they are sharply demarcated from Fungi: fungi are never phagotrophic, always have plate-like cristae, and typically (but not invariably) have chitinous walls in their trophic phase. rDNA phylogeny shows clearly that the Fungi (if restricted to Chytridiomycetes, Zygomycetes, Ascomycetes, and Basidiomycetes) form a single holophyletic

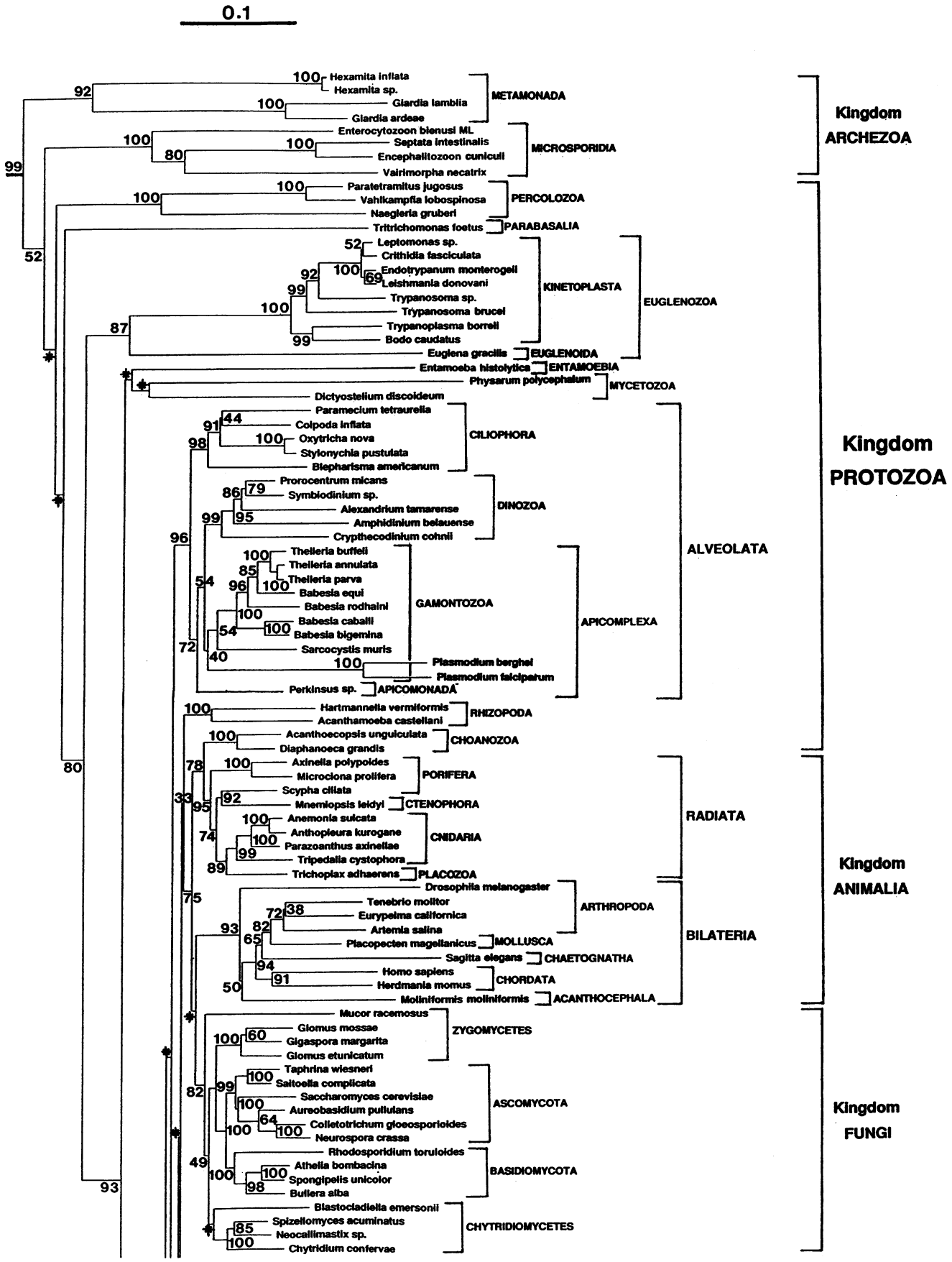
0.1
Hexamita inflata
Hexamita sp.
Giardia lamblia
Giardia ardeae
METAMONADA
Enterocytozoon bienusi ML
Septata intestinalis
Encephalitozoon cuniculi
Vairimorpha necatrix
MICROSPORIDIA
Kingdom ARCHEZOA
Paratetramitus jugosus
Vahlkampfia lobospinosa
Naegleria gruberi
PERCOLOZOA
Tritrichomonas foetus
PARABASALIA
Leptomonas sp.
Crithidia fasciculata
Endotrypanum monterogeii
Leishmania donovani
KINETOPLASTA
Trypanosoma sp.
Trypanosoma brucei
Trypanoplasma borreli
Bodo caudatus
EUGLENOZOA
Euglena gracilis
EUGLENOIDA
Entamoeba histolytica
ENTAMOEBIA
Physarum polycephalum
MYCETOZOA
Dictyostelium discoideum
Paramecium tetraurelia
Colpoda inflata
Oxytricha nova
Stylonychia pustulata
Blepharisma americanum
CILIOPHORA
Prorocentrum micans
Symbiodinium sp.
Alexandrium tamarense
Amphidinium belauense
Crypthecodinium cohnii
DINOZOA
Theileria buffeli
Theileria annulata
Theileria parva
Babesia equi
Babesia rodhaini
Babesia caballi
Babesia bigemina
Sarcocystis muris
GAMONTOZOA
Plasmodium berghei
Plasmodium falciparum
APICOMPLEXA
Perkinsus sp.
APICOMONADA
ALVEOLATA
Kingdom PROTOZOA
Hartmannella vermiformis
Acanthamoeba castellani
RHIZOPODA
Acanthoecopsis unguiculata
Diaphanoeca grandis
CHOANOZOA
Axinella polypoides
Microciona prolifera
PORIFERA
Scypha ciliata
Mnemiopsis leidyi
CTENOPHORA
Anemonia sulcata
Anthopleura kurogane
Parazoanthus axinellae
Tripedalia cystophora
CNIDARIA
Trichoplax adhaerens
PLACOZOA
RADIATA
Drosophila melanogaster
Tenebrio molitor
Eurypelma californica
Artemia salina
ARTHROPODA
Placopecten magellanicus
MOLLUSCA
Sagitta elegans
CHAETOGNATHA
Homo sapiens
Herdmania momus
CHORDATA
Moliniformis moliniformis
ACANTHOCEPHALA
BILATERIA
Kingdom ANIMALIA
Mucor racemosus
Glomus mossae
Gigaspora margarita
Glomus etunicatum
ZYGOMYCETES
Taphrina wiesneri
Saitoella complicata
Saccharomyces cerevisiae
Aureobasidium pullulans
Colletotrichum gloeosporioides
Neurospora crassa
ASCOMYCOTA
Rhodosporidium toruloides
Athelia bombacina
Spongipellis unicolor
Bullera alba
BASIDIOMYCOTA
Blastocladiella emersonii
Spizellomyces acuminatus
Neocallimastix sp.
Chytridium confervae
CHYTRIDIOMYCETES
Kingdom FUNGI

FIG. 1

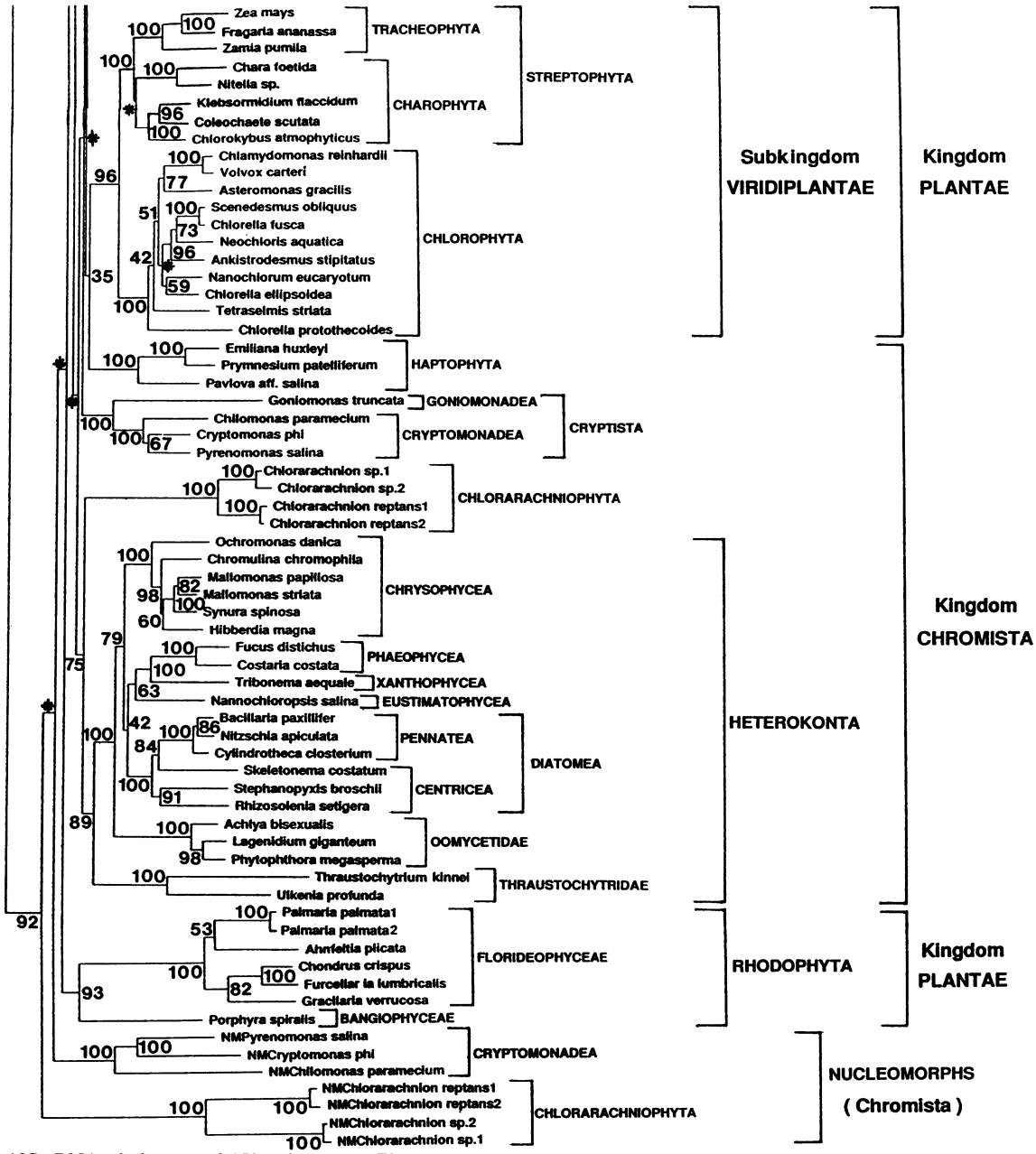

FIG. 1. 18S rRNA phylogeny of 150 eukaryotes. The tree was produced by the neighbor joining algorithm (120), as implemented in Felsenstein's Phylip 3.5 phylogeny package, using the Jukes-Cantor correction and jumbling the input order of species; bootstrap values for 100 replicates are shown. Sequences were obtained from GenBank or EMBL data bases except for eight unpublished ones from our own laboratory (*Axinella polypoides*, *Parazoanthus axinellae*, *Ulkenia profunda*, *Thraustochytrium kinnei*, *Pavlova* affinis *salina*, *Prymnesium patelliferum*, and *Chilomonas paramecium* [nucleus and nucleomorph] [M. P. Allsopp and T. Cavalier-Smith]) and 10 other unpublished sequences (8 *Chlorarachnion* sequences from G. McFadden and U. Maier, *Goniomonas truncata* from G. McFadden, and *Porphyra spiralis* var. *amplifolia* from M. A. Ragan). The initial alignment of a few sequences was by Clustal V (71a); substantial manual improvements and additions of new sequences were done with Genetic Data Environment software. In contrast to most published trees, no parts of the sequences were masked out and excluded from the phylogenetic analysis except for a few nucleotides at each end outside the usual polymerase chain reaction amplification primers (99c), because such masking has a subjective element. The tree is based on 3,400 aligned nucleotide positions. It is rooted by using 6 archaebacteria (*Methanococcus voltae*, *Sulfolobus solfataricus*, *Halobacterium halobium*, *Thermoproteus tenax*, *Pyrodictium occultum*, and *Thermococcus celer*) and 20 eubacteria (*Chlorobium vibrioforme*, *Spirochaeta halophila*, *Leptospira illini*, *Anacystis nidulans*, *Heliobacterium chlorum*, *Sporomusa paucivorans*, *Clostridium ramosum*, *Rhodopseudomonas globiformis*, *Flavobacterium halmophilum*, *Chloroflexus aurantiacus*, *Thermotoga maritima*, *Aquifex pyrophilus*, *Thermus thermophilus*, *Deinococcus radiodurans*, *Corynebacterium variabilis*, *Streptomyces griseus*, *Mycoplasma iowae*, *Mycoplasma coragypsum*, *Chlamydia trachomatis*, and *Planctomyces staleyi*). The instability of a few parts of the tree is emphasized by the fact that the 10 clades marked by asterisks were not present on the majority rule and strict consensus tree used to obtain the bootstrap values; therefore, these values cannot be given for these clades: the bootstrap values for the new, rearranged clades on the consensus tree were all very low (i.e., 4, 17, 20, 21, 27, 35, 48, 49, and 51%) except for two groupings, cryptomonad and *Chlorarachnion* nucleomorphs (59%) and the Percolozoa plus Microsporidia (67%). The other major differences between the tree shown here and the strict consensus tree were that the bilateral animals moved down the tree to the point below the nucleomorphs, the Cryptista joined the heterokont/chlorarachniophyte clade, *Dictyostelium* moved just above a clade consisting of *Physarum* and *Entamoeba*, and *Blastocladiella* moved to just below the *Glomus*/Ascomycota/Basidiomycota clade. The scale indicates the branch length corresponding to 10 changes per 100 nucleotide positions.

branch of the eukaryotic tree and that the mycetozoa are very far removed from them among the protozoa (7, 121, 127) (Fig. 1). Thus, ultrastructure, wall chemistry, feeding mode, and macromolecular sequences are all evidence that Mycetozoa are Protozoa, not Fungi (14, 17, 25).

It seems clear that fungi evolved from Protozoa by the evolution of chitinous walls in the trophic phase: this necessitated a shift from phagotrophy to absorptive nutrition (25). Ultrastructure, wall chemistry, and nutritional mode provide a simple demarcation between protozoa and fungi which corresponds to the traditional one. In my view, this is the biologically soundest place to "cut" the tree between the two kingdoms (17, 25). Margulis (95–99) most idiosyncratically places the cut within the fungi and includes Chytridiomycetes within Protoctista solely because they have a cilium and higher fungi do not: the old "animals move, plants don't" oversimplification in a new guise. But this is highly undesirable, for we know that cilia have been lost many times within Protozoa or within Plantae, but new kingdoms are not created every time this happens. Exclusion of Chytridiomycetes from the fungi is rightly not accepted by mycologists (8, 14) because ciliary loss is too trivial and too negative a character on which to base a kingdom, or even a phylum. It was not the loss of cilia but the origin of the chitinous wall that made fungi what they are: it occasioned the shift from phagotrophy to absorption and enabled mycelial growth (25). This radical innovation is what we should recognize by kingdom status and as the boundary between protozoology and mycology, which is, of course, where it has always been: protozoologists do not study chytridiomycetes, but mycologists do.

The origin of the fungal wall represented a sharper megaevolutionary and nutritional transition than the symbiotic acquisition of chloroplasts: a protozoan could not evolve a wall in the trophic phase without ceasing to be a protozoan, but it could acquire chloroplasts without giving up phagotrophy or radically changing its way of life. That is why the mere presence or absence of chloroplasts is an insufficient basis for defining a kingdom, as the cases of dinoflagellates and euglenoids well show. This is equally true of the problem of demarcating the Protozoa from the second major predominantly photosynthetic kingdom, the Chromista.

DISTINCTION BETWEEN PROTOZOA AND CHROMISTA

The kingdom Chromista Cavalier-Smith, 1981 is a predominantly photosynthetic taxon in which the chloroplasts are typically located not in the cytosol, as in the kingdom Plantae, but in the lumen of the RER, most often in the perinuclear cisterna; moreover, the chloroplasts are separated from the RER lumen by a unique smooth membrane, the periplastid membrane (23, 32), which surrounds and is quite distinct from their two-membraned plastid envelope. The periplastid membrane represents the plasma membrane of a eukaryotic photosynthetic symbiont (for a discussion of its nature, see references 32, 39, 54, 99b, and 123b) that was phagocytosed by a protozoan host during the origin of the Chromista (46) and which entered the RER lumen by fusion of the phagosome membrane with the nuclear envelope (18, 23, 32, 39, 144a). This organelle arrangement is unique to the Chromista and clearly distinguishes photosynthetic chromists not only from Plantae but also from the few photosynthetic protozoa (euglenoids and dinoflagellates) which all have their chloroplasts free in the cytosol, not inside the RER (46). One chromist phylum, Chlorarachniophyta (71), lacks ribosomes on the membrane which surrounds the periplastid membrane: this smooth membrane therefore probably directly represents the original phagosomal membrane which, unlike in other chromists, never fused with the RER. Therefore, in *Chlorarachnion* the chloroplast is not topologically within the RER. For this reason, I earlier excluded them from the Chromista and put them instead in the Protozoa: recent studies showing that the *Chlorarachnion* nucleomorph has three chromosomes as in cryptomonads (99c, 123b), plus the rRNA tree (Fig. 1; where both types of nucleomorphs have a [weak] tendency to form a single clade), support their placement in the Chromista.

Since the kingdom Chromista is relatively unfamiliar to general biologists, its constituent taxa are summarized in Table 2. It will be noted that the kingdom contains 12 classes whose member species have plastids, two classes (Pedinellea and Patelliferea) with some species with and some without plastids, and five classes (Goniomonadea, Bicoecea, Labyrinthulea, Oikomonadea, and Pythiistea) entirely without plastids. Bicoecea, Labyrinthulea, Pythiistea (oomycetes and hyphochytrids), and the aplastidic pedinellids are included in the phylum Heterokonta together with the plastid-bearing Ochrista because, like them, they have an anterior cilium bearing tripartite retronemes (i.e., rigid thrust-reversing tubular ciliary hairs or mastigonemes), which are not found on the cilia of any nonchromist organisms. The 18S rDNA tree (Fig. 1) clearly supports this ultrastructure-based concept of a phylum Heterokonta since it groups oomycetes and Labyrinthulea specifically with the ochrists (7, 127) (sequence data for the fourth subphylum, Bicoecia, are not yet available). The great conservatism in the presence of retronemes in the Chromista (absent only from haptophytes, which are clearly related to Ochrista by their intra-RER chloroplast organization as well as by having a single autofluorescent cilium [32, 45a]; from *Goniomonas*, which is clearly related to cryptomonads by its ejectisomes, periplast, and ciliary transition zones; and from *Chlorarachnion*, which is related to cryptomonads by its nucleomorph and to Flavoretea by its body form) is probably because of their thrust-reversing properties: losing retronemes would change the direction of swimming and thus reverse taxes and be highly disadvantageous (23, 32). The same would be true during the origin of retronemes, of course, so I have suggested that this coincided with the symbiotic acquisition of the chromist chloroplast and facilitated a changeover from a negatively phototactic-positively geotactic phagotroph to a positively phototactic-negatively geotactic phototroph (23, 32). The rarity of this simultaneous acquisition of three radically different structures (retronemes and two extra membranes around the chloroplast) makes this a much more substantial megaevolutionary step than any occurring within either of the kingdoms Protozoa and Chromista and therefore provides the best demarcation line between the predominantly photosynthetic and mainly nonphagotrophic Chromista and the predominantly nonphotosynthetic but phagotrophic Protozoa.

Apart from *Goniomonas*, the only major nonphotosynthetic chromist taxon commonly included in the Protozoa is the heterokont subclass Labyrinthulidae, which was given phylum status in the last protozoologists' classification (89). But labyrinthulids are obviously less closely related to any Protozoa than to the heterokont Thraustochytridae (with which they are now grouped in the class Labyrinthulea and which the rRNA tree [Fig. 1] confirms really are heterokonts); there is no justification for giving them separate phylum status or for retaining them in the Protozoa. Indeed,

TABLE 2. Classification of the kingdom Chromista Cavalier-Smith, 1981 emend.

Subkingdom 1. CHLORARACHNIA subregn. nov.
Phylum 1. CHLORARACHNIOPHYTA Hibberd & Norris, 1984
Class 1. Chlorarachniophycea Hibberd & Norris, 1984
Subkingdom 2. EUCHROMISTA subregn. nov.
Infrakingdom 1. CRYPTISTA Cavalier-Smith 1989, stat. nov.
Phylum 1. CRYPTISTA (syn. Cryptophyta)
Class 1. Cryptomonadea Stein, 1878 stat. nov. Pascher ex Schoenichen, 1925 (syn. Cryptophyceae Pascher, 1914)
Class 2. Goniomonadea nom. nov. pro Cyathomonadea Cavalier-Smith, 1989

Infrakingdom 2. CHROMOBIOTA orthogr. emend. pro. CHROMOPHYTA Cavalier-Smith, 1986
Phylum 1. HETEROKONTA Cavalier-Smith, 1986
Subphylum 1. BICOECIA Cavalier-Smith, 1989
Class 1. Bicoecea orthogr. emend. pro Bicosoecea Cavalier-Smith, 1986
Subphylum 2. LABYRINTHISTA Cavalier-Smith, 1986 stat. nov., 1989
Class 1. Labyrinthulea Olive ex Cavalier-Smith, 1989
Subclass 1. Thraustochytridae Cavalier-Smith, 1989
Subclass 2. Labyrinthulidae Cavalier-Smith, 1989
Subphylum 3. OCHRISTA Cavalier-Smith, 1986
Infraphylum 1. Raphidoista Cavalier-Smith, 1986 emend. stat. nov.
Superclass 1. Raphidomonadia supercl. nov.
Class 1. Raphidomonadea Chadefaud ex Silva, 1980 (syn. Chloromonadea)
Subclass 1. Raphidochloridae subcl. nov.
Subclass 2. Raphidochrysidae subcl. nov.
Superclass 2. Dictyochia Haeckel, 1894 stat. nov. emend.
Class 1. Pedinellea Cavalier-Smith, 1986
Class 2. Silicoflagellatea Borgert, 1891 stat. nov.
Class 3. Oikomonadea cl. nov.
Class 4. Pelagophycea Anderson Andersen and Saunders, 1993 (1a) orthog. emend.
Infraphylum 2. Chrysista Cavalier-Smith, 1986 stat. nov.
Class 1. Chrysophycea Pascher ex Hibberd, 1976
Subclass 1. Chrysomonadidae Saville Kent, 1881 stat. nov.
Subclass 2. Synuridae stat. nov. (= class Synurea Cavalier-Smith, 1986 [syn. Synurophyceae Andersen, 1987])
Subclass 3. Sarcinochrysidae subcl. nov.
Subclass 4. Chrysomeridae subcl. nov.
Class 2. Flavoretea cl. nov. (*Reticulosphaera*)
Class 3. Xanthophycea Allorge ex Fritsch, 1935 (syn. Tribophyceae Hibberd)
Subclass 1. Rhizochloridae subcl. nov.
Subclass 2. Tribophycidae subcl. nov.
Class 4. Phaeophycea Kjellman, 1891 (syn. Melanophyceae Rabenhorst, 1863, Fucophyceae Warming, 1884) orthogr. emend.
Subclass 1. Phaeophycidae Cavalier-Smith, 1986
Subclass 2. Fucophycidae Cavalier-Smith, 1986
Infraphylum 3. Eustigmista infradiv. nov.
Class 1. Eustigmatophycea Hibberd et Leedale, 1971
Infraphylum 4. Diatomea Agardh, 1824 stat. nov.
Class 1. Centricea Schütt, 1896 stat. nov. orthog. emend. (syn. Coscinodiscophyceae Round & Crawford 1990)
Subclass 1. Eucentricidae subcl. nov.
Subclass 2. Corethrophycidae Round and Crawford, 1990
Subclass 3. Rhizosoleniophycidae Round and Crawford, 1990
Class 2. Pennatea Schütt, 1896 stat. nov. orthog. emend. (syn. Fragilariophycidae Round, 1990)
Subclass 1. Araphoidae
Subclass 2. Raphoidae subcl. nov.
Subphylum 4. PSEUDOFUNGI Cavalier-Smith, 1986 emend. 1989
Class 1. Pythiistea Cavalier-Smith, 1986 stat. nov. 1989
Subclass 1. Oomycetidae Winter in Rabenhorst, 1879 stat. nov. Cavalier-Smith, 1989
Subclass 2. Hyphochytridae orthogr. emend. Sparrow ex Dick, 1983 stat. nov. Cavalier-Smith, 1989

Phylum 2. HAPTOPHYTA Hibberd ex Cavalier-Smith, 1986
Class 1. Patelliferea cl. nov. (orders: Isochrysidales, Coccosphaerales, Prymnesiales)
Class 2. Pavlovea Cavalier-Smith, 1986 stat. nov. (order: Pavlovales)

there was no justification for the Labyrinthulidae ever to have been placed in the Protozoa; they do not even feed by phagocytosis: perhaps it was just the obsolete "if it moves, it must be animal" story. Labyrinthulea are obviously not fungi (they have no cell walls and have tubular cristae like all Chromobiota), obviously not plants (they have no plastids), and obviously not protozoa (they are not phagotrophic). They are equally obviously heterokont chromists, where Chromista are defined as eukaryotes with retronemes and/or chloroplasts surrounded by a periplastid membrane within the RER lumen or a smooth endomembrane they are an excellent example of protists that have no place in the classical plant/animal/fungus kingdoms but have an obvious place in the more recently created fourth

kingdom of higher eukaryotes derived from protozoa, namely, Chromista.

The subphylum Pseudofungi (oomycetes plus hyphochytrids) also has a natural place in the heterokont Chromista; they clearly evolved a fungus-like mode of nutrition independently of the kingdom Fungi, as has the opalozoan *Nephromyces* (44, 119a). This convergence is not surprising since it merely requires a wall and the absence of photosynthesis. Indeed, plants in both subkingdoms have evolved a saprophytic or parasitic fungus-like mode of feeding: the colorless parasitic red algae in the Biliphyta, and a variety of saprophytic angiosperms and other green plants. But unlike pseudofungi, these achlorophyllous plants have always retained leukoplasts, possibly because in plants the plastid, not the cytosol, is the site of fatty acid synthesis (39).

Apart from *Goniomonas*, the only chromist classes that are purely phagotrophic, and therefore like typical protozoa in nutrition, are the Bicoecea and Oikomonadea; bicoecids have been studied mainly by botanists, and it is unlikely that protozoologists will object to their inclusion in the "botanical" kingdom Chromista, since they are not even mentioned in the revised classification (89) or in the *Illustrated Guide to the Protozoa* (83) and are commonly lumped with the Chrysomonadea. (*Oikomonas* was also omitted from reference 89 and from the systematic section of reference 83.)

Phagotrophic species are frequent in three of the photosynthetic chromist classes (Pedinellea, Chrysomonadea, and Patelliferea), and one phagotrophic cryptomonad is known, but such a retained ancestral character is of less classificatory importance than the derived characters that they share with other chromists and is therefore insufficient to justify the retention of these three taxa in the Protozoa, any more than does the probable occurrence of phagocytosis in one prasinophyte and one chytrid necessitate the merger of Fungi and Plantae with Protozoa. Cell walls (or frustules) probably evolved polyphyletically within the Chromista and finally abolished phagotrophy in those lineages. The giant kelps of the Phaeophycea (brown algae) represent the pinnacle of chromist evolution and have tissue differentiation at least as complex as any within the kingdom Fungi. Since Plantae, Fungi, Animalia, and Chromista all evolved from Protozoa, it is not surprising that their more lowly members are less easy to separate from protozoa than their peaks of evolution represented by the tree, mushroom, giraffe, and kelp, all of which are so radically different from the average protozoan that one would not want any of them in the same kingdom as *Paramecium*. Recognition of the Chromista simultaneously solved the problem posed by the polyphyly of Whittaker's Plantae and Fungi by providing a proper home for the Phaeophycea and the Pseudofungi, without having to lump them, respectively, with Plantae or Fungi or, alternatively, both together with Protozoa in the catchall Protoctista.

DISTINCTION BETWEEN PROTOZOA AND ANIMALIA

It is easier to draw a sharp line between Protozoa and Animalia than between Protozoa and the two largely photosynthetic kingdoms (Plantae and Chromista). Nonetheless, the boundary is usually placed in the wrong place: Mesozoa are usually included in Animalia rather than Protozoa, Protista, or Protoctista. This in my view (21) makes Animalia polyphyletic. Although Mesozoa are multicellular like true Animalia, the type and arrangement of their cells do not suggest any specific relationship to Animalia sensu stricto. Because of this and because they have tubular cristae like Protozoa, not plate-like cristae as in most animals (including the two most primitive phyla, Porifera and Cnidaria), I transferred the phylum Mesozoa into the kingdom Protozoa (21, 31). Moreover, dicyemid mesozoa, at least, have a double-stranded ciliary necklace (4) like ciliates and opalinids, not a triple-stranded necklace as in invertebrate animals. One cannot therefore define Animalia by multicellularity alone, which is too vague a character, since it has evolved independently numerous times in the history of life. More important is the presence of collagenous connective tissue sandwiched between two dissimilar epithelial cell layers: this, I believe, is the synapomorphy that best defines Animalia, and it is not present in Mesozoa.

Those who have been happy to include kelps in the same kingdom as Protozoa should be even happier to include Mesozoa in the kingdom Protozoa since they are really only one or two steps beyond Opalinida in having a ciliated epithelium rather than a ciliated syncytium and in having segregated germ cells. They show no higher degree of cell differentiation than the multicellular spores of the traditional protozoan phylum Myxosporidia (= Myxozoa). It is sometimes suggested that Myxozoa should be placed in Metazoa (= Animalia) rather than Protozoa because of their multicellular character. But this also must be resisted since it would make Animalia polyphyletic. It is the layered epithelial body organization with collagenous connective tissue (containing a variety of other characteristic proteins, such as fibronectin) that is unique to Animalia, and never found in Protozoa, not multicellularity per se.

DISTINCTION BETWEEN PROTOZOA AND ARCHEZOA

In contrast to the four higher kingdoms derived from Protozoa, the kingdom Archezoa is superficially similar to most Protozoa in that it consists of unicellular phagotrophic or micropinocytotic, nonphotosynthetic eukaryotes which lack a cell wall in the trophic phase. However, in fundamental cellular organization it is much more radically different: Archezoa comprise three phyla (Archamoebae, Metamonada, and Microsporidia), which differ from most Protozoa in having 70S ribosomes, like bacteria, rather than 80S ribosomes as in most other eukaryotes and in never having mitochondria, peroxisomes, hydrogenosomes, or well-developed Golgi dictyosomes. The classification of the Archezoa is shown in Table 3. If the absence of mitochondria, peroxisomes, and dictyosomes in the three phyla were the result of independent secondary losses (and all three organelles have been lost independently in other protists), there would be no justification for grouping these three phyla together in a major taxon or for separating them from Protozoa as a distinct kingdom. However, for the Metamonada and to a lesser extent for the Microsporidia, at least, there is reasonably strong evidence from rDNA phylogeny (121, 127, 128, 142) and the character of their ribosomes (74, 143) for the view (20, 21, 27, 28, 30, 31, 33, 34, 35, 40) that they are primitively without mitochondria, peroxisomes, and dictyosomes and that they represent a surviving relic of a very early stage in eukaryote evolution before these three organelles evolved.

This means that evolution of eukaryotes can be divided into two major phases: first, the origin of the eukaryote cell itself (i.e., the first archezoan, during which the endomembrane system, cytoskeleton, nucleus, and 9+2 cilia evolved [27, 130]); and second, the symbiotic origin of mitochondria and peroxisomes (28, 33, 43) to produce the first energeti-

TABLE 3. Classification of Kingdom Archezoa Cavalier-Smith, 1983 stat. nov. et emend. 1987

Phylum 1. Archamoebae Cavalier-Smith, 1983 (See reference 31 for details.)
Class 1. Pelobiontea Page, 1976 stat. nov. Cavalier-Smith, 1981, emend. 1991
Orders Mastigamoebida Frenzel, 1892 (syn. Rhizo-flagellata Kent, 1880) (e.g., *Mastigamoeba*, *Mastigina*, *Mastigella*, *Pelomyxa*); Phreatamoebida Cavalier-Smith, 1991 (*Phreatamoeba*)

Phylum 2. Metamonada Grassé, 1952 stat. nov. et emend. Cavalier-Smith, 1981
Subphylum 1. Eopharyngia subph. nov.[a]
Class 1. Trepomonadea cl. nov. (cortical microtubules absent from most of cell surface)
Orders Diplomonadida Wenyon, 1926 emend. Brugerolle, 1975 stat. nov.; Enteromonadida Brugerolle, 1975 stat. nov.
Class 2. Retortamonadea Grassé, 1952 stat. nov. (with cortical microtubules over entire body surface)
Order Retortamonadida Grassé, 1952
Subphylum 2. Axostylaria Grassé, 1952 emend. stat. nov.[b]
Class Oxymonadea Grassé, 1952 stat. nov. Margulis, 1974
Order Oxymonadida Grassé, 1952

Phylum 3. Microsporidia[c] Balbiani, 1882 stat. nov. Weiser, 1977
Subphylum 1. Rudimicrospora subphyl. nov. (a broader concept than class Microsporea Sprague, 1977); (polaroplast absent; spores usually spherical, rarely rod shaped)
Class 1. Metchnikovellea Weiser, 1977 (polar tubes lacking an outer honeycomb layer; manubroid, nonspiral)
Order 1. Metchnikovellida Vivier, 1975
Class 2. Minisporea cl. nov. (manubrium absent; polar tube coiled, with honeycomb outer layer)
Order Minisporida Sprague, 1972
Subphylum 2. Polaroplasta subphyl. nov. (polaroplast present; spores usually oval, rarely rod shaped or pyriform)
Class 1. Pleistophorea cl. nov. (multiply by plasmotomy; one spore type)
Order Pleistophorida Stempell, 1906
Class 2. Disporea cl. nov. (multiply by binary fission; disporogenic, i.e., two spore types)
Subclass 1. Unikaryotia subcl. nov. (single nucleus throughout)
Subclass 2. Diplokaryotia subcl. nov. (diplokaryotic, two associated nuclei); e.g., *Nosema*, *Vairimorpha*

[a] Metamonads with one or two tetrakont kinetids, lacking a contractile axostyle, and usually with one or two cytostomes and cytopharynxes; sex unknown.
[b] Metamonads with two, four, or six bikont kinetids joined by a paracrystalline paraxostyle; contractile axostyle typically present; cilia wrapped around body in left-handed spiral; cytopharynx absent, diploid or haploid sexual life cycle. (In contrast to Grassé, Axostylaria and Metamonada now both exclude Parabasalia.)
[c] Microspora Sprague, 1977 is an undesirable phylum name since *Microspora* is a green alga; for a good cladistic treatment of microsporidian diversity, see reference 81d.

cally efficient, aerobically respiring protozoan able to make ATP by oxidative phosphorylation and efficient β-oxidation of lipids (33, 43). The development of a permanent Golgi dictyosome and the changeover from 70S to 80S ribosomes may have occurred later still (38, 39, 43, 45b). The transition from a primitive archezoan obtaining energy by glycolysis to a well-developed, aerobically respiring protozoan involved a much larger number of fundamental changes in cell and macromolecular structure than occurred during the transition between Protozoa and any of the four higher eukaryote kingdoms. For this reason, I am convinced that the distinction between Archezoa and all other eukaryotes should be recognized by the highest possible taxonomic ranking within the Eukaryota. I therefore have grouped the five kingdoms Protozoa, Chromista, Fungi, Animalia, and Plantae into a superkingdom Metakaryota (26, 32, 45b) and also created a superkingdom Archezoa (containing only the kingdom Archezoa). These changes made it necessary to raise both Eukaryota and Bacteria in rank from superkingdom to empire. Table 1 summarized the resulting eight-kingdom system; I believe it to be phylogenetically sounder than Whittaker's five-kingdom system (147) with its three polyphyletic higher kingdoms and to be a better representation of the major megaevolutionary cleavages within the tree of life than Margulis's five-kingdom system (96).

Originally, I treated Archezoa only as a subkingdom of Protozoa. But this was before I was aware of the evidence for 70S ribosomes (such evidence is still not available for Archamoebae [and needs to be confirmed by broader surveys even in the other two phyla], but there are good ultrastructural arguments for their inclusion in Archezoa [36]) or realized that peroxisomes also were uniformly absent; it was also at a time when the idea of the primitiveness of the archezoan phenotype (20, 21) was only a good working hypothesis, rather than one well substantiated by rDNA phylogeny and by the prokaryotic-like features of microsporidian 23S rRNA (143) and *Giardia* 16S rRNA (128). Conservative protozoologists may wish to retain Archezoa as a subkingdom of Protozoa, but in my view there is a tremendous gain in predictive value in making the primary division within eukaryotes that between superkingdoms Archezoa and Metakaryota, and this obviously cannot be done by retaining Archezoa within the same kingdom as Protozoa. All protozoa are fundamentally chimeric in origin, having arisen by the permanent incorporation of symbiotic bacteria into a metamonad archezoan host to form mitochondria (39) and probably also peroxisomes (33); the distinction between archezoa and protozoa lies not in the mere absence or presence of mitochondria and peroxisomes, since several protozoan taxa have independently lost peroxisomes and nearly as many have also totally lost mitochondria or else converted them into hydrogenosomes. Archezoa are defined as eukaryotes that are primitively without mitochondria (21) and peroxisomes (26): thus, they had an autogenous, nonsymbiotic origin (33a) and, unlike all other eukaryotes, are not cellular and genomic chimeras.

Transitional Problems in Narrowing the Definition of Protozoa

Some may object to the retention of the name Protozoa, following the removal of the nutritionally protozoa-like Archezoa and Bicoecea, but it has been common practice throughout taxonomic history when removing minority atyp-

ical groups from established taxa to retain the original name for its majority constituents: well-known examples of this are subphylum Insecta (that once included hydra), Protozoa (that included rotifers), and Animalia (that included protozoa and bacteria). In such cases, the value of historical continuity of well-known names outweighs the temporary confusion caused by the refinement in their meaning by the removal of aberrant minorities. I hope that this will be true for the kingdom Protozoa, which in the refined sense advocated here corresponds closely to historical usage and to most biologists' idea of what protozoa are. Recently, protozoa (with a lowercase p) have been defined simply as phagotrophic protists (60); this certainly corresponds closely to the traditional protozoologist's sphere of interest, but though ecologically useful, it is inadequate for systematic purposes for three reasons: first, the classical problem with euglenoids and dinoflagellates, which are clearly valid taxa even though only some are phagotrophs; second, because the most clear-cut demarcating line between Protozoa and Chromista does not fall exactly along the phagotrophy/nonphagotrophy divide; and third, because phagotrophy is found on both sides of the even more fundamental archezoan/metakaryote distinction. This is not surprising since phagotrophy is an ancestral paraphyletic character that evolved during the origin of eukaryotes (and probably played the key role in that radical transformation of their eubacterial ancestor [27, 35, 37, 130]) and therefore on its own is not a sufficient reason for grouping together organisms to form a major eukaryotic taxon.

However, phagotrophy remains a useful aid to defining protozoology, which I suggest is the study of Protozoa, Archezoa, and phagotrophic chromists. Protozoology thus covers a broader field then the kingdom Protozoa, and protistology covers a broader field still, that of all protists (small p), that is, unicellular, colonial, filamentous, plasmodial, and minimally differentiated multicellular eukaryotes (17). There is value in both the protozoological and the protistological perspectives, depending on the problem in hand; neither classification of biologists corresponds to a single kingdom in the eight-kingdom system, and in this age of glasnost there is no reason why it should.

Exclusion of Parabasalia from Archezoa

Originally, Archezoa included one major taxon now removed from it: the Parabasalia. Parabasalia differ from Archezoa in two important ways: (i) they have exceptionally well-developed dictyosomes, and (ii) they have hydrogenosomes. They also branch higher up the eukaryote rDNA tree (78, 127) (Fig. 1) than true Archezoa; this is consistent with my thesis that they are not primitively amitochondrial and that their hydrogenosomes may have evolved from mitochondria (28, 33). The similarity of the trichomonad hydrogenosomal ferredoxin presequence (78a) to presequences of mitochondrial proteins is consistent with a mitochondrial origin, as is the absence of the peroxisomal type of targeting sequences from trichomonads (79a). In some anaerobic ciliates the hydrogenosomes have crista-like membranes, which gives some support to a possible origin from mitochondria (61c); this, like the presence of peroxisomal targeting sequences in fungal hydrogenosomes (99a), however, is no evidence for the ancestry of parabasalian hydrogenosomes, since hydrogenosomes are almost certainly polyphyletic (28). However, like bacteria and Microsporidia (74, 143), Parabasalia have 70S ribosomes (46b): whether Parabasalia diverged before (86a) or after (Fig. 1) (38, 43) the adictyosomal Percolozoa, which (except for the lyromonads) do have mitochondria, is of key importance for deciding whether they rightly belong in the Archezoa rather than in the Protozoa sensu stricto as I treat them here. The 18S rRNA tree (Fig. 1) at present does not unambiguously resolve this question. A recent study based on several trichomonad longer partial 28S rRNA sequences also did not resolve the issue; Parabasalia branched above Euglenozoa when a *Giardia ardeae* sequence was included but below Euglenozoa when it was excluded (139a).

Leipe et al. (86a) have recently claimed to have shown by rRNA sequence analysis that Parabasalia diverged before the metamonad diplomonads, but this claim is not supported by the data shown in their paper. They tested the effects of using different bacterial outgroups on the early branching order of eukaryote taxa and found four topologically different trees: different outgroups gave different trees, but of the 41 different trials, 22 (over half) gave one or the other of the two trees that had Parabasalia diverging after diplomonads. Thus, their analysis weakly supports the later divergence of the Parabasalia: the opposite to what they claim.

Exclusion of Entamoebia from Archezoa

A second problematic taxon once included in the Archezoa (21), but later excluded from it (36), is the Entamoebidae. Molecular sequence trees give conflicting evidence as to whether they are primitively or secondarily without mitochondria; the rRNA tree supports the idea of a secondary loss of mitochondria and peroxisomes (much more strongly than for Parabasalia), while the elongation factor 1α tree supports their original absence (68a), as do several other characters (43, 103). However, contrary to what is often said about the absence of Golgi dictyosomes in *Entamoeba* spp., there is at least one published micrograph showing a small dictyosome (65). Moreover, like metakaryotes but unlike Archezoa, they have spliceosomal introns (41a). I therefore continue to exclude Entamoebidae from the Archezoa and place them in the kingdom Protozoa in the subkingdom Dictyozoa, as a new phylum Entamoebia: in view of the conflicting evidence, we cannot yet totally exclude the possibility that they might be archezoa after all (43), but detailed study of the rRNA sequence alignment convinces me that they really are secondarily amitochondrial; the 18S rRNA tree suggests that they may have been derived from mycetozoan amoebae.

Are Microsporidia Archezoa or Protozoa?

Unlike Parabasalia, Microsporidia have no hydrogenosomes or permanent well-developed Golgi dictyosomes, so there are no ultrastructural reasons to suspect that they have been misplaced in the kingdom Archezoa and are secondarily derived from Protozoa by the loss of mitochondria and peroxisomes. The fact that, unlike the two archezoan phyla, both of which have free-living members, microsporidia are obligate intracellular parasites of eukaryotes with mitochondria has, however, aroused some skepticism as to their primitively amitochondrial character: could they have suffered extreme parasitic reduction, including the loss not only of mitochondria and peroxisomes but also of lysosomes, cilia, and centrioles (the latter three organelles are present in all other Archezoa but absent from microsporidia)? Initially, the presence of 70S ribosomes in Microsporidia (74, 143) appeared to support their inclusion in the Archezoa since this appeared likely to be an ancestral character derived

directly from bacteria. The same was true for the demonstration that Microsporidia, like bacteria, have no separate 5·8S rRNA (143); the corresponding sequences are included as part of the 23S rRNA molecule.

However, the recent demonstration that trichomonads also have 70S ribosomes (46b) diminishes the force of this argument because of the reasons for thinking that Parabasalia are secondarily amitochondrial, i.e., the presence of double-membraned hydrogenosomes, perhaps derived from mitochondria, and of Golgi dictyosomes. If Parabasalia really are secondarily amitochondrial, then either the transition from 70S to 80S ribosomes occurred after the origin of mitochondria or else it is possible for 70S ribosomes to evolve secondarily from 80S ribosomes. In either case, the 70S ribosomes of microsporidia are not sufficient evidence that microsporidia are primitively amitochondrial. Likewise, the recent establishment of Percolozoa (38, 43) as a phylum of mitochondrion-containing protozoa that probably primitively lack Golgi dictyosomes implies that dictyosomes evolved after mitochondria: thus, their absence from microsporidia, contrary to earlier assumptions (26, 30, 31), cannot be used to support the archezoan status of microsporidia: they might instead belong in the Adictyozoa, together with Percolozoa (indeed, they formed a clade with Percolozoa in the consensus tree from which the bootstrap values for Fig. 1 were taken).

The absence of 5·8S rRNA also is not a strong argument, since a single deletion could remove the RNA processing site from the pre-rRNA that is recognized by the enzyme that cleaves it to generate 5·8S plus 28S rRNA and thus secondarily make their large subunit rRNA resemble bacterial 23S rRNA. Now that several microsporidian small-subunit rRNA sequences are available, it is clear that they share several unique deletions, since pieces are missing that are present in bacteria as well as in all other eukaryotes, making the microsporidian smaller than any nonmitochondrial small-subunit rRNA. Since this small size of the small-subunit rRNA is certainly the result of a secondary shortening and simplification of microsporidian rRNA, it is highly plausible that this is true also for their 23S rRNA. Elsewhere (45b), I have suggested that the gain and loss of mitochondria might be expected to have caused increases and decreases, respectively, in the size of rRNA and the number of attached proteins as well as changes in the rRNA nucleotide sequence, because of the need, when mitochondria are present (but not otherwise), to prevent mitochondrial ribosomal proteins made in the cytosol from binding to and interfering with cytosolic rRNA. Conceivably, therefore, the 70S character of both microsporidian and parabasalian ribosomes might, in part at least, be a secondary response to the very early loss of mitochondria. A study of metamonad (putatively primitively amitochondrial) and of percolozoan ribosomes would usefully test this hypothesis (are they 70S or 80S?) and clarify the significance of the 70S ribosomes of Microsporidia and Parabasalia.

The recent analysis of Leipe et al. (86a) and my own unpublished studies show that the position of Microsporidia on the rRNA tree is not very robust and is sensitive to which bacterial outgroup is chosen, especially if only one bacterium and one microsporidian are included.

Leipe et al. found that microsporidia branch lower down than Parabasalia in 26 of 41 trees. The branching order of Percolozoa, Parabasalia, and Microsporidia in Fig. 1 was different in the bootstrapped consensus tree where Percolozoa and Microsporidia actually formed a clade, but the bootstrap value for this clade (67%) was sufficiently low that one cannot have much confidence that either topology is correct: indeed, the branching order of these three phyla may never be unambiguously resolvable by rRNA sequence trees. The three phyla must have diverged very close to the time of origin of mitochondria. Since Fig. 1 is based on 26 bacteria and 4 microsporidia, it is probably more reliable than that of Sogin's group (86a) which used only 1, 2, 3, or 6 bacteria, only 1 microsporidian, and only 1 percolozoan. When the tree shown in Fig. 1 was rerun with only *Methanococcus voltae* as the bacterial outgroup, however, it did show microsporidia a little below the metamonads.

One reason for considering the possibility that microsporidia may be secondarily amitochondrial is that Vossbrinck and DiMaria (141c) have good evidence for U2, and preliminary evidence for U6, spliceosomal small nuclear RNAs in microsporidia. If, as I have proposed (38a), spliceosomal introns originated from group II introns after the latter were introduced into the nucleus as a result of the symbiotic origin of mitochondria, then this would imply that they must once have had mitochondria. However, although the recent discovery of group II introns in proteobacteria and cyanobacteria (61b) supports one of the key assumptions of this theory of the origin of spliceosomal introns, the other key assumption (that spliceosomal introns are absent from primitively amitochondrial eukaryotes) has still not been sufficiently rigorously tested. Only about a dozen protein-coding genes have so far been sequenced from the metamonad *Giardia*; the fact that none have introns, whereas introns have been found in Percolozoa (117c) although fewer genes have been sequenced, means that they must be rarer than in Percolozoa, but until many more *Giardia* genes are sequenced, it would be premature to conclude that they are totally absent, as this theory predicts.

Clearly, whether Microsporidia should be classified in Archezoa or Protozoa cannot yet be determined with great confidence. But since there is still no strong evidence that they are secondarily amitochondrial, I leave them in the Archezoa. If, however, clear evidence were to be found that they are secondarily amitochondrial, it would be necessary to transfer them from the kingdom Archezoa to the kingdom Protozoa and to place them with the Percolozoa (which themselves have two amitochondrial genera in the new class Lyromonadea; see below) in the subkingdom Adictyozoa, which is characterized by the absence of Golgi dictyosomes.

The view that Microsporidia are more primitive than Archamoebae because they lack cilia (115b) is not well based. Cilia have been lost numerous times during eukaryotic evolution: at least two other amitochondrial taxa (Entamoebia and the parabasalian *Dientamoeba*) have no cilia. The 18S rRNA tree (Fig. 1) confirms that all of these taxa have secondarily lost cilia and supports the view that cilia evolved at the same time as the nucleus (27, 30a), that all nonciliate eukaryotes are ultimately derived from ancestors with cilia, and that mitochondria evolved substantially after cilia in a tetraciliate host (35, 37, 38, 40, 43).

Figure 2 shows the 18 phyla that I include within the kingdom Protozoa and their postulated evolutionary relationships with other organisms.

DIAGNOSIS OF THE KINGDOM PROTOZOA

"Unicellular phagotrophic eukaryotes with mitochondria" would be a very simple definition that would include the vast majority of Protozoa and exclude very few, but it would also include a few Chromista. Such a diagnosis would also not be sufficiently precise to define the kingdom's exact

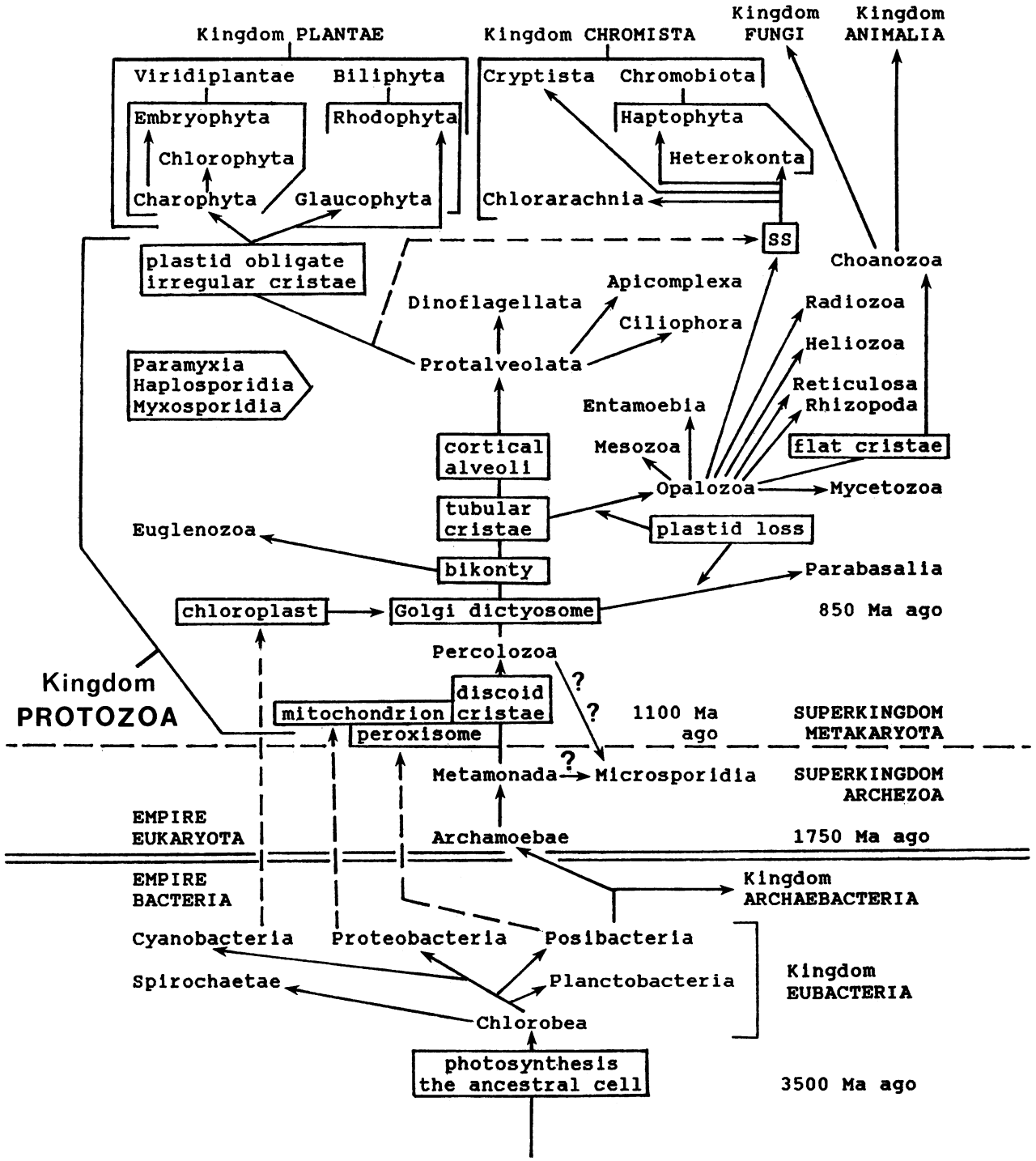

FIG. 2. Phylogenetic relationships between the protozoan and archezoan phyla and the other six kingdoms based on an integration of ultrastructural cladistics, rDNA sequence trees, and the fossil record; for more detailed discussion of protozoan phylogeny, see references 38, 40, 42, and 43. Three phyla are not attached to the tree because of the lack of clear evidence as to where to put them. Probably all three phyla evolved after the origin of tubular cristae. The protozoan phylum Dinozoa is shown as its constituent subphyla: Dinoflagellata and Protalveolata. The dashed lines indicate the four major symbiotic origins of organelles in the history of life: the symbiotic origins of mitochondria, peroxisomes, and chloroplasts and the secondary symbiosis between two eukaryotes (SS) that created the Chromista (39). Three features of this tree are particularly uncertain: (i) the relative branching order of Parabasalia and Percolozoa (published 18S rRNA trees suggest that Parabasalia branched off before, rather than after, Percolozoa, though Fig. 1 suggests the reverse); (ii) the timing of the origin of chloroplasts (if euglenoids obtained their chloroplasts secondarily from another eukaryote (64, 133), rather than by divergence from the ancestral photosynthetic eukaryote (18) as assumed here, then the origin of chloroplasts would have been later than shown, just after the origin of cortical alveoli); and (iii) the position of the Microsporidia: some rRNA trees place microsporidia below Metamonada (86a), but I consider that on present evidence one of the alternatives shown here is more likely. Only a selection of the major eubacterial taxa is shown.

limits. The most simple, yet accurate phylogenetic definition of the kingdom Protozoa is as follows: eukaryotes, other than those that primitively lack mitochondria and peroxisomes (Archezoa), which lack the shared derived characters that define the four higher, derived kingdoms, Animalia, Fungi, Plantae, and Chromista. Clearly, because it has to be distinguished both from the ancestral kingdom Archezoa and from the four kingdoms derived from it, the definition of the kingdom Protozoa is necessarily more complex than is that of the other seven kingdoms of life. Converting the preceding phylogenetic definition into a proper descriptive diagnosis is complicated by the fact that, even when limited to the taxa presently included, the kingdom is distinctly more diverse cytologically than the other eukaryote kingdoms and because within metakaryotes as a whole many characters have been gained and/or lost polyphyletically and convergently (e.g., chloroplasts, mitochondria, peroxisomes, hydrogenosomes, and multicellularity). Nonetheless, a precise diagnosis of the kingdom Protozoa is possible, as follows.

Predominantly unicellular, plasmodial or colonial phagotrophic eukaryotes, wall-less in the trophic state. Primitively possessing mitochondria and peroxisomes (unlike Archezoa); when mitochondria and peroxisomes are both secondarily absent (Parabasalia, Entamoebia, Lyromonadea, and anaerobic ciliates only), hydrogenosomes and/or Golgi dictyosomes are present instead. Ciliary hairs are never rigid and tubular (unlike most chromists); haptonema absent (excludes nonphotosynthetic [94a] haptophytes). Chloroplasts, when present (some euglenoids and dinoflagellates only), contain neither starch nor phycobilisomes (unlike in Plantae), have stacked thylakoids, and usually have three, rather than two, envelope membranes. Chloroplasts are located in the cytosol, never within a smooth periplastid membrane inside either the lumen of the rough endoplasmic reticulum or a fourth smooth membrane (unlike Chromista); ejectisomes never of the double-scroll cryptist type (this excludes the cryptist *Goniomonas*); the few multicellular species have minimal cell differentiation and altogether lack collagenous connective tissue sandwiched between two dissimilar epithelia (unlike Animalia).

It is obvious that such a precise and detailed diagnosis of Protozoa was impossible before the application of electron microscopy to nearly all of the major protist cell types and the use of these data to develop explicit phylogenies (16, 17, 18, 19, 21, 23, 25, 27, 29, 32, 34–38, 132, 133); together with the definitions of the kingdoms Archezoa and Chromista and firmer distinctions between the empires Bacteria and Eukaryota, it thus represents a major contribution of electron microscopy to megasystematics (55, 113).

THE TWO SUBKINGDOMS, TWO BRANCHES, TWO INFRAKINGDOMS, AND SEVEN PARVKINGDOMS OF PROTOZOA

Subkingdoms Adictyozoa and Dictyozoa

Protozoa were earlier divided into four subkingdoms (21). The three trophically unicellular, colonial or plasmodial subkingdoms were separated according to the nature of their mitochondrial cristae (21): Euglenozoa, with discoid mitochondrial cristae (17); Sarcomastigota (a taxon I have abandoned because of its heterogeneity), with tubular mitochondrial cristae or very rarely vesicular or flat cristae; and Choanozoa, with flattened (nondiscoid) cristae. The fourth subkingdom was the multicellular Mesozoa (21). The idea that the divergence between discoid and tubular cristae is the most fundamental one within Protozoa (at one time considered so fundamental as possibly to merit separate kingdom status for Euglenozoa [17]) has been amply confirmed by rDNA phylogenetics: the taxa with discoid cristae all group together and diverge from those with tubular cristae very close to the base of the metakaryotic clade in the rDNA tree (7, 121, 127) (see also Fig. 1). The rDNA tree also shows clearly that the flattened cristae of Fungi and Animalia are quite unrelated to the discoid cristae of the Euglenozoa and must be derived secondarily from tubular cristae, as suggested previously (18). Later I also treated the Parabasalia, which have double-enveloped hydrogenosomes in place of mitochondria (from which they may have evolved [28, 33]), as a separate protozoan subkingdom (35, 37, 38). More recently still, however, I have segregated a new phylum Percolozoa (38) from the Euglenozoa on account of their absence of dictyosomes and their commonly tetrakont character. I regard the absence of dictyosomes as of such phylogenetic importance (38, 43) that I now place the Percolozoa in a separate subkingdom and group all of the other, dictyosome-containing protozoa in the subkingdom Dictyozoa (38). This reduces the emended Euglenozoa in rank, as well as the Parabasalia, Choanozoa, and Mesozoa.

I here propose the new name Adictyozoa for the subkingdom made up of primitively adictyosomal Protozoa. At present Adictyozoa contains only the Percolozoa, but we cannot yet rule out the possibility that certain "archezoa" (e.g., archamoebae or microsporidia) might in the future need to be transferred into it if they proved to be secondarily amitochondrial. Thus, the primary division within Protozoa is between the subkingdoms Adictyozoa (which lack Golgi dictyosomes) and Dictyozoa (which all have Golgi dictyosomes): both subkingdoms have a phylum with discoid mitochondrial cristae (Percolozoa and Euglenozoa), and both have taxa that have lost mitochondria and ones that have lost cilia and centrioles.

New Dictyozoan Branches: Parabasalia and Bikonta

The subkingdom Dictyozoa is here divided into two primary branches: a new branch, Parabasalia, containing only the phylum Parabasalia, which have 70S ribosomes, Golgi dictyosomes that are attached to striated ciliary roots to form parabasal bodies, and a ciliary kinetid typically containing four centrioles (basal bodies); and a new branch Bikonta (the name was informally suggested earlier [43]) made up of 16 phyla that have 80S ribosomes, Golgi dictyosomes not associated with striated ciliary roots, and a ciliary kinetid typically containing only two centrioles. In both branches, the kinetid has been secondarily lost in the ancestors of most species that have no cilia, and in a very few bikont groups (the opalozoan *Phalansterium* and many ciliates) the kinetid is secondarily reduced to a single centriole. Parabasalia have double-membraned hydrogenosomes instead of mitochondria; Bikonta usually have mitochondria, but in some taxa (Entamoebia and a few ciliates) they have been lost, and in several anaerobic ciliates they have been replaced by or converted into hydrogenosomes (61c).

Infrakingdoms Euglenozoa and Neozoa

As discussed previously (43), the primary division within Bikonta is between the phylum Euglenozoa and the other 15 phyla which I grouped recently together into the infrakingdom Neozoa (43). Euglenozoa are apparently unique among

eukaryotes in that all of their nuclear protein-coding genes are subject to *trans*-splicing of miniexons (102b), whereas Neozoa (only a minority of the phyla have been studied in this respect) have typical *cis*-splicing as in higher eukaryotes. In contrast to Euglenozoa and Percolozoa, which have discoid mitochondrial cristae, most neozoan phyla have tubular cristae. Only the phylum Choanozoa (and a few members of other phyla) have flat cristae like those of animals and fungi.

In contrast to Archezoa (three phyla), Percolozoa, Parabasalia, and Euglenozoa, which to students of higher eukaryotes are all peculiar in several different ways, the Neozoa are very similar in cell structure (except for the secondarily amitochondrial taxa) and probably in genomic organization (except for the Ciliophora which have several peculiarities [63b] because of the evolution of the macronucleus) to higher eukaryotes (which themselves all evolved from Neozoa and not from any of the six most primitive and most aberrant eukaryotic phyla).

The New Category Parvkingdom

Because of the diversity and large number of the 15 neozoan phyla, it is desirable to group them into superphyla, and also to group the superphyla into a smaller number of taxa intermediate in rank between infrakingdom and superphylum, in order to show their differing degrees of relatedness and/or similarity. Since there is no established category at this rank, I propose the use of parvkingdom; this follows the precedent of Sibley and Ahlquist (123a), who use the prefix parv- (as in parvclass and parvorder) to signify categories of rank intermediate between those denoted by infra- and super-. The infrakingdom Neozoa is here divided into seven parvkingdoms; two of these are subdivided into superphyla.

Mesozoa as Multicellular Protozoa

Previously, mesozoa were often traditionally regarded as a subkingdom of the kingdom Animalia (147), though Margulis once briefly put them in the Protista (95). Now that they and protozoa are together both separated from Animalia (Animalia is now equivalent to the former subkingdom Metazoa) in their own kingdom, it is appropriate to treat them as a distinct parvkingdom within the Neozoa to emphasize the fact that they are the only multicellular protozoa with multicellular cell differentiation in their trophic phase: the Myxosporidia are multicellular only in their reproductive phase (as are the Dictyostelea, Myxogastrea, and the aggregative ciliate *Sorogena*), but this I think does not justify the separation of any of these taxa as separate subkingdoms, let alone kingdoms.

Myxozoa are Protozoa, not Animalia

Here Myxozoa also are treated as a protozoan parvkingdom (within the subkingdom Dictyozoa and infrakingdom Neozoa) made up of the three phyla Myxosporidia, Haplosporidia, and Paramyxia. The multicellular spores of these parasitic protozoa have led some authors to suggest that they are metazoa (see references in reference 91). However, the resemblance is entirely superficial. The unicellular amoeboid or plasmodial trophic phase of the Myxozoa has nothing in common with the triploblastic multicellular body structure of Animalia. Animals do not even have multicellular spores, and unlike animals, myxozoa have no cilia or flagella. The myxosporidian cnidocysts are no closer to cnidarian nematocysts than are some dinoflagellate extrusomes. Any long, thin, flexible extrusome is likely to acquire a spiral coiling once it reaches a certain length since this is the simplest way to pack it into a cell. The spirality of the unextruded filaments of myxosporidia, cnidaria, some dinoflagellates, and most microsporidia has almost certainly evolved independently four times: there is clear evidence from rDNA phylogeny that microsporidia, dinoflagellates, and cnidaria are almost as far apart from each other on the eukaryotic phylogenetic tree as it is possible to be (47, 121, 127, 143a) (Fig. 1). There is no good reason to think that myxosporidia will turn out to be related to any of these. At present, we also cannot say whether the multicellular spores of the three myxozoan phyla are convergent or reflect a common ancestry: their taxonomic position may need revision when molecular sequence data become available.

The New Parvkingdom Entamoebia

Entamoebidae are the only dictyozoa that totally lack cilia, mitochondria, peroxisomes, and hydrogenosomes. Since they are also unique in having an intranuclear centrosome that is present only during prophase, they are here placed in their own phylum and parvkingdom. Molecular sequence trees (Fig. 1) do not support any specific relationship with the Rhizopoda (represented by *Acanthamoeba* and *Hartmannella* spp. [121, 127]): Fig. 1 suggests that they may have evolved from nonciliated Mycetozoa by the loss of mitochondria and peroxisomes. A nonciliated protostelid of the family Protosteliidae would be the most suitable ancestor; unfortunately, no 18S rRNA sequences are yet available for any protostelid Mycetozoa.

Four Other New Parvkingdoms: Alveolata, Actinopoda, Neosarcodina, and Ciliomyxa

The primitive state for each of the 10 phyla included in these parvkingdoms appears to be a unicellular protozoan with a kinetid containing two centrioles, as in Euglenozoa, not four as in the more primitive Parabasalia and Percolozoa or none as in the Myxozoa and Entamoebia. One parvkingdom, Alveolata (phyla Dinozoa, Ciliophora, and Apicomplexa), characterized by the presence of cortical alveoli or their presumed derivatives, always has tubular mitochondrial cristae. The other three parvkingdoms have a majority of species with tubular and a minority with flat nondiscoid cristae: Actinopoda (phyla Heliozoa and Radiozoa Cavalier-Smith, 1987), characterized by axopodia and often kinetocysts and the absence of cilia in trophic phases; the Neosarcodina (phyla Rhizopoda and Reticulosa), characterized by the absence of both cilia and axopodia in their trophic phases and by the absence of aerial fruiting bodies; and the Ciliomyxa (phyla Opalozoa Cavalier-Smith, 1991, Choanozoa [choanoflagellates: the only neozoan flagellate phylum with flat cristae], and Mycetozoa), which also lack cortical alveoli and either have a ciliated trophic phase or aerial (often multicellular) fruiting bodies containing spores. Of these four parvkingdoms, only Alveolata is supported by very clear-cut ultrastructural synapomorphies and (at present) by molecular sequence data; it is very probably monophyletic. The other three parvkingdoms might be polyphyletic, though need not be; although all three contain at least some species with somewhat or definitely flattened cristae, this is not (contrary to what is sometimes assumed) a certain indication of polyphyly: indeed, it is highly proba-

ble that flat cristae themselves evolved polyphyletically from tubular ones.

I think it useful to retain these three taxa until such time as polyphyly is clearly established and we also have solid positive data to support an improved classification. As a result of the discovery of *Jakoba libera* (114), the distinction between flat and tubular cristae appears to be less fundamental than originally thought, since apart from having flattish rather than tubular cristae, *Jakoba libera* is not radically different from certain other opalozoan (heteromitean or kinetomonadean) flagellates with ventral grooves and three microtubular roots. This point is even more strongly made by the recent comparison (106) of *Jakoba* and the new genus *Reclinomonas* (61d), which has tubular cristae. Both *Jakoba* and *Reclinomonas* clearly have to be included in the same phylum (Opalozoa) (44): so does *Ancyromonas*, also with flat cristae (44). Two other bikont phyla (Rhizopoda and Heliozoa) have some species with flat, and others with tubular, cristae. Although I continue to believe that this is an important systematic distinction (132), we must not assume that it necessarily indicates a polyphyletic origin for these two phyla. Even within the other actinopod phylum (Radiozoa), there are species with flat cristae (somewhat like the flattened tubular cristae of cryptomonads) in contrast to the tubulicristate majority (1). It would appear that the changeover from tubular to flat cristae has occurred several times, though infrequently enough to make crista shape nonetheless a useful systematic character.

These changes overall yield seven distinct parvkingdoms within the infrakingdom Neozoa, namely, Ciliomyxa, Alveolata, Neosarcodina, Actinopoda, Entamoebia, Myxozoa, and Mesozoa. The revised protozoan classification into 18 phyla and 65 classes is shown in Table 4.

PHYLUM PERCOLOZOA

The organisms segregated into this recently established phylum (*Percolomonas*, Heterolobosea, *Psalteriomonas*, *Lyromonas* gen. nov., and *Stephanopogon*) differ from all other Protozoa and resemble Archezoa in lacking Golgi dictyosomes. Except for *Psalteriomonas* and *Lyromonas* (which have no cristae or no mitochondria), they resemble each other in having mitochondrial cristae that are usually flattish and often somewhat discoid like those of Euglenozoa (but usually more irregular and less rigid in appearance), but which are sometimes (*Tetramitus*) a quite irregular mixture of flattish to somewhat tubular cristae, though never regular tubular cristae, as are characteristic of the vast majority of other Protozoa except Euglenozoa and Choanozoa. It is likely that the microbodies of the percolozoa (other than *Psalteriomonas* and *Lyromonas*) are peroxisomes, but cytochemical study to check this is needed. If percolozoans are indeed the first metakaryotes (38, 43), both their peroxisomes and mitochondria could have unusual and surprising properties. Though it is conceivable that they have secondarily lost dictyosomes, it seems more probable that they are primitively without them like the Archezoa (38, 43), but this should not be regarded as a firm conclusion without a great deal more critical study of the group and much more robust phylogeny for the early protozoa. They may be the most ancient true Protozoa: various odd, and somewhat disparate, relics of the days before dictyosomes evolved. Their disparate character is emphasized by the division into two subphyla and four classes (Table 4; Appendixes 1 and 2), even though the number of genera and species so far recognized is quite small: perhaps the paucity of percolozoan species is because dictyosomes actually have some use! Percolozoa include an important pathogen, *Naegleria fowleri*; the whole group deserves much more thorough study, not only for this reason but because they may have much to tell us about the cellular and molecular biology of the most primitive protozoa and metakaryotes. The rRNA tree clearly supports a very early divergence for *Naegleria* spp. and other Heterolobosea among metakaryotes (121, 127) (Fig. 1).

Psalteriomonas (10a) and *Lyromonas* (10, as *Psalteriomonas vulgaris*) differ from other Percolozoa, and resemble Parabasalia, in lacking peroxisomes and mitochondria and having hydrogenosomes instead; nonetheless, their kinetids show that they are clearly most closely related to the Heterolobosea (110). Whether they are primitively or secondarily without mitochondria is unclear. *Psalteriomonas lanterna* (10a) has double-membraned structures that might be either degenerate mitochondria (without cytochrome oxidase) or symbiotic bacteria. Because of these differences from other Percolozoa, a new class, Lyromonadea, is created for them (see below).

PHYLUM AND INFRAKINGDOM EUGLENOZOA

The grouping of euglenoids and kinetoplastans within the phylum Euglenozoa Cavalier-Smith, 1981 is now almost universally accepted (52, 78b, 81, 90, 113, 137, 138). The exclusion of *Stephanopogon* and the Heterolobosea (110), which have sometimes (17) also been included, and their transfer into the new phylum Percolozoa (38, 43), which unlike the Euglenozoa lacks Golgi dictyosomes, makes the phylum much more homogeneous. Both ultrastructural and molecular sequence data support the inclusion of *Diplonema* (which is neither a euglenoid nor a kinetoplastan) in the Euglenozoa (138), even though it has flat plate-like rather than flat discoid cristae, and I here create a new euglenozoan subphylum for it.

PARVKINGDOM ALVEOLATA AND ITS THREE PHYLA

The three phyla grouped here (Dinozoa, Ciliophora, and Apicomplexa) form a major pinnacle of protozoan evolution from the point of view of the structural complexity that can be attained within a single cell. All three phyla have been able to produce individual cells large enough to be visible with the naked eye, and many of them (e.g., the hypotrich ciliates [131]) probably have many more different genes than the simpler animals such as *Drosophila* and the nematode *Caenorhabditis*. Much of this complexity can be attributed to the varied uses to which they have put cortical alveoli, the shared character that distinguishes the group from all other Protozoa. They are here divided into two superphyla.

Superphylum Heterokaryota and Its Sole Phylum, Ciliophora

The phylum Ciliophora (ciliates and suctorians) is so well defined as to require no discussion of its contents. For its internal classification, I have followed Lynn and Small (92) as to classes and subclasses, although there are clear indications, from both molecular and ultrastructural data, that this will need revision. If, as I think likely (16, 38, 42), the Ciliophora are derived from a biciliate *Colponema*-like dinozoan with well-defined cortical alveoli, then the absence of the cortical alveoli in the Karyorelictea is unlikely to be ancestral for the phylum as a whole, and one should question

TABLE 4. Classification of the kingdom Protozoa Goldfuss, 1818 status nov. Owen, 1858/9 emend. Cavalier-Smith, 1987[a]

Subkingdom 1. ADICTYOZOA subking. nov.
Phylum 1. PERCOLOZOA Cavalier-Smith, 1991
Subphylum 1. Tetramitia Cavalier-Smith, 1993
Superclass 1. Percolomonada supercl. nov.
Class 1. Percolomonadea Cavalier-Smith, 1993 (order Percolomonadida Cavalier-Smith, 1993)
Superclass 2. Striatorhiza supercl. nov.
Class 1. Heterolobosea Page and Blanton, 1985 emend. (syn. Acrasea Olive, 1975 emend. Cavalier-Smith, 1987) (orders Schizopyrenida Singh, 1952; Acrasida Shröter, 1886 emend. Page & Blanton, 1985)
Class 2. Lyromonadea cl. nov. (order Lyromonadida ord. nov.)
Subphylum 2. Pseudociliata Cavalier-Smith, 1993
Class 1. Pseudociliatea Cavalier-Smith, 1981 (sole order Pseudociliatida Corliss and Lipscomb, 1982; family Stephanopogonidae Corliss, 1961)

Subkingdom 2. DICTYOZOA Cavalier-Smith, 1991
Branch 1. PARABASALIA new branch
Phylum 1. PARABASALIA Honigberg, 1973 stat. nov. Cavalier-Smith, 1981
Class 1. Trichomonadea Kirby, 1947 stat. nov. Margulis, 1974 (order Trichomonadida Kirby, 1947)
Class 2. Hypermastigea Grassi & Foà, 1911 stat. nov. Margulis, 1974 (orders Lophomonadida Light, 1927; Trichonymphida Poche, 1913)
Branch 2. BIKONTA new branch
Infrakingdom 1. EUGLENOZOA Cavalier-Smith, 1981 stat. nov.
Phylum 1. EUGLENOZOA Cavalier-Smith, 1981
Subphylum 1. Diplonemia subph. nov.
Class 1. Diplonemea cl. nov. (order Diplonemida ord. nov. [*Diplonema* = *Isonema*])
Subphylum 2. Euglenoida Bütschli, 1884 (as Euglenoidina) emend. Senn, 1900 stat. nov.
Class 1. Petalomonadea cl. nov. (order Petalomonadida ord. nov.)
Class 2. Peranemea cl. nov. (orders Ploeotiida ord. nov.; Peranemida Bütschli, 1884 stat. nov.)
Class 3. Aphagea cl. nov.
Subclass 1. Euglenia subcl. nov. (orders Astasida Ehrenberg, 1831 stat. nov.; Eutreptiida Leedale, 1967)
Subclass 2. Rhabdomonadia subcl. nov. (order Rhabdomonadida Leedale, 1967)
Subphylum 3. Kinetoplasta Honigberg, 1963 stat. nov.
Class 1. Kinetoplastea Honigberg, 1963 emend. Vickerman, 1976 stat. nov. Margulis, 1974 (= subcl. Bodonidea Hollande, 1958) (orders Bodonida Hollande, 1952 emend. Vickerman, 1976, Krylov et al., 1980; Trypanosomida Kent, 1880 stat. nov. Hollande, 1952)
Infrakingdom 2. NEOZOA Cavalier-Smith, 1983
Parvkingdom 1. CILIOMYXA parvking. nov.
SUPERPHYLUM 1. OPALOMYXA superphyl. nov.
Phylum 1. OPALOZOA Cavalier-Smith, 1991
Subphylum 1. Proterozoa Cavalier-Smith, 1981 emend. stat. nov. 1993
Class 1. Heteromitea Cavalier-Smith, 1993
Subclass 1. Sarcomonadia Cavalier-Smith, 1993
Superorder 1. Jakobidea Cavalier-Smith, 1993 {orders Cercomonadida Poche, 1913 emend. Grassé, 1952 [*Cercomonas*, *Heteromita*, *Massisteria*, *Discocelis* (141)]; Jakobida Cavalier-Smith, 1993 [*Jakoba* Patterson, 1990]}
Superorder 2. Thaumatomonadidea Cavalier-Smith, 1993 (order Thaumatomonadida Shirkina, 1987)
Superorder 3. Proteomyxidea Lankester, 1885 emend. stat. nov. Cavalier-Smith, 1993 (orders Pseudosporida Cavalier-Smith, 1993; Leucodictyida Cavalier-Smith, 1993)
Subclass 2. Thecomonadia Cavalier-Smith, 1993 {orders Apusomonadida Karpov & Mylnikov, 1989 [*Amastigomonas* and *Apusomonas* (incl. *Rostromonas*, reference 79]; Cryomonadida Cavalier-Smith, 1993 [*Cryothecomonas* (136)]}
Subclass 3. Anisomonadia Cavalier-Smith, 1993 (orders Diphylleida Cavalier-Smith, 1993; Proteromonadida Grassé, 1957 emend. Cavalier-Smith, 1993)
Subclass 4. Phagodinia subcl. nov. {order Phagodinida ord. nov. [*Phagodinium* (81a)]}
Class 2. Telonemea Cavalier-Smith, 1993 (orders Telonemida Cavalier-Smith, 1993; Nephromycida Cavalier-Smith, 1993 [*Nephromyces* Giard, 1888])
Class 3. Cyathobodonea Cavalier-Smith, 1993 (orders Pseudodendromonadida Hibberd, 1985; Spongomonadida Hibberd, 1983 [including Phalansteriida Hibberd, 1983]; Kathablepharida Cavalier-Smith, 1993)
Class 4. Ebridea Lemmermann, 1901 emend. Deflandre, 1936 stat. nov. Loeblich III, 1970 orthog. emend. (order Ebriida Deflandre, 1936)
Class 5. Phytomyxea Engler & Prantl, 1897 orthog. emend. (orders Phagomyxida Cavalier-Smith, 1993; Plasmodiophorida Cook, 1928)
Subphylum 2. Opalinata Wenyon, 1926 stat. nov. emend. Cavalier-Smith, 1993
Class 1. Opalinea Wenyon, 1926 stat. nov. emend. Cavalier-Smith, 1993 (orders Karotomorphida Cavalier-Smith, 1993; Opalinida Poche, 1913 stat. nov. Hall, 1953)
Subphylum 3. Kinetomonada Cavalier-Smith, 1993.
Class 1. Kinetomonadea Cavalier-Smith, 1993 (orders Histionida Cavalier-Smith, 1993; Heliomonadida Cavalier-Smith, 1993)
Subphylum 4. Hemimastigophora Foissner, Blatterer & Foissner, 1988 stat. nov.
Class 1. Hemimastigea Foissner, Blatterer & Foissner, 1988 (order Hemimastigida Foissner, Blatterer & Foissner, 1988 [*Spironema*, *Stereonema*, *Hemimastix*])

Continued on following page

TABLE 4—*Continued*

Phylum 2. MYCETOZOA de Bary, 1873 stat. nov. Engler & Prantl, 1888
Subphylum 1. Eumyxa nomen novum pro Plasmodiogymnomycotina Martin, Alexopoulos & Farr, 1983
Class 1. Protostelea Olive & Stoianovitch, 1966
Class 2. Myxogastrea Fries, 1829 stat. nov.
Subclass 1. Gastromyxia nomen novum pro Myxogastromycetidae
Subclass 2. Stemonitia Ross, 1973 orthog. emend.
Subphylum 2. Dictyostelia Lister, 1909 stat. nov.
Class 1. Dictyostelea Lister, 1909 stat. nov.
SUPERPHYLUM 2. CHOANOZOA Cavalier-Smith, 1983 stat. nov.
Phylum 1. CHOANOZOA Cavalier-Smith, 1981 emend. 1983
Class 1. Choanomonadea Krylov et al., 1980 (syn. Craspedophyceae Chadefaud, 1960, Craspedomonadophyceae Hibberd, 1976; both unsuitable for a purely zoological taxon) (order Choanoflagellida Kent, 1880 emend. Hibberd, 1983 = family Craspedomonadina Stein, 1878)
Parvkingdom 2. ALVEOLATA Cavalier-Smith, 1991 stat. nov.
SUPERPHYLUM 1. MIOZOA Cavalier-Smith, 1987
Phylum 1. DINOZOA Cavalier-Smith, 1981 emend.
Subphylum 1. Protalveolata Cavalier-Smith, 1991
Class 1. Colponemea cl. nov. (order Colponemida ord. nov.)
Class 2. Oxyrrhea Cavalier-Smith, 1987 (order Oxyrrhida orthog. emend. pro Oxyrrhinales Sournia in Taylor, 1990)
Class 3. Ellobiopsea orthog. emend. pro Ellobiophyceae Loeblich III, 1970
Subphylum 2. Dinoflagellata Bütschli, 1885 stat. nov. Cavalier-Smith, 1991 (originally a class) (syn. Cilioflagellata Müller, Dinophyta auct., Dinophyceae Pascher, 1914)
Superclass 1. Syndina supercl. nov.
Class 1. Syndinea Chatton, 1920 stat. nov. Loeblich, 1976 orthog. emend. Corliss, 1984
Superclass 2. Hemidinia supercl. nov.
Class 1. Noctilucea Haeckel, 1866 stat. nov. (order Cystoflagellata Haeckel, 1873 stat. nov. Bütschli, 1887)
Class 2. Haplozooidea Poche, 1911 (syn. Blastodiniphyceae Fensome et al., 1993 orthog. emend.) (order Blastodinida Chatton, 1906)
Superclass 3. Dinokaryota supercl. nov.
Class 1. Peridinea Ehrenberg, 1830 stat. nov. Wettstein, 1901 emend.
Subclass 1. Gymnodinoidia Poche, 1913 stat. nov. (syn. Gymnodiniphycidae Fensome et al., 1993)
Subclass 2. Peridinoidia Poche, 1913 stat. nov. Fritsch, 1935 (syn. Peridiniphycidae Fensome et al., 1993)
Subclass 3. Prorocentroidia Lemmermann, 1899, stat. nov. (syn. Prorocentrophycidae Fensome et al., 1993)
Subclass 4. Desmocapsoidia Pascher, 1941 stat. nov.
Subclass 5. Thoracosphaeroidia subcl. nov.
Class 2. Bilidinea Cavalier-Smith, 1993 (orders Dinophysida Lindemann, 1928 and Nannoceratopsida)
Phylum 2. APICOMPLEXA Levine, 1970 emend.
Subphylum 1. Apicomonada subph. nov.
Class 1. Apicomonadea cl. nov. (orders Perkinsida Levine, 1978; Colpodellida ord. nov. pro Spiromonadida Krylov & Mylnikov, 1986)
Subphylum 2. Gamontozoa subph. nov.
Infraphylum 1. Sporozoa Leuckart, 1879 stat. nov.
Superclass 1. Gregarinia Dufour, 1828 stat. nov.
Class 1. Eogregarinea cl. nov.
Class 2. Neogregarinea cl. nov.
Superclass 2. Coccidia Leuckart, 1879 stat. nov.
Class 1. Coelotrophea cl. nov.
Class 2. Eucoccidea cl. nov.
Infraphylum 2. Hematozoa Vivier, 1982 stat. nov.
Class 1. Haemosporea Danilewsky, 1885 stat. nov. Sleigh, 1989
Class 2. Piroplasmea Wenyon, 1926 stat. nov. Levine, 1971
SUPERPHYLUM 2. HETEROKARYOTA Hickson, 1903 stat. nov.
Phylum 3. CILIOPHORA Doflein, 1901 stat. nov. Copeland, 1956 emend. auct.
Class 1. Spirotrichea Bütschli, 1889 stat. nov.
Subclass 1. Heterotrichia Stein, 1859 stat. nov.
Subclass 2. Choreotrichia Small & Lynn, 1985
Subclass 3. Stichotrichia Small & Lynn, 1985
Class 2. Prostomatea Schewiakoff, 1896
Class 3. Litostomatea Small & Lynn, 1981
Subclass 1. Haptoria Corliss, 1974
Subclass 2. Trichostomatia Bütschli, 1889
Class 4. Phyllopharyngea de Puytorac et al., 1974
Subclass 1. Phyllopharyngia de Puytorac et al., 1974
Subclass 2. Chonotrichia Wallengren, 1895
Subclass 3. Suctoria Bütschli, 1889
Class 5. Nassophorea Small & Lynn, 1981
Subclass 1. Nassophoria Small & Lynn, 1981
Subclass 2. Hypotrichia Stein, 1859 stat. nov.

Continued on following page

Class 6. Oligohymenophorea de Puytorac et al., 1974
Subclass 1. Hymenostomatia Delage & Hérouard, 1896 stat. nov.
Subclass 2. Peritrichia Stein, 1859 stat. nov.
Subclass 3. Astomatia Schewiakoff, 1896
Subclass 4. Apostomatia Chatton & Lwoff, 1928
Subclass 5. Plagiopylia Small & Lynn, 1985
Class 7. Colpodea de Puytorac et al., 1974
Class 8. Karyorelictea Corliss, 1974

Parvkingdom 3. ACTINOPODA Calkins, 1902 stat. nov. (originally a class)
Phylum 1. HELIOZOA Haeckel, 1866 emend. stat. nov. Margulis, 1974
Class 1. Nucleohelea cl. nov. (orders Desmothoracida Hertwig & Lesser, 1874; Actinophryida Hartmann, 1913)
Class 2. Centrohelea Kühn, 1926 (orders Axoplasthelida Febvre-Chevalier, 1984 stat. nov.; Centroplasthelida Febvre-Chevalier, 1984 stat. nov.)
Phylum 2. RADIOZOA Cavalier-Smith, 1987
Subphylum 1. Spasmaria subph. nov.
Class 1. Acantharea Haeckel, 1881 stat. nov.
Subclass 1. Holacanthia subcl. nov. (orders Holacanthida Schewiakoff, 1926; Plegmacantha, Rechetniak, 1981)
Subclass 2. Euacanthia subcl. nov. (3 orders)
Class 2. Sticholonchea Poche, 1913 stat. nov. Petrushevskaja, 1977
Subphylum 2. Radiolaria Müller, 1858 emend. stat. nov.
Class 1. Polycystinea Ehrenberg, 1838 stat. nov.
Subclass 1. Spumellaria Ehrenberg, 1875
Subclass 2. Nassellaria Ehrenberg, 1875
Class 2. Phaeodarea Haeckel, 1879

Parvkingdom 4. NEOSARCODINA parvking. nov.
Phylum 1. RHIZOPODA Dujardin, 1835 stat. nov. Haeckel, 1866 emend.
Class 1. Lobosea Carpenter, 1861 stat. nov. emend.
Subclass 1. Gymnamoebia Haeckel, 1862 stat. nov. (orders Euamoebida Lepşi, 1960; Leptomyxida Pussard & Pons, 1976 emend. Page, 1987; Copromyxida ord. nov.; Acanthopodida Page, 1976; Loboreticulatida Page, 1987).
Subclass 2. Testacealobosia de Saedeleer, 1934 (orders Arcellinida Kent, 1880, Trichosida Möbius, 1889 Himatismenida Page, 1987)
Class 2. Filosea Leidy, 1879 emend.
Subclass 1. Cristidiscoidia Page, 1987 stat. nov. (orders Nucleariida ord. nov.; Fonticulida ord. nov.)
Subclass 2. Cristivesiculatia Page, 1987 stat. nov. (order Vampyrellida Starobogatov ex Krylov et al., 1980)
Subclass 3. Testaceafilosia De Saedeleer, 1934 (order Gromiida Claparède & Lachmann, 1859)
Phylum 2. RETICULOSA Carpenter, 1862 emend. stat. nov. (syn. Granuloreticulosa) de Saedeleer, 1834
Subphylum 1. Athalamia subph. nov.
Class 1. Athalamea Haeckel, 1862 stat. nov. Lee, 1990 (orders Athalamida Haeckel, 1862; Promycetozoida Grell, 1985)
Subphylum 2. Foraminifera (D'Orbigny, 1826) Eichwald, 1830 stat. nov. Mikhalevich, 1980
Class 1. Monothalamea Haeckel, 1862 stat. nov.
Class 2. Polythalamea Ehrenberg, 1838 stat. nov. Mikhalevich, 1980
Subclass 1. Allogromioidia Chapman & Parr, 1936 stat. nov. Mikhalevich, 1980
Subclass 2. Textularidia Lankester, 1885 stat. nov. Mikhalevich, 1980 (orders Ammodiscida; Lituolida)
Subclass 3. Fusulinidia Fursenko, 1958 stat. nov. (orig. order)
Subclass 4. Miliolidia Lankester, 1885 stat. nov. Saidova, 1981 (order Miliolida)
Subclass 5. Rotalidia Lankester, 1885, stat. nov. (orders Nodosariida; Buliminida; Discordida; Spirillinida; Globigerinida; Orbiloidida; Cassidulinida; Certerinida; Robertinida)

Neosarcodina incertae sedes: Class Xenophyophorea[b] Schulze, 1904
Class Schizocladea[b] Cedhagen & Mattson, 1992
Class Holosea cl. nov. (order Luffisphaerida ord. nov.)

Parvkingdom 5. ENTAMOEBIA parvking. nov.
Phylum 1. Entamoebia phyl. nov.
Class 1. Entamoebea Cavalier-Smith, 1991 (order Entamoebida ord. nov.)

Parvkingdom 6. MYXOZOA Grassé, 1970 stat. nov. emend.
Phylum 1. MYXOSPORIDIA Bütschli, 1881 stat. nov. Grassé, 1970
Pseudoclass 1. Myxosporea[c] Bütschli, 1881 stat. nov.
Pseudoclass 2. Actinomyxea[c] Štolc, 1899 stat. nov. (syn. Actinosporea Noble, 1980)
Phylum 2. HAPLOSPORIDIA Caullery & Mesnil, 1899 stat. nov. Corliss, 1984
Class 1. Haplosporea Chatton, 1911 stat. nov. Desportes and Nashed, 1983
Phylum 3. PARAMYXIA Chatton, 1911 stat. nov.
Class 1. Paramyxea Chatton, 1911, stat. nov. emend. Desportes, 1981 (orders Paramyxida Chatton, 1911; Marteiliida Desportes & Ginsburger-Vogel, 1977)

Parvkingdom 7. MESOZOA van Beneden, 1876 stat. nov.
Phylum 1. MESOZOA van Beneden, 1876
Class 1. Rhombozoa van Beneden, 1876 (orders Dicyemida van Beneden; Heterocyemida van Beneden)
Class 2. Orthonectea Giard, 1879 stat. nov. (order Orthonectida Giard, 1879)

[a] A few orders are included for some of the lesser known groups, but orders are omitted for the larger and better studied ones. There is a clear need to divide the Ciliophora into subphyla, but this is not done here since recent work casts doubt on earlier subphyletic classifications. In this table I have attempted to cite the names of the original authors of taxa, the names of those who assigned them to their present rank, and also those who finally emended the taxon to give it its present composition. But for taxa that have undergone multiple emendations, I have not cited earlier emenders. Stat. nov. indicates a rank different from the original one.

[b] Incertae sedes because they are the only classes unstudied by electron microscopy.

[c] May be different phases of same organisms (150).

the common assumption that the Karyorelictea are the most primitive ciliates. The very different extrusomes of karyorelictids compared with other ciliates (117b) supports their status as a separate class, but the absence of typical spindle trichocysts is probably the result of a secondary loss. Since this type of trichocyst is also present in Dinozoa, they were probably also present in the ancestral ciliate; it is therefore unlikely that they evolved only after the divergence of karyorelictids from other ciliates, as Raikov proposed (117b).

Superphylum Miozoa

Miozoa (27) comprise the phyla Dinozoa and Apicomplexa, which have only a single type of haploid nucleus in contrast to the heterokaryotic Ciliophora, which have separate diploid micronuclei and multiploid macronuclei. It is often stated (117a) that their meiosis is unusual in having only a single step, not two as in most other eukaryotes, but how widely true this is is unclear.

Phylum Dinozoa Emended

The phylum most closely allied to Ciliophora by ultrastructural criteria and by rDNA phylogenetics is the Dinozoa. As originally defined (17), this included only Dinoflagellata and *Oxyrrhis*. The major innovation here is to include *Colponema* also, which several authors have proposed as a potential ancestor for both dinoflagellates and ciliates (84a). I have long considered the presence of cortical alveoli (common to *Colponema*, *Oxyrrhis*, dinoflagellates, and most ciliates, as well as to the phylum Glaucophyta [e.g., *Cyanophora*] of the plant subkingdom Biliphyta) to be of key systematic and phylogenetic importance (16, 17, 18, 29, 38). I postulate that cortical alveoli originated once only in evolution and should be used as a positive character defining the Dinozoa, together with the absence of the apical complex (distinguishing them from Apicomplexa), absence of macronuclei (distinguishing them from Ciliophora), and absence of chloroplasts with phycobilisomes (distinguishing them from Glaucophyta in the plant kingdom, which unlike the three alveolate phyla have flattish mitochondrial cristae).

Earlier, I argued, from their presence in both Glaucophyta and dinoflagellates, that alveoli were also present in the flagellate that originally converted a symbiotic cyanobacterium into the first chloroplast (18). The fact that Viridiplantae, Biliphyta, and Dinozoa diverge more or less simultaneously on the 18S rDNA tree (7, 121) is consistent with this thesis. Also consistent is the presence of a *c*-like chlorophyll in a few prasinophyte green algae (148), the similarities of chlorophyll *a*/*b* and *a*/*c* binding proteins (94), and the diversity in the pigment composition (chlorophyll *c* without or with [122] intrathylakoidal phycobilins) of dinoflagellate chloroplasts, since such diversity can be interpreted as a consequence of the initial radiation of the first chloroplast (18). If, however, the euglenoids also obtained their chloroplasts during the same primary symbiosis, as is possible (39) despite the fact that the nuclear 18S rRNA gene shows euglenoids to have diverged on the metakaryote tree long before the other algae (7, 127), rather than secondarily from another eukaryote as proposed by Taylor (133) and Gibbs (64), it would be more likely that cortical alveoli evolved after the origin of chloroplasts (39).

The rDNA evidence that *Oxyrrhis* diverged from Peridinea before any of them did from each other (87) and the completely different but unique mitotic mechanisms of *Oxyrrhis* and dinoflagellates together justify placing them in separate subphyla. The differences between *Oxyrrhis* and *Colponema*, though sufficient to justify their separation in separate classes, are not sufficient to require separate subphyla for them: I have therefore created a new dinozoan subphylum, Protalveolata, to contain all nondinoflagellate alveolate zooflagellates that lack a sporozoan-like apical complex (see later discussion). It seems likely that *Oxyrrhis*, dinoflagellates, and Ciliophora evolved independently from a cortically alveolate *Colponema*-like dinozoan. Since both Syndinea and Glaucophyta probably have normal histones (judging solely by microscopy) and since Bilidinea and Peridinea contain photosynthetic members, it follows that if the chloroplasts in these three last classes are directly related (i.e., by vertical rather than horizontal transmission), then the ancestral dinoflagellate was photosynthetic and nonphotosynthetic dinoflagellates (i.e., Syndinea, Noctilucea, Haplozooidea many Peridinea, and some Bilidinea) are derived; moreover, chloroplasts must have originated in dinoflagellates prior to the loss of histones and evolution of 5′OH uracil in their DNA and also prior to the origin of the dinoflagellate exonuclear spindle. This can be tested by rDNA phylogenetics. The ellobiopsids are here tentatively also placed as a separate class within the Protalveolata because of a preliminary report that the zoospore has flattened cortical vesicles (145); although they have often been treated as dinoflagellates, there is no solid evidence for such an assignment.

In its present use, Dinozoa is not a synonym for dinoflagellates but the name of a broader phylum containing all flagellates with a combination of ampulliform tubular mitochondrial cristae (by contrast, Glaucophyta [80] have flat or irregular cristae like other Plantae) and cortical alveoli but lacking an apical complex. The recent suggestion (151) to use the phylum name Dinozoa instead for the supraphyletic taxon that I designate Alveolata here and elsewhere (38, 40) should not be accepted, since it would be very confusing to refer to the Ciliophora and Apicomplexa as Dinozoa. A name referring to a defining character of the whole group is better. The name Alveolata or, informally, alveolates is now becoming widely used (114a, 115b).

Phylum Apicomplexa Emended

It is reasonable to suppose (for a discussion, see references 38 and 42) that Apicomplexa arose from Protalveolata by evolving the apical complex as an adaptation to ectoparasitism and that the two inner membranes of their pellicular triple-membrane system are homologous with the cortical alveoli of Dinozoa and Ciliophora. The traditional phylum Apicomplexa is here modified by the addition of the predatory zooflagellates *Colpodella*. It is possible that Paramyxea ought also to be included within Apicomplexa on account of their nine-singlet centrioles, which they share uniquely with most Sporozoa, despite the absence of an apical complex; this hypothesis requires testing by 18S rRNA sequence phylogenetics. At present, their treatment as a separate phylum (52), though followed here, rests on distinctly slender grounds. There is now good evidence from the 18S rRNA tree (121, 127) (Fig. 1) that the Dinozoa, Ciliophora, and Apicomplexa together form a true monophyletic clade and, therefore, good evidence for the parvkingdom Alveolata created here. (Originally, Alveolata was ranked as an infrakingdom [38].) Sequence data for Radiozoa are badly needed to check whether or not they may really belong in the Alveolata (73) rather than in their traditional position in the

Actinopoda, where I have left the phylum in my present classification. At present, we cannot rule out the possibility that all Myxozoa are derived from Apicomplexa. Because of this, and because of the possibility that Glaucophyta might have been derived from an extinct photosynthetic protalveolate, we cannot yet say whether Alveolata are holophyletic or paraphyletic.

If chloroplasts first evolved in Protalveolata (41), then Apicomplexa could in principle have diverged from the Dinozoa either before this happened (and therefore never have had photosynthetic ancestors) or after the origin of chloroplasts; in the latter case, their common ancestor must at some stage have secondarily lost photosynthesis. If, however, chloroplasts first evolved even earlier in the common ancestor of euglenoids and higher eukaryotes, then the ancestral protalveolate must have been photosynthetic; in this scenario, the Apicomplexa, like all nonphotosynthetic eukaryote phyla more advanced than Euglenozoa, must have had a photosynthetic protozoan as a very distant ancestor. The recent finding in Apicomplexa of a second organelle genome, which resembles the chloroplast genome in being circular (−35 kb) and in having RNA polymerase genes and an inverted repeat containing its rRNA genes (148a), might be interpreted as evidence for one or the other of the two latter scenarios. This genome is quite distinct from the established apicomplexan mitochondrial genome, which is a linear concatemer with a repeat of 6 kb and which contains the cytochrome *b* and cytochrome oxidase I and III genes and fragmented rRNA genes. However, I suspect that the circular genome might eventually turn out to be located in the mitochondria also, rather than in the mysterious double-enveloped organelles, which have been suggested to be possible relic plastids (148b). At present, there is no definitive evidence that the 35-kb circle is really derived from chloroplast DNA: since at some stage in their early history mitochondria must have contained prokaryotic RNA polymerase genes, they might simply have been retained in Apicomplexa and lost in higher eukaryotes. This seems to me the simplest interpretation, since the 16S rRNA of the 35-kb genome predominantly trees with mitochondria rather than plastids. Clearly, the 35-kb genome deserves much further study, since if it proved instead to be of chloroplast origin, it would help to establish more accurately the relative time of origin of chloroplasts. (Study of the mitochondrial genome in each of the protozoan phyla would be of great phylogenetic interest: not only could it provide valuable phylogenetic data, but it could reveal novel genetic phenomena, as has already happened most abundantly in the euglenozoan Kinetoplastea. At present, substantial molecular data exist for mitochondrial DNA for only four protozoan phyla: Euglenozoa [mainly trypanosomes], Apicomplexa [mainly *Plasmodium*], Ciliophora, and Rhizopoda [*Acanthamoeba*]; mitochondrial DNA has been studied either not at all or only very superficially in the other 12 protozoan phyla that have mitochondria.)

NEW SUPERPHYLUM OPALOMYXA AND ITS TWO PHYLA

Phylum Opalozoa

In contrast to the species-poor Protalveolata, the vast majority of zooflagellates with tubular cristae have no cortical alveoli and have been placed recently instead in the phylum Opalozoa Cavalier-Smith, 1991 (38, 44), which subsumed the earlier phylum Proterozoa Cavalier-Smith, 1981 (17), which was founded to contain the proteromonads (11), opalinids (112), and a large number of tubulicristate taxa such as the cyathobodonids that were omitted from an earlier protozoan classification (89). Hibberd later (70) created the order Pseudodendromonadida to include *Cyathobodo*: Proterozoa, suitably emended, is now a subphylum of Opalozoa (see below).

The phylum Opalozoa has a well-defined ultrastructural "identity" (113, 114a) or basic body plan: its members are predominantly biciliate protozoa with tubular mitochondrial cristae, which totally lack chloroplasts, cortical alveoli, and tubular ciliary hairs. The importance of the presence or absence of cortical alveoli, which has long been discussed by protistologists (16, 133), has been confirmed recently by the fact that the three protozoan phyla grouped recently in the supraphyletic assemblage Alveolata (i.e., Dinozoa, Ciliophora, and Apicomplexa) form a single monophyletic branch on the 18S rRNA tree (Fig. 1) (121, 127). This strongly supports the use of the absence of cortical alveoli in Opalozoa to distinguish them at the phylum level from the Dinozoa. Likewise, the systematic importance of the presence or absence of rigid tubular ciliary hairs has long been accepted by protistologists (16, 17, 32, 90, 113, 113a, 133). Thus, the use of the absence of such hairs from the Opalozoa to differentiate the phylum from the Heterokonta also stresses a differential character state of well-accepted major systematic importance. The 18S rRNA tree also strongly supports the monophyly of the Heterokonta and shows it to be about as ancient as and of comparable phyletic depth to each of the three alveolate phyla (Fig. 1) (121, 127).

By contrast, no 18S or 28S rRNA sequences have yet been published for any Opalozoa, though in my laboratory we are currently sequencing the 18S rRNA from several opalozoan flagellates in order to test the validity of the group. Since the absence of tubular ciliary hairs and of cortical alveoli in Opalozoa is likely to be the ancestral state, however, Opalozoa are probably paraphyletic rather than holophyletic. Since there is no evidence that Opalozoa are polyphyletic, unless such evidence is found in future, it would not be justifiable to subdivide them into several phyla. The ranking of the four major subgroups as subphyla is sufficient recognition of their differences, which though substantial are significantly less so than those that separate, for example, the three alveolate phyla, the four chromist phyla (Cryptista, Heterokonta, Haptomonada, and Chlorarachniophyta), or the three archezoan phyla (Archamoebae, Microsporidia, and Metamonada). To accept the ranking of the order Plasmodiophorida as a phylum (52, 55a) or the order Opalinida as a subphylum (89) or phylum (52) would be unwarranted taxonomic inflation.

Conversely, although there have long been reasons for thinking that opalinids and *Karotomorpha* are more closely related to each other than to any other organisms (11, 112, 133), including them in a single order (Slopalinida Patterson [112]) in my view gives insufficient weight to the substantial change in body plan associated with the evolution of ciliary rows. Patterson also included *Proteromonas* in the Slopalinida. However, in my view *Proteromonas* is much too radically different from *Karotomorpha* to be included in the same order or class.

The major differences are as follows. (i) *Karotomorpha* (like Opalinida) has surface ridges each strengthened by a band of microtubules, whereas *Proteromonas* does not. (ii) *Proteromonas* has rigid tubular body hairs (somatonemes); *Karotomorpha* does not. (iii) *Proteromonas* has one anterior cilium with a paraxial rod and one trailing cilium without a

paraxial rod; *Karotomorpha*, by contrast, has four trailing cilia, none with a paraxial rod. (iv) *Proteromonas* has a compound rhizostyle made up of dissimilar ciliary microtubular roots emanating from both the trailing and anterior cilia and which passes through a tunnel through the nucleus and is attached to the mitochondrion; *Karotomorpha* has no such structure. Its so-called rhizostyle consists of two similar pairs of microtubular roots that come from only two of the cilia and pass below the cell surface, not through the nucleus, and do not contact the mitochondrion. These are such substantial differences in body plan that I have placed *Proteromonas* and *Karotomorpha* in separate orders. The similarities between them (11) are insufficient to establish a close and specific relationship. These similarities are as follows. (i) A transitional region helix is present, but this is also present in many heterokonts, in the heteromitean opalozoan *Cryothecomonas*, in a few euglenoids (137a), and even apparently in some of the archezoan Archamoebae (36): even *Hemimastix* has a slender transitional "helix," but whether this is really homologous with that of other Opalozoa or Heterokonta is not clear. In *Hemimastix* (63), as well as in the other two hemimastigophoran genera, *Spironema* and *Stereonema* (62), this transitional structure appears more as a slender cylinder than as a discrete helix. The transitional helix is therefore either a very ancient ancestral character, and not a synapomorphy for *Proteromonas* and *Karotomorpha*, or a structure that evolved polyphyletically. (ii) The parabasal positions of the dictyosome are similar, but this also is not a synapomorphy for these two genera, since it is found in many other Opalozoa and in Parabasalia. (iii) The single mitochondrion with tubular cristae is a similarity; single mitochondria are found in a variety of other groups, e.g., Euglenozoa and some Prasinophyceae, though I know of no other definite examples with tubular cristae. (iv) The absence of peroxisomes (11), which is true of other gut symbionts (e.g., the fungus *Neocallimastix*, *Entamoeba*, and many ciliates), might be a convergent response to living in guts of low oxygen tension.

Patterson (113a) has grouped Opalinida, *Karotomorpha*, and *Proteromonas* with Heterokonta under the informal name stramenopiles, rather than with Heteromitea, Phytomyxea, Hemimastigophora, and other Opalozoa, as I have (44). There are three reasons why "stramenopiles" is not a good group. First, while I myself even earlier (23) stressed the evolutionary importance of the similarity between the tubular ciliary hairs of heterokonts and the tubular body hairs (somatonemes) of *Proteromonas*, the latter more closely resemble the bipartite ciliary hairs of cryptomonads than the tripartite ciliary hairs of heterokonts (32). If *Proteromonas* were to be grouped with heterokonts, there would, therefore, be at least as much reason to include cryptomonads also in the stramenopiles, which Patterson does not. Therefore, to group *Proteromonas* with the heterokonts to the exclusion of cryptomonads makes no taxonomic sense (especially since cryptomonads and all photosynthetic heterokonts [but not the proteromonads] share an even more important derived character, the presence of a chlorophyll *c*-containing chloroplast located inside a periplastid membrane, which in turn is located inside the RER).

Second, what makes the tubular hairs of heterokonts and cryptomonads such good and stable systematic characters is not their structure per se but their location on the cilia. It is the combination of this location plus their rigid structure that gives them their special property of reversing the thrust of the cilium, which therefore means that they will be very difficult to lose or to gain (because this will alter the direction of swimming and of feeding currents [23]). Thus, although the ciliary hairs of chromists and the somatonemes of *Proteromonas* probably are homologous, their locations are not. In this important positional respect, therefore, the body plans of heterokonts and proteromonads are not actually homologous, so they should not be included in the same phylum.

Third, the inclusion in the stramenopiles of not only *Proteromonas* but also *Karotomorpha* and the Opalinida, which all lack tubular hairs, directly contradicts the initial definition of the stramenopiles and is based on the presumption that these taxa once had such hairs. However, this presumption need not be true: for example, it could be (and I suggest most probably is the case) that *Karotomorpha* and opalinids evolved from a somatoneme-free heteromitean rather than from *Proteromonas* itself. Moreover, there are three pieces of evidence that haptophytes are cladistically closer to heterokonts than is *Proteromonas*, even though they lack rigid tubular ciliary hairs (45a): (i) the chromobiote plastid inside the periplastid membrane inside the endoplasmic reticulum, (ii) a single autofluorescent cilium, and (iii) the intracristal filaments (which appear to be present in chromobiotes and some Dinozoa but are absent from Opalozoa). Yet Patterson excludes haptophytes from the stramenopiles. Although it is not proven that haptomonads arose by the loss of tubular ciliary hairs, the reasons for thinking that they may have done so (45a) are very much stronger than for thinking that *Karotomorpha* and the opalinids or the actinophryids, all of which Patterson (113a) includes in the stramenopiles, did so. For these reasons, I consider the concept of stramenopiles to be taxonomically very unsound. Although it is possible in principle that *Proteromonas* is derived from the Chromista as Patterson assumed, rather than ancestral to them as I have argued (23), in the absence of any evidence that this has happened, *Proteromonas* should be firmly excluded from the kingdom Chromista and retained in the Opalozoa; it is even more important to exclude *Karotomorpha*, Opalinida, and Actinophryida from the Chromista, since these share absolutely no synapomorphies with Chromista. The demarcation between Opalozoa and Heterokonta (23, 32, 100a) is quite clear-cut.

A fourth point about the name stramenopiles is that from the outset its definition was thoroughly confused. Patterson gave two contradictory definitions of it in the same paper (113a). The first restricted it to species having tripartite tubular ciliary hairs; this clearly excludes *Proteromonas*, even though Patterson included it. Furthermore, this definition is exactly the same as for the previously established phylum Heterokonta Cavalier-Smith 1986, which was based on the name Heterokontae that goes back to 1899. Thus, in this first sense the name was a totally redundant new synonym for heterokonts, a name which has long been adopted by numerous authors (e.g., all of the contributors [e.g., 100a], including Patterson himself in the general introduction, in the recent book edited by Patterson and Larsen [115a]). His second definition was wider in that it included not only species with tubular hairs, whether on their body (i.e., *Proteromonas*) or on cilia, but also a variety of species with no trace whatever of tubular hairs but which Patterson speculated had been derived from heterokonts by the loss of tubular hairs, although there is no sound evidence for this speculation. In his subsequent writings, Patterson has implicitly adopted this second definition based on his own speculations (a practice that he passionately condemns in others [114a]) rather than his first definition based on a

synapomorphy. Unlike Heterokonta, stramenopiles is not latinized and thus has no status under either the Zoological or the Botanical Code of Nomenclature. Since the name stramenopiles is both ambiguous and a totally unnecessary new synonym for heterokonts, it is best ignored.

Unlike cryptomonads and *Goniomonas*, *Kathablepharis* has tubular mitochondrial cristae (84). Indeed, it differs from cryptomonads in all significant respects other than the ejectisomes (84, 84b); even these are not identical, for they lack the subsidiary scroll present in Cryptista. I therefore transferred *Kathablepharis* from the Cryptophyceae, where Skuja placed it (123c), to the Opalozoa, specifically, into the new class Cyathobodonea (44).

The boundary between Opalozoa and Mycetozoa (i.e., the classes Protostelea, Myxogastrea, and Dictyostelea) requires some discussion. Some Opalozoa (e.g., *Cercomonas*) are somewhat amoeboid or are amoeboflagellates (*Pseudospora*) like the amoeboflagellate stage of protostelids and myxogastrids, which also have tubular mitochondrial cristae and lack both cortical alveoli and retronemes. The amoeboflagellate phases of mycetozoa also have similar ciliary roots and are therefore rather close to (129), and I suggest may have evolved from, a heteromitean opalozoan such as *Pseudospora*. The essential difference, however, is the evolution of the fruiting body of Mycetozoa: this is the most useful synapomorphy for defining Mycetozoa and separating them from Opalozoa. I have chosen to treat Mycetozoa as a separate phylum because they have made two major changes in way of life: the emphasis on phagotrophic amoebae or plasmodia for feeding, and cellulose or chitin cell walls and fruiting bodies for aerial dispersion of spores. Cell walls in the two fungoid opalozoan taxa (Phytomyxea and *Nephromyces*) are chitinous, not cellulosic. However, having chitinous walls is not evidence for an affinity with fungi, since chitin-walled cysts are common in archezoa and were probably an ancestral state for the first protozoa. Although some authors might prefer to treat Mycetozoa as a fourth subphylum of Opalozoa, I think these changes represent more fundamental differences in body plan and way of life than those seen between the four opalozoan subphyla, which all lack fruiting bodies and three of which have ciliated trophic phases.

There is no reason to place Plasmodiophorida within the Mycetozoa, as has often been done. They lack all three of the most important mycetozoan characteristics: an amoeboid or amoeboflagellate stage, phagotrophy, and aerial fruiting bodies. They also have a very complex extrusome, the Stachel, for penetrating host cells (55a): Mycetozoa never have extrusomes, whereas Opalozoa often do. Furthermore, plasmodiophorid ciliary roots are very different from those of Mycetozoa. Their microplasmodial trophic phase is clearly a secondary adaptation to intracellular plant parasitism and probably did not exist in their free-living ancestor, which may perhaps have been a soil-dwelling heteromitean flagellate, perhaps similar to *Heteromita* itself: *Phagomyxa* seems intermediate between the heteromitean *Pseudospora* and the plasmodiophorids, while *Pseudospora* seems intermediate between *Heteromita* and *Phagomyxa*. Since these four taxa constitute a near continuum, it would be undesirable to place them in separate phyla (unless future work shows their similarities to be convergent). The recent characterization of the nonplasmodial parasite of algae, *Phagodinium* (81a), which I here place in a new opalozoan subclass, provides yet another possible link between Plasmodiophorida and opalozoan flagellates.

Although Heliomonadida, here informally called heliomonads, superficially resemble certain centroplast-containing heliozoa, there is no good reason to think that the axopodia of the two groups are homologous or that they are homologous with the axopodia of pedinellid chromists like *Ciliophrys*, with which they were formerly united as helioflagellates, almost certainly a polyphyletic concept. The centrosome has clearly been a microtubule organizing center as far back as the earliest ciliated eukaryotes (Mastigamoebea [36, 40]); it would not have been difficult for a variety of flagellates to have independently evolved axoplast- or centroplast-nucleated axopodia by extending microtubules from the centrosome into outward extensions of the cell surface. The irregular or quincunx arrangement of those of heliomonads (13) and the hexagonal arrangement in the centroplast heliozoa suggest (though do not prove) that this happened convergently in the two groups. However, the fact that many Heliozoa and Radiolaria have kinetocysts suggests that most, if not all, Actinopoda may have evolved from kinetomonads, but they might have evolved from a nonaxopodial kinetomonad such as *Histiona* or *Ancyromonas*. It is interesting that both Kinetomonada and Actinopoda contain genera with tubular cristae and genera with flat cristae, suggesting that crista shape may be particularly unstable in the kinetocyst-containing protists. In many respects, Histionida appear intermediate between Jakobida and Heliomonada. This justifies the inclusion of all three in the same phylum. The extrusomes of *Jakoba* may possibly be intermediate between those of Cercomonadida and Kinetomonada.

Opalozoa include 19 different orders of zooflagellates (plus Leucodictyida, Opalinida, and Phytomyxea), the largest number in any phylum. It therefore constitutes the primary seat of zooflagellate diversification, which may well have been very rapid following the evolution of the first biciliated zooflagellate with a dictyosome and tubular cristae. There are still very large numbers of zooflagellate genera that have not yet been examined in the electron microscope (116). I suggest that the majority of these will turn out to belong to the Opalozoa, but whether or not additional orders or classes will be needed to accommodate them cannot be predicted, though I shall not be in the least surprised if they are. Altogether I have assigned 34 of the 150 flagellate genera of uncertain taxonomic position discussed by Patterson and Zölffel (116) to the Opalozoa (44), while 14 have been assigned to the phylum Percolozoa (43). All remaining "mystery" flagellate genera that have been studied by electron microscopy have been assigned to phyla: *Colpodella* (including *Dinomonas*) to the Apicomplexa, *Colponema* to the Dinozoa, and *Clathrulina* to the Heliozoa; only *Chlorarachnion* is placed in its own phylum, within the Chromista. This suggests that few, if any, new phyla will be required to accommodate the 100 or more mysterious flagellate genera that remain to be studied by electron microscopy.

Phylum Mycetozoa

Mycetozoa appear to be among the most primitive bikonts, diverging nearly as early as the Euglenozoa, the most early diverging bikont phylum yet located on the 18S rDNA tree (7, 121, 127). By contrast, the Rhizopoda represented by *Acanthamoeba* and *Hartmannella* are relatively recent, apparently diverging near the time of the divergence of the two plant subkingdoms (Viridiplantae and Biliphyta) and the four chromist phyla (Cryptista, Chlorarachniophyta, Heterokonta, and Haptophyta). This supports treatment of Mycetozoa and Rhizopoda as separate phyla. The inclusion

of Mycetozoa in a phylum Rhizopoda (109), not based on any strong positive characters, was only a matter of temporary convenience. The closest relatives of Mycetozoa may be the Opalozoa, judging from similarities in microtubular roots (129). In fact, the only clear separation between Opalozoa and Mycetozoa that can be made is the fruiting bodies of the latter. Several Opalozoa, notably, *Cercomonas* and *Pseudospora*, have strong amoeboid tendencies, and many live in soil and form spore-like cysts; thus, they could readily be ancestral to Mycetozoa.

Though Mycetozoa appear to be monophyletic (129), it is clear that cellular slime molds (a much less misleading name for them would be aggregative amoebae) are polyphyletic, having evolved independently four times: in Mycetozoa (i.e., Dictyostelia), Percolozoa (i.e., Acrasida), and Rhizopoda (independently in Lobosea [i.e., Copromyxida] and Filosea [i.e., Fonticulida]). Plasmodial slime molds like *Physarum* (Myxogastrea; the names Myxomycetes and Myxomycota are best abandoned as they wrongly imply that they are fungi) probably evolved from a ciliated protostelid (129) rather than from plasmodial Lobosea (Rhizopoda) as proposed by Grell (66b), since the latter lack cilia, they could not have been ancestral to Myxogastrea. Dictyostelia may have evolved from a nonciliated protostelid (129).

Like Page (109) and Corliss (52), I think the former Sarcodina must be subdivided into more than one phylum, because the resemblances between different Sarcodina are exceedingly superficial. Corliss suggested 12 phyla, but this is an unnecessarily high degree of splitting since several of his groups can be transferred into other existing phyla in the Protozoa and Chromista, and the major residue can be subdivided into just eight major, rather homogeneous, phyla: Archamoebae, Mycetozoa, Rhizopoda sensu stricto, Entamoebia, Radiozoa, Heliozoa, Reticulosa, and Chlorarachniophyta, of which Radiozoa and Heliozoa are assigned to the parvkingdom Actinopoda and Rhizopoda and Reticulosa are assigned to the parvkingdom Neosarcodina. The similarity of the mycetozoan and many opalozoan ciliary roots is the main reason for including them in the same parvkingdom. However, since their three microtubular roots are probably an ancestral character shared also with archezoan retortamonads and Percolozoa, they do not support a close relationship; the taxon Opalomyxa is probably paraphyletic.

PARVKINGDOM ACTINOPODA AND ITS TWO PHYLA

Whether a taxon Actinopoda should be retained is unclear. Now that we understand the cytoskeletal potential of microtubules, the grouping together of protists solely because they have axopodia (rigid surface projections strengthened by microtubules) is potentially unsound. Thus, there is little doubt from a consideration of other characters that Actinopoda and the chromistan Pedinellea (32) evolved their axopodia independently. Certainly, piroplasm gametes evolved their axopodia independently. Even Actinopoda and Heliozoa, as defined here, are quite possibly polyphyletic, though they may not be. Because one cannot yet be sure that Actinopoda (with pedinellids and heliomonads [44] removed) are polyphyletic, it is best to retain the taxon until there is stronger evidence that it really is polyphyletic. The present taxon Actinopoda excludes not only pedinellids but also the axopodial flagellates *Dimorpha* and *Tetradimorpha*, which are placed in the order Heliomonadida in the Opalozoa (44), and is therefore confined to organisms that totally lack cilia in their trophic phase.

Phylum Radiozoa Emended

Creation of the phylum Radiozoa Cavalier-Smith, 1987 (27) was not a major innovation since it corresponds almost exactly to Radiolaria sensu lato. Though there are indeed profound differences between Acantharia and Radiolaria (as there are, for example, between subphyla Vertebrata and Tunicata, both included in the phylum Chordata), these differences are sufficiently recognized by placing Radiolaria and Acantharia in separate subphyla of Radiozoa. They are united into a single phylum by two synapomorphies: the central capsule and the ability to secrete strontium sulfate (in the acantharian trophic cell to form the acantharian skeleton and in radiolarian swarmers only as intravacuolar crystals). Treating Radiozoa as three separate phyla (52) is unwarranted taxonomic inflation. Recent treatments of protist diversity (52, 98) have made far too little use of the valuable rank of subphylum to show protistan relationships in the form of a nested hierarchy: Table 4, by contrast, has 19 subphyla (and 2 infraphyla and 7 superclasses). Here I have transferred *Sticholonche* from Heliozoa to Radiozoa because it shares non-actin Ca^{2+}-activated myonemes with the Acantharia: it had once been placed with Radiolaria because it was erroneously thought to have a central capsule.

Phylum Heliozoa Emended

Phylum Heliozoa is the phylum of whose monophyly I am least sure, for it is unclear whether the several different axopodial patterns evolved independently (i.e., a polyphyletic origin) or were mutationally transformed into each other. The centrosomal character of the microtubule nucleating center of Centrohelea and the multiple nuclear envelope nucleating centers of the Nucleohelea also can be interpreted both ways, as can the diversity in cristae (as discussed below for Rhizopoda) and the diversity of extrusome types. They do all seem to have extrusomes and probably evolved from an extrusome-containing opalozoan flagellate ancestor or independently from several such ancestors. One obvious candidate would be a cell like *Dimorpha* or *Tetradimorpha* (13), included here in the flagellate phylum Opalozoa (see previous section) rather than in Heliozoa, where they might be assigned alternatively. rDNA sequences (78) suggest that at least one heliozoan is a relatively advanced protist branching in the "photosynthetic" area of the rDNA tree, but considerable caution is necessary because of the serious doubts about the phyletic unity of Heliozoa (126), even with the removal of the pedinellid *Ciliophrys* to the Chromista (23, 31). Our present knowledge does not even firmly exclude the possibility that Radiozoa, Heliozoa, Reticulosa, and Rhizopoda (in the present restricted sense) are together monophyletic, but since no sensible definition of a joint phylum uniting these four taxa can be given, and there is really nothing other than taxonomic inertia to justify their retention in the same phylum, establishment of Rhizopoda, Reticulosa, Heliozoa, and Radiozoa as four phyla will better aid clarity of thought and better stimulate a more thorough definition of the phyla than a messy and indefensible lumping. Until there are molecular data to justify dismemberment of the Heliozoa, and to tell us the true affinities of its constituents, it seems wisest to maintain it as a single phylum: it might, to everyone's surprise, even turn out to be monophyletic! Similarities in the hexagonal axopodial microtubule patterns of certain Heliozoa and most Acantharia, as well as in extrusomes (58, 59), suggest that there might be a direct link

between Heliozoa and Radiozoa and that the Actinopoda (possibly with a somewhat more strictly defined Heliozoa) also might be monophyletic.

NEW PARVKINGDOM NEOSARCODINA

At present, there is no sound basis to decide whether this taxon is monophyletic or polyphyletic. As the borderline between certain filosea and certain athalamean reticulosa, on the one hand and certain lobosea, on the other hand, is not very sharp, I here include all three taxa in the same parvkingdom in order to focus the attention of molecular systematists on the need to better define their relationships. Since this parvkingdom excludes six phyla (Archamoebae, Mycetozoa, Entamoebia, Heliozoa, Radiozoa, and Chlorarachniophyta), one class (Heterolobosea, placed in phylum Percolozoa), one order (Plasmodiophorida, placed in phylum Opalozoa), and several genera (e.g., *Dientamoeba* and *Histomonas*, now placed in phylum Parabasalia; *Chrysamoeba* and others, placed in phylum Heterokonta) that were formerly included in the Sarcodina, it would be confusing to retain that term. Therefore, I propose the new name Neosarcodina for a much more restricted assemblage that might possibly be monophyletic. "Sarcodine" remains, however, a useful informal term for a polyphyletic grade of organization, just as is "flagellate" for a paraphyletic grade of organization, though even such an informal use of the term sarcodine, I suggest, should not include actinopods. But Sarcodina in the traditional sense are certainly polyphyletic, so its use as a formal taxon name can no longer be justified.

Phylum Rhizopoda Emended

Rhizopoda in the present sense is much narrower, and therefore vastly more homogeneous, than phylum Rhizopoda sensu Page, 1987 (109). Compared with his phylum, it excludes six whole classes: Pelobiontida (placed in kingdom Archezoa), Plasmodiophorida (placed in phylum Opalozoa), Mycetozoa (a separate phylum), Granuloreticulosea (separated as the new phylum Reticulosa), Heterolobosea (placed in phylum Percolozoa), and Xenophyophorea (treated as Neosarcodina incertae sedis until electron microscope and/or molecular studies show their real affinities); it also excludes the Entamoebidae (separated as the new phylum Entamoebia). It thus is reduced to but two of his classes, Lobosea and Filosea, and is very close to Rhizopoda sensu Schuster, 1990 (123), which was restricted to Lobosea (but a broader taxon than here) and Filosea. These two classes may or may not be closely related, but I think it likely that the Gromiida and Testacealobosia are directly related and that the testate state was ancestral and the divergence of pseudopod type took place prior to the polyphyletic loss of tests. It is important to determine whether the "cyanelle" of the filosean *Paulinella* is a recently evolved symbiont or a true chloroplast like that of *Cyanophora*. If it were a true chloroplast, then the Rhizopoda might have evolved from an early photosynthetic ancestor of the Biliphyta and Viridiplantae; that would be consistent with the positions of the loboseans *Acanthamoeba* and *Hartmannella* on the 18S rRNA tree close to the base of the green plant and red algal clades (121, 127).

Another possible origin for Rhizopoda is from an amoeboflagellate opalozoan such as the filose amoeboflagellate *Pseudospora* or the order Thaumatomonadida. Thaumatomonads are scaly monads that feed by putting out pseudopods from a ventral groove. They could have evolved into both the testate amoebae (one of which, *Trichosphaerium*, has been claimed still to have a flagellate stage) and the scaly amoebae by losing their cilia and associated cytoskeleton and, in the case of testate amoebae, modifying their scales into tests. Possibly the ancestral rhizopod was testate and developed its primitive pseudopods in two directions, as lobopodia and as filopodia; the gymnamoebae may have evolved by loss of tests from (at least) two different ancestors, one lobose and one filose. It may also be that in sarcodines (perhaps as a result of the much more frequent cytoplasmic streaming?) the shape of mitochondrial cristae has been freer to diversify than in flagellate or well-pellicled protozoa. This, rather than a polyphyletic origin, might account for the greater diversity of cristal morphology in Rhizopoda and Heliozoa (and to a lesser extent, in Radiozoa) than in other protozoan phyla. Rhizopoda sensu Schuster, 1990 differs from the present usage in including *Vahlkampfia* (here treated as a heterolobosean percolozoan: the rRNA tree [Fig. 1] confirms this, as it does their lack of specific relationship to the lobosean gymnamoebae *Hartmannella* and *Acanthamoeba*), Entamoebidae, and even the fungus *Pneumocystis* (56) in the Lobosea.

Neosarcodina incertae sedes

The Xenophyophorea and Schizocladea (46a), unlike all of the other protist classes, have never been studied by electron microscopy, so their assignment is particularly uncertain. The Xenophyophorea are not placed in their customary place in the Rhizopoda because there is no positive evidence for their inclusion, nor is there currently any justification for or against their separation as a distinct phylum (52); like several rhizopods and foraminifera, they incorporate foreign material in their tests, and like the Schizocladea, they may belong in the Rhizopoda (89) or in the Reticulosa (101) or in neither. Both classes are treated as Neosarcodina incertae sedis. A third class not assigned to a phylum is the new class Holosea created here to contain the enigmatic organism *Luffisphaera*, which though it has been studied by electron microscopy (6, 141b), does not obviously (and this is quite exceptional) fit into any established protozoan phylum. It is like a nonamoeboid scaly amoeba!

New Phylum Reticulosa

The name of the eighth former sarcodine phylum, Reticulosa phylum novum, is an earlier and shorter synonym for the Granuloreticulosea and emphasizes their common feature, the reticulopodia, which there is no good reason to homologize with the typical rhizopod pseudopodia (though a few rhizopods do have nongranular reticulopodia) or the axopodia of radiozoa or heliozoa. I have raised Athalamia Haeckel, 1862, and followed Krylov et al. (81b) in raising Foraminifera (D'Orbigny, 1826), to subphylum rank, and therefore the Monothalamea and Polythalamea to classes, ranks that I think better reflect their phenotypic distinctiveness and systematic importance than their traditional treatment as orders. The Polythalamea is equivalent to the Foraminifera of Krylov et al. (81b) that they subdivided into four separate classes, which seems to me unnecessary.

Since the phylum is essentially marine and fundamentally benthic, with its planktonic species being relatively few and also clearly secondarily derived (82), Reticulosa most probably evolved, in contrast to the more planktonic or sessile and predominantly freshwater Heliozoa, from a benthic sediment- or detritus-loving marine opalozoan flagellate with

extrusomes and a tendency to form long thin protoplasmic projections. The recently discovered cercomonadid flagellate, *Massisteria* (115), with branched granulofilose highly elongate pseudopodia would be an excellent candidate for a reticulosan ancestor: it would have to do little more than acquire the capacity to fuse its pseudopodia into nets to become a primitive reticulosan. However, there are probably many other reticulofilose creatures in sediments just waiting to be better characterized and hit the headlines: the "biomyxid" creatures here tentatively grouped in the class Athalamea include granulofilose as well as granuloreticulose taxa and are so neglected and uncharacterised by modern methods (none, apart from *Reticulomyxa* [2], have been studied by electron microscopy) that, when properly studied, they may eventually turn out to be polyphyletic.

PHYLUM AND SUPERPHYLUM CHOANOZOA

The phylum Choanozoa, created in 1981 (17), was emended in 1983 to include only Choanoflagellida (21). A possible relationship to Opalozoa was suggested by the discovery of the flagellate *Jakoba* (114). *Jakoba libera* and *Ancyromonas* (104) are the only plastid-free biflagellates with flat, nondiscoid cristae. It has long seemed likely that biflagellated ancestors of choanoflagellates with plate-like cristae once existed, so it is gratifying that biflagellate protozoa with flat cristae have now been discovered. Following the discovery of *J. libera*, I included it in a modified Choanozoa (38). However, the discovery of *Reclinomonas* (61d, 106), which is quite similar to *Jakoba* but has tubular cristae (106), means that it is better to place *Jakoba* in the Opalozoa with other anisokont tubulicristate flagellates (in the class Heteromitea) rather than in Choanozoa. To convert a platycristate anisokont such as *Jakoba* into a unikont choanoflagellate provided with a periflagellar collar for filter feeding would have involved a radical restructuring of the cytoskeleton to convert the ancestral type of asymmetric three-member root (40) into the radially symmetric choanoflagellate root system. This radical restructuring of the cytoskeleton is therefore a more appropriate synapomorphy for use in defining the phylum Choanozoa than the probably earlier change from tubular to flat cristae which has clearly occurred polyphyletically.

For well over a century it has been considered that sponges evolved from choanoflagellates (75a, 75b), and some zoologists have argued that this is true for the animal kingdom as a whole (17). Both of these ideas, as well as the more recent proposal that the kingdom Fungi also evolved from a choanoflagellate (25), are now strongly supported by rRNA sequences which group Animalia, Choanozoa, and Fungi together as a clade (143a) (see also Fig. 1). A specific phylogenetic link between sponges, chytridiomycete fungi, and choanoflagellates was first proposed at three meetings in 1980 (17, 17a, 19), when the three taxa were collectively grouped in the kingdom Uniflagellata (19). Although it is now clear that these three taxa are cladistically more closed related to each other than Choanozoa are to most other protozoan phyla, I now prefer to keep Choanozoa in the kingdom Protozoa and to retain the boundaries between the kingdoms Protozoa, Fungi, and Animalia between the Choanozoa and sponges and between the Choanozoa and Chytridiomycetes.

New Parvkingdom Ciliomyxa

Because choanoflagellates probably evolved from an opalozoan flagellate, possibly a uniciliate collared one like *Phalansterium*, and because of the increasing evidence that flat cristae have evolved more than once, I group Choanozoa together with the superphylum Opalomyxa in the new parvkingdom Ciliomyxa.

NEW PARVKINGDOM MYXOZOA

Finally, we come to the three parasitic phyla that altogether lack flagellate stages (and therefore adequate clues to their ancestry): Myxosporidia, Paramyxia, and Haplosporidia. At present, their affinities are so obscure that there is little realistic alternative to their treatment as separate phyla. DNA sequence studies should one day reveal their affinities and facilitate a phylogenetically sounder classification. Their multinuclear spores, parasitism, and lack of cilia are the reasons for grouping them in the infrakingdom Myxozoa, but as their spores develop very differently and have different ultrastructure, they seem rather unlikely to be homologous. On the other hand, there is no solid evidence that they are not related more closely to each other than to other protozoa: all that one can really say of the three phyla is that they are all obviously members of the subkingdom Dictyozoa. The validity of their inclusion in a single parvkingdom must be tested by molecular sequencing.

Phylum Myxosporidia

The myxosporidian polar capsule suggests a possible affinity with Dinozoa, but an apicomplexan, opalozoan, or even rhizopod ancestry is each a reasonable possibility. In my view, an origin of myxosporidia from Cnidaria, sometimes mooted (91), involves far too great a degree of parasitic reduction to be contemplated seriously, so myxosporidia should remain firmly in the kingdom Protozoa (which is not here restricted to unicellular organisms) and not be transferred to the Animalia, which also are defined not by pluricellularity but by the presence of a triploblastic collagenous somatic structure (31), which is vastly more complex than the amoeboid and plasmodial myxosporidia. The multicellular spore, like the multicellular sporangia of many Mycetozoa, is an independent adaptation to dispersal and not the evolution of true animal somatic tissue.

Phylum Haplosporidia

Haplosporidia could have evolved from any of the same four phyla as the myxosporidia or even from the myxosporidia themselves; it is to be hoped that molecular methods will enable this handful of species (116a) eventually to be subsumed as a class or order within some other protozoan phylum. If Paramyxea really turn out to belong with Sporozoa in the Apicomplexa, perhaps Haplosporidia will too, as they have some similarity in mode of sporogenesis.

Phylum Paramyxia

Unlike Myxosporidia and Haplosporidia, Paramyxia have centrioles (53a): since these, like those of most Apicomplexa, consist of nine-singlet microtubules rather than triplets, it is possible that they evolved from Apicomplexa by loss of the apical complex and cortical alveoli.

Figure 2, showing the postulated phylogenetic relationships between the 18 protozoan phyla and the seven other

eukaryote kingdoms or subkingdoms did not attach Haplosporidia, Paramyxia, and Myxosporidia to the tree at all, because their relationships are so uncertain.

NEW PROTOZOAN SUBPHYLA, CLASSES, SUBCLASSES, AND ORDERS

Percolozoa

The recently discovered obligately anaerobic flagellates *Psalteriomonas lanterna* (10a) and *P. vulgaris* (10) have been treated previously as Heterolobosea. However, *P. vulgaris* lacks mitochondria, peroxisomes, and an amoeboid stage and is therefore radically different from typical Heterolobosea: in fact, it lacks all synapomorphies that were used to define Heterolobosea. Whether *P. lanterna* has mitochondria or not is unclear; it has double-membrane enveloped structures lacking both cytochrome oxidase and cristae, which have been called mitochondria (10a) simply because they are (like mitochondria of Heterolobosea) surrounded by a cisterna of RER. They might be degenerate mitochondria (10a) or even symbiotic gram-negative bacteria. Moreover, unlike Heterolobosea and all other Percolozoa, both species have hydrogenosomes with an envelope of two membranes like those of Parabasalia. However, unlike Parabasalia, they lack Golgi dictyosomes and have an endonuclear spindle. They therefore clearly belong in Percolozoa, not Parabasalia. Not only do these two species lack several (*P. lanterna*) or all (*P. vulgaris*) of the synapomorphies that characterize Heterolobosea, but also they have two major synapomorphies absent from all other Percolozoa: hydrogenosomes and a unique harp-shaped structure (consisting of microtubules, cristalline material, and microfilaments) underlying their surface groove(s). Because of these two major synapomorphies and the other radical differences between them and Heterolobosea, I have placed both species in a new class, Lyromonadea (named after the lyre-shaped root or support structure for the groove). *P. vulgaris* differs so radically from *P. lanterna* that it should not be in the same genus or family. Unlike *P. lanterna*, it has no amoeboid stage and has one nucleus and one kinetid instead of four; moreover, it lacks the degenerate mitochondrion-like structure. Therefore, I have created a new genus, *Lyromonas*, and a new family, Lyromonadidae, to accommodate *P. vulgaris*. A new order, Lyromonadida, includes both the Lyromonadidae and the Psalteriomonadidae Cavalier-Smith, 1992.

Euglenozoa

Within the Euglenozoa a new class, Diplonemea cl. nov., and order, Diplonemida ord. nov., are required to accommodate the flagellate *Diplonema*, formerly called *Isonema* (138). Since euglenoids are cytologically much more diverse than either *Diplonema* or the kinetoplastids, I have divided them into three new classes: the Aphagea for the nonphagotrophs (those with plastids and the saprotrophic rhabdomonads), the Peranemea for the most advanced phagotrophs with a complex feeding apparatus consisting of supporting rods and vanes, and the Petalomonadea for the less advanced phagotrophs with only an MTR (microtubule root)/pocket type of feeding apparatus like the bodonids and some Aphagea. The Aphagea are divided into two subclasses, Euglenia with plastids and Rhabdomonadia without. This means that Euglenoida and Euglenia are not synonyms and requires that Euglenoida as a whole be ranked as a subphylum. I therefore also create a new subphylum, Diplonemia, for the class Diplonemea and a new subphylum, Kinetoplasta, for the sole class Kinetoplastea. I have chosen the name Euglenia for the plastid-containing euglenoids rather than Euglenophyceae, as suggested earlier (41), because the latter has often been used for euglenoids as a whole. The terms Euglenophyceae and Euglenophyta are best abandoned. No euglenoids are plants, and only about half (the plastid-containing ones) are algae. Indeed, Euglenia are both Protozoa and algae, since the two concepts are not mutually exclusive. Since Algae has long since been abandoned as a taxon because it is polyphyletic, there is no problem caused by the overlap between algae as a grade (i.e., nonembryophyte eukaryotes with plastids) with the paraphyletic taxon Protozoa. Euglenia and photosynthetic Dinoflagellata are both algae and Protozoa. Plastid-free euglenoids and dinoflagellates are Protozoa but not algae. I have adopted the earlier name Astasida (57) for the order containing *Euglena*, rather than Euglenida, since some authors use euglenid as a synonym for all euglenoids (81c). The Bodonida and Trypanosomida are here treated as orders, not suborders.

Opalozoa

The classification of Opalozoa has been treated recently in detail (44; see also the discussion on the phylum earlier in the present review). The only change necessary here is the inclusion of the newly described endoparasite *Phagodinium* (81a) in the class Heteromitea. *Phagodinium* was described as a dinophyte, but since it lacks cortical alveoli, it belongs in Opalozoa rather than Dinozoa and since it has no trace of its own chloroplasts (it can temporarily harbor those of its host, the synurid chrysophyte *Mallomonas*), it is best treated under the Zoological, not the Botanical, Code of Nomenclature. However, it is sufficiently different from all other Opalozoa to be placed in a new order, Phagodinida. The presence of cytoplasmic starch is not a good reason for it having been treated as a dinophyte, since in addition to Dinoflagellata, cytosolic starch is also present in Rhodophyta, Glaucophyta, and the periplastid space of Cryptomonadea. There appears to be no specific reason to place *Phagodinium* in any of these four taxa: each differs from *Phagodinium* in at least two or three major respects. By contrast, the ciliary structure with a transitional helix is quite close to that of the heteromitean subclass Anisomonadia: but since there are three substantial differences from Anisomonadia (presence of cytosolic starch, the endoparasitic habit with multiple fission to form zoospores within a cyst, and the absence of pellicular microtubules except for those of the four ciliary roots), I here create the new subclass Phagodinia for it rather than simply including it within Anisomonadia and modifying its diagnosis. *Phagodinium* resembles *Phagomyxa* in being a phagotrophic endoparasite of algae: it is therefore possible that it is closer to *Phagomyxa*, from which it differs in not having a plasmodial phase, than to Anisomonadia. I chose not to place it with Phagomyxida in the class Phytomyxea partly because this would have involved modifying the diagnosis of the class Phytomyxea and partly because as the ultrastructure of *Phagomyxa* is unknown it seemed preferable to group *Phagodinium* with protozoa that clearly have a similar ciliary ultrastructure. However, the properties of *Phagodinium* suggest that it may be transitional between Heteromitea and Phytomyxea: when the properties of more such organisms are better known, it might prove necessary to merge the two classes. Though I have argued against placing *Phagodinium* in the Dinozoa, the fact that it

has cytoplasmic starch (unusual in Opalozoa) suggests that it may be a relative of the opalozoan from which the Dinozoa are presumed to have evolved by the origin of cortical alveoli (38); since many early diverging dinoflagellates and the protalveolate ellobiopsids both are endoparasites, it seems possible that the ancestral dinozoan might have evolved from an endoparasitic opalozoan. It is sometimes suggested that dinoflagellates obtained their chloroplasts by a secondary symbiosis from a chromobiote alga (64a); though it is by no means clear that this is the case (see reference 41), if it is, we should perhaps consider the possibility that dinoflagellates may have done so not as free-living phagotrophs, as usually assumed, but as endoparasites of chromobiotes, somewhat similarly to *Phagodinium*, temporarily acquiring its host's chloroplasts. If dinoflagellates did indeed obtain their chloroplasts from a photosynthetic host, this would be a remarkable reversal of the usual host-symbiont relationship prior to the symbiotic origin of organelles.

Dinozoa

The inclusion of *Colponema* in the Dinozoa requires a new order, Colponemida ord. nov., since it cannot be included in the order Oxyrrhida. Since Ellobiopsida apparently have tubular cristae and vesicles somewhat resembling cortical alveoli (145); they also probably belong in the Dinozoa but are sufficiently distinct also to be treated as a class, Ellobiopsea. The name Ellobiophyceae Loeblich III 1970 would be totally misleading for a nonphotosynthetic protozoan class. To contrast Colponemea, Oxyrrhea, and Ellobiopsea with the dinoflagellates with their exonuclear mitotic spindles, I group them in the new subphylum Protalveolata (a name serving to indicate that this subphylum probably includes the most primitive organisms with cortical alveoli). For dinoflagellates, the new subphylum Dinoflagellata characterized by exonuclear spindles is created: it contains five classes (Syndinea Chatton, 1920; Noctilucea Haeckel, 1866; Haplozooidea Poche, 1911; Peridinea Ehrenberg, 1830; and the new class Bilidinea). By this use of the subphylum rank, one can emphasize in a balanced way both the profound differences between dinoflagellates and the protoalveolate dinozoa and the more distant common features that they share. The recent discovery that the chloroplasts of Dinophysida (= Dinophysiales) have phycobilins as well as chlorophyll *c* and peridinin (122) indicates a radical difference from typical photosynthetic dinoflagellates; whether these aberrantly pigmented chloroplasts diverged from the typical peridinean ones during the early diversification of chloroplasts in a dinozoan host (18) or whether (122) they are the result of the symbiotic acquisition of cryptomonad chloroplasts, which they resemble, they are sufficiently different from the typical non-phycobilin-containing ones for the taxa possessing them to be placed in a separate class, Bilidinea cl. nov., so as to contrast them with those lacking phycobilins, i.e., the Peridinea. The idea that Dinophysida are very deeply divergent from typical peridinea is quite old (15). Three other groups of dinoflagellates deserve separate class status, including the parasitic class Syndinea, which differ from typical dinoflagellates and resemble all other eukaryotes in having typical histone-rich chromatin throughout their life cycle; and the parasitic class Haplozooidea, which like the free-living *Noctiluca* have histones in their vegetative cells but not in their reproductive cells. *Noctiluca* is so different in many other respects also that it was not originally treated as a dinoflagellate but put in a separate phylum, Noctilucae Haeckel, 1866, here treated as a class Noctilucea, which is here grouped with Haplozooidea in the new superclass Hemidinia, characterized by a life cycle with an alternation between histone-rich and histone-poor nuclei. The two dinoflagellate classes with typical dinokaryotic nuclei that lack histones throughout their life cycle (i.e., Peridinea and Bilidinea) are here grouped in the new superclass Dinokaryota, while a third superclass, Syndina, contains only the Syndinea. In this way, one can recognize the three very different patterns of chromatin organization in dinoflagellates.

The major reclassification of dinoflagellates by Fensome et al. (61a), published shortly before the present review, independently creates a taxon Dinokaryota, but their subdivision Dinokaryota differs from my superclass Dinokaryota in that it includes all dinoflagellates that have histones in at least one stage of their life cycle, i.e., all dinoflagellates except Syndinea; thus, they include *Noctiluca* and Haplozooidea within Dinokaryota, whereas I separate them as superclass Hemidinia. Both classifications stress the importance of the three different chromatin types but group them differently; both groupings are phylogenetically acceptable, but I prefer my stricter definition of Dinokaryota. Their classification agrees with the present one in excluding *Oxyrrhis* from Dinoflagellata, though they rank Dinoflagellata as a division (equivalent to a zoological phylum) and not a subphylum and treat all Dinoflagellata nomenclaturally under the Botanical Code. It seems to me undesirable to use the botanical suffix -phyceae ("algae") for the three totally nonphotosynthetic dinoflagellate classes (Syndinea [Fensome et al. do use this name for a subdivision or subphylum that includes only their class Syndiniphyceae], Noctilucea, and Haplozooidea), though such a suffix is quite acceptable for the Peridiniphyceae, which in their system includes both my Peridinea and Bilidinea (the latter as the subclass Dinophysiphycidae). However, I prefer to use the more neutral protozoological suffixes for all dinozoan taxa and therefore have adopted earlier zoological spellings of the endings of the classes and subclasses. Since six of the eight dinozoan classes are totally nonphotosynthetic, as are about half of the species in the two remaining classes, it seems best to treat the whole phylum under the Zoological rather than the Botanical Code of Nomenclature.

Apicomplexa

The phylum Apicomplexa also requires subdivision into subphyla. Leuckart's original class Sporozoa contained only Gregarinia and Coccidia and is here treated as an infraphylum, characterized by nine-singlet centrioles, complete conoids and conoidal rings, and the general (but not universal) occurrence of sporocysts and oocysts. Vivier's Hematozoa (140), containing Haemosporea and Piroplasmea, not only lacks sporocysts, conoids, and conoidal rings but also has nine-triplet centrioles like nearly all other eukaryotes: the Hematozoa, with this ancestral type of centriole, cannot therefore be derived from Coccidia, which have the derived nine-singlet centriole, contrary to what was often supposed formerly (88), and should be excluded from the Sporozoa altogether to form a separate infraphylum Hematozoa infraphyl. nov. Because of their nine-singlet centrioles (53a), the Paramyxia may be allied with the Sporozoa, but since they differ from Sporozoa in so many other respects, they are here treated as a separate phylum. For the most primitive flagellate apicomplexans *Perkinsus* and *Colpodella* (earlier miscalled *Spiromonas* [116]), a distinct new subphylum, Apicomonada, is created. Unlike Apicomonada, Sporozoa

share anisogamous sexuality followed by schizogony to form sporozooites with the Hematozoa; therefore, these two taxa are here grouped together in a new subphylum, Gamontozoa. Sporozoa and Hematozoa probably evolved independently from an early gamontozoan ancestor. The fact that *Perkinsus* branches on the rRNA tree (Fig. 1) close to the bifurcation between dinoflagellates and Gamontozoa is consistent with the view that an apicomonad was the evolutionary intermediate between Protalveolata and Gamontozoa. *Colpodella* is sufficiently different from *Perkinsus* to require a new order, Colpodellida (see Appendix 2), but not a separate class, so Perkinsida and Colpodellida are both included in the new class Apicomonadea. Gregarinia and Coccidia are each treated as superclasses, and each is subdivided into two classes: a more primitive one of purely extracellular parasites without merogony (Eogregarinea and Coelotrophea, respectively), and a more advanced one with intracellular parasites and merogony (Neogregarinea and Eucoccidea). It appears that intracellular parasitism and merogony evolved independently in the Neogregarinea and Eucoccidea. It may also have evolved independently in the Hematozoa.

Radiozoa

In order to group the Acantharea and Sticholonchea together because of their spasmin-like myonemes (58) and to contrast them with the subphylum Radiolaria, a new subphylum, Spasmaria, is created. Within the Acantharea, new subclasses are created for the two orders with 10 diametral spines (Holacanthia) and for the three orders with 20 radial spines (Euacanthia).

Heliozoa

The diversity of the phylum Heliozoa (59) requires that they be subdivided into two classes, of which one (Nucleohelea cl. nov.) differs in content from previously defined taxa.

For Reticulosa, the new subphyla were sufficiently discussed in an earlier section. Diagnoses of these new classes and orders are given in Appendix 2.

DISCUSSION

The major innovations in the present paper are the following: (i) the more precise delimitation and diagnoses of the kingdom Protozoa and of the phyla Dinozoa and Rhizopoda, including the transfer of Chlorarachniophyta from Protozoa to the kingdom Chromista; (ii) the creation of the subkingdom Adictyozoa and the branch Bikonta; (iii) the creation of the parvkingdoms Ciliomyxa, Neosarcodina, Entamoebia, Myxozoa, and Mesozoa and the superphylum Opalomyxa; (iv) the creation of 20 new protozoan or archezoan classes (Lyromonadea in the Percolozoa; Colponemea, Noctilucea, and Bilidinea in the Dinozoa; Diplonemea, Petalomonadea, Peranemea, and Aphagea in the Euglenozoa; Apicomonadea, Eogregarinea, Neogregarinea, Coelotrophea, and Eucoccidea in the Apicomplexa; Trepomonadea and Retortamonadea in the Metamonada; Minisporea, Pleistophorea, and Disporea in the Microsporidia; Holosea in the Neosarcodina; and Nucleohelea in the Heliozoa); (v) the creation of seven new protozoan superclasses, seven new subphyla, two new infraphyla, 12 new subclasses, 11 new orders, and two new families; and (vi) the creation of two new chromistan subkingdoms and three new chromistan classes (Flavoretea, Patelliferea, and Pavlovea). Formal diagnoses of all new chromistan taxa are given in Appendix 3.

Though 18 protozoan phyla are substantially more than the 7 phyla in the protozoologists' last classification (89), the present system is much more conservative than the approximately 30 phyla suggested informally by Corliss (52) for the taxa here included in the kingdom Protozoa and I believe presents a good balance between excessive lumping or splitting, given our present state of knowledge. This I have achieved partly by extensive use of the category of subphylum, of which my protist system contains over 30 (19 in kingdom Protozoa, 4 in kingdom Chromista, and 4 in kingdom Archezoa, with yet others in Plantae and Fungi), in contrast to that of Levine et al. (89), which had only three, and those of Margulis et al. (98) and Corliss (52), which had none. I fully agree with Corliss (52) and Page (109) that the phylum Sarcomastigophora had to be abandoned but do not think it necessary to create as many new phyla as in Corliss's scheme. Perhaps not all of the present phyla are monophyletic, but I think most of them will prove to be. Probably most of them are even holophyletic (3), though I think that Percolozoa, Opalozoa, Choanozoa, and Dinozoa at least are almost certainly paraphyletic (38).

It should be stressed that the removal of the relatively few Archezoa and phagotrophic chromists from the kingdom Protozoa should not exclude them from the sphere of interest of protozoology. Nobody is better placed to study those important groups than protozoologists. We can, however, perhaps give a new focus to protozoology by defining it as "the study of Protozoa, Archezoa, and phagotrophic chromists," and by leaving all Chlorophyta in the plant kingdom where they belong. Conversely, clear recognition that Mycetozoa and Plasmodiophorida are Protozoa, not fungi, should not prevent both mycologists and protozoologists from studying them. Clearly demarcated boundaries for biological taxa are as scientifically desirable as they are undesirable for scientific research.

The conventional divisions between botany and zoology played no part in formulating the eight-kingdom system (I have a degree in zoology and a professorship in botany). However, from a nomenclatural viewpoint, it is remarkably convenient that almost all organisms traditionally treated under the botanical code fall into the kingdoms Plantae, Fungi, and Chromista, whereas those treated under the zoological code fall into the kingdoms Animalia, Protozoa, and Archezoa. I have proposed, therefore, that in future protist members of the first three kingdoms should be described according to the rules of the botanical code and all protists in the last three kingdoms should be described according to the zoological code (17). For this nomenclatural purpose only, I have called the first three "botanical" kingdoms and the last three "zoological" kingdoms (17). But this terminology was and is for nomenclatural and not taxonomic purposes. The adoption of this proposal would solve most (but not quite all) of the problems posed by the presently overlapping jurisdiction of the distinctly different Botanical and Zoological Codes of Nomenclature discussed recently by Corliss (52a): creation of a separate code of nomenclature for protists would probably cause more problems than it would solve. There is, however, a clear need for some greater harmonization of the two codes and more explicit recognition by both of them of the special problems of applying them to protists. From a phylogenetic perspective, it is clear that botany is polyphyletic, while zoology is paraphyletic. Therefore, according to ultrastrict cladists (111), neither botany nor zoology can even exist! However,

I do not share their aversion to paraphyly and believe that polyphyletic botany and paraphyletic protozoology both have very bright futures. But for nomenclatural purposes, euglenoids and dinoflagellates should in future be treated under the zoological code only: this will reduce the confusion caused by their current treatment under two partially contradictory codes. It also means that we can retain such well-established protozoological names as *Peranema* Dujardin, 1841 and *Entosiphon* Stein, 1878, which are junior homonyms under the Botanical Code (81c).

Though the boundaries of the present kingdom Protozoa seem at present to be rather well defined, it is possible that they may require revision in future. The proposed boundaries with the kingdom Fungi and the subkingdom (or kingdom) Viridiplantae (both indisputably monophyletic taxa) are particularly sharp and so are highly likely to be stable. But the boundaries with the Archezoa, Biliphyta, Animalia, and Chromista, though perfectly well definable, are still open to question and future minor adjustments, since our understanding of detailed phylogeny at the interface between these taxa and Protozoa is not yet definitive. There are four particular sources of uncertainty.

The first concerns the boundary with the Archezoa. A 28S rRNA tree based on partial sequences shows trichomonads as branching within the metakaryotes (78) and therefore supports the inclusion of the Parabasalia within the Protozoa rather than the Archezoa. But recent 18S rRNA trees (86a, 121) have suggested that they branch slightly more deeply than other metakaryotes, raising again the question of whether they belong in the Archezoa, as originally proposed (21). However, in Fig. 1 they appear to branch just within the metakaryotes. Clearly, many more sequences are needed for Parabasalia (so far only one is available), Percolozoa, Euglenozoa, and Metamonada before we can be confident of the branching order in that part of the tree, since taxa represented by a single species have sometimes been placed somewhat incorrectly on the 18S rRNA tree. It is also clear from our own unpublished studies that the branching order in the region of the 18S rRNA tree between metamonads and the mycetozoan *Dictyostelium* is not very robust: it is sensitive to changes in the bacterial outgroup (as Leipe et al. [86a] also show), the species composition of the tree, the weighting or masking of different parts of the sequence, and the algorithm used to calculate the tree. It remains to be seen whether Archamoebae are genuinely Archezoa, and as discussed earlier, we cannot yet even totally rule out the possibility that microsporidia are secondarily amitochondrial Protozoa rather than genuine archezoa.

The second area that calls for more study is the monophyly (or otherwise) of the Chromista, which will be very hard or even impossible to demonstrate by the rRNA sequence trees if, as I have argued (23, 32, 39, 45a), the four phyla diverged during the very origin of the kingdom: although the Chromobiota are very probably monophyletic (45a), a specific relationship between them and the Cryptista and Chlorarachniophyta is still open to question (39, 54, 54a, 93). If chromist monophyly is eventually unambiguously refuted rather than confirmed by future research, then (and only then) I would favor the transfer of the phylum Cryptista and/or Chlorarachniophyta from the Chromista into the Protozoa or their treatment as separate kingdoms (16). Even if the cryptomonads and/or the Chlorarachniophyta were to be returned to the Protozoa, it would be important to maintain a clear distinction between the kingdoms Protozoa and Chromista and to continue to think of Chromista as being a higher kingdom (like Plantae, Fungi, and Animalia) derived from (and therefore evolutionarily continuous with), but nonetheless having evolved into a higher grade of organization than, the kingdom Protozoa.

The third source of uncertainty in the eight-kingdom system is whether the kingdom Plantae is monophyletic. Though I am increasingly confident that chloroplasts had only a single origin from cyanobacteria (39, 54, 102a, 139), it is possible that one of the three plant taxa Viridiplantae, Rhodophyta, and Glaucophyta (most likely Glaucophyta, which are unique among Plantae in having cortical alveoli) may turn out to be more closely related to dinoflagellates than to Rhodophyta: there might then be a case for transferring Glaucophyta to the Protalveolata within the Dinozoa, though I would still prefer its retention in the Plantae as the basal group.

The fourth source of uncertainty concerns the exclusion of Mesozoa from the animal kingdom, which needs to be confirmed (or refuted) by rRNA phylogeny.

ENVOI

The present classification of Protozoa will undoubtedly require further revision. But since it gives a much better treatment than did the previous one (89) of the fantastic cellular diversity of the zooflagellates (45) (here spread across 37 classes in the kingdoms Archezoa [4 classes], Chromista [5 classes], and Protozoa [28 classes] rather than lumped into a single class), within which most of the major steps in eukaryote cell evolution occurred (38), it much better reflects the complex phylogenetic history of the kingdom than did previous classifications and therefore will, I hope, be more stable than they have proved to be. However, about 100 zooflagellate genera, which lack characters visible in the light microscope that can clearly place them in a particular phylum, have not been studied by electron microscopy (116) and therefore cannot be included in the present classification. While many, probably even most, may prove eventually to be assignable to the Opalozoa, we cannot at present rule out the possibility that additional protozoan phyla may one day be needed to accommodate some of them. Apart from these neglected zooflagellates, it is in the former sarcodine phyla, and possibly in some of the suprahyletic groupings, that we should expect to see the most extensive future revisions as new molecular data accrue.

APPENDIX 1. DIAGNOSES OF SUBKINGDOMS, BRANCHES, INFRAKINGDOMS, PARVKINGDOMS, SUPERPHYLA, PHYLA, SUBPHYLA, AND INFRAPHYLA OF THE KINGDOM PROTOZOA

(Note: These are diagnoses, not descriptions, and so are mostly restricted to the characters necessary to separate each taxon from others of the same rank that are classified together in the next-higher-level taxon.)

SUBKINGDOM 1. **ADICTYOZOA subking. nov.**

Protozoa without Golgi dictyosomes

Phylum 1. **Percolozoa Cavalier-Smith, 1991**

Unicellular protozoa lacking Golgi dictyosomes. Mitochondria or (more rarely) hydrogenosomes present. Mitochondria if present having flat, often somewhat discoid or irregularly variable cristae. Hydrogenosomes usually absent (present in *Psalteriomonas* [10a] and *Lyromonas* [10] only).

Name based on the genus *Percolomonas* Fenchel & Patterson, 1986 (61).

Subphylum 1. **Tetramitia Cavalier-Smith, 1993** (43)

Kinetid quadriciliate, biciliate, or absent. Mitochondria and (probably) peroxisomes usually present: if absent (*Lyromonas*) or (possibly) degenerate (*Psalteriomonas*), then hydrogenosomes al-

ways present. Cristae, if present, usually discoid but flexible. Flagellates, amoeboflagellates, or rarely nonflagellate amoebae, with one or four kinetids.

Subphylum 2. **Pseudociliata Cavalier-Smith, 1993** (43)

Numerous kinetids, each with a single cilium; centrioles (following Heywood's recommendation [69a], I use centriole to include both basal bodies and centrioles) arranged in longitudinal rows. Multinucleate. Mitochondria and (probably) peroxisomes always present. Rigid discoid cristae.

SUBKINGDOM 2. **DICTYOZOA Cavalier-Smith, 1991**

Unicellular, plasmodial, colonial, or multicellular protozoa possessing Golgi dictyosomes. Mitochondria typically present, with varied cristal morphology; if mitochondria are absent, hydrogenosomes are commonly (but not always) present instead.

BRANCH 1. **Parabasalia new branch**

Unicellular flagellates (rarely an amoeba) lacking mitochondria, peroxisomes, or glycosomes, but having hydrogenosomes with a double envelope; highly developed Golgi dictyosomes associated with a cross-striated ciliary root form a parabasal body; closed mitosis with exonuclear spindle. Ribosomes 70S. Spliceosomal and self-splicing introns unknown.

Sole phylum: **Parabasalia Honigberg, 1973 stat. nov. Cavalier-Smith, 1981**

Diagnosis as for branch Parabasalia.

BRANCH 2. **Bikonta new branch**

Dictyozoa usually with mitochondria and peroxisomes (if mitochondria absent, then having both macro- and micronuclei [some ciliates] or else an intranuclear centrosome [Entamoebia]); Golgi dictyosomes not associated with a striated fiber; kinetid usually with two centrioles, sometimes with one or very rarely three or four, or absent; chloroplasts, if present (Euglenia and many Dinokaryota only), located in the cytosol and usually having an envelope of three membranes.

INFRAKINGDOM 1. **Euglenozoa Cavalier-Smith, 1981 stat. nov.**

Unicellular Dictyozoa with discoid or (rarely) plate-like flat mitochondrial cristae; flagellates with one to two cilia (rarely up to four), usually with paraxial rods and often simple (nontubular) hairs; a regular array of longitudinal subpellicular microtubules; either peroxisomes or glycosomes (not both) usually present; three asymmetric microtubular ciliary roots; closed mitosis with endonuclear spindle; hydrogenosomes absent. All nuclear protein-coding genes have *trans*-splicing of miniexons to pre-mRNAs to create mature mRNA (134; the possible evolutionary significance of these and other peculiarities of lower eukaryote genomes are discussed in reference 41a). Chloroplasts, if present, have chlorophylls *a* and *b* and an envelope of three membranes but lack starch: located in the cytosol.

Sole phylum: **Euglenozoa Cavalier-Smith, 1981**

Diagnosis as for the infrakingdom Euglenozoa.

Subphylum 1. **Diplonemia subph. nov.**

Phagotrophic flagellates lacking kinetoplasts; two equal cilia without paraxial rods or transitional helix; peroxisomes are not glycosomes; chloroplasts absent; feeding apparatus of the MTR/pocket type and with vanes and two supporting rods; pellicular plates absent; pellicular microtubules evenly spaced; no surface ridges and grooves; pronounced euglenoid movement; plate-like mitochondrial cristae.

Subphylum 2. **Euglenoida Bütschli, 1884 stat. nov.**

Flagellates lacking kinetoplasts; phagotrophic, photosynthetic, or osmotrophic; chloroplasts or leukoplasts present or absent; two or (very rarely) three or four cilia with paraxial rods and nontubular hairs; cilia arise within a pear-shaped reservoir connected to the cell surface by a narrow canal; cilia equal or, very often, one is reduced and does not emerge from the canal; pellicle with glycoprotein strips underlying the plasma membrane and subtended by a short row of microtubules; strips usually folded into a ridge and groove pattern; peroxisomes are not glycosomes; feeding apparatus of the MTR/pocket type, or consisting of vanes and two supporting rods, or absent; discoid mitochondrial cristae; euglenoid movement present or absent.

Subphylum 3. **Kinetoplasta subph. nov.**

Flagellates with one or more kinetoplasts in the mitochondria; cristae usually discoid, occasionally tubular in one phase of the life history; peroxisomes are glycosomes; one or two cilia, usually with paraxial rods; nontubular ciliary hairs present or absent; feeding apparatus of the MTR/pocket type (137); a feeding apparatus consisting of vanes and two supporting rods is absent; phagotrophic, or micropinocytotic; chloroplasts absent; euglenoid movement absent.

INFRAKINGDOM 2. **Neozoa Cavalier-Smith, 1993** (43)

Mitochondrial cristae typically nondiscoid: usually tubular, sometimes vesicular or flat; if discoid, then cilia and pellicle absent. Nuclear protein-coding genes often with short *cis*-spliced spliceosomal introns, not *trans*-spliced to miniexons.

PARVKINGDOM 1. **CILIOMYXA parvkingd. nov.**

Flagellates without cortical alveoli or slime molds with stalked fruiting bodies; glycosomes and hydrogenosomes absent; chloroplasts absent; mitochondria with tubular or nondiscoid flat cristae invariably present. Almost always free-living; occasionally parasitize plants or commensal in animal guts or lumens; never parasites of animal tissues.

SUPERPHYLUM 1. **Opalomyxa superphyl. nov.**

Unicellular or colonial flagellates, multiciliated cells, or nonciliated amoebae or plasmodia in the trophic phase; kinetid with one, two, or four centrioles or absent (Dictyostelea and some protostelids only); mitochondria with tubular cristae or very rarely irregularly flattened (nondiscoid) cristae; peroxisomes present (except in Proteromonadida and Opalinea); mitosis closed with endonuclear spindle, or semiopen or (rarely) open; stalked aerial fruiting bodies typically present if trophic phase is amoeboid or plasmodial.

Phylum 1. **Opalozoa Cavalier-Smith, 1991** (44)

Mitochondrial cristae typically tubular (if flattened [rarely: sole known examples *Jakoba* and *Ancyromonas*], then kinetid anisokont with two cilia, three asymmetric microtubular roots, and lacking both periciliary collar and cryptomonad-type double-scroll ejectisomes); cortical alveoli and aerial spore-bearing fruiting bodies absent; having single kinetid of one to four cilia (usually two) or having many cilia; cilia lack bipartite or tripartite rigid surface hairs; uninucleate, or multinucleate with equivalent nonheterokaryotic nuclei; mostly unicellular flagellates; rarely multiciliated or occasionally plant-parasitic microplasmodia with biciliate swarmers or animal symbionts with a chitin-walled filamentous stage and biciliate swarmers.

Subphylum 1. **Proterozoa Cavalier-Smith, 1991 stat. nov. emend.**

Usually free-living uninucleate flagellates (rarely microplasmodial or filamentous parasites with a biciliate stage) with one or more, usually two, cilia; cell surface (unlike in Opalinea) not extended as narrow folds supported by a vertical row of microtubules, nor with cortical ridges of euglenoid type; axopodia absent; peroxisomes usually present (absent in *Proteromonas*); mitochondrial cristae tubular, or rarely (only *Jakoba*) flattened but not discoid; extrusomes various, but never kinetocysts.

Subphylum 2. **Opalinata Wenyon, 1926 stat. nov. emend. Cavalier-Smith, 1993**

Uninucleate flagellates with four cilia or multinucleate cells with rows of monokinetid cilia that are divided longitudinally; cell surface extended into narrow folds, each supported by a vertical row of microtubules; parasites or commensals of the gut of terrestrial vertebrates; extrusomes absent; peroxisomes probably absent; mitochondrial cristae tubular and unbranched.

Subphylum 3. **Kinetomonada subph. nov.**

Free-living uninucleate flagellates with two or four cilia; with or without axopodia containing an axoneme of microtubules; axopodial axonemes nucleated by an axoplast associated with the exceptionally long ciliary centrioles; extrusomes are kinetocysts with cylindrical substructure; mitochondrial cristae branched tubules (Heliomonadida and probably *Histiona*) or flat (*Ancyromonas*); peroxisomes present.

Subphylum 4. **Hemimastigophora Foissner, Blatterer & Foissner, 1988 stat. nov.** (62, 63)

Uninucleate cell with twofold rotational symmetry having a plicate pellicle with two large pellicular plates, a distinct epiplasm, and subpellicular microtubules; two rows of identical cilia in the grooves between the plates; temporary cytostome at anterior pole;

nucleus not obviously associated with or attached to the centrioles; complex extrusomes shaped like a wine bottle with a nail-like compartment embedded in the neck; centrioles exceptionally short; ciliary transitional plate and a very slender transitional helix or cylinder; tubular mitochondrial cristae, occasionally connecting to a caverna.

Phylum 2. **Mycetozoa de Bary, 1873 stat. nov. Engler & Prantl, 1888** (first treated as a phylum [Myxomycetes] by Haeckel, 1866)

Unicellular or plasmodial, free-living, nonflagellate phagotrophic trophic phases; unicellular or multicellular aerial fruiting bodies bearing one to many spores with cellulose or chitinous walls; mitochondrial cristae tubular; cilia, none to four, in dispersal phase with only one kinetid per cell.

SUPERPHYLUM 2. **Choanozoa Cavalier-Smith, 1981, emend. stat. nov.**

Uniflagellate unicellular or colonial protozoa; mitochondrial cristae with flattened nondiscoid cristae; ciliary root a symmetric cone or radial array of single microtubules.

Sole phylum **Choanozoa Cavalier-Smith, 1981, emend. 1983.**

Trophic cells with a single cilium surrounded by a collar of microvilli (supported internally by actin filaments) that are used to catch bacteria prior to their phagocytosis. Free-living. Unicellular or multicellular.

PARVKINGDOM 2. **Alveolata Cavalier-Smith, 1991 stat. nov.**

Having cortical alveoli or a large cortical membrane-bound cisterna; free-living or parasites on protozoa or animals. Mitochondria with tubular cristae and peroxisomes usually present; in anaerobic ciliates, both are absent or replaced by hydrogenosomes. Mitosis closed or semiopen; spindle endo- or exonuclear. Chloroplasts usually absent; if present, have chlorophylls *a* and *c* and an envelope of three or rarely two membranes; located in cytosol and lack phycobilisomes.

SUPERPHYLUM 1. **Miozoa Cavalier-Smith, 1987**

Nuclei haploid, monomorphic; meiosis with only one step.

Phylum 1. **Dinozoa Cavalier-Smith, 1981**

Flagellates with tubular, often ampulliform mitochondrial cristae and cortical alveoli; kinetid with two anisokont cilia; usually one, rarely several, karyomastigonts per cell; apical complex absent; usually unicells lacking cell walls (but may have cellulose plates inside the alveoli) or rarely walled filamentous or mycelial multicells with limited cell differentiation; chlorophyll *c*-containing chloroplasts often present, but frequently absent; mitosis closed.

Subphylum 1. **Protalveolata Cavalier-Smith, 1991**

Mitotic spindle intranuclear.

Subphylum 2. **Dinoflagellata Bütschli, 1885 stat. nov. Cavalier-Smith, 1991**

Closed mitosis with extranuclear mitotic spindle.

Phylum 2. **Apicomplexa Levine, 1970 emend.**

Unicellular parasites or predators having at some life cycle stage an apical complex; apical complex of polar rings, rhoptries, micronemes, subcortical microtubules and usually a conoid (complete or incomplete); one or more large subplasma membrane, highly compressed, smooth-membraned cisternae usually present in the cell cortex of infective stages; cilia rarely present in the trophic phase, more usually restricted to male microgametes or absent; mitochondrial cristae tubular or much reduced (or even absent).

Subphylum 1. **Apicomonada subphyl. nov.**

Conoid incomplete; predators or parasites; sex unknown; two cilia.

Subphylum 2. **Gamontozoa subphyl. nov.**

Conoid and conoidal rings complete or absent; parasites; anisogamous sexuality with nontrophic cells (male and female gamonts) that generate dissimilar male and female gametes (often by multiple fission: gamogony); syngamy followed by meiosis and multiple fission (shizogony) to generate infective sporozoites (i.e., sporogony); cilia absent or present only on microgametes (one, two, or three per gamete).

Infraphylum 1. **Sporozoa Leuckart, 1879 stat. nov.**

Centrioles with nine-singlet microtubules; centrosome is a centrocone; centrioles present at spindle poles; microgametes with one, two, or three cilia; complete conoid and conoidal rings present; zygote immediately forms a thick wall (oocyst in coccidia, zygocyst or sporocyst in gregarines); carbohydrate stored as amylopectin granules; mitochondrial cristae tubular (broad straight tubules or ampullae with a narrow base as in Dinozoa); extracellular or intracellular parasites of animals.

Infraphylum 2. **Hematozoa Vivier, 1982 stat. nov.**

Intracellular parasites alternating between the gut of blood-sucking arthropods (where syngamy occurs) and the erythrocytes of vertebrates; merogony in vertebrate erythrocytes; unable to store carbohydrate as amylopectin; mitochondrial cristae usually absent; centrosomes are not centrocones; centrioles absent from spindle poles (Haemosporea) or totally absent (Piroplasmea); if present, centrioles have triplet microtubules; conoid and conoidal rings absent; zygote is motile and does not immediately form a thick wall, but penetrates the gut wall of the invertebrate host; microgametes with one cilium, or none.

SUPERPHYLUM 2. **Heterokaryota Hickson, 1903 stat. nov.**

Nuclei dimorphic; separate diploid micronuclei and multiploid (22) macronuclei. Meiosis with two separate divisions.

Phylum 1. **Ciliophora Doflein, 1901 stat. nov. Copeland, 1956 emend. auct.** (= Infusoria auct.)

Numerous mono- or biciliated kinetids arranged in longitudinal rows (as "kineties") with respect to the transverse binary fission axis; heterokaryotic, with diploid micronuclei and usually multiploid (22) macronuclei; mitosis closed with endonuclear spindle; cortical alveoli usually present; mitochondrial cristae tubular, often curved; occasionally in anaerobes mitochondria are replaced by hydrogenosomes (often with a double envelope) or are degenerate or absent.

PARVKINGDOM 3. **Actinopoda Calkins, 1902 stat. nov.**

Unicellular planktonic or benthic protozoa with axopodia but no cilia in the trophic phase; axopodial axonemes of regularly arranged microtubules; mitochondria always present; mitochondrial cristae tubular, vesicular or flattened, never discoid; cortical alveoli absent; ciliated dispersal phase, if present, biciliated; chloroplasts absent.

Phylum 1. **Heliozoa Haeckel, 1866 stat. nov. Margulis, 1974 emend. Corliss, 1984**

Usually planktonic, often large and spherical, unicellular phagotrophs with axopodia with rigid microtubular axonemes; axonemal microtubules, usually in hexagonal arrays, sometimes in double spiral patterns or irregular, never dodecagonal, never quincunx; trophic phase nonciliate; many entirely nonciliate, some with small brief ciliated phases; mitochondrial cristae tubular or flattened; kinetocysts or functionally similar extrusive organelles usually present. Lacking central capsule or spasmin-like myonemes; skeleton siliceous, organic, or absent; do not secrete strontium sulfate.

Phylum 2. **Radiozoa Cavalier-Smith, 1987**

Usually planktonic, often large and spherical, unicellular phagotrophs with axopodia with rigid microtubular axonemes; axopodial microtubules often in dodecagonal array, or hexagonal, or as branching palisades, never spiral; central capsule usually, but not always, present; if central capsule absent, then possessing spasmin-like myonemes; mitochondrial cristae tubular or flattened; large trophic phase is not ciliated. Some with small brief biciliated stages; usually able to secrete strontium sulfate (either in trophic cells or in swarmers).

Subphylum 1. **Spasmaria subphyl. nov.**

Silicious skeleton absent; skeleton, if present (absent in *Sticholonche*), of strontium sulfate (celestite); skeletons with 10 diametral or 20 radial spicules diverging according to Müller's law (58); Ca^{2+}-stimulated contractile myonemes of 3-nm non-actin spasmin-like microfilaments present; myonemes either link spicule tips to the periplasmic cortex (Acantharea) or are attached to the bases of the axopodia and are used for rowing (*Sticholonche*); axopodial axoneme microtubules in hexagonal, or more rarely dodecagonal, arrays; mitochondrial cristae flattened tubules (Acantharia) or rounded tubules (Sticholonchea).

Subphylum 2. **Radiolaria Haeckel, 1887**

Silicious or mixed silica-organic skeleton usually present; cytoplasm divided into ectoplasm and endoplasm by a dense central capsule secreted either within numerous alveoli (in Polycystinea) or within a large cisterna perforated by one large and two small pores (in Phaeodarea); swarmers with strontium sulfate crystals in vacu-

ole; axopodial axoneme microtubules arranged in dodecagonal arrays or as curved branching palisades; obvious spasmin-like myonemes absent; mitochondrial cristae tubular or, rarely, flattened.

PARVKINGDOM 4. **Neosarcodina parvkingd. nov.**

Trophic stage lacks cilia or axopodia and usually has filose, lobose, or reticulose pseudopodia; nearly always free-living; mitochondria always present; cristae usually tubular, rarely flattened or vesicular; hydrogenosomes absent; chloroplasts absent (except possibly in *Paulinella*); ciliated stage, if present, biciliated; cortical alveoli absent; aerial fruiting bodies usually absent, but if (rarely) present then without a stalk (Copromyxidae) or else mitochondrial cristae flat (*Fonticula*).

Phylum 1. **Reticulosa Carpenter, 1862 stat. nov. emend.**

Mainly benthic, mainly marine, phagotrophic protozoa with a nonflagellate trophic phase having finely granular or hyaline reticulopodia or, rarely, finely pointed but nonanastomosing pseudopodia; axopodia absent but reticulopodia often containing irregularly arranged microtubules; gametes usually biciliate; mitochondrial cristae tubular; central capsule, spasmin-like myonemes, and cortical alveoli all absent.

Subphylum 1. **Athalamia subphyl. nov.**

Naked, test absent.

Subphylum 2. **Foraminifera (D'Orbigny, 1826) Eichwald, 1830 stat. nov. Mikhalevich, 1980**

With a test; test single chambered (class Monothalamea) or multichambered (class Polythalamea).

Phylum 2. **Rhizopoda Dujardin, 1835 stat. nov. Haeckel, 1866 emend.**

Nonflagellated, unicellular or plasmodial phagotrophs usually lacking aerial sporangia; usually pseudopodia (lobopodia or filopodia) serve for both locomotion and feeding; microtubules typically absent from nondividing trophic cells; dictyosomes and mitochondria invariably present; cristae usually tubular, rarely vesicular (Cristivesiculatia) or discoid (Cristidiscoidia); cortical alveoli, spasmin-like myonemes, and central capsule all absent; flagellate stages (biciliate) reported only for *Trichosphaerium*; kinetocysts and other extrusomes absent. Multicellular fruiting bodies (if present: Copromyxidae and *Fonticula* only) do not develop from a plasmodium and usually (Copromyxidae) have no stalk.

PARVKINGDOM 5. **Entamoebia parvkingd. nov.**

Amoeboid gut symbionts of animals; cilia and centrioles absent; mitochondria, peroxisomes, and hydrogenosomes absent; dictyosomes small or possibly sometimes absent; chloroplasts absent; closed mitosis with endonuclear spindle; endonuclear centrosome present only during prophase.

Phylum **Entamoebia phyl. nov.**

Diagnosis as for infrakingdom Entamoebia.

PARVKINGDOM 6. **Myxozoa Grassé, 1970 emend. stat. nov.**

Amoeboid parasites of animals with no cilia, no apical complex, no central capsule, and no cortical alveoli or axopodia. Trophic phase unicellular or plasmodial. Mitochondria always present; cristae tubular to irregular; hydrogenosomes and chloroplasts absent. Dictyosomes not associated with a striated fiber; multicellular spores.

Phylum 1. **Myxosporidia Bütschli, 1881 stat. nov. Grassé, 1970 emend. (syn. Myxozoa Grassé, 1970)**

Amoeboid parasites of animals with no trace of cilia or centrioles; having spores of multicellular origin, with one or more polar capsules and sporoplasms; with one, two, or three (rarely more) valves; mitochondrial cristae of irregular, often indistinct, character.

Phylum 2. **Haplosporidia Caullery and Mesnil, 1899 stat. nov. Corliss, 1984**

Nonflagellated unicellular parasites of invertebrates forming spores without polar capsules and having no trace of cilia or centrioles; haplosporosomes present in uninucleate or multinucleate trophic cells; mitochondrial cristae tubular; spores not made of several cells enclosed inside each other.

Phylum 3. **Paramyxia Chatton, 1911 stat. nov.**

Centrioles nine-singlet, but apical complex and inner membrane complex of the pellicle absent; spores of several cells enclosed inside each other; polar capsules absent; dense microneme- or haplosporosome-like bodies dispersed throughout the cytoplasm.

PARVKINGDOM 7. **Mesozoa van Beneden, 1877 stat. nov.**

Multicells with differentiation between ciliated epithelium and internal germ cells; collagenous connective tissue absent; mitochondria with tubular cristae; hydrogenosomes and chloroplasts absent; parasites of animals. Dictyosomes not associated with a striated fiber.

Sole phylum. **Mesozoa van Beneden, 1877**

Diagnosis as for parvkingdom Mesozoa.

APPENDIX 2. DIAGNOSES OF THE NEW PROTOZOAN SUPERCLASSES, CLASSES, SUBCLASSES, ORDERS, AND FAMILIES

Descriptions of the previously established protozoan superclasses, classes, and orders can be found in references 83 and 89.

1. Phylum Percolozoa

Superclass Percolomonada supercl. nov.

Kinetid without striated roots.

Superclass Striatorhiza supercl. nov.

Kinetid with striated roots.

Class Lyromonadea cl. nov.

Flagellates with two pairs of anterior cilia associated with striated microtubular roots similar to those of Schizopyrenida and with four parallel centrioles. Each kinetid is associated with a groove supported by a single broad arc-shaped ribbon of microtubules that is coated on its concave side with a double layer of crystalline material; in contrast to other Tetramitia, between the two ends of the arc of this microtubule-organizing ribbon is a unique band of microfilaments giving the whole the appearance of a harp or lyre (hence the name of the class) (10, 10a). Catalase and peroxisomes are absent; cytochrome oxidase absent, mitochondria absent (*Lyromonas*) or possibly present in a degenerate form that lacks cristae (*Psalteriomonas*). Hydrogenosomes with an envelope of two membranes present. Cysts unknown. Mitosis closed with an intranuclear spindle.

Order Lyromonadida ord. nov.

Diagnosis as for class Lyromonadea.

Family Lyromonadidae fam. nov.

Only one kinetid and one nucleus per cell; no trace of mitochondria.

Type genus *Lyromonas* gen. nov.

Flagellates with no amoeboid phase; single kinetid with four cilia; no trace of mitochondria.

Type species *Lyromonas vulgaris* (Broers et al.) Cavalier-Smith (originally described by Broers et al. (10a) under the name *Psalteriomonas vulgaris*)

Family Psalteriomonadidae Cavalier-Smith, 1993 (43) emended diagnosis.

Four kinetids, four nuclei, and four grooves per cell; also a non-flagellate amoeboid stage consisting of a limax-type amoeba; crista-less organelles resembling degenerate mitochondria or symbiotic gram-negative bacteria present and are surrounded by a cisterna of the RER.

Type genus *Psalteriomonas* Broers, Stumm, Vogels and Brugerolle, 1990

2. Phylum Euglenozoa

Class Diplonemea cl. nov.

Biflagellates lacking pellicular plates but having a feeding apparatus containing both an MTR-pocket and four plicate vanes and two supporting rods. Mitosis with normal chromosome condensation cycle, nucleolar dispersion, and anaphases A and B (all in contrast to Euglenoida).

Order Diplonemida ord. nov.

Diagnosis as for class Diplonemea.

Family Diplonemidae fam. nov.

Diagnosis as for Diplonemida.

Type genus *Diplonema* Griessmann, 1913.

Class Petalomonadea cl. nov.

Strictly bacterivorous, nonphotosynthetic, phagotrophic euglenoids with an aplastic pellicle (137) of a few longitudinally arranged strips; never exhibiting metaboly; feeding apparatus of the MTR-pocket type; supporting rods and vanes absent.

Order Petalomonadida ord. nov.

Diagnosis as for class Petalomonadea.

Type genus *Petalomonas* Stein, 1859

Class Peranemea cl. nov.

Phagotrophic euglenoids with a plastic or aplastic pellicle (137); chloroplasts absent; metaboly absent or present to varying degrees; feeding apparatus with two supporting rods and with vanes.

Order Ploeotiida ord. nov.

Aplastic pellicle; bacterivorous; feeding apparatus of two supporting rods with no internal microtubules and with plicate vanes.

Type genus *Ploeotia* Dujardin, 1841

Order Peranemida ord. nov.

Phagotrophic euglenoids able to eat bacteria or eukaryotic prey; feeding apparatus with four nonplicate vanes and two supporting rods having internal microtubules.

Type genus *Peranema* Dujardin, 1841

Class Aphagea cl. nov.

Nonphagotrophic euglenoids, osmotrophic or with photosynthetic chloroplasts; vestigial feeding apparatus, if present, of MTR-pocket type, lacking vanes or supporting rods.

Subclass Euglenia subcl. nov.

With chloroplasts or colorless plastids.

{Orders Eutreptida Leedale, 1967 (85) orthog. emend.; Astasida Ehrenberg, 1838 (57) stat. nov. [syn. Euglenida, Euglenales Leedale, 1967 (85); including Euglenamorphales Leedale, 1967 (85)] type genus *Astasia* Dujardin, 1841}

Subclass Rhabdomonadia subcl. nov.

Without plastids.

(Sole order Rhabdomonadida Leedale, 1967 [85] orthog. emend.)

3. Phylum Opalozoa

Class Heteromitea Cavalier-Smith, 1993

Subclass Phagodinia subcl. nov.

Phagotrophic intracellular parasites of algae which undergo multiple fission within sporangia; cytoplasmic starch; Golgi dictyosomes numerous; extrusomes and pseudopodia absent; cells uninucleate; zoospores with two divergent cilia with a transitional helix; pellicular microtubules restricted to the four ciliary roots.

Sole order Phagodiniida ord. nov.

Diagnosis as for subclass Phagodinia.

Sole family Phagodiniidae fam. nov.

Diagnosis as for subclass Phagodinia.

Type genus *Phagodinium* Kristiansen, 1993 (81a)

4. Phylum Dinozoa

Class 1. Colponemea cl. nov.

Nonphotosynthetic phagotrophic, unicellular, uninucleate free-living flagellates with two anisokont, not dinokont, cilia; nuclei with normal (not dinokaryotic) chromatin; closed mitosis with endonuclear spindle.

Order Colponemida ord. nov.

Characters as for class Colponemea; extrusomes toxicysts.

Class 2. Oxyrrhea Cavalier-Smith, 1987

Nonphotosynthetic phagotrophic flagellates with two dinokont cilia but without a sulcus or cingulum; closed mitosis with endonuclear spindle and numerous centrosomal plaques; chromatin appears of the normal eukaryote type, not dinokaryotic. Toxicysts absent.

Superclass 1. Syndina supercl. nov.

Centrioles at spindle poles during division; normal histones present throughout life; nonphotosynthetic parasites of invertebrates.

Superclass 2. Hemidinia supercl. nov.

Histones present in larger trophic cells but absent in smaller swarmers; trophic cells lack theca.

Class 1. Noctilucea Haeckel, 1866 stat. nov. (syn. Cystoflagellata, Haeckel, 1874; Rhynchoflagellata, Lankester, 1885; Noctiluciphyceae Fensome et al., 1993)

Nonphotosynthetic free-living phagotrophs; giant vacuolated trophic cells.

Class 2. Haplozooidea Poche, 1911 (syn. Blastodiniphyceae Fensome et al., 1993)

Parasitic, not highly vacuolated.

Sole order Blastodinida.

Superclass 3. Dinokaryota supercl. nov.

Typical histones absent throughout life; commonly, 5'OH uracil partially or entirely replaces thymine; centrioles not present at spindle poles; multiple cytoplasmic channels through nucleus during division; mostly free-living, some parasites; most frequently phototrophic or phagophototrophic, but many aplastidic phagotrophs.

Class 1. Peridinea Ehrenberg, 1830 stat. nov. Wettstein, 1901 emend.

Chloroplasts when present lack phycobilins and have an envelope of three membranes (or rarely two over part of their surface). Body form varies, never dinophysoid.

Subclass 1. Gymnodinoidia Poche, 1913 stat. nov.

With cingulum and sulcus; numerous cortical alveoli in more than six latitudinal series or with a pellicle; thecal plates within the cortical alveoli too thin to be detectable by light microscopy, or absent.

Subclass 2. Peridinoidia Poche, 1913 stat. nov.

With cingulum and sulcus; cortical alveoli contain thick thecal plates, readily detectable by light microscopy, and are arranged in five or six longitudinal series (includes at least some Phytodiniales).

Subclass 3. Prorocentroidia Lemmermann, 1899 stat. nov.

Two apical cilia, one with multiple waves; thecal plates, but no cingulum or sulcus.

Subclass 4. Desmocapsoidia Pascher, 1914 stat. nov.

Two apical ribbon-like cilia, both without multiple waves; theca unknown.

Subclass 5. Thoracosphaeroidia subcl. nov.

Vegetative cell coccoid with calcareous wall; theca absent in motile cells. Sole genus *Thoracosphaera*.

Class 2. Bilidinea Cavalier-Smith, 1993 (published as nomen nudum in reference 38 and defined in reference 41)

Chloroplasts when present have phycobiliprotein pigments inside the thylakoids and an envelope of two membranes; if phycobilin-containing plastids absent, body form is dinophysoid, i.e., with a cingulum, sulcus, and sagittal suture.

(Orders Dinophysida Pascher, 1931; Nannoceratopsida Piel and Evitt, 1980)

5. Phylum Apicomplexa

Class Apicomonadea cl. nov.

Diagnosis as for subphylum Apicomonada.

Order Colpodellida ord. nov. (formerly Spiromonadida Krylov & Mylnikov, 1986 [104a])

Flagellates with two anisokont cilia; ectoparasitic or ectopredatory on other protists; having micronemes, pellicular membranes, and micropores and subpellicular microtubules as in other Apicomplexa; large posterior vacuole with diverse inclusions; with (63a) or without (12) an apical fixation apparatus consisting of an apical ring of microtubules similar to the sporozoan conoid; with (12) or without (63a) contractile vacuoles; with (12) or without (63a) trichocysts like those of dinoflagellates.

Type genus *Colpodella* Cienkowsky, 1865 (formerly miscalled *Spiromonas* [see reference 116]; *Dinomonas vorax* [104a] is now regarded as a synonym for *Alphamonas edax*, which in turn is now assigned to the genus *Colpodella*, not *Alphamonas* [116].)

Colpodella perforans (12) and *C. gonderi* (63a) differ so greatly that they ought to be placed in separate genera and families; this is not done here simply because of the nomenclatural problems that would be involved. The family Spiromonadidae Hollande, 1952 also must be abandoned.

Superclass Gregarinia Dufour, 1828.

Gamonts (except in *Blastogregarinia*) pair and secrete a common gamontocyst wall; male and female gamonts both divide to produce equal-sized amoeboid gametes; male gametes with one cilium, female gametes have no cilia; during mitosis one centriole per centrocone; zygote wall (zygocyst) is also the sporocyst wall (i.e., no intervening sporoblast divisions); parasites of invertebrates.

Class Eogregarinea cl. nov.

Extracellular parasites usually without merogony.

(Orders Blastogregarinida Chatton & Villeneuve, 1936; Archigregarinida Grassé, 1953; Eugregarinida Léger, 1899)

Class Neogregarinea cl. nov.

Intracellular parasites usually with merogony.

(Order Neogregarinida Grassé, 1953)

Superclass Coccidia Leuckart, 1879 stat. nov.

Oogamous with large nonmotile macrogametes (female gamont does not divide) and small microgametes with two or three cilia; during mitosis two centrioles per centrocone; gamonts do not pair or secrete a gamontocyst wall. Zygote directly forms an oocyst which divides into sporoblasts; sporozoites usually enveloped by a sporocyst wall that is entirely distinct from the earlier oocyst wall.

Class 1. Coelotrophea cl. nov.

Extracellular trophic phase with no merogony.

(Order Coelotrophiida Vivier, 1982)

Class 2. Eucoccidea cl. nov.

Intracellular trophic phase with merogony.

(Orders Adeleida Léger, 1911; Eimeriida Léger, 1911)

Class 3. Haemosporea cl. nov.

Microgametes with one cilium; gamonts without axopodia; centriole absent during merogony; sexual stages in blood-sucking dipteran flies; after crossing the gut wall, the kinete forms a thin-walled oocyst; located within a parasitophorous vacuole in the host cytoplasm, not free in the cytosol.

(Order Haemosporida Danilewsky, 1885)

Class 4. Piroplasmea Wenyon, 1926 stat. nov. Levine, 1961

Microgametes without cilia; gamonts with anterior and posterior axopodia with microtubular axonemes; centrioles totally absent; oocyst absent; sexual stages, when known, in blood-sucking ticks; located freely in cytosol of host.

Order Anthemosomida ord. nov.

Sexual stages unknown; pellicular microtubules absent; 5 to 32 merozoites formed by meront. Sole Family Anthemosomatidae Levine, 1980.

Order Ixoplasmida ord. nov.

Sexual stages in ticks; pellicular microtubules present or absent; two or four merozoites formed per meront.

(Families Babesiidae Poche, 1913, Theileriidae du Tort, 1918)

6. Phylum Heliozoa

Class 1. Nucleohelea cl. nov.

Heliozoa without a centroplast or axoplast (e.g., *Clathrulina*, *Actinophrys*, and *Actinosphaerium*); axopodial microtubules nucleate on the nuclear envelope.

Class 2. Centrohelea cl. nov.

Heliozoa with a centrosome (centroplast or axoplast) as the axopodial microtubule nucleating center.

7. Phylum Radiozoa

Class Acantharia Haeckel, 1881 stat. nov.

Subclass 1. Holacanthia subcl. nov.

Acantharia with 10 diametral spines; axopodial microtubular skeleton in dodecagonal array.

Subclass 2. Euacatharia subcl. nov.

Acantharia with 20 radial spines; axopodial microtubular skeleton hexagonal or irregular.

8. Phylum Rhizopoda

Class Lobosea Carpenter, 1961 stat. nov. emend.

Uninucleate, occasionally multinucleate (rarely binucleate) amoebae or rarely plasmodia; pseudopodia lobose, sometimes (Acanthopodida) with filose subpseudopodia. Cilia absent; mitochondrial cristae tubular, often branched; stalked aerial fruiting bodies absent, but in a few cases (Copromyxida) amoebae aggregate to form undifferentiated multicellular fruiting bodies; cilia absent (except for Schaudinn's 19th century claim for biciliate zoospores in *Trichosphaerium sieboldii*).

Order Copromyxida ord. nov.

Diagnosis as for family Copromyxidae Olive & Stoianovitch, 1975.

Class Filosea Leidy, 1879 emend.

Amoebae with hyaline filopodia, often branching, sometimes anastomosing; unlike in Acanthopodida not produced from lobopodia; cilia absent; fruiting bodies absent, except in *Fonticula*; mitochondrial cristae flat (roughly discoid: Cristidiscoidia), vesicular (Cristivesiculoidia), or tubular (Testaceafilosia).

Subclass 1. Cristidiscoidia subcl. nov.

Mitochondrial cristae flat, roughly discoid; test absent; phagotrophic on smaller microorganisms.

Order 1. Nucleariida ord. nov.

Solitary amoebae without fruiting bodies.

Families Nucleariidae Cann & Page, 1979; Pompholyxophyidae Page, 1987

Order 2. Fonticulida ord. nov.

Amoebae which aggregate to form stalked fruiting bodies bearing a sorus with numerous spores.

Family Fonticulidae Worley, Raper and Hohl, 1979

Subclass 2. Cristivesiculatia Page, 1987 stat. nov.

Mitochondrial cristae vesicular; test absent; feed by boring holes into algal or fungal cell walls.

Sole order Vampyrellida Staurobogatov ex Krylov et al., 1980

Families Vampyrellidae Zopf, 1885; Arachnulidae Page, 1987

Subclass 3. Testaceafilosia De Saedeleer, 1934

Diagnosis as sole order Gromiida (89).

8a. **Neosarcodina incertae sedis**

Class Holosea cl. nov. (Gr. *holo*, entire; to signify that the cell surface has no projecting pseudopods, unlike other Neosarcodina)

Free-living uninucleate unicells without cilia, centrioles, pseudopods, or chloroplasts; covered in a rigid scaly test without an opening; mitochondrial cristae tubular.

Order Luffisphaerida ord. nov.

Diagnosis as for class Holosea.

Sole family Luffisphaeridae fam. nov.

Scales hollow with a latticed wall; bowl shaped, often with an additional long cyclindrical, fusiform, or dome-shaped projection.

Type and sole genus: *Luffisphaera* Belcher & Swale, 1975. Four freshwater (6) and three marine (141b) species.

9. Phylum Entamoebia

Class Entamoebea Cavalier-Smith, 1991

Diagnosis as for phylum Entamoebia.

Order Entamoebida ord. nov.

Diagnosis as for phylum Entamoebia.

APPENDIX 3. DIAGNOSES OF NEW CHROMIST TAXA

Subkingdom **CHLORARACHNIA** subregnum novum

Chlorophyll *b* instructa; sine chlorophyll *c*; membrana externa involucri periplastidali sine ribosomis; cilium unicum posterius pilosum, sine mastigonemae tubulatae; centriolum unicum.

With chlorophyll *b*; chlorophyll *c* absent; the outermost membrane around the chloroplast lacks ribosomes on its cytosolic face; kinetid with a single centriole and a posterior cilium with fine nonrigid hairs.

Subkingdom **EUCHROMISTA** subregnum novum

Latin diagnosis as for kingdom Chromista Cavalier-Smith, 1981.

Usually with chlorophyll c_1 and/or c_2 (exception Eustigmista); chlorophyll *b* absent; the outermost membrane around the chloroplast has ribosomes on its cytosolic face; ciliary hairs rigid, and tubular (except in *Goniomonas*).

Infraphylum **Raphidoista** Cavalier-Smith, 1986 emend. stat. nov.

Plastids numerous or absent; perichloroplast RER never attached to the nuclear envelope.

Superclass **Raphidomonadia** superclassis nova.

Diagnosis as for class Raphidomonadea Chadefaud ex Silva, 1980; kinetid with two cilia.

Raphidochloridae subclassis nova (e.g., *Gonyostomum* and *Vacuolaria*)

Sine fucoxanthin.

Fucoxanthin absent; diadinoxanthin the major carotenoid.

Raphidochrysidae subclassis nova (e.g., *Chattonella*, *Fibrocapsa*, *Heterosigma*, *Olisthodiscus*)

Fucoxanthin instructa.

Fucoxanthin the major carotenoid.

Superclass **Dictyochia** Haeckel, 1894 stat. nov. emend.

Kinetid of a single cilium.

Oikomonadea classis nova

Single cilium with one row of tubular mastigonemes; chloroplasts, axopodia, and silica skeleton all absent.

Sarcinochrysidae subclassis nova

Diagnosis as for its sole constituent order, Sarcinochrysidales Gayral and Billard, 1977 as emended by O'Kelly and Billard (106a).

Chrysomeridae subclassis nova

Diagnosis as for its sole constituent order, Chrysomeridales O'Kelly and Billard (106a).

Flavoretea classis nova (*Reticulosphaera*)

Mastigonemae tubulatae unipartitae, nontripartitae.

Tubular ciliary hairs unipartite, not tripartite.

(L. *flavus*, yellow, and *rete*, net; after their color and body form).

Rhizochloridae subclassis nova (orders Chloramoebales and Rhizochloridales; plus Heterogloeaceae and *Mallodendron*)

Cellulae sine muro.

Cells without a cell wall: naked or embedded in mucilage.

Type species *Rhizochloris*

Tribophycidae subclassis nova (orders Mischococcales and Tribonematales; plus *Pleurochoridella* and *Characidiopsis*)

Cellulae muri cellulosi instructae.

Vegetative cells with a cellulose cell wall.

Type species *Tribonema*.

Infradivision **Eustigmista** infradivisio nova.

Diagnosis as for class Eustigmatophyceae Hibberd et Leedale, 1971.

Eucentricidae subclassis nova.

Valvae non-calyptriformes; peripheria unius valvae frustuli processibus unguiformibus non munita.

Valves not calyptriform; and valves lacking unguiform process. Typical centric diatoms excluding Corethrophycidae and Rhizosoleniophycidae.

Raphoidae subclassis nova.

Raphe et sternum praesens.

Raphe and sternum present. Raphid pennate diatoms.

Patelliferea cl. nov. (replaces subclass Prymnesidae Cavalier-Smith, 1986)

(Descriptive name based on L. *patella*, knee-cap, in reference to the patelliform shape of the scales; and *fero*, I bear)

Cilia glabra, aequala vel fere aequala: integumentum squamarum patelliformis.

Cilia equal or subequal, without hairs. Body covered in patelliform scales. Mitotic centrosome not a rhizoplast.

Pavlovea cl. nov. (replaces subclass Pavlovidae Cavalier-Smith, 1986) (typified name based on *Pavlova*)

Cilium unum anterius, pilosum frequens; cilium posterius unum; sine squamae; centrosoma radix amorpha.

Anisokont, with two very unequal cilia, the anterior one often with simple hairs and/or knobbed hairs; scales absent; mitotic centrosome is the amorphous ciliary root which is the rhizoplast (connecting to the nucleus) in interphase.

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ADDENDUM IN PROOF

(i) A new heterokont algal class (Pelagophyceae) has been discovered (1a). Its ciliary characters clearly place it in the superclass Dictyochia. Its bipartite retronemes add to the force of my argument (45a) based on *Reticulosphaera* that heterokont retronemes are not invariably tripartite and that the bipartiteness of cryptomonad retronemes is not a good argument for excluding them from the kingdom Chromista.

(ii) The new flagellate genus *Caecitellus* (D. J. Patterson, K. Nygaard, G. Steinberg, and C. M. Turley J. Mar. Biol. Assoc. U.K., **73**:67–95, 1993) belongs in Opalozoa in the class Heteromitea. The curious new protist *Ministeria* (Patterson et al., J. Mar. Biol. Assoc. U.K., 1993) may possibly belong in the subclass Cristidiscoidia of the rhizopod class Filosea.

(iii) We now have evidence from PCR, cloning, and sequencing for U6 snRNA in the microsporidian *Nosema locustae* (A. Roger, T. Cavalier-Smith, and W. F. Doolittle, unpublished data).

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