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A revised six-kingdom system of life

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ABSTRACT

A revised six-kingdom system of life is presented, down to the level of infraphylum. As in my 1983 system Bacteria are treated as a single kingdom, and eukaryotes are divided into only five kingdoms: Protozoa, Animalia, Fungi, Plantae and Chromista. Intermediate high level categories (superkingdom, subkingdom, branch, infrakingdom, superphylum, subphylum and infraphylum) are extensively used to avoid splitting organisms into an excessive number of kingdoms and phyla (60 only being recognized). The two 'zoological' kingdoms, Protozoa and Animalia, are subject to the International Code of Zoological Nomenclature, the kingdom Bacteria to the International Code of Bacteriological Nomenclature, and the three 'botanical' kingdoms (Plantae, Fungi, Chromista) to the International Code of Botanical Nomenclature. Circumscriptions of the kingdoms Bacteria and Plantae remain unchanged since Cavalier-Smith (1981). The kingdom Fungi is expanded by adding Microsporidia, because of protein sequence evidence that these amitochondrial intracellular parasites are related to conventional Fungi, not Protozoa. Fungi are subdivided into four phyla and 20 classes; fungal classification at the rank of subclass and above is comprehensively revised. The kingdoms Protozoa and Animalia are modified in the light of molecular phylogenetic evidence that Myxozoa are actually Animalia, not Protozoa, and that mesozoans are related to bilaterian animals. Animalia are divided into four subkingdoms: Radiata (phyla Porifera, Cnidaria, Placozoa, Ctenophora), Myxozoa, Mesozoa and Bilateria (bilateral animals: all other phyla). Several new higher level groupings are made in the animal kingdom including three new phyla: Acanthognatha (rotifers, acanthocephalans, gastrotrichs, gnathostomulids), Brachiozoa (brachiopods and phoronids) and Lobopoda (onychophorans and tardigrades), so only 23 animal phyla are recognized. Archezoa, here restricted to the phyla Metamonada and Trichozoa, are treated as a subkingdom within Protozoa, as in my 1983 six-kingdom system, not as a separate kingdom. The recently revised phylum Rhizopoda is modified further by adding more flagellates and removing some 'rhizopods' and is therefore renamed Cercozoa. The number of protozoan phyla is reduced by grouping Mycetozoa and Archamoebae (both now infraphyla) as a new subphylum Conosa within the phylum Amoebozoa alongside the subphylum Lobosa, which now includes both the traditional aerobic lobosean amoebae and *Multicilia*. Haplosporidia and the (formerly microsporidian) metchnikovellids are now both placed within the phylum Sporozoa. These changes make a total of only 13 currently recognized protozoan phyla, which are grouped into two subkingdoms: Archezoa and Neozoa; the latter is modified in circumscription by adding the Discicristata, a new infrakingdom comprising the phyla Percolozoa and Euglenozoa). These changes are discussed in relation to the principles of megasystematics, here defined as systematics that concentrates on the higher levels of classes, phyla, and kingdoms. These principles also make it desirable to rank Archaebacteria as an infrakingdom of the kingdom Bacteria, not as a separate kingdom. Archaebacteria are grouped with the infrakingdom Posibacteria to form a new subkingdom, Unibacteria, comprising all bacteria bounded by a single membrane. The bacterial subkingdom Negibacteria, with separate cytoplasmic and outer membranes, is subdivided into two infrakingdoms: Lipobacteria, which lack lipopolysaccharide and have only phospholipids in the outer membrane, and Glycobacteria, with lipopolysaccharides in the outer leaflet of the outer membrane and phospholipids in its inner leaflet. This primary grouping of the 10 bacterial phyla into subkingdoms is based on the number of cell-envelope membranes, whilst their subdivision into infrakingdoms emphasises their membrane chemistry; definition of the negibacterial phyla, five at least partly photosynthetic, relies chiefly on photosynthetic mechanism and cell-envelope structure and chemistry corroborated by ribosomal RNA phylogeny. The kingdoms Protozoa and Chromista are slightly changed in circumscription by transferring subphylum Opalinata (classes Opalinea, Proteromonadea, Blastocystea cl. nov.) from Protozoa into infrakingdom Heterokonta of the

kingdom Chromista. Opalinata are grouped with the subphylum Pseudofungi and the zooflagellate *Developayella elegans* (in a new subphylum Bigyromonada) to form a new botanical phylum (Bigyra) of heterotrophs with a double ciliary transitional helix, making it necessary to abandon the phylum name Opalozoa, which formerly included Opalinata. The loss of ciliary retronemes in Opalinata is attributed to their evolution of gut commensalism. The nature of the ancestral chromist is discussed in the light of recent phylogenetic evidence.

Key words: Megasystematics, Bacteria, Protozoa, Archezoa, Fungi, Animalia, Plantae, Chromista, Eomycota, Mycetozoa.

CONTENTS

I. INTRODUCTION

The idea that nature can be divided into three kingdoms, mineral, vegetable and animal (Lemery, 1675), was popularized by Linnaeus in the eighteenth century. Although separate kingdoms for fungi (Necker, 1783), Protozoa (Owen, 1858) or bacteria (Enderlein, 1925) were proposed later, the seventeenth century conception of just two kingdoms of life dominated biology for three centuries. The discovery of protozoa (Leeuwenhoek, 1675) and bacteria (Leeuwenhoek, 1683) eventually undermined the two kingdom system. But general agreement that the living world must be classified into at least five kingdoms (Margulis & Schwartz, 1988; Cavalier-Smith, 1989*a*; Mayr, 1990) was reached only after the dramatic discoveries made by electron microscopy in the second half of the twentieth century. These conclusively confirmed the fundamental differences between bacteria and eukaryotes, and revealed the tremendous ultrastructural diversity of protists. Acceptance of the necessity for several kingdoms also owes much to the systematic synthesis of Copeland (1956), and the influential writings of Stanier (1961: Stanier & van Niel, 1962) and Whittaker (1969).

Whether more kingdoms than five are needed (Leedale, 1974), and if so how many, has been debated for over 20 years (Cavalier-Smith, 1978, 1981*a*; Möhn, 1984; Corliss, 1994). Even more important than the sheer number of kingdoms is the fact that the definition and circumscription of the recognized kingdoms are not yet agreed by different systematists. Phylogenetic advances have been so great over the past 30 years, however, that we can now define monophyletic kingdoms, the circumscription of which ought to be widely acceptable and therefore lead to a much desired stability in the macrosystem of life.

Some years ago, I argued that the minimum number of kingdoms suitable for general purposes was six (Cavalier-Smith, 1981*a*), and that in addition to the previously accepted five kingdoms it was necessary to recognize a third botanical kingdom, Chromista (mainly comprising the oomycetes and those numerous algae that, remarkably, have their chloroplasts located within the lumen of the rough endoplasmic reticulum, not in the cytosol as in the plant kingdom). That paper also made major changes in the circumscription of the existing botanical kingdoms Fungi and Plantae. The six-kingdom system of Cavalier-Smith (1981*a*) was later slightly modified (Cavalier-Smith, 1983*a*) by using the older and more widely familiar name Protozoa, rather than Protista, for the basal eukaryotic kingdom [in effect, broadening the kingdom Protozoa of Cavalier-Smith (1981*a*)] and creating a new protozoan subkingdom, Archezoa, for putatively primitively amitochondrial protozoa. Since then the boundaries of the kingdom Protozoa have not been totally stable: the taxon Archezoa was later (Cavalier-Smith, 1987*a*) modified by exclusion of the Parabasala (trichomonads and hypermastigote flagellates), and the revised Archezoa were segregated from Protozoa as a separate kingdom. At the same time the bacterial or prokaryotic taxa Eubacteria and Archaebacteria, treated as subkingdoms by Cavalier-Smith (1981*a*, 1983*a*), were raised in rank to kingdoms, thus creating an eight kingdom system (Cavalier-Smith, 1987*a*, 1989*a*, *b*), which I subsequently advocated (Cavalier-Smith, 1991*a–c*, 1993*a*, 1995*a*) and which has been adopted in certain general works (Gould & Keeton, 1996; Maynard Smith & Szathmary, 1995).

The central purpose of the present review is to reconsider the merits of the earlier six-kingdom system in comparison with the eight-kingdom system. After discussing the advantages and disadvantages of each, it will be concluded that the simpler six-kingdom classification is preferable as a general reference system. Finer phylogenetic discrimination can be achieved by making better use of intermediate-level categories such as subkingdoms and infrakingdoms, without multiplying the number of kingdoms. Some changes in circumscription of four of the six kingdoms are also needed; in the light of recent molecular sequence evidence it is necessary to remove four groups from the Protozoa and to place them in higher kingdoms; thus Microsporidia are transferred to the Fungi, Myxozoa and Mesozoa to the Animalia, and Opalinata to the Chromista. This makes the kingdom Protozoa substantially more homogeneous. The postulated phylogenetic relationships between the kingdoms, subkingdoms, and the most deeply divergent infrakingdoms of the present system are shown in Fig. 1.

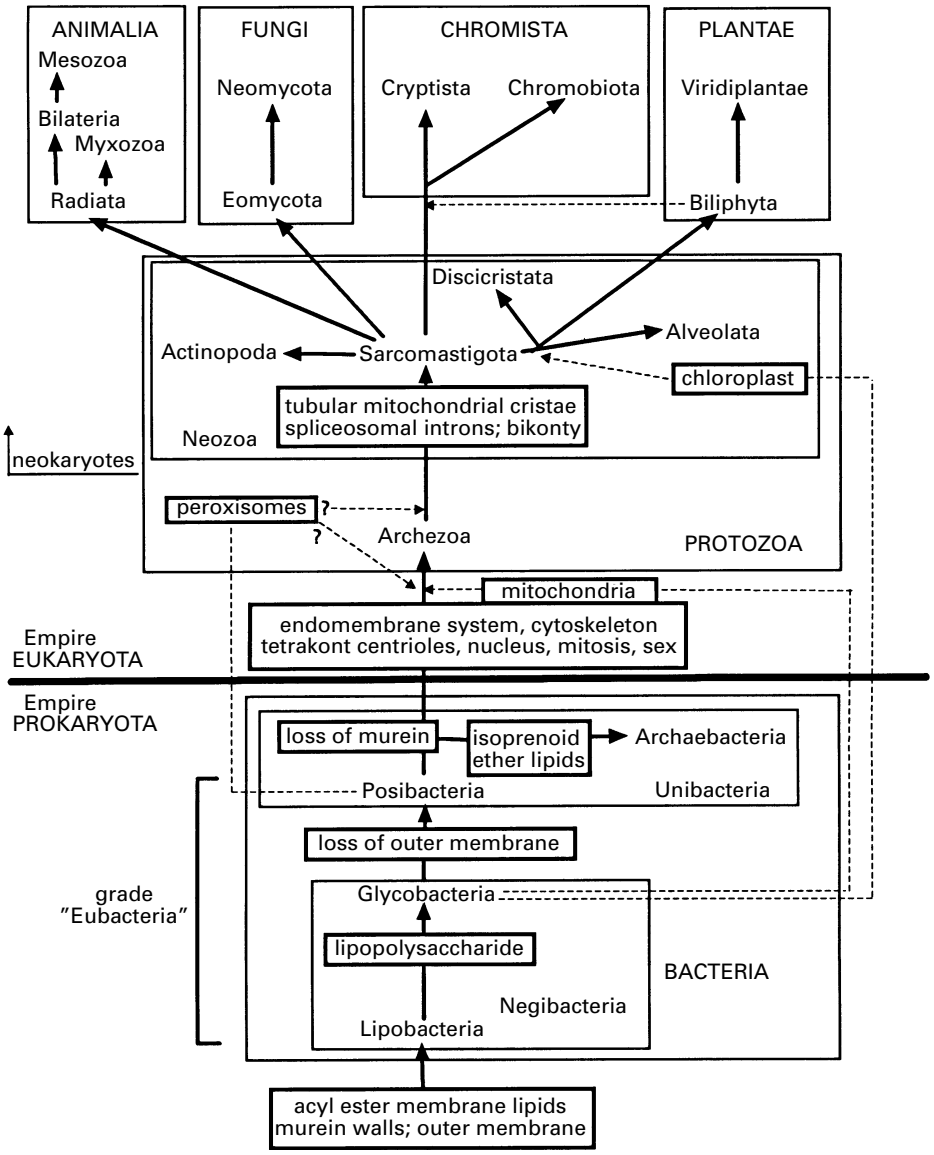

Fig. 1. Postulated phylogenetic relationships between the six kingdoms (upper case) and their subkingdoms. Infrakingdoms are also shown for the two basal paraphyletic kingdoms, as are some key innovations (within heavy boxes). The four major symbiogenetic events in the history of life are shown with dashed arrows: (1) the symbiogenetic origin of mitochondria from an α-proteobacterium, (2) the probable origin of peroxisomes from a posibacterium (Cavalier-Smith, 1990), (3) the monophyletic origin of chloroplasts from a cyanobacterium, and (4) the origin of the ancestral chromist as a eukaryote-eukaryote chimaera between a rhodellophte red algal endosymbiont and a biciliated protist host; whether the host was a protozoan, as assumed here, or a very early plant is unclear. For clarity infrakingdoms are not shown for the four higher holophyletic kingdoms, Animalia, Fungi, Plantae, and Chromista. The diagnoses of the taxa are given in Table 1 and their composition is summarized in Tables 2–7. Chloroplasts are assumed to have originated in the latest common ancestor of Plantae, Discicristata and the alveolate dinoflagellate protozoa (Cavalier-Smith, 1982), and the plastids of euglenoids and alveolates are assumed to have arisen divergently from this primary endosymbiosis. However, the alternative possibility that dinoflagellates and/or euglenoids obtained their chloroplasts secondarily by lateral transfer from a chromobiote and green alga respectively, though considered distinctly less likely, cannot currently be excluded (Cavalier-Smith, 1995*a*); the source of the non-photosynthetic plastid of coccidiomorph Sporozoa is equally uncertain (McFadden & Waller, 1997). For simplicity a fifth important case of symbiogenesis is not shown: secondary symbiogenetic lateral transfers of green algal, possibly ulvophyte (Ishida *et al.*, 1998), chloroplasts into a cercozoan (sarcomastigote) host to create the algal class Chlorarachnea (e.g. *Chlorarachnion*). The least certain features of the tree are the position of its root (some authors favour a root within the Unibacteria rather than the Negibacteria) and of the Discicristata (18S rRNA and EF 1-α trees put them below the Sarcomastigota, whereas other proteins place them as shown).

When establishing the kingdom Chromista (Cavalier-Smith, 1981*a*) I mentioned that, as discussed earlier by Taylor (1978) and Hibberd (1979), there were reasons for thinking that proteromonads may be closer to Chromobiota than to Protozoa. They had some ultrastructural similarities with chromists and some with the opalinids, so it appeared that they might be phenotypically intermediate between chromists and protozoa. In order to make the definition of the Chromista as sharp as possible, I conservatively kept the proteromonads in the kingdom Protozoa, placing them together with opalinids and cyathobodonids in the protozoan group Proterozoa, but recognized that the boundary between chromists and protozoans might require slight adjustment in future. Patterson (1985) subsequently placed proteromonads and opalinids alone together in the order Slopalinida. Later Patterson (1989) argued that slopalinids should be grouped with heterokonts as stramenopiles. I have since argued that the genus *Proteromonas* is less closely related to Opalinida than is the genus *Karotomorpha* and placed the orders Karotomorphida and Opalinida together in the class Opalinea (Cavalier-Smith, 1993*a*, *b*) but put proteromonads in a separate class. As part of a reappraisal of the Proterozoa stimulated by recent rRNA (ribosomal RNA) sequences (Cavalier-Smith & Chao, 1995, 1997) I have created a new class Proteromonadea for the Proteromonadida, abandoned the taxon Proterozoa and grouped Opalinea with Proteromonadea in the subphylum Opalinata (Cavalier-Smith, 1997*a*). In order to increase the homogeneity of the new protozoan subkingdom Neozoa and the zooflagellate phylum Neomonada, I excluded the revised Opalinata from them as a protist taxon *incertae sedis*. Here, I accept the view (Patterson, 1989) that Opalinata (a subphylum now compositionally the same as the order Slopalinida) are probably secondarily modified heterokonts, and therefore place them within the chromist infrakingdom Heterokonta and group them with the heterotrophic subphylum Pseudofungi (Cavalier-Smith, 1986*a*, 1989*b*) to form a new heterotrophic chromist phylum, Bigyra, in which I also place the recently discovered distinctive heterokont protist *Developayella elegans* (Tong, 1995), for which I create a new order, class and subphylum.

In the system of Cavalier-Smith (1981*a*) I refined the kingdom Fungi by removing oomycetes, hyphochytrids, and thraustochytrids and transferring them into the Heterokonta within the new kingdom Chromista, and arguing that Chytridiomycetes, by contrast, should be included in the kingdom Fungi. This circumscription of the kingdom Fungi (see also Cavalier-Smith, 1987*b*) is now almost universally accepted by phylogenetically oriented mycologists (Bruns, White & Taylor, 1991), and adopted by the authoritative *Dictionary of the Fungi* (Hawksworth *et al*., 1995). It will, therefore, come as a considerable surprise to mycologists to learn that the kingdom Fungi now needs to be drastically expanded by the addition of the Microsporidia, a phylum of minute intracellular parasites of animals. Though Microsporidia have chitinous spores and are not phagotrophic, they were traditionally thought of as protozoa rather than fungi because their vegetative cells lack cell walls. Like Opalinata, the Microsporidia lack peroxisomes; but as they also lack mitochondria they were postulated to be archezoan Protozoa (Cavalier-Smith, 1983*a*); however, recent protein sequence data (Li *et al*., 196l; Edlind *et al*., 1996; Keeling & Doolittle, 1996; Roger, 1996; Germot *et al*, 1997) strongly suggest that they are actually highly degenerate fungi, which have secondarily lost mitochondria, like the rumen fungi (Neocallimastigales), which have also been mistakenly classified as protozoa in the past. I here remove the Microsporidia from the Archezoa and place them instead in the kingdom Fungi, where I group them with the Archemycota in a new subkingdom Eomycota. As the higher level classification of the kingdom Fungi needs some revision, I divide it into two new subkingdoms (Eomycota and Neomycota) and create a few other new high level fungal taxa, and also validate some of those proposed earlier (Cavalier-Smith, 1987*b*). I present a detailed classification of the kingdom Fungi, down to the level of subclass.

The third refinement made here to the circumscription of the Protozoa compared with the earlier six-kingdom system (Cavalier-Smith, 1983*a*) is the transfer of the Mesozoa and Myxosporidia into the kingdom Animalia, as briefly indicated earlier (Cavalier-Smith, 1995*b*, Cavalier-Smith *et al*., 1996*a*).

The reasons for the above changes in circumscription of the kingdoms Protozoa, Chromista, Fungi and Animalia will be explained in detail. In view of the numerous changes in high-level classification over the past 15 years, I shall present a detailed summary of this revised six-kingdom classification of the living world, down to the level of infraphylum.

My redefinition of the kingdom Plantae to include all Viridaeplantae (green plants), Glaucophyta, and

Table 1. *The revised six-kingdom system of life*

Empire or Superkingdom 1. PROKARYOTA** Dougherty 1957 stat. nov. Allsopp 1969 (ribosomes in the same compartment as the usually circular chromosome).

Kingdom Bacteria** Cohn 1870 stat. nov. Cavalier-Smith 1983 (syn. Procaryotae Murray 1968: no internal cytoskeleton, endomembrane system, nuclei, mitosis or true sex).

Subkingdom 1. Negibacteria* Cavalier-Smith 1987 (outer membrane present; acyl ester lipids; with small signal-recognition particle RNA).

Infrakingdom 1. Lipobacteria* new infrakingdom (diagnosis: no lipopolysaccharide in outer membrane; murein cell wall).

Infrakingdom 2. Glycobacteria* new infrakingdom (diagnosis: lipolysaccharide in outer membrane; cell wall of murein or, rarely, protein).

Subkingdom 2. Unibacteria* new subkingdom (diagnosis: bacteria with no outer membrane; with large signal-recognition particle RNA as in eukaryotes).

Infrakingdom 1. Posibacteria* Cavalier-Smith 1987 stat. nov. (acyl ester lipids; often with teichoic acids).

Infrakingdom 2. Archaebacteria Woese & Fox 1977 stat. nov. (isoprenoid ether lipids; murein absent).

Empire or Superkingdom 2. EUKARYOTA (cytoskeleton, endomembrane system, nucleus, sex).

Kingdom 1. Protozoa** Goldfuss 1818 stat. nov. Owen 1858 em. (phagotrophs primitively without plastids, collagen or chitinous vegetative cell walls; unicellular, plasmodial or colonial).

Subkingdom 1. Archezoa** Cavalier-Smith 1983 em. (kinetid tetrakont; mitochondria absent; genes unsplit).

Subkingdom 2. Neozoa** Cavalier-Smith 1993 stat. nov. 1997 (kinetid typically bikont; tubular or rarely flat mitochondrial cristae; spliceosomal introns common).

Infrakingdom 1. Sarcomastigota** Cavalier-Smith 1983 stat. nov. em. (kinetid bikont or secondarily unikont or absent; mitochondria usually with tubular cristae, rarely flat (typically non-discoid) or vesicular; cortical alveoli absent; pseudopodia when present non-eruptive; axopodial microtubules if present, not spirally or hexagonally arranged].

Infrakingdom 2. Discicristata new infrakingdom (kinetid tetrakont or bikont; mitochondria or hydrogenosomes present; cristae discoid; genes mostly unsplit; pseudopodia eruptive when present; cortical alveoli and axopodia absent).

Infrakingdom 3. Alveolata Cavalier-Smith 1991 [cortical alveoli (rarely secondarily absent); mitochondrial cristae tubular or ampulliform].

Infrakingdom 4. Actinopoda Calkins 1902 stat. nov. Cavalier-Smith 1996 (axopodia with hexagonal or spirally arranged microtubules; cilia absent or for dispersal only; possibly polyphyletic).

Kingdom 2. Animalia Linnaeus 1758 em. Cavalier-Smith 1995 (unnecessary synonym Metazoa Haeckel 1874) (ancestrally phagotrophic multicells with collagenous connective tissue between two dissimilar epithelia).

Subkingdom 1. Radiata** Linnaeus 1758 stat. nov. em. Cavalier-Smith 1983 (multicellular animals with radial or biradial symmetry; no anus).

Infrakingdom 1. Spongiaria* De Blainville 1816 (choanocytes line body cavity; nervous system primitively absent: sponges).

Infrakingdom 2. Coelenterata* Leuckart 1847 em. auct. (nerve net).

Infrakingdom 3. Placozoa infraking. nov. (without body cavity, gut or nervous system).

Subkingdom 2. Myxozoa Grassé 1970 stat. nov. (secondarily unicellular parasites of bilateral animals; spores multicellular; cilia absent: myxosporidia).

Subkingdom 3. Bilateria Hatschek 1888 stat. nov. Cavalier-Smith 1983 (bilateral, primitively with anus).

Branch 1. Protostomia* Grobben 1908 (blastopore becomes mouth).

Infrakingdom 1. Lophozoa* new infrakingdom (primitively sessile with U-shaped gut and ciliated oral tentacles with coelomic extensions; early ciliated larvae trochophores, later often bivalved).

Infrakingdom 2. Chaetognathi Leuckart 1854 stat. nov. (non-ciliated; thin cuticle not moulted; embryonic enterocoel absent in adult).

Infrakingdom 3. Ecdysozoa new infrakingdom (non-ciliated; thick cuticle that is moulted; haemocoel or pseudocoel).

Infrakingdom 4. Platyzoa new infrakingdom (secondarily acoelomate worms without vascular system; ancestrally ciliated).

Branch 2. Deuterostomia Grobben 1908 (blastopore becomes anus).

Infrakingdom 1. Coelomopora stat. nov. (trimeric coelom, anterior compartment with external pore; enterocoelous; ciliated pelagic larvae; nerve net).

Table 1. (*cont.*)

Infrakingdom 2. Chordonia Haeckel 1874 em. Hatschek 1888 (schizocoelous or coelom absent; larvae tadpole-like; hollow dorsal nerve cord).

Subkingdom 4. Mesozoa Van Beneden 1877 stat. nov. (ciliated multicellular parasites; no nervous system or gut).

Kingdom 3. Fungi Linnaeus 1753 stat. nov. Nees 1817 em. (vegetative and/or spore cell walls of chitin and β-glucan; no phagocytosis).

Subkingdom 1. Eomycota** subking. nov. (diagnosis: hyphae without perforate septa; without dikaryotic phase: cellulae non binucleatae; septa usitate absens; si praesens non perforata).

Subkingdom 2. Neomycota subking. nov. (diagnosis: usually with a dikaryotic phase; hyphae, if present, with perforate septa: cellulae binucleatae plerumque praesentes; endospora aut ascospora aut basidiospora instructa).

Kingdom 4. Plantae Haeckel 1866 em. Cavalier-Smith 1981 (plastids with double envelope in cytosol; starch; no phagocytosis).

Subkingdom 1. Biliphyta* Cavalier-Smith 1981 (phycobilisomes; single thylakoids; starch in cytosol).

Infrakingdom 1. Glaucophyta infraking. nov. (diagnosis: peptidoglycan in plastid envelope: plastidae peptidoglycanum instructae).

Infrakingdom 2. Rhodophyta infraking. nov. (sine peptidoglycano: plastid envelope lacks peptidoglycan).

Subkingdom 2. Viridaeplantae Cavalier-Smith 1981 (chlorophyll *a* and *b*; thylakoids stacked; starch in plastid stroma; ciliary transition nine-fold star: green plants).

Infrakingdom 1. Chlorophyta Cavalier-Smith 1993 (green algae).

Infrakingdom 2. Cormophyta Endlicher 1836 stat. nov. (embryophytes).

Kingdom 5. Chromista Cavalier-Smith 1981 em. (chloroplasts with chlorophyll *c* inside a periplastid membrane within the rough endoplasmic reticulum lumen and/or with rigid bipartite or tripartite ciliary hairs).

Subkingdom 1. Cryptista Cavalier-Smith 1989 (ejectisomes; usually with bipartite hairs on both cilia, nucleomorph and phycobilins; cristae flattened tubules; pellicular plates).

Subkingdom 2. Chromobiota Cavalier-Smith 1991 (cristae tubular; posterior cilium often autofluorescent; no nucleomorph, ejectisomes, pellicular plates or phycobilins).

Infrakingdom 1. Heterokonta Cavalier-Smith 1986 stat. nov. 1995. em. (rigid tripartite or bipartite hairs on anterior cilium only or, rarely, on cell body; no haptonema).

Infrakingdom 2. Haptophyta Cavalier-Smith 1995 (haptonema; hairs unipartite tubules, knob hairs or absent).

* Probably paraphyletic taxon.

** Almost certainly paraphyletic.

The classification of each of the kingdoms is given in more detail in Tables 2–7, with examples of representatives of the major taxa. It is doubtful if the modification of the spelling of the name Archaebacteria in *Bergey's Manual* to *Archaeobacteria* (Gibbons & Murray, 1978) was correct; if archae- was derived from the Greek *archaios* (ancient) then it is a stem and the 'o' should be inserted according to the rules of the botanical code but this is not required by the bacteriological code; but if it was derived from the Greek *arche* (beginning), Archaebacteria is a correctly formed pseudocompound word in which *archae* retains the case ending *-ae*, and as *archae* is a whole word (not the stem *arch-*) it would be incorrect to insert the 'o'. For stability, I prefer to keep the original spelling and to assume that Woese & Fox (1977*a*) made no error. The more recent change to Archaea (Woese, Kandler & Wheelis, 1990) is both totally unnecessary and highly undesirable; its use should be most strongly discouraged (Cavalier-Smith, 1992*b*).

Rhodophyta, and these three groups alone (Cavalier-Smith, 1981*a*), is not yet widely accepted, largely because of a formerly widespread belief in the polyphyletic symbiotic origins of chloroplasts (Mereschkowsky, 1910; Margulis, 1970, 1981; Raven, 1970). But as the theory of the monophyletic symbiogenetic origin of chloroplasts (Cavalier-Smith, 1982), upon which my circumscription of Plantae was based, is increasingly widely accepted (Bhattacharya & Medlin, 1995), and as it logically follows that the three major plant groups are derived from a single photosynthetic common ancestor, I think that it will be only a matter of time before the logic of accepting the monophyly of the kingdom Plantae *sensu* Cavalier-Smith (1981*a*) will also be generally recognized, as it already has been by Ragan & Guttell (1995). I have therefore seen no reason to modify the circumscription of the kingdom Plantae or its subdivision into two subkingdoms (Viridaeplantae and Biliphyta) since Cavalier-Smith (1981*a*), and expect the kingdom to continue to be stable in the future.

An overview of the revised six-kingdom system is given in Table 1, which includes all the supraphyletic taxa, with diagnoses showing how they may be distinguished. In order to save space in the later tables dealing with the phyla, subphyla and infraphyla of each kingdom diagnoses are given only for new or substantially emended taxa. Despite the creation here of five new phyla, I accept only 60 phyla for the entire living world, many fewer than the 92 recognized by Margulis and Schwartz (1988) or the 100 used by Möhn (1984). I am not actually a 'born splitter' (Corliss, 1994). Viruses are best thought of as laterally transmissible parasitic genetic elements, not as living organisms, so I do not attempt to classify these important biological entities here.

II. PHILOSOPHIC PRELIMINARIES

(1) Darwinian evolutionary classification contrasted with Hennigian cladification

Ever since Darwin, it has been accepted that 'the systematist is primarily a morphologist whose task is to discover the true phylogenetic relationships' as Cummings (1916: 254) noted 80 years ago. Darwinian evolutionary classifications take into account both the branching patterns and the degree of change along different branches, and are therefore truly phylogenetic (Mayr & Ashlock, 1991) rather than purely cladistic or purely phenetic. A balanced classification aims to subdivide a taxon like Eukaryota into a number of subordinate taxa of lower rank in such a way as to maximize the degree of similarity within each taxon, while ensuring that none of them is polyphyletic. Since organisms can vary in many different characters, and in ways varying from the trivial to the profound, it is essential to weigh their relative importance. Lamarck (1810) initiated the idea of genealogical classification, but it was Darwin (1859), Haeckel (1866, 1868) and Lankester (1877) who first really popularized the notion of phylogenetic classication as the central goal of systematics. Haeckel (1866) introduced the terms phylogeny, phylum, monophyletic and cladus, whilst Lankester (1977) gave us the concept of a grade and encouraged the English-speaking world to substitute Haeckel's term phylum for subkingdom. These early pioneers of phylogenetic classification used both grades and clades as taxa in their influential classifications.

When I first argued that the kingdoms of life needed reclassifying on phylogenetic principles to make them monophyletic (Cavalier-Smith, 1978), I was complimented by certain cladists who mistakenly thought I was applying the principles of Hennig (1966) because I was using cladistic reasoning and advocating a phylogenetic classification with strictly monophyletic taxa. However, I had not then even heard of Hennig or his confusing redefinition of the term monophyletic, which I do not accept. Like classical phylogeneticists, I use the word monophyletic to include both holophyletic (Ashlock, 1971, i.e. monophyletic *sensu* Hennig, 1966) and paraphyletic. I do, however, accept Hennig's redefinition of Huxley's (1957, 1959) term clade, which was originally defined using the classical, not the Hennigian definition of monophyly. (The earlier use of the word clade in biology by Lankester (1911: 1031) as an anglicization for Haeckel's (1868) taxonomic category of cladus, equivalent in rank to infraphylum in the present system, is no longer current: the Renaissance usage of 'clade' to denote a plague or disaster is even more obsolete.) Hennig and his followers have done much to increase the rigour of phylogenetic analysis, but this advance has been brought at the cost of some verbal confusion and much harmful dogmatism. Furthermore, though cladistic analysis is simply formalized phylogenetic common sense, and should be strongly encouraged, Hennigian taxonomy has two grave defects, one practical and one theoretical.

Practically, it leads to serious instabilities in classification and nomenclature. The great practical merit of a Darwinian evolutionary classification is that it should be much more stable, since whether a taxon is holophyletic or paraphyletic is irrelevant to its classificatory role. There are many thousands of taxa where we do not know whether they are paraphyletic or holophyletic. According to Hennigian principles, whenever any taxa become clearly established as paraphyletic we would have to split them up, abandon their names and invent two or more new taxa. This would be especially nomenclaturally destabilizing for genera, a large proportion of which may be paraphyletic.

Theoretically, the Hennigian attempt to restrict taxa to clades, and forbid paraphyletic groups is incompatible with the basic purpose of phylogenetic classification, even though it misleadingly masquerades under that name. What a biological classification aims to do is to arrange organisms in a hierarchical series of nested taxa, in which each more-inclusive higher-level taxon is subdivided comprehensively into less-inclusive taxa at the next level below. There are two key words here: hierarchical and comprehensive. A set of nested clades is

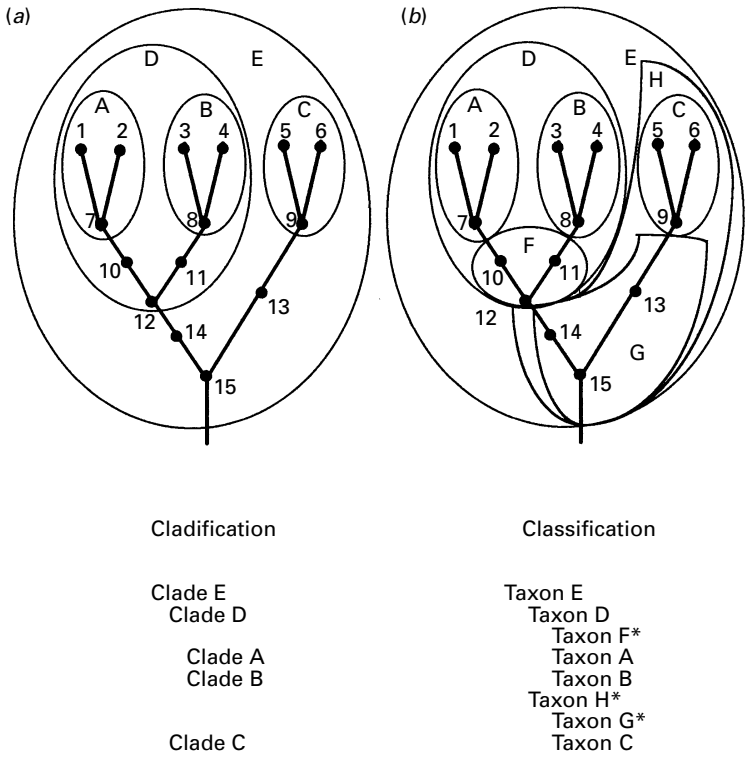

Fig. 2. Contrast between (*a*) cladification and (*b*) classification. From the same phylogenetic tree with holophyletic recent species 1 to 6 and paraphyletic ancestral species 7 to 15 one can produce either a list of nested clades or a comprehensive classification. In the classification taxon D is comprehensively subdivided into three taxa of lower and equal rank (A, B, F); A and B are holophyletic and F is paraphyletic. In the cladification, clade D is subdivided into two clades A and B with the same composition as the holophyletic taxa A and B plus a residue including species 10 to 12 which is not placed in any subgroup of clade D. The cladification is not a comprehensive classification of clade D, because this paraphyletic residue comprising species 10 to 12 is not listed under clade D even though it is part of that clade. The size of this paraphyletic residue could be reduced in an alternative cladification by moving the boundaries of clades A and B from just below species 7 and 8 to just above species 12; but as species 12 is ancestral to both clades A and B, it cannot be included in either. No clade can be comprehensively subdivided into subclades. A cladification can never be a comprehensive classification since it omits the paraphyletic residues which a comprehensive classification must include (taxa F, G and H in this case). The cladification on the left is congruent with the classification on the right. When comprehensively subdivided into taxa of equal rank, it is logically inescapable that every monophyletic taxon (whether holophyletic or paraphyletic) must include at least one paraphyletic taxon. A cladification is a most useful step towards a phylogenetic classification but it does not constitute one in its own right. If paraphyletic taxa are explicitly identified in a phylogenetic classification one can reconstruct the cladification on which it is based by deleting them. But one cannot move from a phylogenetic tree to a classification without exercising taxonomic judgement, which is necessary both in deciding which clades should be treated as taxa and whether the paraphyletic residue should be treated as a single taxon or subdivided. Judgement is likewise needed about the appropriate rank for each taxon.

hierarchical, but because it is not comprehensive at each level in the hierarchy, it is much too incomplete to be properly called a classification. Ernst Mayr (pers. comm.) has recently called such a set of nested clades a cladification. Figure 2 contrasts the way in which a Darwinian evolutionary classification subdivides a phylogenetic tree to produce a comprehensive classification of the organisms making up the tree, whereas Hennigian cladification of the same organisms yields an incomplete subdivision of higher-level groups. Cladists have long accepted that the inability to classify ancestral and many fossil taxa is the Achilles heel of Hennigian classificatory principles, and refer to it as a problem; it is not a problem at all for systematics, but merely a basic defect in the Hennigian ideas on classification. Obviously, if you assert that you must not make paraphyletic groups then you cannot properly classify ancestral species excluded from a particular clade. No comprehensive phylogenetic classification

is even theoretically possible unless one accepts paraphyletic as well holophyletic taxa. The dogma against paraphyletic taxa is logically incompatible with the acceptance of both evolution by descent and the goal of taxonomy as the creation of a comprehensive phylogenetic classification of all organisms, both extant and extinct.

As it is theoretically unsound, and in practice increases nomenclatural and classificatory instability, the dogma against paraphyletic groups has been most detrimental to systematics. Cladists, rightly immensely impressed with the logic and great value of Hennigian principles for phylogenetics, have naturally been reluctant to accept that they can be so fatally flawed when applied to taxonomy. Some do recognize this central flaw, but say that in practice most taxonomists deal only with extant organisms or that it is never possible to recognize ancestors with certainty. However, we sometimes can recognize ancestors and need to classify them. Furthermore, not all ancestors are extinct. In plants, new allopolyploid species are created by hybridization between two ancestral species followed by polyploidization. In several instances, both ancestral species have been unambiguously identified and both are extant. A well known example is the formation of the new grass *Spartina anglica* by allopolyploidy from the ancestors *S. alterniflora* and *S. maritima*. The two ancestors are both perfectly good paraphyletic taxa, and as biological species are natural taxa, distinct from each other and from their descendant species *S. anglica*. Paraphyletic species are therefore as natural as holophyletic ones. Apart from the special case of biological species, paraphyletic and holophyletic taxa are both partly artificial constructs of taxonomists (whether they are asexual species or taxa of higher rank). From such a perspective a holophyletic taxon is not created by the origin of a new synapomorphy, but by the decision of a systematist to subdivide an historically continuous phylogenetic tree at that point. As I have stressed previously (Cavalier-Smith, 1993*a*), every such cutting off of a holophyletic branch necessarily simultaneously generates a paraphyletic stem. If I cut a tree into pieces, each piece, whether it is a terminal piece with one cut or a stem piece with two or more, is both artificial and natural. Each continuous segment owes its continuity to natural processes, whereas its separation from others is the result of human, i.e. artificial intervention. If I group together cut pieces that were never joined on the tree, such joining would be totally unnatural, which is why polyphyletic grouping has long been accepted as much more artificial than a monophyletic grouping. For monophyletic groups (whether paraphyletic or holophyletic), their coherence is the result of historical genetic continuity between all members of the group: it is their discontinuity from other groups that is artificial (except for biological species). The claim that a cladogram or phenogram can be converted into a classification without artificial cuts across the natural phylogenetic continuum (Nelson & Platnick, 1981) is at best a spurious half truth; cladograms and phenograms can be claimed to be as discontinuous as classifications only because the cuts across the phyletic continuum have already been made by classical taxonomists when creating the discrete taxa that form the raw materials for cladistic and phenetic analysis. The claim involves an element of myopic self-deception that allows some cladists to claim falsely that Hennigian systematics is more natural than classical phyletic taxonomy (Stuessy, 1990). No classification can be totally natural.

Whether a taxon is paraphyletic or not is irrelevant to its validity as a taxon. It is also irrelevant to many of the uses to which classifications are put, such as arranging specimens in a museum, organising the chapters in a biology textbook, or providing a convenient label, e.g. bacteria or fungi, for a group of similar organisms. But the distinction is important in certain uses to which classifications are sometimes put. Two common examples are the choice of model systems and the study of group extinction.

A biologist wishing to choose a protozoan as a model system for understanding animal cell biology would be quite mistaken in supposing that as all protozoa are classified in the same kingdom it does not matter which is chosen. Obviously, some members of such a paraphyletic group are cladistically more closely related to a derived taxon than others: for example, choanoflagellates would be much more similar to animal cells than would retortamonads. Likewise, the extinction of all members of a paraphyletic group, in contrast to that for a holophyletic group, does not constitute extinction of the entire lineage. But the mistaken assumption that it does, like the assumption that all members of a group are equally related to another group, are misuses, not uses, of a classification. Where precise phylogenetic relationships are important for a scientific problem, scientists should base their reasoning directly on phylogenies and not use classifications as a crude surrogate for the real thing. The purpose of classification is to provide a simplified reference system that is biologically sound and widely useful.

It should be compatible with the phylogeny, but it cannot serve its central simplifying purpose unless it leaves out some of the fine detail about relationships that are essential for some phylogenetic purposes. One can use a phylogeny as a basis for making a classification, but one cannot logically deduce a fully detailed phylogeny from a classification. Nor is a phylogeny sufficient to give a classification. A phylogeny and a classification must be congruent (i.e. not contradictory) but they are different ways of abstracting from and representing biological relationships. It is highly desirable that taxonomists publish an explicit phylogeny and statement of their phylogenetic assumptions at the same time as their classification, a practice I have long followed (Cavalier-Smith, 1978). This will allow users to use whichever is most appropriate to their needs. In order to alert users of classifications to the sometimes important differences between holophyletic and paraphyletic taxa it would be good practice if, where known, these are clearly distinguished. In Tables 1–7 in this review I mark those taxa that are almost certainly paraphyletic and those which, in my phylogenetic judgement, are probably paraphyletic. Taxa not so identified include not only those known or thought to be holophyletic but also those for which evidence is too little for me to make a judgement; future work is bound to show that some are paraphyletic, and others polyphyletic, and that at least some of those thought to be paraphyletic are actually holophyletic.

(2) Naming taxa and clades

A named taxon such as Protozoa or Plantae has to serve a dual role. Its name acts as a label to refer succinctly to a group of related organisms sufficiently similar to each other that they share a generalized phenotype, by which they can be readily recognized and distinguished from other taxa. Secondly, they are units that can be grouped together hierarchically to create a smaller number of higher taxa. A list of nested clades, such as that in Patterson (1994), can be very useful as a phylogenetic summary, but it is not the same as a classification.

Stability is desirable in nomenclature, so I have tried wherever reasonably possible to retain older and more familiar names. But stability is not a primary value in classification. If it were, we should rigidly retain the oldest classification irrespective of how bad it is! Especially among microorganisms traditional classifications have been so grossly incorrect that they must be changed as science advances. This requires some new names of higher taxa and some shifts in the meaning of old ones. Because classifications are scientific generalizations about the varied properties of organisms, and not just indexes to stored information, they must be changed if new knowledge shows them to be fundamentally wrong. During the past half century, electron microscopy, biochemistry and molecular sequencing have immensely deepened our understanding of biodiversity. After the revolutionary changes occasioned by this have been soundly assimilated, our classifications will become more stable. Thus stability is a valuable outcome of a sound classification, but must not dominate systematics by impeding necessary changes.

It is important to have a brief name for all clades of major evolutionary significance, but one should not make the mistake of supposing that all such clades need to be made into taxa. Nor should one make the converse error, especially common among cladists, of supposing that only clades can be taxa. Taxa and clades should be thought of as two partly overlapping sets that serve somewhat different, but equally essential, biological purposes. In a given classification such as the six-kingdom system advocated here, some taxa (e.g. Fungi, Animalia, Chromista and probably Plantae) are clades, but paraphyletic taxa like Bacteria, Archezoa and Protozoa are not. For example, an important clade which is not a taxon in this system is the clade comprising Animalia, Fungi and Choanozoa, which I named opisthokonta (Cavalier-Smith, 1987*b*); molecular phylogeny has since confirmed the validity of this clade (Cavalier-Smith, 1993*a*; Wainright *et al*., 1993). I deliberately did not create a taxon Opisthokonta or give a diagnosis for it. This is because opisthokonta are far too phenotypically diverse to be useful as a major unit of eukaryote classification. One cladist has even told me that I ought to have created a formal kingdom Opisthokonta. A kingdom Opisthokonta would be much less useful than the existing kingdoms Animalia, Fungi and Protozoa as a way of subdividing the living world into manageable major groups of similar organisms, i.e. in classification as opposed to phylogeny. The cladist's suggestion reflects a misunderstanding of the purposes and functions of classification, which is all too common among cladists, who are typically very much more interested in phylogeny than in classification, and often forget that these are two different branches of systematics. Clades and taxa are non-equivalent concepts, as is shown pictorially in Fig. 3.

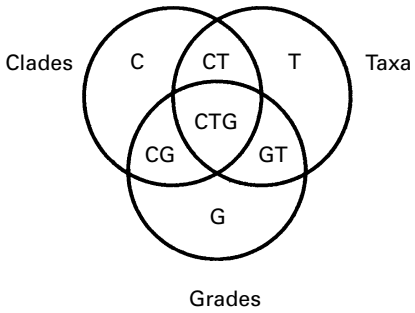

Fig. 3. The non-equivalence of clades and taxa and the non-exclusiveness of either with grades. Biological groups may be simply clades, grades or taxa, or any two or three of these at the same time. Some well known groups, such as vertebrates and eukaryotes, are simultaneously taxa, clades, and important grades of organization (region CTG). Some clearly definable clades should not be made taxa, partly because they do not constitute clearly defined grades of organization (region C) (e.g. opisthokonta, i.e. the clade comprising Fungi, Animalia and Choanozoa (Cavalier-Smith, 1987*a*); or neokaryotes, the clade consisting of all descendants of the most recent common ancestor of Neozoa). Yet other clades are grades of organization, but for other reasons are not made taxa (region CG; e.g. Metakaryota, which was treated as a taxon in the eight kingdom system, but not in the six-kingdom system for reasons discussed in the text). Paraphyletic taxa (regions T and GT) are not clades; but they may be grades of organisation of greater (e.g. Bacteria, Archezoa) or lesser (e.g. Reptilia, Amphibia) importance (region GT) or they may not be grades of organization at all (region T) either because they have secondarily become heterogeneous (e.g. the heterokont 'algal' phylum Ochrophyta by multiple secondary losses of plastids) or because they are too similar to related taxa to be called separate grades of organization (as in paraphyletic species ancestral to allopolyploids, e.g. *Spartina anglica* and *S. maritima*). Though many holophyletic taxa are grades (region CTG, e.g. Tracheophyta) some are not, because of secondary changes that have converted some of their members to a drastically different grade (region CT; e.g. Animalia, where the basic triploblastic body plan has been totally lost by Myxozoa; Heterokonta where the Opalinata have lost the ciliary retroneme synapomorphy that makes other heterokonts an important grade of organization). Finally many important grades are neither clades nor taxa (region G); they may be paraphyletic groups (e.g. eubacteria, invertebrates, fish) or polyphyletic ones (e.g. protists, zooflagellates, amoebae). All seven types of biological groups have valuable roles to play in the description and analysis of organismic diversity; it is a simplistic and harmful error to attempt to restrict the scientifically appropriate use of any of them by naive dichotomies into allowable and forbidden types of group, and still worse to pretend that the three types that are not clades are not even groups at all.

(3) Principles of ranking taxa

Classically, taxonomists reach a consensus on appropriate ranks for taxa based on a broad knowledge of diversity, judgement of degrees of structural disparity, and respect for sensible traditions of what degree of disparity merits a particular rank. Though ranking has a subjective element, it is a very useful device for helping the human mind quickly appreciate the relative position of nested taxa within the taxonomic hierarchy and, roughly, the relative magnitude and significance of the differences between them. It is a mistake to denigrate the process of ranking because it cannot be objectively programmed into a computer.

Human judgement of significance and reasoned choice between alternatives are required throughout science, as Singer (1931: 121–123) perceptively emphasized; the Baconian error of thinking that one should be able to make important discoveries or sensible classifications merely by passing facts 'through a sort of automatic logical mill' (Singer, 1931: 121) is unfortunately widespread amongst cladists.

Even if we were given the true phylogenetic relationships of all organisms, creating a balanced classification would require careful taxonomic judgement in delimiting and ranking taxa: classification should not be based on a dogmatic approach that prohibits paraphyletic taxa or a simple algorithm or rule like that of Hennig (1966), which stated that the categorical rank of a taxon should be determined by its biological age. The latter is practically impossible, as we do not know, and may never know precisely, where the root of the tree of life is located, and there are numerous places in the tree with so much uncertainty about branching order that attempts to apply the rule would be arbitrary and less stable than the classical method of ranking by taxonomists. If, for the sake of argument, we knew that the root of the tree of life was within the cyanobacteria, which is not unreasonable given the palaeontological evidence (Schopf, 1993), then it would be absurd to rank certain cyanobacterial subgroups more highly than the kingdom Animalia or the superkingdom Eukaryota, merely because they diverged earlier. The morphological and chemical uniformity of the cyanobacteria is so marked that they should be ranked only as a phylum, and their subgroups lower still irrespective of their probably immense antiquity; as in most other groups their molecular trees reveal so many rapid bush-like radiations (Turner, 1998) that their branching order would be an almost

worthless guide to ranking, even were it philosophically desirable, which it is not. Significant evolutionary change is highly non-clock like: because of periods of stasis or minimal change despite branching, and an erratic distribution of major innovations across the tree of life, there would be no classificatory value in ranking taxa by their presumed antiquity instead of by the classical criterion of their structural disparity.

(4) The need to weigh and integrate phylogenetic evidence from diverse sources

Systematics is, par excellence, a synthetic science and needs both generalists and specialists. It is a commonplace that excessive reliance on a single line of evidence is dangerous and that not all data are of equal value; but judgement of their appropriate value is both difficult and controversial. Congruence between different types of evidence is the most important criterion for assigning weight to them. But there is no magic formula for doing so and the creation of a macrosystem of life is both an art and a science (Minelli, 1993) – not an art that passively reflects nature, but one which creates a simplified picture of its underlying pattern to further its appreciation by humans. Like any science, macrosystematics advances by trial and error: by intuition and imagination as well as by careful observation, experiment, reasoning and critical discussion. I welcome criticisms of the present system, which attempts to take some account of all types of evidence, but deliberately gives them unequal weight. I have attempted to give reasons for major decisions, but space does not permit this for every detail.

III. IN DEFENCE OF BACTERIA: THE SOLE PRIMARY KINGDOM OF LIFE

It is highly desirable that we keep the long established group of Bacteria as a major taxon in the classification of life. Whether Bacteria are ranked as a kingdom or a superkingdom is less important than the retention of both the name and the concept of bacteria in their classical meaning. However, I shall argue that the advantages of ranking Bacteria as a kingdom rather than a superkingdom outweigh the disadvantages. Before explaining my present views, I need briefly to review some important earlier ideas.

(1) The concept of a bacterium (synonym: prokaryote)

The concept of bacteria was considerably refined by Picken (1960), Stanier (1961) and Stanier & Van Niel (1962). These authors and Echlin & Morris (1965), Allsopp (1969) and Stanier (1970) contrasted the structure of bacteria with that of eukaryotes in 12 major respects: (1) the fundamental differences in the structure and function of bacterial flagella and eukaryotic cilia (a term which, for clarity, embraces also eukaryotic 'flagella'; Cavalier-Smith, 1986*b*); (2) the location of respiratory chains in the cytoplasmic (surface) membrane rather than in mitochondria; (3) the location of photosystems in either the cytoplasmic membrane or free thylakoids rather than chloroplasts; (4) the usual presence of a peptidoglycan cell wall; (5) the absence of an internal cytoskeleton of actin microfilaments and tubulin-containing microtubules; (6) the absence of cytoplasmic motility mediated by ATPase molecular motors such as myosin and dynein; (7) organisation of the chromosome as a nucleoid in the cytoplasm, not as a nucleus; (8) absence of an endomembrane system (endoplasmic reticulum, Golgi complex and lysosomes); (9) absence of phagocytosis, endocytosis and exocytosis; (10) DNA segregation by association with the cell surface, not a mitotic spindle; (11) recombination by parasexual processes, not syngamy and meiosis; (12) inability to harbour cellular endosymbionts.

Each of these twelve character differences between bacteria and eukaryotes is of profound evolutionary importance and significance. Collectively, they are of such overwhelming significance compared with other character differences among organisms, that Stanier, Doudoroff & Adelberg (1963) were entirely correct in proclaiming the bacteria/eukaryote dichotomy as the most profound and significant in the living world. Nothing that has been discovered since then has invalidated their thesis that it outweighs in importance the differences between the eukaryotic kingdoms and equally strongly outweighs the differences between the various groups of bacteria. Since then, my own analyses (Cavalier-Smith, 1981*b*, 1987*c*, 1991*a*, *b*) have added many more such differences of genetic and cellular organisation, ranging from the presence of DNA gyrase in bacteria but not in eukaryotes to the absence of spliceosomes, peroxisomes and hydrogenosomes in bacteria. The total list now extends to about 30 major differences (Cavalier-Smith, 1991*a*, *b*).

(2) Changing views on Archaebacteria

The characterization of Archaebacteria and of their substantial differences from Eubacteria (Woese & Fox, 1977*a*; Woese, 1987) has been of profound importance for our understanding of bacterial diversity. So also have been the methods of rRNA phylogenetics pioneered by Woese (1987; see also Olsen & Woese, 1993) for the progress of phylogenetic knowledge of both bacteria and eukaryotes. I do not wish to minimize the great importance of these very positive contributions to science, but I must emphasize, as I have rather more mildly before (Cavalier-Smith, 1986*c*), that the systematic importance of the distinction between archaebacteria and eubacteria has been greatly exaggerated, a view strongly supported by Mayr (1990). Conversely, the very much greater importance of Stanier's (1961; Stanier & van Niel, 1962) concept of bacteria (or prokaryotes: the two words became synonyms with the acceptance of Stanier's term cyanobacteria for what were previously known as blue-green algae), and of the Bacteria/eukaryote distinction, has been repeatedly attacked by rhetoric and assertion rather than by careful argument. A long talk with George Fox 10 years ago made it clear to me, however, that when he and Woese first started to criticize the concept of a prokaryote (Woese & Fox, 1977*b*) they did not really have Stanier's ideas (1961; Stanier & Niel, 1962) in mind at all, but a naive view then widespread among molecular biologists that *Escherichia coli* was a typical prokaryote and that by studying its molecular biology the whole of bacterial biology would be revealed. This naive view clearly deserved to be criticized. What was most unfortunate about the way that the criticism was made, though, was that it was done as a blanket criticism of the perfectly sound basic idea of what constituted a prokaryote, rather than of the naive views of molecular biologists about the matter. Molecular biologists, being chemically rather than morphologically oriented, never took much notice of Stanier's concepts (1961, 1970; Stanier & Niel, 1962), which were mainly cell biological. Instead they pictured a prokaryote in terms of such molecular genetic features as operon structure and promoter organization in which *E. coli* was known to differ from eukaryotes. Woese and Fox (1977*b*) were correct to criticize the assumption that all bacteria would necessarily have these features, but failed to realize that such details played no role whatever in the basic organismic and cell-biological definition or concept of bacteria. Exaggerating the biological distinctiveness of Archaebacteria was, however, historically useful in that it encouraged a horde of very competent bacteriologists and molecular biologists to study them in the hope that they might unearth some really radical differences in genetic organization from that of *E. coli* and eukaryotes.

What this enormous enterprise has shown, however, is that Archaebacteria are not really very different at all from *E. coli* in the organization of their genes (e.g. in operons) and genomes, or their replication, transcription and translation machinery, as Keeling, Charlebois & Doolittle (1994) have clearly accepted. The first complete genome sequence of eubacteria and an archaebacterium give added force to the view that the genetic organization of all bacteria is fundamentally the same, and radically different from that of eukaryotes (Edgell *et al.*, 1996). Admittedly there are some differences, but these (except for some aspects of transcription) are ones of relatively small detail, mainly of interest to the specialist molecular biologist rather than the general bacteriologist, and these special properties of archaebacteria are mostly shared with eukaryotes, rather than indicative of a 'third form of life'. These differences pale into insignificance in comparison with the immensely more profound differences between the genetic organization of eukaryotes and bacteria, which Stanier (1961, 1970) was largely unaware of, but which I have discussed in detail elsewhere (Cavalier-Smith, 1981*b*, 1987*c*, 1991*a*, *b*, 1993*b*). This conclusion clearly refutes the idea that Archaebacteria and Eubacteria arose independently from a crude precellular progenote with poorly developed genetic mechanisms (Woese & Fox, 1977*b*). That idea was based on the huge differences between the two taxa revealed by 16S rRNA oligonucleotide cataloguing. The way in which this technique severely exaggerates the quantitative differences was trenchantly criticized by Hori, Itoh & Osawa (1982), and the validity of their criticism has been confirmed fully by total rRNA sequences. However, even at the time it was proposed it was probably obvious to any cell biologist, but curiously was not to most molecular biologists, that the idea that eubacteria, archaebacteria and eukaryotes had evolved independently could not possibly have been correct. Long before then it was known that eukaryotes and eubacteria share so many features of cell organization and metabolism that their latest common ancestor must have been a highly developed cell with several thousand genes, and highly developed DNA replication machinery (Edgell & Doolittle, 1997), and therefore could not have been

a 'progenote' with crudely-developed replication, DNA repair, transcription and translation machinery (Woese & Fox, 1977*b*). Belatedly, several authors have become aware of this (e.g. Ouzonis & Kyrpides, 1996).

Woese's (1983) statement that it was impossible for eukaryotes to have evolved from bacteria was a gross overexaggeration. I previously attempted to explain in detail how a bacterium could have evolved into the first eukaryote, and why Woese's criticisms (1983, 1987; see also 1994) of the concept of bacteria are mistaken (e.g. Cavalier-Smith, 1987*c*, 1991*a*, *b*). The evolution of rRNA is important, but one cannot understand bacterial evolution by focusing mainly on just one molecule (e.g. Woese, 1987). Bacteria are cells and organisms, and it is these biological entities (not single molecules) that systematists must classify. Woese (e.g. 1987) has correctly criticized earlier bacterial systematics for its phenetic rather than phylogenetic approach, but this earlier approach was as much through necessity as choice. Ribosomal RNA has added a wealth of new characters without which a phylogenetic approach would have been much more limited. But to understand cell evolution we need to consider very much more than just rRNA.

I have felt obliged to go into this history in some detail because the very early ideas of Woese & Fox (1977*a*, *b*) about the independent and early 'primary' divergence of archaebacteria, eubacteria and eukaryotes from a 'progenote', though not now accepted even by the authors themselves, and the thoroughly mistaken criticism of the concept of bacteria and prokaryotes, have played a larger role in determining the present views of many biologists on the status of archaebacteria, than has a critical assessment of the overall evidence now available to us. I wish, however, to emphasize that my criticisms of these particular early views in no way detracts from the importance of the concept of archaebacteria for understanding cell evolution and biological diversity.

(3) The importance of cell structure in bacterial classification

Though rRNA sequencing has quite clearly caused a revolution, largely most beneficial, in bacterial systematics, it is a great pity that it has sometimes been associated with a neglect (and still worse a positive disparagement) of morphology, which remains crucial for bacterial systematics. Sequence data, morphology, and non-sequence chemical data are complementary types of scientific evidence that have to be integrated in a balanced way for sound systematic decisions. It is historically incorrect to imply that before rRNA sequencing there was no phylogenetically sound bacterial taxonomy (Woese, 1987; 1994): it was even less correct to imply that this was true of microbiology in general, since for eukaryotic microbes there are a wealth of good morphological characters and over a century of good phylogenetic studies and creation of durable higher taxa. Even in bacteria it is a serious mistake to dismiss morphology altogether (Woese, 1987); mere differences in cell shape are indeed often relatively trivial, but ultrastructural morphology in bacteria is exceedingly important and phylogenetically informative. Long before any sequences became available there were several phylogenetically sound groupings of bacteria based on morphology, either alone or in combination with chemical characters.

The three most obvious such groupings are Cyanobacteria, spirochaetes and Gram-positive bacteria, all established in the nineteenth century, and all supported much later first by electron microscopy and then eventually also by rRNA phylogeny (for a summary of the bacterial classification proposed here see Table 2). The phylum Cyanobacteria is exactly the same in definition and circumscription as the Myxophyta of Cohn (1875): rRNA only confirmed what we already knew. For leptospiras and spirochaetes, Stackebrandt & Woese (1981) using trees based on rRNA oligonucleotide cataloguing suggested that they were not related. I never accepted this, because the location, uniquely in bacteria of the flagella in the periplasmic space seemed such a complex and unusual morphological character that it was highly unlikely to have evolved twice. Therefore, my first bacterial classification (Cavalier-Smith, 1987*a*) treated them as a single phylum. Full rRNA sequences agree with the morphological conclusion that this phylum (Spirochaetae) is monophyletic (Olsen, Woese & Overbeek, 1994; Embley, Hirt & Williams, 1995).

This highlights the point often made by systematists, but still not fully appreciated by some molecular biologists, that molecular characters are not inherently superior to morphological ones. There are strong molecular characters (e.g. full 16s rRNA sequences) and weak ones, just as there are strong morphological characters (e.g. the periplasmic location of spirochaete flagella and the structure of cyanobacterial thylakoids) and weaker ones (e.g. rods *versus* cocci). What is still sorely needed in

Table 2. *Classification of the kingdom Bacteria and its 10 phyla*

Subkingdom 1. Negibacteria* Cavalier-Smith 1987 [outer membrane present; acyl ester lipids; murein wall between the two membranes usually present; small recognition particle RNA (based on Proteobacteria only at present); in content nearly the same as the former phylum Gracilicutes].

Infrakingdom 1. Lipobacteria* infrak. nov. (murein cell wall; outer membrane of phospholipids; lipopolysaccharide absent; no flagellar shaft outside outer membrane).

Superphylum‡ 1. Eobacteria* Cavalier-Smith 1982 (as an infrakingdom) stat. nov. (murein walls with ornithine; diaminopimelic acid, cytochrome *aa3,* and RuBisCo absent; flagella absent; small citrate synthetase).

Division or Phylum‡ 1. Heliobacteria Cavalier-Smith 1987 (anaerobic photosynthesisers unable to fix CO_2; bacteriochlorophyll *g* and *g′*; no chlorosomes or *b* cytochromes).

Phylum 2. Hadobacteria Cavalier-Smith 1992 em. (thermophiles or radiation resistant; if photosynthetic fix CO_2).

Subdivision or subphylum‡ 1. Chlorobacteria§ Cavalier-Smith 1992 (aerobic photosynthetic thermophilic gliders with bacteriochlorophyll *c* and *b* cytochromes; e.g. *Heliothrix*, *Chloroflexus*).

Subphylum 2. Deinobacteria§ Cavalier-Smith 1986 (non-photosynthetic; thermophiles or radiation resistant with thick walls, e.g. *Deinococcus*, *Thermus*).

Superphylum 2. Endoflagellata Cavalier-Smith 1992 (flagella in periplasmic space).

Phylum 1. Spirochaetae Ehrenberg 1855 stat. nov.

Subphylum 1. Euspirochaetae subphyl. nov. (murein walls with ornithine, e.g. *Treponema*).

Subphylum 2. Leptospirae subphyl. nov. (murein walls with diaminopimelic acid, e.g. *Leptospira*)

Infrakingdom 2. Glycobacteria infrak. nov. (outer membrane with phospholipid in the inner leaflet and lipolysaccharide in the outer leaflet of the bilayer; RuBisCo present; flagella pass through outer membrane).

Superphylum 1. Pimelobacteria* Cavalier-Smith 1992 (as infrakingdom) stat. nov. (murein wall with diaminopimelic acid).

Phylum 1. Sphingobacteria Cavalier-Smith 1987 (sphingolipids; flagella absent; usually glide)

Subphylum 1. Chlorobibacteria subphyl. nov. (photosynthetic anaerobes with chlorosomes: Chlorobiaceae, e.g. *Chlorobium*).

Subphylum 2. Flavobacteria (non-photosynthetic, e.g. *Flavobacterium*, *Cytophaga*).

Phylum 2. Eurybacteria* phyl. nov. (without sphingolipids or photosynthesis; phylogenetically distinct from proteobacteria; monophyly of this phylum is more uncertain than for any other bacterial phyla, as clear synapomorphies not identified).

Subphylum 1. Selenobacteria* Cavalier-Smith 1992 (as phylum) stat. nov. (non-fusiform; often flagellate, e.g. *Selenomonas*, *Sporomusa*).

Subphylum 2. Fusobacteria subphyl. nov. (fusiform, non-flagellate; *Fusobacterium*, *Leptotrichia*).

Subphylum 3. Fibrobacteria subphyl. nov. (*Fibrobacter*).

Phylum 3. Cyanobacteria Stanier 1973 (oxygenic photosynthesizers with chlorophyll *a*).

Subphylum 1. Gloeobacteria subphyl. nov. (no thylakoids; phycobilisomes on cytoplasmic membrane; *Gloeobacter*).

Subphylum 2. Phycobacteria subphyl. nov. (with thylakoids, e.g. *Anabaena*, *Nostoc*, *Prochloron*).

Phylum 4. Proteobacteria Stackebrandt *et al.* 1986 (without sphingolipids; phylogenetically distinct from Eurybacteria).

Subphylum 1. Rhodobacteria Cavalier-Smith 1987 (as phylum) stat. nov. (photosynthetic purple bacteria and their colourless derivatives; without chlorosomes, phycobilisomes or thylakoids; photosynthetic machinery in cytoplasmic membrane invaginations; bacteriochlorophyll *c* and *d* and purple carotenoid in photosynthetic species; this subphylum and its three major subgroups are delimited primarily by ribosomal RNA similarity as clear synapomorphies have not been found).

Infradivision or infraphylum‡ 1. Alphabacteria Cavalier-Smith 1992 (as class) stat. nov. (with or without photosynthesis, e.g. *Rhodospirillum*, *Rickettsia*, *Agrobacterium*).

Infraphylum 2. Chromatibacteria infraphyl. nov. (with or without photosynthesis; β- and γ-proteobacteria, e.g. *Escherichia*, *Haemophilus*, *Spirillum*, *Chromatium*).

Subphylum 2. Thiobacteria subphyl. nov. (δ- and ϵ-proteobacteria; non-photosynthetic, often sulphur-dependent, e.g. *Desulfovibrio*, *Thiovulum*, *Bdellovibrio*, Myxobacteria, e.g. *Myxococcus*).

Superphylum 2. Planctobacteria superphyl. nov. (wall of protein, not murein peptidoglycan).

Phylum Planctobacteria Cavalier-Smith 1987 (Planctomycetales and Chlamydiae).

Subkingdom 2. Unibacteria new subkingdom (diagnosis: bacteria with single cytoplasmic membrane only, but no outer membrane; large signal recognition particle RNA).

Table 2. (*cont.*)

Infrakingdom 1. Posibacteria* Cavalier-Smith 1987 stat. nov. (acyl ester lipids; murein widespread).
Phylum Posibacteria* Cavalier-Smith 1987.
Subphylum 1. Teichobacteria subphyl. nov. (cell walls, if present, thick and with teichoic acids; includes Firmicutes and Mollicutes; paraphyletic Firmicutes here abandoned as a formal taxon and subdivided between Endobacteria and Actinobacteria).
Infraphylum 1. Endobacteria infraphyl. nov. (spores endospores; DNA low in guanine and cytosine; classes Clostridea Cavalier-Smith 1982, e.g. *Bacillus*, and Mollicutes, e.g. *Mycoplasma*).
Infraphylum 2. Actinobacteria Margulis 1974 (as phylum) stat. nov. (spores exospores; DNA high in guanine and cytosine; e.g. *Corynebacterium*, *Streptomyces*).
Subphylum 2. Togobacteria Cavalier-Smith 1992 (as phylum) stat. nov. (teichoic acids absent; murein wall very thin; external non-lipid toga; obligately anaerobic thermophiles: Thermotogales and *Aquifex*).
Infrakingdom 2. Archaebacteria Woese & Fox 1977 stat. nov. (isoprenoid ether lipids; murein absent).
Phylum Mendosicutes Gibbons & Murray 1978.
Subphylum 1. Euryarcheota Woese, Kandler & Wheelis 1990 stat. nov. (energy metabolism various; not dependent on elemental sulphur).
Infraphylum 1. Halomebacteria Cavalier-Smith 1986 (methanogens or extreme halophiles, e.g. *Halobacterium*, *Methanospirillum*).
Infraphylum 2. Eurytherma infraphy. nov. (non-methanogenic, non-halophilic thermophiles; *Thermoplasma*, Thermococcales, Archaeoglobales).
Subphylum 2. Sulfobacteria Cavalier-Smith 1986 stat. nov. (syn. Crenarcheota Woese, Kandler & Wheelis 1990: energy metabolism depends on elemental sulphur; Sulfolobales and Thermoproteales; Jim Black Pool thermophiles: e.g. *Sulfolobus*, *Pyrobaculum*).

This classification is modified from that in Cavalier-Smith (1992*a*), where its cladistic basis is explained in more detail. To save space diagnoses in Tables 2–7 are given only for new taxa.

* Probably paraphyletic.

† Almost certainly paraphyletic.

‡ All the phyla here should be treated as divisions for formal bacterial nomenclature, and the superphyla as superdivisions, subphyla as subdivisions, and infraphyla as infradivisions. For uniformity with the other kingdoms of life, I have used phylum rather than division in the present paper. The use of division rather than phylum is an historical relic of the origin of the Bacterial Code of Nomenclature from the Botanical Code rather than from the Zoological Code, which impedes a unified approach to biological nomenclature and systematics. As the Botanical Code now allows phyla and states that phylum and division are of equivalent rank, the Bacterial Code ought to be similarly revised. When the Unified Code of Biological Nomenclature, now being planned for the next century (Hawksworth, 1995), is introduced, I hope that it will adopt phylum, not division, for all organisms and that it explicitly recognizes subkingdoms, infrakingdoms, superphyla, subphyla and infraphyla so as to fix their relative rank, and also either superkingdoms or empires or both. By using phylum not division, one is free to use ordinary English terms like subdivision in a general sense with no connotation of rank or risk of their being confused with the taxonomic category subphylum. Haeckel's (1866) name Monera is an unsuitable name for bacteria as it was invented for hypothetical non-nucleate amoebae, which do not exist, and is much less widely known than Bacteria.

§ Originally treated as separate phyla (Cavalier-Smith, 1992*a*); for molecular evidence for their monophyly see Olsen, Woese & Overbeek (1994).

bacterial systematics, and has not been provided by the sequence-oriented school, is the development of a stronger tradition, like that established over two centuries in eukaryotic systematics, of the critical weighing of all available characters, whether molecular, chemical or sequence. Over-emphasis on one molecule and neglect of important morphological evidence is poor systematic practice.

The Gram-positive group, as defined by light microscopy, was slightly heterogeneous. Electron microscopy showed that the vast majority of Gram-positives had only a single bounding membrane, whereas the vast majority of Gram-negatives were bounded by two separate membranes. I have long argued that this morphological difference is of very profound cell biological and evolutionary importance, and therefore coined the word Posibacteria for all eubacteria with a single membrane, because their most numerous members are the true Gram-positive bacteria (Cavalier-Smith, 1987*c*). The taxon Posi-

bacteria was originally both a phylum and a subkingdom but is now ranked as both a phylum and an infrakingdom. Posibacteria is not a synonym for true Gram-positive bacteria (formally Firmicutes or Firmibacteria), as is sometimes incorrectly thought, because it also includes two other taxa: these are mycoplasmas (Mollicutes: another morphologically based group confirmed by rRNA sequences – both sequences and membrane chemistry showed that the archaebacterium *Thermoplasma acidophilum* was originally wrongly included, so morphology had to be supplemented by this extra evidence to refine the group) and Thermotogales. Sequencing showed that mycoplasmas evolved from the branch of Firmicutes with DNA of low guanine and cytosine content by the loss of the murein peptidoglycan wall (Woese, Stackebrandt & Ludwig, 1985). This molecular sequence demonstration that Firmicutes and Mollicutes together are monophyletic is congruent with the morphological fact that both groups have a single membrane, not two as in all other eubacteria. Thus the singleness of the membrane and the acyl ester character of its lipids are more important than the presence or absence of the cell wall that was emphasized in the earlier classification (Gibbons & Murray, 1978).

To contrast all the other eubacteria that are bounded by two distinct lipid membranes with Posibacteria, I named them Negibacteria (Cavalier-Smith, 1987*c*). Negibacteria is not an exact synonym for the vernacular term Gram-negative bacteria, because it includes a few bacteria such as the genus *Deinococcus* that are Gram-positive because they have a thick wall between the two membranes, rather than outside the single membrane as in the majority of Gram-positives. However, it has the same circumscription as the division Gracilicutes (Gibbons & Murray, 1978), but the higher rank of subkingdom. Therefore it could be argued that it was, strictly speaking, an unnecessary new word, like the term Cyanobacteria, a fifth synonym for Myxophyceae, Cyanophyceae, Myxophyta and Cyanophyta. However, as Gracilicutes was of much lower rank, not in wide currency, and there was no informal vernacular equivalent, I thought it useful to create a subkingdom name that would be euphonious in both the vernacular (as negibacteria) and formally (as Negibacteria), and which would contrast the group directly with Posibacteria by using the prefix Negi- and emphasize their bacterial character by using the suffix -bacteria. The importance of euphony is illustrated by the widespread use of the name Archaebacteria in preference to the first formally valid name Mendosicutes (Gibbons & Murray, 1978).

(4) Characters important in the high-level classification of Bacteria

Archaebacteria, like Posibacteria, have only a single bounding membrane. I shall therefore henceforward refer to both groups collectively as Unibacteria. Because of the differences in protein and lipid targeting that it implies, I think that the difference in membrane number between Negibacteria and Unibacteria is the most important morphological difference of all within the Bacteria (Cavalier-Smith, 1987*a*, *c*; 1991*a*, *b*, 1992*a*). By contrast, the differences in morphology between Archaebacteria and Eubacteria are trivial or non-existent. The cells of Archaebacteria and Posibacteria are organized in fundamentally the same way; there are no known morphological differences between them by which all archaebacteria can be distinguished. They have morphologically indistinguishable flagella and gas vacuoles. Archaebacteria are undoubtedly bacteria by all 12 of Stanier's (1961, 1970) criteria listed at the beginning of subsection (1). Their genetic systems are very similar to those of Posibacteria (Doolittle & Brown, 1994; Keeling, Charlebois & Doolittle, 1994), and their cell-division apparatus is fundamentally similar (Wang & Lutkenhaus, 1996).

The complete sequence of the first archaebacterial genome (*Methanococcus jannaschii*: Bult *et al*., 1996) in comparison with those of the eubacteria *Haemophilus influenzae* (a negibacterium: Fleischmann *et al*., 1995) and *Mycoplasma capricolum* (a posibacterium Fraser *et al*., 1995) confirms that both eubacteria and archaebacteria are fundamentally similar in genome organization and gene content, and share nearly 1000 different genes. Many, if not most, of the genes that cannot at present be homologized across the eubacterial/archaebacterial divide may simply be rather rapidly evolving ones, rather than genuinely unique to either group. As Edgell *et al*. (1996) rightly stress, the most striking thing about the eubacterial and archaebacterial genomes is how similar they are; as these authors explain, the apparently high fraction of archaebacterial genes not previously present in databases is almost certainly an artefact of the present low representation of archaebacterial sequences compared with those of eukaryotes and eubacteria, rather than a sign of the uniqueness of archaebacteria as Bult *et al*. (1996) somewhat misleadingly suggested. As more sequence becomes available for the crenarcheote archaebacterium

Sulfolobus solfataricus (Sensen *et al*., 1996) this artefact will be reduced.

If one wished to divide Bacteria into two kingdoms based on morphology then I would argue that Negibacteria and Unibacteria would be a better choice than Eubacteria and Archaebacteria.

In what non-morphological ways do archaebacteria and eubacteria differ that might be sufficient to justify their separation into separate kingdoms? They differ in wall chemistry. The absence of murein in archaebacteria has often been stressed but it is also absent in the posibacterial mycoplasmas and in the negibacterial Planctobacteria. Probably, as I have previously argued (Cavalier-Smith, 1987*a*, *c*), this reflects three independent losses of murein: but this negative character clearly would not even justify their separation as a subkingdom, still less a kingdom. More important is the presence in archaebacteria, but apparently not in eubacteria, of N-linked glycoproteins, a character that is shared with eukaryotes and which I used to argue for a sister group relationship between archaebacteria and eubacteria (Cavalier-Smith, 1987*c*). Given that O-linked glycoproteins are present in a few eubacteria, as well as archaebacteria and eukaryotes, the magnitude of the innovation is not immense. There is also a most interesting difference in the chemistry of the shaft of the flagellum in archaebacteria (Fedorov *et al*., 1994). But none of these differences is so profound individually or collectively to justify a separate kingdom. The well known difference in membrane lipid chemistry (isoprenoid ethers in archaebacteria and acyl esters in eubacteria) is the only other important qualitative chemical character that clearly differentiates archaebacteria from eubacteria. I have long considered that this difference is sufficiently important, coupled with the internal metabolic diversity of archaebacteria, to give the taxon some sort of supraphyletic rank. But why not a subkingdom, infrakingdom or superphylum rather than a separate kingdom? The resemblances between archaebacteria and eukaryotes in transcription factors and some details of rRNA processing, though supportive of a sister relationship between the two groups (i.e. of the clade Neomura: Cavalier-Smith, 1987*c*), involve insufficient differences from the eubacterial pattern to justify any higher rank than infrakingdom or superphylum.

Bacteriologists and molecular biologists seem to have given rather little thought to the possibility of using such taxonomic categories of intermediate rank. Indeed, before the advent of rRNA sequencing bacteriologists had no tradition of using categories of high rank such as phyla; virtually all classifications used only classes, orders and families. There were no phyla, subphyla, or subkingdoms at all: nothing between classes (and precious few of them) and the kingdom Prokaryota. After the recognition of Archaebacteria four phyla were introduced: Gracilicutes, Firmicutes, Mollicutes (formerly a class), and Mendosicutes (Archaebacteria) by Gibbons & Murray (1978). Then suddenly, on the basis just of rRNA sequence divergence, a dozen or more separate bacterial kingdoms were suggested seriously (Woese, 1987), and still seem to be favoured by Olsen, Woese & Overbeek (1994), but have fortunately not been formally adopted by bacterial taxonomists. From a system where major groups such as spirochaetes and Cyanobacteria were given much too low a rank in comparison with eukaryotic taxa, there was a dramatic jump to excessively high ranking of the major bacterial taxa.

The overall system of life must be well balanced if it is to serve the needs of the general biologist as well as those of the specialist in some particular group. It is important for systematics and biology as a whole that ranking within bacteria does not get drastically out of step with that of eukaryotes.

The widespread ranking of Eubacteria and Archaebacteria as separate kingdoms has never been really critically discussed. The main justification usually assumed for such a high rank is the large difference in 16S rRNA sequence between these two groups. This originally so impressed Woese that he thought that archaebacteria must be a radically new form of life (Woese & Fox, 1977*a*). But what does the large difference in this one molecule really amount to biologically? The molecule is over 50% identical between the two taxa and functions in much the same way in both groups. There is no reason to think that most of the differences are of substantial functional significance: they probably are mainly biologically meaningless chemical noise that has accumulated by random mutation and genetic drift of neutral or quasi neutral sequences (Kimura, 1983). As such, they are very important as phylogenetic markers, but do not constitute the sort of organismal characters properly used in the classification and ranking of higher taxa. Quantitative differences in rRNA would be a very arbitrary basis for deciding rank, because rates of rRNA evolution vary considerably, sometimes drastically (up to about 50-fold), in different lineages and do not correlate well with the occurrence of substantial differences in body plan, which have always been

(and ought to continue as) the primary basis for delimiting higher taxa. The differences between archaebacterial and eubacterial rRNA are comparable in magnitude (though actually slightly less than) those that separate the two eukaryote phyla Microsporidia and Basidiomycota, which differ far more from each other morphologically, and in way of life, than do the two bacterial groups, yet are now both included in the kingdom Fungi (see section VII).

Ribosomal RNA on its own provides no sound reason to rank Archaebacteria any higher than a phylum. In fact, I consider that the rRNA differences deserve less weight than do the membrane chemistry, the N-linked glycoproteins, and the presence of a single bounding membrane in determining the rank of Archaebacteria. It is entirely unwarranted to treat the two archaebacterial subphyla as separate kingdoms; erecting a third archaebacterial 'kingdom', Korarchaeota, just on the basis of rRNA divergence, was most unwise, especially as we know next to nothing about the phenotypes of the organisms in question (Barns *et al*., 1996). The discovery of these previously unknown lineages, coupled with the uncertainty of the branching order at the base of the archaebacterial rRNA tree and the known propensity of rRNA for highly misleading non-clock like evolution (see discussion below on microsporidia) should discourage the use of rRNA branching depth alone as a basis for subdividing bacteria or any other organisms. When we know more about the biology of the novel uncultured archaebacteria, it may be appropropriate to subdivide archaebacteria into more than one phylum. But at present I favour a single phylum for all archaebacteria.

The above comments, from a predominantly eukaryotic systematist with extensive experience in ranking higher level microbial taxa, now entering the bacterial field more seriously than before, are intended to be constructive, and should not be construed as personal criticisms of other biologists, despite their critical character in places.

(5) Bacterial subkingdoms and infrakingdoms

Taking both morphology and chemistry into account, we have, in fact, not two but three major groups of bacteria that have to be grouped and ranked: Archaebacteria, Posibacteria and Negibacteria. In deciding groupings and ranks systematists traditionally try to determine which differentiating characters are ancestral and which are derived; for greater weight in grouping is accorded to shared derived characters (synapomorphies) than to shared ancestral ones. This brings me to the vexed question of rooting the tree of life, which is far more difficult than is usually thought and has not yet been unambiguously achieved.

For over a century, most biologists have assumed that eukaryotes evolved from bacteria, not vice versa, both because it was much more reasonable to suppose that the structurally simpler bacterial cell preceded the much more complex eukaryotic one, and because, since the 1950s, that view has been the best interpretation of the now very extensive microbial fossil record (Schopf & Klein, 1992). Woese & Fox (1977*a*), however, relying solely on the rRNA cataloguing evidence and the simple, but invalid assumption of a molecular clock, proposed instead that eukaryotes, archaebacteria and eubacteria were equally old and had evolved independently from the hypothetical progenote. For these three taxa, they coined the novel term Urkingdom to emphasize their hypothesis that the three groups were derived independently as separate 'primary' kingdoms. For the next decade many molecular archaebacteriologists accepted this idea overdogmatically, even though cell biological and palaeontological arguments were always opposed to it.

The situation was radically changed not so much by my detailed arguments about cell evolution (Cavalier-Smith, 1981*a*, *b*, 1987*a*, *c*), and the cladistic considerations that favoured a sister relation between archaebacteria and eukaryotes (Cavalier-Smith, 1987*c*), but by molecular trees for duplicated proteins (Iwabe *et al*., 1989; Gogarten *et al*., 1989). These clearly supported a sister relationship between archaebacteria and eukaryotes and suggested that they had separated from each other a substantial time after the origin of life. If the topology of these trees if correct, they provide cladistic evidence that the eubacterial/eukaryote acyl ester lipids are the ancestral type and the archaebacterial isoprenoid ones are derived, as was argued earlier (Cavalier-Smith, 1987*a*, *c*). If the eubacterial lipids are indeed the ancestral type, then their common possession by Negibacteria and Posibacteria is not a very strong argument for linking them together in the taxon Eubacteria. Eubacteria are a paraphyletic grade, not a holophyletic clade as archaebacteria probably are.

The likelihood that eubacteria are paraphyletic is not in itself a reason for rejecting the taxon; I have treated them as a subkingdom or kingdom for many

years despite believing them to be paraphyletic. But it should cause us to ask seriously whether the best place for the primary subdivision of bacteria is that between archaebacteria and eubacteria or whether it might not be better to place it within the eubacteria between the Negibacteria and Posibacteria. It is really a question of whether one weights more strongly the difference in number of membranes or the differences in membrane chemistry and N-linked glycoproteins. I consider the difference in membrane number to be of more profound evolutionary significance, because there have been a number of changes in membrane chemistry, such as the invention of sterols, but apparently only one changeover between two and one bounding membrane (Cavalier-Smith, 1980, 1987*a*, *c*; 1991*a*, *b*). I therefore here treat Negibacteria and Unibacteria as bacterial taxa of equal rank, and both Posibacteria and Archaebacteria of lower rank but equal to each other.

What rank should be assigned to these three taxa? As Negibacteria are sufficiently diverse in cell structure and/or fundamental biochemistry to merit subdivision into several phyla (eight being adopted here), all three taxa must be ranked at a level above that of phylum. While a reasonable case could be made for treating Negibacteria and Unibacteria as separate kingdoms, I prefer to treat them as subkingdoms of a single kingdom Bacteria, for basically the same reasons given below for the kingdom Protozoa. It provides a simpler and more balanced system for general reference purposes, and maintains a good correspondence between the kingdom name and the vernacular name 'bacteria' that has been in perpetual use for over 150 years and is well known to the layman. If Unibacteria is a subkingdom, it is most appropriate to make Archaebacteria and Posibacteria infrakingdoms. The proposed broad classification of bacteria and its place within the six-kingdom system are summarized in Table 1; the major taxa comprising each infrakingdom are indicated in Table 2.

The subkingdom Negibacteria is here divided into two infrakingdoms based on the chemistry of the cell's outer membrane. One of these I call Glycobacteria, as it has lipopolysaccharide in the outer leaflet of the bilayer of the outer membrane; the other, which lacks lipopolysaccharide and has phospholipid in both leaflets of the outer membrane, I name Lipobacteria, and suggest this is the ancestral condition since lipopolysaccharide synthesis is immensely more complex than phospholipid synthesis, and is therefore unlikely to have been present in the first negibacterium. Infrakingdom Lipobacteria contains the three phyla which lack lipopolysaccharide in their outer membrane; two of these lack flagella and have ornithine rather than diaminopimelic acid (DAPA) in the cross-linking peptide of their murein walls (the photosynthetic Heliobacteria and the partly photosynthetic and partly heterotrophic Hadobacteria), while the Spirochaetae (typical spirochaetes, with ornithine-containing muramopeptides and leptospiras with DAPA) have flagella within the periplasmic space. Glycobacteria are subdivided into two superphyla according to whether they have murein walls or not. The glycobacterial superphylum Pimelobacteria, unchanged in circumscription but slightly reduced in rank compared with my previous classification (Cavalier-Smith, 1992*a*), have murein walls containing diaminopimelic acid and include the two best-known negibacterial phyla, Cyanobacteria and Proteobacteria, as well as the lesser known Sphingobacteria and Eurybacteria. Superphylum Planctobacteria includes only the Planctomycetales and chlamydias (in a single phylum, the Planctobacteria), which lack murein. I have suggested previously that the absence of murein in Planctobacteria is derived; and the presence of murein in the common ancestor of Lipobacteria and Pimelobacteria was the ancestral condition (Cavalier-Smith, 1987*a*). If this and my hypothesis that the lipobacterial lack of lipopolysaccharide is ancestral are both correct, then to understand the early evolution of negibacteria we need to focus attention on the little-studied Lipobacteria. If my view that the root of the tree of life should be placed within the negibacteria is also correct, this means that to understand the earliest divergences in the history of life the study of Lipobacteria will be much more important than that of Archaebacteria. It is, of course, possible that the absence of lipopolysaccharide, flagella, DAPA and RuBisCo in eobacteria is derived (or derived in some but not other taxa), but until there is definite evidence for this we should take seriously the possibility that this was the ancestral state for all bacteria and regard the eobacteria as very good candidates for the earliest diverging cells and thus of great potential evolutionary significance.

The present classification of Bacteria into two subkingdoms, each further subdivided into two infrakingdoms, has a pleasing symmetry and balance. The primary subdivision is according to the number of envelope membranes, and the secondary subdivision of each relies on membrane chemistry.

Each of the four infrakingdoms differs not only in membrane number and or chemistry, but also in cell wall chemistry or structure. Archaebacteria alone have N-linked glycoproteins and sometimes pseudomurein; Posibacteria alone have teichoic acids, and typically very thick murein walls with peptide linkers of very variable chemistry; Glycobacteria have very thin murein layers always with DAPA in the crosslinking peptide; Lipobacteria have usually thin, but sometimes thick, murein walls typically with ornithine in the linker. Thus, the special cytoplasmic membrane chemistry of archaebacteria is given the same taxonomic weight as the special outer membrane chemistry of the glycobacteria. Isoprenoid ethers and lipopolysaccharides are the synapomorphies that can be used to define these two infrakingdoms most clearly. The four-infrakingdom system groups the 10 bacterial phyla recognized here (a slightly lower number than that more tentatively suggested by Woese, 1987) into organismally more homogeneous higher taxa than does the archaebacterial/eubacterial dichotomy: the probably paraphyletic Eubacteria are so much more heterogeneous organismally than archaebacteria, that their splitting into three taxa each of the same rank seems to me a marked improvement and a more balanced classification.

The major phylogenetic assumptions behind the six-kingdom system are explicitly laid out in Fig. 1. Unlike traditional bacterial classifications, the present system does not undervalue the diversity of bacterial body plans in comparison with that of higher kingdoms: as Table 1 makes clear, the kingdom Bacteria has four major subdivisions of rank infrakingdom or above, which is the same as in the kingdom Plantae and more than the kingdoms Fungi and Chromista, which have only two or three respectively. Even Protozoa and Animalia, the most megadiverse kingdoms have, respectively, only six and 11. In this system the taxa Archaebacteria and Archezoa are each treated as one of the 30 major forms of life; this sufficiently emphasizes their distinctiveness – previously Woese and I were probably both a little overenthusiastic in the use of the unnecessarily high rank of kingdom. At the phylum level, Bacteria are the third-most diverse of the six kingdoms. The fact that five out of the eight negibacterial phyla (including more than one in each superphylum) are entirely or partly photosynthetic shows that the diversification of the basic photosynthetic machinery (Pierson & Olson, 1989) played a key role in early cell evolution and is consistent with the view that the first cells were photosynthetic (Woese, 1979; Cavalier-Smith, 1987*a*). The presence of chlorosomes in *Chloroflexus* (a lipobacterium) and Chlorobiaceae (Glycobacteria) suggests that the transition between the two infrakingdoms involved a green photosynthetic bacterium with chlorosomes, thus supporting the idea that these neglected bacteria are of pivotal evolutionary significance (Cavalier-Smith, 1987*a*).

Whether the ranking of Archaebacteria, Posibacteria, Eobacteria and Lipobacteria as infrakingdoms and the abandonment of the taxon Eubacteria will be accepted by others, only time will tell. However, I hope that the reader will agree that it is based on a more careful consideration of what weight should be given to the major differences within Bacteria, than the suggestion that archaebacteria and eubacteria be ranked equally as domains (Woese, Kandler & Wheelis, 1990). These are merely unnecessary new names for the earlier suggested Urkingdoms or 'primary kingdoms' of Woese & Fox (1977*a*) that were not well based. As I stressed previously (Cavalier-Smith, 1986*c*) there are no good reasons to rank the three taxa eukaryotes, archaebacteria and eubacteria equally as kingdoms: merely renaming them domains (Woese, Kandler & Wheelis, 1990) does not lessen the criticisms of equal ranking at all (Cavalier-Smith, 1992*b*); the new category domain is also unnecessary given that we already have superkingdom and empire above the level of kingdom. Equal ranking of these taxa produces a grotesquely unbalanced system for living organisms as a whole. The name eubacteria will, however, continue to be very useful to denote a grade, as are 'invertebrate' and 'fish' in zoology or 'alga' in botany. The suggestion that eubacteria be renamed bacteria (Woese *et al.*, 1990), was exceedingly confusing and should not be followed (Cavalier-Smith, 1992*b*).

Some may object to my placing the Thermotogales and *Aquifex* in the Posibacteria on the grounds that the rRNA tree does not provide evidence for such a grouping. However, the rRNA tree neither provides convincing evidence against such placing nor convincing evidence for any supraphyletic groupings within eubacteria, as has been made clear by Embley, Hirt & Williams (1995). On rRNA trees even all Teichobacteria often do not group together; for their two major subgroups (Endobacteria and Actinobacteria) often, but not always, separate from each other (Olsen, Woese & Overbeek, 1994; Embley, Hirt & Williams, 1995). It appears that the eubacterial phyla (and sometimes also subphyla) underwent an almost simultaneous diversification so

that molecular trees cannot correctly resolve their branching order, or even, in the case of the Teichobacteria, confirm or refute their monophyly. In such a circumstance, it is perfectly proper to base phylogenetic reasoning and classification solely on other evidence, which in some cases may be much stronger than the sequence evidence. I have argued that this major eubacterial radiation most likely was the consequence of the origin of the first photosynthetic eubacterial cell and agree with Woese's (1979) hypothesis that the first bacterial cell was photosynthetic.

For reasons discussed in detail earlier (Cavalier-Smith, 1980, 1987*a*, 1992*a*), I have argued that the first cell was a negibacterium with two membranes, and that Unibacteria are secondarily derived by the loss of the outer membrane. If this is true, Unibacteria and Eukaryota together form a clade that may be referred to by the informal term unimembrana. The distinction between unimembrana and negibacteria is profoundly important for cell evolution and origins. The first cell must either have been unimembranous, as is classically assumed (e.g. Goldacre, 1958; Hargraeves & Deamer, 1978), or negibacterial, as Blobel (1980) and Cavalier-Smith (1987*a*) have suggested. Until we can establish firmly which idea is correct, we shall continue to have a very shaky basis for understanding early cell evolution. It might be thought that a combination of the Iwabe *et al.* (1989) trees and the rRNA trees refute the idea that negibacteria were ancestral since they appear to place the root between Archaebacteria and Posibacteria, i.e. within Unimembrana. But I remain unconvinced that we really know where the root is yet, as do others (e.g. Forterre *et al.*, 1993; Doolittle & Brown, 1994). While the basic logic behind using a duplicate protein gene as the outgroup for the tree of its sister duplicate is good, there is a practical problem arising from the fact that the outgroup tree for one duplicate is a very long branch compared with the branches within the tree for the other duplicate. It is well known that such a very long branch tends to be misplaced on trees (Swofford *et al.*, 1996). Even if the correct position of the root were at the base of the negibacteria, tree-calculation algorithms will tend to misplace the root nearer to the mean position; the more extreme the differences in branch length the greater will be this tendency. The problem is that the gene duplicates so far studied appear to have diverged very strongly from each other very soon after the duplication and prior to the slower divergence of the major taxa. This necessarily means that the outgroup branches are excessively long in comparison with the ideal situation for accurate tree-construction. The possibility of such an artefact, in which the root is placed in the mid position rather than in the correct place, makes the interpretation of all gene duplication trees studied up to now rather uncertain.

It is important to stress that the uncertainty regarding the position of the root of the tree of life does not affect the validity of the bacterial subkingdoms and infrakingdoms proposed here. The uncertainty simply means that we do not know which of them are holophyletic and which are paraphyletic. If I am correct, then Archaebacteria are holophyletic and Posibacteria, Unibacteria, Negibacteria, Glycobacteria and Lipobacteria are all paraphyletic; if, however, the tree of Iwabe *et al.* (1989) is correctly rooted, then Negibacteria would become holophyletic, as would one of Glycobacteria and Lipobacteria (depending on their branching order); the same taxa also become holophyletic if Lake (1988, 1989) is correct and the root lies between the two archaebacterial subphyla; whereas Archaebacteria become paraphyletic. Neither grouping would make them polyphyletic. However certain conceivable branching orders of the eubacterial phyla would make one or more of my taxa polyphyletic and thus necessitate a revision. Accurately determining their branching order will be the best test of monophyly of the major taxa in the present classification. Both this and the fixing of the position of the root may only be possible when a complete genome sequence becomes available for at least one member of each of the nine eubacterial phyla. As four are already published – Proteobacteria [*Haemophilus influenzae* (Fleischmann *et al.*, 1995); also *E. coli* and *Helicobacter pylori*] Cyanobacteria, Spirochaetae, and Posibacteria [*Mycoplasma genitalium* (Fraser *et al.*, 1995)], we might know the answer by the end of the century, provided that whole genomes are sequenced for the other five. It is however possible, as suggested by the 16S rRNA tree, that the radiation was so explosively simultaneous that we shall never be able to resolve the order fully, but I am optimistic that we can.

The fact that if one of these other ideas is correct and I am wrong about the rooting of the tree, more of the bacterial high-level taxa proposed here become holophyletic, means that new knowledge about the rooting can only increase the attractiveness of the present system to strict Hennigian cladists, not decrease it. However, if Archaebacteria are ever shown to be paraphyletic (Lake, 1988, 1989; Baldauf, Palmer & Doolittle, 1996), contrary to the

arguments of both Woese (e.g. 1987) and myself (e.g. 1986*b*, 1987*c*), the taxon should not be abandoned, just because of the dogmas of strict Hennigians.

IV. PROTOZOA, THE BASAL EUKARYOTIC KINGDOM

Treating Archezoa as a subkingdom of Protozoa makes the present kingdom Protozoa very similar in composition to Owen's (1858) original kingdom Protozoa, apart from diatoms now being properly placed with other ochrophyte algae in the kingdom Chromista, and Bacteria being separated into their own kingdom. It maintains in a single kingdom the vast majority of the organisms that have been treated as protozoa over most of the 150 years since Von Siebold (1845) restricted the term to unicellular organisms, as summarized in Table 2. For approaching two centuries, since its invention by Goldfuss (1817), the vernacular term protozoa has been so widely used by biologist and layman alike [for example, by the poet Coleridge (1834)], that there is real value in keeping as close as possible to the historically dominant meaning without compromising the principles of Darwinian classification. Protozoa as a major taxon has, in fact, proved to be more stable than the remarkable variety of different interpretations we have seen over the past 50 years of the far more heterogeneous Protista of Haeckel (1866). Protists in Haeckel's sense of unicellular organisms are now distributed in the present system across all six kingdoms. However, as I stressed previously (Cavalier-Smith, 1981*a*), the vernacular word protist remains a most useful term for designating the polyphyletic grade of unicellular eukaryotes, even though a kingdom Protista or Protoctista (in addition to being polyphyletic) would be much too heterogeneous to be taxonomically meaningful.

(1) Status of Archezoa, early diverging amitochondrial eukaryotes

The original concept of the Archezoa was phylogenetic. It was argued that a symbiotic origin of mitochondria could not have occurred until after the evolution of phagocytosis (Cavalier-Smith, 1983*b*). Since phagocytosis is restricted to eukaryotes (Stanier, 1961, 1970), it followed that the origin of the eukaryote cell and phagocytosis had almost certainly preceded the symbiotic origin of mitochondria (Cavalier-Smith, 1983*b*), not the reverse as had generally been postulated before (Margulis, 1970, 1981). Thus primitively amitochondrial eukaryotic cells must once have existed (Cavalier-Smith, 1983*b*). Although there was no guarantee that they had not all gone extinct after the origin of mitochondria, I considered it rather unlikely that this would have occurred, since niches for anaerobic phagotrophs must always have existed somewhere in the environment, in which amitochondrial eukaryotes would have been at no disadvantage compared with eukaryotes with mitochondria. Therefore, I postulated that at least some of the known amitochondrial eukaryotes were primitively so, and defined the subkingdom Archezoa as comprising all primitively amitochondrial eukaryotes (Cavalier-Smith, 1983*b*). I was perfectly well aware that some amitochondrial eukaryotes had secondarily lost mitochondria (i.e. certain ciliates and fungi) and that some, or conceivably even all, of the taxa that I initially placed in Archezoa might well also have lost mitochondria. But in a Popperian spirit I deliberately chose to frame, what I explicitly referred to as my 'taxonomic hypothesis', in the most strong and therefore most easily refutable form by including in Archezoa all amitochondrial taxa which then had no known mitochondrial ancestry.

(*a*) *Changes in circumscription of the Archezoa*

Later, after coming to the view that all hydrogenosomes had probably evolved from either peroxisomes or mitochondria (Cavalier-Smith, 1987*c*), I decided that the double-membraned hydrogenosomal envelope of Parabasala was therefore probably homologous with the mitochondrial envelope, and therefore removed Parabasala from Archezoa (Cavalier-Smith, 1987*a*). This change in circumscription of the group, contrary to what is sometimes implied, did not change the phylogenetic definition of the group. It was simply a change in view as to whether Parabasala satisfied that definition or not. As previously discussed (Cavalier-Smith, 1993*a*), the evidence for a mitochondrial origin for parabasalan hydrogenosomes was, for a long time, rather equivocal. However, there is no good evidence against it, and what is known about protein-targeting to hydrogenosomes is consistent with the view that their targeting system evolved from that of mitochondria. This is simpler than the alternative idea of an entirely independent symbiogenetic event (Müller, 1992), or an even less likely origin from the endomembrane system. Recent evidence from the proteobacterial affinities of the heat shock protein

hsp 60 of trichomonads (Bui, Bradley & Johnson *et al*., 1996; Germot, Philippe & Le Guyader, 1996; Horner *et al*., 1996; Roger, Clark & Doolitttle, 1996) provides the first strong molecular evidence that Parabasala are indeed secondarily amitochondrial, and therefore gives indirect support for a mitochondrial ancestry for their hydrogenosomes.

To those who believe that classifications should not be based on hypotheses, I simply say that all phylogenetic classifications are based on similar sorts of phylogenetic hypotheses. The degree to which a phylogenetic hypothesis needs to be corroborated before being used as a partial basis for a classification is a matter for scientific judgement by individual systematists. It is neither philosophically correct nor good manners to call the phylogenetic hypotheses of others speculation, and to treat one's own as accepted facts. It is an error to assert that Archezoa were compositionally defined (Patterson, 1988). Although many rRNA trees show Parabasala as branching just below all mitochondrial eukaryotes, some show them instead as branching just above the mitochondria-bearing Percolozoa (Cavalier-Smith, 1993*a*,). The latter position is inconsistent with the idea that Parabasala are primitively amitochondrial archezoa (that is, if mitochondria are monophyletic), but both positions are consistent with the view that they are secondarily amitochondrial; which position is correct is uncertain (Cavalier-Smith & Chao, 1996*a*), but as several protein trees place the divergence of Parabasala lower than that of the Percolozoa, this position is more likely to be correct.

Parabasala are now treated as a subphylum within the protozoan phylum Trichozoa, all of which have hydrogenosomes instead of mitochondria (Cavalier-Smith, 1997*a*). Trichozoa, Percolozoa and Euglenozoa were recently grouped together as the protozoan subkingdom Eozoa to contrast them with the putatively more advanced protozoa placed in the subkingdom Neozoa (Cavalier-Smith, 1997*a*).

The phylogenetic evidence from rRNA that the archamoebae *Entamoeba*, *Phreatamoeba* (Hinkle *et al*., 1994), and *Pelomyxa* (Morin & Mignot, 1996) are all secondarily amitochondrial made it desirable to remove the phylum Archamoebae as a whole from the Archezoa, and to place them in the Neozoa alongside the amoebae with mitochondria (Lobosa and Filosea) (Cavalier-Smith, 1997*a*). The evidence that they are secondarily without mitochondria is relatively clearcut, as they branch well above the deepest branching point of mitochondrial taxa. As they sometimes even group with the Lobosa in maximum-likelihood trees, Lobosa and Archamoebae have each recently been ranked as subphyla within the revised phylum Amoebozoa (Cavalier-Smith, 1997*a*). In addition to the positive cladistic evidence for a mitochondrial ancestor, there is even more direct evidence from hsp 70 sequences that *Entamoeba histolytica* evolved from an ancestor with mitochondria (Clark & Roger, 1995), but in contrast to the situation in Parabasala these were not converted into hydrogenosomes.

Unlike Trichozoa, the Metamonada and Microsporidia have no hydrogenosomes and branch at the very base of the eukaryotic rRNA tree, and it has been widely thought that both taxa are primitively amitochondrial. However, I stressed previously (Cavalier-Smith, 1993*a*; see also Cavalier-Smith & Chao, 1996*a*), that this conclusion might not be correct. The first evidence, apart from their obligate intracellular parasitism, that Microsporidia might be less primitive than metamonads was the discovery of spliceosomal RNA in microsporidia (preliminary data cited in Cavalier-Smith, 1993*a*; DiMaria *et al*., 1996); this makes it possible that Metamonada are the only eukaryotes that primitively lack spliceosomal introns. If spliceosomal introns arose from group II self-splicing introns that entered eukaryotes within the genes of the α-proteobacterium ancestral to mitochondria, as postulated (Cavalier-Smith, 1991*c*), this would imply that microsporidia evolved from ancestors that had mitochondria as previously emphasized (Cavalier-Smith, 1993*a*). Two lines of evidence now strongly support this view and suggest that Microsporidia are really degenerate fungi, and must be removed from the Archezoa. First were trees based on sequences of α-tubulin (Keeling & Doolittle, 1996; Li *et al*., 1996) and β-tubulin (Edlind *et al*., 1996; Li *et al*., 1996; Roger, 1996), which group the Microsporidia very robustly with the Fungi, not the Protozoa. Second, sequence trees for the molecular chaperone hsp70 equally convincingly group the Microsporidia with the Fungi (Germot *et al*., 1997); since the chaperone in question is the mitochondrial type related to that of the α-proteobacteria, there seems little doubt that microsporidia are indeed secondarily amitochondrial. A third, but much less convincing, piece of evidence is that the protein synthesis elongation factor protein EF 1-α of microsporidia has an insertion at exactly the same site as does that of all Fungi, animals and choanozoan protozoa (collectively known as opisthokonta); however, this insertion is a little shorter and only weakly similar in sequence to that in opisthokonta. Moreover the EF 1-α protein sequence tree does not group the microsporidia with the Fungi but places

them near the bottom of the eukaryotic tree, just as does the small subunit rRNA tree.

Thus there is a dramatic conflict between the tubulin and hsp70 trees on the one hand and the EF 1-α and small subunit rRNA trees on the other. Since the two latter trees do not specifically group microsporidia with any other taxa, and in both cases the microsporidial branches are very long, the simplest way to reconcile the data are to suggest that the rRNA and elongation factor genes of microsporidia have undergone a drastically increased rate of evolution compared with all other eukaryotes and that the deep position in which they appear is a grossly misleading systematic error produced by the phylogenetic algorithms, none of which can give the correct tree if branches of sisters differ many-fold in length. There are a number of other instances where rRNA and certain protein trees have been grossly misleading, and tree-calculation algorithms have been proven to give the wrong answer quite reproducibly in certain circumstances (Felsenstein, 1978; Hillis, Huelsenbeck, & Cunningham, 1994; Swofford *et al*., 1996).

Though initially a surprise, the fungal nature of microsporidia is not at all unreasonable. Both groups have spores with walls of chitin, and a fair number of fungi are parasites of animals. A further similarity with higher Fungi is that the vegetative cells do not have Golgi dictyosomes visible in the electron microscope as stacks of cisternae. Higher Fungi are unusual among higher eukaryotes in lacking Golgi stacks; the only Fungi that have Golgi stacks like most other eukaryotes are the Dictyomycotina, which include Chytridiomycetes *sensu stricto*, the most basal group (Cavalier-Smith, 1987*b*). The absence of Golgi stacks in microsporidia suggests that they are not a sister group to the fungi as shown by some of the protein trees but, as Roger (1996) suggests, actually evolved from within the fungi after the allomycete ancestor underwent an evolutionary unstacking of its Golgi cisternae (Cavalier-Smith, 1987*b*).

It has long been known that the mitotic mechanism of microsporidia is remarkably similar to that of ascomycetes (Raikov, 1982). In fact their mitosis is indistinguishable from that of ascomycetes; in both groups mitosis is closed with an intranuclear spindle and a flattened centrosomal plaque at the spindle poles, either embedded in or just outside the nuclear envelope. Most other fungi do not have this type of centrosomal plaque; in basidiomycetes the centrosomes (the recently fashionable fungal term 'spindle pole body' is quite unnecessary) are globular and cytoplasmic, except in Uredomycetidae where they are a unique trilaminar plaque, whereas in Archemycota they are very varied in structure: centrosomal plaques occur in a few Zygomycotina. The similarity of mitotic mechanism between ascomycetes and microsporidia was earlier assumed to be convergent, partly because a similar mechanism is also found in the malaria parasite, *Plasmodium*, which must have evolved it independently from Fungi. The mycetozoan *Dictyostelium* also has centrosomal plaques, though mitosis is semi-open, so it is clear that centrosomal plaques have evolved at least three times following independent losses of centrioles. Though the mitotic mechanism is clearly, therefore, on its own not good evidence for a relationship with ascomycetes and Zygomycotina, it does in conjunction with the tubulin and hsp70 sequences render such a relationship highly probable.

Microsporidia commonly have a binucleate phase in the life cycle, similar to the dikaryophase of ascomycetes and basidiomycetes (Canning, 1990). Because in microsporidia the two nuclei are physically attached via the surfaces of their nuclear envelope this binucleate condition is called diplokaryotic (Canning, 1990). Some protozoologists have suggested that this diplokaryotic condition is a modification of the dikaryotic condition of higher Fungi, and that microsporidia may be derived from higher Fungi (Desportes & Nashed, 1983). However, in my view diplokaryosis is distinct from and convergent with the fungal dikaryophase (Cavalier-Smith, 1995*a*).

The absence of mitochondria and peroxisomes from microsporidia is no reason for excluding them from the Fungi. Both organelles are also absent in the rumen fungi of the order Neocallimastigales; however, these anaerobic fungi do have another respiratory organelle, the hydrogenosome, which may be evolutionarily derived from mitochondria or possibly peroxisomes (Cavalier-Smith, 1987*d*; Marvin-Sikkema *et al*., 1993). Because of the presence of Golgi stacks, centrioles and cilia in Neocallimastigales it is most likely that microsporidia lost mitochondria and peroxisomes independently of them, and had either a zygomycote or a hemiascomycete ancestor (see section VI). The extremely degenerate character of the microsporidian fungi, which caused them to be misclassified for over a century as protozoa, is one of the most striking examples known of evolutionary degeneration, the importance of which was first strongly argued by Dohrn (1875). Such degeneration greatly complicates the task of phylogenetic reconstruction, as

Lankester (1877, 1880) emphasized, since its attendant simplification and loss of ancestral characters is so easily confused with primitive simplicity. Time and again secondarily simplified organisms have been misclassified with genuinely primitive ones.

The clear demonstration that Trichozoa, Archamoebae and Microsporidia are all secondarily amitochondrial leaves only the phylum Metamonada, which appears to have diverged from all other eukaryotes earlier than any others (Cavalier-Smith & Chao, 1996*a*), as possibly primarily amitochondrial. Transferring Microsporidia to the kingdom Fungi, left only Metamonada in the Archezoa, which I now treat as a subkingdom of Protozoa (Cavalier-Smith, 1997*b*), as it was originally (Cavalier-Smith, 1983*a*), rather than as a separate kingdom (Cavalier-Smith, 1987*a*; 1993*a*). If Metamonada lack both mitochondria and peroxisomes primitively (Cavalier-Smith, 1983*a*), then they would be radically different from all higher eukaryotes (collectively designated Metakaryota: Cavalier-Smith, 1987*a*). But it seems increasingly unlikely that they are indeed genuinely primitively amitochondrial, contrary to widespread assumptions. The published evidence that diplomonads may have arisen by the loss of mitochondria (Keeling & Doolittle, 1987; Soltys & Gupta, 1994) is not yet very convincing, but firmer sequence evidence for a secondarily amitochondrial character of the group may soon be forthcoming from heat shock proteins (Roger and Sogin, personal communication) and aminoacyl tRNA synthetases (Hasegawa, personal communication).

If, as seems likely, Metamonada are indeed secondarily amitochondrial, then the distinction between them and the Parabasala becomes less fundamental than I thought when I removed Parabasala from the Archezoa on the grounds that their hydrogenosomes probably arose from mitochondria (which is now generally accepted). Moreover, Archezoa can no longer be distinguished from other eukaryotes on the grounds that they are primitively amitochondrial: we must therefore reconsider the definition and circumscription of the Archezoa and the other protozoan subkingdoms (Cavalier-Smith, 1997*a*). Metamonada and Trichozoa are anaerobic or microaerophilic flagellate protozoa that lack mitochondria and have kinetids that ancestrally have four centrioles, not two as in higher eukaryotes. They also appear to be the two earliest branching eukaryote phyla on several different protein sequence trees (e.g. RNA polymerase; heat shock protein; α-tubulin, β-tubulin). At present there is no evidence that they have any spliceosomal introns, unlike all higher eukaryote phyla.

Given these distinctive features and their apparently early divergence I here revise the subkingdom Archezoa to include both Metamonada and Trichozoa (as it did originally: but now Microsporidia and Archamoebae are excluded). Though it now appears that Archezoa in this revised sense are secondarily without mitochondria, there is no evidence that they ever had peroxisomes. It is possible therefore that they are primarily without peroxisomes, unlike all higher eukaryotes. If this is true and if, as De Duve (1984) and I (Cavalier-Smith, 1987*c*) have argued, peroxisomes are derived symbiogenetically from bacteria, then Archezoa may be less chimaeric in origin than all higher eukaryotes.

In postulating that the presently redefined Archezoa may be primitively without spliceosomal introns and peroxisomes, I do not rule out the possibility that they have lost them, or that some spliceosomal introns may one day be found. I am, as in my earlier discussions of the Archezoa, merely following Lankester's (1877) methodologically sound principle 'that *until we have special reason* to take a different view of any particular case we are bound to make the smallest amount of assumption by assigning to the various groups of organisms the places which they will fit into, on the supposition that they do represent in reality the original progressive series'.

(2) The protozoan subkingdoms: Archezoa and Neozoa

The recent grouping of the phyla Trichozoa, Euglenozoa and Percolozoa together as the subkingdom Eozoa (Cavalier-Smith, 1997*a*) was heavily influenced by the fact that on rRNA trees these three taxa consistently diverge earlier from all other mitochondrial eukaryotes (neokaryotes: Cavalier-Smith, 1993*b*) than the latter do from each other. However the recent demonstration that Microsporidia are Fungi not Archezoa, shows just how grossly misleading the rRNA tree can sometimes be. This highlights early arguments that rRNA gene evolution must be substantially non-clock like and subject to gross systematic biases in evolutionary rate (Cavalier-Smith, 1980). The recent demonstration that the histionid flagellate *Reclinomonas americana* has the most primitive known mitochondrial genome organisation (Lang *et al*., 1997), when coupled with the fact that rRNA trees place *Reclinomonas* above

both Percolozoa or Euglenozoa (Cavalier-Smith & Chao, unpublished) adds further fuel to my earlier suspicions that rRNA trees may place the Euglenozoa substantially too low (Cavalier-Smith, 1993*c*, 1995*a*). *Reclinomonas* is the only known eukaryote to have retained the α-proteobacterial RNA polymerase genes in its mitochondrial genome. It seems unlikely that their replacement by nuclearly encoded phage T3/T7 type RNA polymerases took place more than once in other eukaryotes. This makes it likely that Euglenozoa and Percolozoa arose from neozoan ancestors in which this had already occurred. If true, then the rRNA tree is grossly misleading as to their position and the Eozoa are polyphyletic rather than paraphyletic as earlier suggested (Cavalier-Smith, 1997*a*). In view of this, I here remove the Euglenozoa and Percolozoa from the Eozoa and group them together as a new infrakingdom, Discicristata, which I place in the subkingdom Neozoa.

Such a position is consistent with my earlier argument that the triple enveloped chloroplast envelope of dinoflagellates and euglenoids is a unique shared derived character that links the two groups, and which could have arisen through a primary endosymbiosis, not a secondary endosymbiosis as commonly assumed (Cavalier-Smith, 1982, 1995*a*). The presence of articulins in both alveolates (presently known only in ciliates) and euglenoids is consistent with a closer relationship between Alveolata and Discicristata than is suggested by the 18S rRNA tree. The fact that certain protein trees, notably tubulins and EF 1-α (Baldauf & Palmer, 1993) place Euglenozoa and Percolozoa as a single clade near the green plants and not below the neozoan radiation, is consistent with a primary origin for the euglenoid chloroplast (Cavalier-Smith, 1982) and favours the view that 18S rRNA trees place Discicristata too low, just as they do Microsporidia and Mycetozoa. Though the tetrakont character of some Percolozoa and the absence of Golgi dictyosomes were reasons for suggesting earlier that they might be the most primitive mitochondrial eukaryotes (Cavalier-Smith, 1993*c*), the recent mitochondrial data for *Reclinomonas* suggest that histionids may be more primitive. Detailed studies are needed of mitochondrial genomes and a variety of nuclear protein-coding genes in Percolozoa and a much greater variety of tubulicristate flagellates in order to test this and the present classification more thoroughly.

If Discicristata are really part of the neozoan radiation, as argued here, then the only really early diverging eukaryote phyla are the Metamonada and Trichozoa. To retain separate subkingdoms for these two taxa (Archezoa and Eozoa) seems less useful than to group them together in a single subkingdom, as suggested above. Archezoa thus remains as the basal paraphyletic protozoan and eukaryote subkingdom, as in the original 6-kingdom system (Cavalier-Smith, 1983*a*); but its phylogenetic definition is modified – Archezoa comprise the two phyla that diverged prior to the divergence of all the mitochondria-containing groups. Contrary to what was previously suggested (Cavalier-Smith, 1983*a*), the latest common ancestor of the Archezoa had probably already started to incorporate at least some of the genes from the symbiont that was eventually converted into the mitochondrion, and therefore was probably not a non-chimaeric eukaryote, as postulated earlier (Cavalier-Smith, 1983*b*). Whether this archezoan cenancestor had a fully developed mitochondrion or only a precursor of mitochondria is a semantic rather than a factual issue. In my view however it is proper to call the common ancestor of the trichomonad hydrogenosome and the mitochondrion a mitochondrion since it must have been at least facultatively aerobic with cytochromes and must already have evolved an organelle-specific protein import mechanism of the type shared between the two organelles (Bradley *et al.*, 1997), the best demarcation criterion between an obligate symbiont and an organelle. Therefore trichomonad hydrogenosomes did evolve from mitochondria, as I postulated (Cavalier-Smith, 1987*d*). The suggestion that the accepted common ancestor of these hydrogenosomes and mitochondrion might not be a mitochondrion but merely a precursor of a mitochondrion (Bui, Bradley & Johnson, 1996) is a distinction without substance since no properties are suggested by which the 'non-mitochondrial' precursor might be distinguished from a mitochondrion. Even though one cannot (at least at present) deduce whether or not the mitochondrial protein-import mechanism had evolved prior to the divergence of Metamonada and Trichozoa, it is probably best not to continue to think of Archezoa as being primitively amitochondrial. However, the reasons for postulating that the α-proteobacterium was taken up by an early amitochondrial and non-chimaeric eukaryote after the origin of the cytoskeleton, endomembrane system and phagocytosis (Cavalier-Smith, 1983*b*, 1987*d*) remain compelling, even if no primitively amitochondrial descendants of this early non-chimaeric phase of eukaryote evolution remain.

If Discicristata really occupy the position shown in

Fig. 1, then it is necessary to redefine the term neokaryotes, originally defined as the clade including all eukaryotes that branch higher on rRNA trees than Euglenozoa (Cavalier-Smith, 1993*b*), because this definition no longer defines a clade. I suggest that neokaryote should now signify the clade comprising Neozoa plus the four higher kingdoms of life. Eukaryotes may therefor be divided simply into tetrakont Archezoa, putatively without spliceosomal introns, and the basically bikont neokaryotes, typically with spliceosomal introns (with the exception of the euglenozoan Kinetoplastea, which probably lost them. The present redefinition of both Archezoa and neokaryote make the terms eokaryote (Cavalier-Smith 1993*b*) and megakaryote (Cavalier-Smith, 1995*a*) no longer necessary. Metakaryota (Cavalier-Smith, 1987*a*) are not a taxon in the present system; but following the recognition that microsporidia and Archamoebae are secondarily amitochondrial and branch higher up molecular sequence trees than do Parabasala, the name metakaryotes (Cavalier-Smith, 1987*a*) must now include both microsporidia and archamoebae and, therefore, now designates the clade comprising all eukaryotes other than Metamonada.

(3) Demarcation between the two zoological kingdoms: Protozoa and Animalia

Now that the phylogenetic position of Myxozoa has been established by rRNA phylogeny (Smothers *et al*., 1994; Schlegel *et al*. 1996), it is clear that their vegetative unicellularity is secondarily derived as a result of parasitism. It seems to be only a remote possibility that their position on rRNA trees well within the metazoan animals is a methodological artefact of long-branch attraction. While other molecular data are clearly needed, the present data are convincing enough to make it highly probable that Myxosporidia arose from a multicellular ancestor that possessed not only cell junctions, as do their multicellular spores, but also collagenous connective tissue, a nervous system and a gut. Therefore Myxozoa must be excluded from the kingdom Protozoa and placed within the kingdom Animalia, as stated earlier (Cavalier-Smith, 1995*b*, *c*). Since it is unclear whether they are derived from Cnidaria, as is suggested by their nematocyst-like extrusomes (Weill, 1935; Lom & De Puytorac, 1965; Siddall *et al*., 1995) or are closer to bilateral animals, as is weakly suggested by the rRNA trees, at present Myxozoa are most appropriately excluded from both subkingdoms Radiata and Bilateria and ranked as a third subkingdom of the kingdom Animalia (Cavalier-Smith *et al*., 1996*a*). The great difference in phenotype between Myxozoa and both Radiata and Bilateria also justifies this high rank; to place them in either subkingdom would make it phenotypically too heterogeneous. Long ago Lankester (1877) noted that it was very hard to disprove the idea 'that many of the Protozoa are not descended from Enterozoa by degeneration': it appears that only the Myxozoa have actually done so.

Recent molecular phylogenetic evidence that dicyemid Mesozoa (Katayama *et al*., 1995) and orthonectid Mesozoa (Hanelt *et al*., 1996) are related to bilaterians means that they also have lost a nervous system, gut and collagenous connective tissue, another remarkable example of degeneration raised as a possibility by Lankester. Though the molecular data suggest that the two mesozoan classes may not be directly related to each other (Hanelt *et al*., 1996) the trees lack strong resolution, so the closest relatives of both groups are uncertain and it remains possible that Mesozoa are monophyletic; therefore I retain the phylum Mesozoa until such time as its monophyly is more convincingly disproved, but in view of the radical differences from Bilateria continue to place it in a distinct subkingdom Mesozoa (Cavalier-Smith, 1983*a*, Cavalier-Smith *et al*., 1996*a*). Whether Mesozoa are monophyletic or not, it is now clear that dicyemids, orthonectids and Myxozoa all arose by the loss of the nervous system and of obvious collagenous tissue; thus it is no longer justifiable to use the absence of collagenous connective tissue as a reason for excluding Mesozoa from Animalia (Cavalier-Smith, 1983*a*, 1993*a*). The complex life cycles of the Mesozoa and their copulatory sex have long been reasons for considering that they may have evolved from a flatworm-like ancestor by the loss of the nervous system (Stunkard, 1954; 1972); unfortunately the base of the bilaterian rRNA tree is a bush-like radiation in which no branching orders are clearly resolved, which is consistent with the explosiveness of the early Cambrian radiation seen in the fossil record. The fact that their mitochondrial cristae are tubular not flat, evidence previously cited in support of a protozoan character (Cavalier-Smith, 1983*a*), is not sufficient reason for excluding them from Animalia, since the cristae of flatworms can also be tubular rather than the flat cristae of most animals other than Ctenophora.

The generalization that animals have flat cristae (Taylor, 1978) is clearly an oversimplification. It has

Table 3. *Classification of the kingdom Protozoa† and its 13 phyla*

Subkingdom 1. Archezoa† Cavalier-Smith 1983 em.

Phylum 1. Metamonada Grassé 1952 stat. nov. et em. Cavalier-Smith 1981.

Subphylum 1. Eopharyngia Cavalier-Smith 1993 (e.g. *Giardia*, *Hexamita*, *Trepomonas*, *Chilomastix*).

Subphylum 2. Axostylaria Grassé 1952 stat. nov. em. Cavalier-Smith 1993 (e.g. *Oxymonas*, *Pyrsonympha*).

Phylum 2. Trichozoa Cavalier-Smith 1997.

Subphylum 1. Anaeromonada Cavalier-Smith 1997 (*Trimastix*).

Subphylum 2. Parabasala Honigberg 1973 stat. nov. Cavalier-Smith 1997 (e.g. *Trichomonas*, *Trichonympha*).

Subkingdom 2. Neozoa† Cavalier-Smith 1993 stat. nov. 1997 em.

Infrakingdom 1. Sarcomastigota* Cavalier-Smith 1983 stat. nov. em. [diagnosis: typically sarcodines, flagellates, or amoeboflagellates; usually free-living heterotrophs, rarely chimaeric photophagotrophs with nucleomorphs and periplastid membranes; pseudopods varied, but seldom eruptive; typically aerobes with tubular, less often flat or rarely discoid, mitochondrial cristae and prominent Golgi dictyosomes; cortical alveoli absent; axopodia usually absent (if present with quincunx arrangement); amitochondrial species lack hydrogenosomes and well-developed dictyosomes].

Phylum 1. Neomonada* Cavalier-Smith 1997.

Subphylum 1. Apusozoa Cavalier-Smith 1997 (e.g. *Apusomonas*, *Ancyromonas*, *Jakoba*, *Reclinomonas Ebria*).

Subphylum 2. Isomita Cavalier-Smith 1997 (e.g. *Phalansterium*, *Cyathobodo*, *Kathablepharis*).

Subphylum 3. Choanozoa* Cavalier-Smith 1981 em. 1983 stat. nov. (e.g. *Monosiga*, *Diaphanoeca*, *Corallochytrium*, *Psorospermium*).

Phylum 2. Cercozoa phyl. nov. (syn. Rhizopoda Von Siebold 1845 stat. nov. Haeckel 1886 as emended by Cavalier-Smith 1995*b*, 1997*a*; present phylum emended by addition of Spongomonadida and transfer of Cristidiscoidia to Choanozoa: Cavalier-Smith, 1997*b*.) (diagnosis: unicellular phagotrophic heterotrophs or else photosynthetic algae with green chloroplasts and nucleomorphs within a periplastid membrane located inside a fourth smooth membrane; typically free-living aerobes having peroxisomes and mitochondria with tubular (or very rarely flat or vesicular) cristae; flagellates with two usually anisokont cilia or a single cilium or non-flagellates (usually rhizopods) with a test and/or filose or reticulose pseudopodia or with a green plastid and nucleomorph; cilia without lateral flanges, paraxial rods, transition helix or tubular hairs; cortical alveoli and axopodia absent; heterotrophs have a flexible cell surface without a rigid dense protein layer inside or outside the plasma membrane; distinct cytopharynx absent; silica scales sometimes present but internal silica skeleton absent; extrusomes, if present, isodiammetric or a complex Stachel; often with walled cysts).

Subphylum 1. Phytomyxa Cavalier-Smith 1997 (e.g. *Plasmodiophora*).

Subphylum 2. Reticulofilosa Cavalier-Smith 1997 (e.g. *Chlorarachnion*, *Cryptochlora*).

Subphylum 3. Monadofilosa Cavalier-Smith 1997 (e.g. *Cercomonas*, *Gymnophrys*, *Euglypha*, *Spongomonas*).

Phylum 3. Foraminifera (D'Orbigny 1826) Eichwald 1830 stat. nov. Margulis 1974 (e.g. *Allogromia*, *Ammonia*).

Phylum 4. Amoebozoa Lühe 1913 stat. nov. em. [emended diagnosis: solitary or aggregative amoebae with usually non-eruptive lobose pseudopods, not uncommonly with finer pointed subpseudopodia; mitochondria with tubular cristae, or sometimes absent; Golgi dictyosomes well-developed except in Archamoebae; sometimes with transient or permanent cilia; usually one cilium (rarely two) per kinetid and one kinetid (rarely many) per cell].

Subphylum 1. Lobosa Carpenter 1861 stat. nov. Cavalier-Smith 1997 em. (e.g. *Amoeba*, *Acanthamoeba*, *Arcella*, *Difflugia*, *Multicilia*).

Subphylum 2. Conosa subphyl. nov. (diagnosis: kinetid, when present, with a cone of microtubules which typically subtends the nucleus at its broader end, and with a lateral microtubular ribbon closer to the cell surface; aggregative aerobes with mitochondria or solitary anaerobes lacking mitochondria and peroxisomes).

Infraphylum 1. Archamoebae Cavalier-Smith 1983 stat. nov. (e.g. *Pelomyxa*, *Mastigamoeba*, *Phreatamoeba*, *Entamoeba*).

Infraphylum 2. Mycetozoa De Bary 1859 stat. nov.

Superclass 1. Eumyxa* Cavalier-Smith 1993 stat. nov. (e.g. *Protostelium*, *Physarum*).

Superclass 2. Dictyostelia Lister 1909 stat. nov. (e.g. *Dictyostelium*).

Infrakingdom 2. Discicristata infraking. nov. (diagnosis: mitochondrial cristae typically discoid; pseudopods if present are eruptive lobes; kinetid typically with two centrioles, rarely four or absent).

Phylum 1. Percolozoa Cavalier-Smith 1991.

Subphylum 1. Tetramitia Cavalier-Smith 1993 (e.g. *Percolomonas*, *Naegleria*).

Subphylum 2. Pseudociliata Cavalier-Smith 1993 (*Stephanopogon*).

Phylum 2. Euglenozoa Cavalier-Smith 1981.

Subphylum 1. Plicostoma subphyl. nov. (diagnosis: with pellicular strips and/or a feeding apparatus comprising two or three supporting rods and four or five curved or plicate vanes. (This formalizes the name plicostome, first used by Patterson (1988) for this taxon, even though it is not ideal as the vanes are often not plicate and are absent from petalomonads) [superclasses Diplonemia Cavalier-Smith 1993 stat. nov. (pellicular strips absent, e.g. *Diplonema*) and Euglenoida Bütschli 1884 stat. nov. Cavalier-Smith 1997 (pellicular strips present, e.g. *Euglena*, *Petalomonas*, *Peranema*)].

Subphylum 2. Saccostoma subphyl. nov. (diagnosis: subapical cytostome and cytophyarynx reinforced on one side by microtubules (the so-called microtubular root (MTR)/pocket feeding appararatus: hence the name from latin saccus, bag and stoma mouth), lacking dense reinforcing rods or vanes; without pellicular strips). Classes Kinetoplastea Honigberg 1963 stat. nov. Margulis 1974 (e.g. *Bodo*, *Trypanosoma*, *Leishmania*) and Postgaardea cl. nov. (diagnosis: mitochondria without kinetoplast or cristae: sole genus *Postgaardi*: Simpson *et al.*, 1997).

Table 3. (*cont.*)

Infrakingdom 3. Alveolata Cavalier-Smith 1991 em.
Superphylum 1. Miozoa Cavalier-Smith 1987.
Phylum 1. Dinozoa Cavalier-Smith 1981 em.
Subphylum 1. Protalveolata* Cavalier-Smith 1991 em. (e.g. *Colponema*, *Ellobiopsis*, *Spironema*, *Hemimastix*, *Colpodella*, *Perkinsus*).
Subphylum 2. Dinoflagellata Bütschli 1885 stat. nov. Cavalier-Smith 1991 (e.g. *Noctiluca*, *Crypthecodinium*, *Amphidinium*).
Phylum 2. Sporozoa Leuckart 1879 stat. nov. Cavalier-Smith 1981 (syns Telosporidia Schaudinn 1900; Euspora Levine 1961; Polannulifera Levine 1969; Apicomplexa Levine 1970 pro parte).
Subphylum 1. Gregarinae Haeckel 1866 stat. nov. (e.g. *Monocystis*).
Subphylum 2. Coccidiomorpha Doflein 1901 [Superclasses Coccidia Leuckart 1879 stat. nov. Cavalier-Smith 1993 (e.g. *Sarcocystis*, *Toxoplasma*, *Eimeria*), Ascetospora Sprague 1979 stat. nov. (e.g. *Haplosporidium*, *Paramyxa*) and Hematozoa Vivier 1982 stat. nov. (e.g. *Plasmodium*, *Babesia*)].
Subphylum 3. Manubrispora subphyl. nov. (Diagnosis: plasmodial intracellular parasites of gregarines with inner membrane complex in vegetative cells, but no apical complex, mitochondria, peroxisomes, centrioles or cilia; uninucleate spores usually spherical, with separate membrane-bounded polar body and manubrium/lamellar complex, a thin dense wall; spores formed within walled cyst by plasmotomy; Golgi complex an aggregate of vesicles, associated with centrosomal plaque during closed mitosis; spindle intranuclear: sole class Metchnikovellea Weiser 1977 emend. Cavalier-Smith 1993 to exclude Minisporea, e.g. *Metchnikovella*).
Superphylum 2. Heterokaryota Hickson 1903 stat. nov. Cavalier-Smith 1993.
Phylum Ciliophora Doflein 1901 stat. nov. Copeland 1956 em. auct.
Subphylum 1. Tubulicorticata de Puytorac *et al*. 1992 (e.g. *Loxodes*, *Stylonychia*, *Colpoda*).
Subphylum 2. Epiplasmata de Puytorac *et al*. 1992 (e.g. *Tetrahymena*, *Paramecium*, *Vorticella*).
Subphylum 3. Filocorticata de Puytorac *et al*. 1992 (e.g. *Spathidium*).
Infrakingdom 4. Actinopoda Calkins 1902 stat. nov. Cavalier-Smith 1997.
Phylum 1. Heliozoa Haeckel 1886 stat. nov. Margulis 1974 (e.g. *Actinophrys*, *Acanthocystis*).
Phylum 2. Radiozoa Cavalier-Smith 1987.
Subphylum 1. Spasmaria Cavalier-Smith 1993 (*Sticholonch*e, acantharians, e.g. *Acanthometra*).
Subphylum 2. Radiolaria Müller 1858 emend. stat. nov. Cavalier-Smith 1993 (e.g. *Thallassicolla*, *Aulacantha*).

* Probably paraphyletic.
† Almost certainly paraphyletic.
For a more detailed classification of protozoa to the level of subclass and discussion of its phylogenetic basis see Cavalier-Smith (1993*a*, 1995*b*, 1997*a*, *b*) and Corliss (1994).
The name Archezoa (Cavalier-Smith 1983*a*) is derived from the Greek *arche* meaning 'beginning' or 'first' (as is archetype), not from *archaios* (meaning ancient as in archaeology); the spelling Archaezoa sometimes seen (e.g. Maynard Smith & Szathmary, 1995) is incorrect. When I created the taxon I was perfectly aware that Perty (1852) had used Archezoa in a broader sense; but as nobody since Haeckel (1868 and subsequent editions) had used it at all, I considered that no confusion would arise. In any case, it is often desirable to refine old names rather than to invent totally new ones; any temporary confusion soon fades – nobody now complains that the name Mollusca scarcely overlaps at all with Linnaeus's melange grouped under that name, or that Insecta, Crustacea and Chordata no longer have the same circumscription as they once did, or that Linnaeus included bats among the Primates, and Reptilia and many fish in his Amphibia, whilst his Reptilia included frogs and tortoises but excluded snakes. If we followed those pedantic nomenclaturists who think that all changes in circumscription of higher taxa necessarily require them to be renamed, we would have to change all these and many other established names thus causing undue confusion. As in the present system, Haeckel defined Archezoa as the most primitive Protozoa: but he thought that amoebae were the most primitive organisms and thus, unlike Cavalier-Smith (1983*a*), excluded flagellates from Archezoa. It is now clear that all amoebae are derived from flagellates, contrary to Haeckel's view, so flagellates are more primitive than amoebae, and all non flagellates except *Dientamoeba* are excluded from the present Archezoa.

long been known that those of Ctenophora are distinctly tubular as are those of vertebrate adrenal glands. Clearly, tubular cristae have evolved from the ancestral animal condition of flat cristae several times within the kingdom Animalia. The cristae of myxosporidia are sometimes referred to as flat (Corliss, 1984) and sometimes as tubular (Taylor, 1978), but they are usually not clearly either. They are so different from the flat cristae of choanoflagellates or the tubular cristae of most other neozoan protozoa that it would be more accurate simply to call them irregular. The origin of the irregular cristae of myxozoa from the more typical flat cristae of most lower invertebrates might have been contemporaneous with the secondary origin of their amoeboid plasmodial vegetative state; according to the molecular coevolutionary theory of the origin of mitochondrial cristae, a marked change

in cristal morphology is often a pleiotropic neutral consequence of adaptive changes in the cell surface (Cavalier-Smith, 1997*a*).

The inclusion of Archezoa and the exclusion of Myxozoa and Mesozoa from the kingdom Protozoa means that the kingdom now includes all primitively unicellular phagotrophic non-photosynthetic eukaryotes that have not evolved by the secondary loss of chloroplasts, and is predominantly made up of such organisms. It is thus a relatively homogeneous grade of organisation that will continue to be a very useful major unit of classification. The fact that it is obviously paraphyletic, in contrast to the holophyletic kingdom Animalia, does not lessen its utility.

The division of the phagotrophic zoological world into two distinct kingdoms, the unicellular Protozoa and the ancestrally triploblastic Animalia, appropriately recognizes the fundamental differences in body plan between the two zoological kingdoms. The higher level classification of each is summarized in Tables 3 and 4. Whether one adopts some variant of the five-kingdom system (Whittaker, 1969) or the six-kingdom system (Cavalier-Smith, 1981*a*, 1983*a*), the exclusion of Protozoa from the kingdom Animalia means that Haeckel's term Metazoa is now an unnecessary synonym of Animalia. It is desirable to harmonize the circumscription of the vernacular term animal with that of the formal term Animalia and not to include protozoans within its ambit. Even in the most elementary teaching, it is best not to refer to protozoa as unicellular animals but to encourage the view of protozoa as a different and more primitive form of life than animals, plants or fungi. Calling protozoa unicellular animals not only downplays the great significance of the differences in body plan between the two kingdoms, but helps to condemn the study of protozoa to a position of secondary importance within zoology, which their status as a separate kingdom belies. Zoology is the study of animals and protozoa, each of which have important but distinct roles in the biosphere. The phrase 'unicellular animals' is also particularly inappropriate for the three groups of protozoan algae: Euglenia, Chlorarachnea and photosynthetic dinoflagellates. For the reasons discussed previously (Cavalier-Smith, 1993*a*, 1995*a*), these three photosynthetic groups are too closely related to non-photosynthetic protozoa to be excluded from the kingdom. Since the term algae has long since ceased to refer to a botanical taxon, there is no contradiction in referring to these three groups as protozoan algae or in suggesting that, like all other Protozoa, they should be subject to the Zoological, not to the Botanical Code of Nomenclature (Cavalier-Smith, 1981*a*).

(4) Classification of the Neozoa

(*a*) *The infrakingdom Sarcomastigota*

Neozoan classification is still in a state of flux. Ribosomal RNA sequence studies on a wide variety of zooflagellates currently in progress in our laboratory indicate that the distinction between the infrakingdoms Sarcodina and Neomonada is less clear than it seemed earlier (Cavalier-Smith, 1997*a*). It therefore seems sensible to merge them into a single infrakingdom, for which I adopt the name Sarcomastigota, used earlier (Cavalier-Smith, 1983*a*) for a similar assemblage of flagellates and sarcodines. Unlike my earlier subkingdom Sarcomastigota (Cavalier-Smith, 1983*a*), which was not generally adopted, the present infrakingdom Sarcomastigota includes Choanozoa since the distinction between tubular and flat cristae is less fundamental than was earlier thought (for the reasons, see Cavalier-Smith, 1997*a*); it also excludes the Alveolata and Actinopoda, which are treated as separate infrakingdoms. Here I follow Siddall, Stokes & Burresson (1995) in placing Haplosporidia in the Alveolata; even though this assignment is most uncertain (Cavalier-Smith, 1997*a*), excluding them from the infrakingdom Sarcomastigota makes it phenotypically more uniform.

In the present system Mycetozoa are no longer treated as a separate phylum; instead they are placed within the phylum Amoebozoa, and grouped with the Archamoebae as a new subphylum Conosa, characterized in the flagellate members of the group by a cone of microtubules emanating from the often single centriole and subtending the nucleus. It appears that rRNA trees usually place the Conosa much too low; protein trees place them much higher up near the opisthokonta (animals, fungi, Choanozoa) and in the case of actin group them together. Only two subphyla are recognised in the Amoebozoa: Conosa and Lobosa. The Lobosa comprise the three classes Amoebaea, Testacealobosea and Holomastigea (with sole genus *Multicilia*, which is now placed in the Lobosa as it has cell surface glycostyles similar to those of some Amoebaea: Mikryukov & Mylnikov, 1996*a*). Following these simplifications there are now only four sarcomastigote phyla: two essentially sarcodine (Foraminifera, Amoebozoa) and two predominantly flagellate but with a significant sprinkling of rhizopods or other non-flagellates (Neomonada and Cercozoa).

Table 4. *Classification of the kingdom Animalia and its 23 phyla*

Subkingdom 1. Radiata† Linnaeus 1758 em. stat. nov. Cavalier-Smith 1983 (multicllellular; radial or biradial symmetry; no anus).

Infrakingdom 1. Spongiaria* De Blainville 1816.

Phylum Porifera Grant 1836 (sponges).

Subphylum 1. Hyalospongiae Vosmaer 1886 stat. nov. em. [typically with silicious spicules; sometimes also calcified (i.e. pharetronids; sphinctozoans are probably secondarily without spicules) but without calcareous spicules; mainly demosponges, e.g. *Axinella*, and hexactinellids].

Subphylum 2. Calcispongiae Blainville 1834 stat. nov. (syn. Calcarea Bowerbank 1864 e.g. *Clathrina*, *Sycon*).

Subphylum 3. Archaeocyatha Vologdin 1937 (extinct).

Infrakingdom 2. Coelenterata* Leuckart 1847 em. auct.

Phylum 1. Cnidaria* (corals, sea anemones; jellyfish, hydroids).

Subphylum 1. Anthozoa* Ehrenberg 1831 stat. nov. (e.g. *Sarcophyton*, *Actinia*).

Subphylum 2. Medusozoa Petersen 1979 (e.g. *Hydra*, *Physalia*, *Tripedália*, *Aurelia*).

Phylum 2. Ctenophora Eschscholtz 1829 (comb jellies, e.g. *Beroe*, *Pleurobrachia*).

Infrakingdom 3. Placozoa infrak. nov. (without body cavity, gut or nervous system).

Phylum Placozoa Grell 1971 (*Trichoplax*).

Subkingdom 2. Myxozoa Grassé 1970 stat. nov. Cavalier-Smith 1996 (unicellular non-ciliate parasites with multicellular spores).

Phylum Myxosporidia Bütschli 1881 stat. nov. Grassé 1970 (e.g. *Myxidium*).

Subkingdom 3. Bilateria* Hatschek 1888 stat. nov. Cavalier-Smith 1983 (bilateral animals, primitively with an anus and probably coelom).

Branch 1. Protostomia* Grobben 1908.

Infrakingdom 1. Lophozoa* infrak. nov. (diagnosis: primitively sessile with U-shaped gut and ciliated oral tentacles with coelomic extensions; early ciliated larvae trochophores, later often bivalved).

Superphylum 1. Polyzoa Thompson 1830 stat. nov. em. (diagnosis: no vascular system or longitudinal nerve cords; adult without shell; zooids able to multiply by vegetative budding, often colonially; larval brain disappears at metamorphosis).

Phylum 1. Bryozoa* Ehrenberg 1831 (unnecessary junior synonym: Ectoprocta Nitsche 1870).

Subphylum 1. Gymnolaemata Allman 1856 stat. nov. (e.g. *Bugula*, *Flustra*, *Membranipora*).

Subphylum 2. Lophopoda Dumortier 1835 (syn. Phylactolaemata Allman 1856, e.g. *Plumatella*).

Phylum 2. Kamptozoa Cori 1929.

Subphylum 1. Entoprocta* Nitsche 1870 (e.g. *Loxosoma*, *Pedicellina*, *Urnatella*).

Subphylum 2. Cycliophora Funch & Kristensen 1995 stat. nov. (*Symbion*).

Superphylum 2. Conchozoa superphyl. nov. (diagnosis: vascular system; ancestrally with calcareous shell, primitively bivalved and unhinged).

Phylum 1. Mollusca Linnaeus 1758 em. Lamarck.

Subphylum 1. Bivalvia* Linnaeus 1758 stat. nov. em. auct. (bivalves, e.g. *Mytilus*, *Pecten*: unneccessary junior synonyms Acephala Cuvier; Lipocephala Lankester 1889).

Subphylum 2. Glossophora Lankester 1889 stat. nov. (with radula and head).

Infraphylum 1. Univalvia Linnaeus 1758 stat. nov. em. (diagnosis: non-chambered shell in one piece; tentacles; crystalline style: monoplacophorans, gastropods, scaphopods).

Infraphylum 2. Spiculata infraphyl. nov. (diagnosis: calcareous spicules and/or multiple shell plates; without eyes, tentacles or crystalline style: aplacophorans, caudofoveates, polyplacophorans).

Infraphylum 3. Cephalopoda Cuvier 1797 (single multichambered shell, e.g. octopus, squid, nautilus, cuttlefish).

Phylum 2. Brachiozoa phyl. nov. (diagnosis: with lophophore and vascular system).

Subphylum 1. Brachiopoda* Duméril 1806 (e.g. *Lingula*, *Terebratula*).

Subphylum 2. Phoronida Hatschek 1888 (*Phoronis*, *Phoronopsis*).

Superphylum 3. Sipuncula superphyl. nov. (U-shaped gut, ventral non-ganglionated nerve cord; no vascular system or shell; pelagosphaera larva).

Phylum Sipuncula Raffinesque 1814 stat. nov. Sedgwick 1898 (e.g. *Golfingia*, *Phascolosoma*).

Superphylum 4. Vermizoa superphyl. nov. (diagnosis: coelomate worms with blood and straight gut with anus; ciliated larvae without bivalved shells; two ventrolateral or one primitively paired ventral nerve cord).

[*continued overleaf*

Table 4. (*cont.*)

Phylum 1. Annelida Lamarck 1809 (unnecessary junior synonym Chaetopoda: ancestrally segmented with coelom around gut; chaetae; ganglionated ventral nerve cord).
Subphylum 1. Polychaeta* Grube 1850.
Infraphylum 1. Operculata infraphyl. nov. (diagnosis: live in calcareous tubes with operculum; lacking muscular pharynx, e.g. *Spirorbis*, *Serpula*).
Infraphylum 2. Pharyngata infraphyl. nov. (diagnosis: with muscular pharynx; if tube-dwellers without calcareous operculum: bristleworms, e.g. *Nereis*, *Arenicola*, *Sabella*) .
Subphylum 2. Clitellata auct. (earthworms, leeches).
Subphylum 3. Echiura Sedgwick 1898 orth. em. Stephen 1965 stat. nov. (e.g. *Urechis*, *Bonellia*).
Subphylum 4. Pogonophora Johannson 1937 stat. nov. (Frenulata and Vestimentifera, e.g. *Riftia*).
Phylum 2. Nemertina Oersted 1844 em. Schultze 1850 (unnecessary syn. Rhynchocoela Schulze 1850) (unsegmented coelom around only eversible proboscis: proboscis worms, e.g. *Nemertes*).
Infrakingdom 2. Chaetognathi Leuckart 1854 stat. nov.
Phylum Chaetognatha Hyman 1959 (arrowworms, e.g. *Sagitta*, *Spadella*).
Infrakingdom 3. Ecdysozoa infrak. nov. (diagnosis: with thick cuticle that is moulted; no surface cilia or ciliated larvae; gut, if present, with anus; coelom present only embryonically or absent; ventral nerve cord; usually gonochoristic). Name suggested for this clade minus Loricifera by Aguinaldo *et al*. 1997.
Superphylum 1. Haemopoda superp. nov. (diagnosis: body segmented, with limbs on several segments; adult body cavity a haemocoel that extends into the limbs).
Phylum 1. Arthropoda von Siebold and Stannius 1848 (hard cuticle; jointed limbs moved by muscles).
Subphylum 1. Cheliceromorpha Boudraux 1978.
Infraphylum 1. Pycnogonida Latreille 1810 (sea 'spiders', e.g. *Nymphon*).
Infraphylum 2. Chelicerata Heymons 1901 (arachnids, eurypterids, king crabs).
Subphylum 2. Trilobitomorpha Størmer 1944 stat. nov. (trilobites; trilobitoids).
Subphylum 3. Mandibulata Snodgrass 1938.
Infraphylum 1. Crustacea* Pennant 1777 (e.g. barnacles, crabs, shrimps, copepods, ostracods).
Infraphylum 2. Myriapoda Leach 1814 (centipedes, millipedes, symphylans, pauropods).
Infraphylum 3. Insecta Linnaeus 1758 em. auct. (Unnecessary junior synonym Hexapoda: e.g. springtails, silverfish, locusts, bees, termites, beetles, moths, *Drosophila*).
Phylum 2. Lobopoda phyl. nov. (soft cuticle; unjointed limbs with terminal claws; both muscles and hydraulic pressure involved in locomotion).
Subphylum 1. Onychophora Grube 1853 (e.g. *Peripatus*).
Subphylum 2. Tardigrada Doyère 1840 stat. nov. (water bears, e.g. *Echiniscus*).
Superphylum 2. Nemathelminthes superphyl. nov. (diagnosis: unsegmented worms with spiny mouthparts; vascular system and limbs absent; coelom bounded by epithelium absent).
Phylum Nemathelminthes Gegenbaur 1859 em. (diagnosis as for the superphylum).
Subphylum 1. Scalidorhyncha subphyl. nov. (diagnosis: retractile head covered with circlets of spined scalids).
Infraphylum 1. Priapozoa infraphyl. nov. (diagnosis: unsegmented; larva or adult with cuticular lorica of longitudinal plates into which it can retract: classes Priapula, Loricifera).
Infraphylum 2. Kinorhyncha Reinhard 1887 (superficially segmented; without lorica).
Subphylum 2. Nematoida Rudolphi 1808 (as Nematoidea) orth. em. stat. nov.
Infraphylum 1. Nematoda Gegenbaur 1859 orthog. em. stat. nov. (e.g. *Caenorhabditis*, *Ascaris*).
Infraphylum 2. Nematomorpha Vejovsky 1886 stat. nov. (e.g. *Gordius*, *Nectonema*).
Infrakingdom 4. Platyzoa infraking. nov. [diagnosis: ciliated non-segmented acoelomates or pseudocoelomates lacking vascular system; gut (when present) straight, with or without anus]; possibly neotenously derived from loxosomatid-like entoproct larvae.
Phylum 1. Acanthognatha phyl. nov. (diagnosis: chitinous cephalic bristles or jaws; gut if present with anus or anal pore).
Subphylum 1. Trochata subphyl. nov. (diagnosis: syncytial epidermis with radial tubules penetrating the cuticle; often with paired lemnisci in neck region; epidermis multiciliated and/or non-ciliated; proboscis often functions as introvert; protonephridia flame bulbs; pseudocoelomate; gonochoristic).
Infraphylum 1. Rotifera* Cuvier 1798 stat. nov. (e.g. *Collotheca*, *Asplancha*).
Infraphylum 2. Acanthocephala Rudolphi 1809 stat. nov. (e.g. *Moniliformis*).
Subphylum 2. Monokonta subphyl. nov. (without lemnisci or eversible proboscis; monociliated epidermal cells; protonephridia solenocytes; acoelomate; hermaphrodite: classes Gastrotricha, Gnathostomulida).

Table 4. (*cont.*)

Phylum 2. Platyhelminthes Gegenbaur 1859 em. Minot 1876.
Subphylum 1. Turbellaria† Ehrenberg 1831 em. Ehlers 1864 (usually multiciliated, non-syncytial epidermis retained in adult).
Infraphylum 1. Mucorhabda* infraphyl. nov. (mucoid non-lamellate rhabdoids; protonephridia absent or with two cilia, e.g. catenulids, acoels).
Infraphylum 2. Rhabditophora Ehlers 1985 (unranked) stat. nov. (lamellate rhabdites; protonephridia with many cilia: macrostomids, polyclads, Neoophora).
Subphylum 2. Neodermata Ehlers 1985 (unranked) stat. nov. (larval epidermis shed; replaced by syncytial neodermis).
Infraphylum 1. Trematoda Rudolphi 1808 stat. nov.
Infraphylum 2. Cercomeromorpha Bychowsky 1937 stat. nov. (monogeneans, tapeworms).

Branch 2. Deuterostomia Grobben 1908.

Infrakingdom 1. Coelomopora Marcus 1958 (as superphylum) stat. nov.

Phylum 1. Hemichordata Bateson 1885 stat. nov. orthog. em. auct.
Subphylum 1. Pterobranchia Lankester 1878 stat. nov. (e.g. *Cephalodiscus*; graptolites).
Subphylum 2. Enteropneusta Gegenbaur 1870 stat. nov. (e.g. *Balanoglossus*).
Phylum 2. Echinodermata De Brugière 1789.
Subphylum 1. Homalozoa* Whitehouse 1941.
Subphylum 2. Pelmatozoa Leuckart 1848.
Infraphylum 1. Blastozoa Sprinkle 1973 (e.g. blastoids, cystoids).
Infraphylum 2. Crinozoa Matsumoto 1929 (sea lilies, feather stars).
Subphylum 3. Eleutherozoa Bell 1891.
Infraphylum 1. Asterozoa Von Zittel 1895 (starfish, brittle stars, concentricycloids).
Infraphylum 2. Echinozoa Haeckel in Von Zittell 1895 (sea urchins; sea cucumbers).

Infrakingdom 2. Chordonia Haeckel 1874 em. Hatschek 1888 stat. nov.

Phylum 1. Urochorda Lankester 1877 (urochordates).
Subphylum 1. Tunicata Lamarck 1816 (tunicates).
Infraphylum 1. Ascidiae Blainville 1824 (ascidians, sorberaceans).
Infraphylum 2. Thaliae auct. (salps).
Subphylum 2. Appendicularia Lahille 1890 stat. nov. (larvaceans).
Phylum 2. Chordata Bateson 1885 em.
Subphylum 1. Acraniata* Bleeker 1859.
Infraphylum 1. Cephalochordata Owen 1846 (amphioxus).
Infraphylum 2. Conodonta Eichenberg 1930 stat. nov. (extinct).
Subphylum 2. Vertebrata Cuvier 1812 (unnecessary synonyms: Craniota Haeckel, Craniata auct.).
Infraphylum 1. Agnatha† auct. (lampreys, hagfishes).
Infraphylum 2. Gnathostomata auct. (jawed fish, tetrapods).

Subkingdom 4. Mesozoa subking. nov. (bilateral multicellular parasites with ciliated epithelium; gut, nervous and vascular systems absent).

Phylum Mesozoa van Beneden 1877 (dicyemids, orthonectids).

* Probably paraphyletic.

† Almost certainly paraphyletic.

The present system primarily emphasises classical morphological and embryological data and phylogenetic ideas, but has been revised to take into account recent molecular phylogenetic evidence (reviewed by Ueshima, 1995; see also Cavalier-Smith *et al.*, 1996*a*; Bridge *et al.*, 1995; Winnepenninckx *et al.*, 1995; Winnepenninckx, Backeljau and De Wachter, 1995; Satoh, 1995; Mackey *et al.*, 1996; Garey *et al.*, 1996*a*, *b*)

Cercozoa is a new name for the assemblage of filose and reticulose amoebae and phylogenetically related zooflagellates (including sarcomonads like *Cercomonas*) for which the provisional name Rhizopoda was recently adopted (Cavalier-Smith 1995*a*, *b*, 1997*a*). Because rRNA sequence has shown that flagellate and amoeboid taxa are phylogenetically intermingled, the names Sarcodina and Rhizopoda are now both abandoned as formal names for taxa. They will however remain useful as

non-phylogenetic designations of body form in descriptive and ecological studies, like 'flagellate' or 'alga'; thus 'rhizopod' can continue to be applied in the traditional sense to any amoeba, irrespective of its taxonomic affinity, to contrast it with a flagellate or sporozoan. Neomonada is a rather broad paraphyletic assemblage with many phylogenetically diverse lineages, some more closely related to one or more of the four higher kingdoms of life than to each other. As our knowledge of neozoan phylogeny (in which neomonad diversification played a central role) improves the classification and possibly also the circumscription of the Neomonada will need to change: see Cavalier-Smith (1997*b*). Though Cercozoa form a major clade on rRNA trees (Cavalier-Smith & Chao, 1997 and unpublished), some of them are not phenotypically very distinct from some Neomonada, so the boundary between the two phyla is not morphologically clear cut, and will probably have to be reevaluated as our understanding of these centrally important but much neglected taxa improves.

The class Athalamea was formerly grouped with Foraminifera in the phylum Granuloreticulosa (Lee, 1990) or Reticulosa (Cavalier-Smith, 1993*a*). Electron microscopy of *Penardia* (Mikrjukov & Mylnikov, 1995) and *Gymnophrys* (Mikryukov & Mylnikov, 1996*b*) shows kinetocysts similar to those of some *Cercomonas* and a Golgi associated with the nuclear envelope as in several cercozoans and neomonads. Unlike Foraminifera they have no tests, their pseudopods do not show bidirectional streaming but differ from cercomonad pseudopods primarily in containing some microtubules, their trophic cells have two subparallel centrioles with vestigial ciliary stumps, and they are freshwater, not marine. In the absence of any specific ultrastructural similarities with Foraminifera, the taxon Granuloreticulosa or Reticulosa appears unnatural. Because of their similarities to cercozoan amoeboflagellates, Athalamea (sole order Biomyxida) are here transferred to the subphylum Monadofilosa of the Cercozoa, even though they have flat mitochondrial cristae whereas most cercozoans possess tubular cristae. Foraminifera thus become a phylum in their own right, characterized by granuloreticular pseudopods emanating from one or more pores in their tests and tubular mitochondrial cristae; Granuloreticulosa/Reticulosa are discontinued.

(*b*) *The infrakingdom Alveolata*

The flagellates formerly grouped as the apicomplexan subphylum Apicomonada (Cavalier-Smith, 1993) are here transferred to the subphylum Protalveolata of the flagellate phylum Dinozoa, and the name Apicomplexa is abandoned as an unnecessary junior synonym of the traditional name Sporozoa [Corliss (1971) trenchantly and sensibly criticized the multidudinous unneccessary new names for the Sporozoa *sensu stricto*]. Sporozoa in the present sense are restricted to obligate non-flagellate parasites, and unlike Apicomplexa *sensu* Cavalier-Smith (1993*a*) include three taxa that lack an apical complex: Manubrispora (comprising the metchnikovellids, formerly regarded as microsporidians, but here excluded from Microsporidia because they appear to have a cortical alveolus like that of most sporozoans), Paramyxea, Haplosporidia. Furthermore, as outlined elsewhere (Cavalier-Smith, 1998*a*), the parasitic flagellate *Perkinsus* is probably less closely related to Sporozoa than are some free living flagellates without an apical complex; it appears that its similarities to Sporozoa, which were the reason for grouping them together in the new phylum Apicomplexa (Levine, 1970) are convergent. No molecular evidence is yet available as to the correct phylogenetic position of Manubrispora and Paramyxea, and that for the Haplosporidia is ambiguous (Cavalier-Smith, 1997*a*).

(*c*) *The infrakingdom Actinopoda*

Though the monophyly of Heliozoa is rather dubious, it would be premature to abandon either the taxon Actinopoda or the phylum Radiozoa. The suggestion that Radiozoa are polyphyletic (Amaral Zettler, Sogin & Caron, 1997) may be mistaken: the failure of Acantharia and Radiolaria to group together on their distance trees could simply be an artefact of the especially high evolutionary rate of rRNA in Radiolaria, as clearly indicated by their long branches, which probably places them too low in the tree, an artifact that is even more marked for Mycetozoa and Microsporidia. My own unpublished maximum likelihood analyses indicate that Radiolaria and Acantharia may actually be sister groups and that Radiozoa is probably a valid phylum; the position of Radiolaria, however, is not robust and with some taxon samples they have a tendency to associate instead with other long branches, but not always the same ones as observed by Amaral Zettler, Sogin & Caron (1997).

V. THE KINGDOM ANIMALIA AND ITS 23 PHYLA

The animal kingdom is here divided into only 23 phyla, mostly familiar but a few novel, grouped in four morphologically very distinct subkingdoms (Table 4).

(1) Radiata, the ancestral animal subkingdom

I have used the more ancient term Radiata of Lamarck and Cuvier rather than the more recent Diploblastica (Haeckel, 1866) for the subkingdom comprising the four most primitive animal phyla, even though Radiata originally included also Echinodermata and often other taxa which are now properly classified within Bilateria. The term Diploblastica is entirely inappropriate for this subkingdom since the majority of Radiata are not diploblastic, but triploblastic. Only the cnidarian class Hydrozoa is truly diploblastic. The fact that the cnidarian subphylum Anthozoa (i.e. the majority of Cnidaria) is fundamentally triploblastic was emphasized by Pantin (1960), while the mesogloea of Scyphozoa also frequently contains cells as does that of Ctenophora (Hyman, 1940). Sponges are fundamentally triploblastic, as their mesohyl contains many cells. As sponges are almost certainly the first animals and probably arose directly from choanoflagellate protozoa (Kent, 1881; Cavalier-Smith, 1981*a*; Wainright *et al*., 1993; Cavalier-Smith *et al*., 1996*a*), the kingdom Animalia is ancestrally triploblastic (Cavalier-Smith, 1983*a*) and the diploblastic condition of Hydrozoa alone is a derived simplification. Haeckel's (1866) influential idea that triploblasty evolved from diploblasty is clearly wrong and, to use Hyman's (1959) trenchant phrase (when referring to another phylogenetically incorrect concept of Gephyrea), 'must be obliterated from zoology'. Though the term diploblastic may properly be applied to Hydrozoa, radiates should no longer be referred to as 'diploblasts' (e.g. Christen *et al*., 1991; Ueshima, 1995).

As the mesohyl of sponges is probably both homologous with and ancestral to the mesogloea of Cnidaria it would be sensible to call it mesogloea also.

(2) The derived subkingdom Mesozoa

Since the kingdom Animalia almost certainly evolved by a transition between a colonial choanoflagellate and the first sponge, during which collagenous mesenchymal connective tissue and epithelia first evolved, it is highly improbable that Mesozoa are phyletic links between Protozoa and Animalia. As discussed in section IV molecular evidence indicates that mesozoans are derived from Bilateria by the loss of nervous system and gut. Because of these radical differences I continue to treat them as a separate subkingdom, even though this makes subkingdom Bilateria paraphyletic.

(3) The number of animal phyla

Hyman (1940) recognised only 21 animal phyla or later 23 (1959). Many American college textbooks now recognize about 35, and Möhn (1984) had as many as 38. This taxonomic inflation has mainly come about not by the discovery of new groups or by a clear demonstration that many of Hyman's phyla were polyphyletic and had to be split. Much of it arose by the excessive splitting of the phyla Arthropoda and Aschelminthes of Hyman (1940); in the case of the aschelminths every class has been treated as a separate phylum, not because of convincing evidence that they are phyletically unrelated but merely because their phyletic relationships have been unclear. In essence phylogenetic agnosticism, rather than reasoned arguments, has led to an unnecessary inflation in the number of phyla, which obscures rather than clarifies their evolutionary relationship and makes it more difficult for students to appreciate the diversity of animals than would a system with fewer phyla. In the present system I have therefore taken the bull by the horns – or perhaps I should say the priapulid by the spines – and grouped the aschelminth classes into just two phyla, each of which I think is monophyletic. As a result of also adopting a broader definition of the Annelida and merging brachiopods and phoronids into a single new phylum, Brachiozoa, my present system still has only 23 phyla, even though (unlike Hyman) I accept the phylum Placozoa, treat Urochorda as a separate phylum from Chordata, and segregate tardigrades and onychophorans from Arthropoda as the phylum Lobopoda. I do not accept the recently discovered Cycliophora (Funch & Kristensen, 1995) as a separate monogeneric phylum; *Symbion* is sufficiently similar in body plan to Entoprocta to be grouped with them in an emended phylum Kamptozoa, but in a separate subphylum. Thirteen of the present animal phyla are identical to those of Hyman, and seven are identical with those of the 18 phylum system of

Lankester (1911), though I use the older name Annelida in preference to his Chaetopoda.

(4) A broadened phylum Annelida

The phylum Annelida recognised here is that of Sedgewick (1898) with the addition of the Pogonophora, which were then unknown. Because of their anterior oligomerous coelom and the belief that they lack chaetae, early workers on Pogonophora thought they were unrelated to annelids and closer to deuterostomes, and so deserved their own phylum (Reisinger, 1938). Hyman (1959) accepted this, but at that time it was still not known that early workers were completely ignorant of the posterior segmented chaetiferous portion of the animal's body. Since this was discovered it has been clear that Pogonophora are not deuterostomes, but instead are related to annelids. The closeness of this relationship has been strongly confirmed by sequence data (Winnepenninckx, Backeljau & De Wachter, 1996). It is now evident that Pogonophora are annelids that have lost their gut as a result of the evolution of the capacity to cultivate chemosynthetic bacteria within their body and give up predatory feeding. They probably arose from tubicolous polychaetes. Though loss of the gut and evolution of the ability to cultivate bacteria in a highly developed trophosome are important innovations, Pogonophora retain such a major part of the fundamental annelid body plan (segmentation with chitinous chaetae, haemoglobin-containing vascular system and respiratory tentacles like polychaetes) that there is no longer any justification for treating them as a separate phylum, still less in dividing them into two phyla as has been done. Ranking Pogonophora as a subphylum (within which typical pogomophorans and Vestimentifera can be separate classes) is sufficient recognition of their distinctiveness from Polychaeta and Clitellata, both here ranked as subphyla of the Annelida.

Sedgewick (1898) regarded the class Echiuroidea as 'obviously true Annelids'. He was the first to realize they were radically different from sipunculoids, with which they were previously grouped in the unnatural phylum Gephyrea (which Hyman rightly 'obliterated from zoology'); he first made Sipunculoidea a separate phylum. Hyman (1940) first treated Echiuroida as a separate phylum on the grounds that they are 'unsegmented', whilst at the same time admitted that they might alternatively be appended to the Annelida, 'to which phylum they are undoubtedly related' (Hyman, 1959). Even though Hyman never wrote a volume of her treatise dealing with the echiuroids and annelids and therefore gave no more detailed discussion than that, her unsound decision has been widely followed by textbooks. However, the fact that echiuroids have well developed chaetae, typical trochosphere larvae, and a vascular system and nephridia similar to those of other annelids, leaves little doubt that they are annelids in which the formerly discrete coelomic cavities have become secondarily merged into one. As Sedgewick pointed out, there are traces of segmentation in young *Echiurus*. Loss of septa and partial merger of coelomic cavities is very common in polychaetes and virtually complete merger occurred in leeches (Clark 1964); the almost total suppression of segmentation in adult echiuroids is probably a locomotory adaptation to a relatively sedentary burrowing life in which the proboscis took on an even more important role than in analogous burrowing polychaetes. These adaptations do not constitute a fundamentally different body plan from other annelids. Treatment of the small number of Echiuroida as a subphylum of the Annelida is greatly preferable to making them a phylum in their own right, which obscures their true affinities, and devalues the idea that separate animal phyla should have fundamentally different body plans.

(5) The pseudocoelomate phyla Nemathelminthes and Acanthognatha phyl. nov.

The proper status of the pseudocoelomate aschelminths has always been problematic. But as, Lorenzen (1985) stressed, there are many synapomorphies that can be used to establish real relationships between some of the classes. Therefore it is highly unsatisfactory to rank almost every class as a separate phylum. But, like most zoologists, I do not think that aschelminths as a whole are monophyletic. Instead there seem to be two fundamentally different phylogenetic lineages, which I treat as phyla, and a larger number of goupings of related classes, which I treat as subphyla and infraphyla. Some of these are more strongly supported by existing data than others.

The new subphylum Trochata (comprising rotifers and Acanthocephala) is very strongly supported by the synapomorphies listed in Table 2 [see also Lorenzen (1985) and Nielsen (1995)] as well as by molecular trees (Winnepenninkx *et al.*, 1995*a*; Garey et al. 1996*b*). Ranking Acanthocephala as a phylum (Hyman, 1940) overemphasized the importance of the secondary loss of the gut and cilia; these

degeneracies, the syncytial epidermis, and proboscis hooks are adaptations to parasitism remarkably convergent with those of cestodes, which are also not placed in a separate phylum from their closest free-living ciliated relatives, the Turbellaria. I rank both Rotifera and Acanthocephala as infraphyla so that Bdelloidea and Monogogonta can be classes, as preferred by those who have treated Rotifera as a phylum. Even though Acanthocephala probably arose from rotifers by parasitic degeneration, I prefer not to place them within Rotifera as suggested by Garey *et al.* (1996*b*), since the two groups are phenotypically so different and it would be nomenclaturally confusing to call Acanthocephala rotifers merely because they evolved from them.

The new subphylum Monokonta (comprising gastrotrichs and gnathostomulids) is primarily based on their monociliated epithelial cells (whence the name) and needs testing by gene sequencing. The grouping of Monokonta and Trochata as the new phylum Acanthognatha also needs such testing, as it is not clear whether the chitinous jaws of rotifers and gnathostomulids are homologous or not.

The grouping of Nematoda and Nematomorpha as subphylum Nematoida is an old idea, well supported both by morphology (Lorenzen, 1985) and gene sequences (Winnepenninkx *et al.*, 1995*a*). The similarities between the lorica of longitudinal plates in Loricifera and larval priapulids is the basis for grouping them as the new infraphylum Priapozoa. Priapozoa are grouped with kinorhynchs as the new subphylum Scalidorhyncha, since they all have a retractile head covered with circlets of spined scalids, which seems unlikely to be convergent. Molecular testing is however needed. My grouping of Scalidorhyncha and Nematoida as the Nemathelminthes (an old phylum name) might be questioned on the ground that such a grouping was not evident on a recent rRNA tree (Winnepenninkx *et al.*, 1995*a*). However that tree does not specifically relate either Nematoida or priapulids to any other group, and thus lacks resolution as to their relationship and therefore does not clearly disprove the monophyly of the Nemathelminthes postulated here.

(6) The new phyla Brachiozoa and Lobopoda

Recent rRNA phylogenies show that phoronids probably arose from inarticulate brachiopods by the loss of the shell (Cohen, Gawthrop & Cavalier-Smith, 1997). Since in other respects both groups share a basically similar body plan, phoronids are to brachiopods as slugs are to snails, and do not merit their own phylum. Therefore I rank both groups as subphyla within the new phylum Brachiozoa. Though molecular data support the relationship to arthropods of both Onychophora and Tardigrada (Garey *et al.*, 1996*a*), I separate them from Arthropoda as a new phylum Lobopoda (a name proposed informally by Manton: 1977) because unlike true arthropods they do not have jointed limbs and a rigid cuticle.

(7) Phylogenetic assumptions behind the new bilaterian infrakingdoms and superphyla

Even if we adopt a broad phylum Annelida and recognize only two aschelminth phyla, there are still 17 separate bilaterian phyla, which fall naturally into two branches: Protostomia (13 phyla) and Deuterostomia (4 phyla). Deuterostomia are divisible into two long-standing groups, Coelomopora (Hemichordata and Echinodermata) and Chordonia (Urochorda and Chordata), which I here rank as infrakingdoms, though a rank of superphylum would also be acceptable. For protostomes, however, the number of phyla is so large that we need both infrakingdoms and superphyla in order to group them informatively. I divide them into four new infrakingdoms. Ecdysozoa comprise the three phyla (Arthropoda, Lobopoda, Nemathelminthes) with a moultable cuticle and a very poorly developed or absent coelom (Aguinaldo *et al.*, 1997). As they are undoubtedly more closely related to each other than to the Nemathelminthes I group Lobopoda and Arthropoda together as the new superphylum Haemopoda, because of their shared segmented body and haemocoel that extends into the limbs. This superphylum enables one to indicate that lobopods are closely related to, but not actually arthropods. By adopting the name Lobopoda I do not imply any connection with polychaetes, some of which have been referred to as lobopodial (Sharov, 1966). I do not think there is any direct phylogenetic connection whatever between Haemopoda and Annelida; haemopod limbs and polychaete parapodia have always seemed to me merely analogous, not homologous as assumed by the annelid theory of the origin of arthropods (Snodgrass, 1938; Sharov, 1966). The gulf between Ecdysozoa and Lophozoa, the other major protostome infrakingdom, is immense and very difficult to cross in plausible megaevolutionary steps, which is why I have given both the high rank of infrakingdom.

Lophozoa, the largest protostome infrakingdom, comprise the six protostome phyla that have well developed coelomic cavities plus the Kamptozoa, which do not. Unlike most zoologists, I do not accept Hyman's (1951) view that the absence of a coelom in entoprocts precludes a specific relationship with Bryozoa. Like Nielsen (1995) I think the two groups are related. I suspect that Kamptozoa arose from an early bryozoan by the loss of the coelom. Though I do not altogether rule out the reverse possibility that the coelom first evolved in Bryozoa from a non-coelomate entoproct-like ancestor, I prefer the view that Bilateria were ancestrally coelomate and that the coelom first arose simultaneously with the bilaterian gut by partitioning the anthozoan coelenteron into two parts, as proposed in my invited manuscript for the Systematics Association 1984 meeting on the relationships of lower invertebrates that was rejected by the editors because it was too controversial (Conway Morris *et al.*, 1985). My novel version of the Archicoelomate theory (Ulrich, 1949) of the origin and diversification of bilateral animals was clearly unacceptable to zoologists raised on Hyman's dogmatic view that all non-coelomates were ancestrally so. My present grouping of both Bryozoa and Kamptozoa in the superphylum Polyzoa may be equally unacceptable to many. But I have yet to see a more convincing explanation of the origin of Kamptozoa than by coelomic reduction, possibly from a lophopod-like bryozoan.

Like the resurrection of the Polyzoa, my grouping of the Brachiozoa with the Mollusca as the superphylum Conchozoa may seem a retrograde step, harking back to Cuvier's inclusion of brachiopods in the Mollusca. However, as I argued at the 1984 meeting, Hyman's dogma that Polyzoa, Brachiozoa and Bivalvia are decephalized is probably incorrect. There is no phylogenetic evidence whatever that any of these animals ever had heads. Hyman's dogma was expressed so forcefully as to inhibit dissenting thought: 'lophophorates and deuterostomes as seen today are all decephalized animals of sedentary or sessile habits. It is inconceivable that such types should originate the Bilateria. It appears self-evident that only well-cephalized, active forms can originate definitive bilateral symmetry'. Her view was purely intuitive, unsupported by any phylogenetic reasoning. The fact that the heads of radulate molluscs, annelids, nemathelminths, arthropods and vertebrates are all so different from each other, and lack any recognizable homology, argues strongly that their common ancestor was not strongly cephalized and that each of these groups became cephalized independently. Given that both radiate phyla (Cnidaria and Ctenophora) are uncephalized, there is no phylogenetic reason to think that the ancestral bilaterian was cephalized. I argue that all Lophozoa are ancestrally non-cephalized and that the Bivalvia among the molluscs and the tubicolous Operculata among the annelids are closer to the ancestral state than their more cephalized relatives. The bivalved character of the polyzoan larvae, of brachiopods, and bivalve molluscs suggests that the common ancestor of Polyzoa and Conchozoa had a bivalved larva and sedentary non-cephalized adults. On this view the traditional creeping radulate archimollusc (von Salvini-Plawen, 1990) was not the ancestor of Mollusca as a whole, but just of the subphylum Glossophora (i.e. the radulate molluscs).

The other novel assemblage, the superphylum Vermizoa (a name I proposed at the 1984 meeting) comprises Annelida and Nemertina, both of which have well developed closed vascular systems. Though it is sometimes denied that the rhynchocoel of the nemertines is a true coelom, the presence of the vascular system and gut with an anus makes them much more similar to annelids than to the flatworms, with which many authors have grouped them. Since echiuroids testify to the likelihood that some annelids can lose segmentation, whereas in others the coelom can become occluded by parenchyma (Rieger, 1985), there are no strong morphological reasons against grouping annelids and nemertines in the same superphylum.

Ribosomal RNA trees show that all the lophozoan phyla, except the Sipuncula, are so closely related to each other that their branching order cannot be readily determined. Because of their unique character Sipuncula are placed solitarily in their own lophozoan phylum. The name Lophozoa was chosen on the assumption that the ancestor had a retractile pair of tentacles with an interior coelomic cavity. This is generally agreed for the Bryozoa and Brachiozoa, but the ctenidia of molluscs, the tentacles of Sipuncula, and the gills of operculate polychaetes may, I suggest, also be homologues of the lophophore. It is much more doubtful that the nemertine proboscis has a similar origin, but the possibility cannot be totally dismissed. If the entoprocts are derived from Bryozoa by coelomic occlusion their tentacles also may be derived from those of the lophophore.

The deep divergence between the Ecdysozoa and the Lophozoa is supported by the rRNA trees, which are also inconsistent with an annelid ancestry for

arthropods and Cuvier's old group Articulata, which is clearly poylphyletic. Since arthropods and molluscs appear almost simultaneously at the beginning of the early Cambrian bilaterian fossil record, Ecdysozoa and Lophozoa probably diverged very soon after the origin of the Bilateria. Any theory of how they are related is necessarily highly speculative. The speculation that I presented at the 1984 Systematics meeting, and which I still favour was that haemopod limbs evolved from tentacles of an early solitary polyzoan. I had in mind the solitary loxosomatid entoprocts, which can locomote actively on the tips of their tentacles (Hyman, 1951). The pseudocoel of such an active entoproct could have given rise directly to the haemocoel of the Haemopoda and the pseudocoel of Nemathelminthes. Such an origin involves much less change than the view that arthropods evolved from a coelomate legless worm. Entoproct tentacles can already function similarly to lobopodal limbs and are similarly arranged in two lateral rows. Nemathelminthes could have arisen from an early lobopod through the loss of limbs after adopting a burrowing habit, like snakes, apodans, and caecilians among vertebrates.

The third protostome infrakingdom is the noncoelomate Platyzoa, which contains only the phyla Acanthognatha and Platyhelminthes. Both are ancestrally slender ciliated worms with no vascular system, either acoelomate or pseudocoelomate. Though Hyman (1940–1959) was utterly confident that they represent the ancestral bilaterian condition, I am deeply sceptical of this, as I cannot envisage how they might have evolved from a radiate ancestor. It seems to me more likely that they arose from neotenous entoproct larvae of the creeping type found in *Loxosoma*: even Hyman (1951) noted their remarkable similarity to a rotifer. If such an origin is correct, then no separate coelomic loss need be postulated. An alternative way of subdividing the Protostomia to that in Table 4 would be to transfer Kamptozoa from the Lophozoa to the Platyzoa. Though compatible with my phylogenetic assumptions, I prefer to emphasize the life cycle and trophic similarities of Polyzoa, rather than the loss of the coelom. The rRNA trees are unfortunatly not very helpful in understanding the affinities of the Platyhelminthes; their branches (like those of nematodes) are so long that one must suspect that long-branch artefacts may be placing them lower in the tree than their correct position. When one has a rapid, almost simultaneous radiation, as appears to be the case, coupled with an elevated evolutionary rate in some branches, such systematic biases may easily swamp the true phylogenetic signal and yield a positively incorrect answer.

I do not of course suggest that Ecdysozoa or Platyzoa evolved directly from any extant polyzoan group. The discovery of *Symbion* suggests that the early radiation of Kamptozoa may have been much more varied than we can now guess from the few extant species. Unfortunately the fossil record of this phylum of tiny animals is so poor that it will contribute very little to testing my thesis that similar organisms could have been ancestral to both Ecdysozoa and Platyzoa.

The fourth protostome infrakingdom is the Chaetognathi, which are so different from all other protostome animals that it does not seem possible to group them with any other phylum. Their rRNA sequences also leave them in an isolated position, and give no support to Hyman's view that they are deuterostomes. The protostome infrakingdoms might be further reduced to three by placing chaetognaths in the Ecdysozoa, with which they share some characters, but I prefer to emphasize their uniqueness.

(8) New animal subphyla and infraphyla

Subphyla have long been used in the Arthropoda and Chordata which have the largest number of disparate classes. In other phyla I have made more extensive use of them than is traditional, partly to avoid unnecessary splitting into separate phyla and partly to emphasize that some classes really are more closely related to each other than to others. Most of the subphylum groupings are well accepted and I have often used traditional names; but slight changes in rank are frequent (downward from phylum and upward from class or superclass). Only three subphyla are new both in concept and name: Scalidorhyncha (priapulids, loriciferans and kinorhynchs); Trochata (rotifers and acanthocephalans); Monokonta (gastrotrichs and gnathostomulids).

In phyla with a large number of classes I have used infraphyla much more extensively than usual. Again, some of these, like Cephalopoda, Agnatha and Rotifera, are well known taxa given intermediate ranks in order to include more cladistic information in the classification. Only six are new in both concept and name: the radulate mollusc infraphyla Monoconcha (those with a single shell) and Spiculata (those with multiple shell plates and/or calcareous spicules); the polychaete Operculata and Pharyngata; Priapozoa (priapulids and

loriciferans); and Mucorhabda (turbellarians with non-lamellate rhabdoids). Elementary texts would benefit from using subphyla more extensively than they do, but infraphyla may not be necessary for them, though will be valuable in more specialized works and databases.

VI. THE KINGDOM FUNGI AND ITS FOUR PHYLA

(1) Circumscription of the Fungi

A kingdom Fungi entirely distinct from the kingdom Plantae is now almost universally recognized (Whittaker, 1969; Carlile & Watkinson, 1994). Recently this kingdom has sometimes been referred to instead as Mycota, but this name has probably not been validly published and is entirely unnecessary and much less widely understood. It is increasingly clear that Fungi are more closely related to animals than to plants (Baldauf & Palmer, 1993; Wainright *et al*., 1993); however, it is incorrect to speak of an 'evolutionary link' between fungi and animals (Wainright *et al*., 1993) since the phylogenetic linkage is indirect *via* the choanoflagellate protozoa. As Cavalier-Smith (1987*b*) first suggested, it seems that Fungi evolved from choanoflagellate protozoan ancestors as did animals, but independently.

Molecular evidence strongly supports the restriction of the kingdom Fungi to the taxa summarized in Table 5, including (as discussed in section III above) the unexpected inclusion of the Microsporidia. The earlier circumscription that I advocated previously excluding the Microsporidia (Cavalier-Smith, 1981*a*, 1987*b*) was supported by much rRNA sequence evidence (Bruns, White & Taylor, 1991), and accepted by the authoritative *Dictionary of the Fungi* (Hawksworth *et al*., 1995). Several other taxa have often been treated as Fungi, but are evolutionarily quite unrelated to them. Some recent mycology texts (e.g. Alexopoulos, Mimms & Blackwell, 1996) correctly exclude these elements from the kingdom Fungi, whereas others (e.g. Carlile & Watkinson, 1994) still incorrectly place them in the same kingdom.

The most important of the non-fungal groups widely studied by mycologists and therefore formerly classified as Fungi are the slime moulds, plasmodiophorids, oomycetes, hyphochytrids and thraustochytrids, which all properly belong either in the kingdom Protozoa or in the third botanical kingdom, Chromista, as is fully accepted by Hawksworth *et al*. (1995). These organisms are not fungi but 'fungus-mimics', protists that have convergently become similar to fungi in one or more respects.

The protozoan groups (slime moulds and plasmodiophorids) remain vegetatively amoeboid but have acquired aerial spores for dispersion convergently with fungi. Slime moulds are polyphyletic, but the majority of them form a monophyletic protozoan infraphylum Mycetozoa (Table 2) (i.e. classes Protostelea, Myxogastrea and Dictyostelea); though the myxogastrean *Physarum polycephalum* and the dictyostelid *Dictyostelium discoideum* most often do not group together on 18S rRNA distance trees they usually do so on 18S rRNA maximum-likelihood trees (e.g. Cavalier-Smith, 1995*a*, *b*), which appear to be less misled by the very long *Physarum* branch. Inspection of the alignment shows about seven obvious molecular synapomorphies for the Mycetozoa, and Rusk, Spiegel & Lee (1994) have rRNA sequence evidence that the protostelids are specifically related to dictyostelids and myxogastreans, which is also supported by trees based on sequences of the protein elongation factor EF 1α (Baldauf, & Doolittle, 1997). Molecular evidence is not yet available for the minor groups of non-mycetozoan slime moulds, the acrasids, copromyxids and fonticulids. However, the morphology of their mitochondria and pseudopodia convincingly places Acrasida within the class Heterolobosea (Page, 1987) within the protozoan phylum Percolozoa (Cavalier-Smith, 1993*a*, *c*). While their morphology is less decisive, copromyxids are placed in the protozoan class Lobosea of the phylum Amoebozoa, whilst fonticulids are in the class Cristidiscoidea, now within the subphylum Choanozoa of the protozoan phylum Neomonada (Cavalier-Smith, 1998*a*; formerly Cristidiscoidea were in the Rhizopoda: Page, 1987; Cavalier-Smith, 1993*a*, 1995*b*, 1997*a*); and there is no reason whatever to group them with Fungi. Plasmodiophorids have often been thought to be related to Mycetozoa and therefore were treated as fungi; but molecular evidence clearly shows that they are protozoa that belong in the Cercozoa (formerly Rhizopoda: Cavalier-Smith & Chao, 1997).

In contrast to the foregoing protozoan taxa, oomycetes, hyphochytrids and thraustochytrids all belong in the kingdom Chromista (see section VIII below). They are probably all secondarily non-photosynthetic heterotrophs that have acquired vegetative cell walls convergently with fungi.

The protozoan *Corallochytrium limacisporum* has been erroneously treated as a fungus (Raghu-Kumar, 1987); however, rRNA phylogeny reveals

that it acquired a cell wall and evolved osmotrophy entirely independently of Fungi and heterotrophic chromists, and is actually a protozoan related to choanoflagellates (Cavalier-Smith & Allsopp, 1996). Other Protozoa, notably parasitic Ichthyosporea (now in subphylum Choanozoa: Cavalier-Smith, 1998*a*) have also erroneously been treated as fungi in the past, whereas some Fungi, notably *Pneumocystis* (Edman *et al*., 1988), have mistakenly been thought to be Protozoa.

(2) The trichomycete origin of microsporidia

The key step in the origin of microsporidia apart from the losses of these two organelles was the origin of intracellular parasitism and of the polar tube, the organelle that uniquely distinguishes the phylum from all others. The polar tube is formed within the spore from membranes thought to be of Golgi character, and is typically long and highly coiled. In germinating spores it is extruded and its apex attaches to host cells enabling the sporoplasm to enter the host cell through the tube. Clearly the origin of the polar tube and this unique method of infection was both necessary and sufficient for the origin of intracellular parasitism and the first microsporidian. A necessary corollary of this type of injection mechanism is the digestion of the spore wall and the temporary nakedness of the infective stage. Temporarily naked infective cells are common in Chytridiomycetes, the most primitive fungal group. Furthermore chitin walls have secondarily lost, apparently independently in the vegetative cells of the allomycete *Coelomomyces* and the trichomycete *Amoeboidium*, which both parasitize animals. Thus the secondary loss of chitin walls in fungal parasites of animals is not unprecedented and could also have occurred in the postulated fungal ancestor of microsporidia and thus preadapted it to the origin of the polar tube and an intracellular mode of life. The intracellular stage of microsporidia is not always entirely naked, but sometimes bears a surface coat, which in *Pleistophora* is so thick as almost to resemble a wall.

Elsewhere I propose that the microsporidian polar tube may have evolved from apical spore bodies, which are organelles of certain harpellalean trichomycetes, probably extrusive, that are thought to help the spore attach to the gut lining of their arthropod host (Cavalier-Smith, 1998*b*). The fact that microsporidia mainly infect invertebrates, especially insects, and only very rarely infect protozoa, and are unknown as parasites of Metamonada has long caused some zoologists to be sceptical of the idea that they are primitive eukaryotes. An origin from one of the trichomycetes, which are all obligate endoparasites of arthropods does not suffer from this problem and makes sense ecologically as well as phylogenetically and cytologically (Cavalier-Smith, 1998*b*).

(3) Revision of higher fungal classification

The classification in Table 5 is based on that of Cavalier-Smith (1987*b*) but modified in several important respects, including the subdivision of the kingdom into the two subkingdoms Eomycota and Neomycota. The new names of higher fungal taxa in Cavalier-Smith (1987*b*) were at the editor's insistence not accompanied by proper diagnoses. This is rectified in Table 5 which validates them and all new fungal names introduced here for the first time. Since many mycologists have in recent decades been curiously reluctant to use higher taxa than orders I have thought it desirable to make the present fungal classification more comprehensive than for the other five kingdoms. I therefore include superclasses, classes, subclasses and superroders in order to bring fungal megasystematics more into line with the practice in other kingdoms.

The most important innovations are the creation of the new hemiascomycete class Geomycetes and a major revision of the phylum Archemycota, which includes not only the fungi placed by Bruns, White & Taylor (1991) and many other authors in the nomenclaturally invalid phyla Chytridiomycota and Zygomycota, but also the Laboulbeniales, which were traditionally regarded as ascomycetes. I argued previously (Cavalier-Smith, 1987*b*) that 'Zygomycota' do not differ sufficiently from 'Chytridiomycota' to merit a separate phylum. I am unaware of any mycologist who has seriously argued the case for having two separate phyla: nonetheless this taxonomic inflation has been widely adopted in the past decade.

I suggest that traditional trichomycetes (Lichtwardt, 1986), which are all gut parasites of arthropods, are polyphyletic. Two orders (Eccrinales and Amoebidiales) have Golgi dictyosomes, unlike all other Zygomycotina, so I remove them from the Trichomycetes and Zygomycotina as a new class Enteromycetes. I had earlier (Cavalier-Smith, 1981*a*) divided the traditional Chytridiomycotina into two classes: Chytridiomycetes *sensu stricto* (with Golgi dictyosomes) and Allomycetes (without dictyosomes), both of which I validate in Table 5. I group Chytridiomycetes and Enteromycetes together to

Table 5. *Classification of the kingdom Fungi, its four phyla and* 20 *classes*

Subkingdom 1. Eomycota† subking. nov. (hyphae, when present, usually lacking septa or rarely with imperforate septa; dikarya absent; vegetative walls frequently absent in animal parasites; mitochondria and peroxisomes often absent, sometimes replaced by hydrogenosomes: cellulae non binucleatae; septa usitate absens; si praesens non perforata).

Phylum 1. Archemycota† phyl. nov. (diagnosis: mitochondria and peroxisomes usually present; if absent then possessing hydrogenosomes; vegetative cell walls usually present: mitochondria aut hydrogenosomae praesentes. Name introduced without Latin diagnosis by Cavalier-Smith, 1987*b*).

Subphylum 1. Dictyomycotina† subphyl. nov. (diagnosis: Golgi dictyosome of stacked cisternae: dictyosoma praebens).

Class 1. Chytridiomycetes De Bary 1863 stat. nov. Sparrow 1958 em. Cavalier-Smith 1981 (emended diagnosis: posteriorly ciliated zoospores: sporae ciliis posterioribus instructae).

Subclass 1. Rumpomycetidae subcl. nov. (diagnosis: zoospores with rumposome: zoospora rumposoma instructa; introduced as a class name without Latin diagnosis by Cavalier-Smith, 1987*b*) (orders Chytridiales, Monoblepharidales).

Subclass 2. Spizomycetidae subcl. nov. (diagnosis: rumposome absent: sine rumposoma; introduced as a class name without Latin diagnosis by Cavalier-Smith, 1987*b*) (orders Spizellomycetales, Neocallimastigales).

Class 2. Enteromycetes cl. nov. (diagnosis: cilia absent; parasites of arthropod gut: sine ciliis; intestinum arthropodis incolens) (orders Eccrinales, Amoebidiales).

Subphylum 2. Melanomycotina subphyl. nov. (diagnosis: Golgi cisternae unstacked; melanin-pigmented resting spores common; mitochondria and peroxisomes present: dictyosoma non-praebens: cisternae disjunctae; sporae saepe fuscae).

Infraphylum 1. Allomycotina infraphy. nov. (diagnosis: with uniciliate zoospores.: zoospora cilio unico instructa).

Class 1. Allomycetes cl. nov. (diagnosis: with uniciliate zoospores: zoospora cilio unico instructa; name introduced without Latin diagnosis by Cavalier-Smith, 1981*a*) [orders Blastocladiales, Coelomomycetales ord. nov. (diagnosis: trophic phase without walls: sine muris in statu pabulatorio; sole family Coelomomycetaceae)].

Infraphylum 2. Zygomycotina† infraphyl. nov. (diagnosis: cilia and zoospores absent: sine ciliis).

Superclass 1. Eozygomycetia supercl. nov. (diagnosis: without sporangiospores or aquatic spores; saprophytes or symbionts of vascular plants: sine sporangiosporis aut sporis aquaticis).

Class 1. Bolomycetes cl. nov. (diagnosis: saprophytes with 11–12-singlet centriole; single large propulsive conidia borne on long unbranched conidiophores; zygospores with adjacent beak-like projections; not within a sporocarp; septate mycelia: conidophora instructa; centriolum microtubulos continens) [Basidiobolales ord. nov. (diagnosis as for Bolomycetes) (*Basidiobolus*)].

Class 2. Glomomycetes* cl. nov. (diagnosis: form vesicular arbuscular endomycorhizas with vascular plants; sclerotium-like sporocarps contain chlamydospores (Glomales) or laterally produced zygospores (Endogonales); centrioles, conidia, aerial spores and stalked sporophores absent. Conidiophora et centriolum absens; in radices plantarum crescens; sporae in sporocarpiis subterraniis).

Superclass 2. Neozygomycetia supercl. nov. (diagnosis: sporocarp and centrioles absent; aerial or aquatic aplanospores; saprophytes or parasites of animals: sporangiospora aut sporae aquaticae instructa).

Class 1. Zygomycetes cl. nov. (diagnosis: zygospore wall modified from the gametangial wall, produced between the gametangia; passive aerial asexual spores borne on stalked sporophores; usually saprophytes: murus gametangii in murum zygosporae transiens; sporae aeriae in sporophoro pedicellato).

Subclass 1. Mucoromycetidae Fries 1832 stat. nov. (diagnosis: coenocytic; septa absent in young mycelia; with globose multispored sporangia and/or uni- or oligo- sporic sporangiola; sporophore hypha-like) [orders Mucorales: sporangium with columella; zygospores lacking an investment of sterile hyphae; Mortierellales ord. nov. (diagnosis: sporangium without columella; zygospores often invested by sterile hyphae; chlamydospores: sporangium sine columello; hyphae steriles zygospora saepe investientes; chlamydospora instructates)].

Subclass 2. Meromycetidae subcl. nov. [diagnosis: aerial spores formed in merosporangia or as conidia; globular sporangia absent; mycelia septate with medial plug (Dimargaritales, Kickxellales) or not (Piptocephalaceae, Cuninghamellales); sporophore often vesicular: sporae aeriae in merosporangiis aut conidiae: sporangiae globulosae absens; sporophorum saepe vesiculatum].

Class 2. Zoomycetes cl. nov. (diagnosis: parasites of animals or protozoa; sporangiospores absent: in animaliis aut protozois parasitici; sporangiophora absens).

Subclass 1. Entomycetidae subcl. nov. (diagnosis: endoparasites of invertebrates or protozoa; hyphae syncytial or subdivided by imperforate septa into coenocytic segments; spores propulsive conidia; zygospores lateral

Table 5. (*cont.*)

to gametangia: in animaliis aut protozois parasitici; hyphae septatae aut non-septatae; conidia se praecipitantes) (orders Entomophorales, Zoopagales).

Subclass 2. Pedomycetidae subcl. nov. (diagnosis: symbionts of mandibulate arthropods, attached to their cuticle by foot-like holdfasts; imperforate plugged septa: in arthropodis mandibulatis parasiticae; in cuticula affixi haptero dilatato; septa imperforata).

Superorder 1. Trichomycetalia superord. nov. (diagnosis: endocommensals within the gut; spores are trichospores, formed by budding both asexually and following conjugation or arthrospores formed by hyphal fragmentation: intestinum incolens; trichosporae aut arthrosporae instructae) (orders Harpellales and Asellariales).

Superorder 2. Pyxomycetalia superord. nov. (diagnosis: ectoparasites; differentiated male and female organs; female organ contains endospores and develops from three cells enclosed by a multicellular pseudoperithecium with an apical pore; male organ an antheridium forming spermatia either endogenously or by exogenous budding: ectoparasiticae; pseudoperithecium endosporae continens; antheridium spermatia continens) (orders Laboulbeniales, e.g. *Laboulbenia*; Pyxidiophorales).

Phylum 2. Microsporidia Balbiani 1882 stat. nov. Weiser 1977 (diagnosis: obligate intracellular parasites of animals or rarely protozoa; vegetative cell walls, mitochondria and peroxisomes absent; spores with chitin walls and an extrusive polar tube through which the sporoplasm enters the new host cell).

Class 1. Minisporea Cavalier-Smith 1993 (diagnosis: polar tube with honeycomb outer layer) (e.g. *Chytridiopsis*, *Buxtehudia*, *Hessea*).

Class 2. Microsporea Levine & Corliss 1963 (diagnosis: polaroplast present: spores usually oval, rarely rod-shaped or pyriform).

Subclass 1. Pleistophorea Cavalier-Smith 1993 (diagnosis: multiply by plasmotomy: one spore type: Pleistophorida Stempell 1906, e.g. *Pleistophora*, *Amblyospora*, *Glugea*, *Encephalitozoon*).

Subclass 2. Disporea Cavalier-Smith 1993 (diagnosis: multiply by binary fission; disporogenic, i.e. two spore types, e.g. *Nosema*, *Enterocytozoon*, *Spraguea*, *Caudospora*).

Subkingdom 2. Neomycota subking. nov. (diagnosis: usually with a dikaryotic phase; meiotic products form basidiospores or endospores or divide mitotically once to form ascospores; mitochondria and peroxisomes always present: cellulae binucleatae plerumque praesentes; endospora aut ascospora aut basidiospora instructa).

Phylum 1. Ascomycota Berkeley 1857 stat nov. (diagnosis: meiotic products or their daughters form endospores by subdividing the cytoplasm by membrane, not by budding: sporae intracellulares).

Subphylum 1. Hemiascomycotina* Brefeldt 1891 stat. nov. Ainsworth 1966 (diagnosis: ascocarp absent; usually yeast-like: sine ascocarpo; plerumque in forma fermenti).

Class 1. Taphrinomycetes cl. nov. (diagnosis: cell walls often lack chitin; meiotic products yield four endospores: muri saepe sine chitino endosporae quattuor; name introduced without Latin diagnosis by Cavalier-Smith, 1987*b*) (e.g. *Taphrina*, *Schizosaccharomyces*, *Protomyces*, *Pneumocystis*).

Class 2. Geomycetes cl. nov. (diagnosis: with intracellular cyanobacterial endosymbionts (*Nostoc* sp.); hyphae coenocytic when young; huge pale asexual spores form at hyphal tips; sex unknown: hyphae juvenes sine septis; cyanobacteria intra hyphis incolentes) [Geosiphonales ord. nov. (diagnosis as for Geomycetes) and sole family Geosiphonaceae (*Geosiphon*)].

Class 3. Endomycetes cl. nov. (diagnosis: budding yeasts; chitin only in bud scars; ascogenous hyphae absent; meiotic products four endospores formed by fusion of smooth cytoplasmic membranes around each nucleus separately; ascogenous hyphae and dikaryotic phase absent: fermenti gemmipares; chitinum in muro inter cellulis filiis, non in muri alteri; endosporae quattuor; validates the name introduced without Latin diagnosis by Von Arx, 1967).

Subclass 1. Dipomycetidae subcl. nov. (diagnosis) (e.g. *Dipodascopsis*).

Subclass 2. Saccharomycetidae de Bary 1866 stat. nov. (e.g. *Saccharomyces*, *Candida*, *Endomyces*).

Subphylum 2. Euascomycotina Engler 1897 stat. nov. Ainsworth 1966. (diagnosis: filamentous; chitin throughout the cell walls; ascus vesicle surrounds all four meiotic products; ascogenous hyphae with brief dikaryophase; ascocarp present in sexual forms: chitinum in muris omnibus; ascocarpa plerumque instructae).

Class 1. Discomycetes Fries 1836 stat. nov. Ainsworth 1966 (ascocarp an apothecium).

Subclass 1. Calycomycetidae subcl. nov. (diagnosis) (mostly mazaedial lichens, e.g. *Calicium*, *Coniocybe*).

Subclass 2. Lecomycetidae subcl. nov. (diagnosis: asci inoperculate: asci sine operculis) (most lichen fungi, e.g. *Usnea*, *Lecanora*, *Peltigera*, *Xanthoria*).

Subclass 3. Pezomycetidae subcl. nov. (diagnosis: asci operculate: asci operculati) (e.g. *Peziza*, *Tuber*, *Morchella*, *Rhytisma*).

[*continued overleaf*

Table 5. (*cont.*)

Class 2. Pyrenomycetes Fries 1821 stat. nov. Ainsworth 1966 (ascocarp a perithecium; mycelia septate when young).

Subclass 1. Verrucomycetidae subcl. nov. (diagnosis: pyrenocarpous lichen fungi: fungi lichenis perithecia habentes) (e.g. *Verrucaria*).

Subclass 2. Ostiomycetidae subcl. nov. (diagnosis: non lichen fungi: non lichenes) (e.g. *Neurospora*, *Sordaria*, *Claviceps*, *Nectria*, *Xylaria*, *Daldinia*, some fungi imperfecti).

Class 3. Loculomycetes cl. nov. (diagnosis: ascocarp not apothecial: ascocarpae non apotheciae).

Subclass 1. Dendromycetidae subcl. nov. (diagnosis: lichenized) (e.g. *Arthonia*, *Lecanactis*).

Subclass 2. Loculoascomycetidae Luttrell 1955 (ascocarps pseudothecial within a stroma; asci bitunicate) (e.g. *Dothidea*, *Pleospora*, *Venturia*).

Class 4. Plectomycetes Gwynne-Vaughan 1922 stat. nov. Ainsworth 1966 (ascocarp a cleistothecium; asci unitunicate) (e.g. *Penecillium*, *Aspergillus*, *Eurotium*, *Erysiphe*; most fungi imperfecti).

Phylum 2. Basidiomycota de Bary 1866 em. auct. stat. nov. Moore 1980. [meiotic products form four exospores (ancestrally ballistospores) by budding from the surface of the cell; meiotic endospores absent; dikarya with clamp connections)].

Subphylum 1. Septomycotina*‡ subphyl. nov. (diagnosis: uniporate septa without dolipores; basidia divided by transverse walls, but without long epibasidia; centrosomes flat: septa sine doliporis; muri transversi in basidiis praebens, sine epibasidiis longis; centrosomae complanatae).

Class 1. Septomycetes cl. nov. (diagnosis: centrosomes single layered: centrosomae monostromaticae: name introduced without Latin diagnosis by Cavalier-Smith, 1987*b*; circumscription here narrowed by excluding the non-septate groups now placed in Ustomycetes).

Subclass 1. Sporidiomycetidae Moore 1980 stat. nov. (Erythrobasidiales, Sporidiales).

Subclass 2. Uredomycetidae Brogniart 1824 stat. nov. (flat multilayered centrosomes) [rusts (Uredinales), ballistosporous and some other exosporous yeasts (Septobasidiales)].

Subphylum 2. Orthomycotina subphyl. nov. (diagnosis: uniporate septa with dolipores; centrosomes globular: septa doliporis muri transversi in basidiis praebentes; centrosomae globosae: name introduced without Latin diagnosis by Cavalier-Smith, 1987*b*).

Superclass 1. Hemibasidiomycetia Engler 1897 stat. nov.

Class Ustomycetes Moore 1980 (orders Ustilaginales, Tilletiales).

Superclass 2. Hymenomycetia Fries 1821 stat. nov. et em. (typically with complex basidiocarp; ancestrally with an exposed hymenium, but enclosed in polyphyletically derived subterranean fruiters; soma rarely reduced to yeast phase).

Class 1. Gelimycetes† cl. nov. (diagnosis: basidia divided by vertical walls; gelatinous basidiocarp: muri longitudinali in basidiis praebens; corpus fructorum gelatinosum: name introduced without Latin diagnosis by Cavalier-Smith, 1987*b*) (jelly fungi and related yeasts).

Subclass 1. Tremellomycetidae Fries 1821 stat. nov. Wells 1994 (syn. class Exidiomycetes Moore 1994) subcl. nov. [diagnosis: muri longitudinali in basidiis praebens; corpus fructorum gelatinosus; basidia divided by vertical septa; saprobic jelly fungi (Tremellales) and related yeasts].

Subclass 2. Dacrymycetidae subcl. nov. [diagnosis: basidia furcate; basidia furcata (Dacrymycetales).

Subclass 3. Auromycetidae basidia transversely septate with long epibasidia (Auriculariales): basidia transversaliter septata sed epibasidiiis longis instructa].

Class 2. Homobasidiomycetes Patouillard 1900 (basidia not subdivided; basidiocarp usually non-gelatinous; no yeast phases).

Subclass 1. Clavomycetidae Fries 1821 stat. nov. (e.g. *Clavaria*, *Tulasnella*).

Subclass 2. Pileomycetidae Fries 1821 stat. nov. (e.g. *Fomes*, *Agaricus*, *Coprinus*, *Boletus*, *Lycoperdon*, *Cyathus*; include gasteromycetes, e.g. *Phallus*, *Lycoperdon*, *Cyathus*).

For morphological aspects of the phylogenetic basis of this classification see Cavalier-Smith (1987*b*); for recent molecular sequence evidence on neomycote phylogeny see Sugiyama & Nishida (1995). The nature of the kingdom Fungi and the origin of Microsporidia are discussed in Cavalier-Smith (1998*b*) Some evidence suggests that subphylum Zygomycotina may be polyphyletic (Nagahama *et al.*, 1995; Sugiyama, Nagahama & Nishida, 1996), so it may need to be split in future. Although the names Zygomycotina, Zygomycetes, and Ascomycota have been used widely for years, they appear not to have been validly published, so I validate them here.

* Probably paraphyletic.

† Almost certainly paraphyletic.

form a new subphylum Dictyomycotina, which therefore includes all fungi with well developed Golgi dictyosomes, the ancestral state. Allomycetes are here grouped with the Zygomycotina (here ranked as an infraphylum and emended by removal of the Enteromycetes) in the new subphylum Melanomycotina. The paraphyletic taxon Chytridiomycotina, which has been long used despite never being validly published (for higher taxa mycologists have often ignored the rule of the International Code of Botanical Nomenclature that requires Latin diagnoses for the valid publication of new names) is now abandoned.

The remaining Trichomycetes (Harpellales and Asellariales) are reduced in rank to a superorder (Trichomycetalia: there is no standard mycological suffix for a superorder), and placed in a new zygomycote class exclusively made up of parasites of animals or protozoa, which I call Zoomycetes, and divide into two subclasses. One subclass (Pedomycetidae) includes the Trichomycetalia and a second new superoder (Pyxomycetalia) created for the Laboulbeniales and Pyxidiophorales (Blackwell, 1994), ectoparasites of arthropods formerly regarded as ascomycetes because of the superficial resemblance of their female organs to perithecia. Both Trichomycetalia and Pyxomycetalia attach to their host cuticle by similar foot-like holdfasts (whence the name Pedomycetidae) and have similar imperforate plugged septa and a body form of discrete cells not indefinite hyphae. The other new zoomycete subclass is the Entomycetidae, comprising the orders Entomophorales and Zoopagales, tissue endoparasites of protozoa or invertebrates, which have usually been included in the Zygomycetes. Two other new classes (the saprotrophic Bolomycetes and the endomycorrhizal Glomomycetes) have also been traditionally placed in the Zygomycetes, which are here restricted to Zygomycotina with stalked sporophores bearing passively dispersed aerial spores (e.g. the well-known Mucorales). The present four zygomycote classes are all very much more homogeneous than the traditional Zygomycetes and Trichomycetes. Though there are no sequences yet available for trichomycetes, those for other zygomycetes show their great heterogeneity and justify their subdivision into several classes (Nagahama *et al.*, 1995). The present system, however, emphasizes homogeneity of morphology and does not slavishly follow rRNA trees, since in Fungi these are highly unclocklike, with numerous unusually long branches, and so must in some respects be very misleading.

VII. THE KINGDOM PLANTAE AND ITS FIVE PHYLA

The plant kingdom *sensu* Cavalier-Smith (1981*a*) comprises all organisms possessing plastids with double envelopes that are free in the cytoplasm. Unlike chromists and protozoa, all plants are obligately dependent on plastids and have never lost them even when they have become secondarily nonphotosynthetic: this is probably because they are essential for the synthesis of fatty acids, starch and certain amino acids (Cavalier-Smith, 1993*e*). As the evidence for the single origin of the chloroplast from a cyanobacterium and for the monophyly of Plantae has recently been discussed in detail (Cavalier-Smith, 1995*a*), and the circumscription of Plantae has remained unchanged since Cavalier-Smith (1981*a*), I shall not discuss them further here, except to stress that it is not the presence but the morphology and location of the plastids, together with their obligate nature, that define the kingdom. Table 6 summarizes the classification of the kingdom; from rRNA trees it is clear that the three major lineages, Viridiplantae, Glaucophyta and Rhodophyta diverged almost simultaneously, so closely following the origin of chloroplasts that it is hard to determine their correct branching order or even to corroborate the monophyly of the kingdom by gross sequence similarity; but there is other molecular evidence for the monophyly of Plantae in the present sense (Ragan & Gutell, 1995). The monophyly of Plantae has been questioned on the basis of RNA polymerase sequence trees (Stiller & Hall, 1997), but there are too few taxa yet on these trees to have high confidence in their branching order.

(1) New red algal subphyla

Currently red algae are divided into two classes: the paraphyletic Bangiophyceae and holophyletic Florideophyceae. Bangiophyceae are ultrastructurally so diverse that I divide them into two classes: Bangiophyceae *sensu stricto* (Bangiales, Rhodochaetales) which have pit connections and Rhodellophyceae (Porphyridiales, Cyanidiales, Compsopogonales) which do not. In having pit connections, and on rRNA trees, Bangiales are closer to Florideophyceae (therefore I group them as the new subphylum Macrorhodophtina) than to the Rhodellophyceae, which I place alone in the new subphylum Rhodellophytina. The Porphyridiales are themselves very diverse and should probably be subdivided into more than one order.

Table 6. *Classification of the kingdom Plantae and its five phyla*

Subkingdom 1. Biliphyta* Cavalier-Smith 1981.

Infrakingdom 1. Glaucophyta infrak. nov. (diagnosis: peptidoglycan in plastid envelope: plastidae peptidoglycanum instructae) (glaucophytes).

Phylum Glaucophyta Skuja 1954 (unnecessary syn. Glaucocystophyta Kies & Kremer 1986) (e.g. *Cyanophora*).

Infrakingdom 2. Rhodophyta infrak. nov. (diagnosis: plastid envelope lacks peptidoglycan: sine peptidoglycano).

Phylum Rhodophyta Wettstein 1922 (red algae).

Subphylum 1. Rhodellophytina* subphyl. nov. (diagnosis: unicellular, e.g. *Porphyridium*, or undifferentiated simple or branched uniseriate filaments with a basal disc, e.g. *Stylonema*; pit connections absent: cellulae unicae aut filamentae simplices uniseriatae cum disco basalo: sole class: Rhodellophyceae cl. nov. diagnosis as for subphylum).

Subphylum 2. Macrorhodophytina subphyl. nov. (diagnosis: multicellular with extensive cell differentiation and complex life histories; pseudoparenchymatous or multiseriate filaments or flat sheets of cells; pit connections usually present: thallus multicelllularis; saepe in forma pseudoparenchyma aut filamentae multiseriatae) (classes: Bangiophyceae em.; Floridiophyceae).

Subkingdom 2. Viridiplantae Cavalier-Smith 1981 (green plants).

Infrakingdom 1. Chlorophyta Cavalier-Smith 1993.

Phylum Chlorophyta auct.

Subphylum 1. Chlorophytina subphyl. nov. (diagnosis: cytokinesis without a phragmoplast; multilayered structure (MLS) ciliary roots absent except in *Mesostigma*, *Pterosperma*, *Halosphaera* sine phragmoplastis) (chlorophyte green algae).

Infraphylum 1. Prasinophytae† infraphyl. nov. (diagnosis: usually scaly flagellates with persistent telophase spindle; without phycoplast; Mg 2, 4 divinyl porphyrin often used as a photosynthetic pigment: cellulae cilio aut ciliis instructa, usitate squamatae; fusus persistens in telophaso; sine phycoplasto) [classes Micromonadophyceae Mattox & Stewart 1984 em. with cruciate ciliary roots: orders Mamiellales Moestrup 1984 em., Pyramimonadales em., Mesostigmatales ord. nov. (diagnosis: biflagellates; cruciate ciliary roots with one MLS; tubular ciliary hairs absent: cilia dua; radices microtubulorum in forma crucis cum structura laminarum multarum; cilia sine pilis tubulatis; formerly included in Pyramimonadales); Nephrophyceae Cavalier-Smith 1993, with asymmetric microtubular roots, e.g. *Nephroselmis*, *Pseudoscourfieldia*].

Infraphylum 2. Tetraphytae infraphyl. nov. (diagnosis: cruciate microtubular roots without MLS; telophase spindle collapses; cytokinesis involves a phycoplast: radices microtubulorum in forma crucis; sine structura laminis multis) (e.g. *Ulva*, *Chlamydomonas*, *Chlorella*; type genus *Tetraselmis*).

Subphylum 2. Phragmophytina subphyl. nov. (diagnosis: vegetative cells non-motile; zoospores or sperm with asymmetric ciliary roots typically with MLS; cytokinesis usually involves a phragmoplast) (to avoid ambiguity it is best to call these plants phragmophytes informally, and to reserve the term charophyte strictly for the infraphylum Charophytae).

Infraphylum 1. Charophytae Engler 1887 stat. nov. (haploid multicellular macrophytes with main axis corticated with whorls of lateral branches; with oogonia and antheridia surrounded by sterile cells) (class Charophyceae *sensu stricto*: order Charales: stoneworts – the traditional charophytes).

Infraphylum 2. Rudophytae infraphyl. nov. [diagnosis: antheridia absent or without investing sterile cells: antheridia non cellulis sterilis circumcincta: (descriptive name: suggested vernacular term: rudophytes, meaning simple – Latin rudis – plants)][classes Eophyceae cl. nov. (diagnosis: with biciliate zoospores or sperm: zoosporae aut gametae masculinae ciliis instructae: the name eophyte is chosen because the Chaetophycidae may be ancestral to land plants: Graham, 1993) subclasses Stichophycidae subcl. nov. (diagnosis: sarcinoid or unbranched filaments: cells lack sheathed hairs: thallus non ramosus; cellulae sine setis vaginatis; descriptive name to indicate that the most complex forms have only a single row – stichus – of cells) (orders Chlorokybales, Klebsormidiales); Chaetophycidae cl. nov. (diagnosis: many cells have protective sheathed hairs (chaetae): cellulae setis vaginatis munitae) branched filaments or parenchymatous with sheathed hairs (Coleochaetales); Conjugophyceae auct. (all cells, including gametes, lack cilia) (Desmidiales, Zygnematales)].

Infrakingdom 2. Cormophyta Endlicher 1836 (syn. Embryophyta auct.) stat. nov.

Phylum 1. Bryophyta Braun 1864 em. Eichler 1883 (hornworts, liverworts, mosses).

Subphylum 1. Hepaticae auct. stat. nov. (liverworts, e.g. *Riccia*, *Marchantia*, *Lophocolea*).

Subphylum 2. Anthocerotae auct. stat. nov. (e.g. *Anthoceros*).

Subphylum 2. Musci Linnaeus 1753 stat. nov.

Infraphylum 1. Sphagneae auct. stat. nov. (leaves with large porous dead cells as well as living ones; *Sphagnum*).

Table 6. (*cont.*)

Infraphylum 2. Bryatae infraphyl. nov. (diagnosis: cells in leaves all alive with chloroplasts: cellulae omnis in laminis chloroplastis instructae) (e.g. *Andreaea*, *Bryum*, *Funaria*).

Phylum 2. Tracheophyta phyl. nov. (diagnosis: diploid phase with xylem and phloem: facies diploida xylem et phloem instructa: name introduced without Latin diagnosis by Sinnott, 1935).

Subphylum 1. Pteridophytina† Eichler 1883 stat. nov. (pteridophytes).

Infraphylum 1. Psilophytae infraphl. nov. (sine radicibus; without roots, e.g. *Psilophyton*, *Psilotum*).

Infraphylum 2. Lycophytae auct. stat. nov. (e.g. *Lepidodendron*, lycopods, *Selaginella*, *Isoetes*).

Infraphylum 3. Sphenophytae auct. stat. nov. (e.g. *Sphenophyllum*, horsetails).

Infraphylum 3. Filices* Linnaeus 1753 stat. nov. (ferns).

Subphylum 2. Spermatophytina auct. stat. nov. (seed plants).

Infraphylum 1. Gymnospermae auct. ('seed ferns', cycads, conifers, gnetophytes).

Infraphylum 2. Angiospermae auct. (dicot and monocot flowering plants).

(2) New chlorophyte infraphyla

It has long been accepted that the ancestral green plants are the scaly prasinophyte algae (Mattox & Stewart, 1984), and that there was a basic bifurcation within green algae between the Chlorophytina and Charophytina. Because cormophytes evolved from a charophytine algae, Charophytina have often been treated as a separate phylum (Jeffrey, 1971; Cavalier-Smith, 1993*e*, 1995*a*) to enable Charophyta and Cormophyta to be grouped together as an infrakingdom Streptophyta. It now appears that the prasinophyte *Mesostigma* is cladistically closer to the traditional charophytes than to the other prasinophytes (Melkonian, Marin & Surek, 1995). Because of this and the fact that it is the only prasinophyte with a multilayed structure, it was recently transferred from the Chlorophyta to the Charophyta (Cavalier-Smith, 1995*a*). However, this further diminishes the phenotypic difference between these two major green algal taxa; therefore though the recognition of two green algal phyla is cladistically attractive it creates a boundary at a point where the phenotypic difference seems too slender to justify separate phyla. Therefore I revert to the more traditional simpler system with a single green algal phylum Chlorophyta divided into two new subphyla. Streptophyta remains as a clade name only.

As before (Cavalier-Smith, 1993*e*), Chlorophytina are divided into two major taxa (now ranked as infraphyla) according to their cell division mechanism: Prasinophytae (biciliate or uniciliate with persistent spindle) and Tetraphytae with collapsing spindle and phycoplast. Charophytina are also subdivided into two infraphyla: Charophytae and Coleophytae.

VIII. CHROMISTA, THE THIRD BOTANICAL KINGDOM, AND ITS FIVE PHYLA

Most chromists are algae with chloroplasts containing chlorophylls *a* and *c* which are located not in the cytosol (as in plants or in protozoan algae) but within the lumen of the rough endoplasmic reticulum. In addition to their double chloroplast envelopes, chromistan plastids are surrounded by an additional smooth membrane, the periplastid membrane (Cavalier-Smith, 1989*b*). All chromistan algae are evolutionary chimaeras between a eukaryotic host and a eukaryotic (probably red algal) symbiont; the periplastid membrane is the relic of the red algal plasma membrane. The unique location of plastid plus periplastid membrane within the rough endoplasmic reticulum arose by the fusion of the former phagosomal membrane that first enclosed the endosymbiont with the nuclear envelope (Whatley, John & Whatley, 1979; Cavalier- Smith, 1982, 1986*a*). The evidence for the monophyly of the Chromista is discussed in detail elsewhere (Cavalier-Smith, 1995*a*). Recent molecular evidence (Cavalier-Smith, Allsopp & Chao 1994*a*; Cavalier-Smith & Chao, 1997*a*) makes it clear that chlorarachnean algae, originally excluded from Chromista but temporarily transferred into this kingdom because of misleading rRNA trees (Cavalier-Smith 1993*a*, 1994) are actually Protozoa, as considered previously (Cavalier-Smith 1986*a*).

Chromist monophyly remains controversial for two reasons; one is that the three major chromist groups (Cryptista, Heterokonta and Haptophyta; see Table 7) diverged from each other almost simultaneously with the three major plant lineages, and the six lineages can appear to diverge in almost

Table 7. *Classification of the kingdom Chromista and its five phyla*

Subkingdom 1. Cryptista Cavalier-Smith 1989.
Phylum Cryptophyta Cavalier-Smith 1986 (e.g. *Cryptomonas*, *Goniomonas*).
Subkingdom 2. Chromobiota Cavalier-Smith 1991.
Infrakingdom 2. Heterokonta Cavalier-Smith 1986 stat. nov. 1995 em.
Superphylum 1. Sagenista superphyl. nov. (Diagnosis as for phylum Sagenista Cavalier-Smith 1995*b* p. 1014).
Phylum Sagenista Cavalier-Smith 1995.
Subphylum 1. Bicoecia Cavalier-Smith 1989 [orders Bicoecales Grassé & Deflandre 1952 (*Bicoeca*), Anoecales Cavalier-Smith 1997 (e.g. *Cafeteria*); Pirsoniales ord. nov. [diagnosis: syncytial non-motile trophic phase that feeds on diatoms; with trophosome inside and auxosome outside the frustule; zoospores with non-tubular hairs on cell body: diatomas devorant; in statu pabulatorio cellulae nuclei plures instructae; trophosoma sine nucleis intra frustulo, auxosoma multinucleata extra frustulo; zoosporae pili non-tubulati vestitae) (sole family Pirsoniaceae fam. nov. diagnosis as for order Pirsoniales: type genus *Pirsonia*].
Subphylum 2. Labyrinthista Cavalier-Smith 1986 stat. nov. 1989 (e.g. *Labyrinthula*, *Thraustochytrium*).
Superphylum 2. Gyrista superphyl. nov. (diagnosis: ciliary transition region commonly with a helix – or a double helix/ring system: regio transitoria ciliorum plerumque helicem aut helices praebens).
Phylum 1. Ochrophyta* Cavalier-Smith 1986 (as Ochrista) stat. nov. 1995.
Subphylum 1. Phaeista Cavalier-Smith 1995.
Infraphylum 1. Hypogyrista Cavalier-Smith 1995 (e.g. pedinellids, silicoflagellates, pelagophytes).
Infraphylum 2. Chrysista Cavalier-Smith 1986 stat. nov. 1995 (superclasses Phaeistia Cavalier-Smith 1995, e.g. brown algae, xanthophytes, *Chrysomeris*; Limnistia Cavalier-Smith 1996, e.g. chrysophytes, eustigs, *Oikomonas*, raphidophytes).
Subphylum 2. Diatomeae Dumortier 1821 stat. nov. Cavalier-Smith 1995 (centric and pennate diatoms).
Phylum 2. Bigyra phyl. nov. (diagnosis: ciliary transition region with double helices or concertina-like rings; without plastids: regio transitoria ciliorum annuli in forma concertinae praebens; sine plastidis).
Subphylum 1. Bigyromonada subphyl. nov. (diagnosis: biciliate free-living bacterivorous phagotrophs without cell walls; retronemes on anterior cilium; double ciliary transition helix: cilia dua; murus absens; mastigonemae tubulatae in cilium anterius; pabulum cellulae prokaryotae est; regio transitoria ciliorum annuli in forma concertinae praebens) (*Developayella* Tong 1995).
Subphylum 2. Pseudofungi Cavalier-Smith 1986 em. 1989 (oomycetes, hyphochytrids).
Subphylum 3. Opalinata Wenyon 1926 stat. nov. em. Cavalier-Smith 1993, 1997 (diagnosis: retronemes absent from cilia; gut commensals without peroxisomes: sine mastigonemis tubulatis in ciliis) (*Proteromonas*, *Karotomorpha*, opalinids).
Infrakingdom 2. Haptophyta Cavalier-Smith 1995.
Phylum Haptophyta Cavalier-Smith 1986 (e.g. *Pavlova*, *Prymnesium*).

For a more detailed classification of Ochrophyta and Haptophyta see Cavalier-Smith & Chao (1996*b*) and Cavalier-Smith *et al*. (1996*d*) respectively.
* Probably paraphyletic.
† Almost certainly paraphyletic.

any order on different molecular trees; the other is that all three of the major lineages contain some non-photosynthetic species, and it has been hard to establish whether these are primarily or secondarily photosynthetic. Many authors, following Margulis (1970) and Whatley *et al*. (1979), have assumed that all are primarily non-photosynthetic and that there were three (or even more) independent implantations of eukaryotic algae into unrelated protozoan hosts. In contrast, I have argued that all are secondarily non-photosynthetic and originated by several independent losses of chloroplasts and that all chromists had a common photophagotrophic ancestor – the first eukaryote-eukaryote chimaera that rapidly diverged into the three major lineages (Cavalier-Smith, 1982, 1986*a*). Given our present knowledge of chromist phylogeny, one has to postulate a minimum of six independent losses of chloroplasts within the kingdom.

(1) Multiple losses of chloroplasts by chromists

Evidence for chloroplast loss by chromists is growing; it is strongest for the Heterokonta. We now have strong molecular phylogenetic evidence for three losses (two in pedinellids: Cavalier-Smith, Chao & Allsopp, 1995; Cavalier-Smith & Chao, 1996*b*; and

one in *Oikomonas*: Cavalier-Smith *et al*., 1996*b*) within the predominantly algal phylum Ochrophyta (Cavalier-Smith & Chao, 1996*b*). We have weaker evidence for a third loss in the ancestor of the Pseudofungi (which comprise the classes Oomycetes and Hyphochytrea, traditionally thought of as fungi), which may have evolved from an ochrophyte algal ancestor by plastid loss (Cavalier-Smith *et al*., 1996*b*). Recent maximum likelihood trees for rRNA also suggest that *Goniomonas*, the sole aplastidic genus in the phylum Cryptophyta, may also have arisen by plastid loss (Cavalier-Smith *et al*., 1996*c*). Thus, we have strong evidence from the internal phylogeny of the Heterokonta and Cryptista for three, and some evidence for five, of the six plastid losses required by the theory of a photophagotrophic latest common ancestor for chromists. We lack such internal evidence only for one established chromist group, the phylum Sagenista (bicoecids, thraustochytrids and labyrinthulids), which diverges from other heterokonts near the base of the heterokont clade on rRNA trees, before the earliest divergences within the mostly photosynthetic Ochrophyta. Since molecular trees (contrary to what has been implied by some authors) do not refute the quite strong morphological and chemical evidence for the monophyly of Chromobiota (discussed in detail by Cavalier-Smith, 1994; Cavalier-Smith *et al*., 1996*d*), it is much more parsimonious to postulate that Sagenista also have lost chloroplasts than to suggest that Ochrophyta and Haptophyta acquired indistinguishable fucoxanthin-containing chloroplasts independently, as some authors (e.g. Leipe *et al*., 1994; Bhattacharya & Medlin, 1995) assume.

(2) Transfer of Opalinata to Chromista: the new heterokont phylum Bigyra

When the kingdom Chromista was first established (Cavalier-Smith, 1981*a*) and its origin and systematics discussed in detail (Cavalier-Smith, 1986*a*) it was explicitly recognized that some organisms not then placed in the kingdom might actually belong there if they had secondarily lost both the defining synapomorphic characters that were used to define it (plastids within periplastid membrane inside rough endoplasmic reticulum and bipartite or tripartite ciliary hairs). The possibility that proteromonads were really chromists, not protozoa (Cavalier-Smith, 1981*a*), was suggested by their bipartite tubular body hairs and ciliary transition helix. At present, proteromonads are included with Opalinea, which lack tubular hairs, in the subphylum Opalinata (Cavalier-Smith, 1997*a*). For reasons discussed elsewhere, I have recently left this taxon outside the kingdom Protozoa (Cavalier-Smith, 1997*a*). I now place this subphylum within the Chromista in the infrakingdom Heterokonta. This change is made as a result of the recent discovery of a distinctive new type of heterotrophic heterokont, *Developayella elegans* (Tong, 1995), and a reappraisal of the evolution of the ciliary transition region and cell surface in chromists.

Developayella elegans resembles both Pseudofungi and Opalinata in having a double helix distal to the transverse plate in the ciliary transitional region, which somewhat resembles a concertina in longitudinal section. The structure of the ciliary transition region has proved to be an excellent phylogenetic marker in the past; for example the nine-fold star that characterizes the subkingdom Viridiplantae (Manton, 1965; Cavalier-Smith, 1981*a*). Recently, I have given it considerable weight in revising the higher level classification of the heterokont algal phylum Ochrophyta (earlier spelled Ochrista) (Cavalier-Smith, 1995*a*). I have divided the ochrophyte subphylum Phaeista into two infraphyla: Hypogyrista, characterized by a short single ciliary transitional helix located below (proximal to) the transition plate and Chrysista characterized by a longer single helix above the transition plate. These differences are congruent with other morphological differences and with molecular phylogeny (Cavalier-Smith & Chao, 1996*b*). It therefore seems likely that the double transitional helix is also a strong phylogenetic character. Accordingly, I place *Developayella elegans*, Pseudofungi and Opalinata together to form a new heterotrophic heterokont phylum, the Bigyra; the name refers to the two helices or sets of rings (which interpretation is correct is not entirely clear) in the ciliary transition region (from Latin *bi-*, meaning 'twice', and *gyrus*, meaning 'circle' or 'ring'; pronounced 'Bi- ji-ra'). This change accepts Patterson's (1989) view that the tubular somatonemes of the genus *Proteromonas* evolved from heterokont ciliary retronemes, not *vice versa*, and that the absence of retronemes or somatonemes in Opalinea is the result of secondary loss.

I have long considered that the bipartite hairs of the genus *Proteromonas* and chromists are homologous; Opalinata must either be sisters of Chromista or derived from them. They are unlikely to be directly ancestral to them since they lack both peroxisomes and phagotrophy and are all gut

commensals of tetrapod vertebrates. A free-living heterotrophic flagellate with tubular body hairs like proteromonads but with phagotrophy and peroxisomes would be a suitable host for the origin of chromists as postulated previously (Cavalier-Smith, 1986*a*, 1989*b*); however, flagellates with this exact combination of characters are not currently known and I now accept Patterson's (1989) alternative hypothesis that proteromonads are derived from heterokonts by transfer of ciliary hairs to the cell body. My original interpretation of chromist origins implicitly viewed Proteromonadida as a sister group to Chromista (Cavalier-Smith, 1986*a*); however, I overlooked the fact that the long-known presence of a double transitional helix in Opalinata (Brugerolle & Joyon, 1975) made this assumption inconsistent with my proposal in the same paper that the differing transition region structures of the Heterokonta, Cryptista and Haptophyta arose independently during the divergence of these three taxa from the ancestral chromist. One of these two contradictory phylogenetic hypotheses must be wrong, unless the double helical structures of Opalinata and Heterokonta are convergent rather than homologous, which is possible but seems unlikely. If Opalinata were in fact sisters to, rather than derived from, the chromists the ancestral chromist must have had a double transition helix and this must have been replaced by quite different structures on three separate occasions: the double plates of cryptomonads and haptophytes and the bell-shaped structure of Labyrinthulea.

Originally, I assumed that the bell-shaped structure of Labyrinthulea was derived from the single transitional helix of Chrysista and was related to the oomycete/hyphochytrid double helix, and therefore placed Labyrinthulea within the subphylum Pseudofungi (Cavalier-Smith, 1986*a*). Later (Cavalier-Smith, 1989*b*), I became very sceptical of this relationship and removed Labyrinthulea from Pseudofungi as a separate subphylum Labyrinthista, and suggested that Labyrinthista diverged very early in heterokont evolution before the divergence of the major classes of ochrophyte algae and the origin of Pseudofungi from a xanthophyte-like ochrophyte by the loss of photosynthesis. Recent molecular evidence strongly supports this early divergence of the Labyrinthista (Cavalier-Smith, Allsopp & Chao, 1994*b*; Leipe *et al*., 1994), and shows that Pseudofungi are not specifically related to Labyrinthista and may indeed be secondarily derived by plastid loss from a phaeistan algal ancestor (Cavalier-Smith *et al*., 1996*b*). This early divergence of labyrinthists and later origin of pseudofungi considerably strengthens Patterson's (1989) postulate that the ciliary double transitional helix is a derived condition within the Heterokonta. As there seem no obvious reasons for a replacement of an ancestral double helix by the different structures of the Cryptista, Haptophyta, labyrinthists and hypogyrists, I prefer to accept Patterson's interpretation and to abandon the idea that retronemes evolved from somatonemes.

(3) The origin of ciliary retronemes and the nature of the ancestral chromist

The alternative interpretation for the origin of ciliary retronemes is that they evolved from pre-existing non-tubular ciliary hairs (Cavalier-Smith, 1989*b*). Originally I did not favour this view because one could not envisage a gradual origin for them in this way because of their thrust-reversing properties, whereas a gradual origin for tubular somatonemes was more plausible (for the detailed arguments see Cavalier-Smith, 1986*a*: 317–320). However if one accepts, as I think we now must, a more saltatory origin by a single mutation converting a non-tubular hair into a rigid tube, which is not mechanistically impossible, one can envisage an origin *in situ* on the ciliary surface for functional tubular retronemes. Simple non-tubular hairs suitable as ancestors for retronemes exist in both the Glaucophyta (in *Cyanophora*) and in the dinoflagellate and protalveolate Dinozoa. It is therefore highly probable that such hairs were present in the common ancestor of Plantae and Alveolata in which chloroplasts are postulated to have first evolved (Cavalier-Smith, 1982, 1993*e*, 1995*a*). This favours the idea that the host for the symbiogenetic origin of chromists may not have been a heterotrophic protozoan (Whatley *et al*., 1979; Gibbs, 1981) but an early photosynthetic transitional alga in the actual process of converting the ancestral cyanobacterium into the first chloroplast, a possibility first suggested by Cavalier-Smith (1986*a*) and elaborated in detail by Häuber *et al*. (1994).

The usual tendency of the three major plant groups and three major chromist groups to intermingle on rRNA trees strongly supports the view (Cavalier-Smith, 1989*b*) that the basal diversification of both plants and chromists was virtually simultaneous (Cavalier-Smith, 1986*a*, 1995*a*). If the ancestral host was an early plant that arose after the initial divergence of glaucophytes, rhodophytes and Viridaeplantae, then the kingdom Plantae would

actually be paraphyletic rather than holophyletic. If on the other hand the host was an early photosynthetic intermediate between dinoflagellates and plants, then Plantae would be holophyletic; in the latter case, the host might have contributed the chlorophyll *c* of chromists and the red algal symbiont the phycobilins. Current molecular evidence cannot decide between these two possibilities; nor can it rule out the third possibility that the host was a heterotrophic non-alveolate zooflagellate protozoan, since only a tiny proportion of such protozoa have been placed on the rRNA trees. On many trees, alveolates branch within the plant/chromist assemblage whereas on others they are an outgroup. It is very important for our understanding of algal evolution to determine which position is correct. Unless future research does identify a non-alveolate zooflagellate as an outgroup to chromists, the idea that the host was either an early plant that had not yet abandoned phagotrophy or an early algal alveolate will remain an attractive working hypothesis.

A unique heterokont genus possibly significant in relation to the origin of retronemes is *Pirsonia*. There are several species of these little-known phagotrophic aplastidic chromists; all feed on diatoms by piercing their frustule by means of a non-motile syncytial trophic phase that is bipartite, having a trophosome within the frustule and auxosomes that remain outside: their biciliate zoospores have non-tubular hairs on their cell body and both cilia, in addition to the usual tripartite retronemes on the anterior cilium only (Schnepf & Schweikert, 1997). One species also has dissimilar unipartite tubular hairs on both cilia (Schweikert & Schnepf, 1997); whether or not these are related to retronemes is unclear, as is the phylogenetic position of *Pirsonia* itself. It is sufficiently different from all other heterokonts that I place it in a new order, Pirsoniales (Table 7). Provisionally I include Pirsoniales as a third order of the class Bicoecea of the purely heterotrophic phylum Sagenista (Cavalier-Smith, 1997*c*), on the assumption that it is an early diverging heterokont. However a more derived position within the predominantly photosynthetic phylum Ochrophyta cannot yet be ruled out, even though Ochrophyta at present contain no totally aplastidic heterotrophic biciliate taxa. It is likely that a number of presently ill-characterized heterotrophic protists will eventually be assignable to the Sagenista. Pirsoniids are yet another example of the considerable diversity of non-algal body forms within the kingdom Chromista.

(4) Mitochondrial and cell-surface evolution in chromists

A second difficulty with the idea that Opalinata are an outgroup to the Chromista concerns the evolution of mitochondrial cristae, which are flattened tubules in cryptomonads but rounded tubules in Opalinata and Chromobiota. This problem was avoided originally by assuming that the origin of chromists took place very shortly after the origin of mitochondria, before the morphology of cristae was stabilized, and that cristae developed independently to their modern form in Opalinata, Chromobiota and Cryptista (Cavalier-Smith, 1986*a*). During the past decade, however, molecular evidence has steadily grown for the alternative view (Margulis, 1970; Taylor, 1974) that mitochondria evolved substantially earlier than chloroplasts. In particular, it is increasingly clear that tubular cristae evolved substantially before the origin of chromists and that the cryptist tubules must have undergone secondary flattening (Cavalier-Smith, 1991*d*). In itself, this phylogenetic conclusion poses no problem for the idea that Opalinata are sisters to chromists, since such secondary flattening must have occurred irrespective of whether or not Opalinata are sisters to chromists.

However, my recent mechanistic interpretation of the reasons for this secondary flattening (Cavalier-Smith, 1997*a*) make much more sense if Opalinata are derived from typical heterokonts than if they are the chromist outgroup. Based on recent rRNA evidence that the common ancestor of the four higher kingdoms of life was probably a somewhat amoeboid zooflagellate with a relatively fluid cell surface, I have postulated that cryptist proteinaceous pellicular plates evolved to stabilize the cell surface following the acquisition of a plastid and de-emphasis of phagotrophy, and that mutations causing the associated changes in the plasma membrane pleiotropically affected the mitochondrial cristae by flattening them. If, however, the chromist ancestor already had an extensive cortical investment of microtubules as in Opalinata, there would have been relatively little need for the origin of the cryptist pellicular plates. Moreover, one might have expected such a useful cortical skeleton to have been retained more widely in Chromista if such plates were the ancestral state, as they have been for example in the Euglenozoa. Both the opalinate microtubular bands and the cryptist plates are therefore probably derived within Chromista.

Because my molecular coevolution theory of the significance of the major changes in morphology of

mitochondrial cristae (Cavalier-Smith, 1997*a*) is, at present, only an initial hypothesis, not a well-corroborated interpretation, and because it is still unclear how much earlier mitochondria arose than plastids, these considerations clearly have less weight than those concerning the transition helix phylogeny discussed above. Since, however, they point in the same direction they add some additional strength to the conclusion that Opalinata are probably heterokonts.

(5) Retroneme loss in Opalinata

I have argued that the loss of ciliary retronemes by phagotrophic heterokonts is most unlikely, because it would reverse the direction of their ciliary feeding current and so cause starvation unless accompanied by the evolution of a novel mode of feeding (Cavalier-Smith, 1986*a*). I previously postulated such loss only for the origin of the Haptophyta, in which the origin of the haptonema for feeding could have allowed the loss of retronemes that I have argued were present in the ancestral chromobiote (Cavalier-Smith, 1994). Retroneme loss is also highly unlikely in phototactic heterokont algae or in any other heterokonts with well-developed tactic behaviour, since it would reverse the direction of swimming in response to environmental stimuli. Retroneme loss is therefore highly improbable in any free-living heterokont flagellate.

I postulate that the conversion of retronemes to somatonemes uniquely in *Proteromonas* was possible as a direct consequence of their colonization of the gut of tetrapods. This novel habitat involved two major changes compared with free-living life: first, being surrounded by an isotropic food supply, a ciliary feeding current became irrelevant and the flagellate evolved pinocytotic feeding over its whole body surface, rather than phagocytosis near the ciliary base as in the ancestral heterokont. Whether or not it had already lost phagocytosis and become a saprotroph like pseudofungi before becoming a gut symbiont is less important than the fact that, at some stage, the loss of phagotrophy removed one source of stabilizing selection for the retention of ciliary retronemes. The second change was that entry into an isotropic milieu probably made tactic behaviour redundant, thus removing the second major source of selection for retroneme retention. Thus, selection would no longer have opposed the total loss of retronemes or their movement onto the body surface.

Why were retronemes moved onto the body surface to become somatonemes, not simply lost? In other words what is their function? I suggest that they serve to keep coarse and sharp food particles in the host gut that are too large for the flagellate to ingest away from its cell surface where pinocytosis takes place. This would have two significant advantages: possibly the most important would be to prevent large inedible particles from adhering to the cell surface or simply blocking the pinocytotic uptake of small digestible particles by their very close proximity to the cell surface; this could subtantially increase the effective rate of feeding by pinocytosis. Secondly, the hairs could reduce the chance of damage to the delicate pinocytotic regions of the plasma membrane by either the physical impact or chemically harmful character of large inedible particles or by the attempts of the cell to ingest larger particles than it could handle.

If somatonemes have these advantages to *Proteromonas*, how is it that they were lost by Opalinea? I suggest that their function was replaced by the deep cortical folds that characterise the class Opalinea (both opalinids and the quadriciliate genus *Karotomorpha*). These cortical folds supported by a column of microtubules are convergent with the actin-supported cortical folds of the apicomplexan gregarine protozoa, which are by far the largest protozoan cells that inhabit animal guts and which also feed pinocytotically. This common occurrence of cortical folds in two such disparate groups strongly suggests a similar function. The primary function in both, I suggest, was to keep large inedible particles away from, and to allow rapid access of small edible particles to, the sites of pinocytosis; in Opalinea these are in the troughs of the cortical folds (Patterson, 1985) as they also are in gregarines. Clearly, they also have a skeletal function and have been a mechanical preadaptation that has allowed both opalinids and gregarines to grow to a huge size compared with most protist cells. However, the fact that the folds are also found in the relatively small flagellate *Karotomorpha bufonis* suggests that their primary function was, as postulated here, to prevent the occlusion of and/or damage to the pinocytotic sites by large inedible particles in the host's gut. The fact that the folds replaced the proteromonad somatonemes suggest that they perform these functions more efficiently; possibly they are more rigid and less easily pushed aside by large particles in the churning gut.

The cortical microtubules of *Proteromonas* to which the somatonemes are attached were however a preadaptation for the origin of the microtubular columns that support the cortical folds. The likely

absence of such cortical microtubules in the ancestor of gregarines would explain why their folds are instead supported by actin microfilaments. Likewise the attachment of retronemes to ciliary microtubules was a preadaptation to their subsequent attachment to cortical microtubules.

The loss of somatonemes would in principle be easier than the loss of retronemes as it would not reverse the direction of ciliary hydrodynamic flow. Thus the conversion of retronemes to the somatonemes of *Proteromonas* facilitated their subsequent total loss.

After the present paper was submitted rRNA sequence evidence was published (Silberman *et al*., 1996) showing that *Proteromonas lacertae-viridis* is specifically related to the heterokonts, and is therefore not an outgroup for chromists as a whole. This adds further support to the arguments given above for the view that retronemes were secondarily transferred from the cilia to the cell surface of the ancestral opalinate, and for transferring the Opalinata as a whole into the Heterokonta; however, although *Proteromonas* clearly clusters with the undoubted heterokonts, the published tree does not actually group it with the Pseudofungi as would be expected if the Bigyra are holophyletic, as postulated here. Since however the bootstrap values for the branching order at the base of the Heterokonta are low, and since the deepest branches are rather long, it is possible that some or even all of them are placed too low in the tree, as may be often be true for the Pseudofungi themselves (Cavalier-Smith *et al*., 1996*b*). Additional evidence is therefore needed to test the monophyly of the Bigyra. Silberman *et al*. (1996) also show that the non-ciliated gut symbiont *Blastocystis* is related to *Proteromonas*, which was entirely unexpected. This means that despite the absence of cilia *Blastocystis* should be placed in the Opalinata, and that it is not a fungus or protozoan as previously supposed. As it differs from all other Opalinata in the absence of cilia I place it in a new class, Blastocystea (diagnosis: *sine ciliis* – without cilia). *Blastocystis* is the first chromist known to parasitize humans. These new data are discussed in more detail elsewhere (Cavalier-Smith, 1997*b*).

(6) Are there other unidentified chromists lurking within the kingdom Protozoa?

For the reasons discussed above, the direct loss of retronemes by phagotrophic heterokonts is so improbable that it is unlikely that any of the well characterized zooflagellate protozoa are really heterokonts or cryptophytes that have directly lost retronemes, though such loss is not impossible if an alternative mode of feeding and/or taxis evolved at the same time. The remote possibility that foraminifera are heterokonts (Patterson, 1989) is now excluded by rRNA phylogeny (Pawlowski *et al*., 1994, 1996; Wray *et al*., 1995). Haptophytes are, however, known to be able to lose the haptonema entirely and some have lost the patelliform scales found in most Prymnesiophyceae (Cavalier-Smith *et al*., 1996*d*); moreover a few are non-photosynthetic (Marchant & Thomsen, 1994). A non-photosynthetic haptophyte that had lost both the haptonema and scales could easily be wrongly classified as a protozoan. Although there is no positive reason to think that any such misclassified haptophytes exist, the case of the haptonema-less haptophyte *Reticulosphaera japonensis* that was misclassified as a heterokont (Grell, Heini & Schüller, 1990) before molecular evidence for its true nature was found (Cavalier-Smith *et al*., 1996*d*) should alert one to the possibility that some zooflagellates presently thought of as protozoa might actually be drastically altered haptophytes.

Though the loss of retronemes must be very rare, it is, in principle, much easier for chromists to lose their flagella totally. This has long been known to have occurred in pennate diatoms and probably in *Chrysamoeba*, and has recently been demonstrated for *Pelagococcus* and *Aureococcus*, though as these are all photosynthetic they have not been mistaken for protozoa. If photosynthesis were also lost and the organism had no other characters identifying it as a chromist it could be wrongly treated as a non-flagellate protozoan, just as was done for *Blastocystis*. It is conceivable that at least a few others of the numerous parasitic protists of uncertain taxonomic position listed by Patterson (1994) might actually be chromists. Several hundred protist genera (those listed in Table 5 of Patterson, 1994, minus a few like *Blastocystis*, *Dermocystidium* and *Trimastix*, which have now been put in phyla) are so poorly studied that they cannot with confidence be placed in any of the kingdoms or phyla of the present system, and mostly not yet been placed in any suprageneric taxon. Though the vast majority of these genera will probably turn out to belong somewhere in the infrakingdom Sarcomastigota of the subkingdom Neozoa of the kingdom Protozoa (most I suspect in the Cercozoa), a small minority might turn out to be chromists, once they are studied by molecular phylogenetics.

Patterson (1989) reasonably suggested that *Diplo-*

phrys and *Sorodiplophrys* may actually be non-flagellate thraustochytrids, but molecular evidence is required to confirm or refute this. One other organism (*Corallochytrium limacisporum*) initially treated as a non-flagellate thraustochytrid (Raghu-Kumar, 1987) has recently been shown by rRNA phylogeny to be a non-flagellate choanozoan protozoan instead (Cavalier-Smith & Allsopp, 1996). Earlier Davidson (1982), Patterson & Fenchel (1985) and Patterson (1986) proposed that actinophryid heliozoa were non-flagellate heterokonts; though Patterson (1994) appears no longer to support this unconvincing view, the true position of actinophryids still needs to be checked by rRNA phylogeny. Patterson's (1994) informal unranked group 'stramenopiles' is identical in phylogenetic concept to the infrakingdom Heterokonta. Apart from the exclusion of *Blastocystis*, which prior to the rRNA evidence mentioned above showed no obvious signs of its heterokont affinities, the inclusion of *Reticulosphaera*, which is now known to be an error, and *Commation*, and the uncertainty about the position of *Diplophrys*, Patterson's (1994) 'stramenopiles' is also identical in composition to the infrakingdom Heterokonta as revised here. I do not accept the inclusion of *Commation* in Heterokonta (Thomsen & Larsen, 1993; Patterson, 1994), as I am unconvinced that these protists have bipartite or tripartite retronemes; I have placed *Commation* instead in its own order within the protozoan phylum Neomonada in the class Kinetomonadea (Cavalier-Smith, 1997*a*). In view of the ultrastructural similarities with Heliomonadida, I have grouped Heliomonadida and Commatiida together as the subclass Ramicristia (Cavalier-Smith, 1997*a*). The reasons for not using the unnecessary new synonym stramenopile in preference to the classical term heterokont were expounded previously (Cavalier-Smith, 1993*a*).

In sum, though the Chromista may, in future, need to be augmented slightly by the addition of a few misplaced heterotrophs, it is unlikely that the boundary between Chromista and Protozoa will change substantially, and even possible that both kingdoms will be entirely stable in circumscription in the future.

IX. ENVOI

In the present six-kingdom system the only changes in the circumscription of the six kingdoms from that of Cavalier-Smith (1983*a*) are the transfer of Opalinata from Protozoa to Chromista; of Microsporidia from Protozoa to Fungi; and of Myxozoa and Mesozoa from Protozoa to Animalia. One can expect the boundaries of the six kingdoms to be even more stable in the future. While there remains a need for a yet more thorough testing of the monophyly of Plantae and Chromista, the present six-kingdom system is phylogenetically and taxonomically sounder, for the reasons discussed previously (Cavalier-Smith, 1986*a*, 1993*a*), than the five-kingdom system found in its numerous mutually contradictory variants in most textbooks. It is also distinctly simpler, and so practically more convenient and easier for beginning students and general users to comprehend, than the phylogenetically congruent eight-kingdom system that I advocated from 1987 to 1995. I hope that it will be widely adopted.

The major advances over the past 15 years have been in the phylogenetically sounder definition of the phyla, subphyla and infraphyla, involving many new creations, subdivisions and mergers of major taxa, mainly in the bacteria, protozoa and heterokont Chromista, and in the definition of subkingdoms and infrakingdoms of all kingdoms. There is probably still significant scope for further improvements in these respects, especially amongst the neozoan protozoa, and a need for more detailed testing of the recent changes, but it is likely that the spate of new creations of higher level taxa that has accompanied the recent extension of electron microscopy and molecular phylogeny into previously unexplored territory will be much reduced in the ensuing decades, and we can look forward fairly soon to a period of consolidation and relative stability in the classification and nomenclature of the major groups of life.

X. CONCLUSIONS

1. An outline classification of a revised six-kingdom system of life is presented, down to the level of infraphylum. Intermediate very high level categories (superkingdom, subkingdom, branch, infrakingdom, superphylum, subphylum and infraphylum) are extensively used to avoid splitting organisms into an excessive number of phyla (only 60 being recognized) and kingdoms, and to achieve a balanced system without unwarranted lumping.

2. The circumscription and high level classification of the zoological kingdoms Protozoa and Animalia are modified in the light of recent

molecular phylogenetic evidence that the protist Myxozoa are actually Animalia, not Protozoa, and that mesozoans are bilaterians. Mesozoa are removed from the kingdom Protozoa and placed as a new infrakingdom within the subkingdom Bilateria of the Animalia, which therefore are now subdivided into three subkingdoms: Radiata (phyla Porifera, Cnidaria, Placozoa, Ctenophora), Myxozoa, and Bilateria (bilateral animals: all other phyla).

3. Microsporidia other than the metchnikovellids are transferred from the kingdom Protozoa to the kingdom Fungi.

4. I argue that the need for a simple and general classification of the living world is now best met by placing Archezoa as a subkingdom within the Protozoa, as in my 1983 six-kingdom system, rather than as a separate kingdom as I advocated from 1987 onwards. I group the 13 currently recognized protozoan phyla into two subkingdoms, Archezoa and Neozoa, and four neozoan infrakingdoms. The reasons for these changes are discussed in detail in relation to the principles of megasystematics, here defined as systematics that concentrates on the higher levels of classes, phyla and kingdoms.

5. These principles also make it desirable to rank Archaebacteria as an infrakingdom of the kingdom Bacteria, rather than as a separate kingdom. Archaebacteria are here grouped with the infrakingdom Posibacteria to form a new subkingdom, Unibacteria, comprising all bacteria bounded by a single membrane. The existing bacterial subkingdom Negibacteria, with separate cytoplasmic and outer membranes, is here subdivided into two infrakingdoms, Lipobacteria, which lack lipopolysaccharide and have only phospholipids in the outer membrane, and Glycobacteria, which have lipopolysaccharides in the outer leaflet of the outer membrane and phospholipids in the inner leaflet of its bilayer. Thus, the primary grouping of the 10 bacterial phyla into two subkingdoms is based on the number of cell envelope membranes, whilst their secondary subdivision into four infrakingdoms emphasises their membrane chemistry; the definition of the negibacterial phyla, five of which are at least partly photosynthetic, relies chiefly on photosynthetic mechanism and cell-envelope structure and chemistry corroborated by rRNA phylogeny.

6. The circumscriptions of the kingdoms Protozoa and Chromista are slightly changed by transferring the subphylum Opalinata (classes Opalinea and Proteromonadea) into the kingdom Chromista, specifically to the infrakingdom Heterokonta, where it is grouped with the subphylum Pseudofungi and the recently discovered heterotrophic heterokont *Developayella elegans* (placed here in the new subphylum Bigyromonada) to form a new purely heterotrophic botanical phylum, Bigyra. Bigyra are defined as heterotrophs with a double ciliary transitional helix. This new grouping makes it necessary to abandon the phylum name Opalozoa, in which Opalinata were previously placed. A detailed evolutionary theory is presented to account for the loss of ciliary retronemes in Opalinata as a consequence of their evolution of gut commensalism. Recent phylogenetic evidence for multiple chloroplast losses within the kingdom Chromista strengthens the view that the ancestral chromist was a photophagotroph that evolved by a single symbiogenetic event involving a phagotrophic biciliate host and a red algal endosymbiont; but the monophyly of the Chromista still needs to be tested more.

7. No changes are made to the circumscription of the botanical kingdom Plantae or the kingdom Bacteria, which have both been stable since Cavalier-Smith (1981*a*). New plant subphyla and infraphyla are created.

8. The two zoological kingdoms (Protozoa, Animalia) are subject to the Zooological Code of Nomenclature, the single bacterial kingdom to the Bacteriological Code of Nomenclature, and the three botanical kingdoms (Plantae, Fungi, Chromista) to the Botanical Code of Nomenclature.

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