The Place of Botanical Groups in the Six-kingdom System of Life

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In 1675 Lemery divided things into the animal, vegetable and mineral kingdoms and Leuwenhoek discovered microorganisms. With the growth of microscopy and physiology the idea of the fundamental unity of life developed in the 18th century, and became crystallised into the cell theory by the 1840s after Dujardin realised that protozoan bodies were homologues of animal and plant cells. Suggestions for separate kingdoms for Fungi (Necker 1783), Protozoa (Owen 1858), and Bacteria (Enderlein 1925) were only generally accepted in the last third of the 20th century after high-resolution electron microscopy confirmed the fundamental distinction between bacteria and eukaryotes (higher organisms with nuclei). Electron microscopy also led to the creation of the kingdom Chromista (Cavalier-Smith 1981) to embrace algae with chloroplasts inside a periplastid membrane within the ER lumen and their heterotrophic descendants, such as oomycetes and bicoecids, formerly classified as fungi or Protozoa respectively. Finally DNA sequencing led to the use of computer-based phylogenetic analysis to supplement morphological evidence to allow bacteria and unicellular eukaryotes (protists) to be divided into phylogenetically meaningful phyla. The six-kingdom system dating from 1981 comprises the Empire Prokaryota with the sole kingdom Bacteria and the Empire Eukaryota with five kingdoms: a basal, paraphyletic Protozoa and four derived, holophyletic kingdoms (Animalia, Fungi, Plantae, Chromista.

Bacteria are divided into two subkingdoms: the more ancient Negibacteria with a bounding envelope of two membranes and the Unibacteria with a single surface membrane like that of eukaryotes. Not surprisingly eukaryotes evolved from a unibacterium by evolving phagocytosis through invaginating part of the single surface membrane to form the endomembrane system and internal cytoskeleton. At about the same time (~1 Gy ago) they engulfed and enslaved a negibacterium (α-proteobacterium) to form the mitochondrion and evolved mitosis, a centriole and cilium, and sex. Thereafter they quickly divided into two distinct clades, theunikonts and the bikonts. Unikonts are all heterotrophic and ancestrally retained a single centriole and cilium and diverged into two groups: Amoebozoa (traditional amoebae, including lobose testate amoebae, and slime moulds like Dictyostelium and Physarum) which mostly lost cilia, and opisthokonts with a posterior cilium and second precociously developing young centriole (Choanozoa, Animalia and Fungi). Animals and Fungi each evolved from different choanozoan protozoa: animals, via sponges the most primitive, from choanoflagellates and fungi from relatives of nucleariid filose amoebae that retained a cilium for dispersal but covered their vegetative bodies and branching filopodia with chitinous walls to evolve chytridiomycete rhizoids from which hyphae later evolved. After the loss of cilia by more advanced fungi, a trichomycete-like intestinal parasite generated the anaerobic microsporidia (wrongly classified as Protozoa for over a century) by perfecting its polar injection apparatus and converting mitochondria into mitosomes.

Separation of the unibacterial phylum Archaebacteria (Woese and Fox 1977) from all other bacteria (eubacteria, characterised by ancestrally having murein peptidoglycan walls and anoxygenic photosynthetic carbon dioxide fixation) was a major step forward. Archaebacteria are sisters of eukaryotes, not their ancestors, and are grouped with them as the clade neomura (Cavalier-Smith 1987), since their common ancestor replaced murein by cotranslationally synthesised N-linked glycoproteins. About 20 other major cellular changes occurred during this ‘neomuran revolution’ in association with the change in protein secretion and the replacement of active DNA supercoiling by DNA gyrase by passive supercoiling by core histones. Contrary to what is widely assumed, the root of the tree of life is probably not between Archaebacteria and other bacteria (eubacteria), but within the photosynthetic eubacteria between the Chlorobacteria (green non-sulphur bacteria like Chloroflexus) plus related heterotrophs) and the Cyanobacteria. On this view the last common ancestor of all life was photosynthetic, the primary radiations of life were among photosynthetic organisms, and heterotrophs evolved on numerous occasions by
independent losses of photosynthesis. Cyanobacteria are the only eubacterial phylum that never lost photosynthesis until after one of them was later enslaved to become the first chloroplast. Archaebacteria are probably the youngest of the 10 phyla of bacteria, having evolved from the other, much older unibacterial phylum, the Posibacteria.

Posibacteria comprise two subphyla: the endospore-forming Endobacteria such as Bacillus and Clostridium and the exospore-forming Actinobacteria, which share key features with neomura, such as the proteasome – a cylindrical macromolecular assembly for controlled protein digestion, and probably gave rise to neomura following the loss of their peptidoglycan wall. Following the origin of phagotrophy and the simultaneous origin of the nucleus and the cilium, one lineage evolved a second cilium to generate the ancestral bikont eukaryote. Like the ancestral unikont this was a benthic organism with amoeboïd tendencies, but it developed its pseudopods primarily for feeding, not locomotion (unlike Amoebozoa), for which it relied on its two oppositely pointing and oppositely differentiated cilia. The anterior, younger cilium beat actively to pull it forward and draw prey towards it, whilst the posterior, older cilium uses kinesin-based gliding motility to glide slowly across surfaces. A novel developmental mechanism – ciliary transformation across two cell cycles - arose to allow this structural and functional differentiation, which is also associated with novel ciliary roots of microtubule bands in contrast to the simple ancestral cone of single microtubules largely retained by unikonts. Every cell cycle, the bikont anterior centriole becomes a posterior centriole and its roots are correspondingly modified. This complex mode of development and pattern of cytoskeletal asymmetry is retained by all four major groups of bikonts: Plantae, chromalveolates, Rhizaria and Excavata.

Cyanobacterial enslavement by such an asymmetric host, probably one that had evolved membrane-bounded cortical alveoli and associated cytoskeleton to allow larger cells, generated the last common ancestor of the kingdom Plantae (Glaucophyta, Rhodophyta, Viridiplantae). Cortical alveoli and the peptidoglycan wall of the enslaved cyanobacterium were retained by the strictly unicellular Glaucophyta, but were both lost in the common ancestor of Rhodophyta and Viridiplantae (green plants), each of which independently evolved multicellularity (more than once in Chlorophyta, the green algal ancestors of land plants - Embryophyta: Bryophyta, Tracheophyta ). Chromalveolates, so closely related to Plantae that they often intermingle on single-gene trees, comprise the kingdoms Chromista and the protozoan infrakingdom Alveolata. Most alveolates and a few chromists have cortical alveoli, so I have grouped chromalveolates and Plantae together as the informal group corticates on the assumption that cortical alveoli are homologous throughout this putative clade. Although this requires further testing, the monophyly and holophyly of both the Plantae and the chromalveolates individually is now firmly established. I first argued in 1981/1982 for the single origin of chloroplasts bounded by only two membranes and the monophyly of Plantae (i.e. comprising Glaucophyta, Rhodophyta, Viridiplantae) because the evolutionary origin of protein targeting into a symbiogenetic organelle must be very complex and evolutionarily onerous. My prediction of a common protein-targeting machinery into plastids for all Plantae has been confirmed.

I proposed the clade chromalveolates for the same reason (1999), arguing that all evolved from a single enslavement of a red alga and a single origin of targeting machinery across the extra membranes that consequently separate their chloroplasts from the cytosol. A common targeting process is now established, using an upstream signal sequence for crossing the rough ER membrane and a modified transit sequence for crossing the two membranes of the chloroplast envelope, and also in my theory – not yet proven – the periplastid membrane that is the relic of the red algal plasma membrane. Chromalveolates are now accepted as monophyletic both because of this common membrane topology and targeting machinery but even more so because of the unpredicted replacement of two red algal plastid enzymes (glyceraldehyde phosphate dehydrogenase and fructose bisphosphate aldolase) by paralogues of host origin in the last common ancestor of all chromalveolates. Alveolata comprise two phyla: Ciliophora (ciliates and suctorians) and Myzozoa (Dinozoa and Apicomplexa). Dinozoa include dinoflagellates, about half of which are photosynthetic (many also phagotrophs) and studied by botanists and half purely phagotrophic, having lost
chloroplasts, and the purely heterotrophic protalveolates. Unlike all other chromalveolates dinoflagellates lost the periplastid membrane and have a chloroplast envelope of three membranes that seems to have two targeting mechanisms with distinctly different targeting peptides. Apicomplexa (Sporozoa and Apicomonadea) lost photosynthesis but mostly retained plastids surrounded by four membranes for lipid synthesis.

Chromista comprise subkingdoms Cryptista (usually photosynthetic Cryptophyceae; heterotrophic, phagotrophic Goniomonadea, Leucocryptea) and Chromobiota (Heterokonta and Haptista). Heterokonta comprise three phyla: Ochrophyta that contains all the heterokont algae, e.g. diatoms, phaeophytes, chrysomonads, xanthophytes and two heterotrophic phyla: Pseudofungi (mainly Oomycetes, hyphochytrids) and the deeper branching Bigyra (e.g. bicoecids, Labyrinthulea, Opalinata, actinophryid heliozoa). Haptista are the algal but often also phagotrophic Haptophyta plus two heterotrophic groups – centrohelid heliozoa and telonemid zooflagellates.

Rhizaria, ancestrally characterised by a propensity to form branching filopodia or reticulopodia, comprise two phyla: Retaria (Foraminifera and most Radiolaria), Cercozoa (9 classes; the photophagotrophic Chlorarachnea and 8 heterotrophic). The heterotrophs include numerous free-living phagotrophs such as cercozoans and filose testate amoebae, the plasmodiophorid plant parasites formerly wrongly classified as fungi or slime moulds, and the acetosporan invertebrate parasites. Chlorarachnean chloroplasts are surrounded by four membranes and came from an enslaved green alga, of which the miniaturized nucleus remains as a nucleomorph, as does the red algal nucleus in the Cryptophyceae, while the green algal plasma membrane is now the periplastid membrane.

Excavata are ancestrally characterised by a ventral feeding groove and comprise five phyla: the typically biciliate aerobic zooflagellate Apusozoa and Loukozoa; the typically quadriciliate amoeboflagellate Percolozoa (Heterolobosea and Stephanopogon); the secondarily anaerobic Metamonada with typically four cilia and hydrogenosomes or mitosomes instead of aerobic mitochondria; and the Euglenozoa with two dissimilar cilia possessing dimorphic latticed paraxonemal rods. Euglenozoa comprise Euglenoidea (about half photosynthetic; others phagotrophic or osmotrophic heterotrophs). It is currently controversial whether excavates and Rhizaria are sister groups or not and whether euglenoids and chlorarachneans got their chloroplasts by a single enslavement of a chlorophytine (not charophytine) green alga in their common ancestor (which would imply losses by all other rhizarians and excavates) or by two separate enslavements with fewer losses of plastids. If the former proved correct Rhizaria Excavata would together form a clade – the cabozoa – and there would only be three major bikont lineages Plantae, chromalveolates, and Rhizaria, each ancestrally photosynthetic but all including derived heterotrophic lineages.

In the six-kingdom system the basal eukaryotic kingdom Protozoa is divided into two subkingdoms: Sarcomastigota (Amoeboza, Choanozoa) and Biciliata (infra-kingdoms Alveolata, Rhizaria and Excavata). In my view neither the fundamental distinction between bikonts and unikonts in cytoskeletal organisation, nor strongly supported by key molecular cladistic characters, nor the recognition of the monophyly of chromalveolates require that Protozoa are subdivided into several kingdoms, as the now popular antipathy to paraphyletic taxa is not always justifiable. What the primary bifurcation between unikonts and bikonts does show is that all features shared by animals and plants (e.g. syngamy, meiosis, heterochromatinization) had already evolved in the last common ancestor of all eukaryotes, which we can now reconstruct in detail with high confidence. There appear to be no extant eukaryotes that diverged in the tree of life earlier that did the ancestors of animals and plants, but fungi are distinctly closer to us and share cellular properties with animals not found in plants or chromists, the two major phototrophic kingdoms – only three classes of Protozoa are partly or entirely photosynthetic: chlorarachneans, euglenoids and peridinean dinoflagellates. Clearly the groups most of interest to botanists are widely scattered on the tree of life and botany is a polyphyletic subject – but none the worse for that! As botanists belong in Animalia, all six kingdoms include organisms important for botany.