OPINION: A fungus-like root for the eukaryote tree

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Abstract

In this paper I offer a new interpretation of the early radiation of eukaryotes based on the emergence of major innovations in cell biology that apply uniquely to present day fungi. These emphasised increasingly detailed management of the positioning and distribution of membrane-bound compartments (vacuoles, vesicles and microvesicles) by the filamentous components of the cytoskeleton (microfilaments, intermediate filaments and microtubules); culminating, as far as filamentous fungi are concerned, with emergence of the Spitzenkörper and apical hyphal extension. I interpret *Tappania* fossils to be fully differentiated sclerotia of filamentous fungi, and so believe that the earlier, most ancient, stem eukaryotes must have emerged between 2000 and 1500 million years ago. The primitive eukaryotic stem featured primitive nuclear structures (including the nuclear membrane remaining intact during progress of the division; a characteristic of present day fungi), added the mitochondrion by enslavement of a bacterium, and evolved those aspects of the endomembrane system and cytoskeletal architecture that are also unique characteristics of present day fungi, in the following:

1. **Free cell formation**, by managing positioning of wall- and membrane-forming vesicles to enclose volumes of cytoplasm to subdivide sporangia into spores. This is a possible branch point to plants if the phragmoplast is assumed to be a vestige of free cell formation and the cell wall was adapted to be a polymer of glucose rather than *N*-acetylglucosamine, possibly for economy in usage of reduced nitrogen in organisms abandoning heterotrophy.
2. The beginnings of **filamentous growth**, first to make rhizoids or sporangium necks and stems, in the opisthokont cells of the day.
3. **Cell fusion**, evolving from reversal of wall synthesis procedures and giving rise to cytoplasmic (vegetative) and nuclear (sexual) compatibility/incompatibility systems, including the evolution of gametes.
4. **Septum formation**, initially dependent on a contractile ring of actin as a way to seal rapidly the membrane of damaged opisthokont cells. This is a possible branch point from chytrid-like opisthokont to animal- (choanozoan-) like opisthokont, with the animal stem gradually losing wall and adapting cytoskeletal organisation/vesicle trafficking originally used in wall synthesis to the new function of phagocytosis, and developing disassembly of the nuclear envelope to form the division spindle, cholesterol as the predominant sterol for membrane fluidity, and equatorially contractile cell division.
5. Through this sequence of events the chytrid-like opisthokont evolved towards filamentous fungi and emerged over 1.5 billion years ago as the first crown group of eukaryotes.

1. Introduction

Prokaryotes have dominated the Earth for the bulk of its history; the last universal common ancestor (LUCA) of all organisms alive today must have emerged close to the start of the Archaean Eon, about 3.8 billion years ago, because some of the oldest microbial fossils are fully differentiated, photosynthetic bacteria (cyanobacteria) found in Western Australian sediments that are 3.5×10^9 years old (Schopf, 1993; Derenne *et al*., 2008; Boal and Ng, 2010). On the other hand, eukaryotes are generally thought to have appeared no earlier than about 1.5 billion years ago (but see below). So, for at least the first 2 billion years after the origin of life, the living organisms on the planet were prokaryotes together, presumably, with their associated viruses.

The abundant biological activity in the deep ocean volcanic hydrothermal systems of the present day, most of it being dependent on chemosynthesis rather than photosynthesis, has stimulated the widespread appeal of theories of a 'deep-hot' origin of life (Wächtershäuser, 2006; Alpermann *et al*., 2010). This implies that the pioneer organisms were hyperthermophiles (Stetter, 2006), a notion which builds upon Carl Woese's conclusion that the three domains, now called Eubacteria, Archaea and Eukaryota diverged from LUCA (Woese, 1987; Woese *et al*., 1990). Emerging from these arguments we have what might be called a conventional, or 'textbook' phylogenetic tree of life (for

example see Moore *et al*., 2011; p. 24). Unfortunately, gene trees are ambiguous and the root of the universal tree of life remains controversial (Penny and Poole, 1999).

The most complete reworking of the tree of life is that recently published by Tom Cavalier-Smith (Cavalier-Smith, 2006, 2010a & b). Cavalier-Smith's approach is to integrate palaeontology with comparative study of present day organisms, emphasising key steps in molecular and cellular evolution. Cavalier-Smith (2010a) identifies five successive kinds of cell: (i) The first cells were negibacteria, with cells bounded by two acyl ester phospholipid membranes, divided into the primitive anaerobic Eobacteria without lipopolysaccharide in the outer membrane and more advanced Glycobacteria with lipolysaccharide (e.g. oxygenic Cyanobacteria and Proteobacteria); (ii) unibacteria, with one bounding and no internal membranes, divided into desiccation-resistant posibacteria, ancestors of eukaryotes, and archaebacteria as the youngest bacterial phylum and a sister group (not an ancestor) of eukaryotes; (iii) eukaryotes with endomembranes and mitochondria, (eukaryotes plus archaebacteria make up the neomura); (iv) plants with chloroplasts; (v) chromists with plastids inside the rough endoplasmic reticulum.

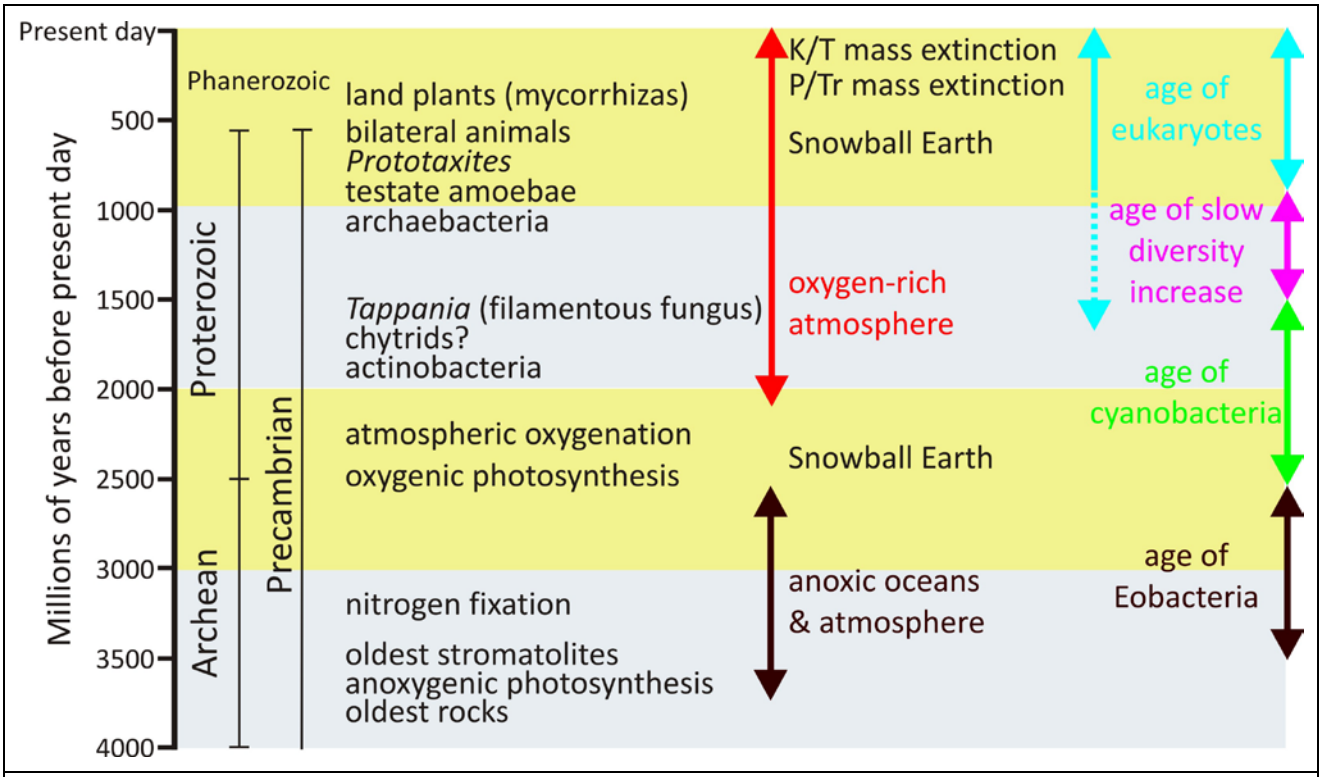

Fig. 1. A geological timescale covering from the time of the oldest rocks (3.8 billion years ago) to the present day, highlighting major geological and evolutionary events and features mentioned in the text, including my interpretation of Cavalier-Smith's four ages of life at extreme right. Note that Cavalier-Smith's age of eukaryotes starts 850-800 million years ago, but as I interpret Tappania fossils to be fully differentiated sclerotia of filamentous fungi I place the origin of stem eukaryotes somewhere between 2000 and 1500 million years ago. Modified and redrawn from Cavalier-Smith, 2010a.

These types of cell are placed into four ages of life as follows (Cavalier-Smith, 2010a; see Figs 1 and 3): (i) the **age of Eobacteria**, an anaerobic phase in which photosynthetic non-sulphur bacteria (and before them extinct stem negibacteria) were the major primary producers. Exclusively anaerobic life probably persisted from about 3.5 billion years ago to just under 2.5 billion years ago (the best date for the origin of photosystem II and start of oxygenic photosynthesis). (ii) The **age of cyanobacteria** (about 2.5-1.5 billion years ago) during which cyanobacteria were the major primary producers (and are now the dominant morphological fossils). (iii) An **age of slow diversity increase** (1.5-0.85 billion years ago) features increasing morphological complexity and colonisation of continental surfaces by both Cyanobacteria and, following loss of the outer membrane,

Posibacteria and the actinomycete Actinobacteria; the latter displaying the greatest morphological complexity. Some of the largest microfossils from this part of the middle Proterozoic have been attributed to eukaryotic algae, filamentous fungi or stem eukaryotes of undefined affinity (Javaux *et al*., 2001), though Cavalier-Smith is sceptical of all such fossil identifications in this period. (iv) The **age of eukaryotes** and obvious macroorganisms (850-800 million years ago to the present).

Cavalier-Smith (2006) argues that eukaryotes derived from an actinobacterial ancestor on the grounds (among others) that current Actinobacteria are the only eubacteria having phosphatidylinositol, which is one of the most important eukaryote phospholipids, required for eukaryote specific cell signalling. "...Thus, eukaryote membrane lipids probably came vertically from an actinobacterial ancestor, archaebacterial lipids originating in their [last common ancestor] after it diverged from eukaryotes." A further aspect of this argument is that shortly after eukaryotes and archaebacteria diverged, the latter group colonised hot, acid environments by evolving the ancestrally hyperthermophilic archaebacteria and later, one archaebacterial lineage evolved biological methanogenesis; (Cavalier-Smith, 2006 pp. 977-978).

2. Towards eukaryotes

A crucial differences between the Cavalier-Smith (2006) model and the 'standard' three domain model of Woese *et al*. (1990) are that the standard model perceives the archaebacteria as an ancient (over 3.5 billion years old) group of prokaryotes which was the ancestor of eukaryotes, whereas Cavalier-Smith views the Archaebacteria as appearing for the first time less than 800 million years ago and being sisters to eukaryotes, rather than ancestors.

Generally speaking I find the Cavalier-Smith model much more convincing because it is based on integration of such a broad range of data (Cavalier-Smith, 2006, 2010a & b); so I accept Cavalier-Smith's basic narrative from the first appearance of living cells about 3.5 billion years ago to the emergence of eukaryotes from an actinobacterial ancestor. I disagree with Cavalier-Smith's version of the origin of eukaryotes which I think is wrong because it is totally dismissive of fungi, and so animal centric that it equates the origin of phagocytosis with the origin of eukaryotes (e.g. "...the origin of phagocytosis by prey engulfment (which indirectly made the eukaryote cell...)..." Cavalier-Smith, 2010a, p. 123). This extreme position is taken without suggesting what selective advantage there might be in the essential intermediate steps towards phagocytosis. Phagocytosis requires water management, precise membrane management of endocytosis and exocytosis, and full cytoskeletal management of enzyme, vesicle and vacuole movement and distribution. Although the selective advantage of the complete process is self-evident now; I can't see nutritional selective advantage(s) for any distant animal-ancestors in partially completed steps of the overall process. However, Martin *et al*. (2003) suggested that osmotrophy had to precede phagotrophy in eukaryote evolution, because without importers, food vacuoles are useless. As all present day fungi are osmotrophs it may be that at least this aspect of the fungal life style is an ancestral feature of the earliest eukaryotes. I will argue below that there are other aspects of the fungal life style that could have contributed to assembly of the array of processes that might have enabled the emergence of phagotrophy in those early eukaryotes from which the animal lifestyle emerged.

3. Rise of the fungi

Although fungal hyphae have few unique morphological features and most fungal structures are poor candidates for preservation as fossils, a respectable fossil record for fungi has been assembled in recent years. Ancient fungal fossils are found in the exquisitely-preserved Devonian Rhynie Chert of Aberdeenshire in the north of Scotland (410 million years old); easily recognisable mycorrhizal fungi from the Glomeromycota and several other fungi have been found associated with the preserved tissues of early vascular plants (Taylor *et al*., 1997, 2004, 2006). glomeromycotan fossils have also been found in mid-Ordovician rocks of Wisconsin (460 million years old) (Redecker *et al*., 2000). The age of these fossil fungi indicates that glomeromycotan fungi were present before the first vascular plants arose, when the land flora consisted of bryophytes, lichens and cyanobacteria. It is reasonable to suppose that arbuscular mycorrhizas

played an important role in the success of early terrestrial plants (Blackwell, 2000; Redecker *et al*., 2000).

Fossil lichens have also been described from the Rhynie Chert (Taylor *et al*., 1997), as well as fine ascomycete specimens showing ascospore development and perithecia (Taylor *et al*., 2004), from which we can infer a fully evolved fungal developmental biology able to produce extreme hyphal differentiation, cell signalling, cell sorting, pattern formation and formation of tissues with different functions, all of which are typical of the present day. Differentiated conidiophores emerging through a plant epidermis are also shown; suggesting a fully adapted plant pathogen at an extremely early stage in plant evolution. Chytrids are "…probably the most common microbial element…" in the Rhynie Chert (Taylor *et al*., 2006) and include fully differentiated chytrid zoosporangia, which suggest the inference that at the time the sediments were laid down the complete cell biology of the chytrids (thallus, rhizoids, free cell formation, motile zoospores, etc.) was fully established. Also among the Rhynie Chert specimens are examples of chytrids parasitising other fungi, showing, again, that 410 million years ago the fungal life style was so firmly established that fungi were parasitising other fungi. The fungus-like lifestyles represented in the Rhynie Chert specimens include some like the Oomycota of the present day. Several of these specimens show an antheridium contacting an oogonium, clearly demonstrating the operation of a fully differentiated sexual reproduction process including differentiated male and female gametes and all that goes with them, including sexual hormones, hormone receptors and male to female cell targeting.

By far the most impressive fungi of the Ordovician/Devonian period are specimens of the fossil genus *Prototaxites*, which were terrestrial organisms found from the mid-Ordovician (460 million years ago) to the early Devonian, suggesting that they lasted a period of at least 40 million years (Hueber, 2001; Boyce *et al*., 2007). These fossils are among the 'nematophyte phytodebris' that constitutes the earliest evidence for terrestrial organisms. This nomenclature was assigned in the middle of the 19th century and has no relevance to present day understanding of taxonomy; though it does indicate that confusion over the identification of the material is over 150 years old (see discussion in Hueber, 2001 and Taylor *et al*., 2010). *Prototaxites* specimens are generally large; over a metre wide (Wellman and Gray, 2000) and up to 8 m tall (Hueber, 2001). Some of the earliest examples were tree-like trunks constructed of interwoven tubes <50 µm in diameter (concentrically arranged in transverse sections), and were interpreted to be small coniferous trees, though we now know that environments at the time *Prototaxites* was fossilised did not (yet) include large vascular plants.

Prototaxites was also so common that it was a major component, both in terms of abundance and diversity, of its habitat and was by far the largest organism then existent. These early terrestrial ecosystems were still dependent on the more ancient primary producers, cyanobacteria, eukaryotic algae, lichens and mosses, liverworts, and bryophytes. Isotope ratio mass spectrometry of individual *Prototaxites* fossils provides the most compelling evidence that *Prototaxites* was a fungus (Boyce *et al*., 2007; Hobbie and Boyce, 2010); I personally consider this evidence conclusive. These analyses show that carbon isotope ratios (^{12}C:^{13}C) of individual *Prototaxites* fossils varied too much for them to be photosynthetic primary producers of any sort (Boyce *et al*., 2007), indicating that *Prototaxites* was a heterotroph (saprotroph) that digested isotopically heterogeneous substrates; it was a consumer and recycler. Hobbie and Boyce (2010) demonstrated a similar large range of carbon isotope values among fungi, particularly saprotrophic fungi, of a present day environment resembling the Early Silurian and Devonian landscapes where *Prototaxites* occurred. Hueber's (2001) critical examination of the microscopic anatomy of *Prototaxites* found similarities with the trimitic system of hyphae evident in present day basidiomycetes; he states: "*Prototaxites* is nomenclaturally valid... This report has a triple purpose: (1) to name, as neotype, a recognizable specimen [of *Prototaxites*] collected by Dawson for which the locality and stratigraphic data are known, (2) to redescribe the genus as structurally composed of three interactive forms of hyphae, i.e. large thin-walled, septate, branching, generative hyphae; large thick-walled, non-septate, skeletal hyphae; and small thin-walled, septate, branching, binding hyphae, which combine to form

a gigantic, phototropic [more likely gravitropic in my view], amphigenous [= a hymenial hyphal layer of present day Ascomycota and Basidiomycota that extends over the entire surface of the spore-producing body], perennial sporophore with saprobic nutrition, and (3) to classify it in the Kingdom Fungi." (Hueber, 2001; abstract, with comments from me in square brackets).

Taking all this evidence together the conclusion is inescapable to me that these enormous fossils, which were the largest land organisms to have lived up to their point in time, were actually giant terrestrial saprotrophic fungi, with affinities (dolipore septa, clamp connections and sterigmata) to present day Basidiomycota. Consequently, I believe that convincing fossil evidence shows that fungi were important, even dominant, members of terrestrial ecosystems approximately 500 million years ago. Well-developed filamentous fungi must have first appeared a long time before that, however. How long would it take the ancestors of the Rhynie Chert water moulds, chytrids, Glomeromycota, lichens and Ascomycota to evolve the capability to form structures microscopically indistinguishable from those of the present day? How long would it take the ancestors of *Prototaxites* to evolve an 8 m tall club-fungus? Guessing could push 'well-developed filamentous fungi' back in time to about 700-800 million years ago. But there are older fossils than that, even though they may be disputed.

Butterfield (2005) assigned fossils extracted from formations in northwestern Canada, the deposition of which has been dated to between 800 and 900 million years ago, to the form-genus *Tappania*; describing the organism as: "…an actively growing, benthic, multicellular organism capable of substantial differentiation. Most notably, its septate, branching, filamentous processes were capable of secondary fusion, a synapomorphy of [trait shared by] the 'higher fungi' [of today]. Combined with phylogenetic, taphonomic and functional morphologic evidence, such 'hyphal fusion' identifies *Tappania* reliably, if not conclusively, as a fungus, probably a sister group to the 'higher fungi' [Dikarya], but more derived than the zygomycetes." (Butterfield, 2005; abstract).

The form genus fossil *Tappania* is widespread, having been found in ancient shoreline carbonaceous shale deposits in Australia, Canada, and China. Specimens fossilised nearly 1.5 billion years ago in shales in northern Australia have been described (Javaux *et al*., 2001). Javaux *et al*. (2001) go no further than to state that the systematic relationships of *Tappania* are uncertain, but its distinctive morphology indicates that: "…the cytoskeletal architecture and regulatory networks that characterize living [eukaryote] protists…" were in place in organisms fossilised 1.5 billion years ago. However, Butterfield (2005) discusses these and other putative pre-Devonian fungi and concludes that "…there is a case to be made for an extended and relatively diverse record of Proterozoic fungi." Cavalier-Smith (2006; pp. 983-984) agrees with Butterfield's (2005) identification of *Tappania* as sporangial entities broken from a branching trophic hyphal network, but does not agree that these fossils are probably fungi. He suggests they could instead be actinobacterial pseudosporangia; I do not find this convincing.

The large spheroidal microfossils shown in these *Tappania* papers are usually described as 'vesicles'. Butterfield's (2005) specimens, after being dissolved into slurry with 30% HF and filtered through a 62 μm mesh sieve, are described as follows: "… from 30 μm … to over 400 μm … in transverse dimension … Processes are typically heteromorphic and range from 0.3 μm … to >4 μm … in diameter. In some instances, simple cylindrical processes may be distributed relatively uniformly over the vesicle surface …; in others, they occur as isolated knoblike buds … or elongate filamentous extensions …. In most cases, however, the processes are further distinguished by distal branching … and a capacity to form closed loops through secondary fusion. This fusion appears to be relatively indiscriminate and gives rise to a wide range of expression: occasionally the processes return directly to the vesicle to form simple loops …; in other cases they have fused either with themselves … or, more commonly, with other processes …, resulting in a distally interconnected network... Multiple layers of process networks are also developed, sometimes to the extent of obscuring the central vesicle …" (Butterfield, 2005; p. 167) (see Fig. 2).

This is quoted in detail because I have spent most of my research life cultivating a basidiomycete fungus (*Coprinopsis cinerea*) which, in common with many other present day ascomycete and basidiomycete soil fungi produces abundant sclerotia in and on mycelial cultures. We have described these: "…Mature aerial sclerotia were dark brown to black, more or less spherical and variable in size although most were in the range 100-250 μm in diameter … three tissue layers were apparent - the outer diffuse layer, the rind and the medulla. The outermost diffuse layer … was composed of apparently dead hyphal cells whose cytoplasm was reduced to membrane fragments and vesiculate structures. Many had crenulate cell walls which may indicate they were damaged during preparation for sectioning. This outer layer, though only loosely attached and often sloughed off during fixation, was always present in mature aerial sclerotia and is therefore regarded as an integral part of their structure." (Waters *et al*., 1975a; p. 201; see also Waters *et al*., 1975b) (see Fig. 2).

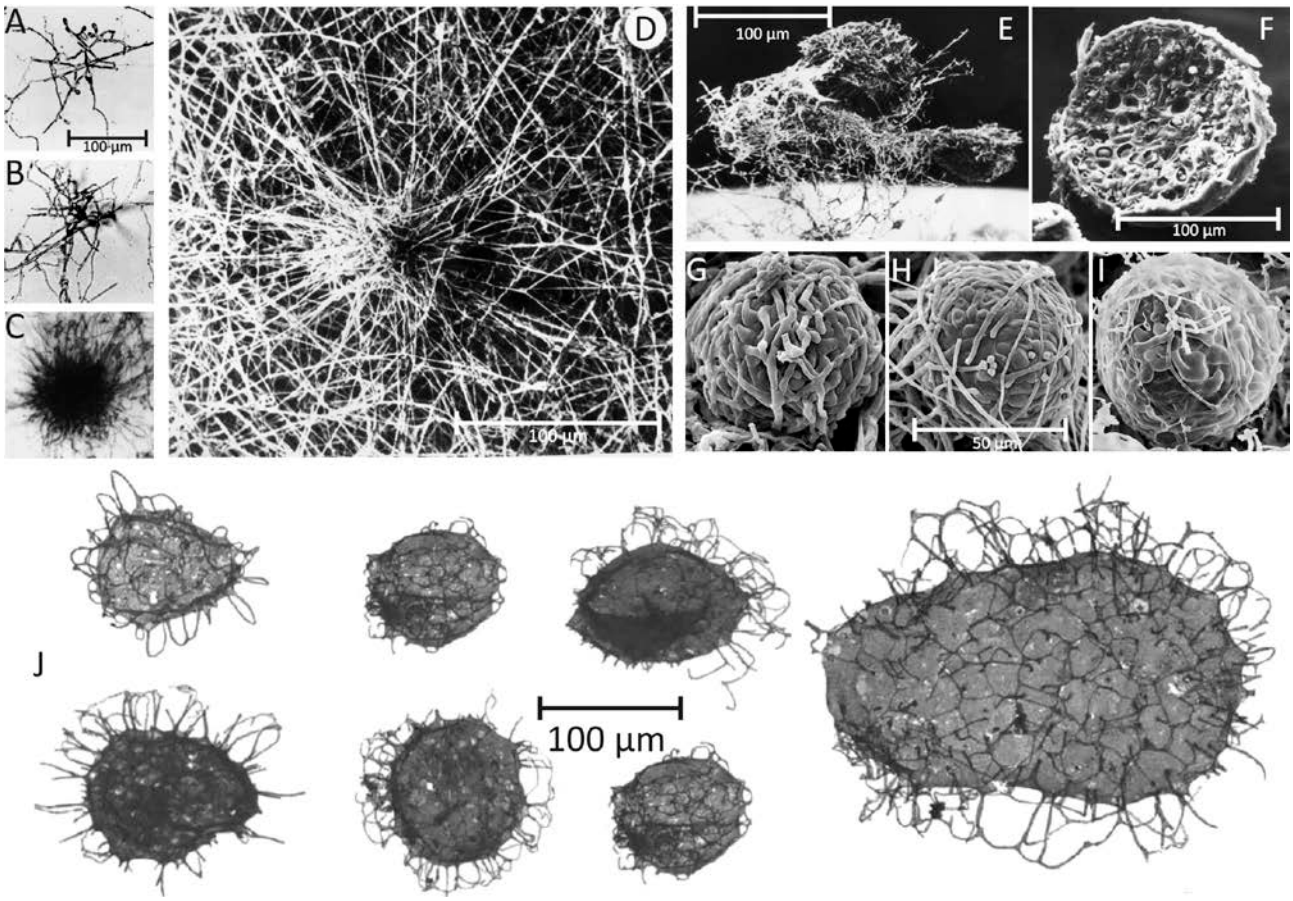

Fig. 2. Comparison of present day fungal sclerotia (top panel) with *Tappania* sp. fossils (bottom panel). **A**-**D**, Culture slide (microcosm) preparations of developing sclerotia of *Coprinopsis cinerea*. **A**: the earliest recognisable stage showing the centre of sclerotium initiation. **B**: increased size of structure as branching proceeds to form a very immature sclerotium. **C**: immature sclerotium showing main body with hyphae of determinate growth and the 'long hyphae' that appear to radiate outwards but are actually targeting inwards towards the developing sclerotium. **D**: Scanning electron micrograph of immature sclerotium at a stage similar to that shown in **C**, which shows more clearly that the long hyphae are part of the long distance nutrient translocation network that supports development of the sclerotium (**A**-**C** are light micrographs; all images from Waters *et al*., (1975a and b) by permission of John Wiley and Sons). **E**: Scanning electron micrograph of a group of three aerial sclerotia of *C. cinerea* in side view, relatively undisturbed and still showing the investing layer of mycelial hyphae and obvious aerial habit. Photographed by Dr H. Waters. The mycelial hyphae that surround the developing sclerotium, which seem so substantial in **D** and **E**, are easily lost during preparation for microscopy. **F**: Scanning electron micrograph of a freeze-fractured aerial sclerotium of *C. cinerea.* This specimen is one of a

collection of sclerotia that were scraped from the surface of a Petri dish culture, suspended in glutaraldehyde fixative, filtered through cotton, dehydrated into acetone, then ground in a mortar and pestle under liquid nitrogen (to fracture the sclerotia and reveal their internal structure); not much of the investing layer of aerial mycelium survives this treatment. Although the chemistry is very different, the physical processes involved in this preparation are quite similar to those recorded for the preparation of *Tappania* fossil specimens (see text). This specimen photographed by Dr F.V. Hereward. **G** to **I**: scanning electron micrographs of the (smaller) sclerotia of *Byssocorticium coprophilum* (MycoBank reference Mb449580), which show more clearly that sclerotia are a 'ball' of filamentous hyphae and that constituent cells (compartments) of those hyphae differentiate as the sclerotium matures. Images reprinted by permission from Dr J.A. Stalpers; they originally appeared on the www.mycobank.org website at this URL: http://www.mycobank.org/MycoTaxo.aspx?Link=T&Rec=449580#Images. **J**: *Tappania* sp. fossils from the Wynniatt Formation on Victoria Island, northwestern Canada. Specimens are generally described as 'vesicles' with 'processes'; some are described as 'now flattened'. These specimens are between 800 and 900 million years old. Images (here adjusted to the same scale) from Butterfield (2005). Note the *Tappania* fossil 'vesicles' have a surface sculpturing beneath the 'processes' similar to that evident in present day fungal sclerotia beneath the investing hyphal layer (particularly evident in images G to I).

Although I've never seen them after a billion years of preservation followed by dissolution into hydrofluoric acid, I have handled a great many fungal sclerotia in various states: fresh, in actively growing cultures including microcosms, desiccated in old stored cultures with collapsed and twisted outer-layer hyphae, fixed for LM and TEM, and critical-point dried for SEM. This first-hand experience convinces me that the *Tappania* 'vesicles' illustrated by Javaux *et al*. (2001) and Butterfield (2005) could have been the sclerotia of filamentous saprotrophic moulds and soil fungi.

If true, this interpretation implies that filamentous moulds with affinities to the Ascomycota and/or Basidiomycota of the present day and able to regulate hyphal branching and hyphal interactions with sufficient finesse to assemble multicellular survival and, perhaps, reproductive structures, were common and widespread 1.5 billion years ago.

4. A fungus-like root for the eukaryote tree

Martin *et al*. (2003, p. 197) suggested that a eukaryotic phylogenetic tree with fungi first would make sense. These authors based their overall tree of life on the standard three-domain model and showed the stem eukaryotes as emerging from within the archaebacteria. I would adhere, as outlined above, to the four ages of life as set out by Cavalier-Smith (2010a) but would start the age of eukaryotes about 1.5 – 2 billion years ago and amend the origin of eukaryotes as follows (Fig. 3).

The eukaryotic stem added the mitochondrion by enslavement of a bacterium (and perhaps added the nucleus by enslavement of an archaean, depending on the timing of divergences of prokaryote groups), and later evolved the endomembrane system and cytoskeletal architecture. The following features, which in the present day are characteristics of fungi, emerged in this temporal sequence:

1. **Free cell formation** the cytoskeletal organisation to manage vesicle and organelle trafficking and particularly the positioning of wall- and membrane-forming vesicles to enclose volumes of cytoplasm to subdivide sporangia into spores (see discussion in Moore *et al*., 2011, pp. 48-50), with adoption of a chitinous cell wall, possibly as an adaptation of the ancestral actinobacterial mechanism for addition of oligosaccharides containing *N*-acetylglucosamine to surface proteins (muramopeptide wall precursors).
    - After this process is established, this is a potential branch point for divergence at the unicellular level to plants and heterokonts, with phragmoplast formation left as a vestige of free cell formation specifically localised at the division spindle equator,

and the early cell wall adapted to be a polymer of glucose rather than *N*-acetylglucosamine, possibly to economise on the demand for reduced nitrogen in an organism that is abandoning heterotrophy in favour of photosynthesis after enslavement of a cyanobacterium.

2. **Filamentous growth** first to make rhizoids and sporangial necks and stalks in chytrid-like opisthokont cells. Limiting wall growth to an apex even in an opisthokont cell involves creation of a coordinated production and distribution system for wall and membrane precursors and enzymes; together with a cytoskeletal delivery system *and* a cytoskeletal tethering system to stabilise the growing wall, weakened by insertion of new precursors, against osmotic stress (for discussion of the situation in present day fungi see Moore *et al*., 2011, pp 137-144; Read *et al*., 2009, 2010; Riquelme *et al*., 2007; Riquelme and Bartnicki-García, 2008; Steinberg, 2007; Steinberg and Schuster, 2011).
3. **Cell fusion** primarily involves adaptation of cell wall construction functions to enable organised disassembly of two cell walls in contact (without risking osmotic stress to either cell), permitting their two cell membranes to make the two cytoplasms coextensive (see Glass *et al*., 2004 for discussion of present day fungi). In the ancient opisthokont cells the selective advantage of cell fusion could have been the opportunity it offers for collaboration in heterokaryons and heteroplasmons. Success could lead to the emergence of cytoplasmic (vegetative) and nuclear (sexual) compatibility/incompatibility systems (self/non-self recognition) which on the one hand would allow cytoplasmically compatible cells to exchange nuclei and form heterokaryons and on the other hand exchange of dissimilar nuclei as a prelude to sexual reproduction and all that means for evolutionary progress.
4. **Septum (cross wall) formation** is primarily a way of protecting the cell from the hazard of loss of cytoplasmic contents following puncture of the osmotically pressurised hydrostatic system. There is, consequently, selective advantage in developing a contractile ring of actin as a way to seal damaged cells rapidly.
    - After all these processes were established at the opisthokont level, this becomes a potential branch point for divergence to animals (chytrid-like to choanozoa-like), gradually losing the rigid wall and adapting the cytoskeletal organisation/vesicle trafficking originally used in wall synthesis and stabilisation to new functions of phagocytosis, locomotion and contractile cell division.

This branch event could also have been the point in time when fungi became (possibly accidentally) fixed on ergosterol as the quantitatively predominant sterol involved with controlling membrane fluidity in contrast to the cholesterol used in animals. The chytrid-like opisthokont now becomes the ‘stem-fungus’ which evolves into ancestral fungi apically-extending with the Spitzenkörper as the ultimate organising centre for hyphal extension and filamentous hyphal morphogenesis. Creating nucleated hyphae to explore and exploit the then extant biofilm and beyond the biofilm to accumulated terrestrial debris of prokaryote growth. This sequence of events (Fig. 3) allowed filamentous fungi to emerge over 1.5 billion years ago as the first crown group of eukaryotes.

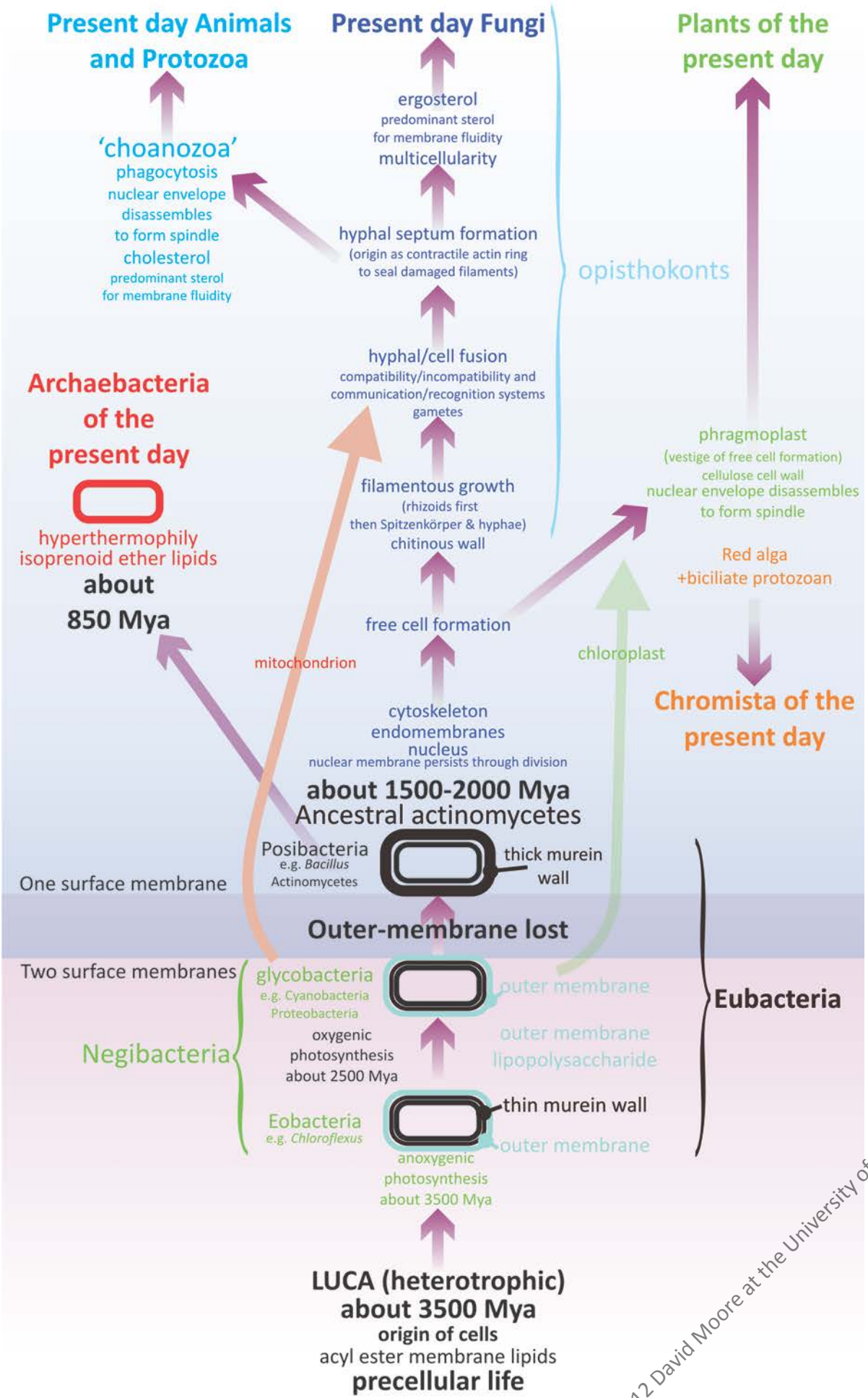

Fig. 3. Emergence of a fungus-like stem for eukaryote evolution. The lower part of this diagram is based on Cavalier-Smith's tree of life (Cavalier-Smith, 2010a; his Fig. 6), which emphasises major evolutionary changes in membrane topology and chemistry, except that the most ancient bacteria are shown here to be heterotrophic descendants of LUCA (the last universal common ancestor). Eukaryotes diverge from actinobacterial ancestors about 1500 to 2000 Mya (million years ago) and the bulk of this illustration deals with eukaryote evolution. Evolution of the most ancient stem eukaryotes are considered to depend on increasingly detailed management of the positioning and distribution of membrane-bound compartments (vacuoles, vesicles and microvesicles) by the filamentous components of the cytoskeleton (microfilaments, intermediate filaments and microtubules). Features which remain in present day organisms as characteristics of modern filamentous fungi.

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Fungal Biology in the Origin and Emergence of Life by David Moore

To be published by Cambridge University Press [ISBN: 9781107652774]; expected to be available from January 2013.

The rhythm of life on Earth includes several strong themes contributed by Kingdom Fungi. So why are fungi ignored when theorists ponder the origin of life on this planet? This book is a mycologist's tale about the origin and emergence of life on Earth based on the most recent research.

There are many books available about the origin of life, most of which have been written by physicists, cosmologists, astronomers, chemists or molecular biologists. They are fascinating stories but they are biologically naïve. Most existing books discuss only animal life, although animals were the last major group of organisms to emerge in evolution and therefore have least to reveal about the origin of life. Some books include discussion of photosynthesis, bacterial and/or plant; but ***none*** discuss fungi, although in terms of biodiversity kingdom fungi is arguably the most numerous and most diverse kingdom on the planet, and exerts the most profound influence on every ecosystem.

The unique feature of this book is that it is based on a proper understanding of the full biodiversity of this planet and the central role of the fungal grade of organisation in the evolution of higher organisms is given due attention. David Moore also points out how aspects of the fungal lifestyle are detectable through the whole of the evolution of life; right back to the chemical evolutionary logic of the biogenic processes that occurred before the origin of life.

This has never been done before, and the main reason it has not been done is that other writers on the topic of the origin and early evolution of life have been, and still are, ignorant of the sheer size, diversity and importance of Kingdom Fungi in the Earth's biosphere today and throughout its evolution.

So what's the book about?

Forget theories in which life originated in a one-off event in an ocean-scale primeval soup, or in a deep, hot place somewhere, or even a warm little pond. Life originated as a biofilm, with precursors for the first slimy films brought together by octillions of drifting aerosol droplets from around the globe acting as dynamic reaction vessels in a chemically diverse ocean and atmosphere. Life's game on Earth first played out in rainwater and seawater trickling through roofs of volcanic caves on the spindrift-washed shore of volcanic islands in an endless shallow stormy sea. In the slime on the volcanic sand the pre-biofilms of 4 billion years ago confined the prebiotic chemistries from many sources of a turbulent planet. Primitive biofilms evolved and those of 2 billion years ago confined together prokaryotes to collaborate to form the first unicellular stem eukaryotes; fostering emergence of eukaryotes, by doing what biofilms do.

The book Fungal Biology in the Origin and Emergence of Life is presented in 13 chapters:

- Chapter 1. Learning from life on Earth in the present day
- Chapter 2. Essentials of fungal cell biology
- Chapter 3. First, make a habitat
- Chapter 4. The building blocks of life
- Chapter 5. An extraterrestrial origin of life?
- Chapter 6. Endogenous synthesis of prebiotic organic compounds on the young Earth
- Chapter 7. Cooking the recipe for life
- Chapter 8. "It's life, Jim..."
- Chapter 9. Coming alive: what happened and where?
- Chapter 10. My name is LUCA
- Chapter 11. Towards eukaryotes
- Chapter 12. Rise of the fungi
- Chapter 13. Emergence of diversity

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