Empire Biota: an Update

Introduction

The newly-discoverd Cavernulacolales (which I shorten to Cavernales) has the distinction of having the fewest derived traits, as seen in the following list showing the number and %:

Rhodobiota	68	18.8
Glaucobiota	34	14
Galdieria	17	12
Cyanidium	21	11
Cyanidioschyzon	12	8.8
Cyanidiococcus	11	7
Cavernales	11	6.8

Huang et al (2024) identify 2 lineages in Cyanidiophycea:

the C-lineage, producing 2 or 4 endospores, being obligately autotrophic, having phytoglycogen as the storage product, lacking linolenic acid, having a chloroplast dividing ring, a low chloroplast-nuclear DNA ratio, low nuclear DNA size, central chloroplast DNA, a low chloroplast DNA value, red storage-glucan iodine reaction, nitrate uptake, % of alpha-1,6 glucose-glucose linkage at 7, storage-glucan branching enzyme 3 b.e., % of salt tolerance 3-4;

the G-lineage (*Galdieria*), producing 2, 4, or 8 endospores, being facultatively autotrophic, having floridean starch as the storage product, linolenic acid, a high chloroplast-nuclear DNA ratio, high nuclear DNA size, peripheral chloroplast DNA, a high chloroplast DNA value, red-violet storage-glucan iodine reaction, absence of nitrate uptake, % of alpha-1,6 glucose-glucose linkage at 6, storage-glucan branching enzyme 2 b.e., % of salt tolerance at 10, without a chloroplast dividing ring, lacking nitrate uptake.

Those features after trophic mode are here presumed to apply generally or entirely for the C-lineage, based on data in Seckback (1994).

Huang et al also say that species in this kingdom cannot be identified reliably as the morphological and physiological traits are too variable at this level, so they are defined molecularly.

The taxonomic placement of strains belonging to the extremophilic *Galdieria maxima* has been controversial due to the inconsistent position inferred from molecular phylogenetic analyses. *Galdieria maxima* nom. inval. was classified in this genus based on morphology and molecular data in the early work, but some subsequent molecular analyses have inferred strains of *G. maxima* to be closely related to *Cyanidioschyzon*. While the ability to uptake various forms of organic carbon for growth is an important physiological trait of *Galdieria*, this strain was identified as an ecologically obligate photoautotroph (i.e., the inability to utilize the natural concentrations of organic carbons) and lacked various gene models predicted as sugar transporters. Based on genomic, morphological, and physiological traits, Liu and colleagues (2020) propose this strain to be a new genus and species, *Cyanidiococcus yangmingshanensis*. Re-evaluation of the 18S rRNA and rbcL gene sequences of the authentic strain of *G. maxima*, IPPAS-P507, with those of *C. yangmingshanensis* suggests that the rbcL sequences of "*G. maxima*" deposited in GenBank correspond to misidentified isolates.

Cavernales was established by Park et al (2023) and contains the genera *Gronococcus, Cavernulacola*, and *Sciadococcus,* found in Naples, Chile, and Taiwan, respectively, representing a distinct lineage—cave-dwelling prerhodophyceans—and therefore a separate kingdom. It has spherical,

walled cells, a single, parietal, cup-shaped chloroplast, 6-8 thylakoid membranes, and phycobilisomes, reproduces by 4 autospores, and is mesophilic.

The age of prerhodophyceans is estimated by molecular clocks to be about 1.5 bln. yrs. (Park et al, 2023), which corroborates my phylogeny and contradicts the standard molecular taxonomy.

Cyanidiofrigus pinteoensis Huang et al 2024 is another newly discovered cyanidiophycean, found in terrestrial habitats and geothermal fumes in Taiwan, Japan, and the Philippines. It lives in cool surroundings by comparison, being thermophilic, having a temperature range of 20-35° C.

Cyanidium+Galdieria are the sister group to red algae in Saunders and Hommersand (2004), but have no basis for it phenotypically. The GB (Golgi Body, aka dictyosome), for instance, occurs in association with the ER, but, as they say themselves, this is common in other eukaryotes and rare in red algae.

Algae are expected to be promising alternative sources of biofuels, foods, and cosmetics (Sumiya & Miyagishima, 2017). *Cyanidioschyzon merolae* is potentially useful for producing high concentrations of desirable biomaterials by metabolic engineering. It is genetically traceable and can thrive at low pH (1–5) and high temperatures (25–50 °C), which are harmful to many other organisms. So it can be suitable for outdoor cultivation without the risk for contamination from other (undesirable) organisms. Recent studies have reported enhanced triacylglycerol (TAG) production, which can be used for biodiesel production, by genetic modification. Cyanidiophyceae have been a popular target for industrial applications such as waste-water treatments, biofuel feedstock, and the sequestration of heavy metal contamination (Huang et al, 2024).

Methods and Materials

I did 3 analyses since the 3rd edition of Empire Biota including the newly established cyanidiophycean genera. PAUP 4 was used, a heuristic search was done using TBR, all characteristics were of equal weight, no topological constraints were in effect, addition sequence was random, and the optimization criterion was ACCTRAN. In PAUP only the DescribeTrees, which gives the number of steps and the CI and RI, is rooted and Fitch is used by default but Wagner was used for the DescribeTrees and the bootstrap as it can be specified for these. The hypothetical ancestor was the outgroup, which was used as the root.

In the 1st there were 36 taxons and 388 characteristics (28 excluded, most because they had proven to be homoplasious), 12.2 rearrangements, 1151 steps, and 3 trees retained, and the CI was .40 and the RI was .38.

In the 2nd linolenic acid was added but, importantly, phytoglycogen as the storage product and extremophily were coded as advanced, whereas they were previously coded as primitive. 44 traits were excluded as they were inapplicable because some groups were united again. There were 38 groups, as Haplosporidia and Paramyxia were added. There were 6.1 mln. rearrangements, 1061 steps, and 33 trees retained, and the CI was .41 and the RI .38.

The 3rd added proteinaceous wall as advanced and the Ascetospora clade was removed to save computer time. There were 3.6 mln. rearrangements, 1046 steps, and 24 retained trees, and the CI was . 42 and the RI .38. 45 characteristics were excluded, largely for animals, when then they had been separated into 2 clades).

Results

For the 1st analysis the strict consensus of note found Neokaryota, Histonia-Marinamebae, Chromobiotes (including Eulobosa), Eusporamebae-Pelamebae, and *Cyanidiococcus*+Cavernales, which was on top. The only bootstrap (always 100 replicates) groups were Neokaryota (60)(included all eukaryotes except the cyanidiophyceans, which were at the bottom), Opisthokonta (64),

Marinamebae (Foraminifera-Actinopoda) (81), Chomerales-Telosporidia (75), Testafilosa (Gromidae-Euglyphida)(83), and Jakomastigota (Jakobida-Polymastigota)(85).

In the 2nd analysis there were the same groups as before in the strict consensus except that Chromobiotes did not contain Eulobosa, and there was a *Cyanidioschyzon*+*Cyanidiococcus*-Cavernales clade, which was at the bottom, an Ascetospora clade, and a Hemimastigota-Euglenobiota clade, and in the majority consensus there was the Proto-Excavata clade (82%) and the Amebobiota clade (55%). The bootstrap groups were *Cyanidiococcus*+Cavernales (55), Opisthokonta (64), Marinamebae (79), Chomerales-Telosporidia (55), Ascetospora (77), Proto-Excavata (56), and Jakomastigota (76).

The strict consensus for the 3rd analysis is given below.

Table 1. Strict Consensus set at 50% for 24 MPTs for Eukaryota, 36x392 (45 excluded).

HypthAnc
Eukaryota
Subcyanidia
Subfilosa
Cyanidioschyzon
Metakaryota
Glaucobiota
Neokaryota
Rhodobiota
Anakaryota
Plasmodiophorae
Histonia-Marinamebae
Histonia
Plantae
Animalia+Fungi
Marinamebae
Foraminifera
Actinopoda
Chromobiotes
Chlorarachnia
Chromobiota
Cryptophycea
MetaChromobiota
Haptophycea
Heterokonta+Dinoflagellata
Cercomonades
Eusporamebae
Dictyostelia
Ciliophora
Chromerales-Telosporidia
Jakobida-Polymastigota
Heterolobosa
Hemimastigota
Euglenobiota
Pelamebae
Eulobosa
Eufilosa

Gymnofilosa
Nucleariidae
Vampyrellidae
Testafilosa
Gromida
Euglyphida
MuriCyanidia
SubGaldieria
Cyanidiococcus+Cavernales
Cyanidium
Galdieria

The following were over 50 in the bootstrap: *Cyanidiococcus*+Cavernales (65), Opisthokonta (60), Marinamebae (78), Chromerales-Telosporidia (52), Proto-Excavata (57), and Jakobida-Polymastigota (81). The cyanidiophyceans were all at the bottom, dinoflagellates were juxtaposed with the other alveolates and between Marinamebae and Nucleariidae, Eusporamebae and Dictyostelia were between Cercomonades and Opisthokonta, and Eulobosa was on top.

Cyanidiophycea are advanced over Rhodophycea in 6 of the 15 molecular analyses that I know of involving these groups, that is, 40%. There are probably groups as primitive as the former but marine and unknown to us, as this clade is terrestrial or freshwater, and the primitive habitat is marine. The molecular estimate for the age of this clade is 1.3 bln. yrs. (Huang et al, 2024). Fossil age estimates for Rhodophycea place it at 1 bln. (based on *Bangomorpha*, Gibson et al, 2017), 1.2 bln. (based on *Bangomorpha,* Javaux, 2007), or 1.6 bln. yrs. (based on *Ramathallus*, Bengston et al, 2017).

Two earlier analyses I did with Rhodobiota separated into Stylonemates, Rhodellae, Porphyridiales, and Eurhodobiota found it monophyletic at 92 and 85 in the bootstrap. Actinopoda was also separated twice into its component clades and was monophyletic at 70 and 57, without Taxopoda. Plants were twice separated into Aquiplantae (the misnamed "Chlorophyta", which necessarily designates all [green] plants) and Streptophyta (or Terriplantae), and a third time into *Pycnococcus* and Coronaplantae (Volvophyta, Ulvophyta, and Streptophyta), after doing an analysis of green algae with a comprehensive data matrix (31x142), which found Aquiplantae to be very much paraphyletic, as would be expected; each time Plantae was monophyletic at 100. I separated Animalia into Metazoa and Choanozoa and Fungi into Chytridiomycota and Amastigomycota and both kingdoms also came out as monophyletic at 100. I also separated Eulobosa into Amebidae, Platyosa, Arcellinidae, and *Trichospherium* on 3 occasions, which showed up as monophyletic in the bootstrap at 66, 53, and 58. (See Empire Biota, 3rd ed., 2023.)

Overall Classification

I incorporate molecular taxonomy where there is moderate or strong support and where there are classical synapomorphies (which may be comparable to a combined analysis), so I recognize Cyanidiophycea (proteinaceous wall and extremophily), Myzobiota (myzocytosis), Amorphea (also called unikonts)(pelamebae, eusporamebae, dictyostelians, and opisthokonts)(posterior-anterior flagellar transformation, CSP [carotenoid synthesis pathway] L [in Eusporamebae, Ascomycota, and Basidiomycota], and 3 myosin features [myosin TH2, class II myosins, SH3 domain tails]). (The Greek name Mycota for Fungi is a misnomer; the name of the science should be manitology, from *manitis* or *manitari,* meaning fungus or mushroom, and phyla should be -manites, -manitaria, or -fungi and for class -morphes. *Pelomyxa* is also a misnomer, so I rename it *Pelamoeba*. I also rename Myxobiota as Sporamoebae.)

The suffix -zoa meaning life is a confusionism. As Confucius said, "The beginning of wisdom is

calling things by their right names" (taxonomicon.nl) and "If names are not correct, language will not be in accordance with the truth of things" (quotegarden.com).

Considered here as belonging to larger groups because of molecular taxonomy and classical evidence, but which did not have resampling support above 50 in my taxonomies, are:

Apusothaumata	Cercobiotes
Colpodella	Telosporidians
Katablepharidae	Cryptomonads
Myxosporidians	Animals
Oxyrhis	Dinoflagellates
Spongomonads	Cercobiotes
Taxopoda	Actinopoda

And 17 groups are of uncertain position:

Aphelidia
Breviates
Ebrids
Ellobiopsids
Collodictyonidae
Colponema
Coproamebae
Fonticula
Guttulinopsis
Hemimastigotes
Ichthyosporea
Microsporidia
Perkinsus
Picomonads
Rozella
Telonema
Trichobiota (Trichomycetes)

Apusothaumata combine Apusomonades with Thaumatomonades, a group found by my analyses.

The domain is considered the highest rank, so in the 3-domain system Metabacteria and Eukaryota together form a super-domain, but this is never mentioned. The term dominion was used as the highest rank by Moore (1974); he recognized 3 of them: Virus, Prokaryota, and Eukaryota.

Eukaryota are probably a chimera, so that they are not related phylogenetically to Metabacteria, and my phylogeny is as follows:

Table 2. Phylogeny for Biota (based on confidence-tree results of prior analysis I performed [see Empire Biota]), but incorporating molecular data [where appropriate] and endosymbiosis)(2 dominions, 27 kgdms.)(all are stat. nov. except some in Diaphoretikes)(geologic time when the taxon emerged is indicated).

dom. Prokaryota Chatton 1925 (1 kgdm., 12 sbks.)(Archeobiotic)
Togabacteria Cav.-Sm. 1993, Pelletier 2012 orthog. corrig.
Deino-Thermi Pelletier 2015 nom. nud.

Rickettsiae da Rocha-Lima 1916, Pelletier 2012 orthog. emend.
Proteinimurus Pelletier 2015, orthog. emend.
Chlamydiae Garrity & Holt 2001
Planctobacteria Cav.-Sm. 1998
Spirochaetes Garrity & Holt 2001
Chloroflexi Garrity & Holt 2001
Chlorobia Garrity & Holt 2001, orthog. emend.
Saprospirae Gross 1911, orthog. emend.Margulis & Schwartz, 1982 (syn. Bacteroidetes Krieg et al, 2010)
Proteobacteria Stackebrandt et al 1988
Cyanobacteria (ex Stanier 1974) Cav.-Sm. 2002
Mollifirmicutes Pelletier 2016
Metabacteria Hori & Osawa 1979, Hori, Itoh, & Osawa 1982 emend.
Nanobdellati Huber et al, 2023 (syn. DPANN Rinke et al, 2013)
Halobacteria Grant et al 2002
Methanaria Pelletier 2015 nom. nud.
Archaeoglobi Garrity & Holt 2001 ex Garrity & Holt 2002
Methanobacteria Boone 2002
Caldaria Möhn 1984
Thermoplasmata Reysenbach 2002
Thermoproteati Guy and Ettema 2024 (syn. TACK Guy and Ettema 2011)
Asgardbacteria nom. nov.
dom. Eukaryota Chatton 1925 (21 hyperkingdoms, 26 kingdoms)
Middle Proterobiotic
Cyanidiophycea Merola 1982, orth. emend.
Glaucophycea Bohlin 1901, orth. emend.
Rhodophycea Ruprecht 1851, orth. emend.
Late Proterobiotic
Plantae (Treviranus 1822) Copeland 1938
Heterokonta Luther 1899, Copeland 1956 orthog. emend.
Cryptophycea Pascher ex Schoenichen 1925, orth. emend.
Haptophycea Christensen ex Silva 1980, orth. emend.
Plasmodiophorae Woronin 1878, orth. emend.
Chlorarachnia Cavalier-Smith 1993
Cercomonades Poche 1913, orthog. emend.
Nucleariidae Cann & Page 1979
Vampyrellidae Zopf 1885
Testafilosa De Saedeleer 1934, orth. emend. Pelletier 2012
Ascetospora Sprague 1979
Amorphea S.M. Adl et al 2012 (syn. Unikonta Cav.-Sm. 1987)(5 spks.)
Eusporamoebae nom. nov.
Dictyostelia Olive 1970
Eulobosa (Deflandre in Grassé, 1952), Pelletier 2015, orth. emend. Pelletier 2015
Pelamoebae nom. nov.
Opisthokonta (Copeland, 1956) Cavalier-Smith, 1992 (2 kgdms.)
Phanerobiotic
Marinamebae Pelletier 2023
Myzobiota Pelletier 2023
Ciliophora Doflein 1901

Jakomastigota Pelletier 2015 nom. nud.
Heterolobosa Page & Blanton 1985, orth. emend. Pelletier 2012
Euglenobiota Pelletier 2023

Marinambeae may have originated in the Late Proterobiotic. Haptophycea (Haptomonades) are indicated as having originated in the Late Proterobiotic according to my phylogenies and molecular data but in the Phanerobiotic according to the fossil record.

In Fungi, the phylogeny by Spatafora et al (2016) is as follows:

Table 3. Spatafora et al's 2016 molecular classification for funguses, focusing on zygomycetes (figures are bootstrap values, Fungi was at 100).

Crypto- (*Rozella allomycis*)	
Meta-	100
Chytridio-	100
Neo-	90
Blastocladio-	100
Ceno-	100
Zoopago-	96
Zoopag.-+Kickxll.	60
Entomophth.	82
Ana-	95
Mucoro-	100
Glom.+Mortll.	68
Mucor.	100
Dikaryo-	100
Asco-	100
Basidio-	100

So in this Zygomycetes are paraphyletic and have 2 divisions: Zoopago- and Mucoro-. Saccharomycetes are falsely synonymized with Hemiascomycetes because Taphrinomycetes, a separate group, are also Hemiascomycetes, and are called Archiascomycetes by Webster and Weber (2007). The independent hemiascomycete lineages of Dipodascaceae, *Dipodascopsis*, and *Endomyces* in Tehler's 1988 cladistic taxonomy are placed in Saccharomycetes (Outline of Ascomycota, Myconet, fieldmuseum.org).

Diaphoretikes (Bikonta) in genotypic phylogeny include *Cyanidioschyzon*, Glaucophycea, red algae, plants, heterokonts, haptophyceans, telosporidians, dinoflagellates, and ciliates, but the DHFR-TS gene fusion, which would basically be the only synapomorphy and may not be a classical trait, occurs in plants, heterokonts, apusomonads, centrohelids, ciliates, and euglenobiotes, so they do not correspond very much.

Togabacteria are here regarded as part of Prokaryota, as no molecular phylogeny places it outside of them, and the ether lipids, which are theresult of LGT from Metabacteria, may be skewing the placement in my phylogenetic phenotypic classification. Out of 21 molecular phylogenies *Thermotoga* comes out as basal and alone 10 times, as related to *Aquifex* 7 times, and elsewhere 4 times. *Aquifex* branches with the Epsilon clade of Proteobacteria (EpsilonProtei) in many other studies (Eveleigh et al, 2013). And Togabacteria have the fewest derived traits of prokaryotes, with 12. Also, the common ancestor to all life was probably hyperthermophilic (Di Giulio, 2003), which provides yet more evidence for them as basal. But contrary to what the abstract for that article says, the basal clade

for eukaryotes, being Cyanidiophycea, is in fact hyperthermophilic, and the one for Metabacteria, being Halobacteria, not Caldaria, is not. Several genera have been added: *Athalassotoga* Itoh et al 2015 and *Mesoaciditoga* Reysenbach et al 2013, creating Mesoaciditogaceae Itoh et al 2015 and Mesoaciditogales Itoh et al 2015; *Tepiditoga* Mori et al 2021 in Petrotogaceae Bhandari & Gupta 2014 in Petrotogales Bhandari & Gupta 2014; and *Pseudothermotoga* Bhandari & Gupta 2014 in Thermotogales. The total is now 14 genera and about 40 species. The phylum name is Thermotogota Reysenbach 2021, and the class name is Thermotogae Reysenbach 2002.

Heliobacteria, which are phototrophic and separate from Firmicutes in my phylogenies, are here considered part of Firmicutes according to molecular phylogeny and some classical traits (lack of outer membrane and formation of heat-resistant endospores).

Proteinimurus does not have bootstrap support in my taxonomies but is often recovered in molecular phylogeny (Proteinimurus and Neosoma are singular; the plurals are *muri* and *somata; monada* is also singular, the plural being *monades*).

The Hydrobacteria-Terrabacteria dichotomy is recovered in Battistuzzi & Hedges (2008), but Eubacteria is monophyletic in their analysis.

Gracilicutes are ambiguously recovered in Gupta (1998a) and Archeota are primitive. And my phylogeny for all life does not include Gracilicutes as monophyletic nor Archeota as primitive.

For convenience there are only 4 kingdoms: Bacteria, Phyta, Fungi, and Zoa. This can also be rendered as 10 kingdoms in 4 superkingdoms, as follows:

Table 4. 10-Kingdom Convenience Classification.

mgk./spk. Bacteria (2 kgdms.)
- Eubacteria (4 sbks.)
  - Togabacteria
  - Gracilicutes
  - Mollicutes
  - Firmicutes
- Metabacteria (3 sbks.)
  - Halobacteria
  - Methanaria
  - Caldaria

mgk. Eukaryota
- spk. Phyta (5 kgdms.)
  - Archeophyta
  - Plantae
  - Chromophyta
  - Chlorarachnia
  - Euglenobiota
- spk. Fungi (1 kgdm.)
  - sbk. Chytriofungi
  - sbk. Amastigofungi
- spk. Zoa (2 kgdms.)
  - Animalia
    - sbk. Unicellularia
    - sbk. Multicellularia
  - Tubulizoa
    - sbk. Flagellata
    - sbk. Plasmodiophorae

sbk. Amebae (Sarcodina)
Lobosa
Filosa
Forams
Actinopoda
sbk. Sporozoa
sbk. Ciliophora

For ease of presentation, there are also 10 kingdoms, that correspond to chapters and are chronologically arranged, as in the book: Bacteria, 4 lamellicristate kingdoms, 4 tubulicristate kingdoms, and 1 discicristate kingdom.

Prokaryotes

There are several newly-discovered groups of Metabacteria. In 2015 there was only 1 Asgard known—*Lokibacterium*, found near Loki's Castle, a hydrothermal vent on the Mid-Oceanic Ridge of the Arctic (or N Atlantic on Gakkel Ridge) at 7717 ft. (2352 m.) deep at 73° N and 8° E, between Svalbard to the N and Norway to the S—but in 2021 there were 18 (Da Cunha et al, 2022). Thorbacteria was discovered in samples from the White Oak River in North Carolina (Seitz et al, 2016). Members are also found in hydrothermal vents in the Gulf of California, hot springs in Yellowstone, terrestrial soil in Colorado, marine water in the Red Sea, freshwater hydric soil in the Great Lakes of N. Am., microbial mats in Shark Bay, W. Australia, and marine sediments in Kattegat Strait and the S. China Sea (MacLeod et al, 2018). Lokibacteria appear more abundant in currently characterized microbiomes, whereas Thorbacteria, Heimdallbacteria, and Odinbacteria appear at relatively lower abundances. Odinbacteria are associated with geothermal environments, while Heimdallbacteria and Lokibacteria are primarily found in marine sediments, and Thorbacteria appear to occur in a diverse range of microbiomes, notably marine, lake, and estuarine sediments. Overall, Asgard is more abundant in methane-rich or hydrothermal environments than terrestrial soil or freshwater. Also, Asgards are almost entirely uncultured, so their characteristics are inferred from metagenomics.

So the 2-domain system (see, for instance, Williams et al, 2019 and Spang et al, 2018)—Eubacteria, misnamed Bacteria, and what I call Neosoma (containing Metabacteria, which are misnamed Archeota (or worse), and Eukaryota), with Neosoma also being misnamed Archeota—is now becoming more widely accepted, because of the discovery of ESPs (eukaryotic signature proteins) in some metabacteria, especially actin, tubulin, and ubiqutin; Heimdallbacteria were found to have histone tails, which occur elsewhere only in Eukaryota (Henneman et al, 2018) and SNF7 (Zaremba et al, 2017), and they encode hallmarks of aerobic eukaryotes, including electron transport chain (ETC) complexes III and IV, heme synthesis, and response to reactive oxygen species (ROS), and their genomes encode CoxD, which regulates the ETC in eukaryotes (Appler, 2024); and ESCRET (endosomal sorting complex required for transport) is found in TACK, Asgard, and Eukaryota (with ESCRET III found only in the latter 2); making Metabacteria paraphyletic, with 7 possible extra kingdoms: Jordbacteria, Odin-Badrabacteria, Lokibacteria (Lokibacteriales-Helbacteriales+Thorbacteriales-Hermodbacteriales), Sifbacteria, Wukongbacteria, Heimdallbacteria 1 (Njord-, Gerd-, Heimdallbacteriales), and Hodbacteria, all Asgard (a taxon established by Kataryzna Zaremba-Niedzwiedzka et al, 2017), the last one being the sister group of eukaryotes (Eme et al, 2023). The name Eukaryomorpha is proposed for Asgard+Eukaryota by Fournier & Poole (2018)(but the plural is *morphes*).

This was foreshadowed by Lake's (1984) eocyte theory and Parkaryota, which contained

Sulfobacteria and Eukaryota. In fact, a cladistic classification based on classical evidence does indeed, not surprisingly, recover a Neosoma (100 in the symmetrical resampling, using TNT) and in which Metabacteria are paraphyletic, the ribosomes in Metabacteria and Eukaryota being very similar, the only difference being the lack of lobes in the former, and there are about 20 other synapomorphies. The outgroup was *Thermosipho*. In the PAUP classification, however, with the hypothetical ancestor as the outgroup, Metabacteria turns up monophyletic at 69 in the bootstrap and 79 in the jacknife, with Neosoma at 100 in the bootstrap and 100 in the jacknife, making a triple play. Eubacteria are paraphyletic in both, with both Gracilicutes and Firmicutes paraphyletic. As alluded to earlier when Eubacteria are analyzed alone, both Gracilicutes and Firmicutes (often falsely used to designate Firmicutes+Mollicutes) are monophyletic, with 70/78 and 89/87, respectively, in bootstrap/jackknife support, and there was only 1 MPT. And contrary to Skophammer et al (2007) and Gupta (2008a and b), Metabacteria in my analyses do not originate in Gram positives. (It is interesting to note that Halobacteria were discovered in 1935 by Schoop and Methanobacteria by Kluywer & Van Niel in 1936 but were recognized as distinct from typical bacteria only in 1977 by Woese & Fox.)

But Da Cunha et al (2022) make a case for caution in the light of possible artifacts that may be at work. And there is also the 1-domain system, proposed by Cavalier-Smith (2002), de Duve (2007), Devos & Reynaud (2010), Reynaud & Devos (2011), Forterre in 2011 and 2013, Cavalier-Smith & Chao (2020), and Devos (2021).

Gribaldo & Brochier-Armanet (2006) say the question of whether neosoman traits are ancestral or derived is solved if Metabacteria, or the neosoman ancestor, arose from within Eubacteria (Gupta, 1998a, 1998b; Cavalier-Smith, 2002). This specifically requires an episode of dramatic evolutionary acceleration in the branch leading to Metabacteria (Gupta, loc. cit.) or to the ancestor of Neosoma (Cavalier-Smith, loc. cit.). Such an event would mask the real origin of neosoman gene sequences and distort universal trees, an argument similar to those made by proponents of chimeric models for the origin of Eukaryota. The trigger for such an event was proposed to have been selection pressure for antibiotic resistance and oxygen sensitivity when the atmosphere went from anaerobic to aerobic (Gupta, loc. cit.), or appearance of neosoman-type histones to protect DNA against thermal denaturation (Cavalier-Smith, loc. cit.).

However, Gribaldo & Brochier-Armanet state that single-point mutations are sufficient to produce drug resistant versions of antibiotic targets. And the histone-like eubacterial HU proteins have replaced the endogenous metabacterial counterparts in Thermoplasmatales, without seemingly triggering any drastic evolutionary acceleration at the genome level. They continue by remarking that the model does not easily explain the replacement of the eubacterial DNA replication apparatus by the totally unrelated neosoman one, and the change in the stereochemistry of the glycerol backbone of eubacterial/eukaryotic lipids in the lineage leading to Metabacteria. And they point out that selection pressure for adaptation to life at high temperature does not seem to be a sufficient trigger for switching from a G3P to G1P glycerol backbone, since thermophilic eubacteria have arisen from mesophilic lineages at least twice and adapted their lipids to mimic metabacterial ones without changing their backbone stereochemistry.

Also, the question of whether neosoman traits are ancestral or derived is solved by cladistic analysis and the comparison of the totals of advanced traits. Indeed, Metabacteria are obviously advanced over Eubacteria, come out as advanced in my analyses, and have more derived traits than Eubacteria: 3 of the 5 clades I used for Metabacteria had over 100 advanced characteristics, while only 1 of the 16 I used for Eubacteria had the same. And Eubacteria as primitive is supported by the 1-domain proponents, and Metabacteria as primitive is an artifact of LBA (Philippe et al, 2000).

Gupta pointed out that Metabacteria (which he calls Archeobacteria) are monophyletic based on protein genes involved with information transfer processes, and that they are polyphyletic based on other protein genes in which they derive from Gram positives, specifically the high GC ones. In his analysis (Table 5), in the former (3b), Gracilicutes are monophyletic, Firmicutes and Mollicutes are

polyphyletic, and in the latter (3a), Gracilicutes are only 30 in the bootstrap, Mollicutes are paraphyletic, and no Firmicutes were included. He used neighbour-joining for both. 3a is the more correct phylogeny because Archeobacteria are manifestly monophyletic or paraphyletic in my analyses.

Gupta as well maintains that eukaryotes are a chimera and unrelated to metabacteria. Indeed, it is difficult to reconcile the endosymbiotic origin of Eukaryota with a phylogenetic relationship with Metabacteria or any part of them, in other words, with an autogenic origin—in fact, it is contradictory.

Table 5. Gupta's Genotypic Classification of Bacteria from 1998 (figures are bootstrap values, 50+).

a) based on EF (elongation factor)-1α/Tu, rooted

Archeobacteria 100
Halobacteria
Thermococcus-Pyrococcus 100
Methanobacteria 100
Thermoplasma
Sulfobacteria 100
Eubacteria 100
Thermotoga maritima
SG 1 59
Mycoplasma A
SG 2 55
Mycoplasma B
Chlorobacteria
Bacteroidetes
Deino-Thermi 99
Cyanobacteria 100
Proteobacteria 74

b) based on HSP (heat shock protein) 70, unrooted

Archeobacteria+Firmicutes 99
Thermotoga maritima+Archeota A 88
SG 2 75
Mycoplasma A+Actinobacteria (high GC) 100
Archeota B+Endospora A (low GC) 75
Mycoplasma B+Endospora B (low GC) 84
Gracilicutes 99
Dn-Th (96)+Cyanobacteria (73) 100
SG 2 86
Cytophaga+Flavobacteria 100
Chlamydiae+Spirochetes 58
Proteobacteria 93

Cavalier-Smith and Devos and Reynaud put forward the idea that Metabacteria evolved instead from Planctobacteria.

The supersmall DPANN (Diapherotrites, Parvbacteria, Aenigmabacteria, Nanobacteria, Nanohalobacteria) are named for their original members and established by Ringe et al in 2013.

Woesebacteria and Pacebacteria were added in 2015 by Cindy Castelle et al. Later added were Alti-, Micro-, Huber-, and Undinbacteria. They are obligate epibiotes of various metabacteria and are supersmall, having an average genome size of only 1.2 Mbp.

By way of comparison the other estimated average genome sizes are Eurybacteria and TACK 1.8, Asgard 3.8, Heimdallbacteria 3.5 (1.6-7.4), and Hodbacteria 5.1 (Eme et al, loc. cit.). The smallest free-living eukaryote is the prasinophycean *Ostreococcus tauri*, at 12.5 (Derelle et al, 2006). The smallest genome overall belongs to the parasitic *Mycoplasma* (Mollicutes): less than 1. The largest known is the New Caledonian forked fern (*Tmeripteris oblanceolata*)(Fernandez et al, 2024) at 160,000 Mbp, beating out *Paris japonica* (in the bunchflower family) at 150,000, discovered in 2010. Stretched they would each be some 100 m. long. The cell sizes of DPANN are below 1 micron (van Wolferen et al, 2022). Cell sizes in prokaryotes range from .1 (mycoplasmas)-10 microns, and in eukaryotes it is 10 to 100 microns (Studying Cells - Cell Size, bio.libretexts.org).

For Eubacteria, the CPR (candidate phyla radiation) clade (containing new groups), which in turn is divided into Microgemonates and Parcubacteria, and PCV (Planctobacteria, Chlamydiae, Verrucomicrobia) are recognized by Hug et al (2016), but CPR has only modest bootstrap support (over 50), but its 2 component superphyla have strong bootstrap values (over 85). The other phyla are presented unclearly so one cannot know how many superphyla, subkingdoms, or kingdoms there might be.

There may be a Hydrobacteria-Terrabacteria dichotomy for eubacteria (Battistuzzi & Hedges, 2008), the former is mostly marine (87 %), while the latter is mostly in arid soils (67%), and they exhibit adaptations to water and land, respectively. Photosynthesis, where it occurs, is anoxygenic in the former and oxygenic in the latter. Spores are commonplace in Terrabacteria and rare in Hydrobacteria (occuring only in Myxospora, part of Proteobacteria). The protein type is I in Terrabacteria and II in Hydrobacteria. The RNA type is I in the former (except for Deino-Thermi which has III), and II in the latter. In the ML for slowly evolving sites based on proteins the bootstrap values are 86 for Hydrobacteria and 81 for Terrabacteria. Acquificae, Togabacteria, and Fusobacteria are the other groups and are basal. I consider Aquificae and Fusobacteria to be of uncertain position. As reported by Battistuzzi & Hedges (loc. cit), Terrabacteria has c. 6000 sp., Hydrobacteria c. 3000, Aquificae 22, Togabacteria 30, and Fusobacteria 32, and Eurybacteria had 243 sp., Crenobacteria 53, and Nanobacteria 1.

The ICNP in its revision of 2023 recognized 4 eubacterial kingdoms: Thermotogati, Pseudomonadati (Hydrobacteria), Fusobacteriati, and Bacillati (Terrabacteria), and 3 metabacterial kingdoms: Nanobdellati (DPANN), Methanobacteriati (Eurybacteria), and Thermoproteati (TACK); -ati signifies kingdom and -ota phylum. Their classification for Eubacteria follows Battistuzzi & Hedges (2008) except for the placement of Aquificae in Hydrobacteria.

Harish & Kurland (2017) deny the chimeric nature of eukaryotes, proposing prokaryotes and eukaryotes descended from a common ancestor based on the reconstruction of a global tree of life with non-reversible and non-stationary models of genomic evolution that root trees intrinsically. They implemented Bayesian-model selection tests and compared the probability support for 4 conflicting tree-of-life theories: Lake's eocyte from 1984, Woese's 3 domains from 1990, Gupta's model, and Mayr's 2 domains (Prokaryota and Eukaryota) from 1998. They find that the most probable theory is Mayr's even though they regard it as phenetic. Eubacteria, Metabacteria, Prokaryota, and Eukaryota all have a posterior probabilty of 1. The authors recognize 3 superkingdoms: Eubacteria, Metabacteria, and Eukaryota, but 2 primordial lineages: Prokaryota (which they call Akaryota) and Eukaryota. Also, the Gupta model they cite ambiguously as 1995 and Gupta et al from 1993, and neither is included in the references, and the more appropriate reference is from 1998. As well, the Prokaryote-Eukaryote dichotomy in Mayr and Harish & Kurland and here is entirely coincidental as it is arrived at from completely different angles. And Mayr was as much of a notorious gradist as Cavalier-Smith, who grossly misrepresented cladistics, and, like other gradists, they pretended their method is phylogenetic.

Endosymbiosis

The concept of symbiosis started with Simon Schwendener in 1867, a Swiss botanist who discovered that lichens consist of a fungus and a photosynthesizer (Martin, Garg, and Simonski, 2015). The German botanist Heinrich Anton de Bary coined the term 'Symbiose' in 1878. German-French botanist and phytogeographer Andreas Schimper in 1883 is sometimes credited with the discovery of endosymbiotic theory, because of a footnote that said: 'If it can be conclusively confirmed that plastids do not arise de novo in egg cells, the relationship between plastids and the organisms within which they are contained would be somewhat reminiscent of a symbiosis. Green plants may in fact owe their origin to the unification of a colorless organism with one uniformly tinged with chlorophyll'.

German pathologist, histologist, and anatomy professor Richard Altmann is often credited with the idea of symbiotic theory for the origin of mitochondria (as in my book, so this is a corrigendum), but Martin et al point out that Altmann's 1890 book shows that he was not interested in mitochondria, and he did not propose their symbiotic origin. He mentioned neither mitochondria (nor their older name, chondriosomes) nor endosymbiosis in his book on 'bioblasts'. To Altmann, everything in eukaryotic cells consisted of bioblasts, including the cytosol, the nucleus, and the chromosomes. His bioblasts corresponded to a chemical organization state of matter that was larger than the molecule but smaller than the cell—'the smallest morphological unit of organized material' (*die kleinste morphologische Einheit der organisirten Materie;* Einheit would literally translate as 'onehood'). They would maybe correspond in size roughly to what we today call macromolecular complexes, but which could not be seen in the (light) microscopes of Altmann's day. He also distinguished autoblasts, cytoblasts, karyoblasts, and somatoblasts, and he developed an histological staining method, and coined the term "nucleic acid" as a synonym or replacement for Friedrich Miescher's "nuclein" when it was found to have acidic properties. Russian botanist Konstantin Merezhkovsky in 1910 did important research in endosymbiosis and coined the term "symbiogenesis" and perhaps first formulated the theory. French zoologist and marine biologist Paul Portier in 1918 presented the first detailed description. Russian botanist Boris Kozo-Polyansky explained it in terms of Darwinian evolution in his book Symbiogenesis: a New Principle of Evolution in 1924. Wallin in 1927 proposed it for mitochondria. More detailed electron microscopic comparisons between cyanobacteria and chloroplasts notably by Hans Ris were published in 1961. And Lynn Margulis popularized, elaborated, and synthesized it in 3 articles in the late '60s.

An important component of endosymbiotic theory is the fact that organelles have retained genomes (Martin et al, 2015). The observation that organelles had DNA at all was one of the key observations that shored up endosymbiotic theory to begin with. Indeed, several autogenous (vertical gene transfer) alternatives to the endosymbiote hypothesis were designed specifically to explain the existence of DNA in organelles. The answer to why organelles retain DNA is satisfactorily explained by John Allen's CoRR model (co-location for redox regulation)(2017), the term introduced in 2003. It proposes that it is so that they can contribute to the expression of components of the respiratory and photosynthetic electron transport chains in order to maintain redox balance in the bioenergetic membrane. Redox balance is the smooth flow of electrons through the electron transport chain. The concept applies both to mitochondria and chloroplasts, because both have electron transport chains that generate proton gradients to drive their respective ATPase. In both electron transport chains, quinones and quinols (reduced quinones) are an essential component.

Syntrophy (metabolic symbiosis) is a frequent property of microbial communities and allows a community as a whole to survive in an environment, even though individual members cannot. For example, methanogens remove the hydrogen waste of fermenting eubacteria, which helps both partners survive in low energy environments. "The mutual dependence between different organisms shared by

all syntrophies is thought to originate in a process of sustained co-evolution. In this process, ancestrally self-sufficient organisms are driven to interdependence by degenerative mutations that erode their metabolic independence. An alternative but underexplored possibility is that syntrophy can emerge spontaneously from serendipitous combinations of organisms with complementary biochemical abilities. If so, syntrophy does not require a shared evolutionary history and is not a degenerative phenomenon." (Libby et al, 2019)

One of the oldest proposals based on explicit syntrophy was that of Searcy (1992, 2003), who stated that eukaryotes derived from a sulfur-mediated symbiosis between an unwalled, sulfur-respiring *Thermoplasma*-like metabacterium and a photo- or chemoautotrophic H2S-utilizing eubacterium. In 2000 Lopez-Garcia & Moreira put forward a syntrophy model based on a tripartite metabolic symbiosis involving a methanogenic metabacterium (future nucleus), a fermentative myxobacterial-like Delta (future eukaryotic cytoplasm) and a metabolically versatile methanotrophic Alpha (future mitochondrion). A refined version later proposed the evolution of the endomembrane and nuclear membrane system by invagination of the Delta membrane. They now adapt the model to contemporary data (the discovery of Asgard), shifting from the original hydrogen and methane-transfer-based symbiosis (HM syntrophy) to a tripartite hydrogen and sulfur-transfer-based model (HS syntrophy) (2020). This scenario contends eukaryotes originated in Early Proterozoic microbial mats from the endosymbiosis of a hydrogen-producing Asgard within a complex sulfate-reducing Delta. Mitochondria are hypothesized to have evolved from versatile, facultatively aerobic, sulfide-oxidizing, and potentially anoxygenic photosynthesizing Alpha endosymbiotes that recycled sulfur in the consortium. The authors claim this HS version accounts for (endo)membrane, nucleus, and metabolic evolution in a realistic ecological context. Three more recent ones are the inside-out (Baum & Baum, 2014); reverse flow (Spang et al, 2019),which involves electron or hydrogen flow from an organoheterotrophic metabacterial host to a eubacterial symbiote, based on genome analyses that suggest Asgards are primarily organoheterotrophs with variable capacity for hydrogen consumption and production; and the entangle–engulf–endogenize (E3), which invokes the participation of one additional eubacterium as facilitator of the eukaryogenetic symbiosis (Imachi et al, 2019) models.

Outside-in models explain the origin of the nucleus and mitochondria as being the result of sequential rounds of phagocytosis and endosymbiosis (Martin et al, 2015). These models invoke 3 partners—host, nucleus, and mitochondria—and envisage the nuclear compartment being derived from an endosymbiote that was engulfed by a host cell. In general, endosymbiotic models are uncommitted as to whether mitochondria were acquired before or after the nucleus. An exception is the syntrophic consortium model, which envisages the simultaneous fusion of a symbiotic community composed of all 3 partners: cytoplasm, nucleus, and mitochondria. A more divergent model is the endospore theory (Gould & Dring, 1979), which holds that the nucleus evolved when a cell enclosed its sister after cell division, similar to the way in which endospores are formed in Endospora. However, there is no evidence of endospore formation or other engulfment processes in Metabacteria, making this hypothesis improbable.

Models have always assumed that the nucleus and endomembrane system evolved within the cytoplasm of a prokaryotic cell, which goes nuclear pores, nuclear envelope, secretory system, and endo- and phagocytosis. Drawing on diverse aspects of cell biology and phylogenetic data, Baun & Baum invert the usual order so that endo- and phagocytosis is first and nuclear pores are last. They propose an ancestral prokaryotic cell, homologous to the modern-day nucleus, extruded membrane-bound blebs beyond its cell wall. These blebs functioned to facilitate material exchange with ectosymbiotic proto-mitochondria. The cytoplasm was then formed through the expansion of blebs around proto-mitochondria, with continuous spaces between the blebs giving rise to the ER (endoplasmic reticulum), which later evolved into the eukaryotic secretory system. Further bleb-fusion steps yielded a continuous plasma membrane, which served to isolate the ER from the environment.

The authors claim the inside-out theory is consistent with diverse kinds of data and provides an

alternative framework by which to explore and understand the dynamic organization of modern eukaryotic cells and also helps to explain a number of previously enigmatic features of cell biology, including the autonomy of nuclei in syncytia and the subcellular localization of N-glycosylation, and makes many predictions, including a novel mechanism of interphase nuclear pore insertion.

The major points to be retained here are that phylogenetic analysis based on classical evidence is just as reliable as the molecular method, the probable chimeric nature of Eukaryota, the difficulty in reconciling lateral gene transfer with vertical gene transfer in eukaryogenesis, the advanced nature of Metabacteria (hence the name), the probable paraphyly of Eubacteria, and the possible near-monophyly of Prokaryota.

Diagnoses

Deino-Thermi: eubacterial taxon with S-layer, RNA polymerase β′ subunit, signal recognition particle protein Ffh/SR54, major sigma factor 70, etc. The first component group is radiation resistant and the second is heat resistant.
Methanaria: metabacterial organisms that carry out methanogenesis.
Asgardbacteria: metabacterial taxon that possesses many ESPs, utilizes the Wood-Ljngdahl metabolic pathway, performs glycolosis, and is mostly obligately anaerobic.
Jakomastigota: unicellular, heterotrophic, eukaryotic organisms, with B and C fibers in the flagellar apparatus.
Pelamoeba: unicellular ameba found in mud at the bottom of freshwater ponds, with short, non-motile flagellum (pseudoflagellum); can develop into giant cell with multiple, separate monokinetids.
Eusporamoebae: organisms with tubular cristae, alternation of monoploid (haploid) and diploid generations, sporic meiosis, a unique plasmodium-sporophore complex, and 3 stages: the reproductive, sporulating; a uninucleate, microscopic, ameboid (usually [anisokont] flagellate) trophic stage, with filose pseudopods; and a plasmodial trophic stage, which is sometimes macroscopic, but still very small (under an inch high), and sometimes pigmented; the sporulating stage has a chitinous or cellulosic wall; bacteria are the primary food source.
Sporamoebae: sporulating amebas, a polyphyletic group, which includes Eusporamebae, Dictyostelia, Acrasia, *Fonticula*, Coproamebae, and *Guttilinopsis*.

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