

SYSTEMATICS OF THE MICROBIAL KINGDOM(S) AND FUNGI

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Keywords: Archea, bacteria, eucaryotes, evolution, fungi, lichens, nomenclature, phylogeny, procaryotes, protocista, species, systematics

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Summary

Carl Woese (1998) wrote: “Microbial life on this planet would remain largely unchanged were all plant and animal life eliminated, but the elimination of microbial life itself would lead in very short order to a completely sterile planet.”

The circumscription of microbial diversity is fraught with difficulty. The organisms by their very nature are small, they encompass a vast diversity of form and function, and after more than two centuries of research we remain largely ignorant as to their emergence, evolution, and full extent. Early attempts to describe diversity were generally incomplete and fragmentary.

The majority of these studies were almost exclusively based on morphological observations, despite the relatively narrow range of characteristics evident, and tended

to focus on individual microbial groups in isolation. Advances in technology, biochemistry, microbial physiology, and electron microscopy enabled us to refine our views, but it was possibly not until the introduction of nucleic acid sequencing methods that the broad sweep of microbial diversity could begin to be assessed and its evolution inferred.

In particular the use of small subunit ribosomal DNA data provided a means by which it was possible for the first time to study all microorganisms. This method has been instrumental in changing our view of the major divisions of life on Earth and in providing insights as to how they came about. In the future it is likely that the increasing quantity of complete genome sequence information derived from microorganisms may lead to refinements, if not a wholesale reevaluation of our views on microbial diversity and evolution.

It is only through analysis of these data that we will be able to assess the true impact and extent of genetic exchange between microorganisms and whether the current linear, step-wise view of microbial diversification should be replaced with a more net-like structure underpinning this process.

1. Introduction

In the late seventeenth century, Antonie van Leeuwenhoek constructed one of the earliest microscopes by grinding glass to create a magnifying lens, and with the creation of this tool the study of microbial life began. Since then our understanding of the diversity and the contribution that microorganisms have made, and continue to make, to life on Earth, has grown steadily.

Yet despite their importance and the advances made particularly during the twentieth century we still know relatively little about the extent and diversity of microbial life on Earth. Estimates suggest that fewer than 2% of all microorganisms have been identified, and we possess detailed knowledge on fewer still. Further, their overall significance and distribution have tended to be overlooked, even by many biologists, yet recent studies have estimated that procaryotes alone constitute more than half of the biomass on Earth.

Microorganisms play a vital role in every aspect of life on our planet. The wide diversity in microbial form and function ensures that microorganisms are ubiquitous in all environments able to sustain life. They are frequently the only form of life able to survive and grow in some of the Earth's most inhospitable habitats with respect to extremes of heat, salt, pressure, and water and nutrient availability (see Figure 1).

Microorganisms encompass a wide range of metabolic activities and frequently exhibit unique characteristics not found in other life forms, for example the ability to fix nitrogen (the conversion of nitrogen gas to ammonia, a vital step in the nitrogen cycle). Not only do microorganisms thrive on an incredible array of energy sources ranging from sunlight to iron, but also some possess the ability to switch their metabolism to survive changing conditions, for example members of the bacterial family *Chromatiaceae* (purple sulphur bacteria) photosynthesize during the day but at night live as heterotrophs.

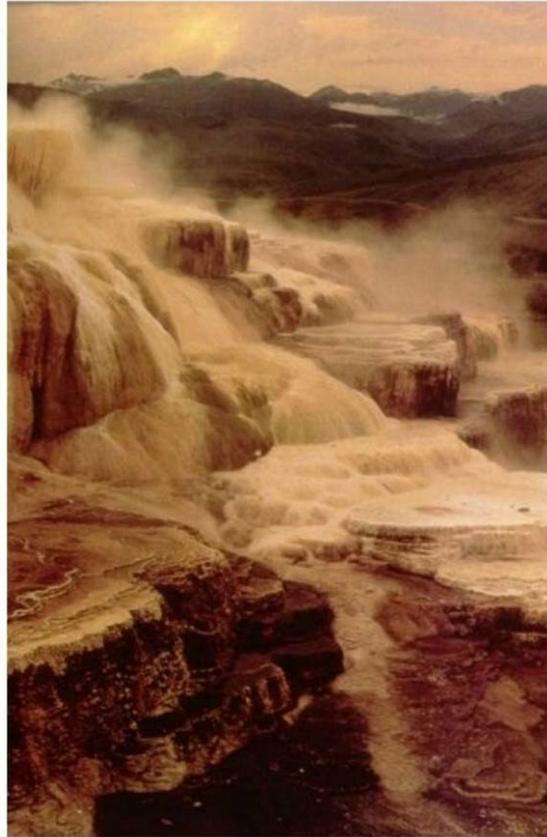


Figure 1. Hot springs at Yellowstone National Park, Montana, US. Microbial forms of life are the only extant organisms found in this near boiling water (produced with permission of CABI Bioscience, UK)

2. The Emergence of the Microbial World

2.1 Timetable of Events

It has long been recognized that microorganisms are the foundation of the biosphere. Microbial life has been present for approximately 4000 million years to 3500 million years of the Earth's 4500-million-year history. The common ancestor, or root to the tree of life, was likely to have been a procaryote from which the bacterial, archaeal, and eucaryotic Domains have arisen. It is a commonly held view that procaryotic, anaerobic, thermophiles were most likely the earliest forms of life as this adaptation appears in what are thought to be the most ancient form of extant life, and this scenario would accord with our knowledge of the atmosphere and temperature of the early Earth. Microfossil evidence positively indicates that cyanobacteria-like organisms, which may have been photosynthetic and oxygen producing, were present as early as 3800 million years before present (MYBP). Between 2800 MYBP and 2000 MYBP, largely through the actions of photosynthetic bacteria, oxygen began to accumulate in the Earth's atmosphere. Between 2000 MYBP and 1500 MYBP early eucaryotes emerged, and evidence of their presence has been found in rocks from about 1400 million years onward. It is assumed that they developed in the oxygenic atmosphere, evolving as a result of procaryotic metabolism.

Domain: BACTERIA

| | |
|---------------|----------------------------------|
| Phylum I: | Aquificae (5 genera) |
| Phylum II: | Thermotogae (5 genera) |
| Phylum III: | Thermodesulfobacteria (1 genus) |
| Phylum IV: | "Deinococcus-Thermus" (3 genera) |
| Phylum V: | Chrysiogenetes (1 genus) |
| Phylum VI: | Chloroflexi (5 genera) |
| Phylum VII: | Thermomicrobia (1 genus) |
| Phylum VIII: | Nitrospira (4 genera) |
| Phylum IX: | Deferribacteres (5 genera) |
| Phylum X: | Cyanobacteria (57 genera) |
| Phylum XI: | Chlorobi (5 genera) |
| Phylum XII: | Proteobacteria (432 genera) |
| Phylum XIII: | Firmicutes (184 genera) |
| Phylum XIV: | Actinobacteria (138 genera) |
| Phylum XV: | Planctomycetes (4 genera) |
| Phylum XVI: | Chlamydiae (5 genera) |
| Phylum XVII: | Spirochaetes (13 genera) |
| Phylum XVIII: | Fibrobacteres (1 genus) |
| Phylum XIX: | Acidobacteria (3 genera) |
| Phylum XX: | Bacteroidetes (53 genera) |
| Phylum XXI: | Fusobacteria (7 genera) |
| Phylum XXII: | Verrucomicrobia (3 genera) |
| Phylum XXIII: | Dictyoglomus (1 genus) |

Domain: ARCHAEA:

| | |
|-------------|---------------------------|
| Phylum I: | Crenarchaeota (22 genera) |
| Phylum II: | Euryarchaeota (48 genera) |
| Phylum III: | Korarchaeota |

Domain: EUKARYA

Rankless lineage: *Opisthokonta*—Platycristate mitochondria, one flagellum positioned at the rear of the cell.

Kingdom: **Fungi** (see *Systematics of Fungi*) + Microsporidia (no mitochondria)

Kingdom: **Metazoa** (animals)

Taxa incertae sedis: Choanoflagellates; Ichthyosporea (DRIPs clade); Nucleariids; *Corallochytrium*.

Rankless lineage: *Chromalveolata*—Tubulocristate mitochondria

Kingdom: **Chromista** ("Stramenopiles"/Heterokonts) (diatoms, various unicellular algal groups—Xanthophyceae, Chrysophyceae, Eustigmatophyceae—macroalgal/seaweed Phaeophyceae; "pseudofungi"—oomycetes, hyphochytriomycetes and the labyrinthulids (slime net fungi); the "protozoan" bisociids, and recently added the opalinids, which lack the tinsel flagellum but share many other characteristics; also included is the human/mamalian pathogen, *Blastocystis*)

| | |
|--|---|
| | <p>Kingdom: Alveolata (incl. Dinoflagellates, apicomplexans and ciliates [and <i>Perkinsus</i>])</p> <p>Taxon incertae sedis: Cryptista (cryptomonads or cryptophytes)</p> <p>Rankless lineage: <i>Euphyta</i>—Platyristate mitochondria; flagellum, when present, anterior.</p> <p>Kingdom: Plantae—unicellular algal groups, including the pathogenic <i>Prototheca</i> which has lost its chloroplasts as well as the multicellular plants.</p> <p>Kingdom: Biliphyta—red algae, glaucophytes.</p> <p>Paraphyletic lineage: <i>Protozoa</i></p> <p>Amoebozoa—Ramicristate mitochondria. amoeboid groups, slime moulds and dictyostelids</p> <p>Discicristata— Heterolobosea (Acrasid slime moulds and amoeboflagellates), Percolomonads, Euglenids & Kinetoplastids (incl. <i>Trypanosoma</i>).</p> <p>Cercozoa —Plasmodiophorids, chlorarachnids, sarcomonads.</p> <p>Deeper branching groups:</p> <p>Phylum: Metamonada (Diplomonads e.g. <i>Giardia</i>, <i>Hexamita</i>)</p> <p>Phylum: Parabasalia (e.g., <i>Trichomonas</i>)</p> <p>Phylum: Archamoebae—amitochondrial amoebae (e.g. <i>Pelomyxa</i>).</p> <p>These are the major lineages, many more lineages which have not, as yet, been sequenced are unassigned to a major lineage.</p> |
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Table 1. Domains of Bacteria, Archaea, and Eukarya

By about 650 MYBP multicellular organisms are thought to have arisen. The first unambiguous fossil record of fungi is dated back to the Ordovician period, 460 MYBP, and molecular clock dating implies that the lineage may be even older still. Protein sequence analyses indicate that major fungal lineages were most probably present about 1000 MYBP. Land plants appeared by 450 MYBP, and fungi have been implicated in their colonization during the Silurian (440 MYBP to 410 MYBP). It has been proposed that mycorrhizal associations of fungi and primitive green plants (Rhyniophytes) enabled these plants to improve uptake of nutrients and water from the soil, which then would only just be forming. Dinoflagellates, based on ultrastructural data and molecular phylogeny, are thought to have originated in the Neoproterozoic (500 MYBP to 900 MYBP), yet organisms classified as ancestors of dinoflagellate date only to the Middle Triassic (circa 240 MYBP). Examination of dinoflagellate-specific biological markers (dinosteranes and 4-methyl-24-ethylcholestane) in concentrated microfossils with known morphology identified ancient dinoflagellate ancestors from the Early Cambrian (~520 MYBP). The first mammals appeared only relatively recently, between 200 million years and 100 million years before present along with flowering plants that are thought to have arisen in the late Jurassic—early Cretaceous period, about 140 MYBP. Controversial claims of the ability to revive bacteria from very old material have been laid. Spores contained within a 250 million year old salt crystal have been successfully cultured, as have bacteria from the intact carcass of a bee trapped in 25-million-year-old

to 30-million-year-old amber.

2.2. Universal Ancestor and Early Eucaryotes

Much speculation exists as to the nature of the earliest form of life, but as described previously, it is likely that it was a procaryote, able to grow in the absence of oxygen at very high temperatures. Increasingly a consensus is emerging that such primitive cellular life-forms may have comprised simple, cell-like structures that also possessed the ability to exchange genetic material. It is thought that the latter attribute may have been one of the principal forces driving on the early diversification of life. Much work has been devoted toward determining the processes by which eucaryotic cells arose. There is broad agreement that this probably occurred approximately 2000 million years before present, most likely from a symbiosis between different procaryotic ancestors. At least three procaryotic components can be traced by comparing molecules in extant procaryotes and eucaryotes. These components are the mitochondria (derived most likely from an early example of the bacterial phylum, *Proteobacteria*), the plastids (from Cyanobacteria), and the nucleocytoplasmic component (from Archaea). The diversity of mitochondrial cristae among eucaryotes may give an insight into some major lineages of evolution.

Essentially there are four main forms; flat (platycristate), tubular (tubulocristate), disc (discicristate), and branched (ramicristate). It now seems that organisms basal in a lineage may show some plasticity in the form of the cristae. An example of this is provided by the ichthyosporeans, basal to the animal and fungal lineage, which have both tubular and flat cristae. The discicristate form is probably the most primitive. Unlike the mitochondria, the acquisition of photosynthesis has occurred repeatedly, and its subsequent loss is not infrequent either. The initial endosymbiosis of a cyanobacterium or prochlorophyte procaryote took place in the chromist, green plant, rhodophyte, and a number of smaller lineages. Subsequently photosynthetic eucaryotes themselves have been endosymbiosed to give rise to other photosynthetic groups such as the dinoflagellates and the euglenids. Evidence for this can be found in the multiple membrane layers surrounding the plastids and occasionally the vestigial nuclear bodies found in the plastids. Other features in eucaryotic cells, for example the cytoskeleton and the membrane-bounded gas vacuoles used for flotation and magnetosomes, have been suggested to be of procaryotic descent. The magnetosome allows cells to orient in the Earth's magnetic field and are similar in size and shape to those found in the brains of bees, homing pigeons, and salmon. It would appear therefore, that some eucaryotic organelles have been directly inherited from procaryotic predecessors without extensive modification. Meanwhile, others may have arisen from intact endosymbiotic bacteria in a degenerative process that resulted in extensive loss of genetic material and transfer of much of the remaining DNA to the eucaryotic nucleus.

3. Microbial Systematics

The study of the Earth's natural history, the description of its diversity and evolutionary pathway, is built on Systematics. This scientific discipline is reliant on diverse types of data on organisms to produce classifications that are logical, robust, and attempt to reflect evolution. The process is crucial, not only because it provides the means

subsequently to identify organisms, but also because it provides a basic framework around which an organism's biology can be studied further.

3.1. Higher Order Classification

Classification of life on Earth at the highest level was traditionally based on the two-kingdom concept, animals (*Animalia*) and plants (*Plantae*), and reflected a basic difference not only in motility but also in nutrition. Only a few cases could be considered ambiguous, such as the sessile sponges and corals, or the motile but undoubtedly photosynthetic unicellular *Euglena*. As more came to be known about the diversity of life, particularly among the unicellular or microscopic forms, such a simplistic division was no longer workable and further higher level groupings were proposed. By the end of the nineteenth century, a separate kingdom for bacteria (*Monera*) had been put forward as had one for the unicellular animals, the *Protozoa* or *Protista*. The recognition of the fungi as a separate kingdom, although proposed several times, really was not accepted until the proposals of Whittaker for the five kingdom system of life, the kingdoms being animals, plants, fungi, protists, and bacteria. Kingdoms were defined by a combination of morphological, nutritional, and biological characteristics. Further techniques such as in electron microscopy in the discovery of the details of cell structure; in biochemistry in understanding metabolic pathways and differences in cell chemistry and physiology; and in the impact of molecular data, the study of genetic material of organisms, were to identify new characteristics. The impact of the latter technology has been greatest in the study of procaryotic diversity, but developments from this area have begun to change our perceptions as to how all life on the planet arose and how we logically group organisms.

For many years our view of higher-order, procaryotic diversity and evolution was hazy, and early treatments of this subject were largely philosophical in nature. Although differences between procaryotes and eucaryotes had long been recognized, this dichotomous view of life was not formalized until the late 1930s. Initially, procaryotes were largely defined on the absence of key characters; however, the definition was refined with advances in biochemistry and cell biology, which allowed for a more objective definition of procaryotes. Although the eucaryotes occupied four of Whittaker's kingdoms and the procaryotes the remaining one, it should be recognized that the difference between eucaryotes and procaryotes was entirely based on differences in cellular organization and structure. As a consequence, some have argued that the classification was artificial and did not reflect phylogeny. The challenge to the five kingdoms and the eucaryote/procaryote view of life came in the late 1970s when the groundbreaking work of Carl Woese and colleagues offered an alternative viewpoint.

In essence these studies, based on a molecular approach, in particular the study of the small subunit of ribosomal RNA (rRNA), challenged the dichotomous view and provided a tool by which to measure diversity. The debate over the significance of this characteristic and how it should be interpreted continues. One thing is certain however: Woese's approach enabled, for the first time, the comparison of "like with like" and as such provided a tool which could be applied to all cellular life forms, both procaryotic and eucaryotic. This represented a major advance over the traditional approaches to

tracing evolutionary events that are reliant on form and function, which are known to be discontinuously distributed throughout the microbial world. Further, genes such as rRNA which perform vital cellular functions are thought more likely to be conserved through time, thus they provide a stable place from which to describe all of life's diversity and project its phylogeny. Using this method, Woese put forward the view that life on Earth falls into three primary lines of evolutionary descent, or Domains, two of which are procaryotic in nature, Archaea and Bacteria; and one eucaryotic, Eucarya.

These Domains, above the traditional level of Kingdoms, are thought to have diverged from one another a long time ago, presumably from an extinct or as yet undiscovered ancestral line or lines. Archaea and Eucarya seem to have arisen from a common line more recently than the divergence of these two groups from the bacteria, although this latter view has been challenged. One thing is certain, however: This phylogenetic framework (see Figure 2) not only provides a partial roadmap for the evolution of microbial life, but it also opens up a whole new conceptual approach to the study and description of microbial diversity.

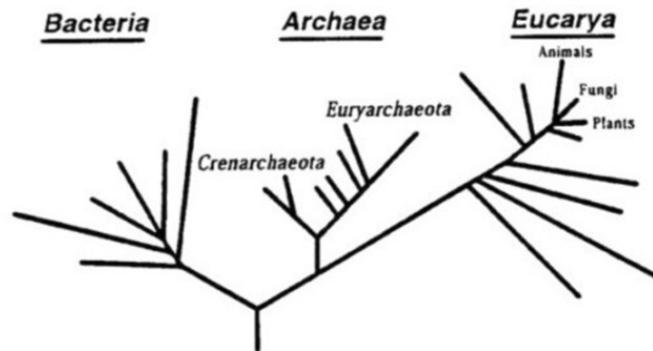


Figure 2. The basal universal phylogenetic tree inferred from comparative analyses of rRNA sequences (from C.E. Woese, 2000)

In parallel to the changing scientific methodology used to study the diversification of microbial life, a radical change in the mathematical methodology of systematics has also occurred: vague assessments of similarity and speculative “trees” were refined through the greater rigor of numerical or phenetic approaches and subsequently through cladistics. The latter has been especially significant in the interpretation of molecular data. As a consequence of these improvements, it has reaffirmed the view that microorganisms are polyphyletic and that other than similarities in scale this is an incredibly diverse, if artificial, group.

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Biographical Sketches

John David has worked in fungal systematics for fifteen years and is manager of CABI Bioscience's Microbial Identification Service and curator of its Biosystematic Reference Collection. He is also an editor of the Ninth Edition of Ainsworth & Bisby's *Dictionary of the Fungi* (2001), with a special interest in the megasystematics of fungi and related protists. His other interests in mycology include nomenclature and the systematics of conidial fungi and lichenized fungi. He obtained a BSc. from Bristol University, and an M.Sc. and Ph.D. from Reading University.

Gerry Saddler is the head of the Diagnostics and Molecular Biology Section of the Scottish Agricultural Science Agency. He has worked for 15 years in bacterial systematics and is a specialist in the taxonomy of actinomycetes and bacterial plant pathogens. He is the Executive Secretary of the International Committee on Systematics of Procaryotes (ICSP) of the International Union of Microbiological Societies (IUMS), formerly the International Committee on Systematic Bacteriology (ICSB), a member of the International Society for Plant Pathology's Committee on Taxonomy of Plant Pathogenic Bacteria, and the Convener of the Systematics and Evolution Group Committee of the Society for General Microbiology. His B.Sc. in Microbiology is from Edinburgh University, and his Ph.D. is from the University of Newcastle upon Tyne.