

## Tracking the record of early life.

### [À la recherche des premières traces de vie]

Emmanuelle J. JAVAUX<sup>1</sup>

Craig P. MARSHALL<sup>2</sup>

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### Introduction

Life may have been present on Earth from about 3.8 Ga or earlier. Based on a combination of geochemical, morphological and sedimentological evidence, the early biosphere included a wide diversity of prokaryotes exhibiting modern metabolisms that thrived in various marine and possibly terrestrial habitats. Among early microorganisms, cyanobacteria played a major role, inventing oxygenic photosynthesis that caused a most profound alteration in our planet. Part of our ongoing work aims to characterize the morphological and geochemical signatures of modern and fossil cyanobacteria and other prokaryotes. Multidisciplinary research, especially on the paleontology of early microorganisms on Earth and the microbiology of extremophiles in recent Earth environments, will allow us to determine the biosignatures needed for advances in paleobiology and astrobiology.

### The early record of life

Tracking the early record of life is not an easy task. Biologists easily differentiate members of the three domains of life: Archaea, Bacteria and Eucarya, using myriad features of molecular and cell biology, but these characters rarely survive fossilization and so are not generally available to the paleontologist. Moreover, geological processes like metamorphism (pressure and thermal alteration), diagenesis, and tectonic activity erase most of the early record of life. Nevertheless, in a few areas of the globe sedimentary rocks are better preserved and contain traces of past life. Microfossils, biomarkers, stromatolites, and isotopes of sedimentary carbon and sulfur all indicate that

microorganisms lived on Earth during the Archean, before 2.5 Ga, and had developed metabolisms similar to many living microbes (Fig. 1). Molecular phylogenies indicate an early branching of the three domains in a tree- or ring-shaped fashion. By calibrating the tree of life with fossils of known affinity, and evidence from biomarkers or isotopes, it appears that prokaryotes evolved before 3.5 to 3.8 Ga, and stem eukaryotes before 2.8 Ga.

Carbon isotopes from the oldest known sedimentary rocks (3.8-3.6 Ga) in the Isua Greenstone succession, Greenland, have values fitting the range of fractionation produced by autotrophic carbon fixation (ROSING, 1999). Fractionated sulfide isotopes in 3.47 Ga sulfate crystals from Northwestern Australia support the existence of mesophilic bacterial sulfate-reducers (SHEN & BUICK, 2004). These studies show that complex cellular biochemistry had already evolved 1 Ga after Earth's accretion. Sedimentary organic matter strongly depleted in the carbon isotope <sup>13</sup>C suggests the presence of archaeal methanogens and bacterial methanotrophs at 2.8 Ga (HAYES *et alii*, 1983). The presence of 2 $\alpha$ -methylhopanes, the biomarker specific to cyanobacteria, in the 2.7 Ga Fortescue shales confirms the antiquity of the domain Bacteria. Cyanobacteria were important primary producers during the Archean and invented oxygenic photosynthesis before 2.7 Ga ago. Several independent lines of geological evidence that suggest significant levels of oxygen accumulated in the atmosphere earlier: in the early Archean. A recent study of sulfur isotopes fractionation has dated this oxygenation event at 2.32 Ga (BEKKER *et alii*, 2004).

Additional records of early Archean life have been reported recently. Abundant and

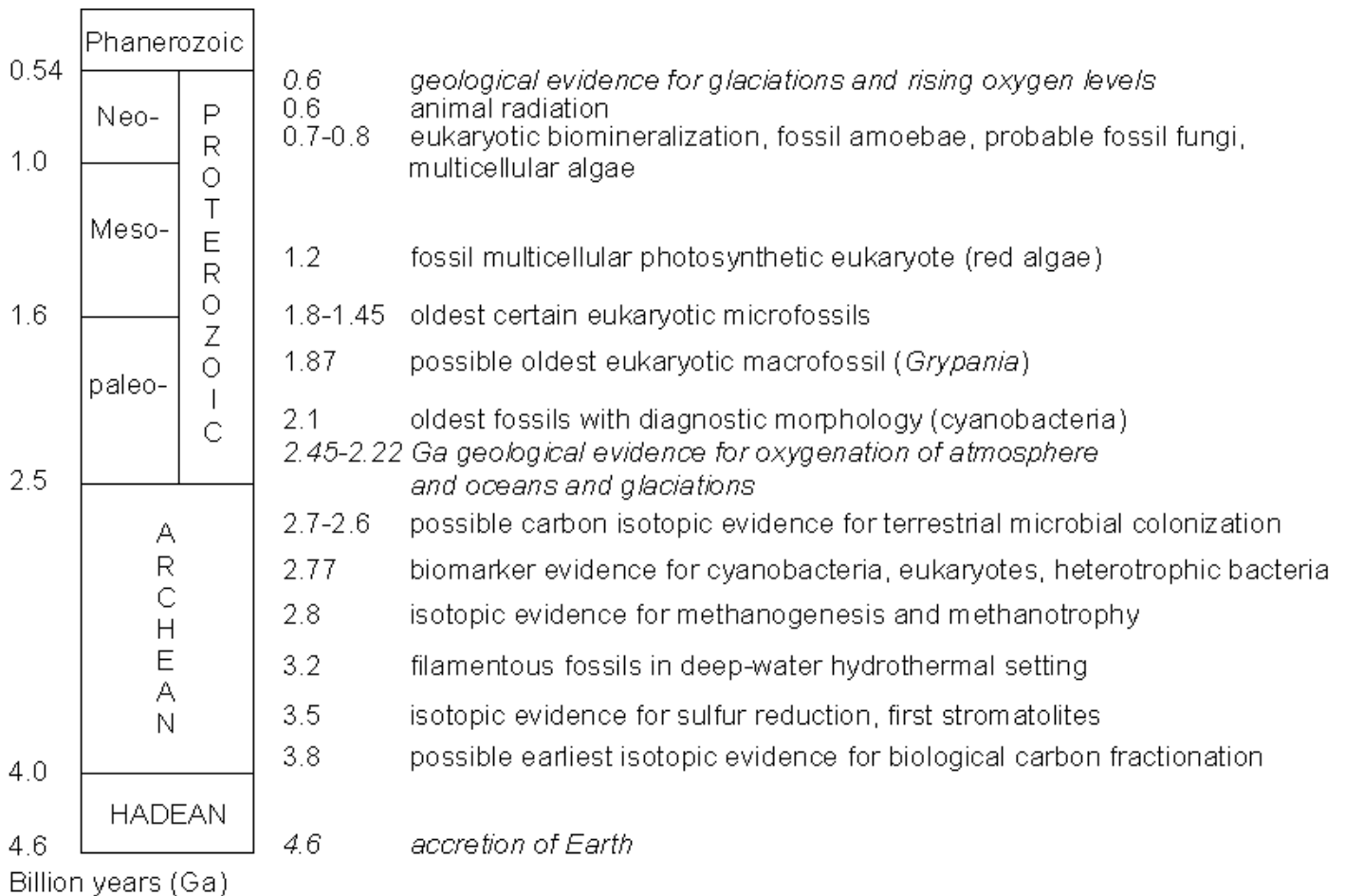
<sup>1</sup> Département de Géologie, Université de Liège, Allée du 6 août, B18, Sart-Tilman, 4000 Liège (Belgium)  
EJ.Javaux@ulg.ac.be

<sup>2</sup> Vibrational Spectroscopy Facility, School of Chemistry, The University of Sydney, NSW 2006 (Australia)  
c.marshall@chem.usyd.edu.au

convincing filamentous fossils have been described from a 3.2 Ga volcanogenic massive sulfide deposit in western Australia, interpreted as having formed like modern deep-water black smokers (RASMUSSEN, 2000). In pillow lavas of the Barberton Greenstone Belt dated 3.5 Ga, micrometer-scale mineralized tubes are suggested to result from microbial etching of basaltic glass along fractures, as seen in modern oceanic crust (FURNES *et alii*, 2004). In rocks dated 3.3-2.5 Ga from the Barberton Mountain Land in South Africa and the Pilbara in Western Australia, rod-shaped or coccoidal carbonaceous structures may be of microbial origin (see review in ALTERMANN & KAZMIERCZAK, 2003). However the biological nature of some of these structures is highly controversial. Abiotic self-assembled filaments of condensed abiotic organic matter can be synthesized and resemble the morphologies of some Archean microfossils (GARCIA-RUIZ *et alii*, 2003) thus suggesting that morphology alone is not sufficient to prove biogenicity. Procedures of sample preparation (such as acid maceration) can also produce structures that appear biological when observed only with the SEM - Scanning Electron Microscope (EDWARDS *et alii*,

2004). Contamination by younger microorganisms may also occur (WESTALL & FOLK, 2003).

In a geothermal setting physico-chemical processes can fractionate carbon isotopes without the intervention of biology. However, in well-dated rocks from well-determined depositional environments the biogenicity of microstructures can be established more decisively when morphology and organic chemistry are considered together with criteria involving degradation and biofabric such as distinctive patterns of cell division, the plasmolysis of cell content, pigmentation gradients in colonies, spatial distribution within a population (HOFMANN, 2004). Paleobiological interpretation is most reliable when careful observations on ancient populations are combined with actualistic studies of microbial populations in comparable modern environments (KNOLL & GOLUBIC, 1992). Development of reliable criteria for biogenicity and instrumentation are crucial for assessing claims of ancient life on earth, as well as when searching for evidence of extraterrestrial life.



**Figure 1:** Geological time-scale with important geological and biological events in the Precambrian (modified from JAVAUX, in press).

Well before the advent of animals at the end of the Proterozoic, cells synthesized eukaryotic

sterols (> 2.7 Ga) (BROCKS *et alii*, 2003), diversified moderately around 1.8-1.3 Ga

(JAVAUX *et alii*, 2001), and evolved multicellularity, sexual reproduction, and photosynthesis (through endosymbiosis with a cyanobacterial ancestor of the chloroplast) by at least as early as 1.2-1.0 Ga (BUTTERFIELD, 2000).

Stromatolites and microbial mats also preserve paleobiological interactions with sediments. In the Proterozoic, the morphological and geochemical fossil record of both prokaryotes and eukaryotes is much better preserved and documented.

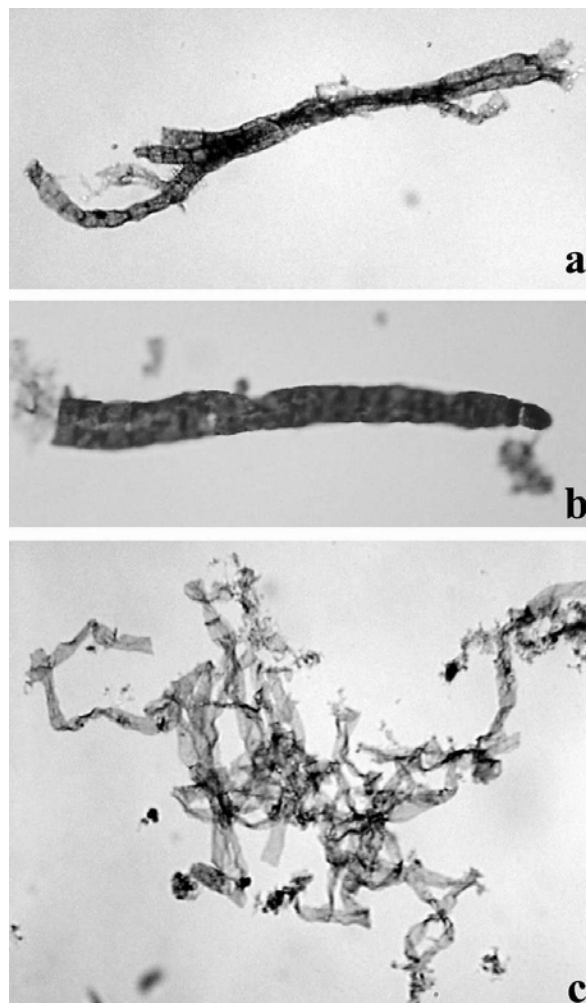
### Scope of research and methodology

The goal of our research on Archean and Proterozoic microfossils from fine-grained sediments is to understand the mechanisms and environmental context of biospheric evolution on the early Earth by identifying the fossils (morphological, ultrastructural or chemical) of early prokaryotes and eukaryotes, determining their biological affinities and/or cellular innovations, and examining their patterns of evolution throughout intervals of environmental change. This approach uses a combination of sedimentary geology, Energy Dispersive X-ray Spectroscopy (EDX) in the SEM, microscopy (transmitted light, scanning electron and transmission electron microscopy) and microchemistry (Micro-FTIR spectroscopy, Laser micro-Raman spectroscopy, laser micro-pyrolysis-GC/MS). Our collaborative research also includes the characterization of resistant biopolymers and cellular structures in a range of living prokaryotes, protists, and fungi by combining microscopy and microchemistry, in order to make comparisons with the same analyses of Proterozoic and Archean microfossils.

### Preservation of prokaryotes in the rock record

The biogenicity of well-preserved organic-walled microfossils (acritarchs) in shale is rarely questionable, but by definition their biological affinities are unknown although most of them are assumed to represent algal remains. However, they probably include many other eukaryotic clades, and also prokaryotic microbes. The potential for the preservation in shale of many protists (unicellular eukaryotes) and most prokaryotes with acid-resistant organic-walls is unknown (review in JAVAUX *et alii*, 2003). Preservation of bacteria other than cyanobacteria may require mineralization by silica (chert), calcium carbonate (calcite, aragonite), calcium phosphate (apatite), iron carbonate (siderite), iron oxide (hematite), and iron sulfide (pyrite) (WESTALL, 1999; BAZYLINSKI & FRANKEL, 2003). Consequently the earliest record of life is commonly preserved as a mineralized cast or mold thus requiring other features in addition to morphology to confirm

the biological nature of the remains. Nevertheless, organic-walled microfossils occur abundantly in shales. They include unicellular and multicellular eukaryotes, cyanobacterial sheaths and most probably other prokaryotic remains.



**Figure 2:** Fossils of probable cyanobacteria, 1.5 Ga Roper Group shales, Australia.

a - *Trachyhystrichoides ovalis* segmented filaments (10  $\mu$ m wide);  
b - filament of probable oscillatorioid cyanobacteria (30  $\mu$ m wide);  
c - filamentous sheaths (10  $\mu$ m wide).

Among early microorganisms, cyanobacteria played a major role, inventing oxygenic photosynthesis and causing a most profound alteration of our planet. Taphonomic experiments (BARTLEY, 1996) show that their sheaths are preserved in the fossil record, in preference to their peptidoglycan-rich cell walls. They are composed of complex polysaccharides and pigments like scytonemin (HOICZYK & HANSEL, 2000). These extracellular fibrillar carbohydrates provide a protective coat for the cells against UV radiation and desiccation as they maintain the cells in a highly hydrated gel-like matrix. This protective coating permits these organisms to adapt to extreme temperatures and desiccation, as in Antarctica

today, but it was probably effective in early Earth environments also. The oldest fossils preserved well enough to be recognized as a member of an extant clade are endolithic cyanobacteria *Eoentophysallis* from the 2.15 Ga Belcher Group, Canada (HOFMAN, 1976). These microfossils are very similar to the living *Entophysallis*, showing a similar packaging of cells in an envelope and a colony surface darkened by pigmentation (KNOLL & GOLUBIC, 1992).

The Roper Shales (~1.5 Ga) of Northern Australia include exquisitely preserved organic-walled microfossils distributed from peritidal to basinal facies (JAVAUX *et alii*, 2001). These fossil assemblages include a moderate diversity of ornamented acritarchs interpreted as the remains of early eukaryotes (JAVAUX *et alii*, 2003, 2004; MARSHALL *et alii*, 2005). Roper leiospheres also display eukaryotic characters (complex wall ultrastructure). Other spherical and filamentous microfossils include probable cyanobacteria (Fig. 2) and possibly other prokaryotes. A combined study of the microscopy and chemistry of these fossils and that of extant prokaryotes (including cyanobacteria from cultures and from extreme environments) is ongoing to determine their potentialities for preservation and the existence of possible biosignatures of prokaryotes in fine-grained sediments.

### Conclusions

Traditionally, paleontologists interested in early life focus their research on permineralized microfossils. However, siliciclastic rocks offer a complementary window on early life; they preserve the ultrastructure of the microfossil walls better over a wider range of facies. Tracking the evolution of cellular innovations and, when possible, determining the systematic relationships of prokaryotic and eukaryotic organisms preserved as fossils will permit the calibration of phylogenetic trees, thus leading to a better understanding of early biospheric evolution. Microbiological studies of extremophiles living in recent environments analogous to early Earth conditions or to past/present conditions elsewhere in the solar system will help to define the limits of life and its biosignatures. Studying the origin and evolution of early eukaryotes will shed light on the processes and conditions required for the development of biological complexity (as defined by eukaryotic cellular architectures) from simple microbial forms. Such data will facilitate the development of suites of biosignatures that may prove applicable in the search for past life on Earth and beyond. Microorganisms have been cycling carbon, sulfur and nitrogen on Earth since the early Archean, as they do today. The planet Mars had early conditions probably similar to early Earth's and thus could have possibly developed

an early biosphere. The signatures of a possible extinct Martian biota would be better preserved in the Martian rock record for it is unaltered by plate tectonics.

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