# Microfossils and Biomolecules in Carbonaceous Meteorites: Possibility of Life in Water-Bearing Asteroids and Comets

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## ABSTRACT

It is well established that carbonaceous meteorites contain water, carbon, biogenic elements and a host of organic chemicals and biomolecules. Several independent lines of evidence indicate that the parent bodies of the CI1 and CM2 carbonaceous meteorites are most probably the C-type asteroids or cometary nuclei. Several of the protein amino acids detected in the meteorites exhibit chirality and have an excess of the *L*-enantiomer -- such as in the amino acids present in the proteins of all known life forms on Earth. Isotopic studies have established that the amino acids and nucleobases in the CI1 and CM2 carbonaceous meteorites are both indigenous and extraterrestrial. Optical and Scanning Electron Microscopy studies carried out by researchers during the past half century have revealed the presence of complex biogenic microstructures embedded in the rock-matrix of many of carbonaceous meteorites similar to extinct life-forms known as acritarchs and hystrichospheres. Carbonaceous meteorites also contain a wide variety of large filaments that exhibit the complex morphologies and correct size ranges of known genera and species of photosynthetic microorganisms such as cyanobacteria and diatoms. However, EDAX investigations have shown that these carbon-rich filaments typically have nitrogen content below the level of detection (<0.5% atomic) of the instrument. EDAX studies of living and dead terrestrial biological materials have shown that nitrogen can be detected in ancient mummies and tissue, hair and teeth of Pleistocene Mammoths. Hence, the absence of detectable nitrogen in the filaments provides direct evidence that they do not represent recent biological contaminants that invaded these meteorite stones after they were observed to fall to Earth. The spectral and fluorescence properties of pigments found in several species of terrestrial cyanobacteria which are similar to some microfossils found in carbonaceous meteorites may provide valuable clues to help search for evidence for biomolecules and life on the icy moons of Jupiter and Saturn, asteroids and comets.

**Keywords:** Microfossils, Meteorites, Biomolecules, Comets, Asteroids, Cyanobacteria, Diatoms, Murchison, Orgueil

## 1. INTRODUCTION

It has been known for over two centuries that carbonaceous chondrites contain carbon and water. Recent isotopic analyses have shown that the carbon and water content of these stones is indigenous and extraterrestrial. Field Emission Scanning Electron Microscopy studies carried out independently in the United States, Russia, and the UK have resulted in the detection of well-preserved remains of recognizable microfossils of filamentous cyanobacteria, diatoms and other algae, acritarchs and hystrichospheres. Investigations at the NASA Marshall Space Flight Center have shown that the nitrogen content of these recognizable remains is almost always below the detection limit of the Energy Dispersive X-Ray Spectrometer instrument. This provides evidence that these remains are indigenous and ancient rather than modern terrestrial biological contaminants. This interpretation is supported by independent studies by meteoriticists in many countries. It is now well established that many carbonaceous meteorites contain extraterrestrial organic chemicals and complex life-critical biomolecules (e.g., chiral protein amino acids, and nucleobases) that are essential to all known forms of life. However, these studies have failed to detect a host of life-critical biomolecules in the carbonaceous meteorites These include 12 of the 20 protein amino acids, 2 of 5 nucleobases, ribose, de-oxyribose and other sugars, DNA, RNA, chlorophyll and other photosynthetic pigments. If the carbonaceous meteorites were contaminated by modern microbes after they landed on Earth, all of these life-critical biomolecules would certainly be present.

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Nanophotonics and Macrophotonics for Space Environments VIII, edited by Edward W. Taylor,
David A. Cardimona, Proc. of SPIE Vol. 9226, 922602 · © 2014 SPIE
CCC code: 0277-786X/14/$18 · doi: 10.1117/12.2065571

The absence of these life-critical biomolecules provides solid evidence that the recognizable microfossils found in freshly fractured interior surfaces of the stones are not modern biological contaminants. While DNA, RNA, ribose and other sugars and chlorophyll, phycobillins and other photosynthetic pigments are absent in the CI1 and CM2 carbonaceous meteorites; these meteorites do contain important fossil biomolecules such as pristane, phytane and porphyrins. These geochemical biomarkers are formed as diagenetic breakdown products of photosynthetic pigments. Porphyrins are tetrapyrroles are present in chlorophyll and the heme porphyrin is the pigment of red blood cells and a cofactor of the protein hemoglobin. Porphyrins are produced by complex enzymatic pathways in a variety of living organisms such as via the C5 (Beale) enzymatic pathway in archaea, algae, bacteria and plants. However chlorophyll and other photosynthetic pigments are notably absent in the meteorites. These biomolecules would certainly be present in the carbonaceous chondrites if they had been contaminated post-arrival by cyanobacteria and diatoms. The extraterrestrial biomolecules found in carbonaceous meteorites provide evidence (but not proof) for the existence of extraterrestrial life. *However, the absence of life-critical protein amino acids, nucleobases and proteins provides proof that these carbonaceous meteorites are not contaminated by modern terrestrial microorganisms.*

The biomolecules and microfossils that are present in cabonaceous meteorites have implications to the possibility that extant or extinct life forms may be found on mars, icy moons, water-bearing asteroids and comets. They may also help guide the design of new space instruments and missions that may be used to remotely search for evidence of life on other bodies of the Solar System.

## 2. REQUIREMENTS FOR LIFE

One of the most interesting discoveries concerning life is the fact that despite the great diversity of living organisms on Earth, they are all very similar at the most fundamental level. All known living organisms on our planet require water, a source of energy and a suite of "biogenic elements" in order to carry out metabolism and form the necessary life-critical biomolecules needed to live, produce life-critical biomolecules, exhibit motility, grow and replicate new cells.

### 2.1 Water

Water is second most abundant molecule (only $H_2$ molecules are more abundant) in the Universe.. Water is also an absolute requirement for all known forms of life. This is largely because the $H_2O$ molecule possesses a unique suite of physical and chemical properties. The water molecule is polar and it has an angular shape due to the paired bonding of the electrons shared between the oxygen and hydrogen atoms. Because of the charge separation and the shape of the dipole water molecule, the oxygen atom has a partial negative charge and the hydrogen atoms a partial positive charge. This makes water molecules behave like tiny magnets. Without this property, water would be a gas at room temperature. This also makes water a good solvent ideally suited for supporting organic chemistry and capable of dissolving most biological molecules. Water has a high dielectric constant ensuring solubility of polar species and flexibility for biological structures stabilized by electrostatic interactions. It also has a high heat capacity and an astonishingly rich phase diagram with fifteen crystalline phases observed in laboratory experiments and eight others predicted theoretically. Water remains in liquid state over a wide range of temperatures. It is now well known that there exists a vast diversity of living organisms that inhabit deep-sea hydrothermal vents at temperatures well above 100 C.

It is also known that carbonaceous meteorites contain ~3%-20% indigenous water, extraterrestrial amino acids, nucleobases and other life-critical biomolecules and it is generally accepted that the parent bodies of the CI1 and CM2 carbonaceous meteorites are water-bearing asteroids or comets.[1,2] Water is the dominant volatile on comets and the icy moons of Jupiter and Saturn. To the great astonishment of many scientists, recent observations by the Spitzer Telescope and the Dawn Mission provided evidence that water ice and organic molecules also exist on the asteroids 24 Themis and 65 Cybele at the outer edge of the asteroid belt **(Fig. 1.a)**.[3,4] Data from the ESA Herschel Space Observatory indicates that water vapor, possibly from ice volcanism, is being spewed into space from Ceres, the largest asteroid (also considered a dwarf planet) of the solar system **(Fig. 1.b)**.[5,6.] The ESA/Rosetta/NavCam image **(Fig. 1.c)** shows water jets being spewed from the comet 67P/Cheryumov-Gerasimenko. It has been shown that the surfaces of asteroids and the nuclei of comets are jet-black (albedo ~ 0.04) and these dark bodies can be heated by absorbed sunlight to temperatures well above the melting point of pure water ice (273 K) even at distances beyond the orbit of the planet Mars. Cracks within the surface would allow the water vapor to rapidly vaporize and vent directly into space. However, if these avenues of escape are impeded as the water re-freezes sealing the surface cracks, pressures within cavities could become high enough to allow liquid water pools and films to exist for periods of time beneath the surface of the asteroid or the comet's crust. During such periods, cyanobacteria, sulfur bacteria, archaea, diatoms, algae or other microorganisms might find conditions suitable for growth. Some of these microorganisms have doubling times of only a few hours even at low temperatures, which would permit sizeable populations to develop in water-bearing asteroids or cometary nuclei in only a matter of days or weeks.

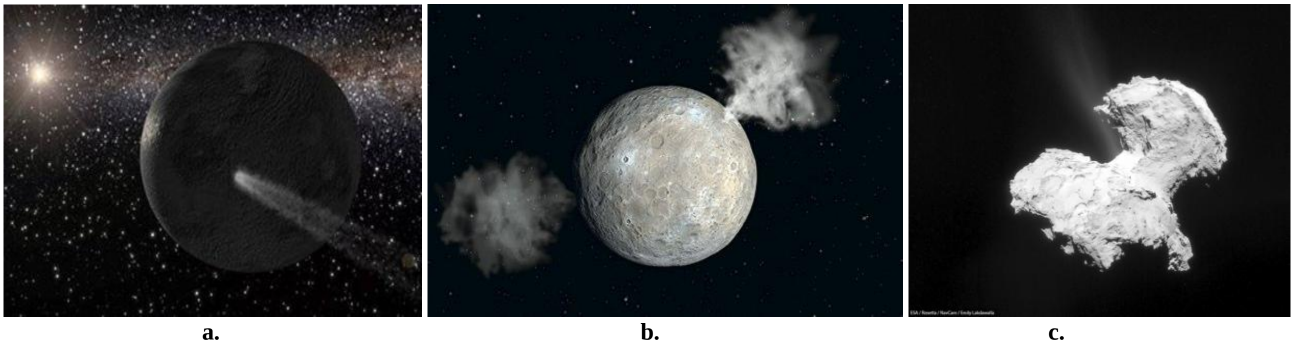


**Fig. 1.** Illustration of water ice and water vapor being spewed **a.** from the asteroid 65 Cybele and **b.** the asteroid or dwarf planet Ceres and **c.** Rosetta/NavCam image showing water jets from the comet 67/P Cheryumov-Gerasimenko. Image Credits: a. *Gabriel Pérez, Instituto de Astrofisica de Canarias, Spain;* **b.** *Pennington Planetarium;* **c.** *ESA/Rosetta/NavCam/Emily Lackawalla*

When salts are present, water remains unfrozen at temperatures far below zero. Microorganisms thrive in the frigid hypersaline Don Juan Pond of Antarctica, cryopegs deep beneath the Siberian permafrost and tidal pools of Southern Patagonia.[7] Water molecules can form a three-dimensional hydrogen bonded network which facilitates its participation in a wide variety of life-critical biochemical processes. When water freezes a slight expansion of hydrogen bond angles results in the solid phase having lower density than the liquid phase and consequently ice floats. This is important to life, because lakes, rivers and oceans freeze from the surface down rather than the bottom up and the icy surface layer provides insulation from sub-zero atmospheric temperatures. This allows large bodies of liquid water to exist deep beneath the surface of frozen lakes, seas or the polar ice caps of Earth. For this reason, liquid water oceans or pools may be present beneath the frozen crusts of the icy moons of Jupiter and Saturn or the frozen surfaces of comets and asteroids. Water is so essential to life that *"Follow the Water"* was adopted as the central strategy of the NASA Astrobiology program searching for evidence for life elsewhere in the Cosmos.

While it is a widely held belief that large bodies of liquid water are necessary for life, this assumption is incorrect. It has long been known that cryptoendolithic lichens and purplish red cyanobacteria (the Red *Gloecapsa* Community) colonize very thin films of liquid water within the photosynthetic regimes (upper layers) of sandstones in the polar regions of Earth.[8] Virtually all cells of prokaryotic and eukaryotic organisms have the ability to be freeze-dried and stored for long periods of time at liquid nitrogen temperatures (lyophilization). Abyzov *et al.* [9,10] established that microorganisms remain alive in a super-long state of anabiosis within ancient layers of the central Antarctic ice sheet above Lake Vostok. Many bacteria and diatoms use special enzymes (ice active substances) to locally melt ice and remain alive in the solid ice of glaciers, polar ice sheets, cryopegs at temperatures below -10 C.[11-13] The novel lactic acid bacterium, *Carnobacterium pleistocenium* isolated from a frozen thermokarst pond in Fox Tunnel. Alaska can grow at -5 C.[15,16]

## 2.1 Energy Sources

Living organisms employ a variety of mechanisms to obtain the energy necessary to carry out metabolism, growth, replication and for the myriad of other vital processes associated with the biochemical machinery of life. The different mechanisms used to harvest energy make it possible to divide organisms into broad groups:

**Autotrophs** grow by carbon fixation - the process by which living organisms convert inorganic carbon from carbon dioxide into the array of organic compounds and biomolecules needed for life. Carbon and Nitrogen are unique in that they cannot be used by living organism until after they have been fixed by other life forms. Inorganic atmospheric $CO_2$

is converted into organic carbon via complex metabolic pathways found only in living organisms. There are presently six known autotrophic carbon fixation pathways.

**Photoautotrophs** perform carbon fixation via the process of photosynthesis with photons as the energy source. By far the most important autotrophic carbon fixation process is oxygenic photosynthesis. In the late 1940's, Melvin Calvin and his team from the Radiation Laboratory of the University of California, Berkeley first described the photosynthetic process. They used radioactive Carbon-14 tags and green algae to decipher the complex metabolic process in which enzymes, proteins and chlorophyll pigments contained in the stroma of chloroplasts use the energy of sunlight to produce sugars and other carbohydrates and release oxygen from water and $CO_2$. Calvin received the Chemistry Nobel Prize in 1961 for the discovery of this fundamental process of life which is now known as the Calvin Cycle.[8] During the first stage of the carbon fixation process the Ribulose-1,5-Biphosphate Carboxylase/Oxygenase enzyme (RuBisCO) incorporates carbon dioxide into the five-carbon sugar ribulose biphosphate (RuBP). The life-critical biomolecule RuBisCO is found in the chloroplasts of cyanobacteria, algae, diatoms and other plants and may well be the most abundant protein in Earth. The coenzyme Adenosone Triphosphate (ATP), the energy carrier in all living cells, is spent as the energy source and NADPH is consumed as the reducing power to add high energy electrons in the last step of the electron chain of light reactions of the complex photosynthetic process in which sugars are produced from $CO_2$.

Five other autotrophic carbon fixation cycles are presently known in living organisms. These include reductive citric acid cycle (Arnon-Buchanan Cycle) which occurs within anoxygenic photosynthetic bacteria (e.g;, the green sulfur bacterium *Chlorobium thiosulfatophilum*); the reductive acetyl Co-A pathway (Wood-Ljungdahl pathway); and the pathways used by the anaerobic and microaerobic Archaea and Crenarchaeota.[19]

Carbon fixation can also occur by chemosynthesis in the absence of light. **Chemotrophs** are organisms that acquire energy by the oxidation of electron donors in their environment. Chemoorganotrophs obtain carbon and energy by consuming organic molecules as food. Chemolithotrophs extract carbon and energy from inorganic molecules. Chemotrophs can be either autotrophic or heterotrophic. Chemoautotrophs can perform carbon fixation and obtain their energy from chemical reactions using molecular hydrogen, methane, hydrogen sulfide elemental sulfur, etc. Oxygen is released as a by-product as the organisms break up the carbon dioxide molecules to synthesize all necessary organic compounds. Most chemoautotrophic life forms such as thermoacidophiles, halophiles, methanogens, sulfur oxidizers, sulfur reducers and archaea thrive in extreme environments like deep crustal rocks and deep-sea hydrothermal vents. Chemoheterotrophs are unable to fix their own carbon and can use sulfur or other inorganic energy sources or organic sources-lipids, proteins or carbohydrates. Many organisms are **Organotrophs**, and obtain their energy and biomolecules by consuming organic matter manufactured by the metabolic process within the cells of other living organisms.

## 2.2 Biogenic Elements

The diversity and complexity of life on Earth is so great and their range of habitats so broad that almost all of the 92 naturally occurring chemical elements on Earth may play a role in some of the different organisms from time to time. But there is a subset of approximately 20 of these elements that are absolutely essential for all known life forms on Earth and therefore have been called "biogenic elements". Water and all of the life-critical biogenic elements are found in carbonaceous meteorites. The ratios of biogenic elements in biological remains change through diagenetic process over geological time periods. Ratios of the biogenic elements carbon, oxygen and nitrogen provide a powerful tool for distinguishing between modern biological contaminants and indigenous microfossils in terrestrial rocks and meteorites.[20]

There are six **Major Biogenic Elements (C, H, O, N, P, S)** that are absolutely essential for all living organisms and abundant in all cells. There are five **Minor Biogenic Elements (Ca, Cl, Mg, K, Na**) that are also life-critical but typically present in cells in relatively small quantities and several **Trace Biogenic Elements (Si, Mn, Fe, Cu, Zn, As, Ni, I, V)** which are present in very small quantities but essential for the chemical reactions and metabolic pathways upon which life depends. These six major biogenic elements make up more than 97% of the matter of all living cells. The first four are by far the most abundant as they are present in the water, and all of the carbohydrates, amino acids and proteins found in every living cell. Phosphorus and sulfur are present in much smaller quantities in living organisms. Phosphorus is essential for life-critical biomolecules such as DNA, RNA, ATP (adenosine triphosphate), NADP (nicotinamide adenine dinucleotide phosphate) and many life-critical proteins and enzymes. Sulfur is essential for the amino acids methionine and cysteine and therefore a key component of most proteins. Nitrogen comprises almost 78% of our atmosphere in the form of diatomic nitrogen ($N_2$) molecules held together by a strong triple bond. Atmospheric nitrogen is a relatively inert molecule that is completely useless to life until it has been converted by the process of nitrogen fixation.[21] Abiotic nitrogen fixation can occur in nature when the enormous energy of lightning strikes breads the triple bond and forms nitrates. Nitrogen fixation can also be achieved by synthetic mechnisms c(e.g., Haber-Bosch, cyanimide or electric arc processes) which usually require very high temperatures. Biological Nitrogen Fixation is by far the dominant mechanism for nitrogen fixation on Earth. Biological Nitrogen Fixation occurs when cyanobacteria, bacteria or archaea accomplish the reduction of the $N_2$ molecule to ammonia, nitrates, or nitrogen dioxide. Cyanobacteria play the dominant role in nitrogen fixation on Earth. This is typically achieved via the nitrogenase enzyme that is found in several genera of heterocystous cyanobacteria (e.g. *Nostoc, Calothrix,* etc.). However, biological nitrogen fixation can also be performed by several species of non-heterocystous cyanobacteria and other diazotrophic microorganisms of the families *Azotobacteracea*, *Rhizobiaceae*, and *Actinomycetaceae*. Biological nitrogen fixation occurs in the heterocystous cyanobacteria, via the nitrogenase enzyme contained within the thick-walled specialized nitrogen fixing cells known as heterocysts. The heterocysts have hyaline, refractive walls that provide well-protected centers in which the nitrogenase enzyme can function. The heterocysts produce three additional cell walls, including one with glycolipid that forms a hydrophobic barrier to oxygen to protect the nitrogenase enzyme, which oxygen inactivates. To provide further protection, the heterocysts themselves lack photosystem II (therefore producing no oxygen) and any of these organisms also up-regulate glycolytic enzymes and produce proteins that scavenge for any remaining oxygen.[22]

### 2.3 Biomolecules

Biomolecules are typically considered to be organic molecules that are produced by living organisms. They include relatively small organic molecules and large biomolecules like RNA, DNA and proteins. Many small biomolecules like amino acids, can be produced by abiotic processes such as Strecker or Miller-Urey synthesis.[23] However, when these small molecules are found on Earth, they apparently resulted result from biology as they exhibit properties (e.g., homochirality and biological fractionation of stable isotopes) that make distinguishable from abiotic molecules. Small biomolecules are often produced by metabolic pathways or enzymatic activity as primary or secondary metabolites. These include proteinogenic amino acids, sugars such as ribose ($C_5H_{10}O_5$), deoxyribose ($C_5H_{10}O_4$) and nitrogenous nucleobases guanine ($C_5H_5N_5O$), cytosine ($C_4H_5N_3O$), adenine ($C_5H_5N_5$), thymine ($C_5H_6N_2O_2$), and uracil ($C_4H_4N_2O_2$). In the canonical Watson-Crick base pairing of DNA, Guanine always pairs with Cytosine and Adenine pairs with thymine (replaced by uracil in RNA). These small biomolecules are "life-critical" as they the fundamental building blocks for the formation the large biomolecules that are essential to all known living organisms.

#### 2.3.1 Chiral Amino Acids and Sugars

Amino acids comprise the basic building blocks of all proteins and enzymes. Several hundred amino acids are known, but only small subset of some twenty or so "proteinogenic" amino acids are the monomers for a host of complex protein polypeptide chains that are essential to life for every organism on Earth. Each of the amino acids consist of four clusters of atoms branching out in three-dimensional space. Amino acids are are composed of an amine ($-NH_2$) functional group linked to a core central $\alpha$-carbon atom bonded to a hydrogen atom, a carboxylic acid (-COOH) group, and a variable -R side chain. The chemical composition of the variable side chain (-R) uniquely defines each distinct amino acid. The location in three-dimensional space of the -R side chain determines the chirality. The location in three-dimensional space of the -R side chain determines the chirality. Chiral molecules are asymmetric and occur in non-superposable mirror images. The term "enantiomers" refers to the two stereoisomers that are related to each other by reflection.

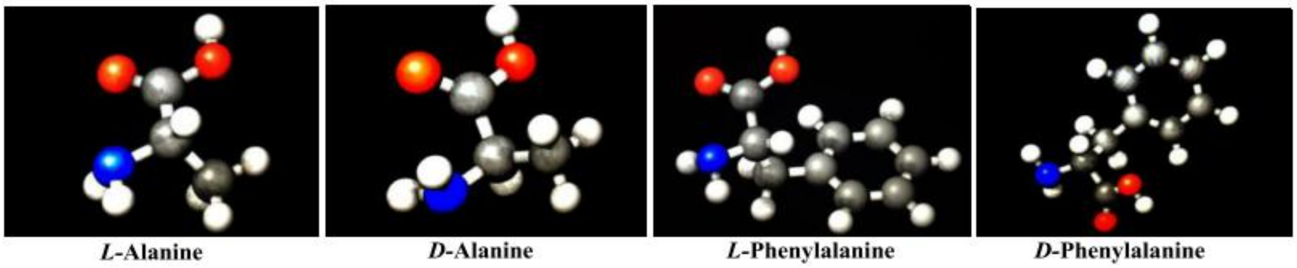

**L**-**Alanine** **D**-**Alanine** **L**-**Phenylalanine** **D**-**Phenylalanine**

**Fig. 2.** Spatial configuration of the *L*- and *D*- enantiomers of the amino acids Alanine ($CH_3NH_2COOH$) and Phenalyalanine $C_6H_5CH_2CH(NH_2)COOH$. (**C**-Grey; **H**-White; **O**-Red; **N**-Blue).

In 1815 the French chemist Jean-Baptiste Biot demonstrated that some organic molecules rotated the plane of polarization of polarized light.[23] Chiral molecules have optical activity are termed "dextrorotary" *D*- or "laevorotary" *L*-based on whether they rotate the plane of a beam of polarized beam in a "right handed" or clockwise (*D*-) manner or in a "left handed" or counterclockwise (*L*-) direction. Since the *L*- and *D*- enantiomers are mirror images of one another they are not superimposable in space in a manner analogous to your right and left hands. (The term "chiral" is from the greek word for "hand".) This phenomenon of chirality was first explained on a molecular basis by Louis Pasteur.[24,25] In 1848 Pasteur suggested that the origin of chiral biomolecules might hold the key to the origin and nature of life. All amino acids found in proteins have the same absolute steric configuration as *L*-glyceraldehyde, with the sole exception of the Glycine. Glycine, the smallest amino acid, has a single hydrogen atom for the -R side chain. It is symmetric and therefore is achiral. In addition to amino acids, sugars and many other biomolecules are asymmetric and exhibit chirality.

There are many different hypotheses for the pre-biotic formation of chiral molecules.[26] These include the action of: A. Linearly polarized light from neutron stars; B. Circularly polarized light from faint stars or planet suaces; C. Inclusion polymerization in chiral clays; D. Parity violation in weak interactions (Vester-Ulbricht hypothesis); E. High pressure acting on racemic mixtures; F. Asymmetric crystals or inorganic surfaces acting as catalysts; G. Soia asymmetric autocatalysis reaction.[27-32] Experiments have shown that most these are amplification processes produce very low yields of *L*-amino acids or *D*-sugars and none have found shown to produce enantiopure or homochiral assemblages as are found in biology.

### 2.3.2 Homochirality and Life

Homochirality of asymmetric biomolecules plays a crucial role in life and it is one of the most distinctive biochemical signatures of Life. It is also one of the most mysterious, since the manner of the origin of homochirality is not at all understood. In every living organism, essentially all amino acids in proteins are the *L*- enantiomer and all of the RNA and DNA molecules have exclusively right-handed pentose sugars (*D*-ribose and *D*-deoxyribose). After the organism dies, the homochirality of amino acids and sugars (which represents a strong signature of life) begins a slow diagenetic process toward racemization. In a racemic mixture the two enantiomers are present in equal numbers (D/L=1.0). The phenomenon of homochirality is crucial for proper folding of the enormously complex life-critical macromolecular biopolymers, such as the right-handed helical RNA and DNA biomolecules that encode the genetic instructions; pigments that harvest and store energy; and proteins and enzymes that catalyze complex metabolic reactions which produce the multitude of biomolecules essential for living organisms.

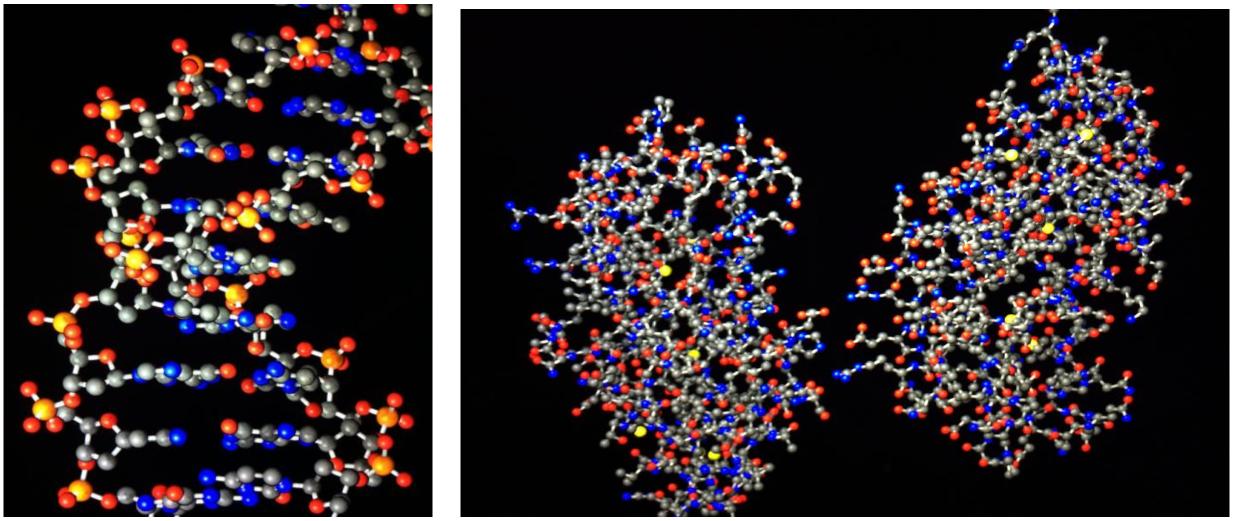
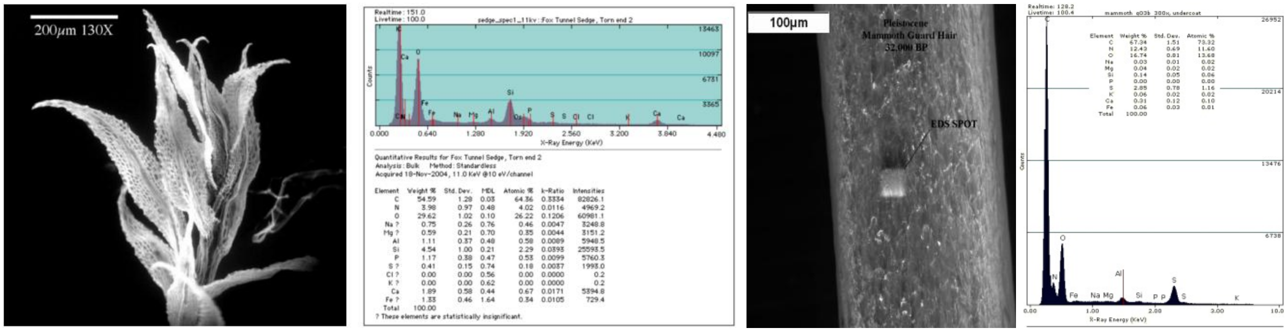


**Fig. 3.** Ball and Stick model of **a.** portion of 5' subunit of DNA molecule and **b.** crystal structure of fragment of of Phycoerythrin pigment from Cyanobacterium *Phormidium tenue.* (**C**-Grey; **H**-White; **O**-Red; **N**-Blue; **P**-Orange; **S**-Yellow).

Proteins are only synthesized by ribosomes within living cells by a strictly regulated and error-checked multi-step gene expression process known as translation. During translation, ribosome complexes within the cytoplasm bind to the messenger RNA (mRNA) and produce a specific amino acid polypeptide chains that subsequently folds into an active protein.[33] Protein molecules can be astonishingly large. Titin, the largest known protein, has a mass of ~3 megadaltons and it is composed of over half a million atoms ($C_{169723}H_{270464}N_{45688}O_{52243}S_{9120}$) folded in an exquisite package that incorporates some 34,350 amino acids.[34] (By definition a Carbon 12 atom has a mass of 12 Daltons.) Proteins are polypeptides that are composed of chains of amino acids, and therefore they contain large amounts of nitrogen. Nitrogen is also present in the nucleobases of the DNA and RNA molecules. Nitrogen atoms are shown as blue spheres in the ball and stick models of the B-DNA dodecamer of 5' subunit of the DNA molecule **(Fig. 3.a.)** and the crystal structure **(Fig. 3.b.)** of 14 kilodalton fragment of the alpha-subunit of phycoerythrin pigment of the Cyanobacterium *Phormidium tenue.*

After death of the organism, the amino acids in proteins begin a long slow process of being destroyed by a variety of biological and biogeochemical processes. Nitrogen is initially removed from biological materials by the process of deamination in which the amine group is removed from amino acids and converted into ammonia. In living organisms, deaminase enzymes usually catalyze the deamination so the components of unneeded amino acids can be recycled or oxidized for energy or incorporation into other biomolecules. Amino acids and proteins within cells are often re-used after consumption by organotrophs. After burial, these biomolecules are usually broken down and the organic nitrogen molecules re-converted back into $N_2$ molecules and returned to the atmosphere. This is the crucial component of the Nitrogen cycle, by which the nitrogen atoms sequestered in amino acids, proteins and other organic nitrogen molecules in living organisms are slowly converted (by bacterial denitrification; anaerobic ammonium oxidation or by the breakdown of nitrogen bearing rocks) back into atmospheric dinitrogen ($N_2$) molecules. This process of the breakdown of amino acids and the removal of organic nitrogen from living cells can be very slow, especially at low temperatures. The U. S. Army Cold Region Research and Engineering Laboratory (CRREL) Permafrost Tunnel near Fox, Alaska is a direct window into the Pleistocene. The CRREL Fox Tunnel contains a frozen record of Pleistocene life. Some of the metazoans (such as tardigrades and moss) are still viable after having been frozen in the permafrost for many millenia. Field Emission Scanning Electron Microscope (FESEM) images are shown for: **Fig. 4.a** living Pleistocene moss[17] from the Fox Tunnel (grown in NASA/NSSTC Astrobiology Laboratory after being frozen for 32,000 years) and **Fig. 4.c.** the guard hair of a Woolly Mammoth. Strong nitrogen peaks are shown in EDAX spectra: **Fig. 4.b.** for edge of leaf (~4% atomic) of Pleistocene sedge (*Carex* sp.) and guard hair of Woolly Mammoth (11.6% atomic). The square spot on the image is beam damage to the mammoth guard hair by the 11 keV electron beam from the EDAX spot data analysis. (This type of beam damage was commonly encountered during studies of living or even long dead cyanobacteria or other biological materials but was almost never seen during EDAX analyses (even at 30 keV) of filamentous microfossils in the carbonaceous meteorites also indicates they are not modern bio-contaminants.



Quantitative Results for Fox Tunnel Sedge, Torn end 2
Analysis: Bulk Method: Standardless
Acquired 18-Nov-2004, 11.0 KeV @10 eV/channel

| Element | Weight % | Std. Dev. | MDL | Atomic % | k-Ratio | Intensities |
|---|---|---|---|---|---|---|
| C | 54.59 | 1.28 | 0.03 | 64.36 | 0.3334 | 82826.1 |
| N | 3.98 | 0.97 | 0.48 | 4.02 | 0.0116 | 4969.2 |
| O | 29.62 | 1.02 | 0.10 | 26.22 | 0.1206 | 60981.1 |
| Na ? | 0.75 | 0.26 | 0.76 | 0.46 | 0.0047 | 3248.8 |
| Mg ? | 0.59 | 0.21 | 0.70 | 0.35 | 0.0044 | 3151.2 |
| Al | 1.11 | 0.37 | 0.48 | 0.58 | 0.0089 | 5948.5 |
| Si | 4.54 | 1.00 | 0.21 | 2.29 | 0.0393 | 25593.5 |
| P | 1.17 | 0.38 | 0.47 | 0.53 | 0.0099 | 5760.3 |
| S ? | 0.41 | 0.15 | 0.74 | 0.18 | 0.0037 | 1993.0 |
| Cl ? | 0.00 | 0.00 | 0.56 | 0.00 | 0.0000 | 0.2 |
| K ? | 0.00 | 0.00 | 0.62 | 0.00 | 0.0000 | 0.2 |
| Ca | 1.89 | 0.58 | 0.44 | 0.67 | 0.0171 | 5394.8 |
| Fe ? | 1.33 | 0.46 | 1.64 | 0.34 | 0.0105 | 729.4 |
| Total | 100.00 | | | | | |

? These elements are statistically insignificant.

| Element | Weight % | Std. Dev. | Atomic % |
|---|---|---|---|
| C | 67.34 | 1.51 | 73.32 |
| N | 12.43 | 0.69 | 11.60 |
| O | 16.74 | 0.81 | 13.68 |
| Na | 0.03 | 0.01 | 0.02 |
| Mg | 0.04 | 0.02 | 0.02 |
| Si | 0.14 | 0.05 | 0.06 |
| P | 0.00 | 0.00 | 0.00 |
| S | 2.85 | 0.78 | 1.16 |
| K | 0.06 | 0.02 | 0.02 |
| Ca | 0.31 | 0.12 | 0.10 |
| Fe | 0.06 | 0.03 | 0.01 |
| Total | 100.00 | | |

**Fig. 4.** Image of **a.** living Pleistocene moss that was grown in the NSSTC Astrobiology Laboratory after being frozen in the permafrost of the Fox Tunnel and **b.** EDAX spectrum of torn edge of Pleistocene sedge shows 4% (atomic) nitrogen. **c.** FESEM image of mammoth guard hair showing beam damage spot and **d.** EDAX data with 11.6% (atomic) nitrogen peak.

**Table I** provides a compilation of the relative abundance of amino acids in terrestrial cyanobacteria and bacteria, a Miocene fly entombed in amber and a Cretaceous Hadrosaur for comparison with amino acids detected in CM2 (Murchison and Murray) and CI1 (Orgueil and Ivuna) carbonaceous meteorites. (Amino acids marked "–" were not detected in these terrestrial fossils or carbonaceous meteorites.) The most abundant amino acids (weight%) in the cyanobacterium *Microcystis* sp. and bacteria (*E. coli* and *Salmonella*) were GLU, ASP, ALA, GLY and LEU, all of which are present at levels above 8%. The next most abundant amino acids in the living cyanobacteria are THR, SER, VAL, ILEU and PRO, which are all at levels above ~5%. Glycine (GLY) is by far the most abundant protein amino acid in the Murchison and Murray (CM2), Orgueil and Ivuna (CI1) carbonaceous meteorites and the next most abundant are

ALA, GLU and ASP. However, the protein amino acids LEU, THR, SER, VAL, ILEU and PRO, which are abundant in all life on Earth, are detected only at trace levels or are totally absent in the CI1 and CM2 carbonaceous meteorites.

**TABLE I**

**Amino Acids in Living Bacteria, Terrestrial Fossils and Carbonaceous Meteorites**

| **Protein Amino Acids** | **Living Bacteria** | | | | **Fossils** | | **Carbonaceous Meteorites** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***Microcystis*** | ***E. coli*** | ***Salm. pull*** | ***Salm. senf*** | ***Fly/ Amber*** | ***Hadrosaur*** | **Murchison CM2** | | Murray CM2 | Orgueil CI1 | Ivuna CI1 |
| | **Wt %** | **Mol/ ALA** | **Mol/ ALA** | **Mol/ ALA** | **Mol/ GLY** | **Mol/ GLY** | **Nmol /g** | **ppb** | **ppb** | **ppb** | **Ppb** |
| *L*-Alanine **ALA** | 10.3 | 1.00 | 1.00 | 1.00 | 0.37 | 0.53 | 15.3 | 956 | 647 | 69 | 157 |
| *D*-Alanine **ALA** | - | - | - | - | | | - | 720 | 617 | 69 | 82 |
| Arginine **ARG** | 4.4 | 0.51 | 0.48 | 0.52 | - | - | - | - | - | - | - |
| L-Aspartic Acid **ASP** | 12.0 | 1.01 | 1.00 | 1.00 | 0.23 | 0.77 | 8.5 | 342 | 65 | 54 | 146 |
| D-Aspartic Acid **ASP** | - | - | - | - | | | - | 100 | 51 | 28 | 30 |
| *L*-Glutamic acid **GLU** | 12.3 | 1.14 | 1.11 | 1.14 | 0.57 | 0.67 | 18.2 | 801 | 261 | 61 | 372 |
| *D*-Glutamic Acid **GLU** | - | - | - | - | | | - | 537 | 135 | 15 | 8 |
| Glycine **GLY** | 8.7 | 0.93 | 1.02 | 0.96 | 1.00 | 1.00 | 45.8 | 2919 | 2110 | 707 | 617 |
| Histidine **HIS** | 1.0 | 0.18 | 0.21 | 0.19 | - | - | - | - | - | - | - |
| Isoleucine **ILEU** | 5.0 | 0.55 | 0.51 | 0.55 | - | - | - | - | - | - | - |
| Leucine **LEU** | 8.2 | 0.83 | 0.78 | 0.78 | - | - | 1.9 | - | - | - | - |
| Lysine **LYS** | 4.4 | 0.56 | 0.59 | 0.56 | - | - | - | - | - | - | - |
| Methionine **MET** | 1.9 | 0.31 | 0.37 | 0.23 | - | - | - | - | - | - | - |
| Phenalyalanine **PHE** | 3.8 | 0.34 | 0.33 | 0.33 | - | - | - | - | - | - | - |
| Proline **PRO** | 4.9 | 0.25 | 0.26 | 0.28 | - | - | 13.5 | - | - | - | - |
| Serine **SER** | 6.6 | 0.41 | 0.48 | 0.43 | 0.56 | 0.91 | 4.7 | - | - | - | - |
| Threonine **THR** | 6.6 | 0.48 | 0.50 | 0.48 | - | 0.41 | - | - | - | - | - |
| Tryptophan **TRY** | - | 0.05 | 0.05 | 0.04 | - | - | - | - | - | - | - |
| Tyrosine **TYR** | 3.4 | 0.12 | 0.15 | 0.08 | - | - | - | - | - | - | - |
| Valine **VAL** | 6.5 | 0.73 | 0.66 | 0.75 | - | 0.24 | 8.6 | - | - | - | - |
| **Non-Protein Amino Acids** | | | | | | | | | | | |
| α-Aminoisobutyric**AIB** | - | - | - | - | - | - | - | 2,901 | 1,968 | 39 | 46 |

The most abundant non-protein amino acids reported in several studies of carbonaceous meteorites are α-aminoisobutyric acid (AIB), Isovaline (IVA) and γ -Aminobutyric Acid (GABA). It has been suggested that the presence of non-protein amino acids in the meteorites proves that the meteorite amino acids were formed by abiotic processes. This hypothesis is not sustainable. Even though these amino acids are not used in proteins, it is an error to conclude that this means they must be abiotic. The amino acids IVA and AIB can be formed on Earth by the diagenetic alteration of ancient biological materials and γ-Aminobutyric Acid is synthesized by lactic acid bacteria and other microorganisms.[35]

As Engel *et al.*[36] have pointed out, only 12 of the 20 life-critical protein amino acids are not found in the carbonaceous meteorites providing evidence that these meteorites have not been contaminated by modern microorganisms. There is no doubt that the Miocene fly and Cretaceous Hadrosaurs are undeniably biological and must have had all protein amino acids while living. They are missing several of the same amino acids that are also absent in the carbonaceous meteorites. Furthermore, several of these missing amino acids (Threonine, Leucine and Isoleucine) are abundant in living cells and should have been detectable if the meteorites were contaminated by living or recent microorganism. Once formed in living organisms, many amino acids and proteins are extremely stable over long periods of time. Ancient proteins have recently been detected in remains of a fly in Miocene Amber, in Cretaceous Hadrosaurs[37] (80 million year old duck-billed dinosaurs) and preserved within organic tubes found in fossils of the Ediacaran *Sabellidites cambriensi* from the Upper Ediacaran (~540 mya) period.[38] Loss of amino acids and the nitrogen atoms that they contain from dead cells certainly does not occur on timescales of years, decades, centuries or even millennia. Therefore, the absence of detectable nitrogen by EDAX studies of microfossils of cyanobacteria, diatoms or bacteria found embedded in the rock matrix of carbonaceous meteorites indicates these recognizable biological remains are not recent microbial contaminants that entered the meteorites after they arrived on Earth.

### 2.3.3 Pigments and the Remote Search for Evidence of Extraterrestrial Life

The process of photosynthesis requires large, complex, nitrogen-rich biomolecules known as pigments. The major pigments of photoautotrophic organisms are:

**Chlorophylls--**greenish pigments that contain the stable ring shaped porphyrin molecule, around which electrons can move freely. The porphyrin ring can gain or lose electrons easily and thus can provide energized electrons to other molecules. This is the fundamental mechanism used by chlorophyll to harvest the energy of sunlight.

Chlorophyll *a*, the most important chlorophyll molecule, is present in all photosynthetic cyanobacteria, algae and plants. It passes its electrons on to molecules that manufacture sugars. Chlorophyll *b* is found primarily in green algae and plants. However, some genera (e.g. *Prochloron, Prochlorococcus* and *Prochlorothrix*) of cyanobacteria use chlorophyll *b* for photosynthesis as they lack phycobilisomes, which are the light harvesting antenna of photosystem II that contain the phycobilin proteins. Chlorophyll *c* occurs in dinoflagellates and the eukaryotic supergroup known as the chromista. The green chlorophyll pigments of the halophilic green alga *Dunaliella salina* can be clearly seen in an image from the International Space Station **(Fig. 5.a)** in ponds surrounding the periphery of the hypersaline Owens Lake, CA.[39]

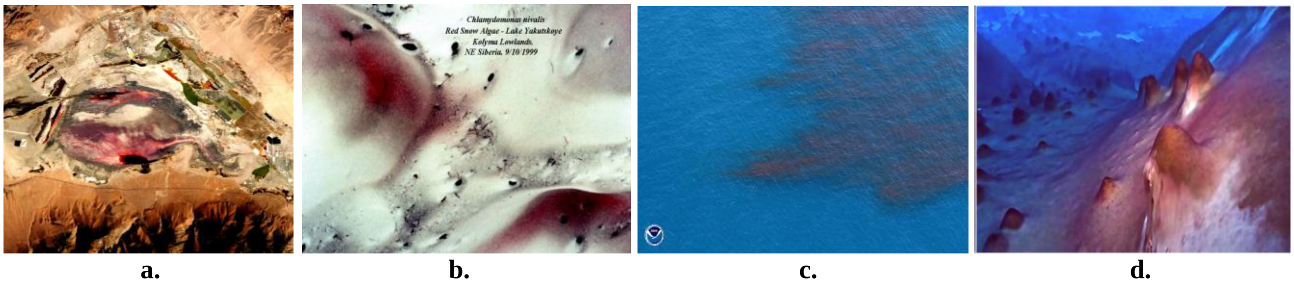


**Fig. 5.** Diverse colors of pigments in microorganisms. **a.** ISS image of Owens Lake, Ca showing green chlorophyll and red carotenoid pigments of *Dunaliella salina*; **b.** red spores of snow algae *Chlamydimonas nivalis* in Siberia; **c.** bloom of marine diatom *Thalassiosira* sp.; **d.** red phycoerythrin pigments in conical stromatolites of cyanobacteria *Phormidium sp.*on floor of Lake Untersee, Antarctica. (Photo Credits: **a.** *NASA/JSC*; **b.** *NOAA;* **c.** *Richard B. Hoover*; **d,** *Dale Andersen*)

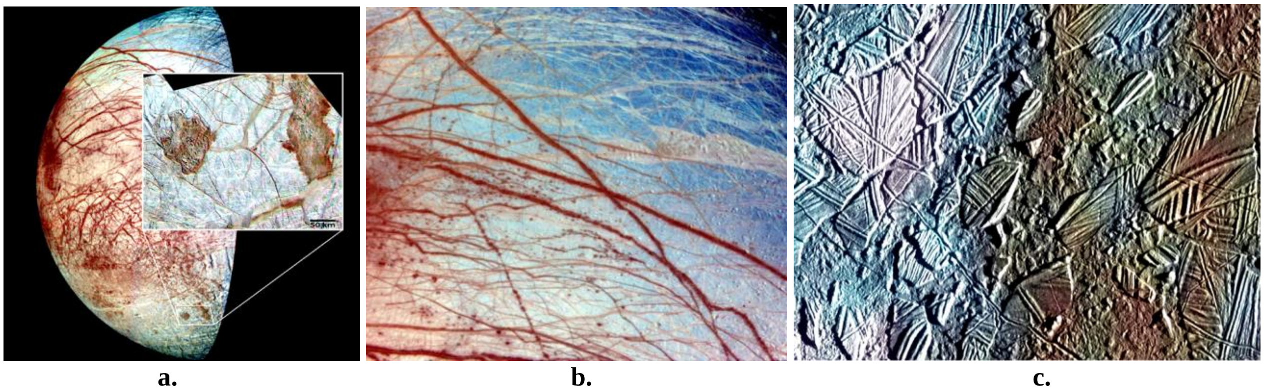


**Fig. 6.** Color images of Europa from the Galileo Spacecraft showing: **a.** Europa with enlargement of Thrace; **b.** Minos Linea; and **c,** Conomara Chaos regions exhibiting brown, orange, and red colorations consistent with pigments of diatoms and cyanobacteria. Image Credits: **a-c.** Arizona State University/NASA**.**

**Carotenoids--**red, orange or yellow carotene containing pigments composed of two small six carbon rings linked by a chain of carbon atoms. Carotenoids are insoluble in water and attach to membranes within cells. They are unable to transfer the energy gained from sunlight directly into the photosynthetic pathway but must transfer their absorbed energy to chlorophyll molecules and are thus termed "accessory pigments." Red and orange carotenoid pigments present in spores of the halophilic green alga *Dunaliella salina* and a novel carotenoid-like pigment found in *Salinobacter ruber* and halophilic archaea that cause the deep red color of hypersaline evaporite lakes such as Owens Lake can also be seen in **Fig. 5.a**.[39,40] Red carotenoid pigments are also seen in spores of the psychrophilic red snow algae *Chlamydomonas nivalis* **(Fig. 5.b)** collected in 1999 by the author from a snow bank during the *International Siberian Astrobiology Expedition***.**[41] Fucoxanthin is another accessory pigment that gives brown color to photosynthetic golden-brown algae known as diatoms. **Figure 5.c** is a NOAA image showing red and brown pigments in a large bloom of the marine planktonic diatom *Thalassiosira sp.* off the coast of Volusia County, Florida on July 25, 2010.

**Phycobilins-**blue or red water-soluble pigments that are the metabolic product of certain porphyrins. They serve as the light harvesting bilane photoreceptor pigments found in cyanobacteria and the chloroplasts of red algae, glaucophytes and cryptomonads. **Phycocyanin** is the phycobilin pigment that gives the blue-green color to many genera and species of cyanobacteria. **Phycoerythrin** is the red pigment that gives color to the eukaryotic red algae (Rhodophyta) and several of the red colored species of Cyanobacteria, such as *Phormidium tenue* and the nitrogen-fixing heterocystous strains of *Anabaena.*[39] Some non-heterocystous red cryptoendolithic species from Antarctica (such as *Gloecapsa spp.*) have c-phycoerythrin pigments as well as phycoerythrocyanin.[40] **Figure 5.d** is an image of large, laminated conical stromatolites that rise to a height of 0.5 growing on the floor of the perennially ice-covered Lake Untersee, Antarctica dominated by *Phormidium* species that contain red phycoerythrin pigments.

The Voyager 1 and Voyager 2 spacecraft obtained color images of the Jovian satellites Europa revealed large brownish areas and intersecting linear features. Higher resolution with better color rendition were then obtained from the Galileo Spacecraft. **Figure 6** shows Galileo images of **a.** the Thrace region;[43] **b.** Minos Linea region[44] and **c.** the Conomara Chaos region[45] of Europa. The blue and white regions are consistent with blue ice and white ice crystals/snow commonly seen in images of glaciers or polar ice sheets on Earth. The red and brown colors have been attributed to "likely contaminants in the ice" [44] or "minerals, such as salt, sulfur compounds, and possibly organic compounds that were previously sprayed on the surface from the ocean below."[45] However, for this hypothesis to be the correct, then the ocean far beneath the thick ice crust of Europa would have to be blood red and filled with water-soluble red minerals, which are unknown in any of the oceans or seas of Earth.

In 1986, Hoover *et al.*[46-48] pointed out that diatoms and other algae are the dominant eukaryotic life forms that inhabit the glaciers, sea ice and polar ice sheets of Earth. They suggested that these pigmented microorganisms were ideally suited to grow and carry out photosynthesis on the ice and in thin films of liquid water in cryptolithic regimes, cryoconite pools or larger bodies of water within comets, Europa or other icy moons of the Solar System. Cyanobacteria and archaea are the dominant types of prokaryotic life forms that inhabit cryptolithic and cryoconite environments and live in glaciers, ice sheets and sediments beneath perennially ice-covered lakes of Earth. The remains of filamentous and heterocystous cyanobacteria have been found in carbonaceous meteorites. These organisms could carry out photosynthesis and the life-critical functions of the carbon- and nitrogen-fixation on comets, water-bearing asteroids or icy moons of the Solar System just as they do on Earth.[49,50] Based upon these observations, the hypothesis has been advanced that the diverse orange, brown and red colors observed on the surface of Europa might be produced by photosynthetic pigments (Chlorophylls, Carotenoids and Phycobilins) contained within algae, diatoms, cyanobacteria, bacteria and archaea. This hypothesis is directly testable by remote observations to search for evidence of the distinctive fluorescence characteristics of these large complex pigment biomolecules.

Occasionally, large bodies of water on Earth do turn red, but this is not due to red minerals. Instead, "red tides" are caused by carotenoid pigments within the dinoflagellate *Karenia brevis* (=*Gymnodinium brevis*) of other phytoplankton that have undergone bloom conditions.[51] Many red cryptoendolithic and heterocystous nitrogen-fixing cyanobacteria contain large quantities of phycoerythrin.[52,53] However, not all cyanobacteria are blue-green, brown, orange or red. Some of the typically red cyanobacteria are almost black to provide protection of spores or increase their photosynthetic efficiency. These include the nitrogen-fixing coccoidal *Gloeocapsa magma* which is responsible for black or dark green stains on roofs as it produces black UV resistant spores in strong sunlight. There are also black filamentous cyanobacteria (e.g., *Phormidium corallyticum* and *Trichodesmium* spp.) responsible for coral black band disease.[54] The complex conformational configurations of the large folded biomolecules of pigments involved in photosynthesis are strongly fluorescent at specific wavelengths and exhibit distinctive relative absorption and emission spectral signatures.[55-57] These response characteristics are not mimicked by minerals or the simple organic molecules that can be formed by abiotic mechanisms. These properties have made it possible to investigate cyanobacteria, diatoms and other algal blooms in lakes, seas and oceans of Earth from space observatories. Therefore, the fluorescence properties of photosynthetic pigments could be used to directly test the hypothesis that the colorations of Europa, asteroids and other solar system bodies are in some cases the result of microorganisms rather than abiotic minerals. These studies could provide direct support of this hypothesis and the remote detection of the complex photosynthetic pigment biomolecules only associated with metabolic energy harvesting pathways of life, would provide clear, convincing and unambiguous evidence for life elsewhere in the Cosmos.

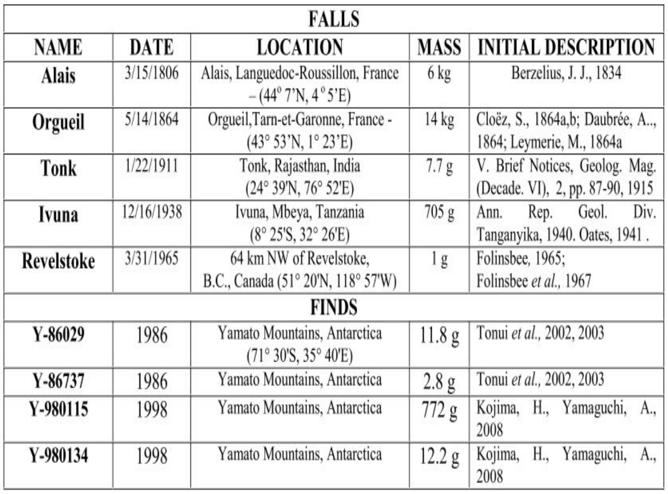
## 3. CARBONACEOUS METEORITES

Carbonaceous chondrites are considered pristine representatives of the protosolar nebula. Except for volatiles their elemental abundances more closely match the elemental composition of the Sun than any other class of chondrites. They are primitive and undifferentiated meteorites thought to have formed in oxygen-rich regions of the early Solar System since most of their metal is not in free form but instead present in form oxide, silicates or sulfides. The most extensive treatment of Carbonaceous Meteorites available to date is the 1975 book of Bartholemew Nagy.[57]

The CI group (named for the Ivuna meteorite that was observed to fall in Tanzania in 1938) are the most primitive and friable of all carbonaceous meteorites. The CI meteorites are all of petrologic type 1. They are distinguished from all other carbonaceous chondrites by a complete absence of chondrules and refractory inclusions, which have been destroyed by extensive aqueous alteration on the parent body and by their high degree (~20%) of indigenous water of hydration. The aqueous alteration took place on the parent bodies of the CI1 meteorites at low temperature (<50 °C) and produced hydrated phyllosilicates similar to terrestrial smectite or montmorillonite clay, carbonates, and iron oxides such as magnetite $Fe_3O_4$ and limonite $Fe_2O_3 \cdot nH_2O$. Sparsely distributed throughout the black meteorite matrix are fragments and crystals of olivine, pyroxene and elemental iron, presolar diamonds, graphite and insoluble organic carbonaceous matter similar to kerogen.[58-60]

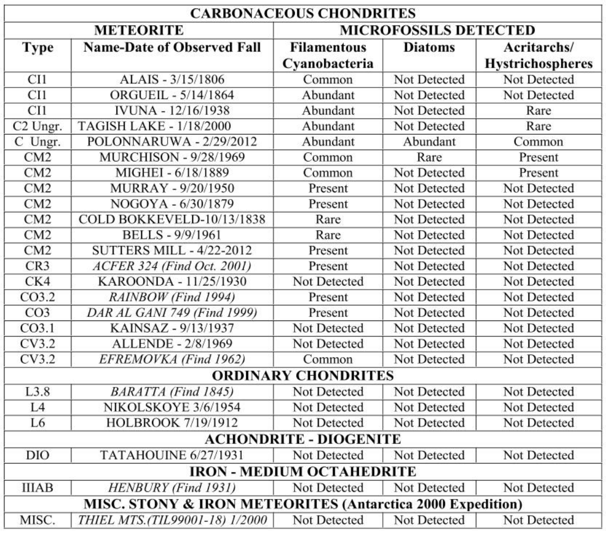
The CI1 carbonaceous chondrites are extremely rare. Although over 35,000 meteorites have been recovered there are only nine CI1 meteorites known on Earth **(Table II)**. Five of them were observed falls: *Alais, Orgueil, Ivuna, Tonk* and *Revelstoke*) and the other four (*Y-86029, Y-86737, Y980115* and *Y-980134*) were collected in the blue ice fields of the Yamato Mountains by Antarctic Expeditions of the National Institute of Polar Research, Japan. The great rarity of the CI1 stones is undoubtedly due to the fact that they are extremely friable micro-regolith breccias. All five CI1 meteorites known before 1986 were collected soon after they were observed to fall. The particulates of the CI1 meteorites are cemented together by water-soluble evaporite minerals such as epsomite ($MgSO_4 \cdot 7H_2O$) and gypsum ($CaSO_4 \cdot 2H_2O$). For this reason, the CI1 meteorite stones disintegrate immediately after they are exposed to liquid water This phenomenon was first observed by Thénard[61] in 1806 during his initial studies of the Alais meteorite that fell in France on Mar. 15, 1806. This property was confirmed by Berzelius[62,63] for Alais and then by Leymerie[64] during his study of the Orgueil meteorite stones that fell in France in 1864. **Table II**I is a compilation of all meteorites studied by Environmental and Field Emission Scanning Electron Microscopy and EDAX at NASA/Marshall Space Flight Center. In 2005, Hoover[65] repeated these experiments with Orgueil and using EDAX analysis and confirmed that the CI1 meteorite stones disaggregate into tiny particles when magnesium sulfate salts that cement their mineral grains together rapidly dissolve.

**TABLE II. CI1 Carbonaceous Meteorites**



| FALLS | | | | |
|---|---|---|---|---|
| **NAME** | **DATE** | **LOCATION** | **MASS** | **INITIAL DESCRIPTION** |
| **Alais** | 3/15/1806 | Alais, Languedoc-Roussillon, France – (44° 7'N, 4° 5'E) | 6 kg | Berzelius, J. J., 1834 |
| **Orgueil** | 5/14/1864 | Orgueil,Tarn-et-Garonne, France - (43° 53'N, 1° 23'E) | 14 kg | Cloëz, S., 1864a,b; Daubrée, A.., 1864; Leymerie, M., 1864a |
| **Tonk** | 1/22/1911 | Tonk, Rajasthan, India (24° 39'N, 76° 52'E) | 7.7 g | V. Brief Notices, Geolog. Mag. (Decade. VI), 2, pp. 87-90, 1915 |
| **Ivuna** | 12/16/1938 | Ivuna, Mbeya, Tanzania (8° 25'S, 32° 26'E) | 705 g | Ann. Rep. Geol. Div. Tanganyika, 1940. Oates, 1941 . |
| **Revelstoke** | 3/31/1965 | 64 km NW of Revelstoke, B.C., Canada (51° 20'N, 118° 57'W) | 1 g | Folinsbee, 1965;<br>Folinsbee *et al.*, 1967 |
| **FINDS** | | | | |
| **Y-86029** | 1986 | Yamato Mountains, Antarctica (71° 30'S, 35° 40'E) | 11.8 g | Tonui *et al.*, 2002, 2003 |
| **Y-86737** | 1986 | Yamato Mountains, Antarctica | 2.8 g | Tonui *et al.*, 2002, 2003 |
| **Y-980115** | 1998 | Yamato Mountains, Antarctica | 772 g | Kojima, H., Yamaguchi, A., 2008 |
| **Y-980134** | 1998 | Yamato Mountains, Antarctica | 12.2 g | Kojima, H., Yamaguchi, A., 2008 |

**TABLE III. Microfossils in Meteorites Investigated at NASA/MSFC**



| CARBONACEOUS CHONDRITES | | | | |
|---|---|---|---|---|
| **METEORITE** | | **MICROFOSSILS DETECTED** | | |
| **Type** | **Name-Date of Observed Fall** | **Filamentous Cyanobacteria** | **Diatoms** | **Acritarchs/ Hystrichospheres** |
| CI1 | ALAIS - 3/15/1806 | Common | Not Detected | Not Detected |
| CI1 | ORGUEIL - 5/14/1864 | Abundant | Not Detected | Not Detected |
| CI1 | IVUNA - 12/16/1938 | Abundant | Not Detected | Rare |
| C2 Ungr. | TAGISH LAKE - 1/18/2000 | Abundant | Not Detected | Rare |
| C Ungr. | POLONNARUWA - 2/29/2012 | Abundant | Abundant | Common |
| CM2 | MURCHISON - 9/28/1969 | Common | Rare | Present |
| CM2 | MIGHEI - 6/18/1889 | Common | Not Detected | Present |
| CM2 | MURRAY - 9/20/1950 | Present | Not Detected | Not Detected |
| CM2 | NOGOYA - 6/30/1879 | Present | Not Detected | Not Detected |
| CM2 | COLD BOKKEVELD-10/13/1838 | Rare | Not Detected | Not Detected |
| CM2 | BELLS - 9/9/1961 | Rare | Not Detected | Not Detected |
| CM2 | SUTTERS MILL - 4/22-2012 | Present | Not Detected | Not Detected |
| CR3 | *ACFER 324 (Find Oct. 2001)* | Present | Not Detected | Not Detected |
| CK4 | KAROONDA - 11/25/1930 | Not Detected | Not Detected | Not Detected |
| CO3.2 | *RAINBOW (Find 1994)* | Present | Not Detected | Not Detected |
| CO3 | *DAR AL GANI 749 (Find 1999)* | Present | Not Detected | Not Detected |
| CO3.1 | KAINSAZ - 9/13/1937 | Not Detected | Not Detected | Not Detected |
| CV3.2 | ALLENDE - 2/8/1969 | Not Detected | Not Detected | Not Detected |
| CV3.2 | *EFREMOVKA (Find 1962)* | Common | Not Detected | Not Detected |
| **ORDINARY CHONDRITES** | | | | |
| L3.8 | *BARATTA (Find 1845)* | Not Detected | Not Detected | Not Detected |
| L4 | NIKOLSKOYE 3/6/1954 | Not Detected | Not Detected | Not Detected |
| L6 | HOLBROOK 7/19/1912 | Not Detected | Not Detected | Not Detected |
| **ACHONDRITE - DIOGENITE** | | | | |
| DIO | TATAHOUINE 6/27/1931 | Not Detected | Not Detected | Not Detected |
| **IRON - MEDIUM OCTAHEDRITE** | | | | |
| IIIAB | *HENBURY (Find 1931)* | Not Detected | Not Detected | Not Detected |
| **MISC. STONY & IRON METEORITES (Antarctica 2000 Expedition)** | | | | |
| MISC. | *THIEL MTS.(TIL99001-18) 1/2000* | Not Detected | Not Detected | Not Detected |

The CM group of carbonaceous chondrites, named for the Mighei meteorite that fell in 1889 in Ukraine, also contain carbon, indigenous extraterrestrial water and a host of complex organic compounds. The Murchison meteorite was observed to fall in Murchison, Australia on Sept. 28, 1969. Originally classified as a CM2 carbonaceous chondrite, Murchison was recently re-classified by Rubin *et al.*[67] as CM2.5 based on the evidence indicating the primary lithology of the meteorite was subjected to a relatively low and uniform degree of aqueous alteration on the parent body.[68] It has been known since the first studies of the Alais meteorite in 1806 that carbonaceous meteorites contain water, carbon and organic chemicals.[63.64] In the 1960's there were diverse reports that the Orgueil, Ivuna and other carbonaceous meteorites contained organics,[69] biogenic hydrocarbons,[70] long chain fatty acids,[71] and possible microfossils.[72] These findings led to serious considerations of problems associated with developing taxonomic systems for extraterrestrial life forms.[73,74]

These early results were highly controversial and it was argued that the hydrocarbons and possible microfossils found in the meteorites were either terrestrial contaminants, carbonaceous coated mineral grains or deformed and misidentified modern pollen grains.[75-79] This conclusion was generally accepted by the scientific community, in spite of the fact that in 1966, the Nobel Laureate in Chemistry (1934) Harold C. Urey[80] reviewed biological materials in meteorites and pointed out that the organic substances in carbonaceous meteorites resemble those in ancient terrestrial rocks but are not similar to modern bio-contaminants. Urey concluded *"If found in terrestrial objects, some substances in meteorites would be regarded as indisputably biological."* Furthermore, some of the forms discovered in Orgueil had the complex morphologies of filamentous cyanobacteria.[81] Timofeev, the Russian pioneer of the study of extinct life forms known as Acritarchs reported the detection of many acritarchs in the Murray carbonaceous meteorite.[82] Timofeev had great expertise in this field and would certainly not have confused pollen grains with acritarchs. In 1967, Tan and VanLandingham[83] presented Transmission Electron Microscopy images of carbonaceous bodies in Orgueil that contained *"*electron dense solid bodies*"* that were aligned along the longitudinal axis and similar in size and orientation to magnetosomes present in magnetotactic bacteria, that were completely unknown at that time.

Since 1996, extensive investigations of biomorphic microstructures and microfossils in meteorites have been conducted at the NASA/Marshall Space Flight Center and at the Paleontology Institute of the Russian Academy of Sciences in Moscow. Table III provides an overview of the different types of meteorites studied at NASA/MSFC along with an assessment of the relative abundance and types of microfossils encountered. Coccoidal forms, small rods, "nanofossils" and other forms that may represent abiotic mineral grains or could have been produced by surface tension on melted mineral drops as well as possible microfossils that do not have recognizably biological characteristics were generally not even imaged or analyzed by EDAX during the Scanning Electron Microscopy studies at NASA/MSFC.

### 3.1 Biomolecules in Carbonaceous Meteorites

Carbonaceous meteorites contain extraterrestrial water and carbon as well as all of the other life-critical biogenic elements. Most of the carbon contained within carbonaceous meteorites is in the form of Insoluble Organic Matter (IOM) similar to Kerogen[58,84] with nominal bulk composition[85] of $C_{100}H_{70}O_{12}N_3S_2$. The carbonaceous meteorites also contain a vast array of suite of organic chemicals and biomolecules. Schmitt-Koplan *et al.*[86] analyzed polar and apolar solvent extracts of uncontaminated, freshly fractured interior fragments of the Murchison meteorite. Using Fourier transform ion cyclotron resonance/mass spectrometry (FTICR/MS) and other advanced instrumentation methods they reported the detection of 46,696 elemental compositions corresponding to 14,197 distinct elemental formulas and suggested that several million different chemical compounds might be present in carbonaceous chondrites. The great majority of these organic compounds may well have been formed by abiotic processes on the parent bodies. However, many of the carbonaceous meteorites also contain evidence for microfossils (Table III) as well as non-racemic and extraterrestrial protein amino acids, nucleobases and large biomolecules that represent the diagenetic breakdown products of photosynthetic pigments which have no known abiotic production mechanisms. Although these complex biomolecules are often attributed to the post-arrival terrestrial contamination, the missing amino acids, nucleobases and other life-critical biomolecules that have never been detected in interior fragments of these meteorites invalidates the recent bio-contamination hypothesis. **Table IV** summarizes many biomolecules found in carbonaceous meteorites along with the missing biomolecules that should be present if the meteorites were contaminated by modern microorganisms. The biomolecules that are missing in the carbonaceous meteorites are just as important as the ones that have been found. They provide clear and convincing proof that the interior portions of these stones that were analyzed have not been contaminated by recent microorganisms.

## TABLE IV. Biomolecules Present and Absent in Carbonaceous Meteorites

| Biomolecule Detected | Biomolecules Not Detected | SIGNIFICANCE |
|---|---|---|
| 8 Extraterrestrial Protein Amino Acids[87-95]<br>**GLY, ALA, GLU, ASP, PRO, LEU, VAL, ILEU** | 12 of 20 Protein Amino Acids<br>**PHE, THR, TRY, MET** | Meteorites not contaminated by living or modern microorganisms |
| **Porphyrins, Pristane, Phytane**[94,96-98] | Chlorins, Chlorophyll, Carotenoids, Phycobilins & other pigments | Ancient diagenetic breakdown products of biological pigments |
| 3 of 5 Nucleobases[99-103]<br>**Adenine, Guanine, and Uracil** | 2 of 5 Nucleobases<br>**Cytosine and Thymine** | **Cytosine→Uracil: Half-Life 17,000 Years**<br>**Thymine→Xanthine: Half-Life 1.3 Ma @ 0 C and pH 7** |

As seen in **Table IV**, only 8 of the 20 life-critical protein amino acids are present in the carbonaceous meteorites investigated by several different scientific teams. With the passage of time the homochirality of amino acids degrades as they become more racemic in dead microorganisms. They also decompose and disappear and their nitrogen is slowly lost and returned to the atmosphere as part of the nitrogen cycle. Simulation experiments by Vallentyne[104] evaluated the relative stability of protein amino acids in fossil shells. He reported the following general trend with regard to the stability of many of the amino acids that constitute proteins:

*SER<THR<MET<LYS<TYR<PHE*<**ILEU<LEU<PRO<VAL<GLY<ALA<GLU**

where the least stable (Italics) is Serine and the most stable (Bold) is Glutamic acid. The unstable acids are also absent in carbonaceous meteorites. The amino acids Serine, Threonine and Phenalynine are relatively abundant in living cyanobacteria and bacteria **(Table I)** but they are absent in carbonaceous meteorites and ancient fossilized remains. There is no known mechanism by which these meteorites could be contaminated by modern biology without including all 20 protein amino acids, DNA, RNA, all nucleobases, sugars, pigments and other biomolecules into the stones.

Biomolecular evidence for ancient photosynthetic microorganisms inhabiting the parent bodies is also provided by the detection of porphyrins, pristane and phytane in several of these stones. Pristane (2,6,10,14-Tetramethylpentadecane) is a C19 isoprenoid hydrocarbon that is the diagenetic breakdown product of phytol.[105] The long phytol chain is a side chain of the chlorin ring component of the photosynthetic pigment chlorophyll. Pristane is possibly the most abundant acyclic isoprenoid in the biosphere and represents a petroleum biogeochemical marker for ancient aquatic environments.[105] Phytane (2,6,10,14-Tetramethylhexadecane) is the C20 acyclic isoprenoid hydrocarbon that is associated with the membranes of thermophilic archaea. Pristane and phytane are both considered to products of the diagenesis of the phytyl side chain of the chlorophyll molecule.[106] Porphyrins are water-soluble nitrogenous biological pigments that are found in living organisms. These heterocyclic compounds are derived from the pyrrole ring and contain a central metal atom - Fe in heme and Mg in chlorophylls. Pristane, phytane and vanadyl porphyrins are found on Earth in ancient sediments, coal and petroleum.[107,108] Hodgson and Baker[94,96,109] reported spectroscopic evidence for pigments in the Orgueil meteorite that are indistinguishable from the vanadyl porphyrins present in bitumen, asphaltene and crude oil. They also reported that the Orgueil sample they studied did not contain chlorins, which are present in all recent biological deposits.[110] The presence of pristane, phytane and vanadyl porphyrins is indicative of photosynthetic organisms that died millions to hundreds of millions or billions of years ago on the meteorite parent body. The absence of photosynthetic pigments provides further evidence that these meteorites have not been contaminated with modern cyanobacteria or diatoms.

Several independent early researchers reported the detection of heterocyclic nitrogen compounds such as purines, pyrimidines and triazines in carbonaceous meteorites.[99-101] More recent studies have established that several carbonaceous chondrites do contain the nitrogenous nucleobases **Adenine, Guanine** and **Uracil** and stable carbon isotope data clearly establishes that the nucleobases in carbonaceous meteorites are extraterrestrial in origin.[102,103,110] However, none of these different investigations have resulted in the detection of the nucleobases **Cytosine** or **Thymine**. By Watson-Crick base pairing rules of DNA, **A**denine always pairs with **T**hymine and **C**ytosine pairs with **G**uanine. (In RNA, the complementary base to **A**denine is **U**racil rather than **T**hymine.) The missing nucleobases, Cytosine and Thymine are unstable. At 0 C, the Cytosine converts to Uracil by spontaneous deamination (releasing ammonia) with a half-life of only 17,000 years.[111] Thymine is much more stable, but it still has a half-life of only 1.3 million years at 0 C and pH 7.0, and it converts to Xanthine, which is present in carbonaceous meteorites.[103,110] If the microfossils found in carbonaceous meteorites are the remains of organisms that died millions of years ago, it would not be surprising for the meteorites to contain no detectable levels of Cytosine and Thymine. However, if the stones were contaminated by living microorganisms within the past several centuries, they should have both Cytosine and Thymine. The *absence of Cytosine* *and Thymine* provides clear and convincing proof that interior regions of these carbonaceous meteorites have not been invaded by living cyanobacteria or other terrestrial microorganisms since they arrived on Earth.

### 3.2 Microfossils in Carbonaceous Meteorites

The search at NASA/MSFC for evidence for microfossils in carbonaceous meteorites was initiated in 1996 immediately after McKay *et al.*[112] reported the discovery of PAH's, biogenic magnetites and possible nanofossils in the Mars meteorite ALH84001. Environmental and Field Emission Scanning Electron Microscope (ESEM and FESEM) studies of uncoated, freshly fractured, interior fragments of carbonaceous meteorite resulted in the detection of large microfossils of cyanobacteria.[113] Similar findings were reported for SEM studies of carbonaceous meteorites conducted by Rozanov and coworkers at the Paleontological and Microbiological Institutes, Russian Academy of Sciences, Moscow.[114] These independent (and subsequent joint) studies resulted in the detection of the remains of a large number of diverse cyanobacteria, acritarchs, hystrichospheres, diatoms and rare unidentified protists. Many of the larrge, complex microfossils found exhibit the sizes and detailed morphologies of known genera and species of cyanobacteria and diatoms.[20,50,115-120] EDAX studies at NASA/MSFC measured relative elemental compositions. In general, the spot analyses and 2-D X-Ray Element Maps revealed that microfossils were typically enriched in carbon and often contain several of the more abundant elements (Si, Fe, Mg, S, O) present in the particular meteorite rock matrix. In the Orgueil meteorite, EDAX data shows many of the filaments have highly carbonaceous sheaths and contain Mg, S, and O. This suggests they were infilled with water-soluble magnesium sulfates after they died and the internal cells were lost. This is not the case for cyanobacterial filaments found in Murchison. It is difficult to account for these composition differences by the modern bio-contamination hypothesis. Since the mineral grains of the Orgueil meteorite disaggregates upon contact with liquid water, the infilling of the filaments with magnesium sulfate must have occurred on the parent body before the stones landed or Earth. Exposure to water after landing would have destroyed the stones. Furthermore, the microfossils in the meteorites are heavily depleted in nitrogen as compared with living or dead organisms on Earth.

Nitrogen is one of the most abundant elements in the Solar System, but it is severely depleted in meteorites.[121,122] Nitrogen is rarely present in microfossils in the meteorites at levels (>0.5% atomic) high enough to be detected by EDAX. However, EDAX studies at NASA/MSFC have shown that modern or even dead dells typically have nitrogen levels of 2% to 20% (atomic) and the absence of nitrogen in meteorite microfossils rules out contamination.

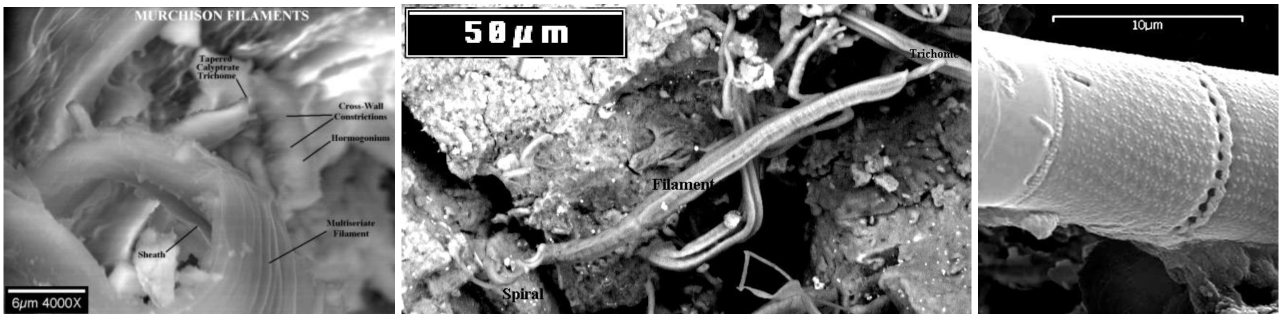


**Fig. 7.a.** Trichomic filamentous cyanobacteria in **a.** Murchison meteorite showing morphotype of *Microcoleus* sp.; tapered *Calothrix sp.* filament with apical cell and calyptra and an escaped hormogonium; **b.** Orgueil meteorite with long *Lyngbya* sp**.** filament with thick sheath and emergent trichome; **c.** Polonnaruwa with filamentous planktonic diatom *Aulacoseira ambigua.* Photo Credits: **a.&b.** *Gregory Jerman and Richard B. Hoover, NASA/MSFC;* **c.** *N. C. Wickramasinghe, University of Buckingham*

**Figure 7** shows images of microfossils of cyanobacteria in **a.** Murchison **b.** Orgueil and **c.** Polonnaruwa meteorites. The Murchison filaments of **Fig.** 7**.a.** are consistent with species of cyanobacteria that grow together in polar environment. These include the multiseriate *Microcoleus chtonoplastes* and *Phomidium* species and tapered filaments of *Calothrix* sp. with apical cell and calyptra; and escaped hormogonium with cell wall constrictions; **b.** Orgueil meteorite long filament with thick sheath and emergent trichome (*Lyngbya* sp**.** morphotype) **c.** Polonnaruwa meteorite with cells of the filamentous planktonic diatom *Aulacoseira ambigua* showing rimoportulae and ringleiste structures.

## 4. SUMMARY

It is well known that all forms of life on Earth require water, a source of energy, and a small group of biogenic elements. It has long been known that water is the dominant volatile on comets and icy moons and recent observations have shown that water is also common on many asteroids. Pristine interior fragments of carbonaceous meteorites have been found to contain all life-critical biogenic elements, water, aqueously altered minerals, organic chemicals, biomolecules and diagenetic breakdown products of photosynthetic pigments. However, photosynthetic pigments such as chlorophylls, carotenoids, phycoerythrin, phycobilins, etc. have never been reported in these stones. Carbonaceous meteorites have also been found to contain a subset of 8 of the 20 life-critical protein amino acids and 3 of the 5 nucleobases necessary to form DNA and RNA molecules. The missing pigments amino acids and nucleobases and the presence of pristane and phytane but the absence of life-critical protein amino acids, nucleobases, pigments and other biomolecules indicates biology but suggests that any microorganisms that inhabited the parent bodies of these meteorites must have perished long before the meteorites arrived on Earth. Furthermore, the missing biomolecules provides clear and convincing evidence that interior regions of these carbonaceous meteorites have not been contaminated by terrestrial organisms. The ESEM and FESEM studies have shown that many carbonaceous meteorites contain recognizable microfossils deeply embedded in the meteorite rock matrix that can be associated with morphotypes of known genera and species of filamentous trichomic and sometimes heterocystous cyanobacteria as well as diatoms and extinct life forms such as acritarchs and hystrichospheres. EDAX studies indicate these microfossils are often encased within carbon-rich sheaths and depleted in nitrogen as compared with recent or Pleistocene organisms. The absence of nitrogen in these microfossils indicates these permineralized remains are of microorganisms that perished long before the meteorites entered the Earth's atmosphere. Consequently they are interpreted as the indigenous remains of extraterrestrial life forms that grew within aqueous environments on the parent body of the meteorite. The detection of the remains of diatoms and nitrogen-fixing, photosynthetic cyanobacteria suggests that the distinctive fluorescence properties of the large, complex photosynthetic biomolecular pigments may provide a sensitive mechanism for the definitive and conclusive remote detection of evidence of life elsewhere in the Cosmos.

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