

Fossils of Cyanobacteria in CI1 Carbonaceous Meteorites: Implications to Life on Comets, Europa and Enceladus

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ABSTRACT

Environmental (ESEM) and Field Emission Scanning Electron Microscopy (FESEM) investigations of the internal surfaces of the CI1 Carbonaceous Meteorites have yielded images of large complex filaments. The filaments have been observed to be embedded in freshly fractured internal surfaces of the stones. They exhibit recognizable features (e.g., the size and size ranges of the internal cells and their location and arrangement within sheaths) that are diagnostic of known genera and species of filamentous trichomic cyanobacteria and other trichomic prokaryotes (such as filamentous sulfur bacteria). ESEM and FESEM studies of living and fossil cyanobacteria show features similar to the filaments found in the meteorites -- uniseriate and multiseriate, branched or unbranched, isodiametric or tapered, polarized or unpolarized filaments with trichomes encased within thin or thick external sheaths. Some of the filaments found in the CI1 meteorites also exhibit specialized cells and structures used by cyanobacteria for reproduction (baecocytes, akinetes and hormogonia), nitrogen fixation (basal, intercalary or apical heterocysts), attachment (pili or fimbriae) or indicative of oscillatoria type locomotion (escaped or coiling hormogonia and flattened and coiled empty sheaths).

Energy dispersive X-ray Spectroscopy (EDS) studies indicate that the Orgueil meteorite filaments are typically carbon-rich sheaths infilled with magnesium sulfate and other minerals characteristic of the CI1 carbonaceous meteorites. However, the size, structure, detailed morphological characteristics and chemical compositions of the meteorite filaments are not consistent with known species of abiotic minerals. The nitrogen content of the meteorite filaments are almost always below the detection limit of the EDS detector. EDS analysis of living and dead biological materials (e.g., filamentous cyanobacteria; bacteria, mummy and mammoth hair and tissues, and fossils of cyanobacteria, trilobites and insects in amber) indicate that nitrogen remains detectable in biological materials for many thousands of years but is undetectable in the truly ancient fossils. These studies have led to the conclusion that the filaments found in the CI1 carbonaceous meteorites are indigenous fossils rather than modern terrestrial biological contaminants that entered the meteorites after arrival on Earth. The $\delta^{13}\text{C}$ and D/H content of amino acids and other organics found in these stones are shown to be consistent with the interpretation that comets represent the parent bodies of the CI1 carbonaceous meteorites. The implications of the detection of fossils of cyanobacteria in the CI1 meteorites to the possibility of life on comets, Europa and Enceladus are discussed.

Keywords: CI1 meteorites, Orgueil, Alais Ivuna, microfossils, cyanobacteria, comets, Europa, Enceladus

1. INTRODUCTION

The CI1 carbonaceous chondrites are the most primitive of all known meteorites in terms of solar elemental abundances and the highest content of volatiles. Carbonaceous chondrites are a major clan of chondritic meteorites that contain indigenous extraterrestrial water, several weight % Carbon, Mg/Si ratios at near solar values, and oxygen isotope compositions that plot below the terrestrial fractionation line. The CI1 classification indicates the meteorites belong to the CI (Ivuna Type) chemical group and are of petrologic Type 1. The CI1 meteorites are distinguished from other carbonaceous chondrites by a complete absence of

chondrules and refractory inclusions (destroyed by extensive aqueous alteration on the parent body) and by their high degree (~20%) of indigenous water of hydration (Endress and Bischoff, 1993, 1996). 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 Fe₃O₄ and limonite Fe₂O₃ · nH₂O. Sparsely distributed throughout the black meteorite matrix are fragments and crystals of olivine, pyroxene and elemental iron, presolar diamonds, graphite and insoluble organic matter similar to kerogen.

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 I**). 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 1986 and 1998 from 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 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 (MgSO₄·7H₂O) and gypsum (CaSO₄·2H₂O). The fact that these stones disintegrate immediately after they are exposed to liquid water was first observed during the initial studies of the Alais meteorite that fell in France on Mar. 15, 1806 (Thénard, 1806; Berzelius, 1834, 1836) and in the Orgueil stones by Leymerie (1864). Hoover (2005b) confirmed that the CI1 meteorite stones disaggregate into tiny particles when water soluble salts that cement mineral grains together in the rock matrix dissolve.

TABLE I. 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; Folinsbee <i>et al.</i> , 1967
FINDS				
Y-86029	1986	Yamato Mountains, Antarctica (71° 30'S, 35° 40'E)	11.8 g	Tonui <i>et al.</i> , 2002, 2003
Y-86737	1986	Yamato Mountains, Antarctica	2.8 g	Tonui <i>et al.</i> , 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

Although ejecta from the moon and Mars are associated with several known meteorites, the parent bodies for vast majority of all meteorites on Earth are asteroids. The high water content, D/H ratios, and the extensive evidence for low-temperature aqueous alteration of the CI1 carbonaceous meteorites indicate significant amounts of liquid water on their parent bodies indicative of water-bearing asteroids or comets.

1.1. Chemical, Mineral and Morphological Biomarkers in CI1 Carbonaceous Meteorites.

A number of biominerals and organic chemicals (which are interpreted as biomarkers when found in Earth rocks) have been detected in CI1 carbonaceous meteorite. These include weak biomarkers such as carbonate globules, magnetites, PAH's, racemic amino acids, sugar alcohols, and short chain alkanes, alkenes and aliphatic and aromatic hydrocarbons. These organic chemicals can all be produced in nature by biological processes but they can also be formed by catalyzed chemical reactions such as Miller-Urey and Fisher-Tropsch synthesis. However, the CI1 meteorites also contain a host of strong biomarkers for which there are no known abiotic production mechanisms. These include magnetites in unusual configurations (framboids and linear chains of magnetosomes), protein amino acids with significant enantiomeric excess, nucleobases (purines and pyrimidines), and diagenetic breakdown products of photosynthetic pigments such as chlorophyll (pristine, phytane, and porphyrins), complex kerogen-like insoluble organic matter and morphological biomarkers with size, size range and recognizable features diagnostic of known orders of *Cyanobacteriaceae* and other prokaryotic microfossils. **Table II** provides a chronological summary of chemical, mineral and morphological biomarkers found by many independent researchers who have studied carbonaceous meteorites since 1806, immediately after the fall of the Alais CI1 carbonaceous meteorite.

TABLE II. Biomarkers in CI1 Carbonaceous Meteorites

METEORITES	WEAK BIOMARKERS	REFERENCE
ALAIS	Carbon, Water, Clay Minerals, Organic Matter ~ humus and lignite with Odor of Bitumen	Thénard (1806); Berzelius (1834)
ALAIS	Sulph-Hydrocarbons	Smith (1876); Roscoe (1864)
ORGUEIL	Organic Matter ~ humus, peat and lignite coal & Clay Minerals,	Daubrée (1864); Cloez, (1864a,b) Pisani, (1864); Bass (1971)
ALAIS, ORGUEIL & IVUNA	Petroleum-like Hydrocarbons, Aliphatic and Aromatic Hydrocarbons and PAH's	Berthelot (1868); Commins & Harrington, (1966); Olson <i>et al.</i> (1967); Nooner&Oró (1967)
ORGUEIL	Amino Acids	Nagy, (1961); Kaplan <i>et al.</i> (1963), Vallentyne (1965); Nagy & Bitz (1963)
ORGUEIL	Normal alkanes	Gelpi & Oró, (1970)
ORGUEIL	“Organized Elements”, Microvessicles	Claus and Nagy (1961, 1962); Claus <i>et al.</i> (1963); Pflug (1984)
STRONG BIOMARKERS		REFERENCE
ORGUEIL	Long-Chain Fatty Acids, Isoprenoids, Kerogen	Nagy & Bitz, (1963); Bitz & Nagy, (1966)
ALAIS, ORGUEIL & IVUNA	Porphyrins, Pristane, Phytane and NorPristane	Kaplan (1963) Hodgson & Baker, (1964); (1969); Oró <i>et al.</i> (1966), Nooner & Oró, (1967); Kissin, (2003)
ORGUEIL & IVUNA	Protein Amino Acids with Enantiomeric Excess	Oró <i>et al.</i> , (1971); Engel <i>et al.</i> . (1982, 2001, 2005) Cronin <i>et al.</i> . (1997); Ehrenfreund <i>et al.</i> , (2001)
ALAIS, IVUNA & ORGUEIL	Nitrogen Heterocycles and Nucleobases - Purines and Pyrimidines	Calvin, 1961, Hayatsu 1964, Hayatsu <i>et al.</i> 1968, 1977; Folsomme <i>et al.</i> (1971; 1973;) Hua <i>et al.</i> , 1986; Stoks & Schwartz, 1981; Glavin & Bada, (2004); Martins <i>et al.</i> , (2008); Callahan <i>et al.</i> (2011).
ALAIS, IVUNA & ORGUEIL	Filamentous Microfossils ~ Trichomic Prokaryotes and Cyanobacteria	Palik, 1962; vanLandingham, 1965; Zhmur (1997); Hoover (1997, 2005a,b, 2006a,b, 2007, 2008, 2009); Hoover <i>et al.</i> (1998, 2003a,b, 2004)

1.2. The Alais CI1 Carbonaceous Meteorite.

Alais was the first carbonaceous meteorite known to science. Two thunderous detonations were heard across southern France at 5:30 P.M on March 15, 1806. Two soft, black stones that emitted a “strong odor of

bitumen” were then observed to fall in small villages near Alais, Languedoc-Roussillon, France – (44° 07'N, 4° 05'E). One black stone of 2 kg mass landed in the small village of St. Étienne de Lolm and a 4 kg stone landed near Valence, France and broke a branch from a fig tree as it fell. Louis Jacques Thénard (1806), the renowned Professor of Chemistry at the College de France in Paris, conducted the first study of the Alais C11 carbonaceous meteorite. He realized that these stones were different from all other meteorites since they had the appearance of solidified clay. Thénard reported that “*when the stones were placed in water they disintegrated immediately and gave off a strong clay-like odor.*” He found the Alais meteorite stones contained 2.5% carbon and oxides of iron, magnesium, and nickel.

The Alais stone was subsequently analyzed by Jöns Jacob Berzelius, the distinguished Swedish organic chemist and mineralogist. Berzelius discovered the elements silicon, selenium, thorium and cerium. He obtained a small fragment of the Alais meteorite from the French mineralogist Lucas. Berzelius (1834, 1836) was initially astonished to find this stone contained water and almost tossed it out as being contaminated. In the English translation provided by Nagy, (1975, p. 45) Berzelius remarked: “*I was so suspicious, because this meteorite contained water that I was about ready to throw my sample away. However, fortunately before I discarded the sample, I reread the record and found certain data which completely agreed with the meteoritic origin of the stone. This intrigued me so that I then carried on the investigation with great interest. The question arose in my mind; does this carbonaceous earth contain humus or a trace of other organic substances? Could this give a hint to the presence of organic formations on other planets?*” Berzelius was the first scientist to recognize that the Alais meteorite consisted mostly of clay-type minerals and he confirmed Thénard’s observation that the Alais stones were destroyed by liquid water: “*These stones are different from all other meteorites because they look like solidified clay and because when they are placed in water they disintegrate and give off a clay-like odor.*” Berzelius concluded that the Alais meteorite contained a portion of metallic iron and nickel (12%) that was attracted to a magnet as well as indigenous extraterrestrial water and carbon, saying: “*some organic matter and 10 per cent of a salt which contained no iron, being a mixture of sulphates of nickel, magnesia, soda, potash and lime with a trace of sulphate of ammonia.*” When he heated the sample it turned brown and “*gave off a tarry odor.*” Berzelius reported “*in water it disintegrates instantaneously to a greyish-green powder which has an odor reminding one of fresh hay.*” He also found it to contain carbon dioxide and a soluble salt containing ammonia.

1.3. The Orgueil C11 Carbonaceous Meteorite.

The Orgueil meteorite is one of the most extensively documented and thoroughly investigated of all known meteorites. At 8:08 P.M. on May 14, 1864 a brilliant fireball illuminated a large region of southern France and thunderous explosions were heard as the blue-white fireball streaked across the sky, turned a dull red color and produced a long thin white smoke trail (Jollois, 1864; d’Esparbés, 1864). The weather was nice on this spring evening in the south of France. Soon after the explosions were heard, a shower of stones fell within an 18 km east-west scatter ellipse between the villages of Orgueil, Campsas and Nohic (Tarn-et-Garonne). The main fall occurred near the village of Orgueil (43° 53’ N; 01° 23’ E) and villagers collected over 20 jet-black stones immediately after the fall. Many of the Orgueil stones had complete fusion crusts and a few were quite large (one with mass ~11 kg). The Orgueil bolide was so spectacular that many villagers at St. Clar thought they were surrounded by flames. The Marquise de Puylaroque (1864) reported that her house looked like “*the interior of a furnace*” and she heard a rumbling noise that sounded like firearms and lasted for 2-3 minutes. The detonations were so violent that some villagers thought the event was an earthquake (Bergé, 1864). Eyewitness reports from all over the region were sent to M. Le Verrier, Director of the Imperial Observatory, and to the eminent geologist Academician G. A. Daubrée. These accounts were published immediately (Daubrée, 1864) and English translations have subsequently been made available (Nagy, 1975). The observations of the fireball and timing of the detonations made it possible to set the upper limit at 30 km for

the altitude at which the bolide exploded. The main part of the meteoritic mass continued to move in its orbit after the explosion leaving “only a few minor pieces of its pre-terrestrial body” (Daubrée and Le Verrier 1864).

M. Leymerie (1864) examined a 211g stone that fell in Campsas and reported that the interior of the Orgueil stones exhibited a “stunning difference” as compared with ordinary stony meteorites: “*The broken surface reveals a dark charcoal colored substance so soft that it can be easily cut with a knife. One can even write with the fragments on a piece of paper. The knife cut creates a smooth and shiny surface which is an indication of fine, paste-like matter. Fragments placed in water disintegrate immediately*” This astonishing observation that the Orgueil CI1 meteorite stones immediately disintegrated into minute particulates when they came in contact with cold water was independently confirmed by Cloëz (1864a) and Pisani (1864). This behavior is similar to that which was previously observed with the Alais CI1 carbonaceous meteorite.

Cloëz (1864a) correctly recognized that the Orgueil meteorite was a breccia composed of microscopic particles cemented together by magnesium sulfate and other water soluble salts. When these salts dissolve in water, the tiny particulates that constitute the Orgueil meteorite are released from the matrix. He found that the Orgueil stones also disintegrate in alcohol, but that the dispersed particles released were not as finely divided and that rate of disintegration in alcohol is slower than in water. These important observations were recently confirmed at the NASA Astrobiology Laboratory using video optical microscopy and Environmental Scanning Electron Microscopy (ESEM) methods. Small samples of Orgueil meteorite stones were placed on a sterile silicon wafer and exposed to a droplet of sterile de-ionized water at 20 °C. Immediate and profuse effervescence was observed. Within a few minutes the samples were completely disaggregated into an assemblage of micron-sized particulates. After the water had evaporated a white residue was found on the silicon wafer substrate around the meteorite mineral grains and particulates. Energy Dispersive X-Ray Spectroscopy (EDS) analyses established that the residue was composed primarily of magnesium, sulfur, and oxygen, which is consistent with magnesium sulfate (Hoover, 2005a; Hoover, 2006a). The Orgueil silicate minerals are more properly designated as serpentine rather than peridotite. The dominant mineral (62.6 %) of the Orgueil meteorite is Chlorite [(Fe, Mg, Al)₆(Si, Al)₄O₁₀(OH)₈] of the clay phyllosilicates mineral group. The other major minerals of the Orgueil meteorite include: 6.7% Epsomite (MgSO₄·7H₂O); 6% Magnetite (Fe₃O₄); 4.6% Troilite (FeS), 2.9% gypsum (CaSO₄·nH₂O) and 2.8% Breunnerite (Fe,Mg)CO₃. Epsomite forms white veins in the meteorite. Epsomite is the most important evaporite mineral that cements the meteorite particulates together in the stones.

In 1868, Pierre Marcellin Berthelot (the famous French chemist who had previously shown in 1860 that all organic compounds contain Carbon, Hydrogen, Oxygen and Nitrogen) experimented with hydrogenation to explore the organic chemistry of the Orgueil meteorite. He discovered that the complex hydrocarbons in the Orgueil stones were analogous to carbonaceous substances of organic origin on Earth (Berthelot, 1868). It is now well known that the total organic content of CI1 and CM2 carbonaceous meteorites consists of 90-95% polymer-type organic matter that is insoluble in common solvents. Nagy (1975) reported that this substance is “*structurally not unlike coal or the aromatic-type kerogen that is the insoluble organic matter encountered in terrestrial sedimentary rocks.*” The complex polymer-like organic matter similar to kerogen in these carbonaceous meteorites is clearly indigenous and constitutes an important biomarker. In terrestrial rocks, Kerogen (as well as pristane, phytane, porphyrins and other biochemical fossils) have long been considered valid biomarkers. Kaplan (1963) reported that the Ivuna CI1 meteorite contained significantly larger quantities of pristane and phytane (diagenetic breakdown components of chlorophyll) than the Orgueil and Alais meteorites. These types of geochemical biomarkers comprise a standard tool for petroleum exploration. They are stable for geologically significant time periods (~ billions of years) and are undeniably biological in origin. The diagenetic and catagenetic processes that alter the original biochemicals are minimal and the basic carbon skeleton remains intact. For this reason, although functional groups (e.g., -OH, =O, etc.) are lost, the chemical structure of these highly stable and ancient biomolecular fossils remains recognizable.

1.4. The Ivuna CI1 Carbonaceous Meteorite

The Ivuna CI1 carbonaceous meteorite fell near Ivuna, Mbeya, Tanzania (8° 25'S, 32° 26'E) in southeast Africa at 5:30 P.M on December 16, 1938. Approximately 705 grams were recovered shortly after the stones were observed to fall. Clayton (1963) investigated carbon isotope abundances in the carbonates of both the Ivuna and Orgueil meteorites and found the Ivuna carbon isotopic values to be virtually identical to those of the Orgueil stones. The $\delta^{13}\text{C}$ value for these meteorites was approximately +60 per mil, which is dramatically different from the either abiotic or biogenic terrestrial carbon. The carbon isotope data provide conclusive evidence that the meteoritic carbon is extraterrestrial in origin and cannot be attributed to terrestrial biological contaminants. The Alais, Ivuna and Orgueil CI1 meteorites have also been found to contain chiral amino acids, nucleobases, pristine, phytane, porphyrins, spectacular magnetite framboids and platelets. They also contain well-preserved, remains of a wide variety of large filaments that are interpreted as the mineralized remains of cyanobacteria and other filamentous trichomic prokaryotes. HPLC studies of amino acids extracted from pristine interior pieces of the Ivuna meteorite resulted in the detection of β -alanine, glycine, and γ -amino-*n*-butyric acid (GABA) at concentrations ranging from \approx 600 to 2,000 parts per billion (ppb). Other α -amino acids such as alanine, α -ABA, α -aminoisobutyric acid (AIB), and isovaline are present in trace amounts ($<$ 200 ppb). Carbon isotopic measurements of β -alanine and glycine and their detection of racemic (D/L \approx 1) alanine and β -ABA were interpreted as indicating that these amino acids were of extraterrestrial origin. The amino acid composition of the CI1 meteorites is strikingly distinct from that of the Murchison and Murray CM2 carbonaceous meteorites. This has been interpreted as indicating that the CI1 meteorites came from different parent bodies than CM2 meteorites. These stones may be the remains of cometary debris.

Half a century ago, Claus and Nagy (1961) microscopically examined the Ivuna and Orgueil meteorites and found them to contain many forms that they originally interpreted as indigenous microfossils. After intense criticism, they designated these bodies as “organized elements” so as to not make any judgment as to their biogenicity. Since they had used standard palynological methods to dissolve the rock matrix in acids in order to extract acid-resistant microfossils, any unseen pollen contaminants (also acid insoluble) on the exterior surfaces of the meteorite would remain intact. They would also have been concentrated in the residue they analyzed. They failed to recognize a pollen grain and erroneously included an image of it in their original paper. This resulted in their work being discredited. It is still widely believed that all of the “organized elements” were either abiotic mineral grains or pollen. Subsequent studies by Rossignol-Strick and Barghoorn (1971) and Rossignol-Strick *et al.* (2005) have confirmed that “organized elements” type microstructures are present in the stones and clearly indigenous to these meteorites. There is no question that they are not pollen grains, but they have simple morphologies and it is difficult to determine whether they are abiotic or biogenic in origin.

1.5. The Tonk and Revelstoke CI1 Carbonaceous Meteorites.

Although the mineralogy and petrology of the Tonk and Revelstoke CI1 meteorites have been carried out, no data has been published on the organic chemistry or chemical or morphological biomarkers that may exist in these meteorites. Only very small samples were recovered for Tonk (7.7 g) and Revelstoke (1 g). No samples were available for this research. After the Tonk meteorite fell, it spent two years at an unknown location in India (Christie, 1913). The Revelstoke meteorite fell on a frozen lake in Canada and remained on the ice for almost two weeks before it was recovered (Folinsbee *et al.*, 1967). The Tonk and Revelstoke meteorites have both been shown to contain hydrated magnesium and calcium sulfates (Christie, 1913; Endress *et al.*, 1994). Larson *et al.* (1974) performed thermomagnetic analysis of all five CI1 meteorites known at the time and found the main phase of magnetite in Revelstoke to be essentially nickel-free Fe_3O_4 . This was in contrast to the other four CI1 meteorites, which all contained magnetite with nickel at 6% or less. Based on the saturation moments, they determined the weight percentage of magnetite in the CI1 meteorites to be: Alais ($5.3 \pm 0.4\%$); Orgueil, ($11.9 \pm 0.8\%$); Ivuna, ($12.2 \pm 0.9\%$); Tonk, ($9.4 \pm 0.6\%$) and Revelstoke, ($7.2 \pm 0.5\%$).

2. MATERIALS, INSTRUMENTS AND METHODS

2.1 Materials: The samples and sources of the CI1 carbonaceous meteorites studied were:

Ivuna CI1 Carbonaceous Meteorite

DuPont Meteorite Collection, Planetary Studies Foundation, Chicago

1 stone: (0.1 g). *Courtesy: Dr. Paul Sipiera*

Alais CI1 Carbonaceous Meteorite

Musée Nationale d'Histoire Naturelle, Paris

1 fragment (0.1 g) *Courtesy: Dr. Claude Perron*

Orgueil CI1 Carbonaceous Meteorite

Musée Nationale d'Histoire Naturelle, Paris

1 stone S219: (0.5 g) *Courtesy: Dr. Claude Perron*

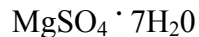
2 stones: (0.6 g & 0.3 g) *Courtesy: Dr. Martine Rossignol-Strick*

DuPont Meteorite Collection, Planetary Studies Foundation, Chicago

2 stones: (0.4 g & 0.1 g). *Courtesy: Dr. Paul Sipiera*

EDS elemental analyses of the Alais, Orgueil and Ivuna filaments revealed that many of them were carbonized sheaths permineralized or infilled with magnesium sulfate minerals similar to epsomite. To evaluate the morphology, chemical composition and size and size range of abiotic mineral structures that might have similar compositions, samples of native fibrous epsomite were obtained. To explore the possibility that abiotic epsomite crystals might mimic filamentous trichomic cyanobacteria or other filamentous trichomic prokaryotes, samples of native epsomite were obtained and their morphological and morphometric characteristics examined using the Hitachi FESEM and the FEI Quanta ESEM and FESEM and elemental composition evaluated using EDS. The native mineral samples studied were:

Native Fibrous Epsomite



Hot Lake, 6.5 km NW of Oroville, Washington – Fibrous Evaporite Sediment: (2 kg).

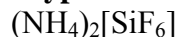
Courtesy: Brent Cunderla, USDI-Bureau of Land Management, Washington

Zaragoza, Spain - 1 stone: (21.5 g)

Courtesy: Keck Museum, Reno, Nevada

None of the epsomite crystals encountered had morphologies that could be confused with biological materials. They exhibited simple crystalline features and they were found to be in a wide range of sizes - whereas the sizes of microorganisms and biological remains are rigorously constrained by genetics.

Cryptohalite



Schoeller Mine, Kladno, Central Bohemia, Czech Republic

1 stone with many Cryptohalite crystals (68.5 g)

Courtesy: Mineralogical Research Co., San Jose, California

Several other terrestrial rocks have been investigated in collaboration with Academician Alexei Rozanov and Marina Astafieva of the Paleontological Institute of the Russian Academy of Sciences. Many of these were found to contain the permineralized and fossilized remains of acritarchs, coccoidal and filamentous trichomic cyanobacteria and purple sulfur bacteria, biofilms and biominerals. These included samples of phosphorites from Khubsugul, Mongolia, bauxites from Russia and Arkansas, oil shales, Shungites and Kukersites from Russia and Siberia, Ongeluk lavas and carbon leader from Gold Mines of South Africa, chimney stones from the Rainbow Deep Sea Hydrothermal Vent and Upper Archean (Lopian) rock from Northern Karelia.

Studies were also carried out using the same Environmental (ESEM) and Field Emission (FESEM) Scanning Electron Microscopes and EDS elemental analysis methods used in the study of the meteorites. The samples included many different genera and species of living, dead and fossilized organisms from native environments and pure cultures and isolates of known genera and species of cyanobacteria and other microbial extremophiles grown in our laboratory. This research was important since the ESEM and FESEM images are often dramatically different from the images obtained by visible light, phase contrast and dark field optical microscopy methods. Living and dead cyanobacteria and filamentous sulfur bacteria; algae, fungi, moss, wood, insects, bones, hair and tissue from Incan and Egyptian mummies and Pleistocene mammoths; diatoms and cyanobacteria from glaciers and deep Vostok Ice, benthic mats, living stromatolites and pure cultures.

The modern and ancient biological materials investigated included:

Modern Cyanobacteria

***Plectonema (Lyngbya) wollei*–Lake Gunterville, Al**

Richard B. Hoover - Collected in May, 2004 (Living Environmental Sample)

***Lyngbya (Leptolyngbya) subtilis* – Lake Michigan,**

Courtesy: Ann St. Amand, Phycotech, Inc. (Fixed Environmental Sample)

***Oscillatoria lud* –UTex Collection LB 1953**

F. T. Haxo - Deposited October, 1972 in UTex Collection as C-43 (Living Axenic Culture)

***Arthrospira platensis* – Carolina Biological Supply (Living Axenic Culture)**

***Calothrix sp.*–Little White River, Oregon,**

Courtesy: Ann St. Amand, Phycotech, Inc. (Fixed Environmental Sample)

Living Microbial Extremophiles

***Carnobacterium pleistocenium* str. FTR-1^T; Fox Tunnel, Alaska**

Richard B. Hoover-Coll. May, 2000 - Pleistocene ice (Living Axenic Culture; Type Strain)

Dried Herbarium Material

***Bangia quadripunctata*, Lyngbye–Collected 5/20/1816 Hoffman Bang, Hals Peninsula**

Courtesy: Dr. Walter van den Bergh, Henri van Heurck Museum, Antwerp, (Dried Type)

Fossilized Cyanobacteria

Upper Archean (Lopian) tufa-genic rocks (2.8 Ga) of Northern Karelia

Courtesy: Dr. Alexei Yu. Rozanov, Paleontological Institute, RAS, Moscow

Hair and Tissues of Egyptian Mummies (2 Kya & 5 Kya) and Mammoths (32 Kya & 40 Kya)

Lyuba Mammoth Tissue & Stomach Milk Samples Courtesy: Dr. Daniel Fisher, Univ. Michigan

Cambrian Trilobites

Perinopsis interstricta & *Perinopsis pygidia* – Cambrian (505 Mya) Wheeler Shale, Utah

Collected by: Richard B. Hoover

2.2. Instruments: The Environmental and Field Emission Scanning Electron Microscopes used were:

ElectroScan Environmental Scanning Electron Microscope (ESEM)

- Secondary Electron Detector (SED); Water vapor (10 Torr vacuum) 90-100,000X
- Noran EDS (Z> Boron)

Hitachi S-4100 Field Emission SEM (FESEM)

- Cold cathode field emission electron gun; 20 - 300,000X;
- Secondary Electron Detector (SED) and Backscattered Electron Detector (BSED);
- KEVEX EDS - Lithium Drifted Silicon detector (Z>Boron)

Hitachi S-3700N Variable Pressure Scanning Electron Microscope

- Tungsten emitter electron gun; 5 - 300,000X; SED & BSED;
- 4 Pi EDS - Silicon Drifted Silicon Detector (Z>Boron)

FEI Quanta 600 (FESEM and ESEM)

- Simultaneous SED and BSED images; 5 - 300,000X
- 4 Pi EDS - Lithium Drifted Silicon detector (Z>Boron)

2.3 Methods: In insure the integrity of the study strict protocols and methods were followed:

In order to exclude the possibility that coating artifacts could be responsible for any of the possible microfossils detected, this research was confined to the study of *uncoated* interior surfaces of freshly fractured fragments of the meteorites. To protect from post-arrival biological contamination effects, all tools, sample holders and stubs were flame sterilized and new microelectronic quality silicon wafers were used as negative controls. To provide additional protection of the samples from biological contamination, for long-term storage the bulk meteorite samples were stored in sealed vials at -80 °C. After the samples had been fractured and the meteorite fragments mounted on electron microscopy stubs and examined, they were subsequently maintained in labeled sealed containers in desiccator cabinets or in sealed vials in the freezer. The fusion crust and old cracks in the stones were carefully avoided. The meteorite samples were placed in the ESEM or FESEM instrument chamber (with the fresh fracture surface up) immediately after the stones were fractured and mounted on the stub. The chamber was then closed and pumped down.

All solvents, acids or other liquids were strictly avoided. Acids and solvents had been commonly used by Nagy, Claus, Timofeyev and many other early investigators in order to extract “acid resistant microfossils” from the host rock. However, this method sometimes resulted in the inadvertent contamination of the sample with acid resistant modern pollen grains that may have been on the external surface or in old fissures within the stones. It should be pointed out that only one samples of all the meteorites studied was found to be seriously contaminated. A single Murchison sample from the Field Museum was found to be heavily contaminated with fungal filaments. They had invaded old cracks in the fusion crust and were not initially noticed. However, many filaments were observed to be above the surface of the stone. The filaments often moved or were damaged when exposed to the electron beam. EDS measurements showed that they had levels of Nitrogen (2-6% atomic) consistent with modern biology rather than with fossilized remains. Visible light imaging showed they were pale blue in color. These filaments provided valuable information to help understand how to distinguish modern bio-contaminants from valid fossils. During extensive studies carried out since 1996 at the NASA/Marshall Space Flight Center and at the Paleontological Institute of the Russian Academy of Sciences in Moscow, not a single pollen grain has been encountered in interior surfaces of the carbonaceous meteorites. While fingerprints, pollen grains, fungi, bacteria, laboratory oils and other contaminants could be encountered on the fusion crust or exposed in old cracks in the stones, the widely accepted belief that the interior surfaces of carbonaceous meteorites are seriously contaminated by modern

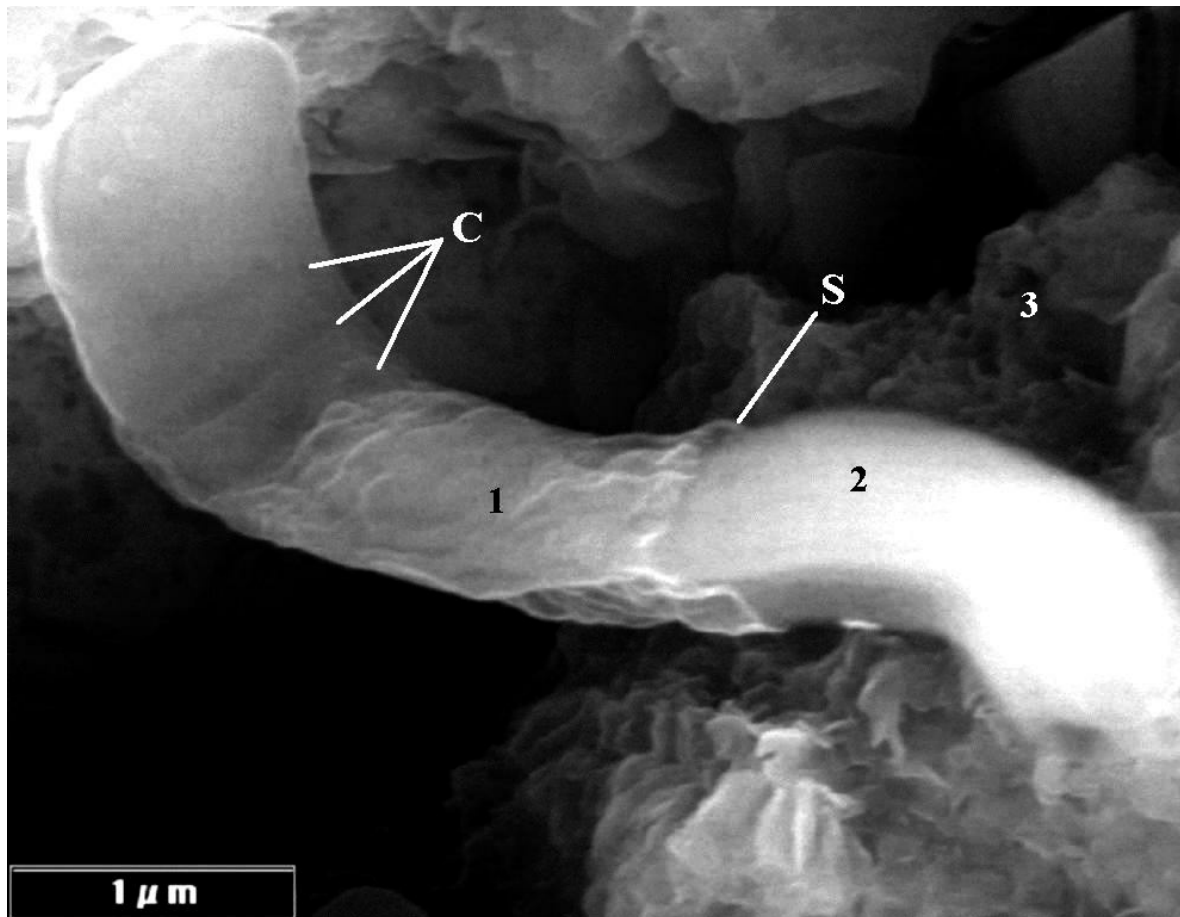
bacteria, fungi, or pollen is not supported by observational data. Hence, it is extremely important that studies of biomarkers such as amino acids, nucleobases, complex organic chemicals and microfossils should be rigorously restricted to interior regions of the freshly fractured stones.

3. OBSERVATIONAL RESULTS AND INTERPRETATIONS

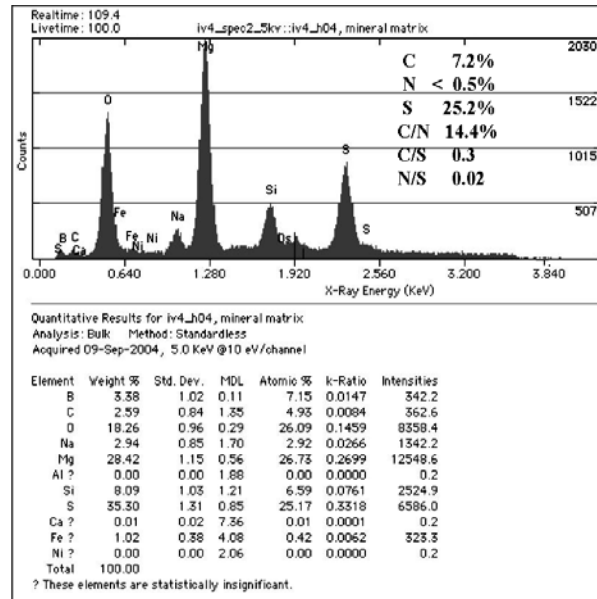
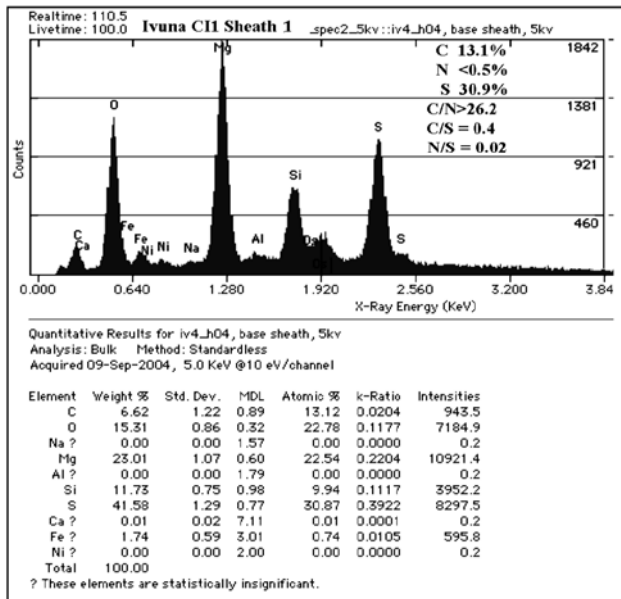
Field Emission Scanning Electron Microscopy (FESEM) studies of the interior surfaces of freshly fractured CI1 carbonaceous meteorites carried out at NASA/MSFC resulted in the detection of a diverse suite of large and complex filamentous microstructures embedded in the matrix of carbonaceous meteorites. Energy dispersive x-ray analysis of these structures reveals that these filaments are permineralized with minerals rich in magnesium and sulfur. Most of the filaments are encased within a carbon-rich external envelope. Images and EDS elemental data for several selected filaments are presented. For convenience, the interpretations of the images are provided in the same section with the figures.

3.1. Images and EDS Spectra of Filaments in the Ivuna CI1 Carbonaceous Meteorite.

Figure 1 provides images and Energy Dispersive X-Ray Spectroscopy elemental data for filaments found embedded in the Ivuna CI1 carbonaceous meteorite.

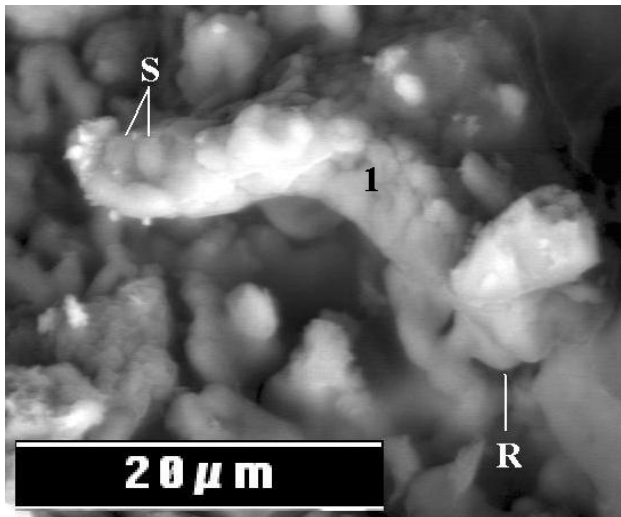


a.

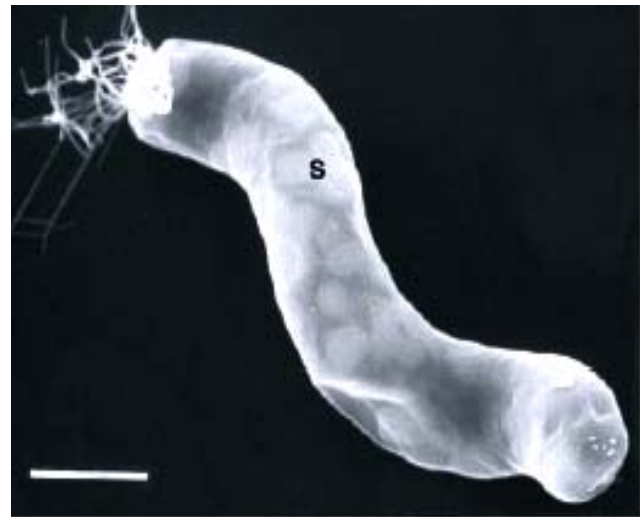


b.

c.



d.



e.

Fig. 1.a. Ivuna CII meteorite filament (0.8 μm diameter) with dark lines C, partially encased in thin carbon-rich sheath. **b.** EDS data of the filament sheath at spot 1 shows biogenic elements N and P (<0.5%). It is enriched in C (13.1%) as compared with nearby meteorite matrix (C 7.2%) at spot 3; **d.** FESEM Backscattered Electron image of an Ivuna filament with N<0.5%; sulfur-rich globules S and rounded terminus R similar in size, morphology and internal composition to **(e.)** giant bacterium “*Titanospirillum velox*” with sulfur (S) globules collected from *Microcoleus* mat of Ebro Delta, Spain. (Scale bar = 5 μm). *Ivuna Meteorite Courtesy: Dr. Paul Sipiera, Dupont Meteorite Collection, Planetary Studies Foundation, Chicago, Illinois. Fig. 1.e. Courtesy: Dr. Riccardo Guerrero*

Fig. 1.a is a FESEM image of a thin uniseriate filament that is flattened at the terminal end. The filament is cylindrical in the lower portion embedded in the meteorite rock matrix. This small, undulatory filament (diameter 0.7 to 1.0 μm) is rich in C, Mg, and S and has no detectable level of nitrogen. The filament is only partially encased within a broken and very thin carbonaceous sheath. The EDS elemental data is shown for **spot 1** on the thin sheath (**Fig. 1.b**) and for **spot 3** on the nearby mineral matrix (**Fig. 1.c**). The sheath has higher carbon content than the matrix or exposed trichome and the biogenic elements N and P are below the 0.5% (atomic) detection limit of the instrument. **Fig. 1.d** is a FESEM image of 5 μm diameter X 25 μm long

spiral filament in the Ivuna meteorite. It contains white globules that are enriched in sulfur as compared with the rest of the filament and the meteorite matrix. A tuft of fine fibrils is visible at the left terminus of the filament and the terminus at the lower right is rounded. **Fig. 1.e** is a FESEM Backscattered Electron image of an Ivuna filament with sulfur-rich globules S and rounded terminus R.

3.1.1. Interpretation of Images and EDS Data for the Ivuna Filaments.

The flattened embedded filament in **Fig. 1.a** is interpreted as the permineralized remains of a partially ensheathed, uniseriate and undulatory trichomic prokaryote. The measured diameter (0.7 - 1.0 μm) as shown by the scale bar of this calibrated FESEM image and the detailed morphology of this Ivuna filament is consistent with some species of the smaller filamentous cyanobacteria. The dark lines **C** near the terminus of the sheath are interpreted as cross-wall constrictions. These are often seen as faint transverse lines in FESEM images, even in images obtained with living cyanobacteria. In this image it is possible to observe what appears to be an extremely thin sheath **S** that has been broken and which covers only the upper portion of the trichome. This interpretation is supported by EDS elemental data indicates the carbon content (13.1 %) at **spot 1** on the thin sheath (**Fig. 1.b**) that is slightly higher than for **spot 2** (9%-not shown) on the exposed trichome but notably higher than for **spot 3** (7.2%) on the nearby mineral matrix (**Fig. 1.c**). Although the sheath has higher carbon content and the less stable biogenic elements N is below the 0.5% (atomic) detection limit of the instrument. The trichome appears to have been completely replaced by infilling magnesium sulfate minerals. The size and morphology of this filament is consistent with those of undulatory trichomic filamentous cyanobacteria such as *Spirulina subtilissima* (filaments 0.6 - 0.9 μm diameter) and *S. laxissima* (0.7 to 0.8 μm in diameter). These cyanobacteria have not been reported as possessing a sheath, but the remains of this extremely thin sheath would have been very difficult to detect, even in a FESEM image, except for the fact that it was broken. It is so thin that it would also be almost impossible to discern by visible light microscopy methods. There also exist very small species of the genus *Limnothrix* that are both undulatory in nature and possess facultative sheaths. Some groups of ensheathed filamentous anoxygenic phototrophic bacteria (photosynthetic flexibacteria) also have a thin sheath and exhibit gliding motility. These include filamentous bacteria of the Phylum Chloroflexi (such as the thermophilic species *Chloroflexus aurantiacus*) which has a thin sheath and trichomes $\sim 0.8 \mu\text{m}$ diameter. Some photoautotrophic bacteria oxidize hydrogen sulfide and deposit it externally as sulfur (e.g., *Oscillochloris trichoides*) and have trichomic filaments ~ 0.8 to $1.4 \mu\text{m}$ diameter.

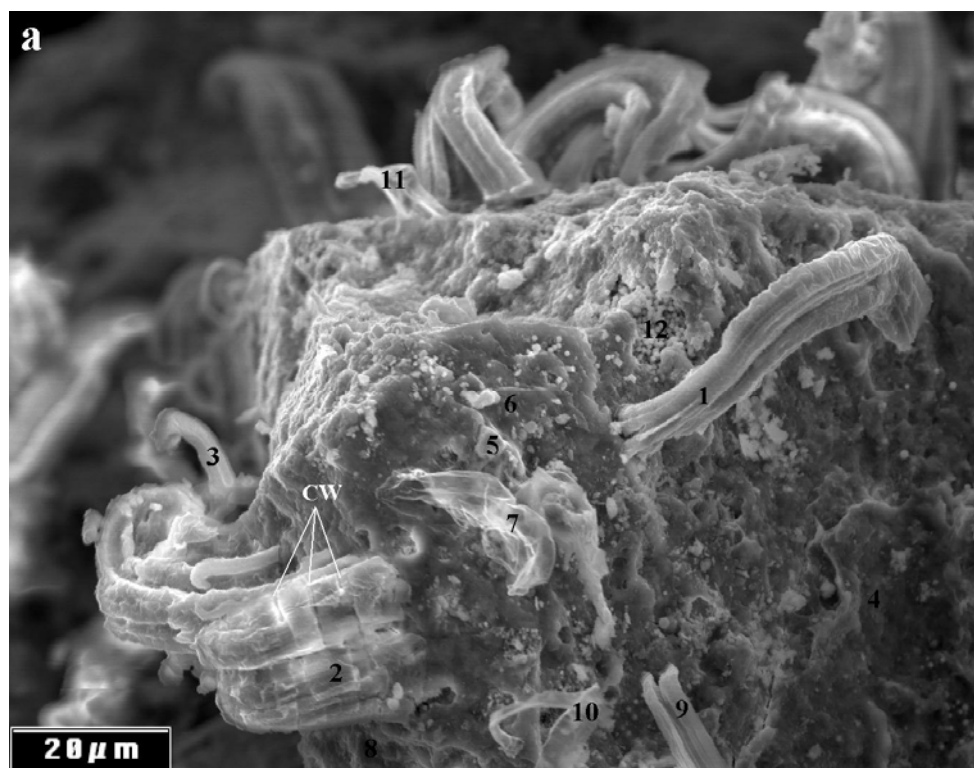
Figure 1.d. is a FESEM Backscattered Electron image of an Ivuna filament sulfur-rich globules S and rounded terminus R. This filament was found embedded in a freshly fractured surface of the Ivuna meteorite. It exhibits a complex suite of features that are very similar to those observed in SEM images of the novel bipolar lophotrichous gram-negative bacterium "*Titanospirillum velox*" (**Fig. 1.e**) which was described by Guerrero *et al* (1999). "*Titanospirillum velox*" is a very large mat-forming bacterium with 3–5 μm diameter X 20–30 μm long filaments. It was collected from a mud sample beneath a *Microcoleus chthonoplastes* mat in the Ebro Delta in Tarragona, Spain. "*T. velox*" swims very rapidly (10 body lengths/sec) with spiral motility, propelled by the lophotrichous tuft of flagella at the cell terminus. It has intracellular elemental sulfur storage globules are seen as white spots in this Scanning Electron Microscope image. This extremophile was grown only in mixed culture with other bacteria, which must be the reason that this unusual organism has not yet been validated. The Bacteriological Code rules of nomenclature requires that a validly named prokaryotic microorganisms must be isolated and grown in *pure culture* and the designated type stain must be deposited in two international culture collections in different countries before the genus and species names can be validated (Tindall *et al.*, 2006). The living organism and the meteorite filament are very similar in length, diameter and spiral configuration. Both have a tuft of small filaments at one pole and rounded end at the other along and internal sulfur globules distributed along the axis of filament. There is also no detectable nitrogen

in this Ivuna filament, which provides evidence that it is indigenous and not a modern biological contaminant. Clearly, there are several morphotypes of known terrestrial microorganisms similar in size and morphology to both of these filaments that were found embedded in the Ivuna meteorite, so it is not possible to ascertain their affiliation to even the genus level. However, they are all undeniably biological in nature, and the absence of nitrogen in the meteorite filaments establish that they could not have invaded the Ivuna meteorite after it entered the Earth's atmosphere on December 16, 1938.

3.2 Images and EDS Spectra of Filaments in the Orgueil CI1 Carbonaceous Meteorite.

Figure 2.a. is a low magnification (1000X) Secondary Electron Detector (SED) FESEM image of a freshly fractured fragment of the Orgueil CI1 meteorite. This fragment is densely populated with several different types of embedded filaments and electron transparent sheaths. Although the field of view of this image is small (~120 μm wide) a wide variety of diverse filamentous microstructures are present. The filaments and sheaths are numbered with the numbers located at the site where the EDS elemental spot data were recorded.

Figure 2.b. shows a 2D X-ray elemental map of this region of the Orgueil meteorite. The large image in the upper left corner is the Backscatter Electron Detector (BSED) image in which the bright spots are high Z elements where clusters and crystallites of magnetite, iron and nickel are concentrated. Other images in the map reveal where relative concentrations Oxygen, Silicon, Magnesium, Sulfur, Iron, Nitrogen; Calcium, and Aluminum are located. The major filaments and sheaths are clearly seen as bright features in the Carbon, Oxygen, Magnesium and Sulfur maps. They appear as dark features in Silicon, Iron, and Nickel maps due to the relatively higher content of these elements in the surrounding Orgueil meteorite rock matrix. In general, the filament and sheath structures are not discernible in the Nitrogen, Phosphorus and Sodium maps, although Filament 1 and sheath 7 can be faintly seen in the Nitrogen map. Empty sheath 7 is wrinkled and electron transparent. It has a relatively high (47%) content of Carbon. Sheath 7 is very unusual. It is one of few filaments found in the Orgueil meteorite to have detectable levels of Nitrogen (1%) and Phosphorus (0.8%).



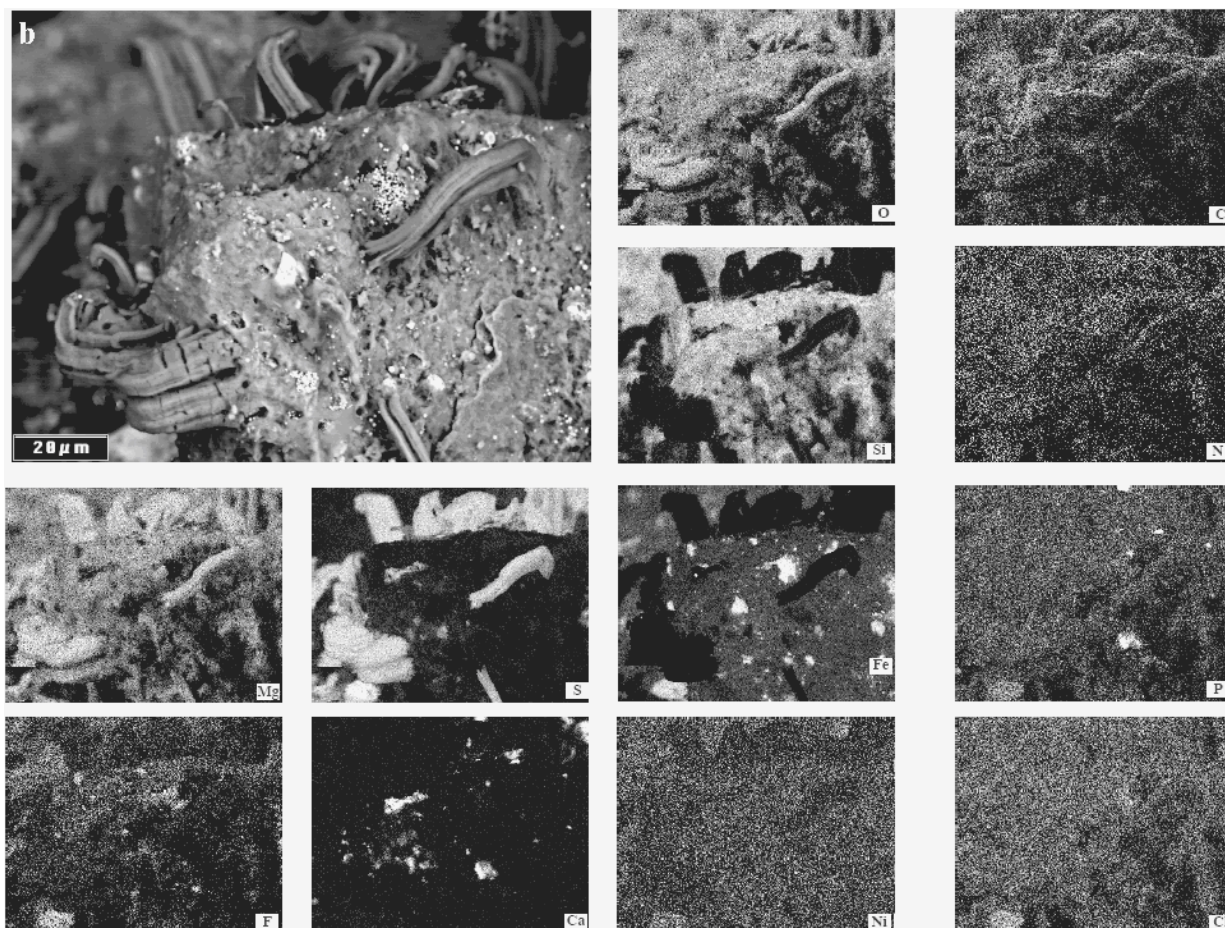


Figure 2.a. Hitachi FESEM Secondary (SED) 1000X image of multiple filaments and sheaths in the Orgueil meteorite. **b.** Backscatter (BSED) image and 2-D x-ray maps show relative distribution of elements O, C, Si, N, Mg, S, Fe, P, F, Ca, Ni and Cl in the filaments as compared with the associated meteorite matrix.
Orgueil Sample Courtesy: Dr. Paul Sipiera, DuPont Meteorite Collection, Planetary Studies Foundation, Chicago

3.1.1 Interpretation and Discussion of Images and EDS Data of the Orgueil Filaments.

Filaments **1** and **2** of **Fig. 2.a** are observed to have sheaths with longitudinal striations that run the length of the filaments. This is characteristic of multiserial cyanobacterial filaments in which multiple parallel oriented trichomes are enclosed within a common homogeneous sheath. These filaments are observed to be attached to and embedded in the Orgueil meteorite rock matrix. The end of filament **1** becomes slightly wider ($\sim 10 \mu\text{m}$) where it enters the rock matrix and it appears to contain four internal trichomes, each with a diameter $\sim 2.5 \mu\text{m}$. Filament **2** is considerably larger ($\sim 20 \mu\text{m}$ dia.) and the longitudinal striations suggest it contains ~ 5 trichomes, each with diameters $\sim 4 \mu\text{m}$ /trichome. Faint transverse lines orthogonal to the long axis of filament **2** are interpreted as cross-wall constrictions and designated **CW**. The longitudinal striations of the long filament **1** and the shorter, curved filament **2** are interpreted as indicating these are multiserial filaments consisting of a bundle of multiple parallel trichomes encased within a common sheath. If the transverse striations **CW** of filament **2** are cross-wall constrictions, this would indicate that the internal cells within each trichome are $\sim 4 \mu\text{m}$ in length and hence they are isodiametric. Therefore, filament **2** is interpreted as being composed of trichomes made up of spherical or cylindrical isodiametric cells of $4 \mu\text{m}$ diameter. This interpretation is consistent with several morphotypes of undifferentiated filamentous cyanobacteria of the Order

Oscillatoriacea. This is consistent with several genera and species within this common cyanobacterial order. These include representatives of the genus *Microcoleus* Desmazières ex Gomont (*Form Genus VIII. Microcoleus* Desmazières 1823) (Castenholz, Rippka & Herdman, 2001; Boone *et al.*, 2001). Reproduction within the order Oscillatoriacea occurs by trichome fragmentation and the production of undifferentiated short trichome segments (hormogonia) by binary fission of the cells in one plane at right angles to the long axis of the trichomes. The small solitary uniseriate filaments **3** and **4** may be interpreted as morphotypes similar to members of the genus *Trichocoleus* Anagnostidis. This genus was separated from the genus *Microcoleus* on the basis of cell size and morphology. Filament **4** is a 2 μm diameter hook-shape filament with a narrowed terminus. Several species of the genus *Trichocoleus* have filaments that are typically in the 0.5 μm to 2.5 μm diameter range (Wehr and Sheath, 2003, pg. 136). Energy Dispersive X-Ray Spectroscopy (EDS) spot spectral data were obtained on the meteorite rock matrix as well as on all of the numbered filaments and sheaths at positions where the numbers are located in the FESEM image.

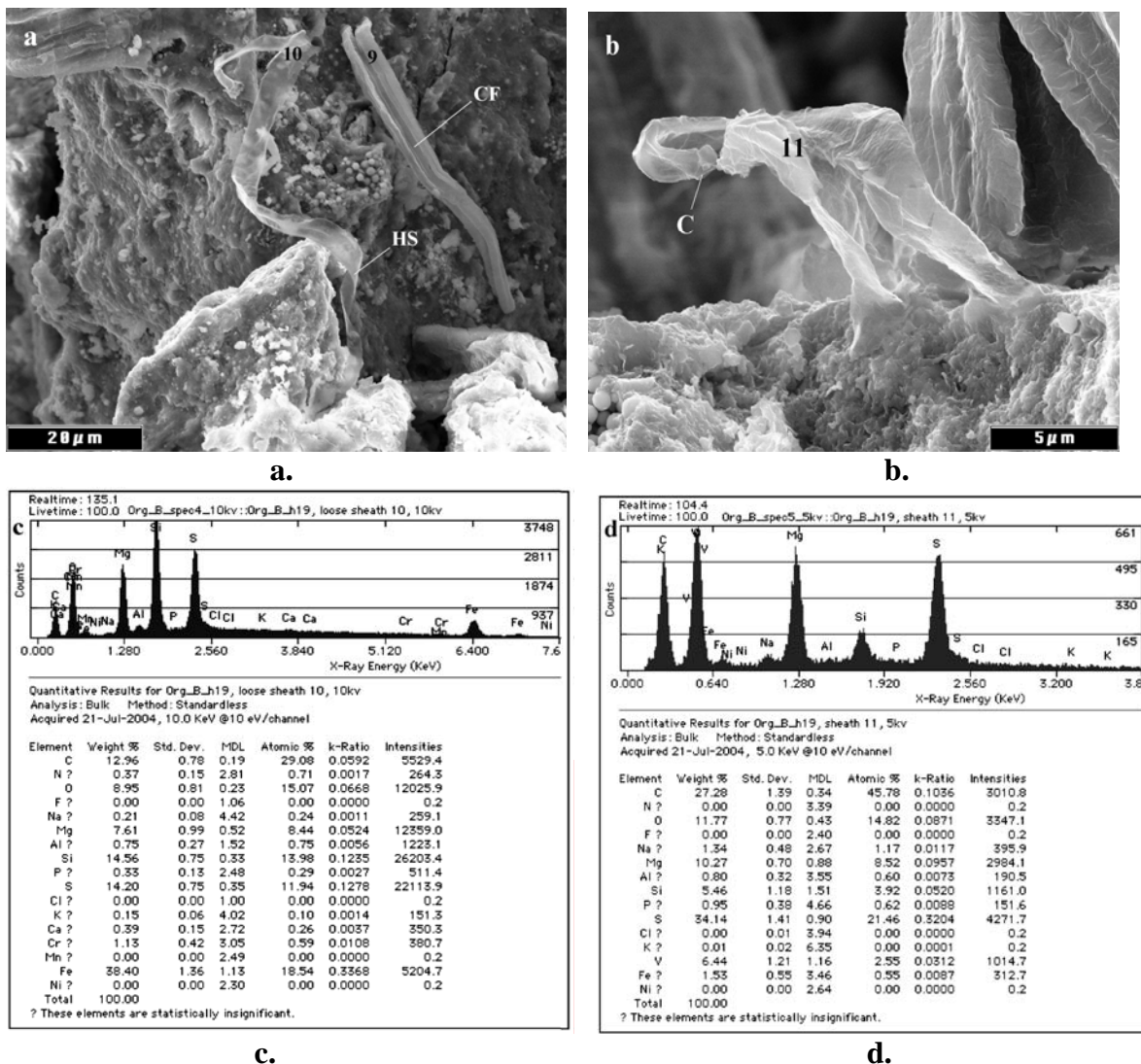


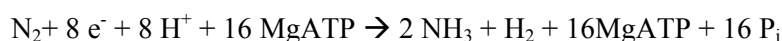
Figure 3. Hitachi FESEM images at 1500X of **a.** collapsed filament **9** and helical coiled empty sheath **10** and **b.** 6000X image of filament **11** showing hook and calyptra or conical apical cell. **c.** EDS spot spectra show elemental compositions **c.** of loose sheath **10** (C 29.1%; N=0.7%) and **d.** sheath **11** (C 47.8%; N<0.5%).
Orgueil Sample Courtesy: Dr. Paul Sipiera, DuPont Meteorite Collection, Planetary Studies Foundation, Chicago

Figure 3.a is a 1500X FESEM SED enlarged image showing the collapsed Filament **9** which is just to the right of a hollow, flattened, twisted and folded sheath **10**. Sheath **10** is 4.6 µm in diameter and it is folded at the top where the EDS spectral data were taken. The flattened portion of Sheath **10** forms a spiral coil near the base where it is attached to the meteorite matrix is similar to helical coiled sheath of *Phormidium stagninum* shown in <http://www.cyanodb.cz//Phormidium/Phormidium.jpg> at the top of the illustration. This type of flattened, coiled hollow sheath is often seen in other genera and species of filamentous cyanobacteria and hence does not constitute a unique diagnostic feature. **Figure 3.b.** provides a much higher magnification (6000X) image of Sheath **11**, which is visible at the top of **Fig. 2.a**. Sheath **11** is a tapered and hooked form with a conical terminal cell or calyptra at the apex. It is 8.5 µm wide where it emerges from the rock matrix and it tapers to 1.5 µm diameter just after the sharp hook.

Figure 3.c. is a 10 keV EDS spectrum taken at spot **10** in the fold of Sheath **10**. It shows detection of low, but measurable level of both Nitrogen (0.7%) and Phosphorus (0.3%) and higher levels of Iron (19%) and Silicon (14%), that may partially be print-thru from the meteorite matrix that lies beneath this electron transparent and carbon-rich sheath. The EDS spectrum at 5 keV for spot **11** on sheath **11** as is shown in (**Fig. 3.d**) reveals this flattened sheath to be highly carbonized (Carbon - 48% atomic), This small filament appears as a bright feature in the carbon map of (**Fig. 2.b**) and as a dark shadow in the Magnesium and Sulfur maps where it crosses in front of the large filaments that are much more heavily mineralized with magnesium sulfate. Filament **11** is also sulfur-rich (21% S), but has Nitrogen below the level of detectability (< ~0.5%).

3.2. Orgueil Filaments with Differentiated Heterocysts

Cyanobacteria are primarily aerobic oxygenic photoautotrophic aquatic microorganisms, but the diazotrophic cyanobacteria are capable of using N₂ as their sole source of nitrogen for growth. Several genera and species of the cyanobacterial orders *Nostocales* and *Stigonematales* use specialized cells (called “heterocytes or “heterocysts”) to fix atmospheric nitrogen (Fogg, 1949, Stewart *et al.*, 1969, Fay *et al.*, 1968). Nitrogen fixation is an unambiguously biological process that is absolutely crucial to all life on Earth. Although nitrogen comprises almost 78% of our atmosphere, it is completely useless to life in its relatively inert molecular form. The biological process of nitrogen fixation occurs by the reduction of gaseous nitrogen molecules (N₂) into ammonia, nitrates, or nitrogen dioxide. Nitrogen is converted into a useable state by nitrogen fixing bacteria such as the heterocystous cyanobacteria. Nitrogen fixation is the process by which the nitrogenase enzyme complex catalyzes the reaction:



Cyanobacteria play a crucial role in nitrogen fixation by converting gaseous dinitrogen from the atmosphere into ammonia NH₄⁺, which can subsequently be converted to nitrite or nitrate ions by nitrifying bacteria. The nitrogen content of living cyanobacteria can be as high as ~10% to 15% by weight. A nitrogen deficiency immediately affects the amount of phycobiliproteins and consequently their photosynthetic light harvesting efficiency. The nitrogenase enzymes that fix nitrogen are extremely sensitive to oxygen. To protect these enzymes from the poisonous oxygen that they are liberating by the photosynthetic process, some species of cyanobacteria encase these enzymes in the highly specialized thick-walled cells known as heterocysts. Some *Anabaena* species provides additional protection by lack of Photosystem 2 in heterocysts (Donze *et al.* 1972).

Heterocysts are encountered in cyanobacterial species belonging to the Orders *Nostocales* and *Stigonematales*. Heterocysts are never present in any species of the other three cyanobacterial orders: *Chroococcales*, *Oscillatoriales*, and *Pleurocapsales*. Furthermore, heterocysts have never been observed in any of the other known trichomic prokaryotes or filamentous sulfur bacteria. Therefore, the detection of heterocysts provides direct evidence that the filaments are not only unambiguously biological but also that they belong to one of these two orders of cyanobacteria. The presence of heterocysts rules out the possibility

that the filaments can be morphotypes of trichomic sulfur bacteria or any other group of filamentous trichomic prokaryotes. The presence or absence of heterocysts and their location, configuration, and proximity to akinetes is a critical diagnostic tool for recognition and classification of cyanobacterial taxa.

Figure 4.a. is a FESEM image of permineralized remains of embedded filaments found in a freshly fractured sample of the Orgueil CII carbonaceous meteorite. Several polarized and tapered filaments with diameters ranging from ~ 1 to 2.5 μm are seen in close proximity to each other. Some of the filaments are fluted and adorned with nodules. They all exhibit smooth basal heterocyst (smooth oval cell) at the base where the filament is attached to the meteorite matrix. These smooth ovoid cells are interpreted as basal heterocysts such as are well known in morphotypes of species of cyanobacteria of the genera *Calothrix* and *Rivularia*. It is noted that most of the known species of the genus *Calothrix* are larger in diameter than the filaments seen in this image. However, **Fig. 4.b.** is a FESEM image of a filament of a modern cyanobacterium (provided by Dr. St. Amand of Phycotech, Inc.) of the genus *Calothrix* from the White River, Washington. This very small (and as yet undescribed) species of *Calothrix* has a diameter ~ 0.8 μm and ovoid basal heterocysts.

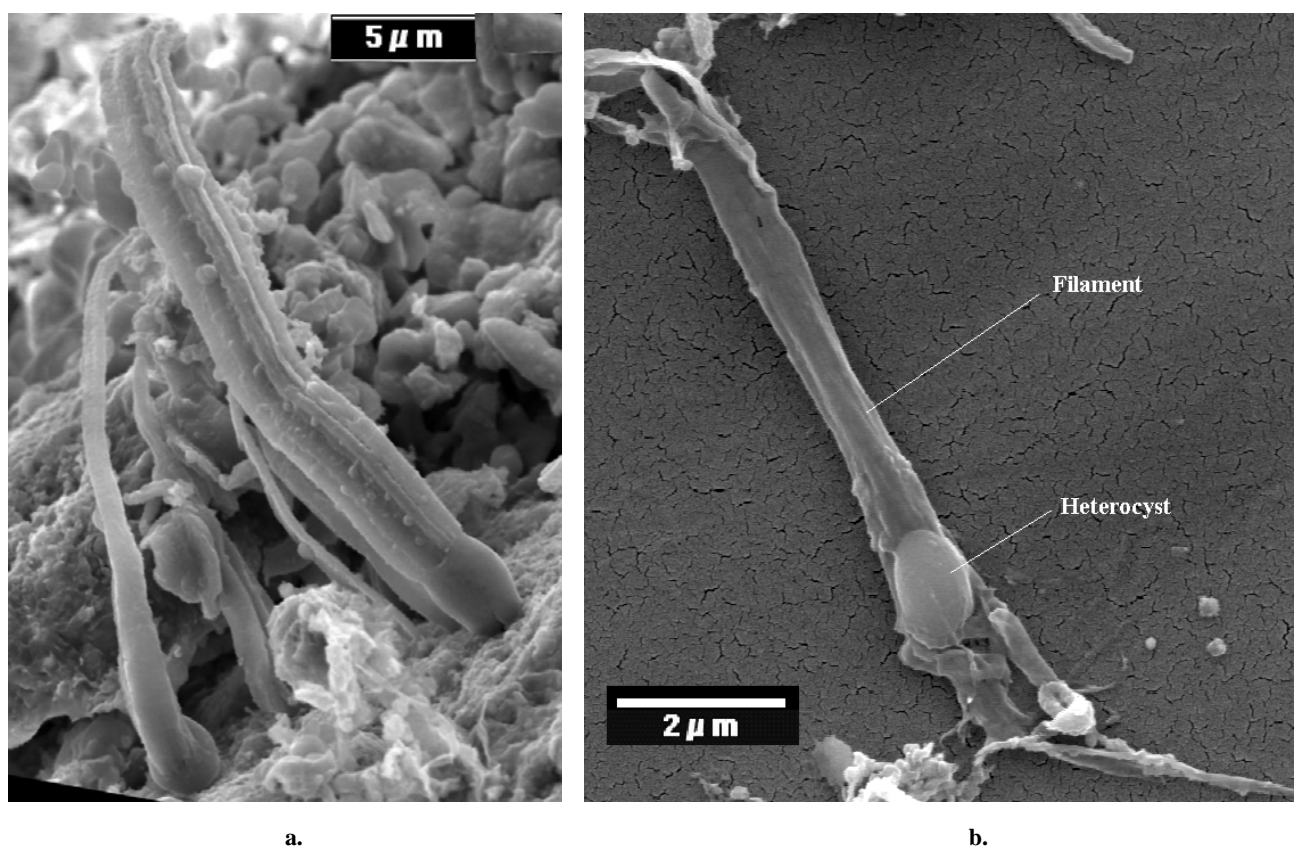


Figure 4.a. FESEM image of permineralized remains in the Orgueil meteorite of polarized tapered filaments (diameter ~ 1 to 2.5 μm) with recognizable heterocysts interpreted as morphotypes of the cyanobacterium *Calothrix spp.* and. **b.** living filament of *Calothrix sp.* with a diameter ~ 0.8 μm and a basal heterocyst from the White River, Washington. *Orgueil Sample Courtesy: Dr. Paul Sipiera, DuPont Meteorite Collection, Planetary Studies Foundation, Chicago Recent Calothrix Sample Courtesy: Dr. Ann St. Amand, Phycotech, Inc.*

Figure 5.a is a Hitachi S4100 FESEM image of helical coiled polarized filament in the Orgueil meteorite. The filament has a conical apex (<1.3 μm) at left end and a bulbous (2.3 μm diameter) heterocyst at the other terminus. The size and morphological features of this filament are characteristic of morphotypes of cyanobacterial species of the genus *Cylindrospermopsis*. **Fig. 5.b.** is an image of a 2.5 μm diameter filament

embedded in Orgueil. This filament has a 4.7 μm diameter bulbous terminal heterocyst and is interpreted as a morphotype of cyanobacteria of the genus *Tolypothrix*. In **Fig. 5.c.**, an image of a living Nostocacean cyanobacterium *Tolypothrix distorta* (grown in pure culture at NASA/NSSTC) is shown for comparison.

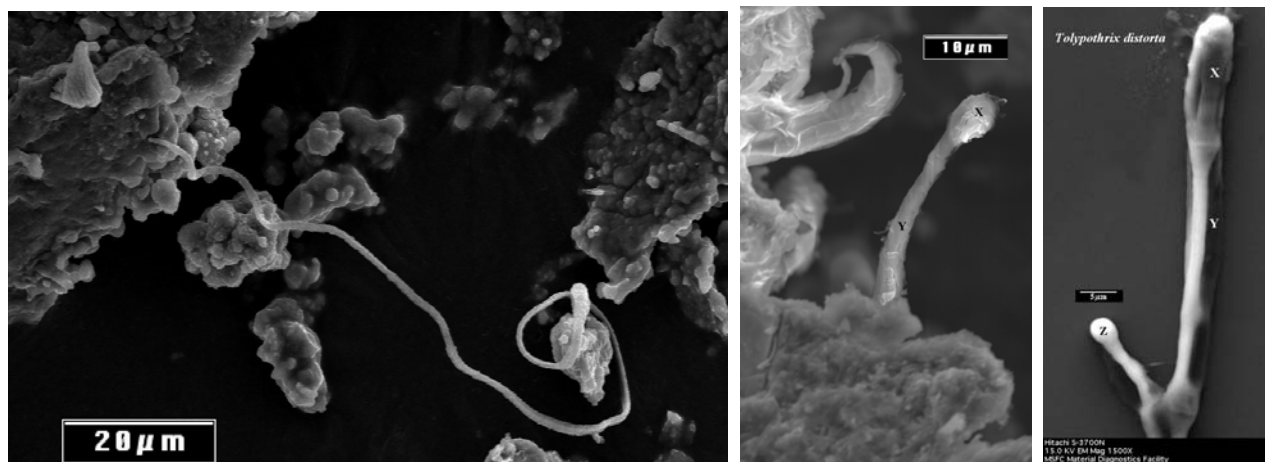


Figure 5.a. Long sinuous, helical coiled and polarized filament with conical apex (<1.3 μm) and terminal heterocyst in the Orgueil meteorite (interpreted as morphotype of the cyanobacterium *Cylindrospermopsis* sp.) and **b.** short embedded filament in Orgueil with apical heterocyst compared with **c.** FESEM image of living *Tolypothrix distorta* grown in pure culture at the NASA/NSSTC Astrobiology Laboratory.

Orgueil Meteorite Sample Courtesy: Dr. Martine Rossignol-Strick, Musée Nationale d'Histoire Naturelle, Paris

It is well established that many species of cyanobacteria are extremely resistant to desiccation. However, they do not carry out active growth and mat building when they are in a dried state. It has been understood since 1864 that Orgueil and other CII carbonaceous meteorites are micro-regolith breccias. They are comprised of minute mineral grains and kerogen globules cemented together by water-soluble salts and are quickly destroyed by exposure to liquid water. Therefore, it is clear that none of the CII carbonaceous meteorite samples could have ever been submerged in pools of liquid water after they arrived on Earth. Liquid water is needed to sustain growth of these large photoautotrophic trichomic filamentous prokaryotes. Liquid water and water substrate interfaces are required for the formation of benthic cyanobacterial mats and cyanobacteria with basal heterocysts such as are seen in these images. These stones could not have been immersed in liquid water such as would be needed for modern cyanobacteria to grow after the meteorites arrived on Earth, or the stones would have been destroyed. Many of the filaments shown in the figures are clearly embedded in the meteorite rock matrix. Therefore, the cyanobacterial filaments found in these meteorites could not have grown there after the meteorites arrived on Earth. Hence, they are interpreted as the indigenous remains of microfossils that were present in the meteorites when they entered the Earth's atmosphere.

This interpretation is also supported by EDS elemental analyses that have been carried out on the filaments, the meteorite rock matrix and living and fossil cyanobacteria and ancient biological materials. The filaments in the CII carbonaceous meteorites have elemental compositions consistent with the composition of the rock matrix. However, they have very different compositions from modern biological materials. Living or recently dead cyanobacteria and other extremophiles have nitrogen levels between 2 and 18% atomic. However, Nitrogen is typically below the 0.5% level of detectability of the EDS. In addition, modern biological materials are usually damaged by exposure to the focused FESEM electron beam during EDS analysis of small spots. This beam damage behavior was not observed in the Orgueil filaments or in any of the Devonian, Cambrian, or Archaean fossils investigated. The Nitrogen level and the C/N and C/S ratios of the meteorite filaments are very similar to coal, kerogen and ancient fossils but very different from living biological matter.

NITROGEN (% Atomic)

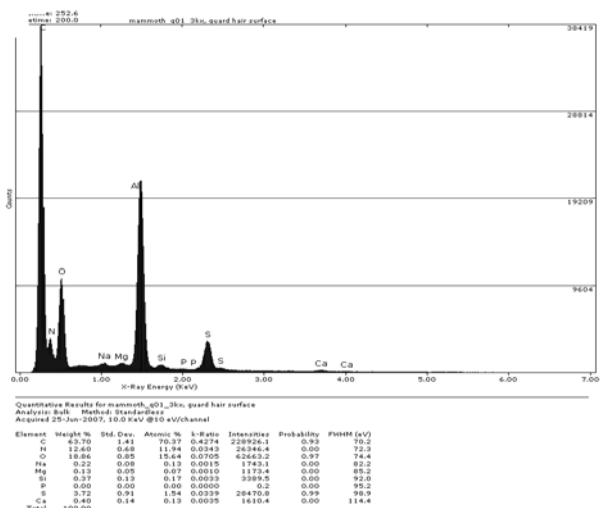
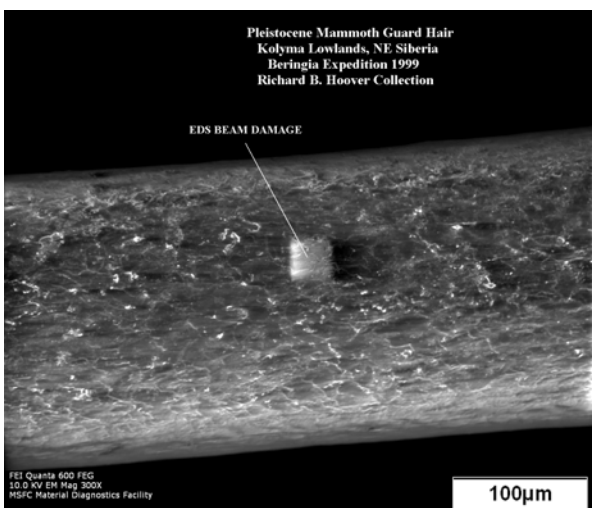
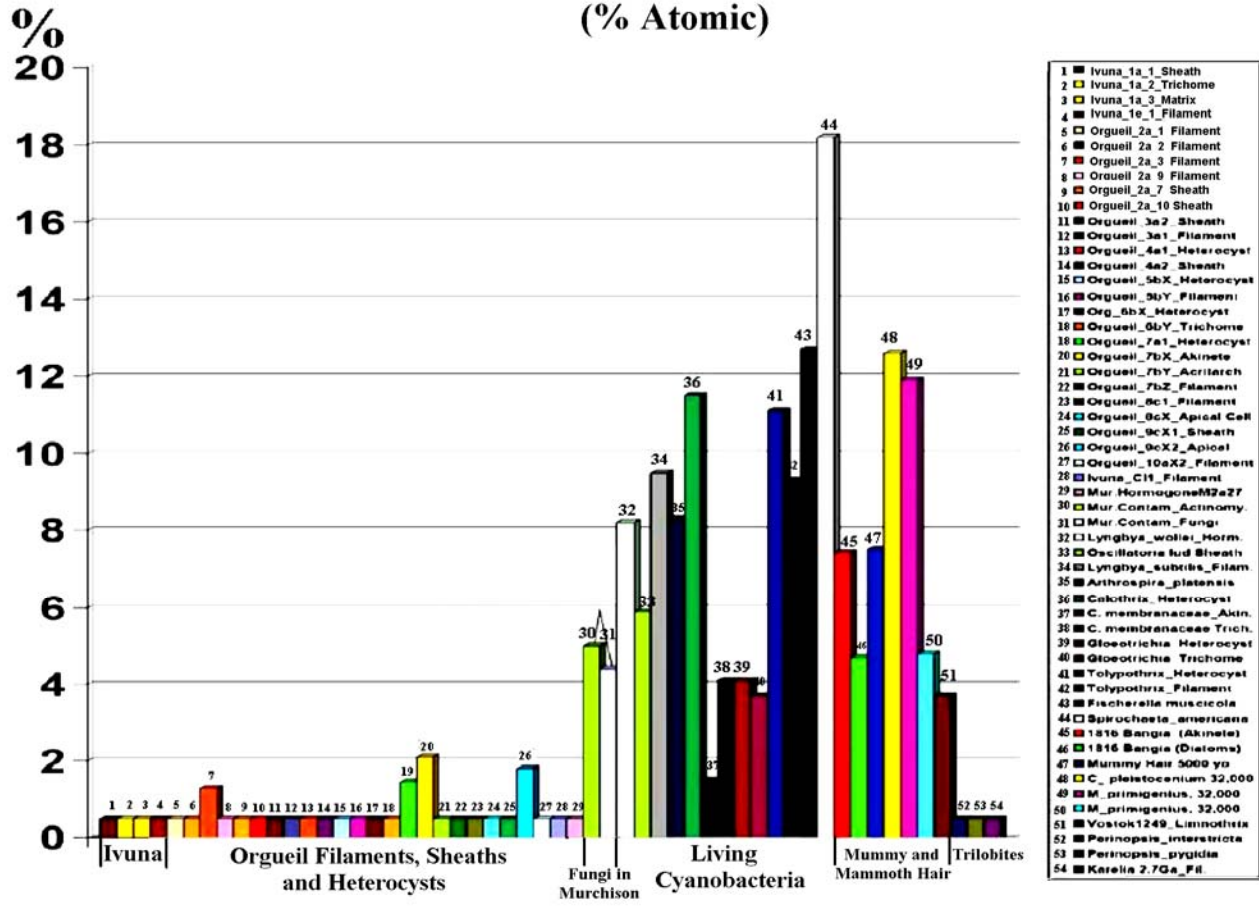


Figure 6.a. EDS data on Nitrogen content of filaments in Ivuna and Orgueil meteorites compared with modern fungal contaminant in Murchison and living, dead and fossil cyanobacteria, mummy/mammoth hair, trilobites & 2.7 Gya cyanobacteria; **b.** Mammoth Hair with beam damage at EDS spot & strong Nitrogen peak (N 11.9% atomic).

Figure 6.a is a compilation of the nitrogen level measured by EDS for a number of the filaments encountered in Ivuna and Orgueil CI1 carbonaceous meteorites compared with modern and ancient terrestrial life forms. The meteorite filaments are typically severely depleted in nitrogen ($N < 0.5\%$) whereas life forms on Earth have nitrogen levels from 2% to 18%. Hoover (2007) has explored the use of Nitrogen levels and biogenic element ratios for distinguishing between modern and fossil microorganisms as a mechanism for recognizing recent biological contaminants in terrestrial rocks and meteorites. Nitrogen is encountered at detectable levels even in the hair and tissues from mummies from Peru (2 Kya) and Egypt (5 Kya) and the hair/tissues of Pleistocene Woolly Mammoths (40-32 Kya). **Fig. 6.b** is a FESEM image of a Guard Hair of a 32,000 year old Pleistocene mammoth. This hair was collected by the author in the Kolyma Lowlands of NE Siberia. The square spot in the image is beam damage (10 KeV electron beam) that occurred during EDS spot analysis. The EDS data revealed a strong (11.94% atomic) peak at the nitrogen $K\alpha$ line (between the Carbon and Oxygen $K\alpha$ lines). Even though the biological material was 32,000 years old, the nitrogen of the proteins was still present. Similar results were obtained for cyanobacterial filaments found in the stomach milk of the 40,000 year old baby mammoth “Lyuba” and for pre-dynastic (5,000 year old) Egyptian mummies. However, truly undeniably biological materials that are truly ancient also had nitrogen levels below the limit of detection with the FESEM EDS detector. The samples studied included fossilized Insects in Miocene (8.4 Mya) Amber, Cretaceous Ammonites (100 Mya); Cambrian trilobites of Wheeler Shale of Utah (505 Mya) and filamentous cyanobacteria from Karelia (2.7 Gya). The detection of nitrogen in Pleistocene biological hair and tissues and the absence of detectable nitrogen in the cyanobacterial filaments in the CI1 carbonaceous meteorites provide clear and convincing evidence that the meteorite filaments are indigenous to the stones and could not be the result of microbial contaminants that invaded the stones after 1806 (Alais); 1864 (Orgueil) or 1938 (Ivuna) when these meteorites landed on Earth.

4. DISCUSSION

4.1 Classification of Carbonaceous Meteorites.

The type meteorites for the different clans of carbonaceous chondrites are CI (Ivuna), CM (Mighei), CO (Ornans), CV (Vigarano), CR (Renazzo) and CK (Karoonda). Wiik (1956) and Van Schmus & Wood (1967) classified carbonaceous chondrites based upon their chemical composition and petrology. In the Wiik classification system, the Group I carbonaceous chondrites have ~7% C, 20% H_2O , and ~22% SiO_2 ; the Wiik Group 2 (e.g. Murchison, Mighei and Cold Bokkeveld) chondrites, have ~ 4% C, 13% H_2O , and 27.5% SiO_2 and the Group 3 (e.g. Mokoai & Felix) have <1% C. Carbonaceous chondrites are further subdivided into petrologic types (1-7). The petrologic type is an indicator of the degree of chemical equilibrium within the meteorite minerals. In this system, the type 3 chondrites have not been significantly altered by either water or thermal metamorphism. Unequilibrated chondrites from lack of thermal metamorphism are of petrologic types 1-3 and types 4 to 7 are increasingly equilibrated due to extended thermal processes. The petrologic types 2 and 1 are found only in the carbonaceous chondrite clan and they have been subjected to an increasing degree of aqueous alteration. Carbonaceous chondrites of petrologic type 1 have been so extensively altered by water that chondrules are entirely absent, even though they have chondritic composition and must have contained chondrules during their early history before the aqueous alteration occurred. The type 2 chondrites have few (somewhat less aqueously altered) chondrules. The chondrules of type 3 are numerous, unaltered and very distinct, whereas those of types 4 to 6 again become more indistinct due to thermal metamorphism and recrystallization. By petrologic type 7 the chondrules are again absent due to thermal destruction.

4.2 Mineralogy, Petrology and Organic Chemistry of C11 Carbonaceous Meteorites.

Cloëz and Pisani conducted the first detailed chemical analysis and study of the mineralogy and the Orgueil C11 meteorite. Pisani (1864) concluded that the Orgueil silicate minerals are more properly designated as serpentine rather than peridotite. Cloëz (1864a,b) found the Orgueil meteorite to be comprised of a soft, black, friable material, with 5.92% carbon, humic substances, magnetite, silicic acid, hygroscopic water (5.2-6.9%) and 8-10% indigenous water of hydration that is liberated only at a temperature > 200 °C. He also found a variety of evaporite minerals -- mainly magnesium, ammonium, calcium and sodium salts. By microscopic and chemical analysis, Cloëz concluded the dominant portion of carbonaceous material in the Orgueil meteorite was a complex polymeric carbon that is insoluble in water. He found the Orgueil Insoluble Organic Matter (IOM) to be similar to humic substances, peat, and coal but unlike living organic matter (TABLE III).

TABLE III. Elemental Composition of the Orgueil IOM, Peat, and Lignite Coal

SAMPLE	CARBON	HYDROGEN	OXYGEN	O/C
Orgueil Insoluble Organic Matter	63.45%	5.98%	30.57%	0.48
Peat from Long (Somme Valley)	60.06%	6.21%	33.73%	0.51
Lignite Coal from Ringkuhl	66.50%	5.33%	28.17%	0.42
Black Matter - Les Landes Sand	60.40%	5.65%	33.65%	0.56
Living Bacteria	6.4%	63%	26%	(~2-10)

Since these early studies a great deal of research has been dedicated to a detailed study of the mineralogy, petrology and organic chemistry of the C11 carbonaceous meteorites (Dufresne and Anders, 1962; Bunch and Chang, 1980; Tomeoka and Buseck, 1988; Nagy, 1975; Kissin, 2003; Sephton, 2005). It is now established that the C11 carbonaceous meteorites contain ~65 wt% of fine scale phyllosilicate aggregates and intergrowths of serpentine and smectite/saponite, 10% magnetite; sulfides including aqueously altered iron-nickel sulfides 7% pyrrhotite ($[\text{Fe,Ni}]_{1-x}\text{S}$), and 5% ferrihydrite ($5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$) and troilite (FeS); recomposed carbonates like Brunnerite (Mg,FeCO_3 (5%); and a small fraction (<1%) of olivine and pyroxene crystallites (Endre and Bischoff, 1996, Bland *et al.*, 2004). The Orgueil meteorite also contains 4.56 Gy magnetites (as individual crystals, framboids and stacks of platelets) and presolar diamonds, silicon carbide and graphite (Huss and Lewis, 1994). Magnetite and pyrite framboids and platelets have been found in all C11 (Alais, Ivuna, and Orgueil) and C2 Ungrouped (Tagish Lake) meteorites that have been investigated during the present research. Spectacular platelets and magnetite framboids with extremely well preserved uniform crystallites are common in the Tagish Lake meteorite. Studies carried out at the Paleontological Institute in Moscow by Academician Alexei Yu. Rozanov has revealed that the framboids in the upper Permian black shales of the Berents Sea shelf are similar in size distribution and characteristics to those found in the carbonaceous meteorites.

Independent studies also have confirmed that the C11 carbonaceous meteorites contain complex insoluble organic matter that is similar to kerogen as is typically encountered in bitumen or coal. Boström and Frederickson (1966) described the Orgueil meteorite as “*bituminous clay with a breccia structure and clastic texture.*” They concluded there were three main stages of mineral formation on the meteorite parent body –

1. Early hot stage with minerals like troilite that are stable a several hundred degrees centigrade.
2. Middle stage with minerals like chlorite and limonite forming below 170 °C
3. Late stage with carbonates and sulphates forming below 50 °C.

Guo *et al.* (2007) used carbonate clumped isotope thermometry to determine the conditions of the aqueous alteration sequence (of calcite to dolomite to bruennerite) as the parent bodies of the carbonaceous meteorites were cooling. They concluded the Orgueil dolomite formed at +26 °C and the bruennerite formed at -6 °C. The Orgueil and Ivuna meteorites appear to have experienced an extended period of aqueous alteration by acidic hydrothermal fluids that completely destroyed the pentlandite ($[\text{Fe,Ni}]_9\text{S}_8$). Pentlandite is present in the Alais and Tonk meteorites, which probably experienced a shorter period of alteration (Bullock *et al.*, 2003, 2005). The dissolved nickel was eventually re-combined with sodium to form sodium nickel sulfate (Ni-bloedite) or iron to form ferrihydrite. These diverse mineral grains and particulates contained within the CI1 carbonaceous meteorites are typically cemented together by epsomite and other water soluble salts.

4.3 Heating of CI1 Carbonaceous Meteorites during Atmospheric Transit.

Immediately after the Orgueil meteorite fell, the villagers collected more than 20 jet-black stones. Many had complete fusion crusts and a few were quite large (one with mass ~11 kg). Leymeri (1864a) related that one of the stones “fell into a farmer’s attic, and this man burned his hand when he touched it.” He described using a knife to cut one of the Orgueil stones soon after the fall: “*The knife cut creates smooth and shiny surfaces which is an indication of a fine, paste-like matter*” (Leymeri, 1864b). These observations indicate that just after they fell, the interior of the Orgueil meteorite stones had the consistency of wet clay. Even though a thin fusion crust was formed on the exterior of the stones by intense heating during the transit through the atmosphere, it is clear that the interior of the stones never became hot. Some Orgueil stones that were found only a few hours after the fall had a thin coating of frost on the outer surface. A frost coating was also reported on some of the Murchison CM2 meteorites found just after it fell in 1969 in Australia. This indicates that the inner portions of the stones remained below zero °C after transit through the atmosphere. The interiors of the stones were apparently protected by ablative cooling during atmospheric transit. Ablative cooling also allows the interior of an Apollo spacecraft to remain cool during re-entry even though the heat-shield becomes very hot. Ablative cooling also appears to keep the inner portions of carbonaceous meteorites frozen as they blaze through the upper atmosphere. This prevents carbon-rich fossils from being destroyed and suggests that living microbes might even be able to remain viable during atmospheric entry.

4.4 Amino Acids and Chiral Biomarkers Modern Bacteria and Carbonaceous Meteorites. A suite of 20 life-critical amino acids are present in the proteins of all living organisms on Earth. These protein amino acids exhibit homochirality, and they are exclusively the *L*-enantiomer. **Table IV** shows the relative abundance of the protein *L*-amino acids present in the slime sheath exopolysaccharide (EPS) of the cyanobacterium *Microcystis aeruginosa* K-3A; in living cells of the bacteria *E. Coli* and *Salmonella* sp. and in ancient terrestrial fossils of a Fly in amber and teeth of a Cretaceous Duck-Billed Hadrosaur. The extraterrestrial amino acids in the CM2 (Murchison, Murray) and CI1 (Orgueil and Ivuna) carbonaceous meteorites are included for comparison (Ehrenfreund *et al.*, Engel *et al.* and Cronin and Pizzarello).

The amino acids of **Table IV** shown in *italics* or marked with “–“ or “*n.d.*” were not detected in these terrestrial fossils and carbonaceous meteorites. Even though there can be no doubt that the Miocene amber encased fly and teeth of Cretaceous Hadrosaurs are undeniably biological, they are missing many of the same amino acids that are also absent in the carbonaceous meteorites. The data shown in **Table IV** indicates that the most abundant (by weight%) amino acids in the cyanobacterium *Microcystis* sp. are GLU, ASP, ALA, GLY and LEU (all above 8%) followed closely by THR, SER, VAL, ILEU and PRO (all above ~5%). However, GLY is by far the most abundant protein amino acid in the Murchison (CM2), Murray (CM2), Orgueil (CI1) and Ivuna (CI1) carbonaceous meteorites and it is followed by 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 either totally absent or detected only at trace levels* in these meteorites. Only 8 of the 20 life-critical protein amino acids are detectable in water/acid extracts of carbonaceous meteorites. As Engel and Macko (2005)

have noted, the missing protein amino acids in the carbonaceous meteorites provide clear and convincing evidence that they have not been contaminated by modern microbial contaminants.

TABLE IV: Amino Acids in Living Bacteria Terrestrial Fossils and Carbonaceous Meteorites

Protein Amino Acids	Living Bacteria				Fossils		Carbonaceous Meteorites				
	<i>Microcystis</i>	<i>E. coli</i>	<i>Salm. pull</i>	<i>Salm. senf</i>	<i>Fly/ Amber</i>	<i>Hadr osaur</i>	Murch. CM2	Murch. CM2	Murray CM2	Orgueil C11	Ivuna C11
	Wt %	Mol/ ALA	Mol/ ALA	Mol/ ALA	Mol/ GLY	Mol/ GLY	Nmol /g	ppb	ppb	ppb	Ppb
<i>L</i> -Alanine ALA	10.3	1.00	1.00	1.00	0.37	0.53	15.3	956	647	69	157
<i>D</i> -Alanine ALA	-	-	-	-			-	720	617	69	82
Arginine ARG	4.4	0.51	0.48	0.52	-	-	-	-	-	-	-
<i>L</i> -Aspartic Acid ASP	12.0	1.01	1.00	1.00	0.23	0.77	8.5	342	65	54	146
<i>D</i> -Aspartic Acid ASP	-	-	-	-			-	100	51	28	30
<i>L</i> -Glutamic acid GLU	12.3	1.14	1.11	1.14	0.57	0.67	18.2	801	261	61	372
<i>D</i> -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	-	-	-	-	-	-	-
Phenylalanine 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											
a-Aminoisobutyric AIB	-	-	-	-				2,901	1,968	39	46
<i>D,L</i> -Isovaline IVA	-	-	-	-							

Table IV shows that several of the amino acids (such as Threonine, Leucine and Isoleucine) that are missing in carbonaceous meteorites and ancient terrestrial fossils are relatively abundant in living bacteria (Howe *et al.*, 1965; Nakagawa *et al.* 1987). If these meteorite stones were contaminated by modern biology, then they should contain all 20 protein amino acids. The most abundant non-protein amino acids in the carbonaceous

meteorites are Isovaline (IVA), α -aminoisobutyric acid (AIB) and γ -Aminobutyric Acid (GABA). Some have suggested that the presence of these non-protein amino acids in the meteorites indicates that the meteorite amino acids were formed by abiotic processes. Even though these amino acids are not used in proteins, it is an error to conclude they must be abiotic. The amino acids IVA and AIB are formed on Earth by the diagenetic alteration of ancient biological materials and γ -Aminobutyric Acid is synthesized by microorganisms.

4.5 Comets as Parent Bodies of CI1 Carbonaceous Meteorites.

The CI1 carbonaceous meteorites are jet-black stones that contain indigenous extraterrestrial water. The albedo of the Orgueil meteorite is extremely low (~ 0.05). It is blacker than asphalt (albedo ~ 0.07) and comparable to the measured albedo of the nuclei of comets and very dark C-type asteroids. The European Space Agency (ESA) Halley Multicolor Camera aboard the Giotto Spacecraft obtained images only 596 km from the centre of the nucleus of comet Halley during the closest approach on March 14, 1986. These images revealed jets and detailed topographic features on the jet-black (albedo ~ 0.04) surface. Lamarre *et al.* (1986) used data from the IKS-Vega spacecraft to determine that the temperature of nucleus of comet Halley was 420 K \pm 60K when the comet was at 0.8 A.U. This result was interpreted as being consistent with “*a thin layer of porous black material covering the comet nucleus.*” The nuclei of comets are extremely complex – they exhibit rugged terrain, smooth rolling plains, deep fractures and are composed of very dark material. Once a comet enters the inner solar system the nucleus becomes hot during perihelion passages and begins to rapidly lose mass. Gounelle *et al.* (2006) used the eyewitness accounts to compute the atmospheric trajectory and orbit of the Orgueil meteoroid and concluded that the orbital plane was close to the ecliptic and that entry into the atmosphere took place at a height of approximately 70 km and an angle of $\sim 20^\circ$. Their calculations indicated the meteoroid had a terminal height of ~ 20 km and pre-atmospheric velocity > 17.8 km/sec. They found the aphelion to be 5.2 AU (the semi-major axis of orbit of Jupiter) and perihelion ~ 0.87 AU, which is just inside the Earth's orbit as would be expected for an Earth-crossing meteorite. This calculated orbit suggests the Apollo Asteroids and the Jupiter-family of comets are likely candidates for the Orgueil parent body (although they did not exclude Halley-type comets).

The ESA Infrared Space Observatory (ISO) showed that water was the primary volatile (75-80 %) of the 40-50 km diameter nucleus of Comet Hale-Bopp. Minor volatile fractions detected (CH_4 , NH_3 and H_2CO) could have come from clathrates (H_2O ice with simple gasses like CO_2 and NH_3 in a stable lattice structure) or result from atmospheric chemistry. ISO found that Hale-Bopp released water vapor, carbon monoxide and carbon dioxide at a rate of 2×10^9 kg/sec. Olivine, which is commonly encountered in meteorites, was detected in the dust. As comets lose ices they develop an inert outer crust from the less volatile material.

Figure 7.a. is a NASA Deep Space 1 composite false color image showing geyser-like jets erupting from the long prolate nucleus (8 km) of comet 19P/Borrelly on Sept. 22, 2001. The colors indicate three orders of magnitude in the intensity of light level of the comet nucleus (red is 1/10, blue 1/100 and purple 1/1000). The red bumps on the nucleus are real and show where the main jet resolves into three distinct narrow jets coming from distinct sources on the comet. These narrow jets are interpreted as supporting the hypothesis that internal pressures are generated by steam produced by melting of internal ices. These gases escape through pores and cracks in the crust. The Deep Space 1 spacecraft found the 8 km long nucleus of Comet 19P/Borrelly had an albedo of 0.01 to 0.03 (Soderbloom *et al.* 2002). The sunlit regions of this comet were also very hot (~ 345 K) and prominent jets were aligned with the orientation of the rotation axis of the nucleus. The nucleus of this comet contained ices of water, carbon dioxide, methane and other volatiles. When the comet rotates and the dark side is moved into the sunlight, ices of the cold nucleus that are near the crust will be heated. As the crust becomes hotter, these volatile ices would first melt and then boil to produce expanding gases. If gas can not escape rapidly enough through the porous black material that covers the nucleus, the pressure will increase. In accordance with the triple-point of water, when the vapor pressure exceeds the 6.12 millibars and the

temperature exceeds 273.16 K, water can exist in liquid phase. Pools and films of liquid water could then form between the crust and the ice and mineral components of the solidly frozen interior of the comet. These pools could provide ideal niches in which cyanobacteria and other microbial extremophiles could thrive. These micro-niches of pools and films of liquid water trapped within pockets in rock and ice are analogous to the cryoconite and ice bubble ecosystems. On Earth, these are known to contain psychrophilic microbial extremophiles, such as those described from the glaciers and frozen Pleistocene thermokarst ponds of Alaska and Siberia and the glaciers and perennially ice covered lakes of the Schirmacher Oasis and Lake Untersee in East Antarctica (Hoover, 2008; Hoover and Pikuta, 2010; Pikuta *et al.* 2005).

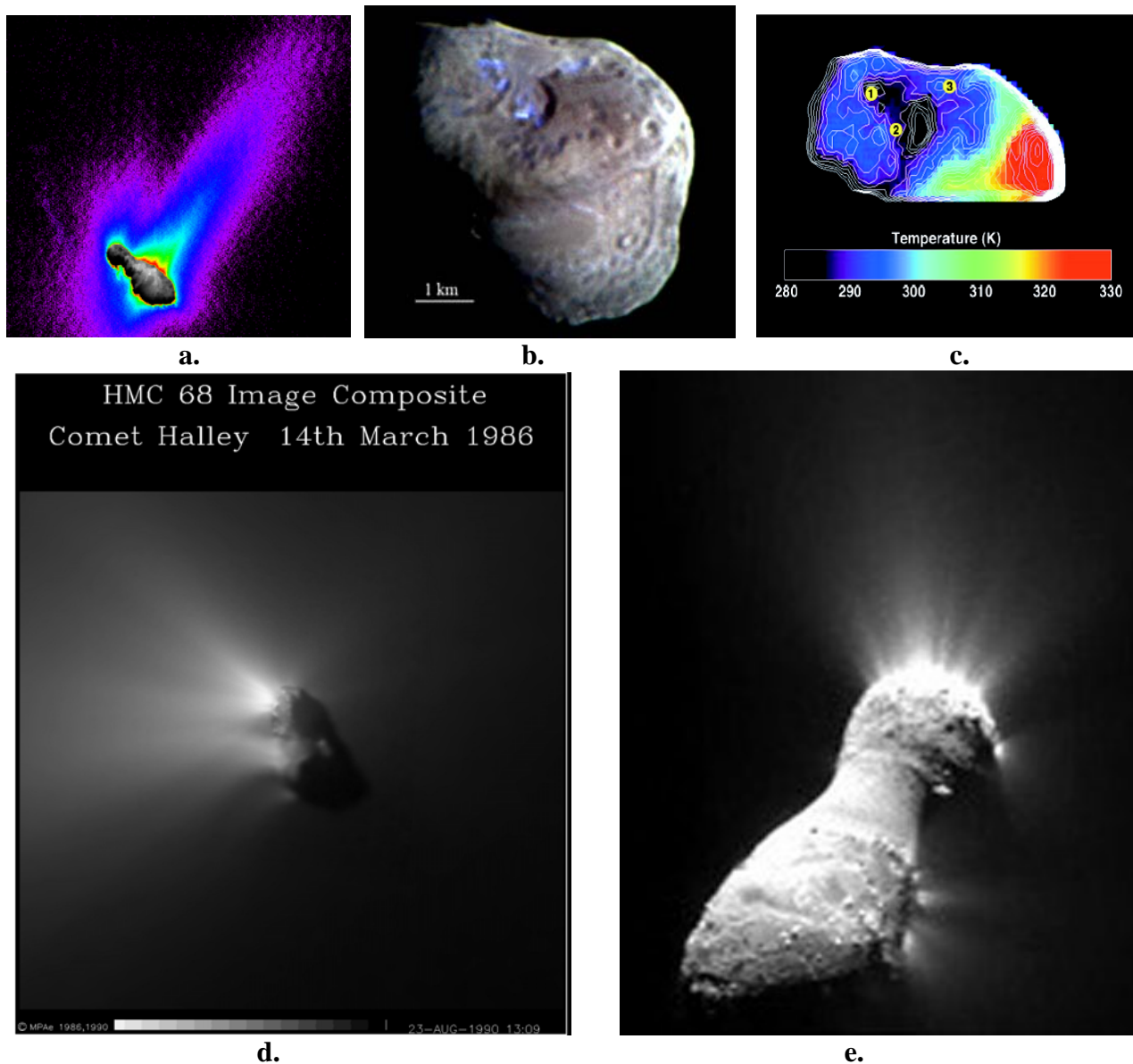


Figure 7. **a.** Deep Space 1 image of Comet P/Borrelly with jets of gas and dust; **b.** Deep Impact image of nucleus of Comet 9P/Tempel 1 shows regions of exposed water ice and **c.** temperature map from Deep Impact IR spectra **d.** Giotto Halley Multicolor Camera (HMC) image showing jets emanating from of the 0.04 albedo nucleus of Comet P/Halley *Image Courtesy: Max Plank Institute for Solar System Research* <http://www.mps.mpg.de/en/projekte/giotto/hmc/> **e.** Deep Impact spacecraft extended mission (EPOXI) image of the nucleus of comet Hartley 2 showing jets of dust and gas. *Image Courtesy: NASA/JPL UMD.*

If the solar heating of the nucleus of a comet liberates volatile gases from the ices faster than they can escape through the porous crust, high pressures that result could cause localized failure of weaker portions of the crust. This could produce violent eruptions of carbon dioxide, water vapor and chunks of crust and particles of ice and dust propelled into space to then form the coma and gas and dust tails of the comet. The expelled dust particulates could also give rise to meteor showers that are seen when the Earth passes through the tail of the comet. From time to time, larger chunks of the ejected crust might survive transit through the Earth's atmosphere and may represent the link between comets and CI1 (and possibly the CM2) carbonaceous meteorites. The fact that the CI1 meteorites contain minerals that were extensively altered by liquid water on the parent body and that the carbonaceous meteorites have been found to contain a large amount of indigenous extraterrestrial water suggest that their parent bodies were very likely either comets or water-bearing asteroids.

The NASA Deep Impact probe obtained the valuable data about the nature of comets during the approach and when the impactor collided with the nucleus of comet 9/P Temple 1 on July 4, 2005. **Figure 7.b** is an image of the comet nucleus provided by Sunshine *et al.* (2005). It shows regions (in blue) where exposed deposits of water ice were near the surface of the comet nucleus. These water ice regions were ~30% brighter than the surrounding areas and may have become exposed when portions of the black crust was blown off into space. Several explosive eruptions were recorded in a video obtained by cameras aboard the spacecraft. **Figure 7.c** shows data from the Deep Impact Mission on the temperature profile of the Temple 1 nucleus at 1.5 AU. As far away from the Sun as the planet Mars, the jet-black nucleus of the comet reaches temperatures as high as 330 K (57 °C). Furthermore, the lowest temperatures measured on the crust were ~ 280 K (7 °C) which is near the temperature at which water ice changes from solid to liquid phase. Before the collision of the impactor, the ambient out gassing of Temple 1 was $\sim 6 \times 10^{27}$ molecules/s of water. However, the free sublimation of ice calculated above (~200 K) was only $\sim 4.5 \times 10^{21}$ molecules/m²/s indicating that the ambient out gassing must have had very significant subsurface sources. The Deep Impact spacecraft also observed numerous events of flaring of the nucleus and eruptions of geyser-like jets as the spacecraft approached the comet prior to the collision of the impactor. The nucleus of comet Hartley 2 was imaged on November 4, 2010, by the extended mission of the Deep Impact Spacecraft (Now called NASA EPOXI Mission). The spacecraft passed within 435 miles of the 2.2 km long nucleus of Hartley 2. It produced images (**Fig. 7.e.**) showing numerous bright jets associated with the release of carbon dioxide, water ice and dust.

The hypothesis that comets are parent bodies for the CI1 carbonaceous meteorites is supported by chemical and mineralogical composition and the physical properties of comets and the meteorites. They have similar albedo, density, and minerals. Furthermore the fact that the CI1 meteorites are micro-regolith breccias whose minerals have undergone extensive low-temperature aqueous alteration is consistent with liquid water and alternating freeze-thaw cycles that must occur on comets as a result of their orbital periods and rotation which presents different of the crust toward the heating effects of Sun when they are in the inner Solar System. The possibility that a comet might represent the parent body of the CI1 carbonaceous meteorites is very significant in view of the detection of microfossils of aquatic photoautotrophs (such as cyanobacteria) in the Alais, Ivuna and Orgueil CI1 meteorites. The evidence for extensive aqueous alteration on the Orgueil parent body and the presence of indigenous extraterrestrial water in the Orgueil meteorite suggests the parent body was either a water-bearing asteroid or a comet. However the Giotto and Vega observations of Halley and the Deep Impact Observations of the nucleus of 9P/Temple-1 have clearly shown that the black nuclei of comets get very hot as they enter the inner regions of the Solar System. Any water bearing asteroid or comet nucleus with an albedo similar to the Orgueil meteorite) would reach a temperature above 100 C at 1AU. At these temperatures, water ice and other volatiles converts to liquid water and steam to produce an expanding cloud of gas and expelled particulates. Any planetesimal orbiting the Sun and possess a gaseous envelope and dust tail is traditionally referred to as a "comet" rather than an asteroid. It has long been known that the annual meteor showers occur when the Earth passes through the orbits of periodic comets (Oct. 21-Orionids-Comet Halley; Aug. 12-Perseids-Comet 1862 III; Nov. 17-Leonids-Comet P/Temple-Tuttle; Dec. 14-Geminids-Comet 3200 Phaeton).

While, asteroids are the likely parent bodies for almost all stony chondrites and nickel-iron meteorites, it is suggested that comets (whose orbits or trajectories cross the orbit of the planet Earth) represent likely parent bodies for water-rich, jet-black, friable micro-regolith breccias that comprise the CI1 carbonaceous meteorites.

4.6 Role of Comets and Carbonaceous Meteorites in the Origin and Evolution of the Earth's Atmosphere, Hydrosphere, and Biosphere

The relationship of comets to carbonaceous meteorites and their role in the origin and evolution of the atmosphere, hydrosphere, and biosphere of Earth has become better understood during the past few decades. The cratered surface of the moon provides clear evidence of the intense Hadean bombardment of the inner planets and moons by comets, asteroids and meteorites during the early history of the Solar System. Watson and Harrison (2005) interpreted the crystallization temperatures of 4.4 Ga Zircons from Western Australia as providing evidence that liquid water oceans were present on the early Earth within 200 million years of the formation of the Solar System. It has recently become more widely recognized that comets played a crucial role in the formation of the atmosphere and oceans of early Earth during the Hadean bombardment. (Delsemme, 1997, 1998; Steel, 1998; Owen, 1997). Wirick *et al.* (2009) discussed similarities and difference between the organic matter of comet 81P/Wild 2 to carbonaceous meteorites and Interstellar Dust Particles.

TABLE V. Deuterium/Hydrogen Ratios
Data extracted from Robert et al. (2000) compilation of Deuterium/Hydrogen ratios

OBJECT	Species	D/H x 10⁻⁶	Reference
Proto-Solar Nebula	H₂	21 ± 5	Geiss and Gloeckler, 1998
Local Interstellar Medium	H	16 ± 1	Linsky <i>et al.</i> , 2003
PLANETS			
Venus (Atmosphere)	H₂O	16,000±200	Donahue <i>et al.</i> , 1982
Earth (Oceans)	H₂O	149 ± 3	Lecuyer <i>et al.</i> , 1998
Mars (Atmosphere)	H₂O	780±80	Owen <i>et al.</i> , 1988
Saturn	H₂	15-35	Griffin <i>et al.</i> , 1996; Owen and Encrenaz, 2003
Jupiter	H₂	21 ± 8	Lellouch <i>et al.</i> , 1996
Neptune	H₂	65 ± 2.5	Feuchtgruber <i>et al.</i> , 1997
Uranus	H₂	55 ± 15	Feuchtgruber <i>et al.</i> , 1998
COMETS			
Comet P/Halley	H₂O	310±30	Eberhardt <i>et al.</i> , 1987, 1995
Comet Hyakutake	H₂O	290±100	Bockelee <i>et al.</i> , 1998
Comet Hale-Bopp	H₂O	330±80	Meier <i>et al.</i> , 1998
CARBONACEOUS METEORITES			
Orgueil CI1 Meteorite	Kerogen	370±6	Halbout <i>et al.</i> , 1990
Orgueil CI1 Meteorite	Amino Acids	315-545	Pizzarello <i>et al.</i> , 1991
Orgueil CI1 Meteorite	Carboxylic Acids	180-310	Pizzarello <i>et al.</i> , 1991
CM, CV & CR Meteorites	Kerogen	370±6	Halbout <i>et al.</i> , 1990
SNC AND STONY METEORITES			
LL 3 Meteorites (Clays)	-OH	780±120	Deloule and Robert, 1995
Mars – SNC Meteorites	H₂O	530±250	Watson <i>et al.</i> , 1994

Sill and Wilkening (1978) proposed that comets may have delivered to Earth the life-critical biogenic elements carbon and nitrogen trapped within the clathrate hydrates of their icy nuclei. Hoyle (1983) and Hoyle and Wickramasinghe (1978, 1981, 1982, 1985) have proposed that comets delivered not only water, biogenic elements and complex organic chemicals to the Earth, but that they also delivered intact and viable microorganisms. Microfossils of cyanobacteria and other filamentous trichomic prokaryotes in the CII carbonaceous meteorites (probably cometary debris) may comprise direct observational data in support of the Hoyle/Wickramasinghe Hypothesis of the role of comets in the exogenous origin of terrestrial life. Eberhardt *et al.* (1987) measured the deuterium/hydrogen ratio of water in comet P/Halley and Delsemme (1998) found the D/H ratio of the water molecules of comets Halley, Hale–Bopp and Hyakutake were consistent with a cometary origin of the Earth's oceans. Dauphas *et al.*, (2000) suggested that ice and water in comets and meteorites helped to cool the Earth's crust and form the early oceans during the Hadean heavy bombardment.

These cosmic bodies are grouped in accordance with their D/H ratio. From this Table, it is clear that the telluric inner planets. The LL3 stony meteorites and SNC meteorites from Mars have high (~500-16,000) D/H ratios and the gas giants, proto-solar nebula, ISM and Galaxies have ratios that very low (~15-65). Clearly, the D/H ratios of these comets (~290-330) and CII, CM, CV and CR carbonaceous meteorites (~180-370) are much closer to the value for the oceans of Earth (~149). These data support the hypothesis that water ices in comets and carbonaceous meteorites may have made significant contributions to the formation of the early oceans of our planet. It is interesting that the D/H ratios of comets are very similar to the ratios measured in the kerogen, amino acids and carboxylic acids of the Orgueil (CI) and other (CM, CV, and CR) carbonaceous meteorites. This supports the view that although stony meteorites are most probably derived from rocky asteroids, the carbonaceous meteorites most probably are derived from comets. The 30 m diameter fast-spinning carbonaceous water-bearing asteroid 1998 KY26 (discovered June 2, 1998) contains 10-20% water. The color and radar reflectivity of Asteroid 1998 KY26 is similar to carbonaceous meteorites and it may be a spent comet. Near IR observations indicated the presence of crystalline water ice and ammonia hydrate on the large Kuiper Belt object (50000) Quaoar with resurfacing suggestive of volcanic out gassing.

5. EVIDENCE OF MICROFOSSILS IN CII METEORITES AND LIFE IN ICE: IMPLICATIONS TO POSSIBLE LIFE ON COMETS, EUROPA, AND ENCELADUS

The possibility life in these frozen worlds has also been enhanced by the detection of evidence of viable microbial life in ancient ice (Abyzov *et al.*, 1998, 2005; Hoover and Pikuta, 2010) and the presence of microfossils of filamentous cyanobacteria and other trichomic prokaryotes in the CII carbonaceous meteorites. The Cassini/Huygens spacecraft has recently obtained data indicating that a vast liquid water ocean may also exist beneath the thick frozen crust of Titan and obtained evidence for cryovolcanic water-ice geysers on Titan and Saturn's moon Enceladus. The possibility that life may exist elsewhere in the Cosmos has been greatly strengthened by new evidence of liquid water on comets and oceans beneath the crusts of the icy moons of Jupiter (Europa, Ganymede or Callisto) and Saturn (Titan and Enceladus). **Figure 8.a** shows the region of the blue and white "tiger stripes of Saturn's spectacular moon Enceladus, which are exhibiting cryovolcanism and spewing water, ice and organics into space. These deep blue and white colors of the Tiger Stripes of Enceladus and as are seen in the Galileo images of Jupiter's moon Europa are typical of glacial ice and snow on Earth as seen in this image of ice bubbles from the Schirmacher Oasis of East Antarctica (**Fig. 8.b**) and are in no way indicative of biology. However, color images of Europa show a wide array of colors that are common in biological pigments but not common in evaporite minerals. These include the red, orange, yellow and ochre colors of the highly fractured regions which are interpreted as pieces of broken the icy crust floating on a liquid water ocean.

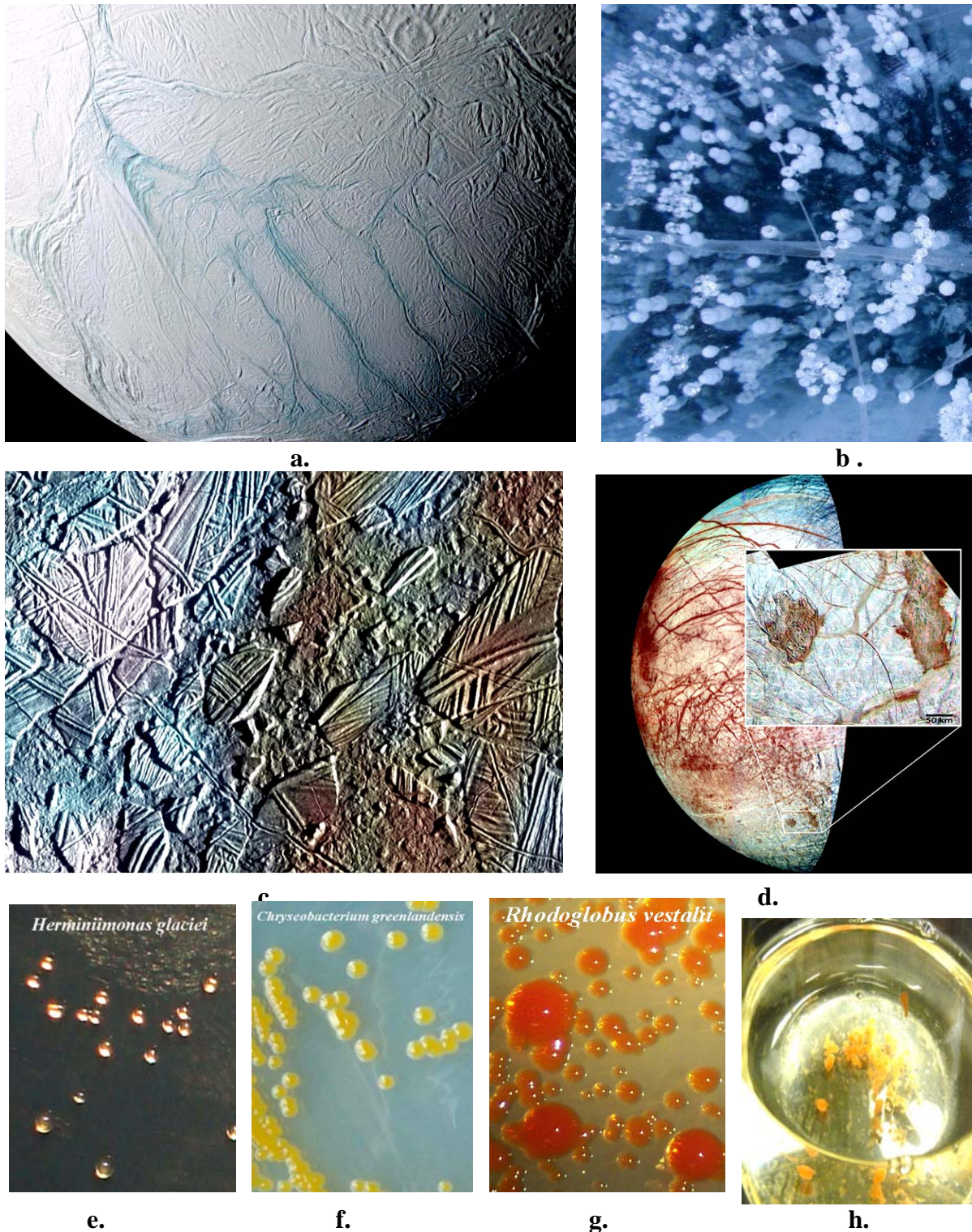


Figure 8. a. Blue and white “Tiger stripes” on Enceladus and b. ice bubbles from Lake Glubokoye, Antarctica. Color images from the Galileo spacecraft of c. the Conamara Chaos region and d. the Thrace region of Europa. Pigmented bacteria from Greenland ice core: e. red *H. glaciei* and f. yellow *C. greenlandensis* and Antarctica g. red *R. vestalii*; and Schirmacher Oasis h. ochre *Hymenobacter* sp. Photos Courtesy: a., c., & d. NASA/JPL/ASU; b. R. B. Hoover; e., f., & g. J. Loveland-Curtze/Penn State and h. Asim K. Bej/UAB

The possibility of life on Europa was discussed by Hoover *et al.* (1986): Chyba *et al.* (2001) and Dalton *et al.* (2003). Hoover *et al.* (1986) argued that the red, yellow, brown, golden brown, green and blue colors detected by the Galileo spacecraft in the Conamara Chaos region (**Fig. 8.c.**) and the deep red lines of the icy crust of Europa (**Fig. 8.d.**) were consistent with microbial pigments. However, since they could not be attributed known evaporite minerals it was argued that they were produced by pigmented microorganisms that grew in ice and water released from the cracks in the crust. More recently (Hoover 2006a, Hoover and Pikuta 2009) have considered the significance of recent discoveries regarding the ability of microorganisms to live in ice and permafrost to the possibility of life on comets and icy moons elsewhere in the Solar System. The possibility of life on Enceladus and the detection of biomarkers in the plumes of water, ice and organic chemicals ejected from the “Tiger Stripes” of Enceladus has been discussed by McKay *et al.*, (2008) and Hoover and Pikuta (2010).

Diatoms are called the golden brown algae due to the color of their chloroplasts, but they appear in a variety of shades from green to gold to dark brown. Cyanobacteria have pigments that allow them to exhibit a wide variety of colors (blue-green, red, orange, brown and black) and colonies of pigmented bacteria (violet, red, orange, yellow, blue, green, brown and black) represent the dominant (in both numbers and biomass) life forms in the ice and permafrost of the polar regions of Earth. Examples are shown of pigmented colonies of microbial extremophiles (Loveland-Curtze *et al.*, 2009) isolated from the ancient Greenland ice cores (*Herminiimonas glaciei* colonies-red) (**Fig. 8.e**) and (“*Chryseobacterium greenlandensis*” colonies-yellow). (**Fig. 8.f.**) **Figure 8.g.** shows red pigmented colonies of the new genus of psychrophile, *Rhodoglobus vestali* isolated from a lake near the McMurdo Ice Shelf, Antarctica (Sheridan *et al.* 2003) and **Figure 8.h.** is an image of red-ochre colonies of *Hymenobacter sp.* isolated from the Schirmacher Oasis Ice Cave (Hoover and Pikuta, 2009, 2010).

6. CONCLUSIONS

Large, complex filaments have been found by Environmental and Field Emission Scanning Electron Microscopy investigations in the freshly fractured interior surfaces of CI1 carbonaceous meteorites. Many are found embedded in or attached to the rock matrix of the meteorites. Filaments are encountered both singly and as components of dense mats of diverse filamentous and coccoidal forms such as are commonly encountered in modern and fossil cyano-bacterial mats on Earth. It is concluded that these filaments and assemblages represent the indigenous carbonized and permineralized remains of microfossils that were present in the meteorites when they entered the Earth’s atmosphere. Many of the Ivuna and Orgueil filaments are isodiametric and their size, size range and morphology can be interpreted as consistent with morphotypes either of known genera and species of cyanobacteria or of other filamentous trichomic prokaryotes, such as trichomic sulfur bacteria. Many other polarized filaments that have been found that are tapered and which exhibit clearly differentiated and distinctive apical and/or basal cells, such as the heterocysts that are known only in the nitrogen-fixing heterocystous cyanobacteria. Many filaments found in the meteorites are attached in a manner (with the basal heterocyst attached to the sediment at the liquid water/substratum interface) similar to that known in species of benthic cyanobacteria that grow on mud or clay sediments. Filamentous cyanobacteria of similar in size and detailed morphology interface. Energy Dispersive X-ray spectroscopy (EDS) data reveals that the filaments in the meteorites typically have external sheaths that are enriched in carbon and infilled with minerals enriched in magnesium and sulfur. These results are interpreted as indicating that the organisms died on the parent body while aqueous fluids were present and the internal cells were replaced by epsomite and other water soluble evaporite minerals dissolved in the liquids circulating through the parent body. The nitrogen level in the meteorite filaments was almost always below the detection limit of the EDS detector (0.5% atomic). However, nitrogen is essential for all amino acids, proteins, and nucleobases in the DNA and RNA of all known microorganisms on Earth. EDS studies of living and dead cyanobacteria and other biological materials using the same instruments have shown that nitrogen is

detectable at levels (2% to 18% atomic) in living and dead biological material, including Pleistocene cyanobacterial filaments and Woolly Mammoths up to 40,000 years old. However, Nitrogen is not found at detectable levels in truly ancient biological materials (e.g., insects in 8 Mya Miocene Amber 8 Mya; Cretaceous ammonites – 100 Mya; Cambrian Trilobites from the Wheeler Shale (505 Mya) or cyanobacterial filaments from Karelia (2.7 Gya). Consequently the absence of nitrogen in the cyanobacterial filaments detected in the CI1 carbonaceous meteorites indicates that the filaments represent the remains of extraterrestrial life forms that grew on the parent bodies of the meteorites when liquid water was present, long before the meteorites entered the Earth's atmosphere.

Therefore, the well-preserved mineralized trichomic filaments with carbonaceous sheaths that have been found embedded in freshly fractured interior surfaces of the Alais, Ivuna, and Orgueil CI1 carbonaceous meteorites are interpreted as the fossilized remains of extraterrestrial prokaryotic microorganisms that grew in liquid regimes on the parent bodies of the meteorites. The detection of valid, indigenous microfossils in carbonaceous meteorites has direct implications to the study of the Origin and Evolution of the Biosphere and the distribution of life in the Cosmos. These results combined with the recent detection of viable microbial extremophiles in ancient permafrost, glaciers and polar ice caps on Earth significantly enhance the possibility that microbial life may thrive today in the Polar Ice Caps and permafrost of Mars, in water-bearing asteroids and in the nuclei of comets (where they may replicate rapidly during perihelion passage and survive long periods of extreme cold during the outer portions of the cometary orbit) and in the surface ice and liquid water oceans of icy moons such as Europa and Enceladus.

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