

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

Richard B. Hoover

Space Science Office, Mail Code 62, NASA/Marshall Space Flight Center, Huntsville, AL 35812

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 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 trichomic cyanobacteria and other trichomic prokaryotes such as the filamentous sulfur bacteria. ESEM and FESEM studies of living and fossil cyanobacteria show similar features in uniseriate and multiseriate, branched or unbranched, isodiametric or tapered, polarized or unpolarized filaments with trichomes encased within thin or thick external sheaths. Filaments found in the CI1 meteorites have also been detected that exhibit structures consistent with the specialized cells and structures used by cyanobacteria for reproduction (baeocytes, akinetes and hormogonia), nitrogen fixation (basal, intercalary or apical heterocysts) and attachment or motility (fimbriae).

Energy dispersive X-ray Spectroscopy (EDS) studies indicate that the meteorite filaments are typically carbon rich sheaths infilled with magnesium sulfate and other minerals characteristic of the CI1 carbonaceous meteorites. The size, structure, detailed morphological characteristics and chemical compositions of the meteorite filaments are not consistent with known species of minerals. The nitrogen content of the meteorite filaments are almost always below the detection limit of the EDS detector. EDS analysis of terrestrial minerals and biological materials (e.g., fibrous epsomite, filamentous cyanobacteria; mummy and mammoth hair/tissues, and fossils of cyanobacteria, trilobites, insects in amber) indicate that nitrogen remains detectable in biological materials for thousands of years but is undetectable in the 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 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 aqueous alteration on the parent body) and by their high degree (~20%) of indigenous water of hydration. The aqueous alteration took place on the parent bodies of the CI1 meteorites at low temperature (<50 °C) and produced hydrated phyllosilicates similar to terrestrial clays, carbonates and

oxides magnetite Fe_3O_4 and limonite $\text{Fe}_2\text{O}_3 \cdot n\text{H}_2\text{O}$. Sparsely distributed throughout the black rock matrix are fragments and crystals of olivine, pyroxene and elemental iron, presolar diamonds and 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 ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) and gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$). The fact that these stones disintegrate immediately after they are exposed to liquid water was observed during the initial studies of the *Alais* meteorite (Thénard, 1806; Berzelius, 1834, 1836) and the *Orgueil* stones (Leymerie, M. 1864). These stones are destroyed and disaggregate into tiny particles as the water soluble salts that cement the insoluble mineral grains together in the rock matrix dissolve (Hoover, 2005).

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., 1864
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	
Revelstoke	3/31/1965	64 km NW of City of Revelstoke, British Columbia, 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> , 2001
Y-86737	1986	Yamato Mountains, Antarctica (71° 30'S, 35° 40'E)	2.8 g	Tonui <i>et al.</i> , 2001
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 the asteroids. The high water content, D/H ratios, and the evidence of extensive aqueous alteration of the CI1 carbonaceous meteorites indicate their parent bodies were either water-bearing asteroids or comets.

1.1. Chemical, Mineral and Morphological Biomarkers in CI1 Carbonaceous Meteorites. A number of biominerals and organic chemicals (that 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 that are produced in nature by biological processes but 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 when research began shortly 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> . (1980, 2003) Cronin <i>et al.</i> . (1997); Ehrenfreund <i>et al.</i> , (2001)
ALAIS, IVUNA & ORGUEIL	Nitrogen Heterocycles and Nucleobases - Purines and Pyrimidines	Hayatsu, <i>et al.</i> 1964, 1968 ; Folsomme <i>et al.</i> 1973; Hua <i>et al.</i> ,1986; Stoks & Schwartz,1981; Glavin & Bada, (2004); Martins <i>et al.</i> ,(2008)
ALAIS, IVUNA & ORGUEIL	Filamentous Microfossils ~ Trichomic Prokaryotes and Cyanobacteria	Hoover (1997); Zhmur <i>et al.</i> (1997); Hoover <i>et al.</i> (1998, 2003a,b; 2004; 2005a,b; 2006a,b; 2007, 2008, 2009; 2008); 2009);

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. Eteinne 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 reknowned Professor of Chemistry at the College de France in Paris, conducted the first study of the Alais CI1 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 discarded it as being contaminated. In the English translation provided by Nagy, (1975, p. 45) he says: “*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 CI1 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 for the altitude at which the bolide exploded at 30 km and to conclude that 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 smooth and shiny surfaces which is an indication of*

fine, paste-like matter. Fragments placed in water disintegrate immediately.” This astonishing observation that the Orgueil stones immediately disintegrated into minute particulates when they came in contact with cold water was independently confirmed by Cloëz (1864a) and Pisani (1864) exhibiting a behavior similar to that of the Alais 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 the disintegration in alcohol takes place more slowly than in water. These very 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 profuse effervescence was observed and within a few minutes the samples were completely disaggregated into an assemblage of micron-sized particulates. Immediately 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 $[(\text{Fe}, \text{Mg}, \text{Al})_6(\text{Si}, \text{Al})_4\text{O}_{10}(\text{OH})_8]$ of the clay phyllosilicates mineral group. The other major minerals of Orgueil include: 6.7% Epsomite ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$); 6% Magnetite (Fe_3O_4); 4.6% Troilite (FeS), 2.9% gypsum ($\text{CaSO}_4 \cdot n\text{H}_2\text{O}$) and 2.8% Breunnerite $(\text{Fe}, \text{Mg})\text{CO}_3$. Epsomite forms white veins in the meteorite and is a significant evaporite mineral that helps cement together the meteorite particulates into the stones.

In 1868, Pierre Marcellin Berthelot The famous French chemist who had shown in 1860 that all organic compounds contain C, H, O, and N experimented with hydrogenation to explore the organic chemistry of the Orgueil CI1 meteorite. He found complex hydrocarbons in Orgueil that were analogous to carbonaceous substance of organic origin on Earth (Berthelot, 1868).

It is now well established that the total organic content of 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 the carbonaceous meteorites is clearly indigenous and constitutes an important biomarker. In terrestrial rocks, Kerogen, isoprenoids, pristane, phytane 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 as they are stable of geologically significant time periods (~ billions of years). On Earth they are undeniably biological in origin. Diagenesis and catagenesis processes that alter the original biochemicals is usually minimal and the basic carbon skeleton remains intact. For this reason, although functional groups (e.g., -OH, =O, etc.) may be lost, the chemical structure derived from biological origins of these stable fossil biomolecules 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 gms 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 Orgueil. The $\delta^{13}\text{C}$ value for these meteorites was approximately +60 per mil, which is dramatically different from the abiotic or biotic terrestrial carbon. This provides conclusive evidence that the meteoritic carbon is extraterrestrial in origin and cannot be associated with terrestrial bio-contaminants. The Alais, Ivuna and Orgueil CI1 meteorites have also been found to contain chiral amino acids, nucleobases, pristine and phytane, spectacular magnetite frambooids and platelets, and the well-preserved and mineralized remains of diverse filaments interpreted as the mineralized remains of cyanobacteria and other trichomic prokaryotes. Amino acid analyses using HPLC of pristine interior pieces of the Orgueil and Ivuna meteorites resulted in the detection of β -alanine, glycine, and γ -amino-*n*-butyric acid (GABA) at concentrations ranging from ≈ 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 the presence of racemic (D/L ≈ 1) alanine and β -ABA have established that these amino acids are extraterrestrial in origin. The amino acid composition of the CI1 meteorites is strikingly distinct from that of the Murchison and Murray CM2 carbonaceous meteorites. This indicates that the CI1 meteorites came from a different parent body than CM2 meteorites, possibly from an extinct comet.

Claus and Nagy (1961) studied the Ivuna and the Orgueil CI1 meteorites and found a large number of forms that they originally interpreted as indigenous microfossils. After intense criticism, they subsequently designated them as “organized elements” so as to not make any judgement as to their biogenicity. Since they used standard palynological methods to dissolve the rock matrix in acids for extracting the insoluble kerogen-like bodies, any unseen pollen contaminants on the exterior surfaces of the meteorite would remain intact and be concentrated in the acid-resistant 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 and it is still widely believed that all of the the “organized elements” they described were either abiotic mineral grains or pollen. Subsequent work by Rossignol-Strick and Barghoorn (1971) revealed that the “organized elements” type microstructures were in fact not pollen grains and were indigenous to the meteorites, but their forms are too simple to make any decision regarding 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 yet been published on the organic chemistry, amino acids and other possible chemical or morphological biomarkers that may be present on these meteorites has ever been published. This is undoubtedly due to the fact that only a tiny amount of the Tonk (7.7 g) and Revelstoke (1 g) meteorites was recovered. 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. It remained on the ice for almost two weeks before the stone was recovered (Folinsbee *et al.*, 1967). Both the Tonk and Revelstoke meteorites have been found 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 that the predominant phase of magnetite in the Revelstoke meteorite was essentially Nickel-free Fe_3O_4 . This was in contrast to the other four CI1 meteorites known at the time, which all contained magnetite with nickel at <6%. Based on their studies of saturation moments, the weight percentage of magnetite for the CI1 meteorites was reported 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 AND METHODS

Samples used in this study were:

Ivuna CI1 Carbonaceous Meteorite

DuPont Meteorite Collection, Planetary Studies Foundation, Chicago

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

Orgueil CI1 Carbonaceous Meteorite

Musée Nationale d'Histoire Naturelle, Paris

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

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

DuPont Meteorite Collection, Planetary Studies Foundation, Chicago

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

EDS elemental analysis of the Orgueil filaments indicated that many of them were carbonized sheaths that infilled with magnesium sulfate minerals. 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 and studied using the Hitachi FESEM and the FEI Quanta ESEM and FESEM.

The abiotic mineral samples investigated were:

Native Fibrous Epsomites



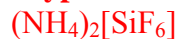
Hot Lake, Oroville, Washington - Fibrous Epsomite Evaporites (6 Stones- 2 kg).

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

Zaragoza, Spain - Fibrous Epsomite - 1 stone: (21.5 gm)

Courtesy: Keck Museum, Reno, Nevada

Cryptohalite



Schoeller Mine, Kladno, Central Bohemia Region, Czech Republic -

1 stone with many Cryptohalite crystals (68.5 gm)

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, bacteria, cyanobacteria and biofilms. 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; and chimney stones from the Rainbow Deep Sea Hydrothermal Vent and Upper Archaean (Lopian) rocks from Northern Karelia.

Modern Cyanobacteria

Plectonema (Lyngbya) wollei–Lake Guntersville, 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 Archaean (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

Coll. Richard B. Hoover

Instruments used in this study 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)

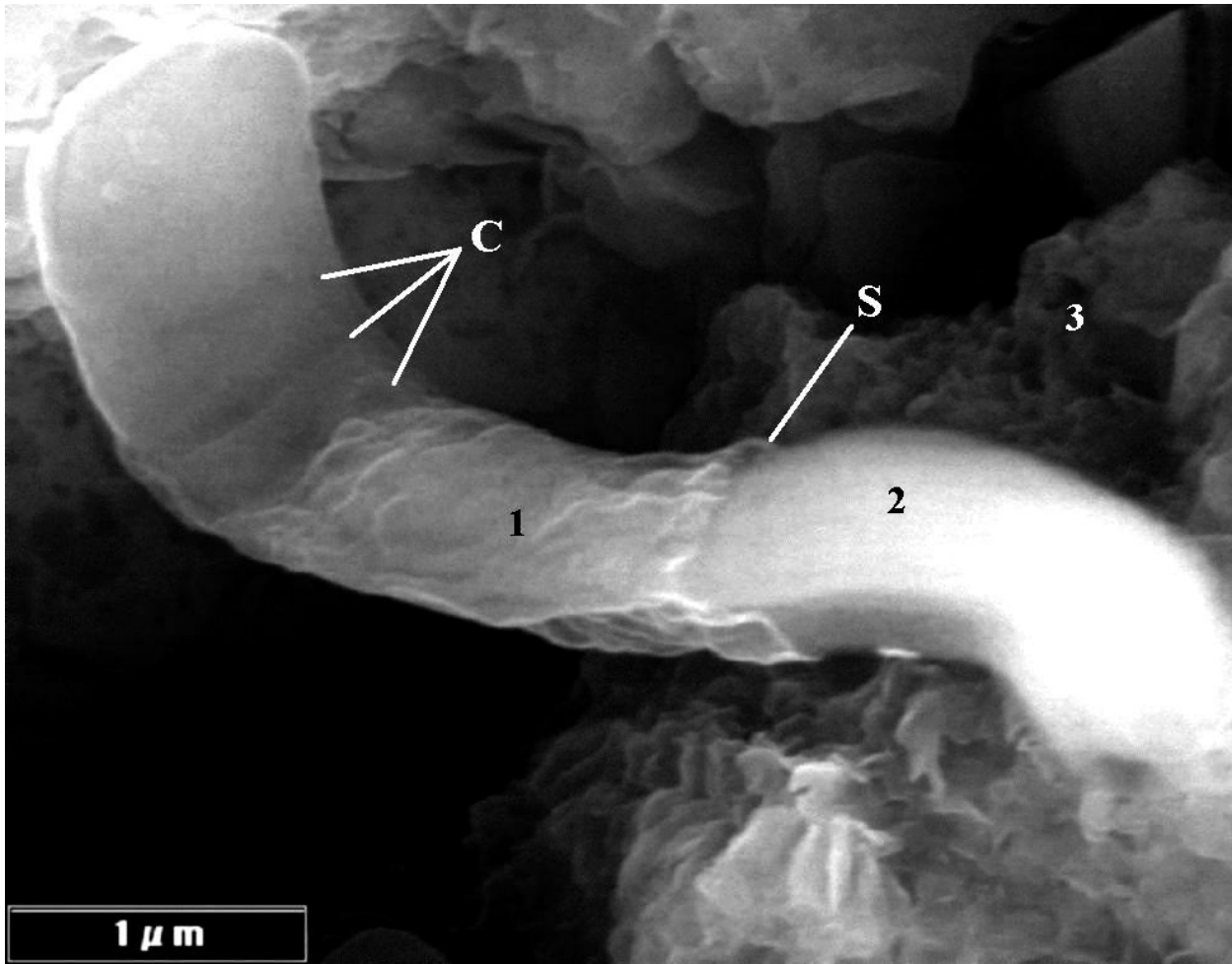
- Simultaneous SED and BSED images; 5 - 300,000X
- 4 Pi EDS - Lithium Drifted Silicon detector (Z>Boron)

In order to minimize the possibility of the detection of coating artifacts or recent contamination by terrestrial biological materials, the study was confined to investigations of uncoated, freshly fractured, interior surfaces of the meteorites. All tools, sample holders and stubs were flame sterilized. Lunar dust samples and silicon wafers were used as negative controls. To further protect the samples from biological contamination, the meteorites were stored in sealed vials at -80°C and after preparation, electron microscopy stubs were kept in sealed containers in desiccator cabinets or in the freezer. The fusion crust and old cracks in the stones were carefully avoided. The meteorite samples were placed in the instrument chamber (with the fresh fracture surface up) a pumped down immediately after the stones were fractured. All solvents, acids or other liquids were strictly avoided. Acids and solvents were used in early studies by other workers 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 could have been on the external surface or in old fissures within the stones. It should be noted that only one seriously Murchison sample was found to be contaminated with fungal filaments (in old cracks in the fusion crust) and not a single pollen grain has been encountered during extensive studies of carbonaceous meteorites carried out since 1996 at the NASA/Marshall Space Flight Center. While fingerprints, pollen grains, fungi, bacteria, laboratory oils and other contaminants could be encountered on the fusion crust or exposed old cracks and fractured surfaces of meteorites, the hypothesis that the interior surfaces carbonaceous meteorites are seriously contaminated by modern bacteria, fungi, or pollen is simply not consistent with the observational results. For this reason, it is extremely important that studies of biomarkers such as amino acids, nucleobases, complex organic chemicals and microfossils should be rigorously restricted to freshly fractured interior portions of the stones. .

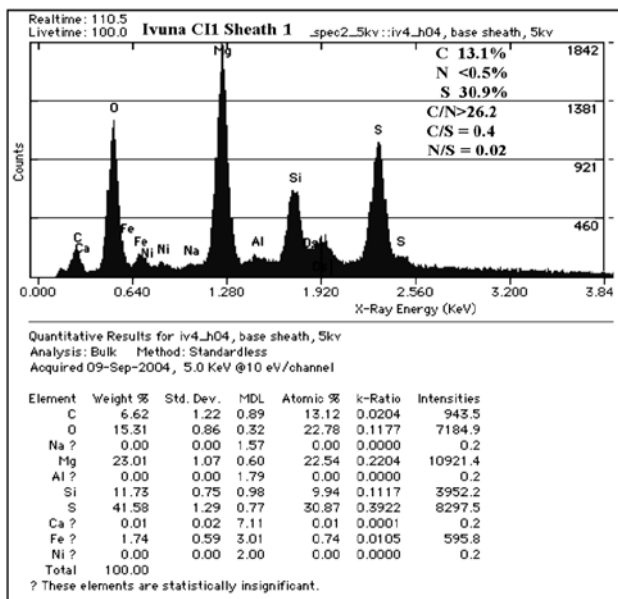
3. OBSERVATIONAL RESULTS AND INTERPRETATIONS

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. To increase readability, the interpretation for each set of images is presented immediately after the Observational Results section for each Figure.

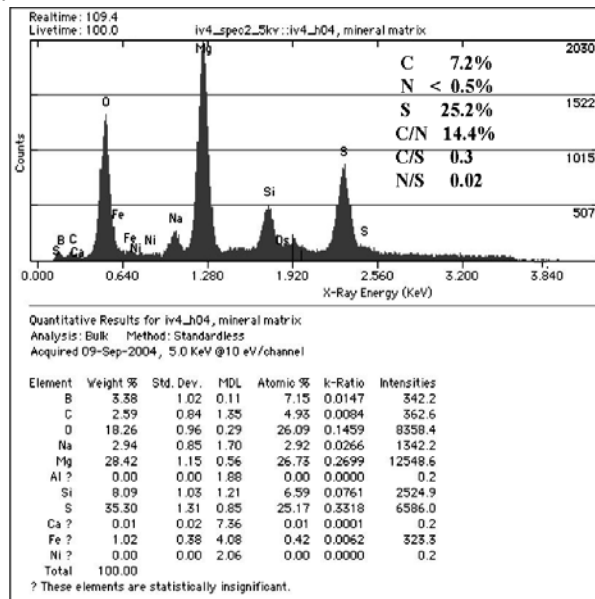
3.1. Images and EDS Spectra of Filaments in the Ivuna CI1 Carbonaceous Meteorite. Figure 1 provides images and Energy Dispersive X-Ray Spectroscopy elemental data for filaments found embedded in the Ivuna CI1 carbonaceous meteorite. **Fig. 1.a** is a FESEM image of a thin uniseriate filament that is 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 depleted in N. The filament is only partially encased within a broken and very thin carbonaceous sheath. 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 and biogenic elements N and P are below the 0.5% detection limit of the instrument. **Fig. 1.d** is a FESEM image of 5 μm diameter X 25 μm long spiral filament Ivuna with white globules that are sulfur-rich 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 that is similar in size and morphology to the giant bacterium “*Titanospirillum velox*”.



a.



b.



c.

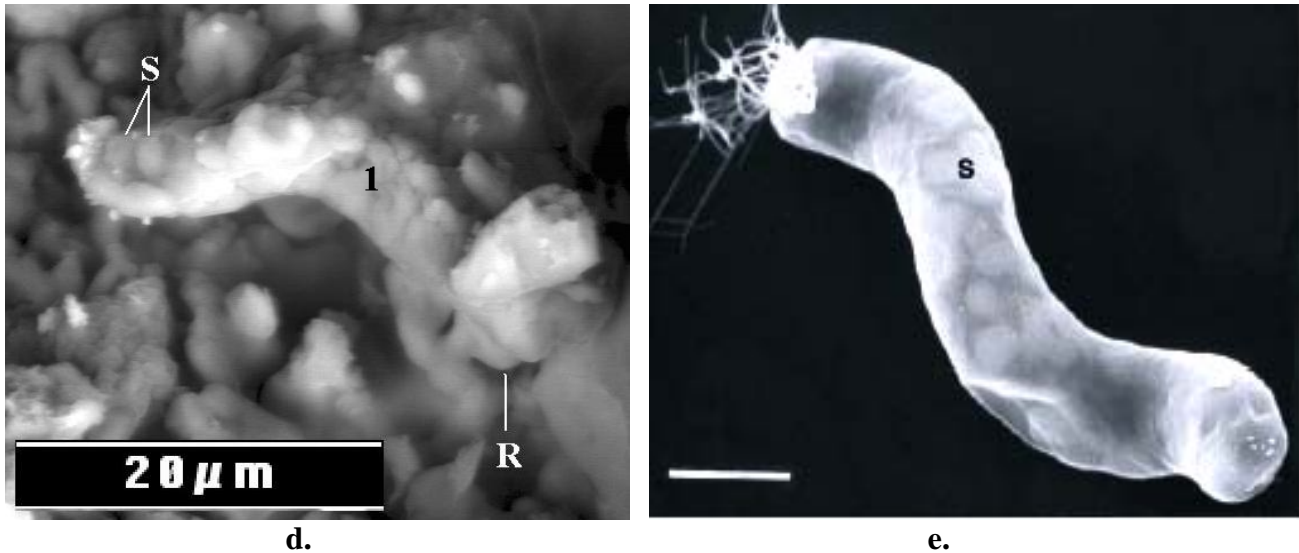


Fig. 1.a. Ivuna CI1 meteorite filament (0.8 μm diameter) with dark lines **C**, partially encased in thin carbon-rich sheath. **b.** EDS elemental data of the filament sheath at spot 1 shows typical biogenic elements Nitrogen and Phosphorus (<0.5%) and Carbon (13.1%) enriched as compared with nearby meteorite matrix (C 7.2%) at spot 3; **d.** FESEM Backscattered Electron image of an Ivuna filament with N<0.5% and sulfur-rich globules **S** and rounded terminus **R** that is 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: Dupont Meteorite Collection, Planetary Studies Foundation; Image 1.e Courtesy: Dr. Riccardo Guerrero

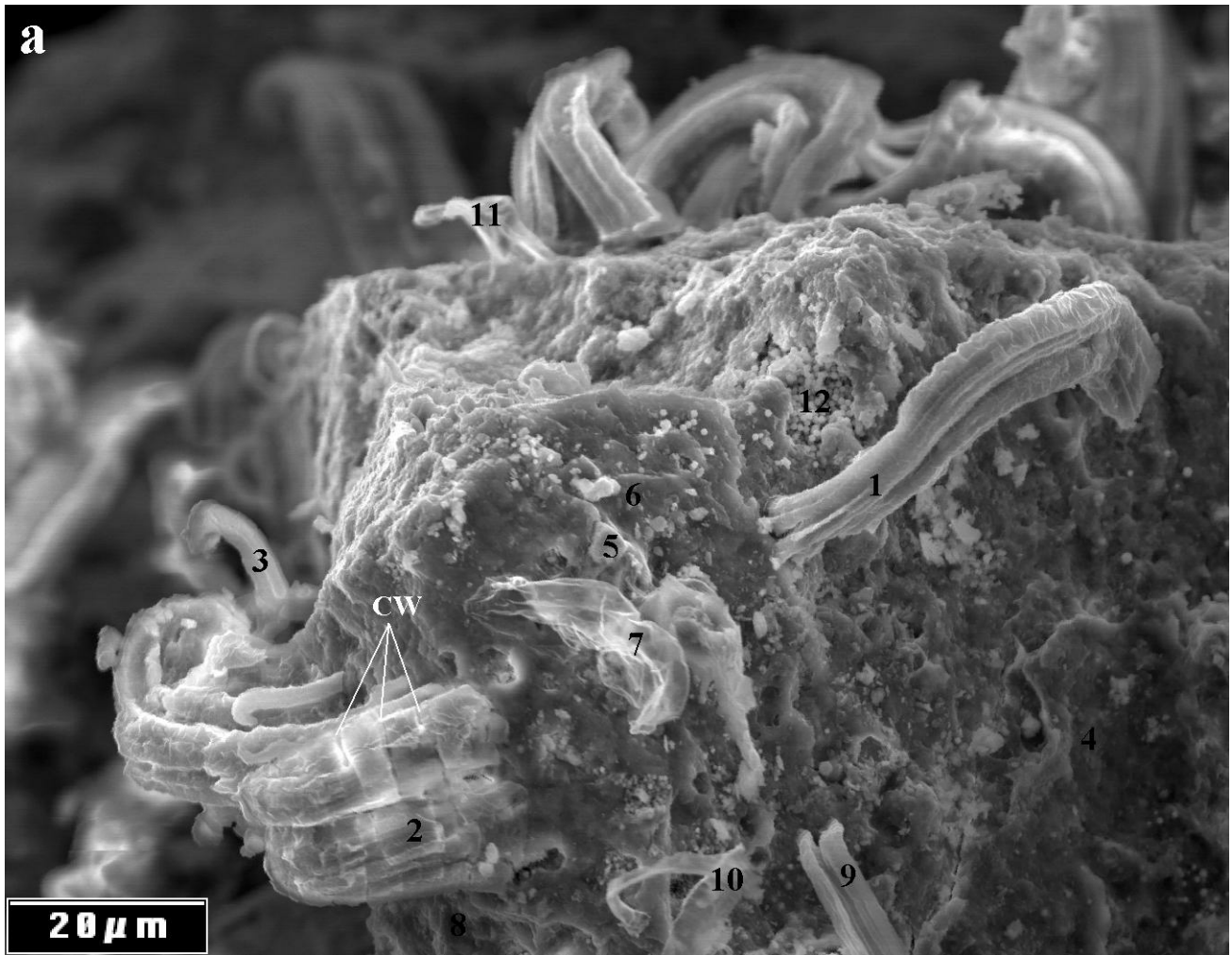
3.1.1. Interpretation of Images and EDS Data of Ivuna Filaments. The flattened embedded filament shown in **Fig. 1.a** is interpreted as the permineralized remains of a partially uniseriate, undulatory, ensheathed trichomic prokaryote. The measured diameter (0.7 - 1.0 μm) as determined from the scale bar of this calibrated FESEM image and the detailed morphology of this Ivuna filament is consistent with some of the smaller filamentous cyanobacteria. The dark lines **C** near the terminus of the sheath are consistent with cross-wall constrictions that are often seen as faint transverse lines in FESEM images obtained with living cyanobacteria. In this image it is possible to see an extremely thin sheath **S** that is broken and covers only the upper portion of the trichome, which appears to have been completely replaced by infilling minerals. The size and morphology of this filament is consistent with filaments of the undulatory trichomic filamentous cyanobacteria *Spirulina subtilissima* (filaments 0.6 - 0.9 μm diameter) and *S. laxissima* (filaments 0.7 to 0.8 μm diameter). These cyanobacteria have not been reported as possessing a sheath, but the sheath seen in this FESEM image is extremely thin and would be very difficult to discern in by visible light microscopy techniques. There are also very small species of the genus *Limnothrix* that are undulatory on nature and possess facultative sheaths. However, it should be pointed out that there also exist groups of ensheathed filamentous anoxygenic phototrophic bacteria (photosynthetic flexibacteria) possess a thin sheath and are capable of gliding motility. There include filamentous representatives of the bacterial Phylum Chloroflexi. The thermophilic species *Chloroflexus aurantiacus* has a thin sheath and trichomes as narrow as 0.8 μm . There also other bacterial photoautotrophs that oxidize hydrogen sulfide and deposit it externally as sulfur (e.g., *Oscillochloris trichoides*) and these trichomic filaments have diameters in the 0.8 to 1.4 μm range.

The length, diameter and spiral configuration and apparent tuft of small filaments at one pole and rounded end at the other along with the internal sulfur globules distributed along the axis of filament (**Fig. 1.d**) found embedded in a freshly fractured surface of the Ivuna meteorite 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. The 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 would explain the fact that this genus and species has not yet been accepted as validly published. The Bacteriological Code rules of nomenclature requires that prokaryotic microorganisms must be isolated and grown in *pure culture* and the designated type strain must be deposited in two international culture collections in two different countries before the genus and species names can be validated (Tindall *et al.*, 2006). The absence of detectable nitrogen content the Ivuna filaments provides evidence that these embedded filaments are indigenous and cannot be dismissed as a modern biological contaminant.

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 freshly fractured fragment of the Orgueil CI1 meteorite that is densely populated with several different types of embedded filaments and electron transparent sheaths. Even though the field of view shown of this image is very small (~120 μm wide) a wide variety of diverse filamentous microstructures are present. To facilitate the description, the filaments and sheaths have been numbered, and all numbers are located on the filament at the site where the EDS elemental spot data were recorded. A 2D X-ray elemental map of this region of the Orgueil meteorite is shown in **Figure 2.b**. The large image in the upper left corner is a Backscatter Electron Detector (BSED) image. The bright spots in this image are high Z elements where clusters and crystallites of magnetite, iron and nickel are concentrated. Other images 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 and they appear as dark features in Silicon, Iron, and Nickel due to the relatively higher content of these elements in the underlying Orgueil meteorite rock matrix. In general, the filament and sheath structures are not discernible in the Nitrogen, Phosphorus and Sodium maps, although Filament **1** can be seen in the Nitrogen map. Empty sheath **7** is wrinkled and electron transparent with a relatively high (47%) content of Carbon. This sheath is unusual in that it is one of the few filaments found in the Orgueil meteorite to have detectable levels of Nitrogen (1%) and Phosphorus (0.8%).

3.1.1 Interpretation and Discussion of Images and EDS Data of the Orgueil Filaments. Filaments **1** and **2** of **Fig. 1.a** are observed to have sheaths with longitudinal striations that run the length of the filaments. This is characteristic of multiseriate trichomic prokaryotic filaments in which multiple parallel oriented trichomes are enclosed within a common homogeneous sheath. These filaments are observed to be either attached to or physically embedded in the Orgueil meteorite matrix. The end of filament **1** becomes slightly wider (~10 μm) where it joins the rock matrix and it appears to contain four internal trichomes, each with a diameter ~2.5 μm . Filament **2** is considerable larger (~20 μm dia.) and the longitudinal striations suggest it contains ~5 trichomes, each with diameters ~4 μm /trichome. Faint transverse lines orthogonal to the long axis of filament **2** are marked **C**.



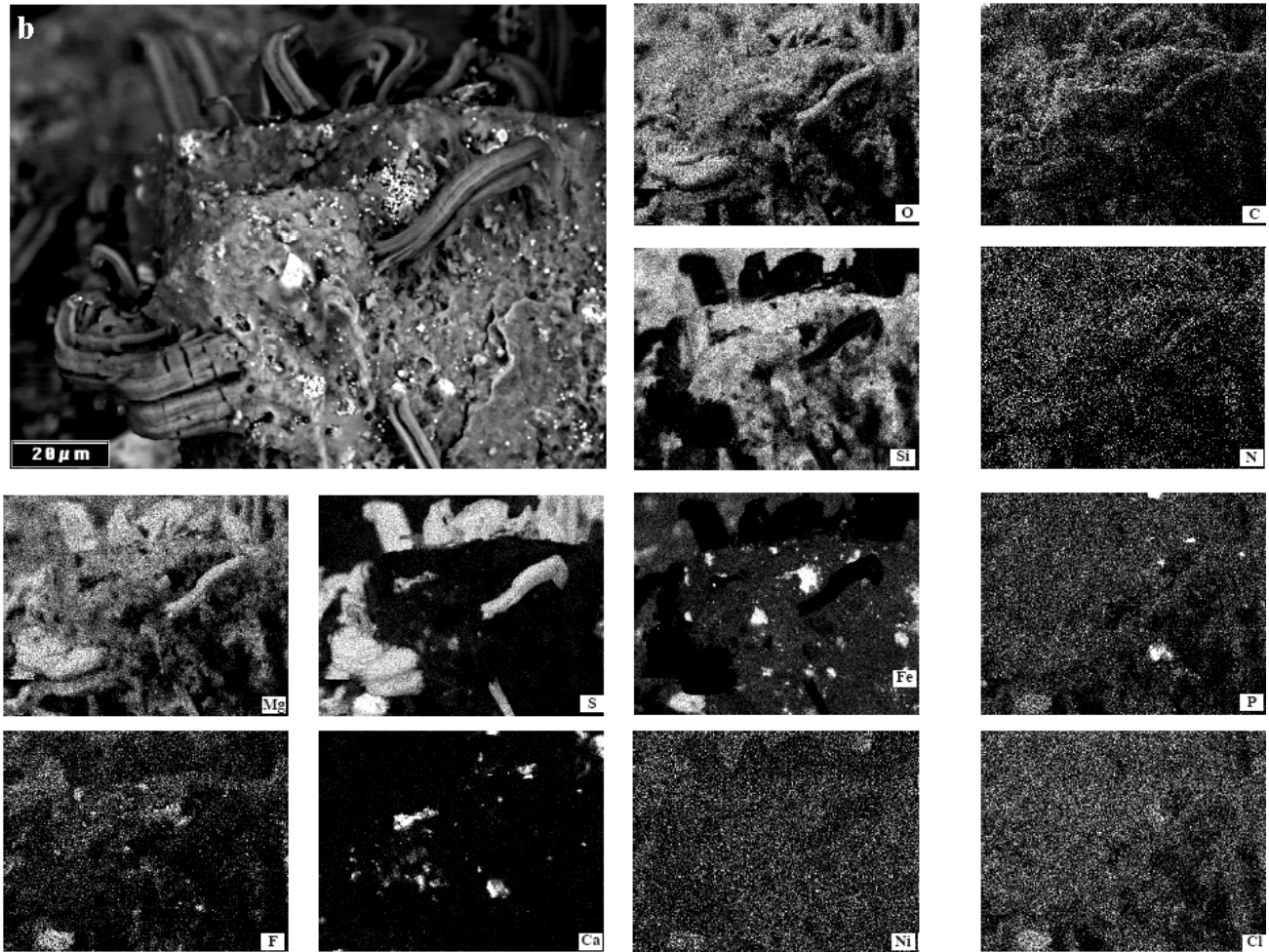


Figure 2.a. Hitachi FESEM Secondary Electron Detector image at 1000 X of multiple filaments and sheaths embedded in Orgueil meteorite matrix and **b.** Backscatter Electron Detector image along with 2-D x-ray maps showing distribution of elements O, C, Si, N, Mg, S, Fe, P, F, Ca, Ni and Cl in filaments for comparison with SED and BSED images.

Orgueil Sample Courtesy: Dr. Paul Sipiera, DuPont Meteorite Collection, Planetary Studies Foundation, Chicago

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 **C** of filament **2** are interpreted as represent cross-wall constrictions, this would indicate that the internal cells within each trichome are $\sim 4 \mu\text{m}$ in length and hence isodiametric. Consequently, the image of filament **2** is interpreted as composed of trichomes made up of spherical or cylindrical isodiametric cells of $4 \mu\text{m}$ diameter. This interpretation is consistent with morphotypes of undifferentiated filamentous cyanobacteria of the Order Oscillatoriaceae. There are many genera and species within this very common cyanobacterial order, including the genus *Microcoleus* Desmazières ex Gomont (*Form Genus VIII. Microcoleus* Desmazières 1823) (Castenholz, Rippka & Herdman, 2001; Boone *et al.*, 2001). Reproduction within this order 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 uniserial filaments **3** and **4** may be interpreted as representing members of the genus *Trichocoleus* Anagnostidis, which was separated from the genus *Microcoleus* on the basis of cell size and morphology. Filament **4** is a $2 \mu\text{m}$ diameter hook-shape filament with a narrowed terminus. Several

species of the genus *Trichocoleus* have filaments 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.

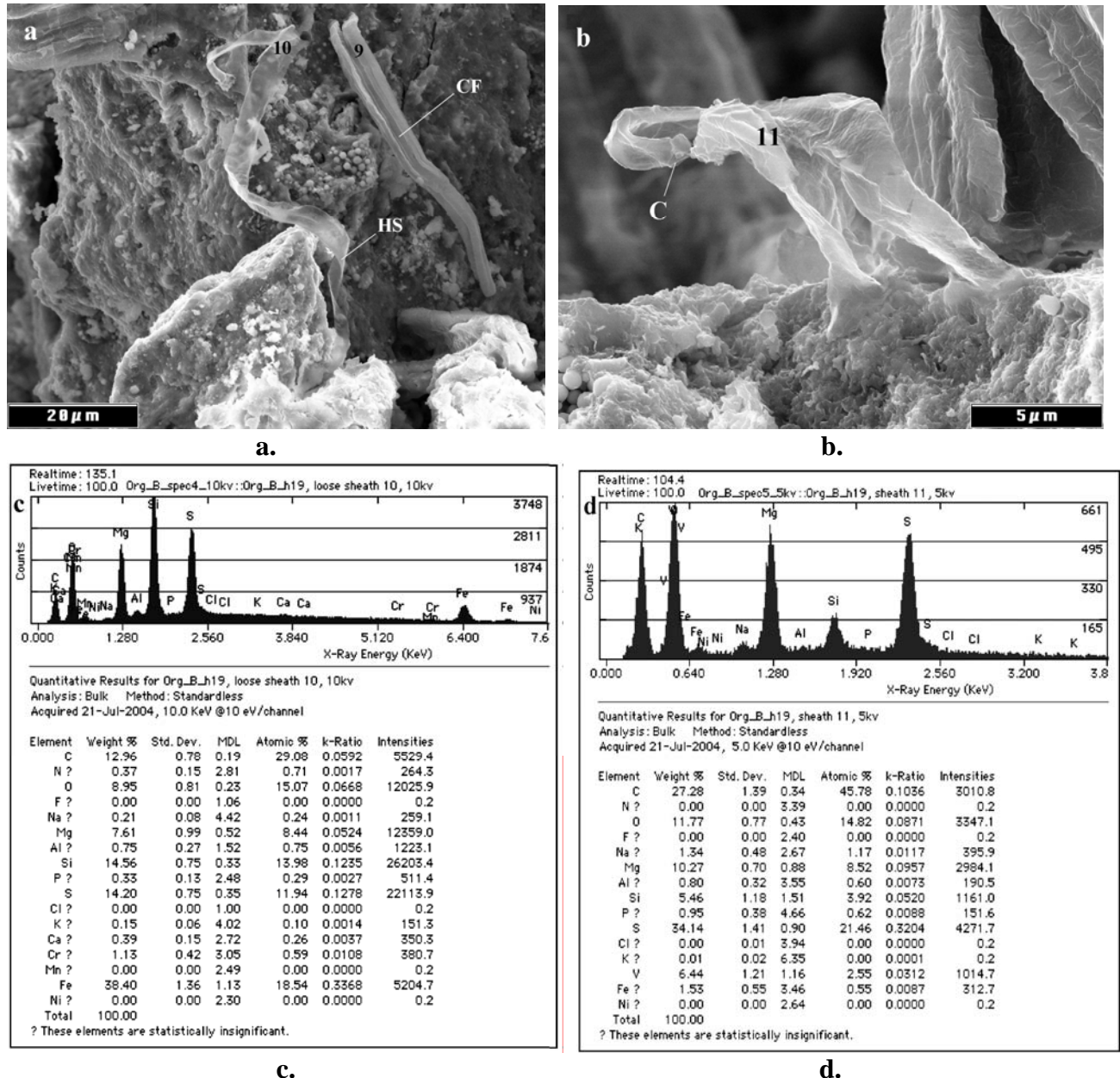


Figure 3. Hitachi FESEM images at 1500X of **a.** collapsed filament **9** and helical coiled empty sheath **10** and **b.** 6000X image of filament **11** showing hook and calyptra or conical apical cell. **c.** EDS spot spectra show elemental compositions **c.** of loose sheath **10** (C 29.1%; N=0.7%) and **d.** sheath **11** (C 47.8%; N<0.5%).

Figure 3.a is a 1500X FESEM SED images of the collapsed Filament **9** and the 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 spectra were taken.

The flattened portion of Sheath **10** forms a spiral coil near the base where it is attached to the meteorite matrix. This is very similar to helical coiled sheath of *Phormidium stagninum* shown in the illustration at <http://www.cyanodb.cz//Phormidium/Phormidium.jpg> illustration. This type of flattened, coiled hollow sheath is often seen in other species of filamentous cyanobacteria and hence does not constitute a unique diagnostic feature. **Figure 3.b.** provides a 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** and shows detection of low, but measurable level of Nitrogen (0.7%) and Phosphorus (0.3%) and higher levels of Iron (19%) and Silicon (14%), which are probably from the meteorite matrix beneath the this, electron transparent carbon-rich sheath. The EDS spectrum at 5 keV for spot **11** on sheath **11** as shown in (**Fig. 3.d**) reveals this flattened sheath to be highly carbonized (48% C 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 as it crosses in front of large filaments more heavily mineralized with magnesium sulfate. Filament **11** is also sulfur-rich (21% S), but has Nitrogen below the level of detectability ($< \sim 0.5\%$).

3.2 Orgueil Filaments with Differentiated Heterocysts. Several genera of the cyanobacterial orders *Nostocales* and *Stigonematales* use specialized cells known as “heterocysts” to fix atmospheric nitrogen. 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 (N_2) into ammonia, nitrates, or nitrogen dioxide. Many species of several genera of cyanobacteria (e.g., *Anabaena*, *Nostoc*, *Calothrix*, *Rivularia*, *Scytonema*, etc.) use highly specialized cells for nitrogen fixation by encapsulating the nitrogenase enzyme in thick-walled protective heterocysts.

Cyanobacteria play the key role in nitrogen fixation on Earth and many genera and species of are capable of diazotrophic growth and nitrogen metabolism. Nitrogen fixation occurs via the nitrogenase enzyme with some other proteins involved in this complex biological process. Since the activity of the nitrogenase enzyme is inhibited by oxygen the enzyme must be protected. In many species it is contained within the thick-walled specialized nitrogen-fixing cells called “heterocysts.” The heterocysts have very distinctive, thick, hyaline, refractive walls that provide well-protected centers in which the nitrogenase enzyme, which is inactivated by oxygen, can carry out its required activity. Heterocysts of cyanobacteria produce three additional cell walls, including one with glycolipids that form a hydrophobic barrier to oxygen. This is crucial since cyanobacteria are aquatic photoautotrophs that evolve oxygen during their photosynthesis. To provide additional protection, the cyanobacterial heterocysts lack photosystem II (Donze *et al.*, 1972). Therefore the heterocysts produce no oxygen and they also up-regulate glycolytic enzymes and produce proteins that scavenge any remaining oxygen. As early as 1949, Fogg recognized that heterocysts are formed from the vegetative cells of the cyanobacteria when the concentration of ammonia or its derivative falls below a critical level and by 1968 it was becoming clear that the heterocysts were the site of nitrogen fixation (Fogg, 1949; Fay *et al.*, 1968; Stewart *et al.*, 1969).

Heterocysts are found in cyanobacteria of the Order *Nostocales* and the Order *Stigonematales*, but they are never found in any of the genera or species of the other three orders (*Chroococcales*, *Oscillatoriales*, or *Pleurocapsales*). Furthermore, heterocysts have not been observed in any the known filamentous sulfur bacteria of any other trichomic prokaryotes. Consequently, the detection of heterocysts provides clear and convincing evidence that the filaments are not only unambiguously biological but that they belong to one of these two orders of cyanobacteria rather than trichomic ensheathed sulfur bacteria or any other group of filamentous trichomic prokaryotes. The presence or absence, location and configuration of heterocysts is a critical diagnostic tool for the recognition and classification of cyanobacterial taxa.

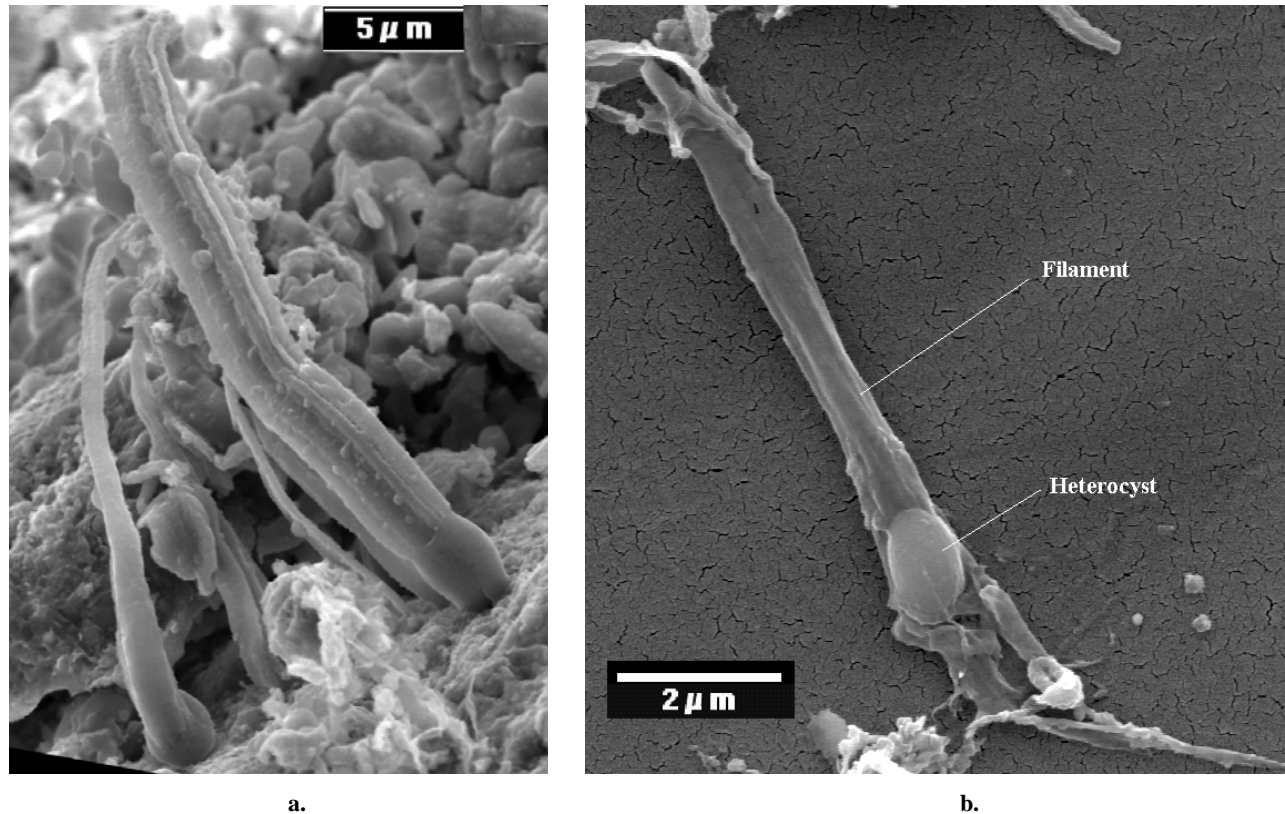


Figure 4.a. FESEM image of permineralized remains in the Orgueil meteorite of polarized tapered filaments (diameter ~ 1 to $2.5 \mu\text{m}$) with recognizable heterocysts interpreted as morphotypes of the cyanobacterium *Calothrix spp.* and **b.** living filament of *Calothrix sp.* with a diameter $\sim 0.8 \mu\text{m}$ and a basal heterocyst from the White River, Washington.

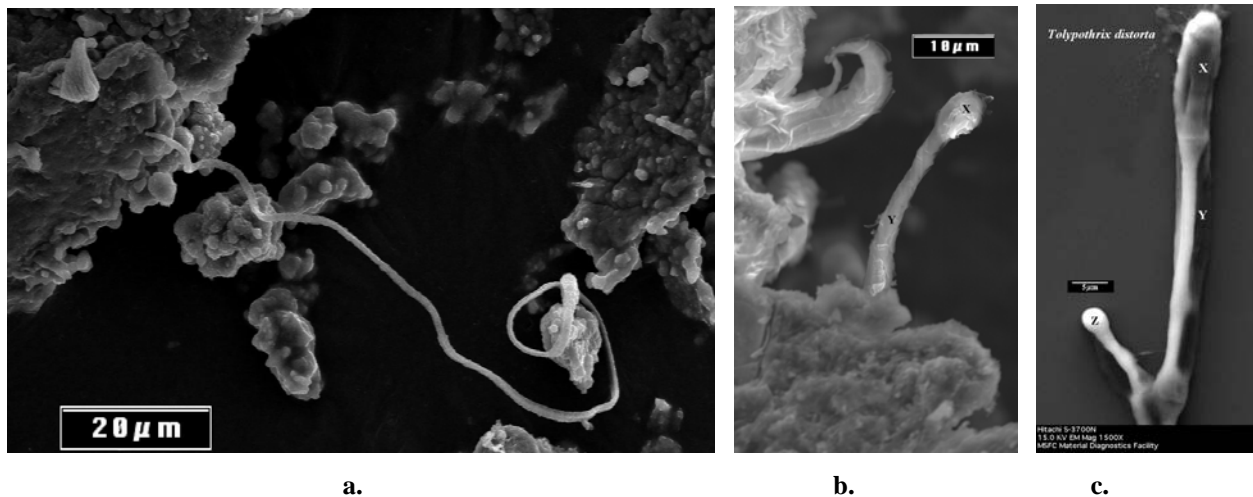


Figure 5. Long sinuous, helical coiled and polarized filament with conical apex ($<1.3 \mu\text{m}$) and terminal heterocyst similar to cyanobacterium *Cylindrospermopsis sp.* in the Orgueil meteorite and **b.** short embedded filament in Orgueil compared with **c.** living *Tolypothrix distorta* grown in pure culture at the NASA/NSSTC Astrobiology Laboratory. *Orgueil Meteorite Sample Courtesy: Dr. Martine Rossignol-Strick, Musée Nationale d'Histoire Naturelle, Paris*

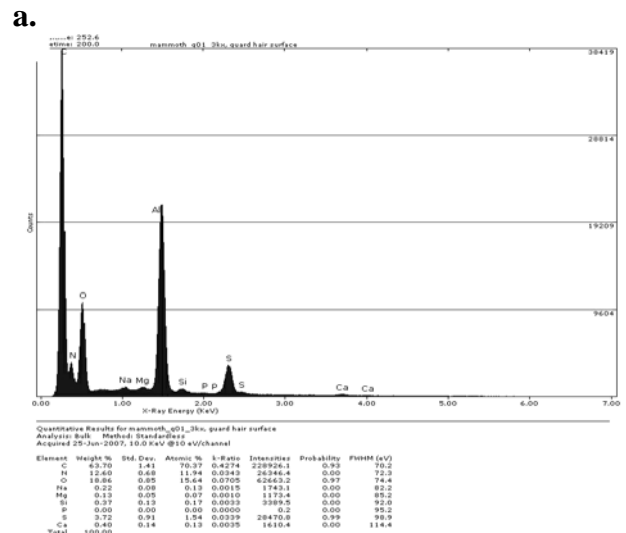
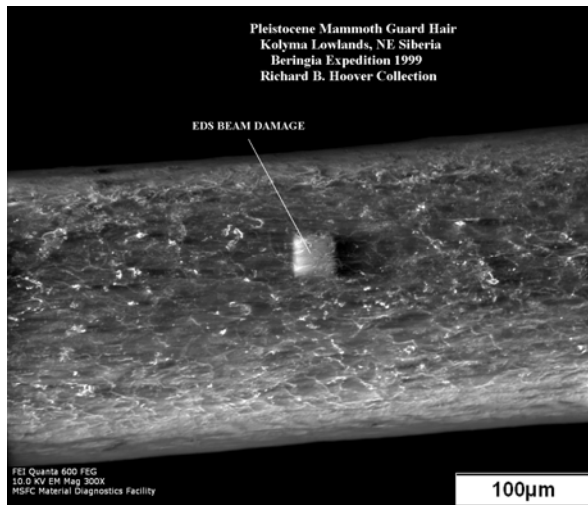
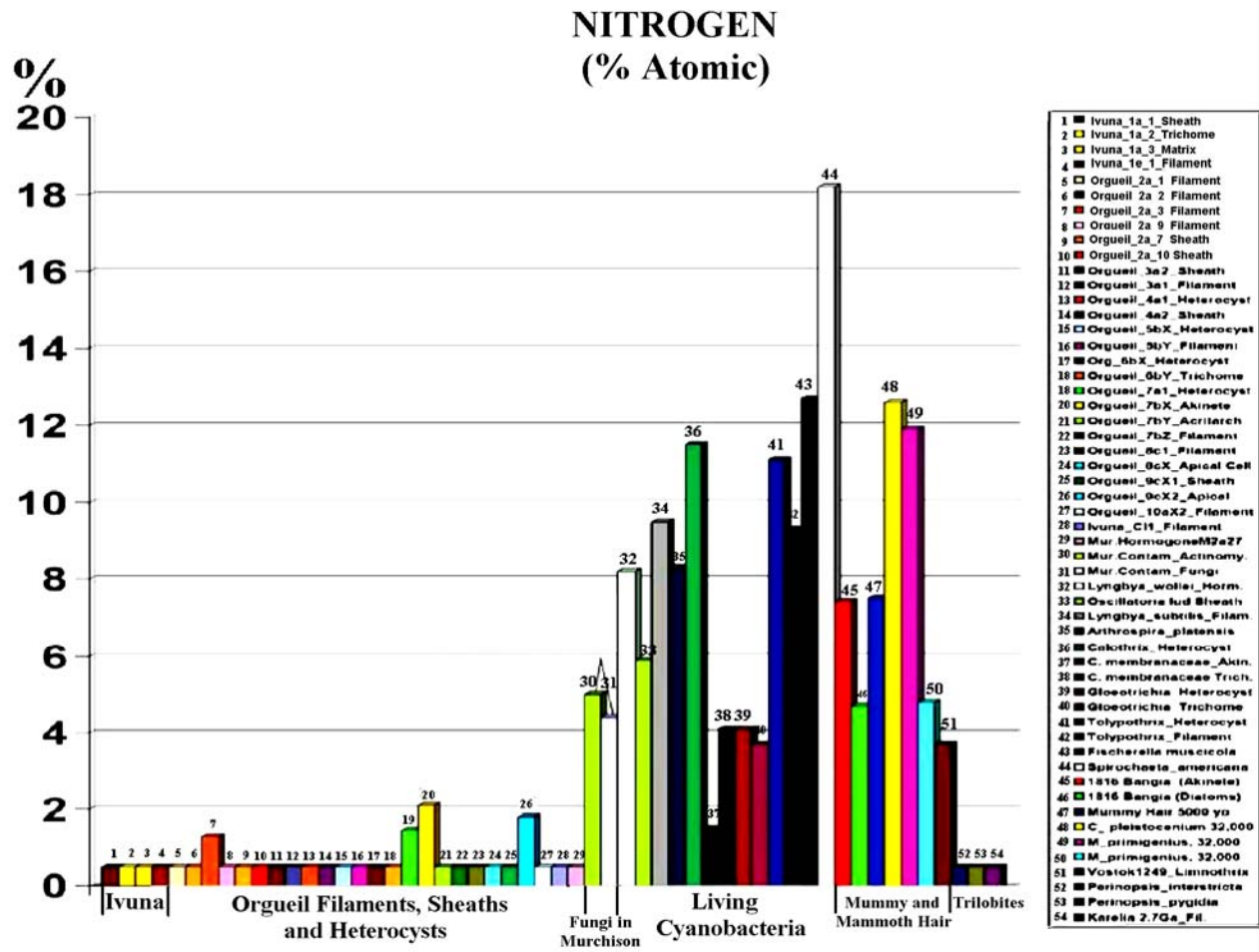
The FESEM image of the mineralized remains of polarized filaments interpreted as morphotypes of the cyanobacterium *Calothrix spp.* found embedded in the Orgueil CI1 carbonaceous meteorite. Several tapering

filaments (diameter ~ 1 to 2.5 μm) and recognizable enlarged cells are seen in close proximity to each other with the smooth basal heterocyst attached to the meteorite matrix (Fig. 4.a). AFESSEM image of a living *Calothrix sp.* (diameter ~ 0.8 μm and basal heterocyst from White River, Washington is shown in **Fig. 4.b**.

Figure 5.a is a Hitachi S4100 FESEM image of helical coiled polarized filament in Orgueil C11 carbonaceous meteorite. The filament has a conical apex (<1.3 μm) at left end and a bulbous (2.3 μm diameter) heterocyst is seen at the other terminus. This filament has size and morphological characteristics of morphotypes of cyanobacteria of 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*. **Fig. 5.c** is an image of a morphotype of living Nostocalean cyanobacterium *Tolypothrix distorta* shown for comparison.

Although many modern cyanobacteria are resistant to desiccation, they do not carry out active growth and mat building when they are in a dried state. However, it has been known since 1864 that the Orgueil meteorite is a microregolith breccia, comprised of minute particulates cemented together by water-soluble salts that are readily destroyed by exposure to liquid water. Therefore, it is suggested that none of the Orgueil samples could have ever been submerged in pools of liquid water needed to sustain the growth of large photoautotrophic cyanobacteria and required for the formation of benthic cyanobacterial mats since the meteorite arrived on Earth. Many of the filaments shown in the figures are clearly embedded in the meteorite rock matrix. Consequently, it is concluded that the Orgueil filaments cannot logically be interpreted as representing filamentous cyanobacteria that invaded the meteorite after its arrival. They are therefore interpreted as the indigenous remains of microfossils that were present in the meteorite rock matrix when the meteorite entered the Earth's atmosphere. EDS elemental analyses carried out on the meteorite rock matrix and on living and fossil cyanobacteria and old and ancient biological materials have shown that the Orgueil filaments have elemental compositions that reflect the composition of the Orgueil meteorite matrix but that are very different from living and old microorganisms and biological filaments. Recently dead cyanobacteria and living cyanobacteria and other modern extremophiles 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 Devonian, Cambrian, or Archaean fossils investigated. The C/N and C/S ratios of the Orgueil filaments are similar to fossilized materials and kerogens but very different from living biological matter, providing further evidence that the Orgueil filaments are not modern biological contaminants.

Figure 6.a is a compilation of the nitrogen level measured by EDS for a number of the filaments encountered in Ivuna and Orgueil C11 carbonaceous meteorites compared with modern and ancient terrestrial life forms. The meteorite filaments are typically severely depleted in nitrogen ($\text{N} < 0.5\%$) whereas life forms on Earth have nitrogen levels from 2% to 15%. Nitrogen is encountered at detectable levels even in the hair/tissue of mummies from Peru (2Kya) and Egypt (5Kya) and the hair and tissues of Pleistocene Mammoths (40-32 Kya). **Fig. 6.b** is a FESEM image the Guard Hair of a 32,000 year old Pleistocene mammoth collected by Hoover in the Kolyma Lowlands of NE Siberia. The square spot in the image is EDS beam damage (10 KeV electron beam) during spot analysis, which revealed a strong peak (11.94% atomic) at the nitrogen $\text{K}\alpha$ line between the Carbon and Oxygen lines. Even though the biological material was 32,000 years old, the nitrogen of the proteins was still present. Similar results have been 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 ancient biological materials (e.g., Cambrian trilobites of Wheeler Shale of Utah (505 Mya) and 2.7 Gya filamentous cyanobacteria from Karelia) have nitrogen levels below the limit of detection with the FESEM EDS detector. These results provide definitive evidence that the filaments in the C11 carbonaceous meteorites are indigenous to the stones and are not the result of microbiota that invaded the stones after they arrived on Earth in 1864 or 1938. Hoover (2007) has discussed 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.



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).

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% H₂O, and ~22% SiO₂; the Wiik Group 2 (e.g. Murchison, Mighei and Cold Bokkeveld) chondrites, have ~4% C, 13% H₂O, and 27.5% SiO₂ 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 re-crystallization. By petrologic type 7 the chondrules are again absent due to thermal destruction.

4.2 Mineralogy, Petrology and Organic Chemistry of CI1 Carbonaceous Meteorites. Cloëz and Pisani conducted the first detailed chemical analysis and study of the mineralogy and the Orgueil meteorite. Pisani (1864) concluded that 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 reported the detection of a variety of evaporite minerals including magnesium, ammonium, calcium and sodium salts. Microscopic and chemical analysis led Cloëz to conclude that the dominant portion of the carbonaceous material within the Orgueil meteorite was in the form of complex polymeric carbon insoluble in water and 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 CI1 carbonaceous meteorites. This work has been summarized in detail by (Tomeoka and Buseck, 1988; Nagy, 1975; Kissin, 2003; Sephton, 2005). It is now established that the CI1 carbonaceous meteorites contain ~65 wt% fine scale phyllosilicate aggregates and intergrowths of serpentine and smectite/saponite, 10% magnetite, sulfides such as aqueously altered iron-nickel sulfides 7% pyrrhotite ([Fe,Ni]_{1-x}S), and 5% ferrihydrite (5Fe₂O₃·9H₂O) and troilite (FeS) as well as 5% recomposed carbonates like Breunnerite (Mg,Fe)CO₃ 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, stacks of platelets) and presolar diamonds, silicon carbide and graphite (Huss and Lewis, 1994). Magnetite and pyrite framboids and platelets are present in the CI1 (Alais, Ivuna, and Orgueil) and C2 Ungrouped (Tagish Lake) carbonaceous meteorites that have been investigated in this 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 framboids are present in the upper Permian black shales of the Berents Sea shelf which are similar in similar size distribution and characteristics as those found in the Alais and other carbonaceous meteorites. Independent studies confirmed the very early findings that the CI1 carbonaceous meteorites contained a complex insoluble organic matter very similar to kerogen as is typically encountered in coal. Boström and Frederickson (1966) described the Orgueil meteorite as a “*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 aqueous alteration sequence (of calcite to dolomite to bruennerite) as the carbonaceous meteorite parent bodies were cooling. They concluded the Orgueil dolomite formed at 26 °C and the bruennerite formed at -6 °C. The Orgueil and Ivuna CI1 meteorites appear to have experienced an extended period of aqueous alteration by acidic hydrothermal fluids that completely destroyed the pentlandite ($[\text{Fe,Ni}]_9\text{S}_8$) which 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 are typically cemented together by epsomite and other water soluble salts.

4.3 Heating of CI1 Carbonaceous Meteorites during Atmospheric Transit. Immediately after the fall of the Orgueil CI1 carbonaceous meteorite, the villagers collected more than 20 jet-black stones. Many of these stones 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 also 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 the interior of the Orgueil stones had the consistency of wet clay-like just after the fall. 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 of the Orgueil stones (as well as those of the Murchison CM2 meteorite that landed in Australia in 1969) were found a few hours after the fall with a thin coating of frost on the outer surface. This finding indicates that the inner portions of the stones were below zero after transit though the atmosphere. The interior portions of these stones were apparently protected by ablative cooling during atmospheric transit in a manner analogous to that experienced during the re-entry of an Apollo Command Module. This is important with regard to the possible transport of bacteria remaining 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 life forms known on Earth. The protein amino acids exhibit homochirality in that they are exclusively the *L*-enantiomer. **Table IV** shows the protein *L*-amino acids in the exopolysaccharide (EPS) slime sheath of the cyanobacterium *Microcystis aeruginosa* K-3A; living cells of the bacteria *E. Coli* and *Salmonella* sp. and ancient terrestrial biology (e.g., a Fly in amber and teeth of a Cretaceous Duck-Billed Hadrosaur) for comparison with extraterrestrial amino acids detected in the Murchison, Murray, Orgueil and Ivuna meteorites (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 either not detected or present at only trace levels in the fossils in terrestrial rocks and carbonaceous meteorites. Even though there is no doubt that the amber encased fly and the Hadrosaur teeth are biological in origin, it is seen that these fossils are also missing several of the same amino acids that absent in the carbonaceous meteorites. Only 8 of the 20 life-critical protein amino acids are detectable in water/acid extracts of carbonaceous meteorites. The fact that several of the amino acids missing in meteorites and ancient terrestrial fossils are abundant in living bacteria provides strong evidence that the meteorites are not contaminated by modern biological materials. If modern bio-contaminants were present, all 20 protein amino acids should be detected.

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

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

The data of **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, in these carbonaceous meteorites, 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.* As has been pointed out by Engel and Macko (2005) these missing protein amino acids provide clear and convincing evidence that the interior portions of the CI1 and CM2 carbonaceous meteorites are not contaminated by modern cyanobacteria, pollen, fingerprints or other microbial contaminants. Isovaline (IVA), α -aminoisobutyric acid (AIB) and γ -Aminobutyric Acid (GABA) are the most abundant non-protein amino acids in carbonaceous meteorites. While they are not protein amino acids it is wrong to conclude that they are not biological in nature. 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 organisms on Earth. However, most protein amino acids are absent in meteorites and terrestrial fossils and only 8 of the 20 life-critical protein amino acids have been found in carbonaceous meteorites using the most sensitive modern methodologies available.

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) and comparable to that of the very dark C-type asteroids and the nuclei of comets. This is blacker than asphalt which has an albedo of ~ 0.07. The European Space Agency Halley Multicolor Camera aboard the Giotto Spacecraft obtained images at the closest approach (00:03:01.84 UT on March 14, 1986) at a distance of 596 km from the centre of the nucleus revealing detailed topographic features on the black (albedo 0.04) surface and jets Lamarre *et al.* (1986) reported that IKS-Vega data indicated the temperature of nucleus of comet Halley was 420 K +/- 60K at 0.8 A.U which was consistent with “*a thin layer of porous black material covering the comet nucleus.*” The Deep Space 1 spacecraft found the 8 km long nucleus of Comet 19P/Borrelly to be very hot (~345 K) with prominent jets aligned with the orientation of the rotation axis of the nucleus and albedo of 0.01 to 0.03 (Soderbloom *et al.* 2002). Ices of water, carbon dioxide, methane and other volatiles in the cold nucleus in proximity to the hot crust would melt and then boil to produce high pressure beneath the crust if gas is released faster than it can escape through the porous crust. In regions where the pressure exceeds the strength of the crust, localized failure of portions of the crust could result in explosive release of the gas giving rise to the observed flaring of comets and the dramatic jets.

Once a comet enters the inner solar system, it becomes hot from solar radiation on the black nucleus and loses mass rapidly. The European Space Agency 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 (CH₄, NH₃ and H₂CO) could have come from clathrates (H₂O ice with simple gasses like CO₂ and NH₃ 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 and detected olivine in the dust. Olivine is commonly encountered in meteorites. As comets lose ices they develop an inert outer crust from the less volatile material. The nuclei of comets are extremely complex – they exhibit rugged terrain, smooth rolling plains, deep fractures and are composed of very dark material. This black crust becomes very hot while the comet is in the inner regions of the Solar System.

Figure 7.a. is a NASA Deep Space 1 spacecraft 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 light level (red is 1/10, blue 1/100 and purple 1/1000 the intensity of the comet nucleus). 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 nucleus. These narrow jets are entirely consistent with the hypothesis that internal pressures generated by steam produced by melting of internal ices which then boil into gases as they are vaporized as heat conducts through hot crust. The NASA Deep Impact probe obtained the valuable data about the nature of comets as it approached and when the impactor collided with the nucleus of comet 9/P Temple 1 on July 4, 2005. **Fig. 7.b** is a Deep Impact image of the nucleus of comet Temple 1. The regions shown in blue are where exposed deposits of water ice that were detected on the surface of the comet nucleus Sunshine *et al.* (2005). These water ice regions were observed to be ~30% brighter than the surrounding areas and probably were exposed when portions of the black crust was blown off into space by the explosive eruptions such as were recorded in a video by the spacecraft. The Deep Impact measurements of the temperature profile of comet P/Temple 1 nucleus at 1.5 AU is shown in **Figure 7.c**. Even as far away from the Sun as Mars the jet-black comet nucleus reaches temperatures as high as 330 K (57 °C). Furthermore, the lowest temperatures measured on the crust were ~ 280 K (7 °C) which is slightly above the temperature at which water ice changes from solid to liquid phase. Prior to the impact, the ambient outgassing 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 outgassing had significant subsurface sources. The Deep Impact spacecraft also observed numerous events of flaring of the nucleus and eruption of geyser-like jets as the comet was approached and before the collision of the impactor. On November 4, 2010, the NASA EPOXI extended mission of the Deep Impact Spacecraft passed within 435 miles of the 2.2 km long nucleus of comet Hartley 2 and revealed bright jets of carbon dioxide gas and dust.

These observations of comets are consistent with the hypothesis that the comet crust impedes the flow of gasses such that pressures develop as ices melt and vaporize in pockets and cavities beneath the crust. This provides the pressures needed to allow water to transition from the solid to the liquid state and then into the gaseous state. This would create micro-niches with pools of liquid water trapped within pockets in rock and ice, very much analogous to the cryoconite and ice bubble ecosystems contained 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). If gas is produced faster than it can escape through the porous crust, it could high pressures resulting in localized failure of weaker portions of the crust and the violent eruption into space of carbon dioxide, water vapor and chunks of crust and particles of ice and dust propelled into space and directed into the dust tail of the comet. These dust particulates could give rise to meteor showers as the comet passes through the tail. From time to time, larger chunks of the ejected may survive passage through the Earth's atmosphere and this could be the link between comets and the 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 stones have been found to contain a large amount of indigenous extraterrestrial water clearly establishes that their parent bodies were most likely comets or water-bearing asteroids. It is now well known that the black nuclei of comets get very hot (significantly above >273 K where water ice melts) as they approach the Sun.

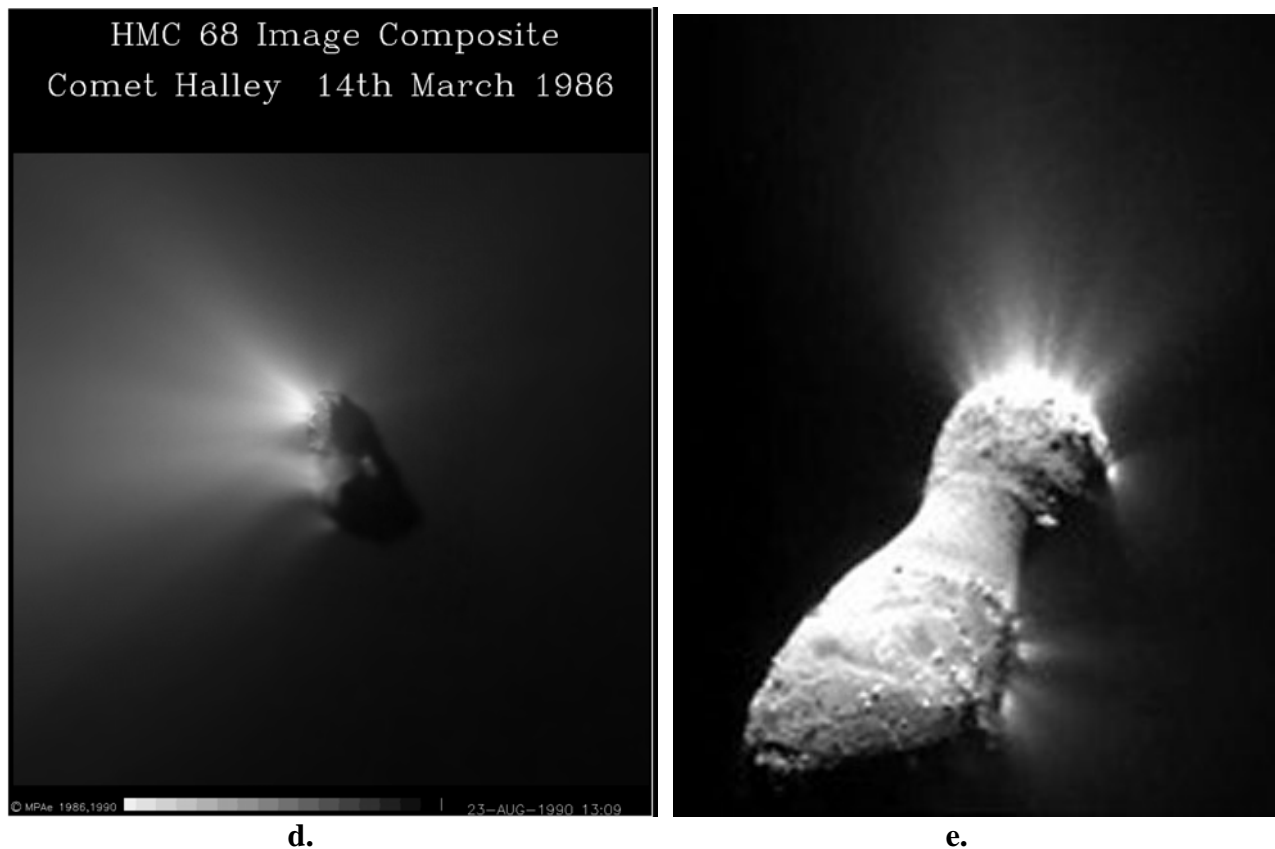
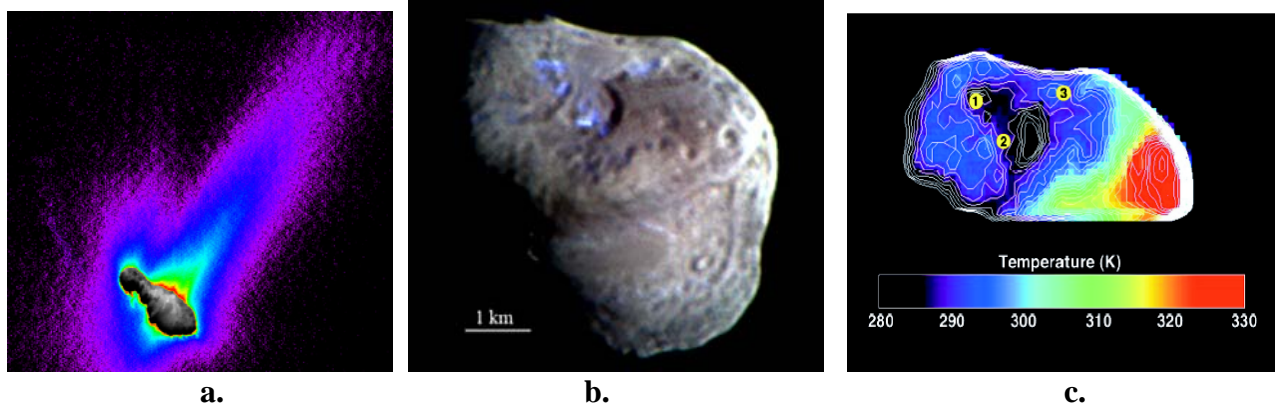


Figure 7. **a.** Deep Space 1 image of Comet P/Borrelly with jets of gas and dust; **b.** Deep Impact image of nucleus of Comet 9P/Tempel 1 shows regions of exposed water ice and **c.** temperature map from Deep Impact IR spectra **d.** Giotto Halley Multicolor Camera (HMC) image showing jets emanating 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).

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 terminal height was ~ 20 km and the pre-atmospheric velocity was > 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 include (although Halley-type comets are not excluded).

The cosmochemistry data for a cometary parent body is entirely consistent with the composition and characteristics of the CI1 meteorites. This suggestion that the parent body of the CI1 carbonaceous meteorites were possibly comets is significant with regard to possible existence of indigenous microfossils in the Alais, Ivuna and Orgueil meteorites. From the extensive evidence of aqueous alteration on the Orgueil parent body and the presence of indigenous water in the Orgueil meteorite it is clear that 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/Tempel-1 have clearly established that these bodies get very hot as they enter the inner regions of the Solar System. It is now clear that any water bearing asteroid with an albedo of the Orgueil meteorite would reach a temperature above 100 C at 1AU. At these temperatures, water ice and other volatiles would be converted to liquid water, steam, and produce an expanding cloud of gas and expelled particulates. Any planetesimal orbiting the Sun and possessing a gaseous envelope and dust tail is traditionally referred to as "comet" rather than an asteroid, and therefore it seems logical that comets represent the most probable parent bodies for these water rich, black meteorites that travel in trajectories that cross the orbit of planet Earth.

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 with 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; Steel, 1998; Owen, 1997).

In 1978, Sill and Wilkening proposed that comets may have delivered life-critical biogenic elements carbon and nitrogen trapped within clathrate hydrates in their icy nuclei. In the same year, Hoyle and Wickramasinghe (1978, 1981, 1982, 1985) have proposed that comets delivered not only water, biogenic elements and complex organic chemicals to the surface of planet Earth, but that they also delivered intact and viable microorganisms. The detection of microfossils of cyanobacteria and other filamentous trichomic prokaryotes in the CI1 carbonaceous meteorites (which are likely cometary crustal remnants) may be interpreted as 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 ratios in the water of comet P/Halley. Delsemme (1998) found that the D/H ratio of the water molecules of comets Halley, Hale-Bopp and Hyakutake were consistent with a cometary origin of the oceans. Dauphas *et al.*, (2000) interpreted the deuterium/hydrogen ratios indicate that the delivery of water and ice to the early Earth during the late Hadean heavy bombardment by comets, asteroids and meteorites helped to cool the Earth's crust and form the early oceans. **Table V** shows data extracted from the Robert *et al.* (2000) compilation of Deuterium/Hydrogen ratios of selected components of the Cosmos.

TABLE V.
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> , 1993
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> , 1995
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 METEORITE			
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

When these bodies are grouped in accordance with their D/H ratio it is easily seen that the telluric inner planets and the LL3 (stony) and SNC (Mars) meteorites have high (~500-16,000) ratios and the gas giants, protosolar nebula, ISM and Galaxies are very low (~15-65). The D/H ratios of the comets (~290-330) and carbonaceous meteorites (~180-370) are much closer to that of Earth (~149) and support the hypothesis that they may have made significant contributions to the formation of the 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 water-bearing asteroids or the nuclei of comets. The 30 m diameter fast-spinning carbonaceous asteroid 1998 KY26 that was discovered on June 2, 1998 has been found to contain 10-20% water. However, the small carbonaceous, water-rich asteroid 1998 KY26 also has color and radar reflectivity 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 suggesting cryovolcanic outgassing. 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. Cassini/Huygens has also detected evidence for cryovolcanic water-ice geysers on Titan and Saturn's moon Enceladus.

5. EVIDENCE OF MICROFOSSILS IN CI1 METEORITES AND LIFE IN ICE: IMPLICATIONS TO POSSIBLE LIFE ON COMETS, EUROPA, AND ENCELADUS

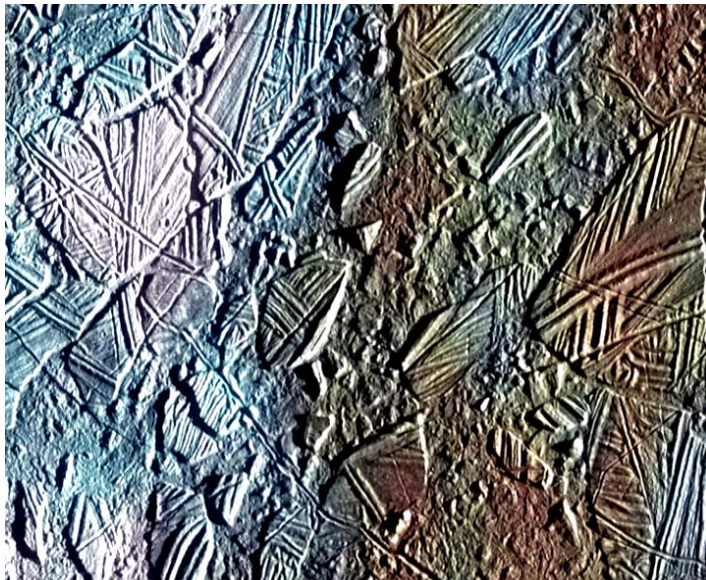
The detection of evidence of viable microbial life in ancient ice (Abyzov *et al.*, 1998, 2003; Hoover and Pikuta, 2010) and the presence of microfossils of filamentous cyanobacteria and other trichomic prokaryotes in the CI1 carbonaceous meteorites has direct implications to possible life on comets and icy moons with liquid water oceans of Jupiter (e.g. Europa, Ganymede or Callisto) and Enceladus (**Fig. 8.a**) Saturn's spectacular moon that is exhibiting cryovolcanism and spewing water, ice and organics into space from the region of the blue and white "tiger stripes."



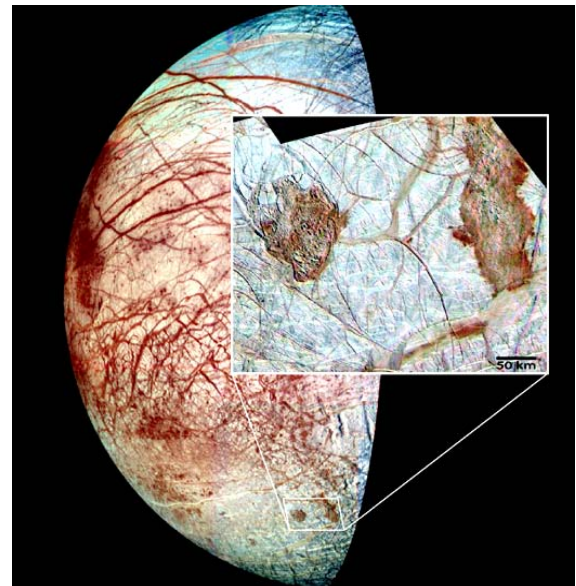
a.



b.



c.



d.

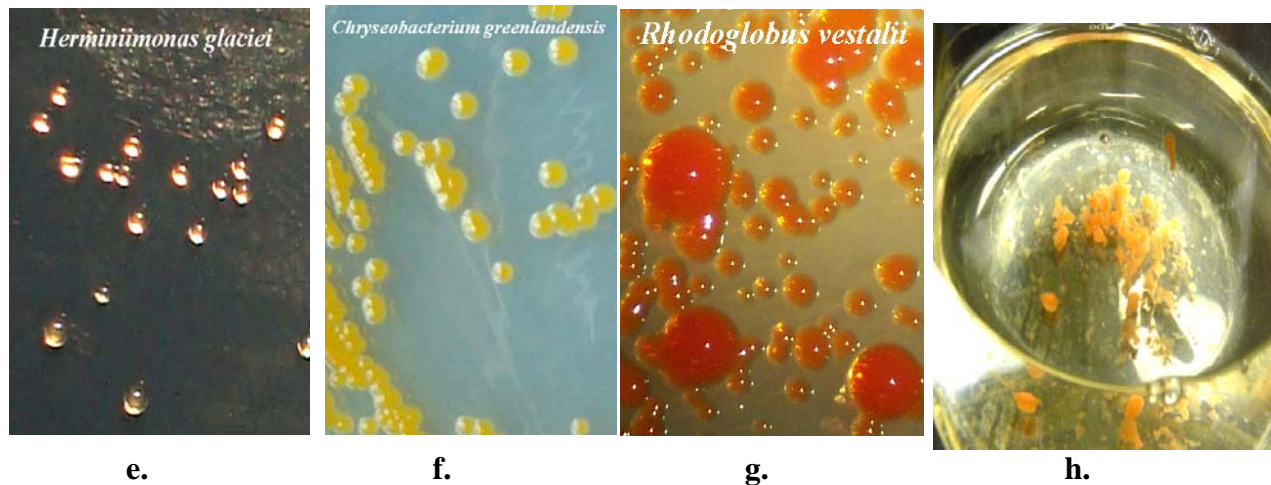


Figure 8.a. Blue and white snow and glacial ice tiger stripes on Saturn's moon Enceladus and **b.** ice bubbles from Lake Glubokoye, Antarctica. Color images from the Galileo spacecraft of **c.** the Conamara Chaos region and **d.** the Thrace region of Europa. Colonies of pigmented bacteria from the deep Greenland glacier ice core: **e.** red *Herminiimonas glaciei* and **f.** yellow *Chryseobacterium greenlandensis*. From the McMurdo Valley ice sheet are colonies of **g.** red *Rhodoglobus vestalii*; and from the Schirmacher Oasis Ice Cave **h.** the red-ochre colony of *Hymenobacter* sp. strain IS118C-5s. Photos Courtesy: **a.,c., & d.** NASA/JPL/Arizona State University; **b.** R. B. Hoover; **e.,f.,&g.** Jennifer Loveland-Curtze/Penn State University and **h.** Asim K. Bej/UAB

Europa exhibits red, orange, yellow and ochre colors and fractured regions indicating the icy crust is floating on a liquid water ocean. The possibility of life on Europa has been discussed by Hoover *et al.* (1986); Chyba *et al.* (2001) and Dalton *et al.* (2003). Hoover *et al.* (1986) argued while deep blue and white colors in the Galileo images of the Jovian moon Europa were typical of glacial ice, ice bubbles and snow on Earth as seen in this image of ice bubbles from the Schirmacher Oasis of East Antarctica (**Fig. 8.b**). 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**) are consistent with microbial pigments rather than evaporite minerals. The 1986 paper suggested that the colors seen in Europa images resulted from microbial life in the upper layers of the ice. A number of more recent studies have been published concerning the significance of ice microbiota to the possibility of life elsewhere in the Solar System.⁷⁶⁻⁸⁰ Diatoms are golden brown and cyanobacteria exhibit a wide range of colors from blue-green to red, orange, brown and black. Bacteria recovered from ice are often pigmented. For example, the extremophiles isolated from the ancient Greenland ice cores produce pigmented colonies. *Herminiimonas glaciei* colonies are red (**Fig. 8.e**) and the colonies of "*Chryseobacterium greenlandensis*" exhibit yellow pigments (**Fig. 8.f**). **Figure 5.c.** shows the red pigmented colonies of the new genus of psychrophile, *Rhodoglobus vestalii* isolated from a lake near the McMurdo Ice Shelf, Antarctica (Sheridan *et al.* 2003). Colonies of *Hymenobacter* sp. (**Fig. 8.d**) isolated from the Schirmacher Oasis Ice Cave are red-ochre in color (Hoover and Pikuta, 2009, 2010). 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).

6. CONCLUSIONS

It is concluded that the complex filaments found embedded in the CI1 carbonaceous meteorites represent the remains of indigenous microfossils of cyanobacteria and other prokaryotes associated with modern and fossil prokaryotic mats. Many of the Ivuna and Orgueil filaments are isodiametric and others tapered, polarized and exhibit clearly differentiated apical and basal cells. These filaments were found in freshly fractured stones and are observed to be attached to the meteorite rock matrix in the manner of terrestrial assemblages of aquatic benthic, epipelagic, and epilithic cyanobacterial communities comprised of species that grow on or in mud or

clay sediments. Filamentous cyanobacteria similar in size and detailed morphology with basal heterocysts are well known in benthic cyanobacterial mats, where they attach the filament to the sediment at the interface between the liquid water and the substratum. The size, size range and complex morphological features and characteristics exhibited by these filaments render them recognizable as representatives of the filamentous *Cyanobacteriaceae* and associated trichomic prokaryotes commonly encountered in cyanobacterial mats. Therefore, the well-preserved mineralized trichomic filaments with carbonaceous sheaths found embedded in freshly fractured interior surfaces of the Alais, Ivuna, and Orgueil C11 carbonaceous meteorites are interpreted as the fossilized remains of prokaryotic microorganisms that grew in liquid regimes on the parent body of the meteorites before they entered the Earth's atmosphere.

The Energy Dispersive X-ray spectroscopy data reveals that the filaments detected in the meteorites typically exhibit external sheaths enriched in carbon 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 purine and pyrimidine nitrogen bases of the nucleotides of all life on Earth.

Extensive EDS studies of living and dead cyanobacteria and other biological materials have shown that nitrogen is detectable at levels between 2% and 18% (atomic) in cyanobacterial filaments from Vostok Ice (82 Kya) and found in stomach milk the mammoth Lyuba (40 Kya); mammoth hair/ tissue (40-32 Kya); pre-dynastic Egyptian and Peruvian mummies (5-2 Kya) and herbarium filamentous diatom sheaths (1815). However, Nitrogen is not detected in ancient biological materials such as fossil insects in Miocene Amber (8 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 C11 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. This finding has direct implications to the distribution of life in the Cosmos and the possibility of microbial life on in liquid water regimes of cometary nuclei as the travel within the orbit of Mars and in icy moons with liquid water oceans such as Europa and Enceladus.

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