

Filaments in Carbonaceous Meteorites: Mineral Crystals, Modern Bio-Contaminants or Indigenous Microfossils of Trichomic Prokaryotes?

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ABSTRACT

Environmental (ESEM) and Field Emission Scanning Electron Microscopy (FESEM) investigations have resulted in the detection of a large number of complex filaments in a variety of carbonaceous meteorites. Many of the filaments were observed to be clearly embedded the rock matrix of freshly fractured interior surfaces of the meteorites. The high resolution images obtained combined with tilt and rotation of the stage provide 3-dimensional morphological and morphometric data for the filaments. Calibrated Energy Dispersive X-ray Spectroscopy (EDS) and 2-D elemental X-ray maps have provided information on the chemical compositions of the filaments and the minerals of the associated meteorite rock matrix. These observations are used to evaluate diverse hypotheses regarding the possible abiotic or biogenic nature of the filaments found embedded in these meteorites.

KEYWORDS: Carbonaceous Meteorites, Orgueil, Murchison, FESEM, EDS, Minerals, Microfossils, Bio-Contaminants, Trichomic Prokaryotes, Filaments

1. INTRODUCTION

In 1996, McKay *et al.*¹ announced the detection by Scanning Electron Microscopy of putative nanofossils in the SNC meteorite ALH84001 from Mars. They were found in close association with a suite of associated biomarkers (carbonate globules, magnetites and polycyclic aromatic hydrocarbon) that were interpreted as enhancing the probability that they were biogenic in origin. However, critics argued that they were not indigenous microfossils but rather abiotic coating artifacts, high temperature minerals or recent bio-contaminants.²⁻⁴ A few weeks after the ALH84001 paper was published, Hoover⁵ initiated a search for evidence of microfossils in the Murchison CM2 carbonaceous meteorite using an Environmental Scanning Electron Microscope (ESEM) at the NASA Marshall Space Flight Center (MSFC). The ESEM was capable of producing high resolution images on uncoated, non-conductive samples of the meteorites. The studies were carried out on uncoated, freshly fractured interior surfaces of the meteorite samples in order to eliminate the possibility that if candidate microfossils were detected they could not be dismissed as coating artifacts or surface contaminants. Rigorous flame sterilization protocols were instigated for all tools, electron microscopy tweezers and mount stubs and the samples were inserted into the ESEM vacuum chamber immediately after mounting to minimize all possibility of contamination during sample preparation. These studies resulted in the detection of a suite of large and complex coccoidal and filamentous microstructures clearly embedded in the associated meteorite rock matrix. The embedded filaments were imaged at several magnifications and diverse orientations by rotation and tilt of the instrument substage. The filament and trichome diameters, sheath thickness, the lengths of the internal cells (when they could be seen directly or inferred from evidence of cross-wall constrictions) were determined by careful comparisons with the calibrated scale bar as provided by the instrument software based for each in accordance with the instrument

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magnification employed. The cellular dimensions and sheath thickness are critical for the identification of many genera and species of cyanobacteria, sulfur-bacteria and other filamentous trichomic prokaryotes. Energy Dispersive X-Ray Spectroscopy (EDS) analysis with electron microscopy element standards and 2-D x-ray maps provided data on the chemical composition of the possible microfossils and proximate meteorite matrix. Visible light images established that some of the candidate microfossils were jet-black and shiny with the appearance of bitumen or kerogen. The EDS quantitative elemental compositions and comparative 2-D X-Ray maps of many of the embedded meteorite filaments revealed the filaments to be permineralized, carbon-rich microstructures or carbonaceous (kerogen-like) electron transparent sheaths in-filled with hydrated magnesium sulfate (e.g., Epsomite $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; Hexahydrite $\text{MgSO}_4 \cdot 6\text{H}_2\text{O}$) or other water-soluble salts. Coccoidal forms and filaments had been found in carbonaceous meteorites by earlier workers and interpreted as indigenous remains of algae, acritarchs, cyanobacteria or other filamentous trichomic prokaryotes.⁵⁻¹²

Entirely independent SEM studies of the Murchison (CM2) and Efremovka (CO2) carbonaceous meteorites were carried out in Moscow by Rozanov and his co-workers at the Paleontological Institute (PIN) and at the Institute of Microbiology (INMI) of the Russian Academy of Sciences. They had detected a large suite filaments in entirely different samples from the meteorite collection of the Vernadsky Institute with similar sizes and morphologies to those found at NASA/MSFC. Their measurements of the sizes, size distributions and detailed morphologies of these well-preserved fossilized filaments, led to the independent interpretation of these filaments as indigenous microfossils of cyanobacteria or other filamentous trichomic prokaryotes.⁶ Later collaborative studies resulted in additional discoveries of large filamentous microstructures.⁷ During the past 14 years, many large, complex filaments, consortia and mat assemblages have been found with undeniable biological characteristics that were interpreted as indigenous microfossils in carbonaceous meteorites during both independent and collaborative SEM, ESEM and FESEM investigations carried out in the United States and Russia.⁸⁻²² Many of the filamentous and coccoidal microstructures found during this research were consistent with forms found half a century ago by earlier researchers (e.g., Nagy, Claus, Fitch, Palik, Timofeyev and van Landingham) and interpreted as indigenous microfossils of blue-green algae (cyanophytes), bacteria, and acritarchs or dismissed as recent bacterial contaminants or pollen grains.²³⁻³²

The filaments detected at NASA/MSFC were imaged with secondary and backscattered electron detectors and analyzed by EDS to obtain spot data and 2D x-ray maps at voltages ranging from 5 keV to 15 keV. Very abundant and extremely well preserved filaments were found in the Orgueil (CI1), Ivuna (CI1) and Murchison (CM2) meteorites. High resolution SEM, ESEM, and FESEM Secondary and Backscattered Electron images and calibrated measurements of the filaments revealed that they exhibited morphology, size and size range were consistent with known and recognizable genera and species of cyanobacteria and other filamentous trichomic prokaryotes. Filamentous microstructures of apparent biogenic origin were notably absent in three of the carbonaceous meteorites studied - Allende (CV3), Kainsaz (CO3), and Karoonda (CK4).

Possible biogenic filaments were never detected in any of the ordinary chondrites, HED basaltic achondrites or iron meteorites investigated during this research effort. The majority of the carbonaceous meteorites studied were observed falls and the stones were recovered soon after their arrival on Earth, but a few were finds with relatively long residence times before they were recovered. Morphological and morphometric data along with EDS elemental analyses of the Carbon, Magnesium, and Sulfur enhancements and O/C and O/S ratios and Nitrogen deficiency of the Orgueil and Ivuna meteorite filaments show they are not modern bio-contaminants.³³ ESEM and FESEM images and EDS analysis of living and fossil cyanobacteria were provided in support of the hypothesis that the forms were both undeniably biological in origin and indigenous to the meteorites. Ancillary evidence from the investigations of other workers was presented concerning the presence in these meteorites of indigenous biomolecules (8 of the 12 protein amino acids, and 3 of the 5 nucleobases of DNA and RNA) and extraterrestrial biomarkers (Pristane, Phytane, Porphyrins). These observations, combined with their failure to detect other life-critical biomolecules in the meteorites (e.g., chlorins, 12 of the 20 protein amino acids, the sugars ribose or de-oxyrribose, and the complementary base pair nucleobases (cytosine and thymine), were interpreted as providing clear and convincing proof that these stones had not been contaminated by modern (post-arrival) biological contaminants. This assessment of the available data led to the conclusion that the filaments found in the Orgueil and Ivuna meteorites were indigenous microfossils that were present in the stones when they entered the earth's atmosphere and hence represented the mineralized remains of extraterrestrial life-forms.

2. INSTRUMENTS AND METHODS

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)

Olympus BH-2 Visible Light Microscope

- High Magnification Dark Field Video Microscopy

Zeiss Phase Contrast/Epifluorescence Microscope

Prior to EDS analysis the instruments were routinely calibrated using the Electron Microscopy Sciences (EMS) Biological Microanalysis Reference Standards. To protect the samples from biological contamination events, all tools, sample holders and mount stubs were flame sterilized prior to use. Lunar dust samples and silicon wafers were used as negative controls. After the meteorite samples mounted on electron microscopy stubs had been examined and were no longer under study, they were returned to their own sealed containers and maintained in the lab in desiccator cabinets. The bulk meteorite specimens that had not been fractured were kept in tightly sealed, sterile vials in the Revco freezer at -80 °C. During the investigations, the meteorite fusion crust and old cracks in the stones were carefully avoided. 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. Solvents, acids or other liquids (such as were used by early researchers to extract “acid resistant microfossils” from the host rock) were avoided as they may have been a source of contamination in these studies. All tools, sample holders and stubs were flame sterilized. Lunar dust samples and silicon wafers were used as negative controls.

3. SAMPLES

The majority of the meteorite samples were provided on loan for this research by museums and meteorite collections from around the world. Several of the L4, L5, and H5 stones (TIL 99001, TIL 99003, TIL 99004, TIL 99009, TIL 99017) were collected by Sipiera, Hoover and other team members in the blue ice fields of the Moulton Escarpment of the Thiel Mountains of Antarctica during the *Antarctica 2000 International Astrobiology Expedition*.³⁴⁻³⁶ Meteorites collected during this expedition are currently deposited in the *Robert A. Pritzker Center for Meteoritics and Polar Studies* of the Field Museum. A few of the samples were purchased from reputable meteorite dealers (Holbrook, Tatahouine, Murchison). The following museums and meteorite collections provided the samples used in this research: *Musée Nationale d'Histoire de Paris*, France (Orgueil, Alais); *Victoria Museum*, Melbourne, Australia (Murchison, Karoonda, Rainbow); *Field Museum*, Chicago, USA (Murchison); *Vernadsky Institute*, Moscow, Russia (Mighei, Murchison, Murray, Nogoya, Kainsaz, Efremovka, Nikolskoye, Barratta); *James M. DuPont Meteorite Collection of the Planetary Studies Foundation*, Chicago, Ill. (Ivuna, Orgueil, Tagish Lake, Acfer 324, Dar al Gani 749, Allende). All of the carbonaceous meteorites examined were found to be extremely heterogeneous with regard to chemical composition and the distribution of the filaments. In some regions of some samples, filaments were found to be very abundant and present as individuals or in thick mats, whereas in other samples of the same meteorite they were scarce. In many meteorite samples, filaments were not detected, even though an extensive search was carried out. The meteorites shown in **boldface** font have been found to contain areas in which abundant filaments are readily found. Samples of the stones in regular font contained filamentous microstructures interpreted as biological remains, but they were not as frequently encountered as in those shown in bold. Filaments that have been interpreted as biogenic in origin have not yet been

detected in any of the stones indicated in *Italics*. It should be noted that a few of the carbonaceous meteorites: *Karoonda* (CK4); *Kainsaz* (CO3); *Allende* (CV3) and all of the Ordinary Chondrites, Achondritic Diogenites and Iron Octahedrites examined exhibited a complete absence of biological-like filamentous microstructures, while filaments were present in the vast majority of the carbonaceous meteorites investigated. Font style is used to indicate relative abundance of filaments in the meteorites:

Bold: Filamentous microstructures with biogenic characteristics found in all samples and abundant in some.

Regular: Filamentous microstructures with biogenic characteristics found in some samples.

Italics: Filamentous microstructures with biogenic characteristics not found in any of the samples examined.

Carbonaceous Chondrites

CI1: *Alais* (Fall, 1806); *Ivuna* (Fall, 1938); *Orgueil* (Fall, 1864)

C2 Ungrouped: *Tagish Lake* (Fall, 2000)

CM2: *Murchison* (Fall, 1969); *Mighei* (Fall, 1889), *Murray* (Fall, 1950); *Nogoya* (Fall, 1879)

CR2: *Acfer 324* (Find, 2001)

CK4: *Karoonda* (Fall, 1930);

CO3: *Rainbow* (Find, 1994); *Dar al Gani 749* (Find, 1999), *Kainsaz* (Fall, 1937)

CV3: *Allende* (Fall, 1969), *Efremovka* (Find, 1962)

Ordinary Chondrites, Achondrites, and Iron Meteorites

L4 Chondrite: *Barratta* (Find, 1845); *TIL 99001*; *TIL 99004*, *TIL 99017* (Find, 2000, Thiel Mountains, Antarctica)

L4-5 Chondrite: *Nikolskoye* (Fall, 1954)

L5 Chondrite: *Thiel Mts. TIL 99009* (Find, 2000, Thiel Mountains, Antarctica)

L/LL6: *Holbrook* (Fall, July 19, 1912)

H5: *TIL 99003* (Find, 2000, Thiel Mountains, Antarctica)

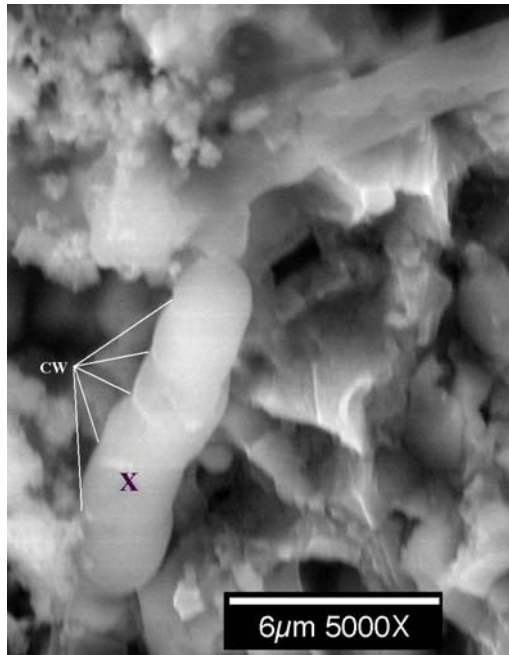
Achondritic Diogenite: *Tatahouine* (Fall, 1931)

Iron (IIIB) Medium Octahedrite: *Henbury*, (Find, 1931, Henbury, Northern Territory, Australia)

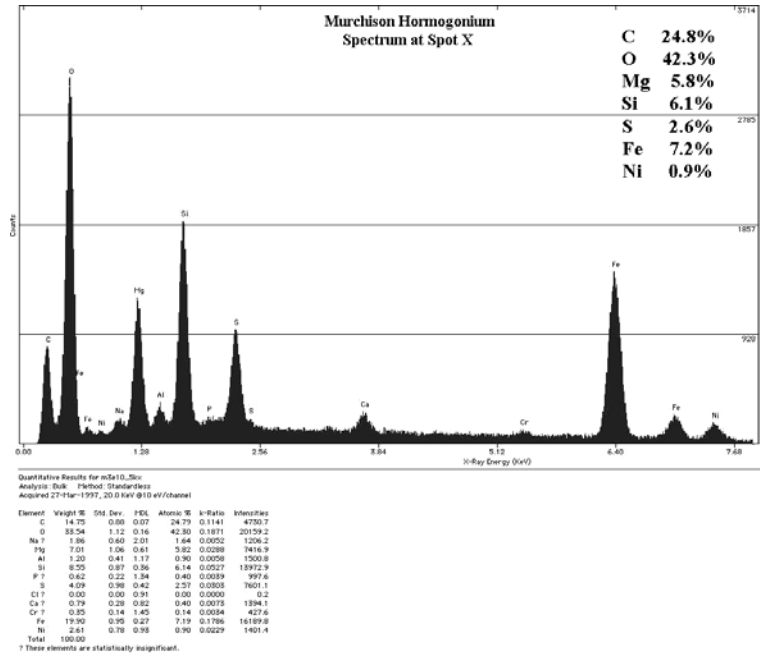
4. RESULTS

Scanning Electron Microscopy studies of freshly fractured interior surfaces of carbonaceous meteorites carried out since 1996 at NASA/MSFC and PIN have resulted in the detection of a wide variety of large and complex filaments, many of which are clearly embedded in the rock matrix of the meteorites. Filaments and filamentous mats have been found to be most abundant in some samples of the *Orgueil*, *Ivuna* and *Alais* (CI1), *Tagish Lake* (C2 Ungrouped) and the *Murchison* (CM2) carbonaceous meteorites. These carbonaceous meteorites are observed falls that were recovered soon after they arrived on Earth. The CI1 meteorites are fragile micro-regolith breccias with mineral grains cemented together by magnesium sulfate or other water soluble salts, and are readily disaggregated by exposure to liquid water. The meteorite samples are extremely heterogeneous. Filaments and mats are can be abundant in one region of a sample and then scarce in nearby areas of the same stone. However, filaments have been notably absent in all of the ordinary chondrites, achondrites, and iron meteorites examined even though most of these meteorites were “Finds” that had been on Earth for long periods of time before recovery. Energy Dispersive X-ray Spectroscopy (EDS) was used to determine elemental composition of the filaments and associated meteorite matrix. The filaments have been found to be typically enriched in carbon as compared with proximate regions of the meteorite. The EDS spot data reveals that nitrogen level of the filaments is very rarely above the detectability limits of the Energy Dispersive X-Ray Spectroscopy instrumentation. Measurements with known standards have shown that the instrument is capable of detecting nitrogen at levels as low as 0.2% under ideal conditions and above 0.5% under worst case conditions. Studies of the nitrogen content as measured by EDS have shown that significantly higher nitrogen levels (2-18%) are present in living cyanobacteria and even in long dead cyanobacteria, diatoms, and other biological materials (hair and tissues of ancient mummies or Pleistocene algae and mammoths) but absent in Miocene and Cambrian fossils.³⁵

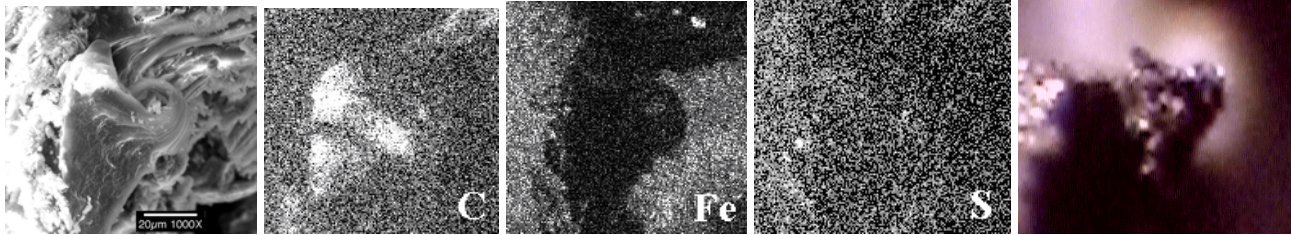
Images and EDS spectra are provided for selected filaments found embedded in the carbonaceous meteorites: *Murchison* CM2 (Fig. 1a.-d.); *Ivuna* CI1 (Fig. 2) and *Orgueil* CI1 (Fig. 1.e., Fig. 3, Fig. 4). Fig. 1.e is an annotated drawing from the 1962 *Nature* paper by Palik, showing filaments she recovered from the *Orgueil* meteorite and interpreted as remnants of algae is provided for comparison with the tapered filament with calyptrate apical cell found in the *Murchison* meteorite.



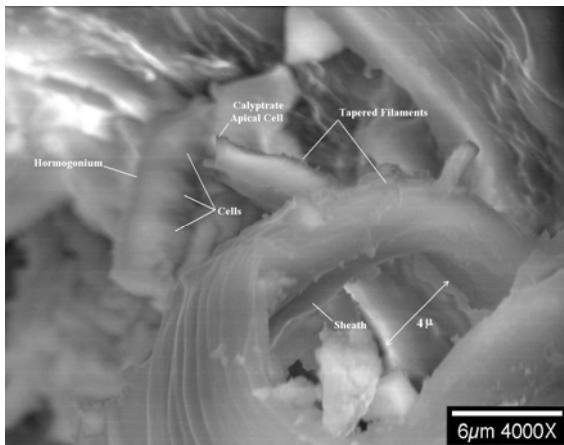
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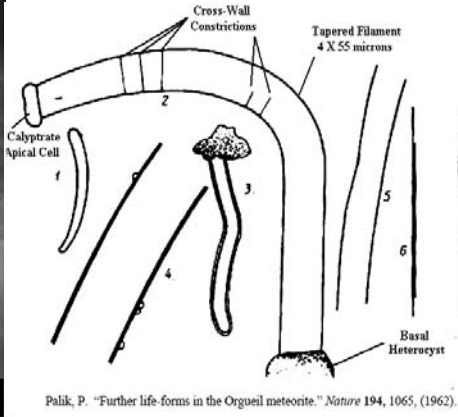
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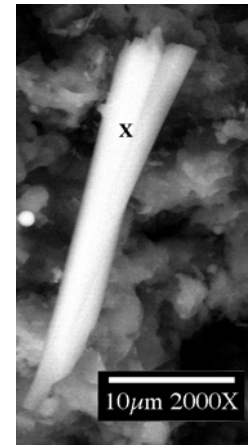
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d.



e.



f.

Fig. 1.a. Cross-Wall CW constrictions of hormogonium in Murchison with indicates cell size $\sim 2.7 \mu\text{m}$ broad; $\sim 1.2 \mu\text{m}$ thick. **b.** EDS spectrum at spot X shows major elements (C, O, Mg, Si, S, Fe, and Ni); **c.** FESEM image of Murchison filaments with 2D X-ray maps in C, Fe, and S and color photograph showing the . **d.** Large Murchison filament with lamellated sheath near hormogonium and $4 \mu\text{m}$ diameter tapered filament with calytrate apical cell that is similar in size and morphology to **e.** Palik³⁴ drawings “algal filaments” in Orgueil with cross-wall constrictions and calytrate apical cell. **f.** abiotic mineral crystal (cf. ferroan epsomite) in Orgueil meteorite *Sample for Images a. to d.* Courtesy: Dr. Bill Birch, Victoria Museum; *Sample for Image f.* Courtesy: Dr Claude Perron, MHNP.

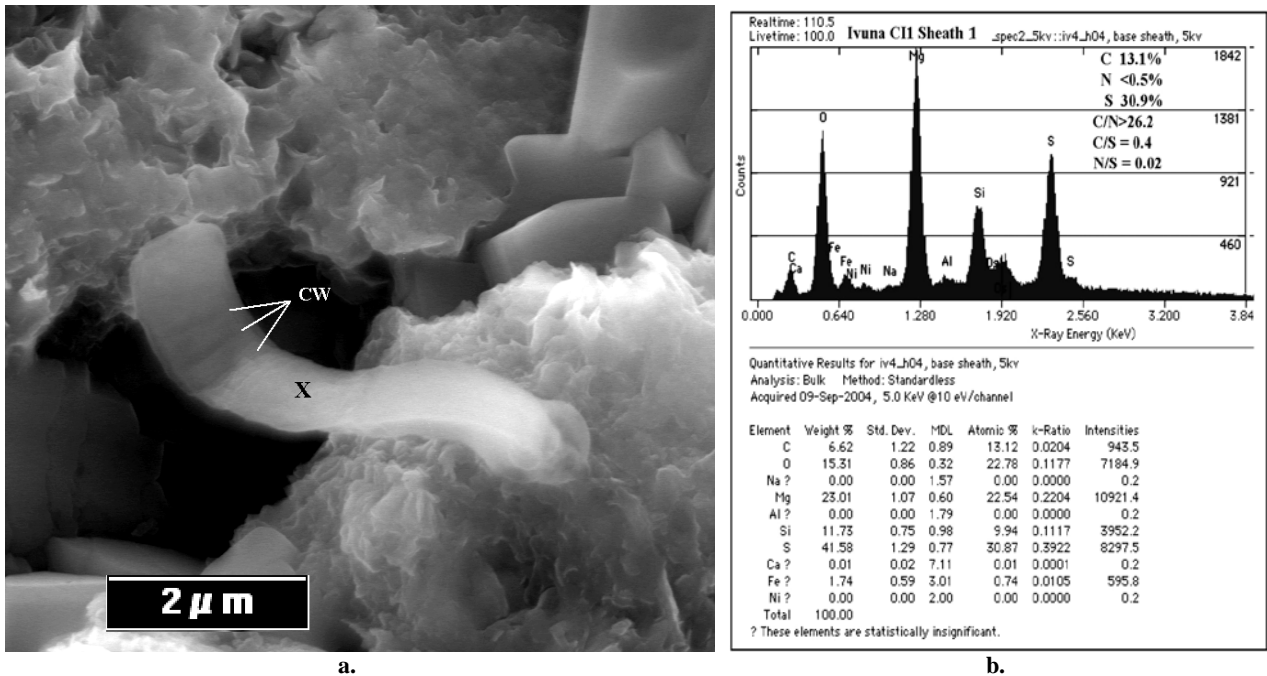


Fig. 2. a. Flattened undulatory $\sim 1.0 \mu\text{m}$ diameter filament with sheath embedded in Ivuna CI1 meteorite annotated to indicate cross-wall CW constrictions. Filament interpreted as morphotype of cyanobacteria (cf. *Spirulina subtilissima*) or sulfur bacteria (cf. *Thioploca minima*). **b.** EDS elemental composition (atomic) at spot X: S 31%; Mg 23%; O 23%; C 13%; Si 10%; Ni < 0.5%. *Ivuna Sample Courtesy: Dr. Paul Sipiera, DuPont Meteorite Collection, Planetary Studies Foundation*

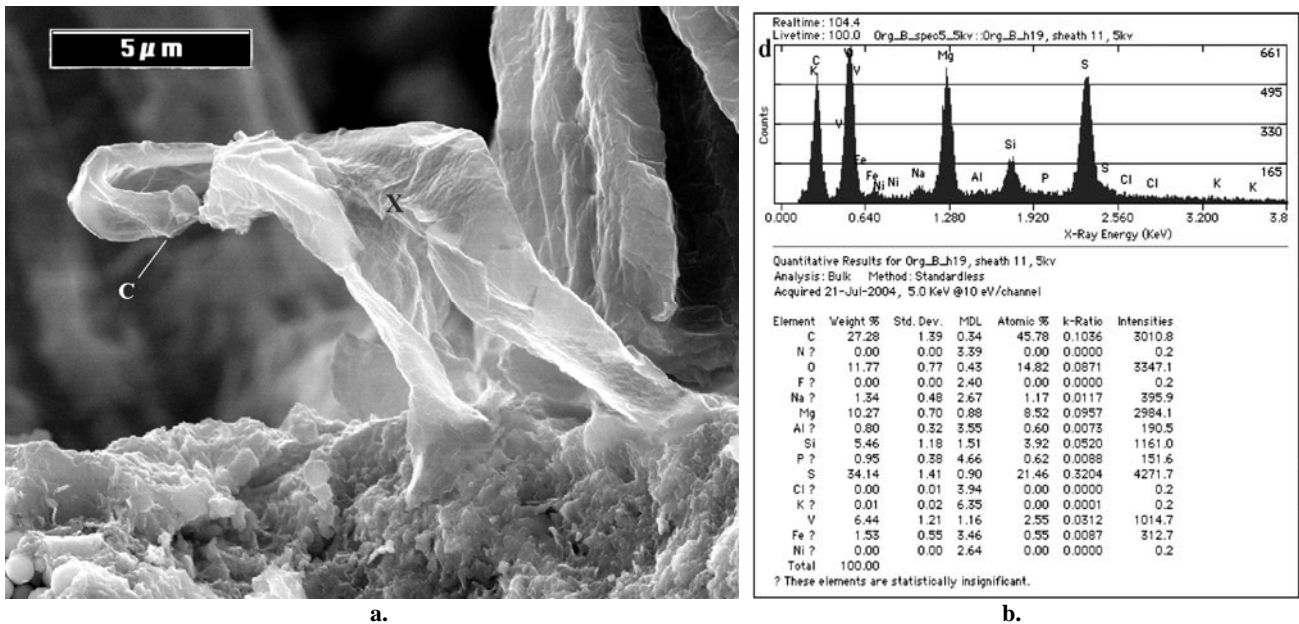


Fig. 3.a. FESEM image (6000X) of hook-shaped, tapered filament with $1.1 \mu\text{m}$ high calyptra C. Uniseriate flattened filament is $8.1 \mu\text{m}$ wide at base indicating circular diameter $\sim 4.5 \mu\text{m}$; tapering to $1.8 \mu\text{m}$ diameter just after the bend consistent with morphotype of *Phormidium dimorphum*. **b.** EDS data at spot X shows filament composition is C 45.8%; N < 0.5%; O 14.8%; Mg 8.5%; Si 3.9%; S 21.5%; V 2.6%. Nitrogen is not detected. *Orgueil Sample Courtesy: Dr. Paul Sipiera, DuPont Meteorite Collection, Planetary Studies Foundation.*

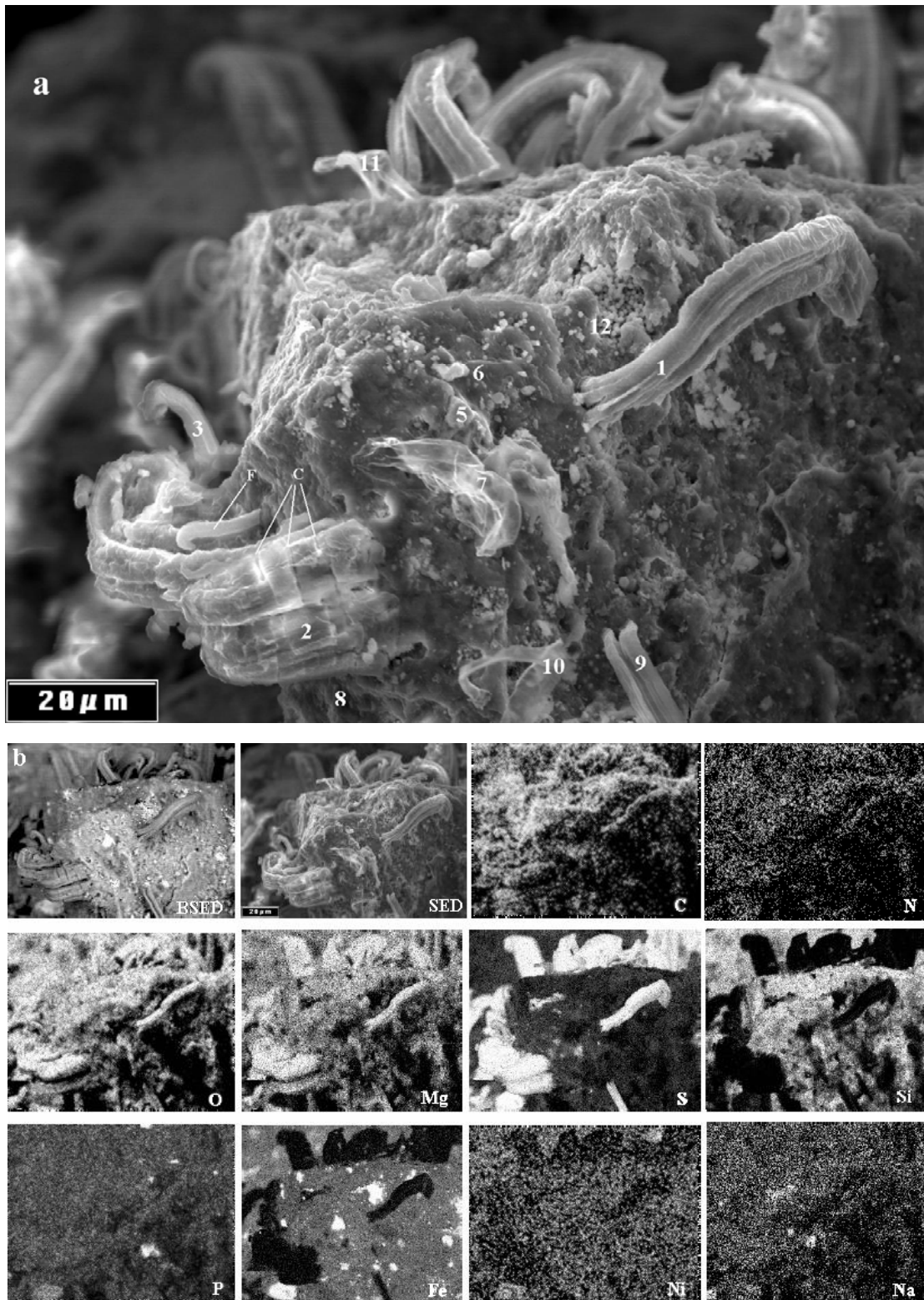


Fig. 4.a. Hitachi FESEM Secondary Electron Detector (SED) image (1000 X) shows loose sheaths and filaments embedded in Orgueil meteorite matrix; **b.** Backscatter Electron (BSED) and Secondary Electron Detector (SED) image and 2-D x-ray maps shows filaments are enriched in C, O, Mg and S consistent with carbonaceous sheaths in-filled with magnesium sulfate. *Orgueil Sample Courtesy: Dr. Paul Sipiera, DuPont Meteorite Collection, Planetary Studies Foundation.*

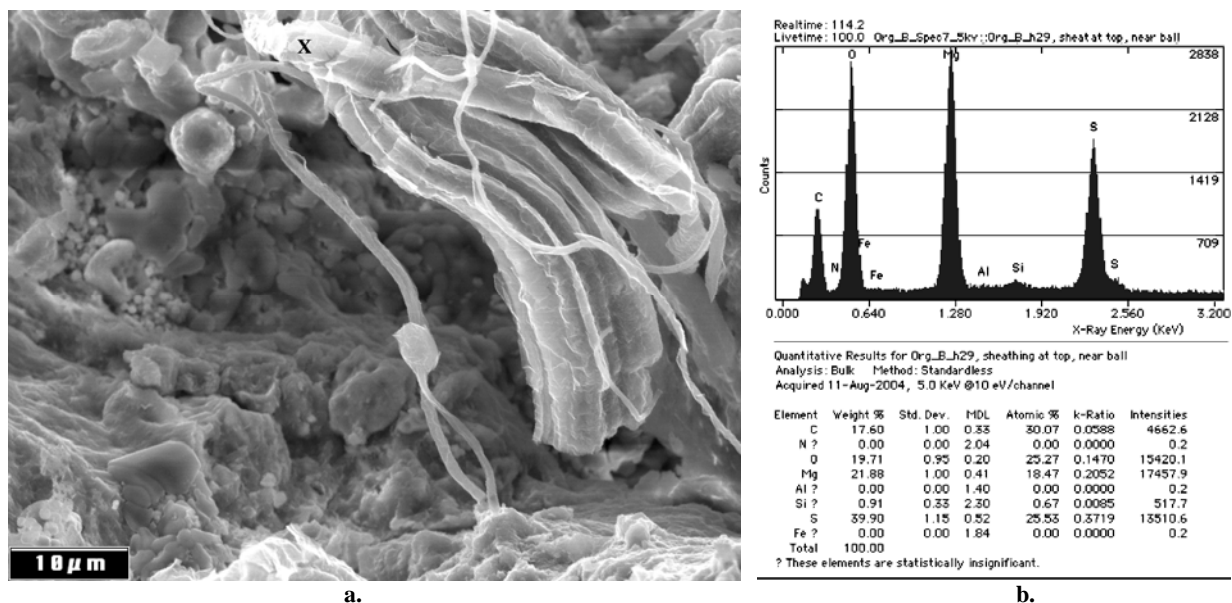


Fig. 5.a. Cyanobacterial mats with branched uniseriate and multiseriate ensheathed filaments and heterocysts in Orgueil CII carbonaceous meteorite as imaged with Hitachi FESEM with Secondary Electron Detector. **b.** EDS elemental composition of filament at spot X (C 30%; O 25%; Mg 18% and S 26%) indicates the carbonaceous sheath is in-filled with magnesium sulfate. *Orgueil Sample Courtesy: Dr. Paul Sipiera, DuPont Meteorite Collection, Planetary Studies Foundation.*

5. DISCUSSION

In 2011, Hoover³⁸ published a review paper in the Journal of Cosmology that described several of these filaments and interpreted them as the permineralized remains of cyanobacteria and other trichomic prokaryotes that because of undetectable nitrogen by EDS were not modern bio-contaminants but present in the meteorites when they entered the Earth's atmosphere. This conclusion led the Editor-in-Chief of the Journal to augment the ongoing peer-review process by inviting critical commentary on the paper from a large number of scientists. While the peer-review and commentary process was underway and the paper still under embargo, it was obtained by the media triggering unprecedented interest by the general public and discussion in the blogosphere. It was reported that there were over 15 million attempts to access the paper shortly after it was released from embargo and placed online at the Journal of Cosmology website.

Several hypotheses were advanced to dismiss or explain these published results and they will now be considered:

Hypothesis I: The filaments are coating artifact or crystallographic edge effects.

Many of the 20 nm to 300 nm forms interpreted by McKay *et al.*¹ as possible fossils of nanobacteria in ALH84001 were dismissed as coating artifacts produced by the heavy metal coatings used to make the samples conductive.^{39, 40} An awareness of this potential problem, when the search for evidence of microfossils in carbonaceous meteorites was initiated at NASA/MSFC in late 1996, an Environmental Scanning Electron Microscope (ESEM) was employed. The ESEM was chosen for the primary reason that uncoated and non-conductive samples could be studied and high spatial resolution images at high magnifications obtained without charging effects. More recently, uncoated meteorite samples have been examined using more sophisticated Field Emission Scanning Electron Microscope (FESEM) instruments. Since the samples were uncoated, it is possible to readily rule out Hypothesis I. Furthermore, the filaments that were encountered were far larger than the “nanobacteria” and exhibited detailed and complex, contoured morphologies and highly differentiated chemical compositions. They could be examined from different angles by tilting and rotating the stage and readily distinguished from crystallographic edge effects that were rarely imaged and analyzed by EDS. ***Hypothesis I is not supported by the sample preparation techniques and observational evidence.***

Hypothesis II: The filaments are abiotic mineral crystals or “ambient inclusion trails”.

In 1995, the International Mineralogical Association Commission on New Minerals and Mineralogical Names adopted the definition set forth by Ernest H. Nickel⁴¹: "A mineral is an element or chemical compound that is normally crystalline and that has been formed as a result of geological processes." Extraterrestrial substances (meteorites, moon rocks, etc.) were considered to be produced by processes similar to those on Earth. Consequently, abiotic, naturally occurring components of extraterrestrial rocks and cosmic dust were also accepted as minerals but biogenic substances (produced entirely by biological processes such as urinary calculi, shells of marine mollusks, etc.) without a geological component were specifically excluded. Mineral species have characteristic chemical compositions, physical properties and highly ordered atomic structure. The modern Nickel-Strunz⁴² classification system as reflected in the extensive on-line *MinDat Database*⁴³ at www.mindat.org which categorizes mineral species in accordance with their chemical compositions. Although the carbonaceous meteorites do contain a large number of mineral crystals, they exhibited sharp edges, facets, and other clearly crystalline structures and chemical compositions that allowed them to be easily identified as crystals of gypsum, elemental iron, magnetite, etc. Martin Brasier, argued that the filaments found in the Orgueil C11 meteorite can be explained as microbial contaminants or abiogenic "ambient inclusion trails" (AIT's) formed by the "forward projection of minerals under gaseous pressure through a solid or liquid medium." Brasier's photo 1b. shows a non-descript, undifferentiated tubular structure that could not possibly be ascribed by a trained phycologist to known genus or species of cyanobacteria. He claims that a small mineral grain at the tip "mimics a heterocyst" but this crystal bears no resemblance to heterocysts as shown in the extensive literature on cyanobacteria or as seen in optical or Scanning Electron Microscopes.

Fig. 1.e. is an ESEM image of a fibrous mineral crystal (probably ferroan epsomite $(\text{Mg,Fe}^{2+})(\text{SO}_4)\cdot 7\text{H}_2\text{O}$) with flat faces and sharp angles in the Orgueil meteorite. The morphology is similar to the crystals of fibrous epsomite, but unlike filamentous trichomic prokaryotes. EDS data shows the crystal contains no detectable Carbon and has dominant elements Oxygen, Magnesium, Sulfur and Iron. Many mineral crystals were encountered during this study, but they often exhibit flat surfaces, facets and sharp angles. These mineral crystals were readily distinguishable from possible biogenic filaments and rarely photographed. On the other hand, contoured, undulatory, tapered, multiseriate, and polarized or differentiated filamentous structures consistent with biology were almost always imaged and analyzed by EDS. For example, **Fig. 1.b.** shows the elemental composition by EDS at spot X for the short constricted hormogonium **Fig. 1.a.** that has emerged from a very long, hollow filament embedded in the Murchison CM2 carbonaceous meteorite. The flattened region of this long filament (not shown) exhibits cross-wall constrictions and has been interpreted as the remains of a cyanobacterial filament of the order Nostocales. The quantitative data for the spectrum at spot X as shown in **Fig. 1.b.** reveals that major elements comprising this filament are **C, O, Mg, Si & S** with Fe and Ni also present. A search of the MinDat database reveals that Chelyabinskite $(\text{Ca,Mg})_3(\text{SO}_4\text{CO}_3)_2[\text{Si}(\text{OH})_6] \cdot 9\text{H}_2\text{O}$ is the only mineral species known that contains the elements C, O, Mg, Si, and S. This rare anthropogenic substance was described in 1988 by Chesnokov *et al.*⁴⁴ as a new species of technogenic mineral that they recovered from burning mine dumps in the Chelyabinsk Oblast of the Urals, Russia. However, since it is anthropogenic, it is not considered a validated mineral species. Furthermore, Chelyabinskite does not contain Iron and Nickel and hence could not be considered a candidate mineral species for this complex meteorite filament even if it were a valid species. ***The Hypothesis that the filaments are abiotic mineral crystals is not supported by the observational evidence.***

Hypothesis III: The filaments are modern biological contaminants.

The EDS data clearly establish that none of these filaments contain detectable levels of Nitrogen. Nitrogen is absolutely essential for all known life forms. Organic nitrogen (formed by the biological enzymatic process of nitrogen fixation) is contained in all DNA and RNA molecules, all amino acids that comprise the life-critical proteins, enzymes and biological pigments. Moore⁴⁵ detected nitrogen at 2,900 ppm (0.29% atomic) in the Alais C11 carbonaceous meteorite and at 2,400 ppm (0.24% atomic) in Orgueil. Gibson *et al.*⁴⁶ analyzed 27 carbonaceous meteorites and found them to contain nitrogen at levels (<0.3%) which is below the level of detectability of the ESEM and FESEM EDS instruments used at NASA/MSFC except under ideal circumstances. Hence, nitrogen was typically not detected by EDS spot analysis or in 2D Elemental X-ray maps made for the meteorite samples at NASA/MSFC. However, nitrogen was easily detected in all of the modern and even many of the very old (Holocene and Pleistocene) biological specimens studied. Even though Nitrogen is present (at 2% to 15% atomic) in all living and dead organisms, it is severely depleted in ancient fossils. Nitrogen enters the geological cycle through the enzymatic fixation of atmospheric N_2 and it is transformed into ammonium. Gallien *et al.*⁴⁷ used Nuclear Reaction Analysis (NRA) to investigate the nitrogen and carbon content of biogenic and abiogenic minerals in Paleozoic shales. They reported the following atomic C/N ratios: Abiotic Devonian

hydrothermal feldspars (C/N = 0.13 - 0.26); Marine bacteria (C/N = 2.9 - 14.3); Biogenic minerals (C/N = 17 - 25); Proterozoic kerogens (C/N = 104 - 167); and Archaean kerogens (C/N = 200 - 500). Studies at MSFC of fossils from the Miocene to the Archaean³⁴ have shown that the nitrogen levels in these ancient biological materials is typically below the EDS detection limit (<0.5%). Since these meteorites are all observed falls, their residence time on Earth is precisely known. The Orgueil meteorite fell in 1864; Ivuna in 1938; and Murchison in 1969. Consequently any living bacteria, cyanobacteria, fungi, or other biological entities that could have invaded the stones after they arrived on Earth would certainly contain detectable levels of Nitrogen. The low level of nitrogen in the filaments provides clear and convincing evidence that they can not be interpreted as modern biological contaminants.

The Hypothesis that the filaments are modern biological contaminants is not supported.

Hypothesis IV: The filaments are indigenous microfossils of cyanobacteria or other trichomic prokaryotes

The Cyanobacteria are an ancient, diverse and abundant group of photosynthetic, oxygenic, aquatic microorganisms. For almost two centuries, the cyanobacteria were considered to be plants ("blue-green algae" or "cyanophytes") and were studied by botanists, algologists or phycologists. They were identified and classified on the basis of the key morphometric and morphological characteristics.⁴⁸⁻⁵¹ and systematically named in accordance with the rules of the International Code of Botanical Nomenclature.⁵² Even though it is now known that cyanobacteria are in fact prokaryotic bacteria rather eukaryotic algae, the cyanobacteria may still be validly classified under the Botanical Code. However, they are also covered by the Bacteriological Code⁵³⁻⁵⁵ and 16S gene sequences are continuing to be added to the database.

Figure 1.a. is an ESEM image of a short trichome segment of six cells with clearly delineated cross-wall (CW) constrictions and rounded ends embedded in the Orgueil CII carbonaceous meteorite. Another similar trichome chain (not shown) is just below it and it is attached to a very long hollow sheath with partially embedded, flattened, section also showing cross-wall constrictions in proximity to structures that represent possible coiling hormogonia. Measurements indicate that the cells in this trichome segment are ~2.7 μm broad and shorter than broad (~1.2 μm thick). Several representatives of the Cyanobacterial genus *Nostoc* form coiling hormogonia and have trichomes within this size range. For example, morphotypes of *Nostoc entophytum* have trichomes that are 2.5 - 3 μm broad and short, barrel-shaped cells. (See Desikachary,⁴⁸ pg. 375). The EDS data reveal that the hormogonium is primarily composed (**Fig. 1.b.**) of this same suite of elements (C, O, Mg, Si, S) as seen in the Ivuna filament of **Fig. 2.**

Fig. 1.c. is an Environmental Scanning Electron Microscope ESEM image of filaments found *in-situ* in the Murchison CM2 carbonaceous meteorite along a visible light image and 2D X-ray maps showing the relative distribution of the elements Carbon, Iron and Sulfur in these forms. It shows a large curved multiserial filament with glycocalyx in close proximity to other tapered filaments. The large coiled filament is clearly visible in the Carbon and Iron maps indicating it is significantly enriched in Carbon. Multiple trichomes within a common sheath are characteristic of the common cyanobacterium *Microcoleus chthonoplastes* Thuret ex Gomont (cells 2.5-6 μm broad) which is consistent with this large curved, multiserial filament. The visible light image in color (taken from a slightly different angle) shows the multiserial filament is Jet-Black, which is consistent with conversion to kerogen. The filament is depleted in Iron but has Sulfur level similar to that of the rest of the meteorite rock matrix, which would not be expected if it were a modern biological contaminant.

Fig. 1.d. is an enlargement of these filaments revealing that the large coiled multiserial filament is layered or lamellated and lies alongside a short trichome with rounded ends. This short trichome contains only three cells and is interpreted as a hormogonium. From this angle the large coiled filament appears to be directly in front of a smaller (4 μm diameter) tapered filament, which has a capitate apical cell with a rounded calyptra at the head. This single filament is consistent with the morphotype of *Microcoleus vaginatus* (Vaucher) Gomont, which has uniserial filaments and cells that are 3.5-7 μm broad with capitate end cells and a flat conical calyptra (see Desikachary, pg. 343.) The size and morphological details of this tapered filament is also consistent with one of the filaments illustrated in 1962 by P. Palik²⁴ of the Microbiological Institute of the Eötvös Lorand University in Budapest. She examined crushed (non-acid treated) samples of the Orgueil meteorite and reported the discovery of several complex filaments (**Fig. 1.e.**) which were carefully illustrated and described. Several of the filaments were curved and had widths from 3 μm to 16 μm . Palik described the central filament in her illustration "Part of the filament is 4 μm wide and 55 μm long. It is covered with a follicle, the apex is rounded off...slightly tapering toward the apex. The apical cell is like a head."

Figure 2.a is a FESEM image of a filament embedded in freshly fractured interior surface of Ivuna C11 meteorite. Measurements and EDS data reveal that this thin, flattened undulatory filament (diameter (0.7 - 1.0 μm) is encased within a thin carbonaceous sheath. The horizontal lines **CW** seen across the filament in the flattened region are consistent with cross-wall constrictions known in SEM images of well-preserved cyanobacteria and other filamentous trichomic prokaryotes. The sheath is broken in the lower area and exposes an interior trichome that has been replaced by magnesium sulfate. The filament is very small and consistent with the smallest non-heterocystous cyanobacteria cf. the Oscillatoriaceae (cf. *Spirulina subtilissima*) or the smaller sulfur bacteria (cf. *Thioploca minima*). **Figure 2.b.** reveals that this Ivuna filament contains the same suite of elements (**C, O, Mg, Si, S**) found in many Orgueil and Murchison. However, this filament also contains Iron can not be ascribed to mineral species found in the *MinDat Database*.

Fig. 3.a is a FESEM image of the hook-shaped, tapered, calyptrate filament **11** with a capitate conical apical cell **C** embedded at the top left of the fragment of the Orgueil C11 carbonaceous meteorite shown in **Fig. 4.a.** This embedded filament is uniseriate and was apparently flattened after internal cells were lost. The flattened filament is 8.1 μm wide at base indicating circular diameter \sim 4.5 μm ; tapers to 1.8 μm diameter just after the bend. This filament has characteristics of the known morphotypes of the planktonic cyanobacterium *Phormidium dimorphum*. Lemmermann which has bent trichomes 3.5 to 5 μm that is prominently attenuated that the capitate tip terminating in a \sim 2 μm broad by \sim 1.5 μm high calyptra (see Desikachary, ⁴⁸ pg. 256). **Fig. 3.b.** provides the EDS data at spot **X** showing the elemental composition of this filament to be: C 45.8%; N<0.5%; O 14.8%; Mg 8.5%; Si 3.9%; S 21.5%; V 2.6%. Nitrogen is not detected, but in this Orgueil filament, Carbon (45.8% atomic) is the most abundant element followed by Sulfur (21.5%); Oxygen (14.8%) and Magnesium (8.5%) and Silicon (3.9%). This particular filament is very unusual in that it also contains 2.5% Vanadium, which is present in CI meteorites at only 49 ppm. This is one of the few filaments ever found in any of the meteorite samples studied to exhibit detectable levels of Vanadium. The chemical composition of this filament reflects the general composition of the meteorite matrix cut with a significantly enhanced level of carbons. The filament does not exhibit crystalline structure and is not consistent in chemical composition with any of the mineral species contained within the MinDat database.

Fig. 4.a. is a Hitachi FESEM Secondary Electron Detector (SED) image (1000 X) showing many filaments and empty sheaths embedded in Orgueil meteorite matrix; **Fig. 4.b.** provides Backscatter Electron (BSED) and Secondary Electron Detector (SED) images along with 2-D x-ray maps showing the relative concentration of chemical elements in the filaments and associated meteorite matrix. The filaments are clearly enriched in C, O, Mg and S and relatively depleted in Silicon, Iron and Nickel. However, their content of Nitrogen, Sodium, and Phosphorus is not very different from the rest of the meteorite rock matrix, which provides additional chemical evidence that they belong in the meteorite and are not modern bio-contaminants. These elemental X-ray maps suggest that the filaments are mainly carbonaceous sheaths in-filled with magnesium sulfate.

Fig. 5.a. is a FESEM image of a thick mat in Orgueil showing a large filament with characteristics similar to known morphotypes of the multiseriate cyanobacteria (cf, *Microcoleus chthonoplaste*) near branched, heterocystous filaments.

Even though, Cyanobacteria occur in some of Earth's most extreme environments - hot springs and geysers, hyper saline and alkaline lakes, hot and cold deserts, and the polar ice caps and small endolithic coccoidal forms are well-known, all cyanobacteria and other filamentous trichomic prokaryotes require liquid water for growth. The filamentous cyanobacteria exhibit a variety of distinct morphologies with precise cell sizes and diagnostic characteristics. Unlike other bacteria and archaea and other bacteria, which are typically classified in pure culture by their physiological, biochemical and phylogenetic properties, the cyanobacteria have historically been classified based upon their size and morphological characteristics. These include the presence or absence of heterocysts, sheath, uniseriate or multiseriate trichomes, true or false branching, arrangement of thylakoids, reproduction by akinetes, binary fission, hormogonia, fragmentation, presence/absence of motility etc. Their antiquity, distribution, structural and chemical differentiation, diversity, morphological complexity and large size compared to most other bacteria, makes the cyanobacteria ideal candidates for morphological biomarkers in meteorites and returned Astromaterials.

The filaments detected in the carbonaceous meteorites have complex morphologies with highly differentiated forms and chemical compositions in a narrowly constrained range of diameters. They were observed to contain cells and linear chains of cells (trichomes) within carbon-rich sheaths to form uniseriate or multiseriate filaments which may be unbranched or branched. Some of the filaments exhibit evidence of motility (escaped hormogonia and twisted or spiral

empty sheath remains); calyptrate or capitate apical cells; specialized cells for nitrogen fixation (basal, intercalary or apical heterocysts or heterocytes); reproduction (akinetes, baeocytes, and linear or coiling hormogonia); and attachment (pili or fimbriae). Many of these characteristics are found only in cyanobacteria, whereas others are present in a wide variety of other gram-positive bacteria, sulfur bacteria or other filamentous trichomic prokaryotes. However, tapered or polarized trichomic filaments and those that have specialized cellular structures for nitrogen fixation (heterocysts) can be clearly recognized as cyanobacterial in nature, in that these features are known only in trichomic filamentous cyanobacteria. These structures are unknown in trichomic filamentous sulfur bacteria such as *Beggiatoa*, *Thioploca*, and *Thiothrix*. Although some of the filaments found in the meteorites could belong to trichomic sulfur bacteria, there are abundant filaments in the carbonaceous meteorites that exhibit specific characteristics diagnostic of known genera and species of filamentous cyanobacteria. The ESEM and FESEM images and EDS data establish that many filaments found embedded in carbonaceous meteorites exhibit chemical composition and morphological/morphometric features consistent with the fossilized remains of filamentous cyanobacteria or other trichomic prokaryotes.

The Hypothesis that the carbonaceous meteorites contain indigenous microfossils of cyanobacteria and other trichomic prokaryotes is supported by the ESEM and FESEM images and EDS spectra data.

6. CONCLUSIONS

During the past half century, very many independent researchers have established that the carbonaceous meteorites contain abundant kerogen-like insoluble organic matter along with an array of organic chemicals and biomolecules that are both indigenous and extraterrestrial.⁵⁶⁻⁷⁹ However, these studies have shown that the carbonaceous meteorites do not contain a great number of essential life-critical biomolecules. Many of these biomolecules are destroyed over geological time periods, but would certainly be present if the meteorites if they were contaminated by modern biological microorganisms if they had invaded the stones after arrival on Earth. Many of the filaments encountered in freshly fractured interior surfaces of the Murchison (CM2), Ivuna (CI1) and Orgueil (CI1) carbonaceous meteorites have been found to be clearly embedded in the mineral matrix and with elemental compositions that reflect the meteorite components but show strong enrichment in carbon. In many cases the filaments are seen to contain cells and trichomes encased within an electron transparent carbonaceous sheath. It has long been known that these carbonaceous meteorites are micro-regolith breccias that are readily disaggregated by exposure to liquid water. Consequently, it is not feasible that aquatic prokaryotes could have invaded the stones after they arrived on Earth, as any stones that landed in any lake, pond or other body of water would have been destroyed and never recovered.

The carbonaceous meteorites contain an extensive array of amino acids (>100), many of which exhibit a moderate to strong excess of the *L*-enantiomer.⁵⁶⁻⁶⁵ Chirality has long been considered a signature of life, with all known life forms using the *D*- sugars in DNA and RNA and *L*- amino acids in all proteins, enzymes and other biomolecules. Protein amino acids are present in the carbonaceous meteorites, and stable isotope studies have shown them to be both indigenous and extraterrestrial. However, only 8 of the 20 life-critical protein amino acids have been found and 12 of the life-critical protein amino acids are missing from the meteorites. Also present are long-chain fatty acids, paraffinic and aliphatic hydrocarbons, isoprenoids, and kerogens.⁶⁵⁻⁶⁸ No polysaccharides are found and the essential sugars for DNA and RNA molecules (ribose and de-oxyribose) are also missing from the meteorites. Biogeochemicals consistent with the diagenetic breakdown products of chlorophyll molecules (pristine, phytane and porphyrins) have been found in carbonaceous meteorites by independent researchers, but they have never reported the detection of either chlorins or chlorophyll-a.⁶⁹⁻⁷¹ Since chlorophyll-a is an abundant photosynthetic molecule in the "blue-green algae", it would certainly be present if these stones were contaminated by modern cyanobacteria. In 1964, Hyatsu⁷² reported the detection of organic nitrogen and nitrogen heterocycles in the Orgueil CI1 carbonaceous meteorite. Several independent researchers have now clearly established that carbonaceous meteorites contain indigenous and extraterrestrial purines, pyrimidines and other nitrogen heterocycles that are the essential for DNA and RNA molecules of all known forms of life.⁷³⁻⁷⁸ It has now been established by several independent studies that many of the CI1 and CM2 carbonaceous meteorites contain indigenous Adenine, Guanine and Uracil. However, Cytosine and Thymine are both missing from the meteorites, and they are both essential for DNA and cytosine is essential for RNA molecules (in RNA Thymine is replaced by Uracil). Nucleobases clearly represent strong signatures of life, but they are not considered to be good biomarkers, as they are unstable and decompose over geological time periods. Levy and Miller⁷⁹ has shown that decomposition of the RNA nucleobases occurs quickly at high temperatures, but they are much more stable at low temperatures. At 0 C, the half-life of the more stable nucleobases (Adenine, Guanine, and Uracil exceeds 1 million years, but the half-life of Cytosine is only 17,000 years. These results are entirely consistent with the hypothesis that ancient

microbial life existed at one time on the parent bodies of these stones, but they are totally inconsistent with the hypothesis that all filaments in the meteorites are abiotic in origin. The recognizable remains of filamentous cyanobacteria and other filamentous trichomic prokaryotes found or if biological in nature they are the result of contamination by recent microorganisms. The images and observational evidence of embedded filaments with the size and morphological characteristics of recognizable and undeniably biological life forms provide direct evidence in strong support of the hypothesis that the filaments are both indigenous to the stones and biological in origin. The Energy dispersive X-Ray Spectroscopy (EDS) data coupled with the extensive *MinDat* data base establish that these filaments are not known species of abiotic minerals. The EDS data reveal that almost all of the filaments in the carbonaceous meteorites have levels below detectability ($N < 0.5\%$) and have carbon-rich sheaths are in-filled with magnesium sulfates. Several of the sheaths have $C > 80\%$ atomic and O/C ratios (0.1 to 0.3) which consistent with kerogen but inconsistent with O/C ratios of living or recently dead biological matter. It is concluded that many of the filaments represent the indigenous permineralized microfossils of cyanobacteria or other trichomic prokaryotes that were present in the carbonaceous meteorites before they entered the Earth's atmosphere.

7. ACKNOWLEDGMENTS

We want to thank Gregory Jerman and James Coston for the extensive Electron Microscopy Support that made this research possible. We also deeply grateful for meteorite samples provided by Dr. Claude Perron and Dr. Martine Rossignol-Strick of the Musée Nationale d'Histoire de Paris (Orgueil and Alais); Dr. William Birch, Victoria Museum, Melbourne, Australia (Murchison); Dr. Paul Sipiera, Dupont Meteorite Collection and the Planetary Studies Foundation, Chicago (Ivuna, Orgueil, Acfer 324, TIL 99001-99019). For many helpful discussions we thank Dr. David McKay, Center for Biomarkers in Astromaterials at NASA/JSC; Academician Eric Galimov, Vernadsky Institute, RAS (Moscow); Academician Georgy Zavarzin and Dr. Ludmila M. Gerasimenko, Institute of Microbiology, RAS (Moscow) and Rosemarie Rippka, Pasteur Institute (Paris).

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