Chapter

Life on Mars: Clues, Evidence or Proof?

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Abstract

The search for life on Mars is one of the main objectives of space missions. At "Pahrump Hills Field Site" (Gale Crater, Mojave target), inside the mudstones of the Murray lacustrine sequence, Curiosity rover found organic materials and lozenge shaped laths considered by NASA as pseudomorphic crystals. Besides it detected mineral assemblages suggesting both oxidizing (hematite) and reducing (magnetite) environments, as well as acidic (diagenetic and/or authigenic jarosite) and neutral (apatite) conditions, that might suggest bacterially mediated reactions. Our morphological and morphometrical investigations show that such diagenetic microstructures are unlikely to be lozenge shapes and, in addition to several converging features, they suggest the presence of remnants of complex algal-like biota, similar to terrestrial procaryotes and/or eukaryotes; possible microorganisms that, on the base of absolute dating criteria used by other scholars, lived on Mars about 2.12 +/−0.36 Ga ago.

Keywords: life on Mars, microalgae, microbialites, Mojave target, Gale Crater, Mars, Curiosity rover

1. Mars planet: environment and geological history

As it is known, Mars is the fourth and the last of the inner and rocky planets of our Solar system. Beyond the "Red Planet", it extends the belt of asteroids just before the giant Giove and the other gaseous external planets of the system. It has a mass: 6.417 x 10^{23} Kg, a density of 3,940 g/cm^3, equatorial diameter of 6,792 Km and a mean temperature of −63°C, a distance from Sol of 228,000,000 km.

Mars orbits the Sun at an average distance of 230 million km and its revolution period is about 687 days; while his solar day is a little longer than ours: 24 hours, 37 minutes and 23 seconds. The Martian axial inclination is 25.19°, which is similar to that of Earth. Due to the discrete eccentricity of its orbit of 0.093, its distance from Earth to opposition can range between about 100 and about 56 million kilometers; only Mercury has a higher eccentricity in the Solar System. However, Mars used to follow a much more circular orbit: about 1.35 million years ago its eccentricity was equivalent to 0.002, which is much lower than the current Earth's. Mars has an eccentricity cycle of 96,000 Earth years compared to Earth's 100,000. Over the past 35,000 years, Martian orbit has become increasingly eccentric due to the gravitational influences of other planets, and the closest point between Earth and Mars will continue to decline over the future time.

The planet is enveloped by a thin atmosphere dominated by the gas carbon dioxide as the 95.3% of the whole. The other chemical elements with their respective rates are: nitrogen (2.7%), Argon (1.6%) and oxygen–carbon monoxide-water steam (0.4%). The weakness of the Martian atmosphere and the lack of a magnetic field do not allow any effective defence against ultraviolet radiations and solar winds.

The environmental and geological history of early Mars is mostly written in its rocks, in their composition and morphological/structural signatures. The first few billion years of Mars' geologic history records surface environments considerably different than the surface today, prompting a succession of coordinated surface and orbiter missions over the past two decades aimed ultimately at determining if Mars ever had an early biosphere. Water is the sine qua non for all life as we understand it and, therefore, past missions have sought environments where water was abundant and possibly long-lived [1, 2].

Mars was formed 4.6 billion years ago, with a history similar to the other three Terrestrial-type planets, i.e. as a result of the condensation of the solar nebula, most probably silicates. Due to the upper distance from the Sun from Earth, during the initial phase of formation in Mars' orbit there was a higher concentration of elements with low boiling points, such as chlorine, phosphorus and sulfur, probably driven away from the inner orbits by the strong solar wind of the young Sun [3].

During the first Martian Era, known as Noachian period, 4.1–3.7 Gys ago, its environment, that had formed, was moderately similar to the one on present Earth. Liquid water was widespread in a neutral environment, volcanic activity and heat flow more vigorous, and atmospheric pressure and temperature were higher than today. Morphological evidences are represented by river delta and river meanders, drainage networks and lakes; such morphologies are accompanied by the occurrence of consistent sedimentary, layered deposits. These conditions may have favoured the spread of life on the surface of Mars [4]. In this period the planet was subject to intense late bombardment, to which Earth was also a victim. In fact, about 60% of the surface has markers dating back to that era, particularly impact craters. The largest of these is located in the northern hemisphere and has a diameter of about 10,000 km, almost half the circumference of the planet. The formation of this crater is probably due to the impact with a big asteroid, which left a deep depression (the Boreal Basin), covering about 40% of the planet, brutally changing the history and environment of planet [5]. Eloquents morphological rest of such big impacts are extensive water flow formations in the Tharsis region; a region subject, towards the end of the era to a very active volcanism and flooded, by a large amount of water.

Slowly, in just over a billion and a half years, Mars went from a warm and humid phase characteristic of the Noachian to that of a cold and on-surface arid planet observable today. This transitional phase occurred during the Hesperian; a period characterized by continuation of intense volcanic activity (like those of Olympus Mons), deposition of evaporitic sedimentary sequences, and catastrophic floods that dug immense canals along the surface [6]. The continuous eruptions brought large amounts of sulphur dioxide and hydrogen sulfide to the surface, changing the large expanses of liquid water into small basins of high acidity water due to the sulphuric acid that formed. Although the disappearance of rivers and lakes is generally considered attributable towards the end of this era, recent dating on Gale Crater outcroppings open up the existence of water lake about billion years ago, during the Amazonian era [7].

One era, this last, from about 3 billion years ago to today, that is characterized by a poor period of meteor bombardment and by a continuation of cold and arid

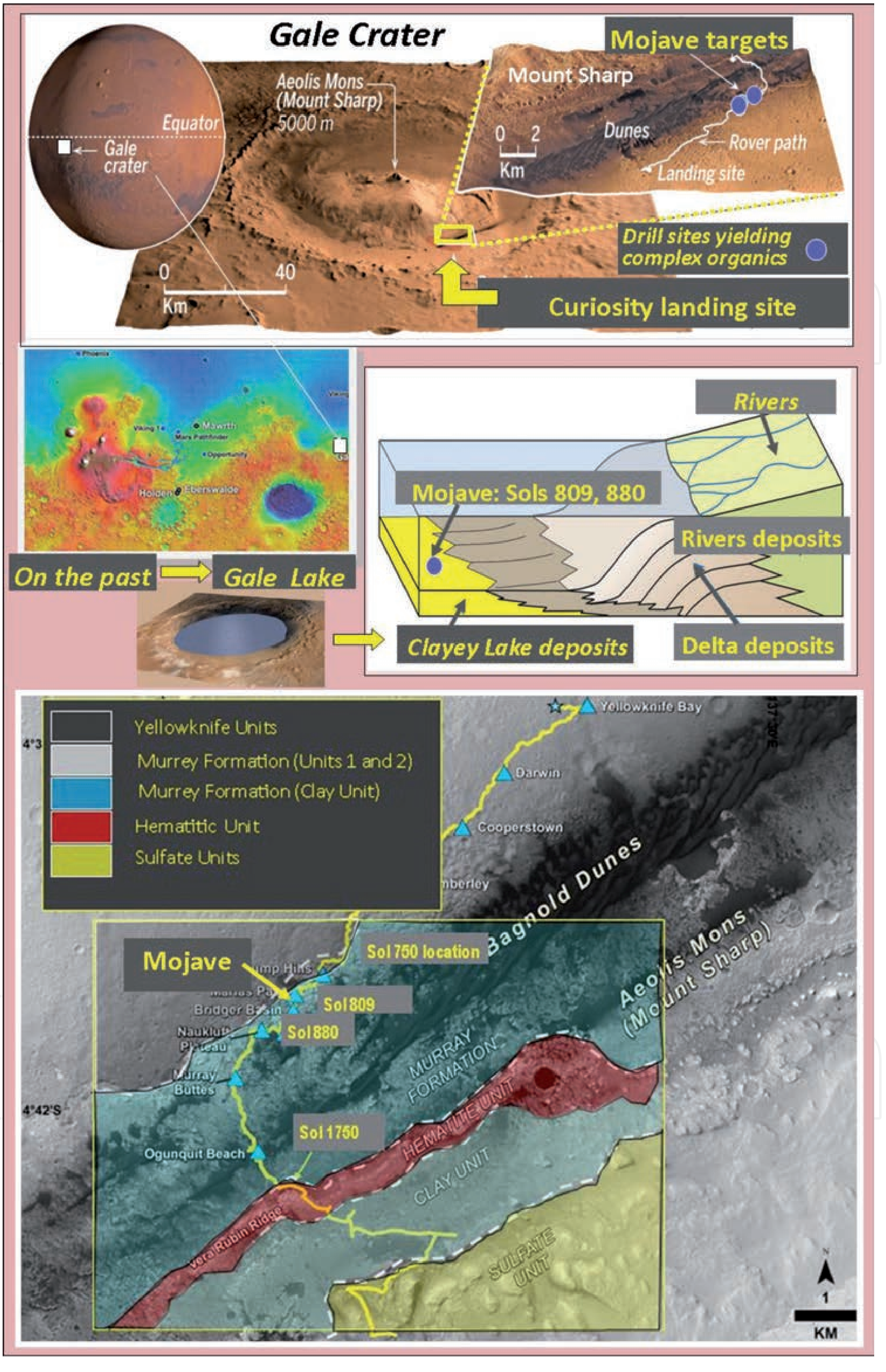


Figure 1.
Pictures show the landing site of NASA Curiosity rover, at GaleCrater, located near the Martian Equator; a site where intertidal and lacustrine clayey deposit have been found, as well as organic molecules and microstructures resembling microbialtes and terrestrial algae have been found. Below: a geological sketching of the ancient lake deposits (from NASA reports, modified).

climatic conditions, until today. Conditions producing surface aridity and deepening of water tables, whose existence has been proven by recent space missions.

In fact, the Martian poles are covered with water ice and permafrost layer extends to latitudes of about 60°, and large amounts of water are believed to be trapped under the thick Martian cryosphere [8]. The formation of Valles Marineris and its spill channels and sink holes, shows that during the early stages of Mars' history there was a large amount of liquid water. The presence of large amount of ground water and ice in the south pole of Mars was confirmed by the European Mars Express probe in January 2004 and by MARSIS radar near the Chryse Planitia region. Radar analyses conducted from 2012 to 2015 by Mars Express revealed presence of liquid salt water under the southern ice cap. In 2015, based on the MRO's monitoring, NASA announced evidence that liquid salt water flows on the surface of Mars in the form of small streams [9].

Recent data of spatial missions and in particular of NASA rovers instrumentation proved on Gale crater the existence of lacustrine and intertidal deltas deposits (**Figure 1**), presence of all the life elements, occurrence of complex organic molecules, sedimentary structures similar to terrestrial microbialites and the existence of an environment favorable to microbial life [1, 10, 11].

Stromatolite-like structures were also found by NASA rovers Opportunity, Spirit and Curiosity on the laminated Martian outcroppings of Meridiani Planum [12–18]. These findings have given strength to the hypothesis of stromatolites presence [19], that could be probably quite widespread from the Noachian to the Hesperian geological era of Martian life.

2. The search of life on Mars

The search for life on Mars, either in the present or in the past history of the "Red Planet", has been the main motivation behind research programs since the 1970s. The first images, highlighting the evidence for past liquid water on Mars, were carried out by orbital images from Mariner 9 [20]. Then, Mars Observer Camera and Mars Reconnaissance Orbiter [21] provided new images of past fluvial networks on its surface, long time ago.

Really, the statement of this question arises from the first debated Viking experiments and, in particular, by a re-analysis of the former Viking Mars Mission. So, Phoenix space probe on 2004, as a follow-up of the first Viking mission, showed the existence of an ice-cemented ground in the northern plains of Mars combined with the presence of perchlorates normally used in terrestrial metabolic pathways of a large number of microorganisms [22–24]. So, perchlorates, found in the ground ice of Mars, is a putative bio-signature resulting by a possible microbial activity on Mars [25] and could suggest a chemosynthetic activity carried out by bacteria on the planet surface, a short time ago [26].

Our knowledge about this topic has been increased considerably as a result of recent NASA missions, Opportunity, Spirit and Curiosity, located at Meridiani Planum, Gusev and Gale Craters, respectively. The Curiosity rover landed inside the Aeolis Crater, informally known as Gale Crater, on August 2012 with a complex set of scientific instruments (MSL), able to detect chemical and mineralogical soil composition, environmental data, and record panoramic and microscopic images of high accuracy, obtaining subsequently several thousands of images. Hence, the MSL scientists discovered a fluvial-lacustrine sequence and fine grained sedimentary rocks containing clay and hydrated minerals, deposited inside an almost neutral lake. These deposits were, then, subjected to two post-genetic more acidic phases, revealing that they contain the elements necessary for life, e.g., H, O, S, C, N, P, and also including Fe, Mg and Mn suitable to support a possible Martian biosphere based on chemolithoautotrophy [1]. In addition, life, if it existed, must have left visible traces of its activity and presence in the sediments, i.e., the rocks, that are now photographed by rovers. Furthermore, microbes and microalgae are the first step on the evolutionary scale of life on Earth and stromatolites are the oldest evidence for them, stretching back at least 3.5 billion years. Hence, these are the structures that may be present if life ever existed on Mars comparable to Earth [19].

The presence of extraterrestrial microorganisms and, in particular, of cyanobacteria, well known as the main builders of terrestrial stromatolites, has been suggested by many authors beginning from the famous discovery of Martian meteorite ALH84001 [27]. This biological approach was further confirmed by some intriguing images of the Martian surface sent by rover Opportunity on 2010, showing a set of rocks partially covered by a dark shiny patina, close to the terrestrial "Desert Varnish" probably formed by the same bacteria that built stromatolites on the Earth [28]. The latest finding is the discovery in CL1 carbonaceous Martian chondrite of some microfossils very close to terrestrial cyanobacteria [29]. All these hypotheses were strengthened by some studies about extremophiles as desert cyanobacteria of the genus Chroccocidiopsis living in extremely hot and cold deserts that sheds a new light on the possible history of Martian microbial life [30, 31]. In particular, it has been pointed out the important role of Cyanobacteria in the formation of organo-sedimentary rocks as one of the most successful and widespread forms of life on Earth [32] because of their great morphological variability and their bio-stabilization capacity on sun-light exposed sedimentary surfaces, but also in environments characterized by extremely low energy light. In this way, the recent discovery of a new photopigment, found within terrestrial stromatolites and named Chlorophyll f, that can absorb light of even lower photon energy until 720 nm [33], suggests that cyanobacteria could alive also in extreme environments as on Mars. Generally, these microorganisms occupy a very broad range of environments including waters of widely different chemistry and composition so that their involvement in sedimentation processes is equally varied [34]. Cyanobacteria, including more than 2000 species [35], as composite microbial associations, dominate microbial mats and are ubiquitous, leaving successful records in sediments and sedimentary rocks.

Morphological study of images reveals evidence of widespread occurrence of micro, meso, and macro structures recalling for some authors early terrestrial forms of life; such as the "blueberries", concretions possibly induced by chemolithoautotrophic bacteria [10, 36, 37]. These strange and complex structures, for which abiological explanation it's hard to find, have strong morphological parallels with terrestrial microbialites/stromatolites [14–16, 18], a conclusion that seems to be supported by morphometric approaches [12, 13]. Other possible biogenic structures have been observed on Mars, recalling those of terrestrial silica deposits in hydrothermal environments [38, 39] or typical structures, known as Microbially Induced Sedimentary Structures (MISS) and generated by microbial mats of intertidal environments [14]. Despite the many observations, mutually supporting a possible microbialite hypothesis, they do not prove the presence of fossil life on Mars, because biologic explanations for their terrestrial counterparts and for their contained microbial structures are often deeply controversial. In fact, such organo-sedimentary structures are sediments, and despite having unusual features at meso and macroscales, somewhere contain controversial microbial remnants of micrometric dimensions; while complex and larger structures, as are evolved and more evident microfossils (generally larger than 0.1 mm) or macrofossils (centimetric) are generally more obvious and indisputable. Finally, in the Martian atmosphere it has been detected traces of methane and formaldehyde, changing seasonally and supporting evidences on the potential habitability of Mars [40].

3. Intriguing sedimentary microstructures, chemicals and complex organics found by Curiosity rover at Gale Crater

At Pahrump Hill (Mojave and Mojave 2 targets, at Sols 809 and 880) a large number of light-toned lozenge-shaped pervasive microstructures having dimensions and shape comparable to rice grains (nicknamed here as "rice grain") were found and regarded by NASA as pseudomorphic sulfate crystals resulting from occasional lake evaporation [7]. In this location, Curiosity detected mineral assemblage suggest, paradoxically, both oxidizing (hematite) and reducing (magnetite) environments, as well as acidic (diagenetic and/or authigenic jarosite) and neutral (apatite) conditions; findings difficult to explain as having an abiological origin and more compatible with a biological origin.

In this work we hypothesize that among the structures imaged by Curiosity at Mojave targets some are suggestive of bacterially mediated reactions or are the remnants of fossilized life forms, and which also agrees with the organic molecules detected just on such target (**Table 1**).

Hence, the objective of this study was to investigate the microstructures observed at Sols 809 and 880 (Mojave and Mojave 2 targets; the same site on successive paths) in order to determine their possible biogenicity. These structures were described by NASA as lozenge shaped, pseudomorphic minerals (https://www.nasa.gov./jpl/msl/pia19077).

First, we studied the morphology of microstructures with reference to possible parallels with primitive terrestrial forms of life, such as stromatolites/microbialites, microfossils and/or algae, taking in account previous similar possible finds observed by Martian rovers. Parallels emerged by a systematic image analysis, considering terrestrial similar forms and sedimentary processes, both syn-genetic and post-genetic, in a given environment.

Second, we morphometrically analyzed the lozenge-shaped structures, here called "rice grains" (**Figure 2**), observed at the same targets, in order to investigate if their microstructures are compatible to sulphate crystals (Gypsum, Jarosite) as well to other minerals contained in these outcroppings or to primitive terrestrial forms of life.

4. Materials and methods

4.1 Morphology

The study was based on Mahli (Mars Hand Lens Imager), ChemCam and Mastacam images uploaded on NASA web site (https://mars.nasa.gov/msl/multimedia/raw-images), and especially those from the Sols range 750–1400. A selection of images (**Table 2**) was made which were considered particularly interesting and have been morphologically analyzed in detail by amplification and color/brightness/contrast modifications, in order to evaluate possible comparison to analogous terrestrial form of life. In particular, Mahli employs a macro lens color camera able to focus on targets at working distances of 2.1 cm to infinity, with a maximum resolution of about 14 μm/pixel. We have used a scale for Mahli images using the relation between motor count and the pixel dimension values provided by NASA and whose pixels are in the range from 20 μm to 30 μm. Consequently, the dimensions of microstructures analyzed in this article are, at least, about 10 time higher than image resolution.

Methanethiol (CH_4S)
Dimethyl sulfide (C_2H_6S)
Thiophene (C_4H_4S)
Methylthiophenes (C_5H_6S)
Benzothiophene (C_8H_6S)
Carbon-chain molecules with 1 to 5 carbons
Benzene (C_6H_6)
Chlorobenzene (C_6H_5Cl)
Alkylbenzenes/benzoate ion ($C_8H_9/C_7H_5O^-$)
Toluene/tropylium ion ($C_7H_8/C_7H_7^+$)
Naphthalene ($C_{10}H_8$)

Table 1.
Carbon-containing compounds revealed by Sample Analysis at Mars (SAM) aboard of Curiosity rover: thiophenic, aromatic and aliphatic molecules in Mojave target.

4.2 Morphometry

4.2.1 Euclidean morphometry: comparison of "rice grains" with various crystals and algae

The purpose of this section was to investigate quantitatively the features referred by NASA as light toned lozenge-shaped microstructures (**Figure 2**). This investigation determined, using a series of metrics, including degree of dispersion, variability in lengths and widths, length/width ratio, fit of lengths to a log-normal distribution, fit of orientations to a rectangular distribution, and morphological analysis, the similarity between the "rice grains" and various abiotic mineral deposits and terrestrial life forms. In particular: images of Gypsum and Jarosite crystals, an image of Feldspar phenocrysts, and a population of terrestrial *Euglena viridis* (O. F. Müller) Ehrenberg, 1830, (**Table 2**) were observed. These images were analyzed using 'Image J' software developed by the National Institute of Health (NIH), Bethesda, USA [42–44].

Each image was magnified to clearly reveal the objects of interest. Images were manipulated using brightness, contrast, sharpening and edge detection to optimize the appearance of the objects and to establish their boundaries. A grid of squares was then superimposed over each image to establish a number of sample fields. Each of the "rice grains", with at least 50% of its area within a sample field, was measured. Various relative measures based on degree of variation, ratios, fit to various distributions and proportions exhibiting a specific morphology, were used because scale measures were not always available for all images or those that were quoted were unreliable.

The following data were obtained from each sample field containing "rice grains": (1) total number of profiles, (2) the maximum length of each profile in relative units, (3) the width of each profile taken at the midpoint, (4) the orientation of each profile, measured as the angle between the horizontal and a line drawn along the maximum length of the profile, (5) the proportion of the profiles which had a fusiform shape and (6) the proportion which exhibited a degree of flexibility or curvature relative to a straight line drawn connecting the two ends of the profile. A number of metrics based on the profiles were analyzed and compared: (1) spatial

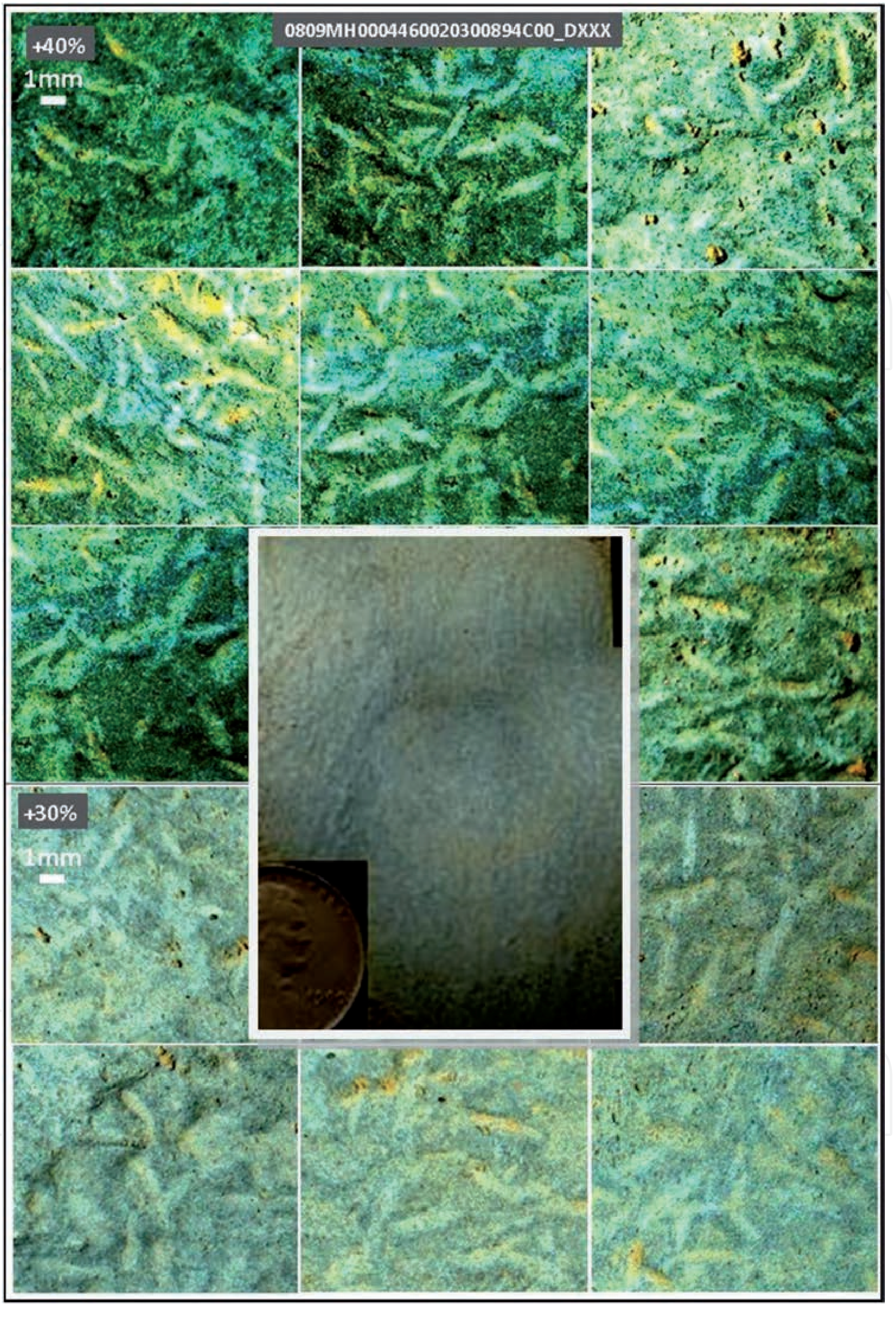


Figure 2.
A set of amplified image samplings (rover image MAHLI, taken at Sol 809) showing chaotically arranged whitish forms, many of which are bezel or fusiform-shaped bodies. Such structures were interpreted by NASA as lozenge-shaped crystals (report PIA 19077; central image). Samplings were obtained by using contrast adjustments of 40% (above) and 30% (below), and the more appropriate luminosity, case by case.

pattern, i.e., whether the profiles were distributed at random, uniformly, or were clustered, (2) degree of variation in length and width, measured as the coefficient of variation (CV) (3) size frequency distribution of lengths, (4) size frequency

Figure	Section	Type	Sol	Source
4	A,B	Terrestrial: Stromatolite	—	Web site
	C	Mars: Concretions	767	Curiosity
	D	Mars: Concretions	871	Curiosity
	E,F	Mars: Concretions	758	Curiosity
5	A′B′	Terrestrial: Microbiolites	—	Web site
	A	Mars: Laminate structures	890	Curiosity
	B	Mars: Laminate structures	810	Curiosity
6	A,D,F	Mars: Filaments	871	Curiosity
	B	Mars: Filaments	758	Curiosity
	C,E	Mars: Filaments	899	Curiosity
	G	Mars: Filaments	598 + 715	Opportunity
	H,I	Mars: Filaments	780	Curiosity
2	All	Mars: 'rice grains'	809	Curiosity
8	All	Mars: 'rice grains'	880	Curiosity
9	Up, Lw L	Mars: Various morphologies	880	Curiosity
	Up inset	Terrestrial: *Euglena*	—	G. Bianciardi
	Lw R	Dasycladales-Epimastopora(f)	—	Web site
10	A,B,D,E	Mars: 'conical bodies' (f)	1103	Opportunity
	C	Terrestrial: Dasyclad (f)	—	Web site
11	A,B,C,L,G	Terrestrial: Dasyclad fragments(f)	—	Web site
	D,E,F,H,I,M	Mars: Shell fragments (f)	1273	Curiosity
	O,Q	Mars 'rice grains'	880	Curiosity
12	1	Terrestrial: Stromatolite filaments (f)		[63]
	2	Terrestrial: *Gymnocodium* (f)	—	[41]
	3	Terrestrial: *Euglena viridis*	—	G. Bianciardi
	4	Terrestrial: Gypsum	—	V. Rizzo
	5	Terrestrial: Jarosite	—	(jarosite4138d, www.dakotamatrix.com)
	6	Terrestrial: Feldspar	—	(1200px-granite-phenocrysts, it.wikipedia.org)
14	A,B,C	Mars: Filaments	880	Curiosity
	D	Mars: Filaments	—	Opportunity
	L	Terrestrial: Algae and Cianobacters	—	A. Munneke
15	—	Terrestrial: tubular septate bodies	—	Hong Hua

Figure	Section	Type	Sol	Source
16	1–15	Terrestrial: Euglena	—	Hong Hua
	A,B	Mars: 'rice grains'	880	

Table 2.
List of terrestrial and Martian images used in the analysis of putative microalgae on Mars. (Abbreviations: Up = Upper, Lw = Lower, R = Right, L = Left, F = Fossil).

distribution of orientations, (5) overall shape of the profiles, i.e., whether fusiform or not fusiform and whether a degree of curvature was present. A Poisson distribution was fitted to the objects from all images. If the objects were distributed at random, then, the probability (P) that the fields contain various numbers of profiles is given by the Poisson distribution [45]. The variance (V) of a Poisson distribution is equal to its mean (M) and hence, the V/M ratio is unity for a random distribution. The V/M ratio can, therefore, be used as an index of "dispersion", uniform distributions having a V/M ratio less than unity and clustered distributions greater than unity. In addition, profile diameters and orientations were used to construct size class-frequency distributions. Two statistical models were fitted to these distributions: (1) a log-normal distribution [46], often used to describe the size distributions of plant populations [47, 48] and (2) a rectangular distribution, to test whether the objects exhibited any preference with regard to orientation. Goodness-of-fit to the various models was tested using the Kolmogorov–Smirnov (KS) test. To study the similarities among the ten images, the data were analyzed using Principal Components Analysis (PCA). The analyses were carried out using the images as variables and the various metrics as defining features. The result of PCA is a scatter plot of the images in relation to the extracted Principal Components (PC) in which the distance between the images reflects their relative similarity or dissimilarity based on the defining metrics. To correlate the location of an image on a PC axis with the specific metrics, correlations (Pearson's 'r') were calculated between the values of each metric from each image and the factor loadings of the case relative to the PC1 and PC2. For example, a significant correlation between a specific metric and PC1 would identify that feature as particularly important in determining the separation of cases along PC1.

4.2.2 Fractal morphometry: comparison of "rice grains" with various crystals and algae

"Rice grains" images were enlarged 10 x by Paint Shop software in order to reach the final size of the sample.

Images were then loaded on paint.net software (https://www.getpaint.net/download.html) in order to extract the specimen of interest. The extracted image was loaded on Digital Image Magnifier (Nikolao Strikos, https://sourceforge.net/projects/digitalimagemag/) in order to apply a Canny edge filter to the image, fixed Sigma, High and Low thresholds were used.

A negative of the skeletonized image was then obtained. The skeletonized image was loaded on Benoit Fractal Analysis software (https://www.trusoft-international.com/) in order to evaluate the Fractal Dimension of the image: Fractal dimension, D0, a measure of the space-filling properties of a structure, was calculated on the skeletonized images by the box-counting method. Briefly, each image was covered by nets of square boxes (from 5 to 100 pixels) and the amount of boxes containing any part of the outline was counted. A log–log graph was plotted on the side length of the square against the number of outline-containing squares, the slope of the

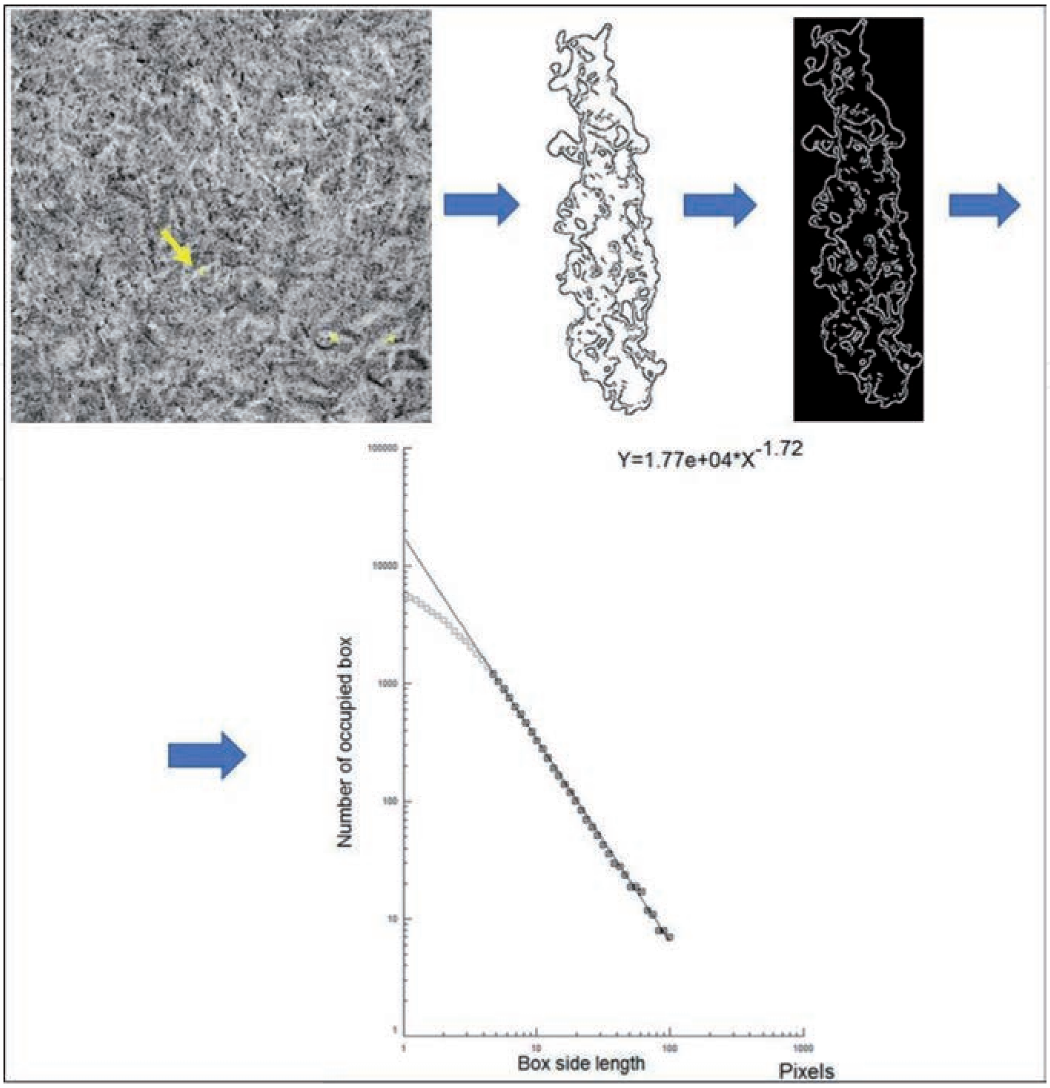


Figure 3.
Top: From a Martian sample (rover image MAHLI, taken at Sol 809, "rice grains"), a single "rice grain" is enlarged, a canny filter is applied and the negative obtained. Bottom: the log log plot is a straight line ($p < 0.01$): the "rice grain" owns a fractal structure or self-similarity, its exponent is the fractal dimension.

linear segment of the graph representing the local fractal dimension of the image (**Figure 3**). The linearity of the log log plot was assessed by the Pearson's correlation test, in order to demonstrate that the "rice grains" owns a fractal structure and fractal dimension may be performed. Variance analysis was used in order to compare fractal dimension of the Martian "rice grains" vs. the mineral (abiologic) gypsum or versus *Euglena viridis*, as a model of a complex (eukaryotic) microorganism.

5. Results

5.1 Environmental and morphological evaluations.

5.1.1 Sedimentological context and possible biogenic structures

Curiosity landed on a flat plain (the Bradbury landing site) to the North of Mount Sharp in August 2012. Subsequently, the rover travelled to reach the extensive strata of a lacustrine sedimentary sequence at the base of Mount Sharp (around Sol 750), detecting along this track a heterogeneous assemblage of sedimentary rocks, representing a fluvial-deltaic-lacustrine environment (the Yellowknife Bay formation). The basal Sheepbed and Gillepsie members of the Yellowknife formation are characterized by silts and mudstones showing mutual unconformity; such sediments were deposited in an intertidal region of a shallow lacustrine environment, representing the latest stage of transport and deposition of fan sediments inside Gale Crater lake [1].

In the first part of the Curiosity survey, interesting chemical and structural data emerged. Hence, with reference to the Sheepbed formation, at John Klein and Cumberland sites, SAM detected organic chemicals referable to molecules of chlorobenzene as well as the occurrence of all the chemical components of life [49]. Very interesting structures and morphologies were also observed, in addition to the many already cited, possible microbialites on the Sheepbed mudstone and structures recalling terrestrial microscopic induced by sedimentary structures, known as MISS, locally present as "erosional remnants," "pocket," "mat chips, "roll ups," "desiccation cracks," and "gas domes" [14]. Other possible microbially induced structures were also observed at this site, e,g., burst bubbles, filaments, mini-atolls, oncoids, domes and many other atypical sedimentary structures known as microbialitic [17, 39, 50], and some of which resemble stromatolites of the "Conophyton" type [18]. However, the morphology together with the chemical data mutually support the presence of ancient life, even if analyzed individually, they suggest alternative abiotic interpretations. For this reason, they will be individually considered, according to the criteria established by Astrobiology Field Laboratory (AFL) at http://mepag.jpl.nasa.gov/reports/index.html, as "possible biosignatures".

All previous described putative organosedimentary macrostructures were not observed when Curiosity moved away from the intertidal zone and entered (at Pahrump Hills location) a clay sedimentary sequence, the Murray formation at the base of the Mount Sharp. This was an area well investigated by the rover between Sols 750 and 930, and where deposits of mudstone and siltstone outcrops reveal a very thin, sub-millimetric lamination, proving the occurrence of a hydrodynamically stable environment. Particularly worthy of attention are the very thin laminated outcroppings which contain a few widely distributed aggregated harder structures [51], some up to several cm in size (**Figures 4** and **5**). Such dendritic, nodular and laminate concretions, comparable to the host rocks, show notable Mg-enhancement and a strong depletion of other major elements [52].

Chemical results indicate that the Gale Crater sediments were strongly influenced by early, subaqueous diagenetic reactions that produced, and sometimes filled a variety of pore types [53]. Since some sulfur was also detected in a dendritic feature, Mg occurrence was likely to be associated with a $MgSO_4$ phase: a mineral present as precipitated cement within sediment pores [2] and an indicator of a local very acid environment, confined to the hardest structures and, intriguingly, in a target (Mojave) where organic molecules, such as kerogen and one of its polyaromatic fragments (thiophene), were found [54]. Considering the lack of contact with host sediment, these harder structures appear not to be transported/deposited elements but diagenetic structures, formed after sediment deposition. In fact, the two step heating experiments made by Martin to date K-Ar ages of primary (at 930°C) and secondary (at 500°C) mineralogical components at Mojave 2 target gave respectively 4.07 +/− 0.03 Ga (associated to detrital plagioclase of lake sediments) and 2.12 +/− 0.36 Ga (associated to sulphates and other secondary components of the diagenetic structures including the "rice grain"). Such data also suggest a post 3 Ga aqueous phase occurred in Gale Crater, at a time after surface fluvial activity on Mars was thought to have largely ceased [7].

Hence, at the landing site of Gale Crater, conditions for the evolution of Martian life beginning with chemotrophic and anaerobic bacteria and which survive in fossilized traces has been hypothesized [11]. Moreover, chemical and textural data

DOI: http://dx.doi.org/10.5772/intechopen.95531

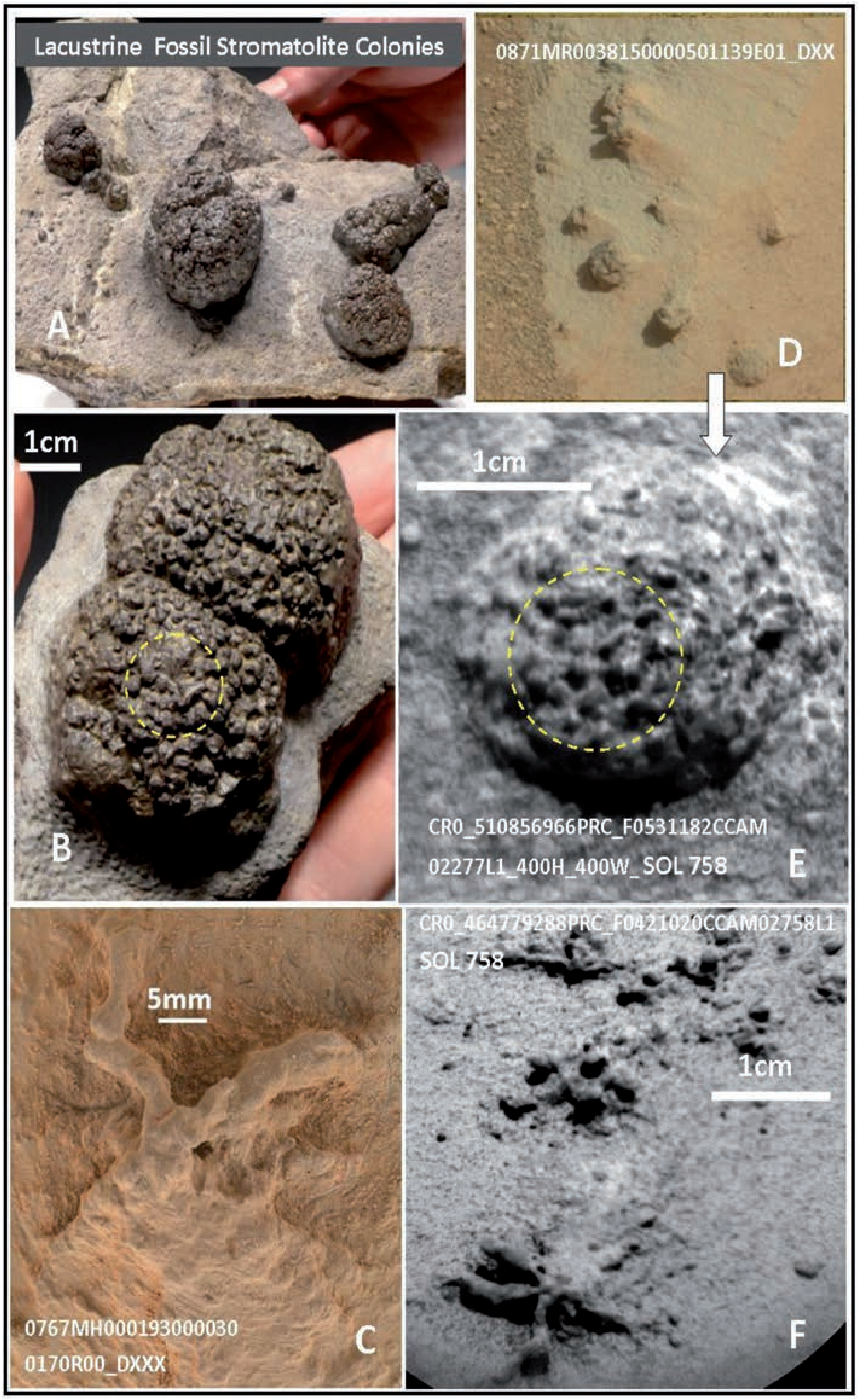


Figure 4.
Concretions found on the Murray Formation, Mars (C-F) in comparison to some Permian lacustrine terrestrial stromatolites (frames A,B; from: https://www.paleodirect.com/strx005-permian-lacustrine-stromatolite-colony-fossil). Both Martian samples and terrestrial stromatolites counterparts show harder structures consisting of lumps/nodules, that developed in close proximity (B, D) or became randomly aligned forming a branched (F) or overlapping structure (C), starting from single point (F and C) or from scattered "germination points" (D-F). One can also observe similarities on their surface pattern (B-E yellow circles) and distribution.

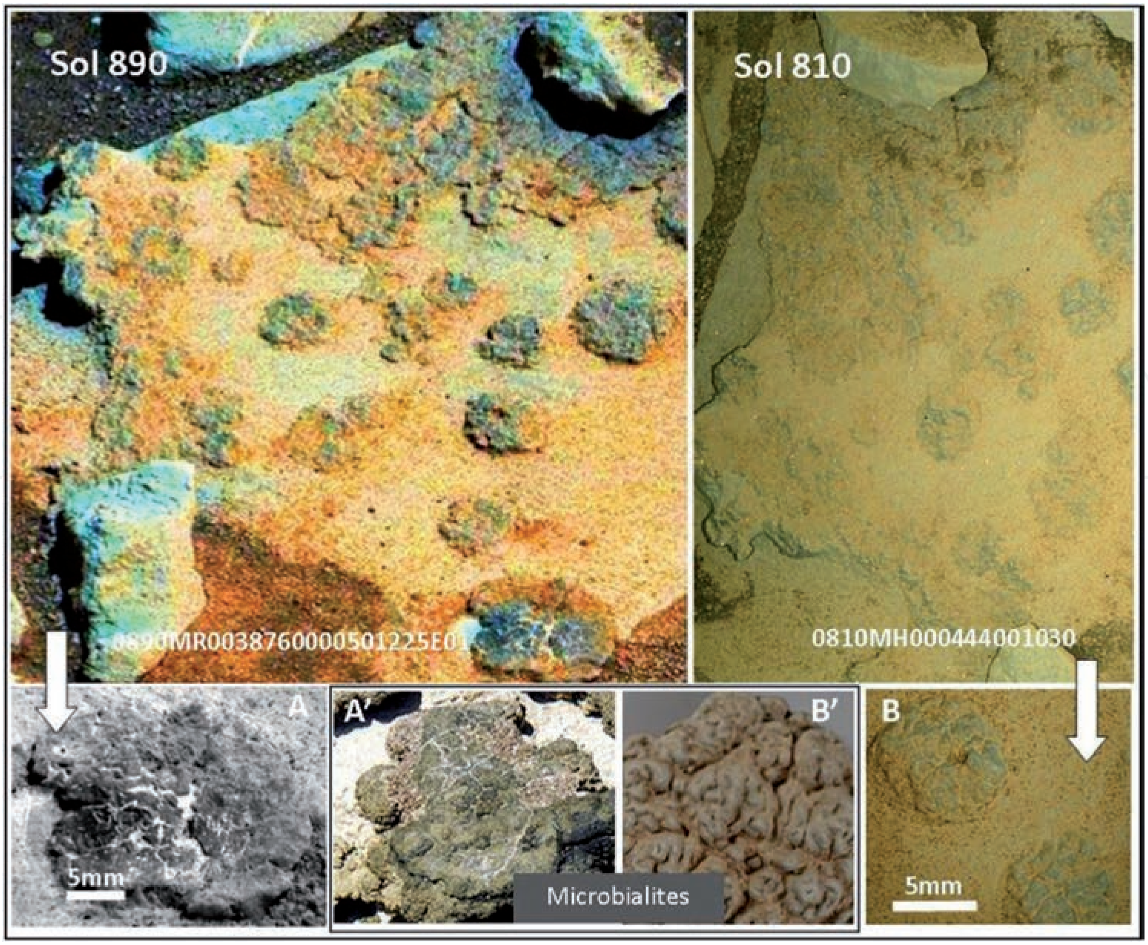


Figure 5.
*The two upper images (taken at the same outcropping, at sols 810 and 890), show irregularly hardened lamination. This case (irregularly developed in layers) is conceptually similar to those shown on **Figure** 2 and shows local transition to nodules (A, B). For this reason these hardened structures may suggest a syngenetic or early diagenetic origin, due to different spatial activity of colonies, rather than the effect of erosion. Note the morphological similarity to terrestrial microbialites (frames A-A' to an encrusting thrombolites; frames B-B' to a cerebrotic surface of stromatolites).*

testify to the presence of almost neutral lake water and to considerable diagenetic variations in pH [52]. The latter may suggest in very locally confined areas, that the acid micro-environment could also be attributed to microbial activity. Hence, the presence of elongated and curved filaments inside these structures and on their surface both in terrestrial stromatolites and Martian samples is particularly noteworthy (**Figures 6** and 7).

5.1.2 Nodular/dendritic harder structures

The harder structures may result from small grain aggregates, randomly dispersed, preferably along diverging alignments from scattered points. As a consequence, they assume several unusual shapes, forming lumps and nodules, and occasionally branched and/or overlapping bodies (called "dendritic"; [55]). The same structures also occur in the laminated sequences typically forming irregular crusts and emerging nodules, marked by an irregular scabrous surface, typical of stromatolites (known as "cerebrotic") and thrombolytic crusts. In these structures and on their surface, both in terrestrial and Martian samples, elongated and curved filaments are sometimes noted (**Figures 6** and 7); most noteworthy, the elongated structures of Martian samples, occasionally exhibit regular septate forms (**Figures 6-9**).

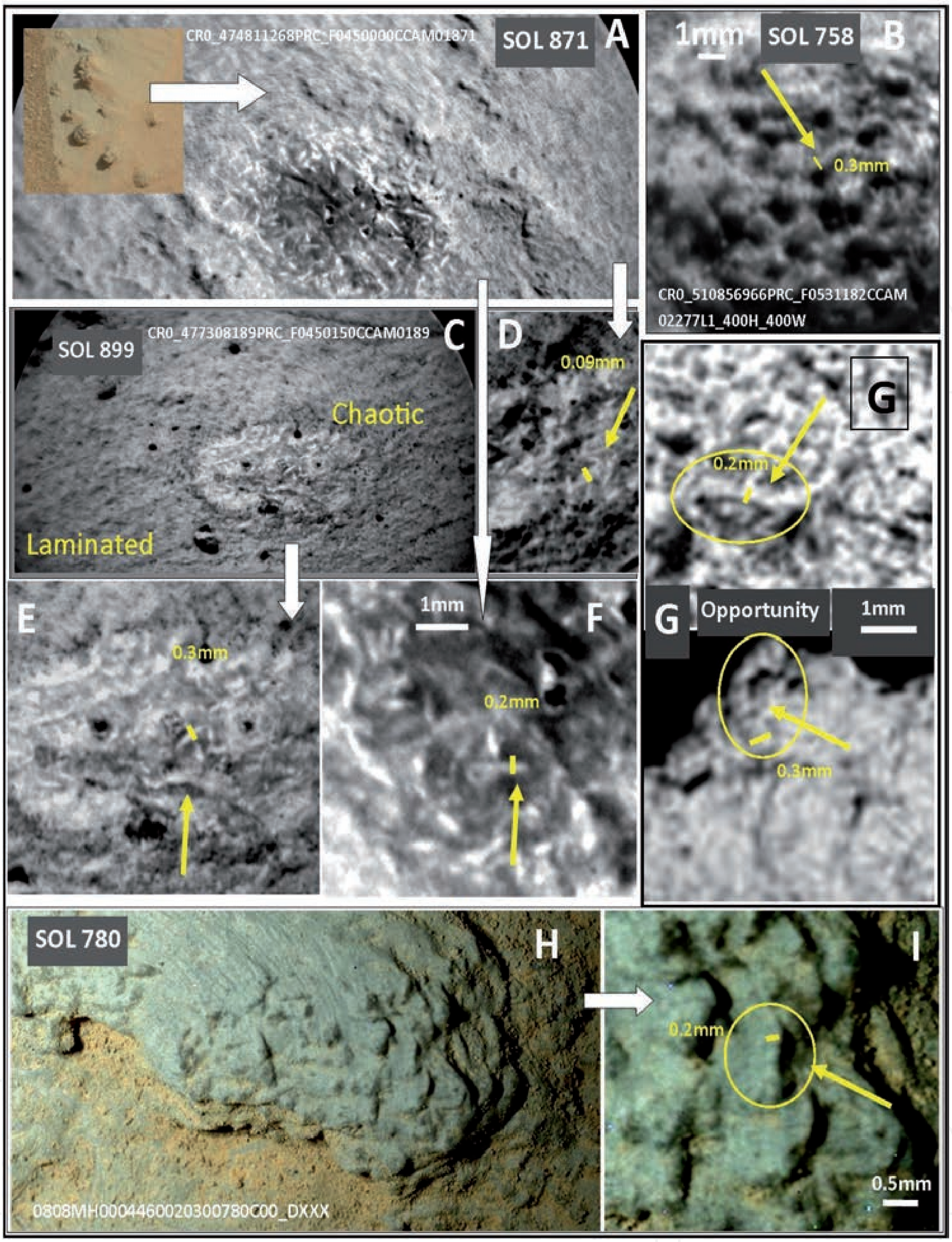


Figure 6.
These images show a series of filamentous/elongated microstructures taken by MAHLI (H,I) and ChemCham (A-B) in the hard concretion of the Murray Formation, in comparison to other Martian filamentous structures taken by Opportunity rover (G). The selected elongated microstructures (arrows and yellow circles are in relief in all frames); they show cross sections of 0.09–0.3 mm and regularly septate interspaces (more evident in G, H and I), forming elongated sinuous (D-I) and intertwined structures (E-I). On note the lateral discordance, from a laminated setting to a more chaotic structure (C), common for terrestrial microbialites.

Such dendritic, nodular and laminate concretions have been investigated in detail by the NASA scientific team [52]. They found, comparable to the host rocks, notable Mg-enhancement and a strong depletion of other major elements; nickel also being reported by ChemCam. Since some sulfur was also detected in a dendritic feature, they interpreted the Mg occurrence as of the presence of $MgSO_4$ phase; a

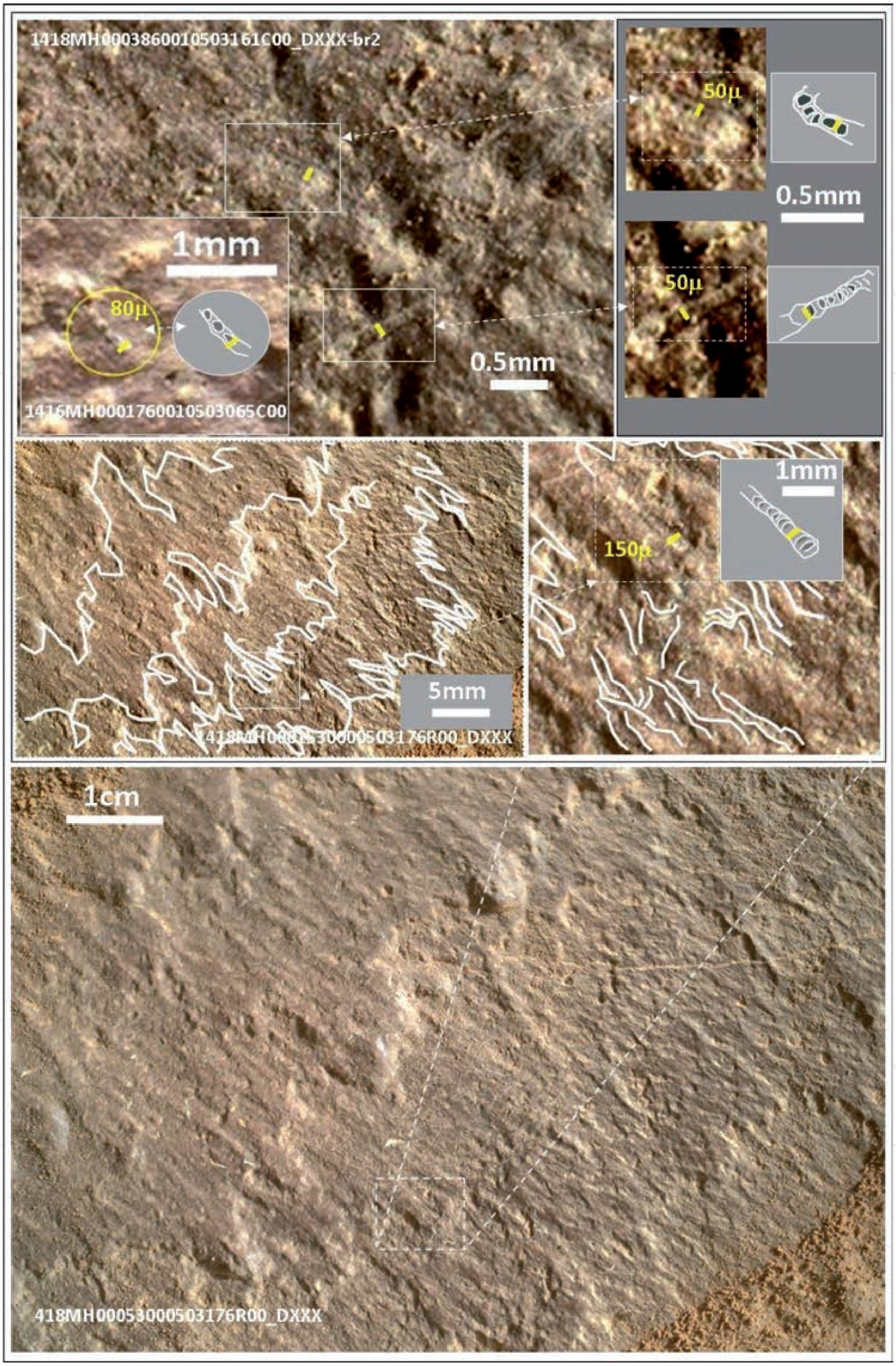


Figure 7.
Harder septate filaments forming thin laminae (Murray Formation; Sols 1416–1418). Images show a series of filaments (as shown in sub-frames) having transverse dimension ranging from 50 to 150 microns. Such filaments stand out from the rock and show a sequence of aligned harder segments, having septate bodies. Their shapes are often sinuous, several millimeters long; in the above images they are in relief, orientated NW-SE, thus determining, due to their hardness, serrated contact between the laminae (white lines).

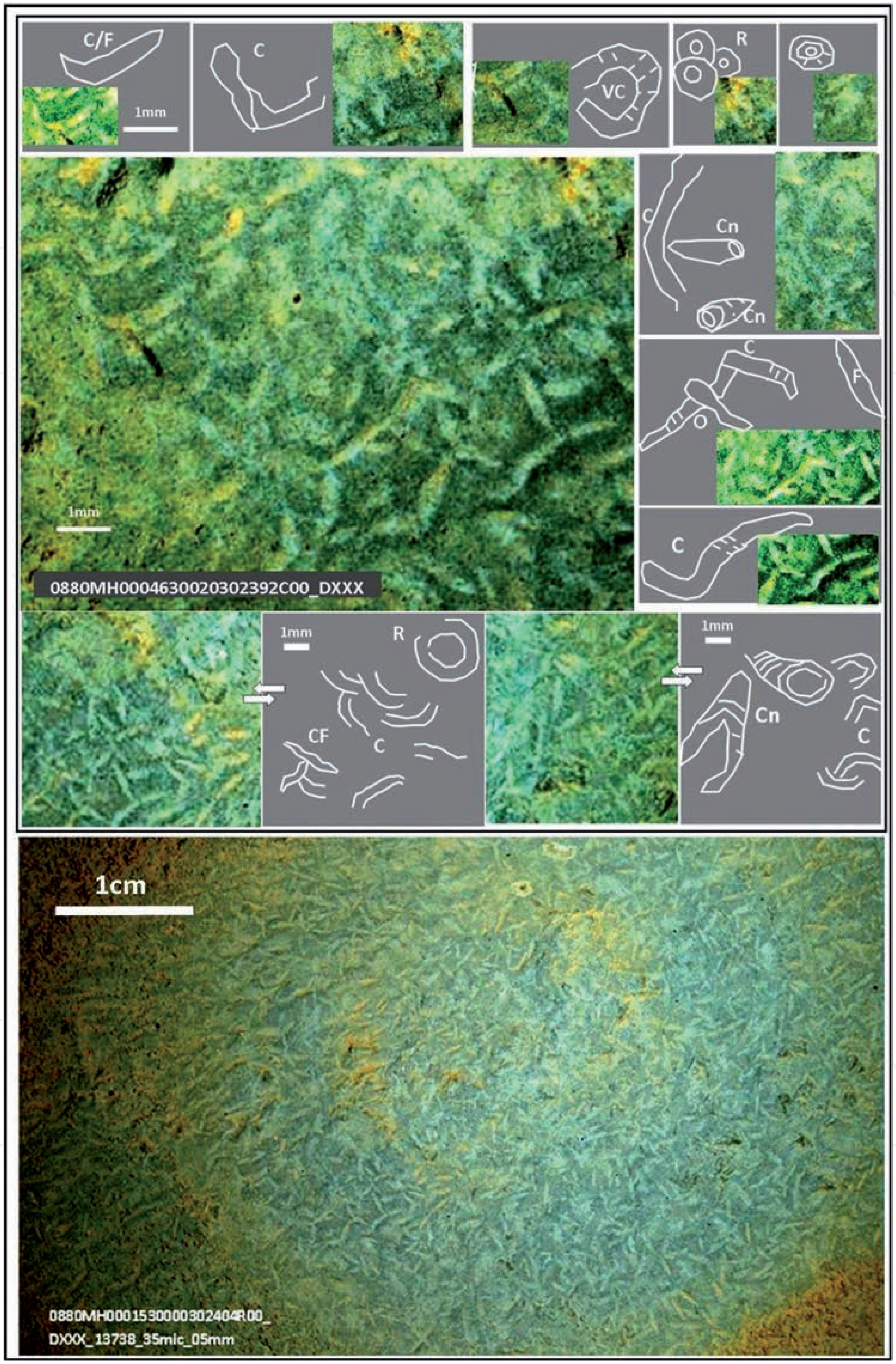


Figure 8.
Morphological interpretation of some of the "rice grains" on an amplified MAHLI image (Sol 880). One can see repetitive curved (C), overlapping (O), fusiform (F), conical (Cn), very curved (VC), ring-shaped (R), curved/fusiform (C/F) shapes and therefore showing significant differences from the expected regular appearance of crystals. Note the presence of shapes (R and Cn types) which we have interpreted as possible transverse sections of conical bodies.

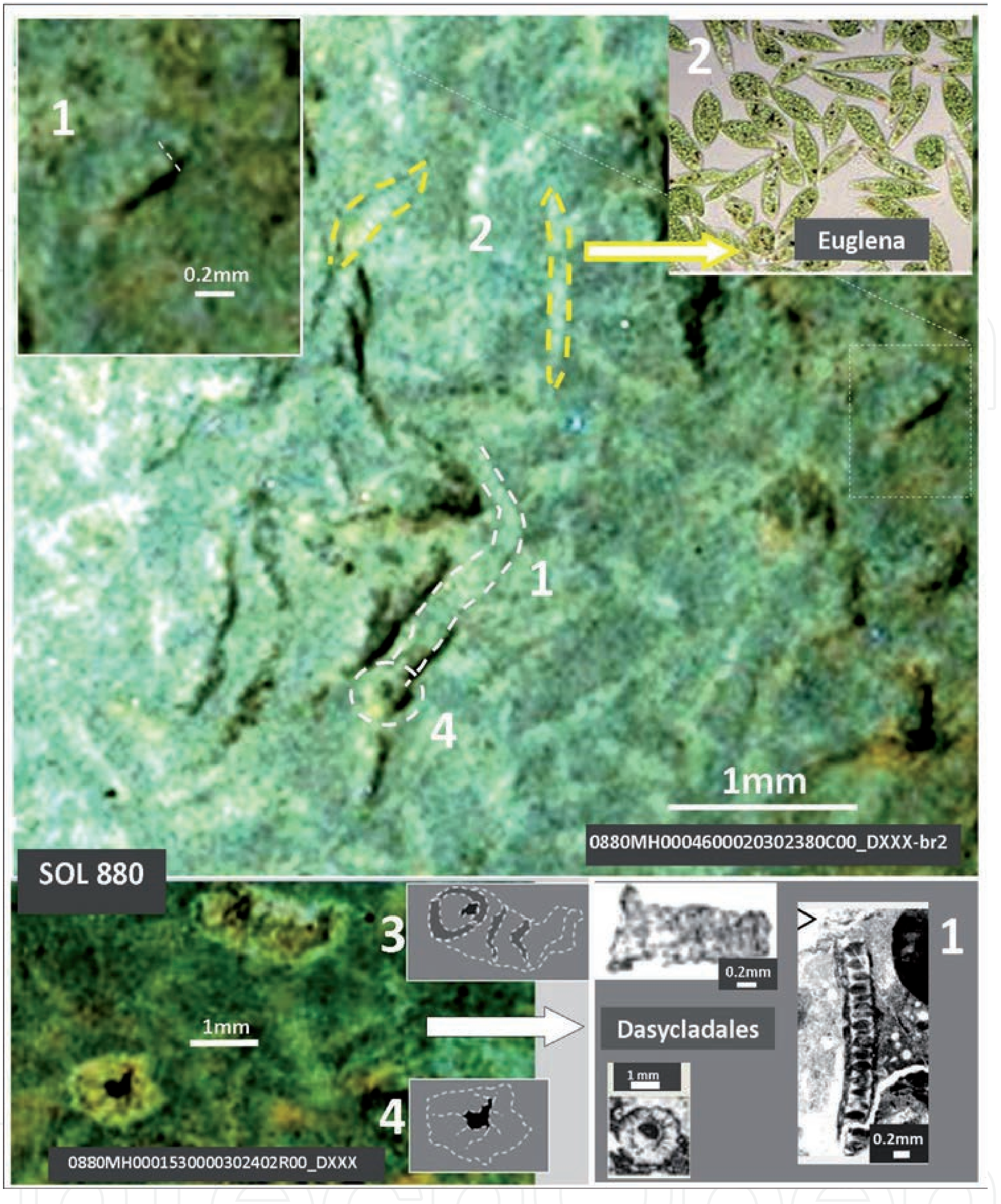


Figure 9.
Highlighting the most common forms observed in MAHLI images at Sols 809 and 880. The amplified image in the center shows the following types: elongated/curved body (1), occasionally septate (frame left on the top; see dashed line), lenticular/curved (2), holed conical (3) and possible transverse sections of previous type bodies (4). Examining possible terrestrial analogues, such microstructures are similar to terrestrial Dasycladales, Euglenoid, or giant filamentous cyanobacteria, rather than crystals. Please note that, the shapes are unambiguously identified by the large number of colored pixels contained.

mineral present as precipitated cement within sediment pores [2], and hence an indicator of a local very acid environment and confined to the hardest structures.

Information regarding the dimensions of such structures, their shape and their layering, in relation to the sedimentary environment and their possible origin, are particularly important. Structures appear to be embedded within sediments of a quite aqueous environment and lack net contacts. Despite their random form and distribution, they show common features and appear as irregular aggregates, composed of globular and/or linear structures, affecting groups of laminae.

The dimension of these aggregates, their unusual structure and distribution and the lacking of net contact with the host sediments, that'suggests that they are not transported/deposited elements, but that their formation was inside the sediment, after deposition, due to local cementation and/or grain rearrangement; conditions likely to be present during diagenesis and still existing in lake waters. In fact, chemical results indicate that Gale Crater sediments were strongly influenced by early, subaqueous diagenetic reactions that produced, and sometimes filled, a variety of pore types [49, 53]. Chemical and textural data indicate almost neutral lake water, while considerable local variations in pH [52]; so, they may suggest micro-environments and related microstructures generated by microbial activity and/or to the presence of local organic material. In addition, at the Mojave target site, traces of thiophene were found by the NASA scientific team, one of the main elements of kerogen; an organic compound that may be related to bacterial metabolism associated with terrestrial microbialites and, commonly, used as one of the main criteria to assess the biogenicity of putative Archean stromatolites [54, 56]. Particularly worthy of attention, both internally and externally, terrestrial stromatolites and Martian samples, both show elongated and curved filaments (**Figures 6** and **7**).

5.1.3 Filamentous microstructures

Occurrence of filamentous structures, detectable by a different color and tone variation, normally appear to be formed by septate bodies having transversal dimensions of 0.05–0.3 mm (**Figures 6** and **7**). These structures are more evident on amplified/slightly blurred images of Martian sediments and on clean/abraded surfaces. Occasionally, they are more resistant and in relief, conditioning the shape of the laminar surface and their mutual contacts (**Figure 7**). They were observed, both as single features as well as intertwined structures and cover the rock surface resulting in a "woven" texture. Similar settings (**Figure 6**, frame G) have been investigated in previous work and interpreted, by visual and numerical approaches on a consistent number of terrestrial sampling analogues, as microbial/stromatolitic structures [12, 13, 17].

5.1.4 Lenticular and conical/tubular structures

MAHLI images taken at Sol 869 show that the lenticular lozenge-shaped "rice grains" observed on brushed surfaces at Sols 809 and 880 (Mojave target), not only occur "on the surface" as harder and whitish structure, but massively affect the entire outcrop, covering about 50% of the lithological mass. Previously, these structures have been interpreted as mineral deposits, e.g. of Gypsum or Jarosite (NASA reports), but subsequently, due to the lack of sufficient amount of crystal, they were interpreted as pseudo-morphic crystals originating from amorphous substances.

In particular, the mineralogical composition of Mojave 2 (Sol 880) shows, in respect to the previous investigated rocks, a variation in mineral composition, exhibiting significant amounts of amorphous material (54%) and minor amount of Plagioclase (24%), Magnetite (4%), Hematite (4%), Jarosite (4%), Phyllosilicates (5%) and Fluorapatite (5%). Such data paradoxically suggest the coexistence of both oxidising (Hematite) and reducing (Magnetite) environments, as well as acidic (Jarosite) and neutral (Fluorapatite) components. Chemical data of this sample suggests the following composition: SiO_2 (49%), FeO (16%), MgO (4%) CaO (4%) and Al_2O_3 (11%), together with other minor components, including Magnetite and Phosphorous [2].

Some of these minor components and minerals, such as Apatite, Magnetite, Ni, Zn and Br (from Curiosity APXS results; in [7]), found at Mojave target, on Earth are generally associated with microbial activity and stromatolites [50, 57] and suggested by AFL report as possible biosignature [58].

Given the lack of mineralogical (CheMin) or chemical (APXS) evidence for calcium sulphates in the Mojave 2 sample (Sol 880), it was assumed that these lenticular bodies represent, on the basis of their morphology, i.e., lenticular gypsum crystals laths, light color compared with the host rock, and penetration vertically into the bedrock, crystals laths that were formed syndepositionally with the Murray mudstone and were later re-dissolved by post depositional fluid flow, forming pseudo-morphic microcrystalline or amorphous substance of unknown composition [2, 7].

It should be noted that the occurrence of microbially precipitated fluorapatite is reported in Jurassic phosphate stromatolites by Sànchez [57] and it is also known that biomineralization processes could give Biologically Induced Mineralization (BIM) and Biologically Controlled Mineralization (BCM), where magnetite is one of major components [59]. Moreover, such structures were found in association with the previously described problematic diagenetic features [55, 60, 61], and hence, a number of controversial features are suggestive of possible biogenic shapes.

Morphological analysis of MAHLI images at sols 809 and 889 (at Mojave1 and Mojave2, respectively) reveal that the "lozenge-shaped sulphates" [51] show chaotic, mainly fusiform/filiform, septate, curved shape; some of which are in relief and resemble terrestrial microalgae (**Figures 8** and **9**).

In particular, considering their shape and dimension, we investigated the structural similarities with Dasycladales algae, giant filamentous Cyanobacteria or Euglenoids. This biological interpretation could be supported by the occurrence of two adjacent 'bright' bodies, present in the same image (Sol 880; **Figure 9**, features 3 and 4). Occurrence in the same target of spherical cross sections (having sharp inner surface and irregular outer edge), could be related to other cones, although less evident and in small amounts (**Figure 8**, Cn and R features). Another conical body, photographed by Opportunity at Meridiani Planum, shows a differentiated skeletal structure in transverse section (a conical thallus?), and possible regular radial laterals attached (of aspondyl type? **Figure 10**). In addition, images at Sol 1273 show transverse and oblique sections of a tube-like bodies associated with regularly jagged discontinuities of their shells (**Figure 11**; features 1–3). Hence, the variety of shapes, present which include septate filamentous structures, is of great biological interest and do not suggest crystal-type structures; and are worthy of morphometric investigation (**Table 2**, **Figure 12**), the results of which are reported in the following section.

5.2 Morphometric results

5.2.1 Euclidean morphometry

The metric data for each image is summarized in the upper section of **Figure 13**. There was considerable variation in all metrics among images with the exception of the fit of lengths to a log-normal distribution, the KS tests suggesting that this distribution fitted the objects of interest in all images. V/M ratio varied from a maximum value of 1.08 (Jar-1) indicating a random distribution of profiles, to a minimum of 0.32 (Euglena) suggesting the majority of profiles exhibit a degree of uniformity in their distribution. Variation in length and width also varied among images being least in the Martian sample and Terrestrial Euglena and greatest in the alga Gymnocodium. Length/width ratios were greatest for Gymnocodium and least for the three gypsum

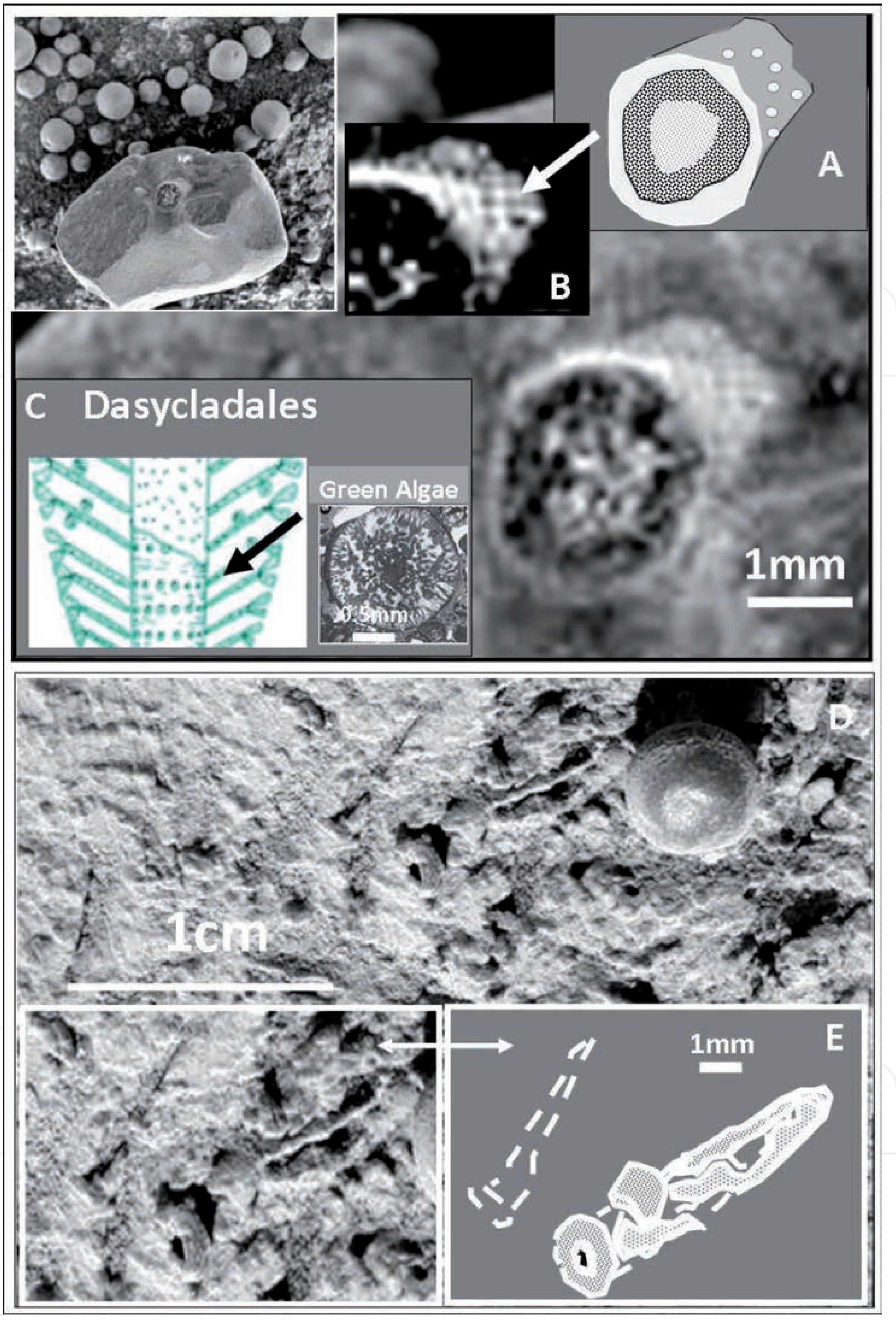


Figure 10.
Different conical bodies, detected at Meridiani Planum by Opportunity; possible biological remnant of fossils "incertae sedis". These microscopic cones have littler bigger dimension. On the top (frames A, B) the cone has similar size and shows an internal zoned structure of a possible algal stem (C), having a number of lateral structures (arrows), resembling (far, not confirmed) terrestrial Dasycladales laterals side. Below (D, E) the cracked cone is littler bigger and show a collar.

crystal samples. Significant departures from a rectangular distribution, suggesting orientation specificity, were observed in the Mars sample and also by the stromatolite algal filaments, Gymnocodium, Jarosite and feldspar phenocrysts. The percentage

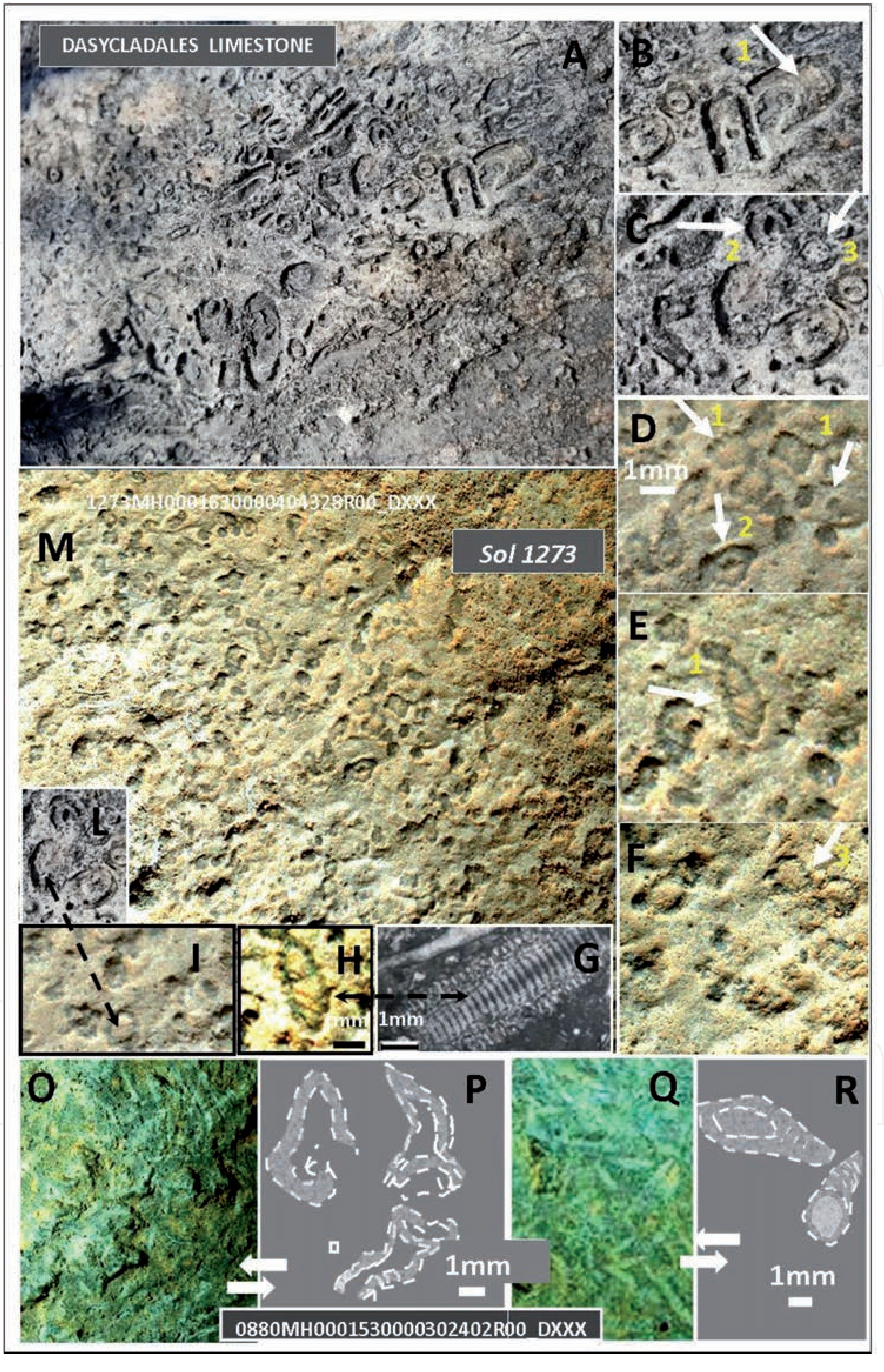


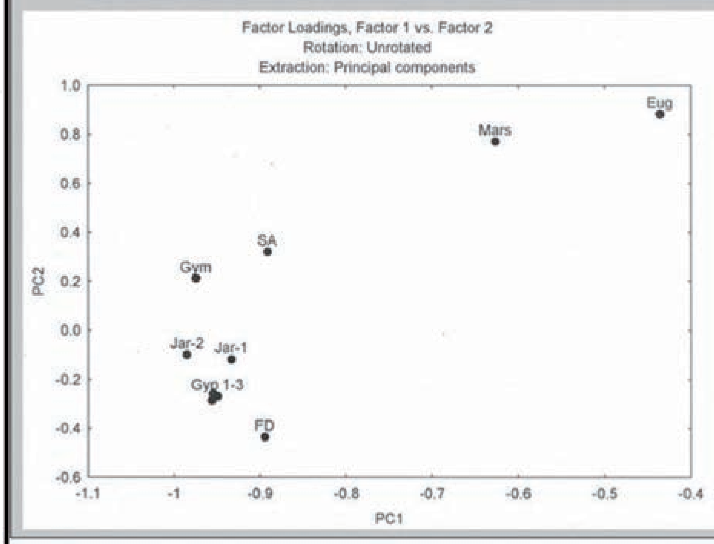
Figure 11.
Comparison of a Dasycladales limestone (b/n pictures) to Martian putative fossils, at Sols 880 (frames O-R) and 1273 (frames D-F, H,I,M). Resembling (far, not confirmed) features of terrestrial Dasycladales include millimetric dimensions, a calcareous (conical or tube-like) stem, occasionally having regular discontinuity (frame G; from [62]), as are undulation or fissures. Such structures are visible on the above b/n pictures, as well on putative Martian fossils where regular transversal lines (arrow 1) or tube-like structures and their transversal sections (arrows 2 and 3) are visible. Careful observation shows knurled shells (for algal laterals). Similar structures appear to be seen in the rover's images (see the arrows), including the knurled shells.

Figure 12.
Fossil and living terrestrial algae together with mineral crystals used to compare with the "rice grains" in ***Figure 2****: (1) Population of fossil stromatolite algal filaments (From: [63], their* ***Figure 5****); (2) Population of fossil algae (Gymnocodium group, possibly a red or green alga) (From: [41], Plate 57 7/8); (3) Sample image of living* Euglena viridis*; (4) Gypsum crystals (Gesso-2), (5) Jarosite crystals (jarosite 4138d, www.dakotamatrix.com), (6) crystals of feldspar (phenocrysts) (1200px-Montblanc-granite-phenocrysts. Mineral from Mineralienatlas.de., authorized).*

of profiles exhibiting a fusiform shape or a degree of curvature also varied among images being generally greatest for the Mars sample and the terrestrial microalgae and least for the mineral deposits.

A PCA of the data resulted in the extraction of two Principal Components (PC's) accounting for 96% of the total variance (PC1 = 77%, PC2 = 19%) indicating that separation of images along PC1 is more significant than along PC2. A plot of the 10 images in relation to PC1 and PC2 is shown in **Figure 13** with significant correlations between the factor loadings of the images on the PC and the various metrics. **Figure 13** shows: (1) that the three images of gypsum crystals (G1–3) have very similar metrics and form a distinct cluster not closely related to the Mars sample or to any of the investigated algae, (2) neither feldspar phenocrysts nor jarosite crystals are closely related to the Mars sample (3) Gymnocodium (Gym) and the Stromatolite Algal filaments (SA) are more closely related to the gypsum crystals in their metrics than Euglena (Eug) and (4) of the studied microalgae. Correlations between factor loadings and the various metrics suggest, the proportion of profiles with a fusiform shape, the proportion of curved profiles and the degree of variation in profile widths are all significantly correlated with PC1 and PC2 and, therefore, are the most important of the metrics distinguishing among images, the Martian sample and terrestrial Euglena displaying the most consistent widths and having a greater proportion of fusiform and curved profiles than the mineral deposits and other terrestrial microalgae.

Image	Metrics N	V\M	CV-L	CV-W	L/W	Fit to LN	Fit to RD	% FF	% Curved
Mars	411	0.78	42%	16%	4.1	0.03	0.09**	64%	36%
SA	79	0.95	95%	25%	6.5	0.08	0.15*	51%	29%
Gym	162	0.48	104%	61%	3.1	0.05	0.30**	64%	32%
Eug	111	0.32	41%	16%	4.4	0.04	0.09	86%	71%
Gyp-1	169	0.35	74%	45%	2.3	0.01	0.07	105%	1%
Gyp-2	159	0.46	73%	63%	1.7	0.04	0.07	17%	0%
Gyp-3	147	0.53	59%	37%	2.0	0.04	0.09	10%	0%
Jar-1	128	1.08	34%	44%	3.7	0.03	0.19**	18%	12%
Jar-2	76	0.48	37%	34%	3.6	0.05	0.11	15%	10%
FD	159	0.65	47%	47%	3.0	0.04	0.33**	1%	1%



A– Euclidean Morphometry

Metrics	PC1	PC2
V/M	-0.13	0.03
CV (lengths)	-0.38	-0.07
CV (widths)	-0.73*	-0.72*
L/W	0.39	0.62
Fit to LN	-0.09	0.20
Fit to RD	-0.26	-0.22
% Fusiform	0.74*	0.95*
% Fus. curved	0.84*	0.94*

B– Fractal Morphometry

Metrics Analyzed structure	N	Mean	SD	
Mars	30	1.570	0.047	
Gypsum	10	1.615	0.034	P < 0.01
E. viridis	30	1.581	0.055	P = n.s.

Figure 13.
Results of Euclidean morphometric investigation on "rice grains". On the top (A) the plot shows results of Euclidean morphometry analysis, differences and similarities, between the 10 images analyzed: PC1 against PC2 (Eug = Euglena, Gyp 1–3 Images of gypsum crystals, FD = Feldspar phenocrysts, Gym = Gymnocodium, Jar1 - Jar2 = Jarosite crystals, SA = Stromatolite algal filaments). Below (B), comparison of fractal dimension values (Mars, "rice grains" vs. Gypsum (P < 0.01) and vs. Euglena viridis (P = n.s.)). Its fractal dimension permit to distinguish them from the mineral negative control, while it is not possible to distinguish the Martian features from the biologic control, perfectly superimposable among them.

5.2.2 Fractal morphometry

Fractal analysis data are summarized in **Figure 13**, bottom, B. Fractal dimension of the Martian "rice grains" is lower than the one of the negative control, gypsum, with high statistical significance ($p < 0.01$). Vice versa, fractal dimension of the Martian "rice grains" overlaps the one of the unicellular alga *Euglena viridis*, positive control ($D = 1.570 + 0.047$ vs. $1.581 + 0.055$, mean + SD, $n = 30$ per each sample).

6. Discussion

The first presence of organic matter on the Red Planet was revealed, even if initially misunderstood, by the Viking's pyrolysis gas chromatography–mass spectrometry (GC–MS) analysis of Martian regolith [64]. More recently, the presence of chloromethane and dichloromethane, as markers of organic matter on Mars, were confirmed by SAM on Curiosity [65]. Since the Viking landers, organic matter has been repeatedly detected in Martian meteorites [66]. Hence, Curiosity rover drilled into the three-billion-year-old mudstones from four areas in Gale Crater and especially at Mojave and Cumberland sites, revealing many organic compounds, including thiophenic, aromatic, and aliphatic compounds, that were released at temperatures from 500° to 820°C [67], and reported in **Table 1**. The variety of different carbon-containing compounds provides evidence of possible macromolecules in the Martian regolith. Interpreting their presence, we can recall the kerogens observed on Mars: they are a type of organic molecule that can be easily associated with life (stromatolites), but, viceversa, it is also present in carbon-rich meteorites, in interplanetary dust particles, and in igneous rocks, where life is not present. Nevertheless, the thiophenes observed on Mars, should be strongly suggestive of life [68], being easily explained as a result of biologically related sulfur incorporation into organic matter during early diagenesis.

Moreover, it has been suggested that biominerals could be important indicators of life and thus could play an important role in the search for past or present life on Mars as on Earth [58, 69, 70]. Organic components themselves (Kerogene and Tiophene) are often associated with biominerals and are believed to play crucial roles in both pre-biotic and biotic reactions. They have been found in the fossil record that date back to the Precambrian and were used on Earth as evidence of the biogenicity of Archean stromatolites [56].

On Mars, and in particular at Mojave targets, morphological observations of dendritic, nodular and laminated, harder structures (and complex organics, as well biominerals occurrence), may suggest a common origin, and may represent possible developmental stages of a single entity. Their variable dimensions, scattered distribution, and uncommon shape, show the same morphology as terrestrial microbialites. In this frame, noteworthy are the small nodular and encrusting microbialites, which are found in a wide range of lacustrine environments and in thin laminated mudstone, and they have been attributed to moderate wave agitation [71]; convincing parallels being visible, in the lake stromatolites of the West Germany lower Permian (Lauterecken Formation); as well as examples of stromatolites in nodular settings, forming larger cemented complexes known in current alkaline (pH > 9) fresh lakes (Salda Lake, Turkey).

In this context, the irregular shapes assumed by the harder structures containing complex organics and biominerals most likely represent a results of bacterial or microalgae extracellular polymeric substances, according to an organic mineralization process present during diagenesis.

The spatial development of stromatolites is important in interpreting their eventual structures. Hence, the basic structure of microbialitic sediments are essentially laminar (in plane), nodular (balls or lumps) and/or elongated (linear). These structures can also merge, respectively resulting in stromatolites, thrombolites, dendrolites and with ever-larger combinations providing all the typical known morphologies. The observed structures and morphologies, shown in **Figures 4**-**11** and all of those described to date in various studies, are all typical of microbialitic world.

In general, the complexity and distinctiveness of biological structures increase with size and degree of biological evolution. There is still controversy on Earth regarding the biogenicity of some primordial microscopic structures and specialists attempt to solve these problems using instrumental insights and further laboratory investigations. These problems are generally related to the presence of possible very ancient microbial structures, having micrometric or sub-micrometric dimensions.

Indeed, microfossils, with a size of hundreds of microns, are more complex and distinctive, and on Earth other investigations are usually not necessary to recognize their biogenic nature.

In terms of "relevance for morphological recognition of biogenic structures", three domains could be distinguished: microbes, microfossils and fossils. Although doubts have often been expressed about the visual unambiguity of Martian microstructures, the described morphologies here described and related to putative "microalgae" should be considered unambiguous. In fact, the Mahli images that we have analyzed most frequently have pixels in the range 20–30 microns. For example, **Figure 9** (taken at Sol 880) which contain the lozenge-shaped bodies and the "cornucopia", have a pixel dimension of about 25 microns whereas the analyzed objects have millimetric or submillimetric dimensions and contain hundreds of colored pixels. As consequence, a single septate partition of the elongated structure shown in **Figure 9** (frame 1), having a dimension of about 0.1 mm, contains more than 16 colored pixels, which enables the septate structures to be unambigously observed.

Such filamentous segmented structures, having a cross section of 0.09–0.30 mm, are common in Martian sediments and have been described in previous papers [16, 72, 73]. In general, we can state that septate filament-like structures are frequent in terrestrial algal-like biota and that the Martian structures, that we have highlighted, are morphologically similar to a wide range of terrestrial counterparts (i.e., Epimastopora green alga; **Figure 14**).

The fossil record of septate bodies and the filaments are abundant, and are mainly characteristic of three big groups, i.e. Oscillatoriopsis, Megathrix, and those phosphatized tubular fossils (**Figure 15**) in the Ediacaran Weng'an Biota [74]. Oscillatoriopsis is characterized by unbranched, unsheathed, uniserate cellular trichome. The cells are uniform in length and diameter within the same trichome with no constrictions at the cell boundaries. Butterfield [75] recognized four species of Oscillatoriopsis according to their diameter, i.e., O. vermiformis Schopf, 1968, 1–3 μm; *O. obtusa* Schopf, 1968, 3–8 μm; O. amadeus Schopf and Blacic, 1971, 8–14 μm and O. longa Timofeev and Hermann, 1979, 14–25 μm.

Megathrix, however, are tubular microfossils typically less than 100 μm wide and several hundred μm long. These tubes, rarely branched, are characterized by evenly spaced transverse cross-walls which are complete or incomplete. Complete cross-walls are corrugated or flat and most are regularly intercalated with incomplete cross-walls. Incomplete cross-walls are flatter or less strongly corrugated than the complete examples and they have central perforations typically of similar size within the same specimen, although the perforation size may vary between specimens. Liu et al. [74] described five species of tubular microfossils from the Ediacaran Doushantuo Formation at Weng'an, Guizhou Province, South China. They also have complete and incomplete cross-walls [76]. However, the diameter of the Doushantuo species (mostly 100 μm – 250 μm in diameter) is much greater than Megathrix longus Yin L. and they all have flat rather than corrugated cross-walls. Of the five Doushantuo species, Ramitubus increscens Liu P. [74] and *Ramitubus decrescens* Liu P. [74] are both characterized by regularly dichotomous branching and rare incomplete cross-walls and by tetragonal tubes while *Crassitubus costatus* Liu P. [74] by a curved cylindrical tube with a longitudinal ridge, while *Quadratitubus orbigoniatus* Xue Y. [77] is characterized by a ridge. Finally, *Sinocyclocyclicus guizhouensis* Xue Y. [77] is most similar to *Megathrix longus* Yin L. except the former has greater diameter and flat cross-walls [76, 77]. We cannot be certain whether the septate filaments have corrugated or flat cross-walls but some ring-shaped (R) in **Figures 8** and **9** may represent central perforations on the cross walls; and very curved (VC) in the same figure have short incomplete walls, all supporting a resemblance to *Megathrix longus* Yin L.. The cornucopia-like

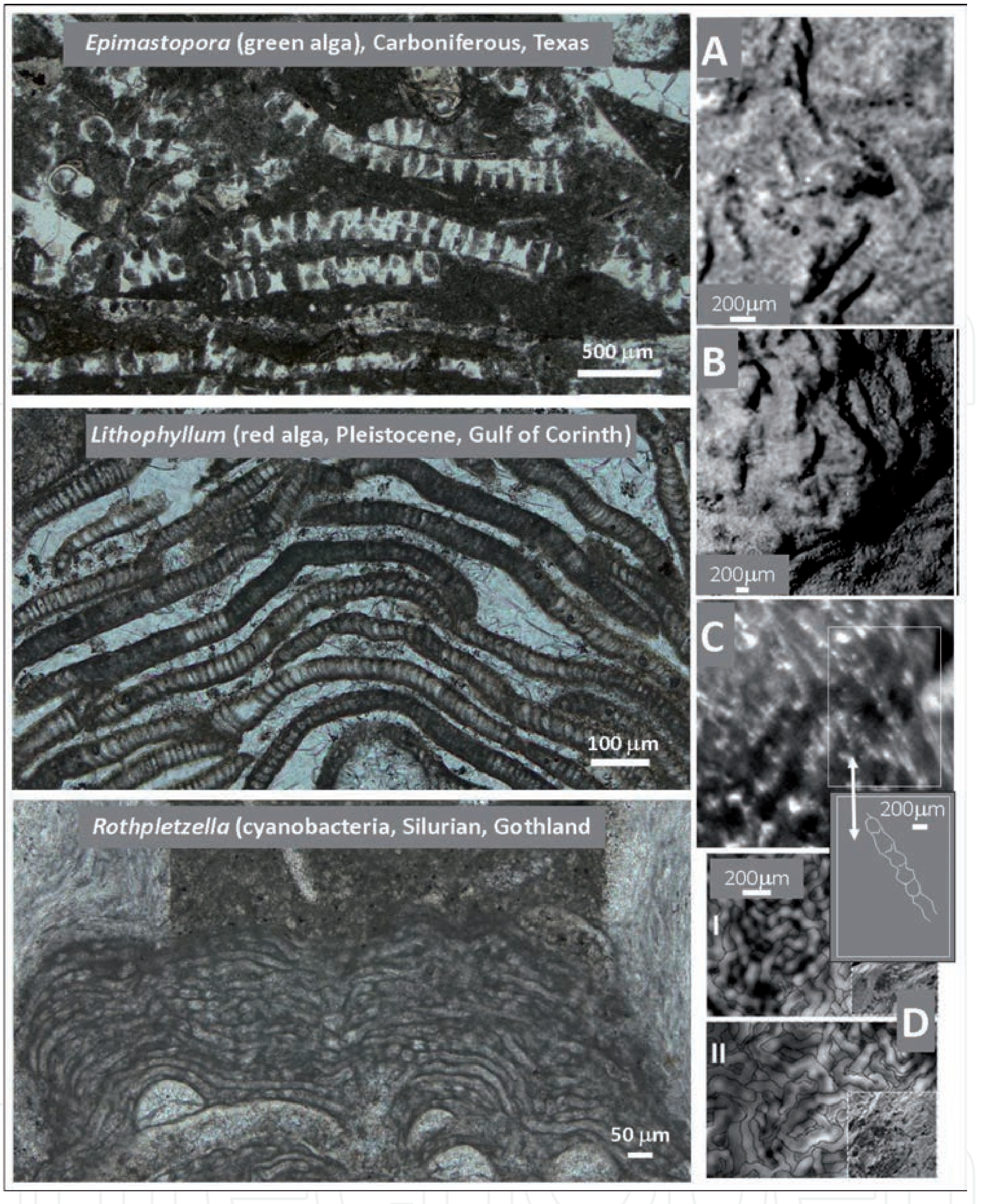


Figure 14.
Septate filament-like structures are biological "in principle" on Earth given a large number of algal structures having similar shape but different dimensions. On the left, three examples of different biota. Frames on the right represent Martian samples of similar structures. Right on the top (A), an enlarged cutting of Sol 880 image showing hard filament, in relief and septate. Frame B represent a reproduction of filamentous structure described on ***Figure 6****, on frames H and I. Frame C is a ChemCam (CR0_631854670PRC_F0781160CCAM03640L1) image catting showing some septate filaments, resembling intertwined filaments of spherules. In D similar structures observed in previous works and compared to some stromatolite similar structures.*

structures may represent oblique sections of tubular fossils with septate filaments such as present in *Megathrix longus* Yin L. or *Sinocyclocyclicus guizhouensis* Xue Y. [77] with possibly cyanobacteria affinity (**Figure 15**).

Moreover, morphometric investigations suggest a PCA and fractal dimensions of the "rice grains" with an affinity far from the mineral deposits studied such as gypsum, jarosite, and feldspar phenocrysts. In effect, phenocrysts are euhedral and angular whereas many of the Martian deposits are fusiform and exhibit a degree

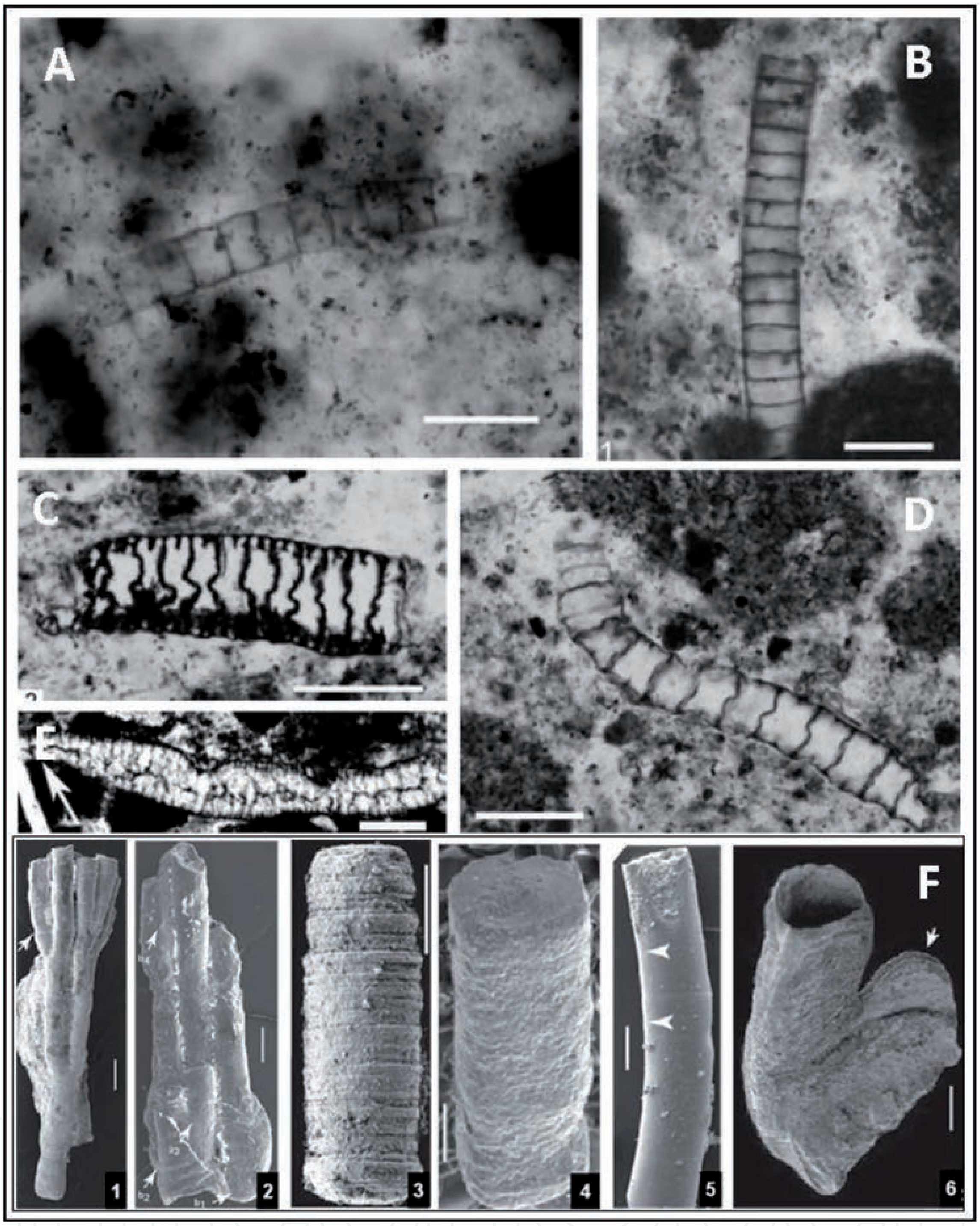


Figure 15.
Microphotographs of a set of tubular septate-likes bodies thin sections, comparable to Martian samples. Frames A and B: Oscillatoriopsis longa *Timofeev and Hermann, 1979; scale bars are 50 μm. Frames C-E:* Megathrix longus *Yin L. from the lower Yurtus and lower Yanjiahe formations; scale bars are 100 μm. Frame F: 1.* Ramitubus increscens *Liu P., 2008 (scale bar is 200 μm); 2.* Ramitubus decrescens *Liu P., 2008 (scale bar is 200 μm); 3.* Sinocyclocyclicus guizhouensis *Xue Y., 1992 (scale bar is 100 μm); 4.* Quadratitubus orbigoniatus *Xue Y., 1992 (scale bar is 100 μm); 5.* Crassitubus costatus *Liu P., 2008 (scale bar is 100 μm); 6.* Yangtzitubus semiteres *Liu P., 2008 (This one is silicified and also from Ediacaran Doishantuo Formation; scale bar is 50 μm).*

of curvature. However, some gypsum and jarosite crystals exhibit a more fusiform shape but only a small proportion exhibited a degree of curvature which itself is regarded as a microbial biosignature [73].

Morphometric comparisons of "rice grains" with Euglena and Dasycladales have also been investigated, due to their morphological affinity. The fossil record of Euglena, however. is rare [79–81] and only a fossil, called Moyenia, has been recorded from Late Ordovician non-marine deposits. This record was suggested by

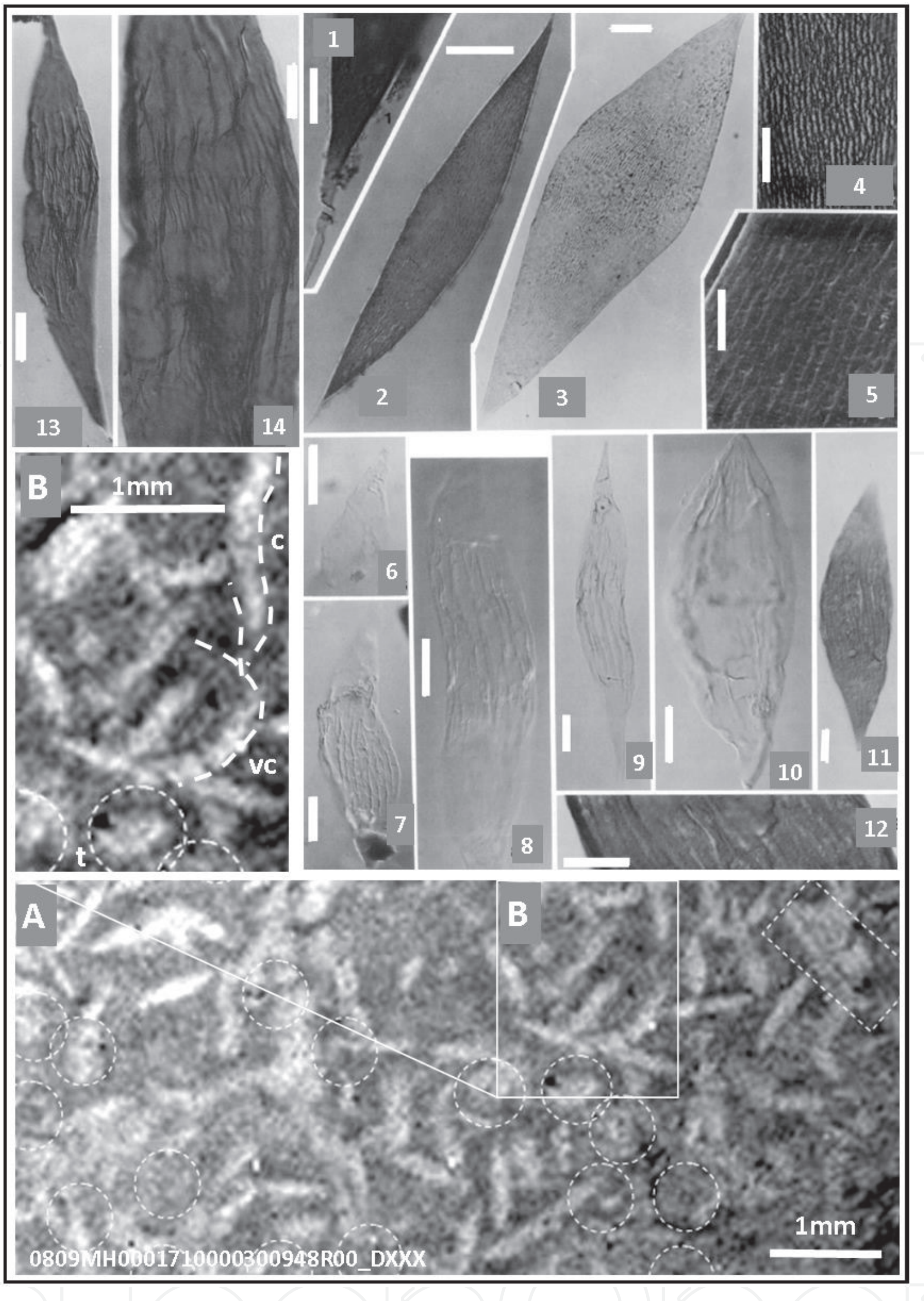


Figure 16.
Structural features of Euglenae (from Loeblich and Tappan [78]) in comparison to the "rice grain" (A,B). "Rice grain" show curved (c) and very curved (vc) structures with holed transversal sections (t). 1–5 - Cleithronetrum cancellatum *Loeblich and Tappan, 1979 1, showing smooth distal portion of process, Scale bar 10 μm; 2, same specimen, showing slightly asymmetrical fusiform shape, Scale bar 30 μm; 3, nearly symmetrical lozenge-shaped outline, Scale bar 20 μm; 4, enlargement of surface of specimen of* ***Figure 2****, showing longitudinal sinuous and anastomosing ridges that bear tiny grana, Scale bar 10 μm; 5; scanning electron microscope photograph, showing partial or complete bridges between and perpendicular to longitudinal ridges and deep pits separating the bridges, Scale bar 5 μm. All from the Mountain Lake Member of the Bromide Formation of Oklahoma. 6,7 -* Eupoikilofusa striata *Staplin, Jansonius and Pocock n. comb. 6, holotype, blunt-tipped polar process that lacks the ridges found on median area of vesicle, Scale bar 20 μm; 7, overall view of holotype, showing longitudinal ridges, Scale bar 20 μm. From the Middle Ordovician, Trenton Formation, Anticosti Island, Canada. 8,9 -* Eupoikilofusa ctenista *Loeblich and Tappan, 1979. 8, holotype, enlargement to show nature and distribution of ridges on vesicle, Scale bar 20 μm; 9, lesser magnification of same, showing pointed processes that lack the ridges found on the vesicle, Scale bar 20 μm. From the Sylvan Shale of Oklahoma,* Eupoikilofusa platynetrella *Loeblich and Tappan, 1979, holotype, showing slightly asymmetrical vesicle and distribution of vesicle ribs, Scale bar 20 μm. From the Sylvan Shale of Oklahoma. 11, 12-*Eupoikilofusa anolota *Loeblich and Tappan, 1979. 11, holotype, showing relatively broad and low discontinuous ridges, Scale bar 20 μm; 12, enlargement of surface to show wall sculpture, Scale bar 10 μm. From the Sylvan Shale of Oklahoma, 13,14 -* Eupoikilofusa parvuligranosa *Loeblich and Tappan, 1979. 13, holotype, nearly symmetrical fusiform vesicle with ridges that die out toward polar processes, Scale bar 20 μm; 14, enlargement of holotype to show sinuous ridges and grana aligned in rows on vesicle, Scale bar 10 μm. From the Sylvan Shale of Oklahoma.*

Colbath & Grenfell to be a possible fossil pellicle (cell wall) of a euglenid, based on their surface morphology, whereby the spiral pattern of ridges on the pellicle resembles that of some photoautotrophic euglenids in Monomorphina Mereschkowsky, 1877 [81]. **Figure 6** is particularly interesting and the cone present could easily be accepted as a fossil but given its incomplete preservation, it could be similar to a portion of Cloudina, a small shell fossil, or an example of other conotubular fossils (**Figure 16**). We emphasise, however, that the degree of variation in profile, width and shape and the degree of curvature of the profiles, both criteria highlighted by Williams [73] with consistency in width and curvature, are indicative of biogenicity and fractal analysis clearly confirms that the "rice grains" cannot be identified with mineral abiological structure as the gypsum, a result with a high statistical significance ($p < 0.01$).

Nevertheless, our conclusion in this paper concerning the analyzed Martian microstructures is not that they can be identified with the terrestrial species described but that the characteristics of shape and degree of complexity, make it probable that on Mars, 2.2 billion years ago, there were complex life forms, analogous to terrestrial eukaryotic cells. From a biological point of view, it is not unlikely that similar forms of life, with so many structural similarities, have developed independently on two different planets.

Results presented in this article can easily be interpreted as a phenomenon of evolutionary convergence, a phenomenon which is extremely widespread in terrestrial life forms. We can recall mammals and octopuses having camera-like eyes with an iris, a lens and a retina or the wings of bats and birds or the shape of sharks and dolphins: analogous environments producing the "same" shapes and structures without any evolutionary linkage. There no any problem for what concerns the time and the environment, on Earth, and, respectively, on Mars. Age of 2.2 billion years ago on Mars it is the same age in which complex, eukaryotic, cells, appeared on Earth, so there is no any problem for the time that could be need on Mars to produce those type of cells. No any problem, also, for the environment: the Earth aging 2.2–2.5 Gy ago is the one at the time of the oxygen crisis and of the "snowball Earth" [82], an anoxic and cold Earth as Mars of that age.

7. Further experimental observations for compelling evidence of life on Mars

Will never be able to definitively prove the existence of life on the Red Planet? The search for definitively proving the presence of life on Mars is one of the outstanding scientific challenges of our time [69, 83, 84]. We have described in this paper how the Curiosity landed region was clearly demonstrated as a fluvial-deltaic-lacustrine environment [2]. There are strong evidences that the whole surface of early Mars was habitable and several biomarkers were found (including microbialite/stromatolite-like structures and *bona fide* microfossils). Orbital and rover data reveal fluvial valley networks, paleolake deposits, alluvial fans/deltas entering these lakes, clearly revealing sustained precipitation during the Noachian, Hesperian and Amazonian periods, since 4.5 to 0.9 Gy ago, when an extensive glacial event resurfaced the paleolakes themselves [85, 86].

Nevertheless we need complex approaches for conclusively establish the presence of life [58] (NASA MEPAG's program at http://mepag.jpl.nasa.gov/reports/index.html), and, in particular, when tests need to be performed by rovers or landers in a planet hundred million kilometers far from Earth. E.g., we can recall the lot of papers supporting the presence of stromatolites on Mars by morphological approaches, as also here discussed.

The meaning of the possible presence of stromatolites on Mars is enormous. Stromatolites result from the activity of different microbial communities and not the product of a single microorganism, suggesting a real ecosystem on the planet if the presence of stromatolites should be definitively proved. But the morphology of stromatolites as indicator of biogenicity may be ambiguous, similar shapes can be produced by both abiotic and biotic processes [87]. In effect, geologists on Earth needs to study macro-, micro- and ultramicroscopic details of the putative stromatolites, as well as their geochemistry, Raman spectroscopy of their constituents, additional chemical, mineralogical (e.g. magnetite and pirite) and isotopic ratios analysis of redox-sensitive heavy elements (mainly of C, Fe, S, N, Mo, Cu, U, Ce), together which the sedimentological, stratigraphic and palaeoenvironmental context examined [69, 88, 89], to arrive at a reasonable conclusion about the biotic or abiotic origin of the hypothesized biostructures. Much more, the presence of carbonaceous matter (e.g., kerogen, bitumen, molecular biomarkers) in the same area needs to be evaluated, together which their isotopic compositions, drilling meters below the surface in order to collect organics that had no destroyed by the UV flux of the Sun in the present-day Mars. Analysis that involve the use of traditional tested technology, as well new experimental and miniaturized biosensors for “in situ” testing. Not a simple work, so it’s not strange that till today it was not possible to reach unanimous agreement among astrobiologists concerning the presence of life on the Red Planet. However, we feel that the next Mars missions by NASA and ESA, together which Mars Sample Return missions, should be able to reach the goal.

8. Conclusion

On Mars, at Gale Crater, a past environment favourable to life and for a broad span of geological time, has been discovered by various authors [1, 2, 49], as well as the occurrence of many micro, meso and macrostructures similar to terrestrial stromatolites, microbialites and algae [18, 50]. All these items are suggestive of possible biological parallels between Earth and Mars. In the present paper, we show morphological and morphometric analyses of the whitish millimetric shapes (we nicknamed “rice grains”), detected by the rover Curiosity at sols 809 and 880 (Mojave target) in the lacustrine Murray Formation (attributed to pseudomorph crystals of sulfate by previous Authors, [7]). Specimen which are incompatibile to Gypsum, Jarosite, or Feldspar crystals, but show a high shape affinity to life forms such as the Euglenoids. Hence, the microstructures investigated in this study, together with chemical and mineralogical converging data of the outcropping where they are embedded, suggest the possible existence of microbial, and/or little more complex life forms, in the past history of Mars.

Acknowledgements

We thank Filippo Barattolo, Professor at University of Naples (IT) and Joan Bucur, Professor at Department of Geology of Babes-Bolyai University (RO) for the support given in the analysis of algal-like biota and in excluding Dasycladales attribution. We are particularly grateful to Prof. Munneke, Professor at Friederich-Alexander University of North Bayern, for providing us the images of the terrestrial septate filaments shown on **Figure 15**. This work would not have been possible without NASA”s images and data availability, for which we are grateful.

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