Evidence of Martian Microalgae at the Pahrump Hills Field Site A Morphometric Analysis

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Abstract

We describe an objective morphometric analysis of images of specimens resembling fossilized microalgae photographed by the rover *Curiosity* in Aeolis Palus, Gale Crater, Mars; the geological remains of an ancient freshwater lake. lozenge microstructures photographed at Pahrump Hills have been described as abiologic crystals or, based on morphology, microalgae. Our analysis show that the Fractal Dimension, D0, and Entropy, D1, of the Martian lozenge microstructures differ from the one of the negative control, gypsum, with high statistical significance ($p < 0.01$; $p< 0.01$). Vice versa, Fractal Dimension of the Martian lozenge algae-like microstructures overlaps and is statistically similar to unicellular alga *Euglena mutabilis*, positive control (p = n.s.). Fractal analysis support the evidence that such microstructures are possible fossils of Martian microalgae.

Key Words: life on Mars, Curiosity rover, Gale crater, microalgae

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1. Water, Stromatolites, Algae, Fungi, Lichens, and the Search for Life on Mars

For decades scientists have been searching on Mars for life and the presence of water; an indispensable requirement for a possible presence of life on the Red Planet.

It is generally believed that during the first Martian Era, known as Noachian period, 4.1–3.7 Gys ago, the environment was similar to that of ancient Earth. Liquid water was widespread, volcanic activity was present, and atmospheric pressure and temperature were higher than today. Morphological evidence of water include river deltas and river-like geological features, drainage networks and lakes. These features are often accompanied by sedimentary, layered deposits. These conditions may have favored the spread of life on the surface of Mars (Bibring et al. 2006).

It is believed that 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 arid planet as observed today. This transitional phase likely occurred during the Hesperian; a period characterized by continuation of intense volcanic activity (a remnant example of which is Olympus Mons), deposition of evaporitic sedimentary sequences, and catastrophic floods that carved immense canals along the surface (Head and Wilson 2001). At the end of that era and during the actual era Amazonian (3.0 Gys ago), surface rivers and lakes began to or had completely disappeared (Martin et al. 2017)

In those early watery environments a number of scientists have found evidence of stromatolites: sedimentary formations that were likely created by photosynthetic cyanobacteria; and paralleling the formation of stromatolites on ancient anoxic Earth 3.5 Gys ago. Based on the analysis of images of the laminated Martian outcroppings by Opportunity and Spirit NASA rovers in Meridiani Planum and Gusev crater, respectively, Rizzo and Cantasano (2009) and Bianciardi et al. (2014, 2015) published compelling evidence of microbiolites and thrombolites. Subsequently, Noffke (2015) and Ruff and Farmer (2017) published similar findings. These findings strongly support the hypothesis that stromatolites were constructed on ancient Mars, and that the formation of these sedimentary structures may have been widespread during the Noachian and the Hesperian geological eras.

In further support is the publication of pictorial evidence of specimens that resemble algae, fungi lichens and domical stromatolites (Joseph et al. 2019, 2020a, 2021; Latif et al. 2021). Certainly one cannot prove the existence of life based on morphology alone (Kaminskyj 2021; Roy 2021). Pictorial images can be highly deceptive. However the amount of data that has been published so far has been impressive and favors the hypothesis that ancient and present day Mars was and is inhabited (Armstrong 2021; Bianciardi et al 2014, 2015; Elewa 2021; Joseph et al. 2019, 2020a,b, 2021; Krupa, 2017; Latif et al. 2021; Noffke 2015; Rizzo et al. 2015, Rizzo & Cantasano 2009, 2017; Rizzo 2020; Ruff & Farmer 2017). This evidence certainly deserves additional investigation and analysis for the presence on Mars of stromatolites, fungi, algae, and lichens using additional and objective methods of investigation (Kaminskyj 2021; Roy 2021) including and especially comparative morphological analysis and for which positive results have been obtained (Armstrong 2021; Bianciardi et al 2014).

2. Martian Microalgae? Morphometric Analysis

In this report we describe objective morphometric analysis of specimens photographed by the rover Curiosity that resemble microalgae. These specimens were observed in Aeolis Palus, Gale Crater; the geological remains of an ancient freshwater lake, an environment that could have been hospitable for microbial life (Fairén et al 2014). It is in this same vicinity and lacustrine environment that Noffke, Rizzo, and others have observed the possible presence of stromatolites and fossils (Elewa 2021; Joseph et al. 2020a,b; Noffke 2015; Rizzo et al., 2015, Rizzo & Cantasano 2017; Rizzo, 2020; Roy 2021).

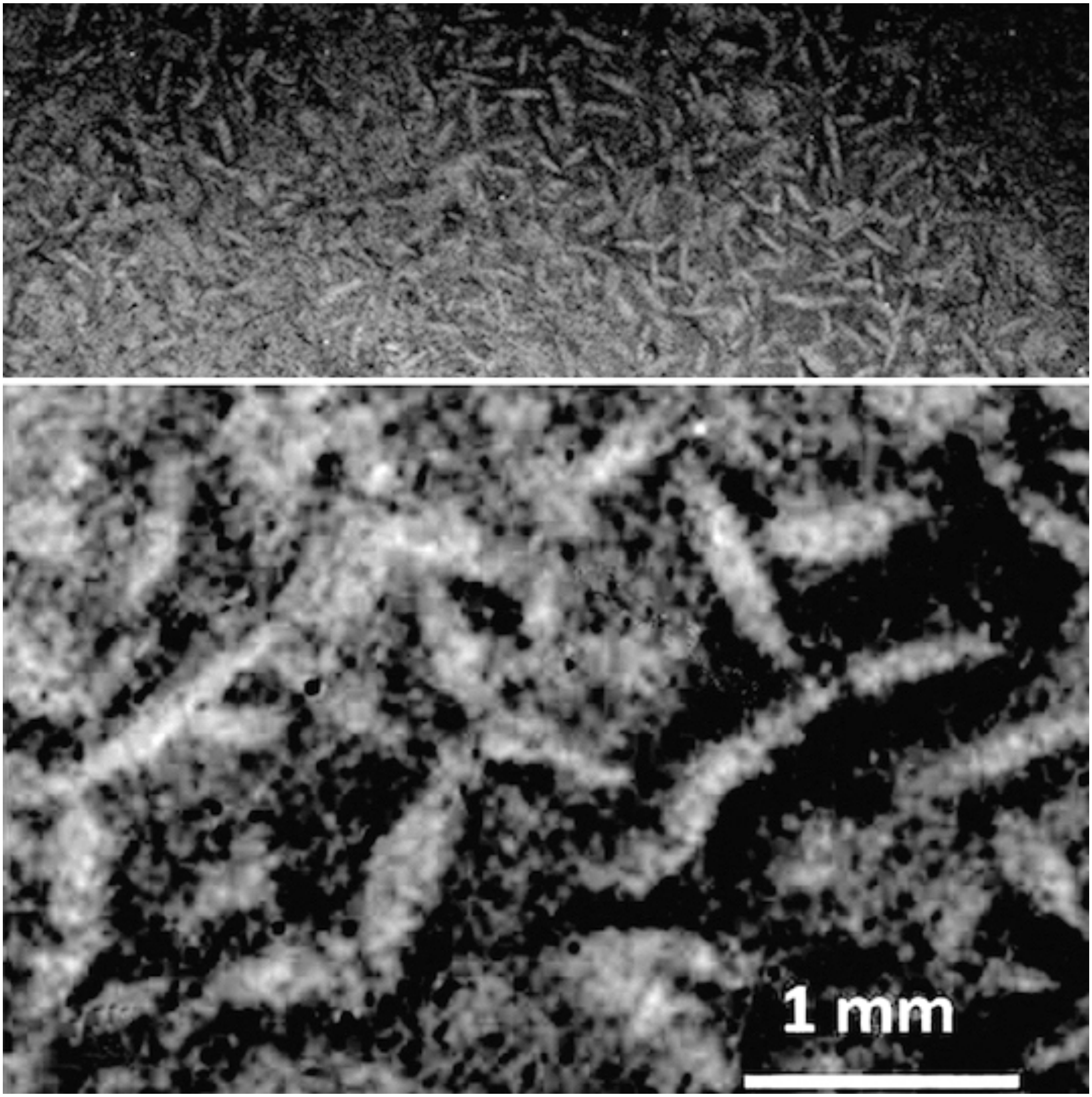

Figure 1. Fossilized impressions of lozenge-shaped algae-like microstructures photographed in the dried lake beds of Gale Crater.

For the purposes of this study several lozenge-shaped algae-like microstructures within the Martian outcrops of the Pahrump Hills Field Site were investigated and analyzed. Some scientists have interpreted those structures as mineral crystals, gypsum (Kah et al. 2018), which, on Earth also harbor algae (Jung et al. 2020). Other scientists have interpreted these as biological structures (Rizzo 2020) that include fungi and algae (Joseph et al. 2020a; Latif et al. 2021) and what may be (the white veins) biological secretions of calcium carbonate and calcium oxalate that are enveloping algae fruiting bodies (Joseph 2021). Directly related to these interpretations are findings in other areas of Mars interpreted as resembling fossilized microalgae and acritarchs (Kaźmierczak 2016, 2020).

The questions before us, therefore, include the following: Do these white veins represent gypsum, or microalgae? To help answer these questions we performed a fractal analysis comparing these Martian microstructures with gypsum and the microalgae Euglena *mutabilis*, an extremophile unicellular flagellate protist that was first discovered in the acidic mine waste of the Berkeley Pit in Butte, Montana and believed to have been uninhabitable. It is believed that Euglena *mutabilis* can consume metal, which makes the iron-rich Red Planet an ideal environment for this and other chemical-mineral-metal eating organisms (Joseph 2021; Robbins, 2021). Moreover, this algae-related species produces oxygen by photosynthesis, and precipitates Fe (Tanaka et al. 2013), thereby forming substrates that other microorganisms may inhabit or utilize as electron donors (Joseph 2021).

Euglena was not an arbitrary choice for this study. As depicted in Figure 1, we chose this organism based on the discovery in the ancient lake beds of Gale Crater of what may be a fossilized conglomeration consisting entirely of formations that are similar to microalgae (Rizzo 2020).

3. Material And Methods

Lozenge-algae-shaped microstructures images from Gale Crater were enlarged 10 x by Paint Shop software. Images were then loaded on paint.net software (https://www.getpaint.net/ download.html) in order to extract the specimens of interest. The extracted image was loaded on Digital Image Magnifier (Nikolao Strikos, https://download.cnet.com/Digital-Image-Magnifier/3000-12511_4- 75452403.html) 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 Dimensions.

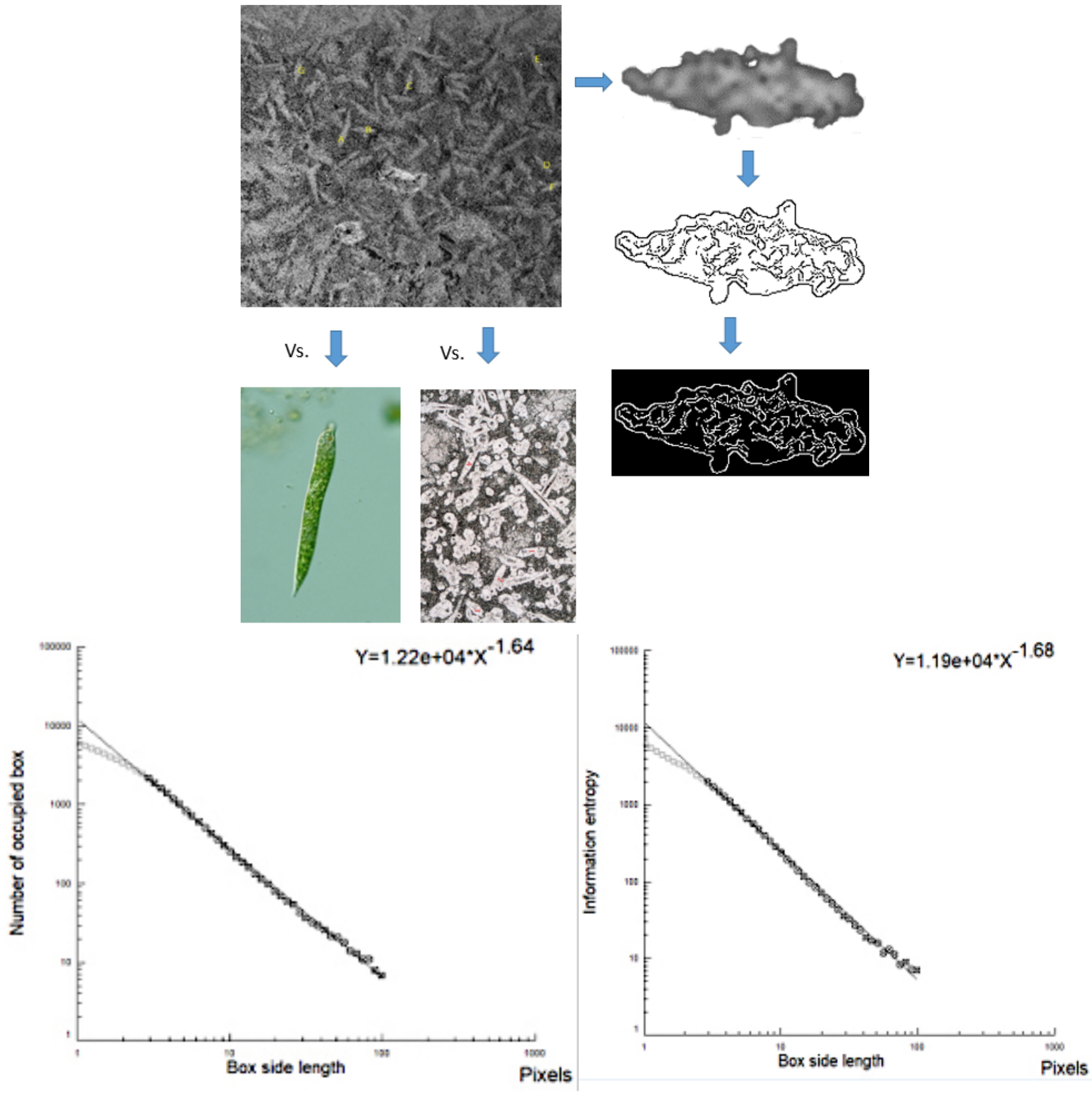

Figure 2. Sol 880 (top, left), lozenge microstructures were extracted and enlarged, a canny filter applied and the negative obtained (center) and the log log plot obtained to determine the fractal dimension (bottom, left) or the entropy (bottom, right). The log log plot is a straight line ($p<0.01$) indicating these microstructures are fractals and the exponent of the straight line is the fractal dimension or entropy, respectively, of the microstructures. The same analysis was performed comparing Euglena *mutabilis* and gypsum crystals (middle, left)

Fractal dimension, D0, a measure of the space-filling properties of a structure, and the Entropy of the image were calculated on the skeletonized images. Briefly, each image was covered by nets of square boxes (from 5 to 100 pixels) and a log–log graph was plotted, the slope of the linear segment of the graph representing the local fractal dimension and the entropy of the image (Figure 4). The linearity of the log log plot was assessed by the Pearson's correlation test, in order to demonstrate that the lozenge microstructures own a fractal structure and fractal dimension/entropy may be performed. Variance analysis was used in order to compare fractal dimension and entropy of the Martian microstructures reported at Pahrump Hills Field Site, Gale crater, Mars (Mojave and Mojave 2 targets, Sols 809 and 880) vs. the mineral (abiologic) gypsum or versus Euglena *mutabilis*, as a model of a complex (eukaryotic) microorganism.

4. Results

Our analysis show that the Fractal Dimension, D0, and Entropy, D1, of the Martian algae-lozenge microstructures differ from the negative control, gypsum, with high statistical significance ($p < 0.01$; $p < 0.01$). Vice versa, fractal dimension of the Martian lozenge microstructures overlaps the one of the unicellular alga Euglena *mutabilis*, while Entropy of E. *mutabilis* and Martian microstructures differ.

Table 1. Comparison of fractal dimension and Entropy values among Martian microstructures (Mojave and Mojave 2 targets, Sols 809 and 880), Euglena *mutabilis* and gypsum sediments. Mean ± SD. The differences between the microstructures of Mars and Gypsum is significant for D0 and D1, both (D0: 1.580 ± 0.04 vs 1.615 ± 0.03 (*), p <0.01; D1: 1.550 ± 0.10 vs. 1.593 ± 0.08(*), p <0.01), while there is a total overlap between the fractal dimension D0 of the biological controls (Euglena *mutabilis*) and Martian microstructures (D0: 1.580 ± 0.04 vs 1.574 ± 0.06, p = n.s.) Entropy values of the biological controls (Euglena *mutabilis*) and Martian microstructures differs (1.550 ± 0.10 vs. 1.60 ± 0.13(*), p <0.01

Samples	Martian microstructures (n=54)	*Euglena mutabilis* (n=32)	Gypsum (n=10)
Fractal Dimension (D0)	1.580 ± 0.04	1.574 ± 0.06	1.615 ± 0.03 *
Entropy (D1)	1.550 ± 0.10	1.60 ± 0.13*	1.593 ± 0.08 *

5. Discussion

There is a great debate as to the ability of life to survive on the surface of Mars (Kaminskyj 2021; Roy 2021). Many scientists favor a habitable subsurface habitat. However, most scientists agree that ancient Mars had lakes, rivers, and possibly two oceans. Because ancient Mars was habitable it is not unreasonable to deduce it was inhabited; particularly given evidence of microbiolites, thrombolites, and stromatolites that may have been built by algae. There is considerable evidence of subglacial lakes of water ice (Lauro et al., 2020; Orosei et al. 2020); and it is possible there may be hydrothermal activity present also today. For example in Eridania basin (Michalski et al, 2017). If various organisms colonized Mars early in its history, it is reasonable to assume their descendants may have evolved and adapted to these changing conditions.

The search for life on Mars, either in the present or in the past history of the "Red Planet" has been an intense area of research for decades. Teams of different scientists have published morphological evidence of stromatolites (Rizzo & Cantasano 2009; Bianciardi et al., 2014, 2015; Noffke 2015; Joseph et al. 2020c; Ruff & Farmer 2016), algae, fungi, bacteria and lichens, including, as documented with sequential photos, life-like specimens that appear to be growing out of the ground and increasing in size (Joseph et al. 2021). However, other scientists have asked for proof and argue that the evidence is not sufficient to make definitive claims that current or past life has been discovered (Roy 2021).

As reported here, we performed an objective comparative morphological statistical analysis of the Martian images obtained by the Rovers on the Red Planet that appear to depict fossilized impressions of algae-like organisms. This morphometric analysis compared the fossilized structures found in the Martian outcrops with gypsum and terrestrial microalgae. The results from this study supports the hypothesis, i.e unicellular algae were fossilized at Pahrump Hills Field Site, Gale crater, Mars (Mojave and Mojave 2 targets, Sols 809 and 880). Based on previous morphological studies (Joseph et al. 2020b; Rizzo, 2020), coupled with other evidence of a variety of fossils that have been observed and reported (DiGregorio 2018; Baucon et al. 2020; Joseph et al. 2020b), and the many reports of formations similar to microbiolites, thrombolites and stromatolites (e.g. Bianciardi et al. 2014, 2015; Noffke 2015; Rizzo & Cantasano 2009, 2017; Ruff & Farmer 2017), evidence of an inhabited Mars is mounting.

Euglena *mutabilis* is a photosynthesizing extremophile that contributes to stromatolite-building and lives in strong acidic watery environment (Tanaka et al. 2013). It is believed that a similar conditions prevailed on Mars 2 billion years ago, in the ancient lakes of Gale Crater (Head & Wilson, 2001; Fairén et al, 2014; Martin, 2017).

We cannot claim to have proven organisms identical to the terrestrial species Euglena *mutabilis* once lived in the lakes of Mars. Nevertheless, the complex comparative morphological analysis performed and reported here, strongly supports that organisms similar to E. *mutabilis* dwelled (and may still dwell) on Mars. These algae-like Martian microstructures, first discovered in 2019 (as reported by Rizzo 2020 and Joseph et al. 2020a), and as analyzed in terms of comparative fractal dimensions and statistical quantitative complexity, supports the possibility that complex life forms, analogous to terrestrial eukaryotic unicellular organisms dwelled on Mars, perhaps 2 billion years ago or more recently, and became fossilized whe the lakes of Gale Crater evaporated into the atmosphere or sank and froze beneath the surface.

Other scientists have reported fossilized formations resembling arcitarchs (Kaźmierczak 2016, 2020) which on Earth evolved over 700 million years (and perhaps over a billion years) ago; and fossilized specimens that resemble burrowing tube worms (DiGregorio 2018; Baucon et al. 2020; Joseph et al. 2020a), and terrestrial Ediacarans and early metazoans (Elewa 2021; Joseph et al. 2020a,b). If the first stromatolites on Mars were created 3.7 billion years ago as reported by Noffke (2015), then we have what appears to be an evolutionary progression that parallels the early evolution of life on Earth leading up to the Cambrian Explosion. Of course we can only speculate. We need more data.

However, if algae-like organisms similar to E. *mutabilis* evolved and became fossilized on Mars, these structural morphological similarities to terrestrial microalgae suggests that they dwelled and evolved in a similar environment on both planets. What we may have is evidence of parallel evolutionary convergence, a phenomenon which is extremely widespread on Earth; an impression based on analogous complexity and not an identification based on morphology alone.

To speculate further: if these are in fact fossils of Martian microalgae, and given estimates that Gale Crater provided a lacustrine environment 2 billion years ago, then it is noteworthy that this corresponds to the same age in which complex eukaryote unicellular organisms may have appeared on Earth during the Great Oxidation event 2.0–2.4 Gys ago. Our results concerning the possible presence of complex unicellular photosynthesizing organisms on Mars are very congruent with what we know about the environment and evolution on Earth.

6. Conclusion

Employing quantitative morphological fractal comparative analysis, we've documented that specimens similar to microalgae may have flourished and became fossilized in Gale Crater.

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