Understanding ancient life: How Martin Brasier changed the way we think about the fossil record

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Critical to our understanding of life on Earth is the ability to judge the validity of claims of very ancient 'fossils'. Martin Brasier's most important contribution to this debate was to establish a framework within which to discuss claims of the 'oldest' life. In particular, Brasier *et al.* (2002) made it clear that the burden of proof must fall on those making the claim of ancient life, not those refuting it. This led to his formulation of the concept of the continuum of morphologies produced by life and non-life, and the considerable challenges of differentiating biogenesis from abiogenesis. Martin Brasier developed a set of criteria for distinguishing life from non-life, and extended the use of many new high-resolution analytical techniques to palaeontological research. He also applied this null hypothesis way of thinking to the origin of animals and the Cambrian explosion (Brasier 2009), leading to him being involved in the development of a series of nested null hypotheses, his "*cone of contention*", to analyse enigmatic fossils more generally. In short, Martin Brasier taught us

how to formulate biological hypotheses in deep time, established the rules for how those hypotheses should be tested, and championed a host of novel analytical techniques to gather the data required. As a consequence, future discussions of enigmatic specimens and very old fossils will be greatly enriched by his contributions.

Running Title: Brasier fossil record

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Palaeontologists work on ancient organisms, and most have preferred organisms or groups of organisms to which they dedicate their time, adding measurably to that area of knowledge. But some palaeontologists do more than that. They survey the big picture. By considering the interplay between palaeontological records and major geological and geochemical events, and by identifying the overarching concepts and paradigms that govern biological evolution, these researchers have significantly changed our perceptions of the fossil record, the history of life, and evolution. For example, George Simpson opened our eyes to the pattern and process paradox (Simpson 1944); Jack Sepkoski transformed our way of looking at the diversity of life on Earth (Sepkoski 1981); Stephen Jay Gould changed how we look at the complex interplay of ontogeny and phylogeny (Gould 1977); David Raup developed innovative ways to measure morphology and reveal underlying evolutionary trends (Raup & Michelson 1965); and Dolf Seilacher highlighted the patterns in animal behaviour that relate

to palaeoenvironmental parameters (Seilacher 1967). The many contributions of Martin Brasier to understanding of the earliest fossil record sit well in this company. By defining criteria for how to identify and analyse ancient fossils robustly, and providing state-of-the-art data from numerous significant stratigraphic levels, Martin Brasier transformed two fields of study: the origin of life, and the origin of animals. Below we outline the nature of his contributions to palaeontological and evolutionary thinking.

Early life and the null hypothesis

Understanding major events in the history of life is one of the driving motivations of the natural sciences, requiring the combination of a unique nexus of disciplines. The origin and radiation of cellular structures in Archaean strata is pertinent to many branches of science, from biology and palaeontology to planetary science and environmental geochemistry. Attempts to identify the very earliest signs of life in ancient strata have stimulated biological research into the genetic controls of cellular evolution (e.g., Cavalier-Smith 2006), development of new geochemical proxies (e.g., Johnston 2011), and high-resolution stratigraphy in deep time (e.g., Van Kranendonk 2012). Integration of findings from these disciplines with evolutionary history requires confident identification of early cellular structures, which is a challenging matter.

Cellular preservation in deep geological time has been recognised since the discovery of early fossil biotas such as the 1.88 Ga Gunflint Chert (Tyler & Barghoorn 1954). There was a natural expectation that older material would be found, and that scientists would be able to repeatedly push the temporal boundaries for the history of life with new discoveries of well-preserved cellular structures (Hofmann 1976; Schopf 1992, 1993). In the very earliest parts of

the stratigraphic record, however, as it becomes increasingly improbable that older fossils will be found, testing and falsifying a null hypothesis of abiogenesis is required before any putative sign of life can be confirmed.

Critical to our understanding of life on Earth is the ability to judge the validity of claims of very ancient fossils. Structures reported from the Apex Chert (3.46 Ga) that were interpreted to occur in sedimentary rocks and be biological in origin (Schopf & Packer, 1987; Schopf, 1992, 1993) were considered compelling candidates for the earliest fossils in publications throughout the 1990s. Martin Brasier's most important contribution to this debate was to characterize those structures in great detail, and also to develop a framework within which claims of the 'oldest' or 'earliest' life should be couched. In his lectures on this subject, Martin referred to the competitive tendency among palaeontologists working on early life as the 'MOFAOTYOF' Principle – *M*y *O*ldest *F*ossils *A*re *O*lder *T*han *Y*our *O*ldest *F*ossils. In particular, Brasier *et al.* (2002) made it clear that the burden of proof must fall on those making the claim of ancient life, not those refuting it:

> "*Ancient filamentous structures should not be accepted as being of biological origin until all possibilities of their non-biological origin have been exhausted. In particular, it is important to note that complex `septate' carbonaceous structures can result from experimental hydrothermal processes*" (Brasier *et al.* 2002, p. 80).

In other words, we should assume that ancient structures resembling fossils such as those in the Apex Chert are abiological until it can be shown beyond reasonable doubt that they are not; rather than the other way around. Brasier (2015) articulates this concept clearly:

> "*This ... allows palaeobiologists to set up a hypothesis which will prevail until proved false….. Any newsworthy, and culturally challenging, interpretation must therefore be tested against a less exciting interpretation. This 'null hypothesis' is usually regarded as the 'most boring explanation'. It is boring precisely because it is thought to have a higher probability of being correct.*" Brasier (2015, p. 9)

This could be thought of as Brasier's razor: "the most boring answer is probably the correct one". This approach also raises the question of what comprises 'reasonable doubt', especially in the adversarial world of academia, and how the probability of a structure being biogenic might be assessed. Martin therefore developed a set of rigorously defined criteria to describe the conditions that must be satisfied, and the evidence required, to reject a null hypothesis of abiogenesis and accept a structure as being reasonable evidence for early life. This approach forces subjective debate to be replaced by objective assessment of whether or not biogenicity criteria are met in any putative fossil under consideration. Further, there is clearly a temporal consideration, with the probability of finding fossilised bacterial life (for example) much more likely and less controversial (and therefore less newsworthy) by the time there are, for instance, abundant trilobites (500 Ma ago) than in very early sediments (3500 Ma ago) when the existence and nature of life is still much debated (Fig. 1). Indeed Brasier (2015) adds that:

> "*For biomorphs older than 3.0 Ga, it seems prudent not to accept them as biogenic until plausible abiogenic origins have been tested and falsified*" (Brasier 2015, p. 10)

This does not mean that putative fossils younger than 3.0 Ga should be treated with less scrutiny than those 3.0 Ga or older. Rather, it means that the older the fossils, the more likely they are to be significant, and this should encourage our scepticism. Palaeontologists in the 1970's were the first to caution on the problem of correctly determining the biogenicity and

127 syngenicity of Precambrian microfossils (Schopf, 1975; Cloud, 1976) and this led to
128 significant advances in developing sets of criteria in an attempt to minimise erroneous
129 interpretations (Schopf & Walter 1983; Buick 1990).
130
131 Brasier's application of the concept of the null hypothesis in palaeobiology, to understand the
132 earliest fossil record, ushered in a paradigm shift in how palaeontologists think about the
133 evidence for the earliest life, towards a norm in which scepticism rather than credulity holds
134 the high ground. It is a mind-set that is entirely in keeping with Martin's own scientific
135 outlook. He described the fossil record as being akin to a card game in which the players are
136 not told the rules (Brasier 2009). It was important, in his view, to question assumptions, to
137 consider how we look at the fossil record, and determine whether we are asking the right
138 questions:

139 "*Human progress towards learning the rules for decoding the fossil record has…been*
140 *slow, requiring trial and error, with lots of questions, intuition and counter-intuition,*
141 *accompanied by oceans of doubt. But then, science, which always rejoices in a good*
142 *question, is a unique system for the measurement of doubt"* (Brasier 2009, p. 34).

143

144 **Distinguishing between biological and abiological**

145 Martin's next step in developing the tools to search for early life was to step away from the
146 world of fossils, and examine in detail abiological structures that could appear to be
147 biological in origin, and then tackle the important question of how to distinguish the two (Fig.
148 2). The recognition that biological products can be very difficult to distinguish from those of
149 physical processes was in itself a critical development since many of the old assumptions
150 regarding 'search criteria' for early life did not yield unique solutions:

151 "*….reports of early microfossils and stromatolites (e.g. Hofmann et al. 1999) are not*
152 *readily distinguishable from self organizing structures (SOS) and have yet to pass the*
153 *null hypothesis, that microfossil- and stromatolite-like structures ... should not be*
154 *accepted as of biological origin until alternative hypotheses for their abiogenic origin*
155 *have been tested and falsified*" (Brasier *et al.* 2006, p. 887).
156

157 He emphasized that, particularly at the interface of competing physical parameters,
158 abiological processes are capable of generating a great range of complex, including self-
159 organising, structures that can appear remarkably biological (Fig. 2). Awareness of this often
160 close similarity between morphologies produced by organisms and abiological physico-
161 chemical processes is important for palaeontologists trying to interpret the Precambrian fossil
162 record.

163 "*Unfortunately, complex structures do not require complex causes, as shown nearly a*
164 *century ago by Thompson (1917). They can arise naturally in physico-chemical systems*
165 *within the realms of 'chaotic' behaviour as Grotzinger & Rothman (1996) showed a*
166 *decade ago with reference to stromatolites... we draw attention to a range of physico-*
167 *chemical gradients that can lead to the formation of macroscopic stromatoloids (figure*
168 *2a) and ripples (b) as well as to microfossil-like structures generated by the growth of*
169 *dendrites (e), ... polygonal crystal rims (g) and spherulites (h)*" (Brasier *et al.* 2006, fig.
170 2 and p. 889).
171

172 An enormous range of abiogenic phenomena, including dendrite growth, mixed fluids and
173 wave fronts, can produce complex structures. An informative example comes from what is
174 now widely known as the "paint stromatolite" (McLoughlin *et al.* 2008). This series of
175 experiments, inspired by structures observed at the Mini car factory outside Oxford, showed

that it was possible to generate stromatolitic deposits, including some morphologies previously considered to be strong indicators of microbial sedimentary activity (e.g. Buick *et al.* 1981), in the complete absence of microbial growth. It is clear that microbes can build the complex morphological structures known as stromatolites through trapping and binding of sediment, phototaxis, and microbially mediated chemical precipitation. However, non-isopachous, wrinkled, and convex upward laminae (Buick *et al.* 1981) can form due to variations in flux rate and viscosity of a flowing colloid (McLoughlin *et al.* 2008). These experiments (McLoughlin *et al.* 2008) demonstrate that colloidal substances (e.g. fine clay particles suspended in sea water; also silica sinters deposited from the vapour phase and in splash zones) can accrete on a substrate to form morphologies previously considered to be biogenic.

Large negative carbon isotope fractionations have also been interpreted as a signature of early life (e.g. Schidlowski 2001). However, Martin Brasier emphasized that context is particularly crucial for isotopic fractionations, which by their very nature must be reported relative to a large terrestrial reservoir, typically the ocean, and with consideration of the processes likely to operate in the environment in which they are found (e.g., fluid-rock interactions). The magnitude of an isotope fractionation alone is not sufficient evidence of biogenicity, and must rather be interpreted in terms of its environmental setting, and this is often where isotopic claims of life from the very early Earth break down (e.g. Mojzsis *et al*. 1996; Bell *et al.* 2015). A number of abiological processes (e.g., atmospheric and hydrothermal synthesis of organic material, or processes such as Fischer-Tropsch synthesis) may also result in carbon isotope fractionations comparable to those of metabolic processes (Horita 2005). The products of such abiological processes were arguably more prevalent on the early Earth than

the products of metabolism, therefore understanding these data in their correct geological context is critical:

> "*isotopically light carbonaceous matter, preserved largely in silica-rich chert, was not only widespread in [Archaean] surface environments, but also intimately connected to numerous, deep hydrothermal dyke systems*" (Brasier *et al.* 2006, p. 887).

Faced with this continuum of morphologies produced by life and non-life, and the considerable challenges of differentiating biology from abiology, Martin actively explored new criteria and approaches to identifying robust traces of life in the early rock record, and even on other planets. Much of his work subsequent to that on the Apex Chert was towards developing so called "lifelines" or "biosignals" for seeking the earliest traces of life in the rock record, and also the earliest evidence of animals in the late Precambrian.

Extrapolation to the extraterrestrial

Martin Brasier strongly advocated that the search for life beyond Earth needed to take account of data generated by studying the earliest putative evidence of life on Earth, and that the Archaean was our testing ground for developing tools and approaches to seeking extra-terrestrial life. Martin first engaged with the field of astrobiology as a micropalaeontologist considering the Mars microfossil debate in the late 1990s, and also as an Earth systems scientist who sought to consider the interaction of life with the surrounding planetary system. He urged extreme caution, arguing that the abiogenic null hypothesis must be our starting point for exploring other planetary surfaces, with the assumption that all candidate biosignatures are abiotic (Brasier *et al*. 2004).

In a series of papers, Martin and colleagues proposed criteria for evaluating potential traces of life beyond Earth, drawing upon experience of working on Precambrian biota in particular. For example, Brasier *et al*. (2004) critically reviewed the biogenicity criteria applied at that time by the palaeontological community, and argued that they were inadequate for evaluating the Apex Chert microtextures. This was followed by further papers offering additional refined biogenicity criteria for both: 1) carbonaceous microfossils (e.g. Brasier *et al*. 2006; Brasier & Wacey 2012); and 2) endolithic microborings,i.e., microtunnels made by rock-boring microbes in substrates including carbonates, siliciclastic grains, and volcanic glass that could one-day provide textural evidence of a subsurface biosphere on Mars (McLoughlin *et al*. 2007). In one of his last astrobiological contributions, Martin stated:

> *"…. no unusual claim for life from a very remote time period, or from a very remote place, should be accepted by the scientific community unless it forms part of a natural population of structures that can be systematically studied. These populations will then need to be placed within an appropriate morphospace, and shown to be distinct in character from any abiogenic mimics that might be expected to occur within the same kind of setting" (Brasier & Wacey 2012, p. 221)*

The importance of multiple lines of evidence

Biogenicity criteria have existed in one form or another for a number of decades, with those advanced by Schopf & Walter (1983) most frequently cited by studies in the 1980s and 1990s. While such criteria were rigorously applied in the years soon after their development, over time they rather slipped from view and a certain amount of complacency regarding Precambrian life set in. In addition, twenty years of technological advances meant that there was an opportunity to build upon the early work of Schopf, Walter and others, and refine and

update these criteria. Martin re-invigorated this line of investigation championing the use of multiple lines of evidence in order to uphold or falsify the null hypothesis of abiogenicity:

> *".....we suggest that the testing of biological signals in very ancient rocks address three main criteria: geological context, biology-like morphology and biology-like processing"* (Brasier *et al.* 2005, p. 80).

Fulfilling the criterion of a geological context plausible for life has been shown to require geological and fabric mapping at a variety of scales, from kilometres to nanometres. Martin's detailed field mapping of the 3.46 Ga Apex Chert around Chinaman Creek, building on that of Van Kranendonk and others (e.g., Van Kranendonk *et al*. 2001), demonstrated that the putative microfossils in this unit were not within a detrital component of bedded chert (Schopf 1993):

> *"Mapping…. shows that putative 'microfossil'-bearing clasts were formed by fracturing and pulverization of earlier fissure-filling deposits and chert, and consistent with formation as hydrobreccia under hydrothermal conditions"* (Brasier *et al.* 2005, p. 81).

Petrographic mapping of host rock and carbonaceous microfabrics was also central to the approach, and was often the primary observation that would lead to additional analyses of geochemical or quantitative morphological data. In the case of the Apex Chert microtextures, it was detailed petrographic re-investigation of the chert microfabrics that led to the first doubt over the biological nature of the microfossils (Brasier *et al*. 2002, 2011). Petrographic observations were used to develop an abiotic framework for the microtextures observed, and to propose a model in which the size, shape and spectrum of morphologies could be related to

the diameter of the silica spherulites and the concentration of impurities in the chert (Brasier *et al*. 2005).

Fulfilling the criteria of biology-like morphology requires more than simple morphological comparison to younger fossil or extant bacteria. Martin advocated the exploration of potential morphospace; i.e., size- and shape-independent mapping of morphology both within microfossils and between them. For example, in well-preserved microfossil assemblages, Martin argued that morphological variation within natural populations is usually less than that of comparable abiological structures, and they will therefore occupy a more restricted morphospace (Brasier *et al*. 2006). There is evidence to support such a view, given that the standard deviation of 'filament' widths is larger for the abiogenic Apex Chert structures when compared, for example, with the biogenic Gunflint Chert assemblage (Brasier *et al*. 2006). Additional information may also come from mapping the areal distribution of putative microfossil filaments. For instance, 'clusters' of filaments that overlap and intertwine are common for microbial communities (e.g. Gunflint Chert) but seem to be rare or lacking in abiogenic assemblages (e.g. Apex Chert). In the case of microtextures from the 3.4 Ga Strelley Pool Formation, additional morphological support for biogenicity was identified:

> *"The microstructures we identify exhibit indicators of biological affinity, including hollow cell lumens, carbonaceous cell walls enriched in nitrogen, taphonomic degradation, organization into chains and clusters…"* (Wacey *et al*. 2011 p. 698).

Addressing the criterion of "biology-like processing", Martin sought evidence for the preservation of 'zones' of microbial processing, and/or evidence for microbial tiering within ecosystems. By this he meant geochemical or morphological evidence for microbial

296 stratification within the putative fossil remains, or evidence for microbial processing by
297 heterotrophs. For example, in the Strelley Pool Formation, occurrence of micron-sized pyrite
298 grains within and around partially degraded carbonaceous cell walls provides evidence for
299 microbial processing by heterotrophic sulfate-reducing bacteria (Wacey *et al*. 2011). This
300 pattern has since been replicated in other younger Precambrian microfossil assemblages (e.g.
301 1.88 Ga Gunflint Formation; Wacey *et al*. 2013).

302

303 Rigorous testing of these criteria of course requires the correct materials and analytical
304 techniques:

305 > *"While the quality of the early fossil record is much better than Darwin might ever*
306 > *have dared to imagine, we must still map out its limits, and push back the boundaries*
307 > *using new techniques to test out ideas both new and old"* (Brasier *et al.* 2015, p.
308 > 4864)

309

310 Martin was quick to recognise opportunities for new technologies to enhance our
311 understanding of the fossil record, while at the same time realising that a single technique
312 could not deliver the 'smoking gun' of unambiguous evidence for early life (Fig. 3). He was
313 one of the first to apply laser Raman micro-spectroscopy to characterize the structure of
314 Archaean carbon, and one of the first to caution its limitations (Brasier *et al.* 2002; Pasteris &
315 Wopenka 2003). Around this time he was also bringing computer aided 'automontage' to the
316 attention of Precambrian palaeontologists, enabling an improved visualisation of three-
317 dimensional structures within thin sections. Notably, this was used to show that some
318 putative microfossils in the Apex Chert were branched, a feature incompatible with very
319 primitive bacteria (Fig. 3a; see also Brasier *et al.* 2002, 2005).

Martin also championed the use of NanoSIMS (nano-scale secondary ion mass spectrometry) in palaeobiology (Wacey *et al.* 2008), recognising that this technique uniquely combined very high spatial resolution (~50 nm) with excellent sensitivity for those light elements and isotopes typical of cellular material and biominerals (e.g. C, N, S, O, P; Wacey *et al*. 2011). More recently, Martin provided the impetus for the development of protocols to analyse putative microfossils in both two and three dimensions at the sub-micrometre scale, using techniques such as TEM (transmission electron microscopy) and FIB-SEM (focused ion beam milling combined with scanning electron microscopy) (Wacey *et al*. 2012, 2015; Brasier *et al*. 2015). These high spatial resolution techniques, combined with careful fabric and morphological mapping, all considered in the context of a well understood geological setting, are now providing greater confidence that ancient life can be accurately distinguished from abiological artefacts (Brasier *et al*. 2015). Notably, this approach has recently dispelled the notion that cell lumina are present in Apex 'microfossils' (Fig. 3b-f), instead:

> *"....carbon is seen wrapped around the margins of the vermiform phyllosilicate–quartz grain boundaries, and is interleaved between vermiform phyllosilicate grains in pseudofossil interiors, along grain boundaries, and along triple junctions of quartz exterior to the pseudofossils"* (Brasier *et al.* 2015 p. 4862).

Such data are also providing unique insights into the younger Precambrian fossil record, revealing for example the 3D organisation of the problematical microfossil *Eosphaera tyleri*, and evidence for heterotrophic degradation by sulfate reducing bacteria of *Gunflintia* sheaths, both from the 1.88 Ga Gunflint Chert (Wacey *et al.* 2013; Brasier *et al.* 2015).

Once liberated by these rigorous approaches Martin Brasier sought to go off the well-worn path in search of new evidence for early life.

> *"There are also new places to look in the fossil record, so that environments long assumed to be barren of life may yet prove to be teeming"* (Brasier *et al.* 2015 p. 4864)

A classic example of this was moving the search for cellular preservation away from cherts and towards other rock types in Archaean cratons. This resulted in the discovery of carbonaceous cells and sheaths preserved in pore space cements of a 3.43 Ga placer-type sandstone (Wacey *et al.* 2011, 2012; Brasier *et al.* 2015). These sorts of results show just how important it is to continually test received wisdom, to refine search criteria, and to look for new ways to analyse old problems.

Developing a generalised null hypothesis

Guided by a null hypothesis in assessing evidence of early life, and armed with a set of rigorous criteria for distinguishing pseudo-fossils from the real thing, Martin applied this way of thinking to other major transitions in the history of life, including the origin of the eukaryotic cell (Brasier 2012), and the origin of animals and the Cambrian explosion of complex animal life (Brasier 2009). Palaeontologists working on the origin of animals face similar problems to those working on the earliest life, since putative early animal fossils are often difficult to recognise, may possess few diagnostic characters, and are typically found in environments with complex and non-uniformitarian geological and taphonomic histories. Many candidate early animal fossils are controversial, with multiple competing hypotheses to explain how they relate to modern organisms. When calibrated against an absolute timescale,

368 it is clear that claims for the earliest animals based on very controversial fossils (Seilacher et
369 al 1998) exceed double the length of the whole known certain fossil record of animals (Fig.
370 1). The same is true for single celled eukaryotes (Fig. 1). It is therefore extremely important
371 to determine what is certain to be true and can form the basis of a null hypothesis, from that
372 which is merely possibly true, when trying to understand the fossil record. This line of
373 thinking is evident in extensive reassessment of the sponge fossil record (Antcliffe *et al*.
374 2014), which retracted previous claims Martin had made about the oldest known sponge
375 spicules in the late Ediacaran of Mongolia (Brasier *et al*. 1997). He was fond of saying that
376 this was his own MOFAOTYOF principle moment, which was particularly sobering because
377 Brasier *et al*. (1997) was one his most highly cited works.

378

379 Throughout the Precambrian there is a general trend of increasing morphological complexity
380 in documented fossils (Knoll *et al*. 2006). However, as with simpler forms, Martin
381 championed the importance of maintaining an abiogenic "starting question" where the burden
382 of proof is on proving the presence of life. If an abiogenic origin can first be rejected, then a
383 prokaryotic affinity becomes the working model, with efforts progressively focused towards
384 demonstrating a higher (eukaryotic) affinity and then a multicellular eukaryotic affinity (Fig.
385 4; Antcliffe & McLoughlin 2008; Brasier 2015).

386 "*Where biogenicity of early fossils is less in question, the chosen null hypothesis may*
387 *then move upwards along a conceptual 'cone of contention' … Such a framework*
388 *requires the rejection of prokaryote (e.g. bacterial) affinities in the first place, and then*
389 *of simple eukaryote (e.g. protozoan and algal) affinities, before moving towards*
390 *acceptance of the much sought-after but highly controversial category of early*
391 *metazoan.*" Brasier (2015, p.10; see also Fig. 4 herein).

392

393 The Ediacaran fossil *Charnia,* known from sections in U.K., Canada, Australia and Russia
394 (e.g. Brasier *et al*. 2012), provides a good example (Figs 4–5). It is clearly not abiogenic
395 because of its consistent morphology across a variety of facies, environments and taphonomic
396 regimes. The maximum size that *Charnia* can achieve—approaching one metre in length—
397 makes a prokaryote affinity impossible given the consistent, replicated morphology that is not
398 facies controlled as it is for prokaryotic colonies such as stromatolites (Bertrand-Sarfati
399 1994). If we accept that *Charnia* has a growth programme more complex than anything a
400 prokaryote is known to achieve, then it is possible to reject the hypothesis of prokaryote
401 affinity and move on to the single celled eukaryote hypothesis. For *Charnia* the eukaryotic
402 null hypothesis (colonial or otherwise) proves very difficult to overcome, and no one has yet
403 demonstrated any compelling case to move beyond it to eukaryotic organisms with true
404 multi-cellularity, since there remains a large number of plausible eukaryotic clades that could
405 form such morphologies (Antcliffe & Hancy 2013). In this way, fossil morphology can be
406 interpreted by the iterative application of the null hypothesis, a process that represents a
407 theoretical structure within which to consider the affinities of any given fossil. It is a
408 powerful approach that can and should be applied to any fossil in deep time, which highlights
409 the limits of our certainty, and the point beyond which we would be breaking 'Brasier's
410 razor'.

411

412 **CONCLUSIONS**

413 The use of a null hypothesis for analysing complex problems is standard practice across the
414 biological sciences. The work of Martin Brasier, particularly through publications concerning
415 the earliest evidence of life on Earth (Brasier *et al*. 2002, 2006; Brasier 2015), formulated
416 rigorous hypotheses concerning Precambrian palaeontology and showed how they could help
417 us understand major events in the history of life.

To test palaeontological hypotheses one must have a series of criteria for the robust identification and analysis of ancient fossils. A palaeontologist must then test for these criteria in candidate fossils using all available investigative techniques to gather data. Martin Brasier developed guidelines for how palaeontological hypotheses should be tested in the Precambrian and in the search for extra-terrestrial life, and in doing so championed the use of many novel analytical techniques to gather the necessary data. Every enigmatic or contentious fossil can be approached in this way.

Martin's rigorous emphasis on application of the null hypothesis and cautious interpretation of ancient fossils went hand in hand with a freedom of thought, and a readiness to explore possibilities that led to creative new approaches to questions such as the environments in which we should seek evidence for the earliest life. Martin Brasier was hugely optimistic about what else we may yet find:

> "*Early geologists like James Hutton (1790) famously reported finding 'no vestige of a beginning'. In recent years, however, we have begun to obtain a much better understanding of the early Earth.... we may perceive a 'vestige of a beginning', ... but there is 'no prospect of an end', as yet, in terms of improving our understanding of the nature and evolution of the early biosphere*." Brasier *et al*. 2006.

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FIGURE CAPTIONS

Fig 1. The "envelope of uncertainty", which captures the time during which the major groups of life may have originated. The deep time end member represents the origin of the clade if the oldest claim from the fossil record is to be believed. The late arrivals end member represents the point in the fossil record by which this clade is certainly recognised and is uncontested in interpretation.

675 **Fig 2.** Examples of continua within apparently biological structures generated by abiogenic
676 processes that produce self-organising structures (SOS). These can arise naturally at
677 interfaces in physico-chemical systems. Symmetry is lost from left to right as morphological
678 complexity increases. Overall SOS size decreases down the figure. With stromatoloids (at a
679 and d) some scientists believe that complex forms (within the 'domain of biological
680 morphology') necessitate a biological component. But the full spectrum of forms is found in
681 both biological and abiological stromatoloid systems; both result from the physics of viscous
682 materials and neither result from behaviour of microbes alone. In well preserved confirmed
683 microfossil assemblages, the morphological variation is usually less than in co-occurring
684 abiogenic structures (at e–h) and so occupies a more restricted domain within the
685 morphospace (from Brasier *et al.* 2006).
686

687 **Fig 3.** Application of imaging techniques to the analysis of putative Precambrian
688 microfossils. (a) Type thin section of the 3.46 Ga Apex chert imaged using *Automontage*,
689 revealing complex branching (arrows) of a microstructure once interpreted to be a microfossil
690 (inset shows original sketch of the 'microfossil' from Schopf (1993)). (b) Light microscope
691 image of a second microfossil-like object from the Apex chert. (c) 3D model (from FIB-SEM
692 data) of the carbonaceous component of the microfossil-like object, shown in the same
693 orientation as (b). (d) 3D model rotated to show a clear branch (arrow) that is hidden from
694 view in the light microscope image. (e) Scanning TEM image of a cross section through a
695 microfossil-like object from the Apex chert. (f) Elemental map of the boxed area in (e) where
696 carbon is shown in yellow, aluminium (representing vermiculite clay) in green, and iron
697 (representing iron oxides) in red. This shows that the distribution of carbon is incompatible
698 with a chain of cells as previously claimed (Schopf 1993) and the object is actually a

699 pseudofossil created by carbon wrapping around mica-like minerals. Images modified from
700 Wacey *et al*. (2015).
701
702 **Fig 4.** A conceptual framework for the critical testing of early fossil claims using the 'cone of
703 contention' structure (from Brasier 2015, after Antcliffe and McLoughlin 2008). Fossils
704 expand in abundance as the fossil record proceeds and as more complex forms emerge. The
705 favoured null hypothesis will shift with time, with (*a*–*e*) illustrating some controversial fossil
706 candidates of progressively younger age. (*a*) Approximately 4.0 Ga prokaryote-like structures
707 from Mars (ALH 84001) (McKay *et al*. 1996) that were challenged by an abiogenic null
708 hypothesis (Schopf 1999); (*b*) 3.46 Ga *Archaeoscillatoriopsis disciformis* from the Apex
709 chert, compared with prokaryotes (Schopf 1999) and later challenged by an abiogenic null
710 hypothesis (Brasier *et al*. 2002); (*c*) 1.88 Ga complex microfossil *Eosphaera* from the
711 Gunflint chert, usually regarded as of problematic affinity (Barghoorn & Tyler 1965) but
712 suggested to be a eukaryote cell colony (Kazmierczak 1979); (*d*) approximately 600 Ma
713 complex microfossil *Megasphaera* from the Doushantuo Formation, whose stem-group
714 metazoan (animal) embryo interpretation (Xiao *et al.* 1998) has been challenged by a
715 prokaryote hypothesis (Bailey *et al*. 2007) and a protistan eukaryote hypothesis (Bengtson *et*
716 *al*. 2012); (*e*) 560 Ma complex megafossil *Charnia*, long regarded as cnidarian but whose
717 animal affinities have been questioned (Antcliffe & Brasier 2007); (*f*) the Carboniferous
718 crinoid whose biogenicity was at first considered moot by Lister (1673) but is now
719 interpreted as the remains of an extinct echinoderm (Brasier 2015). Scale bar is 1 μm (*a*),
720 40 μm (*b*), 10 μm (*c*, *d*), 30 mm (*e*).
721
722 **Fig 5.** A collection of drawings of Ediacaran macrofossils made by Martin Brasier in the
723 course of seeking to better understand the Ediacaran biota. Drawing the specimens in detail

724 provided detailed insights into their morphology. (a) *Vinlandia antecedens*, and (b) *Beothukis*
725 *mistakensis*, from the Conception Group of Newfoundland, Canada. (c) *Charniodiscus*
726 *concentricus* from the Bradgate Formation of Charnwood Forest, U.K. (d) *Dickinsonia*
727 *costata* from the Ediacara Member of the Rawnsley Quartzite, South Australia. (e)
728 *Kimberella quadrata*, a specimen from the White Sea, Russia. (f) *Swartpuntia germsi*,
729 Spitzkopf Member of the Nama Formation, Namibia. (g) *Charnia masoni* (holotype),
730 Bradgate Formation of Charnwood Forest, U.K. At lower right is a summary (*circa* 2004) of
731 the stratigraphic ranges of key Ediacaran taxa (ultimately published in modified form in
732 Brasier & Antcliffe 2004).